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Research Article

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Design, synthesis, antibacterial, antioxidant activity and molecular docking studies of 6-hydroxybenzofuran derivatives

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ABSTRACT

*A series of novel benzofuran derivatives were designed and synthesized through a multistep functional group transformation approach which involves Pechmann condensation of resorcinol followed by a sequence of reaction such as base hydrolysis, activation, dehydration and reduction to afford corresponding acid, amide, nitrile and the Boc protected tryptdiamine-O-analogue derivatives **2-5**. The newly synthesized compounds are characterized by FT-IR, ^{1}HNMR and LC-MS spectral analysis. The synthesized tryptdiamine-O-analogue of benzofuran has skeletal similarity in structure with melatonin and serotonin with only difference in position of hydroxyl group and is found to be a dimer. In-vitro antibacterial and antioxidant studies were carried out for all the synthesized series of compounds **2-5**. Compounds **3**, **4** and **5** emerge as good antibacterial and DPPH scavenging agents. In correlation to antibacterial activity, compounds **2-5** along with serotonin and melatonin are subjected for molecular docking studies with GlcN-6-P synthase as target protein and showed minimum binding energy with good affinity towards target protein thus, they may be considered as good inhibitors of GlcN-6-P synthase. Additionally, Lipinski's rule of five parameters and toxicity parameters were evaluated to predict the bioavailability using online server Molinspiration and Osiris property explorer.*

Keywords: *Tryptdiamine-O-analogue, Reduction, antibacterial, antioxidant activity, Molecular docking studies.*

INTRODUCTION

The benzofuran ring systems bearing various substituents at the C-2 position are widely distributed in nature, e.g., ailanthoidol, is a neolignan derivative, has been reported to have antiviral, antioxidant and antifungal activities [1]. The amide functionality exists in numerous biological [2], pharmaceutical [3] and agrochemical [4] molecules and has prompted in-depth studies in the formation of the amide bond [5]. Amide functionality is widely prevalent in pharmaceuticals and drug candidates as well as various industrial materials including detergents and lubricants [6]. The synthesis of nitriles from their corresponding alkyl or aryl amides is an important functional group transformation widely used for transformation into amides, amines, esters, carboxylic acids etc. in organic synthesis [7]. They are known to be inhibitors of cysteine proteases and were first reported by Hanzlik as inhibitors of the plant protease papain [8]. On the other hand, Melatonin (*N*-acetyl-5-methoxytryptamine) possesses a broad

bioactivities of increasing evidence suggests that melatonin may somehow delay the aging process and/or the progression of age-related disease processes [9], perhaps owing to its ability to eliminate free radicals [10] and Serotonin (5-HT) is a classical neurotransmitter, vasoactive amine best known for its role in the regulation of variety of physiologic states and behaviors, including pain, appetite, mood and sleep [11]. In view of the roles played by these derivatives in synthetic and pharmacological activities, we aimed to design the synthetic route for tryptdiamine-O-analogue derivative of benzofuran, through a multi-step functional group transformation from resorcinol as shown in **Scheme I**. Since benzofuran analogues of tryptamine are not readily available and were not reported, we have made an attempt to synthesize the tryptdiamine-O-analogue with only difference in the position of hydroxyl group and it is a dimer compared to melatonin and serotonin analogues as shown in figure 1. The newly synthesized a series of compounds were screened for their in vitro antibacterial and DPPH scavenging activity. In correlation, in silico molecular docking studies were carried out along with serotonin and melatonin to predict the GlcN-6-P synthase inhibitory activity. Additionally, molecular properties were evaluated to predict oral bioavailability.

