Synthesis, characterization, antimicrobial and *in-silico* studies of (1, 1-dibenzofuran-2-yl) ethyl terephthalamide derivatives

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Abstract

In search of new benzofuran derivatives of biological importance, symmetric terephthalamide derivatives of benzofuran (4a-e) were synthesized from 2-acetylbenzofuran. The synthesis of title compounds follows a sequence of condensation, reduction and coupling reactions. The structures of all the newly synthesized compounds are established by FT-IR, ^{1}H and ^{13}C NMR and MS spectral methods. The formation of amine follows novel reduction method under microwave irradiation.

All the synthesized compounds were subjected to in-vitro antimicrobial studies and found to be moderate antibacterial agent and poor antifungal agents. In correlation, the mode of action of these compounds as antimicrobial agents was determined by the in-silico studies.

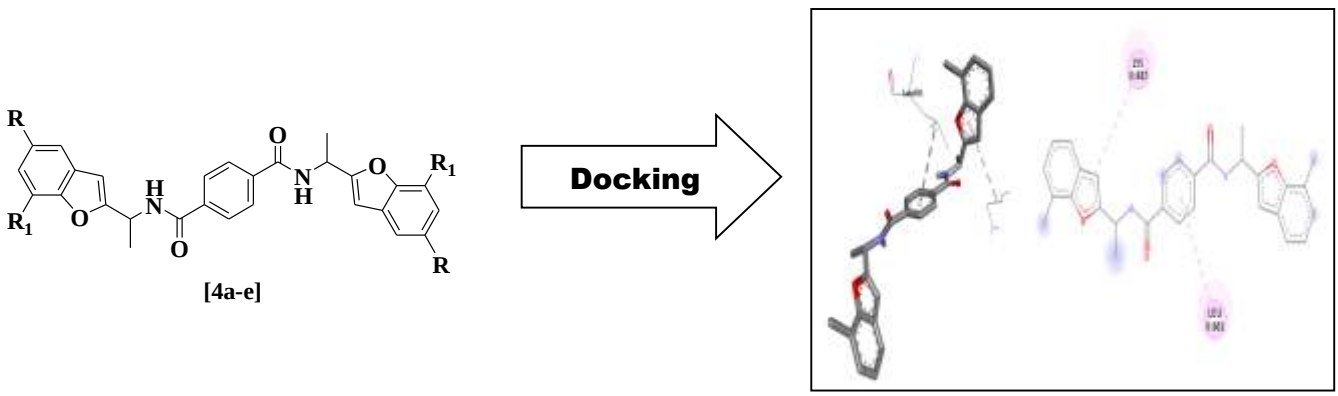
Keywords: 2-Acetylbenzofuran, symmetric, terephthalamide, microwave irradiation, antimicrobial, molecular docking.

Introduction

Benzofurans and their derivatives possess a broad range of important biological activities including anticancer, antibacterial, antifungal and antiviral properties. They have already attracted considerable attention amongst organic and medicinal chemists in the last few years[1,2]. Further, (benzofuran-2-yl) ketoximes shows good antifungal activity[3]. Optically active chiral amines are important as building blocks for pharmaceuticals and as scaffolds for chiral ligands and consequently, many efforts have been devoted to the development of efficient methods for their preparation[4].

For example, reduction of amine precursors with chiral catalysts[5,6], enzymatic kinetic resolution or dynamic kinetic, resolution of racemic amines[7,8] and the direct amination of ketones with trans aminases[9,10] have been developed as the efficient methods for the preparation of optically active chiral amines. On the other hand, amide functionality is the common back bone of numerous organic molecules and natural products that bear diverse chemical and pharmacological features[11,12]. The drug molecules which are having multiple binding sites to bind at more than one docking site of its biological targets exhibit enhanced bioactivity and such multivalent ligand design aimed at variety of drug targets[13-17].

Terephthalamide scaffold is having multiple binding sites known to mimic the α-helical region of Bak peptide which belongs to family Bcl-2 proteins which play crucial role in determining the fate of cell through the process of apoptosis[18,19], oncogenic mutations[20,21] and over expression of Bcl-2 by blocking the apoptotic pathway.



[4a-e]

Graphical Abstract

Therefore, agents that directly mimic the death promoting region BH3 domain of the pro-apoptotic subfamily of Bcl-2 proteins[22] are of potential therapeutic value. Hence, we planned to design and synthesize symmetric terephthalamide derivatives of benzofuran (4a-e). The newly synthesized derivatives were subjected to primary screening like antimicrobial activity studies along with *in-silico* studies.

Material and Methods

Melting points of the synthesized solid compounds were determined with open capillary and are uncorrected. The progress of all reactions was monitored by thin layer chromatography and column chromatographic purifications were performed on silica gel 60-120 and neutral alumina.

FTIR spectra were recorded in KBr pellets by using JASCO FTIR-4100 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ and DMSO-d_6 on JEOL-400 MHz NMR instrument. Chemical shifts are reported in ppm units relative to TMS. Mass spectral data were obtained on AGILENT LC-MS column C-18 instrument with MM+ES+APCI positive mode. Microorganism cultures were procured from National Chemical Laboratory, Pune, India.

Synthesis of 1-(5-bromo-benzofuran-2-yl) ethanone (1b): To a well stirred solution of 5-bromo-salicylaldehyde (10.0 g, 0.012 mol) and K_2CO_3 (10 g) taken in round bottom flask containing acetone (50 mL), bromoacetone (14.3 mL, 0.012 mol) was added drop-wise and mixture was refluxed for 10h. After completion of the reaction as indicated by disappearance of starting material through thin layer chromatography, reaction mixture was poured onto crushed ice and extracted to ethyl acetate (2 x 50 mL). The organic layer was washed with water, dried over anhydrous sodium sulfate and concentrated in vacuum to get crude product.

Finally, the pure compound (1b) was obtained by column chromatography on silica gel using ethyl acetate: petroleum ether (9:1, v/v) as eluent. Similar procedure was adopted for the synthesis of (1a) and (1c-e) derivatives.

Synthesis of 1-(5-bromo-benzofuran-2-yl) ethanone oxime (2b): 5-bromo-2-acetylbenzofuran [1b] (1 g, 0.00418 mol), hydroxylamine hydrochloride (0.43 g, 0.00627 mol) and anhydrous K_2CO_3 (0.86 g, 0.00627 mol) were taken in round bottom flask containing EtOH: H_2O mixture (3:1, 10 mL) and the resulting mixture was refluxed for 5h. Thin layer chromatography monitoring of the reaction indicated the completion.

Then the reaction mixture was poured into ice cold water resulting in separation of product as white solid. The separated white solid was filtered, washed with water and dried. It was recrystallized from EtOH to get pure (2b). Adopting the similar reaction condition and procedure (2a) and (2c-e) derivatives were prepared.

Synthesis of 1-(5-bromo-benzofuran-2-yl) ethanamine (3b): 1-(5-bromo-benzofuran-2-yl) ethanone oxime (2b) (0.5 g, 0.0028 mol) and zinc dust (1.12 g, 0.017 mol) were taken in round bottom flask containing 5 mL of glacial acetic acid. The reaction mixture was irradiated with microwave radiation for 3 mins at regular interval of 10 sec. After completion of the reaction monitored by thin layer chromatography, the reaction mixture was poured into saturated sodium bicarbonate solution (20 mL) and extracted with ethyl acetate layer (2 x 25 mL). The organic layer was washed with water followed by brine solution and dried over anhydrous sodium sulfate.

