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(RESEARCH ARTICLE)

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Design, synthesis, molecular docking and biological evaluation of oxadiazole derivatives

Md. Rahat Raza [1,*], Md. Afaque [2], Saddam Hussain [2], Md. Quamar Niyaz [2], Md. Shamsir Alam [2] and Md. Anwar Salik [2]

[1] *Delhi Institute of Pharmaceutical Sciences and Research, DPSRU (India).*
[2] *School of Pharmacy, Al-Karim University, Katihar (India).*

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Abstract

Purpose: Formulation and analysis of Biological Evalutationof new heterocyclic compounds containing Oxadiazole derivatives.

Objectives: The primary objectives of the project are:1. To synthesize novel oxadiazole derivatives.2. To characterize and evaluate synthesized molecules:i. Determination of melting point; ii. Solubility study; iii. Thin layer chromatography; iv. FT-IR; v. NMR, and3. To examine the in vitro antioxidant activity of the molecules.

Methods: A series of 2,5-substituted oxadiazole were synthesised. The series comprises of 7 compounds. The entire procedure to synthesise the compounds is following: In a round bottom flask, 0.025 mol of 2-(4-chloro-3-nitro benzoyl) benzoic acid, 10 ml of methanol and 0.5g of p-Toluenesulfonic acid were added. Resulting mixture was placed on a water bath for 4 hrs. After the completion of reaction, this mixture was poured in ice for the formation of methyl (2-(4-chloro-3-nitro benzoyl)) benzoate. In an RBF, 0.01mol of methyl (2-(4-chloro-3nitro benzoyl)) benzoate in 15ml methanol was added followed by addition of 0.06mol of hydrazine hydrate. The mixture was refluxed for 3hrs and after completion poured in ice-water. Formed precipitate was washed, filtered and dried and then recrystallized using methanol. In an RBF, 1mmol of acid chloride and 1.30 mmol acid hydrazide was added followed by addition of 10 ml dry THF. The resulting mixture was stirred at room temperature for 6 h. Then, the solvent was evaporated under reduced pressure, small amount of water was added (2 mL) and the formed precipitate (C1-7) was isolated by filtration and dried. For cyclization 1mmol of 2-(4-chloro-3-nitro benzoyl)-N-(4-chlorobenzoyl) benzo hydrazide and 6ml of thionyl chloride was refluxed for 6hrs in water bath at 70°C. After cooling, the ice was added to the solution and formed suspension left standing overnight at 4°C and desired compound was filtered off and dried.

Keywords: Oxadiazole; 2-(4-Chloro-3-Nitrobenzoyl) Benzoic Acid; Biological Evalution

1. Introduction

Antimicrobial resistance (AMR) is one of the main problems of modern medicine. Poor treatment of infections, over-prescription of antibiotics and their inappropriate use by patients have made some of the microorganisms insensitive to currently used drugs. This causes great difficulties in treatment as the antibiotics or other antimicrobial drugs used so far are no longer effective and infections become progressively difficult to treat [1,2]. AMR is an increasingly serious threat to life and public health. Without effective antibiotic therapy, the cost of caring for patients with drug-resistant infections increases, and there is a huge risk during surgery and other medical procedures. Antimicrobial resistance occurs when microorganisms develop the ability to defeat drugs designed to kill them. There is great diversity of

*Corresponding author: Md. Rahat Raza

microbial defense strategies. Antimicrobial resistance can also be acquired, and this is typically the type of resistance we worry about in clinical practice when we encounter bacteria that were initially susceptible but then become resistant to antibiotics [3]. Many factors play a role in acquired drug resistance, but the main driver is antibiotic overuse. Acquired resistance occurs one of two ways, either through bacterial gene mutations or through the acquisition of foreign DNA that encodes resistance genes. Bacteria can reproduce rapidly, leading to evolutionary shifts through random genetic mutations that can be seen in relatively short periods of time. Exposure to antibiotics creates an evolutionary pressure on bacteria and confers a selective survival advantage for the bacteria that have acquired the resistance mutations [4]. Alternatively, bacteria can transfer genetic material from 1 cell to another, a process known as horizontal gene transfer [5]. There are numerous traditional processes of horizontal gene transfer which are: transduction, conjugation, and transformation. Transduction involves genetic material transferred from one bacterium to another by a bacterial virus, also known as a bacteriophage. Transformation is the direct uptake of free genetic material from the environment, usually from a lysed bacterial cell. Conjugation, the process most notable for antibiotic resistance, is the process of sharing small segments of DNA directly between cells. Conjugation can involve circular, extra chromosomal DNA called plasmids, as well as integrative and conjugative elements, which can be integrated into bacterial chromosomes [5, 6]. Plasmids can be disseminated rapidly through bacterial communities. These characteristics allow bacteria to not only rapidly develop changes to their genetic material, which may confer resistance, but to also rapidly share this genetic material. In addition to these classic mechanisms of horizontal gene transfer, a variety of other mobile genetic elements exist that can transfer bacterial resistance traits such as genomic islands, insertion sequences, transposons, integrons, and miniature inverted repeat transposable elements [7]. One of the ways to deal with the AMR problem is the synthesis of new medicinal substances to which microorganisms are sensitive. Researchers around the world are working on new molecules that would stop the development of resistance. Most often, newly formed compounds contain a heterocyclic moiety, and the motifs bearing the oxadiazole ring constitute a large group of potential antimicrobial derivatives. Oxadiazoles are five-membered heterocyclic compounds containing two nitrogen atoms and one oxygen atom in their structure. We can distinguish several isomeric forms of oxadiazole, which occur in the structure of many drugs, e.g., anticancer zibotentan [8] , antimicrobial furamizole [9], antiviral raltegravir [10], ataluren for Duchenne muscular dystrophy [11] and others. Oxadiazoles consist of two carbons, two nitrogens, and one oxygen atom, and they exist in different regioisomeric forms. Oxadiazoles are frequently occurring motifs in druglike molecules, and they are often used with the intention of being bioisosteric replacements for ester and amide functionalities. Oxadiazoles (Figure 1), are of considerable interest in different areas of medicinal and pesticide chemistry and also polymer and material science. The level of interest is clearly shown, as over the past 9 years the number of patent applications containing oxadiazole rings has increased considerably (100%), to a total of 686. Within drug discovery and development, a number of compounds containing an oxadiazole moiety are in late-stage clinical trials, including zibotentan as an anticancer agent and ataluren for the treatment of cystic fibrosis. So far, one oxadiazole containing compound, raltegravir, an antiretroviral drug for the treatment of HIV infection, has been launched onto the marketplace. It is clear that oxadiazoles are having a large impact on multiple drug discovery programs across a variety of disease areas, including diabetes, obesity, inflammation, cancer, and infection. Oxadiazole rings have been introduced into drug discovery programs for several different purposes. In some cases, they have been used as an essential part of the pharmacophore, favorably contributing to ligand binding. In other cases, oxadiazole moieties have been shown to act as a flat, aromatic linker to place substituents in the appropriate orientation, as well as modulating molecular properties by positioning them in the periphery of the molecule.

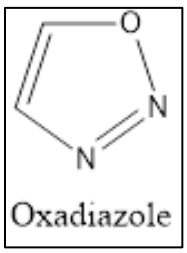


Figure 1 Oxadiazole

2. Materials

The material needed to carry out the project work are divided into software, chemicals, and instruments needed.

2.1. Software used

The ligand structures were made using ChemDraw Ultra 8.0.

