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ORIGINAL ARTICLE

# Biology-oriented drug synthesis (BIODS), in vitro urease inhibitory activity, and in silico studies on ibuprofen derivatives

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## Abstract

Novel ibuprofen derivatives **1**–**19** including ibuprofen hydrazide **1**, and substituted thiourea derivatives **2**–**19** were synthesized and characterized by EI-MS, FAB-MS, HREI-MS, HRFAB-MS, $^{1}$H-, and $^{13}$C-NMR spectroscopic techniques. The synthetic molecules **1**–**19** were examined for their in vitro urease inhibition and were found to display a diversified degree of inhibitory potential in the range of $IC_{50} = 2.96$–$178$ μM as compared to the standard thiourea ($IC_{50} = 21.32 \pm 0.22$ μM). Out of nineteen, thirteen derivatives **2**–**4**, **6**, **7**, **9**, **11**–**15**, **17**, and **18** demonstrated remarkable inhibitory activity with $IC_{50}$ values of $2.96 \pm 1.11$ to $16.1 \pm 1.07$ μM, compound **5** exhibited moderate inhibition with $IC_{50}$ value of $37.3 \pm 0.41$ μM, whereas, compounds **1**, **8**, and **10** demonstrated weak inhibition against urease enzyme. Almost all structural features are participating in the activity; however, limited structure–activity relationship was discussed on the basis of different structural features, i.e., different functional groups and their positions at aryl part. In addition, molecular docking study was performed in order to understand the ligands binding interactions with the active site of urease enzyme.

**Electronic supplementary material** The online version of this article (https://doi.org/10.1007/s11030-019-10032-x) contains supplementary material, which is available to authorized users.

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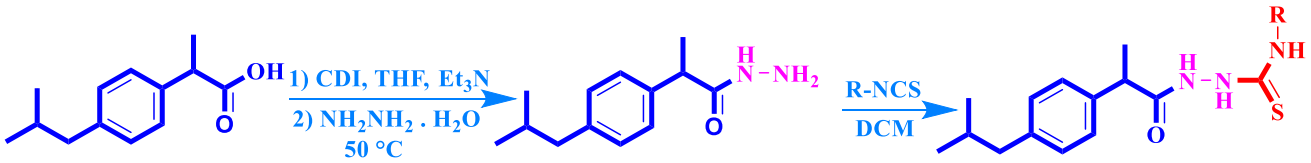
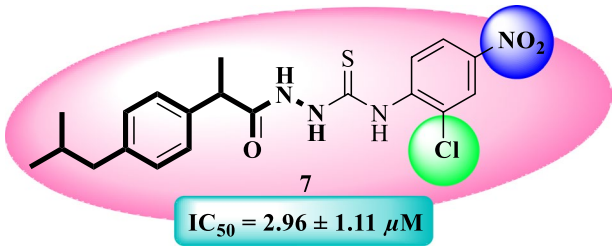
**Graphic abstract**



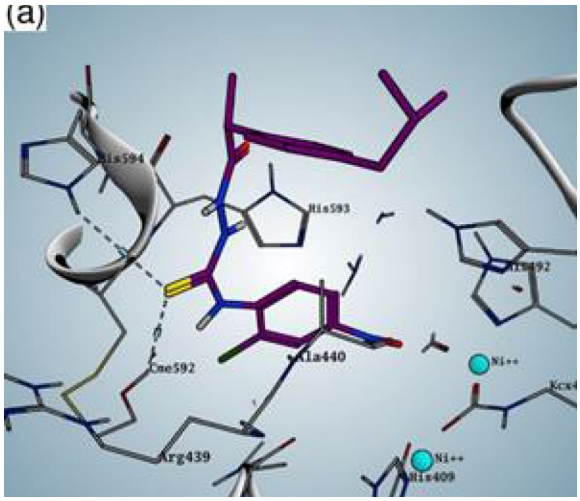

**Scheme-1: Synthesis of ibuprofen derivatives**




**Lead Molecule**

**Standard**

**Thiourea $IC_{50}$ = 21.32 ± 0.22 $\mu$M**




**Keywords** Ibuprofen · Biology-oriented drug synthesis (BIODS) · Urease inhibition · Thiourea derivatives · In silico studies

## Introduction

Ibuprofen (IBP) is the first member of propionic acid derivatives which is most significant nonsteroidal anti-inflammatory drugs (NSAIDs) [1, 2]. It is extensively used for curing osteoarthritis, rheumatoid arthritis, and musculoskeletal pain [3–5]. It was introduced for the first time in 1969 in UK after the approval of FDA in 1947 and then it became available worldwide [6, 7]. In addition to analgesic and anti-inflammatory activities, antimalarial, antimicrobial, anticonvulsant, and antitubercular activities are also reported for *S*-ibuprofen derivatives [8–10]. Only the (*S*) enantiomer of ibuprofen potentially inhibits cyclooxygenase enzyme [11, 12].

Urease is nickel-based metalloenzyme which belongs to the family of phosphotriestreases and amidohydrolases, it is responsible for the production of carbon dioxide and ammonia from the hydrolysis of urea. Urease enzyme of jack bean was crystallized for the first time from plant source *Canavalia ensiformis* in 1926 and mainly found in algae, fungi, bacteria, and plants [13–15]. Each human being is responsible to produce about 10 kg of urea per year due to different metabolic processes. Thus, the alkalinity of urine is increased due to excess formation of ammonia leading to the super-saturation of calcium phosphate resulting in stone formation [16]. Urease enzyme promotes the growth of Gram-negative bacterium, *Helicobacter pylori* (HP), which is involved in many pathological conditions like peptic ulcer, pyelonephritis, hepatic coma, and formation of urinary stones [17, 18]. So, it is essential to introduce new, selective, and potent inhibitors for urease having significant stability, bioavailability, and less toxicity [19–21].

We used biology-oriented drug synthesis (BIODS) approach for the synthesis of library of ibuprofen derivatives in order to evaluate their diversified and unknown biological activities. Previously, using BIODS approach, our research group has reported many lead compounds, such as metronidazole derivatives for their antidiabetic potential by inhibiting $\alpha$-amylase and $\beta$-glucuronidase enzymes, piroxicam derived compounds for their anti-nociceptive activity, flurbiprofen-based compounds for their $\alpha$-amylase inhibitory activity, and *S*-naproxen-based analogs as urease inhibitors (Fig. 1) [22–26].

Since our research group has already reported the diverse biological activities of compounds derived from NSAIDs,

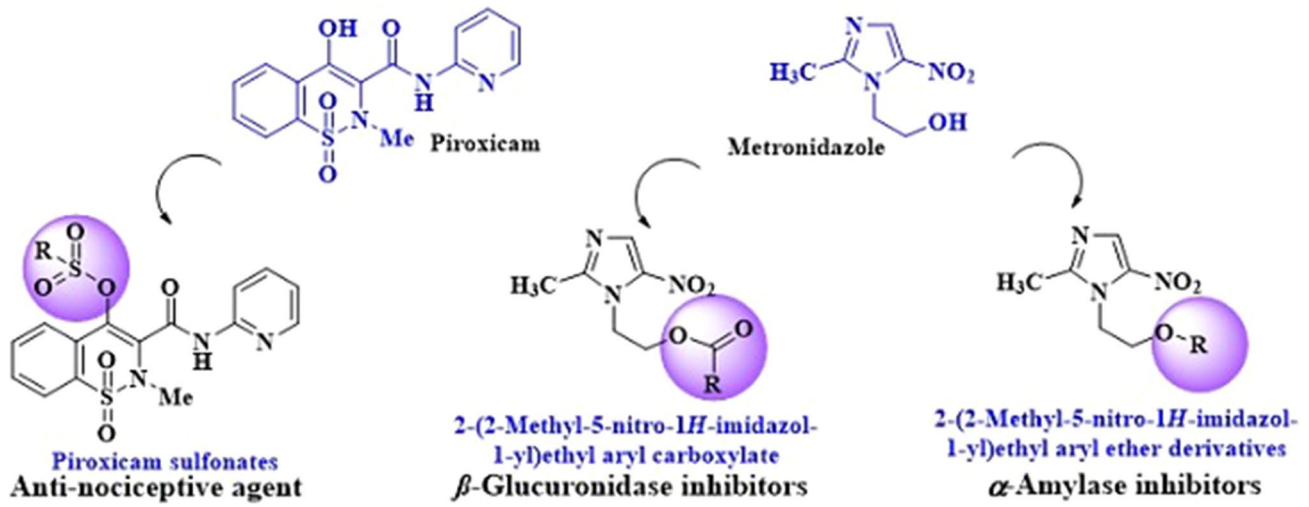



**Fig. 1** Lead candidates derived from commercially available drugs

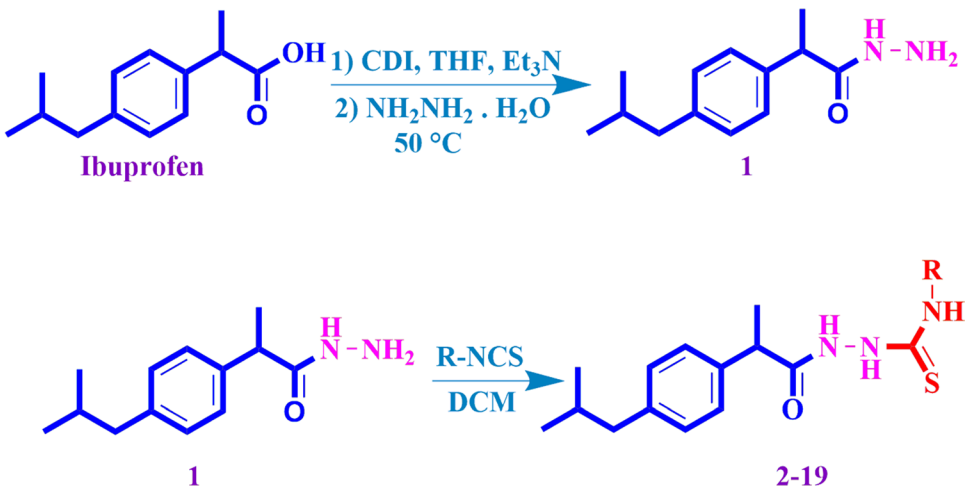
therefore, in continuation of our previous work, we had selected ibuprofen for chemical modifications in order to explore biological potential. It is worth noting that our research group has reported various lead compounds as potential inhibitors of urease enzyme and recently *S*-naproxen derivatives were reported as urease inhibitors. These results encourage us to explore the urease inhibitory activity of newly synthesized derivative of ibuprofen. To the best of our knowledge, the synthetic molecules (**1–19**) are reported for the first time as urease inhibitors.

## Results and discussion

### Chemistry

Novel derivatives (**1–19**) of commercially available drug ibuprofen were synthesized. First, ibuprofen hydrazide was synthesized via one-pot reaction of ibuprofen, hydrazine hydrate, and 1,1-carbonyl diimidazole (CDI) in THF. Further reaction of hydrazide with different substituted phenyl isothiocyanates in dichloromethane with continuous stirring at room temperature afforded compounds **2–19** (Scheme 1). The reaction progress was examined by TLC (hexane:ethyl acetate). Upon completion of reaction, the solvent was evaporated and residue was washed with hot hexane and excess of distilled water to afford pure products. Solid product was crystallized from ethanol. All synthetic compounds **1–19** were characterized by EI-MS, FAB-MS, HREI-MS, HRFAB-MS, $^{1}$H- and $^{13}$C-NMR. Off nineteen molecules, five compounds **1**, **2**, **5**, **13**, and **18** are structurally known [4, 27, 28].

