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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and biological evaluation of some N^4-substituted 5-nitroisatin-3-thiosemicarbazones

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Abstract A series of 5-nitroisatin-3-thiosemicarbazones **2a–2l** was synthesised and evaluated for selected biological activities. The brine shrimp lethality bioassay was carried out to study their in vitro cytotoxicity potential and besides, their antifungal, phytotoxic and urease inhibitory effects were also investigated. Only compound **2j** proved to be active in the brine shrimp assay exhibiting LD_{50} value 1.16×10^{-3} M. Compounds **2a** and **2d** displayed moderate antifungal activity (50 and 40%, respectively) against *M. canis*. Similarly, compound **2l** exhibited moderate activity (40%) against the fungal strain, *A. flavus*. In phytotoxicity assay, all the synthesised compounds including the reference point **2m** showed weak to moderate (20–60%) activity at the highest tested concentrations (1,000 μg and 500 μg/ml, respectively). In urease inhibition assay, compounds **2a**, **2i** and **2k** proved to be potent inhibitors demonstrating pronounced inhibition with IC_{50} values 0.440, 0.901 and 27.880 μM, respectively. These compounds may act as leads for further studies.

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Introduction

Isatin is a naturally occurring product found in many plants including those of the genus *Isatis*. In humans, it is found as a metabolic derivative of adrenaline (Da Silva *et al*., 2001). The biological properties of isatin and its derivatives have been known since long. Isatin itself displayed a number of biological activities including anticonvulsant, anxiogenic and sedative (Pandeya *et al*., 2005). Similarly, isatin derivatives are reported to exhibit broad spectrum chemotherapeutic properties including antiviral, antibacterial, anticonvulsant, cysticidal, herbicidal, anthelmintic, antifungal, antineoplastic, hypotensive and enzymatic inhibition (Aboul-Fadl and Bin-Jubair, 2010; Bal *et al*., 2005; Beauchard *et al.,* 2006; Chen *et al*., 2005; Chiyanzu *et al.,* 2005, 2003; Da Silva *et al.,* 2001; Guzel *et al.,* 2008; Hall *et al.,* 2009; Hyatt *et al.,* 2007; Karali, 2002; Karali *et al.,* 2007, 2002; Khan *et al.,* 2008; Pandeya *et al.,* 2005; Quenelle *et al.,* 2006; Ravichandran *et al.,* 2007; Singh *et al.,* 2010; Terzioglu *et al.,* 2006). Amongst these, isatins-based synthetic thiosemicarbazones have increased a great deal of interest (Bal *et al.,* 2005; Chiyanzu *et al.,* 2005, 2003; Da Silva *et al.,* 2001; Guzel *et al.,* 2008; Karali 2002; Karali, *et al.,* 2007, 2002; Pandeya *et al.,* 2005; Quenelle *et al*., 2006; Terzioglu *et al*., 2006). Incited by this and as a part of our work on isatin derivatives, we recently reported the synthesis of some N^4-aryl-substituted isatins-3-thiosemicarbazones as antiurease compounds (Pervez *et al.,* 2009a, 2007, 2008, 2010). Investigation of the structure–activity relationship (SAR) studies revealed that, in

general, the type and position of the substituents about the phenyl ring at N^4 of the thiosemicarbazone moiety as well as introduction of a nitro function at position-5 of the isatin scaffold played an important role in causing rise in the urease inhibitory potential of these compounds (Pervez *et al.,* 2008, 2009a, 2010). Encouraged by these findings and in order to obtain antiurease compounds with improved efficacy, the study of the attachment of some other groups (alkyl, alkenyl and aryl having one or two substituents about the phenyl ring) to N^4 of the thiosemicarbazone moiety was considered worth pursuing. The work reported herein, therefore, primarily deals with the preparation and description of urease inhibition effects of twelve N^4-substituted 5-nitroisatin-3-thiosemicarbazones. It also describes the effects of the nature of functional groups (alkyl, alkenyl and aryl) attached to N^4 of the thiosemicarbazone moiety on the cytotoxicity, antifungal and phytotoxicity potential of these compounds.

Materials and methods

General

All chemicals and solvents were purchased from Aldrich, Fluka and Merck-Schuchardt. Melting points were determined on cover slips by using a Fisher-Johns melting point apparatus and are uncorrected. Micro (elemental) analyses were performed using a Leco CHNS-9320 (USA) instrument and were within ±0.4% of theoretical limits except where noted otherwise. Infrared (IR) spectra were measured as solids (KBr pellets) on a Shimadzu 8400 or a Shimadzu Prestige-21 FT-IR spectrophotometer. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded in DMSO-d_6 on a 300 MHz Bruker FT-NMR spectrometer using TMS as an internal standard. The chemical shifts (δ) are given in parts per million (ppm) relative to TMS ($\delta = 0.00$) and coupling constants in Hz. Mass spectra were recorded by means of a Finnigan MAT-312 or a JEOL MSRoute mass spectrometer at 70 eV. The homogeneity of the compounds was monitored by thin-layer chromatography (TLC) using Merck silica gel 60 GF_{254} coated glass plates, visualised by iodine vapour or under UV light at 254/366 nm.

Synthesis

General method for the synthesis of 5-nitroisatin-thiosemicarbazones ***2a–2l***

A solution of appropriate *N*-substituted thiosemicarbazide (2.5 mmol) in ethanol (10 ml) was added to a hot solution of 5-nitroisatin (2.5 mmol) in 50% aqueous ethanol (30 ml). After addition of 3–4 drops of glacial acetic acid, the mixture was heated under reflux for 2 h. The product formed during heating was filtered hot and washed thoroughly with hot aqueous ethanol to give the required thiosemicarbazones **2a**–**2l** in pure form.

*N-Hexyl-2-(5-nitro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)-1-hydrazinecarbothioamide (**2a**)*

Yield 82% as yellow crystals; m.p. 240°C (dec.); IR (KBr, cm^{-1}): 3346, 3275 (NH stretching), 1700 (C=O), 1640 (C=N), 1150 (C=S); ^{1}H-NMR (DMSO-d_6, δ, ppm): 0.87 (t, $J = 6.9$ Hz, 3H, $(CH_2)_5\underline{CH_3}$), 1.30–1.31 (m, 6H, $CH_2(\underline{CH_2})_3CH_2CH_3$), 1.16–1.64 (m, 2H, $(CH_2)_4\underline{CH_2}CH_3$), 3.61 (q, $J = 7.8$ Hz, 2H, $\underline{CH_2}(CH_2)_4CH_3$), 7.11 (d, $J = 8.7$ Hz, indole C_7-H), 8.26 (dd, $J = 8.7$, 2.4 Hz, 1H, indole C_6-H), 8.56 (d, $J = 2.4$ Hz, 1H, indole C_4-H), 9.58 (t, $J = 5.7$ Hz, 1H, CS-NH), 11.80 (s, 1H, indole NH), 12.32 (s, 1H, N–NH); EI MS (70 eV) *m/z* (%): 349 (M^+, 11), 321 (19), 273 (3), 206 (16), 192 (94), 190 (10), 189 (2), 178 (9), 159 (22), 144 (25), 117 (14), 115 (18), 103 (22), 100 (43), 56 (100); Anal. calcd. for $C_{15}H_{19}N_5O_3S$: C: 51.56, H: 5.48, N: 20.04; found: C: 51.52, H: 5.48, N: 20.11%.

