DESIGN, SYNTHESIS, MOLECULAR DOCKING AND ANTIDEPRESSANT ACTIVITY OF SOME CHALCONES OF BENZOFURAN

Vinayak M. Shejol[1*], Namrata Singh[1]

Abstract:
Background: In general, the chalcones and benzofuran are exhibiting various biological activities. The evaluation of pharmacological potential of chalcones of benzofuran as antidepressant agents are explored in this research work. To get insight of the intermolecular interactions, the molecular docking studies are performed at active site of MAO-A enzyme. **Aim**: In this study, an attempt has been made to generate new molecular scaffold which contains chalcones of benzofuran. **Methods**: The derivatives was synthesized by conventional reactions and characterized by various spectrometric methods. The derivatives were evaluated for antidepressant activity by using forced swim test (FST). Molecular docking studies of the synthesized derivatives with MAO-A enzyme were performed by using Pyrx software. The pharmacokinetic study of the synthesized compounds were performed by using pkCSM software. **Results**: All the final structures were assigned on the basis of IR, 1H NMR and mass spectra. The antidepressant evaluation exhibited final derivatives 10, 16 and 22 as promising molecules with percentage decrease in immobility duration 64.21, 69.85 and 65.61 respectively. Molecular docking studies are also in agreement with pharmacological evaluation with potent compounds 16 exhibiting dock score -11.5. **Conclusion**: The synthesized compound may have the potential to be developed into an antidepressant agent. The substitution of nitro and chloro groups, i.e., electron releasing groups, at R_1 and R_2 position, showed promising antidepressant efficacy, according to the series of compounds. Therefore, the medicinal chemists who are involved in the creation of MAO-A inhibitors might benefit greatly from our research.

Keywords: Benzofuran, Chalcones, Antidepressant activity, Molecular docking, MAO-A

[1*]Faculty of Pharmacy, Oriental University, Indore-Madhya Pradesh-India, Email: vinayakshejol10@gmail.com

***Corresponding Author:** Vinayak M. Shejol
*Faculty of Pharmacy, Oriental University, Indore-Madhya Pradesh-India, Email: vinayakshejol10@gmail.com

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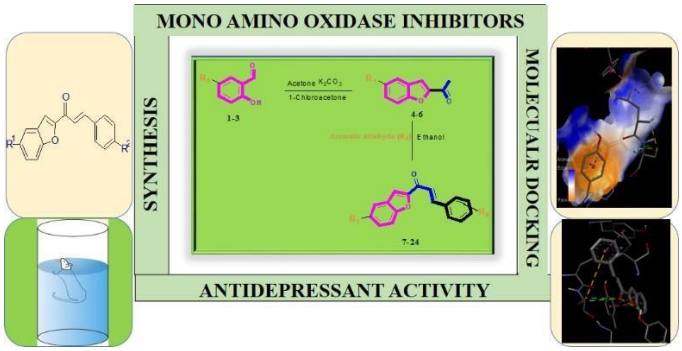
Graphical Abstract



INTRODUCTION

A state characterized by lack of interest, irregular sleep patterns, tiredness, and occasionally suicidal thoughts is referred to as "major depression disorder" (MDD). It is a chronic, perhaps deadly mental illness that is commonly disregarded and improperly managed [1, 2]. Up to 21% of the world's population, according to estimates, suffers from depression [3]. By 2025, the World Health Organization (WHO) projects that problems resulting from stress and the cardiovascular system will make it the second largest cause of mortality. Around the world, the burden of depression and other mental health issues is increasing. In May 2013, the World Health Assembly adopted a resolution calling for a comprehensive, coordinated national approach to mental illnesses. It is quite improbable that someone experiencing a major depressive episode will be able to go on with social, professional, or household activities, except very little [4]. According to epidemiological research, nearly 2/3 of suicide victims are despondent when they take their own lives. Although the precise etiology of depression is unknown, the primary contributing elements are thought to include neurotransmitter unbalance in the brain, hereditary susceptibility, stressful situations in life, and medical issues [5]. At 1870, Perkin had priory studied on benzofuran and Kraemer and Spilker identified it in coal tar in 1890. Numerous literature based on the depression and its symptoms [6]

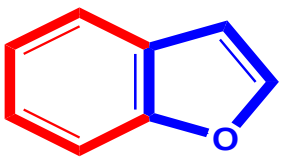


Figure 1: Structure of Benzofuran

There are various compounds containing benzofuran compounds having several therapeutic effects such as Amiodarone, angelicin, bergapten, nodekenetin, xanthotoxin, and usnic acid. These Benzofuran compounds have a vital clinical application value and significant promise for usage in drug development in the future. These compounds have been widely employed in antiarrhythmic, dermatological, and anticancer therapy [7, 8]. It has been discovered that benzofuran derivatives are used in a variety of therapeutic areas like malignancy, psychotic disorders, CNS disorders associated with inflammation, diabetes, hormonal disturbances, renal failure, and cardiovascular disorders. In addition to these, benzofurans have been developed as herbicides, miticides, and arthropocide [9, 10]. A class of organic compounds known as chalcones has a wide range of pharmacological actions, including antiviral, antifungal, and analgesic characteristics. Recently, it has been found that flavonoids have antidepressant properties [11]. A family of enzymes called monoamine oxidases (MAO) biogenic amine oxidation or inactivation is facilitated by CNS enzymes. [12, 13] Monoamine oxidase is inhibited by MAO inhibitors, which increases the availability of monoamine neurotransmitters like noradrenaline and serotonin by limiting their breakdown. Monoamine oxidase has two isoforms: MAO-A and MAO-B. The MAO-B inhibitors could be used to treat Parkinson's disease and Alzheimer's disease and MAO-A inhibitors have been used to treat depression, anxiety, and other mental illnesses. Clinically used antidepressants have a number of drawbacks and adverse effects that necessitate ongoing research into new, effective, and secure medications for the treatment of depression. [14] The majority of synthetic medications used to treat these illnesses have an impact on the system which increases the

concentration of biogenic amines. Chalcone and its derivatives are also of growing interest in academia and industry [15]. The pure chalcone isolates from various plants have undergone clinical trials for the treatment of viruses, tumours, and CNS disorders.

