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Original Research Article

A Research On Unveiling Novel Chemotherapeutic Agents: Design, Synthesis, and Anticancer Evaluation of N-(Substituted Phenyl)-5-(3, 4, 5-Trimethoxyphenyl)-1, 3, 4-Oxadiazol-2-Amine Analogues

Pankaj Yadav[1], Pramod Kumar Goyal[2]

[1]Research Scholar, Maharishi Arvind College of Pharmacy Ambabari, Jaipur, Rajasthan
[2]Professor, Maharishi Arvind College of Pharmacy Ambabari, Jaipur, Rajasthan

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Corresponding author: Pankaj Yadav
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Abstract

A Series of newer 5-substituent 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, 4b & 4f) were evaluated for their anticancer activity in one dose assay and showed moderate activity on various cell lines. Compound N-(4-chlorophenyl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-amine (4b) showed maximum activity with growth percent (GP) of 59.73 on SR (Leukemia), 72.77 on NCI-H522 (Non-Small Cell Lung Cancer A549/ATCC) and mean growth percent (GP) of 96.31. and Compound N-(2-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-amine (4f) showed maximum activity with growth percent (GP) of 66.70 on T-47D (Breast Cancer), 68.96 on NCI-H522 (Non-Small Cell Lung Cancer A549/ATCC), 75.64 on SNB-75 (CNS Cancer) and mean growth percent (GP) of 92.62. Compound N-(4-bromophenyl)-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: Anticancer activity, Lipinski "rule of five", Cancer, 5-substituent N-(substituent phenyl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-amine. Growth percent (GP).

Introduction

Anticancer, or Antineoplastic, drugs are used to treat malignancies, or cancerous growths. Drug therapy may be used alone, or in combination with other treatments such as surgery or radiation therapy, within the broad category of disorders known as cancer, aberrant cells can begin to grow out of supervision, cross usual borders to infiltrate neighboring regions of the body, or expand through other organs. This can occur in practically each organ or tissues within the human body. One of the main ways that malignancy kills people is by the latter phase, known as metastasizing. Other frequent terms for carcinoma are a neoplasm and a malignant tumor.

Any illness known as cancer occurs when some cells inside the body proliferate out of bounds and invade other bodily regions. With countless of cells making up the human being's body, cancer can begin practically anyplace. Human cells typically divide to create new cells as needed by the body by growing and multiplying. Fresh cells replace old ones when they die as a result of aging or injury.

This controlled mechanism can occasionally malfunction, causing damaged or aberrant cells to proliferate and expand where they shouldn't. Tumors are lumps of tissue that can be formed by those cells. Cancerous or benign tumors can both occur.

Malignant tumors can metastasize, or spread into, neighboring tissues, and can also generate new tumors by traveling to far-off regions of the body. A malignant tumor is another term for cancerous growths. While many malignancies grow into solid tumors, blood-related tumors including leukemia's,

Benign tumors do not penetrate or spread to neighboring tissues. Benign tumors seldom grow back after removal, while malignant tumors occasionally do. However, benign tumors can occasionally grow to be rather enormous. Others like malignant tumors in the brain, are potentially fatal or induce severe symptoms. The variations Among Cancer and Normal Cells, Cancerous cells are distinct from normal cells in different ways. Consider cancer cells:

Develop in a lack of signals to do so. Cells that are normal only develop in response to these types of signals.

Signaling that typically signal cells to cease multiplying or die (an action known as programmed cell death, or apoptosis) are ignored. Invade into nearby areas and spread to other areas of the body. Normal cells stop growing when they encounter other cells, and most normal cells do not move around the body.

Drug discovery

Man has found, by trial and error, which compounds/molecules could be for "medicinal purposes" to alleviate symptoms of illness. Modern drug discovery includes key stages such as Programmed selection (choosing a disease to work on), Identification and validation a drug target assay development, identification of a "lead compound", lead optimization, Identification of a candidate, Clinical trials, Release of the drug, Follow up monitoring.

The start of a drug discovery project relies on a "make and test" cycle [1].

