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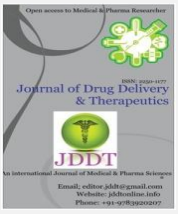

Open Access Research Article

# DESIGN, SYNTHESIS AND DOCKING STUDY OF PYRIDINE DERIVATIVE FOR ANTIDEPRESSANT ACTIVITY

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## ABSTRACT

On the basis of literature survey compound 2-(1*H*-benzo[d]imidazole-1-yl)-*N*-(3-cyano-4,6-diphenylpyridin-2-yl) acetamide (**6**) was designed. Compound **6** and imipramine were virtually studied for their antidepressant activity by performing docking studies using Molegro Virtual Docker (MVD-2013, 6.0) on human MAO (PDB ID: 2BXS). Docking studies revealed its potential antidepressant effect as compared with imipramine. The result of docking study was validated by synthesizing and screening the compound for it's *in vivo* antidepressant activity following forced swim test and locomotor activity using actophotometer. Finally, we conclude that the docking result and experimental results are in good agreement.

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## INTRODUCTION:

Depression is a psychological disorder with a low mood, loss of interest, affecting sense of well being, anorexia, low energy, poor concentration and impaired sleep. This problem may become recurrent and even lead to suicide. According to WHO report depression have become serious health problem affecting about 121 million people worldwide.[1]

Pathophysiology of depression is not clearly understood, but some hypothesis suggests that depression and maniac disorder may be caused by lack of nor adrenaline and serotonin. Most of the synthetic drugs used as an antidepressant act on biogenic amines of the brain. Thus leading to increasing their concentration in respective part of brain. MAO enzyme causes deactivation of biogenic amines. Thus by inhibiting the MAO intracellular concentration of endogenous amines may be increased, which seems to be the major cause of antidepressant effect.

MAO inhibitors available in the market have serious side effects like hypertension, tremors, muscle rigidity and seizures[2]. Hence there is a need of potent antidepressant with less or no side effects.

In medicinal chemistry heterocyclic compound pyridine has been found to be of utmost importance. Pyridine and related compounds had exhibited various biological activities like antimicrobial, anti-inflammatory, anticancer and anti-tubercular activity.[3]

On the basis of above observations we decided to design a novel pyridine derivative, to study it's binding interactions with MAO enzyme by docking studies and to screen the compound for antidepressant activity.

## MATERIALS AND METHODS

### Chemicals:

All the solvents and chemicals were purchased from SD Fine Chem Ltd, Kemphasol, Molychem, and Fisher Scientific Pvt. Ltd. Melting Point of synthesized compounds were determined by Thiele's melting point apparatus and were uncorrected. IR Spectra was recorded on Schimadzu IR AFFINITY -1 spectrophotometer by using KBr pellets. The $^1$H NMR was recorded on BruckerAvance II 400 NMR Spectrometer by using DMSO as a solvent and Tetra Methyl Silane as internal standard, chemical shifts are expressed as δ values in ppm.

### Animals:

Male albino mice of 20-25g were obtained from animal house of PES's Rajaram and Tarabai Bandekar college of Pharmacy, Farmagudi, Goa.

### Molecular docking methodology

The molecular docking study was performed using Molegro Virtual Docker (MVD-2013, 6.0). The crystal structure of the monoamine oxidase - A co-crystallized with clorgyline inhibitor was downloaded from Protein

Data Bank (PDB ID: 2BXS). Molecular docking study of the synthesized compound/ligand was performed in order to understand the various interactions between the ligand and enzyme active site in detail. The molecular docking study was performed for the target compound by using MVD-2013 (Version: 6.0).

The synthesized compound was built using Chemdraw 11.0. The 2D structure was then converted into energy minimized 3D structure and was saved as MDL Molfile (.mol2). The coordinate file and crystal structure of monoamine oxidase - A was obtained from the RCSB PDB website (PDB ID: 2BXS). The protein file was prepared by the removal of water molecules, addition of polar hydrogens and removal of other bound ligands. In the present study, the binding site was selected based on the amino acid residues, which are involved in binding with clorgyline inhibitor of MAO-A as obtained from protein data bank, which would be considered as the probable best accurate region as it is solved by experimental crystallographic data. The docking protocol was carried out for the synthesized compound as listed in Table 1 using MVD-2013 (6.0) software using the standard operating procedures.

**Synthesis:**

**Synthesis of 2-amino-4,6-diphenylpyridine-3-carbonitrile (4):**

A mixture of benzaldehyde (**1,** 0.10612 g, 1 mmol), acetophenone (**2,** 0.12015 g, 1 mmol), malononitrile (**3,** 0.06606 g, 1 mmol) and ammonium acetate (0.6166 g, 8 mmol) were dissolved in toluene (20 mL) and refluxed for about 6 h. The completion of the reaction was monitored by TLC using Chloroform : Ethanol, (0.5:1) as the mobile phase. The resulting solid formed was filtered, washed with ethanol and recrystallized with acetone to get the pure product (**4**).

