

Synthesis of novel Triazinoindole-Based-Thiourea Hybrid: α -Glucosidase Inhibitors and Their Molecular Docking Study

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Abstract

New class of triazinoindole bearing thiosemicarbazide (**1-25**) was synthesized and evaluated for α -glucosidase inhibitory potential. All synthesized analogues exhibited excellent inhibitory potential having IC₅₀ values ranging from 1.30 ± 0.01 to 35.80 ± 0.80 μ M when compared with the standard acarbose having IC₅₀ value 38.60 ± 0.20 μ M. Among series the analogues **1** and **23** was found the most potent having IC₅₀ values 1.30 ± 0.05 and 1.30 ± 0.01 μ M respectively. Structure activity relationship (SAR) was mainly based upon by bring about difference of substituents on phenyl rings. To confirm the binding interactions, molecular docking study was performed. Synthesized analogues were characterized through HREI-MS, ¹H and ¹³C-NMR analysis.

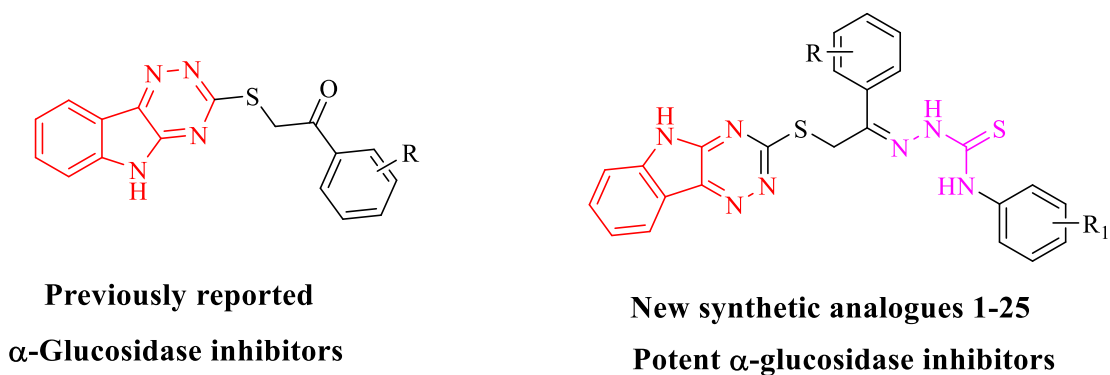
Keyword: Synthesis, triazinoindole, thiosemicarbazide, alpha-glucosidase, molecular docking study, SAR.

1.0. Introduction

Diabetes mellitus is chronic health threatening metabolic disease that is caused due to insufficient insulin secretion and are categorized as hypoglycemia/hyperglycemia [1]. In type-II diabetes mellitus, due to enhanced postprandial glucose level can increase the risk of developing stroke, atherosclerosis and other coronary diseases [2]. In order to treat type-II diabetes and its complications, inhibition of digestive enzyme like α -glucosidase is an effective approach that can reduce the postprandial glucose to reduce the risk factors [3]. In small intestine epithelium cell lining, the α -glucosidase is located which are responsible for conversion of polysaccharides and disaccharides into glucose. α -Glucosidase inhibition is directly associated with blood glucose level and its inhibition is vital due to potential effect of decrease postprandial blood glucose levels [4]. In order to delay the rapid blood glucose production, certain α -glucosidase inhibitors like acarbose and voglibose are clinically used. Though, there are certain side effect that includes abdominal pain, diarrhea and other gastrointestinal disorders in chronic therapy [5]. Therefore, in order to treat postprandial hyperglycemia, the search for efficient and safe α -glucosidase inhibitors is needed.

Triazinoindole scaffolds possess excellent biological potential against malarial and viral diseases. Substituted triazinoindole scaffolds are of considerable interest due to an excellent antihypertensive [7], antidepressant [6], anti-inflammatory [9], anti-hypoxic [10], antifungal and antibacterial activities [8]. In order to treat common cold, selected triazinoindole compounds act as potential drugs [11-14].

Our research group has been working on design and synthesis of heterocyclic compounds in search of potential lead compounds since many years and had found promising results [15-22]. We have already reported triazinoindole analogues as potent α -glucosidase inhibitors [23]. Thus, we decided to screen a library of triazinoindole bearing thiosemicarbazide analogues for α -glucosidase activity.

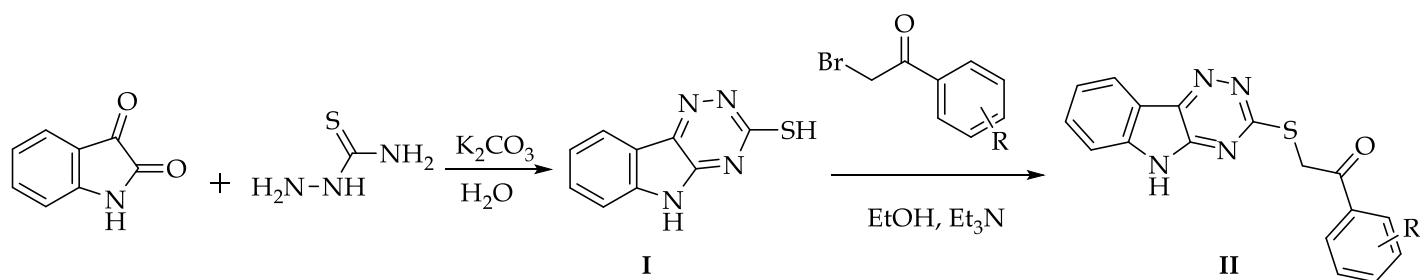
**Figure-1:** Rational of the current work

2.0. Results and discussion

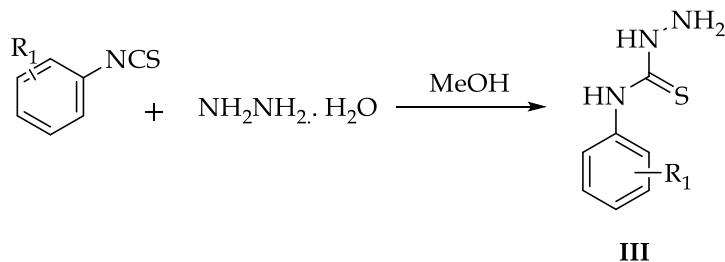
2.1. Chemistry

New class of triazinoindole based thiosemicarbazide analogues (**1-25**) were carried out in three steps.

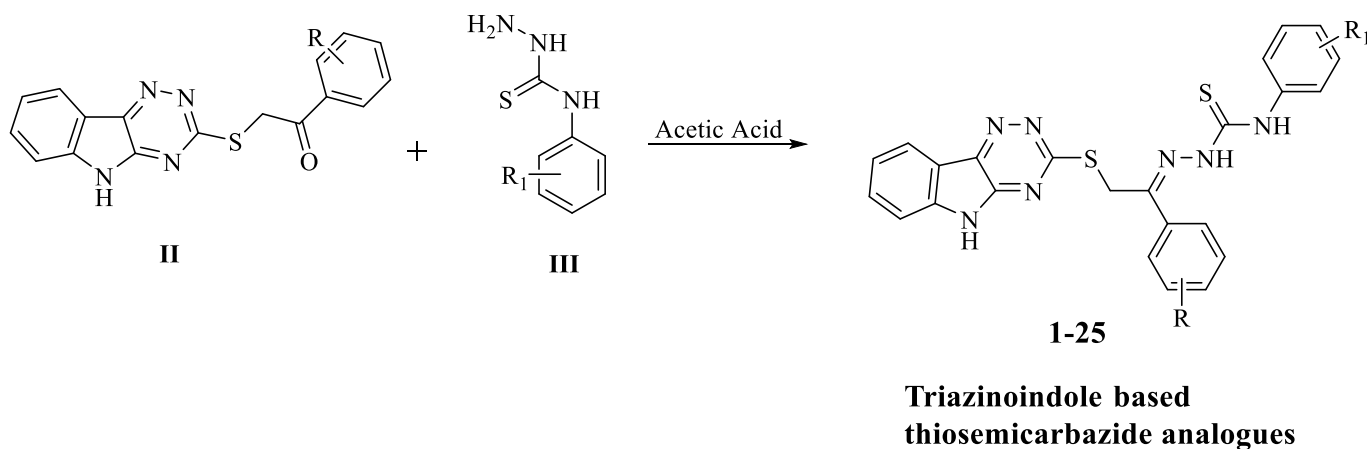
In 1st step, thiosemicarbazide was reacted and refluxed with isatin in H₂O in the presence of potassium carbonate to yield 5*H*-triazinoindole-3-thiol as intermediate product (**I**). The intermediate (**I**) was then mixed and refluxed with different substituted phenacyl bromide in EtOH in the presence of Et₃N to give triazinoindole derivatives as second intermediate product (**II**).

