Chapter 2

Synthesis, Characterization, Antimicrobial, Antidepressant and Antioxidant Activity of Novel Piperamides Bearing Piperidine and Piperazine analogues
INTRODUCTION

Depression is a common psychiatric disorder, often refractive to drug treatment, affecting quality of life and overall productivity [1]. It is characterized by anhedonia or the loss of interest or pleasure in normal daily activities and feelings of sadness [2]. Tricyclics, monoamine oxidase (MAO) inhibitors, selective serotonin re-uptake inhibitors, selective noradrenaline re-uptake inhibitors, serotonin modulators and norepinephrine serotonin modulators are the major antidepressant drugs which are used for the treatment of depressive disorders [3]. Several classes of antidepressants are currently being used due to clinical limitations and adverse effects. In addition, significant proportion of these patients will not be responding to treatment, or will show only partial response. Thus, there is a need for development of efficient and safer drugs, in order to limit the impact of depression on patient’s lives [4, 5].

In recent years, there has been an increased interest in the application of antioxidants to medical treatment as information is constantly gathered linking the development of human diseases to oxidative stress. Recent evidence [6] suggests that free radicals, which are generated in many bioorganic redox processes, may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease, including cancer, arthritis, hemorrhagic shock, autoimmune, inflammatory, cardiovascular, neurodegenerative diseases and age-related degenerative brain diseases [7]. Owing to these facts, synthetic and natural compounds with potential antioxidant activity are receiving increased attention in biological research, medicine and pharmacy [8].

Natural products are always special sources for the discovery of potential drugs with novel structures and varying biological activity. The species of the Piper genus family have shown a great diversity of secondary metabolites. Piperine is a major ingredient of piper species, which have been reported to possess antipyretic, analgesic, insecticidal, anti-inflammatory [9, 10] immune-modulatory, antitumor [11] and antidepressant activities [12]. Antiepilepsirine one derivative of piperine, has been used clinically as an effective anticonvulsant, exhibiting a more potent effect than piperine. Some piperamides were reported as potential antidepressant and
anti-trypanosomal agents. Some researchers have reported MAO inhibitory activity for various piperamide derivatives [13, 14].

**LITERATURE REVIEW**

The Piperamides are abundant in the genus piper and have great ecological and economic importance. Amides are the most common constituents of piper species. Piperamide compounds are neutral (to weakly acidic) as opposed to basic (as in the case of alkaloids). The majority of these compounds are composed of an acid forming an amide where the nitrogen is in a five or a six membered ring or on an isobutyl chain.

Inatani *et al.* [15] have reported the isolation of new phenolic amides, *N*-trans-fomloyl piperidine (1), 7V-5-(4-hydroxy-3-methoxyphenyl)-2E,4E-pentadiQiioy piperidine (2) and *N*-5-(4-hydroxy-3-methoxyphenyl)-2is-pentenoyl piperidine (3) from the fruit of *Piper nigrum* L. the structure determination of each compound was examined by spectroscopic analysis.

![Chemical structures of isolated compounds](image)

Recently, Lian Ee *et al.* [16] identified the pellitorine (4), from the roots of *Piper nigrum* together with known piperamides, piperine (5) and *(E)-1-[3',4'- (methylenedioxy)cinnamoyl]piperidine (6). The pellitorine showed strong cytotoxic activities against HL60 and MCT-7 cell lines. Microbial transformation of piperine (5) gave a new compound 5-[3,4-(methylenedioxy)phenyl]-pent-2-ene piperidine (7). These compounds were isolated using chromatographic methods and their structures were elucidated using MS, IR and NMR techniques.
Wei et al. [17] have reported the new amide alkaloids, named threo-N-isobutyl-4,5-dihydroxy-2E-octanamide (8), threo-1-(1-oxo-4,5-dihydroxy-2E-decaenyl)piperidine (9), erythro-1-(1-oxo-4,5-dihydroxy-2E-decaenyl)piperidine (10), 1-(1,6-dioxo-2E,4E-decadienyl)piperidine (11), 1-[1-oxo-3(3,4-methylene dioxy-5-methoxyphenyl)-2Z-propenyl]piperidine (12) and 1-[1-oxo-5(3,4-methylene dioxyphenyl)-2Z,4E-pentadienyl]pyrrolidine (13), from the roots of *Piper nigrum*. Their structures were elucidated on the basis of spectroscopic analysis and chemical evidence.

Synthetic studies involving amides found in Piper species began in the late 19th century. The earliest syntheses were designed to confirm the structures of isolated compounds. The first synthesis of piperine (5) was described in 1882 [18]
and involved the condensation of piperidine with piperinic acid chloride that had been obtained via hydrolytic digestion of isolated piperine. The total synthesis of piperine was not described until 12 years, later in 1894, Ladenburg and Scholtz [19] established the structure of piperine by total synthesis of piperinic acid. By condensation of piperonal with acetaldehyde, followed by treatment with sodium acetate and acetic anhydride, piperinic acid was obtained. Thus, the structure of piperine was established as piperinic acid piperidide. Other examples of Piperamide syntheses described within the context of structural identification include the synthesis of piperettine, a second component of piperine extracts from *P. nigrum* [20].

The synthesis was described in the literature generally involves very well understood chemistry and share several synthetic features. Typically, the amide linkages are generated via the condensation of an appropriate amine with an acyl halide generated from the corresponding carboxylic acid.

![Chemical structure](image)

The typical introduction of unsaturation into the carbon framework of the acid moiety, the use of phosphonium salts or phosphonate esters in Wittig-type chemistry appears to have been the method of choice in the synthesis of piper amides.

![Chemical structure](image)

The first stereoselective total synthesis of new natural amide alkaloids (8-10) were achieved by Srinivas *et al.* [21] from commercially available starting materials. Wittig olefination, sharpless asymmetric dihydroxylation, epoxidation, a trans regioselective opening of 2,3-epoxy alcohol, Horner–Wadsworth–Emmons (HWE) olefination and amide coupling are the key steps involved in the synthesis. The synthesized compounds are new and evaluated for their *in vitro* anticancer activity.
against colon (HT-29), breast (MCF-7) and lung (A-549) human cancer cell lines. Compound 8 exhibits activity against colon and lung cancer cell line, compound 9 showed activities against colon cancer cell line and compound 10 exhibits activity against lung cancer cell line.

Araujo-Jhior et al. [22] have described a new synthetic route for the preparation of piperamides and analogues (14-16) using an efficient precursor, the Brazilian natural product safrole. The synthesis of the amides as carried out using a safrole was submitted to regioselective hydroboration-oxidation sequence, furnishing the primary alcohol. Treatment of the primary alcohol with pyridinium chlorochromate, in methylene chloride furnished aldehyde. Using the Homer-Wadsworth-Emmons modification of the Wittig reaction the olefination product was formed and the mild hydrolysis aqueous LiOH solution furnished the acid. Finally, treated with thionyl chloride and subsequent addition of the respective amine furnish amides. The target compounds were fully characterized by $^1$H and $^{13}$C NMR studies.
Histone deacetylases (HDACs) play an important role in the promotion of angiogenesis, the regulation of gene expression, oncogenic transformation and cellular differentiation [23]. Thus, HDAC inhibition has generated considerable interest as a potential cancer treatment. Luo et al. [24] have demonstrated a series of piperamide analogues (17-19) synthesis and evaluated for inhibitory activity against histone deacetylases, as well as the cytotoxicity against HCT-116 human colon cancer cell line. Preliminary SAR data reveals that compounds with a hydroxamic acid moiety and a long carbon chain will exhibit good HDAC inhibitory activity and cytotoxicity.
Srinivas et al. [25] have performed a new method of synthesis of piperamides (20). The piper amides were subjected for antibacterial assay against both gram positive and gram negative bacterial strains using minimum inhibitory concentration method. The antibacterial activity was proved to be active against *Staphylococcus aureus* and *Chromobacterium violaceum* strains.

