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# Synthesis, Characterization and In Vitro Anti-Oxidant Activity of Novel Piperazine Derivatives

**Shruti Saraf*[1], Virendra K. Sharma[1]**

[1]School of Pharmacy, LNCT University, Bhopal, 462042, Madhya Pradesh, India.

***Corresponding Author:**

Shruti Saraf

School of Pharmacy, LNCT University, Bhopal, 462042, Madhya Pradesh, India.

Email ID : shrutisaraf06@gmail.com

## ABSTRACT

The objective of this work was to conjugate naphthalene and piperazine nucleuses for designing and synthesis of new heterocyclic compounds. The compounds were also evaluated for their antioxidant action using DPPH and Phosphomolybdenum assay protocols. The synthesized compounds 6a-j were obtained in yield of 64-78% and were soluble in chloroform. the presence of carbon side chain on piperazine ring (1-4 carbon) improved the anti-oxidant potential (6b-6e) whereas increasing the chain length or presence of aromatic substituents reduced the antioxidant action. The results of the antioxidant activity study led us to conclude that linking the two nucleuses indeed proved beneficial for the activity as the compounds were able to inhibit the radical generation in significantly lower doses

***Keywords***: *Antioxidant, Naphthalene, Piperazine, Antidepressant, Tethering*

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## 1. INTRODUCTION

Depression is a mood disorder that causes a persistent feeling of sadness and loss of interest.[1] It can be classified as Disruptive mood dysregulation disorder; Major depressive disorder; Persistent depressive disorder (dysthymia); Premenstrual dysphoric disorder; and Depressive disorder due to another medical condition.[2] All these are characterized by ss, emptiness, or irritable mood, accompanied by somatic and cognitive changes that significantly affect the individual's capacity to function.[3] Depressive disorders affect 3.8% of world populations counting to about 280 million individuals with 5.0% adults and 5.7% adults older than 60 years of age.[4] At its worst, depression can lead to suicide and suicide accounts for about 7 lac deaths every year and is the fourth leading cause of deaths in people of 15-29 years of age.[4]

Antidepressant drugs used in therapy provide symptomatic relief and may occasionally cause abuse and dependency. Hence a continuous endeavor is required to have newer and more effective therapeutic agents that might act as antidepressant. Different causes or pathophysiology might underlie episodes in different patients, or even different episodes in the same patient at different times. Psychosocial stressors and biological stressors (eg, post-partum period) can result in different pathogenesis and respond preferentially to different interventions. Investigations have led to various hypothesis that might lead to depressive disorders. The monoamine hypothesis says that any mechanism that triggers lowering of these amines might cause depressive state. The Hypothalamic–pituitary–adrenal axis changes states that increased plasma cortisol &/or impaired glucocorticoid receptor-mediated feedback inhibition might lead to severe depression. Peripheral cytokine concentrations have also been linked to brain function, wellbeing, and cognition. Neuroplasticity and neurogenesis have also been found to play vital role in depression. A reduced neurogenesis has been linked to severe depressive state.

Heterocyclic rings have been at the center stage of drug research since decades and one such heterocycle viz. piperazine has been found to be an essential scaffold/substitution in several clinically approved antidepressants.[5-9] Fused cyclic nucleuses (either carbocyclic or heterocyclic) have also been an interesting feature in the antidepressant molecules (Figure 1).