**Scheme-1:** Synthesis of triazinoindole derivatives

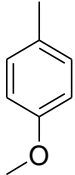
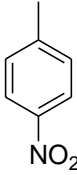
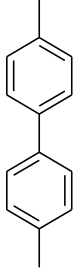
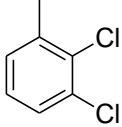
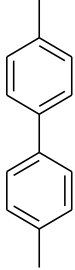
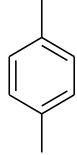
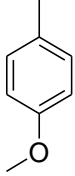
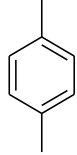
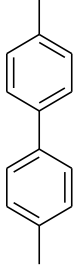
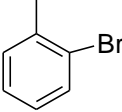
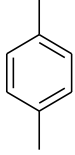
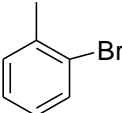
In 2nd step, hydrazine hydrate was reacted and refluxed with different isothiocyanates in methanol to yield thiosemicarbazide derivative as intermediate (**III**).

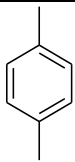
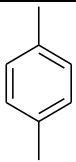
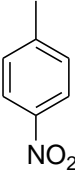
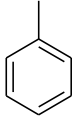
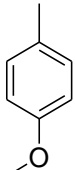
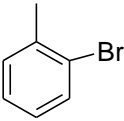
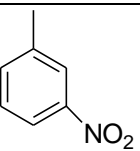
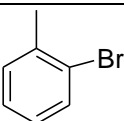
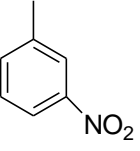
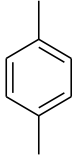
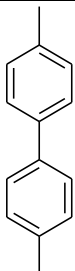
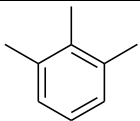
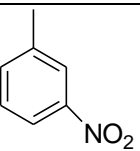
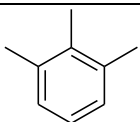
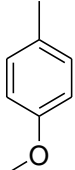
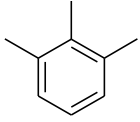
**Scheme-2:** Synthesis of thiosemicarbazide derivatives

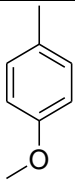
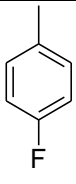
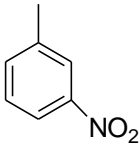
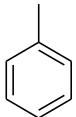
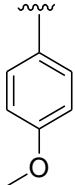
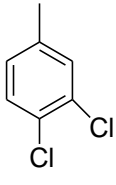
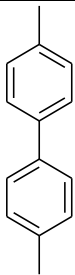
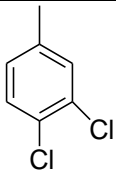
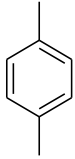
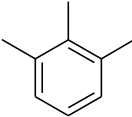
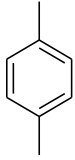
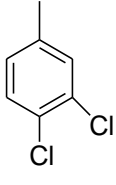
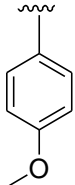
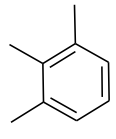
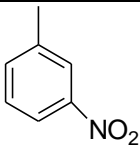
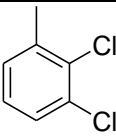
In 3rd step, the intermediate product (**II**) was reacted and refluxed with intermediate product (**III**) in glacial acetic acid to give the final product triazinoindole bearing thiosemicarbazide (**1-25**).

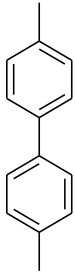
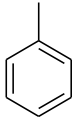
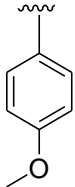
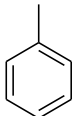
**Scheme-3:** Synthesis of triazinoindole based thiosemicarbazide analogues (**1-25**)**Table-1:** Structure of triazinoindole based thiosemicarbazide analogues and their α -glucosidase activity

S. No	R	R1	IC ₅₀ (μ M)
1			1.30 \pm 0.05

2			2.60 ± 0.05
3			7.5 ± 0.20
4			12.80 ± 0.30
5			5.20 ± 0.30
6			25.3 ± 0.50
7			33.30 ± 0.60

8			17.5 ± 0.30
9			6.80 ± 0.10
10			35.80 ± 0.80
11			26.80 ± 0.70
12			8.30 ± 0.20
13			23.30 ± 0.50
14			7.5 ± 0.20
15			5.80 ± 0.20

16			1.8 ± 0.20
17			8.80 ± 0.20
18			2.30 ± 0.05
19			2.30 ± 0.05
20			7.50 ± 0.20
21			4.80 ± 0.20
22			5.90 ± 0.10
23			1.30 ± 0.01

24			15.3 ± 0.30
25			9.30 ± 0.30
Acarbose		38.60 ± 0.20	

2.2. Biological activity

New class of triazinoindole bearing thiosemicarbazide analogues (**1-25**) were synthesized and was evaluated for α -glucosidase inhibitory potential. All the synthesized scaffolds exhibited outstanding inhibitory potential having IC_{50} values ranging from 1.30 ± 0.01 to $35.80 \pm 0.80 \mu\text{M}$ when compared with standard acarbose having IC_{50} value $38.60 \pm 0.20 \mu\text{M}$. Structure activity relationship mainly based upon by bringing the difference of substituent on phenyl rings.

By comparing compound **1** ($IC_{50} = 1.30 \pm 0.05 \mu\text{M}$) having methoxy moiety at position-4 on one phenyl ring and the two chloro groups at 2,3-position on second phenyl ring with scaffold **18** ($IC_{50} = 2.30 \pm 0.05 \mu\text{M}$) also having methoxy moiety at position-4 on one phenyl ring and the two chloro groups at 3,4-position on second phenyl ring. Inhibition difference in these two scaffolds may be due to two chloro group's which are present at different positions on second phenyl ring. If we compare scaffold **9** (IC_{50} value $6.80 \pm 0.10 \mu\text{M}$) with scaffold **17** (IC_{50} value $8.80 \pm 0.20 \mu\text{M}$), both scaffolds have a nitro moiety on one phenyl ring while in both cases the other phenyl ring is un-substituted. In scaffold **9**, the nitro moiety is present at position-4 while in scaffold **17**; the nitro moiety is present at position-3 on phenyl ring. The potential difference in these two scaffolds may be due to nitro moiety different position on one phenyl ring.

Similarly, by comparing compound **15** ($IC_{50} = 5.80 \pm 0.20 \mu\text{M}$) having methoxy moiety at position-4 on one phenyl ring and the two methyl groups at 2,6-position on second phenyl ring

with scaffold **22** ($IC_{50} = 5.90 \pm 0.10 \mu\text{M}$) also having methoxy moiety at position-4 on one phenyl ring and the two methyl groups at 2,3-position on second phenyl ring. Inhibition difference in these two scaffolds may be due to two methyl group's which are present at different positions on second phenyl ring.

