Design and Synthesis of Novel Quinoxaline Derivatives as Potential Anticancer Targeting Therapeutics.

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To the soul of my Father

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List of Abbreviations

Abl: Abelson Murine Leukemia Viral oncogene.

AcOH: Acetic acid

AML: Acute Myeloid Leukemia.

ATP: Adenosine Triphosphate

BCL-2: B-cell Lymphoma 2.

DES: Diethylstilbestrol

DFG: Aspartate- Phenylalanine- Glycine

DMEDA: N, N'-dimethylethylenediamine.

DMF: Dimethylformamide.

DMSO: Dimethylsulphoxide.

D_2O: Deuterium oxide.

EC: Endothelial cells.

EGFR: Epidermal Growth Factor Receptor.

Ephs: Ephrin Receptors.

FAK: Focal Adhesion Kinase.

FDA: Food and Drug Administration.

FLT3*:* FMS like tyrosine kinase-3

FGFRs: Fibroblast Growth Factor Receptors.

FITC: Fluorescein Isothiocyanate.

GnRH: Gonadotropin-Releasing Hormone.

HCT-116: Human Colon carcinoma.

HEPG-2: Liver Hepatocellular carcinoma.

HUVEC: Human Umbilical Vein Endothelial cells.

ITD: Internal tandem duplication.

JAKs: Janus kinase.

Lck: Lymphocyte-specific protein.

MBC: Metastatic Breast cancer.

MoAbs: Monoclonal antibodies.

mTOR: mammalian Target of Rapamycin.

MCF-7: Human breast adenocarcinoma cell line

NCI: National Cancer Institute.

NRTKs: Non-receptor tyrosine kinases.

NSCLC: Non-small cell lung cancer.

NaI: Sodium Iodide.

PEG: Polyethylene glycol.

PKs: Protein kinases.

PlGF: Placenta Growth Factor.

PDGFR: Platelet-derived Growth Factor Receptor

RCC: Renal cell carcinoma.

RET: Rearranged during transfection.

RT: Room temperature.

RTKs: Receptor Tyrosine Kinases.

SAR: Structure Activity Relationship.

SERMs: Selective Estrogen Receptor Modulators

Src: Sarcoma kinase.

TEA: Triethyl amine

THF: Tetrahydrofuran

VEGFR: Vascular Endothelium Growth Factor Receptor.

µM: Micromole

Abstract

Title of thesis:

"Design & synthesis of novel quinoxaline derivatives as potential anticancer targeting therapeutics"

1-Introduction

Cancer is a major problem in human health and remains the second highest cause of mortality worldwide, with millions of cases every year. A total of 1,658,370 new cancer cases and 589,430 cancer deaths are projected to occur in the United States in 2015. Therefore, current efforts to cure cancer have been focusing on drugs, biological molecules and immune mediated therapies. The use of conventional cytotoxic anti-tumor drugs is based on the theory that rapidly proliferating and dividing cells are more sensitive to these compounds than the normal cells. However, Studies have been focusing recently on targeted therapy to overcome traditional chemotherapy's drawbacks. Targeted therapy refers to a new generation of cancer drugs designed to interfere with a specific target protein that is believed to have a critical role in tumor growth or progression, through targeting one of the six hallmarks of cancer. Sustained tumor angiogenesis is one of the hallmark features of cancer. VEGF is one of the key angiogenic stimulators secreted by the tumor cells to switch on the angiogenic phenotype. Intra-tumor hypoxia, alteration in oncogenes and tumor suppressor genes significantly up regulate VEGF expression. Therefore, targeting VEGFR leading to inhibition of angiogenesis, which further resulted in the arrest of tumor growth. Quinoxaline is a heterocyclic compound containing benzene ring and pyrazine ring. It is proved to be selective ATP competitive inhibitors in many kinases.

2-Rational and Design

In an attempt to discover new quinoxalines analogues with promising anti-tumor activity, our study involved the synthesis of new series of quinoxaline derivatives as type II VEGFR-2 inhibitors based on comprehensive SAR study and structural similarities with Sorafenib as a type II VEGFR-2 inhibitor. Their structures were confirmed by various spectral and micro analytical data (^{13}C NMR, ^{1}H NMR, FT IR, Mass Spectrum, and Elemental Analyses).

