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2,5-Disubstituted thiadiazoles as potent β-glucuronidase inhibitors; Synthesis, *in vitro* and *in silico* studies

Muhammad Taha[a], Noor Barak Almandil[a], Umer Rashid[b], Muhammad Ali[c], Mohamed Ibrahim[a], Mohammed Gollapalli[d], Ashik Mosaddik[a], Khalid Mohammed Khan[a,e]

[a] Department of Clinical Pharmacy, Institute for Research and Medical Consultations (IRMC), Imam Abdulrahman Bin Faisal University, P.O. Box1982, Dammam 31441, Saudi Arabia
[b] Department of Chemistry, COMSATS University, Islamabad, Pakistan
[c] Natural and Medical Plants Science Research Center, University of Nizwa, P.O. Box 33, Birkat Al Mauz, Nizwa 616, Oman
[d] College of Computer Science & Information Technology (CCSIT), Imam Abdulrahman Bin Faisal University, P.O. Box 1982, Dammam 31441, Saudi Arabia
[e] H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan

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ABSTRACT

Twenty-five thiadiazole derivatives **1–25** were synthesized from methyl 4-methoxybenzoate *via* hydrazide and thio-hydrazide intermediates, and evaluated for their potential against β-glucuronidase enzyme. Most of the compounds including **1** (IC_{50} = 26.05 ± 0.60 μM), **2** (IC_{50} = 42.53 ± 0.80 μM), **4** (IC_{50} = 38.74 ± 0.70 μM), **5** (IC_{50} = 9.30 ± 0.29 μM), **6** (IC_{50} = 6.74 ± 0.26 μM), **7** (IC_{50} = 18.40 ± 0.66 μM), and **15** (IC_{50} = 18.10 ± 0.53 μM) exhibited superior activity potential than the standard D-saccharic acid-1,4-lactone (IC_{50} = 48.4 ± 1.25 μM). Molecular docking studies were conducted to correlate the *in vitro* results and to identify possible mode of interaction with enzyme active site.

1. Introduction

Thiadiazole nucleus is one of the most important and well-known heterocyclic nuclei that is present in variety of medicinal agents as well as in natural products. Among four isomers of thiadiazole, the isomer 1,3,4-thiadiazole constitutes an important class of heterocyclic compounds due to its important biological activities. 1,3,4-Thiadiazole nucleus is present as a core structural component in a range of medicinal compounds, agriculture, and other fields of technology [1]. Many 1,3,4-thiadiazoles have been patented in the agricultural field as herbicides and bactericides. Further to this, a broad spectrum of biological potentials related to 1,3,4-thiadiazole nucleus has been reported that includes antimicrobial [2–15], antifungal [16–18], antidiabetic [19], anti-inflammatory [20,21], antileishmanial [22], antituberculosis [23], anticancer [24], anti-HIV [25], antioxidant/radio protective [26], carbonic anhydrase [27], and urease inhibitory activities [28].

β-Glucuronidase is a lysosomal enzyme, its specific task is to catalyze the cleavage of glucuronosyl-*O*-bonds. It degrades glucuronic acid-containing glycosaminoglycan, such as heparan sulfate, chondroitin sulfate and dermatan sulfate. Over-expression of this enzyme has reported in various types of malignancies such as breast, lung, and gastrointestinal tract carcinomas, and melanomas. Elevated level of β-glucuronidase in bronchial tumors has also been observed. Elevated activity of this enzyme was also reported in borderline tuberculoid and lepromatous patients [29].

Immense experience of our research group in the field of computer-aided drug design (CADD) and synthesis of chemical entities as putative drugs for the treatment of various diseases prompted us to further explore new inhibitors for β-glucuronidase as lead compounds. We have already reported various classes of compounds including coumarin diones, thiadiazole containing aryl and *N*-aryl rings, thiazole Schiff bases, and unsymmetrical heterocyclic thiourea as β-glucuronidase inhibitors (Fig. 1) [30–33]. Previously, we identified the 2,5-disubstituted thiadiazoles as new class of β-glucuronidase inhibitors. In the current study, we adopted a chemical transformation which leads to the 2,5-disubstitutes thiazole molecules, in which −NH part of *N*-aryl group is lacking, in order to see its potential effect on the β-glucuronidase inhibitory activity. Furthermore, we used different substitutions on ring B, while keeping the methoxy phenyl ring (Ring A) as permanent feature (Fig. 1). Thus the synthetic compounds having two aryl ring directly linked with thiadiazole scaffold were synthesized and explored for their potential inhibitory activity against β-glucuronidase enzyme. To the best of our knowledge these compounds have never been tested against β-glucuronidase enzyme previously.

E-mail address: mtaha@iau.edu.sa (M. Taha).

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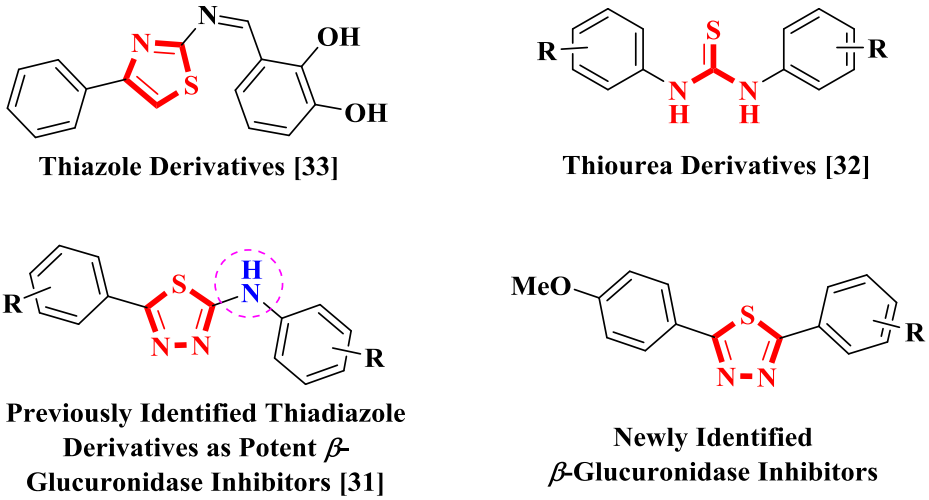


Fig. 1. Previously and newly identified β-glucuronidase inhibitors.

2. Results and discussion

2.1. Chemistry

Methyl 4-methoxybenzoate was refluxed in methanolic hydrazine hydrate solution for 6 h to obtain corresponding hydrazide. Resulting hydrazide upon treatment with Lawson's reagent in toluene was refluxed to yield corresponding thio-analogue. Thiohydrazide was then treated with various aryl aldehydes to form cyclized adducts **1–25** (Table 1) in the presence of $POCl_3$ (Scheme 1).

2.2. β-Glucuronidase inhibition

All the synthetic compounds **1–25** showed excellent β-glucuronidase inhibitory activity in the range of IC_{50} = 6.74 ± 0.26–52.36 ± 1.29 μM compared to the standard D-saccharic acid-1,4-lactone (IC_{50} = 48.4 ± 1.25 μM). Limited structure-activity relationship (SAR) was established by analyzing the effect of varying features such as R group at ring B as shown in Fig. 2.

