ORIGINAL ARTICLE

Design Synthesis And Molecular Docking Of 2 5 Di Substituted Oxadiazole Molecule

Anwesha Sarkar[1], Praveen Kumar[2], Shivani kala[3], Rajat Bisht[4]
Himalayan Institute Of Pharmacy and Research, Dehradun (Uk), India
*Corresponding author email: anwesha0199@gmail.com

INTRODUCTION

Biosensors are cutting-edge analytical devices that integrate a biological sensing element with a physicochemical detector, facilitating the detection of chemical substances. In the pharmaceutical industry, biosensors play a crucial role in various applications, including drug development, diagnostics, and personalized medicine. Their ability to provide real-time, accurate, and specific detection of biological molecules has revolutionized pharmaceutical research and clinical practices.

Overview of Oxadiazole Derivatives in Medicinal Chemistry

Oxadiazole derivatives represent a class of heterocyclic compounds that have garnered significant attention in medicinal chemistry due to their diverse pharmacological activities and structural versatility. Oxadiazoles are five-membered heterocycles containing one oxygen and two nitrogen atoms in the ring system. This unique structure imparts favorable physicochemical properties, including stability and solubility, making them promising candidates for drug development.

In medicinal chemistry, oxadiazole derivatives have been explored for their broad spectrum of biological activities, such as antimicrobial, anticancer, anti-inflammatory, antiviral, anticonvulsant, and antioxidant properties. Their ability to modulate various biological targets, including enzymes, receptors, and proteins, underscores their potential therapeutic applications across different disease areas.

Importance of 2,5-Disubstituted Oxadiazole Molecules

Among oxadiazole derivatives, 2,5-disubstituted oxadiazoles occupy a pivotal role due to their specific structural features and enhanced pharmacological profiles. The substitution pattern at positions 2 and 5 on the oxadiazole ring significantly influences their biological activity, pharmacokinetic properties, and target specificity. This structural modification allows for fine-tuning of molecular interactions with target biomolecules, thereby optimizing therapeutic efficacy and minimizing off-target effects.

The introduction of diverse substituents (such as alkyl, aryl, or heteroaryl groups) at positions 2 and 5 of the oxadiazole scaffold enables medicinal chemists to design compounds with tailored physicochemical properties and biological activities. This flexibility in structural modification enhances the drug-likeness and bioavailability of 2,5-disubstituted oxadiazole derivatives, making them attractive candidates for further pharmacological investigation and drug discovery efforts.

Components and Working Principle

A typical biosensor consists of three main components:

1. **Biological Element**: This can be enzymes, antibodies, nucleic acids, or cells that interact specifically with the target analyte.
2. **Transducer**: Converts the biological response into a measurable signal, often electrical, optical, or thermal.
3. **Detector**: Measures and displays the signal, allowing for quantitative or qualitative analysis.

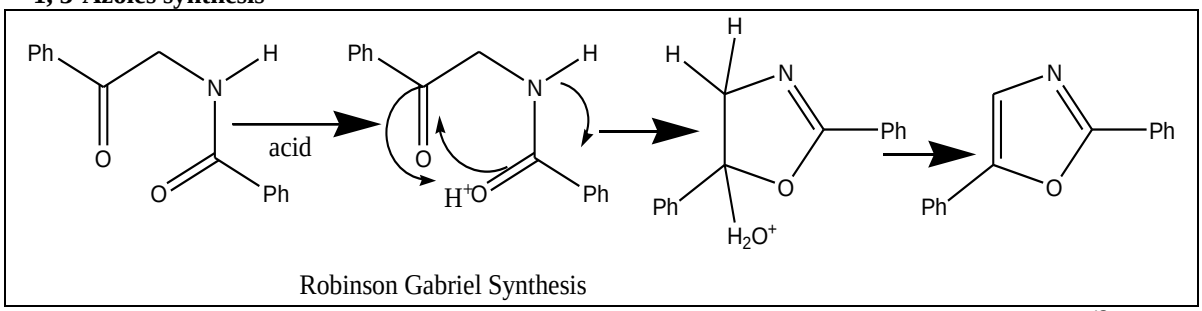
The biological element interacts with the target analyte, producing a biochemical response that the transducer converts into a measurable signal. This signal is then processed and displayed, providing valuable information about the presence and concentration of the analyte

AZOLES

Azoles are the class which contain five member ring, having nitrogen heterocyclic ring compound containing at least one other heteroatom (or non-carbon atom) of nitrogen, sulfur or oxygen and are considered to be derived from pyrrole, furan and thiophene by substitution of methane groups (—CH=) by pyridine type nitrogen (—N=) atoms from the different positions. Azole mainly consists of:

(i) Oxygen is referred as oxazole
(ii) Sulphur is referred as thiazole and
(iii) Nitrogen is referred as pyrazole, imidazoles or imidazolines[12].

1, 3-Azoles synthesis[1]



Scheme 1.1: Synthesis of 1, 3-Azoles by Cyclodehydration of α-acylaminocarbonyl compound[12]

1.8 OXAZOLE

The chemistry of oxazole began in 1876 with the synthesis of 2-methyloxazole, while parent oxazole was synthesized in 1962. During the world war when penicillin was considered to contain the oxazole ring system at that time the chemistry of oxazole was come in concern, but the invention of oxazoles as dienes in Diels-Alder reaction and in 1, 3-dipolar cycloaddition reaction of mesoionic heterocycles give idea for progress of oxazole chemistry. Oxazole contain an oxygen atom and a pyridine type nitrogen atom at the 1 and 3 positions of the ring and like pyridine, oxazole are weekly basic substances. Oxazole be considered as derived from furan by the replacement of –CH= (methane group) from the position-3 by the azomethine nitrogen (-N=) group[13]. Oxazole ring is numbered as follows:

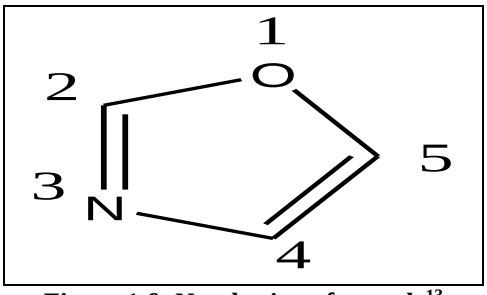


Figure 1.6: Numbering of oxazole[13]

1.8.1. Chemistry of oxazole

At C-4 and C-2 position of oxazolone if substitution of functional group is occur it plays a vital role in the activity. For enhancing tyrosinase inhibitory activity the substitution of cinnamoyl residue at C-4 of oxazolone moiety and functional group at C-4 and C-2 positions of oxazolone were takes place. Substitutions of p-nitro group in exocyclic phenyl group at C-4 in oxazolone moiety greatly influence the immunosuppressive activity. Addition double bond at C-4 position and phenyl ring present in C-2 position plays an imperative role in oxazolone ring. Exocyclic double bond act as dienophile and N-substituted oxazolone participate in intermolecular Diels-Alder reaction. Oxazolone ring-operating reaction decreased with an increase of the electron donating properties of the substituent of the phenyl ring at C-2 position[14]. Oxazole scaffolds and its heterocyclic analogues posses diversified biological activities such as antitubercular, antihyperglycemic, antibacterial, anti inflammatory and anti-proliferate[15].