It was observed in whole study that the phenyl ring substituents nature as well as their positions greatly affect the inhibitory potential of the compound. Docking study was done to understand the binding interactions of the most active scaffolds with the enzyme active site.

2.3. Docking study

Docking studies had been carried out on scaffolds **1** and **23**, which displayed the most potent inhibitory potential among the whole series. Results obtained showed that scaffold **1** is able to form several hydrogen bondings within the cavity. The hydrogen on the nitrogen of triazinoindole forms a hydrogen bonding with backbone (O ϵ 2) of Glu276, the catalytic residue is involved in the hydrolysis reaction, at a distance of 2.16 Å. In the case of compound **1**, one of the nitrogen on the triazine moiety established a hydrogen bonding interaction with the residue of hydrophobic patch Phe300 at a distance of 3.42 Å. The sulfur linkage displayed a hydrogen bonding with the side chain (O) of Glu304 at a distance of 2.23 Å. Interaction involving halide bond was observed between chlorine substituent at *meta* position with the side chain (O) of Thr307 at a distance of 1.89 Å. Aromatic ring containing methoxy substituent forms an electrostatic π -hydrogen interaction with His239 (H ϵ 1) at a distance of 2.64 Å. As for triazine moiety, electrostatic π -hydrogen involving residue of the hydrophobic patch Phe177 is expected to stabilize the ligand-enzyme complex, along side with Tyr71 that forms electrostatic π -hydrogen interaction with one of the hydrogen from triazinoindole moiety at a distance of 3.93 Å.

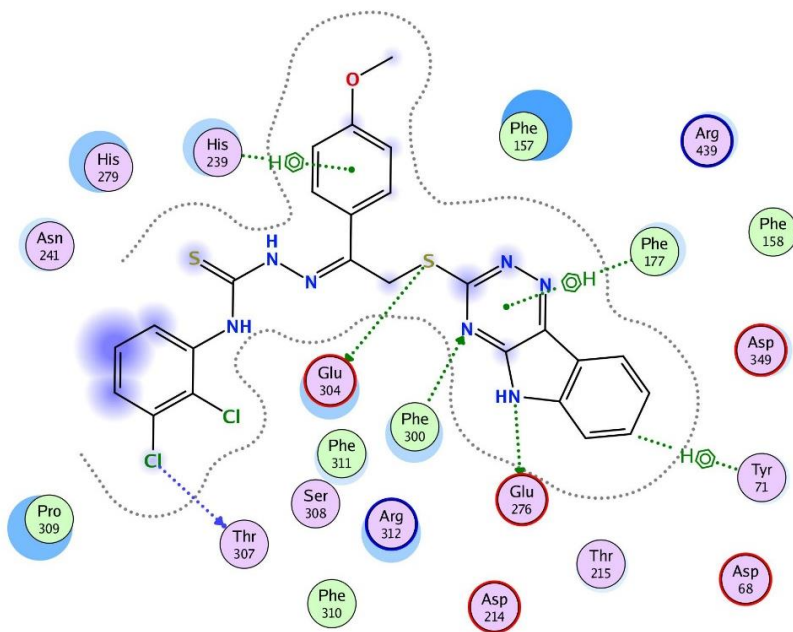


Figure-2: Docking position of compound 1

Analogue **23** docking study reveal that this scaffold is capable to form several hydrogen bonding with in cavity. The residue Glu276 through its backbone (O ϵ 2) participated in hydrogen-bonding interactions with the amino group of triazinoindole compound **23** at a distance of 2.43 Å. As for triazine moiety, electrostatic π -hydrogen involving the side chain of residue Phe157, which is expected to stabilize the ligand-enzyme complex. Another hydrogen forms electrostatic π -hydrogen interaction with Tyr71. It was also observed that His239 is capable to form an electrostatic interaction with the oxygen of the nitro substituent at *meta* position. Aromatic ring consisting of di-chloro substituents were stabilized by electrostatic interaction with Pro309 through electrostatic π -hydrogen interaction.

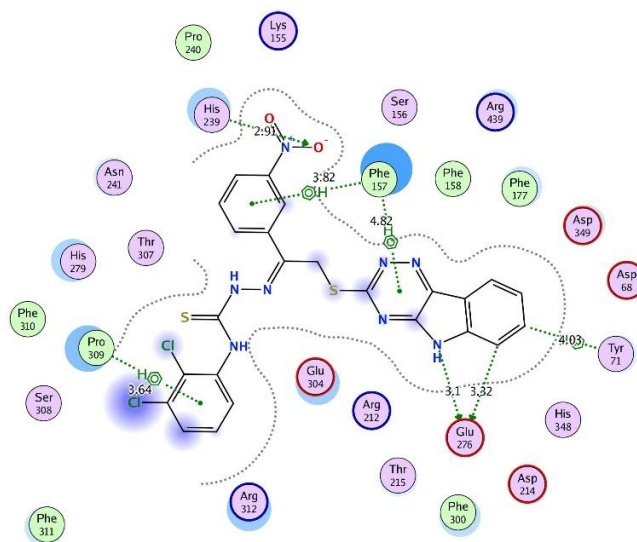


Figure-3: Docking position of compound **23**

Docking results for compound **16** displayed less interaction as compared to compound **1** and **23**. Some of the interaction that remains the same are the interaction of hydrogen on the nitrogen of triazinoindole, which forms a hydrogen bonding with backbone (Oε2) of Glu276 at a distance of 1.84 Å and hydrogen forms electrostatic π -hydrogen interaction with Tyr71 at a distance of 3.98 Å. On the other hand, aromatic ring consisting of fluoro substituent at *para* position displayed an electrostatic interaction with Glu304. It was observed that, CH₃ of methoxy at *para* position of the other aromatic ring displayed π -hydrogen interaction with Phe157 at a distance of 4.12 Å.

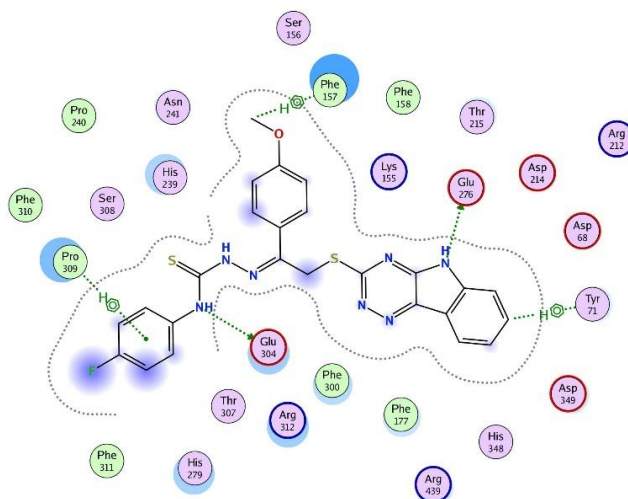


Figure-4: Docking position of compound **16**

3.0. Conclusion

In conclusion, we have synthesized twenty-five analogues of triazinoindole bearing thiosemicarbazide and evaluated against alpha-glucosidase enzyme. All the synthesized scaffolds exhibited outstanding inhibitory potential having IC₅₀ values ranging from 1.30 ± 0.01 to 35.80 ± 0.80 μM when compared with standard acarbose having IC₅₀ value 38.60 ± 0.20 μM. It was confirmed through SAR, that Polar and electron withdrawing groups on phenyl ring has a high influence on the potency of the compounds. Docking study was done to understand the binding interactions of the most active scaffolds.

4.0. Experimental

4.1. General method for the synthesis of triazinoindole bearing thiosemicarbazide analogues (**1-25**)

New class of triazinoindole bearing thiosemicarbazide analogues (**1-25**) were carried out in three steps.

