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Article *in* Letters in Drug Design & Discovery · February 2010

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Synthesis and Urease Inhibitory Properties of Some New N^4-Substituted 5-Nitroisatin-3-thiosemicarbazones

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Received August 06, 2009: Revised October 12, 2009: Accepted October 12, 2009

Abstarct: A series of seventeen N^4-substituted 5-nitroisatin-3-thiosemicarbazones **2a-2q** has been synthesized and screened for *in vitro* urease inhibitory activities. Compounds **2a-2d, 2g, 2i, 2j** and **2q** were found to be potent inhibitors of the enzyme. Of these, **2c** exhibited a potent inhibitory activity with IC_{50} value 16.4 μM and may act as a lead molecule for further studies. Structure-activity relationship studies revealed that electronic effects of the substituents play an important role in the urease inhibitory potential of the synthetic compounds.

Keywords: 5-Nitroisatin, Thiosemicarbazones, Urease Inhibition.

INTRODUCTION

The biological properties of isatin and its derivatives are well known for a long time. Isatin itself has a range of actions such as monoamine oxidase inhibition, anticonvulsant, anxiogenic and sedative activities [1]. Similarly, isatin derivatives are known to have a wide range of pharmacological activities including antibacterial, antifungal, antiviral, antineoplastic, antiulcer, antileishmanial and enzymatic inhibition [1-5]. Amongst these, isatins-derived thiosemicarbazones have attracted a great deal of interest [6-14]. In view of these observations and in continuation of our drug discovery program [15-21], we have recently synthesized a number of N^4-substituted isatin-3-thiosemicarbazones as urease inhibitors with non toxic nature [22, 23]. These findings form a solid basis for further research on such compounds to develop more potent, safe and useful urease inhibitors. Furthermore, structure-activity relationship (SAR) studies revealed that the type and position of the substituents on phenyl ring, substituted at N^4 of the thiosemicarbazone moiety, play an important role in the urease inhibitory potential of these compounds. To further enhance the activity of new antiurease compounds, the study of the combination of substitution at position-5 of the isatin scaffold with attachment of different aryl groups (having one or two substituents about the phenyl ring) at N^4 of the thiosemicarbazone moiety was considered worth pursuing. The present work therefore deals with the synthesis and evaluation of urease inhibitory potential of a series of seventeen N^4-arylsubstituted 5-nitroisatin-3-thiosemicarbazones. We describe here the effects of the nature of aryl groups at N^4 (modified by placement of one or two substituents about the phenyl ring) and the presence of nitro function at position-5 of the isatin scaffold on the urease inhibitory potential of these compounds.

RESULTS AND DISCUSSION

The present work describes the synthesis and *in vitro* evaluation of urease inhibitory activities of seventeen new N^4-subastituted 5-nitroisatin-3-thiosemicarbazones **2a-2q**.

CHEMISTRY

For the synthesis of 5-nitroisatin-thiosemicarbazones, a mixture of 5-nitroisatin, appropriate thiosemicarbazide and aqueous ethanol containing a few drops of glacial acetic acid was refluxed for 2 h (Scheme **1**). The crystalline or amorphous solid formed during refluxing in each case was filtered hot. Thorough washing with hot aqueous ethanol afforded the required compounds **2a-2q** in good to excellent yields (65-96%)**.**

The structures of the synthesized thiosemicarbazones were deduced by analytical and spectroscopic (IR, ^{1}H-NMR, EI MS) data. Satisfactory elemental analyses (± 0.4% of calculated values) were obtained for all the compounds, except where noted otherwise. The IR spectra of **2a-2q** showed bands of the NH stretching of indole and thioamide functions in the 3325-3209 and 3188-3132 cm^{-1} regions. The lactam C = O, azomethine C = N and thioamide C = S stretchings were observed in the 1709-1680, 1627-1600 and 1186-1125 cm^{-1} regions, respectively [24-26]. The ^{1}H-NMR spectra of these compounds exhibited three separate singlets at δ 10.76-11.23, δ 11.83-11.88 and δ 12.50-12.68 for the thiosemicarbazone N^4-H, indole NH and thiosemicarbazone N^2-H, respectively [24, 26, 27]. The indole C_7-H appeared as a doublet at δ 7.12-7.14, while the indole C_6-H, being deshielded due to electron-withdrawing inductive effect of the nitro function at position-5, appeared at δ 8.26-8.29 as a double doublet. Indole C_4-H, experiencing a high deshielding effect due to electron-withdrawing nitro group and C = N function, resonated further downfield as a doublet at δ 8.58-8.70 [28-30]. In some cases, however, overlapping of the indole C_7-H signals was observed as multiplets due to combination with different aromatic protons of the N^4-substituents. The EI mass spectra of **2a-2q** showed molecular ions of different intensity, which confirmed their molecular weights. The major fragmentation pathway involved the cleavage of the exocyclic N-N, NH-CS and endocyclic NH-CO bonds. Compounds **2l**, **2m**, **2p** and **2q** did not show the molecular ion peaks in their spectra. However, the fragments corresponding to thiosemicarbazone moiety, formed by the cleavage of N-N and NH-CS bonds confirmed their structures. The proposed fragmentation pattern of **2c** is presented in Fig. (**1**).