Therefore, in the present investigation, we had prepared the chalcones of benzofuran so as to in crease the antidepressant potential. Our research focused on the discovery of new MAO-A inhibitors which may be used in the management of depression. The scheme of the present study is outlined in **Figure 1** along with the list of substitutions shown in **Table 1**.

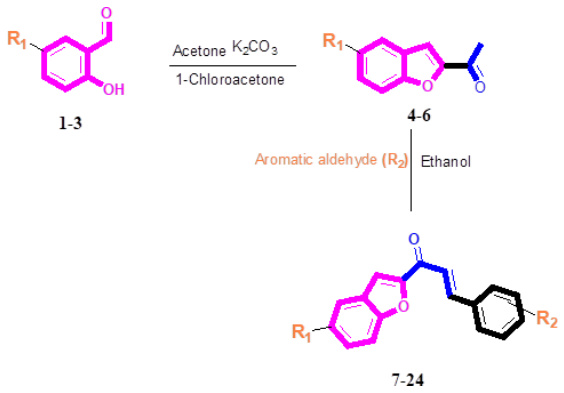


Figure 1: Scheme of synthesis of chalcones of benzofuran

Table 1. List of Substitutions in Synthetic Scheme

Compound	R_1	R_2
7	-H	C_6H_5-
8	-H	3-NO_2- C_6H_4-
9	-H	4-NO_2- C_6H_4-
10	-H	4-Cl- C_6H_4-
11	-H	4-OCH_3- C_6H_4-
12	-H	4-OH,3-OCH_3- C_6H_3-
13	-NO2	C_6H_5-
14	-NO2	3-NO_2- C_6H_4-
15	-NO2	4-NO_2- C_6H_4-
16	-NO2	4-Cl- C_6H_4-
17	-NO2	4-OCH_3- C_6H_4-
18	-NO2	4-OH,3-OCH_3- C_6H_3-
19	-Br	C_6H_5-
20	-Br	3-NO_2- C_6H_4-
21	-Br	4-NO_2- C_6H_4-
22	-Br	4-Cl- C_6H_4-
23	-Br	4-OCH_3- C_6H_4-
24	-Br	4-OH,3-OCH_3- C_6H_3-

MATERIAL AND METHODS

Chemistry

All of the chemicals, medicines, and solvents used for the synthesis process were of laboratory quality SDFine/E.Merck/Loba. The recognized techniques were used to purify the solvents. A small number of the reagent components for the synthesis were purchased from Alfa Aeser in the United Kingdom and Sigma Aldrich in Germany. Vacuum desiccators have been used to dry and recrystallize every remnant. The percentage yield was calculted of recrystallized products.

Using a Thieles tube, the melting points of the compounds were ascertained in open capillaries. The melting points listed below are uncorrected and expressed in the celsius scale (°C). Thin layer chromatography was carried out on microscopic slides (2 x 7.5 cm) coated with silica gel-G which was activated at 110^0C for 30 min in order to monitor the reactions as well as to determine the identity and purity of reactants and products. The spots were visible by exposure to iodine vapours. The Rf values were determined.

At the Dr. Rajendra Gode College of pharmacy Malkapur, the IR spectra of various compounds was done by using KBr pellets. Tetramethylsilane (TMS) was used as the internal standard while a 1H NMR (CDCl3) measurement was made at SAIF, Punjab University, Chandigarh utilising a Bruker Advance-II 400 Spectrometer at 400 MHz. In CDCl3 solution 1H NMR, the chemical shifts (δ) are reported in parts per million (ppm) in relation to TMS. Signal multiplicities are conveyed by the following signal types: singlet (s), doublet (d), triplet (t), quadruplet (q), wide singlet (bs), doublet of doublet (dd), and multiplet (m). At the Waters, Q-TOF ESI-MS spectrometer in the USA, mass spectra (EI-MS) were captured. The Mass spectra of synthesized compounds were recorded on LCMS-ion trap Mass Spectrometer from Punjab University, Chandigarh.

The UV-Visible 1800 double beam spectrophotometer from Shimadzu was used to measure the ultraviolet absorption spectra in methanol. On a Shimadzu, 1S-Furior, affinity spectrometer from the Dr. Rajendra Gode College of pharmacy Malkapur.

Synthesis of 1-(1-benzofuran-2-yl)ethan-1-one (4)

A mixture of salicylaldehyde (0.6 g, 4.97 mmol) and potassium carbonate (0.69 g, 4.97 mmol) in dry acetone (10 mL) was stirred at 25^0C for 1 h. Reaction mixture was cooled at 0–5^0 C, and then chloroacetone (4 mL) was added dropwise. Reaction mixture was stirred at room temperature for ten minutes and then refluxed. Progress of the reaction was monitored by TLC. Upon completion, the reaction mixture was poured on crashed ice. The precipitated solid was filtered, washed with water, and dried. The product was crystallized from ethanol.

Yield: 80 %. mp: 116-118 ºC. R_f: 0.36 (Methanol: Toulene 1:4). IR (KBr, cm^{-1}): 1756.67 (C=O), 1597.37 (C=C), 3078.23 (C-H). ^{1}H NMR (DMSO, ppm): δ 7.05-7.51 (m, 4H, Ar-H), 8.40 (s, 1H, C-H), 2.14 (s, 3H, methyl protons).

Synthesis of 1-(5-nitro-1-benzofuran-2-yl)ethan-1-one (5)

Yield: 73 %. mp: 135-137 ºC. R_f: 0.38 (Methanol: Toulene 1:4). IR (KBr, cm^{-1}): 1735.58 (C=O), 1525.56 (C=C), 3050.78 (C-H), 1356.45 (C-NO_2). ^{1}H NMR (DMSO, ppm): δ 7.06-7.18 (m, 3H, Ar-H), 9.07 (s, 1H, C-H), 2.88 (s, 3H, methyl protons).

