The Effect of Environmental Stress and Selective Glucocorticoid Receptor Modulators on Chicken and Human Leukaemia Cells

Israa Najm Abdullah Al-Ibadi

A thesis submitted to the University Of Salford in partial fulfilment of the requirements for the degree of Doctor of Philosophy in School of Environment & Life Sciences, College of Science & Technology, 2017

Word Count: 56, 135

Table of Contents

1 Introduction and aims 25
1.1 Introduction 25
1.1.1 Cancer 25
1.1.1.1 Cancer definition, prevalence and causes 25
1.1.1.2 Hallmarks of cancer 33
1.1.1.3 Cancer therapy 36
1.1.1.4 Blood Cancer 38
1.1.2 Leukaemia 43
1.1.2.1 Acute lymphoblastic leukaemia (ALL), symptoms, causes and treatment 44
1.1.2.2 Avian lymphoid leukosis 48
1.1.3 Glucocorticoid hormones 49
1.1.3.1 Hypothalamic-pituitary-adrenal axis 50
1.1.3.2 GCs uses in medicine 52
1.1.3.3 Glucocorticoid receptor (GR) 57
1.1.3.3.1 GR domain organisation 58
1.1.3.3.2 Transcriptional regulation by GR 60
1.1.3.3.3 Levels of control of the GR function 61
1.1.3.3.4 GR cofactors 66
1.1.3.3.5 GR post-translational modifications 67
1.1.3.3.6 GR phosphorylation 69
1.1.4 Cell death 73
1.1.4.1 GR role in control of apoptosis 79
1.1.5 Selective glucocorticoid receptor modulators or agonists: 82
1.1.5.1 Overview of SGRM/SGRA 82
1.1.5.1.1 Examples of SGRM 83
1.1.5.1.2 Compound A 87
1.1.5.1.3 Overview of non-steroidal compounds used in the study 94

1.2 Aims of project..96

2 Materials and Methods...98

2.1 Materials...98

2.1.1 Tissue culture reagents..98

2.1.2 Tested compounds, antibodies and kits used in the experiments............99

2.2 Methods..100

2.2.1 Cancer cell lines...100

2.2.1.1 Maintenance of the cells 101

2.2.1.2 Passaging of the cells 102

2.2.1.3 Counting of the cells 103

2.2.1.4 Freezing of the cells 103

2.2.1.5 Thawing of cells...104

2.2.2 Compounds used in the study..104

2.2.3 Proliferation assay (MTS or MTT) to measure cytotoxic effect of the drugs 105

2.2.4 Flow cytometry...106

2.2.4.1 Cell cycle progression analysis -Propidium Iodide staining 106

2.2.4.2 Apoptosis assay - Annexin V-FitC labelling 107

2.2.4.3 ROS measurement- DCFDA staining to measure intracellular ROS ...109

2.2.5 Immunoblotting assay..110

2.2.5.1 Cellular extract preparation and determination of protein concentration 110

2.2.5.2 Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS PAGE) and Western blotting 112

2.2.5.3 Western blot solutions and buffers 113

2.2.6 Quantification of relative gene expression...116

Human primers 116

2.2.6.1 Purification of Total RNA from the cells 117

2.2.6.2 qRT-PCR...120

2.2.7 Detection of nitric oxide by Griess reagent...122

2.2.8 IL-6 and IL-2 detection by ELISA assay...124

2.2.9 Caspase assay……………………………………………………………………………………….127

2.2.10 Ligand-protein binding *in-silico* simulation- Chimera docking……………………128

2.2.10 Drug stability determination……………………………………………………………….128

3 Results……………………………………………………………………………………………………130

3.1 Identification and preliminary characterization of novel compounds………….130

3.2 Ligand-protein binding- *in silico* assay…………………………………………………….132

3.3 Cytotoxicity of DEX and tested compounds…………………………………………….137

3.3.1 Cytotoxicity of tested compounds upon 72h incubation with 10µM to 100µM concentrations……………………………………………………………………………………….137

3.3.2 Cytotoxicity of tested compounds upon 48h incubation with 10µM to 100µM concentrations……………………………………………………………………………………….140

3.3.3 Cytotoxicity of tested compounds upon variable duration of treatment and 0 to 2.5 µM concentrations………………………………………………………………………………142

3.3.3.1 Cytotoxicity profiles of CEM-C7-14 cells……………………………………142

3.3.3.2 Cytotoxicity profiles of CEM-C1-15 cells……………………………………144

3.3.3.3 Cytotoxicity profiles of DT40 cells……………………………………………..145

3.3.3.4 Cytotoxicity of CPDB and C………………………………………………………..146

3.3.3.5 Cytotoxicity of tested compounds on peripheral blood mononuclear cells ……………………………………………………………………………………………….148

3.3.4 Effect of temperature stress on drug response in ALL and PBMCs…………150

3.3.5 Compound cytotoxicity in cancer and non-cancer epithelial cells…………154

3.4 Flow cytometric analysis of cellular effects of studied compounds ……….. ……156

3.4.1 Effects of tested compounds on cell cycle progression………………………..156

3.4.1.1 Effect of tested compounds on CEM-C7-14 cell cycle distribution 156

3.4.1.2 Effect of tested compounds on CEM-C1-15 cell cycle distribution 158

3.4.1.3 Effect of tested compounds on DT40 cell cycle distribution 159

3.4.1.4 Effect of tested compounds on cell cycle distribution of PBMCs.......161

3.4.2 Induction of apoptosis of the leukaemia cells treated with studied compounds……………………………………………………………………………………………….162

3.4.3 Effect of studied compounds on reactive oxygen species (ROS) levels…..165

3.5 Caspase-8- enzyme modulation by tested compounds 170
3.6 Human and chicken proteins similarity..171
3.7 Gene and protein expression analysis 172
3.7.1 Analysis of gene expression and protein levels in CEM-C7-14 cells...........173
3.7.2 Analysis of gene expression and protein levels in CEM-C1-15 cells...........177
3.7.3 Analysis of gene expression and protein levels in DT40 cells.....................180
3.8 Regulation of inflammatory mediators by steroid hormone andthe tested compounds..183
3.8.1 Griess Nitric oxide test...183
3.8.2 Secreted Interleukine 6 modulation...186
3.8.3 Secreted Interleukine 2 modulation...188
3.8.4 Regulation of interleukin genes by activated GR upon tested compounds 190
4 Discussion, conclusions and future work...193
4.1.1 Cytotoxic effects of studied compounds..193
4.1.2 Effects of studied compounds on inflammatory process...........................202
4.1.3 Molecular analysis of investigated agents interactions...............................205
4.2 Conclusions...208
4.3 Future work 211
4.4 References...214
4.5 Supplementary material...247
4.5.1 NMR Spectra...247
4.5.1.1 Dexamethasone 247
4.5.1.2 Compound A 248
4.5.1.3 Tyramine 249
4.5.1.4 Tyramine Hydrochloride 250
4.5.1.5 N-Methyl Tyramine (CPDB) 251
4.5.1.6 Hordenine (CPDC)...252
4.5.2 Quantitative analysis of of apoptosis..253

4.5.3 Western blot images.....254
4.5.3.1 CEM-C7-14.....254
4.5.3.2 CEM-C1-15.....255
4.5.3.3 DT40 Cells.....256
4.5.4 Plots of one selected image of each cell line.....257
4.5.5 Quantifications of individual western blots for each cell line.....258
4.5.6 Blast results.....260
4.5.6.1 GR.....260
4.5.6.2 Actin.....261
4.5.6.3 C-JUN.....262
4.5.6.4 IL-6.....263
4.5.6.5 IL-2.....264

Table of figures

Figure 1 Five years relative survival rates in adolescent cancer suferers in the USA.......26

Figure 2 Cancer survival index for adults patients during (2010-2011) in the United Kingdom. Adapted from Cancer Research UK (2017)b...27

Figure 3 Incidence of childhood cancer in the UK, adapted and modified from Children with cancer UK b(2017)...28

Figure 4 Childhood cancer survival rates in the United States of America adapted from National Cancer Institute at the National Institutes of Health (2017)b. 29

Figure 5 Cancer incidence in the Europe area, Adapted and modified from Cancer Research UK (2017)c. 31

Figure 6 Childhood cancers incidence in Canada, adapted and modified from Ellison & Janz (2015). 32

Figure 7 New blood cancer cases in the United States of America, adaptated from leukemia and lymphoma society, (2017)...38

Figure 8 An estimation of blood cancers in Canada, adapted from the Leukemia & Lymphoma Society of Canada, 2017...39

Figure 9 Children's cancers incidence in the UK in boys and girls. Adapted from Cancer Research UK (2017)c. 40

Figure 10 Haematopoiesis process. 42

Figure 11 Schematic illustration of glucocorticoid (steroid) synthesis in adrenal gland...49

Figure 12 Hypothalamic-pituitary -adrenal axis (HPA). 52

Figure 13 Chemical structures of tested compounds..55

Figure 14 Genomic structure of GR alpha and GR amino acid sequence..........................59

Figure 15 Mode of GR 61

Figure 16 GR Signalling...64

Figure 17 Illustration of the main posttranslational modifications of Glucocorticoid receptor alpha and their corresponding domain location (adapted and modified from Kino (2010)..70

Figure 18 Cell death scheme and main routes 74

Figure 19 Main pathways of apoptosis....77

Figure 20 Caspase independent cell death.....78

Figure 21 Proposed SEGRM mode of action. The figure adapted from Cheng et al. (2014) 90

Figure 22 Chemical structure of tested compounds. 95

Figure 23 Example of the cell cycle profile 107

Figure 24 Diagram of AnnexinV assay workflow 109

Figure 25 Flow cytometry setting for discrimination of apoptotic cells from dead and alive cells....109

Figure 26 Diagram represents process of RNA extraction procedure. 120

Figure 27 The principle of Griess assay....123

Figure 28 Principle of ELISA assay....124

Figure 29 Cartoon illustration of ELISA assay followed. Procedure carried out to quantify levels of secreted interleukins in media of cells treated with the explored drugs. 126

Figure 30 Structures of tested compounds. 130

Figure 31 Auto dock simulation of studied compounds. 134

Figure 32 Ligand - GR interactions. 135

Figure 33 Effect of high drug doses and 72 h treatment duration on viability of examined cells. 139

Figure 34 Effect of high compound doses for 48 h on viability of examined cells. 141

Figure 35 The effect of duration and different doses of drug treatment on CEM-C7-14 viability. 143

Figure 36 The effect of duration and different doses of drug treatment on CEM- C1-15 viability. 144

Figure 37 The effect of duration and different doses of drug treatment on DT40 cells viability. 145

Figure 38 Cytotoxicity analysis of CPDB and CPDC in CEM-C7-14, CEM-C1-15 and DT40 Cells. 147

Figure 39 Cytotoxicity of tested compounds on PBMCs. 149

Figure 40 Cytotoxicity profile of combined high temperature and drug treatment on examined cells..151

Figure 41 Cytotoxicity profile of combined low temperature and drug treatments on examined cells..153

Figure 42 Cytotoxicity of tested compounds in skin and breast malignant cells and non-malignant bronchial cell line. 155

Figure 43 Cell cycle analysis of CEM-C7-14..157

Figure 44 Cell cycle distributions of CEM-C1-15 cells. 159

Figure 45 Cell cycle distributions of DT40 cells...160

Figure 46 Cell cycle distribution of PBMCs. 162

Figure 47 Representative Apoptotic profiles of CEM-C7-14 cell as measured by flow cytometry with AnnexinV-FITC labelled assay...163

Figure 48 Representative Apoptotic profiles of CEM-C1-15 cell as measured by flow cytometry with AnnexinV-FITC labelled assay. 164

Figure 49 Correlation of ROS and RNS in cancer and the impact of their high levels on cell fate, cell growth and genes. Adapted from(Benedetti et al., 2015). 165

Figure 50 Cross talk between ROS level and its adverse effects. Adapted from (Cairns et al., 2011). 166

Figure 51 Flow cytometric analysis of DCFDA fluorescence intensity. 167

Figure 52 Flow cytometry setting of DCFDA staining. 169

Figure 53 Caspase-8 assay. 171

Figure 54 Relative mRNA levels of glucocorticoid receptor target genes in CEM-C7-14 cells. 174

Figure 55 Proteins expression levels in CEM-C7-14 cells..176

Figure 56 Relative mRNA levels of studied genes in CEM-C1-15 cells............................178

Figure 57 Expression levels of studied proteins in CEM-C1-15 cells. 179

Figure 58 Relative mRNA levels of studied genes in DT40 cells. 181

Figure 59 Expression levels of studied proteins in in DT40 cells. 182

Figure 60 Nitric oxide (NO) actions in cancer cell death. 184

Figure 61 Nitric oxide levels upon tested compounds treatment in leukaemia cells. 185

Figure 62 IL-6 levels in CEM-C7-14, CEM-C1-15, DT40 and PBMC cells. 187

Figure 63 IL-2 levels in CEM-C7-14, CEM-C1-15, DT40 and PBMC cells. 189

Figure 64 Quantitative analysis of GR target interleukins gene expression...................192

List of tables

Table 1 The hallmarks of cancer and their representative example cancer 34

Table 2 End sites of metastasis. Available in Carr (2008) 34

Table 3 Examples of cancers and therapeutic approaches. 36

Table 4 Therpeutic agents used in each phase of ALL therapy. 46

Table 5 Common side effects of GCs tratment...56

Table 6 Examples of GR affected genes 65

Table 7 Examples of GR post-translational modifications 71

Table 8 GR effect on BH3-family members 81

Table 9 The known SGRM 85

Table 10 Reported effects of compound A. 93

Table 11 Tissue culture reagents 98

Table 12 Tested compounds, antibodies and kits used in the experiments 99

Table 13 List of the compounds used in the experiments, their molecular weight and the solvents used for in vitro assays 104

Table 14 High salt lysis buffer 113

Table 15 Gel for SDS -Page..114

Table 16 Running and Transfer buffers 114

Table 17 3xSDS Sample buffer 115

Table 18 Human primers...116

Table 19 Chicken primers...117

Table 20 Contents of RNeasy plus Mini kit 118

Table 21 Components of RT-PCR reaction / well setting for RT-PCR 121

Table 22 Amplication protocol. Adapted from primer design.......................................121

Table 23 Components of Griess Reagent..123

Table 24 ELISA kit components..125

Table 25 Solutions used for NMR experiments 129

Table 26 Stability of tested compounds upon various conditions. For details see supplementary data (4.5.1) 132

Table 27 Highest Binding affinities of compounds docked with GR-LBD 135

Table 28 Summary of interactions between tested compounds and GR-LBD 136

Table 29 Human and chicken proteins similarity determined using NCBI Blast tool. 172

Acknowledgment

First of all I would like to acknowledge my government, Ministry of higher education and scientific Research/Republic of Iraq, for believing in me and providing me with this scholarship and all the funding and support that has come with it. Next, I would like to express my sincere thanks to the following people, without whose kind, support, advice and assistance this thesis would not have got off the ground successfully.

Professor Marija Krstic-Demonacos for being my supervisor and for have given me time and logistic supportive whenever I have needed it and to Dr. Constantinos Demonacos for providing me with invaluable guidance, moral support throughout my study,

Dr. Athar Aziz for practical training to flowcytometry, data analysis and for being helpful all the time, Dr. John Hadfield for solid opinion and contribution in chemistry part of the study.

Special thanks to Priyanka Panwar for help in molecular docking and Amjad for a big help in NMR experiments. My gratitude goes to the following PhD students: Basma Talib, Ahmed Azzawi, Basima, Emyr, May Rajab, Sangkab, Hasan, Andrew, Natalie, Arvin, Kubrat, Chrow also Suran and Kamila …you have been good company.

My heartfelt thanks goes to my parent who I haven't seen throughout my study, my mum and dad who have taught me the ambition, for their endless passion and prayers; my brothers (Dr. Ali, Mr Hussein and Mr. Ahmed) and sisters (Dr. Zaineb and Mrs Shaimaa) for their unconditional love and encouragement throughout my life. I also extend my thanks to my colleagues in college of veterinary medicine and surgery/ Al-Qadisiya University/IRQ (Dr. Saffia) for her ongoing support and to my long-life friend Dr.Raafat for being my loyal friend.

Keeping the best to the end, great thanks and continuous love goes to my husband Dr. Shawkat for his huge support and patience along the journey, for kindness and love; and to my special kids; Abbas, Zaid for bearing with me throughout my study, and for my little son Abdullah for keep telling me (Mum you are my best friend), I would like to say sorry if I did not give you enough attention at this hard time, and finally, I thank everyone who has helped me in word or action. Thank you.

Dedication

I dedicated this thesis to:

Mum… whose her eyes guard me…. and her voice guides me.

She has believed in me and let me travel with my dream….

I can only see her face wherever I go, like a fairy she's always with me…

Her spirit has enlightened me….

Cheer up mum; this is kind gift from me.

Israa

Declaration

I hereby certify that Iam the sole author of this thesis and this thesis is my own work and effort. All information in this document has been obtained and presented in accordance with academic rules and ethical conduct and I have fully cited and referenced all material and results that are not original to this work. Neither any part of this thesis nor the thesis has been submitted for a degree to any other university or institution for any award. I declare this is a true copy of my thesis including any final revisions as approved by my thesis review committee.

Israa Najm Abdullah Al-Ibadi

Abbreviations

11b-HSD	11-beta hydroxysteroid dehydrogenase 2
ACT	Activator of thyroid hormone and retinoid receptors
ACTH	Adrenocorticotropic hormone
ACTR	Activator of thyroid hormone and retinoid receptors
AD1	Activation domain 1
AF-2	Activation function-2
AF1	Activation function-1
AKT	Protein kinase B (PKB)
ALL	Acute lymphoblastic leukemia
ALV	Avian leukosis virus
AML	Acute myeloid leukemia
Ang-1	Angiopoeitin-1
AP-1	Activator protein 1
APAF-1	Apoptosis protease activating factor
APK	Acylglycerol kinase
APR	Adverse psychological reactions
APS	Ammonium per sulphate
ARC	Activated recruited cofactor
ARC	Human activator-recruited cofactor
ARF	Alternate reading frame
ASH	American Society of Hematology
ATF1	Activating transcription factor 1
ATF2	Activating transcription factor 2
ATM	Ataxia telangiectasia mutated
ATP	Adenosine triphosphate
ATR	Ataxia telangiectasia RAD 3 related
AURKA	Aurora Kinase A
B-cell	CLL/lymphoma 2 (BCL2)
BAD	BCL2-associated agonist of cell death
Bak	Bcl-2 homologous antagonist killer
BAX	BCL2 associated X, apoptosis regulator
BCL-2	B-cell lymphoma 2
BCL-xL	B-cell lymphoma-extra large
BER	Base excision repair
BET	Betamethasone
BH-3	Bcl-2-homology domain 3
BIC	Bicaudal
BID	BH3 interacting-domain death agonist
Bik	Bcl-2 interacting killer
Bim	Bcl-2-interacting mediator of cell death
BIR	Baculovirus inhibitor repeat
BMDC	Bone marrow-derived dendritic cells
BMSC	Bone marrow stromal cells

BOX1	Bone living cells
BRCA1	Breast cancer type 1 susceptibility protein
BRCA2	Breast cancer type 2 susceptibility protein
BSA	Bovine serum albumin
c-fos	Fos proto-oncogene
c-jun	Jun Proto-Oncogene
C-MYC	v-myc avian myelocytomatosis viral oncogene homologue
C/EBP	CCAAT/enhancer-binding protein
CAP	adenylate cyclase associated protein 1
Caspase	Cysteine-dependent aspartate specific protease
CBG	Corticosteroid-binding globulin
CBP	CREB-binding protein or CREBP
Cdk2	Cyclin E/cyclin-dependent kinase 2
CDKI	Cyclin dependant kinase inhibitors
CDKs	Cyclin dependent kinases
cDNA	Complementary DNA
ChIP	Chromatin immunoprecipitation
Chk1	Checkpoint Kinase 1
Chk2	Checkpoint Kinase 2
CKIs	Cyclin-dependent kinase inhibitor protein (CDIs or CDKIs)
CLL	Chronic lymphocytic leukaemia
CML	Chronic myeloid leukaemia
CNS	Central nervous system
CRD	C-terminal regulatory domain
CREB	cAMP response element-binding protein
CRH /CRF	Corticotrophin-releasing hormone (factor)
CXCL12	CXC-chemokine ligand 12
Cyp44	Cytochrome P450 monooxygenase 44
Cyt c	Cytochrome c
DAU	Daunorubicin
DBD	DNA-binding domain
DCC	Dextran coated charcoal
DD	Death domain
Dex	Dexamethasone
DISC1	Disrupted in schizophrenia 1
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	Dimethylsulphoxide
DNA	Deoxyribonucleic acid
DNA-PK	DNA protein kinase
DOX	Doxorubicin
dsDNA	Double stranded DNA
DTT	Dithiothreitol
DUB	Deubiquitination enzymes
DYRK	Dualspecificity tyrosine-phosphorylation-regulated kinase
E2F	Transcription factor
EAN	Experimental autoimmune neuritis
EDTA	Ethylenediaminetetraacetic acid

EGF	Epidermal growth factor
ELK1	ETS transcription factor
EPI	Epirubicin
ERK	Extracellular regulated MAP kinase
ERK	Extracellular signal-regulated kinase
ERRB2	V-erb-b2 erythoblastic leukaemia viral oncogene homolog2
Ets-1	ETS proto-oncogene 1, transcription facto
FACS	Fluorescence activated cell sorting
FADD	Fas-associated death domain protein
FAS	Fas cell surface death receptor
FCS	Foetal calf serum
Fdr	False discovery rate
FGF2	Fibroblast growth factor 2
FITC	Fluorescein isothiocyanate
FKBP4	FK506-binding protein 4
FKBP5	FK506 binding protein 5
FKBP52	FK506 binding protein 4
G-CSF	Granulocyte colony-stimulating factor
G1	Gap 1
G2	Gap 2
GADD45	Growth arrest and DNA damageinducible
GATA	GatA Glutamyl-tRNA amidotransferase, subunit A
GATA3	GATA binding protein 3
GCs	Glucocorticoids
Gilz	Glucocorticoid-induced leucine zipper
GM	Glioblastoma multiform
GM-CSF	Granulocyte-macrophage colony stimulating factor
GPCRKs	G protein-coupled receptor kinases
GR	Glucocorticoid receptor
GRE	Glucocorticoid response elements
GRIP 1	Glucocorticoid receptor interacting protein 1
GSH	Glutathione
GSK-3	Glycogen synthase kinase 3
GSK-3b	Glycogen synthase kinase 3 beta
GSTM1	Glutathione S-transferase M1
HAT	Histone acetyltransferase
HAUS	Herpes virus associated USP
HAD	Histone deacetylase--
HDAC	Histone deacetylases
HDAC6	Histone deacetylase 6
HDACs	Histone deacetylases
HDM2	Human Double Minute2
HIFI	Hypoxia inducible transcription factor
HPA	Hypothalamus–pituitary-adrenal axis
HPC	Hematopoietic progenitor cell
HSCs	Hematopoietic stem cells
HSD	Hydroxy steroid Dehydrogenase

HSLB	High Salt Lysis buffer
HSP	Heat shock proteins
HSP 23	Heat shock protein 23
HSP70	Heat shock protein 70
HSP90	Heat shock proteins
HTLV-1	Human T-cell leukaemia virus type 1
HTLV-2	Human T-cell leukaemia virus type 2
I-κB	Inhibitor of NK-κB
IAP	Inhibitor of apoptosis
IGF1	Insulin like growth factor
IL	Interleukine
IL-10	Interleukin 10
IL-8	Interleukine 8
IL1	Interleukine 1
IR	Infrared
IRF3	Interferon regulatory factor 3
JAK/STAT	The Janus kinase/signal transducers
JN	C-Jun N-terminal kinase
JNKs	c-Jun N-terminal kinases
LBD	Ligand-binding domain
LC3	Microtubule-associated protein 1A/1B-light chain 3
LF	Leukaemia foundation
LFA-1	Leukocyte function antigen-1
LHSCs	long term hematopoietic stem cells
LP	lymphoid progenitor
LPS	Lipopolysaccharide
Lys	Lysine
M	Mitosis phase
MAC	Mitochondrial outer membrane
MA	Microtubule associated protein
MAPKK	MAPK kinase
MAPKKK	MAPK kinase kinase
MAPKs	Mitogen-Activated Protein Kinases
MCL-1	Multi-domain BCL2 family members
Mcl-1	Myeloid cell leukaemia-1
MDC	Monodansylcadaverine
MDM2	Mouse double minute 2 homolog
MDMX	Murine Double Minute X
MEF2C	Myocyte enhancing factor-2c
MIT	Mitoxantrone
MLKL	mixed lineage kinase domain like pseudokinase
MM	Multiple myeloma
MMP-2	Matrix metalloproteinase-2
MMP-9	Matrix metalloproteinase-9
MMR	Mismatch repair mRNA Messenger RNA
MP	Myeloid progenitor
MPR	Methylprednisolone

MPSC	Multipotent stem cells
mRNA	Messenger Ribonucleic acid
MSCs	Mesenchymal stem cells
MYC	V-myc- myleocytomatosis viral oncogene homolog
N-CoR	Nuclear receptor corepressor 1
NCBI	National Centre for Biotechnology Information
NCI	National Cancer Institute
Nec-1	Necrostatin-1
NES	Nuclear export signals
NF- B	Nuclear factor-kB
NFAT	Nuclear factor of activated T-cells
NFKBIB	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, beta
nGREs	Negative glucocorticoid response elements
NHS	National Health Service
NIH	National Institutes of Health
NK	Natural killer cell
NL1	Nuclear localisation 1
NL2	Nuclear localisation 2
NLS	Nuclear localization signal
NO	Nitric oxide
NR3C1	Nuclear receptor subfamily 3, group C, member 1
NTD	N terminal domain
OCN	Osteoblast late marker osteocalcin
OH	Hydroxylase
P27	Protein 27
p38	MAPK p38 mitogen-activated protein kinases
PBS	Phosphate buffer saline
PCA	Principal components analysis
PCAF	P300/CBP associated factor
PCD	Programmed cell death
Pcd4	Programmed Cell Death 4
PCNA	Proliferating cell nuclear antigen
PE	Phosphatidylethanolamine
PH	Pleckstrin homology
PI	Propidium iodide
PI	Protease inhibitor
PI3K	Phosphatidyl inositol 3-kinase
PIP2	Phosphatidylinositol 4, 5-biphosphate
PIP3	Phosphatidylinositol 3,4,5-triphosphate
PIR	Pirarubicin
PKB	Protein Kinase B
PP5	Protein phosphatase 5
PPID	Peptidylprolyl isomerase D
PPP5C	Protein phosphatase 5 protein
PD-1	Programmed cell death 1
PS	Phosphotidylserine

PTBE	Peritumoral brain edema
PTEN	Phosphatase and tensin homolog
PTMs	Post translational modifications
qRT-PCR	Quantitative real time polymerase chain reaction
Rad3	Related Bad Bcl-2 antagonist of cell death
RIP1	Receptor interacting serine/threonine kinase 1
RIP3	Receptor interacting serine/threonine kinase 1
RN	Ribonucleic acid
RNA pol II	RNA polymerase-II
ROS	Reactive oxygen species
Rpl19	Ribosomal protein L19
Rpm	Round per minute
RT	Room temperature
RUNX3	Runt-related transcription factor 3
S phase	DNAsynthesis
S203	Serine 203
SCF	Stem cell factor
SCT	Stem cells transplantation
SDF-1	Stromal cell-derived factor 1
SDS	Sodium dodecyl sulphate
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SEGRA	Selective glucocorticoid receptor agonist/activator
SGK1	Serum/glucocorticoid regulated kinase 1
SMAC	Second mitochondria derived activator of caspase
SMRT	Silencing mediator of retinoid and thyroid hormone receptor
SNS	Sympathetic nervous system
SRC1	Steroid receptor coactivator-1
SRF	Serum response factor
STAT5	Signal transducer and activator of transcription 5
STIP1	Stress-induced-phosphoprotein 1
SUMO	Small ubiquitin-related modifier1
SUMO1	Small Ubiquitin Related Modifier-1
T-ALL	T-Acute Lymphoblastic Leukemia
T-bet	TBX21 (TBET) T-box 21
TAD	Transcription activation domain
TAFS	TBP-associated factor
TAT3	Tyrosine aminotransferase 3
tBID	BH3 interacting domain death agonist
TBP	Tata box-binding protein
TBS	Tris buffered saline
TCR	T cell antigen receptor
TEMED	Tetra methyl ethylene diamine
TFIIA	Transcription factor two A
TFIIB	Transcription Factor two B
TFIIE	Transcription factor two IE
TFIIF	Transcription factor two F
TFIIH	Transcription factor two Human

Th-1	T-helper cell 1
TIF2	Transcriptional mediators/intermediary factor 2
TNF	Tumor necrosis factor
TNF- α	Tumor necrosis factor- alpha
TNF-R	Tumor necrosis factor receptor
TRADD	TNF receptor-associated death domain
TRADD	TNFRSF1A associated via death domain
TRAP	Thyroid hormone receptor associated protein
TXNIP	Thioredoxin-interacting protein
Ubc9	Ubiquitin-conjugating enzyme E2I
UBCs	E2 ubiquitin-conjugating enzymes
UV	Ultraviolet
VDAC	Voltage-dependent anion channel
VLA-4	Very late antigen-4
WBC	White blood cells
WCR	World Cancer Research Fund International
WHO	World Health Organization
β-GP	β-Glycerol phosphate

Abstract

Glucocorticoids (GCs) play important functions in human physiology, and are commonly prescribed anti-inflammatory and immunosuppressive drugs. GCs are used in treatment of childhood acute lymphocytic leukemia (ALL), however resistance to therapy and side effects highlight the need for further research.

Glucocorticoids exert their function through binding to intracellular protein glucocorticoid receptor (GR). It is believed that the desired apoptotic effect on cancer cells and anti-inflammatory properties of GCs are due to the GR's mediated trans-repression function, and that genes positively regulated by GR may mediate unwanted GCs effects. Thus, this study aimed to investigate compounds that would potentially dissociate transcriptional activation from repression, minimize the side effects and GC resistance, towards improving childhood leukemia therapy.

The recently developed selective GR modulator (SGRM) Compound A (CPDA) and synthetic GC dexamethasone (DEX) were used together with two "single ring" organic compounds; Tyramine (T) and Tyramine hydrochloride (THCl), as well as Compound B and Compound C, to assess their cytotoxic and anti-inflammatory effects. Molecular modelling has indicated that these compounds contact several residues similar to classical GCs. DEX, CPDA, T and THCL all show cytotoxic effect on GC sensitive and GC resistant ALL CEM-C7-14 and CEM-C1-15 cell lines respectively, as well as chicken derived leukemia cells DT40. Compound B and C showed growth stimulatory effects and were not studied further. Leukaemia cells proliferation was mostly inhibited by high

doses and long incubation time, whereas combination of compound treatment with either high or low temperature interfered with this effect. All compounds had marginal growth inhibitory effect on proliferation of normal lung bronchial cells Beas-2b and MCF-C7, whereas T and THCL showed some stimulatory effect on HACAT cells proliferation. Compounds exerted selective and differential effects on cell cycle progression, apoptosis and caspase-8 enzyme activation. Normal peripheral blood mononuclear cells (PBMCs) were used to examine the cytotoxic effect on normal leukocytes. PBMCs were not significantly affected suggesting that tested compounds don't have the growth suppressive effect on normal peripheral white blood cells. Cell type specific, anti-inflammatory action of studied compounds was measured by ROS, nitrite and cytokine production analysis. Evaluation of secretory cytokines IL-6 and IL-2 by ELISA has shown a cell specific regulation of these biomarkers of inflammation. Protein and gene expression of GR target genes and resistance markers was regulated in a drug and cell dependent manner.

These data provided evidence of CPDA, T and THCL capability to inhibit leukemia cells proliferation and alter selected GR target genes expression. Thereby, these compounds show promising characteristics for drug development aiming to potential use in treatment of leukemia and inflammatory conditions.

1 Introduction and aims

1.1 Introduction

1.1.1 Cancer

1.1.1.1 Cancer definition, prevalence and causes

Cancer is over growth of cells and deregulation of the normal mechanisms of cell cycle control leading to formation of solid tumours or leukaemia. Cancer is also characterised by occurrence of metastasis in which the malignant cancer spreads to another area of the body. Tumour formation and metastasis are often associated with the disruption of function of the neighbouring organs and tissues as well as inflammation (Dikaios et al., 2017, Houssami et al., 2017, Palumbo and Russo, 2017, Quidde et al., 2017, Veglia and Gabrilovich, 2017).

The number of people diagnosed with cancer has increased in last 6 years while cancer mortality rates have decreased according to Siegel et al. (2017). Moreover, the five years survival rates have increased in adolescent (15-19) years old cancer sufferers in United States of America's most likely due to developed diagnostic and cancer therapies as seen in the data published by American cancer society (Fig. 1).

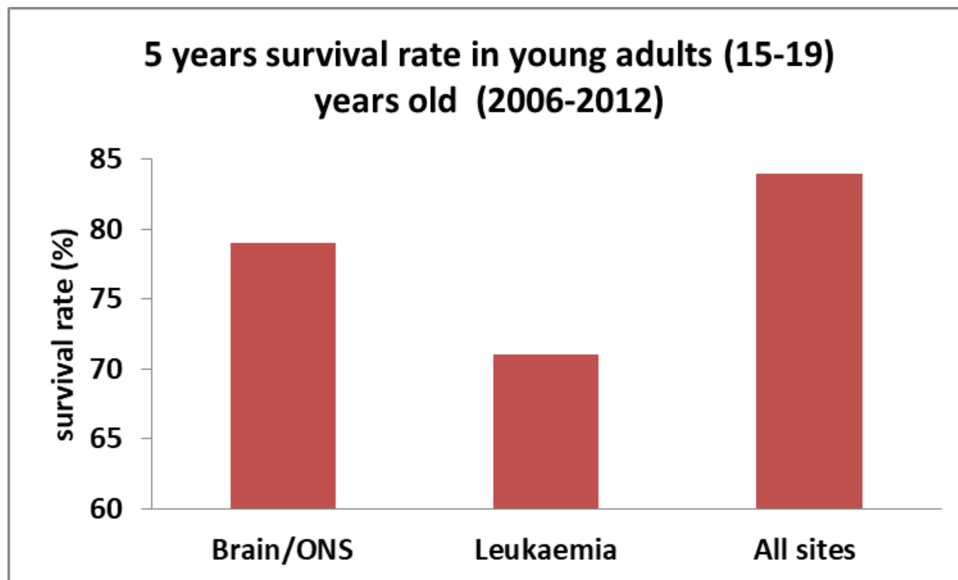


Figure 1 Five years relative survival rates in adolescent cancer suferers in the USA

ONS=other nervous system, adapted and modified from American Cancer Society (2017)

National Institute of Health NIH (2017)a , data revealed there were 356,860 cancer cases and 163,444 cancer related deaths. Cancer occurred in 15,780 people below age of 19, resulting in 1,960 deaths. In the United Kingdom (England and Wales) the cancer survival rates in adults patients (15-99) years old as stated by Cancer Research UK, (2017)a reveal that ten years survival rate was 50%,five years survival rate was 54% and one year survival rate was 70% for all cancer types. The highest survival rates were observed in testis cancers, malignant melanoma, breast cancer, prostate cancer Hodgkin lymphoma and uterus cancer while the low survival rates are recorded in oesophagus, stomach, brain, lung and the lowest survival was reported for pancreas cancer as indicated in bar chart shown in fig (2)

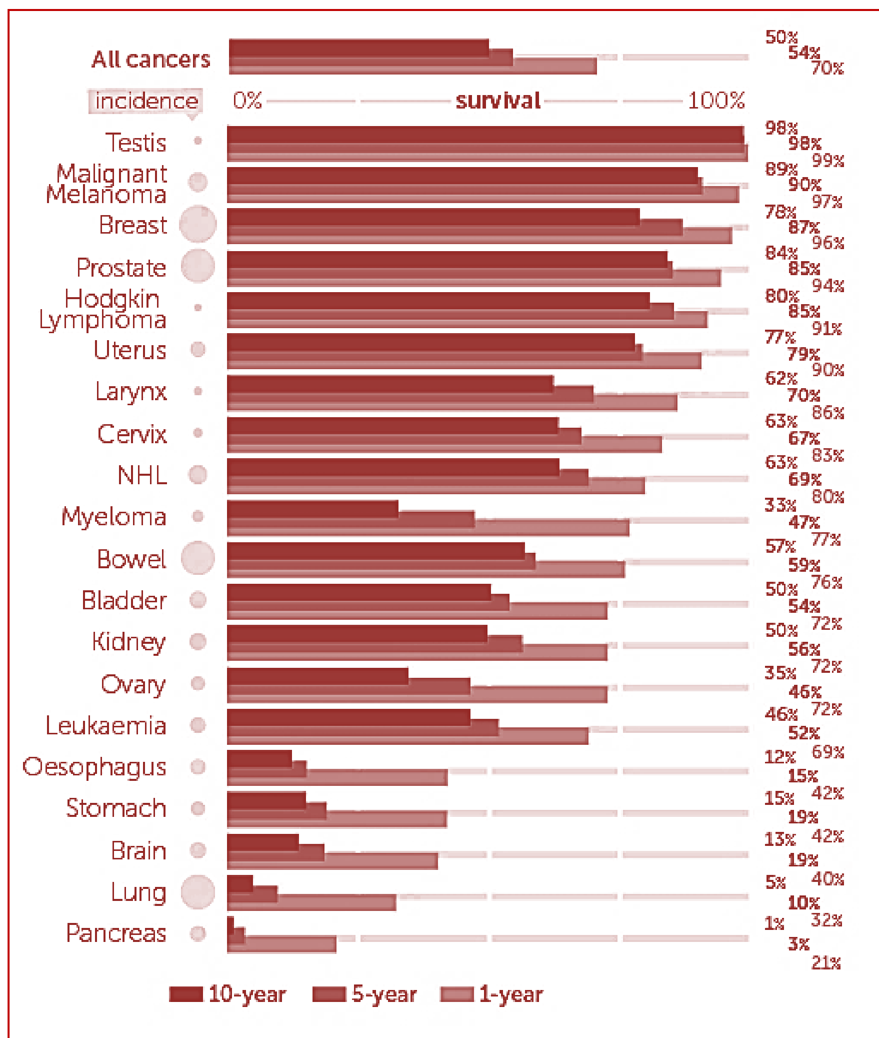


Figure 2 Cancer survival index for adults patients during (2010-2011) in the United Kingdom. Adapted from Cancer Research UK (2017)b.

Pediatric cancers have specific incidence and cure rates. Paediatric leukemia has the highest incidence when compared with other cancers that have been diagnosed in children (0-14) years old, in the United Kingdom, followed by tumours of central nervous system while hepatic tumours incidence is very rare according to the data published by charity Children with Cancer UK, 2017 as shown in fig (3) for the indicated time from 2001 to 2010.

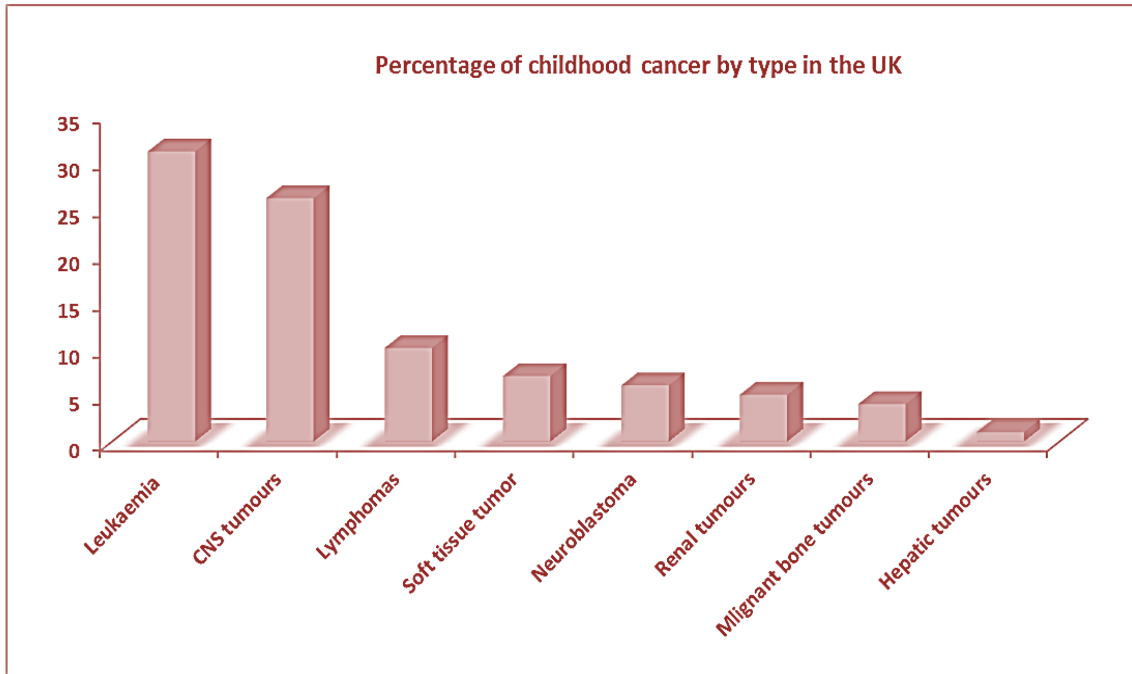


Figure 3 Incidence of childhood cancer in the UK, adapted and modified from Children with cancer UK b(2017).

On the other hand, the five years survival rate of childhood cancer in the United States of America generally refer to a promising survival rates in particular for Hodgkin lymphoma, Wilms tumours and acute lymphoblastic leukemia, however the average survival rates are higher than 50% as shown in Fig.4.

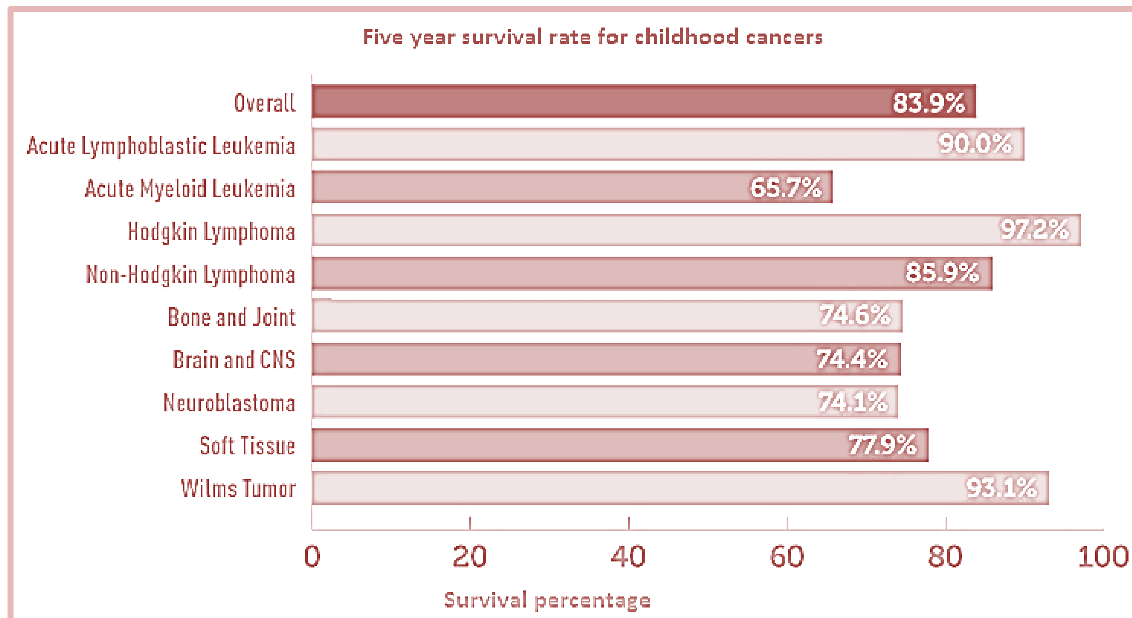


Figure 4 Childhood cancer survival rates in the United States of America adapted from National Cancer Institute at the National Institutes of Health (2017)b.

It has been found that cancer is substantial cause of mortality and causes similar numbers of death as heart disease in US. 8.8 million patients globally died from cancer in 2015, furthermore, cancer is found to be the cause of one per six mortalities across the world. Generally, poor population are at higher risk of cancer due to poor health and environmental conditions, weak immunity, dietary imbalance, pollution and lack of awareness. However the above mentioned numbers are mostly estimates as they are published sporadically for each region (Morris et al., 2016), however, they provide information on the progress of the disease. Leading factors of cancer are either endogenous (inherited) or exposure to exogenous carcinogens (external). External factors can cause gene mutations/epigenetic changes and may include; alcohol, tobacco, radiation (such as direct sun light), chemical carcinogens (heavy metals) and nutrients (aflatoxin contamination), infections (viral or fungal), chronic inflammation and another

factors. Some cancers are caused by external factors however several genetic factors that cause increased susceptibility to cancer have been identified.
In addition to these causes, cancer therapy and even diagnostic approaches can sometime cause cancer. It is believed that paediatric computed tomography (CT) scan exposures can predispose the children to subsequent risk of central nervous system (CNS) tumours and leukaemia (Journy et al., 2016). Chemical therapies predispose some cancer patients to the risk of cardiovascular toxicity as suggested for systemic 5-fluorouracil or capecitabine (Polk et al., 2013).

The suspected causes of cancer can be either genetic or external. Genetic factors are due to inherited genetic make-up, for example mutations in specific genes (BRCA1 or BRCA2) that correlate with a susceptibility to breast cancer. External factors can be due to exposure to radiation, chemical and biological carcinogens. Radiation can be non-ionizing ultraviolet or ionizing radiation, whereas chemical agents include a wide range of toxic chemical substances such as asbestos, tobacco, aflatoxin and arsenic (Pontes, 2017, Palumbo and Russo, 2017, Ozer and Sezerman, 2017, Houssami et al., 2017, Aydin et al., 2017, Yang et al., 2016, Wang et al., 2016b, Schache et al., 2016, Cheng et al., 2016) . Finally biological factors are infection with oncogenic viruses such Human T-cell leukaemia virus type 1 (HTLV-1) and Human T-cell leukaemia virus type 2 (HTLV-2) (Lairmore and Montgomery, 2005), also bacteria and parasites can have carcinogenic effect. Nutrition and exercise play an important role in prevention of cancer development in children; cancer is the number one cause of death by disease. In fact, it is responsible for more deaths than all other diseases in all ages according to World

Cancer Research Foundation WCRF (2017). In addition, the incidence rate of pediatric cancers which exceed 100 occurrences per million in Europe is shown in (fig 5).

.

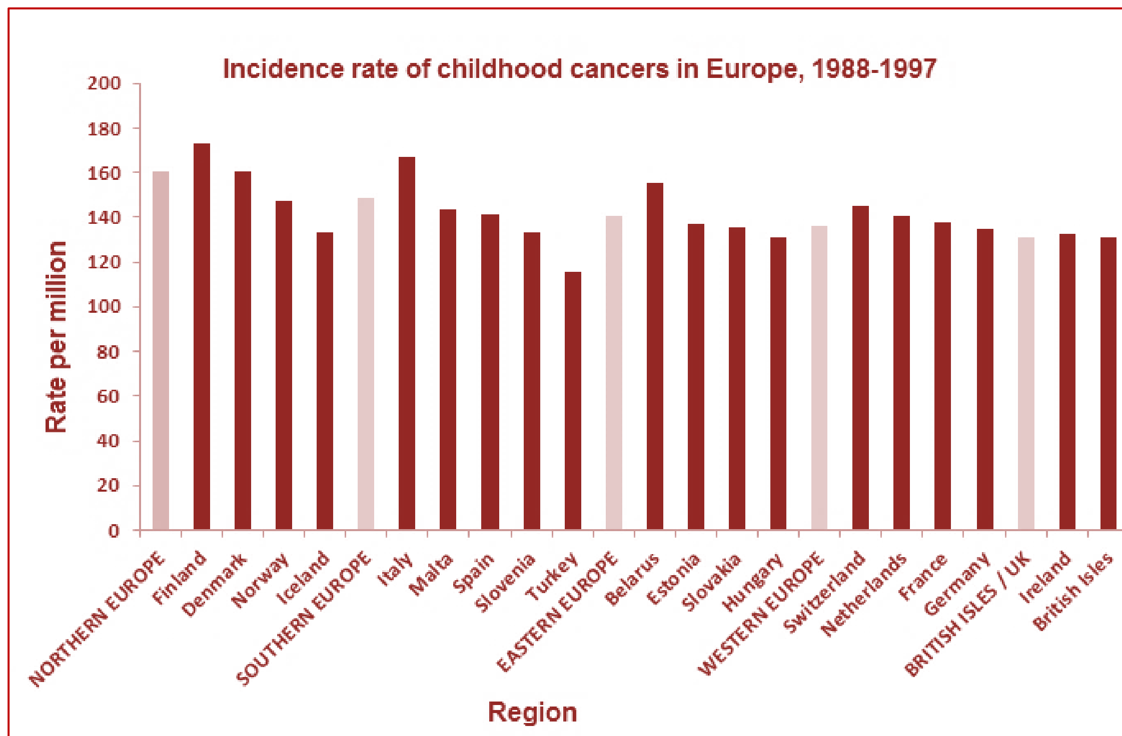


Figure 5 Cancer incidence in the Europe area, Adapted and modified from Cancer Research UK (2017)c.

in Canada, the incidence of childhood cancers are higher among males children rather than females children and the incidence rate increased in less than five years old as explained in (fig. 6).

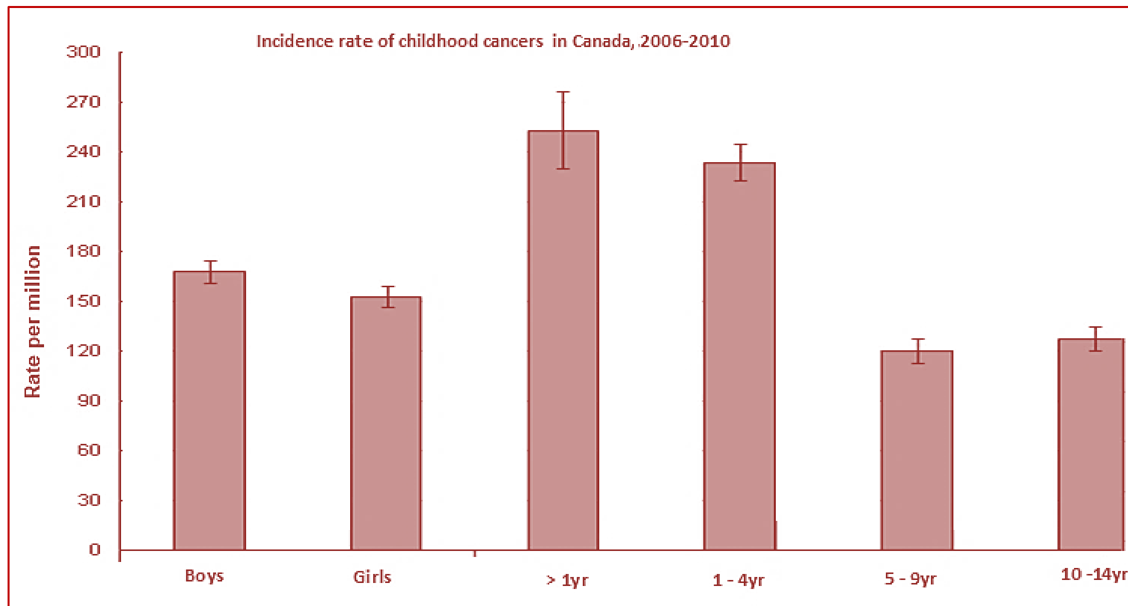


Figure 6 Childhood cancers incidence in Canada, adapted and modified from Ellison & Janz (2015).

The incidence of childhood cancer in the UK has expanded as 1756 cases were recorded within two years from 2012-2014,while this number represents a very low percentage less than 1% Percentage of total cases of cancer, and commonly occur in little ages less than 4 yrs old (Cancer Research UK, 2017)d.

1.1.1.2 Hallmarks of cancer

Cancer cells have different functional characteristics from normal body cells; those features are mostly shared between majorities of known tumours and mainly serve tumour growth, survival and aggressiveness. Those characteristics are named hallmarks of cancer and are: self-sufficiency in growth signals, insensitivity to growth-inhibitory signals, evasion of apoptosis, limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis (Hanahan and Weinberg, 2000). Cancer cells multiply out of control independently of external signalling pathway rather they often develop their own signalling system. Also cancer cells can overwhelm the growth control or checkpoints and growth suppressors' genes effect. Furthermore, they are not undergoing apoptosis and can divide and proliferate uncontrollably. To do so they require blood supply so they adapted their own angiogenesis by enhancing blood supply to the area. Cancer cells have the ability to invade surrounding tissues and metastasise to other parts of the body and start a new tumour which makes the condition complicated and increases the poor outcomes of cancer. Chronic inflammation can also contribute to development of many types of cancer by facilitation of angiogenesis and immune reactions as shown in table 1.

More hallmarks are reported in addition to the main six hallmarks of cancer that make the cancer capable of surviving and spreading throughout the body, these consists of effective metabolism to maintain the growth of tumour cells and the capability to survive the cellular immune defence mechanism as believed by Hanahan and Weinberg (2011), as it is weaken the performance of the immune system to eliminate cancer is weaken via suppression of immunity by $CD4^{+}CD25^{+}$ $FoxP3^{+}$regulatory T cells (Tregs), or

other types of suppressive cells, also production of several immune suppressive cytokines IL-1, IL-6, colony stimulating factor (CSF)-1, IL-8, IL-10, and type I IFNs which enhance cancer (Vinay et al., 2015).

Table 1 The hallmarks of cancer and their representative example cancer

Hallmark of cancer	Example	Reference
self- sufficiency in growth signals	glioblastomas and sarcomas	(Stensjoen et al., 2015)
evading apoptosis	human colorectal adenocarcinoma	(Kadaja-Saarepuu et al., 2008)
sustained angiogenesis	brain cancer	(Jain et al., 2007)
limitless replicative potential	ovarian cancer	(Lengyel, 2010)
tissue invasion and metastasis	osteosarcoma	(Lengyel, 2010)
insensitivity to antigrowth signals	pancreas cancer	(Keleg et al., 2003)

Cancer cells possess genetic abnormalities and have lost control over the cell cycle progression. The severity of cancer is related with metastasis formation which leads to the spread of cancer to the surrounding regions of the body; these locations are specified in table 2 (Carr, 2008).

Table 2 End sites of metastasis. Available in Carr (2008)

Cancer	Site of metastasis
lung cancer	lymph nodes, brain, bone, liver, pancreas
colorectal cancer	adjacent lymph nodes, liver
breast cancer	bone, brain, lung, liver
leukaemia	visceral organs, brain
prostate cancer	bone, adjacent lymph nodes, lung

The metabolic alterations that are seen in cancer cells are often recognized by high cellular intake of glucose and glutamine and excretion of lactate (Zielinski et al., 2017, Schwartz et al., 2017). Specifically, cancer cells metabolism is different from that of normal cells by maintained energy production through high glycolysis and yielding of lactate via fermentation of lactic acid in the cytoplasm of cancer cells independently of oxygen whereas in normal cells metabolism there is moderate levels of glycolysis followed by pyruvate oxidation in mitochondria . This phenomenon is called the Warburg effect (Saunier et al., 2017).

High proliferation rate of cancer cells is modulated by complex pathways such as G protein-coupled receptor kinases (GRKs, GPCRKs) (Nogues et al., 2017) . Interestingly, micro ribonucleic acids (microRNAs) take part in promoting cancer and controlling the signalling network through downregulation of affected genes thereby enhancing tumours progression (Manasa and Kannan, 2017). Chemokines are other regulators which play a pivotal role by negative or positive regulation of carcinogenesis by modulating white blood cells response (Lacalle et al., 2017).Those malignant traits can be affected by drugs applied using a nanoparticles technology to improve the therapy (VanDyke et al., 2016). So called multifunctional nanoparticles, nano platform or nanosomes are being developed to overcome the non-specific distribution of chemotherapy and eliminate the subsequent side effects by aiming to deliver drugs to the target tumour cell (Kouchakzadeh et al., 2017).

Molecular investigation utilizing assays to measure the protein or nucleic acid from cancer cells or cellular assays using fluorescent activated cell sorter are successful tools

to detect the alterations in the candidate gene/ receptor linked to each hallmark of cancer (Menyhart et al., 2016).

1.1.1.3 Cancer therapy

There are four common strategies for treatment of cancer, which are routinely followed and they include surgical removal of the tumour, radiation therapy to stop cancer progression, systemic therapy (chemotherapeutic agents which inhibit cancer growth) and targeted therapy (by administration of compounds that bind with a specific receptor in tumour cells and inhibit cancer) and immunotherapy. Other therapeutic approaches involve stem cell transplant, hyperthermia, photodynamic therapy, blood transfusion and lasers to reduce tumour mass (Baba and Catoi, 2007). Some of recent therapies are shown in table 3.

Table 3 Examples of cancers and therapeutic approaches.

Cancer type	**Therapy approach**	**Targeted pathways**	**Intended outcomes-**	**Reference**
breast cancer	photosensitizer (PS) via photodynamic therapy (PDT)	mannose receptor-mediated endocytosis	efficient and selective killing of cancer cells	(Yin et al., 2017)
liver cancer	Cryoablation therapy	freezing and thawing lead to damage of the cell	safe and effective freezing treatment,	(Yan et al., 2017)
- HeLa, HepG2, A549 and MCF-7 c - H22 tumor-bearing mice	mitochondria-targeted self-assembled nanoparticles amphiphilic triphenylphosphine–quercetin (TPP–Que) conjugates	mitochondrial (intrinsic pathway of PCD)	activation of the mitochondria-mediated apoptosis pathway.	(Xing et al., 2017)
cancer stem cells (CSCs) cancer stem cells (CSCs)	resveratrol	the P450 enzyme CYP1B1	apoptosis of cancer cells	(Ware, 2017)
metastatic prostate cancer	core shell lipid-polymer hybrid nanoparticles (CSLPHNPs) with combined	inhibitor of sphingosine kinase 1 (SK1) FTY720 (fingolimod)	reduce FTY720-induced lymphopenia	(Wang et al., 2017b)

	docetaxel and molecular targeted therapy			
Colorectal cancer (CRC)	target therapy for Transforming growth factor-beta TGF-beta	the inhibition of TGF-β signalling	impair experimental CRC metastasis to the liver	(Villalba et al., 2017)
triple-negative breast cancer TNBC	modified gold-based siRNA nanotherapeutics	target eukaryotic elongation factor 2 kinase (eEF-2K)	Inhibition of TNBC	(Shahbazi et al., 2017)
non-small-cell lung cancer	erlotinib-based doublet targeted therapy	targeting somatic sensitizing mutation in the EGFR-TK	inhibiting TK receptor autophosphorylation (5, 15) and downstream proliferation	(Jett and Carr, 2013)
breast cancer	photosensitizer (PS) via photodynamic therapy (PDT)	mannose receptor-mediated endocytosis	efficient and selective killing of cancer cells	(Yin et al., 2017)
liver cancer	Cryoablation therapy	freezing and thawing lead to damage of the cell	safe and effective freezing treatment,	(Yan et al., 2017)
- HeLa, HepG2, A549 and MCF-7 c - H22 tumor-bearing mice	mitochondria-targeted self-assembled nanoparticles amphiphilic triphenylphosphine–quercetin (TPP–Que) conjugates	Mitochondrial (intrinsic pathway of PCD)	Activation of the mitochondria-mediated apoptosis pathway.	(Xing et al., 2017)
cancer stem cells (CSCs) cancer stem cells (CSCs)	Resveratrol	the P450 enzyme CYP1B1	Apoptosis of cancer cells	(Ware, 2017)
metastatic prostate cancer	Core shell lipid-polymer hybrid nanoparticles (CSLPHNPs) with combined docetaxel and molecular targeted therapy	inhibitor of sphingosine kinase 1 (SK1) FTY720 (fingolimod)	reduce FTY720-induced lymphopenia	(Wang et al., 2017b)
Colorectal cancer (CRC)	target therapy for Transforming growth factor-beta TGF-beta	the inhibition of TGF-β signalling	impair experimental CRC metastasis to the liver	(Villalba et al., 2017)
triple-negative breast cancer TNBC	Modified gold-based siRNA nanotherapeutics	target eukaryotic elongation factor 2 kinase (eEF-2K)	Inhibition of TNBC	(Shahbazi et al., 2017)
non-small-cell lung cancer	erlotinib-based doublet targeted therapy	Targeting somatic sensitizing mutation in the EGFR-TK	inhibiting TKreceptor autophosphorylation (5, 15) and downstream proliferation	(Jett and Carr, 2013)

1.1.1.4 Blood Cancer

Blood cancers are the cancer of cellular components of the blood. Hematological malignancies are initiated from bone marrow of long bones by dysregulation of normal development, altered transition of stem cells into the functioning blood cells and blocking of their indispensable functions. There are three types of hematological cancers, leukemia, lymphoma and myeloma (American society of Hematology, 2017). In USA lymphoma represents the highest number of blood cancers followed by leukemia and myeloma (47%, 36% and 18 % respectively as seen in fig (7).

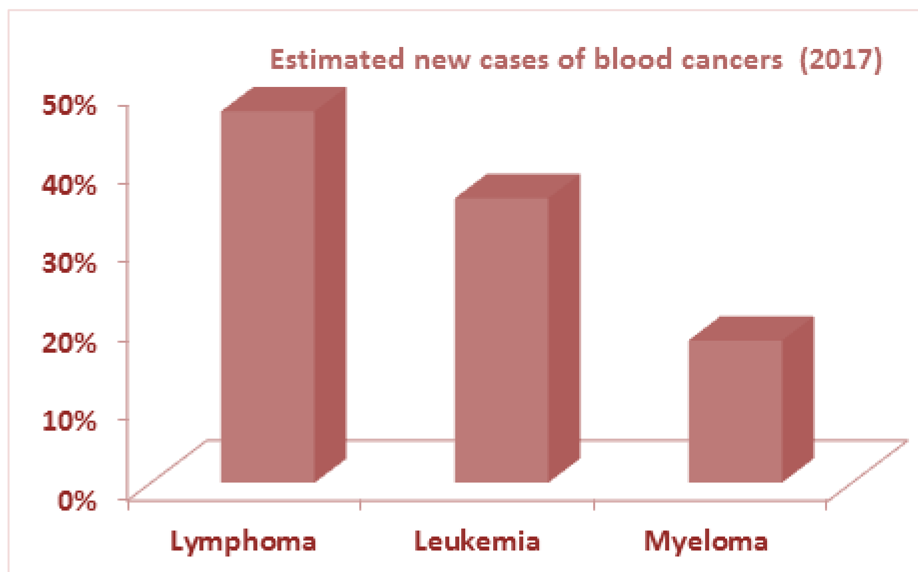


Figure 7 New blood cancer cases in the United States of America, adaptated from leukemia and lymphoma society, (2017)

Similar profiles were observed in Canada with 40%, 27% and 17 %, of lymphoma, leukemia and myeloma cases respectively as shown in fig (8).

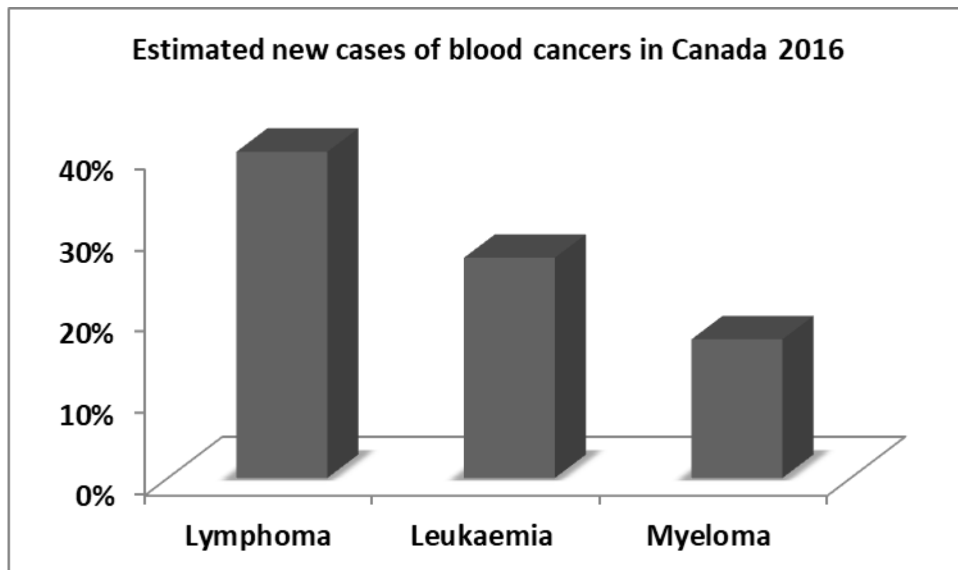


Figure 8 An estimation of blood cancers in Canada, adapted from the Leukemia & Lymphoma Society of Canada, 2017

In the UK, childhood cancers (leukemia, nervous system tumours, lymphomas and soft tissue sarcoma are of high incidence between male children as appear in (fig. 9).

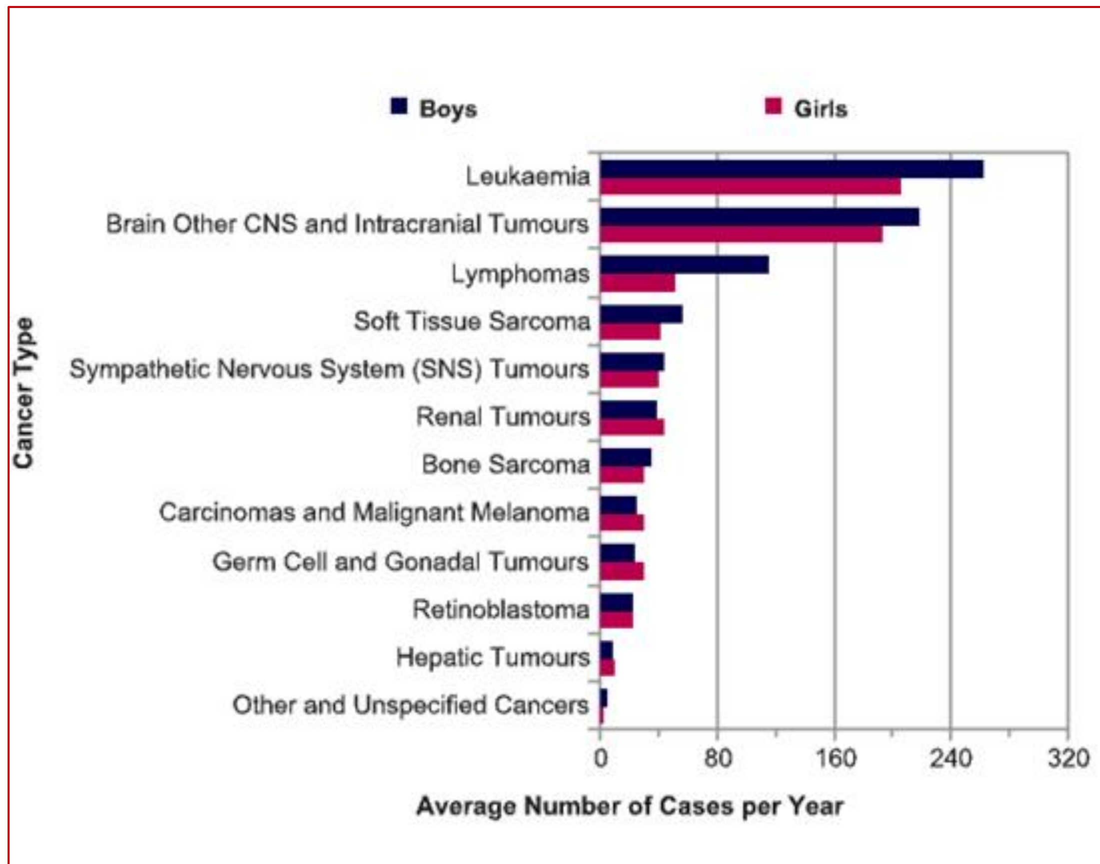


Figure 9 Children's cancers incidence in the UK in boys and girls. Adapted from Cancer Research UK (2017)c.

Blood plays a major role in all vital body functions and is composed of cells and liquid. Blood cells include red blood corpuscles (RBCs), called erythrocytes that aid oxygenation (Frey, 2002) and white blood cells (WBC), named leukocytes that control the immune response including humoral and cellular immunity. WBCs consist of granulocytes (basophils, eosinophils and neutrophils) and agranulocytes (monocytes and lymphocytes). Lymphocytes are the most important immune cells, which exist in two forms (T-lymphocytes or B-lymphocytes), and are low in number in contrast to RBCs. WBC are present in blood, lymph, lymph nodes and immune related organs such as thymus and spleen (Kanekura et al., 2017), therefore in case of acute lymphoblastic leukemia, leukemia cells accumulate in spleen and lead to splenomegaly (Manoharan et

al., 1980). Platelets, which are also called thrombocytes (the name originated from their role in thrombin formation), form the third element of blood cellular components, which mediate coagulation to initiate clot formation together with clot factors in case of injuries(Davis et al., 2016).

Nutrient materials that are dissolved in blood feed all body tissues and organs. Blood contains immunoglobulins, hormones, clot factors and all other secreted materials that are dissolved and carried by plasma, the fluid part of the blood.

Creation of the blood is called haematopoiesis. Haematopoiesis is a process that generates cellular components of the blood; it takes place in the bone morrow, the process begins from hematopoietic stem cells (HSCs)(Ng and Alexander, 2017), which are available in two types; long term (LHSCs) and short term (SHSCs), LHSCs are continuously self-renewed and produce SHSCs, while the last produce multipotent stem cells (MPSC) (Myatt and Lam, 2007) which undergo a series of development processes into to the mature blood cells (Wang and Wagers, 2011).

As stated before, blood cells originate from MPSC which are divided into two types; lymphoid progenitor (LP) or myeloid progenitor (MP). LP develop to lymphocytes, while MP divide into: megakaryocytes (which develop into platelets and red blood cells), myeloblasts (develop into granulocytes and macrophages) and mast cells (fig. 10).

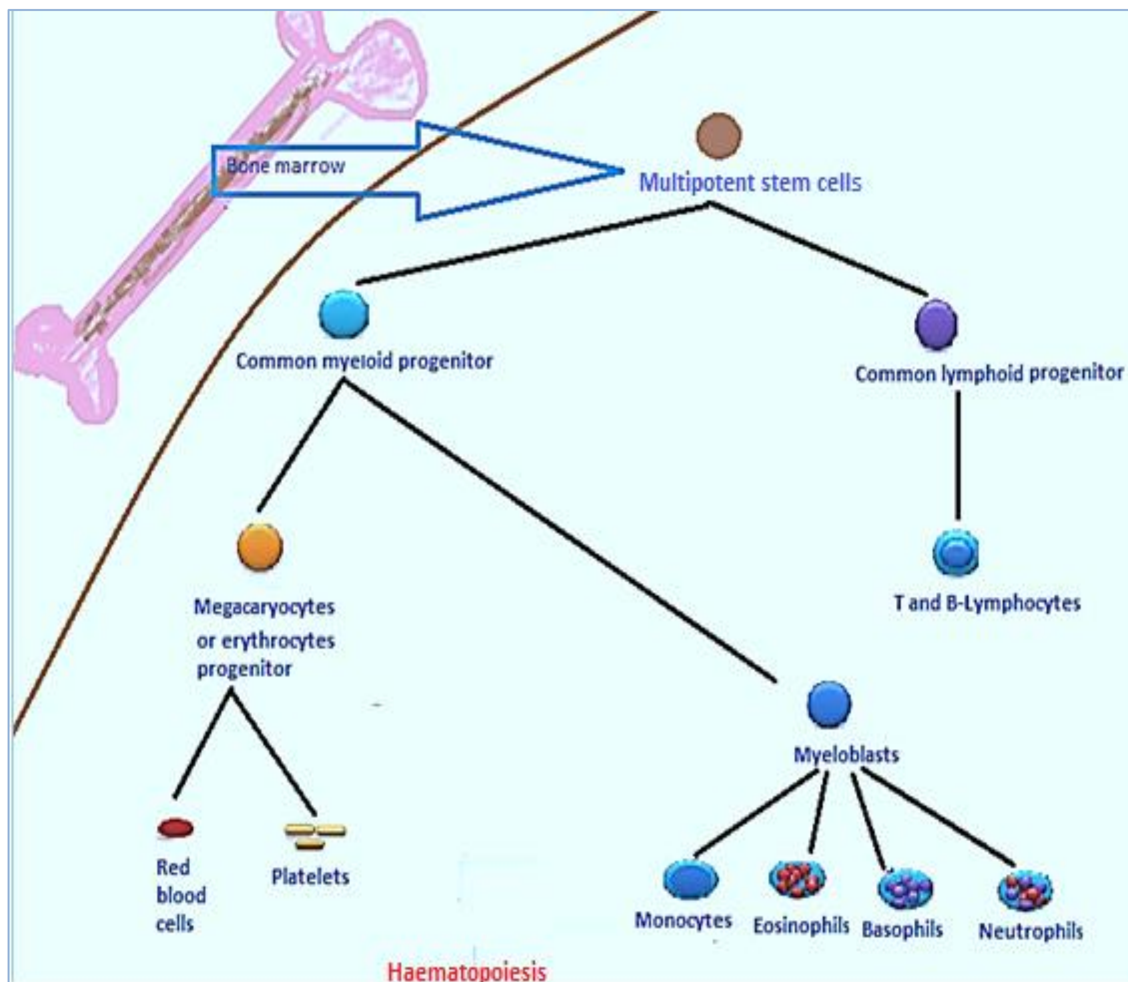


Figure 10 Haematopoiesis process.

Schematic illustration of blood cells' formation.

Blood cells are found in certain numbers and morphology so that any deviation from the natural process is linked to blood disorders such as alterations in the number of the cellular components (more or less cells) or malformation of the shape. For instance in blood cancer such as leukaemia the number of WBC increases substantially (LF, 2017).

1.1.2 Leukaemia

Leukemia is the cancer of white blood cells (leukocytes), characterized by abnormal proliferation of cells, it is the commonest neoplasm in humans, which often starts in bone marrow from the main blood cells progenitor previously mentioned in hematopoiesis. Generally, leukemia originates from either T or B lymphocytes (Horibe et al., 2017, Hung et al., 2017, Iwasa et al., 2017, Kaymak Cihan et al., 2017, Lyu et al., 2017, Richter-Pechanska et al., 2017, Savino and Izraeli, 2017, Thota and Advani, 2017, Villanueva-Lozano et al., 2017, Witkowski et al., 2017, Zhao et al., 2017) .
Estimated number of new cases of leukaemia diagnosed in 2017 in Australia is 3,875 from that 2,358 were predicted to be males and 1,517 were predicted to be females (Cancer Australia ,2017).

It is found in several forms depending on the progress or severity of the disease (acute and chronic leukemia) and type of affected cells (myeloid and lymphoid leukemia).Thus four main types are diagnosed; acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myeloid leukemia (CML) and chronic lymphocytic leukemia (CLL) (National Cancer Institute, 2014).

1.1.2.1 Acute lymphoblastic leukaemia (ALL), symptoms, causes and treatment

Acute lymphocytic leukemia is a malignancy of blood, that arises from dysregulation of normal stem cells (Campos-Sanchez et al., 2011). It is most frequently diagnosed in young people less than 14 years old and characterized by relatively high number of lymphocytes. Childhood ALL has been classified into three major types; B-ALL, T-ALL and NK-ALL. The first type has 10 subtypes, the second has six subtypes and third type does not have any (Tatar et al., 2016). Childhood leukemia represents 80% of leukemia and accounts for 25% of cancers in children (Merck, 2014). ALL represents 25% of cancers that affect children less than 15 years old according to Cancer Information Summaries (National cancer institute, 2017). Symptoms of ALL develop gradually including general weakness, immune suppression, fatigue, swollen lymph nodes, pain in joints and abdomen, night sweats and fever, difficult breathing, bruising and purple skin (Service, 2017). The diagnosis is made upon the clinical signs and laboratory examinations for the whole blood and bone marrow (Children with cancer, 2017).

Although exact causes of ALL are not known some risk factors have been identified. Alterations or mutations of genes that control haematopoiesis and development of stem cells to normal blood cells have been detected in ALL. These include BCR-ABL (Enciso et al., 2015), FLT3 (Fms-like tyrosine kinase 3) (Gilliland and Griffin, 2002), c-Myc (Delgado and Leon, 2010) and HOX (Alharbi et al., 2013). In addition, environmental factors such as radiation (X-rays, gamma rays) as well as chemicals, dietary factors, infections and inflammation have been suggested to play a role in ALL development (Belson et al., 2007).

ALL can be treated by chemotherapy including DNA damaging drugs and DEX to induce complete remission (CR) (Tatar et al., 2016). Radiotherapy is another way to control ALL, however it displays side effects; main side effects of radiotherapy in ALL and brain cancer patients are neurocognitive disorders (Sleurs et al., 2016). Immunotherapy is also applied as patients with ALL are under the risk of recurrent infection, thus agents that target immune system are developed (Moschovi et al., 2016). The poor outcomes in some ALL patients require a novel potential therapy (Ronson et al., 2016), therefore nanomedicine is another choice of treatment. The minimal residual disease (MRD) term is used to describe the response to treatment and refers to the leukemic cells detected after the treatment, MRD usually is measured by FACS and immunophenotyping (Rocha et al., 2016).

Overall survival rate of childhood ALL is 90% and the 5 year survival rate in USA as reported by (Hashkes et al., 2011) was 95.5%; likewise survival rates are 90% in UK (Cancer Research UK, 2016). However, the cure rate from ALL is not globally equal as it depends on the economic factors which play a significant role in the availability of treatments (Lehmann et al., 2016). 11–57% of ALL patients are obese having BMI ≥95%, as a common side effect of lack of exercise and / steroid therapy, those patients are less likely to survive(Tam and Ravussin, 2012). Also, survival rate for older patients (15-69 years) is lower 43.6% in Germany and 37.7 in US % (Pulte et al., 2014). In addition, long term survival is lower and toxicity can lead to secondary cancer development (Cooper and Brown, 2015). ALL treatment protocol (table 4), include three main phases these are; remission induction therapy which is the initial phase which aims to eliminate the vast majority of cancer cells in bone marrow and regenerate the circulating normal

blood cells; consolidation/ intensification therapy which aims to eliminate the further leukemia cells in central nervous system which are not been destroyed from the first phase and the maintenance therapy that aims to avoid reproducing leukemia cells (Mayo Foundation for Medical Education and Research (MFMER), 2017)

Leukemia therapies for each phase are decried in table (American Cancer Society, 2017)

Table 4 Therpeutic agents used in each phase of ALL therapy.

Phase of therapy	Drugs used
remission induction	• vincristine • dexamethasone or prednisone • doxorubicin (adriamycin), daunorubicin, or a similar anthracycline drug • sometimes cyclophosphamide (cytoxan), l-asparaginase, etoposide (vp-16), and/or high doses of methotrexate or cytarabine (ara-c) • philadelphia chromosome's all , imatinib (gleevec) used • intrathecal chemotherapy(methotrexate, but sometimes cytarabine or a steroid such as prednisone) • methotrexate or cytarabine given intravenously • radiation
consolidation therapy(intensification)	• same remission therapy • stem cell transplant (sct)
maintenance therapy	• methotrexate and 6-mercaptopurine (6-mp) • imatinib still given to positive philadelphia chromosome's all

Generally speaking, more than 40 drugs are applied across the various stages of ALL therapy, these are

1. Abitrexate (Methotrexate)
2. Arranon (Nelarabine)
3. Asparaginase Erwinia chrysanthemi
4. Blinatumomab
5. Blincyto (Blinatumomab)
6. Cerubidine (Daunorubicin Hydrochloride)
7. Clafen (Cyclophosphamide)
8. Clofarabine
9. Clofarex (Clofarabine)

10. Clolar (Clofarabine)
11. Cyclophosphamide
12. Cytarabine
13. Cytosar-U (Cytarabine)
14. Cytoxan (Cyclophosphamide)
15. Dasatinib
16. Daunorubicin Hydrochloride
17. Doxorubicin Hydrochloride
18. Erwinaze (Asparaginase Erwinia Chrysanthemi)
19. Folex (Methotrexate)
20. Folex PFS (Methotrexate)
21. Gleevec (Imatinib Mesylate)
22. Iclusig (Ponatinib Hydrochloride)
23. Imatinib Mesylate
24. Marqibo (Vincristine Sulfate Liposome)
25. Mercaptopurine
26. Methotrexate
27. Methotrexate LPF (Methorexate)
28. Mexate (Methotrexate)
29. Mexate-AQ (Methotrexate)
30. Nelarabine
31. Neosar (Cyclophosphamide)
32. Oncaspar (Pegaspargase)
33. Pegaspargase
34. Ponatinib Hydrochloride
35. Prednisone
36. Purinethol (Mercaptopurine)
37. Purixan (Mercaptopurine)
38. Rubidomycin (Daunorubicin Hydrochloride)
39. Sprycel (Dasatinib)
40. Tarabine PFS (Cytarabine)
41. Vincasar PFS (Vincristine Sulfate)
42. Vincristine Sulfate
43. Vincristine Sulfate Liposome
44. Phases of treatment
45. Treatment for acute lymphoblastic leukaemia (ALL) is divided into 3 different phases, find out more about what treatment to expect.(National Cancer Institute, 2017).

