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FULL-LENGTH PAPER

Evaluation of the thiazole Schiff bases as β-glucuronidase inhibitors and their in silico studies

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Abstract Twenty eight (28) derivatives **2–29** were synthesized and four analogs were found to exhibit single-digit IC_{50} values as β-glucuronidase inhibitors. Molecular modeling indicates that three factors: substituent R, lone pair on the nitrogen of azomethine part, and the interactions made by the main skeleton of the molecule, determined the enzyme inhibitory potential of these compounds. The planar conformation of the molecules allows them to fit deep inside the pocket while blocking the entry of other physiological substrates seems to play an important role in their activity.

Keywords Thiazole Schiff bases · β-Glucuronidase inhibitors · Molecular modeling · SAR

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Introduction

Computer-aided rational drug design has made significant contributions in the development of novel therapeutics leading to FDA approval, such as the anti-flu drug Relenza, a neuraminidase inhibitor [1], and the anti-AIDS cyclic urea DMP-450 [2]. During the 1990s high throughput screening (HTS) was introduced in mainstream drug discovery; however, it failed to deliver its high expectations for drug discovery. The foremost concern was the lack of target macromolecular structures of receptors. Experts in computer-aided drug design (CADD) promptly recognized the importance of HTS and developed computer-driven equivalents such as virtual screening. The principle was simple: (1) construct a 3D model of the target site and dock into it as many molecular candidates as possible to estimate how well they might bind in the active site, (2) prioritize and select the most suitable structures, and (3) synthesize the most promising candidates for in vitro screening [3,4].

β-Glucuronidase plays a pivotal role in the hydrolysis of β-glucuronides. Glucuronides are formed in the body during the xenobiotic detoxification process. A large number of toxic compounds are eliminated safely from the body as glucuronides. Since β-glucuronidase hydrolyzes these conjugates, the inhibition of this enzyme may protect the body from the reintroduction of the original xenobiotics [5]. Evidence suggests that inhibiting the β-glucuronidase enzyme has a possible role in controlling different stages in cancer induc-

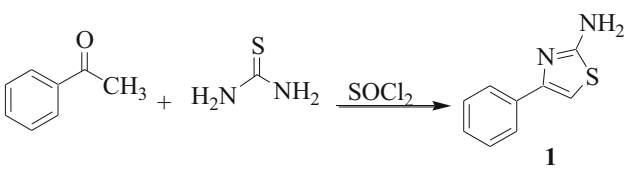
tion [6,7]. β-Glucuronidase (EC 3.2.1.31) is an inducible enzyme elaborated by anaerobic *E. coli, Peptostreptococcus*, *Bacteroides*, and *Clostridia*. Studies have associated bacterial β-glucuronidase primarily to *E. coli* and Enterobacteriaceae. Enhanced activity of this enzyme increases the enterohepatic recirculation of toxins, hormones, drugs, and carcinogens. Recent studies showed that the Gram-positive bacteria in the gastrointestinal tract are also partially involved in β-glucuronidase activity [8]. Initiation of colon cancer is believed to be associated with β-Glucuronidase while its higher levels in intestines are connected with increased risk of colon cancer. These reports emphasize the pharmacological significance for the development of new and specific inhibitors of this enzyme [9,10].

Thiazoles have attracted a lot of interest over the years due to their broad biological activity profiles [11–13]. These molecules have found applications in drug development for the treatment of allergies [14], hypertension [15], schizophrenia [16], inflammation [17], HIV infections [18], and they are also used as cardiotonics [19], sedatives [20], and anesthetics [21]. Additionally, while some new thiazoles possess anti-inflammatory and analgesic properties [22,23], others are potent and selective acetyl CoA carboxylase 2 inhibitors with high promise in Alzheimer's treatment [24,25].

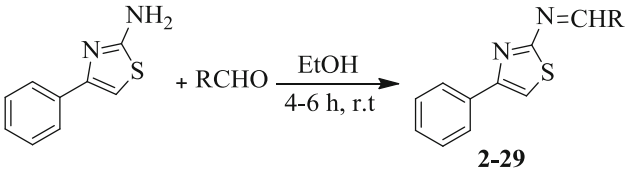
In continuation to our research interests in the development of new and simple leads, thiazole Schiff bases **2–29** were synthesized and screened for their β-glucuronidase inhibitory potentials. Synthetic compounds **2–29** demonstrated varying degrees of β-glucuronidase inhibitory potentials. D-Saccharic acid 1,4-lactone is used as a standard substrate whose IC_{50} value is 48.4 ± 1.25 μM. In addition, molecular modeling was performed to explore the potential binding mode of our newly synthesized thiazole derivatives.

Results and discussion

4-Phenyl-1,3-thiazol-2-amine (**1**), an important intermediate for pharmaceutical industry, was prepared by the condensation of thiourea and acetophenone in the presence of thionyl chloride as an oxidating agent (Scheme 1) [26]. Schiff bases **2–29** were prepared by condensing **1** with a diverse range of aldehydes (Scheme 2). All final products were confirmed by 1H NMR, EI MS, IR, and UV spectroscopy (Table 1).



Scheme 1 Synthesis of 4-Phenyl-1,3-thaizol-2-amine **1**



Scheme 2 Synthesis of Schiff's bases of **1**

SAR studies

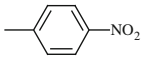
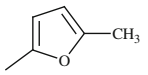
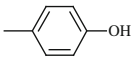
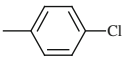
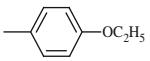
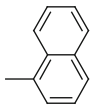
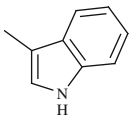
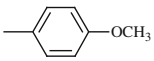
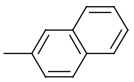
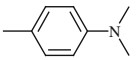
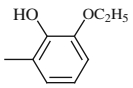
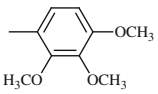
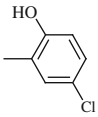
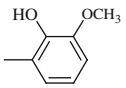
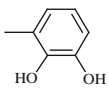
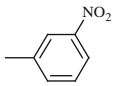
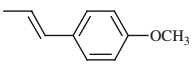
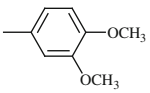
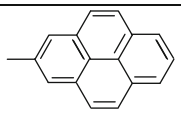
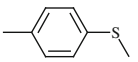
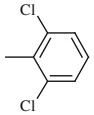
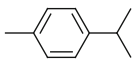
Thiazoles **2–29** were screened against β-glucuronidase following a published protocol [27,28] exhibiting IC_{50} values in the range of 152 ± 3.35 − 4.88 ± 0.12 μM versus that of the standard substrate D-saccharic acid 1,4-lactone (48.4 ± 1.25 μM). The results are shown in Table 2.

Remarkably, compounds **3**, **12**, **17**, and **18** exhibited single-digit micromolar β-glucuronidase inhibition activity, while compounds **2**, **5**, **8**, **9**, **11**, **13**, **14**, **16**, **20**, **21**, **22**, **23**, **24**, **25**, **26**, **27**, **28**, and **29** also showed good β-glucuronidase inhibition activities superior to the standard. Compounds **4**, **10**, **15**, and **19** demonstrated less activity against β-glucuronidase, and compounds **6** and **7** demonstrated no activity at all. In a broader sense, ligands are classified into four groups based on their IC_{50} values as shown in Table 3.