**Scheme 1** Synthesis of ibuprofen derivatives



## In vitro urease inhibitory activity

Ibuprofen hydrazide **1** and synthetic compounds **2**–**19** were screened them for their in vitro urease inhibition. The results listed in Table 1 showed that all compounds were found to be potent with $IC_{50}$ values of $2.96 \pm 1.11$ to $16.1 \pm 1.07$ µM, while, compound **5** displayed moderate inhibition with $IC_{50}$ value of $37.3 \pm 0.41$ µM in comparison with the standard thiourea ($IC_{50} = 21.32 \pm 0.22$ µM). Compounds **16** and **19** were found to be inactive. The general structure of synthetic molecules have been shown in Fig. 2.

## Structure–activity relationship (SAR)

The parent molecule ibuprofen displayed no inhibition against urease enzyme, whereas, hydrazide of ibuprofen **1** ($IC_{50} = 202 \pm 1.23$ µM) was weakly active as compared to the standard thiourea ($IC_{50} = 21.32 \pm 0.22$ µM) might be due to the weak binding interactions of hydrazide functionality. Surprisingly, compound **2** ($IC_{50} = 16.1 \pm 1.07$ µM) with unsubstituted benzene ring and the thiourea functionality was found to be potentially active in comparison to the standard might be due to incorporation of thiourea functionality. Compounds **3**–**19** with substituted benzene ring also exhibited good inhibition against urease. It showed that substitution on benzene ring and incorporation of thiourea moiety is playing significant role in the urease inhibitory activity of synthetic compounds (Fig. 3).

Among chloro-substituted derivatives, compound **3** ($IC_{50} = 5.36 \pm 1.27$ µM) bearing *ortho*-chloro substituent showed potential inhibitory activity as compared to the standard thiourea ($IC_{50} = 21.32 \pm 0.22$ µM). The change in position of chloro group to *meta* and *para*, respectively, in compounds **4** ($IC_{50} = 15.2 \pm 0.98$ µM) and **5** ($IC_{50} = 37.3 \pm 0.41$ µM) resulted in decreased activity as compared to compound **3**. It showed that *ortho*-position is contributing actively might be due to the better binding interactions with the active site of enzyme. The activity was decreased threefold in case of *meta*-substitution, nonetheless, it was more active as compared to standard, however, *para*-substituted compound was least active. The dichloro-substituted derivative **6** ($IC_{50} = 6.16 \pm 1.68$ µM) having chlorine atoms *para* to each other was found to be good inhibitor as compared to compound **4** might be due to the presence of chloro group at *ortho*-position, whereas, it was less active in comparison with compound **3** due to *meta*-substitution. Compound **7** ($IC_{50} = 2.96 \pm 1.11$ µM) showed sevenfold more activity in comparison with the standard thiourea and was most active compound of the series. Compound **7** having chloro and a nitro groups *meta* to each other showed good activity, might be the nitro group having two oxygen atoms resulted in the enhanced activity of this compound due to better binding interactions (Fig. 4).

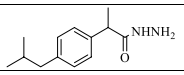
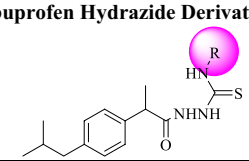
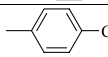
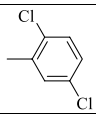
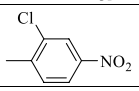
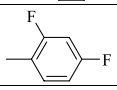
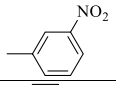
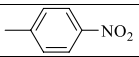
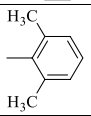
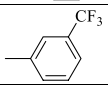
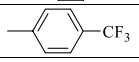
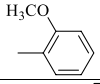
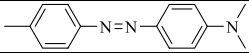
Compound **8** ($IC_{50} = 178 \pm 0.71$ µM) having bromo substituent at *meta*-position showed weak inhibitory potential. Interestingly, compound **9** ($IC_{50} = 10.3 \pm 1.05$ µM) with flouro group at *ortho*-position showed excellent inhibitory activity, however, addition of another fluoro group at *para*-position leads to decreased activity in compound **10** ($IC_{50} = 77.7 \pm 0.77$ µM) might be due to increased inductive effect of two flouro groups or increased steric hindrance which results in less interaction within active site of enzyme. In general, it was observed that halogen substituted at *ortho*-position of aryl ring exhibited good inhibitory potential, addition of halogens or changing their position resulted in loss of activity (Fig. 5).

Interestingly, nitro-substituted compounds showed different pattern, among them compound **12** ($IC_{50} = 6.98 \pm 1.15$ µM) bearing nitro group at *para*-position displayed an increased inhibition in comparison with *meta*-substituted compound **11** ($IC_{50} = 8.72 \pm 0.52$ µM). Both of these compounds were potentially active in comparison with the standard thiourea might be due to the chelation of oxygen atoms with enzyme active site (Fig. 6).

Compounds with mono- and dimethyl substitutions showed varying degree of inhibitory activity. Among them, compound **13** ($IC_{50} = 5.26 \pm 1.35$ µM) with an *ortho*-methyl substitution showed an increased inhibitory potential in comparison with the standard thiourea ($IC_{50} = 21.32 \pm 0.22$ µM) which indicated that methyl group efficiently interacted within the active site of enzyme might be due to hyper-conjugation effect. Nevertheless, dimethyl-substituted derivative **14** ($IC_{50} = 10.5 \pm 5.39$ µM) was found to be less active in comparison with compound **13** might be the inclusion of another methyl substituent at *ortho*-position resulted in increased steric hindrance thus leads to decreased activity. However, both of these compounds were more active as compared to the standard (Fig. 7).

Compounds **15**–**17** were positional isomers of each other bearing trifluoromethyl group at different positions of aryl ring, and all these derivatives were found to be potentially active than the standard thiourea. Among them, compound **15** ($IC_{50} = 9.85 \pm 1.16$ µM) with *ortho*-trifluoromethyl was found to possess excellent inhibitory activity. Nevertheless, compound **17** ($IC_{50} = 8.53 \pm 0.64$ µM) bearing *para* trifluoromethyl group was more active as compared to its *ortho*-substituted analog **15** while *meta*-substituted isomer **16** was completely inactive. Compound **18** ($IC_{50} = 3.5 \pm 3.26$ µM) bearing methoxy group at *ortho*-position demonstrated potent inhibitory activity and was the second most active derivative. The activity of this group might be due to better binding interactions of methoxy group with the active site of enzyme (Fig. 8).

**Table 1** In vitro urease inhibition of compounds (**1**–**19**)

| Compound No. | R | $IC_{50} \pm SEM^a$ ($\mu M$) |
|---|---|---|
| **1** |  | 202 ± 1.23 |
| **Ibuprofen Hydrazide Derivatives**  | | |
| **R** | | |
| **2** |  | 16.1 ± 1.07 |
| **3** |  | 5.36 ± 1.27 |
| **4** |  | 15.2 ± 0.98 |
| **5** |  | 37.3 ± 0.41 |
| **6** |  | 6.16 ± 1.68 |
| **7** |  | 2.96 ± 1.11 |
| **8** |  | 178 ± 0.71 |
| **9** |  | 10.3 ± 1.05 |
| **10** |  | 77.7 ± 0.77 |
| **11** |  | 8.72 ± 0.52 |
| **12** |  | 6.98 ± 1.15 |
| **13** |  | 5.26 ± 1.35 |
| **14** |  | 10.5 ± 5.39 |
| **15** |  | 9.85 ± 1.16 |
| **16** |  | NA[b] |
| **17** |  | 8.53 ± 0.64 |
| **18** |  | 3.5 ± 3.26 |
| **19** |  | NA[b] |
| **Thiourea[c]** | | 21.32 ± 0.22 |

$IC^a_{50}$ (mean ± standard error of mean); NA[b] (not active); Standard[c] (inhibitor for urease activity)

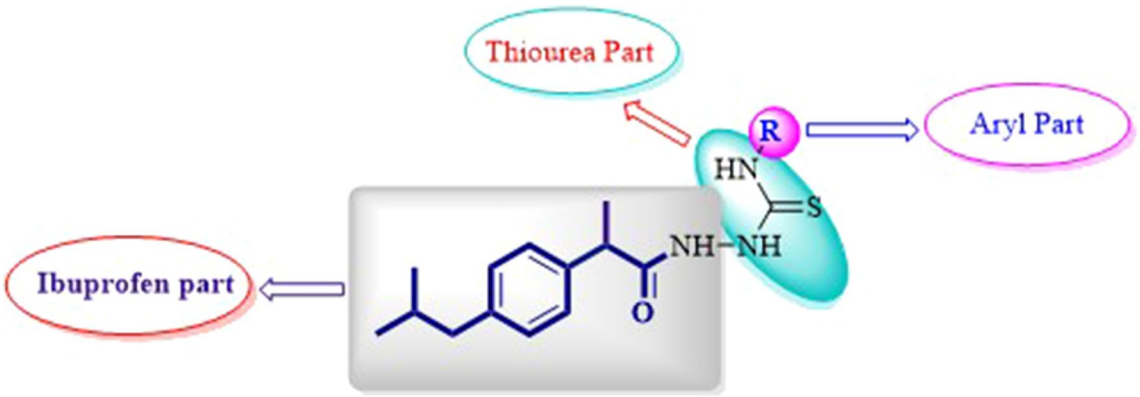



**Fig. 2** General structure of synthetic compounds

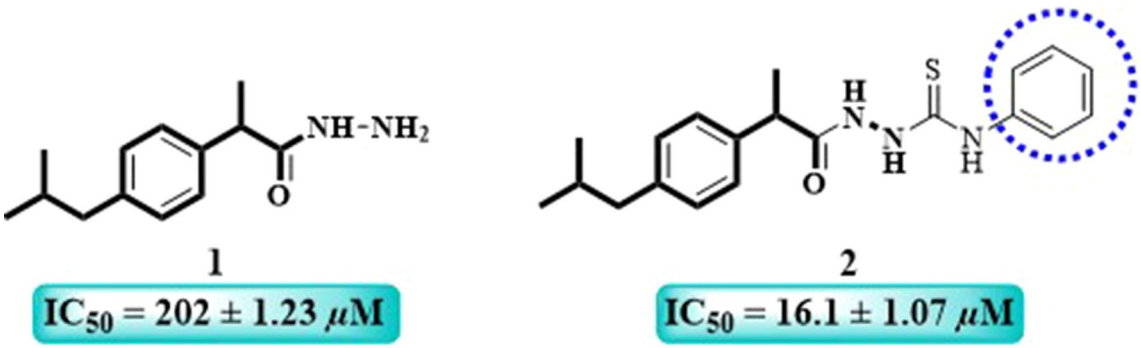



**Fig. 3** SAR of compounds **1**–**2**

## In silico studies

Molecular docking studies were conducted to understand the plausible ligand binding mechanism. The docking studies were carried out by using the crystal structure of Jack Bean urease (PDB 4GY7). Ibuprofen along with the nineteen newly synthesized derivatives was docked. The interaction patterns were understood by visualization of resulting poses.

The top-ranked docked pose of the ibuprofen is presented in Fig. 9a. As evident from the figure the benzene ring of the ibuprofen is stacked against the His593. While the oxygen atom exhibited bi-dentate interaction with one of the nickel atoms. The strategic position of the carboxylic functional group in the binding site of the urease (near the catalytic center) suggests that the modifications in this area might increase the inhibitory potential of the ibuprofen. In this connection, ibuprofen was modified to obtain compound **1**. The compound exhibited additional contacts within the binding site with the Met637. The benzene ring of the compound **1** was stacked against the His593 (Fig. 9b).