Amongst all synthesized derivatives, hydroxy substituted analogs were found to be excellent inhibitors. It was observed that 2-hydroxy substituted derivative **15** (IC_{50} = 18.10 ± 0.53 μM) was found to be more active than 4-hydroxy and 3-hydroxy substituted analogs **1** (IC_{50} = 26.05 ± 0.60 μM) and **2** (IC_{50} = 42.53 ± 0.80 μM), respectively. Which gives a perception that hydroxy at position-2 and -4 interacted more efficiently with the active site of enzyme, while a sharp change in activity was observed when the hydroxy group was at position-3. Amongst the di-hydroxy substituted analogs, 2,4-dihydroxy substituted derivative **6** (IC_{50} = 6.74 ± 0.26 μM) was found to be the most potent analog of the current library. Changing positions of the two hydroxy groups brought changes in the inhibitory potential. Such as 2,3-dihydroxy substituted compound **5** (IC_{50} = 9.30 ± 0.29 μM) showed slightly decreased inhibitory activity as compared to the derivative **6**. Similarly, 2,5- and 3,4-dihydroxy analogs **4** (IC_{50} = 38.74 ± 0.70 μM) and **7** (IC_{50} = 18.40 ± 0.66 μM), respectively, showed superior activity than the standard the standard D-saccharic acid-1,4-lactone (IC_{50} = 48.4 ± 1.25 μM) (Fig. 3). Thus, it can be concluded that di hydroxy analogs were found to have more potential in comparison with mono hydroxy compounds.

Compounds bearing solely methoxy groups such as **3**, **19**, and **23** were found to be completely inactive. However, incorporation of hydroxy with methoxy substitution brought out inhibitory activity. Such as compounds **9** (IC_{50} = 48.95 ± 1.04 μM), **10** (IC_{50} = 38.44 ± 0.76 μM), and **12** (IC_{50} = 42.44 ± 0.88 μM) bearing hydroxy along with methoxy at different positions showed comparable inhibition potential to the standard D-saccharic acid-1,4-lactone (IC_{50} = 48.4 ± 1.25 μM). Among them, compound **10** with 2-hydroxy and 4-methoxy substituents was more active, however, shifting of hydroxy group to position-3 and methoxy to position-5 in compounds **9** and **12**, respectively, resulted in decreased inhibition (Fig. 4).

Amongst the chloro substituted derivatives, 4-chloro substituted derivative **21** (IC_{50} = 39.00 ± 0.88 μM) showed superior activity than 3-chloro substituted analog **8** (IC_{50} = 52.36 ± 1.29 μM). Which indicates that chloro at position-4 is playing a crucial role in the inhibitory potential. All methyl substituted analogs **13**, **14**, and **16** were found to be completely inactive (Fig. 5).

Among heterocyclic ring bearing analogs only compound **18** IC_{50} = 45.72 ± 0.98 μM) having 2-pyridyl ring showed good activity. In general, it was deduced that hydroxy substituted derivatives were found to be more potent than compounds bearing methoxy with hydroxy group. Furthermore, chloro bearing derivatives also showed comparable inhibitory potential to the standard D-saccharic acid-1,4-lactone (IC_{50} = 48.4 ± 1.25 μM).

2.3. Docking studies

Human β-glucuronidase acts as an exoglycosidase in lysosomes and is involved in stepwise degradation of glucuronic acid-containing glycosaminoglycans (GAGs) including heparin sulfate, dermitant sulfate, and chondroitin sulfate. The functional form of GUS is a tetramer of four identical subunits of 75,000 Da [34]. Computational analysis of designed inhibitors by docking simulations was carried out to determine the binding orientation, affinity and the binding energy of the tested inhibitors. These analyses were carried out by using Gold 5.4.1 [35].

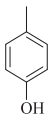
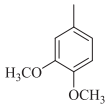
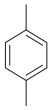
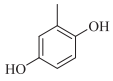
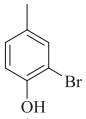
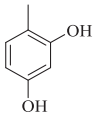
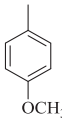
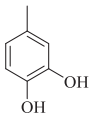
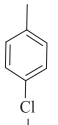
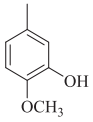
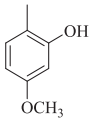
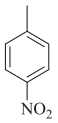
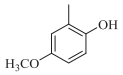
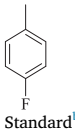
2.4. Validation of GOLD accuracy

In order to check the reliability of the docking method, known physiological substrate *p*-nitrophenyl β-glucuronide was docked into the active site of human β-glucuronidase (1BHG) (http://www.rcsb.org/pdb). The docking results showed that the docked substrate was properly oriented and showed interactions with catalytically important residues Glu451 and Glu540.

2.5. Binding affinities of synthesized compounds

Gold Score was selected as the criteria for the selection of compounds since it serves as fitness function for the orientation and estimates of binding affinity. In general, thiadiazole derivatives with

Table 1
β-Glucuronidase inhibitory potential of compounds **1–25.**

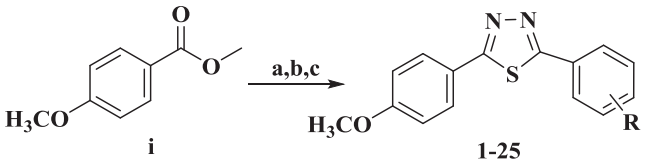
S. No.	R-	IC_{50} ± SEM[a] (μM)	S. No.	R-	IC_{50} ± SEM[a] (μM)
1		26.05 ± 0.60	14		[c]N.A.
2		42.53 ± 0.80	15		18.10 ± 0.53
3		[c]N.A.	16		[c]N.A.
4		38.74 ± 0.70	17		[c]N.A.
5		9.30 ± 0.29	18		45.72 ± 0.98
6		6.74 ± 0.26	19		[c]N.A.
7		18.40 ± 0.66	20		[c]N.A.
8		52.36 ± 1.29	21		39.00 ± 0.88
9		48.95 ± 1.04	22		[c]N.A.
10		38.44 ± 0.76	23		[c]N.A.
11		[c]N.A.	24		[c]N.A.
12		42.44 ± 0.88	25		[c]N.A.
13		[c]N.A.	Standard[b]		48.4 ± 1.25

[a] SEM is the standard error of the mean.
[b] D-saccharic acid-1,4-lactone is standard inhibitor for *β*-glucuronidase activity.
[c] N.A. stands for "Not Active".

R = aryldiols (compounds **4–7**) exhibited highest binding affinities, *i.e.* lower binding energies into the binding site of 1BHG. These compounds fitted properly into the groove of the binding site (Fig. 6).

Compound **5**, **6**, and **7** exhibited GOLD fitness score −60.65, −61.97 and −58.94 respectively. These lower binding energies are mainly attributed to hydrogen bond formation between the diols of the docked molecules and the key amino acid residues. Fig. 6 revealed that aryldiol ring of compounds **5**, **6**, and **7** are involved in hydrogen bonding interactions and formed 3–4 hydrogen bonds with Asp207, Glu451 and Glu541. Van der Waals interaction of thiadiazole ring also enhances the fitting into the active site gorge. Compound **6** anchors itself in such a way that enables a strong hydrogen bond Glu451 and Asp207 at a distance of 2.8 Å and 1.2 Å respectively) *via* its oxygen of the hydroxyl group at 2 and 4-postion of ring B (Fig. 7A). Similar interactions were observed with all compounds containing aryldiol ring (Fig. 7B and C). The superimposed structures of aryldiols are shown in Fig. 7D.

Poor binding affinities are observed with inhibitors **8**, **9**, **10**, and **12** that bear methoxy or chloro substituents on the ring **B**. Binding modes of weakly active compounds (**8** and **9**) and their superimposed structure with the most active compound **5** are shown in Fig. 8A–C.



Scheme 1. Synthesis of 2,5-disubstituted thiadiazoles; *Reagents and Conditions*: (a) $NH_2NH_2{\cdot}H_2O$, MeOH, Reflux, 6 h; (b) Lawesson's Reagent, Toluene, Reflux; (c) Substituted aldehydes, $POCl_3$, Pyridine.

