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SYNTHESIS, DOCKING STUDIES AND DISCOVERY OF NOVEL ANTI-INFLAMMATORY AND ANALGESIC BENZOFURAN DERIVATIVES

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Abstract

A new series of benzofuran derivatives, has been synthesized from 2-amino-6-(2,6-dibromo-4,7-dimethoxy benzofuran-5-yl)-4-(4-methoxyphenyl)nicotinonitrile (5). Some of the new compounds were screened for their anti-inflammatory and analgesic activities. Compounds 8 and 11 showed the greatest anti-inflammatory potency, over the 4 h tested, whereas compound 12b showed the least potency as compared to indomethacin. On the other hand, compound 12b revealed a significant increase in the reaction time/Sec.; suggesting long duration of antinociceptive action. In addition, using the Molsoft ICM 3.5-0a program molecular docking studies of the tested compounds with cyclooxygenase II, complexed with its inhibitor indomethacin (4COX) was performed to predict the affinity and the orientation of the compounds at the active sites of the enzyme.

Keywords: Benzofuran**,** Docking**,** Anti-inflammatory**,** Analgesic.

Introduction

Benzofuran derivatives are of great scientific interest [1] due to their varied biological activities [2–4]. They are widely used in bioorganic and medicinal chemistry with applications in drug discovery. Different benzofuran compounds were reported to possess, antibacterial [5], antifungal [6], anti-inflammatory [7], antidepressant [8] and hypoglycemic [9] activities. The discovery of the existence of two distinct isoforms of COX demonstrated that the side effects of NSAIDs such as gastric irritation, ulcer and gastric perforation are mainly due to the inhibition of the physiological enzyme COX-1 along with the desired blockade of the COX-2 enzyme [10-12]. The structures of COX-1 and COX-2 enzymes and their inhibitors have been thoroughly investigated for more than two decades. This led to the development of many of the selective COX-2 inhibitors, which are almost free of gastric side effects. Recent

studies in this field showed that COX-2 inhibitors constitute an attractive molecular target for the development various chemotherapeutic drugs for the treatment of cancer [13, 14] and neurological diseases such as Alzheimer's disease [15]. Selective COX-2 inhibitors belonging to different structural classes such as vicinal diaryl heterocycles, diaryl carbocycles and heteroaryl ethers have been developed as pharmacophores for anti-inflammatory activity [16]. Soon after the discovery of some selective COX-2 inhibitors namely, celecoxib, rofecoxib, valdecoxib and parecoxib as gastric safe anti-inflammatory drugs, researchers focused extensively on a tricycle template based COX-2 inhibitors [17]. Variations of the central ring of the tricyclic COX-2 inhibitors have already generated a number of potent derivatives bearing thiazole, triazole, imidazole, isoxazoline, pyridine, pyranone, pyridazone, pyrimidine, pyran, indole, benzothiazole, benzotriazole, benzimidazole, and benzopyran central ring scaffolds [18-23].

In the present investigation, we were focused on designing novel COX-2 inhibitors based on benzofuran template.

Materials and Methods

Chemistry

All melting points are uncorrected and were taken on an electro-thermal capillary melting point apparatus using Melting Point, Digital, Advanced, SMP30. The elemental analyses for C, H, and N were done on Vario EL III. Infrared spectra were recorded on a Jasco FT/IR-6100; Fourier transforms, Infrared spectrometer at cm^{-1} scale using the KBr disc technique. ^1H NMR spectra were determined by using a JEOl EX-270 NMR spectrometer. The mass spectra were measured with a Finnigan MAT SSQ-7000 mass spectrometer. Follow-up of the reactions and checking the purity of the compounds were made by TLC on silica gel-precoated aluminum sheets (Type 60, F 254) and the spots were detected by exposure to a UV analysis lamp at λ 254/366 nm for a few seconds.

General procedure for the synthesis of 2-amino-6-(2,6-dibromo-4,7-dimethoxybenzofuran-5-yl)-4-(4-methoxy phenyl) nicotinonitrile (5)

A mixture of compound **4** [24] (0.01 mol) in DMF (25 ml) was added to NH_4OH (50 ml, 25% in water) and the resulting mixture was stirred at room temperature for 12 h. The mixture was extracted with ethyl acetate (100 ml) and the combined organic layer was dried over Na_2SO_4, filtered, concentrated under vacuum, then the resulting solid was crystallized from ethanol to afford compound **5**.

Yield: 85%; Yellow crystalline powder; mp: 185-187 °C, IR (KBr): υ(cm^{-1}) 2,222 (C≡N) and 3,243, 3,330 (NH_2). MS (EI)—*m/z* 559 (M^+, 22 %). ^1H NMR (DMSO-d_6, δ ppm): 3.84 (s, 3H, OCH_3 of benzofuran), 3.86 (s, 3H, OCH_3 of benzofuran), 3.87 (s, 3H, OCH_3 of 4-methoxyphenyl), 5.29 (s, 1H, CH of benzofuran), 6.95-7.39 (m, 4H, Ar-H),

8.26 (s, 1H, CH of pyridine), 8.86 (exch br s, 2H, NH_2). Anal. Calcd. for $C_{23}H_{17}Br_2N_3O_4$ (559): C, 49.40; H, 3.06; N, 7.51; Found: C, 49.30; H, 2.91; N, 7.45.

General procedure for the synthesis of 1-{3-cyano-6-[2,6-dibromo-4,7-dimethoxybenzofuran-5-yl]-4-(4-methoxyphenyl)pyridin-2-yl}-3-phenylthiourea (6)

A mixture of compound **5** (0.01 mol), finely divided sodium metal (0.01 mol) and phenyl isothiocyanate (0.01 mol) was refluxed for 6 h in dry dioxane (50 ml). After cooling, the solvent was concentrated under pressure, then the reaction mixture was poured onto ice-water (40 ml) to give a solid precipitate which was filtered off and crystallized from petroleum ether 60/80 to furnish compound **6**.

Yield: 70%; White crystalline powder; mp: >300 °C, IR (KBr): υ(cm^{-1}) 2,225 (C≡N) and 3,147 (NH). MS (EI)—*m/z* 694 (M^+, 50 %). ^{1}H NMR (DMSO-d_6, δ ppm): 3.85 (s, 3H, OCH_3 of benzofuran), 3.87 (s, 3H, OCH_3 of benzofuran), 3.91 (s, 3H, OCH_3 of 4-methoxyphenyl), 4.69 (exch br s, 1H, NH), 4.94 (exch br s, 1H, NH), 7.11 (s, 1H, CH of benzofuran), 7.26-8.31 (m, 9H, Ar-H), 9.87 (s, 1H, CH of pyridine). Anal. Calcd. For $C_{30}H_{22}Br_2N_4O_4S$ (694): C, 51.89; H, 3.19; N, 8.07; Found: C, 51.80; H, 2.89; N, 7.87.