In 1st step, thiosemicarbazide (10 mmol) was reacted and refluxed with isatin (10 mmol) in H₂O in the presence of potassium carbonate (5 mmol) to yield 5*H*-triazinoindole-3-thiol as intermediate (**I**). Intermediate product (**I**) (5 mmol) was then mixed and refluxed with different substituted phenacyl bromide (5 mmol) in EtOH in the presence of Et₃N to give triazinoindole derivative as second intermediate product (**II**).

In 2nd step, hydrazine hydrate (2 mL) was reacted and refluxed with different isothiocyanates (1 mmol) in methanol to yield thiosemicarbazide derivative as intermediate product (**III**).

In 3rd step, the intermediate (**II**) was reacted and refluxed with equimolar intermediate (**III**) in glacial acetic acid to give the final product triazinoindole bearing thiosemicarbazide.

4.1.1. 2-(2-((5*H*-[1,2,4]triazino[5,6-*b*]indol-3-yl)thio)-1-(4-methoxyphenyl)ethylidene)-*N*-(2,3-dichlorophenyl)hydrazine-1-carbothioamide (**1**)

Yield: 62%: ¹H NMR: (500 MHz, DMSO-*d*₆), δ 13.30 (s, 1H, NH), 11.25 (s, 2H, NH), 7.84 (d, *J* = 7.3 Hz, 2H, Ar), 7.54 (d, *J* = 6.2 Hz, 1H, Ar), 7.45 (s, 1H, Ar), 7.35 (t, *J* = 5 Hz, 2H, Ar), 7.26 (dd, *J* = 1, 6.65 Hz, 1H, Ar), 7.10 (m, 4H, Ar), 5.2 (s, 2H, CH₂), 3.81 (s, 3H, OCH₃). ¹³C NMR

(125 MHz, DMSO-*d*₆): δ 171.9, 165.8, 163.1, 159, 150.9, 141.2, 131.8, 131.8, 130.3, 128.2, 127.0, 126.9, 126.7, 123, 122.3, 121.2, 119.7, 119.7, 118.3 114.1, 114.1, 111, 104, 55.2, 41.2. HREI-MS: m/z calcd for C₂₅H₁₉Cl₂N₇OS₂ [M]⁺ 567.0470, Found: 567.0458.

4.1.2. 2-(2-((5*H*-[1,2,4]triazino[5,6-*b*]indol-3-yl)thio)-1-(4-methoxyphenyl)ethylidene)-*N*-(4-nitrophenyl)hydrazine-1-carbothioamide (**2**)

Yield: 68%: ¹H NMR: (500 MHz, DMSO-*d*₆), δ 11.25 (s, 1H, NH), 10.86 (s, 2H, NH), 8.24 (d, *J* = 7.3 Hz, 2H, Ar), 7.83 (d, *J* = 7.2 Hz, 2H, Ar), 7.79 (d, *J* = 7.65 Hz, 2H, Ar), 7.53 (d, *J* = 6.2 Hz, 1H, Ar), 7.34 (d, *J* = 1H, Ar), 7.10 (t, *J* = 6.3, 1H, Ar), 6.98 (t, *J* = 7.5, 3H, Ar), 4.91 (s, 2H, CH₂), 3.81 (s, 3H, OCH₃). ¹³C NMR (125 MHz, DMSO-*d*₆), δ 171.9, 165.8, 163.1, 159, 150.9, 141.2, 131.8, 131.8, 130.3, 128.2, 127, 126.9, 126.7, 123, 122.3, 121.2, 119.7, 119.7, 118.3 114.1, 114.1, 111, 104, 55.2, 38.2. HREI-MS: m/z calcd for C₂₅H₂₀N₈O₃S₂ [M]⁺ 544.1100, Found: 544.1088.

4.1.3. 2-(2-((5*H*-[1,2,4]triazino[5,6-*b*]indol-3-yl)thio)-1-([1,1'-biphenyl]-4-yl)ethylidene)-*N*-(2,3-dichlorophenyl)hydrazine-1-carbothioamide (**3**)

Yield: 73%: ¹H NMR: (500 MHz, DMSO-*d*₆), δ 11.25 (s, 1H, NH), 10.86 (s, 2H, NH), 8.20 (d, *J* = 7Hz, 1H, Ar), 8.01 (t, *J* = 6.9Hz, 2H, Ar), 7.9 (d, *J* = 7 Hz, 1H, Ar), 7.80 (d, *J* = 6.2 Hz, 1H, Ar), 7.75 (t, *J* = 6.3 Hz, 1H, Ar), 7.76 (m, 3H, Ar), 7.4 (m, 5H, Ar), 7.11 (t, *J* = 6.25 Hz, 1H, Ar), 6.98 (d, *J* = 6.5 Hz, 1H, Ar), 5.2 (s, 2H, CH₂). ¹³C NMR (125 MHz, DMSO-*d*₆), δ 192.8, 166.2, 163.1, 146.4, 144.8, 141, 139.5, 139.4, 138.8, 134.7, 133, 129.0, 129.0, 128.9, 128.4, 127.5, 127.0, 126.9, 126.8, 126.4, 126.2, 122.4, 122.3, 121.4, 119.8, 117.5, 112.6, 111.0, 106.9, 38.2. HREI-MS: m/z calcd for C₃₀H₂₁Cl₂N₇S₂ [M]⁺ 613.0677, Found: 613.0664.

4.1.4. 2-(2-((5*H*-[1,2,4]triazino[5,6-*b*]indol-3-yl)thio)-1-([1,1'-biphenyl]-4-yl)ethylidene)-*N*-(*p*-tolyl)hydrazine-1-carbothioamide (**4**)

Yield: 72%: ¹H NMR: (500 MHz, DMSO-*d*₆), δ 11.25 (s, 2H, NH), 10.86 (s, 1H, NH), 8.2 (d, *J* = 7 Hz, 1H, Ar), 8.01 (m, 2H, Ar), 7.9 (d, *J* = 7 Hz, 1H, Ar), 7.80 (d, *J* = 6.2 Hz, 1H, Ar), 7.7 (m, 5H, Ar), 7.6 (d, *J* = 7.1 Hz, 2H, Ar), 7.4 (m, 3H, Ar), 7.1 (t, *J* = 6.25 Hz, 1H, Ar), 6.9 (d, *J* = 6.5 Hz, 1H, Ar), 4.9 (s, 2H, CH₂), 3.0 (s, 3H, CH₃). ¹³C NMR (125 MHz, DMSO-*d*₆), δ 192.8, 166.2, 163.1, 146.4, 144.8, 141, 139.5, 139.4, 138.8, 134.7, 133, 129.0, 129.0, 128.9, 128.4, 127.5, 127.0, 126.8, 126.8, 126.4, 126.2, 122.4, 122.3, 121.4, 119.8, 117.5, 112.6, 111.0, 106.9, 38.2, 21.3. HREI-MS: m/z calcd for C₃₁H₂₅N₇S₂ [M]⁺ 559.1613, Found: 559.1601.

4.1.5. 2-(2-((5*H*-[1,2,4]triazino[5,6-*b*]indol-3-yl)thio)-1-(4-methoxyphenyl)ethylidene)-*N*-(*p*-tolyl)hydrazine-1-carbothioamide (**5**)

Yield: 73%: ¹H NMR: (500 MHz, DMSO-*d*₆), δ 13.33 (s, 1H, NH), 11.25 (s, 2H, NH), 7.8 (d, *J* = 7.25 Hz, 2H, Ar), 7.79 (d, *J* = 7.2 Hz, 2H, Ar), 7.54 (d, *J* = 6.2 Hz, 2H, Ar), 7.35 (m, 2H, Ar), 7.11 (t, *J* = 6.25 Hz, 2H, Ar), 7.02 (m, 2H, Ar), 5.2 (s, 2H, CH₂), 3.80 (s, 3H, -OCH₃), 2.42 (s, 3H, -CH₃). ¹³C NMR (125 MHz, DMSO-*d*₆), δ 165.8, 163.1, 159.0, 150.9, 141.2, 131.8, 130.3, 127.0, 127.0, 127.0, 126.9, 126.7, 122.3, 122.3, 121.0, 119.7, 119.7, 114.1, 114.1, 114.1, 114.0, 111.0, 104.6, 55.2, 38.2, 22.0. HREI-MS: *m/z* calcd for C₂₆H₂₃N₇OS₂ [M]⁺ 513.1405, Found: 513.1390.