The compound **1** was further modified to yield a series of the ibuprofen hydrazide derivatives (**2**–**19**). Among the halogen-containing compounds, compound **3** ($IC_{50} = 5.36 \pm 1.27$ µM) showed the highest inhibitory activity. The simulated binding pose of the compound **3** is presented in Fig. 9c ($S = -8.25$). As evident from the Fig. 9, the chloro-benzene moiety of the ligand is involved in aromatic interaction with Ala636 (green dashed lines), while the ligand displays hydrogen bonds with His593 and Cme593.

As mentioned earlier in SAR, the combination of other groups with chloro such as compound **7** ($IC_{50} = 2.96 \pm 1.11$ µM) with nitro and chloro groups *meta* to each other was found to be most potent and sevenfold more active as compared to the standard thiourea. Figure 10a presents the binding mode of the compound **7**. The compound **7** exhibited hydrogen bonding interaction with the His593 and Cme593. The benzene ring can be seen stacked against the His593, disrupting the catalytic process.

Among the compounds bearing nitro substitutions, compounds **12** with nitro group at the *para*-position displayed better activity ($IC_{50} = 6.98 \pm 1.15$ µM). The top-ranked simulated pose of the compound **12** suggested that the compound exhibit π-stacking interaction with His539 (Fig. 10b). The contact between His539 and the ligand is

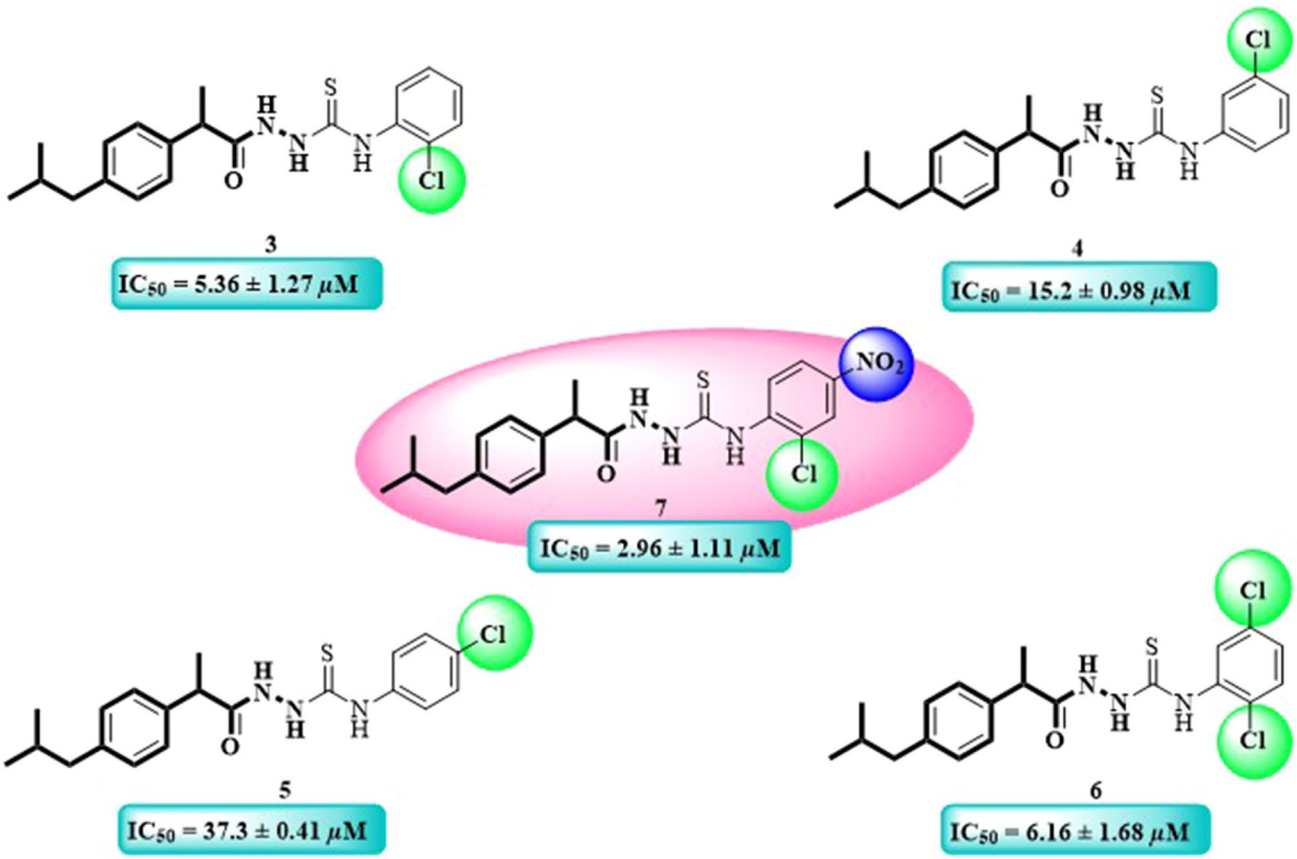



**Fig. 4** SAR of compounds **3**–**7**

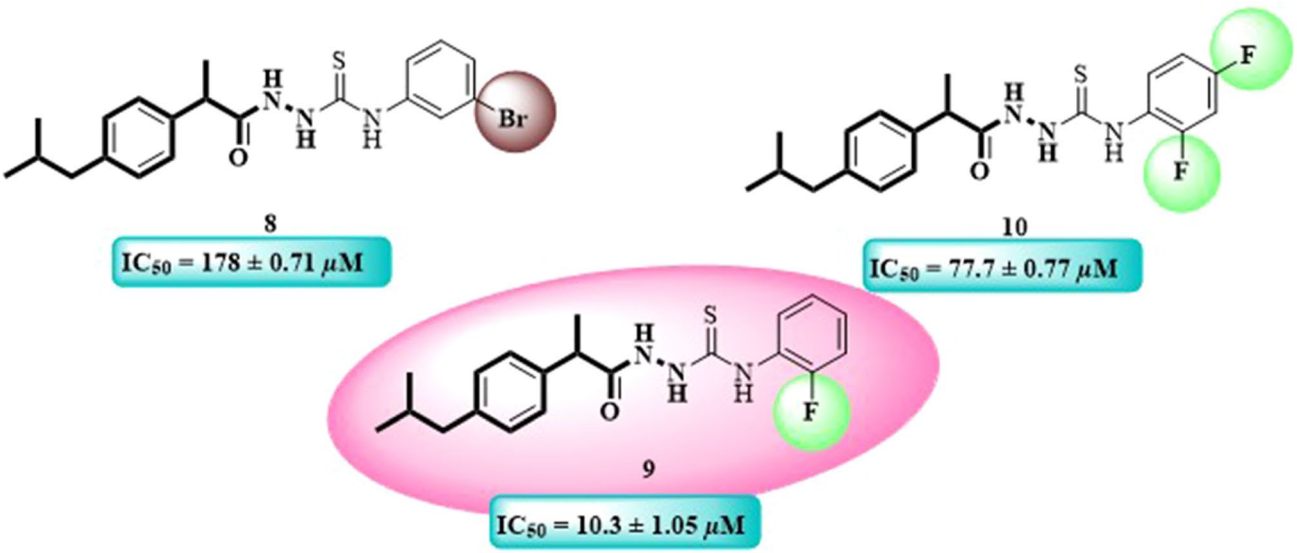



**Fig. 5** SAR of compounds **8**–**10**

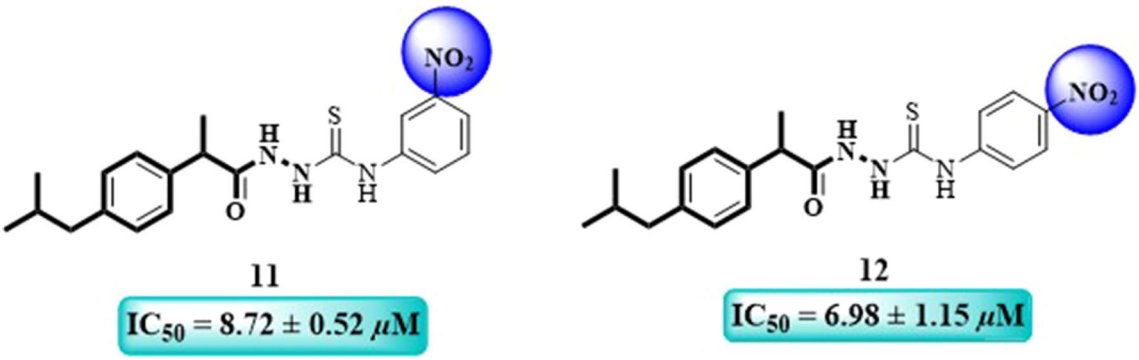



**Fig. 6** SAR of compounds **11**–**12**

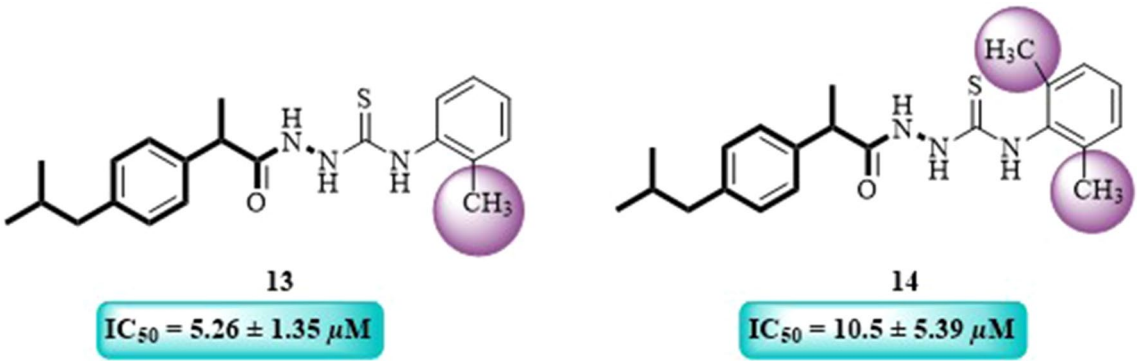



**Fig. 7** SAR of compounds **13**–**14**

further stabilized by the hydrogen bond. Another hydrogen bond is observed between the ligand and Cme592. The *para*-substituted nitro group exhibits hydrogen bonding interaction with the Ni901 at the catalytic center.

Compound **13** ($IC_{50} = 5.26 \pm 1.35$ μM) bearing an *ortho*-methyl substitution demonstrated an increased inhibitory activity which could be attributed to the hyper-conjugation effect. Figure 10c depicted the simulated binding mode of the compound **13** which indicates that methyl group interacted more efficiently with the active site of enzyme. The methyl-benzene ring of the ligand exhibits $\pi$-cation interactions with His409 and His539 which rationalize the observed higher inhibitory activity of **13**.

The binding mode of the compound **18** ($IC_{50} = 3.5 \pm 3.26$ μM) with methoxy substitution is presented in Fig. 10d. The methoxy group of the ligand interacts with the Trp356. Moreover, the ligand exhibits bidentate hydrogen bonds with the Ala436.

The molecular dynamics simulation of the compound **3** was carried out to understand the dynamics of the observed protein–ligand interaction. Figure 11 presents the superimposed coordinates of the active site of the compound **3**-urease complex. As evident from the figure, there is no significant difference in the coordinates of the active site residues and the system presented over all stability.

## Conclusion

Novel derivatives of ibuprofen **1**–**19** were synthesized and screened for their in vitro inhibition against urease enzyme. The synthetic compounds displayed good to moderate inhibition against urease enzyme in comparison with the standard thiourea. All compounds were found to be excellent inhibitors except **16** and **19** which were inactive. Limited structure–activity relationship (SAR) proposed that the variation in the activity of different molecules might be due to the position and nature of the substituted groups. Moreover, the docking study revealed that the synthetic molecules (ligands) have extensive binding interactions with the active site of enzyme. This study identified many lead candidates derived from ibuprofen and further exploration of these molecules for future research to get novel urease inhibitors.

