Synthesis and *in vitro* Bio-activity Evaluation of N^4 -benzyl Substituted 5-Chloroisatin-3-thiosemicarbazones as Urease and Glycation Inhibitors

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Abstract

A series of fifteen N^4 -benzyl substituted 5-chloroisatin-3-thiosemicarbazones **5a–o** were synthesized and screened mainly for their antiurease and antiglycation effects. *Lemna aequinocalis* growth and *Artemia salina* assays were carried out to determine their phytotoxicity and cytotoxicity potential. All the compounds proved to be extremely effective urease inhibitors, demonstrating enzyme inhibition much better than the reference inhibitor, thiourea (IC_{50} values 1.31 ± 0.06 to 3.24 ± 0.15 vs. 22.3 ± 1.12 μ M). On the other hand, eight out of fifteen compounds tested, i.e. **5b**, **5c**, **5h–k**, **5m** and **5n** were found to be potent glycation inhibitors. Of these, five viz. **5c**, **5h–j** and **5n** proved to be exceedingly efficient, displaying glycation inhibition greater than the reference inhibitor, rutin (IC_{50} values 114.51 ± 1.08 to 229.94 ± 3.40 vs. 294.5 ± 1.5 μ M).

Keywords: 5-Chloroisatin, Glycation inhibition, Heterocyclics, Schiff bases, Thiosemicarbazones, Urease inhibition

1. Introduction

Isatin and its derivatives are known to have a diversity of chemotherapeutic activities.^{1–35} Of the isatin derivatives, isatin-thiosemicarbazones have been found to exhibit a range of biological properties, including antiulcer,¹ anticancer,^{1,4,7,12,21–23} antimicrobial,^{1–3,5,7,22,24,25} antituberculosis,^{26–28} antiviral^{1,2,5,7,12,28} and enzyme inhibitory activities.^{1,4,11,17} Incited by these findings and as a part of our synthetic work on bioactive isatin derivatives, we have recently reported the synthesis of numerous N^4 -aryl substituted isatin-3-thiosemicarbazones (thiourea derivatives) as antimicrobial,^{36–39} cytotoxic,^{38–42} phytotoxic,^{38–41,43} antileishmanial⁴⁴ and more importantly antiurease^{36–40,43,45} compounds. SAR studies in the synthesized thiosemicarbazones disclosed that in some cases the nature and location of the substituent on the phenyl ring attached to thiosemicarbazone N^4 and/or the existence of lipophilic/inductively electron-withdrawing functions (Cl, F, F_3CO , NO_2) at po-

sition 5 of the isatin moiety played a significant role in inducing and/or increasing certain activities, including urease inhibition. In view of this and as an extension of our earlier studies^{36–47} aiming to the synthesis of novel isatin derivatives with enhanced or diverse biological activities, we very recently reported the synthesis of a series of N^4 -benzyl substituted 5-nitroisatin-3-thiosemicarbazones (derivatives of thiourea, a substrate-like urease inhibitor) as highly effective urease inhibitors.⁴⁸ Interestingly, all these compounds demonstrated either induced or increased urease inhibitory activity in comparison to the respective N^4 -phenyl substituted derivatives tested in our earlier assays.^{38,45} Furthermore, a number of other derivatives of thiourea are reported to show promising glycation inhibitory activity.^{49–53} Also, some isatin-derived imines (Schiff bases) have been recognized as potent inhibitors of glycation.^{54,55} Prompted by these observations and as a continuation of our previous studies on bioactive isatin

derivatives, we have synthesized a series of fifteen title thiosemicarbazones (a class of Schiff bases) and tested them principally for their antiurease and antiglycation influences. *L. aequinocitalis* growth and *A. salina* assays were carried out to determine their phytotoxic and cytotoxic effects, respectively.

This paper is one of the first to report on N^4 -benzyl substituted isatin-thiosemicarbazones possessing urease and glycation inhibitory potential. In it we limit our discussion to those compounds which have concurrently attached lipophilic/inductively electron-withdrawing chloro substituent at position 5 of the isatin scaffold and the benzyl functions (modified by placing one inductively electron-donating and one or two electron-attracting groups around the phenyl ring) to thiosemicarbazone N^4 .

2. Results and Discussion

This study describes the preparation and *in vitro* testing of antiurease, antiglycation, phytotoxic and cytotoxic potentiality of fourteen presently and one formerly reported²² N^4 -benzyl substituted 5-chloroisatin-thiosemicarbazones **5b–o** and **5a**, respectively.

2. 1. Chemistry of Compounds 5a–o

Appropriate N -substituted thiosemicarbazides **3** were reacted with 5-chloroisatin **4** in 50% ethanol (aq.) to furnish the respective thiosemicarbazones **5a–o** (Scheme 1). The structures of **5a–o** were established by elemental

and spectral analyses. Acceptable elemental data were obtained in all the cases. The infrared (IR) spectra of **5a–o** showed absorption bands in the 3375–3051, 1737–1684, 1620–1599 and 1193–1149 cm^{-1} regions attributed to NH, C=O, C=N and C=S functions, respectively.^{22,56,57} The proton nuclear magnetic resonance (^1H NMR) spectra displayed two separate singlets at δ 7.50–11.36 and 10.84–12.75 for indole NH and thiosemicarbazone $\text{N}^2\text{-H}$. The thiosemicarbazone $\text{N}^4\text{-H}$ appeared as a triplet at δ 8.03–10.91 in all the cases,^{22,27} except **5e**, **5g** and **5h**, wherein it resonated as a singlet at δ 8.26, 10.91 and 10.85, respectively. Similarly, the benzyl CH_2 protons were observed as a doublet at δ 4.40–5.02 in all the cases,^{22,27} except **5b**, **5g** and **5h**; in the case of **5h** they appeared as a singlet at δ 3.79, whilst in **5b** and **5g** they resonated together with the DMSO and DMSO, CH_3 group protons, respectively. The carbon nuclear magnetic resonance (^{13}C NMR) spectra of compounds **5a–o** upheld the IR and ^1H NMR conclusions. Also, the EI (electron impact) mass spectra of all the compounds displayed molecular ions with mixed intensity and the fragments characteristic of isatin and thiosemicarbazone moieties. The main fragmentation path engaged the split of endocyclic NH–CO and exocyclic NH–CS, N–N bonds.⁵⁷ The proposed fragmentation pattern of **5i** is depicted in Figure S1.

2. 2. Biology of Compounds 5a–o

2. 2. 1. Urease Inhibition (*in vitro*)

The synthesized N^4 -benzyl substituted 5-chloroisatin-thiosemicarbazones **5a–o** were tested for their urease

Scheme 1. Reagents and conditions: (i) Et_3N , MeOH, CS_2 , stir, 30°C (internal), 75 min., r.t., 1 h; (ii) CH_3I , MeOH, stir, -10°C , 20 min., r.t., 2 h; (iii) $\text{NH}_2\text{NH}_2\cdot\text{H}_2\text{O}$, EtOH, reflux, 2 h; (iv) 50% EtOH (aq.), reflux, 2 h.

inhibitory effects and found to be extremely effective inhibitors of the enzyme, displaying superb inhibition ($IC_{50} = 1.31 \pm 0.06$ to $4.07 \pm 0.11 \mu\text{M}$) in contrast to the reference inhibitor, thiourea, which showed inhibitory activity with IC_{50} value $22.3 \pm 1.12 \mu\text{M}$ in this assay (Table 1).

