Sulfide-linked 3,4,5-trimethoxyphenyl-thiosemicarbazide/triazole hybrids: Synthesis, antioxidant, antiglycation, DNA cleavage and DNA molecular docking studies

Ashton Bollinger,[a1] Olivia Brandt,[a1] Lauren Stettler,[a] Anna Ream,[a] Vincent Kopysciansky,[a] Caroline Nelson,[a] Allison Slitt,[b] Jian Ma,[b] Lou Ann Tom,[a] Hang Ma,[b] Navindra P. Seeram,[b] Geneive E. Henry[a]*

[a]*Department of Chemistry, Susquehanna University, 514 University Avenue, Selinsgrove, PA 17870, USA*

[b]*Bioactive Botanical Research Laboratory*, *Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, RI 02881, USA*

[1]These authors contributed equally.

***Corresponding author:**

Geneive E. Henry

Department of Chemistry, Susquehanna University, 514 University Avenue, Selinsgrove, PA 17870

Telephone: 570-372-4222

E-mail: henry@susqu.edu

Abstract

Fourteen sulfide-linked 3,4,5-trimethoxyphenyl-thiosemicarbazide/triazole hybrid derivatives containing a variety of structural modifications on the thiosemicarbazide moiety were synthesized. Structure-activity correlations were investigated to determine the influence of structural variations on antioxidant, antiglycation and DNA cleavage activities. Antioxidant activity was determined using the DPPH radical scavenging and CUPRAC assays. The majority of the derivatives displayed moderate to weak DPPH radical scavenging activity (IC_{50} = 19.10 to 106.7 μM) compared to ascorbic acid (IC_{50} =13.72 μM), with derivatives having π-donating substituents being the most effective. A similar trend was observed for the CUPRAC assay All derivatives containing electron-donating substituents were more effective than Trolox at reducing copper(II) ion, with Trolox Equivalent Antioxidant Capacity (TEAC) values ranging from 1.44 to 2.45. However, only the nitro derivative was found to be a promising antiglycation agent, inhibiting the formation of advanced glycation end products (AGEs) by 75% compared to 27% for the aminoguanidine control. In addition, the majority of the derivatives cleaved pBR322 plasmid DNA in the presence of copper(II) acetate. The interaction of the derivatives with DNA was confirmed by molecular docking studies, which revealed that all of the derivatives bind favorably in the minor groove of DNA.

Keywords: Trimethoxyphenyl-thiosemicarbazide hybrids; Antioxidant; Antiglycation; DNA cleavage; DNA molecular docking

Introduction

Oxidative stress occurs when there is an accumulation of reactive oxygen species (ROS) in the body. ROS can be generated by an enzymatic process, or by the reaction of oxygen with molecules found in cells. They can also be introduced to the body via external sources, such as air pollutants, ozone, and chemicals released by industrial plants. ROS disrupt cellular functions by damaging biomacromolecules such as DNA, lipids and proteins. Over time, this damage can result in cancer, inflammation, cardiovascular disease, neurodegeneration and diabetes mellitus. The progression of these diseases can be halted by the intervention of antioxidants. There are several antioxidant mechanisms, including free radical scavenging and prevention of ROS formation by reduction of redox-active transition metal ions such as copper or iron. [1-2]

Polyphenols are the most widely known class of antioxidant compounds. However, several other compounds can act as antioxidants. These include compounds containing aromatic rings that are decorated with other electron donating substituents besides hydroxy groups, such as methoxy and dimethylamino groups.[3-4] In particular, the 3,4,5-trimethoxyphenyl moiety has been proven to significantly enhance antioxidant activity using the DPPH and FRAP assays.[5-6] In addition, sulfur containing compounds such as aryl thiosemicarbazides and sulfide derivatives have been shown to be effective in a variety of antioxidant assays including CUPRAC (CUPric Reducing Antioxidant Capacity), FRAP (Ferric Reducing Antioxidant Power), DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), nitric oxide, and inhibition of lipid peroxidation.[7-14]

Molecular hybridization involves the combination of two or more bioactive units into a single molecule, and is a commonly used strategy in drug design to improve pharmacological properties. This strategy was employed in the synthesis of a series of aryloxy-linked

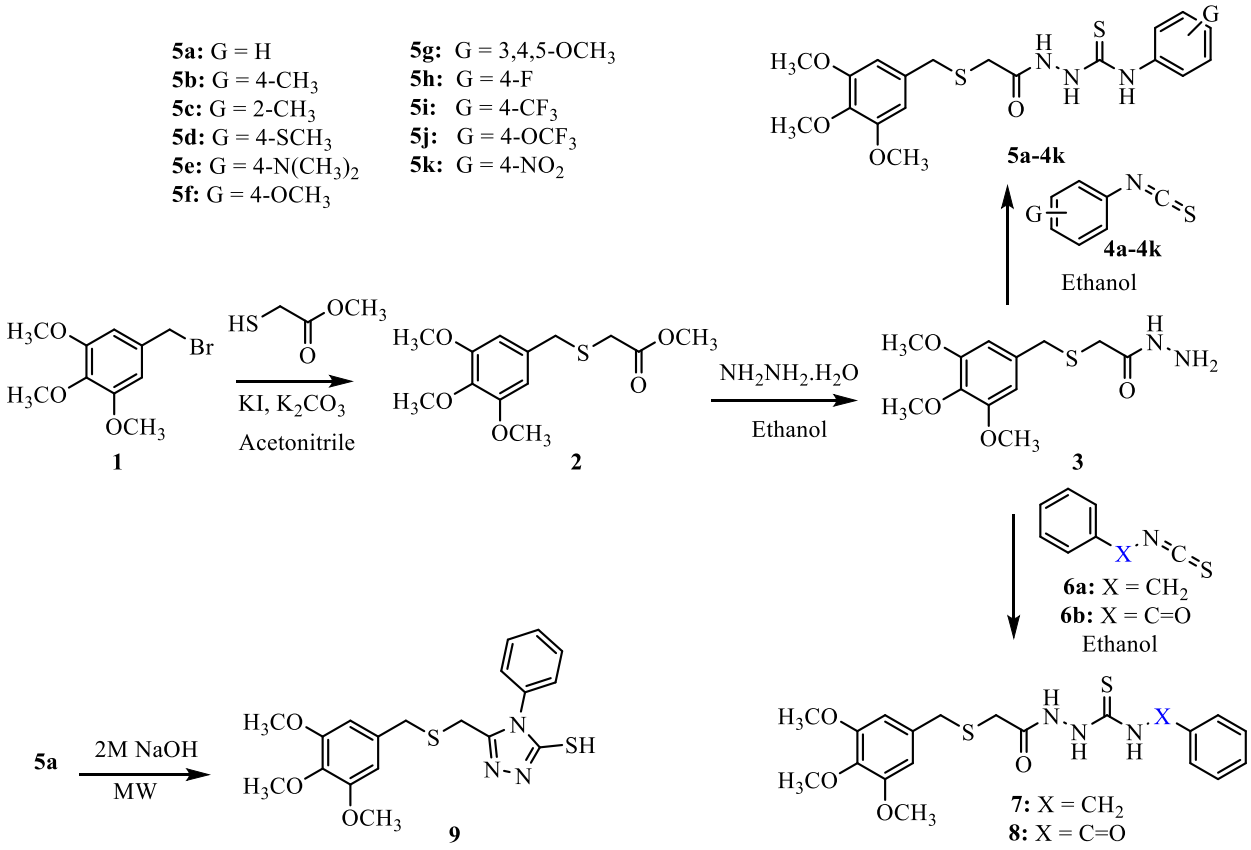
trimethoxybenzyl-thiosemicarbazide hybrid derivatives, which showed strong activity in both DPPH free-radical scavenging and FRAP assays.[5] Using a similar strategy, the current study is focused on the synthesis and biological evaluation of a series of hybrid molecules containing trimethoxyphenyl and aryl thiosemicarbazide units, linked by a dimethylene sulfide bridge (-CH_2SCH_2-). Structure-activity relationships were explored to determine how structural changes in the thiosemicarbazide moiety influence DPPH free radical scavenging, copper(II) ion reduction, antiglycation and oxidative DNA cleavage activities of the hybrid compounds. Structural changes include variation of the substituents on the aryl thiosemicarbazide moiety, replacement of an aryl group with a benzyl or benzoyl group, and conversion of the thiosemicarbazide moiety to a triazole.

