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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH



# Synthesis, in vitro urease inhibitory activity, and molecular docking studies of (perfluorophenyl)hydrazone derivatives

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## Abstract

A series of (perfluorophenyl)hydrazone derivatives **1**–**27** were synthesized by the condensation reaction of (perfluorophenyl) hydrazine with a variety of benzaldehydes. Compounds were structurally characterized by various spectroscopic techniques. All compounds were screened for their urease inhibitory activity which revealed that most of the analogs exhibited significant urease inhibitory activity in the range of $IC_{50} = 14.09 \pm 0.23$–$78.69 \pm 1.56$ μM as compare to the standard thiourea ($IC_{50} = 21.10 \pm 0.31$ μM). Amongst active compounds, derivatives **2** ($IC_{50} = 14.23 \pm 0.21$ μM), **5** ($IC_{50} = 16.78 \pm 0.33$ μM), **7** ($IC_{50} = 15.59 \pm 0.60$ μM), **9** ($IC_{50} = 20.18 \pm 0.78$ μM), **10** ($IC_{50} = 16.13 \pm 0.93$ μM), and **11** ($IC_{50} = 14.09 \pm 0.23$ μM) showed potent inhibitory activity better than the standard thiourea. A limited structure-activity relationship (SAR) was established by rationalized the effect of different groups on the inhibitory potential. Molecular docking study was performed to understand the binding modes of active analogs into the active site of urease enzyme.

**Keywords** Synthesis · (Perfluorophenyl)hydrazones · Schiff bases · Urease inhibition · Structure-activity relationship · Molecular docking

## Introduction

Urease (EC 3.5.1.5.) is a nickel containing metalloenzyme, belongs to the superfamily of amidohydrolases, and phosphotriestreases. It catalyzes the hydrolysis of urea to ammonia and carbamate. Than carbamate molecule suddenly hydrolyzes to form carbonic acid and additional molecule of ammonia (Rashid et al. 2013), (Holm and Sander 1997). Jack bean (*Canavalia ensiformis*) urease enzyme is well-studied and also crystallized (Sujoy and Aparna 2012; Taha et al. 2015). It is widely distributed in a variety of bacteria, fungi, plants, and play an important role in the circulation of nitrogen in nature. The inhibition of urease activity has been extensively studied due to its potential role in disease conditions, such as *Helicobacter pylori*-induced peptic ulcer, urinary lithiasis, pyelonephritis, hepatic coma, and in other infections caused by *Proteus mirabilis* and *Yersinia enterocolitica*. Urease supports the colony formation of *Helicobacter pylori* by increasing the pH of the stomach and therefore plays an important role in the pathogenesis of gastritis and peptic ulcers, as well as cancer (Qin and Cabral 1994; Pope et al. 1998; Rodman 1998). Urease inhibitors are also mixed with fertilizers for controlling the rapid urea degradation by soil bacteria (Sahrawat 1980; Choudhary et al. 2011). Identification of new urease inhibitors is an important and appealing area of research for the medicinal chemist.

Schiff bases, an important class of compounds having azomethine (C=N) functional group also known as imine (Patai 1970). Basically, Schiff bases are formed by the condensation reaction of carbonyl compounds (aldehyde or ketone) with amine to form imine (Da Silva et al. 2011).

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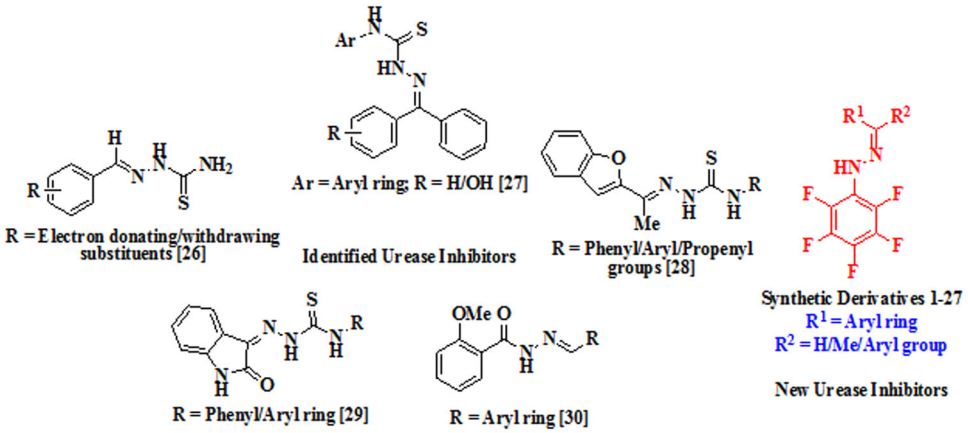



**Fig. 1** Rationale of the current study

Schiff bases have broad spectrum of biological activities such as antifungal, antibacterial, antimalarial, and antiviral properties (Bringmann et al. 2004; Salimon et al. 2010; Hameed et al. 2017). Imine or azomethine groups are present in different natural, natural-derived, and non-natural compounds. The presence of reactive imine group in such types of compounds has been shown to have critical importance to their biological activities (Prakash and Adhikari 2011). Beside a diverse range of biological potential, Schiff bases are reported as significant urease inhibitors (Aslam et al. 2011). Our group has also reported a range of hydrazones bearing aryl and heteroaryl rings as significant urease inhibitors (Fig. 1) (Arshia et al. 2016; Hameed et al. 2015; Pervez et al. 2008). So, in continuation of identifying new inhibitors for urease enzyme, we intended to synthesized a variety of (perfluorophenyl) hydrazone derivatives to evaluate their potential for urease inhibition.

## Results and discussion

### Chemistry

(Perfluorophenyl)hydrazone derivatives **1**–**27** were prepared by reacting perfluorophenylhydrazine with different arylaldehydes/acetophenones/arylketones in ethanol in the presence 2–3 drops of $CH_3COOH$ as catalyst. The reaction was refluxed for 25–45 min to afford desired compounds in good yields (85–92%) (Table 1). The consumption of starting material was monitored through periodic TLC analysis. On completion, the reaction mixture was cooled to room temperature to get precipitate which were filtered and dried in vacuum. The precipitates were crystallized from ethanol. Structure identification was accomplished by using EI-MS and $^1$HNMR spectroscopic techniques (Scheme 1).

### In vitro urease inhibitory activity

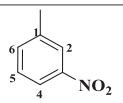
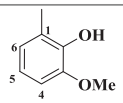
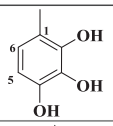
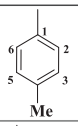
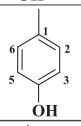
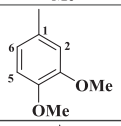
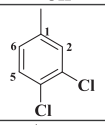
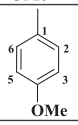
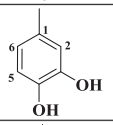
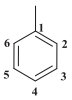
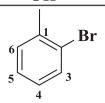
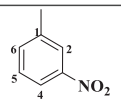
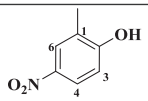
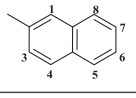
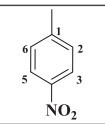
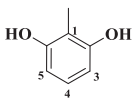
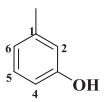
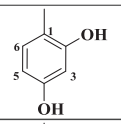
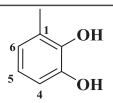
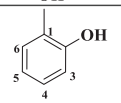
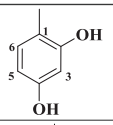
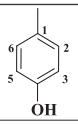
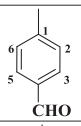
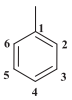
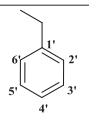
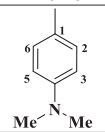
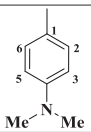
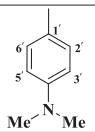
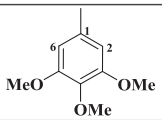
All synthetic (perfluorophenyl)hydrazone derivatives **1**–**27** were subjected for urease inhibitory activity by following the literature protocol (Weatherburn 1967). Except compounds **12**, **21**, **26**, and **27**, all derivatives showed good to moderate urease inhibitory potential in the range of $IC_{50} = 14.09 \pm 0.23$–$78.69 \pm 1.56$ µM as compared to standard thiourea ($IC_{50} = 21.10 \pm 0.31$ µM). Compounds **2** ($IC_{50} = 14.23 \pm 0.21$ µM), **5** ($IC_{50} = 16.78 \pm 0.33$ µM), **7** ($IC_{50} = 15.59 \pm 0.60$ µM), **9** ($IC_{50} = 20.18 \pm 0.78$ µM), **10** ($IC_{50} = 16.13 \pm 0.93$ µM), and **11** ($IC_{50} = 14.09 \pm 0.23$ µM) were found to be more potent than the standard thiourea (Table 2).