Koul and coworkers [26] have described the structure-activity relationship of piperine and its synthetic analogues (21) for their inhibitory potentials of pat hepatic microsomal constitutive and inducible cytochrome P450 (CYP) activities. Thirty eight novel piperamide synthetic analogues were made and relate various modifications in the parent molecule to the inhibition of CYP activities. Two types of monooxygenase reactions arylhydrocarbon hydroxylase (AHH) and 7-methoxycoumarin- O-demethylase (MOCD) have been studied. Modifications were introduced into the piperine molecule: (i) in the phenyl nucleus, (ii) in the side chain and (iii) in the basic moiety. All the compounds have been subjected to such studies and simultaneously an attempt has also been made to arrive at the structure-activity relationship of synthetic analogues.
Piperlonguminine (22) is an efficient inhibitor of melanocyte stimulating hormone. A simple and practical method for the synthesis of an alkaloid piperlonguminine was reported by Lee and his coworkers [27]. It was established by employing Wadsworth-Horner modified Wittig condensation and piperonal with the anion generated from ethyl \((E)-4\text{-diethylphosphono-2-butenoate}\) as a key step. The ester was hydrolyzed to corresponding acid, which was condensed with isobutylamine in presence of boric acid to yield piperlonguminine.

\[
\text{Furan} + \text{Ethyl Acetate} \rightarrow \text{Furan Acid} \rightarrow \text{Imine}
\]

Boll \textit{et al.} [28] have demonstrated the synthesis and molecular structure of piplartine (23). The structure of piplartine was established as \((E)-N-3',4',5',6'\text{-trimethoxycinnamoyl-5,6-dihydro-2(IH)-pyridone}\) by synthesis and by an X-ray crystallographic analysis. The name piperlongumine has also been used for piplartine.

\[
\text{Ammonia} \rightarrow \text{Imine} + \text{Furan}
\]

Ujwal Kumar and coworkers [29] reported the synthesis of a series of piplartine derivatives (24). The synthesized compounds were evaluated for anticancer and antibacterial activities on cell cycle regulation and growth inhibition. The compounds were synthesized \textit{via} Baylis–Hillman reaction. The cytotoxicity of these compounds was examined in two different human tumor cell lines, IMR-32 and HeLa. The antibacterial activity was examined against \textit{Staphylococcus aureus} and
*Pseudomonas aeruginosa*. The rationale to study both anticancer and antibacterial activities with two different cells and bacteria was to examine the unique feature of piplartine for cytostatic property.

Recently, Subba Rao *et al.* [30] have reported the synthesis of piperine-amino acid ester conjugates (25) and study of their cytotoxic activities against human cancer cell lines. The piperine-amino acid ester conjugates were synthesized under mild conditions and cytotoxic studies was carried out against a panel of human cancer cell lines (IMR-32, MCF-7, PC-3, DU-145, Colo-205, and Hep-2). The results indicated that the introduction of D- and L-amino acid side chain to piperine through peptide linkage significantly increased cytotoxic activity.

Araujo-Junior *et al.* [31] have described the synthesis of natural amide alkaloid piperardine (26), \((E,E)-1-[7-(1,3-benzodioxol-5-yl)-1-oxo-2,4-heptadienyl]-piperidine\) (27), which was isolated from the stem of *Piper tuberculatum*. The synthesis was done using safrole, an abundant Brazilian natural product occurring in sassafras oil, as starting material.
The natural product 1-[1-oxo-3(3,4-methylenedioxy-5-methoxyphenyl)-2Z-propenyl] piperidine, isolated from the roots of the *Piper nigrum* [17]. By the reports to our knowledge, no data are available on the synthesis and biological studies about the compound. The main aim of this work was to synthesize the trans-isomer of this compound and its analogues, and to evaluate the antimicrobial, antioxidant and antidepressant activity of synthesized compounds. A recent paper reported that Z-configuration is critical for the biological activity [32]; therefore, in this study the author has design the synthesis of trans-isomer of this compound and its analogues, and to investigate their various biological activities.

**EXPERIMENTAL**

**Materials and methods**

All the chemicals and solvents used were of AR grade. Solvents were used as supplied by commercial sources without any further purification. Elemental analysis (C, H, N) was determined using a Carlo-Erba 1160 elemental analyser. IR spectra were recorded on a JASCO FTIR-8400 spectrophotometer using Nujol mulls. The $^1$H NMR spectra were recorded on Bruker Advance 400 MHz NMR instrument using TMS as internal standard and DMSO-$d_6$ as solvent. Chemical shift are given in part per million (δ-scale). Mass spectra were recorded on Perkin–Elmer LC-MS PE Sciex API/65 Spectrophotometer. Melting points were determined with a Buchi 530 melting point apparatus in open capillaries and are uncorrected. Compound purity was checked by thin layer chromatography (TLC) on precoated silica gel plates (Merck, Kieselgel 60 F254, layer thickness 0.25 mm).
Synthesis

Preparation of (E)-3-(benzo[d][1,3]dioxol-5-yl)acrylic acid (3)

To a solution of piperonal 1 (6.0 g, 40 mmol) in pyridine, piperidine and malonic acid 2 (4.99 g, 48 mmol) were added and the contents were stirred for 24 h, followed by heating on water bath for 6 h. The contents were cooled, poured in to ice-cold water and then acidified with 2 N HCl. The resulting precipitate was collected by filtration and washed with water and air dried. Recrystallization from ethyl acetate:n-hexane (9:1) gave acid (93%) [33]. Yield: 93%.

1H NMR (400 MHz, DMSO-d6) δ: 10.28 (s, 1H, COOH), 7.62 (d, 1H, CH=CH-CO), 7.18 (s, 1H, Ar-H, 3), 7.12 (d, 1H, Ar-H, 5), 6.87 (d, 1H, Ar-H, 6), 6.44 (d, 1H, CH=CH-CO), 5.97 (s, 2H, O-CH2-O). IR (nujol, cm⁻¹): 3480, 1745.


Ethyl 3-(benzo[d][1,3]dioxol-5-yl)acrylate (4)

Sulfuric acid (1.1 eq) was added drop wise into an ethanolic solution of 3 (1 eq) in an ice bath. The solution was refluxed for 4h. The solution was poured over crushed ice, and the precipitate was collected by filtration and dried, obtained the desired compound. Yield: 94%.

1H NMR (400 MHz, DMSO-d6) δ: 7.68 (d, 1H, CH=CH-CO), 7.16 (s, 1H, Ar-H, 3), 7.09 (d, 1H, Ar-H, 5), 6.87 (d, 1H, Ar-H, 6), 6.42 (d, 1H, CH=CH-CO), 5.96 (s, 2H, O-CH2-O), 4.10 (q, 2H, O-CH2-CH3), 1.42 (t, 3H, O-CH2-CH3). IR (nujol, cm⁻¹): 2660, 1745, 960. Anal. calc. for (C12H12O4): C 65.45; H 5.49. found: C 65.17; H 5.41.

