# Synthesis, *in silico* analysis and antidepressant activity of 1,3,4-oxadiazole derivatives

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***Abstract***

*A new class of 1,3,4-oxadiazole derivatives (O1-4) was synthesized and physically characterized by using spectroscopic (IR, $^1$H-NMR, Mass) methods. In silico studies were performed for all the new compounds and were put through ADME screening with Schrodinger suite 2020-4. Some of the compounds were tested for in vivo antidepressant efficacy utilizing the Forced swimming test and Tail suspension test. The final 1,3,4-oxadiazole analogues can potentially be developed into viable antidepressant drugs and might be beneficial as lead compound for pharmaceutical firms based on the in silico and in vivo research.*

**Keywords:** 1,3,4-oxadiazoles, Depression, Imipramine, Molecular docking, Carbohydrazide.

## Introduction

Depression is classified as a mood disorder that results in a prolonged sense of sadness and disinterest. About 350 million people suffer from this disorder as per the WHO findings.[16] Depression is prevalent one among the psychiatric disorders and it is highly recurrent in nature. It is found that over 75% of the depressed patient have more than one depressive episode.[4] Investigations have found genetic and biological factors, environmental characteristics which may lead an individual to experience the episodes of depression.[2]

The important types of depression which have been found out recently are bipolar 2 depression, seasonal depression, recurrent brief depression and atypical depression, It is found that, most of the research is focused on bipolar 2 depression, atypical depression and mixed depression. Some of the major symptoms include decreased interest or pleasure in activities, depressed mode, weight gain / weight loss, insomnia/hypersomnia and fatigue[8]. Presently, the treatment option for depression encompasses pharmacotherapy using novel classes of drugs[10]. The most privileged and well known heterocyclic five membered class is 1,3,4-oxadiazoles. The increased interest is found among the medicinal chemists for 1,3,4-oxadiazoles because of their biological activity and being utilized as a right scaffold in designing of novel drugs with different applications in therapy. Amalgamation of hydrophilic and electron donor characteristics makes the 1,3,4- oxadiazole ring biologically active. In addition, thermal and chemical resistance imparts metabolic stability.[6]

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It is thought that the presence of toxophoric -N=C-O linkage in 1,3,4-oxadiazole is a crucial component in exhibiting these activities[3]. However, these compounds were reported to possess antibacterial[5], cytotoxic[1], anti-proliferative[7], anticonvulsant[17] and anticancer[13] activities.

Based on the above observations, it was contemplated to synthesize a new series of 1,3,4-oxadiazole derivatives and to evaluate for antidepressant activity followed by *in silico* analysis.