SAR studies in the N^4 -benzyl substituted isatin-thiosemicarbazones **5a–o** divulged that in comparison to compound **5a** having no functional group on the phenyl ring of the benzyl group attached to thiosemicarbazone N^4 , all the rest compounds except **5d** and **5k**, regardless of the number, type and place of the functionalities present on the phenyl ring, displayed enhanced enzymatic inhibition (IC_{50} values 1.31 ± 0.06 to 3.05 ± 0.15 vs. $3.24 \pm 0.15 \mu\text{M}$). Compound **5l** bearing chloro substituent at position 3 of the phenyl ring was the most effective urease inhibitor of the series in the present assay, demonstrating two and half, and seventeen times larger activity than compound **5a** and the reference inhibitor, thiourea, respectively ($IC_{50} = 1.31 \pm 0.06$ vs. 3.24 ± 0.15 and $22.3 \pm 1.12 \mu\text{M}$). The next most effective enzyme inhibitor was **5e** possessing methoxy group at position 2 of the phenyl ring. This compound showed a somewhat lesser inhibitory activity than **5l** but is still about two and fifteen times more active than compound **5a** and thiourea (IC_{50} value 1.45 ± 0.23 vs. 1.31 ± 0.06 , 3.24 ± 0.15 and $22.3 \pm 1.12 \mu\text{M}$, respectively). The third most effective inhibitor of the enzyme was compound **5j** having fluoro group at position 4 of the phenyl ring. This compound like **5e** appeared to be a bit less active than **5l** (IC_{50} value 1.77 ± 0.24 vs. $1.31 \pm 0.06 \mu\text{M}$) but about two and twelve times more active than compound **5a** and thiourea (IC_{50} value 1.77 ± 0.24 vs. 3.24 ± 0.15 and $22.3 \pm 1.12 \mu\text{M}$, respectively). The isomeric compounds **5h** and **5i** possessing fluoro functionalities at positions 2 and 3 of the phenyl ring, however, demonstrated comparatively inferior but comparable enzymatic inhibition ($IC_{50} = 2.03 \pm 0.14$ and $2.07 \pm 0.09 \mu\text{M}$, respectively). The remaining relatively more effective inhibitors of the enzyme were the dichloro-substituted derivatives **5n** and **5o**. Among these, **5n** with the substituents at positions 2 and 4 of the phenyl ring displayed about two fold larger enzymatic inhibition in comparison to the monochloro-substituted compound **5k** having the substituent at position 2 of the phenyl ring (IC_{50} value 2.36 ± 0.16 vs. $4.07 \pm 0.11 \mu\text{M}$). On the other hand, **5o** possessing the substituents at positions 3 and 4 of the phenyl ring exhibited distinctly decreased inhibitory activity in contrast to monochloro-substituted compound **5l** (the most potent inhibitor of the series) with the substituent at position 3 of the phenyl ring (IC_{50} value 1.97 ± 0.16 vs. $1.31 \pm 0.06 \mu\text{M}$).

Interestingly, out of fifteen N^4 -benzyl substituted 5-chloroisatin-thiosemicarbazones **5a–o** tested in the present assay, five *viz.* **5e**, **5f**, **5k**, **5l** and **5o** were found to display enhanced urease inhibitory activity when compared with the corresponding N^4 -phenyl substituted 5-chloroisatin-thiosemicarbazones tested in our earlier assay.⁴³ For instance, compounds **5e**, **5k** and **5l** with 2-me-

thoxy-, 2-chloro- and 3-chloro-benzyl functions attached to N^4 of the thiosemicarbazone moiety demonstrated enzyme inhibitory activity with IC_{50} values 1.45 ± 0.23 , 4.07 ± 0.11 and 1.31 ± 0.06 , whereas the corresponding N^4 -phenyl substituted thiosemicarbazones displayed enzymatic inhibition with IC_{50} values 54.76, 54.35 and $38.91 \mu\text{M}$, respectively. Similarly, compounds **5f** and **5n** possessing 3-methoxy- and 2,4-dichloro-benzyl groups at N^4 of the thiosemicarbazone moiety showed inhibitory activity with IC_{50} values 2.63 ± 0.15 and $2.36 \pm 0.16 \mu\text{M}$, while the respective N^4 -phenyl substituted thiosemicarbazones displayed only 22.35 and 26.06% inhibition of the enzyme at $100 \mu\text{M}$ concentration. This indicated that the N^4 -benzyl substituted 5-chloroisatin-thiosemicarbazones **5e**, **5k**, **5l** and more importantly **5f**, **5n** compared with the related N^4 -phenyl substituted 5-chloroisatin-thiosemicarbazones interfered with the enzyme quite efficiently, causing much pronounced enhancement in their enzyme inhibitory potential.

The above results revealed that the attachment of varied benzyl groups (possessing one inductively electron-donating and one or two electron-withdrawing functional groups on the phenyl ring) to N^4 of the thiosemicarbazone moiety caused the molecules to intermingle with the enzyme differently and in some cases more competently. Moreover, exhibition of enhanced enzymatic inhibition by N^4 -benzyl substituted isatin-thiosemicarbazones in contrast to the parallel N^4 -aryl substituted ones may be explained on the basis of their better hydrophobic character, which may play a role in their indiscriminate walk process to the active site, and in the hydrophobic binding near the active site of the enzyme.

Table 1. Inhibition of Jack bean urease by compounds **5a–o**

Compound	R	$IC_{50} \pm \text{SEM} (\mu\text{M})^a$
5a	C_6H_5	3.24 ± 0.15
5b	$2\text{-CH}_3\text{C}_6\text{H}_4$	2.47 ± 0.21
5c	$3\text{-CH}_3\text{C}_6\text{H}_4$	2.55 ± 0.22
5d	$4\text{-CH}_3\text{C}_6\text{H}_4$	3.69 ± 0.15
5e	$2\text{-CH}_3\text{OC}_6\text{H}_4$	1.45 ± 0.23
5f	$3\text{-CH}_3\text{OC}_6\text{H}_4$	2.63 ± 0.15
5g	$4\text{-CH}_3\text{OC}_6\text{H}_4$	2.96 ± 0.19
5h	$2\text{-FC}_6\text{H}_4$	2.03 ± 0.14
5i	$3\text{-FC}_6\text{H}_4$	2.07 ± 0.09
5j	$4\text{-FC}_6\text{H}_4$	1.77 ± 0.24
5k	$2\text{-ClC}_6\text{H}_4$	4.07 ± 0.11
5l	$3\text{-ClC}_6\text{H}_4$	1.31 ± 0.06
5m	$4\text{-ClC}_6\text{H}_4$	3.05 ± 0.15
5n	$2,4\text{-(Cl)}_2\text{C}_6\text{H}_3$	2.36 ± 0.16
5o	$3,4\text{-(Cl)}_2\text{C}_6\text{H}_3$	1.97 ± 0.16
Thiourea ^b		22.3 ± 1.06

^a Standard error of the mean; ^b Standard inhibitor for urease.

2. 2. 2. Antiglycation Activity (*in vitro*)

The synthesized *N*⁴-benzyl substituted 5-chloroisatin-3-thiosemicarbazones **5a–o** were further tested for their antiglycation potentiality, using rutin as the reference inhibitor of glycation. Out of fifteen compounds tested, eight *i.e.* **5b**, **5c**, **5h–k**, **5m** and **5n** proved to be potent antiglycating agents, showing varied antiglycation activity (IC_{50} = 114.51 ± 1.08 to 433.88 ± 2.66 μM). Compounds **5c**, **5h–j** and **5n** displayed superb inhibitory activity even better than the reference inhibitor, rutin (IC_{50} values 114.51 ± 1.08 to 229.94 ± 3.40 vs. 294.5 ± 1.5 μM) (Table 2) and may thus act as convincing leads for further studies.

