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Synthesis of novel inhibitors of β-glucuronidase based on benzothiazole skeleton and study of their binding affinity by molecular docking

Khalid Mohammed Khan [a,*], Fazal Rahim [a], Sobia Ahsan Halim [b], Muhammad Taha [a], Momin Khan [a], Shahnaz Perveen [c], Zaheer-ul-Haq [a,b], Muhammad Ahmed Mesaik [b], M. Iqbal Choudhary [a]

[a] H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan
[b] Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan
[c] PCSIR Laboratories Complex, Karachi, Shahrah-e-Dr. Salimuzzaman Siddiqui, Karachi 75280, Pakistan

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ABSTRACT

Benzothiazole derivatives **1**–**26** have been synthesized and their in vitro β-glucuronidase potential has been evaluated. Compounds **4** (IC_{50} = 8.9 ± 0.25 μM), **5** (IC_{50} = 36.1 ± 1.80 μM), **8** (IC_{50} = 8.9 ± 0.38 μM), **13** (IC_{50} = 19.4 ± 1.00 μM), **16** (IC_{50} = 4.23 ± 0.054 μM), and **18** (IC_{50} = 2.26 ± 0.06 μM) showed β-glucuronidase activity potent than the standard (D-saccharic acid 1,4-lactone, IC_{50} = 48.4 ± 1.25 μM). Compound **9** (IC_{50} = 94.0 ± 4.16 μM) is found to be the least active among the series. All active analogs were also evaluated for cytotoxicity and none of the compounds showed any cytotoxic effect. Furthermore, molecular docking studies were performed using the GOLD 3.0 program to investigate the binding mode of benzothiazole derivatives. This study identifies a novel class of β-glucuronidase inhibitors.

1. Introduction

Benzothiazole, a heterocyclic aromatic molecule with electron rich sulfur and nitrogen atoms, used as a pharmacological agent with a wide variety of biological activities, such as immunomodulatory, immunosuppressive, antitumour, and antiviral properties.[1] The benzothiazole skeleton constitutes an important template for a wide variety of biologically active compounds. This molecule and its derivatives are known to be powerful antitumour agents,[2–7] calmodulin (CaM) antagonists,[8] neurotransmission blocker,[9–11] and neuroprotective agent.[12,13] Benzothiazole-type compounds attracted considerable attention in anticancer drug development.[14–21] Modified benzothiazole derivatives with additional functional groups can likely improve the biological potential of these compounds.

Cantharidin-containing benzothiazoles showed activity against hepatocellular carcinoma, acute breast cancer, leukemia myelogenous, and non-small cell lung carcinoma. The simple benzothiazole nucleus is present in compounds with biological activities such as antimicrobial,[22] antitumoral,[23,24] antimalarial,[25] and antitubercular.[26]

Benzothiazole has been exploited to enhance the donor-acceptor effects in the chromophores.[27] The flexible and synthetically accessible 2-arylbenzothiazole scaffold has provided the basis of the development of a number of new antitumour agents with unusual mechanisms of action in recent years.[28] The fluorinated analogue, 2-(4-amino-3-methylphenyl)-5-fluorobenzothiazole, has emerged as the lead compound against a panel of breast cancer xenografts.[29]

The present work pertained to the synthesis and evaluation of in vitro β-glucuronidase inhibition activity of a series of benzothiazole derivatives **1**–**26**. β-Glucuronidase is an exoglycosidase enzyme which catalyzes the cleavage of glucuronosyl-*O*-bonds.[30] The enzyme is present in many organs and body fluids such as kidney, spleen, bile, serum, urine, respectively.[31,32] Enhanced activity of this enzyme have been reported in a variety of pathological conditions, including urinary tract infection,[33–36] renal diseases,[37] transplantation rejection,[38] epilepsy,[39] neoplasm of bladder,[40] testes,[41] larynx,[41] and breast.[41] Furthermore, β-glucuronidase is reported to be released into the synovial fluid in inflammatory joint diseases, such as rheumatoid arthritis.[42,43] The over-expression of the enzyme is also reported in some hepatic diseases and AIDS.[44] β-Glucuronidase is also found to be involved in the etiology of colon cancer and higher intestinal level of the enzyme associated to higher incidence of colon carcinoma.[45,46] This observation is supported from the fact that the administration of a bacterial β-glucuronidase

* Corresponding author.
E-mail address: hassaan2@super.net.pk (K.M. Khan).

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inhibitor lead to a decrease in carcinogen induced colonic tumors.[44] These reports clearly suggest that the development of specific inhibitors of β-glucuronidase has great pharmacological importance.

In continuation of our ongoing research on the chemistry and bioactivity of new heterocyclic compounds,[47] we carried out the synthesis of benzothiazole derivatives, which is reported herein.

2. Results and discussion

2.1. Chemistry

Benzothiazoles **1–26** were synthesized by reacting commercially available 2-aminothiophenol with different aromatic aldehydes in *N,N*-dimethylformamide (DMF). In a typical reaction, sodium metabisulfite ($Na_2S_2O_5$) was added to a stirring mixture of 2-aminothiophenol (3.12 mmol) and different substituted aromatic aldehydes (3.16 mmol) in DMF under reflux for 2 h.[48] The progress of reaction was monitored by periodic TLC. After completion of the reaction, the reaction mixture was allowed to cool to room temperature, addition of water (30 mL) precipitated a solid, and filtration of the precipitates afforded benzothiazole derivatives 1–**26** in high yields (Scheme 1). Recrystallization from methanol afforded pure products. The structures of compounds **1–26** were determined by using different spectroscopic techniques, including ^{1}H NMR and EI mass spectroscopy.

2.2. Biological activity

Enzyme inhibition is an important tool in controlling the onset and progression of related pathologies. It has been extensively used in drugs discovery and development. In this regard, we have synthesized 26 compounds and screened for their β-glucuronidase enzyme inhibitory potential (Table 1). Out of 26, seven showed remarkable activity against β-glucuronidase. Compound 5-(1,3-benzothiazol-2-yl)-1,2,4-benzenetriol (**18**) showed an excellent activity with an IC_{50} value 2.26 ± 0.06 μM 24-fold more active than the standard D-saccharic acid 1,4-lactone (IC_{50} = 48.4 ± 1.25 μM). Similarly, compound 2-(1,3-benzothiazol-2-yl) phenol (**16**) showed an excellent activity with an IC_{50} value (4.23 ± 0.054 μM), 12-fold better than the standard. The compounds **4**, **5**, **8**, and **13** also exhibited a potent activity.

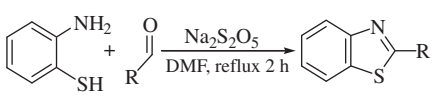
Compounds **4**, **8**, **9**, **10**, **13**, **16**, and **18** showed significant inhibitory activity against β-D-glucuronidase within the range of 2.26 ± 0.06–94.0 ± 4.16 μM. However, the compounds **1**, **11**, **12**, **14**, **19**, and **21**–**23** did not show any inhibitory activity at 0.2 mM concentration. Compounds **2**, **3**, **6**, **7**, **10**, **15**, **17**, **20**, **24**-**26** showed less than 50% inhibition at 0.2 mM concentration.

