RESEARCH PAPER

In Vitro Screening of Inhibition of Mono Amino Oxidase Activity of Some Novel Naphthyloxy Piperazine Derivatives

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Abstract

This study describes the biological evaluation of a new series of naphthyloxy piperazine derivatives (6a–j) designed by conjugating naphthalene and piperazine scaffolds on monoamine oxidase (MAO). The compounds with potent antioxidant activity were screened for *in vitro* MAO inhibition assays employing DNPH spectrophotometry. The results demonstrated that short aliphatic substitutions (ethyl, butyl) on the piperazine ring enhanced both antioxidant potential and MAO inhibitory activity, whereas aromatic substituents reduced efficacy. Compounds 6e emerged as the most potent inhibitors, with IC_{50} values of 0.23 µM for MAO-A and 0.35 µM for MAO-B, respectively. These findings suggest that structural tethering of naphthalene and piperazine nuclei yields multifunctional molecules with dual antioxidant and MAO inhibitory properties, supporting their potential as lead candidates for antidepressant drug development.

Keywords: Naphthyloxy piperazine, depression, monoamine oxidase, DNPH assay, antioxidant

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Introduction

Depression has been reported to be the fourth global burden of disease, with nearly 12% of the global disability-adjusted life years [1]. It is a life-threatening disease which is characteristically different from normal mood swing and short-lived emotional responses to challenges in everyday life. Approximately 280 million people in the world have depression, 5.02% of which comes under the age group of 20 plus years and 5.71% of which comes under the age group of 60–89 years [2].

Monoamine oxidases (MAO), enzymes containing covalently bound flavin adenine dinucleotide (FAD) as the cofactor, are located on the mitochondrial outer membrane where they oxidize various physiologically and pathologically important monoamines. These neurotransmitters and hormones include serotonin, noradrenaline and dopamine that function to regulate movement, emotion, reward, cognition, memory and learning [3-5].

The two isoforms of MAO (MAO-A and MAO-B) are characterized by different affinities for inhibitors and different specificities for substrates [6,7]. MAO-A preferentially metabolizes serotonin, adrenaline, and noradrenaline [8], whereas 2-phenylethylamine and benzylamine are predominantly metabolized by MAO-B

[9]. Tyramine and dopamine are common substrates for both isoenzymes [10]. The therapeutic interest in monoamine inhibitors (MAOIs) covers two major categories: MAO-A inhibitors are used mostly in the treatment of mental disorders, in particular depression and anxiety [11-13], whereas MAO-B inhibitors are used in the treatment of Parkinson's disease and are of interest against Alzheimer's disease [14,15].

Oxidative damage is a consequence of the oxygen dependence of cellular metabolism. The presence of oxygen in the internal media is, on the one hand, crucial for survival; on the other hand, it is a menace producing oxidative damage through the generation of free radical species, which have to be counteracted by the presence of potent enzymatic and non-enzymatic antioxidants. In patients of major depressive disorders, the oxidative stress markers are elevated, and higher baseline levels of F2-isoprostanes, a marker of oxidative stress, is related to a poorer response to antidepressant treatment [16]. On the other hand, patients who respond to the treatments showed reductions in the oxidative markers [16,17].

In our previous study we reported the synthesis and antioxidant activities of ten novel naphtyloxy piperazine derivatives (6a-j) [18] with IC_{50} values ranging between 20-60 µg/mL. In this study, these potent antioxidant

piperazine derivatives were screened for their probable anti-depressant activity by inhibition MAO activity in *in vitro* assay.

Material and Methods

MAO was isolated from brain tissue homogenate; 5-hydroxytryptamine hydrochloride (5-HT) was purchased from Sigma Aldrich; benzylamine and dinitrophenyl hydrazine (DNPH) were purchased from Loba Chemie; all other reagents and chemicals were purchased Central Drug House and Oxford Fine Chemicals and used as procured.

Isolation and preparation of MAO sample

MAO was isolated using brain tissues of chicken. The dissected pieces of the brain tissues were washed with 0.3 M sucrose solution and frozen at − 80 °C for further analysis. The brain tissue (2.5 g) was homogenized in 1:40 (w/v) ratio with 0.3 M ice-cold sucrose solution and centrifuged at 1824 g for 10 min. The supernatant was collected and further centrifuged at 12,768 g for 35 min to obtain crude MAO protein precipitations. This precipitate was resuspended in 250 ml of 0.3 M sucrose solution and mixed with 20 ml of 1.2 M sucrose solution. The precipitate was again centrifuged with 1.2 M sucrose solution at 12,687 g for 40 min followed by a single wash with potassium phosphate buffer (pH 7.60, 100 mM). The pure brain MAO protein precipitate was suspended in 10 ml of potassium phosphate buffer; and stored in aliquots of 1 ml at − 80 °C for subsequent analysis [19,20].

Determination of protein concentration

The protein concentration of MAO precipitate was calculated using Hartree Lowry method [21-24]. Serial dilutions of concentrations 0.03 to 0.15 mg/ml were prepared from the stock solution of 0.3 mg/ml bovine serum albumin (BSA) in potassium phosphate buffer. 1.0 ml of each dilution of standard, protein-containing test and buffer for reference were mixed with 0.90 ml of reagent A (2 g sodium potassium tartrate, 100 g sodium carbonate, 500 ml 1N NaOH, and water to one liter) in separate test tubes. The tubes were incubated for 10 min in water bath at 50 °C, then cooled to room temperature. 0.1 ml of reagent B (2 g sodium potassium tartrate, 1 g copper sulfate, 90 ml H_2O and 10 ml 1N NaOH) was added to each test tube, mixed and incubated for 10 min at room temperature. 3 ml of reagent C (1 vol of Folin–Ciocalteau reagent diluted with 15 vols of water) was added rapidly to each test tube, mixed and again incubated for 10 min in water bath at 50 °C and cooled to room temperature. The final assay volume was 5 ml. Absorbance was measured at 650 nm.

