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Original Research Article

Design, Synthesis, and Anticancer Activity of N-(Substituted Phenyl)-5-(3,4,5-Trimethoxyphenyl)-1,3,4-Oxadiazol-2-Amine Analogues: Exploring Novel Chemotherapeutic Agents

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Abstract

A Series of newer N-(substituent phenyl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-amine analogues 4a-f was subjected to molecular properties prediction by mol soft and Molinspiration software and was synthesized in satisfactory yields. All the compounds followed the Lipinski "rule of five "which makes them potentially active agents. 3 Compound (4a, 4d & 4f) were evaluated for their anticancer activity in one dose assay and showed moderate activity on various cell lines. The compound N-(2,4-dimethylphenyl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-amine (4d) showed maximum activity with mean growth percent (GP) of 97.75 followed by N-(2,4,6-trimethylphenyl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-amine (4f) showed mean GP of more than 85.81. The compound 4f was highly active on T-47D (Breast Cancer) [GP=66.70], NCI-H522 (Non-Small Cell Lung Cancer A549/ATCC) [GP=68.96], SNB-75 (CNS Cancer) [GP=75.64]. The compound 4d showed maximum activity on A498 (Renal Cancer) [GP=78.31], SF-268 (CNS Cancer) [GP=83.14], TK-10 (Renal Cancer) [GP=84.77]. The compound 4d showed maximum activity on SR (Leukemia) [GP=59.73], NCI-H522 (Non-Small Cell Lung Cancer A549/ATCC) [GP=72.77], LOX IMVI (Melanoma) [GP=76.63], MCF7 (Breast Cancer) [GP=81.32]. The maximum activity was observed with 4a on SR (Leukemia) with GP=59.73. The compound N-(phenyl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-amine (4a) could be considered as lead further discovery and could be modified to potentiate the anticancer activity.

Keywords: Molinspiration software, N-(2,4-dimethylphenyl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-amine, cancer, leukemia, efficacy, Lipinski "rule of five,".

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Introduction

Cancer is a condition whereby cells develop abnormally and propagate throughout the body. Cancer can develop practically any place in the human body, which contains millions of cells. Cells in humans regularly expand and multiply (by a process known as cell division) to produce new cells as needed by the body. When cells get old or damaged, they die and are replaced by new cells. This ordered process occasionally fails, allowing abnormal or damaged cells to grow and reproduce when they should not. These cells can develop tumors, which are masses of tissue. Tumors can be malignant or benign.

Cancerous tumors infect neighboring tissues and can move to distant parts of the body to generate new tumors (a process known as metastasis). Cancerous tumours are also known as malignant tumours. Many cancers develop into solid tumors, while blood cancers, such as leukemias, do not. Benign tumors do not spread or infect surrounding tissues. Benign tumors seldom recur after removal, although malignant ones occasionally do. However, benign tumors can grow to be extremely enormous. Some, such as benign brain tumors, can cause severe symptoms or even death.

Characteristics of Normal Cells and Cancer Cells

Cancer cells differ from normal cells in many ways. For instance, cancer cells:

- Expand without receiving instructions to do so. Only in response to these cues do normal cells proliferate.
- Disregard signals that ordinarily instruct cells to cease proliferating or to undergo programmed cell death, also referred to as apoptosis.

- Infiltrate surrounding regions before spreading to other bodily parts. Most normal cells do not travel throughout the body; instead, they cease growing when they come into contact with other normal cells.
- Direct vascular growth in the direction of malignancies. These blood veins remove waste materials from tumors and provide oxygen and nutrition to the tumors..
- Hide from the immune system. The immune system normally eliminates damaged or abnormal cells.
- Trick the immune system into helping cancer cells stay alive and grow. For instance, some cancer cells convince immune cells to protect the tumor instead of attacking it.
- Accumulate multiple changes in their chromosomes, such as duplications and deletions of chromosome parts. Some cancer cells have double the normal number of chromosomes.
- Rely on different kinds of nutrients than normal cells. In addition, some cancer cells make energy from nutrients in a different way than most normal cells. This lets cancer cells grow more quickly.
- Cancer cells frequently rely on these aberrant behaviors so much that they are unable to function normally without them. Because of this, scientists have created treatments that specifically target the aberrant characteristics of cancer cells. Certain cancer treatments, for instance, stop blood vessels from expanding toward tumors, thus depriving the tumor of crucial nutrition.

How Does Cancer Develop?

Cancer is a genetic illness, meaning that alterations to the genes that regulate our cells' growth and division are what cause it.

Genetic changes that cause cancer can happen

- Due to mistakes that arise during cell division.
- Due to DNA damage brought on by dangerous environmental elements including UV radiation from the sun and the toxins in cigarette smoke. (For further information, see our section on cancer causes and prevention.)
- Since our parents passed them on to us.

Normally, damaged DNA cells are eliminated by the body before they become malignant. However, as we age, the body's capacity to do so decreases. This contributes to the increased risk of cancer in later life.

Everybody's cancer is caused by a different combination of genetic alterations. There will be more alterations as the cancer spreads. Different cells within the same tumor may exhibit distinct genetic alterations

Types of Genes that Cause Cancer

Proto-oncogenes, tumor suppressor genes, and DNA repair genes are the three primary gene categories that are typically impacted by the genetic alterations that lead to cancer. These alterations are occasionally referred to as cancer "drivers."

Proto-oncogenes play a role in the proper division and development of cells. On the other hand, these genes may become cancer-causing genes (also known as oncogenes), allowing cells to proliferate and survive when they shouldn't by changing in specific ways or becoming more active than usual.

Additionally, tumor suppressor genes regulate the division and development of cells. Certain mutations in tumor suppressor genes can cause cells to divide uncontrollably. Genes that repair damaged DNA are known as DNA repair genes. Mutations in these genes frequently lead to further mutations in other genes and chromosomal abnormalities such duplications and deletions of chromosomal segments in the cells. When combined, these alterations have the potential to make the cells malignant. Scientists have discovered that specific mutations are frequently present in a variety of cancer forms, as they continue to learn more about the molecular alterations that cause cancer. These days, a wide range of cancer therapies are available that focus on the gene abnormalities that cause cancer. Anybody with cancer that carries the targeted mutation can use some of these treatments, regardless of the cancer's initial growth location.