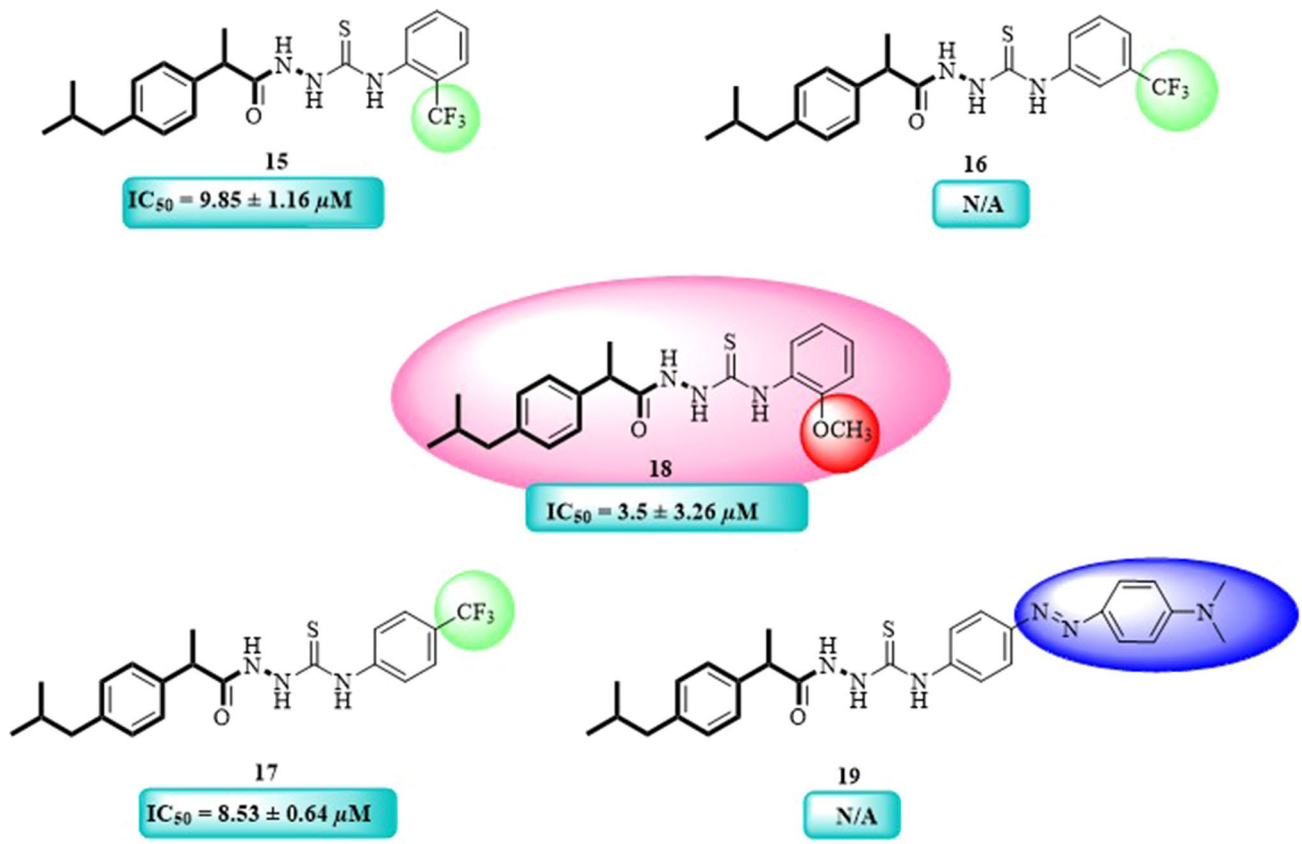



**Fig. 8** SAR of compounds **15**–**19**

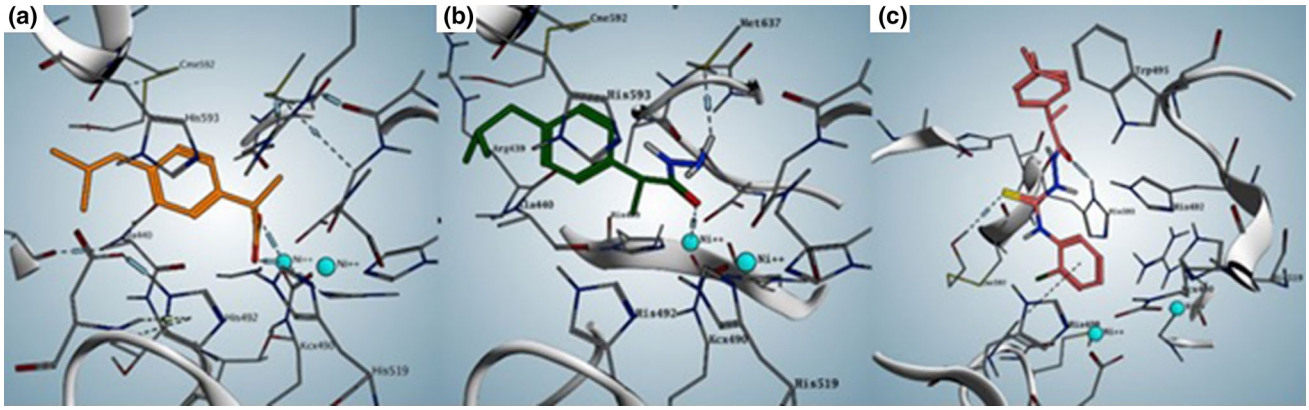



**Fig. 9** The binding mode of the ibuprofen **a** ibuprofen hydrazide; compound **1 b** ibuprofen hydrazide derivative; compound **3 c** hydrogen bonds are presented as blue dashed lines. Green dashes depict the hydrophobic and aromatic interactions. (Color figure online)

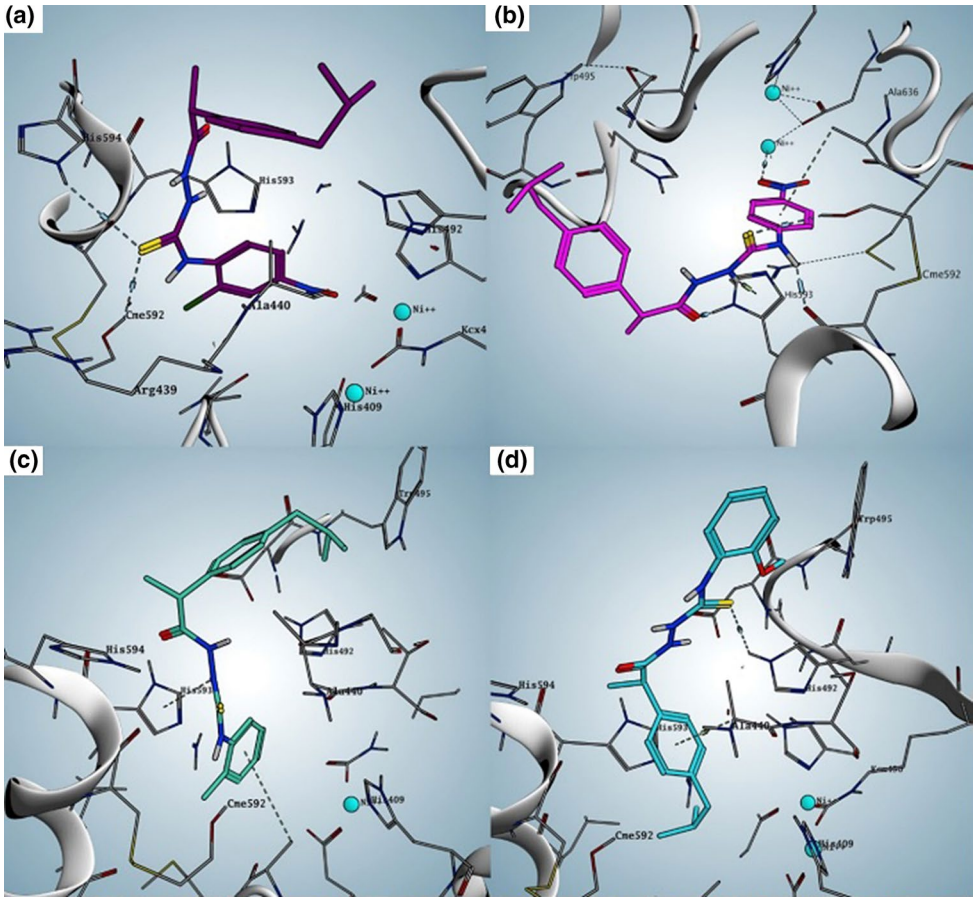



**Fig. 10** The simulated binding mode of the compounds **7** (**a**), **12** (**b**), **13** (**c**), and **18** (**d**). The hydrogen bonds are presented as blue dashed lines. Green dashes depict the hydrophobic and aromatic interactions. (Color figure online)

## Experimental

### Materials and methods

All analytical grade reagents were purchased from Sigma-Aldrich, USA. For thin layer chromatography (TLC), silica gel-coated aluminum plates GF-254 (Merck, Germany) were used. Spots were visualized with a dual wavelength of 254 and 366 nm under ultraviolet light or iodine vapors. Electron impact mass spectra were recorded on MAT 113D and MAT 312 mass spectrometers. The $^{1}$H-NMR spectra were recorded on Bruker AM machines, functioning at 300 and 400 MHz and $^{13}$C-NMR were recorded on 75, 100, and 125 MHz. Chemical shift ($\delta$) values, relative to tetramethylsilane (TMS) as an internal standard, are presented in ppm, and the coupling constant ($J$) values are presented in Hz. Melting point of the compounds were determined on a Stuart® SMP10 melting point apparatus.

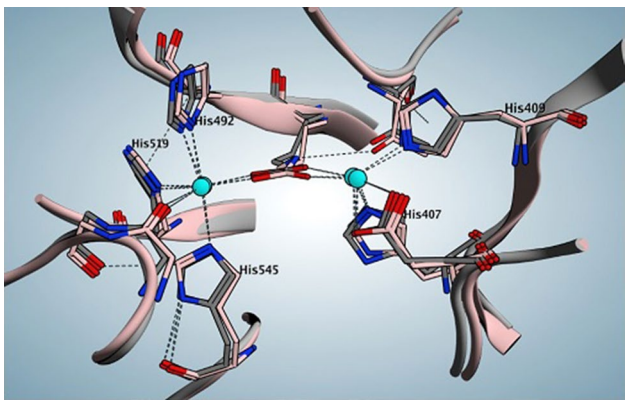



**Fig. 11** The superimposed coordinates of the active site residues, before (gray) and after simulation (pink). The nickel atoms are presented as green balls. (Color figure online)

#### Synthesis of ibuprofen hydrazide (1)

Ibuprofen (1 mmol) and CDI (1.2 mmol) were taken in a round-bottomed flask (100 mL) and dissolved in minimum volume of tetrahydrofuran (THF) in the presence of triethylamine (TEA) to make a saturated solution. The reaction mixture was then stirred for 15 min, followed by the addition of hydrazine hydrate (0.5 mL) and THF (5 mL) and it was further refluxed for 3 h at 60 °C with constant stirring. Reaction progress was monitored by the TLC (7:3; hexane:ethyl acetate) and then after completion of reaction,

reaction mixture was poured onto crushed ice. The precipitates were formed immediately which were filtered, and to afford pure product, precipitates were washed with excess of distilled water, dried in vacuum and crystallized from ethanol. Structure of the compound **1** was elucidated by the EI-MS, FAB-MS, HREI-MS, HRFAB-MS, and $^{1}$H-NMR spectroscopic techniques.