Ethyl 3-(7-nitrobenzo[d][1,3]dioxol-5-yl)acrylate (5)

A cooled nitrating mixture of concentrated nitric acid and concentrated sulfuric acid was added slowly to a cooled solution of 4 (3.30 g, 15 mmol) in glacial acetic acid (4 mL), and the mixture was stirred for about 30 min at 5-10 °C. Stirring was continued for 4h and the reaction mixture was poured into crushed ice. The product which separated as solid was collected, dried, and recrystallized from ethanol. Yield: 86%.

1H NMR (400 MHz, DMSO-d6) δ: 7.48 (d, 1H, CH=CH-CO), 7.42 (s, 1H, Ar-H, 5), 7.27 (s, 1H, Ar-H, 3), 6.51 (d, 1H, CH=CH-CO), 5.96 (s, 2H, O-CH2-O), 4.10 (q, 2H, O-CH2-CH3), 1.42 (t, 3H, O-CH2-CH3). IR (nujol, cm⁻¹):
2662, 1748, 1526, 956. Anal. calc. for (C_{12}H_{11}NO_{6}): C 54.34; H 4.18; N 5.28. found: C 54.17; H 4.10; N 5.49.

*Ethyl 3-(7-aminobenzo[d][1,3]dioxol-5-yl)acrylate (6)*

Compound 5 (2.65 g, 10 mmol) and SnCl$_2$ were dissolved in ethanol and the mixture was stirred under reflux for 2 h. The solution was quenched to pH 7 using saturated NaHCO$_3$ in water, extracted several times with chloroform and dried over anhydrous Na$_2$SO$_4$. The solvent was removed and recrystallization of the product was obtained from ethanol. Yield: 95%. $^1$H NMR (400 MHz, DMSO-$d_6$) δ: 7.42 (d, 1H, CH=CH-CO), 6.94 (s, 1H, Ar-H, 3), 6.86 (s, 1H, Ar-H, 5), 6.49 (d, 1H, CH=CH-CO), 6.37 (s, 2H, NH$_2$), 5.94 (s, 2H, O-CH$_2$-O), 4.12 (q, 2H, O-CH$_2$-CH$_3$), 1.42 (t, 3H, O-CH$_2$-CH$_3$). IR (nujol, cm$^{-1}$): 3120, 2660, 1740, 1310, 946. Anal. calc. for (C$_{12}$H$_{13}$NO$_4$): C 61.27; H 5.57; N 5.95. found: C 61.02; H 5.43; N 5.98.

*Ethyl 3-(7-methoxybenzo[d][1,3]dioxol-5-yl)acrylate (7)*

A solution of sodium nitrite (8 mmol) in water (12 mL) was added portion wise with constant stirring to a solution of the corresponding amine compound 6 (1.88 g, 8 mmol) in hydrochloric acid (1:1), which was cooled in an ice-bath. The solution was allowed to stirring for 30-60 min. To the above reaction mixture, 20 mL of methanol was added portion wise with stirring, and allowed to reflux for 2h. The reaction mixture was extracted with ether and the combined organic portions were dried over anhydrous Na$_2$SO$_4$. Evaporation of the solvent and recrystallization from absolute ethanol gave the product 7. Yield: 78%. $^1$H NMR (400 MHz, DMSO-$d_6$) δ: 7.48 (d, 1H, CH=CH-CO), 7.02 (s, 1H, Ar-H, 5), 6.98 (s, 1H, Ar-H, 3), 6.56 (d, 1H, CH=CH-CO), 6.01 (s, 2H, O-CH$_2$-O), 4.18 (q, 2H, O-CH$_2$-CH$_3$), 3.64 (s, 3H, OCH$_3$), 1.47 (t, 3H, O-CH$_2$-CH$_3$). IR (nujol, cm$^{-1}$): 2668, 1735, 1038, 946. Anal. calc. for (C$_{13}$H$_{14}$O$_5$): C 62.39; H 5.64. found: C 62.17; H 5.62.

*General procedure for the synthesis of (8a-j)*

Solutions of amine (1 eq) in THF and dimethylaluminium chloride (1 eq in THF) were mixed at 0 °C. After the addition was completed, the ice-bath was removed and the solution was stirred for 30 min at room temperature. The ester compound 7 (1 eq) was added into the reaction mixture and stirred for an additional 6
h. Monitor the progress of the reaction by TLC. The reaction mixture was quenched with the addition of aqueous potassium tartrate tetrahydrate slowly. The product was extracted with chloroform (3 x 25 mL). The combined organic extract was washed with brine, dried over anhydrous Na2SO4, and concentrated in vacuo to get the crude product which was next purified by column chromatography on silica employing chloroform: methanol (9:1) as eluted to obtain the pure product.

3-(7-Methoxybenzo[d][1,3]dioxol-5-yl)-1-(piperidin-1-yl)prop-2-en-1-one  
(8a) Yield: 82%, mp: 116-118 °C. 1H NMR (400 MHz, DMSO-d6) δ: 7.55 (d, 1H, CH=CH-CO), 7.08 (s, 1H, Ar-H), 7.03 (s, 1H, Ar-H), 6.78 (d, 1H, CH = CH-CO ), 5.98 (s, 2H, O-CH2-O), 3.76 (s, 3H, OCH3), 3.58-3.40 (m, 4H, N-(CH2)2 ), 1.89-1.66 (m, 6H, N-C- (CH2)3). IR (nujol, cm⁻¹): 2667, 1693, 1035, 938. MS, m/z: 289 (M⁺). Anal. calcd. for (C16H19NO4): C 66.42; H 6.62; N 4.84. found: C 66.36; H 6.58; N 4.88.

1-(4-Hydroxypiperidin-1-yl)-3-(7-methoxybenzo[d][1,3]dioxol-5-yl)prop-2-en-1-one  
(8b) Yield: 75%, mp: 127-130 °C. 1H NMR (400 MHz, DMSO-d6) δ: 7.51 (d, 1H, CH=CH-CO), 7.02 (s, 1H, Ar-H), 6.78 (d, 1H, CH = CH-CO ), 6.01 (s, 2H, O-CH2-O), 4.30 (s, 1H, OH), 3.67 (s, 3H, OCH3), 3.60-3.56 (m, 1H, CH), 3.38-3.12 (m, 4H, N-(CH2)2 ), 1.73-1.49 (m, 4H, N-C- (CH2)2). IR (nujol, cm⁻¹): 3654, 2665, 1697, 1615, 1030, 936. MS, m/z: 305 (M⁺). Anal. calcd. for (C16H19NO5): C 62.94; H 6.27; N 4.59. found: C 62.85; H 6.36; N 4.66.

1-(3-Hydroxypiperidin-1-yl)-3-(7-methoxybenzo[d][1,3]dioxol-5-yl)prop-2-en-1-one  
(8c) Yield: 74%, mp: 118-120 °C. 1H NMR (400 MHz, DMSO-d6) δ: 7.40 (d, 1H, CH=CH-CO), 7.06 (s, 1H, Ar-H), 7.01 (s, 1H, Ar-H), 6.78 (d, 1H, CH = CH-CO ), 5.98 (s, 2H, O-CH2-O), 4.76 (s, 1H, OH), 3.78 (s, 3H, OCH3), 3.57-3.29 (m, 4H, N-(CH2)2), 3.11 (m, 1H, CH), 1.66-1.52 (m, 2H, N-C- CH2), 1.80-1.56 (m, 2H, N-C-CH2). IR (nujol, cm⁻¹): 3642, 2660, 1694, 1617, 1035, 944. MS, m/z: 305 (M⁺). Anal. calcd. for (C16H19NO5): C 62.94; H 6.27; N 4.59. found: C 62.83; H 6.36; N 4.66.