Types of Cancer

Cancer comes in more than a hundred varieties. The organs or tissues where tumors originate are typically used to name different types of cancer. For instance, brain cancer begins in the brain, but lung cancer begins in the lung. Cancers can also be classified according to the kind of cell that gave rise to them, such as squamous or epithelial cells.

You can use our A to Z List of Cancers or the NCI website to search for information on particular cancer types based on where the cancer is located in the body. Additionally, we have data regarding malignancies in children, adolescents, and young adults.

The following are some forms of malignancies that start in particular cell

1. Carcinoma

The most frequent kind of cancer is a carcinoma. Epithelial cells, which coat the body's exterior and interior surfaces, are responsible for their

formation. There are numerous varieties of epithelial cells, which, when examined under a microscope, frequently resemble columns.

Specific designations are given to cancers that originate in distinct types of epithelial cells:

Adenocarcinoma is a type of cancer that develops in mucous-producing epithelial cells. Sometimes, tissues containing this kind of epithelial cell are referred to as glandular tissues. Adenocarcinomas account for the majority of malignancies of the breast, colon, and prostate.

The bottom, or basal (base) layer of the epidermis, or a person's outermost layer of skin, is where basal cell carcinoma starts. cells

Squamous cells are epithelial cells that are found immediately under the skin's outer layer. Squamous cell carcinoma is a form of cancer that arises in these cells. Squamous cells also line the lining of many other organs, including the lungs, stomach, intestines, and kidneys. Scramous cells look flat under a microscope, akin to fish scales. Squamous cell carcinomas are occasionally called epidermoid carcinomas.

A type of epithelial tissue known as transitional epithelium, or urothelium, is where transitional cell carcinoma originates. This tissue is present in the linings of the bladder, ureters, kidneys (renal pelvis), and a few other organs. It is composed of many layers of epithelial cells that have the ability to change size. Transitional cell carcinomas can occur in some kidney, ureter, and bladder malignancies.

2. Sarcomas

Cancers known as sarcomas can develop in the soft tissues of the bone, such as the muscles, fat, blood, and lymph arteries, as well as fibrous tissue (such as ligaments and tendons).

The most frequent cancer of the bone is osteosarcoma. Lipopolysarcoma, Kaposi sarcoma, liposarcoma, malignant fibrous histiocytoma, and dermatofibrosarcoma protuberans are the most prevalent forms of soft tissue sarcoma. Muscle, tendons, fat, blood arteries, lymph vessels, nerves, and the tissue around joints are among the soft tissues in the body where soft tissue sarcoma can develop.

3. Leukemia

Leukemias are cancers that start in the bone marrow's blood-forming tissue. Solid tumors are not formed by these cancers.

Rather, a substantial number of aberrant white blood cells—leukemia cells and leukemic blast cells—accumulate in the bone marrow and circulation, displacing healthy blood cells.

The body may find it more difficult to fight infections, regulate bleeding, and deliver oxygen to its tissues if there is a low concentration of regular blood cells.

Leukemia comes in four basic forms, which are categorized according to the kind of blood cell the cancer begins in (lymphoblastic or myeloid) and the rate at which the illness progresses (acute or chronic). Leukemia grows more slowly in chronic forms and more quickly in acute variants.

4. Lymphoma

Lymphoma is cancer that begins in lymphocytes (T cells or B cells). These are disease-fighting white blood cells that are part of the immune system. In lymphoma, abnormal lymphocytes build up in lymph nodes and lymph vessels, as well as in other organs of the body.

There are two main types of lymphoma:

Hodgkin lymphoma

People with this disease have abnormal lymphocytes that are called Reed-Sternberg cells. These cells usually form from B cells.

Non-Hodgkin lymphoma

This is a large group of cancers that start in lymphocytes. The cancers can grow quickly or slowly and can form from B cells or T cells.

5. Multiple Myeloma

Multiple myeloma is cancer that begins in plasma cells, another type of immune cell. The abnormal plasma cells, called myeloma cells, build up in the bone marrow and form tumors in bones all through the body. Multiple myeloma is also called plasma cell myeloma and Kahler disease.

6. Melanoma

Melanoma is cancer that begins in cells that become melanocytes, which are specialized cells that make melanin (the pigment that gives skin its color). Most melanomas form on the skin, but melanomas can also form in other pigmented tissues, such as the eye.

7. Brain and Spinal Cord Tumors

There are different types of brain and spinal cord tumors. These tumors are named based on the type of cell in which they formed and where the tumor first formed in the central nervous system. For example, an astrocytic tumor begins in star-shaped brain cells called astrocytes, which help keep nerve cells healthy. Brain tumors can be benign (not cancer) or malignant (cancer).

Drug Profile

Oxadiazoles: The compounds having a five member ring containing one oxygen (O) and two

nitrogen (N) are called oxadiazole or in the older literature also called as furadiazle. Oxadiazole ring is considered to be derived from furan ring by replacement of two carbon (-CH=) by two pyridine type nitrogen (-N=). These are four possible isomers of oxadiazole [(I) 1,2,3-oxadiazole, (II) 1,2,4-oxadiazole, (III)1,2,5 oxadiazole, and (IV) 1,3,4 -oxadiazole] depending on the position of nitrogen atom in the ring