All the synthetic compounds **1–26** were also screened for their activity against other hydrolase, for example, α-chymotrypsin and urease and found to be almost inactive, which proves that these are substrate specific inhibitors.

To understand the mechanism of β-glucuronidase inhibition and binding mode of benzothiazole derivatives inside the binding pocket of β-glucuronidase, molecular docking studies were performed.

2.3. Molecular modeling and docking studies

In order to obtain more insight into the binding mode of benzothiazole derivatives within the active site of β-D-glucuronidase and



Scheme 1. Synthesis of benzothiazole derivatives **1**–**26**.

to obtain additional validations for experimental results, molecular docking studies were performed.

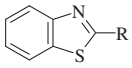
Human β-D-glucuronidase 3-dimensional structure was used for our structure–activity relationship (SAR) studies due to the absence of bovine β-D-glucuronidase structure. The X-ray crystal structure of human β-D-glucuronidase determined by Jain et al.[49] was retrieved from the Protein Data Bank (PDB code 1BHG) for the present docking study. Prior to the docking of benzothiazole derivatives, the known substrate molecule *p*-nitrophenyl β-D-glucuronide[50] was first docked into the active site of β-D-glucuronidase using the docking program GOLD 3.0.[51]

The modeled substrate-bound structure of human β-D-glucuronidase showed that the glycoside bond of *p*-nitrophenyl β-D-glucuronide was properly oriented towards the catalytic residues Glu451 and Glu540 (Fig. 1). It has been proposed for human β-D-glucuronidase that during catalysis, Glu451 acts as the acid/base catalyst while Glu540 serves as the nucleophilic residue.[49] As shown in Figure 1, the inhibitor neatly fits in the active site making various hydrogen bonding interactions with the active site residues including Glu451, His385, and Lys606. All the residues play an important role in binding of the substrate molecule in the active site of β-D-glucuronidase.[49] This modeled protein structure was then used in the prediction of a favorable binding mode of newly synthesized benzothiazole derivatives (as described in material and method section). The predicted binding models of the biologically active compounds are shown in Figure 2.

Analysis of the predicted binding conformations of our most active compound **18** (IC_{50} = 2.26 ± 0.06 μM) revealed that compound **18** can adopt a linear conformation for a better fit to the straight binding groove of β-D-glucuronidase. Visual inspection of the top ranked pose of compound **18** revealed that the hydroxyl groups at *ortho* position mediated strong hydrogen bond interaction with the side chain carboxyl oxygen (Oε1) of Glu451 (1.74 Å). The hydroxyl group at *para* position involved in strong hydrogen bonding interaction with the side chain carboxyl oxygen (OD2) of Asp207 (1.84 Å). The high inhibition constant of compound **18** towards β-D-glucuronidase could be explained by these two strong hydrogen bonds. The predicted binding mode of compound **18** is shown in Figure 2a. Additionally, the indole group of the Trp587 provides hydrophobic interaction to the benzenetriol group of compound **18**. His385, Asn484, Tyr504, His509, Arg600, and Lys606 are the other residues which stabilize the binding of compound in the active site of β-D-glucuronidase.

In contrast, the binding mode of compound **16**, which is the second most active compound (IC_{50} = 4.23 ± 0.054 μM) in the series revealed that the hydroxyl group at *ortho* position is involved in the hydrogen bond interaction with the side chain carboxyl oxygen (Oε1) of Glu451 (1.72 Å). Due to the lack of hydroxyl groups at *meta* and *para* positions of compound **16**, it is unable to form hydrogen bonding interaction with Asp207 (Fig. 2b). The binding mode of compound **8** (IC_{50} = 8.90 ± 0.38 μM) revealed that the hydroxyl group at *ortho* position makes hydrogen bond with the side chain carboxyl of Glu451 (1.66 Å), while the hydroxyl at *meta* position did not adopt favorable conformation for binding with the surrounding residues (Fig. 2c). The observed binding mode of compound **4** showed that it posses hydroxyl group at *para* position which form hydrogen bonding with the side chain hydroxyl oxygen (OD2) of Asp207 (Fig. 2d). Compound **13** showed binding modes similar to compound **16** as the hydroxyl at *ortho* position mediate interaction with the side chain carboxyl oxygen (Oε1) of Glu451 at a distance of 1.88 Å. However, the hydroxyl group at *para* position does not mediate any interaction with the surrounding amino acid residues (Fig. 2e). The observed binding mode of compound **5** revealed that the hydroxyl group at *meta* position engage with the side chain carboxyl oxygen (Oε2) of Glu540 with the

Table 1
In vitro β-glucuronidase of compounds **1**–**26**



Basic Skeleton of Benzothiazole

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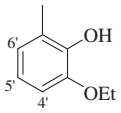
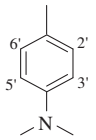
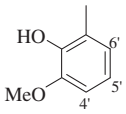
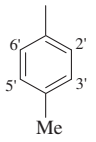
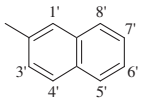
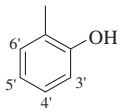
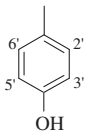
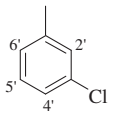
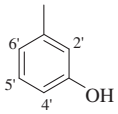
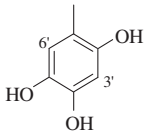
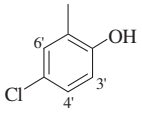
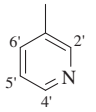
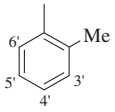
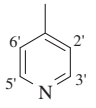
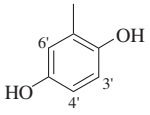
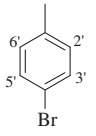
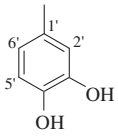
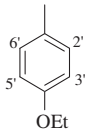
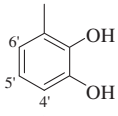
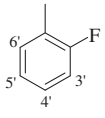
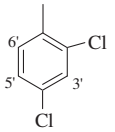
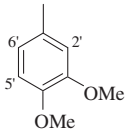
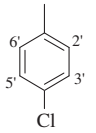
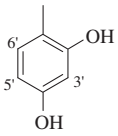
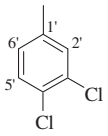
1		NA[b]	14		NA[b]
2		NA[b]	15		NA[b]
3		NA[b]	16		4.23 ± 0.054
4		8.9 ± 0.25	17		NA[b]
5		36.1 ± 1.80	18		2.26 ± 0.06
6		NA[b]	19		NA[b]
7		NA[b]	20		NA[b]
8		8.9 ± 0.38	21		NA[b]
9		94.0 ± 4.16	22		NA[b]
10		NA[b]	23		NA[b]
11		NA[b]	24		NA[b]
12		NA[b]	25		NA[b]

Table 1 (*continued*)

S. No.	R	IC_{50} ± SEM[a] (μM)	S. No.	R	IC_{50} ± SEM[a] (μM)
13		19.4 ± 1.00	26		NA[b]
	D-Saccharic acid 1,4-lactone(st)	48.4 ± 1.25			NA[b]

D-Saccharic acid 1,4-lactone (st) standard inhibitor for β-glucuronidase activity.
[a] SEM is the standard error of the mean.
[b] NA = not active.