The limited structure-activity relationship (SAR) studies in the synthetic thiosemicarbazones **5a–o** indicated that compound **5c** possessing methyl substituent at position 3 of the phenyl ring attached to *N*⁴ of the thiosemicarbazone moiety was the most potent antiglycating agent of the series in the present assay, exhibiting antiglycation activity with IC_{50} value 114.51 ± 1.08 μM. The next most potent antiglycating derivative was **5j** bearing the fluoro substituent at position 4 of the phenyl ring. This compound displayed a bit lesser inhibition of glycation than the most potent derivative **5c** but much larger than the reference inhibitor, rutin (IC_{50} value 168.71 ± 8.58 vs. 114.51 ± 1.08 and 294.5 ± 1.5 μM, respectively). The third most active antiglycating agent was found to be compound **5i** bearing the fluoro substituent at position 3 of the phenyl ring, demonstrating a somewhat lower activity than **5j** but still considerably higher than the reference inhibitor, rutin (IC_{50} = 202.21 ± 0.55 vs. 168.71 ± 8.58 and 294.5 ± 1.5 μM, respectively). The remaining relatively more potent glyca-

tion inhibitors **5h** and **5n** possessing fluoro and chloro substituents at positions 2 and 2,4 (*ortho* and *para*) of the phenyl ring, respectively, showed inhibitory activity with IC_{50} values 229.94 ± 3.40 and 217.71 ± 1.65 μM. The other potent compounds *viz.* **5b** and **5k**, **5m** having methyl and chloro substituents at positions 2 and 2,4 of the phenyl ring, respectively, demonstrated moderate antiglycation activity with IC_{50} values 333.06 ± 9.80 and 343.75 ± 2.76, 433.88 ± 2.66 μM. The rest of the compounds *viz.* **5d–g** and **5l** displayed less than 50% inhibition of glycation and were, therefore, not assessed further for their IC_{50} values. Compound **5a** showed an anomalous behaviour, displaying enhancement in glycation.

The above results showed that the CH₃, F and Cl groups worked as active moieties in causing effective inhibition of glycation. Further, the location and/or the number of the substituents on the phenyl ring played a key role in improving the glycation inhibitory potency of the compounds.

2. 2. 3. Phytotoxicity (*in vitro*)

The synthetic thiosemicarbazones **5a–o** were also tested for their phytotoxic influences at 1000, 100, 10 or 500, 50, 5 μg/mL concentrations. Out of fifteen compounds tested, nine *viz.* **5c**, **5d**, **5h–5m** and **5o** were found to be active, demonstrating weak or non-significant (5–93%) phytotoxic activity at 1000 or 500 μg/mL concentrations in comparison to paraquat (the standard herbicide) which showed 100% plant growth inhibition at 0.015 μg/mL concentration (Table 3).

Table 2. Antiglycation activity of compounds **5a–o**

Compound	R	Concentration (μM)	Inhibition (%)	$IC_{50} \pm SEM^a$ (μM)
5a	C ₆ H ₅	250	–	
5b	2-CH ₃ C ₆ H ₄	500	57.96	333.06 ± 9.80
5c	3-CH ₃ C ₆ H ₄	250	65.32	114.51 ± 1.08
5d	4-CH ₃ C ₆ H ₄	250	37.42	
5e	2-CH ₃ OC ₆ H ₄	250	31.34	
5f	3-CH ₃ OC ₆ H ₄	1000	45.00	
5g	4-CH ₃ OC ₆ H ₄	500	21.96	
5h	2-FC ₆ H ₄	500	59.27	229.94 ± 3.40
5i	3-FC ₆ H ₄	500	68.08	202.21 ± 0.55
5j	4-FC ₆ H ₄	250	66.15	168.71 ± 8.58
5k	2-ClC ₆ H ₄	1000	70.77	343.75 ± 2.76
5l	3-ClC ₆ H ₄	1000	33.22	
5m	4-ClC ₆ H ₄	1000	54.60	433.88 ± 2.66
5n	2,4-(Cl) ₂ C ₆ H ₃	500	78.17	217.71 ± 1.65
5o	3,4-(Cl) ₂ C ₆ H ₃	62.5	19.29	
Rutin^b		1000	86.00	294.50 ± 1.50

^a Standard error of the mean; – Enhancement in glycation; ^b Standard inhibitor of glycation.

Table 3. Growth inhibition of *Lemna aequinocalis* by compounds **5a–o** at different concentrations

Compound	R	1000	100	10	500	50	5
		$\mu\text{g/mL}$ G.I. (%)	$\mu\text{g/mL}$ G.I. (%)	$\mu\text{g/mL}$ G.I. (%)	$\mu\text{g/mL}$ G.I. (%)	$\mu\text{g/mL}$ G.I. (%)	$\mu\text{g/mL}$ G.I. (%)
5a	C ₆ H ₅	–	–	–	00	00	00
5b	2-CH ₃ C ₆ H ₄	00	00	00	–	–	–
5c	3-CH ₃ C ₆ H ₄	06	00	00	–	–	–
5d	4-CH ₃ C ₆ H ₄	25	10	00	–	–	–
5e	2-CH ₃ OC ₆ H ₄	00	00	00	–	–	–
5f	3-CH ₃ OC ₆ H ₄	00	00	00	–	–	–
5g	4-CH ₃ OC ₆ H ₄	00	00	00	–	–	–
5h	2-FC ₆ H ₄	10	05	00	–	–	–
5i	3-FC ₆ H ₄	14	00	00	–	–	–
5j	4-FC ₆ H ₄	93	00	00	–	–	–
5k	2-ClC ₆ H ₄	05	05	00	–	–	–
5l	3-ClC ₆ H ₄	20	00	00	–	–	–
5m	4-ClC ₆ H ₄	25	00	00	–	–	–
5n	2,4-(Cl) ₂ C ₆ H ₃	–	–	–	00	00	00
5o	3,4-(Cl) ₂ C ₆ H ₃	–	–	–	15	00	00

2. 2. 4. Cytotoxicity (*in vitro*)

The synthesized thiosemicarbazones **5a–o** were further screened for their cytotoxicity potential by a brine shrimp (*Artemia salina*) bioassay. All the compounds gave LD₅₀ values larger than 2.43×10^{-4} M to 2.91×10^{-4} M in this assay and were thus considered to be almost inactive.

2. 2. 5. Molecular Docking Studies of Compounds **5a–o**

All the compounds showed interactions with His409, Ala436, His492, Asp494, Arg609, His519, Arg639, His593, Ala636 and His594. Figure 1 represents the putative bind-

Figure 1. The putative binding mode of **5l** (the most active inhibitor; light green colored) in the active site pocket of Jack bean urease.

ing mode of **5l**, the most potent inhibitor of the enzyme Jack bean urease. Hydrogen-bonding interactions were observed between the oxygen and hydrogen atoms of the inhibitors and the amino acid residues Ala436, Ala636, Arg639, Arg609 and His593. The 3-chlorobenzyl group was directed towards amino acids Ala436, Asp494 and His492, whereas the remaining part of the compound was directed towards Arg639 and Ala636.