The solvent was removed under vacuum to get crude amine as yellow oil. The crude amine was purified by column chromatography on neutral alumina using chloroform: methanol (9:1, v/v) as eluent to get pure (3b) as light yellow oil. Above process was followed for preparing the remaining derivatives (3a) and (3c-e).

Synthesis of (1, 1-dibromobenzofuran-2-yl) ethyl terephthalamide (4b): Compound (3b) (0.15 g, 0.00098 mol) in dichloromethane (2 mL) was added to previously cooled (0-5^{o}C) terephtholoylchloride (0.1 g, 0.00049 mol) taken in round bottom flask containing in dichloromethane (2 mL). After complete addition, Et_3N (0.099g, 0.00098 mol) was added and the resulting mixture was stirred at room temperature overnight. After completion of the reaction as indicated by TLC, the reaction mixture was poured into ice cold water, solid precipitated out.

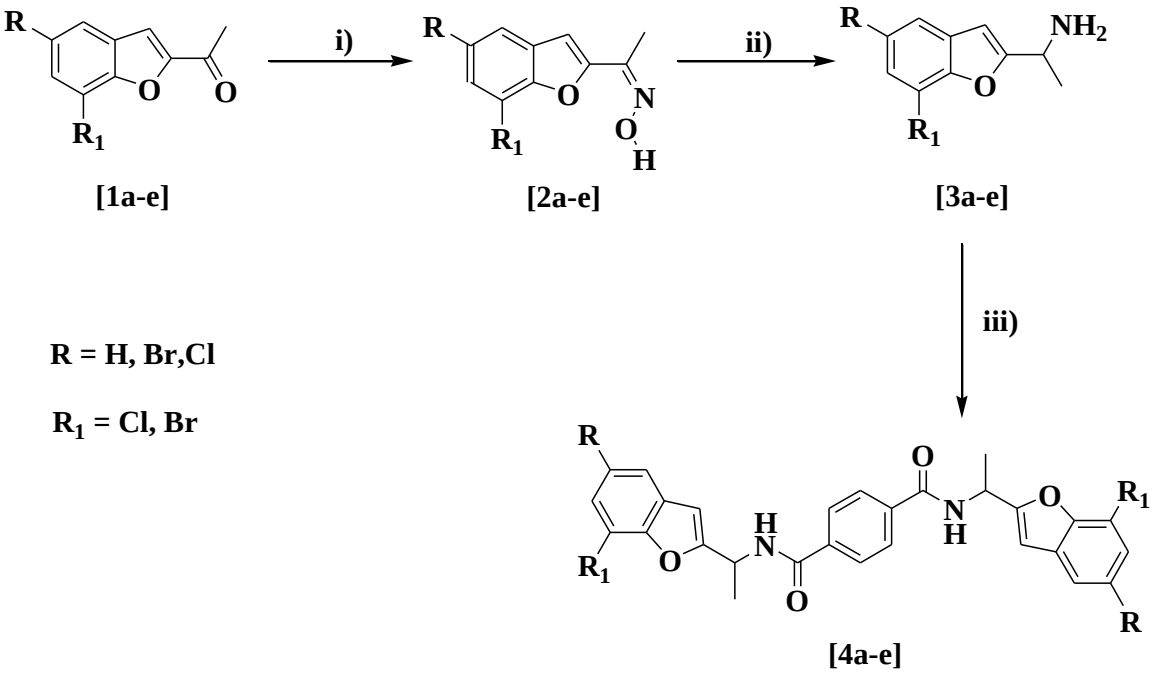
The separated solid was filtered, dried and recrystallized from ethanol. Synthesis of remaining derivatives (4a) and (4c-e) was carried out adopting the similar procedure.

Reagents: i) $NH_2OH.HCl$, K_2CO_3, EtOH, reflux, 5h; ii) Zn dust, AcOH, MWI, 3min; iii) Terephthaloyl chloride, Et_3N, CH_2Cl_2, rt, 4h.

Spectral characterization

1-(1-benzofuran-2-yl) ethanone oxime [2a]: FTIR (KBr, ν cm^{-1}): 3233 (O-H, Oxime, s), 3071 (C-H, Aromatic, m), 2925 (C-H, Alkyl, s), 1557 (C=N, Oxime, m), 1071-1170 (C-O-C, Furan, m). ^{1}H-NMR (400 MHz, $CDCl_3$, δ ppm): 8.45 (s, 1H, N-OH), 7.67-7.65 (d, 1H, J = 7.8 Hz, Ar-H), 7.60-7.58 (d, 1H, J = 7.6 Hz, Ar-H), 7.53-7.38 (m, 1H, Ar-H), 7.31-7.22 (m, 1H, Ar-H), 6.99 (s, 1H, Ar-H), 2.31 (s, 3H, $-CH_3$).

1-(5-bromo-benzofuran-2-yl) ethanone oxime [2b]: FTIR (KBr, ν cm^{-1}): 3432 (O-H, Oxime, s), 3071 (C-H, Aromatic, m), 2925 (C-H, Alkyl, s), 1633 (C=N, Oxime, m), 1033-1170 (C-O-C, Furan, m). ^{1}H-NMR (400 MHz, $CDCl_3$, δ ppm): 8.98 (s, 1H, N-OH), 7.78-7.71 (m, 1H, Ar-H), 7.58-7.55 (d, 1H, J = 7.0 Hz, Ar-H), 7.43-7.39 (d, 1H, J = 9.0 Hz, Ar-H), 6.92 (s, 1H, Ar-H), 2.30 (s, 3H, $-CH_3$). MS: m/z 254.1 (M^{+}).



Scheme 1: Synthetic route for the preparation of (1, 1-dibenzofuran-2-yl) ethyl terephthalamide (4a-e) derivatives

1-(5-chloro-benzofuran-2-yl) ethanone oxime [2c]: ^{1}H-NMR (400 MHz, $CDCl_3$, δ ppm): 8.69 (s, 1H, N-OH), 7.78-7.68 (m, 1H, Ar-H), 7.48-7.39 (m, 2H, Ar-H), 6.99 (s, 1H, Ar-H), 2.31 (s, 3H, -CH_3).

1-(7-bromo-benzofuran-2-yl) ethanone oxime [2d]: ^{1}H-NMR (400 MHz, $CDCl_3$, δ ppm): 8.50 (s, 1H, N-OH), 7.70-7.64 (m, 1H, Ar-H), 7.61-7.55 (m, 1H, Ar-H), 7.52-7.40 (m, 1H, Ar-H), 6.89 (s, 1H, Ar-H), 2.27 (s, 3H, -CH_3).

1-(7-chloro-benzofuran-2-yl) ethanone oxime [2e]: ^{1}H-NMR (400 MHz, $CDCl_3$, δ ppm): 8.59 (s, 1H, N-OH), 7.70-7.68 (m, 1H, Ar-H), 7.60-7.58 (m, 1H, Ar-H), 7.53-7.38 (m, 1H, Ar-H), 6.99 (s, 1H, Ar-H), 2.28 (s, 3H, -CH_3).

