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Synthesis, crystal structure, molecular docking studies and bio-evaluation of some N^4-benzyl-substituted isatin-3-thiosemicarbazones as urease and glycation inhibitors

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Abstract: Fifteen N^4-benzyl-substituted isatin-3-thiosemicarbazones **5a–o** were synthesized and evaluated for their urease and glycation inhibitory potential. *Lemna aequinocitalis* growth and *Artemia salina* assays were also done to determine their phytotoxic and toxic effects. All compounds are potent inhibitors of the urease enzyme, displaying inhibition [half maximal inhibitory concentration (IC_{50}) = 1.08 ± 0.12–11.23 ± 0.19 μM] superior to that of the reference inhibitor thiourea (IC_{50} = 22.3 ± 1.12 μM). Compounds **5c**, **5d**, **5h**, **5j,k** are potent antiglycating agents, showing glycation inhibitory activity better than that of the reference inhibitor rutin (IC_{50} values 209.87 ± 0.37–231.70 ± 6.71 vs. 294.5 ± 1.5 μM). In the phytotoxicity assay, 11 thiosemicarbazones **5a–d**, **5g**, **5h**, **5j–l**, **5n,o** are active, demonstrating 5–100% growth inhibition of *L. aequinocitalis* at the highest tested concentrations (1000 or 500 μg/mL). In the brine shrimp (*A. salina*) lethality bioassay, three derivatives **5b**, **5j** and **5o** are active with median lethal dose (LD_{50}) values of 3.63×10^{-5}, 2.90×10^{-5} and 2.31×10^{-4} M, respectively.

Keywords: antiglycation; antiurease; docking studies; heterocycles; isatin derivatives.

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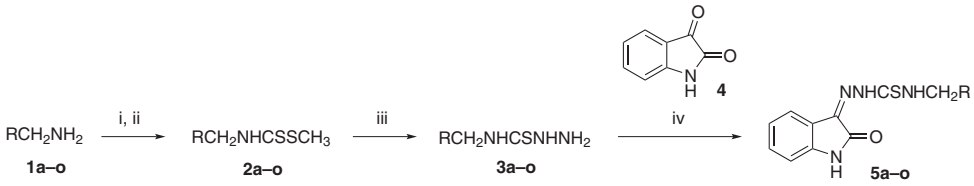
Introduction

Isatin and its derivatives, including thiosemicarbazones exhibit a variety of biological properties [1–25] such as antiulcer [1], anticancer [1, 4, 7, 12, 21, 22], antimicrobial [1–3, 5, 7, 22], antitubercular [23–25], antiviral [1, 2, 5, 7, 12, 25] and enzymatic inhibition [1, 4, 11, 17] activities. We have recently reported the synthesis of a number of N^4-aryl-substituted isatin-3-thiosemicarbazones (thiourea derivatives) as antimicrobial [26, 27], cytotoxic [27–29], phytotoxic [28, 29] and antiurease [27, 28] agents. The structure-activity relationship studies have revealed that in some cases, the kind, number and position of substituents on the phenyl ring attached to the N^4 atom of the thiosemicarbazone moiety play a key role in the biological properties, including urease inhibition. Several thiourea derivatives demonstrate promising antiglycation activity [30–34] and certain isatin-imines (Schiff bases) are potent inhibitors of glycation [35, 36]. Stimulated by the above observations and in continuation of our efforts [37–44] in search of novel isatin-based bioactive organic molecules with diverse pharmacological properties, we have synthesized 15 new title thiosemicarbazones and evaluated them for the first time for urease and glycation inhibitory effects. *Lemna aequinocitalis* growth and *Artemia salina* assays were also done to determine their phytotoxic and toxic influences, respectively.

Results and discussion

Chemistry of compounds 5a–o

Appropriate *N*-substituted thiosemicarbazides **3** were allowed to react with isatin **4** in aqueous ethanol to give the corresponding isatin-3-thiosemicarbazones **5a–o** (Scheme 1) in good to excellent yields (73–95%). Compounds **5a** and **5k** have been reported previously [45, 46]. The structures of the synthesized thiosemicarbazones were confirmed by analytical and spectral data. In addition, X-ray crystallographic analysis for compound **5h** was



a: R = Ph
b: R = 2-$CH_3C_6H_4$
c: R = 3-$CH_3C_6H_4$
d: R = 4-$CH_3C_6H_4$
e: R = 2-$CH_3OC_6H_4$
f: R = 3-$CH_3OC_6H_4$
g: R = 4-$CH_3OC_6H_4$
h: R = 2-FC_6H_4
i: R = 3-FC_6H_4
j: R = 4-FC_6H_4
k: R = 2-ClC_6H_4
l: R = 3-ClC_6H_4
m: R = 4-ClC_6H_4
n: R = 2,4-$(Cl)_2C_6H_3$
o: R = 3,4-$(Cl)_2C_6H_3$

Scheme 1 Reagents and conditions: (i) Et_3N, MeOH, CS_2, 30°C, 75 min, then r.t., 1 h; (ii) CH_3I, MeOH, –10°C, 20 min, then r.t., 2 h; (iii) $NH_2NH_2{\cdot}H_2O$, EtOH, reflux, 2 h; (iv) 50% EtOH, reflux, 2 h.

conducted. The spectral data are in line with the relevant literature [22, 24, 47, 48].

