Synthesis and Potential Anti-Anxiety Activity of Chalcone Benzofuran Derivatives

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Abstract

Background: The molecular scaffold chalcones of benzofuran are exhibiting various biological activities. In this research, we are explore pharmacological potential of chalcones of benzofuran as anti-anxiety agents. To get insight of the intermolecular interactions, the molecular docking studies are performed at active site of GABA-A enzyme.

Aim: In this study, an attempt has been made to design,synthesis and pharmacological evalution of novel molecular scaffold which contains chalcones of benzofuran as an antianxiety activity.

Methods: The spectroscopic analysis were performed for the characterization of various synthesized derivatives. The derivatives were evaluated for anti-anxiety activity by using elevated plus maze test. Molecular docking studies of the synthesized derivatives with GABA-A enzyme were carried on Pyrx online software.

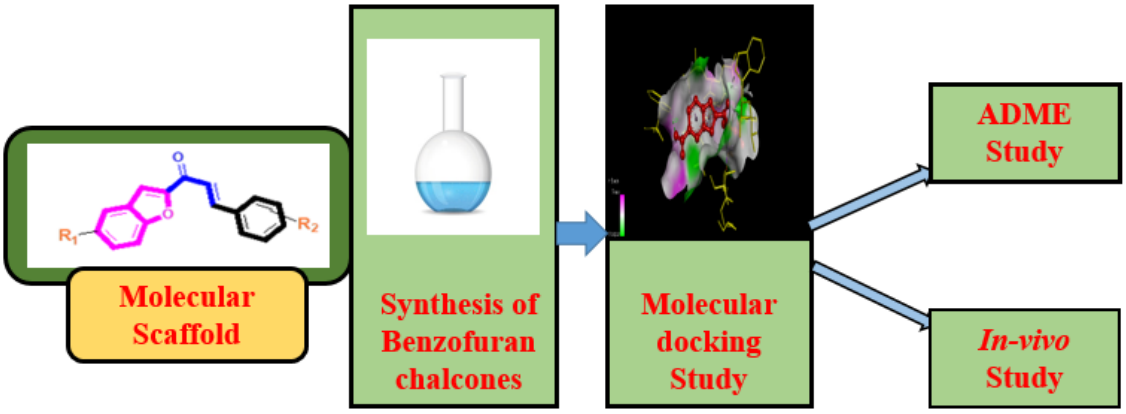
Results: All synthesized derivatives were assigned on the basis of IR, 1H NMR and mass spectra. The antidepressant evaluation exhibited final derivatives 9, 15 and 21 as promising molecules with significant percentage preference to open arm are 60, 67.5 and 65.61 respectively. Molecular docking studies are also in agreement with pharmacological evaluation with potent compound 15 exhibiting dock score -9.0.

Conclusion: Synthesized derivatives may have the potential to be developed into an anti-anxiety agent. The substitution of nitro, i.e., electron withdrawing groups, at R_1 and R_2 position, showed promising anti-anxiety efficacy, according to the series of compounds. Therefore, the medicinal chemists who are involved in the creation of GABA-A inhibitors might benefit greatly from our research.

Keywords: Benzofuran, Chalcones, Anti-anxiety activity, Molecular docking, GABA-A

DOINUMBER:10.48047/NQ.2022.20.19.NQ99374 NEUROQUANTOLOGY2022;20(19):4109-4126

Graphical Abstract



1. INTRODUCTION

Anxiety is neurological disorder of the central nervous system that affect mental condition of human. Anxiety mainly occurs due to chronic stress condition. Stress may leads to imbalance of adrenergic neurotransmitter [1, 2]. According to literature, approximately 40 to 70 percentage of people with depressive disorders and those with anxiety symptoms simultaneously meet the criteria for at least one form of anxiety disorder [3, 4]. The various heterocyclic compounds designated as significant pharmacological effect. As reported in the numerous literature, It was discovered that the benzofuran nucleus was one of the significant and popular heterocyclic rings for the synthesis of bioactive compounds [5]. As per available data, benzofuran derivatives have a wide range of biological effects, including anti-epileptic [6],CNS depressant [7], sedativehypnotic [8], anti-hypertensive [9], analgesic, and anti-inflammatory action [10]. Amiodarone, angelicin, bergapten, nodekenetin, xanthotoxin, and usnic acid are some of the most well-known benzofuran compounds having several pharmacological uses. 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 [11, 12]. 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 [13]. Since Perkin created benzofuran for the first time in 1870 and Kraemer and Spilker discovered it in coal tar in 1890, it has been the subject of intense research. There have been several important monographs on it. [14]