2.1.1. Chemicals used

Table 1 Different chemicals used

S.No	Chemical	Manufacturer/Supplier
1	2-(4-chloro-3-nitrobenzoyl) benzoic acid	Sigma Aldrich Chemistry
2	Thionyl chloride	CDH
3	4-Chlorobenzoyl chloride	Sigma Aldrich Chemistry
4	THF(Tetrahydrofuran)	MERCK
5	4-Nitrobenzoyl chloride	CDH
6	4-Morpholinyl carbonyl chloride	TCI
7	4-Methoxybenzoyl chloride	MERCK
8	4-Bromobenzoyl chloride	MERCK
9	2,4-dichlorobenzoyl chloride	MERCK
10	4-Tert-butylbenzoyl chloride	MERCK

2.2. Instruments used

Table 2 Instruments Used

S. No.	Instrument used	Manufacturer/Supplier
1	Magnetic stirrer with hot plate	Remi 1 MLH
2	UV Transilluminator	MRC Laboratory instruments
3	Fourier Transform Infrared Spectrophotometer	Bruker
4	Melting point apparatus	Sigma scientific
5	UV-Vis Spectrophotometer	Thermofischer

2.3. Methodology

Scheme:

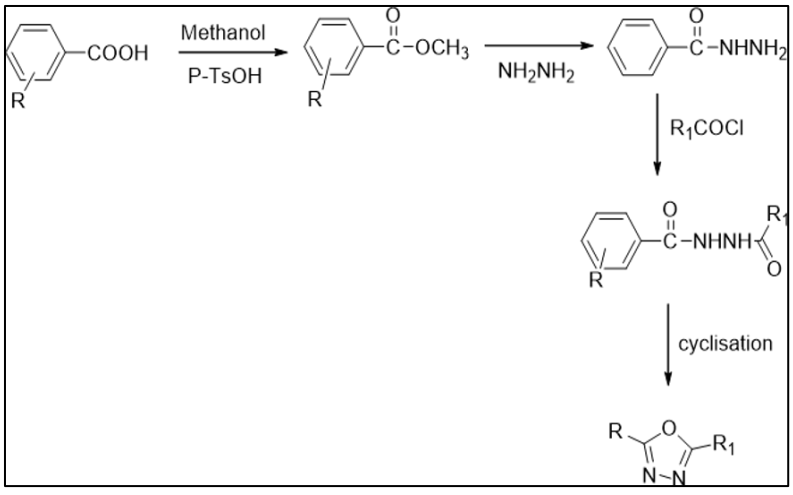


Figure 2 Scheme for synthesis

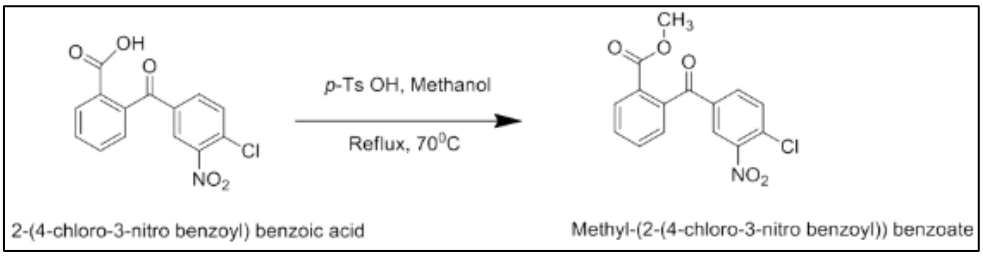
2.4. Synthesis of compounds

A series of 2, 5-substituted oxadiazole were synthesised. The series comprises of 7 compounds. The entire procedure to synthesise the compounds is following:

2.4.1. Procedure

Synthesis of compounds includes 4 steps.

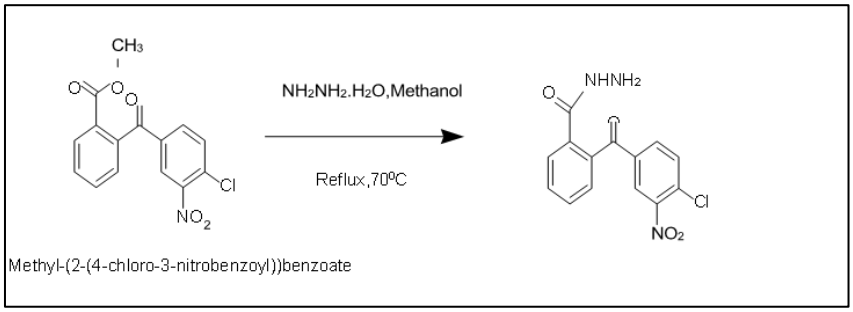
2.4.2. STEP-1



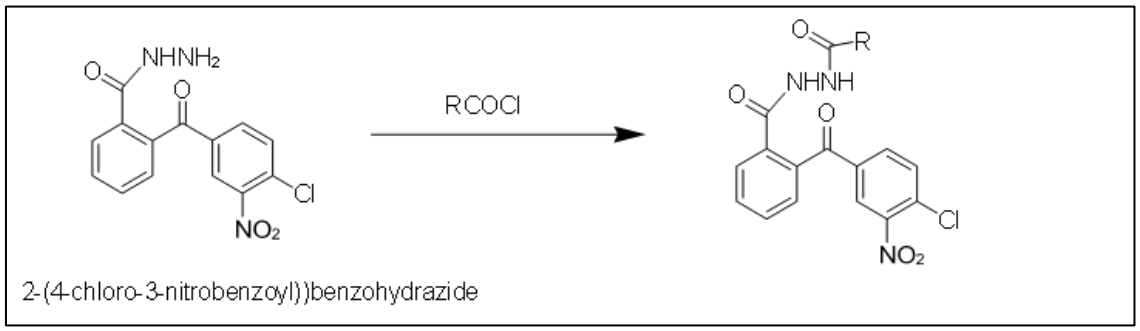
In a round bottom flask, 0.025 mol of 2-(4-chloro-3-nitro benzoyl) benzoic acid, 10 ml of methanol and 0.5g of p-Toluenesulfonic acid were added. Resulting mixture was placed on a water bath for 4 hrs. After the completion of reaction, this mixture was poured in ice for the formation of methyl (2-(4-chloro-3-nitro benzoyl)) benzoate.

2.4.3. STEP-2

In an RBF, 0.01mol of methyl (2-(4-chloro-3nitro benzoyl)) benzoate in 15ml methanol was added followed by addition of 0.06mol of hydrazine hydrate. The mixture was refluxed for 3hrs and after completion poured in ice-water. Formed precipitate was washed, filtered and dried and then recrystallized using methanol.



2.4.4. STEP-3

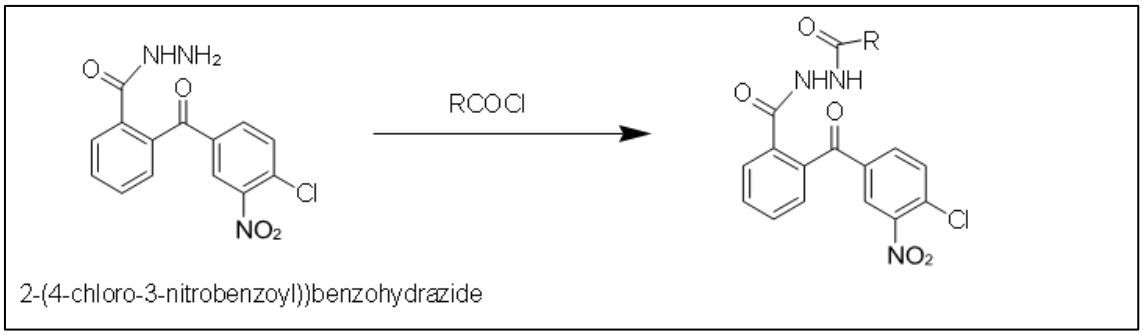


Compound	R
C1	4-chlorophenyl
C2	4-nitrophenyl
C3	4-methoxyphenyl

C4	4-morpholine
C5	4-bromophenyl
C6	2,4-dichloro phenyl
tC7	4-tert-butylphenyl

In an RBF, 1mmol of acid chloride and 1.30 mmol acid hydrazide was added followed by addition of 10 ml dry THF. The resulting mixture was stirred at room temperature for 6 h. Then, the solvent was evaporated under reduced pressure, small amount of water was added (2 mL) and the formed precipitate (C1-7) was isolated by filtration and dried.