General procedure for the synthesis of 6-{2,6-dibromo-4,7-dimethoxybenzofuran -5-yl}-2-[(4-methoxybenzylidene)amino]-4-(4-methoxyphenyl)nicotinonitrile (7)

A mixture of compound **5** (0.001 mol) and 4-anisaldehyde (0.0015 mol) in glacial acetic acid (30 ml) was heated under reflux for 6 h. The reaction mixture was cooled, and the obtained solid was filtered off, washed with petroleum ether, dried, and crystallized from ethanol to furnish compound **7**.

Yield: 70%; Brown crystalline powder; mp: 135-137 °C, IR (KBr): υ(cm^{-1}) 2,219 (C≡N). MS (EI)—*m/z* 677 (M^+, 21.08 %). ^{1}H NMR (DMSO-d_6, δ ppm): 3.62 (s, 3H, OCH_3 of benzofuran), 3.67 (s, 3H, OCH_3 of benzofuran), 3.71 (s, 3H, OCH_3 of 4-methoxyphenyl), 3.75 (s, 3H, OCH_3 of 4-methoxyphenyl), 6.63 (s, 1H, CH of benzofuran), 8.24 (s, 1H, CH of benzylidenimin), 7.25-7.91 (m, 8H, Ar-H), 8.56 (s, 1H, CH of pyridine). Anal. Calcd. for $C_{31}H_{23}Br_2N_3O_5$ (677): C, 54.97; H, 3.42; N, 6.20; Found: C, 54.87; H, 3.36; N, 6.06.

General procedure for the synthesis of *N*-{3-cyano-6-[2,6-dibromo-4,7-dimethoxybenzofuran-5-yl]-4-(4-methoxyphenyl)pyridin-2-yl}formamide (8)

Compound **5** (0.002 mol) in formic acid (10 ml) was heated under reflux for 6 h. After cooling, the reaction mixture was poured onto ice-water (40 ml) to give a solid precipitate which was filtered off and crystallized from methanol to furnish compound **8**.

Yield: 80%; Gray crystalline powder; mp: >300 °C, IR (KBr): υ(cm^{-1}) 1,678 (C=O), 2,207 (C≡N) and 3,143 (NH). MS (EI)—*m/z* 587 (M^+, 10 %). ^{1}H NMR (DMSO-d_6, δ ppm): 3.52 (s, 3H, OCH_3 of benzofuran), 3.56 (s, 3H, OCH_3 of benzofuran), 3.59 (s, 3H, OCH_3 of 4-methoxyphenyl), 6.51 (s, 1H, CH of benzofuran), 7.13-7.84 (m, 4H, Ar-H), 8.12 (s, 1H, CH of pyridine), 8.61 (exch br s, 1H, NH), 8.72 (s, 1H, CHO). Anal. Calcd. for $C_{24}H_{17}Br_2N_3O_5$ (587): C, 49.09; H, 2.92; N, 7.16; Found: C, 48.87; H, 2.78; N, 7.11.

General procedure for the synthesis of *N*-{3-cyano-6-[2,6-dibromo-4,7-dimethoxybenzofuran-5-yl]-4-(4-methoxyphenyl)pyridin-2-yl}-3-oxobutanamide (9)

A mixture of compound **5** (0.005 mol) and ethyl acetoacetate (8 ml) was heated for 6 h at 140–150 °C. Excess ethyl acetoacetate was distilled off under reduced pressure, and the residue was purified by crystallization from ethanol to give compound **9**.

Yield: 65%; Yellow crystalline powder; mp: >300 °C, IR (KBr): υ(cm^{-1}) 1,654, 1,724 (2C=O), 2,207 (C≡N) and 3,143 (NH). MS (EI)—*m/z* 643 (M^+, 30 %). ^{1}H NMR (DMSO-d_6, δ ppm): 2.32 (s, 3H, CH_3 of $COCH_3$), 3.51 (s, 2H, CH_2 of methylene), 3.73 (s, 3H, OCH_3 of benzofuran), 3.75 (s, 3H, OCH_3 of benzofuran), 3.81 (s, 3H, OCH_3 of 4-methoxyphenyl), 6.61 (s, 1H, CH of benzofuran), 8.33 (s, 1H, CH of pyridine), 7.13-7.81 (m, 4H, Ar-H), 9.12 (exch br s, 1H, NH).

Anal. Calcd. for $C_{27}H_{21}Br_2N_3O_6$ (643): C, 50.41; H, 3.29; N, 6.53; Found: C, 50.32; H, 3.19; N, 6.42.

General procedure for the synthesis of *N*-{3-cyano-6-[2,6-dibromo-4,7-dimethoxybenzofuran-5-yl]-4-(4-methoxyphenyl)pyridin-2-yl}benzamide (10)

A mixture of compound **5** (0.01 mol) and benzoyl chloride (0.015 mol) in dimethyl formamide (25 ml) was heated under reflux for 5 h. The reaction mixture was cooled, poured onto ice/water mixture. The separated solid was filtered off, washed with water, dried, and crystallized from ethanol to afford compound **10**.

Yield: 85%; Yellow crystalline powder; mp: 200-205 °C, IR (KBr): υ(cm^{-1}) 1,670 (C=O), 2,119 (C≡N) and 3,175 (NH). MS (EI)—*m/z* 663 (M^+, 18 %). ^{1}H NMR (DMSO-d_6, δ ppm): 3.61 (s, 3H, OCH_3 of benzofuran), 3.64 (s, 3H, OCH_3 of benzofuran), 3.65 (s, 3H, OCH_3 of 4-methoxyphenyl), 6.52 (s, 1H, CH of benzofuran), 8.11 (s, 1H, CH of pyridine), 7.13-8.25 (m, 9H, Ar-H), 9.17 (exch br s, 1H, NH). Anal. Calcd. for $C_{30}H_{21}Br_2N_3O_5$ (663): C, 54.32; H, 3.19; N, 6.33; Found: C, 54.13; H, 3.11; N, 6.21.

General procedure for the synthesis of *N*-{3-cyano-6-[2,6-dibromo-4,7-dimethoxybenzofuran-5-yl]-4-(4-methoxyphenyl)pyridin-2-yl}acetamide (11)

A solution of compound **5** (0.002 mol) in acetic anhydride (10 ml) was heated under reflux for 3 h. After cooling, the solvent was concentrated under reduced pressure, then the reaction mixture was poured onto ice-water (40 ml) to give a solid precipitate which was filtered off and crystallized from petroleum ether 60/80 to furnish compound **11**.