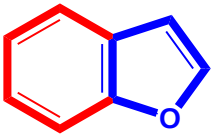
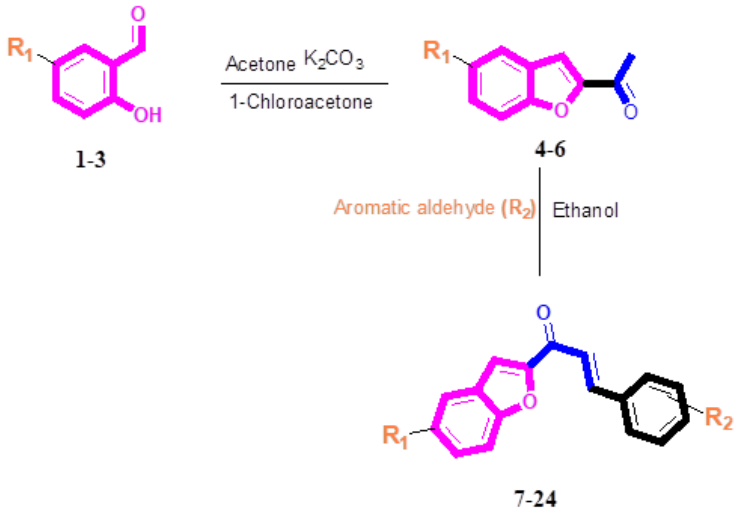


Figure 1: Structure of Benzofuran

In the circuit of fear, where GABA interneurons play crucial roles in the, storage, learning, and extinction of fear, the role of GABA neurons in the control of behaviour has been widely explored. Therapeutic modulators of α_2/α_3 $GABA_A$ receptors, such as TPA023, have shown clinical proof of concept as novel benzodiazepines that are preferable to conventional ones since they lack sedation and have much reduced or no dependence liability [15].

Chalcone and its derivatives are also of growing interest in academia and industry [16]. The pure chalcone isolates from various plants have undergone clinical trials for the treatment of viruses, tumours, and CNS disorders.

So, in the current study, we designed and prepared benzofuran chalcones to increase their anti-anxiety potential. Our study centred on the identification of novel GABA A inhibitors that may be utilised to treat anxiety. The scheme of the present study is outlined in **Scheme 1** along with the list of substitutions shown in **Table 1**.



Scheme 1: Synthetic scheme

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-

2. MATERIALS 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 products produced after purification through recrystallization are used to calculate the percentage yields. 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 $CDCl_3$ 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∘ 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. 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-methoxyphenyl)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-methoxyphenyl)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

Antianxiety activity

Pharmacological activities were performed 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 8th July 2022 at Dept of Pharmacology of Dr. Rajendra Gode College of pharmacy Malkapur. Anti-anxiety activity of the compounds **(7-24)** was evaluated using Elevated plus maze test (EPM). The Elevated plus-maze apparatus was used for evaluation purpose. The apparatus comprised of two open arms (35 × 5 cm) and two closed arms (30× 5× 15cm) that extended from a common central platform (5 × 5 cm). The floor and the walls of each arm were wooden and painted black. The entire maze was elevated to a height of 50 cm above floor level as validated and described by Lister.[12,13] Testing was conducted in a quiet room that was illuminated only by a dim light.

Elevated Plus Maze test

The synthesized compounds (100 mg/kg) and diazepam (2 mg/kg) suspended in aqueous tween 80 (0.5%), were injected as intraperitonealy (i.p.) (n=6), 30 min before their placement on the EPM. To begin a test session, mice were placed on the open arm facing the centre of the maze. An entry into an arm was defined as the animal placing all four paws over the line marking that area. The number of entries and the time spent in the open and closed arms were recorded during a 5-min test period. The percentage of open arm entries (100 × open/total entries) was calculated for each animal. Between each trial, the maze was wiped clean with a damp sponge and dried with paper towels. All the results of the antianxiety activity are given in **Table 2.**

Table 2: Antianxiety activity of synthesized compounds in mice by EPM

Compound No.	No. of entries in open arm	Average time spent in open arm (s)	% preference of open arm	Compound No.	No. of entries in open arm	Average time spent in open arm (s)	% preference of open arm
7	2.75±0.41	27	30.55	**17**	2.5±0.75	28.5	22.72
8	2.75±0.41	28.25	25	**18**	2.5±0.55	32.5	25
9	6±0.35	33.5	60	**19**	5.5±0.55	41.5	50
10	4.5±0.55	35.5	37.5	**20**	2.5±0.55	35.75	20.83
11	6.25±0.73	38.5	62.5	**21**	2±0.35	29.25	65.61
12	6±0.93	34	66.66	**22**	5.5±0.55	36	55
13	4±0.79	42.25	44.44	**23**	1.5±0.55	32.5	16.66
14	2.75±0.41	34.25	22.91	**24**	2.5±0.55	26.25	19.23
15	6.75±0.64	39.25	67.5	**Standard**	7±0.35	42.5	70
16	1.75±0.73	27.75	14.58	**Control**	1.5±0.55	9.75	-

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 GABA A enzyme [17].