1-(2,6-Dimethylpiperidin-1-yl)-3-(7-methoxybenzo[d][1,3]dioxol-5-yl)prop-2-en-1-one  
(8d) Yield: 78%, mp: 112-114 °C. 1H NMR (400 MHz, DMSO-d6) δ: 7.41 (d, 1H, CH=CH-CO), 7.02 (s, 1H, Ar-H), 6.98 (s, 1H, Ar-H), 6.38 (d, 1H, CH = CH-CO ), 5.97 (s, 2H, O-CH2-O), 3.74 (s, 3H, OCH3), 3.11 (m, 2H, CH), 1.82-1.56 (m, 6H, N-
C-(CH₂)₃, 1.27 (t, 6H, N-C-(CH₃)₂). IR (nujol, cm⁻¹): 2657, 1691, 1615, 1031, 966. MS, m/z: 317 (M⁺). Anal. calcd. for (C₁₈H₂₃NO₄): C 68.12; H 7.30; N 4.41. found: C 68.12; H 7.42; N 4.28.

3-(7-Methoxybenzo[d][1,3]dioxol-5-yl)-1-(4-methylpiperidin-1-yl)prop-2-en-1-one (8e) Yield: 80%, mp: 206-208 °C. ¹H NMR (400 MHz, DMSO-d₆) δ: 7.50 (d, 1H, CH=CH-CO), 7.04 (s, 1H, Ar-H), 7.01 (s, 1H, Ar-H), 6.75 (d, 1H, CH = CH -CO), 6.01 (s, 2H, O-CH₂-O), 3.72 (s, 3H, OCH₃), 3.37-3.28 (m, 4H, N-(CH₂)₂), 1.71 (m, 1H, CH), 1.52-1.38 (m, 4H, N-C-(CH₂)₂), 0.98 (d, 3H, CH₃). IR (nujol, cm⁻¹): 2662, 1694, 1618, 1034, 960. MS, m/z: 303 (M⁺). Anal. calcd. for (C₁₇H₂₁NO₄): C 67.31; H 6.98; N 4.62. found: C 67.21; H 6.63; N 4.73.

3-(7-Methoxybenzo[d][1,3]dioxol-5-yl)-1-(piperazin-1-yl)prop-2-en-1-one (8f) Yield: 82%, mp: 198-200 °C. ¹H NMR (400 MHz, DMSO-d₆) δ: 7.40 (d, 1H, CH=CH-CO), 7.08 (s, 1H, Ar-H), 7.02 (s, 1H, Ar-H), 6.71 (d, 1H, CH = CH -CO), 6.03 (s, 2H, O-CH₂-O), 3.65 (s, 3H, OCH₃), 3.21-3.17 (t, 4H, N-(CH₂)₂), 2.79-2.74 (m, 4H, N-C-(CH₂)₂), 1.76 (s, 1H, NH). IR (nujol, cm⁻¹): 3326, 2660, 1690, 1614, 1036, 936. MS, m/z: 290 (M⁺). Anal. calcd. for (C₁₅H₁₈N₂O₄): C 62.06; H 6.25; N 9.65. found: C 62.02; H 6.20; N 9.71.

1-(4-acetyl)piperazin-1-yl)-3-(7-methoxybenzo[d][1,3]dioxol-5-yl)prop-2-en-1-one (8g) Yield: 72%, mp: 212-214 °C. ¹H NMR (400 MHz, DMSO-d₆) δ: 7.56 (d, 1H, CH=CH-CO), 7.04 (s, 1H, Ar-H), 7.01 (s, 1H, Ar-H), 6.50 (d, 1H, CH=CH-CO), 6.07 (s, 2H, O-CH₂-O), 3.86 (s, 3H, OCH₃), 3.62-3.46 (m, 4H, N-C-(CH₂)₂), 3.25-3.18 (m, 4H, N-(CH₂)₂), 2.57 (s, 3H, CH₃). IR (nujol, cm⁻¹): 2660, 1704, 1618, 1038, 922. MS, m/z: 332 (M⁺). Anal. calcd. for (C₁₅H₂₀N₂O₅): C 61.44; H 6.07; N 8.43. found: C 61.33; H 6.02; N 8.43.

3-(7-Methoxybenzo[d][1,3]dioxol-5-yl)-1-(4-(2-methoxyphenyl)piperazin-1-yl)prop-2-en-1-one (8h) Yield: 77%, mp: 218-220 °C. ¹H NMR (400 MHz, DMSO-d₆) δ: 7.42 (d, 1H, CH=CH-CO), 7.07 (s, 1H, Ar-H), 7.03 (s, 1H, Ar-H), 6.83-6.97 (m, 4H, Ar-H), 6.71 (d, 1H, CH=CH-CO), 5.97 (s, 2H, O-CH₂-O), 3.76 (s, 6H, 2 OCH₃), 3.02-2.97 (m, 8H, N-(CH₂)₄). IR (nujol, cm⁻¹): 2668, 1698, 1620, 1038, 938. MS, m/z: 396 (M⁺). Anal. calcd. for (C₂₂H₂₄N₂O₅): C 66.65; H 6.10; N 7.07. found: C 66.42; H 6.24; N 7.01.
3-((7-Methoxybenzo[d][1,3]dioxol-5-yl)-1-(4-phenylpiperazin-1-yl)prop-2-en-1-one \((8i)\) Yield: 78%, mp: 226-228 °C. \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\): 7.56 (d, 1H, \(\text{CH}=	ext{CH}-\text{CO}\)), 7.40 (d, 2H, Ar-H, 1,5), 6.99 (t, 1H, Ar-H, 3), 6.94 (s, 1H, Ar-H), 6.91 (s, 1H, Ar-H), 6.81 (d, 2H, Ar-H 2,4), 6.27 (d, 1H, CH=\(\text{CH}-\text{CO}\) ), 6.01 (s, 2H, O-\(\text{CH}_2\)-O), 3.49 (s, 3H, OCH\(_3\)), 3.16-3.11 (m, 8H, N-(CH\(_2\))\(_4\)). IR (nujol, cm\(^{-1}\)): 2664, 1697, 1614, 1034, 946. MS, m/z: 366 (M\(^+\)). Anal. calcd. for (C\(_{21}\)H\(_{22}\)N\(_2\)O\(_4\)): C 68.84; H 6.05; N 7.65. found: C 68.52; H 6.07; N 7.98.

3-((7-Methoxybenzo[d][1,3]dioxol-5-yl)-1-(4-(pyridin-2-yl)piperazin-1-yl)prop-2-en-1-one \((8j)\) Yield: 75%, mp: 216-220 °C. \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\): 8.13-6.97 (m, 4H, Ar-H), 7.36 (d, 1H, CH=\(\text{CH}-\text{CO}\)), 6.89 (s, 1H, Ar-H), 6.84 (s 1H, Ar-H), 6.42 (d, 1H, CH=\(\text{CH}-\text{CO}\) ), 6.01 (s, 2H, O-\(\text{CH}_2\)-O), 3.76 (s, 3H, OCH\(_3\)), 3.54-3.30 (m, 8H, N-(CH\(_2\))\(_4\)). IR (nujol, cm\(^{-1}\)): 2658, 1699, 1622, 1560, 1032, 930. MS, m/z: 367 (M\(^+\)). Anal. calcd. for (C\(_{20}\)H\(_{21}\)N\(_3\)O\(_4\)): C 65.38; H 5.76; N 11.44. found: C 65.16; H 5.87; N 11.44.