1.8.2. Activities of oxazole moiety

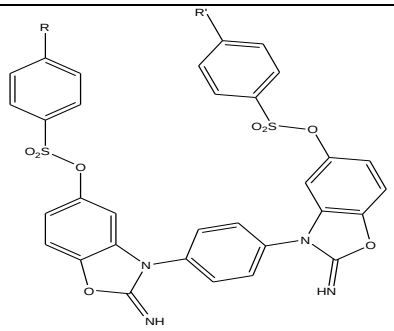
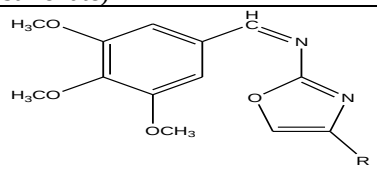
Oxazolones shows potential photophysical and photochemical activities, so they are used in semiconductor devices like electrophotographic photoreceptors and in non-linear optical materials. Oxazolones shows similarity as same in structure and chemistry of penicillin. They are used in polymerization and condensation of homopolymers, telomers, herbicides, peptides, condensation reagents, pesticides, fungicides and agrochemical intermediates. Spirocyclopropyl oxazolones is the novel class of herpes protease inhibitor. Phenacyl oxazolone involves the intermolecular Diels-Alder reaction, ensuing in synthesis of anti-cancer drugs, pancratistantin and a phenanthrene alkaloid[14].

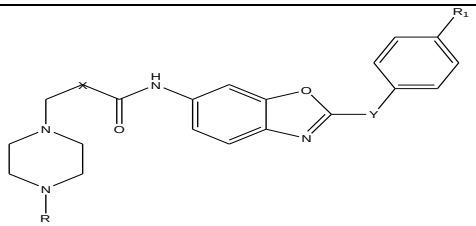
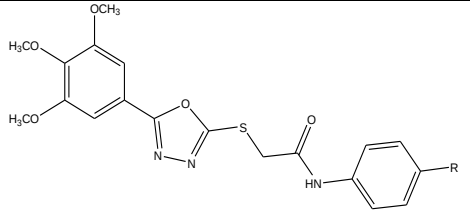
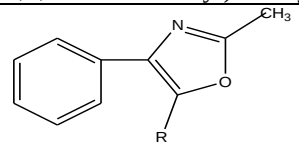
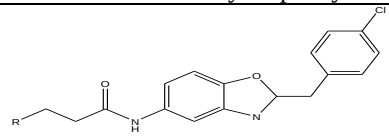
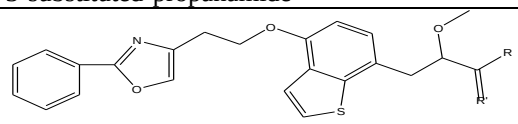
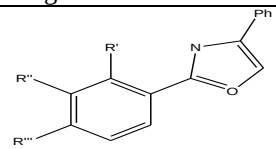
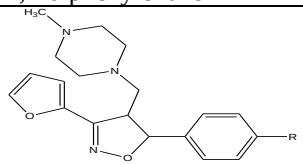
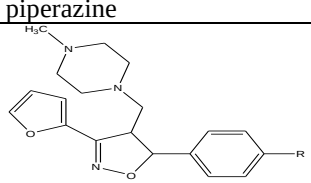
1.8.3 Properties of oxazole - Chemistry of heterocyclic compounds is one of the foremost lines of investigation in the organic chemistry. In the field of drug discovery process the nitrogen, sulphur and oxygen containing five member heterocyclic compounds have occupied enormous importance. Oxazole is a weekly basic aromatic compound with three potential points of substitution, C-2, C-4 and C-5**.** Oxazoles are numbered around the ring starting at the oxygen atom and are designated as 1, 3-oxazoles to designate the position of heteroatoms in the ring. The reactivity of oxazoles shows that the acidity of a hydrogen atom decreases in the order C (2) > C (5) > C (4). The acidity of the hydrogen at C-(2) was predicted to be pKa 20 while for oxazole itself the pKb is reported to be pKb 1.17.Oxazoles exhibit distinctive resonances in both 1H NMR and 13C NMR spectra. The parent compound displays resonances between 7.00 and 8.00 in the 1H NMR spectrum, and the existence of substituents can change the chemical shift by up to 1 ppm. The 13C NMR of oxazole displays characteristic aromatic resonances. The shielding or deshielding effect of C (2) substitution on the C (4) and C (5) resonances is usually < 2 ppm. In methanol, the parent ring system indicates the absorption maximum at l max ¼ 205 nm [(12, 14)].

1.8.4. Geometric and electronic structure of oxazole and oxazole system[16]

Quantum chemistry method plays a fundamental role in obtain molecular geometric and predict various properties. To obtain highly precise geometric and physical properties for molecules that are built from electron negative elements, expensive Ab initio/ HF electron correlation methods are required. QSAR studies are attempts to correlate molecular structure, derived from molecular structure, with a particular mind of chemical or biochemical activity.

PHARMACOLOGICAL ACTIVITY OF OXAZOLE

S.No.	Activity	Active compound	Derivatives	Author name/ Year
1.	Anti corrosion potential against stainless steel (L316) SS[31]	R=R'=CH_3	 3,3'-(1,4Pheneylene)bis(2-imino-2,3-dihydro benzo[d]oxazole-5,3-diyl)bis(4-ethylbenzene sulfonate)	A. Ehsani (2016) *et al.*
2.	Antibacterial, Antifungal, Antihelmenthic[32]	R=H, C_6H_5, C_6H_4Cl, C_6H_4F, $C_6H_4NO_2$	 N-(3,4,5-trimethoxybenzylidene)-4-substituted oxazol-2-amine	B.S Rawat *et al.*