1.1.2.2 Avian lymphoid leukosis

Lymphoid leukosis is a neoplastic disease that is commonly observed in poultry such as chicken. The disease occurs in an adult chicken upon infection with the avian leukosis virus (ALV). The causative retroviruses belong to subgroup A and are from leukosis /sarcoma group (The poultry site, 2014). Leukosis in general indicates transmissible malignant or benign neoplasm commonly found in chickens. Lymphoid leukosis is considered as one of the most common forms of leukosis in poultry. ALV can be divided according to envelope antigen to six subgroups with distinct pathogenesis. The common subtypes are (ALV-A and J). Lymphoid leukosis (LL) is caused by (ALV-A) which is also the causative agent of erythroblastosis (EB), while ALV-J is the etiology of myeloid leukosis (ML), LL and ML can be induced experimentally through C-MYC and C-erbB oncogene stimulation respectively via chimeric viruses (Chesters et al., 2002).

It has been demonstrated that protein 27 (P27) is linked with resistance in chicken breeds by identifying relatively low p27 serum concentrations in resistant breeds when compared to susceptible breeds (Barbour et al., 1999).

The disease in chicken is characterized by the presence of sporadic tumors mainly seen in the primary immune organ in chicken, bursa of fabricious. It is believed that the viral pathogenicity is determined by C-MYC and C-BIC (Hihara et al., 1998). As disease causes significant economic losses novel treatments will be beneficial. In addition, comparison of GR function in chicken and human cells will provide information about evolutionary conservation of its action. Numerous drugs are used for leukemia therapy and of main interest are steroids glucocorticoid hormones (GCs use in medicine is described in (Murayi and Chittiboina, 2016)

1.1.3 Glucocorticoid hormones

Glucocorticoids (GCs) are steroid hormones, lipophilic and hydrophobic in nature as they are derived from cholesterol (see fig. 11).However, GCs synthesis and secretion are orchestrated by circadian system (Dickmeis, 2009).

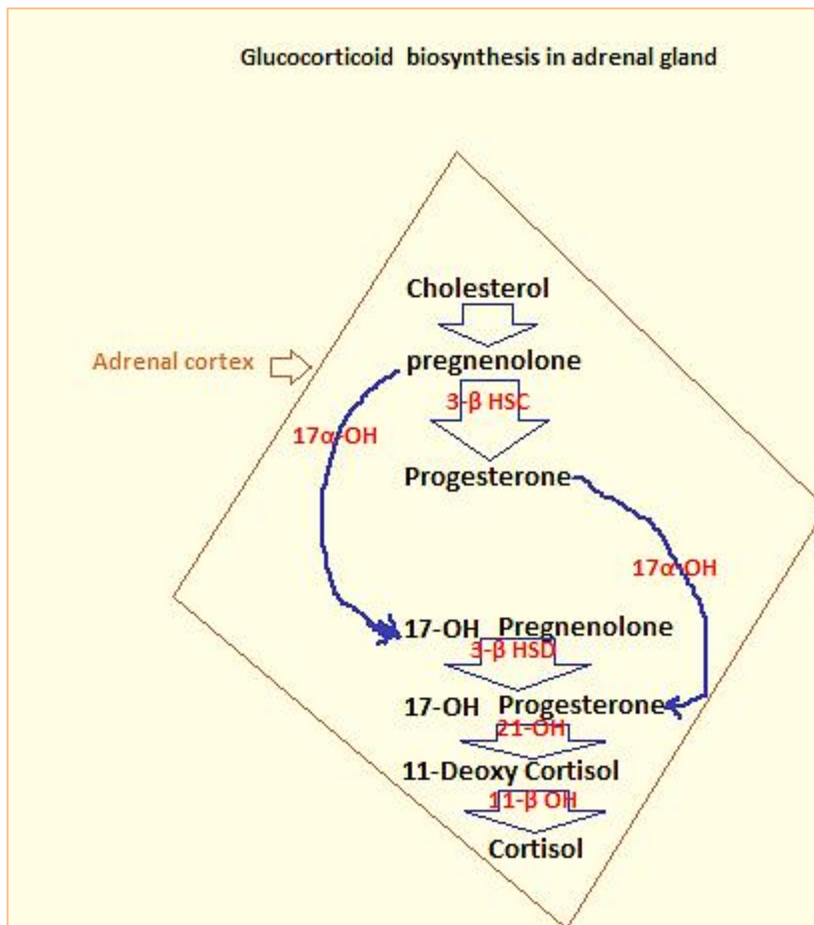


Figure 11 Schematic illustration of glucocorticoid (steroid) synthesis in adrenal gland

The process starts with cholesterol conversion into pregnenolone, the new product that undergoes several hydroxylation reactions ending with cortisol. Adapted and modified from Erhuma (2012). HSD: Hydroxysteroid Dehydrogenase, OH: Hydroxylase

Glucocorticoids are stress hormones produced by the adrenal gland upon stress or stimulation (Zotter et al., 2017) and display immunosuppressive and anti-inflammatory

effects. Stressors, which affect GC production, lead to stimulation of GC receptor (GR) function (Beck et al., 2013a). Stress is usually accompanied by undesired side effects or severe illnesses for instance gastric-ulcer (Wang et al., 2016a) or delay in healing of injury (Jozic et al., 2016), (Chandramohan et al., 2007), or sometimes GCs resistance development (Pazdrak et al., 2016, Barnes, 2010, Goldstein and Ozols, 1994). Stress could be considered as a promoting factor for cancer development (Wang et al., 2016b). Additionally, extensive continuous stress leads to glucocorticoid receptor resistance due to loss of sensitivity of the target tissue to GC hormones, thereby altering the inflammatory regulation function of GR leading to up regulation of pro-inflammatory proteins (Hamdi et al., 2007).

1.1.3.1 Hypothalamic-pituitary-adrenal axis

GCs are secreted from the zona fasciculata of the adrenal cortex in response to stress and this is controlled by hypothalamus-pituitary adrenal axis (HPA) (Fig. 12) that also contains negative feedback loops. The process begins from the hypothalamus which regulates body temperature, metabolic reactions, fatigue, sleep and circadian rhythms (Oster et al., 2006). Exogenous or endogenous stress stimulates the hypothalamus, specifically parvocellular neurosecretory cells of the paraventricular nucleus, to secrete corticotrophin-releasing hormone or factor (CRH / CRF), which activates the anterior lobe of the pituitary gland to synthesize and release adrenocorticotropic hormone (ACTH). This then stimulates the adrenal cortex to release glucocorticoids into blood stream. Glucocorticoids levels are returned to normal through negative feedback loops affecting the hypothalamus and pituitary gland (Deng et al., 2015). The appropriate

response to stress depends on hypothalamus-pituitary-adrenal axis (Fig. 12) and circulated cortisol levels. Adrenal malfunction such as high production of cortisol (hypercortisolism) leads to Cushing's syndrome (Findling and Raff, 2017, Casanovas Taltavull and Pena-Cala, 2017). The low cortisol production leads to Addison's disease (2017, Mozolevska et al., 2016). These GC hormones are affected by corticosteroid-binding globulin (CBG) and 11β-HSD (Clark, 2003). CBG carries and circulates the cortisol throughout the body (Gardill et al., 2012), while 11β-HSD oxidizes and converts cortisol to inactive cortisone (Chapman et al., 2013).

In addition to the indicated role of GCs hormones in stress conditions as stress hormones, normal levels of GCs are of major importance as they regulate a variety of vital process in the body from normal metabolism (Ayyar et al., 2015), muscle tone (Braun and Marks, 2015), performance (Shaashua et al., 2014) , normal bones mineralization (Tack et al., 2016b), homeostasis (Lou et al., 2016), nervous systems in relation to cognition and memory development (Libro et al., 2017), and maintenance of normal liver function (van der Geest et al., 2016), normal growth- development and immunity (Solano et al., 2016); endogenous GCs are shown to have protection effect from LPS-induced sepsis (Li et al., 2015). However over exposure to GCs contributes to impairment of normal physiological functions and endangeres the normal physiology of the affected organs leading to serious pathological changes (Nikolic et al., 2013).

In general, Glucocorticoids exert their action via binding to their steroid receptors in order to achieve their downstream effects (Jaffuel et al., 1999).

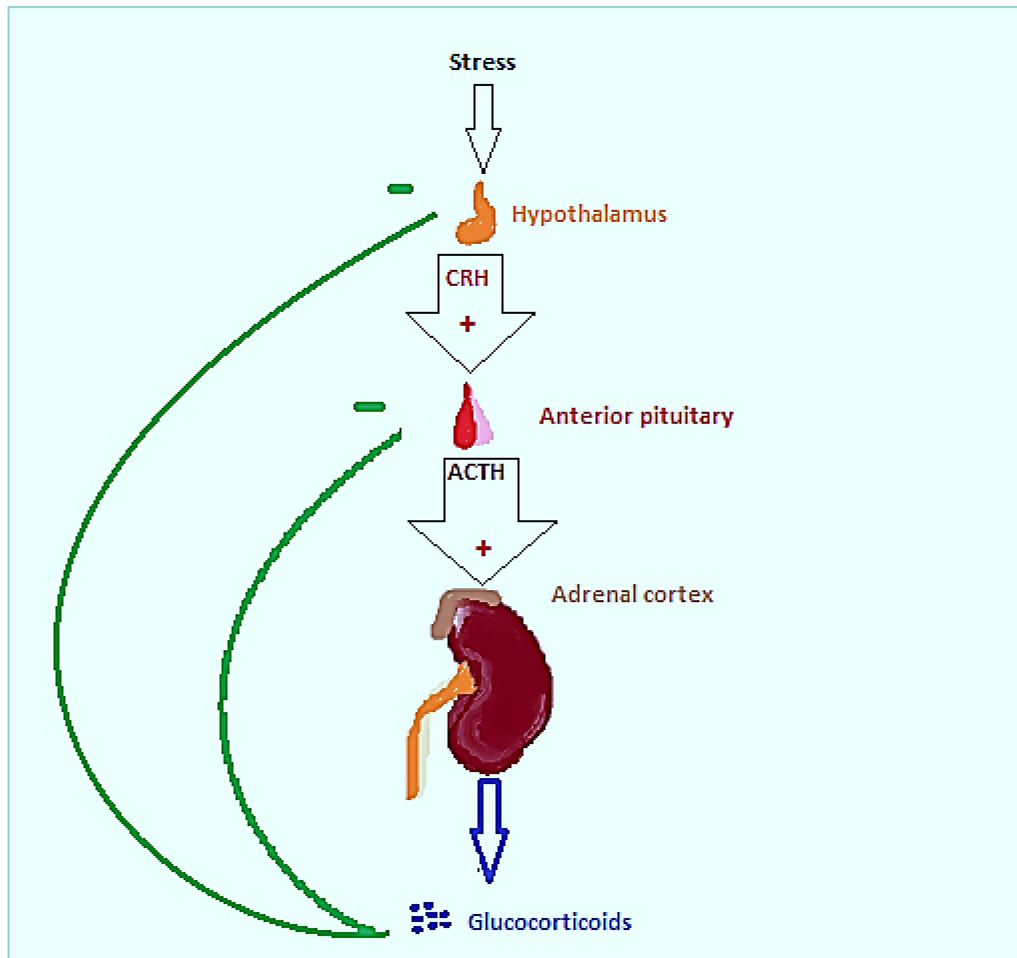


Figure 12 Hypothalamic-pituitary -adrenal axis (HPA).

The hypothalamus secretes the neuro-hormone corticotrophin-releasing hormone (CRH/CRF) and this in turn stimulates secretion of a pituitary hormone adrenocorticotropic hormone (ACTH), which stimulates adrenal gland to release glucocorticoids (GC) from the adrenal cortex. Finally these hormones inhibit the further secretion of hypothalamus hormones via negative feedback loop.

1.1.3.2 GCs uses in medicine

Synthetic glucocorticoid hormones have been prescribed clinically for over six decades, for different inflammatory conditions and allergic related disorders such as; allergic rhinitis, asthma, chronic bronchitis, cystic fibrosis, emphysema, inflammatory bowel disease, multiple sclerosis, (Nuwayhid, 1983, Jobe, 2000, Sauerwald and Rath, 2000, Robson and Hughes, 2003, Baid and Nieman, 2006, Xia et al., 2007, Zhang et al., 2008, Goichot, 2009, Wang et al., 2010, Serra et al., 2012, Dong, 2013) and rheumatic arthritis

(Matsuno, 2016).GCs have been used in cancer therapy either alone or along with chemotherapy for certain types of cancer, because they induce apoptotic of white blood cells and have been part of standard therapy for ALL patients for a number of years (Zhao et al., 2013, Sanchez-Lara et al., 2013, Salvador et al., 2012, Virik et al., 2001, Salmon et al., 1994, Tandan et al., 1990, Gel'berg et al., 1986, Fiegel, 1961, Spiess, 1960).

The main GC used in ALL treatment regimens are; hydrocortisone (HC), prednisolone (PRE), methylprednisolone (MPR), dexamethasone (DEX) and betamethasone (BET) (Styczynski et al., 2002) , However, above described effects on apoptosis are restricted to a few cell types and mostly are observed in white blood cells whereas in other cell types GCs can have no effect or even protect cells from apoptosis. Nevertheless, long term use of glucocorticoids like dexamethasone can negatively affect the body and produce side effects such as: hypertension, glaucoma, osteoporosis, retardation of growth, immune-repression, obesity and accumulation of abdominal fluid (Frenkel et al., 2015); bone abnormalities also follow chronic use of GCs (Tack et al., 2016a). Further side effects include kidney problems with related complications (Singh et al., 2016). Finally, Mons and Beracochea (2016) demonstrated that GCs leads to mental disturbances in case of people who are addicted to alcohol due to persistence stimulation of HPA. Part of metabolic effects of GCs belong to glucose mediated modulation of thioredoxin-interacting protein (TXNIP) which in turn regulates thioredoxin (TRX) by interfering with Ros activity (Apostolopoulos and Morand, 2016, Paredes and Alves, 2016, Wells et al., 2016, Williams et al., 2016, Caplan et al., 2017b, Caplan et al., 2017a, Goto et al., 2017, Kaymak Cihan et al., 2017, Szabo and Kiss, 2017, Tacey et al., 2017) .

More than one drug acts together to treat hematological malignancies (Pei et al., 2016). To increase the chance of complete remission in ALL patients, DEX has been applied in high doses in combination with chemotherapy (Parovichnikova et al., 2003). However this can lead to adverse effects (table 3) as described previously. Regarding effects on nervous system, in spite of the depression like state arising from chronic administration of GCs, (Skupio et al., 2015), the clinical trial of Kadan-Lottick et al. (2009) revealed that no significant neurocognitive malfunction in ALL children treated with DEX was detected.

In addition to uses in leukemia therapy, GCs are used in many other conditions. In patients infected with neuromyelitis optica (NMO) (autoimmune disease) GCs treatment causes downregulation of abnormal monocytes (Zeng et al., 2016). GCs protect the body from septic shock lethal effects (Yende and Thompson, 2016). In another study DEX was employed as a part of treatment regime against Parkinson's disease (PD) due to it anti-inflammatory properties (Tentillier et al., 2016). GC has been used in treatment regime of peritumoral brain oedema (PTBE) (Murayi and Chittiboina, 2016).

GCs act as a physiological trigger of stress but are also used in medicine to treat chronic inflammations and range of other diseases as mentioned above (Madliger and Love, 2016). (Lems et al., 2016).

Dexamethasone (DEX) is the common synthetic GC, which is mainly prescribed as an anti-inflammatory drug although has many other uses. It produces this effect through glucocorticoid receptor (Zotter et al., 2017). This drug differs from natural GC by resistance to 11-beta hydroxysteroid dehydrogenase 2 (11b-HSD2) suppression and is

not regulated by corticosteroid- binding globulin CBG, making DEX (Fig. 13) more stable (Kadmiel and Cidlowski, 2013).

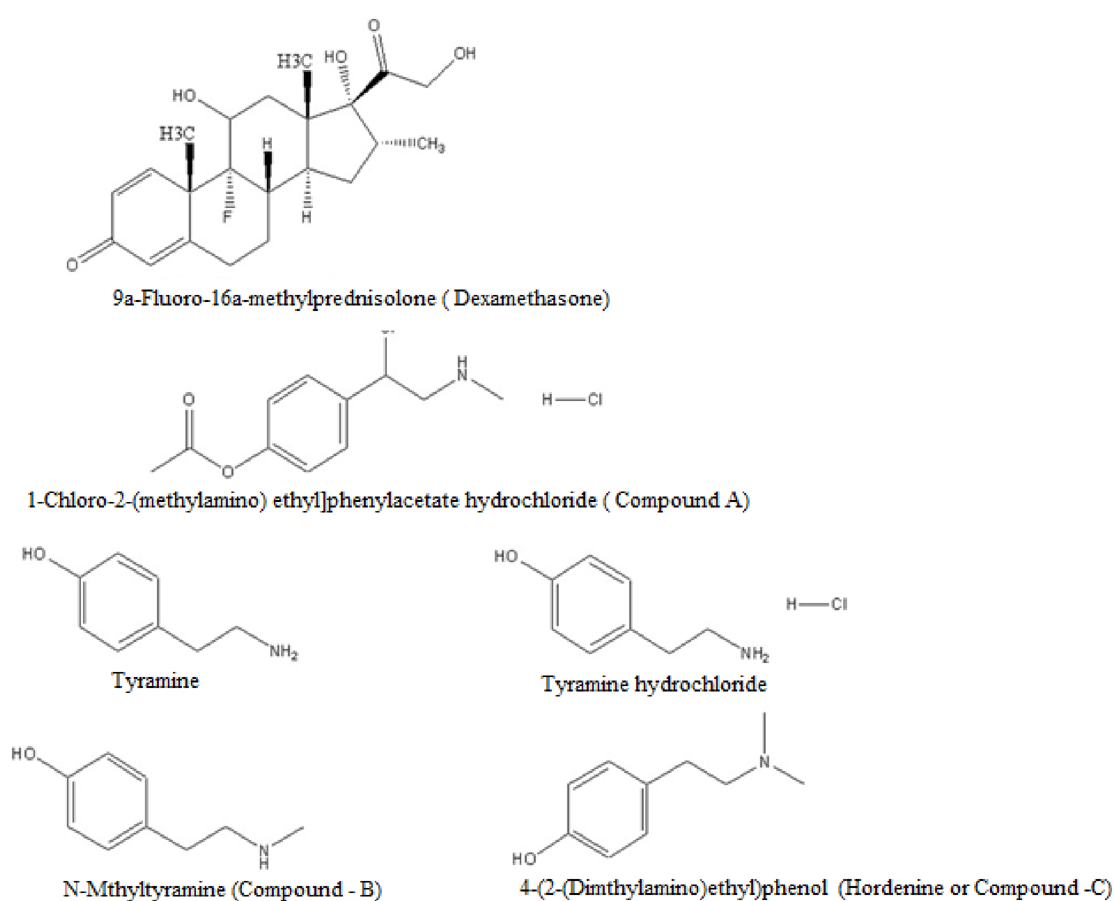


Figure 13 Chemical structures of tested compounds.

DEX can affect invading and metastatic ability of fibro sarcoma cells (Foty et al., 1998), it also demonstrated inhibitory effect on the growth of cells in certain cancers and leukemia (Wu et al., 2006). This is in addition to its ability to treat different autoimmunity disorders (Hu et al., 2012). It can be either given alone or in combination

with other medicines to improve the therapeutic effect (Adem et al., 2016). GR activity is controlled at several levels such as alternative splicing, GR expression, nuclear translocation, transactivation and posttranslational modifications. Deregulation at these levels can lead to GC resistance (Vandevyver et al., 2014). Therefore it is important to develop new compounds that may overcome GC unresponsiveness, sensitize resistant cells and display fewer side effects. GCs common side effects are emphasised in table 5.

Table 5 Common side effects of GCs tratment.

	Adverse effect	Mechanism /physiology	Reference
1	diabetes	apoptosis of islet cells	(Zhang et al., 2016)
2	adrenal suppression	suppression of (HPA) function by glucocorticoid	(Goldbloom et al., 2017)
3	skin thinning/ damage	Dermal atrophy and vasodilation	(Abraham and Roga, 2014)
4	osteoporosis	glucocorticoids reduce bone formation and increase bone resorption	(Lane and Lukert, 1998)
5	glomerular disease	bisphosphonates or active vitamin d metabolites	(Kikuchi et al., 2007)
6	myopathy	catabolism of skeletal muscle by active gr	(Vecht, 1998)
7	avascular necrosis	bone cells apoptosis	(Weinstein et al., 2000)
8	hyperlipidaemia	not known	(Berg and Nilsson-Ehle, 1996)
9	hyperglycaemia	Increase hepatic glucose production	(Tamez-Perez et al., 2015)
10	Hypertension a	sodium retention, resulting in dose-related fluid retention	(Lee and Elwing, 2017)
11	depression	HPA negative feedback dysregulation	(Gobinath et al., 2014)
12	gastritis and peptic ulcers	Suppression of gastric cytoprotective	(Narum et al., 2014)

		prostaglandins	
1	diabetes	apapoptosis of islet cells	(Zhang et al., 2016)
2	adrenal suppression	suppression of (HPA) function by glucocorticoid	(Goldbloom et al., 2017)
3	skin thinning/ damage	Dermal atrophy and vasodiltion	(Abraham and Roga, 2014)
4	osteoporosis	glucocorticoids reduce bone formation and increase bone resorption	(Lane and Lukert, 1998)
5	glomerular disease	bisphosphonates or active vitamin d metabolites	(Kikuchi et al., 2007)
6	myopathy	catabolism of skeletal muscle by active gr	(Vecht, 1998)
7	avascular necrosis	bone cells apoptosis	(Weinstein et al., 2000)
8	hyperlipidaemia		(Berg and Nilsson-Ehle, 1996)
9	hyperglycaemia	Increase hepatic glucose production	(Tamez-Perez et al., 2015)
10	Hypertension and oedma	sodium retention, resulting in dose-related fluid retention	(Lee and Elwing, 2017)
11	depression	HPA negative feedback dysregulation	(Gobinath et al., 2014)
12	gastritis and peptic ulcers	Suppression of gastric cytoprotective prostaglandins	(Narum et al., 2014)

1.1.3.3 Glucocorticoid receptor (GR)

GCs exert their effects on cells by binding to their specific GC receptor, which is called glucocorticoid receptor (GR) encoded by NR3C1 gene located in the chromosome area 5q31.3. GR is nuclear receptor, its transcriptional activity is controlled by binding with its ligand via C-terminal region which is best described as "a pocket " due to the distinct structure that surrounds the ligand (Bledsoe et al., 2002). GR also has two domains, N terminal (NTD) domain and Deoxyribonucleic acid binding region named DBD which

contacts glucocorticoid responsive elements (GREs) in target genes (Gruver-Yates and Cidlowski, 2013).

In the absence of ligand, GR exists in the cytoplasm complexed with other proteins called chaperones heat shock proteins (HSP) that have different molecular weights including HSP23, HSP70 and HSP90. Also immunophilins like FKBP51, FKBP52, Cyp44 and PP5 are part of this complex.

Upon binding with ligand, the activated GR may either influence other genes or proteins in cytoplasm and affect or modulate the pro-inflammation response via interference with the T-cell receptor signalling pathway (De Bosscher et al., 2010). However, main mode of action of the activated GR homodimer is by translocation into the nucleus and binding to a specific consensus sequence in the DNA of GCs target genes. This consensus sequence is called GRE (from glucocorticoid responsive element) and they are located in the promoter region of target genes. Subsequently GR orchestrates the transcription of these genes by activation or repression of genes involved in metabolism, inflammation, apoptosis and numerous other processes (Adcock and Barnes, 1996).

1.1.3.3.1 GR domain organisation

GR is a transcription factor located in cytoplasm in the absence of the hormone, that translocate to the nucleus after hormone activation. GR is member of nuclear hormone receptors super family, named NR3C1 and belongs to a subfamily of steroid receptors. The human GR protein consists of 777 amino acids, and it has several major regions (Weikum et al., 2017b) illustrated in (Fig. 14) These are N (amino) - terminal (variable) domain (involved in transcriptional regulation), a DNA-binding (GRE-binding) domain which contains two zinc fingers and a hinge region that includes one of the nuclear

localization signals and a C (carboxyl) -terminal which is site of ligand–binding domain and is composed of 9 exons (Kumar and Thompson, 2012).

NR3C1 gene has two main different molecular isoforms, GRα that has 777 amino acids and GRβ that has 742 amino acids. GRα is the active isoform, while alternative splicing forms GRβ. There are other RNA splice variants called GRγ, and GRd which differ in their LBD and DBD potency, each one may produce four new forms named A-D by alternative translation (McMaster and Ray, 2008) .

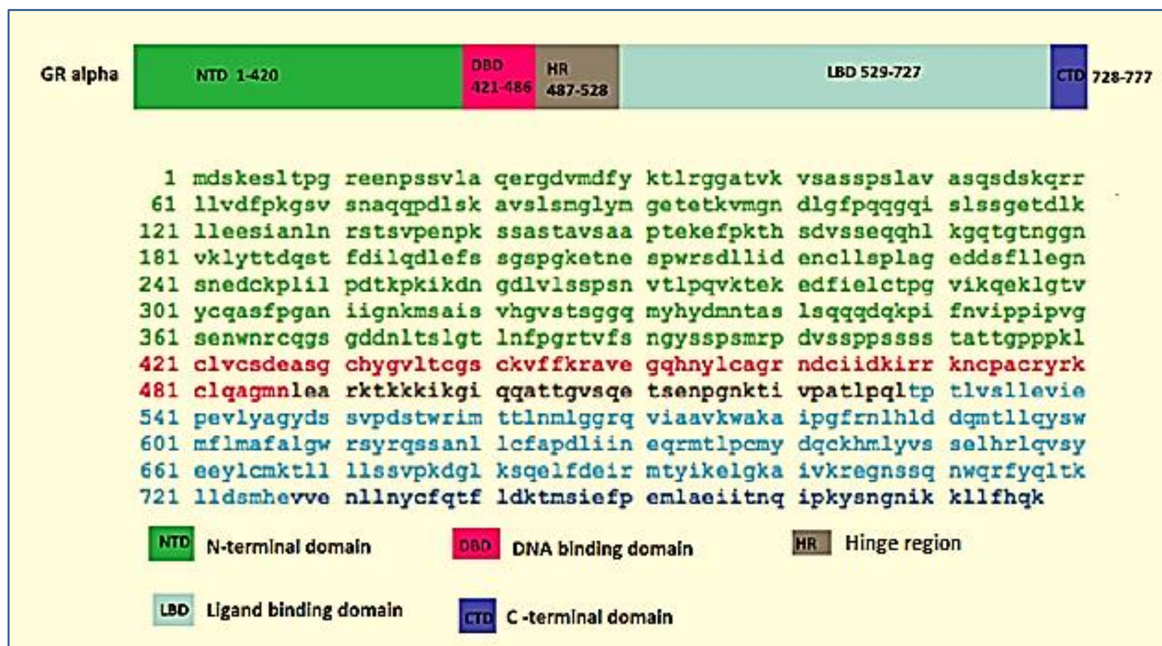


Figure 14 Genomic structure of GR alpha and GR amino acid sequence

GR isoform α-protein segmentation is shown, Glucocorticoid receptor isoform alpha [Homo sapiens] NCBI Reference Sequence: NP_001018087.1

1.1.3.3.2 Transcriptional regulation by GR

GR is a sequence specific transcription factor that is expressed in all tissues /organs /systems of the body. Despite ubiquitous expression, GR regulates transcription in a gene and tissue specific manner. In the absence of hormone GR exists in the cytoplasm complexed with chaperones (HSp 23, HSp70 KDa and HSp90 KDa), via LBD C-terminal domain (Fig.15). Also, there are other proteins in a complex including immunophilins like FKBP5, FKBP52, PPID, Cyp44, STIP1, immunophilin homolog PPP5C. GC hormones pass through the cellular membrane because of their lipophilic structure, and bind GR leading to GR activation and dissociation of specific heat shock proteins and exchanging of FKBP5 by FKBP4. GC-GR complex dimerizes and binds to dynein to translocate to the nucleus as a homodimer consisting of two molecules of GR attached to two molecules of GC (Abraham et al., 2017, Feng et al., 2013, Gross et al., 2011, Yang et al., 2008, Agler et al., 2007, Salmon et al., 1994).

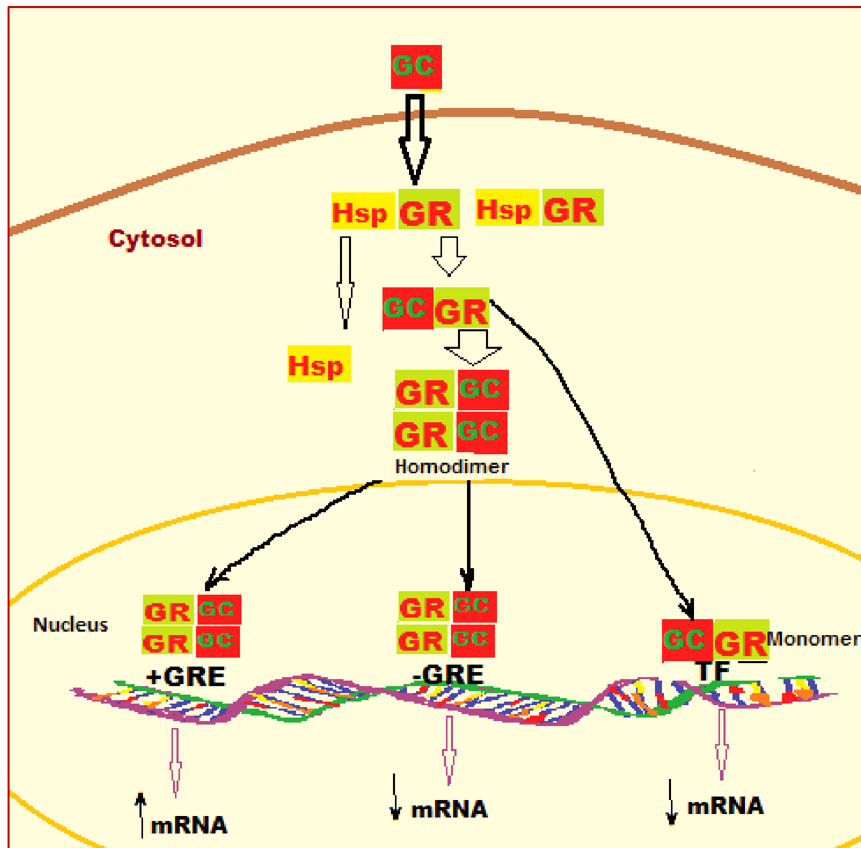


Figure 15 Mode of GR

GCs enter the cell and bind to GR, then GR dissociates from the complexed heat shock proteins. GC-GR complex enters the nucleus and binds to glucocorticoids responsive element in the promoter region of the responsive gene, adapted from Trevor and Deshane (2014).

Glucocorticoid receptor's biological functions are result of GR role in mediation of transcription. GR either positively or negatively regulates its target genes expression, however, this mechanism involves a network of regulators or elements including chromatin and cofactors activation (Meijsing, 2015).

1.1.3.3.3 Levels of control of the GR function

Upon binding with ligand the activated GR regulates expression of target genes and also may have non-genomic effects suggested to take place in the membrane through protein-protein interaction without the need for the nuclear translocation or DNA

binding, though this mechanism and outcomes are not fully understood (Beck et al., 2009b, Beck et al., 2009a, Gossye et al., 2008, De Bosscher et al., 2006, Vanden Berghe et al., 2002). The activated GR homodimer that mentioned in (1.1.3.3.2), translocates into the nucleus and binds to a GRE in the DNA of GC target genes, located in the promoter region of these genes. Subsequently GR orchestrates the transcription of large number of genes (fig.8) by activation or repression of transcription (Ratman et al., 2013, De Bosscher et al., 2000b, De Bosscher et al., 2000a, De Bosscher et al., 1997).

These genes are involved in control of apoptosis, metabolism inflammation and immune response (Hou et al., 2014, Sluyser, 2005). A dimerized GR binds to DNA in the nucleus at its central region through its two zinc fingers; it can either mediate trans-activation or trans-repression depending on the type of the GRE, cofactors involved and type of the cell.

Activated dimer GC-GR regulates more than 100 genes by up regulation of anti-inflammatory proteins such as interleukin IL-10, Annexin1 and inhibitor of NF-κB or down regulation of pro-inflammatory mediators such as IL-6, nuclear factor-κB (NF-κB) or activator protein 1 (AP1) activities, enzymes including mitogen-activated protein kinases (MAPKs) or up regulation of histone acetyltransferases (HAT) and histone deacetylases (HDAC) which modify chromatin (Prusator and Greenwood-Van Meerveld, 2017, Morgan et al., 2016, Hunter et al., 2016, Sevilla et al., 2015, Zou et al., 2013, Sasse et al., 2013, Hu et al., 2013, Zhang et al., 2012, Speksnijder et al., 2012, Nader et al., 2012). In addition GR regulates inflammation via trans repression of pro-inflammatory mediators cytokines (interleukin 1, 2, 3, 4, 5, 6, 11, 13), tumour necrosis factor- alpha

TNF- α, granulocyte-macrophage colony stimulating factor (GM-CSF), chemokines cyclooxygenase and other numerous genes (Grigor'ian et al., 2014)

GR mediated transcriptional activation for instance expression of genes involved in metabolism may bring side effects to patients on long term of high dose GC therapy (Schacke et al., 2004).

GR interacts with its target genes and inflammatory mediators either by GRE or other ways shown in (Fig. 16). Simple mode of action is binding with simple GREs in the GR target genes leading to either activation of transcription if binding is to positive GRE or suppression of transcription if binding to negative GRE in target genes (Newton and Holden, 2007). In composite mode, GR binds to the transcription factors in the composite regions in the DNA of responsive genes then either enhances or inhibits the transcription of the target genes. In the tethering mode of regulation, GR binds the transcription factors that bind positive GRE or negative GRE to either enhance or suppress the transcription. GR mediated transcriptional activation usually involves coactivators (such as CBP or p160) recruitment whereas GR mediated transcriptional repression is often through recruitment of corepressors (such as HDACs) leading to control of general transcriptional machinery (such as TBP and RNA polymerase II) (Latchman, 2001) and (Barnes, 2006).

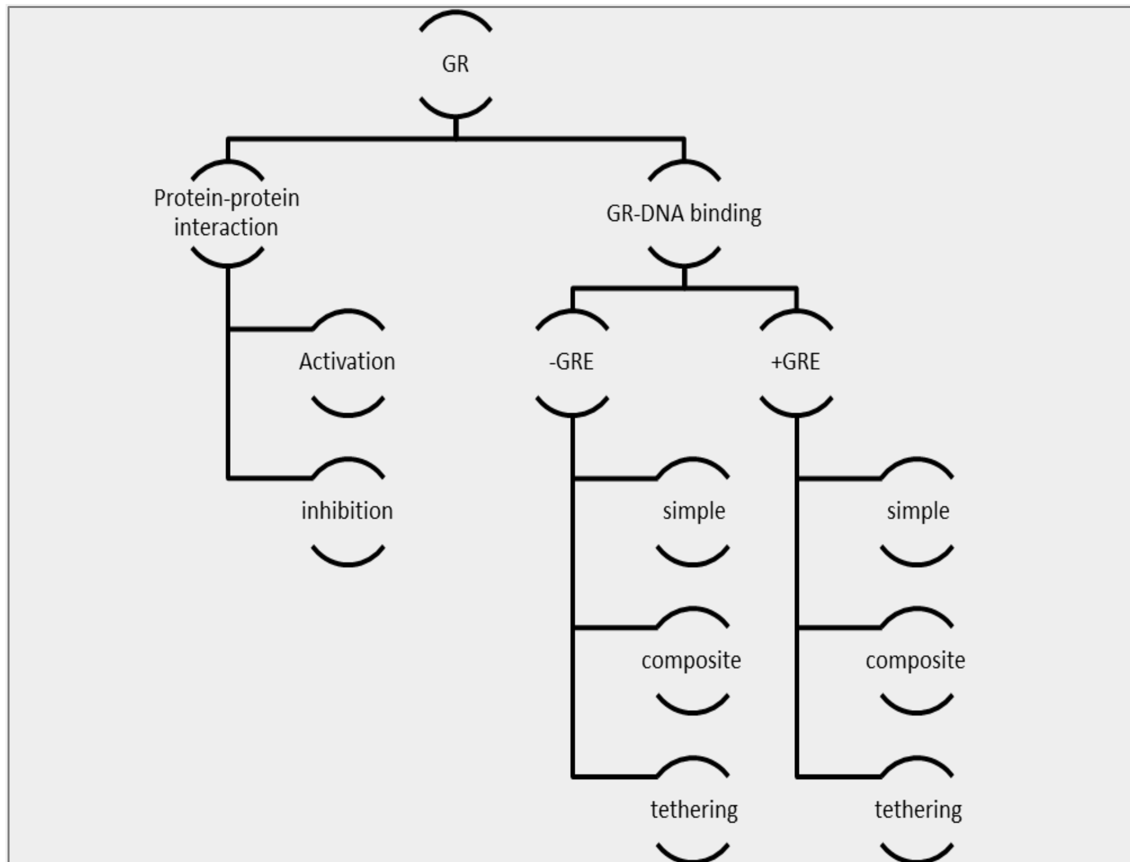


Figure 16 GR Signalling

Main GR interactions in regulation of gene expression adapted and modified from Newton and Holden (2007)

There are many isoforms of the glucocorticoid receptor resulting from alternative RNA splicing and translation initiation of the GR mRNA, which contribute to the diverse effects of glucocorticoid hormones. It has been suggested that the anti-inflammatory action of glucocorticoids occurs via trans-repression of targeted gene by GR while the negative side effects are from the transactivation of targeted genes by GR (De Bosscher, 2010). Therefore separating these two modes of action would lead to enhancement of

therapeutic features, minimize side effects and drug resistance encountered in diseases such as asthma that require prolonged glucocorticoid therapy (Baudy et al., 2012).

GR transrepression actions are carried out mostly by protein–protein binding. GR can down regulate gene expression by interactions between monomeric GR and the other proteins involved in transcription mechanism for example nuclear factor-kappa B (NF-kB) and activator protein1 AP1 (c-jun and c-fos) that regulate the inflammation process (Bladh et al., 2005). As part of anti-inflammatory process GR suppresses the activity of mitogen-activated protein kinases MAPK through negative effect on their phosphorylation (Sotelo-Rivera et al., 2017, Beck et al., 2013a, Beck et al., 2009b, Yoshino et al., 2001, Holler et al., 2000, Vanden Berghe et al., 1998, Bandyopadhyay and Faller, 1997). More examples are in table 6.

Table 6 Examples of GR affected genes

Affected gene	Type of effect	
GLIZ	upregulation	(Ng et al., 2017)
osteogenic genes	repression	(Pico et al., 2016)
beta-catenin and c-myc	upregulation	(Jozic et al., 2017)
metallothionein 2A (MT2A) gene	upregulation	(Sato et al., 2013)
interleukin 11	repression	(Rauch et al., 2010)
skeletal muscle atrophy-associated MuRF1 gene	upregulation	(Waddell et al., 2008)
COX-2-	suppression	(Brewer et al., 2003)
in basal cellular and extracellular PLA2 activity	suppression	(Kol et al., 1998)
interleukin 2	upregulation	(Lamas et al., 1993)

1.1.3.3.4 GR cofactors

Cofactors regulate GR transcription and function and include several categories of regulators: p300, CREB-binding protein (CBP), p300/CBP associated factor (PCAF) that control the acetylation of histones (acetylation facilitates the transcription process), activator of thyroid hormone and retinoid receptors (ACTR), steroid receptor coactivator-1 (SRC1), or other cofactors that can modulate the chromatin in an ATP dependent manner.

Szapary et al. (1999) demonstrated that number of cofactors including transcriptional intermediary factor 2 (TIF2), steroid receptor coactivator 1 (SRC-1), and amplified in breast cancer 1 (AIB1) act as coactivators of GR transcription. Other cellular factors act as corepressors such as silencing mediator for retinoid and thyroid-hormone receptors (SMRT), which is known to facilitate GR's repressive effect. Ronacher et al. (2009) reported that ligand selectivity of GR mediated transactivation and transrepresson can be determined by cofactor recruitment.

Many cofactors cooperate and participate in gene transcription and those are: thyroid hormone receptor associated protein (TRAP), glucocorticoid receptor interacting protein 1 (GRIP 1), activated recruited cofactor (ARC), TATA box-binding protein (TBP), TBP associated factors (TAFS), the general transcription factors (TFIIA, TFIIB, TFIIE, TFIIF, TFIIH), the enzyme RNA polymerase-II (RNA pol II. On the other hand the corepressors complex with GR and suppress the gene expression (through recruiting the enzymes which deacetylate the histones histone deacetylases (HDACs). Examples of corepressors

are nuclear receptor corepressor (N-CoR) silencing mediator of retinoid and thyroid hormone receptor (SMRT).

GR is a positive modulator of signal transducer and activator of transcription 5 (STAT5) genes (Martinez et al., 2015). It also remodels chromatin and modulates the adipogenesis by control of the lipolytic and antilipogenic genes transcription. It can interact with wide range of transcription factors and co-factors, for instance suppression of activation of heat shock transcription factor -1 (Thompson et al., 2005).

1.1.3.3.5 GR post-translational modifications

Proteins are exposed to a covalent process of post-translational modifications (table 7), which includes methylation, sulfation, phosphorylation, lipid addition, glycosylation and other modifications which may modify protein activity (Kim et al., 2006). N-terminal glycosylation is common form of PTM that occurs through addition of oligosaccharides to the expressed protein. PTMs of the glucocorticoid receptor can be considered as one mechanism involved in regulation of GR mediated target gene specificity. Glucocorticoid receptor has been described to undergo methylation (Guo et al., 2017), ubiquitination, acetylation(Murphy et al., 2005, Kovacs et al., 2005, Matthews et al., 2004, Ito et al., 2000) and SUMOylation (Druker et al., 2013). These are important PTMS of GR which affect its function and the best studied is GR phosphorylation (Jovicic et al., 2015), as kinases target GR and lead to enhanced or repressed GR transcriptional regulation (Krstic et al., 1997), and alter the transcriptional activity of GR (Oakley and Cidlowski, 2013). And the phosphorylation sites at GR are sensitive to ligand activation (Kadmiel and Cidlowski, 2013)

PTMs events also involve addition of ubiquitin molecule to certain residues in the affected protein, by aid of ubiquitin enzymes (E-1, E-2 and E-3 enzymes), which achieve activation, conjugation and ligation. This process is followed by degradation by 26S proteasome. GR is ubiquitinated at lysine 419 (K419) and targeted by proteasome-ubiquitination pathway leading to inhibition of its function due to proteasome degradation action.Davies et al. (2011), reported that p300 / tetratricopeptide repeat domain 5 (TTC5) cofactors have the capacity to protect GR against ubiquitin degradation. This can affect GR transcriptional activity.

SUMOylation term is derived from SUMO-1 which stands for Small Ubiquitin-Related Modifier-1 protein, that is covalently linked to the amino acid lysine in GR. SUMOylation in GR occur at K277, K293, K313, K297 and K703, and K721 (K refer to the amino acid lysine) sites which were catalyzed by sumo-conjugating enzyme ubc9. This modification can effect GR transcription, expression, and the mechanism of action, which may be either up or down regulated depending on the SUMOylation site. It has been suggested that SUMOylation may switch off GR activity and GR mediated gene expression (Paakinaho et al., 2014, Druker et al., 2013).

Tian et al. (2002) have identified Ubc-9 targeting GR-NTD; Lysine 277 and Lysine 293, changes had been demonstrated to influence proteins interplay. In addition, the Lysine 703 is an Ubc-9 binding site located in LBD.

Acetylation is the process where the functional acetyl group is added to the amino acid lysine on proteins. This process is controlled by histone acetyltransferase and histone deacetylase (HDACs) enzymes. GR is acetylated at K494 and K495 and that alters its

transcriptional activity. GR modulates inflammation through negative regulation of histone acetylation of inflammatory biomarkers (Ito et al., 2000). Chronic DEX derived bone defects are attributed to crosstalk between GR, and both Histone Deacetylase 6 HDAC6 and osteoblast late marker osteocalcin (OCN)(Rimando et al., 2016). Also HDAC1 and HDAC2 are regulating expression of several genes such as STAT3 (Icardi et al., 2012). The fork head transcription factor FoxA1 regulates glucocorticoid receptor (GR) activity by promoting acetylation at H4K16 (Belikov et al., 2012).

Nitrosylation means the interaction between the sulfur-containing amino acid (such as cysteine Cys in GR) and nitric oxide (NO) or reactive nitrogen species (RNS) generating S-nitrosothiols (such as Cys656), this process interferes with and can change protein function (Martinez-Ruiz and Lamas, 2004). S-nitrosylation impairs GR ligand binding thereby interrupting GR anti-inflammatory therapeutic effect and the response to GCs therapy (Duma et al., 2004, Galigniana et al., 1999).

1.1.3.3.6 GR phosphorylation

Phosphorylation is addition of a new phosphate molecule to the GR on site of serine (Ser) and/or Threonine (Thr). The N-terminal domain residues in the GR have been shown to undergo phosphorylation. GR phosphorylation is linked to GC response or resistance in leukemia cells (Lynch et al., 2010). GR activity can be modulated by its phosphorylation, in particular GR is targeted by glucocorticoid-inducible kinase 1 (SGK1) at Ser422 and Thr256 and there is crosstalk between protein phosphatase 5 (PP5) and GR (Sotelo-Rivera et al., 2017, Zhang et al., 2016, Pazdrak et al., 2016, Li et al., 2016, Carruthers et al., 2015, Hinds et al., 2014, Adzic et al., 2013).

GR activity depends on the level of phosphorylation at numerous sites, which have been

identified at Ser203, Ser211, and Ser226, Tyr8, Ser45, Ser234, and Ser267 sites (fig.17). Phosphorylation of Ser211 is mediated by p38 and CDKs. On the other hand phosphorylation by MAPK, JNK, ERK and GSK-3 has also been described to target GR (Pocuca et al., 1998, Adzic et al., 2009, Lynch et al., 2010, Popovic et al., 2010, Adzic et al., 2013).

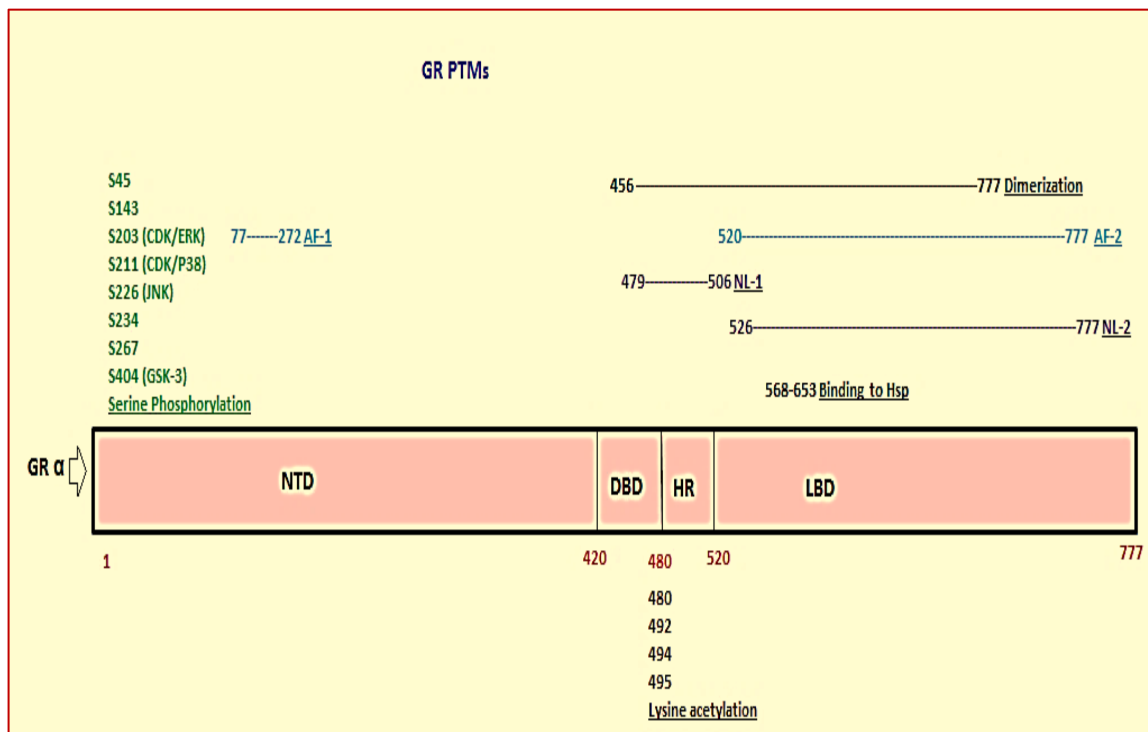


Figure 17 Illustration of the main posttranslational modifications of Glucocorticoid receptor alpha and their corresponding domain location (adapted and modified from Kino (2010)

The cyclin-dependent kinases CDKs [cyclin E/cyclin-dependent kinase 2 (Cdk2) phosphorylate GR at S203 site and cyclin A/Cdk2 phosphorylates S203 and S211 upon ligand activation. Phosphorylation by cyclin A/Cdk2 up regulates GR transcriptional activity. The p38 MAPKs and the c-Jun N-terminal kinases (JNKs) phosphorylation phosphorylate GR at S226 leading to inhibition of its function. The glycogen synthase kinase 3 (GSK-3), ERK and casein kinase II are other kinases involved in GR

phosphorylation. Furthermore GR is phosphorylated at higher level at S211 in hormone treated cells. The phosphorylation mechanism can be reversed through another process called de-phosphorylation; through the action of phosphatase enzymes such as PP1, PP2a, and PP5, which are capable of dephosphorylating GR (Blind and Garabedian, 2008). It has been found that GR phosphorylation at S211 and S226 are regulated by neuroendocrine stress (Simic et al., 2013). Additional possible phosphorylation sites of GR are found to be at S203, S211, S226, S404, S45, S134, S234, S267 and T8 as in (Galliher-Beckley and Cidlowski, 2009)

Table 7 Examples of GR post-translational modifications

PTM	Effect	Reference
GR ubiquitination	down-regulation of glucocorticoid receptor	(Wang and DeFranco, 2005)
reduced histone H3 acetylation of GR promoter I7	reduced GR expression	(Park et al., 2017)
increased DNA methylation at the NGFI-A (nerve growth factor-induced protein A) binding site of the NR3C1	lower NR3C1 expression	(Vukojevic et al., 2014)

promoter in male		
DNA methylation in the 1-F promoter region of the GR gene	glucocorticoid insufficiency or down regulation of cell surface GR expression that, in turn, results in GR resistance (GCR)	(Kantake et al., 2014)
SUMOylation at lysine 297 (K297) and K313 and K721	regulates GR the activity on target genes.	(Druker et al., 2013)
Phosphorylation at GR serine 220 (pSer220GR)	promoted GR activity	(Brossaud et al., 2017)
GR phosphorylation at serine 211 (pGR-211) and at serine 226 (pGR-226)	Correlated with negative activity	(Jovicic et al., 2015)
GR phosphorylation on serine 226 and serine 211	Effect on NOXA and Mcl-1 gene expression and then apoptosis	(Lynch et al., 2010)

1.1.4 Cell death

There are several different types of cell death (fig.18) represented by necrosis, apoptosis, autophagy and necroptosis, which have been classified according to underlying signalling pathway. Cell death types mostly share similar triggers, factors and mediators or under certain circumstances one form can overlap with another such as necroptosis which is a programmed necrosis of cells (Bibel and Barde, 2000). Specific profiles are characteristics of each death type, which enables the pathologist to distinguish between them. Such morphological changes correlated with inflammatory reaction and disease condition as appear in necrotic organs and sometimes accompanied by pathogens (virus, bacteria, fungi, parasites)(Wyllie et al., 1980).

Necrosis is cell damage in response to a physical injury, external trauma or is related to disease or abnormal conditions which cause the plasma membrane to rupture and cells to die (Leist and Jaattela, 2001). Autophagy is the cellular ingestion of itself (auto cannibalization) in which engulfment of the cell cytoplasm and intracellular organelles occurs within organelles called auto phagosomes (Levine and Kroemer, 2008).Necroptosis can be defined as a regulated form of necrosis, which is controlled by RIP1, RIP3 and MLKL and is also affected by PTM of these mediators and their crosstalk with caspase-8. Necroptosis is often associated with several inflammatory disorders

(Christofferson and Yuan, 2010).

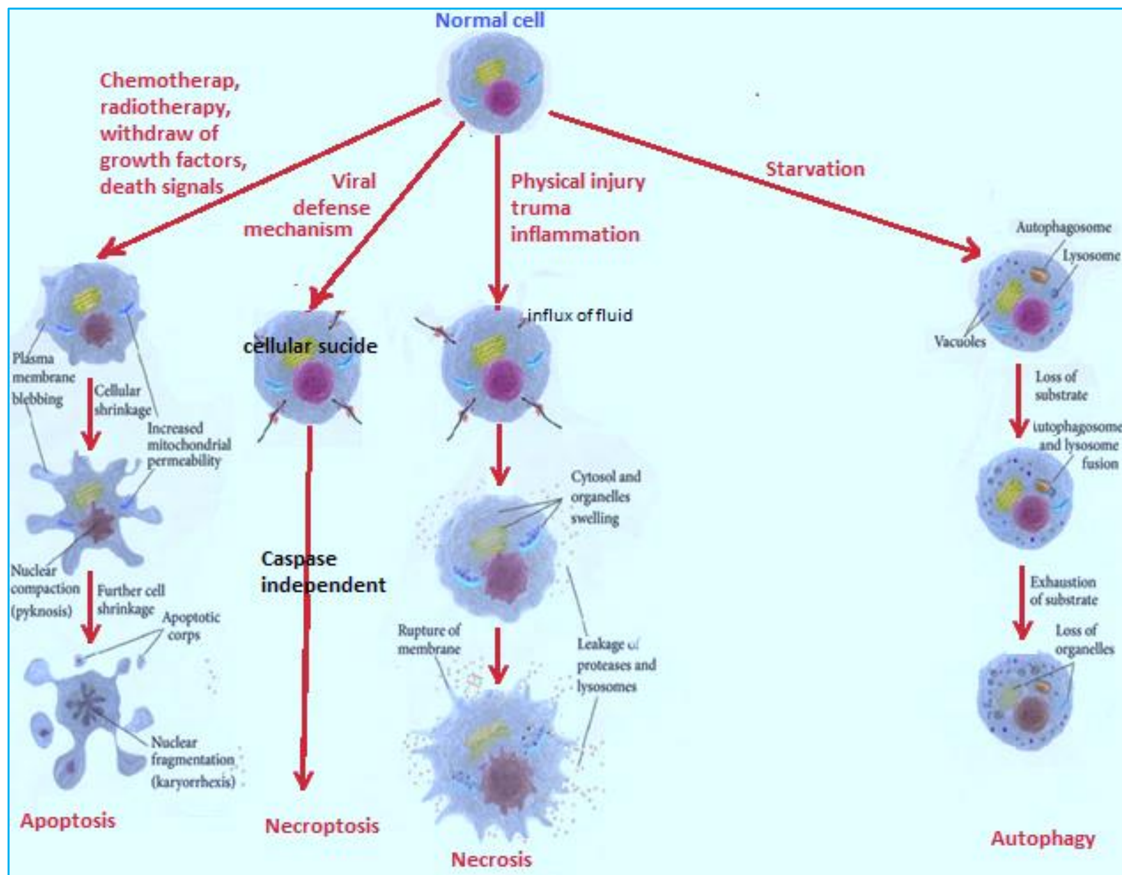


Figure 18 Cell death scheme and main routes

The three types apoptosis, necrosis and autophagy can be microscopically distinguished by the unique cell morphology accompanied each type as updated fromNunes et al. (2014).

The most important type of cell death is Apoptosis, which refers to the programmed cell death (PCD).The term PCD has been first given to this type of cell death by Richard and William in 1964 who explained the disappearance of muscular structures of the silk moth during different life stages in (Lockshin and Williams, 1964).

PCD is essential to maintain the normal development and is controlled by Cysteine Aspartyl Proteases (Caspases). Apoptosis involves a series of biological, chemical, and physical changes leading mostly to beneficial outcomes, for example the apoptosis of cancer cells caused by therapy. The harmful apoptosis is usually a result of malfunctioning and impairments of proper physiological mechanisms in the body.

Apoptosis (shown in fig.19). is controlled by complex pathways named extrinsic and intrinsic according to the type of trigger and signalling pathway although they sometimes happen together upon anticancer agents treatment (Tsai et al., 2016) . Generally, the extrinsic pathway is starting by binding of death ligand with the death receptor (Saralamma et al., 2015), the former transmits the signal inside the affected cell. Death receptor belongs to the TNF family which is characterized by the presence of a death domain; this domain is responsible of translating the outer signal into the programmed death pathway. Examples of cell death ligands are the FasL (CD95L), TNF-α, Apo3L, Apo2L, TRAIL, while examples of death receptors are FasR (APO-1), TNFR1, DR3, DR4 and DR5. The death ligand – death receptor form a complex with CAP proteins/adapters (CAP1, CAP2 (FADD), CAP3, CAP4, TRADD), this complex then interacts with Pro-Casp8 to form death-inducing signaling complex DISC. This complex later activates Caspase-8, once Casp8 becomes active, execution phase will begin by activating three caspases 7,6,and 3 which initiate the beginning of death process (Chen et al., 2016). This process starts by shrinking of the cells, and then disintegration of the DNA and organelles and membrane blebbing, then the apoptosis bodies are separated from the mother cell following by engulfing by phagocytes, to be completely removed from the body. Apoptotic cells exhibit phosphatidyl serine at the external surface of cell membrane. In some occasions DISC directly affects BID cleavage, leading to formation of tBID, which adheres to the mitochondria and follows intrinsic pathway, thus providing a link between the extrinsic and intrinsic processes.

The intrinsic mechanism of apoptosis includes a series of actions that utilize the mitochondria. This pathway is triggered by various biological or non-biological stimuli or so called ' cell stressors' for instance cell ischemia (Wu et al., 2016), DNA damage, ER

stress, stress hormones such as glucocorticoids, chemotherapy, oxidative stress caused by ROS, NO or GSH and pathogens (Mycobacterium) (Tsai et al., 2016, Lin et al., 2015, Yaoxian et al., 2013, Kristen et al., 2013, Seitz et al., 2010). These signals lead to loss of mitochondria membrane potential (surface integrity), and activation of pro-apoptotic BCL-2 members, which regulate the apoptosis by activating the apoptosis initiators Bax and Bak. They make pores in the membrane of the mitochondria to release Cytochrome-C, which forms complex with Apaf-1 and procaspase-9 leading to multiprotein complex “apoptosome“. This apoptosome activates caspase-9, which eventually activates the apoptosis effector caspases resulting in cell death (Tsai et al., 2016). At this stage the apoptotic cell can be distinguished from necrotic and autophagic cells by protruding blebs from the surface and distribution of cytoplasmic and nuclear material in these structures. Subsequently, blebs separate from the cells and then are lysed by macrophages, while the necrotic cells are swollen with damaged cytoplasmic membrane leading to rupture of the cell and diffusion of the cytoplasmic granules to the area leading to diseased conditions. Autophagy cells are identified by cytosolic phagocytic molecule autophagosome which is controlled by autophagy-related genes (ATG) (Wang et al., 2017a).

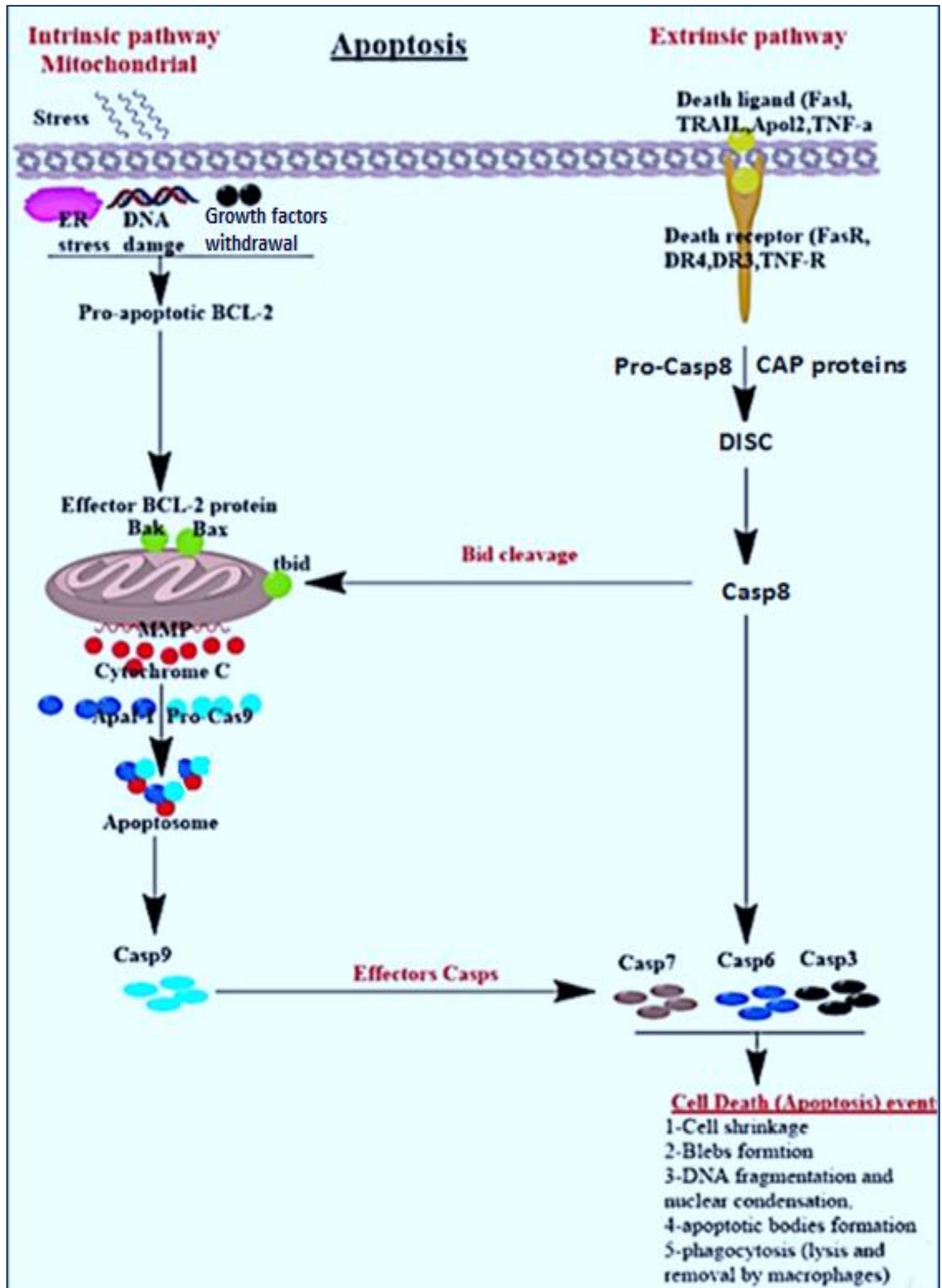


Figure 19 Main pathways of apoptosis

Schematic illustration of intrinsic and extrinsic pathways of apoptosis leading to caspases activation and triggering of cell death (drew using ChemDraw Professional, 2016).

Programmed cell death can be either caspase dependent or caspase independent, the caspase dependent apoptosis is described above. On the other hand, the caspase independent pathway may contribute to cell death by unusual mechanism of apoptosis (Nikoletopoulou et al., 2013). This pathway of cell death is of therapeutic importance in glioma cells which involve the PARP-1/AIF signalling pathway following external radiotherapy due to oxidative damage of DNA's (Zhang et al., 2017). Caspase - independent pathway is illustrated in fig 20

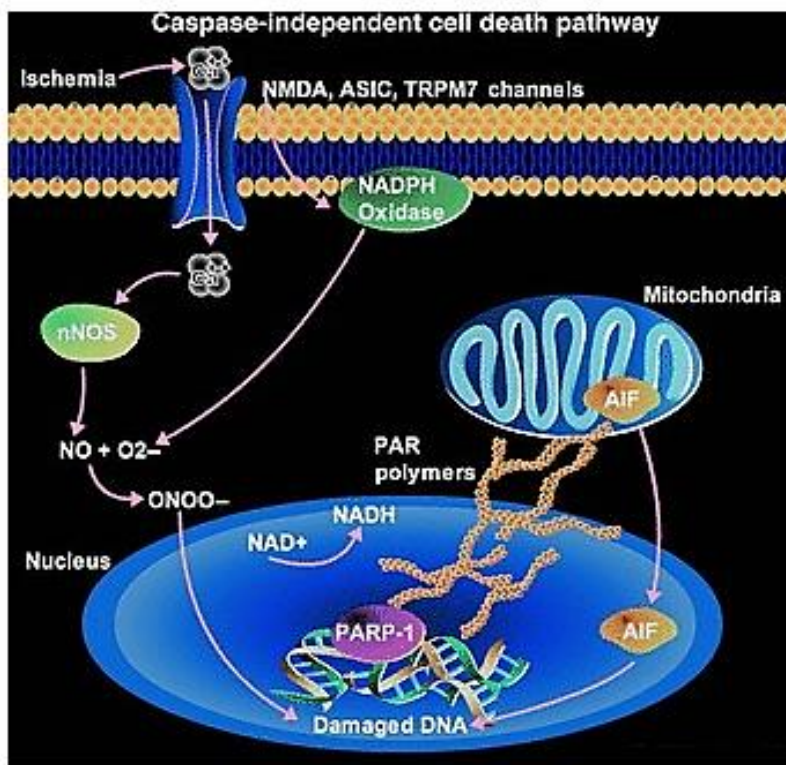


Figure 20 Caspase independent cell death.

The figure illustrates the main mechanisms which lead to caspase independent cell death**.** Ischemia, for instance, lead to penetration of calcium via NMDA, ASIC, or TRPM7 channels, then nitric oxide will be synthesised leading to the damage of DNA of the cells and cell death. Adapted from (Siegel and McCullough, 2011). nNos = Neuronal nitric oxide synthase, ONOO−= peroxynitrite, NO= nitric oxide,O2−=superoxide.

1.1.4.1 GR role in control of apoptosis

GCs are used in treatments of certain types of cancers due to GR ability to induce apoptosis (Schlossmacher et al., 2011). GCs are used to treat blood cancer such as multiple myeloma (MM) and ALL (Kervoelen et al., 2015). GR is capable of inducing apoptosis in a cell specific manner, as not all cells are sensitive to this cytotoxic effect. For example fibroblasts are not affected by DEX while osteocytes are much more sensitive to low concentrations of DEX (Mostafa et al., 2011). Researchers found that DEX can also induce apoptosis in monocytes in a dose dependent manner (Schmidt et al., 1999).

Furthermore, GCs give rise to cell cycle arrest and apoptosis in lymphoid cells (Vayssiere et al., 1997). It is documented that GC stimulate apoptosis in leukemia cells through its steroid receptor. It has also been suggested that both transactivation and trans repression of genes could lead to apoptosis (Saenz et al., 2015, Jing et al., 2015, Liu et al., 2014, Tao et al., 2013, Liu et al., 2013, Guo et al., 2013, Wasim et al., 2012, Heidari et al., 2012, Heidari et al., 2010, Carlet et al., 2010). DEX induces apoptosis in T-lymphocytic leukemia via GR activation of Programmed cell death 1 (PD-1) expression (Xing et al., 2015). It can induce apoptosis by caspase dependent pathway and through changing mitochondrial membrane permeability leading to cellular death. In addition, GC dependent apoptosis leads to activation of pro-apoptotic Bcl-2 protein family members. This family of proteins possesses Bcl-2 Homology (BH) domains (BH1, BH2, BH3, and BH4), and its members can have pro or anti-apoptotic effects (Taylor et al.,

2008). Bcl-2 family (table 8) is important for GC action and modulates GC resistance in ALL (Ploner et al., 2005, Geley et al., 1996, Tsujimoto, 1998).

In particular, B-cell CLL/lymphoma 2 (BCL2) can inhibit GR- initiated- apoptosis and influence cell survival by either modulation of apoptosis or effects on the cell cycle. Also BCL-xL is anti- apoptotic protein and stimulates proliferation of cells by affecting the G0 (resting phase) of the cell cycle, whereas BCL2-associated X protein (BAX) has pro-apoptotic properties through its effect on S-phase. Moreover, apoptosis and cell cycle are regulated by multi-domain BCL2 family members (MCL-1) the anti-apoptotic biomarker. BCL2-associated agonist of cell death (BAD) is pro-apoptotic marker which is the BH3-only group of Bcl-2 family. BH3 Interacting Domain Death Agonist (BID) is pro-apoptotic member of this family, which binds other members to mediate the damage of mitochondria and provide link between intrinsic and extrinsic pathway.

One of the main mediators of cell death induced by GR is proapoptotic and Bcl2-interacting mediator of cell death (Bim). Bim enhances programmed cell death by its BH3 domain and has the ability to antagonize the effects of some anti-apoptotic markers. Alternative splicing produces BIMEL (extra-large), BIML (large) and BIMS (small) isoforms (O'Connor et al., 1998), which consist of 198 aa, 138 aa, and 112 aa respectively, all have BH3 region in their structure (Genes, 2017a).

It is believed that GR mediated apoptosis is linked with Bim expression. Heidari et al. (2012) have studied the relationship between GC-apoptosis and BIM in leukemia cells and their study revealed that apoptosis induction in leukemia cells is accompanied by up-regulation of BIM, C-JUN and Runx2 in GC sensitive cells whereas this interaction are missing in GC resistant cells. This mechanism can be blocked by inhibition of MAPK, thus investigating Bim (which is regulated transcriptionally) and Bim affected targets is

important in order to explain how cell death occurs in different cancers. Another report by Jing et al. (2015) suggested that BIM mediated GC induced apoptosis by specific mechanism involves GR-Bim binding at the internal promoter region of BIM. However other reports indicated that main mode of GR mediated induction of Bim is through indirect mechanisms (Adams and Cory, 2007).

It has been found that Programmed Cell Death 4 (Pcd4) gene was up-regulated upon GC therapy inducing apoptosis. In addition PTMs like phosphorylation which takes place at Ser211 accelerate GR apoptotic efficiency (Lankat-Buttgereit and Goke, 2003).

Table 8 GR effect on BH3-family members

Target protein	**Type of response**	**Type of apoptotic cells**	**Reference**
BAX Bim, Bcl-xL Bak	upregulation upregulation upregulation upregulation	thymocyte apoptosis.	(Prenek et al., 2017)
Bax/Bcl-2 ratio	increased	germ cell apoptosis	(Mukherjee et al., 2015)
Bim	upregulation	multiple myeloma	(Kervoelen et al., 2015)
Bim BCL2	upregulation downregulation	pediatric acute lymphoblastic leukemia cells	(Jing et al., 2015)
BCL2	downregulation	spinal cord injury (SCI)	(Maldonado Bouchard and Hook, 2014)
BCL2	upregulation	Small cell lung cancer	(Schlossmacher et al., 2013)
Bim BCL-XL MCL-1	upregulation	human plasmacytoid dendritic cells	(Hong et al., 2013)
Bim	upregulation	ALL cells	(Beach et al., 2011)
Bim	upregulation	WEHI7.2 and S49.A2 murine lymphoma cell, CEM-C7 cells and in primary murine thymocytes	(Wang et al., 2003)
Bim	upregulation	ALL	(Wang et al., 2010)

However apoptosis does not always have a favourable effect as the early administration of GCs in pregnancy can affect foetus and cause unwanted permanent central nervous system damage due to early apoptosis of nervous cells (Lanshakov et al., 2016). In addition to the crosstalk between glucocorticoid receptor (GR) and T cell antigen receptor (TCR) have been suggested (Jamieson and Yamamoto, 2000)

GR causes downregulation of transcription proinflammatory genes /mediators via suppression of their transcription factors such as AP-1 and NF- κB (Hermoso and Cidlowski, 2003). NF-κB has also attributed to Warburg effect and alteration of metabolism in tumours (Johnson and Perkins, 2012). The NF-κB also modulates the inflammation and immunity (Hoesel and Schmid, 2013). NF-κB contributed to cancer as it is family member of v-Rel oncogen, and NF-κB affected genes are seen to be modulated during many cancers. The stimulation of NF-κB signaling pathway is preceded by inflammatory events or by inflammation accompanied by a developing cancer (Karin, 2009)

1.1.5 Selective glucocorticoid receptor modulators or agonists:

1.1.5.1 Overview of SGRM/SGRA

Glucocorticoids anti-inflammation properties are believed to be due to GR mediated inhibition of transcription of its pro-inflammatory target genes, while GC-induced side effects may be due to GR mediated activation of transcription of its target. However, some negative effects may commence from both transcriptional activation and transcriptional repression. The compounds that can bind GR and activate it to generate selective anti-inflammatory or anti-cancer effects separately from the adverse effects are given the term of selective glucocorticoid receptor modulators (Schacke et al., 2004).

Some of these compounds are non-steroids that are selected to mimic some or all of the steroid beneficial effect (Buijsman et al., 2005).

Recent efforts in GC application in medicine have focused on GR studies and invention of novel compounds that promote the trans-repression actions of GR with dissociation of its positive from negative action on gene expression. The selectivity in steroid receptors targeted therapy has been studied for number of years and first selective modulators of sex hormones were established in seventies (Giannini et al., 2015, Sherman et al., 1970). For example SGRM is Org 214007-0 ,which showed effective anti-inflammatory characteristics and GR binding efficiency in murine model of acute and chronic inflammation without the glucose related side effects (van Lierop et al., 2012).

1.1.5.1.1 Examples of SGRM

Many compounds have been tested for their GR targeting and dissociative characteristics. Another known SGRM is ZK 216348, which has been developed for its potential in cutaneous anti-inflammatory effect in mouse skin cell line. This compound revealed side effects similar to GC in terms of diabetes and inhibition of ACTH, though, less skin lesions accompanied the treatment (Schacke et al., 2004).

Furthermore, ZK 245186 is promising anti-inflammatory agent applied for various skin inflammatory conditions, with minor adverse effects (Schacke et al., 2009). ZK 245186 which is also called BOL-303242-X can not only be used for cutaneous lesions but also for ophthalmic allergic disorders (Zhang et al., 2009a) and eye inflammation, (Baiula et al., 2011) as this SGRM exerts anti-inflammatory effect without increase in intraocular pressure of the treated eye, which was verified in cat eye inflammation model (Kato et

al., 2011). Shafiee et al. (2011) have investigated the anti-inflammatory efficiency of this compound against rabbit dry eye syndrome. This compound which is called Mapracorat was suggested to treat dry eye disease (Cavet et al., 2010) and was tested for transactivation of myocilin which was suggested to cause side effects upon administration of classical steroids in eye. No expression of this biomarker was observed upon administration of Mapracorat (Pfeffer et al., 2010), Proksch et al. (2011). Several other researchers have studied the anti-allergic and anti- inflammatory properties of this compound. Stamer et al. (2013) tested the drug in primary cultures of human trabecular meshwork (TM), (Baiula et al., 2014) in conjunctivitis model of guinea pigs and demonstrated activity against allergic conjunctivitis.

Berger et al. (2017) have found that tetrahydronaphthalenes act as a strong SGRM, while others (Okamoto et al., 1998) reported that EPC-K1 (L-ascorbic acid 2-[3,4-dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-2H-1-benzopyran-6-yl hydrogen phosphate] potassium salt) regulates GR by maintaining GR activity under oxidative conditions. Compound K (C-K), 20-O-D-glucopyranosyl-20(S)-protopanaxadiol), protopanaxadiol ginsenoside metabolite, is another GR ligand which was designed to control lethal bacterial sepsis and regulate the inflammation, via a GR-transrepresson pathway (Yang et al., 2008). In addition, it has a protective role against the acute inflammation experimentally induced by zymosan (Cuong et al., 2009). The up to date SGRM are listed in table 9.

Table 9 The known SGRM

SGRM	Action	Condition	System	Published
Fosdagrocorat (PF-04171327	anti-inflammatory	rheumatoid arthritis	patient	(Shoji et al., 2017)
Sutherlandia frutescens	anti-stress and anti-inflammatory anti-hypertensive	forskolin stimulated conditions	COS-1 cells, adrenal H295R cell model	(Sergeant et al., 2017)
CORT 118335	stress response regulation	forced swim stress (FST)	male rats	(Nguyen et al., 2017)
AZD5423	autoimmune suppressor	Allergen-induced asthma	Healthy and asthmatic people	(Melin et al., 2017)
JTP-117968	anti-inflammatory effects	TAT protein and mRNA Transactivation	rat hepatoma cells primary human hepatocytes	(Kurimoto et al., 2017)
AZD5423	no effect detected	chronic obstructive pulmonary disease COPD	patients	(Kuna et al., 2017)
mapracorat	anti-inflammatory	skin inflammation model	Canine (dog)	(Baumer et al., 2017)
SA22465	drug penetration into meibomian gland tested	eye	Rabbit	(Asano et al., 2017)
C108297	anti obesity and anti-inflammatory	diet induced obesty and LPS induced Inflammation	C57Bl/6 J mice and RAW 264.7 cells	(van den Heuvel et al., 2016)
CORT118335	anti-obesity	diet-induced obesity.	C57BL/6J mice	(Mammi et al., 2016)
Compound -A	nosubstantial otoprotective capacities	noise trauma model	guinea pigs	(Landegger et al., 2016)
Compound -A	no induction of leptin or ob-r in human oa synovial fibroblasts	osteoarthritis	human osteoarthritis synovial fibroblasts	(Malaise et al., 2015)
Compound -A	growth inhibition effect on of colon cancer-derived myofibroblasts	colon cancer	colon cancer-derived myofibroblasts CT5.3hTERT cells	(Drebert et al., 2015)
mapracorat (BOL-303242-X),	anti-inflammatory	ocular inflammation	human ocular cells - Keratocytes	(Spinelli et al., 2014)
compound A	inhibition of immune-inflammatory diabetes	type 1 diabetes	mice	(Saksida et al., 2014)

compound 14, a t-butyl containing derivative-non steroid	anti-inflammatory	collagen-induced arthritis	mouse	(Razavi et al., 2014)
rigid steroid 21-hydroxy-6,19-epoxyprogesterone (21OH-6,19OP)	coadjuvants in the treatment of solid tumors	transrepression assays	*In vitro* - epithelial lung cancer cells-A549 cells	(Orqueda et al., 2014)
CORT108297	normalization of hippocampus parameters-promising therapy for human ALS	human amyotrophic lateral sclerosis (ALS)	Mutant Wobbler mice	(Meyer et al., 2014)
MK-5932	anti-inflammatory with no increase in glucose level	rat contact dermatitis,collagen-induced arthritis and adjuvant-induced arthritis models	In vivo-rat and dog In vitro- human whole blood	(Brandish et al., 2014)
Mapracorat ZK245186 or BOL-303242-X)	powerful anti-inflammatory	inflammatory skin and ocular disorders	Phase II Clinical trials	(Baiula and Spampinato, 2014)
C108297	selectively abrogation of pathogenic GR-dependent processes in the brain	depression	In vivo-rat brain	(Zalachoras et al., 2013)
GW870086X	no side effect on homeostasis	(GC)-induced glaucoma	trabecular meshwork	(Stamer et al., 2013)
Mapracorat	anti-allergic effects	allergic conjunctivitis	human conjunctival epithelial cells (HConEpiC) and human conjunctival fibroblasts (HConF),	(Rauner et al., 2013)
Org 214007-0	anti-inflammatory	insulin resistance	In vitro -THP1 cells ,primary human whole blood cells t In vivo-mice	(Cavet et al., 2013)
ZK209614	ophthalmic anti-inflammatory and anti -allergic effects,	external eye diseases	In vitro transrepression andtransactivation assays	(Kato et al., 2011)

			In vivo-rat and cat	
Compound A (CpdA	anti-inflammatory	experimental autoimmune encephalomyelitis (EAE), multiple sclerosis	In vivo-mice	(van Loo et al., 2009)
L5 (Roohk et al., 2010- tetrahydro-4H-benzo[f]indazol-5-yl]-[4-(trifluoromethyl)phenyl]methanol).	L5 dissociates the pleiotropic effects of the GC	disease-relevant target pathways	n vivo in mice	(Roohk et al., 2010)
AL-438	few side effect on chondrocytes	growth plate chondrocytes	In vitro-murine chondrogenic ATDC5 cell line In vivo-Fetal mouse	(Owen et al., 2007)

1.1.5.1.2 Compound A

Compound A (CPDA) is a dissociated non-steroidal glucocorticoid receptor modifier which was created as a stable analogue, derived from the African shrub plant known as the *Salsola tuberculatiformis Botschantzev (Zhang et al., 2009b)*. Compound A as a novel SGRM, has been suggested to act by separating transcriptional repression from transcriptional activation by GR , thereby regulating the inflammation without the side effects of the conventional GC (Rauner et al., 2011). In most cases, CpdA interacts with various plasma steroid-binding globulins, proteins or enzymes. The chemical structure is 2-((4-acetophenyl)-2-chloro-N-methyl) ethyl ammonium chloride. This particular shrub-derived compound can effectively induce the glucocorticoid receptor's trans-repression action by inhibiting AP-1, CRE/ATF, Elk01, Ets-1, NFATc, and SRF, however it doesn't induce the GR-mediated transactivation (Beck et al., 2013b). It is capable of inducing

the anti- inflammatory or immune-regulatory effects of the glucocorticoid receptor (Liberman et al., 2012). It has been demonstrated that CPDA reduced the growth of colon cancer cells (Drebert et al., 2015) induced suppression of NF_kB, the proinflammatory mediator and its downstream signaling pathway to produce the significant effect, thus providing advantage over the conventional GC therapy which modulates a wide range of inflammatory mediators via the genomic effect to produce both beneficial and harmful effects (Lesovaya et al., 2015).