X-ray crystallographic analysis of compound 5h

Quality crystals of **5h** (Table S1) were cultivated by slow evaporation of the solution in $CHCl_3$. The X-ray analysis shows that compound **5h** crystallizes in monoclinic lattice with the $P2_1/c$ space group. The molecular structure [Oak Ridge Thermal-Ellipsoid Plot (ORTEP) diagram] of **5h** along with crystallographic numbering scheme is presented in Figure 1. The central thiosemicarbazone (*N*-iminothiourea) moiety is nearly planar adopting the S-*cis*/S-*trans* conformation, most probably due to the formation of intramolecular hydrogen bond [N(4)-H(4A)···N(2) 2.269 Å] [46, 49, 50]. The dihedral angles between S(1)-C(9)-N(3)-H(3A), S(1)-C(9)-N(4)-H(4A) and C(9)-N(3)-N(2)-C(8) are –6.45°, –174.20° and 178.73°, respectively. This planarity around the central thiosemicarbazone moiety may be attributed to substantial delocalization of N lone electron pairs onto the thiocarbonyl (C=S) group; this is clearly manifested by short N-C bond lengths [N(3)-C(9) 1.366(3) Å and N(4)-C(9) 1.333(3) Å], signifying the partial double bond character of the N-C bonds. The slightly longer N-C bond is directly connected to the imino (-C=N-) function of the thiosemicarbazone moiety.

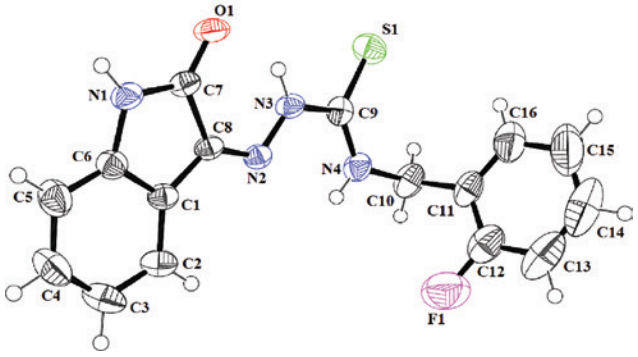


Figure 1 The ORTEP diagram of **5h**.

In crystal, compound **5h** forms a centrosymmetric amide dimer as the major supramolecular interaction, dominating over a thioamide dimer. The three-dimensional (3D) network structure of **5h** is composed of various one-dimensional (1D) tapes (Figure 2A), driven by supramolecular centrosymmetric amide [N(1)-H(1)···O(1) 2.05 Å] and centrosymmetric CH···F [C(4)-H(4)···F(1) 2.61 Å] interactions. These tapes are stacked on each other by means of CH···O [C(2)-H(2)···O(1) 2.71 Å] and π···π [C(6)···C(8) 3.38 Å, C(1)···C(5) 3.34 Å] interactions [51] to provide an overall 3D-network (Figure 2B and C).

In vitro antiurease activity

The N^4-benzyl-substituted isatin-3-thiosemicarbazones **5a–o** were evaluated for their antiurease activity against jack bean urease. Thiourea served as a reference inhibitor in this assay. All compounds are potent inhibitors of the enzyme, exhibiting inhibitory activity [half maximal inhibitory concentration (IC_{50}) = 1.08 ± 0.12–11.23 ± 0.19 μM], which is better than the activity of the reference inhibitor thiourea (IC_{50} = 22.3 ± 1.12 μM) (Table 1). In comparison to compound **5a** having no substituent in the phenyl ring of the benzyl group attached to N^4 of the thiosemicarbazone moiety, all other compounds demonstrate enhanced enzymatic activity (IC_{50} values 1.08 ± 0.12–7.97 ± 0.14 vs. 11.23 ± 0.19 μM). Compound **5f** bearing a methoxy substituent at position 3 of the phenyl ring is the most potent urease inhibitor of the series, displaying 10- and 20-fold more activity than compound **5a** and the reference inhibitor thiourea (IC_{50} = 1.08 ± 0.12 vs. 11.23 ± 0.19 and 22.3 ± 1.12 μM, respectively). The other methoxy-substituted compounds **5e** and **5g** having the substituent at positions 2 and 4 of the phenyl ring are less active