Synthesis of 1-(5-bromo-1-benzofuran-2-yl)ethan-1-one (6)

Following above procedure, a mixture of 5-bromosalicylaldehyde (1 g, 4.97 mmol) and potassium carbonate (0.69 g, 4.97 mmol) in dry acetone (10 mL) was stirred at 25◦ C for 1 h. Reaction mixture was cooled at 0–5∘ C, and then chloroacetone (4 mL) was added dropwise. Reaction mixture was stirred at room temperature for ten minutes and then refluxed to gave the product (6).

Yield: 75 %. mp: 145-147 ºC. R_f: 0.39 (Methanol: Toulene 1:4). IR (KBr, cm^{-1}): 1715.76 (C=O), 1560.76 (C=C), 3090.34 (C-H), 678.98 (C-Br). ^{1}H NMR (DMSO, ppm): δ 6.45-7.05 (m, 3H, Ar-H), 8.13 (s, 1H, C-H), 2.75 (s, 3H, methyl protons).

Synthesis of 1-(1-benzofuran-2-yl)-3-(substituted phenyl)-prop-2-en-1-one

General Procedure for Synthesis of Chalcones:

A solution of 1-benzofuran-2-ethanone (4.18 mmol) and substituted aldehyde (4.18 mmol) in methanol (10 mL) was cooled at 0–5◦ C and then 6 mL of aqueous NaOH (1 mol/L) was added to this solution and stirred at room temperature for 3 h. The reaction mixture was poured on crushed ice. The precipitated solid was filtered after neutralization with diluted HCl and was washed several times with water and then dried. The product was recrystallized from ethanol.

1-(1-benzofuran-2-yl)-3-phenylprop-2-en-1-one (7)

Yield: 76 %. mp: 176-178 ºC. R_f: 0.43 (Methanol: Toulene 1:4). IR (KBr, cm^{-1}): 1756.22 (C=O), 1569.72 (C=C), 3053.72 (C-H). ^{1}H NMR (DMSO, ppm): δ 6.56-7.44 (m, 9H, Ar-H), 6.97 (d, J = 8 Hz, 1H, CO–CH), 7.05 (d, J = 8.1 Hz, 1H, CH–Ar).

1-(1-benzofuran-2-yl)-3-(3-nitrophenyl)prop-2-en-1-one (8)

Yield: 78 %. mp: 184-186 ºC. R_f: 0.63 (Methanol: Toulene 1:4). IR (KBr, cm^{-1}): 1745.34 (C=O), 1578.82 (C=C), 1378.45 (C-NO_2), 3076.72 (C-H). ^{1}H NMR (DMSO, ppm): δ 6.54-7.34 (m, 8H, Ar-H), 6.56 (d, J = 8.3 Hz, 1H, CO–CH), 7.04 (d, J = 7.4 Hz, 1H, CH–Ar).

1-(1-benzofuran-2-yl)-3-(4-nitrophenyl)prop-2-en-1-one (9)

Yield: 76 %. mp: 185-187 ºC. R_f: 0.43 (Methanol: Toulene 1:4). IR (KBr, cm^{-1}): 1731.34 (C=O), 1612.12 (C=C), 1367.73 (C-NO_2), 3123.82 (C-H). ^{1}H NMR (DMSO, ppm): δ 6.67-7.73 (m, 8H, Ar-

H), 6.78 (d, J = 8.5 Hz, 1H, CO–CH), 7.09 (d, J = 7.1 Hz, 1H, CH–Ar).

1-(1-benzofuran-2-yl)-3-(4-chlorophenyl)prop-2-en-1-one (10)
Yield: 82 %. mp: 176-178 ºC. R_f: 0.53 (Methanol: Toulene 1:4). IR (KBr, cm^{-1}): 1738.54 (C=O), 1598.17 (C=C); 696.30 (C-Cl) 3076.47 (C-H). ^{1}H NMR (DMSO, ppm): δ 6.66-7.89 (m, 8H, Ar-H), 6.69 (d, J = 8.5 Hz, 1H, CO–CH), 7.89 (d, J = 7.1 Hz, 1H, CH–Ar). ^{13}C NMR (DMSO-d6, 100 MHz) δ: 111.06, 114.02, 115.56, 121.16, 131.93, 137.83, 143.86 and 146.41 (Ar), 76.73 (C–Cl), 39.91 (CH_3). EI-MS: m/z $[M+H]^+$ 283.87.

1-(1-benzofuran-2-yl)-3-(4-methoxyphenyl)prop-2-en-1-one (11)
Yield: 87 %. mp: 167-169 ºC. R_f: 0.54 (Methanol: Toulene 1:4). IR (KBr, cm^{-1}): 1745.54 (C=O), 1589.56 (C=C), 2967.56 (O-CH_3) 3045.78 (C-H). ^{1}H NMR (DMSO, ppm): δ 6.87-7.82 (m, 8H, Ar-H), 5.89 (d, J = 8.3 Hz, 1H, CO–CH), 7.72 (d, J = 3.1 Hz, 1H, CH–Ar).

1-(1-benzofuran-2-yl)-3-(4-hydroxy-3-methoxy phenyl)prop-2-en-1-one (12)
Yield: 63 %. mp: 178-180 ºC. R_f: 0.52 (Methanol: Toulene 1:4). IR (KBr, cm^{-1}): 1757.51 (C=O), 1578.92 (C=C); 2924.84 (O-CH3), 3567.78 (O-H), 3056.73 (C-H) ^{1}H NMR (DMSO, ppm): δ 6.84-7.72 (m, 7H, Ar-H), 5.71 (s, 1H, OH), 5.73 (d, J = 6.3 Hz, 1H, CO–CH), and 7.72 (d, J = 6.1 Hz, 1H, CH–Ar).

1-(5-nitro-1-benzofuran-2-yl)-3-phenylprop-2-en-1-one (13)
Yield: 78 %. mp: 145-147 ºC. R_f: 0.57 (Methanol: Toulene 1:4). IR (KBr, cm^{-1}): 1754.45 (C=O), 1573.87 (C=C), 1359.89 (C-NO_2), 3061.43 (C-H). ^{1}H NMR (DMSO, ppm): δ 6.73-7.78 (m, 8H, Ar-H), 5.23 (d, J = 5.8 Hz, 1H, CO–CH), 6.89 (d, J = 7.1 Hz, 1H, CH–Ar).