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

Yield 73% as orange crystals; m.p. 230°C (dec.) (lit. (Karali, 2002; Hall *et al.,* 2009) m.p. 235–236°C, 226–230°C (dec.)); IR (KBr, cm^{-1}): 3345, 3220 (NH stretching), 1695 (C=O), 1610 (C=N), 1163 (C=S); ^{1}H-NMR (DMSO-d_6, δ, ppm): 4.27 (t, $J = 5.1$ Hz, 2H, $\underline{CH_2}CH{=}CH_2$), 5.14–5.23 (m, 2H, $CH_2CH{=}\underline{CH_2}$), 5.86–5.98 (m, 1H, $CH_2\underline{CH}{=}CH_2$), 7.11 (d, $J = 8.7$ Hz, 1H, indole C_7-H), 8.25 (dd, $J = 8.7$, 2.4 Hz, 1H, indole C_6-H), 8.55 (d, $J = 2.1$ Hz, 1H, indole C_4-H), 9.75 (t, $J = 6.0$ Hz, 1H, CS-NH), 11.81 (s, 1H, indole NH), 12.36 (s, 1H, N–NH); EI MS (70 eV) *m/z* (%): 305 (M^+, 6), 277 (5), 206 (6), 192 (64), 190 (3), 144 (8), 117 (12), 115 (76), 103 (15), 99 (6), 81 (37), 56 (100); Anal. calcd. for $C_{12}H_{11}N_5O_3S$: C: 47.21, H: 3.63, N: 22.94; found: C: 47.15, H: 3.65, N: 22.98%.

*N-(3-Methylphenyl)-2-(5-nitro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)-1-ydrazinecarbothioamide (**2c**)*

Yield 83% as yellow amorphous solid; m.p. 232°C (dec.); IR (KBr, cm^{-1}): 3300, 3175 (NH stretching), 1680 (C=O), 1610 (C=N), 1151(C=S); ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.36 (s, 3H, CH_3), 7.11 (d, $J = 8.7$ Hz, 1H, indole C_7-H), 7.12 (d, $J = 7.2$ Hz,1H, phenyl C_4-H), 7.32 (t, $J = 7.8$ Hz, 1H, phenyl C_5-H), 7.39-7.42 (m, 2H, phenyl C_2-H, C_6-H),

8.25 (dd, $J = 8.7$, 2.4 Hz, 1H, indole C_6-H), 8.68 (d, $J = 2.1$ Hz, 1H, indole C_4-H), 11.01 (s, 1H, CS-NH), 11.84 (s, 1H, indole NH), 12.51 (s, 1H, N–NH); EI MS (70 eV) *m/z* (%): 355 (M^+, 2), 327 (13), 248 (8), 206 (33), 190 (8), 189 (14), 178 (5), 164 (2), 149 (77), 148 (13), 144 (10), 117 (12), 115 (17), 107 (100), 106 (93), 103 (9), 91 (60), 65 (27); Anal. calcd. for $C_{16}H_{13}N_5O_3S$: C: 54.08, H: 3.69, N: 19.71; found: C: 54.20, H: 3.64, N: 19.65%.

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

Yield 91% as orange yellow crystals; m.p. 238°C (dec.) (lit. (Karali, 2002) m.p. 251–253°C); IR (KBr, cm^{-1}): 3300, 3170 (NH stretching), 1707 (C=O), 1620 (C=N), 1157 (C=S); ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.34 (s, 3H, CH_3), 7.12 (d, $J = 8.7$ Hz, 1H, indole C_7-H), 7.25 (d, $J = 8.4$ Hz, 2H, phenyl C_3-H, C_5-H), 7.45 (d, $J = 8.4$ Hz, 2H, phenyl C_2-H, C_6-H), 8.26 (dd, $J = 8.7$, 2.4 Hz, 1H, indole C_6-H), 8.69 (d, $J = 2.1$ 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* (%): 355 (M^+, 1), 327 (7), 248 (3), 206 (29), 190 (3), 189 (8), 178 (3), 149 (66), 148 (24), 144 (6), 117 (7), 115 (13), 107 (70), 106 (100), 103 (8), 91 (71), 65 (28); Anal. calcd. for $C_{16}H_{13}N_5O_3S$: C: 54.08, H: 3.69, N: 19.71; found: C: 54.01, H: 3.67, N: 19.75%.

*N-(4-Methoxyphenyl)-2-(5-nitro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)-1-hydrazinecarbothioamide (**2e**)*

Yield 87% as dirty golden amorphous solid; m.p. 233°C (lit. (Hall *et al.,* 2009) m.p. 220–248°C (dec.)); IR (KBr, cm^{-1}): 3305, 3180 (NH stretching), 1705 (C=O), 1660 (C=N), 1150(C=S); ^{1}H-NMR (DMSO-d_6, δ, ppm): 3.79 (s, 3H, OCH_3), 7.00 (dd, $J = 6.9$, 2.1 Hz, 2H, phenyl C_3-H, C_5-H), 7.12 (d, $J = 8.7$ Hz, 1H, indole C_7-H), 7.44 (dd, $J = 9.0$, 2.1 Hz, 2H, phenyl C_2-H, C_6-H), 8.26 (dd, $J = 8.7$, 2.4 Hz, 1H, indole C_6-H), 8.69 (d, $J = 2.4$ Hz, 1H, indole C_4-H), 11.00 (s, 1H, CS-NH), 11.84 (s, 1H, indole NH), 12.50 (s, 1H, N–NH); EI MS (70 eV) *m/z* (%): 371 (M^+, 8), 343 (9), 248 (9), 206 (9), 190 (16), 189 (8), 165 (34), 163 (4), 150 (29), 148 (12), 144 (22), 133 (14), 123 (53), 117 (7), 115 (18), 108 (100), 103 (12), 80 (83); Anal. calcd. for $C_{16}H_{13}N_5O_4S$: C: 51.75, H: 3.53, N: 18.86; found: C: 51.82, H: 3.49, N: 18.82%.