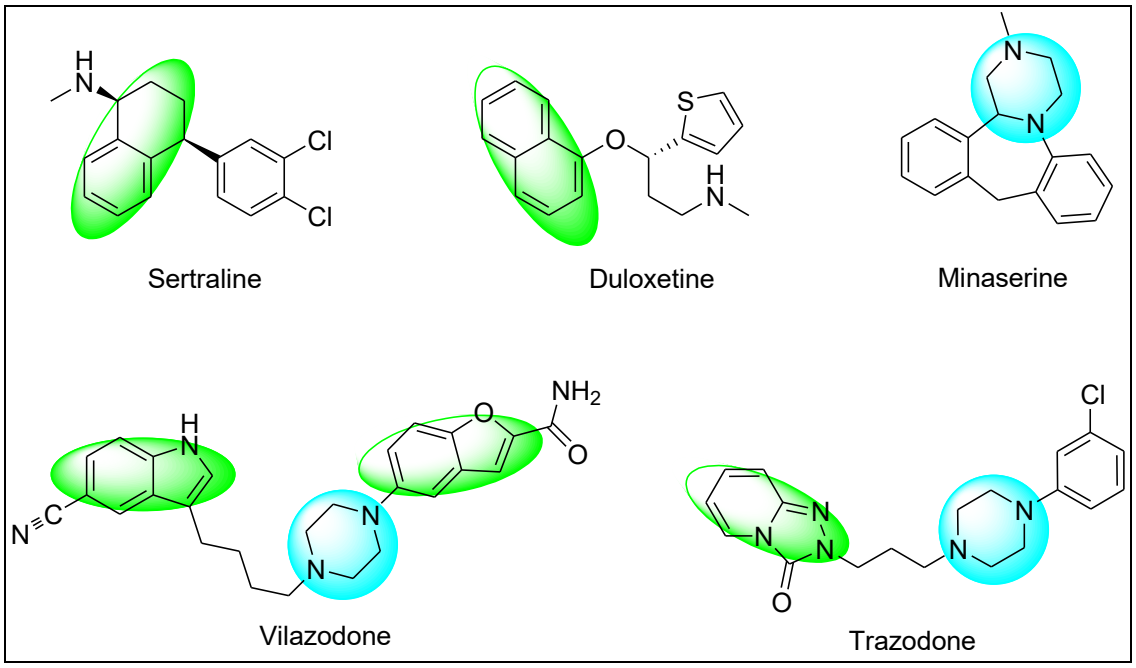



**Figure 1 Clinically used antidepressant with piperazine/fused rings**

Oxidative stress plays vital part in neurodegenerative disorders, diabetes, cancer and inflammation. Studies reveal that depression is associated with lower intake of antioxidants such as vitamins A, C, and E, selenium and zinc, and B vitamins (B6, folate, and B12).[10] Indeed, excessive ROS generation and lack of efficient antioxidant response trigger processes such as inflammation, neurodegeneration, tissue damage, and cell death.[11] Thus, oxidative stress is correlated with the pathogenesis and progression of depression. Hence it was envisioned to tether naphthalene and piperazine nucleuses as one compound and evaluate its congenors for antioxidant action which may be helfpul in comating depression (Figure 2).