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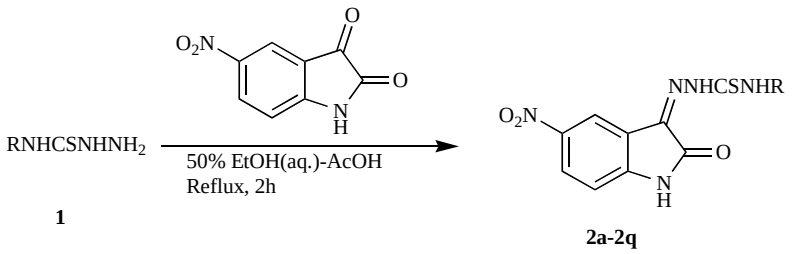

2a	R	=	C_6H_5	**2j**	R	=	2-FC_6H_4
2b	R	=	2-$CH_3C_6H_4$	**2k**	R	=	3-FC_6H_4
2c	R	=	2-$C_2H_5C_6H_4$	**2l**	R	=	2-BrC_6H_4
2d	R	=	4-$C_2H_5C_6H_4$	**2m**	R	=	2-IC_6H_4
2e	R	=	2-$OCH_3C_6H_4$	**2n**	R	=	2,4-$F_2C_6H_3$
2f	R	=	3-$OCH_3C_6H_4$	**2o**	R	=	2,5-$F_2C_6H_3$
2g	R	=	3-$CF_3C_6H_4$	**2p**	R	=	2,6-$F_2C_6H_3$
2h	R	=	4-$CF_3C_6H_4$	**2q**	R	=	2,4-$Cl_2C_6H_3$
2i	R	=	4-$OCF_3C_6H_4$				

Scheme 1. Synthesis of title compounds.

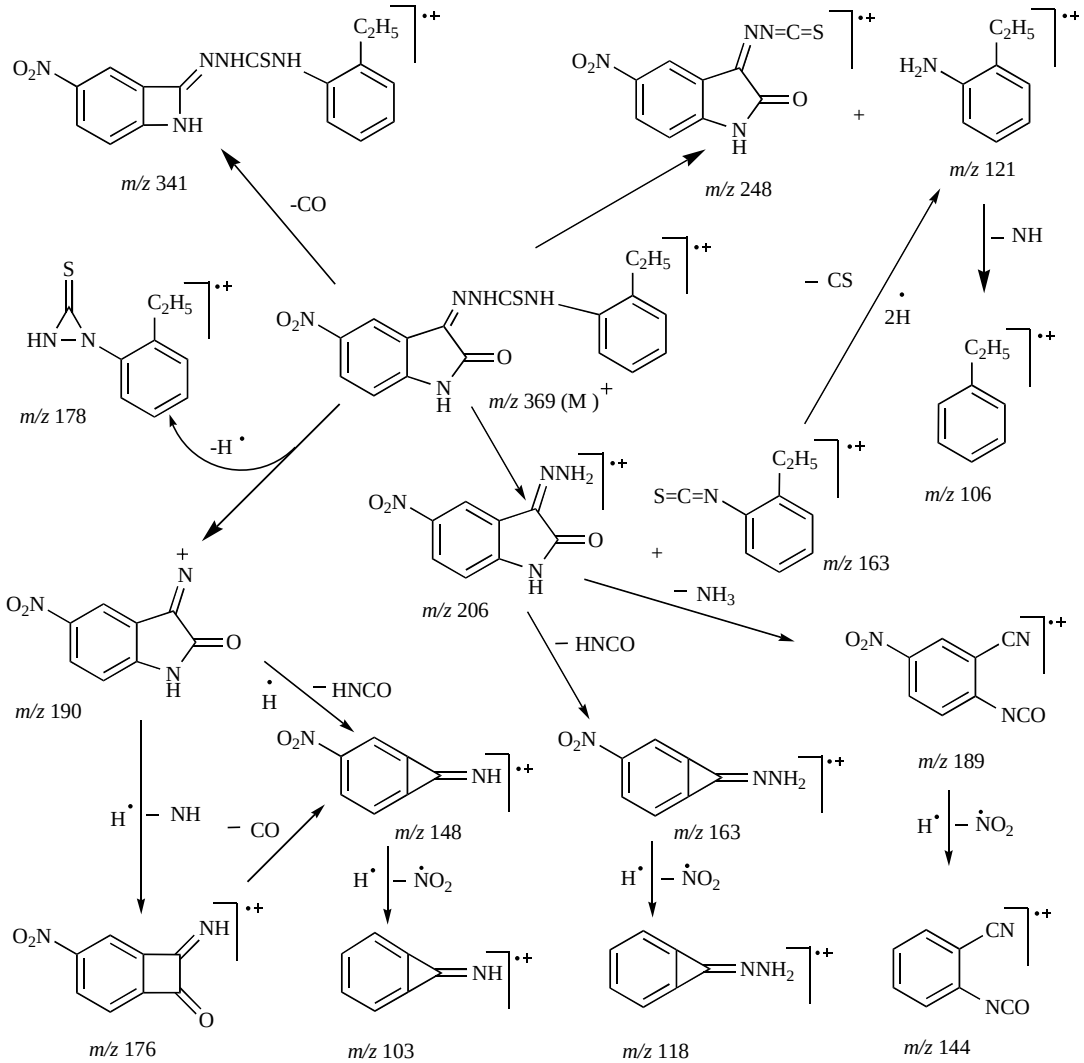

Fig. (1). The proposed fragmentation pattern of compound **2c**.