The three-dimensional structure of GABA-A (PDB code 6CDU; protein data bank) was retrieved from RCSB PDB for docking purposes. Using the autodock vina 1.2.0 programme, the receptor molecule has been optimised and verified [18].The genetic algorithm approach is used by the autodock vina for molecular docking [19].

The scoring system is based on the moiety that is most compatible with the target in terms of energy and interactions between molecules. By using the docking technique, the ligands that were already inside the receptor in bound form were eliminated. For this investigation, all of the ligands were created, docked using flexible docking mode, and atoms in the active site that were 5.0 Å or less from the amino acid residues were chosen. In order to compare the compounds under research with regard to in silico analysis, the common drug diazepam was also included.

Selection of protein and preparation of its structure

Synthesized compounds were analysed by *in silico* method using the crystal structure of GABA A (PDB ID: 6CDU) 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/) 6CDU 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 [20].

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) [21].

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.

3. 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

Synthesized compounds (7-24) were evaluated for antianxiety activity by using elevated plus maze test (EPM) in mice at 100 mg/kg and compared with the standard drug diazepam (2 mg/kg) suspended in aqueous tween 80 (0.5%), were injected as intraperitonealy (i.p.) (*n*=6), 30 min before their placement on the EPM. Compounds 9, 15 and 21 were found to be the most potent derivatives from the series. Some of the compounds 8, 16, 20, and 23 showed moderate activity while some showed lesser activity.

Molecular docking

Molecular docking studies

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

In the Pyrx in Autodock module, docking computations and energy minimization were set. The PDB code for the GABA-A's active sites, 6CDU, was discovered to contain the interacting residues VAL73, ASN60, TRP66, TRP72, GLN62, ILE67, GLU59, GLU64, VAL58, LEU56, VAL58, VAL73, and ILE67.

In order to investigate the molecular interactions and binding modes of titled derivatives, we docked these derivatives with GABA -A enzyme (PDB code 6CDU) using autodock vina 1.2.0 software. The autodock vina uses genetic algorithm method for molecular docking. Prior to carrying out docking the GABA-A enzyme was prepared for docking by removing the ligands and water molecules. The docking was carried out for all synthesized compounds.

Table 3: Inhibitors interactions with GABA -A enzyme (PDB code 6CDU)

Ligands/ Inhibitors	Binding Energy (kcal/mol)	Amino acid interaction		
		With hydrogen bond	Interaction distance (Å)	With hydrophobic bond
7	-8.0	CYS172, TYR435	4.5621 3.21451	TYR326, CYS172,LEU171, ILE199, TYR398
8	-8.7	CYS172	4.5621	TYR398, TYR326, ILE199, LEU171
9	-8.9	LYS90 THR61 VAL73	3.20242 3.21373 3.11616	GLU64, GLU59, VAL73, GLU62, VAL58, ILE67
10	-8.5	TYR444 CYS172 TYR435	3.2444 4.5668 6.213	TYR407, TYR444, GLU216, ILE335 LEU337
11	-7.5	TYR435 CYS172	3.587 5.5321	TYR326,CYS172,LEU 171, ILE316,LEU164,ILE19 9
12	-8.0	ARG36 ARG233	3.2456 5.2145	LEU268,ALA35,VAL1 0,VAL235, ARG42,GLY13,PRO26 5
13	-8.7	SER59 LYS296	5.2456 4.2356	ILE199, TYR326,TYR398,CYS 172
14	-7.9	THR195 THR196	2.2564 2.589	ILE316,ILE199,PHE10 3