2.4.5. STEP-4



2-(4-chloro-3-nitrobenzoyl))benzohydrazide

Compound	**R**
R1	4-chlorophenyl
R2	4-nitrophenyl
R3	4-methoxyphenyl
R4	4-morpholine
R5	4-bromophenyl
R6	2,4-dichloro phenyl
R7	4-tert-butylphenyl

For cyclization 1mmol of 2-(4-chloro-3-nitro benzoyl)-N-(4-chlorobenzoyl) benzo hydrazide and 6ml of thionyl chloride was refluxed for 6hrs in water bath at 70°C. After cooling, the ice was added to the solution and formed suspension left standing overnight at 4°C and desired compound was filtered off and dried.

2.5. Characterisation and evaluation of compounds

After synthesis of all compounds, they were analyzed for characterization according to the following methods:

- **Physical evaluation:** Compounds were evaluated visually to determine their nature, crystalline or amorphous, colour, odor etc.
- **Solubility test:** Compounds were dissolved in a range of solvents to determine their solubility.
- **Melting point determination:** The melting point of the compounds was determined by sealing a small amount of the compounds in a small capillary tube and connecting it to the stem of a thermometer located in the melting point apparatus. The temperature at which the chemical begins to melt in the capillary was measured.
- **Thin-layer chromatography of compounds:** TLC of all compounds was performed on plates coated with silica gel G. Silica gel G was applied via the pour method on clean glass plates to a thickness of approximately 1 mm. The plates were air dried for 15 min, then activated in a hot air oven at 110 °C for 30 min. Chloroform: Methanol (9:1) was used as the mobile phase to analyze the samples and determine the RF value. The spot was then observed in a UV transilluminator.
- **1H-NMR spectral analysis:** The samples were submitted to Panjab University, Chandigarh for NMR spectral analysis. For analysis, DMSO was used as the solvent and TMS as the standard.

2.6. Computational Analysis (Docking)

The entire process of computational analysis, i.e., molecular docking was carried out in the following steps:

2.6.1. Molecular Docking Procedure

All computations were performed on 11th Gen Intel(R) Core (TM) i3-1115G4 @ 3.00GHz 8.00 GB. Windows 11 Home. 64-bit operating system, x64-based processor. Glide 9.1, Phase 3.5, LigPrep 2.6 modules implemented in the Maestro 12.8 [Graphical user interface (GUI)] of Schrodinger's computational chemistry software was used. Docking studies were conducted using the Glide module of Schrodinger Maestro to examine the binding pattern and affinity of potential compounds. The Crystal Structure of carotenoid dehydrosqualene synthase (PDB ID:2ZCS) complexed with bisphosphonate BPH-700 served as the basis of this study. The results of binding affinity and interaction pattern were compared with standard ligand ampicillin.

2.6.2. Protein Preparation

The preparation of protein molecule represents the initial stage in the process of molecular docking. For this purpose, the Protein Preparation Wizard (Prep Wizard) from the Schrodinger suite 2021-22 was employed. In this study, the 3D crystal structure of protein were procured from protein data bank with PDB ID: 2ZCS. The proteins were pre-processed in protein preparation wizard of Maestro. The missing loops and side chains were found using prime and water molecules beyond 3Å were deleted. The protein was optimized for hydrogens and minimized using OPLS4 (Optimized Potentials for Liquid Simulations) force field.

2.6.3. Ligand Preparation

The structure of the ligands was generated in the CDX format using the tool Chem Draw ultraversion 12.0. The compounds were rationally design by keeping in mind the active catalytic binding pocket of S.aureus. The rationally designed compound was prepared by LigPrep module of Maestro. The designed compounds were first imported and then minimized using OPLS4 force field, all possible conformations were generated using the LigPrep module.

2.6.4. Receptor Grid generation

Maestro v12.8 Glid-grid generation module (Schrodinger 2021-22) is used to generate a grid. The purpose of generating the grid was to facilitate the investigation of the docking behaviour of coumarin linked piperazine derivatives within a specific binding site. The grid was specifically generated around the binding site that was already occupied by the co-crystallized ligand. This approach allowed for the exclusion of the co-crystallized ligand from the subsequent docking experiments. By excluding the ligand, it became possible to attach and study the docking of new molecule within the same binding site. During the grid generation process, the Glide-grid molecule was utilized with default settings. The co crystallised ligand was used to define the centre of grid box, which had dimensions of 16 × 16 × 16 Å. No constraints were applied during the grid generation, and the default parameters were used.

2.7. Glide Ligand Docking

The glide docking of the proposed molecules was carried out using the previously prepared receptor grid and the ligand molecules. The favourable interactions between the ligand molecules and the receptor were scored using Glide ligand docking program. All the docking calculations were performed using standard precision (SP) mode and OPLS4 force field. The above docking process was run in a flexible docking mode which automatically generates conformations for each input ligand. The ligand poses generated were passed through a series of hierarchal filters that evaluate the ligand's interaction with the receptor.

2.7.1. Biological evaluation of compounds

Various concentrations were prepared from these for biological evaluation of seven compounds. The concentrations prepared were 20, 40, 60 and 80 µg/mL. Based on the resources available at the institute, antioxidant activity were assessed for a range of compounds.

Antioxidant Activity

Antioxidant activity is defined as the restriction or prevention of nutritional oxidation (particularly lipids and proteins) through oxidative chain reactions. The antioxidant activity of the samples of interest was assessed using two in vitro assay techniques. The results are listed in the table, and here are the procedures used for both tests:

DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging assay

The chemical 2,2-diphenyl-1-picrylhydrazyl (DPPH) is known to be a stable free radical because the extra electron is delocalized throughout the molecule, preventing dimerization, as is the case with most other free radicals. The deep purple color is determined by electron delocalization, which is characterized by an absorption band in the ethanol solution at 517 nm. When a solution of DPPH is combined with a substrate capable of donating hydrogen atoms, the reduced form is formed and the violet color is lost.

The following formula was used to compute the DPPH scavenging capacity:

Scavenging activity percentage = $A-A'$ /A× 100

Where, ⁂ 100 A = Absorbance of positive control (DPPH + methanol),

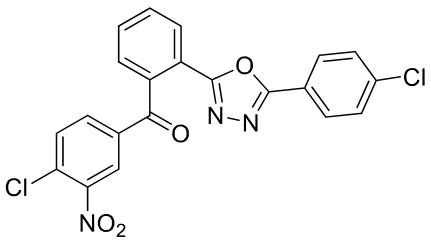
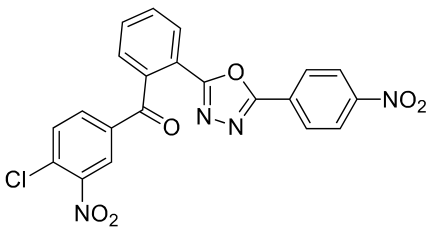
and A' = Absorbance of sample/standard