Yield: 90%; Gray crystalline powder; mp: >300 °C, IR (KBr): υ(cm^{-1}) 1,687 (C=O), 2,105 (C≡N) and 3,201 (NH). MS (EI)—*m/z* 601 (M^+, 60 %). ^{1}H NMR (DMSO-d_6, δ ppm): 2.41 (s, 3H, CH_3 of $COCH_3$), 3.82 (s, 3H, OCH_3 of benzofuran), 3.85 (s, 3H, OCH_3 of benzofuran), 3.87 (s, 3H, OCH_3 of 4-methoxyphenyl), 6.51 (s, 1H, CH of benzofuran), 8.63 (s, 1H, CH of pyridine), 7.12-7.63 (m, 4H, Ar-H), 9.54 (exch br s, 1H, NH). Anal. Calcd. for $C_{25}H_{19}Br_2N_3O_5$ (601): C, 49.94; H, 3.19; N, 6.99; Found: C, 49.87; H, 2.98; N, 6.77.

General procedure for the synthesis of 1-{3-cyano-6-[2,6-dibromo-4,7-dimethoxybenzofuran-5-yl]-4-(4-methoxyphenyl)pyridin-2-yl}substituted (12a, b)

A mixture of compound **5** (0.01 mol) and urea (or thiourea) (0.01 mol) was fused in an oil bath at 180 ^{0}C, for 1-3 h. After cooling and dilution with ethanol (30 ml) the solid product formed was filtered off and crystallized from ethanol to give compounds **12a, b**, respectively.

1-{3-Cyano-6-[2,6-dibromo-4,7-dimethoxybenzofuran-5-yl]-4-(4-methoxyphenyl) pyridin-2-yl}urea (12a)

Yield: 60%; Black crystalline powder; mp: >300 °C, IR (KBr): υ(cm^{-1}) 1,701 (C=O), 2,209 (C≡N), 3,186 (NH) and 3,320, 3,442 (NH_2). MS (EI)—*m/z* 602 (M^+, 43 %). ^{1}H NMR (DMSO-d_6, δ ppm): 3.61 (s, 3H, OCH_3 of benzofuran), 3.65 (s, 3H, OCH_3 of benzofuran), 3.68 (s, 3H, OCH_3 of 4-methoxyphenyl), 6.43 (s, 1H, CH of benzofuran), 8.34 (s, 1H, CH of pyridine), 7.25-7.73 (m, 4H, Ar-H), 9.21 (exch br s, 1H, NH), 10.13 (exch br s, 2H, NH_2). Anal. Calcd. for $C_{24}H_{18}Br_2N_4O_5$ (602): C, 47.86; H, 3.01; N, 9.30; Found: C, 47.78; H, 2.90; N, 9.12.

1-{3-Cyano-6-[2,6-dibromo-4,7-dimethoxybenzofuran-5-yl]-4-(4-methoxyphenyl) pyridin-2-yl}thiourea (12b)

Yield: 85%; Black crystalline powder; mp: 268-270 °C, IR (KBr): υ(cm^{-1}) 2,210 (C≡N), 3,186 (NH) and 3,331, 3,442 (NH_2). MS (EI)—*m/z* 618 (M^+, 13 %). ^{1}H NMR (DMSO-d_6, δ ppm): 3.91 (s, 3H, OCH_3 of benzofuran), 3.95 (s, 3H, OCH_3 of benzofuran), 3.98 (s, 3H, OCH_3 of 4-methoxyphenyl), 6.62 (s, 1H, CH of benzofuran), 8.11 (s, 1H, CH of pyridine), 7.12-7.92 (m, 4H, Ar-H), 9.33 (exch br s, 1H, NH), 10.51 (exch br s, 2H, NH_2). Anal. Calcd. for $C_{24}H_{18}Br_2N_4O_4S$ (618): C, 46.62; H, 2.93; N, 9.06; Found: C, 46.54; H, 2.87; N, 8.91.

General procedure for the synthesis of 2-chloro-*N*-{3-cyano-6-[2,6-dibromo-4,7-dimethoxybenzofuran-5-yl]-4-(4-methoxyphenyl)pyridin-2-yl}acetamide (13)

A solution of **5** (0.001 mol) was allowed to react with chloroacetyl chloride (0.001 mol) in dimethylformamide (20 ml) and stirred at room temperature for 1 h. Then the reaction solution was poured over ice/HCl. The obtained product was filtered and crystallized from ethanol to give compound **13**.

Yield: 90%; Brown crystalline powder; mp: 175-177 °C, IR (KBr): υ(cm^{-1}) 1,739 (C=O), 2,209 (C≡N) and 2,973 (NH). MS (EI)—*m/z* 635 (M^+, 37 %). ^{1}H NMR (DMSO-d_6, δ ppm): 3.92 (s, 3H, OCH_3 of benzofuran), 3.95 (s, 3H, OCH_3 of benzofuran), 3.96 (s, 3H, OCH_3 of 4-methoxyphenyl), 4.13 (s, 2H, CH_2 of CH_2Cl), 6.71 (s, 1H, CH of benzofuran), 8.63 (s, 1H, CH of pyridine), 7.32-8.31 (m, 4H, Ar-H), 9.61 (exch br s, 1H, NH). Anal. Calcd. for $C_{25}H_{18}Br_2ClN_3O_5$ (635): C, 47.24; H, 2.85; N, 6.61; Found: C, 47.11; H, 2.73; N, 6.53.

Docking studies

All docking studies were performed using Internal Coordinate Mechanics (Molsoft ICM 3.5-0a).

Preparation of a small molecule

Compounds **3** [24], **4** [24], **5, 7, 8, 10, 11, 12b** and **13** were built in ChemDraw Ultra version 12.0 and their energy was minimized through Chem3D Ultra version 12.0 / MM2, Job Type: minimum RMS Gradient of 0.100, and saved as MDL MolFile (*.mol).

Generation of Ligand and Enzyme Structures

The crystal structures of cyclooxygenase II (PDB code: 4COX) complex was retrieved from the RCSB Protein Data Bank (http://www.rcsb.org/pdb/ home/home.do). In our investigation, the 3D-coordinates in X-ray crystal structure of cyclooxygenase II in complex with the ligand, indomethacin (PDB entry 4COX) was used as the receptor model in cyclooxygenase II docking simulations (Fig. 1). All bound water ligands, and cofactors were removed from the protein.

