RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

Synthesis of Novel Inhibitors of β-Glucuronidase Based on the Benzothiazole Skeleton and their Molecular Docking Studies

Muhammad Taha[*a,b], Nor Hadiani Ismail[a,b], Syahrul Imran[a,b], Manikandan Selvaraj[c,d], Fazal Rahim[e]

[a]*Atta-ur-Rahman Institute for Natural Product Discovery, Universiti Teknologi MARA (UiTM), Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor, Malaysia*
[b]*Faculty of Applied Science UiTM, 40450 Shah Alam, Selangor, Malaysia*
[c]*Integrative Pharmacogenomics Institute (iPROMISE), Universiti Teknologi MARA (UiTM), Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor Darul Ehsan, Malaysia*
[d]*Faculty of Pharmacy, Universiti Teknologi MARA (UiTM), Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor Darul Ehsan, Malaysia*
[e]*Department of Chemistry, Hazara University, Mansehra-21120, Pakistan*

Abstract

A series of benzothiazole based oxadiazole analogs **1-20** was synthesized by reacting intermediate sulfite adduct with 2-aminothiophenol upon refluxing in DMF for 12 h to afford ester analog **I** which on further refluxing in methanolic hydrazine hydrate solution to afford compound **II**. Compound **II** was then condensed with different aromatic carboxylic acids in $POCl_3$ to synthesize novel benzothiazole based oxadiaxole derivatives **1-20** in good yields. All compounds were screened for β-glucuronidase inhibitory potential. Compounds **7**, **14**, **8** and **17** were found to be the most active analogs among the series with micromolar activities (IC_{50} = 2.16, 4.38, 7.20 and 8.56 μM respectively). While compounds **5**, **10**, **18**, **16**, **1**, **2**, **15**, **11**, and **20** showed moderate activity with IC_{50} value ranging between 14.12-75.14 μM), whereas compounds **3**, **12**, **13**, and **19** were found to be inactive. Further studies showed that they do not possess any cytotoxic properties. Molecular docking studies were done to reveal the binding modes of the synthetic benzothiazole derivatives **1-20** targeting the active site of β-glucuronidase (PDB code: 1BHG).

Keywords: benzothiazole, β-glucuronidase, Oxadiazole, Docking studies

* Correspondence and reprints
E-mail: taha_hej@yahoo.com and muhamm9000@puncakalam.uitm.edu.my, Tel: 0060193098141

1. Introduction

β-Glucuronidase enzyme is present in lysosomes and microsomes tissues, and excretes normally in urine along with cells of the urinary tract [1]. *β*-Glucuronidase is an exoglycosidase enzyme related with breakage of glucuronosyl-*O*-bonds [2]. It is present in different body parts like, body fluids, liver, spleen, kidney, gastric juice, blood cells, bile, lung, urine, muscle, and serum. [3,4]. In some diseases like hepatic diseases, cancer, inflammatory joint, and AIDS, activity of *β*-glucuronidase increases [5]. Endogenous biliary *β*-glucuronidase is related with deconjugation of glucuronides of bilirubin which in terns produce cholelithiasis in human bile [6]. Amplified level of this enzyme has been related with different ailments of urinary tract, such as active pyelonephritis, acute renal necrosis and cancer of the kidney and bladder [7]. In contrast to other enzymes *E. coli* *β*-glucuronidase has higher pH [8]. Deficiency of *β*-glucuronidase in humane has resulted in mucopolysaccharidosis type VII (MPS VII; Sly syndrome), which is characterized by growth of glycosaminoglycans in cells of most tissues [9,10]. Leprosy is a disease found in children of less than 15 years of age caused by *β*-glucuronidase.

The benzothiazole skeleton constitutes an important central template for a wide variety of biologically active compounds, having many pharmacological functions. The benzothiazoles also have immunosuppressive, antiviral properties [11,12], and calmodulin (CaM) antagonists activity [13]. Extensive literature search reveals the antimalarial [14], antifungal [15], anti-inflammatory, analgesic [16], anticandidous [17] and various CNS activities [18] of benzothiazoles.

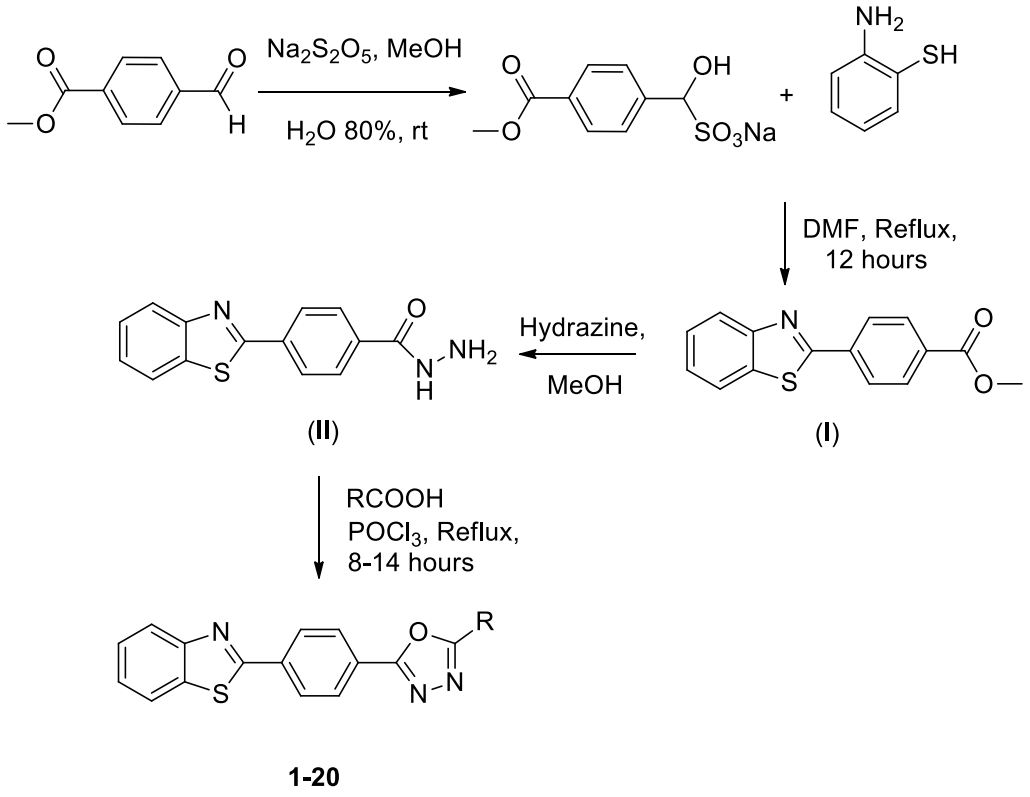
Much attention had been paid to the chemistry and biological activities of 1,3,4-oxadiazole nucleus. Several compounds possessing 1,3,4-oxadiazole scaffold have been recently reported as potential antiproliferative agents [19-21]. Beside anticancer activity, other biological activities have been reported for 1,3,4-oxadiazole derivatives such as antidiabetic [22], antitubercular [23], antifungal [24], anti-inflammatory [25], and antibacterial activities [26]. Moreover, many sulfonamide analogs have been reported as anticancer agents [27-29].

Here in this study we are reporting on synthesis of the hybrid molecule of benzothiazole and oxadiazole, its characterization, *β*-Glucuronidase inhibition and molecular docking studies.

2. Result discussion

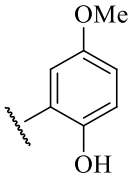
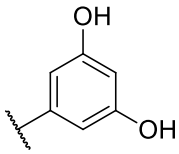
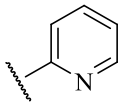
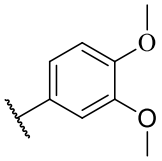
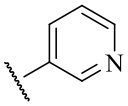
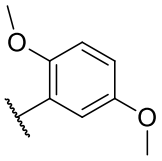
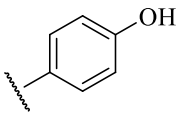
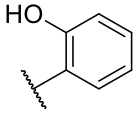
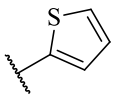
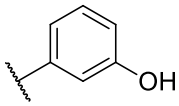
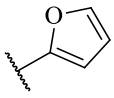
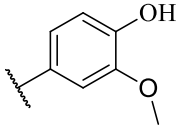
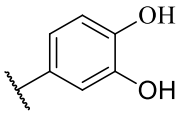
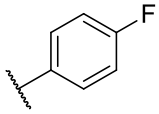
2.1 Chemistry

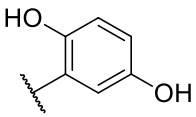
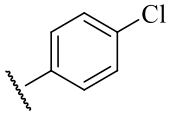
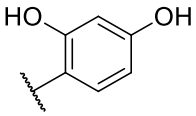
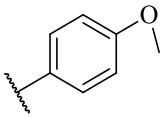
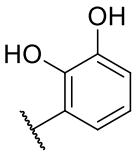
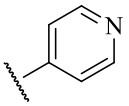
A series of benzothiazole was synthesized according to literature protocol [30]. The intermediate sulfite adduct was refluxed with 2-aminothiophenol in dimethylformamide for 12 h to afford compound **I**. The compound **I** was refluxed in methanolic hydrazine hydrate solution to afford compound **II**. Compound **II** was then condensed with different aromatic carboxylic acids in $POCl_3$ to synthesize novel benzothiazole derivatives **1-20** in good yields [31]. Spectral data including ^{1}H-NMR, ^{13}C NMR, HR MS, elemental analysis and melting points of the synthesized compounds were recorded Scheme-1.



Scheme-1 Synthesis of novel benzothiazole derivatives 1-20

Table 1. β-Glucuronidase inhibitory potential of novel benzothiazole derivatives 1-20.