Results shown in the above table are prioritized and summarized as follows:

It has been envisioned that the aromatic side chain directly attached to azomethine moiety determined the inhibitory potential of compounds **2–29**. Compound **18**, with two hydroxyl groups on the aromatic ring of side chain, proved to be the most potent inhibitor (IC_{50} = 4.88±0.12 μM) among the current series as well as much superior than the standard inhibitor D-saccharic acid 1,4-lactone. Similarly, compound **17** having a hydroxyl group at *ortho* and a chloro at *meta*-position of the phenyl ring also showed an excellent inhibitory potential with an IC_{50} value 5.63 ± 0.16 μM. Compounds **7** and **9** were proved to be exceptions to this generalization since the side chain of these compounds is aliphatic. Compound **12**, without any substitution on the phenyl ring, showed an IC_{50} value 9.77 ± 0.11 μM. Compound **3**, with mono hydroxy substitution at *para* position of the phenyl ring, also showed an excellent activity (IC_{50} = 9.84±0.33 μM) but less than compounds **17** and **18**. Compound **8**, with a *mono* hydroxy substitution at *ortho* position of the phenyl ring, also exhibited an outstanding activity (IC_{50} = 19.1 ± 1.20 μM). Introduction of *meta*-methoxy and *meta*-ethoxy substitutions to compound **8** resulted in compounds **27** and **16**, respectively. Consequent outcomes of their inhibition potential against β-glucuronidase enzyme yielded IC_{50} values in the range of 21.82 ± 0.40 and 21.98 ± 0.32 μM, respectively. However, in compound **17**, where *meta*-position of the aromatic side chain is occupied by a chloro group resulted in much lower IC_{50} value (5.63 ±

Table 1 Schiff bases **2–29** of 4-phenyl-1,3-thiazol-2-amine (**1**)

Comp. No.	R	Comp. No.	R	Comp. No.	R
2		**12**		**22**	
3		**13**		**23**	
4		**14**		**24**	
5		**15**		**25**	
6		**16**		**26**	
7	$CH\text{-}CH_2\text{-}CH_3$	**17**		**27**	
8		**18**		**28**	
9		**19**		**29**	
10		**20**			
11		**21**			

0.16 μM). These findings suggest that decrease in activities of compounds **27** and **16** may be due to internal hydrogen bonding of *ortho*-hydroxyl and *meta*-methoxy/ethoxy groups. However, in case of *meta*-chloro analog **17**, hydrogen bonding element is somewhat diminished (Fig. 1).

Compounds **2** ($IC_{50} = 12.49 \pm 0.18\,\mu M$) and **28** ($IC_{50} = 10.73 \pm 0.17\,\mu M$) containing nitro groups at *para*- and *meta*-positions of the phenyl ring, respectively, showed similar activities. These results are comparable to those explained *vide supra* and suggests that the nature of atoms/groups (electron donating or withdrawing) has minimal or no effect on β-glucuronidase inhibition. Compound **29** having a bulky pyrene side chain ($IC_{50} = 16.15 \pm 0.83\,\mu M$) and compound **14** ($IC_{50} = 37.98 \pm 1.05\,\mu M$) having a 1-naphthalene ring instead of phenyl group showed a slight decline in activity. Compound **9** ($IC_{50} = 21.80 \pm 0.42\,\mu M$) having a vinyl-aromatic and likewise compound **7** ($IC_{50} = 152 \pm 3.35\,\mu M$) with an aliphatic substitution instead of phenyl residue also showed less activity. These observations suggest that the phenyl residue governs the β-glucuronidase inhibition potential of these compounds. Similarly, compound **11** ($IC_{50} = 16.64 \pm 1.37\,\mu M$) having a pyridyl group and compound **21** ($IC_{50} = 12.48 \pm 0.56\,\mu M$) with a *para*-cumenyl group showed an appreciable amount of activity suggesting that an aromatic residue directly attached to azomethine group greatly influenced the activity.

Table 2 In vitro β-glucuronidase inhibitory activities of thiazole Schiff bases

Compounds	$IC_{50} \pm SEM\mu M$	Compounds	$IC_{50} \pm SEM\mu M$	Compounds	$IC_{50} \pm SEM\mu M$
2	12.49 ± 0.18	**11**	16.64 ± 1.37	**21**	12.48 ± 0.56
3	9.84 ± 0.33	**12**	9.77 ± 0.11	**22**	18.43 ± 0.19
4	61 ± 1.05	**13**	12.27 ± 0.43	**23**	14.25 ± 0.72
5	19.86 ± 0.17	**14**	37.98 ± 1.05	**24**	44.14 ± 0.25
6	152 ± 3.35	**15**	51.48 ± 2.46	**25**	19.61 ± 0.32
7	152 ± 3.35	**16**	21.98 ± 0.32	**26**	45.36 ± 0.17
8	19.1 ± 1.20	**17**	5.63 ± 0.16	**27**	21.82 ± 0.40
9	21.8 ± 0.42	**18**	4.88 ± 0.12	**28**	10.73 ± 0.17
10	67.12 ± 2.29	**19**	52.7 ± 1.64	**29**	16.15 ± 0.83
D-Saccharic acid 1,4-lactone[a]	48.4 ± 1 .25	**20**	22.45 ± 0.70		

SEM standard error of the mean
[a] D-Saccharic acid 1,4-lactone, standard for the inhibitory activity for β-glucuronidase

Table 3 Priority order of various ligands based on their IC_{50} values

S. no.	Group	IC_{50} range ± SEM	Compounds (in ascending order w.r.t. IC_{50})
1	1	4–10	**18**, **17**, **12**, **3**, **28**
2	2	12–22	**13**, **21**, **2**, **23**, **29**, **11**, **22**, **8**, **25**, **5**, **9**, **27**, **16**, **20**
3	3[a]	44–52	**14**[b], **24**, **26**, **15**, **19**
4	4	> 61	**4**, **10**, **6**, **7**

[a] The compounds that lie within an IC_{50} range around the standard inhibitor, D-saccharic acid 1, 4-lactone, $IC_{50} = 48.4 \pm 1.25\,\mu M$
[b] Compound **14** has an IC_{50} of 37.98 ± 1.05

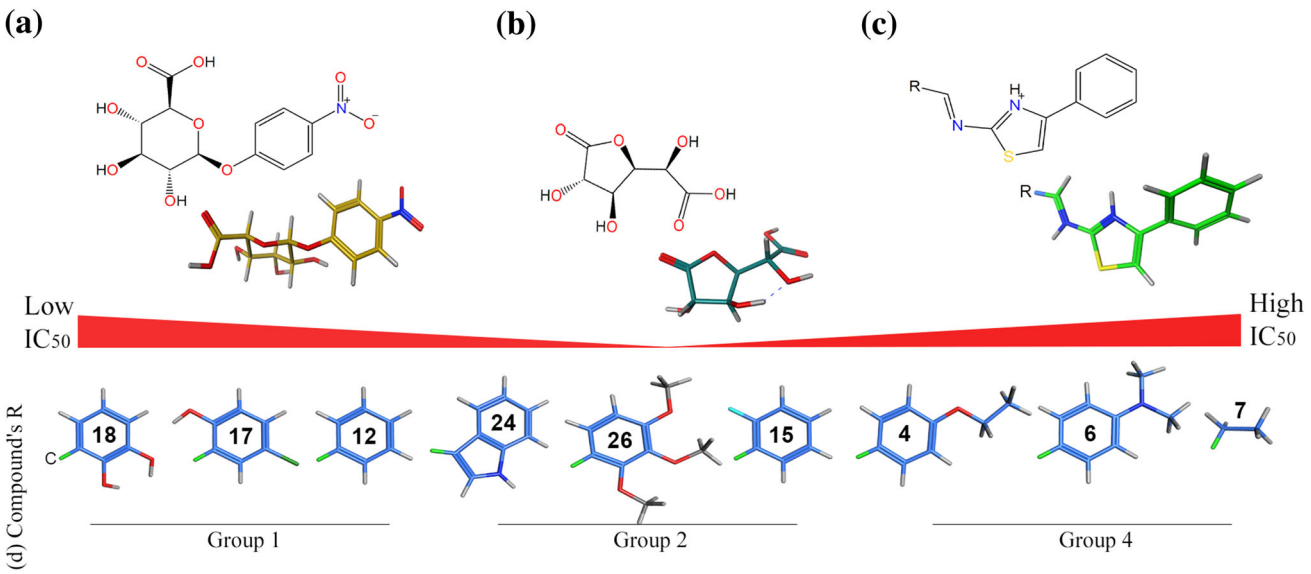


Fig. 1 The known substrate molecule—*p*-nitrophenyl β-D-glucuronide (*a*) and the standard inhibitor—D-saccharic acid 1,4-lactone (*b*) of β-glucuronidase are shown along with our compound 2-amino-4-phenyl-1,3-thiazole R (*c*). The selected derivatives (R) are shown in order of increasing IC_{50} (*d*). Color scheme: Carbons in (*a*), (*b*), (*c*), and (*d*) are colored *gold*, *sea green*, *light green*, and *light blue*. Hydrogen, oxygen, nitrogen, and sulfur molecules are colored *light gray*, *red*, *blue*, and *yellow*, respectively. Chlorine in **17** and Fluorine in **15** (*d*) are colored *green* and *bright blue*