15	-9.0	VAL73 ASN60 TRP66	3.12 3.02 3.24	TRP72,GLN62, ILE67, GLU59,GLU64,VAL58
16	-8.0	SER59 LYS296	4.568 4.897	ILE199,CYS172,TYR326, ILE316,TYR398
17	-8.3	SER15 THR426	2.5687 3.568	ARG42,ALA439,TYR398,TYR60
18	-8.3	SER59 LYS296	4.3256 3.5891	ILE199,TYR326,TYR398,CYS172
19	-8.3	TYR435 CYS172	4.5589 4.2135	TYR326,CYS172,LEU171, ILE316,LEU164,ILE199
20	-7.7	TYR435 CYS172	2.3654 2.5681	TYR326,CYS172,LEU171
21	-8.8	GLU59 TRP66	4.28 5.21	LEU56, VAL58, VAL73, ILE67
22	-7.4	GLY67 CYS406	4.521 4.67	TYR444, TYR407, ARG51, MET445, ALA448, ILE23
23	-8.4	SER59 LYS296	5.236 4.5897	TYR326,TYR398,CYS172
24	-8.4	SER15 THR426	2.6325 2.3651	ARG42,ALA439,TYR398,TYR60
Diazepam (Standard drug)	-7.2	LYS90 TRP72 VAL73	3.28705 3.35126 3.1335	LEU56, VAL58, TRP66

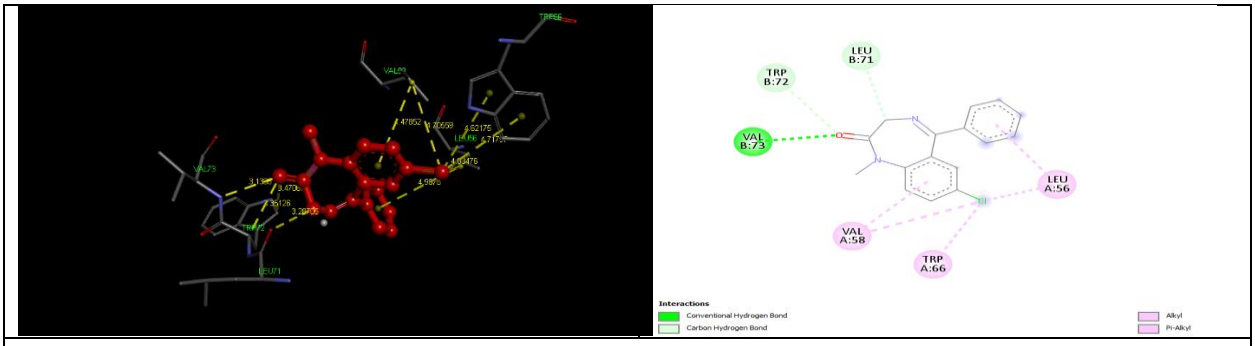
All of the synthetic derivatives have a similar binding mechanism and dock in about the same locations inside the enzyme's active region. **Figure 2** depicts the docking modes of 9, 15, and 21 with all three significant interactions, including hydrogen bonds, hydrophobic interactions, and Van der Waals forces. It was further confirmed by molecular docking data, which showed that the energies for 9, 15, and 21 were, respectively, -8.9 kcal/mol, -9.0 kcal/mol, and -8.8 kcal/mol, the highest in the series of synthesised derivatives and equivalent to the standard drug diazepam with a score of -7.2 kcal/mol.

Compound 9 showed a hydrogen bond with GABA-A at bond distances of 3.20242, 3.21373, and 3.11616 with respect to LYS90, THR61, and VAL73. Hydrophobic interaction with amino acid residue GLU64, GLU59, VAL73, GLU62, VAL58 and ILE67.

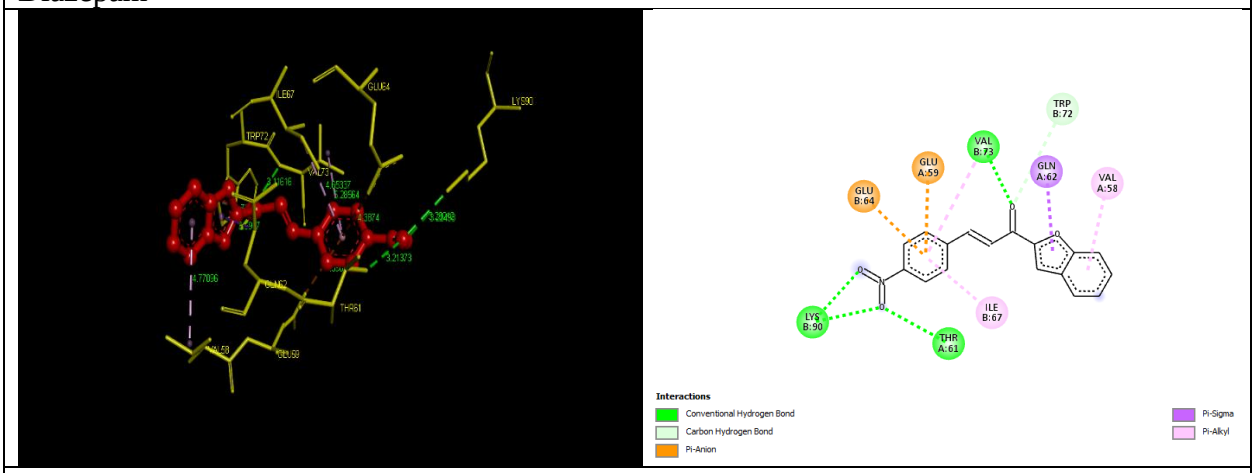
Due to the presence at compound 15 of nitro groups at positions R1 and R2 with hydrogen bonding VAL73, ASN60, and TRP66 showed that it attaches to the active site of the GABA-A enzyme by generating hydrogen bonds (bond lengths: 3.12, 3.02, and 3.24, respectively). It was discovered that the majority of the five and six membered centroid rings which produce hydrophobic contacts include TRP72, GLN62, ILE67, GLU59, GLU64, and VAL58.Compound 21 prominently displayed that it binds to the active site of