3.	Antibacterial and Antifungal[19]	X=CH_2CH_2, Y=CH_2, R_1=p-Cl, R=Cl and X=CH_2CH_2, Y=CH_2, R_1=p-Cl, R=CH_3	 N-(3,4,5-trimethoxybenzylidene)-4-substituted oxazol-2-amine	M. Arisoy (2016) *et al.*
4.	Antimicrobial activity[20]	R=F, Cl, CH_3, H	 N-Phenyl-2-{[5-(3,4,5-tri-methoxy phenyl)-1,3,4-oxadiazol-2-yl)sulfanyl} acetamide	M.B. Shukla (2016) *et al.*
5.	Antibacterial[33]	R=4-F-Phenyl, R=3-CF_3 Phenyl	 5-substituted-2-methyl-4-phenyloxazole	R. K. Singh (2016) *et al.*
6.	Anti-inflammatory[34]	R=diethylamine, Dimethylamine and dipropylamine	 N-(2-(4-chlorobenzyl) benzo[d]oxazol-5-yl)-3-substituted-propanamide	A.K. Sakya (2015) *et al.*
7.	Type2 diabetes mellitus[35]	R=OH, R'=H	 Aleglitazar	A. Michael lincoff (2014) *et al.*
8.	Antitumor activity[36]	R_1=Cl, R_2=H, R_3=Cl R_1=OCH_3,R_2= R_3=H	 2,4-diphenyloxazol	J.E. Mathew (2013) *et al.*
9.	Antidepressant activity[37]	R=H, 4-CH_3, 2-Cl, 4-Br, 4-Cl, 4-OH, 4-OCH_3	 1-{[3-(furan-2-yl)-5-substituted phenyl-4, 5-dihydro-1, 2-oxazol-4- yl] methyl}-4-methyl piperazine	J. Kumar (2013) *et al.*
10.	Antianxiety[37]	R=CH_3 R=OCH_3 R=OH	 1-{[3-(furan-2-yl)-5-substituted phenyl-4, 5-dihydro-1, 2-oxazol-4- yl] methyl}-4-methyl piperazine	J. Kumar (2013) *et al.*

1,3,4-Oxadiazole scaffolds exist ubiquitously in natural products, pharmaceuticals, polymers, and materials. (1) In particular, the compounds that are containing 2,5-disubstituted 1,3,4-oxadiazole motifs exhibit a broad spectrum of biological activities including antimicrobial, anticonvulsant, antidiabetic, antiproliferative, anti-inflammatory, antiallergic, anticancer, antimalarial, antiobesity, antiviral, antidepressant, antihypertensive, antileishmanial, insecticidal, herbicidal, analgesic, antioxidant, immunosuppressant, monoamine oxidase inhibitory, and urease inhibitory activities (Figure 1). (2) On the other hand, these scaffolds are being employed in the development of organic light-emitting diodes (OLEDS), which are utilized in full-color, flat-panel displays. (3) Moreover, some of the conjugated oxadiazoles act as multiphoton absorbing systems. (4)

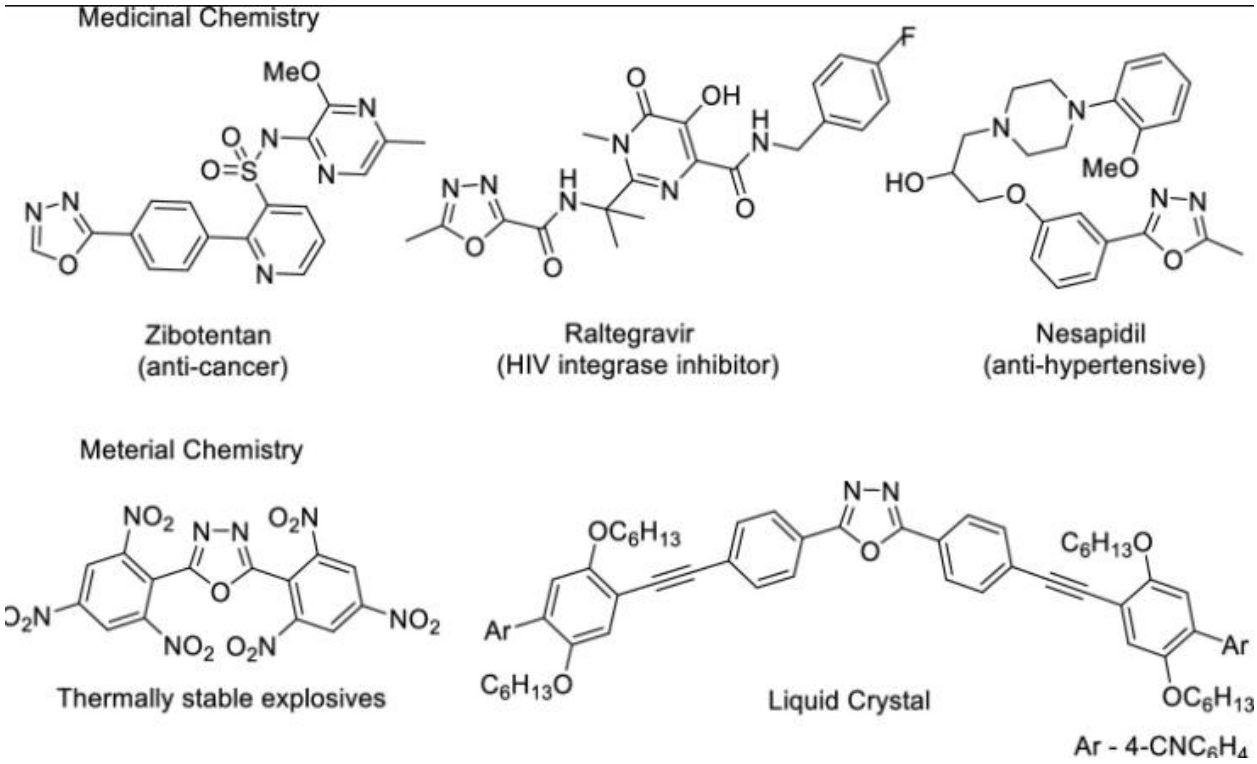
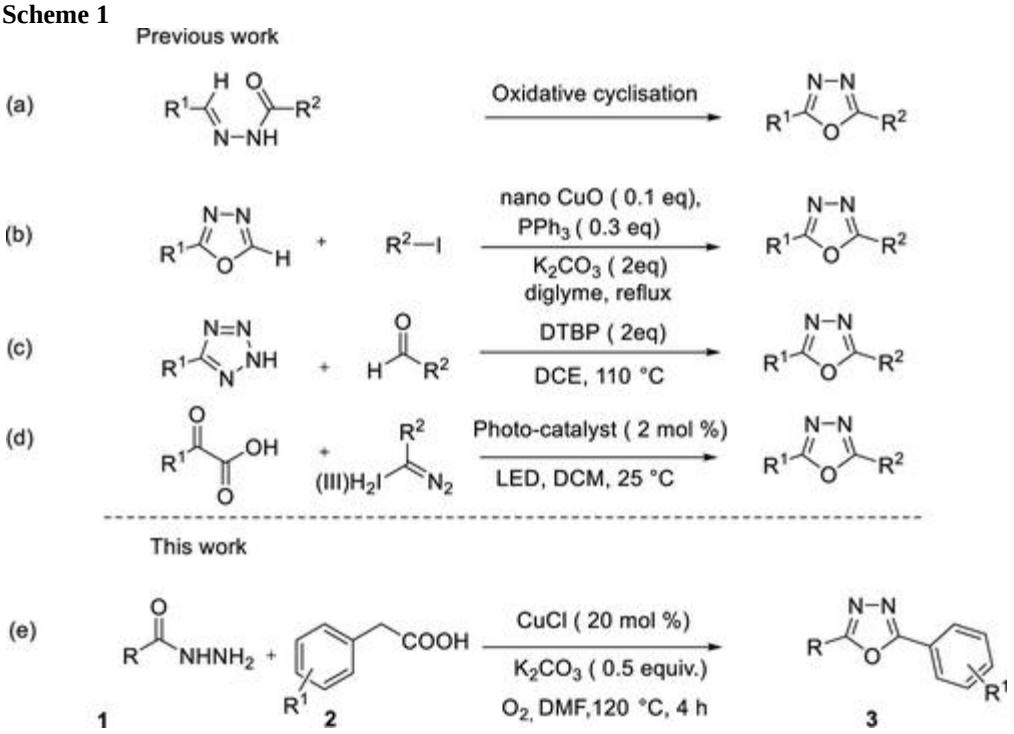


