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α -Amination and the 5-exo-trig cyclization reaction of sulfur-containing Schiff bases with *N*-phenyltriazolinedione and their anti-lipid peroxidation activity

Emel Pelit ^{a, *}, Kosmas Oikonomou ^b, Melek Gul ^c, Dimitra Georgiou ^b,
 Slawomir Szafert ^d, Sotirios Katsamakos ^e, Dimitra Hadjipavlou-Litina ^e,
 Yiannis Elemen ^b

^a Department of Chemistry, Faculty of Arts and Sciences, Kirklareli University, Kirklareli, Turkey

^b Department of Chemistry, University of Ioannina, 45110 Ioannina, Greece

^c Department of Chemistry, Faculty of Arts and Sciences, Amasya University, Amasya, Turkey

^d Department of Chemistry, University of Wrocław, 14 F. Joliot-Curie, 50-383 Wrocław, Poland

^e Department of Pharmaceutical Chemistry, School of Pharmacy, Faculty of Health Sciences, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece

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This work is dedicated to the memory of
 Professor Yiannis Elemen.

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ABSTRACT

Triazolinedione quenches efficiently the 1,2-dipoles from Schiff bases of glycine esters which are formed via a [1,2-*H*]-prototropic shift of their α -hydrogens, and affords the respective α -aminated products in good yields. Competition experiments show a stabilization of the 1,2-dipole from the sulfide substituent. 5-exo-trig cyclization of *N*-PhTAD with Schiff bases of other amino acids gave triazolines. The antioxidative and lipoxygenase inhibitory activities of the novel synthesized compounds were studied.

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1. Introduction

There are few reports on additions of amino acid derivatives to azodicarboxylates to afford 1,2,4-triazolines or their saturated precursors, 1,2,4-triazolidines. Thus, oxidation of *N*-acyl substituted amino acid esters under the Mitsunobu reaction conditions has been reported to proceed through an enolate addition to diisopropylazodicarboxylate (DIAD), at the crucial step. The resulted

intermediate upon cyclization leads to the final 1,2,4-triazolidines and 1,2,4-triazolines [1]. 1,2,4-Triazolines are also formed during the phosphine mediated annulations of *N*-protected imines with diethyl azodicarboxylate (DEAD) [2]. In the presence of a base, the 1,3-dipolar cycloaddition of glycine ester aldimines leads to 1,2,4-triazolines through dehydrogenation of the non-isolated 1,2,4-triazolidine primary adducts [3]. Aldimines of phenylglycine esters [4] and benzylamine [5] react in a 1,3-dipolar cycloaddition manner with DEAD and afford 1,2,4-triazolidines. 1,2,4-Triazolines are formed via a retro-1,3-dipolar cycloaddition of imidazolines in the presence of DEAD [6]. Finally, the nonstabilized azomethine ylides generated from the

* Corresponding author.

E-mail address: epelit@klu.edu.tr (E. Pelit).

desilylation of *N*-[(trimethylsilylmethyl]iminium triflates undergo, in the presence of CsF, a 1,3-dipolar cycloaddition reaction and afford 1,2,4-triazolidines and 1,2,4-triazolines [7]. *N*-substituted amino acids react in a decarboxylative 1,3-dipolar cycloaddition with DEAD to afford 4*N*-substituted-1,2,4-triazolidines [8].

The 1,2,4-triazoline and 1,2,4-triazole moiety is found in biologically active compounds with various activities such as antimicrobial, antifungal, antitubercular, anti-HIV, anti-inflammatory, and antioxidant activities [9]. Therefore there is a continuous need for new derivatives having structural differences in terms of substitution patterns that might affect the biological activity.