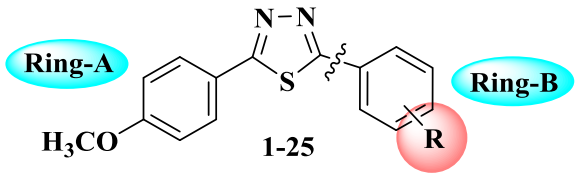


Fig. 2. General representation of compounds **1–25** for SAR analysis.

3. Conclusion

Thiadiazole derivatives **1–25** were synthesized and screened for their potential against *β*-glucuronidase enzyme. A number of compounds such as **1**, **2**, **4**, **5–7**, **10**, **12**, **15**, and **21** exhibited potent *β*-glucuronidase inhibitory potential having IC_{50} values of 6.74 ± 0.26 to 42.53 ± 0.80 μM compared to standard D-saccharic acid-1,4-lactone (IC_{50} = 48.4 ± 1.25 μM). Nevertheless, compounds **8** (IC_{50} = 52.36 ± 1.29 μM) and **9** (IC_{50} = 48.95 ± 1.04 μM) displayed comparable activity with the standard D-saccharic acid-1,4-lactone. Structure-activity relationship revealed that hydroxy containing compounds exhibited superior inhibitory activity as compared to other substituents. The molecular docking studies has identified many structural motifs actively interacting within the enzyme's active site thus taking part in the inhibition. This study has identified some lead molecules those can be used in future research in order to get more potent *β*-glucuronidase inhibitors.

4. Experimental

4.1. Materials and methods

Melting point was measured on Büchi M-560 melting point apparatus and are uncorrected. IR spectra were recorded on a Spectrum One FT-IR spectrometer (Perkin Elmer), using KBr discs and values were signified in cm^{-1}. The ^1H NMR and ^{13}C NMR spectra were measured on a Bruker 500 Ultrashield Plus NMR (500 MHz) in DMSO-d_6, using tetramethylsilane (TMS) as an internal standard, and chemical shifts are expressed in ppm. ESI-MS were determined on Agilent 6330 Ion Trap using positive/negative mode at Faculty of Pharmacy, UiTM Puncak Alam, Malaysia.

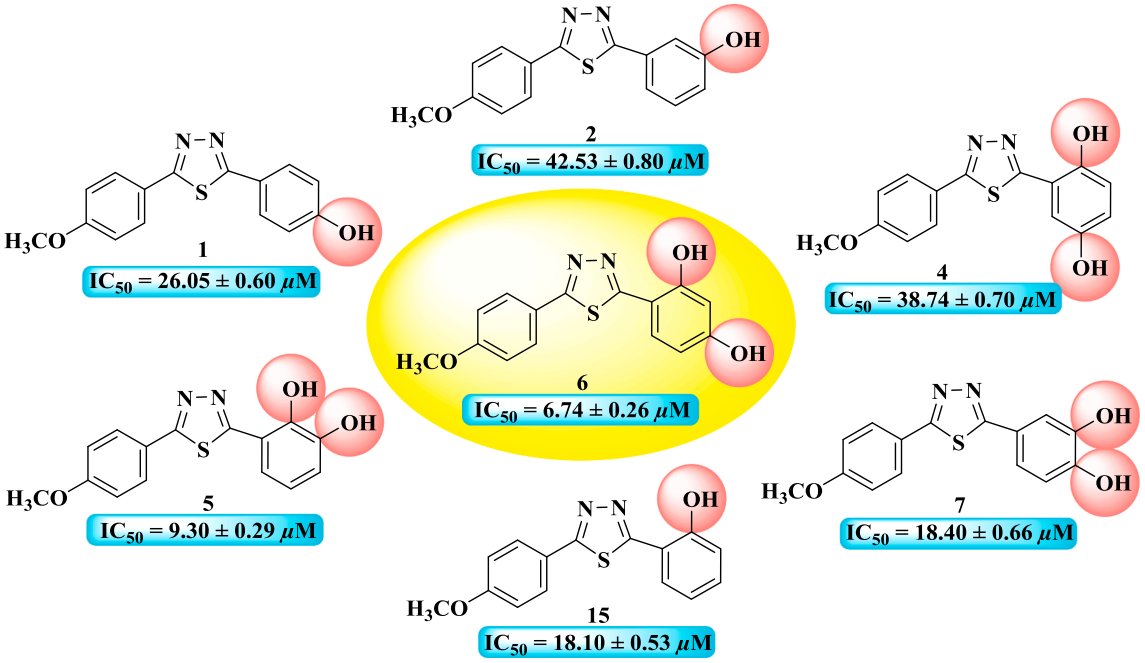


Fig. 3. SAR of hydroxy substituted compounds.

4.2. General procedure for the synthesis of thiadiazole derivatives 1–25

Methyl-4-methoxybenzoate (i) was refluxed with 5 mL of hydrazine hydrate solution in 15 mL of methanol for 6 h. The excess hydrazine and methanol was then removed *in vacuo* to obtain crude product which was then recrystallized from methanol to afford pure 4-methoxybenzohydrazide in 92% yield. Pure 4-methoxybenzohydrazide (20 mmol, 3.32 g) were refluxed with Lawesson's reagent (20 mmol, 8.09 g) of in dry toluene for 8 h to get corresponding 4-methoxybenzothiohydrazide. The crude product was washed with diethyl ether and crystallized from methanol to get pure 4-methoxybenzothiohydrazide in 91% yield. In order to get thiadiazole derivatives **1–25**, a mixture of 4-methoxybenzothiohydrazide (0.5 mmol) and the corresponding aromatic aldehyde (0.5 mmol) were taken together in a round-bottomed flask and was then added drop wise $POCl_3$ (5 mL) carefully. The reaction mixture was heated to reflux for 4–6 h, then cooled to room temperature and poured onto crushed ice. $NaHCO_3$ solution was added and the resulting solid mass precipitated out was filtered, dried, and crystallized from methanol in good to excellent yields.

4.3. 4-(5-(4-Methoxyphenyl)-1,3,4-thiadiazol-2-yl)phenol (1)

White needle shaped crystalline solid; M.p. = 256 °C; R_f: 0.86 (ethyl acetate/hexanes, 3:7); ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.80 (s, 1H, OH), 7.92 (d, 2H, J = 8.0 Hz), 7.52 (d, 2H, J = 8.0 Hz), 7.08 (d, 2H, J = 8.0 Hz), 6.82 (d, 2H, J = 8.5 Hz), 3.83 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ 174.2, 174.2, 160.5, 158.4, 128.8, 128.8, 128.4, 128.4, 126.1, 125.7, 116.3, 116.3, 114.7, 114.7, 55.7, Anal Calcd for $C_{15}H_{12}N_2O_2S$, C, 63.36; H, 4.25; N, 9.85; Found: C, 63.34; H, 4.23; N, 9.84; HREI-MS: m/z Calcd for for $C_{15}H_{12}N_2O_2S$, $[M]^+$ 284.0619; Found 284.0627.

