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Biology-oriented drug synthesis (BIODS), *in vitro* urease inhibitory activity, and *in silico* study of *S*-naproxen derivatives

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ABSTRACT

Current study is based on the biology-oriented drug synthesis (BIODS) of *S*-naproxen (NSAID) derivatives and the evaluation of their urease inhibitory potential. In this regard, a variety of *S*-naproxen derivatives **2–39** including hydrazide **1**, Schiff bases **2–21**, aroyl substituted hydrazides **22–24**, sulfohydrazides **25–34**, 2-mercapto oxadiazole **35**, phenacyl substituted 2-mercapto oxadiazoles **36–39** were synthesized under the umbrella of BIODS by simple chemical transformation of its pharmacophoric carboxylic group. Compounds **1–39** were evaluated for *in vitro* urease inhibitory activity and most of them showed good to moderate inhibitory potential in the range of $IC_{50} = 14.01 \pm 0.23–76.43 \pm 0.8\,\mu M$ as compared to standard acetohydroxamic acid ($IC_{50} = 27.0 \pm 0.5\,\mu M$). Limited structure-activity relationship (SAR) was established in order to rationalize the participation of varying groups (R) in the inhibitory potential of compounds. Molecular docking study on all active compounds was also carried out to decipher the interactions detail of the ligand with the receptors of active site of enzyme.

1. Introduction

S-Naproxen is a non-steroidal antiinflammatory drug (NSAID) of the propionic acid class. It is widely used for the treatment of pain, fever, joint swelling, arthritis, and acute/chronic inflammation conditions [1]. *S*-Naproxen has potential biological activity as compared to the *R*-naproxen [2]. Most of the NSAIDs inhibit inflammation by blocking the cyclooxygenase enzyme (COX) which is involved in the production of prostaglandins synthesis. Cyclooxygenase exist in two forms, COX-I and COX-II. Major cause of inflammation is cyclooxygenase-II (COX-II) enzyme [3]. It is a fact that the nonsteroidal anti-inflammatory drugs (NSAIDs) are frequently prescribed as anti-inflammatory, analgesics, antipyretic agents. Despite their wide acceptance by the medical community, a range of pathologies such as nonspecific colitis, large intestinal ulcers, bleeding, and perforation, are also associated with NSAIDs and these side effects are thought to be due to having a free acidic carboxylic group [4–7]. Other than antiinflammatory and analgesic activities, antifungal and anticancer activities are also reported for *S*-naproxen derivatives [5–10].

Urease (amidohydrolase; EC 3.5.1.5), a nickel-based enzyme from large hetero-polymeric family of amidohydrolases and phosphotriestreases that catalyzes the hydrolysis of urea to ammonia and carbon dioxide. Rate of catalyzed reaction is 10^{14} times faster than the rate of uncatalyzed reaction. Urease enzyme is mainly found in bacteria, fungi, algae, and plants. Urease enzyme of jack bean (*Canavalia ensiformis*) was first time crystallized in 1926 [11–13]. Nitrogen is an important source for the plant growth, therefore, urea has been widely used as nitrogen fertilizer in agriculture. It forms ammonia on hydrolysis which further transformed into ammonium ion. The ammonium ion are than utilized by plants in ideal soil condition at pH 6–6.5. In case of hyperactivity of urease in urea fertilization, increased hydrolysis of urea into volatile ammonia deprives plants from essential nutrients and it also leads to increased ammonia toxicity in air and brings out to major economic damages [11,14]. In human, urease hyperactivity may

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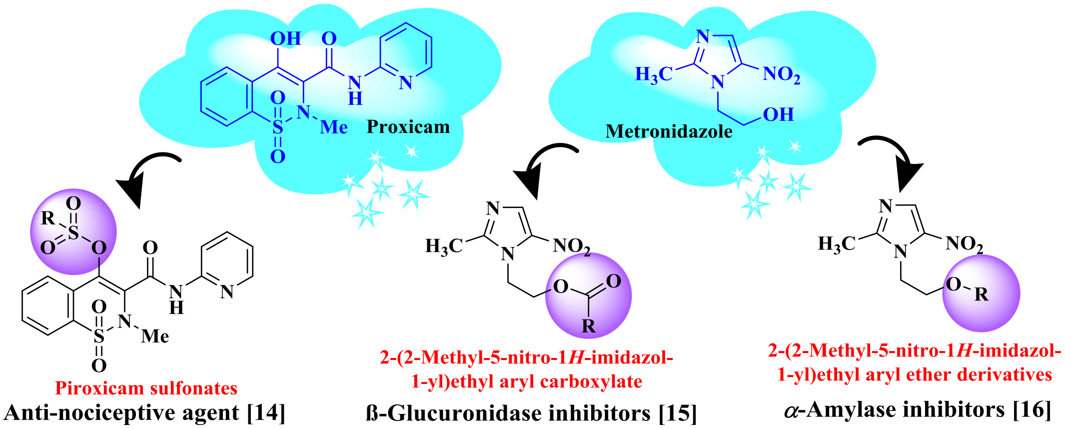


Fig. 1. Lead candidates derived from commercially available drugs piroxicam and metronidazole.

produce several health concerns including hepatic coma, pyelonephritis, gastric and peptic ulcer. Urease enzyme also facilitates the *Helicobacter pylori* (HP), a Gram-negative bacterium, to survive at low pH of stomach and plays a significant role in the development of gastric and peptic ulcer which may eventually cause cancer. Therefore, there is a need to identify new urease inhibitors which should have substantial stability, low toxicity, and good bioavailability [14,15].

The key objective of biology-oriented drug synthesis (BIODS) is to explore the compounds derive from the commercial pharmaceutical drugs for their new and diversified biological potential by adopting simple chemical transformations. Our research group has identified many lead compounds using the BIODS approach, such as piroxicam analogs for anti-nociceptive activity and metronidazole derived compounds for their potential *β*-glucuronidase and *α*-amylase inhibitory activities (Fig. 1) [16–18]. Most recently Kessab & Gedawy reported novel ciprofloxacin derivatives using biology-oriented drug synthesis (BIODS) approach for their anticancer activity, effects on cell cycle profile, caspase-3 mediated apoptosis, topoisomerase II inhibition, and antibacterial activity [19].

Our research group has reported a wide range of classes of compounds as urease inhibitors. So we decided to synthesize a diverse range of *S*-naproxen derivatives by incorporating different pharmacophores on the main scaffold which are known for urease inhibitory activity [20–23]. Furthermore, *S*-naproxen itself possesses fused aromatic ring system that can strongly interact with the transition metals. Since it is strongly believed that urease enzyme contains a catalytically important nickel atom in its active site, so this enzyme is a logical candidate for the inhibition by the synthesized derivative **1–39**. Thus, this report portrayed the BIODS of different analogs including hydrazide, Schiff base, sulfonamide, amide, and oxadiazole of commercially available non-steroidal antiinflammatory drug *S*-naproxen (Scheme 1.), structural characterization, urease inhibitory activity *in vitro*, and molecular docking study.

2. Results and discussion

2.1. Chemistry

S-Naproxen hydrazide **1** was synthesized by treating *S*-naproxen with hydrazine hydrate in the presence of 1,1′-carbonyldiimidazole (CDI) as a coupling agent. In this reaction, carboxylic group of *S*-naproxen reacted with CDI to form reactive intermediate acid imidazole with the liberation of carbon dioxide and imidazole. Then, acid imidazole reacted immediately with hydrazine hydrate to form *S*-naproxen hydrazide **1**. The synthesized *S*-naproxen hydrazide **1** was used as precursor molecule in multiple reactions for getting a variety of *S*-naproxen derivatives **2–39**. First, *S*-naproxen hydrazide **1** was reacted with different benzaldehyde derivatives in the presence of glacial acetic acid to afford Schiff bases **2–21**. As a result of this condensation reaction, two *E* and *Z* geometrical isomers were formed as shown by the NMR spectra. For easy understanding of the NMR data, the chemical shift values of one geometrical isomer which is resonated at few ppm less chemical shift than the other, is represented by the symbol steric (*) (Fig. 2). Both isomers gave the same R_f on TLC plate on different compositions of mobile phase systems such as EtOAc:Hexane and CH_2Cl_2:MeOH.

Secondly, *S*-naproxen hydrazide **1** was coupled with a variety of benzoic acid derivatives in the presence of CDI to obtained aroyl substituted hydrazide derivatives **22–24**. Furthermore, *S*-naproxen sulfohydrazide derivatives **25–34** were synthesized by reacting different aliphatic and aromatic sulfonyl chloride with *S*-naproxen hydrazide **1**. In addition, 2-mercapto oxadiazole **35** was afforded by treating *S*-naproxen hydrazide **1** with carbon disulfide under basic conditions. The resulted 2-mercapto oxadiazole **35** of *S*-naproxen was reacted with a variety of phenacyl bromide to get phenacyl substituted 2-mercapto oxadiazoles **36–39** (Scheme 1.). All synthetic compounds were characterized by various spectroscopic techniques. To the best of our knowledge, compounds **1–3**, **6**, **14**, **17**, **19–22**, **29**, and **35** are structurally known [8,24–26] while rest of the compounds are new.

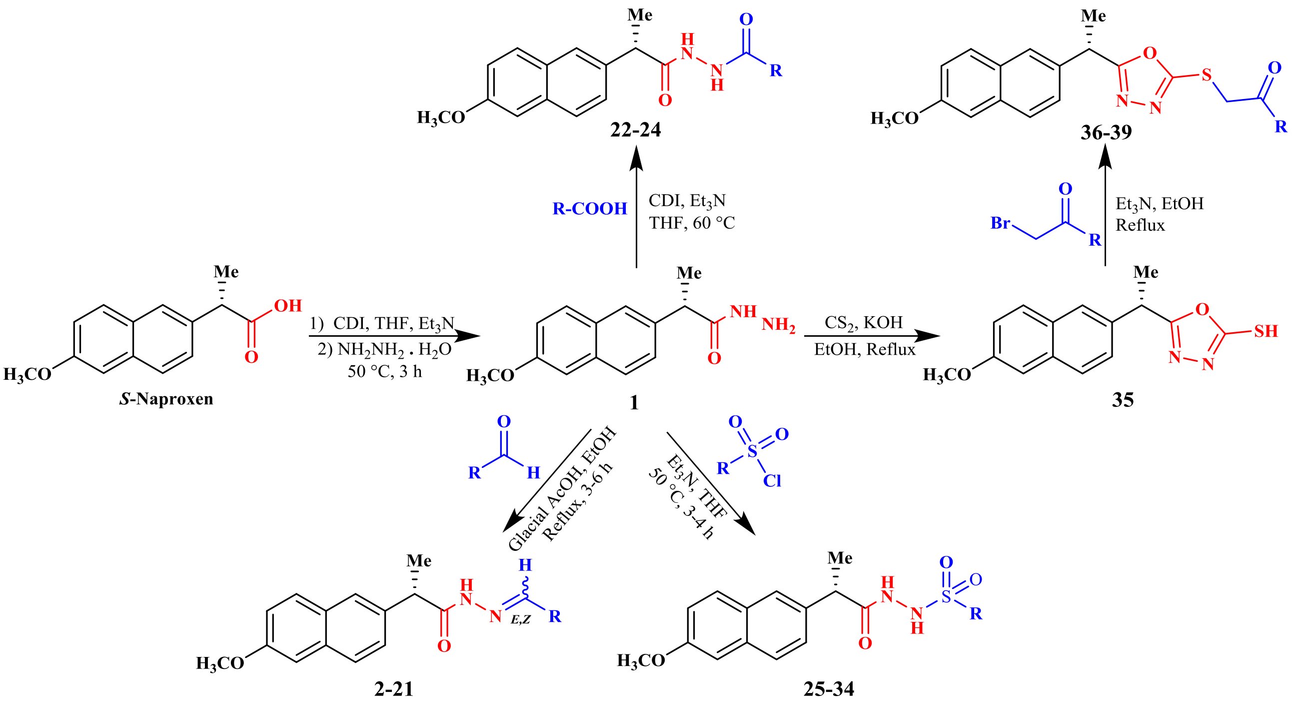
2.2. In vitro urease inhibitory activity

S-Naproxen hydrazide **1** and derivatives **2–39** were evaluated for *in vitro* urease inhibitory activity. Most of the compounds showed urease inhibitory activity with IC_{50} values ranging from 14.01 ± 0.23 to 76.43 ± 0.8 µM as compared to standard acetohydroxamic acid (IC_{50} = 27.0 ± 0.5 µM) (Table 1).

2.3. Structure-activity relationship (SAR)

In order to have a good understanding of structure–activity relationship (SAR), compounds were divided into four categories “**A-D**” based on different pharmacophores incorporated after a variety of chemical transformations. However, in each category, limited SAR was established by looking at different substituted “R” groups. It was assumed that main and persistent structural features in each category such as naphthalene ring, methine, methyl, methoxy, hydrazide moiety, iminic moiety in Schiff bases, sulfone group in sulfonamides, and 2-mercapto thiazole moiety are apparently taking part in the urease inhibitory activity, but the variation in the activities is the resultant of variable features of “R”.

S-Naproxen hydrazide **1** and (*E*,*Z*) Schiff bases of *S*-naproxen hydrazide **2–21** belongs to category “**A**”. *S*-Naproxen hydrazide **1** was found to be inactive, however, most of the Schiff bases except **3**, **8**, **10**, **13**, **14**, **17**, and **18** were found to be active. Compound **2** (IC_{50} = 22.81 ± 0.45 µM) with 2-methoxy substitution was found to



Scheme 1. Synthesis of *S*-Naproxen derivatives (hydrazide, Schiff base, sulfonamide, amide, and oxadiazole) 1–39.

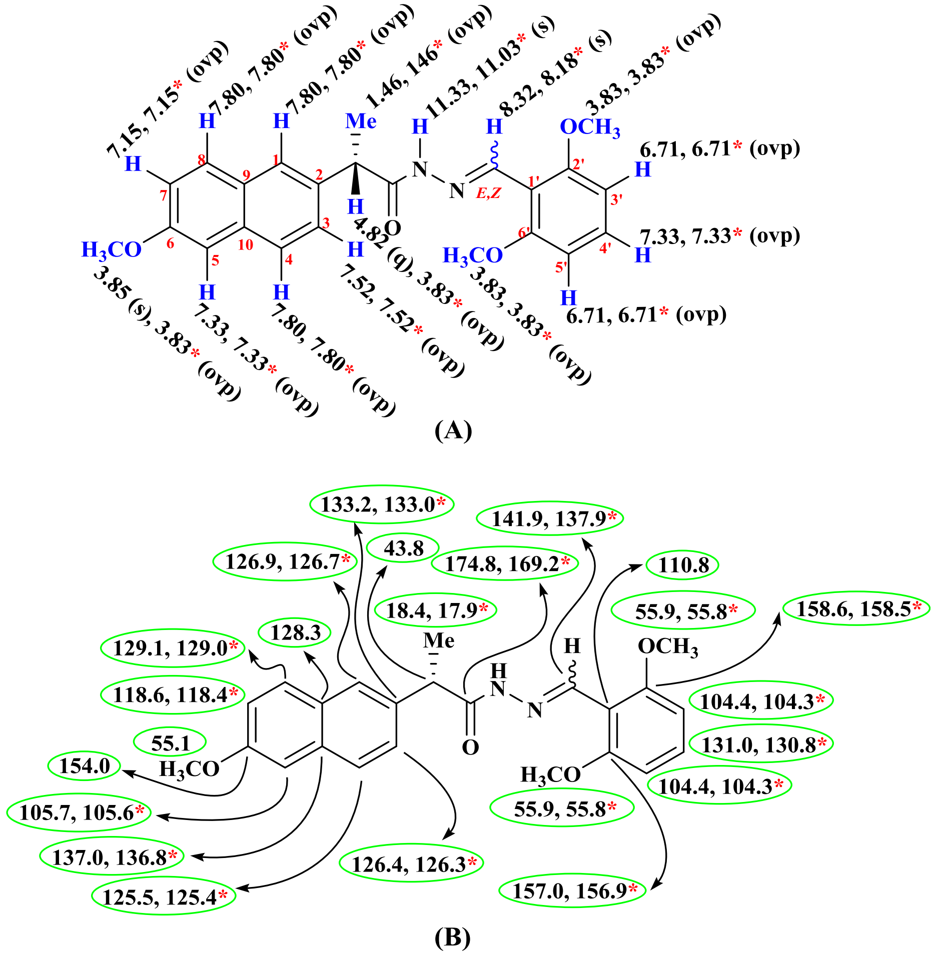


Fig. 2. ^{1}H- (**A**) & ^{13}C NMR (**B**) chemical shifts of two geometrical isomers of representative compound **4**.

Table 1
In vitro urease inhibitory activity of *S*-naproxen derivatives **1–43**.