#### 2-(4-Isobutylphenyl)propanehydrazide (1) [4]

Yield: 72%; M.P.: 60–62 °C; $^{1}$H-NMR (400 MHz, DMSO-$d_6$): $\delta_H$ 9.10 (s, 1H, NH), 7.20 (d, 2H, $J_{2,3/6,5}$ = 8.0 Hz, H-2, H-6), 7.05 (d, 2H, $J_{3,2/5,6}$ = 8.0 Hz, H-3, H-5), 4.15 (s, 2H, $NH_2$), 3.46 (q, 1H, H-2b), 2.39 (d, 2H, $J_{1a,2a}$ = 7.2 Hz, H-1a), 1.78 (m, 1H, H-2a), 1.30 (d, 3H, $J_{3b,2b}$ = 6.8 Hz, H-3b), 0.84 (d, 6H, $J_{3a,2a/4a,2a}$ = 6.8 Hz, H-3a, H-4a); EI-MS *m/z* (% rel abund.): 220 ($M^+$, 65), 188 (57), 161 (100).

### General procedure for the synthesis of substituted thiourea of ibuprofen (2–19)

Ibuprofen hydrazide **1** (0.5 mmol) and corresponding substituted phenyl isothiocyanates (0.5 mmol) were taken into a round-bottomed flask (100 mL) and dissolved in dichloromethane (DCM) (5 mL). Reaction mixture was stirred at room temperature. Reaction progress was monitored by TLC (6:4; hexane:ethyl acetate) analysis. After completion of reaction, the solvent was evaporated *in vacuo* and the residue obtained was washed with distilled water followed by washing with hot hexane. Pure products were obtained by crystallization from ethanol. All compounds were characterized by EI-MS, FAB-MS, HREI-MS, HRFAB-MS, $^{1}$H-, and $^{13}$C-NMR spectroscopic techniques. Spectroscopic data of all known compound were found to be in agreement with the reported data.

#### 2-(2-(4-Isobutylphenyl) propanoyl)-*N*-phenylhydrazine-1-carbothioamide (2) [4]

Yield: 80%; M.P.: 170–172 °C; $^{1}$H-NMR (400 MHz, DMSO-$d_6$): $\delta_H$ 10.05 (s, 1H, NH–CO), 9.56 (s, 1H, NH–CS), 9.37 (s, 1H, NH), 7.41 (d, 2H, $J_{2',3'/6',5'}$ = 7.2 Hz, H-2′, H-6′), 7.32 (t, 2H, $J_{3',2'/3',4'/5',6'/5',4'}$ = 7.8 Hz, H-3′, H-5′), 7.26 (d, 2H, $J_{2,3/6,5}$ = 7.6 Hz, H-2, H-6), 7.15 (t, 1H, $J_{4',3'/4',5'}$ = 7.2 Hz, H-4′), 7.09 (d, 2H, $J_{3,2/5,6}$ = 8.0 Hz, H-3, H-5), 3.67 (q, 1H, H-2b), 2.40 (d, 2H, $J_{1a,2a}$ = 6.8 Hz, H-1a), 1.78 (m, 1H, H-2a), 1.38 (d, 3H, $J_{3b,2b}$ = 6.8 Hz, H-3b), 0.84 (d, 6H, $J_{3a,2a/4a,2a}$ = 6.8 Hz, H-3a, H-4a); EI-MS *m/z* (% rel abund.): 355 ($M^+$, 1), 337 (2), 321 (43), 306 (13), 278 (7), 262 (5), 220 (23), 188 (23), 161 (100).

#### *N*-(2-Chlorophenyl)-2-(2-(4-isobutylphenyl)propanoyl) hydrazine-1-carbothioamide (3)

Yield: 75%; M.P.: 219–221 °C; $^{1}$H-NMR (400 MHz, DMSO-$d_6$): $\delta_H$ 10.16 (s, 1H, NH–CO), 9.76 (s, 1H, NH–CS), 9.14 (s, 1H, NH), 7.60 (s, 1H, H-6′), 7.46 (dd, 1H, $J_{3',4'}$ = 7.8 Hz, $J_{3',5'}$ = 1.0 Hz, H-3′), 7.32 (dt, 1H, $J_{5',4'/5',6'}$ = 7.7 Hz, $J_{5',3'}$ = 1.0 Hz, H-5′), 7.24 (m, 3H, H-2, H-6, H-4′), 7.08 (d, 2H, $J_{3,2/5,6}$ = 8.0 Hz, H-3, H-5), 3.66 (d, 1H, $J_{2b,3b}$ = 5.2 Hz, H-2b), 2.39 (d, 2H, $J_{1a,2a}$ = 7.2 Hz, H-1a), 1.78 (m, 1H, H-2a), 1.38 (d, 3H, $J_{3b,2b}$ = 7.2 Hz, H-3b), 0.84 (d, 6H, $J_{3a,2a/4a,2a}$ = 6.4 Hz, H-3a, H-4a); $^{13}$C-NMR (125 MHz, DMSO-$d_6$): *δc* 181.2 (C=S), 173.3 (C=O), 139.4 (C-1), 138.5 (C-4), 136.2 (C-1′), 129.6 (C-2′), 129.1 (C-6′), 128.7 (C-2, -6), 127.4 (C-3, -5, -4′), 127.2 (C-3′), 126.9 (C-5′), 44.1 (C-1a), 42.8 (C-2b), 29.5 (C-2a), 22.1 (C-3a, -4a), 18.9 (C-3b); FAB$^+$-MS *m/z*: 390 ($M^+$+1); HRFAB$^+$-MS *m/z*: Calcd for $C_{20}H_{25}ON_3ClS$ [390.1411], Found [390.1407].

#### *N*-(3-Chlorophenyl)-2-(2-(4-isobutylphenyl)propanoyl) hydrazine-1-carbothioamide (4)

Yield: 88%; M.P.: 195–197 °C; $^{1}$H-NMR (400 MHz, DMSO-$d_6$): $\delta_H$ 10.08 (s, 1H, NH–CO), 9.74 (s, 1H, NH–CS), 9.48 (s, 1H, NH), 7.61 (s, 1H, H-2′), 7.39 (m, 1H, H-6′), 7.35 (t, 1H, $J_{5',4'/5',6'}$ = 7.8 Hz, H-5′), 7.26 (d, 2H, $J_{2,3/6,5}$ = 8.0 Hz, H-2, H-6), 7.20 (d, 1H, $J_{4',5'}$ = 7.2 Hz, H-4′), 7.09 (d, 2H, $J_{3,2/5,6}$ = 8.0 Hz, H-3, H-5), 3.67 (d, 1H, $J_{2b,3b}$ = 6.0 Hz, H-2b), 2.40 (d, 2H, $J_{1a,2a}$ = 7.2 Hz, H-1a), 1.78 (m, 1H, H-2a), 1.38 (d, 3H, $J_{3b,2b}$ = 6.8 Hz, H-3b), 0.84 (d, 6H, $J_{3a,2a/4a,2a}$ = 6.4 Hz, H-3a, H-4a); $^{13}$C-NMR (100 MHz, DMSO-$d_6$): *δc* 221.8 (C=S), 170.6 (C=O), 139.4 (C-1), 138.5 (C-4), 129.6 (C-3′), 128.7 (C-2, -6, -5′), 127.1 (C-3, -5, -2′, -4′),124.49 (C-6′), 44.1 (C-1a), 42.8 (C-2b), 29.5 (C-2a), 22.1 (C-3a, -4a), 18.2 (C-3b); EI-MS *m/z* (% rel abund.): 389 ($M^+$,2), 355 (27), 340 (9), 312 (8), 220 (26), 188 (26), 161 (100); HREI-MS *m/z*: Calcd for $C_{20}H_{24}ON_3ClS$ [389.1327], Found [389.1329].

#### *N*-(4-Chlorophenyl)-2-(2-(4-isobutylphenyl)propanoyl) hydrazine-1-carbothioamide (5) [27]

Yield: 79%; M.P.: 150–152 °C; $^{1}$H-NMR (400 MHz, DMSO-$d_6$): $\delta_H$ 10.06 (s, 1H, NH–CO), 9.66 (s, 1H, NH–CS), 9.47 (s, 1H, NH), 7.46 (d, 2H, $J_{2',3'/6',5'}$ = 7.6 Hz, H-2′, H-6′), 7.37 (d, 2H, $J_{3',2'/5',6'}$ = 8.4 Hz, H-3′, H-5′), 7.26 (d, 2H, $J_{2,3/6,5}$ = 8.0 Hz, H-2, H-6), 7.08 (d, 2H, $J_{3,2/5,6}$ = 8.0 Hz, H-3, H-5), 3.66 (d, 1H, $J_{2b,3b}$ = 6.8 Hz, H-2b), 2.40 (d, 2H, $J_{1a,2a}$ = 7.2 Hz, H-1a), 1.78 (m, 1H, H-2a), 1.38 (d, 3H, $J_{3b,2b}$ = 6.8 Hz, H-3b), 0.84 (d, 6H, $J_{3a,2a/4a,2a}$ = 6.8 Hz, H-3a, H-4a); EI-MS *m/z* (% rel abund.): 389 ($M^+$, 1), 355 (21), 262 (5), 220 (28), 188 (26), 161 (100).

### *N*-(2,5-Dichlorophenyl)-2-(2-(4-isobutylphenyl)propanoyl) hydrazine-1-carbothioamide (**6**)

Yield: 82%; M.P.: 180–182 °C; $^{1}$H-NMR (400 MHz, DMSO-$d_6$): $\delta_H$ 10.20 (s, 1H, NH–CO), 9.92 (s, 1H, NH–CS), 9.19 (s, 1H, NH), 7.71 (s, 1H, H-6′),7.52 (d, 1H, $J_{3',4'}$ = 8.8 Hz, H-3′), 7.33 (d, 1H, $J_{4',3'}$ = 6.8 Hz, H-4′), 7.26 (d, 2H, $J_{2,3/6,5}$ = 8.0 Hz, H-2, H-6), 7.08 (d, 2H, $J_{3,2/5,6}$ = 8.0 Hz, H-3, H-5), 3.65 (s, 1H, H-2b), 2.40 (d, 2H, $J_{1a,2a}$ = 7.2 Hz, H-1a), 1.78 (m, 1H, H-2a), 1.38 (d, 3H, $J_{3b,2b}$ = 7.2 Hz, H-3b), 0.84 (d, 6H, $J_{3a,2a/4a,2a}$ = 6.4 Hz, H-3a, H-4a); FAB$^+$-MS *m*/*z*: 424 (M$^+$+1); $^{13}$C-NMR (75 MHz, DMSO-$d_6$): *δc* 139.4 (C-1), 138.4 (C-4, 1′), 137.4 (C-5′), 130.8 (C-2′), 130.5 (C-4′), 128.7 (C-2, -6, -3′), 127.1 (C-3, -5, -6′), 44.1 (C-1a), 42.8 (C-2b), 29.5 (C-2a), 22.1 (C-3a, -4a), 18.3 (C-3b); HRFAB$^+$-MS *m*/*z*: Calcd for $C_{20}H_{24}ON_3Cl_2S$ [424.0999], Found [424.1017].