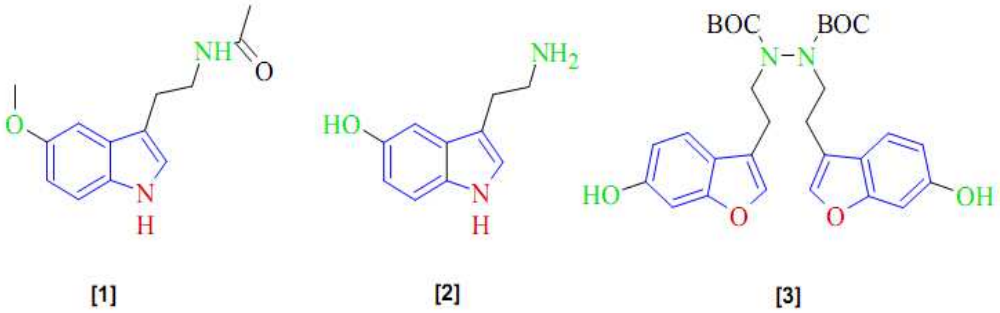
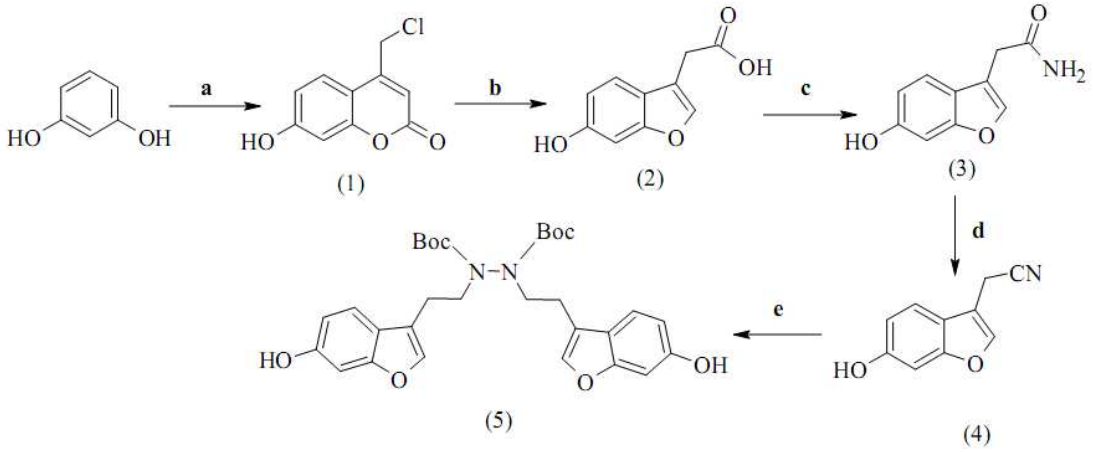


Figure 1: Skeletal similarity between [1] Melatonin, [2] Serotonin and [3] Tryptdiamine-O-analogue derivative of benzofuran

EXPERIMENTAL SECTION

All melting points were determined with open capillary and are uncorrected. The progress of all reactions was monitored by thin layer chromatography using silica gel plates and spots located either by UV or potassium permanganate solution. IR spectra were recorded in KBr pellets by using JASCO FTIR-4100 spectrophotometer. ^{1}H NMR spectra were recorded in $CDCl_3$/DMSO-d6 on JEOL-400 MHz NMR instrument. Chemical shift are reported in ppm units relative to TMS. Mass spectral data were obtained on AGILENT LC-MS column C-18 instrument. UV-Visible absorption was recorded in methanol by using Thermo scientific UV-Vis spectrophotometer. Microorganism cultures were procured from National Chemical Laboratory, Pune, India.



Scheme 1: Synthetic route for the 6-hdroxybenzofuran derivative series. Conditions and reagents: a) Chloroethylacetoacetate, Con.H_2SO_4, 0 °C, 24 h; b) 10% NaOH, 100 °C; c) CDI, CH_2Cl_2, ammonia solution, overnight; d) DMF:$SOCl_2$, 0 °C, 2 h, then overnight at rt; e) $NaBH_4$, $CoCl_2.6H_2O$, $(Boc)_2O$, 3 h

1.1.1. Procedure for synthesis of 4-(chloromethyl)-7-hydroxy-2H-chromen-2-one (1):
Resorcinol (5.0 g, 0.045 mmol) was added to conc.H_2SO_4 (25 mL) which cooled to 0^{o}C in ice bath, after complete dissolution of resorcinol; Chloroethylacetoacetate (8.22 g, 0.049 mmol) was added drop-wise and reaction mixture was stirred at room temperature for 24 hrs. After complete reaction, the reaction mixture was poured into crushed ice; solid separates out. The solid obtained was filtered, washed with water and dried. The product was recrystallized from ethyl acetate. white solid; Yield: 70%; IR (KBr): 3248 cm^{-1} (-OH str), 2921 cm^{-1} (-CH str), 1713 cm^{-1} (>C=O str), 1145 cm^{-1} (C-O-C), 850cm^{-1} (C-Cl str); ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.72 (s, 1H, Ar-OH), 7.86(s, 1H, Ar-H), 7.69-7.67(d, *J* = 8 Hz, 1H, Ar-H), 6.76-6.75 (d, *J* = 6.9 Hz, 1H, Ar-H), 6.76-6.75 (d, *J* = 7.6 Hz, 1H, Ar-H), 5.03 (s, 2H, CH_2); MS: *m/z* 210.1 (M^+).