1-(1-benzofuran-2-yl) ethanamine [3a]: FTIR (KBr, ν cm^{-1}): 3359 (N-H, Asym, s), 3294 (N-H, Sym, s), 3063 (C-H, Aromatic, m), 2959 (C-H, Alkyl, m), 1078-1174 (C-O-C, Furan, m). ^{1}H-NMR (400 MHz, $CDCl_3$, δ ppm): 7.52-7.49 (d, 1H, J = 7.8 Hz, Ar-H), 7.44-7.42 (d, 1H, J = 7.6 Hz, Ar-H), 7.24-7.17 (m, 2H, Ar-H), 6.50 (s, 1H, Ar-H), 4.14-4.09 (q, 1H, J = 7.6 Hz, N-CH), 1.53-1.51 (d, 3H, J = 7.6 Hz, -CH_3), 2.08 (s, 2H, -NH_2). MS: m/z 159.8 (M^+).

1-(5-bromo-benzofuran-2-yl) ethanamine [3b]: FTIR (KBr, ν cm^{-1}): 3397 (N-H, Asym, s), 3235 (N-H, Sym, s), 3068 (C-H, Aromatic, m), 2924 (C-H, Alkyl, m), 1091-1154 (C-O-C, Furan, m). ^{1}H-NMR (400 MHz, $CDCl_3$, δ ppm): 7.65-7.64 (m, 1H, Ar-H), 7.37-7.30 (m, 2H, Ar-H), 6.55 (s, 1H, Ar-H), 5.54 (m, 3H, -NH_2), 4.39-4.35 (q, 1H, J = 6.5 Hz, N-CH), 1.60-1.59 (d, 3H, J = 6.7 Hz, -CH_3). MS: m/z 240 (M^+).

1-(5-chloro-benzofuran-2-yl) ethanamine [3c]: FTIR (KBr, ν cm^{-1}): 3355 (N-H, Asym, s), 3284 (N-H, Sym, s), 3061 (C-H, Aromatic, m), 2955 (C-H, Alkyl, m), 1070-1170 (C-O-C, Furan, m).^{1}H-NMR (400 MHz, $CDCl_3$, δ ppm): 7.66-7.54 (m, 1H, Ar-H), 7.32-7.24 (m, 2H, Ar-H), 6.58 (s, 1H, Ar-H), 4.31-4.24 (q, 1H, J = 7.5 Hz, N-CH), 2.26 (s, 2H, -NH_2), 1.50-1.48 (d, 3H, J = 7.0 Hz, -CH_3).

1-(7-bromo-benzofuran-2-yl) ethanamine [3d]: FTIR (KBr, ν cm^{-1}): 3365 (N-H, Asym, s), 3288 (N-H, Sym, s), 2989 (C-H, Aromatic, m), 2945 (C-H, Alkyl, m), 1010-1144 (C-O-C, Furan, m). ^{1}H-NMR (400 MHz, $CDCl_3$, δ ppm): 7.37-7.55 (m, 3H, Ar-H), 6.50 (s, 1H, Ar-H), 4.44-4.31 (q, 1H, J = 6.5 Hz, N-CH), 1.40-1.38 (d, 3H, J = 7.0 Hz, -CH_3), 1.26 (s, 2H, -NH_2).

1-(7-chloro-benzofuran-2-yl) ethanamine [3e]: FTIR (KBr, ν cm^{-1}): 3350 (N-H, Asym, s), 3289 (N-H, Sym, s), 3055 (C-H, Aromatic, m), 2960 (C-H, Alkyl, m), 1077-1178 (C-O-C, Furan, m). ^{1}H-NMR (400 MHz, $CDCl_3$, δ ppm): 7.35-7.50 (m, 3H, Ar-H), 6.50 (s, 1H, Ar-H), 6.58 (s, 1H, Ar-H), 4.57-4.54 (q, 1H, J = 7.5 Hz, N-CH), 1.50-1.48 (d, 3H, J = 7.0 Hz, -CH_3), 1.33 (s, 2H, -NH_2).

(1, 1-dibenzofuran-2-yl) ethyl terephthalamide [4a]: FTIR (KBr, ν cm^{-1}): 3420 (N-H, Amide, s), 2255 (C-H, Alkyl, m), 1646 (C=O, Amide, s), 1049-1025 (C-O-C, Furan, m). ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 9.10-9.08 (d, 2H, J = 8.0 Hz, CO-NH), 8.00 (s, 4H, Ar-H), 7.60-7.59 (m, 4H, Ar-H), 7.58-7.53 (m, 4H, Ar-H), 6.78 (s, 2H, Ar-H), 5.44-5.40 (m, 2H, N-CH), 1.62-1.60 (d, 6H, J = 6.9 Hz, CH_3). ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 165.032 (C11 & C18), 159.248 (C8 and C28), 154.051 (C2 and C22), 137.858 (C12 and C17), 129.435 (C1 and C21), 129.190 (C16), 128.048 (C15), 127.702 (C14), 127.414 (C13), 123.872 (C4 and C24), 122.764 (C5 and C25), 120.854 (C6 and C26), 110.948 (C3 and C23), 102.232 (C7 and C27),

43.042 (C9 and C19), 18.815 (C10 and C20). MS: m/z 453.0 (M^+).

5, 5-dibromobisbenzofuran-2-yl) ethyl terephthalamide [4b]: FTIR (KBr, ν cm^{-1}): 3380 (N-H, Amide, s), 2248 (C-H, Alkyl, m), 1659 (C=O, Amide, s), 1045-1021 (C-O-C, Furan, m). ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 9.10-9.07 (t, 2H, J = 8.0 Hz, CO-NH), 7.98-7.93 (m, 4H, Ar-H), 7.79 (s, 2H, Ar-H), 7.53-7.49 (m, 2H, Ar-H), 7.39-7.38 (d, 2H, J = 7.1 Hz, Ar-H) 6.76-6.71 (d, 2H, J = 7.1 Hz, Ar-H), 5.41-5.38 (q, 2H, J = 6.3 Hz, N-CH), 1.58-1.39 (d, 6H, J = 6.8 Hz, -CH_3,). ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 165.57 (C11 and C18), 161.52 (C8 and C28), 153.47 (C2 and C22), 136.90 (C12 and C17), 127.99 (C1 and C21), 127.59 (C16), 127.05 (C15), 126.99 (C14), 123.89 (C13), 123.86 (C4 and C24), 115.63 (C5 and C25), 113.59 (C6 and C26), 113.57 (C3 and C23), 102.44 (C7 and C27), 70.29 (C9 and C19), 19.26 (C10 and C20). MS: m/z 611.0 (M^+).

(5, 5-dichlorobisbenzofuran-2-yl) ethyl terephthalamide [4c]: FTIR (KBr, ν cm^{-1}): 3443 (N-H, Amide, s), 2925 (C-H, Aromatic, m), 2872 (C-H, Alkyl, m), 1633 (C=O, Amide, s), 1040-1191 (C-O-C, Furan, m). ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 9.09-9.07 (d, 2H, J = 8.0 Hz, CO-NH), 8.00 (s, 4H, Ar-H), 7.60-7.52 (m, 2H, Ar-H), 7.28-7.20 (m, 4H, Ar-H), 6.78 (s, 2H, Ar-H), 5.45-5.38 (m, 2H, N-CH), 1.61-1.60 (d, 6H, J = 6.9 Hz, -CH_3,).