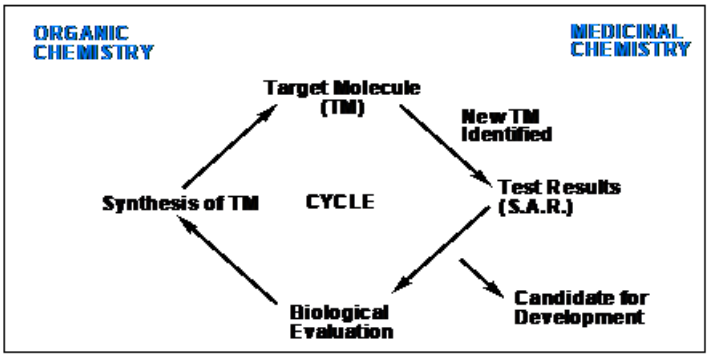


Figure 1:Design cycle of target molecule using organic and medicinal chemistry

The Basics of Cancer

Malignancy micro environment, which includes a range of immune systems, fibroblasts, chemicals, and blood arteries, surrounds cancerous cells inside the tumor's cells. The surrounding environment may be altered by cancer cells, and this can have an impact on the growth and dissemination of cancer.

Cancer cells can be recognized and attacked by immune system cells. However, few cancer cells are able to evade discovery or block an assault. Certain cancer therapies can improve the immune system's capacity to identify and eliminate cancer cells. Everybody's cancer is caused by a different mix of genetic alterations. A person's cancer might react differently to a particular therapy depending on individual variations in genetics. Cancer-causing genetic alterations may be transmitted or result from specific surroundings. Mutations may develop because a result of genetic modifications are also possible cells divide. Most typically, as a person matures, malignancy-causing genetic alterations gradually build up and increase the likelihood of developing carcinoma in later years. Cancerous cells have the ability to separate from their parent tumor and propagate via the bloodstream or lymphatic system to other parts of the human body. Once there, they depart the vessels and grow into new tumors. We call to this as cancer. Whenever cells multiply uncontrolled or infiltrate neighboring tissues, carcinoma is the result.

Mutations in DNA are responsible for the development of cancer. Most DNA alterations that cause cancer are found in regions of DNA known as genes. Genetic modifications is another term for these alterations. Gene that regulates ordinary cell development can turn into oncogenes due to a mutation in their DNA.

Oncogenes are genes that promote uncontrollable cell development because they are unable to be changed off, compared to normal genes.

Neoplasm microenvironment, which includes a range of immune system cells, fibroblasts, chemicals, and arterial blood vessels, surrounds malignant cells inside a malignant tumor.

The surrounding microenvironment may be altered by cancer cells, and this can have an impact on the growth and dissemination of cancer.

Cancer cells can be recognized and attacked by immune system cells. However, some tumor cells are able to evade discovery or block an assault. Certain cancer therapies can improve the immune system's abilities to identify and eliminate cancer cells.

Everybody's cancer is caused by a different mix of genetic alterations. A person's cancer might take in some manner to a particular treatment depending on unique genetic variations.

Cancer-causing genetic alterations can be inherited or result from specific circumstances. Mutations

that arise during cell division can also result in genetic alterations.

Usually, as a person matures, malignancy-causing genetic alterations gradually build up and increase the likelihood of developing cancer in later life.

Cancerous cells have the ability to separate themselves from their parent tumor and circulate via the blood or lymphatic system to distant parts of the body. Once there, they depart from the veins and grow into additional tumors. We refer to this as metastasis.

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

Proto-ontogenesis 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 ontogenesis), enabling cells to proliferate and survive when they shouldn't by changing in certain ways or becoming more active than usual.

Additionally, tumor suppressor genes affect the division and development of cells. Certain tumor inhibitor gene mutations can cause uncontrollably dividing cells.

Genes that healing damaged DNA are known as DNA repair genes. Abnormalities in these genes often cause chromosomal abnormalities and other abnormalities in other genes

Tell blood vessels to grow toward tumors. These blood vessels supply tumors with oxygen and nutrients and remove waste products from tumors.

Hide from the immune system. The immune system normally eliminates damaged or abnormal cells.

Elude the body's defenses and allow cancer cells to proliferate and survive. As an example, certain cancer cells persuade immune cells to defend the tumor rather than to fight it.

Acquire a variety of chromosomal modifications, including chromosome duplications and deletions. Some cancer cells include twice as many chromosomes as healthy cells.

Furthermore, unlike the majority of normal cells, some cancer cells use nutrients in a different method to produce atp. This promotes the faster growth of cancer cells.

Cancer cells are frequently dependent on these aberrant behaviors so much that they are unable to function normally absent them. Because of this, researchers have created treatments that focus on the aberrant characteristics of cancer cells. Certain cancer treatments, for instance, stop the blood vessels from arising near tumors, thus feeding the tumor of needed nutrients

When Cancer Spreads

In metastasis, cancer cells break away from where they first formed and form new tumors in other parts of the body.