Results and Discussion

Chemistry

Fourteen dimethylene sulfide-linked 3,4,5-trimethoxyphenyl-thiosemicarbazide/triazole hybrid derivatives with variations in the thiosemicarbazide moiety were prepared using the synthetic sequence outlined in Scheme 1. The thiosemicarbazide moiety contains an unsubstituted phenyl ring (**5a**), a substituted phenyl ring containing electron-donating or electron-withdrawing substituents such as methyl, methoxy, methylthio, dimethylamino, fluoro, trifluoromethyl, trifluoromethoxy, and nitro groups (**5b-5k**), a benzyl group (**7**), and a benzoyl group (**8**). These derivatives were synthesized in three steps, beginning with the 3,4,5-trimethoxy benzyl bromide (**1**), which was prepared using a published procedure.[15] The trimethoxybenzyl thioglycolate derivative (**2**) was prepared by reacting **1** with methyl thioglycolate in the presence of potassium iodide and potassium carbonate in dry acetonitrile at room temperature. The

corresponding hydrazide (**3**) was obtained by treating **2** with hydrazine hydrate in ethanol. Aryl thiosemicarbazide derivatives **5a-5k** were synthesized by reacting **3** with unsubstituted and substituted aryl isothiocyanates **4a-4k**. Derivatives **7** and **8** were prepared in a similar manner by treating **3** with benzyl isothiocyanate (**6a**) and benzoyl isothiocyanate (**6b**), respectively. In addition, the triazole derivative (**9**) was formed by cyclization of **5a** under basic conditions using sodium hydroxide.[9, 14] All compounds were characterized by NMR and IR spectroscopy, and high resolution mass spectrometry.



Scheme 1. Synthesis of sulfide-linked 3,4,5-trimethoxyphenyl-thiosemicarbazides (**5a-5k**, **7-8**) and triazole (**9**)

Free radical scavenging studies

The free radical scavenging ability of 3,4,5-trimethoxyphenyl-thiosemicarbazide/triazole derivatives **5a-5k** and **7-9** was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, which involves the reduction of the DPPH radical by an antioxidant. The IC_{50} data for the derivatives are presented in Table 1, with ascorbic acid and Trolox as positive controls.

The unsubstituted derivative (**5a**) showed moderate DPPH radical scavenging, with an IC_{50} value of 21.63 μM, and was more effective than the 4-methyl (**5b**) and 2-methyl (**5c**) derivatives, with IC_{50} values of 36.55 μM and 38.24 μM, respectively. Three derivatives containing π-donating substituents showed comparable activity to **5a**. These include the methylthio (**5d**, IC_{50} = 20.96 μM), methoxy (**5f,** IC_{50} = 24.34 μM), and fluoro (**5h**, IC_{50} = 22.11 μM) derivatives. The trimethoxy derivative (**5g**, IC_{50} = 26.24 μM) was slightly less effective than the methoxy derivative. This pattern of additional methoxy groups leading to a reduction in DPPH radical scavenging activity was also observed by Nazarbahjat et al.[14] for a series of sulfide containing thiosemicarbazide derivatives. The dimethylamino derivative (**5e**, IC_{50} = 45.52 μM) was significantly less effective than the trimethoxy derivative, following a pattern reported by Sicak[9] for a series of furan-based thiosemicarbazide derivatives. The trifluoromethyl derivative (**5i**, IC_{50} = 31.16 μM) was more effective than the trifluoromethoxy derivative **(5j,** IC_{50} = 47.36 μM). While it is less effective than derivatives with strong π-donating groups, derivative **5i** has a stronger inductive effect than **5j**, which enhances hydrogen atom donating ability.[7] Meanwhile, the nitro derivative (**5k**, IC_{50} = 38.18 μM) was comparable to the 4-methyl and 2-methyl derivatives. It should be noted that derivatives **5a-5k** were all more effective than Trolox (IC_{50} = 53.43 μM) at scavenging the DPPH radical, but they were less effective than ascorbic acid (IC_{50} = 13.72 μM).

The higher activity for the majority of the derivatives with π-donating substituents suggests that the loss of the hydrogen atom on the nitrogen atom connected to the aromatic ring, accompanied by resonance stabilization, plays an important role in the free radical scavenging mechanism. Derivative **7**, which contains a methylene group between the thiosemicarbazide moiety and the phenyl ring compared to **5a**, had significantly reduced free radical scavenging activity (IC_{50} = 84.41 μM), which supports this hypothesis. Resonance stabilization is partially restored with the incorporation of a carbonyl group in place of the methylene group for compound **8**, which shows comparable activity to the dimethylamino and trifluoromethoxy derivatives. The proposed mechanisms for stabilization of radicals obtained for **5a-5k** and **8** are shown in Figure 1.

Nazarbahjat[14] reported that conversion of a phenyl/aryl thiosemicarbazide derivative to the corresponding triazole led to a slight improvement in free-radical scavenging activity, while Sicak[9] reported both enhancement and decline in activities, depending on the substituent. In this work, the conversion of **5a** to the corresponding triazole **9** resulted in a significant reduction of activity (IC_{50} = 106.69 μM). Since the DPPH mechanism involves hydrogen atom transfer, the decrease in activity of the triazole may be rationalized based on the reduction in the number of NH bonds during ring formation. In addition, electron delocalization across the aromatic ring is limited.[14]

Table 1. DPPH and CUPRAC data for thiosemicarbazide/triazole derivatives (**5a-5k** and **7-9**)

Cmpd.	**Identifier**	**DPPH (IC_{50} μM)**	**CUPRAC (TEAC)**
5a	$-C_6H_5$	21.63±2.66	1.69±0.05
5b	$-C_6H_4$-4-CH_3	36.55±0.71	1.44±0.04
5c	$-C_6H_4$-2-CH_3	38.24±0.42	1.76±0.10
5d	$-C_6H_4$-4-SCH_3	20.96±0.18	1.78±0.04
5e	$-C_6H_4$-4-$N(CH_3)_2$	45.52±1.09	2.45±0.10
5f	$-C_6H_4$-4-OCH_3	24.34±0.14	1.80±0.08
5g	$-C_6H_2$-3,4,5-OCH_3	26.24±0.57	1.74±0.02
5h	$-C_6H_4$-4-F	22.11±0.45	1.66±0.19
5i	$-C_6H_4$-4-CF_3	31.16±0.72	0.78±0.02
5j	$-C_6H_4$-4-OCF_3	47.36±0.71	1.26±0.04
5k	$-C_6H_4$-4-NO_2	38.18±0.86	1.49±0.07
7	$-CH_2-C_6H_5$	84.41±0.59	0.48±0.03
8	$-CO-C_6H_5$	45.50±2.93	0.37±0.03
9	triazole	106.69±6.24	0.61±0.02
Ascorbic acid		13.72±0.59	0.72±0.06
Trolox		53.43±0.29	

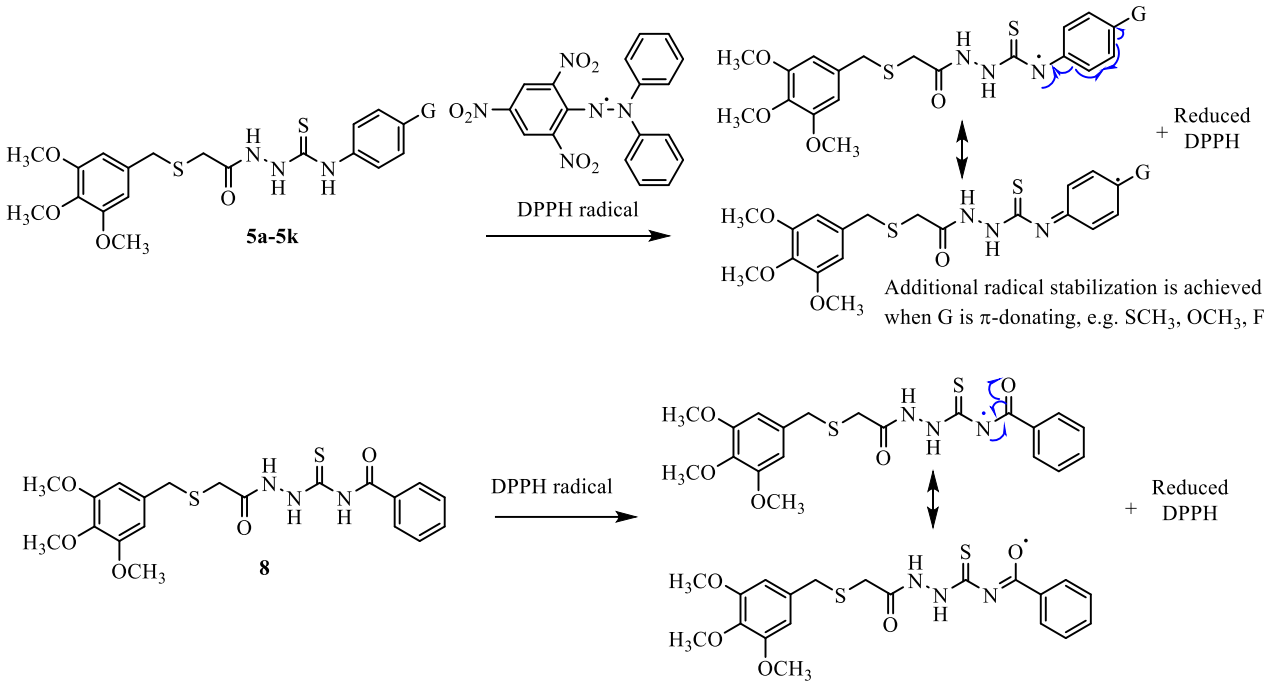


Figure 1. Proposed mechanism for DPPH radical scavenging activity of thiosemicarbazide derivatives (**5a-5k**, **8**).