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

Yield 85% as yellow crystals; m.p. 242°C; IR (KBr, cm^{-1}): 3300, 3210 (NH stretching), 1690 (C=O), 1602 (C=N), 1161(C=S); ^{1}H-NMR (DMSO-d_6, δ, ppm): 7.14 (d, $J = 8.7$ Hz, 1H, indole C_7-H), 7.56 (d, $J = 7.8$ Hz, 1H, phenyl C_6-H), 7.63 (t, $J = 7.5$ Hz, 1H, phenyl C_4-H), 7.80 (t, $J = 7.2$ Hz, 1H, phenyl C_5-H), 7.86 (d, $J = 8.1$ Hz, phenyl C_3-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), 11.08 (s, 1H, CS-NH), 11.85 (s, 1H, indole NH), 12.60 (s, 1H, N–NH); EI MS (70 eV) *m/z* (%): 409 (M^+, 30), 381 (100), 340 (2), 312 (2), 283 (8), 248 (1), 224 (12), 218 (2), 206 (47), 203 (75), 190 (6), 189 (3), 184 (99.7), 178 (21), 176 (8), 163 (5), 161 (31), 149 (29), 148 (5), 145 (45), 144 (15), 117 (8), 115 (41), 103 (31); Anal. calcd. for $C_{16}H_{10}F_3N_5O_3S$: C: 46.95, H: 2.46, N: 17.11; found: C: 46.87, H: 2.42, N: 17.16%.

*N-(4-Fluorophenyl)-2-(5-nitro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)-1-hydrazinecarbothioamide (**2g**)*

Yield 86% as orange crystals; m.p. 276°C (lit. (Karali, 2002) m.p. 275°C); IR (KBr, cm^{-1}): 3295, 3150 (NH stretching), 1700 (C=O), 1650 (C=N), 1140(C=S); ^{1}H-NMR (DMSO-d_6, δ, ppm): 7.12 (d, $J = 8.7$ Hz, 1H, indole C_7-H), 7.28 (t, $J = 7.8$ Hz, 2H, phenyl C_2-H, C_6-H), 7.57–7.61 (m, 2H, phenyl C_3-H, C_5-H), 8.26 (dd, $J = 8.7$, 2.4 Hz, 1H, indole C_6-H), 8.65(d, $J = 2.4$ Hz, 1H, indole C_4-H), 11.06 (s, 1H, CS-NH), 11.85 (s, 1H, indole NH), 12.54(s, 1H, N–NH); EI MS (70 eV) *m/z* (%): 359 (M^+, 13), 331 (59), 301 (3), 248 (3), 206 (18), 190 (5), 189 (3), 178(11), 163 (3), 153 (55), 149 (20), 148 (4), 144 (14), 117 (8), 115 (14), 111 (54), 103 (29), 95 (100), 76 (35), 75 (70); Anal. calcd. for $C_{15}H_{10}FN_5O_3S$: C: 50.14, H: 2.81, N: 19.49; found: C: 50.21, H: 2.77, N: 19.45%.

*N-(4-Bromophenyl)-2-(5-nitro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)-1-hydrazinecarbothioamide (**2h**)*

Yield 88% as orange yellow amorphous solid; m.p. 262°C (lit. (Karali, 2002) m.p. 235–236°C); IR (KBr, cm^{-1}): 3300, 3150 (NH stretching), 1680 (C=O), 1600 (C=N), 1159 (C=S); ^{1}H-NMR (DMSO-d_6, δ, ppm): 7.12 (d, $J = 8.7$ Hz, 1H, indole C_7-H), 7.57-7.65 (m, 4H, phenyl C_2-H, C_3-H, C_5-H, C_6-H), 8.26 (dd, $J = 8.7$, 2.4 Hz, 1H, indole C_6-H), 8.66 (d, $J = 2.4$ Hz, 1H, indole C_4-H), 11.07 (s, 1H, CS-NH), 11.86 (s, 1H, indole NH), 12.58 (s, 1H, N–NH); EI MS (70 eV) *m/z* (%): 393 (3), 391 (3), 248 (6), 215 (75), 213 (71), 206 (48), 190 (9), 189 (17), 173 (82), 171 (86), 163 (3), 149 (16), 148 (2), 144 (11) 134 (29), 117 (9), 115 (31), 103 (19), 92 (46), 91 (20), 65 (100); Anal. calcd. for $C_{15}H_{10}BrN_5O_3S$: C: 42.87, H: 2.40, N: 16.67; found: C: 42.79, H: 2.39, N: 16.73%.

*N-(4-Iodophenyl)-2-(5-nitro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)-1-hydrazinecarbothioamide (**2i**)*

Yield 89% as dirty yellow amorphous solid; m.p. 270°C (dec.); IR (KBr, cm^{-1}): 3304, 3160 (NH stretching), 1697

(C=O), 1622 (C=N), 1159 (C=S); ^{1}H-NMR (DMSO-d_6, δ, ppm): 7.11 (d, $J = 8.7$ Hz, 1H, indole C_7-H), 7.45 (d, $J = 8.7$ Hz, 2H, phenyl C_2-H, C_6-H), 7.79 (dd, $J = 8.7$, 1.8 Hz, 2H, phenyl C_3-H, C_5-H), 8.26 (dd, $J = 8.7$, 2.4 Hz, 1H, indole C_6-H), 8.66 (d, $J = 2.4$ Hz, 1H, indole C_4-H), 11.05 (s, 1H, CS-NH), 11.85 (s, 1H, indole NH), 12.57 (s, 1H, N–NH); EI MS (70 eV) *m/z* (%): 467 (M^+, 5), 439 (21), 261 (52), 248 (23), 219 (95), 206 (19), 190 (21), 189 (14), 178 (6), 176 (5), 163 (5), 159 (14), 150 (4), 148 (2), 144 (25), 134 (32), 117 (8), 115 (25), 103 (13), 92 (86), 65 (100); Anal. calcd. for $C_{15}H_{10}IN_5O_3S$: C: 38.56, H: 2.16, N: 14.99; found: C: 38.62, H: 2.13, N: 14.93%.

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

Yield 80% as yellow amorphous solid; m.p. 275–280°C (dec.); IR (KBr, cm^{-1}): 3285, 3170 (NH stretching), 1701 (C=O), 1624 (C=N), 1157 (C=S); ^{1}H-NMR (DMSO-d_6, δ, ppm): 7.12 (d, $J = 8.7$ Hz, 1H, indole C_7-H), 7.66 (t, $J = 7.8$ Hz, 1H, phenyl C_5-H), 7.77 (dt, $J = 7.8$, 1.2 Hz, 1H, phenyl C_4-H), 7.98 (ddd, $J = 8.1$, 2.1, 1.2 Hz, 1H, phenyl C_6-H) 8.11 (t, $J = 1.5$ Hz, 1H, phenyl C_2-H), 8.27 (dd, $J = 8.7$, 2.4 Hz, 1H, indole C_6-H), 8.63 (d, $J = 2.4$ Hz, 1H, indole C_4-H), 11.17 (s, 1H, CS-NH), 11.88 (s, 1H, indole NH), 12.62 (s, 1H, N–NH); EI MS (70 eV) *m/z* (%): 366 (M^+, 2), 352 (1), 338 (3), 306 (1), 293 (1), 248 (17), 206 (50), 190 (10), 189 (24), 178 (8), 175 (2), 163 (4), 160 (100), 149 (7), 144 (9), 118 (73), 115 (24), 103 (8), 102 (31), 91 (17), 76 (10), 64 (12); Anal. calcd. for $C_{16}H_{10}N_6O_3S$: C: 52.46, H: 2.75, N: 22.94; found: C: 52.53, H: 2.72, N: 22.89%.