4.1.6. 2-(2-((5*H*-[1,2,4]triazino[5,6-*b*]indol-3-yl)thio)-1-([1,1'-biphenyl]-4-yl)ethylidene)-*N*-(2-bromophenyl)hydrazine-1-carbothioamide (**6**)

Yield: 77%: ¹H NMR: (500 MHz, DMSO-*d*₆), δ 11.25 (s, 2H, NH), 10.86 (s, 1H, NH), 8.0 (d, *J* = 7 Hz, 1H, Ar), 7.9 (d, *J* = 7 Hz, 1H, Ar), 7.7 (d, *J* = 6.2 Hz, 1H, Ar), 7.5 (d, *J* = 7 Hz, 2H, Ar), 7.4 (d, *J* = 7.1 Hz, 2H, Ar), 7.36 (m, 3H, Ar), 7.34 (t, *J* = 6.25 Hz, 2H, Ar), 7.24 (d, *J* = 6.6 Hz, 1H, Ar), 7.21 (t, *J* = 7.3 Hz, 1H, Ar), 7.17 (d, *J* = 7 Hz, 1H, Ar), 7 (t, *J* = 6.9 Hz, 1H, Ar), 6.9 (d, *J* = 6.5 Hz, 1H, Ar), 4.9 (s, 2H, CH₂). ¹³C NMR (125 MHz, DMSO-*d*₆), δ 192.8, 166.2, 163.1, 146.4, 144.8, 141, 139.5, 139.4, 138.8, 134.7, 133, 129.0, 129.0, 128.9, 128.4, 127.5, 127.0, 126.8, 126.8, 126.4, 126.2, 122.4, 122.3, 121.4, 119.8, 117.5, 112.6, 111.0, 106.9, 38.2. HREI-MS: *m/z* calcd for C₃₀H₂₂BrN₇S₂ [M]⁺ 623.0561, Found: 623.0550.

4.1.7. 2-(2-((5*H*-[1,2,4]triazino[5,6-*b*]indol-3-yl)thio)-1-(*p*-tolyl)ethylidene)-*N*-(2-bromophenyl)hydrazine-1-carbothioamide (**7**)

Yield: 68%: ¹H NMR: (500 MHz, DMSO-*d*₆), δ 12.5 (s, 2H, NH), 11.25 (s, 1H, NH), 8.28 (d, *J* = 6.45 Hz, 1H, Ar), 8.01 (d, *J* = 6.75 Hz, 3H, Ar), 7.69 (t, *J* = 6.65 Hz, 2H, Ar), 7.5 (d, *J* = 6.97 Hz, 2H, Ar), 7.43 (m, 4H, Ar), 4.8 (s, 2H, CH₂), 2.42 (s, 3H, CH₃). ¹³C NMR (125 MHz, DMSO-*d*₆), δ 171.9, 165.8, 163.1, 151.1, 141.2, 137.2, 131.9, 131.2, 130.4, 130.4, 130.4, 129.3, 129.2, 125.6, 125.6, 125.4, 122.3, 121.2, 119.7, 119.7, 111.0, 109.7, 105.8, 38.2, 20.7. HREI-MS: *m/z* calcd for C₂₅H₂₀BrN₇S₂ [M]⁺ 561.0405, Found: 561.0392.

4.1.8. 2-(2-((5*H*-[1,2,4]triazino[5,6-*b*]indol-3-yl)thio)-1-(*p*-tolyl)ethylidene)-*N*-(*p*-tolyl)hydrazine-1-carbothioamide (**8**)

Yield: 65%: ^1H NMR: (500 MHz, DMSO- d_6): δ 12.5 (s, 2H, NH), 11.4 (s, 1H, NH), 8.25 (d, J = 6.45 Hz, 1H, Ar), 7.98 (d, J = 6.75 Hz, 3H, Ar), 7.69 (m, 2H, Ar), 7.48 (d, J = 6.97 Hz, 2H, Ar), 7.39 (m, 4H, Ar), 4.9 (s, 2H, CH₂), 2.42 (s, 6H, CH₃). ^{13}C NMR (125 MHz, DMSO- d_6): δ 171.9, 165.8, 163.1, 151.1, 141.2, 137.2, 131.9, 131.2, 130.4, 129.3, 129.3, 129.3, 129.2, 125.6, 125.6, 125.4, 122.3, 121.2, 119.7, 119.7, 111.0, 109.7, 105.8, 38.2, 20.7, 20.7. HREI-MS: m/z calcd for C₂₆H₂₃N₇S₂ [M]⁺ 497.1456, Found: 497.1440.

4.1.9. *2-(2-((5H-[1,2,4]triazino[5,6-b]indol-3-yl)thio)-1-(4-nitrophenyl)ethylidene)-N-phenylhydrazine-1-carbothioamide (9)*

Yield: 71%: ^1H NMR: (500 MHz, DMSO- d_6): δ 13.34 (s, 1H, NH), 11.25 (s, 2H, NH), 7.79 (d, J = 6.75 Hz, 3H, Ar), 7.54 (d, J = 5.8 Hz, 3H, Ar), 7.36 (ddd, J = 0.9, 6.4 Hz, 1H, Ar), 7.28 (dd, J = 6.55 Hz, 3H, Ar), 7.11 (m, 1H, Ar), 6.97 (d, J = 6.5 Hz, 2H, Ar), 5.1 (s, 2H, CH₂). ^{13}C NMR (125 MHz, DMSO- d_6): δ 171.9, 165.8, 163.1, 151.1, 141.2, 137.2, 131.9, 131.2, 130.4, 130.4, 130.4, 129.3, 129.3, 129.3, 129.2, 125.6, 125.4, 122.3, 121.2, 119.7, 119.7, 111.0, 105.8, 38.2. HREI-MS: m/z calcd for C₂₄H₁₈N₈O₂S₂ [M]⁺ 514.0994, Found: 514.0980.

4.1.10. *2-(2-((5H-[1,2,4]triazino[5,6-b]indol-3-yl)thio)-1-(4-methoxyphenyl)ethylidene)-N-(2-bromophenyl)hydrazine-1-carbothioamide (10)*

Yield: 71%: ^1H NMR: (500 MHz, DMSO- d_6): δ 13.30 (s, 1H, NH), 11.25 (s, 1H, NH), 10.86 (s, 1H, NH), 7.84 (t, J = 7.2 Hz, 2H, Ar), 7.54 (d, J = 6.2 Hz, 2H, Ar), 7.35 (t, J = 6.35, 2H, Ar), 7.10 (t, J = 6.55, 2H, Ar), 7.03 (d, J = 7.1 Hz, 2H, Ar), 6.99 (m, 2H, Ar), 4.80 (s, 2H, CH₂), 3.81 (s, 3H, OCH₃). ^{13}C NMR (125 MHz, DMSO- d_6): δ 171.9, 165.8, 163.1, 151.1, 141.2, 137.2, 131.9, 131.2, 130.4, 130.4, 130.4, 129.3, 129.2, 125.6, 125.6, 125.4, 122.3, 121.2, 119.7, 119.7, 111.0, 109.7, 105.8, 38.2, 55.2. HREI-MS: m/z calcd for C₂₅H₂₀BrN₇OS₂ [M]⁺ 577.0354, Found: 577.0342.