Copper(II) ion reduction studies

The CUPRAC (CUPric Reducing Antioxidant Capacity) assay involves the reduction of a bis(neocuproine)-Cu(II) ion to the bis(neocuproine)-Cu(I) ion in the presence of an antioxidant.[16] We have previously demonstrated that sulfide containing derivatives of carvacrol and thymol, the major essential oils in thyme and oregano, have the ability to effectively reduce Cu(II) to Cu(I) using the CUPRAC assay, and that the activity was linked to the presence of the sulfide.[12-13] In this work, the CUPRAC assay was performed on the sulfide-linked thiosemicarbazide derivatives using Trolox and ascorbic acid as antioxidant standards. Results are reported as Trolox Equivalent Antioxidant Capacity (TEAC) coefficients (Table 1), which are derived from the ratio of the molar absorptivity (obtained from calibration plots) of the thiosemicarbazide/triazole derivatives compared to that of Trolox (TEAC = $\varepsilon_{sample}/\varepsilon_{Trolox}$).

The phenyl thiosemicarbazide derivative (**5a**), with a TEAC coefficient of 1.69, was more effective at reducing Cu(II) than Trolox. Derivatives **5d**, **5f** and **5g**, containing electron-donating methylthio and methoxy groups, showed only slight improvement in Cu(II) ion reducing capacity (TEAC coefficient = 1.74-1.80) compared to **5a**, suggesting that π-donation does not significantly enhance activity, and additional methoxy groups have negligible effects. Incorporation of a dimethylamino substituent (**5e**) led to almost 1.5 fold increase in activity (TEAC coefficient = 2.45) relative to the parent compound **5a**. This could be due to *N*-oxidation during the redox process. Comparison of the two σ-donating methyl substituted isomers, **5b** and **5c**, indicate that the 2-methyl derivative has comparable activity to the π-donating groups, while the 4-methyl derivative is less effective than the parent compound **5a**. Among the compounds with electron-withdrawing substituents, the order of reactivity was **5h** > **5k** > **5j** > **5i**, representing the fluoro, nitro, trifluoromethoxy and trifluoromethyl derivatives, respectively. The fluoro derivative shows comparable activity to the parent derivative **5a**. A similar outcome was obtained for the DPPH assay. Likewise, the nitro derivative showed comparable activity to the 4-methyl derivative in both the CUPRAC and DPPH assays. The trifluoromethyl derivative (**5i**), with a TEAC value of 0.78, was the least effective among the eleven aryl thiosemicarbazide derivatives. However, the activity was comparable to that of ascorbic acid. Further structural modification of **5a** by incorporation of a methylene (**7**) or carbonyl (**8**) group between the aromatic ring and the nitrogen atom led to a 3.5-4.5 fold decrease in Cu(II) ion reducing capacity. Similar to the DPPH assay, a nitrogen radical may be formed during the Cu(II) ion reduction, and stabilization is affected by the presence of the methylene and carbonyl groups. In addition, conversion of the thiosemicarbazide to a triazole (**9**) led to a 2.8 fold reduction in Cu(II) ion reducing capacity. Based on a previous study,[9] the triazole derivative had been expected to

have better Cu(II) ion reducing capacity than the parent compound **5a,** but it is possible that the loss of potential oxidation sites at the three nitrogen atoms within the triazole ring contributed to the low TEAC value for derivative **9**.

Antiglycation studies

Reducing sugars and their metabolites such as reactive carbonyl species (e.g., methylglyoxal; MGO) interact with proteins to form a group of macromolecules known as advanced glycation end products (AGEs). The formation of AGEs is initiated with an oxidation process known as the Maillard reaction, which further produces a group of keto-amine adducts (i.e. Amadori products). Amadori products interact with amino acids on proteins to form AGEs via multiple reactions including oxidation, dehydration, and polymerization.[17] During this process, several reactive carbonyl species including MGO are generated by oxidative reactions, which can exacerbate the formation of polymerized AGEs. The final formed AGEs products can be measured in fluorescence-based assays.[18] Thus, molecules with antioxidant effects and MGO trapping capacity are potential inhibitors of AGEs. While the literature on antiglycation effects of thiosemicarbazide derivatives is sparse, antiglycation activities of related compounds, including thiosemicarbazones and thioureas have been reported.[19-20]

Given the antioxidant activities of the thiosemicarbazide derivatives, they were evaluated for their potential to inhibit the formation of AGEs. The antiglycation effects of the derivatives (100 μM) in the BSA (bovine serum albumin)-MGO model are shown in Figure 2. Compound **9** was not evaluated due to low availability of material. Thiosemicarbazide derivatives **5a**-**5d**, **5f**, and **5h**-**5j** showed moderate antiglycation effects (16-22%) compared to the positive control aminoguanidine (27%), a known AGEs inhibitor, while the benzoyl derivative **8** (32%) was

slightly more effective. Owing to the common hydrazine group in synthetic intermediate **3** and aminoguanidine, **3** was also included in the study and showed comparable inhibition (33%) to the benzoyl derivative.

The thiosemicarbazide derivative **5k**, which contains a nitro group, showed superior activity to aminoguanidine, inhibiting the formation of AGEs by 75%. As noted before, this derivative was also more effective than Trolox in both the DPPH and CUPRAC assays (IC_{50} = 38.18 μM and TEAC = 1.49, respectively). It was also more effective than ascorbic acid in the CUPRAC assay. However, it was less effective than ascorbic acid in the DPPH assay. To further elucidate its mechanism of action in the antiglycation assay, **5k** was evaluated for reactive carbonyl species scavenging effects in an MGO trapping assay (Table S1, Supplementary Material). However, the compound was inactive in decreasing the MGO level, suggesting that its antiglycation activity was likely attributed to its antioxidant capacity and other factors. Khan et al.[21] have demonstrated that in a series of Schiff base derivatives containing nitro, methyl, methoxy, hydroxy, fluoro, chloro and dimethylamino substituents, the compounds containing nitro substituents had significantly enhanced antiglycation effects. It was suggested that the enhanced activity of derivatives with nitro substituents is attributed to the formation of a zwitterionic complex with the carbonyl group of MGO.

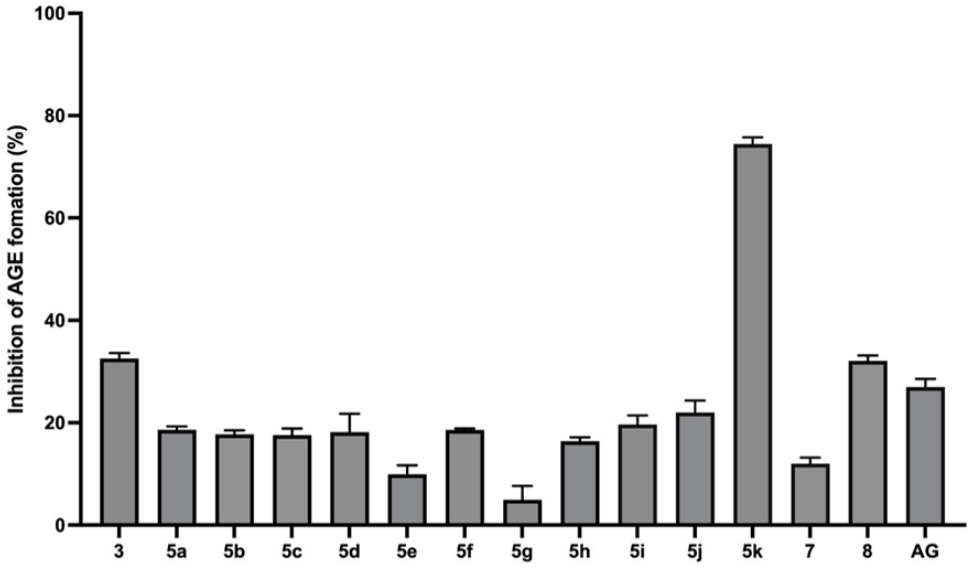


Figure 2. Effects of thiosemicarbazide derivatives (**5a-5k** and **7-8**) and hydrazide (**3**) at a concentration of 100 μM on the formation of AGEs in a BSA-MGO glycation model. Aminoguanidine (AG) was used as the positive control at an equivalent concentration. Statistical data for the fluorescence intensity measurements used to generate the percent inhibition are shown in Fig. S31 (Supplementary Material).

DNA cleavage studies

When the reduction of Cu(II) ion to Cu(I) ion takes place in the presence of oxygen, the Cu(I) ion can be oxidized back to Cu(II), and the redox process generates ROS. Since cancer cells typically have a higher concentration of copper than normal cells, copper-mediated ROS generation in cancer cells can lead to selective damage of biomolecules such as DNA, leading to cell death.[23-25] Plasmid DNA is often used as a model to determine the anticancer potential of small molecules. Based on the demonstrated copper(II) ion reducing capacity of the sulfide-linked trimethoxyphenyl thiosemicarbazide derivatives, their ability to cleave supercoiled DNA to open-circular and/or linear forms in the presence of copper(II) ions was evaluated. Cleavage

products were examined using gel electrophoresis, since cleaved DNA products migrate at different rates on agarose gel compared to the supercoiled DNA.

