Synthesis, Cytotoxic and Phytotoxic Effects of Some New N^4-Aryl Substituted Isatin-3-thiosemicarbazones

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Abstract: A series of N^4-aryl substituted isatin-3-thiosemicarbazones was prepared by the reaction of isatin with an appropriate thiosemicarbazide in ethanol containing a few drops of acetic acid. The newly synthesized compounds were characterized by means of their analytical (CHN) and spectral (IR, ^{1}H-NMR, EIMS) data, and evaluated for their cytotoxicity and phytotoxicity potential. Eleven out of thirteen compounds tested proved to be active in the brine-shrimp lethality bioassay exhibiting significant cytotoxic activity with LD_{50} values ranging from $1.75x10^{-5}$M to $1.91x10^{-4}$M. In phytotoxicity assay, all the synthesized compounds, regardless of the nature of aryl substituents, demonstrated weak to moderate (5-30%) plant growth inhibition at the highest tested concentration (500 µg/mL).

Keywords: Isatin, Isatin Derivatives, Thiosemicarbazones, Isatin-3-thiosemicarbazones, Cytotoxicity, Phytotoxicity.

INTRODUCTION

Isatin and its derivatives are important heterocyclic molecules, which possess numerous types of biological properties including sedative, anticonvulsant, anxiogenic, anthelmintic, antimicrobial, antineoplastic, antiviral, antiplasmodial, antitubercular and enzymatic inhibition [1-9]. In recent years, isatins-thiosemicarbazones are reported to display broad spectrum of medicinal properties such as antimicrobial, antineoplastic, antitubercular, antiulcer, antiviral and enzymatic inhibition [1-3,10-16]. Tempted by this and in continuation of our earlier efforts [17-20] in search of potent organic molecules exhibiting antibacterial, antifungal, cytotoxic, phytotoxic and urease inhibitory activities, we have synthesized a new series of thirteen title thiosemicarbazones by condensation of isatin with appropriate 4-substituted 3-thiosemicarbazides **1** and screened them for their cytotoxic and phytotoxic effects.

RESULTS AND DISCUSSION

The present work describes the synthesis and *in vitro* determination of the cytotoxic and phytotoxic effects of thirteen new N^4-aryl substituted isatin-3-thiosemicarbazones **2a-2m**. It also describes *in vitro* determination of the phytotoxicity potential of a previously reported thiosemicarbazone *i.e.* 2-(2-oxo- 1,2- dihydro- 3*H*-indole- 3-ylidine)- *N*-phenyl-1-hydrazinecarbothioamide [17].

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CHEMISTRY

The synthesis of isatin-thiosemicarbazones was simple and straightforward. A mixture of isatin, appropriate thiosemicarbazide and ethanol containing a few drops of glacial acetic acid was refluxed for 2 h (Scheme **1**). The crystalline solid formed during refluxing in each case was filtered hot. Thorough washing with hot ethanol followed by ether furnished the required compounds **2a-2m** in moderate to excellent yields (54-92%).

The structures of the synthesized thiosemicarbazones **2a-2m** were established through analytical (CHN) and spectral (IR, ^{1}H-NMR, EIMS) data. Satisfactory elemental analysis (± 0.4% of calculated values) was obtained for all compounds except where noted otherwise. The IR spectra of compounds **2a-2m** showed two separate bands resulting from NH stretchings of indole and thioamide functions in the 3316-3200 and 3185-3135 cm^{-1} regions, respectively. The lactam C=O, azomethine C=N and thioamide C=S stretchings were observed in the 1699-1680, 1602-1570 and 1207-1161 cm^{-1} regions, respectively [10,21-23]. The ^{1}H-NMR spectra of **2a-2m** exhibited three singlets at δ 8.15-10.97, δ 9.38-11.29 and δ 12.74-12.94 for the thiosemicarbazone N^4-H, indole NH and thiosemicarbazone N^2-H, respectively [10,21,24,25]. The indole C_7-H appeared as a doublet at δ 6.71-6.95, while the indole C_5-H and C_6-H appeared at δ 7.00-7.37 and δ 7.24-7.63, respectively, as a triplet or a doublet of double doublet. Indole C_4-H, experiencing a deshielding effect caused by inductively electron-withdrawing C=N function, resonated as a doublet at δ 7.61-7.99 [10,25-27]. In certain cases, however, overlapping of two signals corresponding to indole C_5-H and C_6-H was observed as multiplets due to combination with different aromatic protons of N^4-substituents. The EI mass spectra of the thiosemicarbazones showed molecular ions of different intensity, which confirmed their molecular weights. The major fragmentation pathways involved rupture of the exocyclic N-N, NH-CS and endocyclic NH-CO bonds [10].