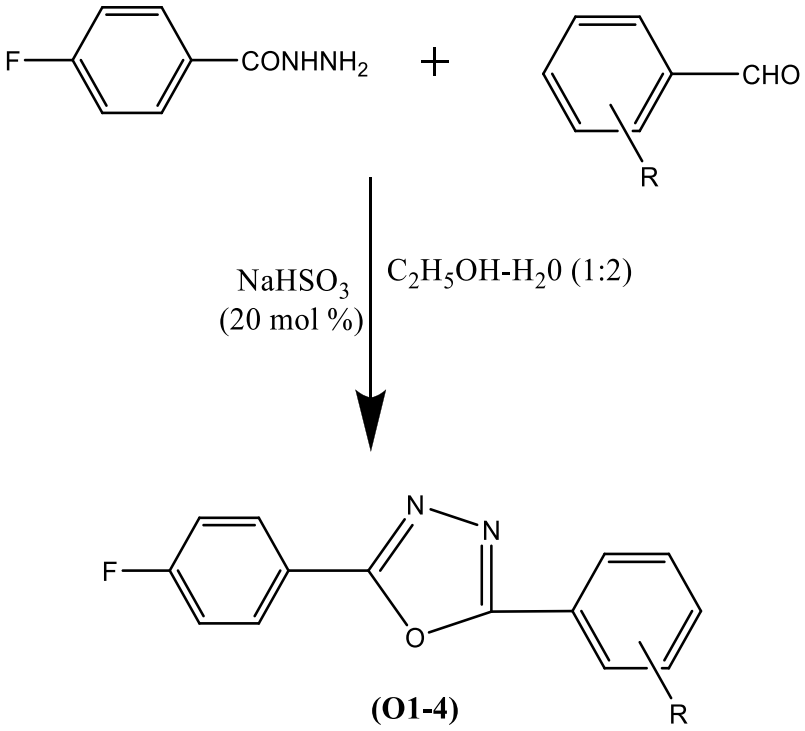
## Material and Methods

The analytical grade solvents utilized were purchased from Sigma Aldrich and HiMedia (India). Melting point determination was conducted using the Equiptronics digital melting point apparatus (Model EQ-730, India). FT-IR spectral analysis was performed using KBr discs on the Alpha Bruker FT-IR Infrared Spectrophotometer (Germany) ($cm^{-1}$). Agilent 400MR DD2 spectrometer (USA) was utilized to collect $^1$H-NMR spectra at 400 MHz with d6-DMSO/$CDCl_3$ as the solvent, with TMS acting as an internal standard. Mass spectra were recorded using the Waters LC-MS/MS (USA). The progress of reactions and purity of the synthesized compounds were assessed through thin-layer chromatography (TLC) on silica gel G plates (stationary phase).

**General procedure for the synthesis of 1,3,4-oxadiazole derivatives (O1-4):** The carbohydrazide (0.01M) was dissolved in ethanol (20ml), in a round bottom flask. To this, solution of aromatic aldehydes (0.01M) in ethanol was added and was diluted with water. Solution of sodium bisulfite (0.01M) in water (10ml) was added gradually and the mixture was refluxed for 10-12 hrs. After completion of the reaction, the contents were poured into the crushed ice, filtered, washed with water and recrystallized from alcohol[12].

**Compound 1 - 2-(4-chlorophenyl)-5-(4-fluorophenyl)-1,3,4-oxadiazole:** M.P.-114-16 $^0$C IR (KBr, $cm^{-1}$) $\nu_{max}$: 1553(C=C), 1590(C=N), 3030 (C-H), $^1$H-NMR (400 $MH_Z$, DMSO-$d_6$) (δ, ppm)**:** 7.32-7.96 (m, Ar-H, 8H). **MS(m/z):** 274.68 (M+).

**Compound 2 - 4-(5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl)-2-methoxyphenol:** M.P.-128-30$^0$C: IR (KBr, $cm^{-1}$) $\nu_{max}$: 1601(C=C), 1543(C=N), 3042 (C-H), 3399 (OH). $^1$H-NMR (400 $MH_Z$, DMSO-$d_6$) (δ, ppm)**:** 3.83 (s, $OCH_3$, 3H), 6.70--7.93 (m, Ar-H, 7H), (s, OH, 1H), **MS(m/z):** 286.26 (M+).




**Scheme 1: Schematic route for the synthesis of 1,3,4-oxadiazoles (O1-4)**

**Compound 3: 2-(3,4-dimethoxyphenyl)-5-(4-fluorophenyl)-1,3,4-oxadiazole:** M.P.-145-47$^0$C: IR (KBr, cm$^{-1}$) $\nu_{max}$: 1556(C=C), 1596(C=N), 2978 (C-H), $^1$H-NMR (400 MHz, DMSO-$d_6$) (δ, ppm)**:** 3.83 (s,2X $OCH_3$, 6H), 6.70-7.94 (m, Ar-H, 7H). **MS(m/z):** 300.29 (M+).

**Compound 4: 2-(4-bromophenyl)-5-(4-fluorophenyl)-1,3,4-oxadiazole:** M.P.-164-66$^0$C: IR (KBr, cm$^1$) $\nu_{max}$: 1591(C=C), 1556(C=N), 3031 (C-H), $^1$H-NMR (400 MHz, DMSO-$d_6$) (δ, ppm)**:** 6.71-7.96 (m, Ar-H, 8H). **MS(m/z):** 319.13 (M+).