GABA-A enzyme by forming hydrogen bond with GLU59 and TRP66 [bond length: 4.28 Å and 5.21 Å]. Hydrophobic interactions were found to be mostly among LEU56, VAL58, VAL73 and ILE67.

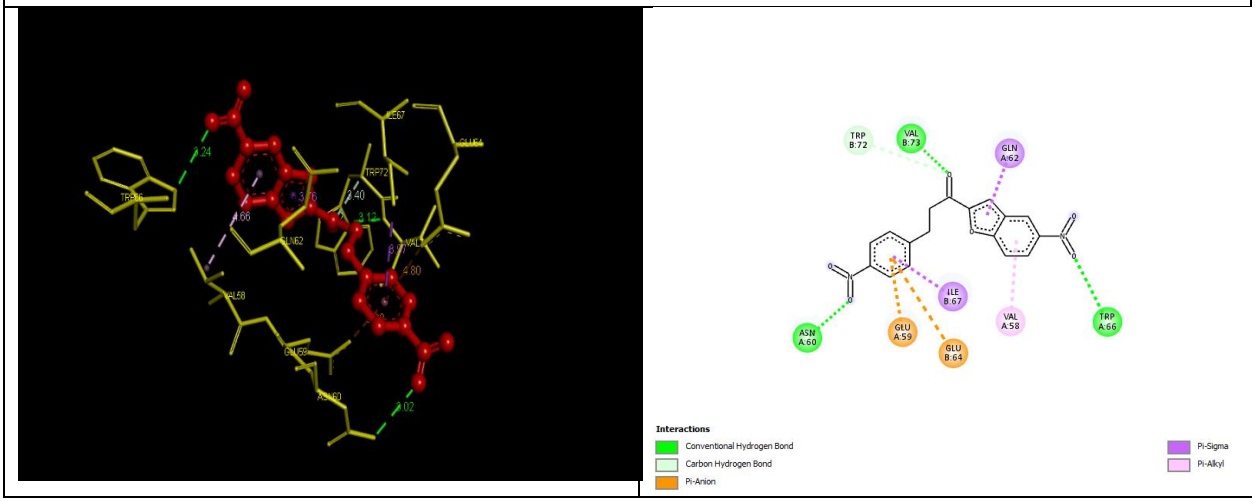
Compounds 9, 15, and 21 had a stronger interaction compared to the reference standard diazepam. Compound 15 further exhibited the important traits of aromaticity, solvent accessible surface area (SAS), ionizability, and hydrogen bonding. All other synthetic compounds were stabilised through interactions between drugs and receptors that involved both hydrogen bonds and hydrophobicity. The ring of all synthetic compounds undergoes an additional hydrophobic contact. After docking with an enzyme residue, the named scaffold may have certain binding interactions.