BIOACTIVITIES

All the synthesized thiosemicarbazones **2a-2q** were tested for their urease inhibitory effects. Thiourea and compound **2r** *i.e.* 2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)-*N*-phenyl-1-hydrazine-carbothioamide, the synthesis of which has been reported elsewhere [21], served as references to evaluate the effects of substituents on the isatin scaffold as well as phenyl ring substituted at N^4 of the thiosemicarbazone moiety of the test compounds on their inhibitory potential. The results presented in Table **1** revealed that as compared to compound **2r**, which has no substituent in the isatin scaffold, substitution of nitro function at its position-5 either enhanced or reduced enzyme activity in certain cases. This inference was supported from the results obtained by us in earlier studies [22, 23]. For example, compounds **2a** and **2b** with no substituent, or methyl group at the *ortho* position of the phenyl ring, respectively, displayed 88.75% and 54.0% inhibition of the enzyme, whereas the corresponding compounds having no nitro group in the isatin moiety were found to exhibit no inhibitory activity at the tested concentration (100 μM). Compounds **2f, 2i** and **2l** having methoxy, trifluoromethoxy and bromo substituents at the *meta*, *para* and *ortho* positions of the phenyl ring were found to display a moderate activity (27.8%, 58.3% and 39.3%, respectively), when compared with the corresponding compounds with no nitro function at position-5 of the isatin scaffold (exhibiting 12%, 49.6% and 18.9% inhibition) at the tested concentration (100 μM). Much enhancement was observed in the case of **2i** with IC_{50} value 86.2 μM. This indicated that the isatin scaffold having strong inductively electron-withdrawing substituent at position-5 interacts with the enzyme more effeciently. To the contrary, compounds **2e, 2h, 2n** and **2o** having 2-methoxy, 4-trifluoromethyl, 2,4-difluoro and 2,5-difluoro substituents, respectively, on the phenyl ring showed no inhibitory effect, whereas the corresponding compounds without nitro group at position-5 of the isatin scaffold exhibited 12.9%, 44.6%, 71.9% and 66.6% inhibition at the tested concentration (100 μM). Compounds **2g, 2j, 2k** and **2p** having 3-trifluoromethyl, 2-fluoro, 3-fluoro and 2,6-difluoro substituents, respectively, on the phenyl ring were found to show a reduced enzymatic activity (77.7%, 76.8%, 49.6% and 24%, respectively), when compared with the corresponding compounds with no nitro group at position-5 of the isatin moiety, displaying 78.2%, 84.9%, 57.3% and 92% inhibition at the tested concentration (100 μM). Relatively, pronounced reduction in the enzyme activity was observed in the cases **2g, 2j** and **2p**. This showed that the simultaneous presence of strong inductively electron-attracting groups in the isatin scaffold as well as the phenyl ring substituted at N^4 of the thiosemicarbazone moiety caused the molecules to interfere with the enzyme activity differently, resulting into either complete loss or strongly marked reduction in the inhibitory potential.

On the whole, out of seventeen compounds tested for their urease inhibitory effects, eight *i.e.* **2a-2d, 2g, 2i, 2j** and **2q** were found to be potent inhibitors for urease activity. Compound **2c** having an inductively electron-donating ethyl substituent at the *ortho* position of the phenyl ring was the most potent one, exhibiting IC_{50} value 16.4 μM, even better than the reference inhibitor *i.e.* thiourea (IC_{50} = 21.0 μM). In contrast, compounds **2b** and **2d** having methyl and ethyl substituents at the *ortho* and *para* positions of the phenyl ring displayed much higher IC_{50} values (187.7 and 76.5 μM, respectively). This clearly indicated that compound **2c**, as compared to **2b** and **2d**, interacts with the enzyme efficiently, resulting into an enhanced inhibitory potential. The next most potent compounds were **2a** with no substituent and **2j** with a fluoro group at the *ortho* position of the phenyl ring, displaying IC_{50} values 32.4 and 49.2 μM, respectively. The remainder *i.e.* **2g**, **2i** and **2q** exhibited a varying degree of activity with IC_{50} values ranging from 86.2 to 170.1 μM.

All ureases regardless of their origin have two Lewis acid nickel ions [31-36] and one to three protein subunits present in varying stoichiometric ratios [37]. A urease inhibitor can therefore interfere with the enzyme activity by interacting either with the

Table 1. Inhibition of Urease by Compounds 2a-2q

Compounds	Inhibition at 200 μM (%)	IC_{50} ± SEM (μM)
2a	88.75	32.36 ± 0.48
2b	54.0	187.7 ± 1.97
2c	94.6	16.4 ± 0.8
2d	66.5	76.5 ± 0.38
2e	NA	-
2f	27.8	-
2g	77.7	91.5 ± 5.5
2h	NA	-
2i	58.3	86.2 ± 2.33
2j	76.8	49.2 ± 4.0
2k	49.6	-
2l	39.3	-
2m	32.8	-
2n	NA	-
2o	NA	-
2p	24.0	-
2q	53.5	170.1± 3.4
2r* [21, 22]	NA	-
Thiourea[§]	-	21.0 ± 0.01

SEM: Standard error of the mean; NA: no inhibitory activity; * tested at 100 μM; [§]reference inhibitor of the enzyme.

metal ions or the protein component. β-Mercaptoethanol (BME), hydroxamic acids (HXAs) and phosphorodiamidates (PPDs), for example, are the synthetic inhibitors, which interact with the enzyme activity by binding to the metal ions of its active site [31,33,36,38]. By contrast, sulphenamide, quinones and heavy metal ions have been reported to inhibit activity of the enzyme by interacting with the thiol groups present in its protein component [39-44]. The exact mechanism of urease inhibition by our compounds **2a-2q** is not known. Apparently, these compounds employ a mechanism of action by exploiting a common transition catalysis state and acting as ligand chelators to form complexes with the two slightly distorted octahedral nickel ions of the enzyme coordinating through carbonylo oxygen, imino nitrogen and thiolato sulphur atoms. Hydrogen bonding or hydrophobic interaction of the enzyme with the coordinated thiosemicarbazones (ligands) may also be the contributing factors in their inhibitory potential. Detailed kinetic studies to get an insight into the mechanism of inhibition are underway, which will be reported in future.

In summary, thirteen out of seventeen synthetic compounds displayed urease inhibitory activity; eight of these *i.e.* **2a**-**2d**, **2g**, **2i**, **2j** and **2q** were found to be potent inhibitors. Compound **2c** demonstrated a potent urease inhibitory activity and may act as a lead compound. These compounds could be potential candidates for further studies. Their urease inhibitory potential was shown to be dependent upon electronic effects of the nitro group at position-5 of the isatin scaffold and the substituents about the phenyl ring substituted at N^4 of the thiosemicarbazone moiety. This combination of electronic effects could be responsible for distortion of the architecture of the emzyme's active site, the mechanism of which remains to be determined. This preliminary structure-activity relationship (SAR) study may serve as a basis for chemical modifications directed towards the development of potential antiurease compounds of medicinal / asgricultural interest.