**Synthesis of 2- chloro-*N*-(3-cyano-4,6-diphenylpyridin-2-yl)acetamide (5):**

A mixture of 2-amino-4,6-diphenylpyridine-3-carbonitrile (**4,** 1.45 g, 1 mmol), triethylamine (1.08 g, 2 mmol) and chloroacetyl chloride (1.2 g, 2 mmol) was dissolved in dichloromethane (20 mL) and stirred for 12 h. The completion of the reaction was monitored by TLC using Chloroform: Methanol (0.5:1) as the mobile phase. The solid thus formed was filtered, washed with water, dried and recrystallized from ethanol to get the pure compound.

**Synthesis of 2-(1*H*-benzo[d]imidazole-1-yl)-*N*-(3-cyano-4,6-diphenylpyridin-2-yl) acetamide (6):**

A mixture of potassium carbonate (0.34 g, 2.5 mmol) and benzimidazole (2 mmol) in dry acetone (20 mL) were stirred on magnetic stirrer for 1 h. The above solution was then added to 2-chloro-N-(3-cyano-4,6-diphenylpyridin-2yl)acetamide (**5**, 0.43 g, 1.25 mmol) and refluxed in round bottom flask for 6-13 h. The completion of reaction was monitored by TLC. After the reaction completion the solvent was removed by vacuum distillation and residue was treated with sodium bicarbonate (5 % w/v) to remove acid impurities. The residue was washed with water, dried and recrystallized from ethanol.

**2-Amino-4,6-diphenylpyridine-3-carbonitrile** (**4**)

Yield: 50%, m. p.: 182$^{o}$C, IR (KBr, cm$^{-1}$): 3464.15 (NH), 3302.13, 3176.76 (C-H aromatic), 2206.57 (CN ), 1573.91 (C=C); $^{1}$HNMR (400 MHz, $CDCl_3$, δ ppm): 7.20 (s, 1H, Ar-H), 7.45-7.49 (m, 3H, Ar-H),7.507.54 (m, 3H,Ar-H), 7.62-7.64 (m, 2H, Ar-H), 7.988.00 (m, 2H, Ar-H), 5.41 (s, 2H, N-H).

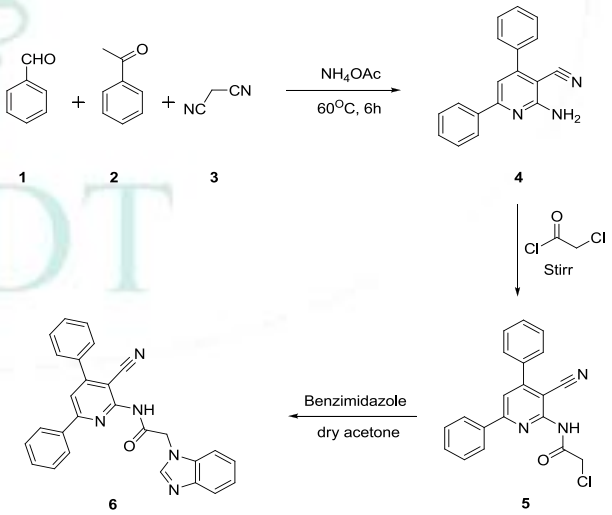
**2-Chloro-*N*-(3-cyano-4,6-diphenylpyridin-2-yl)acetamide** (**5**)

Yield: 64%, m. p.: 172$^{o}$C, IR (KBr, cm$^{-1}$): 3464.15 (NH), 3302.13, 3132.740 (C-H aromatic), 2206.57 (CN ), 1639.49 (C=O), 1604.77 (C=C), 754.17 (C-Cl); $^{1}$HNMR (400 MHz, $CDCl_3$, δ ppm): 7.21 (s, 1H, Ar-H), 7.45-7.48 (m, 3H, Ar-H),7.49-7.54 (m, 3H, Ar-H), 7.627.65 (m, 2H, Ar-H),7.99-8.01 (m, 2H, Ar-H), 5.36 (s, 1H, NH), 3.68 (s, 2H, $CH_2$).

**2-(1*H*-Benzo[d]imidazol-1-yl)-*N*-(3-cyano-4,6-diphenylpyridin-2-yl)acetamide** (**6**)

Yield: 66%, m. p.: 149$^{o}$C, IR (KBr, cm$^{-1}$): 3464.15 (N-H), 3176.76 (C-H aromatic), 2206.57 (CN), 1637.56 (C=O amide), 1573.91 (C=N); $^{1}$HNMR (400 MHz, DMSO- d6, δ ppm): 7.22 (s, 1H, Ar-H), 7.39-7.46 (m, 5H, Ar-H),7.47-7.51 (m, 3H, Ar-H), 7.53-7.59 (m, 5H, Ar-H), 7.61-7.84 (m, 2H, Ar-H), 5.38 (s, 1H, NH), 3.69 (s, 2H, $CH_2$).