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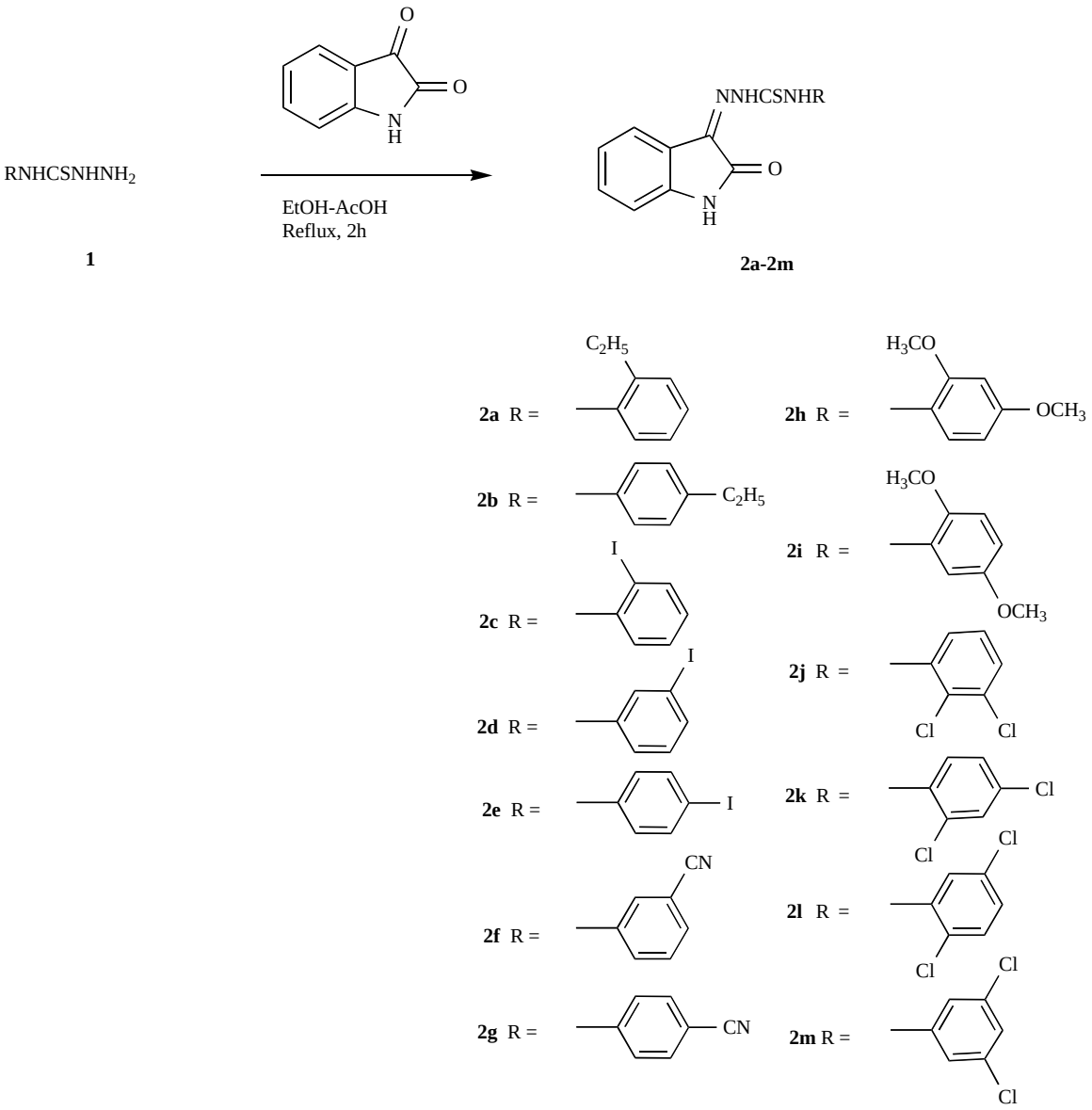

Scheme 1. Synthesis of title compounds **2a-2m**.

Compounds **2c** and **2j-2l** did not show molecular ion peaks in their spectra. However, the fragments corresponding to thiosemicarbazone moiety, formed by N-N and NH-CS bond cleavage, confirmed their structures. The proposed fragmentation pattern of **2a** is depicted in Fig. (**1**). X-ray structures of two representative examples **2a** and **2d** were determined in order to confirm the assigned structures and establish conformations of the synthesized thiosemicarbazones **2a-2m**. Relevant crystal data and details of the structure elucidation have been reported elsewhere [28,29].

BIOLOGICAL TESTING

In Vitro Cytotoxicity

The synthesized thiosemicarbazones **2a-2m** were evaluated for their cytotoxic effects by the brine-shrimp lethality bioassay. All these compounds except **2h** and **2i** displayed promising cytotoxicity (LD_{50} = 1.75 x 10^{-5}M - 1.91 x 10^{-4}M) against *Artemia salina* in the present assay (Table **1**). Compounds **2h** and **2i** with methoxy substituents at positions- 2, 4 and -2, 5 gave a value of LD_{50} >2.80 x10^{-4}M and, therefore, can be considered to be almost inactive. It may be noted that compound **2m** with chloro substituents at positions-3 and -5 of the phenyl ring showed maximum activity (LD_{50} = 1.75 x 10^{-5}M) and, therefore, proved to be the most potent compound in this series. The other chloro-substituted compounds **2j-2l** having substituents at positions-2,3, -2,4 and -2,5, respectively, were found to be less potent giving LD_{50} values of 1.02 x 10^{-4}M, 1.20 x 10^{-4}M and 1.66 x 10^{-4}M. Interestingly, substitution of two chloro substituents at the phenyl ring was found to cause induction of cytotoxic activity, as the respective monochloro-substituted compounds gave a value of LD_{50} > 3.02 x 10^{-4}M in the earlier assay [18] and were thus considered to be almost inactive. To the contrary, the monoiodo-substituted compounds **2c-2e** having substituent at positions-2, -3 and -