4.4. 3-(5-(4-Methoxyphenyl)-1,3,4-thiadiazol-2-yl)phenol (2)

Light yellow granular crystalline solid; M.p. = 249 °C; R_f: 0.76 (ethyl acetate/hexanes, 3:7); ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.40 (s, 1H, OH), 7.90 (d, 2H, J = 8.5 Hz), 7.30 (t, 1H, J = 8.5 Hz), 7.26 (s, 1H), 7.12 (d, 1H, J = 8.0 Hz), 7.04 (d, 2H, J = 8.5 Hz), 6.80 (d, 1H, J = 6.5 Hz, H-4), 3.86(s, 3H, OCH_3); ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.2, 174.2, 160.5, 157.4, 134.8, 130.7, 128.4, 128.4, 125.7, 123.4,

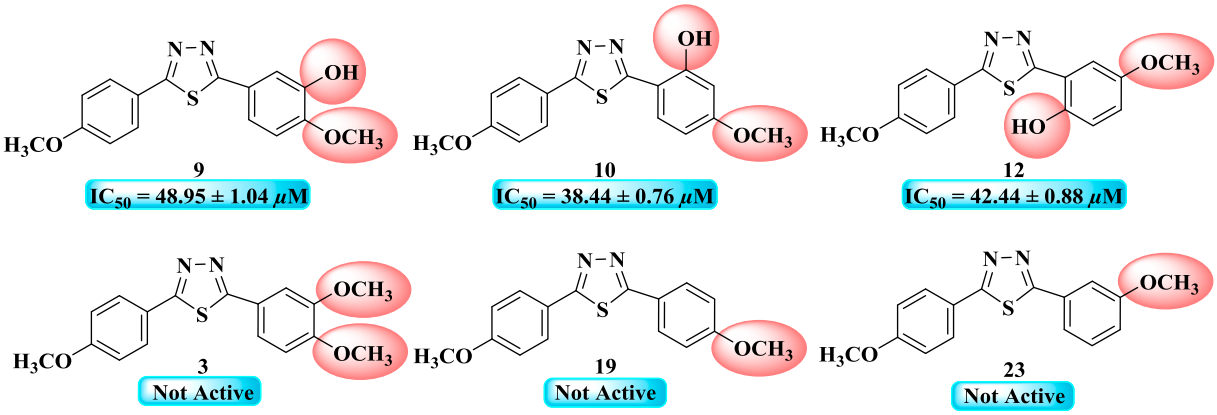


Fig. 4. SAR of hydroxy and methoxy substituted compounds.

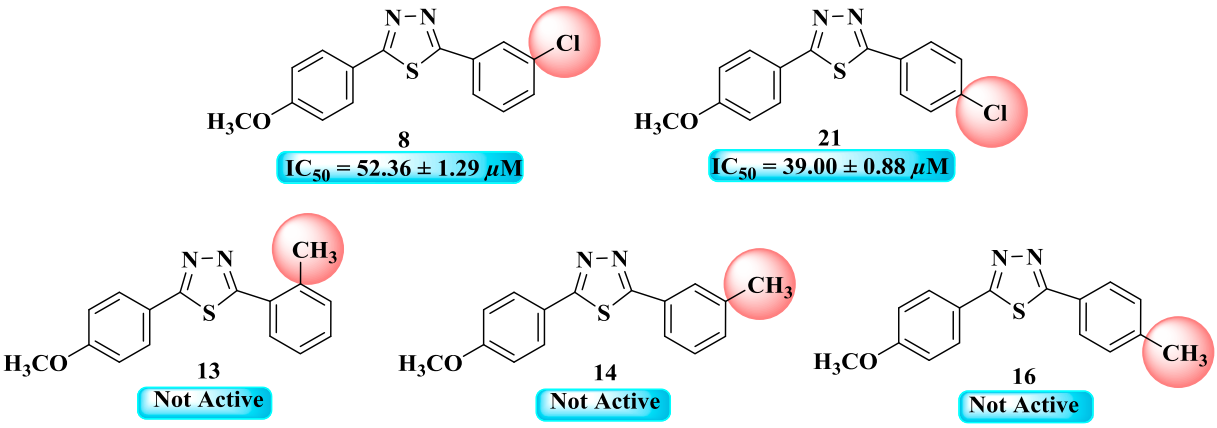


Fig. 5. SAR of chloro and methyl substituted compounds.

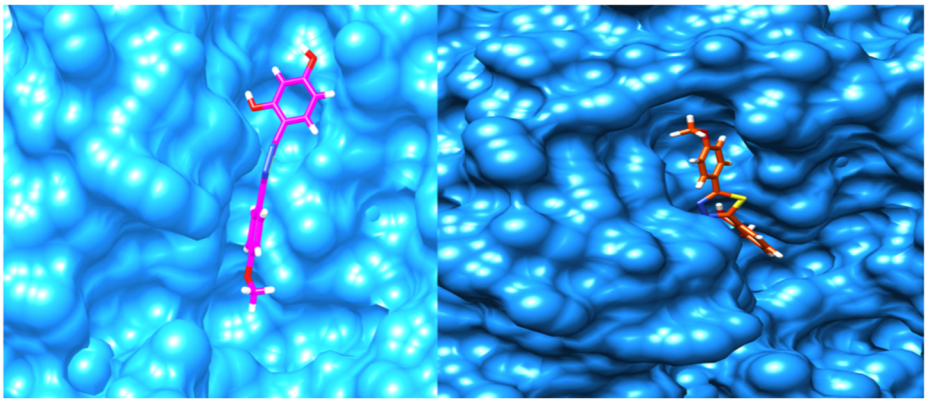


Fig. 6. Modelled mode of binding of (a) Compound **5** (b) Compound **6** in 1BHG active site.

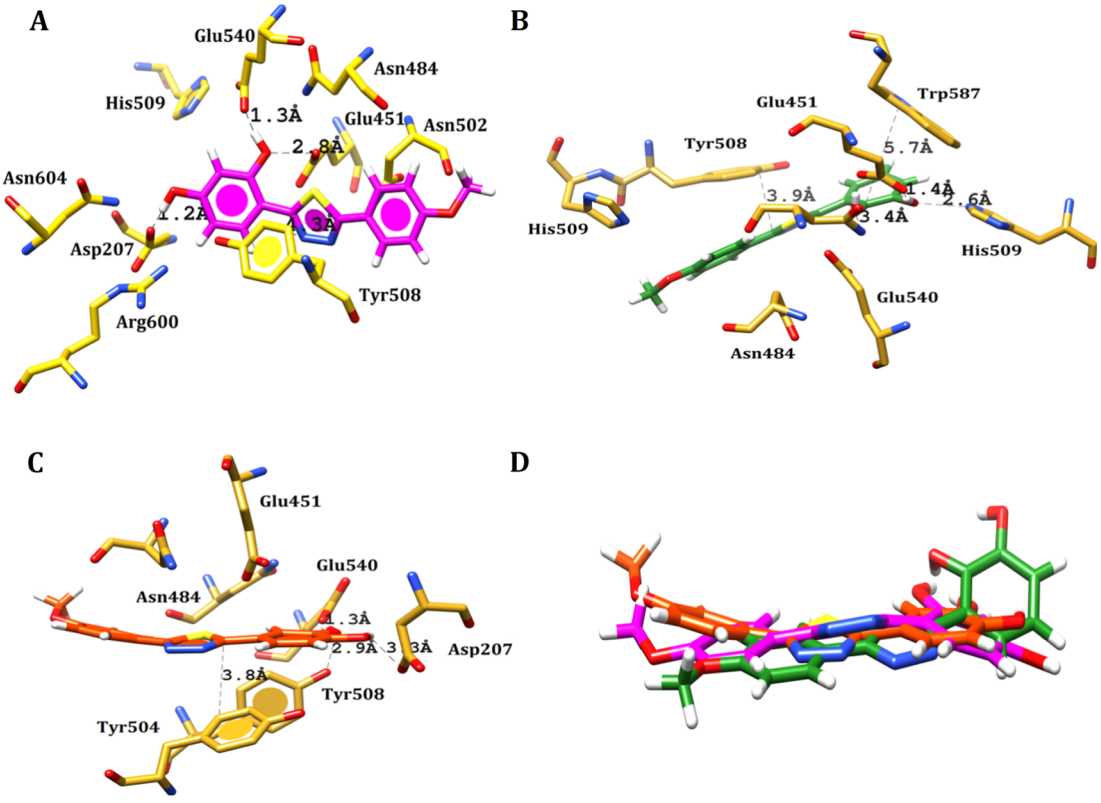


Fig. 7. 3D interactions of compounds **5**, **6**, and **7** and their superimposed structures in the active site of 1BHG.