Compounds **13** (IC_{50}= 12.27 ± 0.43 μM), **23** (IC_{50}= 14.25 ± 0.72 μM), and **20** (IC_{50}= 22.45 ± 0.7 μM) are differently *mono*- and *di*-substituted chloro compounds and their activities suggest that an *ortho*-substitution is better than a *para*-substitution; however, *di-ortho*-chloro substitution decreases the β-glucuronidase inhibitory activity to a great extent. Surprisingly, compound **15** (IC_{50}= 51.48 ± 2.46 μM) with an *ortho*-fluoro residue was found to be the least

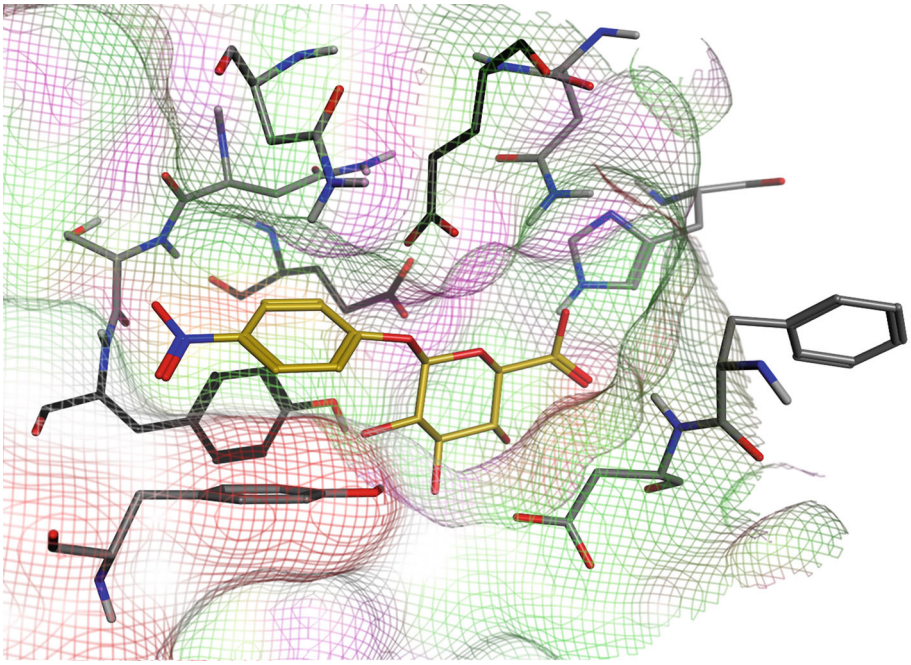


Fig. 2 Orientation of the key catalytic residues GLU451, GLU540, and TYR504 while the predicted binding pose of *p*-nitrophenyl β-glucuronic acid is shown

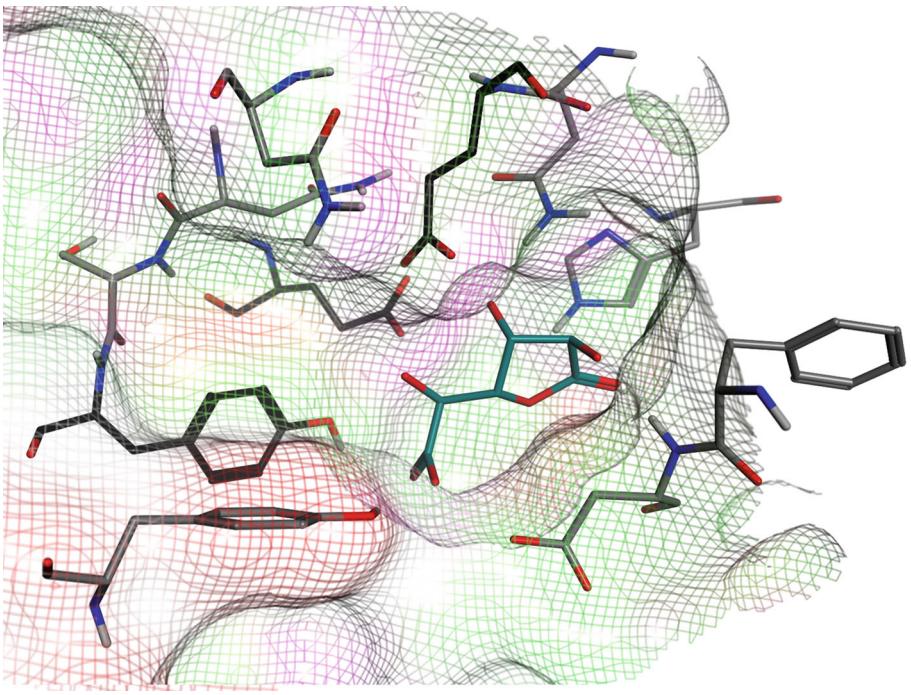


Fig. 3 Standard inhibitor in the active site of enzyme

active compound in halogen-containing compounds. Compound **22** (IC_{50}= 18.43 ± 0.19 μM) having a methyl substituted furan showed better activity as compared to indole-containing compound **24** (IC_{50}= 44.14 ± 0.25 μM).

Conclusively, these observations propose that SAR of compounds is largely dependent upon aromatic ring residue and substitutions over it. Further, internal hydrogen bonding in compounds also proved one factor that resulted in lowering of inhibitory potentials. Compound **18**, with two hydroxyl groups on aromatic ring of side chain, proved to be the most potent inhibitor among the current series.

Molecular modeling studies

Compounds **2–29** were docked into the active site of the enzyme using GOLD 4.1 version [29].

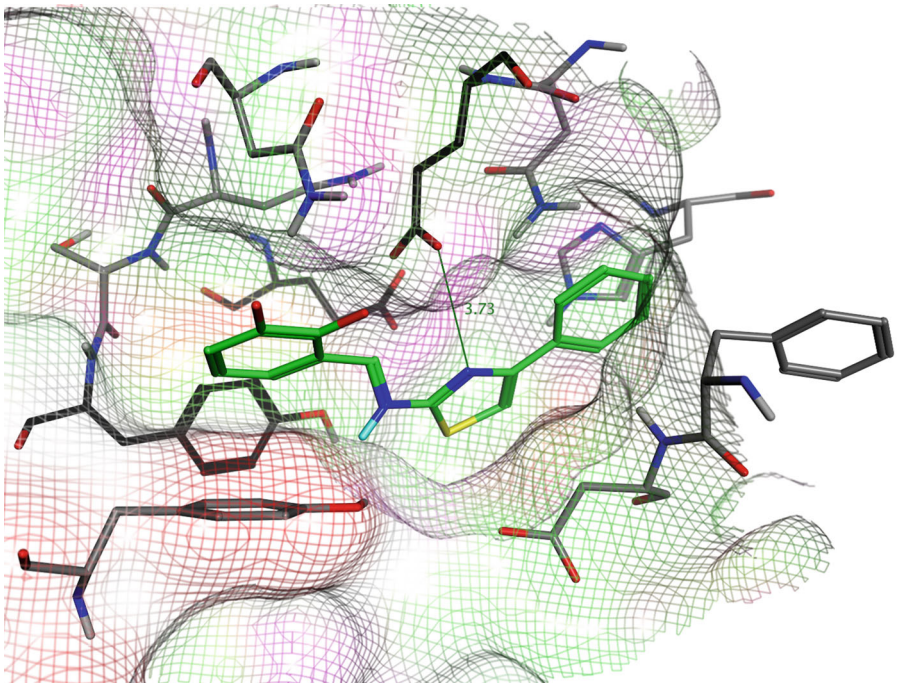


Fig. 4 Compound **18** with the lowest IC_{50} value in the active site of enzyme while blocking completely the entry pathway