***In silico* analysis**

**Ligand preparation:** Chemdraw was used to illustrate the structures of all the synthesized compounds. The Smile strings of each compound are imported into the Maestro utility workspace and underwent analysis using the LigPrep module of the Schrodinger suite 2020-40. The energy minimization was carried out using the OPLS_2005 force field. The ligands underwent various transformations including stereo concoction, ionisation, tautomerism, vitality minimization and geometry optimization. Additionally, the ligands were prepared by removing hydrogen atoms and chiralities[14].

**Protein preparation:** The target protein's crystallized structure, with PDB ID 4n6h, was obtained from PDB and inspected using the Maestro interface. In order to assign the correct bond order, hydrogen atoms that are lacking from the protein's X-ray crystal structure are initially inserted. The extra water molecules and other disruptive ligands were removed. To facilitate further molecular modeling investigations of the protein structure, the protein energy minimization was executed utilizing the OPLS_2005 force field[14].

**Receptor ligand docking:** Using an *in silico* docking analysis, the optimal approaches for the ligand to bind to the active residue of the target protein were identified. Using standard precision algorithm (SP) which is followed by an extra precision (XP) method, the new compounds were then docked into the pocket of the protein target (4n6h). When evaluating the docking data, the binding energy was taken into account. The lowest binding energy or a significantly negative magnitude of binding affinity yields superior confirmation[14].

**Receptor grid generation and docking study:** The protein structures were used to identify the binding cavity instead of the pre-existing ligand WR99210. Grid boxes were created at the center of these binding cavities. To analyze the binding interactions between the receptor protein and ligands, the Glide module of the Schrodinger suite was employed. The docking study utilized the extra precision (XP) mode of Glide[15].

**ADME Predictions:** The evaluation of the ligand molecules' quality was conducted through the execution of ADME calculations utilizing the QikProp module of Schrödinger suite 2020-4 on synthesized compounds. The physicochemical characteristics of a molecule are instrumental in determining its pharmacokinetic conduct[15].

***In vivo* antidepressant screening**

**Selection of Animals:** Swiss albino mice (8-12 weeks old) weighing about 20-25g of either sex were used for this study.

The animals were procured from NUCARE, Paneer, Mangalore. They were grouped appropriately, housed in polypropylene cages and kept in the lab for acclimatization. The cages were maintained at temperature $27^0C \pm 2^0C$ under 12hrs dark/light cycles. *Ad libitum* access to food (standard pellet feed) and purified water was permitted for animals. Ethical approval was granted by the Institutional Animal Ethics Committee (IAEC) to conduct animal experimentation. (Certificate reference no: NGSMIPS/ IAEC/JUNE-2021/227) prior to the beginning of the work and all the investigations were carried out in accordance with the guidelines of CPCSEA, New Delhi, India[9].

**Acute toxicity studies:** Female albino mice weighing 20-25 g were subjected to acute toxicity studies of 1,3,4-oxadiazoles following the guidelines set by OECD in 2001. The mice were kept under standard husbandry conditions and were fasted overnight prior to the experiment. A single dose of 13,4-oxadiazole derivatives was administered to the mice and their mortality was observed for a period of 48 hours to assess short-term toxicity. Based on the results of this assessment, subsequent doses were determined in accordance with OECD guidelines no.425.