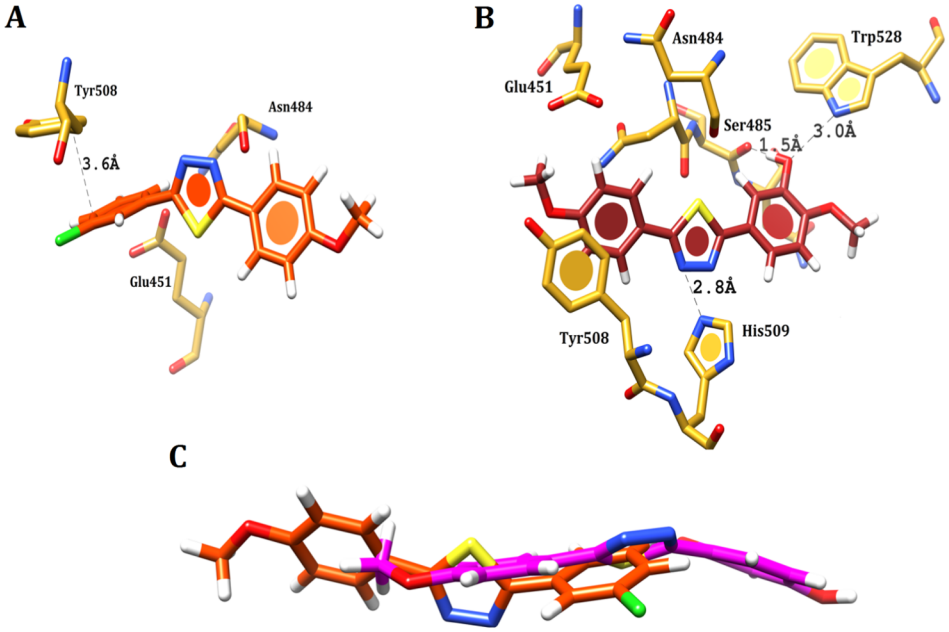


Fig. 8. 3D interactions of weakly compounds **8** and **9** (A-B) and their superimposed structures (C) in the active site of 1BHG.

115.8, 114.7, 114.7, 112.8, 55.7, Anal. Calcd for $C_{15}H_{12}N_2O_2S$, C, 63.36; H, 4.25; N, 9.85; Found: C, 63.34; H, 4.23; N, 9.84; HREI-MS: *m/z* Calcd for for $C_{15}H_{12}N_2O_2S$, $[M]^+$ 284.0619; Found 284.0611.

4.5. 2-(3,4-Dimethoxyphenyl)-5-(4-methoxyphenyl)-1,3,4-thiadiazole (3)

Yellow needle like crystalline solid; M.p. = 220 °C; R_f: 0.72 (ethyl acetate/hexanes, 3:7); ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.94 (d, 2H, J = 8.0 Hz), 7.36 (s, 1H), 7.21 (d, 1H, J = 8.5 Hz), 7.07 (d, 2H, J = 8.5 Hz), 6.76 (d, 1H, J = Hz), 3.86 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3); ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.2, 174.2, 160.5, 150.2, 149.7, 128.4, 128.4, 126.7, 125.7, 120.8, 114.7, 114.7, 112.2, 111.1, 56.1, 56.1, 55.7, Anal Calcd for $C_{17}H_{16}N_2O_3S$, C, 62.18; H, 4.91; N, 8.53; Found C, 62.17; H, 4.90; N, 8.52; HREI-MS: *m/z* Calcd for for $C_{17}H_{16}N_2O_3S$, $[M]^+$ 328.0882; Found 328.0896.

4.6. 2-(5-(4-Methoxyphenyl)-1,3,4-thiadiazol-2-yl)benzene-1,4-diol (4)

Off white rod like solid; M.p. = 282 °C; R_f: 0.76 (ethyl acetate/hexanes, 3:7); ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.10 (s, 1H, OH), 9.15 (s, 1H, OH), 7.92 (d, 2H, J = 9.0 Hz), 7.06 (d, 2H, J = 9.0 Hz), 6.94 (dd, 1H, J = 8.0, J = 2.0 Hz), 6.83 (d, 1H, J = 2.0 Hz), 6.72 (d, 1H, J = 8.0 Hz), (s, 3H, OCH3); ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.2, 174.2, 160.5, 150.0, 147.7, 128.4, 128.4, 125.7, 125.0, 117.7, 117.3, 114.7, 114.7, 114.2, 55.7; Anal. Calcd for $C_{15}H_{12}N_2O_3S$, C, 59.99; H, 4.03; N, 9.33; Found C, 59.98; H, 4.01; N, 9.32; HREI-MS: *m/z* Calcd for for $C_{15}H_{12}N_2O_3S$, $[M]^+$ 300.0569; Found 300.0558.

4.7. 3-(5-(4-Methoxyphenyl)-1,3,4-thiadiazol-2-yl)benzene-1,2-diol (5)

Yellow massive crystalline solid; M.p. = 265 °C; R_f: 0.84 (ethyl acetate/hexanes, 3:7); ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.20 (s, 1H, OH), 9.50 (s, 1H, OH), 7.92 (d, 2H, J = 9.0 Hz), 7.12 (d, 2H, J = 9.0 Hz), 6.93 (dd, 1H, J = 6.5, J = 2.0 Hz), 6.88 (dd, 1H, J = 6.5, J = 2.0 Hz, H-6), 6.76 (t, 1H, J = 6.5 Hz, H-5), 3.85 (s, 3H, OCH3); ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.2, 174.2, 160.5, 143.8, 145.5, 125.2, 125.7, 114.7, 121.4, 128.4, 114.7, 117.2, 128.4, 123.1, 55.7; Anal Calcd for $C_{15}H_{12}N_2O_3S$, C, 59.99; H, 4.03; N, 9.33; Found C, 59.97; H, 4.02; N, 9.31; HREI-MS: *m/z* Calcd for for $C_{15}H_{12}N_2O_3S$, $[M]^+$ 300.0569; Found 300.0576.

4.8. 4-(5-(4-Methoxyphenyl)-1,3,4-thiadiazol-2-yl)benzene-1,3-diol (6)

Yellow square crystalline solid; M.p. = 270 °C; R_f: 0.82 (ethyl acetate/hexanes, 3:7); ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.30 (s, 1H, OH) 9.98 (s, 1H, OH), 7.94 (d, 2H, J = 8.0 Hz), 7.32 (d, 1H, J = 8.5 Hz), 7.06 (d, 2H, J = 8.0 Hz, H-3), 6.39 (dd, 1H, J = 8.5, 2.0 Hz), 6.30 (d, 1H, J = 2.0 Hz), 3.86 (s, 3H, OCH_3); ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.2,174.2,160.5, 156.5, 159.8, 116.2, 125.7, 105.5, 114.7, 109.1, 130.2, 128.4, 114.7, 128.6, 55.9; Anal Calcd for $C_{15}H_{12}N_2O_3S$, C, 59.99; H, 4.03; N, 9.33; Found C, 59.98; H, 4.01; N, 9.32; HREI-MS: *m/z* Calcd for for $C_{15}H_{12}N_2O_3S$, $[M]^+$ 300.0569; Found 300.0582.

