كلية الصيدلة

Discovery of Certain Organic Compounds as Targeted Anticancer Agents

Thesis
Presented by

Maiy Youssef Mohamed Jaballah

BSc. in Pharmaceutical Sciences (May 2002)
MSc. in Pharmaceutical Sciences (Pharmaceutical Chemistry) (December 2007)
Assistant Lecturer of Pharmaceutical Chemistry
Ain Shams University
Submitted in partial fulfillment of the
Doctorate of Philosophy Degree
In Pharmaceutical Sciences (Pharmaceutical Chemistry)
Under the supervision of

Khaled Abouzid Mohamed Abouzid

Professor and Chairman of Pharmaceutical Chemistry
Department
Faculty of Pharmacy
Ain Shams University

Nasser Saad Mohamed Ismail

Professor of Pharmaceutical Chemistry
Faculty of pharmacy -Ain Shams University

Rabah Ahmed Taha Serya

Associate professor of Pharmaceutical Chemistry
Faculty of pharmacy -Ain Shams University

Faculty of Pharmacy

Ain Shams University

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List of Abbreviations

Å: Angstrom
ABL: Abelson tyrosine kinase
ADMET: Absorption, Distribution, Metabolism, Excretion, and Toxicity study
ALK: Anaplastic lymphoma kinase
ANLL: Acute Non-Lymphocytic Leukemia
AIs: Aromatase Inhibitors
AQ Sol: Aqueous solubility
Asp: Aspartate
ATP: Adenine-5'-triphosphate
BBB: Blood brain barrier
BCR: breakpoint cluster region protein
BSA: Bovine serum albumin
BTK: Bruton's Tyrosine Kinase
°C: Celsius
C-Fms: Colony-Stimulating factor-1 receptor
C-kit: v-kit (Hardy-Zuckerman 4 feline) sarcoma viral oncogene
CHARMm: Chemistry at HARvard Macromolecular Mechanics
CNS: Central nervous system
CYP 450: Cytochrome P450
Cys: Cysteine
D_2O: Deuterium oxide
DFG: Aspartate- Phenylalanine- Glycine
DIPEA: Diisopropyl ethylamine
DMF: Dimethyl formamide
DMSO: Dimethyl sulfoxide
DNA: Deoxyribonucleic acid
EI-MS: Electron impact mass spectroscopy
EGFR: Epidermal growth factor receptor
ERA: Estrogen Receptor Antagonists
Fab: Fragment antigen-binding
FDA: Food and Drug Administration
FGFR: Fibroblast growth factor receptor
FLT: FMS-like receptor tyrosine kinase
FT-IR: Fourier transform-Infrared
Glu: Glutamate
HIA: Human intestinal absorption
His: Histidine

List of Abbreviations

Hrs: hours
HUVEC: Human umbilical vein endothelial cells
Hz: Hertz
IC_{50}: Half-maximal inhibitory concentration
IGFR: Insulin-like growth factor receptor
IRK: Insulin receptor kinase
ITK: Interleukin-2-inducible T-cell kinase
JAK: Janus kinase
KDa: Kilo Dalton
KDR: Kinase insert domain receptor
Lys: Lysine
Lck: Lymphocyte-specific protein tyrosine kinase
6-MP: 6-Mercaptopurine
m.p.: Melting point
Min: Minutes
MHz: Mega hertz
µM: Micromole
mmol: Millimole
µl: Microliter
MS: Mass spectroscopy
NCI: National Cancer Institute
NIH: National Institutes of Health
nM: Nanomole
NMR: Nuclear magnetic resonance
NRTK: Non-receptor tyrosine kinase
NSCLC: Non-Small Lung Cell cancer
PARP: Poly ADP ribose polymerase
Pd-C: Palladium on carbon
PDB: Protein data bank
PDGFR: Platelet derived growth factor receptor
PDT: Photodynamic therapy
Phe: Phenyl alanine
PM: Picometre
PPB: Plasma protein binding
Ppm: Part per million
PSA: Polar surface area
Psi: Pound per Square Inch
Raf: v-raf murine sarcoma viral oncogene
Ras: Rat sarcoma

List of Abbreviations

RMSD: Root mean square deviation
RNA: Riboneucleic Acid
rt: Room temperature
RTK: Receptor tyrosine kinase
SAR: Structure activity relationship
SMART: string matching algorithms research tool
SRC: Sarcoma (Schmidt-Ruppin A-2) Viral Oncogene
TEA: Triethyl amine
THF: Tetrahydrofuran
Tie-2: Tyrosine kinase with immunoglobulin-like and EGF-like domains 2
TK: Tyrosine kinase
TKI: Tyrosine kinase inhibitors.
TLC: Thin layer Chromatography
VEGFR: Vascular endothelial growth factor receptor

Abstract

Cancer, or malignant tumor, is a myriad of diseases involving abnormal cell growth with the potential to invade or metastasize to other parts of the body. therefore, there is a need for newer treatment strategies with novel drugs acting at different pathways for treatment of malignancies.