**Forced Swimming Test (FST):** Imipramine 10 mg/kg was used as a standard drug. Mice were given (p.o.) 0.5 ml of the test compounds' suspension (100 mg/kg) and the reference medication (1% aqueous solution of tween 80). A control was also kept, consisting of a 1% aqueous solution of tween 80. Each mouse was made to swim for six minutes in a 10 cm diameter, 25 cm tall open cylindrical container of water that was filled to a height of 18 cm. Prior to the study's first hour, treatment was delivered. The first two minutes will be marked by the animal's first strong struggling. The last 4 minutes of inactivity were tracked. To get the percentage change from the control, the following formula was used[9]:

% Change of immobility = [(Test/control)×100)–100]

**Tail Suspension Test (TST):** In the TST, mice are exposed to an inescapable yet moderately stressful situation, resulting in the absence of any escape-related behavior which is considered as immobility. The synthesized compounds to be tested, at a dosage of 100 mg/kg, were suspended in 1% aqueous solution of tween 80, along with the standard drug (Imipramine (10 mg/kg) subsequently administered orally to the mice in a volume of 0.5 ml per body weight. A control group was also maintained which received 1% aqueous solution of tween 80. On the day of the experiment, the mice were allocated to distinct groups, with a total of six mice in each group. After thirty minutes had elapsed since administration, the mice were individually suspended using paper adhesive tape at a height of 65 cm from the benchtop, with the tape positioned 1 cm away from the tail tip.

The animals were permitted to hang for a duration of six minutes, during which the duration of immobility was recorded over the preceding five minutes. To determine the percentage change from the control, the subsequent formula was implemented[9]:

% Change of immobility = [(Test/control)×100)–100]

**Statistical Analysis:** The data was analyzed and group differences were compared using ANOVA (one-way analysis of variance) and the Dunnett test, both conducted through GraphPad Prism 8 software in this study.

## Results and Discussion

**Chemistry:** Adequate quantities of 1,3,4-oxadiazole derivatives (O1-4) were synthesized by reacting 4-fluoro benzhydrazide with various substituted aromatic aldehydes. The reaction was carried out with small amount of sodium bisulphite, acting as a catalyst and the use of ethanol-water as a solvent system contributed to the satisfactory yield of the desired compounds.

The successful reaction sequence is presented in scheme 1. Through analysis using FT-IR, $^1$H-NMR and Mass spectral data, the newly synthesized compounds were confirmed to possess their designated structures.

***In silico* analysis:** In order to mitigate the occurrence of unsuccessful clinical trials, extensive research was conducted on the physicochemical characteristics of compounds that are commonly of interest. Lipinski's rule is employed to assess the molecular properties of potential compounds, which play a crucial role in determining their suitability as drug candidates. Compounds that adhere to Lipinski's rule of five possess a higher likelihood of being considered as viable drug candidates. All the synthesized compounds exhibited the desired physicochemical properties without any deviations from the standard ranges (Table 1). Furthermore, the synthesized compounds adhere to Lipinski's rule of 5.

The compounds were placed in the binding site groove of PDB:4n6h and their affinity for the receptor, along with their docking score, is presented in table 1. The docking scores of the synthesized compounds range from −5.42 to -6.61 kcal/mol. However, when compared to the standard imipramine, the dock scores were found to be -6.29 kcal/mol. which is comparatively less score when compared to the newly synthesized compounds. The active residues in 4n6h are Met 232, ASN 233, Tyr 229, Trp 274. Compound $O_2$ fits into the binding cleft of 4n6h receptor with dock score of −6.61 kcal/mol. The docking conformations of this compound are represented in figure 1.

**ADMET analysis:** In order for a molecule to be considered a viable drug candidate, it must adhere to Lipinski's rule of five which is determined by its ADME properties: absorption, distribution, metabolism and excretion. In addition, percentage human oral absorption, QPPMDCK, QPlogKhsa, QlogKp, #metab and QPPCaCO parameters have also been studied and are summarized in table 1.

QPPCaCO is a tool that forecasts the apparent Caco-2 cell permeability in nm/sec, which is a crucial parameter for evaluating the gut-blood barrier. Caco-2 cells serve as a representative model for studying this barrier.The QPPCaco-2 predicted values suggest that all the compounds possess favorable intestinal permeability, coupled with an excellent capacity for human oral absorption (> 80%). The synthesized compounds were subjected to ADMET studies which aided in the determination that all the compounds exhibit favorable BBB penetration. Madin-Darby canine kidney (MDCK) serves as an effective mimic of the blood-brain barrier (BBB), aiding in the prediction of BBB penetration.