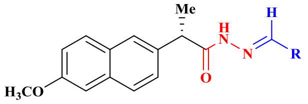
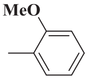
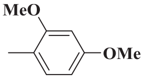
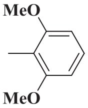
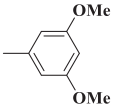
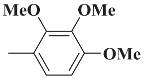
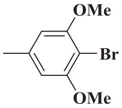
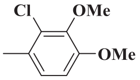
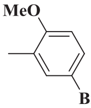
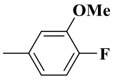
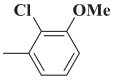
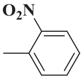
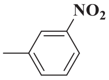
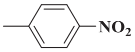
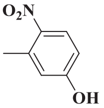
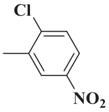
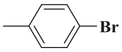
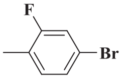
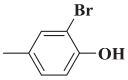
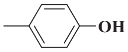
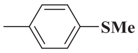
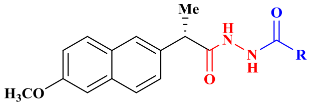
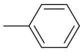
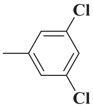
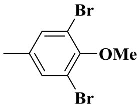
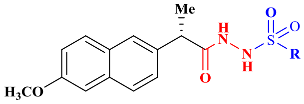
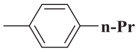
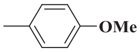
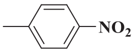
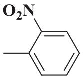
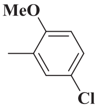
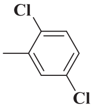
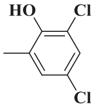
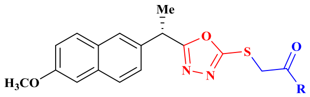
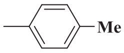
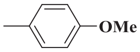
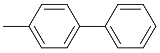
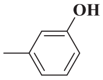
Compounds	R	Urease inhibitory activity IC_{50} ± SEM[a]
Category "A" (Schiff Bases of *S*-Naproxen Hydrazide)		
1	–	NA[b]
2		22.81 ± 0.45
3		NA[b]
4		17.9 ± 0.32
5		33.0 ± 0.7
6		14.01 ± 0.23
7		25.0 ± 0.39
8		NA[b]
9		33.0 ± 0.61
10		NA[b]
11		27.01 ± 0.5
12		23.01 ± 0.48
13		NA[b]
14		NA[b]
15		41.08 ± 0.7
16		53.31 ± 0.51
17		NA[b]
18		NA[b]
19		35.01 ± 0.71
20		28.42 ± 0.51
21		55.10 ± 0.2
Category "B" (*S*-Naproxen Hydrazide Derivatives)		
22		NA[b]
23		22.02 ± 0.42
24		23.01 ± 0.47
Category "C" (*S*-Naproxen Sulfohydrazide Derivatives)		
25	—Me	NA[b]
26	—Et	76.43 ± 0.8
27	—n-Bu	NA[b]
28		NA[b]
29		17.30 ± 0.41
30		74.52 ± 1.7
31		53.70 ± 0.37
32		19.02 ± 0.36
33		24.01 ± 0.47
34		26.11 ± 0.5
Category "D" (Substituted 2-Mercapto Oxadiazoles of *S*-Naproxen)		
35	–	NA[b]
36		NA[b]
37		32.01 ± 0.61
38		NA[b]
39		27.03 ± 0.55

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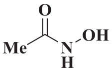
Table 1 (*continued*)

Compounds	R	Urease inhibitory activity IC_{50} ± SEM[a]
Standard[c] = Acetohydroxamic Acid		27.0 ± 0.5

[a]IC_{50} (mean ± standard error of mean); NA[b] (Not Active); Standard[c] (Inhibitor for urease inhibitory activity).

be potent molecule as it showed better urease inhibitory potential than the standard acetohydroxamic acid (IC_{50} = 27.0 ± 0.5 µM). Incorporation of halogen such as bromo *para* to methoxy in compound **9** (IC_{50} = 33.0 ± 0.61 µM), brought decline in the inhibitory activity. Bromo group being larger in size may create steric hindrance to bind with the active site of urease enzyme. Compound **11** (IC_{50} = 27.01 ± 0.5 µM) with 2-chloro-3-methoxy substitutions, showed comparable urease inhibitory activity to the standard. However, combination of fluoro with methoxy was failed to show any activity as in compound **10**. Most important is the derivatives **10** and **11** have methoxy at the same *meta* position, only variation was halogens (F/Cl) at different positions which indicate that halogen are also participating in the inhibitory potential (Fig. 3).

Positional isomers **3–5** having dimethoxy substitutions showed different urease inhibitory activities. Amongst them, compound **4** (IC_{50} = 17.9 ± 0.32 µM) with 2,6-dimethoxy groups was found to be potent, followed by moderately active 3,5-dimethoxy analog **5** (IC_{50} = 33.0 ± 0.7 µM). Comparison of compound **5** with distinctively similar analog **7** (IC_{50} = 25.0 ± 0.39 µM) having an extra *para* bromo group leads to increase in urease inhibitory activity. Nonetheless, 2,4-dimethoxy substituted derivative **3**, was found to be inactive, might attained the conformation which is not suited to fit into the active site of urease enzyme. 2,3,4-Trimethoxy substituted analog **6** (IC_{50} = 14.01 ± 0.23 µM) was found to be the most potent of the whole series **1–39**. Important to observe that replacement of 2-methoxy with 2-chloro as in compound **8**, brought a complete inactivity which revealed the vital role of 2-methoxy group in the inhibitory activity (Fig. 4).

Amongst the nitro substituted Schiff bases **12–16**, 2-nitro substituted compound **12** (IC_{50} = 23.01 ± 0.48 µM) showed potent activity as compared to the standard acetohydroxamic acid (IC_{50} = 27.0 ± 0.5 µM). Its positional isomers **13** and **14** having nitro group at *meta* and *para* positions, respectively, were found to be completely inactive. It revealed that *ortho* position is important for nitro group to take part in the inhibitory potential. Incorporation of hydroxy group at *meta* position in compound **15** (IC_{50} = 41.08 ± 0.7 µM) demonstrated a decreased inhibitory activity. Furthermore, incorporation of chloro group at *para* to nitro, as in compound **16** (IC_{50} = 53.31 ± 0.51 µM), also showed moderate activity (Fig. 5).

4-Hydroxy substituted derivative **20** (IC_{50} = 28.42 ± 0.51 µM) showed inhibitory potential comparable to the standard acetohydroxamic acid. Incorporation of bromo group adjacent to hydroxy as in compound **19** (IC_{50} = 35.01 ± 0.71 µM) leads to a decreased activity as well as replacement of hydroxy to bromo brought a complete inactivity of molecule **17**. Similarly, another *para* bromo, *ortho* fluoro substituted compound **18** was also found to be inactive which means that bromo group is not playing any significant role in the inhibitory activity and in some cases such as in compounds **9** and **19** cause a decreased activity. Furthermore, 4-thiomethyl substituted compound **21** was also found to be moderately active. It suggests that *para* position is more appropriate for hydroxy followed by thiomethyl to demonstrate an inhibitory activity (Fig. 6).

Aroyl substituted *S*-naproxen hydrazide derivatives **22–24** belongs to category "**B**". Inactivity of unsubstituted compound **22** clearly showed that the activities of derivatives **23** (IC_{50} = 22.02 ± 0.42 µM) and **24** (IC_{50} = 23.01 ± 0.47 µM) are due to functional groups such as chloro, bromo, and methoxy attached to benzene moiety which may be involved in some important interactions with the active site of enzyme (Fig. 7).

Category "C" comprises of the *S*-naproxen sulfohydrazide derivatives **25–34**. Among them, the alkyl substituted **25–27** didn't show any significant activity, although, derivative **26** (IC_{50} = 76.43 ± 0.8 µM) having ethyl group as "R", demonstrated moderate potential. The inefficiency of alkyl substituted compounds might be due to inappropriate conformation of compounds for properly fitting into the active site of urease enzyme (Fig. 8).

It is worth noting that the aroyl substituted sulfohydrazide derivatives **29**, and **32–34** having groups such as methoxy, hydroxy, and chloro, showed potent urease inhibitory activity. However, nitro substituted derivatives **30** (IC_{50} = 74.52 ± 1.7 µM) and **31** (IC_{50} = 53.70 ± 0.37 µM) were found to be moderately active, showing the less participation of nitro in the inhibitory potential. Exceptionally, 4-*n*-propyl substituted compound **28** was found to be inactive. Off the potent compounds, 4-methoxy substituted derivative **29** (IC_{50} = 17.30 ± 0.41 µM) was found to be most potent and it is the second most potent of the whole library. Another, 5-chloro-2-methoxy substituted compound **32** (IC_{50} = 19.02 ± 0.36 µM) was found to be the third most potent residue revealing the importance of methoxy group in this category. Companion of activity of compound **32** with 2,5-dichloro substituted derivative **33** (IC_{50} = 24.01 ± 0.47 µM), showed

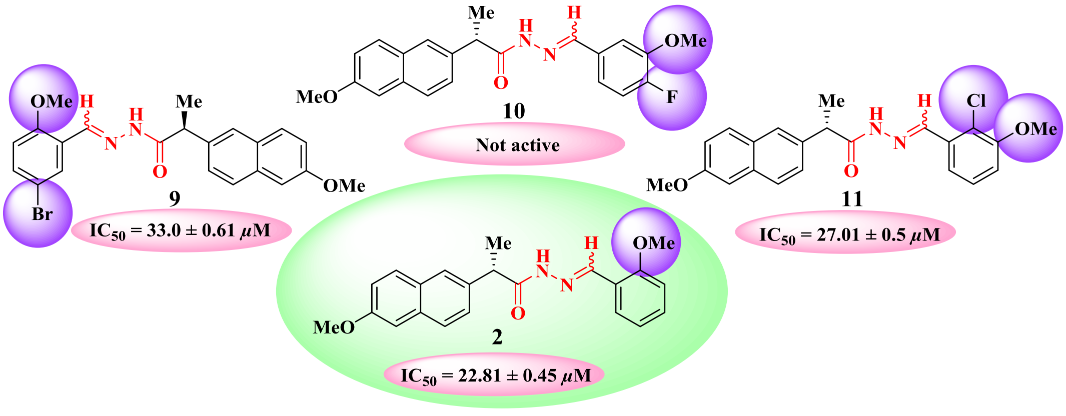


Fig. 3. SAR of compounds **2** and **9–11**.

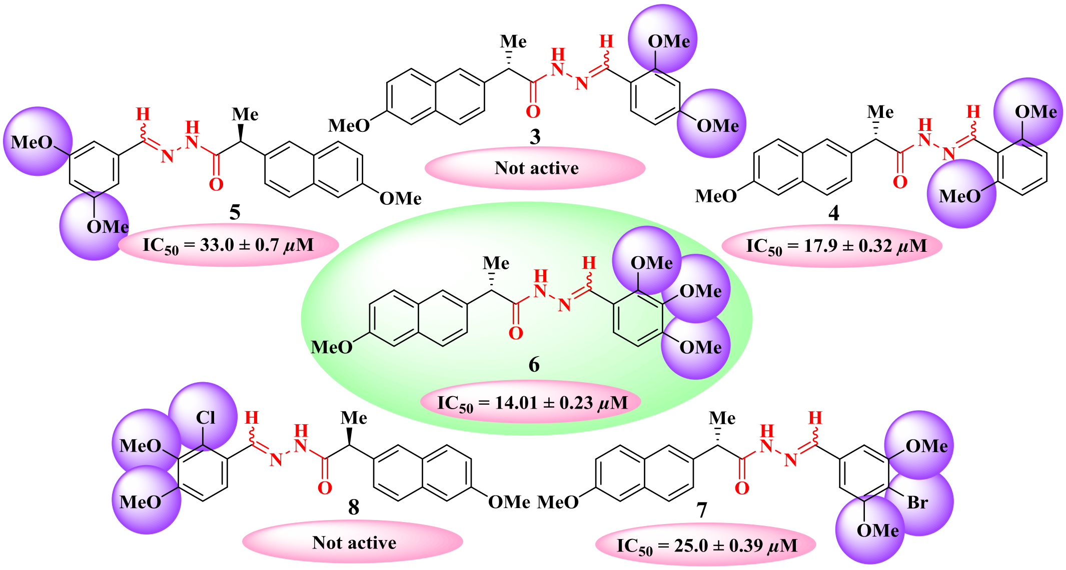


Fig. 4. SAR of compounds **3–8**.

a slight decrease in the activity also confirming the crucial role of methoxy substitution. However, 3,5-dichloro-2-hydroxy substituted compound **34** (IC$_{50}$ = 26.11 ± 0.5 μM) was also found to be a good inhibitor of urease enzyme (Fig. 9).

2-Mercapto oxadiazole **35** and phenacyl substituted 2-mercapto oxadiazoles of *S*-naproxen **36–39** belong to category “D”. Intervening intermediate **35** was found to be inactive, however, its phenacyl substituted analogs **36–39** showed some mixed behaviors. Among them, 4-methyl and 4-phenyl phenacyl substituted derivatives **36** and **38** were found to be inactive. Whilst 4-methoxy and 3-hydroxy substituted compounds **37** (IC$_{50}$ = 32.01 ± 0.61 μM) and **39** (IC$_{50}$ = 27.03 ± 0.55 μM) were demonstrated significant activity which might be due to attainment the right conformation to fit well into the active site of urease enzyme (Fig. 10).

Although the limited SAR revealed some important structural motifs/features which are playing key role in the inhibition of urease enzyme but in order to have a clear picture of the interaction of ligands (synthetic compounds) with the active pocket of enzyme, molecular docking study was also conducted on active compounds by using MOE (Molecular Operating Environment) [27].

3. Molecular docking study

3.1. Interactions detail of ligands and protein

Active site of urease enzyme comprises of both hydrophobic and hydrophilic amino acids. The hydrophobic region is composed of Ala366, 170, Leu319, Lys169, Cys322, and Met 637 while hydrophilic part included Arg339, Glu166, Glu223, Asp494, His323, His324, Asp224, and Asp363. The two Ni ions also played a critical role by linking the key amino acids and ligands. The most promising docked conformation of each compound was evaluated for their binding modes. The docked poses were ranked by the scores from the GBVI/WSA binding free energy calculation. Detailed docking results are listed in Table 2.

The analysis of the binding mode of the most preferred docked conformations revealed that all ligands interacted well over the active

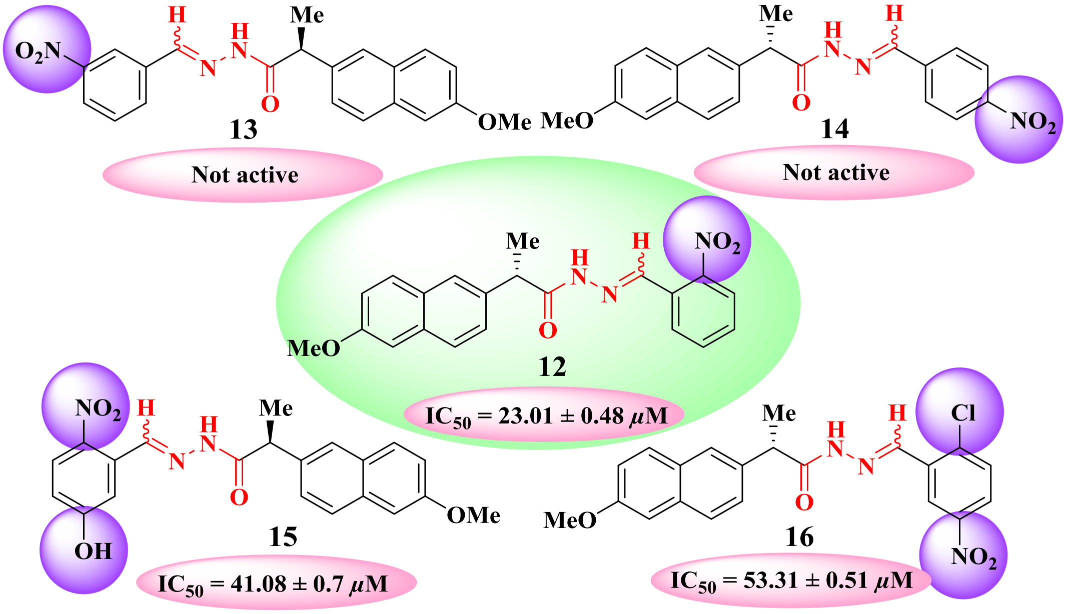


Fig. 5. SAR of compounds **12–16**.

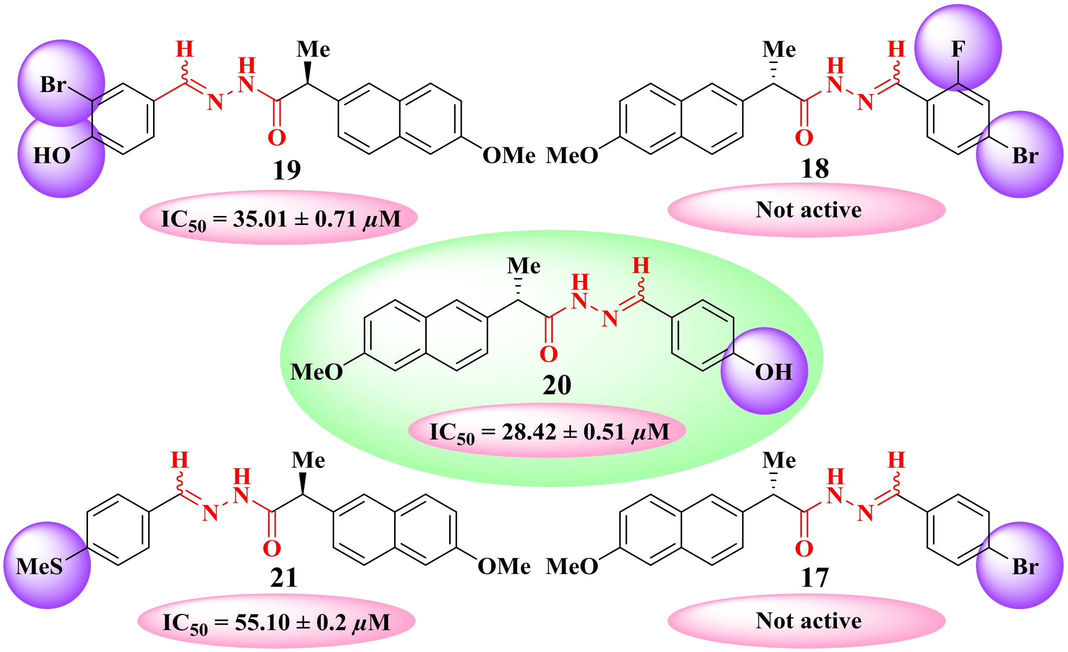


Fig. 6. SAR of compounds **17–21**.

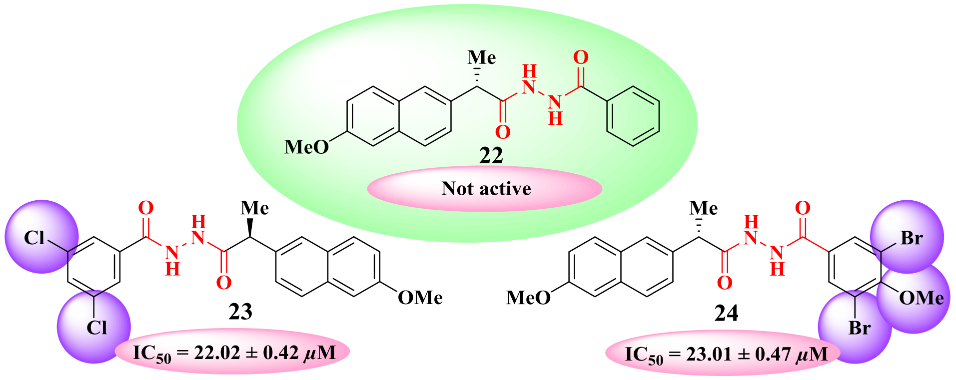


Fig. 7. SAR of compounds **22–24**.

site cavity through NH, O, NO_2, X (halogens), S, and π (pi) system. The binding mode of the ligands with the active site residues showed that almost all the ligands tightly engaged the enzyme using hydrogen bonds, polar bonds, arene-arene, and arene-cation interactions. To discuss the interactions detail of ligands and protein, all the active compounds were classified into four different groups on the basis of their structural similarities. The comparative analysis of ligand-protein interactions for each group is given below.

