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Original Research Article

The Synthesis, Characterisation and Biological Evaluation of Novel Benzofuran Derivatives

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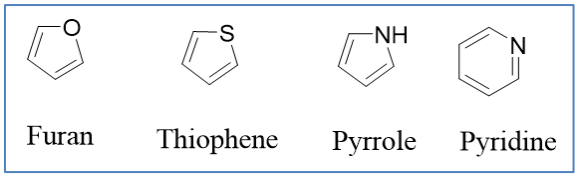
Abstract: As Non-steroidal anti-inflammatory drugs plays a critical role in the management of multiple disorders, the demand of its existence in the clinical therapeutics is crucial. Hence, in this study synthesis, characterisation, and biological evaluation of novel benzofuran derivatives were performed. All synthesized compounds were purified by successive recrystallization from the appropriate solvents. The purity of compounds was checked by the TLC (thin layer chromatography). The characterization was done based on MP (melting point) determination and its properties was examined through TLC, FT-IR, ^{1}H NMR, ^{13}C NMR and mass spectroscopy. The anti-inflammatory activity of novel Benzofuran derivatives was estimated by rat paw oedema in vivo anti-inflammatory assay.

Keywords: Benzofuran, Synthesis, Characterisation, Anti-Inflammatory, Heterocyclic Compounds.

INTRODUCTION

Substances that are heterocyclic have at least one heteroatom—a non-carbon atom—in the ring. Despite the fact that additional elements do participate, the most common heteroatoms are those with five or six members and heteroatoms of nitrogen, sulfur, and oxygen [1, 2]. Heteroatoms give heterocyclic compounds chemical and physical properties that are frequently substantially different from those of their all-carbon-ring analogues. Heterocyclic compounds share a general structure with cyclic organic compounds that solely contained carbon atoms in the rings. Furan, thiophene, pyrrole, and pyridine are the most effective illustrations of simple heterocyclic compounds [3].



Due to their adaptability and distinctive physicochemical features, heterocyclic compounds and heterocyclic fragments are prevalent in many medications and have become a crucial part of medicinal chemistry [4, 5]. These structures can be found in numerous important natural goods and natural

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therapies. Some medications and clinical drug prospects are mostly derived from natural materials that contain benzofuran rings [6]. A fundamental structural component of many physiologically active natural medications and synthetic chemical raw materials is the heterocyclic molecule with a benzofuran ring as the core [7]. Due to its biological activities and potential medicinal applications, benzofuran and its derivatives have garnered a lot of attention. The wide range of clinical uses of benzofuran derivatives reflect the various pharmacological activities of this class of molecules [8].

Some 2-arylbenzofurans that are generated from natural sources also exhibit beneficial biological properties, including anti-cancer, anti-inflammatory, anti-oxidative, and antibacterial activities [9, 10]. A powerful anti-amyloid aggregation activity has recently been discovered in an oral active and blood-brain barrier permeable benzofuran analog, which may offer an alternative treatment for Alzheimer's disease (AD) [11]. The benzofuran analog oxazolidine was also discovered to be a possible multifunctional chemical, and due to its significant anti-proliferative properties, it can be used to treat cancers effectively. [12]. It is anticipated that benzofuran molecules would play a significant role in the treatment of multifactorial disorders. Due to their biological activity and numerous potential uses, benzofuran compounds have drawn the interest of researchers in the fields of chemistry and medicine from all over the world. Therefore, developing an effective way of synthesizing novel benzofuranderivatives is vital and should be characterized on its physical and chemical properties by utilizing standard techniques such as IR, NMR and mass spectroscopy. This research study involves reliable way of synthesizing benzofuran derivatives and determination of its chemical and biological activity.

MATERIALS AND METHODS

MATERIALS USED

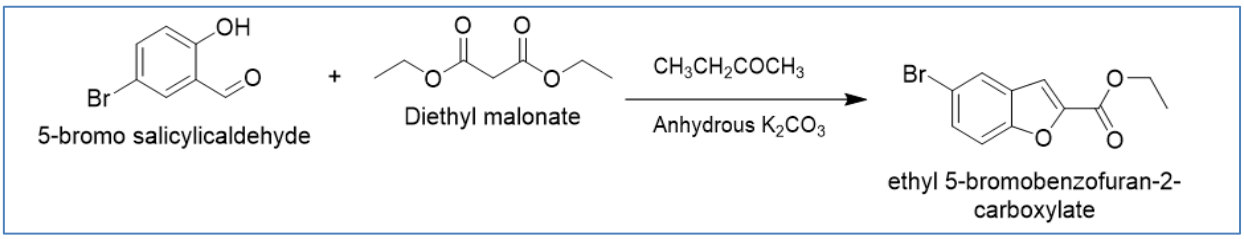
TLC silica-gel plates were purchased from sigma aldrich, Banglore, Karnataka.N-hexane, ethyl acetate, methanol, petroleum ether, Dimethyl sulfoxide (DMSO), Tetra methyl silane (TMS), chloroform, and dichloromethane are solvents were purchased in analytical grade from Merck India, Bangalore. All the other chemicals used in the study were of analytical grade.

METHODS

General Procedure for Synthesized Compounds

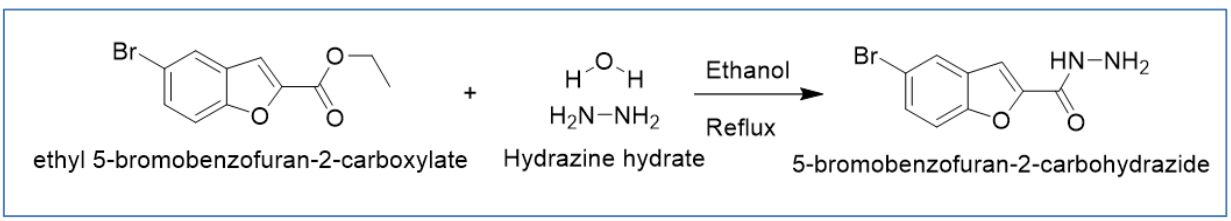
Step 1: Synthesis of ethyl-5-bromobenzofuran-2-carboxylate

Anhydrous potassium carbonate (10 g) was used to treat a mixture of 5 - bromo salicylaldehyde (0.01 mol) and diethylmalonate (0.013 mol) in ethyl methyl ketone (40 mL). The reaction mixture was heated on a steam bath for 10 hours while being refluxed. The remaining salts were dissolved in roughly 200 mL of water, chilled in an ice bath, and carefully acidified with diluted hydrochloric acid after the solvent was distilled off under reduced pressure. The item (1) was extracted using ether, and the etheric extract was then cleaned with saturated sodium bicarbonate solution before being dried over anhydrous calcium chloride [13].



Step 2: Synthesis of 5-bromobenzofuran-2carbohydrazide

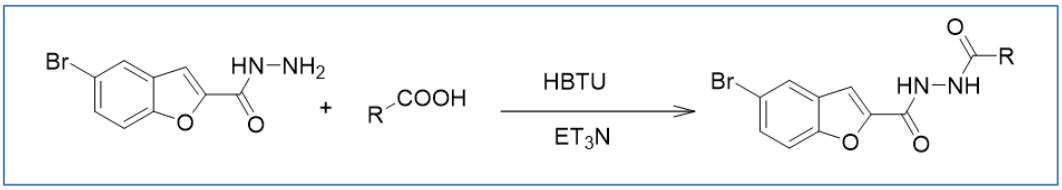
Hydrazine hydrate (25 mL) was added to a solution of ethyl-5-bromobenzofuran-2-carboxylate (0.01 mol) in ethanol (30 mL), and the combination was heated at reflux for 4 hours on a water bath. Extra ethanol was taken out at a lower pressure and diluted with water. In order to create colourless needles, the separated 5- bromobenzofuran-2-carbohydrazide was collected and crystallized from ethanol [14].



Step 3: Synthesis of N substituted 5-bromobenzofuran-2carbohydrazidederivatives

The 5-bromobenzofuran-2-carbohydrazide (1 mmole), different substituted acid (1 mmole), Et3N (3.5 mmol), and HBTU (3.5 mmol) were added to the suspension of 5-bromobenzofuran-2-carbohydrazide in

DMSO (30 mL/ 10 mmol) and stirred at room temperature for 18 hours. The reaction was next diluted with 100 ml of EtOAc, followed by 100 ml each of water and three 100 ml portions of brine for washing. The EtOAc layer was vacuum-concentrated, dried (Na2SO4), and filtered [15].



In-Silico Molecular Docking Studies

Devices and Materials

Docking is frequently used to understand the interaction between the target ligand-receptor and the target lead molecule's binding orientation with its protein receptor in the molecular scenario in modern drug design. It is also frequently used to identify associations between the target components. Bioinformatics tools were used to conduct the research in-silico. Additionally, we make use of offline programs like Marvin sketch, the PubChem database, and the Protein Data Bank (PDB) (www.rcsb.org/pdb). Through Discovery studio, molecular docking investigations were completed [16].

Preparation of Protein

Using the offline program protein data bank (PDB), we were able to achieve the resolution of 1.90Ao for cyclooxygenase II (PDB: 5W58). We removed the crystal water from the protein (5W58), then added the missing hydrogens, protonated, ionized, and minimized the energy. For energy minimization, the SPDBV (swiss protein data bank viewer) force field was used. The Ramachandran plot is used to validate prepared protein [17].

Identification of Active Sites

The Protein-Ligand Interaction Profile (PLIP) offline tool can be found online at https://plip-tool.biotec.tu-dresden.de/plipweb/plip/index to identify the active amino acids that are present in the protein. I discovered the protein's active amino acid from this [18].

Preparation of Ligands

The molecules are created in two- and three-dimensional structures using the Marvin sketch tool. Following the creation of the molecule, the structure was 3D optimized in Marvin Sketch and saved in pdb format [19].

Pharmacological Evaluation

The synthetic substances were tested for their ability to reduce inflammation in vivo.

In Vivo Anti-Inflammatory Screening

Animal Used

Adult Wistar rats in good health weighing between 150 and 200 g were purchased. The animals maintained a 12-hour day and night routine, and the temperature in the animal house ranged from 11 to 20 degrees Celsius. Throughout the trial, the animals were kept in a large, clean cage that was both spacious and airtight. Rat pellets and water from M/s Hindustan Lever Limited, Bangalore, India, were used to feed the animals. According to the CPCSEA, Chennai's suggestions, the experiments were carried out.

Chemicals

Sodium CMC and zaltoprofen

Preparation of Test Compounds

To achieve a final concentration of 50 mg/ml and 100 mg/ml, the entire mixture of the two test compounds was converted into a suspension using 0.3% w/v carboxy methylcellulose suspension.

Acute Oral Toxicity Studies According to OECD 423 Guideline

According to OECD 423 recommendations, research on the acute oral toxicity of a new benzofuran derivative were conducted. Wistar rats weighing between 150 and 200 grams will be used for the investigation, and six of these rats will be used in an acute toxicity limit test at a dose of 1000 mg/kg. The animal's behaviour was observed five hours, twelve hours, and every day following the administration. The animals in both treated groups underwent general behavioral changes for 14 days following the administration, but the animals in the untreated vehicle group and the synthesized compound group showed no significant changes in behaviour. Comparing this visual observation to the negative control group revealed no appreciable change.

After 14 days of medication, neither mortality nor tremors nor convulsions were noticed. The test compounds' acute toxic symptoms and behavioral changes were monitored continuously for 4 hours, and at the 8-hour, 12-hour, and 24-hour marks, the onset of toxic symptoms and severe behavioural alterations were also noted. The mice from all the test substance treated groups were observed for 14 days to determine the delayed onset of hazardous signs and symptoms. Additionally, there was no mortality rate seen, leading to the utilization of all rats for in vivo anti-inflammatory screening [20, 21].