**Antimicrobial activity**

**Microbial strains**

The *in vitro* antimicrobial screening effects of the piperamide compounds were individually tested against a panel of bacteria and fungi including *Staphylococcus aureus* (NCIM 5021), *Bacillus subtilis* (NCIM 2999), *Pseudomonas aeruginosa* (NCIM 5029), *Escherichia coli* (NCIM 2574), *Candida. albicans* (NCIM 3471) and *Aspergillus flavus* (NCIM 524). Microbial strains were cultured overnight at 37 °C in Nutrient and potato dextrose agar medium. All the pure microbial strains obtained from National Chemical Laboratory (NCL), Pune, India.

**Disc diffusion method**

The antimicrobial activity of each compound was determined by agar disc diffusion method [34]. Briefly, a suspension of tested bacterial strains was spread on the nutrient agar medium and potato dextrose agar for fungi. The discs (6 mm in diameter) impregnated with test chemicals each dissolved in concentration (100 µg/mL in DMSO) was placed on the inoculated agar and these plates were kept at 4 °C for 2 h. The plate was incubated at 37 °C for 24 h in case of bacteria and 48 h at
28 °C in case of fungi. Streptomycin (10 µg/disc) and Fluconazole (10 µg/disc) were used as the standard for antibacterial and antifungal activity, respectively. The activity of each sample was performed in triplicate and the zone of inhibition was measured in millimeters.

**Minimum inhibitory concentration (MIC)**

The minimum inhibitory concentration of the synthesized compounds was determined by dilution method [35]. The compounds were dissolved and then diluted using DMSO, two-fold serial concentrations of the compounds were employed to determine the MIC. In this method, the test concentrations of chemically synthesized compounds were made from 5 to 125 µg/mL. The MIC-value was determined as the lowest concentration of the compound that completely inhibited macroscopic growth of microorganism.

**Antioxidant activity**

**DPPH free radical scavenging activity**

The capacity to scavenge the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was monitored according to the Blois method [36]. The test samples (10-100 µL) were mixed with 1 mL of DPPH (0.1 mM) 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 (Elico SL-177, India). The free radical scavenging rate of the reaction solution was calculated as a percentage (%) of DPPH decolouration using the equation

$$ I(\%) = \left( \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \right) \times 100 $$

where $A_{\text{blank}}$ is the absorbance of the control reaction mixture excluding the test compounds, and $A_{\text{sample}}$ is the absorbance of the test compounds. Radical scavenging potential was expressed as IC$_{50}$ value, which represents the sample concentration at which 50 % of the DPPH radicals scavenged. Tests were carried out in triplicate and the results were expressed as mean values ± standard deviations.
Superoxide scavenging assay

Superoxide radical scavenging activity was measured as described by Kovala-Demertzki et al. [37]. The assay is based on the reduction of nitroblue tetrazolium (NBT) by superoxide ions generated by the xanthine-xanthine oxidase (X-XO) system. The reaction system contained 0.2 mM xanthine, 0.6 mM NBT in 0.1 M phosphate buffer of pH 7.8. The tested compounds were dissolved in methanol. The reaction was started by addition of XO (0.07 U mL⁻¹), an activity which allowed to yield the absorbance change between 0.03 and 0.04 per minute, at 560 nm. The extent of NBT reduction was followed spectrophotometrically by measuring the increase in absorbance at 560 nm. All the experiments were replicated three times. The IC₅₀ of each compound was defined as the concentration which inhibited 50% of the NBT reduction by O₂⁻ produced in the X–XO system.

Antidepressant Evaluation

Animals

Swiss albino mice were procured from National Institute of Nutrition, Hyderabad. Swiss albino male mice, weighing between 20 and 25 g, kept under controlled conditions (12 h dark–light cycle, 23–25 ºC temperature and 50–60% humidity) were used. The minimum number of animals and duration of observation required to obtain reproducible data. Behavioral experiments were conducted from 10:00 AM to 2:00 PM. The animals received a standard food pellet and before the experiments they were fasted overnight with water.

All the experimental procedures were carried out in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines. The study was reviewed and approved by the Institutional Animal Ethics Committee (320/CPCSEA), G Pulla Reddy College of Pharmacy, Hyderabad, India.

Forced Swim Test (FST)

This test was performed according to the procedure described by Porsolt et al. [38]. The animals were individually forced to swim in a transparent glass vessel (25
cm high, 15 cm in diameter) filled with (12.5 cm high) water at 21-24 °C. The total duration of immobility (in seconds) was measured during the last 4 min of a single 6 min test session. The test compounds 8a-j (20 mg/kg p.o) was administered 30 minutes before test. Imipramine (20 mg/kg, p.o) was used as the standard drug. Mice were considered immobile when they made no further attempts to escape except the movements necessary to keep the head above the water.

Tail Suspension Test (TST)

Tail suspension test is behavior despair model of depression, employed in rodents to predict antidepressant potential by decreasing immobility period produced by several different classes of antidepressant drugs [39]. Mice were suspended on the edge of the table, 50 cm above the floor, with the help of adhesive tape placed approximately 1 cm from the tip of the tail. The total duration of immobility induced by tail suspension was recorded during a 6 min period. The test compounds 8a-j (20 mg/kg p.o) was administered 30 minutes before test. Imipramine (20 mg/kg, p.o) was used as the standard drug. The animal was considered immobile when it did not show any movement of the body except for those required for respiration and hanged passively.

Measurement of MAO inhibitory activity

Mouse brain mitochondrial fraction was prepared following the procedure described previously [40]. Briefly, the mitochondrial fraction suspended in 9 mL of cold sodium phosphate buffer (10 mmol, pH 7.4, containing 320 mmol sucrose), was mingled at 4 °C for 20 min. The mixture was centrifuged at 15000 g for 30 min at 0 °C, the supernatant re-centrifuged to deposit the protein, which was suspended in the same buffer. The protein concentration was adjusted to 1 mg/mL. Protein concentration was estimated by the method Lowry et al. [41] using bovine serum albumin as the standard. MAO activity was assessed spectrophotometrically as described previously [42]. The assay mixtures contained 4 mmol 5-HT or 2mmol β-PEA as specific substrates for MAO A and B, respectively, the 200 µL of the mitochondrial fraction, and 10 mmol sodium phosphate buffer (pH 7.4) up to a final volume of 1 mL. The reaction was allowed to proceed at 37 °C for 20 min, and stopped by adding 1M HCl (200 µL), the reaction product was extracted with 5 mL
each of the butylacetate (for MAO A assay) or cyclohexane (for MAO B assay). The absorbance of organic phase was measured at 280 nm for MAO A assay and at 242 nm for MAO B assay in a UV-spectrophotometer. Blank samples were prepared by adding 1 M HCl (200 µL) prior to reaction, and worked up subsequently in the same manner. MAO-A and MAO-B values were expressed as nmol/mg protein.

RESULTS AND DISCUSSION

In view of the above findings, it was considered of interest to undertake the synthesis of piperamide derivatives, hoping that these compounds might possess certain antimicrobial, antioxidant and antidepressant activity. The structures of the synthesized compounds were deduced on the basis of $^1$H NMR, IR and mass spectra. The composition of all the compounds was obtained by elemental analysis. The proton magnetic resonance spectra of synthesized compounds were recorded in DMSO-$d_6$. In addition, the chemical shift and multiplicity patterns correlated well with the proposed structures.