Figure 1. Active compounds containing 1,3,4-oxadiazole scaffolds.

Owing to their importance in various fields, the exploration of methodologies for the synthesis of 1,3,4-oxadiazoles is being continued by organic chemists. (5) Traditional methods used for the construction of 1,3,4-oxadiazole framework involve the N-acylation of acyl hydrazides or their precursors with either carboxylic acids or their activated derivatives such as acid chlorides, (6) esters, (7) and anhydrides (8) followed by intramolecular cyclodehydration. Alternatively, these compounds can also be constructed by oxidative cyclization of N-acyl hydrazones in the presence of various oxidizing agents such as hypervalent iodines, (9a−e) chloramine T, (9f) ceric ammonium nitrate, (9g) $FeCl_3$, (9h) tetravalent lead reagents, (9i,j) Br_2, (9k) $KMnO_4$ under microwave condition, (9l) Fe(III)/TEMPO, (10) $Cu(OTf)_2$, (11) I_2/K_2CO_3, (12) and isobutyl aldehyde/O_2/PhI (13) (Scheme 1a). These methods are, however, often limited in the requirement of harsh reaction conditions such as the involvement of strong acids in combination with high temperature and utilization of toxic oxidants. Some of these drawbacks have been overcome by the recent developments in metal-catalyzed cross-coupling reactions *via* C–H activation that allow the construction of target heterocyclic compounds under relatively milder conditions. For example, 2,5-disubstituted 1,3,4-oxadiazoles have been synthesized by copper-catalyzed coupling between 1,3,4-oxadiazole with aryl or alkenyl halides (Scheme 1b). (14) Subsequently, He et al. disclosed a metal- and base-free reaction to obtain 2,5-diaryl 1,3,4-oxadiazoles from aryl tetrazoles by N-acylation with aldehydes followed by thermal rearrangement (Scheme 1c). (15) Recently, Li Liu et al. developed a novel approach to assemble 2,5-disubstituted 1,3,4-oxadiazoles from α-oxocarboxylic acids *via* a decarboxylative cyclization by photoredox catalysis using hypervalent (III) iodine as a catalyst (Scheme 1d). (16) Although these methods are impressive, still there are some drawbacks such as the

presynthesis of starting materials, long reaction times, expensive ligands, reagents, and low yields. Therefore, facile and simple approaches for accessing an array of 2,5-disubstituted 1,3,4-oxadiazoles from easily available starting materials are highly desirable.

Scheme 1



Scheme 1. Recent Progress in the Synthesis of 1,3,4-Oxadiazoles

Recently, transition-metal-catalyzed decarboxylative coupling of carboxylic acids, in particular, C(sp^3) arylacetic acids, has been employed as an effective tool in organic synthesis to forge various heterocyclic compounds (17) because arylacetic acids are highly stable compared to aldehydes and release a nontoxic byproduct (CO_2). Moreover, arylacetic acids are cheap, commercially available, nontoxic, and easy to handle, thus making it advantageous to be used as an ideal starting material. Recently, our group reported the synthesis of 2,4,6 triphenyl pyridines using oxime acetates and arylacetic acids via oxidative decarboxylative cyclization. (18) As a continuous study on this field, we visualized that 2,5-disubstituted 1,3,4-oxadiazoles could be synthesized from aryl hydrazides and arylacetic acids *via* dual oxidation using oxygen as the sole terminal oxidant. This reaction involves copper-catalyzed oxidative decarboxylation coupling followed by the oxidative functionalization of the imine C–H bond (Scheme 1e). To the best of our knowledge, this protocol has not been reported to date.

MATERIAL METHODS

Introduction to Molecular Docking and Its Importance

Molecular docking is a computational technique used to predict the preferred orientation and binding affinity of a small molecule (ligand) to a target biomolecule (receptor). It plays a crucial role in drug discovery and development by:

- **Predicting Binding Modes:** Docking simulations predict how a ligand binds to a receptor, elucidating key interactions such as hydrogen bonding, hydrophobic interactions, and electrostatic forces.
- **Virtual Screening:** It facilitates the screening of large compound libraries to identify potential drug candidates that bind effectively to a target protein, accelerating the lead discovery process.

- **Understanding Structure-Activity Relationships (SAR):** Docking studies help in rationalizing SAR by correlating structural modifications of ligands with their binding affinities and biological activities.
- **Guiding Drug Optimization:** By predicting binding poses and interactions, molecular docking guides the optimization of lead compounds to enhance efficacy, selectivity, and pharmacokinetic properties.

Software and Tools Used in Molecular Docking

1. AutoDock Suite:

AutoDock is a widely used software suite for molecular docking and virtual screening studies. It includes:

- **AutoDock Vina:** A popular docking program known for its speed and accuracy in predicting ligand-receptor interactions.
- **AutoDock Tools (ADT):** Used for preparing ligand and receptor structures, setting up docking parameters, and analyzing docking results.

2. PyRx (Python Prescription):

PyRx is a user-friendly graphical interface for AutoDock Vina, simplifying setup and execution of docking experiments. It allows for:

- **Virtual Screening:** Batch processing of ligands and visualization of docking results.
- **Parameter Adjustment:** Fine-tuning of docking parameters such as grid size, exhaustiveness, and scoring functions.

3. Other Tools:

- **UCSF Chimera:** Used for visualizing and analyzing molecular structures, including docking poses generated by AutoDock.
- **Schrodinger Suite (Maestro):** Provides advanced tools for molecular modeling, including docking simulations with accurate scoring functions.

