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Research paper

Synthesis and evaluation of isatin-β-thiosemicarbazones as novel agents against antibiotic-resistant Gram-positive bacterial species

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE) have caused an increasing mortality rate, which means that antibiotic resistance is becoming an important health issue. In the course to screen new agents for resistant bacteria, we identified that a series of isatin-β-thiosemicarbazones (IBTs) could inhibit the growth of MRSA and VRE. This was the first time that the "familiar" IBT compounds exhibited significant anti Gram-positive pathogen activity. Against a clinical isolated MRSA strain, 20 of the 51 synthesized compounds showed minimum inhibitory concentration (MIC) data of 0.78 mg/L and another 12 novel compounds had MICs of 0.39 mg/L. Moreover, these compounds also inhibited *Enterococcus faecalis* and VRE at similar levels, indicating that IBTs might have different mode of action compared with vancomycin. For these IBTs, comparative field analysis (CoMFA) models were further established to understand the structure–activity relationships in order to design new compounds from steric and electrostatic contributions. This work has suggested that IBTs can be considered as potential lead compounds to discover antibacterial inhibitors to combat drug resistance.

1. Introduction

The antibiotics have contributed enormously to treat bacterial infection because they decrease the mortality rate significantly [1]. However, with the overuse of antibiotics, bacterial resistance appeared gradually. In 1961, the first case of methicillin-resistant *Staphylococcus aureus* (MRSA) was reported, for which only few antimicrobial agents were effective [2]. In 1988, vancomycin resistant *Enterococci* (VRE) was also identified that increased the significance of this problem [3]. It is for decades that antimicrobial resistance has become a public health threat around the world [4]. The emergence of New Delhi metallo-beta-lactamase-1 (NDM-1) in 2010 indicated that "superbug" era was approaching [5]. Nowadays MRSA stands for one of the most serious antibiotic-resistant infections and it causes an increasing mortality in hospital.

Currently, there are only a few clinical drugs available for the treatment of MRSA, including vancomycin, daptomycin, linezolid, tigecycline and ceftobiprole [6–10]. Nevertheless these clinical drugs have limitation themselves. Vancomycin and daptomycin have very complicated chemical structures, and their starting materials rely heavily on biological fermentation; tigecycline and ceftobiprole can be synthesized chemically but their synthetic routes require many steps. On the other hand, these agents have been reported to display some side effects that cannot be neglected, including red-man syndrome, muscle toxicity, myelosuppression and nausea [8], which has hindered their further use. There exists a great need to discover antibacterial agents with totally new chemical structures to defeat the increasing bacterial resistance.

Isatin (Fig. 1A) is an important endogenous natural product with versatile biological activities and it can be found in the mammalian brain, peripheral tissues and body fluids [11]. This simple compound is also a metabolite by marine bacteria from some shrimp

Abbrevations: MRSA, methicillin-resistant *Staphylococcus aureus*; VRE, vancomycin-resistant *Enterococcus*; IBTs, isatin-β-thiosemicarbazones; CoMFA, comparative field analysis; NDM-1, New Delhi metallo-beta-lactamase-1; MAO-B, monoamine oxidase B; MIC, minimum inhibitory concentration; ppm, parts per million; DMSO, dimethyl sulfoxide; TMS, tetramethylsilane; IR, infrared; MHA, Mueller-Hinton agar; MHB, Mueller-Hinton Broth; LOO, leave-one-out; PLS, partial least squares.

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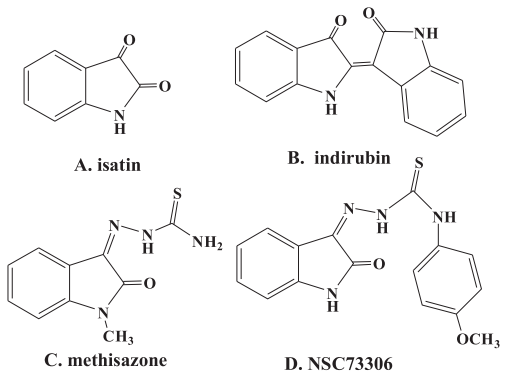


Fig. 1. Structures of isatin (A), indirubin (B), methisazone (C) and NSC73306 (D).

embryos, which makes the embryos remarkably resistant to infection caused by pathogenic fungus [12]. Isatin was also identified as a potent inhibitor of human mitochondrial monoamine oxidase B (MAO-B) [13]. The derivatives of isatin possess a wide range of biological activities, for instance, antimycobacterial, anti-HIV, anticancer, anticonvulsant, antiviral, antiinflammatory and analgesic activities [14–17]. However, there is not any other report that isatin derivatives are desirable inhibitors of MRSA.

There are some drugs that contain isatin as an active scaffold. Two representative examples are indirubin (Fig. 1B) and methisazone (Fig. 1C). Indirubin is a bioactive ingredient from the Chinese traditional medicine Dang Gui Long Hui Wan that has potent antineoplastic activity such as treating chronic myelogenous leukemia [18]. Methisazone, 1-methylisatin β-thiosemicarbazone, is effective against smallpox infection from the 1960s [19]. The structural optimization of methisazone is still under way although their precise viral target and mechanism of action is still unknown [20].

Methisazone has been used as an anti smallpox drug for nearly 50 years, indicating that this type of compounds might be biologically safe. In 1984, Omar et al. synthesized some isatin β-thiosemicarbazone (IBT) compounds and they had not observed their activity against *S. aureus* [21]. Recently, Pervez et al. had also synthesized some IBT derivatives and evaluated their antibacterial activities [22]. The compounds in their study displayed some inhibition against human urease, but their activities upon *S. aureus* were all very weak even at 10,000 mg/L concentration. Also recently, Gottesman et al. found that some IBTs were promising candidates for treating cancer of multidrug resistance by selectively killing P-glycoprotein-expressing cells (NSC73306, Fig. 1D) [23]. However, Gottesman et al. had not evaluated the antibacterial activities for any of their IBT compounds.

In an effort to discover novel anti-MRSA agents, we firstly synthesized 10 (**3-1** to **3-10**) IBT compounds and evaluated their antibacterial activity. Excitingly, one compound (**3-2**) inhibited a clinical isolated MRSA (Chaoyang) strain significantly, with the minimum inhibitory concentration (MIC) value of 0.78 mg/L, which was better than that of vancomycin (1.0 mg/L). This is the first time that the IBTs possess strong anti-MRSA activity in vitro. Encouraged by this result, another 41 (**3–11** to **3–51**) novel IBTs were further synthesized and 12 of them showed MICs of 0.39 mg/L against the tested MRSA strain. The second generation of IBTs was subsequently tested for their broad-spectrum antibacterial activities against other antibiotic-resistant isolates. These compounds also had good efficiency on a tested VRE strain, implying their distinct mode of action. Obviously, the IBTs are very "old" compounds, however, their strong anti-MRSA activity is fairly novel compared with other reported biological activities [19,23]. This study has provided meaningful information to discover novel lead candidates of Gram-positive bacteria therapeutics.

2. Results and discussion

2.1. Chemistry of the IBT compounds and crystal structure

The IBT compounds were synthesized by the straightforward condensation of isatin and thiosemicarbazide, a method which has been used for over 50 years [24]. Compounds **3-4**, **3-6**, **3-7**, **3-8** and **3-10** have been reported elsewhere [25,26], however their characterization data were not available. Compounds **3-5** and **3-9** have been characterized by us, but the publication was in Chinese [27]. We therefore list here the analytical data for all the target compounds. Compound **3-1** was recrystallized from ethanol/dichloromethane to give yellow crystals suitable for X-ray single-crystal diffraction. Cif file for **3-1** is also given in the supplemental data. Generally, the structure of IBT is similar to other reported isatin derivatives, forming an intramolecular H-bond (O1 and the H atom linked to N3) [17,23]. Thus, the IBT structures are not *E* isomers, but *Z* isomers. The crystal structure of **3-1** is somewhat interesting in that it is a duplex crystal. As can be seen, the phenyl ring attached to N4 atom possesses alternative poses (Fig. 2).

2.2. The in vitro antibacterial activities

We initially synthesized 10 IBT compounds (**3-1** to **3-10**) and evaluated their antibacterial activities. The bacterial strains of *S. aureus* (ATCC 6538) and *Bacillus subtilis* (ATCC 6633) were used as standard Gram-positive strains. A clinical isolated strain (Chaoyang) was used for the anti-MRSA assay. It was interesting that these compounds exhibited desirable antibacterial activities. Compound **3-2** displayed the best biological activity, the MICs of which were 0.78 mg/L, 1.56 mg/L and ≤0.78 mg/L for MRSA, *S. aureus* and *B. subtilis*, respectively (Table 1). The other nine compounds also showed good antimicrobial activities, MICs ranging from 1.56 mg/L to 25 mg/L for the tested MRSA and *Staphylococcus* strains. Except **3-3** and **3-6**, all compounds inhibited the *B. subtilis* strain completely at a concentration of no more than 1.56 mg/L. In comparison, the MIC values for clinical antibiotic vancomycin were 1.0 mg/L, 1.0 mg/L and 0.5 mg/L against these bacterial strains. From a view of the structure–activity relationships (for MIC against MRSA Chaoyang), a single halogen atom at 7-position of isatin ring (R_1) gives better activity when R_2 is a hydrogen atom. For such a case when R_1 is a chlorine atom, the compound has the best potency (**3-2**). However, when isatin is substituted at 5-position (R_1), whether there is a halogen or hydrogen atom at R_2 position, the activity becomes weaker. Therefore **3-2** is regarded as a lead compound for further structural optimization.

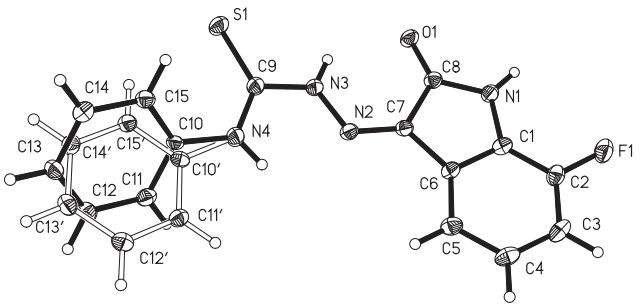
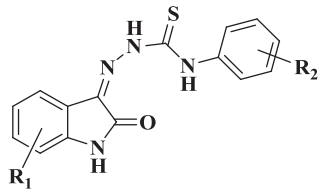


Fig. 2. Duplex crystal structure of IBT compound **3-1**.

Table 1
Chemical structures of the first batch of isatin β-thiosemicarbazone compounds and their antibacterial activities.



Compd.	R_1	R_2	MIC (mg/L) for MRSA Chaoyang	MIC (mg/L) for SA[a] ATCC 6538	MIC (mg/L) for BS[a] ATCC 6633
3-1	7-F	H	1.56	3.12	≤0.78
3-2	7-Cl	H	0.78	1.56	≤0.78
3-3	5-Cl	4-F	3.12	12.5	6.25
3-4	5-Br	H	6.25	6.25	≤0.78
3-5	5-NO_2, 7-Br	4-F	3.12	1.56	≤0.78
3-6	5-F	4-F	25	25	25
3-7	H	H	3.12	3.12	1.56
3-8	H	4-F	3.12	6.25	1.56
3-9	5-Br, 7-Br	4-F	6.25	12.5	1.56
3-10	5-NO_2	4-F	12.5	6.25	25
Vancomycin	–		1.0	1.0	0.5

[a] SA = *Staphylococcus aureus*, BS=*Bacillus subtilis*.

As far as we know, the isatin β-thiosemicarbazone compounds seldom displayed good activities against Gram-positive bacteria. To study the structure–activity relationships, we further synthesized another 41 novel IBT compounds (**3-11** to **3-51**) based on the chemical structure of **3-2** (Table S1). These compounds have halides or methyl substituent at 7-position of the isatin moiety (R_1), and the phenyl ring attached to the thiourea bridge are also substituted by a single group of halides, methyl or methoxy group (R_2). As can be seen in Table S1, the second generation of the IBT compounds demonstrated strong activities, indicating that the structural modifications were successful. The MIC values of compounds **3-11**, **3-12**, **3-15**, **3-16**, **3-22**, **3-24**, **3-29**, **3-30**, **3-32**, **3-35**, **3-36** and **3-37** were 0.39 mg/L against MRAS (Chaoyang) strain, twice as potent as that of **3-2**. Compounds **3-13**, **3-14**, **3-18**, **3-19**, **3-21**, **3-25**, **3-26**, **3-27**, **3-28**, **3-38**, **3-39**, **3-41**, **3-42**, **3-44**, **3-45**, **3-46**, **3-48**, **3-50** and **3-51** had equivalent MICs with **3-2**, which inhibited MRSA (Changyang) strain completely at 0.78 mg/L. In comparison with **3-2**, when R_1 is a halogen atom at 7-position of isatin ring, another halogen atom or methyl group at 4-position of the phenyl ring (R_2) provides an improved MIC; however, if such a R_2 group is at 3-position, some compounds have improved inhibition while some others remain at the same level with **3-2**. When R_1 is a methyl group at 7-position and R_2 is a halogen atom at 4-position, the compound exhibits same activity with **3-2**. It can also be seen that when R_2 is a methoxy group at 2-position, the compound decreases its inhibition significantly, although when R_2 is a methyl group, the activity of the compound is similar to **3-2**.

It was interesting that the anti-MRSA (Chaoyang) activities were better than the activities against *S. aureus* ATCC strain. Compounds **3-29** and **3-30** had the best antibacterial activity against *S. aureus* ATCC strain, the MICs of which were 0.78 mg/L. However, compounds **3-34**, **3-40**, **3-43**, **3-47** and **3-49** could not inhibit *S. aureus* strain completely even at 100 mg/L concentration. The new IBT compounds were also very potent against *B. subtilis*, and 30 of the 41 compounds had MIC values of 0.78 mg/L or less.

To exploit the application of these compounds, we further evaluated the antibacterial activities of the second generation of IBT compounds together with **3-2** against a series of MRSA strains (309-4, 6281, 309-8, 6-42, 8-21, 309-3, 309-1, 309-7, 8-24 and 309-6) from different clinical sources. Besides good inhibition against the MRSA (Chaoyang) strain, the compounds also displayed significant inhibition against other MRSA isolates. Although the MIC values varied among different MRSA strains (Table 2), compounds **3-11**, **3-12**, **3-13**, **3-15**, **3-16**, **3-22**, **3-29**, **3-30**, **3-35** and **3-36** had MICs of ≤1.56 mg/L. This was in agreement with the data for MRSA (Chaoyang) strain. For **3-15**, **3-16** and **3-35**, each of the compounds had the MIC value of 0.39 mg/L against one clinical strain. Compound **3-30** had MICs of 0.39 mg/L against two clinical MRSA strains and compound **3-29** had MIC data of 0.39 mg/L against three MRSA strains. On the other hand, both **3-34** and **3-49** had MICs >100 mg/L against all the different clinical MRSA strains. For the control drug, the MIC values for vancomycin against all the MRSA strains were 1.0 mg/L, and for methicillin, the MICs were >200 mg/L against all the MRSA strains.