4.9. 4-(5-(4-Methoxyphenyl)-1,3,4-thiadiazol-2-yl)benzene-1,2-diol (7)

White, granular crystalline solid; M.p. = 240 °C; R_f: 0.76 (ethyl acetate/hexanes, 3:7); ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.30 (s, 2H, OH), 7.91 (d, 2H, J = 8.0 Hz), 7.40 (s, 1H), 7.08 (d, 2H, J = 8.0 Hz), 6.91 (d, 1H, J = 8.0 Hz), 6.76 (d, 1H, J = 8.0 Hz), 3.85 (s, 3H, OCH_3); ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.2, 174.2, 160.5, 145.8, 147.2, 127.4, 125.9, 114.2, 114.9, 116.1, 121.4, 128.4, 114.9, 128.4, 55.7; Anal Calcd for $C_{15}H_{12}N_2O_3S$, C, 59.99; H, 4.03; N, 9.33; Found C, 59.97; H, 4.01; N, 9.31; HREI-MS: *m/z* Calcd for for $C_{15}H_{12}N_2O_3S$, $[M]^+$ 300.0569; Found 300.0580.

4.10. 2-(3-Chlorophenyl)-5-(4-methoxyphenyl)-1,3,4-thiadiazole (8)

Yellow crystalline solid, M.p. = 221.6 °C; R_f: 0.80 (ethyl acetate/hexanes, 3:7); ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.90 (d, 2H, J = 8.0 Hz), 7.40 (t, 1H, J = 7.5 Hz), 7.32–728 (m, 1H), 7.09 (d, 2H, J = 8.0 Hz), 6.80 (dd, 1H, J = 7.5, 2.0 Hz), 3.86 (s, 3H, OCH_3); ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.2, 174.2, 134.7, 160.5, 134.8, 125.7, 127.3, 114.7, 128.4, 129.1, 128.7, 114.7, 128.4, 129.4, 55.7; Anal Calcd for $C_{15}H_{11}ClN_2OS$, C, 59.50; H, 3.66; N, 9.25; Found C, 59.49; H, 3.65; N, 9.24; HREI-MS: *m/z* Calcd for for $C_{15}H_{11}ClN_2OS$, $[M]^+$ 302.0281; Found 302.0290.

4.11. 2-Methoxy-5-(5-(4-methoxyphenyl)-1,3,4-thiadiazol-2-yl)phenol (9)

Light yellow crystalline solid; M.p. = 235 °C; R_f: 0.72 (ethyl acetate/hexanes, 3:7); ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.55 (s, 1H, OH), 7.92 (d, 2H, J = 8.5 Hz, H-2), 7.30 (s, 1H), 7.07 (d, 1H, J = 8.0 Hz), 7.03 (d, 2H, J = 8.5 Hz), 6.80 (d, 1H, J = 8.0 Hz), 3.82 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3); ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.2, 174.2, 147.4, 160.5, 147.3, 127.1, 125.6, 113.8, 111.3, 114.9, 121.2, 128.6, 114.9, 128.3, 56.2, 55.7; Anal Calcd, $C_{16}H_{14}N_2O_3S$ C, 61.13; H, 4.49; N, 8.91; Found C, 61.12; H, 4.48; N, 8.90; HREI-MS: *m/z* Calcd for for $C_{15}H_{11}ClN_2OS$, $[M]^+$ 314.0725; Found 314.0714.

4.12. 5-Methoxy-2-(5-(4-methoxyphenyl)-1,3,4-thiadiazol-2-yl)phenol (10)

Yellow needle like solid, M.p. = 264 °C; R_f: 0.74 (ethyl acetate/hexanes, 3:7); ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.70 (s, 1H, OH), 7.91 (d, 2H, J = 8.0 Hz), 7.30 (d, 1H, J = 8.5 Hz), 7.08 (d, 2H, J = 8.0 Hz, H-3), 6.42 (dd, 1H, J = 8.5, 2.0 Hz), 6.33 (d, 1H, J = 2.0 Hz), 3.88 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3); ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.2, 174.2, 162.1, 160.7, 156.1, 116.1, 125.7, 104.1, 107.3, 114.7, 129.8, 128.3, 114.9, 128.4, 55.6, 55.6; Anal Calcd, $C_{16}H_{14}N_2O_3S$ C, 61.13; H, 4.49; N, 8.91; Found C, 61.11; H, 4.47; N, 8.91; HREI-MS: *m/z* Calcd for for $C_{15}H_{11}ClN_2OS$, $[M]^+$ 314.0725; Found 314.0732.

4.13. 2-(4-Methoxyphenyl)-5-(pyridin-4-yl)-1,3,4-thiadiazole (11)

Off white crystalline solid, M.p. = 216. °C; R_f: 0.88 (ethyl acetate/hexanes, 3:7); ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.62 (d, 2H, J = 6.0 Hz), 7.90 (d, 2H, J = 8.5 Hz), 7.67 (d, 2H, J = 6.0 Hz), 7.11 (d, 2H, J = 8.5 Hz), 3.86 (s, 3H, OCH_3); ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.2, 174.2, 160.4, 149.7, 149.7, 143.6, 125.7, 114.7, 121.2, 121.2, 128.4, 114.7, 128.4, 55.7; Anal Calcd for $C_{14}H_{11}N_3OS$, C, 62.43; H, 4.12; N, 15.60; Found C, 62.42; H, 4.11; N, 15.59; HREI-MS: *m/z* Calcd for for $C_{14}H_{11}N_3OS$, $[M]^+$ 269.0623; Found 269.0614.

4.14. 4-Methoxy-2-(5-(4-methoxyphenyl)-1,3,4-thiadiazol-2-yl)phenol (12)

Yellow granular crystalline solid, M.p. = 201.8 °C; R_f: 0.76 (ethyl acetate/hexanes, 3:7); ^{1}H NMR (500 MHz, DMSO-d_6): 10.30 (s, 1H, OH), 7.91 (d, 2H, J = 8.5 Hz), 7.10 (d, 1H, J = 8.5 Hz), 7.07 (d, 2H, J = 8.5 Hz), 6.92 (dd, 1H, J = 8.5, 2.0 Hz), 6.85 (d, 1H, J = 2.0 Hz, H-6), 3.88 (s, 3H, OCH_3), 3.82(s, 3H, OCH_3); ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.2, 174.2, 153.6, 160.5, 147.4, 124.6, 125.7, 115.6, 114.7, 117.3, 112.6, 128.3, 114.7, 128.3, 55.6, 55.6; Anal Calcd, $C_{16}H_{14}N_2O_3S$ C, 61.13; H, 4.49; N, 8.91; Found C, 61.12; H, 4.48; N, 8.90; HREI-MS: *m/z* Calcd for for $C_{15}H_{11}ClN_2OS$, $[M]^+$ 314.0725; Found 314.0736.

