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# *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

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# Synthesis and in vitro antiproliferative activity of certain novel pyrazolo[3,4-*b*]pyridines with potential p38 $\alpha$ 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-1H-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<sub>50</sub> = 4.2 and 5.9  $\mu$ M, 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 p38 $\alpha$  kinase, with IC<sub>50</sub> = 0.42, 0.41, 0.13, and 0.64  $\mu$ M, respectively. A docking study was carried out on the p38 $\alpha$  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

**Table of Contents:**

<b>Acknowledgements .....</b>	<b>II</b>
<b>Table of Contents.....</b>	<b>V</b>
<b>List of figures.....</b>	<b>VII</b>
<b>List of tables.....</b>	<b>VIII</b>
<b>List of Abbreviations .....</b>	<b>IX</b>
<b>Abstract.....</b>	<b>XI</b>
<b>1. Introduction .....</b>	<b>1</b>
1.1 Cancer .....	1
1.1.1 Overview .....	1
1.1.2 Development .....	1
1.1.3 Hallmarks of cancer.....	3
1.1.4 Epidemiology .....	5
1.1.5 Etiology .....	6
1.1.6 Treatment.....	6
1.2 Pyrazolo[3,4- <i>b</i> ]pyridines.....	17
1.2.1 Overview .....	17
1.2.2 Types of anticancer compounds with pyrazolo[3,4- <i>b</i> ]pyridines nucleus according to the pharmacological action .....	18
<b>2. Rationale and Design.....</b>	<b>27</b>
2.1 Design process based on exploration of the previously revealed SAR and identification of key interaction with the binding site of p38 kinase inhibitors.....	28
2.2 Structure based design of targeted compounds based on the previous study.....	29
2.3 Primary evaluation of some selected compounds using molecular docking.....	31
2.3.1. Validation of docking protocol.....	31
2.3.2. Molecular docking.....	31
2.4 Synthetic schemes for synthesis of the designed compounds.....	35
2.4.1 Scheme 1 for preparation of 1H-pyrazolo[3,4- <i>b</i> ]pyridines <b>VIa-e</b> .....	35
2.4.2 Scheme 2 for preparation of pyrazolo[3,4- <i>b</i> ]pyridine-5-carbonitriles <b>VIIIa-j</b> .....	37
2.4.3 Scheme 3 for preparation of 2-oxo-dihydrospiro[indoline-3,4'-pyrazolo[3,4- <i>b</i> ]pyridine]-5'-carbonitrile <b>Xa-f</b> .....	38
<b>3. Results and Discussion .....</b>	<b>39</b>
3.1. Chemistry.....	39
3.1.1. Scheme 1a.....	39

3.1.2.	Scheme 1b .....	40
3.1.3.	Scheme 1c.....	40
3.1.4.	Scheme 1d .....	41
3.1.5.	Scheme 2.....	42
3.1.6.	Scheme 3.....	43
3.2.	Biological Evaluation .....	44
3.2.1.	<i>In vitro</i> anti-proliferative activity against HepG2 and Hela cell lines.....	44
3.2.2.	<i>In vitro</i> anti-proliferative activity assay against NCI 60-cell line panel.....	46
3.2.3.	Structure activity relationship (SAR).....	50
3.2.4.	Effect of compound <b>VIIc</b> and <b>Xa</b> on cell cycle distribution of HepG2 cell line after 48hrs incubation.....	50
3.2.5.	Apoptosis assay.....	51
3.2.6.	In-vitro P38 kinase $\alpha$ inhibitory activity.....	52
3.3.	In-silico ADMET study.....	53
3.3.1.	Computer aided ADME.....	53
3.3.2.	Toxicity prediction.....	55
4.	<b>Conclusion</b> .....	56
5.	<b>Experimental</b> .....	57
5.1.	Chemistry .....	57
5.1.1.	Materials and instrumentation.....	57
5.1.2.	Synthesis.....	58
5.2.	Biological evaluation: .....	77
5.2.1.	<i>In vitro</i> anti-proliferative activity against HepG2 and Hela cell lines.....	77
5.2.2.	<i>In vitro</i> anti-proliferative activity assay against NCI 60-cell line panels.....	78
5.2.3.	Cell cycle analysis.....	79
5.2.4.	Apoptosis assay.....	79
5.2.5.	P38 kinase $\alpha$ assay.....	80
5.3.	Molecular docking study .....	80
5.3.1.	Protein preparation for docking.....	80
5.3.2.	Ligand and active compounds preparation for docking.....	80
5.3.3.	Docking process .....	81
6.	<b>References</b> .....	82
7.	<b>Supplementary data</b> .....	88