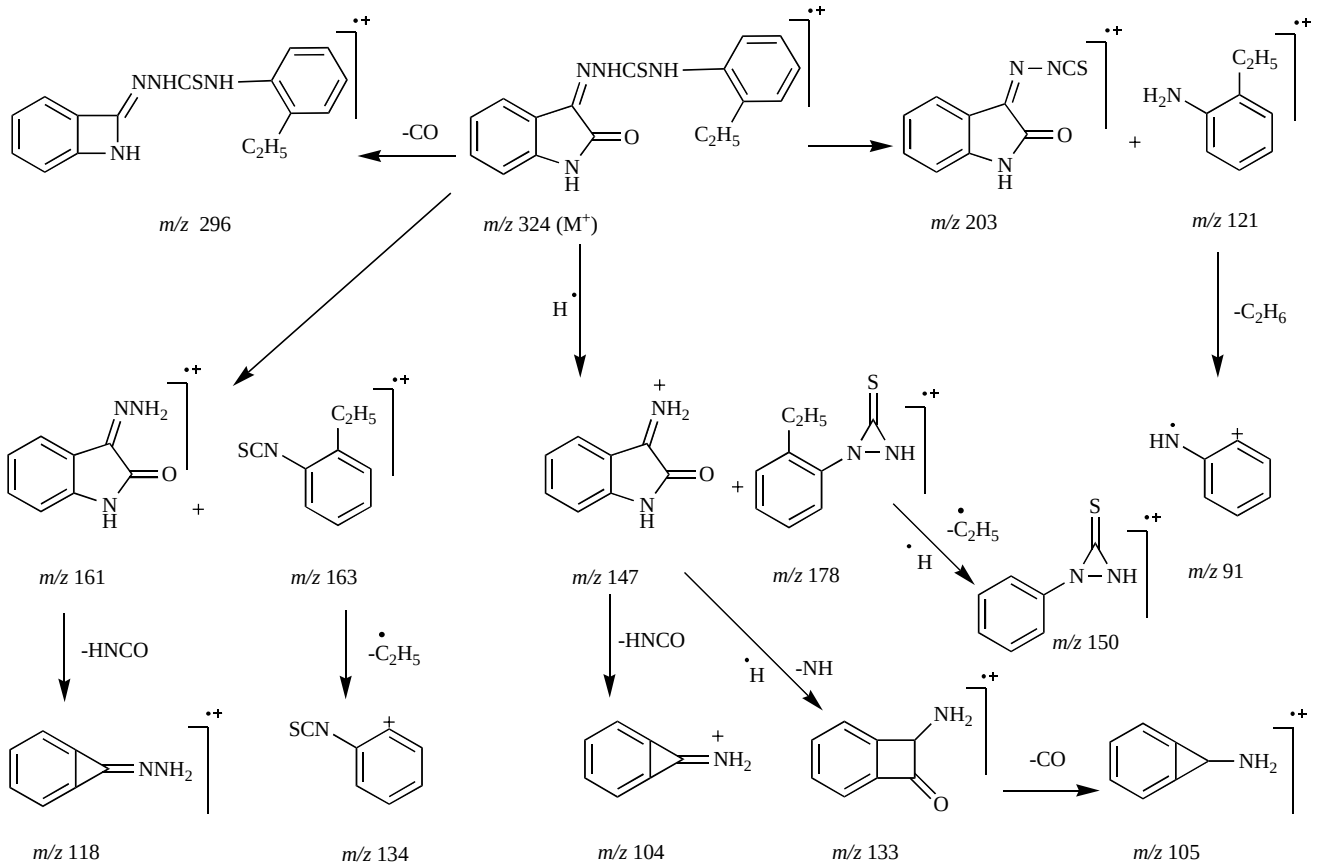

Fig. (1). The proposed fragmentation pattern of **2a**.

4, respectively, exhibited LD_{50} values of 1.59×10^{-4}M , 1.40×10^{-4}M and 1.26×10^{-4}M. Similarly, the cyano compounds having substituents at positions-3 and -4 of the phenyl ring gave LD_{50} values of 1.91×10^{-4} M and 1.51×10^{-4}M, respectively. Further, as compared to compound, 2-(2-oxo-1,2-dihydro-3*H*-indole-3-ylidine)-*N*-phenyl-1-hydrazinecarbothioamide [17] having no substituent at the phenyl ring attached to N^4 of the thiosemicarbazone moiety and giving a value of $LD_{50} > 3.38 \times 10^{-4}$M in the earlier assay [18], substitution of ethyl group at position-2 or -4 of its phenyl ring caused induction of activity **(**1.74×10^{-4}M and 5.10×10^{-5}M, respectively). However, compared with the respective methyl-substituted compounds [18], compound **2b** with an ethyl substituent at position-4 of the phenyl ring exhibited induced activity ($LD_{50} > 3.22 \times 10^{-4}$M $\rightarrow$ 5.10×10^{-5}M), whereas compound **2a** having such a substituent at position-2 of the phenyl ring displayed reduced activity ($LD_{50} = 8.10 \times 10^{-5}$M $\rightarrow$ 1.74×10^{-4}M). These structure-activity relationships (SAR) may serve as a basis for chemical modifications aimed at the development of certain cytotoxic agents of clinical interest.

In Vitro Phytotoxicity

All the synthesized thiosemicarbazones **2a-2m** having one or two substituents about the phenyl ring at N^4 of the thiosemicarbazone moiety were also evaluated for their phytotoxic effects at three different concentrations *i.e.* 500, 50 and 5 µg / mL. These compounds displayed weak or non-significant (5-30 %) inhibition at the highest tested concentration (500 µg/ mL) as compared to the reference point, 2-(2-oxo-1,2-dihydro-3*H*-indole-3-ylidine)-*N*-phenyl-

Table 1. Brine-Shrimp Bioassay of Compounds 2a-2m

Compounds	LD_{50} (M)
2a	1.74×10^{-4}
2b	5.10×10^{-5}
2c	1.59×10^{-4}
2d	1.40×10^{-4}
2e	1.26×10^{-4}
2f	1.91×10^{-4}
2g	1.51×10^{-4}
2h	$> 2.80 \times 10^{-4}$
2i	$>2.80 \times 10^{-4}$
2j	1.02×10^{-4}
2k	1.20×10^{-4}
2l	1.66×10^{-4}
2m	1.75×10^{-5}
2-(2-Oxo-1,2-dihydro-3*H*-indole-3-ylidine)-*N*-phenyl-1-hydrazine-carbothioamide‡	$>3.38 \times 10^{-4}$

‡[17,18].

Table 2. Percent Growth Inhibition of *Lemna aequinocitalis* by Compounds 2a-2m at Different Concentrations*

Compounds	500 (μg/mL) (% G.I. †)	50 (μg/mL) (% G.I. †)	5 (μg/mL) (% G.I. †)
2a	15	15	25
2b	15	10	05
2c	25	20	10
2d	10	20	05
2e	30	20	20
2f	05	05	05
2g	25	20	10
2h	20	20	10
2i	30	25	05
2j	10	10	10
2k	20	20	20
2l	20	15	05
2m	10	10	05
2-(2-Oxo-1,2-dihydro-3*H*-indole-3-ylidine)-*N*-phenyl-1-hydrazinecarbothioamide‡	40	25	15

*The reference compound paraquat shows 100 % growth inhibition at a concentration of 0.015μg/mL; †G.I.: growth inhibition; ‡[17].

1-hydrazinecarbothioamide [17], which demonstrated moderate (40%) inhibition at the same concentration (Table **2**). Similarly, all the synthesized compounds including reference point exhibited weak or non-significant (5-25%) plant growth inhibition at the lowest tested concentration (5 μg/ mL). From the results obtained in this bioassay, it may be concluded that our test compounds showed non-significant inhibition at much higher levels of concentration compared with the standard paraquat, which displayed 100 % inhibition at a concentration of 0.015 μg / mL. Furthermore, the percent growth inhibition values revealed that the type, number and position of the substituents about the phenyl ring at N^4 of the thiosemicarbazone moiety did not significantly affect the phytotoxicity potential of the synthesized compounds at both the highest as well as lowest tested concentrations (500 μg / mL and 5 μg / mL).

MATERIALS AND METHODS

General

All reagents and solvents were used as obtained from the suppliers 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 disks) were run on Shimadzu Prestige-21 FT-IR spectrometer. The ^{1}H-NMR spectra were recorded in $CDCl_3$ and DMSO-d_6 on Bruker (Rhenistetten-Forchheim, Germany) AM 300 and AM 400 spectrometers operating at 300 MHz and 400 MHz, respectively, 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 the purity of the products were checked on TLC plates pre-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 evaluation of the synthesized compounds was done at Dr. Panjwani Center for Molecular Medicine and Drug Research, and H. E. J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Pakistan.