When incubated with DNA in the absence of copper(II) acetate, the derivatives showed no detectable DNA cleavage. As shown in Figure 3, the majority of the sulfide-linked aryl thiosemicarbazide derivatives (**5a-5k**) showed full or partial cleavage of the supercoiled DNA to give the open-circular and/or linear form in the presence of copper(II) ions. Among the derivatives with electron-donating substituents, the dimethylamino derivative (**5e**) appeared to be the most effective, with no evidence of the supercoiled form of the plasmid DNA present, followed by the trimethoxy derivative (**5g**), which showed a small amount of the supercoiled form. Among the derivatives with electron-withdrawing substituents, the fluoro derivative (**5h**) was the most effective, and also showed strong copper(II) reducing activity in the CUPRAC assay. Although the benzyl derivative (**7**) did not show strong copper(II) ion reducing activity, it was more effective at cleaving DNA than some of the aryl thiosemicarbazide derivatives. The benzoyl (**8**) and triazole (**9**) derivatives showed very little or no detectable cleavage relative to the DNA control.

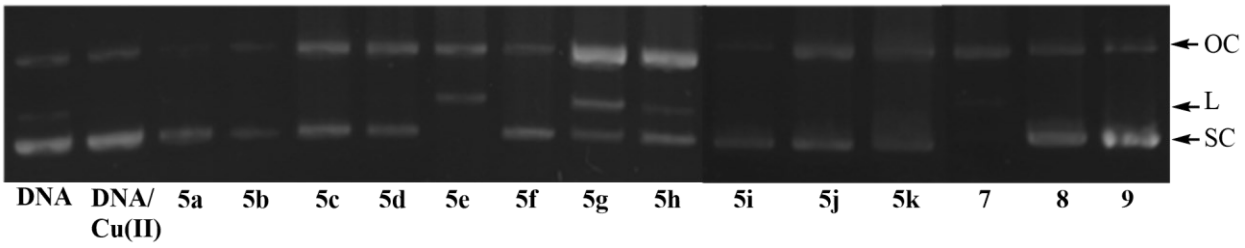


Figure 3. Agarose gel electrophoresis pattern of pBR322 plasmid DNA after treatment with derivatives **5a-5k** and **7-9** (1 mM) in the presence of copper(II) acetate. *Lane 1:* DNA control; *Lane 2:* DNA + $Cu(OAc)_2$; *Lanes 3-17:* DNA + $Cu(OAc)_2$ + test compounds as indicated. OC = open-circular; L = linear; SC = supercoiled.

In order to determine the type of ROS that is responsible for DNA cleavage, the cleavage experiments were carried out in the presence of a variety of ROS scavengers. These include hydroxyl radical scavengers (DMSO and tert-butyl alcohol), superoxide anion scavengers (tiron and potassium iodide), and singlet oxygen scavengers (sodium azide and histidine).[24-25] Owing to its high copper(II) ion reducing and DNA cleavage ability, the dimethylamino derivative (**5e**) was used for this study, Figure 4. In the presence of copper(II) acetate, the derivative (1 mM) completely cleaved plasmid DNA to open-circular and linear forms, with no evidence of the supercoiled form (lane 1), compared to the DNA control (lane 8). In the presence of DMSO (lane 2) and tert-butyl alcohol (lane 3) no significant change was observed, indicating that the hydroxyl radical does not play a role in the cleavage mechanism. Tiron (lane 6) also had no effect on cleavage, but addition of potassium iodide (lane 7) resulted in a small amount of the supercoiled form, suggesting that superoxide anion may play a role in DNA cleavage. Both sodium azide (lane 4) and histidine (lane 5) showed inhibition of DNA cleavage by **5e**, and no linear form was detected, indicating that singlet oxygen was the major ROS species involved in DNA cleavage.

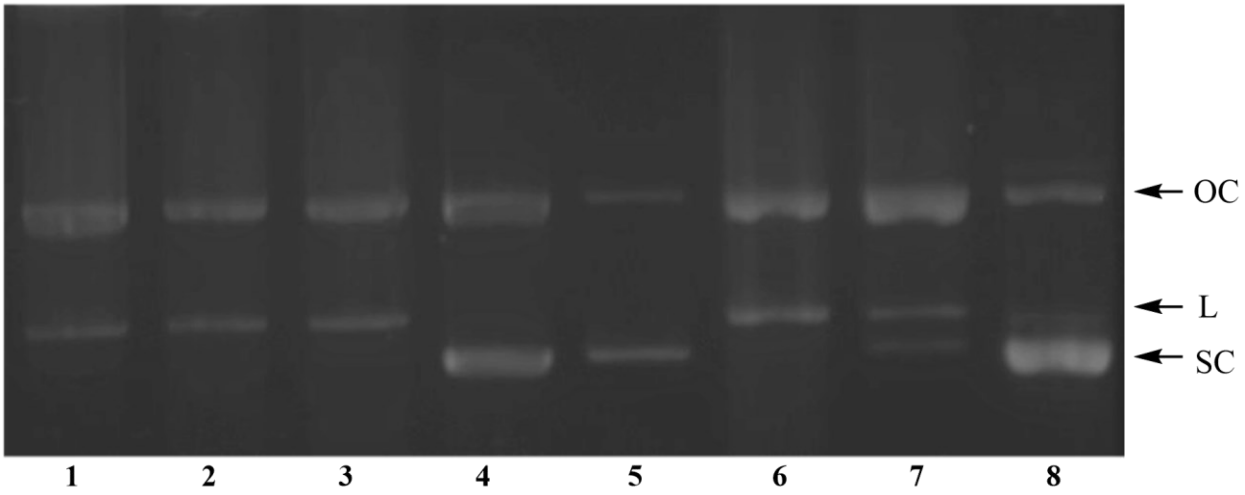


Figure 4. Effect of ROS scavengers on the cleavage of dimethylamino derivative (**5e**). *Lane 1:* DNA + $Cu(OAc)_2$ + **5e**; *Lane 2:* DNA + $Cu(OAc)_2$ + **5e** + DMSO (dimethyl sulfoxide); *Lane 3*: DNA + $Cu(OAc)_2$ + **5e** + *tert*-butyl alcohol*; Lane 4:* DNA + $Cu(OAc)_2$ + **5e** + sodium azide; *Lane 5:* DNA + $Cu(OAc)_2$ + **5e** + histidine; *Lane 6:* DNA + $Cu(OAc)_2$ + **5e** + Tiron; *Lane 7:* DNA + $Cu(OAc)_2$ + **5e** + potassium iodide; *Lane 8:* DNA control.

OC = open-circular; L = linear; SC = supercoiled.

DNA molecular docking studies

To further explore the interactions of the 3,4,5-trimethoxyphenyl-thiosemicarbazide/triazole hybrid derivatives with DNA, molecular docking studies were performed with DNA dodecamer (PDB ID: 1BNA) using AutoDock Vina. The docking affinities of the derivatives range from -7.3 kcal/mol to -7.9 kcal/mol (Table 2), with all compounds bound in the minor groove of DNA. The 3D and 2D interaction maps for the dimethylamino derivative (**5e**), which displays a docking score of -7.6 kcal/mol, are shown in Figure 5. The complex is stabilized by hydrogen bonding interaction between one of the NH bonds on the thiosemicarbazide moiety and the oxygen in the deoxyribose ring of a cytosine containing

nucleotide. Additional stabilization is achieved through hydrophobic interactions involving several DNA residues. The stabilizing interactions for all the derivatives are shown in Table S2 (Supplementary Material).

Table 2. DNA docking data for thiosemicarbazide/triazole derivatives (**5a-5k** and **7-9)**

Compound	DNA docking (kcal/mol)	Compound	DNA docking (kcal/mol)
5a	-7.4	**5h**	-7.8
5b	-7.7	**5i**	-7.8
5c	-7.3	**5j**	-7.4
5d	-7.3	**5k**	-7.4
5e	-7.6	**7**	-7.4
5f	-7.7	**8**	-7.9
5g	-7.5	**9**	-7.4

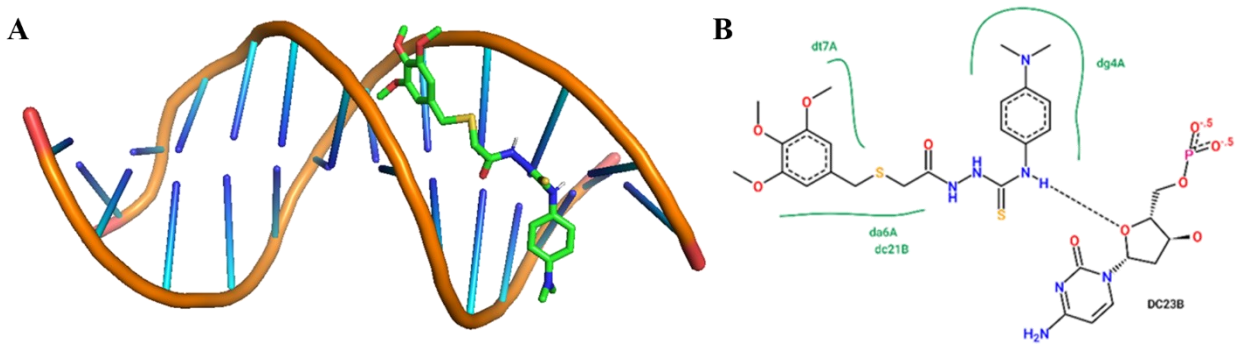


Figure 5. Interactions between dimethylamino derivative **5e** and 1BNA. A) 3D image generated in Pymol; B) 2D image generated using ProteinsPlus.