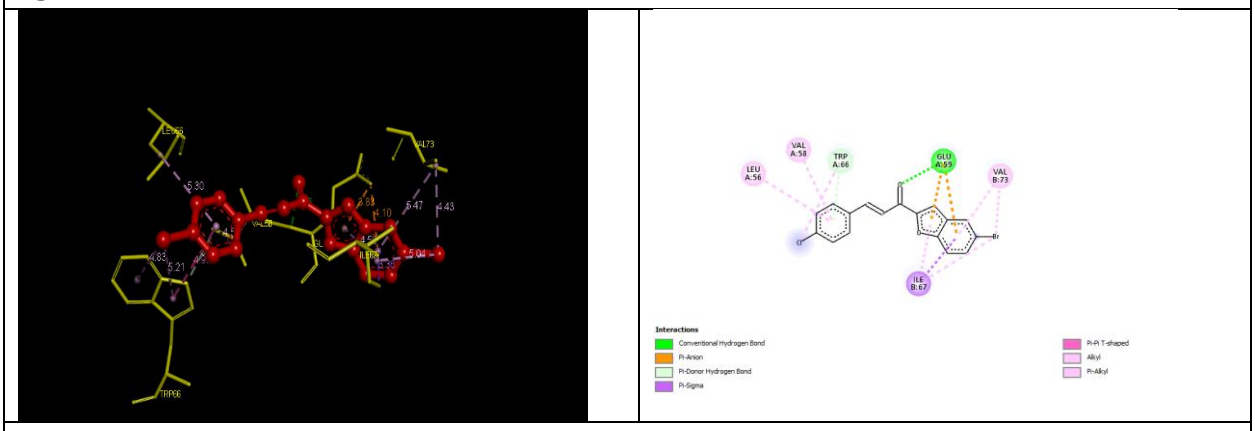
Diazepam



9

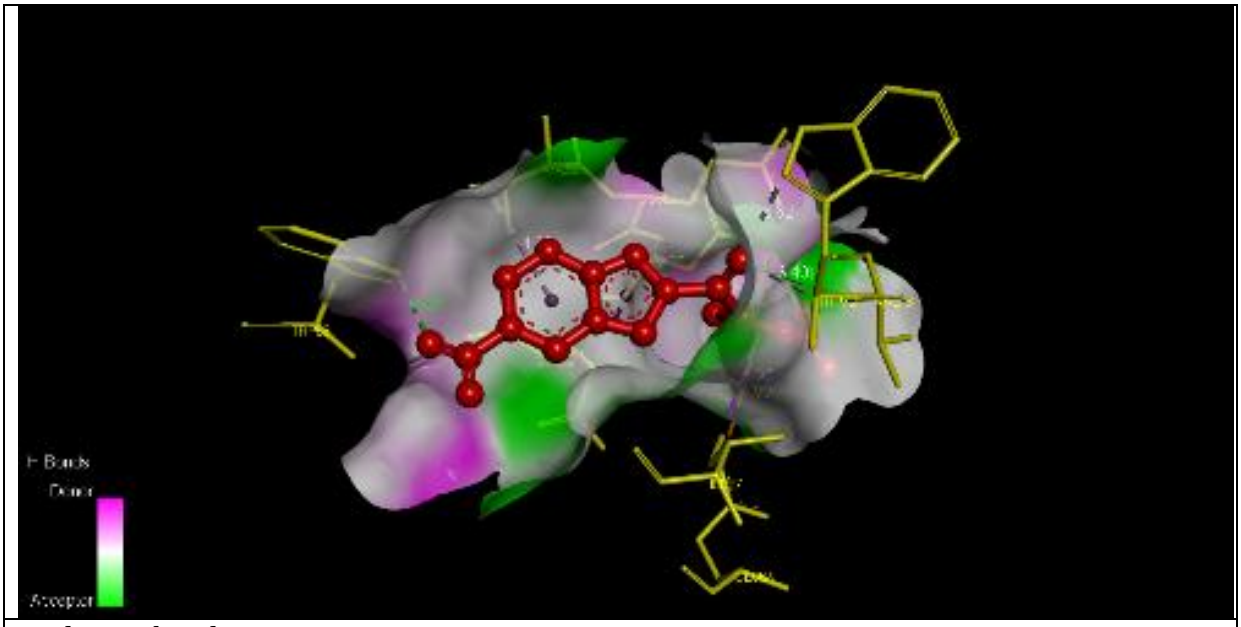


15

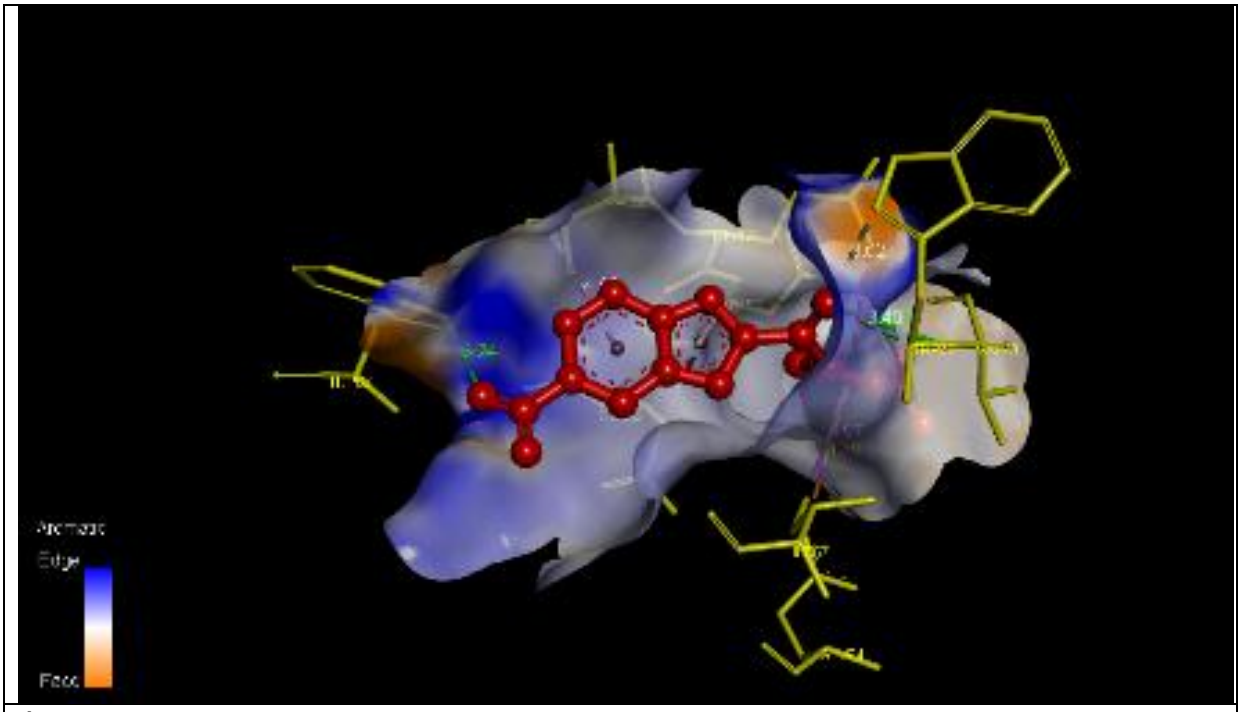


21

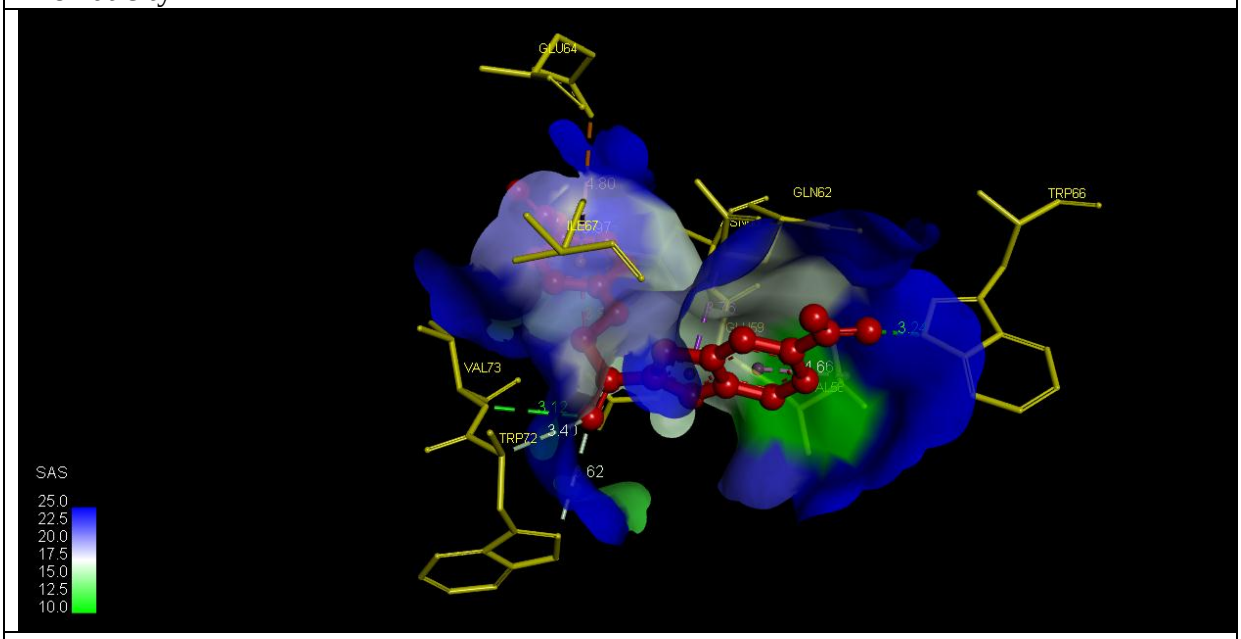
Figure 2: Binding interaction and docked pose of standard drug diazepam, compound (9,15 and 21) targeting GABA-A (PDB ID: 6CDU). The ligand (black) and amino acid residue binding pocket represent in ball & stick