(7, 7-dibromobisbenzofuran-2-yl) ethyl terephthalamide [4d]: FTIR (KBr, ν cm^{-1}): 3319 (N-H, Amide, s), 2928 (C-H, Alkyl, m), 1650 (C=O, Amide, s), 1024-1139 (C-O-C, Furan, m). ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): δ 9.06-9.04 (d, 2H, J = 8.0 Hz, CO-NH), 7.96-7.77 (m, 6H, Ar-H), 7.51-7.35 (m, 4H, Ar-H), 6.74 (s, 2H, Ar-H), 5.38-5.36 (m, 2H, N-CH), 1.57-1.56 (d, 6H, J = 6.9 Hz, -CH_3).

(7, 7-dichlorobisbenzofuran-2-yl) ethyl terephthalamide [4e]: FTIR (KBr, ν cm^{-1}): 3420 (N-H, Amide, s), 2255 (C-H, Alkyl, m), 1642 (C=O, Amide, s), 1049-1155 (C-O-C, Furan, m). ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 9.05-9.07 (d, 2H, J = 8.0 Hz, CO-NH), 7.97-7.75 (m, 6H, Ar-H), 7.61-7.57 (m, 4H, Ar-H), 6.73 (s, 2H, Ar-H), 5.39-5.34 (m, 2H, N-CH), 1.59-1.55 (d, 6H, J = 6.9 Hz, -CH_3).

Antimicrobial Studies: The antibacterial activity was carried out against four different bacterial strains *Klebsiella aerogenes* (NCMI-2098), *Escherichia coli* (NCMI-5051), *Psedomonas desmolyticum* (NCMI-2112) and *Staphylococcus aereus* (NCMI-5022) and antifungal screening was carried out against two antifungal strains *Aspergillus niger* and *Candida albicans*. The newly synthesized molecules were examined for the presence or absence of zone of inhibition on nutrient agar plates by agar diffusion method**.** Ciprofloxacin and Griesofulvin were used as reference for antibacterial and antifungal activity respectively. In this method, the liquefied medium was inoculated with the 18h old cultures (100 µL, 10^{-4} cfu) of the suspension of the microorganisms between 40-50°C and spread evenly to get uniform thickness with depth of 3 to 4 mm. After 20 min, with the help of a sterile cork borer, five cups of each 6 mm diameter were made into each Petri dish (five cups were numbered for the particular compound and standard).

Using strile micropipettes, the standard and the synthesized compound solutions of different concentrations were added into the bored cups. All the plates were then incubated for 24h at 37°C and for 48h at 27°C for antibacterial and antifungal activity respectively. The zone of inhibition developed, if any, was then accurately measured and recorded[23]. Minimum inhibitory concentrations [MICs] of synthesized compounds with Mueller Hinton broth and controls were inoculated with approximately 10^5 colony forming unit/mL (c.f.u/mL) of actively dividing bacterial cells. The growth was monitored visually and spectrophotometrically. The lowest concentration (highest dilution) which inhibits the growth of bacteria was recorded as minimum inhibitory concentration (MIC).

***In silico* ADMET studies:** Pharmacokinetic parameters such as absorption, distribution, metabolism, excretion and toxicity (ADMET) were predicted for the synthesized compounds in order to evaluate their biological activity. In ADMET studies, wherein the active lead molecule which contains undesirable functional groups can be removed based on Lipinski rule[24]. Lipinski's rule of five is commonly used by pharmaceutical chemists in drug design and development to predict oral bioavailability of potential lead or drug molecules.

According to Lipinski's rule of five, a candidate molecule will likely to be orally active if: i) the molecular weight is under 500, ii) the calculated octanol/water partition coefficient (Log P) is less than 5, iii) there were fewer than 5 hydrogen bond donors (OH and NH groups) and, iv) there are less than ten hydrogen bond acceptors (notably N and O). The molecular properties and Lipinski rule of five for the compounds were determined by Molinspiration online server (http://www.molinspiration.com). The toxicity profile and drug-likeness of the compounds were predicted by Osiris software data warrior computer programmer.

***In-silico* molecular docking studies**

Protein preparation: The native crystal structure of GlcN-6-P synthase (X chain) in complex with glucosamine6-phosphate was retrieved from Protein Data Bank (http://www.pdb.org/pdb/home/home.do) with the PDB ID: 2VF5 which was resolved at 2.90 Å using X-ray diffraction[25]. Before protein preparation, the inhibitors, other ligand and water molecules were deleted from the protein to obtain clean protein. The structures thus obtained were optimized classically using CHARMm force field implemented in the DS 3.5. The site in which co-crystallized ligand complexed was identified and the active pocket consisted of 12 amino acid residues as GLY:X:301, SER:X:604, GLY:X:350, VAL:X:605, GLY:X:301,

THR:X:302, ALA:X:602, VAL:X:399, SER:X:401, GLN:X:348, SER:X:349 and ALA:X:400. The grid was centered at the region surrounding important amino acid residues as shown in fig. 1. The center of grid box was set to 30.27, 17.85 and -0.91 and number of points in x, y and z dimensions 26, 28 and 28 Å respectively.

Ligand preparation: The ligands were drawn on Chem Draw Ultra 8.0, assigned with proper 2D orientation and were converted to energy minimized 3D structures using Chem3D Ultra 8.0 and Gasteiger charges[26], nonpolar hydrogen atoms and the rotatable bonds were set by using AutoDock 4.2 tools.

Auto dock Vina[27] was used for docking purpose. During the docking process, a maximum of 10 conformers were considered for each compound. All the AutoDock docking runs were performed in Intel Centrino Core2 Duo CPU @ 2.20GHz of IBM system origin, with 4 GB DDR2 RAM run under Microsoft Windows operating system.

Results and Discussion

Chemistry: Synthetic route for the preparation of symmetric terephthalamide benzofuran (4a-e) derivatives involves four steps and overall sequence is depicted in scheme 1. The key intermediate (1a-e) for the synthesis was prepared by reacting substituted salicylaldehyde and bromoacetone in presence of base under reflux condition. Further, the compound (1a-e) underwent condensation reaction with hydroxylamine hydrochloride in presence of base afforded oxime derivatives of benzofuran (2a-f) in good yield[28,29]. Microwave irradiated reduction of (2a-e) was carried out using Zn dust in acetic acid to get primary amine derivatives of benzofuran (3a-e).

Finally, symmetric terephthalamide derivatives of benzofuran (4a-e) were synthesized by nucleophilic substitution reaction of amine derivatives (3a-e) with terephthaloyl chloride in basic medium. Assigned structures of all the synthesized compounds were confirmed from their FTIR, ^{1}H-NMR, ^{13}C-NMR and LCMS or Mass spectral analysis. The representative FTIR, ^{1}HNMR, ^{13}CNMR and mass spectra of compound (4a) were given in figure 2-5 respectively. The physical characterization data along with percentage of yield in all the steps involved were tabulated in table 1.

Antimicrobial Studies: The antibacterial results of compounds (2a-e), (3a-e) and (4a-e) derivatives of benzofuran were screened at two different concentrations (200 µg/mL). These derivatives exhibited weak to poor activity compared to standard drug ciprofloxacin and MIC for these derivatives were found in the range of 50-200 µg/mL shown in table 2 and table 3 respectively.