Conclusion

In conclusion, fourteen sulfide-linked 3,4,5-trimethoxyphenyl-thiosemicarbazide/triazole hybrid derivatives were synthesized and evaluated for their free-radical scavenging, copper(II) ion reducing, antiglycation and DNA cleavage effects. Derivatives containing electron-donating substituents on the thiosemicarbazide moiety were generally more effective at scavenging the DPPH radical and reducing copper(II) ions than derivatives with electron-withdrawing substituents. However, the nitro derivative was the most promising as an antiglycation agent. Structural modification of the phenyl thiosemicarbazide derivative by insertion of a methylene group, a carbonyl group or cyclization to a triazole reduced the antioxidant activity. Exploration of the DNA cleavage ability indicated that the majority of the derivatives displayed DNA cleavage effects, possibly via singlet oxygen generation. Interactions with a model DNA dodecamer were confirmed by molecular docking studies, which showed that all compounds were minor groove binders. These data indicate that some of the thiosemicarbazide derivatives are effective antioxidant agents. Owing to their DNA cleavage properties and interactions with DNA in silico, these derivatives may be promising as anticancer agents. However, anticancer studies in human cancer cell lines are beyond the scope of this project.

Declaration of Competing Interest

The authors declare that they have no competing financial interests that could influence the work reported in this paper.

Acknowledgments

The authors are thankful to Susquehanna University and University of Rhode Island for funding this work. We are also grateful to Dr. Morgan Olsen and Dr. Douglas Collins from Bucknell University for assistance in acquiring high resolution mass spectrometry data.

Appendix A. Supplementary data

Experimental procedures and spectroscopic data (^{1}H, ^{13}C NMR and HRMS) for all new compounds are available.

Experimental section

Materials and instruments

All chemicals and solvents were purchased from Sigma Aldrich, Fisher Scientific or TCI America. Reactions were monitored by TLC using silica gel plates obtained from Sigma-Aldrich. Microwave reactions were conducted in a CEM Discover SP reactor. Column chromatography was carried out on a Teledyne CombiFlash Rf 200 chromatography system, using RediSep Gold silica gel columns. Samples were eluted with ethyl acetate-hexane solvent gradients, and methanol-water solvent gradients for normal phase and reversed phase chromatography, respectively. Melting points were recorded on RD-MP or Thomas Hoover capillary melting point instruments and values are uncorrected. FTIR spectra were acquired using a Nicolet iS50 spectrometer, equipped with attenuated total reflectance (ATR) apparatus. NMR data (^{1}H, 400 MHz and ^{13}C, 100 MHz) were obtained on a JEOL 400 MHz instrument (NSF MRI: CHE-1625340) using $CDCl_3$ and DMSO-d_6 as solvents. High resolution mass spectrometry data were acquired on an Agilent 6560 ion mobility Q-ToF mass spectrometer with Agilent Jet Spray dual

ESI inlet (NSF MRI: CHE-2018547). Samples were run in positive mode by flow injection analysis in LC-MS grade 50% acetonitrile and 50% water containing 0.1% formic acid.

Experimental Procedures and Analytical Data

Synthesis of methyl 2-((3,4,5-trimethoxybenzyl)thio)acetate (2)

Potassium iodide (3.62 g, 21.8 mmol) was added to a stirred solution of trimethoxybenzyl bromide (4.74 g, 18.2 mmol) in dry acetonitrile (85 mL). After 5 min, methyl thioglycolate (1.80 mL, 20.1 mmol) and potassium carbonate (3.77g, 27.3 mmol) were added to the cloudy reaction mixture, followed by heating under reflux for 22 hours. The acetonitrile was removed in vacuo and the residue was diluted with water (200 mL), followed by extraction with dichloromethane (3 × 50 mL). The combined dichloromethane solution was washed with saturated NaCl (100 mL), dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography using 15% ethyl acetate-hexanes to give compound **2** as an amorphous pale yellow solid (3.73 g, 71.8%)

M.p. 34-36°C; FTIR (ATR), (cm^{-1}): 1722, 1587, 1504, 1420, 1233, 1117.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.56 (s, 2H), 3.85 (s, 6H), 3.82 (s, 3H), 3.78 (s, 2H), 3.73 (s, 3H), 3.12 (s, 2H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 171.0, 153.3, 137.1, 132.8, 106.1, 60.9, 56.1, 52.5, 36.9, 32.2.

HRMS (ESI): *m/z* 325.0505 $[M + K]^+$; calcd. for $C_{13}H_{18}O_5SK$, 325.0512.

Synthesis of 2-((3,4,5-trimethoxybenzyl)thio)acetohydrazide (3)

A solution of **2** (3.04 g, 10.6 mmol) and hydrazine monohydrate (1.00 mL, 20.5 mmol) in absolute ethanol (5 mL) was refluxed for 3 hours, followed by removal of the solvent. The

resulting residue was washed with cold water and filtered to afford a compound **3** as a pure white solid (2.41 g, 79.2%).

M.p. 120-123°C; FTIR (ATR), (cm^{-1}): 3296, 1645, 1621, 1592, 1238, 1127

^{1}H NMR (400 MHz, DMSO-d_6): δ 9.10 (broad s, 1H), 6.59 (s, 2H), 4.23 (broad s, 2H), 3.72 (s, 6H), 3.72 (s, 2H), 3.59 (s, 3H), 2.98 (s, 2H).

^{13}C NMR (100 MHz, DMSO-d_6): δ 168.8, 153.2, 136.8, 134.2, 106.7, 60.5, 56.3, 36.6, 32.8.

HRMS (ESI): *m/z* 309.0883 $[M + Na]^+$; calcd. for $C_{12}H_{18}N_2O_4SNa$, 309.0885.

Synthesis of 3,4,5-trimethoxyphenyl-thiosemicarbazide hybrids (5a-5k and 7-8)

A mixture of compound **3** (0.700 mmol) and the corresponding aryl, benzyl or benzoyl isothiocyanate (0.700 mmol) in absolute ethanol was heated under reflux for 2-6 hours. The ethanol was removed in vacuo to give either solids or oils. The solids were recrystallized with acetonitrile and water, and oils were purified by reversed phase column chromatography using methanol-water mixtures.

N-phenyl-2-(2-((3,4,5-trimethoxybenzyl)thio)acetyl)hydrazine-1-carbothioamide (5a)

White solid (81%); M.p. 127-129°C; FTIR (ATR), (cm^{-1}): 3553, 3459, 3148, 1662, 1594, 1507, 1236, 1121.

^{1}H NMR (400 MHz, DMSO-d_6): δ 10.07 (s, 1H), 9.64 (s, 1H), 8.63 (s, 1H), 7.38 (broad s, 2H), 7.30 (t, 2H, J = 8.4 Hz), 7.13 (t, 1H, J = 8.4 Hz), 6.61 (s, 2H), 3.76 (s, 2H), 3.72 (s, 6H), 3.60 (s, 3H), 3.13 (s, 2H).

^{13}C NMR (100 MHz, DMSO-d_6): δ 181.4, 169.2, 153.3, 139.6, 136.9, 134.0, 128.9, 126.4, 125.6, 106.7, 60.5, 56.3, 36.4, 33.1.

HRMS (ESI): *m/z* 422.1211 $[M + H]^+$; calcd. for $C_{19}H_{24}N_3O_4S_2$, 422.1208.

N-(p-tolyl)-2-(2-((3,4,5-trimethoxybenzyl)thio)acetyl)hydrazine-1-carbothioamide (5b)

White solid (69.3%); M.p. 110-112°C; FTIR (ATR), (cm^{-1}): 3155, 1694, 1591, 1508, 1243, 1124.

^1H NMR (400 MHz, $CDCl_3$): δ 9.76 (s, 1H), 9.01 (s, 1H), 8.31 (s, 1H), 7.25 (d, 2H, *J* = 8.0 Hz), 7.18 (d, 2H, *J* = 8.0 Hz), 6.56 (s, 2H), 3.85 (s, 2H), 3.81 (s, 6H), 3.80 (s, 3H), 3.20 (s, 2H), 2.31 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 178.4, 165.6, 153.4, 137.4, 137.2, 133.8, 132.1, 130.4, 124.9, 106.2, 61.0, 56.3, 37.5, 33.4, 21.2.

HRMS (ESI): *m/z* 436.1372 $[M + H]^+$; calcd. for $C_{20}H_{26}N_3O_4S_2$, 436.1365.