## List of figures:

Figure 1. Stages of tumor developmet .....	2
Figure 2. The Hallmarks of Cancer .....	4
Figure 3 presents the distribution of all-cancer incidence according to world area for both sexes combined .....	5
Figure 4 presents the distribution of all-cancer mortality according to world area for both sexes combined .....	5
Figure 5. Cancer causes .....	6
Figure 6. Tumor angiogenesis .....	8
Figure 7. General structure of pyrazolo[3,4- <i>b</i> ]pyridine .....	17
Figure 8. Schematic representation of the structural characteristics necessary for compound ( <b>41</b> ) as p38 $\alpha$ kinase inhibitor.....	28
Figure 9. Binding mode of compound <b>41</b> to p38 $\alpha$ kinase hinge region through hydrogen bonding and hydrophobic interaction. ....	29
Figure <b>10</b> . Fitness of the essential pharmacophoric features of p38 $\alpha$ kinase inhibitors on the designed compounds revealing the red color that presents the main scaffold. The terminal substituted aromatic moiety occupying the sugar pocket presented in gray shades, cyano group and N of pyridine group with the hinge region presented in pink shades and finally the pyrazolopyridine core with the phosphate binding region presented in cyan shades.....	30
Figure 11. (A) The alignment between the co-crystallized native ligand (blue) and the pose of the same compound retrieved from docking (yellow) at p38 MAPK binding site using CDOCKER. (B) Retrieved docking pose of the ligand showing the same key interactions as reported. ....	31
Figure 12. Cell cycle analysis after 48 hrs incubation of HepG2 cells with the tested compounds. A: compound <b>VIIIc</b> ; B: compound <b>Xa</b> ; C: control .....	51
Figure 13. Effect of compounds <b>VIIIc</b> and <b>Xa</b> over the AV-FITC-positive staining percentages in HepG2 cells. a: Compound <b>VIIIc</b> ; b: Compound <b>Xa</b> ; c: control .....	52
Figure 14. <i>In silico</i> Predictive ADME study. ....	54



**List of tables:**

Table 1. Docking interactions and amino acids residues involved in the binding of some of the designed compounds with p38 $\alpha$ MAPK (PDB code 1A9U) .....	32
Table 2: <i>In vitro</i> anti-proliferative activity of the synthesized .....	45
Table 3. In vitro GI % for compounds <b>VIa-e</b> and <b>VIIIa-e</b> , at 10 $\mu$ M concentration, towards the subpanel cancer cell lines.....	47
Table 4. In vitro GI % for compounds <b>VIIIb-i</b> and <b>Xa-f</b> at 10 $\mu$ M concentration, towards the subpanel cancer cell lines.....	48
Table 5. Inhibitory activity of compounds <b>VIIIc</b> , <b>VIIIh</b> , <b>Xa</b> and <b>Xd</b> against p38 $\alpha$ kinase. ....	53
Table 6. <i>In silico</i> predictive ADME parameters calculated for the newly synthesized compounds. ....	54
Table 7. Toxicity prediction for the newly synthesized compounds. ....	55

***List of abbreviations:***

**Å**: 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 Kinase 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<sub>50</sub>**: 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

**Abstract:**

*Title of thesis:*

***“Synthesis and biological evaluation of various heterocyclic compounds”***

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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-1*H*-pyrazolo[3,4-*b*]pyridine (**VIa**)
2. 3,4-Bis(4-chlorophenyl)-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine (**VIb**)
3. 3-(4-Chlorophenyl)-1-phenyl-4-(*p*-tolyl)-1*H*-pyrazolo[3,4-*b*]pyridine (**VIc**)
4. 3-(4-Chlorophenyl)-4-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine (**VIId**)
5. 3-(4-Chlorophenyl)-1-phenyl-4-(thiophen-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridine (**VIe**)
6. 3-(4-Chlorophenyl)-1,4,6-triphenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (**VIIIa**).
7. 3,4-Bis(4-chlorophenyl)-1,6-diphenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (**VIIIb**).
8. 3-(4-Chlorophenyl)-4-(4-hydroxyphenyl)-1,6-diphenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (**VIIIc**).
9. 3-(4-Chlorophenyl)-4-(4-methoxyphenyl)-1,6-diphenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (**VIIId**).
10. 3-(4-Chlorophenyl)-4-(4-nitrophenyl)-1,6-diphenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (**VIIIe**).
11. 3-(4-Chlorophenyl)-6-(4-methoxyphenyl)-1,4-diphenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (**VIIIIf**).
12. 3,4-Bis(4-chlorophenyl)-6-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (**VIIIg**).
13. 3-(4-Chlorophenyl)-4-(4-hydroxyphenyl)-6-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (**VIIIh**).
14. 3-(4-Chlorophenyl)-4,6-bis(4-methoxyphenyl)-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (**VIIIi**).
15. 3-(4-Chlorophenyl)-6-(4-methoxyphenyl)-4-(4-nitrophenyl)-1-phenyl-1*H*-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  $IC_{50} = 4.2 \mu M$  compared to reference compound (doxorubicin) with  $IC_{50} = 1.7 \mu M$ . On the other side, compound **Xd** has potent activity as anti-proliferative agents against Hela cell line with  $IC_{50} = 5.9 \mu M$  compared to cisplatin  $IC_{50} = 4.8 \mu M$ . 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 $\alpha$  kinase. Compounds **VIIIc**, **VIIIh**, **Xa** and **Xd** exhibited good inhibitory action against cellular pathway regulator p38 $\alpha$  kinase with  $IC_{50} = 0.42, 0.41, 0.13$  and  $0.64 \mu M$ , respectively, which are comparable to those of SB 202190 ( $IC_{50} = 0.188 \mu M$ ).

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**)