3.2. *Interactions of the Schiff bases of S-Naproxen hydrazide (1–21)*

Compound **6** (IC_{50} = 14.01 ± 0.23 μM) was identified as the most active compound of this group on the basis of lowest IC_{50} value. It is clear from Fig. 11c that compound **6** was bound deeply into the binding cavity of urease showing four interactions with the residues Cys322 and His323. Cys322 is involved in two side chain hydrogen donor interaction with the NH of hydrazide and α-CH group. His323 showed two π-hydrogen interactions with the π-electrons of naphthalene moiety of the

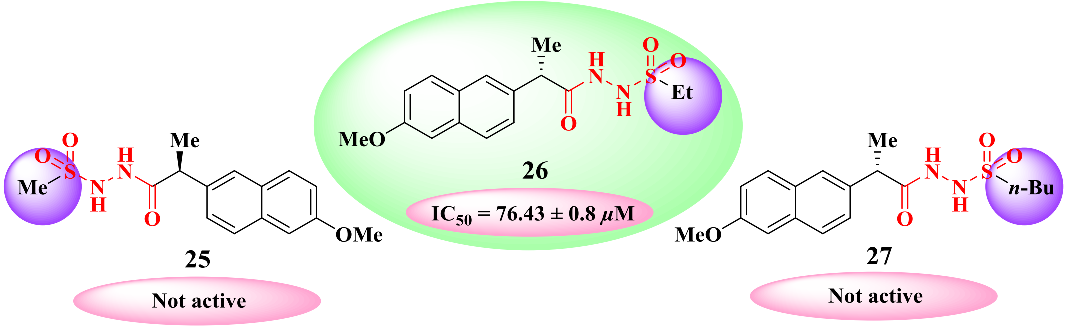


Fig. 8. SAR of compounds **25–27**.

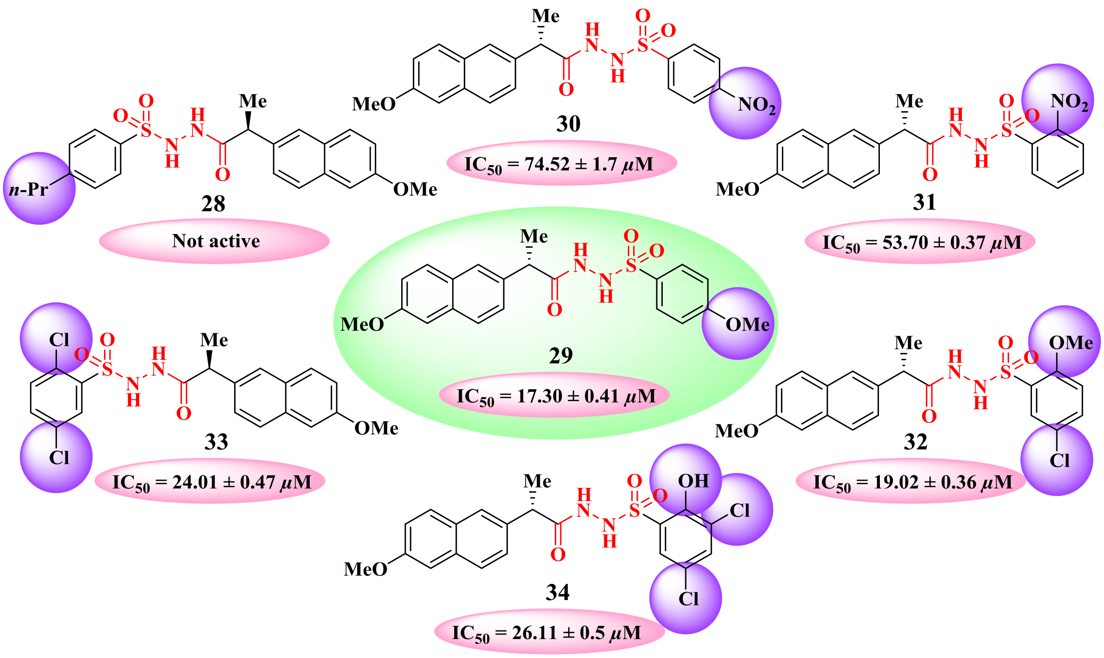


Fig. 9. SAR of compounds **28–34**.

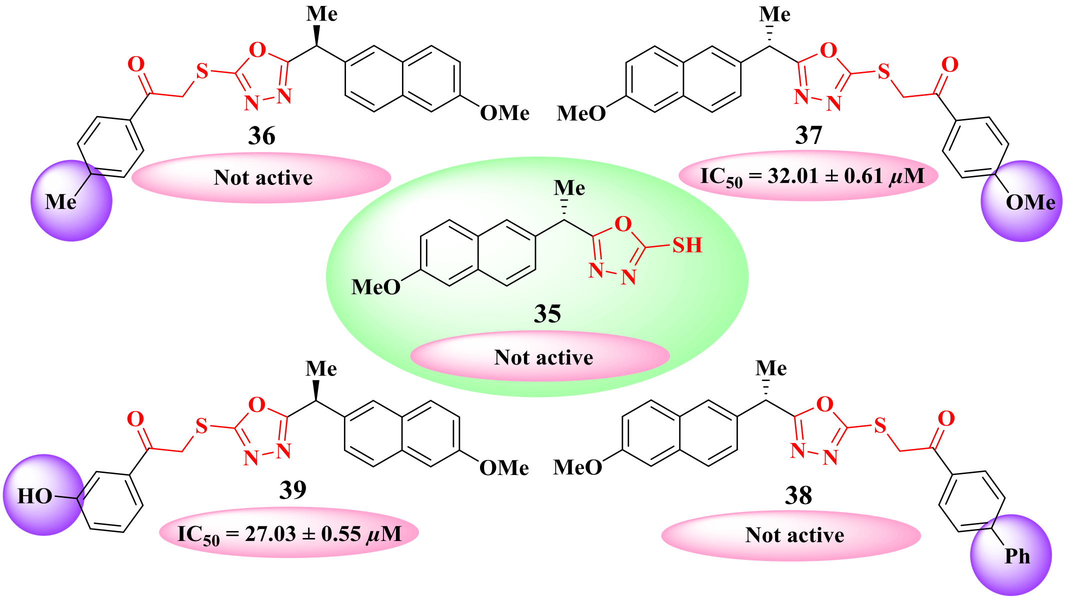


Fig. 10. SAR of compounds **35–39**.

compound. In 3D view, Ala170 was also found in another π-hydrogen interaction with the π-electrons of terminal tri-methoxyphenyl group.

Compound **4** (IC_{50} = 17.9 ± 0.32 μM) is the second most active compound in this group forming three prominent interactions with the binding site residues Cys322 and His323, Lys169, and Ala170 as shown in Fig. 11b. His139 showed induced polar interaction with the side chain hydrogen of compound. Lys169 and Ala170 formed two π-hydrogen interactions with the π systems of compound.

Fig. 11a clearly showed that compound **2** (IC_{50} = 22.81 ± 0.45 μM) is forming three interactions with the residues Cys322 and His323 such as compound **6**. The 3D view also depicts an additional interaction with His324. Compound **12** the fourth most active compound of this category, showed two different interactions with the residues KCX220 and Ni 799 as shown in Fig. 11d. KCX220 showed a hydrogen donor interaction with the hydrogen of naphthalene group. Ala170 was also observed in 3D view that is engaged in a π-hydrogen interaction with the same π system of naphthalene group. Compound **16**, in contrast to its biological activity, presented an unexpected good binding into the active site by interacting with both the Ni ions and Thr171 as listed in Table 2. In this category, the presence of electron rich centers like aromatic rings, NH and C═O groups are the structural features observed for good interaction and active nature of compound. NO_2 and X group containing compounds showed poor results than compounds having single OMe/OH.

3.3. Interactions of the S-Naproxen hydrazide derivatives (22–24)

Two compounds **23** (IC_{50} = 22.02 ± 0.42 μM) and **24** (IC_{50} = 23.01 ± 0.47 μM) in this group were active against urease

Table 2
Interactions detail of the active synthetic compounds.

Interactions detail						
Compounds	Docking Score	Ligand	Receptor	Interaction	Distance	E (kcal/mol)
2	−6.4771	N 14	O CYS 322 (C)	H-donor	2.82	−2.3
		6-ring	5-ring HIS 323 (C)	π-π	3.79	−0.0
		6-ring	5-ring HIS 323 (C)	π-π	3.16	−0.0
4	−10.0610	C 6	NE2 HIS 139 (C)	H-donor	2.74	−0.8
		6-ring	CD LYS 169 (C)	π-H	4.32	−0.6
		6-ring	CA ALA 170 (C)	π-H	3.77	−0.4
5	−5.6526	6-ring	CA CYS 322 (C)	π-H	4.17	−0.6
6	−8.8483	C 11	O CYS 322 (C)	H-donor	2.62	−0.3
		N 14	O CYS 322 (C)	H-donor	3.10	−2.0
		6-ring	5-ring HIS 323 (C)	π-π	3.74	−0.0
		6-ring	5-ring HIS 323 (C)	π-π	3.43	−0.0
7	−5.7874	C 10	OD2 ASP 224 (C)	H-donor	3.39	−0.7
		O 26	NI NI 798 (C)	metal	1.71	−1.6
9	−4.8216	6-ring	CB LYS 169 (C)	π-H	4.11	−0.6
11	−5.5500	C 27	O ALA 366 (C)	H-donor	3.03	−0.8
		C 29	OD2 ASP 363 (C)	H-donor	2.86	−1.1
12	−6.4807	C 1	OX1 KCX 220 (C)	H-donor	2.58	−0.7
		O 25	NI NI 799 (C)	metal	1.66	−1.1
15	−3.9037	6-ring	CE1 HIS 324 (C)	π-H	3.89	−0.6
		6-ring	5-ring HIS 323 (C)	π-π	3.86	−0.0
16	−3.5418	O 30	CA THR 171 (C)	H-acceptor	2.76	−1.3
		O 29	NI NI 798 (C)	metal	2.61	−0.5
		O 29	NI NI 799 (C)	metal	2.16	−1.4
19	−5.6320	O 24	OX1 KCX 220 (C)	H-donor	3.40	−1.4
		BR 25	O MET 367 (C)	H-donor	3.41	−0.4
20	−6.035	6-ring	CA CYS 322 (C)	π-H	4.73	−0.6
		6-ring	CA CYS 322 (C)	π-H	3.79	−0.6
21	−3.7252	6-ring	5-ring HIS 323 (C)	π-π	3.62	−0.0
23	−7.3349	O 18	SG CYS 322 (C)	H-donor	2.32	−8.1
		6-ring	NE2 HIS 222 (C)	π-H	4.30	−0.7
		6-ring	NE2 HIS 222 (C)	π-H	4.16	−1.0
		6-ring	CB CYS 322 (C)	π-H	4.25	−0.8
24	−6.2355	C 28	OD2 ASP 363 (C)	H-donor	2.87	−1.0
		6-ring	CB CYS 322 (C)	π-H	4.38	−0.6
		6-ring	CB CYS 322 (C)	π-H	3.77	−0.7
26	−3.8265	C 3	OD2 ASP 363 (C)	H-donor	3.10	−1.0
29	−6.3576	N 14	O CYS 322 (C)	H-donor	2.68	−4.9
		6-ring	5-ring HIS 323 (C)	π-π	3.62	−0.0
		6-ring	5-ring HIS 323 (C)	π-π	3.68	−0.0
30	−4.0638	O 19	CA CYS 322 (C)	H-acceptor	2.68	−0.9
31	−4.3748	C 6	OD2 ASP 363 (C)	H-donor	2.66	−0.6
32	−6.6649	N 15	O ALA 170 (C)	H-donor	2.97	−3.1
		O 13	NE2 HIS 222 (C)	H-acceptor	3.35	−1.4
		O 17	CE1 HIS 323 (C)	H-acceptor	3.08	−1.0
33	−6.0448	N 14	O ALA 170 (C)	H-donor	2.85	−5.8
		N 15	SD MET 367 (C)	H-donor	3.81	−1.5
		O 17	SG CYS 322 (C)	H-donor	3.03	−1.0
34	−6.1353	O 19	SG CYS 322 (C)	H-donor	2.88	−0.7
		C 20	SG CYS 322 (C)	H-donor	3.60	−1.0
		6-ring	5-ring HIS 323 (C)	π-π	3.47	−0.1
37	−5.2747	S 17	OE2 GLU 166 (C)	H-donor	3.72	−1.0
		O 21	CE1 HIS 324 (C)	H-acceptor	2.60	−0.1
		5-ring	CB ALA 170 (C)	π-H	3.72	−1.0
		6-ring	5-ring HIS 323 (C)	π-π	2.94	−0.1
39	−7.4839	S 17	OD2 ASP 224 (C)	H-donor	3.24	−1.2
		O 21	SG CYS 322 (C)	H-donor	3.20	−0.9
		O 27	OX1 KCX 220 (C)	H-donor	2.67	−3.3
Standard (Acetohydroxamic acid)	−7.3475	O 4	ND1 HIS 323 (C)	H-donor	3.39	−0.9
		C 5	O ALA 170 (C)	H-donor	2.97	−1.0
		C 5	OX1 KCX 220 (C)	H-donor	3.11	−0.9
		O 2	NI NI 798 (C)	metal	1.88	−3.8

enzyme. Compound **23** showed four important interactions with the residues Cys322 and His222 as presented by Fig. 12a. Similarly, compound **24** which have almost similar biological activity, also showed three interactions with Cys322 and Asp363 using π system of naphthalene ring and terminal OMe group (Fig. 12b). The π system of naphthalene ring with attached OMe group was observed as active moiety in this category of compounds.

3.4. Interactions of the S-Naproxen sulfohydrazide derivatives (25–34)

Compound **29** (IC_{50} = 17.30 ± 0.41 µM) demonstrated the highest activity in this group and showed three important interactions with the residues Cys322 and His323 as shown in Fig. 13a. Cys322 formed H-donor interaction with hydrazide moiety and His323 showed π-π interaction with naphthalene ring. An induced polar contact was also found between Met367 and aryl hydrogen of compound in the 3D view.

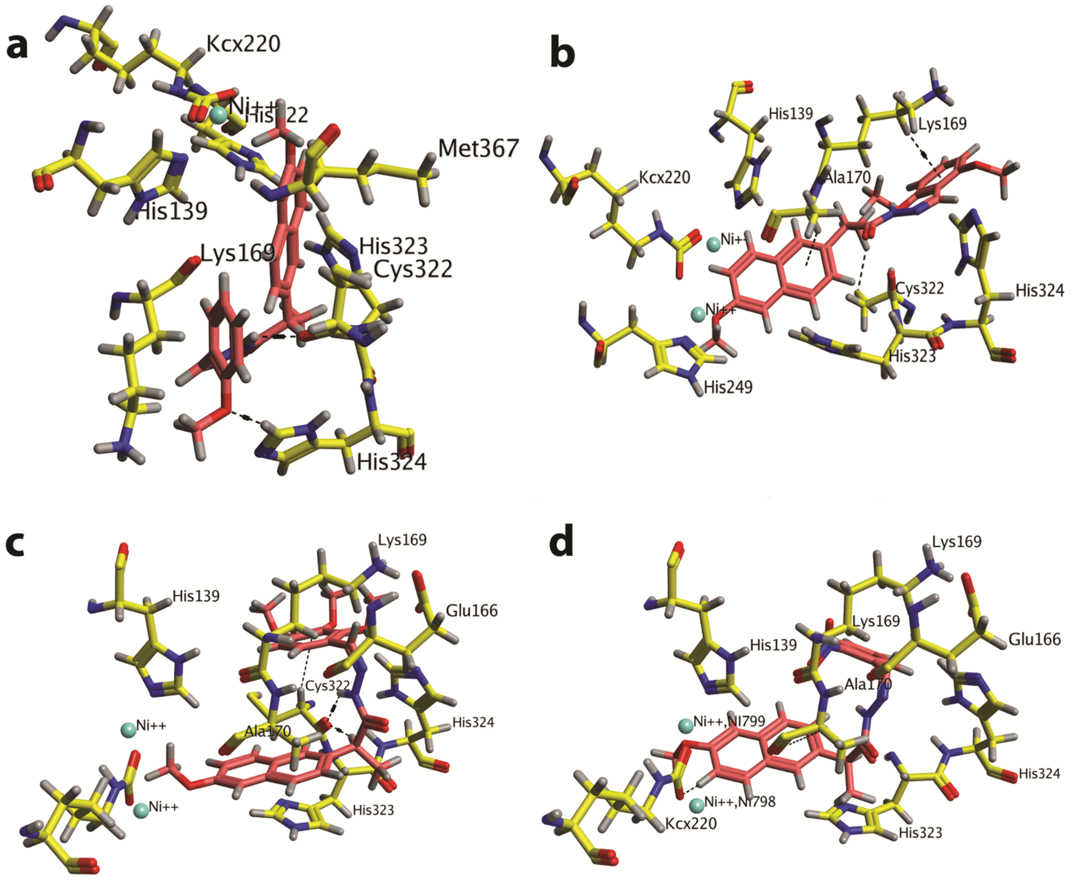


Fig. 11. 3D pictures of the docked conformations, (**a**) compound **2**, (**b**) compound **4**, (**c**) compound **6**, and (**d**) compound **12.**

Compound **32** (IC_{50} = 19.02 ± 0.36 µM) the second most active member of this category was found forming three interactions with the residues Ala170, His222, and His323 as shown in Fig. 13b. Ala170 showed H-donor interaction with hydrazide group, while His222 and His323 formed hydrogen acceptor interaction with sulfohydrazide moiety of the compound. Compound **33** (IC_{50} = 24.01 ± 0.47 µM) is the third most active compound and showed three different interactions with the residues Ala170, Cys322, and Met367 as shown in Fig. 13c. All the three residues are engaged with sulfohydrazide moiety *via* hydrogen donor interactions. A hydrogen-π contact was also found between Lys169 and π-system of compound in the 3D view. Compound **34** (IC_{50} = 26.11 ± 0.5 µM) the fourth most active member also showed three important interactions with Cys322 and His323 as listed in Table 2. The presence of a sulfone group adjacent to hydrazide was observed in improving the interaction mode particularly for the compounds having MeO/Cl groups.