N-(o-tolyl)-2-(2-((3,4,5-trimethoxybenzyl)thio)acetyl)hydrazine-1-carbothioamide (5c)

White solid (76%); M.p. 125-127°C; FTIR (ATR), (cm^{-1}): 3344, 3267, 1664, 1588, 1506, 1240, 1126.

^1H NMR (400 MHz, DMSO-d_6): δ 10.07 (s, 1H), 9.56 (s, 1H), 9.35 (s, 1H), 7.13-7.18 (m, 4H), 6.61 (s, 2H), 3.76 (s, 2H), 3.72 (s, 6H), 3.59 (s, 3H), 3.11 (s, 2H), 2.13 (s, 3H).

^{13}C NMR (100 MHz, DMSO-d_6): δ 182.0, 169.2, 153.2, 138.4, 136.8, 136.4, 134.0, 130.6, 129.4, 127.3, 126.4, 106.7, 60.5, 56.3, 36.4, 33.0, 18.2.

HRMS (ESI): *m/z* 436.1371 $[M + H]^+$; calcd. for $C_{20}H_{26}N_3O_4S_2$, 436.1365.

N-(4-(methylthio)phenyl)-2-(2-((3,4,5-trimethoxybenzyl)thio)acetyl)hydrazine-1-carbothioamide (5d)

White solid (74%); M.p. 104-107°C; FTIR (ATR), (cm^{-1}): 3553, 3112, 1691, 1590, 1495, 1232, 1123.

^{1}H NMR (400 MHz, DMSO-d_6): δ 10.06 (s, 1H), 9.63 (s, 1H), 7.32 (d, 2H, *J* = 8.8 Hz), 7.19 (d, 2H, *J* = 8.48 Hz), 6.61 (s, 2H), 3.75 (s, 2H), 3.72 (s, 6H), 3.59 (s, 3H), 3.12 (s, 2H), 2.42 (s, 3H).

^{13}C NMR (100 MHz, DMSO-d_6): δ 181.4, 169.3,153.2, 136.8, 136.7, 135.0, 134.0, 127.0, 126.3, 106.7, 60.5, 56.3, 36.4, 33.1, 15.6.

HRMS (ESI): *m/z* 468.1089 $[M + H]^+$; calcd. for $C_{20}H_{26}N_3O_4S_3$, 468.1085.

N-(4-(dimethylamino)phenyl)-2-(2-((3,4,5-trimethoxybenzyl)thio)acetyl)hydrazine-1-carbothioamide (5e)

White solid (65%); M.p. 153-155°C; FTIR (ATR), (cm^{-1}): 3536, 3168, 1664, 1591, 1520, 1226, 1124.

^{1}H NMR (400 MHz, DMSO-d_6): δ 10.0 (s, 1H), 9.40 (s, 1H), 7.10 (d, 2H, *J* = 8.4 Hz), 6.64 (d, 2H, *J* = 8.4 Hz), 6.61 (s, 2H), 3.75 (s, 2H), 3.72 (s, 6H), 3.60 (s, 3H), 3.11 (s, 2H), 2.84 (s, 6H).

^{13}C NMR (100 MHz, DMSO-d_6): δ 181.6, 169.0, 153.3, 148.9, 136.9, 134.0, 128.6, 127.2, 112.4, 106.8, 60.5, 56.3, 40.9, 36.4, 33.1.

HRMS (ESI): *m/z* 465.1637 $[M + H]^+$; calcd. for $C_{21}H_{29}N_4O_4S_2$, 465.1630.

N-(4-methoxyphenyl)-2-(2-((3,4,5-trimethoxybenzyl)thio)acetyl)hydrazine-1-carbothioamide (5f)

White solid (74%); M.p. 98-100°C; FTIR (ATR), (cm^{-1}): 3524, 3130, 1660, 1589, 1505, 1239, 1125.

^{1}H NMR (400 MHz, DMSO-d_6): δ 10.03 (s, 1H), 9.52 (s, 1H), 7.22 (d, 2H, *J* = 8.4 Hz), 6.86 (d, 2H, *J* = 8.4 Hz), 6.61 (s, 2H), 3.76 (s, 2H), 3.72 (s, 6H), 3.71 (s, 3H), 3.60 (s, 3H), 3.12 (s, 2H).

^{13}C NMR (100 MHz, DMSO-d_6): δ 181.7, 169.1, 157.3, 153.3, 136.9, 134.0, 132.4, 127.8, 113.9, 106.8, 60.5, 56.3, 55.7, 36.4, 33.1.

HRMS (ESI): *m/z* 452.1320 $[M + H]^+$; calcd. for $C_{20}H_{26}N_3O_5S_2$, 452.1314.

2-(2-((3,4,5-trimethoxybenzyl)thio)acetyl)-N-(3,4,5-trimethoxyphenyl)hydrazine-1-carbothioamide (5g)

Colorless oil (75%); FTIR (ATR), (cm^{-1}): 3262, 1675, 1592, 1504, 1229, 1118.

^{1}H NMR (400 MHz, $CDCl_3$): δ 9.57 (s, 1H), 9.10 (s, 1H), 8.40 (s, 1H), 6.67 (s, 2H), 6.56 (s, 2H), 3.88 (s, 2H). 3.81 (s, 3H), 3.79 (s, 9H), 3.77 (s, 6H), 3.23 (s, 2H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 179.4, 167.2, 153.7, 153.4, 137.3, 136.7, 132.3, 132.0, 106.2, 102.5, 61.0, 60.9, 56.34, 56.2, 37.4, 33.4.

HRMS (ESI): *m/z* 512.1528 $[M + H]^+$; calcd. for $C_{22}H_{30}N_3O_7S_2$, 512.1525.

N-(4-fluorophenyl)-2-(2-((3,4,5-trimethoxybenzyl)thio)acetyl)hydrazine-1-carbothioamide (5h)

White solid (66%); M.p. 97-100°C; FTIR (ATR), (cm^{-1}): 3566, 3303, 3141, 1660, 1594, 1508, 1235, 1123.

^{1}H NMR (400 MHz, $CDCl_3$): δ 9.74 (s, 1H), 9.28 (s, 1H), 8.52 (s, 1H), 7.38 (dd, 2H, *J* = 8.4, 4.8 Hz), 7.05 (t, 2H, *J* = 8.4 Hz), 6.55 (s, 2H), 3.84 (s, 2H), 3.80 (s, 9H), 3.23 (s, 2H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 179.1, 166.2, 161.1 (d, *J* = 246.3 Hz), 153.4, 137.3, 133.0, 132.0, 126.9 (d, *J* = 7.7 Hz), 116.3 (d, *J* = 22.1 Hz), 106.2, 61.0, 56.3, 37.5, 33.5.

HRMS (ESI): *m/z* 440.1117 $[M+H]^+$; calcd. for $C_{19}H_{23}FN_3O_4S_2$, 440.1114.

N-(4-(trifluoromethyl)phenyl)-2-(2-((3,4,5-trimethoxybenzyl)thio)acetyl)hydrazine-1-carbothioamide (5i)

White solid (55%); M.p. 134-135°C; FTIR (ATR), (cm^{-1}): 3364, 3170, 1675, 1593, 1496, 1325, 1099.

^1H NMR (400 MHz, $CDCl_3$): δ 10.05 (s, 1H), 9.97 (s, 1H), 9.11 (s, 1H), 7.67 (d, 2H, *J* = 8.8 Hz), 7.56 (d, 2H, *J* = 8.8 Hz), 6.56 (s, 2H), 3.84 (s, 2H), 3.80 (s, 6H), 3.79 (s, 3H), 3.30 (s, 2H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 177.5, 165.7, 153.4, 141.1, 137.3, 132.0, 127.4 (q, ^2J = 32.6 Hz), 126.2 (q, ^3J = 3.8 Hz), 124.0 (q, ^1J = 270.2), 123.0, 106.2, 61.0, 56.3, 37.7, 33.8.

HRMS (ESI): *m/z* 490.1086 $[M + H]^+$; calcd. for $C_{20}H_{23}F_3N_3O_4S_2$, 490.1082.

N-(4-(trifluoromethoxy)phenyl)-2-(2-((3,4,5-trimethoxybenzyl)thio)acetyl)hydrazine-1-carbothioamide (5j)

Yellow solid (68%); M.p. 116-118°C; FTIR (ATR), (cm^{-1}): 3154, 1695, 1591, 1507, 1200, 1121.

^1H NMR (400 MHz, DMSO-d_6): δ 10.11 (s, 1H), 9.78 (s, 1H), 9.67 (s, 1H), 7.52 (d, 2H, *J* = 8.8 Hz), 7.30 (d, 2H, *J* = 8.8 Hz), 6.62 (s, 2H), 3.77 (s, 2H), 3.73 (s, 6H), 3.60 (s, 3H), 3.14 (s, 2H).

^{13}C NMR (100 MHz, DMSO-d_6): δ 181.5, 169.3, 153.3, 145.7, 138.9, 136.9, 134.0, 128.0, 121.4, 120.6 (q, *J* = 253.9 Hz), 106.8, 60.5, 56.3, 36.4, 33.1.