Current research studies indicate that 2-((4-acetoxyphenyl)-2-chloro-N-methyl) ethyl ammonium chloride has the ability to modulate the immune response of bone marrow-derived dendritic cells (BMDC) by down regulating the pro-inflammatory mediators and $NF\text{-}_kB$ (Barcala Tabarrozzi et al., 2016). Another report, indicated that the compound induced down regulation of nuclear factor $NF\text{-}_kB$ and AP-1, this function enhanced apoptosis of bladder cancer cells and GR trans repression effect (Zheng et al., 2015). This compound can be administered as a replacement for prednisolone therapy in *in vitro* model of human arthritis without the known steroid side effects (Malaise et al., 2015). *In vivo* and *in vitro* application of CPDA on mouse skin model of inflammation revealed potential therapeutic effects without the side effects obtained with GC i.e. keratinocytes atrophy (Klopot et al., 2015). It has been demonstrated experimentally that CPDA can be used systematically for inner ear problems in Guinea pigs (Honeder et al., 2015), it was also used to treat mouse experimental rheumatoid arthritis with less side effects than when GCs are used (Rauner et al., 2013). In cancer studies, CPDA suppressed the proliferation rate of primary T-ALL cells by a GR- dependent mechanism and enhanced GR-trans repression of NF-Kb and AP-1 (Lesovaya et al., 2013).

Sundahl et al. (2015), claimed that CPDA does not show the undesired hyperglycaemic and hyperinsulinemia metabolic disorders, it is also free of HPA negative feedback control while estimation of negative regulation revealed inhibition of several inflammatory mediators mainly IL-6, IL-8 and TNFα.

In vivo and *in vitro* research on CPDA revealed the anti-inflammatory effect similar to DEX-mediated regulation of pro-inflammatory genes (Beck et al., 2013b),(Liberman et al., 2012).Interestingly, CPDA does not cause GR dimerization thus it dissociated GR transactivation effect. This was explained in a study where CPDA suppressed levels of the rat corticosteroid-binding globulin (CBG), adrenocorticotropic hormone (ACTH), and luteinizing hormone and did not cause any expression in tyrosine amino transferase in liver cell lines HepG2 (Robertson et al., 2010). CPDA has been used *in vivo* (rat model) to treat experimental autoimmune neuritis (EAN) successfully with potent anti-inflammatory effects and less pro-inflammatory reaction (Zhang et al., 2009b). The classical GC activation and proposed selective glucocorticoid receptor modulators are presented in fig.21.

Robertson et al. (2010), demonstrated that CPDA had led to reduction in Corticosteroid Binding Globulin and ACTH measurements in laboratory animals (Rat). The transcription of these molecules is inhibited upon glucocorticoids therapy, however the researchers found that CPDA was not capable of inducing overexpression of TAT3 and the coupled CPDA-GR translocate to a nucleus as a single- monomeric pair to produce GR nuclear effect, while still displaying the transrepresson effect represented by downregulation of CBG and ACTH.

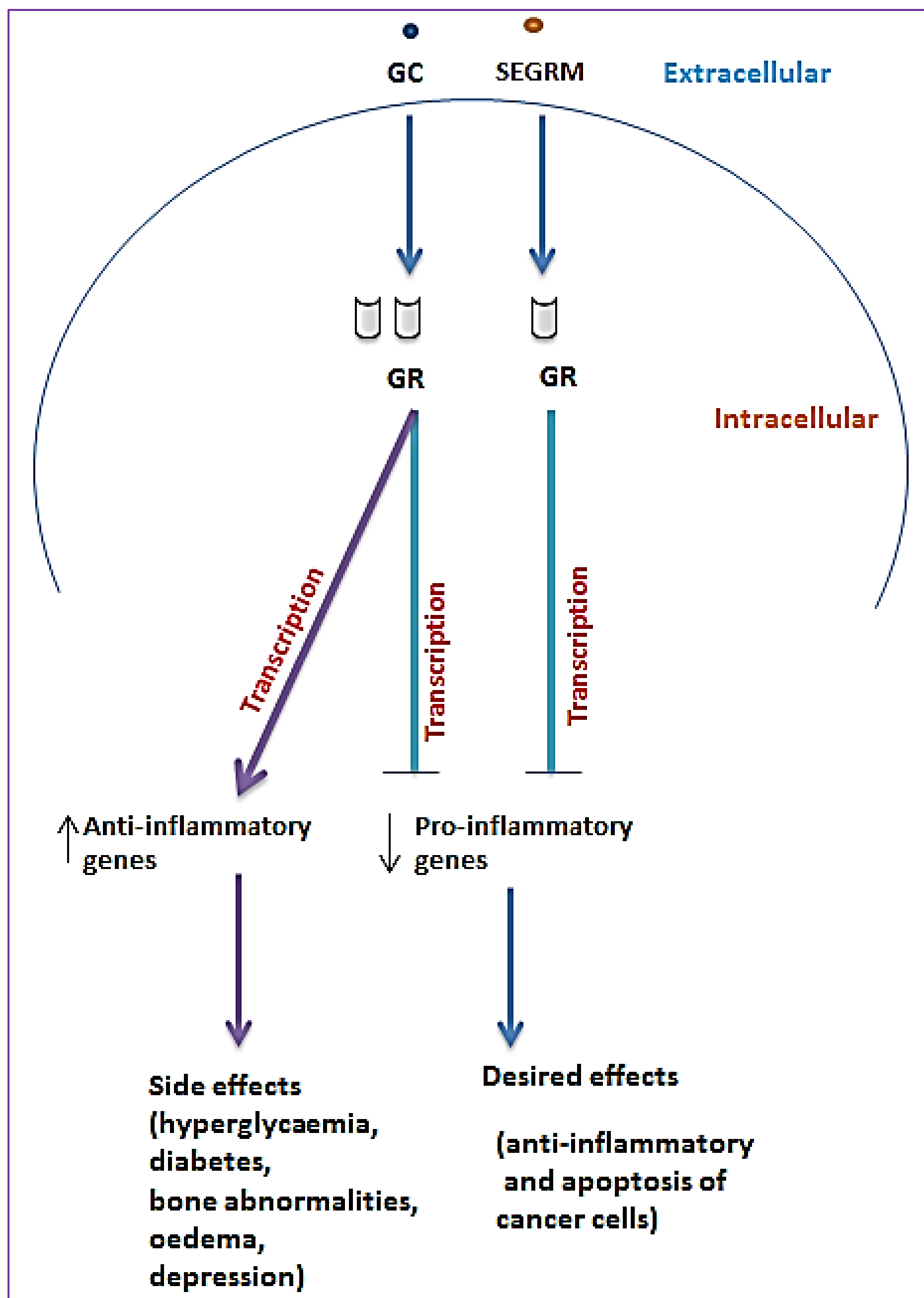


Figure 21 Proposed SEGRM mode of action. The figure adapted from Cheng et al. (2014)

As mentioned above chronic use of synthetic glucocorticoids brings destructive osteoskeletal deformities, hence CPDA was investigated for potential effects on bone forming cells, the osteoblasts which are obtained from bone marrow, in an attempt to detect CPDA role in formation and deformation of bones. The results revealed that

CPDA had efficiently regulated the inflammatory response in these cells via inhibition of cytokines IL1, IL6 and TNF-α similar to DEX, while not promoting or inhibiting bone formation (Rauner et al., 2011).

Macrophage stimulation of cellular immunity and phagocyte recruitment is managed by type 1 T helper (Th1) cells which secrete interferon-gamma, interleukin
IL-2, and tumour necrosis factor (TNF)-beta. Those cells are activated upon bacterial or viral infection. Type 2 Th (Th2) cells create IL-4, IL-5, IL-6, IL-9.IL-10 and IL-13, and are responsible for humoral immunity, eosinophil activation, and inhibition of several macrophage functions. Th2 cells are activated upon parasitic infestation (Romagnani, 1999)and(Romagnani, 2000). The Th1/Th2 cell ratios of human peripheral blood and endometrial T cells are distinguished via fluorescent activated cell sorter by detection of the surface marker CD3. Also the specific intracellular cytokines are expressed by each type , interferon gamma for Th1 and interleukin 4 for Th2 (Saito et al., 1999). Immunity related problems were previously correlated with Th1 cells, so that classical medication are supposed to enhance Th2 rather than Th1, in this context, Liberman et al. (2012), have confirmed that CPDA was not found to encourage the upregulation of GR target genes via GRE (the action normally seen upon GC binding with GR). Reportedly, CPDA beneficial effects are through negative regulation of Th1 and positive regulation of Th2 controller genes (T-bet and GATA3). Importantly, the transactivation function did not occurred via GRE but through p38-MAPK derived phosphorylation of GATA-3.

Beck et al. (2013b), demonstrated that CPDA inhibited the expression of NF-κB target genes through effect on the translocation of NF-κB to the nucleus of tested cells (A549). To estimate the anti-cancer effect of CPDA, Lesovaya et al. (2013), tested it in different blood cancer cell lines (T lymphoma, B Lymphoma and Multiple Myeloma) and primary

acute lymphocytic leukemia cultures. They determined the crosstalk between proteasome and CPDA as cells previously treated with suppressors of proteasome revealed overexpression of GR and downregulation of NF-κB and AP-1 (trans repression function). Rauner et al. (2013), studied mice to compare the side effects on skeleton for both DEX and CPDA. The latter has no effect on Procollagen type I N-terminal propeptide (PINP) in comparison to the significant downregulation of this marker of fibrogenesis upon DEX, nevertheless, a significant anti-inflammatory effect of CPDA has been shown in addition to WBC migration Inhibition and downregulated TNF-α (Suttitheptumrong et al., 2013). This SGRM prevented the development of inflammatory diabetes in mice via regulation of Th1 and Th2 immune cells response (Saksida et al., 2014). Honeder et al. (2015), have added other uses of CPDA in treatment of trauma related ear problems in guinea pigs. CPDA effect on GR signalling have been assessed in mice ear inflammation model and *in vitro* murine keratinocytes cell line and the results demonstrated relevant anti-inflammatory modulation role linked to CPDA (Klopot et al., 2015). Lesovaya et al. (2015), referred to the efficiency of CPDA in the relief of inflammatory and immune disorders, whereas Malaise et al. (2015), studied the effect of CPDA on leptin which is part of GC-GR trans activated genes and usually upregulated after treatment of arthritis with GC and is the main reason of osteoporosis. Their results have confirmed that CPDA produced the anti-arthritic effect while not inducing leptin receptor transcription or overexpression of leptin in fibroblasts taken from inflamed human joints. However most relevant effects of CPDA are shown in table 10.

Table 10 Reported effects of compound A.

	Protein/gene	Effect on expression	
1	IL2P70	activation	(Barcala Tabarrozzi et al., 2016)
2	MCP1	activation	
3	TNF-α	activation	
4	(NF)-κB transcriptional activity	inhibition	(Zheng et al., 2015)
5	activator protein 1 transcriptional activity matrix	inhibition	
6	metalloproteinase-2	inhibition	
7	matrix metalloproteinase-9,	inhibition	
8	interleukin-6,	inhibition	
9	vascular endothelial growth factor,	inhibition	
10	IL-6	inhibition	(Malaise et al., 2015)
11	IL-8	inhibition	
12	MMP-1 MMP-3 protein	inhibition	
13	hepatocyte growth factor	inhibition	(Drebert et al., 2015)
14	IL-1β TNF IL-6	inhibition inhibition inhibition	saksida, 2014 #412}
15	IL-10	activation	
16	IFN-γ (mRNA)	inhibition	
17	IL-17 (mRNA)	inhibition	
18	NF-κB	inhibition	(Rauner et al., 2013)
19	IFN-γ	inhibition	
20	TNF	inhibition	
21	Hsp70 genes	upregulation	(Beck et al., 2013b)
22	IFN-γ	decreases	(Liberman et al., 2012)
23	IL-5 production	increase	
24	TNF-α protein	inhibit	(Rauner et al., 2011)
25	IL4 mRNA	upregulation	(Zhang et al., 2009b)
26	FOX-P3 mRNA	upregulation	

CPDA effect may extend to other steroid receptors. This was suggested by Zheng et al. (2015), who provided evidence that CPDA inhibited the growth of bladder cancer cells and xenografts through GR trans repression pathway (by inhibition of NF-κB and AP-1 transcriptional activities) and suppression of androgen receptor (AR) expression (this steroid receptor is responsible for survival of bladder tumor). Finally, Barcala Tabarrozzi et al. (2016) confirmed that CPDA is efficient in dendritic cells immunity regulation via inhibition of NF-κB transcriptional activity.

1.1.5.1.3 Overview of non-steroidal compounds used in the study

In this study four other compounds have been tested (fig. 22). Compounds were chosen by Dr John Hadfield based on similarity in their chemical structure to Compound A in terms of one carbon ring forming the compound. Tyramine or 4-(2-aminoethyl)phenol or Tyramine monochloride is monoxide derived from tyrosine. Tyramine hydrochloride or 4-(2-aminoethyl)phenol hydrochloride, N-methyltyramine or 4-[2 (methylamino)ethyl]phenol and hordenine or 4-(2-(dimethylamino)ethyl)phenol were investigated for their antiproliferative activity and other parameters believed to be important for potential use of these compounds as anti-cancer therapeutic reagents. Tyramine acts on nervous system alpha-1 (α1) adrenergic receptor and is affected by anti-depressant medications (Ghose, 1980). n-methyltyramine (NMT/ CPDB) functions as an antagonist of alpha-2 (α2) adrenergic receptor (Koda et al., 1999) and is considered as a potent appetite-enhancer (Stohs and Hartman, 2015).

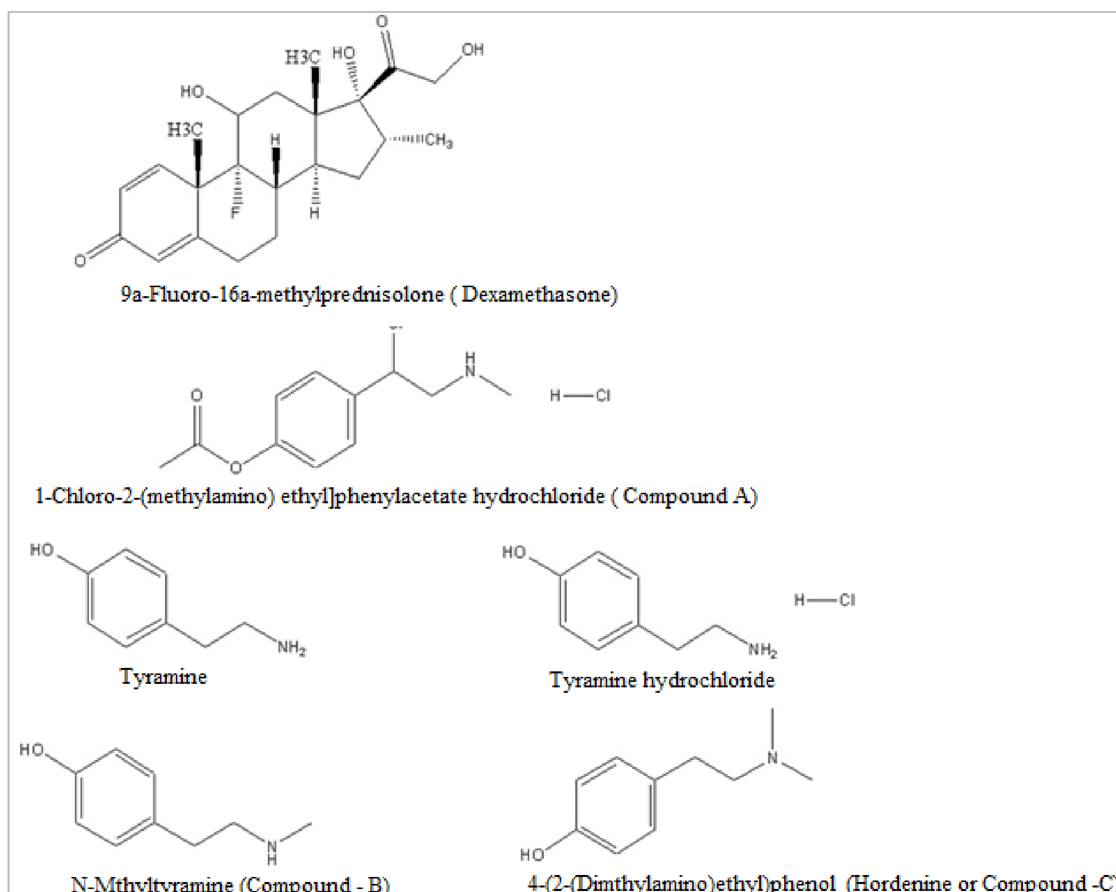


Figure 22 Chemical structure of tested compounds.

NMT was found to promote amylase, lipase, and peptidase production from the pancreas via the Vagovagal reflex (Tsutsumi et al., 2010). Hordenine is available in barley (Ma et al., 2015). It has cutaneous and melanogenesis effects through downregulation of Human cathelicidin antimicrobial peptide (CAMP), and prevents further pigmentation (Kim et al., 2013).Sommer et al. (2017) Virtual screening detected the relationship between hordenin and dopamine D2 receptor as either antagonist or agonist for this receptor. This effect extended to the parasympathetic system and it promoted the secretion of norepinephrine (Hapke and Strathmann, 1995), however no literature has been published yet about the potential role of these compounds in treatment regime of acute childhood leukemia.

1.2 Aims of project

This study was designed to investigate GR as a therapeutic target and explore if either steroids such as dexamethasone or non-steroid synthetic Compound A or similar compounds could be potentially better therapeutic solutions. Furthermore, the project aimed to investigate the effect of tested compounds in human and chicken cells towards developing better compounds for medicine and veterinarian approaches. The specific objectives are:

- To determine cytotoxic effects of GCs and potential GC dissociated compounds (Compound A, Tyramine and Tyramine hydrochloride, Compound B and Compound C) on examined cells (human derived leukemia cells, chicken derived leukemia cells, normal white blood cells, cancerous epithelial cells and normal epithelial cells)
- Experimental system utilised was acute lymphoblastic childhood leukaemia cell line CEM and its two variants CEM-C7-14 and CEM-C1-15 that are sensitive and resistant to glucocorticoids respectively as well as DT40 chicken lymphoblast cell line derived from chicken immune organ (bursa of fabricious) infected with avian lymphoid leukosis. This will allow differential analysis of sensitivity/resistance to GCs as well as comparison of GC actions in two species; Normal peripheral blood polymorph nuclear cells (PBMCs) as well as epithelial normal and cancer cells were used to determine their potential wider effects.

- To determine GR binding potential of studied compounds by using computer modelling techniques as a tool to characterize compounds properties through ligand Protein (GR) simulation using Vina dock tool, Stability studies via NMR aimed to determine the compounds stability.

- To evaluate anti-inflammatory properties of tested compounds by analysis of effects on inflammatory biomarkers such as cytokine interleukins IL-6, IL-10 and IL-2 mRNA levels and secretory interleukins 2 and 6 in response to GC or the investigated compounds treatment and /or stress.

- Molecular evaluation of drug effects on targets mRNA and protein levels using qRT-PCR and SDS PAGE followed by western blot. These experiments will determine effects of the tested compounds on GR as potential markers of GC response; GILZ as a determinants of glucocorticoid response in acute lymphoblastic leukaemia; known markers of apoptosis such as Bcl2-interacting mediator of cell death (Apoptosis Facilitator) (Bim) will be used as control for apoptosis and GR phosphorylation at S211 will be investigated as a important post translational modification of GR .

- To investigate cell death pathways utilized by these compounds and elicit cytotoxic effect by determining effects on cell cycle, apoptosis (Annexin 5), and by following caspase-8, reactive oxygen species and reactive nitrogen species levels.

2 Materials and Methods

2.1 Materials

2.1.1 Tissue culture reagents

Table 11 Tissue culture reagents

Materials	Supplier	Product code
CellTiter 96® AQueous MTS Reagent Powder 250mg	Promega, UK	G1112
CellTiter 96® Non-Radioactive Cell Proliferation Assay (MTT)	Promega	G4000
Chicken serum	Sigma, UK	C5405-500ML
Dextran Coated Charcoal (DCC)	Hyclone, UK	SH30068.03
DMSO (500 mL)	Fisher, UK	10213810
FBS (500 mL)	Labtech, UK	FB-1090/500
L-Glutamine (100 mL)	Labtech, UK	XC-T1715/100
PBS (10X)	Fisher, UK	10214733
Pen-Strep (100 mL)	Labtech, UK	LM-A4118/100
Propidium Iodide	Sigma, UK	P4864-10ML
Ribonuclease A From Bovine Pancreas	Sigma, UK	R5500-10MG
RPMI 1640 (560 mL)	SLS, UK	LZ12-167F24
Trypsin (500 mL)	Labtech, UK	LM-T1705/500

2.1.2 Tested compounds, antibodies and kits used in the experiments

Table 12 Tested compounds, antibodies and kits used in the experiments

Antibodies /compounds/ kit	Supplier	Product code
4-(2-(Dimethylamino) ethyl) phenol (CPDC)	Fluorochem, UK	239138
4-(2-(Methylamino) ethyl) phenol (CPDB)	Fluorochem, UK	222578
Annexin V-FITC Apoptosis Detection Kit,	eBioscience, UK	BMS500FI
Anti-beta Actin antibody	Abcam, UK	(Ab8227)
Bim Antibody (H-191)	Santa Cruz, UK	Sc-11425
Caspase-Glo® 8 Assay	Promega, UK	G8200
Compound A	Enzo Life Sciences, UK	ALX-550-516-M005
Dexamethasone	Enzo Life Sciences, UK	BML-EI126-0001
GR Antibody (H-300)	Santa Cruz, UK	Sc-8992
Griess reagent system	Santa Cruz, UK	
IL-2 Human ELISA Kit	Thermo fisher scientific, UK	EH2IL2
IL-6 Human ELISA Kit	Thermo fisher scientific, UK	EH2IL6
Lipopolysaccharides from Escherichia coli O111:B4	Sigma, UK	L2630-10MG
Phytohemagglutinin PHA-P	Sigma, UK	L9132
RNeasy® Plus Mini	Qiagene, UK	74134
Tyramine	Sigma-Aldrich, UK	T90344-5G
Tyramine Hydrochloride	Sigma-Aldrich, UK	T2879-1G
β-Actin Antibody (N-21)	Santa Cruz, UK	sc-130656

Precision OneStepPLUSTM qRT-PCR Master mix	Primerdesign, UK	
Page Ruler Prestained protein ladder	Fermentas, UK	SM0672
X - Ray film	Fuji Films, UK	Super Rx

2.2 Methods

2.2.1 Cancer cell lines

GCs-sensitive model of acute lymphoblastic leukemia (ALL), CEM-C7-14 and GC-resistant model of acute lymphoblastic leukemia (ALL), CEM-C1-15 cell lines were obtained from Brad E Thompson (The University of Texas, USA). These cell lines were derived from the parental line CCRF-CEM, grown in Dexamethasone to select for resistant and sensitive clones. Both cell lines were derived from 4 years Caucasian female suffering from acute lymphoblastic leukemia. CEM-C1-15 cells display a typical multiple drug resistance (MDR). CEM-C1-15 cell line is GCs -resistant clone obtained by growing CEM-C7-14 cells in dexamethasone (Medh et al., 1998). C7 cells are ALL cell line that undergoapoptosis when incubated with GCs while C1 are not sensitive to apoptosis initiated by GCs (Medh et al., 2003).

DT40 cells were kind gift from Professor Julian Sale (MRC Laboratory of Molecular Biology, Cambridge Biomedical Campus). These DT40 cells are chicken lymphoblast cell line derived from chicken primary immune organ (bursa of fabricious) infected with

avian lymphoid leukosis. Employing chicken DT40 B cell line is of importance due high replication efficiency and for easy monitoring of cell death, growth curve and gene expression (Winding and Berchtold, 2001), which serve as a prototype for experimental biology and molecular biology for the efficiency of homologous gene of interest (Molnar et al., 2014)

Epithelial cell lines; HACAT (Human immortalized keratinocytes primary adherent cell line), MCF-7(Human breast cancer adherent cell line) and BEAS2B (Human normal lung, bronchus epithelial cell line) were obtained from ATCC. Peripheral Blood Mononuclear Cells (PBMC), were kind gift from Dr Lucy Smyth, Lecturer in Human Physiology-University of Salford. A limited number of the PBMCs was available due to experiments and the types of performed assays and ethics application details. Especially the choice of CEM-C7-14 and CEM-C1-15 cell line are good system for GR sensitivity/resistance. Disadvantage of using this system is that extrapolating from cell lines to patients is difficult. However, given thatno good mouse models of ALL are available and time consuming/cost prohibitive, this system is important tool to uncovering potential new and better therapeutic approaches.

2.2.1.1 Maintenance of the cells

Roswell park memorial institute (RPMI)-1640 growth medium (Sigma) supplemented with 10% heat-inactivated foetal calf serum (FCS) and 1% penicillin/streptomycin (Labtech), 2 mmol/L L-glutamine (Labtech) was used to maintain CEM-C7-14 and CEM-C1-15 cells.

DT40 cells growth medium consisted of Dulbecco's Modified Eagle's Medium (DMEM) (ATCC® 30-2002™) supplemented with 7% heat-inactivated foetal calf serum (FCS) and 1% penicillin/streptomycin (Labtech), 2 mmol/L L-glutamine and chicken serum 3% (Sigma –Aldrich) supplemented with 50 µM of 2- mercaptoethanol from Sigma.

Epithelial cells HACAT and MCF-C7 were maintained in Dulbecco's Modified Eagle's Medium (DMEM) (ATCC® 30-2002™) supplemented with 10% FCS and 1% penicillin/streptomycin (Labtech). BEBM medium was used to grow BEAS-2B cells. Cells were transferred to media containing Dextran Coated Charcoal (DCC) treated FBS (from Cyclone) before treatment with compounds. PBMCs were defrosted from -80 freezers, fresh RPMI medium added, spun down using the centrifuge, then the medium replaced by RPMI medium with DCC then treatment added.

Cells were incubated at 37 ^{o}C in the incubator (Galaxy S -Wolf laboratories) in humidified atmosphere of 5% CO_2.

2.2.1.2 Passaging of the cells

Passaging of the cells was carried out 3 times a week for CEM-C7-14, CEM-C1-15 and epithelial cells were spitted twice a week, while DT40 cells were sub cultured every 24-48 h due to high proliferation rate. Cells were diluted in fresh medium to a density of about (0.3 – 1.0) × 10^6 cells/ml in either T 25 or T 75 flasks. Cells were inspected daily and counting was carried out using microscope Motif AE31 to ensure proper density.

2.2.1.3 Counting of the cells

Cells were counted to determine the optimal density. 10 µL was taken directly from cell suspension or diluted with trypan blue (to count the viable cells) and placed on disposable haemocytometer chamber (C-chips of dimensions 25 mm (W) x75 mm (L) x Thickness 1.6 mm from LabTech). Cells were examined under light microscope Motif AE 31 using 10X lens. The average of total number of cells was counted in the four large corner squares and cell concentration calculated according to this formula:

Total cells/ml=(Total cells counted/No. of squares) x dilution factor x 10^4 cells/ml

The viable cells number was measured by Trypan blue exclusion staining based on the fact that dead cells only can take the dye and viable cells exclude it. 1:1 volume of cell suspension and 0.4% trypan blue dye (dilution factor is 2) were mixed in Eppendorf tube and vortexed then incubated for less than 3 min at room temperature, then the indicated volume was taken and cells were counted following above mentioned protocol.

2.2.1.4 Freezing of the cells

To provide low passage numbers for the experiments, cells were cryopreserved by centrifugation at 1500 RPM for 5 mins, supernatant was removed, and then 2 ml sterile media consisting of 90% FCS and 10% DMSO, was added to the pelleted cells and mixed by pipetting. Afterwards the cells suspension was transferred into 2 sterile cryovials, 1ml each. Vials were immediately placed in a freezer box at -80 °C and for longer storage

transferred into the Dewar containing liquid nitrogen.

2.2.1.5 Thawing of cells

Cells were defrosted by placing them in a warm water bath at 37 ^{O}C for less than one minute; the content was transferred to T25 sterile flask containing 4ml fresh media, mixed carefully and kept in incubator for 24 h. Next day, the cells were centrifuged and the media replaced to remove DMSO.

2.2.2 Compounds used in the study

Table 13 List of the compounds used in the experiments, their molecular weight and the solvents used for in vitro assays

CPD Name	Molecular weight g/mol	Solubility	Pubchem ID
Dexamethasone	392.467	100% ethanol	5743
Compound A	264.146	100% ethanol	9838147
Tyramine	137.182	DMSO	5610
Tyramine Hydrochloride	173.64	H_2O	66449
N-Methyltyramine (CPDB)	151.209	H_2O	9727
Hordenine (CPDC)	165.236	H_2O	68313

2.2.3 Proliferation assay (MTS or MTT) to measure cytotoxic effect of the drugs

Proposed project design includes measuring cell proliferation upon treatment with above indicated compounds. Treatment with dexamethasone included doses between 0 µM and 100 µM for MTS assay, according to Petersen et al (2008). Concentrations of 1 µM are applied for Compound A, Tyramine, THCL, CPDB and CPDC throughout experiments.

Heat shock and cold shock were induced by incubating cells with the selected drug for the particular time then exposing them to high temperature 43 ^{o}C for 2 h if heat shock is induced, according to Debi et al., (2010) and Jaattelal et al., (1992). Cold shock was induced by exposing examined cells to 4° C for the same duration.

Proliferation rate of the cells grown in suspension was determined by using (Promega Cell Titer Aqueous One Solution) the tetrazolium compound 3-(4, 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy phenyl)-2-(4-sulfophenyl)-2Htetrazolium (MTS). This assay is chromogenic assay designed to measure cell viability under certain conditions. The principle of this assay is that MTS is converted by the mitochondria of living cells to soluble formazan and the absorbance can be measured to detect the changes in cell viability in response to various therapies, so that the assay measures metabolic activity. Cells were cultured and counted as described above, than 5000 cells in 100 µl were seeded per well in U-shape 96 well plate. Next day, cells were dosed with serial dilutions of the appropriate compound in 100 µl DCC media per well. Plates are incubated in 37 ^{O}C and 5% CO_2, humidified incubator for 72 hours. 20 µl of MTS reagent which is composed of MTS and the electron coupling agent phenazinemethosulfate (PMSF)

(PROMEGA), were added to the wells in dark atmosphere and the samples incubated for 3-4 hours inside tissue culture incubator. The absorbance of the plate is read using spectrophotometer at 490-540 nm.

Proliferation rate of adherent cell lines was determined using tetrazolium dye MTT 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide as described by (Seidl and Zinkernagel, 2013). The same protocol described for MTS assay was followed except that the formation of insoluble formazan needed addition of DMSO to be dissolved and to produce the purple distinct colour. The plate was read using (Multiscan Ascent / Thermo lab Systems) spectrophotometer with ascent software at 520-690 nm absorbances.

2.2.4 Flow cytometry

2.2.4.1 Cell cycle progression analysis -Propidium Iodide staining

Fluorescence Activated Cell Sorter (FACS) was used to assess the effect of studied compounds on cell cycle progression. The Fluorochrome dye Propidium Iodide (PI) that is capable of binding and labelling the DNA was used in this test (Ramirez de Molina et al., 2008) CEM-C7-14, CEM-C1-15, DT40 and PBMCs cells were seeded in 6 well plates at density of 0.5-1x10^6 cells per ml, then tested compound were added to the wells at final concentration of 1 µM, and then incubated for further 48 h. Cells were harvested and counted, then 1 x 10^6 cells are transferred to sterile tube, then washed 2 times with 2 mL of PBS and centrifuged at 1500 rpm for 5 min. Supernatant was discarded and cells were re-suspended in 1 ml 70% ice cold ethanol in PBS, mixed and stored at -20 °C for at less 30 min. Cells were centrifuged, the ethanol was decanted and washed twice with

2 ml PBS. DNA staining was carried out by mixing the pellet with 50 µl of a 100 µg/ml (Ribonuclease A Sigma) then incubated at room temperature for 30 min, followed by addition of 300 µl of a 50 µg/ml PI (Propidium Iodide Sigma). Samples were kept in the dark for 15 min, vortexes and examined by BD FACS verse^{TM}. Data was analysed through PE-A laser channel after adjusting both forward and side scatter of control sample using BD FACS Suite software and the reading was set as seen in fig .23.

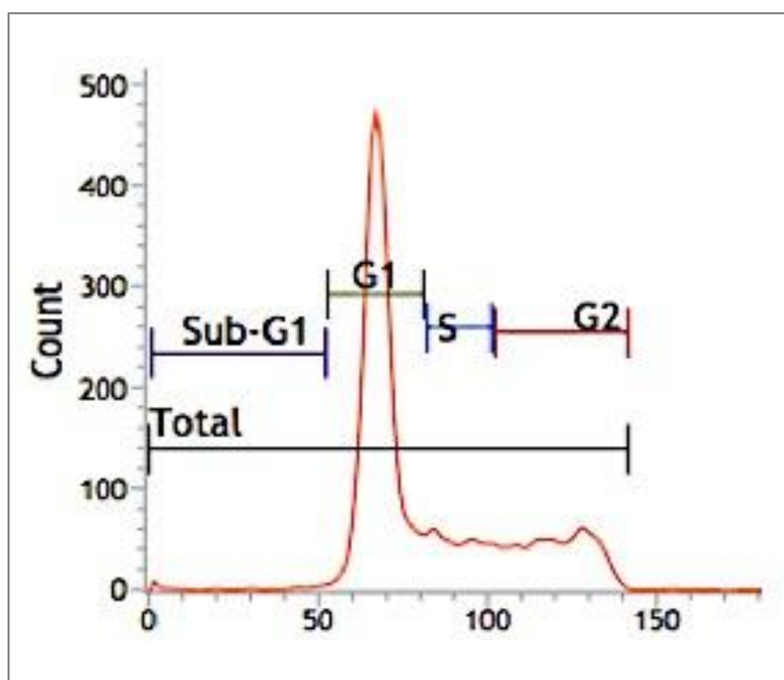


Figure 23 Example of the cell cycle profile

As a result of Propidium Iodide staining of cellular DNA gates were selected and refer to Sub-G1, G0/G1, S and G2/M phase respectively.

2.2.4.2 Apoptosis assay - Annexin V-FitC labelling

Annexin V-FITC labelling kit was purchased from eBioscience (BMS500FI/100) (Cai et al., 2008) and was employed to detect phosphatidylserine on the outer surface of the cell membrane of cells undergoing apoptosis. AnnexinV was found to bind strongly with

phosphatidylserine (PS) located in internal cell membrane in intact cells. In the apoptotic cells' PS is exposed on the external layer of cell membrane (Fig. 24). AnnexinV can bind to the PS-exposing apoptotic cells and can inhibit the pro-coagulant and pro-inflammatory activities (exhibits anti-phospholipase activity) of the dying cell. Fluorescein isothiocyanate (FITC) labelling allows simple detection by FACS analysis. Counterstaining by propidium iodide allows the discrimination of apoptotic cells from dead cells. CEM-C7-14, CEM-C1-15, DT40 and PBMCs cells were seeded in 6 well plates at density of 0.5-1x10^6 cells per well, each well was loaded with 3 ml of cells suspension, then tested compounds were added to the wells at final concentration of 1 µM, followed by incubation for 48 h. Then cells were washed in PBS by pipetting up and down and spun down at 1200 rpm for 4 min. Cell pellet was dissolved in 200 µl of binding buffer provided with the kit. 2-5x10^5/ml of cells was used for analysis. 5 µl of Annexin V-FITC was added to 195 µl of cell suspension, mixed and incubated for 10 min at room temperature. Cells were washed in 200 µl binding buffer and 190 µl of binding buffer with 10 µl propidium iodide (20µg/ml) was added followed by FACS analysis.

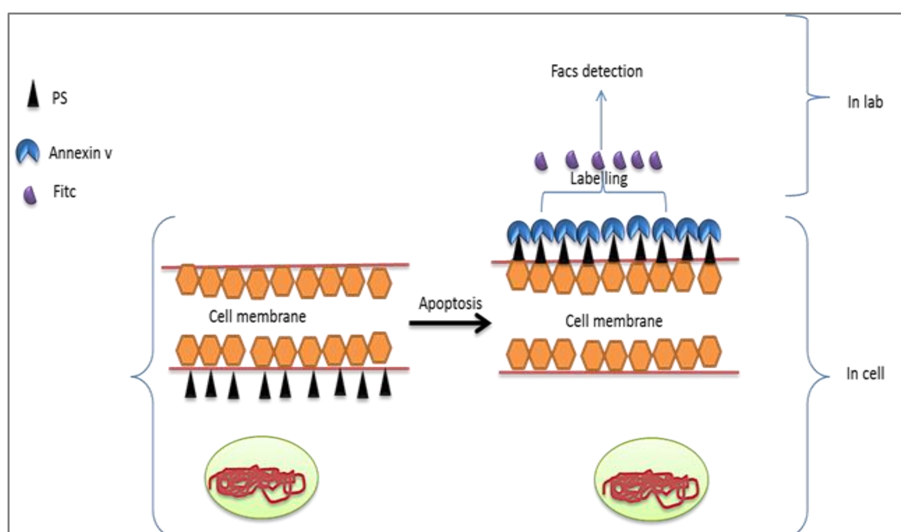


Figure 24 Diagram of AnnexinV assay workflow

Analysis was carried out using BD FACS verse^{TM}, by aid of BD FACS Suite software. Both PE channel and FITC channel of flow cytometry are used to determine apoptosis in cells, the selection was based on the distribution of the cells in the four quarters shown in (fig. 25) and the number was expressed as a percentage of population.

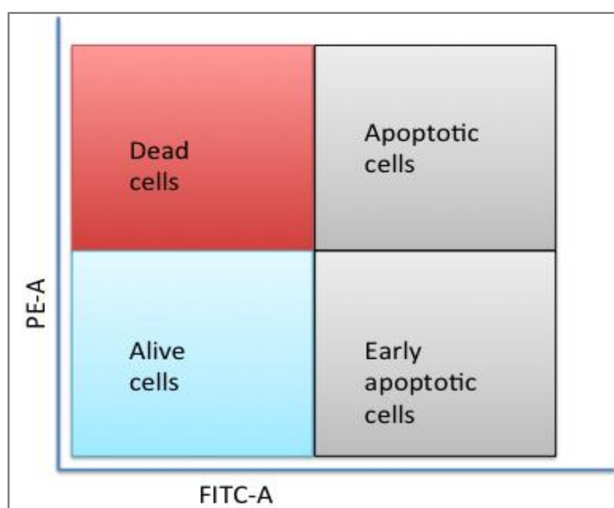


Figure 25 Flow cytometry setting for discrimination of apoptotic cells from dead and alive cells

2.2.4.3 ROS measurement- DCFDA staining to measure intracellular ROS

Reactive Oxygen Species (ROS) were detected using ROS Assay Kit from (Affymetrix eBioscience-Cat. No.88-5930-74), according to (Freemerman et al., 2014). ROS kit was composed of the cell permeant reagent 2’, 7’ –dichlorofluorescin diacetate (DCFDA). DCFDA is a fluorogenic dye that measures hydroxyl, peroxyl and other reactive oxygen species (ROS) activity within the cell. After diffusion into the cell, DCFDA is deacetylated by cellular esterases to a non-fluorescent compound, which is later oxidized by ROS into

2’, 7’ –dichlorofluorescein (DCF). DCF is a highly fluorescent compound, which can be detected by fluorescence spectroscopy with maximum excitation and emission spectra of 495 nm and 529 nm respectively.

CEM-C7-14, CEM-C1-15, DT40 cells and PBMCs were seeded in 6 well plates at density $1x10^6$ cells per well carrying 2 ml, then tested compound were added to the cells at final concentration of 1 µM, and then incubated for further 24 h. 100 µL of 1X ROS assay stain was added to each well and incubated for another 60 min in a 37 °C incubator with 5% CO_2. Then samples were transferred to Corning Falcon Round-bottom polystyrene Tubes and analysed with flow cytometry using FITC channel.

2.2.5 Immunoblotting assay

Immunoblotting procedures were as described previously (Andreou et al., 2012, Demonacos et al., 2001, Shikama et al., 2000) to detect the expression of protein of interest and investigate the intracellular pathways targeted by different compounds. Incubating suspended cells in flask with 1 µM of the selected compound overnight initiated the expected expression. Then cellular proteins extract was prepared, proteins separated using SDS PAGE and expression detected using western blot technique as described below.

2.2.5.1 Cellular extract preparation and determination of protein concentration

High salt lysis buffer (HSLB) was used to make whole cell extract (Table 14). Cell lysates were prepared from cells which grown in flask by spinning in centrifuge 1500 RPM for

5 min, the supernatant was removed and cell pellets washed twice with cold 1XPBS (prepared from dilution of 10XPBS stock which was composed of (80 g NaCl, 2 g KCL, 7.62 g Na_2HPO_4, 0.77 g KH_2PO_4 and H_2O Up to 1 L). Supernatant was removed and 120µl of ice cold HSLB was added to the pellet. In the next step the samples were transferred to sterile Eppendorf tubes and rotated for 20 min on the rotator at 4 ^oC, then centrifuged in micro centrifuge for 15 min at 13000 RPM at 4°C. Supernatant was then transferred to new Eppendorf tubes kept on ice for further analysis.

Protein concentration was measured by adding Bio-Rad Bradford dye (Bio-Rad Protein Assay Dye Reagent Concentrate from BIO-RAD) diluted with distilled water 1:5 (200 µL Bradford reagent was added to 800 µL distilled water). 2µl from protein extract was analysed together with blank sample at 595 nm absorbance. The absorbance was read using Jenway 6305 spectrophotometer. In order to analyse equal amount of protein by electrophoresis, the amount of taken protein was calculated by adjusting the sample of the low reading that equals to 40 µL and the sample with the high reading equals to (low reading x40/sample reading). 3X SDS Laemmle sample buffer (table 16)(composed of 1.87 ml Tris, ph 6.95 (1 M), 3 ml glycerol, 1.5 ml beta mercaptoethanol, 0.6 g SDS, H_2O up to 10 ml 0.01% of bromophenol aliquot) was used as loading buffer and half of volume was added to the sample to reach final volume of 1X SDS sample buffer. Samples were mixed and then incubated for 3 min at 95 °C to denature the proteins for SDS PAGE, or the samples were kept in -20 °C before being analysed by SDS PAGE.

2.2.5.2 Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS PAGE) and Western blotting

This procedure is often used to investigate a protein of interest (Roth et al, 2009). Here the procedure was used to semi-quantitatively determine protein levels of studied proteins. Gel casting apparatus (Mini-PROTEAN 3 System from Bio-Rad laboratories) was used to make gels. Resolving gel (Table 17) was poured between spacer plates and 0.1% SDS was used to overlay the solution that was left for 30 min to polymerize. Then 0.1% SDS was removed and stacking gel (Table 15) was added and a comb was inserted to form wells. The gel was placed in Bio-Rad electrophoresis mini buffer tank then 1XSDS-PAGE running buffer (Table 6) was added. The comb was removed and samples loaded using Hamilton syringe. The protein ladder was used as a molecular weight marker (Page ruler plus pre-stained protein ladder from Fermentas). Electrophoresis was started using electricity supplied by Power Pac Basic at 80 V until the proteins entered the resolving gel, then 110V current was applied until the bromophenol blue stained front reached the end of the gel. The gel was placed on Immobilon-P membrane (MILLIPORE™), that was previously soaked in methanol and then in the transfer buffer, and fitted between two filter papers and two sponges in the transfer cassette. The cassettes were placed in the transfer tank filled with the western transfer buffer (Table 16) in presence of ice holder. The tank was placed on the stirrer and connected with the power supply (Power Pack HCT™) and run at 0.4 A for an hour. Then ice was replaced after one hour, the transfer continued for another hour. The membrane was blocked by incubation on a rocking platform for 1 h in 5% milk /PBS to prevent nonspecific binding to antibodies. In the next stage of incubation the membrane was incubated overnight 4 oC with the primary antibody against the protein of interest by placing the membrane inside 50 ml

sterile universal tubes containing 4 µL Ab in 10 ml of 2.5% milk made in 0.1% Tween/PBS. Then was incubated on a roller at 4 ^{o}C overnight, next day the membrane was washed three times for 10 min each with 0.1% Tween/PBS on a rocking platform. After that, the membrane was incubated with secondary Ab diluted (1: 1000) in 2.5% milk made in 0.1% Tween/PBS dilution for 1 h at room temperature on a rocking platform and washed three times with 0.1% Tween/PBS.

The membrane was developed by soaking in chemiluminescent HRP substrate from Chembio Ltd for 30 seconds and exposing to either G-box to detect blot images or using X-ray film. The bands were quantified using image J software and data normalised to Beta-actin control.

2.2.5.3 Western blot solutions and buffers

Table 14 High salt lysis buffer

Chemicals	Final Concentration
HEPES pH 7.5	100 mM
NaCl	500 mM
EDTA	5 mM
Glycerol	1%
NP-40	0.5%
DTT	1 mM
phenylmethylsulphonyl fluoride PMSF	1 mM
Protease inhibitors (PI) a-Aprotinin b-Leupeptin c-Pepstatine	1 µg/ml
Sodium Orthovanadate (NaOV)	2 mM
β-Glycerol phosphate	20 mM
Sodium Pyrophosphate (NaPPi)	5 mM

Table 15 Gel for SDS -Page

Composition of 7.5% and 12 % gel for SDS-PAGE				
	7.5% gel		12% gel	
Solutions	Separating	Stacking	Separating	Stacking
Water	13.3 ml	6.73 ml	9.1 ml	6.73 ml
Acrylamide	7.0 ml	1.67 ml	11.2 ml	1.67 ml
Tris pH 8.95 (1.5 M)	7.0 ml		7.0 ml	
Tris pH 6.95 (1 M)		1.25 ml		1.25 ml
EDTA (0.2 M)	280 µl	100 µl	280 µl	100 µl
SDS (10%)	280 µl	100 µl	280 µl	100 µl
APS (10%)	157 µl	157 µl	157 µl	157 µl
TEMED	17 µl	17 µl	17 µl	17µl

Table 16 Running and Transfer buffers

Running buffer			
Composition of X10 SDS Running Buffer (Stock) (1 L)		**Composition of X1 SDS Running Buffer (1 L)**	
Reagent	Amount	Reagent	Amount
Glycine	144 g	X10 Running Buffer	100 mL
Tris base	30.2 g	ddH_2O	900 mL
ddH_2O	1 L		

Transfer buffer			
Composition of X10 Western Transfer Buffer WTB stock (1L)		**Composition of X1Western Transfer Buffer WTB (1L)**	
Reagent	Reagent	Amount	Amount
Glycine	X10WTB stock	100 mL	112.5 g
Tris base	Methanol	200 ml	33 g
ddH_2O	ddH_2O	Up to 1 L	1 L

Table 17 3xSDS Sample buffer

Reagent	**Amount**
Tris base PH 6.95(1M)	1.87ml
Glycerol	3 ml (10%)
5% Beta Mercaptoethanol	1.5 ml
SDS	0.6 g
ddH_2O	10 ml
Bromophenol Blue	10 µL

2.2.6 Quantification of relative gene expression

mRNA expression was determined using quantitative real time polymerase reaction qRT-PCR according to (Akimkin et al., 2011). Total RNA was purified by Qiagene RNeasy plus Mini kit. Precision One Step PLUSTM qRT-PCR Mastermix was used according to amplification protocols for DNA Engine Opticon 2 System (Bio Rad). Data were analyzed using Opticon Monitor™ Software version 3.1 and relative mRNA expression calculated according to comparative quantification method ($2^{-\Delta\Delta ct}$). Primers used in this study are listed in Tables 18 and 19.

Table 18 Human primers

Human primers			
Gene symbol	**Accession Number**	**Sense primer**	**Anti-sense primer**
IL2*	NM_000586	CCTATCACTCTCTTTAATCACTACTC	GTTTGTGACAAGTGCAAGACT
IL10	NM_000572	GCTGGAGGACTTTAAGGGTTAC	TGATGTCTGGGTCTTGGTTCT
NFKB1	NM_003998	GTAACTGCTGGACCCAAGGA	CCTCTGTCATTCGTGCTTCC
RPL19	NM_000981	GTTAGACCCCAATGAGACCAATG	GTCACAGGCTTGCGGATGA
BCL2L11	NM_138621	AGAAGATCCTCCCTGCTGTCT	CTTGGGGTTTGTGTTGATTTGTC
NR3C1*	NM_000176	TACGTGGGGGAAAAGAAAGTC	GCCAGATAACACATACATAGGAAAT
JUN*	NM_002228	ACCTAACATTCGATCTCATTCAGTA	TACAGAAGCAATCTACAGTCTCTATT
Il6	NM_000600	GCAGAAAACAACCTGAACCTT	ACCTCAAACTCCAAAAGACCA

* The primer found in mRNA but not in coding area

Table 19 Chicken primers

Chicken primers			
Gene symbol	**Accession Number**	**Sense primer**	**Anti-sense primer**
IL2	NM_204153	TCCCGTGGCTAACTAATCTG	TTTACCGACAAAGTGAGAATCAA
IL10	NM_00100441	GCTGTCACCGCTTCTTCAC	ATCCCGTTCTCATCCATCTTCT
NFKB1	NM_205134	GCGGACAGCACTACATACG	ATCTTTCACATCTTCTTCTTCTACATCAA
NR3C1	NM_001037826	ATCAGGGGACGAGGCTTTAG	TGAGGTTGTGGATGGAGAAGA
JUN	NM_001031289	TATAATAACGCCAAGGTGCTGAA	GTCGGGGGAGGTGAGGAT
RPL19	NM_001030929	GAGGCTCGCAGGTCCAA	CCGTTCACTTCTTGGTCTCTT
IL6	NM_204628	ATGGTGATAAATCCCGATGAAGT	TCTCCATAAACGAAGTAAAGTCTC
BCL2L11	XM_015283427.1	CTTCTTCTTCGTGCGGAGGT	AGAAGCCATTGAGTCCCAGC

2.2.6.1 Purification of Total RNA from the cells

RNeasy plus Mini kit (Qiagene- cat 74134) was used to extract RNA from leukemia cells.

The kit consists of specific tubes and buffers listed in table 20

Table 20 Contents of RNeasy plus Mini kit

Content of RNeasy plus Mini kit
gDNA Eliminator Mini Spin Columns (uncolored) (each in a 2 ml Collection Tube)
Collection Tubes (1.5 ml)
Collection Tubes (2 ml)
Buffer RLT Plus*
Buffer RW1*
Buffer RPE† (concentrate)
RNase-Free Water
Mini Spin Columns (pink) 50 250 (each in a 2 ml Collection Tube)

CEM-C7-14, CEM-C1-15, and DT40 cells were seeded in 6 well plates at density 1-2x10^6cells per ml well, and then tested compounds were added to the cells at final concentration of 1 µM, and then incubated for further 24 h. Later each well content was transferred to a 15 ml universal tube, centrifuged for 5 min at 1500 RPM, then the supernatant was removed and the cells washed twice with sterile phosphate buffer saline. Later on, the cells were disrupted by adding 350 µL of Buffer RLT Plus and vortexed thoroughly, and then the lysate was pipetted directly into a QIAshredder spin column placed in a 2 ml collection tube, and centrifuged for 2 min at maximum speed.

Then the homogenized lysates were transferred into gDNA eliminator spin column placed in a 2 ml collection tube and centrifuged for 30 s at 10,000 rpm (Figure 26). The columns were discarded, and the flow- through was saved. In the next step 350 µl of 70% ethanol was added to the flow- through, and mixed well by pipetting up and down. Next, 700 µL of the sample was transferred to RNeasy spin column placed in a 2 ml collection tube and centrifuged for 15 s at 10, 000 rpm. The flow-through was discarded and 700 µl of RW1 Buffer was added to the RNeasy spin column and centrifuged for 15 s at speed (≥10,000 rpm).Then the flow-through was discarded, and 500 µl of RPE Buffer was added to the RNeasy spin column and centrifuged for 15 s at ≥8000 x *g* (≥10,000 rpm), the flow-through discarded and 500 µl of RPE Buffer were added to the RNeasy spin column and centrifuged for 2 min at speed (≥10,000 rpm). After centrifugation, the RNeasy spin column was removed from the collection tube. Then the RNeasy spin column was placed in a new sterile 2 ml collection tube, then centrifuged at full speed for 1 min then the RNeasy spin column was placed in a new 1.5 ml collection tube and 30–50 µL of RNase-free water were added to the spin column membrane and centrifuged for 1 min at ≥8000 x *g* (≥10, 000 rpm) to elute the RNA. After that, purified RNA was stored at -80 ^{o}C. The concentration of RNA was determined by measuring the absorbance at 260 nm (A260) in a spectrophotometer (Nano drop) by placing 1 µL of purified RNA on the Nano drop pore after calibration with same amount of RNase- free water.

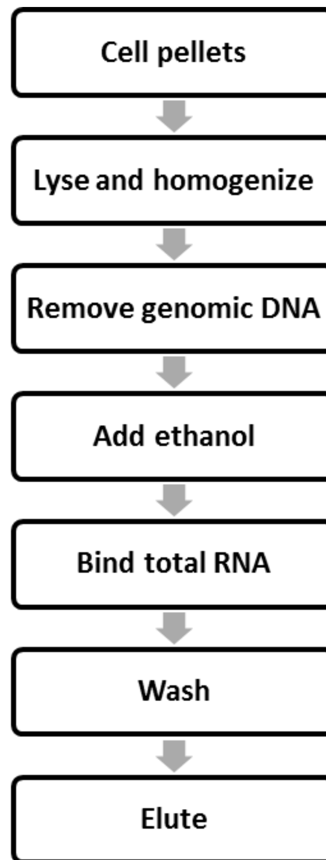


Figure 26 Diagram represents process of RNA extraction procedure.

2.2.6.2 qRT-PCR

Precision OneStepPLUS^{TM} qRT-PCR Mastermix system (from Primerdesign) was used for one step real-time PCR experiment. The Mastermix also contains buffer and $MgCl_2$ necessary for the amplification.

RNA samples, primers mixed with probe, master mix and RNAse free water are mixed in appropriate amounts listed below (tables 21 and 22) according to the manufacturer instructions to the final volume of 20 µL. Reaction was carried out in a specific 96 well plate in duplicate. The plate was centrifuged at 300 RPM for 30 sec then placed immediately in qPCR machine, which had been set according to the instruction provided by supplier (Primerdesign). Data was visualized by opticon monitor software

Table 21 Components of RT-PCR reaction / well setting for RT-PCR

Components	**1 reaction**
Precision OneStepPLUS^{TM} qRT-PCR Mastermix	10 µL
Primer/probe mix	1 µL
Template RNA (25ng)	x µL
RNAse/DNAse free water	x µL
Final volume	20 µL

Table 22 Amplication protocol. Adapted from primer design

	Step	**Time**	**Temperature**
	Reverse transcription	10 min	55 ^{o}C
	Enzyme activation	2 min	95 ^{o}C
Cycling x40	Denaturation	10 s	95 ^{o}C
	DATA COLLECTION	60 s	60 ^{o}C
	Melt curve		

2.2.7 Detection of nitric oxide by Griess reagent

In order to measure nitric oxide in examined cells upon treatment, Griess reagent system was used (Promega Cat.no. G2930), according to Chae et al. (2004). Method has been used extensively to detect nitrite (NO_2^-) in media of treated cells, which is one of two primary, stable and non-volatile breakdown products of nitric oxide. This was carried out on CEM-C7-14, CEM-C1-15, and DT40 cells. Cells were seeded in 6 well plates at density $1x10^6$cells per well carrying 2 ml of media, then tested compounds were added to the cells at final concentration of 1 µM, and then incubated for further 24h or cells were stimulated first by incubating for 24 h with 1 µg/ml Lipopolysaccharide LPS and 10 µg/ml phytohemagglutinin. Later, the well content was transferred to 15 ml universal tube, centrifuged for 5 min at 1500 RPM, then the supernatant transferred to new tube. 50 µl of each experimental sample was added to wells in 96 well plates, after that 50µl of the sulfanilamide solution was dispensed to all wells (materials in table 23). Then samples were incubated for 10 min in the dark at room temperature. Finally, 50 µl of the NED Solution was added to the wells and incubated for another 10 min. Appearance of purple colour of azo compound (as explained in fig. 27) was measured by monitoring absorbance within 30 min in a plate reader at wavelength between (520-550) nm.

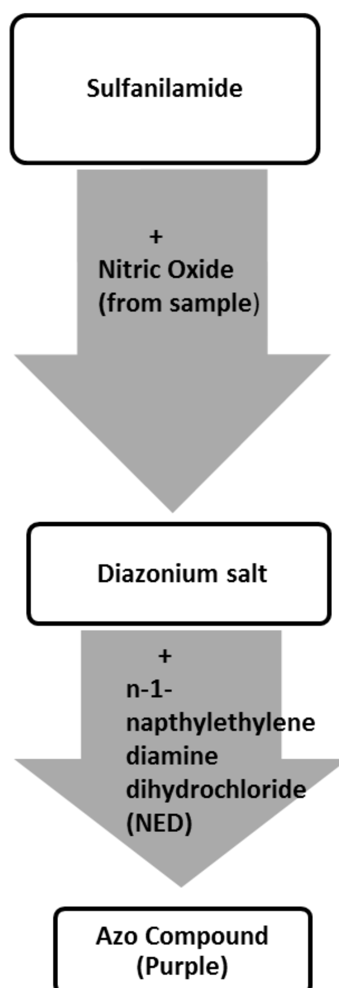


Figure 27 The principle of Griess assay

Table 23 Components of Griess Reagent

Material	Volume
Sulfanilamide	(2 × 25 ml)
N-1-napthylethylenediamine dihydrochloride (NED)	(2 × 25 ml)
Nitrite Standard	(0.1 M Sodium Nitrite)

2.2.8 IL-6 and IL-2 detection by ELISA assay

Secretory Interleukins were detected by capture ELISA method, using The Thermo Scientific Human IL6 and IL-2 kits (Affymetrix -eBioscience) for this experiment for which components were listed in Table 24. Capture assay -sandwich method was followed for this purpose, illustrated in (Fig. 28 and 29).

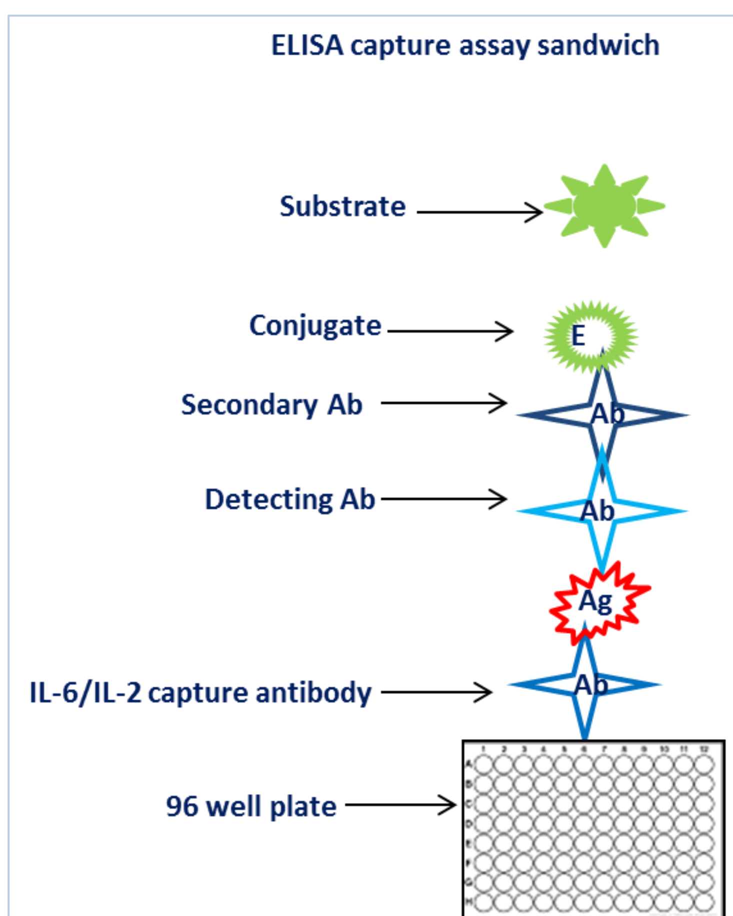


Figure 28 Principle of ELISA assay

The assay was performed in 96 well plate provided with the kit that has been coated with the capture antibody of the interleukin of interest. In this case two cytokines are followed; IL-6 and IL-2.

Table 24 ELISA kit components

96-well Strip Plates pre-coated with a human IL-6/IL-2 capture antibody
Detection antibody
Conjugate
Buffers
Diluent
TMB
Stop buffers
Lyophilized human IL-6/IL-2 standard

CEM-C7-14, CEM-C1-15, DT40 and PBMCs cells were seeded in 6 well plates at density 0.5-1x10^6 cells per ml, each well had 3 ml of media. Then tested compound were added to the wells at final concentration of 1 µM, and then incubated for further 24h or cells were incubated first for 24 h with PHA dose 1 µg/ml then treated with indicated compounds. Later each well content was transferred to 15 ml universal tube, centrifuged for 5 min at 1500 RPM, then the supernatant transferred to new properly labelled tubes. The media of treated cells was either frozen at -80 ^oC for future experiments or processed immediately through ELISA protocol. Pierce Protein Methods was followed as illustrated in flowchart below: plates were loaded with 50 µL of the samples in duplicate, then 50 µL of biotynylated antibody reagent was added. Plate were covered and incubated immediately for 2h at 20-25 ^oC, washed three times and

100 µL of Streptavidin HRP solution (conjugate) was added to all wells. Plates were covered and incbated for 30 min at 20-25 ^{o}C, then the plates were washed three times with Tween PBS (ELISA wash). Next, wells were loaded with 100 µL of TMB substrate and incubated for 30 min at 20-25 ^{o}C in dark. The reaction was stopped by 100 µL of stop buffer, and absorbance read at 450-550 nm at plate reader.

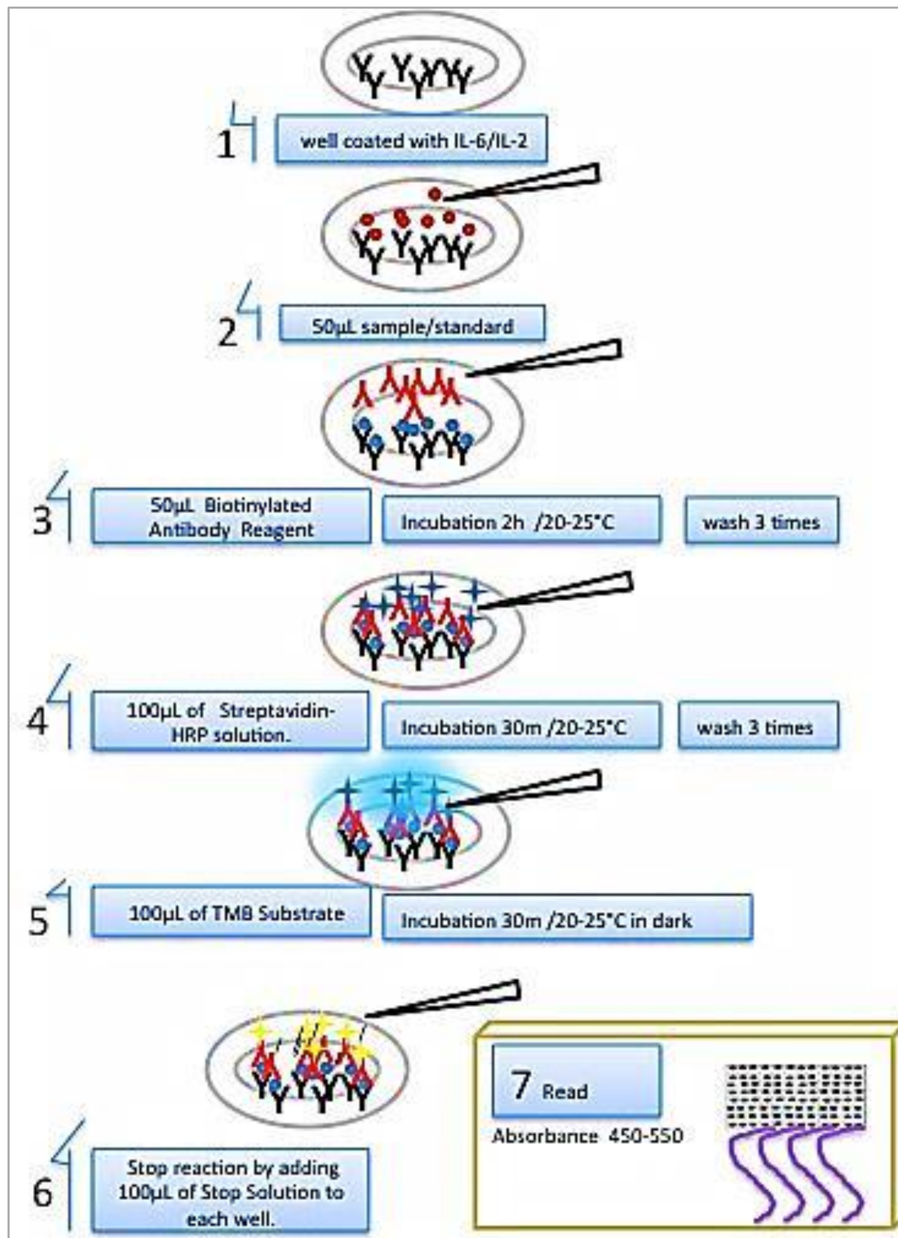


Figure 29 Cartoon illustration of ELISA assay followed. Procedure carried out to quantify levels of secreted interleukins in media of cells treated with the explored drugs.

2.2.9 Caspase assay

Caspase-8 activity was measured by Caspase-Glo 8 Assay from Promega catalogue no.G8200**.** This luminescent assay measures cysteine aspartic acid-specific c protease caspase-8 which is activator of extrinsic pathway of apoptosis. The kit includes reagents for cell lysis, and caspase-8 substrate in luciferase system that produce light signal from reaction. The glow is detected by illuminometer and the density of light is correlated with caspase -8 enzyme activity.The assay is performed in 96 well plate were (15,000) cells per well were seeded in DCC contain media and treated with 1µM of indicated compounds for 48 h. After that reagents are prepared as indicated by manufacturer company and 100µl of Caspase-Glo 8 Reagent (10 units/ml) was added to each well, plates were covered with a plate sealer. The contents of the wells were mixed at 300–500rpm using a plate shaker, then incubated at room temperature for 30 min. After that luminescence measurement was performed by placing the plate in a plate-reading Omega illuminometer according to the setting of illuminometer manufacturer. The substrate cleavage leads to generation of glow and the amount of caspase 8 are represented by RLU which is the signal proportional to the amount of caspase-8 activity present, in which one unit of caspase 8 is the amount of enzyme required to cleave 1 Pmol of the substrate LETD per minute at 30 ^{o}C. The data were obtained through MARS software.

2.2.10 Ligand-protein binding *in-silico* simulation- Chimera docking

Molecular graphics and analyses were performed with the UCSF Chimera 1.10.1 package. Chimera is generated by the resource for Biocomputing, visualization, and informatics at the University of California, San Francisco (supported by NIGMS P41-GM103311). This is an extensive program for interactive visualization and analysis of molecular structures and related data, including density maps, supramolecular assemblies, sequence alignments, docking results, trajectories, and conformational ensembles (UCSF Chimera, 2015). Docking was performed via auto dock vina tool. In this study the desired target protein (GR) was docked with 5 different compounds for which variable binding affinity with GR was obtained.

2.2.10 Drug stability determination

Drug stability was tested via Nuclear Magnetic Resonance Spectroscopy (NMR) by Salford Analytical Services SAS, using Bruker Advance 400 MHz spectrometer. This device contains multinuclear probe (31P to 109Ag) that can be used for a wide range of experiments including chemical characterization, counterfeit drug analysis, active pharmaceutical ingredient (API) determination and purity.

Hydrogen atoms are monitored by NMR, 0 h (control) and reading compared with that recorded after being left 24 h in room temperature, where signals peak represents the

protons of the investigated compound. Less than 5 mg of each compound were dissolved in 1 ml of the suitable solvent then 600 µl transferred to NMR tube and placed in NMR. Data were analysed using Bruker software Topspin 3.5p16. Solvents used for NMR experiments are listed in table 25.

Table 25 Solutions used for NMR experiments

Compound	NMR solvent
Dexamethasone	Acetone
Compound A	Methanol-4 (MeOD)
Tyramine	Deuterium oxide (D_2O)
Tyramine Hydrochloride	Deuterium oxide (D_2O)
N-Methyltyramine (CPDB)	Chloroform d1 (CDCL3)
Hordenine (CPDC)	Deuterium oxide (D_2O)

3 Results

3.1 Identification and preliminary characterization of novel compounds

In order to gain insight of mechanisms of action of SEGRAS and develop new compounds, we have tested compounds that have displayed similar chemical properties to compound A (Fig.30). This part of the study was carried out in collaboration with Dr. John Hadfield (University of Salford), who provided chemical expertise to identify potential novel SEGRA. Dexamethasone was used as control and Compound A as a reference point for testing four new compounds including Tyramine, Tyramine HCl, N-Methyl tyramine (CPDB) and Hordenine (CPDC) (Fig. 30).

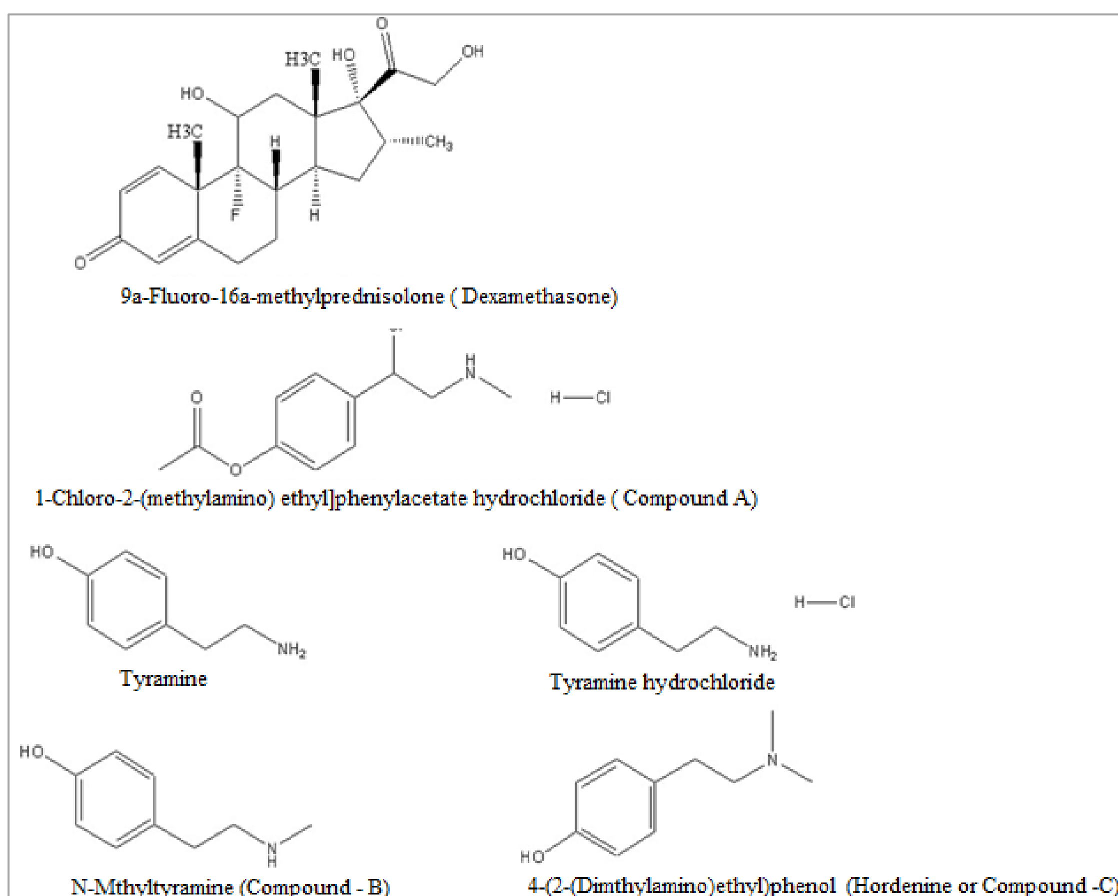


Figure 30 Structures of tested compounds.

The purpose of this part of the study was to determine the stability of tested compounds in indicated solution at certain time point and thermal levels (Table17). Stability of the compounds powder was taken from the manufacturer. Solutions of tested compounds were prepared as described in materials and methods. The first experiment was carried out immediately upon dissolving the stock in suitable solvent and solvent alone was considered the control. Second reading was performed using Nuclear Magnetic Resonance (NMR) 24h later after compounds in indicated solution were kept at room temperature and the third reading was obtained 24 h after storing the solutions at 4 ^{o}C.

The results of Proton NMR Spectroscopy demonstrated that exposing the DEX dissolved in solution at R.T/24 h and 4 ^{o}C did not lead to a visible changes in molecules structure as shown in NMR data as no alterations in profile of NMR resonance signals was observed (see supplementary material 4.5.1). CPDA solution resonance frequencies (peaks) have been unchanged upon 24 h at R.T/24 h and 4 ^{o}C storage conditions. Furthermore (Tyramine, Tyramine hydrochloride, CPDB and CPDC) solutions were all stable at experimental conditions used when compared to control.

To summarize, results revealed that all compounds solutions are stable for short time stored at temperature higher (4 ^{o}C and RT) than optimal (-80 ^{o}C for CPDA and -20 ^{o}C for others) (table 26). Drug stability was tested via NMR and showed that studied compounds were all stable for 24 h in solution making them optimal for experimental analysis.

Table 26 Stability of tested compounds upon various conditions. For details see supplementary data (4.5.1)

Compound	PubChem ID	Chemical formula	NMR Solvent	0h	24h/RT	24h/4C
Dexamethasone (DEX)	5743	$C_{22}H_{29}FO_5$	Acetone	Stable	Stable	Stable
Compound A (CPDA)	9838147	$C_{11}H_{15}Cl_2NO_2$	Methanol-d4 (MeOD)	Stable	Stable	Stable
Tyramine (T)	5610	$C_8H_{11}NO$	Deuterium Oxide D2O	Stable	Stable	Stable
Tyramine Hydrochloride (THCL)	66449	$C_8H_{12}ClNO$	Deuterium Oxide D2O	Stable	Stable	Stable
N-Methyl tyramine (CPDB)	9727	$C_9H_{13}NO$	Chloroform-d1 (CDCl3	Stable	Stable	Stable
Hordenine (CPDC)	68313	$C_{10}H_{15}NO$	Deuterium Oxide (D2O)	Stable	Stable	Stable

3.2 Ligand-protein binding- *in silico* assay

In the next set of experiments *in silico* simulation of ligand-GR binding was carried out in order to determine potential of these compounds to bind the receptor with high affinity and within the ligand-binding pocket (Fig. 31). In study to reveal the binding sites of GR with the antagonist ligand HO-PCBs 4 main residues were detected to form hydrogen interactions at (Glu540) and hydrophobic bonds at (Ile539, Val543 and Trp577) in GR which is an indicator of GR specific function (Liu et al., 2016a).Molecular docking helped to distinguish GR nuclear translocation as indicated by (Liu et al., 2016b) and to design novel GR ligand (Xu et al., 2009).Molecular modelling can assist in designing of new drugs through prediction of the main binding sites with known ligand, where binding

affinities expressed as a numbers and indicate the powerful of contact between the two molecules (Vilar et al., 2017).

Predicted binding affinities and distances indicated this order: Dex>CpdA>T=THCL>CPDB=CPDC (Table 27). The studied compounds interact with some of the residues that also interact with Dex. The molecular docking analysis and related data using Auto Dock Vina tool indicated that ARG611 is conserved in all of contacts identified (Table 28 and Fig.32 - coloured residues) which has been known to enhance GR-ligand binding along with other residues (LEU563, ASN564, GLN570, PHE623, LEU608 (DiSorbo et al., 1980).

The results demonstrated variable predicted binding affinities for tested compounds with GR Furthermore DEX shares similar binding residues in GR with the investigated compounds.This part of research was performed in collaboration with Priyanka Panwar-PhD candidate and Dr. Niroshini Nirmalan (University of Salford).

ttan

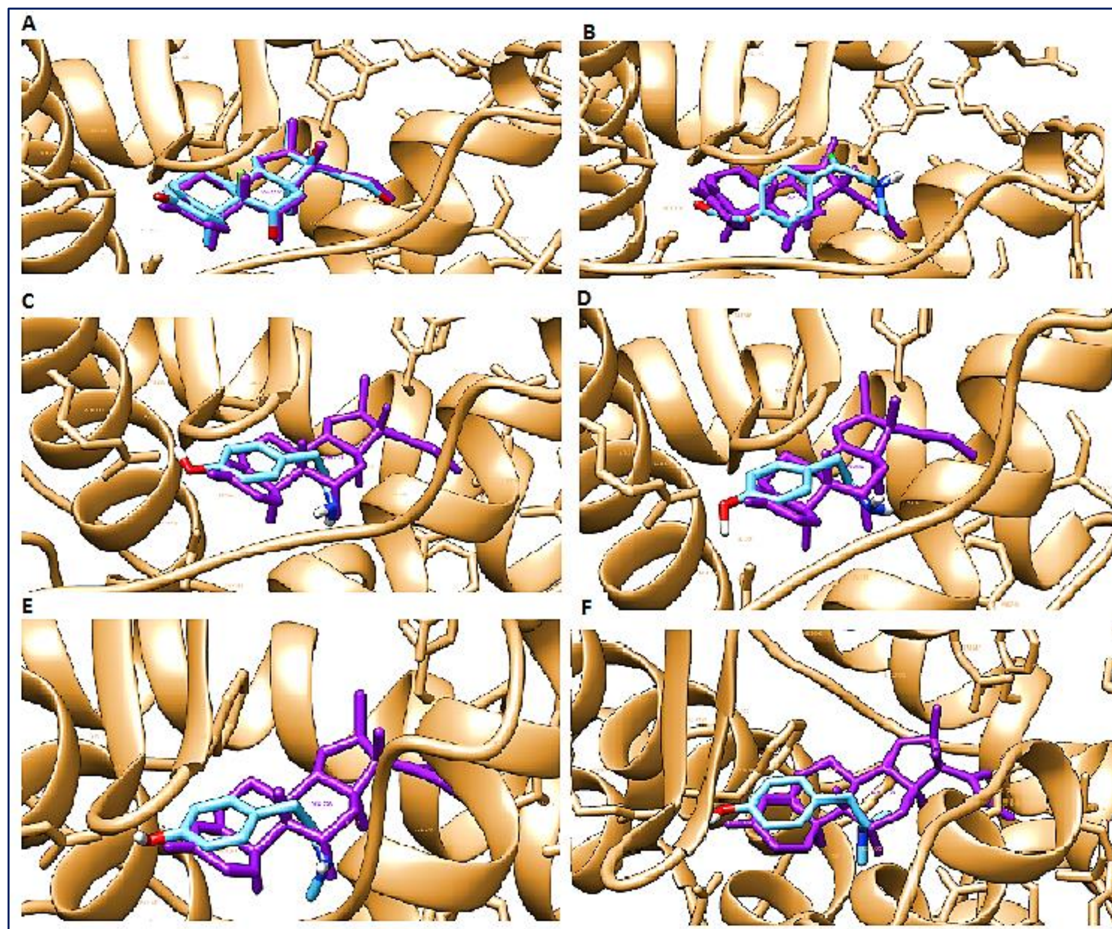


Figure 31 Auto dock simulation of studied compounds.