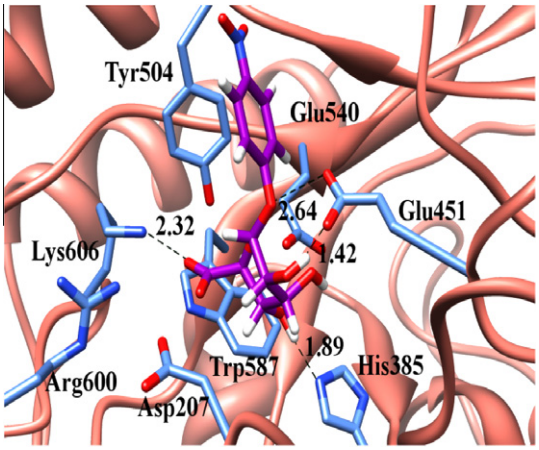


Figure 1. Predicted binding model of *p*-nitrophenyl β-glucuronide in complex with human β-glucuronidase. The black dashed lines indicate hydrogen bonding interactions. The interacting amino acid residues are shown in blue stick.

distance of 1.82 Å (Fig. 2f). The binding mode compound **9** (Fig. 2g) revealed that the hydroxyls at *meta* and *para* positions creates strong hydrogen bonds with two important amino acids residues (Glu451 and Glu540), nevertheless, the compound is not well accommodated in the binding cavity of the enzyme, resulting in low inhibition potency.

The order of inhibitory potential for disubstituted analogs may be in the same way as in monosubstituted. As we know that inhibitory potential of *o* and *p* substituted are higher than *m*-substituted. It means that both *o* and *p* position are more favorable for binding affinity than *m* position. So the order of binding affinity in disubstituted is may be *o,p* > *o,m*

The top ranked poses of inactive compounds are shown in Figure 3. The binding mode analysis of inactive compounds (compounds with low percent inhibition), clearly demonstrated the reason of inactivity. As shown in Figure 3, these compounds do not make any noteworthy hydrogen bond interactions with the surrounding amino acid residues. Hence, the activity of compounds largely depends on the maximum hydrophilic interactions with the active site amino acids of the β-D-glucuronidase.

2.4. Biological assay

β-Glucuronidase (E.C. 3.2.1.31, from bovine liver, G-0251) and *p*-nitrophenyl-β-D-glucuronide (N-1627) were purchased from Sigma Chemical Co. (USA). Sodium carbonate anhydrous from Fluka and all other reagents were obtained from E. Merck and were of analytical grade. Anhydrous $CHCl_3$ and EtOH were dried using the standard methods. All other solvents and reagents were of reagent grade and used directly without purification, except for benzoyl chloride, which was distilled before use.

2.5. Assay for β-glucuronidase

β-Glucuronidase activity was determined by measuring the absorbance at 405 nm of *p*-nitrophenol formed from the substrate by the spectrophotometric method. The total reaction volume was 250 μL. DMSO (100%) was used to dissolve the compound (5 μL), which become 2% in the final assay (250 μL) and the same conditions were used for standard (D-saccharic acid 1,4-lactone). The reaction mixture contained 185 μL of 0.1 M acetate buffer, 5 μL of test compound solution, 10 μL of enzyme solution was incubated at 37 °C for 30 min. The plates were read on a multiplate reader (SpectraMax plus 384) at 405 nm after the addition of 50 μL of 0.4 mM *p*-nitrophenyl-β-D-glucuronide. All assays were run in triplicate.

2.6. Docking protocol

X-ray crystal structure of human β-D-glucuronidase (PDB[52] code 1BHG[49] was retrieved for docking studies. The structure was checked for missing atoms, bonds and contacts. The B-chain of protein and hetero-atoms including cofactors were removed from the original protein data bank file. Hydrogen atoms were added to the enzyme structure by using the Biopolymer module in SYBYL7.3 software package.[53] All the docking calculations were performed on Intel® Xeon® Quad™ Core processor 3.0 GHz Linux workstation running under open SUSE11.0, equipped with GOLD3.0 as the docking software.

2.7. GOLD3.0

Docking studies were performed using the genetic optimization for ligand docking (GOLD) software version 3.0 from the Cambridge Crystallographic Data Centre (CCDC).[51] GoldScore was chosen as a fitness function and the standard default settings were employed in all calculations. The configuration file was defined by the following process: docking site was defined as all atoms within 10 Å of a specified centroid (*x*, *y*, *z* coordinates: 80.43, 84.41, 90.48). For each of the 100 independent genetic algorithm runs, a default maximum of 100,000 genetic operations was performed using the default operator weights and a population size of 100 chromosomes. Operator weights for cross over, mutation and migration were set to 100, 100 and 0, respectively. To allow poor non-bonded contacts at the start of each GA run, the maximum distance between hydrogen donors and fitting points were set to 5.0 Å, and non-bonded VdW energies were selected at cut-off value of 10 Å. All single bonds were treated as rotatable. Results differing by less than 1.5 Å in ligand-all atom RMSDs were clustered together. After docking thirty poses were saved for each ligand. On the basis of score, top scored docked poses were visually inspected for each ligand. The molecular interactions were visualized in CHIMERA version 1.3.[54]

To build a reliable protein model whose active site exists in the probable substrate-bound conformation, the known substrate