The antifungal activity results of compounds (2a-e), (3a-e) and (4a-e) derivatives revealed that these compounds showed poor activity against both tested organisms. The MIC was found to be 2000 µg/mL shown in table 4.

ADMET studies: The compounds (2a-e) and (3a-e) were found in compliance with Lipinski's rule of five recommendations for new chemical entity to have good oral bioavailability with no violations. On the other hand, compounds (4a-e) have more than one violation in molecular weight greater than 500 and AlogP value greater than 5, even though they have positive drug likeness. All the synthesized compounds in the present investigation have negative drug likeness score, hence they should not be considered as drug-like. Toxicity profile showed that the compounds (2b), (3b) and (4b) were found to have high mutagenic effect whereas the other compounds have non-mutagenic, non-irritating with no reproductive effects as tabulated in table 5.

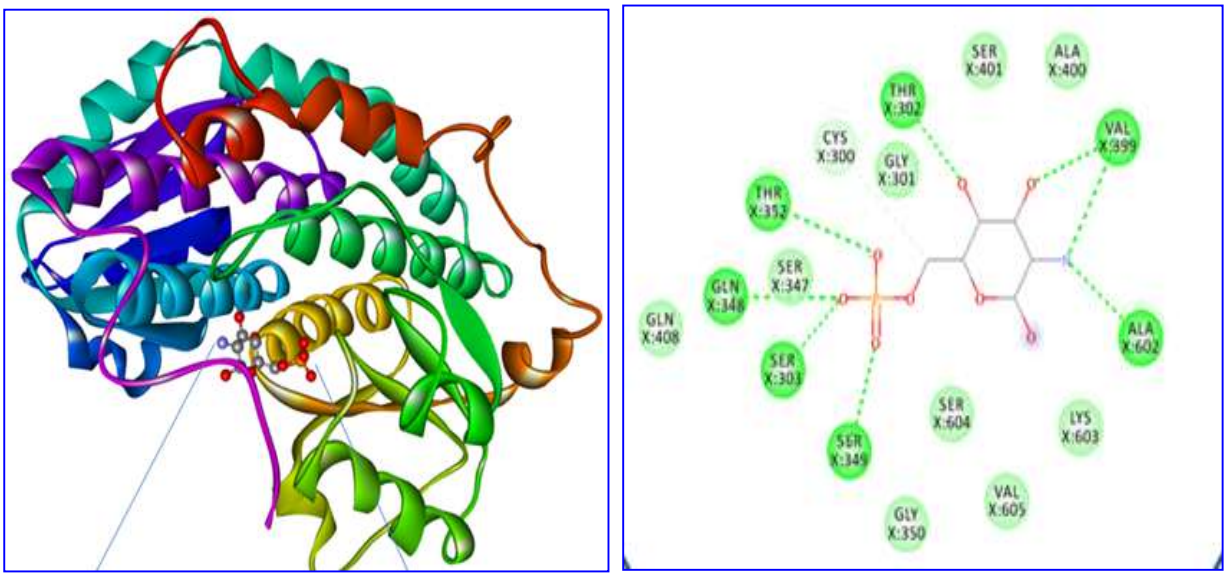
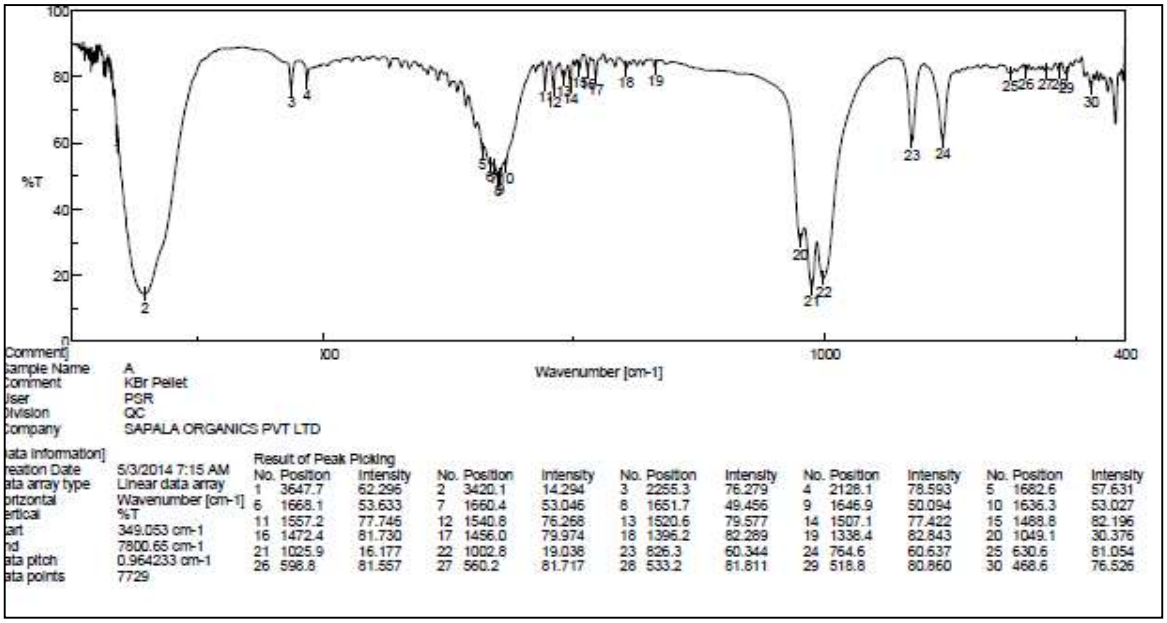


Figure 1: Crystal structure of GlcN-6-P synthase (X chain) in complex with glucosamine-6-phosphate and Ligplot of GlcN-6-P synthase