1.1.2. Procedure for synthesis of (6-hydroxy-1-benzofuran-3-yl) acetic acid (2):
4-(chloromethyl)-7-hydroxy-2*H*-chromen-2-one (4.0 g) and 10% aqueous NaOH (4.0 g in 40 mL water) was refluxed for 4 hrs at 100^{o}C. After completion of the reaction, the reaction mixture was poured into ice cold water containing 10% HCl, solid separates out. The obtained solid was filtered, washed with water and dried to give compound **2**. white crystals; Yield: 90%; IR (KBr): 3430 cm^{-1} (-OH str), 2921 cm^{-1} (-CH str), 1715 cm^{-1} (>C=O str), 1132 cm^{-1} (C-O-C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.35 (s, 1H, COOH), 9.50 (s, 1H, Ar-OH), 7.65 (s, 1H, Ar-H), 7.34-7.32 (d, *J* = 8.2 Hz, 1H, Ar-H), 6.869-6.864 (d, *J* = 1.9 Hz, 1H, Ar-H), 6.74-6.71 (dd, *J* = 6.3, 2.0 Hz, 1H, Ar-H), 3.59 (s, 2H, -CH_2); MS: *m/z* 192.1 (M^+).

1.1.3. Procedure for synthesis of 2-(6-hydroxy-1-benzofuran-3-yl) acetamide (3):
(6-hydroxy-1-benzofuran-3-yl) acetic acid (3.0 g, 0.015 mmol) was taken in DMF (15 mL), 1, 1-carbonydiimadazole (3.79 g, 0.023 mmol) was added at 0^{o}C and reaction mixture was stirred at 0^{o}C for 45-50 mins. Ammonium acetate (3.61 g, 0.046 mmol) and Et_3N (1mL) was added and stirred at room temperature over night. Finally the reaction mixture was diluted with Ethyl acetate and washed with water followed by brine solution. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under vacuum to give crude amide. The crude product was subjected for column chromatography on silica gel (60-120 mesh) using a mixture of petroleum ether and ethyl acetate (4:6) as eluent to obtain pure compound **3**. The single crystal X-ray study of compound **3** has been reported from our laboratory [12]. Brown crystals; Yield: 95%; IR (KBr): 3594 cm^{-1} (-OH str), 3344 cm^{-1} (-NH Asy str), 3192 cm^{-1} (-NH sym str), 2921 cm^{-1} (-CH str), 1660 cm^{-1} (>C=O str), 1134 cm^{-1} (C-O-C); ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.47 (s, 1H, Ar-OH), 7.58 (s, 1H, Ar-H), 7.37-7.35 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.00 & 6.94 (s, 2H, NH_2), 6.85-6.84 (d, *J* = 1.9 Hz, 1H, Ar-H), 6.72 (s, 1H, Ar-H), 3.37 (s, 2H, -CH_2); MS: *m/z* 191.1 (M^+).

1.1.4. Procedure for synthesis of (6-hydroxy-1-benzofuran-3-yl) acetonitrile (4):
2-(6-hydroxy-1-benzofuran-3-yl) acetamide (0.2 g) in DMF (2 mL) was added to a cooled mixture of DMF (0.7 mL) and $SOCl_2$ (0.15 mL) was stirred at 0^{o}C for 2hrs and left stirring at room temperature overnight. After completion of the reaction, the reaction mixture was poured into ice cold water and extracted to organic layer. The organic layer was washed with water followed by brine and dried over anhydrous Na_2SO_4 and concentrated in vacuum to give corresponding nitrile. The crude product was subjected to column chromatography on neutral alumina using petroleum ether and ethyl acetate (8:2) as an eluent to obtain pure amorphous solid **4**. pale yellow solid; Yield: 95%; IR (KBr): 3363 cm^{-1} (Ar-OH str), 2921 cm^{-1} (-CH str), 2264 cm^{-1} (>CN str), 1134 cm^{-1} (C-O-C); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.56 (s, 1H, Ar-H), 7.42-7.40 (d, *J* = 8.4Hz, 1H, Ar-H), 6.996-6.991 (d, *J* = 2.0Hz, 1H, Ar-H), 6.86 (s, 1H, Ar-H), 5.02 (s, 1H, Ar-OH), 3.73 (s, 2H, -CH_2); MS: *m/z* 173.1 (M^+).