Targeted therapies act by blocking essential biochemical pathways or mutant proteins that are required for tumour cell growth and survival. In the past few decades, protein kinases has drawn much attention as being of the most important drug targets for treatment of many diseases; especially cancer. Since, dysregulation and mutation in this family of ezymes play causual role in the development and maintenance of different types of cancer. Particularly, Vascular endothelial growth factor receptor-2 (VEGFR2) which is implemented in the maintanence of angiogenesis.

Angiogensis; the growth of new blood vessels; is considered one of the important hallmarks of cancer. Hence there is a growing interest in VEGFR2 inhibition and its therapeutic implementation in cancer treatment in light of its unique ability to regulate cancer cell proliferation and metastasis through inhibiting angiogenesis, eventually leading to death of tumor cells.

In the current study, a series of pyridazine based scaffolds (**Series 1 to 5**) were designed and synthesized as VEGFR2 inhibitors. The design focused on exploration of the previous revealed SAR studies, bioisosteric modifications of the lead compounds both in market and in clinical studies as well as identification of the key interactions with the binding site *in silico.* The structures of synthesized compounds were confirmed by various spectral and microanalytical data.

Analysis of the VEGFR2 enzyme activity revealed that five of the synthesized compounds; namely (**XXIIb, XXVIb, XXIXa, XXIXb and XXXVII)** exhibited potent VEGFR2 inhibitory activity with IC_{50} values equal to 1.8µM, 1.3µM, 1.4µM, 107nM, 60nM respectively. Moreover, the above compounds were further evaluated for their VEGF-stimulated proliferation of human umbilical vein endothelial cells (HUVEC) revealing moderate to potent inhibition at 10 µM concentration; especially **XXVIb** which displayed 99.82% inhibition.

Abstract

Evaluation of the anti-proliferative activities of the compounds were attempted. Fourteen of the final Compounds **(XIXa, XXVIc, XXIXb, XXIXc, XXXa, XXXb, XXXIb, XLa, XLb, XLe, XLIa, XLIb, XLII, XLIIIa)** were selected by the National Cancer Institute "NCI" for single dose screening program at 10 μM in the full NCI 60 cell panel. While the 6-chloropyridazine derivative **(XXXb)** showed remarkably the lowest cell growth promotion, hence good anti-proliferative activity against different cell lines compared to the rest of the compounds; it was noticed that compounds (**XLa, XLb, XLe, XLIIIa**) demonstrated 71.4, 67.2, 64.6 and 47.2% growth inhibition almost exclusively against breast cancer cell line T47D.

Furthermore, nine of the synthesized compounds were evaluated for their antiproliferative activities against T47D cell line. Most of the investigated compounds showed IC_{50} in low nanomolar range; especially compounds **(XXIIe)** and **(XLd)** ; they displayed IC_{50} values of 0.75 and 0.94 respectively.

Since it was reported that the aromatase enzyme is overexpressed in breast cancer cell line T47D, thus, aromatase inhibitory activity was measured for compounds (**XXIIe)** and **(XLd),** which displayed 89 and 82% inhibitiory activities respectively. These percent inhibition was superior to that of the refrence drug ; Letrozole (aromatase percentage inhibition of 65%). Both compounds **XXIIe** and **XLd** displayed an IC_{50} against aromatase enzyme of 2.6 and 3.1 μM respectively.

To further exploit anti-tumor effect of compound **XXIIe** in T47D cell line; Level of caspase 3 RNA for compound **XXIIe** was measured. Caspase3 levels; one of the crucial mediators of apoptosis in cancer cells, were elevated by 92 thousand folds upon treatment with **XXIIe** compared to the control. These results confirmed that **(XXIIe)** exhibited its antiproliferative activity against breast cancer cell line T47D via induction of apoptosis revealed in elevated Caspase3 levels as well as inhibition of aromatase enzyme

Finally, a thorough Molecular docking, using C-DOCKER protocol in Discovery Studio 3.5 Software, was attempted to investigate the binding mode of the targeted compounds and interpret their variable inhibitory activity. Computer aided ADMET study was also performed using the same software.

Abstract

This study involved the synthesis of the following unavailable reported intermediates:

1. 1-(3,4-Dichlorophenyl)-3-(3-nitrophenyl)urea **(Ia)**
2. 1-(4-Chloro-3-(trifluoromethyl)phenyl)-3-(3-nitrophenyl)urea**(Ib)**
3. 1-(3-Aminophenyl)-3-(3,4-dichlorophenyl)urea **(IIa)**
4. 1-(3-Aminophenyl)-3-(4-chloro-3-(trifluoromethyl)phenyl)urea**(IIb)**
5. 1-(3,4-Dichlorophenyl)-3-(4-nitrophenyl)urea **(IIIa)**
6. 1-(4-Chloro-3-(trifluoromethyl)phenyl)-3-(4-nitrophenyl)urea **(IIIb)**
7. 1-(3,5-Dimethoxyphenyl)-3-(4-nitrophenyl)urea **(IIIc)**
8. 1-(4-Aminophenyl)-3-(3,4-dichlorophenyl)urea **(IVa)**
9. 1-(4-Aminophenyl)-3-(4-chloro-3-(trifluoromethyl)phenyl)urea **(IVb)**
10. 1-(4-Aminophenyl)-3-(3,5-dimethoxyphenyl)urea **(IVc)**
11. 3-(3,4-Dichlorobenzyl)-6-nitrobenzo[d]thiazol-2(3H)-one **(VIa)**
12. 3-(3-Bromobenzyl)-6-nitrobenzo[d]thiazol-2(3H)-one **(VIb)**
13. 6-Amino-3-(3,4-dichlorobenzyl)benzothiazol-2(3H)-one **(VIIa)**
14. 6-Amino-3-(3-bromobenzyl)benzothiazol-2(3H)-one **(VIIb)**
15. 1H-Indazol-5-amine **(IX)**
16. 1,2-Dihydropyridazine-3,6-dione **(X)**
17. 3,6-Dichloropyridazine **(XI)**
18. 3-Chloro-6-hydrazinylpyridazine **(XV)**
19. 6-Chloro-[1,2,4]triazolo[4,3-b]pyridazin-3-amine **(XVI**)
20. 6-[1,2,4]Triazolo[4,3-b]pyridazin-3-amine**(XVIII)**
21. 6-(4-Nitrophenyl)pyridazin-3(2H)-one **(XX)**
22. 6-(4-Aminophenyl)pyridazin-3(2H)-one **(XXI)**
23. 3-Nitro acetophenone **(XXIII)**
24. 6-(3-Nitrophenyl)pyridazin-3(2H)-one **(XXIV)**
25. 6-(3-Aminophenyl)pyridazin-3(2H)-one (XXV)
26. N-(4-((6-Chloropyridazin-3-yl)oxy)phenyl)acetamide **(XXVII)**
27. 6-(4-Aminophenoxy)pyridazin-3(2H)-one**(XXVIII)**
28. 4-((6-Chloropyridazin-3-yl)amino)benzoic acid **(XXXVIII)**

29. 6-Methoxy-N-(4-nitrophenyl)pyridazin-3-amine **(XXXV)**

30. N1-(6-methoxypyridazin-3-yl)benzene-1,4-diamine **(XXXVI)**

Also, it comprised the following new intermediates:

1. 1-(3,5-Dimethoxyphenyl)-3-(3-nitrophenyl)urea **(Ic)**
2. 1-(3-Aminophenyl)-3-(3,5-dimethoxyphenyl)urea **(IIc)**
3. N-(6-Chloropyridazin-3-yl)-1H-indazol-5-amine **(XXXII)**

In addition, the study involved the synthesis and the characterization of the following new-targeted compounds:

1. 1-(3,5-Dimethoxyphenyl)-3-(pyridazin-3-yl)urea **(XIVa)**
2. 1-(4-Chloro-3-(trifluoromethyl)phenyl)-3-(pyridazin-3-yl)urea **(XIVb)**
3. 1-(6-Chloro-[1,2,4]triazolo[4,3-b]pyridazin-3-yl)-3-(3,4-dichlorophenyl)urea **(XVII)**
4. 1-(3,4-Dichlorophenyl)-3-(6-morpholino-[1,2,4]triazolo[4,3-b]pyridazin-3-yl)urea **(XIXa)**
5. 1-(4-Chloro-3-(trifluoromethyl)phenyl)-3-(6-morpholino-[1,2,4]triazolo[4,3-b]pyridazin-3-yl)urea **(XIXb)**
6. 1-(3-Methoxyphenyl)-3-(6-morpholino-[1,2,4]triazolo[4,3-b]pyridazin-3-yl)urea**(XIXc)**
7. 1-(3,4-Dichlorophenyl)-3-(4-(6-oxo-1,6-dihydropyridazin-3-yl)phenyl)urea **(XXIIa)**
8. 1-(4-Chloro-3-(trifluoromethyl)phenyl)-3-(4-(6-oxo-1,6-dihydropyridazin-3-yl)phenyl) urea **(XXIIb)**
9. 1-(4-Chloro-2-methylphenyl)-3-(4-(6-oxo-1,6-dihydropyridazin-3-yl)phenyl)urea **(XXIIc)**
10. 1-(4-Chlorophenyl)-3-(4-(6-oxo-1,6-dihydropyridazin-3-yl)phenyl)urea **(XXIId)**
11. 1-(4-(6-Oxo-1,6-dihydropyridazin-3-yl)phenyl)-3-(4-(trifluoromethyl)phenyl)urea **(XXIIe)**
12. 1-(4-(6-Oxo-1,6-dihydropyridazin-3-yl)phenyl)-3-(3-(methoxy)phenyl)urea **(XXIIf)**
13. 1-(3,4-Dichlorophenyl)-3-(3-(6-oxo-1,6-dihydropyridazin-3-yl)phenyl) urea**(XXVIa)**