4.15. 2-(4-Methoxyphenyl)-5-o-tolyl-1,3,4-thiadiazole (13)

Yellow elongated crystalline solid, M.p. = 201 °C; R_f: 0.90 (ethyl acetate/hexanes, 3:7); ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.90 (d, 2H, J = 8.5 Hz), 7.58 (d, 1H, J = 8.0 Hz), 7.11 (d, 2H, J = 8.5 Hz), 7.30–7.26 (m, 2H), 3.86 (s, 3H, OCH_3), 2.49 (s, 3H, CH_3); ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.2, 174.2, 160.5, 137.1, 125.7, 136.8, 114.7, 127.3, 128.4, 129.4, 114.7, 128.4, 126.1, 128.5, 55.7, 18.6; Anal Calcd $C_{16}H_{14}N_2OS$, C, 68.06; H, 5.00; N, 9.92; Found C, 68.05; H, 5.01; N, 9.91; HREI-MS: *m/z* Calcd for for $C_{16}H_{14}N_2OS$, $[M]^+$ 282.0827; Found 282.0838.

4.16. 2-(4-Methoxyphenyl)-5-m-tolyl-1,3,4-thiadiazole (14)

Dark yellow granular solid, M.p. = 201 °C; R_f: 0.86 (ethyl acetate/hexanes, 3:7); ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.92 (d, 2H, J = 8.5 Hz), 7.72 (dd, 1H, J = 8.0, 2.0 Hz), 7.64 (d, 1H, J = 2.0 Hz), 7.34 (t, 1H, J = 7.0 Hz), 7.17 (d, 1H, J = 20 Hz), 7.10 (d, 2H, J = 8.5 Hz), 3.84(s, 3H, OCH_3), 2.46 (s, 3H, CH_3); ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.2, 174.2, 160.5, 133.3, 125.7, 138.8, 114.7, 128.4, 127.8, 130.3, 114.7, 128.4, 129.1, 129.2, 55.7, 21.5; Anal Calcd $C_{16}H_{14}N_2OS$, C, 68.06; H, 5.00; N, 9.92; Found C, 68.04; H, 5.02; N, 9.90; HREI-MS: *m/z* Calcd for for $C_{16}H_{14}N_2OS$, $[M]^+$ 282.0827; Found 282.0832.

4.17. 2-(5-(4-Methoxyphenyl)-1,3,4-thiadiazol-2-yl)phenol (15)

Yellow amorphous solid, M.p. = 182.5 °C; R_f: 0.75 (ethyl acetate/hexanes, 3:7); ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.20 (s, 1H, OH), 7.93 (d, 2H, J = 9.0H), 7.53 (d, 1H, J = 7.5, H-3), 7.32 (t, 1H, J = 8.5 Hz), 7.06 (d, 2H, J = 9.0 Hz), 6.93–6.88 (m, 2H), 3.86 (s, 3H, OCH_3); ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.2, 174.2, 160.5, 155.1, 130.0, 128.8, 128.4, 128.4, 125.7, 123.6, 121.7, 117.7, 114.7, 114.7, 55.7, Anal. Calcd for $C_{15}H_{12}N_2O_2S$, 63.36; H, 4.25; N, 9.85; Found: C, 63.34; H, 4.23; N, 9.84; HREI-MS: *m/z* Calcd for for $C_{15}H_{12}N_2O_2S$, $[M]^+$ 284.0619; Found 284.0632.

4.18. 2-(4-Methoxyphenyl)-5-p-tolyl-1,3,4-thiadiazole (16)

Light yellow granular crystalline solid, M.p. = 226. °C; R_f: 0.84 (ethyl acetate/hexanes, 3:7); ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.90 (d, 2H, J = 8.5 Hz), 7.55 (d, 2H, J = 8.0 Hz), 7.24 (d, 2H, J = 8.0 Hz), 7.10 (d, 2H, J = 8.5 Hz), 3.86 (s, 3H, OCH_3), 2.40 (s, 3H, CH_3), ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.2, 174.2, 160.5, 131.1, 130.4, 129.4, 129.4, 128.4, 128.4, 127.3, 127.3, 114.7, 114.7, 55.7, 21.4; Anal Calcd, $C_{16}H_{14}N_2OS$, C, 68.06; H, 5.00; N, 9.92; Found C, 68.04; H, 5.02; N, 9.90; HREI-MS: *m/z* Calcd for for $C_{16}H_{14}N_2OS$, $[M]^+$ 282.0827; Found 282.0821.

4.19. 2-Bromo-4-(5-(4-methoxyphenyl)-1,3,4-thiadiazol-2-yl)phenol (17)

Yellow crystalline solid, M.p. = 230 °C; R_f: 0.75 (ethyl acetate/hexanes, 3:7); ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.40 (s, 1H, OH), 7.80 (d, 2H, J = 8.5 Hz), 7.84 (s, 1H), 7.52 (d, 1H, J = 8.0 Hz), 7.08 (d, 2H, J = 9.0 Hz), 7.02 (d, 1H, J = 8.0 Hz), 3.84 (s, 3H, OCH_3); ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.2, 174.2, 160.5, 156.5, 134.4, 128.4, 128.4, 128.2, 127.8, 125.7, 118.5, 114.7, 114.7, 114.2, 55.7; Anal Calcd for $C_{15}H_{11}BrN_2O_2S$, C, 49.60; H, 3.05; N, 7.71 Found C, 49.58; H, 3.04; N, 7.70; HREI-MS: *m/z* Calcd for for $C_{15}H_{11}BrN_2O_2S$, $[M]^+$ 361.9725; Found 361.9736.

4.20. 2-(4-Methoxyphenyl)-5-(pyridin-2-yl)-1,3,4-thiadiazole (18)

White elongated crystalline solid, M.p. = 206 °C; R_f: 0.81 (ethyl acetate/hexanes, 3:7); ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.62 (d, 1H, J = 5.0 Hz), 7.96 (d, 1H, J = 8.0 Hz), 7.91 (d, 2H, J = 8.5 Hz), 7.84–7.80 (m, 1H, H-4), 7.55 (t, 1H, J = 8.0 Hz), 7.09 (d, 2H, J = 8.5 Hz), 3.87 (s, 3H, OCH_3); ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.2, 174.2, 160.5, 157.3, 149.1, 137.1, 128.4, 128.4, 125.7, 124.1, 123.7, 114.7, 114.7, 114.2, 55.7; Anal Calcd $C_{14}H_{11}N_3OS$, C, 62.43; H, 4.12; N, 15.60; Found C, 62.42; H, 4.11; N, 15.59; HREI-MS: *m/z* Calcd for for $C_{14}H_{11}N_3OS$, $[M]^+$ 269.0623; Found 269.0614.

4.21. 2,5-Bis(4-methoxyphenyl)-1,3,4-thiadiazole (19)

Yellow amorphous solid, M.p. = 214 °C; R_f: 0.65 (ethyl acetate/hexanes, 3:7); ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.90 (d, 2H, J = 8.5 Hz), 7.66 (d, 2H, J = 8.0 Hz), 7.06 (d, 2H, J = 9.0 Hz), 7.03 (d, 2H, J = 8.0 Hz), 3.86 (s, 3H, OCH_3), 3.82 (s, 3H, CH_3); ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.2, 174.2, 160.5, 160.5, 128.4, 128.4, 128.4, 128.4, 125.7, 125.7, 114.7, 114.7, 114.7, 114.7, 55.7, 55.7; Anal Calcd

$C_{16}H_{14}N_2O_2S$, C, 64.41; H, 4.73; N, 9.39; Found C, 64.40; H, 4.71; N, 9.37; HREI-MS: *m/z* Calcd for for $C_{16}H_{14}N_2O_2S$, $[M]^+$ 298.0776; Found 298.0762.