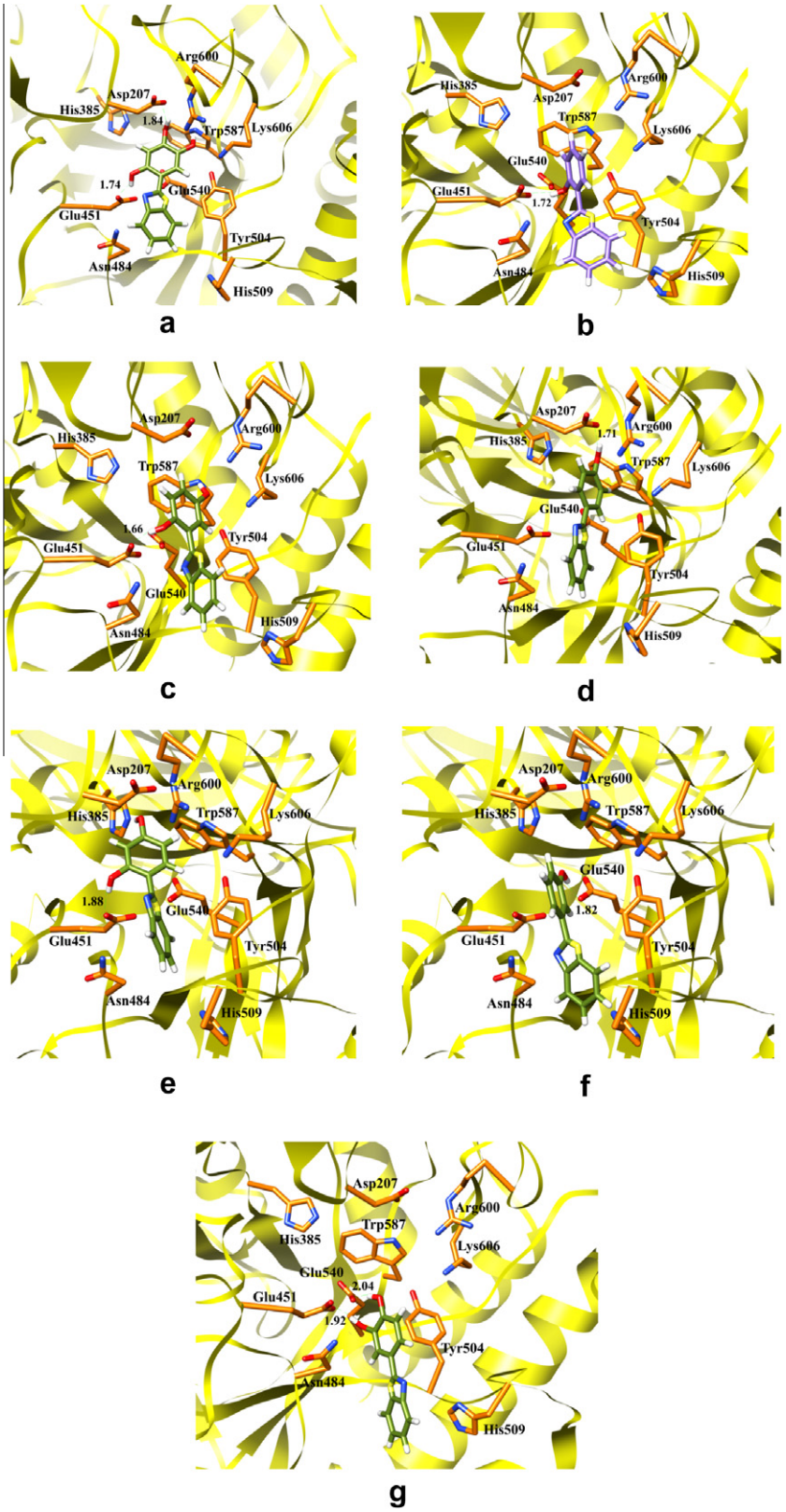


Figure 2. The putative binding modes and molecular interactions of the benzothiazole derivatives in the active site of β-D-glucuronidase: compounds **18** (2a), **16** (2b), **8** (2c), **4** (2d), **13** (2e), **5** (2f) and **9** (2 g). The interacting amino acid residues are shown in orange. The ligands are shown in green stick.

p-nitrophenyl β-glucuronide[50] was first docked into the active site of β-glucuronidase using GOLD. From the docking results, a high-scoring docked pose of *p*-nitrophenyl β-glucuronide having the glucuronyl moiety oriented properly toward the key residues Glu451 and Glu540 involved in the enzyme catalysis reaction was chosen to represent its most probable binding mode. The obtained docked model was further subjected to energy minimization for refinement. Prior to energy minimization, the Glu451

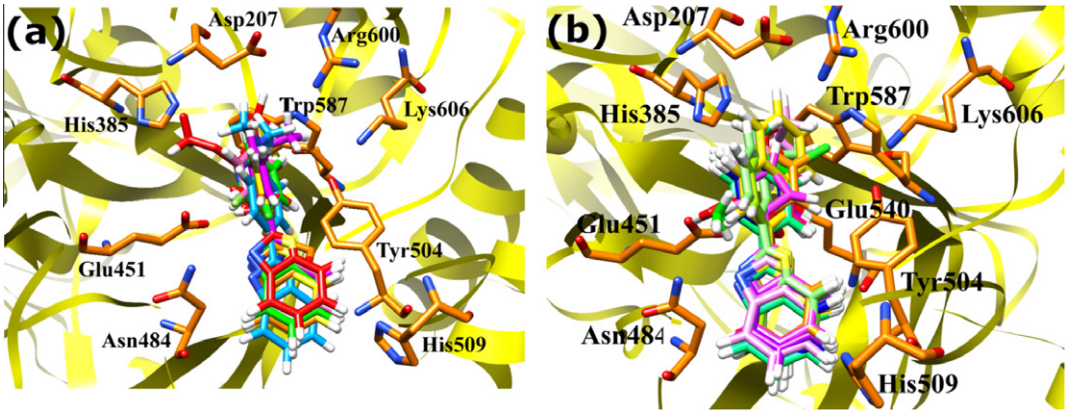


Figure 3. The binding modes and molecular interactions of the benzothiazole derivatives in the active site of β-D-glucuronidase: (a) compounds with −ve % inhibition, (b) compounds with low % inhibition.

Table 2
Predicted binding scores of the newly synthesized benzothiazole derivatives and standard β-glucuronidase inhibitor (*p*-nitrophenyl-β-glucuronide) obtained by GOLD scoring function

Compounds	Score
p-Nitrophenyl β-glucuronide	58.37
1	53.45
2	49.79
3	48.17
4	**51.81**
5	**51.9**
6	49.28
7	45.78
8	**53.95**
9	**51.36**
10	49.68
11	48.59
12	51.25
13	**54.72**
14	45.19
15	47.91
16	**55.92**
17	52.09
18	**56.97**
19	50.6
20	49.2
21	51.72
22	50.47
23	49.16
24	54.55
25	47.77
26	49.35

The scores of the most active compounds are highlighted in bold.

residue in the active site was assigned a protonated form that was consistent with its role in acting as a proton donor during catalysis.[49] In addition, a structural subset was specified as the ligand and the amino acid residues within a 10 Å radius of the ligand for energy minimization which eventually occurred through a two-step minimization, (i) only the ligand and the side chains of the subset to relax and then (ii) only all the atoms of the subset to relax. The energy minimization calculations were performed in SYBYL 7.1 using the Tripos force field by Powell method, a distance-dependent dielectric constant of 1*r*, and a non-bonded cutoff of 8 Å. The minimization was terminated when the energy gradient convergence criterion of 0.01 kcal/mol Å was reached.

The modeled complex structure of β-glucuronidase with the substrate *p*-nitrophenyl β-glucuronide was used for the prediction of a favorable binding mode of our newly synthesized compounds to β-glucuronidase by docking (Table 2).