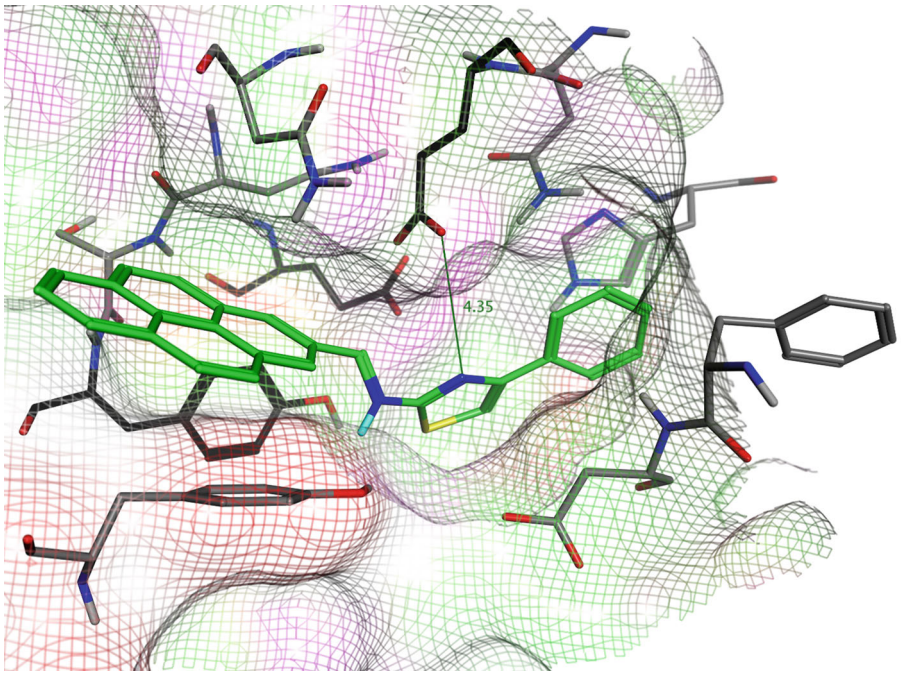


Fig. 5 Compound **29** docked into the active site of enzyme with the pyrene side chain bulged out from the pocket

Orientation of the key catalytic residues GLU451, GLU540, and TYR504 and the predicted binding pose of the standard substrate *p*-nitrophenyl β-glucuronic acid are shown in Fig. 2 while the predicted binding pose of the inhibitor is shown in Fig. 3. D-Saccharic acid 1,4-lactone fails to completely mask the catalytic residues thereby allowing *p*-nitrophenyl β-D-glucuronide to gain access to the catalytic residues. The location of the active site pocket and its topology indicates that ligands may gain access to it from the left side.

Contrarily, compounds **2–29** fit deep in the pocket, but unlike D-saccharic acid 1,4-lactone, the standard inhibitor, they extend toward the entry way and masked the catalytic residues of enzyme (Fig. 4). Striking analogy among the predicted binding poses for our compounds suggested that the main body of the molecule, i.e., phenylthiazolyl part of

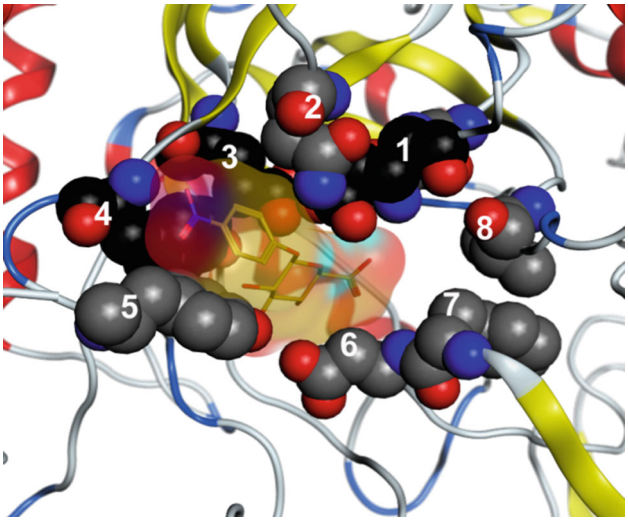


Fig. 6 Residues of the active site showing the predicted binding pose of the known substrate

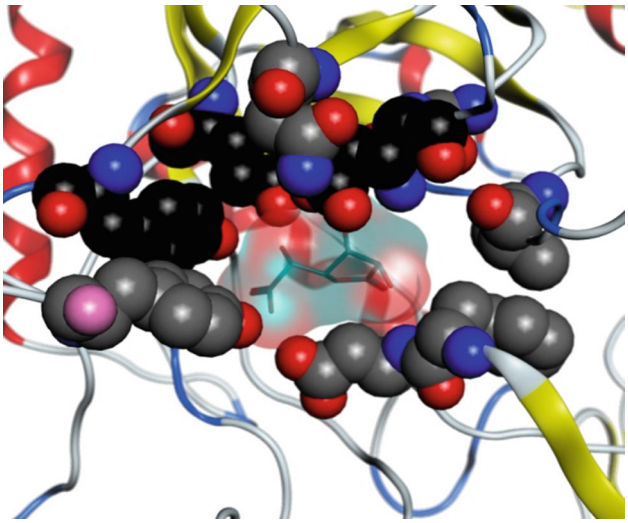


Fig. 7 Binding pose of standard inhibitor

the molecules is the first to gain access to the hydrophobic regions of the pocket and has the least impact on the IC_{50} variation. Therefore, it seems that the following three factors: (i) the substituent R on the compound, (ii) the direction of the lone pair on the nitrogen of azomethine part, and (iii) the distance between thiazole nitrogen and GLU451, determined the IC_{50} of these compounds. Compound **18** with two hydroxyl groups succeeds in making contacts with catalytic residues, resulting in a better accommodation of the ligand inside the pocket (Fig. 4). These extra interactions of polar groups such as hydroxyl on the side-chain residue resulted in the lowest IC_{50} value for compound **18**. On the other hand, compounds **12**, **14**, **21**, **25**, and **29** containing non-polar aromatic groups as their side chains lack such dipolar interactions and, therefore, cause reduced affinity (Figs. 5, 6, 7).

Molecular modeling studies showed that the presence of two hydroxyl groups on an aromatic ring in the side chain of compound **18** provided extra interactions for hydrogen bonding inside the active site pocket of enzyme.

Another important aspect is the long planar conformation, as for compound **29** that allowed it to properly fit deep inside the pocket, yet blocking the entry path. Moreover, the single covalent bond between thiazole and benzene ring in the main skeleton of the molecule provided more conformational flexibility, while allowing it to create interactions that stabilize the molecule within the pocket (Fig. 8).

Conclusions

In conclusion, a combined effort of chemistry and modeling studies identify a new class of β-glucuronidase inhibitors; and compound **18** was found to be the most interesting inhibitor with IC_{50} value of $4.8 \pm 0.12\,\mu M$. Two hydroxyl groups on benzene ring provided extra interactions within the pocket of enzyme by means of hydrogen bonding. Compounds **17**, **12**, **3**, **28**, **13**, **21**, and **2** also exhibited excellent inhibitory potentials; however, the compounds with non-polar displayed a relatively higher inhibitory concentrations due to lack of additional non-covalent interactions. These compounds may serve as viable lead molecules against β-glucuronidase inhibition for future research.

Experimental general

Melting points were determined on a Büchi 434 melting point apparatus and were uncorrected. Chemicals were purchased from Alfa Aesar and used without purification. Solvents were distilled through standard procedures. Solvents NMR experiments were performed on Avance Bruker AM 300, 400, and 500 MHz using deuterated solvents such as CD_3OD and $CDCl_3$. Splitting patterns were as follows: s, singlet; d, doublet; dd, double doublets; t, triplet; and m, multiplet. Chemical shifts are reported in δ (ppm) and coupling constants are given in Hz. Ultraviolet (UV) spectra were recorded on Perkin-Elmer Lambda-5 UV/Vis spectrometer 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 spectrometer, Germany. 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.

