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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

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

Humayun Pervez · Naveeda Saira · Mohammad Saeed Iqbal · Muhammad Yaqub · Khalid Mohammed Khan

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Abstract A series of N^4-aryl-substituted 5-fluoroisatin-3-thiosemicarbazones **3a–3l** was synthesized 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. Seven compounds i.e. **3a**, **3d**, **3f**, **3g**, **3h**, **3j** and **3k** proved to be active in the brine shrimp assay, displaying promising cytotoxicity ($LD_{50} = 6.89 \times 10^{-5}$–$2.79 \times 10^{-4}$ M). Amongst these, **3a** and **3h** were found to be the most active ones ($LD_{50} = 6.89 \times 10^{-5}$ and 9.79×10^{-5} M, respectively). Compounds **3i**, **3j** and **3 k** displayed moderate (40 %) antifungal activity against one or two fungal strains i.e. *A. flavus* and/or *M. canis*. In phytotoxicity assay, all the synthesized compounds, including the reference point **2m** showed weak-to-moderate (15–70 %) activity at the highest tested concentration (500 μg/mL). In urease inhibition assay, compounds **3f**, **3g** and **3j** proved to be the most potent inhibitors, demonstrating relatively a higher degree of enzymatic inhibition with IC_{50} values ranging from 37.7 to 47.3 μM.

H. Pervez (✉) · N. Saira · M. Yaqub
Organic Chemistry Division, Institute of Chemical Sciences, Bahauddin Zakariya University, Multan 60800, Pakistan
e-mail: pdhpervez@hotmail.com

M. S. Iqbal
Department of Chemistry, FC College, Lahore 54600, Pakistan

K. M. Khan
H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan

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Introduction

It is evident from the literature that isatin and its derivatives are associated with a wide variety of biological activities like analgesic, anticonvulsant, antimicrobial, anti-inflammatory, antiglycation, antineoplastic, antiplasmodial, antituberculosis and antiviral (Aboul-Fadl and Bin-Jubair, 2010; Bal *et al.,* 2005; Banerjee *et al.,* 2011; 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.,* 2011, 2009; Hyatt *et al.,* 2007; Jarrahpour *et al.,* 2007; Karali *et al.,* 2007; Khan *et al.,* 2009; Pandeya *et al.,* 2005; Patel *et al.,* 2006; Pirrung *et al.,* 2005; Quenelle *et al.,* 2006; Ravichandran *et al.,* 2007; Singh *et al.,* 2010; Smitha *et al.,* 2008; Terzioglu *et al.,* 2006; Vine *et al.,* 2009). Among isatin derivatives, isatin-derived thiosemicarbazones have been reported to exhibit diverse chemotherapeutic activities, including antimicrobial, antituberculosis, antiulcer, cytotoxicity and enzymatic inhibition (Aboul-Fadl and Bin-Jubair, 2010; Bal *et al.,* 2005; Chiyanzu *et al.,* 2005, 2003; Da Silva *et al.,* 2001; Hall *et al.,* 2011, 2009; Karali *et al.,* 2007; Pandeya *et al.,* 2005; Pirrung *et al.,* 2005; Quenelle *et al.,* 2006; Terzioglu *et al.,* 2006; Vine *et al.,* 2009). In view of these facts and as a part of our synthetic work on the potential biologically active isatin derivatives, we have recently synthesized a number of 5-(un)-substituted N^4-aryl-substituted isatin-3-thiosemicarbazones as antibacterial, antifungal, cytotoxic, phytotoxic and antiurease compounds (Pervez *et al.*, 2007, 2008, 2009, 2010, 2011a, b, 2012a, b). Structure–activity relationship (SAR) studies revealed that in certain cases, the nature and position of the substituents about phenyl ring

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attached to N^4 of the thiosemicarbazone moiety and the presence of inductively electron-withdrawing groups (nitro, trifluoromethoxy, chloro) at position-5 of the isatin scaffold played an important role in the inducement and/or enhancement of different activities. Moreover, it has been reported by some other workers that certain N^4-aryl-substituted 5-bromo- and 5-chloro-isatin 3-thiosemicarbazones were found to exhibit favourable cytotoxic and antifungal activities (Karali *et al.,* 2002; Pandeya *et al.,* 1999). In view of this and in continuation of our work in search of new isatin-based bioactive compounds with improved efficacy, the study of the combination of halogen substitution at position-5 of the isatin scaffold with attachment of a variety of aryl substituents to N^4 of the thiosemicarbazone moiety was considered worth pursuing. The present work, therefore, deals with the synthesis and biological evaluation of a series of twelve N^4-aryl-substituted 5-fluoroisatin-3-thiosemicarbazones. It describes the effects of the type of aryl functions (modified by placement of one or two substituents about the phenyl ring) at N^4 of the thiosemicarbazone moiety as well as the presence of fluoro substituent at position-5 of the isatin scaffold on the cytotoxic, antifungal, phytotoxic and urease inhibitory properties of these compounds.

Results and discussion

This study illustrates the synthesis and in vitro determination of the cytotoxic, antifungal, phytotoxic and urease inhibitory potential of twelve N^4-aryl-substituted 5-fluoroisatin-3-thiosemicarbazones **3a–3l**.

Chemistry

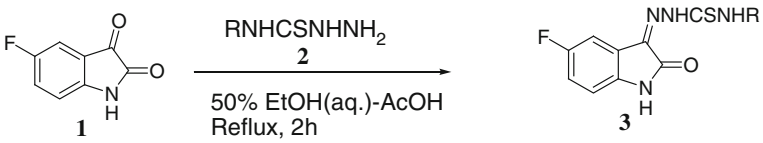
5-Fluoroisatin **1** was reacted with appropriate *N*-substituted thiosemicarbazides **2** in aqueous ethanol (50 %) containing a few drops of glacial acetic acid to give the corresponding 5-fluoroisatin-3-thiosemicarbazones **3a–3l** (Scheme 1) in good-to-excellent yields (72–93 %). The structures of all the synthesized compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR, EIMS and elemental (C, H, N) analyses. The IR spectra of **3a–3l** showed the absorption bands of NH stretching in the 3460–3200 and 3195–3072 cm^{-1} regions. The absorption bands of lactam C=O, azomethine C=N and thioamide C=S stretchings appeared in the 1691–1684, 1619–1539 and 1279–1244 cm^{-1} regions, respectively (Karali, 2002; Naumov and Anastasova, 2001; Omar *et al.,* 1984; Petrov *et al.,* 1986). The ^{1}H NMR spectra of **3a–3l** displayed three separate singlets at δ 10.63–11.01, δ 11.26–11.31 and δ 12.68–12.84 attributed to thiosemicarbazone N^4–H, indole NH and thiosemicarbazone N^2–H, respectively (Karali, 2002; Laatsch *et al.,* 1984; Naumov and Anastasova, 2001; Nizamuddin *et al.,* 1999; Omar *et al.,* 1984; Petrov *et al.,* 1986). The ^{13}C NMR spectra of **3a–3l** supported the IR and ^{1}H NMR findings (Guzel *et al.,* 2008; Karali *et al.,* 2007). Furthermore, the electron impact mass spectra (EIMS) of the synthesized thiosemicarbazones **3a–3l** demonstrated molecular ions of different intensities, confirming their molecular weights. The major fragmentation pathway involved the rupture of exocyclic N–N, NH–CS and endocyclic NH–CO bonds. The proposed fragmentation pattern of **3f** is illustrated in Fig. 1.