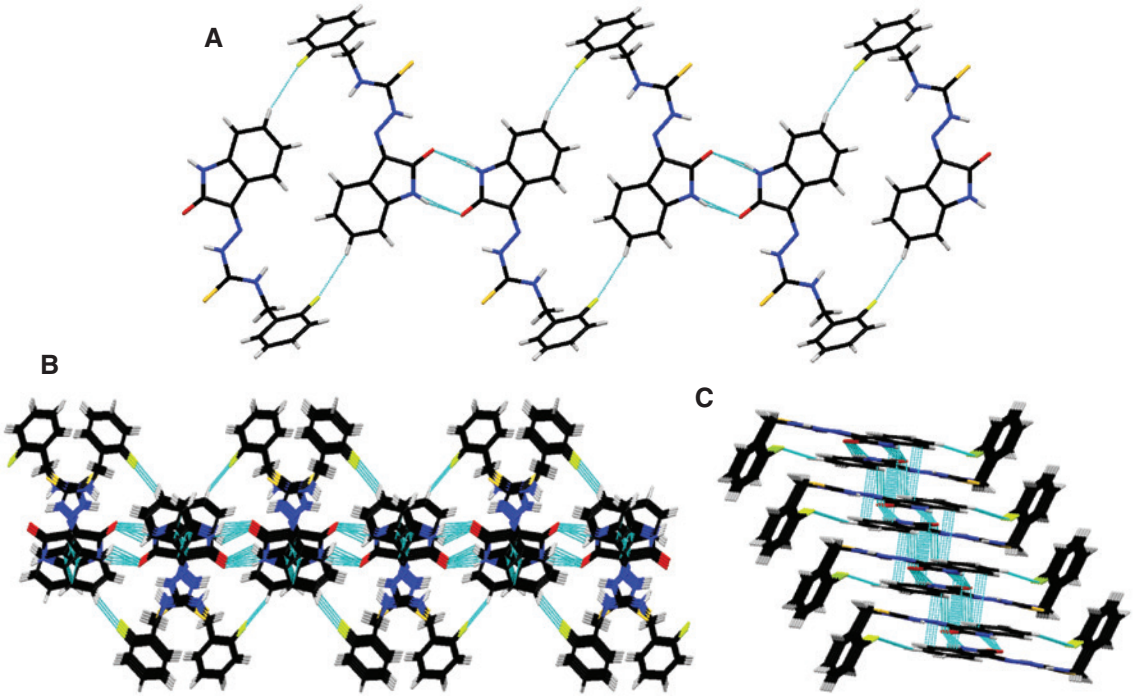


Figure 2 The crystal packing of compound **5h**. (A) 1D-tapes viewed along the c-axis, (B) 3D-network viewed along the c-axis, (C) 3D-network viewed along the b-axis.

Table 1 Inhibition of urease by compounds **5a–o**.

Compound	$IC_{50} \pm SEM$ (μm)
5a	11.23 ± 0.19
5b	1.22 ± 0.17
5c	1.62 ± 0.13
5d	2.17 ± 0.11
5e	1.51 ± 0.26
5f	1.08 ± 0.12
5g	3.11 ± 0.13
5h	7.97 ± 0.14
5i	2.62 ± 0.15
5j	1.38 ± 0.15
5k	2.53 ± 0.06
5l	2.32 ± 0.21
5m	3.46 ± 0.18
5n	3.34 ± 0.14
5o	2.69 ± 0.12
Thiourea[a]	22.3 ± 1.06

SEM, Standard error of the mean. [a]Reference inhibitor of urease enzyme.

with IC_{50} values of 1.51 ± 0.26 and 3.11 ± 0.13 μM, respectively. The next most potent antiurease compound is **5b** possessing the methyl substituent at position 2 of the phenyl ring. This compound exhibits slightly lower inhibitory activity than **5f** but is more active than the reference inhibitor thiourea (IC_{50} value 1.22 ± 0.17 vs. 1.08 ± 0.12 and 22.3 ± 1.12 μM, respectively). Comparison of the antiurease activity of compound **5b** with that of closely related methyl-substituted compounds **5c** and **5d** having the substituent at positions 3 and 4 of the phenyl ring, respectively, reveals that methyl substitution at position 2 of the phenyl is more favorable than at positions 3 and 4. Compound **5c** is less active than **5b** but considerably more active than **5d** (IC_{50} value 1.62 ± 0.13 vs. 1.22 ± 0.17 and 2.17 ± 0.11 μM, respectively). The N^4-benzyl-substituted isatin-thiosemicarbazones tested in the present assay, **5a–i** and **5k–m**, are more active in comparison to the corresponding N^4-phenyl-substituted isatin-thiosemicarbazones tested in our earlier assays [27, 28].

In vitro antiglycation activity

The thiosemicarbazones **5a–o** were further evaluated for their glycation inhibitory potential using rutin as a reference inhibitor. Seven compounds **5b–d**, **5f**, **5h**, **5j**,**k**, proved to be potent inhibitors of glycation. Compounds **5c**, **5d**, **5h**, **5j**,**k** show antiglycation activity even better than the reference inhibitor rutin (Table 2). Compound **5d** bearing a methyl substituent at position 4 of the phenyl ring attached to N^4 of the thiosemicarbazone moiety is the most potent antiglycating agent of the series, displaying inhibition of glycation with IC_{50} value of 209.87 ± 0.37 μM.

Table 2 Antiglycation activity of compounds **5a–o**.

Compound	Concentration (μm)	Inhibition (%)	IC_{50} ± SEM (μm)
5a	500	40.98	
5b	1000	62.89	605.26 ± 2.24
5c	500	57.97	231.70 ± 6.71
5d	500	70.12	209.87 ± 0.37
5e	1000	18.26	
5f	1000	64.72	522.68 ± 9.10
5g	125	28.45	
5h	1000	60.26	224.34 ± 0.47
5i	1000	25.54	
5j	250	53.67	217.94 ± 1.98
5k	1000	59.78	228.55 ± 2.30
5l	125	13.71	
5m	500	10.32	
5n	500	42.04	
5o	1000	39.57	
Rutin[a]	1000	86.00	294.5 ± 1.50

[a]Standard inhibitor of glycation.

The isomer **5c** having the substituent at position 3 of the phenyl ring has the IC_{50} value of 231.70 ± 6.7 μM. This compound is significantly more active than the corresponding methoxy-substituted derivative **5f**. The next most potent antiglycating agent is compound **5j** possessing a fluoro substituent at position 4 of the phenyl ring. This compound exhibits slightly less inhibition than the most active derivative **5d** but a great deal more than the reference inhibitor rutin. Overall, compounds with the CH_3, F and Cl substitutions cause effective inhibition of glycation.

In vitro phytotoxicity

Phytotoxic potential of compounds **5a–o** was determined by *L. aequinocitalis* growth bioassay. Eleven compounds, **5a–d**, **5g**, **5h**, **5j–l**, **5n,o**, are active in this assay, displaying weak or non-significant (5–100%) growth inhibition of *L. aequinocitalis* at the highest tested concentrations (1000 or 500 μg/mL) in comparison to the standard drug, paraquat, which shows 100% plant growth inhibition at 0.015 μg/mL concentration.