Since the chemical structure of IBT is quite different from that of vancomycin, this family of novel anti-MRSA inhibitors might have distinct mode of action. Bearing this in mind, we hence tested the compounds against *Enterococcus faecalis* and VRE strains. As can be seen from Table 2, the MIC of vancomycin for *E. faecalis* was 2 mg/L and for VRE-309 was >8 mg/L. Surprisingly, all the IBTs showed equal or even better inhibition against VRE compared with the inhibition against *E. faecalis*. For compounds **3-11**, **3-15**, **3-16**, **3-18**, **3-19**, **3-27**, **3-32**, **3-35**, **3-36** and **3-37**, their MICs for VRE were 1.56 mg/L and their MICs for *E. faecalis* were 3.12 mg/L. It was exciting that for compounds **3-29** and **3-30**, their MICs against *E. faecalis* and VRE were both 1.56 mg/L.

The compounds were also screened their inhibition against Gram-negative *Pseudomonas aeruginosa* (PAO1) and *Klebsiella pneumoniae* (KP-249). However, no compounds could inhibit these bacteria strains completely even at 100 mg/L concentration. Thus it seems the IBT compounds are active upon the Gram-positive bacteria, whether they are susceptible or resistant strains towards the clinical online antibiotics methicillin and vancomycin. It is also interesting that the MICs of the IBT compounds for resistant strains are sometimes lower than those for the susceptible strains, which deserves further investigation on their inhibition mechanism.

2.3. Three dimensional structure–activity relationships

Comparative field analysis (CoMFA) is a tool to generate 3D contour models to quantitatively analyze the structure–activity relationships of bioactive compounds by steric and electrostatic

Table 2
Selected MIC data (mg/L) of the second generation of IBT compounds against *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, clinical isolated VRE and various MRSA strains.

Compd.	R_1	R_2	*E. faecalis*	VRE-309	PA	KP-249	MRSA										
							Chaoyang	309-4	6281	309-8	6-42	8-21	309-3	309-1	309-7	8-24	309-6
3-2	7-Cl	H	6.25	3.12	>100	>100	0.78	1.56	1.56	1.56	3.12	3.12	6.25	6.25	0.78	1.56	6.25
3-11	7-Cl	4-Cl	3.12	1.56	>100	>100	0.39	0.78	1.56	1.56	0.78	0.78	1.56	0.78	0.78	1.56	1.56
3-12	7-Cl	4-CH_3	6.25	3.12	>100	>100	0.39	0.78	1.56	0.78	0.78	0.78	0.78	0.78	0.78	1.56	1.56
3-13	7-Cl	2-CH_3	6.25	3.12	>100	>100	0.78	1.56	1.56	1.56	0.78	0.78	1.56	1.56	0.78	1.56	1.56
3-15	7-Br	4-F	3.12	1.56	>100	>100	0.39	0.78	1.56	0.78	0.78	0.78	0.78	0.78	0.39	1.56	1.56
3-16	7-Br	4-Cl	3.12	1.56	>100	>100	0.39	0.78	1.56	0.78	0.78	0.78	0.78	0.78	0.39	1.56	1.56
3-18	7-CH_3	4-Cl	3.12	1.56	>100	>100	0.78	1.56	3.12	1.56	1.56	1.56	3.12	1.56	1.56	1.56	3.12
3-19	7-CH_3	4-F	3.12	1.56	>100	>100	0.78	1.56	3.12	1.56	1.56	1.56	1.56	1.56	0.78	1.56	1.56
3-22	7-F	3-CH_3	3.12	3.12	>100	>100	0.39	0.78	1.56	0.78	0.78	0.78	0.78	0.78	0.78	1.56	1.56
3-27	7-Cl	3-Cl	3.12	1.56	>100	>100	0.78	0.78	1.56	1.56	0.78	1.56	1.56	1.56	0.78	1.56	3.12
3-29	7-F	3-F		1.56	>100	>100	0.39	0.39	1.56	0.78	0.39	0.78	0.78	0.78	0.39	0.78	1.56
3-30	7-F	3-Cl	1.56	1.56	>100	>100	0.39	0.78	1.56	0.78	0.39	0.78	0.78	0.78	0.39	0.78	1.56
3-32	7-Cl	3-F	3.12	1.56	>100	>100	0.39	0.78	3.12	1.56	0.78	1.56	1.56	1.56	0.78	1.56	1.56
3-34	7-Br	2-OCH_3	>100	>100	>100	>100	25	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
3-35	7-Br	3-F	3.12	1.56	>100	>100	0.39	0.78	1.56	0.78	0.78	0.78	0.78	0.78	0.39	0.78	1.56
3-36	7-Br	3-Cl	3.12	1.56	>100	>100	0.39	0.78	1.56	1.56	0.78	1.56	1.56	1.56	0.78	1.56	1.56
3-37	7-Br	4-Br	3.12	1.56	>100	>100	0.39	0.78	3.12	1.56	0.78	1.56	1.56	1.56	0.78	1.56	1.56
3-40	7-I	2-OCH_3	>100	>100	>100	>100	6.25	12.5	25	12.5	6.25	12.5	25	12.5	6.25	12.5	25
3-49	7-F	2-OCH_3	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
Van[b]			2.0	>8.0	ND[a]	ND		1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Met[b]			ND	ND	ND	ND		>200	>200	>200	>200	>200	>200	>200	>200	>200	>200
Cip[b]			ND	ND	1.0	2.0		ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

[a] ND = not determined.
[b] Van = cancomycin, Met = methicillin, Cip = ciprofloxacin.

contributions [28]. Since the MIC data against MRSA (Chaoyang) were determined for all the IBTs, they were used to calculate the biological data *D* for the molecular simulation. The leave-one-out q^2 is 0.601 at the optimum components of 6, and the non-cross-validated r^2 is 0.903, with a standard error of estimate of 0.165 and an F value of 55.963. The steric and electrostatic contributions are 49.6% and 50.4%, respectively. From Table S3, it can be seen that except **3-3**, the activities of all the other 5 IBTs in the test set were reasonably predicted, suggesting that the CoMFA model had predictive ability.

Compound **3-2** was used to illustrate the steric and electrostatic contour maps. For the steric contour map (Fig. 3A), a bulky group is favorable for better anti-MRSA activity in the green contour region and such a group is likely to decrease the inhibition in the yellow contour space. For the electrostatic contour map (Fig. 3B), in the blue contour region, an increase in the positive charge will result in an increase of activity, whereas in the red contour region, negative charge is favorable to enhance the activity. From the 3D contour maps, it can be seen a halogen atom is a key structural feature at the 7-position of isatin for desirable anti-MRSA activity of this series of IBT compounds, from both the steric and the electrostatic requirements. Thus we will design new molecules that have a $-CF_3$ or $-OH$ group at 7-position of isatin ring to see if more potent biological activity can be observed. On the other hand, a steric group is unfavorable at *meta*-position of the phenyl ring (for the R_2 substituent), this can be explained by the fact that compounds **3-34**, **3-40** and **3-49** all have a $-OCH_3$ group at this position and their activities are very weak. The CoMFA contour map has provided valuable information for further structural optimization of this family of new anti-MRSA inhibitors.

From our structure–activity relationship analysis, a halogen substitution at 7-position of isatin is a key structural requirement for IBTs to possess good anti-MRSA activity. Although the compounds reported by Omar and Pervez et al. are very close to our IBT compounds, they do not have any halogens at this position [21,22]. This might be one reason that the previously reported IBTs do not have strong activity against *S. aureus*.

3. Conclusion

Invention of novel antibiotics is a big challenge owing to the limited resource from microbial metabolites. With the prevalence of diseases caused by superbug infection, this task has turned to be more and more urgent for medicinal research. We present here for the first time that some novel isatin-β-thiosemicarbazones are strong inhibitors of Gram-positive bacteria, especially for MRSA and VRE.

The IBTs studied here were active against the selected Gram-positive bacteria and non-active against the tested Gram-negative strains. This “new” family of antibacterial compounds exhibited desirable activity against dozens of clinical isolated MRSA strains, and some of them also had potent inhibition against a VRE strain. This suggests that the IBTs might inhibit the Gram-positive bacteria through a different mechanism compared with vancomycin, which deserves further investigation. Further structural optimization is under way to get compounds with enhanced activity based on the 3D structure–activity relationships. The present research herein has provided meaningful information to discover anti-MRSA agents for potential clinical use.

4. Experimental section

4.1. General synthesis and instruments

The substituted isatins **1** and substituted *N*-phenylhydrazinecarbothioamide **2** were commercially procured from Alfa-Aesar, Sigma–Aldrich, J&K Chemical or Shanghai Aladdin Reagents. The solvents and other materials for the organic synthesis were purchased from some local chemical suppliers in Tianjin. All solvents and liquid reagents were dried by standard methods in advance and distilled before use. Melting points were determined using an X-4 melting apparatus and were uncorrected. ^{1}H NMR spectra were obtained using a 300 MHz Bruker AC-P300 spectrometer or a 400 MHz Varian Mercury Plus 400 spectrometer. The chemical shift values (δ) for the NMR spectra were reported as parts

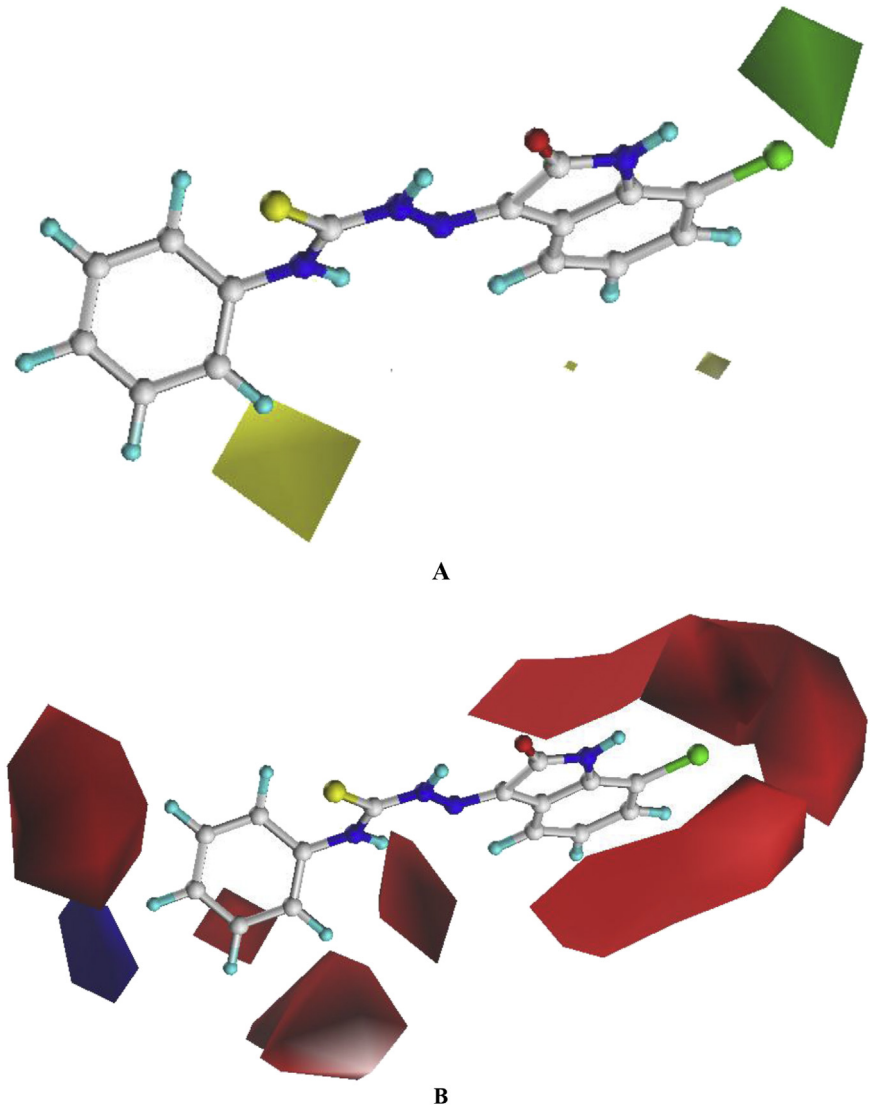


Fig. 3. Three-dimensional contour maps for the CoMFA model of the IBT compounds. Sterically favored and disfavored regions are shown in green and yellow in map **A**. Electrostatic favored and disfavored regions are shown in blue and red in map **B**. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

per million (ppm), using deuterated dimethyl sulfoxide (DMSO-d_6) as the solvent and tetramethylsilane (TMS) as an internal reference standard. The infrared (IR) spectra were recorded with a Bruker Equinox 55 spectrophotometer in KBr pellets. Elemental analyses were determined on a Yanaca CHN Corder MT-3 elemental analyzer. Mass spectra were recorded on a Thermo Finnigan LCQ Advantage LC/mass detector instrument. Single-crystal X-ray diffraction analyses were performed on a Bruker Smart 1000 CCD diffractometer.

4.2. Test compounds preparation

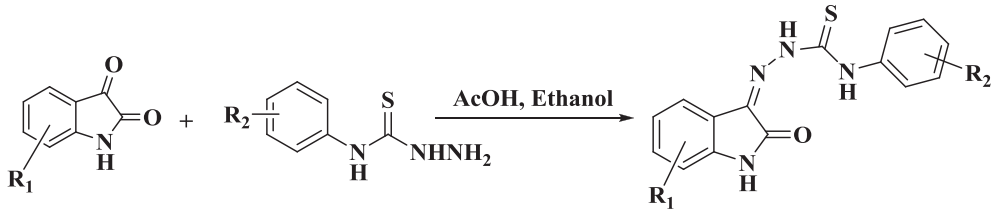
The chemical synthesis route of the target IBT compounds is illustrated in Scheme 1. The IBTs **3** were synthesized by the reaction of isatin **1** and *N*-phenylhydrazinecarbothioamide **2** in an aqueous solution of ethanol and acetic acid under reflux [22,23]. Generally, equivalent **1** and **2** was dissolved in a solution of acetic acid/water/alcohol solution, and then the mixture was refluxed for approximate 5–6 h. The reaction mixture was subsequently cooled to room temperature and in most cases a yellow or orange solid precipitated out. This precipitate was filtered, washed with water, and then dried to give a crude product. Recrystallization from ethanol gave pure solid in high yields.