Biology

In vitro cytotoxicity

All the synthesized thiosemicarbazones **3a–3l** were screened for their cytotoxic activity by a brine shrimp (*Artemia salina*) lethality bioassay. Compound **3m** i.e. 2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)-*N*-phenyl-1-hydrazinecarbothioamide, the synthesis of which has been reported elsewhere (Pervez *et al.,* 2007), served as reference point (without a substituent in the isatin scaffold as well as on the phenyl ring) to assess the influence of

Scheme 1 Synthesis of title compounds **3a–3l**



3a	R = C_6H_5	**3g**	R = 3-FC_6H_4
3b	R = 2-$F_3CC_6H_4$	**3h**	R = 4-FC_6H_4
3c	R = 3-$F_3CC_6H_4$	**3i**	R = 2,4-$(F)_2C_6H_3$
3d	R = 4-$F_3CC_6H_4$	**3j**	R = 2,6-$(F)_2C_6H_3$
3e	R = 4-$F_3COC_6H_4$	**3k**	R = 3,5-$(F)_2C_6H_3$
3f	R = 2-FC_6H_4	**3l**	R = 2-F-4-BrC_6H_3

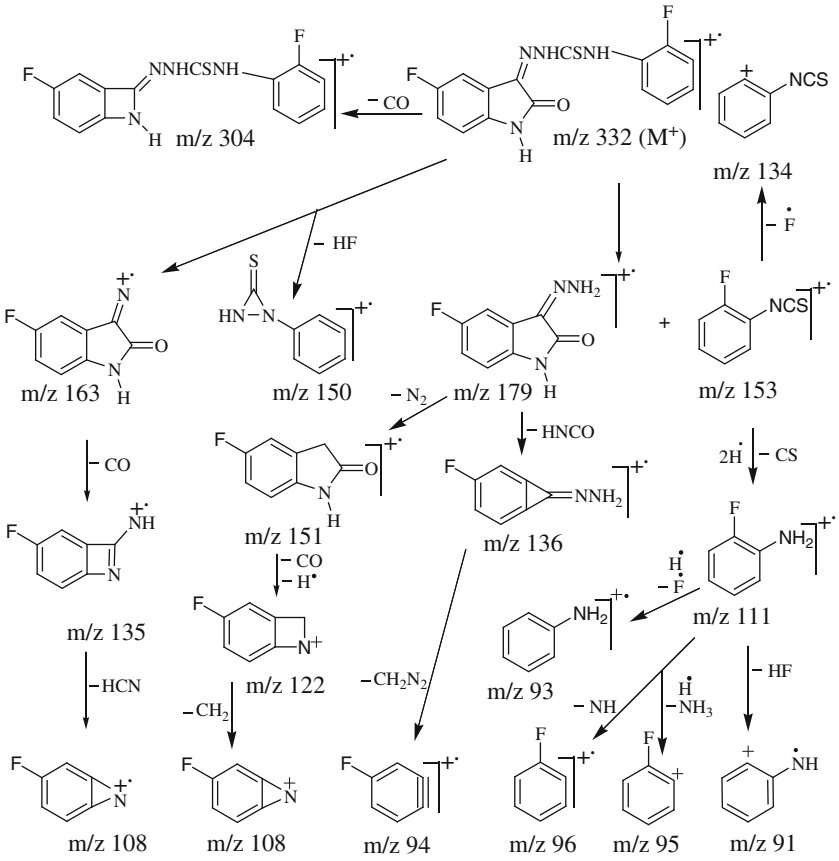


Fig. 1 The proposed fragmentation pattern of **3f**

Table 1 Brine shrimp bioassay for compounds **3a–3l**

Compounds	LD_{50}(M)
3a	6.89×10^{-5}
3b	$>5.24 \times 10^{-5}$
3c	$>5.24 \times 10^{-5}$
3d	1.76×10^{-4}
3e	$>5.03 \times 10^{-5}$
3f	2.79×10^{-4}
3g	1.21×10^{-4}
3h	9.79×10^{-5}
3i	$>5.71 \times 10^{-5}$
3j	1.15×10^{-4}
3k	1.07×10^{-4}
3l	$>4.91 \times 10^{-5}$
3m (Pervez *et al.,* 2007, 2008)	$>3.38 \times 10^{-4}$

substituents about the isatin moiety as well as the phenyl ring attached to N^4 of the thiosemicarbazone part of the test compounds on their cytotoxicity potential. The results presented in Table 1 revealed that compared with the reference compound **3m,** compound **3a** having fluoro group alone in the isatin scaffold displayed induction of cytotoxic activity. However, in the rest cases, combined substitution of fluoro and some other substituents in the aromatic ring of the isatin scaffold and on the phenyl ring attached to N^4 of the thiosemicarbazone moiety, respectively, caused either elimination or reduction in the activity. This inference was supported by the results obtained in our earlier studies (Pervez *et al.,* 2008, 2009). For example, compound **3a** having inductively electron-withdrawing fluoro substituent at position-5 of the isatin scaffold displayed promising cytotoxic activity ($LD_{50} = 6.89 \times 10^{-5}$ M), whereas the corresponding compound **3m** i.e. the reference point possessing no fluoro group in the isatin moiety was found to be almost inactive ($LD_{50} = >3.38 \times 10^{-4}$ M) (Pervez *et al.,* 2008). On the contrary, compounds **3b, 3c**, **3e** and **3i** with 2-trifluoromethyl, 3-trifluoromethyl, 4-trifluoromethoxy and 2,4-difluoro substituents about the phenyl ring showed almost no cytotoxic effects ($LD_{50} = >5.24 \times 10^{-5}, >5.24 \times 10^{-5}, >5.03 \times 10^{-5}$ and $>5.71 \times 10^{-5}$ M, respectively), whereas the corresponding compounds without fluoro group at position-5 of the isatin scaffold gave LD_{50} values of 1.36×10^{-4}, 2.20×10^{-5}, 1.80×10^{-5} and 2.00×10^{-5} M, respectively (Pervez *et al.,* 2009). Also, compared with compound **3f** ($LD_{50} = 2.79 \times 10^{-4}$ M), the dihalogeno-substituted compound **3l** possessing fluoro and bromo substituents at positions-2 and -4 of the phenyl ring, respectively, exhibited no cytotoxic effect ($LD_{50} = >4.91 \times 10^{-5}$ M) in the present assay. Similarly,

compounds **3d**, **3f**, **3g**, **3j** and **3k** with 4-trifluoromethyl, 2-fluoro, 3-fluoro, 2,6-difluoro and 3,5-difluoro substituents about the phenyl ring showed reduced cytotoxic activity ($LD_{50} = 1.76 \times 10^{-4}$, 2.79×10^{-4}, 1.21×10^{-4}, 1.15×10^{-4} and 1.07×10^{-4} M, respectively) in comparison to the corresponding compounds (without fluoro group at position-5 of the isatin moiety), which gave LD_{50} values of 1.17×10^{-4}, 3.10×10^{-5}, 4.60×10^{-5}, 2.00×10^{-5} and 2.10×10^{-5} M (Pervez *et al.,* 2009). Relatively, much pronounced reduction in the cytotoxic activity was observed in the case of **3k** ($LD_{50} = 2.10 \times 10^{-5} \rightarrow 1.07 \times 10^{-4}$ M). This showed that the simultaneous presence of inductively electron-attracting groups in the isatin scaffold as well as the phenyl ring substituted at N^4 of the thiosemicarbazone moiety caused either elimination or reduction in the cytotoxicity potential. However, compared with monofluoro-substituted compounds **3f** and **3g**, compound **3h** having fluoro substituent at position-4 of the phenyl ring displayed promising activity ($LD_{50} = 9.79 \times 10^{-5}$ M) in the present assay.

The exact mechanism of cytotoxic activity exhibited by the test thiosemicarbazones **3a–3l** is not known. However, it is generally accepted that the mode of cytotoxic action of this class of agents involves the inhibition of ribonucleoside diphosphate reductase (RDR), an enzyme containing a diferric centre involved in the rate-limiting step of DNA synthesis. Notably, it is the capability of these compounds to act as transition metal chelating agents that escorts to their cytotoxic activity. The chelation of iron (Fe) from intracellular Fe pools results in the inhibition of RDR. This was thought to be the major mechanism involved in the inhibition of RDR by thiosemicarbazones, in which the diferric Fe centre is required to stabilize the tyrosyl radical and is deemed essential for the enzymatic activity (Danuta *et al.,* 2009).

On the whole, out of twelve compounds tested for cytotoxic activity, seven i.e. **3a**, **3d**, **3f–3h**, **3j** and **3k** proved to be active, exhibiting promising cytotoxicity ($LD_{50} = 6.89 \times 10^{-5}$–$2.79 \times 10^{-4}$ M) against *Artemia salina.* The remaining compounds i.e. **3b, 3c**, **3e, 3i** and **3l** gave LD_{50} values ranging from $>4.91 \times 10^{-5}$ to $>5.71 \times 10^{-5}$ M in the present assay and, therefore, can be considered as inactive. These preliminary results indicate that structural modifications may lead to the development of certain novel cytotoxicants with improved efficacy.