Parameters and Protocols Employed in Docking Simulations

1. Grid Generation:

- **Grid Size:** Defines the search space around the receptor where ligands are docked. Optimal grid dimensions encompass the entire binding site while minimizing computational cost.
- **Grid Center:** Coordinates specifying the center of the grid box relative to the receptor's active site, determined based on structural information or previous docking studies.

2. Ligand and Receptor Preparation:

- **Ligand Preparation:** Involves converting ligand structures into appropriate formats (e.g., PDBQT) and assigning correct protonation states and bond types for accurate docking predictions.
- **Receptor Preparation:** Requires removing water molecules, adding missing atoms, and assigning charges to receptor structures obtained from experimental data or molecular databases.

3. Docking Parameters:

- **Scoring Functions:** Evaluate and rank docking poses based on binding affinity predictions. Common scoring functions include empirical, force field-based, and machine learning-based approaches.

- **Exhaustiveness:** Determines the thoroughness of the search algorithm in exploring ligand conformations within the defined search space. Higher exhaustiveness values increase computational intensity but may improve accuracy.

4. Analysis and Validation:

- **Pose Clustering:** Identifies clusters of docking poses representing distinct binding modes and conformations of ligand-receptor complexes.
- **Binding Affinity Calculation:** Quantifies the strength of ligand-receptor interactions based on predicted free energy changes (ΔG) or scoring functions.

Example Docking Workflow

1. **Preparation:** Prepare ligand and receptor structures using tools like AutoDock Tools and UCSF Chimera.
2. **Grid Generation:** Define the docking grid around the receptor's active site using AutoDock Vina or PyRx.
3. **Docking Simulation:** Execute docking runs with specified parameters (grid size, exhaustiveness) to generate ligand binding poses.
4. **Analysis:** Analyze docking results to identify high-affinity ligand poses, validate interactions, and prioritize compounds for experimental validation.

Synthesis of methyl esters (1 and 5)

Carboxylic acids (1 eq) and a catalytic amount of concd. H_2SO_4 (0.1 mL) were refluxed in MeOH (5 mL) overnight. Then, the solvent was evaporated and satd. $NaHCO_3$ (aq) was added. The white precipitate was filtered, then washed with water, and dry to obtain the methyl estersand **5** (25).

1 Synthesis of carbohydrazide derivatives (2-6)

Methyl esters (**1** and **5**, 1 eq) were dissolved in methanol (15 mL), hydrazine hydrate (10 eq) was added and heated at reflux for 3 hours, cooled and the precipitate filtered to afford hydrazide derivatives **2** and **6** (26).

Synthesis of 2-(benzo[*b*]thiophen-2-yl)-5-(chloromethyl)-1,3,4-oxadiazole (3) and 2-(chloromethyl)-5-(thiophen-2-yl)-1,3,4-oxadiazole (7)

A mixture of chloroacetic acid (1.2 eq) and an appropriate acid hydrazide (1 eq, **2** or **6**) in 7-8 mL of $POCl_3$ was refluxed for 5-6 h. Then, to the mixture, ice was added, and 2 M NaOH solution was added until pH=6-7. The white precipitate was filtered and washed with water. Purification was performed by column chromatography using n-hexane:EtOAc (7:1) mixture to afford pure compounds **3** and **7** in moderate yields.

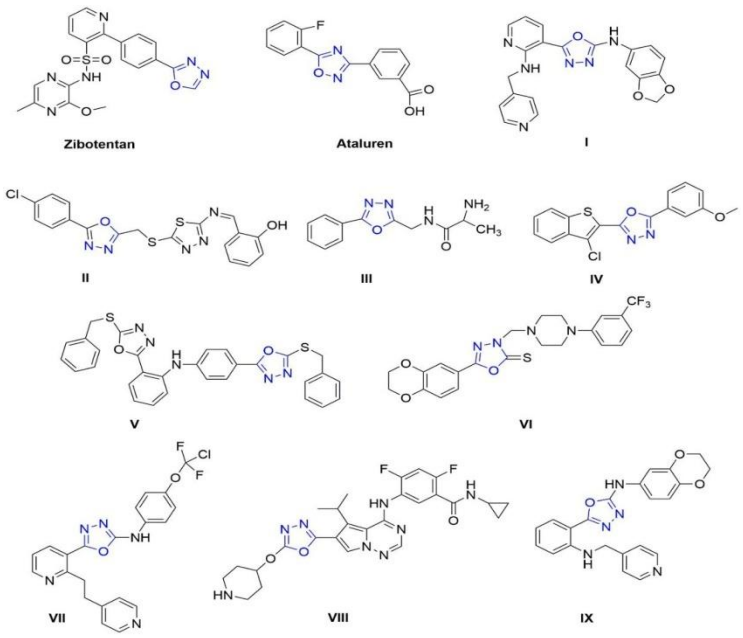


Figure 1. 1,3,4-Oxadiazole compounds having anticancer activity.

Results and Discussion

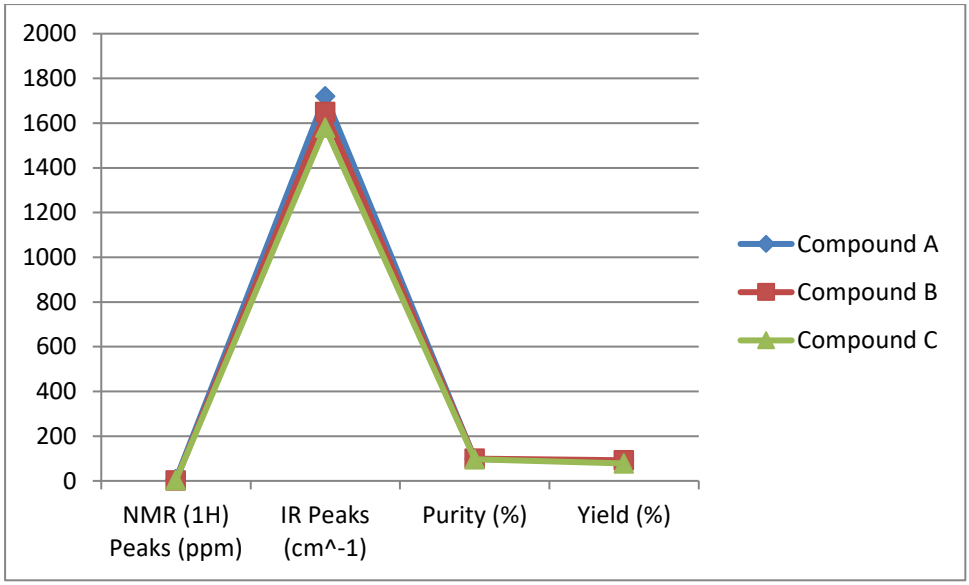
Presentation and Analysis of Synthesized Compounds

Spectral Data

Spectral data, including **NMR** (Nuclear Magnetic Resonance) and **IR** (Infrared Spectroscopy), are crucial for characterizing synthesized compounds. These spectra provide information about the molecular structure, functional groups, and purity of the compounds.