LogKp is a crucial factor in determining the extent of dermal penetration for a drug. The estimated LogKp values for compounds (O1-4) strongly suggest that these drugs can be effectively administered through the skin. Qikprop additionally calculates the quantity of potential metabolic reactions, aiding in the prediction of the drug's accessibility to the desired location. This value is represented as #metab, with a suggested range of 1-8. All of the derivatives are confined within the range of 1-6.

LogKHSA is utilized to assess the efficacy of drug binding to human serum albumin. A higher binding efficiency implies that the drug can diffuse more effectively. However, compounds (O1-4) deviate from the recommended range, suggesting a potential strong binding to human serum albumin. The permeability and solubility of the drug candidate are crucial factors that determine its absorption. Consequently, these factors also play a significant role in determining the bioavailability. All the synthesized compounds exhibit a favorable percentage of human oral absorption, exceeding 80%. Given their high percentage of human oral absorption, any violation of Lipinski's rule of five will not impact the bioavailability.

**Anti-depressant activity:** Two compounds derived from the synthesized 1,3,4-oxadiazole derivatives were selected and they underwent *in vivo* screening for anti-depressant effects through FST and TST. The results indicate that each of the compounds examined displayed promising activity.

In the FST model of antidepressant activity, compound O3 displayed the highest antidepressant efficacy followed by O2. Compound O2 reduced the immobility time by 40%, whereas O2 reduced it by 34.48%. In the TST model, compounds O2 and O3 displayed the highest antidepressant efficacy with a reduction in the immobility by 33% and 47.27% respectively. The data indicates that despite both models displaying a shared behavioral measure of despair, there may be distinct differences in the underlying pathophysiology.

**Table 1**
**Lipinski rule of 5 and dock scores of compounds (O1-4)**

| Comp | MW | Log p | Donor HB | Accept HB | Rule of Five | Dock scores |
|---|---|---|---|---|---|---|
| **Acceptable range** | **≤500** | **> 5** | **≤5** | **≤10** | **<5** | - |
| O1 | 274.68 | 4.026 | 0 | 2.5 | 0 | -5.429 |
| O2 | 286.26 | 3.081 | 1 | 4 | 0 | **-6.613** |
| O3 | 300.28 | 3.64 | 0 | 4 | 0 | -5.523 |
| O4 | 319.13 | 4.11 | 0 | 2.5 | 0 | -5.441 |
| Imipramine | 280.41 | 4.42 | 0 | 2.5 | 0 | -6.296 |

**MW-** Molecular weight; **Log p –** lipophilicity; **Donor HB -** Estimated number of hydrogen bonds; **Accept HB-** Estimated number of hydrogen bonds.