3.5. *Interactions of the 2-Mercapto oxadiazoles of S-Naproxen (35–39)*

Compound **37** (IC_{50} = 32.01 ± 0.61 µM) and **39** (IC_{50} = 27.03 ± 0.55 µM) were proved to be an active analogs of this category. It is clear from Fig. 13d that compound **37** is showing four

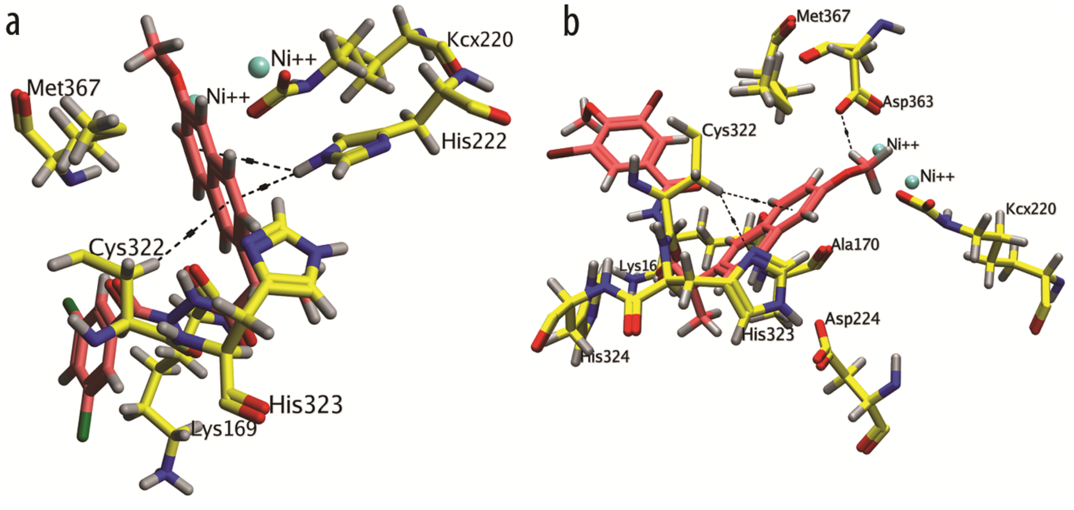


Fig. 12. 3D pictures of the docked conformations, (**a**) compound **23**, (**b**) compound **24**.

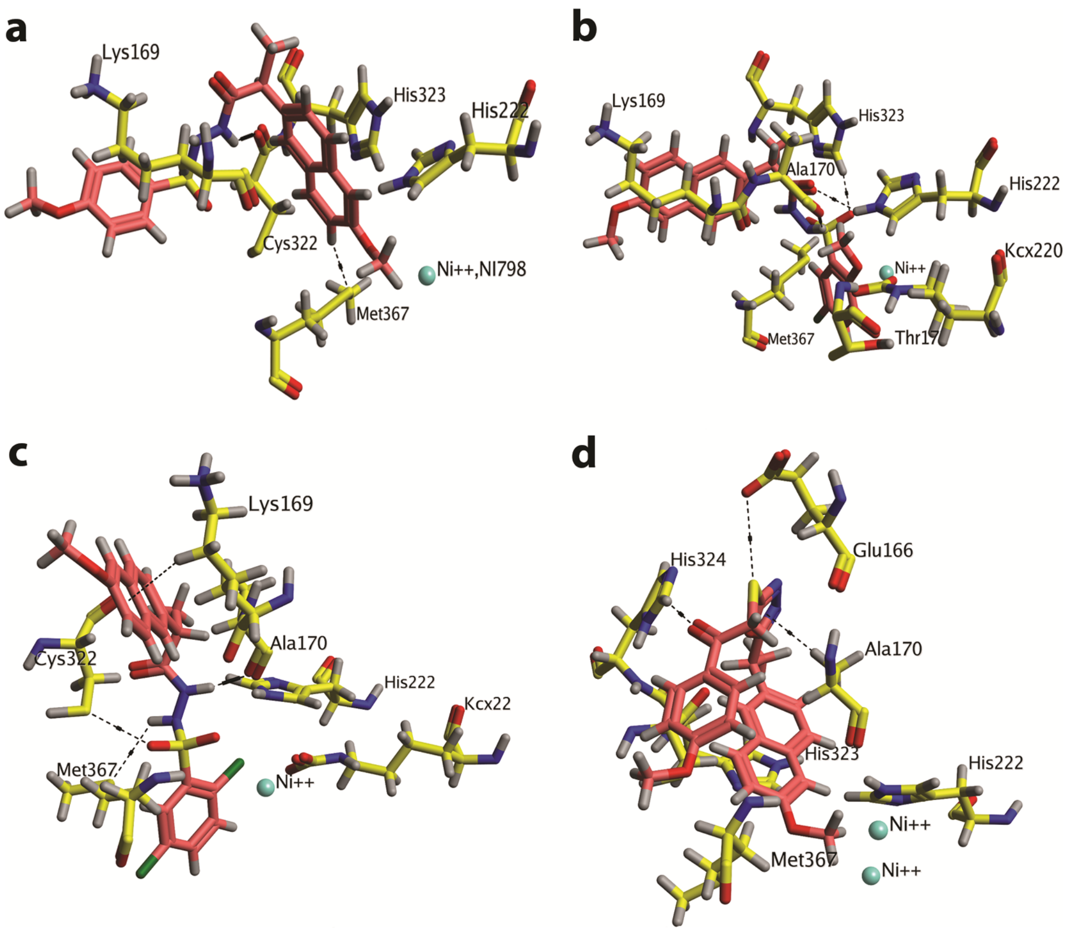


Fig. 13. 3D pictures of the docked conformations, (**a**) compound **29**, (**b**) compound **32**, (**c**) compound **33**, and (**d**) compound **37**.

interactions with residues Glu166, Ala170, His323, and His324 in the binding cavity. Glu166 is involved in side chain H-donor interaction with the sulfur bonded to oxadiazole moiety of compound. Ala170 showed a π-hydrogen interactions with the π-electrons of oxadiazole ring of the compound. His323 was found in another π-π interaction with the π-electrons of the same oxadiazole group. Similarly, His324 established a hydrogen acceptor bond with C═O group through its imidazole ring.

Compound **39** also showed considerable interactions with the active site residues. The binding mode of compound **39** is shown in Fig. 14a. Compound **39** showed interactions with Asp224, Cys322, and KCX220. Asp224 formed H-donor interaction with the S of oxadiazole moiety of compound while Cys322 formed two interactions with C═O and π system of naphthalene ring. KCX220 was observed to be involved in the hydrogen bonding with the terminal methoxybenzene group. The presence of oxadiazole ring in the members of this category was observed in improving the interaction mode of active compounds.

Comparing the binding mode of the active compounds with that of the standard acetohydroxamic acid, it was observed that almost all the active synthetic compounds impart nearly similar or slightly superior interaction strength. In this *in silico* inhibition study, the standard acetohydroxamic acid showed four energetically highly favorable interactions with the active site residues, His323, Ala170, and KCX220 as well as with the Ni ion of the active site (Ni 798) as shown in Fig. 14b. The interactions detail given in Table 2 clearly predict the inhibiting capability of all synthetic derivatives for urease enzyme. The correlation coefficient value calculated for the docking scores of active compounds and their biological activities is given in Fig. 15.

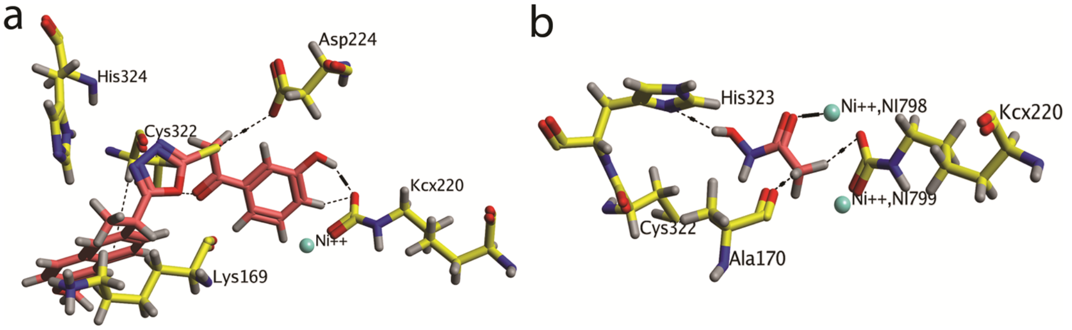


Fig. 14. 3D pictures of the docked conformations, (**a**) compound **39** and (**b**) standard acetohydroxamic acid.

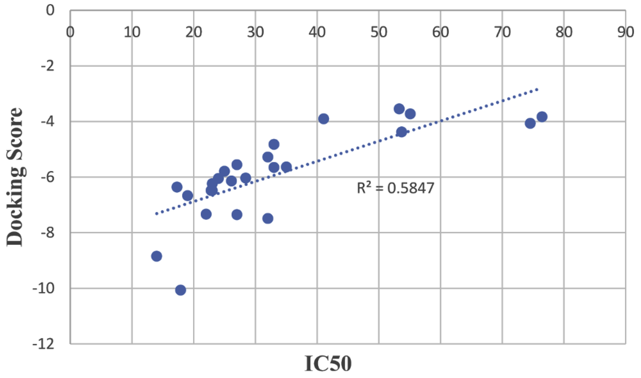


Fig. 15. The correlation graph between the calculated docking scores and IC_{50} values.

3.6. Conclusion

Synthetic *S*-naproxen derivatives including hydrazide, Schiff bases, aroyl and sulfohydrazides, 2-mercapto oxadiazole, phenacyl substituted 2-mercapto oxadiazoles, showed significant *in vitro* urease inhibitory activity as compared to standard acetohydroxamic acid. Limited SAR and *in silico* studies identified a range of structural motifs of the active synthetic compounds those are taking part in urease enzyme inhibition. This study has identified a number of lead compounds which can serve for future research in order to identify potent urease inhibitors.

4. Experimental

4.1. Materials and methods

Reagents were purchased from Sigma-Aldrich, USA. Thin layer chromatography was carried out on pre-coated silica gel, GF-254 (Merck, Darmstadt, Germany). Spots were visualized under ultraviolet light at 254, 366 nm or iodine vapor. EI- and HREI-MS spectra were recorded on MAT 312 and MAT 113D mass spectrometers. The ^{1}H-, ^{13}C NMR were recorded on Bruker AM spectrometers, operating at 300 and 400 MHz. The chemical shift are presented in ppm (δ), relative to tetramethylsilane (TMS) as an internal standard and the coupling constant (J) are in Hz. CHN analyses were carried out on a Carlo Erba Strumentazione-Mod-1106, Italy. Melting points of the compounds were determined on a Stuart® SMP10 melting point apparatus and are uncorrected.

4.2. Procedure for the synthesis of S-Naproxen hydrazide (1)

S-Naproxen (10 mmol) was taken in a round-bottommed flask (250 mL) and dissolved in a minimum volume of tetrahydrofuran (THF) (usually 10 mL) to make a saturated solution. Then, CDI (11 mmol) and triethylamine (10 mmol) were added into the reaction mixture and stirred until ceasing of effervescence. After that, THF (20 mL) and hydrazine hydrate (5 mL) were added and refluxed the reaction mixture for 3 h with continuous stirring. Progress of the reaction was determined by thin layer chromatography (TLC; Hexane:ethylacetate (6:4)). After completion, the reaction mixture was poured onto crushed ice. Immediately, precipitates appeared which were filtered, washed with distilled water, dried in air and crystallized from ethanol to afford pure product in good yield.

4.2.1. (S)-2-(6-Methoxynaphthalen-2-yl)propanehydrazide (1)

White solid; Yield: 61%; R_f: 0.73; M.p.: 146–148 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.18 (s, 1H, NH), 7.76 (d, $J_{8,7}$ = 9.2 Hz, 1H, H-8), 7.73 (d, $J_{4,3}$ = 8.4 Hz, 1H, H-4), 7.69 (bd.s, 1H, H-1), 7.44 (dd, $J_{3,1}$ = 1.6 Hz, $J_{3,4}$ = 8.4 Hz, 1H, H-3), 7.25 (d, $J_{5,7}$ = 2.4 Hz, 1H, H-5), 7.13 (dd, $J_{7,5}$ = 2.4 Hz, $J_{7,8}$ = 8.8 Hz, 1H, H-7), 4.17 (s, 2H, NH_2), 3.84 (s, 3H, OCH_3), 3.66 (q, $J_{CH,CH3}$ = 7.2 Hz, 1H, CH), 1.40 (d, $J_{CH3,CH}$ = 7.2 Hz, 3H, CH_3); EI-MS *m/z* (% rel. abund.): 244 (M^+, 80), 185 (100); Anal. Calcd for $C_{14}H_{16}N_2O_2$: C = 68.83; H = 6.60; N = 11.47; Found: C = 68.81; H = 6.62; N = 11.49.

4.3. General procedure for the synthesis of Schiff bases of S-Naproxen hydrazide (2–21)

S-Naproxen hydrazide **1** (0.5 mmol) and substituted benzaldehydes (0.5 mmol) were dissolved in ethanol into a round-bottommed flask (100 mL). Glacial acetic acid (3–5 drops) was added into the reaction mixture and refluxed for 3–8 h. Reaction progress was monitored *via* periodic TLC (Hexane:ethylacetate (6:4)). After completion, the reaction mixture was poured onto the crushed ice. Resulting precipitates were filtered, washed with distilled water, and dried in air. Products were crystallized from ethanol.

4.3.1. (E,Z)-(S)-N′-(2-Methoxybenzylidene)-2-(6-methoxynaphthalen-2-yl)propanehydrazide (2)

White solid; Yield: 81%; R_f: 0.82; M.p.: 151–153 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.52 (s, 1H, NH), 11.20 (s, 1H, NH^*), 8.52 (s, 1H, N=CH), 8.22 (s, 1H, $N{=}CH^*$), 7.83 (ovp, 8H, H-1, H-1^*, H-4, H-4^*, H-8, H-8^*, H-6′, H-$6'^*$), 7.48 (ovp, 2H, H-4′, H-$4'^*$), 7.39 (ovp, 2H, H-3, H-3^*), 7.27 (d, $J_{5,7}$ = 2.4 Hz, 1H, H-5), 7.23 (d, $J_{5,7}$ = 2.4 Hz, 1H, H-5^*), 7.15 (ovp, 2H, H-7, H-7^*), 7.07 (t, $J_{5',4'/5',6'}$ = 8.0 Hz, 2H, H-5′, H-$5'^*$), 7.00 (ovp, 2H, H-3′, H-$3'^*$), 4.76 (q, $J_{CH,CH3}$ = 7.2 Hz, 1H, CH), 3.84 (s, 3H, OCH_3), 3.81 (ovp, 3H, 6H, OCH_3, OCH_3'), 3.78 (ovp, 4H, CH^*, $OCH_3'^*$), 1.47 (ovp, 6H, CH_3, CH_3^*); EI-MS *m/z* (% rel. abund.): 362 (M^+, 22), 185 (100); Anal. Calcd for $C_{22}H_{22}N_2O_3$: C = 72.91; H = 6.12; N = 7.73; Found: C = 72.92; H = 6.14; N = 7.71.

4.3.2. (E,Z)-(S)-N′-(2,4-Dimethoxybenzylidene)-2-(6-methoxynaphthalen-2-yl)propanehydrazide (3)

White solid; Yield: 56%; R_f: 0.81; M.p.: 180–182 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.38 (s, 1H, NH), 11.06 (s, 1H, NH^*), 8.42 (s, 1H, N=CH), 8.13 (s, 1H, $N{=}CH^*$), 7.79 (ovp, 6H, H-1, H-1^*, H-4, H-4^*, H-8, H-8^*), 7.68 (d, $J_{6',5'}$ = 8.4 Hz, 2H, H-6′, H-$6'^*$), 7.47 (ovp, 2H, H-3, H-3^*), 7.26 (d, $J_{5,7}$ = 2.4 Hz, 1H, H-5), 7.23 (d, $J_{5,7}$ = 2.4 Hz, 1H, H-5^*), 7.14 (ovp, 2H, H-7, H-7^*), 6.62 (ovp, 4H, H-3′, H-$3'^*$, H-5′, H-$5'^*$), 4.76 (q, $J_{CH,CH3}$ = 7.2 Hz, 1H, CH), 3.84 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3^*), 3.80 (s, 3H, OCH_3'), 3.79 (s, 3H, $OCH_3'^*$), 3.78 (ovp, 7H, CH^*, OCH_3'', $OCH_3''^*$), 1.46 (ovp, 6H, CH_3, CH_3^*); EI-MS *m/z* (% rel. abund.): 392 (M^+, 57), 185 (100); Anal. Calcd for $C_{23}H_{24}N_2O_4$: C = 70.39; H = 6.16; N = 7.14; Found: C = 70.37; H = 6.14; N = 7.15.

4.3.3. (E,Z)-(S)-N′-(2,6-Dimethoxybenzylidene)-2-(6-methoxynaphthalen-2-yl)propanehydrazide (4)

White solid; Yield: 91%; R_f: 0.87; M.p.: 183–185 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.33 (s, 1H, NH), 11.03 (s, 1H, NH^*), 8.32 (s, 1H, N=CH), 8.18 (s, 1H, $N{=}CH^*$), 7.80 (ovp, 6H, H-1, H-1^*, H-4, H-4^*, H-8, H-8^*), 7.52 (ovp, 2H, H-3, H-3^*), 7.33 (ovp, 4H, H-5, H-5^*, H-4′, H-$4'^*$), 7.15 (ovp, 2H, H-7, H-7^*), 6.71 (ovp, 4H, H-3′, H-$3'^*$, H-5′, H-$5'^*$), 4.82 (q, $J_{CH,CH3}$ = 7.2 Hz, 1H, CH), 3.85 (s, 3H, OCH_3), 3.83 (ovp, 16H, OCH_3^*, OCH_3', $OCH_3'^*$, OCH_3'', $OCH3''^*$, CH^*), 1.46 (ovp, 6H, CH_3, CH_3^*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 174.8, 169.2^*, 158.6, 158.5^*, 157.0, 156.9^*, 154.0, 141.9, 137.9^*, 137.0, 136.8^*, 133.2, 133.0, 131.0, 130.8^*, 129.1, 129.0^*, 128.3, 126.9, 126.7^*, 126.4, 126.3^*, 125.5, 125.4^*, 118.6, 118.4^*, 110.8, 105.7, 105.6^*, 104.4, 104.3^*, 55.9, 55.8^*, 55.1, 43.8, 18.4, 17.9^*; EI-MS *m/z* (% rel. abund.): 392 (M^+, 57), 185 (100); HREI-MS Calcd for $C_{23}H_{24}N_2O_4$ *m/z* 392.1736, found = 392.1759; Anal. Calcd for $C_{23}H_{24}N_2O_4$: C = 70.39; H = 6.16; N = 7.14; Found: C = 70.41; H = 6.18; N = 7.12.