*N-(2,4-Dimethoxyphenyl)-2-(5-nitro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)-1-hydrazinecarbothioamide (**2k**)*

Yield 85% as orange red crystals; m.p. 260°C (dec.); IR (KBr, cm^{-1}): 3280, 3160 (NH stretching), 1680 (C=O), 1610 (C=N), 1143 (C=S); ^{1}H-NMR (DMSO-d_6, δ, ppm): 3.80 (s, 6H, OCH_3), 6.58 (dd, $J = 8.7$, 2.7 Hz, 1H, phenyl C_5-H), 6.69 (d, $J = 2.7$ Hz, 1H, phenyl C_3-H), 7.14 (d, $J = 8.7$ Hz, 1H, indole C_7-H), 7.28 (d, $J = 8.4$ Hz, 1H, phenyl C_6-H), 8.27 (dd, $J = 8.7$, 2.4 Hz, 1H, indole C_6-H), 8.67 (d, $J = 2.4$ Hz, 1H, indole C_4-H), 10.65 (s, 1H, CS-NH), 11.82 (s, 1H, indole NH), 12.47 (s, 1H, N–NH); EI MS (70 eV) *m/z* (%): 401 (M^+, 24), 373 (36), 358 (1), 342 (1), 248 (31), 206 (29), 195 (58), 190 (21), 189 (29), 178 (19), 153 (76), 149 (7), 144 (19), 138 (100), 131 (11), 117 (6), 115 (30), 110 (34), 103 (8), 95 (28) 78 (8), 52 (12); Anal. calcd. for $C_{17}H_{15}N_5O_5S$: C: 50.87, H: 3.77, N: 17.45; found: C: 50.79, H: 3.75, N: 17.53%.

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

Yield 82% as light orange red amorphous solid; m.p. 276°C; IR (KBr, cm^{-1}): 3300, 3230 (NH stretching), 1700 (C=O), 1610 (C=N), 1109 (C=S); ^{1}H-NMR (DMSO-d_6, δ, ppm): 3.72 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 6.89 (dd, $J = 9.0$, 3.0 Hz, 1H, phenyl C_4-H), 7.07 (d, $J = 9$ Hz, 1H, phenyl C_3-H), 7.14 (d, $J = 8.7$ Hz, 1H, indole C_7-H), 7.20 (d, $J = 3$ Hz, 1H, phenyl C_6-H), 8.28 (dd, $J = 8.7$, 2.4 Hz, 1H, indole C_6-H), 8.59 (d, $J = 2.4$ Hz, 1H, indole C_4-H), 10.74 (s, 1H, CS-NH), 12.32 (s, 1H, indole NH), 12.48 (s, 1H, N–NH); EI MS (70 eV) *m/z* (%): 401 (M^+, 3), 373 (4), 206 (21), 195 (38), 190 (2), 189 (9), 180 (49), 176 (1), 153 (41), 148 (2), 144 (7), 138 (100), 115 (20), 107 (5), 103 (10), 95 (34), 76 (14); Anal. calcd. for $C_{17}H_{15}N_5O_5S$: C: 50.87, H: 3.77, N: 17.45; found: C: 50.92, H: 3.73, N: 17.40%.

Biological testing

Cytotoxicity in vitro

A hatching tray was filled with sea water (38 g sea salt/l water, pH 7.4). Brine shrimp (*Artemia salina* Leach) eggs were sprinkled in one half of the tray and that part was covered with a lid. Other half of the tray was left open under light for 1–2 days at 30 ± 3°C. The eggs hatched and larvae moved from the dark to enlightened area through the pores made between the two halves of the tray. Brine shrimp larvae were collected with the help of Pasteur pipette. Test samples were dissolved in DMF and different volumes of the prepared solutions were disposed off in three test tubes, each corresponding to a different concentration (500, 100 and 20 μg). The solvent was evaporated to dryness overnight. After ensuring the complete evaporation of solvent, sea water was added into the test tubes to a total volume of 5 ml and 2–4 days old larvae were transferred from hatching tray to the test tubes containing samples. Incubation was done at 30 ± 3°C for 24 h under illumination. The number of survivors were then counted and recorded (Rahman *et al.*, 1999). The test tubes supplemented with DMF and etoposide (the anticancer drug) served as negative and positive controls, respectively. The controls were also processed in the same way as the test samples were. Data were analysed by EZ-Fit 5 software from Perrella Scientific, Inc.

Antifungal activity in vitro

Antifungal activities of all the compounds were studied against five fungal cultures. Sabouraud dextrose agar (Oxoid, Hampshire, England) was seeded with 10^5 (cfu) ml^{-1}

fungal spore suspensions and transferred to petri plates. Discs soaked with 20 µl (200 µg/ml in DMSO) of all the compounds were placed at different positions on the agar surface. The plates were incubated at 27–29°C for 7 days. The results were recorded (Hussain *et al.,* 2003) as zone of inhibition (mm) and compared with standard drugs, miconazole and amphotericin B.

Phytotoxicity in vitro

Phytotoxic activity of all the synthesised compounds was determined according to the modified literature protocol (McLaughlin *et al.,* 1991) using MeOH and paraquat as negative and positive controls, respectively.