1-(5-nitro-1-benzofuran-2-yl)-3-(3-nitrophenyl)prop-2-en-1-one (14)
Yield: 83 %. mp: 167-169 ºC. R_f: 0.52 (Methanol: Toulene 1:4). IR (KBr, cm^{-1}): 1756.32 (C=O), 1621.67 (C=C), 1373.76 (C-NO_2), 3045.69 (C-H). ^{1}H NMR (DMSO, ppm): δ 6.72-7.73 (m, 7H, Ar-H), 5.62 (d, J = 6.3 Hz, 1H, CO–CH), 6.59 (d, J = 8.1 Hz, 1H, CH–Ar).

1-(5-nitro-1-benzofuran-2-yl)-3-(4-nitrophenyl)prop-2-en-1-one (15)
Yield: 73 %. mp: 172-174 ºC. R_f: 0.62 (Methanol: Toulene 1:4). IR (KBr, cm^{-1}): 1756.34 (C=O), 1647.51 (C=C), 1354.89 (C-NO_2), 3073.81 (C-H). ^{1}H NMR (DMSO, ppm): δ 6.52-7.61 (m, 7H, Ar-H), 5.69 (d, J = 6.1 Hz, 1H, CO–CH), 6.81 (d, J = 7.2 Hz, 1H, CH–Ar).

1-(5-nitro-1-benzofuran-2-yl)-3-(4-chlorophenyl)prop-2-en-1-one (16)
Yield: 67 %. mp: 180-182 ºC. R_f: 0.52 (Methanol: Toulene 1:4). IR (KBr, cm^{-1}): 1753.11 (C=O), 1643.58 (C=C), 1487.56 (C-NO_2), 2974.23 (C-H). ^{1}H NMR (DMSO, ppm): δ 6.96-8.51 (m, 7H, Ar-H), 6.98 (d, J = 6.7 Hz, 1H, CO–CH), 7.38 (d, J = 6.8 Hz, 1H, CH–Ar). 13C NMR (DMSO-d6, 100 MHz) δ: 110.65, 111.09, 115.78, 116.74, 122.48, 128.89, 141.63 and 143.41 (Ar), 71.43 (C–Cl), 39.01 (CH_3). EI-MS: m/z [M+H]+ 328.38.

1-(5-nitro-1-benzofuran-2-yl)-3-(4-methoxy phenyl)prop-2-en-1-one (17)
Yield: 68 %. mp: 156-158 ºC. R_f: 0.54 (Methanol: Toulene 1:4). IR (KBr, cm^{-1}): 1756.34 (C=O), 1643.45 (C=C), 1487.56 (C-NO_2), 3074.23 (C-H), 2976.57 (O-CH3). ^{1}H NMR (DMSO, ppm): δ 6.78-8.20 (m, 7H, Ar-H), 6.43 (d, J = 6.2 Hz, 1H, CO–CH), 7.21 (d, J = 6.1 Hz, 1H, CH–Ar).

1-(5-nitro-1-benzofuran-2-yl)-3-(4-hydroxy-3-methoxyphenyl)prop-2-en-1-one (18)
Yield: 72 %. mp: 162-164 ºC. R_f: 0.46 (Methanol: Toulene 1:4). IR (KBr, cm^{-1}): 1768.62 (C=O), 1642.69 (C=C), 1452.71 (C-NO_2), 3041.65 (C-H), 2951.49 (O-CH3), 3554.86 (O-H). ^{1}H NMR (DMSO, ppm): δ 6.78-8.20 (m, 7H, Ar-H), 5.76 (s, 1H, OH), 6.43 (d, J = 6.2 Hz, 1H, CO–CH), 7.21 (d, J = 6.1 Hz, 1H, CH–Ar).

1-(5-bromo-1-benzofuran-2-yl)-3-phenyl)prop-2-en-1-one (19)
Yield: 68 %. mp: 145-147 ºC. R_f: 0.45 (Methanol: Toulene 1:4). IR (KBr, cm^{-1}): 1723.53 (C=O), 1650.45 (C=C), 640.56 (C-Br), 3083.58 (C-H). ^{1}H NMR (DMSO, ppm): δ 6.67-7.53 (m, 8H, Ar-H), 5.76 (d, J = 5.6 Hz, 1H, CO–CH), 6.89 (d, J = 6.8 Hz, 1H, CH–Ar).

1-(5-bromo-1-benzofuran-2-yl)-3-(3-nitrophenyl)prop-2-en-1-one (20)
Yield: 71 %. mp: 158-160 ºC. R_f: 0.56 (Methanol: Toulene 1:4). IR (KBr, cm^{-1}): 1767.57 (C=O), 1647.45 (C=C), 637.62 (C-Br), 1452.71 (C-NO_2) 3032.23 (C-H). ^{1}H NMR (DMSO, ppm): δ 6.42-7.51 (m, 7H, Ar-H), 5.82 (d, J = 5.4 Hz, 1H, CO–CH), 6.71 (d, J = 7.8 Hz, 1H, CH–Ar).

1-(5-bromo-1-benzofuran-2-yl)-3-(4-nitrophenyl)prop-2-en-1-one (21)
Yield: 68 %. mp: 167-169 ºC. R_f: 0.56 (Methanol: Toulene 1:4). IR (KBr, cm^{-1}): 1767.57 (C=O), 1651.51 (C=C); 671.56 (C-Br), 1487.91 (C-NO_2) 3083.58 (C-H). ^{1}H NMR (DMSO, ppm): δ 6.71-7.41 (m, 8H, Ar-H), 5.86 (d, J = 5.4 Hz, 1H, CO–CH), 6.61 (d, J = 6.4 Hz, 1H, CH–Ar).