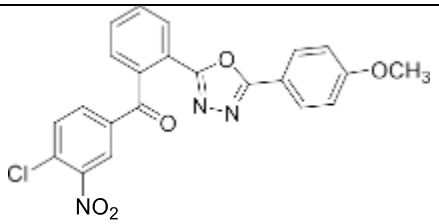
3. Results and discussion

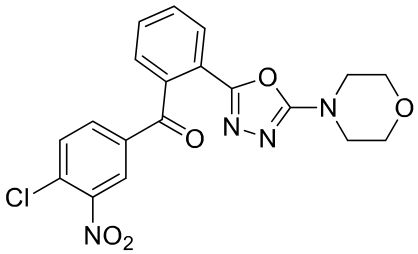
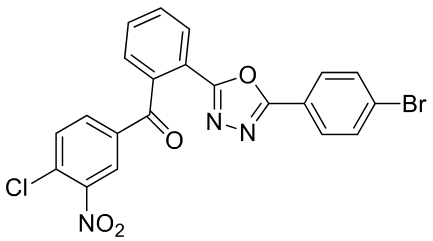
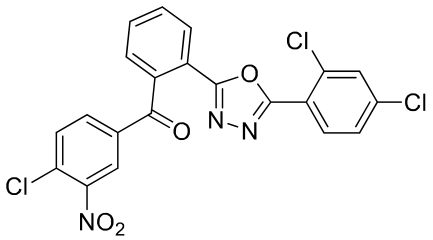
3.1. Synthesis and characterisation

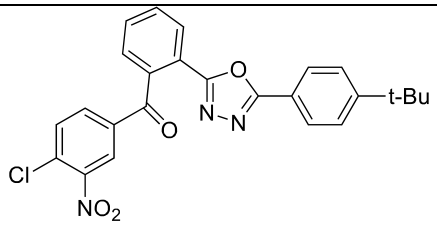
All the compounds that were synthesised, their physical properties, chemical properties, and analytical observations are mentioned in this chapter. The data in the table exhibits various properties of the compounds as examined.

Table 3 Preliminary characterization

Code	StructureandIUPAC Name	M.W (gm/ mol)	% Yield	M.P. (ºC)	Solubility	Rf
R1	 (4-chloro-3-nitrophenyl)(2-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2- yl)phenyl)methanone	440.24	62.5	260-270	DMSO, Methanol, Hexane	0.83
R2	 (4-chloro-3-nitrophenyl)(2-(5-(4-nitrophenyl)-1,3,4-oxadiazol-2- yl)phenyl)methanone	450.79	66.5	260-270	Methanol, DMSO.	0.79
R3		435.82	63	260-270	Methanol, DMSO.	0.76

	 (4-chloro-3-nitrophenyl)(2-(5-(4- methoxyphenyl)-1,3,4-oxadiazol- 2-yl)phenyl)methanone					

Code	StructureandIUPAC Name	M.W (gm/ mol)	% Yield	M.P. (ºC)	Solubility	Rf
R4	 (4-chloro-3-nitrophenyl)(2-(5- morpholino-1,3,4-oxadiazol-2- yl)phenyl)methanone	414.80	55	70-80	Methanol, DMSO.	0.8
R5	 (2-(5-(4-bromophenyl)-1,3,4- oxadiazol-2-yl)phenyl)(4-chloro- 3- nitrophenyl)methanone	484.69	31.14	75-80	Methanol, DMSO.	0.79
R6	 (4-chloro-3-nitrophenyl)(2-(5- (2,4-dichlorophenyl)-1,3,4- oxadiazol-2- yl)phenyl)methanone	474.68	65	110-125	Methanol, DMSO.	0.75
R7		461.90	75.16	290-300	Methanol, DMSO.	0.77

