Faculty of Pharmacy

Design and Synthesis of Benzothiazole Derivatives Having Potential Targeted Anticancer Activity

Thesis

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List of Abbreviations

Abbreviation	Full term
AcOH	Acetic acid.
AIF	Apoptosis Inducing Factor.
AML	Acute myeloid leukemia.
Apfa-1	Apoptotic protease activating factor 1.
Apo2L	Apoptosis-inducing ligand 2.
ATP	Adenosine triphosphate.
Bak	BCL-2 homologous antagonist/killer.
Bax	BCL2-associated protein.
BCL-2	Beta-cell chronic lymphocytic leukemia/lymphoma 2.
BH	BCL-2 Homolgy Domain.
BID	BH3-interacting domain death agonist.
BRD-4	Bromodomain-containing 4.
C-DOCKER	CHARMm-based docker.
CHARMm:	Chemistry at HARvard Macromolecular Mechanics.
CLL	Chronic lymphocytic leukemia.
^{13}C NMR	Carbon-13 Nuclear Magnetic Resonance.
DCC	*N,N'*-Dicyclohexylcarbodiimide.
DCM	Dichloromethane.
DED	Death effector domain.
DEL	Double expressing lymphoma.
DIABLO	Direct IAP binding protein with low pI.
DISC	Death Inducing Signaling Complex.
DHL	Double hit lymphoma.
DMAP	4-Dimethylaminopyridine.
DMF	Dimethylformamide.

DMSO	Dimethylsulfoxide.
D_2O	Deuterium oxide.
DR	Death receptor
EDC.HCl	N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride.
EI-MS	Electron Ionization Mass Spectrometry.
EtOAc	Ethyl acetate.
EtOH	Ethanol.
FADD	Fas associated death domain.
FDA	Food and Drug Administration.
FL	Follicular lymphoma.
FPP	Field Point Pattern.
HB	Hydrogen bond.
HBA	Hydrogen bond acceptor.
HBTU	N,N,N′,N′-tetramethyl-O-(1H-benzotriazol-1-yl)uranium hexafluorophosphate.
HCCs	Hepatocellular carcinoma cells.
^{1}H NMR	Proton Nuclear Magnetic Resonance.
HOBT	*N*-Hydroxybenzotriazole.
hrs	Hours.
Hz	Hertz.
IAPs	Inhibitor of apoptosis proteins.
IC_{50}	Half-maximal inhibitory concentration.
Ki	The inhibitor constant.
NHL	Non-Hodgkin lymphoma.
NSCLC	Non-small cell lung cancer.
MCL-1	Myeloid cell leukemia-1.
MeOH	Methanol.
MOMP	Mitochondrial outer membrane permeabilization.
m.p.	Melting Point.
m/z	Mass-to-charge ratio.

M+	Molecular ion.
µM	Micromole.
mmol	Millimole.
MS	Mass spectroscopy.
Mwt	Molecular Weight.
NCI	National Cancer Institute.
NMR	Nuclear Magnetic Resonance.
PDB	Protein Data Bank.
PARAs	Pro-apoptotic receptor agonists.
PPI	Protein-protein interaction.
Ppm	Part per million.
PUMA	P53-upregulated modulator of apoptosis.
RMSD	Root Mean Square Deviation.
rt	Room temperature.
SAR	Structure activity relationship.
SCLC	Small cell lung cancer.
SLL	Small lymphocytic lymphoma.
SMAC	Second mitochondria-derived activator of caspase.
TEA	Triethylamine.
THF	Tetrahydrofuran.
TLC	Thin layer Chromatography.
TMS	Tetramethylsilane.
TNF	Tumor necrosis factor.
TR-FRET	Time-resolved Fluorescence Energy Transfer.
TRAIL	Tumor necrosis factor related apoptosis-inducing ligand.

Abstract

Cancer is a disease in which a group of abnormal cells grow uncontrollably by disregarding the normal rules of cell division. Normal cells are constantly subjects to signals that dictate weather the cell should divide and differentiate to another cell or die. Apoptosis is a normal physiological process which is very crucial to maintain tissue homeostasis**.** Dysregulated apoptosis can lead to various diseases as cancer. Thus, evasion of apoptosis stands out as a key hallmark of cancer cells. BCL-2 family of proteins is the key modulator of the mitochondrial apoptotic pathway. Therefore, the balance between the anti-apoptotic (BCL-2, BCL-XL and MCL-1) and pro-apoptotic (BAK, BAX, BAD, PUMA and NOXA) members of this family will govern cell fate. Overexpression of anti-apoptotic BCL-2 members is implicated in the progression of many human cancers as well as the emerging resistance to various anti-cancer agents including targeted therapies. Indeed, inhibition of the anti-apoptotic BCL-2 members by small molecule BH3 mimetics may provide an excellent approach in cancer therapy.