Carrageenan Induced Rat Paw Oedema Method

Following is a breakdown of the rats into three groups of six each.

Group I: Prevention of disease 1ml/kg of oral CMC at 0.5%.

Group II: Animals were given 1000 mg/kg of zaltoprofen in a 0.5% CMC solution orally.

Groups III to VI: Animals were given modest and high doses of a synthetic chemical that was suspended in a CMC 0.5% solution.

Carrageenan-induced paw oedema on rats was used to assess the anti-inflammatory efficacy. Wistar rats (150- 200 g) were used in all groups to test each group's anti-inflammatory activity (n = 6). The first group of rats was employed as a drug-free control; group II was given Zaltoprofen 25 mg/kg; and groups III to VI were given the test substance in low and high doses. The synthetic substance (1000 mg/kg) was dissolved in distilled water with a 0.5% solution of carboxymethyl cellulose (CMC). Oral administration of a 0.5% solution of CMC in distilled water will serve as the control. The reference medication will be zaltoprofen 25 mg/kg. A 0.1 ml injection of 1% w/v carrageenan solution was given to the animals' left hind paws 30 minutes after the medication was given. After injecting the phlogistic drugs for 0, 30, 60, 80, or 120 minutes, the inflammation was assessed using a plethysmograph at a predetermined time interval and compared to that of the control. Comparing the current rise in paw volume to the zero-minute value allowed for the calculation of the increase. The average growth in paw volume and its % inhibition is also calculated.

RESULT AND DISCUSSION

Synthetic Work

After treating ethyl methyl ketone with anhydrous potassium carbonate, the reaction between diethylmalonate and 5-bromo salicylaldehyde produced the ethyl-5-bromobenzofuran-2-carboxylate (1). When the acquired product of compound 1 is dissolved in ethanol and heated under reflux for 4 hours on a water bath, the resulting combination yields the matching ethyl-5-bromobenzofuran-2-carboxylate derivatives (2) (Scheme 1). By repeatedly recrystallizing the chemicals and intermediates from ethanol, all of the substances were cleaned. In contrast, the distinctive broad absorption peaks C=O for CONH were found in the range of 1720-1690 cm-1, 3350-3157 cm-1 for NH, 1489-1464 cm-1 for CH2, 1379-1344 cm-1 for CH3, and 800-700 cm-1 for aromatic rings. The IR spectrum of the final synthesized compounds displayed absorption bands around 3300-3156 cm-1 for amide NH. These Compounds 1H NMR spectra likewise showed appropriate peaks at matching ppm. The produced compounds' 1H NMR spectra showed singlet signals at 9.9–10.5 for H of NH and at 7.5–8.5 for H of aromatic ring. The 13C NMR spectra of the synthesized compounds showed a signal for carbonyl carbon at 160–175 and a signal for aromatic carbon at 120–145. The assigned structures were supported by the appropriate molecular ion peaks in the LC-MS spectra. A rat model and docking experiments were used to conduct in vivo anti-inflammatory research on all the produced drugs.

