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Isoindolin-1-one derivatives as urease inhibitors: Design, synthesis, biological evaluation, molecular docking and *in-silico* ADME evaluation

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ABSTRACT

An efficient, one-pot and four-component synthesis of a new series of 2,3-disubstituted isoindolin-1-ones is described and their Jack bean urease inhibitory activities are evaluated. Heating a mixture of 1,1-bis(methylthio)-2-nitroethene, a 1,2-diamine, a 2-formylbenzoic acid and a primary amine in EtOH for 3.5 h afforded the corresponding 2,3-disubstituted isoindolin-1-ones in good to excellent yields. All sixteen synthesized isoindolin-1-one derivatives **5a–p** showed urease inhibitory activity. Among them, **5c** showed the most urease inhibitory activity ($IC_{50} = 10.07 \pm 0.28$ µM) being over 2-fold more potent than thiourea ($IC_{50} = 22.01 \pm 0.10$ µM) and 10-fold than hydroxyurea ($IC_{50} = 100.00 \pm 0.02$ µM) as the standard inhibitors, respectively. Also, results from molecular docking studies were in good agreement with those obtained from *in vitro* tests.

1. Introduction

Urease (urea amidrolase, EC 3.5.1.5) is a large heteropolymeric enzyme with two nickel ions in its active site [1–3] which hydrolyze urea to ammonia and carbon dioxide at a rate approximately 10^{14} times of the uncatalyzed reaction with carbamate as the intermediate. Researches have shown that *Helicobacter pylori* plays an important role in gastric lymphoma, gastric carcinoma and peptic ulcer disease [4]. Produced ammonia by urease provides *Helicobacter pylori* a suitable environment for growth and reproduction in the harsh pH conditions of the stomach by increasing pH in the aqueous medium [5]. It follows logically that inhibition of active site of urease can be a promising action for preventing adverse effects of ureolytic bacterial infections in humans [6]. With this in mind, synthetic and medicinal chemists have developed different types of effective inhibitors such as hydroxamic acid derivatives [7a], semicarbazones [7b], Schiff bases [7c], urea derivatives [7d], dihydropyrimidines [7e], piperazines [7f], barbituric- and thiobarbituric acid derivatives [7g], benzimidazoles [7h], biscoumarines [7i] and sulfonamides [7j]. *Helicobacter pylori* urease is found in a variety of fungi, plants, algae and bacteria [8,9].

Many efforts have been taken to synthetize valuable heterocyclic scaffolds through multi-component reactions (MCRs) [10]. Approaching simple efficient synthetic methods for the preparation of compounds containing two or more heterocyclic rings is an interesting challenge for modern organic synthesis [11].

Isoindolinones are fascinating nitrogen-containing heterocycles which are fundamental building blocks in many prevalent natural products (Fig. 1) [12]. They have a broad spectrum of biological activities including antimicrobial [13], anti-viral [14], antitumor [15], anti HIV [16], hypnotic [17], anxiolytic and muscle relaxant [18], antipsychotic [19] and anti-diabetic properties [20].

There are several approaches for the synthesis of isoindolin-1-ones [21–24]. Some methods are based upon 2-formylbenzoic acids [25]. But, the reaction of ketene aminals with 2-formylbenzoic acids to provide isoindolin-1-ones has not been reported yet. It must be noted that ketene aminals have received great attention because of their ability to behave as bisnucleophilic substrates [26,27]. In addition, they have been widely used in multicomponent reactions to construct five- or six-membered and fused polycyclic compounds including spiropyrano acenaphthylene derivatives [28], pyrrolo[1,2-*a*]fused[1,3] diazaheterocycles [29], indene-fused heterocyclic compounds [26d,30] imidazo[1,2-*a*]pyridines and pyrido[1,2-*a*]pyrimidine [31], chromeno [2,3-*d*]pyrimidine-2,4-diones [32] 1,3-diazaheterocycle-fused [1,2-*b*] isoquinolin-1(2*H*)-one [26a] and bicyclic pyridines [26c]. These fused structures have been known as herbicides, pesticides [33], anti-anxiety [34], antileishmanial [35] and antibacterial agents [36].

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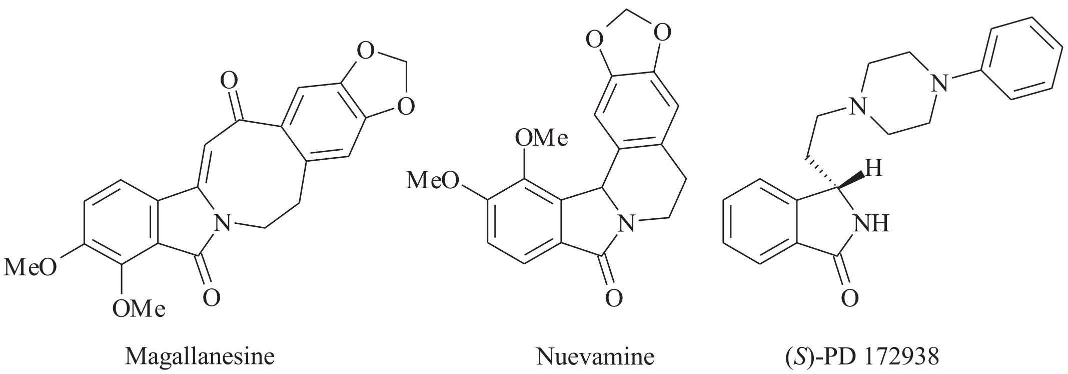


Fig. 1. Examples of fused alkaloids and medicinally important compounds with an isoindolin-1-one core structure.

There are several reports in literature concerning urease inhibitors having nitroalkene moiety in their structures [37]. On the other hand, compounds having imidazoles, benzimidazoles and indole heterocycles have been shown to possess urease inhibitory activities [38] Thus, we have focused on the synthesis of a new series of isoindole-benzimidazole-nitroalkenes and evaluation of these new compounds as urease inhibitors.

2. Results and discussion

2.1. Chemistry

In extension of our preceding researches on the development of efficient synthesis of biologically active organic compounds [39] and as part of our recent studies focused on synthesis of 2,3-disubstituted isoindolin-1-one derivatives [40], we turn our attention to obtain a new class of these compounds by use of the *in situ* generated nitroketene aminals. In this paper, we present a new one-pot and four-component reaction between 1,1-bis(methylthio)-2-nitroethene **1**, 1,2-diamines **2**, 2-formylbenzoic acids **3** and primary amines **4**. Thus, the reaction between **1** and a 1,2-diamine **2** proceeded in EtOH under reflux conditions and completed within 3 h. After nearly complete conversion to the corresponding nitroketene aminal as was indicated by TLC monitoring, a 2-formylbenzoic acid **3** and a primary amine **4** were added to the mixture which was refluxed for further 30 min. TLC and ^{1}H NMR analysis of the reaction mixture confirmed the formation of the corresponding 2,3-disubstituted isoindolin-1-ones **5** in good to excellent yields (Scheme 1). To determine the generality of the reaction, we applied 1,1-bis(methylthio)-2-nitroethene **1**, 1,2-diamines **2a–c**, 2-formylbenzoic acids **3a**,**b** and also various primary amines **4a–h** (Scheme 1). All the reactions were carried out by use of a 1:1:1:1 M ratio of the four components in boiling EtOH and went to completion within 3.5 h to afford the corresponding 2,3-disubstituted isoindolin-1-ones **5a–p** in 68–90% yields (Fig. 2).

The structures of the isolated products were deduced on the basis of their IR, ^{1}H-, and ^{13}C NMR spectroscopy, mass spectrometry and elemental analysis. Partial assignments of these resonances are given in the Experimental Part.

A reasonable mechanism for the four-component reaction is illustrated in Scheme 2. The reaction may be initiated by condensation of 1,1-bis(methylthio)-2-nitroethene **1** and the diamine **2** to produce nitroketene aminal **6**. On the other hand, amine **4** condenses with 2-formylbenzoic acid **3** to generate imine **7**. Nucleophilic addition of nitroketene aminal **6** from *C*-atom on the imine function of **7** gives adduct **8**. Next, an intramolecular amidation may occur through nucleophilic attack of the amine moiety on the adjacent carboxylic acid to close the isoindolin-1-one ring skeleton and form **9**. Finally, an imine-enamine tautomerization takes place to afford the isolated compounds **5**.