3-Chemistry

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

1. 3-Methylquinoxalin-2-(1H)-one **(I)**
2. 2-chloro-3-methylquinoxaline **(II)**
3. Quinoxaline-2,3(1H,4H)-dione **(X)**
4. 2, 3-Dichloroquinoxaline **(XI)**
5. Quinoxalin-2(1H)-one **(XIX)**
6. 2-chloroquioxaline **(XX)**

Also, it involved the following unavailable reported intermediates:

1. 3-((3-Methylquinoxalin-2-yl) amino) benzoic acid **(III).**
2. N^1-(3-methylquinoxalin-2-yl)benzene-1,4-diamine **(IV).**
3. 3-((3-Chloroquinoxalin-2-yl) amino) benzoic acid **(XII).**
4. N^1-(3-chloroquinoxalin-2-yl) benzene-1,4-diamine **(XIII).**
5. 3-(Quinoxalin-2-ylamino)benzoic acid **(XXI).**
6. N^1-(quinoxalin-2-yl)benzene-1,4-diamine **(XXII).**

Moreover, these new target compounds were synthesized:

1. 3-((3-Methylquinoxalin-2-yl)amino)-N-phenylbenzamide **(VIa).**
2. *N*-(4-chlorophenyl)-3-((3-methylquinoxalin-2-yl)amino)benzamide **(VIb).**
3. N-(4-methoxyphenyl)-3-((3-methylquinoxalin-2-yl)amino)benzamide **(VIc).**
4. N-(4-((3-methylquinoxalin-2-yl)amino)phenyl) benzamide **(VIIa).**
5. 4-Chloro-N-(4-((3-methylquinoxalin-2-yl)amino)phenyl)benzamide **(VIIb).**

6. 4-Methoxy-N-(4-((3-methylquinoxalin-2-yl)amino)phenyl)benzamide**(VIIc).**
7. 4-Methyl-N-(4-((3-methylquinoxalin-2-yl)amino)phenyl)benzamide **(VIId).**
8. 1-(4-((3-Methylquinoxalin-2-yl)amino)phenyl)-3-phenylthiourea **(VIIIa).**
9. 1-(4-((3-Methylquinoxalin-2-yl)amino)phenyl)-3-phenylurea **(VIIIb)**.
10. 1-(4-Chlorophenyl)-3-(4-((3-methylquinoxalin-2-yl)amino)phenyl)urea **(VIIIc).**
11. 1-(4-((3-Methylquinoxalin-2-yl)amino)phenyl)-3-(m-tolyl)urea **(VIIId)**.
12. 1-(3-Methoxyphenyl)-3-(4-((3-methylquinoxalin-2-yl)amino)phenyl)urea **(VIIIe).**
13. 4-Methyl-N-(4-((3-methylquinoxalin-2-yl)amino)phenyl)benzenesulfonamide **(IXa).**
14. N-(4-((3-methylquinoxalin-2-yl)amino)phenyl)-4-nitrobenzenesulfonamide **(IXb)**.
15. 3-((3-Chloroquinoxalin-2-yl) amino)-N-phenylbenzamide **(XVa).**
16. N-(4-chlorophenyl)-3-((3-chloroquinoxalin-2-yl) amino) benzamide **(XVb).**
17. 3-((3-Chloroquinoxalin-2-yl) amino)-N-(4-methoxyphenyl) benzamide **(XVc).**
18. *N*-(4-((3-chloroquinoxalin-2-yl) amino) phenyl) benzamide **(XVIa).**
19. 4-Chloro-N-(4-((3-chloroquinoxalin-2-yl) amino) phenyl) benzamide **(XVIb).**
20. N-(4-((3-chloroquinoxalin-2-yl) amino) phenyl)-4-methoxybenzamide **(XVIc).**
21. *N*-(4-((3-chloroquinoxalin-2-yl) amino) phenyl)-4-methylbenzamide **(XVId).**
22. 1-(4-((3-Chloroquinoxalin-2-yl) amino) phenyl)-3-phenylthiourea **(XVIIa).**
23. 1-(4-((3-Chloroquinoxalin-2-yl) amino) phenyl)-3-phenylurea **(XVIIb).**
24. 1-(4-Chlorophenyl)-3-(4-((3-chloroquinoxalin-2-yl) amino) phenyl) urea **(XVIIc).**
25. 1-(4-((3-Chloroquinoxalin-2-yl) amino) phenyl)-3-(m-tolyl) urea **(XVIId).**
26. 1-(4-((3-Chloroquinoxalin-2-yl) amino) phenyl)-3-(3-methoxyphenyl) urea **(XVIIe).**
27. 1-(4-((3-Chloroquinoxalin-2-yl)amino)phenyl)-3-(4-methoxyphenyl)urea **(XVIIf).**
28. N-(4-((3-chloroquinoxalin-2-yl) amino) phenyl)-4-methylbenzenesulfonamide **(XVIIIa**).
29. N-(4-((3-chloroquinoxalin-2-yl)amino)phenyl)-4-nitrobenzenesulfonamide **(XVIIIb**).
30. N-(phenyl)-3-(quinoxalin-2-ylamino)benzamide derivatives **(XXIVa).**
31. N-(4-methoxyphenyl)-3-(quinoxalin-2-ylamino)benzamides)**(XXIVb).**
32. 1-(3-Methoxyphenyl)-3-(4-(quinoxalin-2-ylamino) phenyl) urea **(XXV).**