Vina Docking results Reveal the tested Compounds (Blue) superimposed with original DEX (purple) from crystal GR-DEX (4UDC). (A) DEX, (B) CPDA, (C)T (D)THCL (E) CPDB,(F)CPDC

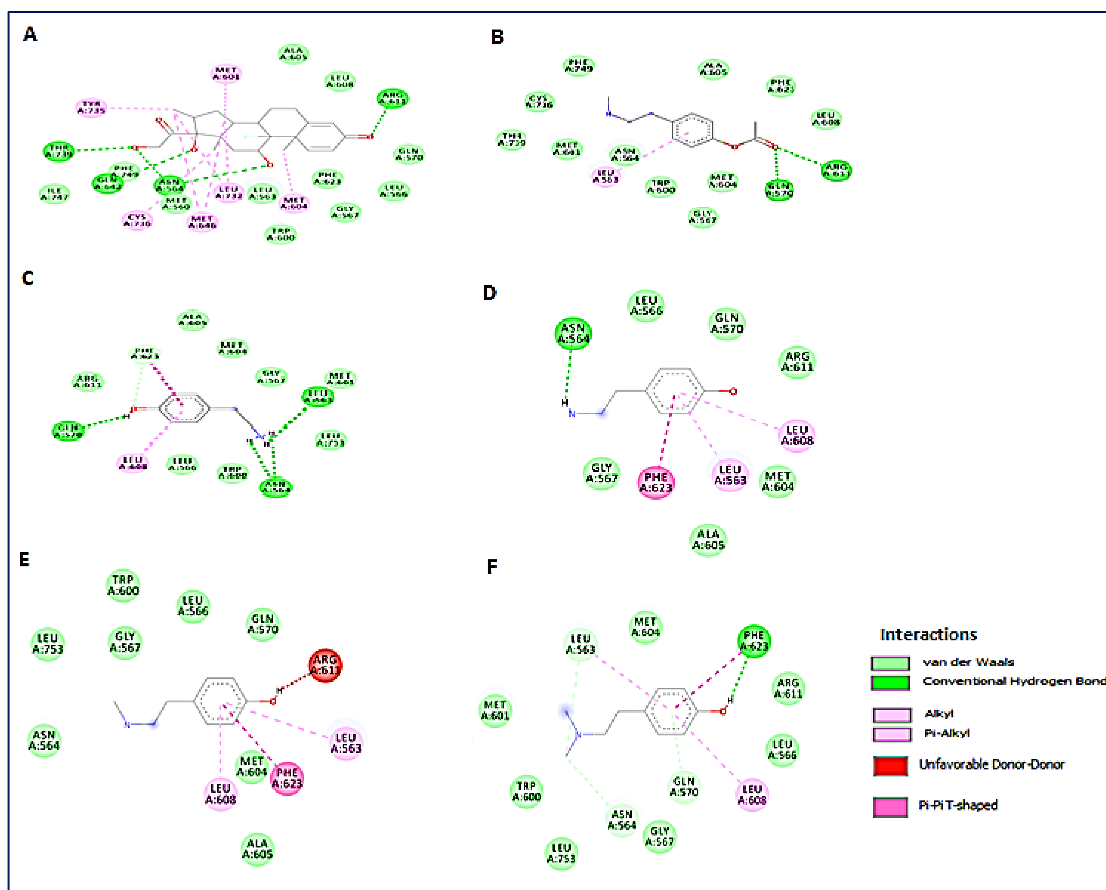


Figure 32 Ligand - GR interactions.

Illustration of the binding sites with GR. (A)DEX, (B)CPDA,(C)T (D)THCL (E) CPDB,(F)CPDC. *In silico* simulation was carried out using chimera 1.10.2 package for interactive visualization and analysis of molecular structures (Auto Dock Vina tool) whereas Discovery studio 4.5 was used to visualize the amino acids involved in ligand docking.

Table 27 Highest Binding affinities of compounds docked with GR-LBD

Compound	kcal/mol
DEX	- 12.8
CPDA	-6.2
T	-5.6
THCL	-5.6
CPDB	-5.9
CPDC	-5.9

Table 28 Summary of interactions between tested compounds and GR-LBD

CPD	Deep green conventional hydrogen bonds	Light green-van der Waals interactions	Pale green-carbon hydrogen bond	Pink - PI-Alkyl	Deep pink-PI-PI T shaped
Dex	*ARG611, ASN564, GLN642, THR739	MET646, ALA605, LEU608, GLN570, LEU566, GLY567, LEU753, LEU563, PHE749, MET 560, ILE747, TRP600, PHE623, ALA 605.LEU608, LEU566, THR739, MET560, GLN642		TYR 735, CYS 736, LEU 732, MET601, MET 604, TRO 600, CYS736, MET646, LEU732, MET604, TRP600, ALA605, MET640	
CpdA	GLN570, ARG611	ALA605, PHE623, LEU608, PHE749 CYS736, THR739, MET601, ASN564 TRP600, MET604, GLY567. MET691, BOG501, ASP687, GLU688, LYS695		LEU563	
T	LEU563, ASN564, GLN570	ARG611, LEU566,TRP600 LEU753, MET601, GLY567 MET604, ALA605	PHE623	LEU608	PHE623
THCL	ASN564, LEU563 PHE623 ARG611 GLN570		PHE623	LEU608, MET604, LEU566	PHE623

Colors indicate five types of interactions as displayed in fig.32. The table represents residues in GR that bind with the tested compounds distributed according to the binding potency, Van der Waals strength (0.4-4.0 KJ/mol) Hydrogen bonds potency (12-30 KJ/mol). Ionic interactions (20 KJ/mol) and hydrophobic interactions (<40 KJ/mol).

3.3 Cytotoxicity of DEX and tested compounds

The synthetic steroid compound Dexamethasone (DEX) and other organic compounds were tested to determine the concentration and incubation time needed to induce cell death. For this purpose MTS or MTT assays were used to assess *in vitro* cytotoxicity of DEX, CPDA, T, and THCL. Leukemia cells were treated with different concentrations of DEX ranging from 0 to 100 µM at three different incubation times of 24 h, 48 h and 72 h to assess cell type dependent effects and drug specific effect for each compound.

3.3.1 Cytotoxicity of tested compounds upon 72h incubation with 10µM to 100µM concentrations

The overall effect of long incubation time with the starting dose of 100 µM concentration of studied compounds on CEM-C7-14 cells was analysed using MTS cytotoxicity assay (Fig. 33). Cell proliferation was inhibited by increasing doses of DEX, CPDA and T (Fig. 33 A, D and G). Curiously, THCL inhibited cell growth at low concentration but promoted growth rate at the high dose of 100 µM (Fig. 33 J).
In C7 cells, DEX inhibited the growth to approximately less than 40% proliferating cells at all doses (Fig. 33 A, compare lanes 1 to lanes 2-6), IC_{50} <10 µM., Similarly, the addition of CPDA inhibited the growth significantly at all concentrations to approximately 60% of proliferating cells (Fig. 33 D, compare lanes 1 to lanes 2-6), IC_{50} > or =100 µM. T displayed cytotoxic effects by inhibiting the growth in a dose dependent manner and reaching the rate of less than 20% living cells at high doses (Fig. 33 G, compare lane 1 to lane 6), IC_{50} > or = 10 µM. Surprisingly, THCL seems to affect cells in a different way as

the high dose of 100µM promoted the growth while all other smaller doses suppressed the cell growth (Fig. 33 J, compare lane 1 to lanes 2-5 and to lane 6).

CEM-C1-15 growth rate was also affected by high concentrations of the compounds and longer durations in a similar manner to C7 cells (Fig. 33 B, E, H and K). Despite the fact that those cells are known to be GC resistant, significantly reduced growth was observed potentially due to use of compounds at doses higher than 10 µM for the indicated incubation period. Compounds displayed following IC_{50} values: DEX (IC_{50} <10 µM), CpdA (IC_{50} > or =100 µM) and T (IC_{50} > or = 10 µM). DEX, CpdA and T caused diminished C1 cell viability at all doses tested (Fig. 33 B, E and H, compare line 1 to all other lanes) whereas THCL inhibited the growth rate at doses lower than 100 µM (Fig. 33 K, compare lane 1 to lanes 2-5 and 6).

Similarly, the cytotoxic effect on DT40 cells was observed upon incubation with the high doses of DEX (IC_{50} =12.5 µM), CPDA and T (IC_{50} =12.5 µM), and THCL at doses less than 100 µM (Fig. 33 C, F, I and L).

To conclude, all compounds inhibited growth of CEM-C7-14, CEM-C1-15 and DT40 cells at most doses studied and at long duration of treatment at 72 hrs.

However, given potential toxicity of these doses to other tissues and long durations of treatment, a series of further tests was undertaken to determine optimal/minimal dose and duration.

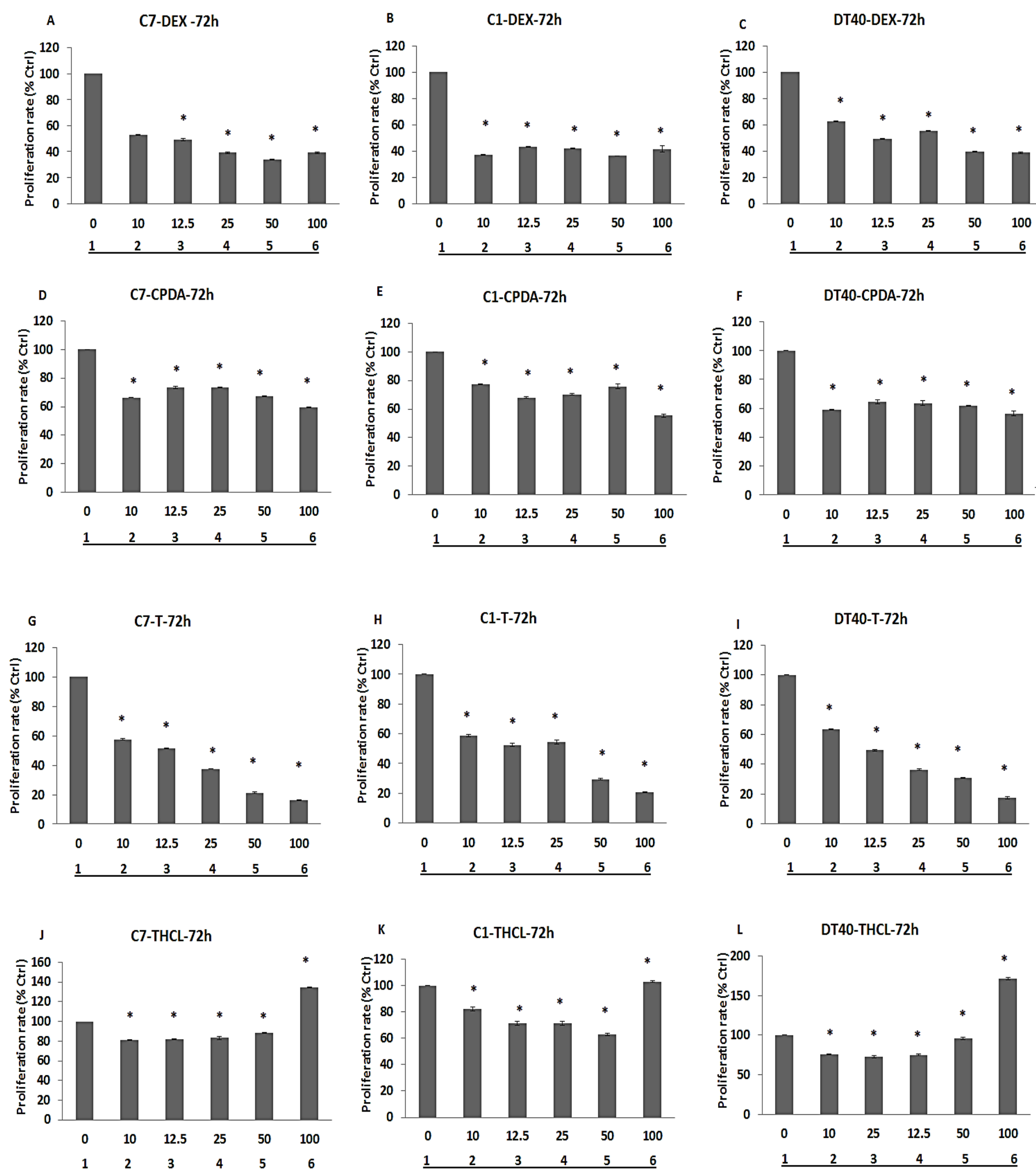


Figure 33 Effect of high drug doses and 72 h treatment duration on viability of examined cells.

CEM-C7-14, CEM-C1-15 and DT40 cells were treated with tested compounds. (A, B, C) DEX; (D, E, F) CPDA; (G, H, I) T; (J, K, L) THCL Compounds were incubated with starting high dose of 100 µM with indicated cell lines. MTS assay was carried out 72h later to detect the effect of the compound on the proliferation of leukemia cells. Data shown are representative of three experiments in triplicates. Error bars represent standard error of means. *$P<0.05$.

3.3.2 Cytotoxicity of tested compounds upon 48h incubation with 10μM to 100μM concentrations

In this set of experiments duration of treatment was lowered from 72 h

to 48 h. In GC sensitive C7 cells all compounds inhibited the growth to a certain extent (Fig. 34 A, D, G, and J). T had the strongest effect (around 60% of cell death, Fig. 34 G, compare lane 1 to 6) whereas inhibitory effect for other compounds varied between 40-50% mostly in dose dependent manner (Fig. 34 A, D and J compare lanes 1 to lanes 2-6).

In GC resistant CEM-C1-15 cells, most compounds when used for 48 hours inhibited C1 growth moderately (Fig. 34 B, E, H and K). In general, Dex, CpdA and T mediated growth inhibition was weaker in C1 cells than in C7 cells (compare middle panels with the left panel, except in the case of THCL which had the strongest inhibitory effect of all compounds in C1 cells and marginally better inhibition of growth in C1 versus C7 cells. In DT40 cells upon 48 h treatment DEX and T had weak inhibitory effect on growth rate, whereas CPDA and THCL showed stronger cytotoxic effect with THCl showing the most potent dose dependent effect similarly to effect in C1 cells (Fig. 34, compare right panels to middle panels).

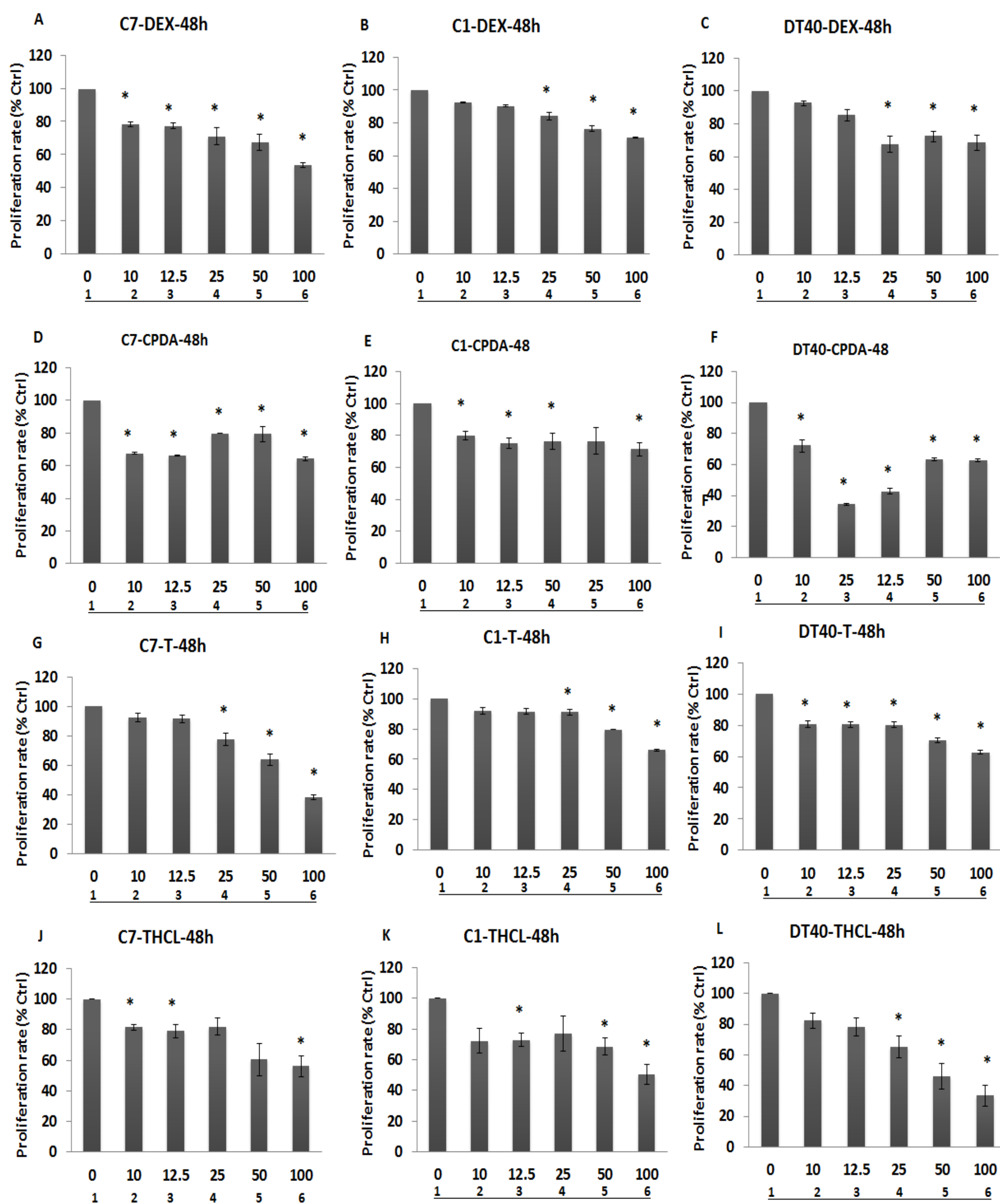


Figure 34 Effect of high compound doses for 48 h on viability of examined cells.

CEM-C7-14, CEM-C1-15 and DT40 cells were treated with tested compounds. (A, B, C) DEX; (D, E, F) CPDA; (G, H, I) T; (J, K, L) THCL compounds were incubated with starting high dose of 100 µM with indicated cell lines. MTS assay were carried out 48 h later to detect the effect of the compound on the proliferation of leukemia cells. Data shown are representative of three experiments in triplicates. Error bars represent standard error of means. *P<0.05.

3.3.3 Cytotoxicity of tested compounds upon variable duration of treatment and 0 to 2.5 μM concentrations

3.3.3.1 Cytotoxicity profiles of CEM-C7-14 cells

In order to investigate the growth inhibitory effect of lower doses of the studied compounds, and determine the cytotoxic doses and duration of the drugs, MTS assays were employed. In the first series of experiments CEM-C7-14 cells were treated with 0-2.5 μM concentrations of compounds using various durations including 24 h, 48 h and 72 h to reveal the effect of time, dose and cancer type as seen in Fig. 35. The tested substances exhibited concentration-dependent inhibitory effects on the proliferation of the examined CEM-C7-14 cell line. In GCs sensitive CEM-7-14 cells (Fig. 35) treated with DEX, CPDA and T growth inhibitory effect can be observed at 48 h that becomes stronger at 72 h of treatment duration (Fig. 35, compare panels A,B and C, black light grey and dark grey bars). THCL effect is significant mostly at high doses and long duration of treatment (Fig. 35, panel C).

24 h duration of DEX treatment did not produce any IC_{50} apart from a minor growth inhibition which can be seen upon certain doses of all compounds (Fig. 35, black columns). Therefore longer incubation is needed to allow the drugs to exert their effects.

48h incubation time with DEX has a moderate inhibitory effect on C7 cells at all doses tested but the survival rate was higher than 50% and IC_{50} couldn’t be calculated (Fig. 35 A, light grey columns); CPDA and T induced inhibition of cell growth similar to that of

DEX (Fig. 35 B and C, light grey columns). THCL cytotoxicity was associated with the high concentrations of the drug (Fig. 35 D, light grey columns).

Increase in the incubation time to 72 h resulted in clear growth inhibitory effect on cells treated with both DEX and CPDA (Fig. 35 A and B,- dark grey columns) as all concentrations inhibited the growth significantly; IC_{50} therefore was estimated to be IC_{50}<0.039 µM for DEX and CpdA. Regarding T (Fig. 35 C, dark grey columns)-all doses inhibited the growth significantly and IC_{50} was around 0.039 µM. THCL (Fig. 35 D, dark grey columns) inhibited the growth significantly at doses 2.5, 1.25, 0.63 µM but lower doses had no effect; IC_{50} was estimated at 0.625 µM.

Dose dependent effect for CPDA has been observed. At high doses and long incubation times CPDA shows GR independent effects that are likely due to the metabolites of the compound (Wust et al., 2009)

.

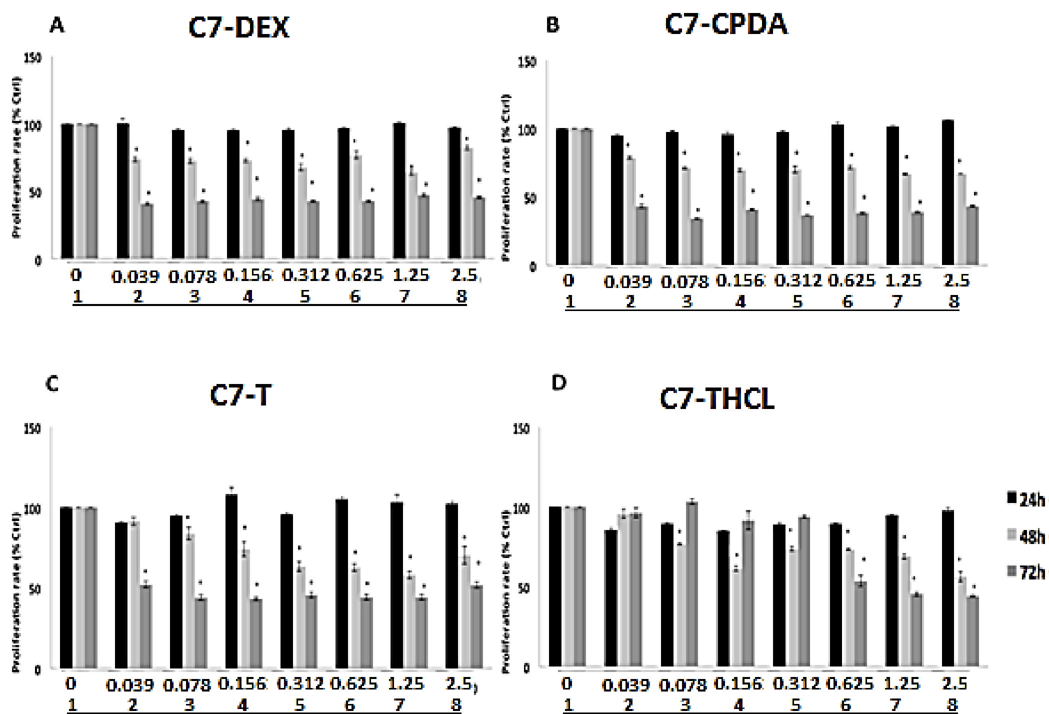


Figure 35 The effect of duration and different doses of drug treatment on CEM-C7-14 viability.

CEM-C7-14 cells were treated with (A) DEX, (B) CPDA, (C) T, and (D) THCL. Indicated cell lines were incubated with starting high dose 2.5 µM of studied compounds. MTS assay were carried out at different time points 24, 48 or 72 h later to detect the effect of the compound on the proliferation of leukemia cells. Data shown are representative of three independent experiments in triplicates. Error bars represent standard error of mean,*P<0.05.

3.3.3.2 Cytotoxicity profiles of CEM-C1-15 cells

Analysis of GC resistant acute childhood leukemia cell line CEM-C1-15 under the same conditions as above indicated that overall these cells are more likely to survive 24 and 48 h, or treatment than CEM-C7-14 GC sensitive cells. Overall, results indicated that long incubation and high concentrations of drugs had slight cytotoxic effect (Fig. 36).

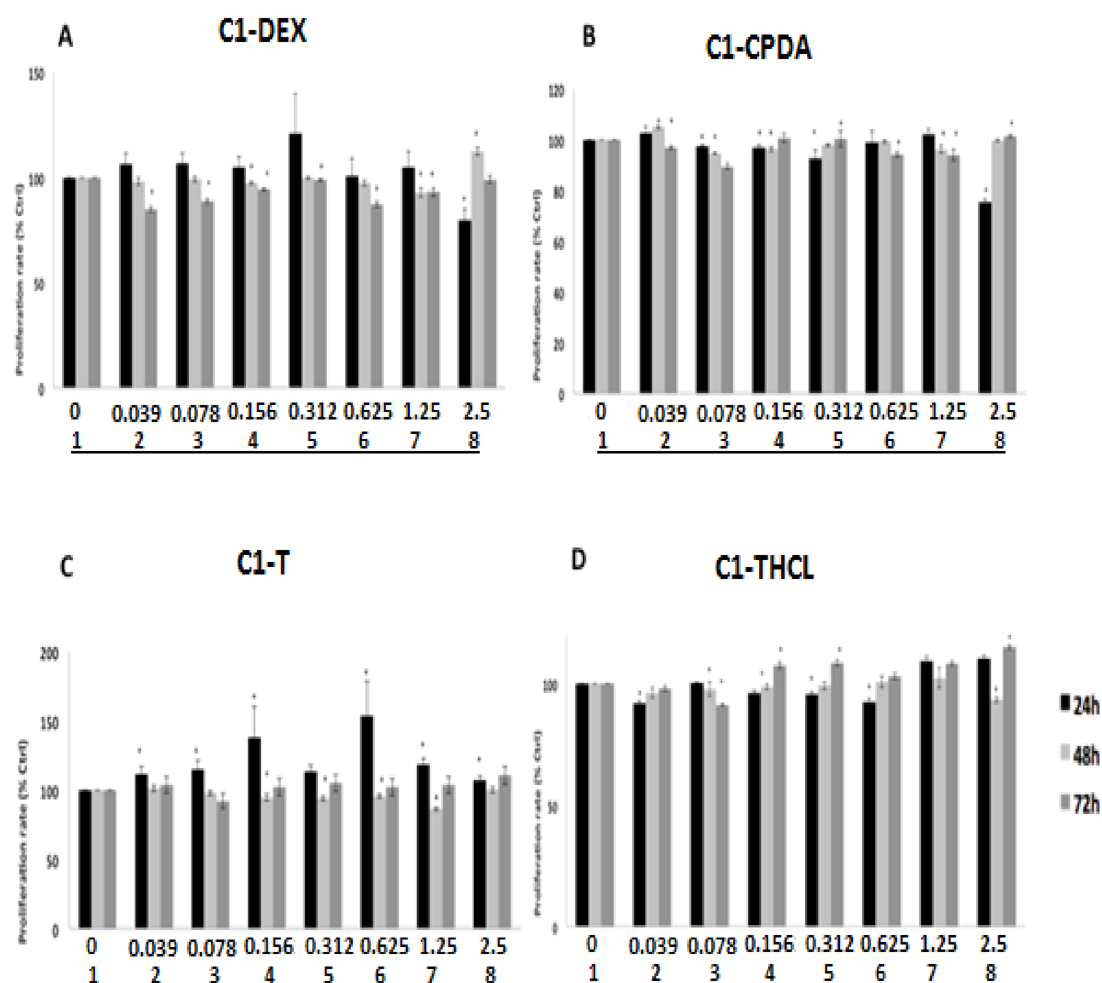


Figure 36 The effect of duration and different doses of drug treatment on CEM- C1-15 viability.

CEM-C1-15 cells treated with (A) DEX, (B) CPDA, (C) T, and (D) THCL. Compounds were incubated with starting high dose 2.5 µM with indicated cell line. MTS assay were carried out at

different time points 24, 48 or 72 h later to detect the effect of the compound on the proliferation of leukemia cells. Data shown are representative of three independent experiments in triplicates. Error bars represent standard error of mean,*P<0.05.

3.3.3.3 Cytotoxicity profiles of DT40 cells

Analysis of compound effect on chicken derived lymphoma DT40 cells suggested that the anti-proliferative activity of all the four compounds DEX, CPDA, T, THCL mostly increased with longer incubation time, however cytotoxic effect was small (in the range of 20-30%) or non-existent (Fig. 37). All compounds showed similar effects with the strongest cytotoxicity displayed by Dex at IC_{50} 0.626 µM 72 h, CpdA at 1.25 µM at 72 h, T at 0.313 µM at 48 h and THCl at 0.078 µM at 72 h of treatment (Fig. 37).

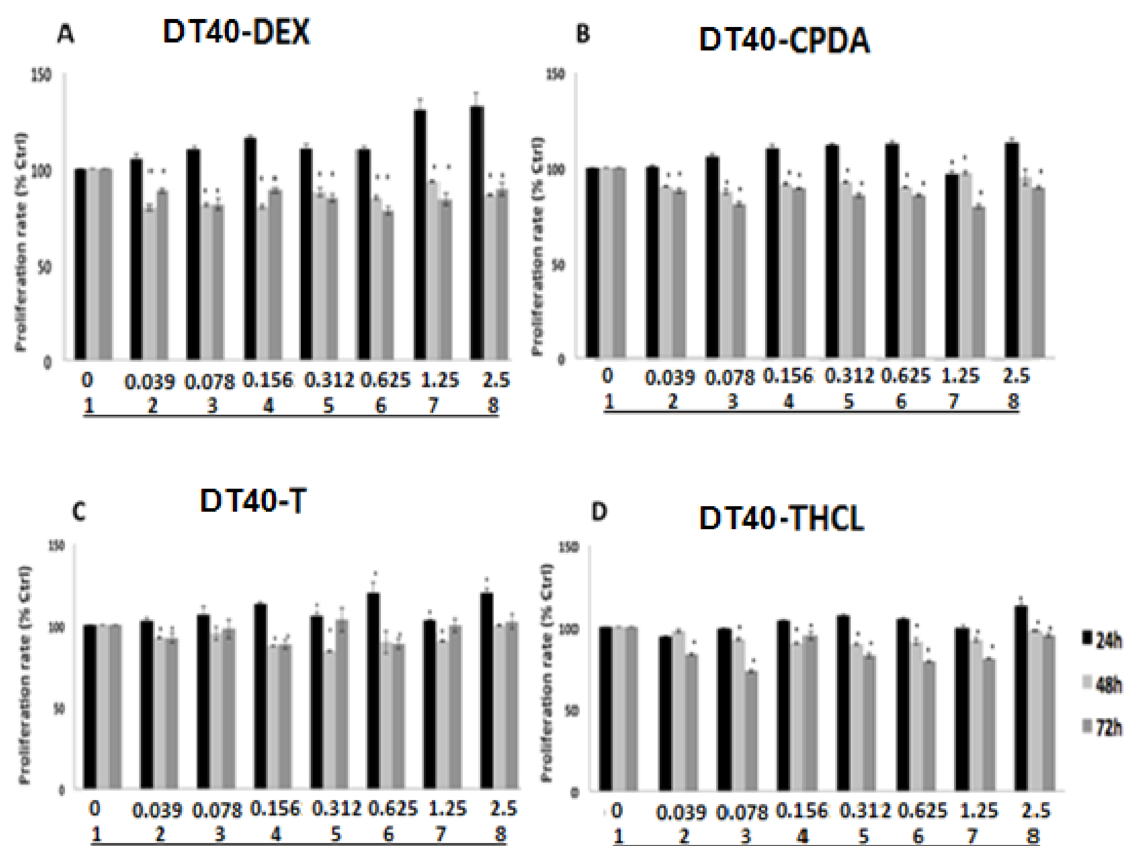


Figure 37 The effect of duration and different doses of drug treatment on DT40 cells viability.

DT40 cells treated with (A) DEX, (B) CPDA, (C) T, and (D) THCL. Compounds were incubated with starting high dose 2.5 µM and serial dilutions with indicated cell line. MTS assay were carried out at different time

points 24, 48 or 72h later to detect the effect of the compound on the proliferation of leukemia cells. Data shown are representative of three independent experiments in triplicates. Error bars represent standard error of mean,*P<0.05.

3.3.3.4 Cytotoxicity of CPDB and C

In the next set of experiments, analysis of the activity of new compounds with similar chemical structure to CPDA, named CPDB and CPDC was carried out (chemical structure displayed in (Fig. 38). Both compounds were shown to promote cell growth at certain durations/concentrations in C7 and C1 cells (Fig. 38 panels A-D). CPD B and C suppressed DT40 cell growth at certain concentrations with the strongest effect observed at 48 h treatment above 0.156 µM concentration. However, there was growth stimulatory effect of CPD C at lower concentrations at 48 h treatment (Fig. 38 F, light grey bars).

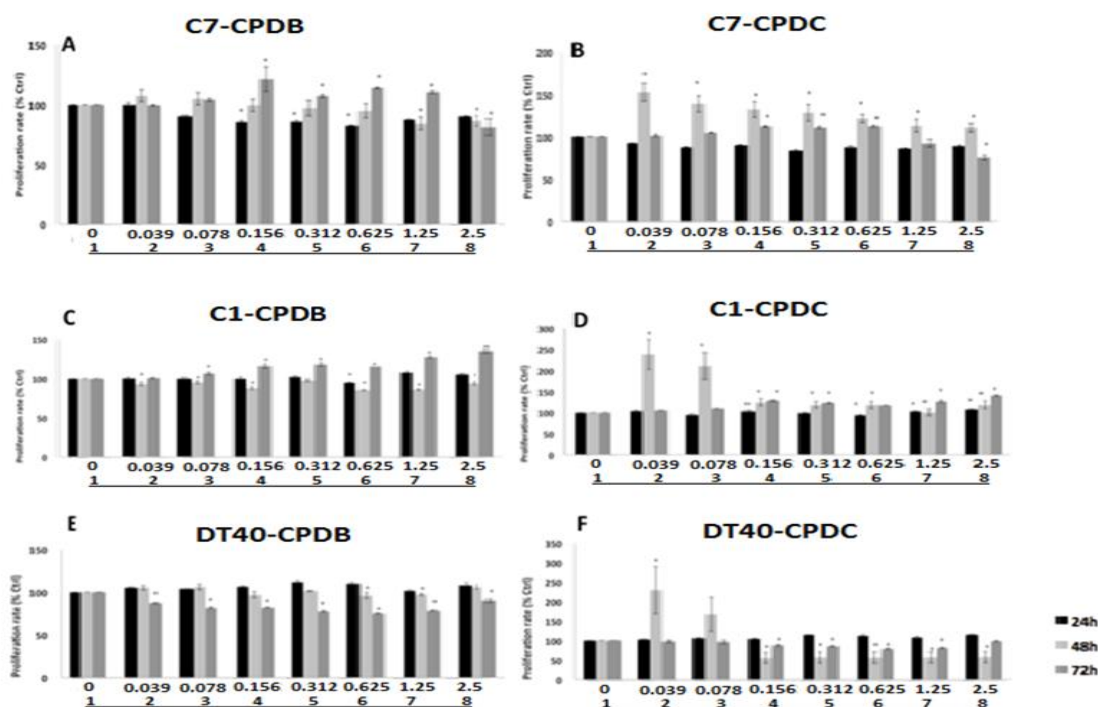


Figure 38 Cytotoxicity analysis of CPDB and CPDC in CEM-C7-14, CEM-C1-15 and DT40 Cells.

(A and B) C7, (C &D) C1, (E&F) DT40 cells treated with CPDB or CPDC. Compounds were incubated with starting high dose 2.5 µM with indicated cell line MTS assay were carried out at different time points 24, 48 or 72 h later to detect the effect of the compound on the proliferation of leukemia cells. Data shown are representative of three independent experiments in triplicates. Error bars represent standard error of mean. *P<0.05.

Altogether, cells sensitivity to 72h exposure and 100 µM doses of compounds was high, and all cell lines proliferation was inhibited by high doses of steroid and non-steroid treatment for long duration. CEM-C7-14 cells were sensitive to shorter duration (48 h) and high doses of compounds (starting from 100 µM), whereas C1 and DT40 were less sensitive to short incubation, however THCL showed strongest inhibitory effect. Upon treatments with starting doses of 2.5 µM and different incubation times a dose and time dependent growth inhibition effect on CEM-C7-14 and C1-15 cells was observed, and the effect was reduced by decreasing these two factors. C1 cell line displayed less sensitivity

than C7 to cytotoxic actions of compounds. This growth inhibition was mostly time dependent in DT40 cells. Thus, the compounds effect was cell, dose and time dependent. CPDB and CPDC produced both inhibitory and stimulatory effect on examined cells at different doses.

3.3.3.5 Cytotoxicity of tested compounds on peripheral blood mononuclear cells

In order to test the potential cytotoxic effect of tested compounds on normal white blood cells, the viability of peripheral blood mononuclear cells (PBMCs) was analyzed (Fig. 39 A). No effect was observed of either hormone or non-steroid treatment of PBMCs for 48h using 1 µM drug concentration (Fig. 39). Effects of DEX on PBMCs are commonly studied upon cells exposure to pro-inflammatory signals (Skendros et al., 2008). Therefore Phytohemagglutinin (PHA) was used for this purpose. PHA stimulation caused some elevation in survival rate of treated groups on its own (Fig. 39 compare lanes 1 and 2). DEX and CPDB treatment led to a loss of significant PHA caused increase in viability (Fig. 39 B, compare lanes 1 and 2 to lanes 3 and 7), whereas CPDA, T and THCL didn’t change substantially the effect of PHA, whereas CPDC showed further substantial stimulatory effect (Fig. 39 B, compare lanes 1 and 2 to lanes 4, 5, 6 and 8).

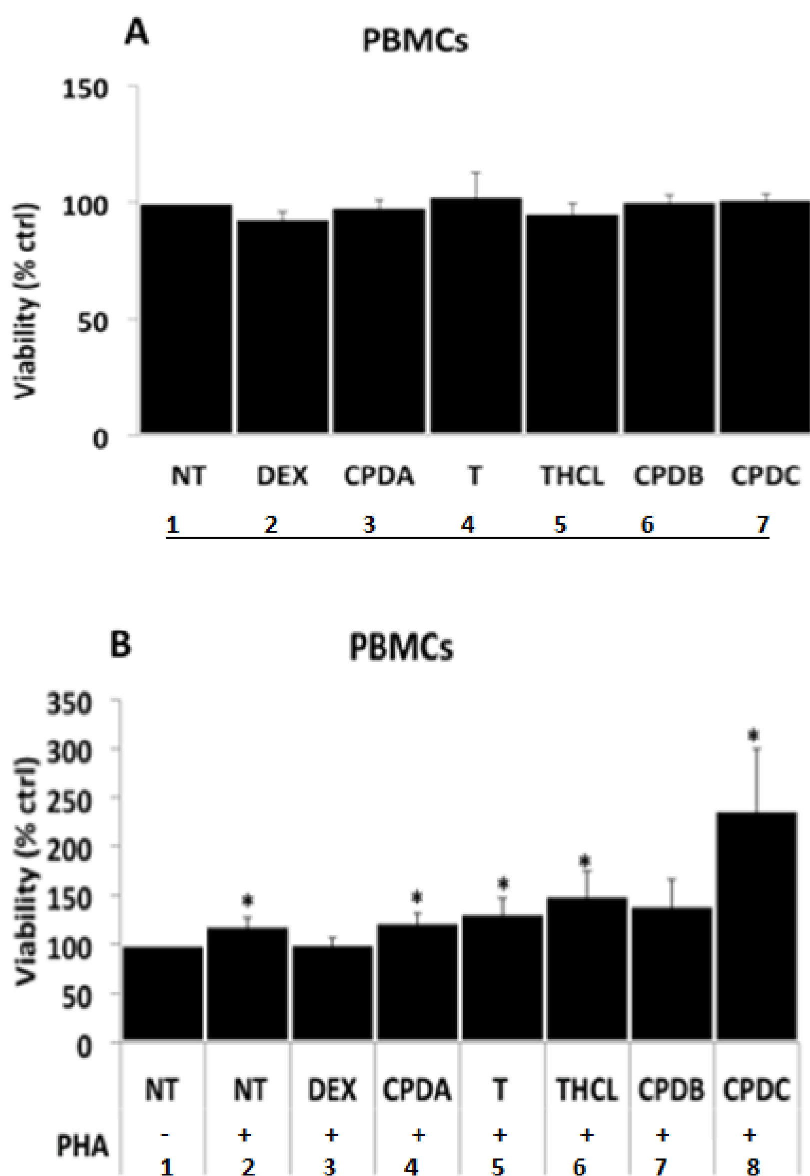


Figure 39 Cytotoxicity of tested compounds on PBMCs.

The effect of DEX, CPDA, T, THCL, CPDB, CPDC on PBMCs viability (A) No stimulation (B) PHA stimulation 1µg/ml. Compounds were incubated at dose 1 µM with indicated cells. MTS assay were carried out 48h later to detect the effect of the compound on the viability of normal. Data shown are representative of three independent experiments in triplicates. Error bars represent standard error of mean,*P<0.05.

3.3.4 Effect of temperature stress on drug response in ALL and PBMCs

The purpose of this experiment was to determine the therapeutic effect of combination of low temperature or high temperature with the studied drugs. Temperature variation was chosen as it is known environmental stressor in both Human and animals beings are exposed to rise and fall in surrounding temperature that may affect various processes including enzymatic reactions, heat responsive genes, response to drugs (Kapila et al., 2016), immunity (Franci et al., 1996) and impact intracellular proteins' profile (Somal et al., 2015). In study of hyperthermia effect in rats (Matic et al., 1989) found a significant reduction in glucocorticoid binding and a slight increase in binding affinity in hyper-thermic rats (41 ^{o}C) as compared to the controls. Heat stress could lead to thermal injury and activate multiple signalling pathways that involve heat shock proteins , inflammatory chemokines, and other pro-inflammatory mediators such as NF-kB, STAT3, and HIf-1a which regulate the cellular growth (Maghsudlu and Farashahi Yazd, 2017). It has been found that heat stress at 42°C applied to Riverine Buffalo's mammary epithelial cells inhibited the growth and survival of these cells as it induced both apoptosis and necrosis; and the effect were extended to gene transcription , as 153 genes were shown to be upregulated and 8 genes were down regulated upon heat shock (Kapila et al., 2016)

Therefore high and low temperatures were applied in combination with drug treatment to elucidate the effects on PBMCs. The results indicated that high temperature itself caused significant decrease in viability of the tested cells (Fig. 40, compare lanes 1 and 2). When combined with heat stress, DEX produced growth suppression effect similar to

heat (Fig. 40, compare lanes 1 and 2 to lane 3 in A, B, C, D). All other compounds reduce the growth in varying degrees except Tyramine, which did not show growth inhibition effect in presence of high temperature conditions.

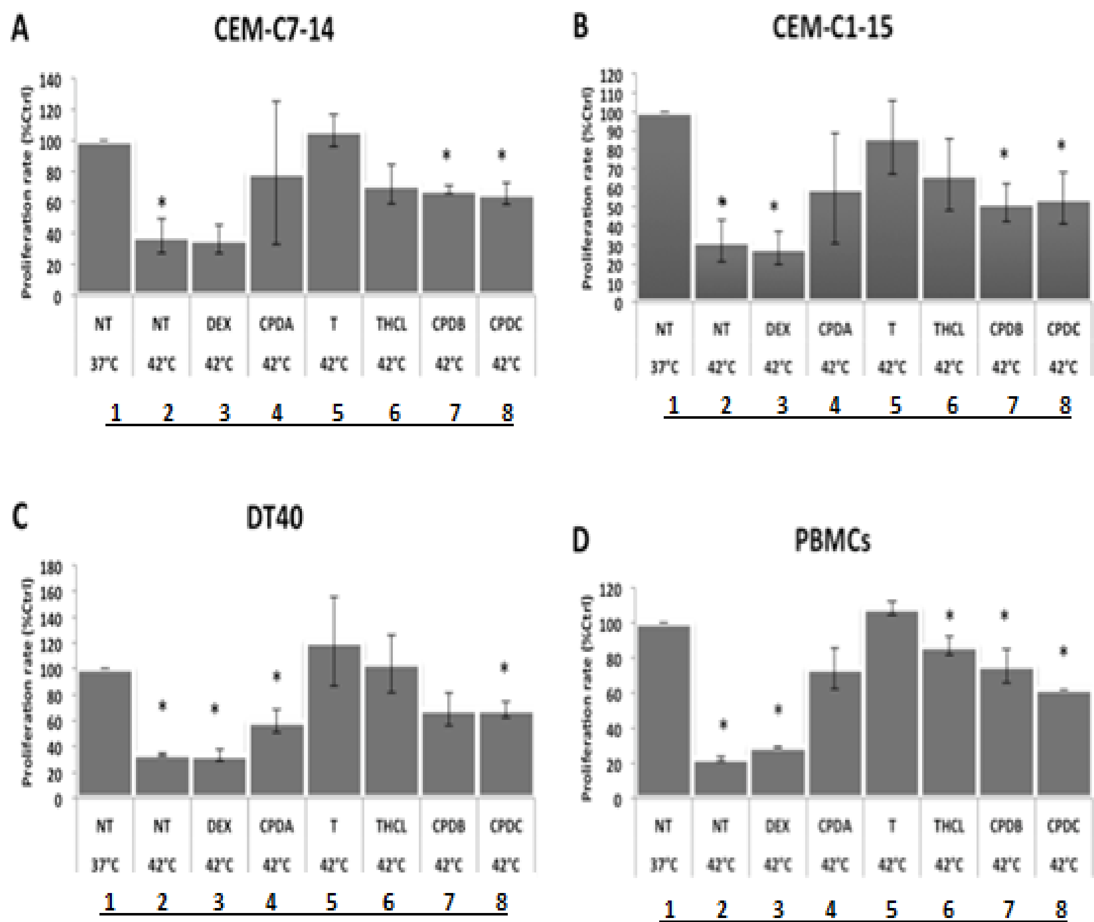


Figure 40 Cytotoxicity profile of combined high temperature and drug treatment on examined cells.

(A) CEM-C7-14. (B) CEM-C1-15 (C) DT40 cells. (D) PBMCs
The cells were exposed to 42 ^{0}C/2 h, then treated with 1µM DEX, CPDA, T, THCL and incubated at 37 ^{O}C .MTS assay were carried out 72h later to detect the effect of the compound and heat on the proliferation of leukemia cells and PBMCs. Data shown are representative of three experiments in triplicates. Error bars represent standard error of means *P<0.05.

Low temperature is considered a type of treatment that create a hypothermia conditions such as ice cold intravenous fluid that is given to patients with cardiac arrest. This therapy helps to improve the physiological parameters such as acid-base

balance. When it is applied at 4 ^{0}C for more than half of an hour it was found to be optimum to lower the internal body temperature to less than 37 ^{0}C (Fisher et al., 2017) . Therefore cells were incubated at 4 ^{0}C and treated with studied drugs. Viable number of cells exposed to a cold shock dropped to lower than 50% of control in all cell types studied (which was 37 ^{O}C) (Fig. 41, compare lanes 1 and 2). Combination of cold and DEX didn't have any different effect than cold alone (Fig. 41, compare lanes 1 and 2 to lane 3). CPDA, CPD B and CPDC treatment caused partial recovery from the effect of cold in all cell types studied except in PBMCs treated with CPDB and C1 cells treated with CPDA where it led to some, but not significant difference when compared to control. T and THCL caused complete recovery from the effect of cold. (Fig. 41, compare lanes 1 and 2 to lanes 5 and 6 across cell lines).

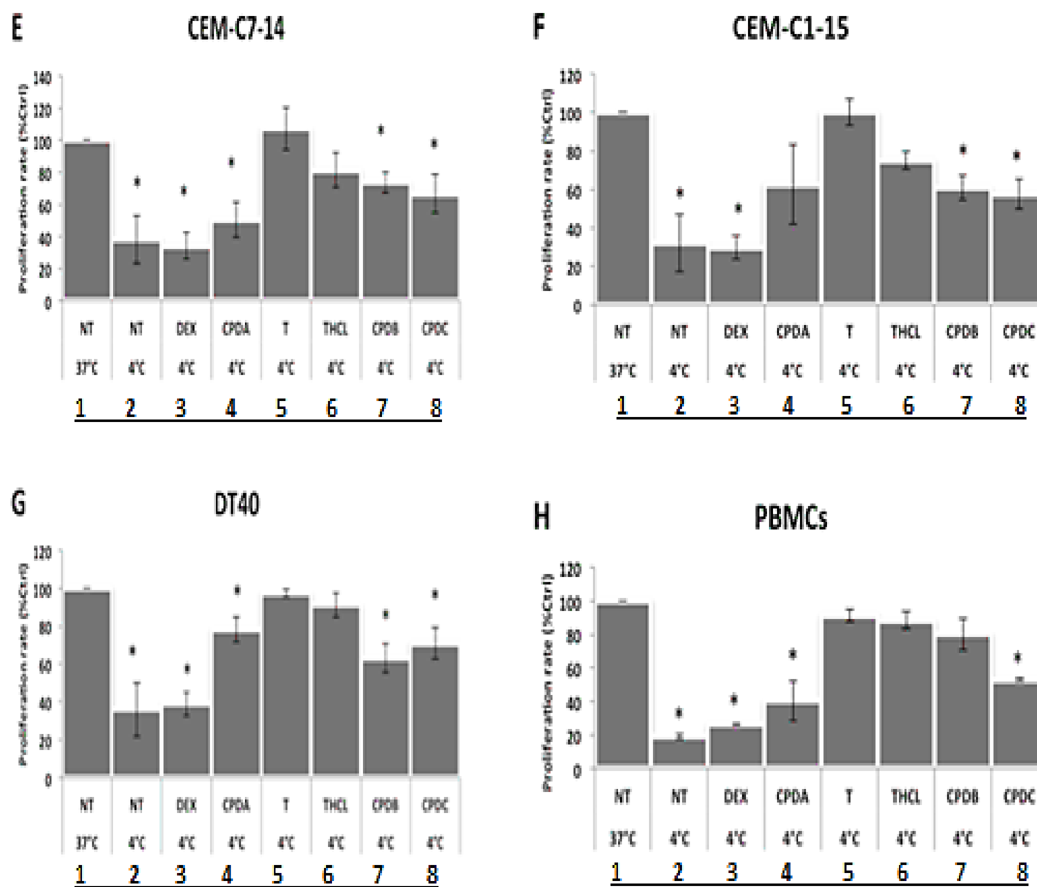


Figure 41 Cytotoxicity profile of combined low temperature and drug treatments on examined cells.

(E) CEM-C7-14. (F) CEM-C1-15 (G) DT40 cells. (H) PBMCs
The cells were exposed to 4 ^{0}C/2 h, then treated with 1 µM DEX, CPDA, T, THCL and incubated at 37 ^{O}C .MTS assay were carried out 72h later to detect the effect of the compound and heat on the proliferation of leukemia cells and PBMCs. Data shown are representative of three experiments in triplicates. Error bars represent standard error of means *P<0.05.

3.3.5 Compound cytotoxicity in cancer and non-cancer epithelial cells

This part of study was designed to investigate potential cytotoxic effects of studied compounds on other types of cells. For this purpose skin cancer cells HACAT, breast cancer cells MCF-7 and bronchial cell line from normal lung (BEAS-2B), were used. Results of 1 μM treatment for 48 h revealed that DEX and CPDA have no significant inhibitory effect on HACAT and HACAT cells viability while BEAS-2B proliferation rate was inhibited upon DEX and CPDA therapy. T and THCL increased viability of MCF-7 and HACAT cells (Fig. 42 A).

Analysis of the effect of 1 μM treatment for 72 h demonstrated that all studied drugs have significantly inhibited viability of MCF-7 while viability of HACAT cells was suppressed by DEX and CPDA but enhanced by T and THCL. All the compounds suppress BEAS-2B proliferation rate to the half or less of control with 72 h duration of treatment (Fig. 42 B).

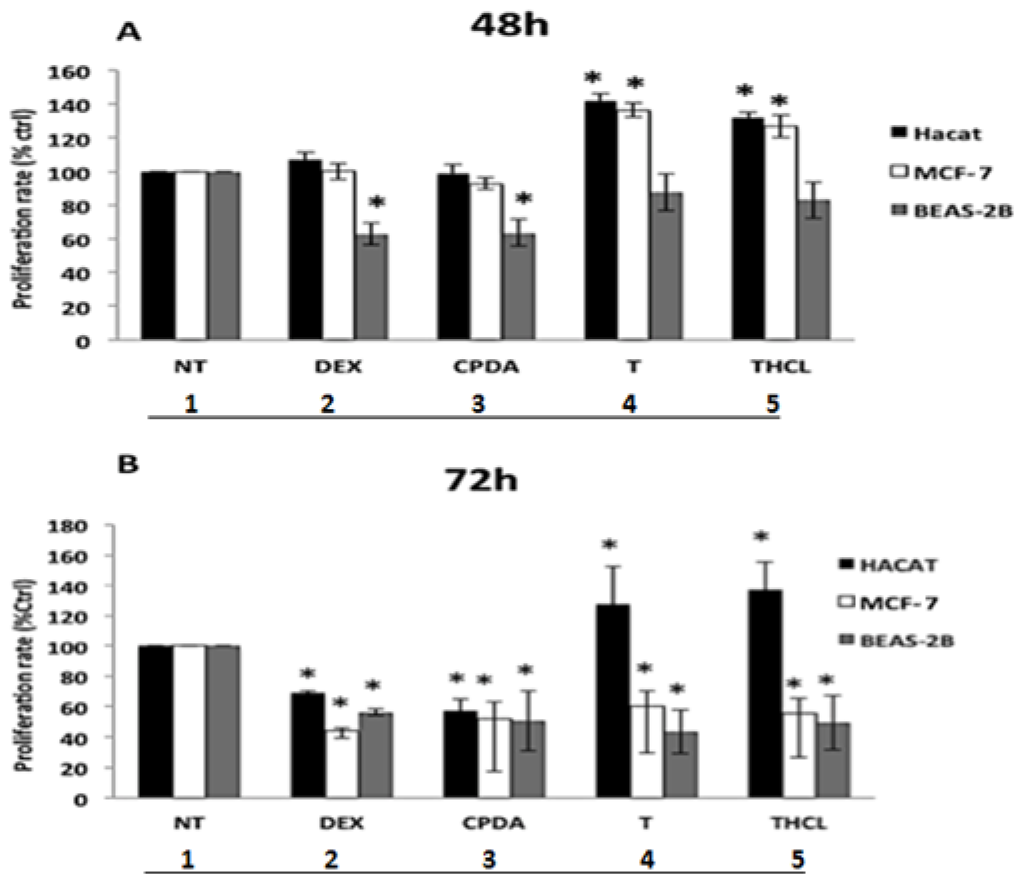


Figure 42 Cytotoxicity of tested compounds in skin and breast malignant cells and non-malignant bronchial cell line.

(A) The effect of 1 µM treatment on epithelial cells viability for 48h. (B) The effect of 1 µM treatment on epithelial cells viability for 72 h.
HACAT (dark grey), MCF-7 (white) and BEAS-2B (light grey) adherent were treated with 1 µM DEX, CPDA, T and THCL. MTS assay were carried out 72 h later to detect the effect of the compound on the proliferation of epithelial cells. Data shown are representative of three experiments in triplicates. Error bars represent standard error of means *P<0.05.

3.4 Flow cytometric analysis of cellular effects of studied compounds

Flow cytometry technique was chosen to study cell cycle progression and cell fate in detail and investigate what pathways to death were utilized by cells when treated with individual compounds. Cells were stained with propidium iodide (PI) to determine effects on cell cycle progression and total cell death. Annexin V analysis was used to determine effects on apoptosis. ROS was followed to investigate the possibility that the tested compound might act by altering ROS pathways.

3.4.1 Effects of tested compounds on cell cycle progression

3.4.1.1 Effect of tested compounds on CEM-C7-14 cell cycle distribution

CEM-C7-14 cells were treated with indicated compounds for 48 h, and then DNA content of the cells was stained with PI to elucidate potential effects on cell cycle progression (Fig. 43). A significant up-regulation of sub-G1 phase was observed in cells treated with DEX and CPDA compounds (Fig. 43, A black and grey bars lanes 2 and 3), except in the case of T (Fig. 43, A lane 4), while THCL (Fig. 43, A lane 5) caused non-significant expansion of the sub- G1 stage of cell cycle progression, which is indicatory of cytotoxic effect of the drugs.

Changes in other cell cycle phases were investigated and particularly in G1 phase (Fig. 43 B) a statistically significant decrease in G1 phase upon all treatments was observed (Fig. 43,B compare lane 1 to lanes 2-5). There was no increase in S phase, (Fig. 43, C

compare lane 1 to lanes 2-5). G2/M phase (D) has shown a non-significant decrease upon DEX and CPDA (lanes 1 and 2). Taken together, DEX and tested compounds increased cell death and decreased G1 cell population in CEM-C7 cell line.

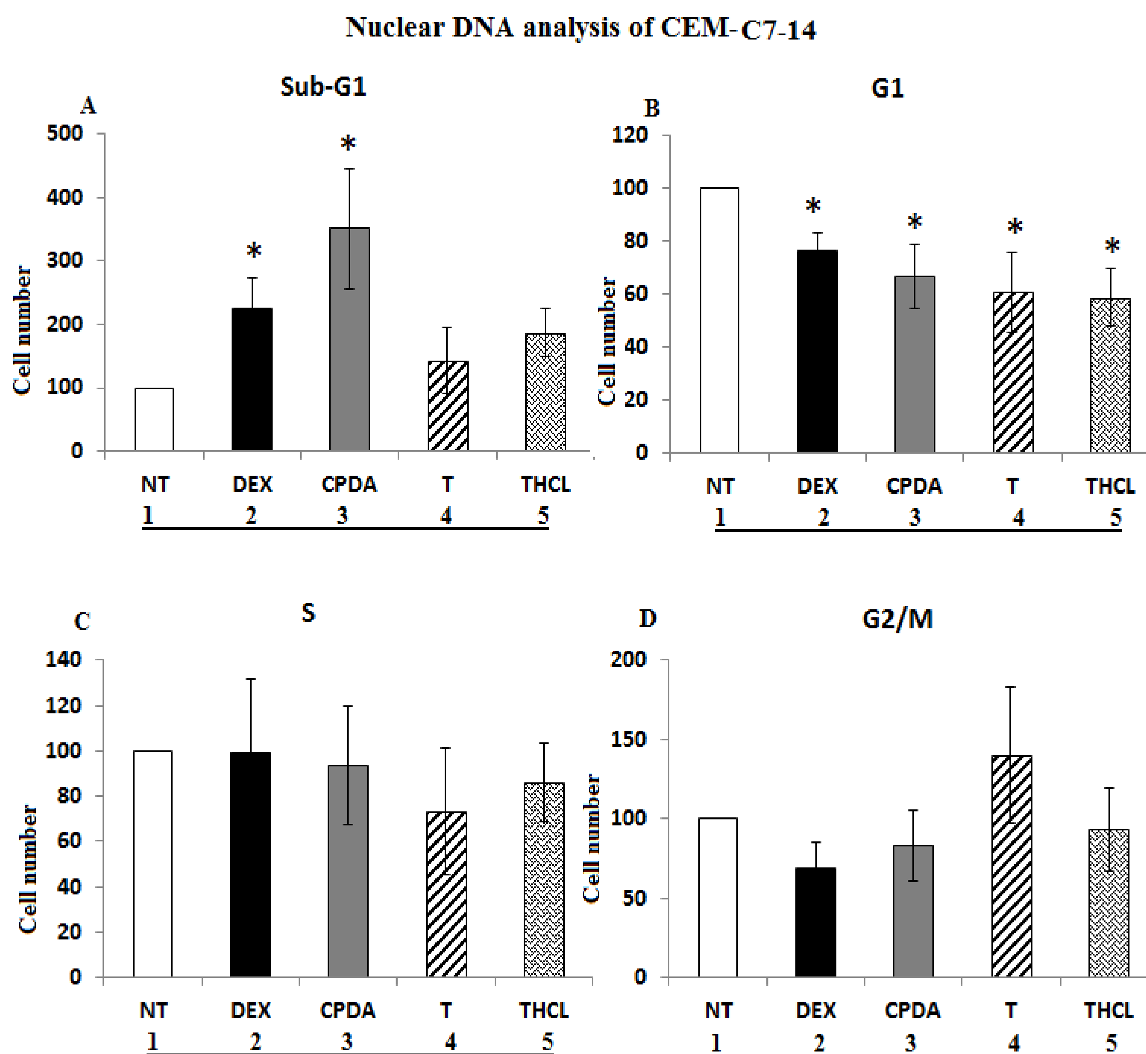


Figure 43 Cell cycle analysis of CEM-C7-14.

Fluorescence activated cell sorter (FACS) analysis of CEM - C7-14 cells. (A) Sub-G1, (B) G1, (C) S phase and (D) G2/M. cells were treated with 1 µM of DEX, CPDA, T or THCL for 48 h, and then stained with propidium iodide. The analysis was performed using BD FACS verse TM, by aid of BD FACS Suite software, through PE-A channel. Data shown are representative of minimum of three experiments. Error bars represent standard error of means *P<0.0

3.4.1.2 Effect of tested compounds on CEM-C1-15 cell cycle distribution

CEM-C1-15 cells were treated as indicated in materials and methods and in the previous section, and then DNA content of the cells was stained with fluorescent dye PI to elucidate cell cycle progress of the cells (Fig. 44). Small insignificant increase in the sub-G1 population of C1 was observed upon all treatments except when T was used (A, compare 4 with lanes 1-5). G1 phase generally decreased or was unchanged (B lanes 1-5). S phase cell population revealed insignificant decrease upon DEX and T and unchanged upon DEX and THCL, also insignificant decreased have been observed upon DEX, CPDA and THCL in G2 cell population (C, lanes 2, 3 and 5). Relative ratios of different cell cycle phases was altered in DEX versus CPD A treated cells.

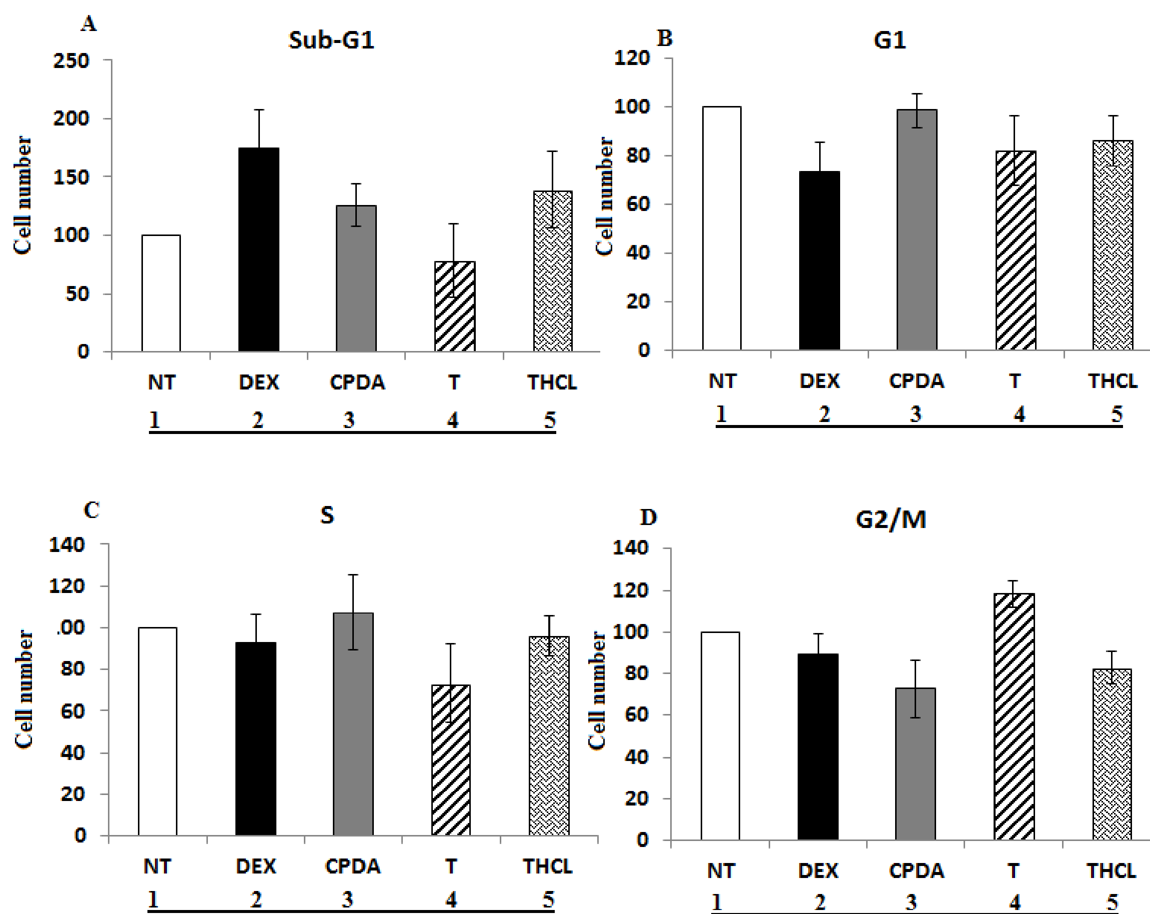


Figure 44 Cell cycle distributions of CEM-C1-15 cells.

Fluorescence activated cell sorter (FACS) analysis of CEM -C1-15 cells. (A) Sub-G1, (B) G1, (C) S phase and (D) G2/M; cells were treated with 1 µM of DEX, CPDA, T or THCL for 48 h, and then stained with propidium iodide. The analysis was performed using BD FACS verse TM by aid of BD FACS Suite software. Data shown are representative of minimum three experiments. Error bars represent standard error of means *P<0.05.

3.4.1.3 Effect of tested compounds on DT40 cell cycle distribution

DT40 cells were treated as indicated in materials and methods and previous section, and then DNA content of the cells was stained with fluorescent dye to determine effect of compounds on cell cycle progression (Fig. 45). Sub-G1 phase was up regulated

insignificantly upon DEX, CPDA and THCL (A ,lanes 2,3 and 5); insignificant down-regulation of G1 has been seen upon DEX,T and THCL (B lanes 2,4 and 5); There were no significant changes in S phase comparing to not treated cells (C, compare lane 1 and 2,3,5) except insignificant down-regulation upon T (C, lane 5); in addition no significant decrease has been recorded upon DEX and T treatments comparing to control in G2/M phase, except in cells treated with CPDA where significant downregulation was observed (D compare lane 1 to lanes 2-5).

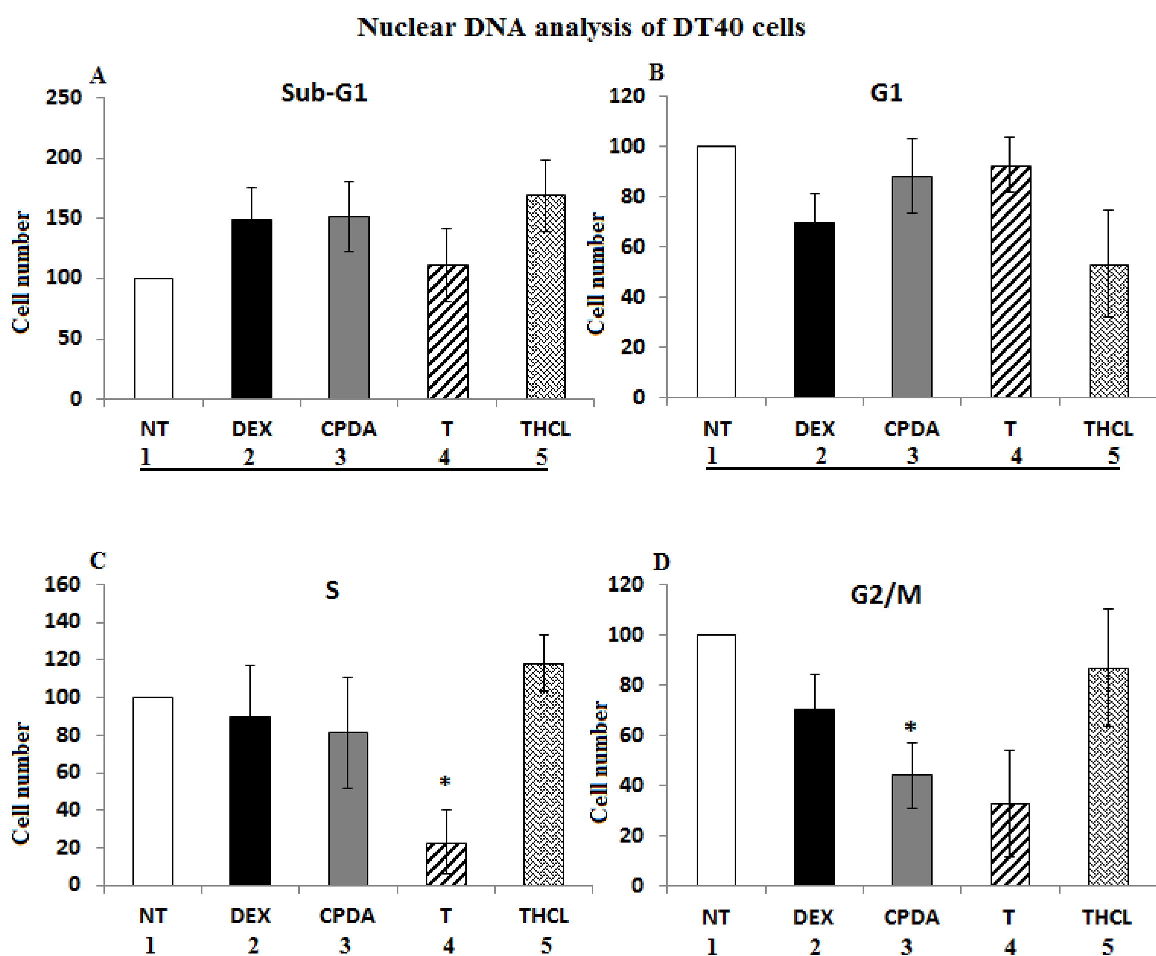


Figure 45 Cell cycle distributions of DT40 cells.

Fluorescence activated cell sorter (FACS) analysis of DT40 cells. (A) sub-G1, (B) G1, (C) S phase and (D) G2/M; cells were treated with 1 µM of DEX, CpdA, T or THCL for 48 h, and then stained with propidium iodide. The analysis was performed using BD FACS verse TM by aid of BD FACS Suite software. Data shown are representative of minimum three experiments. Error bars represent standard error of means *P<0.05.

3.4.1.4 Effect of tested compounds on cell cycle distribution of PBMCs

PBMCs were treated as indicated in materials and methods, and then DNA content of the cells was stained with PI to analyze cell cycle progression of the cells upon different treatments (Fig. 46 A and B).

Sub-G1 phase was lower than control in PBMCs treated with all tested compounds (Fig. 46 A compare lane 1 to lanes 2-5), and G1 increased in comparison to not treated cells (Fig. 46, B compare lane 1 to lanes 2-5). However no statistically significant changes were detected and this experiment was preliminary as it was performed twice due to lack of available samples. As expected, S and G phases were not observed in those cells, as they are not proliferating.

To conclude, the cell cycle of GC-sensitive, GC-resistant and DT40 analyzed cell populations displayed substantial alterations upon tested compounds. In particular sub-G1 phase increased substantially in C7 upon treatments with Dex, CpdA, T and THCl and to a lesser extent in C1 cells treated with Dex, CpdA and THCl, although it was reduced in PBMCs. DT40 cells showed increase in Sub-G1 and G1 cell cycle phases when treated with THCl.

The current results demonstrate that the hormone treatment and tested compounds induced cell death in C7 and C1 cells and that different compounds exerted selective and differential effect of cell cycle progression.

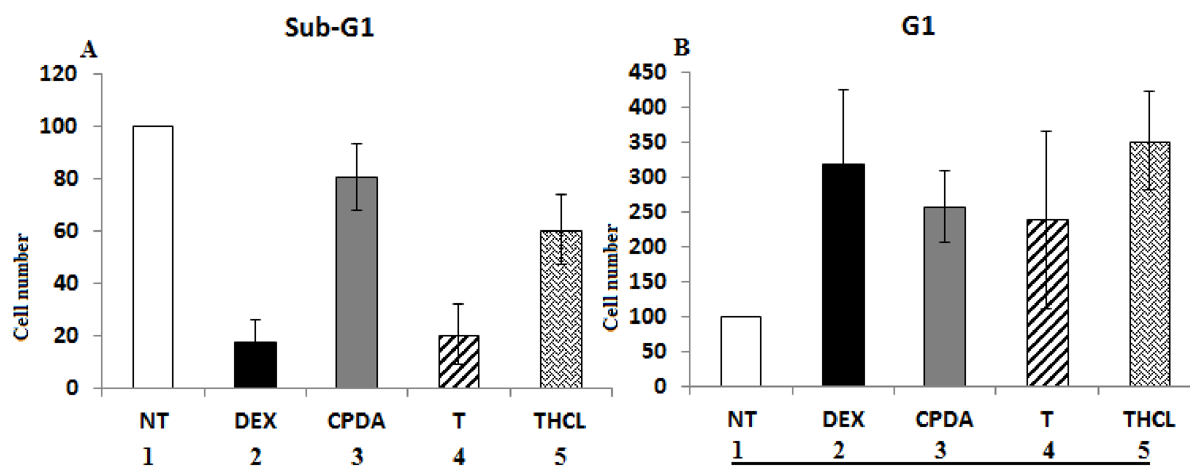


Figure 46 Cell cycle distribution of PBMCs.

Fluorescence activated cell sorter (FACS) analysis of PBMCs. (A) sub-G1, (B) G1 phase; cells were treated with 1 µM of DEX, CpdA, T or THCL for 48 h, and then stained with propidium iodide. The analysis was performed using BD FACS verse TM by aid of BD FACS Suite software. Data shown are representative of two experiments. Error bars represent standard error of means.

3.4.2 Induction of apoptosis of the leukaemia cells treated with studied compounds

The purpose of this part of the study was to determine molecular basis of cancer cell death caused by steroid treatment and tested compounds. For this reason the GC resistant CEM-C1-15 and the GC sensitive CEM-C7-14, DT40 and normal PBMCs were exposed to the synthetic glucocorticoid and tested compounds. Given that MTS assays and FACS/PI staining indicated cytotoxic potential of certain compounds, it was important to investigate what pathway to cell death these compounds induce. Phosphatidyl serine (PS) expression investigated by Fluorochrome-labeled AnnexinV (A5) in presence of PI was employed as described in materials and methods to determine whether one of these pathways was apoptosis.

Total apoptosis was assessed and quantified (see supplementary material 4.5.2) in examined leukemic and normal cells. In C7 cells (Fig. 47 A, B, C , D) DEX and CPDA treatments were found to induce apoptosis. There was insignificant increase in apoptosis in C1 (fig. 48) and DT40 cells treated with all compounds. Also PBMCs cells (based on two experiments) showed a non-significant increase in apoptotic cells upon other compounds treatment rather than DEX which showed tendency to reduce apoptotic cell death in PBMCs. Thus, all tested compounds have different levels of apoptosis induction depending on the compound and cell type. However, given the variation in the data these preliminary results need to be strengthened with additional repetitions of the experiments.

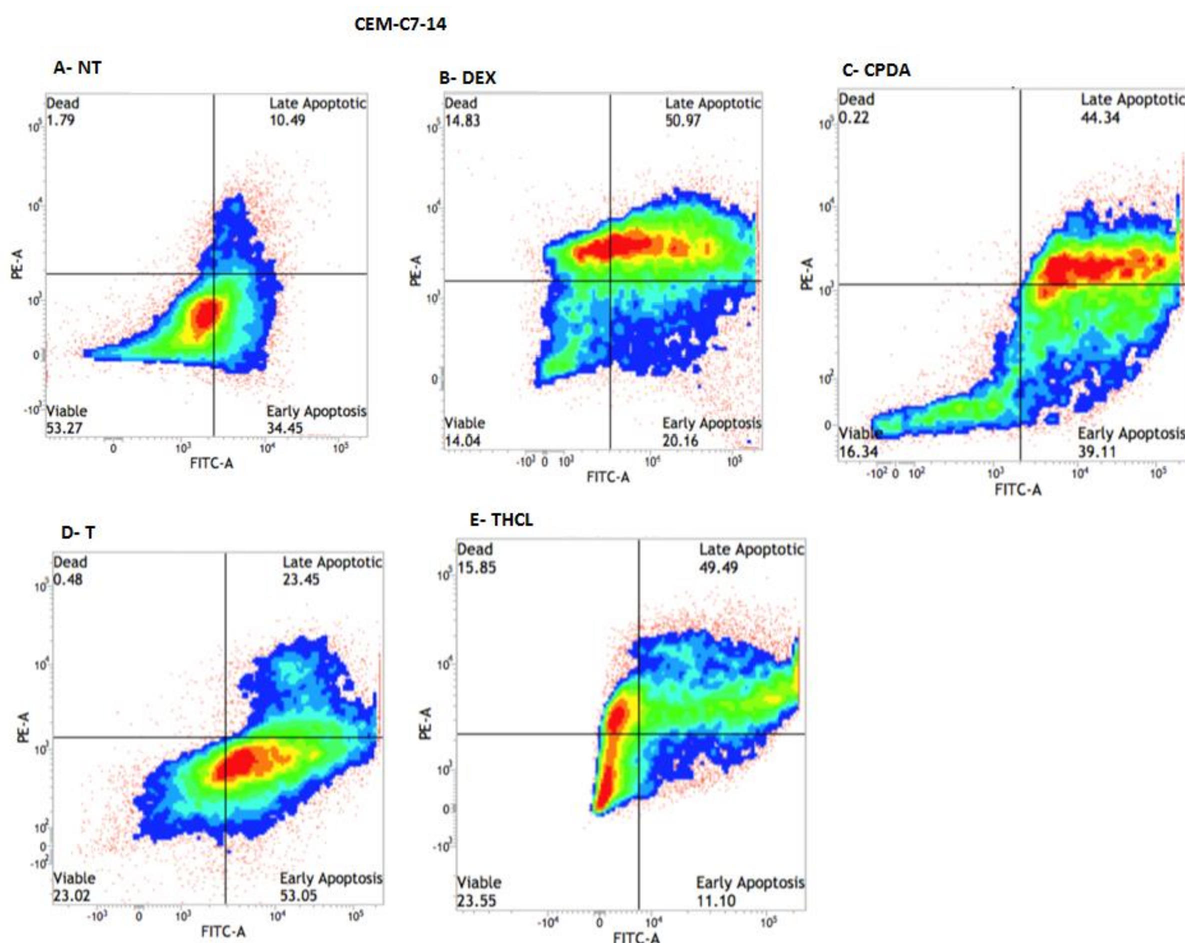


Figure 47 Representative Apoptotic profiles of CEM-C7-14 cell as measured by flow cytometry with AnnexinV-FITC labelled assay

The profile of ALL cells used to detect the apoptosis in leukemia cells upon different treatments at dose 1 µM/48 h. Each square has 4 quarters, upper left quarter indicates dead cells; upper right quarter indicates late apoptotic cells; lower right quarter represents early apoptotic cells; lower left quarter represents viable cells. C7 cells analysis was shown.

