Molecular Design and Synthesis of Certain Quinoxaline Derivatives with Potential Anticancer Activity

Thesis
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List of Abbreviations

5-FU: 5-Fluorouuracil

6-MP: 6-Mercaptopurine

ATP: Adenosine triphosphate

BC: Before Christ

DFG: Aspartate- Phenylalanine- Glycine

DMF: Dimethyl formamide

DNA: Deoxyribo Neucleic Acid

EC: Endothelial cells

FDA: Food and Drug Administration

FGFR: Fibroblast growth factor receptor

FT-IR: Fourier transform-Infrared

GIST: gastrointestinal stromal tumors

GOLD: Genetic optimization of ligand docking

HUVEC: Human umbilical vein endothelial cell

IBX: 2-Iodoxybenzoic acid

KDa: Kilodalton

KDR: Kinase insert domain

MHz: Mega hertz

MS: Mass spectroscopy

NCI: National Cancer Institute.

NMR: Nuclear magnetic resonance

NRTK: Non- receptor tyrosine kinase

PDGFR: Platelet-derived growth factor receptor

PIGF: Placental growth factor

rt: Room temperature

RTK: Receptor tyrosine kinase

SAR: Structure Activity Relationship

SARMs: Selective androgen receptor modulators

SBSSA: Silica-bonded S-sulfonic Acid

SERMs: Selective estrogen receptor modulators

SMI: Small molecule inhibitors

TCA: Trichloro acetic acid

TEA: Triethyl amine

THF: Tetrahydrofuran

TK: Tyrosine kinase

TLC: Thin layer Chromatography

USA: United States of America

VEGFR: Vascular endothelial growth factor receptor

WHO: World Health Organization

Y: Tyrosine amino acid

µM: Micromole

Abstract:

1. Introduction

Cancer is one of the most common causes of death, taking nearly 7 million lives each year worldwide. Due to the low selectivity and the high side effects seen by the traditional chemotherapeutic agents, tremendous efforts are being exerted to get more selective anticancer agents. This required thorough study of signal transduction pathways that holds the promise of efficacy with minimal toxicity. Vascular endothelial growth factor receptor (VEGFR) was identified as one of the efficient targets for evolving new anticancer agents having the desired selectivity on cancerous cells. By targeting VEGFR, angiogenesis is greatly inhibited leading to the death of the tumor cells.

2. Rationale and Design

In our current study, new quinoxaline derivatives were explored for its activity against VEGFR-2. The targeted compounds were designed as type-II inhibitors based on comprehensive SAR study. Synthesis of the designed quinoxaline-based compounds was accomplished and their structures were confirmed by various spectral and micro analytical data.

3. Chemistry

This thesis comprises the synthesis of the following reported starting materials and intermediates:

1. Quinoxaline-2,3(1H,4H)-dione **(I)**.
2. 6-Nitroquinoxaline-2,3(1H,4H)-dione **(II)**.
3. 2,3-Dichloro-6-nitroquinoxaline **(III)**.
4. Quinoxalin-2(1H)-one **(XI).**

5. 6-Nitroquinoxalin-2(1H)-one **(XII).**
6. 2-Chloro-6-nitroquinoxaline **(XIII).**
7. 1-(4-Nitrophenyl)-3-phenylurea **(XIXa)**
8. 1-(3-Chlorophenyl)-3-(4-nitrophenyl)urea **(XIXb)**
9. 1-(4-Aminophenyl)-3-phenylurea **(XXa)**
10. 1-(4-Aminophenyl)-3-(3-chlorophenyl)urea **(XXb)**

Also, the synthesis and the characterization of the following new intermediate compounds was achieved:

1. 3-Chloro-N-(4-methoxyphenyl)-6-nitroquinoxalin-2-amine **(IV).**
2. 3-((4-Methoxyphenyl)amino)-7-nitroquinoxalin-2(1H)-one **(V).**
3. 7-Amino-3-((4-methoxyphenyl)amino)quinoxalin-2(1H)-one **(VI).**
4. N-(4-Methoxyphenyl)-6-nitroquinoxalin-2-amine **(XIVa).**
5. N-(4-Chlorophenyl)-6-nitroquinoxalin-2-amine **(XIVb).**
6. N^2-(4-Methoxyphenyl)quinoxaline-2,6-diamine **(XVa).**
7. N^2-(4-Chlorophenyl)quinoxaline-2,6-diamine **(XVb).**

Moreover, the targeted compounds synthesized were:

1. 1-(2-((4-Methoxyphenyl)amino)-3-oxo-3,4-dihydroquinoxalin-6-yl)-3-phenylurea **(VIIa).**
2. 1-(2-((4-Methoxyphenyl)amino)-3-oxo-3,4-dihydroquinoxalin-6-yl)-3-phenylthiourea **(VIIb).**
3. 1-(3-Chlorophenyl)-3-(2-((4-methoxyphenyl)amino)-3-oxo-3,4-dihydroquinoxalin-6-yl)urea **(VIIc).**
4. 1-(2-((3-Methoxyphenyl)amino)-3-oxo-3,4-dihydroquinoxalin-6-yl)-3-(p-tolyl)urea **(VIId).**
5. 1-Cyclohexyl-3-(2-((4-methoxyphenyl)amino)-3-oxo-3,4-dihydroquinoxalin-6-yl)urea **(VIIe).**
6. N-(2-((4-Methoxyphenyl)amino)-3-oxo-3,4-dihydroquinoxalin-6-yl)benzenesulfonamide **(VIIIa).**