A cancer that has spread from the place where it first formed to another place in the body is called metastatic cancer. The process by which cancer cells spread to other parts of the body is called metastasis.

Metastatic cancer has the same name and the same type of cancer cells as the original, or primary, cancer. For example, breast cancer that forms a metastatic tumor in the lung is metastatic breast cancer, not lung cancer.

Under a microscope, metastatic cancer cells generally look the same as cells of the original cancer. Moreover, metastatic cancer cells and cells of the original cancer usually have some molecular features in common, such as the presence of specific chromosome changes.

In some cases, treatment may help prolong the lives of people with metastatic cancer. In other cases, the primary goal of treatment for metastatic cancer is to control the growth of the cancer or to relieve symptoms it is causing. Metastatic tumors can cause severe damage to how the body functions, and most people who die of cancer die of metastatic disease

Tissue Changes that are Not Cancer

Not every change in the body’s tissues is cancer. Some tissue changes may develop into cancer if they are not treated, however. Here are some examples of tissue changes that are not cancer but, in some cases, are monitored because they could become cancer:

Hyperplasia occurs when cells within a tissue multiply faster than normal and extra cells build up. However, the cells and the way the tissue is organized still look normal under a microscope. Hyperplasia can be caused by several factors or conditions, including chronic irritation.

Dysplasia is a more advanced condition than hyperplasia. In dysplasia, there is also a buildup of extra cells. But the cells look abnormal and there are changes in how the tissue is organized. In general, the more abnormal the cells and tissue look, the greater the chance that cancer will form. Some types of dysplasia may need to be monitored or treated, but others do not. An example of dysplasia is an abnormal mole (called a dysplastic nevus) that forms on the skin. A dysplastic nevus can turn into melanoma, although most do not

Anti-cancer Agents

Antineoplastic agents are drugs used for the treatment of cancer. Antineoplastic drugs are most effective against rapidly dividing tumor cells.

The main goal of Antineoplastic agents is to eliminate the cancer cells without affecting normal tissues (the concept of differential sensitivity). In reality, all cytotoxic drugs affect normal tissues as well as malignancies- aim for a favorable therapeutic index (aka therapeutic ratio).

$$\text{Therapeutic Index} = LD_{50}/ED_{50}$$

A therapeutic index is the lethal dose of a drug for 50% of the population (LD_{50}) divided by the minimum effective dose for 50% of the population (ED_{50})

Classification

1. Alkylating agents

a) Nitrogen mustards: Melphalan, Cyclophosphamide, Ifosamide
b) Alkyl Sulphonate: Busulfan
c) Nitrosoureas: Chlorozotocin, Carmustine, Fotemustine.
d) Ethyleneimenines: Triethylene Thiophosphoramide (Thio-TEPA)
e) Triazene: Dacarbazine
f) Methyl Hydrazines: Procarbazine, Dacarbazine
g) Platinum Co-ordination complexes: Cisplatin, Carboplatin, Oxaliplatin

2. Antimetabolites

a) Pyrimdine analogues: 5-Flurouracil (5-FU), Capectitabine, Cytarabine
b) Purine Analogues: 6-Mercaptopurine, 6-Thioguanine, Fludarabine
c) Folic acid analogues: Aminopterin, Methotrexate

3. Natural Products

a) Plant products

i. Vinca alkaloids: Vincristine, Vinblastine
ii. Taxol derivatives: Paclitaxel, Docetaxel
iii. Epipodophyllo toxins: Etoposide, Teniposide
iv. Camptothecin Derivatives: Irinotecan, Topotecan

b) Microorganism Products

i. Antibiotics: Doxorubicin, Bleomycin
ii. Enzymes: L-asparaginase, Pegaspargase

4. Miscellaneous

Hydroxy urea, Carboplastin, Cisplastin

5. Drugs altering hormonal milieu

a) Glucocorticoids: Prednisone
b) Antiandrogen: Flutamide
c) Estorgenic derivatives: Ethinyl estradiol, Fosfestrol
d) Antiestorgen: Tamoxifen
e) 5-Alpha reductase inhibitor:Finasteride
f) GnRH Analogues: Naferewlin, Gosereline
g) Progestines: Hydroxyprogesterone acetate

Chemotherapy

Classification based on the mechanism of action

Antimetabolites

Drugs that interfere with the formation of key biomolecules including nucleotide, the building blocks of DNA.

Genotoxic Drugs

Drugs that alkylate or intercalate the DNA causing the loss of its function.

Plant-derived inhibitors of mitosis

These agents prevent proper cell division by interfering with the cytoskeletal components that enable the cell to divide.