1-(5-bromo-1-benzofuran-2-yl)-3-(4-chlorophenyl) prop-2-en-1-one (22)
Yield: 72 %. mp: 156-158 ºC. R_f: 0.64 (Methanol: Toulene 1:4). IR (KBr, cm^{-1}): 1739.79 (C=O), 1544.98 (C=C), 678.94 (C-Br), 837.11 (C-Cl), 3136.25 (C-H). ^{1}H NMR (DMSO, ppm): δ 6.87-8.93 (m, 7H, Ar-H), 6.56 (d, J = 6.2 Hz, 1H, CO–CH), and 6.67 (d, J = 6.5 Hz, 1H, CH–Ar). ^{13}C NMR (DMSO-d6, 100 MHz) δ: 114.55, 121.67, 122.56, 135.71, 146.29 and 148.32 (Ar), 77.30 (C–Cl), 40.81 (CH_3). EI-MS: m/z [M+H]+ 362.72.

1-(5-bromo-1-benzofuran-2-yl)-3-(4-methoxyphenyl)prop-2-en-1-one (23)
Yield: 67 %. mp: 157-159 ºC. R_f: 0.53 (Methanol: Toulene 1:4). IR (KBr, cm^{-1}): 1789.43 (C=O), 1578.87 (C=C); 623.86 (C-Br), 3014.64 (C-H), 2822.15 (O-CH3); ^{1}H NMR (DMSO, ppm): δ 6.31-8.31 (m, 7H, Ar-H), 6.43 (d, J = 5.2 Hz, 1H, CO–CH), 6.45 (d, J = 6.1 Hz, 1H, CH–Ar).

1-(5-bromo-1-benzofuran-2-yl)-3-(4-hydroxy-3-methoxyphenyl)prop-2-en-1-one (24)
Yield: 76 %. mp: 172-174 ºC. R_f: 0.63 (Methanol: Toulene 1:4). IR (KBr, cm^{-1}): 1757.76 (C=O), 1645.64 (C=C); 677.81 (C-Br), 3056.61 (C-H), 2822.15 (O-CH_3), 3589.52 (O-H). ^{1}H NMR (DMSO, ppm): δ 6.-8.31 (m, 7H, Ar-H), 5.65 (s, 1H, OH), 6.43 (d, J = 5.2 Hz, 1H, CO–CH), 6.45 (d, J = 6.1 Hz, 1H, CH–Ar).

Pharmacology

Antidepressant activity

Pharmacological activities were carried out in accordance to the OECD[16] guidelines and the Institutional Animal Ethical Committee (IAEC) guidelines. The protocols were authorized under Sanction no. IAEC; 1336/ac/10/CPCSEA, dated 9th August, 2022 at Department of Pharmacology, Dr Rajendra Gode College of Pharamcy, Malkapur.

The antidepressant activity of the compounds **(7-24)** was evaluated using Forced Swimming Test (behavioral despair test). [17] The antidepressant activity was carried out on albino mice (20-25g) of either sex as experimental animals. Chlorgyline was used as standard drug. The synthesized compounds (100 mg/kg) and Chlorgyline (20 mg/kg) suspended in aqueous tween 80 (0.5%), were injected as intraperitonealy (i.p.) (n=6). After 1/2 hr, the mouse was dropped into the glass cylindrical container (diameter 10 cm, height 25 cm), containing approximately 20 cm of water at 25 ± 1◦C temperature. Water was replaced between every trial. Each mouse left for 6 minute at the end of the first 2 min; the animals showing initial vigorous struggling were immobile. The immobility times of each mouse were measured over the period of 4 min. Each mouse was judged immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water. Conventional antidepressants decreased the immobility time in this test. Percentage decrease in immobility duration in test and standard drugs relative to controls is calculated and detailed in **Table 2**.

Table 2: Antidepressant activity of synthesized compounds in mice by FST

Compound No.	Duration of immobility (sec.) (mean ±SEM)	Percentage decrease in immobility duration (% DID)	Compound No.	Duration of immobility (sec.) (mean ±SEM)	Percentage decrease in immobility duration (% DID)
7	55.25±4.51	45.83333	17	50±3.91	50.98039
8	38.75±1.49	62.0098	18	73.75±5.58	27.69608
9	52.75±4.44	48.28431	19	72±5.11	29.41176
10	36.5±5.1	64.21569	20	46±6.17	54.90196
11	77.25±3.79	24.26471	21	43±3.70	57.84314
12	70.25±3.03	31.12745	22	33.25±2.48	67.40196
13	68.75±2.78	32.59804	23	37±1.77	63.72549
14	57.25±4.28	43.87255	24	60.75±6.14	40.44118
15	77.25±4.34	24.26471	Standard	27.75±1.65	72.79412
16	30.75±2.32	69.85294	Control	102±6.37	-

Statistical analysis was performed using one-way analysis of variance (ANOVA) with Dunnett's test. $n = 6$; dose = 100 mg/kg. Values are represented as mean ± S.E.M. Values are significant at $***P < 0.001$, compared with control group.

Molecular study

Molecular docking platform

Pyrx in autodock vina software was used to conduct a molecular docking analysis on all synthesized compounds that were chosen as ligands against the target monoamine oxidase A (MAO-A) enzyme [18].

Selection of protein and preparation of its structure

Synthesized compounds were analysed by *in silico* method using the crystal structure of monoamine oxidase B (PDB ID: 2BXS) were downloaded in PDB format from the RCSB protein data bank (www.rcsb.org) with resolution 3.15 Å selected for the present study. The structure of the protein target was prepared, refined and visualize using discovery studio visualizer 2021 (https://discover.3ds.com/). (https://www.ebi.ac.uk/thornton-srv/software/LigPlus/) 2BXS is a complex structure containing chains A, and B whereas chain A was used to prepare macromolecules and other co-crystallized water molecules and non-standard residue, were removed and added the polar hydrogen atom. Energy minimization and addition of missing amino acid residue done using Swiss-Pdb viewer (https://spdbv.vital-it.ch/). We used autodock vina, to build geometry optimization and to add polar hydrogen, gasteiger charges as well as Kollman charges [19].

Selection of ligands and preparation of its structure

The chem sketch software was used to draw structure of all synthesized compounds. Energy minimization and geometrical confirmation done by the PyRx-virtual screening tool. All ligands were put into the PyRx virtual screening programme using the Open Babel control and converted into the PDB format. Additionally, to obtain atomic coordinates for molecules, the Autodock Vina tool (http://vina.scripps.edu/) assists in identifying the torsion root, correcting torsion angles, altering charges and universal force field optimization (UFF) [20].