2.2. Biological evaluation

2.2.1. Jack bean urease inhibitory activity of 2,3-disubstituted isoindolin-1-ones 5

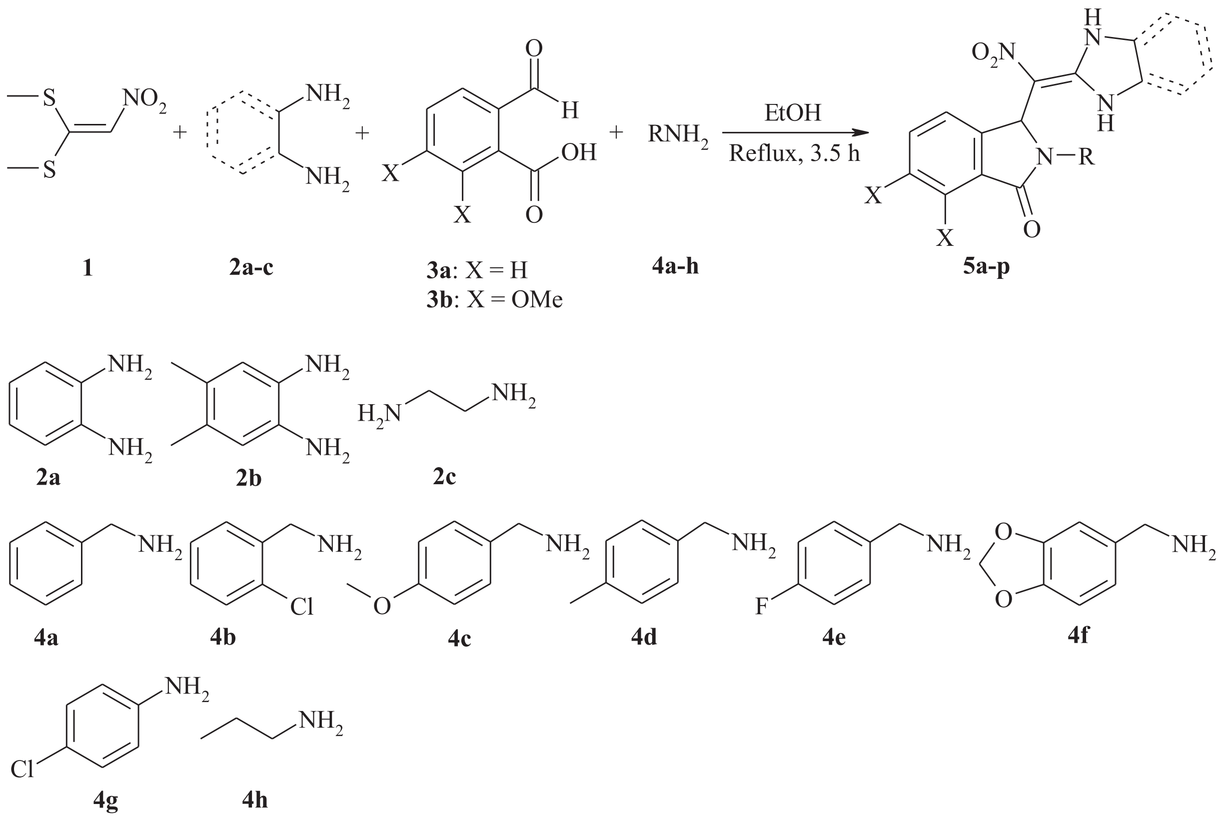
The synthesized 2,3-disubstituted isoindolin-1-one derivatives **5a–p** were tested for their urease inhibitory activity. Thiourea and hydroxyurea were taken as the reference drugs (Table 1). All isoindolin-1-one compounds **5a–p** showed very good activities with IC_{50} values of 10.07–43.27 µM, in comparison to the standard drugs thiourea (IC_{50} = 22.01 µM) and hydroxyurea (IC_{50} = 100.00 µM). The most potent compound, **5c** (IC_{50} = 10.07 µM) bearing *ortho*-chloro substituent on the *N*-benzyl moiety, represented over two and ten times more potent than the reference compounds, thiourea and hydroxyurea, respectively. Compounds **5f** and **5g** (IC_{50} = 38.24 µM, 43.27 µM) with *para*-methyl and *para*-methoxy substituents on the *N*-benzyl moiety and two methyl groups on the benzimidazole ring showed the least inhibitory activity.

2.3. Docking studies

Molecular docking studies were performed to consider the binding mode of 2,3-disubstituted isoindolin-1-one derivatives **5a–p** at the active site of urease. Compound **5c** can adopt a conformation for a better fit into the active site of urease as shown in (Fig. 3). The results show three hydrogen bonds between amino acid residues and functional groups of compound **5c**. The carbonyl oxygen atom in isoindolin-1-one moiety of compound **5c** forms two hydrogen bonds with Arg439 and Cme592 amino acids, and nitrogen of benzimidazole part with Arg439. Also, the hydrophobic interactions were observed between **5c** and Ala436, His593, Ala440, His519, Asp633, Arg609, Ala636, Gly550, Met637 and Gln635 amino acids. As depicted in Fig. 3, the phenylene moiety of isoindolin-1-one ring of compound **5c** has been positioned in vicinity of two nickel ions (Ni841, Ni842) with binding distances of 3.21 Å, 3.29 Å, respectively.

Changing the 1,3-azole-2-yliden(nitro)methyl moiety on C3 of isoindolin-1-ones essentially affects the activities. Derivatives having unsubstituted 1,3-benzimidazole (**5a**, **5b**, **5c**, **5m** and **5n**) showed better activities in comparison to isoindolin-1-ones having 5,6-dimethyl-1,3-benzimidazole (**5f** and **5g**). These two methyl groups on the benzimidazole ring probably prevent proximity of the observed least inhibitor isoindolin-1-ones **5f** and **5g** to nickel ions (Fig. 3). The molecular docking outcomes confirmed these results, also the distance and interaction of the nickel ions with the phenyl ring is important and increases the urease inhibitory effect. Isoindolin-1-ones having tetrahydro-2*H*-imidazole (**5h**, **5i**, **5k**, **5l** and **5o**) showed moderate activities.

Changing the *N*-substituent slightly affects the activity. However, 2-chlorobenzyl increases inhibitory activity, while 4-fluorobenzyl decreases it (comparison of **5c**, **5a** and **5m**). Presence of two methoxy groups on the C6 and C7 of isoindolinone slightly decreases the inhibitory activity of the compound (**5a** vs **5b**).



Scheme 1. Synthesis of 2,3-disubstituted isoindolin-1-ones **5a–p**.

2.4. *In-silico ADME evaluation*

The ADME properties of the prepared isoindolin-1-one derivatives **5a–p** were computed using SwissADME online (http://www.swissadme.ch/index.php) toolkit [41]. The evaluated parameters are given in Table 2. This *in-silico* study under Lipinski's rule of five determined the drug-likeness of the synthesized compounds compared with the known drugs [42]. Good oral bioavailability of tested compounds is evaluated on the basis of logP (< 5), MW (< 500), HBA (≤ 10), and HBD (< 5) values. Lipophilicity is estimated by logP in which P is the octanol-water partition coefficient. As can be seen from the table, all compounds have logP values in the range. The molecular flexibility is determined by the number of rotatable bonds (nROTB) which should be < 10. All the evaluated numbers are in the range of 4–6. The surface contribution of polar fragments is estimated by the topological polar surface area (TPSA). High TPSA values (> 140 Å^2) is related to low blood-brain barrier (BBB) penetration, and poor membrane permeability [43]. All compounds have TPSA values in the range of 90.19–127.11 Å^2 (Table 2) indicating good permeability of the evaluated compounds in the cellular plasma membrane. The total number of hydrogen bond donors (HBD) should be < 5, and the total number of hydrogen bond acceptors (HBA) should be ≤ 10. All compounds have HBDs of 2 and HBAs range of 4–6. On the basis of Veber rule [44] by the number of rotatable bonds ≤ 10 and TPSA < 140 Å^2, or sum of HBD and HBA < 12, all compounds were expected to have good oral bioavailability. Finally, consistent with the ADME findings, no violations were expected for the synthesized compounds (except for **5p**), affording positive drug-likeness values.