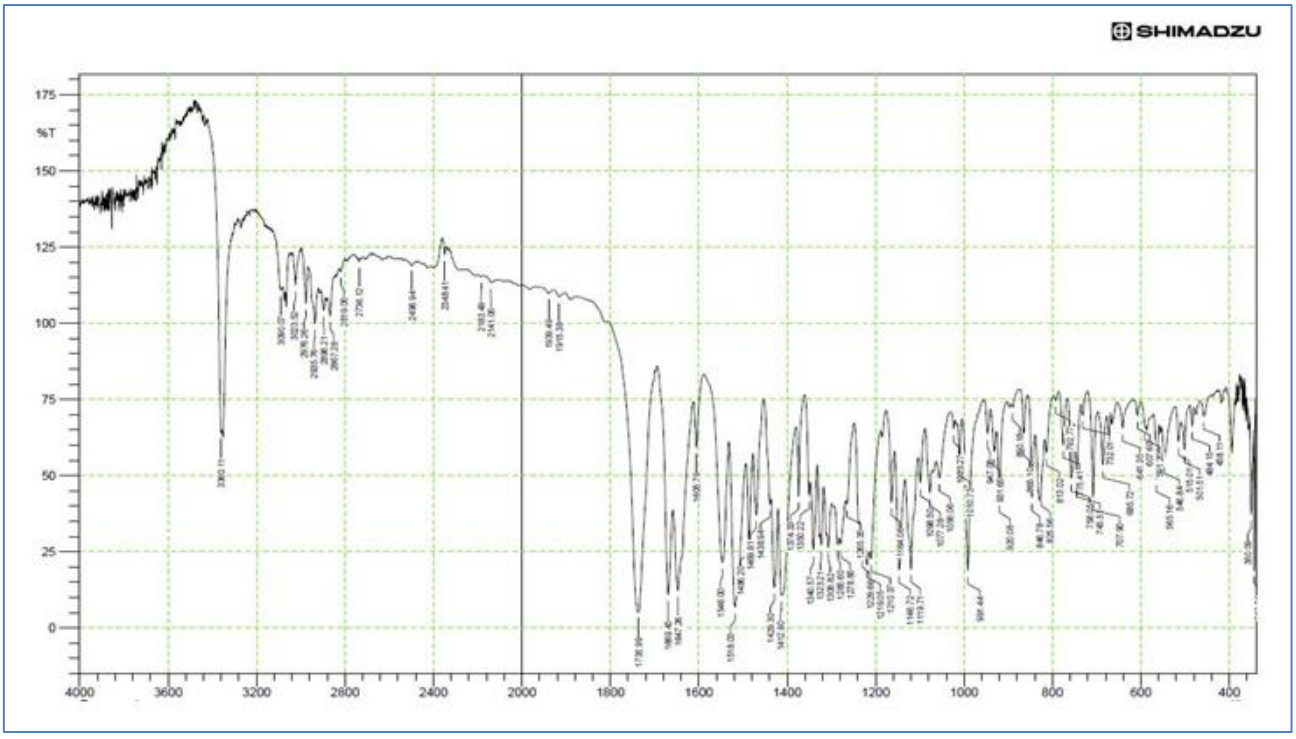


Figure 1: IR spectra for compound BA

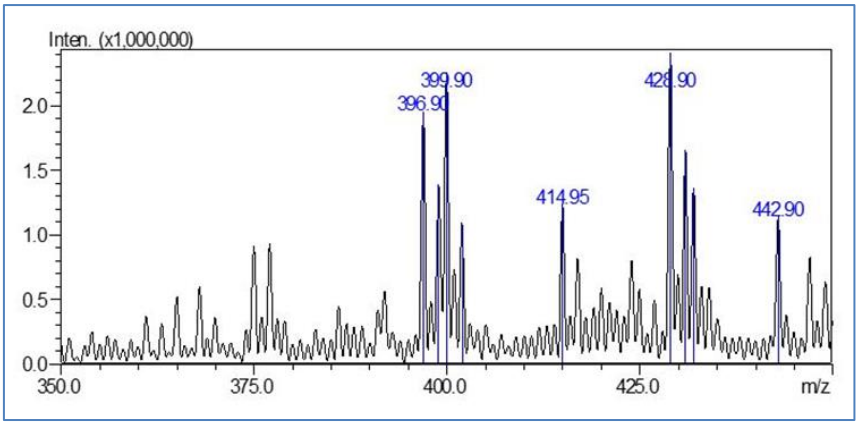


Figure 2: Mass spectra for compound BA

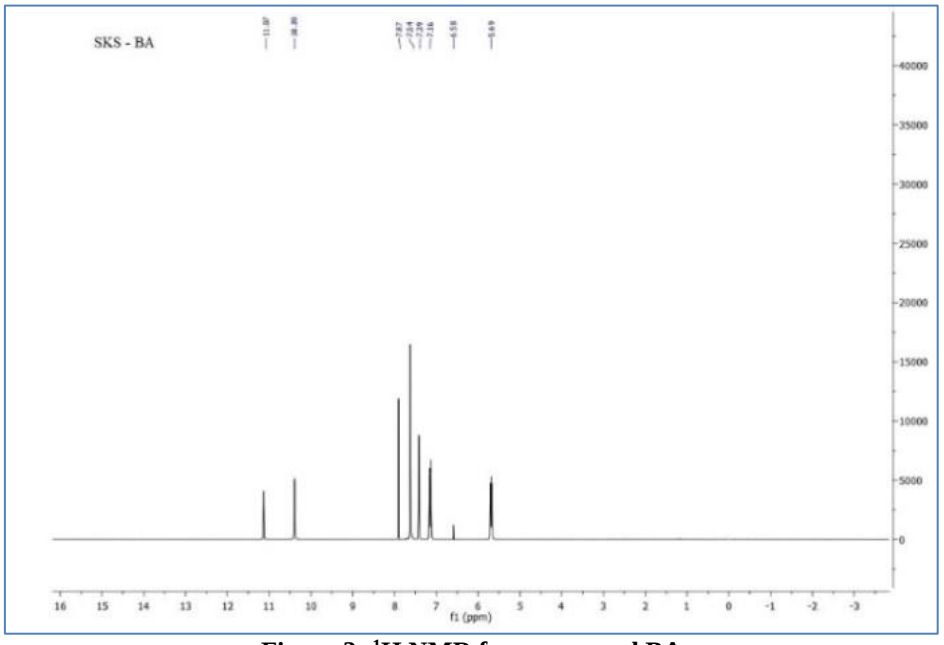


Figure 3: ^{1}H NMR for compound BA

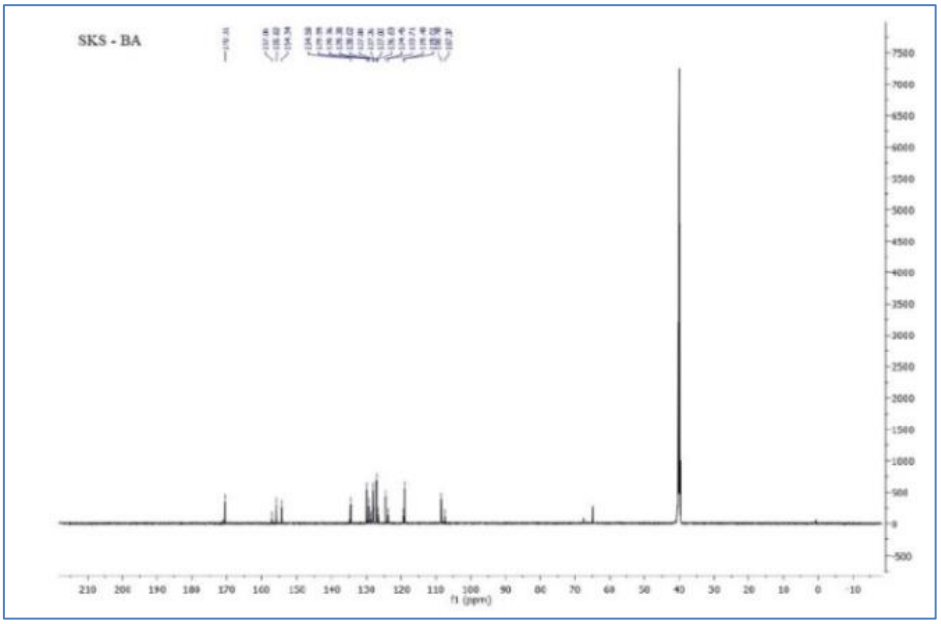


Figure 4: ^{13}C NMR for compound BA

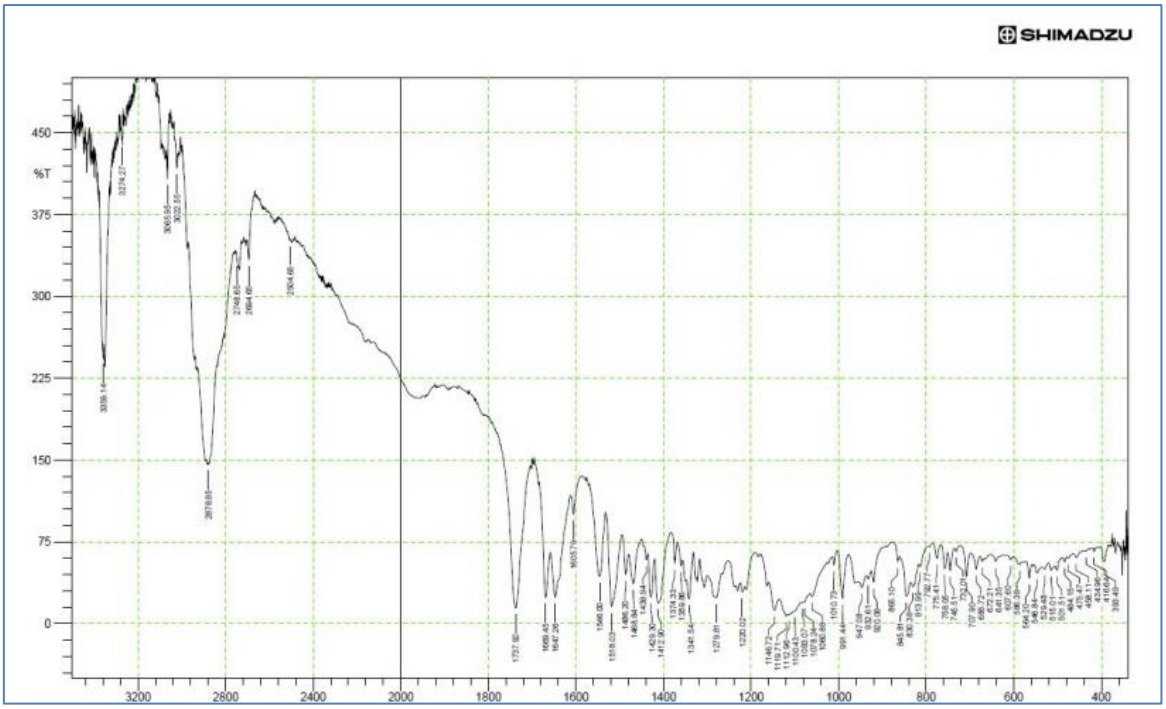


Figure 5: IR spectra for compound BB

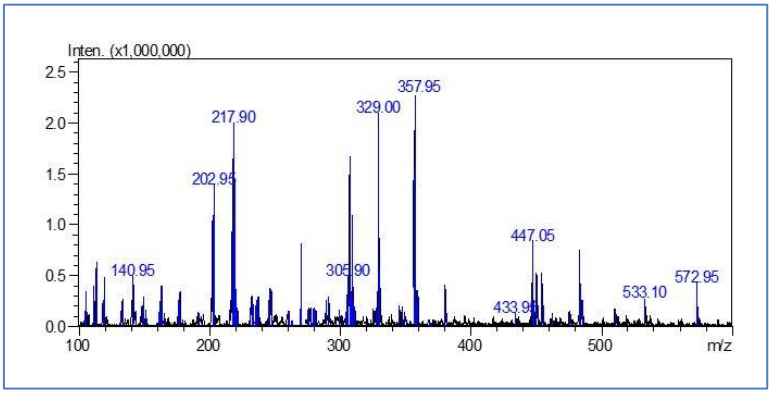


Figure 6: Mass spectra for compound BB

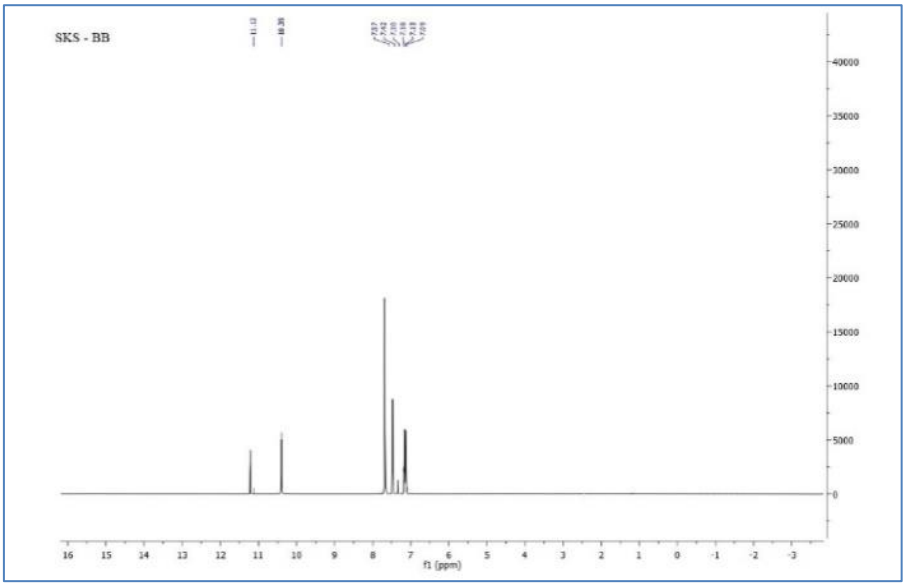


Figure 7: ^{1}H NMR for compound BB

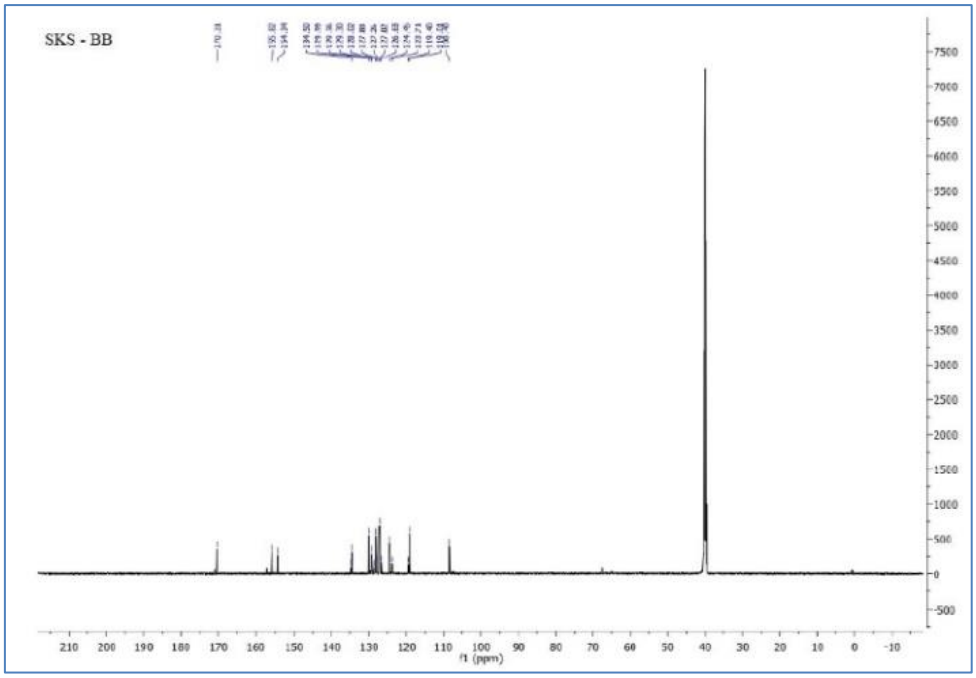


Figure 8: ^{13}C NMR spectra for compound BB

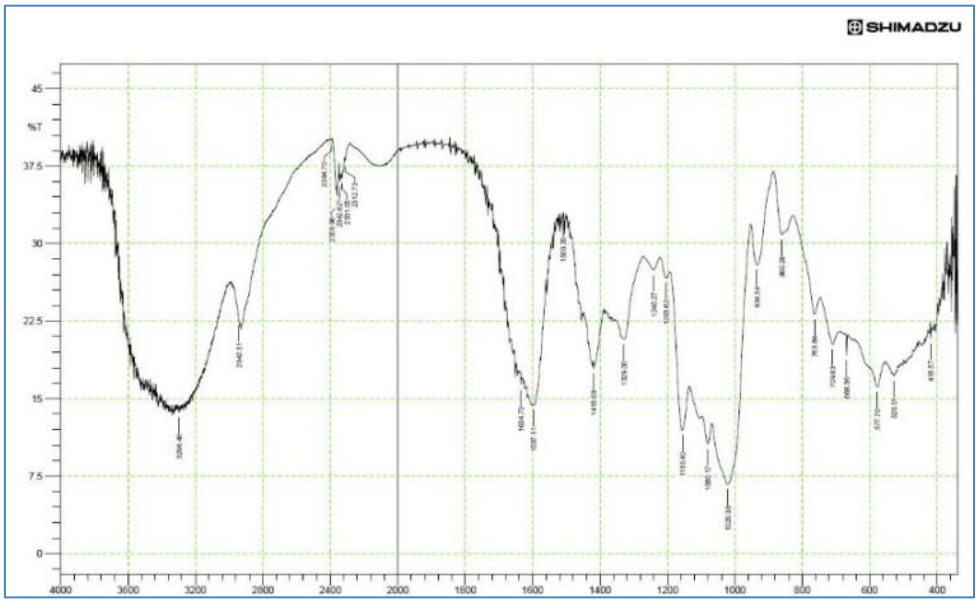


Figure 9: IR spectra for compound BF

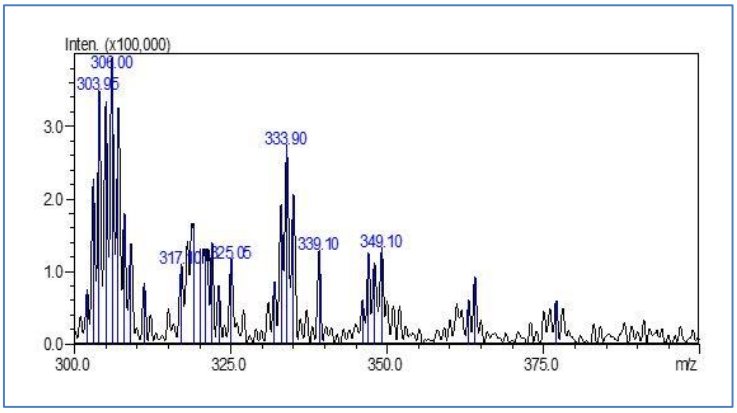


Figure 10: Mass spectra for compound BF

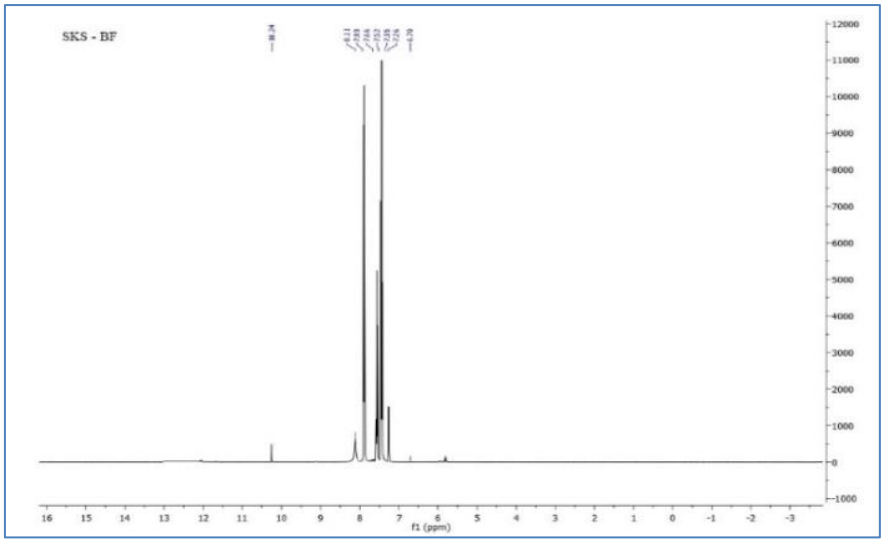


Figure 11: ^{1}H NMR for compound BF

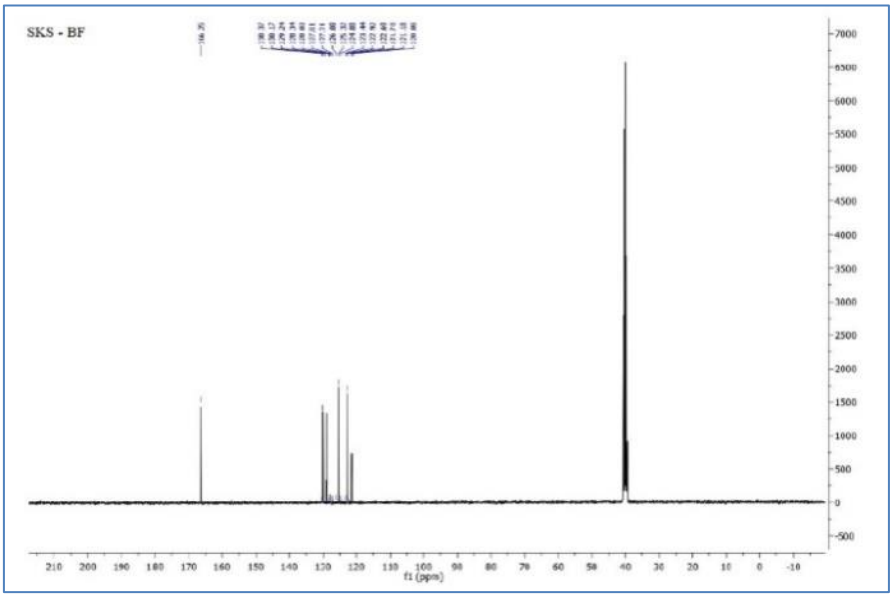


Figure 12: ^{13}C NMR for compound BF

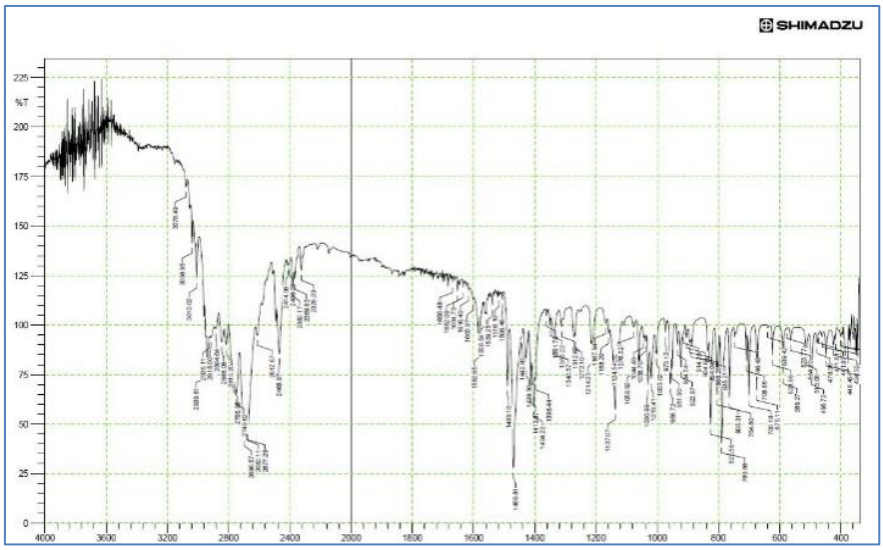


Figure 13: IR spectra for compound BM

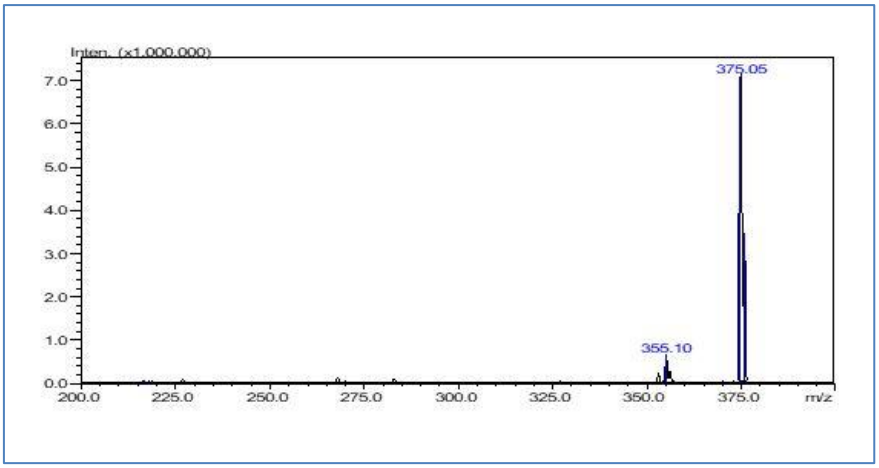


Figure 14: Mass spectra for compound BM