3.2. Molecular Docking Studies

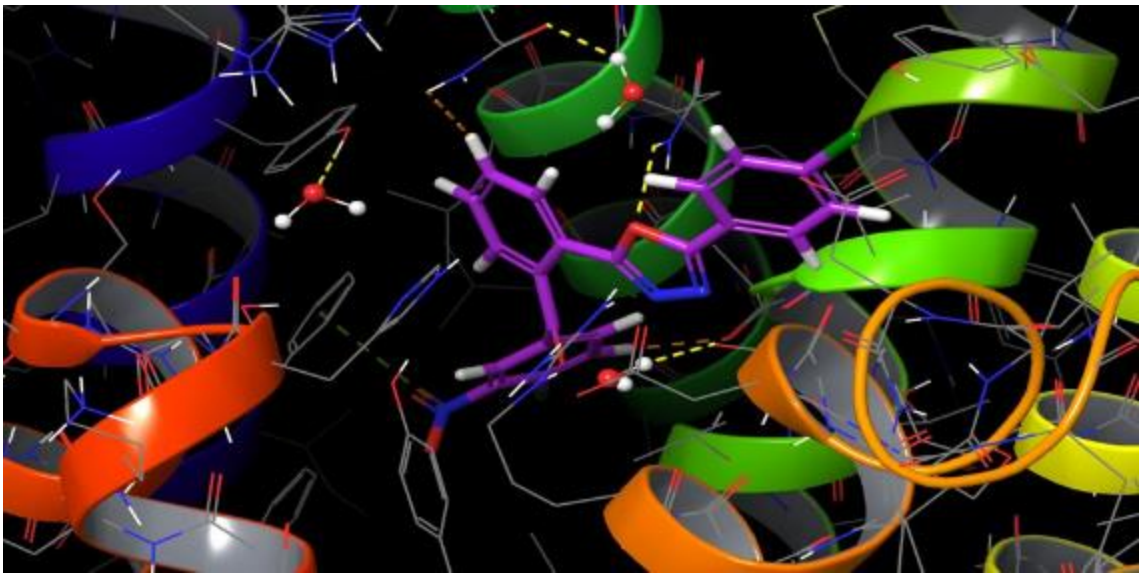


Figure 3 3D interaction diagram of compound R1 with PDB ID: 2ZCS

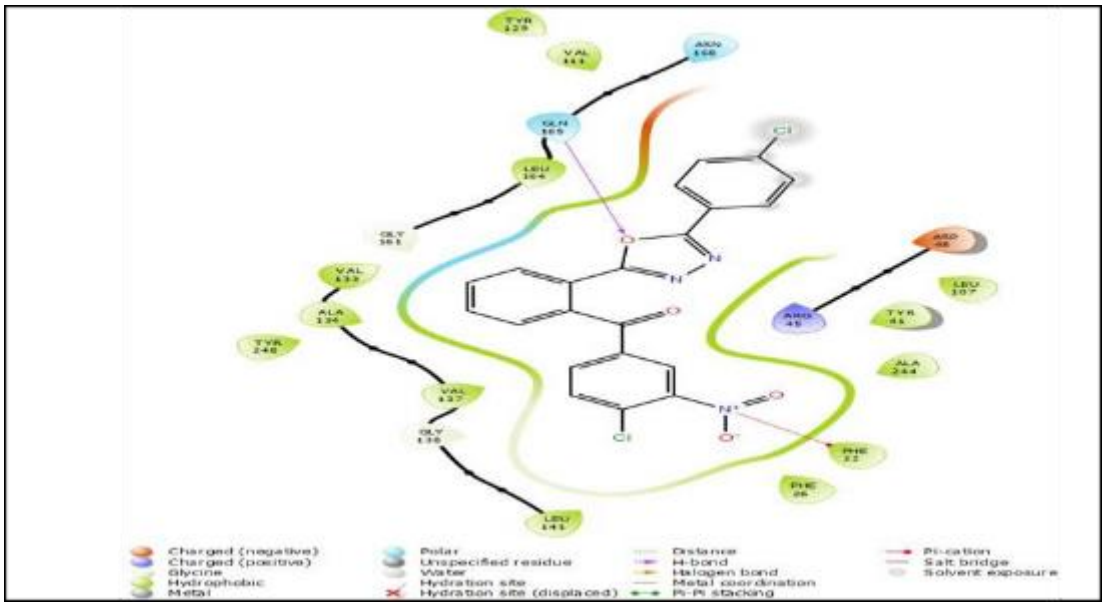


Figure 4 2D representation of compound R1 with PDB ID: 2ZCS

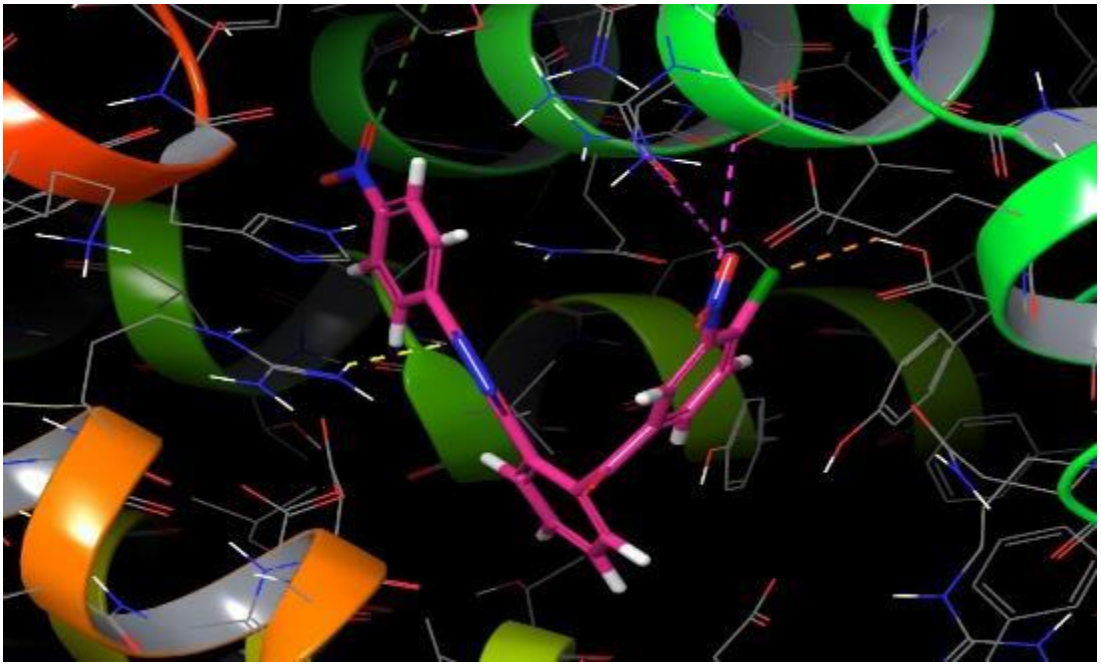


Figure 5 3D interaction diagram of compound R2 with PDB ID: 2ZCS