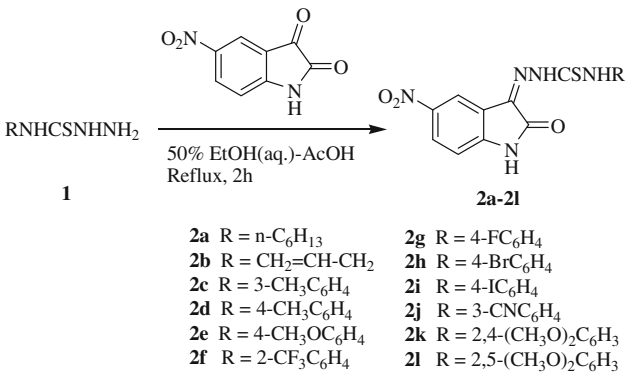
Urease inhibitory activity in vitro

The modified Berthelot (phenol-hypochlorite) method (Weatherburn, 1967) was used for the determination of antiurease activity of the synthesised compounds **2a–2l**. Briefly, 200 µl of phosphate buffer ($K_2H_2PO_4$ + KOH, pH 7) containing 1 unit of Human urease (Gesellschaft für Biochemica und Diagonistica mbH, Germany) enzyme was mixed with 230 µl of phosphate buffer and 20 µl of the test and thiourea (reference inhibitor of urease) solutions in DMF containing 0.01–100 µM concentrations (three test tubes for each concentration). DMF (20 µl) tested alone as a control did not have any inhibitory effect on the activity of the enzyme. The reaction mixture was then incubated at 25°C for 5 min. After the incubation period, 400 µl of urea stock solution (20 mM) was added. Calibration mixture was prepared with no urea solution. All the test tubes were further incubated for 10 min at 40°C to accomplish urea hydrolysis. This was followed by the addition of 1,150 µl phenol-hypochlorite reagent (freshly prepared by mixing 450 µl phenol reagent (phenol + sodium nitroprusside) and 700 µl alkali reagent (NaOH + NaOCl)). The tubes were again incubated at 56°C for 25 min. After 5 min of cooling, absorbance of the blue coloured complex thus formed was noted at 625 nm and the percentage inhibition was calculated as the difference of absorbance values with and without the test compounds (Nagata *et al.*, 1992). IC_{50} values were calculated using EZ-Fit 5 software from Perrella Scientific, Inc.

Results and discussion

Chemistry

5-Nitroisatin, appropriate thiosemicarbazide and 50% aqueous ethanol containing a catalytic amount of glacial acetic acid were heated under reflux for 2 h (Scheme 1).



Scheme 1 Synthesis of title compounds **2a–2l**

The crystalline or amorphous solid formed during heating in each case was filtered hot. Thorough washing with 50% hot aqueous ethanol afforded the target thiosemicarbazones **2a–2l** in good to excellent yields (73–91%).

The structures of all the synthesised compounds **2a–2l** were determined by analytical and spectral (IR, ^{1}H-NMR, EIMS) methods. The infrared (IR) spectra of **2a–2l** displayed absorption bands in the 3346–3210 and 3180–3150, 1707–1680, 1660–1600 and 1163–1109 cm^{-1} regions attributed to NH, C=O, C=N and C=S functions, respectively (Karali, 2002; Naumov and Anastasova, 2001; Omar *et al.,* 1984; Petrov *et al.,* 1986). The proton nuclear magnetic resonance (^{1}H-NMR) spectra of compounds **2c–2l** demonstrated the N^4-H and N^2-H protons of the thiosemicarbazone moiety (δ 10.65–11.17 and δ 12.47–12.62, respectively) and the indole NH proton (δ 11.82–12.32) as three separate singlets (Karali, 2002; Laatsch *et al.,* 1984; Nizamuddin *et al.,* 1999; Omar *et al.,*1984). Similarly, the ^{1}H-NMR spectra of **2a** and **2b** exhibited two separate singlets at δ 11.80–11.81 and δ 12.32–12.36, respectively, for the indole NH and thiosemicarbazone N^2-H. However, the thiosemicarbazone N^4-H resonance in these cases was observed as a triplet at δ 9.58–9.75 due to CH_2 coupling. The indole C_7-H resonated as a doublet at δ 7.11–7.14, while the indole C_6-H, being deshielded due to electron-attracting inductive effect of the nitro group at position-5, appeared at δ 8.25–8.28 as a double doublet. Indole C_4-H experienced a deshielding effect due to inductively electron-withdrawing nitro and C=N functions and resonated further downfield as a doublet at δ 8.55–8.69 (Baron *et al.,*1990; Eshba *et al.,* 1987; Karali, 2002; Laatsch *et al.,* 1984). The electron impact mass spectra (EIMS) of all the synthesised compounds (except **2h**) displayed molecular ions with different intensities. The structure of compound **2h** was, however, confirmed by the presence of the fragments corresponding to thiosemicarbazone moiety, formed by cleavage of N–N and NH–CS bonds. The proposed fragmentation pattern of **2f** is illustrated in Fig. 1. X-ray structure of **2a** was determined in order to confirm the

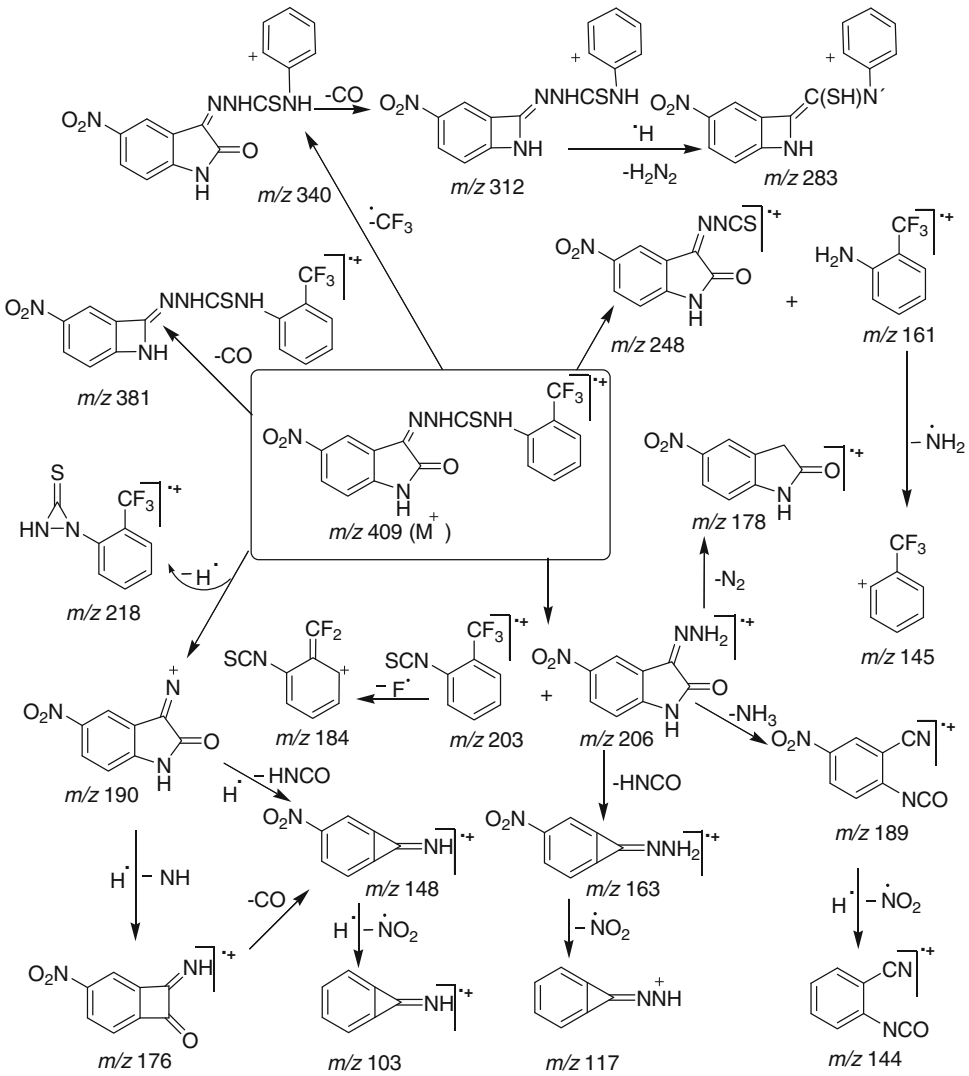


Fig. 1 The proposed fragmentation pattern of compound **2f**

assigned structures and establish conformations of the synthesised thiosemicarbazones **2a**–**2l**. Relevant crystal data and details of the structure elucidation have been reported elsewhere (Pervez *et al.,* 2009b).