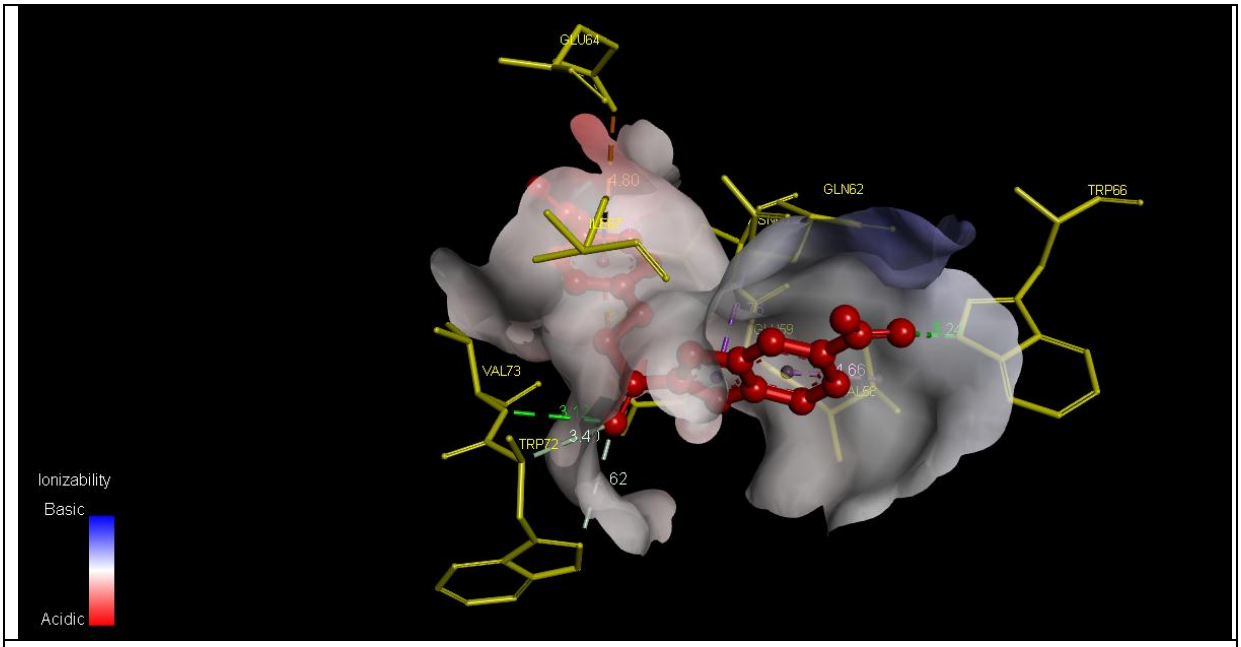
Hydrogen bonding



Aromaticity



SAS



Ionizability

Figure 3: Hydrogen bonding, aromaticity, solvent accessible surface (SAS) and ionizability of Compound 15

Based on visual inspection, computational docking of compounds on targeting GABA-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 GABA-A. Additionally, it showed that diazepam had a similar binding pattern to GABA-A.

ADME Properties

Based on the ADMET studies (Table 3), 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. All compounds are high percentage of intestinal absorption. Therefore, all compounds may show antidepressant activity. Only compounds 19 and 23 are violated Lipinski's rule.

Table 3: 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

4. CONCLUSION

In summary, we have labelled to developed and synthesized novel structure of chalcones of benzofuran for antianxiety potential. The compounds were successfully synthesized following a two step 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 antianxiety activity. Among all derivatives tested in the present study, compounds 9, 15 and 21 exhibiting promising antianxiety effect comparable to that of the standard drug diazepam. Molecular docking studies are also in agreement with the pharmacological evaluation with potent compounds exhibiting dock score of -9.0. From the series of compounds it was found that the substitution of nitro group i.e. electron releasing group at R_1 and R_2 position exhibited promising antianxiety activity. Therefore, the medicinal chemists who are involved in the creation of GABA-A inhibitors might benefit greatly from our research.

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