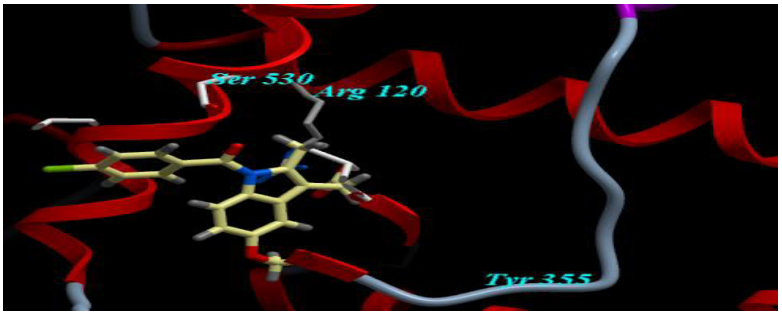


Fig. 1: Binding mode of the original ligand Indomethacin into its binding site of cycloxygenase II, it has ICM score -81.49, and forms 5 H bonds with Arg120, Tyr355 and Ser530 (Table 3).

Docking using Molsoft ICM 3.5-0a program.

The conversion of our PDB file into an ICM object involves the addition of hydrogen bonds, assignment of atom types, and charges from the residue templates, then perform ICM small molecule docking through a setup the receptor, review and adjust a binding site makes receptor maps, then start docking simulation, followed by displaying the results. ICM stochastic global optimization algorithm attempts to find the global minimum of the energy function that included five grid potentials describing the interaction of the flexible ligand with the receptor and internal conformational energy of the ligand, during this process a stack of alternative low energy conformations is saved. All inhibitors were compared according to the best binding free energy (minimum) obtained among all the run.

Biological activity

Animals: The rats used in this study were procured from the Animal House Colony at the National Research Centre (NRC), Egypt. Animals were allowed to adapt to the laboratory environment for one week before experimentation. Male Wistar rats weighing 150-200 g were housed at 24±1 °C on a 12:12 h light dark cycle, and with free access to food and tap water. Eight hours before the experiment, only tap water was available to the rats. All experiments were performed between 10 a.m. and 5 p.m. The study was conducted in compliance with the ethical guidelines of the Ethics Committee of the National Research Centre- Egypt and in accordance with the recommendations of the proper care and use of laboratory animals.

1- Anti-inflammatory activity

The anti-inflammatory testing was performed according to the method of Winter et al [25]. Paw edema was induced in rats by subcutaneous (s.c.) injection of 0.1 ml of 1% (w/v) freshly prepared carragennan in distilled water in the sub-plantar region of their left hind paws. Edema was measured with the use of a plethysmometer (model 7140, Ugo Basile, Italy) at 1 h intervals up to 4 h after carrageenin injection. A group of rats was left without any treatment, but given a respective volume of the solvent (distilled water), and was kept as control. Compounds **3** [24], **4** [24], **5, 7, 8, 10, 11, 12b** and **13** were orally administered to rats at doses of 30 mg/kg. Indomethacin was orally given to rats in a dose of 20 mg/kg as a reference drug.

Edema rate and inhibition rate of each group were calculated at the above-mentioned time intervals as follows:

Edema (%) = Vt - Vo/Vo

Inhibition (%) = Ec - Et/Ec

Where, Vo is the volume before carrageenin injection (ml), Vt is the volume at *t* hour after carrageenin injection (ml), Ec is the edema rate of the control group, and Et is the edema rate of the treated group.

2- Analgesic activity

The experiment was carried out as described by Turner [26] using a hot-plate apparatus, (model 7280, UGO Basile-Italy). The plate temperature was maintained at 53 ± 0.5 ºC.

Seventy-two rats were divided into 12 groups of 6 animals each. The reaction time of rats to the thermal stimulus was the time interval between placing the animal on the hot-plate and when it licked its hind paw or jumped. Reaction time was measured prior to compound and drug treatment (0 min).

Group 1 was kept as normal control and was treated orally with the vehicle alone (5 ml/kg). Rats of the 2nd group (reference group) were orally treated with indomethacin in a dose of 20 mg/kg b.wt., while compounds number **3** [24], **4** [24], **5, 7, 8, 10, 11, 12b** and **13** were orally administered to rats of groups 3-12 at doses of 30 mg/kg b.wt.

The reaction time was again measured at 30 min and repeated at 60 and 90 min after treatment. To avoid tissue damage to the rat paws, cut-off time for the response to the thermal stimulus was set at 60 s. The reaction time was calculated for each compound and drug-treated group.

Statistical Analysis

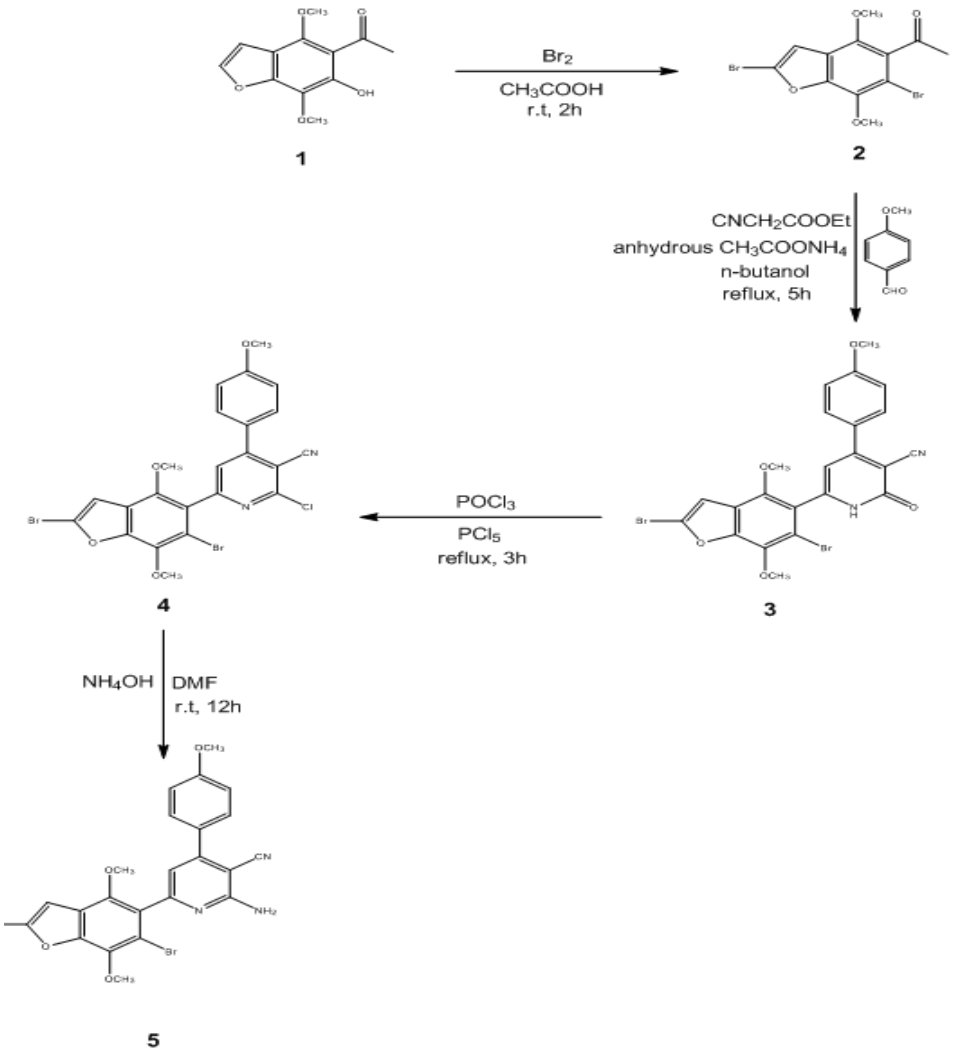
Results were analyzed using analytical software (SPSS statistics 17.0, Chicago, USA) and data were expressed as means ± standard error.