MATERIAL AND METHODS

General

All reagents and solvents were used as supplied by the supplier or recrystallized / redistilled as necessary. Melting points were taken on a Fisher-Johns melting point apparatus and are uncorrected. Elemental analyses were performed on a Leco CHNS-9320 elemental analyzer. Infrared spectra (KBr discs) were run on a Shimadzu 8400 or a Shimadzu Prestige-21 FT-IR spectrometer. The ^{1}H-NMR spectra were recorded in DMSO-d_6 on Bruker (Rheniststetten-Forchheim, Germany) AM 300 spectrometer operating at 300 MHz, using TMS as an internal standard. ^{1}H chemical shifts are reported in δ (ppm) and coupling constants in Hz. The electron impact mass spectra (EI MS) were determined with a Finnigan MAT-312 and a JEOL MSRoute mass spectrometer. The progress of the reaction and purity of the products were checked on TLC plates coated with Merck silica gel 60 GF_{254} and the spots were visualized under ultraviolet light at 254 and 366 nm and / or spraying with iodine vapours. *In vitro* biological screening of the synthesized compounds was done at the Department of Chemistry, The Islamia University of Bahawalpur, Pakistan and Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Pakistan.

Synthesis

General Procedure for the Preparation of 5-Nitroisatin-thiosemicarbazones (2a-2q)

To a hot solution of 5-nitroisatin (2.5 mmol) in 50% aqueous ethanol (30 mL) containing a catalytic amount of glacial acetic acid was added the appropriate thiosemicarbazide (2.5 mmol) dissolved in ethanol (10 mL) and the reaction mixture was then heated under reflux for 2 h. The crystalline or amorphous solid formed during heating was collected by suction filtration. Thorough washing with hot aqueous ethanol afforded the desired compounds **2a-2q** in pure form.

The different compounds were characterized as under:

2-(5-Nitro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)-N-phenyl-1-hydrazinecarbothioamide (2a)

Yield 69% as yellow crystals; m.p. 258 °C (d) (lit. m.p. 251-254 °C [6]); IR (KBr, cm^{-1}): 3307, 3245, 3180 (NH stretching), 1695 (C=O), 1620 (C=N), 1530, 1350 (NO_2), 1155 (C=S); ^{1}H-NMR (DMSO-d_6, δ, ppm): 7.13 (d, *J* = 8.7 Hz, 1H, indole C_7-H), 7.31 (tt, *J* = 8.7, 1.8 Hz, 1H, phenyl C_4-H), 7.45 (t, *J* = 8.1 Hz, 2H, phenyl C_3-H, C_5-H), 7.58 (d, *J* = 8.1 Hz, 2H, phenyl C_2-H, C_6-H), 8.28 (dd, *J* = 8.7, 2.7 Hz, 1H, indole C_6-H), 8.70 (d, *J* = 2.4 Hz, 1H, indole C_4-H), 11.09 (s, 1H, CS-NH), 11.86 (s, 1H, indole NH), 12.55 (s, 1H, N-NH); EI MS (70 ev) *m/z* (%): 341 ([M^+], 15), 313 (69), 283 (3), 248 (17), 206 (38), 190 (16), 189 (46), 178 (8), 163 (7), 144 (12), 135 (45), 118 (18), 115 (32), 103 (9), 93 (100), 77 (41), 66 (27), 51 (14); (Found: C, 52.88; H, 3.22; N, 20.47%. Calc. for $C_{15}H_{11}N_5O_3S$: C, 52.79; H, 3.23; N, 20.53%).

N-(2-Methylphenyl)-2-(5-nitro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)-1-hydrazinecarbothioamide (2b)

Yield 80% as yellow crystals; m.p. 226 °C (d); IR (KBr, cm^{-1}): 3310, 3227, 3188 (NH stretching), 1709 (C=O), 1624 (C=N), 1510, 1340 (NO_2), 1155 (C=S); ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.25 (s, 3H, CH_3), 7.13 (d, *J* = 8.7 Hz, 1H, indole C_7-H), 7.26-7.36 (m, 4H, phenyl C_3-H, C_4-H, C_5-H, C_6-H), 8.27 (dd, *J* = 8.7, 2.7 Hz, 1H, indole C_6-H), 8.66 (d, *J* = 2.4 Hz, 1H, indole C_4-H), 11.00 (s, 1H, CS-NH), 11.83 (s, 1H, indole NH), 12.50 (s, 1H, N-NH); EI MS (70 ev) *m/z* (%): 355 ([M^+], 10), 327 (38), 297 (3), 248 (3), 206 (24), 190 (5), 189 (3), 178 (8), 176 (4), 164 (12), 163 (7), 149 (63), 148 (20), 144 (11), 117 (38), 106 (100), 103 (17), 91 (77), 77 (69), 65 (68), 51 (75); (Found: C, 53.90; H, 3.65; N, 19.80%. Calc. for $C_{16}H_{13}N_5O_3S$: C, 54.08; H, 3.66; N, 19.72%).

N-(2-Ethylphenyl)-2-(5-nitro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)-1-hydrazinecarbothioamide(2c)

Yield 83% as yellow amorphous solid; m.p. 192 °C; IR (KBr, cm^{-1}): 3318, 3186 (NH stretching), 1701 (C=O), 1627 (C=N), 1541, 1341 (NO_2), 1136 (C=S); ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.16 (t, *J* = 7.5 Hz, 3H, $CH_2\underline{CH_3}$), 2.62 (q, *J* = 7.5 Hz, 2H, $\underline{CH_2}CH_3$), 7.13 (d, *J* = 8.7 Hz, 1H, indole C_7-H), 7.24-7.38 (m, 4H, phenyl C_3-H, C_4-H, C_5-H, C_6-H), 8.27 (dd, *J* = 8.7, 2.4 Hz, 1H, indole C_6-H), 8.67 (d, *J* = 1.8 Hz, 1H, indole C_4-H), 10.99 (s, 1H, CS-NH), 11.83 (s, 1H, indole NH), 12.50 (s, 1H, N-NH); EI MS (70 ev) *m/z* (%): 369 ([M^+], 6), 341 (21), 248 (4), 206 (26), 191 (2), 189 (4), 178 (40), 176 (5), 163 (65), 148 (37), 144 (12), 121 (43), 118 (13),106 (100), 103 (32), 77 (79), 51 (38); (Found: C, 55.15; H, 4.08; N, 19.04%. Calc. for $C_{17}H_{15}N_5O_3S$: C, 55.28; H, 4.07; N, 18.97%).