Plant-derived topoisomerase inhibitors

To poisomerases unwind or relegate DNA during replication.

Cancer Statics

Cancer is a leading cause of death worldwide, accounting of nearly 10 million deaths in 2020.

- **Lungs**: 1.80 million deaths
- **Colon and rectum** : 9,16,000 deaths
- **Liver**: 8,30,000 deaths
- **Stomach**: 7,69,000 deaths
- **Breast**: 6,85,000 deaths

Each year, approximately 4,00,000 children develop cancer

Medication 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

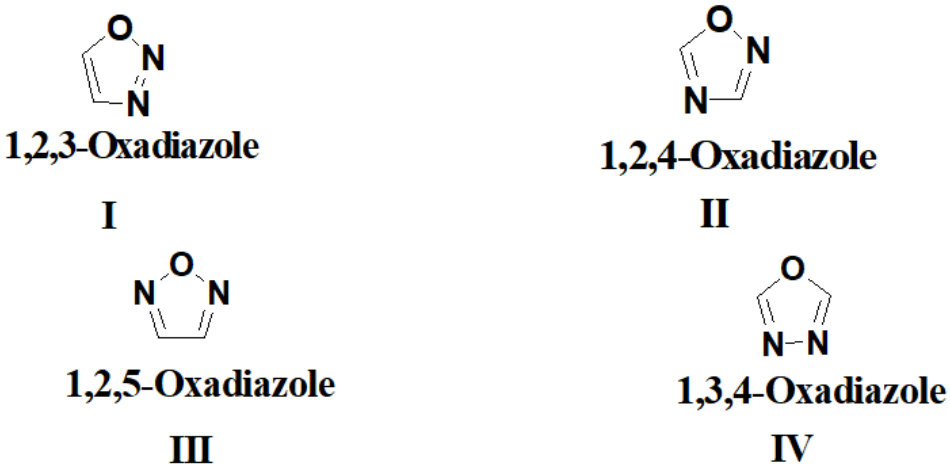


Figure 2:

Table 1: Characteristics of heterocyclic nucleus

Structure	
IUPAC name	1,3,4 –oxadiazole
Molecular formula	$C_2H_2N_2O$
Molecular weight	70

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.

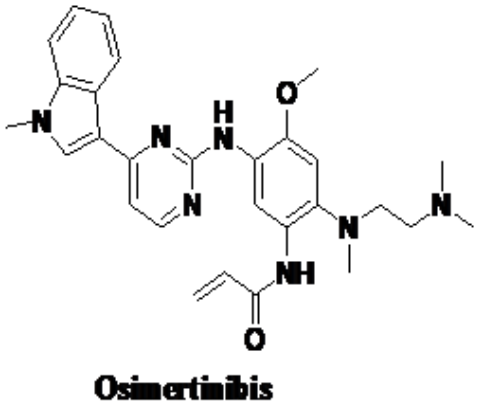


Figure 3:

- 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.

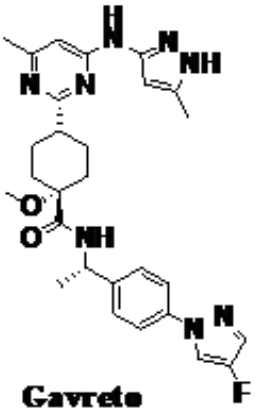


Figure 4:

- 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).

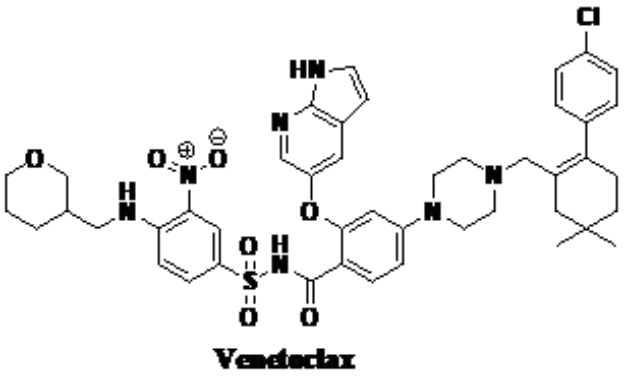


Figure 5:

- 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.

