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Synthesis, biological evaluation and docking studies of some novel isatin-3-hydrazonothiazolines

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Abstract

New series of thirty nine 5-trifluoromethoxy/fluoro/chloro-isatin 3-hydrazonothiazolines **5a-n, 6a-o** and **7a-j** were synthesized by cyclization of the corresponding intermediate N^4-aryl-substituted isatin-3-thiosemicarbazones **3** (prepared by condensation of appropriate isatin **1** with appropriate N^4-aryl-substituted 3-thiosemicarbazides **2**) with 4-chlorophenacyl bromide **4** in absolute ethanol or ethanol-benzene mixture and screened for their cytotoxicity, phytotoxicity, antifungal and urease inhibitory potential. All the synthesized compounds were found to be almost inactive in the brine shrimp (*Artemia salina*) bioassay, demonstrating IC_{50} values $>1.62 \times 10^{-4} - 2.17 \times 10^{-4}$ M. In phytotoxicity assay, out of thirty nine compounds tested, six i.e. **5i**, **6h**, **6i**, **6k**, **7c** and **7h** proved to be active, showing weak or non-significant (5-30%) activity at the highest tested concentration (500 µg/mL). Similarly, in antifungal assay, twenty six compounds i.e. **5a, 5b, 5d-f**, **5h-j**, **5m**, **6a**, **6b**, **6d**, **6j**, **6l-o, 7a, 7b** and **7d-j** were found to be active against one, two, or three selected fungal strains, exhibiting weak or non-significant inhibition (10-30%). Of these, **6d** and **6o** displayed relatively better activity profile in terms of the number of organisms inhibited. On the other hand, in urease inhibition bioassay, all the synthesized hydrazonothiazolines proved to be potent enzyme inhibitors, demonstrating inhibitory activity with IC_{50} values ranging from 3.70 ± 0.62 to 849 ± 2.26 µM.

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Compounds **5c, 5g-i, 5k, 5n, 6b, 6c, 6i, 6k, 6l, 6n, 6o, 7a, 7e, 7i** and **7j** were, however, found to be relatively very much potent, displaying outstanding enzymatic activity (IC_{50} = 3.70 ± 0.62 – 20.9 ± 0.57 μM) even better than the reference inhibitor, thiourea (IC_{50} = 22.3 ± 1.12 μM) and may thus act as valid leads for further studies. Molecular docking studies of the synthesized isatin-thiazolines **5a-n**, **6a-0** and **7a-j** were also carried out to elucidate their relationship with the binding pockets of the enzyme. This study offers the first example of exhibition of urease inhibitory potential by isatin-thiazolines and as such provides a solid basis for further research on these compounds to develop more potent antiurease compounds of medicinal /agricultural interest.

Keywords: Cytotoxicity, Hydrazones, Hydrazonothiazolines, Isatin, Phytotoxicity, Thiazoline, Urease inhibition, Urease inhibitors

Introduction

The literature survey indicates that isatin and its derivatives possess a wide spectrum of biological properties.[1-20] Among isatin derivatives, isatin-thiosemicarbazones are reported to possess diverse pharmacological activities, including antiulcer,[1] analgesic and anti-inflammatory,[1,7] anticancer,[1,4,7,12,21,22] antimicrobial,[1,2,3,5,7,22] antituberculosis,[23-25] antiviral,[1,2,5,7,12,25] and enzymatic inhibition.[1,4,11,17] Stimulated by this and as a part of our research work aiming to the synthesis of isatin-based organic compounds of medicinal/agricultural interest, we recently reported the synthesis of a number of 5-(un)-N^4-aryl-substituted isain-3-thiosemicarbazones as antimicrobial, cytotoxic, phytotoxic and more importantly antiurease compounds.[26-33] Investigation of the structure-activity relationship (SAR) studies in the synthesized compounds revealed that in certain cases, the type and position of different substituents about the phenyl ring attached to N^4 of the thiosemicarbazone part[26,27,29] and/or the presence of an inductively electron-withdrawing group (NO_2, F_3CO, F, or Cl) at position-5 of the isatin moiety[28,30-33] played a key role in the inducement and/ or increment of different activities. Moreover, it has been reported by some other authors that certain 5-bromoisatin-

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3-hydrazonothiazolines, prepared by cyclization of the respective thiosemicarbazones with phenacyl bromides, exhibited high cytotoxicity at low concentrations when tested for antiviral activity against MDCK cells. The presence of more than one lipophilic functions in these compounds was considered responsible for such a display of activity.[34] Likewise, 5-bromo isatin-derived 3-hydrazonothiazolidinones, obtained by cyclization of the corresponding thiosemicarbazones with ethyl 2-bromopropionate, have been found to show favourable cytotoxicity.[21] Also, certain 5-fluoroisatin-derived 3-hydrazonothiazolidinones, obtained by condensation of 5-fluoroisatin with different thiazolidin-4-ones, have been claimed to possess antimicrobial activity.[35] In view of these points, it was envisioned that the simultaneous presence of different lipophilic groups at position-5 of the isatin scaffold and at the N atom of thiazoline moiety in the isatin-thiazoline hybrids would result in an increase in the selected activities. Thus, the present work to synthesize three new series of thirty nine title hydrazonothiazolines (derived by cyclization of avariety of N^4-aryl-substituted 5-trifluoromethoxy/fluoro/chloro-isatin 3-thiosemicarbazones with 4-chlorophenacyl bromide) and screen them for their cytotoxicity, phytotoxicity, antifungal and specially urease inhibitory potential was accomplished. It is pertinent to mention here that such a category of isatin derivatives has been scarcely investigated before by other researchers for their antiurease potential. The work presented herein illustrates the influences of the type of aryl groups (modified by placing one, two, or three functionalities on the phenyl ring) attached to N atom of the thiazoline moiety as well as the presence of inductively electron-attracting/lipophilic trifluoromethoxy, fluoro and chloro groups at position-5 of the isatin part on the cytotoxic, phytotoxic, antifungal and urease inhibitory properties of the synthsized isatin-thiazoline hybrids. This study furnishes some motivating and attention-grabbing results, which are reported herein.

Results and discussion

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The present study describes the synthesis and *in vitro* evaluation of cytotoxic, phytotoxic, antifungal and urease inhibitory potential of thirty seven currently and two previously reported[36,37] isatin-hydrazonothiazolines **5a-n**, **6a-d**, **6f-h**, **6j-o**, **7a-j** and **6e**, **6i**, respectively.

Chemistry of compounds 5a-n, 6a-o and 7a-j

The designed isatin-hydrazonothiazolines **5-7** were prepared in 73-91% yield from 5-substituted isatins **1** by first reacting them with equimolar quantities of appropriate N^4-substituted 3-thiosemicarbazides **2** in aqueous ethanol containing a catalytic amount of glacial acetic acid, and then by treating the resultant intermediate thiosemicarbazones **3** with 4-chlorophenacyl bromide **4** in absolute ethanol or ethanol-benzene mixture at reflux temperature (Scheme 1).

The structures of the synthesized isatin-3-hydrazonothiazolines **5a-n, 6a-o** and **7a-j** were established on the basis of elemental (CHN) and spectral (IR, ^{1}HNMR, EIMS) analyses. Satisfactory elemental analyses were obtained for all the compounds synthesized in this study. Also, the spectral data were in ageement with the relevant literature.[21,34,38] The IR spectra of isatin-thiazolines **5a-n, 6a-o** and **7a-j** showed a single band resulting from NH stretching of indole in the 3261-3008 cm^{-1} region. The lactam C=O and azomethine C=N were observed in the 1739-1693 and 1631-1558 cm^{-1} regions, respectively.[21,34,38] The ^{1}H-NMR spectra of **5a-n, 6a-o** and **7a-j** did not show the down field signals of thiosemicarbazone N^4-H and N^2-H,and displayed one or two separate singlets at δ 10.39-10.75 and δ 10.35-10.77, respectively, for indole NH. The thiazoline ring C_5-H resonated either as a singlet at δ 7.01-7.22 or two separate singlets at δ 6.90-7.12 or a multiplet at δ 6.95-7.13.[21,34,38] Furthermore, the ^{1}H-NMR spectra of compounds **6c, 6d, 6f, 6o, 7a, 7c, 7d** and **7f-j** exhibited duplicate signals, confirming the presence of two isomers. It is suggested that in these compounds, the restricted rotation about the azomethine (C=N) linkage and the partial double bond character of the lactam C-N bond, induced by delocalization of the nitrogen lone pair of electrons onto the carbonyl oxygen, led to the formation of E and Z isomers.[21,38] The EI mass spectra of **5a-n, 6a-o** and **7a-j** showed

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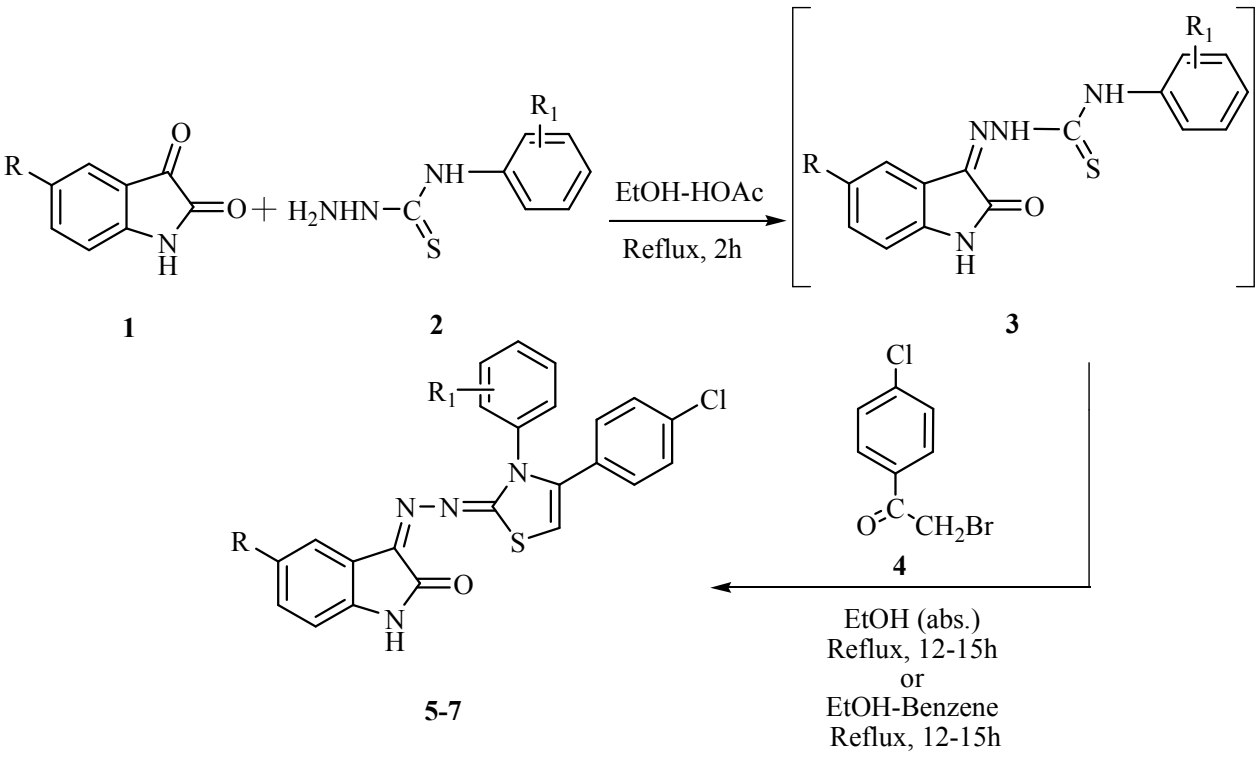
molecular ions of different intensity peculiar to the isatin and thiazoline moieties. The key fragmentation pathways involved the N-N, N-C, and N-CO bonds fission.[38] The proposed fragmentation patterns of compounds **5c, 6g** and **7f**, representing each series of isatin-thiazoline hybrids, are illustrated in Figures 1, 2 and 3, respectively. X-ray structures of two representative examples **6e** and **6i** were determined in order to prove the allocated structures and to substantiate conformations of the synthesized isatin-hydrazonothiazolines. The related crystallographic data and refinement details of **6e** and **6i** have been reported somewhere else.[36,37]



1 **2** **3** **4** **5-7**

5a	R=OCF_3 ; R_1=2-CH_3	**5n**	R=OCF_3 ; R_1=2,4,6-$(Cl)_3$	**6m**	R=F ; R_1=2,5-$(Cl)_2$
5b	R=OCF_3 ; R_1=3-CH_3	**6a**	R=F ; R_1=2-CH_3	**6n**	R=F ; R_1=2,6-$(Cl)_2$
5c	R=OCF_3 ; R_1=4- CH_3	**6b**	R=F ; R_1=3-CH_3	**60**	R=F ; R_1=2,4,6-$(Cl)_3$
5d	R=OCF_3 ; R_1=2-O CH_3	**6c**	R=F ; R_1=4-CH_3	**7a**	R=Cl ; R_1=2-CH_3
5e	R=OCF_3 ; R_1=3-OCH_3	**6d**	R=F ; R_1=2-OCH_3	**7b**	R=Cl ; R_1=3-CH_3
5f	R=OCF_3 ; R_1=4-OCH_3	**6e**	R=F ; R_1=3-OCH_3	**7c**	R=Cl ; R_1=4-CH_3
5g	R=OCF_3 ; R_1=2-F	**6f**	R=F ; R_1=4-OCH_3	**7d**	R=Cl ; R_1=2-OCH_3

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5h	R=OCF_3 ; R_1=3-F	**6g**	R=F ; R_1=2-F	**7e**	R=Cl ; R_1=3-OCH_3
5i	R=OCF_3 ; R_1=4-F	**6h**	R=F ; R_1=3-F	**7f**	R=Cl ; R_1=4-OCH_3
5j	R=OCF_3 ; R_1=2,4-$(Cl)_2$	**6i**	R=F ; R_1=4-F	**7g**	R=Cl ; R_1=2-F
5k	R=OCF_3 ; R_1=2,5-$(Cl)_2$	**6j**	R=F ; R_1=2,4-$(F)_2$	**7h**	R=Cl ; R_1=3-F
5l	R=OCF_3 ; R_1=2,6-$(Cl)_2$	**6k**	R=F ; R_1=2,6-$(F)_2$	**7i**	R=Cl ; R_1=4-F
5m	R=OCF_3 ; R_1=3,4-$(Cl)_2$	**6l**	R=F ; R_1=2,4-$(Cl)_2$	**7j**	R=Cl ; R_1=2,4-$(F)_2$

Scheme 1. Synthesis of title hydrazonothiazolines **5a-n, 6a-o** and **7a-j**.

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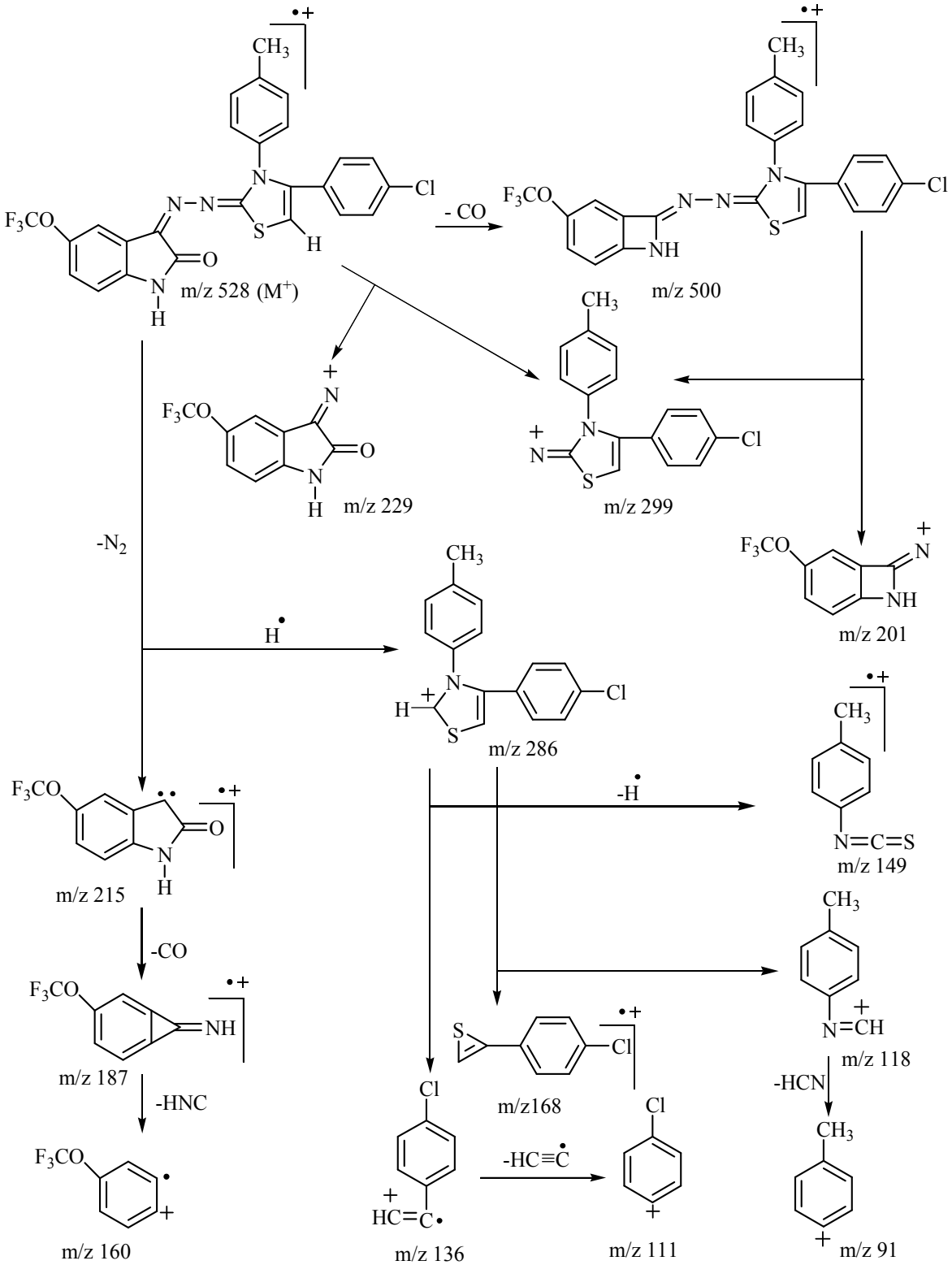