Comp.	R	IC_{50} ± SEM[a] [μM]	Comp.	R	IC_{50} ± SEM[a] [μM]
1		**18.38 ± 0.60**	**11**		**39.15 ± 1.25**
2		**31.21 ± 0.75**	**12**		**N. A.[b]**
3		**N. A.[b]**	**13**		**N. A.[b]**
4		**14.12 ± 0.48**	**14**		**4.38 ± 0.20**
5		**74.37 ± 1.68**	**15**		**32.42 ± 1.10**
6		**24.17 ± 1.05**	**16**		**17.30 ± 0.70**
7		**2.16 ± 0.25**	**17**		**8.56 ± 0.49**

8		**7.20 ± 0.35**	**18**		**15.40 ± 0.75**
9		**19.68 ± 0.95**	**19**		**N. A.[b]**
10		**14.89 ± 0.46**	**20**		**75.14 ± 2.49**
	D-Saccharic acid 1,4-lactone			**48.4 ± 1.25**	

[a]SEM is the standard error of the mean, [b]N.A. No activity

3.0. *β*-Glucuronidase and pharmacokinetic properties evaluation

In the continuation of our effort for enzyme inhibition [32] we synthesized novel derivatives of benzothiazole **1-20**. All synthesized compounds were evaluated for *β*-glucuronidase inhibitory potential. The *β*-glucuronidase inhibitory activity performed in this study involves mixing synthesized compounds with substrate (*p*-nitrophenyl-*β*-D-glucuronide) and *β*-glucuronidase enzyme to determine the ability of the compounds synthesized to prevent *β*-glucuronidase from breaking down the substrate (*p*-nitrophenyl-*β*-D-glucuronide) into *β*-D-glucuronide. Compounds **7**, **14**, **8** and **17** were found to be the most active analogs among the series with IC_{50} ranging 2.16-8.56 μM respectively. Whereas compound **5**, **10**, **18**, **16**, **1**, **11**, **2**, **15**, and **20** showed good to moderate activity with IC_{50} value ranging between 14.12-75.14 μM. On the other hand compound **3**, **12**, **13,** and **19** were found inactive (**Table 1**). Further studies showed that all compounds were not cytotoxic.

The twenty benzothiazole derivatives **1-20** were optimized using Ligprep and their pharmacokinetic properties were evaluation using QikProp for three descriptors: the predicted aqueous solubility (QP log S), the predicted octanol/water partition coefficient (QP logP), and

the predicted Caco-2 cell permeability (QPPcaco) considering their recommended range (**Table 2**).

Table 2: Optimization of novel benzothiazole derivatives 1-20

Compounds	QPlog P $_{o/w}$[a]	QPlog S[b]	QP P_{caco}[c] [nms^{-1}]
1	4.501	- 6.698	1027.072
2	4.204	-5.942	1531.164
3	3.888	-5.621	1310.952
4	4.319	-6.541	733.849
5	4.843	-6.472	2339.252
6	4.168	-5.544	2180.364
7	3.622	-6.229	265.417
8	3.664	-6.199	312.746
9	3.664	-6.2	312.305
10	3.722	-6.177	375.562
11	3.569	-6.253	223.569
12	5.141	-6.965	2420.136
13	5.083	-6.835	2375.661

14	4.414	-6.484	1029.629
15	4.319	-6.539	735.78
16	4.42	-6.839	731.947
17	5.184	-6.918	2422.332
18	5.45	-7.312	2422.529
19	5.002	-6.688	2421.15
20	3.886	-5.618	1308.768

[a] Predicted octanol/water partition coefficient (QP log $P_{o/w}$): range of recommended values= - 2.0 - + 6.5.
[b] Predicted aqueous solubility (QP log S): values less than -6 or greater than -1 are undesirable.
[c] Predicted apparent Caco-2 cell permeability (QP P_{caco}): value < 25 is poor.

3.1. Molecular docking studies

Molecular docking studies were done to reveal the binding modes of the **1-20** synthetic benzothiazole derivatives targeting the active site of *β*-glucuronidase (PDB code: 1BHG). In compound **7** the hydroxyl group at meta and para position in the phenyl ring forms hydrogen bond with Trp528, Gln524 and Asn486 respectively. While the ring forms hydrophobic interaction with Tyr487 and Leu501. The phenyl-oxadiazole group forms hydrophobic interaction via weak van der Waals interactions with side chains non-polar group of His509, Tyr505, Tyr508, Asn484, Ser503 and Asn502. The thiazole group of the benzothiazole ring forms π- π interaction with Tyr504, while the benzene ring forms cation-π interaction with

Lys606. Additionally, the benzothiazole ring forms hydrophobic mediated van der Waals contact with side chains of Arg600, Asp207, Glu451 and Glu540 (**Figure-1a)**.

Compound **14** being the second most active compound, forms stable interaction with active site residues of β-D-glucuronidase. Where, the benzene ring of the benzothiazole ring and the benzene ring of the other end of the compound **14** form π- π interaction with Tyr505 and Trp587, respectively. While the hydroxyl group attached to the benzene ring forms hydrogen bond interaction with Tyr508 and non-polar side chains of Lys606, Glu451, Asn450, Asp207, His385, Asn604 and Glu540 form albeit weak van der Waals interactions with phenyl-oxadiazole. Similar hydrophobic interactions are also found between the benzothiazole ring and with side chains of Gln524, Ser503, His509, Ser503 Asn484, Asn502 and Tyr504 as in **Figure-1b**.

Compound **8** is the third most active compound among the benzothiazole-phenyl-oxadiazole derivatives and the predicted preferred bind pose of compound **8** in the active site of β-D-glucuronidase showed that the benzothiazole ring system forms π-π interaction with Tyr504, while the same ring system forms van der Waals interaction with non-polar side chains of residues such as Tyr508, Arg600, Asp207, Lys606, Trp587, Glu540, Glu451, Asn484. Additionally, the middle benzene ring of the compound **8** forms similar van der Waals interaction with side chains of Ser503, His509, Asn502, and Tyr508. The phenyl-oxadiazole group at the other end forms non-polar contacts through weak van der Waals interactions with side chains of Leu501, Tyr487 and Tyr505.While the meta hydroxyl group forms hydrogen bond interaction with Gln524 and Trp528 (**Figure-1c)**.

Compound **17** is the fourth most active compound among the synthesized benzothiazole-phenyl-oxadiazole derivatives. The predicted binding mode of compound **17** showed that the flurophenyl oxadiazole forms weak hydrophobic interaction with Leu501, Tyr505, Trp528, Tyr487 and van der Waals interactions with Gln524, Asn486, Ser485, Asn484, Ser505 and Asn502. The thiazole group of the benzothiazole ring forms π-π interaction with Tyr504, while the benzene ring forms cation-π interaction with Lys606 similar as that observed in compound **7**. Furthermore, the central benzene ring forms van der Waals interaction with non-polar part of Glu451 and Tyr508. While the benzothiazole ring system was involved in weak hydrophobic contacts with side chains of Arg600, Glu540 and Asp207 as shown in **Figure-1d**.

Binding mode analysis shows that the inactivity of these compounds largely depends on the hydrophobic substituted group, while the active site residues are of hydrophilic in nature. The binding mode of the potent compounds illuminates the activity profile, where the hydrophilic nature of these compounds form stable hydrogen bond network with the key hydrophilic residues in the active site. Consistently, the activity profile of the compounds directly depends on magnitude of hydrogen bonding.

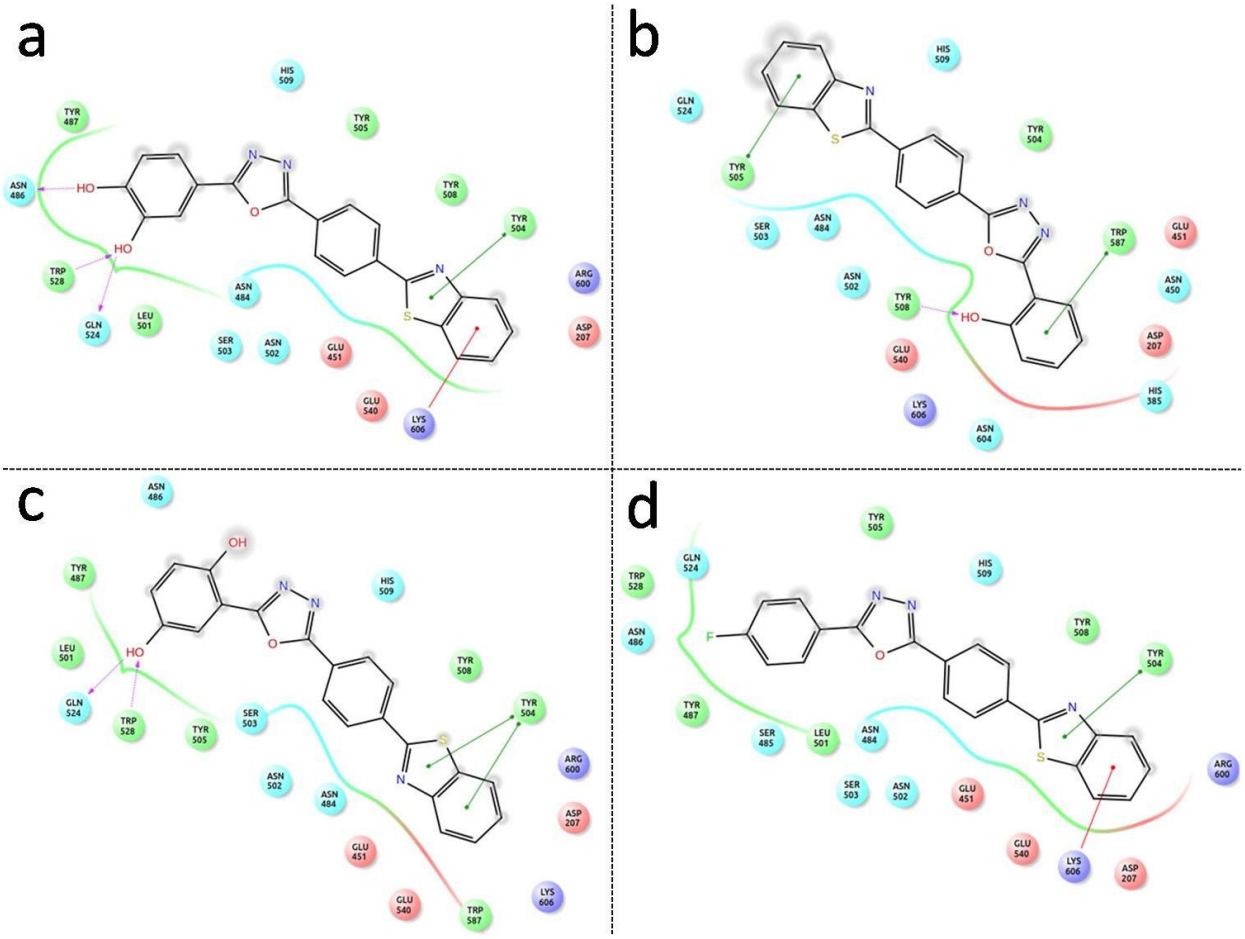


Figure1: Graphical illustration of predicted binding modes of active benzothiazole derivatives.