N-(4-Ethylphenyl)-2-(5-nitro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)-1-hydrazinecarbothioamide(2d)

Yield 96% as yellow amorphous solid; m.p. 214 °C (d); IR (KBr, cm^{-1}): 3319, 3175 (NH stretching), 1703 (C=O), 1625 (C=N), 1540, 1344 (NO_2), 1153 (C=S); ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.21 (t, *J* = 7.5 Hz, 3H, $CH_2\underline{CH_3}$), 2.64 (q, *J* = 7.5 Hz, 2H, $\underline{CH_2}CH_3$), 7.12 (d, *J* = 8.7 Hz, 1H, indole C_7-H), 7.28 (d, *J* = 8.4 Hz, 2H, phenyl C_3-H, C_5-H), 7.48 (d, *J* = 8.1 Hz, 2H, phenyl C_2-H, C_6-H), 8.27 (dd, *J* = 8.7, 2.4 Hz, 1H, Indole C_6-H), 8.68 (d, *J* = 2.4 Hz, 1H, indole C_4-H), 11.02 (s, 1H, CS-NH), 11.84 (s, 1H, indole NH), 12.52 (s, 1H, N-NH); EI MS (70 ev) *m/z* (%): 369 ([M^+], 4), 341 (19), 326 (1), 311 (1), 296 (0.5), 248 (26), 206 (21), 191 (7), 189

(18), 178(3), 176 (4), 163 (27), 148 (36), 144 (12), 131 (10), 121 (45), 118 (3), 106 (100), 103 (5), 90 (4), 77 (11), 53 (4); (Found: C, 55.04; H, 4.06; N, 18.91%. Calc. for $C_{17}H_{15}N_5O_3S$: C, 55.28; H, 4.07; N, 18.97%).

N-(2-Methoxyphenyl)-2-(5-nitro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)-1-hydrazinecarbothioamide (2e)

Yield 88% as light orange crystals; m.p. 266 °C (d); IR (KBr, cm^{-1}): 3262, 3169 (NH stretching), 1701 (C=O), 1626 (C=N), 1543, 1342 (NO_2), 1186 (C=S); ^{1}H-NMR (DMSO-d_6, δ, ppm): 3.38 (s, 3H, OCH_3), 7.02 (ddd, *J* = 7.5, 7.5, 1.2 Hz, 1H, phenyl C_4-H), 7.12-7.17 (m, 2H, indole C_7-H and phenyl C_3-H), 7.34 (ddd, *J* = 7.5, 7.5, 1.5 Hz, 1H, phenyl C_5-H), 7.49 (dd, *J* = 7.8, 1.5 Hz, 1H, phenyl C_6-H), 8.28 (dd, *J* = 8.7, 2.4 Hz, 1H, indole C_6-H), 8.64 (d, *J* = 2.4 Hz, 1H, indole C_4-H), 10.76 (s, 1H, CS-NH), 11.83 (s, 1H, indole NH), 12.50 (s, 1H, N-NH); EI MS (70 ev) *m/z* (%): 371 ([M^+], 33), 343 (100), 313 (4), 248 (14), 206 (23), 191 (3), 189 (8), 180 (3), 178 (12), 165 (51), 148 (44), 144 (24), 123 (58), 117 (10), 108 (87), 103 (24), 77 (45), 51 (57); (Found: C, 51.66; H, 3.51; N, 18.94%. Calc. for $C_{16}H_{13}N_5O_4S$: C, 51.75; H, 3.50; N, 18.87%).

N-(3-Methoxyphenyl)-2-(5-nitro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)-1-hydrazinecarbothioamide (2f)

Yield 65% as orange yellow crystals; m.p. 258 °C (d); IR (KBr, cm^{-1}): 3304, 3211, 3188 (NH stretching), 1693 (C=O), 1624 (C=N), 1535, 1344 (NO_2), 1159 (C=S); ^{1}H-NMR (DMSO-d_6, δ, ppm): 3.79 (s, 3H, OCH_3), 6.88 (dd, *J* = 8.1, 1.8 Hz, 1H, phenyl C_4-H), 7.12 (d, *J* = 8.7 Hz, 1H, indole C_7-H), 7.18-7.25 (m, 2H, phenyl C_2-H, C_6-H), 7.35 (t, *J* = 8.1Hz, 1H, phenyl C_5-H), 8.27 (dd, *J* = 8.7, 2.4 Hz, 1H, indole C_6-H), 8.69 (d, *J* = 2.4 Hz, 1H, indole C_4-H), 11.03 (s, 1H, CS-NH), 11.85 (s, 1H, indole NH), 12.55 (s, 1H, N-NH); EI MS (70 ev) *m/z* (%): 371 ([M^+], 30), 343 (81), 313 (5), 248 (16), 206 (25), 191 (4), 189 (8), 178 (11), 176 (5), 165 (71), 163 (5), 149 (48), 148 (45), 144 (24), 123 (74), 118 (5), 103 (23), 77 (100), 51 (56); (Found: C, 51.67; H, 3.49; N, 18.92%. Calc. for $C_{16}H_{13}N_5O_4S$: C, 51.75; H, 3.50; N, 18.87%).