Figure 1. The proposed fragmentation pattern of compound **5c.**

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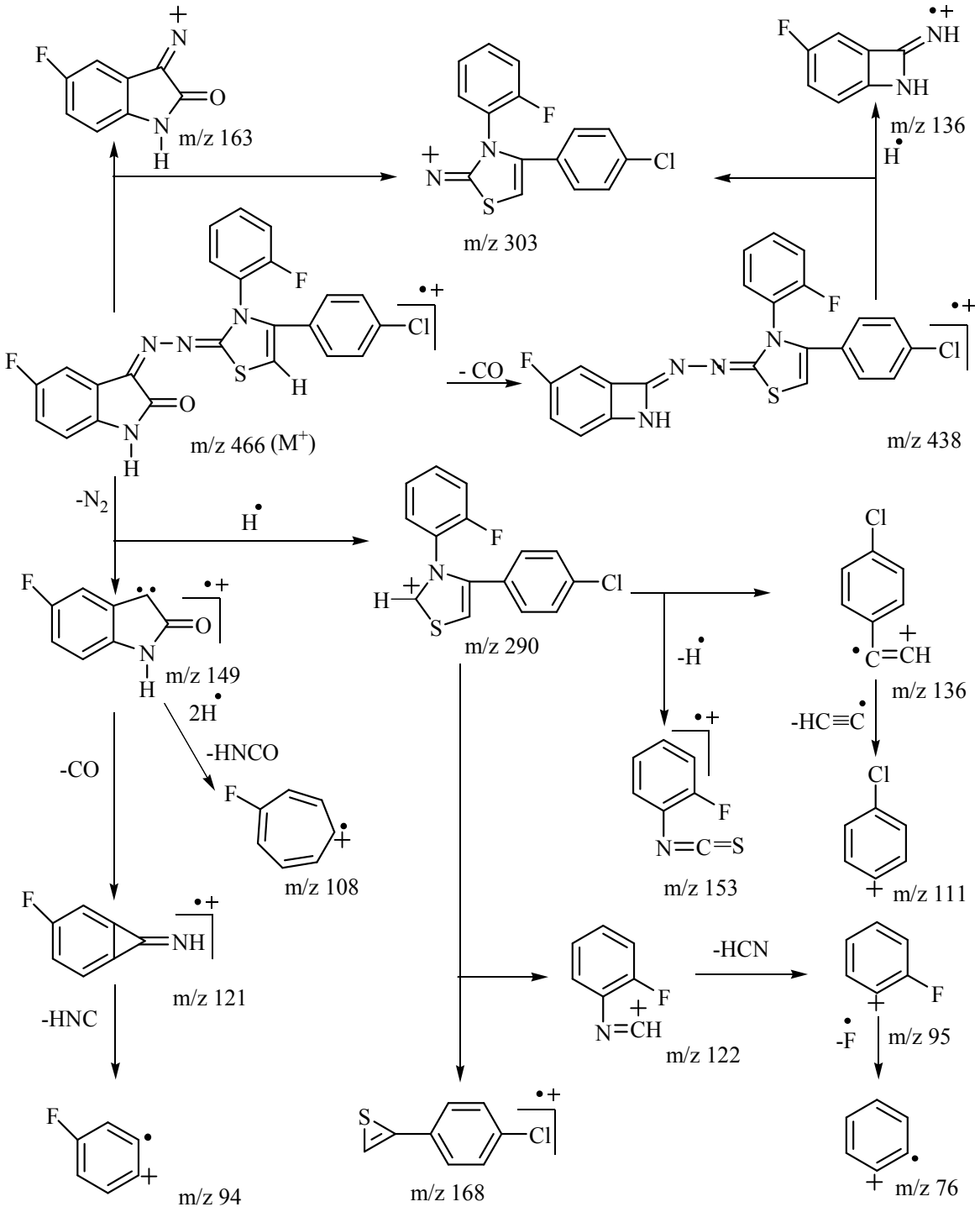

Figure 2. The proposed fragmentation pattern of compound **6g.**

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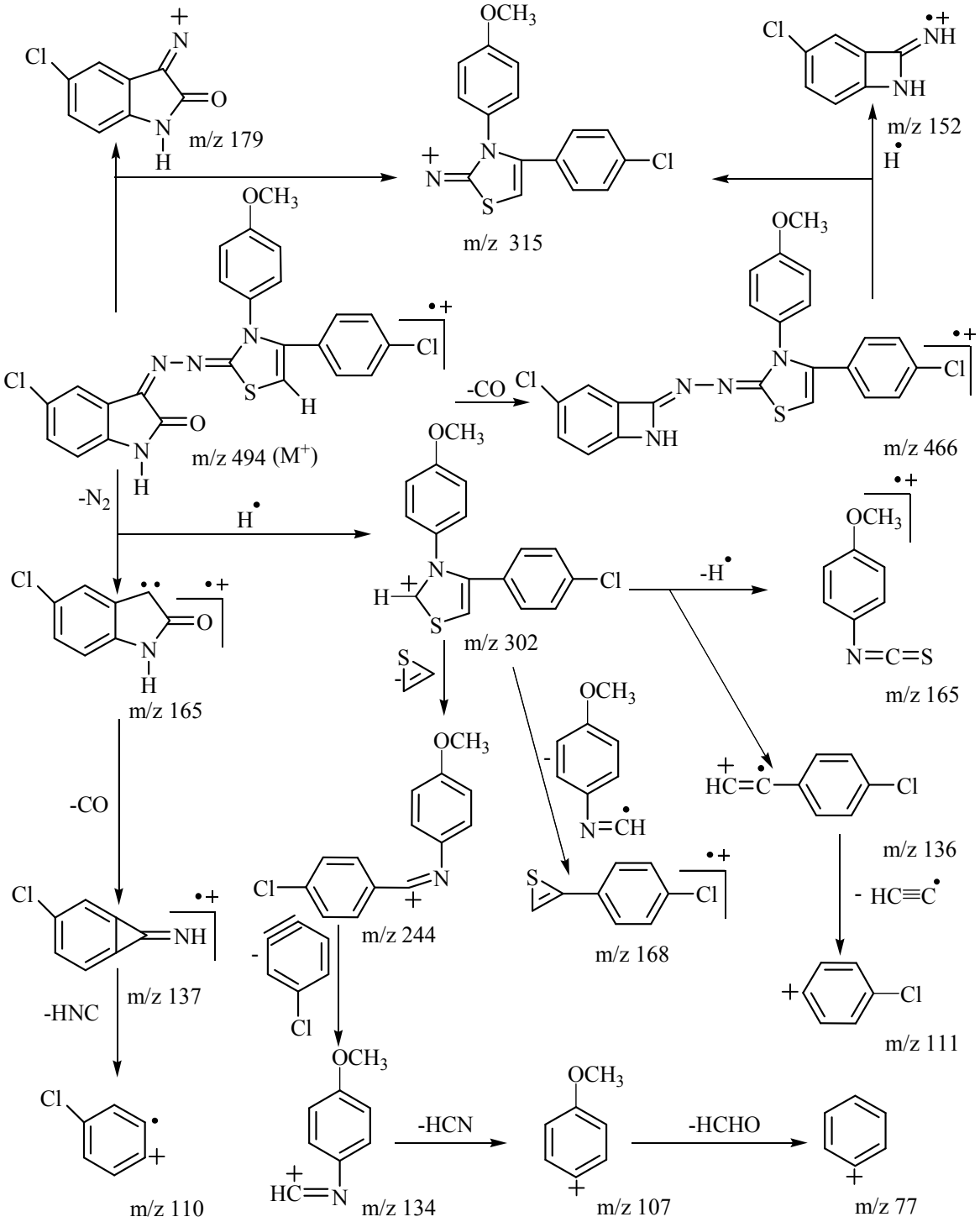

Figure 3. The proposed fragmentation pattern of compound **7f.**

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Biology

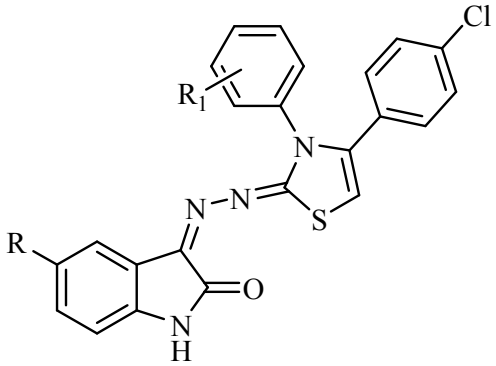
Cytotoxicity (*in vitro*) of compounds 5a-n, 6a-o and 7a-j

The synthesized isatin-3-hydrazonothiazolines **5a-n, 6a-o** and **7a-j** were screened for their cytotoxic effects by a brine shrimp (*Artemia salina*) lethality bioassay using etoposide, a standard anticancer drug as a reference point for comparison to the trial compounds. All the compounds of this series gave LD_{50} values >1.62 x 10^{-4} − 2.17 x 10^{-4} M in the present assay when tested at 100, 10 and 1 µg/mL concentrations and thus can be considered to be almost inactive.

Phytotoxicity (*in vitro*) of compounds 5a-n, 6a-o and 7a-j

The synthetic compounds **5a-n, 6a-o** and **7a-j** were further screened for their phytotoxicity potential at 500, 50 and 5 µg/mL concentrations. Out of thirty nine compounds tested, only six i.e. **5i**, **6h**, **6i**, **6k**, **7c** and **7h** appeared to be active, exhibiting weak or non-significant (5-30%) plant growth inhibition at the highest tested concentration (500 µg/mL) compared to the standard drug, paraquat, which displayed 100% inhibition at 0.015 µg/mL concentration (Table 1).

Table 1. Growth inhibition of *Lemna aequinocitalis* by compound **5a-n, 6a-o** and **7a-j*, **** at different concentrations



Compound	R	R_1	% Growth inhibition		
			500 µg/mL	50 µg/mL	5 µg/mL
5i	OCF_3	4-F	05	00	00

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6h	F	3-F	05	00	00
6i	F	4-F	05	00	00
6k	F	2,6-$(F)_2$	15	00	00
7c	Cl	4-CH_3	18	00	00
7h	Cl	3-F	30	00	00

*The reference compound, paraquat, shows 100% growth inhibition at 0.015 µg/mL

Antifungal activity (*in vitro*) of compounds 5a-n, 6a-o and 7a-j

Antifungal activity of the synthesized isatin-hydrazonothiazolines **5a-n, 6a-o** and **7a-j** was determined against five fungal cultures i.e. *Candida albicans, Aspergillus flavus, Microsporum canis, Fusarium solani* and *Candida glabrata* at 200 µg /mL in DMSO (Table 2). Of the thirty nine compounds tested, twenty six i.e. **5a, 5b, 5d-f**, **5h-j**, **5m**, **6a**, **6b**, **6d**, **6j**, **6l-o, 7a, 7b** and **7d-j** were found to be active against one, two, or three selected pathogens, displaying varied but low or non-significant inhibition (10-30%). Among these, **6d** and **6o** bearing fluoro group at position-5 of the isatin scaffold and methoxy and chloro functions, respectively, at positions-2 (*ortho*) and -2,4,6 (*ortho, para, ortho*) of the phenyl ring attached to N atom of the thiazoline moiety exhibited relatively better activity profile in terms of the number of organisms affected.

Table 2. Antifungal activity (*in vitro*) of compounds **5a-n, 6a-o** and **7a-j*, ** ** (% inhibition)

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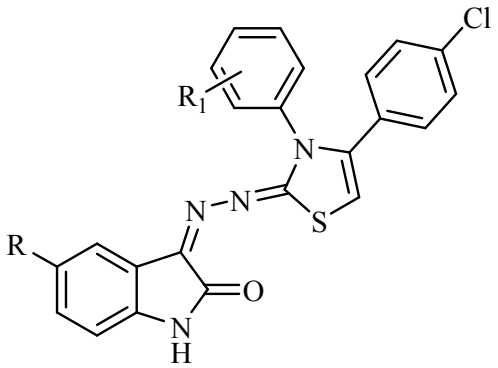

Compound	R	R_1	Microbial species				
			C. albicans	*A. flavus*	*M. canis*	*F. solani*	*C. glabrata*
5a	OCF_3	2-CH_3	00	00	20	00	00
5b	OCF_3	3-CH_3	00	00	20	25	00
5d	OCF_3	2-OCH_3	00	00	00	10	00
5e	OCF_3	3-OCH_3	00	00	20	00	00
5f	OCF_3	4-OCH_3	00	00	00	10	00
5h	OCF_3	3-F	00	00	25	00	00
5i	OCF_3	4-F	00	00	00	30	00
5j	OCF_3	2,4-$(Cl)_2$	00	00	00	25	00
5m	OCF_3	3,4-$(Cl)_2$	00	00	00	10	00
6a	F	2-CH_3	00	00	00	25	00
6b	F	3-CH_3	00	00	20	00	00
6d	F	2-OCH_3	00	20	20	20	00
6j	F	2,4-$(F)_2$	00	00	10	00	00
6l	F	2,4-$(Cl)_2$	00	20	30	00	00
6m	F	2,5-$(Cl)_2$	00	00	10	00	00
6n	F	2,6-$(Cl)_2$	00	00	30	00	00
6o	F	2,4,6-$(Cl)_3$	00	10	20	25	00
7a	Cl	2-CH_3	00	00	20	00	00
7b	Cl	3-CH_3	00	00	20	00	00
7d	Cl	2-OCH_3	00	20	00	00	00
7e	Cl	3-OCH_3	00	00	20	00	00
7f	Cl	4-OCH_3	00	00	25	00	00
7g	Cl	2-F	00	00	25	00	00
7h	Cl	3-F	00	00	20	00	00
7i	Cl	4-F	00	00	25	00	00
7j	Cl	2,4-$(F)_2$	00	00	00	20	00
Standard Drug**			A	B	C	D	E

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*Concentration used 200 µg/mL; ** Standard drugs (MIC in µg/mL): A = Miconazole (110.8 µg/mL); B= Amphotericin B (20 µg/mL); C = Miconazole (98.4 µg/mL); D = Miconazole (73.25 µg/mL); E= Miconazole (110.8 µg/mL); 00: absence of measurable inhibitory action

Urease inhibition (*in vitro*) of compounds 5a-n, 6a-o and 7a-j

The synthesized 5-trifluoromethoxy/fluoro/chloro-isatin 3-hydrazonothiazolines **5a-n**, **6a-o** and **7a-j**, respectively, were tested for their antiurease activity against Jack bean urease. Thiourea served as reference standard in this assay. All the compounds proved to be potent inhibitors of the enzyme, exhibiting inhibitory activity with IC_{50} values of 3.70 ± 0.62 – 849 ± 2.26 µM. Compounds **5c, 5g-i, 5k, 5n, 6b, 6c, 6i, 6k, 6l, 6n, 6o, 7a, 7e, 7i** and **7j** were, however, found to be relatively very much potent, displaying excellent activity (IC_{50} values 3.70 ± 0.62 – 20.9 ± 0.57 µM) even better than the reference inhibitor, thiourea (IC_{50} = 22.3 ± 1.12 µM) and may thus act as compelling leads for further studies. The remaining compounds i.e. **5a, 5b, 5d-f, 5j, 5l, 5m, 6a, 6d-h, 6j, 6m, 7b-d** and **7f-h** showed a varying degree of activity with IC_{50} values of 24.6 ± 2.14 – 849 ± 2.26 µM (Table 3).

The structure-activity relationship (SAR) studies in the case of 5-trifluoromethoxyisatin derivatives **5a-n** revealed that compound **5k** having chloro substituents at positions-2 and -5 of the phenyl ring attached to N atom of the thiazoline moiety was the most potent urease inhibitor of the series, demonstrating several fold more activity (IC_{50} = 3.70 ± 0.62 µM) than the reference inhibitor, thiourea (IC_{50} = 22.3 ± 1.12 µM). Comparison of the urease inhibitory potential of compound **5k** with that of closely related dichloro-substituted compounds **5j, 5l** and **5m** indicated that chloro substitution at positions-2,5 (*ortho ,meta*) of the phenyl ring was more favourable than at positions-2,4 (*ortho, para*), -2,6 (*ortho, ortho*) and -3,4 (*meta, para*), respectively. The dichloro–substituted derivatives **5j, 5l** and **5m** displayed relatively much lower activity (IC_{50} = 44.5 ± 3.15, 129 ± 5.91 and 134 ± 2.21 µM, respectively) in the present assay. This clearly showed that compound **5k** compared to **5j**, **5l** and **5m** interfered with the enzyme in a different fashion. Interestingly, the only trichloro–substituted compound tested in this assay i.e. **5n** bearing the substituents at positions-2,4,6

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(*ortho, para, ortho*) of the phenyl ring demonstrated much higher activity in contrast to the dichloro-substituted compounds **5j** and **5l** with the substituents at positions-2,4 (*ortho, para*) and -2,6 (*ortho, ortho*) of the phenyl ring, respectively (IC_{50} value 14.1 ± 1.31 vs. 44.5 ± 3.15 and 129 ± 5.91 μM). Chlorine is both electron-donating by mesomeric effect (+M) and electron-withdrawing by inductive effect (-I). Much higher enzyme inhibitory activity presented by compound **5n** in the present assay clearly indicated that the ultimate or overall electron-withdrawing effect of the three chloro functions increased its inhibitory potential, though not exclusively. The next most potent urease inhibitor was compound **5g** having a fluoro group at position-2 of the phenyl ring, showing inhibitory activity with IC_{50} value of 7.27 ± 0.33 μM. This compound was found to be three fold more active than the reference inhibitor, thiourea, but two fold less active than the most potent urease inhibitor **5k**. The other highly active monofluoro-substituted derivatives **5h** and **5i**, possessing the substituent at positions-3 and -4 of the phenyl ring exhibited enzymatic inhibition with IC_{50} values of 12.0 ± 0.83 and 11.7 ± 0.07 μM, respectively. This clearly indicated that compound **5g** in comparison to **5h** and **5i** meddled with the enzyme differently and more competently. The remaining relatively very much potent urease inhibitor was compound **5c** with a methyl substituent at position-4 of the phenyl ring attached to N atom of the thiazoline moiety. This compound was found to be two fold more active than the reference inhibitor (thiourea) but three times less active than the most potent urease inhibitor of the series i.e. compound **5k** (IC_{50} value 10.3 ± 0.51 vs. 22.3 ± 1.12 and 3.70 ± 0.62 μM, respectively). The other methyl-substituted compounds **5a** and **5b** bearing the substituent at positions-2 and -3 of the phenyl ring displayed relatively much lower inhibitory activity (IC_{50} values 336 ± 5.45 and 427 ± 2.89 μM, respectively). These results showed that steric hindrance played a significant role in reducing the inhibitory potential of the compounds.