1.1.5. Procedure for synthesis of Bis- tert-butyl-2-(Di-6-hydroxybenzofuran-3-yl) ethylcarbamate (5):
To a solution of (6-hydroxy-1-benzofuran-3-yl) acetonitrile (0.1 g, 0.00057 mmol) in ice cooled Methanol (5 mL), $CoCl_2.6H_2O$ (0.01 g, 0.000057 mmol) and $(Boc)_2O$ (0.1 g, 0.00057 mmol) was added. To this $NaBH_4$ (0.043 g, 0.011 mmol) was added portion wise, black precipitate was formed with effervescence and was left stirring at room temperature for 4-5 hrs. After completion of the reaction, the reaction mixture was poured into water and extracted to EtOAc. The organic layer was washed with water, dried over anhydrous Na_2SO_4 and solvent was removed under vacuum to give corresponding Boc protected diamine derivative. The obtained residue was purified by column chromatography on neutral alumina using Pet ether: Ethyl acetate (9:1) as eluent to get pure semi solid of **5**. pale yellow semi solid; Yield: 85%; IR (KBr): 3108 cm^{-1} (Ar-H str), 2921 cm^{-1} (-CH str), 1755 cm^{-1} (>C=O str), 1077-1174 cm^{-1} (C-O-C); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.52-7.50 (d, *J* = 8.41 Hz, 2H, Ar-H), 7.47 (s, *J* = 7.56 Hz, 2H, Ar-H), 7.329-7.325 (d, *J* = 1.9Hz, 2H, Ar-H), 7.09-7.06 (dd, *J* = 7.07 Hz, 2H, Ar-H), 4.62 (s, 2H, Ar-OH), 3.44-3.43 (t, *J* = 6.2Hz, 4H, CH_2), 2.88-2.84 (t, *J* = 6.7Hz, 4H, CH_2), 1.57 (s, 18H, -CH_3); MS: *m/z* 552.1 (M^+).

1.2. Biological studies

1.2.1.Antibacterial activity

The antibacterial activity was carried out against 24 hour mature broth cultures of four different bacterial strains *Klebsiella aerogenes* (NCMI-2098), *Escherichia coli* (NCMI-5051), *Psedomonas aeruginosa (*NCMI-2242) and *Staphylococcus aereus* (NCMI-5022) by cup plate method [13]. The synthesized molecules were examined for the presence or absence of zone of inhibition on nutrient agar plates. Nutrient agar (NA) plates were swabbed (sterile L-Shaped glass rod) with 100 µL of 24 hours old broth culture of respective bacteria. Using the sterile cork borer, wells (6mm) were made into the each petri-plate. The stock solutions of compounds were prepared at concentration of 2000µg/mL. Two different concentrations of compounds **(1-5)** (50 and 100µg/ well) were used to assess the dose dependent activity of the compound and loaded using sterile micropipettes. Ciprofloxacin (5µg/ 50µL) was used as standard drug for antibacterial activity. DMSO was used as solvent control. The zone of inhibition was compared with standard drug after incubation for 24-36 hrs at 37^{o}C.

1.2.2.Antioxidant activity

All the compounds **2-5** were tested for 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity [14] with ascorbic acid as standard. This method is based on the reduction of free radical DPPPH by free radical scavengers. Test compounds (1 mg/mL) were prepared in 50% methanol. Different concentrations of 240, 480, 720, 960 and 1200 µg/mL were added to each test tubes and volume was made upto 860 µL using 50% methanol. To this 140 µL of DPPH (0.04% in 50% methanol) was added and mixture was incubated at 37^{o}C for 30 min. The scavenging activity on DPPH radical was determined by measuring the absorbance at 520 nm. Ascorbic acid used as control and 95% methanol was used as blank.

1.3. In silico molecular docking studies and molecular properties:

The synthesized compounds **2-5** along with serotonin and melatonin were subjected for comparative and automated molecular docking studies. The native crystal structure of GlcN-6-P synthase (X chain) in complex with glucosamine-6-phosphate was retrieved from Protein Data Bank (http://www.pdb.org/pdb/home/home.do) with the (PDB ID: 2VF5) which was resolved at 2.90A^{o} using X-ray diffraction [15]. The active pocket was identified and Ligplot for the protein was downloaded from PDB. Discovery studios 4.0 was used to prepare 2vf5 by removing ligand, water molecules and hydrogens were added. The site in which co-crystallized ligand complexed was identified and grid was centered at the region surrounding important amino acid residues as shown in Fig.1.The center of grid box was set to 36.16, 13.0 & -1.16 and number of points in x, y & z dimensions 52, 54 & 52 respectively. The ligands were drawn on Chem Draw Ultra 8.0, assigned with proper 2D orientation and were converted to energy minimized 3D structures using PRODRG server [16] and Gasteiger charges [17], nonpolar hydrogen atoms and the rotatable bonds were set by using AutoDock 4.2 tools. The Lamarckian Genetic Algorithm (LGA) was chosen to search for the best conformers. During the docking process, a maximum of 10 conformers were considered for each compound.