3. Conclusion

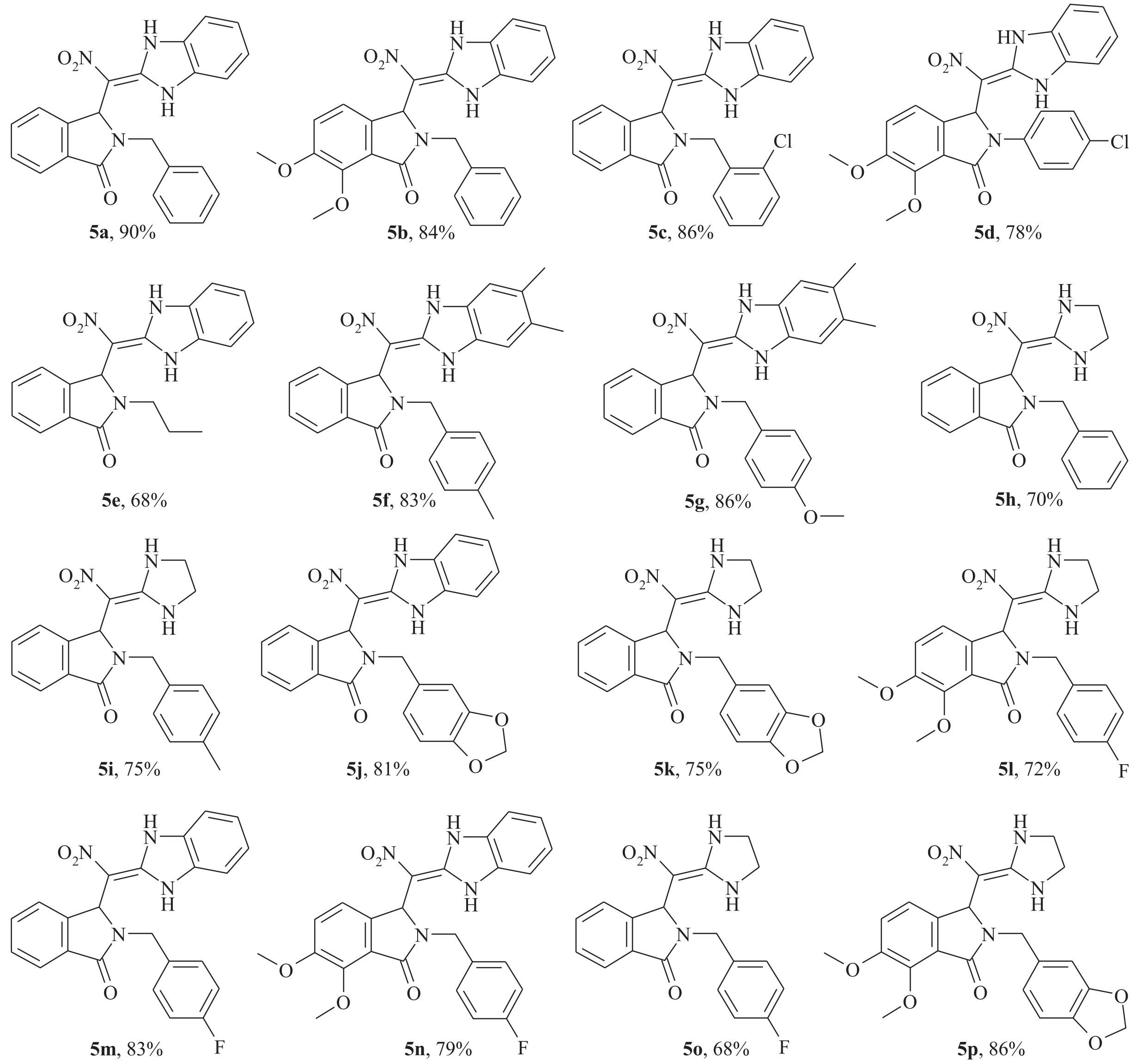
In conclusion, we have synthesized a new series of 2,3-disubstituted isoindolin-1-ones through a one-pot and four-component reaction and evaluated their urease inhibitory activity. The simplicity, commercially available starting materials, short reaction times, catalyst-free and mild conditions, easy work-up without any need to chromatography purification processes and high yields of the products are the main advantages of this reaction. All compounds **5a–p** showed very good potential against urease. Among them, compound **5c** was the most potent compound over two and ten times more potent than the reference compounds, thiourea and hydroxyurea, respectively. Also, the docking studies outcomes confirmed the results of the *in vitro* assay. In this paper we have introduced a new class of compounds with urease inhibitory activity which are more active than our previous reports [45].

4. Experimental part

General. All chemicals were purchased from Merck (Germany) and were used without further purification. Melting points were measured on an Electrothermal 9100 apparatus and are not corrected. Elemental analyses for C, H and N were performed using a Heraeus CHN-O-Rapid analyzer. Mass spectra were recorded on an Agilent Technologies (HP) 5973 mass spectrometer operating at an ionization potential of 20 eV. IR spectra were recorded on a Shimadzu IR-460 spectrometer. ^{1}H and ^{13}C NMR spectra were measured (DMSO-d_6 solution) with Bruker DRX-500 AVANCE (at 500.1 and 125.8 MHz) spectrometer with TMS as an internal standard.

4.1. *General procedure for the preparation of compounds **5**, exemplified with **5a***

A solution of 1,1-bis(methylthio)-2-nitroethene **1** (0.165 g, 1 mmol) and 1,2-phenylenediamin **2a** (0.108 g, 1 mmol) in EtOH (5 mL) was magnetically stirred for 3 h under reflux conditions. After nearly complete conversion to the corresponding nitroketene aminal as was indicated by TLC monitoring, 2-formylbenzoic acid **3a** (0.150 g, 1 mmol), and benzylamine **4a** (0.107 g, 1 mmol) were added to the mixture which was refluxed for further 30 min. After completion of the reaction


5a, 90% **5b**, 84% **5c**, 86% **5d**, 78%

5e, 68% **5f**, 83% **5g**, 86% **5h**, 70%

5i, 75% **5j**, 81% **5k**, 75% **5l**, 72%

5m, 83% **5n**, 79% **5o**, 68% **5p**, 86%

Fig. 2. Synthesis of 2,3-disubstituted isoindolin-1-one derivatives **5a–p**.

according to the TLC analysis, the mixture was cooled to ambient temperature, the precipitated product was filtered and washed with EtOH (5 mL) to afford pure **5a** (0.358 g, 90%) as a white powder.

*4.1.1. 2-Benzyl-3-[1,3-dihydro-2H-1,3-benzimidazol-2-yliden(nitro)methyl]-1-isoindolinone (**5a**)*

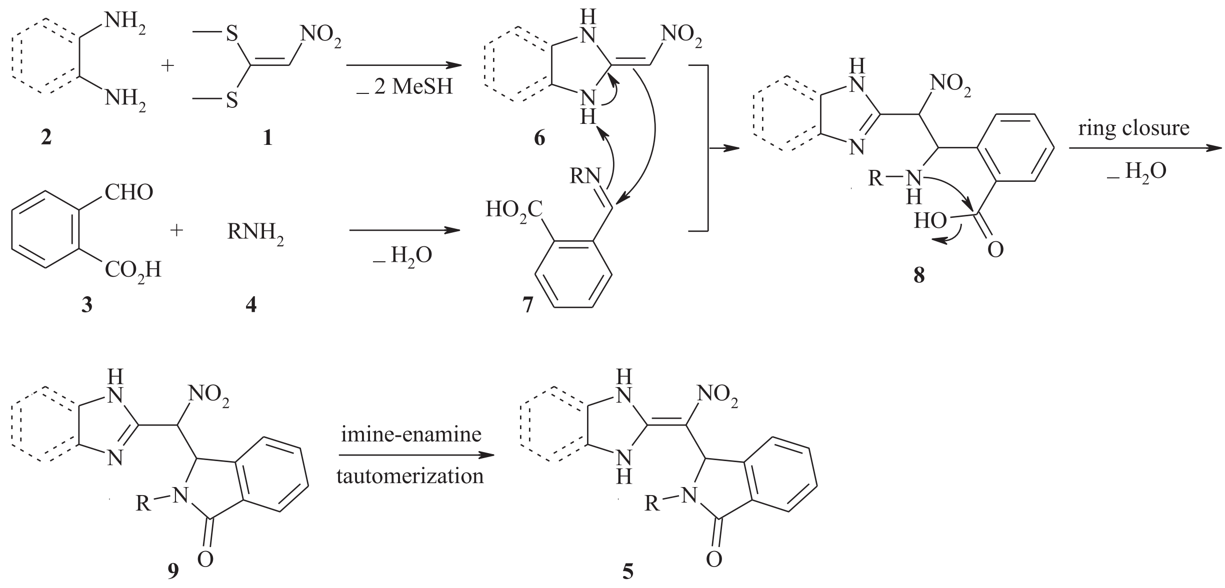
White powder; yield: 90%, m.p. 218 °C, IR (KBr) (ν_{max}/cm^{-1}): 3245 and 3185 (NH), 1688 (C═O), 1623, 1563, 1496, 1464, 1409, 1331, 1233, 1175, 1049, 946, 823, 741, 697, 667, 642, 621. ^{1}H NMR (500.1 MHz, DMSO-d_6): δ 13.20–12.60 (br, 2H, 2NH), 7.75 (d, J = 7.5 Hz, 1H, CH), 7.55–7.48 (m, 3H, 3CH), 7.47 (t, J = 7.3 Hz, 1H, CH), 7.40 (d, J = 7.5 Hz, 1H, CH), 7.32–7.28 (m, 2H, 2CH), 7.12–7.05 (m, 5H, 5CH), 5.74 (s, 1H, NCH), 4.87 and 4.12 (2d, ^2J = 15.0 Hz, 2H, NCH$_2$). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 167.8 (NC═O), 148.0 (CN$_2$), 144.4, 137.4 and 132.8 (3C), 131.1 (CH), 130.7 (br, 2C), 128.1 (2CH), 127.6 (CH), 127.4 (2CH), 126.9 (CH), 123.5 (2CH), 122.5 and 121.6 (2CH), 111.9 (br, 2CH), 100.5 (C—NO$_2$), 57.7 (NCH), 43.9 (NCH$_2$). EI-MS: m/z (%) = 399 (20) [M$^+$ + 1], 351 (18), 322 (6), 269 (12), 246 (6), 222 (12), 183 (100), 154 (22), 124 (6), 91 (16), 74 (1), 56 (30). Anal. Calcd for $C_{23}H_{18}N_4O_3$ (398.42): C, 69.34; H, 4.55; N, 14.06. Found: C, 69.11; H; 4.29, N, 13.85%.