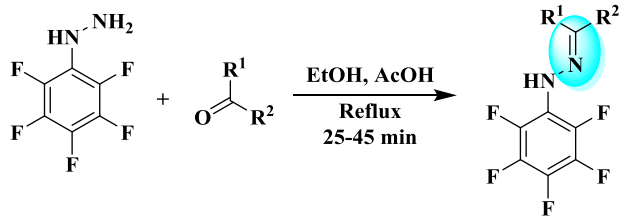
### Structure-activity relationship (SAR)

Limited SAR was established by analyzing the effect of varying groups $R_1$ and $R_2$ on the inhibitory potential. Bioactivity data presented in Table 2 suggested that most of the active analogs have hydroxy group/groups in the structure.

Amongst the mono-hydroxy substituted analogs, compound **3** ($IC_{50} = 23.54 \pm 0.24$ µM) and **9** ($IC_{50} = 20.18 \pm 0.78$ µM) having *para* and *meta* hydroxy substituted benzene ring ($R^1$) and hydrogen atom as $R^2$, showed comparable activity than standard ($IC_{50} = 21.10 \pm 0.31$ µM). *Ortho* hydroxy substituted analog **24** ($IC_{50} = 36.24 \pm 0.44$ µM) and *para* hydroxy substituted analog **25** ($IC_{50} = 33.88 \pm 0.82$ µM) with an extra methyl group ($R^2$) showed moderate activities. The additional non-polar methyl group might be creating some hindrance for the compounds to bind with the active site of urease enzyme. Dihydroxy analogs such as **5** ($IC_{50} = 16.78 \pm 0.33$ µM), **10** ($IC_{50} = 16.13 \pm 0.93$ µM), and **11** ($IC_{50} = 14.09 \pm 0.23$ µM), having hydroxy groups at different positions of benzene ring, were found to be the potent analogs. However, one dihydroxy substituted compound **22**

**Table 1** $R^1$ and $R^2$ substitutions of synthetic (perfluorophenyl)hydrazone derivatives **1–27**

| Compounds | $R^1$ | $R^2$ | Compounds | $R^1$ | $R^2$ |
|---|---|---|---|---|---|
| **1** |  | H | **15** |  | H |
| **2** |  | H | **16** |  | H |
| **3** |  | H | **17** |  | H |
| **4** |  | H | **18** |  | H |
| **5** |  | H | **19** |  | Me |
| **6** |  | H | **20** |  | Me |
| **7** |  | H | **21** |  | Me |
| **8** |  | H | **22** |  | Me |
| **9** |  | H | **23** |  | Me |
| **10** |  | H | **24** |  | Me |
| **11** |  | H | **25** |  | Me |
| **12** |  | H | **26** |  |  |
| **13** |  | H | **27** |  |  |
| **14** |  | H | - | - | - |




**Scheme 1** Synthesis of (perfluorophenyl)hydrazone derivatives **1–27**

**Table 2** In vitro urease inhibitory activity of compounds **1–27**

| Compounds | $IC_{50}$ + SEM (μM) | Compounds | $IC_{50}$ + SEM (μM) |
|---|---|---|---|
| **1** | 25.93 ± 0.96 | **15** | 27.19 ± 0.57 |
| **2** | 14.23 ± 0.21 | **16** | 35.61 ± 1.04 |
| **3** | 23.54 ± 0.24 | **17** | 49.35 ± 0.56 |
| **4** | 39.43 ± 0.11 | **18** | 42.56 ± 0.35 |
| **5** | 16.78 ± 0.33 | **19** | 40.76 ± 0.92 |
| **6** | 30.75 ± 0.76 | **20** | 33.36 ± 0.27 |
| **7** | 15.59 ± 0.60 | **21** | NA |
| **8** | 23.32 ± 0.15 | **22** | 29.30 ± 0.35 |
| **9** | 20.18 ± 0.78 | **23** | 32.37 ± 0.41 |
| **10** | 16.13 ± 0.93 | **24** | 36.24 ± 0.44 |
| **11** | 14.09 ± 0.23 | **25** | 33.88 ± 0.82 |
| **12** | NA[b] | **26** | NA |
| **13** | 78.69 ± 1.56 | **27** | NA |
| **14** | 56.67 ± 0.86 | Std = Thiourea | 21.10 ± 0.310 |

*SEM* standard error of the mean, *NA* not active, *Std* standard inhibitor for urease enzyme

($IC_{50} = 29.30 \pm 0.35$ μM) was exceptionally showed less potential might be due to not fulfilling the conformational requirement to fit well into the active site of enzyme. Another dihydroxy substituted compound **23** ($IC_{50} = 32.37 \pm 0.41$ μM) with distinctively similar structure to the most active analog **1** ($IC_{50} = 14.09 \pm 0.23$ μM) of this series **1**, but having additional methyl group ($R^2$), was found to be moderately active. Trihydroxy substituted analog **2** ($IC_{50} = 14.23 \pm 0.21$ μM) was found to be second most active of this series (Fig. 2). It was observed that compounds bearing more hydroxy groups found to be more active might be due to increased number of interactions by the hydroxy group with the active site of enzyme.

*Meta* and *para* nitro substituted analogs **1** ($IC_{50} = 25.93 \pm 0.96$ μM) and **8** ($IC_{50} = 23.32 \pm 0.15$ μM), respectively, showed inhibitory potential comparable to standard. However, incorporation of hydroxy group with nitro as in compound **7** ($IC_{50} = 15.59 \pm 0.60$ μM), leads to potent inhibitory activity. Similarly, incorporation of non-polar methyl group ($R^2$) as in compound **20** ($IC_{50} = 33.36 \pm 0.27$ μM) brings out the decreased activity (Fig. 3).

It is rationalized from the limited SAR that compounds with hydroxy substitutions were found to be more active. More the number of hydroxy groups more the inhibitory potential. After hydroxy, nitro group is also playing an important role in the activity. In addition to that activity results presented in the Table 2 also suggested that compounds with methoxy, *N*,*N*-dimethyl, and halogens substitutions were found to be moderately active. Since, in order to get a clear picture of the participation of different groups in the binding interaction with the active site of enzyme, molecular docking study was conducted.

## Molecular docking studies

Top ten compounds with high inhibition were selected for molecular docking studies with the enzyme urease (3LA4) through MOE software. It was observed that all the compounds represented excellent binding affinity within the active site of enzyme as compared to the standard thiourea (Table 3). From these, compounds **2**, **5**, **10**, and **11** exhibited outstanding binding affinity with Gibb's free binding energy ($\Delta G$) value of −8.72, −7.53, −7.63, and −7.22 kcal $mol^{-1}$ with inhibition constant (*Ki*) rate of 0.73, 5.00, 4.25, and 8.26 μM, respectively, as compared to thiourea with Gibb's free energy value of only −0.89 kcal $mol^{-1}$ as shown in Table 3. Thus, molecular docking studies substantiated that high ranked compounds in urease inhibition test also exhibited high affinity for active site of enzyme.