Synthesis

General Procedure for the Preparation of Isatin-thiosemicarbazones (2a-2m)

To a hot solution of isatin (5 mmol) in ethanol (10 mL) containing a few drops of glacial acetic acid was added the appropriate thiosemicarbazide (5 mmol) dissolved in ethanol (10 mL) under stirring. The reaction mixture was then heated under reflux for 2 h. The crystalline solid formed during heating was collected by suction filtration. Thorough washing with hot ethanol followed by ether gave the required compounds **2a-2m** in pure form.

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

Yield 88% as yellow crystals; m.p. >212 °C (decomp.); (Found: C, 63.15; H, 4.95; N, 17.28. Calcd for $C_{17}H_{16}N_4OS$: C, 62.96; H, 4.94; N, 17.28%); IR (KBr, cm^{-1}): 3316, 3184, 3165, 3145 (NH stretching), 1695 (C = O), 1593 (C = N), 1541 (NH bending), 1161 (C = S); ^{1}H-NMR ($CDCl_3$): δ 1.31(t, *J* = 7.5 Hz, 3H, CH_3), 2.74 (q, *J* = 7.5 Hz, 2H, CH_2), 6.95 (d, *J* = 7.8 Hz, 1H, indole C_7-H), 7.15 (t, *J* = 7.5 Hz, 1H, indole C_5-H), 7.30-7.41 (m, 3H, indole C_6-H, phenyl C_3-H, C_4-H), 7.61 (d, *J* = 7.5 Hz, 1H, indole C_4-H), 7.77-7.83 (m, 2H, phenyl C_5-H, C_6-H), 8.35 (s, 1H, CS-NH), 9.38 (s, 1H, indole NH), 12.88 (s, 1H, N-NH); EI MS (70 eV) *m/z* (%): 326 (M^+ +2, 2), 325 (M^+ +1, 4), 324 (M^+,11), 296 (100), 203 (9), 178 (48), 163 (55), 161 (49), 150 (16), 147

(17), 134 (4), 133(14), 132 (20), 121 (17), 118 (34), 105(12), 104 (31), 91 (18), 77 (35).

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

Yield 75% as yellow crystals; m.p. >238 °C (decomp.); (Found: C, 63.16; H, 4.96; N, 17.27. Calcd for $C_{17}H_{16}N_4OS$: C, 62.96; H, 4.94; N, 17.28%); IR (KBr, cm^{-1}): 3305, 3200, 3165 (NH stretching), 1695 (C = O), 1593 (C = N), 1541 (NH bending), 1202 (C = S); ^{1}H-NMR ($CDCl_3$): δ 1.20 (t, *J* = 7.6 Hz, 3H, CH_3), 2.60 (q, *J* = 7.6 Hz, 2H, CH_2), 6.88 (d, *J* = 7.8 Hz, 1H, indole C_7-H), 6.97- 7.10 (m, 2H, phenyl C_2-H, C_6-H), 7.13-7.20 (m, 3H, indole C_5-H and phenyl C_3-H, C_5-H), 7.28-7.39 (m, 1H, indole C_6-H), 7.63 (d, *J* = 7.6 Hz, 1H, indole C_4-H), 8.15 (s, 1H, CS-NH), 9.42 (s, 1H, indole NH), 12.80 (s, 1H, N-NH); EI MS (70 eV) *m/z* (%): 325 (M^+ +1, 3), 324 (M^+, 20), 296 (96), 203 (6), 163 (16), 161 (20), 150 (37), 147 (21), 134 (3), 132 (22), 118 (29), 104 (86), 91 (35), 77 (100), 51 (41).

N-(2-Iodophenyl)-2-(2-oxo-1,2-dihydro-3H-indol-3-ylidene)-1- hydrazinecarbothioamide (2c)

Yield 92% as yellow crystals; m.p. >270 °C (decomp.); (Found: C, 42.81; H, 2.62; N, 13.27. Calcd for $C_{15}H_{11}IN_4OS$: C, 42.65; H, 2.61; N, 13.27%); IR (KBr, cm^{-1}): 3226, 3184 (NH stretching), 1685 (C = O), 1590 (C = N), 1544 (NH bending), 1165 (C = S); ^{1}H-NMR (DMSO-d_6): δ 6.95 (d, *J* = 7.8 Hz, 1H, indole C_7-H), 7.08-7.15 (m, 2H, phenyl C_4-H, C_5-H), 7.37 (ddd, *J* = 7.8, 1.2 Hz, 1H, indole C_5-H), 7.47 (ddd, *J* = 8.1, 1.2 Hz, 1H, indole C_6-H), 7.53 (dd, *J* = 8.1, 1.8 Hz,1H, phenyl C_6-H), 7.73 (d, *J* = 7.5 Hz, 1H, indole C_4-H), 7.96 (dd, *J* = 8.1, 0.9 Hz, 1H, phenyl C_3-H), 10.79 (s, 1H, CS-NH), 11.28 (s, 1H, indole NH), 12.81 (s, 1H, N-NH); EI MS (70 eV) *m/z* (%): 394 (2), 294 (49), 266 (100), 219 (9), 161 (2), 149 (21), 144 (5), 118 (21), 104 (4), 90 (7), 77 (4).

N-(3-Iodophenyl)-2-(2-oxo-1,2-dihydro-3H-indol-3-ylidene)-1-hydrazinecarbothioamide (2d)

Yield 84% as dark yellow crystals; m.p. >230 °C (decomp.); (Found: C, 42.79; H, 2.62; N, 13.28. Calcd for $C_{15}H_{11}IN_4OS$: C, 42.65; H, 2.61; N, 13.27%); IR (KBr, cm^{-1}): 3280, 3166 (NH stretching), 1689 (C = O), 1570 (C = N), 1523 (NH bending), 1163 (C = S); ^{1}H-NMR ($CDCl_3$): δ 6.92 (d, *J* = 7.9 Hz, 1H, indole C_7-H), 7.00-7.15 (m, 2H, indole C_5-H and phenyl C_6-H), 7.27-7.41 (m, 2H, indole C_6-H and phenyl C_5-H), 7.57-7.74 (m, 2H, phenyl C_2-H, C_4-H), 7.79 (d, *J* = 7.8 Hz, 1H, indole C_4-H), 8.50 (s, 1H, CS-NH), 9.42 (s, 1H, indole NH), 12.85 (s, 1H, N-NH); EI MS (70 eV) *m/z* (%): 423 (M^+ +1, 2), 422 (M^+, 12), 394 (77), 276 (2), 261(58), 244 (18), 219 (100), 203 (43), 161 (21), 145 (25), 134 (24), 104 (11), 92 (65), 78 (61), 63 (62).