In vitro toxicity

Toxicity potential of compounds **5a–o** was determined by a brine shrimp (*A. salina*) lethality bioassay, using etoposide (a standard anticancer drug) as a reference. Compounds **3b**, **3j** and **3o** are active in this assay, demonstrating moderate to weak toxicity [median lethal dose (LD_{50}) = 3.63×10^{-5}, 2.90×10^{-5} and 2.31×10^{-4} M, respectively]. The remaining compounds gave LD_{50} values >2.64–3.22×10^{-4} M and, therefore, can be considered to be almost inactive.

Molecular docking studies of compounds 5a–o

Molecular docking studies were performed as previously reported [52–58]. Docking studies of compound **5f** against urease was carried out to gain further structural insights of the binding mode and possible interactions with the active site of the enzyme. Binding mode analysis of compound **5f** with urease, selected after Hyde assessment, is given in Figure 3. Docking studies support the experimental results that inhibitors bind with the catalytic site of the enzyme. The interactions within the active pocket of the receptor for its ligand are very important in the design of inhibitors. The π-stacked interactions are suggested for 3-methoxyphenyl ring of the inhibitor in the active site with residues His519, His593 and Arg609. 2-Oxoindolin-3-ylidene moiety is oriented in the hydrophobic pocket of the active site, where amino acid residues Ala636 and Met637 are present. Moreover, polar interactions are observed by the central thiosemicarbazone moiety and the amino acid residues Arg439, Ala436 and Ala440.

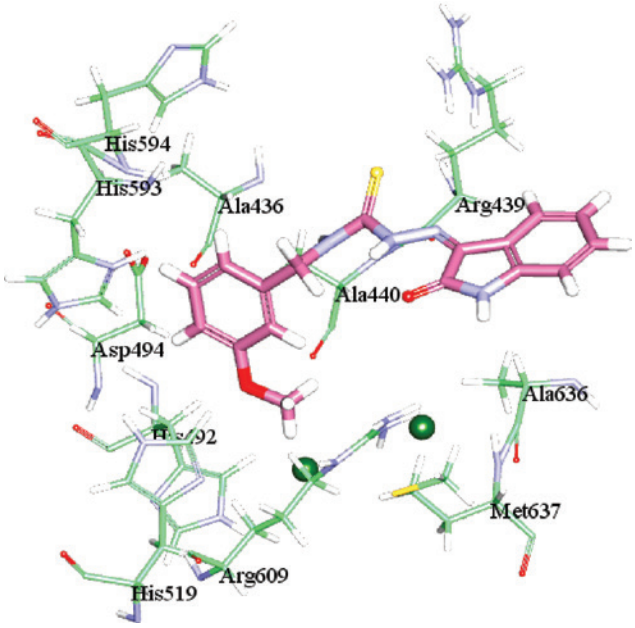


Figure 3 A possible binding mode of compound **5f** to urease. Carbon atoms of **5f** are colored pink, while that of protein are light green; oxygen, sulfur and nitrogen atoms are colored red, yellow and blue, respectively. The two nickel ions in the active site are represented as small dark-green spheres.

Conclusions

All compounds **5a–o** are extremely potent inhibitors of the urease enzyme. In addition, compounds **5c**, **5d**, **5h**, **5j,k** inhibit glycation quite convincingly. The glycation inhibitory activity of N^4-benzyl-substituted isatin-3-thiosemicarbazones is described here for the first time.

Experimental

Melting points (uncorrected) were determined on a Fisher-Johns melting point apparatus. Elemental analyses were performed on a Leco CHNS-9320 (USA) elemental analyzer. Fourier transform-infrared (FT-IR) spectra (KBr discs) were recorded on a Shimadzu Prestige-21 FT-IR spectrophotometer. Proton nuclear magnetic resonance (^{1}H NMR) spectra were measured in dimethyl sulfoxide-d6 (DMSO-d_6) on Bruker Spectrospin 300 and Bruker AVANCE 400 spectrometers operating at 300 MHz and 400 MHz, respectively. Carbon-13 nuclear magnetic resonance (^{13}C NMR) spectra were recorded in DMSO-d_6 on Bruker AVANCE 300 operating at 75 MHz. Electron impact mass spectra were obtained at 70 eV on MAT312 and JEOL JMS-600 mass spectrometers. X-ray crystallographic data were taken on a Bruker Kappa APEXII charge coupled device (CCD) instrument. Compounds **5a** and **5k** have been reported previously [45, 46].

Synthesis of isatin-thiosemicarbazones 5a–o

N-substituted thiosemicarbazides **3a–o** were synthesized in accordance with the literature route [59].

A solution of thiosemicarbazide **3** (5 mmol) in ethanol (10 mL) was added to a hot solution of isatin **4** (5 mmol) in 50% aqueous ethanol (15 mL), and the mixture was heated under reflux for 2 h. The resultant crystalline precipitate was filtered and washed with hot aqueous ethanol to afford the desired compounds **5a–o**.