Biology

Cytotoxicity in vitro

The synthesised compounds **2a**–**2l** were subjected to cytotoxicity evaluation by the brine shrimp lethality bioassay. Compound **2m** i.e. 1-(5-nitro-2-oxoindolin-3-ylidene)-4-phenylthiosemicarbazide, the synthesis of which has been reported elsewhere (Pervez *et al.,* 2010), served as a reference point to evaluate the effects of different substituents (alkyl, alkenyl and aryl having one or two functions about the phenyl ring) at N^4 of the thiosemicarbazone moiety on cytotoxicity potential of these compounds. From the data recorded in Table 1, it is evident that all the compounds of this series except **2j** gave LD_{50} values greater than 1.07×10^{-3}–1.63×10^{-3} M against *Artemia salina* in this assay and, therefore, can be considered to be almost inactive. Compound **2j** with a cyano group at position-3 of the phenyl ring, however, proved to be an active cytotoxic agent in the present series exhibiting LD_{50} value 1.16×10^{-3} M.

Antifungal activity in vitro

Antifungal activity of the synthesised compounds **2a**–**2l** was determined against five fungal cultures *i.e. Candida albicans, Aspergillus flavus, Microsporum canis, Fusarium solani* and *Candida glabrata* (Table 2) at 200 μg/ml in DMSO. Here too, compound **2m** served as a reference point to assess the effects of different substituents attached to N^4 of the thiosemicarbazone moiety on antifungal potential of these compounds. All the compounds except **2i** were found to be active against one or more pathogens

Table 1 Brine shrimp bioassay for compounds **2a**–**2m**

Compounds	LD_{50} (M)
2a	$>1.43 \times 10^{-3}$
2b	$>1.63 \times 10^{-3}$
2c	$>1.40 \times 10^{-3}$
2d	$>1.40 \times 10^{-3}$
2e	$>1.34 \times 10^{-3}$
2f	$>1.22 \times 10^{-3}$
2g	$>1.39 \times 10^{-3}$
2h	$>1.19 \times 10^{-3}$
2i	$>1.07 \times 10^{-3}$
2j	1.16×10^{-3}
2k	$>1.24 \times 10^{-3}$
2l	$>1.24 \times 10^{-3}$
2m[a]	$>1.47 \times 10^{-3}$

[a] Pervez *et al.*, 2010

Table 2 Antifungal activity (in vitro) of compounds **2a**–**2m*** (% inhibition)

Compounds	Microbial species				
	C. albicans	*A. flavus*	*M. canis*	*F. solani*	*C. glabrata*
2a	00	00	50	00	00
2b	00	00	20	00	00
2c	00	00	00	30	00
2d	00	30	40	30	00
2e	00	20	00	00	00
2f	00	00	20	00	00
2g	00	00	20	20	00
2h	00	20	00	00	00
2i	00	00	00	00	00
2j	00	20	20	15	00
2k	00	20	20	00	00
2l	00	40	20	00	00
2m	00	20	00	00	00

*Concentration used 200 μg/ml of DMSO

exhibiting varied inhibition (15–50%). Compounds **2a** and **2b** having n-hexyl and allyl substituents at N^4 of the thiosemicarbazone moiety were found to display 50 and 20% inhibition of *M. canis*, respectively, whereas **2m** with phenyl group at the same position displayed no activity against the said fungal strain. Amongst the aryl-substituted compounds **2c** and **2d** having methyl substituents at different positions of the phenyl ring, compound **2c** with *meta*-substituent demonstrated 30% inhibition against *F. solani,* whereas the *para*-substituted compound **2d** displayed relatively much better activity profile (30, 40 and 30%) against *A. flavus, M. canis* and *F. solani,* respectively. Compound **2e** having methoxy substituent at *para* position of the phenyl ring, however, showed 20% inhibition against *A. flavus* only. Similarly, compound **2f** having trifluoromethyl function at *ortho* position of the phenyl ring exhibited 20% inhibition against *M. canis* only. Amongst the halogenated compounds **2g**–**2i**, compounds **2g** and **2h** with fluoro and bromo groups at *para* position were active against *M. canis* and *F. solani,* and *A. flavus*, respectively, demonstrating 20% inhibition, whereas compound **2i** having iodo substituent at the same position was inactive against all the fungi. Also, similar to compound **2d,** compound **2j** with cyano substituent at *mata* position of the phenyl ring exhibited relatively better activity profile (20, 20 and 15%, respectively) against the same fungal strains i.e. *A. flavus, M. canis* and *F. solani.* Of the remainder N^4-aryl-substituted compounds **2k** and **2l** having methoxy substituents at *ortho*, *para* (2, 4) and *ortho*, *meta* (2, 5) positions of the phenyl ring, respectively, compound **2l** was found to show relatively more inhibition against *A. flavus* (40 vs. 20%). Interestingly, the reference compound **2m** having no substituent about its phenyl ring also displayed 20% inhibition of *A. flavus*, thus clearly indicating that the electronic effects of different substituents about the phenyl ring attached to N^4 of the thiosemicarbazone moiety did not play a significant role in enhancing the capability of certain active compounds to exhibit added inhibition against this fungal strain. On the contrary, both **2k** and **2l** demonstrated 20% inhibition of *M. canis*, whereas as mentioned earlier, **2m** was found to be completely inactive against this fungal culture.

Notably, some fungi, especially *C. albicans* and *C. glabrata* were resistant to all the tested compounds, whereas the others i.e. *A. flavus* and *F. solani* showed resistance to most of the tested compounds. None of the compounds of the present series displayed significant antifungal activity; however, the negative findings or display of weak or moderate activities do not preclude from further investigations of these compounds against other fungal strains.

Phytotoxicity in vitro

The synthesised 5-nitroisatin-3-thiosemicarbazones **2a**–**2l** were also tested for their phytotoxicity potential at 1000, 100 and 10 μg/ml concentrations. The results presented in Table 3 revealed that all the compounds except **2a** and **2b** exhibited weak or non-significant (20–35%) plant growth inhibition at the highest tested concentration (1,000 μg/ml). Compounds **2a** and **2b** having n-hexyl and allyl substituents at N^4 of the thiosemicarbazone moiety, however, displayed moderate inhibition (60 and 50%, respectively) at the same tested concentration. Out of ten compounds possessing different substituents about the phenyl ring attached to N^4 of the thiosemicarbazone moiety, eight i.e.