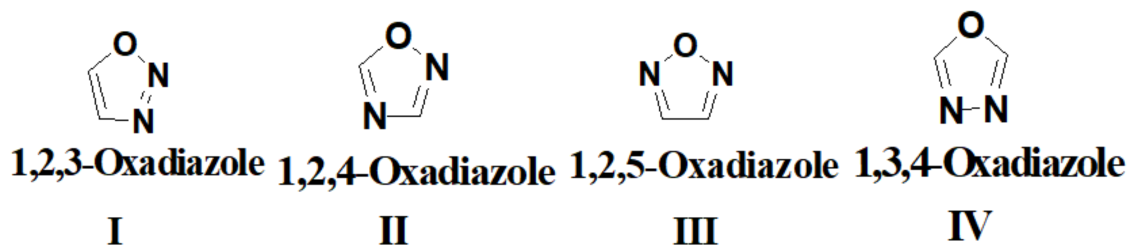
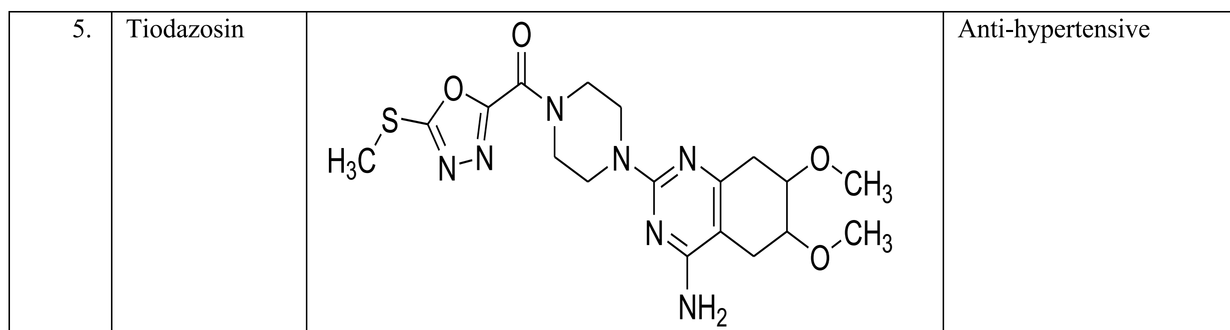


Figure 1:

Table 1: Drug Containing 1, 3, 4-Oxadiazole Moiety

S.NO.	Name	Structure	Biological activity
1.	Raltegravir		Anti –HIV
2.	Zibotentan		Anticancer
3.	Furamizole		Antibiotic
4.	Nesapidil		Antihypertensive

5.	Tiodazosin		Anti-hypertensive

Recent development on anticancer agents

Discovery and development of anticancer agents are the key focus of several pharmaceutical companies as well as non-profit government and non-government organization, like the National Cancer Institute (NCI) In the United States, the European Organization for research and Treatment of Cancer (EORTC), and the British Cancer Research Campaign (CRC)

Recent Drugs

- Osimertinibis a drug for the treatment of lung cancer. it was approved in 18 December 2020 by the food and drug administration for the treatment of adjuvant therapy after tumor resection in patients with non-small cell lung cancer (NSCLC) whose tumors have epidermal growth factor receptor (EGFR) axon 19 deletions or axon 21 L858R mutations.

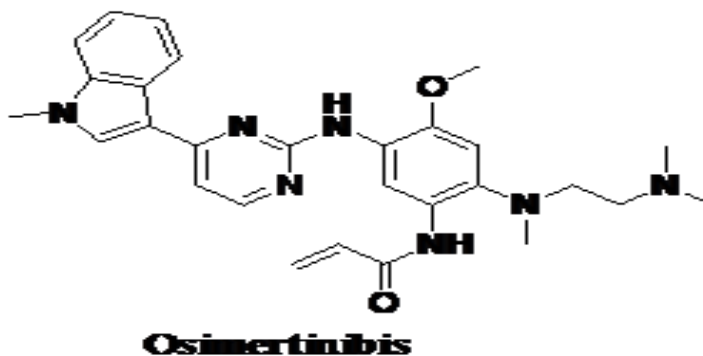


Figure 2:

- Pralsetinib (GAVRETO) a drug for the treatment of thyroid cancer it is approved in 1 December 2020 by the US FDA for the treatment of metastatic *RET*-mutant modularly thyroid cancer (MTC) who require systemic therapy or *RET* fusion-positive thyroid cancer.

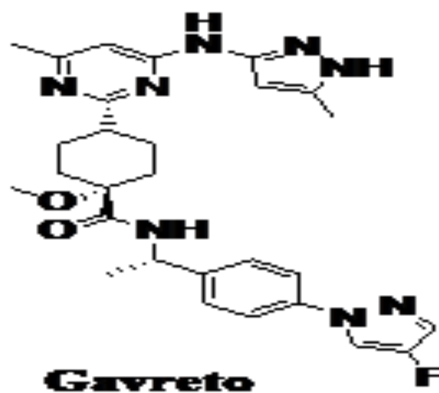


Figure 3:

- Venetoclax is an Anti-cancer drug it is combination with azacitidine, Decitabine, or low-dose Cytarabine (LDAC) it was approved in October 16, 2020 by the FDA for the treatment of newly-diagnosed acute myeloid leukemia (AML).

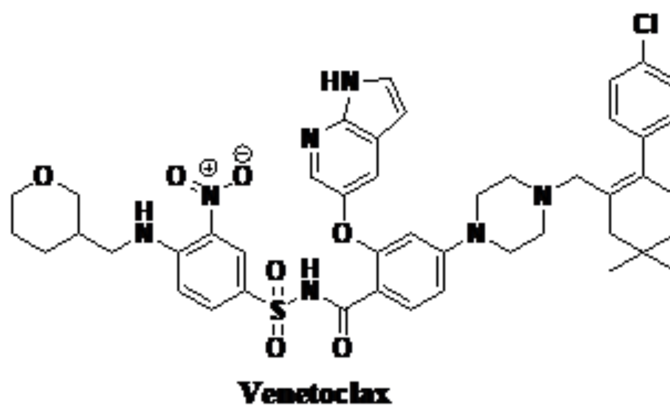


Figure 4:

- Azacitidine tablets for continued treatment of patients with acute myeloid leukemia. It is complete remission (CR) or complete remission with incomplete blood count recovery (CRI) following intensive induction chemotherapy and is not able to complete intensive curative therapy it was approved in September 1, 2020 by the food and drug administration.

Figure 5:

- Selinexor in combination with Bortezomib and Dexamethasone for the treatment of adult patients with multiple myeloma who have received at least one prior therapy. It is an orally administered whose disease is refractory to at least two Proteasome inhibitors, at least two Immunomodulatory agents, and an anti-CD38 monoclonal antibody. It was approved in December 18, 2020, by the Food and Drug Administration.