### *N*-(2-Chloro-4-nitrophenyl)-2-(2-(4-isobutylphenyl) propanoyl)hydrazine-1-carbothioamide (**7**)

Yield: 83%; M.P.: 166–168 °C; $^{1}$H-NMR (400 MHz, DMSO-$d_6$): $\delta_H$ 10.29 (s, 1H, NH–CO), 10.14 (s, 1H, NH–CS), 9.30 (s, 1H, NH), 8.33 (s, 1H, H-3′), 8.18 (broad s, 2H, H-5′,6′), 7.27 (d, 2H, $J_{2,3/6,5}$ = 8.0 Hz, H-2, H-6), 7.09 (d, 2H, $J_{3,2/5,6}$ = 8.0 Hz, H-3, H-5), 3.67 (s, 1H, H-2b), 2.40 (d, 2H, $J_{1a,2a}$ = 7.2 Hz, H-1a), 1.78 (m, 1H, H-2a), 1.39 (d, 3H, $J_{3b,2b}$ = 6.8 Hz, H-3b), 0.84 (d, 6H, $J_{3a,2a/4a,2a}$ = 6.4 Hz, H-3a, H-4a); $^{13}$C-NMR (75 MHz, DMSO-$d_6$): *δc* 180.5 (C=S), 170.1 (C=O), 142.2 (C-4′), 139.5 (C-1), 138.3 (C-4), 128.8(C-2, -6), 128.3 (C-2′), 127.1 (C-3, -5, -6′), 124.4 (C-3′), 122.3 (C-5′), 44.1 (C-1a), 42.9 (C-2b), 29.5 (C-2a), 22.1 (C-3a, -4a), 18.3 (C-3b); FAB$^+$-MS *m*/*z*: 435 (M$^+$+1); HRFAB$^+$-MS *m*/*z*: Calcd for $C_{20}H_{24}O_3N_4ClS$ [435.1262], Found [435.1258].

### *N*-(3-Bromophenyl)-2-(2-(4-isobutylphenyl)propanoyl) hydrazine-1-carbothioamide (**8**)

Yield: 92%; M.P.: 215–217 °C; $^{1}$H-NMR (400 MHz, DMSO-$d_6$): $\delta_H$ 10.07 (s, 1H, NH–CO), 9.73 (s, 1H, NH–CS), 9.46 (s, 1H, NH), 7.73 (s, 1H, H-2′), 7.45 (d, 1H, $J_{4',5'}$ = 8.0 Hz, H-4′), 7.29 (ovp. m, 4H, H-5′, H-6′, H-2, H-6), 7.09 (d, 2H, $J_{3,2/5,6}$ = 8.0 Hz, H-3, H-5), 3.66 (d, 1H, $J_{2b,3b}$ = 6.4 Hz, H-2b), 2.40 (d, 2H, $J_{1a,2a}$ = 7.2 Hz, H-1a), 1.79 (m, 1H, H-2a), 1.38 (d, 3H, $J_{3b,2b}$ = 7.2 Hz, H-3b), 0.84 (d, 6H, $J_{3a,2a/4a,2a}$ = 6.4 Hz, H-3a, H-4a); $^{13}$C-NMR (100 MHz, DMSO-$d_6$): *δc* 173.0, (C=S), 165.0 (C=O), 140.7 (C-1), 139.4 (C-4), 138.5 (C-1′), 129.9 (C-3′), 128.7 (C-2, -6, -5′), 127.3 (C-4′), 127.1 (C-3, -5, -2′, -6′), 44.1 (C-1a), 42.8 (C-2b), 29.5 (C-2a), 22.1 (C-3a, -4a), 18.2 (C-3b); FAB$^+$-MS *m*/*z*: 434 (M$^+$+1); HRFAB$^+$-MS *m*/*z*: Calcd for $C_{20}H_{25}ON_3BrS$ [434.0911], Found [434.0902].

### *N*-(2-Fluorophenyl)-2-(2-(4-isobutylphenyl)propanoyl) hydrazine-1-carbothioamide (**9**)

Yield: 68%; M.P.: 195–197 °C; $^{1}$H-NMR (400 MHz, DMSO-$d_6$): $\delta_H$ 10.12 (s, 1H, NH–CO), 9.73 (s, 1H, NH–CS), 9.22 (s, 1H, NH), 7.39 (s, 1H, H-3′), 7.26 (d, 3H, $J_{2,3/6,5/6',5'}$ = 7.6 Hz, H-2, H-6, H-6′), 7.18 (m, 2H, H-4′, H-5′), 7.08 (d, 2H, $J_{3,2/5,6}$ = 8.0 Hz, H-3, H-5), 3.64 (s, 1H, H-2b), 2.40 (d, 2H, $J_{1a,2a}$ = 7.2 Hz, H-1a), 1.78 (m, 1H, H-2a), 1.38 (d, 3H, $J_{3b,2b}$ = 6.8 Hz, H-3b), 0.84 (d, 6H, $J_{3a,2a/4a,2a}$ = 6.8 Hz, H-3a, H-4a); $^{13}$C-NMR (125 MHz, DMSO-$d_6$): *δc* 139.4 (C-4), 128.7 (C-2,6), 127.2 (C-3, -5), 126.9 (C-5′), 123.9 (C-4′), 115.7 (C-6′), 115.5 (C-3′), 44.2 (C-1a), 42.8 (C-2b), 29.6 (C-2a), 22.1 (C-3a, -4a), 18.3 (C-3b); FAB$^+$-MS *m*/*z*: 374 (M$^+$+1); HRFAB$^+$-MS *m*/*z*: Calcd for $C_{20}H_{25}ON_3FS$ [374.1708], Found [374.1702].

### *N*-(2,4-Difluorophenyl)-2-(2-(4-isobutylphenyl)propanoyl) hydrazine-1-carbothioamide (**10**)

Yield: 71%; M.P.: 230–232 °C; $^{1}$H-NMR (400 MHz, DMSO-$d_6$): $\delta_H$ 10.11 (s, 1H, NH–CO), 9.75 (s, 1H, NH–CS), 9.24 (s, 1H, NH), 7.34 (s, 1H, H-3′), 7.28 (dd, 1H, $J_{5',6'}$ = 9.8 Hz, $J_{5',3'}$ = 2.2 Hz, H-5′), 7.25 (d, 2H, $J_{2,3/6,5}$ = 7.6 Hz, H-2, H-6), 7.08 (ovp. m, 3H, H-3, H-5, H-6′), 3.62 (s, 1H, H-2b), 2.40 (d, 2H, $J_{1a,2a}$ = 7.2 Hz, H-1a), 1.78 (m, 1H, H-2a), 1.37 (d, 3H, $J_{3b,2b}$ = 7.2 Hz, H-3b), 0.84 (d, 6H, $J_{3a,2a/4a,2a}$ = 6.4 Hz, H-3a, H-4a); $^{13}$C-NMR (75 MHz, DMSO-$d_6$): *δc* 173.0, (C=S), 164.0 (C=O), 162.7 (C-2′), 160.4 (C-4′), 139.3 (C-1), 138.5 (C-4), 128.7 (C-2, -6), 127.2 (C-3, -5), 126.9 (C-6′), 125.4 (C-1′), 104.1 (C-5′), 103.7 (C-3′), 44.1 (C-1a), 42.8 (C-2b), 29.5 (C-2a), 22.1 (C-3a, -4a), 18.3 (C-3b); EI-MS *m*/*z* (% rel abund.): 391 (M$^+$, 1), 357 (42), 342 (10), 314 (13), 220 (21), 188 (23), 161 (100); HREI-MS *m*/*z*: Calcd for $C_{20}H_{23}ON_3F_2S$ [391.15453], Found [391.1530].

### 2-(2-(4-Isobutylphenyl)propanoyl)-*N*-(3-nitrophenyl) hydrazine-1-carbothioamide (**11**)

Yield: 74%; M.P.: 160–162 °C; $^{1}$H-NMR (400 MHz, DMSO-$d_6$): $\delta_H$ 10.12 (s, 1H, NH–CO), 9.89 (s, 1H, NH–CS), 9.81 (s, 1H, NH), 8.47 (s, 1H, H-2′), 7.99 (d, 1H, $J_{4',5'}$ = 6.4 Hz, H-4′), 7.94 (d, 1H, $J_{6',5'}$ = 7.2 Hz, H-6′), 7.62 (t, 1H, $J_{5',4'/5',6'}$ = 8.2 Hz, H-5′), 7.27 (d, 2H, $J_{2,3/6,5}$ = 8.0 Hz, H-2, H-6), 7.09 (d, 2H, $J_{3,2/5,6}$ = 8.0 Hz, H-3, H-5), 3.68 (d, 1H, $J_{2b,3b}$ = 6.4 Hz, H-2b), 2.40 (d, 2H, $J_{1a,2a}$ = 6.8 Hz, H-1a), 1.78 (m, 1H, H-2a), 1.39 (d, 3H, $J_{3b,2b}$ = 7.2 Hz, H-3b), 0.84 (d, 6H, $J_{3a,2a/4a,2a}$ = 6.8 Hz, H-3a, H-4a); $^{13}$C-NMR (125 MHz, DMSO-$d_6$): *δc* 178.0, (C=S), 164.0 (C=O), 145.7 (C-3′), 140.4 (C-4), 139.4 (C-1), 138.5 (C-1′), 129.2 (C-6′), 128.8 (C-2, -6), 127.2 (C-3, -5, -5′), 119.3 (C-4′, -2′), 44.1 (C-1a), 42.9 (C-2b), 29.6 (C-2a), 22.1 (C-3a, -4a), 18.3

(C-3b); FAB$^+$-MS *m/z*: 401 ($M^+$+1); HRFAB$^+$-MS *m/z*: Calcd for $C_{20}H_{25}O_3N_4S$ [401.1641], Found [401.1647].

#### 2-(2-(4-Isobutylphenyl)propanoyl)-*N*-(4-nitrophenyl) hydrazine-1-carbothioamide (12)

Yield: 85%; M.P.: 170–172 °C; $^1$H-NMR (400 MHz, DMSO-$d_6$): $\delta_H$ 10.31 (s, 1H, NH–CO), 10.02 (s, 1H, NH–CS), 9.78 (s, 1H, NH), 8.19 (d, 2H, $J_{3',2'/5',6'}$ = 9.2 Hz, H-3′, H-5′), 7.86 (d, 2H, $J_{2',3'/6',5'}$ = 9.2 Hz, H-2′, H-6′), 7.26 (d, 2H, $J_{2,3/6,5}$ = 8.0 Hz, H-2, H-6), 7.09 (d, 2H, $J_{3,2/5,6}$ = 8.0 Hz, H-3, H-5), 3.68 (s, 1H, H-2b), 2.40 (d, 2H, $J_{1a,2a}$ = 7.2 Hz, H-1a), 1.79 (m, 1H, H-2a), 1.39 (d, 3H, $J_{3b,2b}$ = 6.8 Hz, H-3b), 0.84 (d, 6H, $J_{3a,2a/4a,2a}$ = 6.8 Hz, H-3a, H-4a); FAB$^+$-MS *m/z*: 401 ($M^+$+1); $^{13}$C-NMR (75 MHz, DMSO-$d_6$): *δc* 180.7 (C=S), 173.0 (C=O), 145.7 (C-4′), 139.4 (C-1), 139.2 (C-4), 138.4 (C-1′), 128.7 (C-2, -6), 127.1 (C-3, -5, -2′, -6′), 124.7 (C-3′, -5′), 44.1 (C-1a), 42.7 (C-2b), 29.5 (C-2a), 22.1 (C-3a, -4a), 18.3 (C-3b); HRFAB$^+$-MS *m/z*: Calcd for $C_{20}H_{25}O_3N_4S$ [401.1646], Found [401.1647].