N-(4-Iodophenyl)-2-(2-oxo-1,2-dihydro-3H-indol-3-ylidene)-1-hydrazinecarbothioamide (2e)

Yield 81% as yellow crystals; m.p. >250 °C (decomp.); (Found: C, 42.80; H, 2.62; N, 13.26. Calcd for $C_{15}H_{11}IN_4OS$: C, 42.65; H, 2.61; N, 13.27%); IR (KBr, cm^{-1}): 3324, 3226 (NH stretching), 1680 (C = O), 1602 (C = N), 1555 (NH bending), 1163 (C = S); ^{1}H-NMR ($CDCl_3$): δ 6.71 (d, *J* = 8.5 Hz, 1H, indole C_7-H), 6.87-6.97 (m, 2H, phenyl C_2-H, C_6-H), 7.03-7.17 (m, 1H, indole C_5-H), 7.29-7.37 (m, 1H, indole C_6-H), 7.50-7.67 (m, 2H, phenyl C_3-H, C_5-H), 7.70 (d, *J* = 8.5 Hz, 1H, indole C_4-H), 8.50 (s,1H, CS-NH), 9.42 (s, 1H, indole NH), 12.85 (s, 1H, N-NH); EI MS (70 eV) *m/z* (%): 424 (M^+ +2, 1), 423 (M^+ +1, 4), 422 (M^+, 17), 394 (80), 276 (1), 261(63), 244 (35), 219 (100), 203 (30), 161 (29), 150(11), 147 (3), 144 (26), 132 (6), 134 (19), 104 (14), 92 (27), 76 (13), 65 (19).

N-(3-Cyanophenyl)-2-(2-oxo-1,2-dihydro-3H-indol-3-ylidene)-1-hydrazinecarbothioamide (2f)

Yield 54% as bright yellow crystals; m.p. >282 °C (decomp.); (Found: C, 59.94; H, 3.44; N, 21.82. Calcd for $C_{16}H_{11}N_5OS$: C, 59.81; H, 3.43; N, 21.81%); IR (KBr, cm^{-1}): 3300, 3251 (NH stretching), 1689 (C = O), 1587 (C = N), 1521 (NH bending), 1170 (C = S); ^{1}H-NMR (DMSO-d_6) δ 6.95 (d, *J* = 7.8 Hz, 1H, indole C_7-H), 7.12 (t, *J* = 7.5 Hz, 1H, indole C_5-H), 7.39 (t, *J* = 7.8 Hz, 1H, phenyl C_5-H), 7.63 (t, *J* = 7.8 Hz, 1H, indole C_6-H), 7.73-7.77 (m, 2H, phenyl C_4-H, C_6-H), 7.99 (d, *J* = 8.1 Hz, 1H, indole C_4-H), 8.13 (s, 1H, phenyl C_2-H), 10.97 (s, 1H, CS-NH), 11.29 (s, 1H, indole NH), 12.90 (s, 1H, N-NH); EI MS (70 eV) *m/z* (%):322 (M^+ +1, 3), 321 (M^+, 20), 293 (77), 203 (20), 161 (43), 160 (45), 150 (16), 147 (3), 145 (26), 132 (25), 118 (100), 104 (44), 91 (39), 77 (24), 65 (13), 51 (34).

N-(4-Cyanophenyl)-2-(2-oxo-1,2-dihydro-3H-indol-3-ylidene)-1-hydrazinecarbothioamide(2g)

Yield 71% as orange yellow crystals; m.p. >278 °C (decomp.); (Found: C, 59.96; H, 3.44; N, 21.81. Calcd for $C_{16}H_{11}N_5OS$: C, 59.81; H, 3.43; N, 21.81%); IR (KBr, cm^{-1}): 3300, 3265, 3244 (NH stretching), 1699 (C = O), 1587 (C = N), 1529 (NH bending), 1195 (C = S); ^{1}H-NMR ($CDCl_3$): δ 6.92 (d, *J* = 8.3 Hz, 1H, indole C_7-H), 7.07-7.15 (m, 3H, indole C_5-H and phenyl C_2-H, C_6-H), 7.32-7.39 (m, 1H, indole C_6-H), 7.62-7.74 (m, 2H, phenyl C_3-H, C_5-H), 7.98 (d, *J* = 8.1 Hz, 1H, indole C_4-H), 9.80 (s, 1H, indole NH), 12.94 (s, 1H, N-NH); EI MS (70 eV) *m/z* (%): 322 (M^+ +1, 4), 321(M^+, 22), 293 (95), 203 (19), 161 (51), 160 (56), 150 (24), 147 (4), 145 (25), 132 (29), 118 (100), 104 (49), 91 (36), 77 (27), 65 (11), 51 (29).

N-(2, 4-Dimethoxyphenyl)-2-(2-oxo-1,2-dihydro-3H-indol-3-ylidene)-1-hydrazinecarbothioamide (2h)

Yield 76% as deep yellow crystals; m.p. >256 °C (decomp.); (Found: C, 57.46; H, 4.50; N, 15.74. Calcd for $C_{17}H_{16}N_4O_3S$: C, 57.30; H, 4.49; N, 15.73 %); IR (KBr, cm^{-1}): 3265, 3185, 3135 (NH stretching), 1689 (C = O), 1585 (C = N), 1541 (NH bending), 1161 (C = S); ^{1}H-NMR (DMSO-d_6): δ 3.80 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 6.57 (dd, *J* = 8.7, 2.4 Hz, 1H, phenyl C_5-H), 6.68 (d, *J* = 2.7 Hz, 1H, phenyl C_3-H), 6.94 (d, *J* = 7.8 Hz, 1H, indole C_7-H), 7.10 (t, *J* = 7.8 Hz, 1H, indole C_5-H), 7.37 (ddd, *J* = 7.8, 1.2 Hz, 1H, indole C_6-H), 7.51 (d, *J* = 8.4 Hz, 1H, phenyl C_6-H), 7.70 (d, *J* = 7.2 Hz, 1H, indole C_4-H), 10.30 (s, 1H, CS-NH), 11.24 (s, 1H, indole NH), 12.74 (s, 1H, N-NH); EI MS (70 eV) *m/z* (%): 358 (M^+ +2, 5), 357 (M^+ +1, 14), 356 (M^+, 63), 328 (100), 295 (2), 195 (43), 153 (79), 150 (8), 147 (4), 138 (85), 132 (8), 118 (23), 104 (11), 91 (8), 77 (8), 65 (5).