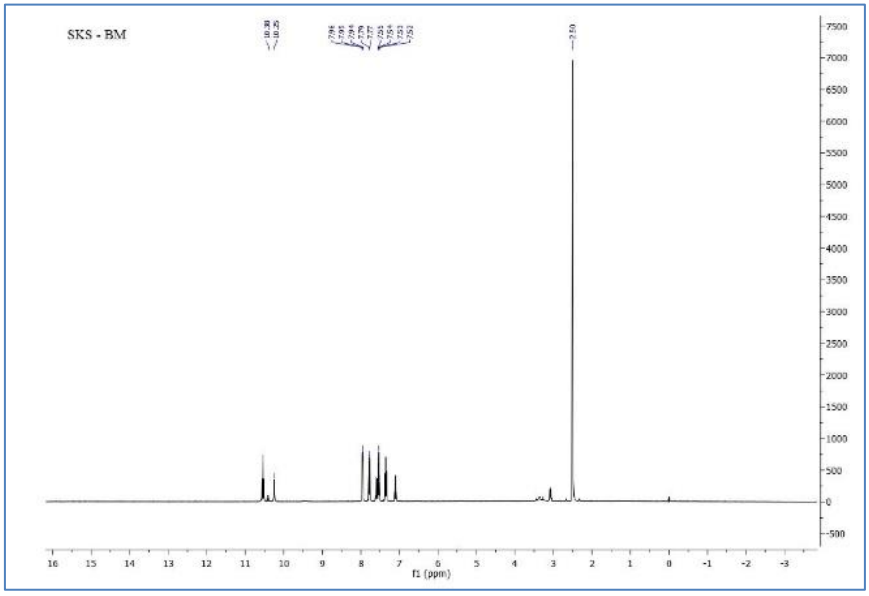


Figure 15: ^{1}H NMR for compound BM

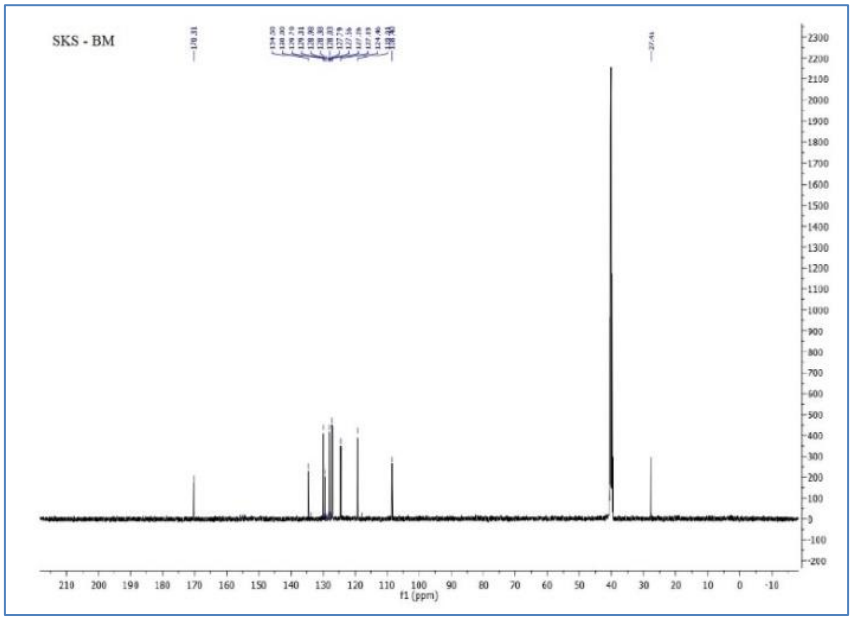


Figure 16: ^{13}C NMR for compound BM

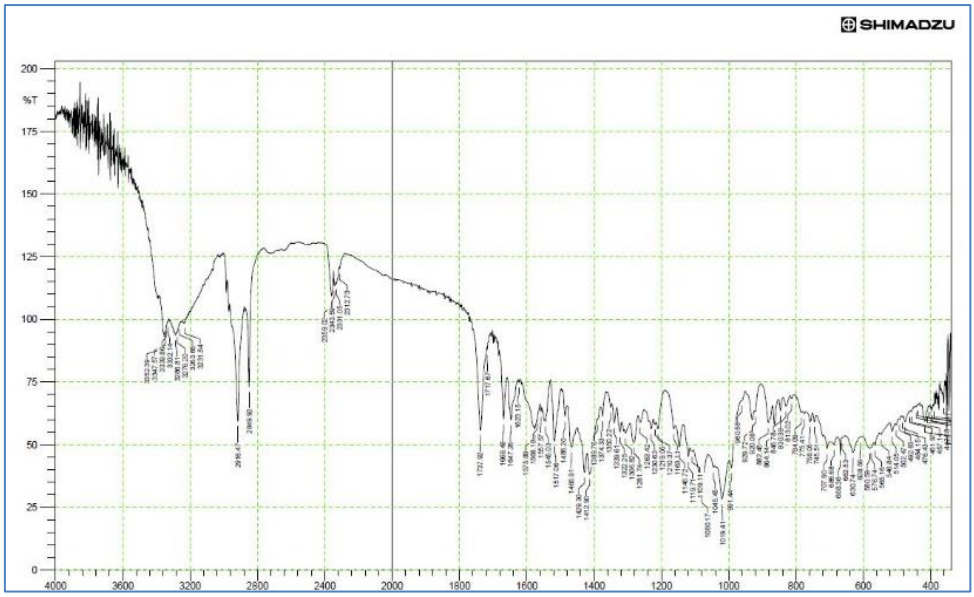


Figure 17: IR spectra for compound BT

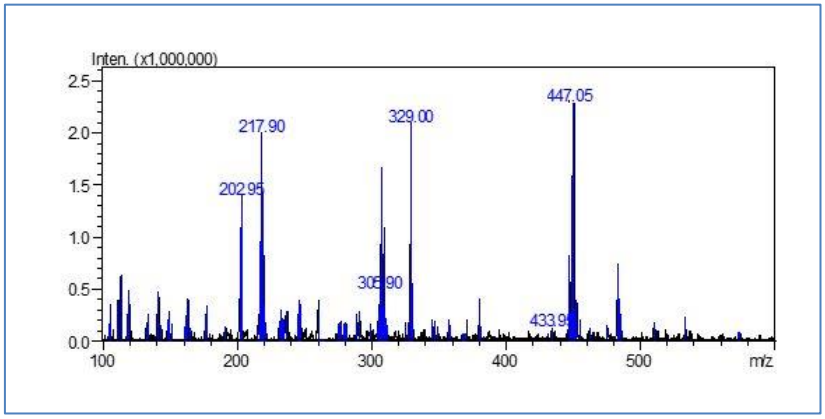


Figure 18: Mass spectra for compound BT

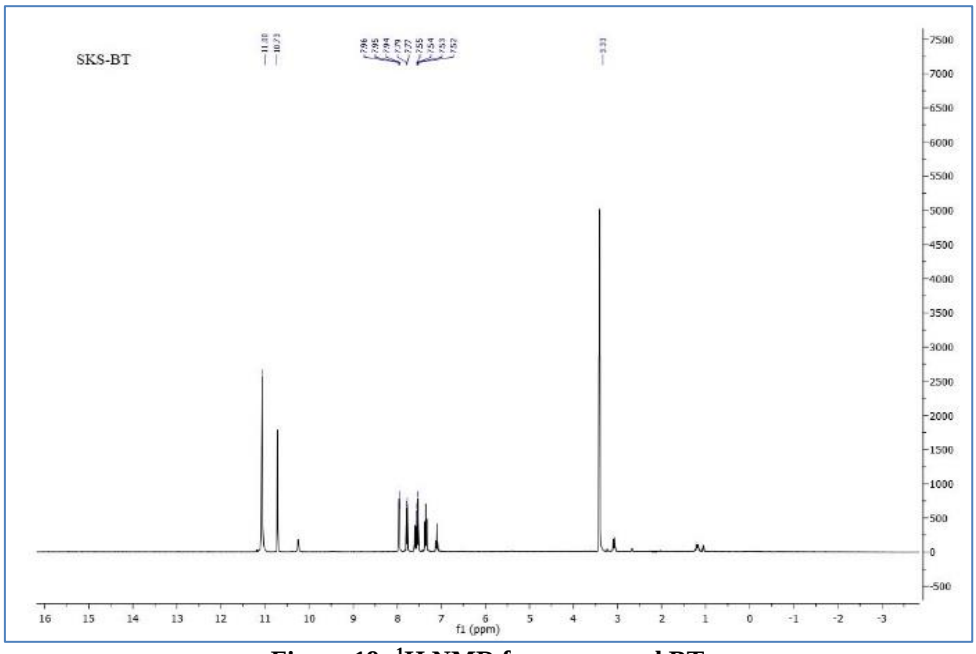


Figure 19: ^{1}H NMR for compound BT

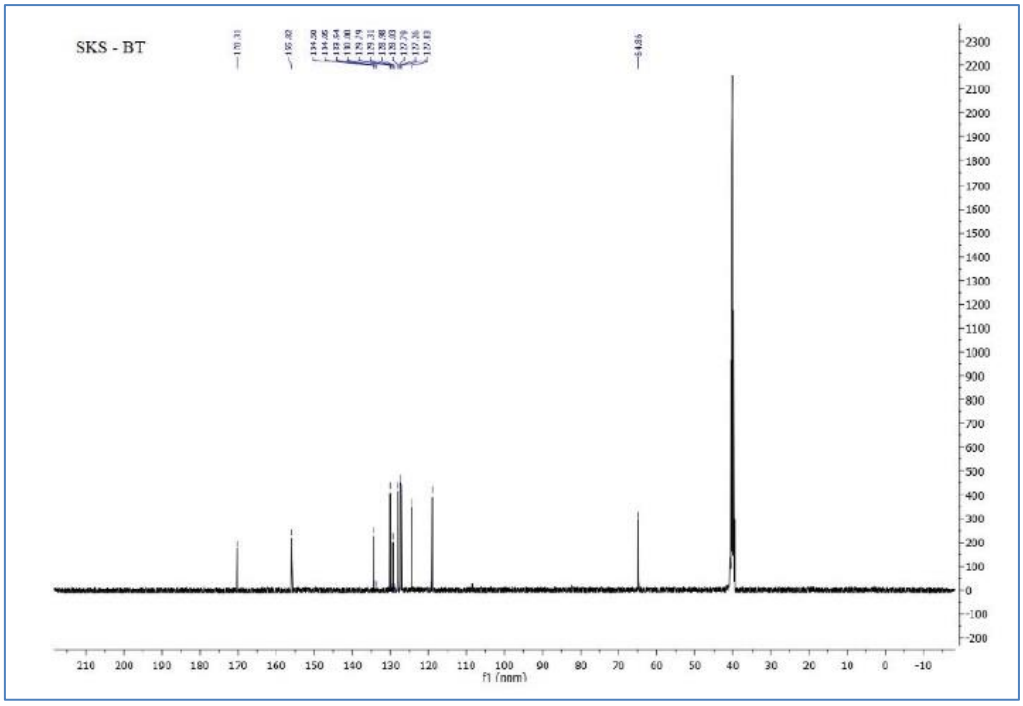


Figure 20: ^{13}C NMR for compound BT

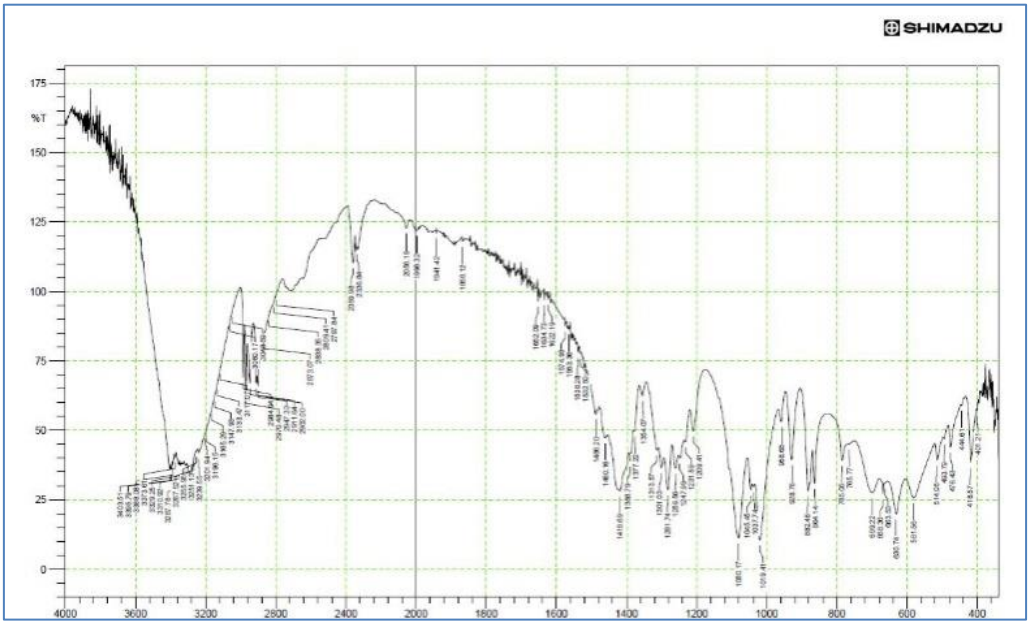


Figure 21: IR spectra for compound BS

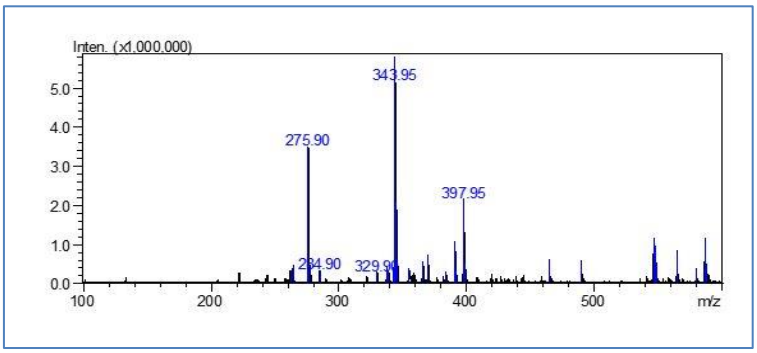


Figure 22: Mass spectra for compound BS

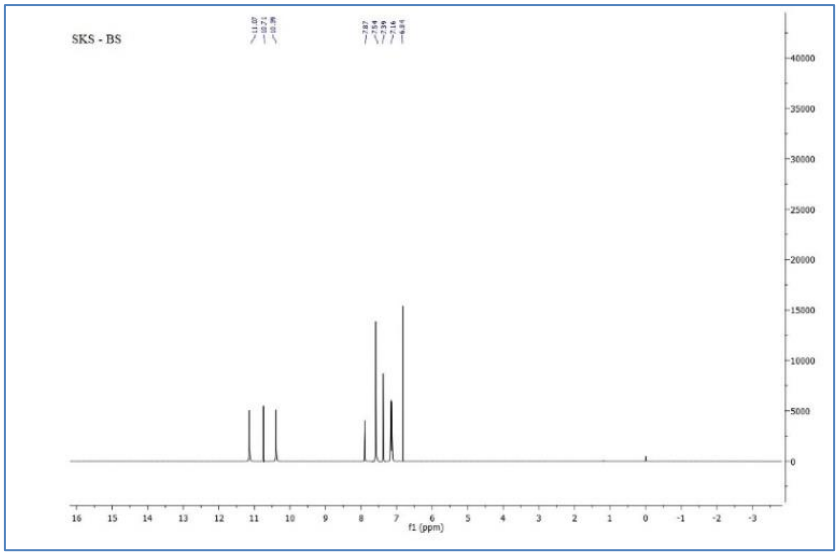


Figure 23: ^{1}H NMR for compound BS

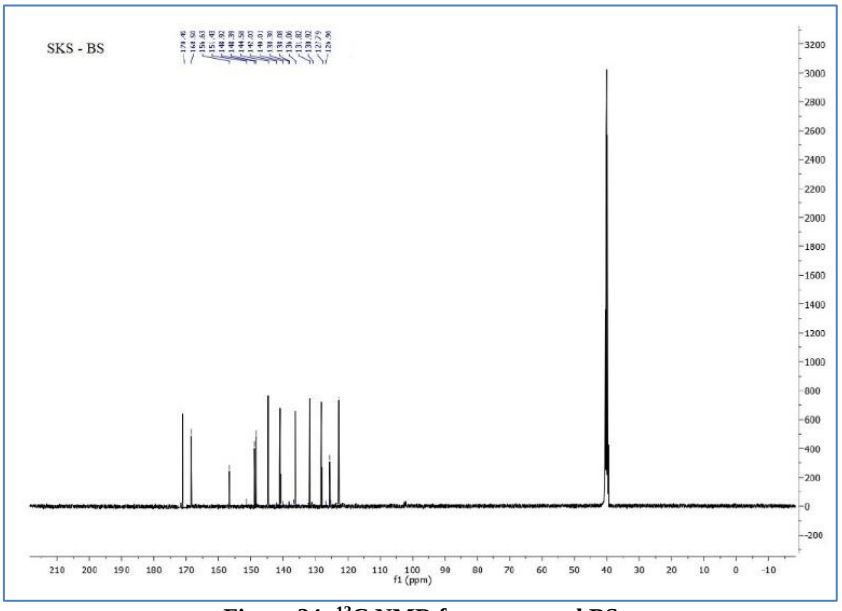


Figure 24: ^{13}C NMR for compound BS

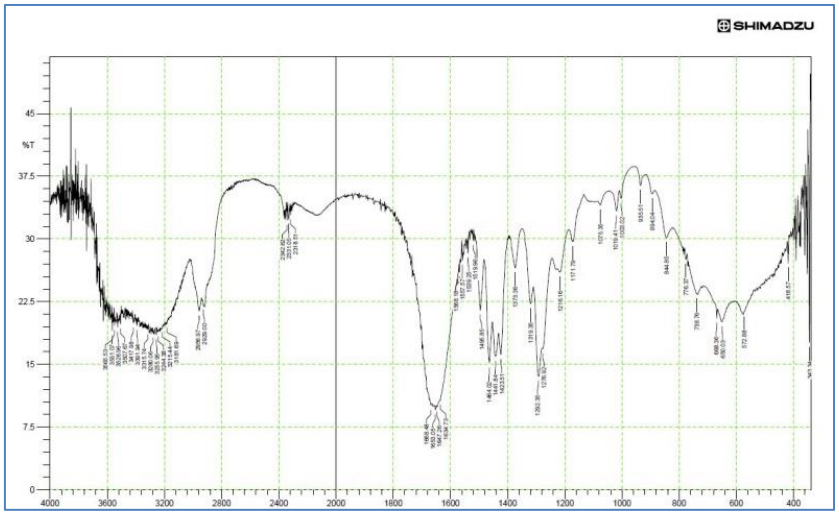


Figure 25: IR spectra for compound BO

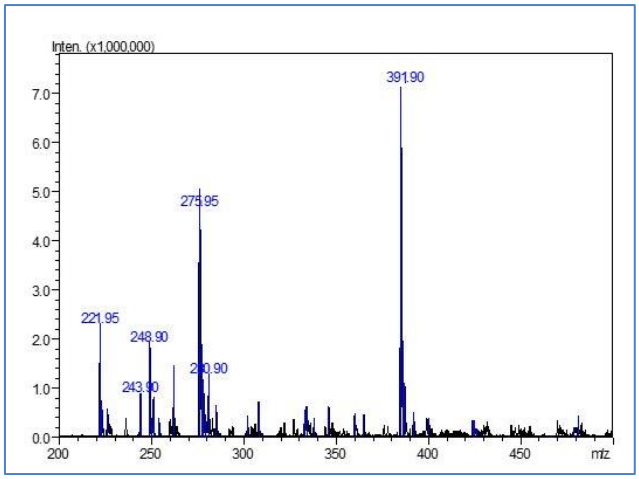


Figure 26: Mass spectra for compound BO

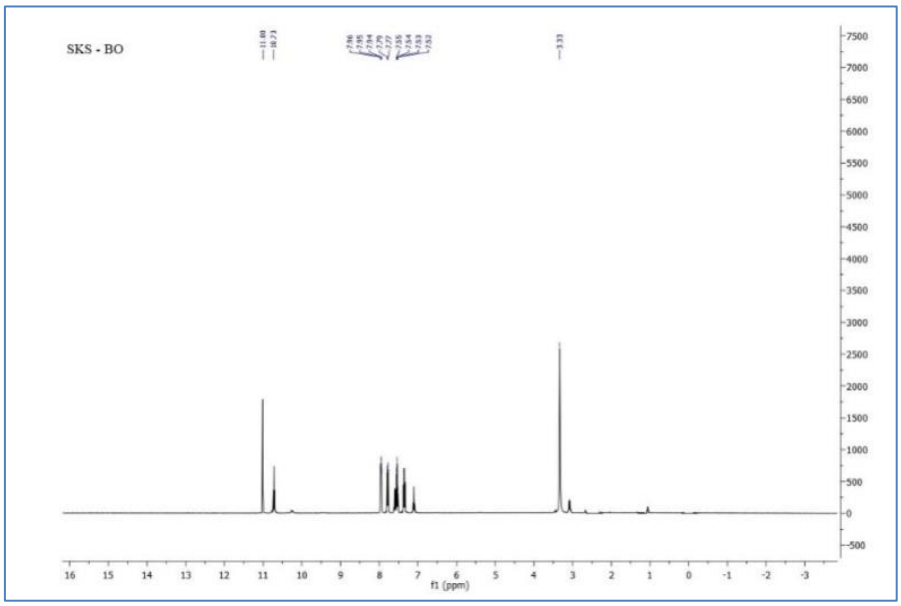


Figure 27: ^{1}H NMR for compound BO

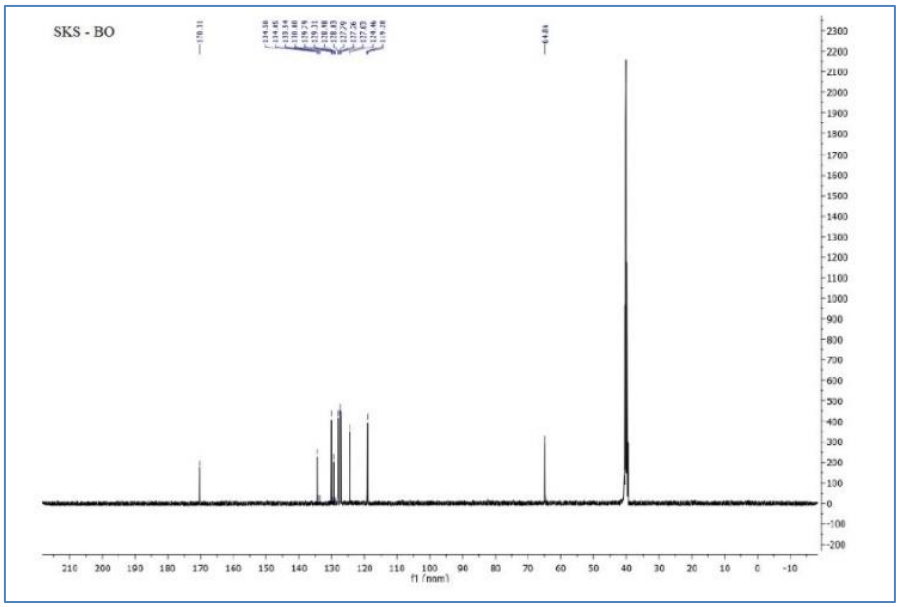
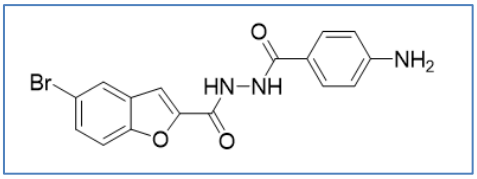