2-(5-Nitro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)-N-[3-(trifluoromethyl)phenyl]-1-hydrazinecarbothioamide (2g)

Yield 95% as orange amorphous solid; m.p. 228 °C (d); IR (K-Br, cm^{-1}): 3300, 3225 (NH stretching), 1695 (C=O), 1615 (C=N), 1541, 1352 (NO_2), 1168 (C=S); ^{1}H-NMR (DMSO-d_6, δ, ppm): 7.12 (d, *J* = 8.7 Hz, 1H, indole C_7-H), 7.64-7.72 (m, 2H, phenyl C_5-H, C_6-H), 7.98 (d, *J* = 7.2 Hz, 1H, phenyl C_4-H), 8.03 (s, 1H, phenyl C_2-H), 8.27 (dd, *J* = 8.7, 2.4 Hz, 1H, indole C_6-H), 8.64 (d, *J* = 2.4 Hz, 1H, indole C_4-H), 11.19 (s, 1H, CS-NH), 11.87 (s, 1H, indole NH), 12.63 (s, 1H, N-NH); EI MS (70 ev) *m/z* (%): 409 ([M^+], 30), 381 (100), 248 (11), 218 (6), 206 (32), 203 (62), 191 (2), 189 (3), 178 (12), 177 (13), 161 (66), 149 (17), 145 (71), 144 (16), 117 (5), 103 (15), 76 (15); (Found: C, 47.08; H, 2.43; N, 17.04%. Calc. for $C_{16}H_{10}F_3N_5O_3S$: C, 46.94; H, 2.44; N, 17.11%).

2-(5-Nitro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)-N-[4-(trifluoromethyl)phenyl] -1-hydrazinecarbothioamide (2h)

Yield 87% as light orange amorphous solid; m.p. 260 °C (d); IR (KBr, cm^{-1}): 3300, 3175 (NH stretching), 1680 (C=O), 1600 (C=N), 1541, 1344 (NO_2), 1163 (C=S); ^{1}H-NMR (DMSO-d_6, δ, ppm): 7.14 (d, *J* = 8.7 Hz, 1H, indole C_7-H), 7.82 (d, *J* = 8.4 Hz, 2H, phenyl C_2-H, C_6-H), 7.92 (d, *J* = 8.4 Hz, 2H, phenyl C_3-H, C_5-H), 8.29 (dd, *J* = 8.7, 2.4 Hz, 1H, indole C_6-H), 8.68 (d, *J* = 2.4 Hz, 1H, indole C_4-H), 11.23 (s, 1H, CS-NH), 11.88 (s, 1H, indole NH), 12.66 (s, 1H, N-NH); EI MS (70 ev) *m/z* (%): 409 ([M^+], 3), 381 (16), 248 (1), 206 (46), 203 (100), 191 (2), 189 (5), 178 (7), 177 (6), 161 (51), 149 (10), 145 (65), 144 (7) 117 (4), 103 (12); (Found: C, 47.09; H, 2.43; N, 17.06%. Calc. for $C_{16}H_{10}F_3N_5O_3S$: C, 46.94; H, 2.44; N, 17.11%).

2-(5-Nitro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)-N-[4-(trifluoromethoxy)phenyl]-1-hydrazinecarbothioamide (2i)

Yield 87% as pale yellow amorphous solid; m.p. 250 °C (d); IR (KBr, cm^{-1}): 3320, 3188 (NH stretching), 1703 (C=O), 1626 (C=N), 1540, 1346 (NO_2), 1155 (C=S); ^{1}H-NMR (DMSO-d_6, δ, ppm): 7.12 (d, *J* = 8.7 Hz, 1H, indole C_7-H), 7.45 (d, *J* = 8.4 Hz, 2H, phenyl C_2-H, C_6-H), 7.73 (d, *J* = 9.0 Hz, 2H, phenyl C_3-H, C_5-H), 8.27 (dd, *J* = 8.7, 2.4 Hz, 1H, indole C_6-H), 8.64 (d, *J* = 2.4 Hz, 1H, indole C_4-H), 11.11 (s, 1H, CS-NH), 11.86 (s, 1H, indole NH), 12.58 (s, 1H, N-NH); EI MS (70 ev) *m/z* (%); 425 ([M^+], 6), 397 (22), 248 (2), 234 (2), 219 (29), 206 (13), 202 (9), 190 (5), 189 (3), 178 (6), 177 (18), 163 (5), 149 (12), 144 (15), 133 (15), 117 (5),108 (31), 103 (21), 92 (8), 69 (100); (Found: C, 45.05; H, 2.36; N, 16.53%. Calc. for $C_{16}H_{10}F_3N_5O_4S$: C, 45.18; H, 2.35; N, 16.47%).

N-(2-Fluorophenyl)-2-(5-nitro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)-1-hydrazinecarbothioamide (2j)

Yield 78% as orange crystals; m.p. 254 °C (d); IR (KBr, cm^{-1}): 3316, 3167, 3132 (NH stretching), 1695 (C=O), 1622 (C=N), 1529, 1340 (NO_2), 1165 (C=S); ^{1}H-NMR (DMSO-d_6, δ, ppm): 7.14 (d, *J* = 8.7 Hz, 1H, indole C_7-H), 7.27-7.52 (m, 4H, phenyl C_3-H, C_4-H, C_5-H, C_6-H), 8.28 (dd, *J* = 8.7, 2.4 Hz, 1H, indole C_6-H), 8.63 (d, *J* = 2.1 Hz, 1H, indole C_4-H), 10.98 (s, 1H, CS-NH), 11.85 (s, 1H, indole NH), 12.59 (s, 1H, N-NH); EI MS (70 ev) *m/z* (%): 359 ([M^+], 11), 331 (44), 206 (28), 190 (4), 178 (7), 168 (7), 163 (3), 153 (62), 149 (13), 144 (13), 117 (10), 111 (39), 103 (43), 95 (42), 75 (100) (Found: C, 50.39; H, 2.80; N, 19.40%. Calc. for $C_{15}H_{10}FN_5O_3S$: C, 50.14; H, 2.79; N, 19.50%).