In vitro antifungal activity

The synthetic compounds **3a–3l** were also screened for their antifungal activity against six fungal cultures i.e. *Trichophyton longifusus, Candida albicans, Aspergillus flavus, Microsporum canis, Fusarium solani* and *Candida glabrata* (Table 2) at 200 μg/mL in DMSO. All the compounds were found to be active against one or more fungi, exhibiting varied inhibition (10–40 %). Compound **3a** with no substituent on the phenyl ring attached to N^4 of the thiosemicarbazone moiety was found to be active against *M. canis* only, displaying 20 % inhibition. To the contrary, compounds **3b–3d** having trifluoromethyl substituents at different positions of the phenyl ring were active against more than one fungal strains. Of these, compound **3c** with a *meta* substituent was found to be the most active one, showing 30, 30 and 25 % inhibition of *A. flavus*, *M. canis* and *F. solani*, respectively. Next active to it was compound **3d** having a *para* substituent, demonstrating 20, 25 and 5 % inhibition of the same fungal strains. However, the *ortho*-substituted compound **3b** was found to be active against *A. flavus* and *M. canis* only, exhibiting 15 and 20 % inhibition. Similarly, compound **3e** having trifluoromethoxy substituent at *para* position of the phenyl ring was found to be active against these two fungi, showing 10 and 30 % inhibition, respectively. Amongst the mono-halogenated compounds **3f–3h**, compound **3f** possessing fluoro substituent at *ortho* position of the phenyl ring was found to be active against two fungal strains i.e. *M. canis* and *F. solani*, demonstrating 30 and 20 % inhibition, respectively. Also, the *para*-substituted compound **3h** was found to be active against these fungi, displaying 15 and 10 % inhibition. However, the *meta*-substituted compound **3g** was found to be the most active one, exhibiting 10, 25 and 15 % inhibition of three fungal strains i.e. *A. flavus*, *M. canis* and *F. solani*, respectively. Similarly, in the cases of di-halogenated compounds **3i–3l**, compound **3i** having fluoro substituents at the *ortho* and *para* positions was found to be the most active one, showing inhibitory activity (40, 35 and 15 %, respectively) against *A. flavus*, *M. canis* and *F. solani*. Compared to this, compound

Table 2 In vitro antifungal activity of compounds **3a–3l*** (% inhibition)

Compounds	Microbial species					
	T. longifusus	*C. albicans*	*A. flavus*	*M. canis*	*F. solani*	*C. glabrata*
3a	00	00	00	20	00	00
3b	00	00	15	20	00	00
3c	00	00	30	30	25	00
3d	00	00	20	25	05	00
3e	00	00	10	30	00	00
3f	00	00	00	30	20	00
3g	00	00	10	25	15	00
3h	00	00	00	15	10	00
3i	00	00	40	35	15	00
3j	00	00	40	40	00	00
3k	00	00	00	40	00	00
3l	00	00	00	20	10	00

* Concentration used 200 μg/mL of DMSO

3j with fluoro substituents at *ortho* positions was active against *A. flavus* and *M. canis*, displaying 40 % inhibition, whereas compound **3k** possessing fluoro substituents at *meta* positions showed 40 % inhibition against *M. canis* only. The remainder di-halogenated compound **3l** with fluoro and bromo substituents at *ortho* and *para* positions of the phenyl ring, respectively, exhibited 20 and 10 % inhibition of *M. canis* and *F. solani*. Although there is no ready explanation for this variation in activity, it may be related to the ability of the compounds to enter the cell or their ability to react at the unknown target site(s) in the microorganisms. According to Overtone's concept of cell permeability, the lipid membrane that surrounds the cell favours the permeation of only lipid-soluble compounds. So, liposolubility/lipophilicity is an important factor controlling the antifungal activity. Delocalization of electrons over the whole molecule increased the lipophilicity. The increased lipophilicity in turn enhanced penetration of the compounds into the cell membranes, thus further restricting proliferation of the microorganisms (Al-Amiery *et al*., 2012). Furthermore, like cytotoxicity, antifungal activity of the present compounds could be based on the deactivation of ribonucleoside diphosphate reductase (RDR). Importantly, it is the ability of the test thiosemicarbazones **3a–3l** to act as transition metal chelators coordinating with ferrous ions of the RDR, thus inhibiting its activity (Pandeya *et al.,* 1999).

Conclusively, compound **3a** having no substituent about its phenyl ring displayed 20 % inhibition of *M. canis* only, whereas the remainder compounds displayed relatively better activity profile in terms of percentage of inhibition and/or the number of fungal strains inhibited. This clearly indicates that the effects of different substituents about the phenyl ring attached to N^4 of the thiosemicarbazone moiety played an important role in enhancing the capability of the compounds to exhibit either induced or increased inhibitory activity. Notably, three of the six fungi tested i.e. *T. longifusus, C. albicans* and *C. glabrata* were resistant to all the tested compounds, while the two others i.e. *A. flavus* and *F. solani* showed resistance to some of them. Furthermore, none of the compounds of the present series exhibited significant antifungal activity; however, the negative findings or display of weak or moderate activities against certain selected fungal strains in the present assay do not prevent from further investigation of these compounds against other fungal strains. Also, further investigations on the SAR and appropriate chemical modifications among the synthesized thiosemicarbazones is likely to provide more effective compounds with improved efficacy.

In vitro phytotoxicity

The synthesized thiosemicarbazones **3a–3l** were further screened for their phytotoxic effects at 500, 50 and 5 μg/mL concentrations. Here too, compound **3m** i.e. 2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)-*N*-phenyl-1-hydrazinecarbothioamide served as reference point to evaluate the effects of fluoro function at position-5 of the isatin scaffold as well as other substituents about the phenyl ring attached to N^4 of the thiosemicarbazone moiety on the plant growth inhibitory potential of these compounds. All compounds except **3a** displayed weak or non-significant (15–35 %) plant growth inhibition at the highest tested concentration (500 μg/mL). Compound **3a** having flluoro substituent at position-5 of the isatin scaffold, however, exhibited moderate (70 %) inhibition at this concentration (Table 3). On the other hand, three compounds i.e. **3c**, **3j** and **3k**, out of twelve compounds tested, showed no plant growth inhibition, whereas the rest, including reference point displayed non-significant (5–20 %) inhibition at the lowest tested concentration (5 μg/mL).

From the results obtained in this assay, it may be concluded that our compounds, in general, exhibited either no or non-significant plant growth 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, compared with the reference compound **3m** having no substituent in the isatin scaffold as well as about the phenyl ring attached to N^4 of the thiosemicarbazone moiety and displaying moderate (40 %) plant growth inhibition at the highest tested concentration (500 μg/mL), the percent inhibition values exhibited by our test compounds revealed that the nature, number and

Table 3 Percentage growth inhibition of *Lemna aequinocitalis* by compounds **3a–3l** at different concentrations

Compounds	500 (μg/mL) (% G.I.)	50 (μg/mL) (% G.I.)	5 (μg/mL) (% G.I.)
3a	70	15	10
3b	25	10	05
3c	30	10	00
3d	25	15	10
3e	35	20	10
3f	35	25	20
3g	30	25	15
3h	35	25	10
3i	30	20	10
3j	30	10	00
3k	15	10	00
3l	30	25	10
3m (Pervez *et al.,* 2007, 2012b)	40	25	15

The reference compound paraquat shows 100 % growth inhibition at a concentration of 0.015 g/mL

G.I. growth inhibition

position of the substituents did not affect the phytotoxic inhibitory potential to a great extent.

In vitro urease inhibitory activity

All the synthetic thiosemicarbazones **3a–3l** were also subjected to urease inhibition studies. Thiourea and compound **3m** i.e. 2-(2-oxo-1,2-dihydro-3*H*-indole-3-ylidine)-*N*-phenyl-1-hydrazinecarbothioamide served as reference points in this assay. The results given in Table 4 demonstrated that compared with compound **3 m** having no substituent in the isatin part as well as on the phenyl ring, substitution of fluoro function at position-5 of the isatin scaffold alone or in combination with different aryl groups (possessing one or two substituents about the phenyl ring) at N^4 of the thiosemicarbazone moiety caused either induction or enhancement of enzymatic activity in certain cases at the tested concentrations (200 or 500 μM). This inference receives support from the results obtained by us in early investigations (Pervez *et al.,* 2008, 2009). For example, compound **3a** bearing fluoro substituent at position-5 of the isatin scaffold displayed induced activity (53 % with IC_{50} value 342.93) at 500 μM concentration as compared to the corresponding compound **3m** having no fluoro substituent at position-5 of the isatin moiety, which showed no inhibitory activity at the tested concentration(100 μM). Furthermore, compounds **3d**, **3g** and **3k** possessing 4-trifluoromethyl, 3-fluoro and 3,5-difluoro substituents about the phenyl ring exhibited enhanced activity (53.4 % with IC_{50} value 90.40, 79.8 % with IC_{50} value 45.3 and 80.2 % with IC_{50} value 150.70, respectively) as compared to the corresponding compounds bearing no fluoro substituent at position-5 of the isatin scaffold, which exhibited 44.6 %, 57.3 % with IC_{50} value 50.6 and 38.8 % inhibition at the tested concentration (100 μM). Much pronounced enhancement in the enzymatic activity was observed in the cases of **3d** and **3k** (44.6 → 53.4 % with IC_{50} value 90.40 and 38.8 → 80.2 % with IC_{50} value 150.70, respectively). To the contrary, compounds **3b**, **3c**, **3e**, **3f**, **3i** and **3j** having 2-trifluoromethyl, 3-trifluoromethyl, 4-trifluoromethoxy, 2-fluoro, 2,4-difluoro and 2,6-difluoro functions on the phenyl ring were found to display reduced activity (77.3 % with IC_{50} value 50.53, 92.6 % with IC_{50} value 59.86, 18.9 %, 94.6 % with IC_{50} value 37.7, 22.1 % and 72.5 % with IC_{50} value 47.3, respectively), when compared with the respective compounds without fluoro group at position-5 of isatin part, which demonstrated 63.1 % with IC_{50} value 33.1, 78.2 % with IC_{50} value 20.6, 49.6 %, 84.9 % with IC_{50} value 20.6, 71.9 % with IC_{50} value 47.6 and 92.0 % with IC_{50} value 33.1 inhibition of the enzyme at the tested concentration (100 μM). Much pronounced reduction occurred in the cases of **3c**, **3f** and **3i** (78.2 % with IC_{50} value 20.6 → 92.6 % with IC_{50} value 59.86, 84.9 % with IC_{50} value 20.6 → 94.6 % with IC_{50} value 37.7 and 71.9 % with IC_{50} value 47.6 → 22.1 %, respectively). This indicated that the simultaneous presence of diverse inductively electron-attracting groups in the isatin moiety as well as on the phenyl ring attached to N^4 of the thiosemicarbazone part caused the molecules to intermingle with the enzymatic activity differently, resulting into increment or decrement in their inhibitory potential. Overall, all the synthesized compounds **3a–3l** tested for their urease inhibitory potential displayed enzymatic inhibition. Of these, eight i.e. **3a–3d**, **3f**, **3g**, **3j** and **3k** were found to be potent inhibitors. Compounds **3f**, **3g** and **3j** having one or two fluoro substituents at positions-2, -3 and -2,6 of the phenyl ring were found to be the most potent ones, showing IC_{50} values 37.7, 45.3 and 47.3, respectively. The remainder compounds i.e. **3a–3d** and **3k** exhibited varying degree of activity with IC_{50} values ranging from 50.53 to 342.93 μM.