***N*-(2-Methylbenzyl)-2-[2-oxo-2,3-dihydro-1*H*-indol-3-ylidene]-1-hydrazinecarbothioamide (5b)** Yellow crystals; yield 73%; mp 240°C; IR: 3304, 3190 (NH), 1687 (C=O), 1616 (C=N), 1168 cm^{-1} (C=S); ^{1}H NMR: δ 2.50 (s, 3H, CH_3), 4.74 (d, J = 6.0 Hz, 2H, benzyl CH_2), 6.92 (d, J = 8.0 Hz, 1H, isatin C7-H), 7.02–7.26 (m, 5H, benzyl C3-H, C4-H,C5-H,C6-H, isatin C5-H), 7.28–7.40 (m, 1H, isatin C6-H), 7.72 (d, J = 7.6 Hz, 1H, isatin C4-H), 9.77 (t, J = 6.0 Hz, 1H, CSNH), 11.30 (s, 1H, isatin NH), 12.76 (s, 1H, NNH); ^{13}C NMR: δ 19.2 (CH_3), 45.6 (CH_2), 111.5, 120.4, 121.4, 122.8, 126.1, 127.1, 127.1, 127.6, 130.3, 131.7, 132.5, 137.8, 142.8, 163.1, 178.2; MS: m/z 324 (M$^+$, 4), 179 (37), 161 (100), 136 (20), 121 (46), 118 (10), 91 (69), 77 (22), 65 (13%). Anal. Calcd for $C_{17}H_{16}N_4OS$: C, 62.96; H, 4.94; N, 17.28. Found: C, 62.78; H, 4.83; N, 16.87.

***N*-(3-Methylbenzyl)-2-[2-oxo-2,3-dihydro-1*H*-indol-3-ylidene]-1-hydrazinecarbothioamide (5c)** Yellow crystals; yield 79%; mp 209°C; IR: 3358, 3248 (NH), 1693 (C=O), 1599 (C=N), 1149 cm^{-1} (C=S); ^{1}H NMR: δ 2.50 (CH_3), 3.30 (benzyl CH_2), 6.96 (d, J = 8.0 Hz, 1H, isatin C7-H), 7.15 (t, J = 7.6 Hz, 1H, isatin C5-H), 7.19–7.57 (m, 5H, benzyl C2-H, C4-H, C5-H, C6-H, isatin C6-H), 7.78 (d, J = 7.6 Hz, 1H, isatin C4-H), 10.75 (s, 1H, CSNH), 11.36 (s, 1H, isatin NH), 12.68 (s, 1H, NNH); ^{13}C NMR: δ 21.5 (CH_3), 47.7 (CH_2), 111.5, 120.4, 121.4, 122.8, 125.0, 128.1, 128.4, 128.7, 131.7, 132.5, 137.8, 138.8, 142.8, 163.1, 178.1; MS: m/z 324 (M$^+$, 6), 178 (28), 147 (79), 132 (10), 120 (77), 118 (74), 105 (100), 91 (65), 77 (75), 65 (36%). Anal. Calcd for $C_{17}H_{16}N_4OS$: C, 62.96; H, 4.94; N, 17.28. Found: C, 62.86; H, 4.89; N, 17.22.

***N*-(4-Methylbenzyl)-2-(2-oxo-2,3-dihydro-1*H*-indol-3-ylidene)-1-hydrazinecarbothioamide (5d)** Yellow fluffy crystals; yield 78%; mp 200°C; IR: 3300, 3196 (NH), 1683 (C=O), 1600 (C=N), 1153 cm^{-1} (C=S); ^{1}H NMR: δ 2.26 (s, 3H, CH_3), 4.80 (d, J = 6.0 Hz, 2H, benzyl CH_2), 6.89 (d, J = 8.8 Hz, 1H, isatin C7-H), 7.07 (t, J = 7.6 Hz, 1H, isatin C5-H), 7.13 (d, J = 7.6 Hz, 2H, benzyl C2-H, C6-H), 7.24 (d, J = 8.0 Hz, 2H, benzyl C3-H, C5-H), 7.34 (t, J = 7.6 Hz, 1H, isatin C6-H), 7.64 (d, J = 7.6 Hz, 1H, isatin C4-H), 9.73 (t, J = 6.0 Hz, 1H, CSNH), 11.18 (s, 1H, isatin NH), 12.62 (s, 1H, NNH); ^{13}C NMR: 21.2 (CH_3), 55.5, 111.5, 114.1, 120.4, 121.3, 122.7, 127.8, 129.3, 131.7, 132.4, 136.6, 142.8, 163.1, 178.0; MS: m/z 324 (M$^+$, 7), 194 (13), 178 (38), 161(20), 147 (100), 132 (13), 120 (67), 118 (62), 105 (91), 91 (38), 77 (39), 65 (14%). Anal. Calcd for $C_{17}H_{16}N_4OS$: C, 62.96; H, 4.94; N, 17.28. Found: C, 62.87; H, 4.89; N, 17.20.

***N*-(2-Methoxybenzyl)-2-[2-oxo-2,3-dihydro-1*H*-indol-3-ylidene]-1-hydrazinecarbothioamide (5e)** Yellow crystals; yield 76%; mp 205°C; IR: 3360, 3140 (NH), 1685 (C=O), 1598 (C=N), 1184 cm^{-1} (C=S); ^{1}H NMR: δ 3.83 (s, 3H, CH_3), 4.37 (d, J = 5.0 Hz, 2H, benzyl CH_2), 6.87 (d, J = 8.0 Hz, 1H, isatin C7-H), 6.91 (t, J = 8.4 Hz, 1H, benzyl C5-H), 7.01 (t, J = 8.0 Hz, 2H, benzyl C4-H, isatin C5-H), 7.21–7.27 (m, 2H, benzyl C3-H, C6-H), 7.33 (t, J = 8.0 Hz, 1H, isatin C6-H), 7.53 (t, J = 6 Hz, 1H, CSNH), 8.05 (d, J = 7.6 Hz, 1H, isatin C4-H), 10.30 (s, 1H, isatin NH), 10.67 (s, 1H, NNH); ^{13}C NMR: δ 55.8 (CH_2), 70.2 (CH_3), 110.8, 111.0, 116.1, 120.6, 122.0, 125.7, 127.3, 128.3, 128.7, 132.1, 134.0, 143.4, 155.4, 157.2, 165.2; MS: m/z 340 (M$^+$, 2), 235 (100), 221 (30), 178 (56), 163 (16), 145 (27), 120 (100), 118 (32), 105 (100), 93 (41), 77 (43), 58 (89%). Anal. Calcd for $C_{17}H_{16}N_4O_2S$: C, 60.00; H, 4.71; N, 16.47. Found: C, 59.89; H, 4.67; N, 16.39.