DNPH spectrophotometry

Potassium phosphate buffer (pH 7.60, 25 mM, 200 µL, control) or 200 µL of various concentrations of the piperazine derivatives (25, 50, 100, 200 and 400 µg/mL, test) and 200 µL of MAO protein homogenates were mixed and incubated for 20 min at 37 °C. Then 200 ml of 0.016 M benzylamine in buffer (for detecting MAO-B) and 150 ml of 0.02 M 5-HT (for detecting MAO-A) were added to the above mixture and incubated for 60 min. After this 400 ml of 2 M DNPH in 1 M HCl was added. After incubation for 40 min at room temperature, 2 ml of 1.25 M NaOH containing 5 g/L of Triton X-100 was added and the reaction mixture was kept for an additional 30 min at room temperature. Finally, absorption was measured at 465 nm for MAO-B and 425 nm for MAO-A. The inhibition of MAO activity was calculated [25].

Results and Discussion

In order to determine the concentration of protein in the MAO protein precipitate, calibration curve of BSA was used (Figure 1). The concentration of protein the precipitate was found to be 169.59 ± 0.631 µg/mL.

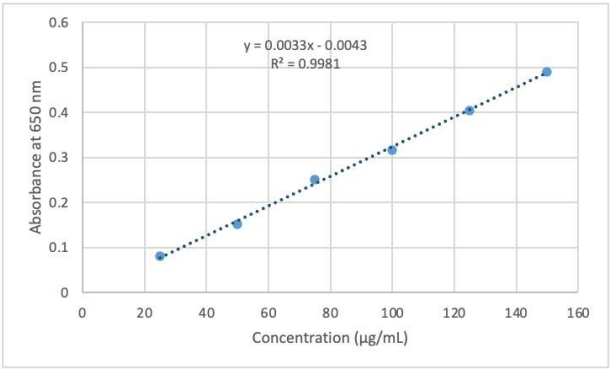

Figure 1. Standard Curve of BSA

The DNPH (2,4-dinitrophenylhydrazine) assay for MAO inhibition detects inhibitors by measuring the aldehydes produced when MAO oxidizes amine substrates (such as benzylamine or serotonin) into hydrazones. These hydrazones are then measured spectrophotometrically, often after a NaOH treatment to create quinones with strong absorbance, indicating enzyme activity. Therefore, a decrease in color with an inhibitor shows its efficacy. Quantifying the MAO-dependent product production is a simple, dependable, and sensitive way to screen MAO inhibitors [25].

From the absorbances in presence of test samples and control, the percent inhibition of MAO-A and MAO-B was calculated using the equation % inhibition = [{(A_0-

$A_1)/A_0\}$ × 100], where, A_0 = Absorbance of control and A_1 = Absorbance of test sample [22]. The percent inhibition of MAO-A and MAO-B are presented in Figure 2 and 3 respectively.

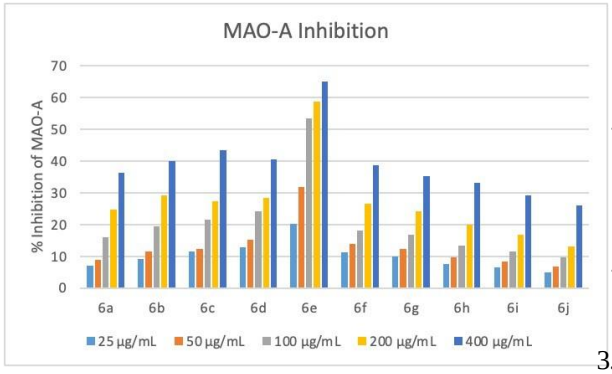

Figure 2. % Inhibition of MAO-A by naphthyloxy piperazine derivatives

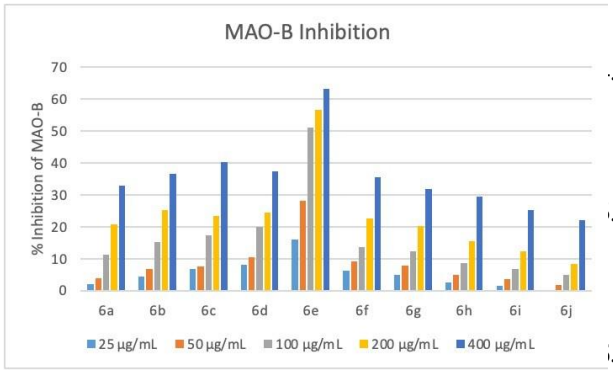

Figure 3. % Inhibition of MAO-A by naphthyloxy piperazine derivatives

The IC_{50} values compounds 6a-j ranged from 0.23 µM to 268.20 µM for MAO-A inhibition and 0.35 µM to 2148.58 µM for MAO-B inhibition. The most potent compounds 6e and 6c were having butyl and ethyl substitutions on the piperazine ring.

Conclusion

The conjugation of naphthalene and piperazine moieties produced a novel class of heterocyclic derivatives with significant antioxidant and MAO inhibitory activity. Structure–activity relationship analysis revealed that short aliphatic substitutions on the piperazine ring were optimal for enhancing biological activity, while longer chains or aromatic groups diminished potency. Among the synthesized compounds, 6e and 6c demonstrated the strongest inhibition of MAO-A and MAO-B, highlighting their promise as dual-acting agents capable of reducing oxidative stress and modulating monoaminergic neurotransmission. These results provide a strong foundation for further pharmacological evaluation and *in vivo* studies to establish their therapeutic potential as antidepressant leads.

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