Receptor grid preparation

A mesh appears at the top of the protein structure. The size of the grid will be adjusted according to the binding pocket of the receptor at coordinate X, Y, and Z were set around the centroid of the active site to center X= 21.8060, Y= 119.8855, Z= 52.6093 and dimension coordinates at X= 50.9684, Y= 43.6562, Z= 45.3702. Further, PyRx in Autodock Vina will start. However, the protein-ligand interaction was analyzed using digital studio visualizer (DSV) 2021 (https://discover.3ds.com/).

Prediction of ADME properties

The synthesized compounds were evaluated in order to predict their ADME characteristics. The several ADME parameters investigated includes TPSA, the quantity of rotatable bonds, molecular volume, number of hydrogen acceptors, the number of hydrogen donors and Lipinski rule violations were calculated by using SWISSADME online tool.

RESULT AND DISCUSSION

Synthesis

The present work deals with synthesis and characterization of several chalcones of benzofuran derivatives. For this, two different steps were carried out. In the first step, different substituted salicyldehyde (1-3) was reacted with 1-chloroacetone to yield the 3-hydrazinylidene1,3-dihydro-2H-indol-2-one (4-6). In the next step the chalcones (7-24) were synthesized by the reaction between benzofuran and substituted aromatic aldehydes. NMR spectra of this compound exhibited prominent signals at δ 6.39 ppm and 5.57 ppm corresponding to the proton present at double bonded carbon atoms. The aromatic protons belonging to fused benzene ring and substituted benzene ring was exhibited around d 6.45 to 8.37 ppm presenting eight protons. The IR spectrum provides with an appearance of ketone group at amine functional group at 1756.67 cm−1 while the respective nitro, bromo, methoxy and hydroxy group at 1378 cm−1 , 678 cm−1 , 2967 cm−1 and 3567 cm−1 respectively. The ^{13}C NMR Spectra showed the signals majorly in the range of 111-145 for C-C group and methyl group at 39. The EI-MS of all compounds displayed the [M + H]+ confirming their molecular weight.

Pharmacology

The compounds (7-24) were evaluated for antidepressant activity by forced swim test (FST) in mice at dose of 100 mg/kg and compared with the standard drug Chlorgyline (20 mg/kg). The standard chorgyline reduced immobility times to 72.79% at a dose level of 20 mg/kg. In our research all the synthesized derivatives can produce significant reduction in the immobility time when compared to the standard drug. Compounds 10, 16

and 22 were found to be the most potent derivatives from the series, showing percentage decrease in immobility duration 64.21, 69.85 and 67.40 respectively. Some of the compounds 9, 20, 21, and 24 showed moderate activity while some showed lesser activity.

Molecular docking

Molecular docking studies

The docking score and binding energy of all compounds targeting MAO-A and interaction of amino acid residue with bonding distance are shown in **Table 3.**

The reference standard binding energy of Clorgyline is -9.7 kcal/mol. The presence of chlorine group in Clorgyline which interacts with amino acid along with conventional hydrogen bonding CYS323 and hydrophobic interaction with LEU337,PHE208,ILE335, GLU216 and TYR407 (**Figure 2**).

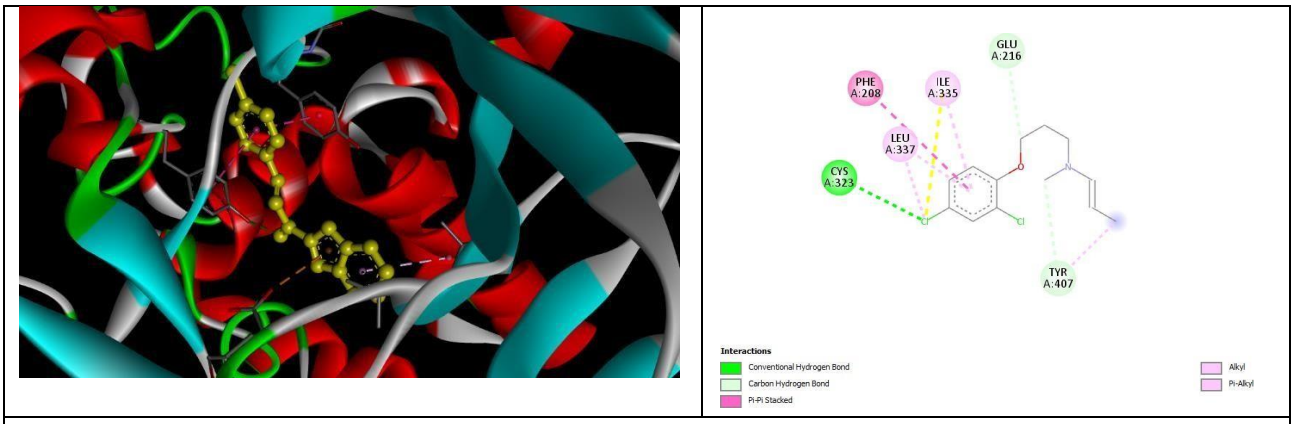
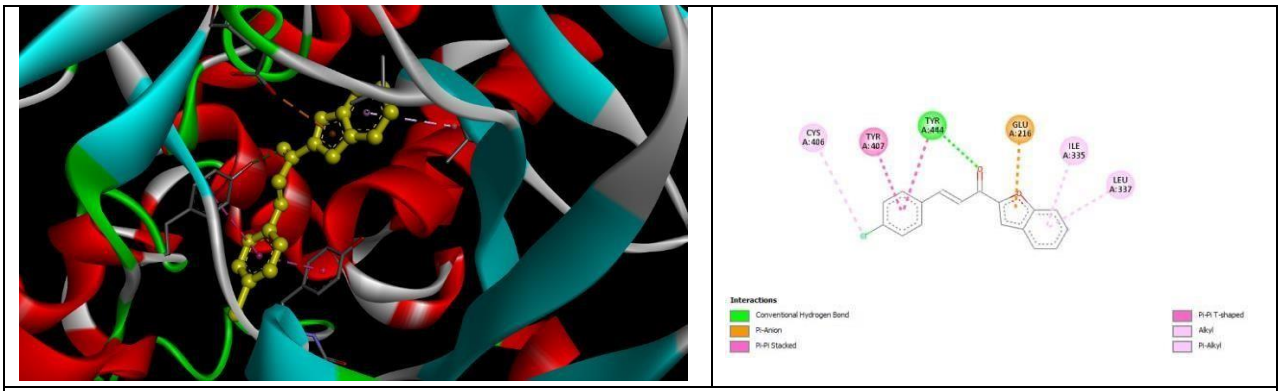
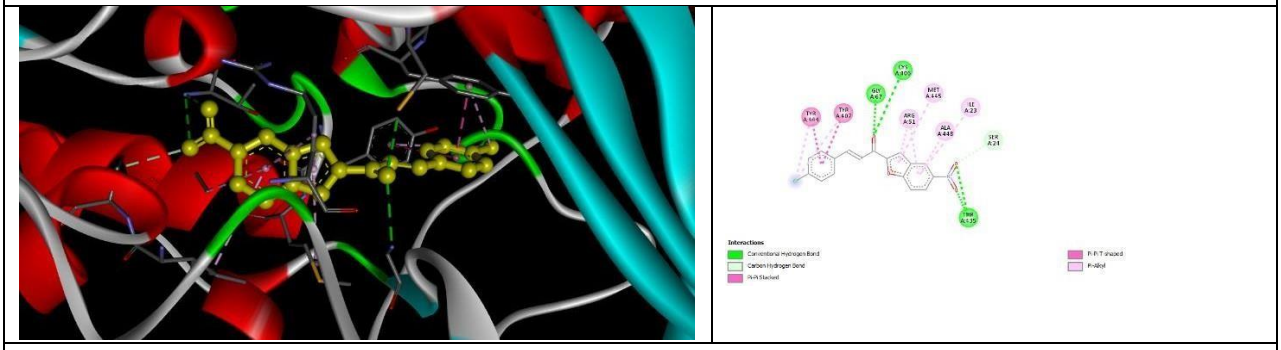