**Scheme 1.** Synthesis of pyridine derivative




***In-vivo* studies:**

**Animals:**

Male albino mice weighing between 20-25 gm were used. Animals were maintained under standard conditions in the animal house of Ponda Eduation Society's Rajaram and Tarabai Bandekar College of Pharmacy, Goa. The animals were housed at 24± 2 $^{0}$C with 12 :12 h light and dark cycle. They had free access to food and water. The animals were acclimatised for a period of seven days before study. The study was approved by Institutional Animal Ethics Committee with resolution number PESRTBCOP/IAEC;clear/2015-16/R-12

**Acute toxicity studies:**

Acute oral toxicity studies of compound **6** was carried out on Swiss albino mice weighing between 25-30g according to OECD guideline No. 423. The animals were then observed continuously for the first 4 h for any behavioural changes and for mortality if any at the end of 72 h.The doses were found to be safe at 200 mg/kg when administered orally and is used for antidepressant activity and locomotor activity.

**Antidepressant activity**

Antidepressant activity of compound **6** was evaluated by force swimming test[5] and locomotor activity using actophotometer and was compared with reference standard imipramine. A total of 18 animals were used in study. They were divided into 3 groups of 6 animals each.

**Treatment groups**

Group 1: Normal control (saline)

Group 2: Compound **6** (200 mg/kg, p.o.)

Group 3: Imipramine (10 mg/kg, p.o.)[4]

**Forced swim test**: Animals were moved from animal house to laboratory in their own cages and allowed to adapt to the laboratory conditions for 1-2 hr. Mice were individually forced to swim in bucket (25 × 25cm) containing fresh water to a height of 15 cm and maintained at 26°±1°C. At this height of water, animals were not able to support themselves by touching the bottom or the side walls of the chamber with their hind-paws or tail. Water in the chamber was changed after subjecting each animal to Forced swimming test because "used water" has been shown to alter the behavior. Each animal showed vigorous movement during initial 2 min period of the test. The duration of immobility was manually recorded during the next 4 min of the total 6 min testing period.

**Locomotor activity:**The locomotor activity was monitored by using actophotometer. Before subjecting the animal for locomotor task they were individually placed in activity meter and the total activity count was registered for 10 min. the locomotor activity was expressed in terms of total photo beams count / 10 min per animal.

## RESULTS AND DISCUSSION

**Docking study**

Molecular docking study of compound **6** and imipramine was performed using Molegro Virtual Docker (MVD-2013, 6.0). The crystal structure of mono amine oxidase - A was obtained from the RCSB PDB website (PDB ID: 2BXS). The PDB structure 2BXS bound to the inhibitor clorgyline shows a true binding site for each of the subunits. An essential feature of the binding site is the conservation of hydrogen bondings and the aromatic π-π stacking interactions. The active pocket consists of amino acid residues such as Ala68, Ala409, Arg51, Asp64, Cys406, Glu436, Gly49, Gly67, Gly301, Gly405, Gly426, Gly447, Ile23, Lys305, Met300, Met445, Phe352, Ser223, Ser403, Thr52, Thr183, Thr435, Trp397, Tyr69, Tyr407, Val65 and Val182. Hence, to identify other residual interactions of the compound **6,** a grid box of 10.0 Å was constructed. Clorgyline being a known inhibitor, the center of this site was considered as the center of search space for docking.

The docking results reveals that for compound **6,** the Mol-Dock score was -183.47, while for reference standard imipramine it is -112.101. The best orientation poses of the docked compound **6** and imipramine are shown in Figure 1. It was clearly observed that imipramine exhibited one hydrogen bond with Tyr407, while compound **6** displayed four hydrogen bonds with Tyr407, Tyr69, Ala68 and Met445.

**Chemistry**

The title compound was synthesised as outlined in the Scheme 1. The compound **4** was obtained by condensation and cycloaddition of benzaldehyde, acetophenone and malononitrile. Compound **4** was further treated with chloroacetyl chloride to afford compound **5**. Finally, compound **5** was reacted with benzimidazole to afford the title compound **6**.The formation of all the synthesized compounds were ascertained by IR and $^{1}$HNMR spectral data.