In vitro β-glucuronidase assay protocol

β-Glucuronidase activity was determined by measuring the absorbance at 405 nm of *p*-nitrophenol, formed from the sub-

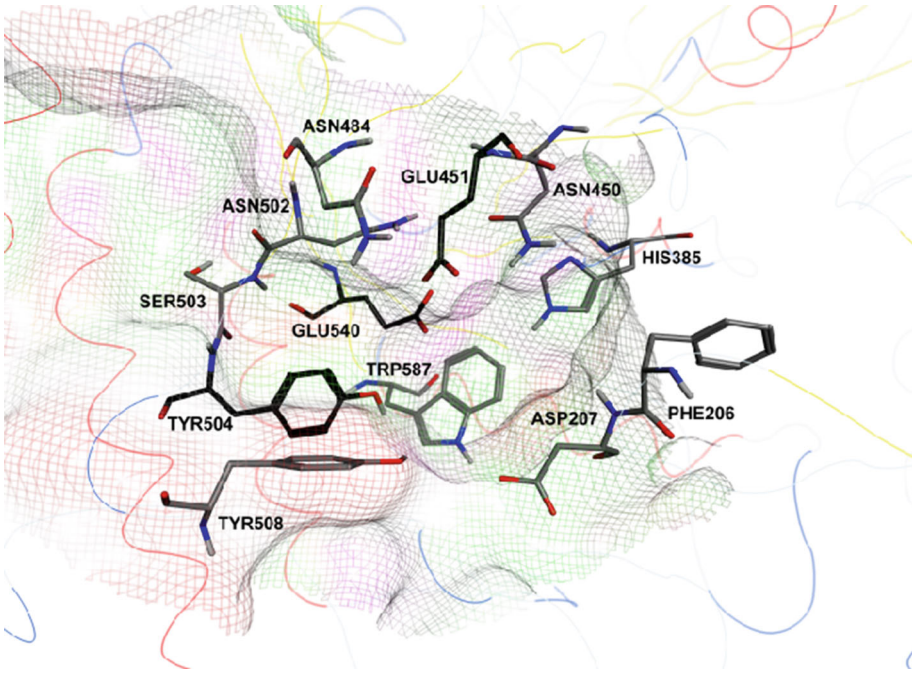


Fig. 8 Active site pocket of β-glucuronidase and possible direction of ligand entry

strate, by the spectrophotometric method. The total reaction volume was 250 μL. The reaction mixture containing 185 μL of 0.1 M acetate buffer, 5 μL of test compound solution, and 10 μL of enzyme solution was incubated at 37 °C for 30 min. The plates were read on a multiplate reader (SpectraMax plus 384, Applied Biosystem, CA, USA) at 405 nm after the addition of 50 μL of 0.4 mM *p*-nitrophenyl-β-D-glucuronide [27,28]. All the assays were run in triplicate.

Procedure for the synthesis of 4-phenyl-1,3-thiazol-2-amine

To a mixture of acetophenone (0.2 mol) and thiourea (0.4 mol), thionyl chloride (0.2 mol) as oxidizing agent was added and the reaction mixture was heated on a steam bath for 24 h. The progress of reaction was monitored by TLC; after the completion of reaction, the crude product was washed by *n*-hexane and crystallized from ethanol.

General procedure for the synthesis

To a mixture of 2-amino-4-phenyl thiazole (0.52 g, 3 mmol) and aldehydes (3 mmol), ethanol (15 mL) was added. The progress of reaction was monitored by TLC; after the completion of reaction, solvent was removed under vacuum on a rotary evaporator. The resulting product was crystallized from ethanol.

N-(4-Nitrobenzylidene)-4-phenylthiazol-2-amine (**2**)

Yield: 2.51 g (55 %); m.p. = 135.1°C; R_f = 0.64; (Hex:EtOAc, 7:3); UV (MeOH): λ_{max} 258 (log ε = 2.76) nm; IR (KBr): ν_{max} 2851, 1627, 1605, 1346 cm^{-1}, ^{1}H NMR (300 MHz, CD_3OD): δ 9.18 (s, 1H, =C<u>H</u>–S), 8.42 (s, 1H, N=C<u>H</u>), 8.39 (d, $J_{3',2'} = J_{5',6'}$ = 7.8Hz, 2H, H-3′, H-5′, Ar–<u>H</u>′), 8.14 (d, $J_{2',3'} = J_{6',5'}$ = 7.6 Hz, 2H, H-2′, H-6′, Ar–<u>H</u>′), 7.60 (m, 5H, H-2, H-3, H-4, H-5, H-6, Ar–<u>H</u>); EI MS: *m*/*z* (rel. abund. %), 309 (47.8), 262 (12.15), 176 (45.23).

4-(((4-Phenylthiazol-2-yl)imino)methyl)phenol (**3**)

Yield: 1.31 g (73 %); m.p. = 111.4°C; R_f = 0.91; (Hex:EtOAc, 7:3); UV (MeOH): λ_{max} 225 (log ε = 2.37) nm; IR (KBr): ν_{max} 3635, 3051, 1665, 1326 cm^{-1}, ^{1}H NMR (400 MHz, CD_3OD): δ 8.8 (s, 1H, =C<u>H</u>–S), 7.78 (s, 1H, N=C<u>H</u>), 6.92 (d, $J_{3',2'} = J_{5',6'}$ = 8.0Hz, 2H, H-3′, H-5′, Ar–<u>H</u>′), 6.77 (d, $J_{2',3'} = J_{6',5'}$ = 7.8Hz, 2H, H-2′, H-6′, *Ar*–<u>H</u>′), 7.59 (m, 5H, H-2, H-3, H-4, H-5, H-6, Ar–<u>H</u>); EI MS: *m*/*z* (rel. abund. %), 280 (36.4), 263 (6.8), 176 (35.0).

N-(4-Ethoxybenzylidene)-4-phenylthiazol-2-amine (**4**)

Yield: 1.19 g (26 %); m.p. = 117.4°C; R_f = 0.88; (Hex:EtOAc, 7:3); UV (MeOH): λ_{max} 236 (log ε = 2.79) nm; IR (KBr): ν_{max} 2980, 1671, 1331, 1245, cm^{-1}, ^{1}H NMR (300 MHz, CD_3OD): δ 8.5 (s, 1H, =C<u>H</u>–S), 7.78 (s, 1H, N=C<u>H</u>), 7.02 (d, $J_{3',2'} = J_{5',6'}$ = 7.7Hz, 2H, H-3′, H-5′, Ar–<u>H</u>′), 7.48 (d, $J_{2',3'} = J_{6',5'}$ = 7.8Hz, 2H, H-2′, H-6′, Ar–<u>H</u>′), 7.64 (m, 5H, H-2, H-3, H-4, H-5, H-6, Ar–<u>H</u>), 3.98 (q, 2H, $–CH_2$), 1.35 (s, 3H, $–CH_3$); EI MS: *m*/*z* (rel. abund. %), 308 (75.3), 263 (43.3), 208 (20.8).

N-(4-Methoxybenzylidene)-4-phenylthiazol-2-amine (**5**)

Yield: 0.59 g (69 %); m.p. = 116.4°C; R_f = 0.76; (Hex:EtOAc, 7:3); UV (MeOH): λ_{max} 232 (log ε = 2.35) nm.; IR (KBr): ν_{max} 1652, 1605, 1305, 1250, cm^{-1}, ^{1}H NMR (300 MHz, CD_3OD): δ 8.87 (s, 1H, =C<u>H</u>–S), 7.97 (s, 1H, N=C<u>H</u>), 7.33 (d, $J_{2',3'} = J_{6',5'}$ = 8.0Hz, 2H, H-2′, H-6′, Ar–<u>H</u>′), 6.88 (d, $J_{3',2'} = J_{5',6'}$ = 7.8Hz, 2H, H-3′, H-5′, Ar–<u>H</u>′), 7.53 (m, 5H, H-2, H-3, H-4, H-5, H-6, Ar–<u>H</u>); EI MS: *m*/*z* (rel. abund. %), 294 (100), 263 (6.8), 279 (7.20).