***N*-(3-Methoxybenzyl)-2-[2-oxo-2,3-dihydro-1*H*-indol-3-ylidene]-1-hydrazinecarbothioamide (5f)** Yellow fluffy crystals; yield 74%; mp 170°C; IR: 3350, 3194 (NH), 1686 (C=O), 1600 (C=N), 1151 cm^{-1} (C=S); ^{1}H NMR: δ 3.74 (s, 3H, CH_3), 4.85 (d, J = 4.5 Hz, 2H, benzyl CH_2), 6.83 (d, J = 9.0 Hz, 1H, isatin C7-H), 6.85–6.95 (m, 3H, benzyl C2-H, C4-H, C6-H), 7.09 (t, J = 9.0 Hz, 1H, benzyl C5-H), 7.26 (t, J = 9.0 Hz, 1H, isatin C5-H), 7.36 (t, J = 9.0 Hz, 1H, isatin C6-H), 7.65 (d, J = 8.0 Hz, 1H, isatin C4-H), 9.79 (t, J = 6.0 Hz, 1H, CSNH), 11.22 (s, 1H, isatin NH), 12.66 (s, 1H, NNH); ^{13}C NMR: δ 47.1 (CH_2), 55.0 (CH_3), 111.0, 112.2, 113.2, 119.5, 119.9, 120.9, 122.3, 129.3, 131.2, 132.0, 139.9, 142.3, 159.2, 162.6, 177.7; MS: m/z 340 (M$^+$, 7), 194 (24), 161 (18), 147 (93), 136 (92), 121 (95), 91 (100), 77 (95), 65 (55%). Anal. Calcd for $C_{17}H_{16}N_4O_2S$: C, 60.00; H, 4.71; N, 16.47. Found: C, 59.82; H, 4.67; N, 16.38.

***N*-(4-Methoxybenzyl)-2-[2-oxo-2,3-dihydro-1*H*-indol-3-ylidene]-1-hydrazinecarbothioamide (5g)** Yellow fluffy crystals; yield 82%; mp 230°C; IR: 3277, 3178 (NH), 1684 (C=O), 1605 (C=N), 1157 cm^{-1} (C=S); ^{1}H NMR: δ 3.72 (s, 3H, CH_3), 4.79 (d, J = 6.0 Hz, 2H, benzyl CH_2), 6.89 (d, J = 8.8 Hz, 2H, benzyl C3-H, C5-H), 6.91 (d, J = 8.0 Hz, 1H, isatin C7-H), 7.07 (t, J = 7.6 Hz, 1H, isatin C5-H), 7.30 (d, J = 8.8 Hz, 2H, benzyl C2-H, C6-H), 7.34 (td, J = 8.0, 0.8 Hz, 1H, isatin

C6-H), 7.64 (d, J = 7.6 Hz, 1H, isatin C4-H), 9.70 (t, J = 6.0 Hz, 1H, CSNH), 11.18 (s, 1H, isatin NH), 12.61 (s, 1H, NNH); ^{13}C NMR: 47.2 (CH_2), 55.5 (CH_3), 111.5, 114.1, 120.4, 121.3, 122.7, 129.3, 130.8, 131.7, 132.4, 142.8, 158.9, 163.1, 177.8; MS: m/z 340 (M^+, 15), 194 (55), 161 (47), 147 (52), 136 (52), 121 (100), 104 (13), 91 (17%). Anal. Calcd for $C_{17}H_{16}N_4O_2S$: C, 60.00; H, 4.71; N, 16.47. Found: C, 59.65; H, 4.66; N, 16.42.

***N*-(2-Fluorobenzyl)-2-[2-oxo-2,3-dihydro-1*H*-indol-3-ylidene]-1-hydrazinecarbothioamide (5h)** Yellow fluffy crystals; yield 79%; mp 210°C; IR: 3352, 3188 (NH), 1685 (C=O), 1610 (C=N), 1159 cm^{-1} (C=S); ^{1}H NMR: δ 4.92 (d, J = 6.0 Hz, 2H, benzyl CH_2), 6.93 (d, J = 8.0 Hz, 1H, isatin C7-H), 7.10 (t, J = 8.0 Hz, 1H, isatin C5-H), 7.17–7.21 (m, 2H, benzyl C3-H, C6-H), 7.24–7.39 (m, 3H, benzyl C4-H, C5-H, isatin C6-H), 7.66 (d, J = 6.0 Hz, 1H, isatin C4-H), 9.77 (t, J = 6.0 Hz, 1H, CSNH), 11.24 (s, 1H, isatin NH), 12.71 (s, 1H, NNH); ^{13}C NMR: δ 41.0 (CH_2), 111.1, 114.9, 115.2, 119.9, 120.9, 122.3, 124.2, 124.3, 124.9, 125.1, 128.9, 128.9, 131.3, 132.2, 142.4, 161.4, 162.6, 178.03; MS: m/z 328 (M^+, 10), 182 (13), 147 (51), 124 (52), 118 (31), 109 (100), 104 (24), 90 (16), 83 (47), 77 (32%). Anal. Calcd for $C_{16}H_{13}FN_4OS$: C, 58.54; H, 3.96; N, 17.07. Found: C, 58.38; H, 3.87; N, 16.98.