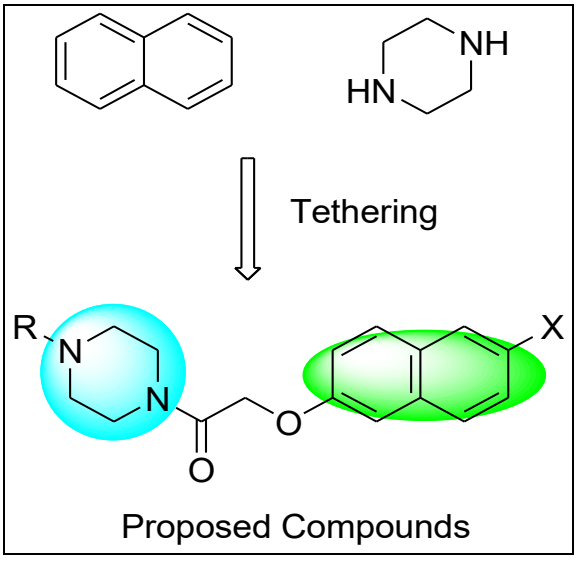
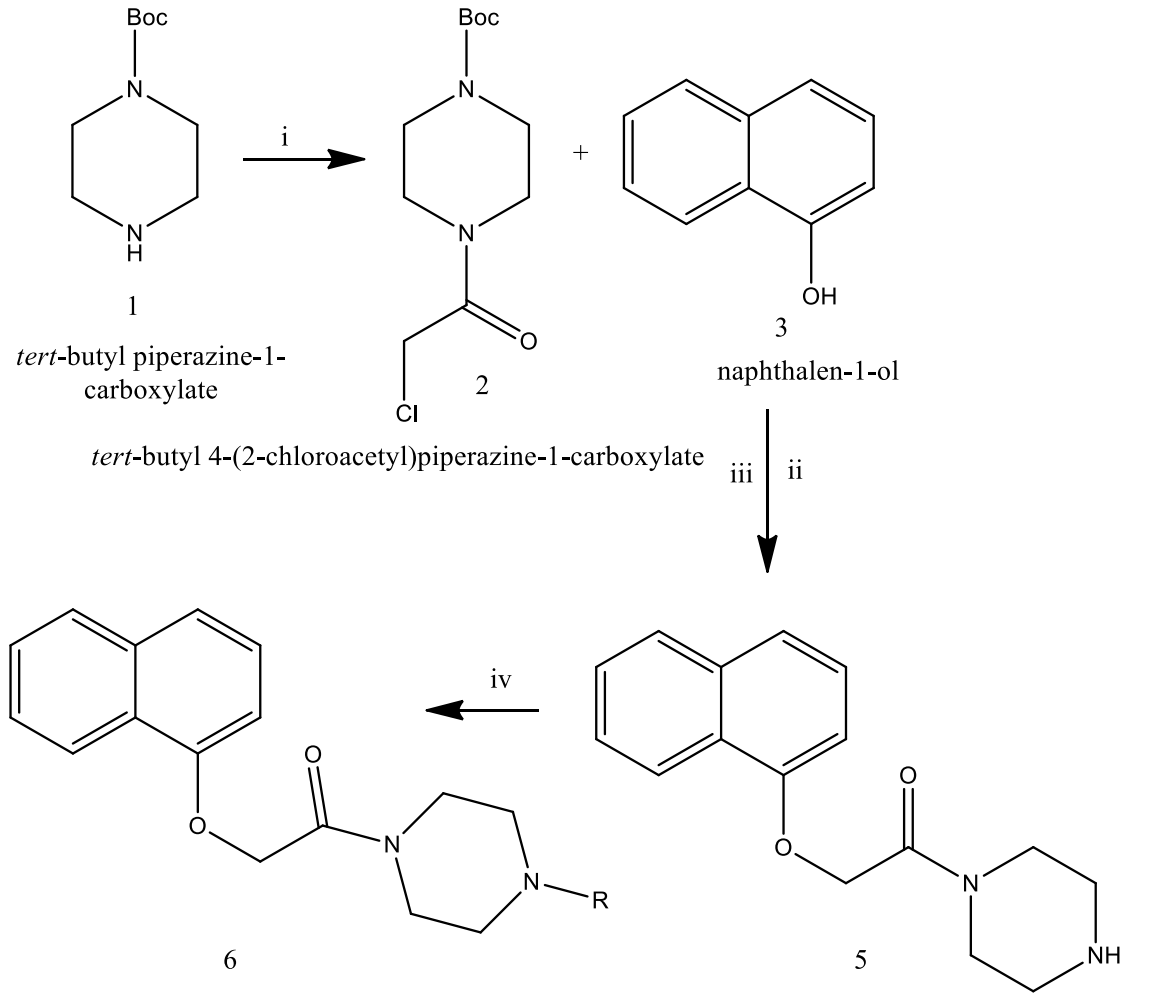



**Figure 2 Structure of the proposed compounds**

## 2. MATERIAL AND METHODS

The reaction was proceeded as per scheme 1.




**Scheme 1. Synthesis pathway for piperazine derivatives**

**Synthesis of tert-butyl 4-(2-chloroacetyl)piperazine-1-carboxylate, 2**

1-Boc piperazine (10.0 mmol) and potassium carbonate (12.0 mmol) were added to dichloromethane (30 mL), and stirred at room temperature for 0.5 h. The reaction mixture was cooled to 0°C using an ice bath and then chloroacetyl chloride (11.0 mmol) was added dropwise in order to maintain the temperature at 0–5°C. After the reaction was complete (TLC examination), the solvent was evaporated under reduced pressure, the residue was dissolved in water (30 mL), and filtered off (washed with water 3 times ×10 mL) under reduced pressure to give product 2 as a solid in 70–99% yield. The product was used in the next step without further purification.