Table 4 Inhibition of Jack bean urease by compounds **3a–3l**

Compounds	Percentage (%) of inhibition at 200 μM	Percentage (%) of inhibition at 500 μM	$IC_{50} \pm SEM$ (μM)
3a		53.00	342.93 ± 1.58
3b	77.3		50.53 ± 0.31
3c		92.6	59.86 ± 0.24
3d	53.4		90.4 ± 0.66
3e		18.9	NA
3f		94.6	37.7 ± 0.16
3g	79.8		45.3 ± 0.16
3h		45.6	NA
3i		22.1	NA
3j		72.5	47.3 ± 0.08
3k		80.2	150.7 ± 1.17
3l	21.9		NA
3m (Pervez *et al.,* 2007, 2008)[a]			
Thiourea[b]	97.1	99.0	21.0 ± 0.11

NA No inhibitory activity

[a] Reference thiosemicarbazone screened against human urease at 100 μM concentration

[b] Reference inhibitor of urease enzyme

All ureases, regardless of their source, contain in addition to two nickel ions (Benini *et al.,* 1998, 2001, 2004, 2000; Ciurli *et al.,* 1999; Krajewska and Zaborska, 2007a, 2007b), one to three protein subunits present in varying stoichiometric ratios (*Mobley et al.,* 1995). Thus, an anti-urease compound can intermingle with the enzymatic activity by interacting either with the nickel ions or the protein constituent. β-Mercaptoethanol, hydroxamic acids and phosphorodiamidates, for example, are the synthetic inhibitors, which interact with the urease activity by

binding to the nickel ions present at its active site [Ciurli *et al.,* 1999; Kuhler *et al.,* 1995; Nagata *et al.,* 1993; Zaborska *et al.,* 2007), whereas sulphenamide, quinones and heavy metal ions have been reported to inhibit its activity by intermingling with the sulphhydryl (S–H) functions present in the protein component (Krajewska, 2008; Krajewska and Zaborska, 2007a, 2007b; Krajewska *et al.,* 2004; Kuhler *et al.,* 1995; Nagata *et al.,* 1993 Krajewska and Zaborska, 2007a). Although the actual mechanism of urease inhibitory activity exhibited by our trial compounds **3a–3l** is not documented, it is intriguing to carry out investigations as regards detailed kinetics of such interaction. Apparently, 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 nickel (II) of the enzyme, suggesting that the thiolate binds directly to the nickel ion(s). Diverse non-covalent interactions, including hydrogen bonds and hydrophobic contacts may stabilize such enzyme-inhibitors chelate interactions and thus contribute towards their inhibitory potential (Amtul *et al.,* 2002). Detailed kinetic investigations to get an insight into the mechanism of inhibition are underway, the results of which will be reported in due course of time.

In summary, a series of twelve 5-fluoroisatin-3-thiosemicarbazones **3a–3l** has been synthesized and characterized by analytical and spectral data. The synthesized thiosemicarbazones were screened for their in vitro cytotoxic, antifungal, phytotoxic and urease inhibitory effects. Seven out of twelve compounds tested proved to be active in the brine shrimp bioassay, displaying promising cytotoxicity. In antifungal assay, all the compounds showed weak-to-moderate activity against one or more fungi. However, the negative findings or demonstration of weak or moderate activity against the selected fungal strains do not preclude from further investigations of these compounds against some other pathogens. In phytotoxicity bioassay, the synthesized thiosemicarbazones showed weak-to-moderate activity at the highest tested concentration. The urease inhibition screening results have substantiated that eight out of twelve compounds tested appeared as potent urease inhibitors; three of these i.e. **3f**, **3g** and **3j** proved to be the most potent ones and may act as leads for further studies. These compounds, exhibiting no significant phytotoxic activity at the highest tested concentration, invited attention to their usefulness as potent soil ureases inhibitors, as they could be mixed with fertilizers in small quantities to increase the overall efficacy of nitrogen utilisation. In general, the urease inhibitory activity was found to be dependent upon electronic effects of the fluoro function at position-5 of the isatin scaffold as well as different inductively electron-attracting substituents about the phenyl ring attached to N^4 of the thiosemicarbazone moiety.

Experimental section

Chemistry

All chemicals and solvents were purchased from Aldrich, Fluka and Merck-Schuchatdt. Melting points were determined on cover slips using a Fisher-Johns melting point apparatus and are uncorrected. Elemental (C, H, N) analyses were performed on a Leco CHNS-9320 (USA) elemental analyser and were in full agreement with the proposed structures within ±0.4 % of the theoretical limits, except where noted otherwise. Infrared (IR) spectra (KBr discs) were run on Shimadzu Prestige-21 FT-IR spectrometer. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded in DMSO-d_6 on Bruker (Rhenistetten-Forchheim, Germany) AM 300 spectrometer, operating at 300 MHz and using TMS as an internal standard. The chemical shifts (δ) are reported in parts per million (ppm) and coupling constants in Hz. Carbon-13 nuclear magnetic resonance (^{13}C NMR) spectra were recorded at 75 MHz with the same internal standard. The electron impact mass spectra (EI MS) were determined with MAT-312, JEOL MSRoute and JEOL JMS 600 mass spectrometers. 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.

*General procedure for the preparation of 5-fluoroisatin-thiosemicarbazons (**3a–3l**)*

To a solution of 5-fluoroisatin **1** (2.5 mmol) in 50 % aqueous ethanol (10 mL) containing a catalytic amount of glacial acetic acid, the appropriate thiosemicarbazide **2** (2.5 mmol) dissolved in ethanol (10 mL) was added under stirring. 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 (50 %) furnished the target compounds **3a–3l** in pure form.

*N-Phenyl-2-(5-fluoro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)-1-hydrazinecarbothioamide (**3a**)* Yield 93 % as yellow powder; m.p. 218–220 °C (lit. (Karali *et al.,* 2007) m.p. 218 °C); IR (KBr, cm^{-1}): 3460, 3195 (NH), 1691 (C=O), 1595 (C=N), 1265 (C=S); ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm, 6.95 (dd, J = 8.7, 4.2 Hz, 1H, indole C_7–H), 7.22 (td, J = 8.7, 2.7 Hz, 1H, indole C_6–H), 7.29

(t, $J = 7.5$ Hz, 1H, phenyl C_4–H), 7.45 (t, $J = 7.5$ Hz, 2H, phenyl C_3–H, C_5–H), 7.61 (d, $J = 8.1$ Hz, 2H, phenyl C_2–H, C_6–H), 7.65 (dd, $J = 8.4$, 2.7 Hz, 1H, indole C_4–H), 10.85 (s, 1H, CSNH), 11.26 (s, 1H, indole NH), 12.68 (s, 1H, NNH); ^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm, 108.11, 108.46, 112.10, 112.21, 117.47, 117.79, 121.41, 125.63, 126.21, 128.43, 138.27, 138.74, 156.67, 159.82, 162.76, 176.30; EI MS (70 eV) *m/z* (%), 314 (M^+, 34), 286 (100), 254 (3), 221 (14), 197 (6), 179 (40), 163 (15), 162 (29), 151 (17), 15 (20), 149 (23), 136 (53), 135 (71), 122 (36), 108 (25), 96 (6), 95 (7), 91 (16), 77 (48), 65 (14). Anal calcd. for $C_{15}H_{11}FN_4OS$: C, 57.31; H, 3.53; N, 17.82. Found: C, 57.20; H, 3.52; N, 17.79.