All the synthetic derivatives **5a–o** were docked inside the active site of the enzyme. No specific interactions were noticed with Ni atoms by any compound; however, aromatic interactions were observed in the active pocket of the enzyme, as shown in Figure 2. This suggests that all the compounds exhibit inhibitory activity by blocking the entrance of the catalytic domain in the enzyme. The docking scores were obtained in the range of -19.57 to -16.27 and are given in Table 4.

Figure 2. Common binding modes of all the inhibitors inside the active site pocket of the urease enzyme.

2. 2. 5. 1. ADME Profile of *N*⁴-Benzyl Substituted

5-Chloroisatin-3-thiosemicarbazones 5a–o

Drug-likeness is a qualitative property of chemicals evaluated with respect to factors like bioavailability. In order to evaluate drug-likeness of the synthetic derivatives 5a–o, ADME (Absorption, Distribution, Metabolism and Excretion) properties were taken into account. The parameters selected for the purpose were octanol-water distribution coefficients (Slog *P* and log *P*), the log solubility in water (log *S*), the number of hydrogen bond acceptors (HBAH), the number of hydrogen bond donors (HBDH) and the topological polar surface area (TPSA). Along with *in silico* evaluation of ADME profile, free binding energies were also calculated for all the derivatives. The TPSA values indicated that all the compounds 5a–o can be easily penetrable in the form of drugs, as they showed polar surface area in the range of 95–107 Å². Typically, for molecules to penetrate the blood–brain barrier and, therefore, to act as receptors, an area less than 120 Å² is usually needed and molecules with a polar surface area of greater than 120–140 Å² tend to be poor in permeating cell membranes.⁵⁸ In general, compounds with molecular weight less than 500, HBAH less than 10, HBDH less than 5 and a log*P* value of less than 5 are treated as orally bioavailable with favorable ADME profile.^{59,60} The determined ADME properties of compounds 5a–o are given in Table 4. All the trial compounds exhibited favorable ADME properties and no violation of Lipinski's rule was observed. According to Lipinski's rule of 5, only two violations are allowed in the molecular docking studies.^{61–63}

3. Experimental

3. 1. General

Melting points (uncorrected) were measured on a Fisher–Johns melting point apparatus. Elemental analyses

were carried out using a Leco CHNS-9320 (USA) instrument. IR (KBr discs) spectra were recorded with a Shimadzu Prestige-21 or Shimadzu 8400 spectrophotometer. ¹H NMR spectra were obtained in CDCl₃/DMSO-*d*₆ with Bruker Avance/Spectrospin 300 and Bruker Avance 400 spectrometers, using tetramethylsilane (TMS) as the internal standard. ¹³C NMR spectra were determined in DMSO-*d*₆ with a Bruker Avance 300 spectrometer, operating at 75 MHz. EI (electron impact) mass spectra were obtained on MAT-312 and JEOL MSRoute mass spectrometers. All the chemicals and reagents used in the present study were purchased from Merck-Schuchardt, Fluka and Sigma-Aldrich.

3. 2. Synthesis of *N*-Substituted Thiosemicarbazides 3

The *N*-substituted thiosemicarbazides used in this study were synthesized according to the synthetic route reported in the literature.⁶³

3. 3. Synthesis of Isatin-thiosemicarbazones 5a–o

Appropriate thiosemicarbazides 3 (5 mmol) were treated with 5-chloroisatin 4 (5 mmol) in 50% aqueous ethanol (25 mL) under reflux for 2 h. The solid formed during heating in each case was filtered and washed thoroughly with hot aqueous ethanol, affording the required compounds 5a–o in pure form.

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

Yellow crystals; Yield: 81%; m.p. 270–272 °C (lit.²² m.p. 245 °C); IR (KBr, cm^{−1}): 3375, 3159 (NH), 1691 (C=O), 1618 (C=N), 1164 (C=S); ¹H NMR (DMSO-*d*₆, 400 MHz): δ 4.87 (d, *J* = 6.4 Hz, 2H, benzyl CH₂), 6.93 (d, *J* = 8.4 Hz, 1H, indole C₇-H), 7.24–7.28 (m, 1H, benzyl C₄-H),

Table 4. Free binding energy and ADME profile of compounds 5a–o

Compound	Free binding energy	log <i>P</i>	log <i>S</i>	Slog <i>P</i>	M. Wt.	HBAH	HBDH	TPSA
5a	−17.8283	4.987	−5.7985	2.9269	344.826	6	3	97.61
5b	−19.3512	5.283	−6.2724	3.2353	358.853	6	3	97.61
5c	−19.5795	5.322	−6.2724	3.2353	358.853	6	3	97.61
5d	−16.5393	5.285	−6.2724	3.2353	358.853	6	3	97.61
5e	−16.9345	4.941	−5.8488	2.9355	374.852	7	4	106.84
5f	−17.4872	4.980	−5.8488	2.9355	374.852	7	4	106.84
5g	−18.5219	4.943	−5.8488	2.9355	374.852	7	4	106.84
5h	−16.7386	5.138	−6.0934	3.066	362.816	6	3	97.61
5i	−17.3204	5.177	−6.0934	3.066	362.816	6	3	97.61
5j	−16.9446	5.140	−6.0934	3.066	362.816	6	3	97.61
5k	−19.2379	5.577	−6.5328	3.5803	379.271	6	3	97.61
5l	−17.1999	5.616	−6.5328	3.5803	379.271	6	3	97.61
5m	−16.3547	5.579	−6.5328	3.5803	379.271	6	3	97.61
5n	−16.2771	6.206	−7.267	4.2337	413.716	6	3	97.61
5o	−17.3941	6.206	−7.267	4.2337	413.716	6	3	97.61

7.31–7.36 (m, 4H, benzyl C₂-H, C₃-H, C₅-H, C₆-H), 7.37 (dd, $J_1 = 7.4$ Hz, $J_2 = 2.4$ Hz, 1H, indole C₆-H), 7.71 (d, $J = 7.7$ Hz, 1H, indole C₄-H), 9.90 (t, $J = 6.4$ Hz, 1H, CSNH), 11.29 (s, 1H, indole NH), 12.48 (s, 1H, NNH); ¹³C NMR (DMSO-*d*₆, 75 MHz): δ 47.18 (CH₂), 112.53 (CH), 120.46 (CH), 121.86, 126.46, 127.03 (CH), 127.26 (CH), 128.29 (CH), 130.41 (CH), 130.80, 138.22, 140.96, 162.38, 177.71; EI-MS (70 eV) *m/z* (%): 346/344 (M⁺, 13/36), 318/316 (8/20), 270/268 (9/25), 195 (19), 181 (20), 164 (13), 152 (33), 131 (17), 106 (57), 91 (100), 77 (16), 65 (35). Anal. Calcd. for C₁₆H₁₃ClN₄OS (344): C, 55.81; H, 3.78; N, 16.28. Found: C, 55.68; H, 3.75; N, 16.21.