A series of new piperamide derivatives (Scheme1) was synthesized with the readily available piperonal 1, which was treated with malonic acid 2 in pyridine, and followed by acidification with dilute hydrochloric acid giving carboxylic acid 3. This material was subjected to esterification using sulphuric acid in ethanol to afford the product 4. The IR spectra showed mainly absorption bands at 1745 and 960 cm$^{-1}$ assigned to $\nu$C=O and $\delta$CH=CH functionalities, respectively. The $^1$H NMR spectrum showed a triplet signal at $\delta$ 1.42 and quartet signal at $\delta$ 4.10 which supported the presence of ethyl group. In the $^1$H NMR spectrum, the presence of methylenedioxy moiety was indicated by signal at $\delta$ 5.96.

Subsequent nitration of 4 with nitrating mixture led to compound 5 and followed by reduction of the nitro moiety of the resulting 5 using SnCl$_2$/EtOH [43] yields the compound 6. The IR spectrum of compound 5 shows the characteristic band at 1526 cm$^{-1}$ due to the nitro group. IR spectrum of compound 6 revealed the presence of amine group at 3120 cm$^{-1}$ and its $^1$H NMR spectrum showed singlet at $\delta$ 6.37 for amine group.
Scheme 1: The reaction sequence for the synthesis of novel piperamide derivatives (8a-j). Reagents and conditions: (i) pyridine, piperidine, 24 h; (ii) Conc. H$_2$SO$_4$, EtOH, 4 h; (iii) nitrating mixture, 5-10 °C; (iv) SnCl$_2$, EtOH, NaHCO$_3$, 2 h; (v) NaNO$_2$, HCl, 30-60 min, MeOH, reflux, 2 h; (vi) dimethylaluminium chloride, piperidine/piperazine derivatives, THF, 0 °C.

The amine group of 6 was converted to methoxy moiety in 7 by diazotization in the presence of sodium nitrite at 0 °C and subsequent addition of methanol at reflux. Compound 7 gave correct values in elemental analysis. A comparative study of spectral data on compounds 6 and 7, revealed that the absence of a signal for amine (at δ 6.37, s, 2H) and appeared a signal at δ 3.64 (s, 3H) in the $^1$H NMR spectra of the product 7, thus confirming the presence of methoxy group.
The resulting compound 7 was coupling with different piperidine and piperazine derivatives, in THF in presence of dimethylaluminium chloride [44], afforded the corresponding piperamides 8a-j. TLC was run throughout the reaction to optimize the reaction for purity and completion. Compounds 8a-j gave correct values in elemental analysis. The IR spectrum of compound 8f shows the absorption band at 3326 cm$^{-1}$ is attributed to N–H stretching of the piperazine ring and the compound 8b shows the characteristic band at 3654 cm$^{-1}$ due to the hydroxyl (OH) group. The IR spectra of 8a-j shows the characteristic bands in the region 1704–1690 and 1038–1030 cm$^{-1}$ which indicates the presence of carbonyl and methoxy groups, respectively.

The $^1$H NMR spectrum of each compound in the series 8a-j showed singlet at δ 3.62–3.96 which revealed the presence of methoxy group and singlet at δ 5.97-6.03 due to methylenedioxy group. The two doublets centered at δ 7.55-7.36 and 6.78–6.27, which could be accounted for a trans double bond conjugated to the carbonyl group. The $^1$H NMR spectrum of compound 8a showed the characteristic signals for a piperidine ring at δ 3.58-3.40 (m, 4H, N-(CH$_2$)$_2$) and 1.89-1.66 (m, 6H, N-C-(CH$_2$)$_3$), together with the singlet at δ 5.94 and 3.76, due to the presence of methylenedioxy and methoxy group, respectively. The aromatic singlets at δ 7.08 and 7.03, indicating the presence of a 1,3,4,5-tetrasubstituted benzene ring. The signal due to OH in compound 8b appeared as singlet at δ 4.30 [45]. The Mass spectra of compounds are in agreement with their assigned structures. All the spectra exhibit parent peaks due to molecular ions (M$^+$).

Representative NMR and Mass spectra of the synthesized compounds are given at the end of this chapter.

Antimicrobial activity

In this work, novel series of piperamide compounds were synthesized. Synthetic scheme-1 illustrates the way used for the synthesis of target compounds. All the synthesized compounds were screened for their anti-bacterial activity against S. aureus, B. subtilis, E. coli and P. aeruginosa and anti-fungal activity against A. flavus and C. albicans. The antimicrobial activity of each compound was carried out
by agar disc diffusion method and the minimum inhibitory concentration was determined by dilution method. The results are presented in Table 1.

Table 1: Antimicrobial activity of the synthesized compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>Gram positive (MIC in µg/mL)</th>
<th>Gram negative (MIC in µg/mL)</th>
<th>fungi (MIC in µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. aureus</td>
<td>B. subtilis</td>
<td>E. coli</td>
</tr>
<tr>
<td>8a</td>
<td>10(24)</td>
<td>13(26)</td>
<td>15(30)</td>
</tr>
<tr>
<td>8b</td>
<td>10(50)</td>
<td>07(50)</td>
<td>11(&gt;100)</td>
</tr>
<tr>
<td>8c</td>
<td>08(50)</td>
<td>11(62)</td>
<td>07(&gt;100)</td>
</tr>
<tr>
<td>8d</td>
<td>11(28)</td>
<td>12(30)</td>
<td>10(50)</td>
</tr>
<tr>
<td>8e</td>
<td>12(24)</td>
<td>10(25)</td>
<td>10(40)</td>
</tr>
<tr>
<td>8f</td>
<td>14(30)</td>
<td>10(50)</td>
<td>11(20)</td>
</tr>
<tr>
<td>8g</td>
<td>11(14)</td>
<td>16(14)</td>
<td>12(18)</td>
</tr>
<tr>
<td>8h</td>
<td>14(16.4)</td>
<td>16(15)</td>
<td>09(20)</td>
</tr>
<tr>
<td>8i</td>
<td>12(18.3)</td>
<td>14(18.0)</td>
<td>10(25)</td>
</tr>
<tr>
<td>8j</td>
<td>14(15.2)</td>
<td>12(15.5)</td>
<td>11(22)</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>11.6(05)</td>
<td>10.4(05)</td>
<td>14.5(05)</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>Nt</td>
<td>Nt</td>
<td>Nt</td>
</tr>
</tbody>
</table>

* the values given are means of three experiments; Nt denotes not tested
The values given in parenthesis are MIC in µg/mL.

Most of the synthesized compounds showed good antibacterial activity. Among them, compounds 8g and 8j exhibited the most potent antibacterial activity against S. aureus and B. subtilis. Antibacterial data of compounds 8a-j against B. subtilis reveals that compound 8g exhibits the most potent antibacterial activity. Its activity (MIC = 14.2 µg/mL) was better than that of other compounds. Moreover, the activities of compounds 8h and 8j against S. aureus and B. subtilis were comparable to 8g.

All compounds did not show any improvement of activity against Gram-negative bacteria in comparison to Gram-positive bacteria. However, the compounds 8g, 8h and 8j showed comparable activity against Gram negative bacteria with respect to the other synthetic compounds. Compound 8g has N-substitution acetyl
group in piperazine nucleus exhibited high activity against all tested bacterial strains. When the acetoxy group was replaced by phenyl/2-methoxy phenyl group the activity was slightly diminished.

If, it further take into account on the substituent effect, in general the compound with piperazine group had shown less potency than the acetyl, 2-methoxyphenyl and pyridyl substituents. The piperizine derived compounds showed excellent antibacterial activity indicating that a wide range of alkyl and acyl substituents were well tolerated on the piperazine N4-position for proper fit at the potential receptor site [46].