High ranked compounds in molecular docking studies as well as in in vitro testing (i.e., compounds **2**, **5**, **10**, and **11**) demonstrated metal interaction with Ni841 and Ni842 through OH groups, conventional hydrogen bonds with Ala440, KCX490, Asp633, and CME592 also due to OH groups and $\pi$-$\pi$ stacking with His593 through perfluorophenyl ring (Fig. 1). It was observed that the top most compound in docking studies, i.e., compound **2** interacts with Ni841 and Ni842 through OH groups at position 3 and 4 simultaneously in a bidentate manner each. It also exhibits conventional hydrogen bonds with Ala440, KCX490, His492 and Asp633 and $\pi$-$\pi$ stacking with His593 as shown in Fig. 4.

Binding poses of compound **2** (pink) and compound **11** (yellow) with the active site of enzyme Jack bean urease (3LA4) were compared with the binding pose of thiourea (cyan) in Fig. 5a. Hydrophobic surface and active site cavity of the enzyme with the docking pose of compound **2** (green) was also represented in Fig. 5b.

## Physicochemical properties and pharmacokinetics prediction

Early prediction of physicochemical and ADMET (Absorption, Distributation, Metabolism, Excretion, and Toxicity) properties of lead molecules has become very useful in the drug discovery and development process later

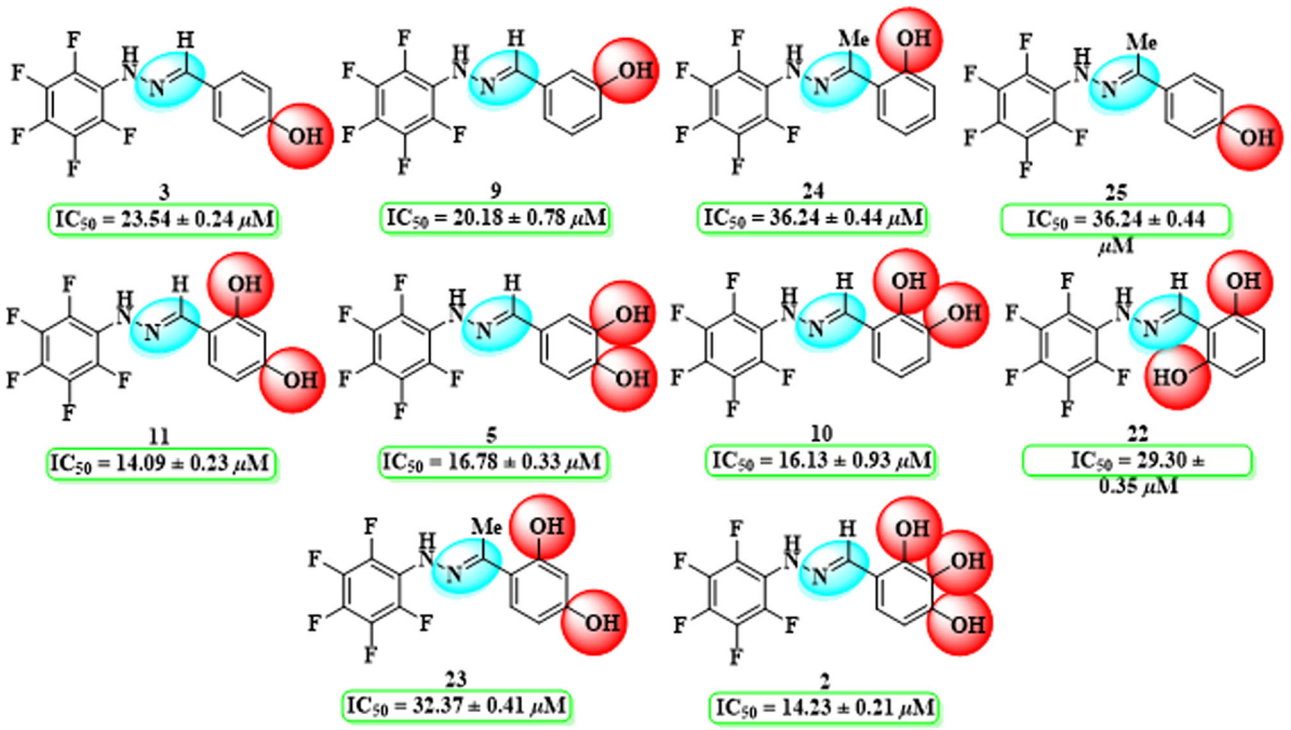



**Fig. 2** SAR of compounds bearing hydroxy substitutions

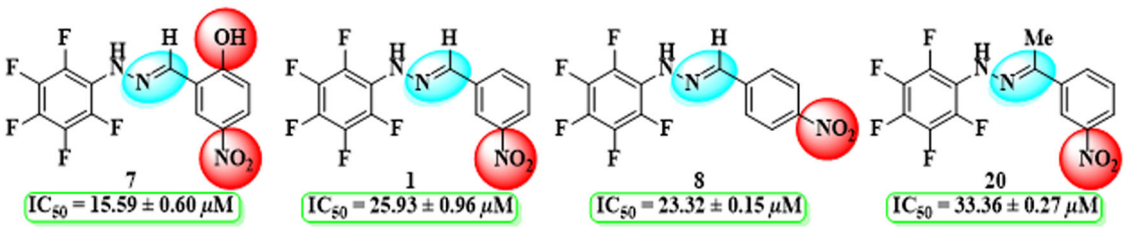



**Fig. 3** SAR of compounds bearing nitro substitutions

**Table 3** Gibb's free binding energy (Δ*G*) and inhibition constant (*ki*) values of top ten compounds studied through MOE

| Compounds | MOE[a] | *Ki* (μM) | Compounds | MOE[a] | *Ki* (μM) |
|---|---|---|---|---|---|
| **1** | −4.99 | 316.23 | **8** | −5.10 | 256.8 |
| **2** | −8.72 | 0.73 | **9** | −5.95 | 64.74 |
| **3** | −6.31 | 36.12 | **10** | −7.63 | 4.25 |
| **5** | −7.53 | 5.00 | **11** | −7.22 | 8.26 |
| **7** | −6.65 | 20.80 | **15** | −3.81 | 2078.55 |
| **Thiourea** | −0.89 | 236287.03 | | | |

*Note*: [a]Gibb's free binding energy values (Δ*G*) in kcal $mol^{-1}$

on. Lipinsiki's rule of five (RO5), molar refractivity (MR), polar surface area (PSA), octanol water partition coefficient (logP) were determined through Mcule and PreADMET online softwares. It was observed that the entire selected compounds followed RO5 with no violation. Generally, drugs with log*P* value below zero are injectable, 0–3 are orally administered, 3–4 are transdermal, and high value produces toxic build up in fatty tissue. According to these criteria only compound **2** has almost oral absorption, others have transdermal absorption except compounds **1** and **8** that represented undesirable bioaccumulation in fatty tissue, highlighted in Table 4. Another extension in RO5 to improve the prediction of drug-likeness is MR which should be 40–130. PSA values are important for the determination of blood brain barrier (BBB) penetration. According to Waterbeemd the cutoff value is 90 $Å^2$ or less. Except compound **7**, all the compounds showed BBB penetration.