HRMS (ESI): *m/z* 506.1039 $[M + H]^+$; calcd. for $C_{20}H_{23}F_3N_3O_5S_2$, 506.1031.

N-(4-nitrophenyl)-2-(2-((3,4,5-trimethoxybenzyl)thio)acetyl)hydrazine-1-carbothioamide (5k)

Yellow solid (78%); M.p. 156-158°C; FTIR (ATR), (cm^{-1}): 3190, 3114, 1659, 1574, 1505, 1322, 1113.

^{1}H NMR (400 MHz, DMSO-d_6): δ 10.16 (s, 1H), 10.07 (s, 1H), 9.89 (s, 1H), 8.18 (d, 2H, J = 8.4 Hz), 7.85 (d, 2H, J = 8.4 Hz), 6.62 (s, 2H), 3.77 (s, 2H), 3.72 (s, 6H), 3.60 (s, 3H), 3.15 (s, 2H).

^{13}C NMR (100 MHz, DMSO-d_6): δ 181.1, 169.5, 153.3, 146.1, 144.0, 136.9, 133.9, 125.1, 124.3, 106.8, 60.5, 56.4, 36.4, 33.0.

HRMS (ESI): *m/z* 467.1053 $[M + H]^+$; calcd. for $C_{19}H_{23}N_4O_6S_2$, 467.1059.

N-benzyl-2-(2-((3,4,5-trimethoxybenzyl)thio)acetyl)hydrazine-1-carbothioamide (7)

White solid (81%); M.p. 152-153°C; FTIR (ATR), (cm^{-1}): 3346, 3295, 3129, 1672, 1588, 1457, 1231, 1129.

^{1}H NMR (400 MHz, DMSO-d_6): δ 9.92 (s, 1H), 9.39 (s, 1H), 8.44 (s, 1H), 7.23 (m, 5H), 6.60 (s, 2H), 4.68 (d, 2H, J = 6.0 Hz), 3.73 (s, 2H), 3.71 (s, 6H), 3.59 (s, 3H), 3.07 (s, 2H).

^{13}C NMR (100 MHz, DMSO-d_6): δ 182.5, 169.2, 153.2, 139.7, 136.8, 134.0, 128.6, 127.5, 127.2, 106.7, 60.5, 56.3, 47.2, 36.3, 32.9.

HRMS (ESI): *m/z* 436.1371 $[M + H]^+$; calcd. for $C_{20}H_{26}N_3O_4S_2$, 436.1365.

N-(2-(2-((3,4,5-trimethoxybenzyl)thio)acetyl)hydrazine-1-carbonothioyl)benzamide (8)

White solid (62%); M.p. 178-181°C; FTIR (ATR), (cm^{-1}): 3232, 1662, 1644, 1592, 1435, 1236, 1124.

^{1}H NMR (400 MHz, DMSO-d_6): δ 12.69 (s, 1H), 11.74 (s, 1H), 10.99 (s, 1H), 7.92 (d, 2H, *J* = 7.6 Hz), 7.62 (t, 1H, *J* = 7.6 Hz,), 7.49 (t, 2H, *J* = 7.6 Hz), 6.63 (s, 2H), 3.80 (s, 2H), 3.73 (s, 6H), 3.59 (s, 3H), 3.27 (s, 2H).

^{13}C NMR (100 MHz, DMSO-d_6): δ 177.0, 168.6, 166.5, 153.3, 136.9, 134.0, 133.7, 132.3, 129.3, 129.0, 106.8, 60.5, 56.3, 36.6, 32.5.

HRMS (ESI): *m/z* 472.0969 $[M + Na]^+$; calcd. for $C_{20}H_{23}N_3NaO_5S_2$, 472.0977.

Synthesis of 4-phenyl-5-(((3,4,5-trimethoxybenzyl)thio)methyl)-4H-1,2,4-triazole-3-thiol (9)

A mixture of compound **5a** (120 mg, 0.300 mmol) and 2M NaOH (2.00 mL) was heated in a microwave reactor (300W, 110°C) for 45 minutes. After cooling, the mixture was diluted with water (10 mL) and acidified with 10% aqueous HCl. The resulting precipitate was filtered and recrystallized from a mixture of acetonitrile and water to give compound **9** as an off-white solid (47.2 mg, 41.0%).

M.p. 181-185°C; FTIR (ATR), (cm^{-1}): 1591, 1505, 1418, 1244, 1120.

^{1}H NMR (400 MHz, DMSO-d_6): δ 7.49 (m, 3H), 7.34 (d, 2H, *J* = 8.4 Hz), 6.47 (s, 2H), 3.70 (s, 6H), 3.60 (s, 3H), 3.55 (s, 2H), 3.49 (s, 2H).

^{13}C NMR (100 MHz, DMSO-d_6): δ 168.7, 153.2, 150.0, 136.9, 134.0, 133.5, 130.0, 129.8, 128.8, 106.7, 60.5, 56.3, 36.0, 25.4.

HRMS (ESI): *m/z* 404.1113 $[M + H]^+$; calcd. for $C_{19}H_{22}N_3O_3S_2$, 404.1103.

DPPH free radical scavenging assay

Solutions of 3,4,5-trimethoxyphenyl-thiosemicarbazide derivatives (**5a-5k** and **7-9**) and the positive controls ascorbic acid and Trolox were prepared in methanol at concentrations

ranging from 5.00×10^3-1.95×10^1 μM. Test solutions (5.00 μL) were added to 96 well plates, followed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical solution (203 μM in methanol, 245 μL). The plates were placed in a dark cupboard for 30 minutes, followed by measuring the absorbance at 515 nm using a BioRad iMark microplate reader. For the control sample, methanol (5.00 μL) was used instead of the test samples. To correct for background absorbance, blank samples were prepared by using 250 μL of methanol. All experiments were carried out in triplicate.

Prior to determining IC_{50} values, the percent scavenging activity at each concentration was calculated using the following equation:

$$\%\text{Scavenging activity} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

$A_{control}$ = average absorbance of DPPH solution without sample or standard; A_{sample} = average absorbance of test sample after 30 min. Data are expressed as the mean value ± standard deviation (S.D.).

CUPRAC (CUPric Reducing Antioxidant Capacity) assay

Test solutions for the CUPRAC assay were prepared as follows: 7 tubes each containing 1 mL each of nanopure H_2O, 7.5 mM neocuproine in 95% ethanol, 1 M aqueous NH_4Ac and 10 mM aqueous $CuCl_2$ solutions. Additional reagents (100 μL total) were placed in the tubes as follows, using 5 mM stock solutions of test samples (TS), prepared in absolute ethanol: Tube 1, Blank (100 μL H_2O); Tube 2 (5 μL TS, 95 μL H_2O); Tube 3 (10 μL TS, 90 μL H_2O); Tube 4 (15 μL TS, 85 μL H_2O); Tube 5 (20 μL TS, 80 μL H_2O); Tube 6 (25 μL TS, 75 μL H_2O); Tube 7 (30 μL TS, 70 μL H_2O). Final concentrations of test samples ranged from 6.10 to 36.6 μM. Trolox and ascorbic

acid were used as positive controls, and prepared similarly. The samples were vortexed for 30 seconds, covered and allowed to sit at room temperature for 30 minutes, prior to measuring the absorbance at 450 nm (Carey UV-Vis spectrometer). The measurements were performed in triplicate for each concentration, and average absorbance values were used to generate calibration plots. The Trolox Equivalent Antioxidant Capacity (TEAC) coefficient for this assay was determined by relating the molar absorptivity, ε (obtained from the slopes of the calibration plots), of the test samples to that of Trolox as follows: ε Test samples/ε Trolox.

Antiglycation assay in BSA-methylglyoxal model

Stock solutions of bovine serum albumin (BSA; 25 mg/mL) and MGO (100 mM) were prepared in phosphate buffer solution (0.1 M; pH 7.4) and test samples (100 μM) were co-incubated with the BSA-MGO solution at 37 °C for 2 weeks. Aminoguanidine (AG; at 100 μM) served as a positive control. After incubation, the formation of MGO-induced AGEs was measured using a Spectra Max M2 spectrometer (Molecular Devices, Sunnyvale, CA, USA) at excitation and emission wavelengths of 340 nm and 435 nm, respectively. The inhibition level was calculated using the following equation: % inhibition = [1- (fluorescence intensity of solution with treatment/fluorescence intensity of control solution)] x 100%.

DNA cleavage assays

DNA cleavage activity of the sulfide-linked 3,4,5-trimethoxyphenyl-thiosemicarbazide/triazole derivatives was determined by gel electrophoresis using double-stranded circular plasmid DNA, pBR322. The assay medium (20.0 μL) consisted of DNA (250 ng, 1.00 μL), copper(II) acetate (10.0 mM, 2.00 μL), test samples (10.0 mM in DMF, 2.00 μL)

and pH 7.2 Tris-HCl buffer (25.0 mM, 15.0 μL). The DNA control contained DNA only with buffer (19.0 μL), while the DNA-copper(II) acetate control had DNA, copper(II) acetate, and buffer (17.0 μL). For the ROS scavenging experiments, the assay buffer contained 1 μL of scavenger/quencher (10 mM) and 4 μL of copper(II) acetate instead of 2 μL. The samples were incubated at 37˚C for 90 minutes, followed by the addition of 1X DNA loading dye (5.00 μL). Electrophoresis of the samples was performed on 1% agarose for 90 minutes at 90V in pH 8 TBE buffer. Gels were stained with GelRed® nucleic acid stain (3X, water) for 15 minutes and visualized at 302 nm using a Western blot imager (Azure Biosystems). Each experiment was performed at least two times.