**Synthesis of Tert-butyl 2-(naphthalen-1-yloxy)-1-(piperazin-1-yl)ethanone, 4**

1-naphthol 3 (10.0 mmol), piperazine-1-carboxylate 2 (11.0 mmol), potassium carbonate (11.0 mmol) and a catalytic amount of potassium iodide were added to acetone (30 mL). The resulting mixture was stirred under reflux at 80 ◦C for 25–30 h. After the reaction was complete (TLC examination), the solvent was evaporated under reduced pressure. The residue was dissolved in 30 mL water, filtered with suction, washed three times with water, and dried to obtain product 4. The product was used in the next step without further purification.

**Synthesis of 2-(naphthalen-1-yloxy)-1-(piperazin-1-yl)ethanone, 5**

Tert-butyl 2-(naphthalen-1-yloxy)-1-(piperazin-1-yl)ethanone 4 (10.0 mmol) and trifluoroacetic acid (13.4 mmol) were added to dichloromethane (30 mL) and the reaction was stirred at room temperature for 1 h. TLC examination indicated the reaction was complete. The excess dichloromethane and trifluoroacetic acid were evaporated under vacuo to obtain product 5. The product was used in the next step without further purification.

**General method for synthesis of 4-piperazine substituted derivatives, 6a-j**

2-(naphthalen-1-yloxy)-1-(piperazin-1-yl)ethanone 5 (10.0 mmol), potassium carbonate (11.0 mmol) and a catalytic amount of potassium iodide were added to acetonitrile (30 mL) and the reaction mixture was refluxed for 0.5 h. The appropriate substituted alkyl (10.0 mmol) halide was then added and refluxing was continued for 24 h. After the reaction was complete as indicated by TLC examination, the solvent was evaporated under reduced pressure to give a residue, to which water (30 mL) was added. The resulting suspension was extracted with dichloromethane (3 × 10 mL) and the combined solvents dried by $MgSO_4$. The drying agent was filtered off and the solvent was removed under reduced pressure to give the appropriate crude products 6a–u which were purified using column chromatography (methanol:dichloromethane, 1:50) to give the appropriate target compounds 6a–j.

*2-(naphthalen-1-yloxy)-1-(piperazin-1-yl)ethanone, 6a*

Yield: 78%; Melting point (°C): 155; FTIR ($cm^{-1}$): 3619.87 (N-H Stretch), 2888.32 (Ar C-C Stretch), 1739.85 (C=O Stretch), and 1426.28 (C-N Stretch); proton NMR (δ, ppm): 7.04-8.12 Aromatic protons, 4.72 ether proton, 2.92-3.52 piperazine H and 2.14 amine proton; m/z: 272.3 (M+2)

*1-(4-methylpiperazin-1-yl)-2-(naphthalen-1-yloxy)ethanone, 6b*

Yield: 68%; Melting point (°C): 125; FTIR ($cm^{-1}$): 2985.13 (Ar C-C Stretch), 1740.14 (C=O Stretch), and 1426.07 (C-N Stretch); proton NMR (δ, ppm): 7.04-8.12 Aromatic protons, 4.72 ether proton, 2.87-3.49 piperazine H and 2.50 methyl proton; m/z: 284.5 (M+)

*1-(4-ethylpiperazin-1-yl)-2-(naphthalen-1-yloxy)ethanone, 6c*

Yield: 74%; Melting point (°C): 123; FTIR ($cm^{-1}$): 2886.60 (Ar C-C Stretch), 1740.74 (C=O Stretch), and 14256.88 (C-N Stretch); proton NMR (δ, ppm): 7.04-8.12 Aromatic protons, 4.72 ether proton, 2.65-3.44 piperazine H, 1.05 methyl proton and 2.52 methylene proton; m/z: 299.6 (M+1)