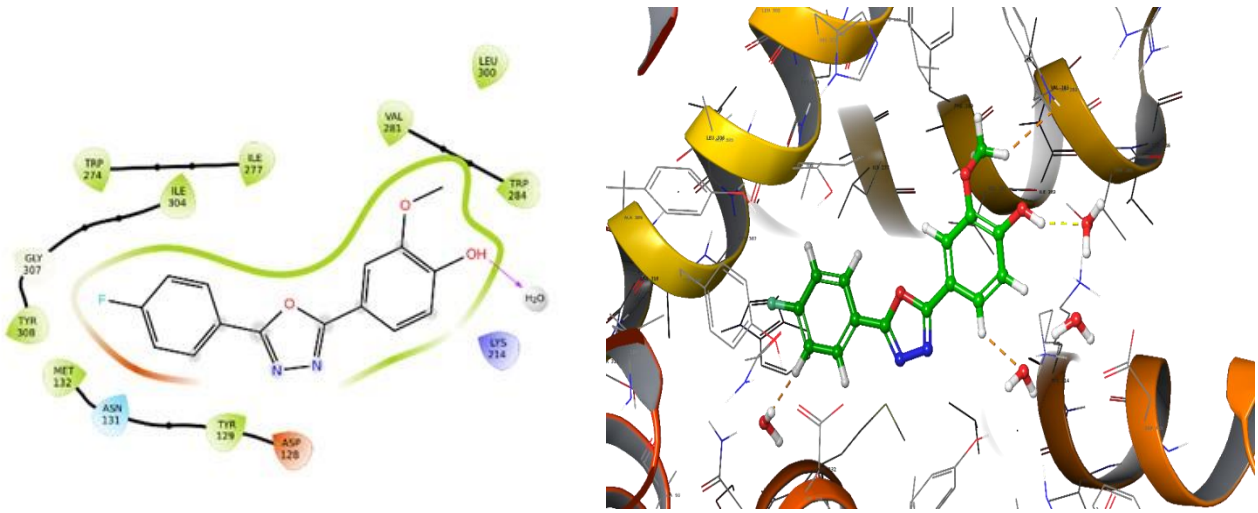



**Figure 1: 2D and 3D interaction of compound O2.**

**Table 2**
**ADME properties of compounds (O1-4)**

| Comp | QPPCaco | QPlogBB | QPPMDCK | QPlogKp | #metab | QPlogKhsa | %Human Oral Absorption |
|---|---|---|---|---|---|---|---|
| **Acceptable arrange** | <25 poor >500 Great | (− 3 to 1.2) | <25 poor >500 Great | (− 8.0–− 10.0) | (1–8) | (− 1.5–1.5) | > 80% High, <25% low |
| O1 | 3292.675 | 0.392 | 8015.634 | -1.27 | 1 | 0.378 | 100 |
| O2 | 992.993 | -0.436 | 888.533 | -2.25 | 3 | 0.19 | 100 |
| O3 | 3293.66 | 0.079 | 3247.185 | -1.296 | 3 | 0.191 | 100 |
| O4 | 3292.945 | 0.403 | 8619.343 | -1.273 | 1 | 0.404 | 100 |
| Imipramine | 2129.702 | 0.658 | 1239.092 | -2.3 | 6 | 0.735 | 100 |

**Table 3**
**Antidepressant activity of 1,3,4-oxadiazoles by Forced Swimming Test (FST) and Tail Suspension Test (TST)**

| Comp | Dose (mg/kg) | TST | | FST | |
|---|---|---|---|---|---|
| | | Duration of immobility (s) | % change in immobility | Duration of immobility (s) | % change in immobility |
| O2 | 2.88 | 2.88 | 69.25±2.25*** | 32.93 | 61.75±1.60** |
| O3 | 2.88 | 2.88 | 99.28±4.12* | 47.27 | 71.25±2.38*** |
| Control | -- | -- | 210.25±2.25 | -- | 179.08±2.89 |
| Imipramine (Std) | 30 | 30 | 75.58±2.31* | 35.95 | 89.23±3.58* |

Values are expressed as mean ± SD for 6 animals; ***p < 0.001, *p < 0.001 and *p < 0.05 Vs control

This could explain the moderate activity observed in the compounds O3 and O2 during the FST and TST respectively. The antidepressant data is showed in table 3.

## Conclusion

A convenient and a very easy cost-effective synthesis of 1,3,4-oxadizole derivatives was achieved using $NaHSO_3$ and a solvent system of ethanol and water. In recent times, the green chemistry approach has gained popularity due to its ability to reduce environmental pollution and to minimize the use of toxic cyclizing reagents. Water, in particular, has shown great potential in certain organic reactions. To investigate this further, the reactions were conducted using water and 20 mol% $NaHSO_3$.

The ethanol-water solvent system proved to be effective in this particular process. Additionally, the reaction conditions were advantageous in reducing the time intervals required for the reactions. Some of the tested compounds displayed good anti-depressant activity.

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