***N*-(3-Fluorobenzyl)-2-[2-oxo-2,3-dihydro-1*H*-indol-3-ylidene]-1-hydrazinecarbothioamide (5i)** Yellow fluffy crystals; yield 80%; mp 215°C; IR: 3300, 3184 (NH), 1682 (C=O), 1600 (C=N), 1192 cm^{-1} (C=S); ^{1}H-NMR: δ 4.87 (d, J = 6.0 Hz, 2H, benzyl CH_2), 6.92 (d, J = 8.0 Hz, 1H, isatin C7-H), 7.08 (t, J = 8.0 Hz, 2H, isatin C5-H, benzyl C5-H), 7.15–7.20 (m, 2H, benzyl C2-H, C6-H), 7.33–7.41 (m, 2H, benzyl C4-H, isatin C6-H), 7.64 (d, J = 7.2 Hz, 1H, isatin C4-H), 9.81 (t, J = 6.0 Hz, 1H, CSNH), 11.20 (s, 1H, isatin NH), 12.67 (s, 1H, NNH); ^{13}C NMR: δ 47.18 (CH_2), 111.56, 114.09, 114.33, 114.62, 120.39, 121.37, 122.77, 123.79, 123.83, 130.65, 130.76, 131.75, 132.71, 141.82, 141.91, 142.87, 161.01, 163.12, 164.23, 178.36; MS: m/z 328 (M^+, 77), 300 (45), 252 (34), 203 (13), 182 (52), 161 (13), 147 (100), 132 (15), 124 (95), 118 (39), 109 (93), 104 (19), 83 (14), 77 (12%). Anal. Calcd for $C_{16}H_{13}FN_4OS$: C, 58.54; H, 3.96; N, 17.07. Found: C, 58.43; H, 3.87; N, 17.02.

***N*-(4-Fluorobenzyl)-2-[2-oxo-2,3-dihydro-1*H*-indol-3-ylidene]-1-hydrazinecarbothioamide (5j)** Yellow fluffy crystals; yield 74%; mp 230°C; IR: 3374, 3248 (NH), 1699 (C=O), 1608 (C=N), 1163 cm^{-1} (C=S); ^{1}H NMR: δ 3.30 (DMSO, benzyl CH_2), 6.95 (d, J = 8.0 Hz, 1H, isatin C7-H), 7.17 (tt, J = 8.0, 2.4 Hz, 2H, benzyl C3-H, C5-H), 7.42 (dd, J = 8.0, 2.4 Hz, 2H, benzyl C2-H, C6-H), 7.54–7.61 (m, 2H, isatin C5-H,C6-H), 7.85 (d, J = 7.2 Hz, 1H, isatin C4-H), 10.93 (s, 1H, CSNH), 11.38 (s, 1H, isatin NH), 12.74 (s, 1H, NNH); ^{13}C-NMR: δ 107.9, 108.3, 112.7, 121.1, 121.5, 126.6, 130.9, 131.8, 141.3, 160.3, 162.4, 163.5, 175.9; MS: m/z 328 (M^+, 7), 182 (41), 161 (15), 147 (72), 132 (15), 124 (65), 118 (56), 109 (100), 104 (44), 90 (27), 83 (60), 77 (49%). Anal. Calcd for $C_{16}H_{13}FN_4OS$: C, 58.54; H, 3.96; N, 17.07. Found: C, 58.45; H, 3.87; N, 17.01.

***N*-(3-Chlorobenzyl)-2-[2-oxo-2,3-dihydro-1*H*-indol-3-ylidene]-1-hydrazinecarbothioamide (5l)** Yellow crystals; yield 86%; mp 220°C; IR: 3350, 3142 (NH), 1695 (C=O), 1605 (C=N), 1148 cm^{-1} (C=S); ^{1}H NMR: δ 3.30 (DMSO, benzyl CH_2), 6.91 (d, J = 7.6 Hz, 1H, isatin C7-H), 7.24 (t, J = 7.6 Hz, 1H, isatin C5-H), 7.53 (d, J = 7.8 Hz, 1H, benzyl C2-H) 7.64–7.72 (m, 3H, benzyl C4-H, C5-H, C6-H), 7.99–8.01 (m, 2H, isatin C4-H, C6-H), 9.76 (t, J = 6.0 Hz, 1H, CSNH), 11.21 (s, 1H, isatin NH), 12.73 (s, 1H, NNH); ^{13}C NMR: δ 93.6, 113.0, 114.2, 122.1, 123.7, 124.9, 130.3, 131.2, 133.6, 134.7, 139.6, 141.6, 162.3, 176.2; MS: m/z 346/344 (M^+, 2/5), 200/198 (7/16), 147 (100), 142/140 (25/73), 132 (20), 127/125 (44/97), 118 (83), 104 (69), 89 (78), 77 (93%). Anal. Calcd for $C_{16}H_{13}ClN_4OS$: C, 55.73; H, 3.77; N, 16.26. Found: C, 55.69; H, 3.69; N, 16.20.