*4.1.2. 2-Benzyl-3-[1,3-dihydro-2H-1,3-benzimidazol-2-yliden(nitro)methyl]-6,7-dimethoxy-1-isoindolinone (**5b**)*

White powder; yield: 84%, m.p. 196 °C. IR (KBr) (ν_{max}/cm^{-1}): 3254 and 3120 (NH), 1687 (C═O), 1618, 1561, 1499, 1455, 1414, 1356, 1299, 1236, 1186, 1137, 1083, 1046, 1006, 955, 892, 785, 763, 736, 698. ^{1}H NMR (500.1 MHz, DMSO-d_6): δ 13.00–12.60 (br, 2H, NH), 7.57–7.44 (br s, 2H, 2CH), 7.31–7.28 (m, 2H, 2CH), 7.15 (d, J = 8.5 Hz, 1H, CH), 7.13–7.04 (m, 5H, 5CH), 7.02 (d, J = 8.5 Hz, 1H, CH), 5.61 (s, 1H, NCH), 4.82 and 4.04 (2d, ^2J = 15.0 Hz, 2H, NCH$_2$), 3.95 and 3.81 (2 s, 6H, 2OCH$_3$). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 166.1 (NC═O), 151.7 (C), 148.0 (CN$_2$), 146.0, 137.5 and 137.4 (3C), 130.7 (br, 2C), 128.1 (2CH), 127.5 (2CH), 126.8 (CH), 125.1 (C—C═O), 123.5 (2CH), 116.6 and 116.2 (2CH), 111.8 (br, 2CH), 101.0 (C—NO$_2$), 61.6 (OCH$_3$), 56.7 (NCH), 56.3 (OCH$_3$), 43.9 (NCH$_2$). EI-MS: m/z (%) = 458 (< 1) [M$^+$], 425 (5), 297 (41), 282 (86), 251 (10), 206 (25), 190 (9), 165 (9), 143 (70), 106 (17), 91 (100), 78 (10), 65 (17), 51 (7). Anal. Calcd for $C_{25}H_{22}N_4O_5$ (458.47): C, 65.49; H, 4.84; N, 12.22. Found: C, 65.35; H, 4.66; N, 12.04%.

*4.1.3. 2-(2-Chlorobenzyl)-3-[1,3-dihydro-2H-1,3-benzimidazol-2-yliden(nitro)methyl]-1-isoindolinone (**5c**)*

White powder; yield: 86%, m.p. 202 °C. IR (KBr) (ν_{max}/cm^{-1}): 3408



Scheme 2. The proposed mechanism for the formation of 2,3-disubstituted isoindolin-1-ones **5**.

Table 1
The urease inhibitory activity of isoindolin-1-ones **5a–p**.

Entry	Isoindoline **5**	IC_{50} (μM) ± SEM	Docking energy (kcal/mol)
1	**5a**	12.14 ± 1.30	−5.71
2	**5b**	13.22 ± 0.05	−5.37
3	**5c**	10.07 ± 0.28	−6.34
4	**5d**	19.20 ± 1.04	−5.51
5	**5e**	16.41 ± 1.15	−5.56
6	**5f**	38.24 ± 0.02	−4.53
7	**5g**	43.27 ± 1.01	−4.25
8	**5h**	24.18 ± 0.03	−5.08
9	**5i**	24.66 ± 0.15	−4.77
10	**5j**	19.04 ± 0.93	−5.13
11	**5k**	22.37 ± 0.10	−5.49
12	**5l**	23.16 ± 1.13	−5.44
13	**5m**	12.95 ± 1.0	−5.68
14	**5n**	12.98 ± 1.04	−5.40
15	**5o**	21.01 ± 0.05	−5.50
16	**5p**	23.73 ± 1.00	−5.10
17	Thiourea	22.01 ± 0.10	
18	Hydroxyurea	100.00 ± 0.02	

Thiourea and hydroxyurea (reported as standards urease inhibitor).
SEM: Standard error of the mean.

and 3238 (NH), 1669 (C═O), 1622, 1577, 1467, 1417, 1336, 1297, 1233, 1177, 1093, 1061, 949, 913, 880, 844, 745, 693. ^{1}H NMR (500.1 MHz, DMSO-d_6): δ 13.30–12.55 (br, 2H, NH), 7.87 (d, J = 7.6 Hz, 1H, CH), 7.84 (d, J = 7.4 Hz, 1H, CH), 7.72–7.67 (m, 1H, CH), 7.62 (t, J = 7.5 Hz, 1H, CH), 7.57 (t, J = 7.3 Hz, 1H, CH), 7.43–7.39 (m, 1H, CH), 7.38–7.27 (m, 2H, 2CH), 7.31 (d, J = 7.9 Hz, 1H, CH), 7.28 (d, J = 7.3 Hz, 1H, CH), 7.19–7.07 (m, 2H, 2CH), 5.82 (s, 1H, NCH), 5.03 and 4.37 (2d, ^2J = 15.7 Hz, 2H, NCH_2). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 167.9 (NC═O), 148.0 (CN_2), 144.4, 134.4, 134.0 and 132.5 (4C), 131.3 (CH), 130.7 (2C), 129.8, 129.0, 127.6, 126.9 and 123.7 (5CH), 123.5 (2CH), 122.5 and 121.7 (2CH), 112.1 (br, 2CH), 100.4 (C–NO_2), 57.9 (NCH), 41.5 (NCH_2). EI-MS: m/z (%) = 434 (< 1) [M^+ ^{37}Cl], 432 (1) [M^+ ^{35}Cl], 416 (3), 399 (8), 385 (11), 291 (10), 256 (68), 236 (100), 219 (21), 160 (9), 143 (67), 125 (72), 104 (15), 89 (24), 76 (29), 63 (18), 50 (13). Anal. Calcd for $C_{23}H_{17}ClN_4O_3$ (432.87): C, 63.82; H, 3.96; N, 12.94. Found: C, 63.94; H, 4.18; N, 12.76%.

4.1.4. 2-(4-Chlorophenyl)-3-[1,3-dihydro-2H-1,3-benzimidazol-2-yliden (nitro)methyl]-6,7-dimethoxy-1-isoindolinone (*5d*)