For any ligands to complete drug discovery process good pharmacokinetics and toxicity profile are essential to become a good drug molecule [18]. Hence we screened our compounds **2-5** for Lipinski's rule of five and toxicity parameters. The Lipinski rule [19] describes drug's pharmacokinetics such as absorption, distribution, metabolism, excretion and toxicity ("ADMET"). The compound is orally bioavailable if they obey the following criteria: log p (≤ 5), molecular weight (≤500), hydrogen bond acceptors (≤10), and hydrogen bond donors (≤5). Molecules that violate more than one of these rules may have problems with bioavailability [20]. Therefore, this rule establishes some structural parameters relevant to the theoretical prediction of the oral bioavailability and is widely used in designing new drugs. The percentage of absorption was estimated using the Equation: % ABS = 109 − (0.345 × TPSA) [21]. The calculations of ADMET property was achieved for the molecules **2-5** including serotonin, melatonin and ciprofloxacin through molinspiration and organic chemistry portal web based application for predicting Insilico ADMET. 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.

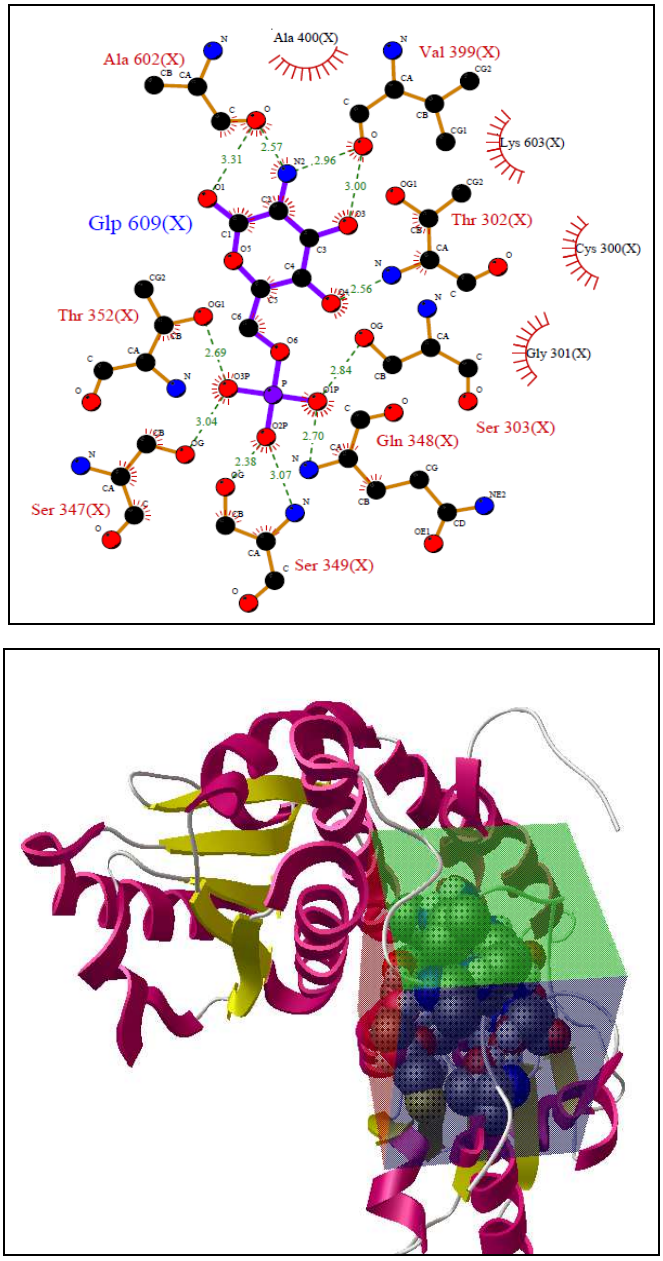


Figure 2: Ligplot of GlcN-6-P synthase with ligand GlcN-6-phosphate and Grid map of GlcN-6-p synthase