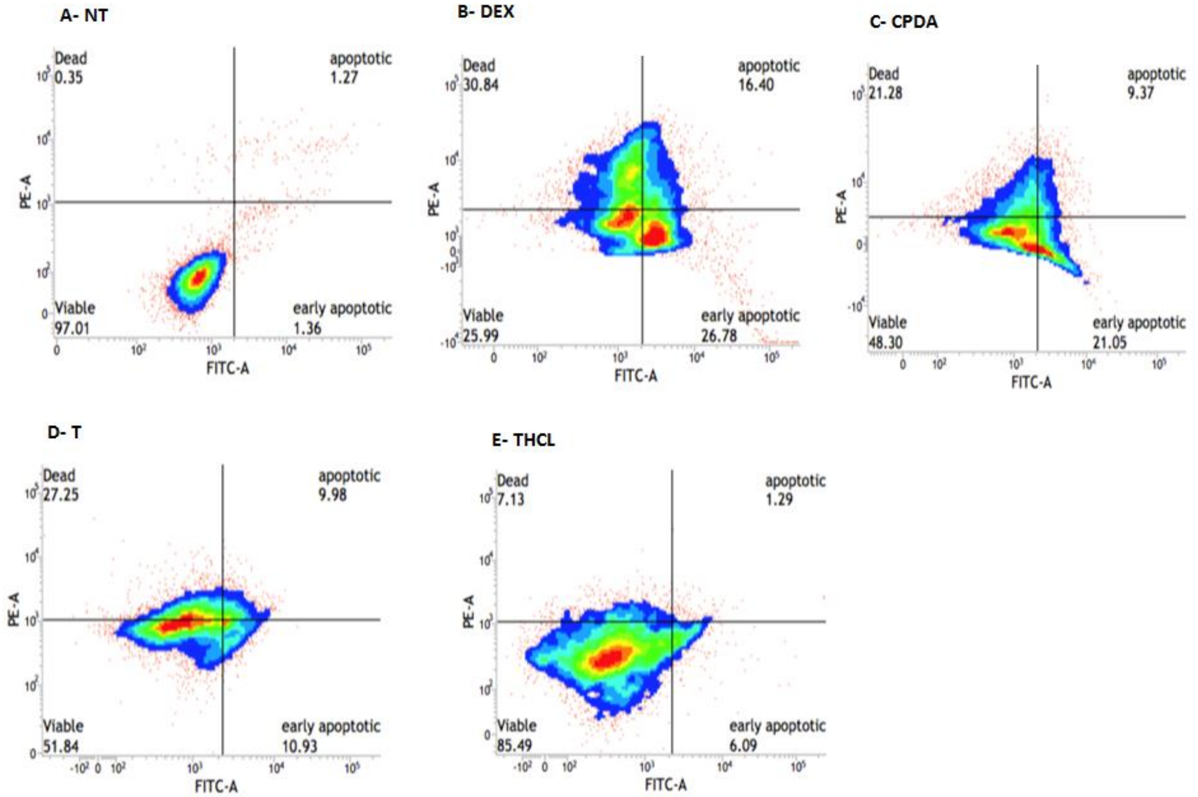


Figure 48 Representative Apoptotic profiles of CEM-C1-15 cell as measured by flow cytometry with AnnexinV-FITC labelled assay.

The profile of ALL cells used to detect the apoptosis in leukemia cells upon different treatments at dose 1 μM/48 h. Each square has 4 quarters, upper left quarter indicates dead cells; upper right quarter indicates late apoptotic cells; lower right quarter represents early apoptotic cells; lower left quarter represents viable cells. C1 cells analysis was shown.

3.4.3 Effect of studied compounds on reactive oxygen species (ROS) levels

Tumour cells normally maintain high levels of ROS due to higher metabolic activity than normal cells (Liou and Storz, 2010). Most chemotherapeutic agents induce cancer cell death and can modulate ROS levels. The purpose of this assay was to investigate if tested compounds modulate ROS through ROS-sensing signalling pathways. Reactive oxygen species (Fig. 49) are believed to be involved in damaging healthy cells, chronic inflammation and cellular biogenic molecules destruction high RO and reactive oxygen species RNS and can lead to development of cancer (Kruk and Aboul-Enein, 2017).

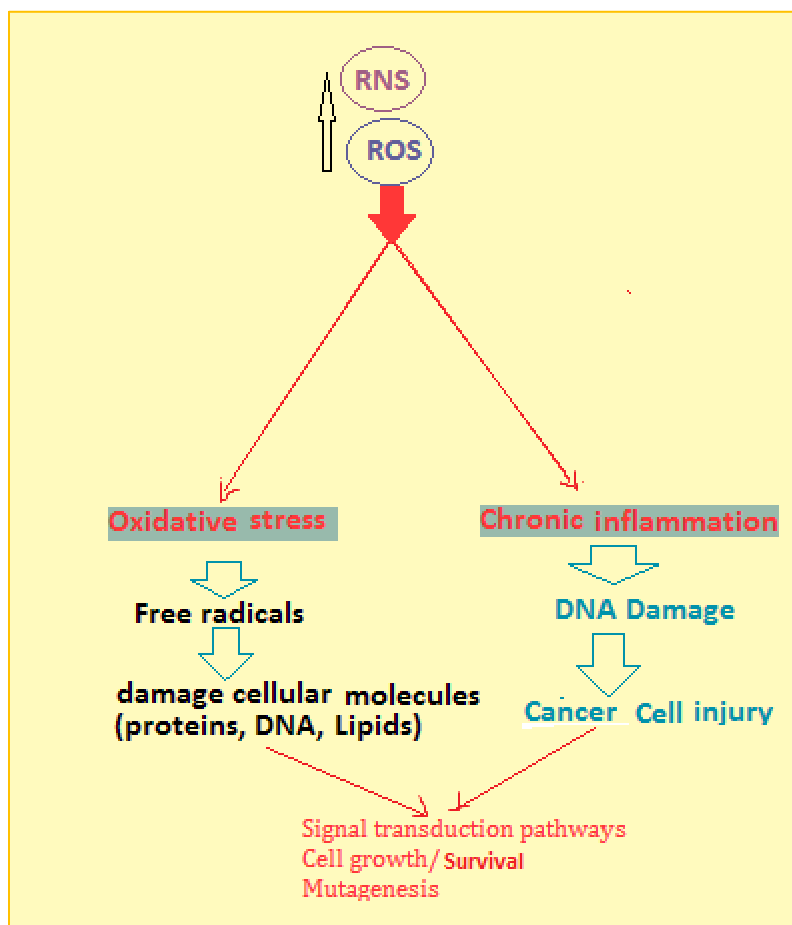


Figure 49 Correlation of ROS and RNS in cancer and the impact of their high levels on cell fate, cell growth and genes. Adapted from(Benedetti et al., 2015).

High ROS levels have been linked to cellular damage or cancer development due to the effect of free radicals on the cell or its vital biogenic components and the effects are proportional to the concentrations of ROS (as shown in fig. 50). Herein low levels are useful to maintain normal cell growth, whereas increasing amounts of ROS leads to dysregulation of normal growth while cancer cells are adapted to normalise ROS levels by antioxidants as a tool to prevent ROS induced cell death (Woo et al., 2017, Teppo et al., 2017, Liou and Storz, 2010).

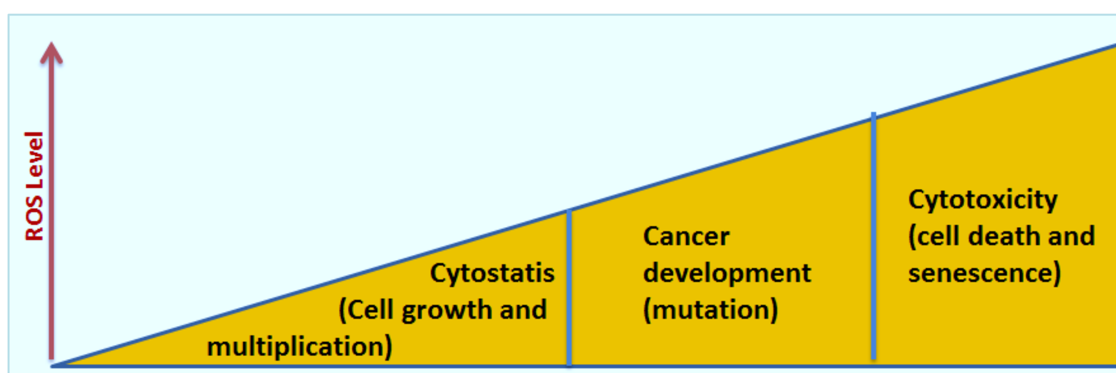


Figure 50 Cross talk between ROS level and its adverse effects. Adapted from (Cairns et al., 2011).

*Cytostasis means growth inhibition.

ROS levels were significantly lower upon DEX and CPDA treatment in CEM-C7-14 cells (Fig. 51 A, lanes 2 and 3). However the opposite was observed in CEM-C1-15 cell line where ROS levels did not change with any treatments (Fig. 51 B lanes 1-5). CPDA and THCL showed tendency to inhibit ROS production in DT40 cells (Fig. 51 C lanes 3 and 5). In PBMCs ROS levels were not substantially affected. The results indicated that ROS levels were regulated in a cell dependent manner upon treatment with tested compounds and linked to the compound ability to induce the death in cell specific manner given that different effect were observed in three cell line. Representative examples of ROS profiles in cells upon different treatments are shown in Fig. (52), where the changes in cellular ROS (shown as positive ROS) was calculated according to the control setting.

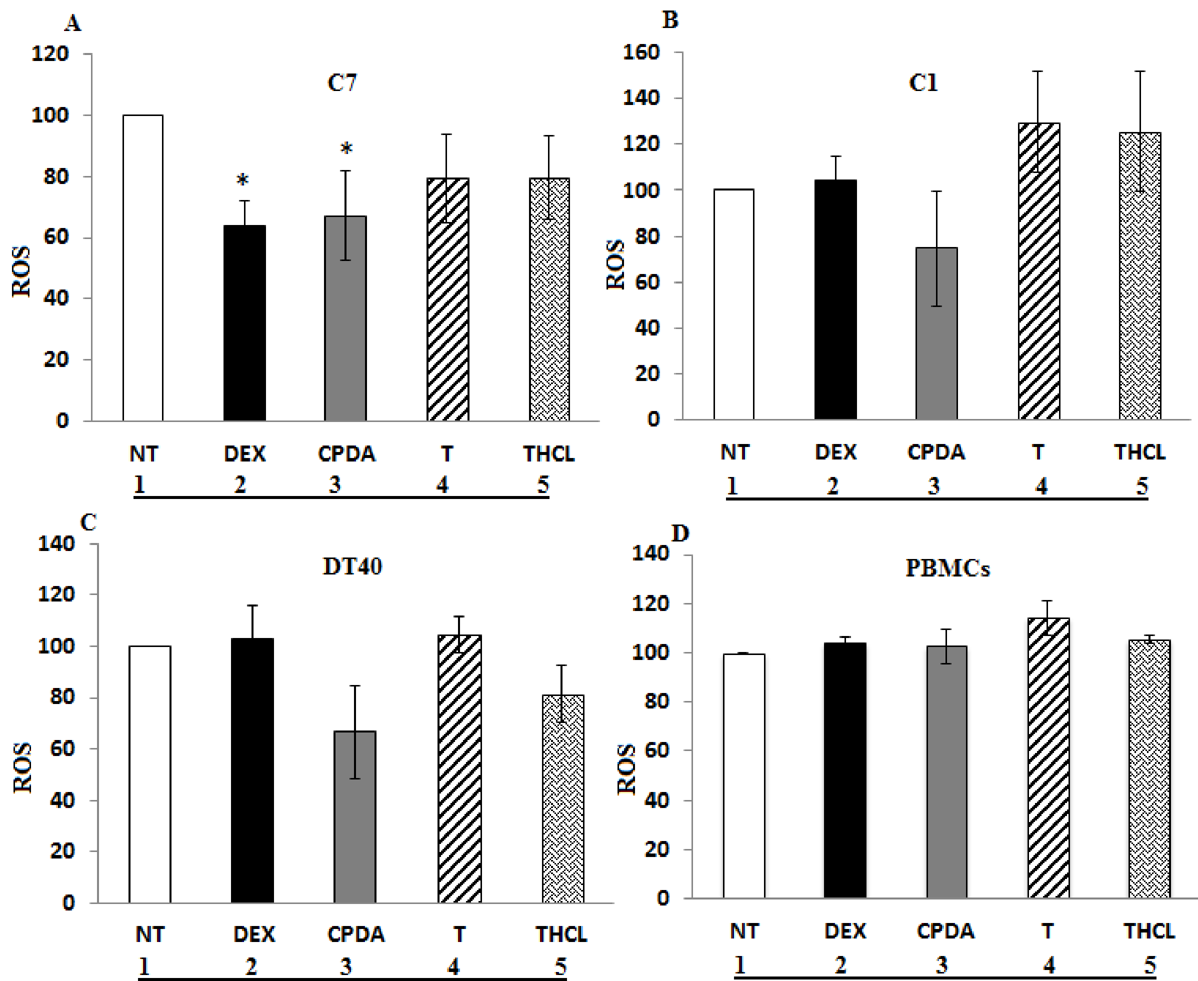
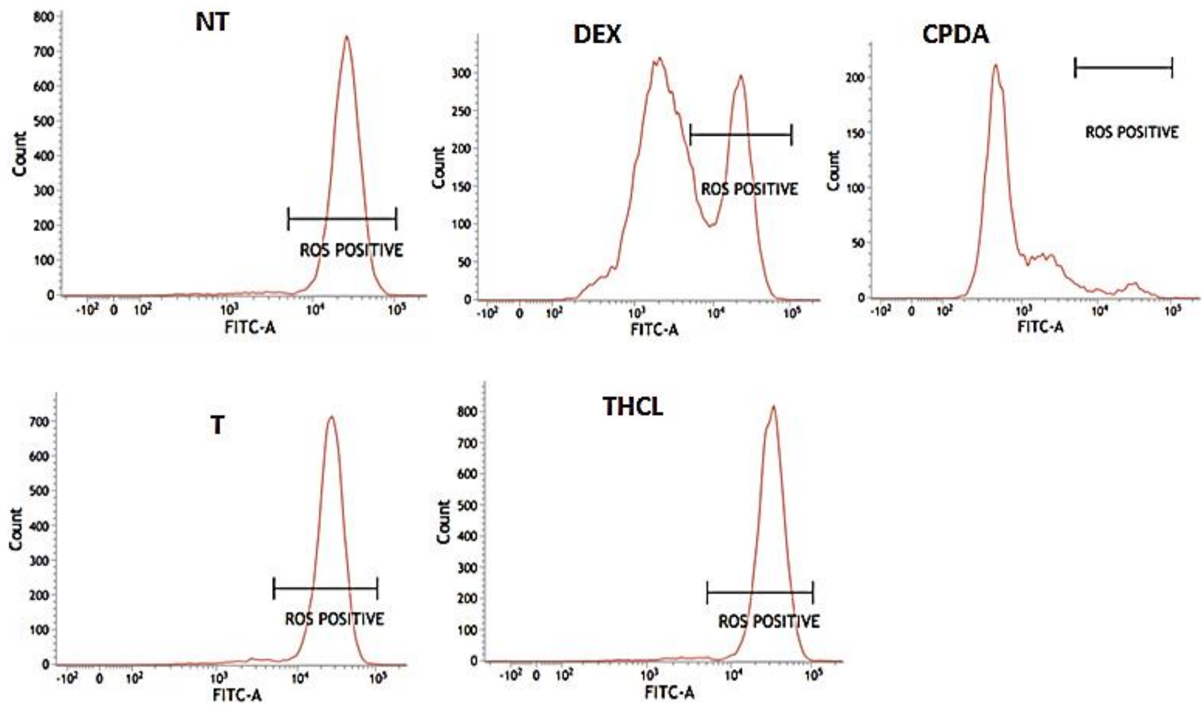
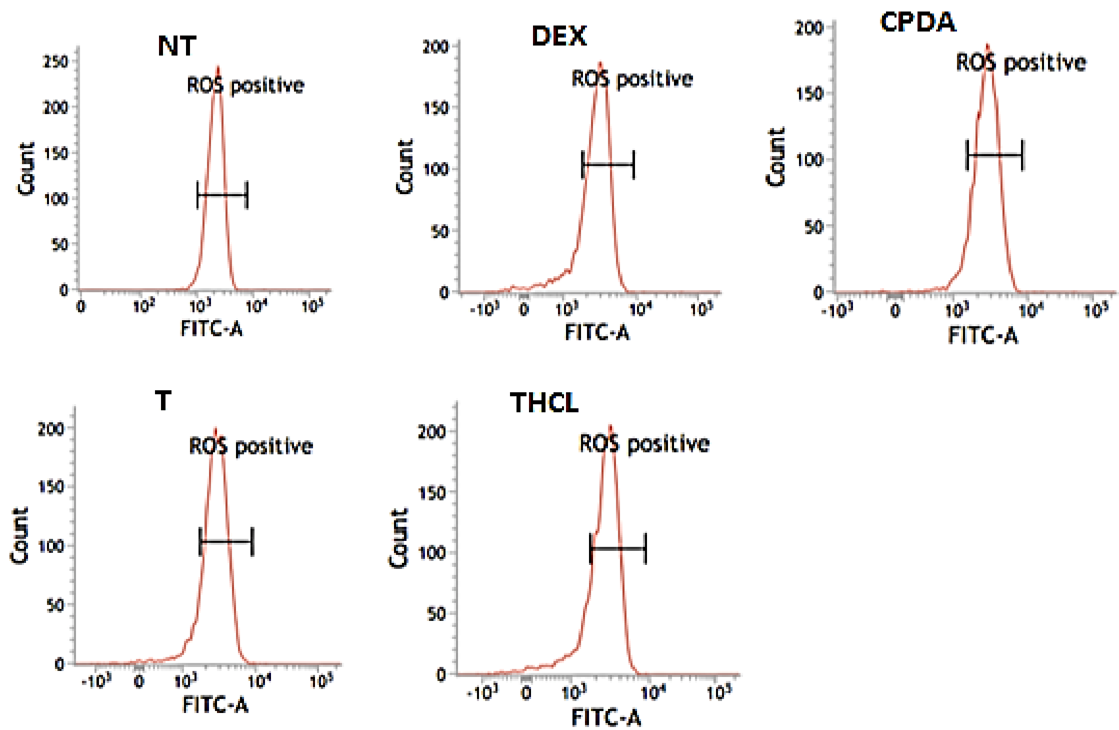


Figure 51 Flow cytometric analysis of DCFDA fluorescence intensity.

Quantification ROS levels as detected by DCFDA (2, 7dichlorodihydrofluoresceindiacetate) fluorescence intensity (measure of ROS). CEM-C7-14 cells (GR- sensitive), CEM-C1-15 (GR- resistant), DT40 cells and PBMCs cells were treated with 1 µM DEX, CPDA, T, and THCL for 24 h. Cells were analysed using BD FACS verse TM by aid of BD FACS Suite software. PI was used to exclude apoptotic and necrotic cells from analysis Data shown of A, B and C are representative of three experiments, and in D two experiments. Error bars represent standard error of means *$P<0.05$.


A CEM-C7-14
NT
DEX
CPDA
T
THCL
ROS POSITIVE
Count
FITC-A


B CEM-C1-15
NT
DEX
CPDA
T
THCL
ROS positive
Count
FITC-A

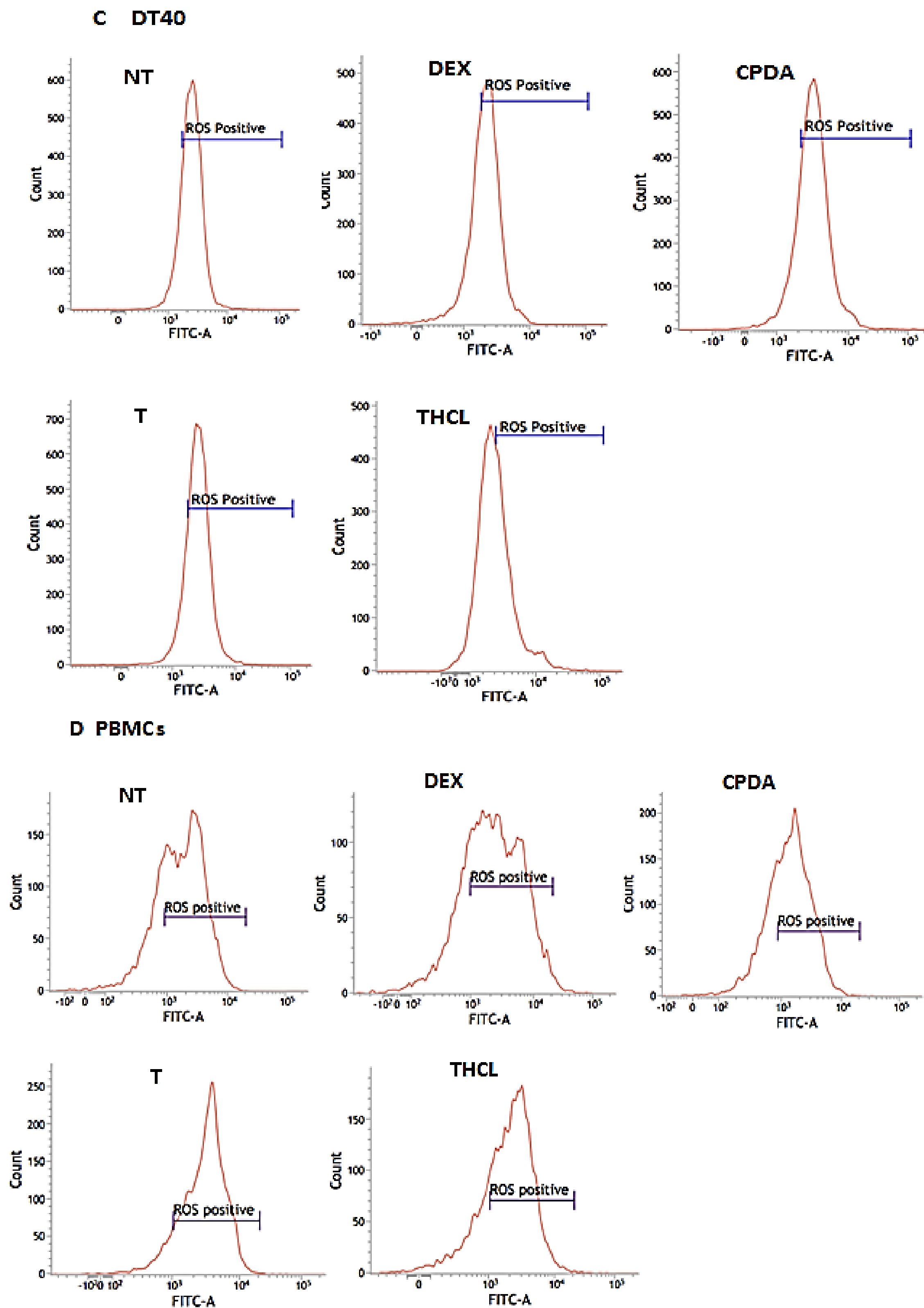


Figure 52 Flow cytometry setting of DCFDA staining.

A. CEM-C7-14, B. CEM-C1-15, C. DT40 CELLS, D. PBMCs
ROS are tested at FITC-A Channe First calibration was done by setting the forward and side scatter according to the control sample;next samples were measured at same wavelengths, the gating for ROS samples positive began from 10^3 and same setting were applied to all tested samples.

3.5 Caspase-8- enzyme modulation by tested compounds

Cysteine aspartate protease 8 (Caspase-8- is) a marker of apoptosis, and has been found to be activated upon apoptosis induced by the death receptor pathway; hence caspase-8 assay was carried out to gain insight into cell death pathways potentially induced by tested ligands by measuring Caspase-8 enzyme activity. Caspase 8 activity increased significantly in GC-sensitive C7 cells upon DEX, T, and THCL whereas CPDA lead to decrease in caspase-8 (Fig. 53, compare black bars).

In C1 cells DEX and T showed tendency to insignificantly decrease the activity of Caspase-8, while, CPDA demonstrated substantial significant inhibition of caspase-8 level, whereas THCL had no effect (Fig. 53, dark grey bars).

In DT40 cells DEX, CPDA and T had no major effects on caspase-8 activity; however there was tendency of Caspase-8 increase upon THCL treatment (Fig. 53, compare white bars). In PBMCs Caspase-8 was only significantly downregulated upon treatment with DEX while other tested compounds have no significant effect (Fig. 53, compare light grey bars).

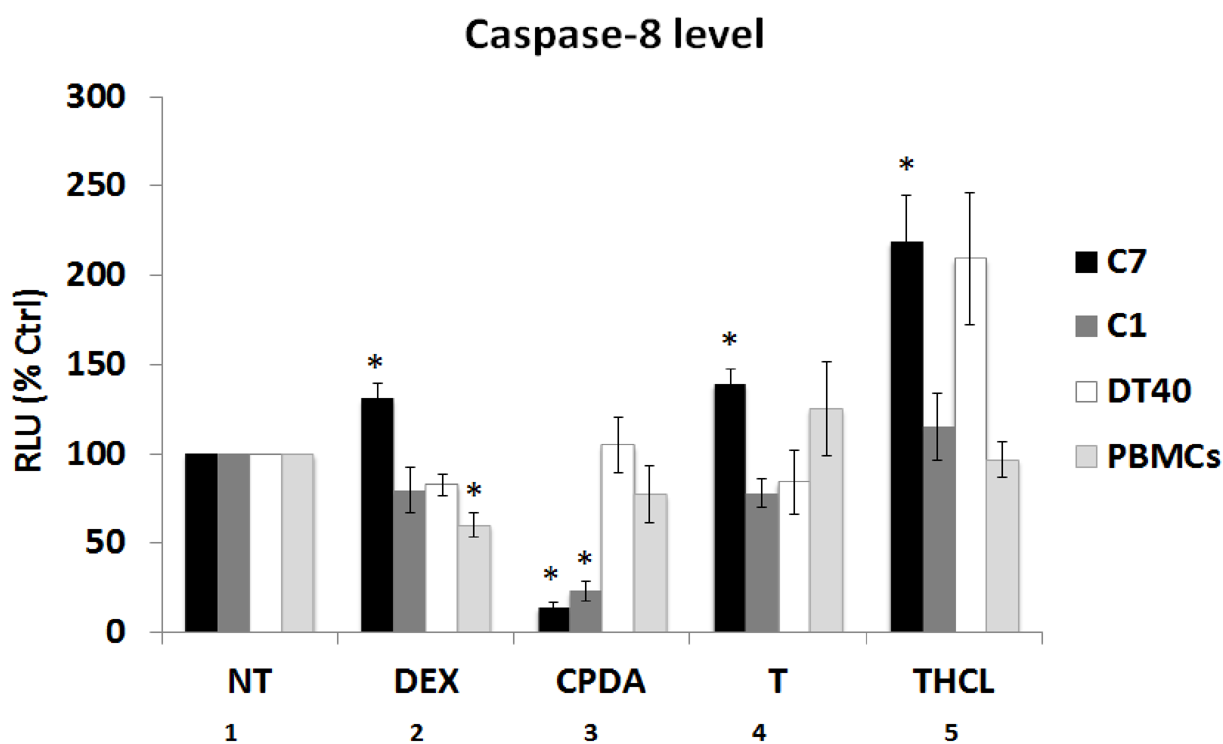


Figure 53 Caspase-8 assay.

Caspase-8 activity was measured in CEM-C7-14, CEM-C1-15, DT40 cells and PBMCs after 48 h of treatment with 1µM of tested compounds. Relative luminescence units were measured using illuminometer and standardized according to control (NT) group. Data shown are representative of three experiments in triplicates. Error bars represent standard error of means *P<0.05.

3.6 Human and chicken proteins similarity

The purpose of this part of investigation was to provide background about the molecular assays which have been used in this studyand to analyseand compare the effect of tested compounds on range of selected proteins/biomarkers. According to (Boratyn et al., 2012), Basic Local Alignment Search Tool in NCBI BLAST was used to detect the similarity between (chicken) [Gallus gallus] and human proteins [Homo sapiens] (table 28).

Table 29 Human and chicken proteins similarity determined using NCBI Blast tool.

Blast results for each protein are in supplementary material

Protein	*Gallus gallus*		*Homo sapiens*		% of similarity
	ID	Length	ID	Length	
GR	NP001032915.1	772aa	ADP91252.1	777aa	74
BIM	XP015138913.1	314aa	NP619532.1	135aa	55
C-JUN	P18870.2	314aa	NP002219.1	331aa	82
Actin	CAA25004.1	375aa	P60709.1	375aa	99
IL-6	ADL14564.1	241aa	AAD13886.1	212aa	95
IL-2	CAE17662.1	143aa	CAA25742.1	153aa	33

3.7 Gene and protein expression analysis

In order to obtain further insight in molecular basis of observed cytotoxic effects of studied drugs, we analysed protein and gene expression levels of the GR and its target genes. For this purpose we employed western blot and qRT-PCR analysis to monitor GR and auto regulation of its own levels (Veneris et al., 2017), BIM as it is a known pro-apoptotic GR target (Prenek et al., 2017), C-JUN (Weikum et al., 2017a) and NF-$_{\kappa}$B (Bekhbat et al., 2017) that are involved in negative crosstalk with GR in the GC-sensitive (CEM-C7-14), GC-resistant (CEM-C1-15) and DT40 cells. To closely analyse the effect of GR on its targets, we determined protein and mRNA expression profiles upon treatment of these cells with DEX, CPDA, T, and THCL for 24 h. The time points were selected based on studies performed by previous members of our and other laboratories, which determined this duration as

optimal Chen (2012) and Qattan (2014). Actin was used as an internal control for the measurements of protein levels, and RPL-19 as an internal control for gene expression analysis.

3.7.1 Analysis of gene expression and protein levels in CEM-C7-14 cells

Known glucocorticoid receptor targets were analysed as indicators of leukemia cell response to DEX and the tested compounds CPDA, T, THCL respectively. To assess the role of the tested compounds in apoptosis, BIM the Bcl-2 family pro-apoptotic member that is involved in the regulation of glucocorticoid-induced apoptosis, mRNA levels were measured. In addition GR, c-Jun and NF-κB mRNA levels were tested in all cell lines treated with studied compounds. To investigate whether these GR targets were regulated by GR at the transcriptional level in cells treated with investigated compounds qRT-PCR was performed to quantify the mRNA levels (Fig. 54). The mRNA levels of GR and BIM showed trend of upregulation following DEX, CPDA, T and THCL treatments, however only Dex treatment led to significant change (Fig. 54 A and C). C-Jun levels increased in cells treated with all studied compounds except with DEX which caused downregulation (Fig. 54, B). NF-kB mRNA expression has displayed a reduction in DEX and THCL treated cells, whereas it showed trend to increase upon CPDA and T treatment (Fig. 54, D).

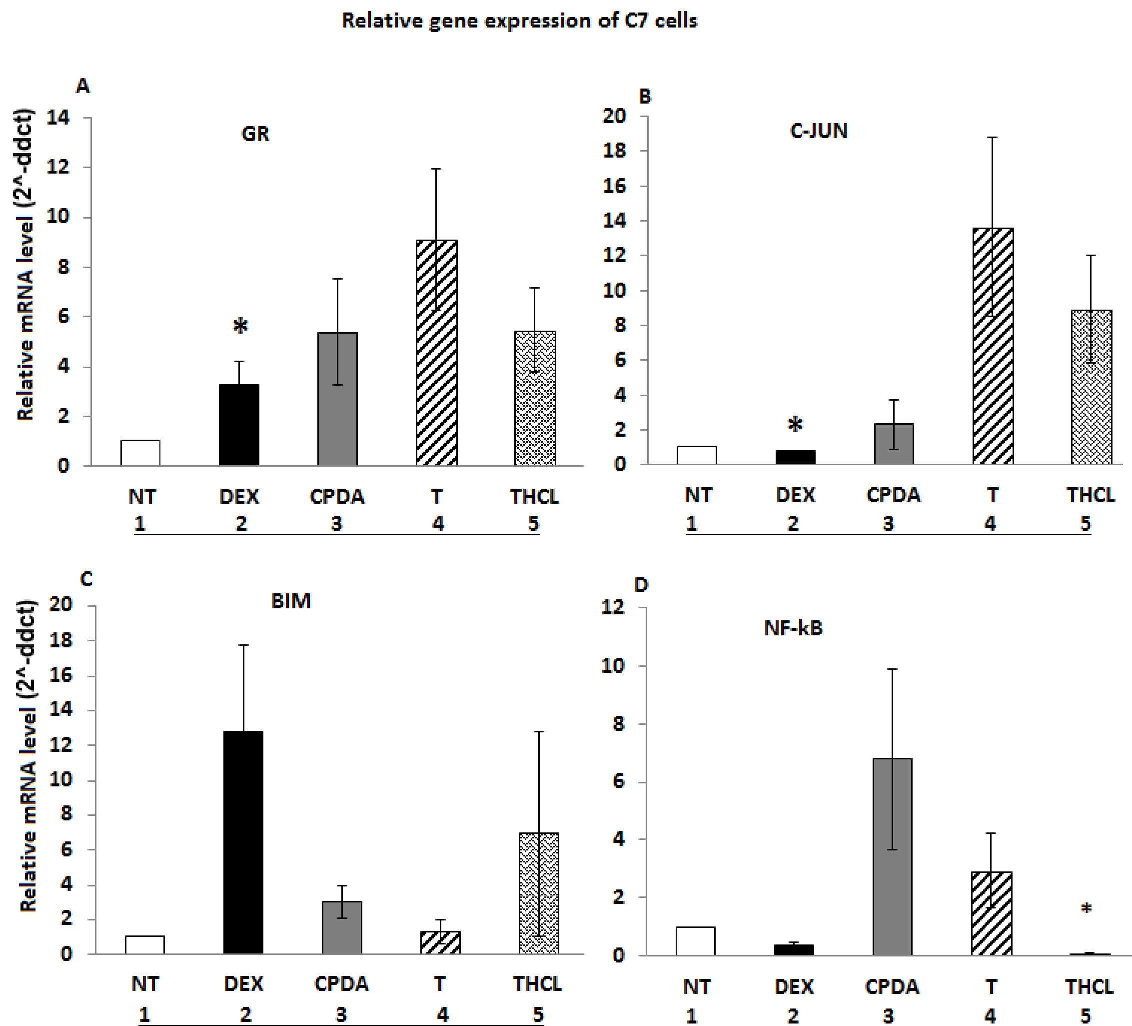


Figure 54 Relative mRNA levels of glucocorticoid receptor target genes in CEM-C7-14 cells.

CEM- C7-14 **cells were treated with 1µM DEX, CPDA, T, and THCL for 24 h and the mRNA levels of (A) GR, (B) C-JUN, (C)BIM and (D)NF-kB (normalized to RPL-19) were determined by quantitative real-time PCR. Data shown are representative of three experiments in triplicates. Error bars represent standard error of means *P<0.05. GR**

In order to analyse and compare if protein levels follow of mRNA levels of these genes, western blot analysis was carried out in CEM-C7-14 cells treated with 1 µM DEX, CPDA, T and THCL. In addition to protein levels of GR, BIM and c-JUN phosphorylated GR at serine 211 (S211) was also followed. GR phosphorylation at serine 211 was followed as an additional control mechanism implicated in the regulation of glucocorticoid GR induced apoptosis. Experiments were repeated three times (see supplementary figure 4.5.3.1) and

values of densitometrically quantified bands were normalized to those obtained for actin, which was used as a loading control. Results were plotted as fold change over the value acquired for the untreated sample (NT) (Fig. 55). Due to the poor quality of some blots limited conclusions were obtained. Nevertheless, the results showed an increase in protein levels of the total GR in C7 cells upon all investigated agents and significantly upon DEX (Fig. 55 A lane 2). On the other hand GR phosphorylated at Ser211 protein levels increased in cells treated with all compounds, however due to large variations results were not significant (Fig. 55 C). Protein levels of JUN showed tendency to be up-regulated upon DEX, CPDA and THCL and somewhat in T treated cells (Fig. 55 B). BIM (Fig. 55 D) protein levels were found to be upregulated upon treatment with all compounds, however change was not significant.

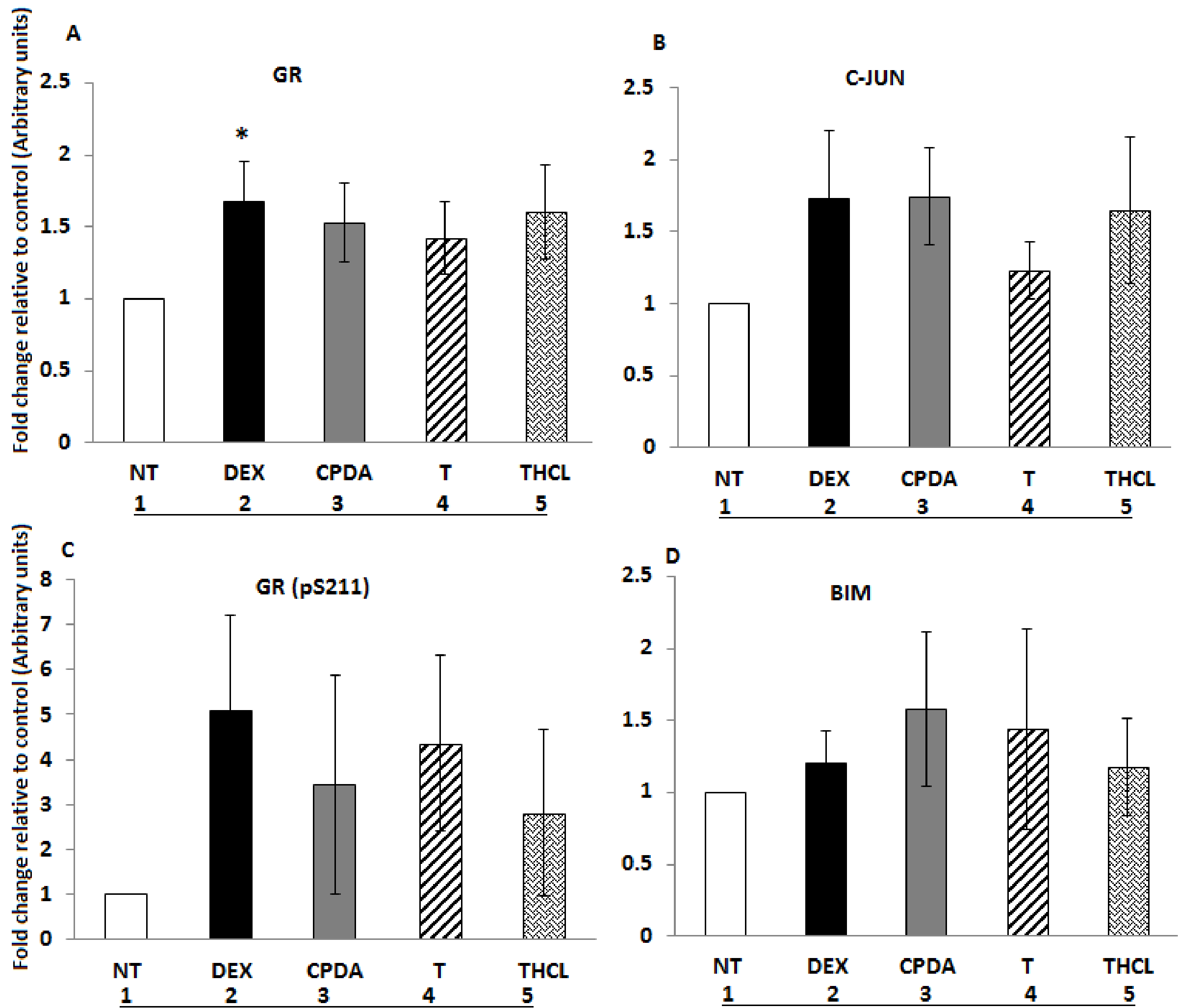


Figure 55 Proteins expression levels in CEM-C7-14 cells.

A Western blot analysis of the GR, C-JUN, GR (pS211) and BIM was carried out, with actin as a control in CEM-C7-14 cells cultured with 1 µM DEX 24 h. (A) GR, (B) JUN, (C) GR (pS211) and BIM. Protein levels obtained from three experiments were quantified by Image J, normalized to actin and presented as a histogram. Error bars represent standard error of means *P<0.05.

3.7.2 Analysis of gene expression and protein levels in CEM-C1-15 cells

An increase in BIM mRNA gene expression was observed in the resistant CEM-C1-15 upon CPDA, T and THCl treatment (Fig. 56 C, lanes 3,4,5); in contrast BIM mRNA level were reduced significantly in DEX treated cells. In resistant CEM-C1-15 cells levels of GR (A) and C-JUN (B) mRNA were reduced in DEX treated cells whereas NF-κb levels (D) increased in most treatments except in the presence of THCl where some inhibition was observed; however significance was not achieved.

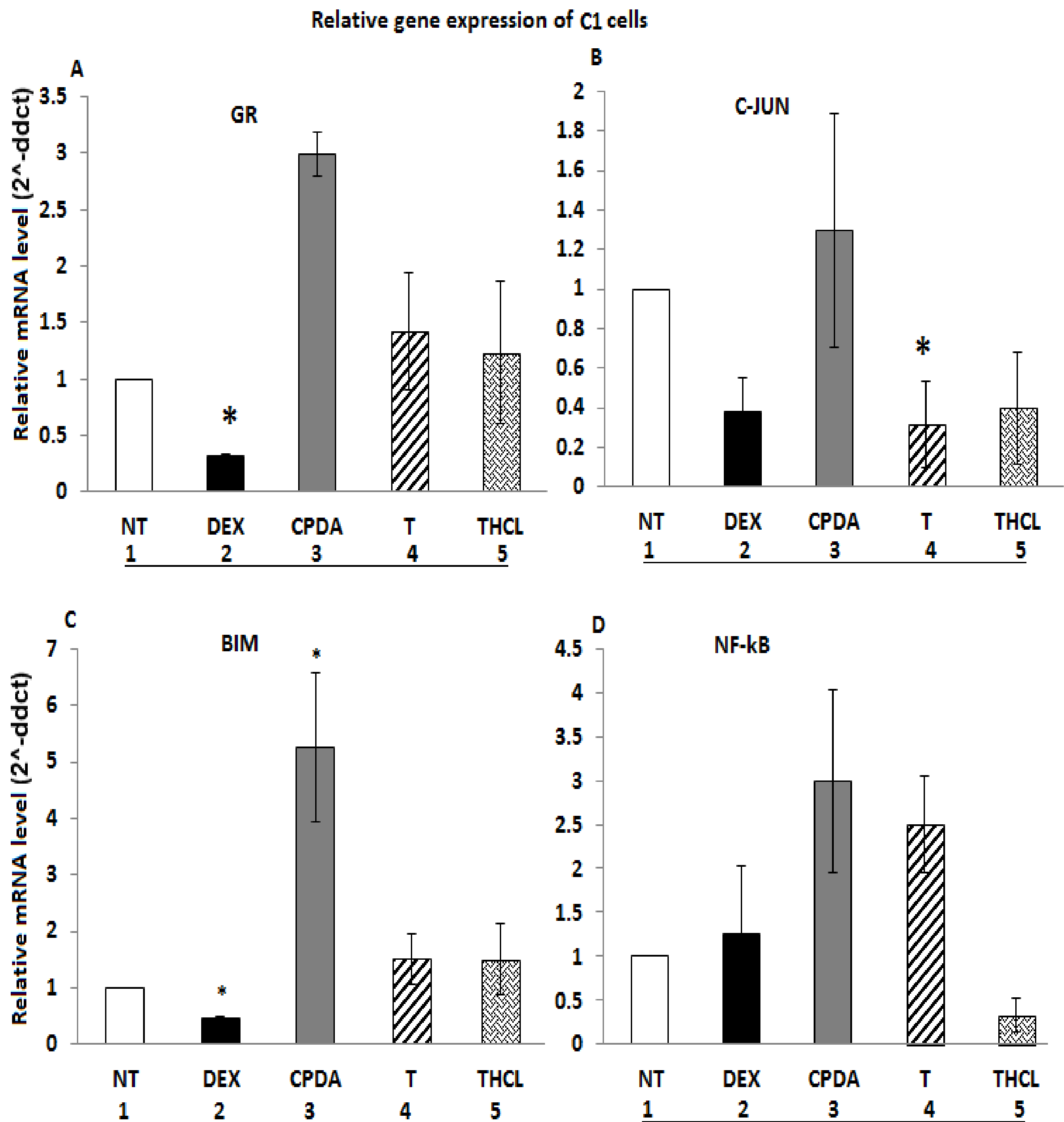


Figure 56 Relative mRNA levels of studied genes in CEM-C1-15 cells.

CEM- C7-14 cells were treated with 1 µM DEX, CPDA, T, and THCL for 24 h and the mRNA levels of (A) GR, (B) C-JUN, (C) BIM and (D) NF-kB (normalized to RPL-19) were determined by quantitative real-time PCR. Data shown are representative of three experiments in triplicates. Error bars represent standard error of means $^*P<0.05$.

The endogenous GR, JUN and GR (pS211) protein levels were analyzed in CEM-C1-15 cells treated with 1μM DEX, CPDA, T or THCL for 24h (Fig. 57). GR protein levels were mostly unchanged in cells treated with all tested compounds (Fig. 57, A lanes 2-5). S211GR protein level showed downward trend in cells exposed to all treatments (C). C-JUN (B) upregulation trend was detected in CEM-C1-15 cells treated with DEX, T and THCL in spite of insignificancy.

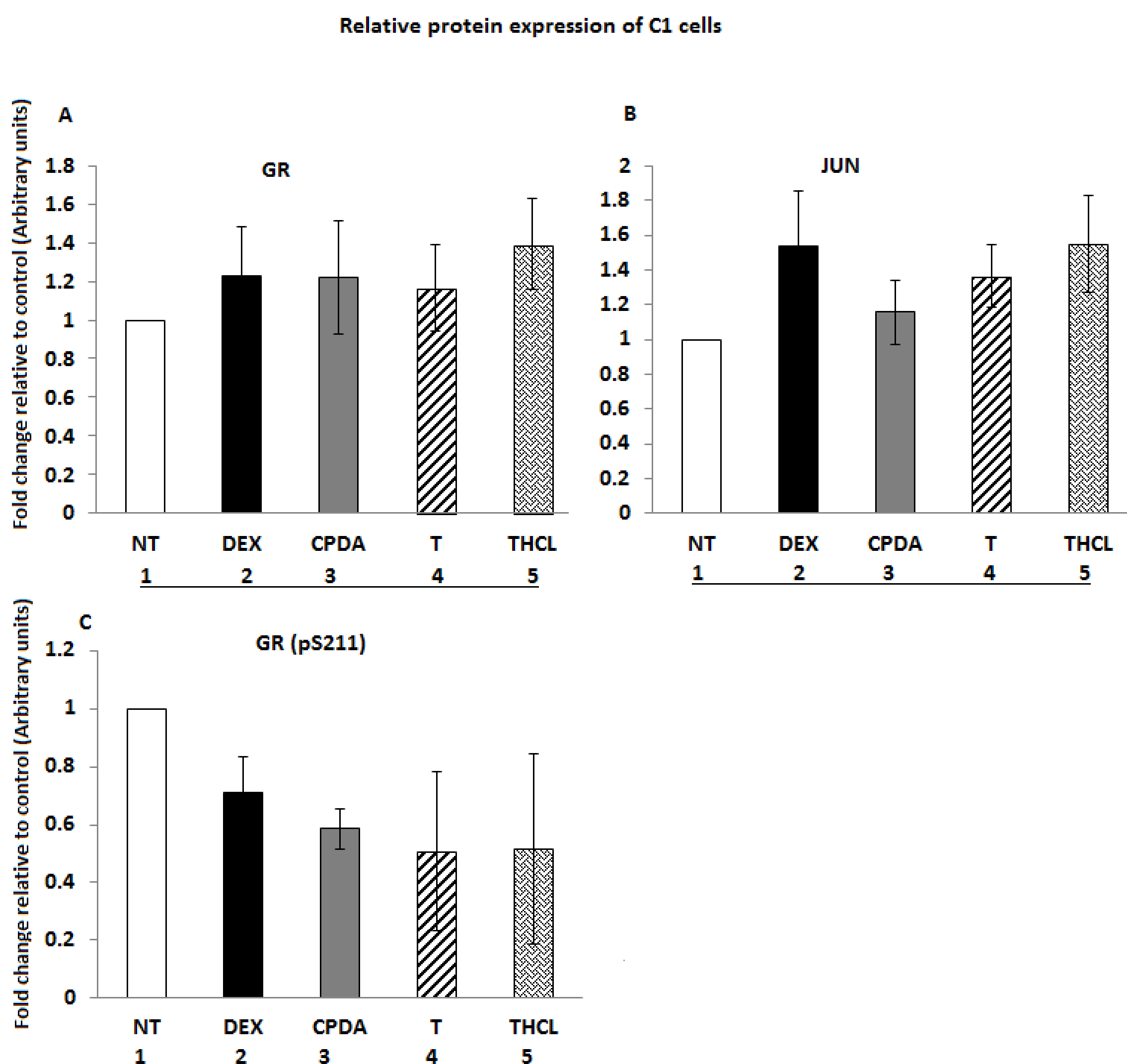


Figure 57 Expression levels of studied proteins in CEM-C1-15 cells.

A Western blot analysis of the GR, C-JUN, and GR (pS211) was carried out, with actin as a control in CEM-C1-

15 cells cultured with 1µM DEX 24h. (A) GR, (B) JUN and (C) GR (pS211). Protein levels obtained from three experiments were quantified by Image J, normalized to actin and presented as a histogram. Error bars represent standard error of means *P<0.05.

3.7.3 Analysis of gene expression and protein levels in DT40 cells

In chicken DT40 cells mRNA expression profiles were followed in cells treated with tested compounds. GR and c-Jun mRNA levels were mostly downregulated in cells treated with DEX, CpdA and T (A and B).There was downregulation observed in NF-κB gene expression in T treated cells (Fig. 58, D), whereas BIM mRNA was downregulated in CPDA and T treated cells (Fig. 58, C lanes 3 and 4).THCl caused large variation in expression of most genes studied leading to inconclusive results.

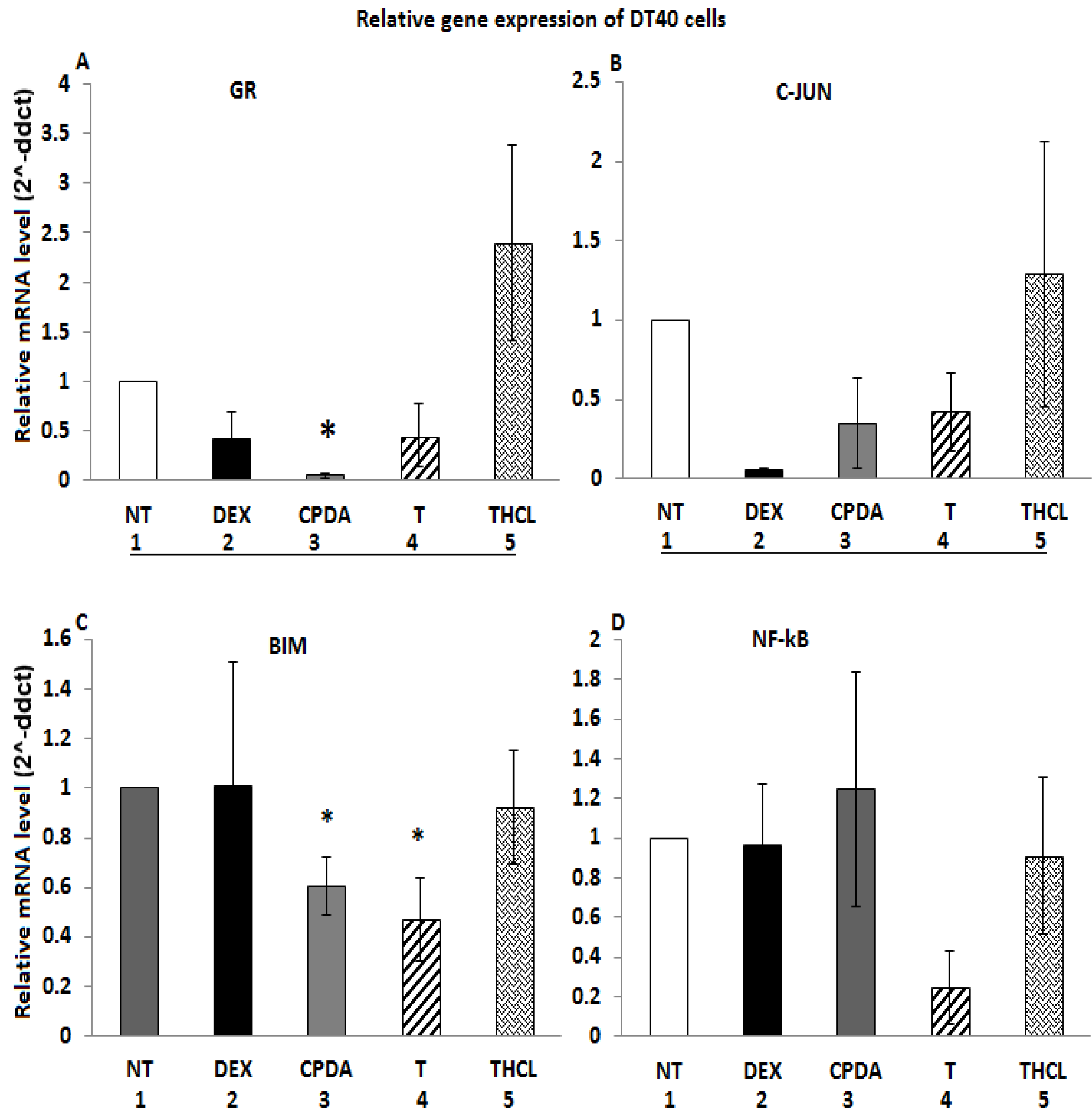


Figure 58 Relative mRNA levels of studied genes in DT40 cells.

DT40 cells were treated with 1 µM DEX, CPDA, T, and THCL for 24 h and the mRNA levels of of (A) GR, (B) C-JUN, (C) BIM and (D) NF-kB (normalized to RPL-19) were determined by quantitative real-time PCR. Data shown are representative of three experiments in triplicates. Error bars represent standard error of means *P<0.05.

Whole protein lysates of DT40 cells were used to analyse protein levels by western blot analysis. DT40 cells treated with DEX, CPDA, T, THCL showed that GR protein levels were generally upregulated with CPDA, T THCL (Fig. 59, A 3-5). C-JUN levels increased upon all

the treatments (Fig. 59, B 2-5). GR (pS211) showed tendency towards upregulation upon CPDA, T and significantly increased upon THCL (Fig. 59, C lane 5). Although chicken GR and human GR have 77% similarly at the level of amino acids , crucial expression may be missed due to using human specific antibodies against chicken GR, C-JUN and S211.

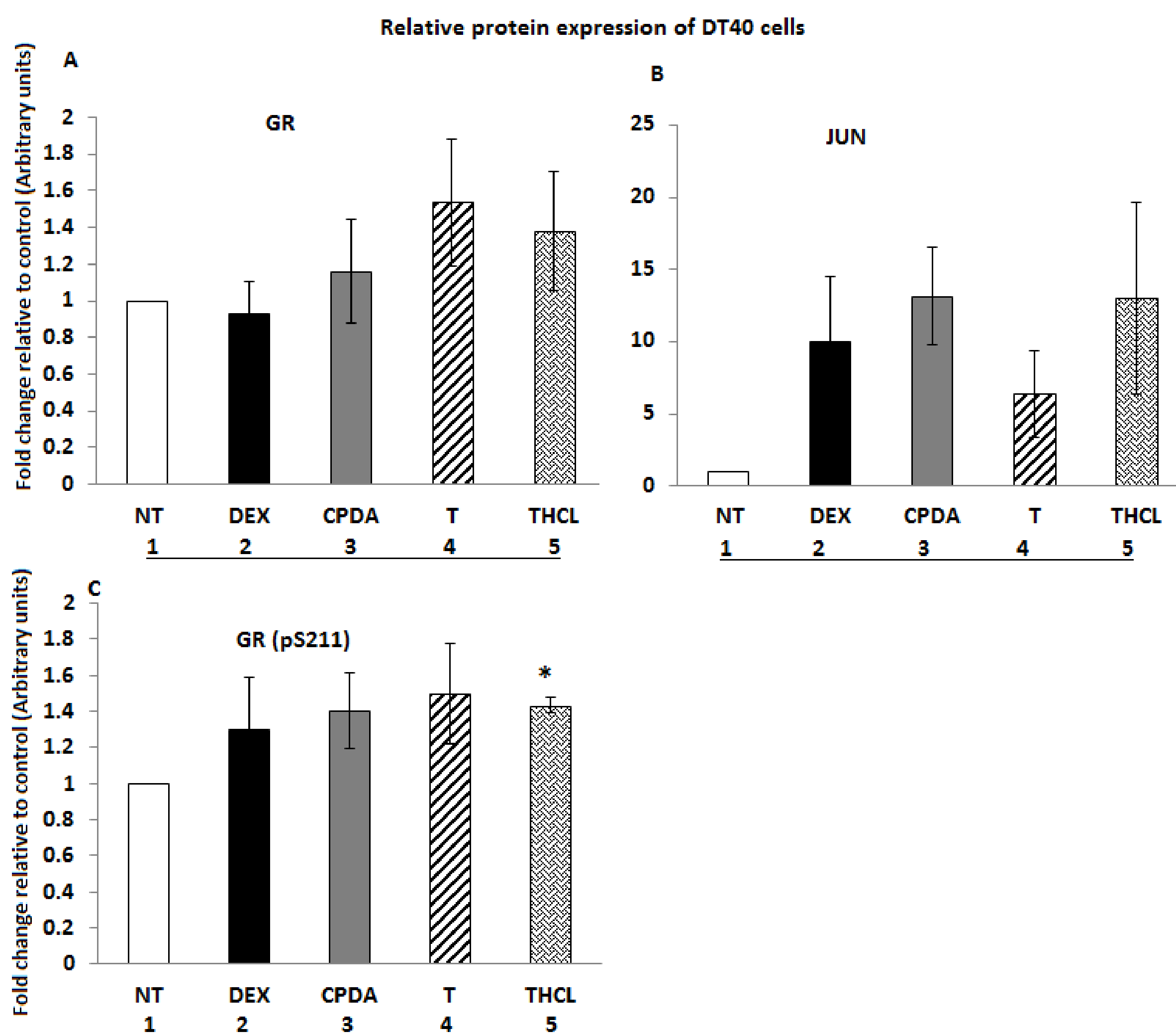


Figure 59 Expression levels of studied proteins in in DT40 cells.

A Western blot analysis of the GR, C-JUN, and GR (pS211) was carried out, with actin as a control in DT40 cells cultured with 1 µM DEX 24 h.(A) GR, (B) JUN and (C) GR (pS211) . Protein levels obtained from three experiments were quantified by Image J, normalized to actin and presented as a histogram. Error bars represent standard error of means *P<0.05.

3.8 Regulation of inflammatory mediators by steroid hormone andthe tested compounds

Cancers are often preceded by inflammation or infection (Robinson et al., 2017, Shimizu et al., 2017, Trehanpati and Vyas, 2017), and some anticancer drugs yield antiinflammatory effect . GR is known to play major role in regulation of pro and anti-inflammatory pathways upon DEX intake. Thus, this part of study was designed to investigate potential anti-inflammatory properties of tested compounds in the examined cells by measuring activity of secretory interleukines to address the alterations in GR function towards pro and anti-inflammatory mediators.

3.8.1 Griess Nitric oxide test

Nitric oxide NO which includes Nitrite and Nitrate has been linked with the severity of inflammatory diseases (Nandeesha et al., 2015). The construction of this reactive oxygen species is controlled by nitric oxide synthase (NOS) gene. Three types of NOS are present in the bodies: inducible, neuronal and endothelial (iNOS, nNOS and eNOS respectively) (Alderton et al., 2001).In cancer studies it has been shown to play role in tumor growth and tumor suppression (Choudari et al., 2013. As explained in (fig . 60) NO canmodulate cancer development by interfering and impairing apoptosis in addition to its role in enhancing the angiogenesis, therefore designing therapeutic agents that supress NO will be of great value for cancer patients (Ito et al., 2015).

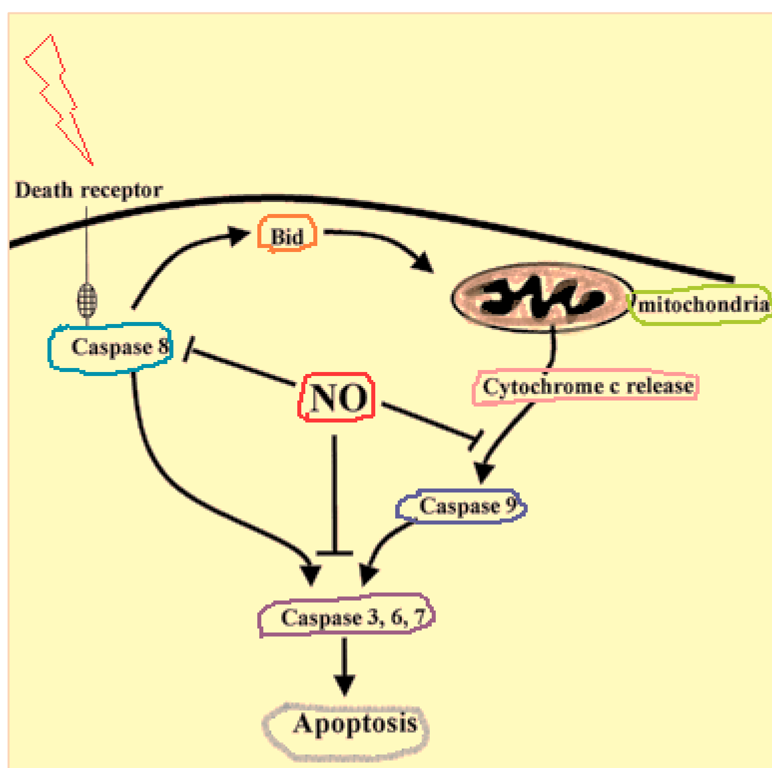


Figure 60 Nitric oxide (NO) actions in cancer cell death.

The aim of this part of the study was to investigate nitric oxide regulation upon steroid hormone and other compounds treatment under different conditions, which may provide additional evidence to explain the effect of the studied compounds on inflammatory processes and cancers. The cells were treated as described in materials and methods and subjected to Griess assay. In general, in the absence of inflammatory stimulus, NO_2^- levels were not significantly altered by tested compounds in C7, C1 and DT40 cells (Fig. 61, compare lane 1 to lanes 2-5, panels A, B and C) respectively.

In separate experiments cells were previously treated with PHA-LPS to induce inflammatory stimulation condition. Surprisingly, NO_2^- levels show significant downregulation by PHA-LPS stimulation, while, CPDA slightly decreased nitrite levels, however NO_2^- not highly affected by DEX,T,THCL in GC-responsive C7 cells (Fig. 61 B lanes 3, 4, 5,6), whereas NO_2^- levels where not significantly changed upon normal conditions in GC-resistant C1 cells (Fig. 61 C and D).looking at DT40 cells, NO_2^- upregulated upon DEX in normal conditions (Fig. 61 E),

similarly the levels increased upon DEX and CPDA in DT40 cells treated with inflammatory stimulus (Fig. 61 F). We conclude that NO is regulated in C7 and DT40 cells in response to drugs.

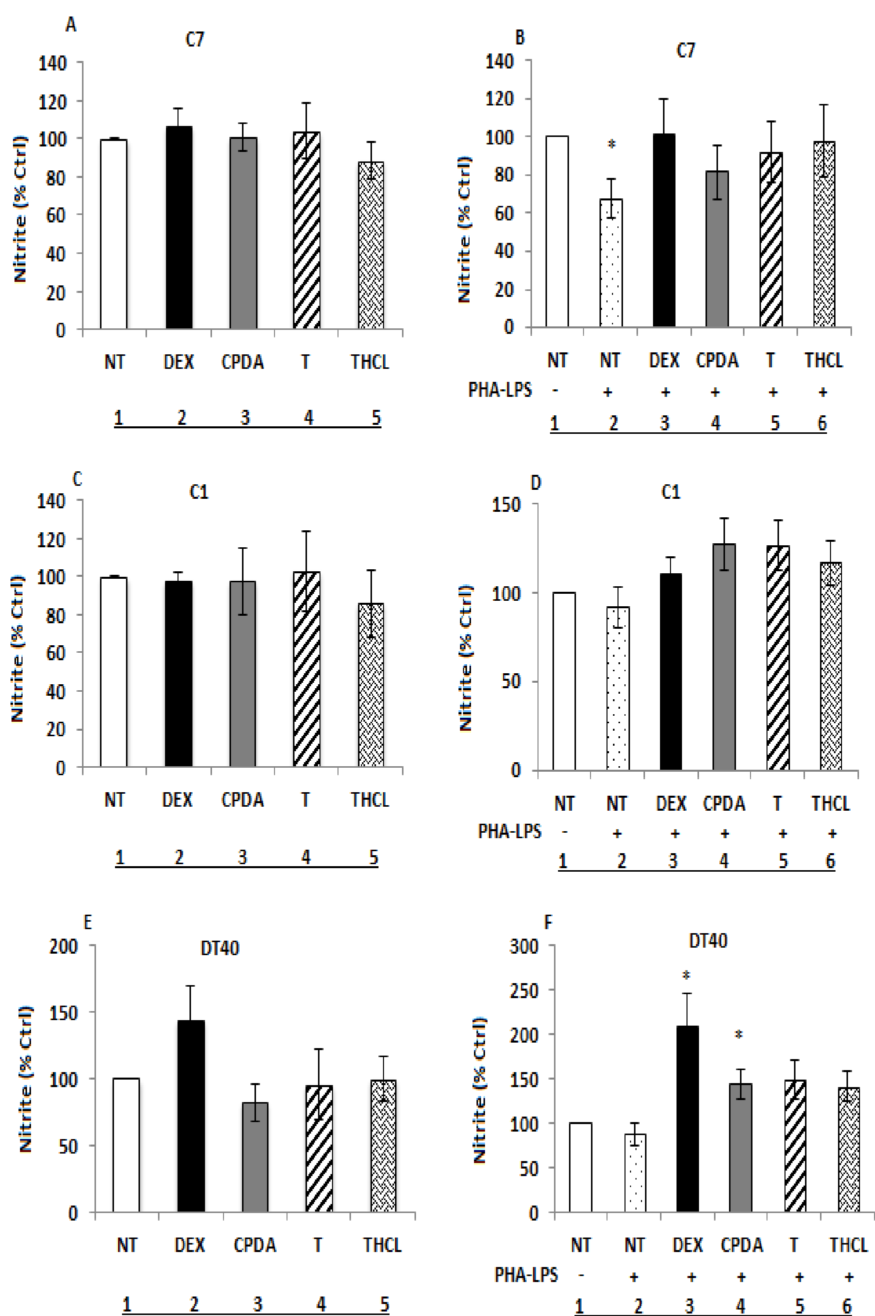


Figure 61 Nitric oxide levels upon tested compounds treatment in leukaemia cells.

Cells were evaluated by Griess reagent system as described in materials and method. Total NO_2^- level in media of leukemia cells was measured upon treatment with tested compounds. C7, C1 and DT40 cells were treated with 1 µM compounds for 24 h (A, C, E) without PHA-LPS stimulation and (B, D, F) with PHA- LPS pretreatment. Data shown are representative of three experiments in triplicates. Error bars represent standard error of means *P<0.05.

3.8.2 Secreted Interleukine 6 modulation

Interleukins 6 (IL-6) is an important inflammatory cytokine and mediates specific cells maturation and differentiation (for example B-cells and hepatocytes); it is correlated with both acute and chronic inflammatory conditions (Genecards, 2017). IL-6 receptor subunits are encoded by IL-6R gene(Genes, 2017b). IL-6 levels are known to be regulated by GCs and GR (Dittrich et al., 2012). Therefore the purpose of this part of the study is to investigate the effect of tested compounds on IL-6 regulation by GR.

ALL, DT40 and PBMCs cells were treated with steroids or non-steroid compounds, cell culture media was collected and either stored at -80 ^{o}C or immediately processesed and subjected to enzyme linked immunosorbent assay (ELISA). IL-6 levels were evaluated using ELISA kit I. IL-6 levels showed trend of modest downregulation in CEM-7-14 treated with all tested compounds, however they are showing statisticaly insignificant effects (Fig. 62 A). IL-6 was marginally downregulated in C1 cells and to a greater extent in PBMCs (Fig. 62 B and D). There were no changes in IL-6 levels in DT40 cells treated with the studied agents. These experiments were repeated in cells stimulated with Phytohemagglutinin (PHA) to induce inflammatory response.In the C7 cells and PBMCs, PHA treatment led to increase in IL-6, whereas in C1 cells there was marginal but significant decrease; there was trend to decrease IL-6 levels in DT40 (Fig. 62 E to H). In stimulated C7 cells Dex increased IL-6 levels; in stimulated PBMCs most compounds showed no major effect, except that T inhibited IL-6 levels (Fig. 62 E and H). In C1 cells all compounds restored normal levels of IL-6 whereas in DT40 there were no major effects (Fig. 62 B and C).

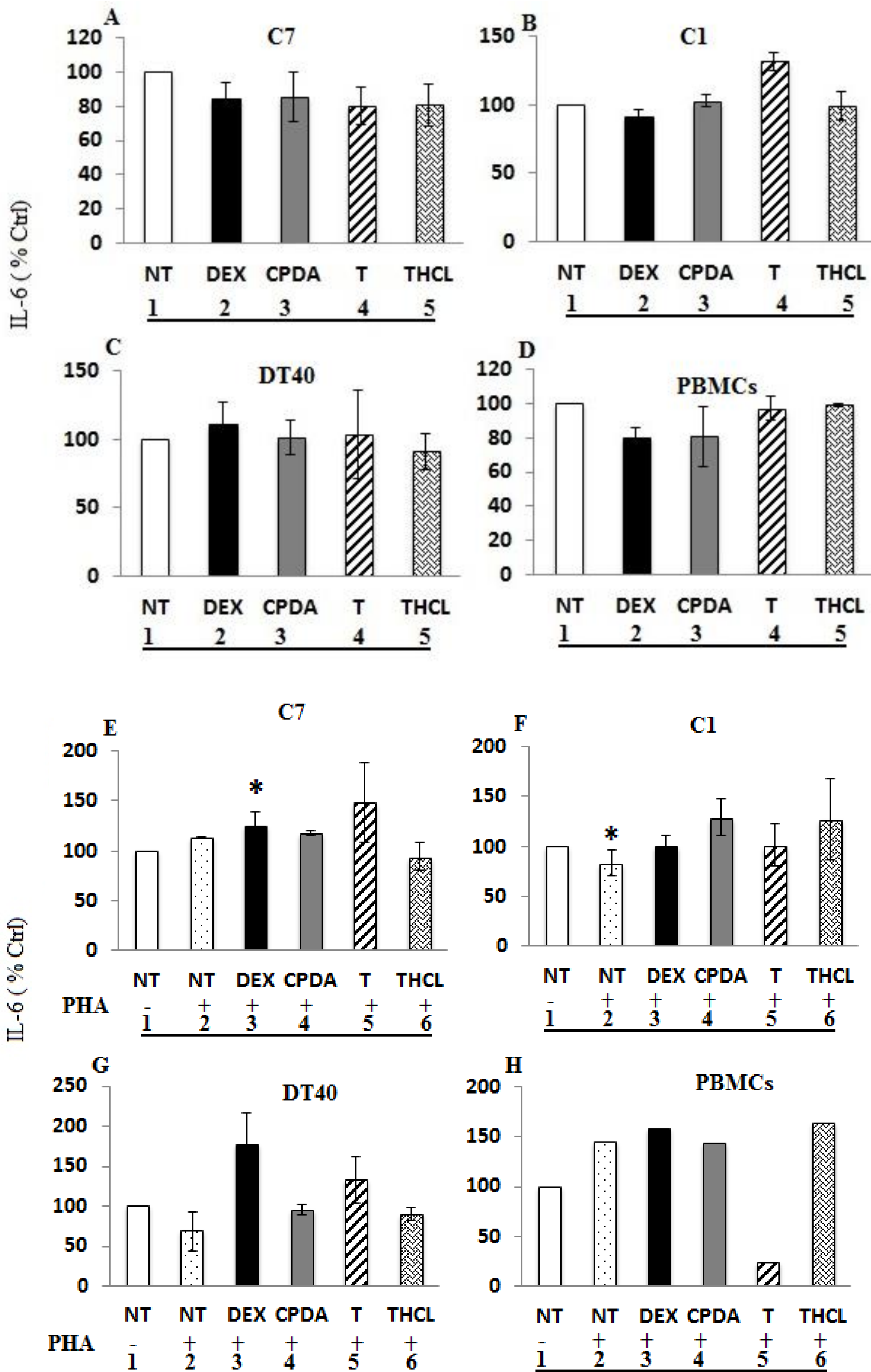


Figure 62 IL-6 levels in CEM-C7-14, CEM-C1-15, DT40 and PBMC cells.

Cells were treated with 1 µM DEX, CPDA,T,THCL for 24 h. E-H are stimulated cells by PHA 1 µg/ml overnight before indicated treatments. Media was collected and IL-6 production was determined by ELISA assay as described in Materials and Methods. IL-6 values were normalized to the control. Data shown of A, B, C, D, E, F

are representative of three experiments performed in duplicate, G represents two experiments performed in duplicate and H represents one experiment performed in duplicate. Error bars represent standard error of means *P<0.05.

3.8.3 Secreted Interleukine 2 modulation

Interleukine 2 is an inflammatory cytokine and has been chosen to test anti inflammatory activity of tested compounds in several reports (Becker et al., 2014). Also, serum interleukine-2 receptor has been linked with steroid resistance in patients and used as a prediction tool (Youssef et al., 2011).

Overall secretory IL-2 levels without stimulation showed tendency to decrease upon T treatment in C7, C1,DT40, PBMCs cells (Figure 63, A to D, compare lanes 1 and 4) . DEX showed trend of downregulating IL-2 in C7, DT40 and PBMCs with (Fig. 63, A, C and D, compare lanes 1 and 2). CPDA caused suppression of IL-2 in C1, DT40 and PBMCs (Fig. 63, B, C and D compare lanes 1 and 3), whereas THCl caused suppression of IL-2 levels in C7, C1 and PBMCs (Fig. 63, A, B and D compare lanes 1 and 5), however no significance was reached. PHA stimulation led to a small increase in all cells except DT40, with significant changes observed only in PBMCs. In PBMCs, all tested compounds reversed PHA effect .

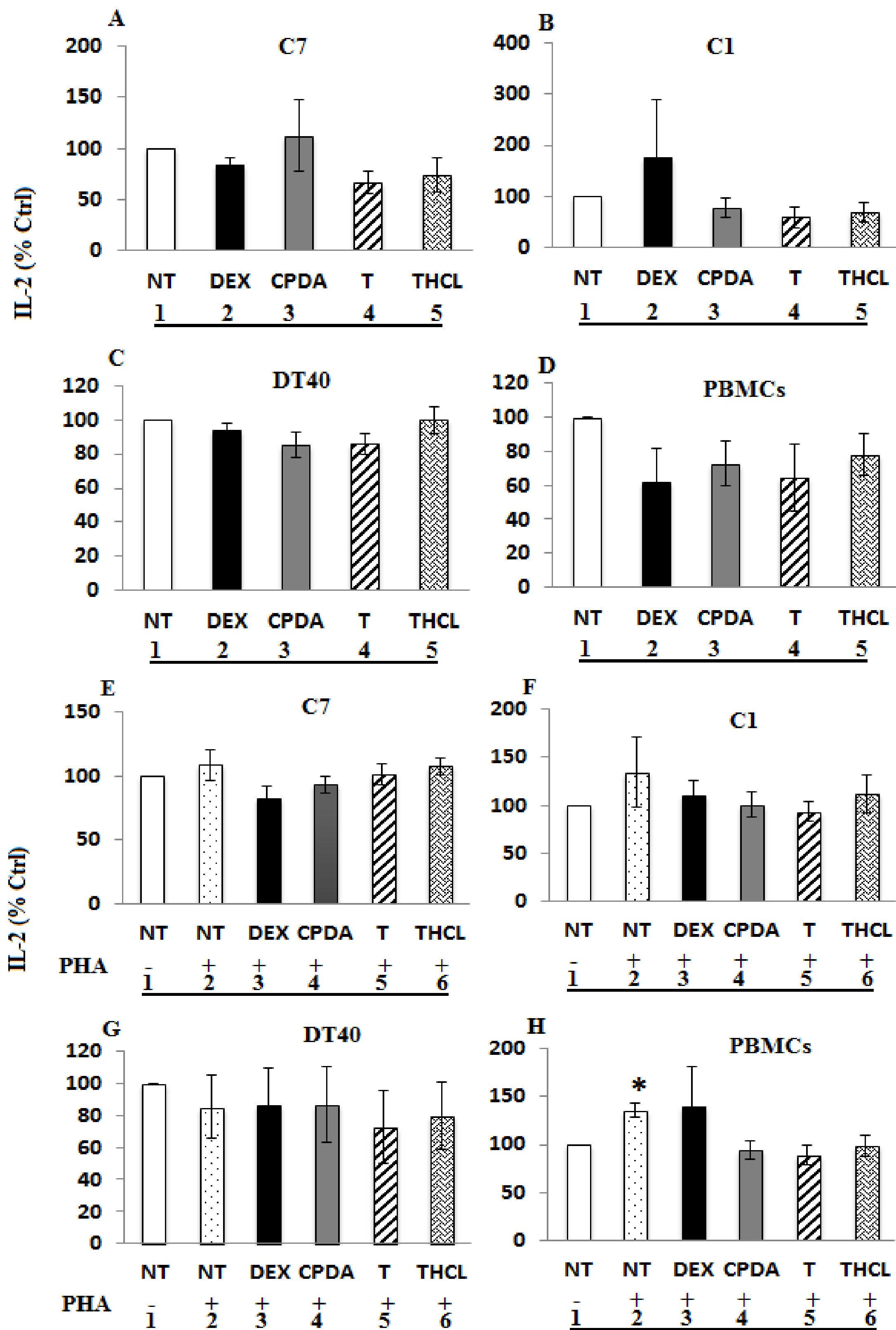


Figure 63 IL-2 levels in CEM-C7-14, CEM-C1-15, DT40 and PBMC cells.

Cells were treated with 1 μM DEX, CPDA,T,THCL for 24h. . E-H are stimulated cells by PHA 1 μg/ml overnight before indicated treatments. Media was collected immediately and IL-6 production was determined by ELISA assay as described in Materials and Methods. IL-2 values normalized to the control. Data shown are representative of three experiments in duplicate. Error bars represent standard error of means *P<0.05.

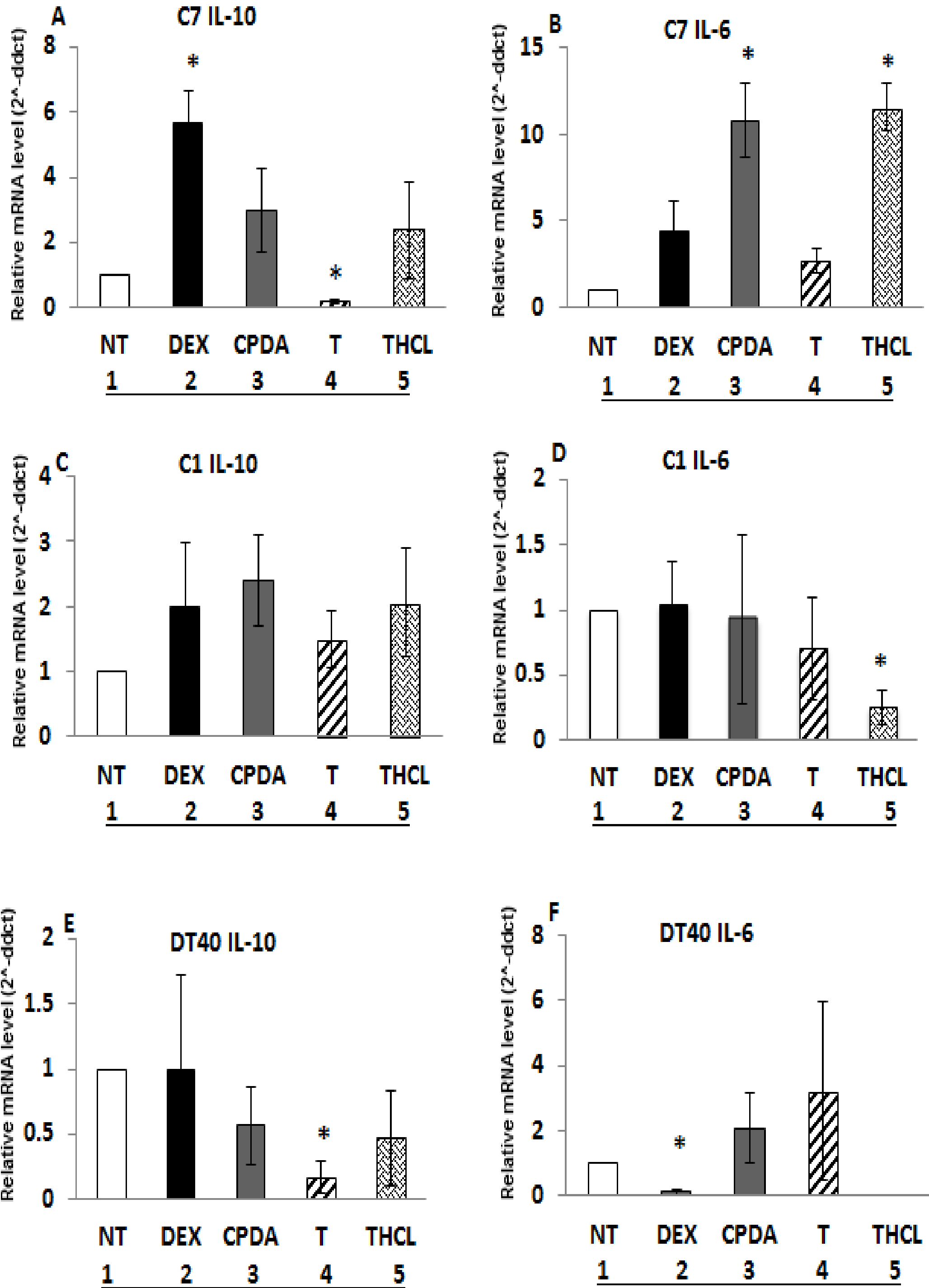
3.8.4 Regulation of interleukin genes by activated GR upon tested compounds

To investigate the role of the tested compounds on the regulation of interleukins production, quantitative measuring of mRNA levels was carried out for DEX, CPDA, T, THCL treated or untreated cells. The qPCR results for interleukins in leukemia cells are shown in Fig. 64.