*2-(naphthalen-1-yloxy)-1-(4-propylpiperazin-1-yl)ethanone, 6d*

Yield: 71%; Melting point (°C): 121; FTIR ($cm^{-1}$): 2975.19 (Ar C-C Stretch), 1737.45 (C=O Stretch), and 1427.18 (C-N Stretch); proton NMR (δ, ppm): 7.04-8.12 Aromatic protons, 4.72 ether proton, 2.68-3.44 piperazine H, 2.43, 1.57 methylene proton and 0.89 methyl proton; m/z: 314.5 (M+2)

*1-(4-butylpiperazin-1-yl)-2-(naphthalen-1-yloxy)ethanone, 6e*

Yield: 67%; Melting point (°C): 122; FTIR ($cm^{-1}$): 2983.71 (Ar C-C Stretch), 1740.21 (C=O Stretch), and 1426.22 (C-N Stretch); proton NMR (δ, ppm): 7.04-8.12 Aromatic protons, 4.72 ether proton, 2.68-3.44 piperazine H, 2.419, 1.47, 1.31 methylene proton and 0.92 methyl proton; m/z: 326.7 (M+)

*2-(naphthalen-1-yloxy)-1-(4-pentylpiperazin-1-yl)ethanone, 6f*

Yield: 72%; Melting point (°C): 115; FTIR ($cm^{-1}$): 2875.08 (Ar C-C Stretch), 1739.58 (C=O Stretch), and 1426.26 (C-N Stretch); proton NMR (δ, ppm): 7.04-8.12 Aromatic protons, 4.72 ether proton, 2.68-3.44 piperazine H, 2.44, 1.49, 1.31 methylene proton and 0.89 methyl proton; m/z: 341.5 (M+1)

*2-(naphthalen-1-yloxy)-1-(4-phenylpiperazin-1-yl)ethanone, 6g*

Yield: 70%; Melting point (°C): 153; FTIR ($cm^{-1}$): 2985.37 (Ar C-C Stretch), 1740.57 (C=O Stretch), and 1425.98 (C-N Stretch); proton NMR (δ, ppm): 6.92-8.12 Aromatic protons, 4.73 ether proton, and 3.29-3.44 piperazine H; m/z: 346.7 (M+)

*2-(naphthalen-1-yloxy)-1-(4-p-tolylpiperazin-1-yl)ethanone, 6h*

Yield: 75%; Melting point (°C): 158; FTIR ($cm^{-1}$): 2879.07 (Ar C-C Stretch), 1740.63 (C=O Stretch), and 1426.04 (C-N Stretch); proton NMR (δ, ppm): 6.91-8.12 Aromatic protons, 4.73 ether proton, and 3.29-3.44 piperazine H and 2.65 methyl proton; m/z: 361.6 (M+1)

*1-(4-benzylpiperazin-1-yl)-2-(naphthalen-1-yloxy)ethanone, 6i*

Yield: 72%; Melting point (°C): 155; FTIR ($cm^{-1}$): 2983.80 (Ar C-C Stretch), 1740.09 (C=O Stretch), and 1426.00 (C-N Stretch); proton NMR (δ, ppm): 7.04-8.12 Aromatic protons, 4.72 ether proton, and 2.67-3.46 piperazine H and 3.66 benzyl methylene proton; m/z: 361.5 (M+1)

*1-(4-(4-ethylphenyl)piperazin-1-yl)-2-(naphthalen-1-yloxy)ethanone, 6j*

Yield: 69%; Melting point (°C): 160; FTIR ($cm^{-1}$): 2873.77 (Ar C-C Stretch), 1740.37 (C=O Stretch), and 1426.04 (C-N Stretch); proton NMR (δ, ppm): 6.69-8.12 Aromatic protons, 4.73 ether proton, 3.29-3.44 piperazine H, 2.69 methylene proton and 1.22 methyl proton; m/z: 376.6 (M+2)

**Antioxidant activity**

*DPPH Scavenging Assay*

The antioxidant action of the synthesized compounds was determined using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay [21].