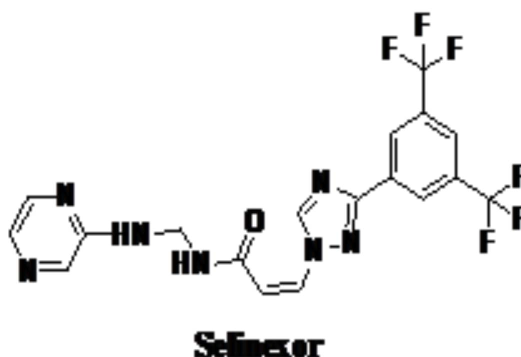


Figure 6:

Plan of Work

Scheme

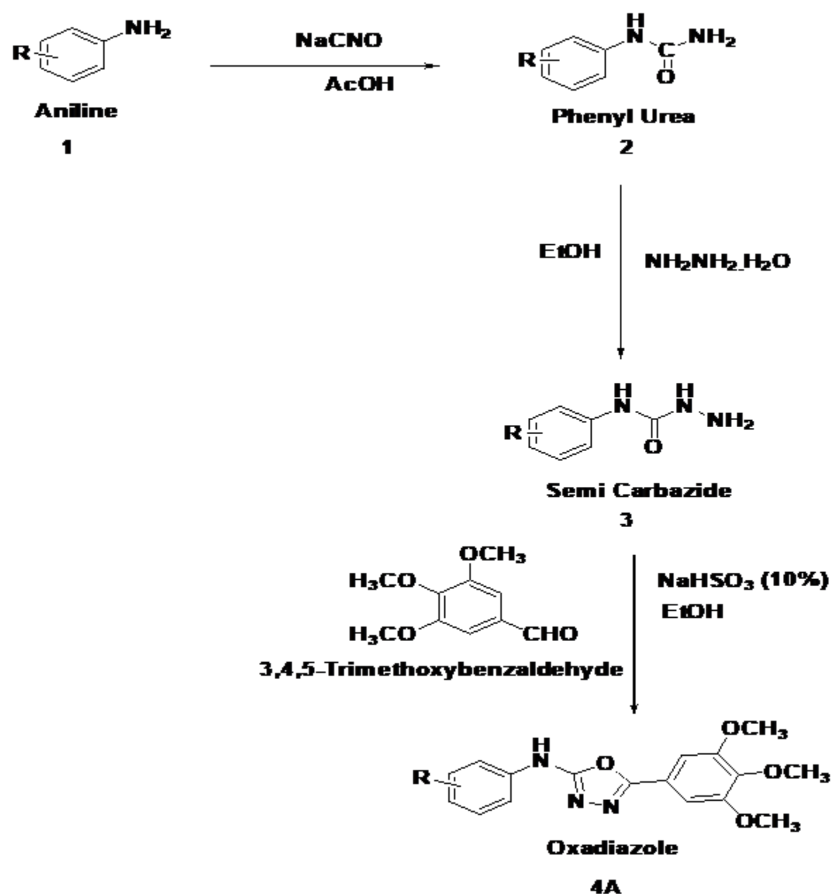


Figure 7:

R= 4-OCH_3; 2-CH_3; 4-CH_3; 2,4-CH_3; 2,6-CH_3; 2,4,6-CH_3

Scheme: Protocol for synthesis of 1,3,4-oxadiazole analogues (4a-f).

General Method of Synthesis

Step I: General method for the Synthesis of Phenyl urea analogues (2a-f)

The substituent aniline (0.08 mol) was dissolved in 20 ml of glacial acetic acid and 10 ml of hot water, to this sodium cyanide (0.05 mol; 3.25 g) in 80 ml of hot water was added with stirring .it was then allowed to stand for 40 min, then cooled in ice bath and filtered with suction, dried and recrystallized from boiling water to obtain substituent phenyl urea. The purity of the compound was checked by TLC using chloroform + methanol ((9:1) as mobile phase.

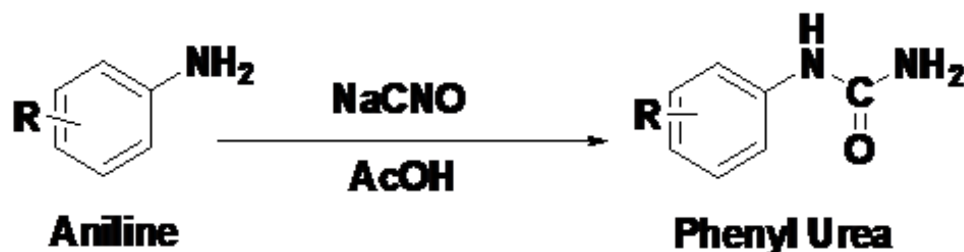


Figure 8:

Step II: Synthesis of substituent phenyl semi carbazide analogue (3a-f)

Equimolar quantities (0.05mol) of substituent phenyl urea and hydrazine hydrate (0.05mol, 2.5ml) in ethanol were refluxed for 48-50 hr. with stirring. The two third volume of alcohol was distilled by vacuum distillation and then poured into crushed ice. The precipitate was filtered, washed with water and dried. The solid was recrystallized from 50 ml of 90% ethanol to obtain semi carbazide analogues the purity of the semi carbazide was checked by TLC using chloroform+ methanol (9:1) as mobile phase.