Moreover, all of the synthesized compounds exhibited a moderate antifungal activity for *C. albicans* and *A. flavus* with MIC values of 14-100 and 15.8-75 μg/mL, respectively. Among the synthesized compounds, compound 8j was the most potent one and exhibited remarkably good antifungal activity against the tested fungi. The MIC value of compound 8j against *C. albicans* was 14μg/mL, which were 7-fold more potent than that of compounds 8b and 8c. It is of interest that compound 8g was found to exhibit the significant *in vitro* anti-fungal activity with MICs of 15 and 22μg/mL against *C. albicans* and *A. flavus*, respectively.

The results of antimicrobial activity illustrate that the presence of electron donating group, -OCH\(_3\) on the methylenedioxyphenyl ring, in the compounds 8a-j showed the antibacterial as well as antifungal activities. Further, the presence of electron withdrawing group, -COCH\(_3\) in compound 8g enhances the antibacterial activity to a greater extent.

In short, author made an attempt to enhance the biological activity by increasing the volume of the substituents attached to the piperazine ring system led to different biological potency, depending on the nature, position and number of the atoms or groups introduced, whereas, high potency has been observed in the final scaffolds due to the presence of piperazine systems with acetyl group. This has thrown open a new era for exploring suitably designed, new scaffold in molecules as potential antifungal / antibacterial drugs.
Antioxidant activity

The capacity to scavenge the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was monitored according to the Blois method. DPPH radical can be used in preliminary screening of compounds capable of scavenging reactive oxygen species, since these nitrogen radicals are much more stable and easier to handle than oxygen free radicals. The use of DPPH in antioxidant assays is well documented probably because of its rapid reaction, reliability and independent of sample polarity [47]. Initially, free radical scavenging activities of the newly prepared piperamide compounds were evaluated based on the DPPH decoloration range.

All the compounds showed interesting antioxidant activity compared to the standard (Table 2). Compound 8b was found to be the most potent antioxidant with the least values of IC\textsubscript{50} (8.3±0.02 µg/mL, inhibition concentration) and the least active among the series being compound 8i with the largest values of IC\textsubscript{50} (36.9±0.17 µg/mL). Table 2 gives an account for this antioxidant evaluation and indicates that compounds were endowed with substantial scavenging properties towards DPPH with potencies of 8b > 8c > 8d > 8h > 8a.

Antioxidant activity of the compounds is related with their electron or hydrogen radical releasing ability to DPPH so that they become stable diamagnetic molecules. This might be the reason for the higher antioxidant activity of the above-mentioned compounds. Compounds having hydroxy and methoxy groups are found relatively more active e.g., compounds 8b and 8c (hydroxy derivative) are more active than 8h (dimethoxy derivative). The compounds 8b and 8c showed significant difference in radical scavenging activity due to bearing a hydroxy group at different position. The coupling of 2,6-dimethylpiperidine showed much better activity than the 4-methylpiperidine. The IC\textsubscript{50} values were found to vary from 8.3 to 36.9µg/mL showed a wide range of variations in the reactivity of the samples. The wide variations in free radical scavenging activities may be due to the variations in the proton-electron transfer by the compounds due to difference in their structures and stability. An insight to the structure–activity relationship gives an idea that activity generally increases with number and strength of electron donating groups.
Table 2: Results of antioxidant activity of the synthesized compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>DPPH</th>
<th>Superoxide</th>
</tr>
</thead>
<tbody>
<tr>
<td>8a</td>
<td>14.9±0.11</td>
<td>18.2±0.17</td>
</tr>
<tr>
<td>8b</td>
<td>8.3±0.02</td>
<td>12.8±0.08</td>
</tr>
<tr>
<td>8c</td>
<td>13.4±0.37</td>
<td>16.9±0.14</td>
</tr>
<tr>
<td>8d</td>
<td>13.8±0.03</td>
<td>15.8±0.13</td>
</tr>
<tr>
<td>8e</td>
<td>15.3±0.19</td>
<td>19.8±0.36</td>
</tr>
<tr>
<td>8f</td>
<td>20.1±0.07</td>
<td>38.4±0.04</td>
</tr>
<tr>
<td>8g</td>
<td>23.2±0.16</td>
<td>21.9±0.16</td>
</tr>
<tr>
<td>8h</td>
<td>14.7±0.11</td>
<td>19.4±0.19</td>
</tr>
<tr>
<td>8i</td>
<td>36.9±0.17</td>
<td>55.3±0.17</td>
</tr>
<tr>
<td>8j</td>
<td>19.3±0.08</td>
<td>18.5±0.26</td>
</tr>
<tr>
<td>AA</td>
<td>12.6±0.43</td>
<td>Nt</td>
</tr>
<tr>
<td>BHA</td>
<td>Nt</td>
<td>13.4±0.29</td>
</tr>
</tbody>
</table>

*a* ascorbic acid; *b* butylated hydroxyanisole; *Nt* denotes not tested

Values are means of triplicate determinations

Superoxide radical scavenging activity was measured as described by reported method. Generation of reactive oxygen species (ROS) and free radicals *in vivo* is involved in a wide range of human diseases. ROS, including superoxide anion, hydrogen peroxide and hydroxyl radical are byproducts of a variety of pathways of aerobic metabolism [48]. These are unstable and react readily with a wide range of biological substrates, such as lipids, DNA and protein molecules, consequently resulting in the cell damage [49]. The enzymatic superoxide anion radical was generated by a xanthine and xanthine oxidase reaction system.

The piperamide derivatives 8a-j were found to be moderate to weak superoxide radical scavenger. The IC$_{50}$ values of these compounds were in the range 12.8 to 55.35 µg/mL. Favorable superoxide radical scavenging was found for compounds 8a, 8b, 8c, 8d and 8j ranging from 12.8 to 18.5µg/mL. Compound 8b has substituted hydroxyl group attached to piperidine ring, showed the highest activity among the piperamide compounds 8a-j, while compounds 8f and 8i showed less activity. This indicates that selective modification of the hydroxy part of 8b decreases the radical scavenging potency.
Antidepressant activity

Forced swim test and tail suspension test

The piperamide derivatives (8a-j) were screened for their antidepressant activity, in two behavioral tests, modified porsolt forced swimming test (FST) and tail suspension test (TST). Antidepressant activity was assessed as mean immobility time in seconds and data has been presented as Mean ± S.E.M. The obtained data on the antidepressant activity of the compounds and reference drug are given in Table 3.