Results and discussion

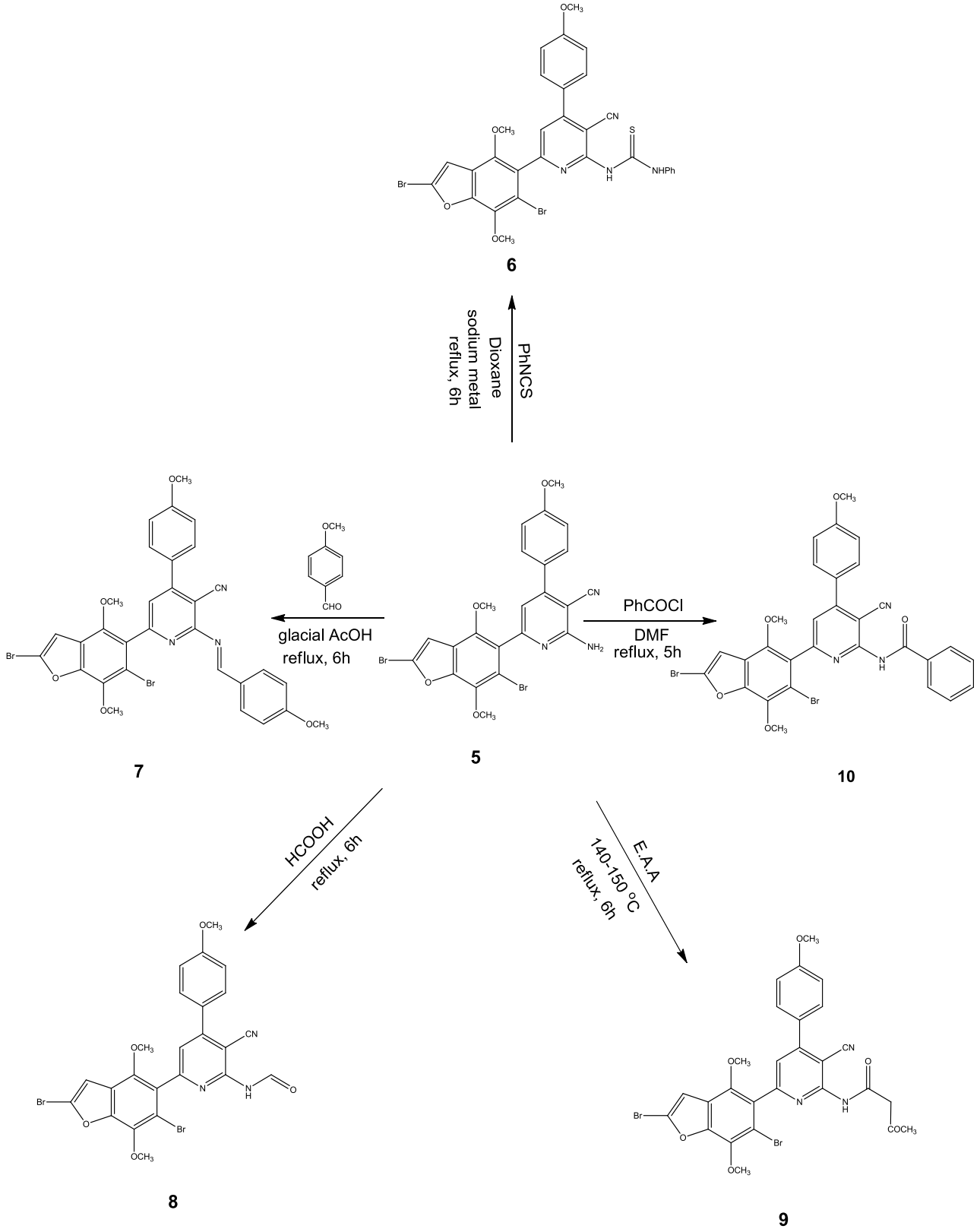
Chemistry

Herein we report the synthesis and characterization of a series of benzofuran derivatives. Compound 2-chloro-6-(2,6-dibromo-4,7-dimethoxybenzofuran-5-yl)-4-(4-methoxyphenyl)nicotinonitrile **(4)** [24] treated with NH_4OH to give 2-amino-6-(2,6-dibromo-4,7-dimethoxybenzofuran-5-yl)-4-(4-methoxyphenyl)nicotinonitrile **(5)** (Scheme 1). The latter compound **(5)** was used as a precursor for synthesizing a number of new benzofuran derivatives. Thus, the reaction of compound **(5)** with phenyl isothiocyanate gave the corresponding analogue 1-{3-cyano-6-[2,6-dibromo-4,7-dimethoxybenzofuran-5-yl]-4-(4-methoxyphenyl)pyridin-2-yl}-3-phenylthiourea **(6)** (Scheme 2). Furthermore, compound **(5)** was allowed to react with 4-anisaldehyde in glacial acetic acid to give the compound 6-{2,6-dibromo-4,7-dimethoxybenzofuran-5-yl}-2-[(4-methoxybenzylidene)amino]-4-(4-methoxyphenyl)nicotinonitrile **(7)** (Scheme 2). Also, compound (**5**) was condensed with formic acid to give the corresponding compound *N*-{3-cyano-6-[2,6-

dibromo-4,7-di methoxybenzofuran-5-yl]-4-(4-methoxyphenyl)pyridin-2-yl}formamide **(8)** (Scheme 2). In addition, compound *N*-{3-cyano-6-[2,6-dibromo-4,7-dimethoxybenzofuran-5-yl]-4-(4-methoxyphenyl)pyridin-2-yl}-3-oxobutanamide **(9)** was synthesized by the reaction of compound **(5)** with ethyl acetoacetate (Scheme 2). At the same time, the reaction of compound **(5)** with benzoyl chloride in refluxing dimethyl formamide yielded the derivative *N*-{3-cyano-6-[2,6-dibromo-4,7-dimethoxybenzofuran-5-yl]-4-(4-methoxyphenyl)pyridin-2-yl}benzamide**(10)** (Scheme 2). ^{1}H NMR (DMSO-d_6) spectra of compound **(6)** revealed signals at δ 4.69 and 4.94 ppm representing 2NH groups, and revealed signals at δ 7.26-8.31 ppm representing Ar-H. ^{1}H NMR (DMSO-d_6) spectra of compound **(7)** revealed signals at δ 3.62, 3.67, 3.71 and 3.75 ppm for 2OCH_3 of benzofuran and 2OCH_3 of 4-methoxyphenyl, respectively. ^{1}H NMR (DMSO-d_6) spectra of compound **(8)** revealed signals at δ 8.61 ppm representing NH group. IR spectra of compound **(9)** exhibited characteristic absorption bands at 1,654, 1,724 cm^{-1} due to the respective (2C=O), also IR spectra of compound **(10)** exhibited a characteristic absorption band at 1,670 cm^{-1} due to the respective (C=O).