N-(3-Fluorophenyl)-2-(5-nitro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)-1-hydrazinecarbothioamide (2k)

Yield 86% as orange crystals; m.p. 238 °C (d); IR (KBr, cm^{-1}): 3305, 3150 (NH stretching), 1695 (C=O), 1625 (C=N), 1533, 1340 (NO_2), 1153 (C=S); ^{1}H-NMR (DMSO-d_6, δ, ppm): 7.09-7.17 (m, 2H, indole C_7-H and phenyl C_5-H), 7.47-7.60 (m, 3H, phenyl C_2-H, C_4-H, C_6-H), 8.26 (dd, *J* = 8.4, 2.4 Hz, 1H, indole C_6-H), 8.65 (d, *J* = 2.4 Hz, 1H, indole C_4-H), 11.08 (s, 1H, CS-NH), 11.86 (s, 1H, indole NH), 12.58 (s, 1H, N-NH); EI MS (70 ev) *m/z* (%): 359 ([M^+], 16), 331 (58), 301 (2), 248 (10), 206 (27), 190 (11), 189 (8), 178 (12), 176 (5), 163 (4), 153 (72), 149 (18), 148 (3), 144 (17), 117 (7), 111 (100), 103 (19), 95 (83), 76 (43); (Found: C, 50.30; H, 2.78; N, 19.42%. Calc. for $C_{15}H_{10}FN_5O_3S$: C, 50.14; H, 2.79; N, 19.50%).

N-(2-Bromophenyl)-2-(5-nitro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)-1-hydrazinecarbothioamide (2l)

Yield 77% as yellow crystals; m.p. 242 °C (d); IR (KBr, cm^{-1}): 3264, 3227 (NH stretching), 1699 (C=O), 1620 (C=N), 1529, 1334 (NO_2), 1146 (C=S); ^{1}H-NMR (DMSO-d_6, δ, ppm): 7.12 (d, *J* = 8.7 Hz, 1H, indole C_7-H), 7.31-7.36 (m, 1H, phenyl C_5-H), 7.48-7.50 (m, 2H, phenyl C_4-H, C_6-H), 7.77 (d, *J* = 8.7, 1H, phenyl C_3-H), 8.28 (dd, *J* = 8.7, 2.4 Hz, 1H, indole C_6-H), 8.65 (d, *J* = 2.1 Hz, 1H, indole C_4-H), 11.11 (s, 1H, CS-NH), 11.85 (s, 1H, indole NH), 12.55 (s, 1H, N-NH); EI MS (70 ev) *m/z* (%): 391 (5), 393(7) 340 (94), 311 (100), 281 (16), 264 (46), 215 (98), 248 (23), 213 (94), 206 (98), 191 (6), 189 (24), 171 (74), 163 (10), 149 (49), 144 (21), 134 (37) 118 (6), 103 (18); (Found: C, 43.05; H, 2.39; N, 16.75%. Calc. for $C_{15}H_{10}BrN_5O_3S$: C, 42.86; H, 2.38; N, 16.67%);

N-(2-Iodophenyl)-2-(5-nitro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)-1-hydrazinecarbothioamide (2m)

Yield 87% as light orange crystals; m.p. 246 °C (d); IR (KBr, cm^{-1}): 3320, 3210 (NH stretching), 1702 (C=O), 1605 (C=N), 1529, 1335 (NO_2), 1125 (C=S); ^{1}H-NMR (DMSO-d_6, δ, ppm): 7.12-7.18 (m, 2H, indole C_7-H and phenyl C_4-H), 7.42 (dd, *J* = 7.8, 1.5 Hz, 1H, phenyl C_6-H), 7.50 (ddd, *J* = 7.5, 7.5, 1.2 Hz, 1H, phenyl C_5-H), 7.97 (d, *J* = 7.8 Hz, 1H, phenyl C_3-H), 8.28 (dd, *J* = 8.7, 2.4 Hz,

1H, indole C_6-H), 8.67 (d, *J* = 2.4 Hz, 1H, indole C_4-H), 11.12 (s, 1H, CS-NH), 11.85 (s, 1H, indole NH), 12.54 (s, 1H, N-NH); EI MS (70 ev) *m/z* (%): 439 (9), 340 (38), 311 (10), 261 (54), 248 (20), 219 (10), 206 (21), 190 (22), 189 (14), 178 (6), 176 (6), 163 (4), 150 (49), 149 (32), 148 (6), 144 (31), 117 (10), 103 (17), 92 (85), 65 (100); (Found: C, 38.72; H, 2.15; N, 15.04%. Calc. for $C_{15}H_{10}IN_5O_3S$: C, 38.54; H, 2.14; N, 14.99%).

N-(2,4-Difluorophenyl)-2-(5-nitro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)-1-hydrazinecarbothioamide (2n)

Yield 87% as yellow crystals; m.p. 260 °C (d); IR (KBr, cm^{-1}): 3313, 3209, 3184, 3169 (NH stretching), 1690 (C=O), 1624 (C=N), 1530, 1341 (NO_2), 1161 (C=S); ^{1}H-NMR (DMSO-d_6, δ, ppm): 7.12-7.23 (m, 2H, indole C_7-H and phenyl C_3-H), 7.40-7.58 (m, 2H, phenyl C_5-H, C_6-H), 8.29 (dd, *J* = 8.7, 2.4 Hz, 1H, indole C_6-H), 8.62 (d, *J* = 2.4 Hz, 1H, indole C_4-H), 10.94 (s, 1H, CS-NH), 11.86 (s, 1H, indole NH), 12.61 (s, 1H, N-NH); EI MS (70 ev) *m/z* (%): 377 ([M^+], 34), 349 (100), 319 (6), 248 (7), 206 (35), 191 (3), 189 (8), 186 (5), 178 (18), 176 (8), 171 (79), 163 (5), 149 (28), 148 (6), 144 (25), 129 (73), 117 (8), 103 (32), 101 (60), 77 (24), 63 (67); (Found: C, 47.61; H, 2.40; N, 18.63%. Calc. for $C_{15}H_9F_2N_5O_3S$: C, 47.75; H, 2.39; N, 18.57%).