***N*-(2-Methylbenzyl)-2-[5-chloro-2-oxo-2,3-dihydro-*H*-indol-3-ylidene]-1-hydrazinecarbothioamide (5b)**

Yellow crystals; Yield 81%; m.p. 230–232 °C; IR (KBr, cm⁻¹): 3363, 3173 (NH), 1697 (C=O), 1605 (C=N), 1159 (C=S); ¹H NMR (DMSO-*d*₆, 400 MHz): δ 2.50 (DMSO, CH₃), 3.30 (DMSO, benzyl CH₂), 6.90 (d, $J = 8.3$ Hz, 1H, indole C₇-H), 7.30 (td, $J_1 = 6.8$ Hz, $J_2 = 2.5$ Hz, 1H, benzyl C₅-H), 7.45–7.55 (m, 3H, benzyl C₃-H, C₄-H, C₆-H), 7.76 (d, $J = 8.0$ Hz, 1H, indole C₆-H), 7.94 (d, $J = 1.8$ Hz, 1H, indole C₄-H), 10.91 (t, $J = 7.5$ Hz, 1H, CSNH), 11.36 (s, 1H, indole NH), 12.62 (s, 1H, NNH); ¹³C NMR (DMSO-*d*₆, 75 MHz): δ 113.03 (CH), 114.15, 122.16, 122.35, 123.65 (CH), 128.21 (CH), 129.25 (CH), 130.68 (CH), 131.12, 132.71 (CH), 133.40 (CH), 137.45, 141.57, 162.23, 177.55; EI-MS (70 eV) *m/z* (%): 360/358 (M⁺, 2/7), 330 (2), 282 (5), 209 (3), 195 (3), 181 (42), 178 (35), 152 (31), 145 (18), 120 (62), 105 (100), 91 (32), 77 (61), 65 (18), 51 (28). Anal. Calcd. for C₁₇H₁₅ClN₄OS (358): C, 56.98; H, 4.19; N, 15.64. Found: C, 56.75; H, 4.15; N, 15.59.

***N*-(3-Methylbenzyl)-2-[5-chloro-2-oxo-2,3-dihydro-*1H*-indol-3-ylidene]-1-hydrazinecarbothioamide (5c)**

Yellow fluffy crystals; Yield 80%; m.p. 202–204 °C; IR (KBr, cm⁻¹): 3361, 3192 (NH), 1697 (C=O), 1600 (C=N), 1153 (C=S); ¹H NMR (DMSO-*d*₆, 300 MHz): δ 2.30 (s, 3H, CH₃), 4.85 (d, $J = 8.4$ Hz, benzyl CH₂), 6.94 (d, $J = 8.4$ Hz, 1H, indole C₇-H), 7.07–7.26 (m, 4H, benzyl C₂-H, C₄-H, C₅-H, C₆-H), 7.38 (dd, $J_1 = 8.4$ Hz, $J_2 = 2.1$ Hz, 1H, indole C₆-H), 7.73 (d, $J = 2.1$ Hz, 1H, indole C₄-H), 9.90 (t, $J = 6.0$ Hz, 1H, CSNH), 11.31 (s, 1H, indole NH), 12.49 (s, 1H, NNH); ¹³C NMR (DMSO-*d*₆, 75 MHz): δ 111.86 (CH), 112.17 (CH), 112.65 (CH), 115.79, 120.81 (CH), 121.69, 126.53, 129.76 (CH), 130.83 (CH), 132.08, 141.41, 156.89, 160.14, 162.33, 178.62; EI-MS (70 eV) *m/z* (%): 360/358 (M⁺, 2/5), 330 (3), 282 (6), 209 (5), 181 (58), 152 (37), 138 (16), 120 (81), 105 (100), 91 (34), 77 (59), 65 (21), 51 (22). Anal. Calcd. for C₁₇H₁₅ClN₄OS (358): C, 56.98; H, 4.19; N, 15.64. Found: C, 56.88; H, 4.14; N, 15.58.

***N*-(4-Methylbenzyl)-2-[5-chloro-2-oxo-2,3-dihydro-*1H*-indol-3-ylidene]-1-hydrazinecarbothioamide (5d)**

Yellow crystals; Yield 87%; m.p. 210–212 °C; IR (KBr, cm⁻¹): 3300, 3194 (NH), 1684 (C=O), 1599 (C=N), 1157

(C=S); ¹H NMR (DMSO-*d*₆, 400 MHz): δ 2.29 (s, 3H, CH₃), 4.65 (d, $J = 5.0$ Hz, 2H, benzyl CH₂), 6.94 (d, $J = 7.5$ Hz, 1H, indole C₇-H), 7.07–7.33 (m, 4H, benzyl C₂-H, C₃-H, C₅-H, C₆-H), 7.38 (dd, $J_1 = 7.5$ Hz, $J_2 = 2.0$ Hz, 1H, indole C₆-H), 7.73 (d, $J = 2.0$ Hz, 1H, indole C₄-H), 9.89 (t, $J = 7.5$ Hz, 1H, CSNH), 11.31 (s, 1H, indole NH), 12.48 (s, 1H, NNH); ¹³C NMR (DMSO-*d*₆, 75 MHz): δ 20.67 (CH₃), 46.95 (CH₂), 112.52 (CH), 113.69 (CH), 120.46 (CH), 121.85, 126.46, 126.95 (CH), 127.28 (CH), 128.82, 135.16, 136.14, 140.93, 162.38, 177.68; EI-MS (70 eV) *m/z* (%): 360/358 (M⁺, 2/7), 331 (2), 281 (5), 205 (3), 191 (3), 181/179 (13/19), 151 (29), 137 (40), 125 (55), 111 (81), 97 (96), 83 (77), 71 (86), 57 (100). Anal. Calcd. for C₁₇H₁₅ClN₄OS (358): C, 56.98; H, 4.19; N, 15.64. Found: C, 56.74; H, 4.13; N, 15.56.

***N*-(2-Methoxybenzyl)-2-[5-chloro-2-oxo-2,3-dihydro-*1H*-indol-3-ylidene]-1-hydrazinecarbothioamide (5e)**

Yellow crystals; Yield 86%; m.p. 275–277 °C; IR (KBr, cm⁻¹): 3356, 3093 (NH), 1737 (C=O), 1606 (C=N), 1182 (C=S); ¹H NMR (DMSO-*d*₆, 400 MHz): δ 3.85 (s, 3H, CH₃), 4.40 (d, $J = 5.0$ Hz, 2H, benzyl CH₂), 6.88–7.03 (m, 3H, benzyl C₃-H, C₆-H, indole C₇-H), 7.23–7.30 (m, 2H, benzyl C₄-H, C₅-H), 7.39 (dd, $J_1 = 7.5$ Hz, $J_2 = 2.0$ Hz, 1H, indole C₆-H), 7.61 (s, 1H, indole C₄-H), 8.26 (s, 1H, CSNH), 10.60 (s, 1H, indole NH), 10.84 (s, 1H, NNH); ¹³C NMR (DMSO-*d*₆, 75 MHz): δ 55.82 (CH₃), 111.04 (CH), 112.01 (CH), 117.25 (CH), 120.66, 125.24 (CH), 126.15 (CH), 127.22 (CH), 128.29 (CH), 128.76, 131.39, 132.45, 142.06, 155.17, 157.20, 165.03; EI-MS (70 eV) *m/z* (%): 358 (3), 221 (3), 206 (2), 195 (100), 179 (69), 163 (45), 136 (42), 121 (79), 104 (10), 91 (73), 77 (12), 65 (10), 51 (6). Anal. Calcd. for C₁₇H₁₅ClN₄O₂S (374): C, 54.55; H, 4.01; N, 14.97. Found: C, 54.42; H, 3.95; N, 14.89.