Herein, our research objective is to design, synthesize and biologically evaluate novel inhibitors targeting BCL-2 with a promising anti-cancer activity. The design process aimed to target BCL-2 BH3 binding groove and started by identification of the key interactions between BCL-2 binding groove and a previously reported BCL-2 inhibitors following, rational modification of the lead compound was proposed and a series of novel benzothiazole-based derivatives were suggested and finally molecular modeling studies including field alignment and docking were performed to investigate the predicted binding modes and binding affinities of the designed compounds.

The designed compounds were synthesized, purified and structurally confirmed by different analytical and spectral techniques.

The study involved the synthesis of the following reported intermediates:

1) Ethyl 4-fluoro-3-nitrobenzoate **(I).**
2) 1-((3-Fluorobenzyl)oxy)-4-nitrobenzene **(IVa).**
3) 1-((3-Chlorobenzyl)oxy)-4-nitrobenzene **(IVb)**.

4) 1-((4-Chlorobenzyl)oxy)-4-nitrobenzene **(IVc)**.
5) 1-((4-Bromobenzyl)oxy)-4-nitrobenzene **(IVd)**.
6) 1-((4-Methylbenzyl)oxy)-4-nitrobenzene **(IVe)**.
7) 4-((3-Fluorobenzyl)oxy) aniline **(Va)**.
8) 4-((3-Chlorobenzyl)oxy) aniline **(Vb)**.
9) 4-((4-Chlorobenzyl)oxy) aniline **(Vc)**.
10) 4-((4-Bromobenzyl)oxy)aniline **(Vd)**.
11) 4-((4-Methylbenzyl)oxy)aniline **(Ve)**.
12) 4-Nitro-1-(4-bromophenoxy)benzene **(VIa)**.
13) 4-Nitro-1-(3-fluoro-4-chlorophenoxy)benzene **(VIb)**.
14) 4-(4-Bromophenoxy)aniline **(VIIa)**.
15) 4-(3-Fluoro-4-chloro phenoxy) aniline **(VIIa)**.
16) Ethyl 2-aminobenzo[*d*]thiazole-6-carboxylate **(VIII)**.
17) 6-Nitrobenzo[*d*]thiazol-2-amine **(XIX)**.
18) *N*-(6-Nitrobenzo[*d*]thiazol-2-yl)naphthalene-2-sulfonamide **(XX)**.
19) *N*-(6-Aminobenzo[*d*]thiazol-2-yl) naphthalene-2-sulfonamide **(XXI)**.

Also, it comprised the synthesis of the following new intermediates:

1) Ethyl 3-nitro-4-(phenethylamino)benzoate **(II)**.
2) 3-Nitro-4-(phenethylamino)benzoic acid **(III)**.
3) 1-((4-Trifluoromethylbenzyl)oxy)-4-nitrobenzene **(IVf)**.
4) 4-((4-Triflouromethylbenzyl)oxy)aniline **(Vf).**
5) Ethyl 2-(naphthalene-2-sulfonamido)benzo[*d*]thiazole-6-carboxylate **(IX)**.
6) 2-(Naphthalene-2-sulfonamido)benzo[*d*]thiazole-6-carboxylic acid **(X)**.
7) Ethyl 2-(3-nitro-4-(phenethylamino) benzamido)benzo[*d*]thiazole-6-carboxylate **(XIV)**.
8) 2-(3-Nitro-4-(phenethylamino)benzamido)benzo[*d*]thiazole-6-carboxylic acid **(XV)**.

Furthermore, the study involved the synthesis and characterization of the following new final compounds:

1) *N*-(6-(4-Phenylpiperazine-1-carbonyl)benzo[*d*]thiazol-2-yl)naphthalene-2-sulfonamide **(XIa)**.