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

Yield 78% as yellow crystals; m.p. >258 °C (decomp.); (Found: C, 57.45; H, 4.50; N, 15.73. Calcd for $C_{17}H_{16}N_4O_3S$:

C, 57.30; H, 4.49; N, 15.73 %); IR (KBr, cm^{-1}): 3282, 3246, 3210 (NH stretching), 1691 (C = O), 1590 (C = N), 1544 (NH bending), 1163 (C = S); ^{1}H-NMR (DMSO-d_6): δ 3.72 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 6.83 (dd, *J* = 9.0, 3.0 Hz, 1H, phenyl C_4-H), 6.95 (d, *J* = 7.8 Hz, 1H, indole C_7-H), 7.07 (d, *J* = 9.0 Hz, 1H, phenyl C_3-H), 7.12 (t, *J* = 7.5 Hz, 1H, indole C_5-H), 7.38 (t, *J* = 7.5 Hz , 1H, indole C_6-H), 7.66 (d, *J* = 7.5 Hz, 1H, indole C_4-H), 7.74 (d, *J* = 2.7 Hz, 1H, phenyl C_6-H), 10.43 (s, 1H, CS-NH), 11.27 (s, 1H, indole NH), 12.81 (s, 1H, N-NH); EI MS (70 eV) *m/z* (%): 357 (M^+ +1 , 7), 356 (M^+ ,33), 328 (100), 297 (22), 195 (27), 181 (70), 161 (17), 150 (18), 147 (9), 145 (28), 138 (82), 132 (22), 118 (27), 104 (50), 91 (15), 77 (41), 65 (21), 51 (38).

N-(2, 3-Dichlorophenyl)-2-(2-oxo-1,2-dihydro-3H-indol-3-ylidene)-1-hydrazinecarbothioamide (2j)

Yield 85% as yellow crystals; m.p. >290 °C (decomp.); (Found: C, 49.45; H, 2.75; N, 15.34. Calcd for $C_{15}H_{10}Cl_2N_4OS$: C, 49.32; H, 2.74; N, 15.34 %); IR (KBr, cm^{-1}): 3244, 3230 (NH stretching), 1695 (C = O), 1585 (C = N), 1527 (NH bending), 1207 (C = S); ^{1}H-NMR (DMSO-d_6): δ 6.94 (d, *J* = 7.8 Hz, 1H, indole C_7-H), 7.10 (t, *J* = 7.5 Hz, 1H, indole C_5-H), 7.37 (ddd, *J* = 7.68, 0.86 Hz, 1H, indole C_6-H), 7.44 (t, *J* = 9.0 Hz, 1H, phenyl C_5-H), 7.55 (dd, *J* = 7.8, 1.5 Hz, 1H, phenyl C_4-H), 7.65 (dd, *J* = 8.7, 1.5 Hz, 1H, phenyl C_6-H), 7.69 (d, *J* = 7.5 Hz, 1H, indole C_4-H), 10.88 (s, 1H, CS-NH), 11.26 (s, 1H, indole NH), 12.88 (s, 1H, N-NH); EI MS (70 eV) *m/z* (%): 338 (12), 329 (100), 301 (3), 203 (29), 185 (35), 161 (44), 160 (7), 150 (24), 147 (23), 145 (43), 132 (31), 118 (39), 104 (59), 91 (23), 77 (39), 65 (9), 51 (34).

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

Yield 87% as deep yellow crystals; m.p. >272 °C (decomp.); (Found: C, 49.46; H, 2.74; N, 15.33. Calcd for $C_{15}H_{10}Cl_2N_4OS$: C, 49.32; H, 2.74; N, 15.34 %); IR (KBr, cm^{-1}): 3300, 3261 (NH stretching), 1695 (C = O), 1579(C = N), 1533 (NH bending), 1165 (C = S); ^{1}H-NMR (DMSO-d_6): δ 6.94 (d, *J* = 7.8 Hz, 1H, indole C_7-H), 7.10 (t, *J* = 7.5 Hz 1H, indole C_5-H), 7.37 (t, *J* = 7.6 Hz, 1H, indole C_6-H), 7.50 (dd, *J* = 8.8, 1.5 Hz, 1H, phenyl C_5-H), 7.60 (d, *J* = 8.5 Hz, 1H, phenyl C_6-H), 7.69 (d, *J* = 7.5 Hz, 1H, indole C_4-H), 7.76 (d, *J* = 2.23 Hz, 1H, phenyl C_3-H), 10.75 (s, 1H, CS-NH), 11.24 (s, 1H, indole NH), 12.88 (s, 1H, N-NH); EI MS (70 eV) *m/z* (%): 340 (4), 338 (21), 337 (5), 329 (100), 301 (3), 203 (22), 185 (36), 161 (42), 160 (10), 150 (27), 147 (21), 145 (37), 132 (32), 118 (31), 104 (54), 91 (16), 77 (29), 65 (7), 51 (21).

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

Yield 83% as yellow crystals; m.p. >270 °C (decomp.); (Found: C, 49.44; H, 2.75; N, 15.34. Calcd for $C_{15}H_{10}Cl_2N_4OS$: C, 49.32; H, 2.74; N, 15.34 %); IR (KBr, cm^{-1}): 3244, 3200 (NH stretching), 1699 (C = O), 1589 (C = N), 1533 (NH bending), 1200 (C = S); ^{1}H-NMR (DMSO-d_6): δ 6.94 (d, *J* = 7.8 Hz, 1H, indole C_7-H), 7.10 (t, *J* = 7.2 Hz, 1H, indole C_5-H), 7.37 (ddd, *J* = 7.5, 1.1 Hz, 1H, indole C_6-H), 7.46 (dd, *J* = 8.66, 2.53 Hz,1H, phenyl C_4-H), 7.63 (d, *J* = 8.6 Hz, 1H, phenyl C_3-H), 7.68 (d, *J* = 7.4 Hz, 1H, indole C_4-H), 7.74 (d, *J* = 2.5 Hz, 1H, phenyl C_6-H), 10.81 (s, 1H, CS-NH), 11.27 (s, 1H, indole NH), 12.89 (s, 1H, N-NH); EI MS (70 eV) *m/z* (%): 338 (12), 337 (3), 329 (100), 301 (3), 203 (17), 185 (32), 161 (28), 160 (6), 150 (21), 147 (27), 145 (34), 132 (25), 118 (23), 104 (41), 91 (12), 77 (22), 65 (6), 51 (18).