To determine the toxicity of these compounds ames mutagenecity test and oral rat $LD_{50}$ values were estimated for the selected compounds by using TEST (Toxicity Estimation Software Tool) software. With ames mutagenicity test, compounds **3**, **7**, and **9** were found positive, highlighted in red (Table 4). According to Hodge and Sterner scale oral rat $LD_{50}$ values ranging from 1–50 mg $kg^{-1}$ are

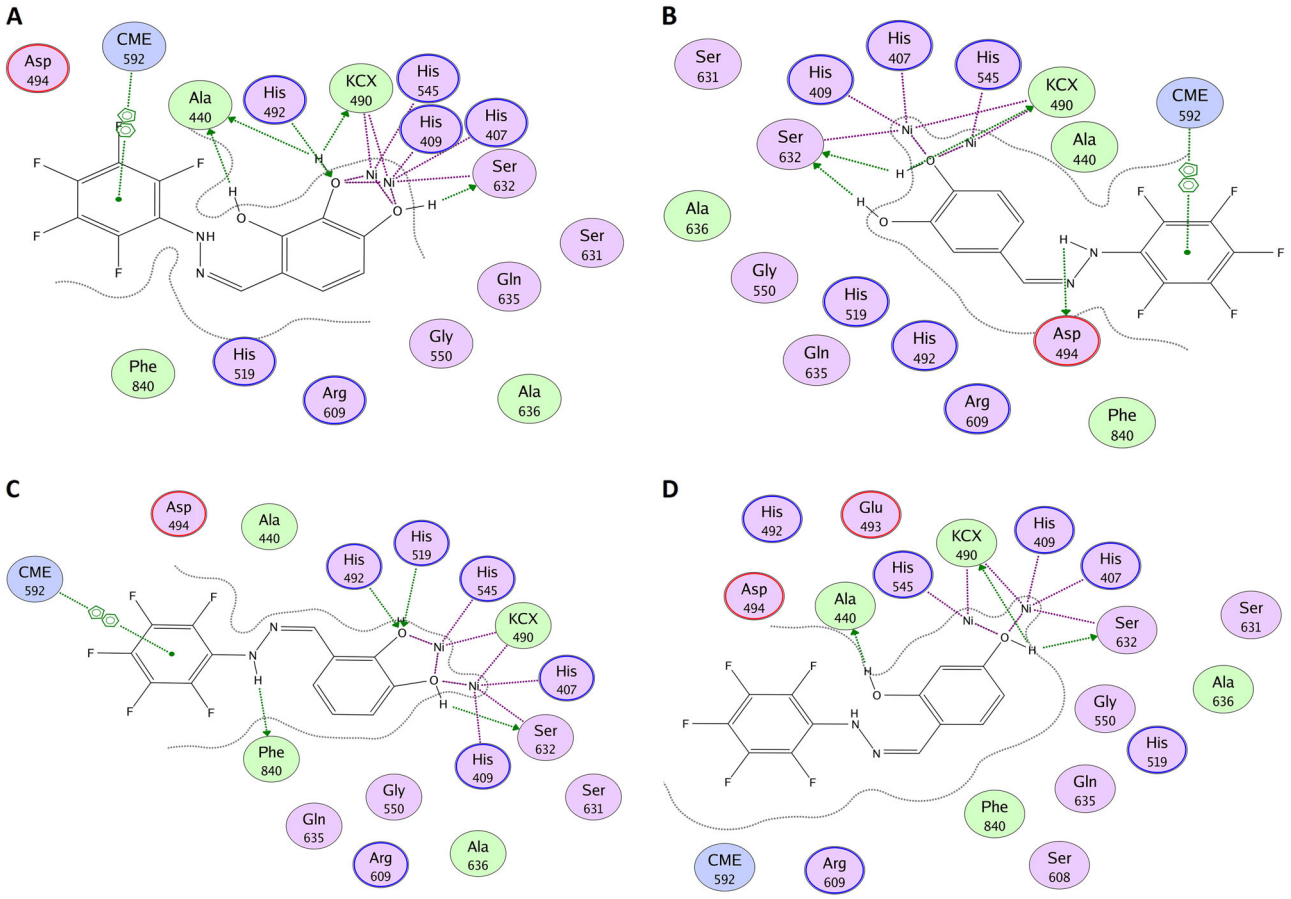



**Fig. 4** 2D Binding pose representation of compounds **2**, **5**, **10**, and **11** with the active site of enzyme Jack bean urease (3LA4)

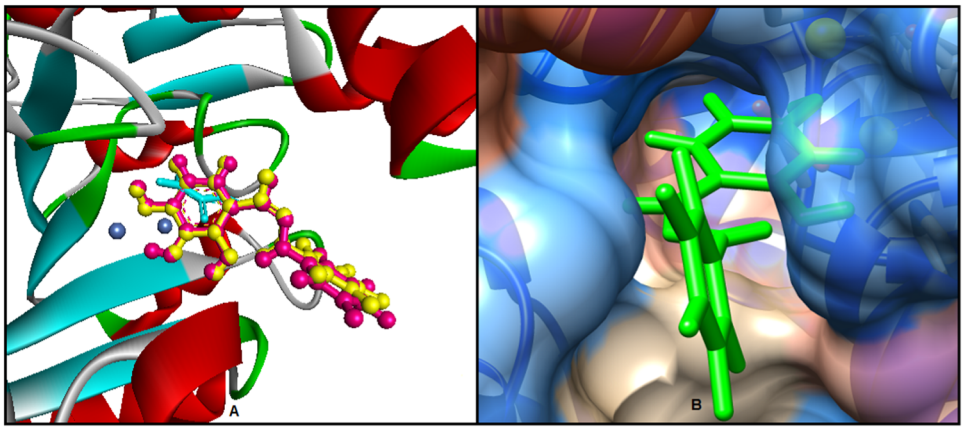



**Fig. 5** **a** 3D binding pose representation of compound **2** (pink), compound **11** (yellow) and thiourea (cyan) with the active site of enzyme Jack bean urease (3LA4) with two nickel atoms in gray color. **b** Hydrophobic surface and the active site cavity of the enzyme with two yellow balls representing nickel atom docked with compound **2** in green color

highly toxic, 50–500 mg $kg^{-1}$ are moderately toxic and 500–5000 mg $kg^{-1}$ are slightly toxic. Referring to this scale most of the compounds were slightly toxic except compounds **1**, **5**, and **7** (highlighted in red) which showed moderate toxicity as shown in Table 4. Keeping all the properties in view, compounds **2**, **10**, **11**, and **15** were found to display excellent physicochemical properties that execute the criteria of drug-likeness as shown in Table 4.

Combining in vitro and in silico studies, we emphasized that compound **2** (having 2,3,4-trihydroxy benzene ring as $R^1$) and compound **11** (having 2,5-dihydroxy benzene as $R^1$) were found to be most efficient and potent inhibitors of urease enzyme with excellent physicochemical and drug-like properties. Compound **2** has molecular weight of 334 g $mol^{-1}$, $IC_{50}$ value of 14.23 µM, $\Delta G$ value of −8.72 Kcal $mol^{-1}$, *Ki* rate of 0.73 µM, log*P* value of

**Table 4** Physicochemical properties and pharmacokinetics prediction of top ten compounds

| Compounds | MW | RO5[a] | MR[b] | Mut[c] | logP[d] | | PSA[e] | | $LD_{50}$[f] | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Value | Bio-availability | Value | BBB | Value | Toxicity |
| **1** | 331 | 0 | 72.5 | −ve | 4.2 | Accumulate | 70.2 | +ve | 174.8 | Moderate |
| **2** | 334 | 0 | 69.7 | −ve | 3.0 | Oral Abs. | 85.0 | +ve | 698.6 | Slight |
| **3** | 302 | 0 | 65.7 | +ve | 3.6 | Transdermal | 44.6 | +ve | 515.8 | Slight |
| **5** | 318 | 0 | 67.7 | −ve | 3.3 | Transdermal | 64.8 | +ve | 367.4 | Moderate |
| **7** | 347 | 0 | 74.5 | +ve | 4.0 | Transdermal | 90.4 | −ve | 304.0 | Moderate |
| **8** | 331 | 0 | 72.5 | −ve | 4.3 | Accumulate | 70.2 | +ve | 826.1 | Slight |
| **9** | 302 | 0 | 65.7 | +ve | 3.6 | Transdermal | 44.6 | +ve | 560.0 | Slight |
| **10** | 318 | 0 | 67.7 | −ve | 3.3 | Transdermal | 64.8 | +ve | 920.0 | Slight |
| **11** | 318 | 0 | 67.7 | −ve | 3.3 | Transdermal | 64.7 | +ve | 1131.2 | Slight |
| **15** | 316 | 0 | 70.2 | −ve | 3.9 | Transdermal | 33.6 | +ve | 727.3 | Slight |

[a]Lipinski's rule of five (RO5) violation

[b]Molar refractivity

[c]Ames Mutagenecity test

[d]Ghose-Crippen octanol water coefficient

[e]Polar surface area

[f]Oral rat LD50 (mg $kg^{-1}$)

3.0, $LD_{50}$ value of 698.6 mg $kg^{-1}$ and non-mutagenic. Likewise compound **11** has also exhibited almost similar properties which signify the consequence of these compounds in pre-clinical and clinical trials.