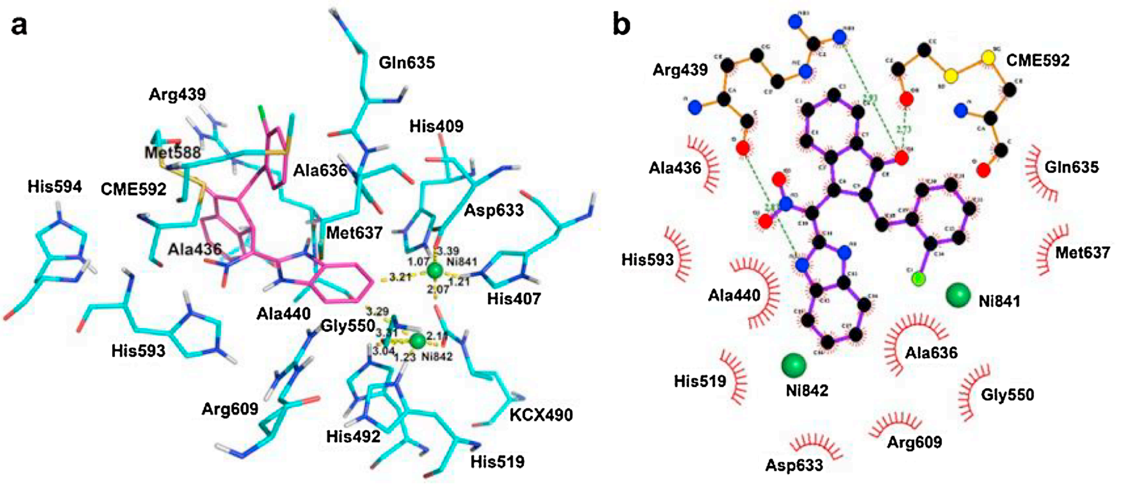
White powder; yield:78%, m.p. 201 °C. IR (KBr) (ν_{max}/cm^{-1}): 3318 and 3224 (NH), 1734 (C═O), 1596, 1498, 1498, 1459, 1422, 1338, 1296, 1269, 1229, 1168, 1128, 1062, 1038, 974, 903, 849, 826, 735, 717, 640. ^{1}H NMR (500.1 MHz, DMSO-d_6): δ 13.23–12.85 (br, 2H, 2NH), 7.61–7.57 (m, 2H, 2CH), 7.35–7.30 (m, 3H, 3CH), 7.23 (d, J = 8.8 Hz, 2H, 2CH), 7.12 (d, J = 8.1 Hz, 1H, CH), 6.90 (d, J = 8.8 Hz, 2H, 2CH), 6.66 (s, 1H, NCH), 3.95 and 3.85 (2 s, 6H, $2OCH_3$). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 166.3 (NC═O), 151.8 (C), 147.6 (CN_2), 146.9, 144.2, 141.2 and 138.2 (4C), 130.7 (2C), 128.8 (2CH), 123.7 (2CH), 122.7 (C − C═O), 119.7 and 118.9 (2CH), 115.8 (2CH), 112.1 (2CH), 102.0 (C–NO_2), 61.5 (OCH_3), 56.7 (NCH), 56.6 (OCH_3). EI-MS: m/z (%) = 480 (< 1) [M^+ ^{37}Cl], 478 (< 1) [M^+ ^{35}Cl], 353 (6), 336 (10), 323 (92), 307 (7), 291 (26), 276 (9), 251 (8), 236 (6), 221 (5), 208 (8), 193 (100), 179 (6), 163 (8), 143 (43), 118 (7), 77 (9), 63 (7). Anal. Calcd for $C_{24}H_{19}ClN_4O_5$ (478.89): C, 60.19; H, 4.00; N, 11.70. Found: C, 60.01; H, 3.86; N, 11.52%.

4.1.5. 3-[1,3-Dihydro-2H-1,3-benzimidazol-2-yliden)(nitro)methyl]-2-propyl-1-isoindolinone (*5e*)

White powder; yield:68%, m.p. 196 °C. IR (KBr) (ν_{max}/cm^{-1}): 3249 and 3054 (NH), 1673 (C═O), 1623, 1574, 1466, 1410, 1332, 1297, 1260, 1233, 1182, 1070, 958, 933, 834, 747, 696. ^{1}H NMR (500.1 MHz, DMSO-d_6): δ 13.45–12.90 (br, 2H, 2NH), 7.69 (d, J = 7.5 Hz, 1H, CH), 7.63–7.55 (br s, 2H, 2CH), 7.52–7.42 (m, 2H, 2CH), 7.39–7.29 (m, 3H, 3CH), 5.89 (s, 1H, NCH), 3.57 (dt, ^2J = 13.6, ^3J = 7.2 Hz, ABX_2, 1H, CH), 2.98 (dt, ^2J = 13.6, ^3J = 6.4 Hz, ABX_2, 1H, CH), 1.65–1.35 (m, 2H, CH_2), 0.81 (t, J = 7.0 Hz, 3H, CH_3). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 167.6 (NC═O), 148.3 (CN_2), 144.2 and 133.2 (2C), 130.9 (CH), 130.8 (2C), 127.5 (CH), 123.6 (2CH), 122.3 and 121.5 (2CH), 112.1 (br, 2CH), 100.5 (C–NO_2), 57.6 (NCH), 41.4 (NCH_2), 21.4 (CH_2), 11.4 (CH_3). EI-MS: m/z (%) = 350 (< 1) [M^+], 317 (4), 277 (10), 263 (39), 248 (7), 234 (15), 205 (13), 189 (36), 174 (48), 160 (100), 143 (95), 118 (28), 104 (32), 90 (16), 76 (37), 50 (17). Anal. Calcd for $C_{19}H_{18}N_4O_3$ (350.38): C, 65.13; H, 5.18; N, 15.99. Found: C, 64.78; H, 5.06; N, 15.73%.

4.1.6. 3-[(5,6-Dimethyl-1,3-dihydro-2H-1,3-benzimidazol-2-yliden)(nitro) methyl]-2-(4-methylbenzyl)-1-isoindolinone (*5f*)

White powder; yield: 83%, m.p. 234 °C. IR (KBr) (ν_{max}/cm^{-1}): 3229 and 3036 (NH), 1671 (C═O), 1617, 1561, 1471, 1416, 1389, 1320,



a) 3D illustration of compound **5c** and the corresponding interactions with amino acid residues and nickel ions.

b) Interactions of compound **5c** with amino acid residues and nickel ions at urease active site. Hydrogen bonds and hydrophobic interactions are shown as green dashed lines and red circles, respectively.

Fig. 3. Binding pocket of urease with binding position of the best compound **5c** is shown in the center.

1275, 1243, 1196, 1174, 1126, 1053, 945, 916, 851, 809, 685, 743, 694. ^{1}H NMR (500.1 MHz, DMSO-d_6): δ 12.84–12.30 (br, 2H, 2NH), 7.72 (d, J = 7.5 Hz, 1H, CH), 7.50 (t, J = 7.0 Hz, 1H, CH), 7.45 (t, J = 7.2 Hz, 1H, CH), 7.36 (d, J = 7.0 Hz, 1H, CH), 7.28 (br s, 2H, 2CH), 6.93 (d, J = 8.0 Hz, 2H, 2CH), 6.88 (d, J = 8.0 Hz, 2H, 2CH), 5.70 (s, 1H, NCH), 4.84 and 3.99 (2d, ^2J = 15.0 Hz, 2H, NCH$_2$), 2.33 (s, 6H, 2CH$_3$), 2.14 (s, 3H, CH$_3$). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 167.7 (NC=O), 147.5 (CN$_2$), 144.5, 136.0, 134.3 and 132.8 (4C), 132.0 (br, 2C), 131.0 (CH), 129.3 (br, 2C), 128.7 (2CH), 127.6 (CH), 127.5 (2CH), 122.4 and 121.6 (2CH), 112.2 (br, 2CH), 100.5 (C–NO$_2$), 57.6 (NCH), 43.6 (NCH$_2$), 20.5 (CH$_3$), 19.8 (2CH$_3$). EI-MS: m/z (%) = 440 (< 1) [M$^+$], 404 (12), 393 (53), 364 (13), 289 (18), 274 (15), 251 (57), 236 (65), 222 (10), 171 (55), 156 (64), 120 (31), 105 (100), 91 (28), 77 (33), 65 (13), 51 (14). Anal. Calcd for $C_{26}H_{24}N_4O_3$ (440.50): C, 70.89; H, 5.49; N, 12.72. Found: C, 70.71; H, 5.21; N, 12.35%.

*4.1.7. 3-[(5,6-Dimethyl-1,3-dihydro-2H-1,3-benzimidazol-2-yliden)(nitro)methyl]-2-(4-methoxybenzyl)-1-isoindolinone (**5g**)*

White powder; yield 86%, m.p. 230 °C. IR (KBr) (ν_{max}/cm^{-1}): 3433 and 3268 (NH), 1670 (C=O), 1616, 1573, 1471, 1417, 1367, 1330, 1300, 1243, 1173, 1104, 1045, 950, 925, 850, 820, 782, 742, 695. ^{1}H NMR (500.1 MHz, DMSO-d_6): δ 12.85–12.40 (br, 2H, NH), 7.73 (d, J = 8.0 Hz, 1H, CH), 7.50 (t, J = 7.0 Hz, 1H, CH), 7.46 (t, J = 7.3 Hz, 1H, CH), 7.37 (d, J = 8.0 Hz, 1H, CH), 7.28 (br s, 2H, 2CH), 6.95 (d, J = 8.5 Hz, 2H, 2CH), 6.61 (d, J = 8.5 Hz, 2H, 2CH), 5.64 (s, 1H, NCH), 4.84 and 3.96 (2d, ^2J = 14.7 Hz, 2H, NCH$_2$), 3.56 (s, 3H, OCH$_3$), 2.33 (s, 6H, 2CH$_3$). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 167.6 (NC=O), 158.2 (C), 147.3 (CN$_2$), 144.4, 133.7 and 132.5 (3C), 132.1 (br, 2C), 131.0 (CH), 129.1 (br, 2C), 128.9 (2CH), 127.5, 122.4 and 121.6 (3CH), 113.5 (2CH), 112.3 (br, 2CH), 100.4 (C–NO$_2$), 57.5 (NCH), 54.7 (OCH$_3$), 43.3 (NCH$_2$), 19.8 (2CH$_3$). EI-MS: m/z (%) = 456 (< 1) [M$^+$], 409 (2), 378 (4), 321 (4), 304 (17), 291 (100), 275 (10), 262 (25), 246

Table 2
Computed ADME properties of **5a–p** derivatives.