Figure 28: ^{13}C NMR for compound BO

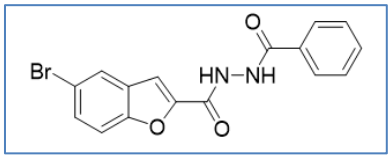
Characterization of Synthesised Compounds

N'-(4-aminobenzoyl)-5-bromobenzofuran-2-carbohydrazide (BA)



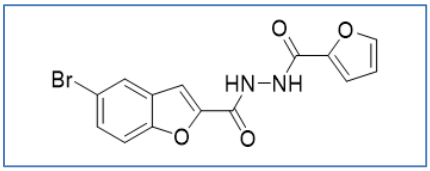
Chemical Formula	**: $C_{16}H_{12}BrN_3O_3$**
Colour	: Orange solid
MP	: 167 – 173^{O}C
Mass	Actual mass: 374 Found mass: 396 (M+23)
IR KBr pellet (cm^{-1})	: 3360 (NH stretching amine); 2348 (CH stretching aromatic); 1647 (C=O stretching amide); 1412 (CN bending); 846 (Aromatic ring); 707 (C-Br stretching).
^{1}H NMR (400 MHz, DMSO) δ	: 11.07, 10.39, 7.87, 7.54, 7.39, 7.16, 6.58, 5.69.
^{13}C NMR (126 MHz, DMSO) δ	: 170.31, 154.34, 134.70, 129.36, 129.30, 128.79, 128.02, 127.88, 127.26, 127.02, 126.63, 124.45, 123.71, 119.01, 108.40, 107.37.
Elemental Analysis	: C, 51.36; H, 3.23; Br, 21.35; N, 11.23; O, 12.83

N'-benzoyl-5-bromobenzofuran-2-carbohydrazide (BB)



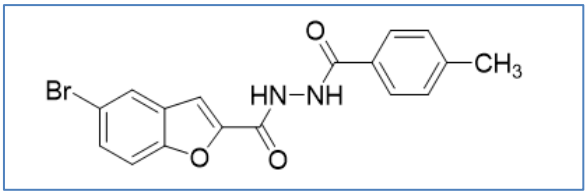
Chemical Formula	**: $C_{16}H_{11}BrN_2O_3$**
Colour	: Yellow solid
MP	: 187 – 191^{O}C
Mass	Actual mass: 358 Found mass: 357.95