4.2.1. (Z)-2-(7-Fluoro-2-oxoindolin-3-ylidene)-N-phenylhydrazinecarbothioamide (**3-1**)

Yield 67%; m.p.: 222–224 °C; yellow solid; ^{1}H NMR (DMSO-d_6, 400 MHz), δ 12.82 (s, 1H, NNH), 11.85 (s, 1H, NH), 10.96 (s, 1H, SCNH), 7.70 (d, 1H, J = 8.0 Hz, Ar_1–H_4), 7.66 (d, 2H, J = 8.0 Hz, Ar_2–H_2 and Ar_2–H_6), 7.61 (d, 1H, J = 8.4 Hz, Ar_1–H_6), 7.50 (t, 2H, J = 8.0 Hz, Ar_2–H_3 and Ar_2–H_5), 7.36 (t, 1H, J = 8.4 Hz, Ar_2–H_4), 7.19 (t, 1H, J = 7.5 Hz, Ar_1–H_5); Elemental analysis calculated for $C_{15}H_{11}FN_4OS$, C: 57.31, H: 3.53, N: 17.82, found C: 56.92, H: 3.64, N: 17.81.

4.2.2. (Z)-2-(7-Chloro-2-oxoindolin-3-ylidene)-N-phenylhydrazinecarbothioamide (**3-2**)

Yield 75%; m.p.: 239–241 °C; yellow solid; ^{1}H NMR (DMSO-d_6, 300 MHz), δ 12.74 (s, 1H, NNH), 11.71 (s, 1H, NH), 10.91 (s, 1H, SCNH), 7.76 (d, 1H, J = 8.0 Hz, Ar_1–H_4), 7.60 (d, 2H, J = 8.1 Hz, Ar_2–H_2 and Ar_2–H_6), 7.44 (m, 3H, J = 8.4 Hz, Ar_1–H_5, Ar_2–H_3 and Ar_2–H_5), 7.29 (t, 1H, J = 7.5 Hz, Ar_1–H_6), 7.14 (t, 1H, J = 6.9 Hz, Ar_2–H_4); Elemental



Scheme 1. Synthesis route of the target isatin β-thiosemicarbazone (IBT) compounds.

analysis calculated for $C_{15}H_{11}ClN_4OS$, C: 54.46, H: 3.35, N: 16.94, found C: 54.10, H: 3.54, N: 16.66; ESI-MS *m*/*z*: 329, $[M-H]^-$.

*4.2.3. (Z)-2-(5-Chloro-2-oxoindolin-3-ylidene)-N-(4-fluorophenyl) hydrazinecarbothio amide (**3-3**)*

Yield 66%; m.p.: 268–270 °C; brown solid; ^{1}H NMR (DMSO-d_6, 400 MHz), δ 12.62 (s, 1H, NNH), 11.36 (s, 1H, NH), 10.90 (s, 1H, SCNH), 7.86 (s, 1H, isatin ArH), 7.62–7.58 (m, 2H, ArH), 7.42–7.39 (m, 1H, isatin ArH), 7.31 (t, 2H, *J* = 8.8 Hz, ArH), 6.97 (d, 1H, *J* = 8.4 Hz, isatin ArH); Elemental analysis calculated for $C_{15}H_{10}ClFN_4OS$, C: 51.65, H: 2.89, N: 16.06, found C: 51.25, H: 3.14, N: 16.26; ESI-MS *m*/*z*: 347, $[M-H]^-$.

*4.2.4. (Z)-2-(5-Bromo-2-oxoindolin-3-ylidene)-N-phenylhydrazinecarbothioamide (**3-4**)*

Yield 58%; m.p.: 230–232 °C; yellow solid; ^{1}H NMR (DMSO-d_6, 400 MHz), δ 12.61 (s, 1H, NNH), 11.36 (s, 1H, NH), 10.91 (s, 1H, SCNH), 8.02 (s, 1H, isatin ArH), 7.60 (d, 2H, ArH), 7.53 (d, 1H, isatin ArH), 7.46 (t, 2H, *J* = 8.8 Hz, ArH), 7.30 (d, 1H, *J* = 8.4 Hz, isatin ArH), 6.91 (d, 1H, ArH); Elemental analysis calculated for $C_{15}H_{11}BrN_4OS$, C: 50.16, H: 3.09, N: 15.60, found C: 49.86, H: 3.12, N: 15.26.

*4.2.5. (Z)-2-(7-Bromo-5-nitro-2-oxoindolin-3-ylidene)-N-(4-fluorophenyl)hydrazine carbothioamide (**3-5**)*

Yield 74%; m.p.: 268–269 °C; yellow solid; ^{1}H NMR (DMSO-d_6, 400 MHz), δ 12.53 (s, 1H, NNH), 12.21 (s, 1H, NH), 11.14 (s, 1H, SCNH), 8.69 (d, 1H, *J* = 2.1 Hz, isatin ArH), 8.46 (d, 1H, *J* = 2.1 Hz, isatin ArH), 7.60–7.57 (m, 2H, ArH), 7.30 (t, 2H, *J* = 7.8 Hz, ArH); Elemental analysis calculated for $C_{15}H_9BrFN_5O_3S$, C: 41.11, H: 2.07, N: 15.62, found C: 41.12, H: 2.32, N: 15.62; ESI-MS *m*/*z*: 439, $[M-H]^-$.

*4.2.6. (Z)-2-(5-Fluoro-2-oxoindolin-3-ylidene)-N-(4-fluorophenyl) hydrazinecarbothio amide (**3-6**)*

Yield 70%; m.p.: 279–280 °C; brown-yellow solid, ^{1}H NMR (DMSO-d_6, 400 MHz), δ 12.69 (s, 1H, NNH), 11.27 (s, 1H, NH), 10.86 (s, 1H, SCNH), 7.62–7.59 (m, 3H, isatin ArH&ArH), 7.28 (t, 2H, J_1 = 8.8 Hz, ArH), 7.24–7.19 (m, 1H, isatin ArH), 6.69–6.93 (m, 1H, isatin ArH); Elemental analysis calculated for $C_{15}H_{10}F_2N_4OS$, C: 54.21, H: 3.03, N: 16.86, found C: 54.55, H: 2.95, N: 16.74; ESI-MS *m*/*z*: 332, $[M-H]^-$.

*4.2.7. (Z)-2-(2-Oxoindolin-3-ylidene)-N-phenylhydrazinecarbothioamide (**3-7**)*

Yield 80%; m.p.: 233–235 °C; orange solid; ^{1}H NMR (DMSO-d_6, 300 MHz), δ 12.79 (s, 1H, NNH), 11.25 (s, 1H, NH), 10.82 (s, 1H, SCNH), 7.79–7.35 (m, 6H, 5Ar$_2$–H and Ar$_1$–H$_4$), 7.27 (t, 1H, *J* = 7.2 Hz, Ar$_1$–H$_6$), 7.11 (t, 1H, *J* = 7.5 Hz, Ar$_1$–H$_5$), 6.94 (d, 1H, *J* = 8.1 Hz, Ar$_1$–H$_7$); IR (KBr, ν/cm^{-1}): 3299 (NN–H), 3176 (CON–H), 3059 (Ar–H), 1693 (–CONH–), 1620 (C=N), 1541 and 1463 (Ar–C=C), 1149 (C=S), 1029 (N–N); Elemental analysis calculated for $C_{15}H_{12}N_4OS$, C: 60.79, H: 4.08, N: 18.91, found C: 60.88, H: 4.28, N: 18.75; ESI-MS *m*/*z*: 295, $[M-H]^-$.

*4.2.8. (Z)-N-(4-Fluorophenyl)-2-(2-oxoindolin-3-ylidene) hydrazinecarbothioamide (**3-8**)*

Yield 69%; m.p.: 242–244 °C; yellow solid; ^{1}H NMR (DMSO-d_6, 300 MHz), δ 12.81 (s, 1H, NNH), 11.26 (s, 1H, NH), 10.82 (s, 1H, SCNH), 7.76 (d, 1H, *J* = 7.2 Hz, Ar$_1$–H$_4$), 7.63–7.58 (m, 2H, Ar$_2$–H$_3$ and Ar$_2$–H$_5$), 7.38 (t, 1H, *J* = 7.5 Hz, Ar$_1$–H$_6$), 7.26 (t, 2H, *J* = 8.7 Hz, Ar$_2$–H$_2$ and Ar$_2$–H$_6$), 7.11 (t, 1H, *J* = 7.5 Hz, Ar$_1$–H$_5$), 6.95 (d, 1H, *J* = 7.8 Hz, Ar$_1$–H$_7$); Elemental analysis calculated for $C_{15}H_{11}FN_4OS$, C: 57.31, H: 3.53, N: 17.82, found C:57.03, H: 3.77, N: 18.00; ESI-MS *m*/*z*: 313, $[M-H]^-$.

*4.2.9. (Z)-2-(5,7-Dibromo-2-oxoindolin-3-ylidene)-N-(4-fluorophenyl)hydrazinecarbo thioamide (**3-9**)*

Yield 83%; m.p.: 264–265 °C; yellow solid; ^{1}H NMR (DMSO-d_6, 300 MHz), δ 12.56 (s, 1H, NNH), 11.70 (s, 1H, NH), 10.95 (s, 1H, SCNH), 8.01 (s, 1H, isatin ArH), 7.82 (s, 1H, isatin ArH), 7.61–7.57 (m, 2H, ArH), 7.32–7.26 (m, 2H, ArH); Elemental analysis calculated for $C_{15}H_9BrN_4O_5$, C: 38.16, H: 1.92, N: 11.87, found C: 38.47, H: 2.12, N: 12.05; ESI-MS *m*/*z*: 472, $[M-H]^-$.

*4.2.10. (Z)-N-(4-Fluorophenyl)-2-(5-nitro-2-oxoindolin-3-ylidene) hydrazinecarbothio amide (**3-10**)*

Yield 71%; m.p.: 284–286 °C; orange solid; ^{1}H NMR (DMSO-d_6, 400 MHz), δ 12.56 (s, 1H, NNH), 11.87 (s, 1H, NH), 11.09 (s, 1H, SCNH), 8.68 (d, 1H, *J* = 2.4 Hz, isatin ArH), 8.30 (dd, 1H, J_1 = 2.0 Hz, J_2 = 8.4 Hz, isatin ArH), 7.61–7.57 (m, 2H, ArH), 7.29 (t, 2H, *J* = 8.4 Hz, ArH), 7.15 (d, 1H, *J* = 8.8 Hz, isatin ArH); Elemental analysis calculated for $C_{15}H_{10}FN_5O_3S$, C: 50.14, H: 2.81, N: 19.49, found C: 50.30, H: 2.58, N: 19.66; ESI-MS *m*/*z*: 359, $[M-H]^-$.

*4.2.11. (Z)-2-(7-Chloro-2-oxoindolin-3-ylidene)-N-(4-chlorophenyl)hydrazinecarbo thioamide (**3-11**)*

Yield 76%; m.p.: 251–253 °C; yellow solid; ^{1}H NMR (DMSO-d_6, 400 MHz), δ 12.78 (s, 1H, NNH), 11.73 (s, 1H, NH), 10.93 (s, 1H, SCNH), 7.74 (d, 1H, *J* = 7.6 Hz, Ar$_1$–H$_4$), 7.67–7.65 (d, 2H, *J* = 4.4 Hz, Ar$_2$–H$_3$ and Ar$_2$–H$_5$), 7.51–7.48 (d, 2H, *J* = 8.8 Hz, Ar$_2$–H$_2$ and Ar$_2$–H$_6$), 7.46–7.44 (d, 1H, *J* = 8.0 Hz, Ar$_1$–H$_6$), 7.16–7.12 (t, 1H, *J* = 8.0 Hz, Ar$_1$–H$_5$); ^{13}C NMR (DMSO-d_6, 101 MHz), δ 176.3, 162.7, 139.7, 137.7, 131.8, 131.2, 130.8, 127.6, 123.5, 121.9, 119.8, 118.4, 115.1; IR: (KBr, ν/cm^{-1}), 3312 (NN–H), 3175 (CON–H), 3065 (Ar–H), 1691 (–CONH–), 1618 (C=N), 1589 and 1476 (Ar–C=C), 1168 (C=S), 1135 (N–N); Elemental analysis calculated for $C_{15}H_{10}Cl_2N_4OS$, C: 49.33, H: 2.76, N: 15.34, found C: 49.10, H: 2.95, N: 15.05; ESI-MS *m*/*z*: 362.9, $[M-H]^-$.

*4.2.12. (Z)-2-(7-Chloro-2-oxoindolin-3-ylidene)-N-(p-tolyl) hydrazinecarbothioamide (**3-12**)*

Yield 72%; m.p.: 252–254 °C; yellow solid; ^{1}H NMR (DMSO-d_6, 400 MHz), δ 12.72 (s, 1H, NNH), 11.70 (s, 1H, NH), 10.83 (s, 1H, SCNH), 7.77 (d, 1H, J = 7.2 Hz, Ar_1–H_4), 7.49–7.44 (m, 3H, Ar_2–H_3, Ar_2–H_5 and Ar_1–H_6), 7.26–7.24 (d, 2H, J = 7.6 Hz, Ar_2–H_2 and Ar_2–H_6), 7.15 (t, 1H, J = 7.6 Hz, Ar_1–H_5), 2.35 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 101 MHz); δ 176.3, 162.7, 139.6, 135.8, 135.4, 131.4, 130.7, 128.8, 125.5, 123.5, 122.0, 119.8, 115.1, 20.6; IR: (KBr, ν/cm^{-1}), 3287 (NN–H), 3167 (CON–H), 3069 (Ar–H), 1698 (–CONH–), 1624 (C=N), 1544 and 1472 (Ar–C=C), 1294 (C=S), 1140 (N–N); Elemental analysis calculated for $C_{16}H_{13}ClN_4OS$, C: 55.73, H: 3.80, N: 16.25, found C: 55.64, H: 3.86, N: 16.08; ESI-MS m/z: 343.1, $[M - H]^-$.