Binding modes of ***a.*** *compound* ***7*** *shown in stick form,* ***b.*** *Compound* ***14***, ***c.*** *Compound* ***8*** *and* ***d.*** *Compound* ***17****. Key residues are represented in spear and labeled. The hydrogen bond interactions are represented by magenta arrow, π-π interactions are shown in green color lines and cation-π interactions are shown in red color lines. Compounds are represented in line form.*

Conclusions

Successively, our molecular docking studies evidently demonstrate the interaction pattern of our synthetic compounds in correspondence with β-glucuronidase inhibitory assay. Analysis of the binding mode clearly shows the presence of hydrophilic group i.e. hydroxyl moiety plays a key

role in the activity data. Additionally, π-π and cation-π interaction along with hydrophobic interaction do also influence the activity profile. The compounds were not showing any cytotoxicity activity.

3. Experimental

3.1 General

NMR experiments were performed on UltraShield Bruker FT NMR 500 MHz, CHN analyses were performed on a Carlo Erba Strumentazion-Mod-1106, Italy. 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.

3.2 *β*-glucuronidase inhibition activity

β-glucuronidase (E.C. 3.2.1.31 from bovine liver, G-0251) inhibition activity of the compounds had been evaluated by using method as reported in [33]. In this study, *D*-saccharic acid 1,4-lactone had been used as a standard drug [34].

3.3 Cytotocity

In vitro cytotoxicity assays were performed as described by Taha *et al.* (2015)[35]. Briefly, the cells (1x104/well) were seeded in 96-well microtiter plates (Nunc) and allowed to grow for 24 h before treatment. After 24 h of incubation, the cells were treated with six different concentrations (0.1-100 μg/mL) of test compounds, in three replicates. The plates were incubated for 72 h at 37°C in a 5% CO_2 incubator. A stock solution was obtained by dissolving the test compounds in DMSO. Further dilution to different tested concentrations were then carried out ensuring that the final concentration of DMSO in the test and control wells was not in excess of 1% (v/v). No effect due to the DMSO was observed. Doxorubicin was used as the positive control. The well containing untreated cells was the negative control. At the end of the incubation period, the media were replaced with medium containing 50 μg/mL of Neutral Red. The plates were incubated for another 3 h to allow for uptake of the vital dye into the lysosomes of viable and injured cells. After the incubation period, the media were removed and cells were washed with the neutral red washing solution. The dye was eluted from the cells by adding 200 μL of Neutral Red resorb solution and incubated for 30 min at room temperature with rapid agitation on a

microtiter plate shaker. Dye absorbance was measured at 540 nm using a spectrophotometer ELISA plate reader.

3.4 Protein Preparation for Docking

The human *β-D*-glucuronidase structure (PDB code: 1BHG) [36] was downloaded from the PDB database and optimized using protein preparation wizard in Maestro [37]. The initial crystal structure were preprocessed for assigning bond order, addition of hydrogen, adding missing disulfide bonds and deleting B-chain, co-factors and water molecules. Further the structure was refined by minimizing hydrogens of altered species, pH 7 was set and a restrained energy minimization was done with converge heavy atoms to RMSD 0.30 Å.

3.5 Ligand Preparation

All the twenty benzothiazole-phenyl-oxadiazole derivatives 3D structures were sketched using the Schrodinger Maestro [37] using build option and the structure were further optimized using Ligprep application with OPLS_2005 forcefield, generation of possible states of pH 7.0 ± 2.0.Generating possible tautomers, with all combinations of stereoisomer and with lowest energy conformation.

3.6 Rapid pharmacokinetic predictions of Benzothiazole-phenyl-oxadiazole derivatives

In silico pharmacokinetic properties were evaluated for the twenty benzothiazole derivatives using the QikProp program, implemented in Maestro [38]. Particular, three descriptors were taken into consideration for evaluation: the predicted aqueous solubility (QP log S), the predicted octanol/water partition coefficient (QP logP), and the predicted Caco-2 cell permeability (QPPcaco).

3.7 Docking studies

Docking studies were performed using Glide: A complete solution for ligand-receptor docking in small molecule drug discovery suite [37]. Initially, receptor grid generation was used to generate grid on the β-D-glucuronidase protein structure were the grid box was centered on catalytic residue Glu540 by supplying X,Y,Z coordinates: 81.875, 81.601 and 88.010 of β-D-glucuronidase with 12 Å radius respectively. The standard precision (SP) mode was chosen

during the Glide docking process and Glide gscore was considered during analysis. While, GOLD (Genetic Optimization for Ligand Docking) version 5.1 [39] was used as docking validation tool. Gold scoring function was chosen as a fitness function. The genetic algorithm (GA) with search efficiency of 100% was set. The specified centroid (x, y, z coordinates: 80.43, 84.41, 90.48) with a cavity 12 Å radius was defined as active site. Results divergent by less than 1.5 Å in ligand-all atom RMSDs were clustered together. Best clusters and top rank scored binding mode analyzed in Pymol [40].

3.8 Synthesis of methyl 4-(benzo[*d*]thiazol-2-yl)benzoate (I)

2-aminobenzenethiol (6.63 g, 53 mmol) and 4-formylbenzoic acid methyl ester (6.73 g, 41 mmol) were dissolved in DMF (40 mL). Sodium metabisulfite (7.79 g, 41 mmol) was added and reaction mixture was refluxed at 130 °C for 6 h. Then reaction mixture was poured into ice water and the solid product was filtered. Crystallization of crude product from ethanol gave methyl 4-(benzo[*d*]thiazol-2-yl)benzoate (**I**) and yielded 10.20 g (92.5 %) of White solid, R_f = 0.57 (ethyl acetate:hexane 2:8); M.p. 228 °C; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 8.26 (d, 2H, J = 8.5 Hz), 8.21 (d, 2H, J = 8.5 Hz), 8.10 (d, 1H, J = 8.0 Hz), 8.08 (d, 1H, J = 8.0 Hz), 7.61 (dt,1H, J = 6.5, J = 1.5 Hz), 7.45 (dt,1H, J = 8.0, J = 1.0 Hz), 3.98 (s, 3H, CH_3); ^{13}C-NMR (DMSO-d_6, 125 MHz): δ 52.88, 122.99, 123.71, 126.53, 127.41, 127.41, 127.95, 130.63, 130.63, 132.17, 135.25, 137.24, 153.98, 166.09, 166.45; Anal. Calcd for $C_{15}H_{11}NO_2S$, C = 66.89; H = 4.12; N 5.20; found C = 66.87; H = 4.13; N 5.22; EI MS *m/z* (% rel. abund.): 269.

3.9 Synthesis of 4-(benzo[*d*]thiazol-2-yl)benzohydrazide (II)

Mixture of compound **I** (8.07 g, 30 mmol) and hydrazine hydrate (10 mL, 95%) in ethanol (50 mL) was refluxed for 12 h. The solvent and excess of hydrazine hydrate were evaporated and the residue (**II**) was washed with water, filtered, dried, and then crystallized from ethanol and yielded White solid, R_f = 0.32 (ethyl acetate:hexane 9:1); (7.35 g, 91%). m.p. 325 °C; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 9.82 (s, 1H, NH), 8.28 (d, 2H, J = 8.5 Hz), 8.25 (d, 2H, J = 8.0 Hz), 8.11 (d, 1H, J = 8.0 Hz), 8.06 (d, 1H, J = 8.0 Hz), 7.64 (dt,1H, J = 6.5, J = 2.0 Hz), 7.52 (dt,1H, J = 8.0, J = 1.0 Hz), 3.91 (br. s, 2H, NH_2); ^{13}C-NMR (DMSO-d_6, 150 MHz): δ 122.93, 123.56, 126.33, 127.31, 127.31, 127.62, 128.48, 128.48, 135.13, 135.42, 136.05, 153.99, 165.44, 166.87;

Anal. Calcd for $C_{14}H_{11}N_3OS$, C = 62.43; H = 4.12; N 15.60; found C = 62.44; H = 4.13; N 15.62; EI MS *m/z* (% rel. abund.):269.

3.9.1 General procedure for synthesis of oxadiazole benzohydrazones (1-20)

A mixture of compound (**II**) (0.25 mmol) and various aromatic acid (0.25 mmol) in $POCl_3$ (5 ml) was refluxed for 8-14 h at 120 °C. The mixture was cooled and poured onto crushed ice. It was neutralized with $NaHCO_3$ solution and the resulting solid mass precipitated out was filtered, dried, and crystallized [17].