IR KBr pellet (cm^{-1})	: 3354 (NH stretching amine); 2304 (CH stretching aromatic); 1647 (C=O stretching amide); 1412 (CN bending); 845 (Aromatic ring); 707 (C-Br stretching).
^{1}H NMR (400 MHz, DMSO) δ	: 11.12, 10.39, 7.57, 7.42, 7.30, 7.18, 7.13, 7.09.
^{13}C NMR (126 MHz, DMSO) δ	: 170.31, 155.82, 154.34, 134.70, 134.50, 129.99, 129.36, 129.30, 128.79, 127.88, 127.26, 126.63, 124.45, 123.71, 119.40.
Elemental Analysis	: C, 53.50; H, 3.09; Br, 22.25; N, 7.80; O, 13.36

5-bromo-N'-(furan-2-carbonyl) benzofuran-2-carbohydrazide (BF)



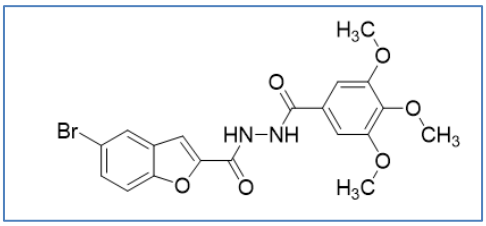
Chemical Formula	**: $C_{14}H_9BrN_2O_4$**
Colour	: Brown solid
MP	: 152 – 156^{O}C
Mass	Actual mass: 349 Found mass: 349
IR KBr pellet (cm^{-1})	: 3296 (NH stretching amine); 2331 (CH stretching aromatic); 1634 (C=O stretching amide); 1418 (CN bending); 850 (Aromatic ring); 709 (C-Br stretching).
^{1}H NMR (400 MHz, DMSO) δ	: 10.24, 8.11, 7.93, 7.66, 7.52, 7.35, 7.26, 6.70.
^{13}C NMR (126 MHz, DMSO) δ	: 166.25, 130.37, 129.24, 128.34, 127.81, 127.21, 126.08, 125.32, 124.88, 123.44, 122.92, 122.69, 121.70, 121.18.
Elemental Analysis	: C, 48.16; H, 2.60; Br, 22.89; N, 8.02; O, 18.33

5-bromo-N'-(4-methylbenzoyl) benzofuran-2-carbohydrazide (BM)