2) *N*-(6-(4-(2-Fluorophenyl)piperazine-1-carbonyl)benzo[*d*]thiazol-2-yl)naphthalene-2-sulfonamide **(XIb)**.
3) *N*-(6-(4-(2-Methoxyphenyl)piperazine-1-carbonyl)benzo[*d*]thiazol-2-yl)naphthalene-2-sulfonamide **(XIc)**.
4) *N*-(6-(4-(4-Chlorophenyl)piperazine-1-carbonyl)benzo[*d*]thiazol-2-yl)naphthalene-2-sulfonamide **(XId)**.
5) *N*-(6-(4-(3,4-Dichlorophenyl)piperazine-1-carbonyl)benzo[*d*]thiazol-2-yl)naphthalene-2-sulfonamide **(XIe)**.
6) *N*-(6-(4-Benzhydrylpiperazine-1-carbonyl)benzo[*d*]thiazol-2-yl)naphthalene-2-sulfonamide **(XIf)**.
7) (*E*)-*N*-(6-(4-Cinnamylpiperazine-1-carbonyl)benzo[*d*]thiazol-2-yl)naphthalene-2-sulfonamide**(XIg)**.
8) *N*-(6-(4-(Tetrahydrofuran-2-carbonyl)piperazine-1-carbonyl)benzo[*d*]thiazol-2-yl)naphthalene-2-sulfonamide **(XIh)**.
9) *N*-(6-(4-(Benzo[d]dioxol-5-ylmethyl)piperazine-1-carbonyl)benzo[*d*]thiazol-2-yl)naphthalene-2-sulfonamide **(XIi)**.
10) *N*-(4-((3-Fluorobenzyl)oxy)phenyl)-2-(naphthalene-2-sulfonamido)benzo[*d*] thiazole-6-carboxamide **(XIIa)**.
11) *N*-(4-((3-Chlorobenzyl)oxy)phenyl)-2-(naphthalene-2-sulfonamido)benzo[*d*] thiazole-6-carboxamide **(XIIb)**.
12) *N*-(4-((4-Chlorobenzyl)oxy)phenyl)-2-(naphthalene-2-sulfonamido)benzo[*d*] thiazole-6-carboxamide **(XIIc)**.
13) *N*-(4-((4-Bromobenzyl)oxy)phenyl)-2-(naphthalene-2-sulfonamido)benzo[*d*] thiazole-6-carboxamide **(XIId).**
14) *N*-(4-((4-Methylbenzyl)oxy)phenyl)-2-(naphthalene-2-sulfonamido)benzo[*d*] thiazole-6-carboxamide **(XIIe)**.
15) *N*-(4-((4-Trifluormethylbenzyl)oxy)phenyl)-2-(naphthalene-2-sulfonamido)benzo [*d*]thiazole-6-carboxamide ***(*XIIf)**.
16) *N*-(4-(4-Chloro-3-fluorophenoxy)phenyl)-2-(naphthalene-2-sulfonamido) benzo[*d*]thiazole-6-carboxamide **(XIIIa)**.
17) *N*-(4-(4-Bromophenoxy)phenyl)-2-(naphthalene-2-sulfonamido)benzo[*d*] thiazole-6-carboxamide **(XIIIb)**.

18) *N*-(6-(4-(2-Methoxyphenyl) piperazine-1-carbonyl) benzo [*d*] thiazol-2-yl)-3-nitro-4-(phenethylamino)benzamide **(XVIa)**.
19) *N*-(6-(4-(3,4-Dichlorophenyl)piperazine-1-carbonyl)benzo[*d*]thiazol-2-yl)-3-nitro-4-(phenethylamino)benzamide **(XVIb)**.
20) *N*-(6-(4-Benzhydrylpiperazine-1-carbonyl)benzo[*d*]thiazol-2-yl)-3-nitro-(phenethylamino)benzamide **(XVIc)**.
21) **(***E***)-***N*-(6-(4-Cinnamylpiperazine-1-carbonyl)benzo[*d*]thiazol-2-yl)-3-nitro-4-(phenethylamino)benzamide **(XVId)**.
22) 3-Nitro-4-(phenethylamino)-*N*-(6-(4-(tetrahydrofuran-2-carbonyl)piperazine-1-carbonyl)benzo[*d*]thiazol-2-yl)benzamide **(XVIe)**.
23) *N*-(4-((3-Chlorobenzyl)oxy)phenyl)-2-(3-nitro-4-(phenethylamino)benzamido) benzo[*d*]thiazole-6-carboxamide **(XVIIa)**.
24) *N*-(4-((4-Bromobenzyl)oxy)phenyl)-2-(3-nitro-4-(phenethylamino)benzamido) benzo[*d*]thiazole-6-carboxamide **(XVIIb)**.
25) *N*-(4-((4-Methylbenzyl)oxy)phenyl)-2-(3-nitro-4-(phenethylamino)benzamido) benzo[*d*]thiazole-6-carboxamide **(XVIIc)**.
26) *N*-(4-((4-Trifluoromethylbenzyl)oxy)phenyl)-2-(3-nitro-4-(phenethylamino)benzamido)benzo[*d*]thiazole-6-carboxamide **(XVIId)**.
27) *N*-(4-(4-Chloro-3-fluorophenoxy)phenyl)-2-(3-nitro-4-(phenethylamino)benzamido)benzo[*d*]thiazole-6-carboxamide **(XVIIIa)**.
28) *N*-(4-(4-Bromophenoxy)phenyl)-2-(3-nitro-4-(phenethylamino)benzamido benzo[*d*]thiazole-6-carboxamide **(XVIIIb)**.
29) *N*-(6-(3-Phenylureido)benzo[*d*]thiazol-2-yl)naphthalene-2-sulfonamide **(XXIIa**).
30) *N*-(6-(3-(3-Chlorophenyl)ureido)benzo[*d*]thiazol-2-yl)naphthalene-2-sulfonamide **(XXIIb)**.
31) *N*-(6-(3-(3-Bromophenyl)ureido)benzo[*d*]thiazol-2-yl)naphthalene-2-sulfonamide **(XXIIc)**.
32) *N*-(6-(3-(3-Methoxyphenyl)ureido)benzo[*d*]thiazol-2-yl)naphthalene-2-sulfonamide **(XXIId)**.
33) *N*-(6-(3-(*m*-tolyl)ureido)benzo[*d*]thiazol-2-yl)naphthalene-2-sulfonamide **(XXIIe)**.