4.1.11. *2-(2-((5H-[1,2,4]triazino[5,6-b]indol-3-yl)thio)-1-(3-nitrophenyl)ethylidene)-N-(2-bromophenyl)hydrazine-1-carbothioamide (11)*

Yield: 70%: ^1H NMR: (500 MHz, DMSO- d_6): δ 13.30 (s, 1H, NH), 11.25 (s, 2H, NH), 8.71 (m, 2H, Ar), 8.37 (d, J = 6.5 Hz, 1H, Ar), 8.33 (d, J = 6.35, 1H, Ar), 8.21 (m, 2H, Ar), 7.95 (s, 1H, Ar), 7.75 (t, J = 6.6 Hz, 1H, Ar), 7.55 (d, J = 6.25 Hz, 1H, Ar), 7.36 (t, J = 6.15 Hz, 1H, Ar), 7.11 (t, J = 6.2, 1H, Ar), 6.9 (d, J = 6.5, 1H, Ar), 5.5 (s, 2H, CH₂). ^{13}C NMR (125 MHz, DMSO- d_6): δ 171.9, 165.8, 163.1, 151.1, 141.2, 137.2, 131.9, 131.2, 130.4, 130.4, 130.4, 129.3, 129.3,

129.3, 129.2, 125.6, 125.4, 123.3, 121.5, 119.7, 119.7, 111.0 105.8, 38.2. HREI-MS: m/z calcd for C₂₄H₁₇BrN₈O₂S₂ [M]⁺ 592.0099, Found: 592.0084.

4.1.12. 2-(2-((5H-[1,2,4]triazino[5,6-b]indol-3-yl)thio)-1-(3-nitrophenyl)ethylidene)-N-(p-tolyl)hydrazine-1-carbothioamide (**12**)

Yield: 70%: ¹H NMR: (500 MHz, DMSO-*d*₆): δ 11.25 (s, 2H, NH), 10.86 (s, 1H, NH), 7.80 (m, 3H, Ar), 7.68 (s, 1H, Ar), 7.54 (d, *J* = 4.8 Hz, 2H, Ar), 7.35 (t, *J* = 6.4, 1H, Ar), 7.25 (m, 3H, Ar), 7.11 (t, *J* = 6.3, 1H, Ar), 6.98 (m, 1H, Ar), 4.8 (s, 2H, CH₂), 1.9 (s, 3H, CH₃). ¹³C NMR (125 MHz, DMSO-*d*₆): δ 171.9, 165.8, 163.1, 151.1, 141.2, 137.2, 131.9, 131.2, 130.4, 130.4, 130.4, 129.3, 129.3, 129.3, 129.2, 125.6, 125.4, 122.3, 121, 119.7, 119.7, 111.0 105.8, 38.2, 21.0. HREI-MS: m/z calcd for C₂₅H₂₀N₈O₂S₂ [M]⁺ 528.1151, Found: 528.1136.

4.1.13. 2-(2-((5H-[1,2,4]triazino[5,6-b]indol-3-yl)thio)-1-([1,1'-biphenyl]-4-yl)ethylidene)-N-(2,6-dimethylphenyl)hydrazine-1-carbothioamide (**13**)

Yield: 64%: ¹H NMR: (500 MHz, DMSO-*d*₆): δ 11.25 (s, 2H, NH), 10.86 (s, 1H, NH), 8.0 (d, *J* = 7 Hz, 1H, Ar), 7.9 (d, *J* = 7 Hz, 1H, Ar), 7.7 (d, *J* = 6.2 Hz, 1H, Ar), 7.5 (d, *J* = 7, 2H, Ar), 7.4 (d, *J* = 7.1 Hz, 2H, Ar), 7.36 (m, 3H, Ar), 7.34 (d, *J* = 6.25 Hz, 2H, Ar), 7.21 (d, *J* = 7.3 Hz, 1H, Ar), 7.17 (d, *J* = 7 Hz, 1H, Ar), 7 (t, *J* = 6.9 Hz, 1H, Ar), 6.9 (d, *J* = 6.5 Hz, 1H, Ar), 4.9 (s, 2H, CH₂), 1.88 (s, 6H, CH₃). ¹³C NMR (125 MHz, DMSO-*d*₆): δ 192.8, 166.2, 163.1, 146.4, 144.8, 141, 139.5, 139.4, 138.8, 134.7, 133.5, 129.0, 129.0, 128.9, 128.4, 127.5, 127.0, 126.9, 126.8, 126.4, 126.2, 122.4, 122.3, 121.4, 119.8, 117.5, 112.6, 111.0, 106.9, 38.2, 21.0, 21.0. HREI-MS: m/z calcd for C₃₂H₂₇N₇S₂ [M]⁺ 573.1769, Found: 573.1755.

4.1.14. 2-(2-((5H-[1,2,4]triazino[5,6-b]indol-3-yl)thio)-1-(3-nitrophenyl)ethylidene)-N-(2,6-dimethylphenyl)hydrazine-1-carbothioamide (**14**)

Yield: 70%: ¹H NMR: (500 MHz, DMSO-*d*₆): δ 12.57 (s, 2H, NH), 11.28 (s, 1H, NH), 8.36 (t, *J* = 6.5 Hz, 1H, Ar), 8.6(s, 1H, Ar), 8.28 (d, *J* = 6.4, 2H, Ar), 7.96 (m, 3H, Ar), 7.56 (d, *J* = 6.75, 2H, Ar), 7.43 (m, 2H, Ar), 5(s, 2H, CH₂), 1.91 (s, 6H, CH₃). ¹³C NMR (125 MHz, DMSO-*d*₆): δ 171.9, 165.8, 163.1, 151.1, 141.2, 137.2, 131.9, 131.2, 130.4, 130.4, 130.4, 129.3, 129.3, 129.3, 129.2, 125.6, 125.4, 122.3, 121, 119.7, 119.7, 111.0, 105.8, 38.2, 22.1 22.1. HREI-MS: m/z calcd for C₂₆H₂₂N₈O₂S₂ [M]⁺ 542.1307, Found: 542.1293.

4.1.15. 2-(2-((5H-[1,2,4]triazino[5,6-b]indol-3-yl)thio)-1-(4-methoxyphenyl)ethylidene)-N-(2,6-dimethylphenyl)hydrazine-1-carbothioamide (**15**)

Yield: 75%: ^1H NMR: (500 MHz, DMSO- d_6): δ 11.25 (s, 2H, NH), 10.86 (s, 1H, NH), 7.84 (d, J = 7.25 Hz, 3H, Ar), 7.54 (d, J = 6.2 Hz, 1H, Ar), 7.34 (d, J = 6.5 Hz, 1H, Ar), 7.10 (t, J = 6.3, 1H, Ar), 6.99 (t, J = 7.2 Hz, 5H, Ar), 4.4 (s, 2H, CH₂), 3.81 (s, 3H, OCH₃), 2.3 (s, 6H, CH₃). ^{13}C NMR (125 MHz, DMSO- d_6): δ 165.8, 163.1, 159.0, 150.9, 141.2, 131.8, 130.3, 127.0, 127.0, 127.0, 126.9, 126.7, 122.3, 122.3, 121.0, 119.7, 119.7, 114.1, 114.1, 114.1, 114.0, 111.0, 104.6, 55.2, 38.2, 22.2, 22.2. HREI-MS: m/z calcd for C₂₇H₂₅N₇OS₂ [M]⁺ 527.1562, Found: 527.1551.