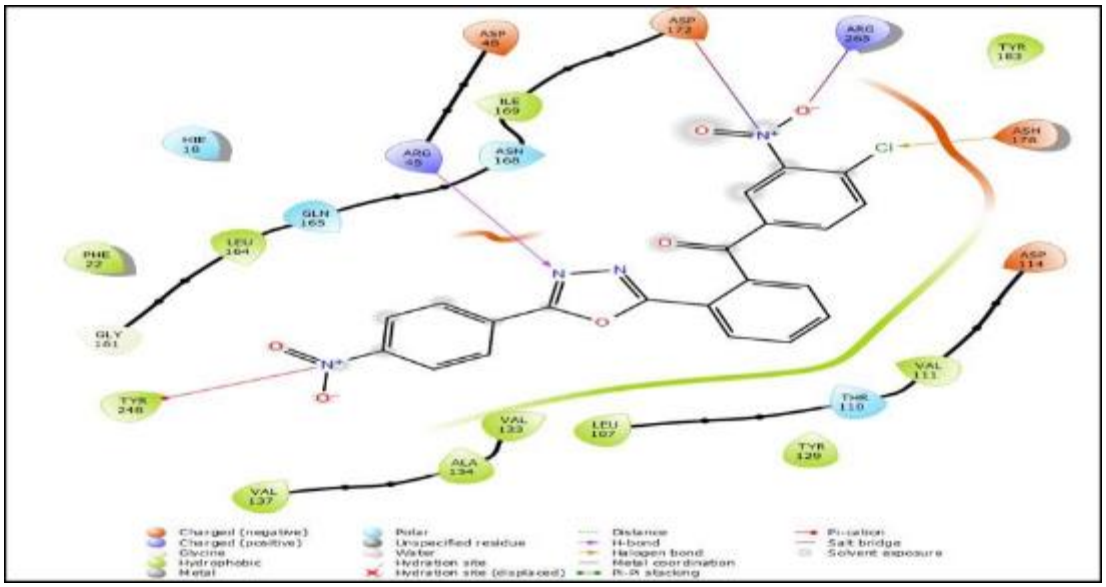


Figure 6 2D representation of compound R2 with PDB ID: 2ZCS

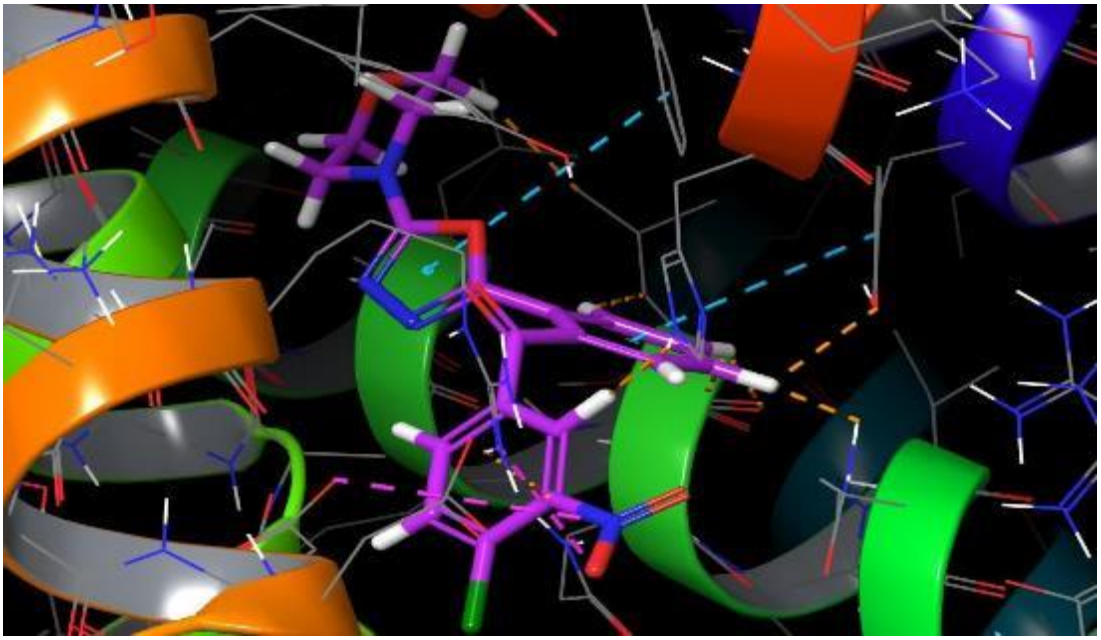


Figure 7 3D interaction diagram of compound R4 with PDB ID: 2ZCS

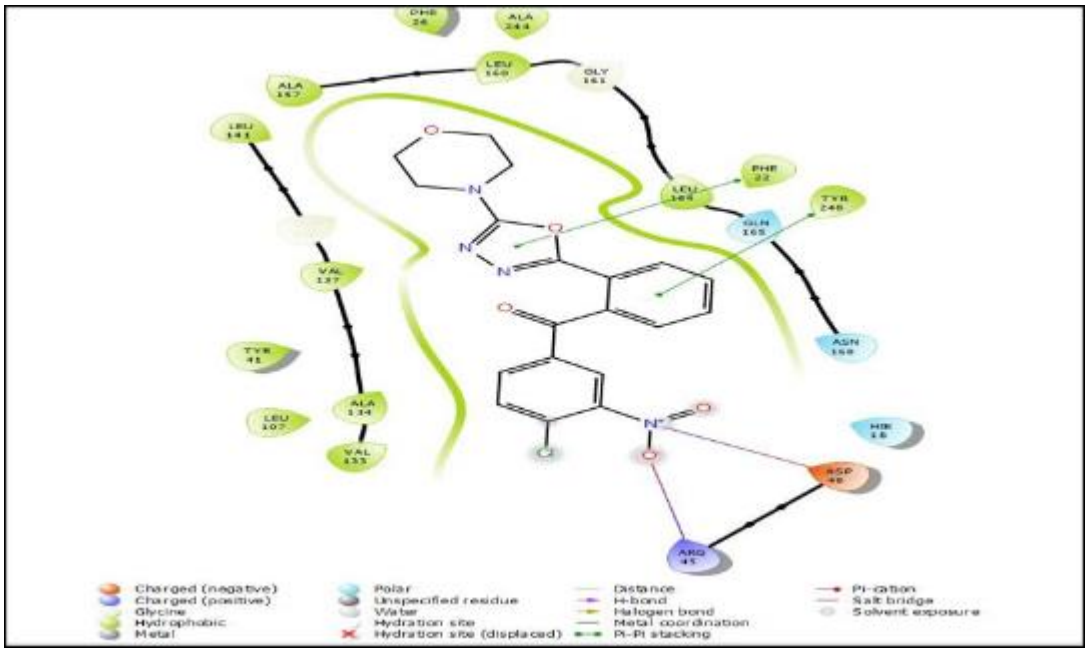


Figure 8 2D representation of compound R4 with PDB ID: 2ZCS

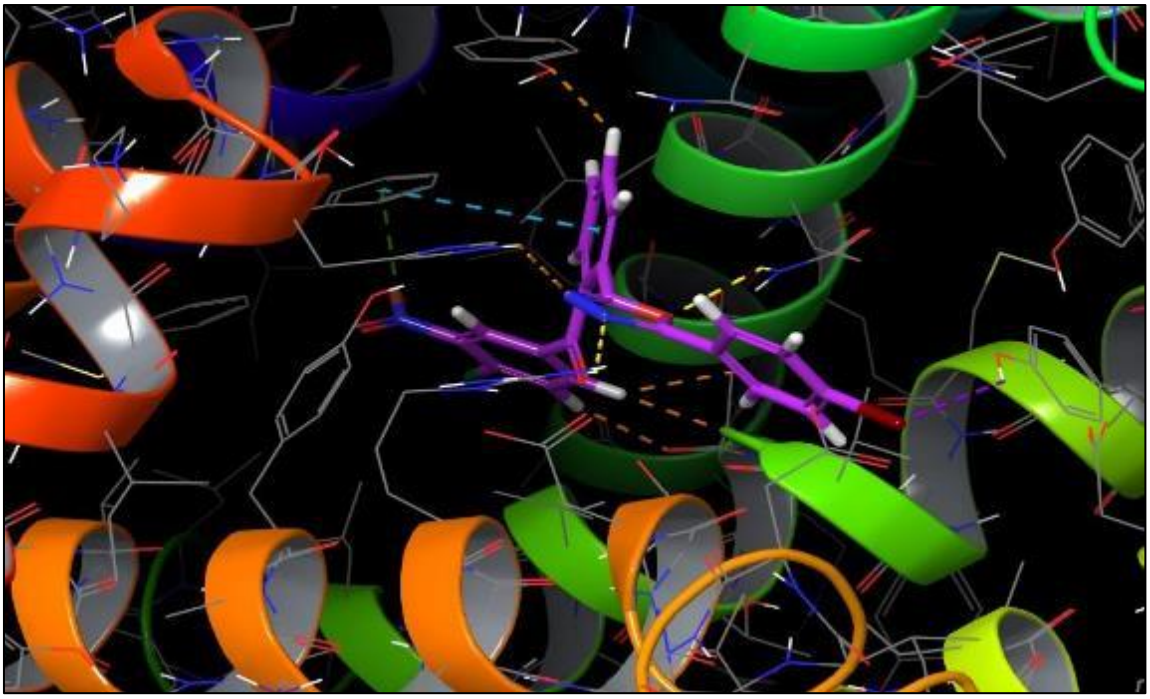