#### 2-(2-(4-Isobutylphenyl)propanoyl)-*N*-(*o*-tolyl) hydrazine-1-carbothioamide (13) [27]

Yield: 82%; M.P.: 195–197 °C; $^1$H-NMR (400 MHz, DMSO-$d_6$): $\delta_H$ 10.04 (s, 1H, NH–CO), 9.44 (s, 1H, NH–CS), 9.16 (s, 1H, NH), 7.26 (d, 2H, $J_{2,3/6,5}$ = 8.0 Hz, H-2, H-6), 7.19 (m, 4H, H-3′, H-4′, H-5′, H-6′), 7.07 (d, 2H, $J_{3,2/5,6}$ = 8.0 Hz, H-3, H-5), 3.64 (d, 1H, $J_{2b,3b}$ = 6.8 Hz, H-2b), 2.39 (d, 2H, $J_{1a,2a}$ = 6.8 Hz, H-1a), 2.11 (s, 3H, $CH_3$-2′), 1.77 (m, 1H, H-2a), 1.37 (d, 3H, $J_{3b,2b}$ = 6.8 Hz, H-3b), 0.84 (d, 6H, $J_{3a,2a/4a,2a}$ = 6.8 Hz, H-3a, H-4a); EI-MS *m/z* (% rel abund.): 369 ($M^+$, 1), 335 (30), 262 (5), 220 (26), 188 (25), 174 (72), 161 (100).

#### *N*-(2,6-Dimethylphenyl)-2-(2-(4-isobutylphenyl)propanoyl) hydrazine-1-carbothioamide (14)

Yield: 72%; M.P.: 180–182 °C; $^1$H-NMR (400 MHz, DMSO-$d_6$): $\delta_H$ 10.06 (s, 1H, NH–CO), 9.39 (s, 1H, NH–CS), 9.01 (s, 1H, NH), 7.26 (d, 2H, $J_{2,3/6,5}$ = 8.0 Hz, H-2, H-6), 7.04 (ovp. m, 5H, H-3, H-5, H-3′, H-4′, H-5′), 3.63 (q, 1H, H-2b), 2.39 (d, 2H, $J_{1a,2a}$ = 7.2 Hz, H-1a), 2.10 (s, 6H, $CH_3$-2′, $CH_3$-6′), 1.77 (m, 1H, H-2a), 1.38 (d, 3H, $J_{3b,2b}$ = 6.8 Hz, H-3b), 0.83 (d, 6H, $J_{3a,2a/4a,2a}$ = 6.8 Hz, H-3a, H-4a); $^{13}$C-NMR (75 MHz, DMSO-$d_6$): *δc* 181.4 (C=S), 173.1 (C=O), 139.3 (C-1), 138.7 (C-4), 136.8 (C-1′), 136.6 (C-2′), 136.4 (C-6′), 128.7 (C-2, -6), 127.4 (C-3′, -5′), 127.1 (C-3, -5), 126.7 (C-4′), 44.1 (C-1a), 42.8 (C-2b), 29.5 (C-2a), 22.1 (C-3a, -4a), 18.3 (C-3b), 17.8 (C-2′, -6′, $CH_3$); EI-MS *m/z* (% rel abund.): 383 ($M^+$, 2), 349 (49), 220 (9), 188 (100); HREI-MS *m/z*: Calcd for $C_{22}H_{29}ON_3S$ [383.2041], Found [383.2031].

#### 2-(2-(4-Isobutylphenyl)propanoyl)-*N*-(2-(trifluoromethyl) phenyl)hydrazine-1-carbothioamide (15)

Yield: 85%; M.P.: 185–187 °C; $^1$H-NMR (400 MHz, DMSO-$d_6$): $\delta_H$ 10.12 (s, 1H, NH–CO), 9.76 (s, 1H, NH–CS), 9.05 (s, 1H, NH), 7.67 (m, 2H, H-3′, H-4′), 7.47 (m, 2H, H-5′, H-6′), 7.26 (d, 2H, $J_{2,3/6,5}$ = 8.0 Hz, H-2, H-6), 7.07 (d, 2H, $J_{3,2/5,6}$ = 8.0 Hz, H-3, H-5), 3.64 (s, 1H, H-2b), 2.39 (d, 2H, $J_{1a,2a}$ = 6.8 Hz, H-1a), 1.77 (m, 1H, H-2a), 1.37 (d, 3H, $J_{3b,2b}$ = 6.8 Hz, H-3b), 0.83 (d, 6H, $J_{3a,2a/4a,2a}$ = 6.8 Hz, H-3a, H-4a); $^{13}$C-NMR (75 MHz, DMSO-$d_6$): *δc* 139.3 (C-1), 138.5 (C-4), 132.4 (C-1′), 132.1 (C-5′), 128.7 (C-2, -6), 127.1 (C-3, -5, 3′), 126.0 (C-4′, -6′), 125.9 (C-2′, C-$CF_3$), 44.1 (C-1a), 42.7 (C-2b), 29.5 (C-2a), 22.0 (C-3a, -4a), 18.2 (C-3b); FAB$^+$-MS *m/z*: 424 ($M^+$+1); HRFAB$^+$-MS *m/z*: Calcd for $C_{21}H_{25}ON_3F_3S$ [424.1671], Found [424.1670].

#### 2-(2-(4-Isobutylphenyl)propanoyl)-*N*-(3-(trifluoromethyl) phenyl)hydrazine-1-carbothioamide (16)

Yield: 59%; M.P.: 248–250 °C; $^1$H-NMR (400 MHz, DMSO-$d_6$): $\delta_H$ 10.08 (s, 1H, NH–CO), 9.79 (s, 1H, NH–CS), 9.65 (s, 1H, NH), 7.86 (s, 1H, H-2′), 7.77 (d, 1H, $J_{4',5'}$ = 8.4 Hz, H-4′), 7.55 (t, 1H, $J_{5',4'/5',6'}$ = 7.8 Hz, H-5′), 7.49 (m, 1H, H-6′), 7.27 (d, 2H, $J_{2,3/6,5}$ = 8.0 Hz, H-2, H-6), 7.09 (d, 2H, $J_{3,2/5,6}$ = 8.0 Hz, H-3, H-5), 3.67 (d, 1H, $J_{2b,3b}$ = 6.4 Hz, H-2b), 2.40 (d, 2H, $J_{1a,2a}$ = 7.2 Hz, H-1a), 1.78 (m, 1H, H-2a), 1.38 (d, 3H, $J_{3b,2b}$ = 6.8 Hz, H-3b), 0.84 (d, 6H, $J_{3a,2a/4a,2a}$ = 6.8 Hz, H-3a, H-4a); $^{13}$C-NMR (75 MHz, DMSO-$d_6$): *δc* 188.3 (C=S), 172.8 (C=O), 139.9 (C-1), 139.4 (C-4), 138.5 (C-1′), 129.2 (C-3′), 128.7 (C-2, -6, -2′, -5′), 127.2 (C-3, -5, -4′, -6′), 110.0 (C-$CF_3$), 44.1 (C-1a), 42.8 (C-2b), 29.5 (C-2a), 22.1 (C-3a, -4a), 18.3 (C-3b); EI-MS *m/z* (% rel abund.): 423 ($M^+$, 2), 389 (72), 374 (27), 346 (35), 262 (24), 220 (45), 203 (94), 188 (63), 161 (100); HREI-MS *m/z*: Calcd for $C_{21}H_{24}ON_3F_3S$ [423.1619], Found [423.1592].

#### 2-(2-(4-Isobutylphenyl)propanoyl)-*N*-(4-(trifluoromethyl) phenyl)hydrazine-1-carbothioamide (17)

Yield: 81%; M.P.: 170–172 °C; $^1$H-NMR (400 MHz, DMSO-$d_6$): $\delta_H$ 10.09 (s, 1H, NH–CO), 9.82 (s, 1H, NH–CS), 9.59 (s, 1H, NH), 7.71 (d, 4H, *J* = 16.8 Hz, H-2′, H-3′, H-5′, H-6′), 7.26 (d, 2H, $J_{2,3/6,5}$ = 7.6 Hz, H-2, H-6), 7.09 (d, 2H, $J_{3,2/5,6}$ = 8.0 Hz, H-3, H-5), 3.67 (s, 1H, H-2b), 2.40 (d, 2H, $J_{1a,2a}$ = 7.2 Hz, H-1a), 1.78 (m, 1H, H-2a), 1.38 (d, 3H, $J_{3b,2b}$ = 6.8 Hz, H-3b), 0.84 (d, 6H, $J_{3a,2a/4a,2a}$ = 6.8 Hz, H-3a, H-4a); $^{13}$C-NMR (125 MHz, DMSO-$d_6$): *δc* 142.9 (C-4), 139.4 (C-1, -4′), 138.5 (C-1′), 128.8 (C-2, -6, -2′, -6′), 127.2 (C-3, -5, -3′, -5′), 125.1 (C-$CF_3$), 44.1 (C-1a), 42.8 (C-2b), 29.6 (C-2a), 22.1 (C-3a, -4a), 18.3 (C-3b); FAB$^+$-MS *m/z*:

424 ($M^+$+1); HRFAB$^+$-MS *m/z*: Calcd for $C_{21}H_{25}ON_3F_3S$ [424.1677], Found [424.1670].

#### 2-(2-(4-Isobutylphenyl)propanoyl)-*N*-(2-methoxyphenyl) hydrazine-1-carbothioamide (18) [27]

Yield: 88%; M.P.: 110–112 °C; $^1$H-NMR (400 MHz, DMSO-$d_6$): $\delta_H$ 10.20 (s, 1H, NH–CO), 9.72 (s, 1H, NH–CS), 8.12 (s, 1H, NH), 7.27 (d, 2H, $J_{2,3/6,5}$ = 8.0 Hz, H-2, H-6), 7.10 (m, 4H, H-3, H-5, H-5′, H-6′), 7.02 (d, 1H, $J''_{34}$ = 8.0 Hz, H-3′), 6.91 (t, 1H, $J''_{45/4,3}$ = 7.8 Hz, H-4′), 3.73 (s, 3H, $OCH_3$), 3.69 (d, 1H, $J_{2b,3b}$ = 6.8 Hz, H-2b), 2.40 (d, 2H, $J_{1a,2a}$ = 6.8 Hz, H-1a), 1.78 (m, 1H, H-2a), 1.39 (d, 3H, $J_{3b,2b}$ = 6.8 Hz, H-3b), 0.83 (d, 6H, $J_{3a,2a/4a,2a}$ = 6.8 Hz, H-3a, H-4a); FAB$^+$-MS *m/z*: 386 ($M^+$+1).

#### *N*-(4-((4-(dimethylamino)phenyl)diazenyl) phenyl)-2-(2-(4-isobutylphenyl)propanoyl) hydrazine-1-carbothioamide (19) [28]

Yield: 90%; M.P.: 215–217 °C; $^1$H-NMR (400 MHz, DMSO-$d_6$): $\delta_H$ 10.10 (s, 1H, NH–CO), 9.70 (s, 1H, NH–CS), 7.74 (m, 4H, H-2′, H-3′, H-5′, H-6′), 7.64 (m, 2H, H-2″, H-6″), 7.27 (d, 2H, $J_{2,3/6,5}$ = 8.0 Hz, H-2, H-6), 7.09 (d, 2H, $J_{3,2/5,6}$ = 7.6 Hz, H-3, H-5), 6.83 (d, 2H, $J''''_{3,2/5,6}$ = 8.8 Hz, H-3″, H-5″), 3.69 (s, 1H, H-2b),3.04 (s, 6H, N-$(CH_3)_2$, 2.41 (d, 2H, $J_{1a,2a}$ = 6.8 Hz, H-1a), 1.79 (pent, 1H, H-2a), 1.39 (d, 3H, $J_{3b,2b}$ = 6.8 Hz, H-3b), 0.85 (d, 6H, $J_{3a,2a/4a,2a}$ = 6.4 Hz, H-3a, H-4a); ESI$^+$-MS *m/z*: 503 ($M^+$+1); HRESI$^+$-MS *m/z*: Calcd for $C_{28}H_{35}SON_6$ [503.2583], Found [503.2588].