***N*-(3-Methoxybenzyl)-2-[5-chloro-2-oxo-2,3-dihydro-*1H*-indol-3-ylidene]-1-hydrazinecarbothioamide (5f)**

Yellow crystals; Yield 85%; m.p. 212–214 °C; IR (KBr, cm⁻¹): 3371, 3180 (NH), 1699 (C=O), 1605 (C=N), 1157 (C=S); ¹H NMR (DMSO-*d*₆, 300 MHz): δ 3.74 (s, 3H, CH₃), 4.87 (d, $J = 6.3$ Hz, 2H, benzyl CH₂), 6.84 (dd, $J_1 = 8.1$ Hz, $J_2 = 2.4$ Hz, 1H, benzyl C₄-H), 6.92–6.95 (m, 3H, indole C₇-H, benzyl C₂-H, C₆-H), 7.27 (t, $J = 8.1$ Hz, 1H, benzyl C₅-H), 7.38 (dd, $J_1 = 9.0$ Hz, $J_2 = 2.4$ Hz, 1H, indole C₆-H), 7.73 (d, $J = 2.1$ Hz, 1H, indole C₄-H), 9.90 (t, $J = 6.3$ Hz, 1H, CSNH), 11.32 (s, 1H, indole NH), 12.49 (s, 1H, NNH); ¹³C NMR (DMSO-*d*₆, 75 MHz): δ 113.04 (CH), 114.14, 115.88 (CH), 122.17, 123.63 (CH), 124.43 (CH), 126.31, 126.52, 129.30 (CH), 130.86 (CH), 131.30, 133.44 (CH), 141.61, 162.21, 177.97; EI-MS (70 eV) *m/z* (%): 376/374 (M⁺, 3/9), 298 (3), 209 (17), 194 (42), 181 (69), 178 (11), 152 (64), 138 (40), 136 (98), 121 (100), 109 (44), 106 (22), 91 (87), 77 (77), 65 (64), 51 (43). Anal. Calcd. for C₁₇H₁₅ClN₄O₂S (374): C, 54.55; H, 4.01; N, 14.97. Found: C, 54.45; H, 3.96; N, 14.92.

***N*-(4-Methoxybenzyl)-2-[5-chloro-2-oxo-2,3-dihydro-1*H*-indol-3-ylidene]-1-hydrazinecarbothioamide (5g)**

Yellow fluffy crystals; Yield 89%; m.p. 190–192 °C; IR (KBr, cm^{-1}): 3369, 3267 (NH), 1687 (C=O), 1620 (C=N), 1164 (C=S); ^1H NMR (DMSO- d_6 , 400 MHz): δ 2.50 (DMSO, CH_3 , benzyl CH_2), 3.45 (DMSO, benzyl CH_2), 6.91 (d, $J = 8.3$ Hz, 1H, indole C_7 -H), 7.14 (td, $J_1 = 7.6$ Hz, $J_2 = 1.8$ Hz, 1H, benzyl C_5 -H), 7.42–7.55 (m, 3H, benzyl C_2 -H, C_3 -H, C_6 -H), 7.95–7.99 (m, 2H, indole C_4 -H, C_6 -H), 10.91 (s, 1H, CSNH), 11.36 (s, 1H, indole NH), 12.60 (s, 1H, NNH); ^{13}C NMR (DMSO- d_6 , 75 MHz): δ 20.66 (CH_3), 40.34 (CH_2), 100.00, 113.02 (CH), 114.16, 122.20, 123.66 (CH), 128.88 (CH), 129.23 (CH), 129.86 (CH), 130.97, 133.36 (CH), 138.83 (CH), 140.76, 141.54, 162.23, 177.33; EI-MS (70 eV) m/z (%): 374 (M^+ , 3), 298 (2), 209 (4), 194 (55), 181 (33), 178 (10), 161 (59), 152 (30), 136 (64), 134 (38), 121 (100), 109 (26), 106 (22), 91 (38), 77 (77), 65 (28), 51 (44). Anal. Calcd. for $\text{C}_{17}\text{H}_{15}\text{ClN}_4\text{O}_2\text{S}$ (374): C, 54.55; H, 4.01; N, 14.97. Found: C 54.42; H, 3.96; N, 14.90.

***N*-(2-Fluorobenzyl)-2-[5-chloro-2-oxo-2,3-dihydro-1*H*-indol-3-ylidene]-1-hydrazinecarbothioamide (5h)**

Orange fluffy crystals; Yield 87%; m.p. 260–262 °C; IR (KBr, cm^{-1}): 3375, 3116 (NH), 1697 (C=O), 1605 (C=N), 1161 (C=S); ^1H NMR (DMSO- d_6 , 400 MHz): δ 3.79 (s, 2H, benzyl CH_2), 6.85–6.92 (m, 2H, benzyl C_6 -H, indole C_7 -H), 7.20–7.37 (m, 3H, benzyl C_3 -H, C_4 -H, C_5 -H), 7.51 (d, $J = 7.8$ Hz, 1H, indole C_6 -H), 8.02 (s, 1H, indole C_4 -H), 10.85 (s, 1H, CSNH), 11.36 (s, 1H, indole NH), 12.62 (s, 1H, NNH); ^{13}C NMR (DMSO- d_6 , 75 MHz): δ 55.23 (CH_2), 111.16 (CH), 111.68 (CH), 112.98 (CH), 114.15, 117.61 (CH), 122.15, 123.77 (CH), 129.13 (CH), 130.87, 133.36 (CH), 139.32, 141.47, 159.17, 162.28, 175.99; EI-MS (70 eV) m/z (%): 364/362 (M^+ , 2/4), 336/334 (2/4), 288/286 (1/3), 195 (4), 181 (25), 167 (20), 152 (27), 138 (16), 124 (73), 109 (100), 83 (52), 75 (25), 61 (27), 57 (20). Anal. Calcd. for $\text{C}_{16}\text{H}_{12}\text{FClN}_4\text{OS}$ (362): C, 53.04; H, 3.31; N, 15.47. Found: C, 52.91; H, 3.28; N, 15.39.

***N*-(3-Fluorobenzyl)-2-[5-chloro-2-oxo-2,3-dihydro-1*H*-indol-3-ylidene]-1-hydrazinecarbothioamide (5i)**

Yellow crystals; Yield 77%; m.p. 242–244 °C; IR (KBr, cm^{-1}): 3242, 3205 (NH), 1692 (C=O), 1605 (C=N), 1157 (C=S); ^1H NMR (DMSO- d_6 , 400 MHz): δ 4.90 (d, $J = 7.5$ Hz, 2H, benzyl CH_2), 6.95 (d, $J = 8.4$ Hz, 1H, indole C_7 -H), 7.07–7.23 (m, 3H, benzyl C_2 -H, C_5 -H, C_6 -H), 7.37–7.46 (m, 2H, benzyl C_4 -H, indole C_6 -H), 7.72 (d, $J = 2.5$ Hz, 1H, indole C_4 -H), 9.94 (t, $J = 7.5$ Hz, 1H, CSNH), 11.32 (s, 1H, indole NH), 12.52 (s, 1H, NNH); ^{13}C NMR (DMSO- d_6 , 75 MHz): δ 46.64 (CH_2), 112.56 (CH), 113.63 (CH), 113.74 (CH), 113.96 (CH), 114.08 (CH), 119.34 (CH), 120.45, 123.23 (CH), 126.47, 130.22 (CH), 130.35 (CH), 130.47, 141.06, 141.15, 141.26, 162.38, 164.07, 177.88; EI-MS (70 eV) m/z (%): 364/362 (M^+ , 19/49), 336/334 (12/33), 288/286 (7/23), 197/195 (5/17), 183/181 (52/81), 154/152 (13/37), 140/138 (5/12), 124 (76), 109 (100), 83 (12), 75

(7), 61 (7), 57 (3). Anal. Calcd. for $\text{C}_{16}\text{H}_{12}\text{FClN}_4\text{OS}$ (362): C, 53.04; H, 3.31; N, 15.47. Found: C, 52.90; H, 3.27; N, 15.39.