2-(5-Fluoro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)-N-[2-(trifluoromethyl)phenyl]-1-hydrazinecarbothioamide ***(3b)*** Yield 75 % as yellow fluffy crystals; m.p. 247–248 °C; IR (KBr, cm^{-1}): 3300, 3200 (NH), 1684 (C=O), 1580 (C=N), 1279 (C=S); ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm, 6.94 (dd, $J = 8.7$,4.2 Hz, 1H, indole C_7–H), 7.22 (td, $J = 8.7$, 2.7 Hz, 1H, indole C_6–H), 7.53 (dd, $J = 8.1$, 2.7 Hz, 1H, indole C_4–H), 7.56–7.64 (m, 2H, phenyl C_3–H, C_4–H), 7.77–7.86 (m, 2H, phenyl C_5–H, C_6–H), 10.85 (s, 1H, CSNH), 11.29 (s, 1H, indole NH), 12.75 (s, 1H, NNH); ^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm, 107.98, 108.32, 112.24, 112.35, 117.67, 117.99, 121.25, 121.38, 121.60, 125.23, 126.54, 126.59, 126.66, 126.94, 128.42, 132.15,133.23, 136.82, 138.95, 156.68, 159.83, 162.79, 178.56; EI MS (70 eV) *m/z* (%), 382 (M^+, 13), 354 (65), 334 (3), 313 (2), 285 (4), 256 (13), 219 (4), 196 (11), 184 (78), 179 (23), 163 (10), 151 (30), 150 (45), 136 (27), 135 (35), 122 (100), 108 (54), 96 (20), 95 (55), 94 (21), 91 (5), 75 (37). Anal calcd. for $C_{16}H_{10}F_4N_4OS$: C, 50.26; H, 2.64; N, 14.65. Found: C, 50.19; H, 2.63; N, 14.60.

2-(5-Fluoro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)-N-[3-(trifluoromethyl)phenyl]-1-hydrazinecarbothioamide ***(3c)*** Yield 72 % as orange powder; m.p. 219–220 °C; IR (KBr, cm^{-1}): 3248, 3202(NH), 1697 (C=O), 1547 (C=N), 1244 (C=S); ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm, 6.94 (dd, $J = 8.7$, 4.2 Hz, 1H, indole C_7–H) 7.22 (td, $J = 8.7$, 2.7 Hz, 1H, indole C_6–H), 7.63 (dd, $J = 8.1$, 2.4 Hz, 1H, indole C_4–H), 7.66–7.71 (m, 2H, phenyl C_5–H, C_6–H), 8.00–8.06 (m, 2H, phenyl C_2–H, C_4–H), 11.01 (s, 1H, CSNH), 11.31 (s, 1H, indole NH), 12.78 (s, 1H, NNH); ^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm, 108.13, 108.48, 112.19, 112.30, 117.68, 118.00, 121.12, 121.25, 121.66, 121.71, 121.76, 121.81, 122.13, 122.50, 122.55, 125.74, 128.90, 129.28, 129.57, 132.17, 132.21, 138.88, 138.90, 139.10, 156.68, 159.83, 162.77, 176.43; EI MS (70 eV) *m/z* (%), 382 (M^+, 20), 354 (100), 218 (4), 204 (19), 184 (10), 179 (21), 168 (30), 163 (9), 151 (19), 150 (35), 145 (48), 136 (16), 135 (20), 122 (50), 108 (8), 96 (9), 95 (23), 94 (8), 75 (17). Anal calcd. for $C_{16}H_{10}F_4N_4OS$: C, 50.26; H, 2.64; N, 14.65. Found: C, 50.20; H, 2.63; N, 14.60.

2-(5-Fluoro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)-N-[4-(trifluoromethyl)phenyl]-1-hydrazinecarbothioamide ***(3d)*** Yield 82 % as yellow fluffy crystals; m.p. 249–250 °C; IR (KBr, cm^{-1}): 3285, 3187 (NH), 1694 (C=O), 1608 (C=N), 1271 (C=S); ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm, 6.94 (dd, $J = 8.7$, 4.2 Hz, 1H, indole C_7–H), 7.25 (td, $J = 9.3$, 2.7 Hz, 1H, indole C_6–H), 7.63 (dd, $J = 8.1$, 2.7 Hz, 1H, indole C_4–H), 7.81 (d, $J = 8.7$ Hz, 2H, phenyl C_2–H, C_6–H), 7.95 (d, $J = 8.4$ Hz, 2H, phenyl C_3–H, C_5–H), 11.01 (s, 1H, CSNH), 11.30 (s, 1H, indole NH), 12.80 (s, 1H, NNH); ^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm, 108.28, 108.46. 112.22, 112.27, 117.79, 117.95, 121.14, 121.20, 123.25, 125.47, 125.51, 125.54, 125.93, 126.14, 132.26, 132.28, 138.90, 141.99, 157.46, 159.04, 162.78, 176.28; EI MS (70 eV) *m/z* (%), 382 (M^+, 32), 354 (100), 218 (5), 204 (19), 179 (22), 168 (28), 163 (9), 151 (17), 150 (34), 145 (49), 136 (23), 135 (25), 122 (61), 108 (31), 96 (12), 95 (31), 94 (12), 75 (18). Anal calcd. for $C_{16}H_{10}F_4N_4OS$: C, 50.26; H, 2.64; N, 14.65. Found: C, 50.13; H, 2.63; N, 14.69.

2-(5-Fluoro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)-N-[4-(trifluoromethoxy)phenyl]-1-hydrazinecarbothioamide ***(3e)*** Yield 80 % as light orange powder; m.p. 244–245 °C; IR (KBr, cm^{-1}): 3306, 3183(NH), 1695 (C=O), 1580 (C=N), 1250 (C=S); ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm, δ 6.94 (dd, $J = 8.4$, 4.2 Hz, 1H, indole C_7–H), 7.21 (td, $J = 9.3$, 2.7 Hz, 1H, indole C_6–H), 7.45 (d, $J = 8.4$ Hz, 2H, phenyl C_2 –H, C_6–H), 7.60 (dd, $J = 8.1$, 2.7 Hz, 1H, indole C_4–H), 7.75 (d, $J = 8.7$ Hz, 2H, phenyl C_3–H, C_5–H), 10.92 (s, 1H, CSNH), 11.28 (s, 1H, indole NH), 12.73 (s, 1H, NNH), 12.73 (s, 1H, NNH); ^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm, 108.09, 108.43, 112.16, 112.27, 117.60, 117.92, 121.11, 121.18, 121.31, 127.37, 131.98, 132.02, 137.45, 138.83, 145.89, 145.91, 156.67, 159.82, 162.76, 176.51; EI MS (70 eV) *m/z* (%), 398 (M^+, 26), 370 (100), 313 (3), 235 (3), 220 (18), 179 (19), 163 (9), 151 (19), 150 (38), 136 (16), 135 (20), 122 (58), 108 (36), 96 (13), 95 (42), 94 (9), 75 (11). Anal calcd. for $C_{16}H_{10}F_4N_4O_2S$: C, 48.24; H, 2.53; N, 14.07. Found: C, 48.22; H, 2.54; N, 14.01.

2-(5-Fluoro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)-N-(2-fluorophenyl)-1-hydrazinecarbothioamide ***(3f)*** Yield 80 % as yellow fluffy crystals; m.p. 260 °C; IR (KBr, cm^{-1}): 3300, 3169, 3101 (NH), 1696 (C=O), 1619 (C=N), 1264 (C=S); ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm, 6.95 (dd, $J = 8.4$, 4.2 Hz, 1H, indole C_7–H), 7.22 (td, $J = 9.3$, 2.4 Hz, 1H, indole C_6–H), 7.29–7.43 (m, 3H, phenyl C_4–H, C_5–H, C_6–H), 7.48 (d, $J = 7.8$ Hz, phenyl C_3–H), 7.55 (dd,

$J = 8.1$, 2.4 Hz, indole C_4–H), 10.78 (s, 1H, CSNH), 11.28 (s, 1H, indole NH), 12.74 (s, 1H, NNH); ^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm, 107.96, 108.30, 112.18, 112.29, 112.91, 116.17, 117.59, 117.90, 121.25, 121.38, 124.43, 124.47, 126.19, 126.35, 129.13, 129.23, 130.16, 132.06, 132.11, 138.87, 138.88, 155.62, 156.66, 158.91, 159.81, 162.72, 178.00; EI MS (70 eV) *m/z* (%), 332 (M^+, 9), 304 (100), 284 (5), 221 (4), 197 (3), 179 (12), 168 (13), 150 (16), 135 (29), 122 (46), 108 (27), 96 (11), 95 (20), 91 (3), 83 (21), 75 (18). Anal calcd. for $C_{15}H_{10}F_2N_4OS$: C, 54.21; H, 3.03; N, 16.86. Found: C, 54.16; H, 3.03; N, 16.84.