IL-10 mRNA levels increased in C7 cells treated with DEX, CPDA and THCl (A, lanes 2,3 and 5) and decreased upon T treatment (A, lane 4); in the same cell line, IL-6 mRNA levels increased significantly upon CPDA and THCl treatment (Fig. 64 B, lanes 3 and 4). CEM-C1-15 qPCR results to measure mRNA for interleukins indicated that IL-10 gene mRNA levels were up-regulated upon DEX, CPDA, T, and THCL treatments (Fig. 64 C), however none were significant changes. IL-6 gene expression was significantly downregulated upon THCL in GC resistant C1 cell line (Fig. 64 D, lane 5).

qPCR results for interleukins mRNA levels in DT40 cells are shown in (Fig. 64, E and F). IL10 mRNA levels were significantly downregulated upon treatment with tyramine (Fig. 64, E lane 3), whereas IL6 gene expression was downregulated upon treatment with DEX and THCl (Fig. 64, F lane2).

In CEM-C7-14, IL-2 mRNA levels increased significantly upon DEX treatment and changes were not significant in the presence of other compounds (Fig. 64 G lanes 2-3), In CEM-C1-15, IL-2 mRNA levels were significantly upregulated upon CPDA treatment (H), and in DT40 cells IL2 were not significantly changed upon DEX, CPDA and T, although the large increase was detected upon THCL due to substantial data variation.



Relative interleukines genes expression
A
C7 IL-10
B
C7 IL-6
C
C1 IL-10
D
C1 IL-6
E
DT40 IL-10
F
DT40 IL-6
Relative mRNA level (2^-ddct)
NT DEX CPDA T THCL
1 2 3 4 5

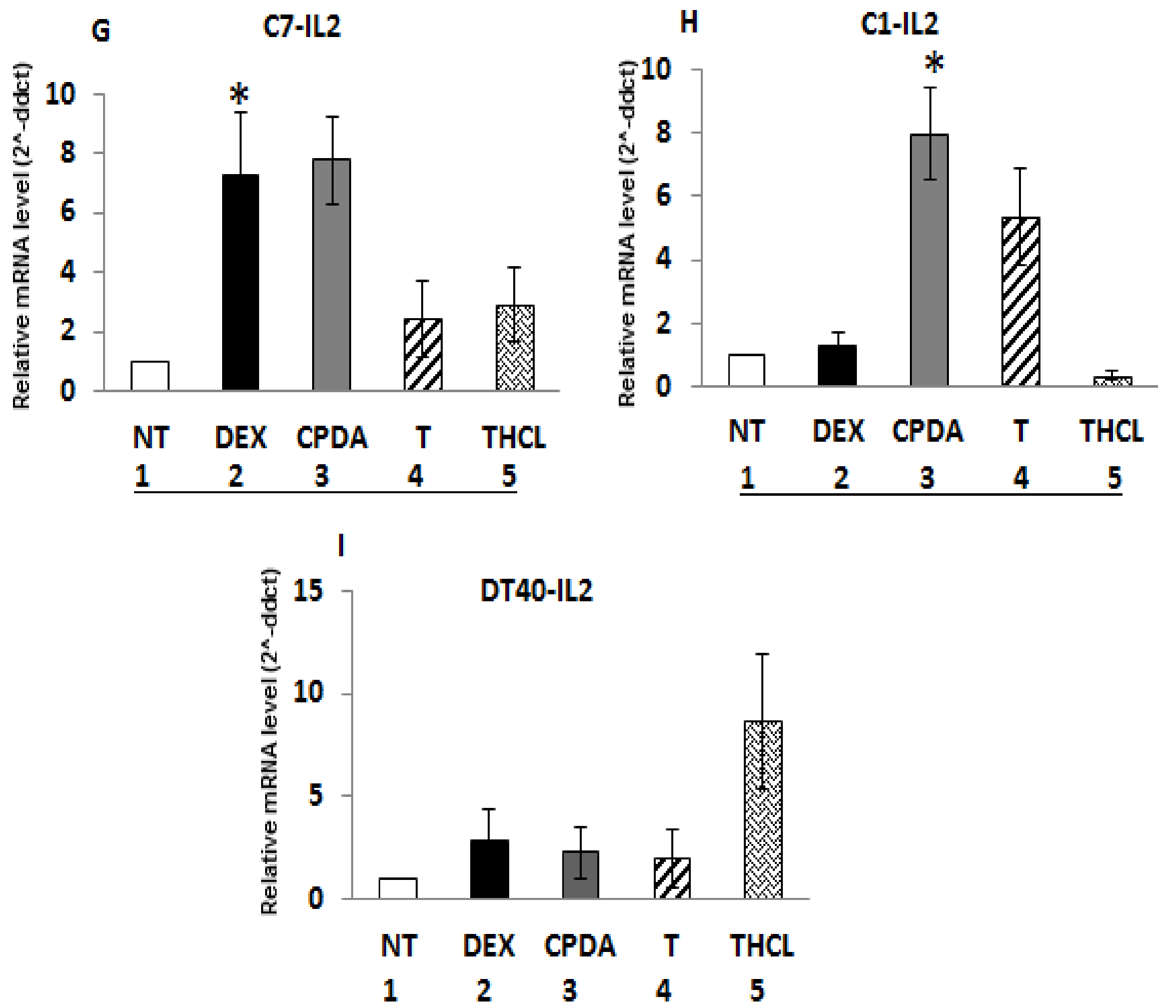


Figure 64 Quantitative analysis of GR target interleukins gene expression.

CEM-C7-14, CEM-C1-15 and DT40 cells were treated with 1 µM DEX, CPDA, T, or THCL for 24 h and the mRNA levels of IL-6 , IL-10, and IL-2 (normalized to RPL-19) were determined by quantitative real-time PCR. Data are presented as means± S.E.M. of three experiments. *P<0.05.

4 Discussion, conclusions and future work

4.1.1 Cytotoxic effects of studied compounds

The molecular basis of the glucocorticoids induced side effects and glucocorticoids resistance are not well understood. In addition, drug induced pathways to cell death mechanism such as necroptosis; autophagy or apoptosis are not well defined. In this study synthetic glucocorticoid DEX was used as a classical GC, and Compound A as dissociated GC to develop novel potential non-steroidal compounds that were tested for cytotoxic and anti-inflammatory properties and to learn more about glucocorticoid function.

We modeled in silico how four compounds that had similar structure to CpdA fit in the ligand binding pocket of the GR and observed that these compounds contact similar residues to dex and CpdA. This suggests that some of the effects of these compounds may be through GR binding. Computer modelling predicted that Dex, CpdA, and THCL all bind Arg 611 through hydrogen bond whereas T interaction with this residue is through van der waals interactions. This amino acid is crucial for ligand binding through A ring in DEX . Asn 564 is predicted to be contacted by Dex, T and THCl through conventional hydrogen bond, whereas its contact with CpdA is through van der Waals interactions. GCs form hydrogen bonds with C11 OH group to contact asparagine (Asn/N) 564. Asn 564 mutation affects both steroid binding, transcriptional activation and transcriptional repression but is more important for activation (Blind et al., 2012). Compounds can affect gene expression through affecting GR conformation that can affect other domains function and cofactor binding as observed for this family of ligand dependent transcription factors (Wong et al., 2001,

Kraichely et al., 2000). However, they may also act through affecting other regions of GR or through non-GR mediated mechanisms (Yemelyanov et al., 2008).

Computer- aided drug design based on ligand-protein interaction analysis is a predictive tool used previously in generation of novel compounds. There are numerous examples of this approach used in biomedical research and medicine that after an *in-vitro* and *in-vivo* validation had impact in clinical field (Sliwoski et al., 2014). For example Alzheimer's therapy tacrine and its derived compounds which act as inhibitors of acetylcholinesterase (Zhou et al., 2015) have been optimized by aid of computer modeling for potential binding sites. Other examples include 5-fluorouracil (5-FU)(Dobritzsch et al., 2001) and the discovery of carbonic anhydrase inhibitor dorzolamide (Ali et al., 2015).

A computer docking between GR and the tested compounds was carried out to obtain prediction about the degrees of their interactions with GR as similar binding points with DEX (tables 27 and 28 and fig. 31, 32).The natural occurring mutations in ligand binding domain of glucocorticoid receptor are the source of many illnesses, from them Cushing's syndrome, immunity disorders and tumors. Numerous residues have been identified to be mutated in the ligand binding region including G507C, M601L, M604P, M646T, Y735S, C736S, and L753F results in blocking van deer Waal's bonds and the binding activity of GR with the ligand. The other mutations affect bonds within the receptor itself which are called the hydrophobic bonds and lead to unstable protein, these are P541A, I559D, C638Y, V729I, Y764N, and F774A (Bledsoe et al., 2002). Hydrophobic bonds are defined as interactions between the non-polar molecules such as backbone amide hydrogens of the protein (Alderson et al., 2017). Backbone amide hydrogens N-H refers to the hydrogens atoms that contact the nitrogen atoms in the proteins and can be exchanged with water making the protein flexible (Persson and Halle, 2015)

.It has been found that Y 735 makes hydrophobic interaction with the carbon ring of DEX

and any change of this residue can weaken the transactivation function of GR (Stevens et al., 2003)

The mutation A610V in ligand binding domain of glucocorticoid receptor activates GR and promotes ligand interaction and improves GR function (Reyer et al., 2016). In addition S637, P639, A641, G642, and L647 in LBD are important residues to bind with the ligand and for transactivation activity (Robin-Jagerschmidt et al., 2000).

Compounds stability analysis demonstrated that tested compounds were stable if stocks were kept at manufacturer's preferred temperature this temperature is 4 ^{o}C for DEX, T, THCL, CPDB, CPDC stock powder. Solutions of DEX, T, THCL, CPDB, CPDC need to be stored at -20 ^{o}C, and the stock powder of CPDA have to be kept at -80^{0}C, also CPDA solution needs to be stored at -80 ^{o}C (Table 26).

It has been reported that most substances undergo changes in their chemical and physical properties due to the effect of temperature on chemical reaction (Elfarra and Hwang, 1996, Totomi et al., 1995). However our results indicated that DEX in solution was stable at 4 ^{o}C and 20 ^{o}C, in agreement with Watson et al. (2005), where they measured the stability of DEX in liquid at 4 °C, 23 °C, and 37 °C for 0, 2, 4, 8, 24, 48, 96, and 192 h by High Performance Liquid Chromatograph HPLC and confirmed it stability under these conditions. Furthermore another published data suggested that DEX can resist even temperatures higher than 40 °C using HPLC with no significant changes in the peak as an indicator of its stability according to Selerity Technologies Inc Inc (2004) finding , CPDA stock solutions in ethanol are stable for up to 1 month if kept at -80°C (ENZO, 2017). However no records were found for Tyramine, Tyramine hydrochloride, CPDB and CPDC stability from their supplier website and literature search.

Cellular cytotoxicity analysis has suggested that novel compounds can potentially serve as new inhibitors of leukemic cell proliferation and induced death at varying extents depending on concentration and duration of exposure. Tested compounds inhibited growth of CEM-C7-14, CEM-C1-15 and DT40 cells at high doses and 72h (Fig. 33), while at 48 h the compounds inhibited the growth of C7 to a limited extent however DT40 and C1 cells were less sensitive (Fig. 33). Upon lower doses starting from 2.5 µM maximal concentration and time course of 24 h, 48 h and 72 h, a time dependent growth inhibition was observed in C7, C1 and DT40 cells (Figs. 35, 36, 37). Application of high doses of studied compounds to leukemia reduced their growth rate whereas cellular viability was reduced at lower concentrations too. Obtained results suggest that CEM-C7-14 are significantly more sensitive to steroid treatment and C1 cells are more resistant to low doses of steroid hormone in agreement with Thompson et al. (2005). Thus, high concentrations of synthetic GCs may be needed to overcome GC-resistance, however this approach may cause toxic side effects.

However, a subcellular changes needs to be investigated to recognize if cells died from apoptosis or other type of cell death. Likely, CPDA produced same effect; as all doses inhibited the growth significantly. CPDA may act via GR to exert the growth interaction function, and may interfere with the growth factors and disrupt cancer cells' proliferation. T produced a similar profile and THCL at certain doses (0.039, 0.156, 0.312 and 0.625)

µM at 24 h incubation (Fig .36). No significant effect of hormone or non-steroid compounds was detected on normal peripheral blood cells viability PBMCs (Fig. 39) suggesting they may not affect normal blood cells.

Compound A has been shown to promote GR inhibition of transcription function but not

activation by GR homodimers (Lesovaya et al., 2015). In addition, CPDA was shown to regulate the inflammatory response through Th1/Th2 (Th1) immunity and promote humoral (Th2) immunity) route and initiate GATA-3 expression by enhancing the phosphorylation of indicated protein by p38-MAPK (Liberman et al., 2012) CPD A was also reported to induce growth inhibition and apoptosis of CEM and K562 leukemia cells, to downregulate AP-1 and NF-Kb function but not to activate FKBP51 gene transcription. (Lesovaya et al., 2011). Lesovaya et al. (2013), found that CEM and NCEB lymphoma cell lines previously treated with proteasome-inhibitor (Bortezomib) promoted and enhanced CPDA function leading to further down regulation of gene transcription.

Anti-proliferative role of GC in leukemia cells has been well documented (Hu et al., 2013, Pace and Miller, 2009); therefore the possibility that tested compounds can inhibit leukemia cells in a similar way to a Dexamethasone was explored.

High temperature stress applied to the cells could inhibit their growth while its combination with tested compounds did not increase the growth inhibition effect (Fig. 40). Combination of DEX or CPDA with increased temperature inhibited cell viability suggesting that this could be another means of inducing apoptosis in resistant cells

Cold shock demonstrated similar effect as the heat shock (Fig. 41). These results suggest that temperature variations may affect drug response and could find application in veterinary and medical treatments to allow for efficient drug use. The thermal stress has already been studied in animals; for example toads that are maintained in relatively high temperature expressed elevation in corticosterone hormone as a response to stress (Narayan and Hero, 2014). Acute or chronic thermal stress has impact on HPA axis as it seem to increase GCs secretion and negatively affect HPA axis and the correlated

physiological activities. This is evident in milk production decline upon high temperature conditions observed in cattle (Aggarwal, 2012). In goat it has led to alterations in HPA axis and downstream biological immune regulators; ACP, ALP, T3, T4 and calcium levels which is essential for their life (Sejian and Srivastava, 2010).

Leukemia chemotherapy affects numerous cells and tissues in patients. In order to assess effects of studied compounds on epithelial cells, compounds ability to induce epithelial cells death was investigated. Our findings regarding DEX effects are in agreement with Petersen et al. (2008) who found that DEX inhibits Human lens epithelial cells (HLECs) growth. In addition, dexamethasone is effective inhibitor of glioma cells (Ismail et al., 2016, Koibuchi et al., 2014, Ni Chonghaile et al., 2006, Yague et al., 2009, Yu et al., 2010) . In our study at 48 h incubation time we observed that DEX could inhibit breast cancer cells MCF-7, while, DEX and CPDA have no significant inhibitory effect on HACAT and MCF-7 cells viability, but it affected BEAS-2B survival. T and THCL showed growth promotion effect on MCF-7 and HACAT (Fig. 42 A), at 72 h duration of treatment all tested compounds inhibited MCF-7 viability, however, HACAT cells proliferation was suppressed by DEX and CPDA and enhanced by T and THCL; BEAS-2B proliferation rate was inhibited by all tested agents (Fig. 42 B). These findings suggest that the studied compounds may have broader applications as growth inhibitory agents for blood cancers and other cancers. It also points to needs to modify compounds further to eliminate potential pro-proliferative effects on some cell types.

Our results suggested that investigated compounds are cytotoxic in cancer cells model of study. However, pathways to cell death were not possible to be investigated with MTS and MTT methods. Therefore we employed additional techniques to analyse what type of cell death pathway is activated and what cell cycle effects each individual compound has in

sensitive and resistant to GC leukemia. Therefore cell cycle progress is monitored first using FACS analysis combined with PI staining.

Analysis revealed that tested compounds can induce SUB-G1 arrest in GC-sensitive C7 cells and inhibit G1 phase in same cell line significantly with alteration in G2/M phase. This revealed an important effect of compounds onthe cell cycle progression of GC sensitive leukemia cells (Fig. 43). Further our results demonstrated that tested compounds were able to interfere to certain extent with the cell cycle progress of the GC resistant cell line C1, as no significant increase in S phase or G2/M were demonstrated with any of tested compound as an indicator of impairment of the multiplication of the resistant leukemia cells (fig.44).These results are in agreement with (Bindreither et al., 2014) who found that 1 µM of Dex was able to induce apoptosis in T-chALL CCRF-CEM-C7H2 cells incubated for 24 and 48 h . In DT40 cells, the tested compounds have reshaped the cell cycle by diminishing the cells that are going into G2/M which is a sign of effect on a growth inhibition system (Fig. 35). Interestingly, those compounds did not demonstrate an adverse effect on normal human PBMCs as they did not stimulate their growth or induce their death as an initial indicator for their safety application in normal white blood cells (Fig. 46). Taken together, these results demonstrated that steroid and non-steroid compounds regulated cell cycle in a drug and cell type dependent way, suggesting that their cytotoxic effects may be through different mechanisms. It is mostly possible that SUB-G1 maximized by intervention of GR and the growth inhibitory effect depend on the amount of expressed GR, i.e.(auto-induction), as upregulated GR accompanied by programmed death of sensitive cells T-ALL (Ramdas et al., 1999).

In order to learn more about the type of the cell death induced by the studied compounds, AnnexinV assay was used to detect the apoptosis in the cells. The tested compounds were

able to induce apoptosis in GCs- sensitive leukemia cells and in chicken leukemia cells while CPDA and THCL displayed apoptosis in GCs resistant cells (Fig. 47, 48 and supplementary material) although different levels of apoptosis were detected with different compounds. Apoptosis was shown to occur in lymphocytes that possess active GR, except in some GC resistant clones such as C1 which could be sensitized by activating protein kinase A pathways to boost GR action (Medh et al., 1998)

Our finding of apoptosis induction with DEX treatment is in agreement with (Zong and Thompson, 2006) who demonstrated that GCs were able to induce apoptosis of both sensitive and resistant ALL clones to varying degrees. GCs application induced programmed cell death of C7 cell line after more than 24 h of incubation as DEX demonstrated to arrest cell cycle in G1/G0 (Thompson et al., 1999)

Belvisi et al. (2001), attributed the contrast in apoptotic profile between C1 and C7 to the alteration of anti-apoptotic genes in these clones of cells. Expression of different sets of genes was identified between C1 and C7 cells that maybe linked with GC resistance.
Bcl-2 Interacting Mediator of Cell Death BIM as we mentioned before is the apoptosis biomarker of leukemia cells upon GC therapy, in contrary BCL2 supports survival. The *in vivo* study demonstrated that KLF13 and MYB genes were upregulated in patient-derived xenograft (PDX) taken from GC responsive patients and that GR were bound to BIM gene only in sensitive but not in resistant group. GR was also bound to GRE in KLF13 to activate this gene transcription. Activated KLF13 itself downregulate the transcription of MYB by binding to the promoter region of this gene (Jing et al., 2015).
Glucocorticoids induction of apoptosis in white blood cells is intensively studied and crucial pathways such as Bcl2 family members (BIM), BCL2 Family Apoptosis Regulator(MCl-1) and

NADPH Oxidase Activator 1 (NOXA1) have been implicated in this pathway (Yamashita et al., 2017, Guzauskas et al., 2017, Yeo et al., 2016, Warris et al., 2016, Vundamati and Bostrom, 2016, Polak et al., 2016, Quadri et al., 1997, Miyoshi et al., 1997). However, only a few reports have investigated the role of GCs in other cell death or pro-survival pathways such as necroptosis and autophagy. Caspase -8 is at the crossroads between apoptotic and necroptosis pathways and its activity was examined in set of experiments. Caspase-8 is related to apoptotic activity of therapeutic substances. We found that Cas-8 reading increased with steroid treatment in C7 cells (Fig. 53) as DEX induces apoptosis via Cas-8 activation, in line with Marchetti et al. (2003). Contrarily, its levels significantly dropped in PBMCs upon DEX as an indicator of absence of apoptosis induction in normal white blood cells. Caspase-8 increased in C7 and DT40 cells upon THCL treatment (Fig. 38) oppositely, it decreased upon CPDA in ALL cells. This indicates potential involvement of CPDA in additional pathway that may decide cell fate such as necroptosis. Joanny et al. (2012), demonstrated that steroid and non-steroid ligands of GR positively regulate dual specificity phosphatase 1 (DUSP1) gene hereby regulate the inflammation in mice. Given that necroptosis is linked both to inflammation through Nf-KB pathway and cell death through Caspase -8 pathway it would be important to further investigate this observation.

In order to learn more about effects of studied compounds on cellular processes linked to cell death or inflammation, we measured cellular ROS levels. Although certain pathways are well described, ROS role in these processes is dependent on the context. Our results suggest that ROS levels are down-regulated in drug and cell dependent manner. DEX treatment led to inhibition of ROS in C7 cells (Fig. 51 A) which suggests crosstalk between GCs and ROS. The results agreed with Sanner et al. (2002), who found that synthetic steroids down-regulated levels of reactive oxygen species of inflamed platelets obtained

from humans whereby confirming their role in control the inflammation. Interestingly, most treatments did not affect GC-resistant C1 cells (Fig. 51 B). No significant ROS changes in DT40 cells and PBMCs were observed (Fig. 51 C and D). Dandona et al. (1999), correlated ROS inhibition by DEX with the anti-inflammatory traits and immune-regulatory action. The current outcomes indicated that steroid DEX inhibited ROS and that this action goes through GR which is consistent with Marumo et al. (1998) who demonstrated that DEX treatment for 24h decreased ROS levels in human aortic smooth muscle cells.

4.1.2 Effects of studied compounds on inflammatory process

Tested compounds activity against inflammation was followed by analysis of biomarkers of inflammation. Classical GCs are currently used as potent anti-inflammatory drugs and they act through GR. Previous experiments provided evidence that non-steroidal compounds such as Compound A may act via GR, thereby anti-inflammatory capacity of studied compounds was analyzed.

Classical glucocorticoids such as DEX control inflammation by inhibiting pro-inflammatory biomarkers and activating anti-inflammatory mediators (Coutinho and Chapman, 2011). We measured secretory cytokines from media of treated cells. IL-6 levels although showing downward trend in media from C7 unstimulated cells were not changed significantly (Fig. 62 A). In PHA stimulated cells significant increase in IL-6 was observed in Dex treated C7 cells and decrease in C1 PHA stimulated cells (Fig. 62 E and F). (Vicennati et al., 2002) determined that steroid hormones administration repressed IL-6, which is in agreement with observation in C7 unstimulated cells. However, PHA stimulation did not increase IL-6

levels in C1 and DT40 cells but led to its decrease. As IL-6 is proinflammatory cytokine PHA should lead to its increase and Dex treatment to its decrease. This perhaps indicates deregulation of inflammatory response in leukemia since first, Dex didn't suppress IL-6 levels in stimulated C7 and C1 cells and second, PHA treatment didn't lead to its significant increase in any of the cell lines.

Although not significant, secretory IL-2 level showed downward trend in T treated C7, C1, DT40, and PBMCs cells (Fig. 63). Similarly, DEX negatively regulated IL-2 in C7, DT40 and PBMCs (Fig. 63 A, C, D) in line with Boumpas et al. (1991) who found that DEX treatment of human T lymphocytes suppressed IL-2 proteins level. However, given that only significant observation was caused by loss of IL-2 increase caused by compounds treatment in PHA stimulated PBMCs (Fig.63 H), this again indicates potential deregulation of normal inflammatory response in leukemia cells.

In order to determine if analysed compounds affected the cytokines transcriptional levels, mRNA expression levels of IL-10, IL6 and IL-2 genes were quantified upon cells treatment with tested compounds. The results revealed that in DEX treated C7 cells, IL-10 mRNA levels were increased (fig. 64 A) which is consistent with findings of Mozo et al. (2004). IL-10 modulation is controlled by GR which is found to promote up-regulation of this secretory cytokine in dendritic cells (Sondergaard et al., 2015) as GCs are known to regulate immune response and caused increase in IL-10 level in human dendritic cell (Franchimont, 2004). However, IL-10 mRNA levels revealed no significant elevation upon CPDA and THC1 in C7 cells (Fig. 64, A). In a same manner it showed a significant downregulation upon T treatment in this cell as an indicator of dissociation of GR transactivation function, although the upregulation of IL-6 mRNA levels upon CPDA and THCl treatment (Fig. 64 B), and in GC resistant cells IL-6 mRNA levels were shown to be downregulated upon THCL only (Fig. 64 D) and in DT40 cells IL-10 was down-regulated with CPDA,T and THCL (fig. 64 E), while IL-6

expression was reduced upon DEX and THCL (Fig. 64, F).

Glucocorticoid receptor and inflammatory cytokines are described to crosstalk as IL-6 and IL-10 control Stat3 transcription and Stat3 acts as a cofactor for GR through protein-protein interaction (Zhang et al., 1997). Hardin et al. (1994), demonstrated that dexamethasone inhibited IL-6 gene expression in three human multiple myeloma cell lines, thus the effect on IL-6 can be correlated with the cell line.

The results are in agreement with Visser et al. (1998), who found that DEX enhanced regulation of IL-10 gene and protein in whole blood cells cultures and Hua et al. (2012) who indicated that dexamethasone treatment led to positive expression of intracellular IL-10 in CD5+ B cells and IL-10 concentration in supernatants of CD5+ B cells isolated from patients with Primary Immune Thrombocytopenia.

IL-2 gene mRNA was significantly upregulated in CEM-C7-14 upon DEX (Fig. 64 G), and upon CPDA in CEM-C1-15 (Fig. 48 G) and not significantly changed upon DEX, CPDA and T, although the large variation was detected upon THCL treatment (upregulation in C7 and DT40 cells and downregulation in C1 cells). Although, Northrop et al. (1992) demonstrated that GC treatment suppress IL-2 gene in mice. Given that leukemia cells were not exposed to inflammatory stimulus these experiments will have to be expanded in future to analyse how cytokine expression changes in inflammation and in animal models.

Nitric oxide may be the cause or the consequence of cancer it can either enhance or suppress the tumour progress or can be an indicator to successful therapy (Xu et al., 2002). NO levels were measured in presence and absence of stimulus. We found that without stimulation NO_2^- level was not changed upon treatment with tested chemicals (Fig. 61).

NO_2^- significantly decreased in C7 cells stimulated with combination of PHA and LPS treatments; DEX is known to be inhibitor of nitric oxide production in both smooth muscles and macrophages (Korhonen et al., 2002, Marumo et al., 1993) which is not found in our results, potentially highlighting the difference between cell type, normal versus leukemia cells or in vitro versus in vivo studies. Dex effect is limited to stimulated cells as suggested by Korhonen et al. (2002) as they indicated that in mice macrophages treated with lipopolysaccharide additional dexamethasone treatment caused downregulation in NO levels due to reduction of inducible nitric-oxide synthase iNOS mRNA levels. Ito et al. (2015), have attributed the anti-cancer activity of a Toll-like receptor (TLR7) activator agent called imiquimod to the inhibition of iNOS.

4.1.3 Molecular analysis of investigated agents interactions

In order to analyse molecular changes in examined cells upon tested compounds, a subset of selected genes expression was tested. Tested compounds affect GR in specific way reportedly not affecting GR mediated repression whereas GR mediated transcriptional activation is not stimulated. Given that GR repressive effects on cytokines were analysed in the previous section, in the next set of experiments selected genes that are stimulated by GR such as C-JUN and marker for apoptosis BIM, also NF-kB genes were followed. In CEM-C7-14 cells (Fig. 54) the relative mRNA expression of GR was increased significantly whereas C-JUN was shown to be downregulated upon DEX treatment. NF-kB gene expression was significantly downregulated upon THCL incubation. In C1 cells (Fig. 56) GR gene expression was significantly downregulated upon DEX while C-JUN was significantly downregulated upon T. BIM gene expression was significantly reduced upon DEX and increased upon CPDA.

DT40 cells has a different profile of genes expression as GR gene expression was significantly downregulated upon CPDA while significant downregulation of BIM was observed with CPDA and T in these cells.

GR is a target of interest for lymphoid cancers' medications due to its apoptotic initiating characteristic which was not shared with other steroid receptors (Pufall, 2015). Opposite to acute leukemia, multiple myeloma does not respond to DEX induced apoptosis, where Bharti et al. (2004) demonstrated that DEX treatment ere not interact with NF-κB in MM function. GR and NF-κB crosstalk occurs at several levels. GCs inhibit pro-inflammatory activation function of the stress transcription factor NF-κB by activation of the inhibitor of this transcription factor IκB in murine cells (Auphan et al., 1995). Inhibitor of NF-κB destabilizes it and blocks its translocation to the nucleus. STAT6 positively regulates IκB while GR was not reported to regulate NF-κB through IκB in Hela cells line (Nelson et al., 2003). GR inhibits the pro-inflammatory response by inhibiting NF-κB-mediated transcriptional control of the pro-inflammatory genes such as intercellular adhesion molecule-1 (ICAM-1). This occurs by direct interaction of GR with the DNA or destabilizing NF-κB from translocation rather than by changing the configuration of the pro-inflammatory proteins complexed with NF-κB in histiocytic lymphoma U-937 cells (Liden et al., 2000).

In order to determine how GR target genes are controlled at protein level, SDS PAGE followed by western blot was used to determine the levels of subset of relevant targets. In C7 cells GR, GR (pS211), C-JUN, and BIM were followed. GR protein levels increased upon treatment with DEX, this correlated with the growth inhibition seen upon DEX. All compounds showed trend to upregulate GR total levels, GR (pS211), BIM and c-Jun levels (fig. 55). The results demonstrated that tested compounds are likely to activate GR which is compatible with computer modelling results.

Total GR protein levels were unchanged in C1 cells (Fig. 45) treated with the tested compounds which confirm their resistance to glucocorticoid therapy. No compounds showed significant change in C-Jun or GR (Ps211) levels.

In addition, our findings describe novel effects in chicken cell line 'DT40' as model of avian lymphocytic leukemia which give rise to the ability of treating this poultry disease by current compounds. Further *in vivo* experiments are required to fulfil this purpose.

4.2 Conclusions

This project investigated possibility to improve the conventional glucocorticoid therapy to treat leukemia and inflammatory conditions. Several compounds were used and different assays employed to test their cellular effects. Examined chemicals are stable in solution at 4^0C and 25^0C and molecular modelling suggests they contact some of the residues already demonstrated to be contacted by synthetic glucocorticoid dexamethasone.

Leukemia cell proliferation is regulated by studied compounds that show differential cytotoxic effects depending on the cell type, doses, incubation time and temperature. CPDA and DEX downregulate proliferation of Beas-2b whereas T and THCL increased proliferation of MCF-C7 and HACAT.

C7 cell cycle progression is regulated by tested compounds with significant increase observed in sub-G1 phase of DEX and CpdA treated cells and decrease in G1 phase of cells treated by all compounds; no significant changes were observed in C1 and PBMCs. In DT40 cells significant downregulation was observed in S phase of T treated cells and in G2/M of CPD A treated cells.

ROS levels were regulated in cell dependent manner upon treatment with tested compounds. In particular total ROS levels were downregulated upon DEX and CPDA treatment in GCs sensitive cells and not changed in CEM-C1-15 cell line which reveals the difference between those two clones in a molecular response.

The tested agents have differential effects on Caspase-8 enzyme. Dex upregulated Caspase-8 in all cell lines which refer to the involvement of DEX in caspase-dependent type of cell death through intrinsic pathway of apoptosis (mitochondrial) and recruiting of Bim,

whereas CpdA seems to be act through caspase independent pathway of programming cell death may be via ER stress and ROS suppression in GC sensitive cells.

Relative gene expression of GR, C-JUN, BIM, NF-κB, and GR, C-JUN,BIM proteins expression were altered in studied cells and the results correlate the response in C7 cells to treatments as it effect through GR and the affected network in genes and protein level.

NO_2^- levels show downregulation in C7 but not in C1 cells stimulated by pro inflammatory signal and this downregulation is abolished in C7 cells treated with studied compounds. DEX and CPDA upregulated NO_2^- in DT40 cells treated with inflammatory stimulus. We conclude that NO is regulated in C7 cells exposed to pro inflammatory stimulus in response to drugs.

In stimulated C7 cells Dex increased secreted IL-6 levels, but PHA stimulation didn't change IL-6 levels. In C1 cells all compounds restored normal levels of secreted IL-6 whereas in DT40 there was no major effect. In unstimulated C7 cell line, IL-6 mRNA levels increased upon all compounds treatment and significantly upon CPDA and THCl treatment. IL-6 gene expression was significantly downregulated upon THCL treatment in unstimulated C1 and in DT40 cells incubated with Dex.

Secretory IL-2 levels in PBMCs increased with PHA treatment and all tested compounds reversed PHA effect. However in leukemia cells inflammatory response was deregulated as no significant changes were observed in PHA treated cells. In unstimulated C7 cells Dex induced IL-2 mRNA whereas CpdA induced it in C1 cells.

IL-10 mRNA levels increased in non-stimulated C7 cells treated with DEX and decreased upon T treatment. In unstimulated DT40 cells IL10 mRNA levels were significantly downregulated upon treatment with tyramine.

In summary, tested compounds produced cytotoxic effects similar to DEX particularly in GC-sensitive C7 cells. These compounds are likely to act through GR, according to the computer modelling results. And according to other results, they may be suitable for use as an anti-inflammatory and anticancer compounds as demonstrated through MTS/FACS and ELISA results. Additionally, preliminary results indicate that compounds likely don't affect significantly normal PBMCs cells. Thus further experiments need to be carried out on these compounds as next step towards introducing them to the medicinal use.

4.3 Future work

Future experiments will need to confirm preliminary results obtained for some experiments, mostly experiments employing PBMCs in need to provide more replicates of the data. This would be done by repeating cellular assays to compare the cytotoxic effect of the compounds on normal white blood cells, on the other PBMCs obtained from patients suffering from chronic and acute inflammatory conditions. This will be a suitable tool to investigate the anti-inflammatory effect of the studied compounds in human conditions. Furthermore PBMCs derived from GC treated patients and GCs resistant patients would be desirable model to investigate the GR dissociation effect of these compounds. Normal PBMCs from animals would also be useful to understand the compounds effects on animal white blood cells and for comparison purpose for their effect between two species (human and chicken).

Anti-cancer or anti-leukemic effect could be in depth investigated by carrying out the experiments which have been performed across the thesis on primary leukemia cells. On cells derived from ALL sufferers who respond or not to GC therapy and serve a GC sensitive/ resistant model of study.

It is important to perform animal research in order to gain that knowledge of the wider drug effects and side effects. For this reason, the local topical therapeutic or toxic effect can be carried out in animal experimental model of inflammatory skin disease or skin lesion; also *In vivo* drug test using chick embryo or chorioallantoic membrane infected with chicken lymphoid leucosis could be used to investigate the compounds effect on the avian species. In addition the compounds need to be tested in mouse models of leukemia such as xenografts or patient derived xenografts for cytotoxic effects as well as mouse model of acute and chronic inflammation. Althoughome of these in vivo experiments of compounds'

anti-inflammatory effect are under way in collaborators laboratory in Novosibirsk institute in Russia.

Furthermore, the antibacterial, anti-fungal and anti-parasitic effect would be easily investigated in petri dishes /microbiology lab. Few experiments may be repeated in different optimum conditions to get accurate description of cell death mechanism, for instance Apoptosis assay (Annexin5 and Caspase-8).

In addition, ligand-receptor binding studies would be necessary to confirm that compounds act through the GR. In addition,the identified binding sites could be mutated and their effects studied using luciferase assays or qRT- PCR in cells overexpressing these mutant GR derivatives would be useful to test array of genes that are activated or suppressed by GR. However, Chip assay can help more to identify the interaction of GR with GRE in selected genes under the effect of tested compound. Similarly, further exploration of a large array of genes is required in leukemia cells sensitive and resistant to glucocorticoid treatment. This could be achieved using either microarray analysis or RNA sequencing or PCR array to quantify the expression of large number of genes and discover the signalling pathway induced by each compound.

Drugs effects on interleukins should be extended to stimulated cells or inflamed cells as mentioned before to analyse a specific stimulator for the examined cancer cells that should induce inflammatory response and using multiple new sets of specific primers and multiplex Human Cytokine ELISA Plate Array (Chemiluminescencelarge) kit that consists of one pre-coated plate and can measure 32 cytokines for 3 human samples.

In parallel, Western blot experiments needs to be repeated with a different set of antibodies and following a several proteins which are thought to be affected by

glucocorticoid treatment. In addition, the post translational modifications of these proteins could be followed to unveil the alterations in their signalling pathway upon treatments. Chicken specific antibodies could be generated and used for DT40 cells experiments to assure the better quality of blots.

Ultimately, from above mentioned protocols the exact effect can be determined and validated then could be transferred into clinical trials if no side effects and adverse effects were demonstrated.

4.4 References

ABRAHAM, A. & ROGA, G. 2014. Topical steroid-damaged skin. *Indian J Dermatol,* 59**,** 456-9.

ABRAHAM, Y., GERRITS, B., LUDWIG, M. G., REBHAN, M. & GUBSER KELLER, C. 2017. Exploring Glucocorticoid Receptor Agonists Mechanism of Action Through Mass Cytometry and Radial Visualizations. *Cytometry B Clin Cytom,* 92**,** 42-56.

ADCOCK, I. M. & BARNES, P. J. 1996. Ligand-induced differentiation of glucocorticoid receptor (GR) trans-repression and transactivation. *Biochem Soc Trans,* 24**,** 267S.

ADEM, J., ERAY, M., EEVA, J., NUUTINEN, U. & PELKONEN, J. 2016. Timing determines dexamethasone and rituximab induced synergistic cell death. *Mol Immunol,* 75**,** 200-2.

ADZIC, M., DJORDJEVIC, J., DJORDJEVIC, A., NICIFOROVIC, A., DEMONACOS, C., RADOJCIC, M. & KRSTIC-DEMONACOS, M. 2009. Acute or chronic stress induce cell compartment-specific phosphorylation of glucocorticoid receptor and alter its transcriptional activity in Wistar rat brain. *J Endocrinol,* 202**,** 87-97.

ADZIC, M., LUKIC, I., MITIC, M., DJORDJEVIC, J., ELAKOVIC, I., DJORDJEVIC, A., KRSTIC-DEMONACOS, M., MATIC, G. & RADOJCIC, M. 2013. Brain region- and sex-specific modulation of mitochondrial glucocorticoid receptor phosphorylation in fluoxetine treated stressed rats: effects on energy metabolism. *Psychoneuroendocrinology,* 38**,** 2914-24.

AGGARWAL, A. 2012. Heat Stress and Hormones. *Heat Stress and Animal Productivity.* Springer India.

AGLER, M., PRACK, M., ZHU, Y., KOLB, J., NOWAK, K., RYSECK, R., SHEN, D., CVIJIC, M. E., SOMERVILLE, J., NADLER, S. & CHEN, T. 2007. A high-content glucocorticoid receptor translocation assay for compound mechanism-of-action evaluation. *J Biomol Screen,* 12**,** 1029-41.

AKIMKIN, V., BINDEL, F., HOFERER, M., STING, R., POLLEY, B., HANEL, A. & HAFEZ, H. M. 2011. One-step RT-qPCR with an internal control system for the detection of turkey rotaviruses in faecal samples. *J Virol Methods,* 177**,** 112-7.

ALDERSON, T. R., CHARLIER, C., TORCHIA, D. A., ANFINRUD, P. & BAX, A. 2017. Monitoring Hydrogen Exchange During Protein Folding by Fast Pressure Jump NMR Spectroscopy. *J Am Chem Soc*.

ALDERTON, W. K., COOPER, C. E. & KNOWLES, R. G. 2001. Nitric oxide synthases: structure, function and inhibition. *Biochem J,* 357**,** 593-615.

ALHARBI, R. A., PETTENGELL, R., PANDHA, H. S. & MORGAN, R. 2013. The role of HOX genes in normal hematopoiesis and acute leukemia. *Leukemia,* 27**,** 1000-8.

ALI, B. M., ZAITONE, S. A., SHOUMAN, S. A. & MOUSTAFA, Y. M. 2015. Dorzolamide synergizes the antitumor activity of mitomycin C against Ehrlich's carcinoma grown in mice: role of thioredoxin-interacting protein. *Naunyn Schmiedebergs Arch Pharmacol,* 388**,** 1271-82.

ANDREOU, K., RAJENDRAN, R., KRSTIC-DEMONACOS, M. & DEMONACOS, C. 2012. Regulation of CXCR4 gene expression in breast cancer cells under diverse stress conditions. *Int J Oncol,* 41**,** 2253-9.

APOSTOLOPOULOS, D. & MORAND, E. F. 2016. It hasn't gone away: the problem of glucocorticoid use in lupus remains. *Rheumatology (Oxford)*.

ASANO, N., UEDA, K. & KAWAZU, K. 2017. Penetration Route of the Selective Glucocorticoid Receptor Agonist SA22465 and Betamethasone into Rabbit Meibomian Gland Based on Pharmacokinetics and Autoradiography. *Drug Metab Dispos,* 45**,** 826-833.

AUPHAN, N., DIDONATO, J. A., ROSETTE, C., HELMBERG, A. & KARIN, M. 1995. Immunosuppression by glucocorticoids: inhibition of NF-kappa B activity through induction of I kappa B synthesis. *Science,* 270**,** 286-90.

AYDIN, M., BOZKURT, A., CIKMAN, A., GULHAN, B., KARABAKAN, M., GOKCE, A., ALPER, M. & KARA, M. 2017. Lack of evidence of HPV etiology of prostate cancer following radical surgery and higher frequency of the Arg/Pro genotype in Turkish men with prostate cancer. *Int Braz J Urol,* 43**,** 36-46.

AYYAR, V. S., ALMON, R. R., JUSKO, W. J. & DUBOIS, D. C. 2015. Quantitative tissue-specific dynamics of in vivo GILZ mRNA expression and regulation by endogenous and exogenous glucocorticoids. *Physiol Rep,* 3.

BABA, A. I. & CATOI, C. 2007. *Comparative Oncology.* Bucharest.

BAID, S. K. & NIEMAN, L. K. 2006. Therapeutic doses of glucocorticoids: implications for oral medicine. *Oral Dis,* 12**,** 436-42.

BAIULA, M., BEDINI, A., BALDI, J., CAVET, M. E., GOVONI, P. & SPAMPINATO, S. 2014. Mapracorat, a selective glucocorticoid receptor agonist, causes apoptosis of eosinophils infiltrating the conjunctiva in late-phase experimental ocular allergy. *Drug Des Devel Ther,* 8**,** 745-57.

BAIULA, M. & SPAMPINATO, S. 2014. Mapracorat, a novel non-steroidal selective glucocorticoid receptor agonist for the treatment of allergic conjunctivitis. *Inflamm Allergy Drug Targets,* 13**,** 289-98.

BAIULA, M., SPARTA, A., BEDINI, A., CARBONARI, G., BUCOLO, C., WARD, K. W., ZHANG, J. Z., GOVONI, P. & SPAMPINATO, S. 2011. Eosinophil as a cellular target of the ocular anti-allergic action of mapracorat, a novel selective glucocorticoid receptor agonist. *Mol Vis,* 17**,** 3208-23.

BANDYOPADHYAY, R. S. & FALLER, D. V. 1997. Regulation of c-jun gene expression in endothelial cells by the protein kinase inhibitor staurosporine. *Endothelium,* 5**,** 95-105.

BARBOUR, E. K., BOULJIHAD, M., HAMDAR, B., SAKR, W., EID, A. & SAFIEH-GARABEDIAN, B. 1999. Dynamics of protein 27 of avian leukosis virus and transforming growth factor beta2 in lymphoid leukosis susceptible and resistant broiler chicken breeding stock. *Vet Res Commun,* 23**,** 191-200.

BARCALA TABARROZZI, A. E., ANDREONE, L., DECKERS, J., CASTRO, C. N., GIMENO, M. L., ARIOLFO, L., BERGUER, P. M., ANTUNICA-NOGUEROL, M., LIBERMAN, A. C., VETTORAZZI, S., TUCKERMANN, J. P., DE BOSSCHER, K. & PERONE, M. J. 2016. GR-independent down-modulation on GM-CSF bone marrow-derived dendritic cells by the selective glucocorticoid receptor modulator Compound A. *Sci Rep,* 6**,** 36646.

BARNES, P. J. 2006. Corticosteroid effects on cell signalling. *Eur Respir J,* 27**,** 413-26.

BARNES, P. J. 2010. Mechanisms and resistance in glucocorticoid control of inflammation. *J Steroid Biochem Mol Biol,* 120**,** 76-85.

BAUDY, A. R., REEVES, E. K., DAMSKER, J. M., HEIER, C., GARVIN, L. M., DILLINGHAM, B. C., MCCALL, J., RAYAVARAPU, S., WANG, Z., VANDERMEULEN, J. H., SALI, A., JAHNKE, V., DUGUEZ, S., DUBOIS, D., ROSE, M. C., NAGARAJU, K. & HOFFMAN, E. P. 2012. Delta-9,11 modification of glucocorticoids dissociates nuclear factor-kappaB inhibitory efficacy from glucocorticoid response element-associated side effects. *J Pharmacol Exp Ther,* 343**,** 225-32.

BAUMER, W., ROSSBACH, K. & SCHMIDT, B. H. 2017. The selective glucocorticoid receptor agonist mapracorat displays a favourable safety-efficacy ratio for the topical treatment of inflammatory skin diseases in dogs. *Vet Dermatol,* 28**,** 46-e11.

BEACH, J. A., NARY, L. J., HIRAKAWA, Y., HOLLAND, E., HOVANESSIAN, R. & MEDH, R. D. 2011. E4BP4 facilitates glucocorticoid-evoked apoptosis of human leukemic CEM cells via upregulation of Bim. *J Mol Signal,* 6**,** 13.

BECK, I. M., CLARISSE, D., BOUGARNE, N., OKRET, S., HAEGEMAN, G. & DE BOSSCHER, K. 2013a. Mitogen- and stress-activated protein kinase 1 MSK1 regulates glucocorticoid response element promoter activity in a glucocorticoid concentration-dependent manner. *Eur J Pharmacol,* 715**,** 1-9.

BECK, I. M., DREBERT, Z. J., HOYA-ARIAS, R., BAHAR, A. A., DEVOS, M., CLARISSE, D., DESMET, S., BOUGARNE, N., RUTTENS, B., GOSSYE, V., DENECKER, G., LIEVENS, S., BRACKE, M., TAVERNIER, J., DECLERCQ, W., GEVAERT, K., VANDEN BERGHE, W., HAEGEMAN, G. & DE BOSSCHER, K. 2013b. Compound A, a selective glucocorticoid receptor modulator, enhances heat shock protein Hsp70 gene promoter activation. *PLoS One,* 8**,** e69115.

BECK, I. M., VANDEN BERGHE, W., GERLO, S., BOUGARNE, N., VERMEULEN, L., DE BOSSCHER, K. & HAEGEMAN, G. 2009a. Glucocorticoids and mitogen- and stress-activated protein kinase 1 inhibitors: possible partners in the combat against inflammation. *Biochem Pharmacol,* 77**,** 1194-205.

BECK, I. M., VANDEN BERGHE, W., VERMEULEN, L., YAMAMOTO, K. R., HAEGEMAN, G. & DE BOSSCHER, K. 2009b. Crosstalk in inflammation: the interplay of glucocorticoid receptor-based mechanisms and kinases and phosphatases. *Endocr Rev,* 30**,** 830-82.

BECKER, K., SCHROECKSNADEL, S., GOSTNER, J., ZAKNUN, C., SCHENNACH, H., UBERALL, F. & FUCHS, D. 2014. Comparison of in vitro tests for antioxidant and immunomodulatory capacities of compounds. *Phytomedicine,* 21**,** 164-71.

BEKHBAT, M., ROWSON, S. A. & NEIGH, G. N. 2017. Checks and balances: The glucocorticoid receptor and NFkB in good times and bad. *Front Neuroendocrinol,* 46**,** 15-31.

BELSON, M., KINGSLEY, B. & HOLMES, A. 2007. Risk factors for acute leukemia in children: a review. *Environ Health Perspect,* 115**,** 138-45.

BELVISI, M. G., WICKS, S. L., BATTRAM, C. H., BOTTOMS, S. E., REDFORD, J. E., WOODMAN, P., BROWN, T. J., WEBBER, S. E. & FOSTER, M. L. 2001. Therapeutic benefit of a dissociated glucocorticoid and the relevance of in vitro separation of transrepression from transactivation activity. *J Immunol,* 166**,** 1975-82.

BENEDETTI, S., NUVOLI, B., CATALANI, S. & GALATI, R. 2015. Reactive oxygen species a double-edged sword for mesothelioma. *Oncotarget,* 6**,** 16848-65.

BERG, A. L. & NILSSON-EHLE, P. 1996. ACTH lowers serum lipids in steroid-treated hyperlipemic patients with kidney disease. *Kidney Int,* 50**,** 538-42.

BERGER, M., REHWINKEL, H., SCHMEES, N., SCHACKE, H., EDMAN, K., WISSLER, L., REICHEL, A. & JAROCH, S. 2017. Discovery of new selective glucocorticoid receptor agonist leads. *Bioorg Med Chem Lett,* 27**,** 437-442.

BHARTI, A. C., SHISHODIA, S., REUBEN, J. M., WEBER, D., ALEXANIAN, R., RAJ-VADHAN, S., ESTROV, Z., TALPAZ, M. & AGGARWAL, B. B. 2004. Nuclear factor-kappaB and STAT3 are constitutively active in CD138+ cells derived from multiple myeloma patients, and suppression of these transcription factors leads to apoptosis. *Blood,* 103**,** 3175-84.

BIBEL, M. & BARDE, Y. A. 2000. Neurotrophins: key regulators of cell fate and cell shape in the vertebrate nervous system. *Genes Dev,* 14**,** 2919-37.

BINDREITHER, D., ECKER, S., GSCHIRR, B., KOFLER, A., KOFLER, R. & RAINER, J. 2014. The synthetic glucocorticoids prednisolone and dexamethasone regulate the same genes in acute lymphoblastic leukemia cells. *BMC Genomics,* 15**,** 662.

BLADH, L. G., LIDEN, J., DAHLMAN-WRIGHT, K., REIMERS, M., NILSSON, S. & OKRET, S. 2005. Identification of endogenous glucocorticoid repressed genes differentially regulated by a glucocorticoid receptor mutant able to separate between nuclear factor-kappaB and activator protein-1 repression. *Mol Pharmacol,* 67**,** 815-26.

BLEDSOE, R. K., MONTANA, V. G., STANLEY, T. B., DELVES, C. J., APOLITO, C. J., MCKEE, D. D., CONSLER, T. G., PARKS, D. J., STEWART, E. L., WILLSON, T. M., LAMBERT, M. H., MOORE, J. T., PEARCE, K. H. & XU, H. E. 2002. Crystal structure of the glucocorticoid receptor ligand binding domain reveals a novel mode of receptor dimerization and coactivator recognition. *Cell,* 110**,** 93-105.

BLIND, R. D. & GARABEDIAN, M. J. 2008. Differential recruitment of glucocorticoid receptor phospho-isoforms to glucocorticoid-induced genes. *J Steroid Biochem Mol Biol,* 109**,** 150-7.

BLIND, R. D., PINEDA-TORRA, I., XU, Y., XU, H. E. & GARABEDIAN, M. J. 2012. Ligand structural motifs can decouple glucocorticoid receptor transcriptional activation from target promoter occupancy. *Biochem Biophys Res Commun,* 420**,** 839-44.

BORATYN, G. M., SCHAFFER, A. A., AGARWALA, R., ALTSCHUL, S. F., LIPMAN, D. J. & MADDEN, T. L. 2012. Domain enhanced lookup time accelerated BLAST. *Biol Direct,* 7**,** 12.

BOUMPAS, D. T., ANASTASSIOU, E. D., OLDER, S. A., TSOKOS, G. C., NELSON, D. L. & BALOW, J. E. 1991. Dexamethasone inhibits human interleukin 2 but not interleukin 2 receptor gene expression in vitro at the level of nuclear transcription. *J Clin Invest,* 87**,** 1739-47.

BRANDISH, P. E., ANDERSON, K., BALTUS, G. A., BAI, C., BUNGARD, C. J., BUNTING, P., BYFORD, A., CHIU, C. S., CICMIL, M., CORCORAN, H., EULER, D., FISHER, J. E., GAMBONE, C., HASBUN-MANNING, M., KUKLIN, N., LANDIS, E., LIFSTED, T. Q., MCELWEE-WITMER, S., MCINTOSH, I. S., MEISSNER, R. S., MIAO, J., MITCHELL, H. J., MUSSELMAN, A., SCHMIDT, A., SHIN, J., SZCZERBA, P., THOMPSON, C. D., TRIBOULEY, C., VOGEL, R. L., WARRIER, S. & HERSHEY, J. C. 2014. The preclinical efficacy, selectivity and pharmacologic profile of MK-5932, an insulin-sparing selective glucocorticoid receptor modulator. *Eur J Pharmacol,* 724**,** 102-11.

BRAUN, T. P. & MARKS, D. L. 2015. The regulation of muscle mass by endogenous glucocorticoids. *Front Physiol,* 6**,** 12.

BREWER, J. A., KHOR, B., VOGT, S. K., MUGLIA, L. M., FUJIWARA, H., HAEGELE, K. E., SLECKMAN, B. P. & MUGLIA, L. J. 2003. T-cell glucocorticoid receptor is required to suppress COX-2-mediated lethal immune activation. *Nat Med,* 9**,** 1318-22.

BROSSAUD, J., ROUMES, H., HELBLING, J. C., MOISAN, M. P., PALLET, V., FERREIRA, G., BIYONG, E. F., REDONNET, A. & CORCUFF, J. B. 2017. Retinoic acid increases glucocorticoid receptor phosphorylation via cyclin-dependent kinase 5. *Mol Cell Neurosci,* 82**,** 96-104.

BUIJSMAN, R. C., HERMKENS, P. H., VAN RIJN, R. D., STOCK, H. T. & TEERHUIS, N. M. 2005. Non-steroidal steroid receptor modulators. *Curr Med Chem,* 12**,** 1017-75.

CAI, J., YU, F., LI, F. & WANG, S. Z. 2008. [Application of 99Tc(m)-annexinV in predicting the therapeutic effect of chemical agent on lung cancer]. *Zhongguo Yi Xue Ke Xue Yuan Xue Bao,* 30**,** 595-8.

CAIRNS, R. A., HARRIS, I. S. & MAK, T. W. 2011. Regulation of cancer cell metabolism. *Nat Rev Cancer,* 11**,** 85-95.

CAMPOS-SANCHEZ, E., TOBOSO-NAVASA, A., ROMERO-CAMARERO, I., BARAJAS-DIEGO, M., SANCHEZ-GARCIA, I. & COBALEDA, C. 2011. Acute lymphoblastic leukemia and developmental biology: a crucial interrelationship. *Cell Cycle,* 10**,** 3473-86.

CAPLAN, A., FETT, N., ROSENBACH, M., WERTH, V. P. & MICHELETTI, R. G. 2017a. Prevention and management of glucocorticoid-induced side effects: A comprehensive review: Infectious complications and vaccination recommendations. *J Am Acad Dermatol,* 76**,** 191-198.

CAPLAN, A., FETT, N., ROSENBACH, M., WERTH, V. P. & MICHELETTI, R. G. 2017b. Prevention and management of glucocorticoid-induced side effects: A comprehensive review: Ocular, cardiovascular, muscular, and psychiatric side effects and issues unique to pediatric patients. *J Am Acad Dermatol,* 76**,** 201-207.

CARLET, M., JANJETOVIC, K., RAINER, J., SCHMIDT, S., PANZER-GRUMAYER, R., MANN, G., PRELOG, M., MEISTER, B., PLONER, C. & KOFLER, R. 2010. Expression, regulation and function of phosphofructo-kinase/fructose-biphosphatases (PFKFBs) in glucocorticoid-induced apoptosis of acute lymphoblastic leukemia cells. *BMC Cancer,* 10**,** 638.

CARR, E. R. 2008. Cancer Nursing: A Solid Foundation for Practice, 3rd Edition. *CHAPTER 2, BIOLOGY OF CANCER. .* USA: WESTERN SCHOOLS.

CAVET, M. E., HARRINGTON, K. L., WARD, K. W. & ZHANG, J. Z. 2010. Mapracorat, a novel selective glucocorticoid receptor agonist, inhibits hyperosmolar-induced cytokine release and MAPK pathways in human corneal epithelial cells. *Mol Vis,* 16**,** 1791-800.

CAVET, M. E., VOLHEJN, S., HARRINGTON, K. L. & ZHANG, J. Z. 2013. Anti-allergic effects of mapracorat, a novel selective glucocorticoid receptor agonist, in human conjunctival fibroblasts and epithelial cells. *Mol Vis,* 19**,** 1515-25.

CHAE, S. Y., LEE, M., KIM, S. W. & BAE, Y. H. 2004. Protection of insulin secreting cells from nitric oxide induced cellular damage by crosslinked hemoglobin. *Biomaterials,* 25**,** 843-50.

CHANDRAMOHAN, Y., DROSTE, S. K. & REUL, J. M. 2007. Novelty stress induces phospho-acetylation of histone H3 in rat dentate gyrus granule neurons through coincident signalling via the N-methyl-D-aspartate receptor and the glucocorticoid receptor: relevance for c-fos induction. *J Neurochem,* 101**,** 815-28.

CHAPMAN, K., HOLMES, M. & SECKL, J. 2013. 11beta-hydroxysteroid dehydrogenases: intracellular gate-keepers of tissue glucocorticoid action. *Physiol Rev,* 93**,** 1139-206.

CHEN, D. W.-C. 2012. *Integrative Modelling of Glucocorticoid Induced Apoptosis with a Systems Biology Approach* Doctor of Philosophy, University of Manchester.

CHEN, F., ZHENG, S. L., HU, J. N., SUN, Y., HE, Y. M., PENG, H., ZHANG, B., MCCLEMENTS, D. J. & DENG, Z. Y. 2016. Octyl Ester of Ginsenoside Rh2 Induces Apoptosis and G1 Cell Cycle Arrest in Human HepG2 Cells by Activating the Extrinsic Apoptotic Pathway and Modulating the Akt/p38 MAPK Signaling Pathway. *J Agric Food Chem*.

CHENG, Q., MORAND, E. & YANG, Y. H. 2014. Development of novel treatment strategies for inflammatory diseases-similarities and divergence between glucocorticoids and GILZ. *Front Pharmacol,* 5**,** 169.

CHENG, X. J., LIN, J. C. & TU, S. P. 2016. Etiology and Prevention of Gastric Cancer. *Gastrointest Tumors,* 3**,** 25-36.

CHESTERS, P. M., HOWES, K., PETHERBRIDGE, L., EVANS, S., PAYNE, L. N. & VENUGOPAL, K. 2002. The viral envelope is a major determinant for the induction of lymphoid and myeloid tumours by avian leukosis virus subgroups A and J, respectively. *J Gen Virol,* 83**,** 2553-61.

CHILDREN WITH CANCER, U. 2017. Acute lymphoblastic leukaemia.
CHRISTOFFERSON, D. E. & YUAN, J. 2010. Necroptosis as an alternative form of programmed cell death. *Curr Opin Cell Biol,* 22**,** 263-8.
CLARK, A. R. 2003. MAP kinase phosphatase 1: a novel mediator of biological effects of glucocorticoids? *J Endocrinol,* 178**,** 5-12.
COOPER, S. L. & BROWN, P. A. 2015. Treatment of pediatric acute lymphoblastic leukemia. *Pediatr Clin North Am,* 62**,** 61-73.
COUTINHO, A. E. & CHAPMAN, K. E. 2011. The anti-inflammatory and immunosuppressive effects of glucocorticoids, recent developments and mechanistic insights. *Mol Cell Endocrinol,* 335**,** 2-13.
CUONG, T. T., YANG, C. S., YUK, J. M., LEE, H. M., KO, S. R., CHO, B. G. & JO, E. K. 2009. Glucocorticoid receptor agonist compound K regulates Dectin-1-dependent inflammatory signaling through inhibition of reactive oxygen species. *Life Sci,* 85**,** 625-33.
DANDONA, P., MOHANTY, P., HAMOUDA, W., ALJADA, A., KUMBKARNI, Y. & GARG, R. 1999. Effect of dexamethasone on reactive oxygen species generation by leukocytes and plasma interleukin-10 concentrations: a pharmacodynamic study. *Clin Pharmacol Ther,* 66**,** 58-65.
DAVIES, L., PARASKEVOPOULOU, E., SADEQ, M., SYMEOU, C., PANTELIDOU, C., DEMONACOS, C. & KRSTIC-DEMONACOS, M. 2011. Regulation of glucocorticoid receptor activity by a stress responsive transcriptional cofactor. *Mol Endocrinol,* 25**,** 58-71.
DAVIS, R. P., MILLER-DOREY, S. & JENNE, C. N. 2016. Platelets and coagulation in infection. *Clin Transl Immunology,* 5**,** e89.
DE BOSSCHER, K. 2010. Selective Glucocorticoid Receptor modulators. *J Steroid Biochem Mol Biol,* 120**,** 96-104.
DE BOSSCHER, K., HAEGEMAN, G. & ELEWAUT, D. 2010. Targeting inflammation using selective glucocorticoid receptor modulators. *Curr Opin Pharmacol,* 10**,** 497-504.
DE BOSSCHER, K., SCHMITZ, M. L., VANDEN BERGHE, W., PLAISANCE, S., FIERS, W. & HAEGEMAN, G. 1997. Glucocorticoid-mediated repression of nuclear factor-kappaB-dependent transcription involves direct interference with transactivation. *Proc Natl Acad Sci U S A,* 94**,** 13504-9.
DE BOSSCHER, K., VANDEN BERGHE, W. & HAEGEMAN, G. 2000a. Mechanisms of anti-inflammatory action and of immunosuppression by glucocorticoids: negative interference of activated glucocorticoid receptor with transcription factors. *J Neuroimmunol,* 109**,** 16-22.
DE BOSSCHER, K., VANDEN BERGHE, W. & HAEGEMAN, G. 2006. Cross-talk between nuclear receptors and nuclear factor kappaB. *Oncogene,* 25**,** 6868-86.
DE BOSSCHER, K., VANDEN BERGHE, W., VERMEULEN, L., PLAISANCE, S., BOONE, E. & HAEGEMAN, G. 2000b. Glucocorticoids repress NF-kappaB-driven genes by disturbing the interaction of p65 with the basal transcription machinery, irrespective of coactivator levels in the cell. *Proc Natl Acad Sci U S A,* 97**,** 3919-24.
DELGADO, M. D. & LEON, J. 2010. Myc roles in hematopoiesis and leukemia. *Genes Cancer,* 1**,** 605-16.
DEMONACOS, C., KRSTIC-DEMONACOS, M. & LA THANGUE, N. B. 2001. A TPR motif cofactor contributes to p300 activity in the p53 response. *Mol Cell,* 8**,** 71-84.
DENG, Q., RIQUELME, D., TRINH, L., LOW, M. J., TOMIC, M., STOJILKOVIC, S. & AGUILERA, G. 2015. Rapid Glucocorticoid Feedback Inhibition of ACTH Secretion Involves Ligand-

Dependent Membrane Association of Glucocorticoid Receptors. *Endocrinology,* 156**,** 3215-27.
DICKMEIS, T. 2009. Glucocorticoids and the circadian clock. *J Endocrinol,* 200**,** 3-22.
DIKAIOS, N., ATKINSON, D., TUDISCA, C., PURPURA, P., FORSTER, M., AHMED, H., BEALE, T., EMBERTON, M. & PUNWANI, S. 2017. A comparison of Bayesian and non-linear regression methods for robust estimation of pharmacokinetics in DCE-MRI and how it affects cancer diagnosis. *Comput Med Imaging Graph,* 56**,** 1-10.
DITTRICH, A., KHOURI, C., SACKETT, S. D., EHLTING, C., BOHMER, O., ALBRECHT, U., BODE, J. G., TRAUTWEIN, C. & SCHAPER, F. 2012. Glucocorticoids increase interleukin-6-dependent gene induction by interfering with the expression of the suppressor of cytokine signaling 3 feedback inhibitor. *Hepatology,* 55**,** 256-66.
DOBRITZSCH, D., SCHNEIDER, G., SCHNACKERZ, K. D. & LINDQVIST, Y. 2001. Crystal structure of dihydropyrimidine dehydrogenase, a major determinant of the pharmacokinetics of the anti-cancer drug 5-fluorouracil. *EMBO J,* 20**,** 650-60.
DONG, Z. 2013. [Nasal glucocorticoids are the major medicine for chronic rhinosinusitis]. *Zhonghua Er Bi Yan Hou Tou Jing Wai Ke Za Zhi,* 48**,** 98-9.
DREBERT, Z., BRACKE, M. & BECK, I. M. 2015. Glucocorticoids and the non-steroidal selective glucocorticoid receptor modulator, compound A, differentially affect colon cancer-derived myofibroblasts. *J Steroid Biochem Mol Biol,* 149**,** 92-105.
DRUKER, J., LIBERMAN, A. C., ANTUNICA-NOGUEROL, M., GEREZ, J., PAEZ-PEREDA, M., REIN, T., INIGUEZ-LLUHI, J. A., HOLSBOER, F. & ARZT, E. 2013. RSUME enhances glucocorticoid receptor SUMOylation and transcriptional activity. *Mol Cell Biol,* 33**,** 2116-27.
ELFARRA, A. A. & HWANG, I. Y. 1996. Effects of pH, temperature, and chemical structure on the stability of S-(purin-6-yl)-L-cysteine: evidence for a novel molecular rearrangement mechanism to yield N-(purin-6-yl)-L-cysteine. *Chem Res Toxicol,* 9**,** 654-8.
ENCISO, J., MENDOZA, L. & PELAYO, R. 2015. Normal vs. Malignant hematopoiesis: the complexity of acute leukemia through systems biology. *Front Genet,* 6**,** 290.
ENZO. 2017. *Compound A Glucocorticoid receptor modulator*

[Online]. Available: http://www.enzolifesciences.com/ALX-550-516/compound-a/ [Accessed].
ERHUMA, A. M. 2012. *Glucocorticoids: Biochemical Group That Play Key Role in Fetal Programming of Adult Disease*.
FENG, Y., FAMUYIDE, M. & BHATT, A. J. 2013. Dexamethasone decreases insulin-like growth factor-I and -II via a glucocorticoid receptor dependent mechanism in developing rat brain. *Neuro Endocrinol Lett,* 34**,** 624-34.
FIEGEL, G. 1961. [Modified chemotherapy of malignant tumors with glucocorticoids and sex hormones]. *Dtsch Med J,* 12**,** 344-9.
FISHER, C. J., NIU, C., FOLTZ, W., CHEN, Y., SIDOROVA-DARMOS, E., EUBANKS, J. H. & LILGE, L. 2017. ALA-PpIX mediated photodynamic therapy of malignant gliomas augmented by hypothermia. *PLoS One,* 12**,** e0181654.
FOTY, R. A., CORBETT, S. A., SCHWARZBAUER, J. E. & STEINBERG, M. S. 1998. Dexamethasone up-regulates cadherin expression and cohesion of HT-1080 human fibrosarcoma cells. *Cancer Res,* 58**,** 3586-9.
FRANCHIMONT, D. 2004. Overview of the actions of glucocorticoids on the immune response: a good model to characterize new pathways of immunosuppression for new treatment strategies. *Ann N Y Acad Sci,* 1024**,** 124-37.

FRANCI, O., AMICI, A., MARGARIT, R., MERENDINO, N. & PICCOLELLA, E. 1996. Influence of thermal and dietary stress on immune response of rabbits. *J Anim Sci,* 74**,** 1523-9.
FRENKEL, B., WHITE, W. & TUCKERMANN, J. 2015. Glucocorticoid-Induced Osteoporosis. *Adv Exp Med Biol,* 872**,** 179-215.
FREY, B. 2002. Transfusion in premature infants impairs production and/or release of red blood cells, white blood cells and platelets. *J Paediatr Child Health,* 38**,** 265-7.
GALLIHER-BECKLEY, A. J. & CIDLOWSKI, J. A. 2009. Emerging roles of glucocorticoid receptor phosphorylation in modulating glucocorticoid hormone action in health and disease. *IUBMB life,* 61**,** 979-986.
GARDILL, B. R., VOGL, M. R., LIN, H. Y., HAMMOND, G. L. & MULLER, Y. A. 2012. Corticosteroid-binding globulin: structure-function implications from species differences. *PLoS One,* 7**,** e52759.
GEL'BERG, I. S., ZHAROVINA, M. M., ZAEKO, II, ZUBRITSKAIA, R. M. & TSISHKEVICH, F. K. 1986. [Effect of glucocorticoids on the vitamin allowance of the body during chemotherapy of tuberculosis]. *Probl Tuberk***,** 31-5.
GELEY, S., HARTMANN, B. L., HALA, M., STRASSER-WOZAK, E. M., KAPELARI, K. & KOFLER, R. 1996. Resistance to glucocorticoid-induced apoptosis in human T-cell acute lymphoblastic leukemia CEM-C1 cells is due to insufficient glucocorticoid receptor expression. *Cancer Res,* 56**,** 5033-8.
GENECARDS, T. G. H. G. D. 2017. IL6 Gene(Protein Coding) -Interleukin 6, http://www.genecards.org/cgi-bin/carddisp.pl?gene=IL6. Weizmann Institute of Science.
GENES, A. O. G. A. C. I. O. A. H. 2017a. BCL2L11 (BCL2-like 11 (apoptosis facilitator)).
GENES, N. U. N. L. O. M. 2017b. IL6R gene.
GHOSE, K. 1980. Decreased tyramine sensitivity after discontinuation of amitriptyline therapy. An index of pharmacodynamic half-life. *Eur J Clin Pharmacol,* 18**,** 151-17.
GIANNINI, A., RUSSO, E., MANNELLA, P. & SIMONCINI, T. 2015. Selective steroid receptor modulators in reproductive medicine. *Minerva Ginecol,* 67**,** 431-55.
GILLILAND, D. G. & GRIFFIN, J. D. 2002. The roles of FLT3 in hematopoiesis and leukemia. *Blood,* 100**,** 1532-42.
GOBINATH, A. R., MAHMOUD, R. & GALEA, L. A. 2014. Influence of sex and stress exposure across the lifespan on endophenotypes of depression: focus on behavior, glucocorticoids, and hippocampus. *Front Neurosci,* 8**,** 420.
GOICHOT, B. 2009. [Side effects of glucocorticoids and specialist in internal medicine]. *Rev Med Interne,* 30 Suppl 4**,** S299-301.
GOLDBLOOM, E. B., MOKASHI, A., CUMMINGS, E. A., ABISH, S., BENSELER, S. M., HUYNH, H. Q., WATSON, W. & AHMET, A. 2017. Symptomatic adrenal suppression among children in Canada. *Arch Dis Child,* 102**,** 338-339.
GOLDSTEIN, L. J. & OZOLS, R. F. 1994. *Anticancer drug resistance : advances in molecular and clinical research,* Boston, Kluwer Academic.
GOSSYE, V., HAEGEMAN, G. & DE BOSSCHER, K. 2008. Therapeutic implications of the nuclear factor-kappaB/nuclear receptor cross-talk. *Front Biosci,* 13**,** 4122-43.
GOTO, K., NAKAI, K., FUJII, H. & NISHI, S. 2017. The Effects of Plasma Exchange on Severe Vasculitis with Diffuse Alveolar Hemorrhage. *Intern Med,* 56**,** 55-59.
GRIGOR'IAN, G. A., DYGALO, N. N., GEKHT, A. B., STEPANICHEV, M. & GULIAEVA, N. V. 2014. [Molecular and cellular mechanisms of depression. Role of glucocorticoids, cytokines, neurotransmitters, and trophic factors in genesis of depressive disorders]. *Usp Fiziol Nauk,* 45**,** 3-19.

GROSS, K. L., OAKLEY, R. H., SCOLTOCK, A. B., JEWELL, C. M. & CIDLOWSKI, J. A. 2011. Glucocorticoid receptor alpha isoform-selective regulation of antiapoptotic genes in osteosarcoma cells: a new mechanism for glucocorticoid resistance. *Mol Endocrinol,* 25**,** 1087-99.
GRUVER-YATES, A. L. & CIDLOWSKI, J. A. 2013. Tissue-specific actions of glucocorticoids on apoptosis: a double-edged sword. *Cells,* 2**,** 202-23.
GUO, Q., SUN, Y., YU, S., BAI, H., ZHAO, J., ZHUO, M. & WANG, J. 2017. Programmed cell death-ligand 1 (PD-L1) expression and fibroblast growth factor receptor 1 (FGFR1) amplification in stage III/IV lung squamous cell carcinoma (SQC). *Thorac Cancer,* 8**,** 73-79.
GUO, X., ZHOU, C. Y., LI, Q., GAO, J., ZHU, Y. P., GU, L. & MA, Z. G. 2013. Rapamycin sensitizes glucocorticoid resistant acute lymphoblastic leukemia CEM-C1 cells to dexamethasone induced apoptosis through both mTOR suppression and up-regulation and activation of glucocorticoid receptor. *Biomed Environ Sci,* 26**,** 371-81.
GUZAUSKAS, G. F., VILLA, K. F., VANHOVE, G. F., FISHER, V. L. & VEENSTRA, D. L. 2017. Risk-Benefit Analysis of Pediatric-Inspired Versus Hyperfractionated Cyclophosphamide, Vincristine, Doxorubicin, and Dexamethasone Protocols for Acute Lymphoblastic Leukemia in Adolescents and Young Adults. *J Adolesc Young Adult Oncol,* 6**,** 53-61.
HAMDI, H., GODOT, V., MAILLOT, M. C., PREJEAN, M. V., COHEN, N., KRZYSIEK, R., LEMOINE, F. M., ZOU, W. & EMILIE, D. 2007. Induction of antigen-specific regulatory T lymphocytes by human dendritic cells expressing the glucocorticoid-induced leucine zipper. *Blood,* 110**,** 211-9.
HANAHAN, D. & WEINBERG, R. A. 2000. The hallmarks of cancer. *Cell,* 100**,** 57-70.
HANAHAN, D. & WEINBERG, R. A. 2011. Hallmarks of cancer: the next generation. *Cell,* 144**,** 646-74.
HAPKE, H. J. & STRATHMANN, W. 1995. [Pharmacological effects of hordenine]. *Dtsch Tierarztl Wochenschr,* 102**,** 228-32.
HARDIN, J., MACLEOD, S., GRIGORIEVA, I., CHANG, R., BARLOGIE, B., XIAO, H. & EPSTEIN, J. 1994. Interleukin-6 prevents dexamethasone-induced myeloma cell death. *Blood,* 84**,** 3063-70.
HASHKES, P. J., WRIGHT, B. M., LAUER, M. S., WORLEY, S. E., TANG, A. S., ROETTCHER, P. A. & BOWYER, S. L. 2011. Survival rates of children with acute lymphoblastic leukemia presenting to a pediatric rheumatologist in the United States. *J Pediatr Hematol Oncol,* 33**,** 424-8.
HEIDARI, N., HICKS, M. A. & HARADA, H. 2010. GX15-070 (obatoclax) overcomes glucocorticoid resistance in acute lymphoblastic leukemia through induction of apoptosis and autophagy. *Cell Death Dis,* 1**,** e76.
HEIDARI, N., MILLER, A. V., HICKS, M. A., MARKING, C. B. & HARADA, H. 2012. Glucocorticoid-mediated BIM induction and apoptosis are regulated by Runx2 and c-Jun in leukemia cells. *Cell Death Dis,* 3**,** e349.
HERMOSO, M. A. & CIDLOWSKI, J. A. 2003. Putting the brake on inflammatory responses: the role of glucocorticoids. *IUBMB Life,* 55**,** 497-504.
HIHARA, H., MAEDA, M., NAKAMURA, K., ISHINO, S., TSUKAMOTO, K., YUASA, N. & SHIRAI, J. 1998. Rapid induction of lymphoid leukosis and ascites by avian leukosis virus from a lymphoid leukosis cell line. *J Vet Med Sci,* 60**,** 77-85.
HOESEL, B. & SCHMID, J. A. 2013. The complexity of NF-kappaB signaling in inflammation and cancer. *Mol Cancer,* 12**,** 86.
HOLLER, N., ZARU, R., MICHEAU, O., THOME, M., ATTINGER, A., VALITUTTI, S., BODMER, J. L., SCHNEIDER, P., SEED, B. & TSCHOPP, J. 2000. Fas triggers an alternative, caspase-

8-independent cell death pathway using the kinase RIP as effector molecule. *Nat Immunol,* 1**,** 489-95.

HONEDER, C., ENGLEDER, E., SCHOPPER, H., KRAUSE, M., LANDEGGER, L. D., PLASENZOTTI, R., GABOR, F., GSTOETTNER, W. & ARNOLDNER, C. 2015. Evaluation of the selective glucocorticoid receptor agonist compound A for ototoxic effects. *Laryngoscope,* 125**,** E149-55.

HONG, Y., WU, J., ZHAO, J., WANG, H., LIU, Y., CHEN, T., KAN, X., TAO, Q., SHEN, X., YAN, K. & ZHAI, Z. 2013. miR-29b and miR-29c are involved in Toll-like receptor control of glucocorticoid-induced apoptosis in human plasmacytoid dendritic cells. *PLoS One,* 8**,** e69926.

HORIBE, K., YUMURA-YAGI, K., KUDOH, T., NISHIMURA, S., ODA, M., YOSHIDA, M., KOMADA, Y., HARA, J., TAWA, A., USAMI, I., TANIZAWA, A., KATO, K., KOBAYASHI, R., MATSUO, K., HORI, H. & JAPAN ASSOCIATION OF CHILDHOOD LEUKEMIA STUDY, J. 2017. Long-term Results of the Risk-adapted Treatment for Childhood B-Cell Acute Lymphoblastic Leukemia: Report From the Japan Association of Childhood Leukemia Study ALL-97 Trial. *J Pediatr Hematol Oncol*.

HOU, W., GUAN, J., LU, H., DONG, Q., HAN, Y. & ZHANG, R. 2014. The effects of dexamethasone on the proliferation and apoptosis of human ovarian cancer cells induced by paclitaxel. *J Ovarian Res,* 7**,** 89.

HOUSSAMI, N., BERNARDI, D., PELLEGRINI, M., VALENTINI, M., FANTO, C., OSTILLIO, L., TUTTOBENE, P., LUPARIA, A. & MACASKILL, P. 2017. Breast cancer detection using single-reading of breast tomosynthesis (3D-mammography) compared to double-reading of 2D-mammography: Evidence from a population-based trial. *Cancer Epidemiol,* 47**,** 94-99.

HU, A., JOSEPHSON, M. B., DIENER, B. L., NINO, G., XU, S., PARANJAPE, C., ORANGE, J. S. & GRUNSTEIN, M. M. 2013. Pro-asthmatic cytokines regulate unliganded and ligand-dependent glucocorticoid receptor signaling in airway smooth muscle. *PLoS One,* 8**,** e60452.

HU, Y., TIAN, W., ZHANG, L. L., LIU, H., YIN, G. P., HE, B. S. & MAO, X. M. 2012. Function of regulatory T-cells improved by dexamethasone in Graves' disease. *Eur J Endocrinol,* 166**,** 641-6.

HUA, F., JI, L., ZHAN, Y., LI, F., ZOU, S., WANG, X., SONG, D., MIN, Z., GAO, S., WU, Y., CHEN, H. & CHENG, Y. 2012. Pulsed high-dose dexamethasone improves interleukin 10 secretion by CD5+ B cells in patients with primary immune thrombocytopenia. *J Clin Immunol,* 32**,** 1233-42.

HUNG, S. H., RANKIN, A., VIRJI-BABUL, N., PRITCHARD, S., FRYER, C. & CAMPBELL, K. L. 2017. Associating Physical Activity Levels with Motor Performance and Physical Function in Childhood Survivors of Acute Lymphoblastic Leukemia. *Physiother Can,* 69**,** 57-64.

HUNTER, R. G., SELIGSOHN, M., RUBIN, T. G., GRIFFITHS, B. B., OZDEMIR, Y., PFAFF, D. W., DATSON, N. A. & MCEWEN, B. S. 2016. Stress and corticosteroids regulate rat hippocampal mitochondrial DNA gene expression via the glucocorticoid receptor. *Proc Natl Acad Sci U S A,* 113**,** 9099-104.