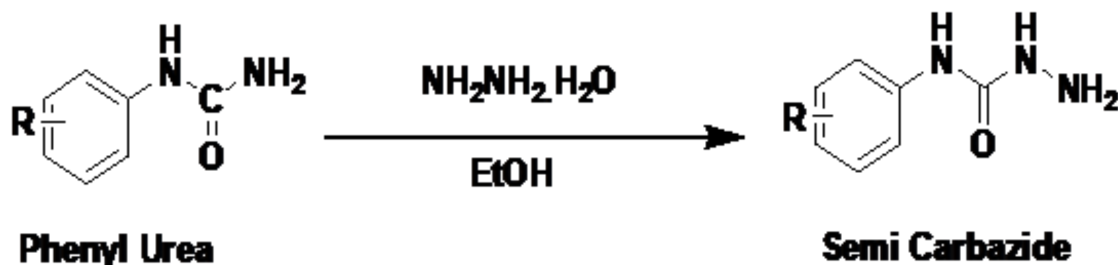


Figure 9:

Step III: General method for the synthesis of 5-substituent N-(substituent phenyl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-amine analogues (4a-f)

The substituent phenyl semi carbazide (0.05smol) and 3,4,5-Trimethoxyaldehyde (0.001mol,0.175g) was refluxed 10-15 hr. using 10% NaHSO3 and ethanol water solvent. The two third volume of alcohol was distilled by vacuum distillation and then poured into crushed ice. The precipitate was filtered, washed with water and dried. The solid was recrystallized from 50 ml of 90% ethanol to obtain the final product. The purity of the compound was monitored by thin layer chromatography using chloroform+ methanol (9:1) as mobile phase.

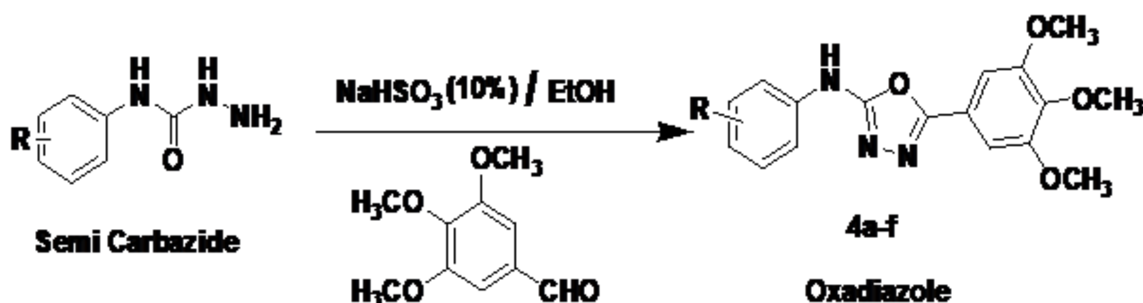


Figure 10:

Table 2: Physical Data of Synthesized compound (4a-f):-

Compounds	R/Ar	% Yield	Mp(°C)	R_f Value
4a	Phenyl	71.09	140-142	0.724
4b	2-Methylphenyl	90.12	130-132	0.48
4c	4- Methylphenyl	89.75	118-120	0.72
4d	2,4- Dimethylphenyl	61.38	125-127	0.59
4e	2,6- Dimethylphenyl	62.82	95-97	0.58
4f	2,4,6- Trimethylphenyl	68.96	108-110	0.71

Identification and characterization of compounds

1. Determination of melting point range.
2. TLC analysis.
3. Spectral analysis.

Synthesized intermediate and final molecules will be characterized using IR, ^{1}H NMR and Mass spectroscopy [9,6]

IR Spectral Analysis

All synthesized molecules were characterized to determine the characteristic functional group in the molecule.

^{1}H NMR Spectral Analysis

One of the representative molecules was characterized to determine the number & type of hydrogen atoms in the molecule.

^{13}C NMR Spectral Analysis

One of the representative molecules was characterized to determine the number & type of carbon atoms in the molecules.

Mass Spectroscopy

Synthesized molecules were characterized to determine the molecular weight in the molecule.

Spectral Analysis

N-(2-methylphenyl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-amine (4b)

^{1}H NMR (300 MHz): δ ppm 8.31(1H, S, ArNH), 6.88-7.84(6H, M, ArNH)

^{13}C NMR (75 MHz): δ ppm 120.14, 122.15, 126.03, 126.10, 126.56, 128.35, 129.02, 129.16, 130.10, 135.17, 136.51, 137.69, 153.95

N-(4-methylphenyl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-amine (4c)

^{1}H NMR (300 MHz): δ ppm 8.28(1H, S, ArNH), 6.99-7.63 (6H, M, ArH), 6.99 (1H, S, ArH), 2.14 (6H, S, CH_3)

^{13}C NMR (75 MHz): δ ppm 18.10-18.16, 125.55-125.87, 126.29-126.37, 127.57-127.75, 128.15-128.28, 129.00-129.52, 132.95,134.18, 135.11, 135.39-135.54, 136.13-136.27, 153.51, 155.74

N-(2,4-dimethylphenyl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-amine (4d)

^{1}H NMR (300 MHz): δ ppm 9.07 (1H, S, ArNH), 8.40 (2H, d, J=9Hz, ArH), 8.30 (1H, S, ArH), 7.10-7.67 (4H, M, ArH)

^{13}C NMR (75 MHz): δ ppm 114.76-115.05, 122.09-122.19, 127.58, 128.69, 129.11, 130.70, 133.14, 134.33, 135.21, 135.56, 152.94, 159.49

MS (*m/z*): 205 (100, M^{+}), 185 (22), 124 (30), 129 (95), 125(75), 91 (40), 58 (38).

N-(2,6-dimethylphenyl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-amine (4e)

^{1}H NMR (300 MHz): δ ppm 8.40(1H, d, J=8.4Hz, ArH),8.27(1H, S, ArH), 7.65(1H, d, J=2.1Hz, ArH), 7.48(2H, d, J=2.1Hz, ArH), 6.86 (2H, d, J=2.1Hz, ArH), 3.72(3H, S, OCH_3)

^{13}C NMR (75 MHz): δ ppm 55.16, 113.60-113.77, 120.25, 122.21, 127.57, 128.68, 129.09, 130.81, 131.80, 133.08, 134.22, 135.16, 153.03, 155.11.

N-(2,4,6-trimethylphenyl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-amine (4f)

^{1}H NMR (300 MHz): δ ppm 9.14(1H, S, ArNH, 8.39(d, J=3.3Hz), 8.24(1H, S, ArH), 7.32(2H, d, J=3.3Hz, ArH), 7.35-7.76(H4, M, ArH)

^{13}C NMR (75 MHz): δ ppm 120.70, 121.62, 126.29, 127.58, 128.26, 128.72-129.12, 130.63, 133.21, 134.41, 135.82, 137.95, 152.75

MS (*m/z*): 205 (100, M^{+}), 175 (22), 144 (30), 159 (95), 135(75), 95 (40), 48 (38).