2-(5-Fluoro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)-N-(3-fluorophenyl)-1-hydrazinecarbothioamide ***(3g)*** Yield 74 % as orange fluffy crystals; m.p. 256 °C; IR (KBr, cm^{-1}): 3304, 3163,3103 (NH), 1697 (C=O), 1595 (C=N), 1261 (C=S); ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm, 6.93 (dd, $J = 8.4$, 4.2 Hz, 1H, indole C_7–H), 7.11–7.17 (m, 1H, phenyl C_5–H), 7.24 (td, $J = 8.7$, 4.3 Hz, 1H, indole C_6–H), 7.44–7.53 (m, 2H, phenyl C_2–H, C_6–H), 7.69 (dd, $J = 8.4$, 4.2 Hz, 2H, indole C_4–H, phenyl C_2–H), 10.81 (s, 1H, CSNH), 11.29 (s, 1H, indole NH), 12.73 (s, 1H, NNH); ^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm, 108.22, 108.48, 112.10, 112.18, 112.26, 112.35, 112.68, 112.89, 117.67, 117.91, 121.18, 121.22, 121.25, 129.93, 130.02, 132.00, 132.04, 132.85, 139.90, 140.01, 157.07, 159.43, 160.40, 162.77, 162.81, 176.16; EI MS (70 eV) *m/z* (%), 332 (M^+, 4), 304 (26), 197 (14), 179 (14), 168 (20), 163 (4), 153 (27), 151 (14), 150 (18), 136 (27), 135 (22), 122 (66), 111 (34), 108 (40), 96 (32), 95 (100), 94 (20), 84 (22), 83 (77), 75 (63), 69 (15), 57 (49). Anal calcd. for $C_{15}H_{10}F_2N_4OS$: C, 54.21; H, 3.03; N, 16.86 Found: C, 54.17; H, 3.04; N, 16.84.

2-(5-Fluoro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)-N-(4-fluorophenyl)-1-hydrazinecarbothioamide ***(3h)*** Yield 82 % as orange powder; m.p. 265–266 °C (lit. (Karali *et al.,* 2007) m.p. 246–248 °C); IR (KBr, cm^{-1}): 3355, 3155 (NH), 1692 (C=O), 1540 (C=N), 1258 (C=S); ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm, 6.94 (dd, $J = 8.4$,4.2 Hz, 1H, indole C_7–H), 7.18–7.31 (m, 3H, phenyl C_2–H, C_6–H, indole C_6–H), 7.58–7.62 (m, 3H, phenyl C_3–H, C_5–H, indole C_4–H), 10.87 (s, 1H, CSNH), 11.82 (s, 1H, indole NH), 12.68 (s, 1H, NNH); ^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm, 108.14, 108.31. 112.15, 112.21, 115.10, 115.25, 117.58, 117.74, 121.28, 121.34, 127.89, 127.94, 131.74, 131.76, 134.62, 134.64, 138.76, 157.45, 159.03, 159.25, 160.86, 162.75, 176.69; EI MS (70 eV) *m/z* (%), 332 (M^+, 20), 304 (80), 221 (4), 197 (9), 179 (20), 168 (32), 163 (10), 151 (25), 150 (28), 136 (40), 135 (35), 122 (90), 108 (48), 96 (49), 95 (100), 80 (68), 75 (62). Anal calcd. for $C_{15}H_{10}F_2N_4OS$: C, 54.21; H, 3.03; N, 16.86. Found: C, 54.21; H, 3.05; N, 16.84.

N-(2,4-Difluorophenyl)-2-(5-fluoro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)-1-hydrazinecarbothioamide ***(3i)*** Yield 78 % as yellow fluffy crystals; m.p. 259–260 °C; IR (KBr, cm^{-1}): 3300, 3217(NH), 1694 (C=O), 1539 (C=N), 1263 (C=S); ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm, 6.95 (dd, $J = 8.4$, 4.2 Hz, 1H, indole C_7–H), 7.15–7.25 (m, 2H, indole C_6–H, phenyl C_6–H), 7.43 (td, $J = 9.0$, 2.7 Hz, 1H, phenyl C_5–H), 7.50–7.58 (m, 2H, phenyl C_3–H, indole C_4–H), 10.72 (s, 1H, CSNH), 11.28 (s, 1H, indole NH), 12.76 (s, 1H, NNH); ^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm, 104.31, 104.65, 104.98, 107.98, 108.32, 111.39, 111.43, 111.68, 111.73. 112.24, 112.34, 117.68, 118.00, 121.24, 121.36, 122.92, 122.97, 123.09, 123.13, 131.44, 131.57, 132.28, 132.32, 138.96, 155.77, 155.94, 156.86, 159.09, 159.26, 159.34, 159.49, 159.83, 162.61, 162.75, 178.33; EI MS (70 eV) *m/z* (%), 350 (M^+, 31), 322(100), 179 (6), 172 (9), 163 (30), 151 (5), 150 (8), 136 (5), 135 (5), 122 (11), 108 (9), 95 (3), 75 (3). Anal calcd. for $C_{15}H_9F_3N_4OS$: C, 51.43; H, 2.59; N, 15.99. Found: C, 51.44; H, 2.58; N, 16.00.

N-(2,6-Difluorophenyl)-2-(5-fluoro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)-1-hydrazinecarbothioamide ***(3j)*** Yield 90 % as orange powder; m.p. 274–275 °C; IR (KBr, cm^{-1}): 3360, 3194(NH), 1694 (C=O), 1583 (C=N), 1246 (C=S); ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm, 6.95 (dd, $J = 8.7$, 4.2 Hz, 1H, indole C_7–H), 7.20–7.30 (m, 3H, indole C_4–H, C_6–H, phenyl C_4–H), 7.46–7.56 (m, 2H, phenyl C_3–H, C_5–H), 10.63 (s, 1H, CSNH), 11.29 (s, 1H, indole NH), 12.84 (s, 1H, NNH); ^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm, 107.94, 108.28, 111.90, 112.13, 112.21, 112.24, 112.35, 115.63, 115.85, 116.06, 117.75, 118.07, 121.16, 121.28, 129.66, 129.79, 129.92, 132.70, 132.74, 139.04, 139.06, 156.64, 156.88, 156.94, 159.79, 160.18, 160.24, 162.69, 178.66; EI MS (70 eV) *m/z* (%), 350 (M^+, 28), 322 (100), 282 (2), 221 (4), 196 (8), 179 (20), 171 (21), 163 (9), 151 (15), 150 (32), 136 (17), 135 (23), 122 (57), 108 (61), 101 (20), 96 (9), 95 (22), 94 (13), 75 (16). Anal calcd. for $C_{15}H_9F_3N_4OS$: C, 51.43; H, 2.59; N, 15.99. Found: C, 51.39; H, 2.60; N, 15.91.

N-(3,5-Difluorophenyl)-2-(5-fluoro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)-1-hydrazinecarbothioamide ***(3k)*** Yield 77 % as orange powder; m.p. 259–260 °C; IR (KBr, cm^{-1}): 3290, 3072 (NH), 1693 (C=O), 1602 (C=N), 1265 (C=S): ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm, 6.93 (dd, $J = 8.7$, 4.2 Hz, 1H, indole C_7–H), 7.14–7.25 (m, 2H, indole C_6–H, phenyl C_4–H), 7.55–7.63 (m, 3H, indole C_4 – H, phenyl C_2–H, C_6 –H), 10.89 (s, 1H, CSNH), 11.31 (s, 1H, indole NH), 12.78 (s, 1H, NNH); ^{13}C NMR (75 MHz,

DMSO-d_6) δ, ppm, 101.01, 101.22, 101.42, 107.81, 107.86, 107.98, 108.04, 108.29, 108.49, 112.22, 112.28, 117.84, 118.03, 121.03, 121.11, 132.36, 132.39, 138.95, 140.72, 140,83, 140.94, 157.29, 159.18, 160.79, 160.91, 162.75, 162.85, 175.93; EI MS (70 eV) *m/z* (%), 350 (M^+, 30), 321 (100), 220 (2), 196 (9), 179 (20), 168 (29), 163 (4), 151 (17), 150 (27), 136 (18), 135 (19), 122 (50), 108 (24), 96 (9), 95 (18), 94 (9), 82 (26). Anal calcd. for $C_{15}H_9F_3N_4OS$: C, 51.43; H, 2.59; N, 15.99. Found: C, 51.36; H, 2.61; N, 16.01.