RESULTS AND DISCUSSION

1.4. Chemistry

Novel tryptamine-O-analogue (Bis- tert-butyl-2-(Di-6-hydroxybenzofuran-3-yl) ethylcarbamate) of benzofuran was synthesized through a multistep functional group transformation reaction from resorcinol. The first step in the sequence involves pechmann condensation of resorcinol with chloroethylacetoacetate in presence of con.H_2SO_4 to give synthesis 4-(chloromethyl)-7-hydroxy-2*H*-chromen-2-one **1**. Further the compound **1** undergoes ring opening reaction followed by SN2 displacement of chloride ion in presence of base to give corresponding acetic acid derivative of benzofuran **2**. Then the acid derivative **2** was made active to facilitate nucleophilic substitution by ammonia using 1, 1'-carbonyldiimidazole (CDI) to afford corresponding primary amide derivative of benzofuran **3**. The single crystal X ray study of compound **3** has been reported from our laboratory [12]. Subsequent dehydration

of **3** by DMF and $SOCl_2$ mixture give corresponding nitrile derivative **4**. The compound **4** undergo reduction in presence of $CoCl_2.6H_2O$ and $NaBH_4$ followed by protection with $(Boc)_2O$ to give tryptdiamine-O-analogue of benzofuran **5.** Structure of newly synthesized derivatives was confirmed by FT-IR, 1HNMR and LC-MS spectral data.

1.5. Biological evaluation

1.5.1.Antibacterial activity

In-vitro antibacterial screening results shown in table 1 revealed that all compounds **2-5** exhibited good activity. Among the tested compounds, compound **3** & **5** show good activity against all the bacterial strains whereas the compound **(4)** shows activity against three bacterial strains except *S.aureus* compared with standard drug Ciprofloxacin.

Table 1: Antimicrobial activity of synthesized derivatives (2-5)

Compound	Zone of Inhibition in mm				
	Conc in µg/50µL	***K.aerogenes***	***E.coli***	***P.desmolyticum***	***S. aureus***
2	50	2.67	0.67	0.67	1.67
	100	5.67	1.67	2.00	3.67
3	50	2.67	2.33	1.33	1.33
	100	3.67	4.33	2.67	2.33
4	50	1.67	1.67	1.33	-
	100	3.67	3.67	3.67	-
5	50	2.33	2.67	2.00	1.33
	100	3.67	7.00	4.67	3.67
Standard	5	16.33	18.67	15.33	17.67

Table 2: DPPH assay of synthesized derivatives (2-5)

Compounds	Concentration in µg/mL	% inhibition	IC_{50}
2	120	13.7	100 µg/mL
	240	18.6	
	360	29.6	
	480	41.8	
	600	62.4	
3	120	22.0	50 µg/mL
	240	27.4	
	360	33.0	
	480	46.4	
	600	55.3	
4	120	40.1	187 µg/mL
	240	51.8	
	360	60.3	
	480	65.1	
	600	71.4	
5	120	39.4	425 µg/mL
	240	44.9	
	360	47.0	
	480	49.5	
	600	52.4	

1.5.2.DPPH free radical scavenging activity

DPPH Free radical scavenging activity method results shown in table 2 indicate that all compounds **2-5** exhibits good DPPH scavenging activity. Among the tested compounds **2**, **3**, and **4** exhibits promising radical scavenging activity with IC_{50} of 100, 50, & 187µg/mL respectively.