Figure 2 Binding interaction and docked pose of clorgyline targeting MAO-A (PDB ID:2BXS). The ligand (Pink) and amino acid residue (Red) binding pocket represent in ball & stick

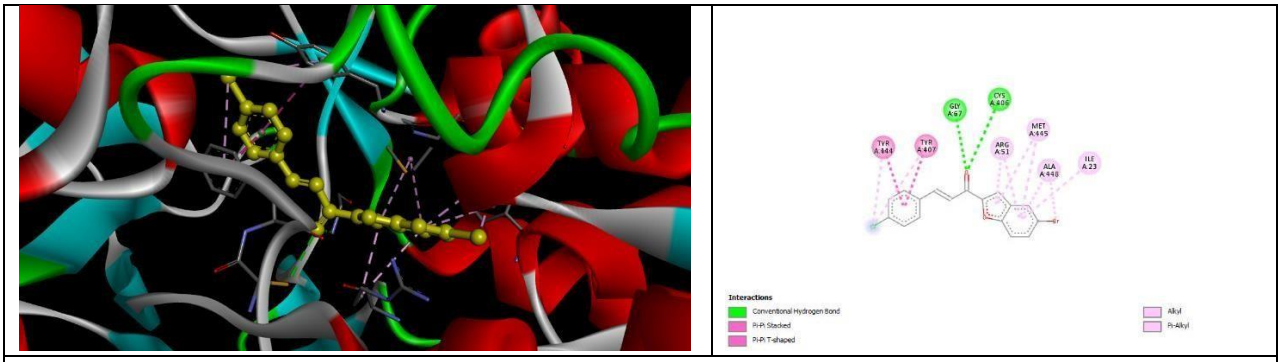
Analysis of binding affinity of selected synthesized ligands in the ranges of -10.2 to -12.1 kcal/mol. from docked result, it is observed that 7-24 exhibit highest binding affinity in complex with MAO-A enzyme, amongst compounds 10, 16 and 22 showed docking score -10.9 kcal/mol, -11.5 kcal/mol and -11.3 kcal/mol as compared to other compounds. The RMSD value of these three compounds are near to zero.



10



16



22

Figure 3 Synthesized compounds binding interaction with MAO-A (PDB ID: 2BXS) visualized by DSV

Based on visual inspection, computational docking of compounds on targeting MAO-A substantially involves many types of interactions, including hydrogen bonds and hydrophobic bonds, alkyl, pi-stacking, and pi-alkyl interaction for the stable complex with MAO-A. Additionally, it showed that clorgyline had a similar binding pattern to MAO-A.

From all the docked compounds it was found out that the compounds 10, 16 and 22 were suitably positioned in the pocket of MAO-A. The keto group (=O) of 10 interacted with conventional hydrogen bonding with amino acid TYR444. Moreover, chorine group of 10 is attributed pi-alkyl hydrophobic bonding with CYS406. Further, ring residue of 10 is attributed to the formation pi-anion, pi-pi-stacked and pi-pi T-shaped bonding with TYR407, TYR444, GLU216, ILE335 and LEU337. Compound 16 has fitted in the pocket region of MAO-A enzyme. The presence of keto group has interacted with conventional hydrogen bonding with amino acid residue GLY7 and CYS406. Additionally, NO_2 group of 16 has attributed to formation hydrogen bonding with THR435. Compound 16 has hydrophobic interaction with TYR444, TYR407, ARG51, MET445, ALA448, ILE23 and SER24. Further, docking study visualized and revealed that compound 22 has prominently fitted with MAO-A pocket region. The presence of keto group interacted with GLY67 and CYS406 with hydrogen bonding and the centroid benzene ring interacted with hydrophobic region is attributed to the formation of the pi-alkyl and pi-cationic & anionic interaction and the binding pocket formed by TYR444, TYR407, ARG51, MET445, ALA448 and ILE23. **(Figure 3)**