Among 5-fluoroisatin derivatives **6a-o**, compounds **6i** and **6l** having fluoro and chloro substituents at positions-4 (*para*) and -2,4 (*ortho, para*) of the phenyl ring attached to N atom of the thiazoline moieties were the most potent urease inhibitors of the series, presenting excellent and almost the

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same inhibitory activity (IC_{50} values 8.20 ± 0.01 and 8.40 ± 0.23 μM, respectively). Comparison of the urease inhibitory potential of compound **6i** with that of closely related fluoro-substituted derivatives **6g** and **6h** showed that fluoro substitution at position-4 of the phenyl ring was more favourable than at positions-2 and -3. The fluoro-substituted compounds **6g** and **6h** exhibited relatively much lower activity (IC_{50} values 26.1 ± 1.11 and 32.6 ± 3.42 μM, respectively) in the present assay. Also, comparison of the antiurease potential of compound **6l** with that of **6m** and **6n** indicated that chloro substitution at positions-2,4 (*ortho, para*) was more favourable than at -2,5 (*ortho, meta*) and -2,6 (*ortho, ortho*). The dichloro-substituted derivative **6n** compared to **6l** displayed lower but still exciting activity (IC_{50} value 12.7 ± 0.62 vs. 8.40 ± 0.23 μM), while **6m** demonstrated relatively much lower activity with IC_{50} value of 33.1 ± 2.88 μM. Similarly, the trichloro-substituted compound **6o** having the chloro substituents at positions-2,4,6 (*ortho, para, ortho*) of the phenyl ring exhibited lower but still stimulating inhibitory activity (IC_{50} = 14.3 ± 0.12 μM) in comparison to the corresponding dichloro-substituted compounds **6l** and **6n**, showing enzyme inhibition with IC_{50} values of 8.40 ± 0.23 and 12.7 ± 0.62 μM, respectively. In case of difluoro-substituted compounds tested in this assay, compound **6k** bearing the fluoro substituents at positions-2,6 (*ortho, ortho*) of the phenyl ring was found to show much higher inhibitory potential compared to compound **6j** having the substituents at positions-2,4 (*ortho, para*) (IC_{50} value 11.0 ± 0.86 vs. 66.6 ± 5.40 μM). This indicated that compound **6k** in contrast to **6j** intermingled with the enzyme in a different manner, resulting into much marked enhancement in enzyme inhibitory potential. Amongst methyl-substituted compounds **6a-6c**, compound **6c** possessing the substituent at positions-4 of the phenyl ring was found to be the most active one, displaying enzyme inhibition with IC_{50} value of 11.4 ± 1.51 μM. The next most active derivative was **6b** with the substituent at position-3 of the ring, which showed inhibitory potential with IC_{50} value of 15.0 ± 1.92 μM. The remainder derivative **6a** having the substituent at position-2 of the phenyl ring exhibited much lower inhibitory activity with IC_{50} value of 275 ± 4.99 μM. This indicated that in case of **6a**, steric hindrance played a pivotal role

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in decreasing the inhibitory potential of the compound. The above results demonstrated that compounds **6g** and **6h** in contrast to **6i,** and **6m** in comparison to **6l**, **6n** and **6o,** interacted with the enzyme differently, resulting into reduction in the inhibitory potential to a smaller or greater extent.

The results given in the Table revealed that compared to compounds **5a-n** having trifluoromethoxy substituent at position-5 of the isatin part, substitution of fluoro group at the same position in the case of **6a-o** caused either a decrement or an increment in the enzymatic activity in certain cases. For example, compound **6c** having a methyl substituent at position-4 of the phenyl ring attached to N atom of the thiazoline moiety showed inhibition of the enzyme with IC_{50} value of 11.4 ± 1.51 μM, whereas the respective compound **5c** bearing trifluoromethoxy function at position-5 of the isatin moiety displayed more inhibitory activity (IC_{50} = 10.3 ± 0.51 μM). Similarly, compounds **6f-h** possessing methoxy and fluoro substituents at positions -4 and -2, -3 of the phenyl ring, respectively, were found to demonstrate reduced activity (IC_{50} values 849 ± 2.26, 26.1 ± 1.11 and 32.6 ± 3.42 μM) in comparison to the corresponding 5-trifluoromethoxyisatin derivatives **5f-h**, which exhibited inhibitory activity with IC_{50} values of 102 ± 5.82, 7.27 ± 0.33 and 12.0 ± 0.83 μM, respectively. Also, compound **6m** possessing chloro functions at positions -2 and -5 of the phenyl ring was found to show decreased activity (IC_{50} = 33.1 ± 2.88 μM) when compared with the respective compound **5k** having trifluoromethoxy substituent at position-5 of the isatin moiety, which displayed enzyme inhibition with IC_{50} value 3.70 ± 0.62 μM. Much marked decrement in enzyme inhibitory activity was found to occur in case of compounds **6g**, **6h** and **6m**, respectively, when compared with the corresponding 5-trifuoromethoxyisatin-derived hydrazonothiazolines **5g, 5h** and **5k** (IC_{50} values 7.27 ± 0.33 → 26.1 ± 1.11 μM, 12.0 ± 0.83 → 32.6 ± 3.42 μM and 3.70 ± 0.62 → 33.1 ± 2.88 μM, respectively). On the contrary, compounds **6a** and **6b** bearing methyl function at positions -2 and -3 of the phenyl ring, respectively, displayed increased inhibitory activity (IC_{50} values 275 ± 4.99 and 15.0 ± 1.92 μM, respectively) in contrast to the respective compounds **5a** and **5b**, showing enzyme inhibition with IC_{50} values of 336 ± 5.45 and 427 ± 2.89 μM. Similarly, compounds **6d** and **6e**

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having methoxy substituent at positions-2 and -3 of the phenyl ring displayed increased enzyme inhibition with IC_{50} values 40.1 ± 5.32 and 29.3 ± 1.17 μM, respectively, when compared with the corresponding compounds **5d** and **5e** bearing trifluoromethoxy group at position-5 of the isatin scaffold, demonstrating inhibition of the enzyme with IC_{50} values of 42.9 ± 4.67 and 42.6 ± 1.40 μM. Furthermore, the monofluoro-substituted derivative **6i** possessing the substituent at position-4 of the phenyl ring showed enhanced enzyme inhibition with IC_{50} value of 8.20 ± 0.01 μM in comparison to the corresponding 5-trifluoromethoxyisatin-derived hydrazonothiazoline **5i**, which displayed inhibitory activity with IC_{50} value of 11.7 ± 0.07 μM. Also, the dichloro-substituted compounds **6l** and **6n** having the substituents at positions-2,4 (*ortho, para*) and -2,6 (*ortho, ortho*) exhibited enhanced enzyme inhibition (IC_{50} values 8.40 ± 0.23 and 12.7 ± 0.62 μM, respectively) in contrast to the respective compounds **5j** and **5l** bearing trifluoromethoxy residue at position-5 of the isatin moiety, demonstrating inhibitory activity with IC_{50} values of 44.5 ± 3.15 and 129 ± 5.91 μM. Relatively, marked increment in enzyme inhibitory activity (IC_{50} values 427 ± 2.89 → 15.0 ± 1.92 μM, 44.5 ± 3.15 → 8.40 ± 0.23 μM and 129 ± 5.91 → 12.7 ± 0.62 μM, respectively) occurred in case of compounds **6b, 6l** and **6n**. Among the remaining compounds of the series i.e. **6j** and **6k**, compound **6j** having fluoro substituents at positions-2 and -4 of the phenyl ring exhibited inhibitory activity with IC_{50} value of 66.6 ± 5.40 μM. In contrast, **6k** possessing the same substituents at positions -2 and -6 of the phenyl ring showed much enhanced activity (IC_{50} = 11.0 ± 0.86 μM). These results showed that the presence of fluoro group (exercising both –ve inductive and +ve mesomeric effects) at position-5 of the isatin scaffold as well as the nature, number and position of different functions present in the phenyl ring caused the molecules to meddle with the enzyme in a different fashion.

In case of 5-chloroisatin-derived hydrazonothiazolines **7a-j**, **7a** bearing methyl group at position-2 of the phenyl ring attached to N atom of the thiazoline moiety was found to be the most potent antiurease compound, showing enzymatic activity (IC_{50} = 9.20 ± 0.77 μM) much higher than the

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reference inhibitor, thiourea (IC_{50} = 22.3 ± 1.12 μM). Comparison of the antiurease activity of compound **7a** with that of closely related methyl-substituted compounds **7b** and **7c** having the substituent at positions-3 and -4 of the phenyl ring, respectively, revealed that methyl substitution at position-2 of the phenyl ring was more favourable than at -3 and -4. Compound **7b** was found to be > two fold less active than **7a** but slightly more active than **7c** (IC_{50} value 24.6 ± 2.14 vs. 9.20 ± 0.77 and 25.3 ± 3.20 μM, respectively). This showed that compounds **7b** and **7c** compared to **7a** interfered with the enzyme much less efficiently, resulting in a decrement in their inhibitory potential. The next most potent antiurease compound was **7e** possessing methoxy substituent at position-3 of the phenyl ring. This compound exhibited slightly less inhibitory activity (IC_{50} =10.3 ± 0.38 μM) than the most potent inhibitor **7a** (IC_{50} = 9.20 ± 0.77 μM) but much more than the reference inhibitor, thiourea (IC_{50} = 22.3 ± 1.12 μM). The other potent methoxy-substituted compounds **7d** and **7f** having the substituent at position-2 and -4 of the phenyl ring attached to N atom of the thiazoline moiety, however, displayed relatively much lower activity with IC_{50} values of 38.6 ± 1.50 μM and 81.3 ± 7.99 μM, respectively. This clearly demonstrated that compound **7e** intermingled with the enzyme differently and much more competently. The remaining relatively much more potent urease inhibitor was compound **7i** bearing a fluoro substituent at position-4 of the phenyl ring. This compound was found to be slightly less active than the most potent urease inhibitor of the series i.e. compound **7a** but two fold more active than the reference inhibitor, thiourea (IC_{50} value 11.3 ± 0.32 vs. 9.20 ± 0.77 and 22.3 ± 1.12 μM, respectively). The other fluoro-substituted compound **7g** possessing the substituent at position-2 of the phenyl ring

Table 3. Inhibition of Jack been urease by compounds **5a-n, 6a-o** and **7a-j**

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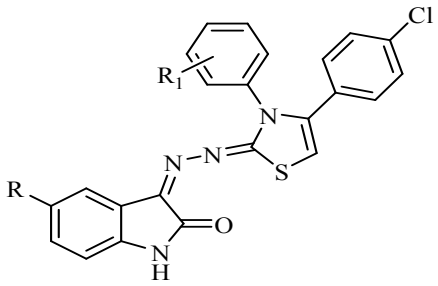

Compound	R	R_1	IC_{50} ± SEM (μM)	Compound	R	R_1	IC_{50} ± SEM (μM)
5a	OCF_3	2-CH_3	336 ± 5.45	**6g**	F	2-F	26.1 ± 1.11
5b	OCF_3	3-CH_3	427 ± 2.89	**6h**	F	3-F	32.6 ± 3.42
5c	OCF_3	4-CH_3	10.3 ± 0.51	**6i**	F	4-F	8.20 ± 0.01
5d	OCF_3	2-OCH_3	42.9 ± 4.67	**6j**	F	2,4-$(F)_2$	66.6 ± 5.40
5e	OCF_3	3-OCH_3	42.6 ± 1.40	**6k**	F	2,6-$(F)_2$	11.0 ± 0.86
5f	OCF_3	4-OCH_3	102 ± 5.82	**6l**	F	2,4-$(Cl)_2$	8.40 ± 0.23
5g	OCF_3	2-F	7.27 ± 0.33	**6m**	F	2,5-$(Cl)_2$	33.1 ± 2.88
5h	OCF_3	3-F	12.0 ± 0.83	**6n**	F	2,6-$(Cl)_2$	12.7 ± 0.62
5i	OCF_3	4-F	11.7 ± 0.07	**6o**	F	2,4,6-$(Cl)_3$	14.3 ± 0.12
5j	OCF_3	2,4-$(Cl)_2$	44.5 ± 3.15	**7a**	Cl	2-CH_3	9.20 ± 0.77
5k	OCF_3	2,5-$(Cl)_2$	3.70 ± 0.62	**7b**	Cl	3-CH_3	24.6 ± 2.14
5l	OCF_3	2,6-$(Cl)_2$	129 ± 5.91	**7c**	Cl	4-CH_3	25.3 ± 3.20
5m	OCF_3	3,4-$(Cl)_2$	134 ± 2.21	**7d**	Cl	2-OCH_3	38.6 ± 1.50
5n	OCF_3	2,4,6-$(Cl)_3$	14.1 ± 1.31	**7e**	Cl	3-OCH_3	10.3 ± 0.38
6a	F	2-CH_3	275 ± 4.99	**7f**	Cl	4-OCH_3	81.3 ± 7.99
6b	F	3-CH_3	15.0 ± 1.92	**7g**	Cl	2-F	25.6 ± 2.91
6c	F	4-CH_3	11.4 ± 1.51	**7h**	Cl	3-F	32.0 ± 0.69
6d	F	2-OCH_3	40.1 ± 5.32	**7i**	Cl	4-F	11.3 ± 0.32
6e	F	3-OCH_3	29.3 ± 1.17	**7j**	Cl	2,4-$(F)_2$	20.9 ± 0.57
6f	F	4-OCH_3	849 ± 2.26	**Thiourea***			22.3 ± 1.12

*Reference inhibitor of urease enzyme

showed inhibitory activity with IC_{50} value of 25.6 ± 2.91 μM. On the contrary, compound **7h** with the fluoro substituent at position-3 of the phenyl ring displayed reduced enzymatic activity with IC_{50} value of 32.0 ± 0.69 μM. Finally, the only difluoro-substituted compound tested in the present assay

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i.e. **7j** having fluoro substituents at positions-2,4 (*ortho, para*) of the phenyl ring, though found to be markedly less active than the monofluoro-substituted compound **7i** (20.9 ± 0.57 vs. 11.3 ± 0.32 μM), but was still more active than the monofluoro-substituted compound **7g** (IC_{50} = 25.6 ± 2.91 μM) as well as the reference inhibitor, thiourea (IC_{50} = 22.3 ± 1.12 μM). Fluorine is both strongly electron-attracting by inductive effect (-I) and electron-donating by mesomeric effect (+M). Noticeably higher inhibitory activity shown by compounds **7g**, **7i** and **7j** compared to **7h** in the present assay is attributed, though not exclusively, to the overall or ultimate electron-donating influences of the fluoro functions present at position-2 (*ortho*) and -4 (*para*) of the phenyl ring attached to N atom of the thiazoline moiety.

The results given in the Table further revealed that compared with the 5-fluoroisatin-derived thiazolines **6a-o**, thiazolines of this series exhibited either increased or decreased enzyme inhibition in certain cases. For example, compound **7a** possessing a methyl group at position-2 of the phenyl ring attached to N atom of the thiazoline moiety showed inhibitory activity with IC_{50} value 9.20 ± 0.77 μM, whereas the respective compound **6a** displayed enzymatic inhibition with IC_{50} value of 275 ± 4.99 μM. Similarly, compounds **7d-f** bearing methoxy functions at position-2, -3 and -4 of the phenyl ring, respectively, showed enhanced activity (IC_{50} values 38.6 ± 1.50, 10.3 ± 0.38 and 81.3 ± 7.99 μM) compared to the corresponding compounds **6d-f**, demonstrating enzyme inhibition with IC_{50} values of 40.1 ± 5.32, 29.3 ± 1.17 and 849 ± 2.26 μM, respectively. Also, the monofluoro-substituted compounds **7g** and **7h** having the substituent at position-2 and -3 of the phenyl ring, respectively, displayed enhanced activity (IC_{50} values 25.6 ± 2.91 and 32.0 ± 0.69 μM) in comparison to the respective compounds **6g** and **6h**, exhibiting enzyme inhibitory activity with IC_{50} values of 26.1 ± 1.11 and 32.6 ± 3.42 μM, respectively. Furthermore, the difluoro-substituted compound **7j** with the substituents at positions-2 and -4 of the phenyl ring showed increased activity (IC_{50} = 20.9 ± 0.57 μM) in contrast to the corresponding compound **6j**, displaying inhibition of the enzyme with IC_{50} value of 66.6 ± 5.40 μM. Marked increment was found to occur in case of

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compounds **7a, 7e, 7f** and **7j**. To the contrary, compounds **7b** and **7c** possessing the methyl substituent at position-3 and -4 of the phenyl ring, respectively, exhibited decreased enzyme inhibitory activity (IC_{50} values 24.6 ± 2.14 and 25.3 ± 3.20 μM) compared to the corresponding compounds **6b** and **6c**, showing activity with IC_{50} values of 15.0 ± 1.92 and 11.4 ± 1.51 μM. Also, compound **7i** bearing the fluoro function at position-4 of the phenyl ring showed reduced inhibitory activity (IC_{50} value 11.3 ± 0.32 μM) in contrast to the respective compound **6i**, exhibiting enzyme inhibition with IC_{50} value of 8.20 ± 0.01 μM. Relatively, pronounced reduction in the enzymatic activity was observed in the case of **7c**.

The above results showed that the simultaneous presence of varied inductively electron-withdrawing groups at position-5 of the isatin scaffold and the variously substituted aryl functions at N atom of the thiazoline moiety caused the isatin-thiazoline hybrids to meddle with the enzyme differently and sometimes relatively much more competently.

Molecular docking studies of compounds 5a-n, **6a-o and 7a-j**

Molecular docking studies of isatin-hydrazonothiazolines **5a-n**, **6a-o** and **7a-j** were performed using LeadIT to investigate the binding modes of inhibitors as well as interactions in the active site. The structures were docked to the crystallographic structure of Jack bean urease PDB ID: 3LA4 having bi-nickel center in its active site. After reproducing the co-crystallized reference ligand into the active site of receptor, all the derivatives were docked inside the active site. Most of the compounds were found interacting deep inside with the active site residues and showed coordinated interactions with the nickel ions in the active site of the receptor. The active site of the receptor contains the amino acids residues His409, Ala436, Arg439, Ala440, His492, Asp494, His519, His593, His594, Arg609, Ala636 and Met637.