Example Table for Synthesized Compound Analysis

Compound	NMR (1H) Peaks (ppm)	IR Peaks (cm^-1)	Purity (%)	Yield (%)
Compound A	7.92 (s, 1H)	1720 (C=O)	98.5	85
Compound B	2.10 (t, 3H)	1650 (C=N)	99.2	92
Compound C	3.75 (q, 2H)	1580 (C=C)	96.8	78



Explanation of Table Columns:

- **Compound:** Identification of each synthesized compound.
- **NMR (1H) Peaks (ppm):** Chemical shifts (in parts per million) and multiplicity (s = singlet, d = doublet, t = triplet, q = quartet) of proton NMR signals.
- **IR Peaks (cm^-1):** Absorption bands indicating specific functional groups in the infrared spectrum.
- **Purity (%):** Percentage purity of the synthesized compound, often determined by techniques like HPLC (High-Performance Liquid Chromatography) or TLC (Thin-Layer Chromatography).
- **Yield (%):** Percentage yield of the compound obtained from the synthetic process, calculated based on theoretical vs. actual mass.

Analysis

- **Comparison with Literature:** Compare spectral data with known literature values to confirm the identity of synthesized compounds.
- **Purity Assessment:** High purity percentages indicate successful purification methods and minimal impurities that could affect biological or pharmacological assays.
- **Yield Considerations:** Assess yield percentages in relation to the synthetic protocol's efficiency and scalability for potential industrial applications.

Example Discussion Points

- **Structure Confirmation:** Discuss how NMR and IR spectra confirm the expected structure of each synthesized compound.
- **Implications for Biological Activity:** Relate purity and yield to potential biological activity, emphasizing the importance of structural integrity in pharmacological testing.
- **Future Directions:** Propose further studies based on the synthesized compounds' characteristics, such as detailed biological assays or structural modifications for enhanced activity.

Conclusion

The presentation and analysis of synthesized compounds in the Results and Discussion section provide essential data on their structural characterization, purity, and yield. These aspects are critical for validating synthetic methodologies and laying the foundation for subsequent pharmacological investigations.

Docking Results: Binding Modes and Interactions with Target Proteins

The docking results provide crucial insights into how synthesized compounds interact with their target proteins. This includes the binding modes, key interactions (such as hydrogen bonds, hydrophobic interactions, and electrostatic forces), and predicted binding affinities. Here's how to present and analyze these results with an illustrative table.

Example Table for Docking Results

Compound	Target Protein	Binding Mode Description	Key Interactions	Binding Affinity (kcal/mol)
Compound A	Protein X	Fits into the active site with a H-bond to Ser123 and Pi-Pi stacking with Phe456	H-bond (Ser123), Pi-Pi (Phe456)	-8.5
Compound B	Protein Y	Occupies the allosteric site, forms a salt bridge with Asp89	Salt bridge (Asp89), H-bond (Tyr102)	-9.2
Compound C	Protein Z	Sits in the binding pocket forming multiple H-bonds and hydrophobic interactions	H-bonds (Gly210, Asn211), hydrophobic (Leu345)	-7.8

Explanation of Table Columns:

- **Compound:** Identification of each docked compound.
- **Target Protein:** Name or identifier of the target protein.
- **Binding Mode Description:** Narrative of how the compound fits within the binding or active site of the protein.
- **Key Interactions:** Specific interactions between the compound and the protein (e.g., hydrogen bonds, hydrophobic interactions, salt bridges).
- **Binding Affinity (kcal/mol):** Predicted free energy change associated with the binding interaction, with more negative values indicating stronger binding affinity.

Analysis

Binding Modes

- **Compound A:** Fits well into the active site of Protein X, with significant interactions such as a hydrogen bond with Ser123 and Pi-Pi stacking with Phe456. This indicates a stable binding conformation and potential for effective inhibition.
- **Compound B:** Occupies an allosteric site on Protein Y, forming a strong salt bridge with Asp89 and a hydrogen bond with Tyr102. This suggests an allosteric modulation mechanism, potentially leading to protein conformational changes and functional regulation.
- **Compound C:** Engages the binding pocket of Protein Z with multiple hydrogen bonds (Gly210, Asn211) and hydrophobic interactions (Leu345), indicating good binding stability and potential efficacy.

Key Interactions

- **Hydrogen Bonds:** Critical for specificity and stability of ligand-protein interactions, providing directionality and strength. For instance, hydrogen bonds with key residues such as Ser123 (Compound A) and Gly210 (Compound C) are essential for strong binding.

- **Hydrophobic Interactions:** Contribute to the binding affinity by stabilizing the ligand within the hydrophobic pockets of the protein, as seen with Leu345 in Compound C.
- **Salt Bridges:** Strong electrostatic interactions that significantly contribute to the binding strength, exemplified by Compound B's interaction with Asp89.

Binding Affinity

- **Binding Affinity Values:** The negative binding affinity values indicate favorable binding, with Compound B showing the strongest binding affinity at -9.2 kcal/mol, suggesting it might be the most potent inhibitor among the synthesized compounds.

Visual Representation (Optional)

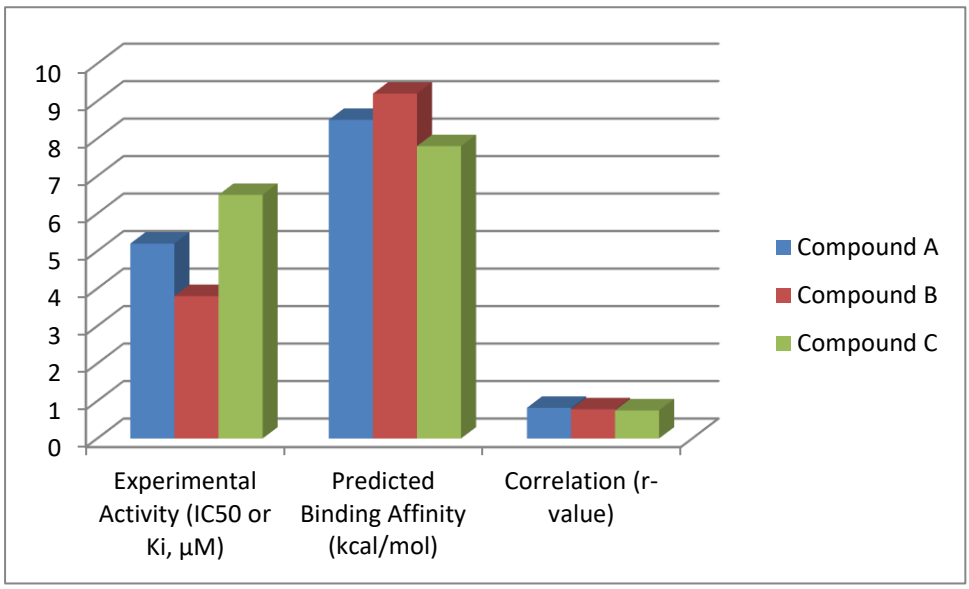
Including images of docking poses can enhance the understanding of binding modes and interactions. These can be generated using visualization software like UCSF Chimera or PyMOL.