## Experimental

### Materials and methods

NMR experiments were performed on Bruker Avance AM 300 and 400 MHz machine. Electron impact mass spectra (EIMS) were recorded on a Finnigan MAT-311A, Germany. CHN analyses were carried out a Carlo Erba Strumentazione-Mod-1106, Italy. Thin layer chromatography (TLC) was performed on pre-coated silica gel aluminum plates (Kieselgel 60, 254, E. Merck, Germany). Chromatograms were visualized by UV at 254 and 365 nm. 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH), ethylene diaminetetraacetic acid (EDTA), ascorbic acid, *tris*-HCl buffer, ferrous sulfate, ferric chloride ($FeCl_3$), sulfuric acid, *O*-phenenthroline, potassium phosphate (mono phosphate and diphosphate), ammonium molybdate, hydrogen peroxide ($H_2O_2$), and ethanol were of analytical grade and purchased from Sigma Aldrich, USA.

### General procedure for the synthesis of compounds 1–27

(Perfluorophenyl)hydrazone derivatives **1**–**27** were prepared by reacting perfluorophenylhydrazine (1 mmol) with different substituted aldehydes and acetophenones (1 mmol) in absolute ethanol (15 mL) with 2–3 drops of $CH_3COOH$ under reflex up to 25–45 min. Reaction completion was monitored through TLC analysis and the transparent colored reaction mixture was kept at room temperature for cooling and dried up to afford crude products **1**–**27**. Compounds were crystallized in ethyl acetate. Reaction yield is in the range of 85–92%. For structure conformations, the synthesized compounds were inspected using EI-MS and $^1$HNMR analysis.

#### 1-(3-Nitrobenzylidene)-2-(perfluorophenyl)hydrazine (1)

Yield: 0.30 g (90%); $^1$H-NMR: (300 MHz, DMSO-$d_6$): $\delta$ 10.66 (s, 1H, –NH), 8.40 (s, 1H, –H-2), 8.20 (s, 1H, *Vin*. H), 8.16 (d, 1H, $J_{4,5} = 8.5$ Hz, H-4), 8.02 (d, 1H, $J_{6,5} = 8.5$ Hz, H-6), 7.70 (t, 1H, $J_{5,(4,6)} = 8.5$ Hz, H-5); EI-MS: *m/z* (rel. abund. %) 331 ($M^+$, 75), 182 (100), 155 (40), 76 (38); Anal. Calcd for $C_{13}H_6F_5N_3O_2$ (331.20): C, 47.14; H, 1.83; N, 12.69; Found: C, 47.12; H, 1.82; N, 12.70.

#### 4-((2-(Perfluorophenyl) hydrazono) methyl)benzene-1,2,3-triol (2)

Yield: 0.26 g (78%); $^1$H-NMR: (300 MHz, DMSO-$d_6$): $\delta$ 10.49 (s, 1H, –NH), 10.22 (s, 1H, –OH), 9.34 (br.s, 1H, –OH), 8.44 (br.s, 1H, -OH), 8.20 (s, 1H, *Vin*. H), 6.67 (d, 1H, $J_{6,5} = 8.5$ Hz, H-6), 6.35 (d, 1H, $J_{5,6} = 8.5$ Hz, H-5); EI-MS: *m/z* (rel. abund. %) 334 ($M^+$, 100), 183 (70), 124 (93), 7946); Anal.Calcd for $C_{13}H_7F_5N_2O_3$ (334.2): C, 46.72; H, 2.11; N, 8.38; Found: C, 46.71; H, 2.13; N, 8.35.

#### 4-((2-(Perfluorophenyl) hydrazono) methyl) phenol (3)

Yield: 0.27 g (84%); $^1$H-NMR: (300 MHz, DMSO-$d_6$): $\delta$ 10.00 (s, 1H, -NH), 9.79 (br.s, 1H, –OH), 8.01 (s, 1H, *Vin*. H), 7.40 (d, 2H, $J_{2,3/6,5} = 8.5$ Hz, H-2/6), 6.78 (d, 2H, $J_{3,2/5,6} = 8.5$ Hz, H-3/5); EI-MS: *m/z* (rel. abund. %) 302 ($M^+$, 100), 182 (40), 118 (73), 76 (66); Anal. Calcd for $C_{13}H_7F_5N_2O$ (302.19): C, 51.67; H, 2.33; N, 9.27; Found: C, 51.66; H, 2.30; N, 9.29.

#### 1-(3,4-Dichlorobenzylidene)-2-(perfluorophenyl)hydrazine (4)

Yield: 0.33 g (95%) ;$^1$H-NMR: (300 MHz, DMSO-$d_6$): $\delta$ 10.75 (s, 1H, –NH), 8.44 (s, 1H, *Vin*. H), 7.85 (d, 1H, $J_{5,6} = 8.7$ Hz, H-5), 7.66 (d, 1H, $J_{2,6} = 2.0$ Hz, H-2), 7.45 (dd, 1H, $J_{6,2} = 2.0$,$J_{6,5} = 8.7$ Hz, H-6); EI-MS: *m/z* (rel. abund. %) 356 (M+2, 50), 354 ($M^+$, 72), 319 (37), 183 (100), 172 (36), 155 (37); Anal. Calcd for $C_{15}H_{11}F_5N_2O_2$ (346.25): C, 52.03; H, 3.20; N, 8.09; Found: C, 52.01; H, 3.18; N, 8.06.

#### 4-((2-(Perfluorophenyl)hydrazono)methyl) benzene-1,2-diol (5)

Yield: 0.26 g (82%); $^1$H-NMR: (300 MHz, DMSO-$d_6$): $\delta$ 9.90 (br.s, 2H, –OH), 9.67 (s, 1H, –NH), 7.94 (s, 1H, *Vin*. H), 7.09 (d, 1H, $J_{2,6} = 1.5$ Hz, H-2), 6.80 (d, 1H, $J_{5,6} = 8.0$ Hz, H-5), 6.80 (dd, 1H, $J_{6,2} = 1.5$,$J_{6,5} = 8.0$ Hz, H-6); EI-MS: *m/z* (rel. abund. %) 318 ($M^+$, 100), 183 (40), 137 (64), 109 (60); Anal. Calcd for $C_{13}H_7F_5N_2O_2$ (318.20): C, 49.07; H, 2.22; N, 8.80; Found: C, 49.04; H, 2.25; N, 8.76.