Figure 9 3D interaction diagram of compound R5 with PDB ID: 2ZCS

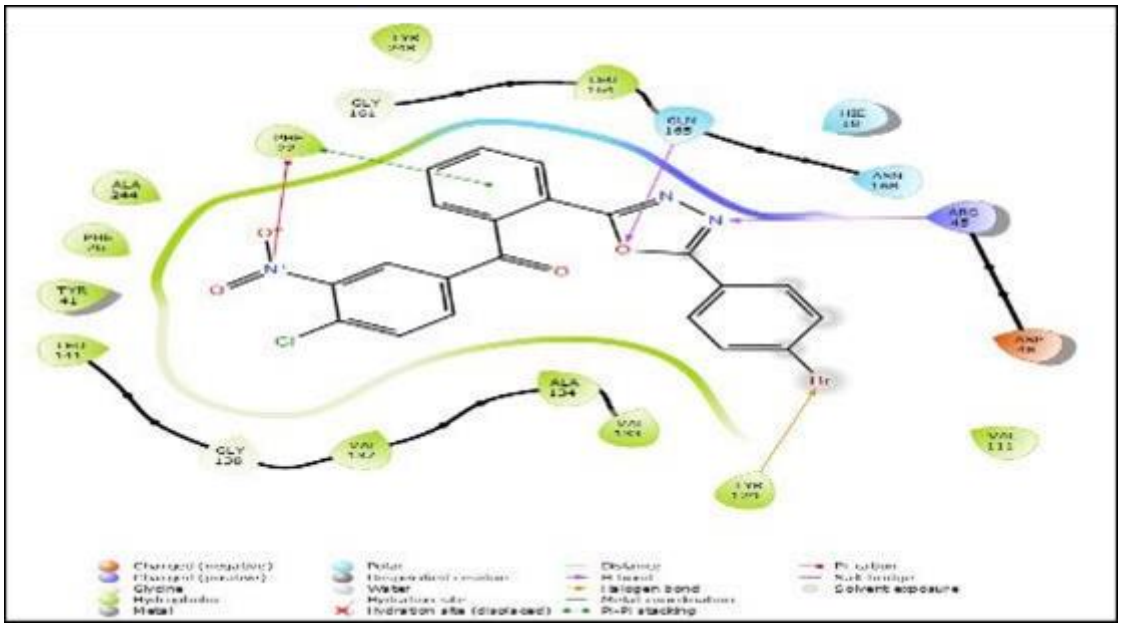


Figure 10 2D representation of compound R5 with PDB ID: 2ZCS

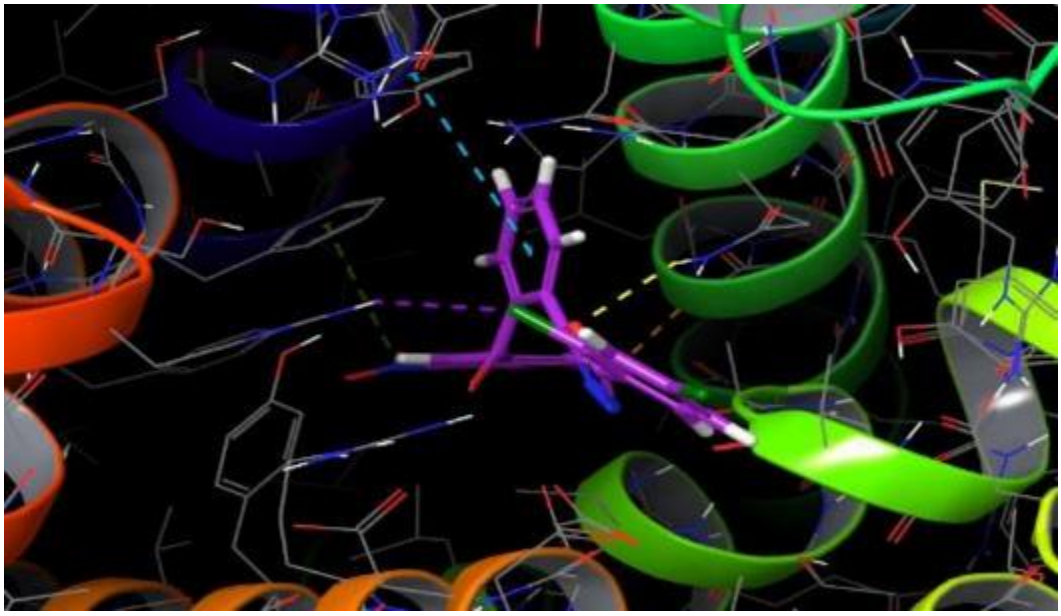


Figure 11 3D interaction diagram of compound R6 with PDB ID: 2ZCS

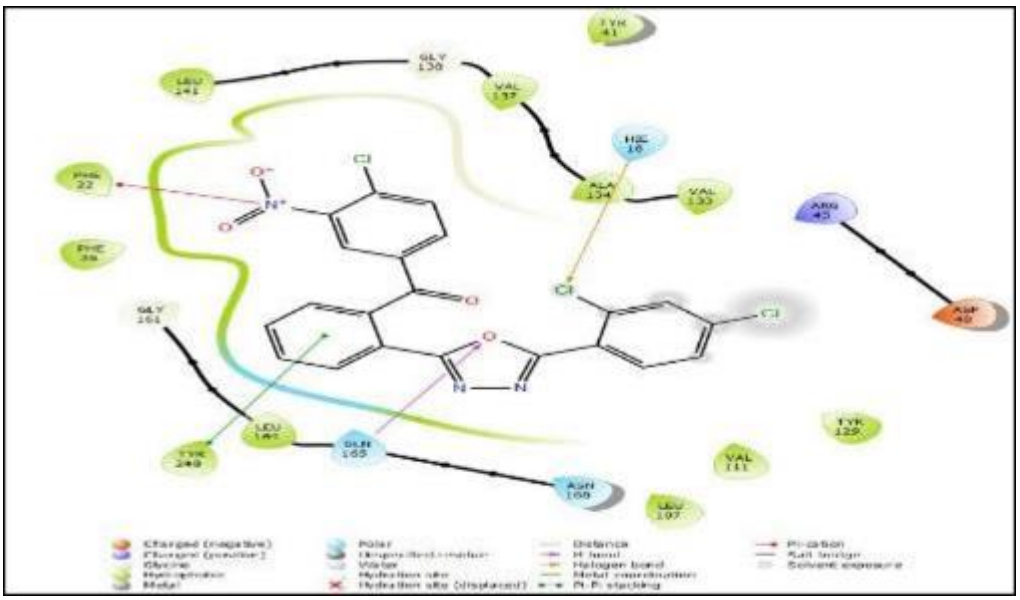


Figure 12 2D representation of compound R6 with PDB ID: 2ZCS.