Table 3 Percent growth inhibition of *Lemna aequinocitalis* by compounds **2a–2m** at different concentrations

Compounds	1000 (μg/ml) (% G.I.)	100 (μg/ml) (% G.I.)	10 (μg/ml) (% G.I.)
2a	60	35	25
2b	50	35	25
2c	25	20	10
2d	30	20	15
2e	25	15	10
2f	25	15	10
2g	20	15	05
2h	20	15	10
2i	25	10	05
2j	25	15	05
2k	30	25	15
2l	35	30	25
2m[a]	20[b]	10[b]	05[b]

The reference compound paraquat shows 100% growth inhibition at a concentration of 0.015 μg/ml

G.I. growth inhibition

[a] Tested at 500, 50 and 5 μg/ml concentrations; [b] corresponding G.I. values

2c–2f and **2i–2l** were found to exhibit relatively greater inhibition of plant growth (25–35%), when compared with the reference point **2m** (having un-substituted phenyl ring), which displayed 20% plant growth inhibition. On the other hand, all the synthesised compounds including **2m** demonstrated weak or non-significant (5–25%) plant growth inhibition at the lowest tested concentration (10 μg/ml).

From the results obtained in this assay, it may be concluded that compared with the standard drug, paraquat, which displayed 100% inhibition at a concentration of 0.015 μg/ml, our test compounds exhibited weak or non-significant inhibition at much higher concentration. Furthermore, the percent growth inhibition values displayed by our trial compounds revealed that the nature or type of the substituents at N^4 of the thiosemicarbazone moiety did not significantly affect their phytotoxicity potential at both the highest as well as lowest tested concentrations (1,000 and 10 μg/ml).

Urease inhibition in vitro

Urease inhibitory effects of the synthesised thiosemicarbazones **2a–2l** were determined according to the protocol of the Department of Chemistry, The Islamia University of Bahawalpur, Pakistan. The results given in Table 4 demonstrated that all the compounds tested in this assay except **2g** exhibited enzymatic activity at almost all the tested concentrations (100, 10, 1, 0.1, 0.01 μM). Compound **2d** and the reference point **2m**, however, did not show inhibitory activity at 0.1 and 10 μM concentrations, respectively, whereas compound **2g** showed an anomalous behaviour displaying increment in the enzymatic activity at all the tested concentrations. The results collected in Table 4 also revealed that as compared to compound **2m** with a phenyl group at N^4 of the thiosemicarbazone moiety, **2a** and **2b** having n-hexyl and allyl functions at the same position exhibited enhanced enzyme inhibitory activity at the highest tested concentration (100 μM).

Table 4 Inhibition of human urease by compounds **2a–2m**

Compounds	Inhibition at 100 μM (% ± SD)	Inhibition at 10 μM (% ± SD)	Inhibition at 1 μM (% ± SD)	Inhibition at 0.1 μM (% ± SD)	Inhibition at 0.01 μM (% ± SD)	IC_{50} (μM)
2a	40.9 ± 2.571	27 ± 1.000	37 ± 1.527	58 ± 1.616	60 ± 1.327	0.440
2b	15.9 ± 0.950	8 ± 1.001	12 ± 1.000	15 ± 0.577	21.6 ± 1.184	
2c	34 ± 3.000	22 ± 2.640	13.9 ± 0.854	5.8 ± 1.026	6.5 ± 2.157	
2d	7 ± 0.577	4 ± 0.416	4 ± 1.386	NA	8 ± 1.517	
2e	46.6 ± 1.527	38.6 ± 0.529	33.6 ± 1.527	44 ± 1.000	47.6 ± 0.577	
2f	21 ± 1.184	20.9 ± 0.173	30.9 ± 1.001	23 ± 1.154	21.6 ± 0.635	
2g	↑	↑	↑	↑	↑	
2h	4 ± 0.896	8 ± 0.577	6.6 ± 0.550	4.5 ± 0.814	18.9 ± 1.000	
2i	50 ± 3.055	47 ± 1.442	52 ± 1.157	51 ± 2.200	57 ± 0.577	0.901
2j	8.9 ± 1.792	12.6 ± 2.281	1.8 ± 1.205	16 ± 2.804	20.7 ± 1.123	
2k	37.9 ± 2.475	53.5 ± 2.200	61 ± 3.605	66.8 ± 2.362	68 ± 1.258	27.880
2l	11 ± 1.500	17.8 ± 1.069	15.6 ± 0.692	25.9 ± 0.173	34.5 ± 2.590	
2m	6 ± 0.320	NA	0.65 ± 0.224	5.8 ± 0.763	8.8 ± 1.616	
Thiourea[a]	94.33 ± 1.003	8.09 ± 2.006	20.92 ± 0.501	14.54 ± 1.504	8.16 ± 0.501	50.105

↑ Increment in enzymatic activity, *NA* no inhibitory activity

[a] Reference urease inhibitor

Much pronounced enhancement was observed in the case of **2a** (6.0% → 40.9% with IC_{50} value 0.440 μM). Similarly, compared with **2m**, all the synthesised N^4-aryl-substituted thiosemicarbazones except **2d** and **2h** displayed increased inhibitory activity at the same tested concentration. Relatively, much increment was observed in the cases of **2c**, **2e**, **2i** and **2k** (34.0, 46.6, 50.0% with IC_{50} value 0.901 μM and 37.9% with IC_{50} value 27.880 μM, respectively). To the contrary, compound **2h** was found to exhibit slight decrement in the inhibitory activity (6% → 4%), whereas **2d** displayed almost the same activity as of the reference point **2m** (7 vs. 6%).

Overall, out of 12 compounds tested for their urease inhibitory potential, 11 i.e. **2a**–**2f** and **2h**–**2l** exhibited enzymatic inhibition. Of these, three i.e. **2a**, **2i** and **2k** were found to be potent inhibitors of urease activity. These compounds may act as leads and could be the potential candidates for further studies.

All ureases, regardless of their origin, possess besides two coordinated Ni(II) ions (Benini *et al.,* 1998, 2000, 2001, 2004; Ciurli *et al.,*1999; Krajewska and Zaborska, 2007a, b), one, two or three closely related protein subunits (Mobley *et al.,* 1995). Thus, a urease inactivator intermingles with the enzyme activity by contacting either with the Ni(II) ions or the protein constituent. *β*-Mercaptoethanol (BME), hydroxamic acids (HXAs) and phosphorodiamidates (PPDs), for example, are the synthetic inhibitors, which were reported to interact with the enzyme activity by binding to the Ni(II) ions present at its active site (Ciurli *et al.,* 1999; Kuhler *et al.,* 1995; Nagata *et al.,* 1993; Zaborska *et al.,* 2007). In contrast, sulphenamide, quinones and heavy metal ions have been found to influence enzymatic activity by intermingling with the thiol (S–H) functions present in its protein component (Krajewska 2008; Krajewska and Zaborska, 2007a, b; Krajewska *et al.,* 2004; Kuhler *et al.,* 1995; Nagata *et al.,* 1993; Zaborska *et al.,* 2007).

