بسم الله الرحمن الرحيم

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تم رفع هذه الرسالة بواسطة / سامية زكى يوسف

بقسم التوثيق الإلكتروني بمركز الشبكات وتكنولوجيا المعلومات دون أدنى مسئولية عن محتوى هذه الرسالة.

ملاحظات: لا يوجد

Synthesis and biological evaluation of various heterocyclic compounds

Thesis
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Aya Ahmed Farahat

Pre-requisite Premaster Courses and Exams

Besides the work presented in this thesis, the candidate has attended and successfully passed the following general and special postgraduate courses in Pharmaceutical Chemistry:

- Statistics
- Instrumental Analysis
- Computer Sciences
- Physical Chemistry
- Pharmaceutical Chemistry
- Drug spectroscopy
- Selected Topics in Pharmaceutical Chemistry
- Drug Stereochemistry

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FULL PAPER

ARCH PHARM DPhG

Synthesis and in vitro antiproliferative activity of certain novel pyrazolo[3,4-*b*]pyridines with potential p38α MAPK-inhibitory activity

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Abstract

Novel series of pyrazolo[3,4-*b*]pyridines **9a–j** and **14a–f** were prepared via a one-pot three-component reaction. Compounds **9a–j** were synthesized by the reaction of 3-(4-chlorophenyl)-1-phenyl-1*H*-pyrazol-5-amine (**4**) with benzoyl acetonitriles **3a,b** and aldehydes **5a–e**, whereas the spiro derivatives **14a–f** were synthesized by the reaction of pyrazole derivative **4** with **3a–c** and indoline-2,3-diones **10a,b**. Screening of the antiproliferative activity of **9a–j** and **14a–f** revealed that **14a** and **14d** were the most potent analogues against HepG2 and HeLa cells, with IC_{50} = 4.2 and 5.9 μM, respectively. Moreover, compounds **9c** and **14a** could promote cell cycle disturbance and apoptosis in HepG2 cells, as evidenced by DNA flow cytometry and Annexin V-FITC/PI assays. Cell cycle analysis of **9c** and **14a** indicated a reduction in HepG2 cells in the G1 phase, with arrest in the S phase and the G2/M phase, respectively. Also, **9c** and **14a** are good apoptotic inducers in the HepG2 cell line. Furthermore, compounds **9h** and **14d** stood out as the most efficient anti-proliferative agents in the NCI 60-cell line panel screening, with mean GI % equal to 60.3% and 55.4%, respectively. Additionally, **9c**, **9h**, **14a**, and **14d** showed good inhibitory action against the cellular pathway regulator p38α kinase, with IC_{50} = 0.42, 0.41, 0.13, and 0.64 μM, respectively. A docking study was carried out on the p38α kinase active site, showing a binding mode comparable to that of reported p38 mitogen-activated protein kinase inhibitors. These newly discovered pyrazolo[3,4-*b*] pyridines could be considered as potential candidates for the development of newly targeted anticancer agents.

KEYWORDS

apoptosis, cytotoxic, enzyme inhibition, *N*-heterocycles, p38 kinase

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List of abbreviations:

A˚: Angstroms

ADMET: Absorption, Distribution, Metabolism, Excretion, and Toxicity study

ADT: Androgen deprivation therapy

AIs: Aromatase inhibitors

ALK: Anaplastic lymphoma kinase

Anal.calc.: analytical calculations

Asp: Aspartate

ATP: Adenosine-5'- triphosphate

BBB: Blood brain barrier

BMD: Bone mineral density

C-Docker: CHARMm Docker

CAR: Chimeric antigen receptor

CDK: Cyclin-dependent kinases.

CHARMm: Chemistry at HARvard Macromolecular Mechanics

CMGC: Cyclin-dependent kinases (CDKs), Mitogen-activated protein kinases (MAP kinases), Glycogen synthase kinases (GSK) and CDK-like kinases.

CYP2D6: Cytochrome P450 2D6.

DMF: Dimethyl formamide.