N-(4-(Dimethylamino)benzylidene)-4-phenylthiazol-2-amine (**6**)

Yield: 2.7 g (60 %); m.p. = 164.7°C; R_f = 0.71; (Hex:Et OAc, 7:3); UV (MeOH): λ_{max} 340 (log ε = 2.21) nm; IR (KBr): ν_{max} 2920, 1662, 1594, 1328 cm^{-1}, ^{1}H NMR (300 MHz, CD_3OD): 9.06 (s, 1H, =C<u>H</u>–S), δ 7.21 (s, 1H, N=C<u>H</u>), 7.24 (d, $J_{3',2'} = J_{5',6'}$ = 8.0Hz, 2H, H-3′, H-5′, Ar–<u>H</u>′), 7.73 (d, $J_{2',3'} = J_{6',5'}$ = 7.8Hz, 2H, H-2′, H-6′, Ar–<u>H</u>′), 7.55 (m, 5H, H-2, H-3, H-4, H-5, H-6, Ar–<u>H</u>), 3.29 (s, 6H, –CH_3); EI MS: *m*/*z* (rel. abund. %), 307 (46.4), 134 (18.5), 176 (6.2).

N-Butylidene-4-phenylthiazol-2-amine (**7**)

Yield: 0.38 g (60.3 %); m.p. = 203°C; R_f = 0.83; (Hex:EtOAc, 7:3); UV (MeOH): λ_{max} 241 (log ε = 2.91) nm; IR (KBr): ν_{max} 2964, , 1662, , 1592 1351 cm^{-1}, ^{1}H NMR (300 MHz, CD_3OD): δ 9.13 (s, 1H,=C<u>H</u>–S), 7.78 (s, 1H, N=C<u>H</u>), 1.83 (m, 2H, –CH_2), 1.03 (t, –CH_3), 7.67 (m, 5H, H-2, H-3, H-4, H-5, H-6, Ar–<u>H</u>); EI MS: *m*/*z* (rel. abund. %), 216 (17.0), 201 (12.3), 156 (20.6).

2-(((4-Phenylthiazol-2-yl)imino)methyl)phenol (**8**)

Yield: 0.59 g (72 %); m.p. = 133°C; R_f = 0.87; (Hex: EtOAc, 7:3); UV (MeOH): λ_{max} 241 (log ε = 2.64) nm; IR (KBr): ν_{max} 2993, 1690, 1603, 1328, cm^{-1}, ^{1}H NMR (400 MHz, CD_3OD): δ 8.70 (s, 1H, =C<u>H</u>–S), 7.78 (s, 1H, N=C<u>H</u>), 7.28 (m, 5H, H-2, H-3, H-4, H-5, H-6, Ar–<u>H</u>), 7.16 (m, H-3′, H-4′, H-5′, H-6′, 4H, Ar–<u>H</u>′); EI MS: *m*/*z* (rel. abund. %), 280 (20.8), 263 (7.5), 176 (35.0).

N-((E)-3-(4-Methoxyphenyl)allylidene)-4-phenylthiazol-2-amine (**9**)

Yield: 0.90 g (99 %); m.p. = 197.3°C; R_f = 0.81; (Hex: EtOAc, 7:3); UV (MeOH): λ_{max} 244 (log ε = 2.07) nm; IR (KBr): ν_{max} 1726, 1664, 1348, 1110 cm^{-1}, ^{1}H NMR (300 MHz, CD_3OD): δ 9.12 (s, =C<u>H</u>–S), 7.64 (s, 1H, N=C<u>H</u>), 7.04 (d, $J_{3',2'} = J_{5',6'}$ = 7.8 Hz, 2H, H-3′, H-5′Ar–<u>H</u>′), 7.95 (d, $J_{2',3'} = J_{6',5'}$ = 7.9Hz, 2H, H-2′, H-6′, Ar–<u>H</u>′), 7.42 (m, H-2, H-3, H-4, H-5, H-6, 5H, Ar–<u>H</u>); EI MS: *m*/*z* (rel. abund. %), 322 (28.1), 323 (7.2), 291 (14.9).

N-(4-(Methylthio)benzylidene)-4-phenylthiazol-2-amine (**10**)

Yield: 0.47 g (46.2 %); m.p. = 176.2°C; R_f = 0.81; (Hex:EtOAc, 7:3); UV (MeOH): λ_{max} 241.6 (log ε = 2.67) nm; IR (KBr): ν_{max} 2958, 1663, 1590, 1349, cm^{-1}, ^{1}H NMR (300 MHz, CD_3OD): δ 9.17 (s, 1H, =C<u>H</u>–S), 7.89 (s, 1H, N=C<u>H</u>), 7.57 (d, $J_{2',3'} = J_{6',5'}$ = 8.0Hz, 2H, H-2′, H-6′, Ar–<u>H</u>′), 7.38 (d, $J_{3',2'} = J_{5',6'}$ = 7.8Hz, 2H, H-3′, H-5′, Ar–<u>H</u>′), 7.43 (m, H-2, H-3, H-4, H-5, H-6, 5H, Ar–<u>H</u>); EI MS: *m*/*z* (rel. abund. %), 310 (9.0), 311 (3.5), 263 (5.4).

4-Phenyl-*N*-(pyridin-4-ylmethylene)thiazol-2-amine (**11**)

Yield: 0.46 g (61.3 %); m.p. = 171.8°C; R_f = 0.85; (Hex:EtOAc, 7:3); UV (MeOH): λ_{max} 239 (log ε = 2.62) nm; IR (KBr): ν_{max} 2924, 1659, 1604, 1286, cm^{-1}, ^{1}H NMR (500 MHz, CD_3OD): δ 8.73 (s, 1H, =C<u>H</u>–S), 7.96 (s, 1H, N=C<u>H</u>) 7.36 (d, $J_{3',2'} = J_{5',6'}$ = 7.9Hz, 2H, H-3′, H-5′, Ar–<u>H</u>′), 7.84 (d, $J_{2',3'} = J_{6',5'}$ = 7.6Hz, 2H, H-2′, H-6′, Ar–<u>H</u>′), 7.59 (m, 4H, H-2, H-3, H-5, H-6, Ar–<u>H</u>), 7.18 (t, 1H, H − 4′, Ar<u>H</u>′); EI MS: *m*/*z* (rel. abund. %), 265 (42.0), 267 (67.5), 253 (7.0).

N-Benzylidene-4-phenylthiazol-2-amine (**12**)

Yield: 0.21 g (27 %); m.p. = 192.4°C; R_f = 0.84; (Hex: EtOAc, 7:3); UV (MeOH): λ_{max} 240 (log ε = 2.42) nm; IR (KBr): ν_{max} 2857, 1662, 1605, 1250, cm^{-1}, ^{1}H NMR (400 MHz, CD_3OD): δ 8.80 (s, 1H, =C<u>H</u>–S), 7.78 (s, 1H, N=C<u>H</u>), 7.58 (m, 5H, H-2′, H-3′, H-4′, H-5′, H-6′, Ar–<u>H</u>′), 7.41 (m, 5H, H-2, H-3, H-4, H-5, H-6, Ar–<u>H</u>); EI MS: *m*/*z* (rel. abund. %), 264 (100), 186 (4.6), 108 (6.3).

N-(2-Chlorobenzylidene)-4-phenylthiazol-2-amine (**13**)

Yield: 0.47 g (60 %); m.p. = 146.4°C; R_f = 0.85; (Hex: EtOAc, 7:3); UV (MeOH): λ_{max} 238 (log ε = 2.43) nm; IR (KBr): ν_{max} 1660, 1607, 1328, 754cm^{-1}, ^{1}H NMR (300 MHz, CD_3OD): δ 8.83 (s, 1H, =C<u>H</u>–S), 7.81 (s, 1H, N=C<u>H</u>), 7.54 (m, 2H, H-3′, H-6′, Ar–<u>H</u>'), 7.43 (m, 2H, H-4′, H-5′, Ar–<u>H</u>′), 7.32 (m, 5H, H-2, H-3, H-4, H-5, H-6, Ar–<u>H</u>); EI MS: *m*/*z* (rel. abund. %), 298 (35.5), 301 (8.3), 221 (10.1).