**Table 1: Moldock scores and other energy calculations of compound 6 and imipramine by MVD-2013 against 2BXS**

| Compound | MolDock Score (Kcal/mol) | E- Inter (Protein-ligand) | H Bond No. | | Heavy Atoms count | LE1 | LE3 | Docking Score (Kcal/mol) | Rerank Score (Kcal/mol) |
|---|---|---|---|---|---|---|---|---|---|
| **Imipramine** | -112.101 | -134.749 | 1 | -1.94795 | 21 | -5.33816 | -4.26125 | -111.345 | -89.4863 |
| **6** | -183.47 | -206.105 | 4 | -7.39705 | 33 | -5.55969 | -3.82814 | -180.712 | -126.329 |

***In-vivo* activity:**

**Forced swim test:**

The antidepresant activity of compound **6** was carried by force swim test and the results are depicted in Figure 2. In this test the reference standard imipramine at a dose level of 10 mg/kg showed more significant decrease in the immobility time to 71.00±4.171 as compared to control (169.2±4.826). The synthesized pyridine derivative showed a significant decrease in the immobility time to 130.0±8.278 as compared to control.

**Locomotor activity:**

The locomotor activity of compound **6** was carried by using actophotometer and results are shown in Figure 3. The test results showed that the reference standard drug imipramine at a dose level of 20mg/kg produced increased in the locomotor activity (621.8±18.13) compared to control (307.3±12.71). The synthesized pyridine derivative showed a significant increase in the locomotor activity to 585.8±24.88 as compared to control.

## CONCLUSION:

Compound **6** exhibited better antidepressant potential and the docking study results are validated by *in-vivo* activity.

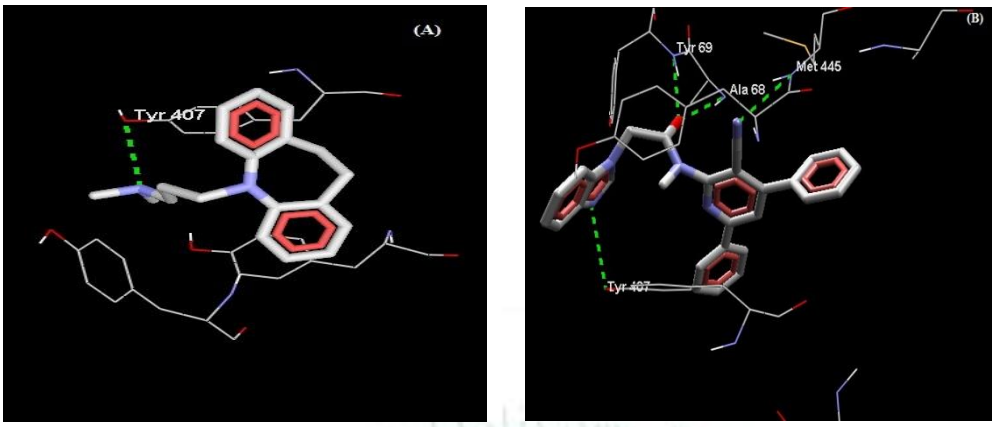
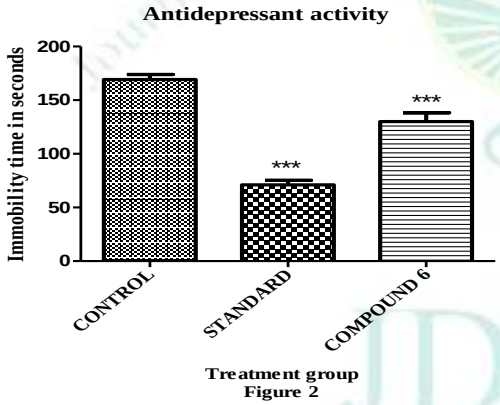



Figure 1. Showing the compounds docked in best of its conformation into the binding site of 2BXS (A) binding mode of imipramine forming 1H bond with Tyr407; (B) binding mode of compound **6** forming forming 4H bonds with Tyr407, Ala68, Tyr69 and Met445.



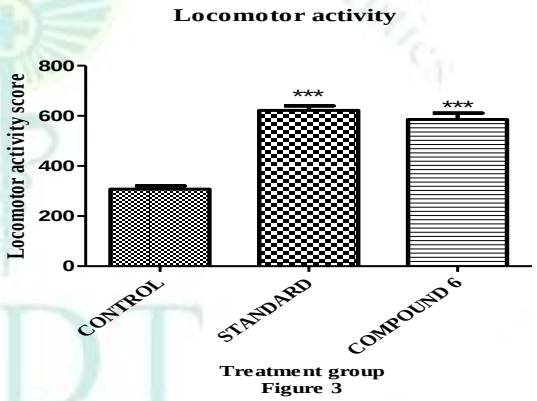




Figure 2 and 3: Values are expressed as mean±SEM. Number of animals=6. *** P<0.001 compared to respective vehicle treated control group. Results were analyzed by one-way ANOVA fallowed by Dunnett's multiple comparison test.

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