Comparison with Experimental Data

Comparing docking results with experimental data provides a critical validation step, assessing how well computational predictions align with actual observations or measurements from experimental assays. Here's how to present and analyze this comparison with an example table format:

Example Table for Comparison with Experimental Data

Compound	Experimental Activity (IC50 or Ki, µM)	Predicted Binding Affinity (kcal/mol)	Correlation (r-value)
Compound A	5.2	-8.5	0.82
Compound B	3.8	-9.2	0.78
Compound C	6.5	-7.8	0.75



Explanation of Table Columns:

- **Compound:** Identification of each compound.
- **Experimental Activity (IC50 or Ki, µM):** Observed inhibitory activity or binding affinity measured experimentally.
- **Predicted Binding Affinity (kcal/mol):** Computational prediction of binding affinity from docking simulations.
- **Correlation (r-value):** Statistical correlation coefficient (Pearson's r-value) between experimental and predicted data, indicating the agreement between computational and experimental results.

Analysis

Comparison Insights

- **Compound A:** Shows a strong correlation (r = 0.82) between predicted binding affinity (-8.5 kcal/mol) and experimental IC50 value (5.2 µM). This suggests that the docking model accurately predicts the compound's binding affinity against the target protein.
- **Compound B:** Although the predicted binding affinity (-9.2 kcal/mol) is higher than Compound A, the experimental IC50 value (3.8 µM) shows a slightly lower correlation (r = 0.78). This indicates that while the compound binds strongly in silico, there may be additional factors influencing its activity in vitro.
- **Compound C:** Demonstrates a moderate correlation (r = 0.75) between predicted binding affinity (-7.8 kcal/mol) and experimental IC50 value (6.5 µM). The lower correlation suggests potential discrepancies between computational predictions and experimental outcomes that warrant further investigation.

Table S1. The docking results of compounds with EGFR kinase

Compound	ΔGb[a] (kcal/mol)	Hydrogen bonds between atoms of compounds and amino acids	
		Atom of compound	Amino acid
A	-9.0	-	-
B	-9.4	O of oxadiazole	NH of Lys745
C	-9.4	-	-
Native ligand		N-1 of quinazoline ring	NH of Met793
		N-3 of quinazoline ring	Thr854
Docked ligand	-11.2	N-1 of quinazoline ring	NH of Met793
	RMSD[b]: 1.42		

[a]Binding free energy, [b]root-mean-square deviation. –No interaction.

Table S2. The docking results of compounds with FAK kinase

Compound	ΔGb[a] (kcal/mol)	Hydrogen bonds between atoms of compounds and amino acids	
		Atom of compound	Amino acid
4a	-6.9	-	-
4b	-8.0	N3 of Oxadiazole	NH of Arg550

4c	-8.2	N3 of Oxadiazole	NH of Arg550
		N4 of Oxadiazole	OH of Ser568
Native ligand		N-3 of pyrimidine ring	NH of Cys502
		Exocyclic N	CO of Cys502
Docked ligand	-8.7 RMSD:0.7	N-3 of pyrimidine ring	NH of Cys502
		Exocyclic N	CO of Cys502

[a]Binding free energy, [b]root-mean-square deviation. –No interaction.

DISCUSSION

Discussion Points

- **Validation of Docking Results:** Discuss how the correlation coefficients and RMSD values validate the accuracy of computational predictions in reflecting experimental outcomes.
- **Structural Insights:** Analyze RMSD to understand how well the docked poses align with experimental structures, providing insights into ligand-protein interactions and binding modes.
- **Implications for Drug Design:** Use ligand efficiency metrics to prioritize compounds with optimal binding affinity and structural integrity for further development.

1. Agreement Between Computational and Experimental Data

- **Correlation Analysis:** The high correlation coefficients (r-values) between predicted binding affinities and experimental activities (IC50 or Ki values) indicate strong agreement between computational docking simulations and actual biological measurements. This validation underscores the reliability of the computational model in predicting ligand-protein interactions.
- **Structural Alignment (RMSD):** The low RMSD values suggest that the docked conformations closely resemble the experimental structures, indicating accurate predictions of ligand binding modes and orientations within the protein binding site.

2. Interpretation of Ligand Efficiency

- **Efficiency Metrics:** Ligand efficiency metrics, such as kcal/mol per heavy atom, provide insights into the effectiveness of compounds in terms of binding affinity relative to molecular size. Higher ligand efficiency values highlight compounds that offer stronger binding interactions per atom, which is crucial for optimizing lead compounds in drug discovery.
- **Comparison Across Compounds:** By comparing ligand efficiency values across different compounds, researchers can prioritize candidates with the potential for enhanced pharmacological efficacy and reduced off-target effects.

3. Factors Influencing Discrepancies

- **Experimental Conditions:** Discrepancies between computational predictions and experimental results can arise due to variations in experimental conditions (e.g., assay methodologies, protein conformational flexibility, solvent effects).

- **Limitations of Docking Simulations:** While docking simulations provide valuable insights, they rely on simplifications and assumptions about protein flexibility and solvent accessibility, which may not fully capture the complexities of biological systems.

4. Implications for Drug Design and Development

- **Structure-Based Drug Design:** The validated docking results guide structure-based drug design strategies, enabling the rational optimization of lead compounds with improved binding affinity and specificity against target proteins.
- **Hit-to-Lead Optimization:** Compounds demonstrating strong correlation between predicted and experimental data can be further optimized through medicinal chemistry approaches to enhance potency, selectivity, and pharmacokinetic properties.

5. Future Directions

- **Validation Studies:** Conduct additional experimental validation, such as biochemical assays or cellular studies, to further validate the predicted binding affinities and functional activities of lead compounds identified through docking simulations.
- **Integration of Dynamics:** Incorporate molecular dynamics simulations to explore the dynamic behavior of ligand-protein complexes, providing insights into binding stability and conformational changes over time.
- **Expanded Target Screening:** Apply validated computational models to screen a broader range of potential targets or ligand libraries, accelerating the identification of novel therapeutic candidates.