3.9.1.1 2-(5-(4-(benzo[d]thiazol-2-yl)phenyl)-1,3,4-oxadiazol-2-yl)-4-methoxyphenol (1)

Yellow solid. Yield: 85.1%. m.p.: 279-280 °C; ^{1}HNMR (500 MHz, DMSO-d_6): δ 10.26 (s, 1H, OH), 8.29 (d, 2H, $J_{2',6'/5',3'}$ = 8.0 Hz, H-2′,H-6′), 8.20 (d, 1H, $J_{4,5}$ = 8.0 Hz, H-4), 8.15 (d, 2H, $J_{3',5'/2',6'}$ = 8.0 Hz, H-3′,H-5′), 8.10 (d, 1H, $J_{7,6}$ = 7.5 Hz, H-7), 7.63 (dt, 1H, $J_{5,7}$ = 2.0, $J_{5(4,6)}$ = 7.5 Hz, H-5), 7.55 (dt, 1H, $J_{6,4}$ = 2.0, $J_{6(5,7)}$ = 7.5 Hz, H-6), 7.11 (d, 1H, $J_{6'',4''}$ = 2.0 Hz, H-6′′), 6.90 (dd,1H, $J_{4'',6''}$ = 2.0, $J_{4'',3''}$ = 8.0 Hz, H-4′′), 6.84 (d, 1H, $J_{3'',4''}$ = 8.0 Hz, H-3′′), 3.77 (s, 3H, OCH_3); ^{13}C-NMR (150 MHz, DMSO-d6): δ 165.37, 162.52, 154.38, 154.25, 152.79, 152.38, 137.23, 136.39, 128.47, 128.47, 126.72, 126.72, 126.54, 126.24, 125.31, 123.47, 122.60, 118.16, 116.72, 114.81, 110.68, 56.23; HREI-MS: m/z calcd for $C_{22}H_{15}N_3O_3S$ [M]+ 401.0834; Found 401.0838; Anal. Calcd for $C_{22}H_{15}N_3O_3S$, C, 65.82; H, 3.77; N, 10.47; found C, 65.81; H, 3.79; N, 10.45;

3.9.1.2 2-(4-(benzo[d]thiazol-2-yl)phenyl)-5-(pyridin-2-yl)-1,3,4-oxadiazole (2)

Brown solid. Yield: 83.3%. m.p.: 281-282 °C; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 8.69 (d, 1H, $J_{6'',5''}$ = 6.0 Hz, H-6′′), 8.30 (d, 2H, $J_{2',6'/5',3'}$ = 8.5 Hz, H-2′,H-6′), 8.20 (d, 1H, $J_{4,5}$ = 8.5 Hz, H-4), 8.17 (d, 2H, $J_{3',5'/2',6'}$ = 8.5 Hz, H-3′,H-5′), 8.10 (d, 1H, $J_{7,6}$ = 8.5 Hz, H-6), 8.00 (d, 1H, $J_{3'',4''}$ = 7.0 Hz, H-3′′), 7.91 (t, 1H, $J_{5''(6'',4'')}$ = 7.0 Hz, H-5′′), 7.64 (t, 1H, $J_{5(4,6)}$ = 6.5 Hz, H-5), 7.52 (t, 1H, $J_{6(5,7)}$ = 7.0 Hz, H-6), 7.49 (t, 1H, $J_{4''(3'',5'')}$ = 7.0 Hz, H-4′′); ^{13}C-NMR (150 MHz, DMSO-d6): δ 167.20, 165.47, 162.42, 152.48, 148.46, 142.01, 139.58, 137.43, 136.29, 128.50, 128.50, 128.50, 126.92, 126.92, 126.59, 126.44, 125.48, 123.70, 122.70, 119.84; HREI-MS: m/z calcd for

$C_{20}H_{12}N_4OS$ [M]+ 356.0732; Found 356.0735; Anal. Calcd for $C_{20}H_{12}N_4OS$, C, 67.40; H, 3.39; N, 15.72; Found C, 67.41; H, 3.40; N, 15.70;

3.9.1.3 2-(4-(benzo[d]thiazol-2-yl)phenyl)-5-(pyridin-3-yl)-1,3,4-oxadiazole (3)

Light brown solid. Yield: 75.3%. m.p.: 275-266 °C; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 8.96 (s, 1H, H-2″), 8.60 (d, 1H, $J_{6'',5''}$ = 5.0 Hz, H-6″), 8.24 (d, 2H, $J_{2',6'/5',3'}$ = 8.5 Hz, H-2′,H-6′), 8.19 (d, 1H, $J_{4,5}$ = 8.5 Hz, H-4), 8.15 (d, 1H, $J_{5'',6''}$ = 5.0 Hz, H-5″), 8.11 (d, 2H, $J_{3',5'/2',6'}$ = 8.5 Hz, H-3′,H-5′), 8.09 (d, 1H, $J_{7,6}$ = 7.0 Hz, H-7), 7.60 (t, 1H, $J_{5(4,6)}$ = 7.0 Hz, H-5), 7.51 (t, 1H, $J_{6(5,7)}$ = 7.0 Hz, H-6), 7.45 (t, 1H, $J_{4''(3'',5'')}$ = 7.0 Hz, H-4″); ^{13}C-NMR (150 MHz, DMSO-d6): δ 165.38, 162.39, 156.14, 152.49, 149.27, 148.44, 137.53, 136.49, 135.71, 128.48, 128.48, 128.24, 126.87, 126.87, 126.49, 126.32, 125.38, 123.67, 123.61, 122.56; HREI-MS: m/z calcd for $C_{20}H_{12}N_4OS$ [M]+ 356.0732; Found 356.0729; Anal. Calcd for $C_{20}H_{12}N_4OS$, C, 67.40; H, 3.39; N, 15.72; Found C, 67.41; H, 3.41; N, 15.71;

3.9.1.4 4-(5-(4-(benzo[d]thiazol-2-yl)phenyl)-1,3,4-oxadiazol-2-yl)phenol (4)

Red solid. Yield: 80.2%. m.p.: 288-289 °C; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 10.60 (s, 1H, OH), 8.26 (d, 2H, $J_{2',6'}$ /$_{3',5'}$ = 7.5 Hz, H-2′,H-6′), 8.20 (d, 1H, $J_{4,5}$ = 8.0 Hz, H-4), 8.10 (d, 2H, $J_{3',5'}$ /$_{2',6'}$ = 7.5 Hz, H-3′,H-5′), 8.08 (d, 1H, $J_{7,6}$ = 8.0 Hz, H-7), 7.60-7.56 (m, 3H, H-5, H-2″, H-6″), 7.51 (t, 1H, $J_{6(5,7)}$ = 7.5 Hz, H-6), 6.89 (d, 2H, $J_{3'',5''/2'',6''}$ = 7.5 Hz, H-3″,H-5″); ^{13}C-NMR (150 MHz, DMSO-d_6): δ 165.49, 165.49, 162.32, 160.76, 152.43, 137.48, 136.39, 128.69, 127.42, 126.72, 126.54, 126.41, 125.49, 123.60, 122.68, 117.38, 115.78; HREI-MS: m/z calcd for $C_{21}H_{13}N_3O_2S$ [M]+ 371.0728; Found 371.0736; Anal. Calcd for $C_{21}H_{13}N_3O_2S$, C, 67.91; H, 3.53; N, 11.31; Found C, 67.89; H, 3.52; N, 11.30;

3.9.1.5 2-(4-(benzo[d]thiazol-2-yl)phenyl)-5-(thiophen-2-yl)-1,3,4-oxadiazole (5)

Dark brown solid. Yield: 78.4%. m.p.: 276-278 °C; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 8.29 (d, 2H, $J_{2',6'/3',5'}$ = 8.0 Hz, H-2′, H-6′), 8.19 (d, 1H, $J_{4,5}$ = 7.0 Hz, H-4), 8.12 (d, 2H, $J_{3',5'/2',6'}$ = 8.0 Hz, H-3′,H-5′), 8.08 (d, 1H, $J_{7,6}$ = 8.0 Hz, H-7), 7.69(d, 1H, $J_{3'',5''}$ = 6.0 Hz, H-3″), 7.63 (dt, 1H, $J_{5,7}$ = 2.0, $J_{5(4,6)}$ = 7.5 Hz, H-5), 7.51-7.48 (m, 2H, H-6, H-5″), 7.16 (dd, 1H, $J_{4'',5''}$ = 4.5 Hz, $J_{4'',3''}$ = 6.0 Hz, H-4″); ^{13}C-NMR (150 MHz, DMSO-d6): δ 165.92, 162.36, 160.37, 152.31, 137.53, 136.49, 131.39, 130.53, 130.14, 128.30, 128.30, 127.47, 126.62, 126.62, 126.19, 126.42, 125.48, 123.68,

122.57; HREI-MS: m/z calcd for $C_{19}H_{11}N_3OS_2$ [M]+ 361.0344; Found 361.0347; Anal. Calcd for $C_{19}H_{11}N_3OS_2$, C, 63.14; H, 3.07; N, 11.63; Found C, 63.13; H, 3.08; N, 11.65;

3.9.1.6 2-(4-(benzo[d]thiazol-2-yl)phenyl)-5-(furan-2-yl)-1,3,4-oxadiazole (6)

Brown solid. Yield: 79.7%. m.p.: 289-290 °C; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 8.30 (d, 2H, $J_{2',6'/3',5'}$ = 8.0 Hz, H-2′, H-6′), 8.18 (d, 1H, $J_{4,5}$ = 7.0 Hz, H-4), 8.14 (d, 2H, $J_{3',5'/2',6'}$ = 8.0 Hz, H-3′,H-5′), 8.10 (d, 1H, $J_{7,6}$ = 8.0 Hz, H-7), 7.72 (d, 1H, $J_{3'',5''}$ = 6.5 Hz, H-3″), 7.60 (dt, 1H, $J_{5,7}$ = 2.0, $J_{5(4,6)}$ = 7.0 Hz, H-5), 7.54-7.51 (m, 2H, H-6, H-5″), 7.19 (dd, 1H, $J_{4'',5''}$ = 4.0 Hz, $J_{4'',3''}$ = 6.0 Hz, H-4″); ^{13}C-NMR (150 MHz, DMSO-d_6): δ 165.96, 162.85, 162.36, 152.42, 149.83, 144.02, 137.53, 136.37, 128.51, 128.51, 126.98, 126.62, 126.62, 126.43, 125.68, 123.62, 122.86, 112.61, 111.18; HREI-MS: m/z calcd for $C_{19}H_{11}N_3O_2S$ [M]+ 345.0572; Found 345.0575; Anal. Calcd for $C_{19}H_{11}N_3O_2S$, C, 66.07; H, 3.21; N, 12.17; Found C, 66.08; H, 3.19; N, 12.16;

3.9.1.7 4-(5-(4-(benzo[d]thiazol-2-yl)phenyl)-1,3,4-oxadiazol-2-yl)benzene-1,2-diol (7)