4.1.16. 2-(2-((5H-[1,2,4]triazino[5,6-*b*]indol-3-yl)thio)-1-(4-methoxyphenyl)ethylidene)-*N*-(4-fluorophenyl)hydrazine-1-carbothioamide (**16**)

Yield: 74%: ^1H NMR: (500 MHz, DMSO- d_6): δ 12.5 (s, 2H, NH), 10.86 (s, 1H, NH), 8.28 (d, J = 6.5 Hz, 1H, Ar), 7.90 (d, J = 7.3 Hz, 2H, Ar), 7.66 (m, 1H, Ar), 7.55 (d, J = 6.75 Hz, 1H, Ar), 7.43 (d, J = 6.4 Hz, 2H, Ar), 7.39 (m, 1H, Ar), 7.08 (d, J = 7.3 Hz, 2H, Ar), 7.02 (d, J = 6.75 Hz, 2H, Ar), 4.94 (s, 2H, CH₂), 3.46 (s, 3H, OCH₃). ^{13}C NMR (125 MHz, DMSO- d_6): δ 191.5, 166.3, 163.4, 163.3, 146.7, 146.3, 140.9, 140.2, 131.5, 131.1, 130.8, 130.6, 129.2, 128.7, 128.6, 122.4, 122.2, 121.7, 121.3, 117.5, 114.0, 113.9, 112.6, 55.5, 38.0. HREI-MS: m/z calcd for C₂₅H₂₀FN₇OS₂ [M]⁺ 517.1155, Found: 517.1143.

4.1.17. 2-(2-((5H-[1,2,4]triazino[5,6-*b*]indol-3-yl)thio)-1-(3-nitrophenyl)ethylidene)-*N*-phenylhydrazine-1-carbothioamide (**17**)

Yield: 69%: ^1H NMR: (500 MHz, DMSO- d_6): δ 11.27 (s, 2H, NH), 10.86 (s, 1H, NH), 8.6 (s, 1H, Ar), 8.36 (d, J = 6.5, 3H, Ar), 8.19 (m, 1H, Ar), 7.9 (d, J = 5.2, 1H, Ar), 7.75 (m, 2H, Ar), 7.55 (d, J = 6.15 Hz, 2H, Ar), 7.36 (t, J = 6.35, 1H, Ar), 7.18 (t, J = 6.3, 1H, Ar), 6.98 (d, J = 6.4, 1H, Ar), 4.9 (s, 2H, CH₂). ^{13}C NMR (125 MHz, DMSO- d_6): δ 171.9, 165.8, 163.1, 151.1, 141.2, 137.2, 131.9, 131.2, 130.4, 130.4, 130.4, 129.3, 129.3, 129.3, 129.2, 125.6, 125.4, 122.3, 121.2, 119.7, 119.7, 111.0, 105.8, 38.2. HREI-MS: m/z calcd for C₂₄H₁₈N₈O₂S₂ [M]⁺ 514.0994, Found: 514.0980.

4.1.18. 2-(2-((5H-[1,2,4]triazino[5,6-*b*]indol-3-yl)thio)-1-(4-methoxyphenyl) ethylidene)-*N*-(3,4-dichlorophenyl)hydrazine-1-carbothioamide (**18**)

Yield: 71%: ^1H NMR: (500 MHz, DMSO- d_6): δ 12.6 (s, 2H, NH), 11.25 (s, 1H, NH), 8.28 (d, J = 6.45 Hz, 1H, Ar), 8.10 (d, J = 7.3 Hz, 2H, Ar), 7.69 (m, 2H, Ar), 7.61 (s, 1H, Ar), 7.55 (d, J = 6.7 Hz, 1H, Ar), 7.43 (t, J = 6.3 Hz, 2H, Ar), 7.12 (d, J = 7.35 Hz, 2H, Ar), 4.9 (s, 2H, CH₂), 3.81 (s, 3H, OCH₃). ^{13}C NMR (125 MHz, DMSO- d_6): δ 171.9, 165.8, 163.1, 159, 150.9, 141.2,

131.8, 131.8, 130.3, 128.2, 127, 126.9, 126.7, 123, 121.3, 121.2, 119.7, 119.7, 118.3 114.1, 114, 111, 104, 55.2, 38.2. HREI-MS: m/z calcd for C₂₅H₁₉Cl₂N₇OS₂ [M]⁺ 567.0470, Found: 567.0458.

4.1.19. 2-(2-((5*H*-[1,2,4]triazino[5,6-*b*]indol-3-yl)thio)-1-([1,1'-biphenyl]-4-yl)ethylidene)-*N*-(3,4-dichlorophenyl)hydrazine-1-carbothioamide (**19**)

Yield: 77%: ¹H NMR: (500 MHz, DMSO-*d*₆): δ 12.5 (s, 2H, NH), 11.3 (s, 1H, NH), 8.27 (d, *J* = 6.5 Hz, 1H, Ar), 8.21 (d, *J* = 6.95 Hz, 2H, Ar), 7.91 (d, *J* = 6.9 Hz, 2H, Ar), 7.80 (d, *J* = 6.2 Hz, 2H, Ar), 7.69 (d, *J* = 7Hz, 2H, Ar), 7.55 (m, 4H, Ar), 7.47(m, 3H, Ar), 5.03 (s, 2H, CH₂). ¹³C NMR (125 MHz, DMSO-*d*₆): δ 192.8, 166.2, 163.1, 146.4, 144.8, 141, 139.5, 139.4, 138.8, 134.7, 133, 129.0, 129.0, 128.9, 128.4, 127.5, 127.0, 126.9, 126.8, 126.4, 126.2, 122.6, 122.3, 121.4, 119.8, 117.5, 112.6, 111.0, 106.9, 38.2. HREI-MS: m/z calcd for C₃₀H₂₁Cl₂N₇S₂ [M]⁺ 613.0677, Found: 613.0664.

4.1.20. 2-(2-((5*H*-[1,2,4]triazino[5,6-*b*]indol-3-yl)thio)-1-(*p*-tolyl)ethylidene)-*N*-(2,6-dimethylphenyl)hydrazine-1-carbothioamide (**20**)

Yield: 68%: ¹H NMR: (500 MHz, DMSO-*d*₆): δ 11.25 (s, 2H, NH), 10.86 (s, 1H, NH), 7.79 (d, *J* = 6.6 Hz, 2H, Ar), 7.53 (d, *J* = 4.1 Hz, 3H, Ar), 7.35 (t, *J* = 6.45 Hz, 1H, Ar), 7.23 (d, *J* = 6.5 Hz, 3H, Ar), 7.10 (t, *J* = 6.3 Hz, 1H, Ar), 6.97 (d, *J* = 6.5, 1H, Ar), 2.3(s, 6H, CH₃), 4.8 (s, 2H, CH₂), 1.91 (s, 3H, CH₃). ¹³C NMR (125 MHz, DMSO-*d*₆): δ 171.9, 165.8, 163.1, 151.1, 141.2, 137.2, 131.9, 131.2, 130.4, 129.3, 129.3, 129.2, 129.2, 125.6, 125.6, 125.4, 122.3, 121.2, 119.7, 119.7, 111.0, 109.7, 105.8, 38.2, 21.0, 21.0, 20.7. HREI-MS: m/z calcd for C₂₇H₂₅N₇S₂ [M]⁺ 511.1613, Found: 511.1600.

4.1.21. 2-(2-((5*H*-[1,2,4]triazino[5,6-*b*]indol-3-yl)thio)-1-(*p*-tolyl)ethylidene)-*N*-(3,4-dichlorophenyl)hydrazine-1-carbothioamide (**21**)

Yield: 68%: ¹H NMR: (500 MHz, DMSO-*d*₆): δ 11.25 (s, 2H, NH), 10.86 (s, 1H, NH), 7.77 (d, *J* = 6.4 Hz, 2H, Ar), 7.54 (d, *J* = 4.8 Hz, 2H, Ar), 7.32 (t, *J* = 6.2 Hz, 1H, Ar), 7.23 (d, *J* = 6.5 Hz, 3H, Ar), 7.10 (t, *J* = 6.25 Hz, 2H, Ar), 6.97 (s, 1H, Ar), 4.9 (s, 2H, CH₂), 1.91 (s, 3H, CH₃). ¹³C NMR (125 MHz, DMSO-*d*₆): δ 171.9, 165.8, 163.1, 159, 150.9, 141.2, 131.8, 131.8, 130.3, 128.2, 127, 126.9, 126.7, 123, 121.3, 121.2, 119.7, 119.7, 118.3 114.1, 114, 111, 104, 38.2, 20.1. HREI-MS: m/z calcd for C₂₅H₁₉Cl₂N₇S₂ [M]⁺ 551.0520, Found: 551.0510.