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

Yield 80% as deep yellow crystals; m.p. >270 °C (decomp.); (Found: C, 49.43; H, 2.73; N, 15.36. Calcd for $C_{15}H_{10}Cl_2N_4OS$: C, 49.32; H, 2.74; N, 15.34 %); IR (KBr, cm^{-1}): 3315, 3244, 3221 (NH stretching), 1695 (C = O), 1587 (C = N), 1541 (NH bending), 1172 (C = S); ^{1}H-NMR (DMSO-d_6): δ 6.94 (d, *J* = 8.1 Hz, 1H, indole C_7-H), 7.12 (t, *J* = 7.5 Hz, 1H, indole C_5-H), 7.38 (t, *J* = 7.5 Hz, 1H, indole C_6-H), 7.50 (t, *J* =1.8 Hz, 1H, phenyl C_4-H), 7.76 (d, *J* = 7.2 Hz, 1H, indole C_4-H), 7.87 (d, *J* = 1.8 Hz, 2H, phenyl C_2-H,C_6-H), 10.90 (s, 1H, CS-NH), 11.29 (s, 1H, indole NH), 12.91 (s, 1H, N-NH); EI MS (70 eV) *m/z* (%): 366 (M^+ +1, 14), 338 (59), 203 (43), 186 (15), 161 (77), 160 (14), 150 (54), 147 (29), 145 (61), 132 (60), 118 (57), 104 (100), 91 (30), 77 (59), 65 (16), 51 (45).

BIOLOGICAL TESTING

In Vitro Cytotoxicity

Brine-shrimp (*Artemia salina* Leach) eggs were hatched in a shallow rectangular plastic dish (22x32 cm) filled with artificial sea water, which was prepared with a commercial salt mixture (Instant Ocean, Aquarium System, Inc., Mentor, Ohio, USA) and double-distilled water. An unequal partition was made in the plastic dish with the help of a perforated device. Approximately 50 mg of eggs were sprinkled into the large compartment, which was darkened, while the smaller compartment was opened to ordinary light. After two days, nauplii were collected by a pipette from the lighted side. A sample of the test compound was prepared by dissolving 2 mg of each compound in 2 mL of methanol. From this stock solution, 500, 50 and 5 µL were transferred to 9 vials, three for each dilution, and one vial was kept as control having 2 mL of methanol. The solvent was allowed to evaporate overnight. After two days, when shrimp larvae were ready, 1 mL of sea water and 10 shrimps were added to each vial (30 shrimps/ dilution) and the volume was adjusted with sea water to 5 mL per vial. After 24 h, the number of survivors was counted [30,31]. Data were analyzed by a Finney computer program to determine the LD_{50} values [32].

Phytotoxicity Bioassay

This test was performed according to the modified literature protocol [30]. The test compounds were incorporated into sterilized E-medium at different concentrations *i.e.* 5, 50, and 500 µg/mL in methanol. Sterilized conical flasks were inoculated with compounds of the desired concentrations prepared from the stock solution and allowed to evaporate overnight. Each flask was inoculated with 20 ml of sterilized E-medium and then ten *Lemna aequinocitalis* Welv, each containing a rosette of three fronds were placed on media. Other flasks were supplemented with methanol serving as negative control and reference inhibitor *i.e.* parquet serving as positive control.

Treatments were replicated three times and the flasks incubated at 30 °C in a Fisons Fi-Totron 600 H growth cabinet for seven days, 9000 lux light intensity, 56+10 relative humidity, and 12 h day length. Growth of *L. aequinocitalis* in flasks containing the compounds was determined by counting the number of fronds per dose and growth inhibition was calculated with reference to negative control.

CONCLUSIONS

We have demonstrated the potential of N^4-aryl substituted isatin-3- thiosemicarbazones to exhibit cytotoxic and phytotoxic activities. Further investigation on structure-activity relationships (SAR) and appropriate chemical modifications amongst the synthesized thiosemicarbazones is likely to furnish more effective cytotoxic and phytotoxic agents.

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REFERENCES

[1] Pandeya, S. N.; Smitha, S.; Jyoti, M.; Sridhar, S. K. Biological activities of isatin and its derivatives. *Acta Pharm*., **2005**, *55*, 27-46 and references therein.

[2] Da Silva, J. F. M.; Garden, S. J.; Pinto, A.C. The chemistry of isatins: A review from 1975 to 1999. *J. Braz. Chem. Soc.*, **2001**, 273-324 and references therein.

[3] Chiyanzu, I.; Clarkson, C.; Smith, P. J.; Lehman, J.; Gut, J.; Rosenthal, P. J.; Chibale, K. Design, synthesis and anti-plasmodial evaluation *in vitro* of new 4-aminoquinoline isatin derivatives. *Bioorg. Med. Chem.*, **2005**, *13*, 3249-3261.

[4] Chen, Li-Rung; Wang, Yu-Chin; Lin, Y. W.; Chou, Shan-Yen; Chen, Shyh-Fong; Liu, L. T.; Wu, Ying-Ta; Kuo, Chih-Jung; Chen, T. Shieh-Shung; Juang, Shin-Hun. Synthesis and evaluation of isatin derivatives as effective SARS coronavirus 3CL protease inhibitors. *Bioorg. Med. Chem. Lett.*, **2005**, *15*, 3058-3062.

[5] Beauchard, A.; Ferandin, Y.; Frere, S.; Lozach, O.; Blairvacq, M.; Meijer, L.; Thiery, V.; Besson, T. Synthesis of novel 5-substituted indirubins as protein kinases inhibitors. *Bioorg. Med. Chem.*, **2006**, *14*, 6434-6443.

[6] Hyatt, J. L.; Moak, T.; Hatfield, M. -J.; Tsurkan, L.; Edwards, C. C.; Wierdl, M.; Danks, M. K.; Wadkins, R. M.; Potter, P. M. Selective inhibition of carboxylesterases by isatins,indole-2,3-diones. *J. Med. Chem.*, **2007**, *50*, 1876-1885.

[7] Ravichandran, V.; Mohan, S.; Kumar, K. S. Synthesis and antimicrobial activity of Mannich bases of isatin and its derivatives with 2-[(2,6-dichlorophenyl)amino]phenylacetic acid. *Arkivoc*, **2007**, *XIV*, 51-57.

[8] Aboul-Fadi, T.; Bin-Jubair, F. A. S. Anti-tubercular activity of isatin derivatives. *Int. J. Res. Pharm. Sci.*, **2010**, *1*, 113-126 and references therein.

[9] Singh, U. K.; Pandeya, S. N.; Singh, A.; Srivastava, B. K.; Pandey, M. Synthesis and antimicrobial activity of Schiff's and *N*-Mannich bases of isatin and its derivatives with 4-amino-*N*-carbamimidoyl benzene sulfonamide. *Int. J. Pharm. Sci. Drug Res.*, **2010**, *2*, 151-154.