N-(2,5-Difluorophenyl)-2-(5-nitro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)-1-hydrazinecarbothioamide (2o)

Yield 76% as golden yellow crystals; m.p. 250 °C (d); IR (KBr, cm^{-1}): 3325, 3215 (NH stretching), 1700 (C=O), 1625 (C=N), 1543, 1335 (NO_2), 1125 (C=S); ^{1}H-NMR (DMSO-d_6, δ, ppm): 7.12 (d, *J* = 8.7 Hz, 1H, indole C_7-H), 7.25-7.33 (m, 1H, phenyl C_6-H), 7.39-7.49 (m, 2H, phenyl C_3-H, C_4-H), 8.27 (dd, *J* = 8.7, 2.4 Hz, 1H, indole C_6-H), 8.58 (d, *J* = 2.1 Hz, 1H, indole C_4-H), 10.99 (s, 1H, CS-NH), 11.86 (s, 1H, indole NH), 12.62 (s, 1H, N-NH); EI MS (70 ev) *m/z* (%): 377 ([M^+], 35), 349 (100), 319 (4), 248 (6), 206 (28), 190 (7), 189 (3), 178 (14), 177 (6), 171 (62), 163 (4), 149 (26), 148 (3), 144 (25), 129 (53), 117 (8), 113 (27), 103 (34), 101 (67), 76 (31), 63 (59); (Found: C, 47.60; H, 2.40; N, 18.64%. Calc. for $C_{15}H_9F_2N_5O_3S$: C, 47.75; H, 2.39; N, 18.57%).

N-(2,6-Difluorophenyl)-2-(5-nitro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)-1-hydrazinecarbothioamide (2p)

Yield 84% as light orange amphrous solid; m.p. 270 °C (d); IR (KBr, cm^{-1}): 3298, 3175 (NH stretching), 1697 (C=O), 1622 (C=N), 1522, 1339 (NO_2), 1173 (C=S); ^{1}H-NMR (DMSO-d_6, δ, ppm): 7.13 (d, *J* = 8.7 Hz, 1H, indole C_7-H), 7.27 (t, *J* = 8.1 Hz, 2H, phenyl C_3-H, C_5-H), 7.47-7.56 (m, 1H, phenyl C_4-H), 8.29 (dd, *J* = 8.7, 2.4 Hz, 1H, indole C_6-H), 8.59 (d, *J* = 1.8 Hz, 1H, indole C_4-H), 10.83 (s, 1H, CS-NH), 11.86 (s, 1H, indole NH), 12.68 (s, 1H, N-NH); EI MS (70 ev) *m/z* (%): 349 (5), 206 (23), 176 (6), 171 (58), 163 (5), 149 (19), 129 (13), 117 (6), 113 (10), 103 (30), 77 (27), 63 (100), 51 (33); (Found: C, 47.89; H, 2.38; N, 18.50%. Calc. for $C_{15}H_9F_2N_5O_3S$: C, 47.75; H, 2.39; N, 18.57%).

N-(2,4-Dichlorophenyl)-2-(5-nitro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)-1-hydrazinecarbothioamide (2q)

Yield 83% as yellow amorphous solid; m.p. 230 °C (d); IR (KBr, cm^{-1}): 3240, 3160 (NH stretching), 1693 (C=O), 1627 (C=N), 1525, 1346 (NO_2), 1165 (C=S); ^{1}H-NMR (DMSO-d_6, δ, ppm): 7.14 (d, *J* = 8.7 Hz, 1H, indole C_7-H), 7.54 (s, 2H, phenyl C_5-H, C_6-H), 7.81 (d, *J* = 1.2 Hz, 1H, phenyl C_3-H), 8.28 (dd, *J* = 8.7, 2.4 Hz, 1H, indole C_6-H), 8.62 (d, *J* = 2.4 Hz, 1H, indole C_4-H), 11.08 (s, 1H, CS-NH), 11.86 (s, 1H, indole NH), 12.60 (s, 1H, N-NH); EI MS (70 ev) *m/z* (%): 381 (12), 374 (85), 376 (35), 248 (17), 206 (49), 203 (64), 191 (6), 189 (13), 178 (14), 163 (65), 161 (100), 149 (24), 148 (8), 144 (36), 133 (53), 117 (11), 103 (28), 90 (46), 75 (46); (Found: C, 44.12; H, 2.21; N, 17.16%. Calc. for $C_{15}H_9Cl_2N_5O_3S$: C, 43.90; H, 2.20; N, 17.07%).

Urease Inhibitory Activity (*In Vitro*)

Reaction mixtures comprising 25 μL of enzyme (Jack bean urease) solution and 55 μL of buffers containing 100 mM urea were incubated with 5 μL of test compounds (0.2 mM concentration) at 30° C for 15 min in 96-well plates. Urease activity was evaluated by measuring ammonia production using the indophenol method as described by Weatherburn [45]. Briefly, 45 μL each of phenol reagent (1 % w/v phenol and 0.005 % w/v sodium nitroprusside) 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, using a microplate reader (Molecular Devices, 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 SoftMax Pro software (Spectra Max Plus Molecular Devices, USA). All the assays were performed at pH 8.2 (0.01 M K_2HPO_4. $3H_2O$, 1 mM EDTA and 0.01M LiCl). Percentage inhibition was calculated from the formula 100-($OD_{testwell}$ / $OD_{control}$) x 100. Thiourea was used as the reference inhibitor of urease along with compound **2r**.

ACKNOWLEDGEMENTS

We acknowledge partial funding of this research work and the award of Indigenous Ph. D. scholarship to NM by the Higher Education Commission, Pakistan.

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