Compd	MW(g/mol)	HBA	HBD	nROTB	Lipinski, violation	TPSA(Å^2)	Log Po/w (iLOGP)	Log Po/w (MLOGP)
5a	398.41	3	2	4	Yes, 0	97.71	2.88	3.08
5b	458.47	5	2	6	Yes, 0	116.17	3.04	1.65
5c	432.86	3	2	4	Yes, 0	97.71	2.95	3.55
5d	478.88	5	2	5	Yes, 0	116.17	3.33	2.19
5e	350.37	3	2	4	Yes, 0	97.71	2.55	1.59
5f	440.49	3	2	4	Yes, 0	97.71	3.34	2.89
5g	456.49	4	2	5	Yes, 0	106.94	3.53	2.37
5h	350.37	3	2	4	Yes, 0	90.19	2.32	2.01
5i	364.40	3	2	4	Yes, 0	90.19	2.62	1.43
5j	442.42	5	2	4	Yes, 0	116.17	2.92	2.66
5k	394.38	5	2	4	Yes, 0	108.65	2.54	1.61
5l	428.41	6	2	6	Yes, 0	108.65	2.59	1.00
5m	416.40	4	2	4	Yes, 0	97.71	3.01	3.45
5n	476.46	6	2	6	Yes, 0	116.17	3.23	2.02
5o	368.36	4	2	4	Yes, 0	90.19	2.60	2.39
5p	454.43	7	2	6	Yes, 1 N or O > 10	127.11	3.04	0.25

(13), 233 (10), 219 (12), 207 (4), 188 (11), 171 (25), 156 (29), 133 (21), 121 (27), 104 (21), 91 (19), 77 (29), 65 (11). Anal. Calcd for $C_{26}H_{24}N_4O_4$ (456.50): C, 68.41; H, 5.30; N, 12.27. Found: C, 68.24; H, 5.26; N, 12.03%.

*4.1.8. 2-Benzyl-3-[nitro(tetrahydro-2H-imidazol-2-yliden)methyl]-1-isoindolinone **(5h)***

White powder; yield: 70%, m.p. 158 °C. IR (KBr) (ν_{max}/cm^{-1}): 3406 and 3337 (NH), 1685 (C═O), 1589, 1531, 1468, 1415, 1383, 1287, 1241, 1185, 1128, 1095, 1002, 931, 905, 822, 746, 700. ^{1}H NMR (500.1 MHz, DMSO-d_6): δ 8.80–8.30 (br, 2H, 2NH), 7.68 (d, J = 7.0 Hz, 1H, CH), 7.49 (t, J = 7.5 Hz, 1H, CH), 7.42 (t, J = 7.5 Hz, 1H, CH), 7.30–7.19 (m, 6H, 6CH), 5.40 (s, 1H, NCH), 4.79 and 4.05 (2d, ^2J = 15.3 Hz, 2H, NCH_2), 3.80–3.40 (m, 4H, $2CH_2$). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 167.6 (NC═O), 160.9 (CN_2), 144.8, 137.6 and 132.7 (3C), 131.0 (CH), 128.2 (2CH), 127.6 (2CH), 127.3, 126.9, 122.3 and 121.3 (4CH), 100.6 (C–NO_2), 57.9 (NCH), 43.7 (CH_2CH_2), 43.3 (NCH_2). EI-MS: m/z (%) = 350 (1) [M$^+$], 286 (3), 257 (5), 236 (8), 213 (4), 185 (5), 167 (6), 149 (22), 137 (13), 123 (13), 111 (16), 97 (28), 81 (52), 69 (100), 57 (56), 43 (74). Anal. Calcd for $C_{19}H_{18}N_4O_3$ (350.38): C, 65.13; H, 5.18; N, 15.99. Found: C, 64.95; H, 5.25; N, 15.80%.

*4.1.9. 2-(4-Methylbenzyl)-3-[nitro(tetrahydro-2H-imidazol-2-yliden)methyl]-1-isoindolinone **(5i)***

White powder; yield: 75%, m.p. 218 °C. IR (KBr) (ν_{max}/cm^{-1}): 3404 and 3332 (NH), 1691 (C═O), 1597, 1517, 1467, 1412, 1347, 1281, 1143, 1090, 999, 948, 916, 801, 749, 701, 671, 617. ^{1}H NMR (500.1 MHz, DMSO-d_6): δ 8.80–8.25(br, 2H, 2NH), 7.68 (d, J = 7.0 Hz, 1H, CH), 7.49 (t, J = 7.0 Hz, 1H, CH), 7.42 (t, J = 7.5 Hz, 1H, CH), 7.27 (d, J = 7.0 Hz, 1H, CH), 7.09 (d, J = 8.0 Hz, 2H, 2CH), 7.07 (d, J = 8.0 Hz, 2H, 2CH), 5.36 (s, 1H, NCH), 4.78 and 3.96 (2d, ^2J = 15.0 Hz, 2H, NCH_2), 3.70–3.40 (m, 4H, $2CH_2$), 2.26 (s, 3H, CH_3). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 167.5 (NC═O), 160.9 (CN_2), 144.8, 136.0, 134.7 and 132.7 (4C), 131.0 (CH), 128.8 (2CH), 127.7 (2CH), 127.3, 122.3 and 121.4 (3CH), 100.6 (C–NO_2), 57.8 (NCH), 43.4 (br, CH_2CH_2), 43.1 (NCH_2), 20.7 (CH_3). EI-MS: m/z (%) = 364 (3) [M$^+$], 347 (63), 329 (32), 316 (47), 297 (5), 282 (9), 251 (73), 236 (56), 213 (19), 198 (20), 160 (12), 130 (20), 120 (39), 105 (100), 91 (33), 77 (45). Anal. Calcd for $C_{20}H_{20}N_4O_3$ (364.40): C, 65.92; H, 5.53; N, 15.37. Found: C, 65.78; H, 5.46; N, 15.30%.

*4.1.10. 2-{(Benzo[d][1,3]dioxol-6-yl)methyl}-3-[1,3-dihydro-2H-1,3-benzimidazol-2-yliden(nitro)methyl]-1-isoindolinone **(5j)***

White powder; yield: 81%, m.p. 242 °C. IR (KBr) (ν_{max}/cm^{-1}): 3117 and 3026 (NH), 1675 (C═O), 1620, 1562, 1494, 1448, 1413, 1296, 1234, 1172, 1127, 1094, 1063, 1037, 981, 932, 878, 851, 825, 805, 738, 692, 657. ^{1}H NMR (500.1 MHz, DMSO-d_6): δ 13.20–12.46 (br, 2H, 2NH), 7.75 (d, J = 7.4 Hz, 1H, CH), 7.55–7.44 (m, 4H, 4CH), 7.42 (d, J = 7.4 Hz, 1H, CH), 7.34–7.28 (m, 2H, 2CH), 6.62 (s, 1H, CH), 6.57 (d, J = 7.9 Hz, 1H, CH), 6.51 (d, J = 7.6 Hz, 1H, CH), 5.88 (s, 1H, NCH), 5.75 and 5.72 (2 s, 2H, OCH_2O), 4.80 and 4.03 (2d, ^2J = 15.0 Hz, 2H, NCH_2). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 168.2 (NC═O), 148.5 (CN_2), 147.6, 146.6, 144.9 and 133.3 (4C), 131.7 (2C), 131.6 (CH), 131.2 (C), 128.1 (CH), 124.0 (2CH), 123.0, 122.1 and 121.4 (3CH), 112.4 (br, 2CH), 108.6 and 108.3 (2CH), 101.2 (OCH_2O), 101.1 (C–NO_2), 58.0 (NCH), 44.2 (NCH_2). EI-MS: m/z (%) = 442 (< 1) [M$^+$], 395 (12), 366 (2), 281 (12), 265 (29), 247 (17), 219 (17), 150 (10), 135 (100), 105 (10), 77 (29), 51 (10). Anal. Calcd for $C_{24}H_{18}N_4O_5$ (442.43): C, 65.15; H, 4.10; N, 12.66. Found: C, 65.07; H, 3.97; N, 12.58%.