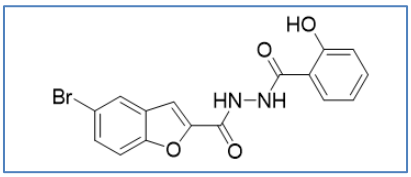
Chemical Formula	**: $C_{17}H_{13}BrN_2O_3$**
Colour	: Yellow solid
MP	: 166 – 170^{O}C
Mass	Actual mass: 373 Found mass: 375 (M+2)
IR KBr pellet (cm^{-1})	: 3078 (NH stretching amine); 2326 (CH stretching aromatic); 1616 (C=O stretching amide); 1404 (CN bending); 825 (Aromatic ring); 708 (C-Br stretching).
^{1}H NMR (400 MHz, DMSO) δ	: 0.38, 10.25, 7.96, 7.95, 7.94, 7.79, 7.77, 7.55, 7.54, 7.53, 7.52, 2.50.
^{13}C NMR (126 MHz, DMSO) δ	: 170.31, 134.50, 133.75, 130.00, 129.79, 128.38, 128.03, 127.79, 127.56, 127.26, 127.03, 124.46, 119.01, 117.86, 108.40, 27.46.
Elemental Analysis	: C, 54.71; H, 3.51; Br, 21.41; N, 7.51; O, 12.86

5-bromo-N'-(3, 4, 5-trimethoxybenzoyl) benzofuran-2-carbohydrazide (BT)



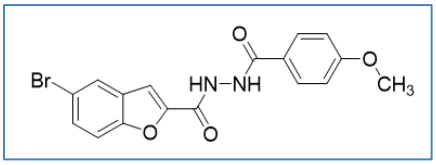
Chemical Formula	**: $C_{19}H_{17}BrN_2O_6$**
Colour	: Yellow solid
MP	: 184 – 188^{O}C
Mass	Actual mass: 448 Found mass: 447 (M – 1)
IR KBr pellet (cm^{-1})	: 3352 (NH stretching amine); 2312 (CH stretching aromatic); 1623 (C=O stretching amide); 1412 (CN bending); 830 (Aromatic ring); 707 (C-Br stretching).
^{1}H NMR (400 MHz, DMSO) δ	: 11.00, 10.73, 7.96, 7.95, 7.94, 7.79, 7.77, 7.55, 7.54, 7.53, 7.52, 3.33.
^{13}C NMR (126 MHz, DMSO) δ	: 170.31, 155.82, 134.50, 134.05, 133.54, 130.00, 129.79, 129.31, 128.98, 128.03, 127.79, 127.26, 127.03,64.86.
Elemental Analysis	: C, 50.80; H, 3.81; Br, 17.79; N, 6.24; O, 21.37

5-bromo-N'-(2-hydroxybenzoyl) benzofuran-2-carbohydrazide (BS)



Chemical Formula	**: $C_{16}H_{11}BrN_2O_4$**
Colour	: brown solid
MP	: 161 – 163^{O}C
Mass	Actual mass: 375 Found mass: 398 (M+23)
IR KBr pellet (cm^{-1})	: 3403 (NH stretching amine); 2336 (CH stretching aromatic); 1622 (C=O stretching amide); 1418 (CN bending); 785 (Aromatic ring); 699 (C-Br stretching).
^{1}H NMR (400 MHz, DMSO) δ	: 11.07, 10.71, 10.39, 7.87, 7.54, 7.39, 7.16, 6.84.
^{13}C NMR (126 MHz, DMSO) δ	: 170.45, 168.50, 156.63, 151.43, 148.92, 148.39, 144.58, 142.03, 140.01, 138.30, 138.08, 136.06, 131.82, 130.92, 127.79, 126.96.
Elemental Analysis	: C, 51.22; H, 2.96; Br, 21.30; N, 7.47; O, 17.06

5-bromo-N'-(4-methoxybenzoyl) benzofuran-2-carbohydrazide (BO)



Chemical Formula	**: $C_{17}H_{13}BrN_2O_4$**
Colour	: brown solid
MP	: 161 – 163^{O}C
Mass	Actual mass: 389 Found mass: 390 (M+1)
IR KBr pellet (cm^{-1})	: 3551 (NH stretching amine); 2331 (CH stretching aromatic); 1634 (C=O stretching amide); 1423 (CN bending); 844 (Aromatic ring); 705 (C-Br stretching).
^{1}H NMR (400 MHz, DMSO) δ	: 11.00, 10.73, 7.96, 7.95, 7.94, 7.79, 7.77, 7.55, 7.54, 7.53, 7.52, 3.33.
^{13}C NMR (126 MHz, DMSO) δ	: 170.31, 134.50, 134.05, 133.54, 130.00, 129.79, 129.31, 128.98, 128.03, 127.79, 127.26, 127.03, 124.46, 64.86.
Elemental Analysis	: C, 52.46; H, 3.37; Br, 20.53; N, 7.20; O, 16.44

Molecular Docking

The C dock module of Discovery studio carried out an in-silico docking analysis of the proposed compounds to the enzyme's active sites to ascertain the binding affinities of the ligands. In order to determine the proposed compounds' anti-inflammatory cyclooxygenase II inhibitory activity, they were docked towards cyclooxygenase II (PDB: 5W58). When compared to Zaltoprofen, which has cyclooxygenase II inhibitory activity as an anti-inflammatory, all the drugs showed good receptor affinities. Table 1 displays the Glide scores of docking investigations conducted against cyclooxygenase II (PDB: 5W58). Because aromatic heterocyclic rings are present, it is clear from the in-silico docking results that lipophilic factors dominate the interactions.

Compound BF, one of the docked compounds, has a noteworthy docking score of -9.77 K/cal. When compared to the common medication Ibuprofen, the molecule 3d exhibits a pi-pi interaction between Tyr 896 and benzofuran. The molecule BT displays three hydrogen bonds with the amino acids Ser 681, Met 675, and Thr 866 along with a substantial docking score of -9.49 K/cal. The remaining docked molecule has one or two hydrogen bond interactions and a docking score range of 6 to 9 K/cal. The docking poses of all the proposed compounds are shown in Figures 29–35.

Name	**C docker energy**	**Binding interaction energy**
BB	-9.20	28.44
BA	-6.12	25.94
BS	-7.09	24.44
BM	-9.40	29.58
BO	-6.15	26.89
BF	-9.77	31.12
BT	-9.49	29.18
Ibuprofen	-8.20	25.64