4.3.4. (E,Z)-(S)-N′-(3,5-Dimethoxybenzylidene)-2-(6-methoxynaphthalen-2-yl)propanehydrazide (5)

White solid; Yield: 87%; R_f: 0.84; M.p.: 183–185 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.54 (s, 1H, NH), 11.28 (s, 1H, NH^*), 8.10 (s, 1H, N=CH), 7.80 (ovp, 7H, N=CH*, H-1, H-1*, H-4, H-4*, H-8, H-8*), 7.48 (d, $J_{3,4}$ = 9.2 Hz, 2H, H-3, H-3*), 7.27 (d, $J_{5,7}$ = 2.4 Hz, 1H, H-5), 7.24 (d, $J_{5,7}$ = 2.4 Hz, 1H, H-5*), 7.15 (ovp, 2H, H-7, H-7*), 6.79 (ovp, 4H, H-2′, H-2′*, H-6′, H-6′*), 6.52 (s, 1H, H-4′), 6.49 (s, 1H, H-4′*), 4.74 (q, $J_{\mathrm{CH,CH3}}$ = 6.8 Hz, 1H, CH), 3.84 (s, 3H, OCH$_3$), 3.82 (ovp, 4H, CH*, OCH$_3$*), 3.77 (s, 6H, OCH$_3$′, OCH$_3$″), 3.74 (s, 6H, OCH$_3$′*, OCH$_3$″*), 1.47 (ovp, 6H, CH$_3$, CH$_3$*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 174.9, 169.8, 160.5, 160.5, 146.4, 142.1, 137.3, 136.2, 129.0, 128.8*, 128.4, 126.7, 126.2, 125.3, 125.2*, 118.6, 118.5*, 105.6, 105.6, 104.7, 104.3*, 102.1, 101.9*, 55.2, 55.2, 55.1, 43.8, 41.0*, 18.7, 18.4*; EI-MS m/z (% rel. abund.): 392 (M$^+$, 80), 185 (100); HREI-MS Calcd for $C_{23}H_{24}N_2O_4$ m/z 392.1736, found = 392.1753; Anal. Calcd for $C_{23}H_{24}N_2O_4$: C = 70.39; H = 6.16; N = 7.14; Found: C = 70.38; H = 6.18; N = 7.15.

4.3.5. *(E,Z)-(S)-2-(6-Methoxynaphthalen-2-yl)-N′-(2,3,4-trimethoxybenzylidene)propanehydrazide (6)*

White solid; Yield: 63%; R$_f$: 0.84; M.p.: 137–139 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.49 (s, 1H, NH), 11.14 (s, 1H, NH*), 8.33 (s, 1H, N=CH), 8.08 (s, 1H, N=CH*), 7.80 (ovp, 6H, H-1, H-1*, H-4, H-4*, H-8, H-8*), 7.59 (d, $J_{6′,5′}$ = 8.7 Hz, 1H, H-6), 7.51 (ovp, 3H, H-3, H-3*, H-6′*), 7.27 (d, $J_{5,7}$ = 2.4 Hz, 1H, H-5), 7.24 (d, $J_{5,7}$ = 2.4 Hz, 1H, H-5*), 7.15 (ovp, 2H, H-7, H-7*), 6.93 (ovp, 2H, H-5′, H-5′*), 4.77 (q, $J_{\mathrm{CH,CH3}}$ = 7.2 Hz, 1H, CH), 3.84 (s, 3H, OCH$_3$), 3.83 (ovp, 25H, CH*, OCH$_3$, OCH$_3$*, OCH$_3$′, OCH$_3$′*, OCH$_3$″, OCH$_3$″*, OCH$_3$‴, OCH$_3$‴*), 1.47 (ovp, 6H, CH$_3$, CH$_3$*); EI-MS m/z (% rel. abund.): 422 (M$^+$, 57), 185 (100); Anal. Calcd for $C_{24}H_{26}N_2O_5$: C = 68.23; H = 6.20; N = 6.63; Found: C = 68.25; H = 6.23; N = 6.65.

4.3.6. *(E,Z)-(S)-N′-(4-Bromo-3,5-dimethoxybenzylidene)-2-(6-methoxynaphthalen-2-yl)propanehydrazide (7)*

White solid; Yield: 88%; R$_f$: 0.79; M.p.: 225–227 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.62 (s, 1H, NH), 11.41 (s, 1H, NH*), 8.18 (s, 1H, N=CH), 7.81 (ovp, 7H, N=CH*, H-1, H-1*, H-4, H-4*, H-8, H-8*), 7.50 (ovp, 2H, H-3, H-3*), 7.27 (d, $J_{5,7}$ = 2.4 Hz, 1H, H-5), 7.24 (d, $J_{5,7}$ = 2.4 Hz, 1H, H-5*), 7.15 (ovp, 2H, H-7, H-7*), 6.98 (s, 2H, H-2′, H-6′), 6.94 (s, 2H, H-2′*, H-6′*), 4.76 (q, $J_{\mathrm{CH,CH3}}$ = 7.2 Hz, 1H, CH), 3.88 (ovp, 19H, CH*, OCH$_3$′, OCH$_3$′*, OCH$_3$″, OCH$_3$″*, OCH$_3$, OCH$_3$*), 1.49 (d, $J_{\mathrm{CH3,CH}}$ = 7.2 Hz, 3H, CH$_3$), 1.44 (d, $J_{\mathrm{CH3,CH}}$ = 7.2 Hz, 3H, CH$_3$*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 175.0, 169.9, 156.6, 156.6, 141.5, 134.9, 129.1, 128.9, 126.8, 126.8, 125.4, 125.1, 118.7, 118.6, 105.7, 103.2, 102.9, 56.4, 56.4, 55.1, 43.8, 41.2*, 18.9, 18.4*; EI-MS m/z (% rel. abund.): 470 (M$^+$, 29), 472 (M + 2, 28), 185 (100); HREI-MS Calcd for $C_{23}H_{23}BrN_2O_4$ m/z 470.0841, found = 470.0835; Anal. Calcd for $C_{23}H_{23}BrN_2O_4$: C = 58.61; H = 4.92; N = 5.94; Found: C = 58.64; H = 4.94; N = 5.92.

4.3.7. *(E,Z)-(S)-N′-(2-Chloro-3,4-dimethoxybenzylidene)-2-(6-methoxynaphthalen-2-yl)propanehydrazide (8)*

White solid; Yield: 50%; R$_f$: 0.75; M.p.: 106–108 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.64 (s, 1H, NH), 11.33 (s, 1H, NH*), 8.49 (s, 1H, N=CH), 8.92 (s, 1H, N=CH*), 7.80 (ovp, 7H, H-1, H-1*, H-4, H-4*, H-8, H-8*, H-6′), 7.66 (d, $J_{6′,5′}$ = 8.8 Hz, 1H, H-6′), 7.48 (ovp, 2H, H-3, H-3*), 7.27 (d, $J_{5,7}$ = 2.4 Hz, 1H, H-5), 7.24 (d, $J_{5,7}$ = 2.4 Hz, 1H, H-5*), 7.17 (ovp, 4H, H-7, H-7*, H-5′, H-5′*), 4.76 (q, $J_{\mathrm{CH,CH3}}$ = 7.2 Hz, 1H, CH), 3.88 (s, 3H, OCH$_3$′), 3.85 (s, 3H, OCH$_3$′*), 3.84 (s, 3H, OCH$_3$), 3.83 (ovp, 4H, CH*, OCH$_3$*), 3.73 (s, 3H, OCH$_3$″), 3.73 (s, 3H, OCH$_3$″*), 1.48 (d, $J_{\mathrm{CH3,CH}}$ = 7.2 Hz, 3H, CH$_3$), 1.45 (d, $J_{\mathrm{CH3,CH}}$ = 7.2 Hz, 3H, CH$_3$*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 174.8, 169.7, 157.0, 156.9*, 154.6, 154.2*, 144.5, 142.6, 138.8, 137.0, 136.5*, 133.2, 133.0*, 129.1, 129.0*, 128.4, 128.3*, 126.8, 126.7*, 126.7, 126.2*, 125.5, 125.4*, 124.6, 124.4*, 121.9, 121.8*, 118.6, 118.5*, 112.0, 111.9*, 105.6, 60.1, 56.2, 56.1*, 44.0, 40.7*, 18.5, 18.4*; EI-MS m/z (% rel. abund.): 426 (M$^+$, 42), 428 (M + 2, 13), 185 (100); HREI-MS Calcd for $C_{23}H_{23}ClN_2O_4$ m/z 426.1346, found = 426.1343; Anal. Calcd for $C_{23}H_{23}ClN_2O_4$: C = 64.71; H = 5.43; N = 6.56; Found: C = 64.73; H = 5.45; N = 6.53.

4.3.8. *(E,Z)-(S)-N′-(5-Bromo-2-methoxybenzylidene)-2-(6-methoxynaphthalen-2-yl)propanehydrazide (9)*

White solid; Yield: 57%; R$_f$: 0.86; M.p.: 178–180 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.64 (s, 1H, NH), 11.31 (s, 1H, NH*), 8.44 (s, 1H, N=CH), 8.11 (s, 1H, N=CH*), 7.84 (d, $J_{6′,4′}$ = 2.4 Hz, 1H, H-6′), 7.81 (d, $J_{6′,4′}$ = 2.4 Hz, 1H, H-6′*), 7.80 (ovp, 6H, H-1, H-1*, H-4, H-4*, H-8, H-8*), 7.54 (ovp, 2H, H-4′, H-4′*), 7.47 (ovp, 2H, H-3, H-3*), 7.27 (d, $J_{5,7}$ = 2.4 Hz, 1H, H-5), 7.25 (d, $J_{5,7}$ = 2.4 Hz, 1H, H-5*), 7.15 (ovp, 2H, H-7, H-7*), 7.06 (d, $J_{3′,4′}$ = 8.8 Hz, 1H, H-3′), 7.02 (d, $J_{3′,4′}$ = 8.8 Hz, 1H, H-3′*), 4.72 (q, $J_{\mathrm{CH,CH3}}$ = 7.2 Hz, 1H, CH), 3.84 (s, 3H, OCH$_3$), 3.82 (s, 3H, OCH$_3$*), 3.81 (s, 3H, OCH$_3$′*), 3.77 (ovp, 4H, CH*, OCH$_3$′*), 1.47 (ovp, 6H, CH$_3$, CH$_3$*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 174.9, 169.7, 157.1, 157.0*, 156.7, 156.5*, 140.2, 137.2, 136.5, 136.4*, 133.4, 133.2*, 133.1, 133.0*, 129.1, 128.9*, 128.4, 128.3*, 127.2, 126.8, 126.7*, 126.5, 126.1*, 125.5, 125.4*, 124.5, 124.3*, 118.6, 118.5*, 114.3, 114.2*, 105.7, 105.6*, 56.08, 56.02*, 55.1, 44.0, 41.1*, 18.5, 18.3*; EI-MS m/z (% rel. abund.): 440 (M$^+$, 22), 442 (M + 2, 18), 185 (100); HREI-MS Calcd for $C_{22}H_{21}BrN_2O_3$ m/z 440.0736, found = 440.0745; Anal. Calcd for $C_{22}H_{21}BrN_2O_3$: C = 59.87; H = 4.80; N = 6.35; Found: C = 59.89; H = 4.82; N = 6.38.

4.3.9. *(E,Z)-(S)-N′-(4-Fluoro-3-methoxybenzylidene)-2-(6-methoxynaphthalen-2-yl)propanehydrazide (10)*

White solid; Yield: 45%; R$_f$: 0.83; M.p.: 185–187 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.54 (s, 1H, NH), 11.29 (s, 1H, NH*), 8.15 (s, 1H, N=CH), 7.82 (ovp, 7H, N=CH*, H-1, H-1*, H-4, H-4*, H-8, H-8*), 7.49 (ovp, 2H, H-3, H-3*), 7.42 (ovp, 2H, H-5, H-5*), 7.27 (ovp, 8H, H-7, H-7*, H-2′, H-2′*, H-5′, H-5′*, H-6′, H-6′*), 4.76 (q, $J_{\mathrm{CH,CH3}}$ = 7.2 Hz, 1H, CH), 3.90 (s, 3H, OCH$_3$′), 3.85 (ovp, 10*H*, OCH$_3$′, OCH$_3$′*, CH*, OCH$_3$*), 1.48 (d, $J_{\mathrm{CH3,CH}}$ = 7.2 Hz, 3H, CH$_3$), 1.44 (d, $J_{\mathrm{CH3,CH}}$ = 7.2 Hz, 3H, CH$_3$*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 174.9, 169.8, 157.0, 156.9*, 154.1, 153.9*, 150.6, 147.5*, 145.7, 141.4*, 137.4, 136.6*, 131.3, 131.2*, 129.0, 128.9*, 128.4, 128.3*, 126.8, 126.7*, 125.4, 125.3*, 120.7, 120.6*, 120.1, 120.0*, 118.6, 118.5*, 116.2, 116.0*, 110.7, 110.6*, 105.6, 55.99, 55.91*, 55.1, 43.9, 41.0*, 18.7, 18.4*; EI-MS m/z (% rel. abund.): 380 (M$^+$, 70), 185 (100); HREI-MS Calcd for $C_{22}H_{21}FN_2O_3$ m/z 380.1536, found = 380.1547; Anal. Calcd for $C_{22}H_{21}FN_2O_3$: C = 69.46; H = 5.56; N = 7.36; Found: C = 69.43; H = 5.58; N = 7.38.

4.3.10. *(E,Z)-(S)-N′-(2-Chloro-3-methoxybenzylidene)-2-(6-methoxynaphthalen-2-yl)propanehydrazide (11)*

White solid; Yield: 91%; R$_f$: 0.92; M.p.: 187–189 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.75 (s, 1H, NH), 11.46 (s, 1H, NH*), 8.59 (s, 1H, N=CH), 8.30 (s, 1H, N=CH*), 7.80 (ovp, 6H, H-1, H-1*, H-4, H-4*, H-8, H-8*), 7.57 (d, $J_{6′,5′}$ = 7.6 Hz, 1H, H-6′), 7.49 (ovp, 3H, H-5′, H-5′*, H-6′*), 7.38 (ovp, 2H, H-3, H-3*), 7.27 (d, $J_{5,7}$ = 2.4 Hz, 1H, H-5), 7.23 (d, $J_{5,7}$ = 2.4 Hz, 1H, H-5*), 7.17 (ovp, 4H, H-7, H-7*, H-4′, H-4′*), 4.77 (q, $J_{\mathrm{CH,CH3}}$ = 6.8 Hz, 1H, CH), 3.84 (ovp, 13H, OCH$_3$, OCH$_3$*, OCH$_3$′, OCH$_3$′*, CH*), 1.48 (ovp, 6H, CH$_3$, CH$_3$*); ^{13}C NMR (75 MHz, DMSO-d_6): δ 175.2, 170.0, 157.1, 157.0*, 155.0, 142.8, 138.9*, 137.0, 136.5*, 133.3, 133.1*, 132.7, 132.6*, 129.1, 129.0*, 128.5, 128.4*, 127.9, 127.8*, 126.9, 126.8*, 126.7, 126.3*, 125.7, 125.5*, 121.5, 121.4*, 118.7, 118.6*, 118.2, 118.0*, 113.6, 113.2*, 105.7, 56.3, 55.2, 44.1, 40.9*, 18.5, 18.4*; EI-MS m/z (% rel. abund.): 396 (M$^+$, 31), 398 (M + 2, 8), 185 (100); HREI-MS Calcd for $C_{22}H_{21}ClN_2O_3$ m/z 396.1241 found is 396.1245; Anal. Calcd for $C_{22}H_{21}ClN_2O_3$: C = 66.58; H = 5.33; N = 7.06; Found: C = 66.60; H = 5.35; N = 7.08.

4.3.11. *(E,Z)-(S)-2-(6-Methoxynaphthalen-2-yl)-N′-(2-nitrobenzylidene)propanehydrazide (12)*

White solid; Yield: 85%; R$_f$: 0.84; M.p.: 151–153 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.86 (s, 1H, NH), 11.55 (s, 1H, NH*), 8.58 (s, 1H, N=CH), 8.27 (s, 1H, N=CH*), 8.04 (ovp, 4H, H-3′, H-3′*, H-6′, H-6′*), 7.80 (ovp, 8H, H-1, H-1* H-4, H-4*, H-8, H-8*, H-5′, H-5′*), 7.65

(ovp, 2H, H-4′, H-4′*), 7.48 (ovp, 2H, H-3, H-3*), 7.27 (d, $J_{5,7}$ = 2.4 Hz, 1H, H-5), 7.24 (d, $J_{5,7}$ = 2.4 Hz, 1H, H-5*), 7.15 (ovp, 2H, H-7, H-7*), 4.75 (q, $J_{\mathrm{CH,CH3}}$ = 7.2 Hz, 1H, CH), 3.85 (s, 3H, OCH$_3$), 3.83 (ovp, 4H, CH*, OCH$_3^{*}$), 1.49 (d, $J_{\mathrm{CH3,CH}}$ = 7.2 Hz, 3H, CH$_3$), 1.46 (d, $J_{\mathrm{CH3,CH}}$ = 7.2 Hz, 3H, CH$_3^{*}$); EI-MS *m/z* (% rel. abund.): 377 (M^{+}, 95), 185 (100); ^{13}C NMR (150 MHz, DMSO-d_6): δ 175.4, 170.2*, 157.2, 157.1*, 148.1, 148.0*, 141.9, 138.0*, 136.9, 136.5*, 133.7, 133.5*, 133.1, 130.6, 130.3*, 129.2, 129.0*, 128.7, 128.5*, 128.4, 128.3*, 127.9, 126.9, 126.8*, 126.6, 126.2*, 125.7, 125.5*, 124.6, 124.5*, 118.7, 118.6*, 105.7, 55.2, 44.0, 40.8*, 18.6, 18.5*; HREI-MS Calcd for $C_{21}H_{19}N_3O_4$ *m/z* 377.1376 found is 377.1370; Anal. Calcd for $C_{21}H_{19}N_3O_4$: C = 66.83; H = 5.07; N = 11.13; Found: C = 66.81; H = 5.05; N = 11.15.