The structures of the ligands were constructed using standard bond lengths and angles from the SYBYL7.3 fragment library. Partial charges were assigned according to the Gasteiger–Hückel method.[55] Geometry optimizations were performed using the Tripos force field[53] with a distance-dependent dielectric and the Powell conjugate gradient algorithm.

3. Conclusions

In conclusion we have synthesized 26 benzothiazole derivatives and tested their inhibitory potential against β-glucuronidase. Seven compounds were identified as potent β-glucuronidase inhibitors. Compounds **18**, **16**, **13**, **8**, **4**, and **5** exhibited a significant activity with IC_{50} values 2.2–36.1 μM. While compound **9** (IC_{50} = 94.0 ± 4.16 μM) is found to be the least active among the series. Subsequently molecular docking strategy was carried out to understand the binding pattern of these compounds. The binding mode analysis revealed that the hydrophilic character of the compound is responsible for the biological activity. All potent inhibitors were subjected for cytotoxicity assay, none of the analogs showed any cytotoxic effect. The proposed scaffold of β-glucuronidase inhibitors offers the possibility of convenient further modifications that could give rise to lead structures with improved inhibitory activity and selectivity towards the enzyme.

3.1. Cytotoxicity assays using 3T3-L1 and CC-1 cell-lines and MTT

In vitro cytotoxicity assays were performed as described by Scholz et al.[56], using the 3T3-L1 mouse embryo fibroblast cell line (American Type Culture Collection 'ATCC', Manassas, VA 20108, USA), and CC-1 cells, a rat Wistar hepatocyte cell line (European Collection of Cell Cultures, Salisbury, UK). The CC-1 cells were suspended in Minimum Essential Medium Eagle (MEM) supplemented with 10% FBS, 2 mM glutamine, 1% non-essential amino acids and, 20 mM HEPES. While the 3T3-L1 cells were suspended in Dulbecco's Modified Eagle's Medium (DMEM) formulated with 10% FBS. Using flat bottomed plates, both cell-lines were plated at a concentration of 6×10^4 cells/mL and incubated for 24 h at 37 °C and 5% CO_2 environment. After removal of media, cells were challenged with three different concentrations (1.0, 5.0, and 20 μg/mL) of compounds in triplicates and were then further incubated for 48 h at 37 °C in CO_2 incubator. Following exposure to each compound, cells viability was assessed by using 0.5 mg/mL of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) for 4 h followed by removal of supernatant and addition of DMSO to solubilize the formazan complex. Plates were read at 540 nm after one minute shaking

and readings were processed using MS Excel software. Results were expressed as means ± SD of triplicate readings.

4. Material and methods

NMR experiments were performed on Avance Bruker AM 300 MHz machine. CHN Analysis was performed on a Carlo Erba Strumentazion-Mod-1106, Italy. Ultraviolet (UV) spectra were recorded on Perkin–Elmer Lambda-5 UV/vis spectrophotometer in MeOH. Infrared (IR) spectra were recorded on JASCO IR-A-302 spectrometer as KBr (disc). Electron impact mass spectra (EI MS) were recorded on a Finnigan MAT-311A (Germany) mass spectrometer. Thin layer chromatography (TLC) was performed on pre-coated silica gel aluminum plates (Kieselgel 60, 254, E. Merck, Germany). Chromatograms were visualized by UV at 254 and 365 nm.

4.1. General procedure for the synthesis of compounds 1–26

In a typical procedure, benzothiazoles **1–26** were synthesized by mixing together commercially available 2-aminothiophenol (3.12 mmol) and different aromatic aldehydes (3.16 mmol) in *N*,*N*-dimethylformamide (DMF) 10 mL, sodium metabisulfite $Na_2S_2O_5$ (0.61 g) was added to a stirring mixture. The reaction mixture was refluxed for 2 h and the progress of the reaction was monitored by TLC. After completion of the reaction, mixture was allowed to cool to room temperature, addition of water (30 mL), product which precipitated as a solid, after filtration afforded the benzothiazole derivatives **1–26** in high yields. Recrystallization from methanol afforded pure product.

4.1.1. 2-(1,3-Benzothiazol-2-yl)-6-ethoxyphenol (1)

^{1}H NMR: (500 MHz, CD_3OD): δ 8.00 (m, 2H, H-4/4′), 7.56 (td, 1H, $J_{5,7}$ = 1.0, $J_{5(6,4)}$ = 8.0 Hz, H-5), 7.48 (dd, 1H, $J_{6',4'}$ = 1.0 Hz, $J_{6',5'}$ = 8.0 Hz, H-6′), 7.45 (td, 1H, $J_{6,4}$ = 1.0 Hz, $J_{6(7,5)}$ = 7.0 Hz, H-6), 7.10 (dd, 1H, $J_{7,5}$ = 1.0 Hz, $J_{7,6}$ = 8.0 Hz, H-7), 6.93 (t, 1H, $J_{5'(6',4')}$ = 8.0 Hz, H-5′), 4.18 (q, 2H, J = 7.0 Hz, –OCH_2), 1.45 (t, 3H, J = 7.0 Hz, –CH_3); MS: *m/z* (rel. abund.%), 271 (M^+, 42), 214 (100), 186 (82), 160 (33), 109 (42), 144(79), 69 (64), 63 (47).

4.1.2. 2-(1,3-Benzothiazol-2-yl)-6-methoxyphenol (2)

^{1}H NMR: (500 MHz, CD_3OD): δ 7.94 (s, 1H, H-4), 7.93 (s, 1H, H-7), 7.7 (d, 1H, $J_{2',6'}$ = 2.0 Hz, H-2′), 7.52 (dd, 1H, $J_{7,5}$ = 2.0, $J_{7,6}$ = 7.5 Hz, H-6′), 7.49 (dd, 1H, $J_{5,7}$ = 2.0, $J_{5,6}$ = 7.5 Hz, H-5), 7.38 (td, 1H, $J_{6,4}$ = 1.0, $J_{6(5,7)}$ = 7.5 Hz, H-6), 6.91 (d, 1H, $J_{5',6'}$ = 8.0 Hz, H-5′), 3.97 (s, 3H, –OCH_3); MS: *m/z* (rel. abund.%), 257 (M^+, 100), 213 (58), 185 (32), 159 (7), 107 (23), 81(18), 68 (43).

4.1.3. 2-(1-Naphthyl)-1,3-benzothiazole (3)

^{1}H NMR: (500 MHz, CD_3OD): δ 8.59 (d, 1H, $J_{1',3'}$ = 1.5 Hz, H-1′), 8.21 (dd, 1H, $J_{5',7'}$ = 2.0, $J_{5',6'}$ = 8.5 Hz, H-5′), 8.02 (m, 4H, H-4/7/4′/7′), 7.94 (t, 1H, $J_{3'(2',4')}$ = 8.5 Hz, H-3′), 7.58 (m, 2H, H-2′/6′), 7.55 (td, 1H, $J_{5,7}$ = 1.5, $J_{5(4,6)}$ = 8.0 Hz, H-5), 7.44 (td, 1H, $J_{6,4}$ = 1.0, $J_{6(5,7)}$ = 8.0 Hz, H-6); MS: *m/z* (rel. abund.%), 261 (M^+, 100), 130 (16), 108 (24), 69 (23).