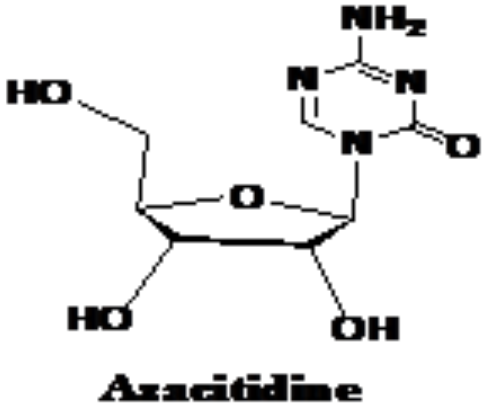
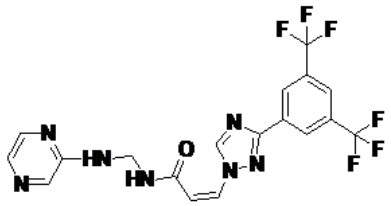


Figure 6:

- 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.



Selinexor

Figure 7:

Molecular Properties Predictions

Among the pharmacokinetics properties, a low and highly variable bioavailability is indeed the main reason for stopping further development of the drugs. Thus, prediction of bioavailability and bioavailability-related properties, such as solubility, lipophilicity are important before actual synthesis, in order to reduce enormous wastage of expensive chemicals and precious time. An in silico model for predicting oral bioavailability is very important, both in the early stage of drug discovery to select the most promising compounds for further optimization and in the later stage to identify candidates for further clinical development. In present investigation a series of oxadiazole analogues were subjected to molecular properties prediction, drugs-likeness by Mol-inspiration & Mol-Soft (MolSoft, 2007) software's, lipophilicity and solubility parameter by using ALOGPS 2.1 program to filter the compounds for further synthesis and biological evaluation

Molecular Properties and Drug-likeness

A molecular property is a complex balance of various structural features which determine whether a particular molecule is similar to the known drugs. It generally means "molecules which contain functional group and have physical properties consistent with most of the known drugs". These properties, mainly hydrophobicity, molecular size, flexibility and presence of various pharmacophoric features influence the behavior of molecules in a living organism, including bioavailability. Thus in order to achieve good oral drugs we have subjected a series of 1,3,4-oxadiazole derivatives for the prediction of lipophilicity, solubility and Lipinski "Rule of Five" and other properties for filtering compound for subsequent synthesis and antimicrobial screening

Lipophilicity

The ALOGPS method is part of the ALOGPS 2.1 program used to predict lipophilicity and aqueous solubility of compounds. Log P values are used where, P is the partition coefficient of the molecule in the Water-octanol system.

The lipophilicity calculations within this program are based on the associative neural network approach and the efficient partition algorithm

- LogKow (Kow-WIN) program estimates the log octanol/water partition coefficient (logP) of organic chemicals and using an atom/fragment contribution method developed
- XLOGP2 is an atom additive method applying corrections

Solubility

Drug solubility is one of the important factors, which affect the movement of a drug from the site of administration into the blood.

Investigation of the rate limited steps of human oral absorption of 238 drugs showed that the absorption of a drug is usually very low if the calculated solubility is <0.0001 mg/l

Absorption, Polar Surface Area and "Rule of Five" Properties

- Molecular properties such as membrane permeability and bioavailability is always associated with some basic molecular descriptors such as logP (partition coefficient), molecular weight (MW), or hydrogen bond acceptors and donors counts in a molecule
- Lipinskin used these molecular properties in formulating his "Rule of Five". The rule state that most molecular with good membrane permeability have logP ≤5, molecular weight ≤500, number of hydrogen bond acceptor ≤10, and number of hydrogen bond donors ≤5. This rule is widely used as a filter for drug-like properties
- Number of Rotatable Bonds is important for conformational changes of molecules under study and ultimately for the binding of receptors or channels
- Molecular Polar Surface Area (PAS) is a very useful parameter for the prediction of drug transport properties. PAS is a sum of surface of polar and volume is inversely proportional to %ABS. The percentage of absorption was estimated using the Equation: %ABS = 109-(0.345×TPSA)
- Drug-likeness model score: A combined effect of physico-chemical properties, pharmacokinetics and pharmacodynamics of compound and is represented by a numerical value was computed by (MolSoft, 2007) software for the (07) molecules under study