4.3.12. *(E,Z)-(S)-2-(6-Methoxynaphthalen-2-yl)-N′-(3-nitrobenzylidene) propanehydrazide (13)*

White solid; Yield: 56%; R$_f$: 0.83; M.p.: 193–195 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.80 (s, 1H, NH), 11.51 (s, 1H, NH*), 8.47 (s, 2H, H-2′, H-2′*), 8.30 (s, 1H, N=CH), 8.23 (ovp, 2H, H-3′, H-3′*), 8.09 (ovp, 2H, H-6′, H-6′*), 7.98 (s, 1H, N=CH*), 7.81 (ovp, 8H, H-1, H-1*, H-4, H-4*, H-8, H-8*, H-5′, H-5′*), 7.49 (d, $J_{3,4}$ = 8.7 Hz, 2H, H-3, H-3*), 7.27 (d, $J_{5,7}$ = 2.4 Hz, 1H, H-5), 7.23 (d, $J_{5,7}$ = 2.4 Hz, 1H, H-5*), 7.15 (ovp, 2H, H-7, H-7*), 4.79 (q, $J_{\mathrm{CH,CH3}}$ = 6.8 Hz, 1H, CH), 3.86 (ovp, 4H, CH*, OCH$_3$), 3.81 (s, 3H, OCH$_3^{*}$), 1.49 (ovp, 6H, CH$_3$, CH$_3^{*}$); ^{13}C NMR (75 MHz, DMSO-d_6): δ 175.2, 170.1*, 148.2, 145.2*, 144.2, 140.2*, 137.0, 136.4, 136.1*, 133.2, 133.1*, 133.0, 132.9*, 130.4, 130.3*, 129.1, 128.9*, 128.4, 128.3*, 126.8, 126.8*, 126.5, 126.2*, 125.5, 125.4*, 124.0, 123.8*, 120.9, 120.7*, 118.7, 118.6*, 114.5, 105.7, 55.1, 43.9, 41.1*, 18.5, 18.4*; EI-MS *m/z* (% rel. abund.): 377 (M^{+}, 58), 185 (100); HREI-MS Calcd for $C_{21}H_{19}N_3O_4$ *m/z* 377.1376 found is 377.1389; Anal. Calcd for $C_{21}H_{19}N_3O_4$: C = 66.83; H = 5.07; N = 11.13; Found: C = 66.80; H = 5.05; N = 11.15.

4.3.13. *(E,Z)-(S)-2-(6-Methoxynaphthalen-2-yl)-N′-(4-nitrobenzylidene) propanehydrazide (14)*

Off white solid; Yield: 78%; R$_f$: 0.88; M.p.: 171–173 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.82 (s, 1H, NH), 11.56 (s, 1H, NH*), 8.28 (ovp, 5H, N=CH, H-3′, H-3′*, H-5′, H-5′*), 7.98 (s, 1H, N=CH*), 7.93 (ovp, 4H, H-2′, H-2′*, H-6′, H-6′*), 7.80 (ovp, 6H, H-1, H-1*, H-4, H-4*, H-8, H-8*), 7.84 (d, $J_{3,4}$ = 2.4 Hz, 2H, H-3, H-3*), 7.27 (d, $J_{5,7}$ = 2.4 Hz, 1H, H-5), 7.23 (d, $J_{5,7}$ = 2.4 Hz, 1H, H-5*), 7.15 (dd, $J_{7,5}$ = 2.4 Hz, $J_{7,8}$ = 8.8 Hz, 1H, H-7), 7.11 (dd, $J_{7,5}$ = 2.4 Hz, $J_{7,8}$ = 8.8 Hz, 1H, H-7*), 4.81 (q, $J_{\mathrm{CH,CH3}}$ = 7.2 Hz, 1H, CH), 3.88 (ovp, 4H, CH*, OCH$_3$), 3.82 (s, 3H, OCH$_3^{*}$), 1.49 (ovp, 6H, CH$_3$, CH$_3^{*}$); EI-MS *m/z* (% rel. abund.): 377 (M^{+}, 81), 185 (100); Anal. Calcd for $C_{21}H_{19}N_3O_4$: C = 66.83; H = 5.07; N = 11.13; Found: C = 66.81; H = 5.09; N = 11.11.

4.3.14. *(E,Z)-(S)-N′-(5-Hydroxy-2-nitrobenzylidene)-2-(6-methoxynaphthalen-2-yl) propanehydrazide (15)*

Light green solid; Yield: 66%; R$_f$: 0.89; M.p.: 248–250 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.86 (s, 1H, NH), 11.55 (s, 1H, NH*), 11.04 (s, 2H, OH, OH*), 8.68 (s, 1H, N=CH), 8.42 (s, 1H, N=CH*), 8.02 (ovp, 2H, H-3′, H-3′*), 7.81 (ovp, 6H, H-1, H-1* H-4, H-4*, H-8, H-8*), 7.47 (ovp, 3H, H-3, H-3*, H-6′), 7.36 (d, $J_{6′,5′}$ = 2.4 Hz, 1H, H-6′*), 7.27 (d, $J_{5,7}$ = 2.4 Hz, 1H, H-5), 7.25 (d, $J_{5,7}$ = 2.4 Hz, 1H, H-5*), 7.15 (ovp, 2H, H-7, H-7*), 6.95 (ovp, 2H, H-4′, H-4′*), 4.77 (q, $J_{\mathrm{CH,CH3}}$ = 7.2 Hz, 1H, CH), 3.85 (s, 3H, OCH$_3$), 3.83 (ovp, 4H, CH*, OCH$_3^{*}$), 1.48 (m, 6H, CH$_3$, CH$_3^{*}$); ^{13}C NMR (100 MHz, DMSO-d_6): δ 175.2, 170.1*, 162.2, 162.1*, 157.1, 157.0*, 142.6, 139.6, 138.7*, 136.7, 136.4*, 133.2, 133.1*, 131.9, 131.7*, 129.0, 128.9*, 128.4, 128.3*, 127.9, 126.8, 126.7*, 126.6, 126.2*, 125.6, 125.4*, 118.6, 118.5*, 117.0, 116.8*, 113.2, 112.9*, 105.6, 55.1, 43.9, 40.6*, 18.4, 18.3*; EI-MS *m/z* (% rel. abund.): 393 (M^{+}, 44), 185 (100); HREI-MS Calcd for $C_{21}H_{19}N_3O_5$ *m/z* 393.1325 found is 393.1312; Anal. Calcd for $C_{21}H_{19}N_3O_5$: C = 64.12; H = 4.87; N = 10.68; Found: C = 64.14; H = 4.89; N = 10.71.

4.3.15. *(E,Z)-(S)-N′-(2-Chloro-5-nitrobenzylidene)-2-(6-methoxynaphthalen-2-yl)propanehydrazide (16)*

White solid; Yield: 76%; R$_f$: 0.90; M.p.: 236–238 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.98 (s, 1H, NH), 11.66 (s, 1H, NH*), 8.68 (d, $J_{6′,4′}$ = 2.4 Hz, 1H, H-6′), 8.61 (d, $J_{6′,4′}$ = 2.8 Hz, 1H, H-6′*), 8.59 (s, 1H, N=CH), 8.26 (s, 1H, N=CH*), 8.21 (ovp, 2H, H-4′, H-4′*), 7.81 (ovp, 8H, H-1, H-1*, H-4, H-4*, H-8, H-8*, H-3′, H-3′*), 7.49 (ovp, 2H, H-3, H-3*), 7.28 (ovp, 2H, H-5, H-5*), 7.15 (ovp, 2H, H-7, H-7*), 4.75 (q, $J_{\mathrm{CH,CH3}}$ = 7.2 Hz, 1H, CH), 3.84 (s, 3H, OCH$_3$), 3.82 (ovp, 4H, CH*, OCH$_3^{*}$), 1.50 (d, $J_{\mathrm{CH3,CH}}$ = 7.2 Hz, 3H, CH$_3$), 1.46 (d, $J_{\mathrm{CH3,CH}}$ = 7.2 Hz, 3H, CH$_3^{*}$); EI-MS *m/z* (% rel. abund.): 411 (M^{+}, 31), 413 (M + 2, 14), 185 (100); Anal. Calcd for $C_{21}H_{18}ClN_3O_4$: C = 61.24; H = 4.41; N = 10.20; Found: C = 61.26; H = 4.43; N = 10.23.

4.3.16. *(E,Z)-(S)-N′-(4-Bromobenzylidene)-2-(6-methoxynaphthalen-2-yl) propanehydrazide (17)*

White solid; Yield: 58%; R$_f$: 0.91; M.p.: 187–189 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.59 (s, 1H, NH), 11.31 (s, 1H, NH*), 8.15 (s, 1H, N=CH), 7.85 (s, 1H, N=CH*), 7.80 (ovp, 6H, H-1, H-1*, H-4, H-4*, H-8, H-8*), 7.61 (ovp, 8H, H-2′, H-2′*, H-3′, H-3′*, H-5′, H-5′*, H-6′, H-6′*), 7.47 (d, $J_{3,4}$ = 8.0 Hz , 2H, H-3, H-3*), 7.27 (d, $J_{5,7}$ = 2.4 Hz, 1H, H-5), 7.23 (d, $J_{5,7}$ = 2.4 Hz, 1H, H-5*), 7.15 (ovp, 2H, H-7, H-7*), 4.77 (q, $J_{\mathrm{CH,CH3}}$ = 7.2 Hz, 1H, CH), 3.84 (s, 3H, OCH$_3$), 3.82 (ovp, 4H, CH*, OCH$_3^{*}$), 1.47 (ovp, 6H, CH$_3$, CH$_3^{*}$); EI-MS *m/z* (% rel. abund.): 410 (M^{+}, 21), 412 (M + 2, 20), 185 (100); Anal. Calcd for $C_{21}H_{19}BrN_2O_2$: C = 61.33; H = 4.66; N = 6.81; Found: C = 61.36; H = 4.68; N = 6.83.

4.3.17. *(E,Z)-(S)-N′-(4-Bromo-2-fluorobenzylidene)-2-(6-methoxynaphthalen-2-yl)propanehydrazide (18)*

White solid; Yield: 68%; R$_f$: 0.94; M.p.: 183–185 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.71 (s, 1H, NH), 11.42 (s, 1H, NH*), 8.34 (s, 1H, N=CH), 8.03 (s, 1H, N=CH*), 7.88 (ovp, 8H, H-1, H-1*, H-4, H-4*, H-8, H-8*, H-6′, H-6′*), 7.63 (ovp, 2H, H-3, H-3*), 7.51 (ovp, 4H, H-3′, H-3′*, H-5′, H-5′*), 7.27 (d, $J_{5,7}$ = 2.0 Hz, 1H, H-5), 7.23 (d, $J_{5,7}$ = 2.0 Hz, 1H, H-5*), 7.15 (ovp, 2H, H-7, H-7*), 4.77 (q, $J_{\mathrm{CH,CH3}}$ = 7.2 Hz, 1H, CH), 3.84 (s, 3H, OCH$_3$), 3.82 (ovp, 4H, CH*, OCH$_3^{*}$), 1.48 (ovp, 6H, CH$_3$, CH$_3^{*}$); ^{13}C NMR (100 MHz, DMSO-d_6): δ 175.1, 169.9, 161.4, 158.8, 156.9*, 138.4, 136.8*, 136.3, 134.5*, 133.2, 133.0*, 129.0, 128.9*, 128.4, 128.3*, 128.2, 128.1*, 127.6, 126.8, 126.7*, 126.6, 126.1*, 125.6, 125.4*, 121.3, 121.2*, 119.4, 119.2*, 118.6, 118.5*, 105.6, 55.1, 44.7, 40.7*, 18.4; EI-MS *m/z* (% rel. abund.): 428 (M^{+}, 53), 430 (M + 2, 49), 185 (100); HREI-MS Calcd for $C_{21}H_{18}BrFN_2O_2$ *m/z* 428.0536 found is 428.0544; Anal. Calcd for $C_{21}H_{18}BrFN_2O_2$: C = 58.76; H = 4.23; N = 6.53; Found: C = 58.78; H = 4.25; N = 6.55.

4.3.18. *(E,Z)-(S)-N′-(3-Bromo-4-hydroxybenzylidene)-2-(6-methoxynaphthalen-2-yl)propanehydrazide (19)*

White solid; Yield: 89%; R$_f$: 0.85; M.p.: 120–122 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.44 (s, 1H, NH), 11.14 (s, 1H, NH*), 10.74 (s, 1H, OH), 10.66 (s, 1H, OH*), 8.04 (s, 1H, N=CH), 7.79 (ovp, 9H, N=CH*, H-1, H-1*, H-4, H-4*, H-5, H-5*, H-8, H-8*), 7.47 (ovp, 4H, H-2′, H-2′*, H-6′, H-6′*), 7.26 (dd, $J_{3,1}$ = 2.0 Hz, $J_{3,4}$ = 11.6 Hz, 2H, H-3, H-3*), 7.14 (ovp, 2H, H-7, H-7*), 6.97 (d, $J_{5′,6′}$ = 8.4 Hz, 2H, H-5′, H-5′*), 4.73 (q, $J_{\mathrm{CH,CH3}}$ = 7.2 Hz, 1H, CH), 3.84 (s, 3H, OCH$_3$), 3.82 (ovp, 4H, CH*, OCH$_3^{*}$), 1.46 (d, $J_{\mathrm{CH3,CH}}$ = 7.2 Hz, 3H, CH$_3$), 1.44 (d, $J_{\mathrm{CH3,CH}}$ = 7.2 Hz, 3H, CH$_3^{*}$); EI-MS *m/z* (% rel.abund.): 426 (M^{+}, 26), 428 (M + 2, 25), 185 (100); Anal. Calcd for $C_{21}H_{19}BrN_2O_3$: C = 59.03; H = 4.48; N = 6.56; Found: C = 59.06; H = 4.50; N = 6.58.

4.3.19. *(E,Z)-(S)-N′-(4-Hydroxybenzylidene)-2-(6-methoxynaphthalen-2-yl)propanehydrazide(20)*

White solid; Yield: 82%; R$_f$: 0.79; M.p.: 164–166 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.30 (s, 1H, NH), 11.03 (s, 1H, NH*), 9.83 (s, 1H, OH), 9.82 (s, 1H, OH*), 8.06 (s, 1H, N=CH), 7.79 (ovp, 7H,

N=CH*, H-1, H-1*, H-4, H-4*, H-8, H-8*), 7.48 (ovp, 6H, H-3, H-3*, H-2′, H-2′*, H-6′, H-6′*), 7.26 (d, $J_{5,7} = 2.4$ Hz, 1H, H-5), 7.22 (d, $J_{5,7} = 2.4$ Hz, 1H, H-5*), 7.14 (ovp, 2H, H-7, H-7*), 6.79 (ovp, 4H, H-3′, H-3′*, H-5′, H-5′*), 4.75 (q, $J_{CH,CH3} = 7.2$ Hz, 1H, CH), 3.84 (s, 3H, OCH_3), 3.82 (ovp, 4H, CH*, OCH_3^*), 1.46 (ovp, 6H, CH_3, CH_3^*); EI-MS *m/z* (% rel. abund.): 348 (M^+, 47), 185 (100); Anal. Calcd for $C_{21}H_{20}N_2O_3$: C = 72.40; H = 5.79; N = 8.04; Found: C = 72.43; H = 5.81; N = 8.06.

4.3.20. (E,Z)-(S)-2-(6-Methoxynaphthalen-2-yl)-N′-(4-(methylthio)benzylidene)propanehydrazide (21)

White solid; Yield: 53%; R_f: 0.84; M.p.: 164–166 °C;^{1}H NMR (400 MHz, DMSO-d_6): δ 11.48 (s, 1H, NH), 11.20 (s, 1H, NH*), 8.13 (s, 1H, N=CH), 7.83 (s, 1H, N=CH*), 7.80 (ovp, 6H, H-1, H-1*, H-4, H-4*, H-8, H-8*), 7.59 (ovp, 4H, H-2′, H-2′*, H-6′, H-6′*), 7.47 (d, $J_{3,4} = 8.0$ Hz, 2H, H-3, H-3*), 7.29 (ovp, 6H, H-5, H-5*, H-3′, H-3′*, H-5′, H-5′*), 7.15 (ovp, 2H, H-7, H-7*), 4.77 (q, $J_{CH,CH3} = 7.2$ Hz, 1H, CH), 3.84 (s, 3H, OCH_3), 3.82 (ovp, 4H, CH*, OCH_3^*), 1.47 (ovp, 6H, CH_3, CH_3^*); EI-MS *m/z* (% rel. abund.): 378 (M^+, 55), 185 (100); Anal. Calcd for $C_{22}H_{22}N_2O_2S$: C = 69.83; H = 5.88; N = 7.43; Found: C = 69.83; H = 5.89; N = 7.42.

4.4. General procedure for the synthesis of aroyl substituted S-Naproxen hydrazides (22–24)

Benzoic acids (0.5 mmol) were dissolved in a minimum volume of tetrahydrofuran (THF) solvent to make a saturated solution. Then, CDI (0.7 mmol) and triethylamine (0.5 mmol) were added into the reaction mixture and stirred until the effervescence ceased. After that, THF (10 mL) and *S*-naproxen hydrazide **1** (0.5 mmol) were also added and refluxed the reaction mixture for 4–6 h with continuous stirring. Progress of the reaction was determined by TLC analysis (Hexane:ethylacetate (6:4)). After completion, the reaction mixture was poured onto crushed ice. Precipitates were formed which were filtered, washed with distilled water, and dried in air. Crude product was crystallized from ethanol to afford pure products.