4.1.4. 4-(1,3-Benzothiazol-2-yl)phenol (4)

Mp 220–224 °C, ^{1}H NMR: (500 MHz, CD_3OD): δ 7.92 (m, 4H, H-4/7/2′/6′), 7.48 (td, 1H, $J_{5,7}$ = 1.0, $J_{5(6,4)}$ = 7.0 Hz, H-5), 7.39 (td, 1H, $J_{6,4}$ = 1.0, $J_{6(7,5)}$ = 7.0 Hz, H-6), 6.90 (d, 2H, $J_{3',2'/5',6'}$ = 9.0 Hz, H-3′/5′); MS: *m/z* (rel. abund.%), 227 (M^+, 100), 198 (7), 107 (38), 69 (30), 63 (11).

4.1.5. 3-(1,3-Benzothiazol-2-yl)phenol (5)

Mp 130–132 °C, ^{1}H NMR: (500 MHz, CD_3OD): δ 7.99 (m, 2H, H-4′/4), 7.53 (m, 3H, H-5/7/2′), 7.44 (td, 1H, $J_{6,4}$ = 1.0, $J_{6(5,7)}$ = 8.0 Hz, H-6), 7.35 (t, 1H, $J_{5'(4',6')}$ = 8.0 Hz, H-5′), 6.97 (dd, 1H, $J_{7,5}$ = 1.0, $J_{7,6}$ = 8.0, $J_{7,6}$ = 2.5 Hz, H-6′); MS: *m/z* (rel. abund.%), 227 (M^+, 100), 198 (13), 173 (4), 113 (17), 108 (60), 84 (17), 69 (65), 63 (29).

4.1.6. 2-(1,3-Benzothiazol-2-yl)-4-chlorophenol (6)

^{1}H NMR: (500 MHz, CD_3OD): δ 8.04 (s, 1H, H-4), 8.02 (s, 1H, H-7), 7.90 (d, 1H, $J_{6',4'}$ = 2.5 Hz, H-6′), 7.57 (td, 1H, $J_{5,7}$ = 1.5 Hz, $J_{5(4,6)}$ = 8.0 Hz, H-5), 7.47 (td, 1H, $J_{6,4}$ = 1.0 Hz, $J_{6(5,7)}$ = 8.0 Hz, H-6), 7.37 (dd, 1H, $J_{4',6'}$ = 2.5 Hz, $J_{4',3'}$ = 9.0 Hz, H-4′), 7.04 (d, 1H, $J_{3',4'}$ = 9.0 Hz, H-3′); MS: *m/z* (rel. abund.%), 261 (M^+, 100), 198 (21), 108 (6).

4.1.7. 2-(2-Methylphenyl)-1,3-benzothiazole (7)

^{1}H NMR: (500 MHz, CD_3OD): δ 7.95 (m, 2H, H-4, 7), 7.72 (d, 1H, $J_{6',5'}$ = 7.5 Hz, H-6′), 7.56 (dt, 1H, $J_{5,7}$ = 1.0, $J_{5(4,6)}$ = 8.0 Hz, H-5), 7.39 (td, 1H, $J_{6,4}$ = 1.0, $J_{6(5,7)}$ = 8.0 Hz, H-6), 7.42 (m, 2H, H-3′,5′), 7.34 (td, 1H, $J_{4',6'}$ = 1.0, $J_{4'(3',5')}$ = 7.3 Hz, H-4′), 2.59 (s, 3H, CH_3); MS: *m/z* (rel. abund.%), 225 (M^+, 100), 107 (18), 88 (20).

4.1.8. 2-(1,3-Benzothiazol-2-yl)-1,4-benzenediol (8)

Mp 192–194 °C, ^{1}H NMR: (500 MHz, CD_3OD): δ 7.99 (d, 2H, $J_{4,5}$ = $J_{7,6}$ = 8.0 Hz, H-4/7), 7.54 (td, 1H, $J_{5,7}$ = 1.0, $J_{5(4,6)}$ = 8.0 Hz, H-5), 7.44 (td, 1H, $J_{6,4}$ = 1.0, $J_{6(5,7)}$ = 8.0 Hz, H-6), 7.23 (d, 1H, $J_{6',4'}$ = 1.0 Hz, H-6′), 6.88 (s, 2H H-3′/4′); MS: *m/z* (rel. abund.%), 242 (M^+, 100), 185 (49), 108 (15).

4.1.9. 4-(1,3-Benzothiazol-2-yl)-1,2-benzenediol (9)

Mp 189–191 °C, ^{1}H NMR: (500 MHz, CD_3OD): δ 7.93 (d, 1H, $J_{4,5}$ = 8.0 Hz, H-4), 7.92 (d, 1H, $J_{7,6}$ = 10 Hz, H-7), 7.52 (d, 1H, $J_{2',6'}$ = 2.0 Hz, H-2′), 7.49 (dt, 1H, $J_{5,7}$ = 1.5, $J_{5(4,6)}$ = 8.0 Hz, H-5), 7.44 (dd, 1H, $J_{6',2'}$ = 2.5, $J_{6',5'}$ = 8.0 Hz, H-6′), 7.38 (td, 1H, $J_{6,4}$ = 1.0 Hz, $J_{6(5,7)}$ = 8.0 Hz, H-6), 6.40 (d, 1H, $J_{5',6'}$ = 8.0 Hz, H-5′); MS: *m/z* (rel. abund.%), 242 (M^+, 100), 185 (14), 108 (17), 69 (16).

4.1.10. 3-(1,3-Benzothiazol-2-yl)-1,2-benzenediol (10)

^{1}H NMR: (500 MHz, CD_3OD): δ 8.00 (dd, 2H, $J_{4,6/7,5}$ = 1.5, $J_{4,5/7,6}$ = 8.0 Hz, H-4/7), 7.55 (td, 1H, $J_{5,7}$ = 1.0, $J_{5(4,6)}$ = 8.0 Hz, H-5), 7.46 (td, 1H, $J_{6,4}$ = 0.5, $J_{6(5,7)}$ = 8.0 Hz, H-6), 7.31 (dd, 1H, $J_{6',4'}$ = 1.5, $J_{6',5'}$ = 8.0 Hz, H-6′), 6.95 (dd, 1H, $J_{4',6'}$ = 1.5, $J_{4',5'}$ = 8.0 Hz, H-4′), 7.04 (t, 1H, $J_{5'(4',6')}$ = 8.0 Hz, H-5′); MS: *m/z* (rel. abund.%), 242 (M^+, 100), 185 (43), 108 (10).