### In vitro urease inhibitory assay

Urease inhibitory potentials of ibuprofen derivatives **1**–**19** were performed by reported method [29]. Reaction mixture comprising enzyme solution (25 µL) and buffer solution (55 µL) containing urea (100 mM) were incubated with test compounds (5 µL and 1 mM concentration) at 30 °C for 15 min in 96-well plate. By using the indophenols method, ammonia production was measured to determine the urease activity. Alkali reagent (70 µL; 0.5% w/v NaOH and 0.1% active chloride NaOCl) and phenol reagent (45 µL; 1% w/v phenol and 0.005% w/v sodium nitroprusside) were added to each well. After 50 min, the absorbance at 630 nm was measured using a microplate reader (Molecular Device, USA). All steps were performed thrice in a final volume of 200 µL. By using SoftMax Pro software (Molecular Device, USA), the results (change in absorbance per min) were calculated. Assays were performed at pH 8.2 (0.01 M $K_2HPO_4{\cdot}3H_2O$, 1 mM EDTA and 0.01 M LiCl). Thiourea was used as standard inhibitor of urease. From the following formula, percentage inhibitions were calculated:

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

### Molecular docking assay

Molecular docking studies were carried out to predict the molecular basis of the observed urease inhibition demonstrated by the newly synthesized ibuprofen hydrazide derivatives. In this connection, the crystal structure of the Jack Bean urease under the accession code 4GY7 was retrieved from RCSB-PDB [30]. The protein preparation module in MOE v2018.0101 was implemented to fill in the missing loops and atoms, assign bond orders, and for the treatment of formal charges using AMBER 10:EHT force field. The prepared structure was further used to establish the binding mode of the newly synthesized derivatives. The global search coordinates were generated using the centroids of the residues Kcx-490, His-492, His-519, His-545, Asp-633, Ni-901, and Ni-902. The primary placement method was Triangle Matcher, and the London dG was selected as placement scoring method. The Rigid Receptor protocol was used for refinement of the initially generated 30 poses. The top-ranked five poses scored by the GBVI/WSA dG methods were retrieved for subsequent analysis. The top-ranked binding pose of each compound was analyzed visually using the Protein–Ligand Interaction Profiler (PLIP) web server [31]. All the visuals were rendered using Chimera and MOE.

The compounds were sketched using the builder module in MOE. The molecules were charged and minimized at neutral pH using the MMFFF 94x force field [32].

A short production run of 500 ps was carried out in cases of compounds **3** and **7** to optimize the docking poses generated by MOE. The system was initially solvated using TIP3P water model under periodic boundary conditions. The cut-off for the non-bonded interactions was set as 8 Å. The system was initially minimized to remove the steric clashes followed by the gradual increase in the temperature up to 300 K. The pressure was set to 1 atm. The trajectories were saved 2 fs.

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## References

1. Hung KK, Graham CA, Lo RS, Leung YK, Leung LY, Man SY, Woo WK, Cattermole GN, Rainer TH (2018) Oral paracetamol and/or ibuprofen for treating pain after soft tissue injuries: single centre double-blind, randomised controlled clinical trial. PLoS ONE 13:e0192043. https://doi.org/10.1371/journal.pone.0192043.g002

2. Southey ER, Soares-Weiser K, Kleijnen J (2009) Systematic review and meta-analysis of the clinical safety and tolerability of ibuprofen compared with paracetamol in paediatric pain and fever. Curr Med Res Opin 25:2207–2222. https://doi.org/10.1185/03007990903116255
3. Gokoglu E, Yılmaz E, Gokoglu E, Baran AU (2016) Study of binding properties between two new ibuprofen and naproxen based acyl hydrazone derivatives and trypsin. J Fluoresc 26:113–119. https://doi.org/10.1007/s10895-015-1690-2
4. Mohamed SK, Albayati MR, Younes SH, Abed-Alkareem MG (2013) Functionalization of ibuprofen core structure compound: synthesis of new potential chemotherapeutic agents incorporating ibuprofen substructure. Chem Sci J 4:1–10. https://doi.org/10.0001/(aj).v3i0.718.g879
5. Sujith K, Kalluraya B, Adhikari A, Ravikumar J (2011) Microwave mediated synthesis of non-carboxylic analogues of ibuprofen with improved pharmacological activity. Chin Chem Lett 22:508–510. https://doi.org/10.1016/j.cclet.2010.12.013
6. Aranda J, Thomas R (2006) Systematic review: intravenous ibuprofen in preterm newborns. Semin Perinatol 30:114–120. https://doi.org/10.1053/j.semperi.2006.04.003
7. Rainsford KD (2009) Ibuprofen: pharmacology, efficacy and safety. Inflammopharmacology 17:275–342. https://doi.org/10.1007/s10787-009-0016-x
8. Liu Z, Wang X, Zhang H, Zhang S, Li Y, Liu Y, Peng D (2017) Synthesis and antiinflammatory effects of a series of novel 9-*O*-substituted berberine derivatives. Med Chem Res 26:672–679. https://doi.org/10.1007/s00044-017-1787-z
9. Neeraja P, Srinivas S, Mukkanti K, Dubey PK, Pal S (2016) 1*H*-1,2,3-Triazolyl-substituted 1,3,4-oxadiazole derivatives containing structural features of ibuprofen/naproxen: their synthesis and antibacterial evaluation. Bioorg Med Chem Lett 26:5212–5217. https://doi.org/10.1016/j.bmcl.2016.09.059
10. Xin G, Wang Y, Guo X, Huang B, Du D, He S, Chen Q (2013) Synthesis of diosgenin-ibuprofen derivatives and their activities against insulin dependent diabetes mellitus. Chem Pharm Bull 61:532–538. https://doi.org/10.1248/cpb.c12-01024
11. Morgado PI, Miguel SP, Correia IJ, Aguiar-Ricardo A (2017) Ibuprofen loaded PVA/chitosan membranes: a highly efficient strategy towards an improved skin wound healing. Carbohyd Polym 159:136–145. https://doi.org/10.1016/j.carbpol.2016.12.029
12. Sagdinc S, Pir H (2009) Spectroscopic and DFT studies of flurbiprofen as dimer and its Cu(II) and Hg(II) complexes. Spectrochim Acta A Mol Biomol Spectrosc 73:181–194. https://doi.org/10.1016/j.saa.2009.02.022
13. Krajewska B (2002) Ureases: roles, properties and catalysis. Wiadomości Chemiczne 56:223–253
14. Mobley H, Island MD, Hausinger RP (1995) Molecular biology of microbial ureases. Microbiol Rev 59:451–480
15. Sumner JB (1926) The isolation and crystallization of the enzyme urease preliminary paper. J Biol Chem 69:435–441
16. Belzer C, Kusters J, Kuipers E, Van Vliet A (2006) Urease induced calcium precipitation by *Helicobacter* species may initiate gallstone formation. Gut 55:1678–1679. https://doi.org/10.1136/gut.2006.098319
17. Griffith DP, Musher D, Itin C (1976) Urease. The primary cause of infection-induced urinary stones. Invest Urol 13:346
18. Mumtaz A, Arshad J, Saeed A, Nawaz MAH, Iqbal J (2018) Synthesis, characterization and urease inhibition studies of transition metal complexes of thioureas bearing ibuprofen moiety. J Chil Chem Soc 63:3934–3940
19. Abdullah MA, Abuo-Rahma GEDA, Abdelhafez ESM, Hassan HA, El-Baky RMA (2017) Design, synthesis, molecular docking, anti-Proteus mirabilis and urease inhibition of new fluoroquinolone carboxylic acid derivatives. Bioorg Chem 70:1–11. https://doi.org/10.1016/j.bioorg.2016.11.002
20. Benini S, Rypniewski WR, Wilson KS, Mangani S, Ciurli S (2004) Molecular details of urease inhibition by boric acid: insights into the catalytic mechanism. J Am Chem Soc 126:3714–3715. https://doi.org/10.1021/ja049618p
21. Vassiliou S, Grabowiecka A, Kosikowska P, Yiotakis A, Kafarski P, Berlicki Ł (2008) Design, synthesis, and evaluation of novel organophosphorus inhibitors of bacterial ureases. J Med Chem 51:5736–5744. https://doi.org/10.1021/jm800570q
22. Khan M, Alam A, Khan KM, Salar U, Chigurupati S, Wadood A, Ali F, Mohammad JI, Riaz M, Perveen S (2018) Flurbiprofen derivatives as novel $\alpha$-amylase inhibitors: Biology-oriented drug synthesis (BIODS), in vitro, and in silico evaluation. Bioorg Chem 81:157–167. https://doi.org/10.1016/j.bioorg.2018.07.038
23. Mohiuddin G, Khan KM, Salar U, Lodhi MA, Wadood A, Riaz M, Perveen S (2019) Biology-oriented drug synthesis (BIODS), in vitro urease inhibitory activity, and in silico study of *S*-naproxen derivatives. Bioorg Chem 83:29–46. https://doi.org/10.1016/j.ejmech.2016.11.031
24. Salar U, Khan KM, Taha M, Ismail NH, Ali B, Perveen S, Ghufran M, Wadood A (2017) Biology-oriented drug synthesis (BIODS): *in vitro* $\beta$-glucuronidase inhibitory and in silico studies on 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl) ethyl aryl carboxylate derivatives. Eur J Med Chem 125:1289–1299. https://doi.org/10.1016/j.ejmech.2016.11.031
25. Taha M, Imran S, Ismail NH, Selvaraj M, Rahim F, Chigurupati S, Ullah H, Khan F, Salar U, Javid MT (2017) Biology-oriented drug synthesis (BIODS) of 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl) ethyl aryl ether derivatives, in vitro $\alpha$-amylase inhibitory activity and in silico studies. Bioorg Chem 74:1–9. https://doi.org/10.1016/j.bioorg.2017.07.001
26. Ullah S, Saeed M, Halimi SMA, Fakhri MI, Khan KM, Khan I, Perveen S (2016) Piroxicam sulfonates biology-oriented drug synthesis (BIODS), characterization and anti-nociceptive screening. Med Chem Res 25:1468–1475. https://doi.org/10.1007/s00044-016-1571-5
27. Amir M, Kumar S (2007) Synthesis and evaluation of anti-inflammatory, analgesic, ulcerogenic and lipid peroxidation properties of ibuprofen derivatives. Acta Pharm 57:31–45. https://doi.org/10.2478/v10007-007-0003-y
28. Manjunatha K, Poojary B, Lobo PL, Fernandes J, Kumari NS (2010) Synthesis and biological evaluation of some 1,3,4-oxadiazole derivatives. Eur J Med Chem 45:5225–5233. https://doi.org/10.1016/j.ejmech.2010.08.039
29. Arshad T, Khan KM, Rasool N, Salar U, Hussain S, Asghar H, Ashraf M, Wadood A, Riaz M, Perveen S, Taha M (2017) 5-Bromo-2-aryl benzimidazole derivatives as non-cytotoxic potential dual inhibitors of $\alpha$-glucosidase and urease enzymes. Bioorg Chem 72:21–31. https://doi.org/10.1016/j.bioorg.2017.03.007
30. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyaloy IN, Bourne PE (2000) The protein data bank. Nucleic Acids Res 28:235–242
31. Salentin S, Schreiber S, Haupt VJ, Adasme MF, Schroeder M (2015) PLIP: fully automated protein-ligand interaction profiler. Nucleic Acids Res 43:W443–W447. https://doi.org/10.1093/nar/gkv315
32. Halgren TA (1996) Merck molecular force field. I. Basis, form, scope, parameterization, and performance of MMFF94. J Comput Chem 17:490–519. https://doi.org/10.1002/(SICI)1096-987X(199604)17:5/6%3C490:AID-JCC1%3E3.0.CO;2-P