The actual mechanism of urease inhibition by our trial compounds **2a**–**2l** is not documented. These compounds seem to be mechanism-based urease inhibitors and inhibit the enzyme through a chelate interaction, which binds to its active site in a normal substrate-like mode. This process appears to be similar to charge-transfer transitions, which occur between thiolate of *β*-mercaptoethanol and Ni (II) of the enzyme, suggesting that the thiolate binds directly to the nickel ion(s). Different non-covalent interactions including hydrogen bonds and hydrophobic contacts may stabilise such enzyme-inhibitors chelate interactions and thus contribute towards their inhibitory potential. Additionally, inhibitory potency may also depend upon electron-affinity of the coordinating atoms. Furthermore, bulky groups attached to the pharmacophore (the actual ligand-chelator) may reduce activity of the inhibitors, as the lack of bulky attachment makes it easier for the urease inhibitors to enter the substrate-binding pocket as well as avoid unfavourable steric interactions with the amino acid residues in their vicinity (Amtul *et al.,* 2002). The results that we have observed in the cases of potent thiosemicarbazones **2a**, **2i** and **2k** are consistent with these hypotheses. The SAR studies demonstrated that the differential inhibitory potential, as exhibited by these potent compounds, depended primarily on the electronic and/or steric effects of the alkyl or aryl substituents attached to N^4 of the thiosemicarbazone moiety. For example, compared with the reference point **2m** having un-substituted phenyl group at N^4, compound **2a** possessing an electron-donating n-hexyl substituent at the same position displayed much pronounced inhibitory potential (IC_{50} value 0.440 μM). This might be attributed to the promotion of electron-availability on N^4 by the n-hexyl function. The unshared electron-pair on N^4 is fully available for delocalization over the thiocarbonyl sulphur atom, so as to enhance its electron-affinity for coordination with the electrophilic nickel ions present at the active site of the enzyme. In the case of **2m**, reduction in electron-availability on N^4 occurs by the partial delocalization of unshared electron-pair over the benzene ring-carbon atoms via their *p* orbitals. This caused reduction in the electron-affinity of the thiocarbonyl sulphur atom, which took part in coordination/chelation with the nickel ions of the enzyme's active site, making a relatively less stable enzyme-inhibitor chelate and thus resulting into a much pronounced decrement in inhibitory activity. To the contrary, compound **2i** having iodo substituent at *para* position of the phenyl ring exhibited a high degree of inhibitory potential with IC_{50} value 0.901 μM. Iodo group is both electron-donating by mesomeric effect and electron-attracting by inductive effect. However, the larger positive mesomeric effect overweighs the smaller negative inductive effect. The IC_{50} value indicates that by comparison, the electron-donating mesomeric effect of the iodo substituent increases the inhibitory potential of the compound because of much reduction in delocalization of the N^4 unshared electron-pair over the benzene ring-carbon atoms. The unshared electron-pair on N^4 is now more available for delocalization over the adjacent electron-withdrawing thiocarbonyl group, thus increasing the electron-affinity of the sulphur atom and making a relatively more stable enzyme-ligand complex. In view of this, it was expected that substitution of two strong electron-donating groups at *ortho* and *para* positions of the phenyl ring would have given a more potent urease inhibitor with IC_{50} value lower than that of compound **2i**. However, quite interestingly, compound **2k** with methoxy substituents at *ortho* and *para* positions of the phenyl ring attached to N^4 of the thiosemicarbazone moiety displayed much higher IC_{50} value (27.880 μM). This effect can be attributed to the

steric bulk introduced by methoxy group at *ortho* position of the phenyl ring, which overweighed the positive mesomeric effects of the substituents in part and thus played an important role in decreasing the inhibitory potential of the compound. Expectedly, because of steric hindrance of the substituent at *ortho* position, the side chains of the enzyme may not be able to efficiently access the compound/ligand for binding. This conclusion is supported by the much smaller IC_{50} value given by compound **2i** having a bulky iodo substituent at *para* position of the phenyl ring, which exhibited either no or much less steric hindrance. Furthermore, contrary to compound **2k**, the corresponding mono-substituted compound possessing a methoxy substituent at *ortho* position of the phenyl ring did not show any inhibitory activity in our earlier assay (Pervez *et al*., 2010). However, compared with the reference compound **2m**, the corresponding mono-substituted compound **2e** with a methoxy substituent at *para* position of the phenyl ring was found to display much enhanced inhibitory activity in the present assay. Similarly, as compared to **2m**, the respective di-substituted compound **2l** having methoxy substituents at *ortho* and *meta* (2,5) positions of the phenyl ring exhibited enhanced inhibitory activity at all the tested concentrations in the present assay, but with percent inhibitory values lower than that of compound **2e**. This lessening in inhibitory potential is attributed to the fact that the mesomeric effects are similar at *ortho* and *para* positions but different for the substituents at *meta* position. These results strongly suggest that mesomeric effects of the substituents at *ortho* and *para* positions of the phenyl ring play a pivotal role in the inhibition of urease. Nonetheless, extensive studies are required to elucidate the mechanism by which isatins-derived thiosemicarbazones exhibit the urease inhibitory activity.

Conclusions

Conclusively, all the synthesised compounds of the present series except **2j** were found to be almost inactive in the brine shrimp bioassay. In antifungal assay, 11 out of 12 compounds showed weak to moderate activity against one or more selected fungi. The negative findings or exhibition of weak or moderate activities, however, do not prevent from further investigations of these compounds against other fungal cultures. In phytotoxicity bioassay, all the synthesised thiosemicarbazones displayed weak activity at the highest tested concentration. Nevertheless, in the enzyme inhibition assay, 11 out of 12 compounds displayed urease inhibitory activity; three of these i.e. **2a**, **2i** and **2k** proved to be potent urease inhibitors exhibiting exciting activity and may represent valid lead compounds for further studies. These compounds, having shown no significant phytotoxic influences at the highest tested concentration, invited attention to their effectiveness as potent inactivators of soil ureases, as they could be combined with fertilizers in small amounts to increase the overall efficiency of nitrogen utilisation. The SAR studies revealed that the urease inhibitory potential of the test compounds depended mainly upon electronic effects of the substituents attached to N^4 of the thiosemicarbazone moiety. Furthermore, these studies may serve as a basis for chemical modifications aimed at the development of potential bioactive compounds of agricultural/medicinal interest.

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