4.1.11. 2-(2,4-Dichlorophenyl)-1,3-benzothiazole (11)

^{1}H NMR: (300 MHz, CD_3OD): δ 8.35 (d, 1H, $J_{6',5'}$ = 8.7 Hz, H-6′), 8.16 (dd, 1H, $J_{4,6}$ = 1.0, $J_{4,5}$ = 7.2 Hz, H-4), 8.12 (dd, 1H, $J_{7,5}$ = 1.0, $J_{7,6}$ = 8.7 Hz, H-7), 7.75 (d, 1H, $J_{3',5'}$ = 2.1 Hz, H-3′), 7.60 (m, 3H, H-5′/5/6), MS: *m/z* (rel. abund.%), 279 (M^+, 100), 108 (85), 69 (90).

4.1.12. 2-(3,4-Dimethoxyphenyl)-1,3-benzothiazole (12)

^{1}H NMR: (500 MHz, CD_3OD): δ 7.96 (m, 2H, H-4/7), 7.70 (d, 1H, $J_{2',6'}$ = 2.0 Hz, H-2′), 7.62 (dd, 1H, $J_{6',2'}$ = 2.0, $J_{6',5'}$ = 8.5 Hz, H-6′), 7.51 (td, 1H, $J_{5,7}$ = 1.0, $J_{5(4,6)}$ = 7.0 Hz, H-5), 7.41 (td, 1H, $J_{6,4}$ = 1.0, $J_{6(5,7)}$ = 7.0 Hz, H-6), 7.08 (d, 1H, $J_{5',6'}$ = 8.5 Hz, H-5′), 3.94 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3); MS: *m/z* (rel. abund.%), 271 (M^+, 100), 185 (25), 108 (9).

4.1.13. 4-(1,3-Benzothiazol-2-yl)-1,3-benzenediol (13)

Mp 188–190 °C, ^{1}H NMR: (500 MHz, CD_3OD): δ 7.95 (d, 1H, $J_{4,5}$ = 7.5 Hz, H-4), 7.91 (d, 1H, $J_{7,6}$ = 8.0 Hz, H-7), 7.62 (d, 1H, $J_{6',5'}$ = 8.0 Hz, H-6′), 7.50 (dt, 1H, $J_{5,7}$ = 1.5, $J_{5(4,6)}$ = 8.5 Hz, H-5), 7.39 (td, 1H, $J_{6,4}$ = 1.0, $J_{6(5,7)}$ = 8.0 Hz, H-6), 6.43 (dd, 1H, $J_{5',3'}$ = 2.0, $J_{5',6'}$ = 8.0 Hz, H-5′), 6.40 (d, 1H, $J_{3',5'}$ = 2.0 Hz, H-3′); MS: *m/z* (rel. abund.%), 242 (M^+, 100), 185 (43), 108 (10).

4.1.14. 4-(1,3-Benzothiazol-2-yl)-*N*,*N*-dimethylaniline (14)

^{1}H NMR: (500 MHz, CD_3OD): δ 7.47 (m, 4H, H-4/7/2′/6′), 7.49 (dt, 1H, $J_{5,7}$ = 1.0, $J_{5(4,6)}$ = 8.0 Hz, H-5), 7.35 (dt, 1H, $J_{6,4}$ = 1.0, $J_{6(5,7)}$ = 8.0 Hz, H-6), 6.83 (d, 2H, $J_{3',2'/5',6'}$ = 8.0 Hz, H-3′/5′), 3.06 (s,

6H, 2xCH_3); MS: *m/z* (rel. abund.%), 254 (M^+, 100), 210 (15), 127 (25), 108 (11).

4.1.15. 2-(4-Methylphenyl)-1,3-benzothiazole (15)

^{1}H NMR: (500 MHz, CD_3OD): δ 7.99 (m, 2H, H-4/7), 7.97 (d, 2H, $J_{2',3'/6',5'}$ = 8.0 Hz, H-2′/6′), 7.51 (dt, 1H, $J_{5,7}$ = 1.0, $J_{5(6,4)}$ = 7.0 Hz, H-5), 7.41 (dt, 1H, $J_{6,4}$ = 1.0, $J_{6(7,5)}$ = 7.0 Hz, H-6), 6.90 (d, 2H, $J_{3',2'/5',6'}$ = 8.0 Hz, H-3′/5′), 2.42 (s, 3H, CH_3); MS: *m/z* (rel. abund.%), 225 (M^+, 100), 108 (23), 82 (14), 69 (37).

4.1.16. 2-(1,3-Benzothiazol-2-yl)phenol (16)

^{1}H NMR: (500 MHz, CD_3OD): δ 8.02 (br d, 1H, $J_{4,5}$ = 4.5 Hz, H-4), 8.00 (dd, 1H, $J_{7,5}$ = 1.0, $J_{7,6}$ = 5.0 Hz, H-7), 7.84 (dd, 1H, $J_{6',4'}$ = 1.5, $J_{6',5'}$ = 8.0 Hz, H-6′), 7.55 (dt, 1H, $J_{5',3'}$ = 1.0, $J_{5'(4',6')}$ = 8.0 Hz, H-5′), 7.46 (td, 1H, $J_{4',6'}$ = 1.0, $J_{4'(3',5')}$ = 8.0 Hz, H-4′), 7.40 (td, 1H, $J_{5,7}$ = 1.0, $J_{5(6,4)}$ = 7.5 Hz, H-5), 7.04 (dd, 1H, $J_{3',5'}$ = 1.0, $J_{3',4'}$ = 8.0 Hz, H-3′), 7.00 (td, 1H, $J_{6,4,}$ = 1.0, $J_{6(5,7)}$ = 7.0 Hz, H-6); MS: *m/z* (rel. abund.%), 227 (M^+, 90), 199 (100), 108 (37), 69 (99).

4.1.17. 2-(3-Chlorophenyl)-1,3-benzothiazole (17)

^{1}H NMR: (500 MHz, CD_3OD): δ 8.1 (t, 1H, $J_{2'(4',6')}$ = 2.0 Hz, H-2′), 7.99 (m, 3H, H-4/7/4′), 7.53 (m, 3H, H-5/5′/6′), 7.45 (dt, 1H, $J_{6,4}$ = 1.5, $J_{6(7,5)}$ = 7.5 Hz, H-6); MS: *m/z* (rel. abund.%), 245 (M^+, 100), 210 (11), 108 (69), 82 (29).

4.1.18. 5-(1,3-Benzothiazol-2-yl)-1,2,4-benzenetriol (18)

Mp 194–196 °C, ^{1}H NMR: (500 MHz, CD_3OD): δ 7.94 (d, 1H, $J_{4,5}$ = 8.0 Hz, H-4), 7.89 (d, 1H, $J_{7,6}$ = 8.0 Hz, H-7), 7.49 (dt, 1H, $J_{5,7}$ = 1.0, $J_{5(4,6)}$ = 8.0 Hz, H-5), 7.38 (td, 1H, $J_{6,4}$ = 1.0, $J_{6(5,7)}$ = 8.0 Hz, H-6), 7.14 (s, 1H, H-6′), 6.44 (s, 1H, H-3′); MS: *m/z* (rel. abund.%), 259 (M^+, 100), 185 (14), 108 (14), 69 (31).