N-(4-Bromo-2-fluorophenyl)-2-(5-fluoro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)-1-hydrazinecarbothioamide ***(3l)*** Yield 80 % as orange fluffy crystals; mp 209–210 °C; IR (KBr, cm^{-1}): 3310, 3171(NH), 1691 (C=O),1580 (C=N), 1271 (C=S); ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm, 6.94 (dd, J = 8.7, 4.2 Hz, 1H, indole C_7–H), 7.22 (td, J = 9.3, 2.7 Hz, 1H, indole C_6–H), 7.45–7.54 (m, 3H, phenyl C_3–H, C_5–H, C_6–H), 7.74 (dd, J = 9.3, 2.7 Hz, 1H, indole C_4–H), 10.76 (s, 1H, CSNH), 11.30 (s, 1H, indole NH), 12.78 (s, 1H, NNH); ^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm, 107.99, 108.20, 112.22, 112.28, 117.73, 117.92, 119.39, 119.58, 120.20, 120.27, 121.16, 121.24, 125.97, 126.06, 127.63, 127.66, 131.64, 132.35, 132.38, 138.92, 156.17, 157.26, 158.18, 159.15, 162.68, 177.96; EI MS (70 eV) *m/z* (%), 412/410 (M^+, 15/14), 384/382 (100/95), 233/231 (27/26), 214 (27), 191/189 (21/23), 179 (37), 168 (68), 163 (35), 151 (31), 150 (52), 136 (40), 135 (47), 122 (97), 108 (78), 96 (17), 95 (37), 94 (45), 75 (16). Anal calcd. for $C_{15}H_9BrF_2N_4OS$: C, 43.81; H, 2.21; N, 13.62. Found: C, 43.72; H, 2.20; N, 13.51.

Biology

Cytotoxicity in vitro

Brine shrimp (*Artemia salina* leach) eggs were hatched in a shallow rectangular plastic dish (22 × 32 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 2 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 2 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 (McLaughlin *et al.,* 1991; Meyer *et al.,* 1982). Data were analysed by a Finney computer program to determine the LD_{50} values (Finney, 1971).

Antifungal activity in vitro

Antifungal activities of all the compounds were studied against six 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 seven 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

This bioassay was performed according to a modified protocol (McLaughlin *et al.,* 1991). 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 a negative control and the reference inhibitor i.e. paraquat as the positive one. Treatments were replicated three times and the flasks incubated at 30 °C in Fisons Fi-Totron 600 H growth cabinet for 7 days, 9,000 lux light intensity, 56 ± 10 rh (relative humidity), and 12 h day length. Growth of *Lemna aequinocitalis* in compounds-containing flasks was determined by counting the number of fronds per dose and growth inhibition was calculated with reference to negative control.

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 and 0.5 mM concentrations) at 30 °C for 15 min in 96-well plates. Urease inhibitory activity was determined by measuring ammonia production using the indophenol method as described by Weatherburn (Weatherburn, 1967). In brief, 45 µL each of phenol reagent (1 % w/v phenol and

0.005 % w/v sodium nitropruside) 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 Device, USA). All reactions were performed in triplicate in a final volume of 200 μL. The results (change in absorbance per min) were processed using SoftMax Pro software (Molecular Device, USA). All the assays were performed at pH 8.2 (0.01 M K_2HPO_4. $3H_2O$, 1 mM EDTA and 0.01 M LiCl). Percentage inhibitions were calculated from the formula $100 - (OD_{testwell}/OD_{control}) \times 100$. Thiourea was used as the standard inhibitor of urease.

Conclusions

We have reported the potential of N^4-aryl-substituted 5-fluoroisatin-3-thiosemicarbazones to exhibit cytotoxic and antiurease activities. Based on the preliminary data presented in Tables 1 and 4, and in terms of further development and structure–activity relationship (SAR) studies, simultaneous substitution of different inductively electron-withdrawing groups at position-5 of the isatin scaffold and on the phenyl ring attached to N^4 of the thiosemicarbazone moiety certainly warrants further studies. Work in this regard along with extended SAR studies will be reported in the very near future.

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References

Aboul-Fadl T, Bin-Jubair FAS (2010) Anti-tubercular activity of isatin derivatives. Int J Res Pharm Sci 1:113–126

Al-Amiery AA, Al-Majedy YK, Ibrahim HH, Al-Tamimi AA (2012) Antioxidant, antimicrobial, and theoretical studies of the thiosemicarbazone derivative Schiff base 2-(2-imino-1-methylimidazolidin-4-ylidene)hydrazinecarbothioamide (IMHC). Org Med Chem 2:4–10

Amtul Z, Rahman AU, Siddiqui RA, Choudhary MI (2002) Chemistry and mechanisim of urease inhibition. Curr Med Chem 9:1323–1348

Bal TR, Anand B, Yogeeswari P, Sriram D (2005) Synthesis and evaluation of anti-HIV activity of isatin β-thiosemicarbazone derivatives. Bioorg Med Chem Lett 15:4451–4455

Banerjee D, Yogeeswari P, Bhat P, Thomas A, Srividya M, Sriram D (2011) Novel isatinyl thiosemicarbazones derivatives as potential molecule to combat HIV-TB co-infection. Eur J Med Chem 46:106–121

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

Benini S, Rypniewski WR, Wilson KS, Ciurli S, Mangani S (1998) The complex of *Bacillus pasteurii* urease with β-mercaptoethanol from X-ray data at 1.65-Å resolution. J Biol Inorg Chem 3:268–273

Benini S, Rypniewski WR, Wilson KS, Miletti S, Ciurli S, Mangani S (2000) The complex of *Bacillus pasteurii* urease with acetohydroxamate anion from X-ray data at 1.55 Å resolution. J Biol Inorg Chem 5:110–118

Benini S, Rypniewski WR, Wilson KS, Ciurli S, Mangani S (2001) Structure-based rationalization of urease inhibition by phosphate: novel insights into the enzyme mechanism. J Biol Inorg Chem 6:778–790

Benini S, Rypniewski WR, Wilson KS, Mangani S, Ciurli S (2004) Molecular details of urease inhibition by boric acid: insights into the catalytic mechanism. J Am Chem Soc 126:3714–3715

Chen L-R, Wang Y-C, Lin YW, Chou S-Y, Chen S-F, Liu LT, Wu Y-T, Kuo C-J, Chen T-S, Juang S-H (2005) Synthesis and evaluation of isatin derivatives as effective SARS coronavirus 3CL protease inhibitors. Bioorg Med Chem Lett 15:3058–3062

Chiyanzu I, Hansell E, Gut J, Rosenthal PJ, McKerrow JH, Chibale K (2003) Synthesis and evaluation of isatins and thiosemicarbazone derivatives against cruzain falcipain-2 and rhodesain. Bioorg Med Chem Lett 13:3527–3530

Chiyanzu I, Clarkson C, Smith PJ, Lehman J, Gut J, Rosenthal PJ, Chibale K (2005) Design, synthesis and anti-plasmodial evaluation in vitro of new 4-aminoquinoline isatin derivatives. Bioorg Med Chem 13:3249–3261

Ciurli S, Benini S, Rypniewski WR, Wilson KS, Miletti S, Mangani S (1999) Structural properties of the nickel ions in urease: novel insights into the catalytic and inhibition mechanisms. Coord Chem Rev 190–192:331–355

Da Silva JFM, Garden SJ, Pinto AC (2001) The chemistry of isatins: a review from 1975 to 1999. J Braz Chem Soc 12:273–324

Danuta SK, Patricia Q, Desrr D (2009) Thiosemicarbazones: the new wave in cancer treatment. Future Med Chem 1:1143–1151

Finney DJ (1971) Probit Analysis, 3rd edn. Cambridge University Press, London

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

Hall MD, Salam NK, Hellawell JL, Fales HM, Kensler CB, Ludwig JA, Szakacs G, Hibbs DE, Gottesman MM (2009) Synthesis, activity, and pharmacophore development for isatin-β-thiosemicarbazones with selective activity toward multidrug-resistant cells. J Med Chem 52:3191–3204