Light brown solid. Yield: 91.3%. m.p.: 294-295 °C; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 11.02 (s, 2H, 2xOH), 8.26 (d, 2H, $J_{2',6'/3',5'}$ = 8.0 Hz, H-2′, H-6′), 8.20 (d, 1H, $J_{4,5}$ = 8.0 Hz, H-4), 8.11 (d, 2H, $J_{3',5'/2',6'}$ = 8.0 Hz, H-3′,H-5′), 8.08 (d, 1H, $J_{7,6}$ = 8.0 Hz, H-7), 7.60 (dt, 1H, $J_{5,7}$ = 2.0, $J_{5(4,6)}$ = 7.5 Hz, H-5), 7.51 (dt, 1H, $J_{6,4}$ = 2.0, $J_{6(5,7)}$ = 7.5 Hz, H-6), 7.02 (d, 1H $J_{3'',4''}$ = 8.0 Hz, H-4″), 6.89 (d, $J_{6'',5''}$ = 8.0 Hz, H-6″), 6.76 (t, 1H, $J_{5''(4'',6'')}$ = 7.5 Hz, H-5″); ^{13}C-NMR (150 MHz, DMSO-d_6): δ 165.89, 165.48, 162.52, 152.37, 148.27, 148.12, 137.53, 136.19, 128.47, 128.47, 126.98, 126.98, 126.50, 126.41, 125.43, 123.60, 122.63, 121.44, 119.17, 117.62, 112.30; HREI-MS: m/z calcd for $C_{21}H_{13}N_3O_3S$ [M]+ 387.0678; Found 387.0685; Anal. Calcd for $C_{21}H_{13}N_3O_3S$, C, 65.11; H, 3.38; N, 10.85; Found C, 65.13; H, 3.36; N, 10.84;

3.9.1.8 2-(5-(4-(benzo[d]thiazol-2-yl)phenyl)-1,3,4-oxadiazol-2-yl)benzene-1,4-diol (8)

Pale yellow solid, Yield: 76.5%. m.p.: 287-289 °C; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 10.20 (s, 1H, OH), 9.25 (s, 1H, OH), 8.25 (d, 2H, $J_{2',6'/3',5'}$ = 8.0 Hz, H-2′,H-6′), 8.18 (d, 1H, $J_{4,5}$ = 8.0 Hz, H-4), 8.11 (d, 2H, $J_{3',5'/2',6}$ = 8.0 Hz, H-3′,H-5′), 8.07 (d, 1H, $J_{7,6}$ = 7.5 Hz, H-7), 7.60 (t, 1H, $J_{5(4,6)}$ = 7.5 Hz, H-5), 7.51 (dt, 1H, $J_{6(5,7)}$ = 7.0 Hz, H-6), 7.0 (d, 1H, $J_{6'',4''}$ = 2.0 Hz, H-6″), 6.76-6.73 (m, 2H, H-3″, H-4″); ^{13}C-NMR (150 MHz, DMSO-d_6): δ 165.57, 162.48, 154.25, 153.11, 152.49, 152.19, 137.33, 136.32, 128.48, 128.48, 126.97, 126.97, 126.31, 126.14, 125.68, 123.73,

122.50, 120.15, 118.16, 115.58, 111.13; HREI-MS: m/z calcd for $C_{21}H_{13}N_3O_3S$ [M]+ 528.2161; Found 528.2167; Anal. Calcd for $C_{21}H_{13}N_3O_3S$, C, 65.11; H, 3.38; N, 10.85; Found C, 65.09; H, 3.40; N, 10.84;

3.9.1.9 4-(5-(4-(benzo[d]thiazol-2-yl)phenyl)-1,3,4-oxadiazol-2-yl)benzene-1,3-diol (9)

Light brown solid. Yield: 86.3%. m.p.: 297-298 °C; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 11.20 (s, 1H, OH), 10.15 (s, 1H, OH), 8.25 (d, 2H, $J_{2',6'/3',5'}$ = 8.0 Hz, H-2′,H-6′), 8.19 (d, 1H, $J_{4,5}$ = 7.5 Hz, H-4), 8.14 (d, 2H, $J_{3',5'/2',6}$ = 8.0 Hz, H-3′,H-5′), 8.09 (d, 1H, $J_{7,6}$ = 7.5 Hz, H-7), 7.60 (dt, 1H, $J_{5,7}$ = 2.0, $J_{5(4,6)}$ = 7.5 Hz, H-5), 7.51 (dt, 1H, $J_{6,4}$ = 2.0, $J_{6(5,7)}$ = 7.5 Hz, H-6), 7.32 (d, 1H, $J_{6'',5''}$ = 8.5 Hz, H-6″), 6.94 (dd, 1H, $J_{5'',3''}$ = 2.0 Hz, $J_{5'',6''}$ = 8.5 Hz, H-5″), 6.31 (d, 1H, $J_{3'',5''}$ = 2.0 Hz, H-3″); ^{13}C-NMR (150 MHz, DMSO-d_6): δ 165.51, 162.51, 159.43, 159.17, 155.85, 152.49, 137.45, 136.32, 129.78, 128.56, 128.56, 126.94, 126.94, 126.63, 126.47, 125.31, 123.58, 122.72, 109.19, 105.18, 104.26; HREI-MS: m/z calcd for $C_{21}H_{13}N_3O_3S$ [M]+ 387.0678; Found 387.0682; Anal. Calcd for $C_{21}H_{13}N_3O_3S$, C, 65.11; H, 3.38; N, 10.85; Found C, 65.12; H, 3.39; N, 10.83;

3.9.1.10 3-(5-(4-(benzo[d]thiazol-2-yl)phenyl)-1,3,4-oxadiazol-2-yl)benzene-1,2-diol (10)

Pale yellow solid. Yield: 89.3%. m.p.: 298-299 °C; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 11.29 (s, 1H, OH), 11.20 (s, 1H, OH), 8.26 (d, 2H, $J_{2',6'/3',5'}$ = 8.0 Hz, H-2′, H-6′), 8.21 (d, 1H, $J_{4,5}$ = 8.0 Hz, H-4), 8.12 (d, 2H, $J_{3',5'/2',6}$ = 8.0 Hz, H-3′, H-5′), 8.09 (d, 1H, $J_{7,6}$ = 8.0 Hz, H-7), 7.60 (dt, 1H, $J_{5,7}$ = 2.0, $J_{5(4,6)}$ = 7.5 Hz, H-5), 7.52 (dt, 1H, $J_{6,4}$ = 2.0, $J_{6(5,7)}$ = 7.5 Hz, H-6), 7.29 (d, 1H, $J_{2'',6''}$ = 2.0 Hz, H-2″), 6.96 (dd, 1H, $J_{6'',2''}$ = 2.0 Hz, $J_{6'',5''}$ = 7.5 Hz, H-6″), 6.79 (d, 1H, $J_{5'',6''}$ = 7.5 Hz, H-5″);^{13}C-NMR (150 MHz, DMSO-d_6): δ 165.51, 162.39, 155.78, 152.31, 148.84, 147.47, 137.49, 136.31, 128.36, 128.36, 126.95, 126.95, 126.69, 126.24, 125.58, 123.80, 122.75, 121.91, 120.87, 118.12, 112.53; HREI-MS: m/z calcd for $C_{21}H_{13}N_3O_3S$ [M]+ 387.0678; Found 387.0681; Anal. Calcd for $C_{21}H_{13}N_3O_3S$, C, 65.11; H, 3.38; N, 10.85; Found C, 65.10; H, 3.36; N, 10.87;

3.9.1.11 5-(5-(4-(benzo[d]thiazol-2-yl)phenyl)-1,3,4-oxadiazol-2-yl)benzene-1,3-diol (11)

White solid. Yield: 92.6%. m.p.: 295-296 °C; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 9.30 (s, 2H, 2xOH), 8.28 (d, 2H, $J_{2',6'/3',5'}$ = 8.0 Hz, H-2′,H-6′), 8.20 (d, 1H, $J_{4,5}$ = 8.0 Hz, H-4), 8.12 (d, 2H, $J_{3',5'/2',6}$ = 8.5 Hz, H-3′,H-5′), 8.09 (d, 1H, $J_{7,6}$ = 8.0 Hz, H-7), 7.60(t, 1H, $J_{5(4,6)}$ = 7.5 Hz, H-5), 7.52 (t, 1H, $J_{6(5,7)}$ = 7.5 Hz, H-6), 6.60 (d, 2H, $J_{2'',6''/4''}$ = 2.0 Hz, H-2″H-6″), 6.26 (s, 1H, H-4″);

^{13}C-NMR (150 MHz, DMSO-d_6): δ 165.49, 164.54, 162.41, 161.68, 161.68, 152.44, 137.63, 136.22, 128.70, 128.70, 126.72, 126.72, 126.50, 126.32, 126.16, 125.39, 123.69, 122.52, 109.75, 109.75, 103.87; HREI-MS: m/z calcd for $C_{21}H_{13}N_3O_3S$ [M]+ 387.0678; Found 387.0675; Anal. Calcd for $C_{21}H_{13}N_3O_3S$, C, 65.11; H, 3.38; N, 10.85; Found C, 65.09; H, 3.40; N, 10.84;

3.9.1.12 2-(4-(benzo[d]thiazol-2-yl)phenyl)-5-(3,4-dimethoxyphenyl)-1,3,4-oxadiazole (12)

Brown solid m.p.: 276-277 °C; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 8.25 (d, 2H, $J_{2',6'/3',5'}$ = 8.0 Hz, H-2′,H-6′), 8.20 (d, 1H, $J_{4,5}$ = 8.0 Hz, H-4), 8.12 (d, 2H, $J_{3',5'/2',6}$ = 8.0 Hz, H-3′,H-5′), 8.09 (d, 1H, $J_{7,6}$ = 8.0 Hz, H-7), 7.60 (t, 1H, $J_{5(4,6)}$ = 7.0 Hz, H-5), 7.52 (t, 1H, $J_{6(5,7)}$ = 7.0 Hz, H-6), 7.37 (d, 1H, $J_{2'',6''}$ = 2.0 Hz, H-2″), 7.28 (d, 1H, $J_{6'',5''}$ = 7.0 Hz, H-6″), 7.09 (d, 1H, $J_{5'',6''}$ = 7.0 Hz, H-5″) 3.86 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3); ^{13}C-NMR (150 MHz, DMSO-d_6): δ 165.81, 165.48, 162.32, 152.28, 152.16, 151.48, 137.47, 136.39, 128.51, 128.51, 126.94, 126.94, 126.69, 126.41, 125.58, 123.78, 122.60, 121.21, 120.15, 115.53, 107.69, 56.79, 56.72; HREI-MS: m/z calcd for $C_{23}H_{17}N_3O_3S$ [M]+ 415.0991; Found 415.0987; Anal. Calcd for $C_{23}H_{17}N_3O_3S$, C, 66.49; H, 4.12; N, 10.11; Found C, 66.51; H, 4.14; N, 10.13;