#### 1-(2-Bromobenzylidene)-2-(perfluorophenyl)hydrazine (6)

Yield: 0.33 g (90%); $^1$H-NMR: (300 MHz, DMSO-$d_6$): $\delta$ 10.70 (s, 1H, –NH), 8.45 (s, 1H, *Vin*. H), 7.85 (dd, 1H, $J_{3,5} = 1.2$, $J_{3,4} = 8.0$ Hz, H-3), 7.64 (d, 1H, $J_{6,5} = 8.0$ Hz, H-6), 7.39 (t, 1H, $J_{4(3,5)} = 8.0$ Hz, H-4), 7.24 (t, 1H, $J_{5(4,6)} = 8.0$ Hz, H-5); EI-MS: *m/z* (rel. abund. %) 366 (M+2, 56), 364 ($M^+$, 53), 285 (100), 183 (82), 155 (41); Anal. Calcd for $C_{13}H_6BrF_5N_2$ (365.10): C, 42.77; H, 1.66; N, 7.67; Found: C, 42.75; H, 1.64; N, 7.71.

#### 4-Nitro-2-((2-(perfluorophenyl)hydrazono)methyl)phenol (7)

Yield: 0.32 g (92%); $^1$H-NMR: (300 MHz, DMSO-$d_6$): $\delta$ 10.61 (s, 1H, –NH), 8.44 (d, 1H, $J_{6,4} = 2.5$ Hz, H-6), 8.41 (s, 1H, *Vin*. H), 8.05 (dd, 1H, $J_{4,6} = 2.5$, $J_{4,3} = 9.0$ Hz, H-4), 6.98(d, 1H, $J_{3,4} = 9.0$ Hz, H-3); EI-MS: *m/z* (rel. abund. %) 347 ($M^+$, 92), 183 (100), 155 (26); Anal. Calcd for $C_{13}H_6F_5N_3O_3$ (347.20): C, 44.97; H, 1.74; N, 12.10; Found: C, 45.01; H, 1.72; N, 12.07.

#### 1-(4-Nitrobenzylidene)-2-(perfluorophenyl)hydrazine (8)

Yield: 0.27 g (82%); $^1$H-NMR: (300 MHz, DMSO-$d_6$): $\delta$ 10.81 (s, 1H, -NH), 8.24 (d, 2H, $J_{3,2/5,6} = 8.7$ Hz, H-3/5), 8.18 (s, 1H, *Vin*. H), 7.82 (d, 2H, $J_{2,3/6,5} = 8.7$ Hz, H-2/6); EI-MS: *m/z* (rel. abund. %) 331 ($M^+$, 100), 182 (65), 155 (24), 76 (13); Anal.Calcd for $C_{13}H_6F_5N_3O_2$(331.20): C, 47.14; H, 1.83; N, 12.69; Found: C, 47.17; H, 1.80; N, 9.65.

#### 3-((2-(Perfluorophenyl) hydrazono)methyl)phenol (9)

Yield: 0.26 g (86%); $^1$H-NMR: (300 MHz, DMSO-$d_6$): $\delta$ 10.23 (s, 1H, –NH), 9.50 (s, 1H, –OH), 8.02 (s, 1H, *Vin*. H), 7.18 (t, 1H, $J_{5(4,6)} = 8.0$ Hz, H-5), 7.04 (br.s, 1H, H-2),6.98 (d, 1H, $J_{6,5} = 8.0$ Hz, H-6), 6.74 (dd, 1H, $J_{4,6} = 1.5$, $J_{4,3} = 8.0$ Hz, H-4); EI-MS: *m/z* (rel. abund. %) 302 ($M^+$, 100), 183 (68), 83 (55); Anal. Calcd for $C_{13}H_7F_5N_2O$ (302.20): C, 51.67; H, 2.33; N, 9.27; Found: C, 51.69; H, 2.31; N, 9.29.

#### 3-((2-(Perfluorophenyl)hydrazono)methyl)benzene-1,2-diol (10)

Yield: 0.28 g (88%); $^1$H-NMR: (300 MHz, DMSO-$d_6$): $\delta$ 10.42 (s, 1H, –NH), 10.08 (s, 1H, –OH), 10.02 (s, 1H, -OH), 8.32 (s, 1H, *Vin*. H), 6.85 (d, 1H, $J_{6,5} = 7.5$ Hz, H-6), 6.76 (d, 1H, $J_{4,5} = 7.5$ Hz, H-4), 6.70 (t, 1H, $J_{5(4,6)} = 7.5$ Hz, H-5); EI-MS: *m/z* (rel. abund. %) 318 ($M^+$, 100), 183 (65), 135 (74), 108 (71); Anal. Calcd for $C_{13}H_7F_5N_2O_2$ (318.05): C, 49.07; H, 2.22; N, 8.80; Found: C, 49.04; H, 2.18; N, 8.84.

#### 4-((2-(Perfluorophenyl)hydrazono)methyl)benzene-1,3-diol (11)

Yield: 0.28 g (88%); $^1$H-NMR: (300 MHz, DMSO-$d_6$): $\delta$ 10.54 (s, 1H, –NH), 10.17 (s, 1H, –OH), 9.80 (s, 1H, –OH), 8.24 (s, 1H, *Vin*. H), 7.20 (d, 1H, $J_{6,5} = 7.5$ Hz, H-6), 6.32 (dd, 1H, $J_{5,3} = 2.0$,$J_{5,6} = 8.5$ Hz, H-5), 6.27 (s, 1H, H-3); EI-MS: *m/z* (rel. abund. %) 318 ($M^+$, 15), 183 (100), 136 (24); Anal. Calcd for $C_{13}H_7F_5N_2O_2$ (318.20): C, 49.07; H, 2.22; N, 8.80; Found: C, 49.06; H, 2.25; N, 8.83.

#### 4-((2-(Perfluorophenyl)hydrazono)methyl)benzaldehydes (12)

Yield: 0.26 g (83%); $^1$H-NMR: (300 MHz, DMSO-$d_6$): $\delta$ 10.66 (s, 1H, –NH), 9.98 (s, 1H, –CHO), 7.99 (s, 1H, *Vin*. H), 7.92 (d, 2H, $J_{2,3/6,5} = 8.7$ Hz, H-2/6), 6.79 (d, 2H, $J_{3,2/5,6} = 8.7$ Hz, H-3/5); EI-MS: *m/z* (rel. abund. %) 314 ($M^+$, 51), 183 (100), 103 (29); Anal. Calcd for $C_{14}H_7F_5N_2O$(314.21): C, 53.52; H, 2.25; N, 8.92; Found: C, 53.55; H, 2.22; N, 8.90.

### *N, N*-Dimethyl-4-((2-(perfluorophenyl)hydrazono)methyl) aniline (13)

Yield: 0.26 g (79%); $^1$H-NMR: (300 MHz, DMSO-$d_6$): $\delta$ 9.89 (s, 1H, –NH), 7.99 (s, 1H, *Vin*. H), 7.41 (d, 2H, $J_{2,3/6,5} = 8.7$ Hz, H-2/6), 6.70 (d, 2H, $J_{3,2/5,6} = 8.7$ Hz, H-3/5), 2.93 (s, 6H, N($CH_3$)$_2$); EI-MS: *m/z* (rel. abund. %) 329 ($M^+$, 100), 147 (62), 120 (20); Anal. Calcd for $C_{15}H_{12}F_5N_3$ (329.27): C, 54.72; H, 3.67; N, 12.76; Found: C, 54.70; H, 3.69; N, 12.75.

### 1-(Perfluorophenyl)-2-(3,4,5-trimethoxybenzylidene) hydrazine (14)

Yield: 0.33 g (88%); $^1$ H-NMR: (300 MHz, DMSO-$d_6$): $\delta$ 10.28 (s, 1H, –NH), 8.00 (s, 1H, *Vin*. H), 6.67(s, 2H, H-2/6), 3.78 (s, 6H, 2-$OCH_3$), 3.66 (s, 3H, $OCH_3$); EI-MS: *m/z* (rel. abund. %) 376 ($M^+$, 100), 161 (42), 167 (24), 152 (28); Anal. Calcd for $C_{13}H_7F_5N_2O_3$ (334.2): C, 51.07; H, 3.48; N, 7.44; Found: C, 51.04; H, 3.50; N, 7.45.