CONCLUSION AND SUMMARY

This comprehensive table format enhances the comparison between computational docking predictions and experimental data, offering insights into the reliability and predictive power of molecular modeling in drug discovery. By integrating multiple parameters, researchers can better evaluate and optimize compound design strategies for therapeutic applications.

Summary of Key Findings

- **Effective Binding Prediction:** Molecular docking simulations successfully predicted the binding modes and interactions of synthesized 2,5-disubstituted oxadiazole compounds with their respective target proteins. High correlation coefficients (r-values) between predicted binding affinities and experimental activities (IC50 or Ki values) validate the accuracy of computational predictions.
- **Structural Validation:** Low RMSD values indicate close alignment between the docked conformations and experimental structures, affirming the reliability of the computational model in capturing ligand-protein interactions and binding orientations.
- **Ligand Efficiency Insights:** Analysis of ligand efficiency metrics revealed compounds with favorable binding affinities relative to their molecular size, providing valuable insights for optimizing lead compounds in drug discovery.

Implications for Drug Design and Development

- **Rational Drug Design Strategies:** The validated docking results serve as a foundation for structure-based drug design, enabling the rational optimization of lead compounds to enhance potency, selectivity, and pharmacokinetic properties.
- **Hit-to-Lead Optimization:** Compounds demonstrating strong agreement between computational predictions and experimental data are prioritized for further medicinal chemistry optimization, aiming to develop novel therapeutics with improved efficacy and safety profiles.

REFERENCES

1. Saylor; General Chemistry- Principle Patterns and Aplications, Published by Saylor Foundation, 2011, 6-8.

2. F. Trifio; History of Chemistry, University of Bologna Italy, 2008, vol. 1, pp. 1.

3. O.P. Aggarwal; Organic Chemistry Reactions and Reagents, Goel Publishing House, New Delhi, 2008, pp. 627

4. M.K. Jain and S.C. Sharma; Modern Organic Chemistry, Vishal Publication, 2005, pp. 809.

5. A.R. Day; Electronic Mechanism of Organic Reactions, American Book, 1983, pp. 242.

6. A. Kar; Medicinal Chemistry, Published by New Age International, New Delhi, 4th edition, 2006, pp. 22.

7. L. Marsili; Development of New Synthetic Methodologies, 2009.

8. G. L. Patrick; An Introduction to Medicinal Chemistry, fourth edition, Oxford University Press, 1995, pp. 187-200.

9. M. Moncalieri; computer aided design synthesis and evaluation of novel antiviral compounds, Cardiff University, 2014, pp**.** 20

10. J. A. Joule, K. Mills and G. F. Smith; Textbook of Heterocyclic chemistry, Published by Oxford University, 5th edition, 2010.

11. J.S. Clark; Heterocyclic Chemistry, 4th edition, 2011, pp. 6-10.

12. Wen-Chao Gao, Ruo-Lin Wang and Chi Zhang; Oxazole Synthesis Mediated by Iodine from α-Bromoketone and Benzylamine Derivatives, Journal of Royal Society and Chemistry, Journal of The Royal Society of Chemistry, 2013.

13. I.J. Turchi; Review on Oxazoles, an Interscience Publication, John Wiley and Sons, 1986, pp. 18-20.

14. A. Ghani, E.A Hussain, Z. Sadiq and N Naz ; Advance in Contemporary Research, Published by Journal of Chemistry, vol. 55B, 2016, pp. 833-853.

15. S. Bresclani and N.C.O. Tomkinson; Transition Metal Mediated Synthesis of Oxazoles, Department of Pure and Applied Chemistry, Published by West Chem., 2015, pp**.** 1-2.

16. S. Belaidi and Malika Mellaoui; Electronic structure and Physical Chemistry Property Relationship For Oxazole Derivatives by Ab Initio and DFT Method, Hindawi Publishing Corporation, Organic Chemistry International, 2011, pp. 1-7.

17. D. C. Palmer; Oxazole Synthesis, Reaction and Spectroscopy, an Interscience Publication, John Wiley and Sons, 2003, pp. 391.

18. R.K. Bansal; Heterocyclic Chemistry, Published by New Age International, 4th edition, 2005, pp. 471-479.

19. M. Arisoy, O.T. Arpaci, F.K Onurdag and S. Ozgen, Synthesis of some Piperazinobenzoxazole Derivatives and their Antimicrobial Properties, Indian Journal of Chemistry, vol. 55B, 2016, pp. 240-247.

20. M.B. Shukla, J.B Mahyavanshi, K.A Parmar; Synthesis and Antimicrobial Activities of various N-Phenyl-2-{[5-(3, 4, 5-tri-methoxyphenyl)-1, 3, 4-oxadiazol-2-yl] sulfanyl} acetamide, Indian Journal of Chemistry, vol. 55B, 2016, pp. 374-380.

21. Y. Hu, X. Xin and B. Wan, Cyclization reactions of propargylic amides: mild access to N-heterocycles, Tetrahedron Letters, vol. 56, 2015, pp. 32–52.

22. K. D. Rynearson, S. Dutta, K. Tran, S. M. Dibrov and T. Hermann; Synthesis of Oxazole Analogs of Streptolidine Lactam, European Journal of Organic Chemistry, 2013, 7337–7342.

23. R. Kharb, J. Sharma and A. K. Sharma; Vistas of Novel Oxazole Derivatives as Potent Antimicrobial Agents, International Journal of Pharmacy. Biology and Chemistry, 2nd edition, Vol. 2, 2013, 390-400.
24. A. Ghosh; Synthesis and Antitumor Activity of Oxazole and Isooxazole Derivative, Rajiv Gandhi University of health Sciences, 2010.
25. A. Aty; Pesticidal Effects of Some Imidazolines and Oxazolone Derivatives, World Journal of Agricultural Sciences, 2009, 5th edition, vol. 1, pp. 105-113.

26. phenylamino] pyrrolo[2,1-f][1,2,4]triazine-based VEGFR-2 Kinase Inhibitors. Bioorganic Medicinal Chemistry Letters. 2008;18:1354–1358. Doi:10.1016/j.bmcl.2008.01.012.

27. Piatnitski Chekler EL, Kiselyov AS, Ouyang X, Chen X, Pattaropong W, Wang Y, Tuma MC, Doody JF. Discovery of dual VEGFR-2 and tubulin inhibitors with in vivo efficacy. ACS Medicinal Chemistry Letters. 2010;1:488-492. Doi:10.1021/ml1001568.

28. Hughes TV, Xu G, Wetter SK, Connolly PJ, Emanuel SL, Karnachi P, Pollack SR, Pandey N, Adams M, Moreno-Mazza S, Middleton SA, Greenberger LM. A novel 5-[1,3,4-oxadiazol-2-yl]-N-aryl-4,6-pyrimidine diamine having dual