3.9.1.13 2-(4-(benzo[d]thiazol-2-yl)phenyl)-5-(2,5-dimethoxyphenyl)-1,3,4-oxadiazole (13)

Orange solid. Yield: 81.7%. m.p.: 274-275 °C; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 8.26 (d, 2H, $J_{2',6'/3',5'}$ = 8.5 Hz, H-2′,H-6′), 8.20 (d, 1H, $J_{4,5}$ = 8.5 Hz, H-4), 8.14 (d, 2H, $J_{3',5'/2',6}$ = 8.0 Hz, H-3′,H-5′), 8.10 (d, 1H, $J_{7,6}$ = 8.0 Hz, H-7), 7.61 (dt, 1H, $J_{5,7}$ = 2.0 Hz, $J_{5(4,6)}$ = 7.5 Hz, H-5), 7.52 (dt, 1H, $J_{6,4}$ = 2.0 Hz, $J_{6(5,7)}$ = 7.5 Hz, H-6), 6.90 (d, 2H, $J_{2'',6'',/4''}$ = 2.0 Hz, H-2″, H-6″), 6.62 (d, 1H, $J_{4''/6'',2''}$ = 2.0 Hz, H-4″), 3.83 (s, 6H, 2xOCH_3); ^{13}C-NMR (150 MHz, DMSO-d_6): δ 165.49, 162.41, 156.17, 155.25, 152.49, 151.34, 137.33, 136.32, 128.48, 128.48, 126.94, 126.94, 126.61, 126.46, 125.58, 123.71, 122.54, 118.12, 117.47, 115.65, 115.38, 56.72, 56.01; HREI-MS: m/z calcd for $C_{23}H_{17}N_3O_3S$ [M]+ 415.0991; Found 415.0996; Anal. Calcd for $C_{23}H_{17}N_3O_3S$, C, 66.49; H, 4.12; N, 10.11; Found C, 66.47; H, 4.14; N, 10.09;

3.9.1.14 2-(5-(4-(benzo[d]thiazol-2-yl)phenyl)-1,3,4-oxadiazol-2-yl)phenol (14)

Brownish solid. Yield: 96.2%. m.p.: 265-266 °C; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 10.56 (s, 1H, OH), 8.29 (d, 2H, $J_{2',6'/3',5'}$ = 8.0 Hz, H-2′,H-6′), 8.20 (d, 1H, $J_{4,5}$ = 7.5 Hz, H-4), 8.13 (d, 2H, $J_{3',5'/2',6}$ = 8.0 Hz, H-3′,H-5′), 8.10 (d, 1H, $J_{7,6}$ = 8.0 Hz, H-7), 7.60-7.56 (m, 2H, H-6, H-5″), 7.52

(dt, 1H, $J_{6,4}$ = 2.0, $J_{6(5,7)}$ = 7.5 Hz, H-6), 7.31 (t, 1H, $J_{4''(5'',3'')}$ = 2.0 Hz, H-4′′), 6.95-6.90 (m, 2H, H-3′′, H-6′′), H-5′′); ^{13}C-NMR (150 MHz, DMSO-d_6): δ 165.57, 162.32, 158.48, 155.72, 152.52, 137.49, 136.19, 131.74, 130.01, 128.40, 128.40, 126.95, 126.95, 126.62, 126.47, 125.38, 123.60, 122.69, 119.84, 118.76, 111.62; HREI-MS: m/z calcd for $C_{21}H_{13}N_3O_2S$ [M]+ 371.0728; Found 371.0722; Anal. Calcd for $C_{21}H_{13}N_3O_2S$, C, 67.91; H, 3.53; N, 11.31; Found C, 67.93; H, 3.51; N, 11.32;

3.9.1.15 3-(5-(4-(benzo[d]thiazol-2-yl)phenyl)-1,3,4-oxadiazol-2-yl)phenol (15)

Dark brown solid. Yield: 76.2%. m.p.: 278-279 °C; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 9.90 (s, 1H, OH), 8.29 (d, 2H, $J_{2',6'/3',5'}$ = 8.5 Hz, H-2′,H-6′), 8.21 (d, 1H, $J_{4,5}$ = 8.5 Hz, H-4), 8.13 (d, 2H, $J_{3',5'/2',6}$ = 8.0 Hz, H-3′,H-5′), 8.10 (d, 1H, $J_{7,6}$ = 8.5 Hz, H-7), 7.60 (t, 1H, $J_{5(4,6)}$ = 8.0 Hz, H-5), 7.51 (t, 1H, Hz, $J_{6(5,7)}$ = 7.5 Hz, H-6), 7.32 (t, 1H, $J_{5''(4'',6'')}$ = 7.0 Hz, H-5′′), 7.25 (s, 1H, H-2′′), 7.11 (d, 1H, $J_{4'',5''}$ = 7.5 Hz, , H-4′′), 6.89 (dd, 1H, $J_{6'',2''}$ = 2.0 Hz, $J_{6'',5''}$ = 7.0 Hz, H-6′′); ^{13}C-NMR (150 MHz, DMSO-d_6): δ 165.87, 165.37, 162.49, 158.51, 152.38, 137.53, 136.28, 130.90, 128.30, 128.30, 126.72, 126.72, 126.94, 126.42, 126.25, 125.49, 123.71, 122.68, 121.13, 118.32, 114.27. HREI-MS: m/z calcd for $C_{21}H_{13}N_3O_2S$ [M]+ 371.0728; Found 371.0731; Anal. Calcd for $C_{21}H_{13}N_3O_2S$, C, 67.91; H, 3.53; N, 11.31; Found C, 67.92; H, 3.54; N, 11.32;

3.9.1.16 4-(5-(4-(benzo[d]thiazol-2-yl)phenyl)-1,3,4-oxadiazol-2-yl)-2-methoxyphenol (16)

Dark brown solid. Yield: 88.4%. m.p.: 291-292 °C; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 9.60 (s, 1H, OH), 8.29 (d, 2H, $J_{2',6'/3',5'}$ = 8.0 Hz, H-2′,H-6′), 8.20 (d, 1H, $J_{4,5}$ = 8.0 Hz, H-4), 8.11 (d, 2H, $J_{3',5'/2',6}$ = 8.0 Hz, H-3′,H-5′), 8.08 (d, 1H, $J_{7,6}$ = 8.5 Hz, H-7), 7.64 (dt, 1H, $J_{5,7}$ = 2.0 Hz, $J_{5(4,6)}$ = 7.5 Hz, H-5), 7.53 (dt, 1H, $J_{6,4}$ = 2.0 Hz, $J_{6(5,7)}$ = 7.5 Hz, H-6); 7.35 (d, 1H, $J_{2'',6''}$ = 2.0 Hz, H-2′′), 7.16 (dd, 1H, $J_{6'',2''}$ = 2.0 Hz, $J_{6'',5''}$ = 7.5 Hz, H-6′′), 6.90 (d, 1H, $J_{5'',6''}$ = 8.0 Hz, H-5′′), 3.82 (s, 3H, OCH_3); ^{13}C-NMR (150 MHz, DMSO-d_6): δ 165.95, 165.49, 162.38, 152.41, 150.82, 149.78, 137.53, 136.39, 128.40, 128.40, 126.82, 126.82, 126.49, 126.34, 125.47, 123.69, 122.87, 121.94, 117.50, 115.61, 111.78, 56.34; HREI-MS: m/z calcd for $C_{22}H_{15}N_3O_3S$ [M]+ 401.0834; Found 401.0837; Anal. Calcd for $C_{22}H_{15}N_3O_3S$, C, 65.82; H, 3.77; N, 10.47; Found C, 65.83; H, 3.76; N, 10.48.

3.9.1.17 2-(4-(benzo[d]thiazol-2-yl)phenyl)-5-(4-fluorophenyl)-1,3,4-oxadiazole (17)

Yellow solid. Yield: 86.4%. m.p.: 271-272 °C; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 8.29 (d, 2H, $J_{2',6'} = J_{6',2'} = 8.0$ Hz, H-2′,H-6′), 8.20 (d, 1H, $J_{4,5} = 8.0$ Hz, H-4), 8.12 (d, 2H, $J_{3',5'} = J_{3',5'} = 8.0$ Hz, H-3′,H-5′), 8.09 (d, 1H, $J_{7,6} = 8.0$ Hz, H-7), 7.74 (dd, 2H, $J_{3'',5''/2'',6''} = 7.0$ Hz, $J_{3'',5''/F} = 5.5$ Hz, H-3″ H-5″), 7.60 (t, 1H, $J_{5(4,6)} = 7.5$ Hz, H-5), 7.51 (t, 1H, $J_{6(5,7)} = 7.5$ Hz, H-6), 7.71 (dd, 2H, $J_{3'',5''/2'',6''} = 5.5$ Hz, $J_{3'',5''/F} = 5.5$ Hz, H-3″ H-5″), 7.36 (t, 2H, $J_{2'',6''/3'',5'',F} = 7.0$ Hz, H-2″,H-6″); ^{13}C-NMR (150 MHz, DMSO-d_6): δ 166.37 (d, J = 223.2 Hz), 165.26, 162.45, 152.38, 137.53, 136.19, 129.82, 129.82, 128.60, 128.60, 126.82, 126.82, 126.60, 126.45, 125.47, 123.68, 123.51, 122.63, 116.23, 116.17, 116.17; HREI-MS: m/z calcd for $C_{21}H_{12}FN_3OS$ [M]+ 373.0685; Found 373.0690; Anal. Calcd for $C_{21}H_{12}FN_3OS$, C, 67.55; H, 3.24; N, 11.25; Found C, 67.54; H, 3.22; N, 11.28;