DMF-DMA: Dimethyl formamide- Dimethyl acetal

DMSO: Dimethyl sulfoxide

DYRK1A: Dual Specificity Tyrosine Phosphorylation Regulated Kina se 1A

EI-MS: Electron impact mass spectroscopy

EGFR: Epidermal growth factor receptor

Equiv.: Equivalent

FT-IR: Fourier transform-Infrared

GSK-3: Glycogen synthase kinase-3

Hela: cervical cancer cells named after Henrietta Lacks

Hrs: Hours

Hz: Hertz

IC_{50}: Half-maximal inhibitory concentration

LHRH: Luteinizing hormone-releasing hormone

Lys: Lysine

MAPK: Mitogen activated protein kinase

MCF-7: Michigan Cancer Foundation-7

Met: Methionine

MDM2: Mouse double minute 2 homolog

MHz: Mega hertz

mmole: millimole

MP: Melting point

MS: Mass Spectroscopy

MTAs: Microtubule-targeting agents

mTOR: Mammalian target of rapamycin

NCI: National Cancer Institute

NMR: Nuclear magnetic resonance

NSCLC: Non-Small Lung Cell cancer

PDB: Protein Data Bank

PI3Ks: Phosphoinositide 3-kinases

Pim -1: Proviral integration site for Moloney murine leukemia virus-1

PI/RNase: Propidium iodide

Ppm: Part per million

PSA: Polar surface area

RMSD: Root mean square deviation

Rt: Room temperature

RTK: Receptor tyrosine kinase

SAR: Structure activity relationship

SERMs: Selective estrogen receptor modulators

S1P: sphingosine-1-phosphate

TEA: Triethyl amine

Thr: Threonine

TLC: Thin layer Chromatography

U.V: Ultraviolet

VEGFR: Vascular endothelial growth factor receptor

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Cancer is a huge global health concern and is the leading cause of human mortality exceeded only by cardiovascular diseases. Malignant tumor an alternative name to cancer which is a large group of diseases involving loss of control on normal cell growth and function with the capability of spreading to other body parts. A major problem in treating cancer is the fact that it is not a single disease. Different defects in cellular function lead to formation of more than 200 different types of cancer each needs particular diagnosis and treatment.

In this study, a series of pyrazolo[3,4-*b*]pyridines have been designed and synthesized as anticancer compounds. The design based on investigation of the reported SAR studies, bioisosteric modifications of the lead compound and distinguishing the key interactions with the binding site *in silico*. Synthesis of the new compounds was then accomplished and their structures were confirmed by various spectral and microanalytical data.

This study involved the synthesis and the characterization of the following novel compounds:

1. *3-(4-Chlorophenyl)-1,4-diphenyl-1H-pyrazolo[3,4-b]pyridine (**VIa**)*
2. *3,4-Bis(4-chlorophenyl)-1-phenyl-1H-pyrazolo[3,4-b]pyridine (**VIb**)*
3. *3-(4-Chlorophenyl)-1-phenyl-4-(p-tolyl)-1H-pyrazolo[3,4-b]pyridine (**VIc**)*
4. *3-(4-Chlorophenyl)-4-(4-methoxyphenyl)-1-phenyl-1H-pyrazolo[3,4-b]pyridine (**VId**)*
5. *3-(4-Chlorophenyl)-1-phenyl-4-(thiophen-2-yl)-1H-pyrazolo[3,4-b]pyridine (**VIe**)*
6. *3-(4-Chlorophenyl)-1,4,6-triphenyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (**VIIIa**).*
7. *3,4-Bis(4-chlorophenyl)-1,6-diphenyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (**VIIIb**).*
8. *3-(4-Chlorophenyl)-4-(4-hydroxyphenyl)-1,6-diphenyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (**VIIIc**).*
9. *3-(4-Chlorophenyl)-4-(4-methoxyphenyl)-1,6-diphenyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (**VIIId**).*
10. *3-(4-Chlorophenyl)-4-(4-nitrophenyl)-1,6-diphenyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (**VIIIe**).*
11. *3-(4-Chlorophenyl)-6-(4-methoxyphenyl)-1,4-diphenyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (**VIIIf**).*
12. *3,4-Bis(4-chlorophenyl)-6-(4-methoxyphenyl)-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (**VIIIg**).*
13. *3-(4-Chlorophenyl)-4-(4-hydroxyphenyl)-6-(4-methoxyphenyl)-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (**VIIIh**).*
14. *3-(4-Chlorophenyl)-4,6-bis(4-methoxyphenyl)-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (**VIIIi**).*
15. *3-(4-Chlorophenyl)-6-(4-methoxyphenyl)-4-(4-nitrophenyl)-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (**VIIIj**).*
16. *3'-(4-Chlorophenyl)-2-oxo-1',6'-diphenyl-1',7'-dihydrospiro[indoline-3,4'-pyrazolo[3,4-b]pyridine]-5'-carbonitrile (**Xa**).*
17. *5-Chloro-3'-(4-chlorophenyl)-2-oxo-1',6'-diphenyl-1',7'-dihydrospiro[indoline-3,4'-pyrazolo[3,4-b]pyridine]-5'-carbonitrile (**Xb**).*
18. *3',6'-Bis(4-chlorophenyl)-2-oxo-1'-phenyl-1',7'-dihydrospiro[indoline-3,4'-pyrazolo[3,4-b]pyridine]-5'-carbonitrile (**Xc**).*
19. *5-Chloro-3',6'-bis(4-chlorophenyl)-2-oxo-1'-phenyl-1',7'-dihydrospiro[indoline-3,4'-pyrazolo[3,4-b]pyridine]-5'-carbonitrile(**Xd**).*
20. *3'-(4-Chlorophenyl)-6'-(4-methoxyphenyl)-2-oxo-1'-phenyl-1',7'-dihydrospiro[indoline-3,4'-pyrazolo[3,4-b]pyridine]-5'-carbonitrile (**Xe**).*
21. *5-Chloro-3'-(4-chlorophenyl)-6'-(4-methoxyphenyl)-2-oxo-1'-phenyl-1',7'-dihydrospiro[indoline-3,4'-pyrazolo[3,4-b]pyridine]-5'-carbonitrile (**Xf**).*