***N*-(4-Fluorobenzyl)-2-[5-chloro-2-oxo-2,3-dihydro-1*H*-indol-3-ylidene]-1-hydrazinecarbothioamide (5j)**

Orange crystals; Yield 86%; m.p. 252–254 °C; IR (KBr, cm^{-1}): 3350, 3059 (NH), 1703 (C=O), 1600 (C=N), 1149 (C=S); ^1H NMR (DMSO- d_6 , 300 MHz): δ 4.86 (d, $J = 7.5$ Hz, 2H, benzyl CH_2), 6.93 (d, $J = 8.4$ Hz, 1H, indole C_7 -H), 7.15–7.22 (m, 2H, benzyl C_2 -H, C_6 -H), 7.36–7.44 (m, 3H, benzyl C_3 -H, C_5 -H, indole C_6 -H), 7.71 (d, $J = 2.4$ Hz, 1H, indole C_4 -H), 9.92 (t, $J = 6.0$ Hz, 1H, CSNH), 11.31 (s, 1H, indole NH), 12.49 (s, 1H, NNH); ^{13}C NMR (DMSO- d_6 , 75 MHz): δ 113.05 (CH), 114.16, 122.06, 123.76 (CH), 124.06 (CH), 125.08 (CH), 125.94 (CH), 129.98 (CH), 132.46, 133.52 (CH), 139.70, 141.59, 162.29, 164.87, 176.23; EI-MS (70 eV) m/z (%): 364/362 (M^+ , 1/3), 288/286 (1/3), 197/195 (1/3), 183/181 (20/135), 154/152 (12/36), 124/122 (69/19), 109 (100), 97 (21), 83 (32), 75 (29), 61 (39), 57 (25). Anal. Calcd. for $\text{C}_{16}\text{H}_{12}\text{FClN}_4\text{OS}$ (362): C, 53.04; H, 3.31; N, 15.47. Found: C, 52.88; H, 3.28; N, 15.42.

***N*-(2-Chlorobenzyl)-2-[5-chloro-2-oxo-2,3-dihydro-1*H*-indol-3-ylidene]-1-hydrazinecarbothioamide (5k)**

Yellow fluffy crystals; Yield 89%; m.p. 230–232 °C; IR (KBr, cm^{-1}): 3358, 3174 (NH), 1693 (C=O), 1610 (C=N), 1193 (C=S); ^1H NMR (DMSO- d_6 , 400 MHz): δ 4.94 (d, $J = 9.0$ Hz, 2H, benzyl CH_2), 6.96 (d, $J = 8.5$ Hz, 1H, indole C_7 -H), 7.29–7.41 (m, 4H, benzyl C_3 -H, C_4 -H, C_5 -H, C_6 -H), 7.41 (d, $J = 8.5$ Hz, 1H, indole C_6 -H), 7.73 (s, 1H, indole C_4 -H), 9.92 (t, $J = 7.5$ Hz, 1H, CSNH), 11.34 (s, 1H, indole NH), 12.57 (s, 1H, NNH); ^{13}C NMR (DMSO- d_6 , 75 MHz): δ 44.97 (CH_2), 112.58 (CH), 120.50 (CH), 126.49, 126.91 (CH), 127.12 (CH), 127.64 (CH), 128.13 (CH), 129.16 (CH), 130.26, 130.75, 135.08, 141.05, 162.40, 178.20; EI-MS (70 eV) m/z (%): 352/350 (10/10), 345/343 (36/89), 199/197 (10/20), 183/181 (19/33), 167/165 (14/16), 154/152 (11/28), 140/138 (41/22), 125 (100), 106 (9), 89 (13), 75 (10), 63 (8), 51 (5). Anal. Calcd. for $\text{C}_{16}\text{H}_{12}\text{Cl}_2\text{N}_4\text{OS}$ (378): C, 50.79; H, 3.17; N, 14.81. Found: C, 50.65; H, 3.13; N, 14.72.

***N*-(3-Chlorobenzyl)-2-[5-chloro-2-oxo-2,3-dihydro-1*H*-indol-3-ylidene]-1-hydrazinecarbothioamide (5l)**

Yellow crystals; Yield 80%; m.p. 228–230 °C; IR (KBr, cm^{-1}): 3333, 3065 (NH), 1705 (C=O), 1605 (C=N), 1174 (C=S); ^1H NMR (DMSO- d_6 , 300 MHz): δ 4.88 (d, $J = 6.3$ Hz, 2H, benzyl CH_2), 6.95 (d, $J = 8.4$ Hz, 1H, indole C_7 -H), 7.32–7.43 (m, 5H, benzyl C_2 -H, C_4 -H, C_5 -H, C_6 -H, indole C_6 -H), 7.72 (d, $J = 8.5$ Hz, 1H, indole C_4 -H), 9.95 (t, $J = 6.3$ Hz, 1H, CSNH), 11.33 (s, 1H, indole NH), 12.51 (s, 1H, NNH); ^{13}C NMR (DMSO- d_6 , 75 MHz): δ 47.07 (CH_2), 113.08 (CH), 120.95 (CH), 122.33, 126.49 (CH), 126.94 (CH), 127.50 (CH), 130.74 (CH), 131.00, 131.58, 133.41, 141.33, 141.55, 162.90, 178.77; EI-MS (70 eV) m/z (%):

380/378 (M^+ , 2/3), 304/302 (2/3) 198 (16), 181 (52), 152 (50), 140 (76), 138 (40), 125 (100), 113 (17), 106 (13), 89 (80), 75 (58), 63 (58), 51 (43). Anal. Calcd. for $C_{16}H_{12}Cl_2N_4OS$ (378): C, 50.79; H, 3.17; N, 14.81. Found: C, 50.68; H, 3.12; N, 14.72.

***N*-(4-Chlorobenzyl)-2-[5-chloro-2-oxo-2,3-dihydro-1*H*-indol-3-ylidene]-1-hydrazinecarbothioamide (5m)**

Orange crystals; Yield 84%; m.p. 280–282 °C; IR (KBr, cm^{-1}): 3290, 3053 (NH), 1688 (C=O), 1611 (C=N), 1180 (C=S); 1H NMR (DMSO- d_6 , 400 MHz): δ 4.87 (d, J = 7.5 Hz, 2H, benzyl CH_2), 6.95 (d, J = 7.5 Hz, 1H, indole C_7 -H), 7.37–7.41 (m, 5H, benzyl C_2 -H, C_3 -H, C_5 -H, C_6 -H, indole C_6 -H), 7.72 (s, 1H, indole C_4 -H), 9.95 (t, J = 7.5 Hz, 1H, CSNH), 11.32 (s, 1H, indole NH), 12.50 (s, 1H, NNH); ^{13}C NMR (DMSO- d_6 , 75 MHz): δ 46.49 (CH_2), 112.56 (CH), 120.44 (CH), 121.82, 126.46, 128.25 (CH), 129.15 (CH), 130.46 (CH), 130.94, 131.58, 137.26, 141.00, 162.38, 177.77; EI-MS (70 eV) m/z (%): 380/378 (M^+ , 22/32), 352/350 (13/20), 211/209 (5/13), 200/198 (29/58), 195 (25), 181 (92), 165 (19), 152 (46), 140 (46), 125 (100), 106 (9), 89 (15), 77 (10), 61 (12). Anal. Calcd. for $C_{16}H_{12}Cl_2N_4OS$ (378): C, 50.79; H, 3.17; N, 14.81. Found: C, 50.64; H, 3.13; N, 14.75.