3.9.1.18 2-(4-(benzo[d]thiazol-2-yl)phenyl)-5-(4-chlorophenyl)-1,3,4-oxadiazole (18)

Brown solid, Yield: 78.7%. m.p.: 268-269 °C; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 8.29 (d, 2H, $J_{2',6'/3',5'} =$ 8.0 Hz, H-2′,H-6′), 8.20 (d, 1H, $J_{4,5} = 8.0$ Hz, H-4), 8.11 (d, 2H, $J_{3',5'/2',6} = 8.0$ Hz, H-3′,H-5′), 8.10 (d, 1H, $J_{7,6} = 7.5$ Hz, H-7), 7.77 (d, 2H, $J_{2'',6''/3'',5''} =$ 8.5 Hz, H-2″,H-6″), 7.60 (t, 1H, $J_{5(4,6)} = 7.5$ Hz, H-5), 7.56 (t, 1H, $J_{6(5,7)} = 7.5$ Hz, H-6), 7.52 (d, 2H, $J_{3'',5''/2'',6''} =$ 8.0 Hz, H-3″,H-5″); ^{13}C-NMR (150 MHz, DMSO-d_6): δ 165.57, 162.48, 152.42, 137.53, 137.41, 136.31, 129.47, 129.47, 128.34, 128.34, 128.12, 127.79, 127.79, 126.93, 126.93, 126.49, 126.24, 125.38, 123.67, 122.54, 122.54; HREI-MS: m/z calcd for $C_{21}H_{12}ClN_3OS$ [M]+ 389.0390; Found 389.0395; Anal. Calcd for $C_{21}H_{12}ClN_3OS$, C, 64.70; H, 3.10; N, 10.78; Found C, 64.71; H, 3.09; N, 10.76;

3.9.1.19 2-(4-(benzo[d]thiazol-2-yl)phenyl)-5-(4-methoxyphenyl)-1,3,4-oxadiazole (19)

Light brown solid. Yield: 85.9%. m.p.: 285-286 °C; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 8.29 (d, 2H, $J_{2',6'/3',5'} = 8.0$ Hz, H-2′,H-6′), 8.20 (d, 1H, $J_{4,5} = 8.0$ Hz, H-4), 8.12 (d, 2H, $J_{3',5'/2',6} = 8.5$ Hz, H-3′,H-5′), 8.09 (d, 1H, $J_{7,6} = 7.5$ Hz, H-7), 7.70 (d, 2H, $J_{2'',6''/3'',5''} =$ 8.5 Hz, H-2″,H-6″), 7.64 (t, 1H, $J_{5(4,6)} = 7.0$ Hz, H-5), 7.51 (t, 1H, $J_{6(5,7)} = 7.0$ Hz, H-6), 7.08 (d, 2H, $J_{3'',5''/2'',6''} =$ 8.0 Hz, H-3″,H-5″), 3.80 (s, 3H, OCH_3); ^{13}C-NMR (150 MHz, DMSO-d_6): δ 165.47, 162.42, 162.19, 152.48, 137.43, 136.29, 128.50, 128.50, 127.07, 127.07, 126.92, 126.92, 126.59, 126.59, 126.44, 125.48, 123.70, 122.70, 121.54, 113.88, 113.88, 56.03; HREI-MS: m/z calcd for $C_{22}H_{15}N_3O_2S$

[M]+ 385.0885; Found 385.0880; Anal. Calcd for $C_{22}H_{15}N_3O_2S$, C, 68.55; H, 3.92; N, 10.90; Found C, 68.56; H, 3.93; N, 10.91.

3.9.1.20 2-(4-(benzo[d]thiazol-2-yl)phenyl)-5-(pyridin-4-yl)-1,3,4-oxadiazole (20)

Brown solid. Yield: 86.1%. m.p.: 280-281 °C; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 8.67 (d, 2H, $J_{2'',6''/3'',5''}$ = 6.0 Hz, H-2″,H-6″), 8.27 (d, 2H, $J_{2',6'/3',5'}$ = 8.5 Hz, H-2′,H-6′), 8.20 (d, 1H, $J_{4,5}$ = 8.5 Hz, H-4), 8.11 (d, 2H, $J_{3',5'/2',6}$ = 8.5 Hz, H-3′,H-5′), 8.09 (d, 1H, $J_{7,6}$ = 8.0 Hz, H-7), 7.71 (d, 2H, $J_{3'',5''/2'',6''}$ = 6.0 Hz, H-3″,H-5″), 7.64 (dt, 1H, $J_{5,7}$ = 2.0 Hz, $J_{5(4,6)}$ = 7.5 Hz, H-5), 7.53 (dt, 1H, $J_{6,4}$ = 2.0 Hz, $J_{6(5,7)}$ = 7.5 Hz, H-6); ^{13}C-NMR (150 MHz, DMSO-d_6): δ 165.48, 162.32, 152.49, 150.20, 137.45, 136.19, 133.37, 128.53, 128.53, 128.53, 126.96, 126.96, 126.50, 126.50, 126.21, 125.47, 123.75, 123.43, 122.61, 122.61; HREI-MS: m/z calcd for $C_{20}H_{12}N_4OS$ [M]+ 356.0732; Found 356.0729; Anal. Calcd for $C_{20}H_{12}N_4OS$, C, 67.40; H, 3.39; N, 15.72; Found C, 67.41; H, 3.38; N, 15.73.

Acknowledgment

Authors would like to acknowledge The Ministry of Agriculture (MOA) Malaysia and Universiti Teknologi MARA for the financial support under MOA grant file No. 100-RMI/ MOA 16/6/2 (1/2013) and Atta-ur-Rahman Institute for Natural product Discovery (RiND) to provide excellent designed lab and facility for the research and all technical and non-technical staff for a lot of support for this work. The computational part of this project was funded through the "TRGS" (grant no. 600-RMI/TRGS 5/3 (1/2014)-3) by the Ministry of Higher Education Malaysia (MOHE).

References

1. Glaser, J. H.; Sly, W. S. b-Glucuronidase deficiency mucopolysaccharidosis: Methods for enzymatic diagnosis. J. Lab. Clin. Med. 82 (1973) 969-977.

2. Sperker, B.; Backman, J. T.; Kromer, K. The role of β-glucuronidase in drug disposition and drug targeting in humans. Clin. Pharmacokinet. 33 (1997) 18-24.

3. Fishman, W. H. Wakabayyashi, M. "Metabolic conjugation and metabolic hydrolysis". Academic Press, New York 1970, pp 519.

4. Paigen, K. Mammalian β-glucuronidase: genetics, molecular biology, and cell biology. Progress in Nucleic Acid Res. Mol. Biol. 37 (1989) 155-205.

5. Holiman, P.C.H.; Hertog, M.G.L.; Katan, M.B. Analysis and health effects of flavonoids. Food Chemistry 57 (1996) 43-46.

6. Ho, K. J.; Hsu, S. C.; Chen, J. S.; Ho, L. H. C. Human biliary glucuronidase: correlation of its activity with deconjugation of bilirubin in the bile. Eur. J. Clin. Invest. 16 (1986) 361-367.

7. Gonick, H. C.; Kramer, H. J.; Schapiro, A. E. Urinary β-glucuronidase activity in renal disease. Archives of Internal Medicine, 132 (1973) 63-69.

8. Ho, Y. C.; Ho, K. J. Differential quantitation of urinary β-glucuronidase of human and bacterial origins. J. Urol. 134 (1985) 1227-1230.

9. Sly, W. S.; Quinton, B. A.; McAlister, W. H.; Rimoin, D. L. β-Glucuronidase deficiency: report of clinical, radiologic, and biochemical features of a new mucopolysaccharidosis. The Journal of Pediatrics 82 (1973) 249-257.

10. Hall, C.W.; Cantz, M.; Neufeld, E.F. A β-Glucuronidase deficiency mucopolysaccharidosis: studies in cultured fibroblasts. Archives of Biochemistry and Biophysics 155 (1973) 32-38.

11. Carroll, A.R.; Scheuer, P.J. Kuanoniamines A, B, C, and D: pentacyclic alkaloids from a tunicate and its prosobranch mollusk predator Chelynotus semperi. Jounal of Organic Chemistry 55 (1990) 4426-4431.

12. Gunawardana, G.P.; Kohmoto, S.; Gunasekera, S.P.; McConnell, O.J.; Koehn, F.E. Dercitine, a new biologically active acridine alkaloid from a deep water marine sponge, Dercitus sp. Journal of American Chemical Society 110 (1988) 4856-4858.

13. Tanaka, T.; Umekawa, H.; Saitoh, M.; Ishikawa, T.; Shin, T.; Ito, M.; Itoh, H.; Kawamatsu, Y.; Sugihara, H.; Hidaka, H. Modulation of calmodulin function and of Ca2+-induced smooth muscle contraction by the calmodulin antagonist, HT-74. Mol. Pharmacol. 29 (1986) 264-269.

14. Bowyer, P.W.; Gunaratne, R.S.; Grainger, M.; Withers-Martinez, C.; Wickramsinghe, S. R.; Tate, E.W.; Leatherbarrow, R.J.; Brown, K.A.; Holder, A.A.; Smith, D.F. Molecules incorporating a benzothiazole core scaffold inhibit the N-myristoyltransferase of Plasmodium falciparum. Biochem. J. 408 (2007) 173-180.

15. Mittal, S.; Samota, M.K.; Kaur, J.; Seth, G. Synthesis, spectral, and antifungal evaluation of phosphorylated and thiophosphorylated benzothiazole derivatives. Phosphorus, Sulfur and Silicon and the Related Elements 182 (2007) 2105-2113.

16. Gurupadayya, B.M.; Gopal, M.; Padmashali, B.; Manohara, Y.N. Synthesis and pharmacological evaluation of azetidin-2-ones and thiazolidin-4-ones encompassing benzothiazole. Indian J. Pharm. Sci. 70 (2008) 572-577.

17. Pozas, R.; Carballo, J.; Castro, C.; Rubio, J. Synthesis and in vitro antitrypanosomal activity of novel nifurtimox analogues. Bioorg. Med. Chem. Lett. 15 (2005) 1417-1421.