4.1.22. 2-(2-((5H-[1,2,4]triazino[5,6-b]indol-3-yl)thio)-1-(4-methoxyphenyl)ethylidene)-N-(2,3-dimethylphenyl)hydrazine-1-carbothioamide (**22**)

Yield: 62%: ¹H NMR: (500 MHz, DMSO-*d*₆): δ 11.25 (s, 2H, NH), 10.86 (s, 1H, NH), 7.79 (d, *J* = 6.6 Hz, 2H, Ar), 7.51 (d, *J* = 4.5 Hz, 3H, Ar), 7.30 (t, *J* = 6.45 Hz, 1H, Ar), 7.20 (d, *J* = 6.7 Hz, 3H, Ar), 7.10 (t, *J* = 6.3 Hz, 1H, Ar), 6.93 (d, *J* = 6.5, 1H, Ar), 4.8 (s, 2H, CH₂), 3.8 (s, 3H, OCH₃), 2.3 (s, 6H, CH₃). ¹³C NMR (125 MHz, DMSO-*d*₆): δ 171.9, 165.8, 163.1, 151.1, 141.2, 137.2, 131.9, 131.2, 130.4, 129.3, 129.3, 129.3, 129.2, 125.6, 125.6, 125.4, 122.3, 121.2, 119.7, 119.7, 111.0, 109.7, 105.8, 55.2, 38.2, 21.0, 21.0. HREI-MS: *m/z* calcd for C₂₇H₂₅N₇OS₂ [M]⁺ 527.1562, Found: 527.1551.

4.1.23. 2-(2-((5H-[1,2,4]triazino[5,6-b]indol-3-yl)thio)-1-(3-nitrophenyl)ethylidene)-N-(2,3-dichlorophenyl)hydrazine-1-carbothioamide (**23**)

Yield: 65%: ¹H NMR (500 MHz, DMSO-*d*₆): δ 11.29 (s, 2H, NH), 10.86 (s, 1H, NH), 8.72 (d, *J* = 6.4 Hz, 1H, Ar), 8.37 (m, 1H, Ar), 8.23 (d, *J* = 6.2, 1H, Ar), 7.96 (s, 1H, Ar), 7.75 (m, 3H, Ar), 7.56 (d, *J* = 6.25, 1H, Ar), 7.37 (t, *J* = 6.25, 1H, Ar), 7.12 (t, *J* = 6.25, 1H, Ar), 6.98 (d, *J* = 6.5, 1H, Ar), 4.8 (s, 2H, CH₂). ¹³C NMR (125 MHz, DMSO-*d*₆): δ 171.9, 165.8, 163.1, 159, 150.9, 141.2, 131.8, 131.8, 130.3, 129.2, 127, 126.9, 126.7, 123, 122.3, 121.2, 119.7, 119.7, 118.3, 114.1, 114, 111, 104, 38.2. HREI-MS: *m/z* calcd for C₂₄H₁₆Cl₂N₈O₂S₂ [M]⁺ 582.0215, Found: 582.0202.

4.1.24. 2-(2-((5H-[1,2,4]triazino[5,6-b]indol-3-yl)thio)-1-([1,1'-biphenyl]-4-yl)ethylidene)-N-phenylhydrazine-1-carbothioamide (**24**)

Yield: 74%: ¹H NMR (500 MHz, DMSO-*d*₆): δ 11.27 (s, 2H, NH), 10.86 (s, 1H, NH), 8.2 (d, *J* = 6.8 Hz, 2H, Ar), 7.75 (m, 7H, Ar), 7.55 (d, *J* = 6.2 Hz, 1H, Ar), 7.49 (t, *J* = 6.3 Hz, 3H, Ar), 7.39 (m, 3H, Ar), 7.19 (t, *J* = 6.25 Hz, 1H, Ar), 6.98 (d, *J* = 6.45 Hz, 1H, Ar), 5.03 (s, 2H, CH₂). ¹³C NMR (125 MHz, DMSO-*d*₆): δ 192.8, 166.2, 163.1, 146.4, 144.8, 141, 139.5, 139.4, 138.8, 134.7, 133, 129.0, 129.0, 128.9, 128.4, 127.5, 127.0, 126.8, 126.8, 126.4, 126.2, 122.4, 122.3, 121.4, 119.8, 117.5, 112.6, 111.0, 106.9, 38.2. HREI-MS: *m/z* calcd for C₃₀H₂₃N₇S₂ [M]⁺ 545.1456, Found: 545.1439.

4.1.25. 2-(2-((5H-[1,2,4]triazino[5,6-b]indol-3-yl)thio)-1-(4-methoxyphenyl)ethylidene)-N-phenylhydrazine-1-carbothioamide (**25**)

Yield: 73%; ^1H NMR (500 MHz, DMSO- d_6): δ 11.25 (s, 2H, NH), 10.6 (s, 1H, NH), 7.84 (d, $J = 7.3$ Hz, 3H, Ar), 7.54 (d, $J = 6.25$ Hz, 1H, Ar) 7.4 (m, 1H, Ar), 7.36 (t, $J = 6.4$ Hz, 1H, Ar), 7.11 (t, $J = 6.75$ Hz, 2H, Ar), 6.99 (t, $J = 7.1$ Hz, 5H, Ar), 4.9 (s, 2H, CH₂), 3.84 (s, 3H, OCH₃). ^{13}C NMR (125 MHz, DMSO- d_6): δ 171.9, 165.8, 163.1, 159.0, 150.9, 141.2, 131.8, 130.4, 127.0, 126.9, 126.9, 126.9, 126.7, 122.3, 121.2, 119.7, 119.7, 114.2, 114.0, 114.0, 114.0, 111.0, 104.6, 55.2, 38.2. HREI-MS: m/z calcd for C₂₅H₂₁N₇OS₂ [M]⁺ 499.1249, Found: 499.1233.

4.2. Alpha-glucosidase assay protocol

The α -glucosidase inhibition activity was performed with slight modifications as given by Fazal *et al.*, [24]. A total volume of 100 μL reaction mixture contained, 70 μL 50 mM phosphate buffer pH 6.8, 10 μL (0.5 mM in methanol) test compound, followed by the addition of 10 μL (0.057 units, Sigma Inc.) enzyme solution in the buffer. The contents were mixed, pre-incubated for 10 min at 37 °C and pre-read at 400 nm. The reaction was initiated by the addition of 10 μL of 0.5 mM substrate (p-nitrophenyl glucopyranoside, Sigma Inc.). After 30 min of incubation at 37 °C, the absorbance of p-nitrophenol was measured at 400 nm using the Synergy HT 96-well plate reader, BioTek, USA. Acarbose was used as positive control. All experiments were carried out in triplicates (mean \pm SEM, $n = 3$). Percent inhibition was calculated by the following equation:

$$\text{Inhibition (\%)} = (\text{Abs of Control} - \text{Abs of Test} / \text{Abs of Control}) \times 100$$
 Active compound solutions were suitably diluted and their inhibition studies were determined. Data obtained was used for the determination of IC₅₀ values (concentration at which there is 50 % enzyme inhibition) using EZ-Fit Enzyme Kinetics Software (Perrella Scientific Inc. Amherst, USA).

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