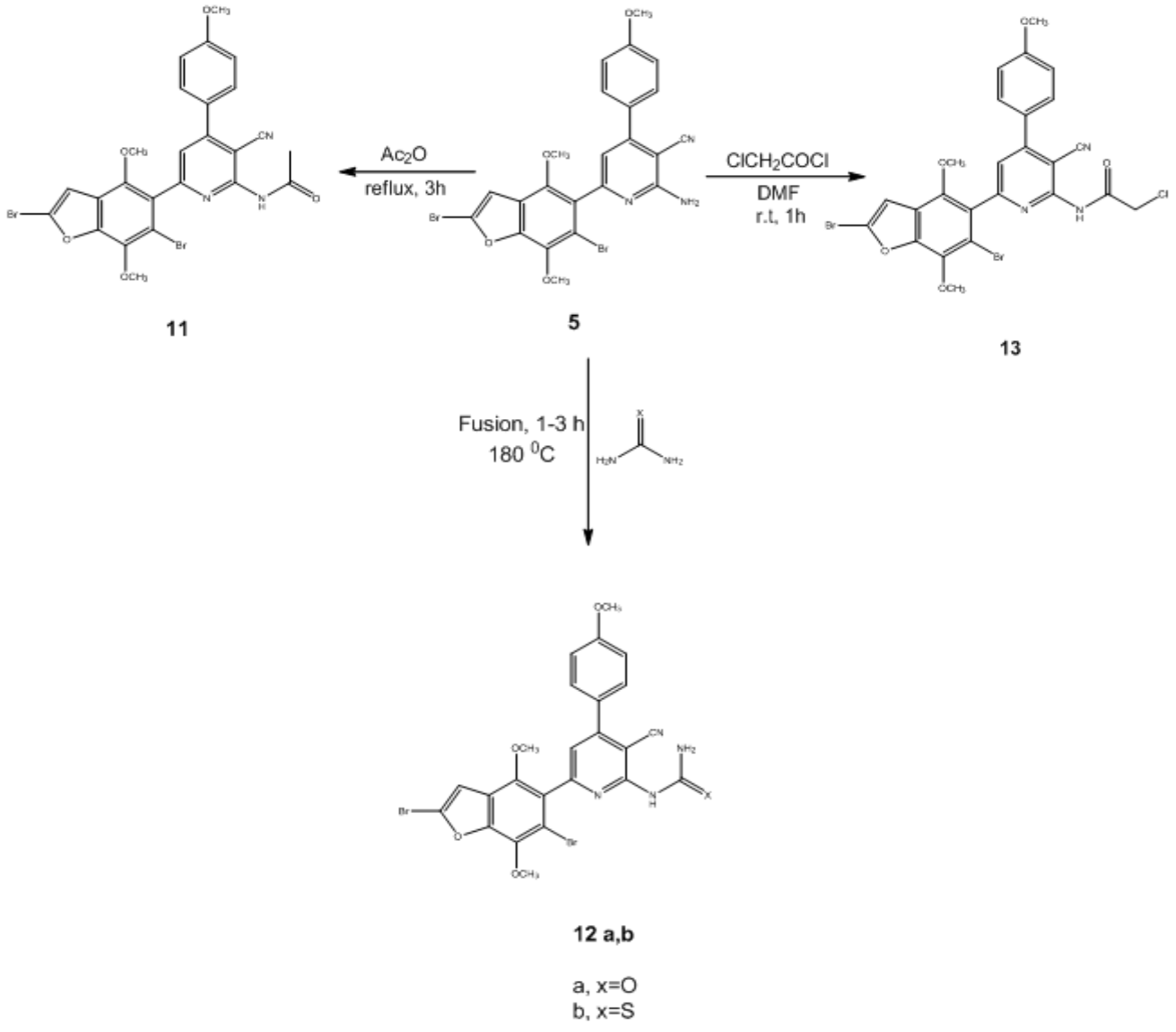
Scheme-1



Scheme 2

Further treatment of compound **(5)** with acetic anhydride gave the corresponding acetamide derivative *N*-{3-cyano-6-[2,6-dibromo-4,7-dimethoxybenzofuran-5-yl]-4-(4-methoxyphenyl)pyridin-2-yl}acetamide **(11)** (Scheme 3), while the reaction of compound **(5)** with urea and/or thiourea gave the corresponding derivatives 1-{3-cyano-6-[2,6-dibromo-4,7-dimethoxybenzofuran-5-yl]-4-(4-methoxyphenyl)pyridin-2-yl}substituted **(12a,b)** (Scheme 3).

Compound 2-chloro-*N*-{3-cyano-6-[2,6-dibromo-4,7-dimethoxybenzofuran-5-yl]-4-(4-methoxyphenyl)pyridin-2-yl}acetamide **(13)** was prepared by the reaction of compound **(5)** with chloroacetyl chloride in dimethyl formamide (Scheme 3). IR spectra of compound **(11)** exhibited characteristic absorption band at 1,687 cm^{-1} due to the respective (C=O). IR spectra of compound **(12a)** exhibited a characteristic absorption band at 1,701 cm^{-1} due to the respective (C=O), while the mass spectrum of compound **(12b)** showed the molecular ion peak $[M]^+$ at m/z ≈ 618 (13%). IR spectra of compound **(13)** exhibited characteristic absorption band at 1,739 cm^{-1} due to the respective (C=O).



Scheme-3

Biological activity

1. Carragennan induced inflammation in rats.

Some synthesized compounds were evaluated for their possible anti-inflammatory effects in a rat model of carrageenan-induced paw edema. This animal model is widely used for determining the acute phase of inflammation. (Table 1) is showing the effect of compounds **3** [24], **4 [**24], **5, 7, 8, 10, 11, 12b** and **13** on carrageenan-induced paw

edema in rats in comparison to a non-steroidal anti-inflammatory drug that inhibits prostaglandin, indomethacin, as a reference drug.