***N*-(2,4-Dichlorobenzyl)-2-[5-chloro-2-oxo-2,3-dihydro-1*H*-indol-3-ylidene]-1-hydrazinecarbothioamide (5n)**

Yellow crystals; Yield 75%; m.p. 248–250 °C; IR (KBr, cm^{-1}): 3300, 3051 (NH), 1687 (C=O), 1606 (C=N), 1159 (C=S); 1H NMR ($CDCl_3$, 400 MHz): δ 5.02 (d, J = 6.0 Hz, 2H, benzyl CH_2), 6.83 (d, J = 8.4 Hz, 1H, indole C_7 -H), 7.24–7.26 (m, 1H, benzyl C_5 -H), 7.29 (dd, J_1 = 8.4 Hz, J_2 = 2.0 Hz, 1H, indole C_6 -H), 7.43 (d, J = 2.0 Hz, 1H, benzyl C_3 -H), 7.45 (d, J = 8.4 Hz, 1H, benzyl C_6 -H), 7.53 (d, J = 2.0 Hz, 2H, indole C_4 -H, CSNH), 8.03 (brs, 1H, indole NH), 12.69 (s, 1H, NNH); ^{13}C NMR (DMSO- d_6 , 75 MHz): δ 45.11 (CH_2), 113.05, 120.98 (CH), 122.23 (CH), 127.00 (CH), 127.79 (CH), 129.09, 129.90 (CH), 131.01 (CH), 131.62, 132.76, 132.98, 134.86, 141.54, 162.86, 178.70; EI-MS (70 eV) m/z (%): 379/377 (17/22), 199/197 (12/18), 181 (52), 176/174 (30/52), 166 (11), 159 (100), 124 (34), 111 (29), 102 (26), 89 (42), 75 (54), 63 (42), 51 (28). Anal. Calcd. for $C_{16}H_{11}Cl_3N_4OS$ (412): C, 46.60; H, 2.67; N, 13.59. Found: C, 46.44; H, 2.63; N, 13.54.

***N*-(3,4-Dichlorobenzyl)-2-[5-chloro-2-oxo-2,3-dihydro-1*H*-indol-3-ylidene]-1-hydrazinecarbothioamide (5o)**

Orange fluffy crystals; Yield 74%; m.p. 272–274 °C; IR (KBr, cm^{-1}): 3300, 3192 (NH), 1689 (C=O), 1608 (C=N), 1157 (C=S); 1H NMR ($CDCl_3$, 400 MHz): δ 4.94 (d, J = 6.0 Hz, 2H, benzyl CH_2), 6.82 (d, J = 8.4 Hz, 1H, indole C_7 -H), 7.24 (m, $CDCl_3$, benzyl C_6 -H), 7.29 (dd, J_1 = 8.4 Hz, J_2 = 2.0 Hz, 1H, indole C_6 -H), 7.43 (d, J = 8.0 Hz, 1H, benzyl C_5 -H), 7.47 (d, J = 1.6 Hz, 1H, benzyl C_2 -H), 7.50 (brs, 1H, CSNH), 7.54 (d, J = 2.0 Hz, indole C_4 -H), 8.03 (brs, 1H, indole NH), 12.75 (s, 1H, NNH); ^{13}C NMR

(DMSO- d_6 , 75 MHz): δ 46.56 (CH_2), 113.06, 120.93 (CH), 122.25 (CH), 126.97 (CH), 128.18 (CH), 129.71 (CH), 130.05 (CH), 131.00, 131.34, 131.63, 139.98, 141.53, 162.87, 178.37; EI-MS (70 eV) m/z (%): 414/412 (M^+ , 2/2), 384/382 (2/2), 234/232 (9/8), 195 (9), 161 (58), 159 (100), 138(31), 111 (26), 102 (22), 89 (43), 75 (48), 63 (47), 51(27). Anal. Calcd. for $C_{16}H_{11}Cl_3N_4OS$ (412): C, 46.60; H, 2.67; N, 13.59. Found: C, 46.50; H, 2.63; N, 13.52.

3. 4. Biological Assays

In vitro antiurease, antiglycation, phytotoxicity and cytotoxicity potential of the synthesized thiosemicarbazones was determined according to the literature protocols.^{64,55,43,42}

3. 5. Molecular Docking Studies

Molecular docking studies were performed in accordance with the protocol used in our recently published article.⁶⁵

4. Conclusions

In this study, a series of fifteen N^4 -benzyl substituted 5-chloroisatin-3-thiosemicarbazones **5a–o** were synthesized and screened primarily for urease and glycation inhibitory potential. All the synthetic thiosemicarbazones emerged as highly effective urease inhibitors, demonstrating enzymatic inhibition even better than the reference inhibitor, thiourea, used in the assay. These compounds, by displaying non-significant phytotoxic influences, draw a great deal of consideration towards their effectiveness as important inhibitors of soil ureases because they might be combined with fertilizers in minute amounts to greatly improve the usefulness of nitrogen consumption. Also, being non-toxic, they could prove to be promising candidates for orally effective remedial agents used for the treatment of certain clinical conditions induced by microbial ureases. In antiglycation assay, five out of fifteen compounds tested, *i.e.* **5c**, **5h–j** and **5n** were recognized as extremely effective inhibitors of glycation, showing inhibitory activity much larger than the reference inhibitor, rutin, and may thus act as credible leads for further studies.

This study presents the first example of exhibiting glycation inhibition by N^4 -benzyl substituted isatin-thiosemicarbazones and offers a firm base for additional studies on such compounds to build up more persuasive and safer glycation inhibitors of pharmaceutical interest.

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Povzetek

Sintetizirali smo serijo petnajstih N^4 -benzil substituiranih 5-kloroizatin 3-tiosemikarbazonov **5a–o** in preverili njihove antiureazne in antiglikacijske učinke. Da bi ugotovili fitotoksični in citotoksični potencial pripravljenih spojin, smo izvedli teste rasti organizmov *Lemna aequinocitalis* in *Artemia salina*. Za vse spojine smo ugotovili, da so izjemno učinkoviti inhibitorji ureaz, saj so testirani encim inhibirale mnogo bolje kot referenčni inhibitor tiosečnina (IC_{50} vrednosti 1.31 ± 0.06 do 3.24 ± 0.15 proti $22.3 \pm 1.12 \mu\text{M}$ za tiosečnino). Po drugi strani pa smo za osem testiranih spojin (t.j. **5b**, **5c**, **5h–k**, **5m** in **5n**) izmed petnajstih ugotovili, da so zelo močni inhibitorji glikacije. Od teh se jih je pet (t.j. **5c**, **5h–j** in **5n**) izkazalo za izjemno učinkovite, saj so glikacijo inhibirali celo bolj kot referenčni inhibitor rutin (IC_{50} vrednosti 114.51 ± 1.08 do 229.94 ± 3.40 proti $294.5 \pm 1.5 \mu\text{M}$ za rutin).