Overall, the structures of the compounds are too large to be accommodated in the active pocket of the enzyme; therefore, some part lies within the bottom, while the rest interacts with the mid gorge

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area. The putative binding modes of the most active compounds among the three series i.e. **5a-n**, **6a-o** and **7a-j** are given below:

Binding mode analysis of compounds 5a-n, **6a-o and 7a-j**

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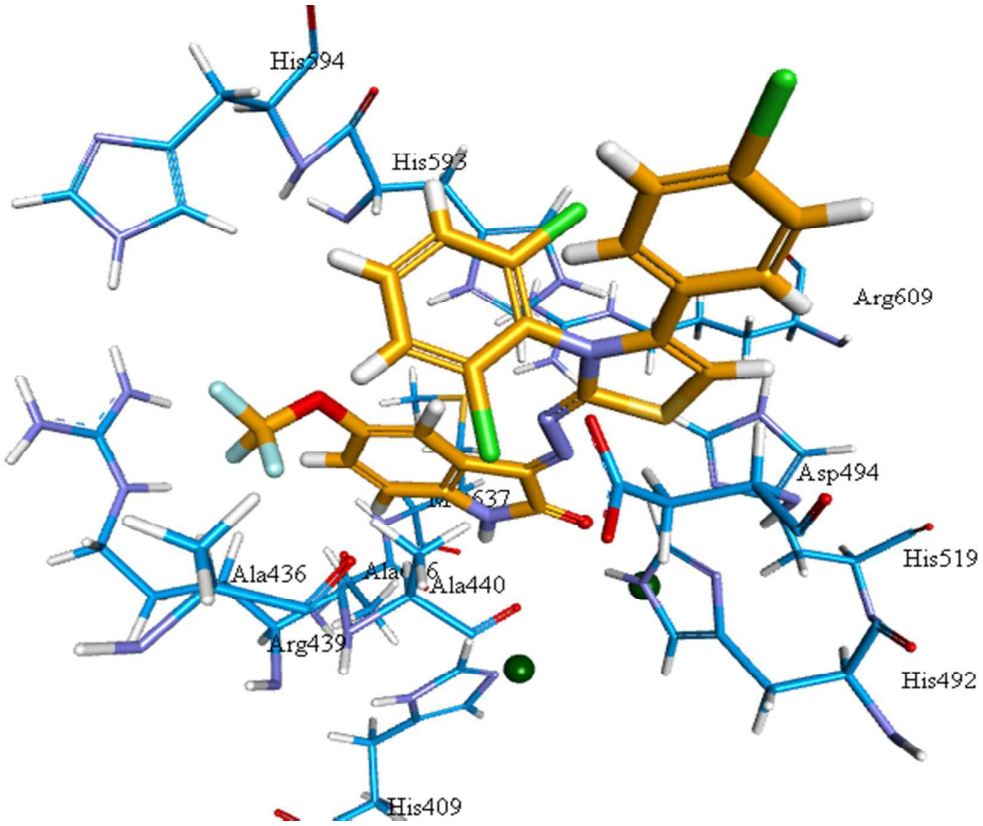
Among 5-trifluoromethoxyisatin-hydrazonothiazolines **5a-n**, **5k** was found to be the most potent inhibitor of Jack bean urease. It was directed deep towards the nickel ion present inside the active site of the receptor, as shown in Figure 4. Its 5-trifluoromethoxyisatin part was located towards bottom of the binding pocket, whereas the 4-chlorophenyl and 2,5-dichlorophenyl substituents attached to C and N atoms of the thiazoline moiety, respectively, were directed more towards entrance of the active site. In all the compounds studied, the 5-trifluoromethoxy isatin scaffold did not form direct bond with the Ni center. The 2,5-dichlorophenyl substituent attached to C atom of the thiazoline moiety showed π-π interactions with the imidazole ring of His593. Additionally, non-covalent molecular interactions were found between 5-trifluoromethoxy isatin moiety and Arg609 in the active site, while the hydrogen bonding was noticed between imidazole ring of His492 and the



carbonyl-O of the 5-trifluoromethoxy isatin part of compound **5k**. Figure 4 reports a prioritized binding pose of the potent inhibitor **5k**, and it is selected after Hyde assessment and visualization.

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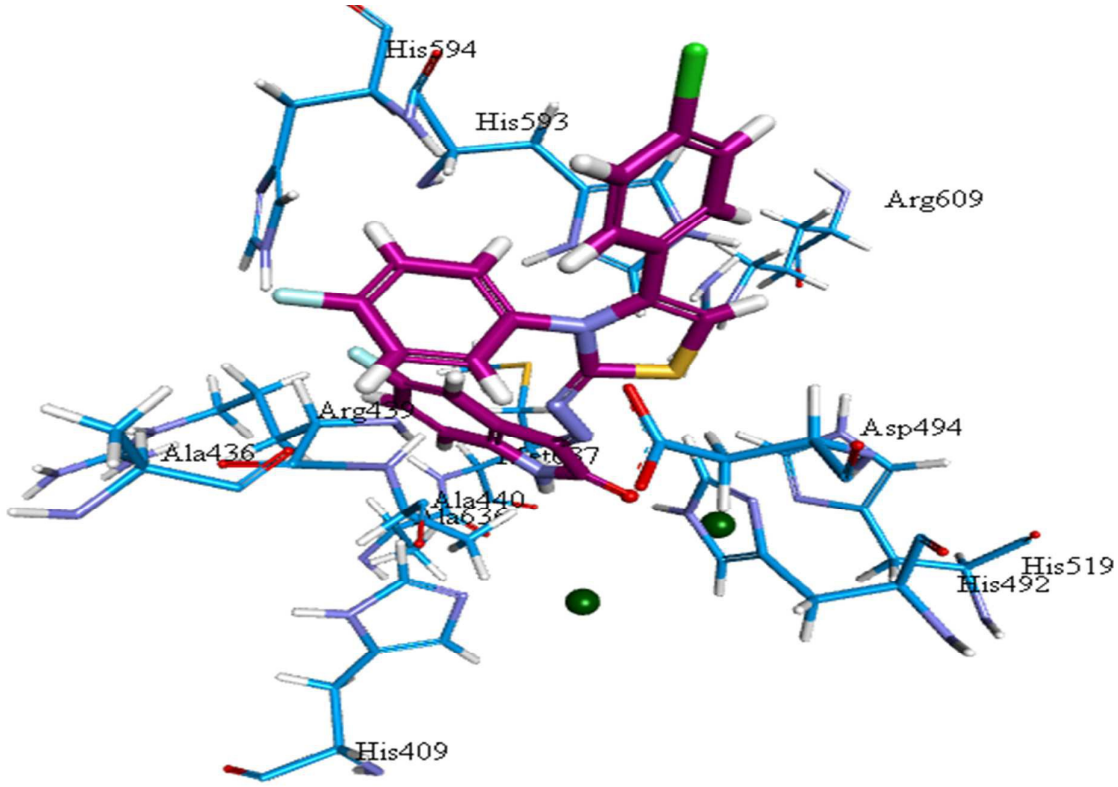
Figure 4. The putative binding mode of compound **5k** bound to the active site of Jack bean urease. Carbon atoms of the compound are golden coloured and that of protein are blue. Dark green spheres are two nickel ions.

Binding mode analysis of compounds 6a-o

As evident from the *in vitro* results, amongst 5-fluoroisatin-hydrazonothiazolines **6a-o**, **6i** was the most active inhibitor of Jack bean urease. The docking studies revealed that it adopts best orientation inside the active pocket by forming hydrogen bonding with the amino acid residues of His492, His593 and His594. The 5-fluoroisatin part of the compound was directed into the pocket towards the nickel ions. Furthermore, the 4-chlorophenyl substituent attached to C and 4-fluorophenyl one to N atoms of the thiazoline moiety was aligned towards His594 and Asp494, respectively. Additionally, π-charge interactions were found with amino acid Asp494, which stabilized the pose. The thiazoline part of the compound showed π-π interactions with the imidazole ring of His594. The putative binding pose of compound **6i** was selected after Hyde assessment and visualization, and is



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depicted in Figure 5.

Figure 5. The putative binding mode of compound **6i** bound to the active site of Jack bean urease. Carbon atoms of the compound are magenta coloured and that of protein are blue. Dark green spheres are two nickel ions.

Binding mode analysis of compounds 7a-j

Docking studies supported the experimental results that potent inhibitors inhibit the catalytic site of the enzyme. The docking studies revealed the stable hydrogen bonding interactions between the oxygen and hydrogen atoms of the inhibitors and the amino acid residues Arg439, His593 and Arg609. Figure 6 reports the prioritized binding mode of the most potent inhibitor **7a;** the pose was selected after Hyde assessment and visualization of 30 lowest energy docked conformations. The 5-chloroisatin part of the molecule was oriented towards amino acids His409, Ala436, Arg439, Ala440 and His593 where π-π interactions were observed with His593 in the active pocket; the 4-chlorophenyl and 2-methylphenyl substituents attached to C and N atoms of the thiazoline moiety, respectively, were inclined towards Asp494.

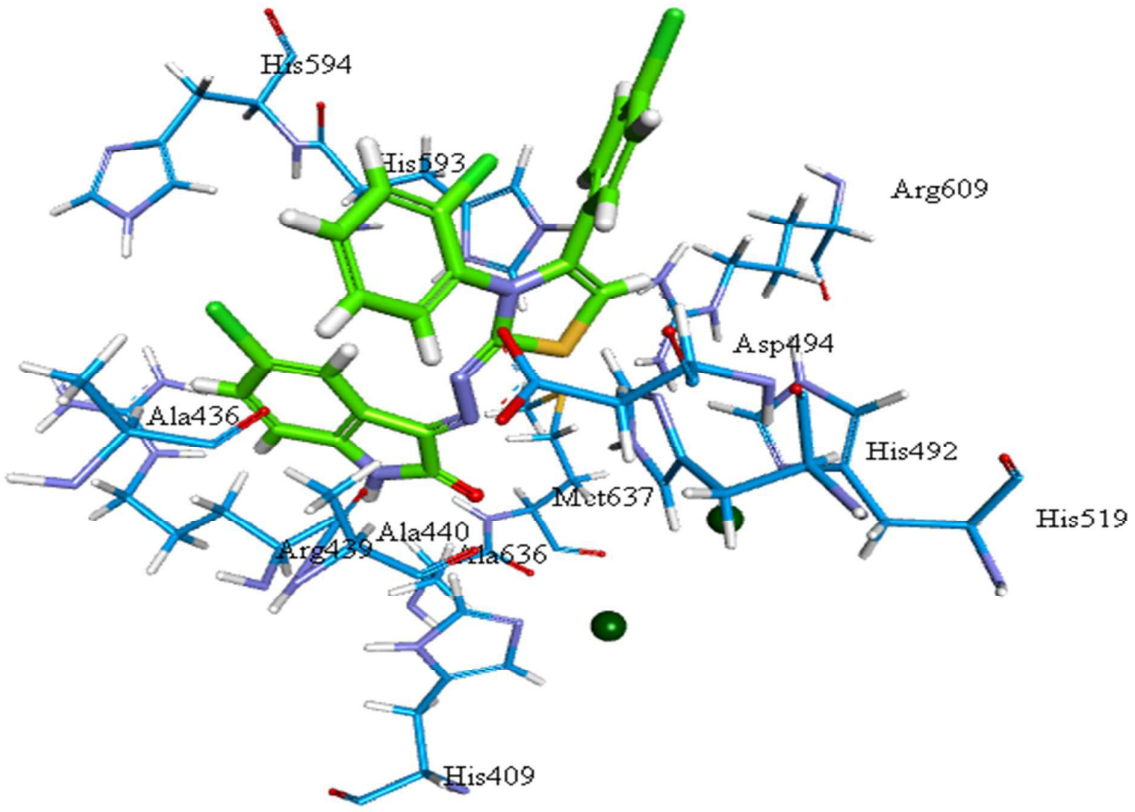

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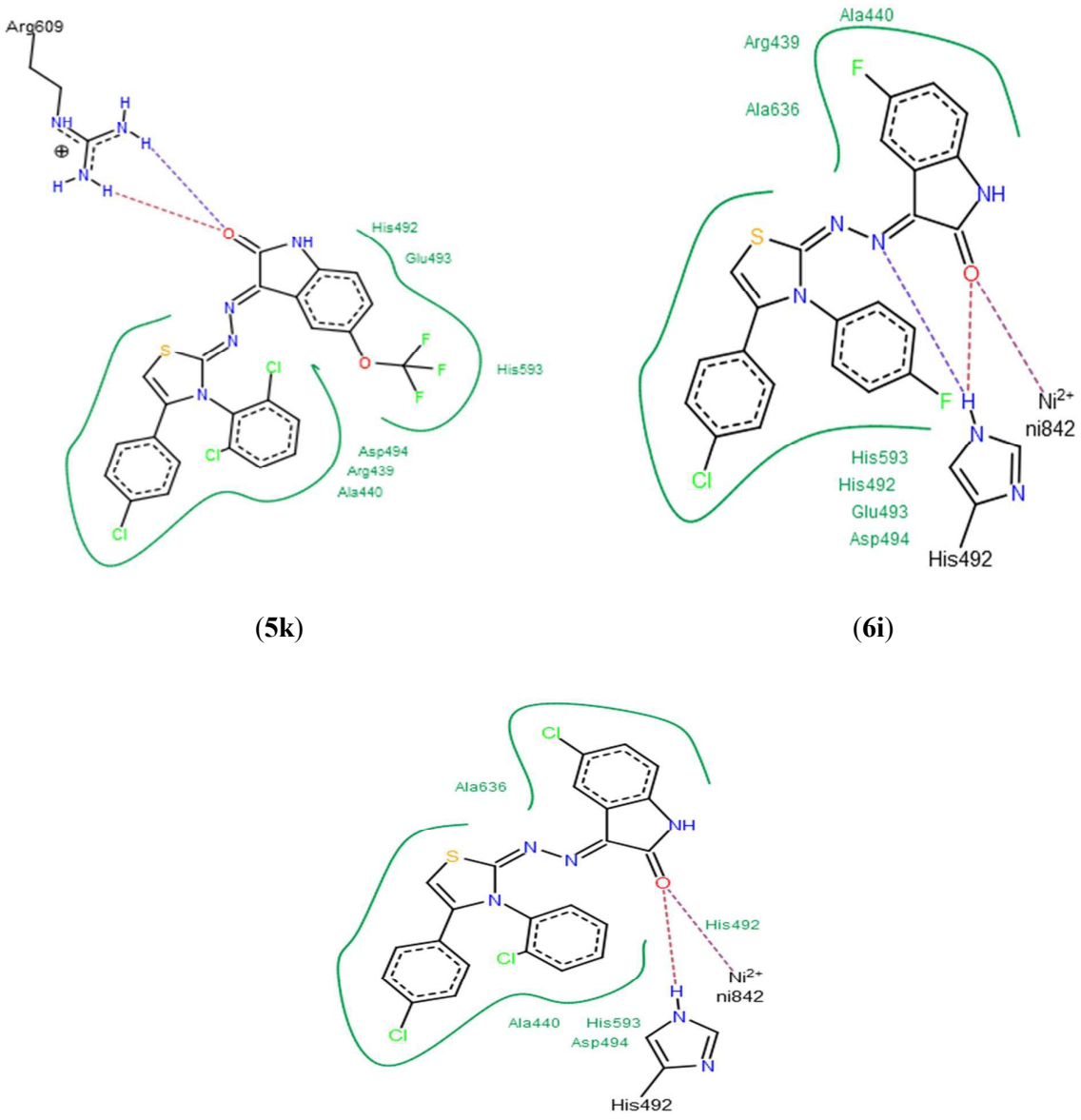
Figure 6. The putative binding mode of compound **7a** bound to the active site of Jack bean urease. Carbon atoms of the compound are light green coloured and that of protein are blue. Dark green spheres are two nickel ions.



(**5k**) (**6i**)

(**7a**)

Figure 7. 2D Interaction diagrams of the selected docked conformations for most potent inhibitors. Hydrogen bond interactions are indicated with dotted lines and hydrophobic interactions are shown with green lines. Metal interactions were observed by Nickel ions.

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Conclusions

Conclusively, three new series of thirty nine isatin-3-hydrazonothiazolines have been synthesized in this study and evaluated for their *in vitro* cytotoxic, phytotoxic, antifungal and urease inhibitory effects. All the compounds proved to be almost inactive in the brine shrimp (*Artemia salina*) lethality bioassay. However, in phytotoxicity bioassay, six compounds were found to be active, exhibiting weak or non-significant (5-30%) activity at the highest tested concentration (500µg/mL). Similarly, in antifungal assay, out of thirty nine compounds tested, twenty six proved to be active, showing weak or non-significant activity (10-30%) against one, two, or three fungal species. On the other hand, the urease inhibition testing results have demonstrated that all the thirty nine compounds tested appeared as potent urease inhibitors; seventeen of these i.e. **5c, 5g-i, 5k, 5n, 6b, 6c, 6i, 6k, 6l, 6n, 6o, 7a, 7e, 7i** and **7j** proved to be relatively highly potent ones and may act as convincing leads for further studies. These compounds, being non toxic, could be potent candidates for orally effective therapeutic agents used for the treatment of certain clinical conditions induced by microbial ureases. Also, by demonstrating weak or non-significant phytotoxicity at the highes tested concentration, they invite much attention to their utility as potent inhibitors of soil ureases, as they could be mixed with fertilizers in small quantities to enhance the overall effectiveness of nitrogen utilization. Generally, urease inhibitory potential of the sunthesized isatin-thiazolines was found to be dependent upon electronic effects of the substituents at position-5 of the isatin scaffold and on the phenyl ring attached to N atom of the thiazoline moiety. In order to understand the binding mechanism, molecular docking studies were performed for compounds **5a-n**, **6a-o** and **7a-j**, which elucidated their relationship with the binding pockets of urease. Based on the data presented in Table 3 and in terms of further development and SAR studies, simultaneous substitution of diverse inductively electron-withdrawing groups at position-5 of the isatin scaffold and the aryl substituents (modified by placing one or more lipophilic functions about the phenyl ring) at N as well as C-4 atoms of the thiazoline moiety in the hybrid molecules certainly warrants investigations.