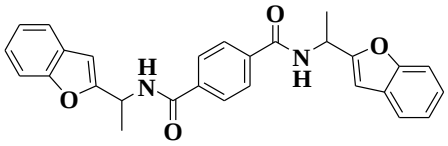
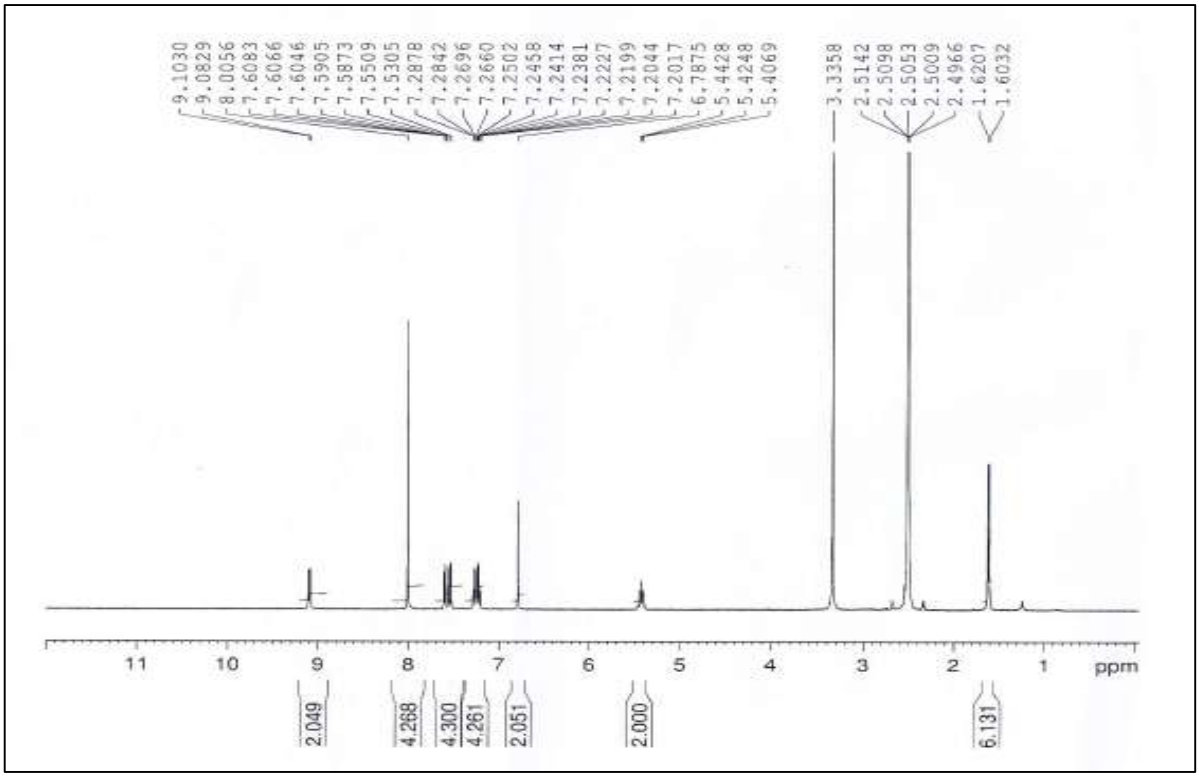


Figure 2: FTIR spectrum of (1, 1-dibenzofuran-2-yl) ethyl terephthalamide (4a) in KBr



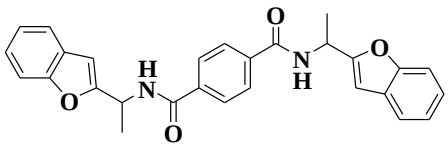


Figure 3: ^{1}H-NMR spectrum of (1, 1-dibenzofuran-2-yl) ethyl terephthalamide (4a) in DMSO-d_6

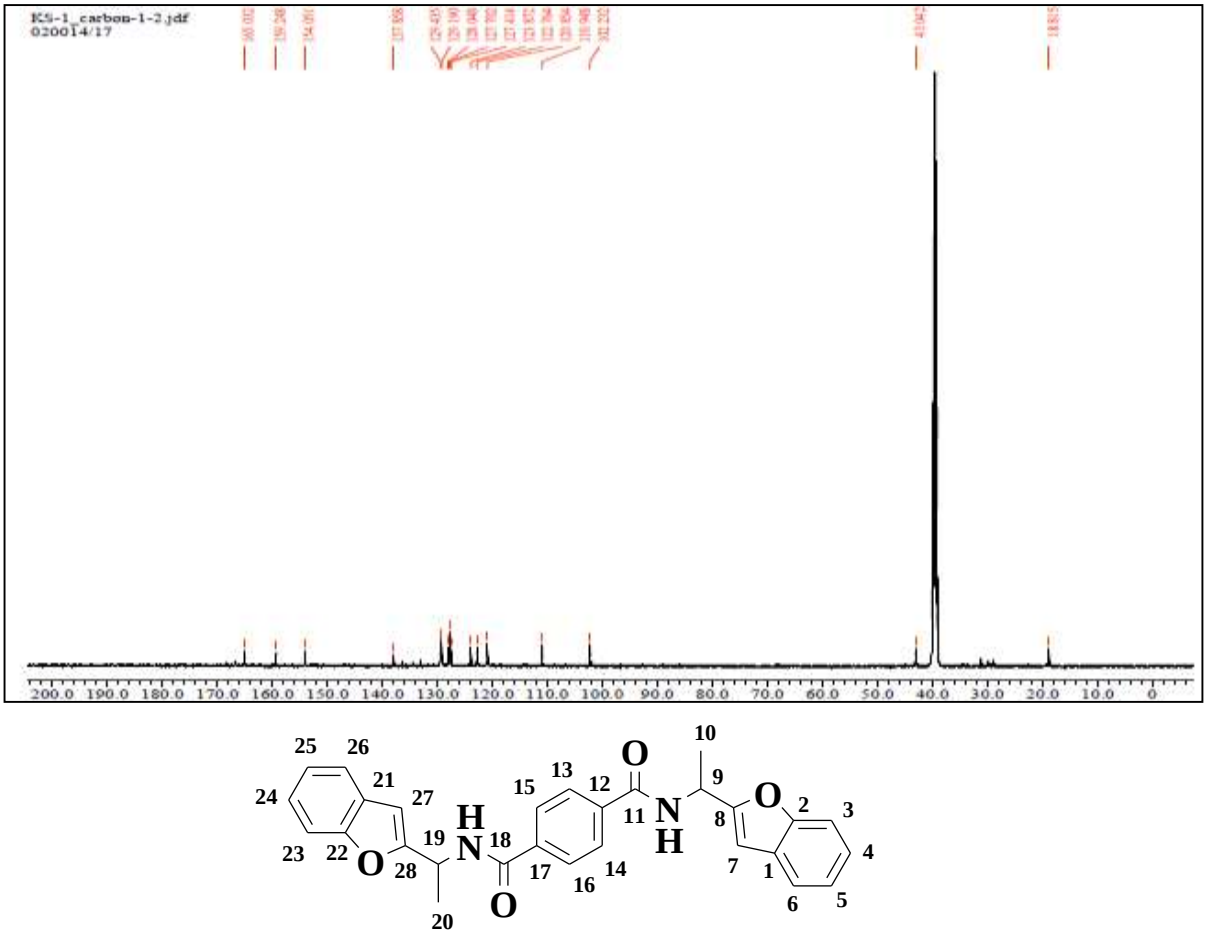


Figure 4: ^{13}C-NMR spectrum of (1, 1-dibenzofuran-2-yl) ethyl terephthalamide (4a) in DMSO-d_6