***N*-(4-Chlorobenzyl)-2-[2-oxo-2,3-dihydro-1*H*-indol-3-ylidene]-1-hydrazinecarbothioamide (5m)** Yellow fluffy crystals; yield 85%; mp 250°C; IR: 3300, 3188 (NH), 1680 (C=O), 1604 (C=N), 1155 cm^{-1} (C=S); ^{1}H NMR: δ 4.86 (d, J = 6.0 Hz, 2H, benzyl CH_2), 6.94 (d, J = 8.0 Hz, 1H, isatin C7-H), 7.09 (t, J = 7.6 Hz, 1H, isatin C5-H), 7.52–7.66 (m, 5H, benzyl C2-H. C3-H, C5-H, C6-H, isatin C6-H), 7.65 (d, J = 6.0 Hz, 1H, isatin C4-H), 9.83 (t, J = 6.0 Hz, 1H, CSNH), 11.23 (s, 1H, isatin NH), 12.68 (s, 1H, NNH); ^{13}C NMR: δ 46.5 (CH_2), 111.1, 119.9, 120.9, 122.3, 128.2, 129.2, 1313, 131.5, 132.2, 137.4, 142.4, 162.6, 177.8; MS: m/z 346/344 (M^+, 3/1), 198(16), 147 (92), 140 (70), 125 (100), 118 (72), 104 (50), 90 (50), 77 (92%). Anal. Calcd for $C_{16}H_{13}ClN_4OS$: C, 55.73; H, 3.77; N, 16.26. Found: C, 55.69; H, 3.77; N, 16.26.

***N*-(2, 4-Dichlorobenzyl)-2-[2-oxo-2,3-dihydro-1*H*-indol-3-ylidene]-1-hydrazinecarbothioamide (5n)** Yellow crystals; yield 82%; mp 265°C; IR: 3360, 3172 (NH), 1701 (C=O), 1599 (C=N), 1184 cm^{-1} (C=S); ^{1}H NMR: δ 4.45 (d, J = 5.6 Hz, 2H, benzyl CH_2), 6.88 (d, J = 7.6 Hz, 1H, isatin C7-H), 7.02 (t, J = 8.0 Hz, 1H, isatin C5-H), 7.32–7.45 (m, 3H, benzyl C5-H, C6-H, isatin C6-H), 7.61 (d, J = 2.0 Hz, 1H, benzyl C3-H), 7.85 (t, J = 6 Hz, 1H, CSNH), 8.06 (d, J = 7.6.Hz, 1H, isatin C4-H), 10.38 (s, 1H, isatin NH), 10.69 (s, 1H, NNH)); ^{13}C NMR: δ 41.0 (CH_2), 110.9, 116.1, 122.0, 125.8, 127.8, 129.0, 130.3, 132.3, 132.6, 133.2, 134.6, 136.4, 143.5, 155.7, 165.2; MS: m/z 364/362 (3/5), 329/327 (4/11), 186 (10), 166 (15), 161 (100), 140 (26), 132 (18), 104 (17%). Anal. Calcd for $C_{16}H_{12}Cl_2N_4OS$: C, 50.66; H, 3.17; N, 14.78. Found: C, 50.48; H, 3.14; N, 14.77.

***N*-(3, 4-Dichlorobenzyl)-2-[2-oxo-2,3-dihydro-1*H*-indol-3-ylidene]-1-hydrazinecarbothioamide (5o)** Yellow crystals; yield 95%; mp 230°C; IR: 3348, 3217 (NH), 1707 (C=O), 1610 (C=N), 1190 cm^{-1} (C=S); ^{1}H NMR: δ 4.84 (d, J = 6.0 Hz, 2H, benzyl CH_2), 6.92 (d, J = 8 Hz, 1H, isatin C7-H), 7.08 (t, J = 7.6 Hz, 1H, isatin C5-H), 7.34–7.36 (m, *2*H, isatin C6-H, benzyl C6-H), 7.59–7.64 (m, 3H, benzyl C2-H, C5-H, isatin C4-H), 9.81 (t, J = 6.0 Hz, 1H, CSNH), 11.20 (s, 1H, isatin NH), 12.68 (s, 1H, NNH)); ^{13}C NMR: δ 46.6 (CH_2), 111.6, 120.3, 121.3, 122.8, 128.2, 129.8, 130.0, 130.9, 131.3, 131.8, 132.8, 140.1, 142.9, 163.1, 178.4; MS: m/z 380/378 (M^+, 16/22), 352/350 (11/15), 234/232 (16/15), 203 (42), 176/174 (38/62), 161/159 (58/86), 147 (100), 144/142 (29/25), 140 (69), 118 (65), 104/102 (31/17), 91/89 (19/25), 77/75 (29/25), 63/61 (24/13%). Anal. Calcd for $C_{16}H_{12}Cl_2N_4OS$: C, 50.66; H, 3.17; N, 14.78. Found: C, 50.49; H 3.15; N, 14.76.

Crystallographic data collection and structural refinement

A crystal of **5h** was mounted on a thin glass fiber at room temperature and the reflection data were collected on a Bruker Kappa APE XII CCD diffractometer equipped with graphite monochromated MoKα radiation (λ = 0.71073 Å). The data were corrected for Lorentz and polarization effects. The structure was solved using SHELXS-97 [51]. A final refinement on F^2 was carried out by full-matrix least-squares techniques using SHELXL-97 [51].

Biological assays

In vitro antiurease, antiglycation, phytotoxic and toxic activities of thiosemicarbazones **5a–o** were performed by using the reported methods [52, 36, 38, 41].

Molecular docking studies

Docking studies of compounds **5a–o** were carried out in accordance with the previously described protocols [52–58].

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