*4.2.13. (Z)-2-(7-Chloro-2-oxoindolin-3-ylidene)-N-(o-tolyl) hydrazinecarbothioamide (**3-13**)*

Yield 72%; m.p.: 241–243 °C; yellow solid; ^{1}H NMR (DMSO-d_6, 400 MHz), δ 12.73 (s, 1H, NNH), 11.70 (s, 1H, NH), 10.79 (s, 1H, SCNH), 7.72 (d, 1H, J = 6.8 Hz, Ar_1–H_4), 7.45 (d, 1H, J = 8.0 Hz, Ar_1–H_6), 7.34–7.30 (m, 4H, Ar_2–H_3, Ar_2–H_4, Ar_2–H_5 and Ar_1–H_5), 2.27 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 101 MHz), δ 177.1, 162.7, 139.7, 137.3, 135.4, 131.4, 130.7, 130.5, 128.3, 127.4, 126.2, 123.5, 122.1, 119.6, 115.1, 17.6; IR: (KBr, ν/cm^{-1}), 3314 (NN–H), 3175 (CON–H), 3143 (Ar–H), 1692 (–CONH–), 1622 (C=N), 1587 and 1545 (Ar–C=C), 1295 (C=S), 1193 (N–N); Elemental analysis calculated for $C_{16}H_{13}ClN_4OS$, C: 55.73, H: 3.80, N: 16.25, found C: 55.82, H: 3.64, N: 16.28; ESI-MS m/z: 379.0, $[M + Cl]^-$.

*4.2.14. (Z)-2-(7-Chloro-2-oxoindolin-3-ylidene)-N-(m-tolyl) hydrazinecarbothioamide (**3-14**)*

Yield 72%; m.p.: 219–221 °C; yellow solid; ^{1}H NMR (DMSO-d_6, 400 MHz), δ 12.72 (s, 1H, NNH), 11.71 (s, 1H, NH), 10.83 (s, 1H, SCNH), 7.76 (d, 1H, J = 7.2 Hz, Ar_1–H_4), 7.63–7.60 (m, 3H, J = 6.8 Hz, Ar_1–H_6, Ar_2–H_2 and Ar_2–H_6), 7.32 (t, 1H, J = 7.6 Hz, Ar_2–H_3), 7.15–7.09 (m, 2H, J = 8.4 Hz, Ar_1–H_5 and Ar_2–H_4), 2.35 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 101 MHz), δ 176.1, 162.7, 139.6, 138.2, 137.7, 131.4, 130.7, 128.2, 126.8, 126.0, 123.4, 122.7, 122.0, 119.8, 115.1, 20.9; IR: (KBr, ν/cm^{-1}), 3314 (NN–H), 3197 (CON–H), 3148 (Ar–H), 1693 (–CONH–), 1622 (C=N), 1545 and 1487 (Ar–C=C), 1248 (C=S), 1172 (N–N); Elemental analysis calculated for $C_{16}H_{13}ClN_4OS$, C: 55.73, H: 3.80, N: 16.25; found C: 55.90, H: 3.68, N: 16.25; ESI-MS m/z: 343.0, $[M - H]^-$.

*4.2.15. (Z)-2-(7-Bromo-2-oxoindolin-3-ylidene)-N-(4-fluorophenyl)hydrazinecarbo thioamide (**3-15**)*

Yield 74%; m.p.: 254–256 °C; yellow solid; ^{1}H NMR (DMSO-d_6, 400 MHz), δ 12.78 (s, 1H, NNH), 11.59 (s, 1H, NH), 10.93 (s, 1H, SCNH), 7.78 (d, 1H, J = 7.6 Hz, Ar_1–H_4), 7.67–7.65 (d, 2H, J = 8.4 Hz, Ar_2–H_3 and Ar_2–H_5), 7.57 (t, 1H, J = 8.0 Hz, Ar_1–H_6), 7.51–7.48 (d, 2H, J = 8.4 Hz, Ar_2–H_2 and Ar_2–H_6), 7.08 (t, 1H, J = 8.0 Hz, Ar_1–H_6); ^{13}C NMR (DMSO-d_6, 101 MHz); δ 176.3, 162.7, 141.4, 137.3, 133.7, 132.0, 130.1, 128.3, 127.3, 123.8, 121.9, 120.2, 103.3; IR: (KBr, ν/cm^{-1}) 3310 (NN–H), 3129 (CON–H), 3011 (Ar–H), 1696 (–CONH–), 1621 (C=N), 1590 and 1535 (Ar–C=C), 1435 (C=S), 1160 (N–N); Elemental analysis calculated for $C_{15}H_{10}BrFN_4OS$, C: 45.82, H: 2.56, N: 14.25, found C: 45.59, H: 2.85, N: 14.05.

*4.2.16. (Z)-2-(7-Bromo-2-oxoindolin-3-ylidene)-N-(4-chlorophenyl)hydrazinecarbothioamide (**3-16**)*

Yield 75%; m.p.: 255–257 °C; yellow solid; ^{1}H NMR (DMSO-d_6, 400 MHz), δ 12.79 (s, 1H, NNH), 11.60 (s, 1H, NH), 10.94 (s, 1H, SCNH), 7.79 (d, 1H, J = 6.8 Hz, Ar_1–H_4), 7.68–7.66 (d, 2H, J = 8.0 Hz, Ar_2–H_3 and Ar_2–H_5), 7.58 (d, 1H, J = 7.6 Hz, Ar_1–H_6), 7.52–7.49 (d, 2H, J = 7.6 Hz Hz, Ar_2–H_2 and Ar_2–H_6), 7.10 (t, 1H, J = 7.6 Hz, Ar_1–H_5); ^{13}C NMR (DMSO-d_6, 101 MHz), δ 176.3, 162.7, 141.5, 137.3, 133.5, 132.0, 130.2, 128.3, 127.3, 123.8, 121.9, 120.3, 103.4; IR: (KBr, ν/cm^{-1}), 3308 (NN–H), 3122 (CON–H), 3056 (Ar–H), 1696 (–CONH–), 1620 (C=N), 1590 and 1534 (Ar–C=C), 1240 (C=S), 1158 (N–N); Elemental analysis calculated for $C_{15}H_{10}BrClN_4OS$, C: 43.98, H: 2.46, N: 13.68, found C: 43.69, H: 2.54, N: 13.52; ESI-MS m/z: 408.9, $[M - H]^-$.

*4.2.17. (Z)-2-(7-Methyl-2-oxoindolin-3-ylidene)-N-(p-tolyl) hydrazinecarbothioamide (**3-17**)*

Yield 73%; m.p.: 247–249 °C; yellow solid; ^{1}H NMR (DMSO-d_6, 400 MHz), δ 12.82 (s, 1H, NNH), 11.32 (s, 1H, NH), 10.74 (s, 1H, SCNH), 7.63 (d, 1H, J = 7.2 Hz, Ar_1–H_4), 7.50–7.48 (d, 2H, J = 8.0 Hz, Ar_2–H_3 and Ar_2–H_5), 7.25–7.19 (m, 3H, J = 7.6 Hz, Ar_1–H_6, Ar_2–H_2 and Ar_2–H_6), 7.03 (t, 1H, J = 7.6 Hz, Ar_1–H_5), 2.34 (s, 3H, Ar_2–CH_3), 2.248 (s, 3H, Ar_1–CH_3); ^{13}C NMR (DMSO-d_6, 101 MHz), δ 176.2, 163.1, 141.0, 135.9, 135.3, 132.5, 132.5, 128.8, 125.5, 122.3, 120.4, 119.6, 118.8, 20.6, 15.9; IR: (KBr, ν/cm^{-1}), 3302 (NN–H), 3164 (CON–H), 3063 (Ar–H), 1693 (–CONH–), 1626 (C=N), 1585 and 1478 (Ar–C=C), 1211 (C=S), 1135 (N–N); Elemental analysis calculated for $C_{17}H_{16}N_4OS$, C: 62.94, H: 4.97, N: 17.27, found C: 62.70, H: 5.15, N: 17.28; ESI-MS m/z: 323.1, $[M - H]^-$.

*4.2.18. (Z)-N-(4-Chlorophenyl)-2-(7-methyl-2-oxoindolin-3-ylidene)hydrazinecarbothioamide (**3-18**)*

Yield 77%; m.p.: 251–253 °C; yellow solid; ^{1}H NMR (DMSO-d_6, 400 MHz), δ 12.91 (s, 1H, NNH), 11.35 (s, 1H, NH), 10.86 (s, 1H, SCNH), 7.69–7.63 (m, 3H, J = 6.4 Hz, Ar_1–H_4, Ar_2–H_3 and Ar_2–H_5), 7.51–7.43 (m, 2H, J = 7.6 Hz, Ar_2–H_2 and Ar_2–H_6), 7.22 (d, 1H J = 7.6 Hz, Ar_1–H_6), 7.05 (t, 1H J = 8.0 Hz, Ar_1–H_5), 2.26 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 101 MHz), δ 176.3, 163.1, 141.2, 137.4, 132.9, 132.7, 130.0, 128.2, 127.2, 122.3, 120.5, 119.5, 118.1, 15.8; IR: (KBr, ν/cm^{-1}), 3310 (NN–H), 3173 (CON–H), 3057 (Ar–H), 1696 (–CONH–), 1626 (C=N), 1595 and 1539 (Ar–C=C), 1209 (C=S), 1155 (N–N); Elemental analysis calculated for $C_{16}H_{13}ClN_4OS$, C: 55.73, H: 3.80, N: 16.25, found C: 56.01, H: 4.10, N: 16.46; HRMS(MALDI) m/z: calculated. 345.0577, found 345.0565, $[M + H]^+$.

*4.2.19. (Z)-N-(4-Fluorophenyl)-2-(7-methyl-2-oxoindolin-3-ylidene)hydrazinecarbothioamide (**3-19**)*

Yield 70%; m.p.: 242–244 °C; yellow solid; ^{1}H NMR (DMSO-d_6, 400 MHz), δ 12.89 (s, 1H, NNH), 11.33 (s, 1H, NH), 10.84 (s, 1H, SCNH), 7.68–7.66 (m, 2H, J = 8.0 Hz, Ar_2–H_3 and Ar_2–H_5), 7.61 (d, 1H J = 7.2 Hz, Ar_1–H_4), 7.49–7.48 (m, 2H, J = 4.4 Hz, Ar_2–H_2 and Ar_2–H_6), 7.20 (d, 1H J = 7.2 Hz, Ar_1–H_6), 7.03 (t, 1H J = 7.6 Hz, Ar_1–H_5), 2.24 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 101 MHz), δ 176.3, 163.1, 141.1, 137.4, 132.9, 132.7, 130.0, 128.2, 127.2, 122.3, 120.5, 119.4, 118.8, 15.8; IR: (KBr, ν/cm^{-1}), 3308 (NN–H), 3165 (CON–H), 3052 (Ar–H), 1695 (–CONH–), 1625 (C=N), 1572 and 1539 (Ar–C=C), 1209 (C=S), 1153 (N–N); Elemental analysis calculated for $C_{16}H_{13}FN_4OS$, C: 58.52, H: 3.99, N: 17.06, found C: 58.75, H: 3.66, N: 17.45.

*4.2.20. (Z)-2-(7-Fluoro-2-oxoindolin-3-ylidene)-N-(o-tolyl) hydrazinecarbothioamide (**3-20**)*

Yield 79%; m.p.: 230–232 °C; yellow solid; ^{1}H NMR (DMSO-d_6, 400 MHz), δ 12.75 (s, 1H, NNH), 11.78 (s, 1H, NH), 10.69 (s, 1H, SCNH), 7.61 (d, 1H, J = 7.2 Hz, Ar_1–H_4), 7.33–7.30 (m, 5H, J = 8.0 Hz, Ar_1–H_5, Ar_1–H_6, Ar_2–H_3, Ar_2–H_4 and Ar_2–H_5), 7.14 (m, 1H, J = 5.2 Hz, Ar_1–H_5), 2.26 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 101 MHz), δ 177.1, 162.4, 137.3, 135.4, 130.3, 129.1, 128.3, 127.4, 126.7, 123.2, 123.2, 123.0, 117.9, 117.8, 117.2, 17.6; IR: (KBr, ν/cm^{-1}), 3242 (NN–H), 3176 (CON–H), 3059 (Ar–H), 1694 (–CONH–), 1646 (C=N), 1541 and 1463 (Ar–C=C), 1149 (C=S), 1029 (N–N); Elemental analysis calculated for $C_{16}H_{13}FN_4OS$, C: 58.52, H: 3.99, N: 17.06, found C:

58.40, H: 4.03, N: 17.30; ESI-MS *m/z*: 327.1, $[M - H]^-$.

*4.2.21. (Z)-2-(7-Fluoro-2-oxoindolin-3-ylidene)-N-(4-fluorophenyl)hydrazinecarbothioamide (**3-21**)*

Yield 78%; m.p.: 241–243 °C; yellow solid; ^{1}H NMR (DMSO-d_6, 400 MHz), δ 12.78 (s, 1H, NNH), 11.80 (s, 1H, NH), 10.89 (s, 1H, SCNH), 7.63–7.59 (m, 3H, J = 7.2 Hz, Ar_1–H_4, Ar_2–H_3 and Ar_2–H_5), 7.32–7.25 (m, 3H, J = 9.2 Hz, Ar_1–H_6, Ar_1–H_2 and Ar_1–H_6), 7.14 (m, 1H J = 4.4 Hz, Ar_1–H_5); ^{13}C NMR (DMSO-d_6, 101 MHz), δ 176.7, 161.2, 162.5, 148.0, 134.7, 131.5, 129.3, 128.0, 123.2, 122.9, 118.0, 117.3, 115.2; IR: (KBr, ν/cm^{-1}), 3294 (NN–H), 3178 (CON–H), 3046 (Ar–H), 1695 (–CONH–), 1646 (C=N), 1515 and 1479 (Ar–C=C), 1167 (C=S), 1137 (N–N); Elemental analysis calculated for $C_{15}H_{10}FClN_4OS$, C: 54.21, H: 3.03, N: 16.86, found C: 54.44, H: 3.00, N: 16.57; ESI-MS *m/z*: 331.0, $[M - H]^-$.

*4.2.22. (Z)-2-(7-Fluoro-2-oxoindolin-3-ylidene)-N-(m-tolyl) hydrazinecarbothioamide (**3-22**)*

Yield 79%; m.p.: 230–232 °C; yellow solid; ^{1}H NMR (DMSO-d_6, 400 MHz), δ 12.75 (s, 1H, NNH), 11.81 (s, 1H, NH), 10.85 (s, 1H, SCNH), 7.65 (d, 1H, J = 7.2 Hz, Ar_1–H_4), 7.45–7.43 (m, 2H, J = 6.8 Hz, Ar_1–H_5 and Ar_1–H_6), 7.34–7.28 (m, 2H, J = 6.8 Hz, Ar_2–H_3 and Ar_2–H_4), 7.16–7.11 (m, 2H, J = 7.2 Hz, Ar_2–H_2 and Ar_2–H_6), 2.36 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 101 MHz), δ 176.1, 162.5, 148.0, 138.2, 137.7, 131.3, 129.2, 128.2, 126.8, 123.2, 123.1, 122.9, 122.7, 118.0, 117.8, 20.9; IR: (KBr, ν/cm^{-1}) 3298 (NN–H), 3179 (CON–H), 3048 (Ar–H), 1695 (–CONH–), 1644 (C=N), 1593 and 1548 (Ar–C=C), 1247 (C=S), 1149 (N–N); HRMS(MALDI) *m/z*: calculated for $C_{16}H_{13}FN_4OS$ 329.0872, found 329.0865, $[M + H]^+$.