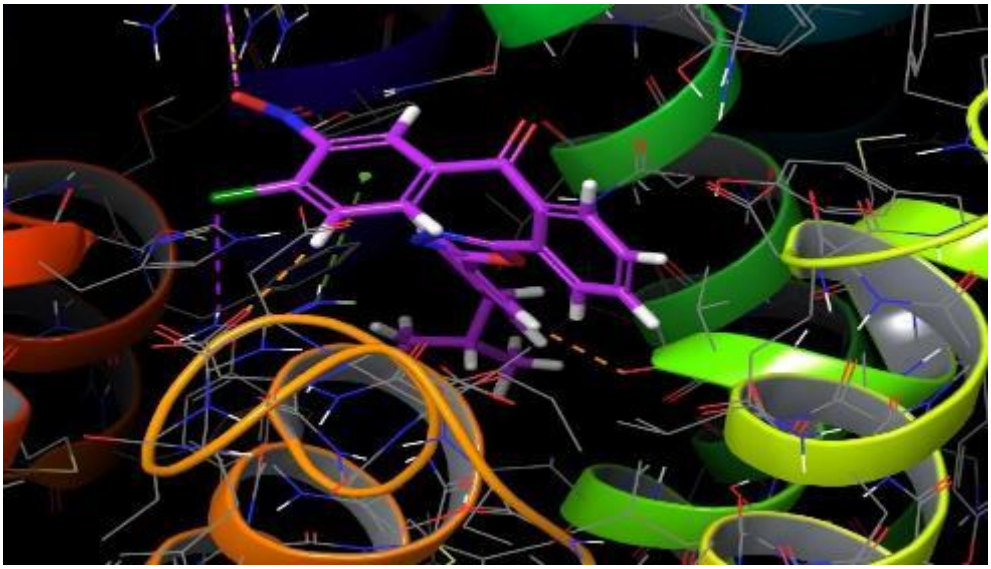


Figure 13 3D interaction diagram of compound R7 with PDB ID: 2ZCS

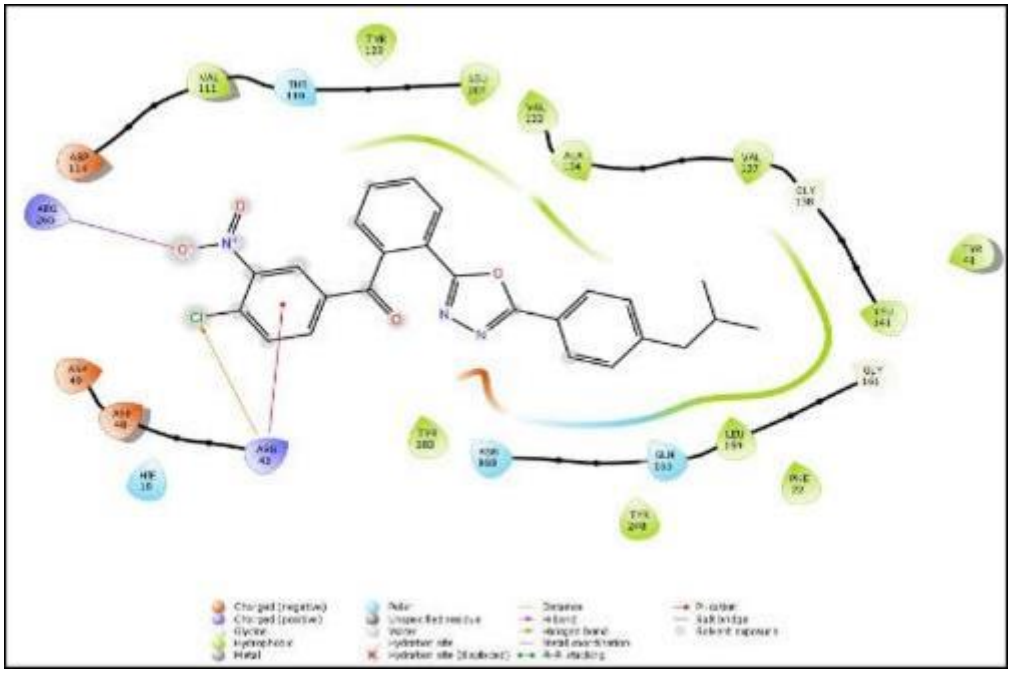


Figure 14 2D representation of compound R7 with PDB ID: 2ZCS

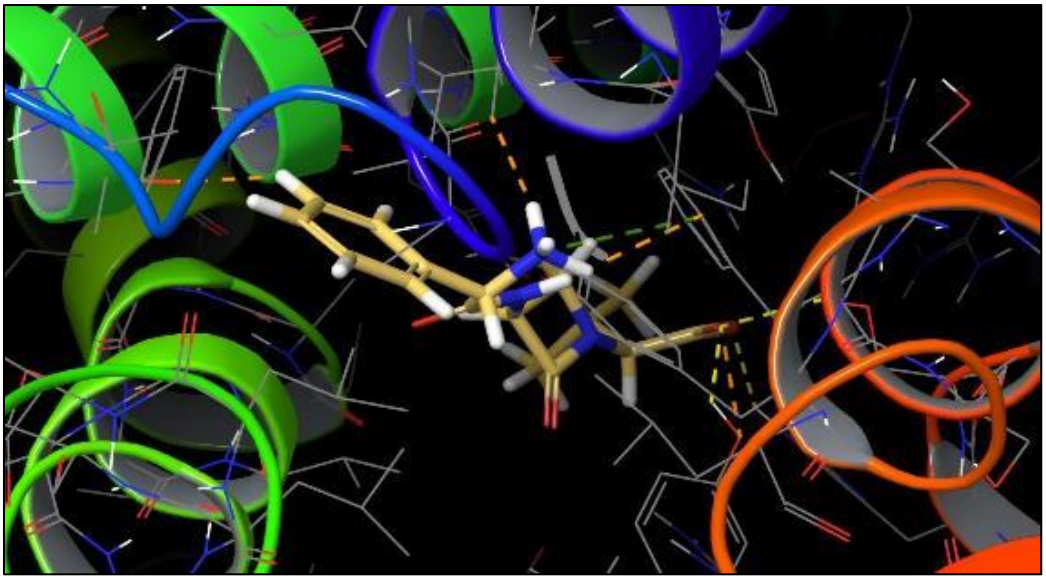


Figure 15 3D interaction diagram of ampicillin (standard drug) with PDB ID: 2ZCS

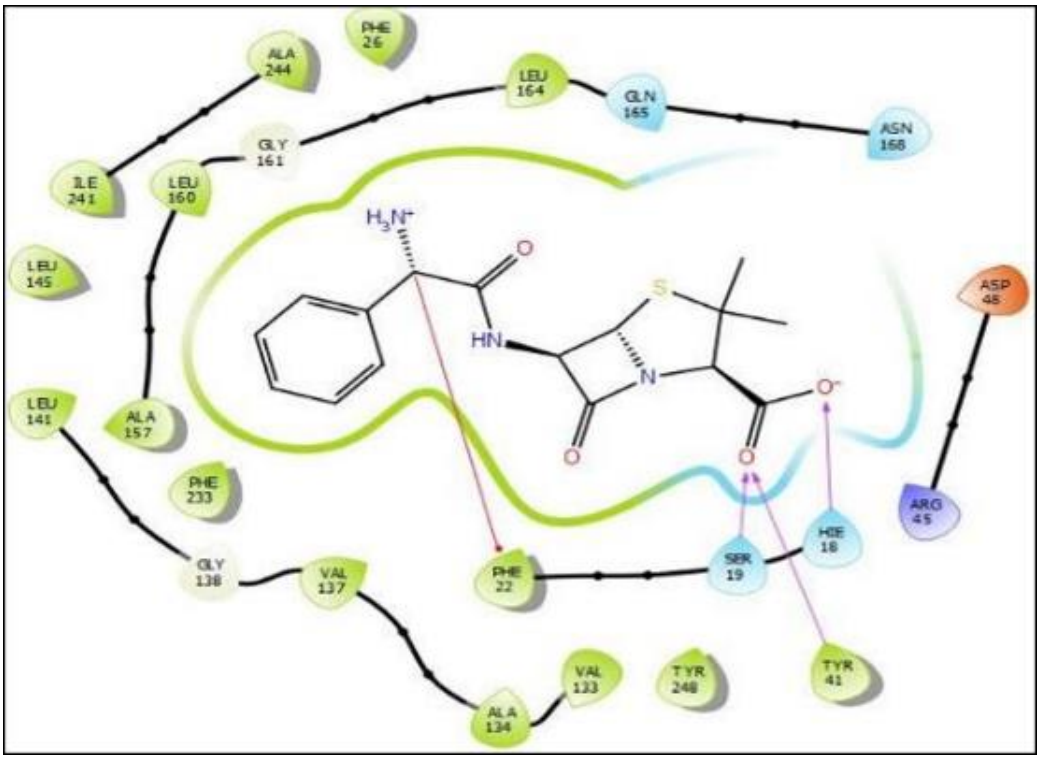


Figure 16 2D representation of ampicillin (standard drug) with PDB ID: 2ZCS

Table 4 Binding affinity of compounds

S.No.	Compound codes	Bindingaffinity(kcal/mol)
1	Ampicillin	-8.54
2	R1	-7.19
3	R2	-5.58
4	R4	-5.98

5	R5	-8.01
6	R6	-7.09
7	R7	-5.71

Table 5 Antioxidant activity of compound DPPH assay (concentration after 20 mins)

Compounds	Concentrations(µg/ml)			
	20	40	60	80
R1	32.33	32.70	34.21	42.85
R2	1.50	15.41	19.54	27.06
R3	34.58	38.72	38.72	56.01
R4	48.87	52.25	57.14	72.55
R5	68.42	74.81	75.56	78.57
R6	06.76	13.90	17.29	19.92
R7	55.26	56.76	59.39	60.90
AA	94.36	94.36	94.36	94.73

Table 6 Antioxidant activity of compound DPPH assay (concentration after 40 mins)

Compounds	Concentrations(µg/ml)			
	20	40	60	80
R1	46.99	48.49	49.62	56.76
R2	07.51	22.55	29.32	32.70
R3	44.36	46.99	52.25	66.16
R4	60.15	64.28	66.16	83.45
R5	74.81	76.69	77.06	79.32
R6	09.39	18.42	21.80	24.06
R7	56.76	63.53	68.04	68.79
AA	94.36	94.73	94.73	95.11

4. Conclusion

To conclude, in this project a series of 2,5-substituted oxadiazole derivatives were synthesized using efficient, green and simple protocol. The method used has the advantage of having short reaction time, simple and clean work-up procedure, high yields and broad substrates scope. In this series, 7 different oxadiazole derivatives have been synthesized and tested for their activity using various analytical and biological evaluation methods. Initially, reaction was monitored using chromatography techniques, which include TLC and the spot were visualized by UV transilluminator. The compounds were found to be soluble in varying solvent ranging from methanol to hexane and partially soluble in chloroform. The practical yield of most of the compounds was in the range 50-75% and the color of the compounds was shades of yellow while the 2,4-dicholobenzoyl chloride substituted oxadiazole derivatives were found to be black in color. The compounds were mainly crystalline and highly hygroscopic. The protein carotenoid dehydrosqualene synthase (PDB ID: 2ZCS) was taken for in-silico docking studies and the ligand attached to it was ampicillin. R1 and R5 were found to be comparable to the standard ligand i.e., -8.54. Further analysis was conducted in the form of FTIR and 1H NMR. Compound tested for antioxidant activity using DPPH assay and it was found that R5, R4 and R7 have

significant antioxidant activity. For future studies, antibacterial properties of the compounds can be tested as docking activities for compounds at the active site was found to be comparable to the standard. Therefore, further in-vitro antibacterial assay and animal studies can be conducted to determine the antimicrobial activity.

Compliance with ethical standards

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Md. Rahat Raza is a M. Pharm (Pharmaceutical Chemistry) student at Department of Pharmaceutical Chemistry, Delhi Institute of Pharmaceutical Sciences and Research, DPSRU, Authors are grateful to an eminent and Prof. P.K. Sahoo, Director, DIPSAR, for supplying the required facilities for carrying out this work.

Disclosure of conflict of interest

The authors have no conflict of interest.

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