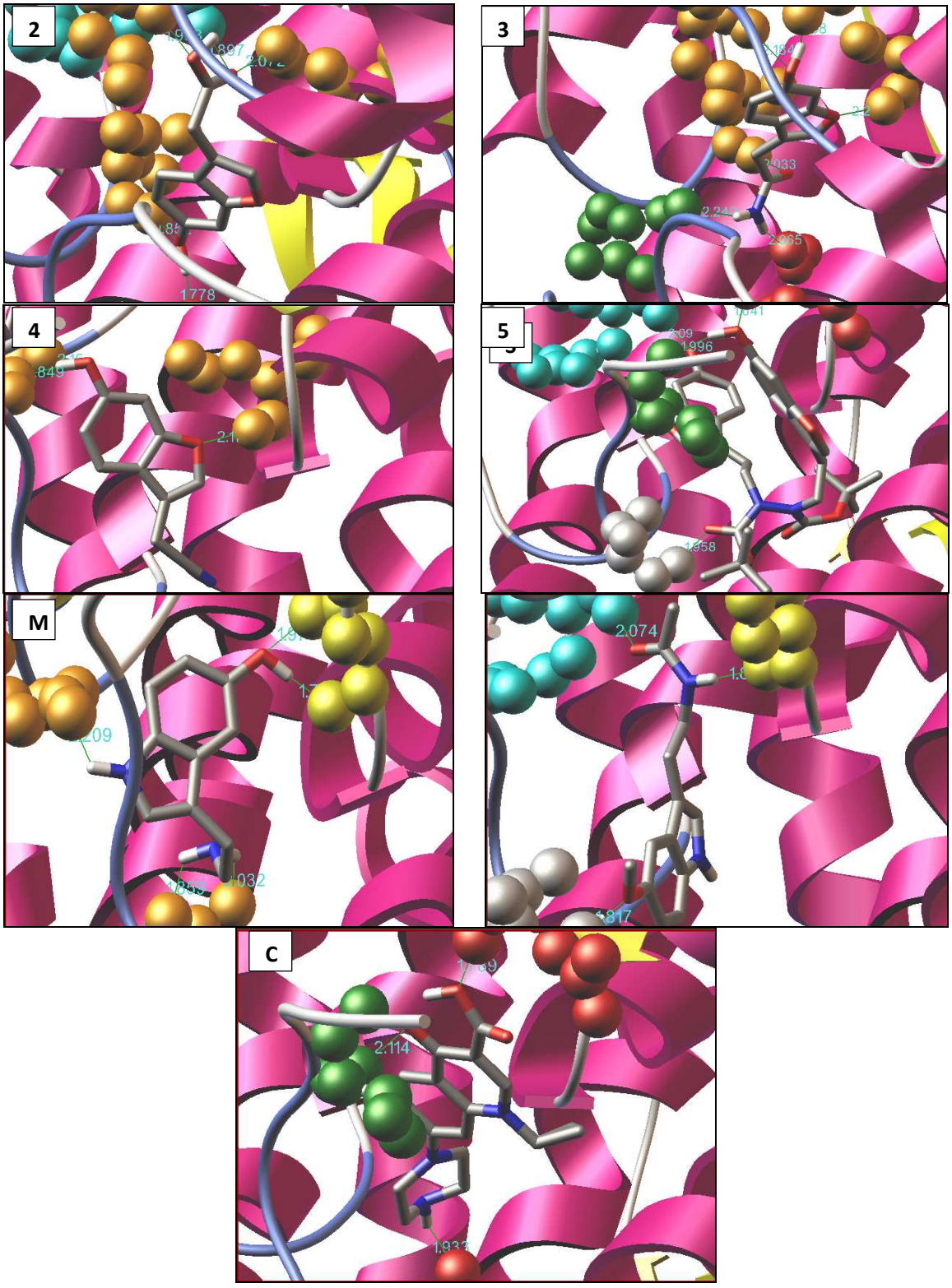


Figure 3: Binding modes of all the synthesized [2] acid, [3] amide, [4] nitrile, [5] tryptdiamine-O-analogue molecules including the considered standard drug [C], [S] serotonin and [M] melatonin with GlcN-6-P synthase

Table 3: Molecular docking results with GlcN-6-P synthase

Molecule	Binding Energy	Ligand efficiency	Torsional Energy	RMS	H-bonds	Bonding	Bond length (Å)
2	-5.23	-0.37	1.19	0.0	5	1::DRG1:2VF5::X:GLU488:OE2:H 1::DRG1:2VF5::X:SER349:HN:O 1::DRG1:2VF5::X:SER303:HG:O 1::DRG1:2VF5::X:SER401:HN:O 1::DRG1:2VF5::X:GLN348:HN:O	1.74 1.89 2.16 2.07 1.90
3	-6.31	-0.45	0.89	0.0	6	2::DRG2:2VF5::X:VAL399:O:H 2::DRG2:2VF5::X:SER347:OG:H 2::DRG2:2VF5::X:SER303:HN:O 2::DRG2:2VF5::X:GLU488:OE2:H 2::DRG2:2VF5::X:SER401:HN:O 2::DRG2:2VF5::X:SER349:HN:O	1.70 2.18 2.12 2.06 2.24 2.02
4	-5.65	-0.43	0.6	0.0	3	9::DRG9: 2VF5::X:SER349:OG:H 9::DRG9: 2VF5::X:SER303:HN:O 9::DRG9: 2VF5::X:SER349:HN:O	2.15 1.85 2.17
5	-6.08	-0.15	3.88	0.0	4	6::DRG6: 2VF5::X:GLN348:HN:O 6::DRG6: 2VF5::X:ALA602:HN:O 6::DRG6: 2VF5::X:VAL605:O:H 6::DRG6:2VF5::X:ASP354:HN:O	1.64 1.99 2.09 1.95
Ciprofloxacin	-6.02	-0.25	1.19	0.0	3	1:: Ciprofloxacin:2VF5::X:GLU488:OE2:H 1:: Ciprofloxacin:2VF5::X:VAL605:HN:O 1:: Ciprofloxacin:2VF5::X:ASP354:HN:O	1.78 2.11 1.93