*4.2.23. (Z)-2-(7-Fluoro-2-oxoindolin-3-ylidene)-N-(3-methoxyphenyl)hydrazinecarbothioamide (**3-23**)*

Yield 75%; m.p.: 230–232 °C; yellow solid; ^{1}H NMR (DMSO-d_6, 400 MHz), δ 12.79 (s, 1H, NNH), 11.80 (s, 1H, NH), 10.85 (s, 1H, SCNH), 7.66 (d, 1H, J = 7.6 Hz, Ar_1–H_4), 7.37–7.29 (m, 3H, J = 6.8 Hz, Ar_1–H_5, Ar_1–H_6 and Ar_2–H_5), 7.25 (d, 1H, J = 8.0 Hz, Ar_2–H_6), 7.18–7.12 (m, 1H, J = 8.4 Hz, Ar_2–H_4), 6.89–6.86 (m, 1H, J = 6.8 Hz, Ar_2–H_2), 3.80 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6, 101 MHz), δ 176.0, 162.5, 159.1, 148.1, 145.6, 139.4, 131.5, 129.1, 123.2, 122.9, 118.0, 117.9, 117.6, 111.6, 111.1, 55.2; IR: (KBr, ν/cm^{-1}), 3302 (NN–H), 3162 (CON–H), 3080 (Ar–H), 1695 (–CONH–), 1595 (C=N), 1541 and 1454 (Ar–C=C), 1148 (C=S), 1049 (N–N); Elemental analysis calculated for $C_{16}H_{13}FN_4O_2S$, C: 55.80, H: 3.81, N: 16.27, found C: 55.84, H: 4.03, N: 16.13; ESI-MS *m/z*: 343.1, $[M - H]^-$.

*4.2.24. (Z)-2-(7-Bromo-2-oxoindolin-3-ylidene)-N-(p-tolyl) hydrazinecarbothioamide (**3-24**)*

Yield 78%; m.p.: 254–256 °C; yellow solid; ^{1}H NMR (DMSO-d_6, 400 MHz), δ 12.71 (s, 1H, NNH), 11.57 (s, 1H, NH), 10.84 (s, 1H, SCNH), 7.80 (d, 1H, J = 7.6 Hz, Ar_1–H_4), 7.57 (d, 1H, J = 8.0 Hz, Ar_1–H_6), 7.48–7.46 (d, 2H, J = 8.0 Hz, Ar_2–H_3 and Ar_2–H_5), 7.25–7.23 (d, 2H, J = 8.0 Hz, Ar_2–H_2 and Ar_2–H_6), 7.07 (t, 1H, J = 8.0 Hz, Ar_1–H_5), 2.34 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 101 MHz), δ 176.2, 162.7, 141.3, 135.7, 135.4, 133.6, 131.5, 128.8, 125.5, 123.8, 122.0, 120.2, 103.3, 20.6; IR: (KBr, ν/cm^{-1}), 3310 (NN–H), 3125 (CON–H), 3050 (Ar–H), 1694 (–CONH–), 1595 (C=N), 1535 and 1479 (Ar–C=C), 1152 (C=S), 1132 (N–N); Elemental analysis calculated for $C_{16}H_{13}BrN_4OS$, C: 49.37, H: 3.37, N: 14.39, found C: 49.12, H: 3.42, N: 14.16; ESI-MS *m/z*: 380.9, $[M - H]^-$.

*4.2.25. (Z)-2-(7-Bromo-2-oxoindolin-3-ylidene)-N-(o-tolyl) hydrazinecarbothioamide (**3-25**)*

Yield 72%; m.p.: 241–243 °C; yellow solid; ^{1}H NMR (DMSO-d_6, 400 MHz), δ 12.71 (s, 1H, NNH), 11.57 (s, 1H, NH), 10.79 (s, 1H, SCNH), 7.75 (d, 1H, J = 6.8z, Ar_1–H_4), 7.57 (d, 1H, J = 7.6 Hz, Ar_1–H_6), 7.33–7.29 (m, 4H, J = 8.4 Hz, Ar_1–H_5, Ar_2–H_2, Ar_2–H_3 and Ar_2–H_5), 7.07 (t, 1H, J = 7.6 Hz, Ar_1–H_6), 2.25 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 101 MHz), δ 177.1, 162.7, 141.4, 137.3, 135.4, 133.6, 131.6, 130.3, 128.4, 127.5, 126.2, 123.8, 122.1, 120.1, 103.1, 17.6; IR: (KBr, ν/cm^{-1}), 3310 (NN–H), 3161 (CON–H), 3057 (Ar–H), 1692 (–CONH–), 1619 (C=N), 1580 and 1472 (Ar–C=C), 1190 (C=S), 1153 (N–N); HRMS(MALDI) *m/z*: calculated for $C_{16}H_{13}BrN_4OS$ 386.9915, found 386.9913, $[M - H]^-$.

*4.2.26. (Z)-2-(7-Bromo-2-oxoindolin-3-ylidene)-N-(3-methoxyphenyl)hydrazinecarbothioamide (**3-26**)*

Yield 79%; m.p.: 206–208 °C; yellow solid; ^{1}H NMR (DMSO-d_6, 400 MHz), δ 12.74 (s, 1H, NNH), 11.58 (s, 1H, NH), 10.85 (s, 1H, SCNH), 7.81 (d, 1H, J = 7.2 Hz, Ar_1–H_4), 7.57 (d, 1H, J = 8.0 Hz, Ar_1–H_6), 7.36–7.23 (m, 3H, J = 8.0 Hz, Ar_1–H_5, Ar_2–H_2 and Ar_2–H_6), 7.08 (t, 1H, J = 8.0 Hz, Ar_2–H_3), 6.87 (d, 1H, J = 7.6 Hz, Ar_2–H_4), 3.79 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6, 101 MHz), δ 175.9, 162.7, 159.1, 141.4, 139.4, 133.6, 131.7, 129.3, 123.8, 122.0, 120.3, 117.5, 111.6, 111.1, 103.3, 55.2; IR: (KBr, ν/cm^{-1}), 3246 (NN–H), 3144 (CON–H), 3065 (Ar–H), 1698 (–CONH–), 1620 (C=N), 1539 and 1492 (Ar–C=C), 1156 (C=S), 1129 (N–N); Elemental analysis calculated for $C_{16}H_{13}BrN_4O_2S$, C: 47.42, H: 3.23, N: 13.82, found C: 47.29, H: 3.40, N: 13.68; ESI-MS *m/z*: 404.9, $[M - H]^-$.

*4.2.27. (Z)-2-(7-Chloro-2-oxoindolin-3-ylidene)-N-(3-chlorophenyl)hydrazinecarbothioamide (**3-27**)*

Yield 75%; m.p.: 246–248 °C; yellow solid; ^{1}H NMR (DMSO-d_6, 400 MHz), δ 12.79 (s, 1H, NNH), 11.74 (s, 1H, NH), 10.95 (s, 1H, SCNH), 7.79–7.64 (m, 3H, J = 8.0 Hz, Ar_1–H_4, Ar_1–H_6, and Ar_2–H_4), 7.51–7.35 (m, 3H, J = 7.6 Hz, Ar_1–H_5, Ar_2–H_2, and Ar_2–H_6), 7.15 (t, 1H, J = 8.0 Hz, Ar_2–H_3); ^{13}C NMR (DMSO-d_6, 101 MHz), δ 176.2, 162.7, 139.8, 137.3, 132.4, 131.9, 130.1, 128.3, 127.3, 125.8, 124.0, 123.5, 121.8, 119.8, 115.2; IR: (KBr, ν/cm^{-1}), 3320 (NN–H), 3165 (CON–H), 3050 (Ar–H), 1691 (–CONH–), 1621 (C=N), 1586 and 1525 (Ar–C=C), 1164 (C=S), 1137 (N–N); Elemental analysis calculated for $C_{15}H_{10}Cl_2N_4OS$, C: 49.33, H: 2.76, N: 15.34, found C: 49.75, H: 3.02, N: 15.63; ESI-MS *m/z*: 363.0, $[M - H]^-$.

*4.2.28. (Z)-2-(7-Chloro-2-oxoindolin-3-ylidene)-N-(3-methoxyphenyl)hydrazinecarbothioamide (**3-28**)*

Yield 70%; m.p.: 219–221 °C; yellow solid; ^{1}H NMR (DMSO-d_6, 400 MHz), δ 12.74 (s, 1H, NNH), 11.72 (s, 1H, NH), 10.86 (s, 1H, SCNH), 7.77 (d, 1H, J = 7.6 Hz, Ar_1–H_4), 7.45 (d, 1H, J = 8.0 Hz, Ar_1–H_6), 7.34 (t, 1H, J = 8.0 Hz, Ar_1–H_5), 7.29 (s, 1H, Ar_2–H_6), 7.24 (d, 1H, J = 7.6 Hz, Ar_2–H_4), 7.14 (t, 1H, J = 7.6 Hz, Ar_2–H_3), 6.87 (d, 1H, J = 6.4 Hz, Ar_2–H_2), 3.79 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6, 101 MHz), δ 176.0, 162.7, 159.1, 139.7, 139.4, 131.5, 130.7, 129.1, 126.5, 121.9, 119.9, 117.7, 115.1, 111.6, 111.1, 55.2; IR: (KBr, ν/cm^{-1}), 3302 (NN–H), 3179 (CON–H), 3018 (Ar–H), 1695 (–CONH–), 1624 (C=N), 1594 and 1542 (Ar–C=C), 1204 (C=S), 1149 (N–N); Elemental analysis calculated for $C_{16}H_{13}ClN_4O_2S$, C: 53.26, H: 3.63, N: 15.53, found C: 53.45, H: 3.59, N: 15.34; ESI-MS *m/z*: 359.0, $[M - H]^-$.

*4.2.29. (Z)-2-(7-Fluoro-2-oxoindolin-3-ylidene)-N-(3-fluorophenyl)hydrazinecarbothioamide (**3-29**)*

Yield 76%; m.p.: 230–232 °C; orange solid; ^{1}H NMR (DMSO-d_6, 400 MHz), δ 12.83 (s, 1H, NNH), 11.82 (s, 1H, NH), 10.94 (s, 1H, SCNH), 7.65–7.63 (m, 2H, J = 7.2 Hz, Ar_1–H_4 and Ar_1–H_6), 7.53–7.45 (m, 2H, J = 8.0 Hz, Ar_1–H_5 and Ar_2–H_4), 7.31 (t, 1H, J = 8.8 Hz, Ar_2–H_5), 7.17–7.12 (m, 2H, J = 4.0 Hz, Ar_2–H_2 and Ar_2–H_6); ^{13}C NMR (DMSO-d_6, 101 MHz), δ 176.2, 162.5, 148.1, 145.6, 140.1, 131.8, 129.8, 129.4, 123.2, 122.8, 121.3, 118.2, 117.5, 112.6, 112.4; IR: (KBr, ν/cm^{-1}), 3286 (NN–H), 3160 (CON–H), 3068 (Ar–H), 1696 (–CONH–), 1644 (C=N), 1602 and 1540 (Ar–C=C), 1213 (C=S), 1148 (N–N); Elemental analysis calculated for $C_{15}H_{10}F_2N_4OS$, C:

54.21; H: 3.03; N: 16.86, found C: 54.07, H: 3.27, N: 17.06; ESI-MS *m/z*: 331.1, $[M - H]^-$.

4.2.30. (Z)-N-(3-Chlorophenyl)-2-(7-fluoro-2-oxoindolin-3-ylidene)hydrazinecarbothioamide (**3-30**)

Yield 69%; m.p.: 210–211 °C; yellow solid; ^{1}H NMR (DMSO-d_6, 400 MHz), δ 12.82 (s, 1H, NNH), 11.81 (s, 1H, NH), 10.93 (s, 1H, SCNH), 7.79 (d, 1H, J = 7.2 Hz, Ar_1–H_4), 7.66–7.62 (m, 2H, J = 4.8 Hz, Ar_1–H_5 and Ar_1–H_6), 7.46 (t, 1H, J = 8.0 Hz, Ar_2–H_3), 7.35–7.27 (m, 3H, J = 6.8 Hz, Ar_2–H_2, Ar_2–H_4 and Ar_2–H_6); ^{13}C NMR (DMSO-d_6, 101 MHz), δ 176.2, 162.4, 148.0, 145.7, 142.2, 139.8, 132.6, 129.9, 125.8, 124.0, 123.2, 120.6, 118.1, 117.4, 115.3; IR: (KBr, ν/cm^{-1}), 3313 (NN–H), 3167 (CON–H), 3077 (Ar–H), 1692 (–CONH–), 1645 (C=N), 1589 and 1530 (Ar–C=C), 1210 (C=S), 1161 (N–N); Elemental analysis calculated for $C_{15}H_{10}FClN_4OS$, C: 51.65, H: 2.89, N: 16.06, found C: 51.45, H: 2.68, N: 16.05; ESI-MS *m/z*: 347.0, $[M - H]^-$.

4.2.31. (Z)-2-(7-Fluoro-2-oxoindolin-3-ylidene)-N-(4-methoxyphenyl)hydrazinecarbothioamide (**3-31**)

Yield 77%; m.p.: 255–257 °C; yellow solid; ^{1}H NMR (DMSO-d_6, 400 MHz), δ 12.72 (s, 1H, NNH), 11.79 (s, 1H, NH), 10.81 (s, 1H, SCNH), 7.63 (d, 1H, J = 6.8 Hz, Ar_1–H_4), 7.47–7.45 (m, 2H, J = 8.0 Hz, Ar_2–H_3 and Ar_2–H_5), 7.30 (t, 1H, J = 9.2 Hz, Ar_1–H_5), 7.13 (d, 1H, J = 4.0 Hz, Ar_1–H_6), 7.00–6.98 (m, 2H, J = 8.0 Hz, Ar_2–H_2 and Ar_2–H_6), 3.79 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6, 101 MHz), δ 176.5, 162.5, 157.4, 148.0, 131.1, 129.2, 129.0, 127.2, 123.2, 117.9, 117.9, 117.6, 113.8, 55.2; IR: (KBr, ν/cm^{-1}) 3329 (NN–H), 3189 (CON–H), 3041 (Ar–H), 1698 (–CONH–), 1643 (C=N), 1597 and 1521 (Ar–C=C), 1239 (C=S), 1158 (N–N); Elemental analysis calculated for $C_{16}H_{13}FN_4O_2S$, C: 55.80, H: 3.81, N: 16.27, found C: 55.57, H: 3.59, N: 16.08; ESI-MS *m/z*: 343.1, $[M - H]^-$.