Anticancer Screening

Following oxadiazole synthesis, their structural data was uploaded to the NCI's official website in order to select potential drugs for anticancer screening.

NCI employs its own compound selection process for successful drug development, which is based on the uniqueness of the heterocyclic ring system, drug-like properties using the concept of privileged scaffolds, structure based on computer-aided drug design, and so on. Avoid structures with difficult linkages or functional groups.

One Dose Assay

When performing the anticancer screening, the NCI US protocol was followed. All substances submitted to the NCI 60 Cell screen were tested at a single high dose (10 M) on over 60 different cancer cell lines, including those for leukaemia, melanoma, lung, colon, CNS, ovarian, renal, prostate, and breast cancer. Only substances that pass a small number of cell line threshold inhibition tests will proceed to the full 5-dose experiment.

The threshold inhibition criteria for progression to the 5-dose screen were chosen based on a careful examination of previous DTP screening results in order to effectively capture drugs with anti-proliferative activity. The threshold criteria may change as more data becomes available

Interpretation of One-Dose Data

The one-dose data will be displayed as a mean graph showing the percent growth of treated cells, just like the data from the 5-dose assay. The One-dose assay value represents growth in comparison to the no-drug control and the number of cells at time zero. This allows for the detection of lethality (values ranging from 0 to 100) as well as growth inhibition (values less than 0). This methodology is used in the 5-dose assay, which is described further below. A value of 100, for example, indicates that there is no growth inhibition. A value of 40 indicates a 60% growth restriction. A result of 0 indicates that no net growth occurred during the trial. A value of -40 corresponds to 40% lethality. A value of -100 indicates that all cells are dead. The data in the One-dose mean graph can be used in a COMPARE analysis

Five-Dose Assay

The 60-cell panel is tested with five concentration levels of compounds that significantly restrict growth in the One-Dose Screen

Methodology of the in vitro cancer screen

- Human tumor cell lines from the cancer screening panel are cultured in RPMI 1640 medium with 5% foetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells are plated at densities ranging from 5,000 to 40,000 cells/well in 96-well 100L microtiter plates, depending on the doubling time of specific cell lines.
- Before adding experimental medicines, the microtiter plates are incubated for 24 hours at 37° C, 5% CO2, 95% air, and 100% relative humidity after cell inoculation. After 24 hours, two plates of each cell line are fixed in situ with TCA to reflect a measurement of the cell population at the time of drug addiction (Tz).
- Prior to use, experimental medicines are solubilized in dimethylsulfoxide 400 times higher than the required final maximum test

concentration. An aliquot of frozen concentrate is thawed and diluted with complete medium containing 50 g/m1 gentamicin to twice the desired final maximum test concentration at the time of drug addiction.

- Additional four, 10-fold- or 12-log serial dilutions are created to produce a total of five drug concentrations plus control. The required final drug concentrations are obtained by adding aliquots of 100 L of each of these various drug dilutions to the relevant microtiter wells, which already contain 100 L of medium.
- Following drug administration, the plates are incubated for 48 hours at 37 °C, 5% CO2, 95% air, and 100% relative humidity. The addition of cold TCA terminates the adhering cell test. Cells are gently fixed in place and incubated for 60 minutes at 4°C after carefully adding 50 L of cool, 50% (w/v) TCA (final concentration, 10% TCA). The plates are rinsed with tap water five times, air dried.
- Each well is filled with a 0.4% (w/v) sulforhodamine B (SRB) (100L) solution in 1% acetic acid, and the plates are incubated at room temperature for 10 minutes. The plates are air dried after staining, and the unbound dye is removed by washing them five times in 1% acetic acid. After solubilizing the bound dye with 10 mM trizma base, the absorbance is measured at 515 nm with an automatic plate reader. The procedure for suspension cells is the same, with the exception that the test is completed by gently adding 50 L of 80% TCA (final concentration, 16% TCA) to fix settled cells at the bottom of wells
- Using the seven absorbance measurements [time zero, (Tz), control growth, (C), and test growth in the presence of (Imp at the five concentration levels, (Ti)], the percentage growth at each of the drug concentration levels is calculated. Calculating percentage growth inhibition is as follows:
  [(Ti-Tz)/(C-Tz)] x 100 for concentrations for which Ti>/=Tz
  [(Ti-Tz)/Tz] x 100 for concentrations for which Ti<rz.
- Three dosage response parameters have been determined for each experimental drug. The drug concentration that results in a 50% reduction in the net protein increase (as shown by SRB staining) in control cells during drug incubation is referred to as the growth inhibition of 50% (GI50). The GI50 is calculated as [(Ti-Tz)/(C-Tz)] x 100 = 50.

Ti = Tz is used to determine the medication concentration that inhibits total growth. The LC50 (concentration of drug resulting in a 50% drop in the measured protein at the end of the drug treatment as compared to the beginning) is calculated from [(Ti-Tz)/Tz] x 100 = -50 and indicates a net loss of cells after treatment.

If the level of activity is met, values for each of these three parameters are calculated; however, if the effect is not realised or is exceeded, the parameter value is expressed as greater or less than the maximum or minimum concentration tested

Interpretation of One –Dose Data

The one dose data will be reported as a mean graph of the percent growth of treatedcells. The number reported for the one dose assay is growth relative to the no drug control and relative to the time zero number of cells. This allows detection of both growth inhibition (values between 0-100) and lethality (value less than 0). For example, a value of 100 means no growth inhibition. A value of 40 would mwan 60% growth inhibition.