Table 3: Change in duration of immobility in forced swim test (FST) and Tail suspension test (TST) in male albino mice treated with single dose vehicle, test compounds (20 mg/kg p.o) and Imipramine (20 mg/kg p.o) treated groups

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mean duration of immobility (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FST</td>
</tr>
<tr>
<td>Control</td>
<td>75.33±3.35</td>
</tr>
<tr>
<td>Imipramine</td>
<td>32.17±2.44</td>
</tr>
<tr>
<td>8a</td>
<td>32.15±2.12</td>
</tr>
<tr>
<td>8b</td>
<td>30.8±1.30</td>
</tr>
<tr>
<td>8c</td>
<td>35.4±2.2</td>
</tr>
<tr>
<td>8d</td>
<td>43.33±3.44</td>
</tr>
<tr>
<td>8e</td>
<td>51.6±1.7</td>
</tr>
<tr>
<td>8f</td>
<td>71±1.91</td>
</tr>
<tr>
<td>8g</td>
<td>55.83±4.97</td>
</tr>
<tr>
<td>8h</td>
<td>59.16±4.10</td>
</tr>
<tr>
<td>8i</td>
<td>66.2±5.80</td>
</tr>
<tr>
<td>8j</td>
<td>67.6±3.64</td>
</tr>
</tbody>
</table>

a all data are expressed in mean ± SEM (n=10)
b p<0.001 vs normal control
c p<0.01 vs normal control

In the FST and TST, the treatment with doses of 20 mg/kg of 8a-j showed significantly decreased the immobility time. Acute treatment with the compounds 8a, 8b, 8c and 8d promoted a decrease in the immobility time when compared to normal control group and these effects were similar to standard treated group. Test compounds 8a and 8b were more effective than standard treated group in both FST
and TST. The immobility time of animals treated with the other compounds 8f, 8i and 8j did not statistically differ from control values. However, compounds 8e, 8g and 8h shown moderate decrease in duration of immobility and this effect was significant (p<0.001) difference when compared to standard treated group.

The behavioral despair tests, FST and TST are effective at predicting the activity of a wide variety of antidepressants [50, 51]. It has good predictive value for antidepressant potency in humans [52], as they are sensitive and selective for clinically used antidepressant drugs. It discriminates antidepressants from neuroleptics and anxiolytics [53] and also provides a useful model to study neurobiological and genetic mechanisms underlying stress and antidepressant responses [54]. FST was designed by Porsolt as a primary screening test for antidepressant compounds, concluded that the immobility time observed in the test reflected a state of lowered mood or hopelessness in animals; thus, this animal model is the most widely used tool for preclinical screening of putative antidepressant agents [55, 56]. It is still one of the best models for this procedure. The main advantage of this method is low-cost, fast and reliable model to test potential antidepressant treatments with a strong predictive validity.

Tail suspension test is widely employed animal model for testing antidepressant activity in mice. The same way as observed in FST, the TST is based on the fact that animals subjected to the short term inescapable stress of being suspended by their tail will develop an immobile posture [57]. The total duration of immobility induced by tail suspension was measured according to the method described by Steru et al. [39]. TST is less stressful than FST and has greater pharmacological sensitivity [58]. A wide variety of antidepressants, and compounds with potential antidepressant activity reduce the duration of immobility in the TST [59, 60].

Remarkably, TST has been shown to be sensitive to an array of antidepressant treatments, including tricyclics, SSRIs, monoamine oxidase inhibitors, atypical antidepressants and electroconvulsive therapy [61, 62].
An obvious advantage of this test is its ability to detect a broad spectrum of antidepressants irrespective of their underlying mechanism; it is inexpensive, methodologically unsophisticated and easily amenable to automation [63].

Some of the hypotheses concerning the mechanism of antidepressant activity are based on the ability of drugs to influence the turnover of neurotransmitters [12]. For several years, natural or synthetic compounds with potential antidepressant action have been studied and the piperamides are examples of these. The piperamides are the most important active constituents in piper species. The natural piperamides like piperine, Antiepilepsirine, Laetispicine, Piplartine etc, showed wide range of antidepressant activities [64, 65].

The prepared compounds consists of three important components, viz. methoxy group substituted methylenedioxyphenyl (MDP) ring, side chain with double bond and a basic piperidine/piperazine moiety attached through a carbonyl amide linkage to side chain. These results suggested that piperidine group essential for the pharmacological activity. A large number of piperidine containing compounds are biologically and medicinally important [66]. The replacement of piperidine by piperazine ring decreases the antidepressant activity in behavioral tests. Furthermore, an MDP ring appears to be a common functional group of several naturally occurring compounds of pharmacological importance [67]. Compound 8b bearing hydroxy group in the 4-position of the piperidine moiety was more active than compounds with a hydroxy substituent at position 3. On the other hand, coupling of substituted piperidine containing 2,6-dimethyl/4-methyl group showed significant difference. The replacement of the acetyl group on the piperazine moiety at the N-position by a phenyl or pyridyl group decreases the antidepressant activity.

**MAO inhibitory activity**

The in vitro inhibition activities against MAO-A and MAO-B of selected compounds 8a, 8b and 8c was investigated spectrophotometrically using mouse brain homogenate as source of MAO. Table 4 revealed that compounds 8a, 8b and 8c showed a slightly more potent inhibitory effect against MAO-A than MAO-B. The discrepancy in the inhibitory effects of compounds 8a, 8b and 8c may be due to the differences in experimental conditions and in the substrate of the MAO enzyme.
Regulation of MAO-A activity has been thought to be an effective approach for the treatment of depression and anxiety, while regulation of MAO-B appears to be helpful in the prevention and adjunct treatment of Parkinson’s disease [68, 69]. These findings suggested that antidepressant effects of piperamides in animal models of immobility tests may be related to the inhibitory activity of MAO. The confirmation of therapeutic potential and explanation of molecular mechanism by which investigated compounds exerted their action require further pharmacological studies.

### Table 4: In vitro inhibition activity of compounds 8a, 8b, 8c and fluoxetine on MAO activity in mouse brain

<table>
<thead>
<tr>
<th>Compound</th>
<th>MAO activity (nmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MAO-A</td>
</tr>
<tr>
<td>Control</td>
<td>23.59 ± 2.71</td>
</tr>
<tr>
<td>8a</td>
<td>17.09 ± 2.26c</td>
</tr>
<tr>
<td>8b</td>
<td>14.46 ± 1.83b</td>
</tr>
<tr>
<td>8c</td>
<td>16.63 ± 3.49c</td>
</tr>
<tr>
<td>Clorgyline</td>
<td>17.05 ± 2.52</td>
</tr>
</tbody>
</table>

In conclusion, a series of piperamide derivatives was prepared and tested for their potential antimicrobial, antioxidant and antidepressant activity. These compounds offer the advantage of a facile synthesis. The results of the study demonstrate that out of ten synthesized piperamide derivatives, six compounds showed antidepressant activity by forced swim test and tail suspension test. Meanwhile compounds 8a, 8b and 8c inhibited mouse brain MAO-A and MAO-B activities. Modifications of 8a molecule may thus proved useful in the development of selective antidepressant agent. On the basis of above observations, the author will further modify to improve the antidepressant activity of piperamides.

---

**CONCLUSION**

In conclusion, a series of piperamide derivatives was prepared and tested for their potential antimicrobial, antioxidant and antidepressant activity. These compounds offer the advantage of a facile synthesis. The results of the study demonstrate that out of ten synthesized piperamide derivatives, six compounds showed antidepressant activity by forced swim test and tail suspension test. Meanwhile compounds 8a, 8b and 8c inhibited mouse brain MAO-A and MAO-B activities. Modifications of 8a molecule may thus proved useful in the development of selective antidepressant agent. On the basis of above observations, the author will further modify to improve the antidepressant activity of piperamides.
Figure 1: $^1$H NMR spectrum of compound 8a

Figure 2: $^1$H NMR spectrum of compound 8b
Figure 3: $^1$H NMR spectrum of compound 8f

Figure 4: LC-MS of compound 8c
Chapter 2

Piperamide derivatives

Figure 5: LC-MS of compound 8g

Figure 6: LC-MS of compound 8j
Figure 7: IR spectrum of compound 8a

Figure 8: IR spectrum of compound 8b
REFERENCES


[34] S.H. Gillespie, Medical Microbiology-Illustrated Butterworth Heinemann Ltd. United Kingdom, 1994, pp 234.


Chapter 2

Piperamide derivatives