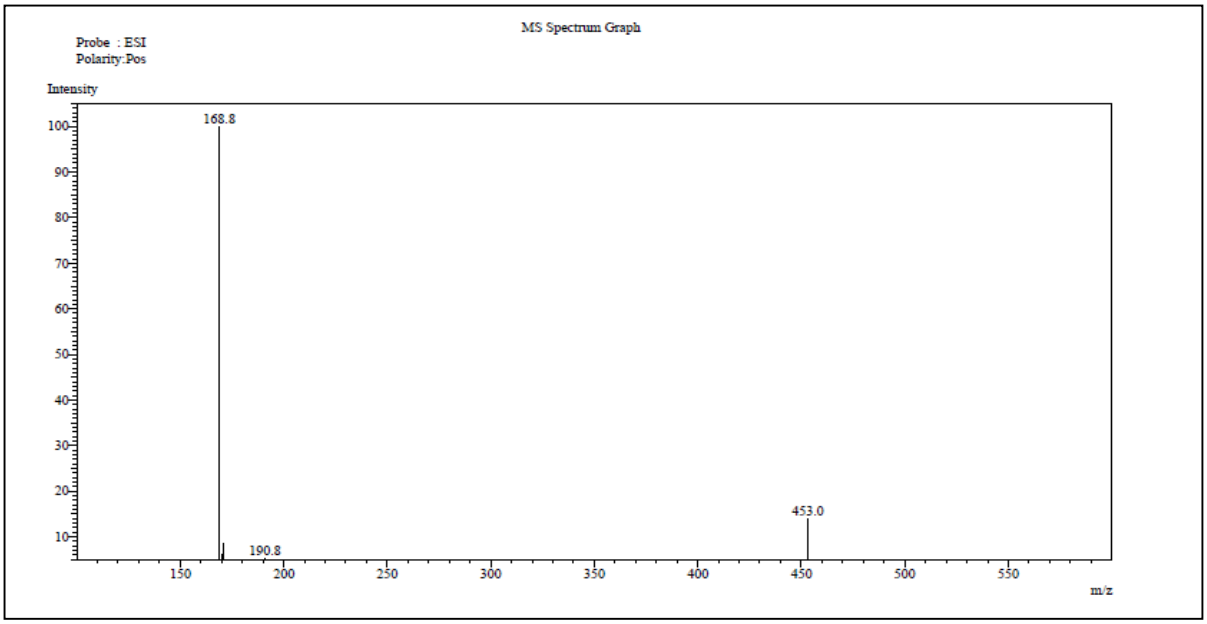


Figure 5: Mass spectrum of (1, 1-dibenzofuran-2-yl) ethyl terephthalamide (4a)

Table 1
Physical characterization data of Compounds (2a-e), (3a-e) and (4a-e)

Entry	Mol. Formula (Mol.Wt)	Nature	Yield (%)	Melting point (^{o}C)
2a	$C_{10}H_9NO_2$ (175.1)	White crystals	85	203-205
2b	$C_{10}H_8BrNO_2$ (254.08)	Pale yellow crystals	80	215-217
2c	$C_{10}H_8ClNO_2$ (209.63)	White crystals	80	207-209
2d	$C_{10}H_8ClNO_2$ (209.63)	Yellow solid	65	210-212
2e	$C_{10}H_8BrNO_2$ (254.08)	Pale yellow solid	70	220-222
3a	$C_{10}H_{11}NO$ (161.2)	Yellow oil	90	-
3b	$C_{10}H_{10}BrNO$ (240.1)	Pale yellow oil	75	-
3c	$C_{10}H_{10}ClNO$ (195.65)	Yellow oil	65	-
3d	$C_{10}H_{10}ClNO$ (195.65)	Yellow oil	65	-
3e	$C_{10}H_{10}BrNO$ (240.1)	Yellow oil	65	-
4a	$C_{28}H_{24}N_2O_4$ (452.5)	White solid	80	>300
4b	$C_{28}H_{22}Br_2N_2O_4$ (610.29)	White solid	75	>300
4c	$C_{28}H_{22}Cl_2N_2O_4$ (521.39)	White solid	70	>300
4d	$C_{28}H_{22}Cl_2N_2O_4$ (521.39)	White solid	65	>300
4e	$C_{28}H_{22}Br_2N_2O_4$ (610.29)	White solid	70	>300

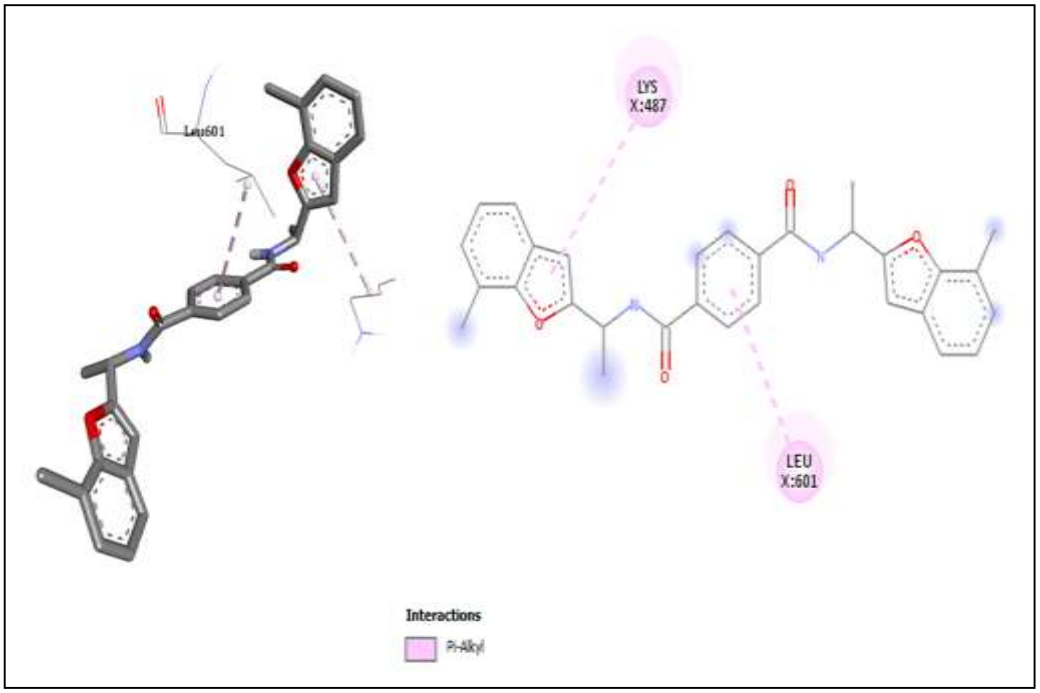


Figure 6: Binding modes in 3D and 2D of the compound (4d) with GlcN-6-P synthase.

Molecular docking: *In silico* studies with GlcN-6-P synthase reveal that all the compounds exhibited interaction with one or the other amino acid residues in the active site. Table 6 depicts the result of docked complexes of (2a-e); (3a-e) and (4a-e) results reveal that all the compounds have binding energies in the range of -5.6 to -8.7 Kcal/mol compared to ciprofloxacin and GlcN-6-P with binding energies -7.9 and -7.6 Kcal/mol respectively. (1, 1-dibesnzofuran-2-yl) ethyl terephthalamide derivatives (4a-e) result reveals that the compound (4d) has emerged as a better inhibitor with least binding energy of -8.7 Kcal/mol with two interactions with the active site amino acid residue as shown in figure 6.