4.22. 2-(Furan-2-yl)-5-(4-methoxyphenyl)-1,3,4-thiadiazole (20)

Off white needle like crystalline solid, M.p. = 206.8 °C; R_f: 0.82 (ethyl acetate/hexanes, 3:7); ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.91(d, 2H, J = 9.0 Hz), 7.84 (s, 1H, H-3), 7.07 (d, 2H, J = 9.0 Hz), 6.91 (s, 1H), 6.64 (dd, 1H, J = 5.0, 2.0 Hz), 3.83 (s, 3H, OCH_3); ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.2, 174.2, 160.5, 146.1, 146.1, 128.4, 128.4, 125.7, 114.7, 114.7, 112.1, 112.1, 55.7,; Anal Calcd $C_{13}H_{10}N_2O_2S$, C, 60.45; H, 3.90; N, 10.85; Found C, 60.45; H, 3.90; N, 10.85; HREI-MS: *m/z* Calcd for for $C_{13}H_{10}N_2O_2S$, $[M]^+$ 258.0463; Found 258.0450.

4.23. 2-(4-Chlorophenyl)-5-(4-methoxyphenyl)-1,3,4-thiadiazole (21)

Light yellow solid, M.p. = 214 °C; R_f: 0.68 (ethyl acetate/hexanes, 3:7); ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.92 (d, 2H, J = 9.0 Hz), 7.76 (d, 2H, J = 8.5 Hz), 7.54 (d, 2H, J = 8.5 Hz), 7.07 (d, 2H, J = 9.0 Hz), 3.84 (s, 3H, OCH_3); ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.2, 174.2, 160.5, 134.2, 131.5, 129.2, 129.2, 128.8. 128.8, 128.4, 128.4, 125.7, 114.7, 114.7, 55.7; Anal Calcd for $C_{15}H_{11}ClN_2OS$, C, 59.50; H, 3.66; N, 9.25; Found C, 59.48; H, 3.65; N, 9.23; HREI-MS: *m/z* Calcd for for $C_{15}H_{11}ClN_2OS$, $[M]^+$ 302.0281; Found 302.0270.

4.24. 2-(4-Methoxyphenyl)-5-(pyridin-3-yl)-1,3,4-thiadiazole (22)

Yellow granular solid, M.p. = 218 °C; R_f: 0.76 (ethyl acetate/hexanes, 3:7); ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.61 (d, 1H, J = 2.0 Hz), 8.16 (d, 1H, J = 8.0 Hz), 7.93 (d, 2H, J = 8.5 Hz), 7.51 (dd, 1H, $J_{5,4}$ = 8.0, 5.0 Hz), 7.08 (d, 2H, J = 8.5 Hz), 3.88 (s, 3H, OCH_3); ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.2, 174.2, 160.5, 148.8, 147.8, 134.0, 133.3, 128.8. 128.8, 125.7, 124.0, 114.7, 114.7, 55.7; Anal Calcd for $C_{14}H_{11}N_3OS$, C, 62.43; H, 4.12; N, 15.60; Found C, 62.42; H, 4.11; N, 15.58; HREI-MS: *m/z* Calcd for for $C_{14}H_{11}N_3OS$, $[M]^+$ 269.0623; Found 269.0630.

4.25. 2-(3-Methoxyphenyl)-5-(4-methoxyphenyl)-1,3,4-thiadiazole (23)

Yellow fine crystalline solid, M.p. = 218 °C; R_f: 0.72 (ethyl acetate/hexanes, 3:7); ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.92 (d, 2H, J = 9.0 Hz), 7.38 (t, 1H, J = 7.5 Hz), 7.28–723 (m, 2H), 7.08 (d, 2H, J = 9.0 Hz), 6.81 (dd, 1H, J = 7.5, 2.0 Hz, H-6), 3.86 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3); ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.2, 174.2, 160.5, 161.0, 134.4, 130.1, 128.8. 128.8, 125.7, 123.1, 114.7, 114.7, 114.2, 111.2, 55.7, 55.7; Anal Calcd for $C_{16}H_{14}N_2O_2S$, C, 64.41; H, 4.73; N, 9.39; Found C, 64.40; H, 4.72; N, 9.37; HREI-MS: *m/z* Calcd for for $C_{16}H_{14}N_2O_2S$, $[M]^+$ 298.0776; Found 298.0784.

4.26. 2-(4-Methoxyphenyl)-5-(4-nitrophenyl)-1,3,4-thiadiazole (24)

White square crystaline solid, M.p. = 240 °C; R_f: 0.80 (ethyl acetate/hexanes, 3:7); ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.30 (d, 2H, J = 8.0 Hz), 8.02 (d, 2H, J = 8.0 Hz), 7.94 (d, 2H, J = 8.5 Hz), 7.09 (d, 2H, J = 8.5 Hz), 3.85 (s, 3H, OCH_3); ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.2, 174.2, 160.5, 147.8, 139.5, 128.8. 128.8, 128.4, 128.4, 125.7, 124.4, 124.4, 114.7, 114.7, 55.7; Anal Calcd for $C_{15}H_{11}N_3O_3S$, C, 57.50; H, 3.54; N, 13.41; Found C, 57.49; H, 3.53; N, 13.40; HREI-MS: *m/z* Calcd for for $C_{15}H_{11}N_3O_3S$ $[M]^+$ 313.0521; Found 313.0513.

4.27. 2-(4-Fluorophenyl)-5-(4-methoxyphenyl)-1,3,4-thiadiazole (25)

Yellow amorphous solid, M.p. = 255 °C; R_f: 0.86 (ethyl acetate/hexanes, 3:7); ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.90 (d, 2H, J = 8.5 Hz), 7.82 (t, 2H, J = 7.0 Hz), 7.30 (t, 2H, J = 7.0 Hz), 7.09 (d, 2H, J = 8.5 Hz), 3.87 (s, 3H, OCH_3); ^{13}C NMR (126 MHz, DMSO) δ 174.2, 174.2, 162.0, 160.5, 135.1, 128.8, 128.4, 127.5, 126.4, 125.7, 115.5, 115.5, 114.7, 114.7, 55.7; Anal Calcd for $C_{15}H_{11}FN_2OS$ C, 62.92; H, 3.87; N, 9.78; Found C, 62.91; H, 3.85; N, 9.76; HREI-MS: *m/z* Calcd for for $C_{15}H_{11}FN_2OS$, $[M]^+$ 286.0576; Found 286.0590.

4.28. Docking studies

Docking studies were carried out using GOLD 5.4.1. GOLD uses the Genetic algorithm (GA). This method allows a partial flexibility of protein and full flexibility of ligand. To initialize the in-silico studies, high resolution crystal structures of proteins were retrieved from the PDB (PDB ID 1BHG, 2.6 Å resolution). Hydrogen atoms were added to enzyme structure. The B-chain of protein and hetero-atoms including cofactors were removed from the crystal structure [30]. To start the docking simulations, a protein model was built by performing docking on choosing a known substrate *p*-nitrophenyl β-glucuronide into the binding site. This model was then subjected to energy minimization and further used for docking studies of the synthesized compounds. There were four possible scoring functions: Gold score, Chemscore, Astex Statistical Potential (ASP) and ChemPLP. GoldScore performs a force field-based scoring function and is made up of four components: 1. Protein-ligand hydrogen bond energy (external H-bond); 2. Protein-ligand van der Waals energy (external vdw); 3. Ligand internal van der Waals energy (internal vdw); 4. Ligand intramolecular hydrogen bond energy (internal-H-bond). Binding site was defined as the residues within 10 Å from the ligand. No water was present in any binding site. The default docking protocol was applied (1.09 auto settings, 10 GA) and the best pose saved. Each experiment was then repeated 10 times. Other docking parameters were set to the software's default values. The view of the docking results and analysis of their surface with graphical representations were done using UCSF Chimera package [36].

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