Project Plan

Scheme

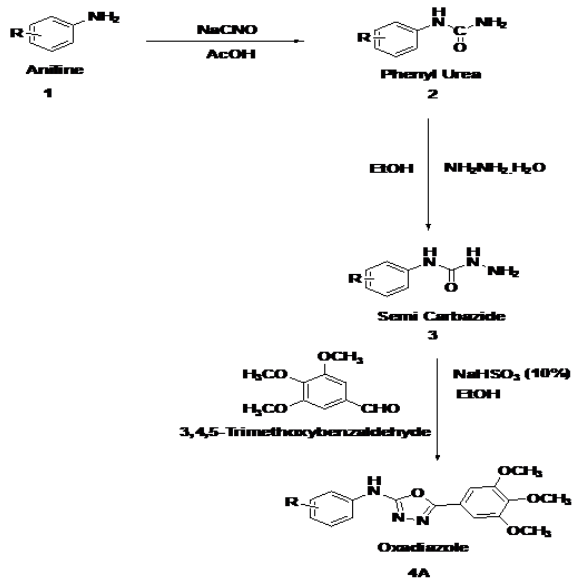


Figure 8:

R= 4-Br; 2-Cl; 3-Cl; 4-Cl; 4-F; 2-OCH_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.

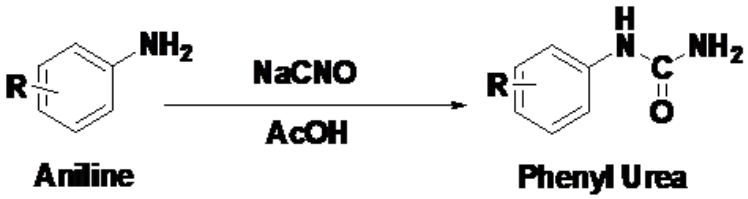


Figure 9:

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.

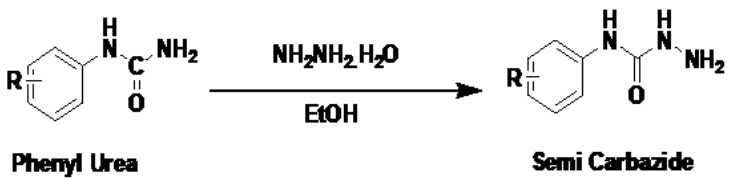


Figure 10:

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.

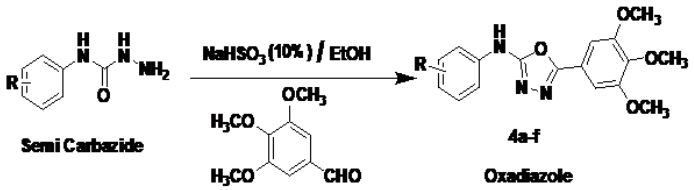


Figure 11:

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

Compounds	R	% Yield	Mp(ºC)	R_f Value
4a	4-Bromophenyl	58.23	207-2011	0.624
4b	2-Chlorophenyl	70.08	142-144	0.714
4c	3-Chlorophenyl	61.73	125-127	0.685
4d	4-Chlorophenyl	72.21	164-167	0.628
4e	4-Fluorophenyl	85.18	155-158	0.740
4f	2-Methoxyphenyl	68.53	158-161	0.546

Identification and characterization of compounds

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

Synthesized intermediate and final molecules were characterized using IR, ^{1}H NMR and Mass spectroscopy.

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-(4-bromophenyl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-amine (4a).

^{1}H NMR (300 MHz): δ ppm: 7.44 (d, 2H, *J*=2.4Hz, ArH), 7.85 (d, 2H, *J*=2.4 Hz, ArH), 7.57-7.93 (m, 7H, ArH), 8.72 (s, 1H, ArNH).

^{13}C NMR (100 MHz,): *δ* 169.6 (C), 150.8 (CH), 140.5 (C), 129.7 (CH), 125.8 (CH), 124.6 (C), 98.0 (CH), 21.4 (CH_3).

MS (*m/z*): 215 (100, M^+), 175 (22), 134 (30), 119 (95), 115(75), 91 (40), 56 (38).

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

^{1}H NMR (300 MHz): δ ppm: 8.21-8.20 (d, 1H, *J*=2.4Hz, $ArNO_2$), 8.34-8.31 (d, 2H, *J*=9.9Hz, ArH), 8.84 (m, 7H, ArH).

^{13}C NMR (100 MHz, $CDCl_3$): *δ* 165.8 (C), 150.8 (CH), 128.7 (C), 127.0 (CH), 125.4 (CH), 124.3 (CH), 98.4 (CH).

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

N-(4-fluorophenyl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-amine (4e).

^{1}H NMR (300 MHz): δ ppm: 8.90 (s, 1H, ArNH), 7.45-8.39 (m, 1 H, ArH), 11.52 (s, 1H, ArOH);

^{13}C NMR (75 MHz): δ 155.31, 153.21, 133.74, 133.68, 128.01, 127.82, 127.65, 127.34, 125.96, 125.76, 125.57, 124.86, 122.64, 120.94;

N-(2-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-amine (4f).