Table 2
Antibacterial activity of (2a-e), (3a-e) and (4a-e) derivatives of scheme 1

Compound	Zone of Inhibition in mm (µg/mL)			
	K.aerogenes	***E.coli***	***P.desmolyticum***	***S. aureus***
	200 µg	**200 µg**	**200 µg**	**200 µg**
2a	4.67±0.33**	4.33±0.33**	4.67±0.33**	4.33±0.33**
2b	6.00±0.33**	6.50±0.33**	4.67±0.33**	4.33±0.33**
2c	5.33±0.33**	5.10±0.06**	4.67±0.33**	4.67±0.33**
2d	3.00±0.00	6.10±0.06**	5.00±0.00	5.13±0.03**
2e	1.33±0.33**	3.13±0.09**	3.13±0.09**	2.10±0.06**
3a	4.33±0.33**	6.67±0.33**	5.60±0.33**	5.67±0.33**
3b	3.67±0.33*	2.67±0.33**	4.67±0.33**	5.10±0.06**
3c	5.10±0.03**	7.00±0.33*	5.33±0.33**	4.67±0.33**
3d	4.67±0.33**	4.33±0.33**	4.67±0.33**	4.33±0.33**
3e	3.67±0.33**	3.67±0.33**	2.67±0.33**	2.33±0.33**
4a	1.67±0.33**	2.10±0.06**	2.00±0.00	2.13±0.03**
4b	1.33±0.33**	3.13±0.09**	3.13±0.09**	2.10±0.06**
4c	1.67±0.33**	1.67±0.33*	3.10±0.06**	3.67±0.03**
4d	3.17±0.06**	3.00±0.00	4.10±0.06**	2.47±0.03**
4e	2.67±0.33**	3.67±0.33**	4.33±0.33**	2.67±0.03
Control	-	-	-	-
Std	35±0.24**	34±0.32**	30±0.32**	34±0.32**

Values are the mean ± SE of clear zone in mm. Symbols represent statistical significance, **P* < 0.05, ***P* < 0.01 as compared with the control group.

Table 3
Minimum Inhibitory Concentration of (2a-e), (3a-e) and (4a-e) derivatives of benzofuran

Compound	MIC in µg/mL			
	K.aerogenes	***E.coli***	***P.desmolyticum***	***S. aureus***
2a	50	50	50	100
2b	50	50	50	50
2c	50	50	50	50
2d	50	100	50	50
2e	200	50	100	100
3a	100	100	100	200
3b	100	100	100	100
3c	100	100	100	100
3d	100	100	200	100
3e	100	100	100	200
4a	200	100	100	100
4b	100	200	100	100
4c	100	200	200	200
4d	200	100	100	200
4e	100	100	100	50
Ciprofloxacin	5	5	5	5

Table 4
Minimum Inhibitory Concentration and antifungal activity of (2a-e), (3a-e) and (4a-e) derivatives

Compound	MIC µg/mL	Zone of Inhibition in mm	
		A. niger	***C. Albicans***
2a	2000	-	0.5±0.00
2b	2000	0.5±0.00	-
2c	2000	0.5±0.00	1±0.09**
2d	2000	0.5±0.09**	-
2e	2000	-	0.5±0.00
3a	NF	-	-
3b	NF	-	-
3c	NF	-	-
3d	NF	-	-
3e	NF	-	-
4a	2000	-	1±0.33**
4b	NF	-	-
4c	2000	-	0.5±0.00
4d	NF	-	-
4e	NF	-	--
Griseofulvin	200	3.67±0.06**	6.33±0.23**
Control	-	-	-

Note: "-" Not active, NF- "Not Found". Values are the mean ± SE of clear zone in mm.
Symbols represent statistical significance, **P* < 0.05, ***P* < 0.01 as compared with the control group.

Table 5
***In-silico* ADMET of (2a-e), (3a-e) and (4a-e) derivatives**

Entry	HBA	HBD	MW	ALogP	nrotb	Violations	TPSA (Å^2)	Drug likeness	Mutagenic	Tumorigenic	Reproductive	Irritant
2a	3	1	175.19	2.47	1	0	45.73	-0.73	None	None	None	None
2b	3	1	254.08	3.25	1	0	45.73	-2.52	High	None	None	None
2c	3	1	209.63	3.12	1	0	45.73	-0.61	None	None	None	None
2d	3	1	254.08	3.23	1	0	45.73	-2.52	None	None	None	None
2e	3	1	209.63	3.10	1	0	45.73	-0.61	None	None	None	None
3a	2	2	161.20	0.37	1	0	39.16	-0.63	None	None	None	None
3b	2	2	240.10	1.15	1	0	39.16	-2.42	High	None	None	None
3c	2	2	165.65	1.02	1	0	39.16	-0.52	None	None	None	None
3d	2	2	240.10	1.13	1	0	39.16	-2.42	None	None	None	None
3e	2	2	195.65	1.00	1	0	39.16	-0.52	None	None	None	None
4a	6	2	452.51	5.14	6	1	84.48	2.82	None	None	None	None
4b	6	2	610.30	6.94	6	2	84.48	1.03	High	None	None	None
4c	6	2	521.40	6.68	6	2	84.48	2.89	None	None	None	None
4d	6	2	610.30	6.94	6	2	84.48	1.03	None	None	None	None
4e	6	2	521.40	6.63	6	2	84.48	2.89	None	None	None	None

HBA: Hydrogen Bond Acceptor, **HBD:** Hydrogen Bond Donor, **MW:** Molecular Weight, **Alogp:** Logarithm of partition b/w n-octanol and water, **nrotb:** No. rotatable bonds, **TPSA:** Topological Polar Surface Area

Conclusion

We have synthesized novel symmetric terephthalamide derivatives of benzofuran from 2-acetylbenzofuran through a sequence of condensation, reduction under microwave irradiation followed by coupling reaction. The structures of newly synthesized molecules were well supported by their spectral studies. The antibacterial activity of synthesized compounds reveals that they are moderately good active while antifungal activity results revealed that they are poor antifungal agents compared to standard drugs. The *in silico* studies of the compounds reveal that they are having very good binding affinity towards the GlN-6-phosphate synthase, hence they may be considered as potent antibacterial agents.

The synthesized symmetric terephthalamide derivative can be used for the study of fluorescence polarization assay, acts as multidentate ligand and also for the study of optical activity.

Table 6
Molecular docking results of (2a-e), (3a-e) and (4a-e) derivatives with GlcN-6-P synthase

Entry	Binding energy (Kcal/mol)	H-bonds	No. of interactions	Active site residues of GlcN-6-P synthase interacting with ligands
2a	-6.3	2	2	SER:X:349, THR:X:352
2b	-6.2	1	2	SER:X:349, THR:X:352
2c	-6.5	1	4	SER:X:349, GLY:X:356, GLN:X:348, LEU:X:601
2d	-6.0	2	4	THR:X:302, SER:X:349, SER:X347, THR:X:352
2e	-6.0	1	3	THR:X:302, SER:X:349, SER:X347, THR:X:352
3a	-5.7	1	2	SER:X:347, SER:X:349
3b	-5.6	1	2	SER:X:347, SER:X:349
3c	-6.1	1	1	SER:X:347
3d	-5.9	3	4	SER:X:349, SER:X:347, THR:X:352
3e	-5.7	2	4	SER:X:349, SER:X:347, THR:X:352
4a	-8.2	0	2	LYS:X:487, LEU:X:601
4b	-7.9	0	2	LYS:X:487, LEU:X:601
4c	-8.1	1	7	GLU:X:488, LEU:X:484, TYR:X:304, LEU:X:480, ILE:X:326
4d	-8.7	0	2	LYS:X:487, LEU:X:601
4e	-8.2	0	2	LYS:X:487, LEU:X:601
CIPRO	-7.9	4	7	CYS:X:300, VAL:X:605, SER:X:347, SER:X:349, GLN:X:348, THR:X:302, GLU:X:488
GlcN-6-P	-7.6	8	16	THR:X:352, GLN:X:348, SER:X:347, SER:X:303, SER:X:349, CYS:X:300, GLY:X:301, SER:X:604, GLY:X:350, VAL:X:605, GLY:X:301, THR:X:302, ALA:X:602, VAL:X:399, SER:X:401, ALA:X:400

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