7. N-(2-((4-Methoxyphenyl)amino)-3-oxo-3,4-dihydroquinoxalin-6-yl)-4-methylbenzenesulfonamide **(VIIIb).**
8. 2-((2-((4-Methoxyphenyl)amino)-3-oxo-3,4-dihydroquinoxalin-6-yl)carbamoyl)benzoic acid **(IX).**
9. N-(2-((4-Methoxyphenyl)amino)-3-oxo-3,4-dihydroquinoxalin-6-yl)acetamide**(X).**
10. 1-(2-((4-Methoxyphenyl)amino)quinoxalin-6-yl)-3-phenylthiourea **(XVIa).**
11. 1-(2-((4-Chlorophenyl)amino)quinoxalin-6-yl)-3-phenylurea **(XVIb).**
12. 1-(2-((4-Methoxyphenyl)amino)quinoxalin-6-yl)-3-(m-tolyl)urea **(XVIc).**
13. 1-Cyclohexyl-3-(2-((4-methoxyphenyl)amino)quinoxalin-6-yl)urea **(XVId).**
14. 1-(2-((4-Chlorophenyl)amino)quinoxalin-6-yl)-3-cyclohexylurea **(XVIe).**
15. N-(2-((4-Methoxyphenyl)amino)quinoxalin-6-yl)benzenesulfonamide **(XVIIa).**
16. N-(2-((4-Methoxyphenyl)amino)quinoxalin-6-yl)-4-methylbenzenesulfonamide **(XVIIb).**
17. N-(2-((4-Chlorophenyl)amino)quinoxalin-6-yl)-4-methylbenzenesulfonamide **(XVIIc).**
18. N-(2-((4-Chlorophenyl)amino)quinoxalin-6-yl)acetamide **(XVIII).**
19. 1-(4-((6-Nitroquinoxalin-2-yl)amino)phenyl)-3-phenylurea **(XXIa)**
20. 1-(3-Chlorophenyl)-3-(4-((6-nitroquinoxalin-2-yl)amino)phenyl)urea **(XXIb)**

4. Biological evaluation

The biological evaluation was accomplished by testing both anticancer activity and enzyme inhibition activity. The anticancer activity of the synthesized compounds (**VIIc**, **VIIe , VIIIb, X** , **XVId, XVIIb)** was evaluated at the national cancer institute (NCI), Maryland, USA. They were challenged against 60 cancer cell lines at 10 μM. Unfortunately, they showed no significant anti-cancer activity. The twenty final compounds were evaluated for their VEGFR-2 inhibition activity. The evaluation was performed in KINEXUS Corporation, Canada. Enzyme inhibition results revealed the promising activity of the quinoxaline nucleus that can be considered for synthesis of new effective anti-angiogenic drugs. In

particular, compound **IX**, which showed 69% inhibition, was the most powerful candidate. Most of other compounds activity was between 10% and 29%.

5. Molecular modeling study

Finally, a thorough Molecular docking using GOLD software was attempted to investigate the binding mode of the targeted compounds and interpret the biological results. The unexpected low results were interpreted using Field alignment study.

1. Introduction

1.1. Cancer

1.1.1. Overview

The first description of cancer is found in an Egyptian papyrus and dates back to approximately 1600 BC. It was regarded as an incurable disease until the nineteenth century, when surgical removal was made more efficient by anaesthesia, improved techniques and histological control. [1]

Cancer is a collective term used for a group of diseases that is characterized by the loss of control of the growth, division, and spread of a group of cells, leading to a primary tumor that invades and destroys adjacent tissues. It may also spread to other regions of the body through a process known as metastasis, which is the cause of 90% of cancer deaths. The terms cancer and tumor, however, are more commonly accepted. A major problem in treating cancer is the fact that it is not a single disease. There are more than 200 different cancers resulting from different cellular defects, and so a treatment that is effective in controlling one type of cancer may be ineffective on another.[2]

Over the past 30 years, significant progress has been achieved in understanding the molecular basis of cancer. The accumulation of this basic knowledge has established that cancer is a variety of distinct diseases and that defective genes cause these diseases. Further, gene defects are diverse in nature and can involve either loss or gain of gene functions.[3]

1.1.2. Causes of cancer

Possibly as many as 30 % of cancers are caused by smoking, while another 30% are diet relates. Carcinogenic chemicals in smoke, food and the environment may cause cancer by inducing gene mutations or interfering with normal cell differentiation. The birth of a cancer (carcinogenesis) can be initiated by a chemical – usually a mutagen- but other