### 2-Methoxy-6-((2-(perfluorophenyl) hydrazono)methyl) phenol (15)

Yield: 0.29 g (87%); $^1$H-NMR: (300 MHz, DMSO-$d_6$): $\delta$ 10.42 (s, 1H, –OH), 10.03 (s, 1H, –NH), 8.36 (s, 1H, *Vin*. H), 7.05 (d, 1H, $J_{6,5} = 7.0$ Hz, H-6), 6.94 (d, 1H, $J_{4,5} = 7.0$ Hz, H-4), 6.80 (t, 1H, $J_{5(4,6)} = 7.0$ Hz, H-5), 3.78 (s, 3H, $OCH_3$); EI-MS: *m/z* (rel. abund. %) 332 ($M^+$, 85), 149 (57), 135 (100), 83 (81); Anal. Calcd for $C_{14}H_9F_5N_2O_2$ (332.23): C, 50.61; H, 2.73; N, 8.43; Found: C, 50.58; H, 2.76; N, 8.44.

### 1-(4-Methylbenzylidene)-2-(perfluorophenyl)hydrazine (16)

Yield: 0.25 g (83%); $^1$H-NMR: (300 MHz, DMSO-$d_6$): $\delta$ 10.20 (s, 1H, –NH), 8.07 (s, 1H, *Vin*. H), 7.48 (d, 2H, $J_{2,3/6,5} = 8.0$ Hz, H-2/6), 7.20 (d, 2H, $J_{3,2/5,6} = 8.0$ Hz, H-3/5), 2.30 (s, 3H, –$CH_3$);MS: *m/z* (rel. abund. %) 300 ($M^+$, 100), 183 (26), 118 (25), 91 (56); Anal. Calcd for $C_{14}H_9F_5N_2$ (300.23): C, 56.01; H, 3.02; N, 9.33; Found: C, 56.04; H, 3.00; N, 9.36.

### 1-(3,4-Dimethoxybenzylidene)-2-(perfluorophenyl) hydrazine (17)

Yield: 0.32 g (92%);$^1$ H-NMR: (300 MHz, DMSO-$d_6$): $\delta$ 10.13 (s, 1H, –NH), 8.03 (s, 1H, *Vin*. H), 7.20 (br.s, 1H, H-2), 7.07 (dd, 1H, $J_{6,2} = 1.2$, $J_{6,5} = 8.5$ Hz, H-6), 6.96 (d, 1H, $J_{5,6} = 7.5$ Hz, H-5), 3.76 (s, 6H, (–$OCH_3$)$_2$); EI-MS: *m/z* (rel. abund. %) 346 ($M^+$, 100), 164 (17), 137 (17); Anal. Calcd for $C_{15}H_{11}F_5N_2O_2$ (346.25): C, 52.03; H, 3.20; N, 8.09; Found: C, 52.05; H, 3.23; N, 8.05.

### 1-(4-Methoxybenzylidene)-2-(perfluorophenyl)hydrazine (18)

Yield: 0.25 g (83%); $^1$H-NMR: (300 MHz, DMSO-$d_6$): $\delta$ 10.10 (s, 1H, –NH), 8.05 (s, 1H, *Vin*. H), 7.54 (d, 2H, $J_{2,3/6,5} = 8.5$ Hz, H-2/6), 6.96 (d, 2H, $J_{3,2/5,6} = 8.5$ Hz, H-3/5), 3.76 (s, 3H, –$OCH_3$); EI-MS: *m/z* (rel. abund. %) 316 ($M^+$, 100), 183 (44), 134 (100), 77 (100); Anal. Calcd for $C_{14}H_9F_5N_2O$(316.23): C, 53.17; H, 2.87; N, 8.86; Found: C, 56.04; H, 3.00; N, 9.36.

### 1-(Perfluorophenyl)-2-(1-phenylethylidene) hydrazine (19)

Yield: 0.133 g (89%); $^1$H-NMR: (400 MHz, DMSO-$d_6$): $\delta$ 8.78 (s, 1H, –NH), 7.72 (d, 2H, $J_{2,3/6,5} = 7.2$ Hz, H-2/6), 7.39-7.33 (m, 3H, H-3/4/5), 2.30 (s, 3H, $CH_3$); EI-MS: *m/z* (relative. abundence. %) 300 ($M^+$, 100), 182 (8), 155 (12), 118 (92), 103 (6), 77 (95), 69 (3), 51 (12); Anal. Calcd for $C_{14}H_9F_5N_2$ (300.23): C, 56.01; H, 3.02; N, 9.33; Found: C,56.00, H, 3.05, N, 9.30.

### 1-(1-(3-Nitrophenyl) ethylidene)-2-(perfluorophenyl) hydrazine (20)

Yield: 0.162 g (92%); $^1$H-NMR: (400 MHz, DMSO-$d_6$): $\delta$ 9.12 (s, 1H, –NH), 8.49 (s, 1H, H-2), 8.18 (d, 1H, $J_{4,5} = 8$ Hz, H-4), 8.135 (d, 1H, $J_{6,5} = 7.6$ Hz, H-6), 7.684 (t, 1H, $J_{5,(4.6)} = 8$ Hz, H-5), 2.364 s (s, 3H, $CH_3$); EI-MS: *m/z* (relative. abundence. %) 345 ($M^+$, 100), 328 (4), 298 (9), 182 (22), 163 (44), 155 (12), 117 (65), 92 (5),76 (23), 50 (4); Anal. Calcd for $C_{14}H_8F_5N_3O_2$, (345.22); C, 48.71; H, 2.34; N, 12.17; Found: C, 48.70; H, 2.35; N, 12.18

### 1-(1-(Naphthalen-2-yl) ethylene)-2-(perfluorophenyl) hydrazine (21)

Yield: 0.152 g (86%); $^1$H-NMR: (400 MHz, DMSO-$d_6$): $\delta$ 8.90 (s, 1H, –NH), 8.16 (s, 1H, H-1), 7.997-7.952 (m, 2H, H-3/4), 7.894-7.854 (m, 2H, H-5/8), 7.514 (t, 2H, $J_{6,(5,7)/7,(6,8),} = 7.2$ Hz, H-6/7), 2.42 (s, 3H, $CH_3$); EI-MS: *m/z* (relative. abundence. %) 350 ($M^+$, 86), 335 (2), 222 (2), 181 (7), 168 (71), 153 (15), 127 (100), 101 (3),77 (5), 44 (1); Anal. Calcd for $C_{18}H_{11}F_5N_2$ (350.29), C, 61.72; H, 3.17; N, 8.00; Found: C,61.75; H, 3.15; N, 8.03.