4.2.32. (Z)-2-(7-Chloro-2-oxoindolin-3-ylidene)-N-(3-fluorophenyl)hydrazinecarbothioamide (**3-32**)

Yield 76%; m.p.: 24**3**-245 °C; yellow solid; ^{1}H NMR (DMSO-d_6, 400 MHz), δ 12.80 (s, 1H, NNH), 11.73 (s, 1H, NH), 10.95 (s, 1H, SCNH), 7.76 (d, 1H, J = 7.6 Hz, Ar_1–H_4), 7.63 (d, 1H, J = 11.2 Hz, Ar_1–H_6), 7.52–7.45 (m, 3H, J = 7.6 Hz, Ar_1–H_5, Ar_2–H_3 and Ar_2–H_4), 7.17–7.12 (m, 2H, J = 8.0 Hz, Ar_2–H_2 and Ar_2–H_6); ^{13}C NMR (DMSO-d_6, 101 MHz), δ 176.2, 162.8, 160.3, 140.0, 139.8, 131.9, 130.9, 129.9, 125.5, 121.9, 121.4, 119.9, 115.2, 112.6; IR: (KBr, ν/cm^{-1}), 3282 (NN–H), 3136 (CON–H), 3017 (Ar–H), 1698 (–CONH–), 1623 (C=N), 1591 and 1530 (Ar–C=C), 1248 (C=S), 1179 (N–N); Elemental analysis calculated for $C_{15}H_{10}ClFN_4OS$, C: 51.65, H: 2.89, N: 16.06, found C: 51.55, H: 3.05, N: 16.01; ESI-MS *m/z*: 347.0, $[M - H]^-$.

4.2.33. (Z)-2-(7-Chloro-2-oxoindolin-3-ylidene)-N-(4-methoxyphenyl)hydrazinecarbothioamide (**3-33**)

Yield 78%; m.p.: 251–253 °C; yellow solid; ^{1}H NMR (DMSO-d_6, 400 MHz), δ 12.69 (s, 1H, NNH), 11.70 (s, 1H, NH), 10.82 (s, 1H, SCNH), 7.75 (d, 1H, J = 7.6 Hz, Ar_1–H_4), 7.47–7.44 (m, 3H, J = 7.2 Hz, Ar_1–H_5, Ar_2–H_6 and Ar_2–H_4), 7.14 (t, 1H, J = 8.0 Hz, Ar_2–H_3), 7.01–6.98 (m, 2H, J = 9.2 Hz, Ar_2–H_2 and Ar_2–H_6), 3.791 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6, 101 MHz), δ 176.5, 162.7, 157.4, 139.6, 131.3, 131.1, 130.6, 127.2, 123.5, 122.0, 119.7, 115.1, 113.5, 55.2; IR: (KBr, ν/cm^{-1}), 3330 (NN–H), 3240 (CON–H), 3128 (Ar–H), 1702 (–CONH–), 1623 (C=N), 1586 and 1518 (Ar–C=C), 1239 (C=S), 1133 (N–N); Elemental analysis calculated for $C_{16}H_{13}ClN_4O_2S$, C: 53.26, H: 3.63, N: 15.53, found C: 53.42, H: 3.90, N: 15.75; ESI-MS *m/z*: 378.9, $[M + NH_4]^+$.

4.2.34. (Z)-2-(7-Bromo-2-oxoindolin-3-ylidene)-N-(2-methoxyphenyl)hydrazinecarbothioamide (**3-34**)

Yield 70%; m.p.: 270–272 °C; yellow solid; ^{1}H NMR (DMSO-d_6, 400 MHz); δ 12.746 (s, 1H, NNH), 11.581 (s, 1H, NH), 10.521 (s, 1H, SCNH), 7.779 (d, 1H, J = 7.6 Hz, Ar_1–H_4), 7.721 (d, 1H, J = 7.2 Hz, Ar_1–H_6), 7.590 (d, 1H, J = 7.6 Hz, Ar_2–H_5), 7.319 (t, 1H, J = 7.6 Hz, Ar_1–H_5), 7.163 (d, 1H, J = 8.0 Hz, Ar_2–H_5), 7.089 (t, 1H, J = 8.0 Hz, Ar_2–H_3), 7.025 (t, 1H, J = 7.6 Hz, Ar_2–H_4), 3.872 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6, 101 MHz); δ 176.1, 162.7, 153.1, 141.4, 133.7, 131.7, 127.6, 126.9, 126.5, 123.9, 121.9, 120.0, 119.9, 111.9, 103.3, 55.7; IR: (KBr, ν/cm^{-1}), 3294 (NN–H), 3162 (CON–H), 3066 (Ar–H), 1691 (–CONH–), 1652 (C=N), 1544 and 1479 (Ar–C=C), 1224 (C=S), 1139 (N–N); Elemental analysis calculated for $C_{16}H_{13}BrN_4O_2S$, C: 47.42, H: 3.23, N: 13.82, found C: 47.22, H: 3.35, N: 13.83; ESI-MS *m/z*: 405.0, $[M - H]^-$.

4.2.35. (Z)-2-(7-Bromo-2-oxoindolin-3-ylidene)-N-(3-fluorophenyl)hydrazinecarbothioamide (**3-35**)

Yield 76%; m.p.: 243–245 °C; yellow solid; ^{1}H NMR (DMSO-d_6, 400 MHz), δ 12.81 (s, 1H, NNH), 11.59 (s, 1H, NH), 10.96 (s, 1H, SCNH), 7.80 (d, 1H, J = 7.6 Hz, Ar_1–H_4), 7.64 (d, 1H, J = 6.8 Hz, Ar_1–H_6), 7.58 (d, 1H, J = 5.2 Hz, Ar_2–H_4), 7.53–7.46 (m, 2H, J = 6.8 Hz, Ar_1–H_5 and Ar_2–H_5), 7.16–7.07 (m, 2H, J = 7.6 Hz, Ar_2–H_2 and Ar_2–H_6); ^{13}C NMR (DMSO-d_6, 101 MHz), δ 176.2, 162.7, 141.5, 139.9, 133.8, 132.1, 129.9, 129.8, 123.8, 121.9, 121.3, 120.3, 112.6, 112.4, 103.4; IR: (KBr, ν/cm^{-1}), 3233 (NN–H), 3132 (CON–H), 3058 (Ar–H), 1694 (–CONH–), 1638 (C=N), 1548 and 1488 (Ar–C=C), 1182 (C=S), 1133 (N–N); Elemental analysis calculated for $C_{15}H_{10}BrFN_4OS$, C: 45.82, H: 2.56, N: 14.25, found C: 45.65, H: 2.65, N: 14.44; ESI-MS *m/z*: 392.9, $[M - H]^-$.

4.2.36. (Z)-2-(7-Bromo-2-oxoindolin-3-ylidene)-N-(3-chlorophenyl)hydrazinecarbothioamide (**3-36**)

Yield 78%; m.p., 232–234 °C; yellow solid; ^{1}H NMR (DMSO-d_6, 400 MHz), δ 12.80 (s, 1H, NNH), 11.60 (s, 1H, NH), 10.95 (s, 1H, SCNH), 7.79–7.77 (m, 2H, J = 8.0 Hz, Ar_1–H_4 and Ar_2–H_6), 7.65 (d, 1H, J = 7.6 Hz, Ar_1–H_6), 7.58 (d, 1H, J = 8.0 Hz, Ar_2–H_4), 7.48 (t, 1H, J = 8.0 Hz, Ar_1–H_5), 7.35 (d, 1H, J = 8.0 Hz, Ar_2–H_2), 7.61 (d, 1H, J = 8.0 Hz, Ar_2–H_3), ^{13}C NMR (DMSO-d_6, 101 MHz), δ 176.2, 162.8, 141.5, 139.8, 133.8, 132.6, 132.1, 129.9, 125.9, 125.0, 124.06, 123.8, 121.9, 120.3, 103.4; IR: (KBr, ν/cm^{-1}), 3320 (NN–H), 3222 (CON–H), 3121 (Ar–H), 1697 (–CONH–), 1618 (C=N), 1587 and 1528 (Ar–C=C), 1219 (C=S), 1160 (N–N); Elemental analysis calculated for $C_{15}H_{10}BrClN_4OS$, C: 43.98, H: 2.46, N: 13.68, found C: 43.78, H: 2.57, N: 13.57. ESI-MS *m/z*: 408.9, $[M - H]^-$.

4.2.37. (Z)-2-(7-Bromo-2-oxoindolin-3-ylidene)-N-(4-bromophenyl)hydrazinecarbothioamide (**3-37**)

Yield 73%; m.p.; 247–249 °C; yellow solid; ^{1}H NMR (DMSO-d_6, 400 MHz), δ 12.78 (s, 1H, NNH), 11.59 (s, 1H, NH), 10.92 (s, 1H, SCNH), 7.78 (d, 1H, J = 7.2 Hz, Ar_1–H_4), 7.66–7.57 (m, 5H, J = 7.6 Hz, Ar_1–H_6, Ar_2–H_2, Ar_2–H_3, Ar_2–H_5 and Ar_2–H_6), 7.08 (t, 1H, J = 8.0 Hz, Ar_1–H_5); ^{13}C NMR (DMSO-d_6, 101 MHz), δ 176.3, 162.8, 141.5, 137.8, 133.8, 132.0, 131.2, 127.6, 123.8, 121.9, 120.2, 118.4, 103.3; IR: (KBr, ν/cm^{-1}), 3302 (NN–H), 3240 (CON–H), 3021 (Ar–H), 1693 (–CONH–), 1626 (C=N), 1585 and 1534 (Ar–C=C), 1211 (C=S), 1153 (N–N); Elemental analysis calculated for $C_{15}H_{10}Br_2N_4OS$, C: 39.67, H: 2.22, N: 12.34, found C: 39.46, H: 2.68, N: 12.25; ESI-MS *m/z*, 452.9 $[M - H]^-$.

4.2.38. (Z)-2-(7-Bromo-2-oxoindolin-3-ylidene)-N-(4-methoxyphenyl)hydrazinecarbothioamide (**3-38**)

Yield 76%; m.p.: 249–251 °C; yellow solid; ^{1}H NMR (DMSO-d_6, 400 MHz), δ 12.70 (s, 1H, NNH), 11.59 (s, 1H, NH), 10.81 (s, 1H, SCNH), 7.80 (d, 1H, J = 6.8 Hz, Ar_1–H_4), 7.58 (d, 1H, J = 7.6 Hz, Ar_1–H_6), 7.49–7.46 (d, 2H, J = 8.0 Hz, Ar_2–H_3 and Ar_2–H_5), 7.09 (t, 1H, J = 7.2 Hz, Ar_1–H_5), 7.01–6.99 (d, 2H, J = 8.0 Hz, Ar_2–H_2 and Ar_2–H_6), 3.80 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6, 101 MHz), δ 176.0, 162.7, 157.4, 141.3, 133.6, 131.4, 131.2, 127.2, 123.8, 122.1, 120.2, 113.6,

103.3, 55.2; IR: (KBr, ν/cm^{-1}), 3302 (NN–H), 3261 (CON–H), 3047 (Ar–H), 1695 (–CONH–), 1612 (C=N), 1595 and 1542 (Ar–C=C), 1258 (C=S), 1148 (N–N); Elemental analysis calculated for $C_{16}H_{13}BrN_4O_2S$, C: 47.42, H: 3.23, N: 13.82, found C: 47.63, H: 3.40, N: 13.89; ESI-MS m/z: 405.0, $[M - H]^-$.

*4.2.39. (Z)-N-(2-Fluorophenyl)-2-(7-iodo-2-oxoindolin-3-ylidene) hydrazinecarbothioamide (**3-39**)*

Yield 75%; m.p.: 252–254 °C; yellow solid; ^{1}H NMR (DMSO-d_6, 400 MHz), δ 12.82 (s, 1H, NNH), 11.29 (s, 1H, NH), 10.77 (s, 1H, SCNH), 7.75–7.72 (m, 2H, J = 4.8 Hz, Ar_1–H_4 and Ar_2–H_6), 7.50 (t, 1H, J = 6.8 Hz, Ar_1–H_5), 7.43–7.33 (m, 2H, J = 6.8 Hz, Ar_2–H_2 and Ar_2–H_5), 7.28 (t, 1H, J = 7.6 Hz, Ar_2–H_3), 6.93 (t, 1H, J = 6.8 Hz, Ar_2–H_4); ^{13}C NMR (DMSO-d_6, 101 MHz), δ 177.9, 162.7, 145.2, 139.9, 132.6, 130.1, 129.0, 126.4, 124.4, 124.2, 121.2, 120.5, 116.1, 115.9, 76.7; IR: (KBr, ν/cm^{-1}), 3319 (NN–H), 3182 (CON–H), 3060 (Ar–H), 1696 (–CONH–), 1613 (C=N), 1575 and 1542 (Ar–C=C), 1198 (C=S), 1146 (N–N); HRMS(MALDI) m/z: calculated for $C_{15}H_{10}FIN_4OS$ 438.9526, found 438.9526, $[M - H]^-$.

*4.2.40. (Z)-2-(7-Iodo-2-oxoindolin-3-ylidene)-N-(2-methoxyphenyl)hydrazinecarbothioamide (**3-40**)*

Yield 71%; m.p.: 264–266 °C; yellow solid; ^{1}H NMR (DMSO-d_6, 400 MHz), δ 12.74 (s, 1H, NNH), 11.27 (s, 1H, NH), 10.49 (s, 1H, SCNH), 7.79 (d, 1H, J = 7.2 Hz, Ar_1–H_4), 7.74–7.70 (m, 2H, J = 8.4 Hz, Ar_1–H_6 and Ar_2–H_5), 7.30 (t, 1H, J = 7.2 Hz, Ar_1–H_5), 7.15 (d, 1H, J = 8.0 Hz, Ar_2–H_2), 7.09 (t, 1H, J = 7.6 Hz, Ar_2–H_3), 6.93 (t, 1H, J = 7.6 Hz, Ar_2–H_4), 3.86 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6, 101 MHz), δ 176.1, 162.7, 153.0, 145.1, 139.8, 132.1, 127.6, 126.9, 126.4, 124.2, 121.2, 120.3, 120.0, 111.7, 76.6, 55.7; IR: (KBr, ν/cm^{-1}), 3284 (NN–H), 3176 (CON–H), 3056 (Ar–H), 1689 (–CONH–), 1610 (C=N), 1544 and 1476 (Ar–C=C), 1253 (C=S), 1179 (N–N); Elemental analysis calculated for $C_{16}H_{13}IN_4O_2S$, C: 42.49, H: 2.90, N: 12.39, found C: 42.43, H: 3.05, N: 12.66; ESI-MS m/z: 451.0, $[M - H]^-$.