We have reported recently on the 1,3-dipolar cycloaddition of sulfide containing imines of glycine imines to *N*-phenylmaleimide [10] and fullerene-[60], C₆₀ [11], and the antioxidant activity for some of the cycloadducts. Encouraged by the above results and having an interest in the chemistry and synthetic applications of *N*-phenyltriazolinedione (NPhTAD) [12], a *cis*-locked cyclic diazene [13], we decided to test it as a dipolarophile in a dipolar cycloaddition reaction with the above imines of glycine esters. Triazolinediones [14] are very reactive electrophiles that are used in ene [15], Diels–Alder [16], aromatization [17], polymer modification [18], and initiation of radical addition [19] reactions, together with their extensive use in the synthesis of polyureas [20]. In addition, the urazole ring of PhTAD in the addition products can be transformed to an azo or amino group [21].

Lipoxygenases are a family of non-heme iron-containing dioxygenases [22]. LOXs are connected with several inflammation related diseases such as allergic asthma, cancer, cardiovascular, and skin diseases [23]. There is also increasing interest in antioxidants due to prevention of harmful effects of free radicals in human body [24]. Therefore in an attempt to extend our biological interest to this new group of compounds, we decided to test most of them for their anti-lipid peroxidation activity and as possible lipoxygenase inhibitors.

2. Results and discussion

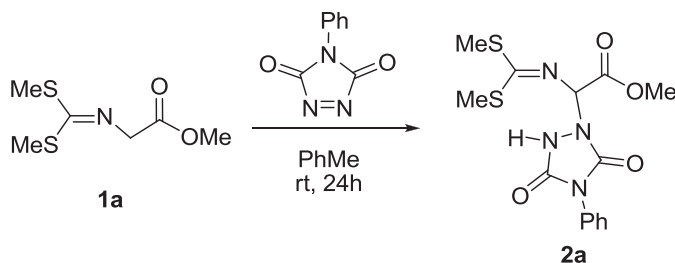
Having in mind the possible synthesis of sulfide substituted triazolidines or triazolines, we were surprised to find that imine of glycine ester **1a**, react with PhTAD at room temperature in PhMe to afford α -amination adduct **2a** after 24hr, Scheme 1, albeit in a very low yield, ~3%.

α -Aminations of a variety of compounds with azodicarboxylates are known in the literature, mainly in their

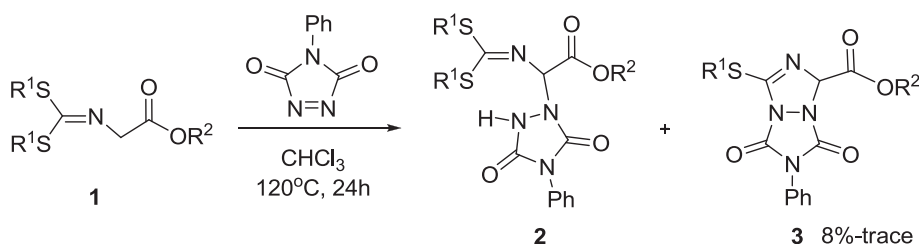
catalytic and asymmetric versions, due to the importance of the stereoselective synthesis of aminated compounds. Carbonyl compounds are a major class of substrates for α -amination [25], and so asymmetric aminations of aldehydes [26], ketones [27], keto- [28] and cyano-esters [29] have been reported. Recently, a nice work of a AgOAc catalyzed asymmetric amination of glycine Schiff bases with azodicarboxylates was reported to afford enantio-merically enriched α -aminated products [30]. The stereoselectivity was induced by an asymmetric ligand present in the reaction. AgOAc/Taniaphos complex was acting as the catalyst and conjugated to the imine. Acetate anion was liberated and acted as a base for H _{α} abstraction. The resulted metalated azomethine ylide dipole then underwent electrophilic addition to azodicarboxylate. To our knowledge, this is the only example of α -amination of amino acid Schiff bases and for this reason, we continued the work with the initial finding of the synthesis of α -aminated glycine Schiff base **2a**.