Table 4: Comparative docking result of compounds 5 with melatonin and serotonin

Molecule	Binding Energy	Ligand efficiency	Torsional Energy	RMS	H-bonds	Bonding	Bond length (Å)
Ser	-7.02	-0.54	1.19	0.0	5	10::DRG10: 2VF5::X:SER349:OG:H 10::DRG10: 2VF5::X:SER401:OG:H 10::DRG10: 2VF5::X:SER349:OG:H 10::DRG10: 2VF5::X:CYS300:HN:O	2.20 1.97 1.70 1.89 2.03
Mel	-6.06	-0.36	1.19	0.0	3	10::DRG10: 2VF5::X:SER349:O:H 8::DRG8: 2VF5::X:GLN348:HN:O 8::DRG8: 2VF5::X:ALA602:HN:O 8::DRG8: 2VF5::X:CYS300:O:H	1.80 2.07 1.81
5	-6.08	-0.15	3.88	0.0	4	6::DRG6: 2VF5::X:GLN348:HN:O 6::DRG6: 2VF5::X:ALA602:HN:O 6::DRG6: 2VF5::X:VAL605:O:H 6::DRG6:2VF5::X:ASP354:HN:O	1.64 1.99 2.09 1.95

Table 5a: In silico ADMET properties of synthesized compounds

Comp.	Mutagenic	Tumerogenic	Irritation	Reproductive effect
2	No	No	No	No
3	No	No	No	No
4	No	No	No	No
5	No	No	No	No
Standard	No	No	No	No

Table 5b: Lipinski's parameters of synthesized compounds

Mol. No	Lipinski's Parameters					TPSA (Å²)	% ABS	cLogP	Solubility	Drug-likeness	Drug score
	HBA	HBD	MW	miLogP	Violations						
2	4	2	192.17	1.113	0	70.667	84.61	1.25	-2.48	-1.09	0.54
3	4	3	191.186	0.598	0	76.462	82.62	0.85	-2.53	-1.07	0.59
4	3	1	173.171	1.652	0	57.16	89.28	1.77	-3.13	-9.41	0.45
5	10	2	552.624	5.525	2	125.822	65.60	5.41	-6.76	-32.1	0.14
C	6	2	331.347	-0.701	0	74.569	83.28	0.86	-3.58	2.55	0.81

HBA-Hydrogen bond acceptor; HBD-Hydrogen bond donor

1.6. In silico study of Molecular docking and molecular properties

In silico studies of ligand molecules **2-5** along with serotonin and melatonin were carried out to support invitro activity with GlcN-6-P synthase. The result reveals that all the ligands forms H-bonds with one or the other amino acid residue in the receptor active pocket as shown in figure 3. All ligand molecules showed encouraging minimum binding energy. Among the docked ligands, **2, 3, 4** & **5** showed that their binding energies are -5.23, -6.31, -5.65 & -6.06 kJ/mol respectively compared to standard with binding energy -6.02 kJ/mol. The compounds **3** & **5** have more binding energy than the ciprofloxacin and hence they are considered as good inhibitors of GlcN-6-P synthase. In addition, synthesized tryptdiamine-O-analogue has comparable binding energy of -6.06 kJ/mol with that of serotonin and melatonin binding energies -7.02 and -6.06 kJ/mol respectively as shown in table 3 & 4. Hence these synthesized compounds can be considered as potential antibacterial agents.

Additionally, molecular properties result as documented in table 5a & b, shows that all the compounds meet the Lipinski's rule of five except the tryptdiamine-O-analogue suggesting that these compounds theoretically does not have problem with oral bioavailability.

CONCLUSION

The present work provides simple, high yielding and easy access to synthesize Bis- tert-butyl-2-(Di-6-hydroxybenzofuran-3-yl) ethylcarbamate containing benzofuran moiety which is O-analogue of tryptamine derivative via sequence functional group transformation reactions. The structures of synthesized molecules were well supported by their spectral studies. The result of *in vitro* antibacterial and DPPH scavenging activities indicates that the compounds **2-5** emerged as active compounds. In silico studies showed that compounds **3** & **5** have more binding energies and more binding affinity towards target protein compared to standard drug and compound **5** has comparable binding energy compared to serotonin and melatonin analogues. From these results, compounds **2-5** appear to be interesting and have widened the scope of developing the tryptdiamine-O-analogue as promising antibacterial agents, neurotransmitter and useful as a ligands in coordination chemistry.

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