*4.2.41. (Z)-2-(7-Iodo-2-oxoindolin-3-ylidene)-N-(o-tolyl) hydrazinecarbothioamide (**3-41**)*

Yield 74%; m.p.: 241–243 °C; yellow solid; ^{1}H NMR (DMSO-d_6, 400 MHz), δ 12.73 (s, 1H, NNH), 11.27 (s, 1H, NH), 10.78 (s, 1H, SCNH), 7.77–7.72 (m, 2H, J = 8.4 Hz, Ar_1–H_4 and Ar_2–H_6), 7.34–7.27 (m, 4H, J = 7.6 Hz, Ar_1–H_5, Ar_2–H_2, Ar_2–H_3, and Ar_2–H_5), 6.93 (t, 1H, J = 7.6 Hz, Ar_2–H_4), 2.26 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 101 MHz), δ 177.1, 162.7, 145.0, 139.7, 137.3, 135.4, 132.1, 130.3, 128.4, 127.4, 126.2, 124.1, 121.3, 120.5, 76.4, 17.6; IR: (KBr, ν/cm^{-1}) 3304 (NN–H), 3240 (CON–H), 3057 (Ar–H), 1698 (–CONH–), 1611 (C=N), 1587 and 1541 (Ar–C=C), 1288 (C=S), 1152 (N–N); Elemental analysis calculated for $C_{16}H_{13}IN_4OS$, C: 44.05, H: 3.00, N: 12.84; found C: 44.00, H: 3.05, N: 12.60; ESI-MS m/z: 434.9, $[M - H]^-$.

*4.2.42. (Z)-N-(3-Chlorophenyl)-2-(7-iodo-2-oxoindolin-3-ylidene) hydrazinecarbothioamide (**3-42**)*

Yield 78%; m.p.: 251–253 °C; yellow solid; ^{1}H NMR (DMSO-d_6, 400 MHz), δ 12.81 (s, 1H, NNH), 11.31 (s, 1H, NH), 10.94 (s, 1H, SCNH), 7.80–7.78 (m, 2H, J = 4.4 Hz, Ar_1–H_4 and Ar_2–H_6), 7.74 (d, 1H, J = 8.0 Hz, Ar_1–H_6), 7.66 (d, 1H, J = 8.0 Hz, Ar_2–H_4), 7.47 (t, 1H, J = 8.0 Hz, Ar_1–H_5), 7.35 (d, 1H, J = 8.0 Hz, Ar_2–H_2), 6.94 (t, 1H, J = 8.0 Hz, Ar_2–H_3); ^{13}C NMR (DMSO-d_6, 101 MHz), δ 176.2, 162.7, 145.1, 139.9, 139.8, 132.6, 132.4, 129.9, 125.8, 125.0, 124.1, 121.1, 120.8, 76.6; IR: (KBr, ν/cm^{-1}), 3311 (NN–H), 3163 (CON–H), 3059 (Ar–H), 1696 (–CONH–), 1611 (C=N), 1589 and 1541 (Ar–C=C), 1236 (C=S), 1151 (N–N); Elemental analysis calculated for $C_{15}H_{10}ICIN_4OS$, C: 39.45, H: 2.21, N: 12.27, found C: 39.25, H: 2.40, N: 12.07; ESI-MS m/z: 454.9, $[M - H]^-$.

*4.2.43. (Z)-2-(7-Iodo-2-oxoindolin-3-ylidene)-N-(3-methoxyphenyl)hydrazinecarbothioamide (**3-43**)*

Yield 68%; m.p.: 166–168 °C; yellow solid; ^{1}H NMR (DMSO-d_6, 400 MHz), δ 12.75 (s, 1H, NNH), 11.28 (s, 1H, NH), 10.83 (s, 1H, SCNH), 7.81 (d, 1H, J = 6.8 Hz, Ar_1–H_4), 7.74 (d, 1H, J = 8.0 Hz, Ar_1–H_6), 7.36–7.29 (m, 2H, J = 8.0 Hz, Ar_1–H_5 and Ar_2–H_6), 7.24 (d, 1H, J = 8.0 Hz, Ar_2–H_4), 6.93 (t, 1H, J = 8.0 Hz, Ar_2–H_3), 6.86 (d, 1H, J = 6.4 Hz, Ar_2–H_2), 3.79 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6, 101 MHz), δ 176.0, 162.7, 159.1, 145.0, 139.8, 139.4, 132.2, 129.1, 124.1, 121.2, 120.8, 117.5, 111.6, 111.1, 76.5, 56.2; IR: (KBr, ν/cm^{-1}), 3246 (NN–H), 3133 (CON–H), 3059 (Ar–H), 1700 (–CONH–), 1610 (C=N), 1539 and 1477 (Ar–C=C), 1207 (C=S), 1153 (N–N); Elemental analysis calculated for $C_{16}H_{13}IN_4O_2S$, C: 42.49, H: 2.90, N: 12.39, found C: 42.65, H: 3.10, N: 12.13; ESI-MS m/z: 379.1, $[M + Cl]^-$.

*4.2.44. (Z)-2-(7-Iodo-2-oxoindolin-3-ylidene)-N-(m-tolyl) hydrazinecarbothioamide (**3-44**)*

Yield 76%; m.p.: 256–258 °C; yellow solid; ^{1}H NMR (DMSO-d_6, 400 MHz), δ 12.73 (s, 1H, NNH), 11.28 (s, 1H, NH), 10.82 (s, 1H, SCNH), 7.81 (d, 1H, J = 7.2 Hz, Ar_1–H_4), 7.73 (d, 1H, J = 7.6 Hz, Ar_1–H_6), 7.44–7.42 (m, 2H, J = 8.0 Hz, Ar_2–H_4 and Ar_2–H_6), 7.32 (t, 1H, J = 8.0 Hz, Ar_1–H_5), 7.10 (d, 1H, J = 7.2 Hz, Ar_2–H_2), 6.93 (t, 1H, J = 7.6 Hz, Ar_2–H_3), 2.35 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 101 MHz), δ 176.1, 162.7, 145.0, 139.8, 138.2, 137.7, 132.1, 128.2, 126.8, 126.0, 124.1, 122.7, 121.3, 120.7, 76.5, 20.9; IR: (KBr, ν/cm^{-1}), 3304 (NN–H), 3225 (CON–H), 3076 (Ar–H), 1691 (–CONH–), 1610 (C=N), 1547 and 1480 (Ar–C=C), 1247 (C=S), 1181 (N–N); Elemental analysis calculated for $C_{16}H_{13}IN_4OS$, C: 44.05, H: 3.00, N: 12.84, Found C: 43.86, H: 3.27, N: 12.73; HRMS(MALDI) m/z: calculated 434.9776, found 434.9775, $[M - H]^-$.

*4.2.45. (Z)-N-(4-Chlorophenyl)-2-(7-iodo-2-oxoindolin-3-ylidene) hydrazinecarbothioamide (**3-45**)*

Yield 74%; m.p.: 258–260 °C; yellow solid, ^{1}H NMR (DMSO-d_6, 400 MHz); δ 12.79 (s, 1H, NNH), 11.29 (s, 1H, NH), 10.91 (s, 1H, SCNH), 7.78 (d, 1H, J = 8.8 Hz, Ar_1–H_4), 7.74 (d, 1H, J = 7.6 Hz, Ar_1–H_6), 7.67–7.65 (m, 2H, J = 8.8 Hz, Ar_2–H_3 and Ar_2–H_5), 7.51–7.48 (m, 2H, J = 8.4 Hz, Ar_2–H_2 and Ar_2–H_6), 6.93 (t, 1H, J = 8.0 Hz, Ar_1–H_5); ^{13}C NMR (DMSO-d_6, 101 MHz), δ 176.3, 162.7, 145.1, 139.9, 137.3, 132.4, 130.1, 128.3, 127.3, 124.1, 121.2, 120.7, 76.5; IR: (KBr, ν/cm^{-1}), 3304 (NN–H), 3129 (CON–H), 3060 (Ar–H), 1696 (–CONH–), 1612 (C=N), 1591 and 1535 (Ar–C=C), 1240 (C=S), 1159 (N–N); HRMS(MALDI) m/z: calculated for $C_{15}H_{10}ICIN_4OS$ 454.9230, found 454.9232, $[M - H]^-$.

*4.2.46. (Z)-N-(4-Bromophenyl)-2-(7-iodo-2-oxoindolin-3-ylidene) hydrazinecarbothioamide (**3-46**)*

Yield 71%; m.p.: 261–263 °C; yellow solid; ^{1}H NMR (DMSO-d_6, 400 MHz), δ 12.80 (s, 1H, NNH), 11.30 (s, 1H, NH), 10.91 (s, 1H, SCNH), 7.79–7.75 (m, 2H, J = 4.0 Hz, Ar_1–H_4 and Ar_1–H_6), 7.63–7.63 (m, 4H, J = 2.4 Hz, Ar_2–H_2, Ar_2–H_3, Ar_2–H_5, and Ar_2–H_6), 6.97–6.93 (m, 1H, J = 6.8 Hz, Ar_1–H_5); ^{13}C NMR (DMSO-d_6, 101 MHz); δ 176.3, 162.7, 145.1, 139.9, 137.8, 132.5, 131.2, 127.6, 124.1, 121.2, 120.7, 118.4, 75.5; IR: (KBr, ν/cm^{-1}), 3304 (NN–H), 3129 (CON–H), 3060 (Ar–H), 1696 (–CONH–), 1612 (C=N), 1591 and 1535 (Ar–C=C), 1240 (C=S), 1159 (N–N); Elemental analysis calculated for $C_{15}H_{10}IBrN_4OS$, C: 35.95, H: 2.01, N: 11.18, found C: 36.13, H: 2.14, N: 11.11; ESI-MS m/z: 500.7, $[M - H]^-$.

*4.2.47. (Z)-2-(7-Iodo-2-oxoindolin-3-ylidene)-N-(4-methoxyphenyl)hydrazinecarbothioamide (**3-47**)*

Yield 79%; m.p.: 256–258 °C; yellow solid; ^{1}H NMR (DMSO-d_6, 400 MHz), δ 12.70 (s, 1H, NNH), 11.26 (s, 1H, NH), 10.78 (s, 1H, SCNH), 7.79 (d, 1H, J = 7.2 Hz, Ar_1–H_4), 7.72 (d, 1H, J = 7.6 Hz, Ar_1–H_6), 7.48–7.46 (m, 2H, J = 8.4 Hz, Ar_2–H_3 and Ar_2–H_5),

7.00–6.98 (m, 2H, J = 8.4 Hz, Ar_2–H_2 and Ar_2–H_6), 6.92 (t, 1H, J = 7.6 Hz, Ar_1–H_5), 3.79 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6, 101 MHz), δ 176.4, 162.7, 157.4, 144.9, 139.7, 131.9, 131.1, 127.2, 124.1, 121.3, 120.6, 113.5, 76.4, 55.2; IR: (KBr, ν/cm^{-1}), 3304 (NN–H), 3163 (CON–H), 3058 (Ar–H), 1695 (–CONH–), 1611 (C=N), 1538 and 1475 (Ar–C=C), 1248 (C=S), 1134 (N–N); Elemental analysis calculated for $C_{16}H_{13}IN_4O_2S$, C: 42.49, H: 2.90, N: 12.39, found C: 42.70, H: 2.67, N: 12.36; ESI-MS m/z: 218.0, $[M - H]^-$.

4.2.48. (Z)-2-(7-Iodo-2-oxoindolin-3-ylidene)-N-(p-tolyl) hydrazinecarbothioamide (**3-48**)

Yield 74%; m.p.: 260–262 °C; yellow solid; ^{1}H NMR (DMSO-d_6, 400 MHz), δ 12.72 (s, 1H, NNH), 11.28 (s, 1H, NH), 10.82 (s, 1H, SCNH), 7.80 (d, 1H, J = 6.4 Hz, Ar_1–H_4), 7.73 (d, 1H, J = 7.6 Hz, Ar_1–H_6), 7.48–7.46 (m, 2H, J = 7.2 Hz, Ar_2–H_3 and Ar_2–H_5), 7.24–7.22 (m, 2H, J = 7.2 Hz, Ar_2–H_2 and Ar_2–H_6), 6.93 (t, 1H, J = 7.2 Hz, Ar_1–H_5), 2.34 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 101 MHz), δ 176.2, 162.7, 145.0, 139.7, 135.8, 135.4, 132.0, 128.8, 125.5, 124.1, 121.3, 120.7, 76.5, 20.6; IR: (KBr, ν/cm^{-1}); 3306 (NN–H), 3165 (CON–H), 3126 (Ar–H), 1693 (–CONH–), 1612 (C=N), 1595 and 1536 (Ar–C=C), 1240 (C=S), 1153 (N–N); Elemental analysis calculated for $C_{16}H_{13}IN_4OS$ C: 44.05, H: 3.00, N: 12.84, found C: 43.94, H: 3.26, N: 12.78; ESI-MS m/z: 434.9, $[M - H]^-$.

4.2.49. (Z)-2-(7-Fluoro-2-oxoindolin-3-ylidene)-N-(2-methoxyphenyl)hydrazinecarbothioamide (**3-49**)

Yield 77%; m.p.: 236–238 °C; yellow solid; ^{1}H NMR (DMSO-d_6, 400 MHz), δ 12.76 (s, 1H, NNH), 11.80 (s, 1H, NH), 10.51 (s, 1H, SCNH), 7.79 (d, 1H, J = 7.6 Hz, Ar_1–H_4), 7.55 (d, 1H, J = 7.2 Hz, Ar_1–H_6), 7.33–7.28 (m, 2H, J = 8.4 Hz, Ar_1–H_5 and Ar_2–H_5), 7.16–7.10 (m, 2H, J = 7.2 Hz, Ar_2–H_2 and Ar_2–H_3), 7.01 (t, 1H, J = 7.2 Hz, Ar_2–H_4), 3. 86 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6, 101 MHz), δ 176.1, 162.4, 153.0, 148.1, 145.8, 131.4, 129.3, 127.6, 126.9, 123.3, 122.8, 120.0, 118.0, 117.0, 111.7, 55.7; IR: (KBr, ν/cm^{-1}), 3314 (NN–H), 3178 (CON–H), 3077 (Ar–H), 1693 (–CONH–), 1600 (C=N), 1574 and 1483 (Ar–C=C), 1251 (C=S), 1161 (N–N); HRMS(MALDI) m/z: calculated for $C_{16}H_{13}FN_4O_2S$ 345.0821, found 345.0817, $[M + H]^+$.