*4.1.11. 2-{(Benzo[d][1,3]dioxol-6-yl)methyl}-3-[nitro(tetrahydro-2H-imidazol-2-yliden)methyl]-1-isoindolinone **(5k)***

White powder; yield: 75%, m.p. 206 °C. IR (KBr) (ν_{max}/cm^{-1}): 3404 and 3320 (NH), 1672 (C═O), 1588, 1527, 1487, 1440, 1405, 1347, 1280, 1241, 1206, 1170, 1147, 1086, 1038, 996, 949, 910, 870, 825, 799, 764, 748, 703. ^{1}H NMR (500.1 MHz, DMSO-d_6): δ 9.20–7.99 (br, 2H, 2NH), 7.70 (d, J = 7.4 Hz, 1H, CH), 7.51 (t, J = 7.4 Hz, 1H, CH), 7.44 (t, J = 7.4 Hz, 1H, CH), 7.29 (d, J = 7.4 Hz, 1H, CH), 6.84 (d, J = 7.8 Hz, 1H, CH), 6.74 (s, 1H, CH), 6.68 (d, J = 7.8 Hz, 1H, CH), 5.98 and 5.97 (2 s, 2H, OCH_2O), 5.39 (s, 1H, NCH), 4.71 and 4.00 (2d, ^2J = 15.3 Hz, 2H, NCH_2), 3.68 (br s, 4H, $2CH_2$). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 168.0 (NC═O), 161.4 (CN_2), 147.7, 146.7, 145.3, 133.2 and 132.0 (5C), 131.6, 127.9, 122.9, 121.9, 121.6, 108.6 and 108.4 (7CH), 101.3 (OCH_2O), 101.2 (C–NO_2), 58.3 (NCH), 43.97 (br, CH_2CH_2), 43.89 (NCH_2). EI-MS: m/z (%) = 394 (< 1) [M$^+$], 368 (15), 280 (26), 265 (8), 254 (15), 239 (9), 183 (8), 167 (9), 150 (20), 135 (31), 124 (18), 111 (18), 97 (42), 83 (45), 69 (60), 57 (83), 43 (100). Anal. Calcd for $C_{20}H_{18}N_4O_5$ (394.39): C, 60.91; H, 4.60; N, 14.21. Found: C, 60.77; H, 4.46; N, 14.08%.

*4.1.12. 2-(4-Fluorobenzyl)-6,7-dimethoxy-3-[nitro(tetrahydro-2H-imidazol-2-yliden)methyl]-1-isoindolinone **(5l)***

White powder; yield: 72%, m.p. 192 °C. IR (KBr) (ν_{max}/cm^{-1}): 3409 and 3238 (NH), 1657 (C═O), 1590, 1536, 1493, 1433, 1396, 1352, 1305, 1259, 1221, 1153, 1097, 1077, 1041, 999, 974, 938, 897, 847, 811, 768, 726, 659. ^{1}H NMR (500.1 MHz, DMSO-d_6): δ 9.10–8.05 (br, 2H, 2NH), 7.22 (dd, $^3J_{HH}$ = 8.1, $^4J_{FH}$ = 6.0 Hz, 2H, 2CH), 7.15 (d, J = 8.2 Hz, 1H, CH), 7.13 (dd, $^3J_{HH}$ = 8.8, $^3J_{FH}$ = 8.8 Hz, 2H, 2CH), 6.91 (d, J = 8.2 Hz, 1H, CH), 5.26 (s, 1H, NCH), 4.66 and 4.09 (2d, ^2J = 15.2 Hz, 2H, NCH_2), 3.91 and 3.81 (2 s, 6H, $2OCH_3$), 3.66 (br s, 4H, $2CH_2$). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 166.4 (NC═O), 162.0 (d, $^1J_{FC}$ = 174.3 Hz, C–F), 160.8 (CN_2), 152.0, 146.4 and 138.4 (3C), 134.4 (d, $^4J_{FC}$ = 2.7 Hz, C), 130.1 (d, $^3J_{FC}$ = 8.2 Hz, 2CH), 125.2 (C), 116.8 and 116.6 (2CH), 115.4 (d, $^2J_{FC}$ = 21.4 Hz, 2CH), 101.5 (C–NO_2), 62.1 (OCH_3), 57.3 (NCH), 56.8 (OCH_3), 43.8 (br, CH_2CH_2), 43.5 (NCH_2). EI-MS: m/z (%) = 428 (< 1) [M$^+$], 411 (19), 393 (24), 380 (25), 350 (6), 288 (6), 272 (5), 258 (7), 202 (12), 109 (100), 83 (12), 44 (18). Anal. Calcd for $C_{21}H_{21}FN_4O_5$ (428.42): C, 58.87; H, 4.94; N, 13.08. Found: C, 58.96; H, 5.00; N, 12.85%.

*4.1.13. 2-(4-Fluorobenzyl)-3-[1,3-dihydro-2H-1,3-benzimidazol-2-yliden(nitro)methyl]-1-isoindolinone **(5m)***

White powder; yield: 83%, m.p. 234 °C. IR (KBr) (ν_{max}/cm^{-1}): 3404 and 3245 (NH), 1668 (C═O), 1620, 1565, 1507, 1464, 1421, 1388, 1334, 1300, 1221, 1179, 1149, 1096, 1057, 1024, 1003, 949, 912, 882, 850, 825, 740, 695, 671. ^{1}H NMR (500.1 MHz, DMSO-d_6): δ 13.15–12.55 (br, 2H, 2NH), 7.76 (d, J = 7.3 Hz, 1H, CH), 7.61–7.50 (m, 3H, 3CH), 7.48 (t, J = 7.3 Hz, 1H, CH), 7.43 (d, J = 7.3 Hz, 1H, CH), 7.35–7.28 (m, 2H, 2CH), 7.11 (dd, $^3J_{HH}$ = 8.1, $^4J_{FH}$ = 5.8 Hz, 2H, 2CH), 6.92 (dd, $^3J_{HH}$ = 8.6, $^3J_{FH}$ = 8.6 Hz, 2H, 2CH), 5.77 (s, 1H, NCH), 4.78 and 4.25 (2d, ^2J = 15.3 Hz, 2H, NCH_2). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 168.3 (NC═O), 161.7 (d, $^1J_{FC}$ = 242.5 Hz, C–F), 148.5 and 144.9 (2C), 134.2 (d, $^4J_{FC}$ = 2.7 Hz, C), 133.2 (C), 131.7 (2C), 131.3 (CH), 129.9 (d, $^3J_{FC}$ = 8.1 Hz, 2CH), 128.1 (CH), 124.1 (2CH), 123.0 and 122.1 (2CH), 115.3 (d, $^2J_{FC}$ = 21.3 Hz, 2CH), 112.4 (br, 2CH), 101.1 (C–NO_2), 58.3 (NCH), 43.7 (NCH_2). EI-MS: m/z (%) = 416 (< 1) [M$^+$], 369 (3), 291 (3), 261 (4), 240 (70), 219 (7), 143 (6), 124 (6), 109 (100), 83 (6), 63 (3). Anal. Calcd for $C_{23}H_{17}FN_4O_3$ (416.41): C, 66.34; H, 4.11; N, 13.45. Found: C, 66.26; H, 4.03; N, 13.24%.

*4.1.14. 2-(4-Fluorobenzyl)-3-[1,3-dihydro-2H-1,3-benzimidazol-2-yliden(nitro)methyl]-6,7-dimethoxy-1-isoindolinone **(5n)***

White powder; yield: 79%, m.p. 195 °C. IR (KBr) (ν_{max}/cm^{-1}): 3358 and 3274 (NH), 1674 (C═O), 1621, 1562, 1490, 1463, 1422, 1377, 1329, 1301, 1263, 1229, 1190, 1151, 1079, 1048, 1010, 954, 920, 823, 753, 685. ^{1}H NMR (500.1 MHz, DMSO-d_6): δ 13.10–12.26 (br, 2H, 2NH), 7.52–7.44 (br s, 2H, 2CH), 7.21–7.13 (m, 4H, 4CH), 7.11 (d, J = 8.1 Hz, 1H, CH), 6.96 (dd, $^3J_{HH}$ = 8.8, $^3J_{FH}$ = 8.8 Hz, 2H, 2CH), 6.94 (d, J = 8.1 Hz, 1H, CH), 6.09 (s, 1H, NCH), 4.74 and 4.07 (2d,

^2J = 15.3 Hz, 2H, NCH_2), 3.93 and 3.79 (2 s, 6H, $2OCH_3$). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 166.7 (NC=O), 161.6 (d, $^1J_{FC}$ = 242.2 Hz, C—F), 151.9 (C), 150.6 (CN_2), 145.6 (C), 134.7 (d, $^4J_{FC}$ = 2.3 Hz, C), 131.7 and 130.8 (2C), 130.0 (d, $^3J_{FC}$ = 8.1 Hz, 2CH), 125.6 (C), 122.3 (2CH), 117.1 and 116.6 (2CH), 115.2 (d, $^2J_{FC}$ = 21.0 Hz, 2CH), 113.1 (br, 2CH), 103.9 (C—NO_2), 62.1 (OCH_3), 57.6 (NCH), 56.8 (OCH_3), 43.7 (NCH_2). EI-MS: *m/z* (%) = 476 (< 1) [M^+], 417 (8), 402 (2), 298 (39), 280 (34), 253 (100), 238 (9), 223 (57), 208 (77), 193 (22), 168 (18), 140 (9), 119 (12), 90 (12), 63 (12). Anal. Calcd for $C_{25}H_{21}FN_4O_5$ (476.46): C, 63.02; H, 4.44; N, 11.76. Found: C, 62.93; H, 4.37; N, 11.64%.