4-Bilological Evaluation:

The targeted compounds were submitted to test their *in-vitro* anticancer activity by screening against 3 tumor cell lines namely (HCT-116, HEPG-2 and MCF-7). Out of the titled compounds, 5 showed promising activity (**VIId, VIIIa, VIIIc, VIIIe, XVa**).

The enzymatic activity of the synthesized compounds was assessed against VEGFR-2 tyrosine kinases at Bio Science Corporation (BPS). Unfortunately, our compounds showed weak activity against VEGFR-2 (most of the compounds activities were between 2% and 21%), which couldn't explain the potent antiproliferative activity of compounds (**VIId, VIIIa, VIIIc, VIIIe, XVa).** Moreover, **VIIIc** was selected as the most potent compound and was further selected to investigate its effect on cell cycle progression, induction of apoptosis, and expression of cleaved caspase-3 using Human colon carcinoma cell line (HCT-116).

5-Docking Studies

Molecular docking using CDOCKER protocol was attempted for initial design and to investigate the binding mode of the targeted compounds and interpret the biological results.

1. Introduction

1.1. Cancer Overview

Cancer is a major problem in human health and remains the second highest cause of mortality worldwide, with millions of cases every year [1]. Before 1950, surgery was most preferred means of treatment. After 1960, radiation therapy started being used to control local disease. However, over time it was realized that neither surgery nor radiation or the two in combination could adequately control the metastatic cancer and that, for treatment to be effective, therapy needed to reach every organ of the body. Therefore, current efforts to cure cancer have been focusing on drugs, biological molecules and immune mediated therapies [2] . To date, cancer remains one of the most life-threatening diseases.

Each year the American Cancer Society estimates the numbers of new cancer cases and deaths that will occur in the United States in the current year and compiles the most recent data on cancer incidence, mortality, and survival. A total of 1,658,370 new cancer cases and 589,430 cancer deaths were projected to occur in the United States in 2015 [3].

1.2 Causes of Cancer:

Since the development of malignancy is a complex multistep process, many factors may affect the likelihood that cancer will develop, and it is overly simplistic to speak of single causes of most cancers. Nonetheless, many agents, including radiation, chemicals, and viruses, have been found to induce cancer in both experimental animals and humans.

Radiation and many chemical carcinogens act by damaging DNA and inducing mutations. These carcinogens are generally referred to as initiating agents. Some of the initiating agents that contribute to human cancers include solar ultraviolet radiation (the major cause of skin cancer), carcinogenic chemicals in tobacco smoke, and aflatoxin (a potent liver carcinogen produced by some molds that contaminate improperly stored supplies of peanuts and other grains). The carcinogens in tobacco smoke are the major identified causes