4.2.50. (Z)-N-(3-Fluorophenyl)-2-(7-iodo-2-oxoindolin-3-ylidene) hydrazinecarbothioamide (**3-50**)

Yield 74%; m.p.: 234–236 °C; yellow solid; ^{1}H NMR (DMSO-d_6, 400 MHz), δ 12.81 (s, 1H, NNH), 11.29 (s, 1H, NH), 10.92 (s, 1H, SCNH), 7.79 (d, 1H, J = 7.2 Hz, Ar_1–H_4), 7.73 (d, 1H, J = 7.6 Hz, Ar_1–H_6), 7.63 (d, 1H, J = 10.8 Hz, Ar_2–H_4), 7.52–7.44 (m, 2H, J = 8.0 Hz, Ar_2–H_2 and Ar_2–H_6), 7.12 (t, 1H, J = 7.2 Hz, Ar_1–H_5), 6.93 (t, 1H, J = 7.6 Hz, Ar_2–H_3), 3. 86 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6, 101 MHz), δ 176.1, 162.7, 160.3, 145.1, 140.0, 139.9, 132.5, 129.9, 124.1, 121.2, 121.1, 120.8, 112.8, 112.1, 76.5; IR: (KBr, ν/cm^{-1}), 3301 (NN–H), 3163 (CON–H), 3055 (Ar–H), 1693 (–CONH–), 1611 (C=N), 1547 and 1524 (Ar–C=C), 1219 (C=S), 1147 (N–N); Elemental analysis calculated for $C_{15}H_{10}IFN_4OS$, C: 40.92, H: 2.29, N: 12.73, found C: 40.90, H: 2.15, N: 12.61; ESI-MS m/z: 438.9, $[M - H]^-$.

4.2.51. (Z)-N-(4-Fluorophenyl)-2-(7-iodo-2-oxoindolin-3-ylidene) hydrazinecarbothioamide (**3-51**)

Yield 78%; m.p.: 259–261 °C; yellow solid; ^{1}H NMR (DMSO-d_6, 400 MHz), δ 12.76 (s, 1H, NNH), 11.29 (s, 1H, NH), 10.89 (s, 1H, SCNH), 7.79 (d, 1H, J = 7.6 Hz, Ar_1–H_4), 7.74 (d, 1H, J = 8.0 Hz, Ar_1–H_6), 7.62–7.58 (m, 2H, J = 5.6 Hz, Ar_2–H_3 and Ar_2–H_5), 7.30–7.26 (m, 2H, J = 4.4 Hz, Ar_2–H_2 and Ar_2–H_6), 6.94 (t, 1H, J = 8.0 Hz, Ar_1–H_5); ^{13}C NMR (DMSO-d_6, 101 MHz), δ 176.7, 162.7, 145.0, 139.8, 134.7, 132.2, 128.0, 127.9, 124.1, 121.2, 120.6, 115.2, 76.5; IR: (KBr, ν/cm^{-1}), 3247 (NN–H), 3164 (CON–H), 3059 (Ar–H), 1697 (–CONH–), 1610 (C=N), 1541 and 1478 (Ar–C=C), 1236 (C=S), 1152 (N–N); HRMS(MALDI) m/z: calculated for $C_{15}H_{10}IFN_4OS$, 438.9526, found 438.9529, $[M - H]^-$.

4.3. Bacterial strains and in vitro antibacterial susceptibility and MIC determination

The Gram-positive bacterial species and strains used in this study include *S. aureus* (ATCC 6538), MRSA clinical isolates (Chaoyang, 309-4, 6281, 309-8, 6-42, 8-21, 309-3, 309-1, 309-7, 8-24 and 309-6), *B. subtilis* (ATCC 6633), *E. faecalis* and VRE (VRE-309). The Gram-negative bacteria species and strains used in this study are *P. aeruginosa* (PAO1) and *K. pneumoniae* (KP-249). MRSA (309-4, 309-8, 309-3, 309-1, 309-7 and 309-6) and VRE-309 are clinical isolated strains from the 309th hospital of Chinese People's Liberation Army in Beijing. MRSA (Chaoyang) is a clinical isolated strain from Chaoyong hospital in Beijing. MRSA (6281, 6-42, 8-21 and 8-24) are clinical isolated strains from the 306th hospital of Chinese People's Liberation Army in Beijing. *E. faecalis* is a clinical isolated vancomycin-susceptible strain from Frederick Ausubel's lab (Harvard Medical School). KP-249 is a clinical isolated *K. pneumoniae* strain from 309th hospital of Chinese People's Liberation Army in Beijing.

The procedure for the antimicrobial bioassay was similar to our published method [29]. All the IBT compounds were prepared into 4 mg/mL stock solution using sterile dimethyl sulfoxide (DMSO). The stock solution was serially diluted by sterile DMSO. The microbes were stored as glycerol stocks in a −80 °C refrigerator and streaked onto Mueller-Hinton agar (MHA) for colony growth at 37 °C before use. The antibacterial activities and MICs were determined in flat bottom, 96-well microtiter plates using a broth microdilution protocol modified from the Clinical and Laboratory Standards Institute M7-A6, M-38A and M-27A2 methods. Briefly, single colonies of bacteria were picked from MHA plate and adjusted to approximately 10^5 CFU/mL with Mueller-Hinton Broth (MHB) as bacterial suspension. Aliquots 2 μL of 2-fold serial dilution of each compound (in DMSO) were added to each row on 96-well plate containing 78 μL of bacteria suspension in each well. The 96-well plate was incubated aerobically at 37 °C for 16 h before results were recorded. MICs were defined as the lowest concentrations of the compounds that can inhibit visible bacterial growth after 16 h incubation. The MICs were tested twice in triplicate. For the bioassays, vancomycin was used as a control drug for *S. aureus*, MRSA, *B. subtilis*, *E. faecalis* and VRE, methicillin was used as a control drug for MRSA, while ciprofloxacin was used as a control drug for *P. aeruginosa* and *K. pneumoniae*.

4.4. Comparative field analysis

Chemical structures of the compounds were built within Sybyl 7.3 (Tripos Inc., St Louis, MO) based on the crystal structure of **3-1**. All the molecules were assigned Gasteiger-Hückel charges and minimized by the Tripos force field when convergence reached 0.001 kcal/mol/Å. The molecules were superimposed using **3-2** as the template. All the parameters were used the default value within comparative field analysis (CoMFA) module and the column filtering was set to 2.0 kcal/mol. Compounds **3-1**, **3-3**, **3-15**, **3-18**, **3-33** and **3-47** were randomly selected into the test set and the other 44 compounds were used in the training set (**3-49** was not included because its MIC could not be determined). The "leave-one-out" (LOO) cross validation method was applied to determine the optimum number of partial least squares (PLS) components. The biological activity data were expressed in terms of D using the following formula

$$D = -\log_{10}[\mathrm{MIC}/(M_w \times 1000)]$$

where MIC is the data for MRSA (Changyang) and M_w is the molecular weight of the inhibitor. The plot of predictive biological activity versus the experimental biological activity from the non-crossvalidated CoMFA analysis for the training set is shown in Fig. S1. The predictive biological activity versus the experimental biological activity for the test set is show in Table S1.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2015.06.047.

References

[1] P.D. Goldsworthy, A.C. McFarlane, Howard Florey, Alexander Fleming and the fairy tale of penicillin, Med. J. Aust. 176 (2002) 176–178.
[2] M. Barber, Methicillin-resistant *staphylococci*, J. Clin. Pathol. 14 (1961) 385–393.
[3] A.H. Uttley, C.H. Collins, J. Naidoo, R.C. George, Vancomycin-resistant *enterococci*, Lancet 1 (1988) 57–58.
[4] L.B. Rice, Federal funding for the study of antimicrobial resistance in nosocomial pathogens: no ESKAPE, J. Infect. Dis. 197 (2008) 1079–1081.
[5] K.K. Kumarasamy, M.A. Toleman, T.R. Walsh, J. Bagaria, F. Butt, R. Balakrishnan, U. Chaudhary, M. Doumith, C.G. Giske, S. Irfan, P. Krishnan, A.V. Kumar, S. Maharjan, S. Mushtaq, T. Noorie, D.L. Paterson, A. Pearson, C. Perry, R. Pike, B. Rao, U. Ray, J.B. Sarma, M. Sharma, E. Sheridan, M.A. Thirunarayan, J. Turton, S. Upadhyay, M. Warner, W. Welfare, D.M. Livermore, N. Woodford, Lancet Infect. Dis. 10 (2010) 597–602.
[6] G.M. Eliopoulos, Microbiology of drugs for treating multiple drug-resistant Gram-positive bacteria, J. Infect. 59 (2009) S17–S24.
[7] M.E. Stryjewski, G.R. Corey, New treatments for methicillin-resistant *Staphylococcus aureus*, Curr. Opin. Crit. Care 15 (2009) 403–412.
[8] M.D. Johnson, C.F. Decker, Antimicrobial agents in treatment of MRSA infections, Dis. Mon. 54 (2008) 793–800.
[9] D.L. Paterson, Clinical experience with recently approved antibiotics, Curr. Opin. Pharmacol. 6 (2006) 486–490.
[10] I.M. Gould, M.Z. David, S. Esposito, J. Garau, G. Lina, T. Mazzei, G. Peters, New insights into meticillin-resistant *Staphylococcus aureus* (MRSA) pathogenesis, treatment and resistance, Int. J. Antimicrob. Agents 39 (2012) 96–104.
[11] A. Medvedev, O. Buneeva, V. Glover, Biological targets for isatin and its analogues: implications for therapy, Biologics 1 (2007) 151–162.
[12] M.S. Gil-Turnes, M.E. Hay, W. Fenical, Symbiotic marine bacteria chemically defend crustacean embryos from a pathogenic fungus, Science 246 (1989) 116–118.
[13] C. Binda, M. Li, F. Hubalek, N. Restelli, D.E. Edmondson, A. Mattevi, Insights into the mode of inhibition of human mitochondrial monoamine oxidase B from high-resolution crystal structures, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 9750–9755.
[14] J.F.M. Silva, S.J. Garden, A.C. Pinto, The chemistry of isatins: a review from 1975 to 1999, J. Braz. Chem. Soc. 12 (2001) 273–324.
[15] S.N. Pandeya, S. Smitha, M. Jyoti, S.K. Sridhar, Biological activities of isatin and its derivatives, Acta Pharm. 55 (2005) 27–46.
[16] K.L. Vine, L. Matesic, J.M. Locke, M. Ranson, D. Skropeta, Cytotoxic and anticancer activities of isatin and its derivatives: a comprehensive review from 2000-2008, Anticancer Agents Med. Chem. 9 (2009) 397–414.
[17] H.M. Zhang, H. Dai, P.J. Hanson, H. Li, H. Guo, X. Ye, M.G. Hemida, L. Wang, Y. Tong, Y. Qiu, S. Liu, F. Wang, F. Song, B. Zhang, J.G. Wang, L.X. Zhang, D. Yang, Antiviral activity of an isatin derivative via induction of PERK-Nrf2-mediated suppression of cap-independent translation, ACS Chem. Biol. 9 (2014) 1015–1024.
[18] Z. Xiao, Y. Hao, B. Liu, L. Qian, Indirubin and meisoindigo in the treatment of chronic myelogenous leukemia in China, Leuk. Lymphoma 43 (2002) 1763–1768.
[19] D.J. Bauer, Chemoprophylaxis of smallpox and treatment of vaccinia gangrenosa with 1-methylisatin 3-thiosemicarbazone, Antimicrob. Agents Chemother. 5 (1965) 544–547.
[20] M.C. Pirrung, S.V. Pansare, K.D. Sarma, K.A. Keith, E.R. Kern, Combinatorial optimization of isatin-beta-thiosemicarbazones as anti-poxvirus agents, J. Med. Chem. 48 (2005) 3045–3050.
[21] A.M. Omar, N.H. Eshba, H.M. Salama, Syntheses of some substituted isatin-beta-thiosemicarbazones and isatin-beta-hydrazonothiazoline derivatives as potential antiviral and antimicrobial agents, Arch. Pharm. 317 (1984) 701–709.
[22] H. Pervez, Z.H. Chohan, M. Ramzan, F. Nasim, K.M. Khan, Synthesis and biological evaluation of some new N^4-substituted isatin-3-thiosemicarbazones, J. Enzyme Inhib. Med. Chem. 24 (2009) 437–446.
[23] M.D. Hall, K.R. Brimacombe, M.S. Varonka, K.M. Pluchino, J.K. Monda, J. Li, M.J. Walsh, M.B. Boxer, T.H. Warren, H.M. Fales, M.M. Gottesman, Synthesis and structure-activity evaluation of isatin-β-thiosemicarbazones with improved selective activity toward multidrug-resistant cells expressing P-glycoprotein, J. Med. Chem. 54 (2011) 5878–5889.
[24] D.J. Bauer, P.W. Sadler, The structure-activity relationships of the antiviral chemotherapeutic activity of isatin-β-thiosemicarbazone, Br. J. Pharmacol. Chemother. 15 (1960) 101–110.
[25] I.J. Kang, L.W. Wang, T.A. Hsu, A. Yueh, C.C. Lee, Y.C. Lee, C.Y. Lee, Y.S. Chao, S.R. Shih, J.H. Chern, Isatin-β-thiosemicarbazones as potent herpes simplex virus inhibitors, Bioorg. Med. Chem. Lett. 21 (2011) 1948–1952.
[26] N. Karali, A. Guersoy, F. Kandemirli, N. Shvets, F.B. Kaynak, S. Oezbey, V. Kovalishyn, A. Dimoglo, Synthesis and structure-antituberculosis activity relationship of 1H-indole-2,3-dione derivatives, Bioorg. Med. Chem. 15 (2007) 5888–5904.
[27] H.D. Li, J.L. Shang, H.Z. Tan, J.G. Wang, Y.H. Li, Z.M. Li, Design, synthesis of 3-substituted benzoyl hydrazone and 3-(substituted thiourea)yl isatin derivatives and their inhibitory activities of AHAS, Chem. J. Chin. Univ. 31 (2010) 935–956.
[28] R.D. Cramer, D.E. Patterson, J.D. Bunce, Comparative molecular field analysis (CoMFA). 1. Effect of shape on binding of steroids to carrier proteins, J. Am. Chem. Soc. 110 (1988) 5959–5967.
[29] J.L. Shang, H. Guo, Z.S. Li, B. Ren, Z.M. Li, H.Q. Dai, L.X. Zhang, J.G. Wang, Synthesis and evaluation of novel sulfenamides as novel anti methicillin-resistant Staphylococcus aureus agents, Bioorg. Med. Chem. Lett. 23 (2013) 724–727.