18. Rana, A.; Siddiqui, N.; Khan, S. A.; Ehtaishamul Haque, S.; Bhat, M. A. N-{[(6-Substituted-1, 3-benzothiazole-2-yl) amino] carbonothioyl}-2/4-substituted benzamides: Synthesis and pharmacological evaluation. Eur. J. Med. Chem. 43 (2008) 1114-1122.

19. Rashid, M.; Husain, A.; Mishra, R., Synthesis of benzimidazoles bearing oxadiazole nucleus as anticancer agents. Eur. J. Med. Chem. 54 (2012) 855-866.

20. Husain, A.; Rashid, M.; Mishra, R.; Parveen S.; Shin, D.-S.; Kumar, D., Benzimidazole bearing oxadiazole and triazolo-thiadiazoles nucleus: design and synthesis as anticancer agents. Bioorg. Med. Chem. Lett. 22 (2012) 5438-5444.

21. Zhang, S.; Luo, Y.; He, L.-Q.; Liu, Z.-J.; Jiang, A.-Q.; Yang, Y.-H.; Zhu, H.-L., Synthesis, biological evaluation, and molecular docking studies of novel 1,3,4-oxadiazole derivatives possessing benzotriazole moiety as FAK inhibitors with anticancer activity. Bioorg. Med. Chem. 21 (2013) 3723-3729.

22. Hadady, Z.; Toth, M.; Somsak, L., C-(β-D-Glucopyranosyl) heterocycles as potential glycogen phosphorylase inhibitors. ARKIVOC 7 (2004) 140-149.

23. Ahsan, M.J.; Samy, J.G.; Khalilullah, H.; Nomani, M.S.; Saraswat, P.; Gaur, R.; Singh, A., Molecular properties prediction and synthesis of novel 1,3,4-oxadiazole analogues as potent antimicrobial and antitubercular agents. Bioorg. Med. Chem. Lett. 21 (2011) 7246-7250.

24. Liu, F.; Luo, X.Q.; Song, B.A.; Bhadury, P.S.; Yang, S.; Jin, L.H.; Xue, W.; Hu, D.Y., Synthesis and antifungal activity of novel sulfoxide derivatives containing trimethoxyphenyl substituted 1,3,4-thiadiazole and 1,3,4-oxadiazole moiety. Bioorg. Med. Chem. 16 (2008) 3632-3640.

25. Jakubkiene, V.; Burbuliene, M.M.; Udrenaite, E.; Garaliene, V.; Vainilavicius, P., (6-methyl-2-methylsulfanyl-4-oxo-3,4-dihydro-3-pyrimidinyl)acetic acid and related compounds exhibiting anti-inflammatory activity. Pharmazie 57 (2002) 610-613.

26. Desai, N.C.; Bhatt, N.; Somani, H.; Trivedi A., Synthesis, antimicrobial and cytotoxic activities of some novel thiazole clubbed 1,3,4-oxadiazoles. Eur. J. Med. Chem. 67 (2013) 54-59.

27. Ghorab, M.M.; Shaaban, M.A.; Refaat, H.M.; Heiba, H.I.; Ibrahim, S.S., Anticancer and radiosensitizing evaluation of some new pyranothiazole-Schiff bases bearing the biologically active sulfonamide moiety. Eur. J. Med. Chem. 53 (2012) 403-407.

28. Wang, X.-M.; Xu, J.; Li, Y.-P.; Li, H.; Jiang, C.-S.; Yang, G.-D.; Lu, S.-M.; Zhang, S.-Q., Synthesis and anticancer activity evaluation of a series of [1,2,4]triazolo[1,5-a]pyridinylpyridines in vitro and in vivo. Eur. J. Med. Chem. 67 (2013) 243-251.

29. Xu, F.; Xu, H.; Wang, X.; Zhang, L.; Wen, Q.; Zhang, Y.; Xu, W.; Discovery of N-(3-((7H-purin-6-yl)thio)-4-hydroxynaphthalen-1-yl)-sulfonamide derivatives as novel protein kinase and angiogenesis inhibitors for the treatment of cancer: Synthesis and biological evaluation. Part III. Bioorg. Med. Chem. 22 (2014) 1487-1495.

30. Taha, M.; Ismail, N.H. ; Lalani, S.; Fatmi, M.Q.; Siddiqui, S.; Khan, K.M.; Imran, S.; Choudhary, M. I.. Synthesis of novel inhibitors of α-glucosidase based on the benzothiazole skeleton containing benzohydrazide moiety and their molecular docking studies. Eur. J. Med. Chem. 92 (2015) 387-400.

31. Zawawi, N.K.N.A.; Taha, M.; Ahmat, N.; Wadood, A.; Ismail, N.H.; Rahim, F. ; Ali, M.; Norishah, A.; Khan, K.M. Novel 2,5-disubtituted-1,3,4-oxadiazoles with benzimidazole backbone: A new class of β-glucuronidase inhibitors and in silico studies. Bioorg. Med. Chem. 23(2015) 3119-3125.

32. a) Taha, M.; Shah, S. A. A.; Khan, A.; Arshad, F.; Ismail, N. H.; Afifi, M.; Imran, S.; Choudhary, M. I. Synthesis of 3, 4, 5-trihydroxybenzohydrazone and evaluation of their urease inhibition potential. Arabian J. Chem. (2015); b) Imran, S.; Taha, M.; Ismail, N. H.; Kashif, S. M.; Rahim, F.; Jamil, W.; Wahab, H.; Khan, K. M. Synthesis, In vitro and Docking Studies of New Flavone Ethers as α-Glucosidase Inhibitors. Chem. Biol. Drug. Des. (2015); c) Imran, S.; Taha, M.; Ismail, N. H.; Kashif, S. M.; Rahim, F.; Jamil, W.; Hariono, M.; Yusuf, M.; Wahab, H. Synthesis of novel flavone hydrazones: in-vitro evaluation of α-glucosidase inhibition, QSAR analysis and docking studies. European J. Med. Chem. 105 (2015) 156-170; d) Imran, S.; Taha, M.; Ismail, N. H.; Fayyaz, S.; Khan, K. M.; Choudhary, M. I. Synthesis, biological evaluation, and docking studies of novel thiourea derivatives of bisindolylmethane as carbonic anhydrase II inhibitor. Bioorg. Chem 62 (2015) 83-93; e) Taha, M.; Ismail, N. H.; Javaid, K., Imran, S.; Wadood, A., Ali, M.; Khan, K. M.; Saad, S. M.; Rahim, F.; Choudhary, M. I. (2015). Evaluation of 2-indolcarbohydrazones as potent α-glucosidase inhibitors, in silico studies and DFT based stereochemical predictions. Bioorg. Chem. 63 (2015) 24-35; f) Taha, M.; Ismail, N. H.; Khan, A., Shah, S. A. A.; Anwar, A.; Halim, S. A.; Fatmi, M. Q.; Imran, S.; Rahim, F.; Khan, K. M. (2015). Synthesis of novel derivatives of oxindole,

their urease inhibition and molecular docking studies. Bioorg. Med. Chem. Lett. 25(2015) 3285-3289

33. Taha, M.; Ismail, N. H.; Imran, S.; Selvaraj, M.; Rashwan, H.; Farhanah, F. U.; Rahim, F.; Kesavanarayanan, K. S.; Ali, M.. Synthesis of benzimidazole derivatives as potent β-glucuronidase inhibitors. Bioorg. Chem. 61 (2015) 36-44.

34. Taha, M.; Ismail, N. H.; Imran, S.; Selvaraj, M.; Rahim, A.; Ali, M.; Siddiqui S.; Rahim F.; Khan, K. M. Synthesis of novel benzohydrazone–oxadiazole hybrids as β-glucuronidase inhibitors and molecular modeling studies. Bioorg. Med. Chem. 23(2015) 7394-7404.

35. Taha, M.; Ismail, N. H.; Imran, S.; Wadood, A., Rahim, F.; Ali, M.; Rehman, A. U. (2015). Novel quinoline derivatives as potent in vitro α-glucosidase inhibitors: in silico studies and SAR predictions. MedChemComm, 6(10), 1826-1836

36. Jain, S.; Drendel, W.B.; Chen, Z.W.; Mathews, F.S.; Sly, W.S.; Grubb, J.H. Structure of human beta-glucuronidase reveals candidate lysosomal targeting and active-site motifs. Nat. Struct. Biol., 3 (1996) 375-381.

37. Schrödinger Release 2015-1**:** Maestro, version 10.1, Schrödinger, LLC, New York, NY, 2015.

38. Maestro, Version 9.2, Schrodinger, LLC, New York, NY, USA, 2011.

39. Verdonk, M.L.; Cole, J.C.; Hartshorn, M.J.; Murray, C.W.; Taylor, R.D. Improved protein-ligand docking using GOLD. Proteins 52 (2003) 609-623

40. PyMOL, Molecular Graphics System L, NY, USA, (2010) PyMOL New York, US: Schrodinger] and Maestro visualization.

Captions for figures and tables

Scheme-1 Synthesis of novel benzothiazole derivatives 1-20

Figure1: Graphical illustration of predicted binding modes of active benzothiazole derivatives.

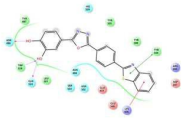
*Binding modes of **a.** compound **7** shown in stick form, **b.** Compound **14**, **c.** Compound **8** and **d.** Compound **17**. Key residues are represented in spear and labeled. The hydrogen bond interactions are represented by magenta arrow, π-π interactions are shown in green color lines and cation-π interactions are shown in red color lines. Compounds are represented in line form.*

Table 1. β-Glucuronidase inhibitory potential of novel benzothiazole derivatives 1-20.

Table 2: Optimization of novel benzothiazole derivatives 1-20

Synthesis of Novel Inhibitors of β-Glucuronidase Based on the Benzothiazole Skeleton and their Molecular Docking Studies

Muhammad Taha, Nor Hadiani Ismail, Syahrul Imran, Manikandan Selvaraj, Fazal Rahim



Benzothiazole derivatives (**1-20**) evaluated for *β*-Glucuronidase inhibitory activity