DNA Molecular Docking Studies

The X-ray crystal structure of B-DNA (PDB ID: 1BNA) dodecamer d(CGCGAATTCGCG)$_2$ was obtained from the Protein Data Bank (http://www.rcsb.org/pdb). In order to prepare the DNA for docking, polar hydrogens were added and Kollman charges were assigned. Avogadro software was used to generate the structures of the sulfide-linked 3,4,5-trimethoxyphenyl-thiosemicarbazide/triazole derivatives, which were subjected to energy optimization using a steepest-descent algorithm. Open Babel software was used to add polar hydrogens and convert pdb files to pdbqt. Docking analyses were carried out using AutoDock Tools version 1.5.6 and AutoDock Vina programs.[26] The grid box included the entire B DNA structure, and nine conformational images were created for each ligand. The output files for the lowest energy conformers were exported to PyMol for display of the 3D structure of the DNA-ligand complexes. 2D interaction diagrams were generated using ProteinsPlus server.

References

1. A.M. Pisoschi, A. Pop, F. Iordache, L. Stanca, G. Predoi, A.I. Serban. Oxidative stress mitigation by antioxidants - An overview on their chemistry and influences on health status. Eur. J. Med. Chem. 209 (2021) 112891.
2. B.L. Tan, M.E. Norhaizan, W-P-P. Liew, H.S. Rahman. Antioxidant and oxidative stress: A mutual interplay in age-related diseases. Front. Pharmacol. 9 (2018) 1162.
3. J. Chen, J. Yang, L. Ma, J. Li, N. Shahzad, C.K. Kim. Structure-antioxidant activity relationship of methoxy, phenolic hydroxyl, and carboxylic acid groups of phenolic acids. Sci. Rep. 10 (2020) 2611.
4. P. Szabados-Furjesi, D. Pajtas, A. Barta, E. Csepanyi, A. Kiss-Szikszai, A. Tosaki, I. Bak. Synthesis, in vitro biological evaluation, and oxidative transformation of new flavonol derivatives: The possible role of the phenyl-N,N-dimethylamino group. Molecules. 23 (2018) 3161.
5. H.S. Kareem, N. Nordin, T. Heidelberg, A. Abdul-Aziz, A. Ariffin. Conjugated oligo-aromatic compounds bearing a 3,4,5-trimethoxy moiety: Investigation of their antioxidant activity correlated with a DFT study. Molecules. 21 (2016) 224.
6. H.S. Kareem, A. Ariffin, N. Nordin, T. Heidelberg, A. Abdul-Aziz, K.W. Kong, W.A. Yehye. Correlation of antioxidant activities with theoretical studies for new hydrazone compounds bearing a 3,4,5-trimethoxy benzyl moiety. Eur. J. Med. Chem. 103 (2015) 497-505.
7. H.I. Narkhede, A.S. Dhake, A.R. Surana. Synthesis and screening of thiosemicarbazide-dithiocarbamate conjugates for antioxidant and anticancer activities. Bioorg. Chem. 124 (2022) 105832.

8. P.T. Acharya, Z.A. Bhavsar, D.J. Jethava, D.B. Patel, H.D. Patel. A review on development of bio-active thiosemicarbazide derivatives: Recent advances. J. Mol. Struct. 1226 (2021) 12926.
9. Y. Sicak. Design and antiproliferative and antioxidant activities of furan-based thiosemicarbazides and 1,2,4-triazoles: their structure-activity relationship and SwissADME predictions. Med. Chem. Res. 30 (2021) 1557-1568.
10. S. Sazeli, A.R. Nath, M.H. Ahmad, N.W.M. Zulkifli, M.R. Johan, W.A. Yehye, L.H. Voon. Semicarbazide and thiosemicarbazide containing butylated hydroxytoluene moiety: new potential antioxidant additives for synthetic lubricating oil. RSC Adv. 11 (2021) 7138.
11. I.V. Smolyaninov, D.A. Burmistrova, M.V. Arsenyev, M.A. Polovinkina, N.P. Pomortseva, G.K. Fukin, A.I. Poddel'sky, N.T. Berberova. Synthesis and antioxidant activity of new catechol thioethers with the methylene linker. Molecules. 27 (2022) 3169.
12. M.H. Havasi, A.J. Ressler, E.L. Parks, A.H. Cocolas, A. Weaver, N.P. Seeram, G. E. Henry. Antioxidant and tyrosinase docking studies of heterocyclic sulfide derivatives containing a thymol moiety. Inorganica Chim. Acta. 505 (2020) 119495.
13. A. Cocolas, E. Parks, A. Ressler, M. Havasi, N.P. Seeram, G.E. Henry. Heterocyclic β-keto sulfide derivatives of carvacrol: synthesis and copper(II) ion reducing capacity. Bioorg. Med. Chem. Lett. 29 (2019) 126636.
14. N. Nazarbahjat, N. Nordin, Z. Abdullah, M.A. Abdulla, W.A. Yehye, S.N.A. Halim, C.H. Kee, A. Ariffin. New thiosemicarbazides and 1,2,4-triazolethiones derived from 2-(ethylsulfanyl) benzohydrazide as potent antioxidants. Molecules. 19 (2014) 11520-11537.

15. S.B. Rossington, J.A. Hadfield, S.D. Shnyder, T.W. Wallace, K.J. Williams. Tubulin-binding dibenz[c,e]oxepines: Part 2.1 Structural variation and biological evaluation as tumor vasculature disrupting agents. Bioorg. Med. Chem. 25 (2017) 1630-1642.
16. M. Özyürek, K. Güclü, E. Tütem, K.S. Başkan, E. Erçağ, S.E. Çelik, S. Baki, L. Yildiz, S. Karaman, R. Apak. A comprehensive review of CUPRAC methodology. Anal. Methods. 3 (2011) 2439-2453.
17. W-J. Yeh, S-M. Hsia, W-H. Lee, C-H. Wu. Polyphenols with antiglycation activity and mechanisms of action: A review of recent findings. J. Food Drug Anal. 25 (2017) 84-92.
18. H. Ma, S.L. Johnson, W. Liu, N.A. DaSilva, S. Meschwitz, J.A. Dain, N.P. Seeram. Evaluation of polyphenol anthocyanin-enriched extracts of blackberry, black Raspberry, blueberry, cranberry, red raspberry, and strawberry for free radical scavenging, reactive carbonyl species trapping, anti-glycation, anti-β-amyloid aggregation, and microglial neuroprotective effects. Int. J. Mol. Sci. 19 (2018) 461.
19. A. Imran, M.T. Shehzad, S.J.A. Shah, M. Laws, T. Al-Adhami, K.M. Rahman, I.A. Khan, Z. Shafiq, J. Iqbal. Development, molecular docking, and in silico ADME evaluation of selective ALR2 inhibitors for the treatment of diabetic complications via suppression of the polyol pathway. ACS Omega. 7 (2022) 26425-26436.
20. C.S. Shantharam, D.M.S. Vardhan, R. Suhas, M.B. Sridhara, D.C. Gowda. Inhibition of protein glycation by urea and thiourea derivatives of glycine/proline conjugated benzisoxazole analogue - Synthesis and structure-activity studies. Eur. J. Med. Chem. 60 (2013) 325-332.

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

22. X. Li, H. Wang, Z. Lu, X. Zheng, W. Ni, J. Zhu, Y. Fu, F. Lian, N. Zhang, J. Li, H. Zhang, F. Mao. Development of multifunctional pyrimidinylthiourea derivatives as potential anti-Alzheimer agents. J. Med. Chem. 59 (2016) 8326-8344.

23. S.U. Rehman, H. Zubair, T. Sarwar, M.A. Husain, H.M. Ishqi, S. Nehar, M. Tabish. Redox cycling of Cu(II) by 6-mercaptopurine leads to ROS generation and DNA breakage: possible mechanism of anticancer activity. Tumour Biol. 36 (2015) 1237-44.

24. S. Khan, A.M. Malla, A. Zafar, I. Naseem. Synthesis of novel coumarin nucleus-based DPA drug-like molecular entity: In vitro DNA/Cu(II) binding, DNA cleavage and pro-oxidant mechanism for anticancer action. PLOS ONE. 12 (2017) e0181783.

25. S.M.A. Monro, K.M. Cottreau, C. Spencer, J.R. Wentzell, C.L. Graham, C.N. Borissow, D.L. Jakeman, S.A. McFarland. Copper-mediated nuclease activity of jadomycin B. Bioorg. Med. Chem. 19 (2011) 3357-3360.

26. O. Trott, A.J. Olson. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. J. Comput. Chem. 31 (2010) 455-461.