Table 1: Anti-inflammatory activity of testing compounds against carrageenan-induced paw edema in rats, (n = 6).

Group	1 hour			2 hours			3 hours			4 hours		
	Edema (%)	Inhibition (%)	Potency (%)	Edema (%)	Inhibition (%)	Potency (%)	Edema (%)	Inhibition (%)	Potency (%)	Edema (%)	Inhibition (%)	Potency (%)
Control	58.5^{b}±1.14	0	----	82.3^{b}±2.13	0	----	86.1^{b}±6.26	0	----	83.0^{b}±7.22	0	----
Indomethacin	31.7^{a}±2.28	45.81	100	43.2^{a}±1.66	47.51	100	44.6^{ab}±2.69	48.18	100	41.9^{a}±2.08	49.48	100
3	51.8^{b}±4.68	11.42	24.9	56.6^{a}±4.26	31.22	65.7	67.5^{ab}±4.17	21.69	45.0	69.5^{b}±5.19	16.25	32.8
4	53.9^{b}±4.22	7.74	16.9	74.0^{b}±6.25	10.15	21.4	74.6^{b}±5.34	13.35	27.7	70.4^{b}±6.04	15.11	30.5
5	45.1±2.07	22.86	49.9	59.5^{a}±5.36	27.79	58.5	66.9^{ab}±3.26	22.36	46.4	68.8^{b}±3.91	17.05	34.5
7	52.2^{b}±3.28	10.76	23.5	56.7^{a}±4.26	31.17	65.6	67.4^{ab}±6.30	21.80	45.3	70.2^{b}±4.66	15.47	31.3
8	50.2^{b}±4.44	14.10	30.8	54.7^{a}±2.00	33.51	70.5	59.9^{a}±2.60	30.50	63.3	56.3^{a}±3.67	27.55	65.1
10	55.0^{b}±3.61	5.98	13.1	61.1^{ab}±2.55	25.84	54.4	67.3^{ab}±3.61	21.86	45.4	68.3^{b}±5.59	17.74	35.9
11	44.8±3.80	23.42	51.1	54.9^{a}±1.35	33.27	70.0	59.8^{a}±4.02	30.58	63.5	58.8^{a}±2.64	29.12	58.9
12b	55.1^{b}±3.28	5.68	12.4	68.7^{b}±3.07	16.49	34.7	81.8^{b}±2.61	5.02	10.4	80.1^{b}±5.33	3.51	7.1
13	47.1^{b}±2.28	19.37	42.3	58.1^{a}±4.72	29.38	61.8	63.6^{ab}±2.87	26.14	54.3	62.0^{ab}±1.65	25.27	51.1

[a] P<0.05: Statistically significant from control (Dunnett's test).

[b] P<0.05: Statistically significant from indomethacin (Dunnett's test).

Intra-plantar injection of carrageenan in rats led to increase in paw volume denoting edema in the control non-treated group as shown in (Table 1). It was noticed that most of the synthesized derivatives in doses of 30 mg/kg significantly decreased the paw edema rate all over the 4 h in comparison to the control non-treated group. The anti-inflammatory potencies of the tested compounds **3** [24], **4 [**24], **5, 7, 8, 10, 11, 12b** and **13** were calculated by comparing their inhibition rate at different time intervals with those obtained from animals receiving indomethacin as a standard anti-inflammatory drug. The anti-inflammatory potencies of the tested compounds after 4 h as compared to indomethacin, arranged in descending order, were 65.1, 58.9, 51.1, 35.9, 34.5, 32.8, 31.3, 30.5 and 7.1 for compounds **8, 11, 13, 10, 5, 3** [24], **7, 4** [24] and **12b**, respectively. Administration of indomethacin significantly decreased paw edema starting from the first hour and was persistent till the end of the experiment. The inhibitory effect of indomethacin on paw edema was 45.81, 47.51, 48.18, and 49.48% at the 1st, 2nd, 3rd and 4th h, respectively.

It is noteworthy to mention that derivatives **8** and **11** showed the greatest anti-inflammatory potency all over the 4 h while derivative **12b** showed the least potency as compared to indomethacin. Moreover, derivatives **8** and **11** showed the highest potency as compared to indomethacin after 2 h (70.5% and 70.0%, respectively).

2. Central analgesic activity in mice (Hot-plate test)

The central analgesic activity of compounds **3** [24], **4 [**24], **5, 7, 8, 10, 11, 12b** and **13** was determined using the hot-plate method. From the data shown in (Table 2), it was noticed that derivative **12b** revealed a significant increase in reaction time/Sec.; denoting antinociceptive effect starting after 60 min of administration till 90 min.

The antinociceptive effect of derivatives **5, 8** and **10** were prominent after 90 min of administration. Animal group administered indomethacin as a reference drug, showed a significant analgesic effect as compared to the control group after 60 and 90 min of administration.

Table-2: Central analgesic activity of testing compounds (hot plate method).

Group	Dose (mg/kg)	Reaction time (sec.)			
		0 min	30 min	60 min	90 min
Control	**----**	11.1±0.21	12.6±0.62	12.8±0.20ǂ	12.6±0.53ǂ
Indomethacin	**20**	10.6±0.04	14.7±2.01	17.3±2.06*	18.1±1.99*
3	**30**	10.8±0.18	16.1±0.56	16.5±0.82	16.9±0.72
4	**30**	10.5±0.22	15.9±0.92	16.5±0.50	17.1±0.43
5	**30**	11.0±0.48	12.5±0.72	16.6±0.90	17.9±1.33*
7	**30**	11.7±0.32	13.5±0.65	14.7±0.50	14.4±0.54
8	**30**	10.5±0.20	14.0±1.11	15.9±0.91	17.9±1.34*
10	**30**	11.1±0.80	15.6±1.30	15.8±1.37	17.9±1.51*
11	**30**	10.8±0.60	13.3±0.54	16.4±0.60	16.5±0.77
12b	**30**	11.2±0.70	16.6±1.55	19.6±1.67*	20.0±1.24*
13	**30**	11.3±0.49	13.0±0.41	13.1±0.36	13.9±0.48

Values represent the mean ± S.E. of six rats for each group.
* P< 0.05: Statistically significant from control (Dunnett's test).
ǂ P< 0.05: Statistically significant from indomethacin (Dunnett's test).