^{1}H NMR (300 MHz): δ ppm: 3.76 (s, 3H, $ArOCH_3$), 3.86 (s, 6H, $ArOCH_3$), 7.13 (s, 2H, ArH), 7.16-7.88 (m, 7H, ArH), 8.89 (s, 1H, NH).
^{13}C NMR (100 MHz): *δ* 174.5(C), 147.8 (CH), 117.3 (C), 141.0 (CH), 121.4 (CH), 127.7 (CH), 87.4 (CH_3).

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 addition (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

Value is calculated for each of these three parameters. if the level of activity is reached, however if the effect is not reached or exceed, the value for that parameter is expressed as greater or less than the maximum or minimum conc. test.

Interpretation of One –Dose Data

The one dose data will be reported as a mean graph of the percent growth of treated cells. 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 man 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 [1].

Table 3: Anticancer activity of oxadiazole analogues

Panel/Cell Line	Growth % in One Dose Assay		
	4a	**4b**	**4f**
Leukemia			
CCRF-CEM	102.49	86.21	99.78
HL-60(TB)	90.65	99.3	98.45
K-562	94.74	91.71	100.46
MOLT-4	86.98	93.95	76.91
RPMI-8226	102.71	101.25	96.57
SR	87.53	59.73	84.6
Non-Small Cell Lung Cancer			
A549/ATCC	88.89	97.15	80.11
EKVX	95.63	114.45	94.09
HOP-62	98.88	89.2	91.24
HOP-92	100.28	101.17	97.89
NCI-H226	89.85	93.76	94.14
NCI-H23	96.86	92.94	94.39
NCI-H322M	87.7	93.41	90.59
NCI-H460	103.14	102.8	98.53
NCI-H522	85.65	72.77	68.96
Colon Cancer			
COLO 205	110.58	97.41	103.29
HCC-2998	90.56	91.8	91.83
HCT-116	102.1	91.87	79.42
HCT-15	106.93	104.98	100.15
HT29	97.93	88.7	82.11
KM12	99.22	94.43	94.58
SW-620	104.88	102.98	104.1
CNS Cancer			
SF-268	83.14	90.07	82.02
SF-295	105.35	101.83	95.62
SF-539	99.38	95.66	93.52
SNB-19	94.46	95.64	95.78
SNB-75	90.67	98	75.64

U-251	95.6	91.62	80.32
Melanoma			
LOX IMVI	94.41	76.63	93.72
MALME-3M	88.14	87.22	87.87
M14	105.45	104	104.01
MDA-MB-435	103.74	108.75	96.5
SK-MEL-2	93.56	106.5	93.25
SK-MEL-28	105.78	109.81	105.94
SK-MEL-5	91.23	97.52	94.87
UACC-257	97.19	94.9	97.54
UACC-62	94.14	96.4	87.68
Ovarian Cancer			
IGROV1	87.64	92.92	86.35
OVCAR-3	98.35	100.62	107.26
OVCAR-4	96.12	100.12	97.9
OVCAR-5	99.89	96.33	98.31
OVCAR-8	102.85	94.28	100.35
NCI/ADR-RES	103.09	99.52	94.68
SK-OV-3	107.71	105.94	95.81
Renal Cancer			
786-0	105.63	103.12	94.66
A498	78.31	106.19	70.38
ACHN	103.19	102.05	101.5
CAKI-1	100.3	101.45	94.23
RXF 393	94.57	108.97	92.9
SN12C	93.79	101.57	97.52
TK-10	84.77	85.19	91.31
UO-31	86.77	87.36	82.78
Prostate Cancer			
PC-3	95.55	98.23	87.46
DU-145	97.6	101.91	104.96
Breast Cancer			
MCF7	90.44	81.32	95.52
MDA-MB-231/ATCC	94.68	101.35	94.05
HS 578T	98.75	105.72	97.8
BT-549	100.02	99.09	101.61
T-47D	91.88	88.02	66.7
MDA-MB-468	96.57	100.94	96.59
Mean	96.25	96.31	92.62
Range	32.27	54.72	40.56

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 (4a, 4b 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
3a	96.25	32.27	A498 (Renal Cancer)	78.31	21.96
			SF-268 (CNS Cancer)	83.14	16.86
			TK-10 (Renal Cancer)	84.77	15.23
			NCI-H522(Non-Small Cell Lung Cancer A549/ATCC)	85.65	14.35
			UO-31(Renal Cancer)	86.77	13.23
			MOLT-4 (Leukemia)	86.98	13.02
3c	96.31	54.72	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) TK-10 (Renal Cancer)	81.32 85.19	18.68 14.81
3f	92.62	40.56	T-47D (Breast Cancer) NCI-H522(Non-Small Cell Lung Cancer A549/ATCC) SNB-75 (CNS Cancer) MOLT-4 (Leukemia) HCT-116 (Colon Cancer)	66.70 68.96 75.64 76.91 79.42	33.3 31.04 24.36 23.09 20.58

Findings and Summary

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-(4-bromophenyl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-amine (4a).