Table 3: Inhibitors interactions with the α-amylase binding (PDB ID: 3BC9)

Ligands/ Inhibitors	**Binding Energy (kcal/mol)**	**Amino acid interaction**	
		With hydrogen bond	**With hydrophobic bond**
7	-10.5	CYS172, TYR435	TYR326, CYS172, LEU171, ILE199, TYR398
8	-10.8	CYS172	TYR398, TYR326, ILE199, LEU171
9	-11.6	THR426, SER15, ARG36	ILE264, ALA35, LEU268, TYR393, ALA263, ARG42, LYS271, PRO265
10	-10.9	TYR444, CYS172,TYR435	TYR407, TYR444, GLU216, ILE335 LEU337
11	-10.5	TYR435,CYS172	TYR326,CYS172,LEU171, ILE316,LEU164,ILE199
12	-10.2	ARG36,ARG233, LEU33,ILE14	LEU268,ALA35,VAL10,VAL235, ARG42,GLY13,PRO265
13	-11.1	SER59, LYS296,TYR435	ILE199, TYR326,TYR398,CYS172
14	-10.7	THR195,THR196,TRP119	ILE316,ILE199,PHE103
15	-12.1	SER15, TYR435	ARG42, TYR398,TYR60
16	-11.5	SER59,LYS296,TYR435	ILE199,CYS172,TYR326, ILE316,TYR398
17	-11.1	SER15,THR426,TYR435, GLY58	ARG42,ALA439,TYR398,TYR60
18	-10.7	SER59, LYS296,TYR435	ILE199,TYR326,TYR398,CYS172

19	-10.7	TYR435,CYS172	TYR326,CYS172,LEU171, ILE316,LEU164,ILE199
20	-11.1	TYR435,CYS172	TYR326,CYS172,LEU171
21	-11.8	TYR435,CYS172	ILE316,LEU164,ILE199
22	-11.3	GLY67, CYS406	TYR444, TYR407, ARG51, MET445, ALA448, ILE23
23	-11.0	SER59, LYS296	TYR326,TYR398,CYS172
24	-10.7	SER15,THR426,TYR435, GLY58	ARG42,ALA439,TYR398,TYR60
Clorgyline	-9.7	CYS323	LEU337, PHE208,ILE335, GLU216, TYR407

The molecular interaction of some compounds 12,13, 14,15,17,18,19,20,21,23 and 24 were also found to target some amino acid residue as reference standard compound.

ADME Properties

Based on the ADMET studies (**Table 4**), all the selected compounds obey Lipinski's rule. Followed, all compounds are an acceptable range for TPSA, Log P, and BBB parameters, and also, ligands are satisfied % HIA, bioavailability score, and total clearance. Further, human intestinal absorption (HIA, %) of synthesized compounds having in the ranges of 98.66 to 100.

Table 4: ADME and toxicity profiles of ligands with high docking scores

ADME Properties	Molecular Formula	Molecular Weight [g/mol]	Log P	TPSA [A^0]	HB Donor	HB Acceptor	Aqueous Solubility [log mol/L]	Human Intestinal Absorption (%)	Blood brain barrier
7	C17H1202	248.28	4.3289	30.21	0	2	-4.708	95.95	0.172
8	C17H11NO4	293.27	4.2371	76.03	0	4	-5.257	93.699	-0.308
9	C17H11NO4	293.27	4.2371	76.03	0	4	-5.262	93.497	-0.302
10	C17H11CLO2	282.72	4.9823	30.21	0	2	-5.348	94.541	0.17
11	C18H14O3	278.30	4.3375	39.44	0	3	-4.924	97.096	0.098
12	C18H14O4	294.30	4.0431	59.67	1	4	-4.567	94.341	-0.142
13	C17H11NO4	293.27	4.2371	76.03	0	4	-5.153	95.322	-0.327
14	C17H10N2O6	338.27	4.1453	121.85	0	6	-5.512	100	-0.867
15	C17H10N2O6	338.27	4.1453	121.85	0	6	-5.518	100	-0.867
16	C17H10CLNO4	327.72	4.8905	76.03	0	4	-5.725	93.913	-0.536
17	C18H13NO5	323.30	4.2457	85.26	0	5	-5.502	92.304	-0.573
18	C18H13NO6	339.30	3.9513	105.49	1	6	-4.323	98.66	-0.632
19	C17H11BrO2	327.17	5.0914	30.21	0	2	-5.545	94.581	0.137
20	C17H10BrNO4	372.17	4.9996	76.03	0	4	-5.764	92.543	-0.553
21	C17H10BrNO4	372.17	4.9996	76.03	0	4	-5.769	92.059	-0.553
22	C17H10BrCLO2	361.62	4.7448	30.21	0	2	-5.972	91.351	0.142
23	C18H13BrO3	357.20	5.1	39.44	0	3	-5.384	93.199	0.284
24	C18H13BrO4	373.20	4.8056	59.67	1	4	-4.409	90.843	0.03

All compounds are high percentage of intestinal absorption. Depression is a brain disease so to require brain targeted medicines. All the compounds are easily crosses the blood brain barrier. Therefore, all compounds may show antidepressant activity. Only compounds 19 and 23 are violated Lipinski's rule.

Conclusion

In summary, we have described the design and synthesis of chalcones of benzofuran for antidepressant potential. The compounds were successfully synthesized following a twostep reaction to yield eighteen derivatives as 1-(1-benzofuran-2-yl)-3-(substituted phenyl)-prop-2-en-1-one (7–24). All the spectral studies were in good agreement with the final structures of the titled derivatives. All synthesized compounds were evaluated for antidepressants activity in FST. Among all derivatives tested in the present study, compounds 10, 16 and 22 exhibiting promising antidepressant activity comparable to that of the standard drug chlorgyline. Molecular docking studies are also in agreement with the pharmacological evaluation with potent compounds exhibiting dock score of -11.5. From the series of compounds it was found that the substitution of nitro and chloro group i.e. electron withdrawing group at R_1 and R_2 position exhibited promising antidepressant activity. Therefore, the medicinal chemists who are involved in the

creation of MAO-A inhibitors might benefit greatly from our research.

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