N-(Naphthalen-1-ylmethylene)-4-phenylthiazol-2-amine (**14**)

Yield: 0.33 g (36 %); m.p. = 148.2°C; R_f = 0.78; (Hex:Et OAc, 7:3); UV (MeOH): λ_{max} 222 (log ε = 2.54) nm.; IR (KBr): ν_{max} 3062, 1654, 1617, 1213, cm^{-1}, ^{1}H NMR (300

MHz, CD_3OD): δ 9.06 (s, 1H, =CH–S), 7.99 (s, 1H, N=CH), 7.37 (m, 4H, H-2, H-3, H-5, H-6, Ar–H), 7.60 (m, 1H, H-4, Ar–H), 7.75 (m, 1H, H-6′, Ar–H′), 7.33(d, $J_{2',3'}$ = 8.0Hz, 1H, H − 2′, Ar − H′), 7.78 (d, 1H, 4′Ar–H′), 7.57 (t, 1H, H-3′, Ar–H′), 8.12 (m, 1H, H-8′, Ar–H′), 8.06 (t,1H, H-7′, Ar–H′), 7.93 (d, $J_{5',6'}$ = 7.9Hz, 1H, H-5′, Ar–H′); EI MS: m/z (rel. abund. %), 314 (23.1), 315 (6.2), 273 (8.8).

N-(2-Fluorobenzylidene)-4-phenylthiazol-2-amine (**15**)

Yield: 0.31 g (37.3 %); m.p. = 217.5°C; R_f = 0.91; (Hex:EtOAc, 7:3); UV (MeOH): λ_{max} 24 (log ε = 2.92) nm; IR (KBr): ν_{max} 1663, 1613, 1335, 1087cm^{-1}, ^{1}H NMR (300 MHz, CD_3OD): δ 8.74 (s, 1H, =CH–S), 7.63 (s, 1H, N=CH), 7.55 (m, 2H, H-4′, H-6′, Ar–H′), 7.52 (m, 1H, H-4, Ar–H), 7.38 (m, 2H, H-3′, H-5′, Ar–H′), 7.38 (m, 4H, H-2, H-3, H-5, H-6, Ar–H); EI MS: m/z (rel. abund. %), 282 (44.6), 284 (29.2), 241 (17.6).

2-Ethoxy-6-(((4-phenylthiazol-2-yl)imino)methyl)phenol (**16**)

Yield: 0.45 g (47.3 %); m.p. = 132°C; R_f = 0.80; (Hex: EtOAc, 7:3); UV (MeOH): λ_{max} 238 (log ε = 2.94) nm; IR (KBr): ν_{max} 1693, 1217, 1271, 1615cm^{-1}, ^{1}H NMR (300 MHz, CD_3OD): δ 9.02 (s, 1H, =CH–S), 7.16 (s, 1H, N=CH), 6.98 (d or dd $J_{4',5'}$ = $J_{6',5'}$ = 8.0Hz, 2H, H-4′, *H*-6′, Ar–H′), 7.50 (m, 1H, H-5′, Ar–H′), 7.45 (m, 5H, H-2, H-3, H-4, H-5, H-6, Ar–H); EI MS: m/z (rel. abund. %), 324 (77.4), 309 (47.6), 263 (8.8).

4-Chloro-2-(((4-phenylthiazol-2-yl)imino)methyl)phenol (**17**)

Yield: 0.68 g (70.6 %); m.p. = 115.7°C; R_f = 0.89; (Hex:EtOAc, 7:3); UV (MeOH): λ_{max} 240 (log ε = 2.91) nm; IR (KBr): ν_{max} 2875, 1616, 1275, 540 cm^{-1}, ^{1}H NMR (300 MHz, CD_3OD): δ 9.13 (s, 1H, =CH–S), 7.78 (s, 1H, N=CH), 6.83 (d, $J_{3',4'}$ = 7.9Hz, 1H, H-3′, Ar–H′), 7.04 (s, 1H, H-6′, Ar–H′), 7.48 (m = 1H, H-4′Ar–H′), 7.48 (m = 1H, H-4, Ar–H), 7.42 (m, 4H, H-2, H-3, H-5, H-6, Ar–H); EI MS: m/z (rel. abund. %), 314 (77.8), 315 (18.1), 297 (34.6).

3-(((4-Phenylthiazol-2-yl)imino)methyl)benzene-1,2-diol (**18**)

Yield: 0.15 g (38 %); m.p. = 142°C; R_f = 0.77; (Hex: EtOAc, 7:3); UV (MeOH): λ_{max} 239 (log ε = 2.64) nm; IR (KBr): ν_{max} 3285, 1695, 1617, 1282, cm^{-1}, ^{1}H NMR (300 MHz, CD_3OD): δ 8.91 (s, 1H, =CH–S), 7.78 (s, 1H, N=CH), 7.43 (m = 3H, H-4′, H-5′, H-6′, Ar–H′), 7.32 (m, 5H, H-2, H-3, H-4, H-5, H-6, Ar–H); EI MS: m/z (rel. abund. %), 296 (43.3), 218 (4.12), 176 (34.7).

N-(3,4-Dimethoxybenzylidene)-4-phenylthiazol-2-amine (**19**)

Yield: 0.68 g (71.5 %); m.p. = 119°C; R_f = 0.85; (Hex:Et OAc, 7:3); UV (MeOH): λ_{max} 234.2 (log ε = 2.99) nm; IR (KBr): ν_{max}, 1680, 1271, 1135, 1013 cm^{-1}, ^{1}H NMR (300 MHz, CD_3OD): δ 9.02 (s, 1H, =CH–S), 7.83 (s, 1H, N=CH), 7.13 (s, 1H, H-2′, Ar–H′), 6.84 (d, $J_{5',6'}$ = 7.9Hz, 1H, H-5′, Ar–H′), 7.37 (d, $J_{6',5'}$ = 8.0Hz, 1H, H-6′, Ar–H′), 7.54 (m, 5H, H-2, H-3, H-4, H-5, H-6, Ar–H); EI MS: m/z (rel. abund. %), 324 (13.6), 325 (2.12), 224 (11.1).

N-(2,6-Dichlorobenzylidene)-4-phenylthiazol-2-amine (**20**)

Yield: 0.13 g (26 %); m.p. = 162.7°C; R_f = 0.80; (Hex:Et OAc, 7:3); UV (MeOH): λ_{max} 239.4 (log ε = 2.55) nm; IR (KBr): ν_{max} 1622, 1202, 779, 696, cm^{-1}, ^{1}H NMR (300 MHz, CD_3OD): δ 9.14 (s, 1H, =CH–S), 7.78 (s, 1H, N=CH), 7.30 (m, 3H, H-3′, H-4′, H-5′, Ar–H′), 7.66 (m, 5H, H-2, H-3, H-4, H-5, H-6, Ar–H); EI MS: m/z (rel. abund. %), 333 (3.42), 297 (38.6), 255 (3.64).

N-(4-Isopropylbenzylidene)-4-phenylthiazol-2-amine (**21**)

Yield: 0.16 g (38 %); m.p. = 158.2°C; R_f = 0.80; (Hex: EtOAc, 7:3); UV (MeOH): λ_{max} 239.8 (log ε = 2.53) nm; IR (KBr): ν_{max} 2960, 1659, 1617, 1282, cm^{-1}, ^{1}H NMR (500 MHz, CD_3OD): δ 8.89 (s, 1H, =CH–S), 7.93 (s, 1H, N=CH), 7.33 (d, $J_{3',2'}$ = $J_{5',6'}$ = 8.0Hz, = 8.0Hz, 2H, H-3′, H-5′, Ar–H′), 7.52 (d, $J_{2',3'}$ = $J_{6',5'}$ = 7.8Hz, = 7.8Hz, 2H, H-2′, H-6′, Ar–H′), 2.97 (d, $J_{1'',2''}$ = 7.7 Hz, 6H, $CH(CH_3)_2$), 2.89 (m, 1H, $CH(CH_3)_2$) 7.52 (m, 5H, H-2, H-3, H-4, H-5, H-6, Ar–H); EI MS: m/z (rel. abund %), 306 (72.8), 263 (34.2), 291 (5.4).