INC, S. T. 2004. THERMAL STABILITY OF API’S UNDER HIGH TEMPERATURE HPLC CONDITIONS, in High Temperature Liquid Chromatography, Technical Note 807.

ISMAIL, F. S., MOINFAR, Z., PROCHNOW, N., DAMBACH, H., HINKEROHE, D., HAASE, C. G., FORSTER, E. & FAUSTMANN, P. M. 2016. Dexamethasone and levetiracetam reduce hetero-cellular gap-junctional coupling between F98 glioma cells and glial cells in vitro. *J Neurooncol*.

ITO, H., ANDO, T., OGISO, H., ARIOKA, Y. & SEISHIMA, M. 2015. Inhibition of induced nitric oxide synthase enhances the anti-tumor effects on cancer immunotherapy using TLR7 agonist in mice. *Cancer Immunol Immunother,* 64**,** 429-36.
ITO, K., BARNES, P. J. & ADCOCK, I. M. 2000. Glucocorticoid receptor recruitment of histone deacetylase 2 inhibits interleukin-1beta-induced histone H4 acetylation on lysines 8 and 12. *Mol Cell Biol,* 20**,** 6891-903.
IWASA, M., MIURA, Y., FUJISHIRO, A., FUJII, S., SUGINO, N., YOSHIOKA, S., YOKOTA, A., HISHITA, T., HIRAI, H., ANDOH, A., ICHINOHE, T. & MAEKAWA, T. 2017. Erratum to: Bortezomib interferes with adhesion of B cell precursor acute lymphoblastic leukemia cells through SPARC up-regulation in human bone marrow mesenchymal stromal/stem cells. *Int J Hematol*.
JAIN, R. K., DI TOMASO, E., DUDA, D. G., LOEFFLER, J. S., SORENSEN, A. G. & BATCHELOR, T. T. 2007. Angiogenesis in brain tumours. *Nat Rev Neurosci,* 8**,** 610-22.
JAMIESON, C. A. & YAMAMOTO, K. R. 2000. Crosstalk pathway for inhibition of glucocorticoid-induced apoptosis by T cell receptor signaling. *Proc Natl Acad Sci U S A,* 97**,** 7319-24.
JETT, J. R. & CARR, L. L. 2013. Targeted therapy for non-small cell lung cancer. *Am J Respir Crit Care Med,* 188**,** 907-12.
JING, D., BHADRI, V. A., BECK, D., THOMS, J. A., YAKOB, N. A., WONG, J. W., KNEZEVIC, K., PIMANDA, J. E. & LOCK, R. B. 2015. Opposing regulation of BIM and BCL2 controls glucocorticoid-induced apoptosis of pediatric acute lymphoblastic leukemia cells. *Blood,* 125**,** 273-83.
JOANNY, E., DING, Q., GONG, L., KONG, P., SAKLATVALA, J. & CLARK, A. R. 2012. Anti-inflammatory effects of selective glucocorticoid receptor modulators are partially dependent on up-regulation of dual specificity phosphatase 1. *Br J Pharmacol,* 165**,** 1124-36.
JOBE, A. H. 2000. Glucocorticoids in perinatal medicine: misguided rockets? *J Pediatr,* 137**,** 1-3.
JOHNSON, R. F. & PERKINS, N. D. 2012. Nuclear factor-kappaB, p53, and mitochondria: regulation of cellular metabolism and the Warburg effect. *Trends Biochem Sci,* 37**,** 317-24.
JOURNY, N., ROUE, T., CARDIS, E., LE POINTE, H. D., BRISSE, H., CHATEIL, J. F., LAURIER, D. & BERNIER, M. O. 2016. Childhood CT scans and cancer risk: impact of predisposing factors for cancer on the risk estimates. *J Radiol Prot,* 36**,** N1-7.
JOVICIC, M., MARIC, N. P., SOLDATOVIC, I., LUKIC, I., ANDRIC, S., MIHALJEVIC, M., PAVLOVIC, Z., MITIC, M. & ADZIC, M. 2015. The role of glucocorticoid receptor phosphorylation in the model of negative affective states. *World J Biol Psychiatry,* 16**,** 301-11.
JOZIC, I., VUKELIC, S., STOJADINOVIC, O., LIANG, L., RAMIREZ, H. A., PASTAR, I. & TOMIC CANIC, M. 2016. Stress Signals, Mediated by Membranous Glucocorticoid Receptor, Activate PLC/PKC/GSK-3beta/beta-catenin Pathway to Inhibit Wound Closure. *J Invest Dermatol*.
JOZIC, I., VUKELIC, S., STOJADINOVIC, O., LIANG, L., RAMIREZ, H. A., PASTAR, I. & TOMIC CANIC, M. 2017. Stress Signals, Mediated by Membranous Glucocorticoid Receptor, Activate PLC/PKC/GSK-3beta/beta-catenin Pathway to Inhibit Wound Closure. *J Invest Dermatol,* 137**,** 1144-1154.
KADAJA-SAAREPUU, L., LAOS, S., JAAGER, K., VIIL, J., BALIKOVA, A., LOOKE, M., HANSSON, G. C. & MAIMETS, T. 2008. CD43 promotes cell growth and helps to evade FAS-

mediated apoptosis in non-hematopoietic cancer cells lacking the tumor suppressors p53 or ARF. *Oncogene,* 27**,** 1705-15.
KADAN-LOTTICK, N. S., BROUWERS, P., BREIGER, D., KALEITA, T., DZIURA, J., LIU, H., CHEN, L., NICOLETTI, M., STORK, L., BOSTROM, B. & NEGLIA, J. P. 2009. A comparison of neurocognitive functioning in children previously randomized to dexamethasone or prednisone in the treatment of childhood acute lymphoblastic leukemia. *Blood,* 114**,** 1746-52.
KADMIEL, M. & CIDLOWSKI, J. A. 2013. Glucocorticoid receptor signaling in health and disease. *Trends Pharmacol Sci,* 34**,** 518-30.
KANEKURA, T., SEISHIMA, M., HONMA, M., ETOU, T., ETO, H., OKUMA, K., OKUBO, Y., YAMAGUCHI, Y., KAMBARA, T., MABUCHI, T., SUGA, Y., MORITA, A., YAMANISHI, K., TSURUTA, D., ITOH, K., YAMAJI, K. & IKEDA, S. 2017. Therapeutic depletion of myeloid lineage leukocytes by adsorptive apheresis for psoriatic arthritis: Efficacy of a non-drug intervention for patients refractory to pharmacologics. *J Dermatol*.
KANTAKE, M., YOSHITAKE, H., ISHIKAWA, H., ARAKI, Y. & SHIMIZU, T. 2014. Postnatal epigenetic modification of glucocorticoid receptor gene in preterm infants: a prospective cohort study. *BMJ Open,* 4**,** e005318.
KAPILA, N., SHARMA, A., KISHORE, A., SODHI, M., TRIPATHI, P. K., MOHANTY, A. K. & MUKESH, M. 2016. Impact of Heat Stress on Cellular and Transcriptional Adaptation of Mammary Epithelial Cells in Riverine Buffalo (Bubalus Bubalis). *PLoS One,* 11**,** e0157237.
KARIN, M. 2009. NF-kappaB as a critical link between inflammation and cancer. *Cold Spring Harb Perspect Biol,* 1**,** a000141.
KATO, M., HAGIWARA, Y., ODA, T., IMAMURA-TAKAI, M., AONO, H. & NAKAMURA, M. 2011. Beneficial pharmacological effects of selective glucocorticoid receptor agonist in external eye diseases. *J Ocul Pharmacol Ther,* 27**,** 353-60.
KAYMAK CIHAN, M., KARABULUT, H. G., YURUR KUTLAY, N., ILGIN RUHI, H., TUKUN, A. & OLCAY, L. 2017. Association Between N363S and BclI Polymorphisms of the Glucocorticoid Receptor Gene (NR3C1) and Glucocorticoid Side Effects During Childhood Acute Lymphoblastic Leukemia Treatment. *Turk J Haematol*.
KELEG, S., BUCHLER, P., LUDWIG, R., BUCHLER, M. W. & FRIESS, H. 2003. Invasion and metastasis in pancreatic cancer. *Mol Cancer,* 2**,** 14.
KERVOELEN, C., MENORET, E., GOMEZ-BOUGIE, P., BATAILLE, R., GODON, C., MARIONNEAU-LAMBOT, S., MOREAU, P., PELLAT-DECEUNYNCK, C. & AMIOT, M. 2015. Dexamethasone-induced cell death is restricted to specific molecular subgroups of multiple myeloma. *Oncotarget,* 6**,** 26922-34.
KIKUCHI, Y., IMAKIIRE, T., YAMADA, M., SAIGUSA, T., HYODO, T., KUSHIYAMA, T., HIGASHI, K., HYODO, N., YAMAMOTO, K., SUZUKI, S. & MIURA, S. 2007. Effect of risedronate on high-dose corticosteroid-induced bone loss in patients with glomerular disease. *Nephrol Dial Transplant,* 22**,** 1593-600.
KIM, S. C., LEE, J. H., KIM, M. H., LEE, J. A., KIM, Y. B., JUNG, E., KIM, Y. S., LEE, J. & PARK, D. 2013. Hordenine, a single compound produced during barley germination, inhibits melanogenesis in human melanocytes. *Food Chem,* 141**,** 174-81.
KIM, Y., SUN, Y., CHOW, C., POMMIER, Y. G. & SIMONS, S. S., JR. 2006. Effects of acetylation, polymerase phosphorylation, and DNA unwinding in glucocorticoid receptor transactivation. *J Steroid Biochem Mol Biol,* 100**,** 3-17.
KINO, T. 2010. The Glucocorticoid Receptor Brain Immune Media Ltd.
KLOPOT, A., BAIDA, G., BHALLA, P., HAEGEMAN, G. & BUDUNOVA, I. 2015. Selective Activator of the Glucocorticoid Receptor Compound A Dissociates Therapeutic and

Atrophogenic Effects of Glucocorticoid Receptor Signaling in Skin. *J Cancer Prev,* 20**,** 250-9.
KODA, H., YOKOO, Y., MATSUMOTO, N., SUWA, Y., FUKAZAWA, H., ISHIDA, H., TSUJI, K., NUKAYA, H. & KURIYAMA, K. 1999. Antagonistic effect of N-methyltyramine on alpha2-adrenoceptor in mice. *Jpn J Pharmacol,* 81**,** 313-5.
KOIBUCHI, F., RITOH, N., AOYAGI, R., FUNAKOSHI-TAGO, M. & TAMURA, H. 2014. Dexamethasone suppresses neurosteroid biosynthesis via downregulation of steroidogenic enzyme gene expression in human glioma GI-1 cells. *Biol Pharm Bull,* 37**,** 1241-7.
KOL, S., BEN-SHLOMO, I., PAYNE, D. W., ANDO, M., ROHAN, R. M. & ADASHI, E. Y. 1998. Glucocorticoids suppress basal (but not interleukin-1-supported) ovarian phospholipase A2 activity: evidence for glucocorticoid receptor-mediated regulation. *Mol Cell Endocrinol,* 137**,** 117-25.
KORHONEN, R., LAHTI, A., HAMALAINEN, M., KANKAANRANTA, H. & MOILANEN, E. 2002. Dexamethasone inhibits inducible nitric-oxide synthase expression and nitric oxide production by destabilizing mRNA in lipopolysaccharide-treated macrophages. *Mol Pharmacol,* 62**,** 698-704.
KOUCHAKZADEH, H., SOUDI, T., HESHMATI AGHDA, N. & SHOJAOSADATI, S. A. 2017. Ligand-modified biopolymeric nanoparticles as efficient tools for targeted cancer therapy. *Curr Pharm Des*.
KOVACS, J. J., MURPHY, P. J., GAILLARD, S., ZHAO, X., WU, J. T., NICCHITTA, C. V., YOSHIDA, M., TOFT, D. O., PRATT, W. B. & YAO, T. P. 2005. HDAC6 regulates Hsp90 acetylation and chaperone-dependent activation of glucocorticoid receptor. *Mol Cell,* 18**,** 601-7.
KRAICHELY, D. M., SUN, J., KATZENELLENBOGEN, J. A. & KATZENELLENBOGEN, B. S. 2000. Conformational changes and coactivator recruitment by novel ligands for estrogen receptor-alpha and estrogen receptor-beta: correlations with biological character and distinct differences among SRC coactivator family members. *Endocrinology,* 141**,** 3534-45.
KRISTEN, A. V., ACKERMANN, K., BUSS, S., LEHMANN, L., SCHNABEL, P. A., HAUNSTETTER, A., KATUS, H. A. & HARDT, S. E. 2013. Inhibition of apoptosis by the intrinsic but not the extrinsic apoptotic pathway in myocardial ischemia-reperfusion. *Cardiovasc Pathol,* 22**,** 280-6.
KRSTIC, M. D., ROGATSKY, I., YAMAMOTO, K. R. & GARABEDIAN, M. J. 1997. Mitogen-activated and cyclin-dependent protein kinases selectively and differentially modulate transcriptional enhancement by the glucocorticoid receptor. *Mol Cell Biol,* 17**,** 3947-54.
KRUK, J. & ABOUL-ENEIN, H. Y. 2017. Reactive Oxygen and Nitrogen Species in Carcinogenesis: Implications of Oxidative Stress on the Progression and Development of Several Cancer Types. *Mini Rev Med Chem,* 17**,** 904-919.
KUMAR, R. & THOMPSON, E. B. 2012. Folding of the glucocorticoid receptor N-terminal transactivation function: dynamics and regulation. *Mol Cell Endocrinol,* 348**,** 450-6.
KUNA, P., AURIVILLIUS, M., JORUP, C., PROTHON, S., TAIB, Z. & EDSBACKER, S. 2017. Efficacy and Tolerability of an Inhaled Selective Glucocorticoid Receptor Modulator - AZD5423 - in Chronic Obstructive Pulmonary Disease Patients: Phase II Study Results. *Basic Clin Pharmacol Toxicol*.
KURIMOTO, T., TAMAI, I., MIYAI, A., KOSUGI, Y., NAKAGAWA, T., YAMAMOTO, Y., DEAI, K., MISAKI, S., BESSHO, Y., NEGORO, T., YAMAGUCHI, T., HATA, T. & MATSUSHITA, M. 2017. JTP-117968, a novel selective glucocorticoid receptor modulator, exhibits

improved transrepression/transactivation dissociation. *Eur J Pharmacol,* 803**,** 179-186.
LACALLE, R. A., BLANCO, R., CARMONA-RODRIGUEZ, L., MARTIN-LEAL, A., MIRA, E. & MANES, S. 2017. Chemokine Receptor Signaling and the Hallmarks of Cancer. *Int Rev Cell Mol Biol,* 331**,** 181-244.
LAIRMORE, M. D. & MONTGOMERY, A. 2005. Isolation and confirmation of human T-cell leukemia virus type 2 from peripheral blood mononuclear cells. *Methods Mol Biol,* 304**,** 113-23.
LAMAS, M., SANZ, E., MARTIN-PARRAS, L., ESPEL, E., SPERISEN, P., COLLINS, M. & SILVA, A. G. 1993. Glucocorticoid hormones upregulate interleukin 2 receptor alpha gene expression. *Cell Immunol,* 151**,** 437-50.
LANDEGGER, L. D., HONEDER, C., ZHU, C., SCHOPPER, H., ENGLEDER, E., GABOR, F., GSTOETTNER, W. & ARNOLDNER, C. 2016. Noise trauma and systemic application of the selective glucocorticoid receptor modulator compound A. *J Negat Results Biomed,* 15**,** 10.
LANE, N. E. & LUKERT, B. 1998. The science and therapy of glucocorticoid-induced bone loss. *Endocrinol Metab Clin North Am,* 27**,** 465-83.
LANKAT-BUTTGEREIT, B. & GOKE, R. 2003. Programmed cell death protein 4 (pdcd4): a novel target for antineoplastic therapy? *Biol Cell,* 95**,** 515-9.
LANSHAKOV, D. A., SUKHAREVA, E. V., KALININA, T. S. & DYGALO, N. N. 2016. Dexamethasone-induced acute excitotoxic cell death in the developing brain. *Neurobiol Dis,* 91**,** 1-9.
LATCHMAN, D. S. 2001. Transcription factors: bound to activate or repress. *Trends Biochem Sci,* 26**,** 211-3.
LEE, C. & ELWING, J. 2017. Pulse dose steroids in severe pulmonary arterial hypertension secondary to systemic lupus erythematosus. *SAGE Open Med Case Rep,* 5**,** 2050313X17707153.
LEHMANN, L., EL-HADDAD, A. & BARR, R. D. 2016. Global Approach to Hematologic Malignancies. *Hematol Oncol Clin North Am,* 30**,** 417-32.
LEIST, M. & JAATTELA, M. 2001. Four deaths and a funeral: from caspases to alternative mechanisms. *Nat Rev Mol Cell Biol,* 2**,** 589-98.
LEMS, W. F., BAAK, M. M., VAN TUYL, L. H., LODDER, M. C., DIJKMANS, B. A. & BOERS, M. 2016. One-year effects of glucocorticoids on bone density: a meta-analysis in cohorts on high and low-dose therapy. *RMD Open,* 2**,** e000313.
LENGYEL, E. 2010. Ovarian cancer development and metastasis. *Am J Pathol,* 177**,** 1053-64.
LESOVAYA, E., YEMELYANOV, A., KIRSANOV, K., POPA, A., BELITSKY, G., YAKUBOVSKAYA, M., GORDON, L. I., ROSEN, S. T. & BUDUNOVA, I. 2013. Combination of a selective activator of the glucocorticoid receptor Compound A with a proteasome inhibitor as a novel strategy for chemotherapy of hematologic malignancies. *Cell Cycle,* 12**,** 133-44.
LESOVAYA, E., YEMELYANOV, A., SWART, A. C., SWART, P., HAEGEMAN, G. & BUDUNOVA, I. 2015. Discovery of Compound A--a selective activator of the glucocorticoid receptor with anti-inflammatory and anti-cancer activity. *Oncotarget,* 6**,** 30730-44.
LESOVAYA, E. A., YEMELYANOV, A. Y., KIRSANOV, K. I., YAKUBOVSKAYA, M. G. & BUDUNOVA, I. V. 2011. Antitumor effect of non-steroid glucocorticoid receptor ligand CpdA on leukemia cell lines CEM and K562. *Biochemistry (Mosc),* 76**,** 1242-52.
LEVINE, B. & KROEMER, G. 2008. Autophagy in the pathogenesis of disease. *Cell,* 132**,** 27-42.
LF, L. F. 2017. Related blood disorders.

LI, C. C., MUNITIC, I., MITTELSTADT, P. R., CASTRO, E. & ASHWELL, J. D. 2015. Suppression of Dendritic Cell-Derived IL-12 by Endogenous Glucocorticoids Is Protective in LPS-Induced Sepsis. *PLoS Biol,* 13**,** e1002269.

LIBERMAN, A. C., ANTUNICA-NOGUEROL, M., FERRAZ-DE-PAULA, V., PALERMO-NETO, J., CASTRO, C. N., DRUKER, J., HOLSBOER, F., PERONE, M. J., GERLO, S., DE BOSSCHER, K., HAEGEMAN, G. & ARZT, E. 2012. Compound A, a dissociated glucocorticoid receptor modulator, inhibits T-bet (Th1) and induces GATA-3 (Th2) activity in immune cells. *PLoS One,* 7**,** e35155.

LIBRO, R., BRAMANTI, P. & MAZZON, E. 2017. Endogenous glucocorticoids: role in the etiopathogenesis of Alzheimer's disease. *Neuro Endocrinol Lett,* 38**,** 1-12.

LIDEN, J., RAFTER, I., TRUSS, M., GUSTAFSSON, J. A. & OKRET, S. 2000. Glucocorticoid effects on NF-kappaB binding in the transcription of the ICAM-1 gene. *Biochem Biophys Res Commun,* 273**,** 1008-14.

LIN, C. H., HONG, Y. C. & KAO, S. H. 2015. Aeroallergen Der p 2 induces apoptosis of bronchial epithelial BEAS-2B cells via activation of both intrinsic and extrinsic pathway. *Cell Biosci,* 5**,** 71.

LIOU, G. Y. & STORZ, P. 2010. Reactive oxygen species in cancer. *Free Radic Res,* 44**,** 479-96.

LIU, S., LUO, Y., FU, J., ZHOU, J. & KYZAS, G. Z. 2016a. Molecular docking and 3D-QSAR studies on the glucocorticoid receptor antagonistic activity of hydroxylated polychlorinated biphenyls. *SAR QSAR Environ Res,* 27**,** 87-99.

LIU, Y., GE, J., LI, Q., GU, L., GUO, X., MA, Z. G. & ZHU, Y. P. 2013. Anisomycin induces apoptosis of glucocorticoid resistant acute lymphoblastic leukemia CEM-C1 cells via activation of mitogen-activated protein kinases p38 and JNK. *Neoplasma,* 60**,** 101-10.

LIU, Y., GE, J., LI, Q., GUO, X., GU, L., MA, Z. G., LI, X. H. & ZHU, Y. P. 2014. Low-dose anisomycin sensitizes glucocorticoid-resistant T-acute lymphoblastic leukemia CEM-C1 cells to dexamethasone-induced apoptosis through activation of glucocorticoid receptor and p38-MAPK/JNK. *Leuk Lymphoma,* 55**,** 2179-88.

LIU, Y. L., JANG, S., WANG, S. M., CHEN, C. H. & LI, F. Y. 2016b. Investigation on critical structural motifs of ligands for triggering glucocorticoid receptor nuclear migration through molecular docking simulations. *J Biomol Struct Dyn,* 34**,** 1214-31.

LOCKSHIN, R. A. & WILLIAMS, C. M. 1964. Programmed cell death—II. Endocrine potentiation of the breakdown of the intersegmental muscles of silkmoths. *Journal of Insect Physiology*

10**,** 643-649.

LOU, Y., ZHANG, F., LUO, Y., WANG, L., HUANG, S. & JIN, F. 2016. Serum and Glucocorticoid Regulated Kinase 1 in Sodium Homeostasis. *Int J Mol Sci,* 17.

LYNCH, J. T., RAJENDRAN, R., XENAKI, G., BERROU, I., DEMONACOS, C. & KRSTIC-DEMONACOS, M. 2010. The role of glucocorticoid receptor phosphorylation in Mcl-1 and NOXA gene expression. *Mol Cancer,* 9**,** 38.

LYU, X., LI, J., YUN, X., HUANG, R., DENG, X., WANG, Y., CHEN, Y. & XIAO, G. 2017. miR-181a-5p, an inducer of Wnt-signaling, facilitates cell proliferation in acute lymphoblastic leukemia. *Oncol Rep,* 37**,** 1469-1476.

MA, J., WANG, S., HUANG, X., GENG, P., WEN, C., ZHOU, Y., YU, L. & WANG, X. 2015. Validated UPLC-MS/MS method for determination of hordenine in rat plasma and its application to pharmacokinetic study. *J Pharm Biomed Anal,* 111**,** 131-7.

MADLIGER, C. L. & LOVE, O. P. 2016. Employing individual measures of baseline glucocorticoids as population-level conservation biomarkers: considering within-individual variation in a breeding passerine. *Conserv Physiol,* 4**,** cow048.

MAGHSUDLU, M. & FARASHAHI YAZD, E. 2017. Heat-Induced Inflammation and Its Role in Esophageal Cancer. *J Dig Dis*.

MALAISE, O., RELIC, B., QUESADA-CALVO, F., CHARLIER, E., ZEDDOU, M., NEUVILLE, S., GILLET, P., LOUIS, E., DE SENY, D. & MALAISE, M. G. 2015. Selective glucocorticoid receptor modulator compound A, in contrast to prednisolone, does not induce leptin or the leptin receptor in human osteoarthritis synovial fibroblasts. *Rheumatology (Oxford),* 54**,** 1087-92.

MALDONADO BOUCHARD, S. & HOOK, M. A. 2014. Psychological stress as a modulator of functional recovery following spinal cord injury. *Front Neurol,* 5**,** 44.

MAMMI, C., MARZOLLA, V., ARMANI, A., FERACO, A., ANTELMI, A., MASLAK, E., CHLOPICKI, S., CINTI, F., HUNT, H., FABBRI, A. & CAPRIO, M. 2016. A novel combined glucocorticoid-mineralocorticoid receptor selective modulator markedly prevents weight gain and fat mass expansion in mice fed a high-fat diet. *Int J Obes (Lond),* 40**,** 964-72.

MANASA, V. G. & KANNAN, S. 2017. Impact of microRNA dynamics on cancer hallmarks: An oral cancer scenario. *Tumour Biol,* 39**,** 1010428317695920.

MANOHARAN, A., CATOVSKY, D., GOLDMAN, J. M., LAURIA, F., LAMPERT, I. A. & GALTON, D. A. 1980. Significance of splenomegaly in childhood acute lymphoblastic leukaemia in remission. *Lancet,* 1**,** 449-52.

MARCHETTI, M. C., DI MARCO, B., CIFONE, G., MIGLIORATI, G. & RICCARDI, C. 2003. Dexamethasone-induced apoptosis of thymocytes: role of glucocorticoid receptor-associated Src kinase and caspase-8 activation. *Blood,* 101**,** 585-93.

MARTINEZ, C. S., PIAZZA, V. G., DIAZ, M. E., BOPARAI, R. K., ARUM, O., RAMIREZ, M. C., GONZALEZ, L., BECU-VILLALOBOS, D., BARTKE, A., TURYN, D., MIQUET, J. G. & SOTELO, A. I. 2015. GH/STAT5 signaling during the growth period in livers of mice overexpressing GH. *J Mol Endocrinol,* 54**,** 171-84.

MARUMO, T., NAKAKI, T., NAGATA, K., MIYATA, M., ADACHI, H., ESUMI, H., SUZUKI, H., SARUTA, T. & KATO, R. 1993. Dexamethasone inhibits nitric oxide synthase mRNA induction by interleukin-1 alpha and tumor necrosis factor-alpha in vascular smooth muscle cells. *Jpn J Pharmacol,* 63**,** 361-7.

MARUMO, T., SCHINI-KERTH, V. B., BRANDES, R. P. & BUSSE, R. 1998. Glucocorticoids inhibit superoxide anion production and p22 phox mRNA expression in human aortic smooth muscle cells. *Hypertension,* 32**,** 1083-8.

MATIC, G., TRAJKOVIC, D., SUSA, M., DAMJANOVIC, S. & PETROVIC, J. 1989. In vitro evidence for modification of rat liver glucocorticoid receptor binding properties and transformation by hyperthermia. *J Steroid Biochem,* 32**,** 263-70.

MATSUNO, H. 2016. Assessment of Distal Radius Bone Mineral Density in Osteoporosis Patients Receiving Denosumab, Including Those with Rheumatoid Arthritis and Those Receiving Oral Glucocorticoids. *Drugs R D*.

MATTHEWS, J. G., ITO, K., BARNES, P. J. & ADCOCK, I. M. 2004. Defective glucocorticoid receptor nuclear translocation and altered histone acetylation patterns in glucocorticoid-resistant patients. *J Allergy Clin Immunol,* 113**,** 1100-8.

MCMASTER, A. & RAY, D. W. 2008. Drug insight: selective agonists and antagonists of the glucocorticoid receptor. *Nat Clin Pract Endocrinol Metab,* 4**,** 91-101.

MEDH, R. D., SAEED, M. F., JOHNSON, B. H. & THOMPSON, E. B. 1998. Resistance of human leukemic CEM-C1 cells is overcome by synergism between glucocorticoid and protein kinase A pathways: correlation with c-Myc suppression. *Cancer Res,* 58**,** 3684-93.

MEDH, R. D., WEBB, M. S., MILLER, A. L., JOHNSON, B. H., FOFANOV, Y., LI, T., WOOD, T. G., LUXON, B. A. & THOMPSON, E. B. 2003. Gene expression profile of human lymphoid CEM cells sensitive and resistant to glucocorticoid-evoked apoptosis. *Genomics,* 81**,** 543-55.

MEIJSING, S. H. 2015. Mechanisms of Glucocorticoid-Regulated Gene Transcription. *Adv Exp Med Biol,* 872**,** 59-81.

MELIN, J., PROTHON, S., KLOFT, C., CLETON, A., AMILON, C., JORUP, C., BACKMAN, P., OLSSON, B. & HAMREN, U. W. 2017. Pharmacokinetics of the Inhaled Selective Glucocorticoid Receptor Modulator AZD5423 Following Inhalation Using Different Devices. *AAPS J,* 19**,** 865-874.

MENYHART, O., HARAMI-PAPP, H., SUKUMAR, S., SCHAFER, R., MAGNANI, L., DE BARRIOS, O. & GYORFFY, B. 2016. Guidelines for the selection of functional assays to evaluate the hallmarks of cancer. *Biochim Biophys Acta,* 1866**,** 300-319.

MEYER, M., GONZALEZ DENISELLE, M. C., HUNT, H., DE KLOET, E. R. & DE NICOLA, A. F. 2014. The selective glucocorticoid receptor modulator CORT108297 restores faulty hippocampal parameters in Wobbler and corticosterone-treated mice. *J Steroid Biochem Mol Biol,* 143**,** 40-8.

MIYOSHI, H., OHKI, M., NAKAGAWA, T. & HONMA, Y. 1997. Glucocorticoids induce apoptosis in acute myeloid leukemia cell lines with A t(8;21) chromosome translocation. *Leuk Res,* 21**,** 45-50.

MOLNAR, J., POTI, A., PIPEK, O., KRZYSTANEK, M., KANU, N., SWANTON, C., TUSNADY, G. E., SZALLASI, Z., CSABAI, I. & SZUTS, D. 2014. The genome of the chicken DT40 bursal lymphoma cell line. *G3 (Bethesda),* 4**,** 2231-40.

MORGAN, D. J., POOLMAN, T. M., WILLIAMSON, A. J., WANG, Z., CLARK, N. R., MA'AYAN, A., WHETTON, A. D., BRASS, A., MATTHEWS, L. C. & RAY, D. W. 2016. Glucocorticoid receptor isoforms direct distinct mitochondrial programs to regulate ATP production. *Sci Rep,* 6**,** 26419.

MORRIS, M., QUARESMA, M., PITKANIEMI, J., MORRIS, E., RACHET, B. & COLEMAN, M. P. 2016. Do cancer survival statistics for every hospital make sense? *Lancet Oncol,* 17**,** 1192-4.

MOSCHOVI, M., ADAMAKI, M. & VLAHOPOULOS, S. A. 2016. Progress in Treatment of Viral Infections in Children with Acute Lymphoblastic Leukemia. *Oncol Rev,* 10**,** 300.

MOSTAFA, N. Z., ULUDAG, H., VARKEY, M., DEDERICH, D. N., DOSCHAK, M. R. & EL-BIALY, T. H. 2011. In vitro osteogenic induction of human gingival fibroblasts for bone regeneration. *Open Dent J,* 5**,** 139-45.

MOZO, L., SUAREZ, A. & GUTIERREZ, C. 2004. Glucocorticoids up-regulate constitutive interleukin-10 production by human monocytes. *Clin Exp Allergy,* 34**,** 406-12.

MUKHERJEE, A., HALDAR, C. & VISHWAS, D. K. 2015. Melatonin prevents dexamethasone-induced testicular oxidative stress and germ cell apoptosis in golden hamster, Mesocricetus auratus. *Andrologia,* 47**,** 920-31.

MURAYI, R. & CHITTIBOINA, P. 2016. Glucocorticoids in the management of peritumoral brain edema: a review of molecular mechanisms. *Childs Nerv Syst*.

MURPHY, P. J., MORISHIMA, Y., KOVACS, J. J., YAO, T. P. & PRATT, W. B. 2005. Regulation of the dynamics of hsp90 action on the glucocorticoid receptor by acetylation/deacetylation of the chaperone. *J Biol Chem,* 280**,** 33792-9.

MYATT, S. S. & LAM, E. W. 2007. Promiscuous and lineage-specific roles of cell cycle regulators in haematopoiesis. *Cell Div,* 2**,** 6.

NADER, N., NG, S. S., WANG, Y., ABEL, B. S., CHROUSOS, G. P. & KINO, T. 2012. Liver x receptors regulate the transcriptional activity of the glucocorticoid receptor: implications for the carbohydrate metabolism. *PLoS One,* 7**,** e26751.

NARAYAN, E. J. & HERO, J. M. 2014. Acute thermal stressor increases glucocorticoid response but minimizes testosterone and locomotor performance in the cane toad (Rhinella marina). *PLoS One,* 9**,** e92090.

NARUM, S., WESTERGREN, T. & KLEMP, M. 2014. Corticosteroids and risk of gastrointestinal bleeding: a systematic review and meta-analysis. *BMJ Open,* 4**,** e004587.

NELSON, G., WILDE, G. J., SPILLER, D. G., KENNEDY, S. M., RAY, D. W., SULLIVAN, E., UNITT, J. F. & WHITE, M. R. 2003. NF-kappaB signalling is inhibited by glucocorticoid receptor and STAT6 via distinct mechanisms. *J Cell Sci,* 116**,** 2495-503.

NEWTON, R. & HOLDEN, N. S. 2007. Separating transrepression and transactivation: a distressing divorce for the glucocorticoid receptor? *Mol Pharmacol,* 72**,** 799-809.

NG, A. P. & ALEXANDER, W. S. 2017. Haematopoietic stem cells: past, present and future. *Cell Death Discov,* 3**,** 17002.

NG, H. P., JENNINGS, S., WANG, J., MOLINA, P. E., NELSON, S. & WANG, G. 2017. Non-canonical Glucocorticoid Receptor Transactivation of gilz by Alcohol Suppresses Cell Inflammatory Response. *Front Immunol,* 8**,** 661.

NGUYEN, E. T., STREICHER, J., BERMAN, S., CALDWELL, J. L., GHISAYS, V., ESTRADA, C. M., WULSIN, A. C. & SOLOMON, M. B. 2017. A mixed glucocorticoid/mineralocorticoid receptor modulator dampens endocrine and hippocampal stress responsivity in male rats. *Physiol Behav,* 178**,** 82-92.

NI CHONGHAILE, T., CONCANNON, C. G., SZEGEZDI, E., GORMAN, A. M. & SAMALI, A. 2006. Dexamethasone inhibits apoptosis in C6 glioma cells through increased expression of Bcl-XL. *Apoptosis,* 11**,** 1247-55.

NIH, N. C. I. A. T. N. I. O. H. 2017. Cancer Statistics.

NIKOLETOPOULOU, V., MARKAKI, M., PALIKARAS, K. & TAVERNARAKIS, N. 2013. Crosstalk between apoptosis, necrosis and autophagy. *Biochim Biophys Acta,* 1833**,** 3448-59.

NIKOLIC, I., VUJICIC, M., SAKSIDA, T., BERKI, T., STOSIC-GRUJICIC, S. & STOJANOVIC, I. 2013. The role of endogenous glucocorticoids in glucose metabolism and immune status of MIF-deficient mice. *Eur J Pharmacol,* 714**,** 498-506.

NOGUES, L., REGLERO, C., RIVAS, V., NEVES, M., PENELA, P. & MAYOR, F., JR. 2017. G-Protein-Coupled Receptor Kinase 2 as a Potential Modulator of the Hallmarks of Cancer. *Mol Pharmacol,* 91**,** 220-228.

NORTHROP, J. P., CRABTREE, G. R. & MATTILA, P. S. 1992. Negative regulation of interleukin 2 transcription by the glucocorticoid receptor. *J Exp Med,* 175**,** 1235-45.

NUNES, T., BERNARDAZZI, C. & DE SOUZA, H. S. 2014. Cell death and inflammatory bowel diseases: apoptosis, necrosis, and autophagy in the intestinal epithelium. *Biomed Res Int,* 2014**,** 218493.

NUWAYHID, B. 1983. Obstetrics and gynecology-important advances in clinical medicine: glucocorticoids and lecithin-sphingomyelin ratio. *West J Med,* 139**,** 520-1.

O'CONNOR, L., STRASSER, A., O'REILLY, L. A., HAUSMANN, G., ADAMS, J. M., CORY, S. & HUANG, D. C. 1998. Bim: a novel member of the Bcl-2 family that promotes apoptosis. *EMBO J,* 17**,** 384-95.

OAKLEY, R. H. & CIDLOWSKI, J. A. 2013. The biology of the glucocorticoid receptor: new signaling mechanisms in health and disease. *J Allergy Clin Immunol,* 132**,** 1033-44.

OKAMOTO, K., TANAKA, H., MAKINO, Y. & MAKINO, I. 1998. Restoration of the glucocorticoid receptor function by the phosphodiester compound of vitamins C and E, EPC-K1 (L-ascorbic acid 2-[3,4-dihydro-2,5,7,8-tetramethyl-2-(4,8,12-

trimethyltridecyl)-2H-1-benzopyran-6 -yl hydrogen phosphate] potassium salt), via a redox-dependent mechanism. *Biochem Pharmacol,* 56**,** 79-86.
ORQUEDA, A. J., DANSEY, M. V., ESPANOL, A., VELEIRO, A. S., BAL DE KIER JOFFE, E., SALES, M. E., BURTON, G. & PECCI, A. 2014. The rigid steroid 21-hydroxy-6,19-epoxyprogesterone (21OH-6,19OP) is a dissociated glucocorticoid receptor modulator potentially useful as a novel coadjuvant in breast cancer chemotherapy. *Biochem Pharmacol,* 89**,** 526-35.
OSTER, H., DAMEROW, S., KIESSLING, S., JAKUBCAKOVA, V., ABRAHAM, D., TIAN, J., HOFFMANN, M. W. & EICHELE, G. 2006. The circadian rhythm of glucocorticoids is regulated by a gating mechanism residing in the adrenal cortical clock. *Cell Metab,* 4**,** 163-73.
OWEN, H. C., MINER, J. N., AHMED, S. F. & FARQUHARSON, C. 2007. The growth plate sparing effects of the selective glucocorticoid receptor modulator, AL-438. *Mol Cell Endocrinol,* 264**,** 164-70.
OZER, B. & SEZERMAN, U. 2017. An integrative study on the impact of highly differentially methylated genes on expression and cancer etiology. *PLoS One,* 12**,** e0171694.
PACE, T. W. & MILLER, A. H. 2009. Cytokines and glucocorticoid receptor signaling. Relevance to major depression. *Ann N Y Acad Sci,* 1179**,** 86-105.
PALUMBO, E. & RUSSO, A. 2017. Chromosome Imbalances in Cancer: Molecular Cytogenetics Meets Genomics. *Cytogenet Genome Res*.
PAREDES, S. & ALVES, M. 2016. [Management and Treatment of Glucocorticoid-Induced Hyperglycemia]. *Acta Med Port,* 29**,** 556-563.
PARK, S. W., LEE, J. G., SEO, M. K., LY, N. N., LEE, C. H., CHO, H. Y., HEIN, L. T., CHOI, A. J., KIM, G. M. & KIM, Y. H. 2017. Epigenetic modification of glucocorticoid receptor promoter I7 in maternally separated and restraint-stressed rats. *Neurosci Lett,* 650**,** 38-44.
PAROVICHNIKOVA, E. N., SAVCHENKO, V. G., ISAEV, V. G., SOKOLOV, A. N., MOSKOV, V. I., KLIASOVA, G. A., GAL'TSEVA, I. V., USTINOVA, E. N., GRIBANOVA, E. O. & LAPIN, V. A. 2003. [Superhigh doses of dexamethasone in treatment of refractory forms of acute lymphoblast leukemia of adults]. *Ter Arkh,* 75**,** 21-3.
PAZDRAK, K., STRAUB, C., MAROTO, R., STAFFORD, S., WHITE, W. I., CALHOUN, W. J. & KUROSKY, A. 2016. Cytokine-Induced Glucocorticoid Resistance from Eosinophil Activation: Protein Phosphatase 5 Modulation of Glucocorticoid Receptor Phosphorylation and Signaling. *J Immunol,* 197**,** 3782-3791.
PEI, S., MINHAJUDDIN, M., D'ALESSANDRO, A., NEMKOV, T., STEVENS, B. M., ADANE, B., KHAN, N., HAGEN, F. K., YADAV, V. K., DE, S., ASHTON, J. M., HANSEN, K. C., GUTMAN, J. A., POLLYEA, D. A., CROOKS, P. A., SMITH, C. & JORDAN, C. T. 2016. Rational Design of a Parthenolide-based Drug Regimen That Selectively Eradicates Acute Myelogenous Leukemia Stem Cells. *J Biol Chem,* 291**,** 21984-22000.
PERSSON, F. & HALLE, B. 2015. How amide hydrogens exchange in native proteins. *Proc Natl Acad Sci U S A,* 112**,** 10383-8.
PETERSEN, A., CARLSSON, T., KARLSSON, J. O., JONHEDE, S. & ZETTERBERG, M. 2008. Effects of dexamethasone on human lens epithelial cells in culture. *Mol Vis,* 14**,** 1344-52.
PFEFFER, B. A., DEWITT, C. A., SALVADOR-SILVA, M., CAVET, M. E., LOPEZ, F. J. & WARD, K. W. 2010. Reduced myocilin expression in cultured monkey trabecular meshwork cells induced by a selective glucocorticoid receptor agonist: comparison with steroids. *Invest Ophthalmol Vis Sci,* 51**,** 437-46.

PICO, M. J., HASHEMI, S., XU, F., NGUYEN, K. H., DONNELLY, R., MORAN, E. & FLOWERS, S. 2016. Glucocorticoid receptor-mediated cis-repression of osteogenic genes requires BRM-SWI/SNF. *Bone Rep,* 5**,** 222-227.

PLONER, C., SCHMIDT, S., PRESUL, E., RENNER, K., SCHROCKSNADEL, K., RAINER, J., RIML, S. & KOFLER, R. 2005. Glucocorticoid-induced apoptosis and glucocorticoid resistance in acute lymphoblastic leukemia. *J Steroid Biochem Mol Biol,* 93**,** 153-60.

POCUCA, N., RUZDIJIC, S., DEMONACOS, C., KANAZIR, D. & KRSTIC-DEMONACOS, M. 1998. Using yeast to study glucocorticoid receptor phosphorylation. *J Steroid Biochem Mol Biol,* 66**,** 303-18.

POLAK, A., KILISZEK, P., SEWASTIANIK, T., SZYDLOWSKI, M., JABLONSKA, E., BIALOPIOTROWICZ, E., GORNIAK, P., MARKOWICZ, S., NOWAK, E., GRYGOROWICZ, M. A., PROCHOREC-SOBIESZEK, M., NOWIS, D., GOLAB, J., GIEBEL, S., LECH-MARANDA, E., WARZOCHA, K. & JUSZCZYNSKI, P. 2016. MEK Inhibition Sensitizes Precursor B-Cell Acute Lymphoblastic Leukemia (B-ALL) Cells to Dexamethasone through Modulation of mTOR Activity and Stimulation of Autophagy. *PLoS One,* 11**,** e0155893.

POLK, A., VAAGE-NILSEN, M., VISTISEN, K. & NIELSEN, D. L. 2013. Cardiotoxicity in cancer patients treated with 5-fluorouracil or capecitabine: a systematic review of incidence, manifestations and predisposing factors. *Cancer Treat Rev,* 39**,** 974-84.

PONTES, J., JR. 2017. Lack of evidence of HPV etiology of prostate cancer following radical surgery and higher frequency of the Arg/Pro genotype in Turkish men with prostate cancer. *Int Braz J Urol,* 43**,** 47.

POPOVIC, N., RUZDIJIC, S., KANAZIR, D. T., NICIFOROVIC, A., ADZIC, M., PARASKEVOPOULOU, E., PANTELIDOU, C., RADOJCIC, M., DEMONACOS, C. & KRSTIC-DEMONACOS, M. 2010. Site-specific and dose-dependent effects of glucocorticoid receptor phosphorylation in yeast Saccharomyces cerevisiae. *Steroids,* 75**,** 457-65.

PRENEK, L., BOLDIZSAR, F., KUGYELKA, R., UGOR, E., BERTA, G., NEMETH, P. & BERKI, T. 2017. The regulation of the mitochondrial apoptotic pathway by glucocorticoid receptor in collaboration with Bcl-2 family proteins in developing T cells. *Apoptosis,* 22**,** 239-253.

PROKSCH, J. W., LOWE, E. R. & WARD, K. W. 2011. Ocular pharmacokinetics of mapracorat, a novel, selective glucocorticoid receptor agonist, in rabbits and monkeys. *Drug Metab Dispos,* 39**,** 1181-7.

PRUSATOR, D. K. & GREENWOOD-VAN MEERVELD, B. 2017. Amygdala-mediated mechanisms regulate visceral hypersensitivity in adult females following early life stress: importance of the glucocorticoid receptor and corticotropin-releasing factor. *Pain,* 158**,** 296-305.

PUFALL, M. A. 2015. Glucocorticoids and Cancer. *Adv Exp Med Biol,* 872**,** 315-33.

PULTE, D., JANSEN, L., GONDOS, A., KATALINIC, A., BARNES, B., RESSING, M., HOLLECZEK, B., EBERLE, A., BRENNER, H. & GROUP, G. C. S. W. 2014. Survival of adults with acute lymphoblastic leukemia in Germany and the United States. *PLoS One,* 9**,** e85554.

QATTAN, M. 2014. *The role of inducible transcription factors and microenvironment in leukemia.* Doctor of Phylosophy, The University of Manchester.

QUADRI, S. A., ISLAM, S., QADRI, A. N. & AHMAD, M. 1997. Aziridinyl steroid-induced lesions in DNA and apoptosis in promyelocytic leukemia cells. *Biochem Mol Biol Int,* 43**,** 1353-65.

QUIDDE, J., DENNE, L., KUTSCHEIDT, A., KINDLER, M., KIRSCH, A., KRIPP, M., PETERSEN, V., SCHULZE, M., SERAPHIN, J., TUMMES, D., ARNOLD, D. & STEIN, A. 2017. Baseline and On-Treatment Markers Determining Prognosis of First-Line Chemotherapy in

Combination with Bevacizumab in Patients with Metastatic Colorectal Cancer. *Oncol Res Treat,* 40**,** 21-26.
RAMDAS, J., LIU, W. & HARMON, J. M. 1999. Glucocorticoid-induced cell death requires autoinduction of glucocorticoid receptor expression in human leukemic T cells. *Cancer Res,* 59**,** 1378-85.
RAMIREZ DE MOLINA, A., GALLEGO-ORTEGA, D., SARMENTERO-ESTRADA, J., LAGARES, D., GOMEZ DEL PULGAR, T., BANDRES, E., GARCIA-FONCILLAS, J. & LACAL, J. C. 2008. Choline kinase as a link connecting phospholipid metabolism and cell cycle regulation: implications in cancer therapy. *Int J Biochem Cell Biol,* 40**,** 1753-63.
RATMAN, D., VANDEN BERGHE, W., DEJAGER, L., LIBERT, C., TAVERNIER, J., BECK, I. M. & DE BOSSCHER, K. 2013. How glucocorticoid receptors modulate the activity of other transcription factors: a scope beyond tethering. *Mol Cell Endocrinol,* 380**,** 41-54.
RAUCH, A., SEITZ, S., BASCHANT, U., SCHILLING, A. F., ILLING, A., STRIDE, B., KIRILOV, M., MANDIC, V., TAKACZ, A., SCHMIDT-ULLRICH, R., OSTERMAY, S., SCHINKE, T., SPANBROEK, R., ZAISS, M. M., ANGEL, P. E., LERNER, U. H., DAVID, J. P., REICHARDT, H. M., AMLING, M., SCHUTZ, G. & TUCKERMANN, J. P. 2010. Glucocorticoids suppress bone formation by attenuating osteoblast differentiation via the monomeric glucocorticoid receptor. *Cell Metab,* 11**,** 517-31.
RAUNER, M., GOETTSCH, C., STEIN, N., THIELE, S., BORNHAEUSER, M., DE BOSSCHER, K., HAEGEMAN, G., TUCKERMANN, J. & HOFBAUER, L. C. 2011. Dissociation of osteogenic and immunological effects by the selective glucocorticoid receptor agonist, compound A, in human bone marrow stromal cells. *Endocrinology,* 152**,** 103-12.
RAUNER, M., THIELE, S., SINNINGEN, K., WINZER, M., SALBACH-HIRSCH, J., GLOE, I., PESCHKE, K., HAEGEMAN, G., TUCKERMANN, J. P. & HOFBAUER, L. C. 2013. Effects of the selective glucocorticoid receptor modulator compound A on bone metabolism and inflammation in male mice with collagen-induced arthritis. *Endocrinology,* 154**,** 3719-28.
RAZAVI, H., RIETHER, D., HARCKEN, C., BENTZIEN, J., DINALLO, R. M., SOUZA, D., NELSON, R. M., KUKULKA, A., FADRA-KHAN, T. N., PACK, E. J., JR., ZUVELA-JELASKA, L., PELLETIER, J., PANZENBECK, M., TORCELLINI, C. A., PROUDFOOT, J. R., NABOZNY, G. H. & THOMSON, D. S. 2014. Discovery of a potent and dissociated non-steroidal glucocorticoid receptor agonist containing an alkyl carbinol pharmacophore. *Bioorg Med Chem Lett,* 24**,** 1934-40.
REYER, H., PONSUKSILI, S., KANITZ, E., POHLAND, R., WIMMERS, K. & MURANI, E. 2016. A Natural Mutation in Helix 5 of the Ligand Binding Domain of Glucocorticoid Receptor Enhances Receptor-Ligand Interaction. *PLoS One,* 11**,** e0164628.
RICHTER-PECHANSKA, P., KUNZ, J. B., HOF, J., ZIMMERMANN, M., RAUSCH, T., BANDAPALLI, O. R., ORLOVA, E., SCAPINELLO, G., SAGI, J. C., STANULLA, M., SCHRAPPE, M., CARIO, G., KIRSCHNER-SCHWABE, R., ECKERT, C., BENES, V., KORBEL, J. O., MUCKENTHALER, M. U. & KULOZIK, A. E. 2017. Identification of a genetically defined ultra-high-risk group in relapsed pediatric T-lymphoblastic leukemia. *Blood Cancer J,* 7**,** e523.
ROBERTSON, S., ALLIE-REID, F., VANDEN BERGHE, W., VISSER, K., BINDER, A., AFRICANDER, D., VISMER, M., DE BOSSCHER, K., HAPGOOD, J., HAEGEMAN, G. & LOUW, A. 2010. Abrogation of glucocorticoid receptor dimerization correlates with dissociated glucocorticoid behavior of compound a. *J Biol Chem,* 285**,** 8061-75.
ROBIN-JAGERSCHMIDT, C., WURTZ, J. M., GUILLOT, B., GOFFLO, D., BENHAMOU, B., VERGEZAC, A., OSSART, C., MORAS, D. & PHILIBERT, D. 2000. Residues in the ligand

binding domain that confer progestin or glucocorticoid specificity and modulate the receptor transactivation capacity. *Mol Endocrinol,* 14**,** 1028-37.
ROBINSON, K., LETLEY, D. P. & KANEKO, K. 2017. The Human Stomach in Health and Disease: Infection Strategies by Helicobacter pylori. *Curr Top Microbiol Immunol,* 400**,** 1-26.
ROBSON, P. & HUGHES, A. 2003. Glucocorticoids in palliative medicine: is osteoporosis a problem? *Palliat Med,* 17**,** 645-6.
ROCHA, J. M., XAVIER, S. G., DE LIMA SOUZA, M. E., ASSUMPCAO, J. G., MURAO, M. & DE OLIVEIRA, B. M. 2016. Current Strategies for the Detection of Minimal Residual Disease in Childhood Acute Lymphoblastic Leukemia. *Mediterr J Hematol Infect Dis,* 8**,** e2016024.
ROMAGNANI, S. 1999. Th1/Th2 cells. *Inflamm Bowel Dis,* 5**,** 285-94.
ROMAGNANI, S. 2000. T-cell subsets (Th1 versus Th2). *Ann Allergy Asthma Immunol,* 85**,** 9-18; quiz 18, 21.
RONACHER, K., HADLEY, K., AVENANT, C., STUBSRUD, E., SIMONS, S. S., JR., LOUW, A. & HAPGOOD, J. P. 2009. Ligand-selective transactivation and transrepression via the glucocorticoid receptor: role of cofactor interaction. *Mol Cell Endocrinol,* 299**,** 219-31.
RONSON, A., TVITO, A. & ROWE, J. M. 2016. Treatment of Relapsed/Refractory Acute Lymphoblastic Leukemia in Adults. *Curr Oncol Rep,* 18**,** 39.
ROOHK, D. J., VARADY, K. A., TURNER, S. M., EMSON, C. L., GELLING, R. W., SHANKARAN, M., LINDWALL, G., SHIPP, L. E., SCANLAN, T. S., WANG, J. C. & HELLERSTEIN, M. K. 2010. Differential in vivo effects on target pathways of a novel arylpyrazole glucocorticoid receptor modulator compared with prednisolone. *J Pharmacol Exp Ther,* 333**,** 281-9.
SAENZ, G. J., HOVANESSIAN, R., GISIS, A. D. & MEDH, R. D. 2015. Glucocorticoid-mediated co-regulation of RCAN1-1, E4BP4 and BIM in human leukemia cells susceptible to apoptosis. *Biochem Biophys Res Commun,* 463**,** 1291-6.
SAITO, S., TSUKAGUCHI, N., HASEGAWA, T., MICHIMATA, T., TSUDA, H. & NARITA, N. 1999. Distribution of Th1, Th2, and Th0 and the Th1/Th2 cell ratios in human peripheral and endometrial T cells. *Am J Reprod Immunol,* 42**,** 240-5.
SAKSIDA, T., VUJICIC, M., NIKOLIC, I., STOJANOVIC, I., HAEGEMAN, G. & STOSIC-GRUJICIC, S. 2014. Compound A, a selective glucocorticoid receptor agonist, inhibits immunoinflammatory diabetes, induced by multiple low doses of streptozotocin in mice. *Br J Pharmacol,* 171**,** 5898-909.
SALMON, S. E., CROWLEY, J. J., GROGAN, T. M., FINLEY, P., PUGH, R. P. & BARLOGIE, B. 1994. Combination chemotherapy, glucocorticoids, and interferon alfa in the treatment of multiple myeloma: a Southwest Oncology Group study. *J Clin Oncol,* 12**,** 2405-14.
SALVADOR, C., MEISTER, B., CRAZZOLARA, R. & KROPSHOFER, G. 2012. Management of hypertriglyceridemia in children with acute lymphoblastic leukemia under persistent therapy with glucocorticoids and L-asparaginase during induction chemotherapy. *Pediatr Blood Cancer,* 59**,** 771.
SANCHEZ-LARA, K., HERNANDEZ, D., MOTOLA, D. & GREEN, D. 2013. [Association between overweight, glucocorticoids and metabolic syndrome in cancer patients under chemotherapy]. *Nutr Hosp,* 28**,** 182-7.
SANNER, B. M., MEDER, U., ZIDEK, W. & TEPEL, M. 2002. Effects of glucocorticoids on generation of reactive oxygen species in platelets. *Steroids,* 67**,** 715-9.

SARALAMMA, V. V., NAGAPPAN, A., HONG, G. E., LEE, H. J., YUMNAM, S., RAHA, S., HEO, J. D., LEE, S. J., LEE, W. S., KIM, E. H. & KIM, G. S. 2015. Poncirin Induces Apoptosis in AGS Human Gastric Cancer Cells through Extrinsic Apoptotic Pathway by up-Regulation of Fas Ligand. *Int J Mol Sci,* 16**,** 22676-91.
SASSE, S. K., MAILLOUX, C. M., BARCZAK, A. J., WANG, Q., ALTONSY, M. O., JAIN, M. K., HALDAR, S. M. & GERBER, A. N. 2013. The glucocorticoid receptor and KLF15 regulate gene expression dynamics and integrate signals through feed-forward circuitry. *Mol Cell Biol,* 33**,** 2104-15.
SATO, S., SHIRAKAWA, H., TOMITA, S., TOHKIN, M., GONZALEZ, F. J. & KOMAI, M. 2013. The aryl hydrocarbon receptor and glucocorticoid receptor interact to activate human metallothionein 2A. *Toxicol Appl Pharmacol,* 273**,** 90-9.
SAUERWALD, A. & RATH, W. 2000. [Lung maturation therapy with glucocorticoids in threatened premature labor. Considerations of risk-benefit in evidence-based medicine]. *Z Geburtshilfe Neonatol,* 204**,** 203-9.
SAUNIER, E., ANTONIO, S., REGAZZETTI, A., AUZEIL, N., LAPREVOTE, O., SHAY, J. W., COUMOUL, X., BAROUKI, R., BENELLI, C., HUC, L. & BORTOLI, S. 2017. Resveratrol reverses the Warburg effect by targeting the pyruvate dehydrogenase complex in colon cancer cells. *Sci Rep,* 7**,** 6945.
SAVINO, A. M. & IZRAELI, S. 2017. Interleukin-7 signaling as a therapeutic target in acute lymphoblastic leukemia. *Expert Rev Hematol*.
SCHACHE, A. G., POWELL, N. G., CUSCHIERI, K. S., ROBINSON, M., LEARY, S., MEHANNA, H., RAPOZO, D., LONG, A., CUBIE, H., JUNOR, E., MONAGHAN, H., HARRINGTON, K. J., NUTTING, C. M., SCHICK, U., LAU, A. S., UPILE, N., SHEARD, J., BROUGHAM, K., WEST, C. M., OGUEJIOFOR, K., THOMAS, S., NESS, A. R., PRING, M., THOMAS, G. J., KING, E. V., MCCANCE, D. J., JAMES, J. A., MORAN, M., SLOAN, P., SHAW, R. J., EVANS, M. & JONES, T. M. 2016. HPV-Related Oropharynx Cancer in the United Kingdom: An Evolution in the Understanding of Disease Etiology. *Cancer Res,* 76**,** 6598-6606.
SCHACKE, H., SCHOTTELIUS, A., DOCKE, W. D., STREHLKE, P., JAROCH, S., SCHMEES, N., REHWINKEL, H., HENNEKES, H. & ASADULLAH, K. 2004. Dissociation of transactivation from transrepression by a selective glucocorticoid receptor agonist leads to separation of therapeutic effects from side effects. *Proc Natl Acad Sci U S A,* 101**,** 227-32.
SCHACKE, H., ZOLLNER, T. M., DOCKE, W. D., REHWINKEL, H., JAROCH, S., SKUBALLA, W., NEUHAUS, R., MAY, E., ZUGEL, U. & ASADULLAH, K. 2009. Characterization of ZK 245186, a novel, selective glucocorticoid receptor agonist for the topical treatment of inflammatory skin diseases. *Br J Pharmacol,* 158**,** 1088-103.
SCHLOSSMACHER, G., PLATT, E., DAVIES, A., MEREDITH, S. & WHITE, A. 2013. Glucocorticoid receptor-mediated apoptosis in small-cell lung cancer requires interaction with BCL2. *Endocr Relat Cancer,* 20**,** 785-95.
SCHLOSSMACHER, G., STEVENS, A. & WHITE, A. 2011. Glucocorticoid receptor-mediated apoptosis: mechanisms of resistance in cancer cells. *J Endocrinol,* 211**,** 17-25.
SCHMIDT, M., PAUELS, H. G., LUGERING, N., LUGERING, A., DOMSCHKE, W. & KUCHARZIK, T. 1999. Glucocorticoids induce apoptosis in human monocytes: potential role of IL-1 beta. *J Immunol,* 163**,** 3484-90.
SCHWARTZ, L., SUPURAN, C. T. & ALFAROUK, K. O. 2017. The Warburg Effect and the Hallmarks of Cancer. *Anticancer Agents Med Chem,* 17**,** 164-170.
SEITZ, S. J., SCHLEITHOFF, E. S., KOCH, A., SCHUSTER, A., TEUFEL, A., STAIB, F., STREMMEL, W., MELINO, G., KRAMMER, P. H., SCHILLING, T. & MULLER, M. 2010.

Chemotherapy-induced apoptosis in hepatocellular carcinoma involves the p53 family and is mediated via the extrinsic and the intrinsic pathway. *Int J Cancer,* 126**,** 2049-66.
SEJIAN, V. & SRIVASTAVA, R. S. 2010. Interrelationship of endocrine glands under thermal stress: effect of exogenous glucocorticoids on mineral, enzyme, thyroid hormone profiles and phagocytosis index of Indian goats. *Endocr Regul,* 44**,** 101-7.
SERGEANT, C. A., AFRICANDER, D., SWART, P. & SWART, A. C. 2017. Sutherlandia frutescens modulates adrenal hormone biosynthesis, acts as a selective glucocorticoid receptor agonist (SEGRA) and displays anti-mineralocorticoid properties. *J Ethnopharmacol,* 202**,** 290-301.
SERRA, H. A., ROGANOVICH, J. M. & RIZZO, L. F. 2012. [Glucocorticoids: examples of translational medicine; from molecular aspects to bedside]. *Medicina (B Aires),* 72**,** 158-70.
SERVICE, N. H. 2017. Acute lymphoblastic leukaemia
SEVILLA, L. M., LATORRE, V., CARCELLER, E., BOIX, J., VODAK, D., MILLS, I. G. & PEREZ, P. 2015. Glucocorticoid receptor and Klf4 co-regulate anti-inflammatory genes in keratinocytes. *Mol Cell Endocrinol,* 412**,** 281-9.
SHAASHUA, L., ROSENNE, E., NEEMAN, E., SORSKI, L., SOMINSKY, L., MATZNER, P., PAGE, G. G. & BEN-ELIYAHU, S. 2014. Plasma IL-12 levels are suppressed in vivo by stress and surgery through endogenous release of glucocorticoids and prostaglandins but not catecholamines or opioids. *Psychoneuroendocrinology,* 42**,** 11-23.
SHAFIEE, A., BUCOLO, C., BUDZYNSKI, E., WARD, K. W. & LOPEZ, F. J. 2011. In vivo ocular efficacy profile of mapracorat, a novel selective glucocorticoid receptor agonist, in rabbit models of ocular disease. *Invest Ophthalmol Vis Sci,* 52**,** 1422-30.
SHAHBAZI, R., ASIK, E., KAHRAMAN, N., TURK, M., OZPOLAT, B. & ULUBAYRAM, K. 2017. Modified gold-based siRNA nanotherapeutics for targeted therapy of triple-negative breast cancer. *Nanomedicine (Lond)*.
SHERMAN, M. R., CORVOL, P. L. & O'MALLEY, B. W. 1970. Progesterone-binding components of chick oviduct. I. Preliminary characterization of cytoplasmic components. *J Biol Chem,* 245**,** 6085-96.
SHIKAMA, N., CHAN, H. M., KRSTIC-DEMONACOS, M., SMITH, L., LEE, C. W., CAIRNS, W. & LA THANGUE, N. B. 2000. Functional interaction between nucleosome assembly proteins and p300/CREB-binding protein family coactivators. *Mol Cell Biol,* 20**,** 8933-43.
SHIMIZU, T., CHIBA, T. & MARUSAWA, H. 2017. Helicobacter pylori-Mediated Genetic Instability and Gastric Carcinogenesis. *Curr Top Microbiol Immunol,* 400**,** 305-323.
SHOJI, S., SUZUKI, A., CONRADO, D. J., PETERSON, M. C., HEY-HADAVI, J., MCCABE, D., ROJO, R. & TAMMARA, B. K. 2017. Dissociated Agonist of Glucocorticoid Receptor or Prednisone for Active Rheumatoid Arthritis: Effects on P1NP and Osteocalcin Pharmacodynamics. *CPT Pharmacometrics Syst Pharmacol*.
SIEGEL, C. & MCCULLOUGH, L. D. 2011. NAD+ depletion or PAR polymer formation: which plays the role of executioner in ischaemic cell death? *Acta Physiol (Oxf),* 203**,** 225-34.
SIEGEL, R. L., MILLER, K. D. & JEMAL, A. 2017. Cancer Statistics, 2017. *CA Cancer J Clin,* 67**,** 7-30.
SIMIC, I., ADZIC, M., MARIC, N., SAVIC, D., DJORDJEVIC, J., MIHALJEVIC, M., MITIC, M., PAVLOVIC, Z., SOLDATOVIC, I., KRSTIC-DEMONACOS, M., JASOVIC-GASIC, M. & RADOJCIC, M. 2013. A preliminary evaluation of leukocyte phospho-glucocorticoid

receptor as a potential biomarker of depressogenic vulnerability in healthy adults. *Psychiatry Res,* 209**,** 658-64.
SINGH, J. A., HOSSAIN, A., KOTB, A. & WELLS, G. 2016. Risk of serious infections with immunosuppressive drugs and glucocorticoids for lupus nephritis: a systematic review and network meta-analysis. *BMC Med,* 14**,** 137.
SKENDROS, P., SARANTOPOULOS, A., TSELIOS, K. & BOURA, P. 2008. Chronic brucellosis patients retain low frequency of CD4+ T-lymphocytes expressing CD25 and CD28 after Escherichia coli LPS stimulation of PHA-cultured PBMCs. *Clin Dev Immunol,* 2008**,** 327346.
SKUPIO, U., TERTIL, M., SIKORA, M., GOLDA, S., WAWRZCZAK-BARGIELA, A. & PRZEWLOCKI, R. 2015. Behavioral and molecular alterations in mice resulting from chronic treatment with dexamethasone: relevance to depression. *Neuroscience,* 286**,** 141-50.
SLEURS, C., DEPREZ, S., EMSELL, L., LEMIERE, J. & UYTTEBROECK, A. 2016. Chemotherapy-induced neurotoxicity in pediatric solid non-CNS tumor patients: An update on current state of research and recommended future directions. *Crit Rev Oncol Hematol,* 103**,** 37-48.
SLIWOSKI, G., KOTHIWALE, S., MEILER, J. & LOWE, E. W., JR. 2014. Computational methods in drug discovery. *Pharmacol Rev,* 66**,** 334-95.
SLUYSER, M. 2005. *Application of apoptosis to cancer treatment,* Dordrecht, Springer.
SOCIETY, A. C. 2017. Cancer Facts and Statistics. USA.
SOLANO, M. E., HOLMES, M. C., MITTELSTADT, P. R., CHAPMAN, K. E. & TOLOSA, E. 2016. Antenatal endogenous and exogenous glucocorticoids and their impact on immune ontogeny and long-term immunity. *Semin Immunopathol,* 38**,** 739-763.
SOMAL, A., AGGARWAL, A. & UPADHYAY, R. C. 2015. Effect of thermal stress on expression profile of apoptosis related genes in peripheral blood mononuclear cells of transition Sahiwal cow. *Iran J Vet Res,* 16**,** 137-43.
SOMMER, T., HUBNER, H., EL KERDAWY, A., GMEINER, P., PISCHETSRIEDER, M. & CLARK, T. 2017. Identification of the Beer Component Hordenine as Food-Derived Dopamine D2 Receptor Agonist by Virtual Screening a 3D Compound Database. *Sci Rep,* 7**,** 44201.
SONDERGAARD, J. N., POGHOSYAN, S., HONTELEZ, S., LOUCHE, P., LOOMAN, M. W., ANSEMS, M. & ADEMA, G. J. 2015. DC-SCRIPT Regulates IL-10 Production in Human Dendritic Cells by Modulating NF-kappaBp65 Activation. *J Immunol,* 195**,** 1498-505.
SOTELO-RIVERA, I., COTE-VELEZ, A., URIBE, R. M., CHARLI, J. L. & JOSEPH-BRAVO, P. 2017. Glucocorticoids curtail stimuli-induced CREB phosphorylation in TRH neurons through interaction of the glucocorticoid receptor with the catalytic subunit of protein kinase A. *Endocrine*.
SPEKSNIJDER, N., CHRISTENSEN, K. V., DIDRIKSEN, M., DE KLOET, E. R. & DATSON, N. A. 2012. Glucocorticoid receptor and myocyte enhancer factor 2 cooperate to regulate the expression of c-JUN in a neuronal context. *J Mol Neurosci,* 48**,** 209-18.
SPIESS, H. 1960. [The effect of glucocorticoids on the course of experimental tuberculosis under the influence of chemotherapy]. *Monatsschr Kinderheilkd,* 108**,** 203-4.
SPINELLI, S. L., XI, X., MCMILLAN, D. H., WOELLER, C. F., RICHARDSON, M. E., CAVET, M. E., ZHANG, J. Z., FELDON, S. E. & PHIPPS, R. P. 2014. Mapracorat, a selective glucocorticoid receptor agonist, upregulates RelB, an anti-inflammatory nuclear factor-kappaB protein, in human ocular cells. *Exp Eye Res,* 127**,** 290-8.

STAMER, W. D., HOFFMAN, E. A., KURALI, E. & KRAUSS, A. H. 2013. Unique response profile of trabecular meshwork cells to the novel selective glucocorticoid receptor agonist, GW870086X. *Invest Ophthalmol Vis Sci,* 54**,** 2100-7.
STENSJOEN, A. L., SOLHEIM, O., KVISTAD, K. A., HABERG, A. K., SALVESEN, O. & BERNTSEN, E. M. 2015. Growth dynamics of untreated glioblastomas in vivo. *Neuro Oncol,* 17**,** 1402-11.
STEVENS, A., GARSIDE, H., BERRY, A., WATERS, C., WHITE, A. & RAY, D. 2003. Dissociation of steroid receptor coactivator 1 and nuclear receptor corepressor recruitment to the human glucocorticoid receptor by modification of the ligand-receptor interface: the role of tyrosine 735. *Mol Endocrinol,* 17**,** 845-59.
STOHS, S. J. & HARTMAN, M. J. 2015. A review of the receptor binding and pharmacological effects of N-methyltyramine. *Phytother Res,* 29**,** 14-6.
STYCZYNSKI, J., WYSOCKI, M., DEBSKI, R., BALWIERZ, W., ROKICKA-MILEWSKA, R., MATYSIAK, M., BALCERSKA, A., KOWALCZYK, J., WACHOWIAK, J., SONTA-JAKIMCZYK, D. & CHYBICKA, A. 2002. Cross-resistance to five glucocorticoids in childhood acute lymphoblastic and non-lymphoblastic leukemia samples tested by the MTT assay: preliminary report. *Acta Biochim Pol,* 49**,** 93-8.
SUNDAHL, N., BRIDELANCE, J., LIBERT, C., DE BOSSCHER, K. & BECK, I. M. 2015. Selective glucocorticoid receptor modulation: New directions with non-steroidal scaffolds. *Pharmacol Ther,* 152**,** 28-41.
SUTTITHEPTUMRONG, A., KHUNCHAI, S., PANAAMPON, J., YASAMUT, U., MORCHANG, A., PUTTIKHUNT, C., NOISAKRAN, S., HAEGEMAN, G., YENCHITSOMANUS, P. T. & LIMJINDAPORN, T. 2013. Compound A, a dissociated glucocorticoid receptor modulator, reduces dengue virus-induced cytokine secretion and dengue virus production. *Biochem Biophys Res Commun,* 436**,** 283-8.
SZABO, M. Z. & KISS, E. 2017. [Recent advances in the treatment of large vessel vasculitides]. *Orv Hetil,* 158**,** 5-12.
SZAPARY, D., HUANG, Y. & SIMONS, S. S., JR. 1999. Opposing effects of corepressor and coactivators in determining the dose-response curve of agonists, and residual agonist activity of antagonists, for glucocorticoid receptor-regulated gene expression. *Mol Endocrinol,* 13**,** 2108-21.
TACEY, A., PARKER, L., GARNHAM, A., BRENNAN-SPERANZA, T. C. & LEVINGER, I. 2017. The effect of acute and short term glucocorticoid administration on exercise capacity and metabolism. *J Sci Med Sport*.
TACK, L. J., TATSI, C., STRATAKIS, C. A. & LODISH, M. B. 2016a. Effects of Glucocorticoids on Bone: What we can Learn from Pediatric Endogenous Cushing's Syndrome. *Horm Metab Res*.
TACK, L. J., TATSI, C., STRATAKIS, C. A. & LODISH, M. B. 2016b. Effects of Glucocorticoids on Bone: What we can Learn from Pediatric Endogenous Cushing's Syndrome. *Horm Metab Res,* 48**,** 764-770.
TAM, C. S. & RAVUSSIN, E. 2012. Energy balance: an overview with emphasis on children. *Pediatr Blood Cancer,* 58**,** 154-8.
TAMEZ-PEREZ, H. E., QUINTANILLA-FLORES, D. L., RODRIGUEZ-GUTIERREZ, R., GONZALEZ-GONZALEZ, J. G. & TAMEZ-PENA, A. L. 2015. Steroid hyperglycemia: Prevalence, early detection and therapeutic recommendations: A narrative review. *World J Diabetes,* 6**,** 1073-81.
TANDAN, R., TAYLOR, R., DICOSTANZO, D. P., SHARMA, K., FRIES, T. & ROBERTS, J. 1990. Metastasizing thymoma and myasthenia gravis. Favorable response to

glucocorticoids after failed chemotherapy and radiation therapy. *Cancer,* 65**,** 1286-90.
TAO, Y., GAO, L., WU, X., WANG, H., YANG, G., ZHAN, F. & SHI, J. 2013. Down-regulation of 11beta-hydroxysteroid dehydrogenase type 2 by bortezomib sensitizes Jurkat leukemia T cells against glucocorticoid-induced apoptosis. *PLoS One,* 8**,** e67067.
TATAR, A. S., NAGY-SIMON, T., TOMULEASA, C., BOCA, S. & ASTILEAN, S. 2016. Nanomedicine approaches in acute lymphoblastic leukemia. *J Control Release,* 238**,** 123-38.
TAYLOR, R. C., CULLEN, S. P. & MARTIN, S. J. 2008. Apoptosis: controlled demolition at the cellular level. *Nat Rev Mol Cell Biol,* 9**,** 231-41.
TENTILLIER, N., ETZERODT, A., OLESEN, M. N., RIZALAR, F. S., JACOBSEN, J., BENDER, D., MOESTRUP, S. K. & ROMERO-RAMOS, M. 2016. Anti-Inflammatory Modulation of Microglia via CD163-Targeted Glucocorticoids Protects Dopaminergic Neurons in the 6-OHDA Parkinson's Disease Model. *J Neurosci,* 36**,** 9375-90.
TEPPO, H. R., SOINI, Y. & KARIHTALA, P. 2017. Reactive Oxygen Species-Mediated Mechanisms of Action of Targeted Cancer Therapy. *Oxid Med Cell Longev,* 2017**,** 1485283.
THOMPSON, C. F., QURAISHI, N., ALI, A., TATA, J. R., HAMMOND, M. L., BALKOVEC, J. M., EINSTEIN, M., GE, L., HARRIS, G., KELLY, T. M., MAZUR, P., PANDIT, S., SANTORO, J., SITLANI, A., WANG, C., WILLIAMSON, J., MILLER, D. K., YAMIN, T. T., THOMPSON, C. M., O'NEILL, E. A., ZALLER, D., FORREST, M. J., CARBALLO-JANE, E. & LUELL, S. 2005. Novel heterocyclic glucocorticoids: in vitro profile and in vivo efficacy. *Bioorg Med Chem Lett,* 15**,** 2163-7.
THOMPSON, E. B., MEDH, R. D., ZHOU, F., AYALA-TORRES, S., ANSARI, N., ZHANG, W. & JOHNSON, B. H. 1999. Glucocorticoids, oxysterols, and cAMP with glucocorticoids each cause apoptosis of CEM cells and suppress c-myc. *J Steroid Biochem Mol Biol,* 69**,** 453-61.
THOTA, S. & ADVANI, A. 2017. Inotuzumab ozogamicin in relapsed b-cell acute lymphoblastic leukemia. *Eur J Haematol*.
TIAN, S., POUKKA, H., PALVIMO, J. J. & JANNE, O. A. 2002. Small ubiquitin-related modifier-1 (SUMO-1) modification of the glucocorticoid receptor. *Biochem J,* 367**,** 907-11.
TOTOMI, N., MAKINO, K., INOUE, S. & KONDO, T. 1995. Permeability of lipid-coated polymer capsule membranes to solutes: effects of temperature and chemical structure of solutes. *J Microencapsul,* 12**,** 263-71.
TREHANPATI, N. & VYAS, A. K. 2017. Immune regulation by T regulatory cells in HBV related Inflammation and cancer. *Scand J Immunol*.
TREVOR, J. L. & DESHANE, J. S. 2014. Refractory asthma: mechanisms, targets, and therapy. *Allergy,* 69**,** 817-27.
TSAI, Y. F., HUANG, C. W., CHIANG, J. H., TSAI, F. J., HSU, Y. M., LU, C. C., HSIAO, C. Y. & YANG, J. S. 2016. Gadolinium chloride elicits apoptosis in human osteosarcoma U-2 OS cells through extrinsic signaling, intrinsic pathway and endoplasmic reticulum stress. *Oncol Rep,* 36**,** 3421-3426.
TSUJIMOTO, Y. 1998. Role of Bcl-2 family proteins in apoptosis: apoptosomes or mitochondria? *Genes Cells,* 3**,** 697-707.
TSUTSUMI, E., KANAI, S., OHTA, M., SUWA, Y. & MIYASAKA, K. 2010. Stimulatory effect of N-methyltyramine, a congener of beer, on pancreatic secretion in conscious rats. *Alcohol Clin Exp Res,* 34 Suppl 1**,** S14-7.
VAN DEN HEUVEL, J. K., BOON, M. R., VAN HENGEL, I., PESCHIER-VAN DER PUT, E., VAN BEEK, L., VAN HARMELEN, V., VAN DIJK, K. W., PEREIRA, A. M., HUNT, H., BELANOFF,

J. K., RENSEN, P. C. & MEIJER, O. C. 2016. Identification of a selective glucocorticoid receptor modulator that prevents both diet-induced obesity and inflammation. *Br J Pharmacol,* 173**,** 1793-804.
VAN DER GEEST, R., OUWENEEL, A. B., VAN DER SLUIS, R. J., GROEN, A. K., VAN ECK, M. & HOEKSTRA, M. 2016. Endogenous glucocorticoids exacerbate cholestasis-associated liver injury and hypercholesterolemia in mice. *Toxicol Appl Pharmacol,* 306**,** 1-7.
VAN LIEROP, M. J., ALKEMA, W., LASKEWITZ, A. J., DIJKEMA, R., VAN DER MAADEN, H. M., SMIT, M. J., PLATE, R., CONTI, P. G., JANS, C. G., TIMMERS, C. M., VAN BOECKEL, C. A., LUSHER, S. J., MCGUIRE, R., VAN SCHAIK, R. C., DE VLIEG, J., SMEETS, R. L., HOFSTRA, C. L., BOOTS, A. M., VAN DUIN, M., INGELSE, B. A., SCHOONEN, W. G., GREFHORST, A., VAN DIJK, T. H., KUIPERS, F. & DOKTER, W. H. 2012. Org 214007-0: a novel non-steroidal selective glucocorticoid receptor modulator with full anti-inflammatory properties and improved therapeutic index. *PLoS One,* 7**,** e48385.
VAN LOO, G., SZE, M., BOUGARNE, N., PRAET, J., MCGUIRE, C., ULLRICH, A., HAEGEMAN, G., PRINZ, M., BEYAERT, R. & DE BOSSCHER, K. 2009. Antiinflammatory Properties of a Plant-Derived Nonsteroidal, Dissociated Glucocorticoid Receptor Modulator in Experimental Autoimmune Encephalomyelitis. *Endocr Rev,* 30**,** 931.
VANDEN BERGHE, W., DE BOSSCHER, K., VERMEULEN, L., DE WILDE, G. & HAEGEMAN, G. 2002. Induction and repression of NF-kappa B-driven inflammatory genes. *Ernst Schering Res Found Workshop***,** 233-78.
VANDEN BERGHE, W., PLAISANCE, S., BOONE, E., DE BOSSCHER, K., SCHMITZ, M. L., FIERS, W. & HAEGEMAN, G. 1998. p38 and extracellular signal-regulated kinase mitogen-activated protein kinase pathways are required for nuclear factor-kappaB p65 transactivation mediated by tumor necrosis factor. *J Biol Chem,* 273**,** 3285-90.
VANDEVYVER, S., DEJAGER, L. & LIBERT, C. 2014. Comprehensive overview of the structure and regulation of the glucocorticoid receptor. *Endocr Rev,* 35**,** 671-93.
VANDYKE, D., KYRIACOPULOS, P., YASSINI, B., WRIGHT, A., BURKHART, E., JACEK, S., PRATT, M., PETERSON, C. R. & RAI, P. 2016. Nanoparticle Based Combination Treatments for Targeting Multiple Hallmarks of Cancer. *Int J Nano Stud Technol,* Suppl 4**,** 1-18.
VAYSSIERE, B. M., DUPONT, S., CHOQUART, A., PETIT, F., GARCIA, T., MARCHANDEAU, C., GRONEMEYER, H. & RESCHE-RIGON, M. 1997. Synthetic glucocorticoids that dissociate transactivation and AP-1 transrepression exhibit antiinflammatory activity in vivo. *Mol Endocrinol,* 11**,** 1245-55.
VECHT, C. J. 1998. Steroid myopathy in cancer patients. *Neurology,* 51**,** 918.
VEGLIA, F. & GABRILOVICH, D. I. 2017. Dendritic cells in cancer: the role revisited. *Curr Opin Immunol,* 45**,** 43-51.
VENERIS, J. T., DARCY, K. M., MHAWECH-FAUCEGLIA, P., TIAN, C., LENGYEL, E., LASTRA, R. R., PEJOVIC, T., CONZEN, S. D. & FLEMING, G. F. 2017. High glucocorticoid receptor expression predicts short progression-free survival in ovarian cancer. *Gynecol Oncol,* 146**,** 153-160.
VICENNATI, V., VOTTERO, A., FRIEDMAN, C. & PAPANICOLAOU, D. A. 2002. Hormonal regulation of interleukin-6 production in human adipocytes. *Int J Obes Relat Metab Disord,* 26**,** 905-11.
VILAR, S., SOBARZO-SANCHEZ, E., SANTANA, L. & URIARTE, E. 2017. Molecular Docking and Drug Discovery in beta-Adrenergic Receptors. *Curr Med Chem*.
VILLALBA, M., EVANS, S. R., VIDAL-VANACLOCHA, F. & CALVO, A. 2017. Role of TGF-beta in metastatic colon cancer: it is finally time for targeted therapy. *Cell Tissue Res*.
VILLANUEVA-LOZANO, H., TREVINO-RANGEL, R. J., RENPENNING-CARRASCO, E. W. & GONZALEZ, G. M. 2017. Successful treatment of Talaromyces amestolkiae

pulmonary infection with voriconazole in an acute lymphoblastic leukemia patient. *J Infect Chemother*.
VINAY, D. S., RYAN, E. P., PAWELEC, G., TALIB, W. H., STAGG, J., ELKORD, E., LICHTOR, T., DECKER, W. K., WHELAN, R. L., KUMARA, H. M., SIGNORI, E., HONOKI, K., GEORGAKILAS, A. G., AMIN, A., HELFERICH, W. G., BOOSANI, C. S., GUHA, G., CIRIOLO, M. R., CHEN, S., MOHAMMED, S. I., AZMI, A. S., KEITH, W. N., BILSLAND, A., BHAKTA, D., HALICKA, D., FUJII, H., AQUILANO, K., ASHRAF, S. S., NOWSHEEN, S., YANG, X., CHOI, B. K. & KWON, B. S. 2015. Immune evasion in cancer: Mechanistic basis and therapeutic strategies. *Semin Cancer Biol,* 35 Suppl**,** S185-98.
VIRIK, K., KARAPETIS, C., DROUFAKOU, S. & HARPER, P. 2001. Avascular necrosis of bone: the hidden risk of glucocorticoids used as antiemetics in cancer chemotherapy. *Int J Clin Pract,* 55**,** 344-5.
VISSER, J., VAN BOXEL-DEZAIRE, A., METHORST, D., BRUNT, T., DE KLOET, E. R. & NAGELKERKEN, L. 1998. Differential regulation of interleukin-10 (IL-10) and IL-12 by glucocorticoids in vitro. *Blood,* 91**,** 4255-64.
VUKOJEVIC, V., KOLASSA, I. T., FASTENRATH, M., GSCHWIND, L., SPALEK, K., MILNIK, A., HECK, A., VOGLER, C., WILKER, S., DEMOUGIN, P., PETER, F., ATUCHA, E., STETAK, A., ROOZENDAAL, B., ELBERT, T., PAPASSOTIROPOULOS, A. & DE QUERVAIN, D. J. 2014. Epigenetic modification of the glucocorticoid receptor gene is linked to traumatic memory and post-traumatic stress disorder risk in genocide survivors. *J Neurosci,* 34**,** 10274-84.
VUNDAMATI, D. & BOSTROM, B. 2016. Prolonged Response in Patient With Multiply Relapsed B-cell Acute Lymphoblastic Leukemia and Monosomy-7 to Bortezomib, Lenalidomide, and Dexamethasone. *J Pediatr Hematol Oncol,* 38**,** 467-9.
WADDELL, D. S., BAEHR, L. M., VAN DEN BRANDT, J., JOHNSEN, S. A., REICHARDT, H. M., FURLOW, J. D. & BODINE, S. C. 2008. The glucocorticoid receptor and FOXO1 synergistically activate the skeletal muscle atrophy-associated MuRF1 gene. *Am J Physiol Endocrinol Metab,* 295**,** E785-97.
WANG, J., DAVIS, S., ZHU, M., MILLER, E. A. & FERRO-NOVICK, S. 2017a. Autophagosome formation: Where the secretory and autophagy pathways meet. *Autophagy***,** 1-2.
WANG, J., LU, J., BOND, M. C., CHEN, M., REN, X. R., LYERLY, H. K., BARAK, L. S. & CHEN, W. 2010. Identification of select glucocorticoids as Smoothened agonists: potential utility for regenerative medicine. *Proc Natl Acad Sci U S A,* 107**,** 9323-8.
WANG, L., LUO, P., ZHANG, F., ZHANG, Y., WANG, X., CHANG, F., ZHANG, Y., TANG, H. & XIA, Z. 2016a. Toll-like receptor 4 protects against stress-induced ulcers via regulation of glucocorticoid production in mice. *Stress***,** 1-8.
WANG, L. D. & WAGERS, A. J. 2011. Dynamic niches in the origination and differentiation of haematopoietic stem cells. *Nat Rev Mol Cell Biol,* 12**,** 643-55.
WANG, Q., ALSHAKER, H., BOHLER, T., SRIVATS, S., CHAO, Y., COOPER, C. & PCHEJETSKI, D. 2017b. Core shell lipid-polymer hybrid nanoparticles with combined docetaxel and molecular targeted therapy for the treatment of metastatic prostate cancer. *Sci Rep,* 7**,** 5901.
WANG, X. & DEFRANCO, D. B. 2005. Alternative effects of the ubiquitin-proteasome pathway on glucocorticoid receptor down-regulation and transactivation are mediated by CHIP, an E3 ligase. *Mol Endocrinol,* 19**,** 1474-82.
WANG, Z., LI, Z., YE, Y., XIE, L. & LI, W. 2016b. Oxidative Stress and Liver Cancer: Etiology and Therapeutic Targets. *Oxid Med Cell Longev,* 2016**,** 7891574.