The product was prepared from 4-bromobenzaldehyde and phenyl semi carbazide and characterized by the ^{1}H NMR spectra.

The ^{1}H NMR spectra of the compound showed a doublet at 7.85 ppm for 2 proton of ArH, a multiplet at 7.57-7.93 ppm for 7 proton of ArH and a singlet at 8.72 ppm for 1 proton of ArNH.

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

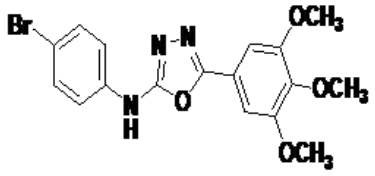


Figure 12:

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

The product was prepared from 4-chlorobenzaldehyde and phenyl semicarbazide and characterized by the ^{1}H NMR spectra.

The ^{1}H NMR spectra of the compound showed a doublet at 8.21-8.20 ppm for 1 proton of $ArNO_2$, a doublet at 8.34-8.31 ppm for 2 proton of ArH and a multiplet at 8.84 ppm for 7 proton of ArH. On the basis of NMR Spectra data the following structure was assigned to the compound

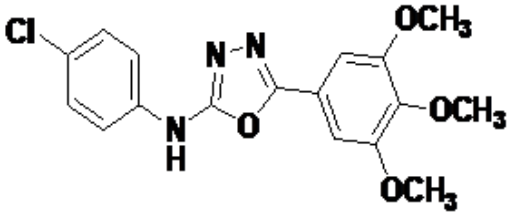


Figure 13:

N-(4-fluorophenyl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-amine (4e).
The product was prepared from 4-fluorobenzaldehyde and phenyl semicarbazide and characterized by the ^{1}H NMR spectra. The ^{1}H NMR spectra of the compound showed a singlet at 8.90 ppm for 1 proton of ArNH, a multiplet at 7.45-8.39 ppm for 1 proton of ArH and a singlet at 11.52 ppm for 1 proton of ArOH. The ^{13}C NMR spectra of the compound showed a ppm 155.31, 153.21, 133.74, 133.68, 128.01, 127.82, 127.65, 127.34, 125.96, 125.76, 125.57, 124.86, 122.64, 120.94. On the basis of NMR Spectra data the following structure was assigned to the compound.

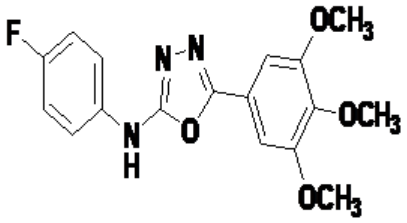


Figure 14:

N-(2-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-amine (4f).
The product was prepared from *2-methoxy*benzaldehyde and 4 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 3.86 ppm for 6 proton of Ar-OCH_3 and a singlet at 7.13 ppm for 2 proton of ArH, a multiplet at 7.16-7.88 ppm for 7 proton of ArH and a singlet at 8.89 ppm for 1 proton of NH.
On the basis of NMR Spectra data the following structure was assigned to the compound.

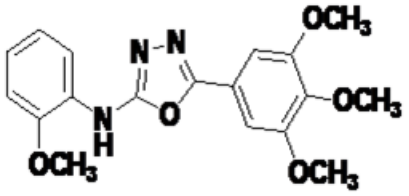


Figure 15:

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, IMC038525, 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 3,4,5-trimethoxy 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 (Lys254) binding with naphthalene ring. In case of the compounds 4b they exhibited the H-bond with crucial residue Thr179 binding to N-atom oxadiazole ring, The compounds 4a, 4b and 4c 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 (Asn258) 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-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-amine (4f) showed maximum activity with mean growth percent (GP) of 92.62

followed by N-(4-bromophenyl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-amine (4a) with mean GP of 96.25 while N-(4-chlorophenyl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-amine (4b) showed mean GP of more than 96.31. 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 4a 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 4b 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 4b on SR (Leukemia) with GP=59.73. The anticancer activity of the compounds is given in Table 5.2 in biological screening section.

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