A value of 0 means no net growth over the course of the experiment. A value of -40 would mean 40% lethality, A value of -100 means all cells are dead

Table 3: Anticancer activity of oxadiazole analogues

Panel/Cell Line	Growth % in One Dose Assay	
	4d	4f
Leukemia		
CCRF-CEM	86.21	99.78
HL-60(TB)	99.3	98.45
K-562	91.71	100.46
MOLT-4	93.95	76.91
RPMI-8226	101.25	96.57
SR	59.73	84.6
Non-Small Cell Lung Cancer		
A549/ATCC	97.15	80.11
EKVX	114.45	94.09
HOP-62	89.2	91.24
HOP-92	101.17	97.89
NCI-H226	93.76	94.14
NCI-H23	92.94	94.39

NCI-H322M	93.41	90.59
NCI-H460	102.8	98.53
NCI-H522	72.77	68.96
Colon Cancer		
COLO 205	97.41	103.29
HCC-2998	91.8	91.83
HCT-116	91.87	79.42
HCT-15	104.98	100.15
HT29	88.7	82.11
KM12	94.43	94.58
SW-620	102.98	104.1
CNS Cancer		
SF-268	90.07	82.02
SF-295	101.83	95.62
SF-539	95.66	93.52
SNB-19	95.64	95.78
SNB-75	98	75.64
U-251	91.62	80.32
Melanoma		
LOX IMVI	76.63	93.72
MALME-3M	87.22	87.87
M14	104	104.01
MDA-MB-435	108.75	96.5
SK-MEL-2	106.5	93.25
SK-MEL-28	109.81	105.94
SK-MEL-5	97.52	94.87
UACC-257	94.9	97.54
UACC-62	96.4	87.68
Ovarian Cancer		
IGROV1	92.92	86.35
OVCAR-3	100.62	107.26
OVCAR-4	100.12	97.9
OVCAR-5	96.33	98.31
OVCAR-8	94.28	100.35
NCI/ADR-RES	99.52	94.68
SK-OV-3	105.94	95.81
Renal Cancer		
786-0	103.12	94.66
A498	106.19	70.38
ACHN	102.05	101.5
CAKI-1	101.45	94.23
RXF 393	108.97	92.9
SN12C	101.57	97.52
TK-10	85.19	91.31
UO-31	87.36	82.78
Prostate Cancer		
PC-3	98.23	87.46
DU-145	101.91	104.96
Breast Cancer		
MCF7	81.32	95.52
MDA-MB-231/ATCC	101.35	94.05
HS 578T	105.72	97.8
BT-549	99.09	101.61
T-47D	88.02	66.7
MDA-MB-468	100.94	96.59
Mean	97.75	85.81
Range	56.69	76.68

Table 4: In vitro anticancer activity of 5-substituent N-(substituent phenyl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-amine analogues (4a-f) on most sensitive cell lines with growth inhibition of compounds (4d and 4f)

Compound	60 cell lines assay in one dose 10^{-5} M conc.				
	Mean GP	Rang of GP	The most sensitive cell line	Growth Percent	Growth Inhibition
4d	97.75	56.69	SR (Leukemia)	59.73	40.27
			NCI-H522(Non-Small Cell Lung Cancer A549/ATCC)	72.77	27.23
			LOX IMVI (Melanoma)	76.63	23.37
			MCF7 (Breast Cancer)	81.32	18.68
			TK-10 (Renal Cancer)	85.19	14.81
4f	85.81	76.68	T-47D (Breast Cancer)	66.7	33.3
			NCI-H522(Non-Small Cell Lung Cancer A549/ATCC)	68.96	31.04
			SNB-75 (CNS Cancer)	75.64	24.36
			MOLT-4 (Leukemia)	76.91	23.09
			HCT-116 (Colon Cancer)	79.42	20.58

Result and Conclusion

Chemistry of Synthesis compound

In the first step substituent aniline (0.08 mol) was dissolved in 20 ml of glacial acetic acid and 10 ml of hot water, to this sodium cyanide (0.05 mol; 3.25 g) in 80 ml of hot water was added with stirring.it was then allowed to stand for 40 min, then cooled in ice bath and filtered with suction, dried and recrystallized from boiling water to obtain substituent phenyl urea. The purity of the compound was checked by TLC using chloroform + methanol ((9:1) as mobile phase. Second step equimolar quantities (0.05mol) of substituent phenyl urea and hydrazine hydrate (0.05mol, 2.5ml) in ethanol were refluxed for 48-50 hr. with stirring. The two third volume of alcohol was distilled by vacuum distillation and then poured into crushed ice. The precipitate was filtered, washed with water and dried. The solid was recrystallized from 50 ml of 90% ethanol to obtain semi carbazide analogues the purity of the semi carbazide was checked by TLC using chloroform+ methanol (9:1) as mobile phase. Third Step the substituent phenyl semi carbazide (0.05smol) and 3,4,5-Trimethoxyaldehyde (0.001mol,0.175g) was refluxed 10-15 hr. using 10% NaHSO3 and ethanol water solvent. The two third volume of alcohol was distilled by vacuum distillation and then poured into crushed ice. The precipitate was filtered, washed with water and dried. The solid was recrystallized from 50 ml of 90% ethanol to obtain the final product. The purity of the compound was monitored by thin layer chromatography using chloroform+ methanol (9:1) as mobile phase.

Spectral characterization of synthesized compounds

N-(2-methylphenyl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-amine (4b)

The product was prepared from 2-methylbenzaldehyde and phenyl semi carbazide and characterized by the ^{1}H NMR spectra.

The ^{1}H NMR spectra of the compound showed a doublet at 8.31 ppm for 2 proton of ArH, a multiplet at 6.88-7.84ppm for 6 proton of ArH and a singlet at 7.63 ppm for 1 proton of ArNH.

On the basis of NMR Spectra data the following structure was assigned to the compound

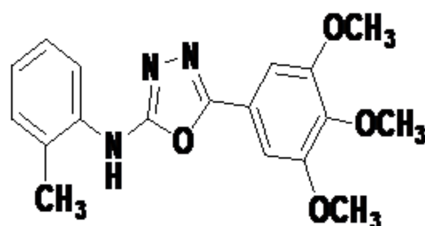


Figure 11:

N-(2,4-dimethylphenyl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-amine (4d)
The product was prepared from 2,4-dimethylbenzaldehyde and phenyl semicarbazide and characterized by the ^{1}H NMR spectra.