WANG, Z., MALONE, M. H., HE, H., MCCOLL, K. S. & DISTELHORST, C. W. 2003. Microarray analysis uncovers the induction of the proapoptotic BH3-only protein Bim in multiple models of glucocorticoid-induced apoptosis. *J Biol Chem,* 278**,** 23861-7.
WARE, W. R. 2017. Natural Cancer Therapy and Prevention Targeted on Cancer Cells and Cancer Stem Cells Based on the Cytochrome P45O Enzyme CYP1B1: A Commentary. *Altern Ther Health Med*.
WARRIS, L. T., VAN DEN AKKER, E. L., BIERINGS, M. B., VAN DEN BOS, C., ZWAAN, C. M., SASSEN, S. D., TISSING, W. J., VEENING, M. A., PIETERS, R. & VAN DEN HEUVEL-EIBRINK, M. M. 2016. Acute Activation of Metabolic Syndrome Components in Pediatric Acute Lymphoblastic Leukemia Patients Treated with Dexamethasone. *PLoS One,* 11**,** e0158225.
WASIM, M., MANSHA, M., KOFLER, A., AWAN, A. R., BABAR, M. E. & KOFLER, R. 2012. Promyelocytic leukemia zinc finger protein (PLZF) enhances glucocorticoid-induced apoptosis in leukemic cell line NALM6. *Pak J Pharm Sci,* 25**,** 617-21.
WATSON, D. G., LIN, M., MORTON, A., CABLE, C. G. & MCARTHUR, D. A. 2005. Compatibility and stability of dexamethasone sodium phosphate and ketamine hydrochloride subcutaneous infusions in polypropylene syringes. *J Pain Symptom Manage,* 30**,** 80-6.
WCRF, W. C. R. F. 2017.
WEIKUM, E. R., DE VERA, I. M. S., NWACHUKWU, J. C., HUDSON, W. H., NETTLES, K. W., KOJETIN, D. J. & ORTLUND, E. A. 2017a. Tethering not required: the glucocorticoid receptor binds directly to activator protein-1 recognition motifs to repress inflammatory genes. *Nucleic Acids Res*.
WEIKUM, E. R., KNUESEL, M. T., ORTLUND, E. A. & YAMAMOTO, K. R. 2017b. Glucocorticoid receptor control of transcription: precision and plasticity via allostery. *Nat Rev Mol Cell Biol*.
WEINSTEIN, R. S., NICHOLAS, R. W. & MANOLAGAS, S. C. 2000. Apoptosis of osteocytes in glucocorticoid-induced osteonecrosis of the hip. *J Clin Endocrinol Metab,* 85**,** 2907-12.
WELLS, E., KAMBHAMPATI, M., DAMSKER, J. M., GORDISH-DRESSMAN, H., YADAVILLI, S., BECHER, O. J., GITTENS, J., STAMPAR, M., PACKER, R. J. & NAZARIAN, J. 2016. Vamorolone, a dissociative steroidal compound, reduces pro-inflammatory cytokine expression in glioma cells and increases activity and survival in a murine model of cortical tumor. *Oncotarget*.
WILLIAMS, E. L., STIMPSON, M. L., COLLINS, P. L., ENKI, D. G., SINHA, A., LEE, R. W. & DHANDA, A. D. 2016. Development and validation of a novel bioassay to determine glucocorticoid sensitivity. *Biomark Res,* 4**,** 26.
WINDING, P. & BERCHTOLD, M. W. 2001. The chicken B cell line DT40: a novel tool for gene disruption experiments. *J Immunol Methods,* 249**,** 1-16.
WITKOWSKI, M. T., HU, Y., ROBERTS, K. G., BOER, J. M., MCKENZIE, M. D., LIU, G. J., LE GRICE, O. D., TREMBLAY, C. S., GHISI, M., WILLSON, T. A., HORSTMANN, M. A., AIFANTIS, I., CIMMINO, L., FRIETZE, S., DEN BOER, M. L., MULLIGHAN, C. G., SMYTH, G. K. & DICKINS, R. A. 2017. Conserved IKAROS-regulated genes associated with B-progenitor acute lymphoblastic leukemia outcome. *J Exp Med*.
WONG, C. W., KOMM, B. & CHESKIS, B. J. 2001. Structure-function evaluation of ER alpha and beta interplay with SRC family coactivators. ER selective ligands. *Biochemistry,* 40**,** 6756-65.

WOO, S. M., KWON, S. C., KO, S. G. & CHO, S. G. 2017. Barley grass extract causes apoptosis of cancer cells by increasing intracellular reactive oxygen species production. *Biomed Rep,* 6**,** 681-685.

WU, G. J., CHEN, J. T., TSAI, H. C., CHEN, T. L., LIU, S. H. & CHEN, R. M. 2016. Protection of Dexmedetomidine Against Ischemia/Reperfusion-Induced Apoptotic Insults to Neuronal Cells Occurs via an Intrinsic Mitochondria-Dependent Pathway. *J Cell Biochem*.

WU, P., DAVIES, F. E., HORTON, C., JENNER, M. W., KRISHNAN, B., ALVARES, C. L., SASO, R., MCCORMACK, R., DINES, S., TRELEAVEN, J. G., POTTER, M. N., ETHELL, M. E. & MORGAN, G. J. 2006. The combination of cyclophosphomide, thalidomide and dexamethasone is an effective alternative to cyclophosphamide - vincristine - doxorubicin - methylprednisolone as induction chemotherapy prior to autologous transplantation for multiple myeloma: a case-matched analysis. *Leuk Lymphoma,* 47**,** 2335-8.

WUST, S., TISCHNER, D., JOHN, M., TUCKERMANN, J. P., MENZFELD, C., HANISCH, U. K., VAN DEN BRANDT, J., LUHDER, F. & REICHARDT, H. M. 2009. Therapeutic and adverse effects of a non-steroidal glucocorticoid receptor ligand in a mouse model of multiple sclerosis. *PLoS One,* 4**,** e8202.

WYLLIE, A. H., KERR, J. F. & CURRIE, A. R. 1980. Cell death: the significance of apoptosis. *Int Rev Cytol,* 68**,** 251-306.

XIA, R., CHE, B. & ZHANG, Z. 2007. [Simultaneous identification of fifteen glucocorticoids in Chinese traditional medicine preparations by liquid chromatography-mass spectrometry]. *Se Pu,* 25**,** 926-9.

XING, K., GU, B., ZHANG, P. & WU, X. 2015. Dexamethasone enhances programmed cell death 1 (PD-1) expression during T cell activation: an insight into the optimum application of glucocorticoids in anti-cancer therapy. *BMC Immunol,* 16**,** 39.

XING, L., LYU, J. Y., YANG, Y., CUI, P. F., GU, L. Q., QIAO, J. B., HE, Y. J., ZHANG, T. Q., SUN, M., LU, J. J., XU, X., LIU, Y. & JIANG, H. L. 2017. pH-Responsive de-PEGylated nanoparticles based on triphenylphosphine-quercetin self-assemblies for mitochondria-targeted cancer therapy. *Chem Commun (Camb)*.

XU, W., LIU, L. Z., LOIZIDOU, M., AHMED, M. & CHARLES, I. G. 2002. The role of nitric oxide in cancer. *Cell Res,* 12**,** 311-20.

XU, Y., ZHANG, T. & CHEN, M. 2009. Combining 3D-QSAR, docking, molecular dynamics and MM/PBSA methods to predict binding modes for nonsteroidal selective modulator to glucocorticoid receptor. *Bioorg Med Chem Lett,* 19**,** 393-6.

YAGUE, J. G., GARCIA-SEGURA, L. M. & AZCOITIA, I. 2009. Selective transcriptional regulation of aromatase gene by vitamin D, dexamethasone, and mifepristone in human glioma cells. *Endocrine,* 35**,** 252-61.

YAMASHITA, Y., TAMURA, S., OIWA, T., KOBATA, H., KURIYAMA, K., MUSHINO, T., MURATA, S., HOSOI, H., NISHIKAWA, A., HANAOKA, N. & SONOKI, T. 2017. Successful Intrathecal Chemotherapy Combined with Radiotherapy Followed by Pomalidomide and Low-Dose Dexamethasone Maintenance Therapy for a Primary Plasma Cell Leukemia Patient. *Hematol Rep,* 9**,** 6986.

YAN, Q. H., XU, D. G., SHEN, Y. F., YUAN, D. L., BAO, J. H., LI, H. B. & LV, Y. G. 2017. Observation of the effect of targeted therapy of 64-slice spiral CT combined with cryoablation for liver cancer. *World J Gastroenterol,* 23**,** 4080-4089.

YANG, C. S., CHEN, X. & TU, S. 2016. Etiology and Prevention of Esophageal Cancer. *Gastrointest Tumors,* 3**,** 3-16.

YANG, C. S., KO, S. R., CHO, B. G., SHIN, D. M., YUK, J. M., LI, S., KIM, J. M., EVANS, R. M., JUNG, J. S., SONG, D. K. & JO, E. K. 2008. The ginsenoside metabolite compound K, a novel agonist of glucocorticoid receptor, induces tolerance to endotoxin-induced lethal shock. *J Cell Mol Med,* 12**,** 1739-53.

YAOXIAN, W., HUI, Y., YUNYAN, Z., YANQIN, L., XIN, G. & XIAOKE, W. 2013. Emodin induces apoptosis of human cervical cancer hela cells via intrinsic mitochondrial and extrinsic death receptor pathway. *Cancer Cell Int,* 13**,** 71.

YEMELYANOV, A., CZWORNOG, J., GERA, L., JOSHI, S., CHATTERTON, R. T., JR. & BUDUNOVA, I. 2008. Novel steroid receptor phyto-modulator compound a inhibits growth and survival of prostate cancer cells. *Cancer Res,* 68**,** 4763-73.

YENDE, S. & THOMPSON, B. T. 2016. Evaluating Glucocorticoids for Sepsis: Time to Change Course. *JAMA,* 316**,** 1769-1771.

YEO, K. K., GAYNON, P. S., FU, C. H., WAYNE, A. S. & SUN, W. 2016. Bortezomib, Dexamethasone, Mitoxantrone, and Vinorelbine (BDMV): An Active Reinduction Regimen for Children With Relapsed Acute Lymphoblastic Leukemia and Asparaginase Intolerance. *J Pediatr Hematol Oncol,* 38**,** 345-9.

YIN, J., ZHANG, Q., CAI, Y., LI, Q. Y., HAO, L. N., MA, Z. & WANG, X. J. 2017. Targeted Delivery of Mannose-Conjugated BODIPY Photosensitizer by Nanomicelles for Photodynamic Breast Cancer Therapy. *Chemistry*.

YOSHINO, T., KISHI, H., NAGATA, T., TSUKADA, K., SAITO, S. & MURAGUCHI, A. 2001. Differential involvement of p38 MAP kinase pathway and Bax translocation in the mitochondria-mediated cell death in TCR- and dexamethasone-stimulated thymocytes. *Eur J Immunol,* 31**,** 2702-8.

YOUSSEF, D. M., ELBEHIDY, R. M., ABDELHALIM, H. S. & AMR, G. E. 2011. Soluble interleukine-2 receptor and MDR1 gene expression levels as inflammatory biomarkers for prediction of steroid response in children with nephrotic syndrome. *Iran J Kidney Dis,* 5**,** 154-61.

YU, J., ROH, S., LEE, J. S., YANG, B. H., CHOI, M. R., CHAI, Y. G. & KIM, S. H. 2010. The Effects of Venlafaxine and Dexamethasone on the Expression of HSP70 in Rat C6 Glioma Cells. *Psychiatry Investig,* 7**,** 43-8.

ZALACHORAS, I., HOUTMAN, R., ATUCHA, E., DEVOS, R., TIJSSEN, A. M., HU, P., LOCKEY, P. M., DATSON, N. A., BELANOFF, J. K., LUCASSEN, P. J., JOELS, M., DE KLOET, E. R., ROOZENDAAL, B., HUNT, H. & MEIJER, O. C. 2013. Differential targeting of brain stress circuits with a selective glucocorticoid receptor modulator. *Proc Natl Acad Sci U S A,* 110**,** 7910-5.

ZENG, Q., DONG, X., RUAN, C., HU, B., LUO, Y., LUO, Z., XU, L., ZHOU, H., WANG, R. & YANG, H. 2016. CD14+CD16++ monocytes are increased in patients with NMO and are selectively suppressed by glucocorticoids therapy. *J Neuroimmunol,* 300**,** 1-8.

ZHANG, C., LI, L., ZHAO, B., JIAO, A., LI, X., SUN, N. & ZHANG, J. 2016. Ghrelin Protects against Dexamethasone-Induced INS-1 Cell Apoptosis via ERK and p38MAPK Signaling. *Int J Endocrinol,* 2016**,** 4513051.

ZHANG, H. Q., YAU, Y. F., WONG, M. S., MAN, O. Y., HE, Y. Y., CHAN, N. & LI, M. 2008. Chinese medicine formula DSQRL versus glucocorticoids for the treatment of experimental pulmonary fibrosis. *J Ethnopharmacol,* 116**,** 318-24.

ZHANG, J. Z., CAVET, M. E., VANDERMEID, K. R., SALVADOR-SILVA, M., LOPEZ, F. J. & WARD, K. W. 2009a. BOL-303242-X, a novel selective glucocorticoid receptor agonist, with full anti-inflammatory properties in human ocular cells. *Mol Vis,* 15**,** 2606-16.

ZHANG, L., YAN, J., LIU, Y., ZHAO, Q., DI, C., CHAO, S., JIE, L., LIU, Y. & ZHANG, H. 2017. Contribution of caspase-independent pathway to apoptosis in malignant glioma induced by carbon ion beams. *Oncol Rep,* 37**,** 2994-3000.

ZHANG, Y., SHENG, H., QI, J., MA, B., SUN, J., LI, S. & NI, X. 2012. Glucocorticoid acts on a putative G protein-coupled receptor to rapidly regulate the activity of NMDA receptors in hippocampal neurons. *Am J Physiol Endocrinol Metab,* 302**,** E747-58.

ZHANG, Z., JONES, S., HAGOOD, J. S., FUENTES, N. L. & FULLER, G. M. 1997. STAT3 acts as a co-activator of glucocorticoid receptor signaling. *J Biol Chem,* 272**,** 30607-10.

ZHANG, Z., ZHANG, Z. Y. & SCHLUESENER, H. J. 2009b. Compound A, a plant origin ligand of glucocorticoid receptors, increases regulatory T cells and M2 macrophages to attenuate experimental autoimmune neuritis with reduced side effects. *J Immunol,* 183**,** 3081-91.

ZHAO, J., DAI, Y. H., XI, Q. S. & YU, S. Y. 2013. A clinical study on insomnia in patients with cancer during chemotherapy containing high-dose glucocorticoids. *Pharmazie,* 68**,** 421-7.

ZHAO, T., MA, F. & YIN, F. 2017. Role of polymorphisms of GSTM1, GSTT1 and GSTP1 Ile105Val in childhood acute lymphoblastic leukemia (ALL) risk: an update meta-analysis. *Minerva Pediatr*.

ZHENG, Y., ISHIGURO, H., IDE, H., INOUE, S., KASHIWAGI, E., KAWAHARA, T., JALALIZADEH, M., REIS, L. O. & MIYAMOTO, H. 2015. Compound A Inhibits Bladder Cancer Growth Predominantly via Glucocorticoid Receptor Transrepression. *Mol Endocrinol,* 29**,** 1486-97.

ZHOU, A., HU, J., WANG, L., ZHONG, G., PAN, J., WU, Z. & HUI, A. 2015. Combined 3D-QSAR, molecular docking, and molecular dynamics study of tacrine derivatives as potential acetylcholinesterase (AChE) inhibitors of Alzheimer's disease. *J Mol Model,* 21**,** 277.

ZIELINSKI, D. C., JAMSHIDI, N., CORBETT, A. J., BORDBAR, A., THOMAS, A. & PALSSON, B. O. 2017. Systems biology analysis of drivers underlying hallmarks of cancer cell metabolism. *Sci Rep,* 7**,** 41241.

ZONG, W. X. & THOMPSON, C. B. 2006. Necrotic death as a cell fate. *Genes Dev,* 20**,** 1-15.

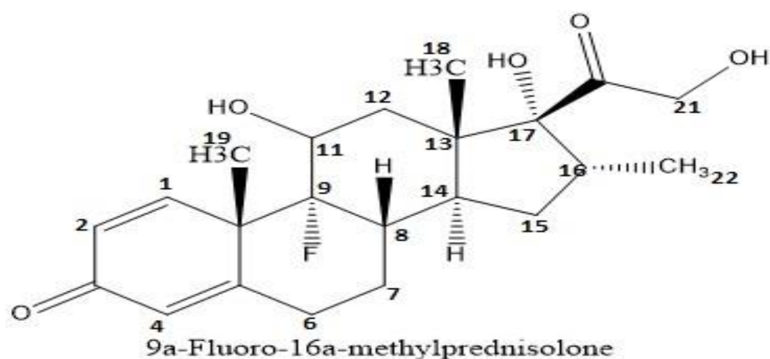
ZOTTER, Z., NAGY, Z., PATOCS, A., CSUKA, D., VESZELI, N., KOHALMI, K. V. & FARKAS, H. 2017. Glucocorticoid receptor gene polymorphisms in hereditary angioedema with C1-inhibitor deficiency. *Orphanet J Rare Dis,* 12**,** 5.

ZOU, H., JIANG, Z., LI, R., JIA, Y., YANG, X., NI, Y. & ZHAO, R. 2013. p53 cooperates with Sp1 to regulate breed-dependent expression of glucocorticoid receptor in the liver of preweaning piglets. *PLoS One,* 8**,** e70494.

4.5 Supplementary material

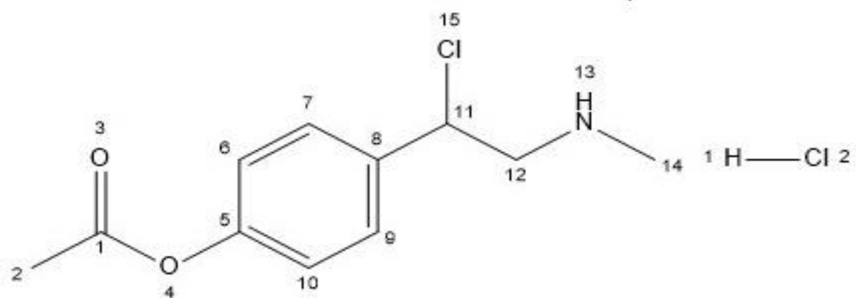
4.5.1 NMR Spectra

4.5.1.1 Dexamethasone



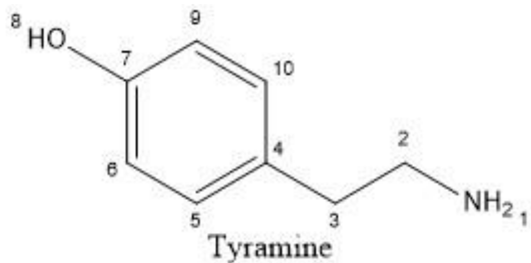
1. NMR analysis of Dex PROTON 64/ hydrogen profile of control (A) 0h (B) 24h/RT and (C) 24h/4°C.

4.5.1.2 Compound A



2. NMR analysis of CPDA PROTON 64/ hydrogen profile of control (A) 0h (B) 24h/RT and (C) 24h/4°C.

4.5.1.3 Tyramine



A

HOD

0h

acetone

5,10 6,9 2 3

B

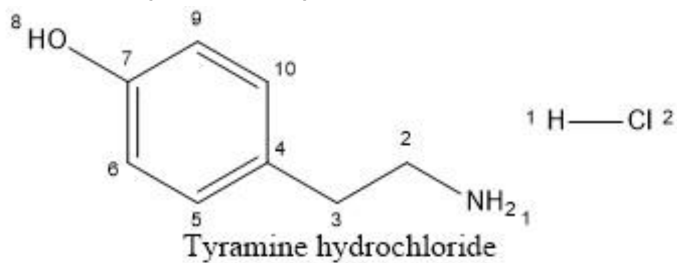
24h/RT

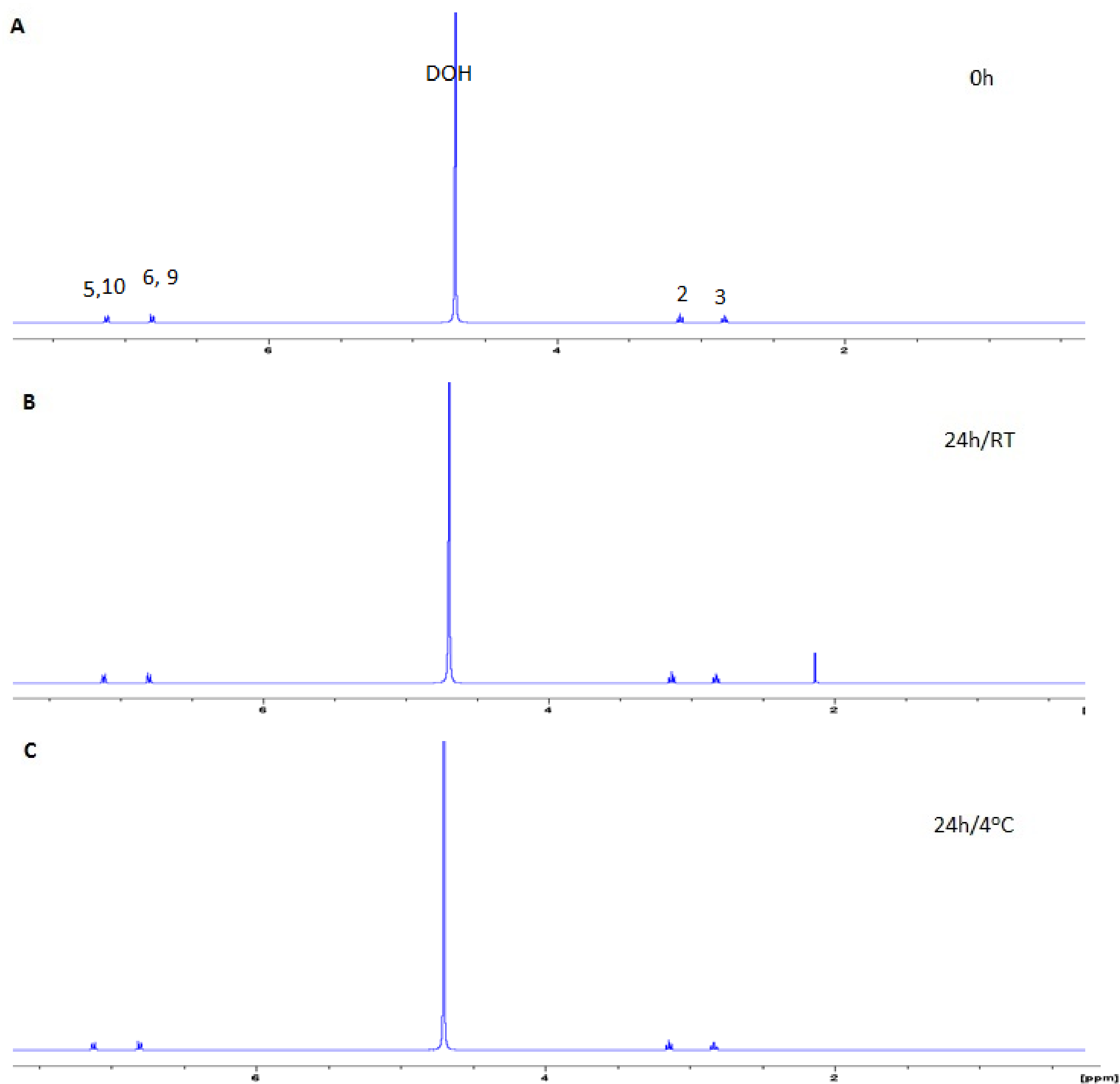
C

24h/4ºC

3. NMR analysis of Tyrmine PROTON 64/ hydrogen profile of control (A) 0h (B) 24h/RT and (C) 24h/4°C.

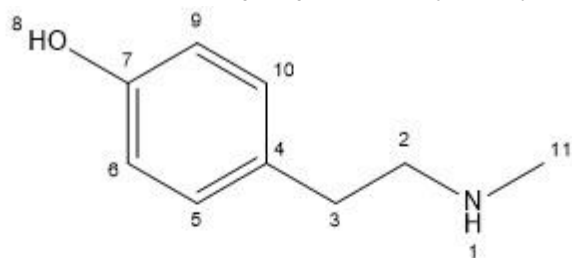
4.5.1.4 Tyramine Hydrochloride





4. NMR analysis of Tyramine hydrochloride PROTON 64/ hydrogen profile of control (A) 0h (B) 24h/RT and (C) 24h/4°C.

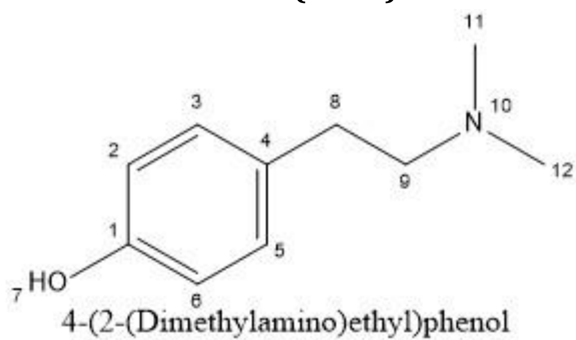
4.5.1.5 N-Methyl Tyramine (CPDB)



N-Methyltyramine

5. NMR analysis of CPDB PROTON 64/ hydrogen profile of control (A) 0h (B) 24h/RT and (C) 24h/4°C.

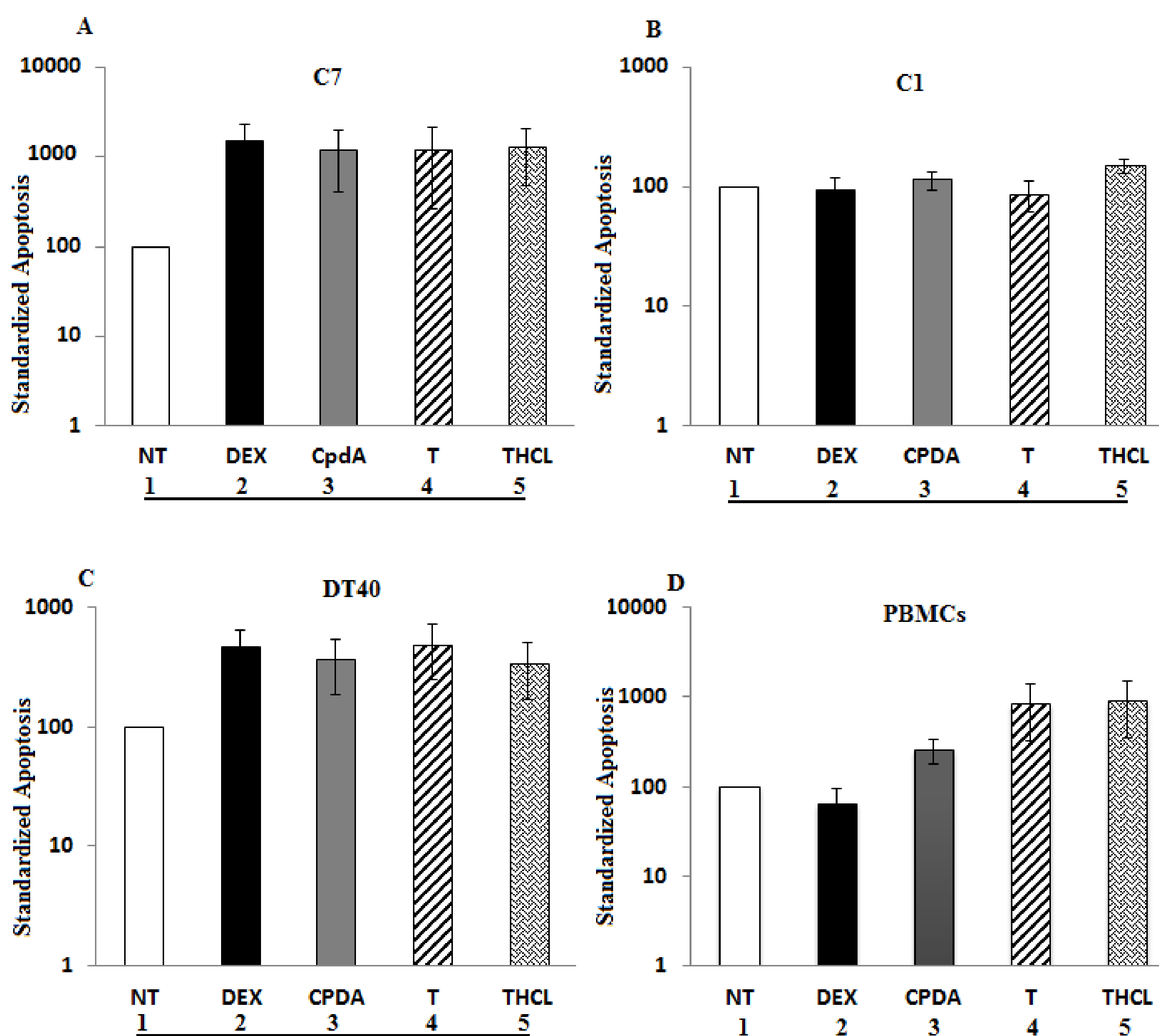
4.5.1.6 Hordenine (CPDC)



6. NMR analysis of CPDC PROTON 64/ hydrogen profile of control (A) 0h (B) 24h/RT and (C) 24h/4°C.

4.5.2 Quantitative analysis of of apoptosis

Annexin V, FITC labelled

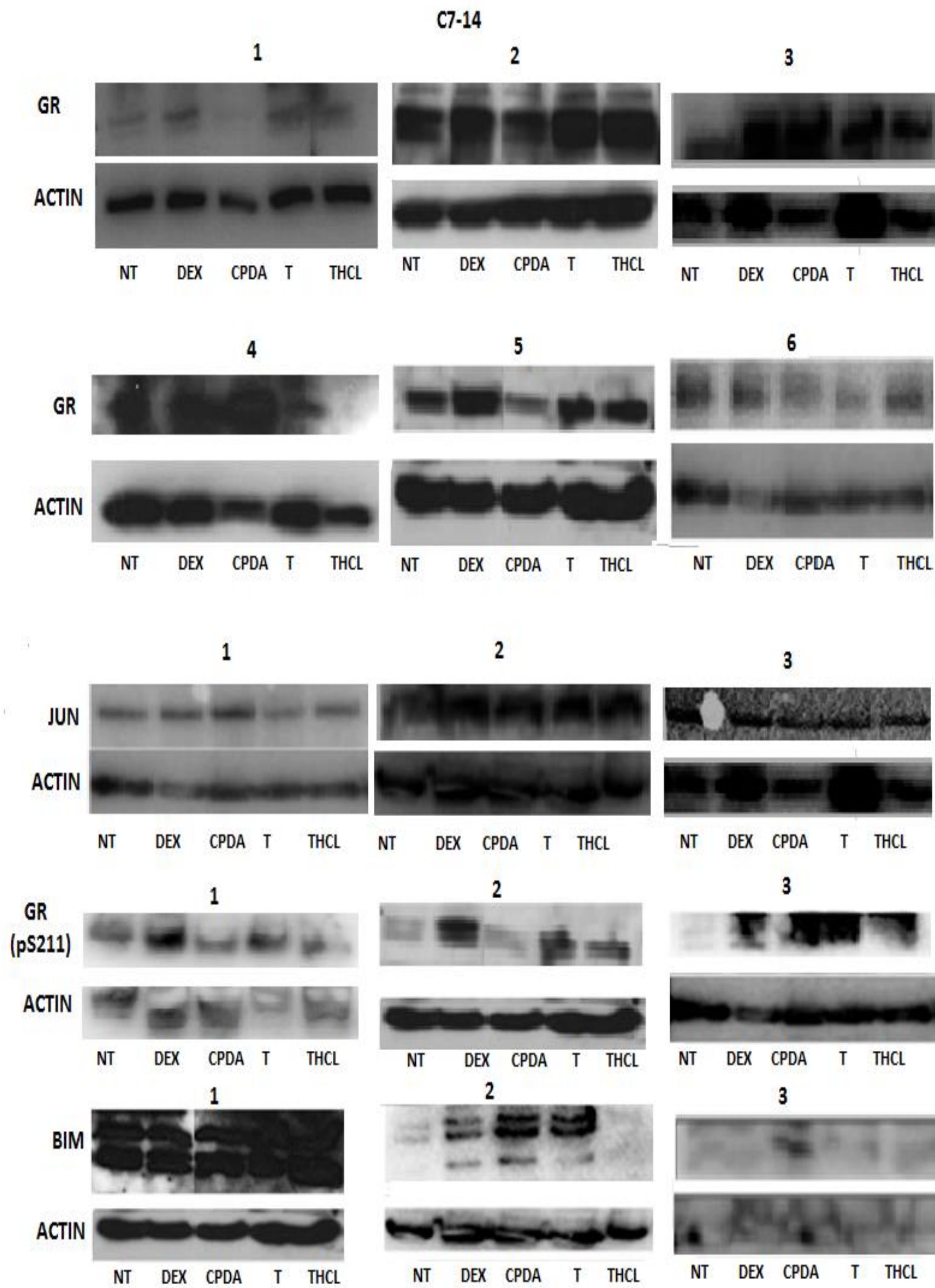


Quantitative analysis of of apoptosis using flow cytometric results and Annexin V staining.

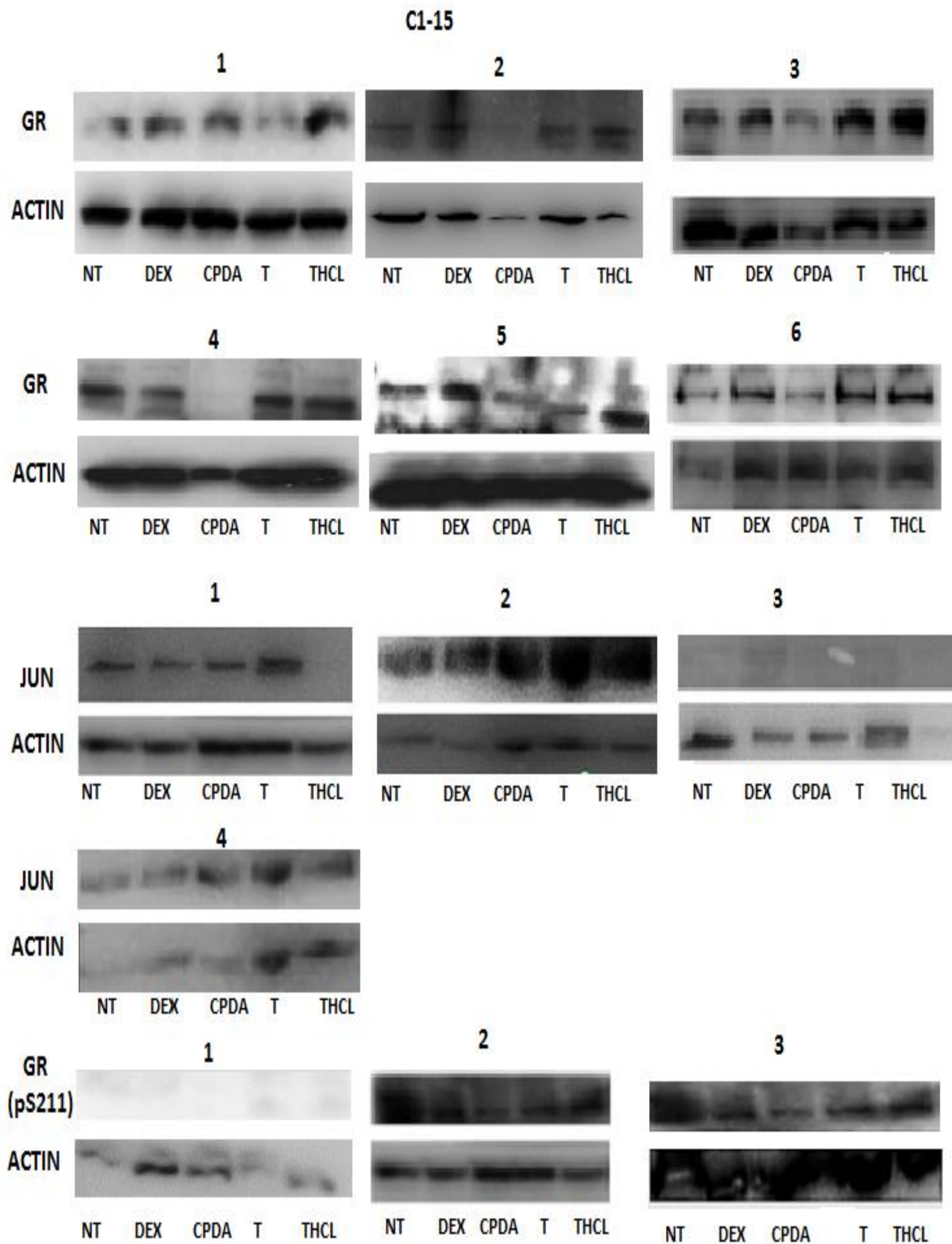
CEM - C7-14, CEM-C1-15, DT40 cells and PBMCs Cells were treated with 1 µM concentration for 48 hrs with DEX, CPDA, T, THCL, and then AnnexinnV-FITC-PI kit was used to detect apoptotic cells. Analysis was performed using BD FACS verse TM by aid of BD FACS Suite software. Data shown are representative of three or more experiments. Error bars represent standard error of means *$P<0.05$.

4.5.3 Western blot images

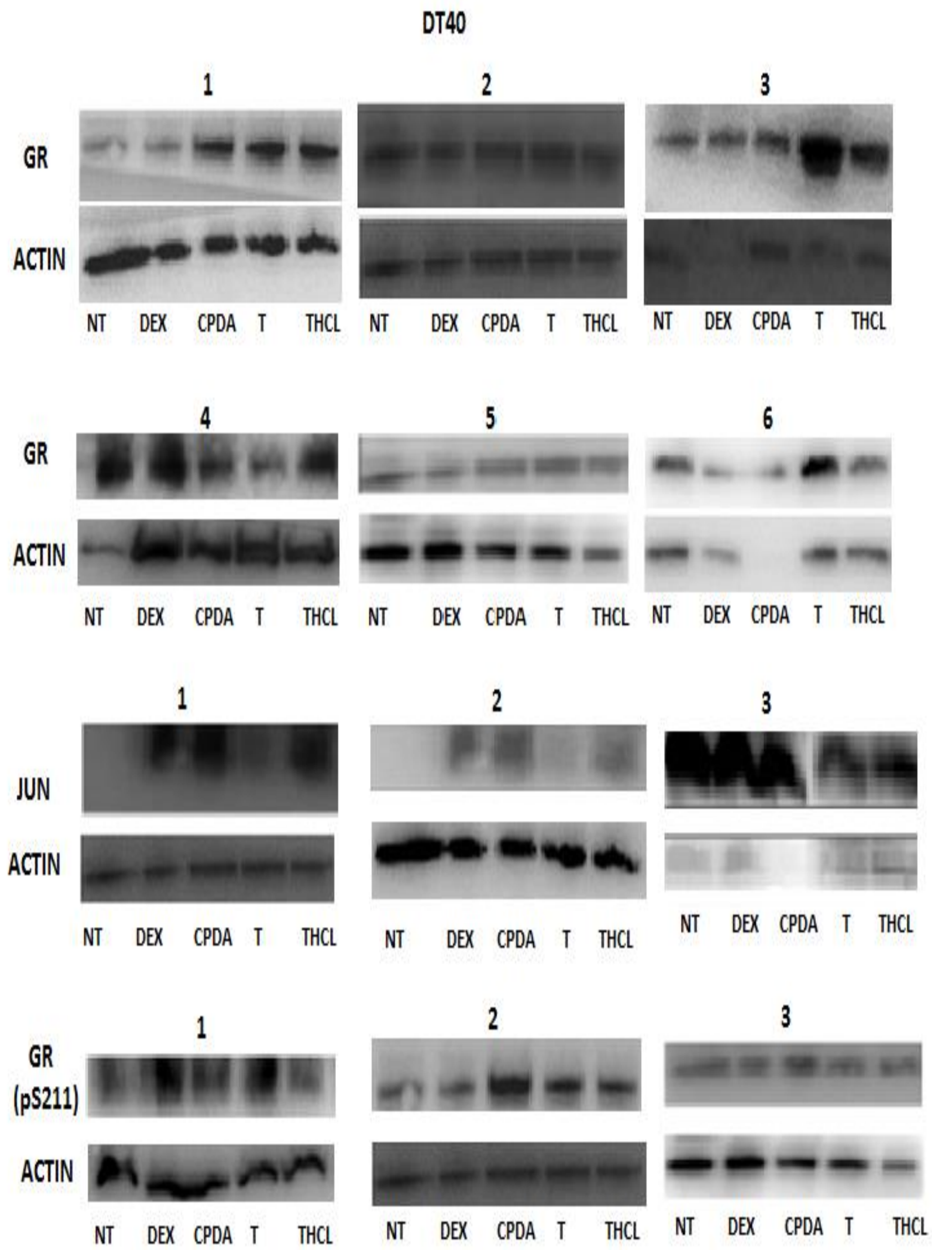
4.5.3.1 CEM-C7-14



4.5.3.2 CEM-C1-15

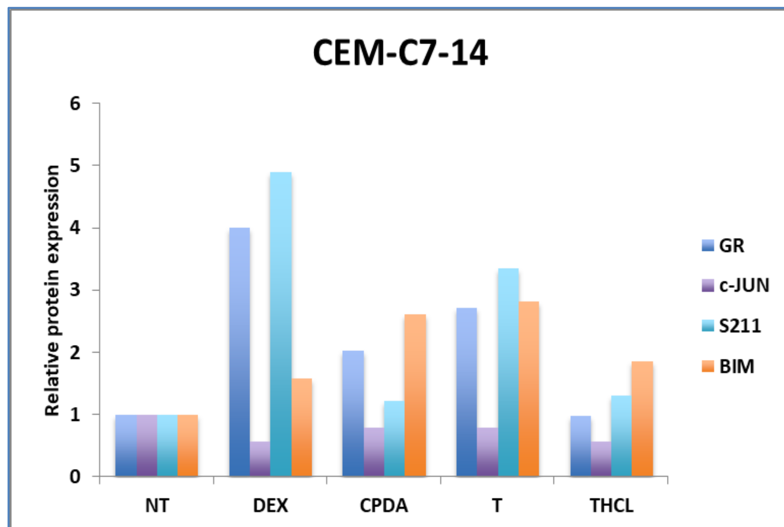


4.5.3.3 DT40 Cells

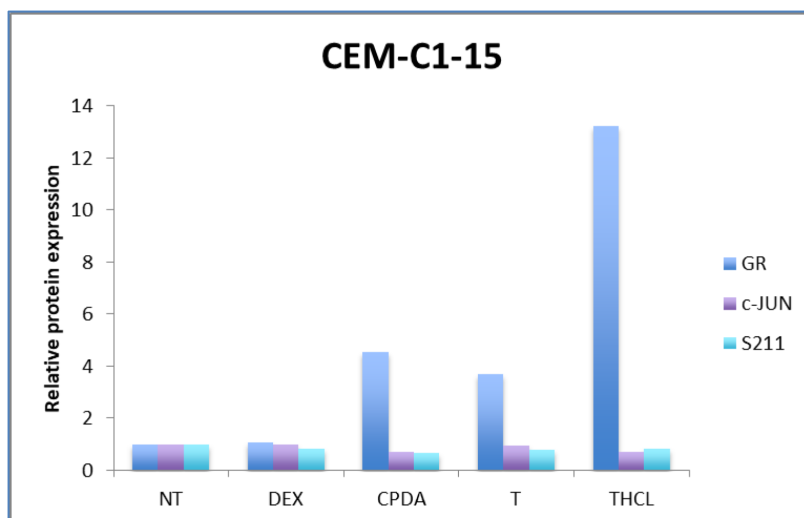


4.5.4 Plots of one selected image of each cell line

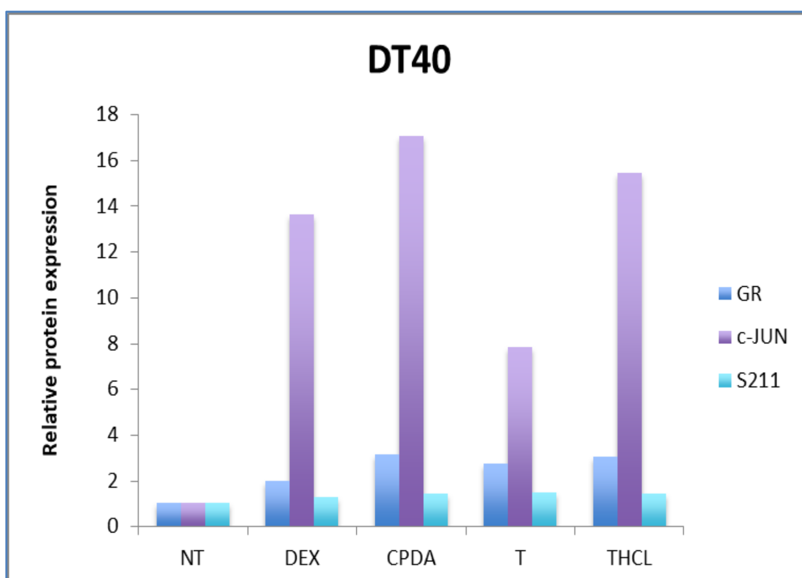
A-C7



B-C1

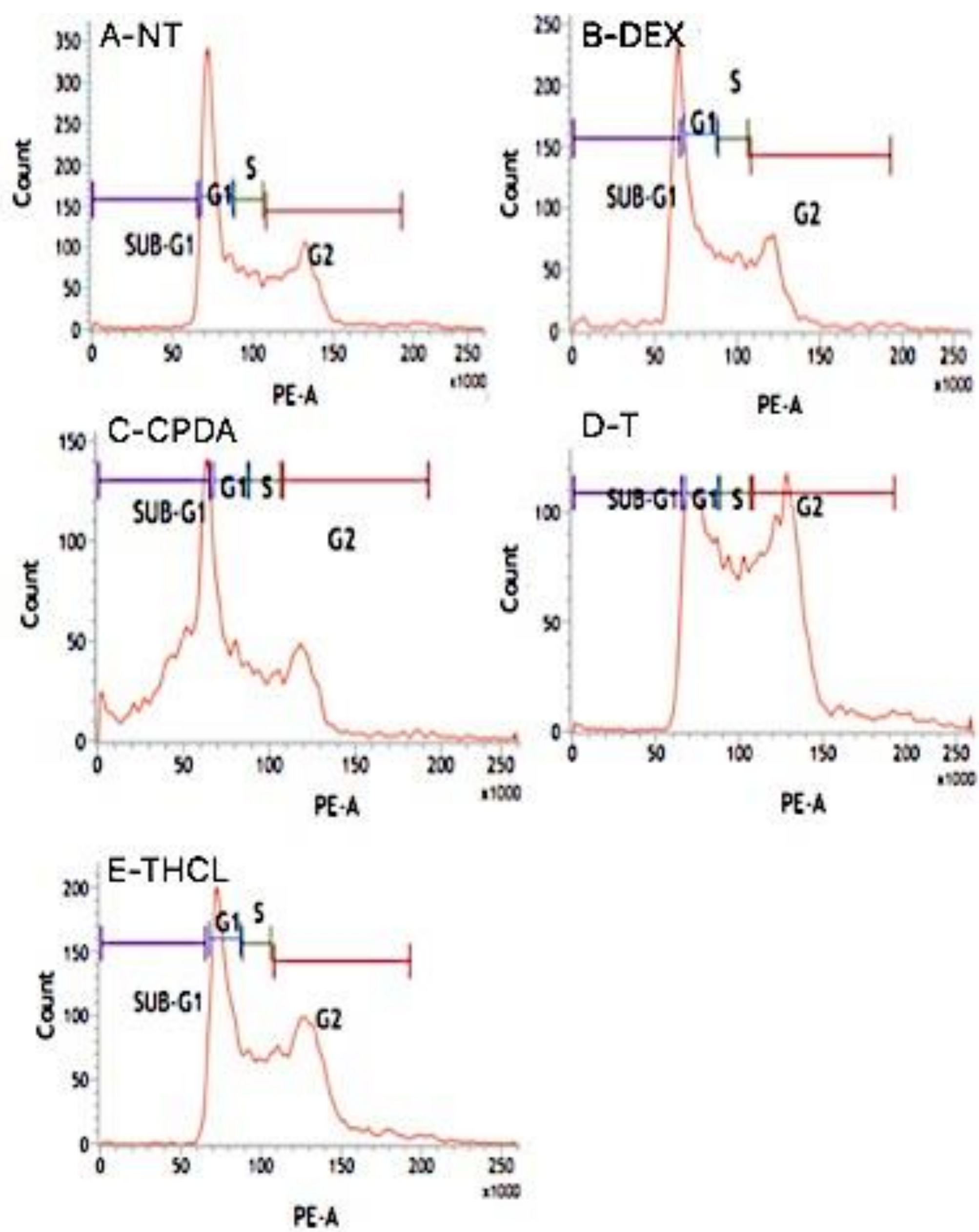


D-DT40



4.5.5 Quantifications of individual western blots for each cell line

Column1	Column2	Column3	Column4	Column5	Column6	Column7	Column8	Column9	Column10	Column11	Column12
C7	1-GR										
NT	1	1	1	1	1	1	1	1	1	1	
DEX	1.987616113	4.006828011	1.277665987	1.26876869	2.272137299	3.140983351	1.315566366	2.834338473	1.186100832	1.1925521	
CPDA	1.291451317	2.027300107	1.028943039	1.810446839	2.845084826	3.043309577	0.19264222	3.376634741	1.777393118	0.816634789	
T	1.123513865	2.705875864	1.005063515	1.250191433	0.905627928	2.915997495	1.522364843	3.252591248	0.903453337	0.996553079	
THCL	1.319535676	0.984952289	1.231692924	1.524291488	0.878644891	2.29407247	1.70981711	3.612399411	1.197839393	0.794785843	
	2- c-JUN										
NT	1	1	1	1	1	1	1				
DEX	2.442915341	0.558424096	1.757221055	4.303829268	3.236625877	0.666502246	0.351098024				
CPDA	2.811009869	0.792537114	2.023690712	15.91906257	2.063279226	1.00919946	0.133815915				
T	1.400041617	0.782143039	1.360683496	10.98365601	1.872520344	0.733991521	0.188192796				
THCL	1.335625361	0.567611721	1.239294155	4.087492441	1.561274838	1.083572402	0.07089006				
	3-S211				4-BIM						
NT	1	1	1	NT	1	1	1				
DEX	8.859384617	1.498340895	4.894009747	DEX	1.58486875	0.835065202	1.196668166				
CPDA	8.307440029	0.81375424	1.218919062	CPDA	2.618923918	0.822419727	1.290434638				
T	8.122241021	1.590537293	3.342351192	T	2.811125472	0.504205823	1.001331209				
THCL	6.490762479	0.663022462	1.312298732	THCL	1.847347463	0.933753705	0.745938425				
C1	1-GR										
NT	1	1	1	1	1	1	1	1	1	1	1
DEX	0.692257426	1.288700818	1.050460844	1.140964448	1.217523912	1.120697356	4.65537567	1.089828228	1.060871673	0.600756023	1.551617619
CPDA	1.7012735	1.189427125	0.464955437	1.12254599	1.181651627	0.533009692	0.769232218	0.928549086	4.561996023	0.431849771	2.033783389
T	2.097257212	0.984512458	0.130664566	1.028561943	1.39756828	1.148260997	0.934302756	0.892762716	3.722203234	0.979741851	1.233779028
THCL	3.161249571	1.308462031	0.025944142	1.807832269	1.404259541	1.16916597	1.227946744	1.356044852	13.22769557	0.871617791	2.962830295
	C-JUN								S211	S211-	
NT	1	1	1	1	1	1		NT	1	1	
DEX	1.002369999	0.860170457	1.676097534	2.839586117	1.285572531	0.100818884		DEX	0.83394312	0.584984511	
CPDA	0.711611464	0.670607107	1.692538682	1.438763281	1.276289348	0.120045426		CPDA	0.653417413	0.5172371	
T	0.944611167	1.158310062	1.354293836	2.089002793	1.272972281	0.05718961		T	0.783184692	0.23122435	
THCL	0.696220482	1.303794652	5.024834618	2.153597232	2.048658939	0.044401821		THCL	0.845239931	0.186986574	
DT40	1-GR										
replicate no	1	2	3	4	5	6	7	8	9	10	
NT	1	1	1	1	1	1	1	1	1	1	
DEX	0.660207918	1.989244209	1.064417027	0.721028277	0.697276461	1.658806557	0.981291382	1.274039231	1.003437541	2.415297707	
CPDA	47.45326041	3.130956259	2.584086211	0.61035139	1.193259099	2.97266345	1.21735073	0.224351605	1.062779135	1.580259564	
T	2.294122733	2.748500788	1.024104041	0.598614005	1.18980693	2.837354517	1.284325827	0.200373478	2.673220879	5.027031641	
THCL	1.299882591	3.031558593	1.106418152	0.199713398	1.386966002	2.838844407	1.244854375	0.221875138	2.435007086	4.352993358	
	2-c-JUN				3-S211						
NT	1	1	1		NT	1	1	1	1		
DEX	13.61399115	1.09670767	15.24963099		DEX	0.695798343	1.163909264	2.103864973	1.227897282		
CPDA	17.05954959	6.33617713	15.9966991		CPDA	1.061241366	1.243953573	2.021149914	1.279280744		
T	7.836997367	0.488283494	10.5927409		T	0.851237755	1.211919529	2.034459126	1.89990873		
THCL	15.43358968	0.467682038	23.15808968		THCL	1.363808567	1.549387197	1.376838069	1.453737684		



Cell cycle profiles. The figure represents the setting and selection of SUB-G1, G1, S,G2 in CEM-C7-14

4.5.6 Blast results

4.5.6.1GR

BLAST Results

Blast 2 sequences

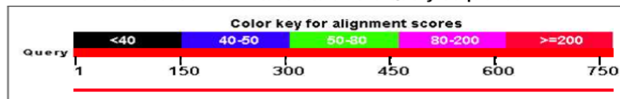
NP_001032915:glucocorticoid receptor [Gallus...

RID	YAS6EBKA114 (Expires on 09-25 01:05 am)		
Query ID	NP_001032915.1	**Subject ID**	ADP91252.1
Description	glucocorticoid receptor [Gallus gallus]	**Description**	glucocorticoid receptor [Homo sapiens] See details
Molecule type	amino acid	**Molecule type**	amino acid
Query Length	772	**Subject Length**	777
		Program	BLASTP 2.5.0+

New Analyze your query with SmartBLAST

Graphic Summary

Distribution of 1 Blast Hits on the Query Sequence



Descriptions

Sequences producing significant alignments:

Description	Max score	Total score	Query cover	E value	Ident	Accession
glucocorticoid receptor [Homo sapiens]	1180	1180	100%	0.0	74%	ADP91252.1

Alignments

glucocorticoid receptor [Homo sapiens]
Sequence ID: **ADP91252.1** Length: 777 Number of Matches:

See 59 more title(s)
Range 1: 1 to 777

Score	Expect	Method	Identities	Positives	Gaps	Frame
1180 bits(3053)	0.0()	Compositional matrix adjust.	581/780(74%)	660/780(84%)	11/780(1%)	

Features:

```
Query  1    MDSKELLKSSDQDETRKNALMSTKGGIVMDFHPPFRGGASAQAPVSAAPLPVSSQSDSAQ  60
            MDSKE L        E   +++++ + G VMDF+    RGGA+ +    S+  L V+SQSDS Q
Sbjct  1    MDSKESLTPGR--EENPSSVLAQERGDVMDFYKTLRGGATVKVSASSPSLAVASQSDSKQ  58

Query  61   QPALADFSKGLVNNVPPPDLSKAVSLSMGLYMGETDAKVMGNDLGFSQQGQTGISSGETD  120
            +  L DF KG V+N   PDLSKAVSLSMGLYMGET+ KVMGNDLGF QQGQ  +SSGETD
Sbjct  59   RRLLVDFPKGSVSNAQQPDLSKAVSLSMGLYMGETETKVMGNDLGFPQQGQISLSSGETD  118

Query  121  FRLLEESIASLNKSSSLAEDAKGAVSSEA---PLEPDFVGMAGRGPLEPGASVQSQVGSN  177
             +LLEESIA+LN+S+S+ E+ K + S+      P E +F          E    ++ Q G+N
Sbjct  119  LKLLEESIANLNRSTSVPENPKSSASTAVSAAPTEKEFPKTHSDVSSEQ-QHLKGQTGTN  177

Query  178  GGSLKLSSEDQSTLDILQDLELPSVSPGKEPNGSPWRLDPLLDDGGLLSPISA-DDTFLL  236
            GG++KL + DQST DILQDLE  S SPGKE N SPWR D L+D+  LLSP++  DD+FLL
Sbjct  178  GGNVKLYTTDQSTFDILQDLEFSSGSPGKETNESPWRSDLLIDENCLLSPLAGEDDSFLL  237

Query  237  EGNLGEDCKPPILSDTKPKINDRGDLLPSS--KMPMPQVKTEKEDFIELCTPG-IKQENV  293
            EGN  EDCKP IL DTKPKI D GDL+ SS    + +PQVKTEKEDFIELCTPG IKQE +
Sbjct  238  EGNSNEDCKPLILPDTKPKIKDNGDLVLSSPSNVTLPQVKTEKEDFIELCTPGVIKQEKL  297

Query  294  GPIYCQANFSGSNMLGTKVSAISIHGVSTSGGQMYHYDLNTASLSQQQDQKPIFNIIPSL  353
            G +YCQA+F G+N++G K+SAIS+HGVSTSGGQMYHYD+NTASLSQQQDQKPIFN+IP +
Sbjct  298  GTVYCQASFPGANIIGNKMSAISVHGVSTSGGQMYHYDMNTASLSQQQDQKPIFNVIPPI  357

Query  354  PAGSENWNRCQGSGDEALAPLGTLNLSGRPAFSNGYSSPGLRSDVSSSPSTT-SATAGPP  412
            P GSENWNRCQGSGD+ L  LGTLN  GR  FSNGYSSP +R DVSS PS++ +AT GPP
Sbjct  358  PVGSENWNRCQGSGDDNLTSLGTLNFPGRTVFSNGYSSPSMRPDVSSPPSSSSTATTGPP  417

Query  413  PKLCLVCSDEASGCHYGVLTCGSCKVFFKRAVEGQHNYLCAGRNDCIIDKIRRKNCPACR  472
            PKLCLVCSDEASGCHYGVLTCGSCKVFFKRAVEGQHNYLCAGRNDCIIDKIRRKNCPACR
Sbjct  418  PKLCLVCSDEASGCHYGVLTCGSCKVFFKRAVEGQHNYLCAGRNDCIIDKIRRKNCPACR  477

Query  473  YRKCLQAGMNLEARKTKKKIKGIQQTTATGTREAAEAAGNKSVVPASLPQLTPTLVSLLE  532
            YRKCLQAGMNLEARKTKKKIKGIQQ T   ++E +E  GNK++VPA+LPQLTPTLVSLLE
Sbjct  478  YRKCLQAGMNLEARKTKKKIKGIQQATTGVSQETSENPGNKTIVPATLPQLTPTLVSLLE  537

Query  533  VIEPEVLYSGYDSTLPDSSWRIMSTLNMLGGRQVVAAVKWAKAIPGFRNLHLDDQMTLLQ  592
            VIEPEVLY+GYDS++PDS+WRIM+TLNMLGGRQV+AAVKWAKAIPGFRNLHLDDQMTLLQ
Sbjct  538  VIEPEVLYAGYDSSVPDSTWRIMTTLNMLGGRQVIAAVKWAKAIPGFRNLHLDDQMTLLQ  597

Query  593  YSWMFLMAFALGWRSYKQSNGNLLCFAPDLIINEQRMNLPCMYEQCKHMLMVARELSRLQ  652
            YSWMFLMAFALGWRSY+QS+ NLLCFAPDLIINEQRM LPCMY+QCKHML V+ EL RLQ
Sbjct  598  YSWMFLMAFALGWRSYRQSSANLLCFAPDLIINEQRMTLPCMYDQCKHMLYVSSELHRLQ  657

Query  653  VSYEEYLCMKTLLLLSTIPKEGLKSQTLFEEIRMTYIKELGKAIVKREGNSSQNWQRFYQ  712
            VSYEEYLCMKTLLLLS++PK+GLKSQ LF+EIRMTYIKELGKAIVKREGNSSQNWQRFYQ
Sbjct  658  VSYEEYLCMKTLLLLSSVPKDGLKSQELFDEIRMTYIKELGKAIVKREGNSSQNWQRFYQ  717

Query  713  LTKLLDSMHDVVENLLSFCFQTFLDKSMSIEFPEMLAEIISNQIPKYSNGNIKKLLFHQK  772
            LTKLLDSMH+VVENLL++CFQTFLDK+MSIEFPEMLAEII+NQIPKYSNGNIKKLLFHQK
Sbjct  718  LTKLLDSMHEVVENLLNYCFQTFLDKTMSIEFPEMLAEIITNQIPKYSNGNIKKLLFHQK  777
```

4.5.6.2 Actin

BLAST Results

Blast 2 sequences

CAA25004:beta-actin [Gallus gallus]

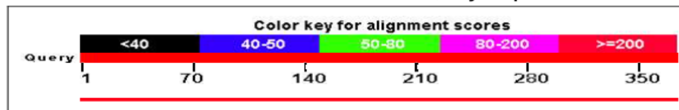
RID YAT5VU9611N (Expires on 09-25 01:22 am)

Query ID	CAA25004.1	**Subject ID**	P60709.1
Description	beta-actin [Gallus gallus]	**Description**	RecName: Full=Actin, cytoplasmic 1; AltName: Full=Beta-actin; Contains: RecName: Full=Actin, cytoplasmic 1, N-terminally processed See details
Molecule type	amino acid	**Molecule type**	amino acid
Query Length	375	**Subject Length**	375
		Program	BLASTP 2.5.0+

New Analyze your query with SmartBLAST

Graphic Summary

Distribution of 1 Blast Hits on the Query Sequence



Descriptions

Sequences producing significant alignments:

Description	Max score	Total score	Query cover	E value	Ident	Accession
RecName: Full=Actin, cytoplasmic 1; AltName: Full=Beta-actin; Contains: RecName: Full=Actin, cytoplasmic 1, N-terminally processed	784	784	100%	0.0	99%	P60709.1

Alignments

RecName: Full=Actin, cytoplasmic 1; AltName: Full=Beta-actin; Contains: RecName: Full=Actin, cytoplasmic 1, N-terminally processed
Sequence ID: **P60709.1** Length: 375 Number of Matches:

See 214 more title(s)
Range 1: 1 to 375

Score	Expect	Method	Identities	Positives	Gaps	Frame
784 bits(2025)	0.0()	Compositional matrix adjust.	374/375(99%)	375/375(100%)	0/375(0%)	

Features:

```
Query  1    MDDDIAALVVDNGSGMCKAGFAGDDAPRAVFPSIVGRPRHQGVMVGMGQKDSYVGDEAQS  60
            MDDDIAALVVDNGSGMCKAGFAGDDAPRAVFPSIVGRPRHQGVMVGMGQKDSYVGDEAQS
Sbjct  1    MDDDIAALVVDNGSGMCKAGFAGDDAPRAVFPSIVGRPRHQGVMVGMGQKDSYVGDEAQS  60

Query  61   KRGILTLKYPIEHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAPLNPKANREKMT  120
            KRGILTLKYPIEHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAPLNPKANREKMT
Sbjct  61   KRGILTLKYPIEHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAPLNPKANREKMT  120

Query  121  QIMFETFNTPAMYVAIQAVLSLYASGRTTGIVMDSGDGVTHTVPIYEGYALPHAILRLDL  180
            QIMFETFNTPAMYVAIQAVLSLYASGRTTGIVMDSGDGVTHTVPIYEGYALPHAILRLDL
Sbjct  121  QIMFETFNTPAMYVAIQAVLSLYASGRTTGIVMDSGDGVTHTVPIYEGYALPHAILRLDL  180

Query  181  AGRDLTDYLMKILTERGYSFTTTAEREIVRDIKEKLCYVALDFEQEMATAASSSSLEKSY  240
            AGRDLTDYLMKILTERGYSFTTTAEREIVRDIKEKLCYVALDFEQEMATAASSSSLEKSY
Sbjct  181  AGRDLTDYLMKILTERGYSFTTTAEREIVRDIKEKLCYVALDFEQEMATAASSSSLEKSY  240

Query  241  ELPDGQVITIGNERFRCPEALFQPSFLGMESCGIHETTFNSIMKCDVDIRKDLYANTVLS  300
            ELPDGQVITIGNERFRCPEALFQPSFLGMESCGIHETTFNSIMKCDVDIRKDLYANTVLS
Sbjct  241  ELPDGQVITIGNERFRCPEALFQPSFLGMESCGIHETTFNSIMKCDVDIRKDLYANTVLS  300

Query  301  GGTTMYPGIADRMQKEITALAPSTMKIKIIAPPERRYSVWIGGSILASLSTFQQMWISKQ  360
            GGTTMYPGIADRMQKEITALAPSTMKIKIIAPPER+YSVWIGGSILASLSTFQQMWISKQ
Sbjct  301  GGTTMYPGIADRMQKEITALAPSTMKIKIIAPPERKYSVWIGGSILASLSTFQQMWISKQ  360

Query  361  EYDESGPSIVHRKCF  375
            EYDESGPSIVHRKCF
Sbjct  361  EYDESGPSIVHRKCF  375
```

4.5.6.3 C-JUN

BLAST Results

Blast 2 sequences

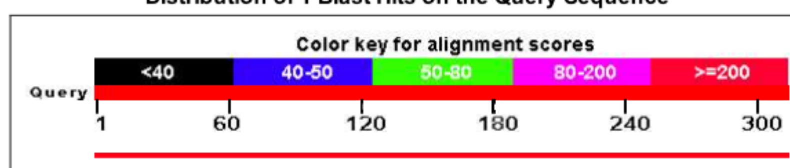
NP_001032915:glucocorticoid receptor [Gallus...

RID	YASUFHUD11N (Expires on 09-25 01:15 am)		
Query ID	P18870.2	**Subject ID**	NP_002219.1
Description	RecName: Full=Transcription factor AP-1; AltName: Full=Proto-oncogene c-Jun	**Description**	transcription factor AP-1 [Homo sapiens] See details
Molecule type	amino acid	**Molecule type**	amino acid
Query Length	314	**Subject Length**	331
		Program	BLASTP 2.5.0+

New Analyze your query with SmartBLAST

Graphic Summary

Distribution of 1 Blast Hits on the Query Sequence



Descriptions

Sequences producing significant alignments:

Description	Max score	Total score	Query cover	E value	Ident	Accession
transcription factor AP-1 [Homo sapiens]	508	508	100%	0.0	82%	NP_002219.1

Alignments

transcription factor AP-1 [Homo sapiens]
Sequence ID: **NP_002219.1** Length: 331 Number of Matches: 1

See 14 more title(s)
Range 1: 1 to 331

Score	Expect	Method	Identities	Positives	Gaps	Frame
508 bits(1308)	0.0()	Compositional matrix adjust.	272/331(82%)	290/331(87%)	17/331(5%)	

Features:

```
Query  1    MSAKMEPTFYEDALNASFAPPESGGYGYNNAKVLKQSMTLNLSDAASSLKPHLRNKNADI  60
            M+AKME TFY+DALNASF P ESG YGY+N K+LKQSMTLNL+D   SLKPHLR KN+D+
Sbjct  1    MTAKMETTFYDDALNASFLPSESGPYGYSNPKILKQSMTLNLADPVGSLKPHLRAKNSDL  60

Query  61   LTSPDVGLLKLASPELERLIIQSSNGLITTTPTPTQFLCPKNVTDEQEGFAEGFVRALAE  120
            LTSPDVGLLKLASPELERLIIQSSNG ITTTPTPTQFLCPKNVTDEQEGFAEGFVRALAE
Sbjct  61   LTSPDVGLLKLASPELERLIIQSSNGHITTTPTPTQFLCPKNVTDEQEGFAEGFVRALAE  120

Query  121  LHNQNTLPSVTSAAQPVSGG--MAP----VSSMAGGGSFNTSLHSEPPVYANLSNFNPNA  174
            LH+QNTLPSVTSAAQPV+G   +AP    V+  +G G F+ SLHSEPPVYANLSNFNP A
Sbjct  121  LHSQNTLPSVTSAAQPVNGAGMVAPAVASVAGGSGSGGFSASLHSEPPVYANLSNFNPGA  180

Query  175  LNS---APNYNANGMGY--------APQHHINPQMPVQHPRLQALKEEPQTVPEMPGETP  223
            L+S   AP+Y A G+ +         P HH+  QMPVQHPRLQALKEEPQTVPEMPGETP
Sbjct  181  LSSGGGAPSYGAAGLAFPAQPQQQQPPHHLPQQMPVQHPRLQALKEEPQTVPEMPGETP  240

Query  224  PLSPIDMESQERIKAERKRMRNRIAASKCRKRKLERIARLEEKVKTLKAQNSELASTANM  283
            PLSPIDMESQERIKAERKRMRNRIAASKCRKRKLERIARLEEKVKTLKAQNSELASTANM
Sbjct  241  PLSPIDMESQERIKAERKRMRNRIAASKCRKRKLERIARLEEKVKTLKAQNSELASTANM  300

Query  284  LREQVAQLKQKVMNHVNSGCQLMLTQQLQTF  314
            LREQVAQLKQKVMNHVNSGCQLMLTQQLQTF
Sbjct  301  LREQVAQLKQKVMNHVNSGCQLMLTQQLQTF  331
```

4.5.6.4 IL-6

BLAST Results

Blast 2 sequences

ADL14564:interleukin-6 [Gallus gallus]

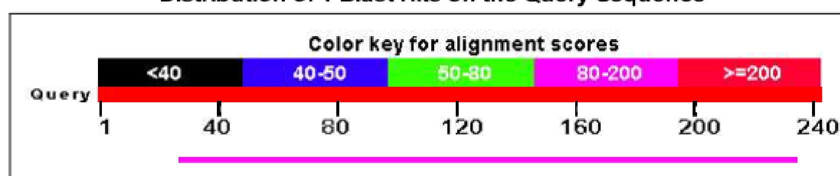
RID YATE5HKA11N (Expires on 09-25 01:26 am)

Query ID	ADL14564.1	**Subject ID**	AAD13886.1
Description	interleukin-6 [Gallus gallus]	**Description**	interleukin-6 [Homo sapiens] See details
Molecule type	amino acid	**Molecule type**	amino acid
Query Length	241	**Subject Length**	212
		Program	BLASTP 2.5.0+

New Analyze your query with SmartBLAST

Graphic Summary

Distribution of 1 Blast Hits on the Query Sequence



Descriptions

Sequences producing significant alignments:

Description	Max score	Total score	Query cover	E value	Ident	Accession
interleukin-6 [Homo sapiens]	136	136	85%	1e-44	37%	AAD13886.1

Alignments

interleukin-6 [Homo sapiens]
Sequence ID: **AAD13886.1** Length: 212 Number of Matches:

See 27 more title(s)
Range 1: 10 to 212

Score	Expect	Method	Identities	Positives	Gaps	Frame
136 bits(342)	1e-44()	Compositional matrix adjust.	78/210(37%)	115/210(54%)	11/210(5%)	

Features:

```
Query  28   GPVAL-LPLLLPLLLPPAAAVPLPAAADSSGEVGLEEEAGARRALLDCEPLARVLR---D  83
            GPVA  L LLL L            A    GE   +  A  R+ L   E + + +R   D
Sbjct  10   GPVAFSLGLLLVL-------PAAFPAPVPPGEDSKDVAAPHRQPLTSSERIDKQIRYILD  62

Query  84   RAVQLQDEMCKKFTVCENSMEMLVRNNLNLPKVTEEDGCLLAGFDEEKCLTKLSSGLFAF  143
                L+ E C K  +CE+S E L  NNLNLPK+ E+DGC  +GF+EE CL K+ +GL  F
Sbjct  63   GISALRKETCNKSNMCESSKEALAENNLNLPKMAEKDGCFQSGFNEETCLVKIITGLLEF  122

Query  144  QTYLEFIQETFDSEKQNVESLCYSTKHLAATIRQMVINPDEVVIPDSAAQKSLLANLKSD  203
            + YLE++Q  F+S ++   ++  STK L   +++   N D +  PD     SLL  L++
Sbjct  123  EVYLEYLQNRFESSEEQARAVQMSTKVLIQFLQKKAKNLDAITTPDPTTNASLLTKLQAQ  182

Query  204  KDWIEKITMHLILRDFTSFMEKTVRAVRYL  233
              W++ +T HLILR F  F++ ++RA+R +
Sbjct  183  NQWLQDMTTHLILRSFKEFLQSSLRALRQM  212
```

4.5.6.5 IL-2

BLAST Results

Blast 2 sequences

CAE17662:Interleukin-2 [Gallus gallus]

RID YATPHD9011N (Expires on 09-25 01:30 am)

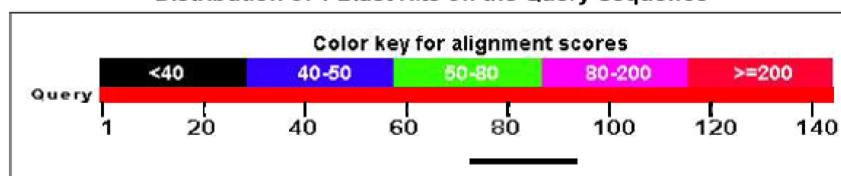
Query ID CAE17662.1
Description Interleukin-2 [Gallus gallus]
Molecule type amino acid
Query Length 143

Subject ID CAA25742.1
Description human interleukin 2 [Homo sapiens]
See details
Molecule type amino acid
Subject Length 153
Program BLASTP 2.5.0+

New Analyze your query with SmartBLAST

Graphic Summary

Distribution of 1 Blast Hits on the Query Sequence



Descriptions

Sequences producing significant alignments:

Description	Max score	Total score	Query cover	E value	Ident	Accession
human interleukin 2 [Homo sapiens]	14.2	14.2	14%	0.86	33%	CAA25742.1

Alignments

human interleukin 2 [Homo sapiens]

Sequence ID: **CAA25742.1** Length: 153 Number of Matches:

See 26 more title(s)

Range 1: 118 to 138

Score	Expect	Method	Identities	Positives	Gaps	Frame
14.2 bits(25)	0.86()	Compositional matrix adjust.	7/21(33%)	9/21(42%)	0/21(0%)	

Features:

```
Query  73   GEVVTLKKETEDDTEIKEEFV  93
            G   T   E  D+T    EF+
Sbjct  118  GSETTFMCEYADETATIVEFL  138
```