4.4.1. (S)-N′-2-(6-Methoxynaphthalen-2-yl)propanoylbenzohydrazide (22)

White solid; Yield: 38%; R_f: 0.85; M.p.: 185–186 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.26 (s, 1H, NH), 10.31 (s, 1H, NH), 7.85 (d, $J_{2',3'/6',5'} = 7.2$ Hz, 2H, H-2′, H-6′), 7.79 (ovp, 3H, H-1, H-4, H-8), 7.56 (ovp, 2H, H-3, H-4′), 7.48 (t, $J_{3',2'/5',6'} = J_{3',4'/5',4'} = 8.2$ Hz, 2H, H-3′, H-5′), 7.27 (d, $J_{5,7} = 2.4$ Hz, 1H, H-5), 7.15 (dd, $J_{7,5} = 2.4$ Hz, $J_{7,8} = 8.8$ Hz, 1H, H-7), 3.88 (ovp, 4H, CH, OCH_3), 1.48 (d, $J_{CH3,CH} = 6.8$ Hz, 3H, CH_3); EI-MS *m/z* (% rel. abund.): 348 (M^+, 9), 185 (100); Anal. Calcd for $C_{21}H_{20}N_2O_3$: C = 72.40; H = 5.79; N = 8.04; Found: C = 72.43; H = 5.81; N = 8.06.

4.4.2. (S)-3,5-Dichloro-N′-2-(6-methoxynaphthalen-2-yl)propanoylbenzohydrazide (23)

White solid; Yield: 40%; R_f: 0.91; M.p.: 215–216 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.58 (s, 1H, NH), 10.27 (s, 1H, NH), 7.85 (s, 3H, H-2′, H-4′, H-6′), 7.79 (ovp, 3H, H-1, H-4, H-8), 7.50 (dd, $J_{3,1} = 1.6$ Hz, $J_{3,4} = 8.4$ Hz, 1H, H-3), 7.28 (d, $J_{5,7} = 2.4$ Hz, 1H, H-5), 7.15 (dd, $J_{7,5} = 2.4$ Hz, $J_{7,8} = 8.8$ Hz, 1H, H-7), 3.88 (ovp, 4H, CH, OCH_3), 1.48 (d, $J_{CH3,CH} = 7.2$ Hz, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.6, 162.7, 157.0, 157.0, 136.4, 135.5, 134.3, 133.2, 131.2, 129.0, 128.3, 126.5, 126.4, 126.1, 126.1, 125.4, 118.5, 105.6, 55.1, 42.9, 18.2; EI-MS *m/z* (% rel. abund.): 416 (M^+, 20), 418 (M + 2, 4), 185 (100); HREI-MS Calcd for $C_{21}H_{18}Cl_2N_2O_3$ *m/z* 416.0694 found is 416.0701; Anal. Calcd for $C_{21}H_{18}Cl_2N_2O_3$: C = 60.45; H = 4.35; N = 6.71; Found: C = 60.43; H = 4.37; N = 6.73.

4.4.3. (S)-3,5-Dibromo-4-methoxy-N′-2-(6-methoxynaphthalen-2-ylpropanoyl)benzohydrazide (24)

Off white solid; Yield: 39%; R_f: 0.92; M.p.: 208–210 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.51 (s, 1H, NH), 10.25 (s, 1H, NH), 8.11 (s, 2H, H-2′, H-6′), 7.79 (ovp, 3H, H-1, H-4, H-8), 7.50 (dd, $J_{3,1} = 0.8$ Hz, $J_{3,4} = 10.0$ Hz, 1H, H-3), 7.28 (d, $J_{5,7} = 2.4$ Hz, 1H, H-5), 7.15 (dd, $J_{7,5} = 2.4$ Hz, $J_{7,8} = 8.8$ Hz, 1H, H-7), 3.87 (ovp, 4H, CH, OCH_3), 3.84 (s, 3H, OCH_3), 1.47 (d, $J_{CH3,CH} = 7.2$ Hz, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.6, 164.6, 157.0, 156.2, 136.4, 133.5, 133.2, 130.7, 129.1, 128.3, 126.6, 125.4, 121.3, 118.6, 117.6, 105.6, 60.6, 55.1, 42.9, 18.2; EI-MS *m/z* (% rel. abund.): 534 (M^+, 23), 536 (M + 2, 46), 538 (M + 4, 23), 185 (100); HREI-MS Calcd for $C_{22}H_{20}Br_2N_2O_4$ *m/z* 535.9946 found is 535.9974; Anal. Calcd for $C_{22}H_{20}Br_2N_2O_4$: C = 49.28; H = 3.76; N = 5.22; Found: C = 49.30; H = 3.78; N = 5.24.

4.5. General procedure for the synthesis of S-Naproxen sulfohydrazide derivatives (25–34)

S-Naproxen hydrazide **1** (0.5 mmol), sulfonyl chloride derivative (0.5 mmol), and triethylamine (0.5 mmol) were taken in THF (10 mL) into a round-bottommed flask (100 mL). Reaction mixture was heated at 50 °C for 2–5 h with continuous stirring. Completion of reaction was monitored *via* TLC (Hexane:ethylacetate (6:4)). After completion, the reaction mixture was poured onto the crushed ice, resulted in precipitates formation which were filtered, washed with distilled water, and dried in air. Products were crystallized from ethanol.

4.5.1. (S)-N′-(2-(6-Methoxynaphthalen-2-yl)propanoyl)methanesulfohydrazide (25)

White solid; Yield: 63%; R_f: 0.85; M.p.: 156–158 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.37 (d, $J_{NH,NH} = 3.2$ Hz, 1H, NH), 9.42 (d, $J_{NH,NH} = 3.2$ Hz, 1H, NH), 7.76 (ovp, 3H, H-1, H-4, H-8), 7.45 (dd, $J_{3,1} = 1.2$ Hz, $J_{3,4} = 8.4$ Hz, 1H, H-3), 7.25 (d, $J_{5,7} = 2.4$ Hz, 1H, H-5), 7.15 (dd, $J_{7,5} = 2.4$ Hz, $J_{7,8} = 8.8$ Hz, 1H, H-7), 3.85 (s, 3H, OCH_3), 3.80 (q, $J_{CH,CH3} = 7.2$ Hz, 1H, CH), 2.79 (s, 3H, CH_3-1′), 1.44 (d, $J_{CH3,CH} = 7.2$ Hz, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.0, 157.0, 136.1, 133.2, 129.1, 128.3, 126.7, 126.1, 125.3, 118.6, 105.6, 55.1, 42.7, 40.0, 18.0; EI-MS *m/z* (% rel. abund.): 322 (M^+, 38), 185 (100); HREI-MS Calcd for $C_{15}H_{18}N_2O_4S$ *m/z* 322.0987 found is 322.0979; Anal. Calcd for $C_{15}H_{18}N_2O_4S$: C = 55.89; H = 5.63; N = 8.69; Found: C = 55.91; H = 5.66; N = 8.71.

4.5.2. (S)-N′-(2-(6-Methoxynaphthalen-2-yl)propanoyl)ethanesulfohydrazide (26)

Off white solid; Yield: 71%; R_f: 0.87; M.p.: 153–155 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.28 (d, $J_{NH,NH} = 2.8$ Hz, 1H, NH), 9.37 (d, $J_{NH,NH} = 2.8$ Hz, 1H, NH), 7.78 (ovp, 3H, H-1, H-4, H-8), 7.44 (dd, $J_{3,1} = 1.6$ Hz, $J_{3,4} = 8.8$ Hz, 1H, H-3), 7.27 (d, $J_{5,7} = 2.4$ Hz, 1H, H-5), 7.15 (dd, $J_{7,5} = 2.4$ Hz, $J_{7,8} = 8.8$ Hz, 1H, H-7), 3.84 (s, 3H, OCH_3), 3.78 (q, $J_{CH,CH3} = 6.8$ Hz, 1H, CH), 2.90 (q, $J_{CH2,CH3} = 7.2$ Hz, 2H, CH_2), 1.43 (d, $J_{CH3,CH} = 6.8$ Hz, 3H, CH_3), 1.22 (t, $J_{CH3,CH2} = 7.2$ Hz, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.0, 157.0, 136.1, 133.2, 129.0, 128.2, 126.6, 126.1, 125.3, 118.6, 105.6, 55.1, 45.7, 42.6, 18.0, 7.8; EI-MS *m/z* (% rel. abund.): 336 (M^+, 50), 185 (100); HREI-MS Calcd for $C_{16}H_{20}N_2O_4S$ *m/z* 336.1144 found is 336.1134; Anal. Calcd for $C_{16}H_{20}N_2O_4S$: C = 57.13; H = 5.99; N = 8.33; Found: C = 57.16; H = 5.97; N = 8.34.

4.5.3. (S)-N′-(2-(6-Methoxynaphthalen-2-yl)propanoyl)butane-1-sulfohydrazide (27)

White solid; Yield: 70%; R_f: 0.76; M.p.: 126–128 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.31 (d, $J_{NH,NH} = 2.4$ Hz, 1H, NH), 9.38 (d, $J_{NH,NH} = 2.4$ Hz, 1H, NH), 7.77 (ovp, 3H, H-1, H-4, H-8), 7.44 (dd, $J_{3,1} = 1.2$ Hz, $J_{3,4} = 8.4$ Hz, 1H, H-3), 7.26 (d, $J_{5,7} = 2.4$ Hz, 1H, H-5), 7.14 (dd, $J_{7,5} = 2.4$ Hz, $J_{7,8} = 8.8$ Hz, 1H, H-7), 3.84 (s, 3H, OCH_3), 3.79 (q, $J_{CH,CH3}$ = 6.8 Hz, 1H, CH), 2.83 (t, $J_{1',2'}$ = 8.0 Hz, 2H, CH_2-1′), 1.65 (m, 2H, CH_2-2′), 1.43 (d, $J_{CH3,CH}$ = 6.8 Hz, 3H, CH_3), 1.28 (m, 2H, CH_2-3′), 0.79 (t, 3H, CH_3-4′); ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.0, 157.0, 136.1, 133.2, 129.0, 128.3, 126.6, 126.1, 125.3, 118.6, 105.6, 55.1, 50.8, 42.7, 24.7, 20.8, 18.0, 13.3; EI-MS m/z (% rel. abund.): 364 (M^+, 87), 185 (100); HREI-MS Calcd for $C_{18}H_{24}N_2O_4S$ m/z 364.1457 found is 364.1441; Anal. Calcd for $C_{18}H_{24}N_2O_4S$: C = 59.32; H = 6.64; N = 7.69; Found: C = 59.35; H = 6.66; N = 7.67.

4.5.4. (S)-N′-(2-(6-Methoxynaphthalen-2-yl)propanoyl)-4-propylbenzenesulfohydrazide (28)

White solid; Yield: 30%; R_f: 0.87; M.p.: 149–151 °C; ^1H NMR (400 MHz, DMSO-d_6): δ 10.26 (s, 1H, NH), 9.67 (s, 1H, NH), 7.73 (ovp, 2H, H-4, H-8), 7.55 (s, 1H, H-1), 7.45 (d, $J_{2',3'/6',5'}$ = 8.0 Hz, 2H, H-2′, H-6′), 7.28 (d, $J_{5,7}$ = 2.0 Hz, 1H, H-5), 7.25 (dd, $J_{3,1}$ = 1.6 Hz, $J_{3,4}$ = 8.8 Hz, 1H, H-3), 7.15 (dd, $J_{7,5}$ = 2.4 Hz, $J_{7,8}$ = 8.8 Hz, 1H, H-7), 7.03 (d, $J_{3',2'/5',6'}$ = 8.0 Hz, 2H, H-3′, H-5′), 3.86 (ovp, 5H, OCH_3, CH_2-1″), 3.67 (q, $J_{CH,CH3}$ = 7.2 Hz, 1H, CH), 1.52 (m, 2H, CH_2-2″), 1.23 (d, $J_{CH3,CH}$ = 7.2 Hz, 3H, CH_3), 0.84 (t, $J_{CH3'',CH2''}$ = 7.2 Hz, 3H, CH_3-3″); ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.8, 157.0, 147.3, 136.0, 135.6, 133.2, 129.0, 128.2, 128.2, 127.5, 127.5, 126.4, 126.3, 125.3, 118.5, 105.6, 55.1, 42.5, 36.8, 23.3, 17.7, 13.3; EI-MS m/z (% rel. abund.): 426 (M^+, 35), 185 (100); HREI-MS Calcd for $C_{23}H_{26}N_2O_4S$ m/z 426.1613 found is 426.1611; Anal. Calcd for $C_{23}H_{26}N_2O_4S$: C = 64.77; H = 6.14; N = 6.57; Found: C = 64.79; H = 6.13; N = 6.55.

4.5.5. (S)-4-Methoxy-N′-(2-(6-methoxynaphthalen-yl)propanoyl)benzenesulfohydrazide (29)

White solid; Yield: 74%; R_f: 0.91; M.p.: 163–166 °C; ^1H NMR (400 MHz, DMSO-d_6): δ 10.26 (d, $J_{NH,NH}$ = 3.6 Hz, 1H, NH), 9.37 (d, $J_{NH,NH}$ = 3.6 Hz, 1H, NH), 7.74 (ovp, 2H, H-4, H-8), 7.56 (s, 1H, H-1), 7.46 (d, $J_{2',3'/6',5'}$ = 8.8 Hz, 2H, H-2′, H-6′), 7.28 (ovp, 2H, H-3, H-5), 7.15 (dd, $J_{7,5}$ = 2.4 Hz, $J_{7,8}$ = 8.8 Hz, 1H, H-7), 6.73 (d, $J_{3',2'/5',6'}$ = 8.8 Hz, 2H, H-3′, H-5′), 3.85 (s, 3H, OCH_3), 3.69 (ovp, 4H, CH,OCH_3), 1.25 (d, $J_{CH3,CH}$ = 7.2 Hz, 3H, CH_3); EI-MS m/z (% rel. abund.): 414 (M^+, 6), 185 (100); Anal. Calcd for $C_{21}H_{22}N_2O_5S$: C = 60.86; H = 5.35; N = 6.76; Found: C = 60.84; H = 5.37; N = 6.74.

4.5.6. (S)-N′-(2-(6-Methoxynaphthalen-2-yl)propanoyl)-4-nitrobenzenesulfohydrazide (30)

Yellow solid; Yield: 50%; R_f: 0.81; M.p.: 190–192 °C; ^1H NMR (400 MHz, DMSO-d_6): δ 10.48 (s, 1H, NH), 10.30 (s, 1H, NH), 7.98 (dd, $J_{3',2'/5',6'}$ = 8.8 Hz, 2H, H-3′, H-5′), 7.74 (ovp, 4H, H-4, H-8, H-2′, H-6′), 7.53 (s, 1H, H-1), 7.27 (ovp, 2H, H-3, H-5), 7.15 (dd, $J_{7,5}$ = 2.4 Hz, $J_{7,8}$ = 8.8 Hz, 1H, H-7), 3.86 (s, 3H, OCH_3), 3.66 (q, $J_{CH,CH3}$ = 6.8 Hz, 1H, CH), 1.25 (d, $J_{CH3,CH}$ = 6.8 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.2, 157.1, 149.5, 144.2, 135.8, 133.2, 129.0, 129.0, 128.9, 128.2, 126.5, 126.2, 125.3, 123.5, 123.5, 118.7, 105.6, 55.1, 42.5, 17.5; EI-MS m/z (% rel. abund.): 429 (M^+, 6), 185 (100); HREI-MS Calcd for $C_{20}H_{19}N_3O_6S$ m/z 429.0995 found is 429.0986; Anal. Calcd for $C_{20}H_{19}N_3O_6S$: C = 55.94; H = 4.46; N = 9.78; Found: C = 55.92; H = 4.48; N = 9.80.

4.5.7. (S)-N′-(2-(6-Methoxynaphthalen-2-yl)propanoyl)-2-nitrobenzenesulfohydrazide (31)

Dark brown solid; Yield: 88%; R_f: 0.83; M.p.: 182–184 °C; ^1H NMR (400 MHz, DMSO-d_6): δ 10.34 (d, $J_{NH,NH}$ = 2.8 Hz, 1H, NH), 10.08 (d, $J_{NH,NH}$ = 2.4 Hz, 1H, NH), 7.74 (ovp, 4H, H-4, H-8, H-4′, H-5′), 7.58 (d, $J_{3',4'}$ = 8.4 Hz, 1H, H-3′), 7.51 (s, 1H, H-1), 7.27 (d, $J_{5,7}$ = 2.0 Hz, 1H, H-5), 7.20 (d, $J_{6',5'}$ = 8.4 Hz, 1H, H-6′), 7.15 (dd, $J_{3,1}$ = 2.4 Hz, $J_{3,4}$ = 8.8 Hz, 1H, H-3), 7.13 (dd, $J_{7,5}$ = 2.0 Hz, $J_{7,8}$ = 8.4 Hz, 1H, H-7), 3.86 (s, 3H, OCH_3), 3.64 (q, $J_{CH,CH3}$ = 6.8 Hz, 1H, CH), 1.23 (d, $J_{CH3,CH}$ = 6.8 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.5, 157.0, 147.5, 135.8, 134.4, 133.2, 132.0, 131.3, 130.8, 129.0, 128.2, 126.5, 126.3, 125.3, 124.3, 118.6, 105.6, 55.1, 42.5, 17.7; EI-MS m/z (% rel. abund.): 429 (M^+, 2), 185 (100); HREI-MS Calcd for $C_{20}H_{19}N_3O_6S$ m/z 429.0995 found is 429.0986; Anal. Calcd for $C_{20}H_{19}N_3O_6S$: C = 55.94; H = 4.46; N = 9.78; Found: C = 55.91; H = 4.48; N = 9.80.