The free radical scavenging activity of the synthesized molecules was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH. The test samples (100 $\mu$L, 10-100 µg/mL) were prepared in DMSO and were mixed with 1.0 mL of DPPH solution and filled up with methanol to a final volume of 4 mL. Absorbance of the resulting solution was measured at 517 nm in a visible spectrophotometer. Ascorbic acid was used as the reference compound. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the following formula:

$$\% \, inhibition = \frac{(Ao - At)}{Ao} \, X \, 100$$

where $Ao$ is the absorbance of the control (blank, without sample) and $At$ is the absorbance in the presence of the test samples. All tests were performed in triplicate and the results were expressed as mean values ± standard deviations.

*Hydroxy radical scavenging activity*

Various concentrations (100 µL) of test solutions (10-100 µg/mL) were taken and 1 mL of iron EDTA solution, 0.5mL of EDTA solution, 1 mL of DMSO and 0.5mL of ascorbic acid were added to it. The mixture was incubated in a boiling water bath at 80 to 90°C for 15 min. After incubation, 1 mL of ice cold TCA and 3mL of Nash reagent were added and the reaction mixture was incubated at room temperature for 15 min. The absorbance was read at 412 nm [22]. The % hydroxyl radical scavenging activity is calculated by the following formula

$$\% \, HRSA = \frac{Abs \, control - Abs \, sample}{Abs \, control} \times 100$$

Where, HRSA is the Hydroxyl Radical Scavenging Activity, Abs control is the absorbance of control and Abs sample is the absorbance of the test solution.

## 3. RESULTS AND DISCUSSION

### Chemical Characterization

The synthesis of the target compounds was achieved by first synthesizing 1-substituted piperazine nucleus, followed by the substitution of the naphthalene nucleus to it, deprotecting the 4-N positions and eventually substitution of the desired groups on the 4-position. The synthesized compounds 6a-j were obtained in yield of 64-78% and were soluble in chloroform and exhibited the stretching of carbonyl (C=O), aromatic C-H, C-C, C=C, and C-N confirming the presence of all the expected functional groups in the molecules. The $^{1}$HNMR spectra revealed the presence of protons of naphthalene ring (8-9 ppm), aromatic ring (6.6-7.8 ppm), piperazine (5-6 ppm) and methylene (5-6 ppm).

### DPPH Scavenging

The anti-oxidant ability of the synthesized compounds was assessed using their ability to scavenge DPPH free radical and the hydroxy radical. It was found that the presence of carbon side chain on piperazine ring (1-4 carbon) improved the anti-oxidant potential (6b-6e) whereas increasing the chain length or presence of aromatic subsitutents reduced the antioxidant action. It was also seen that molecules having a substituted benzene side chain were more potent than unsubstituted ones (Table 3).

**Table 3. $IC_{50}$ of DPPH radical and hydroxy radical scavenging action of 6a-j**

| Sampe | $IC_{50}$ | |
|---|---|---|
| | DPPH | HRSA |
| 6a | 48.83 ± 0.369 | 49.71 ± 0.360 |
| 6b | 46.01 ± 0.852 | 48.73 ± 0.341 |
| 6c | 40.84 ± 0.719 | 44.01 ± 0.752 |
| 6d | 39.77 ± 0.662 | 38.40 ± 0.186 |
| 6e | 28.19 ± 0.255 | 30.15 ± 0.211 |

| | | |
|---|---|---|
| 6f | 48.54 ± 0.456 | 49.32 ± 0.732 |
| 6g | 56.35 ± 0.398 | 57.17 ± 0.318 |
| 6h | 51.47 ± 0.341 | 53.29 ± 0.255 |
| 6i | 58.27 ± 0.386 | 55.81 ± 0.456 |
| 6j | 52.84 ± 0.350 | 54.58 ± 0.350 |
| Ascorbic Acid | 18.20 ± 0.033 | 17.04 ± 0.651 |

## 4. CONCLUSION

The objective of this work was to synthesize naphthalene tethered piperazine derivatives for improved anti-oxidant function. The compounds could be synthesized in four distinct steps in sufficient yield and purity. The results of the antioxidant activity study led us to conclude that linking the two nucleuses indeed proved beneficial for the activity as the compounds were able to inhibit the radical generation in significantly lower doses. Further molecular modelling studies are warranted to establish the plausible mechanisms that might be involved in the anti-oxidant action of the compounds.

## 5. ACKNOWLEDGEMENTS

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