N-((5-Methylfuran-2-yl)methylene)-4-phenylthiazol-2-amine (**22**)

Yield: 0.02 g (90 %); m.p. = 150°C; R_f = 0.74; (Hex: EtOAc, 7:3); UV (MeOH): λ_{max} 240 (log ε = 2.60) nm; IR (KBr): ν_{max} 1659, 1618, 1335, 1266cm^{-1}, ^{1}H NMR (300 MHz, CD_3OD): δ 8.82 (s, 1H, =CH–S), 7.69 (s, 1H, N=CH), 7.17 (d, $J_{2',3'}$ = 7.9Hz, 1H, H-2′, Ar–H′), 6.23 (d, $J_{3',2'}$ = 7.8Hz, 1H, H-3′, Ar–H′), 1.28 (s, 3H,–CH_3), 7.46 (m, 5H, H-2, H-3, H-4, H-5, H-6, Ar–H); EI MS: m/z (rel. abund. %), 268 (14.1), 253 (4.2), 190 (4.0).

N-(4-Chlorobenzylidene)-4-phenylthiazol-2-amine (**23**)

Yield: 0.16 g (40 %); m.p. = 216.8°C; R_f = 0.71; (Hex:EtOAc, 7:3); UV (MeOH): λ_{max} 240.4 (log ε = 2.67)

nm; IR (KBr): ν_{max} 1620, 1663, 1347, 773 cm^{-1}, ^{1}H NMR (300 MHz, CD_3OD): δ 8.91 (s, 1H, =CH–S), 7.98 (s, 1H, N=CH), 7.52 (d, $J_{3',2'} = J_{5',6'} = 8.0$ Hz, 2H, H-3′, H-5′, Ar–H′), 7.18 (d, $J_{2',3'} = J_{6',5'} = 7.8$ Hz, 2H, H-2′, H-6′, Ar–H′), 7.52 (m, 5H, H-2, H-3, H-4, H-5, H-6, Ar–H); EI MS: m/z (rel. abund. %), 298 (57.7), 263 (19.0), 220 (3.4).

N-((1H-Indol-3-yl)methylene)-4-phenylthiazol-2-amine (**24**)

Yield: 0.55g (62 %); m.p. = 139.4°C; R_f = 0.78; (Hex: EtOAc, 7:3); UV (MeOH): λ_{max} 293.8 (log ε = 3.07) nm; IR(KBr): ν_{max} 3171, 1635, 1577, 1335cm^{-1}, ^{1}H NMR (300 MHz, CD_3OD): δ 9.08 (s, 1H, =CH–S), 8.08 (s, 1H, N=CH), 8.13 (m, 4H, H-4, H-5, H-6, H-7), 7.46 (m, 5H, H-2, H-3, H-4, H-5, H-6, Ar–H); EI MS:m/z (rel. abund. %), 303 (94.4), 225 (4.5), 262 (20.2).

N-(Naphthalen-2-ylmethylene)-4-phenylthiazol-2-amine (**25**)

Yield: 0.24 g (26 %); m.p. = 141.7°C; R_f = 0.76; (Hex: EtOAc, 7:3); UV (MeOH): λ_{max} 282.6 (log ε = 2.85) nm; IR (KBr): ν_{max}, 3051, 1681, 1621, 1338 cm^{-1}, ^{1}H NMR (300 MHz, CD_3OD): δ 9.16 (s, 1H, =CH–S), 8.92 (s, 1H, N=CH), 7.37 (m, 4H, H-2, H-3, H-5, H-6, Ar-H), 7.60 (m, 1H, H-4, Ar-H), 7.75 (m, 1H, H-6′, Ar–H′), 7.73(d, $J_{2',3'}$ = 8.0 Hz, 1H, H-2′, Ar–H′), 7.87 (d, $J_{4',3'}$ = 8.0 Hz, 1H, 4′, Ar–H′), 7.57 (t, 1H, H-3′, Ar–H′), 8.12 (m, 1H, H-8′, Ar–H′), 7.95 (d, $J_{5',6'}$ = 7.9Hz, 1H, H-5′, Ar–H′), 7.93(t, 1H, H-7′, Ar–H′); EI MS: m/z (rel. abund. %), 314 (57.1), 236 (10.4), 186 (5.0).

4-Phenyl-*N*-(2,3,4-trimethoxybenzylidene)thiazol-2-amine (**26**)

Yield: 0.06 g (12 %); m.p. = 119°C; R_f = 0.81; (Hex: EtOAc, 7:3); UV (MeOH): λ_{max} 237.2 (log ε = 2.53) nm; IR (KBr): ν_{max} 1664, 1351, 1283, 1202, , cm^{-1}, ^{1}H NMR (300 MHz, CD_3OD): δ 9.11 (s, 1H, =CH–S), 8.56 (s, 1H, N=CH), 7.13 (d, $J_{6',5'}$ = 8.0Hz, 1H, H-6′, Ar–H′), 6.78 (d, $J_{5',6'}$ = 8.0Hz, 1H, H-5′, Ar–H′), 3.83(s, 6H, O–CH_3), 7.46 (m, 5H, H-2, H-3, H-4, H-5, H-6, Ar–H); EI MS: m/z (rel. abund. %), 354 (43.6), 323 (2.5), 279 (13.1).

2-Methoxy-6-(((4-phenylthiazol-2-yl)imino)methyl)phenol (**27**)

Yield: 0.07 g (17 %); m.p. = 128.1°C; R_f = 0.77; (Hex:EtOAc, 7:3); UV (MeOH): λ_{max} 240.8 (log ε = 2.88) nm; IR (KBr): ν_{max} 3276, 1694, 1272, 1220 cm^{-1}, ^{1}H NMR (300 MHz, CD_3OD): δ 9.26 (s, 1H, =CH–S), 8.56 (s, 1H, N=CH), 7.38 (m, 3H, H-4′, H-5′, H-6′, Ar–H′), 3.72 (s, 3H, O–CH_3), 7.53 (m, 5H, H-2, H-3, H-4, H-5, H-6, Ar–H); EI MS: m/z (rel. abund. %), 310 (71.7), 279 (10.4), 293 (8.8), 232 (4.5).

N-(3-Nitrobenzylidene)-4-phenylthiazol-2-amine (**28**)

Yield: 0.04 g (10 %); m.p. = 126.6°C; R_f = 0.87; (Hex: EtOAc, 7:3); UV (MeOH): λ_{max} 243.6 (log ε = 2.69) nm; IR (KBr): ν_{max} 1698, 1611, 1350, 1203, cm^{-1}, ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.03 (s, 1H, =CH–S), 8.81 (s, 1H, N=CH), 8.68 (s, 1H, H-2′, Ar–H′), 8.34 (d, $J_{6',5'}$ = 7.8Hz, 1H, H-6′, Ar–H′), 7.98 (d, $J_{4',5'}$ = 7.8Hz, 1H, H-4′, Ar–H′), 7.77(m, 2H, H-5′, H-6′, Ar–H′), 7.67 (m, 5H, H-2, H-3, H-4, H-5, H-6, Ar–H); EI MS: m/z (rel. abund. %), 309 (41.3), 262 (9.7), 176 (16.2).

4-Phenyl-*N*-(pyren-2-ylmethylene)thiazol-2-amine (**29**)

Yield: 0.08 g (13 %); m.p. = 148°C; R_f = 0.88; (Hex: EtOAc, 7:3); UV (MeOH): λ_{max} 241.6 (log ε = 2.52) nm; IR (KBr): ν_{max}, 2695, 1643, 1614, 1086cm^{-1}, ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.13 (s, 1H, =CH–S), 8.36 (s, 1H, N=CH), 7.51 (m, 9H, H-2′, H-3′, H-4′, H-5′, H-6′, H-7′, H-8′, H-9′, H-10′, Ar–H′), 7.40 (m, 5H, H-2, H-3, H-4, H-5, H-6, Ar–H); EI MS: m/z (rel. abund. %), 390 (29.4), 313 (4.8), 388 (37.2).

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