Hall MD, Brimacombe KR, Varonka MS, Pluchino KM, Monda JK, Li J, Walsh MJ, Boxer MB, Warren TH, Fals HM, Gottesman MM (2011) Synthesis and structure–activity evaluation of isatin-β-thiosemicarbazones with improved selective activity toward multidrug-resistant cells expressing P-glycoprotein. J Med Chem 54:5878–5889

Hussain M, Hussain MT, Rama NH, Hameed S, Malik A, Khan KM (2003) Synthesis and antimicrobial activities of some isocoumarin and dihydroisocoumarin derivatives. Nat Prod Res 17:207–214

Hyatt JL, Moak T, Hatfield MJ, Tsurkan L, Edwards CC, Wierdl M, Danks MK, Wadkins RM, Potter PM (2007) Selective inhibition of carboxylesterases by isatins, indole-2,3-diones. J Med Chem 50:1876–1885

Jarrahpour A, Khalili D, Clercq ED, Salmi C, Brunel JM (2007) Synthesis, antibacterial, antifungal and antiviral activity evaluation of some new *bis*-Schiff bases of isatin and their derivatives. Molecules 12:1720–1730

Karali N (2002) Synthesis and primary cytotoxicity evaluation of new 5-nitroindole-2, 3-dione derivatives. Eur J Med Chem 37:909–918

Karali N, Terzioglu N, Gursoy A (2002) Synthesis and primary cytotoxicity evaluation of new 5-bromo-3-substituted hydrazono-1*H*-2-indolinones. Arch Pharm 335:374–380

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

Khan KM, Khan M, Ali M, Taha M, Rasheed S, Perveen S, Choudhary MI (2009) Synthesis of bis-Schiff bases of isatins and their antiglycation activity. Bioorg Med Chem 17:7795–7801

Krajewska B (2008) Mono-(Ag, Hg) and di-(Cu, Hg) valent metal ions effects on the activity of jack bean urease. Probing the modes of metal binding to the enzyme. J Enzym Inhib Med Chem 23(4):535–542

Krajewska B, Zaborska W (2007a) Double mode of inhibition–inducing interactions of 1,4-naphthoquinone with urease: arylation versus oxidation of enzyme thiols. Bioorg Med Chem 15:4144–4151

Krajewska B, Zaborska W (2007b) Jack bean urease: the effect of active-site binding inhibitors on the reactivity of enzyme thiol groups. Bioorg Chem 35:355–365

Krajewska B, Zaborska W, Chudy M (2004) Multi-step analysis of Hg^{2+} ion inhibition of jack bean urease. J Inorg Biochem 98: 1160–1168

Kuhler TC, Fryklund J, Bergman NA, Weilitz J, Lee A, Larsson H (1995) Structure–activity relationship of omeprazole and analogues as *Helicobacter pylori* urease inhibitors. J Med Chem 38:4906–4916

Laatsch H, Thomson RH, Cox PJ (1984) Spectroscopic properties of violacein and related compounds: crystal structure of tetramethyl violacein. J Chem Soc Perkin Trans 2:1331–1339

McLaughlin JL, Chang CJ, Smith DL (1991) Bench-top bioassays for the discovery of bioactive natural products: an update. In: Rahman AU (ed) Studies in natural products chemistry, vol 9. Structure and chemistry (Part B)Elsevier, Amsterdam, pp 383–409

Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nicholas DE, McLaughlin JL (1982) Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med 45:31–34

Mobley HLT, Island MD, Hausinger RP (1995) Molecular biology of microbial ureases. Microbiol Rev 59(3):451–480

Nagata K, Satoh H, Iwahi T, Shimoyama T, Tamura T (1993) Potent inhibitory action of the gastric proton pump inhibitor lansoprazole against urease activity of *Helicobacter pylori*: unique action selective for *H. pylori* cells. Antimicrob Agents Chemother 37:769–774

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

Nizamuddin N, Khan MH, Alauddin S, Haque R (1999) 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-trazino[5,6-*b*]indoles. Indian J Chem 38B:501–504

Omar AME, Eshba NH, Salama HM (1984) Synthesis of some substituted isatin-β-thiosemicarbazones and isatin-β-hydrazono-thiazoline derivatives as potential antiviral and antimicrobial agents. Arch Pharm 317:701–709

Pandeya SN, Sirram D, Nath G, DeClerck E (1999) Synthesis antibacterial, antifungal and anti-HIV activities of Shiff and Mannich bases derived from isatin derivatives and *N*-[4-(4′-chlorophenyl)thiazol-2yl] thiosemicarbazides. Eur J Pharm Sci 9:25–31

Pandeya SN, Smitha S, Jyoti M, Sridhar SK (2005) Biological activities of isatin and its derivatives. Acta Pharm 55:27–46

Patel A, Bari S, Talele G, Patel J, Sarangapani M (2006) Synthesis and antimicrobial activity of some new isatin derivatives. Iran J Pharm Res 4:249–254

Pervez H, Iqbal MS, Tahir MY, Choudhary MI, Khan KM (2007) Synthesis of some N^4-substituted isatin-3-thiosemicarbazones. Nat Prod Res 21:1178–1186

Pervez H, Iqbal MS, Tahir MY, Nasim FH, Choudhary MI, Khan KM (2008) In vitro cytotoxic, antibacterial, antifungal and urease inhibitory activities of some N^4-substituted isatin-3-thiosemicarbazones. J Enzym Inhib Med Chem 23:848–854

Pervez H, Chohan ZH, Ramzan M, Nasim FH, Khan KM (2009) Synthesis and biological evaluation of some new N^4-substituted isatin-3-thiosemicarbazones. J Enzym Inhib Med Chem 24: 437–446

Pervez H, Manzoor N, Yaqub M, Khan A, Khan KM, Nasim FH, Choudhary MI (2010) Synthesis and urease inhibitory properties of some new N^4-substituted 5-nitroisatin-3-thiosemicarbazones. Lett Drug Des Discov 7:102–108

Pervez H, Ramzan M, Yaqub M, Khan KM (2011a) Synthesis, cytotoxic and phytotoxic effects of some new N^4-aryl substituted isatin-3-thiosemicarbazones. Lett Drug Des Discov 8:452–458

Pervez H, Saira N, Iqbal MS, Yaqub M, Khan KM (2011b) Synthesis and toxicity evaluation of some N^4-aryl substituted 5-trifluoromethoxyisatin-3-thiosemicarbazones. Molecules 16(8):6408–6421

Pervez H, Manzoor N, Yaqub M, Khan A, Nasim FH, Khan KM (2012a) Synthesis and biological evaluation of some N^4-substituted 5-nitroisatin-3-thiosemicarbazones. Med Chem Res 21: 2251–2262

Pervez H, Ramzan M, Yaqub M, Nasim FH, Khan KM (2012b) Synthesis and biological evaluation of some new N^4-aryl substituted 5-chloroisatin-3-thiosemicarbazones. Med Chem 8(3):505–514

Petrov I, Grupce O, Stafilov T (1986) The nitrogen-hydrogen stretching region of some imides and thioimides. J Mol Struct 142:275–278

Pirrung MC, Pansare SV, Sarma KD, Keith KA, Kern ER (2005) Combinatorial optimization of isatin-β-thiosemicarbazones as anti-poxvirus agents. J Med Chem 48:3045–3050

Quenelle DC, Keith KA, Kern ER (2006) *In vitro* and in vivo evaluation of isatin-β-thiosemicarbazone and marboran against vaccinia and cowpox virus infections. Antivir Res 71:24–30

Ravichandran V, Mohan S, Kumar KS (2007) Synthesis and antimicrobial activity of Mannich bases of isatin and its derivatives with 2-[(2,6-dichlorophenyl)amino]phenylacetic acid. Arkivoc 14:51–57

Singh UK, Pandeya SN, Singh A, Srivastava BK, Pandey M (1010) 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 2:151–154

Smitha S, Pandeya SN, Stables JP, Ganapathy S (2008) Anticonvulsant and sedative-hypnotic activeties of *N*-acetyl/methyl isatin derivatives. Sci Pharm 76:621–636

Terzioglu N, Karali N, Gursoy A, Pannecouque C, Leysen P, Paeshuyse J, Neyts J, De Clercq E (2006) Synthesis and primary antiviral activity evaluation of 3-hydrazono-5-nitro-2-indolinone derivatives. Arkivoc 1:109–118

Vine KL, Matesic L, Locke JM, Ranson M, Skropeta D (2009) Cytotoxic and anticancer activities of isatin and its derivatives: a comprehensive review from 2000–2008. Anti Cancer Agents Med Chem 9:397–414

Weatherburn MW (1967) Phenol-hypochlorite reaction for determination of ammonia. Anal Chem 39:971–974

Zaborska W, Krajewska B, Kot M, Karcz W (2007) Quinone-induced inhibition of urease: elucidation of its mechanism by probing thiol groups of the enzyme. Bioorg Chem 35:233–242