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Experimental

General procedure

Chemical materials and solvents were obtained from Merck-Schuchatdt, Fluka and Sigma-Aldrich. Melting points were determined on a Fisher-Johns melting point apparatus and are not corrected. Elemental analyses were performed by using a Leco CHNS-9320 (USA) elemental analyzer. The data obtained from all the synthesized compounds were found to be satisfactory. FT-IR spectra (KBr) were run on a Shimadzu 8400 or Thermo Scientific Nicolet 6700 FTIR spectrophotometer using ATR facility. ^{1}H-NMR spectra were recorded in C_2D_6SO on Bruker (Rhenistetten-Forchheim, Germany) AM 300, Bruker Avance AV300 and Bruker Spectrospin 300 spectrometers, operating at 300 MHz and using Si $(CH_3)_4$ as an internal standard. Mass spectra were obtained on MAT-312 and JEOL JMS-600H mass spectrometers.

Synthesis

General procedure for the synthesis of isatin-3-thiosemicarbazones 3

An appropriate 5-substututed isatin **1** (0.01mol), N^4-substituted thiosemicarbazide **2** (0.01mol) and 50% aqueous ethanol (40 mL) containing a catalytic amount of acetic acid were mixed and heated under reflux for 2h. The solid obtained in each case during heating was filtered and washed thoroughly with hot 50% aqueous ethanol to give the desired thiosemicarbazones **3**, which were used as such in the next step without further purification.[30,32,33,39,40]

General procedure for the synthesis of isatin-3-hydrazonothiazolines 5-7

An appropriate isatin-3-thiosemicarbazone **3** (0.005 mol) was mixed with 4-chlorophenacyl bromide **4** (0.005 mol) in absolute ethanol (25 mL) or in a mixture of ethanol-benzene and heated under reflux for 12-15 h.The refluxate was concentrated on a rotary evaporator and the precipitare thus formed in

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each case was filtered. Thorough washing with warm diethyl ether or n-hexane followed by crystallization from ethanol-water furnished the target compounds **5-7** in pure form.

The different compounds are characterized as under:

(3*Z*)-5-(Trifluoromethoxy)-1*H*-indole-2,3-dione-3-{[(2*Z*)-4-(4-chlorophenyl)-3-(2-methylphenyl)-1,3-thiazol-2(3*H*)-ylidene]hydrazone} 5a

Yield 82% as orange brown crystals; m.p. 248-250 °C; IR (KBr, cm^{-1}): 3122 (NH stretching), 1717 (C=O), 1618 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.08 (s, 3H, CH_3), 6.84 (d, J = 9.1 Hz, 1H, indole C_7-H), 7.13-7.16 (m, 2H, N-phenyl C_4-H, C_5-H), 7.17 (s, 1H, thiazoline =CH), 7.27 (d, J = 9.1 Hz, 2H, phenyl C_2-H, C_6-H) 7.29 (dd, J = 9.1 Hz, 1H, indole C_6-H), 7.31-7.38 (m, 4H, N-phenyl C_6-H, phenyl C_3-H, C_5-H, indole C_4-H), 7.42 (d, J = 7.4 Hz, 1H, *N*-phenyl C_3-H), 10.66 (s, 1H, indole NH); EIMS (70eV): m/z (%): 530 (M^++2, 41), 529 (M^++1, 31), 528 (M^+, 100), 499 (5), 301 (7), 300 (19), 299 (39), 298 (40), 297 (77), 288 (8), 287 (12), 286 (48), 285 (23), 284 (81), 240 (14), 228 (7), 187 (6), 174 (6), 170 (5), 168 (12), 162 (19), 139 (5), 134 (6), 133 (7), 91 (14), 77 (5); Anal calcd. for $C_{25}H_{16}ClF_3N_4O_2S$ (528): C 56.76, H 3.03, N 10.06; found: C 56.77, H 2.98, N 10.11.

(3*Z*)-5-(Trifluoromethoxy)-1*H*-indole-2,3-dione-3-{[(2*Z*)-4-(4-chlorophenyl)-3-(3-methylphenyl)-1,3-thiazol-2(3*H*)-ylidene]hydrazone} 5b

Yield 88% as orange yellow fluffy crystals; m.p. 268-270 °C; IR (KBr, cm^{-1}): 3111 (NH stretching), 1709 (C=O), 1622 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.28 (s, 3H, CH_3), 6.83 (d, J = 8.2 Hz, 1H, indole C_7-H), 7.05 (s, 1H, *N*-phenyl C_2-H), 7.09 (s, 1H, thiazoline =CH), 7.13-7.37 (m, 9H, *N*-phenyl C_4-H, C_5-H, C_6-H, phenyl C_2-H, C_3-H, C_5-H, C_6-H, indole C_4-H, C_6-H), 10.59 (s, 1H, indole NH); EIMS (70eV): m/z (%): 530 (M^++2, 47), 529 (M^++1, 34), 528 (M^+, 100), 501 (6), 500 (16), 500 (7), 300 (6), 299 (11), 298 (6), 288 (10), 287 (7), 286 (32), 285 (6), 284 (30), 228 (6), 187 (4), 170 (5), 168 (12), 149 (13), 133 (4), 91 (9), 69 (9); Anal calcd. for $C_{25}H_{16}ClF_3N_4O_2S$ (528): C 56.76 H 3.03 N 10.06; found: C 56.70, H 3.00, N 10.00.

(3*Z*)-5-(Trifluoromethoxy)-1*H*-indole-2,3-dione-3-{[(2*Z*)-4-(4-chlorophenyl)-3-(4-

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methylphenyl)-1,3-thiazol-2(3*H*)-ylidene]hydrazone} 5c

Yield 92% as orange yellow fluffy crystals; m.p. 276-278 °C; IR (KBr, cm^{-1}): 3072 (NH stretching), 1701 (C=O), 1622 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.31 (s, 3H, OCH_3), 6.83 (d, J = 8.4 Hz, 1H, indole C_7-H), 7.11 (s, 1H, thiazoline =CH), 7.16 (dd, J = 8.3, 1.4 Hz, 1H, indole C_6-H), 7.23 (d, J = 8.4 Hz, 2H, *N*- phenyl C_2-H, C_6-H), 7.28 (d, J = 8.4 Hz, 2H, *N*-phenyl C_3-H, C_5-H),7.30 (d, J = 8.4 Hz, 2H, phenyl C_2-H, C_6-H), 7.38 (d, J = 8.5 Hz, 2H, phenyl C_3-H, C_5-H), 7.39 (d, J = 2.0 Hz, 1H, indole C_4-H), 10.66 (s, 1H, indole NH); EIMS (70eV) m/z (%): 530 (M^++2, 76), 529 (M^++1, 81), 528 (M^+, 100), 502 (11), 501 (13), 500 (37), 499 (13), 468 (8), 301 (8), 300 (10), 299 (18), 298 (14), 288 (22), 287 (16), 286 (95), 285 (19), 284 (69), 228 (12), 215 (5), 187 (7), 174 (11), 169 (6), 168 (28), 149 (40), 133 (8), 91 (18), 69 (12); Anal calcd. for $C_{25}H_{16}ClF_3N_4O_2S$ (528): C 56.76, H 3.03, N 10.06; found: C 56.72, H 3.05, N 10.11.

(3*Z*)-5-(Trifluoromethoxy)-1*H*-indole-2,3-dione3-{[(2*Z*)-4-(4-chlorophenyl)-3-(2-methoxyphenyl)-1,3-thiazol-2(3*H*)-ylidene]hydrazone} 5d

Yield 88% as orange fluffy crystals; m.p. 250-252 °C; IR (KBr, cm^{-1}): 3217 (NH stretching), 1720 (C=O), 1607 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 3.37 (s, 3H, OCH_3), 6.84 (d, J = 8.4 Hz, 1H, indole C_7-H), 6.99 (dd, J = 7.7, 0.9 Hz, 1H, indole C_6-H), 7.03 (s, 1H, thiazoline =CH), 7.08 (d, J = 8.4 Hz, 2H, phenyl C_2-H, C_6-H), 7.2 (td, J = 8.6, 1.8 Hz, 2H, *N*-phenyl C_4-H, C_5-H), 7.34 (d, J = 8.5 Hz, 3H, phenyl C_3-H, C_5-H, *N*-phenyl C_6-H), 7.40 (dd, J = 8.3, 1.5 Hz, 1H, *N*-phenyl C_3-H), 7.45 (d, J = 1.6 Hz, 1H, indole C_4-H), 10.57 (s, 1H, indole NH); EIMS (70eV) m/z (%): 546 (M^++2, 40), 545 (M^++1, 31), 544 (M^+, 100), 516 (6), 485 (11), 302 (39), 300 (74), 287 (8), 285 (11), 229 (5), 228 (8), 215 (3), 201 (4), 187 (4), 168 (6), 150 (3), 136 (4), 132 (5), 122 (4), 92 (4), 77 (5); Anal calcd. for $C_{25}H_{16}ClF_3N_4O_3S$ (544): C 55.10, H 2.94, N 10.28; found: C 55.07, H 2.92, N 10.20.

(3*Z*)-5-(Trifluoromethoxy)-1*H*-indole-2,3-dione3-{[(2*Z*)-4-(4-chlorophenyl)-3-(3-methoxyphenyl)-1,3-thiazol-2(3*H*)-ylidene]hydrazone} 5e

Yield 91% as orange yellow fluffy crystals; m.p. 259-261 °C; IR (KBr, cm^{-1}): 3261 (NH stretching), 1719 (C=O), 1605 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 3.77 (s, 3H, OCH_3), 6.73 (dd, J = 7.7, 1.1

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Hz, 1H, *N*-phenyl C_4-H), 6.86 (d, *J* = 8.5 Hz, 1H, indole C_7-H), 6.90 (dd, *J* = 8.4, 2.0 Hz, 1H, indole C_6-H), 7.07 (s, 1H, thiazoline =CH), 7.17 (t, *J* = 2.1 Hz, 1H, *N*-phenyl C_2-H), 7.22-7.26 (m, 4H, *N*-phenyl C_5-H, C_6-H, phenyl C_2-H, C_6-H), 7.36 (d, *J* = 8.6 Hz, 2H, phenyl C_3-H, C_5-H), 7.38 (d, *J* = 1.8 Hz, 1H, indole C_4-H), 10.63 (s, 1H, indole NH); EIMS (70eV) m/z (%): 546 (M^++2, 46), 545 (M^++1, 36), 544 (M^+, 100), 517 (6), 516 (13), 515 (7), 483 (3), 317 (6), 315 (16), 302 (43), 300 (24), 287 (3), 244 (5), 228 (3), 215 (3), 201 (4), 187 (4), 170 (4), 168 (11), 165 (14), 136 (4), 133 (5), 92 (6), 77 (5); Anal calcd. for $C_{25}H_{16}ClF_3N_4O_3S$ (544): C 55.10, H 2.94, N 10.28; found: C 54.97, H 2.90, N 10.28.

(3*Z*)-5-(Trifluoromethoxy)-1*H*-indole-2,3-dione3-{[(2*Z*)-4-(4-chlorophenyl)-3-(4-methoxyphenyl)-1,3-thiazol-2(3*H*)-ylidene]hydrazone 5f

Yield 91% as orange yellow fluffy crystals; m.p. 256-258 °C; IR (KBr, cm^{-1}): 3210 (NH stretching), 1719 (C=O), 1618 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 3.76 (s, 3H, OCH_3), 6.85 (d, *J* = 8.4 Hz, 1H, indole C_7-H), 6.92 (d, *J* = 8.9 Hz, 2H, *N*-phenyl C_2-H, C_6-H), 7.05 (s, 1H, thiazoline =CH), 7.22 (d, *J* = 8.6 Hz, 3H, phenyl C_2-H, C_6-H, indole C_6-H), 7.27 (d, *J* = 8.9 Hz, 2H, *N*-phenyl C_3-H, C_5-H), 7.36 (d, *J* = 8.6 Hz, 2H, phenyl C_3-H, C_5-H), 7.37 (d, *J* = 2.0 Hz, 1H, indole C_4-H), 10.59 (s, 1H, indole NH); EIMS (70eV) m/z (%): 546 (M^++2, 42), 545 (M^++1, 30), 544 (M^+, 100), 518 (15), 517 (112), 516 (34), 515 (6), 503 (5), 501 (12), 316 (7), 315 (9), 314 (6), 302 (27), 301 (17), 300 (14), 244 (8), 201 (5), 187 (4), 174 (7), 168 (21), 165 (14), 150 (8), 133 (7), 92 (5), 77 (6); Anal calcd. for $C_{25}H_{16}ClF_3N_4O_3S$ (544): C 55.10, H 2.94, N 10.28; found: C 55.00, H 2.94, N 10.23.

(3*Z*)-5-(Trifluoromethoxy)-1*H*-indole-2,3-dione-3-{[(2*Z*)-4-(4-chlorophenyl)-3-(2-fluorophenyl)-1,3-thiazol-2(3*H*)-ylidene]hydrazone} 5g

Yield 84% as yellow crystals; m.p. 256-258 °C; IR (KBr, cm^{-1}): 3221 (NH stretching), 1724 (C=O), 1624 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 6.84 (d, *J* = 8.4 Hz, 1H, indole C_7-H), 7.16 (s, 1H, thiazoline =CH), 7.19-7.22 (m, 2H, *N*-phenyl C_5-H, C_6-H), 7.30 (d, *J* = 8.5 Hz, 3H, phenyl C_2-H, C_6-H, indole C_6-H), 7.35-7.42 (m, 3H, phenyl C_3-H, C_5-H, indole C_4-H), 7.50-7.57 (m, 1H, *N*-phenyl C_4-H), 7.69 (t, *J* = 7.2 Hz, 1H, *N*-phenyl C_3-H), 10.71 (s, 1H, indole NH); EIMS (70eV) m/z (%):

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534 (M^++2, 84), 533 (M^++1, 64), 532 (M^+, 100), 504 (21), 503 (7), 292 (32), 291 (17), 290 (80), 266 (7), 241 (8), 232 (16), 215 (8), 187 (9), 170 (8), 168 (19), 153 (35), 136 (12), 133 (6), 95 (5), 69 (12); Anal calcd. for $C_{24}H_{13}ClF_4N_4O_2S$ (532): C 54.08, H 2.44, N 10.52; found: C 54.04, H 2.46, N 10.49.

(3*Z*)-5-(Trifluoromethoxy)-1*H*-indole-2,3-dione-3-{[(2*Z*)-4-(4-chlorophenyl)-3-(3-fluorophenyl)-1,3-thiazol-2(3*H*)-ylidene]hydrazone} 5h

Yield 86% as orange yellow crystals; m.p. 248-250 °C; IR (KBr, cm^{-1}): 3219 (NH stretching), 1720 (C=O), 1606 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 6.84 (d, J = 8.4 Hz, 1H, indole C_7-H), 7.13 (s, 1H, thiazoline =CH), 7.18 (dd, J = 8.4, 1.7 Hz, 1H, indole C_6-H), 7.22 (dd, J = 7.9, 0.9 Hz, 1H, *N*-phenyl C_6-H), 7.25-7.29 (m, 1H, *N*-phenyl C_5-H), 7.33 (d, J = 8.7 Hz, 2H, phenyl C_2-H, C_6-H), 7.36 (d, J = 1.7 Hz, 1H, indole C_4-H), 7.40 (d, J = 8.7 Hz, 2H, phenyl C_3-H, C_5-H), 7.44-7.49 (m, 1H, *N*-phenyl C_4-H), 7.59 (dt, J = 9.5. 2.1 Hz, 1H, *N*-phenyl C_4-H), 10.69 (s, 1H, indole NH); EIMS (70eV) m/z (%): 534 (M^++2, 93), 533 (M^++1, 77), 532 (M^+, 100), 506 (10), 505 (11), 504 (25), 503 (12), 304 (7), 303 (13), 302 (6), 293 (7), 292 (44), 291 (23), 290 (90), 289 (8), 288 (30), 241 (11), 232 (16), 215 (15), 187 (14), 170 (8), 168 (20), 153 (23), 136 (19), 133 (7), 95 (13), 69 (13); Anal calcd. for $C_{24}H_{13}ClF_4N_4O_2S$ (532): C 54.08, H 2.44, N 10.52; found: C 54.06, H 2.49, N 10.54.

(3*Z*)-5-(Trifluoromethoxy)-1*H*-indole-2,3-dione-3-{[(2*Z*)-4-(4-chlorophenyl)-3-(4-fluorophenyl)-1,3-thiazol-2(3*H*)-ylidene]hydrazone} 5i

Yield 88% as orange yellow crystals; m.p. 274-276 °C; IR (KBr, cm^{-1}): 3221 (NH stretching), 1724 (C=O), 1624 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 6.84 (d, J = 8.5 Hz, 1H, indole C_7-H), 7.11 (s, 1H, thiazoline =CH), 7.16 (dd, J = 8.5, 1.7 Hz, 1H, indole C_6-H), 7.27 (d, J = 8.6 Hz, 2H, phenyl C_2-H, C_6-H), 7.35 (d, J = 8.6 Hz, 2H, phenyl C_3-H, C_5-H), 7.41 (dd, J = 8.5, 1.9 Hz, 2H, *N*-phenyl C_2-H, C_6-H), 7.51 (d, J = 1.5 Hz, 1H, indole C_4-H), 7.54 (t, J = 8.7 Hz, 2H, *N*-phenyl C_3-H, C_5-H), 10.67 (s, 1H, indole NH); EIMS (70eV) m/z (%): 534 (M^++2, 32), 533 (M^++1, 30), 532 (M^+, 100), 504 (11), 503 (7), 303 (5), 292 (11), 291 (8), 290 (33), 288 (10), 232 (7), 215 (5), 187 (6), 168 (9), 153 (15), 136 (4), 133 (4), 95 (6), 69 (9); Anal calcd. for $C_{24}H_{13}ClF_4N_4O_2S$ (532): C 54.08, H 2.44, N 10.52; found: C 54.06, H 2.46, N 10.55.