The biological assessment was accomplished by testing the anticancer activity, enzyme inhibition activity, cell cycle and apoptosis assay. The anticancer activity of the newly synthesized compounds against HepG2 and Hela cell lines revealed that compound **Xa** has potent activity as anti-proliferative agents against HepG2 cell line with IC_{50} = 4.2 µM compared to reference compound (doxorubicin) with IC_{50} = 1.7 µM. On the other side, compound **Xd** has potent activity as anti-proliferative agents against Hela cell line with IC_{50} = 5.9 µM compared to cisplatin IC_{50} = 4.8 µM. Cell cycle and apoptosis assay of compounds **(VIIIc** and **Xa)** was performed at the Diagnostic and Confirmatory lab in the Holding Company for Biological Products and Vaccines (VACSERA), Giza, Egypt. Both compounds **VIIIc** and **Xa** promoted cell cycle disturbance and apoptosis in HepG2 cells as evidenced by the DNA flow cytometry and Annexin V-FITC/PI assays. Furthermore, (**VIIIh**) and (**Xd**) stood out as the most efficient anti-proliferative agents against NCI 60-cell line panel screening with mean GI % equals to 60.3 and 55.4%, respectively.

The enzyme inhibition assay was performed at the Diagnostic and Confirmatory lab in the Holding Company for Biological Products and Vaccines (VACSERA), Giza, Egypt. The enzymatic inhibitory activity of the synthesized compounds was assessed against the cellular pathway regulator p38*α* kinase. Compounds **VIIIc, VIIIh, Xa** and **Xd** exhibited good inhibitory action against cellular pathway regulator p38α kinase with IC_{50} = 0.42, 0.41, 0.13 and 0.64 µM, respectively, which are comparable to those of SB 202190 (IC_{50} = 0.188 µM).

Finally, Computer aided ADME study and toxicity prediction were also performed using Accelrys Discovery Studio® 2.5 software.

1. Introduction

1.1 Cancer

1.1.1 Overview

Cancer was defined as a disease in which some of the body's cells grow abnormally and uncontrollably which can spread to other parts of the body. Cancer cells ignore the normal guidelines of cell division, resulting in uncontrolled cell growth and proliferation. When this proliferation continues and spreads it can result in patient death. About 90% of death in cancer patients is due to tumor spreading which is called metastasis [1].

Modern cancer biology developed a principle that all mammalian cells virtually share almost identical molecular networks that control cell proliferation, differentiation and cell death. This principle stating that normal cells are transformed into cancers as a result of changes in these networks at the molecular, biochemical and cellular level, thus suggesting a limited number of ways this disturbance can occur for each cell [1].

In the past 50 years, cancer researches provided us with a clear perception about the development of cancer cells. Cancer is a genetic disease that is caused by changes to genes which control the way our cells function, especially how they grow and divide. Proteins produced due to these changes (DNA mutations) disrupt the accuracy of cellular balance between cell division and dormancy, resulting in continuous cell division forming cancers [1].

1.1.2 Development

Development of cancer in the body cells is a multistep process comprising mutation and cell selection with accelerating capacity for proliferation, survival, invasion, and metastasis. (**Figure 1)**