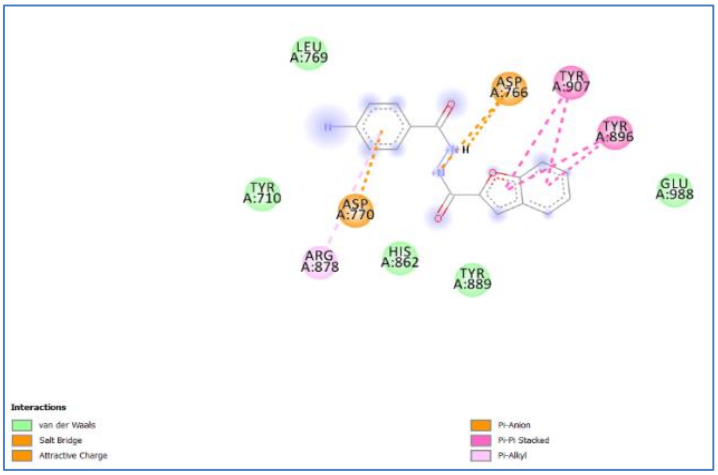


Figure 29: 2D docking pose of compound BA

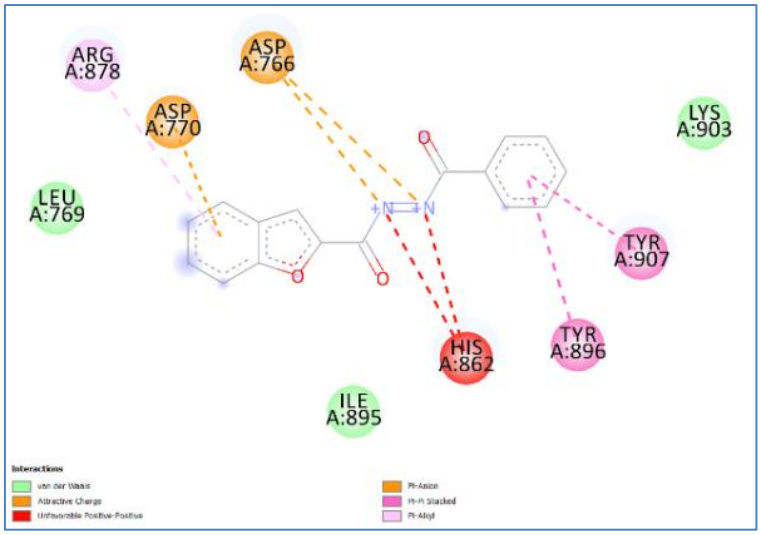


Figure 30: 2D docking pose of compound BB

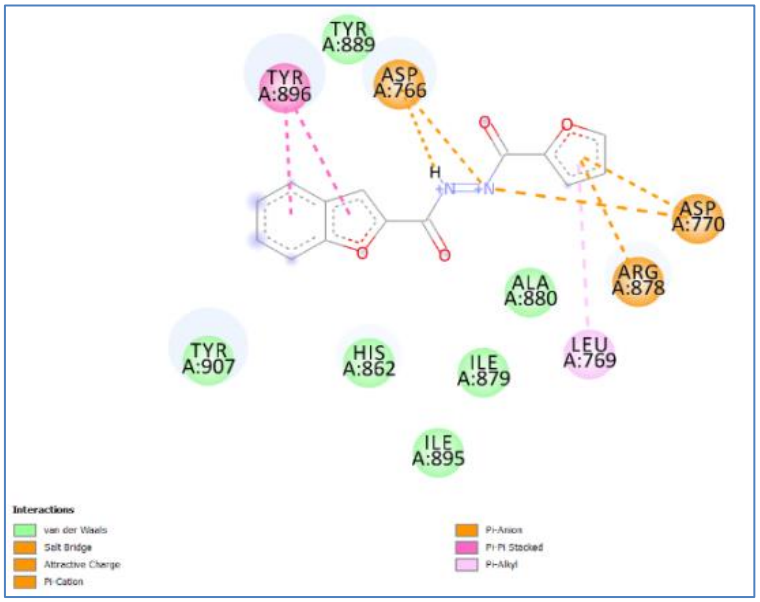


Figure 31: 2D docking pose of compound BF

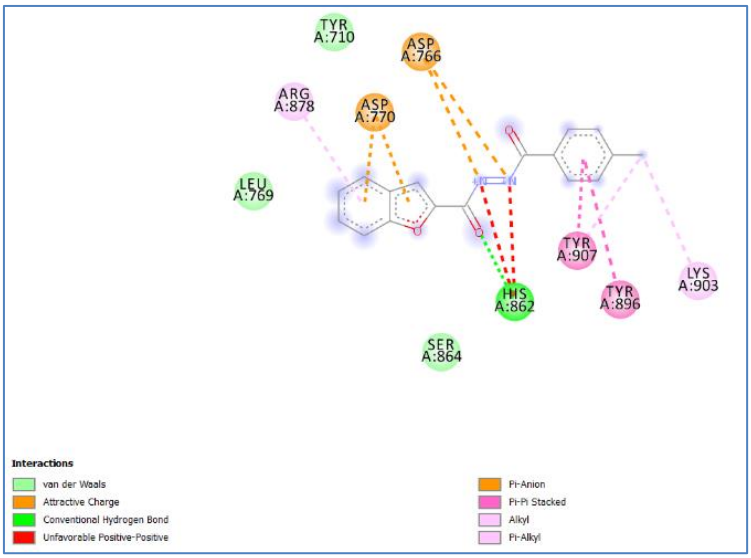


Figure 32: 2D docking pose of compound BM

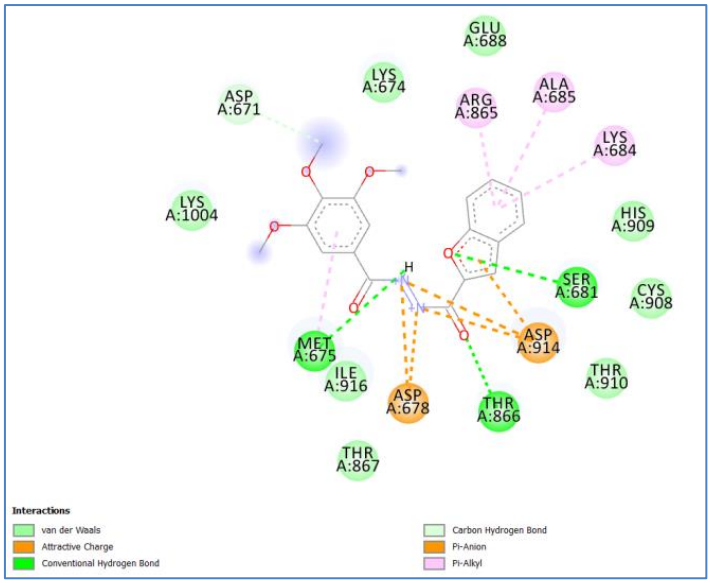


Figure 33: 2D docking pose of compound BT

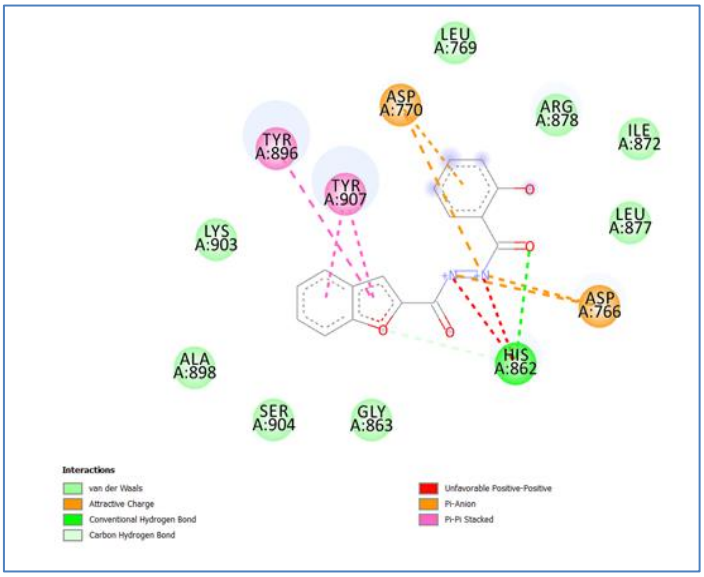


Figure 34: 2D docking pose of compound BS

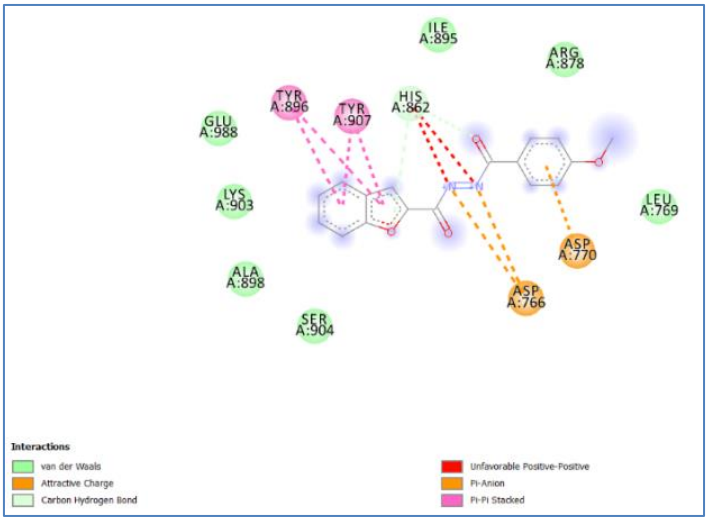


Figure 35: 2D docking pose of compound BO

Pharmacological Screening

Acute Oral Toxicity

Compounds BF and BT were chosen for assessment of their in vivo anti-inflammatory effects based on docking score. Acute oral toxicity testing of the substances BT and BF was done in Wistar rats. Acute toxicity experiments were initiated at a dose of 2000 mg/kg in accordance with OECD standards 423. The tables 1 and 2 present the findings.

Animal Marking	Bodyweight (g)		Drug	Dose (mg)	Volume (mL)	Bodyweight change (g)				
	Day 0	Day 1				Day 7	Weight change	Day 14	Weight change	Mortality
Head	28.5	28	BF	56	0.28	28.9	0.9	28.9	0.9	0
Tail	30	29.5		59	0.29	30	0.5	30	0.5	
Body	31	30.5		61	0.3	31	0.5	31	0.5	
Head	28.9	28	BT	56	0.28	28.4	0.4	28.5	0.5	0
Body	28	27.5		55	0.27	28	0.5	29	1.5	
Tail	29	28.5		57	0.28	28.8	0.3	29.4	1.9	

Table 1: Acute oral toxicity results of a prodrug of zaltoprofen in Wistar rats at the dose 2000 mg/kg

Sl. No.	Parameters	min	0 min	hr	hr	hr	hr	4 hr
1	Hyperactivity	0	0	0	0	0	0	0
2	Piloerection	0	0	0	0	0	0	0
3	Twitching	0	0	0	0	0	0	0
4	Rigidity	0	0	0	0	0	0	0
5	Irritability	0	0	0	0	0	0	0
6	Jumping	0	0	0	0	0	0	0
7	Clonic convulsions	0	0	0	0	0	0	0
8	Tonic convulsions	0	0	0	0	0	0	0
9	Ptosis	0	0	0	0	0	0	0
10	Sedation	0	0	0	0	0	0	0
11	Sleep (Loss of R.R)	0	0	0	0	0	0	0
12	Loss of traction	0	0	0	0	0	0	0
13	Loss of pinna reflex	0	0	0	0	0	0	0
14	Catatonia	0	0	0	0	0	0	0
15	Ataxia	0	0	0	0	0	0	0
16	Loss of muscle tone	0	0	0	0	0	0	0
17	Algesia	0	0	0	0	0	0	0
18	Straub's tail	0	0	0	0	0	0	0
19	Laboured respiration	0	0	0	0	0	0	0
20	Cyanosis	0	0	0	0	0	0	0
21	Blanching	0	0	0	0	0	0	0
22	Reddening	0	0	0	0	0	0	0
23	Abnormal secretion	0	0	0	0	0	0	0
24	Death	0	0	0	0	0	0	0

Table 2: Evaluation parameters of acute toxicity study

The acute toxicity investigation revealed that the chemicals BF and BT were safe for animals to consume. Animals treated with the chemicals BT and BF at a dose of 2000 mg/kg p. o. exhibited no aberrant clinical symptoms, changes in body weight, or gross necropsies in the animals. The LD50 value of the substances BF and BT was determined to be >2000 mg/kg based on the findings.

Anti-Inflammatory Screening

In the current work, rat paw oedema caused by carrageenan was used to test the anti-inflammatory activity of the compounds BF and BT. Ibuprofen was typically utilized. All of the substances demonstrated a reduction in edema volume when compared to control. Table 3 displays the research findings. It was discovered that all substances had modest to moderate action. Highly considerable anti- inflammatory action was seen in the test group. According to the findings, the chemical BF has a good level of inhibition (91% vs. 88.6% for Ibuprofen, the gold standard). The chemical BT inhibits the growth of tumors by 87.7%.

S. No	Groups		Body Wts(gm)	Dose (ml)	Paw Volume					% increase Paw Volume			
					0 min	15 min	30 min	60 min	120 min	15 min	30 min	60 min	120 min
1.	Control	H	149	1.4	0.16	0.2	0.24	0.28	0.3	25	50	75	87.5
		B	195	1.9	0.18	0.24	0.26	0.3	0.32	33	44.4	66.6	77.7
		T	175	1.7	0.16	0.2	0.24	0.28	0.3	25	50	75	87.7
		RH	136	1.3	0.18	0.26	0.3	0.34	0.36	44	66	88.8	100
		Mean(%IPL)								31.75	52.6	76.35	88.175
		%Inhibition											
2.	Standard	H	187	1.8	0.16	0.19	0.2	0.19	0.18	18.7	25	18.7	12.5
		B	180	1.8	0.14	0.17	0.19	0.18	0.15	21.4	35.7	28.5	7.1
		T	135	1.3	0.14	0.19	0.2	0.19	0.16	35.7	42.8	35.7	14.2
		RH	163	1.6	0.16	0.2	0.21	0.19	0.15	25	31.2	18.7	6.2
		Mean(%IPL)								25.2	33.675	25.4	10
		%Inhibition								20.6	35.9	66.75	88.6
3.	Test compound BF 1000 mg/kg	H	183	1.8	0.14	0.18	0.17	0.16	0.15	28.5	21.4	14.2	7.1
		B	240	2.4	0.12	0.14	0.17	0.14	0.13	16.6	41.6	16.6	8.3
		T	178	1.7	0.14	0.18	0.19	0.16	0.15	28.5	35.7	14.2	7.1
		RH	166	1.6	0.11	0.14	0.13	0.13	0.12	27.2	18.1	18.18	9.09
		Mean(%IPL)								25.2	29.2	15.795	7.8975
		%Inhibition								20.6	4.48	79.31	91
4.	Test compound BT 1000 mg/kg	H	166	1.6	0.16	0.2	0.19	0.2	0.18	25	18.7	25	12.5
		B	159	1.5	0.14	0.19	0.18	0.19	0.15	35.7	28.5	35.7	7.1
		T	184	1.8	0.12	0.18	0.17	0.16	0.14	50	41.6	33.3	16.6
		RH	180	1.8	0.14	0.18	0.19	0.17	0.15	28.5	35.7	21.4	7.14
		Mean(%IPL)								34.8	31.125	28.85	10.835
		%inhibition								9.6	40.8	62.9	87.7

Table 3: Anti-inflammatory activity of compound BF and BT

DISCUSSION

Following the synthetic methods indicated in the scheme allowed for the creation of the aforementioned molecules. With the aid of straightforward methods, the title compounds were successfully synthesized. It had a strong yield and was chemically stable. The distinct spot-on TLC and crisp melting point demonstrated that title compounds were produced in their purest form. By repeatedly recrystallizing the synthesized compounds from the right solvents, they were made purer. The resulting compounds' IR spectra revealed absorption bands for amide NH around 3367 cm-1 and a distinctive broad absorption peak. In accordance with the assigned structures, these compounds also displayed appropriate peaks at corresponding ppm in their 1H-NMR spectra and corresponding molecular ion peaks in their LC-MS spectra. The structure of the title compounds was determined through the analysis of IR, 1H NMR, and LC-MS spectra.

Utilizing the software Discovery Studio, molecular docking investigations were conducted on all of the suggested molecules. All of the investigated compounds exhibit a substantial docking score when compared to the reference medication Ibuprofen. The compounds BF and BT were exposed to in vivo anti-inflammatory action using carrageenan-induced rat paw oedema technique based on docking score. The investigated drugs initially displayed less % inhibition. The investigated compounds also shown comparable percent inhibition at two hours, but after that, they displayed maximal percent inhibition in comparison to the parent molecule.

CONCLUSION

The results of the inquiry suggest that the most effective anti-inflammatory substance using the carrageenan-induced rat paw oedema method is a benzofuran derivative. With an oral LD50 value >2000 mg/kg in mice, the acute oral toxicity data of compounds BF and BT demonstrated the safety of these molecules. As a result, the current investigation is successful in finding the benzofuran derivatives' potential anti-inflammatory properties. To better understand the molecular processes behind COX 2 inhibition, more research is needed.

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