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(3*Z*)-5-(Trifluoromethoxy)-1*H*-indole-2,3-dione-3-{[(2*Z*)-4-(4-chlorophenyl)-3-(2,4-dichlorophenyl)-1,3-thiazol-2(3*H*)-ylidene]hydrazone} 5j

Yield 78% as orange brown crystals; m.p. 258-260 °C; IR (KBr, cm^{-1}): 3130 (NH stretching), 1715 (C=O), 1618 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 6.85 (d, J = 9.0 Hz, 1H, indole C_7-H), 7.16 (s, 1H, thiazoline =CH), 7.16-7.20 (m, 1H, indole C_6-H), 7.31 (d, J = 8.7 Hz, 2H, phenyl C_2-H, C_6-H), 7.44 (d, J = 8.7 Hz, 2H, phenyl C_3-H, C_5-H), 7.55 (d, J = 2.0 Hz, 1H, indole C_4-H), 7.62 (dd, J = 8.6, 2.3 Hz, 1H, *N*-phenyl C_5-H), 7.84 (d, J = 2.3 Hz, 1H, *N*-phenyl C_3-H), 7.89 (d, J = 8.6 Hz, 1H, *N*-phenyl C_6-H), 10.71 (s, 1H, indole NH); EIMS (70eV) m/z (%): 584 (M^++1, 94), 583 (M^+, 26), 582 (M^+-1, 100), 555 (13), 553 (11), 549 (9), 547 (12), 521 (23), 519 (30), 376 (16), 374 (13), 344 (16), 342 (49), 341 (11), 319 (10), 318 (8), 292 (7), 291 (8), 245 (14), 215 (12), 205 (22), 203 (30), 188 (9), 187 (12), 170 (9), 168 (24), 136 (9), 133 (7), 69 (13); Anal calcd. for $C_{24}H_{12}Cl_3F_3N_4O_2S$ (583): C 49.36, H 2.06, N 9.60; found: C 49.36, H 2.03, N 9.57.

(3*Z*)-5-(Trifluoromethoxy)-1*H*-indole-2,3-dione-3-{[(2*Z*)-4-(4-chlorophenyl)-3-(2,5-dichlorophenyl)-1,3-thiazol-2(3*H*)-ylidene]hydrazone} 5k

Yield 86% as orange brown crystals; m.p. 266-268 °C; IR (KBr, cm^{-1}): 3121 (NH stretching), 1717 (C=O), 1620 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 6.86 (d, J = 8.3 Hz, 1H, indole C_7-H), 7.16 (s, 1H, thiazoline =CH), 7.18-7.33 (m, 1H, indole C_6-H), 7.35 (d, J = 8.6 Hz, 2H, phenyl C_2-H, C_6-H), 7.45 (d, J = 8.6 Hz, 2H, phenyl C_3-H, C_5-H), 7.60 (d, J = 2.5 Hz, 1H, indole C_4-H), 7.67 (d, J = 8.7 Hz, 2H, *N*-phenyl C_3-H, C_4-H), 8.16 (d, J = 2.4 Hz, *N*-phenyl C_6-H), 10.72 (s, 1H, indole NH); EIMS (70eV) m/z (%): 584 (M^++1, 99), 583 (M^+, 32), 582 (M^+-1, 100), 549 (20), 547 (29), 521 (19), 519 (24), 484 (24), 344 (15), 342 (39), 340 (41), 319 (12), 318 (11), 205 (10), 203 (14), 168 (14), 136 (12), 69 (11); Anal calcd. for $C_{24}H_{12}Cl_3F_3N_4O_2S$ (583): C 49.36, H 2.06, N 9.60; found: C 49.33, H 2.03, N 9.59.

(3*Z*)-5-(Trifluoromethoxy)-1*H*-indole-2,3-dione-3-{[(2*Z*)-4-(4-chlorophenyl)-3-(2,6-dichlorophenyl)-1,3-thiazol-2(3*H*)-ylidene]hydrazone} 5l

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Yield 83% as orange yellow fluffy crystals; m.p. 262-264 °C; IR (KBr, cm^{-1}): 3225 (NH stretching), 1717 (C=O), 1605 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 6.86 (d, J = 8.4 Hz, 1H, indole C_7-H), 7.13-7.18 (m, 2H, indole C_4-H, C_6-H), 7.20 (s, 1H, thiazoline =CH), 7.30 (d, J = 8.6 Hz, 2H, phenyl C_2-H, C_6-H), 7.45 (d, J = 8.6 Hz, 2H, phenyl C_3-H, C_5-H), 7.57 (t, J = 9.0 Hz, 1H, *N*-phenyl C_4-H), 7.70 (dd, J = 8.7, 1.1 Hz, 2H, *N*-phenyl C_3-H, C_5-H), 10.73 (s, 1H, indole NH); EIMS (70eV) m/z (%): 586 (M^++3, 61), 584 (M^++1, 100), 583 (M^+, 40), 582 (M^+-1, 100), 521 (27), 520 (11), 519 (46), 484 (7), 344 (21), 343 (10), 342 (67), 341 (12), 340 (40), 319 (7), 293 (8), 292 (7), 291 (9), 284 (7), 215 (8), 205 (18), 203 (27), 187 (9), 168 (22), 136 (16), 69 (11); Anal calcd. for $C_{24}H_{12}Cl_3F_3N_4O_2S$ (583): C 49.36, H 2.06, N 9.60; found: C 49.34, H 2.01, N 9.54.

(3*Z*)-5-(Trifluoromethoxy)-1*H*-indole-2,3-dione-3-{[(2*Z*)-4-(4-chlorophenyl)-3-(3,4-dichlorophenyl)-1,3-thiazol-2(3*H*)-ylidene]hydrazone} 5m

Yield 84% as orange yellow crystals; m.p. 276-278 °C; IR (KBr, cm^{-1}): 3163 (NH stretching), 1699 (C=O), 1622 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 6.87 (d, J = 8.5 Hz, 1H, indole C_7-H), 7.01 (s, 1H, thiazoline =CH), 7.24-7.29 (m, 4H, *N*-phenyl C_6-H, phenyl C_2-H, C_6-H, indole C_6-H), 7.36 (d, J = 1.6 Hz, 1H, indole C_4-H), 7.42 (d, J = 8.6 Hz, 2H, phenyl C_3-H, C_5-H), 7.64 (d, J = 8.6 Hz, 1H, *N*-phenyl C_5-H), 7.91 (d, J = 2.4 Hz, IH, *N*-phenyl C_2-H), 10.62 (s, 1H, indole NH); EIMS (70eV) m/z (%): 584 (M^++1, 92), 583 (M^+, 32), 582 (M^+-1, 100), 556 (14), 554 (15), 532 (8), 344 (14), 342 (48), 341 (10), 340 (47), 284 (7), 215 (12), 203 (10), 187 (11), 168 (12), 136 (7), 69 (11); Anal calcd. for $C_{24}H_{12}Cl_3F_3N_4O_2S$ (583): C 49.36, H 2.06, N 9.60; found: C 49.30, H 2.03, N 9.66.

(3*Z*)-5-(Trifluoromethoxy)-1*H*-indole-2,3-dione3-{[(2*Z*)-4-(4-chlorophenyl)-3-(2,4,6-trichlorophenyl)-1,3-thiazol-2(3*H*)-ylidene]hydrazone} 5n

Yield 81% as orange yellow crystals; m.p. 280-282 °C; IR (KBr, cm^{-1}): 3167 (NH stretching), 1711 (C=O), 1624 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 6.87 (d, J = 8.5 Hz, 1H, indole C_7-H), 7.16-7.24 (m, 3H, indole C_4-H, indole C_6-H, thiazoline =CH), 7.30 (d, J = 8.6 Hz, 2H, phenyl C_2-H, C_6-H), 7.50 (d, J = 8.6 Hz, 2H, phenyl C_3-H, C_5-H), 7.98 (s, 2H, *N*-phenyl C_3-H, C_5-H), 10.75 (s, 1H, indole NH); EIMS (70eV) m/z (%): 620 (M^++2, 17), 618 (M^+, 32), 584 (94), 582 (100), 555 (13), 553 (11),

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549 (9), 547 (12), 521 (23), 519 (30), 376 (16), 374 (13), 344 (16), 342 (49), 341 (11), 319 (10), 318 (8), 292 (7), 291 (8), 245 (14), 215 (12), 205 (22), 203 (30), 188 (9), 187 (12), 170 (9), 168 (24), 136 (9), 133 (7), 69 (13); Anal calcd. for $C_{24}H_{11}Cl_4F_3N_4O_2S$ (618): C 46.60, H 1.78, N 9.06; found: C 46.60, H 1.77, N 9.02.

(3*Z*)-5-Fluoro-1*H*-indole-2,3-dione3-{[(2*Z*)-4-(4-chlorophenyl)-3-(2-methylphenyl)-1,3-thiazol-2(3*H*)-ylidene]hydrazone} 6a

Yield 79% as bright orange crystals; m.p. 276-278 °C; IR (KBr, cm^{-1}): 3179 (NH stretching), 1705 (C=O), 1624 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.08 (s, 3H, CH_3), 6.72 (dd, J = 8.7. 4.5 Hz, 1H, indole C_7-H), 6.89 (dd, J = 9.3, 2.4 Hz, 1H, N-phenyl C_6-H), 6.99 (dd, J = 8.7, 2.7 Hz, 1H, N-phenyl C_3-H), 7.15 (s, 1H, thiazoline =CH), 7.28 (d, J = 8.7 Hz, 2H, phenyl C_2-H, C_6-H), 7.33-7.46 (m, 6H, N-phenyl C_4-H, C_5-H, phenyl C_3-H, C_5-H, indole C_4-H, indole C_6-H), 10.45 (s, 1H, indole NH); EIMS (70eV) m/z (%): 464 (M^++2, 39), 463 (M^++1, 28), 462 (M^+, 100), 433 (6), 300 (17), 299 (43), 298 (38), 297 (82), 288 (13), 287 (16), 286 (64), 285 (24), 284 (100), 240 (16), 225 (8), 168 (18), 162 (25), 136 (11), 134 (11), 121 (17), 108 (15), 91 (19), 89 (10), 65 (17); Anal calcd. for $C_{24}H_{16}ClFN_4OS$ (462): C 62.27, H 3.46, N 12.11; found: C 62.25, H 3.43, N 12.10.

(3*Z*)-5-Fluoro-1*H*-indole-2,3-dione-3-{[(2*Z*)-4-(4-chlorophenyl)-3-(3-methylphenyl)-1,3-thiazol-2(3*H*)-ylidene]hydrazone} 6b

Yield 87% as orange fluffy crystals; m.p. 278-280 °C; IR (KBr, cm^{-1}): 3217 (NH stretching), 1720 (C=O), 1624 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.27 (s, 3H, CH_3), 6.75 (dd, J = 8.4, 4.2 Hz, 1H, indole C_7-H), 7.02-7.08 (m, 3H, thiazoline =CH, *N*-phenyl C_4-H, C_6-H), 7.13-7.28 (m, 6H, *N*-phenyl C_2-H, C_5-H, phenyl C_2-H, C_6-H, indole C_4-H, C_6-H), 7.35 (d, J = 8.4 Hz, 2H, phenyl C_3-H, C_5-H) 10.39 (s, 1H, indole NH); EIMS (70eV): m/z (%): 464 (M^++2, 37), 463 (M^++1, 29), 462 (M^+, 100), 434 (13), 299 (15), 288 (13), 286 (44), 284 (25), 228 (7), 168 (16), 149 (21), 136 (7), 121 (8), 91 (13), 65 (8); Anal calcd. for $C_{24}H_{16}ClFN_4OS$ (462): C 62.27, H 3.46, N 12.11; found: C 62.24, H 3.43, N 12.13.

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(3*Z*)-5-Fluoro-1*H*-indole-2,3-dione3-{[(2*Z*)-4-(4-chlorophenyl)-3-(4-methylphenyl)-1,3-thiazol-2(3*H*)-ylidene]hydrazone} 6c

Yield 89% as bright orange yellow fluffy crystals; m.p. 278-280 °C; IR (KBr, cm^{-1}): 3217 (NH stretching), 1717 (C=O), 1606 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.31, 2.34 (2s, 3H, CH_3), 6.75, 6.79 (2d, J = 8.4, 4.2 Hz, 1H, indole C_7-H), 6.95-7.13 (m, 2H, indole C_6-H, thiazoline =CH), 7.18-7.49 (m, 9H, *N*-phenyl C_2-H, C_3-H, C_5-H, C_6-H, phenyl C_2-H, C_3-H, C_5-H, C_6-H, indole C_4-H), 10.48, 10.52 (2s, 1H, indole NH); EIMS (70eV): m/z (%): 464 (M^+ +2, 96), 463 (M^+ +1, 82), 462 (M^+, 100), 461 (13), 436 (13), 435 (15), 434 (34), 433 (20), 402 (9), 301 (112), 300 (16), 299 (28), 298 (16), 288 (37), 287 (25), 286 (91), 285 (21), 284 (58), 240 (9), 228 (15), 170 (12), 168 (31), 149 (37), 136 (8), 133 (8), 121 (9), 91 (24), 65 (17); Anal calcd. for $C_{24}H_{16}ClFN_4OS$ (462): C 62.27, H 3.46, N 12.11; found: C 62.23, H 3.44, N 12.15.

(3*Z*)-5-Fluoro-1*H*-indole-2,3-dione-3-{[(2*Z*)-4-(4-chlorophenyl)-3-(2-methoxyphenyl) -1,3-thiazol-2(3*H*)-ylidene]hydrazone} 6d

Yield 88% as orange crystals; m.p. 274-276 °C; IR (KBr, cm^{-1}): 3209 (NH stretching), 1719 (C=O), 1606 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 3.58, 3.61 (2s, 3H, OCH_3), 6.73 (dd, J = 8.4, 4.2 Hz, 1H, indole C_7-H), 6.95-7.07 (m, 4H, thiazoline =CH, *N*-phenyl C_3-H, C_4-H, C_5-H), 7.17 (dd, J = 8.7 Hz, phenyl C_2-H, C_6-H), 7.26 (d, J = 8.7 Hz, 1H, *N*- phenyl C_6-H), 7.32 (d, J = 8.7 Hz, 2H, phenyl C_3-H, C_5-H), 7.36-7.52 (m, 2H, indole C_4-H, C_6-H), 10.35, 10.44 (2s, 1H, indole NH); EIMS (70eV) m/z (%): 480 (M^+ +2, 44), 479 (M^+ +1, 24), 478 (M^+, 100), 450 (6), 419 (12), 304 (9), 303 (10), 302 (36), 301 (14), 300 (58), 287 (8), 286 (10), 285 (13), 272 (12), 270 (16), 168 (9), 136 (7), 121 (7), 108 (9), 77 (6); Anal calcd. for $C_{24}H_{16}ClFN_4O_2S$ (478): C 60.19, H 3.37, N 11.70; found: C 60.17, H 3.33, N 11.73.

(3*Z*)-5-Fluoro-1*H*-indole-2,3-dione-3-{[(2*Z*)-4-(4-chlorophenyl)-3-(3-methoxyphenyl) -1,3-thiazol-2(3*H*)-ylidene]hydrazone} 6e

Yield 88% as orange brown crystals; m.p. 276-278 °C; IR (KBr, cm^{-1}): 3171 (NH stretching), 1693 (C=O), 1607 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 3.73 (s, 3H, OCH_3), 6.76 (dd, J = 8.7, 4.5 Hz,

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1H, indole C_7-H), 6.91 (dd, *J* = 7.8, 1.2 Hz, 1H, *N*-phenyl C_6-H), 6.97-7.05 (m, 2H, *N*-phenyl C_4-H, C_5-H), 7.11 (s, 1H, thiazoline =CH), 7.19 (t, *J* = 2.1 Hz, 1H, *N*- phenyl C_2-H), 7.27 (dd, *J* = 9.0, 2.7 Hz, 1H, indole C_6-H), 7.32 (d, *J* = 8.7 Hz, 2H, phenyl C_2-H, C_6-H), 7.36 (d, *J* = 8.7 Hz, 2H, phenyl C_3-H, C_5-H), 7.41 (dd, *J* = 8.7, 2.1 Hz, 1H, indole C_4-H), 10.51 (s, 1H, indole NH); EIMS (70eV) m/z (%): 480 (M^+ +2, 81), 479 (M^+ +1, 67), 478 (M^+, 100), 451 (10), 450 (21), 449 (11), 317 (11), 316 (11), 315 (25), 304 (29), 303 (18), 302 (92), 301 (12), 300 (42), 287 (6), 244 (9), 168 (15), 165 (19), 136 (8), 121 (6), 108 (5), 92 (7), 77 (7); Anal calcd. for $C_{24}H_{16}ClFN_4O_2S$ (478): C 60.19, H 3.37, N 11.70; found: C 60.10, H 3.36, N 11.71.

(3*Z*)-5-Fluoro-1*H*-indole-2,3-dione3-{[(2*Z*)-4-(4-chlorophenyl)-3-(4-methoxyphenyl)-1,3-thiazol-2(3*H*)-ylidene]hydrazone} 6f

Yield 90% as orange crystals; m.p. 270-272°C; IR (KBr, cm^{-1}): 3177 (NH stretching), 1693 (C=O), 1607 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 3.75, 3.77 (2s, 3H, OCH_3), 6.76 (dd, *J* = 8.7, 4.5 Hz, 1H, indole C_7-H), 6.90, 6.93 (2s, 1H, thiazoline =CH), 6.98-7.01 (m, 3H, *N*-phenyl C_2-H, C_6-H, indole C_6-H), 7.13-7.29 (m, 4H, phenyl C_2-H, C_6-H, *N*-phenyl C_3-H, C_5-H), 7.32-7.41 (m, 3H, phenyl C_3-H, C_5-H, indole C_4-H), 10.38, 10.45 (s, 1H, indole NH); EIMS (70eV) m/z (%): 480 (M^+ +2, 44), 479 (M^+ +1, 35), 478 (M^+, 100), 452 (14), 451 (14), 450 (29), 449 (9), 435 (10), 316 (10), 315 (16), 304 (10), 303 (12), 302 (33), 301 (19), 300 (18), 286 (11), 284 (8), 244 (13), 168 (21), 165 (14), 150 (11), 136 (10), 135 (9), 121 (8), 108 (12), 77 (7); Anal calcd. for $C_{24}H_{16}ClFN_4O_2S$ (478): C 60.19, H 3.37, N 11.70; found: C 60.17, H 3.33, N 11.73.