Docking analysis

Compounds **3** [24], **4** [24], **5, 7, 8, 10, 11, 12b** and **13** were used for docking study. All the calculations were performed using Internal coordinate Mechanics (Molsoft ICM 3.5-0a). Molecular modeling docking study is performed and ICM score values [27-29] combined with hydrogen bonds formed with the surrounding amino acid residues help to predict the correct binding geometry for each binder at the active site. In order to compare the binding affinity of the newly synthesized compounds, we docked compounds **3** [24], **4** [24], **5, 7, 8, 10, 11, 12b** and **13** into the empty binding site of cyclooxygenase II (4COX), with its bound inhibitor indomethacin. As shown in (Table 3), indomethacin (legend) reveals an ICM score of -81.49 and forms 5 H bonds with Arg120, Tyr355 and Ser530 (Fig. 1), the target compounds elicited binding affinities (ICM scores range from -54.67 to -79.90). Compounds **8, 11, 13** showed activity as anti-inflammatory agents probably due to their high ICM scores, which ranged from -77.09 to -79.90, for example compound **8** reveals an ICM score -79.90, and form 5 H bonds with Cys41, Arg44 and Cys47 (Fig. 2), however compounds **12b, 7, 10, 5, 3** [24], **4** [24], are biologically inactive as anti-inflammatory agents probably due to their low ICM scores of ranges from -54.67 to -75.24, for example compound **4** [24] reveals an ICM score -54.67 forms 2 H bonds with Val228 and Asn537 (Fig. 3).

Table 3: ICM Scores of indomethacin, the compounds, and hydrogen bonds formed with amino acid residues and their lengths.

Compounds	ICM scores	No. of hydrogen bonds	Involved group of amino acid	Atom of ligand involved	Length of hydrogen bond (A)
Indomethacin	-81.49	5	Arg120 Arg120 Arg120 Tyr355 Ser530	MO3 MO3 MO4 MO4 MO2	1.99 2.22 1.61 2.29 2.04
3	-60.83	1	Tyr385	MO5	1.70
4	-54.67	2	Val228 Asn537	MO4 MO4	2.64 1.46
5	-65.60	1	Tyr385	MO4	1.76
7	-72.36	4	Gln203 Thr212 His214 Asn382	MO5 MN2 MN3 MN2	2.54 2.78 2.06 2.40
8	-79.90	5	Cys41 Arg44 Arg44 Cys47	MO2 MN3 MO3 MO4	2.65 2.57 2.19 1.73

			Cys41	MH3	1.66
10	-65.81	1	Arg120	MO3	2.79
11	-78.37	7	Thr212	MN3	1.40
			Thr 212	MN3	1.60
			Arg 222	MO5	2.71
			Arg 222	MO5	2.27
			Gln 289	MO5	1.44
			Trp 387	MO1	2.60
			Asn 382	MH2	1.85
12b	-75.24	7	Thr 212	MO2	2.25
			Thr 212	MO2	2.50
			Asn 382	MN4	2.72
			Trp 387	MO4	2.68
			Asn 382	MH2	1.60
			Tyr 385	MH8	1.34
			Tyr 385	MH9	1.28
13	-77.09	2	Arg 222	MO4	2.51
			Gln 289	MO4	1.23

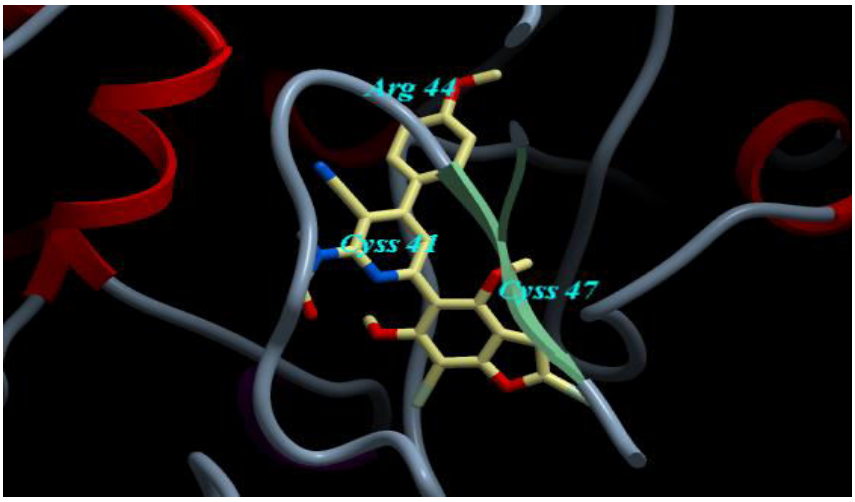


Fig. 2: Binding mode of compound 8 into its binding site of cycloxygenase II, it has ICM score -79.90, and form 5 H bonds with Cys41, Arg44 and Cys47 (Table 3).

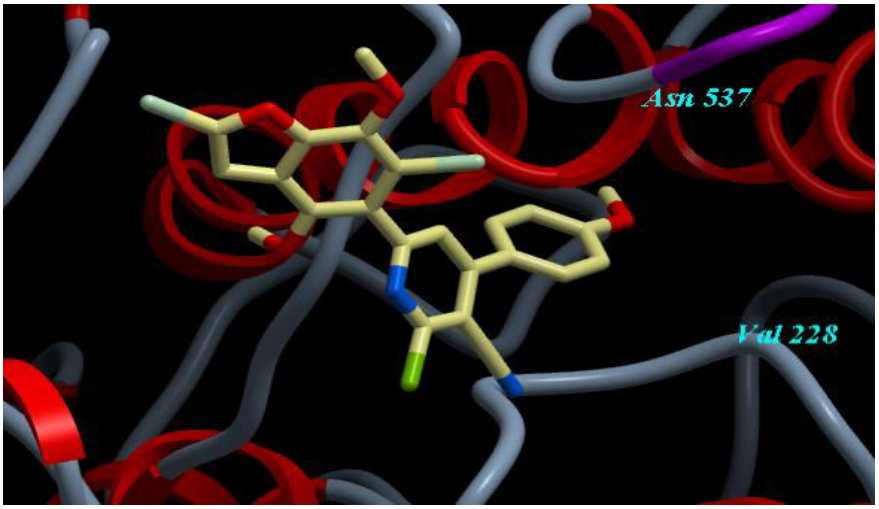


Fig. 3: Binding mode of compound 4 into its binding site of cycloxygenase II, it has ICM score -54.67 forms 2 H bonds with Val228 and Asn537 (Table 3)

Conclusion

A novel series of some new benzofuran derivatives was synthesized and evaluated as anti-inflammatory and analgesic agents. Anti-inflammatory activity results exhibited that, derivatives **8** and **11** showed the greatest anti-inflammatory potency all over the 4 h (Table 1). On the other hand, **12b** showed the least potency as compared to indomethacin (Table 1). However, it was noticed that derivative **12b** revealed a significant increase in reaction time/Sec.; denoting antinociceptive effect starting after 60 min of administration till 90 min (Table 2).

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Conflict of interests

No conflict of interest is there to declare.

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