4.5.8. (S)-5-Chloro-2-methoxy-N′-(2-(6-methoxynaphthalen-2-yl)propanoyl)benzenesulfohydrazide (32)

White solid; Yield: 55%; R_f: 0.91; M.p.: 200–203 °C; ^1H NMR (400 MHz, DMSO-d_6): δ 10.26 (s, 1H, NH), 9.52 (s, 1H, NH), 7.71 (d, $J_{8,7}$ = 8.8 Hz, H-1, H-8), 7.67 (d, $J_{4,3}$ = 8.4 Hz, 1H, H-4), 7.60 (dd, $J_{3,1}$ = 2.8 Hz, $J_{3,4}$ = 8.8 Hz, 1H, H-3), 7.51 (s, 1H, H-1), 7.46 (d, $J_{6',4'}$ = 1.6 Hz, 1H, H-6′), 7.23 (d, $J_{5,7}$ = 2.4 Hz, 1H, H-5), 7.02 (d, $J_{4',3'}$ = 9.2 Hz, 1H, H-4′), 7.12 (ovp, 2H, H-7, H-3′), 3.90 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.63(q, $J_{CH,CH3}$ = 6.8 Hz, 1H, CH), 1.21 (d, $J_{CH3,CH}$ = 6.8 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.1, 156.9, 156.5, 135.9, 134.3, 133.1, 131.2, 130.5, 129.0, 128.2, 126.5, 125.9, 125.1, 122.8, 118.5, 114.9, 105.6, 56.7, 55.1, 42.4, 18.1; EI-MS m/z (% rel. abund.): 448 (M^+, 20), 450 (M + 2, 7), 185 (100); HREI-MS Calcd for $C_{21}H_{21}ClN_2O_5S$ m/z 448.0860 found is 448.0850; Anal. Calcd for $C_{21}H_{21}ClN_2O_5S$: C = 56.19; H = 4.72; N = 6.24; Found: C = 56.21; H = 4.74; N = 6.26.

4.5.9. (S)-2,5-Dichloro-N′-(2-(6-methoxynaphthalen-2-yl)propanoyl)benzenesulfohydrazide (33)

White solid; Yield: 57%; R_f: 0.90; M.p.: 215–217 °C; ^1H NMR (400 MHz, DMSO-d_6): δ 10.36 (s, 1H, NH), 10.21 (s, 1H, NH), 7.72 (ovp, 3H, H-4, H-8, H-6′), 7.64 (ovp, 2H, H-3′, H-4′), 7.56 (s, 1H, H-1), 7.25 (d, $J_{5,7}$ = 2.4 Hz, 1H, H-5), 7.20 (dd, $J_{3,1}$ = 0.8 Hz, $J_{3,4}$ = 8.4 Hz, 1H, H-3), 7.13 (dd, $J_{7,5}$ = 2.4 Hz, $J_{7,8}$ = 8.8 H_Z, 1H, H-7), 3.84 (s, 3H, OCH_3), 3.66 (q, $J_{CH,CH3}$ = 6.8 Hz, 1H, CH), 1.24 (d, $J_{CH3,CH}$ = 6.8 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.6.0, 157.0, 133.2, 135.8, 133.8, 133.3, 133.2, 131.3, 130.9, 130.2, 129.0, 128.2, 126.6, 125.9, 125.2, 118.5, 105.7, 55.1, 42.4, 18.0; EI-MS m/z (% rel. abund.): 452 (M^+, 7), 454 (M + 2, 5), 456 (M + 4, 1), 185 (100); HREI-MS Calcd for $C_{20}H_{18}Cl_2N_2O_4S$ m/z 452.0364 found is 452.0341; Anal. Calcd for $C_{20}H_{18}Cl_2N_2O_4S$: C = 52.99; H = 4.00; N = 6.18; Found: C = 52.96; H = 4.02; N = 6.16.

4.5.10. (S)-3,5-Dichloro-2-hydroxy-N′-(2-(6-methoxynaphthalen-2-yl)propanoyl)benzenesulfohydrazide (34)

White solid; Yield: 85%; R_f: 0.83; M.p.: 253–255 °C; ^1H NMR (400 MHz, DMSO-d_6): δ 10.74 (s, 1H, NH), 10.41 (s, 1H, OH), 9.74 (s, 1H, NH), 7.77 (d, $J_{4',6'}$ = 2.8 Hz, 1H, H-4′), 7.73 (ovp, 2H, H-4, H-8), 7.57 (s, 1H, H-1), 7.39 (d, $J_{6',4'}$ = 2.8 Hz, 1H, H-6′), 7.25 (d, $J_{5,7}$ = 2.0 Hz, 1H, H-5), 7.22 (dd, $J_{3,1}$ = 1.6 Hz, $J_{3,4}$ = 8.4 Hz, 1H, H-3), 7.13 (dd, $J_{7,5}$ = 2.0 Hz, $J_{7,8}$ = 8.8 Hz, 1H, H-7), 3.84 (s, 3H, OCH_3), 3.68 (q, $J_{CH,CH3}$ = 7.2 Hz, 1H, CH), 1.27 (d, $J_{CH3,CH}$ = 7.2 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.7, 157.0, 150.7, 135.8, 133.8, 133.1, 129.0, 128.26, 128.20, 127.9, 126.6, 126.0, 125.2, 123.6, 122.2, 118.6, 105.6, 55.1, 42.4, 18.0; EI-MS m/z (% rel. abund.): 468 (M^+, 27), 470 (M + 2, 20), 472 (M + 4, 4), 185 (100); HREI-MS Calcd for $C_{20}H_{18}Cl_2N_2O_5S$ m/z 468.0313 found is 468.0318; Anal. Calcd for $C_{20}H_{18}Cl_2N_2O_5S$: C = 51.18; H = 3.87; N = 5.97; Found: C = 51.20; H = 3.89; N = 5.95.

4.5.11. Procedure for the synthesis of 2-Mercapto oxadiazole of S-Naproxen (35)

S-Naproxen hydrazide **1** (5 mmol) and potassium hydroxide (5 mmol) were dissolved in ethanol (30 mL) into a 100 mL round-bottommed flask and heated at 60 °C for 15 min. Then, carbon disulphide (5 mL) was added into the reaction mixture and refluxed the reaction mixture for 4 h with constant stirring. Reaction progress was checked by TLC analysis (Hexane:ethylacetate (6:4)). After completion, reaction mixture was poured onto crushed ice. White precipitates were immediately appeared which were filtered, washed with distilled water, and dried in air. Compound was crystallized from ethanol.

4.5.12. *(S)-5–1-(6-Methoxynaphthalen-2-yl)ethyl)-1,3,4-oxadiazole-2-thiol (35)*

White solid; Yield: 90%; R$_f$: 0.92; M.p.: 192–194 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 14.39 (s, 1H, SH), 7.83 (ovp, 2H, H-4, H-8), 7.76 (s, 1H, H-1), 7.40 (dd, $J_{3,1}$ = 1.8 Hz, $J_{3,4}$ = 8.7 Hz, 1H, H-3), 7.30 (d, $J_{5,7}$ = 2.4 Hz, 1H, H-5), 7.18 (dd, $J_{7,5}$ = 2.4 Hz, $J_{7,8}$ = 8.7 Hz, 1H, H-7), 4.48 (q, $J_{CH,CH3}$ = 7.2 Hz, 1H, CH), 3.85 (s, 3H, OCH$_3$), 1.64 (d, $J_{CH3,CH}$ = 7.2 Hz, 3H, CH$_3$); EI-MS *m/z* (% rel. abund.): 286 (M$^+$, 100), 185 (100); Anal. Calcd for $C_{15}H_{14}N_2O_2S$: C = 62.92; H = 4.93; N = 9.78; Found: C = 62.95; H = 4.91; N = 9.76.

4.6. *General procedure for the synthesis of phenacyl substituted 2-Mercapto oxadiazoles of S-Naproxen (36–39)*

Compound **35** (0.5 mmol) and triethyl amine (0.5 mmol) were dissolved in ethanol (10 mL) into a 100 mL round-bottommed flask. Phenacyl bromides (0.5 mmol) were added and refluxed the reaction mixture for 3–5 h. Periodic TLC (Hexane:ethylacetate (6:4)) was taken to monitor the reaction progress. After the completion, reaction mixture was poured onto the crushed ice and precipitates were appeared immediately. The obtained precipitates were filtered, washed with distilled water followed by hexane, and dried in *vacuo*. Products were crystallized from ethanol.

4.6.1. *(S)-2-((5-(1-(6-Methoxynaphthalen-2-yl)ethyl)-1,3,4-oxadiazol-2-yl)thio)-1-(p-tolyl)ethan-1-one (36)*

Off white solid; Yield: 62%; R$_f$: 0.85; M.p.: 113–115 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.88 (d, $J_{2',3'/6',5'}$ = 8.4 Hz, 2H, H-2′, H-6′), 7.78 (ovp, 2H, H-4, H-8), 7.69 (s, 1H, H-1), 7.34 (ovp, 3H, H-3, H-3′, H-5′), 7.28 (d, $J_{5,7}$ = 2.4 Hz, 1H, H-5), 7.16 (dd, $J_{7,5}$ = 2.4 Hz, $J_{7,8}$ = 8.8 Hz, 1H, H-7), 4.98 (s, 2H, CH$_2$), 4.57 (q, $J_{CH,CH3}$ = 7.2 Hz, 1H, CH), 3.85 (s, 3H, OCH$_3$), 2.38 (s, 3H, CH$_3$), 1.67 (d, $J_{CH3,CH}$ = 7.2 Hz, 3H, CH$_3$); ^{13}C NMR (100 MHz, DMSO-d_6):δ 191.8, 169.7, 163.0, 157.3, 144.4, 135.3, 133.2, 132.3, 129.3, 129.3, 129.1, 129.0, 128.4, 128.4, 127.2, 125.7, 125.5, 118.8, 105.7, 55.1, 40.1, 36.2, 21.1, 19.1; EI-MS *m/z* (% rel. abund.): 418 (M$^+$, 90), 119 (100); HREI-MS Calcd for $C_{24}H_{22}N_2O_3S$ *m/z* 418.1351 found is 418.1344; Anal. Calcd for $C_{24}H_{22}N_2O_3S$: C = 68.88; H = 5.30; N = 6.69; Found: C = 68.90; H = 5.32; N = 6.71.

4.6.2. *(S)-2-((5-(1-(6-Methoxynaphthalen-2-yl)ethyl)-1,3,4-oxadiazol-2-yl)thio)-1-(4-methoxyphenyl)ethan-1-one (37)*

White solid; Yield: 23%; R$_f$: 0.82; M.p.: 93–95 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.87 (d, $J_{2',3'/6',5'}$ = 8.8 Hz, 2H, H-2′, H-6′), 7.69 (d, $J_{8,7}$ = 9.2 Hz, 1H, H-8), 7.68 (d, $J_{4,3}$ = 8.4 Hz, 1H, H-4), 7.61 (s, 1H, H-1), 7.25 (dd, $J_{3,1}$ = 1.6 Hz, $J_{3,4}$ = 8.8 Hz, 1H, H-3), 7.19 (d, $J_{5,7}$ = 2.4 Hz, 1H, H-5), 7.07 (dd, $J_{7,5}$ = 2.8 Hz, $J_{7,8}$ = 8.8 Hz, 1H, H-7), 6.96 (dd, $J_{3',2'/5',6'}$ = 9.2 Hz, 2H, H-3′, H-5′), 4.87 (s, 2H, CH$_2$), 4.50 (q, $J_{CH,CH3}$ = 7.2 Hz, 1H, CH), 3.76 (s, 3H, OCH$_3$′), 3.75 (s, 3H, OCH$_3$), 1.58 (d, $J_{CH3,CH}$ = 7.2 Hz, 3H, CH$_3$); ^{13}C NMR (100 MHz, DMSO-d_6):δ 191.8, 169.7, 163.6, 163.1, 157.3, 135.3, 133.4, 130.7, 130.7, 129.1, 128.3, 127.8, 127.2, 125.7, 125.5, 118.8, 114.0, 114.0, 105.7, 55.6, 55.1, 40.0, 36.2, 19.1; EI-MS *m/z* (% rel. abund.): 434 (M$^+$, 64), 135 (100); HREI-MS Calcd for $C_{24}H_{22}N_2O_4S$ *m/z* 434.1300 found is 434.1287; Anal. Calcd for $C_{24}H_{22}N_2O_4S$: C = 66.34; H = 5.10; N = 6.45; Found: C = 66.36; H = 5.12; N = 6.47.

4.6.3. *(S)-1-([1,1′-Biphenyl]-4-yl)-2-((5-(1-(6-methoxynaphthalen-2-yl)ethyl)-1,3,4-oxadiazol-2-yl)thio)ethan-1-one (38)*

Yellow solid; Yield: 56%; R$_f$: 0.94; M.p.: 153–155 °C; ^{1}H NMR (400 MHz, DMSO-d_6):): δ 8.06 (d, $J_{2',3'/6',5'}$ = 8.0 Hz, 2H, H-2′, H-6′), 7.83 (d, $J_{3',2'/5',6'}$ = 8.4 Hz,2H, H-3′, H-5′), 7.78 (ovp, 4H, H-4, H-8, H-2″, H-6″), 7.70 (s, 1H, H-1), 7.53 (t, $J_{3'',2''/5'',6''=}$ $J_{3'',4''/5'',4''}$ = 7.2 Hz,2H, H-3″, H-5″), 7.45 (d,$J_{4'',3''/4'',5''}$ = 7.2 Hz ,1H, H-4″), 7.28 (dd, $J_{3,1}$ = 1.6 Hz,$J_{3,4}$ = 8.8 Hz, 1H, H-3), 7.28 (d, $J_{5,7}$ = 2.4 Hz ,1H, H-5), 7.15(dd, $J_{7,5}$ = 2.4 Hz, $J_{7,8}$ = 8.8 Hz, 1H, H-7), 5.05 (s, 2H, CH$_2$), 4.60 (q, $J_{CH,CH3}$ = 7.2 Hz, 1H, CH), 3.84 (s, 3H, OCH$_3$), 1.68 (d, $J_{CH3,CH}$ = 7.2 Hz, 3H, CH$_3$). ^{13}C NMR (100 MHz, DMSO-d_6):δ 191.8, 169.8, 163.0, 157.3, 145.1, 138.6, 135.3, 133.7, 133.4, 129.1, 129.1, 129.0, 129.0, 128.8, 128.5, 128.3, 127.3, 127.0, 127.0, 126.9, 126.9, 125.7, 125.5, 118.8, 105.7, 55.1, 45.7, 36.3, 19.1; EI-MS *m/z* (% rel. abund.): 480 (M$^+$, 28), 286 (12), 185 (36), 181 (100); HREI-MS Calcd for $C_{29}H_{24}N_2O_3S$ *m/z* 480.1508 found is 480.1517; Anal. Calcd for $C_{29}H_{24}N_2O_3S$: C = 72.48; H = 5.03; N = 5.83; Found: C = 72.50; H = 5.05; N = 5.81.

4.6.4. *(S)-1-(3-Hydroxyphenyl)-2-((5-(1-(6-methoxynaphthalen-2-yl)ethyl)-1,3,4-oxadiazol-2-yl)thio)ethan-1-one (39)*

Brown solid; Yield: 29%; R$_f$: 0.83; M.p.: 152–154 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.87 (s, 1H, OH), 7.78 (ovp, 2H, H-4, H-8), 7.70 (s, 1H, H-1), 7.45 (d, $J_{6',5'}$ = 8.0 Hz, 1H, H-6′), 7.35 (ovp, 3H, H-4′, H-2′, H-5′), 7.28 (d, $J_{5,7}$ = 2.0 Hz, 1H, H-5), 7.16 (dd, $J_{3,1}$ = 2.4 Hz, $J_{3,4}$ = 9.2 Hz, 1H, H-3), 7.07 (dd, $J_{7,5}$ = 2.0 Hz, $J_{7,8}$ = 8.8 Hz, 1H, H-7), 4.97 (s, 2H, CH$_2$), 4.58 (q, $J_{CH,CH3}$ = 7.2 Hz, 1H, CH), 3.85 (s, 3H, OCH$_3$), 1.67 (d, $J_{CH3,CH}$ = 7.2 Hz, 3H, CH$_3$); ^{13}C NMR (100 MHz, DMSO-d_6):δ 191.8, 169.7, 163.0, 157.6, 157.3, 136.2, 135.3, 133.3, 129.9, 129.1, 128.3, 127.3, 125.8, 125.2, 120.9, 119.3, 118.8, 114.4, 105.7, 55.1, 40.3, 36.2, 19.1; EI-MS *m/z* (% rel. abund.): 420 (M$^+$, 74), 185 (100); HREI-MS Calcd for $C_{23}H_{20}N_2O_4S$ *m/z* 420.1144 found is 420.1140; Anal. Calcd for $C_{23}H_{20}N_2O_4S$: C = 65.70; H = 4.79; N = 6.66; Found: C = 65.73; H = 4.77; N = 6.64.

5. *In vitro* urease inhibitory assay

Urease inhibitory potential of *S*-naproxen derivatives **1**–**39** were performed by using reported method [28]. Reaction mixtures comprising of enzyme solution 25 µL and buffer solution 55 µL containing urea 100 mM were incubated with test compounds 5 µL (1 mM concentration) for 15 min at 30 °C in 96-well plates. Ammonia production was measured by using the indophenols method in order to determine the urease activity. Briefly, each of phenol reagent 45 µL (1% w/v phenol and 0.005% w/v sodium nitroprusside) and alkali reagent 70 µL (0.5% w/v NaOH and 0.1% active chloride NaOCl) were added to each well. The increasing absorbance was measured at 630 nm after 50 min using a microplate reader (Molecular Device, USA). All steps were performed thrice in a final volume of 200 µL. The results (change in absorbance per min) were calculated by using SoftMax Pro software (Molecular Device, USA). Assays were performed at pH 8.2 (0.01 M $K_2HPO_4{\cdot}3H_2O$, 1 mM EDTA and 0.01 M LiCl). Acetohydroxamic acid was used as the standard inhibitor of urease. Percentage inhibitions were calculated from the following formula:

$$\%\text{Inhibition} = 100 - (\text{OD}_{\text{testwell}}/\text{OD}_{\text{control}}) \times 100$$

6. Molecular docking

To decipher the binding mode of the synthetic compounds **1**–**39** into the active site of urease enzyme, molecular modeling study was performed by using MOE (Molecular Operating Environment) [27]. The three dimensional structures of the compounds were generated with the help of builder tool, implemented in MOE software. The generated compounds were 3D protonated and energy minimized using the default parameters of the MOE (gradient: 0.05, Force Field: MMFF94X). All the compounds were then saved in Molecular Data Base (mdb) file format for further evaluation of compounds in molecular docking study.

3D structure of the target protein was retrieved from the protein databank (PDB ID 4ubp). The retrieved protein was opened in MOE, water molecules were removed and 3D protonation was done. After 3D protonation, protein was energy minimized to get a stable conformation of the protein by using the default parameters of MOE. Placement: Triangle Matcher, Rescoring 1: London dG, Refinement: Forcefield, Rescoring 2: GBVI/WSA are the parameters MOE, used for docking studies. Ten conformations were allowed to be formed for each ligand and the top ranked conformations were selected for further analysis on the basis of docking score.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2018.10.021.

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