(3*Z*)-5-Fluoro-1*H*-indole-2,3-dione-3-{[(2*Z*)-4-(4-chlorophenyl)-3-(2-fluorophenyl)-1,3-thiazol-2(3*H*)-ylidene]hydrazone} 6g

Yield 67% as bright orange crystals; m.p. 272-274 °C; IR (KBr, cm^{-1}): 3146 (NH stretching), 1715 (C=O), 1600 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 6.74 (dd, *J* = 8.7, 4.5 Hz, 1H, indole C_7-H), 7.06-7.09 (m, 3H, thiazoline =CH, *N*-phenyl C_4-H, C_5-H), 7.15-7.23 (m, 2H, *N*-phenyl C_3-H, C_6-H), 7. 25 (d, *J* = 8.7 Hz, 2H, phenyl C_2-H, C_6-H), 7.38 (d, *J* = 8.7 Hz, 3H, phenyl C_3-H, C_5-H, indole C_6-H), 7.46 (d, *J* = 9.9 Hz, 1H, indole C_4-H), 10.41 (s, 1H, indole NH); EIMS (70eV): m/z (%): 468

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(M^++2, 43), 467 (M^++1, 23), 466 (M^+, 100), 438 (11), 437 (7), 305 (7), 304 (10), 303 (18), 292 (29), 291 (16), 290 (80), 288 (11), 232 (16), 170 (8), 168 (19), 153 (14), 149 (14), 136 (19), 135 (8), 134 (8), 133 (8), 121 (14), 108 (12), 95 (17), 75 (10); Anal calcd. for $C_{23}H_{13}ClF_2N_4OS$ (466): C 59.16, H 2.79, N 12.00; found: C 59.19, H 2.77, N 11.98.

(3*Z*)-5-Fluoro-1*H*-indole-2,3-dione-3-{[(2*Z*)-4-(4-chlorophenyl)-3-(3-fluorophenyl)-1,3-thiazol-2(3*H*)-ylidene]hydrazone} 6h

Yield 87% as orange crystals, m.p. 278-280 °C; IR (KBr, cm^{-1}): 3192 (NH stretching), 1707 (C=O), 1624 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 6.76 (dd, J = 9.3, 4.2 Hz, 1H, indole C_7-H), 7.03 (dd, J = 6.9, 2.1 Hz, 2H, *N*-phenyl C_5-H, C_6-H), 7.15 (s, 1H, thiazoline =CH), 7.31 (d, J = 8.4 Hz, 2H, phenyl C_2-H, C_6-H), 7.35-7.47 (m, 4H, phenyl C_3-H, C_5-H, indole C_4-H, C_6-H), 7.55-7.66 (m, 1H, *N*-phenyl C_4-H), 7.70 (td, J = 7.5, 1.5 Hz, 1H, N- phenyl C_2-H), 10.53 (s, 1H, indole NH); EIMS (70eV): m/z (%): 468 (M^++2, 87), 467 (M^++1, 72), 466 (M^+, 100), 440 (8), 439 (8), 438 (20), 437 (8), 304 (7), 292 (49), 291 (24), 290 (89), 289 (6), 241 (11), 234 (12), 233 (12), 232 (25), 170 (10), 168 (26), 153 (41), 149 (13), 136 (17), 121 (14), 108 (12), 95 (9), 75 (9); Anal calcd. for $C_{23}H_{13}ClF_2N_4OS$ (466): C 59.16, H 2.79, N 12.00; found: C 59.17, H 2.79, N 12.01.

(3*Z*)-5-Fluoro-1*H*-indole-2,3-dione-3-{[(2*Z*)-4-(4-chlorophenyl)-3-(4-fluorophenyl)-1,3-thiazol-2(3*H*)-ylidene]hydrazone} 6i

Yield 88% as orange yellow crystals; m.p. 298-300 °C; IR (KBr, cm^{-1}): 3177 (NH stretching), 1705 (C=O), 1624 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 6.75 (dd, J = 8.7, 4.5 Hz, 1H, indole C_7-H), 7.03 (td, J = 9.3, 2.7 Hz, 1H, indole C_6-H), 7.09 (dd, J = 9.0, 2.7 Hz, 1H, indole C_4-H), 7.11 (s, 1H, thiazoline =CH), 7.29 (d, J = 8.7 Hz, 2H, phenyl C_2-H, C_6-H), 7.37 (t, J = 8.7 Hz, 2H, *N*- phenyl C_2-H, C_6-H), 7.41 (d, J = 8.7 Hz, 2H, phenyl C_3-H, C_5-H), 7.55 (dd, J = 8.7, 4.5 Hz, 2H, *N*-phenyl C_3-H, C_5-H), 10.50 (s, 1H, indole NH); EIMS (70eV) m/z (%): 468 (M^++2, 75), 467 (M^++1, 55), 466 (M^+, 100), 440 (8), 439 (9), 438 (18), 437 (11), 305 (6), 304 (8), 303 (12), 292 (35), 291 (19), 290 (82), 288 (22), 234 (7), 232 (16), 170 (8), 168 (19), 153 (26), 149 (13), 136 (9), 134 (7), 133 (6), 121

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(12), 108 (6), 95 (14), 75 (7); Anal calcd. for $C_{23}H_{13}ClF_2N_4OS$ (466): C 59.16, H 2.79, N 12.00; found: C 59.16, H 2.77, N 11.99.

(3*Z*)-5-Fluoro-1*H*-indole-2,3-dione-3-{[(2*Z*)-4-(4-chlorophenyl)-3-(2,4-difluorophenyl)1,3-thiazol-2(3*H*)-ylidene]hydrazone} 6j

Yield 91% as orange yellow crystals; m.p. 298-300 °C; IR (KBr, cm^{-1}): 3184 (NH stretching), 1707 (C=O), 1606 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 6.75 (dd, J = 8.4, 4.2 Hz, 1H, indole C_7-H), 7.01 (d, J = 9.3, Hz, 2H, indole C_4-H, C_6-H), 7.13 (s, 1H, thiazoline =CH), 7.32 (d, J = 8.4, 3H, phenyl C_2-H, C_6-H, *N*-phenyl C_6-H), 7.44 (d, J = 8.4 Hz, 2H, phenyl C_3-H, C_5-H), 7.55 (td, J = 8.4, 2.4 Hz, 1H, *N*-phenyl C_5-H), 7.82-7.86 (m, 1H, *N*-phenyl C_3-H), 10.50 (s, 1H, indole NH); EIMS (70eV) m/z (%): 486 (M^++2, 56), 485 (M^++1, 40), 484 (M^+, 100), 456 (12), 310 (28), 308 (75), 259 (6), 250 (14), 171 (19), 168 (14), 149 (12), 136 (10), 135 (8), 121 (11), 111 (11); Anal calcd. for $C_{23}H_{12}ClF_3N_4OS$ (484): C 56.97, H 2.48, N 11.56; found: C 56.99, H 2.44, N 11.57.

(3*Z*)-5-Fluoro-1*H*-indole-2,3-dione-3-{[(2*Z*)-4-(4-chlorophenyl)-3-(2,6-difluoro-phenyl)1,3-thiazol-2(3*H*)-ylidene]hydrazone} 6k

Yield 78% as orange brown crystals; m.p. 278-280 °C; IR (KBr, cm^{-1}): 3185 (NH stretching), 1722 (C=O), 1622 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 6.76 (dd, J = 8.4, 4.2 Hz, 1H, indole C_7-H), 6.96-7.07 (m, 2H, indole C_4-H, C_6-H), 7.18 (s, 1H, thiazoline =CH), 7.29 (d, J = 8.4, 2H, phenyl C_2-H, C_6-H), 7.39 (t, J = 8.7 Hz, 2H, *N*-phenyl C_3-H, C_5-H), 7.45 (d, J = 8.4 Hz, 2H, phenyl C_3-H, C_5-H), 7.62-7.74 (m, 1H, *N*-phenyl C_4-H), 10.54 (s, 1H, indole NH); EIMS (70eV) m/z (%): 486 (M^++2, 43), 485 (M^++1, 31), 484 (M^+, 94), 456 (11), 455 (18), 453 (20), 344 (23), 343 (14), 342 (64), 341 (13), 340 (63), 310 (41), 309 (19), 308 (97), 294 (13), 284 (14), 282 (14), 259 (11), 258 (10), 252 (11), 250 (38), 205 (27), 203 (45), 177 (16), 171 (61), 170 (44), 168 (98), 162 (18), 156 (18), 150 (19), 149 (65), 145 (18), 140 (15), 139 (32), 138 (41), 137 (15), 136 (100), 135 (38), 134 (41), 133 (40), 125 (15), 123 (16), 122 (22), 121 (98), 113 (32), 111 (83), 108 (70), 101 (26), 100 (14), 94 (56),

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89 (57); Anal calcd. for $C_{23}H_{12}ClF_3N_4OS$ (484): C 56.97, H 2.48, N 11.56; found: C 56.96, H 2.49, N11.58.

(3*Z*)-5-Fluoro-1*H*-indole-2,3-dione-3-{[(2*Z*)-4-(4-chlorophenyl)-3-(2,4-dichlorophenyl)1,3-thiazol-2(3*H*)-ylidene]hydrazone} 6l

Yield 86% as orange crystals; m.p. 270-272 °C; IR (KBr, cm^{-1}): 3171 (NH stretching), 1708 (C=O), 1624 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 6.76 (dd, J = 8.4, 4.2 Hz, 1H, indole C_7-H), 6.89 (dd, J = 9.0, 2.7 Hz, 1H, indole C_4-H), 7.05-7.11 (m, 1H, indole C_6-H), 7.15 (s, 1H, thiazoline =CH), 7.33 (d, J = 8.4 Hz, 2H, phenyl C_2-H, C_6-H), 7.44 (d, J = 8.4 Hz, 2H, phenyl C_3-H, C_5-H), 7.68 (dd, J = 8.4, 2.4 Hz, 1H, *N*-phenyl C_5-H), 7.91 (d, J = 7.5 Hz, 1H, *N*-pheny C_6-H), 7.89 (d, J = 2.1 Hz, 1H, *N*-phenyl C_3-H), 10.54 (s, 1H, indole NH); EIMS (70eV) m/z (%): 518 (M^++1, 88), 517 (M^+, 22), 516 (M^+ -1, 87), 455 (20), 553 (23), 344 (26), 342 (86), 340 (83), 319 (27), 284 (20), 282 (22), 205 (39), 203 (63), 170 (30), 169 (16), 168 (95), 162 (34), 149 (58), 147 (16), 145 (31), 139 (24), 138 (27), 136 (76), 135 (34), 134 (55), 133 (38), 121 (100), 111 (64), 109 (54), 108 (61), 94 (47); Anal calcd. for $C_{23}H_{12}Cl_3FN_4OS$ (517): C 53.33, H 2.32, N 10.82; found: C 53.34, H 2.34, N 10.84.

(3*Z*)-5-Fluoro-1*H*-indole-2,3-dione-3-{[(2*Z*)-4-(4-chlorophenyl)-3-(2,5-dichlorophenyl)-1,3-thiazol-2(3*H*)-ylidene]hydrazone} 6m

Yield 87% as orange brown crystals; m.p. 274-276 °C; IR (KBr, cm^{-1}): 3225 (NH stretching), 1697 (C=O), 1590 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 6.76 (dd, J = 8.4, 4.2 Hz, 1H, indole C_7-H), 6.98 (dd, J = 8.7, 3.0 Hz, 1H, indole C_4-H), 7.03 (td, J = 9.3, 2.7 Hz, 1H, indole C_6-H), 7.20 (s, 1H, thiazoline =CH), 7.31 (d, J = 8.4, 2H, phenyl C_2-H, C_6-H), 7.34-7.48 (m, 4H, *N*-phenyl C_3-H, C_6-H, phenyl C_3-H, C_5-H), 7.59-7.74 (m, 1H, *N*-phenyl C_4-H), 10.58 (s, 1H, indole NH); EIMS (70eV): m/z (%): 518 (M^++1, 45), 517 (M^+, 34), 516 (M^+-1, 34), 455 (6), 343 (37), 342 (38), 341 (40), 340 (42), 319 (20), 284 (10), 282 (12), 205 (38), 203 (54), 187 (14), 180 (17), 179 (35), 177 (9), 170 (15), 168 (47), 163 (12), 162 (30), 150 (17), 149 (43), 147 (25), 145 (40), 141 (24), 139 (71), 137 (29), 136 (100), 135 (35), 134 (50), 133 (34), 122 (41), 121 (84), 111 (89), 109 (80), 108 (96), 107 (23), 101

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(34), 100 (24), 95 (52), 75 (67); Anal calcd. for $C_{23}H_{12}Cl_3FN_4OS$ (517): C 53.33, H 2.32, N 10.82; found: C 53.32, H 2.30, N 10.85.

(3*Z*)-5-Fluoro-1*H*-indole-2,3-dione-3-{[(2*Z*)-4-(4-chlorophenyl)-3-(2,6-dichlorophenyl)1,3-thiazol-2(3*H*)-ylidene]hydrazone} 6n

Yield 88% as reddish brown crystals; m.p. 272-274 °C; IR (KBr, cm^{-1}): 3177 (NH stretching), 1703 (C=O), 1620 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 6.76 (dd, J = 8.7, 4.5 Hz, 1H, indole C_7-H), 6.91 (dd, J = 8.7, 3.0 Hz, 1H, indole C_4-H), 7.03 (td, J = 9.3, 2.7 Hz, 1H, indole C_6-H), 7.13 (s, 1H, thiazoline =CH), 7.31 (d, J = 8.4, 2H, phenyl C_2-H, C_6-H), 7.45 (d, J = 8.7 Hz, 2H, phenyl C_3-H, C_5-H), 7. 63 (t, J = 7.2 Hz, 1H, *N*-phenyl C_4-H), 7.76 (d, J = 7.5 Hz, 2H, *N*-phenyl C_3-H, C_5-H), 10.41 (s, 1H, indole NH); EIMS (70eV): m/z (%): 518 (M^+ +1, 11), 516 (M^+ -1, 13), 340 (12), 319 (13), 284 (7), 282 (9), 205 (8), 203 (10), 180 (21), 168 (28), 164 (8), 162 (31), 149 (20), 141 (28), 139 (100), 138 (14), 136 (46), 134 (28), 133 (11), 121 (32), 113 (18), 111 (70), 109 (35), 108 (23), 107 (12), 94 (16), 75 (59); Anal calcd. for $C_{23}H_{12}Cl_3FN_4OS$ (517): C 53.33, H 2.32, N 10.82; found: C 53.33, H 2.34, N 10.82.

(3*Z*)-5-Fluoro-1*H*-indole-2,3-dione-3-{[(2*Z*)-4-(4-chlorophenyl)-3-(2,4,6-trichlorophe -nyl)-1,3-thiazol-2(3*H*)-ylidene]hydrazone} 6o

Yield 85% as brown crystals; m.p. 258-260 °C; IR (KBr, cm^{-1}): 3195 (NH stretching), 1701 (C=O), 1631 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 6.76 (dd, J = 8.7, 4.5 Hz, 1H, indole C_7-H), 6.86 (dd, 1H, J = 8.7, 3.0 Hz indole C_4-H), 7.05 (td, J = 8.7, 2.7 Hz, 1H, indole C_6-H), 7.22 (s, 1H, thiazoline =CH), 7.32 (d, J = 8.7 Hz, 2H, phenyl C_2-H, C_6-H), 7.49 (d, J = 8.7 Hz, 2H, phenyl C_3-H, C_5-H), 8.06 (s, 2H, *N*-phenyl C_3-H, C_5-H), 10.43, 10.57 (2s, 1H, indole NH); EIMS (70eV) m/z (%): 552 (M^+, 3), 550 (M^+ -2, 3), 518 (76), 516 (53), 344 (23), 342 (57), 340 (56), 319 (24), 284 (19), 282 (19), 205 (44), 203 (70), 179 (29), 170 (25), 169 (17), 168 (85), 162 (43), 149 (66), 147 (23), 145 (36), 141 (23), 139 (90), 138 (26), 136 (71), 135 (38), 134 (55), 133 (37), 124 (20), 122 (39), 121 (88), 113 (29), 111 (100), 109 (53), 108 (71), 94 (55), 89 (50), 75 (73); Anal calcd. for $C_{23}H_{11}Cl_4FN_4OS$ (552): C 50.00, H 1.99, N 10.14; found: C 50.03, H 1.99, N 10.16.

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(3*Z*)-5-Chloro-1*H*-indole-2,3-dione-3-{[(2*Z*)-4-(4-chlorophenyl)-3-(2-methylphenyl)1,3-thiazol-2(3*H*)-ylidene]hydrazone} 7a

Yield 85% as orange yellow fluffy crystals; m.p. 288-290 °C; IR (KBr, cm^{-1}): 3215 (NH stretching), 1719 (C=O), 1615 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.49 (s, 3H, CH_3), 6.76 (2d, *J* = 8.4, 8.1 Hz, 1H, indole C_7-H), 6.94-7.03 (m, 1H, *N*-phenyl C_4-H), 7.07 (s, 1H, thiazoline =CH), 7.18 (d, *J* = 8.4 Hz, 2H, *N*-phenyl C_3-H, C_6-H), 7. 25 (td, *J* = 8.4, 2.1 Hz, 1H, *N*-phenyl C_5-H), 7.32 (d, *J* = 8.7 Hz, 2H, phenyl C_2-H, C_6-H), 7.35 (d, *J* = 8.7 Hz, 2H, phenyl C_3-H, C_5-H), 7. 42 (d, *J* = 9.0 Hz, 1H, indole C_6-H), 7.47, 7.50 (2s, 1H, indole C_4-H), 10.46, 10.54 (2s, 1H, indole NH); EIMS (70eV) m/z (%): 480 (M^++2, 78), 478 (M^+, 100), 301 (19), 300 (29), 299 (54), 288 (25), 287 (18), 286 (100), 284 (58), 228 (32), 180 (17), 178 (27), 170 (17), 168 (50), 152 (18), 151 (13), 150 (25), 149 (57), 139 (35), 137 (17), 136 (21), 133 (19), 111 (21), 91 (98), 89 (25); Anal calcd. for $C_{24}H_{16}Cl_2N_4OS$ (478): C 60.13, H 3.34, N 11.69; found: C 60.15, H 3.34, N 11.70.