4.1.19. 2-(3-Pyridinyl)-1,3-benzothiazole (19)

^{1}H NMR: (300 MHz, CD_3OD): δ 9.27 (d, 1H, $J_{2',6'}$ = 3.5 Hz, H-2′), 8.76 (dd, 1H, $J_{6',4'}$ = 2.5, $J_{6',5'}$ = 8.0 Hz, H-6′), 8.47 (td, 1H, $J_{4'(6',2')}$ = 3.5, $J_{4',5'}$ = 6.0 Hz, H-4′), 8.19 (d, 1H, $J_{4,5}$ = 8.7 Hz, H-4), 8.12 (d, 1H, $J_{7,6}$ = 8.7 Hz, H-7), 7.63 (m, 3H, H-5′,5,6); MS: *m/z* (rel. abund.%), 212 (M^+, 100), 186 (22), 108 (19), 69 (46).

4.1.20. 2-(4-Pyridinyl)-1,3-benzothiazole (20)

Mp 138–140 °C, ^{1}H NMR: (300 MHz, CD_3OD): δ 8.79 (m, 2H, H-2′/6′), 8.24 (br d, 1H, $J_{4,5}$ = 7.5 Hz, H-4), 8.15 (br d, 1H, $J_{7,6}$ = 7.2 Hz, H-7), 8.02 (m, 2H, H-3′/5′), 7.63 (dt, 1H, $J_{5,7}$ = 1.2, $J_{5(4,6)}$ = 7.2 Hz, H-5), 7.58 (dt, 1H, $J_{6,4}$ = 1.2, $J_{6(5,7)}$ = 7.2 Hz, H-6); MS: *m/z* (rel. abund.%), 212 (M^+, 100), 186 (13), 108 (25), 69 (40).

4.1.21. 2-(4-Bromophenyl)-1,3-benzothiazole (21)

^{1}H NMR: (500 MHz, CD_3OD): δ 8.02 (m, 2H, H-4/7), 7.99 (d, 2H, $J_{3',2'/5',6'}$ = 9.0 Hz, H-3′/5′), 7.70 (d, 2H, $J_{2',3'/6',5'}$ = 9.0 Hz, H-2′/6′), 7.53 (dt, 1H, $J_{5,7}$ = 1.0 Hz, $J_{5(6,4)}$ = 8.0 Hz, H-5), 7.43 (dt, 1H, $J_{6,4}$ = 1.0, $J_{6(7,5)}$ = 8.0 Hz, H-6); MS: *m/z* (rel. abund.%), 291 (M^+, 79), 289 (100), 210 (33), 108 (63).

4.1.22. 2-(4-Ethoxyphenyl)-1,3-benzothiazole (22)

^{1}H NMR: (500 MHz, CD_3OD): δ 8.00 (m, 2H, H-4/7), 7.95 (d, 2H, $J_{3',2'/5',6'}$ = 9.0 Hz, H-3′/5′), 7.49 (dt, 1H, $J_{5,7}$ = 1.0 Hz, $J_{5(6,4)}$ = 7.0 Hz, H-5), 7.38 (dt, 1H, $J_{6,4}$ = 1.0, $J_{6(7,5)}$ = 7.0 Hz, H-6), 7.95 (d, 2H, $J_{2',3'/6',5'}$ = 9.0 Hz, H-2′/6′), 3.01 (q, 2H, J = 7.0 Hz, OCH_2), 3.01 (t, 3H, J = 7.0 Hz, CH_3); MS: *m/z* (rel. abund.%), 255 (M^+, 68), 227 (100), 198 (25), 108 (28).

4.1.23. 2-(2-Fluorophenyl)-1,3-benzothiazole (23)

^{1}H NMR: (400 MHz, CD_3OD): δ 8.40 (dt, 1H, $J_{5,7}$ = 1.6, $J_{5(6,4)}$ = 8.0 Hz, H-5), 8.11 (d, 1H, $J_{4,5}$ = 8.0 Hz, H-4), 7.93 (d, 1H, $J_{7,6}$ = 8.0 Hz, H-7), 7.43 (m, 3H, H-6/3′/6′), 7.24 (m, 2H, H-4′/5′); MS: *m/z* (rel. abund.%), 229 (M^+, 100), 108 (71), 69 (62).

4.1.24. 2-(2-Thienyl)-1,3-benzothiazole (24)

^{1}H NMR: (500 MHz, CD_3OD): δ 8.18 (m, 1H, H-4′), 7.97 (d, 2H, $J_{4,5/7,6}$ = 9.0 Hz, H-4/7), 7.72 (dd, 1H, $J_{3',5'}$ = 1.5, $J_{3',4'}$ = 6.5 Hz, H-3′), 7.60 (dd, 1H, $J_{5',3'}$ = 2.0, $J_{5',4'}$ = 6.5 Hz, H-5′), 7.51 (dt, 1H, $J_{5,7}$ = 1.0, $J_{5(6,4)}$ = 7.5 Hz, H-5), 7.51 (t, 1H, $J_{6(5,7)}$ = 7.5 Hz, H-6); MS: *m/z* (rel. abund.%), 216 (M^+, 100), 108 (45), 81 (33), 69 (77).

4.1.25. 2-(4-Chlorophenyl)-1,3-benzothiazole (25)

^{1}H NMR: (500 MHz, CD_3OD): δ 8.09 (d, 2H, $J_{3',2'/5',6'}$ = 8.5 Hz, H-3′/5′), 8.02 (d, 1H, $J_{4,5}$ = 7.5 Hz, H-4), 7.93 (d, 1H, $J_{7,6}$ = 7.5 Hz, H-7), 7.55 (d, 2H, $J_{2',3'/6',5'}$ = 8.5 Hz, H-2′/6′), 7.53 (m, 1H, H-5), 7.43 (dt, 1H, $J_{6,4}$ = 1.0, $J_{6(7,5)}$ = 7.5 Hz, H-6); MS: *m/z* (rel. abund.%), 244 (M^+, 64), 210 (8), 108 (65), 69 (100).

4.1.26. 2-(3,4-Dichlorophenyl)-1,3-benzothiazole (26)

^{1}H NMR: (500 MHz, CD_3OD): δ 8.27 (d, 1H, $J_{2',6'}$ = 2.5 Hz, H-2′), 8.05 (m, 3H, 4/7 6′), 7.70 (d, 1H, $J_{5',6'}$ = 8.5 Hz, H-5′), 7.55 (dt, 1H, $J_{5,7}$ = 1.0, $J_{5(4,6)}$ = 7.0 Hz, H-5), 7.45 (dt, 1H, $J_{6,4}$ = 1.0, $J_{6(5,7)}$ = 7.0 Hz, H-6); MS: *m/z* (rel. abund.%), 279 (M^+, 98), 244 (22), 108 (77), 82 (41).

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