### 2-(1-(2-(Perfluorophenyl) hydrazono) ethyl) benzene-1,3-diol (22)

Yield: 0.147 g (88%); $^1$H-NMR: (400 MHz, DMSO-$d_6$): $\delta$ 10.03 (s, 2H, –OH-2/6), 8.69 (s, 1H, -NH), 6.96 (t, 1H, $J_{4,(3,5)} = 8$ Hz, H-4), 6.25 (d, 2H, $J_{3,4/5,4} = 8$ Hz, H-3/5), 2.348 (s, 3H, $CH_3$); EI-MS: *m/z* (relative. abundence. %) 332 ($M^+$, 100), 315 (26), 222 (6), 181 (14), 150 (42), 122

(6), 107 (9), 81 (6), 42 (5); Anal. Calcd for $C_{14}H_9F_5N_2O_2$ (332.23), C, 50.61; H, 2.73; N, 8.43; Found: C, 50.60; H, 2.75; N, 8.41

#### 4-(1-(2-(Perfluorophenyl) hydrazono) ethyl) benzene-1,3-diol (23)

Yield: 0.151 g (90%);$^1$H-NMR: (400 MHz, DMSO-$d_6$): $\delta$ 12.12 (s, 1H, –OH), 9.75 (s, 1H, –OH), 8.867 (s, 1H, –NH), 7.382 (d, 1H, $J_{6,5} = 8.8$ Hz, H-6), 6.32 (dd, 1H, $J_{5,3/5,6} = 2.4$ Hz, 8.4 Hz, H-5), 6.218 (s, 1H, H-3) 2.357 (s, 3H, $CH_3$); EI-MS: *m/z* (relative. abundence. %) 332 ($M^+$, 100), 315 (14), 222 (6), 181 (16), 116 (7), 150 (46), 135 (8), 81 (8), 69 (4), 42 (8); Anal. Calcd for $C_{14}H_9F_5N_2O_2$ (332.23), C, 50.61; H, 2.73; N, 8.43; Found: C, 50.60; H, 2.75;N, 8.40

#### 2-(1-(2-(Perfluorophenyl) hydrazono)ethyl)phenol (24)

Yield: 0.149 g (85%); $^1$H-NMR: (400 MHz, DMSO-$d_6$): $\delta$ 8.44 (s, 1H, –OH), 8.42 (s, 1H, –NH), 8.092 (d, 1H, $J_{3,4} =$ 8 Hz, H-3), 7.982 (d, 1H, $J_{6,5} = 8.8$ Hz, H-6), 7.76 (t, 1H, $J_{4,(3,5)} = 7.2$ Hz, H-4), 7.59 (t, 1H, $J_{5,(4,6)} = 8.8$ Hz, H-5), 2.90 (s, 3H, $CH_3$); EI-MS: *m/z* (relative. abundence. %) 348 ($M^+$, 100), 329 (8.6), 306 (2.0), 288 (2.2), 183 (11), 174 (5), 153 (3), 140 (8), 114 (4), 64 (11), 44 (9); Anal. Calcd for $C_{14}H_9F_5N_2O$ (316.23), C, 53.17; H, 2.87; N, 8.86; Analyzed: C, 53.20; H, 2.85; N,8.85

#### 4-(1-(2-(Perfluorophenyl) hydrazono)ethyl)phenol (25)

Yield: 0.148 g (92%); $^1$H-NMR: (400 MHz, DMSO-$d_6$): $\delta$ 10.65 (s, 1H, –NH), 7.82 (d, 2H, $J_{3,2/5,6} = 8.5$ Hz, H-3/5), 6.82 (d, 2H, $J_{2,3/6,5} = 8.5$ Hz, H-2/6), 2.35 (s, 3H, $CH_3$); EI-MS: *m/z* (relative. abundence. %) 316 ($M^+$, 1.7), 287 (18), 231 (2.4), 211 (1.8), 150 (85), 121 (100), 93 (78), 80 (44.5), 65 (38), 48 (15); Anal. Calcd for $C_{14}H_9F_5N_2O$ (316.23), C, 53.17; H, 2.87; N, 8.86; Found: C, 53.20; H, 2.85; N, 8.85

#### 1-(1,2-Diphenylethylidene)-2-((perfluorophenyl)methyl) hydrazine (26)

Yield: 0.176 g (92%); $^1$H-NMR: (400 MHz, DMSO-$d_6$): $\delta$ 9.18 (s, 1H, -NH), 7.71 (d, 2H, $J_{2',3'/6',5'} = 7.2$ Hz, H-2′/6′), 7.303 (m, 5H, H-2/3/4/5/6), 7.22 (m, 2H, H-3′/5′), 7.18 (t, 1H, $J_{4',(3',5')} = 15.6$ Hz, H-4′), 4.309 (s, 2H, $CH_2$); EI-MS: *m/z* (relative. abundence. %) 376 ($M^+$, 100), 285 (4), 193 (53), 182 (49), 155 (8), 103 (3), 91 (33), 65 (3); Anal. Calcd for $C_{21}H_{15}F_5N_2$ (390.35), C, 64.62; H, 3.87; N, 7.18; Found: C, 64.65; H, 3.90; N, 7.20

#### 4,4′-((2-(Perfluorophenyl) hydrazono)methylene)bis(*N*,*N*-dimethylaniline) (27)

Yield: 0.208 g (92%); $^1$H-NMR: (400 MHz, DMSO-$d_6$): $\delta$ 10.66 (s, 1H, –NH), 7.58 (d, 4H, $J_{2,3/6,5/2',3'/6',5'} = 8.8$ Hz, H-2/6/2′/6′), 6.74 (d, 4H, $J_{3,2/5,6/3',2'/5',6'} = 8.8$ Hz, H-3/5/3′/5′), 3.08 (s, 12H, -N($CH_3$)$_2$2); EI-MS: *m/z* (relative. abundence. %) 448 ($M^+$, 10), 268 (100), 251 (42), 224 (59), 148 (92); Anal. Calcd for $C_{23}H_{21}F_5N_4$(448.43), C, 61.60; H, 4.72; N, 12.49; Found: C, 61.62; H, 4.70; N, 12.50.

### Urease inhibition assay (In vitro)

Reaction mixture comprising on 25 µL of enzyme (*Canavalia ensiformis* urease) solution and 55 µL of buffers, containing 100 mM urea, were incubated with 5 µL of test compounds (0.5 mM concentration) at 30 °C for 15 min in 96-well plates. Urease activity was determined by measuring ammonia production by using the indophenol method, as described by Weatherburn (Weatherburn 1967). Briefly, 45 µL each phenol reagent (1% w/v phenol and 0.005% w/v sodium nitroprussside) and 70 µL of alkali reagent (0.5% w/v NaOH and 0.1% active chloride NaOCl) were added to each well. The increasing absorbance at 630 nm was measured after 50 min by using a microplate reader (Spectra Max, Molecular Devices, CA, USA). All reactions were performed in triplicate in a final volume of 200 µL. The results (change in absorbance per min) were processed by using soft Max Pro software (Molecular Devices, CA, USA). The entire assays were performed at pH 6.8. Percentage inhibitions were calculated from the formula 100-($OD_{testwell}/OD_{control}$) × 100. Thiourea was used as the standard inhibitor of urease (Abid et al. 2010).

### In silico studies

The molecular construction of the compounds was performed using ChemBioDraw Ultra 14 suite (PerkinElmer Inc.) and converted into 3D conformations by ChemBio3D (Mills 2006). Crystal structure of the enzymes, Jack bean urease (PDB: 3LA4) (Balasubramanian and Ponnuraj 2010) was retrieved from Protein Data Bank (Berman et al. 2000). Thiourea (PubChem-2723790) was taken as a standard ligand. The target proteins were prepared by the addition of hydrogen, removal of water and the removal of co-crystallized ligands. All other parameters were used with the default settings. The compounds were docked using MOE (Molecular operating environment) software (Da Silva et al. 2011). The structures of the compounds were energy minimized using MMFF94x forcefield and gradient: 0.05. The active sites of the enzymes were obtained by site finder and

the compounds were docked by the default parameters, i.e, Placement: Trainagular Matcher, Rescoring1: London dG, Refinement: Forcefield and Rescoring2: Affinity dG. For each ligand thirty conformations were generated. Topmost interaction was selected with lowest Gibb's free energy value. The images in 2D were captured through MOE ligand binding interaction. 3D images were taken using Discovery Studio Visualizer and Chimera 1.11 software (Pettersen et al. 2004).

Physicochemical properties and pharmacokinetics studies were also performed through computational tools. RO5, MR, PSA, and octanol water partition coefficient (logP) were determined through Mcule, Inc (Moura et al. 2012) and PreADMET online softwares (Menna-Barreto et al. 2005). Oral rat LD50 values and ames mutagenicity test were analyzed using TEST (Shalini et al. 2010).

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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