(3*Z*)-5-Chloro-1*H*-indole-2,3-dione-3-{[(2*Z*)-4-(4-chlorophenyl)-3-(3-methylphenyl)-1,3-thiazol-2(3*H*)-ylidene]hydrazone} 7b

Yield 86% as orange yellow fluffy crystals; m.p. 290-292 °C; IR (KBr, cm^{-1}): 3224 (NH stretching), 1719 (C=O), 1619 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.27 (s, 3H, CH_3), 6.76 (d, *J* = 8.1, Hz, 1H, indole C_7-H), 7.04-7.08 (m, 2H, *N*-phenyl C_4-H, thiazoline =CH), 7.15-7.27 (m, 6H, *N*-phenyl C_2-H, C_4-H, C_6-H, phenyl C_2-H, C_6-H, indole C_6-H), 7.35 (d, *J* = 8.4 Hz, 2H, phenyl C_3-H, C_5-H), 7. 40 (d, *J* = 2.1 Hz, 1H, indole C_4-H), 10.52 (s, 1H, indole NH); EIMS (70eV) m/z (%):479 (M^++1, 88), 478 (M^+, 78), 301 (16), 300 (19), 299 (41), 288 (23), 287 (15), 286 (80), 284 (37), 228 (27), 180 (19), 178 (25), 170 (17), 169 (13), 168 (42), 165 (13), 152 (24), 151 (15), 150 (15), 149 (54), 139 (34), 138 (17), 137 (21), 136 (16), 133 (23), 124 (15), 111 (17), 102 (14), 91 (100), 89 (28); Anal calcd. for $C_{24}H_{16}Cl_2N_4OS$ (478): C 60.13, H 3.34, N 11.69; found: C 60.14, H 3.36, N 11.66.

(3*Z*)-5-Chloro-1*H*-indole-2,3-dione-3-{[(2*Z*)-4-(4-chlorophenyl)-3-(4-methylphenyl)-1,3-thiazol-2(3*H*)-ylidene]hydrazone} 7c

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Yield 87% as orange yellow fluffy crystals; m.p. 284-286 °C; IR (KBr, cm^{-1}): 3218 (NH stretching), 1720 (C=O), 1625 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.31, 2.34 (2s, 3H, CH_3), 6.76, 6.80 (2d, *J* = 8.4, 8.1, Hz, 1H, indole C_7-H), 7.11 (s, 1H, thiazoline =CH), 7.17-7.26 (m, 3H, *N*-phenyl C_2-H, C_6-H, indole C_6-H), 7.29-7.42 (m, 7H, *N*-phenyl C_3-H, C_5-H, phenyl C_2-H, C_3-H, C_5-H, C_6-H, indole C_4-H), 10.59, 10.60 (2s, 1H, indole NH); EIMS (70eV) m/z (%): 479 (M^+ +1, 100), 477 (93), 301 (14), 300 (15), 299 (33), 288 (16), 286 (70), 284 (32), 228 (22), 178 (15), 168 (35), 149 (38), 91 (68), 89 (16); Anal calcd. for $C_{24}H_{16}Cl_2N_4OS$ (478): C 60.13, H 3.34, N 11.69; found: C 60.13, H 3.33, N 11.71.

(3*Z*)-5-Chloro-1*H*-indole-2,3-dione-3-{[(2*Z*)-4-(4-chlorophenyl)-3-(2-methoxyphenyl) -1,3-thiazol-2(3*H*)-ylidene]hydrazone} 7d

Yield 91% as bright orange crystals; m.p. 250-252 °C; IR (KBr, cm^{-1}): 3096 (NH stretching), 1721 (C=O), 1619 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 3.60, 3.63 (2s, 3H, OCH_3), 6.77 (d, *J* = 8.4 Hz, 1H, *N*-phenyl C_3-H), 6.83 (d, *J* = 8.4 Hz, 1H, indole C_7-H), 7.00-7.12 (m, 2H, *N*-phenyl C_6-H, thiazoline =CH), 7.19 (t, *J* = 7.5 Hz , 2H, *N*-phenyl C_4-H, C_5-H), 7.25 (d, *J* = 8.1 Hz, phenyl C_2-H, C_6-H), 7.35 (d, *J* = 8.4 Hz, 1H, indole C_6-H), 7. 46 (d, *J* = 8.1 Hz, 2H, phenyl C_3-H, C_5-H), 7.50 (s, 1H, indole C_4-H), 10.59, 10.73 (2s, 1H, indole NH); EIMS (70eV) m/z (%): 496 (M^++2, 38), 494 (M^+, 59), 462 (16), 303 (13), 302 (60), 301 (19), 300 (100), 287 (15), 286 (30), 285 (25), 284 (13), 272 (21), 270 (38), 229 (12), 180 (12), 178 (29), 170 (13), 168 (39), 165 (29), 152 (17), 151 (18), 150 (31), 149 (17), 139 (19), 138 (21), 137 (25), 136 (24), 134 (15), 133 (23), 132 (23), 124 (23), 123 (20), 122 (31), 108 (27), 102 (17), 92 (40), 91 (31), 89 (31); Anal calcd. for $C_{24}H_{16}Cl_2N_4O_2S$ (494): C 58.18, H 3.23, N 11.31; found: C 58.17, H 3.24, N 11.30.

(3*Z*)-5-Chloro-1*H*-indole-2,3-dione3-{[(2*Z*)-4-(4-chlorophenyl)-3-(3-methoxyphenyl) 1,3-thiazol-2(3*H*)-ylidene]hydrazone} 7e

Yield 85% as orange crystals; m.p. 296-298 °C; IR (KBr, cm^{-1}): 3195 (NH stretching), 1720 (C=O), 1614 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 3.72 (s, 3H, OCH_3), 6.77, 6.80 (2d, *J* = 8.4, 8.1 Hz, 1H,

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indole C_7-H), 6.93 (td, *J* = 8.4, 2.4 Hz, 1H, *N*-phenyl C_5-H), 7.03 (dd, *J* = 8.4, 2.4 Hz, 1H, *N*-phenyl C_6-H), 7.10, 7.12 (2s, 1H, thiazoline =CH), 7.14-7.19 (m, 1H, *N*-phenyl C_4-H), 7.21 (t, *J* = 2.0 Hz, 1H, *N*- phenyl C_2-H), 7.25 (dd, *J* = 8.1, 2.4 Hz, 1H, indole C_6-H), 7.34 (d, *J* = 8.4 Hz, 2H, phenyl C_2-H, C_6-H), 7.40 (d, *J* = 8.4 Hz, 2H, phenyl C_3-H, C_5-H), 7.51 (d, *J* = 2.1 Hz, 1H, indole C_4-H), 10.58 (s, 1H, indole NH); EIMS (70eV) m/z (%): 496 (M^+ +2, 37), 494 (M^+, 52), 315 (20), 304 (16), 302 (52), 300 (28), 244 (11), 178 (12), 170 (11), 168 (33), 165 (45), 152 (13), 151 (11), 137 (17), 136 (16), 133 (12), 124 (12), 107 (10), 92 (41), 89 (16), 77 (100); Anal calcd. for $C_{24}H_{16}Cl_2N_4O_2S$ (494): C 58.18, H 3.23, N 11.31; found: C 58.16, H 3.22, N 11.33.

(3*Z*)-5-Chloro-1*H*-indole-2,3-dione3-{[(2*Z*)-4-(4-chlorophenyl)-3-(4-methoxyphenyl)-1,3-thiazol-2(3*H*)-ylidene]hydrazone} 7f

Yield 86% as orange crystals; m.p. 294-296 °C; IR (KBr, cm^{-1}): 3224 (NH stretching), 1718 (C=O), 1614 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 3.76 (s, 3H, OCH_3), 6.77, 6.84 (2d, *J* = 8.4, 8.1 Hz, 1H, indole C_7-H), 7.02 (d, *J* = 9.0 Hz, 2H, *N*-phenyl C_2-H, C_6-H), 7.10 (s, 1H, thiazoline =CH), 7.20-7.25 (m, 2H, phenyl C_2-H, C_6-H), 7.31 (dd, *J* = 8.4, 2.1 Hz, 1H, indole C_6-H), 7.34 (d, *J* = 9.0 Hz, 2H, *N*-phenyl C_3-H, C_5-H), 7.38 (d, *J* = 8.4 Hz, 2H, phenyl C_3-H, C_5-H), 7.44, 7.46 (2d, *J* = 2.1, 1.8 Hz, 1H, indole C_4-H), 10.60, 10.77 (2s, 1H, indole NH); EIMS (70eV) m/z (%): 496 (M^++2, 76), 494 (M^+, 100), 468 (18), 466 (30), 317 (16), 316 (25), 315 (34), 304 (25), 303 (23), 302 (72), 301 (35), 300 (30), 258 (12), 257 (16), 246 (11), 244 (42), 214 (12), 180 (17), 179 (14), 177 (26), 170 (28), 169 (19), 168 (75), 165 (66), 152 (27), 151 (23), 150 (52), 147 (12), 139 (32), 138 (13), 137 (20), 136 (12), 134 (20), 133 (25), 125 (11), 124 (22), 122 (13), 115 (12), 111 (16), 92 (40), 89 (21), 77 (61); Anal calcd. for $C_{24}H_{16}Cl_2N_4O_2S$ (494): C 58.18, H 3.23, N 11.31; found: C 58.19, H 3.23, N 11.35.

(3*Z*)-5-Chloro-1*H*-indole-2,3-dione3-{[(2*Z*)-4-(4-chlorophenyl)-3-(2-fluorophenyl)-1,3-thiazol-2(3*H*)-ylidene]hydrazone} 7g

Yield 86% as yellowish brown crystals; m.p. 290-292 °C; IR (KBr, cm^{-1}): 3125 (NH stretching), 1720 (C=O), 1599 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 6.76 (dd, *J* = 8.4, Hz, 1H, indole C_7-H), 7.14 (s, 1H, thiazoline =CH), 7.21 (td, *J* = 8.4, 2.1 Hz, 2H, *N*-phenyl C_4-H, C_5-H), 7.26-7.31 (m, 3H, phenyl

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C_2-H, C_6-H, indole C_6-H), 7.36 (d, J = 2.4 Hz, 1H, indole C_4-H), 7.41 (d, J = 8.4 Hz, phenyl C_3-H, C_5-H), 7.58 (m, 1H, *N*-phenyl C_6-H), 7.67 (t, J = 7.2 Hz, 1H, *N*-phenyl C_3-H), 10.49, 10.59 (2s, 1H, indole NH); EIMS (70eV) m/z (%): 484 (M^++2, 63), 483 (M^++1, 28), 482 (M^+, 92), 456 (6), 454 (6), 419 (6), 304 (9), 292 (36), 291 (18), 290 (100), 241 (21), 234 (10), 232 (37), 180 (10), 178 (16), 170 (24), 169 (15), 168 (73), 167 (10), 165 (27), 154 (9), 153 (87), 152 (13), 150 (11), 139 (33), 138 (27), 137 (37), 136 (48), 134 (19), 133 (33), 124 (14), 122 (8), 113 (9), 111 (26), 110 (9), 102 (12), 95 (28), 89 (21), 75 (33); Anal calcd. for $C_{23}H_{13}Cl_2FN_4OS$ (482): C 57.14, H 2.69, N 11.59; found: C 57.13, H 2.66, N 11.60.

(3*Z*)-5-Chloro-1*H*-indole-2,3-dione3-{[(2*Z*)-4-(4-chlorophenyl)-3-(3-fluorophenyl)-1,3-thiazol-2(3*H*)-ylidene]hydrazone} 7h

Yield 88% as orange crystals; m.p. 258-260 °C; IR (KBr, cm^{-1}): 3008 (NH stretching), 1718 (C=O), 1584 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 6.76, 6.79 (2d, J = 8.4, 8.1 Hz, 1H, indole C_7-H), 7.07-7.11 (m, 2H, *N*-phenyl C_6-H, thiazoline =CH), 7.18-7.32 (m, 4H, *N*-phenyl C_2-H, C_4-H, C_5-H, indole C_6-H), 7.34 (d, J = 8.4 Hz, 2H, phenyl C_2-H, C_6-H), 7.40 (d, J = 8.4 Hz, 2H, phenyl C_3-H, C_5-H), 7.42 (s, 1H, indole C_4-H), 10.52, 10.58 (2s, 1H, indole NH); EIMS (70eV) m/z (%): 484 (M^++2, 17), 483 (M^++1, 7), 482 (M^+, 22), 292 (11), 290 (34), 232 (21), 195 (15), 178 (8), 170 (8), 168 (21), 167 (10), 165 (14), 153 (16), 152 (8), 141 (52), 140 (18), 139 (100), 138 (25), 136 (20), 125 (13), 124 (13), 113 (30), 112 (10), 111 (84), 110 (11), 102 (14), 95 (41), 89 (17), 75 (70); Anal calcd. for $C_{23}H_{13}Cl_2FN_4OS$ (482): C 57.14, H 2.69, N 11.59; found: C 57.10, H 2.67, N 11.64.

(3*Z*)-5-Chloro-1*H*-indole-2,3-dione3-{[(2*Z*)-4-(4-chlorophenyl)-3-(4-fluorophenyl)-1,3-thiazol-2(3*H*)-ylidene]hydrazone} 7i

Yield 87% as brown crystals; m.p. 249-251 °C; IR (KBr, cm^{-1}): 3020 (NH stretching), 1739 (C=O), 1558 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 6.76, 6.78 (2d, J = 8.4, 8.1 Hz, 1H, indole C_7-H), 7.07, 7.11 (2s, 1H, thiazoline =CH), 7.21 (d, J = 8.7 Hz, 2H, phenyl C_2-H, C_6-H), 7.25 (dd, J = 8.4, 2.1 Hz, 2H, *N*-phenyl C_2-H, C_6-H),7.31-7.41 (m, 5H, *N*- phenyl C_3-H, C_5-H, phenyl C_3-H, C_5-H, indole C_6-H), 7.54 (m, 1H, indole C_4-H), 10.51, 10.57 (2s, 1H, indole NH); EIMS (70eV) m/z (%): 484 (M^++2,

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66), 483 (M^++1, 65), 467 (17), 454 (6), 305 (11), 304 (17), 303 (25), 292 (33), 291 (13), 290 (99), 288 (18), 241 (11), 234 (15), 232 (41), 178 (19), 170 (14), 169 (9), 168 (45), 167 (10), 165 (21), 154 (14), 153 (73), 152 (9), 150 (11), 141 (19), 139 (87), 138 (25), 137 (26), 136 (35), 134 (118), 133 (17), 125 (13), 124 (14), 121 (12), 115 (12) 111 (52), 102 (13), 95 (100), 89 (24), 75 (68); Anal calcd. for $C_{23}H_{13}Cl_2FN_4OS$ (482): C 57.14, H 2.69, N 11.59; found: C 57.14, H 2.66, N 11.58.

(3*Z*)-5-Chloro-1*H*-indole-2,3-dione-3-{[(2*Z*)-4-(4-chlorophenyl)-3-(2,4-difluorophenyl)-1,3-thiazol-2(3*H*)-ylidene]hydrazone} 7j

Yield 73% as orange crystals; m.p. 238-240 °C; IR (KBr, cm^{-1}): 3100 (NH stretching), 1703 (C=O), 1614 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 6.78 (d, J = 8.1 Hz, 1H, indole C_7-H), 7.08 (s, 1H, thiazoline =CH), 7.13-7.19 (m, 2H, *N*-phenyl C_5-H, C_6-H), 7.25 (d, J = 8.4 Hz, 2H, phenyl C_2-H, C_6-H), 7.30 (dd, J = 8.4, 2.4 Hz, 1H, indole C_6-H), 7.39-7.45 (m, 3H, phenyl C_3-H, C_5-H, indole C_4-H), 7.65 (m, 1H, *N*-phenyl C_3-H), 10.50, 10.60 (2s, 1H, indole NH); EIMS (70eV) m/z (%): 502 (M^++2, 46), 501 (M^++1, 14), 500 (M^+, 62), 310 (31), 309 (14), 308 (100), 303 (11), 259 (10), 252 (10), 250 (49), 180 (12), 178 (25), 171 (70), 170 (17), 169 (17), 168 (62), 167 (14), 165 (31), 152 (14), 150 (13), 140 (13), 139 (41), 138 (29), 137 (43), 136 (41), 134 (18), 133 (20), 125 (11), 124 (17), 115 (10), 113 (49), 111 (68), 102 (18), 101 (17), 100 (10), 89 (42), 75 (52); Anal calcd. for $C_{23}H_{12}Cl_2F_2N_4OS$ (500): C 55.09, H 2.40, N 11.18; found: C 55.07, H 2.42, N 11.20.

Biological assays

Cytotoxicity (*in vitro*)

Cytotoxic activity was determined by a brine shrimp (*Artemia salina*) lethality bioassay described in our published research paper.[30] Etoposide, a potent anticancer drug, was used as a reference standard.

Phytotoxicity (*in vitro*)

Phytotoxicity potential was determined in accordance with the literature protocol given in our published research article.[33]

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Antifungal activity (*invitro*)

Antifungal studies were carried out according to the literature protocol provided in our published research paper.[31]

Urease inhibition (*in vitro)*

Urease inhibitory activity was determined in accordance with the literature protocol given in our published research article.[41]

Molecular docking studies

Structure selection and preparation

Molecular docking studies were conducted to investigate the putative interactions of compounds **5a-n**, **6a-o** and **7a-j** in complex with the urease enzyme. In order to perform efficient docking studies, the crystallographic structure of Jack bean urease (PDB ID: 3LA4) was obtained from the RCSB PDB database.[42] Prior to molecular docking studies, the structures of the enzyme and the compounds were prepared as follows. The enzyme structure was protonated with the Protonate 3D[43] algorithm implemented in the molecular modeling tool MOE.[44] The structure was energy minimized using Amber99 force field, including all the crystallographic solvent molecules. The backbone atoms were restrained with a small force in order to avoid collapse of the binding pockets during energy minimization calculations. After minimization, the water molecules and co-crystallized bound compounds were removed.

Compounds preparation

The 3D structural coordinates were generated for all the compounds using MOE followed by assignment of protonation and ionization states in physiological pH range by using the "wash"

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module. Afterwards, the compounds' structures were energy minimized with the MMFF94x force field for docking studies.[44]

Docking studies

Docking studies of all the compounds were performed using LeadIT from BioSolveIT, GmbH Germany.[45] Receptor was loaded by Load or Prepare Receptor utility of the LeadIT software, while selecting the metal ions as part of the receptor. The binding site for Jack bean urease was defined in 8.0 Å spacing of the amino acid residues surrounding the bound phosphate. By FlexX utility of LeadIT, docking of the compounds was performed. Default docking parameters were not modified and top 30 highest scoring docked positions were kept for further analysis. The Discovery studio visualizer v4 was used for visualizing the results.[46] Using HYDE visual affinity[47] program of LeadIT, the binding mode analysis of the docked poses was evaluated. Binding free energy i.e. ΔG was determined for each pose. Poses with lowest ΔG values were considered as the most stable ones with highest affinity for interaction with the receptor.

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Graphical abstract

Synthesis, biological evaluation and docking studies of some novel isatin-3-hydrazonothiazolines

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