[10] Karali, N. Synthesis and primary cytotoxicity evaluation of new 5-nitroindole-2, 3-dione derivatives. *Eur. J. Med. Chem.*, **2002**, *37*, 909-918.

[11] Chiyanzu, I.; Hansell, E.; Gut, J.; Rosenthal, P. J.; McKerrow, J. H.; Chibale, K. Synthesis and evaluation of isatins and thiosemicarbazone derivatives against cruzain falcipain-2 and rhodesain. *Bioorg. Med. Chem. Lett*., **2003**, *13*, 3527-3530.

[12] Bal, T. R.; Anand, B.; Yogeeswari, P.; Sriram, D. Synthesis and evaluation of anti-HIV activity of isatin β-thiosemicarbazone derivatives. *Bioorg. Med. Chem. Lett*., **2005**, *15*, 4451- 4455.

[13] Terzioglu, N.; Karali, N.; Gursoy, A.; Pannecouque, C.; Leysen, P.; Paeshuyse, J.; Neyts, J.; De Clercq, E. Synthesis and primary antiviral activity evaluation of 3-hydrazono-5-nitro-2-indolinone derivatives. *Arkivoc*, **2006**, 109-118.

[14] Quenelle, D. C.; Keith, K. A.; Kern, E. R. *In vitro* and *in vivo* evaluation of isatin-β-thiosemicarbazone and marboran against vaccinia and cowpox virus infections. *Antiviral Res*., **2006**, *71*, 24-30.

[15] Karali, N.; Gursoy A.; Kandemirli, F.; Shvets, N.; Kaynak, F. B.; Ozbey, S.; Kovalishyn, V.; Dimoglo, A. Synthesis and structure-antituberculosis activity relationship of 1*H*-indole-2,3-dione derivatives. *Bioorg. Med. Chem*., **2007**, *15*, 5888-5904.

[16] Guzel, O.; Karali, N.; Salman, A. Synthesis and antituberculosis activity of 5-methyl/trifluoromethoxy-1*H*-indole-2,3-dione 3-thiosemicarbazone derivatives. *Bioorg. Med. Chem.*, **2008**, *16*, 8976-8987.

[17] Pervez, H.; Iqbal, M.S.; Tahir, M. Y.; Choudhary, M. I.; Khan, K. M. Synthesis of some N^4-substituted isatin-3-thiosemicarbazones. *Nat. Prod. Res*., **2007**, *21*, 1178-1186.

[18] Pervez, H.; Iqbal, M. S.; Tahir, M. Y.; Nasim, F. H.; Choudhary, M. I.; Khan, K. M. *In vitro* cytotoxic, antibacterial, antifungal and urease inhibitory activities of some N^4-substituted isatin-3-thiosemicarbazones. *J. Enz. Inhib. Med. Chem*., **2008**, *23*, 848-854.

[19] Pervez, H.; Chohan, Z. H.; Ramzan, M.; Nasim, F. H.; Khan, K. M. Synthesis and biological evaluation of some new N^4-substituted isatin-3-thiosemicarbazones. *J. Enz. Inhib. Med. Chem.*, **2009**, *24*, 437-446.

[20] Pervez, H.; Manzoor, N.; Yaqub, M.; Khan, A.; Khan, K. M.; Nasim, F. H.; Choudhary, M. I. Synthesis and urease inhibitory properties of some new N^4-substituted 5-nitroisatin-3-thiosemicarbazones. *Lett. Drug. Des. Discov*., **2010**, *7*, 102-108.

[21] Omar, A. M. E.; Eshba, N. H.; Salama, H. M. Synthesis of some substituted isatin-β-thiosemicarbazones and isatin-β-hydrazonothiazoline derivatives as potential antiviral and antimicrobial agents. *Arch. Pharm*., **1984**, *317*, 701-709.

[22] Petrov, I.; Grupce, O.; Stafilov, T. The nitrogen-hydrogen stretching region of some imides and thioimides. *J. Mol. Struct*., **1986**, *142*, 275-278

[23] Naumov, P.; Anastasova, F. Experimental and theoretical vibrational study of isatin, its 5-(NO_2, F, Cl, Br, I, CH_3) analogues and the isatinato anion. *Spectrochim. Acta*, **2001**, *57A*, 469-481.

[24] Nizamuddin, Khan, M. H.; Alauddin, S.; Haque, R. Synthesis and fungicidal activity of some 2-arylamino-1,3,4-thiadiazino[6,5-*b*] indoles and 2-aryl-1 ,3,4-oxadiazolo-[2,3-*c*]-1 ,2,4-triazino[5,6-*b*] indoles. *Indian J. Chem.*, **1999**, *38B*, 501-504.

[25] Laatsch, H.; Thomson, R. H.; Cox, P. J. Spectroscopic properties of violacein and related compounds: Crystal structure of tetramethyl violacein. *J. Chem. Soc. Perkin Trans 2*, **1984**, 1331-1339.

[26] Baron, M. L.; Martin, L. L.; Rae, I. D.; Simmonds, P. M.; Woolcock, M. L. Relaxation processes in aromatic methyl groups. II. Methyl-methyl nuclear overhauser enhancements. *Aust. J. Chem*., **1990**, *43*, 741-747.

[27] Eshba, N. H.; Salama, H. M.; Labouta, I. M.; Omar, A. M. E. Synthesis of some substituted 1,2,4- triazino [5,6-b] indole derivatives as potential antiviral anticancer agents. *Pharmazie*, **1987**, *42*, 664-666.

[28] Pervez, H.; Yaqub, M.; Ramzan, M.; Tahir, M. N.; Iqbal, M. S. 4-(2-Ethylphenyl)-1-(2-oxoindolin-3-ylidene)thiosemicarbazides. *Acta Cryst*., **2010**, *E66*, o1609.

[29] Pervez, H.; Yaqub, M.; Ramzan, M.; Tahir, M. N. 4-(3-Iodophenyl)-1-(2-oxoindolin-3-ylidene) thiosemicarbazides. *Acta Cryst*., **2010**, *E66*, o1629.

[30] McLaughlin, J. L.; Chang, C. J.; Smith, D. L. In: Atta-ur-Rahman, editor. Studies in natural products chemistry; Bench top bioassays for the discovery of bioactive products: Structure and chemistry (part B). Amsterdam: Elsevier; **1991**; p. 383.

[31] Meyer, B. N.; Ferrigni, N. R.; Putnam, J. E.; Jacobsen, L. B.; Nicholas, D. E.; McLaughlin, J. L. Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Medica*, **1982**, *45*, 31-34.

[32] Finney, D. J. Probit Analysis. 3rd ed. London: Cambridge University Press; **1971**.