The ^{1}H NMR spectra of the compound showed a doublet at 8.30 ppm for 1 proton of $ArNO_2$, a doublet at 8.40 ppm for 2 proton of ArH and a multiplet at 7.10-7.67 ppm for 4 proton of ArH.
On the basis of NMR Spectra data the following structure was assigned to the compound

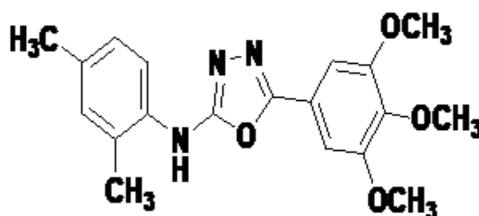


Figure 12:

N-(2,6-dimethylphenyl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-amine (4e)
The product was prepared from 2,6-dimethylbenzaldehyde and phenyl semicarbazide and characterized by the ^{1}H NMR spectra.
The ^{1}H NMR spectra of the compound showed a singlet at 8.40ppm for 1 proton of ArNH, a multiplet at 6.86 - 7.39 ppm for 1 proton of ArH and a singlet at 3.72ppm for 1 proton of ArOH.
The ^{13}C NMR spectra of the compound showed a δ ppm 55.16, 113.60-113.77, 120.25, 122.21, 127.57, 128.68, 129.09, 130.81, 131.80, 133.08, 134.22, 135.16, 153.03, 155.11.
On the basis of NMR Spectra data the following structure was assigned to the compound

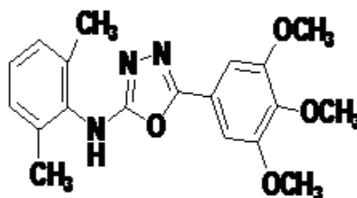


Figure 13:

N-(2,4,6-trimethylphenyl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-amine (4f)
The product was prepared from 2,4,6-trimethylbenzaldehyde and phenyl semicarbazide and characterized by the ^{1}H NMR spectra.
The ^{1}H NMR spectra of the compound showed a singlet at 3.76 ppm for 3 proton of Ar-OCH_3, a singlet at 8.39ppm for 6 proton of Ar-OCH_3 and a singlet at 8.24ppm for 1 proton of ArH, a multiplet at 7.35-7.76ppm for 4 proton of ArH and a singlet at 8.24ppm for 1 proton of NH.
On the basis of NMR Spectra data the following structure was assigned to the compound

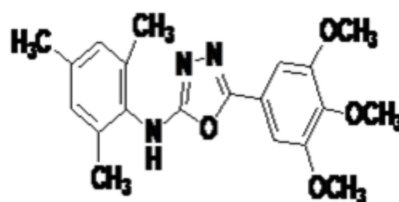


Figure 14:

Molecular docking

Oxadiazole derivatives are a class of anti-proliferative agents, privileged scaffold and have been reported as newer tubulin inhibitors [5]. In the present studies, the oxadiazole analogues were designed, based on the core skeleton of anti-tubulin agent, IMC0387525, hence we selected the tubulin as a potential target of the oxadiazole analogues (4a-f). Tubulin active site consists of large hydrophobic cavity which can accommodate a range of smaller to larger scaffolds. 1,3,4-Oxadiazole derivatives comprising of different substitutions like phenyl on one side ring comprising of chloro, hydroxyl, methoxy, dimethoxy, trimethoxy and nitro on the other side were tested for anticancer studies.

The binding mode analyses of these compounds were studied using Glide. According to the docking simulation frame work, compounds 4a-f were well accommodated in the colchicine binding site, while remaining compounds 4a-f were differed in their binding modes with respect to the compounds 4a-f. Binding site of colchicine in tubulin enzyme consists of hydrophic cavity, lined with a few

active residues like Lys254, Cys241, Lys352, THR179, Ala250 and Ala317. For the compound 4f, the presence 2,4,6-trimethyl seems to be unfavorable for its potency despite of exhibiting H-bond binding with O-atom in phenyl ring; H-bond (Thr179) binding with NH group; and π-Cationic (Lys352) binding with phenyl ring. In case of the compounds 4d they exhibited the H-bond with crucial residue H-bond (Pro175) binding to N-atom oxadiazole ring, The compounds 4d and 4f are similar binding modes for π-Cationic (Asn258) binding with naphthalene ring, in case the compound 4f they exhibited of with crucial residue π-Cationic (Lys352) binding with phenyl ring.

Anticancer Screening of synthesized compounds

The anticancer screening was carried out as per the NCI US protocol. All compounds submitted to the NCI 60 Cell screen were tested initially at a single high dose (10^{-5} M) on leukemia, Melanoma, Lung, Colon, CNS, Ovarian, renal, prostate, and Breast Cancer cell Lines, nearly 60 in number. Compound *N-(2,4-dimethylphenyl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-amine* (4d) showed maximum activity with mean growth percent (GP) of 97.75 followed by *N-(2,4,6-trimethylphenyl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-amine* (4f) showed mean GP of more than 85.81. The compound 4f was highly active on T-47D (Breast Cancer) [GP=66.70], NCI-H522 (Non-Small Cell Lung Cancer A549/ATCC) [GP=68.96], SNB-75 (CNS Cancer) [GP=75.64]. The compound 4d showed maximum activity on A498 (Renal Cancer) [GP=78.31], SF-268 (CNS Cancer) [GP=83.14], TK-10 (Renal Cancer) [GP=84.77]. The compound 4d showed maximum activity on SR (Leukemia) [GP=59.73], NCI-H522 (Non-Small Cell Lung Cancer A549/ATCC) [GP=72.77], LOX IMVI (Melanoma) [GP=76.63], MCF7 (Breast Cancer) [GP=81.32].

The maximum activity was observed with 4d on SR (Leukemia) with GP=59.73. The anticancer activity of the compounds is given in Table 5.2 in biological screening section.

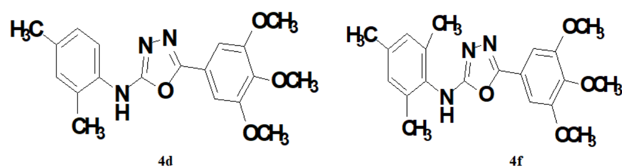


Figure 15:

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