4.1.15. 2-(4-Fluorobenzyl)-3-[nitro(tetrahydro-2H-imidazol-2-yliden)methyl]-1-isoindolinone (5o)

White powder; yield: 68%, m.p. 207 °C. IR (KBr) (ν_{max}/cm^{-1}): 3407 and 3233 (NH), 1671 (C=O), 1583, 1531, 1509, 1468, 1386, 1305, 1218, 1202, 1178, 1157, 1091, 990, 953, 905, 877, 827, 788, 740, 697, 670. ^1H NMR (500.1 MHz, DMSO-d_6): δ 9.20–7.95 (br, 2H, 2NH), 7.70 (d, *J* = 7.4 Hz, 1H, CH), 7.51 (t, *J* = 7.4 Hz, 1H, CH), 7.44 (t, *J* = 7.4 Hz, 1H, CH), 7.29 (d, *J* = 7.4 Hz, 1H, CH), 7.23 (dd, $^3J_{HH}$ = 8.4, $^4J_{FH}$ = 5.9 Hz, 2H, 2CH), 7.12 (dd, $^3J_{HH}$ = 8.9, $^3J_{FH}$ = 8.9 Hz, 2H, 2CH), 5.40 (s, 1H, NCH), 4.71 and 4.17 (2d, ^2J = 15.2 Hz, 2H, NCH_2), 3.67 (br s, 4H, $2CH_2$). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 168.1 (NC=O), 161.8 (d, $^1J_{FC}$ = 242.5 Hz, C—F), 161.4 (CN_2), 145.3 (C), 134.3 (d, $^4J_{FC}$ = 2.8 Hz, C), 133.1 (C), 131.6 (CH), 130.1 (d, $^3J_{FC}$ = 8.2 Hz, 2CH), 127.9, 122.9 and 121.8 (3CH), 115.4 (d, $^2J_{FC}$ = 21.5 Hz, 2CH), 101.1 (C—NO_2), 58.4 (NCH), 43.8 (br, CH_2CH_2), 43.5 (NCH_2). EI-MS: *m/z* (%) = 368 (2) [M^+], 351 (59), 333 (26), 320 (53), 292 (14), 250 (11), 239 (12), 228 (14), 211 (10), 198 (14), 171 (11), 130 (9), 109 (100), 96 (41), 83 (14), 44 (14). Anal. Calcd for $C_{19}H_{17}FN_4O_3$ (368.37): C, 61.95; H, 4.65; N, 15.21. Found: C, 61.87; H, 4.57; N, 15.05%.

4.1.16. 2-{(Benzo[d][1,3]dioxol-6-yl)methyl}-6,7-dimethoxy-3-[nitro(tetrahydro-2H-imidazol-2-yliden)methyl]-1-isoindolinone-1-isoindolinone (5p)

White powder; yield: 86%, m.p. 189 °C. IR (KBr) (ν_{max}/cm^{-1}): 3451 and 3387 (NH), 1684 (C=O), 1592, 1519, 1490, 1439, 1404, 1364, 1337, 1295, 1243, 1194, 1154, 1128, 1079, 1038, 996, 968, 822, 858, 816, 759, 727, 695, 662. ^1H NMR (500.1 MHz, DMSO-d_6): δ 9.10–8.10 (br, 2H, NH), 7.15 (d, *J* = 8.2 Hz, 1H, CH), 6.91 (d, *J* = 8.2 Hz, 1H, CH), 6.83 (d, *J* = 7.9 Hz, 1H, CH), 6.73 (s, 1H, CH), 6.66 (d, *J* = 7.8 Hz, 1H, CH), 5.98 and 5.97 (2 s, 2H, OCH_2O), 5.25 (s, 1H, NCH), 4.65 and 3.95 (2d, ^2J = 14.9 Hz, 2H, NCH_2), 3.91 and 3.81 (2 s, 6H, $2OCH_3$), 3.67 (br s, 4H, $2CH_2$). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 166.3 (NC=O), 161.4 (CN_2), 152.0, 147.7, 146.7, 146.4, 138.4, 132.0 and 125.3 (7C), 121.6, 116.9, 116.6, 108.6 and 108.4 (5CH), 101.6 (C—NO_2), 101.3 (OCH_2O), 62.1 (OCH_3), 57.2 (NCH), 56.8 (OCH_3), 44.0 (NCH_2), 43.9 (br, CH_2CH_2). EI-MS: *m/z* (%) = 454 (< 1) [M^+], 434 (8), 418 (12), 404 (8), 326 (6), 313 (9), 299 (9), 283 (9), 270 (7), 256 (7), 190 (8), 177 (8), 164 (7), 150 (22), 135 (100), 121 (11), 105 (10), 91 (7), 77 (24), 63 (9), 44 (20). Anal. Calcd for $C_{22}H_{22}N_4O_7$ (454.44): C, 58.15; H, 4.88; N, 12.33. Found: C, 58.01; H, 4.70; N, 12.24%.

4.2. Jack bean urease inhibitory activity

Urease inhibitory activity of compounds (5a-p) were performed according to procedures described in the literature [46,47]. All chemicals were obtained from Merck and were of analytical grade. Sodium nitroprusside and Jack bean urease (EC 3.5.1.5) was purchase from Sigma (St. Louis, MO, USA). Ultra-pure water (HPLC grade, Duksan, Korea) was utilized in all experiments. Potassium phosphate buffer solution (100 mM; pH 7.4) was prepared in doubled distilled water. The absorbance spectra were recorded on a Synergy H1 Hybrid multi-mode microplate reader ((BioTek, Winooski, VT, USA).

Urease inhibitory assay of the synthesized compounds **5a–p** were performed at the concentration of 0–10 mg/mL using the modified Berthelot spectrophotometric method and measured as the absorbance at 625 nm. In each set of experiments the assay was performed in triplicate (n = 3). All results were compared with thiourea and hydroxyurea as the reference standard inhibitors. Briefly, the reaction mixtures, included urea (850 µL), compounds **5a–p** (100 µL, 0–10 mg/mL), and phosphate buffer (100 mM, pH 7.4) to obtain the total value of 985 µL. Then, 15 µL of the jack bean urease were added and incubated at 37 °C, the enzymatic reactions began and the jack bean urease activity was determined by measuring the released ammonia produced after 30 min. In the next step, 500 µL of phenol reagent (solution A; containing 5.0 g phenol and 25.0 mg sodium nitroprusside in 500 mL distilled water) and 500 µL alkali reagents (solution B; containing 2.5 g sodium hydroxide and 4.2 mL sodium hypochlorite (5% chlorine) in 500 mL distilled water) were moved to each well plate then added 100 µL of the incubated solution was added to the mixture of solutions (A,B), and again was incubated at 37 °C for 30 min. The absorbance was read at 625 nm, all tests were performed at least three times (n = 3).

Finally, the percentage of urease inhibition activity was calculated as:

$$\text{Inhibition}\,(\%) = [1 - (S/C)] \times 100$$

S is the absorbance of the evaluated sample (synthesized compounds **5a–p** or positive control) in the presence of enzyme, and C (control) is the absorbance of the solvent in the presence of enzyme. Data were expressed as mean ± standard error of the mean (SEM), and then the IC_{50} values were calculated using GraphPad Prism 5 software (GraphPad Software, Inc., SanDiego, CA) (Table 1).

4.3. Molecular docking study

Molecular docking studies were done for all the synthesized compounds **5a–p**. AutoDockTools (ADT) program 1.5.6. was used to prepare input files (protein and ligands) [48]. In the receptor all water molecules and ions were removed, non-polar hydrogens were merged and polar hydrogens were added and Kollman charges were assigned. The crystallographic structure of Jack bean urease enzyme was retrieved from PDB database (PDB ID: 3LA4) (www.rcsb.org) with resolution of 2.05 Å. The number of docking runs for each ligand was 50 runs. The grid spacing was 0.375 nm in each dimension, and to compute the grid maps, AutoGrid 4.2 was used with a grid box of 50 × 50 × 50 Å (X, Y and Z) points around the active site [48]. It should be noted that non-standard protein residues (KCX and CME) and the nickel ion were included in the binding site characterization. All of the ligand structure was sketched by Marvin sketch v.5.7 (Marvin package, Chemaxon Company) [49]. Docking with a maximum number of 25 × 10^6 energy evaluations were performed. The best conformation possessing lowest binding energy values, hydrogen bond and hydrophobic bond were analyzed and chosen the best docking pose by using LigPlot v.1.1.0 [50] and discovery studio v.3.5 [51].

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