At first, we tried to find the optimal reaction conditions. The yield of **2a** remained low (~6%) even at elevated reaction temperatures (up to 180 °C) with toluene as the solvent. We examined PhCH₃, THF, CH₂Cl₂, and CHCl₃ as solvents under neutral, acidic (10% CH₃COOH) and basic (10% NEt₃) conditions at different temperatures. Finally it was found that the product yields were improved substantially when the reaction was performed in CH₂Cl₂, at 100 °C, or in CHCl₃ at 120 °C (reaction in a screw capped tube) under neutral conditions for 24 h, with comparable yields. In general, the reaction was performed by placing the desired quantity of the imines of glycines, **1**, dissolved in dry CHCl₃ in a screw-capped test-tube flushed with Ar. To this solution, solid PhTAD (2 mol/equivalent with respect to the imine) was added in one portion. Then the solution was stirred at 120 °C and the reaction was stopped after 24 h (Scheme 2). After that period of time, in most of the cases, the initially red (from PhTAD) solution was decolorized or color-faded, and this was taken as evidence of total PhTAD consumption. Then the solvent was removed in the rotary evaporator and aminated products **2** were isolated by column chromatography on SiO₂ with EtOAc/hexanes mixtures as the eluant. Products and yields are listed in Table 1. Furthermore, cyclization products **3** were observed with low yields (8%-trace). The highest yield (8%) was observed with **3a**. Compound **3b** was observed with 4% yield. However, the other cyclization products were obtained with 2%-trace yields.

Products **2** were characterized by ¹H and ¹³C NMR, and FTIR spectroscopies and by HRMS spectrometry.



Scheme 1. α -Amination of imine **1a** by PhTAD.



Scheme 2. General reaction scheme for the synthesis of the α -aminated glycine ester Schiff bases **2**.

Table 1
Isolated yields of products **2**.^a

Entry	Adduct	R ¹	R ²	Yield % ^b
1	2a	Me	Me	61
2	2b	Et	Me	55
3	2c	Pr	Me	56
4	2d	^t Pr	Me	40
5	2e	Bu	Me	49
6	2f	^t Bu	Me	42
7	2g	^{sec} Bu	Me	46
8	2h	Bn	Me	15
9	2i	Me	Et	35
10	2j	Me	^t Bu	30
11	2k	Me	Bn	58

^a All the reactions were performed with 0.290 mmol of imine **1**, 0.725 mmol of PhTAD, in 5 mL of dry CHCl₃, at 120 °C for 24 h.

^b Isolated yields of products **2** after chromatographic purification.

Characteristic resonances to be mentioned from their ¹H NMR spectra are: a) the NH signal at ~8.30 ppm, as a single broad peak, b) the H_α hydrogen as a single peak at ~6.45 ppm, c) the non-equivalence of the two sulfide substituents, and d) the diastereotopicity of methylene hydrogens in the EtO- and BnO- groups (entries 9 and 11). The above mentioned non-equivalence of the sulfide substituents was evident also from the ¹³C NMR spectra and

the respective carbon atoms appeared as twin peaks for the *syn* and *anti* substituent. The methinic C_α appears at ~73 ppm, and the iminic carbon resonates at ~170 ppm.

In the case of **2a** and **3a**, crystals of adequate quality for X-ray diffraction analysis [31] were obtained from slow evaporation of CHCl₃/hexanes, 1:1 v/v, Figs. 1 and 2. All the spectroscopic data obtained agree with the structures.

To gain more information about the transformation, we performed intermolecular competition experiments using equimolar mixtures of two imines **1**, keeping the amount of PhTAD at the half of the stoichiometric molar ratio. Thus starting from a 1:1:0.5 M ratio of imine **1a**:imine **1b**:PhTAD (Scheme 3), and applying the same conditions mentioned above, we have calculated the molar ratio of the two resulted adducts **2a** and **2b**, from the ¹H NMR spectra of the crude reaction mixture, by integration of appropriate peaks. The molar ratio of the products reflects to the ratio of their formation rates. With the above competitions, we wished to reveal any steric influence that the sulfide substituent has on the rate of α -amination. For this reason, one imine was kept constant, **1a**, and gradually changed the size of the –SR alkyl chain of the competitor imine, **1b**. Competing couples used in this study and calculated molar ratios of the α -aminated products are shown in Table 2.

The results from Table 2 clearly show that there is a correlation between the size of the substituent and the rate

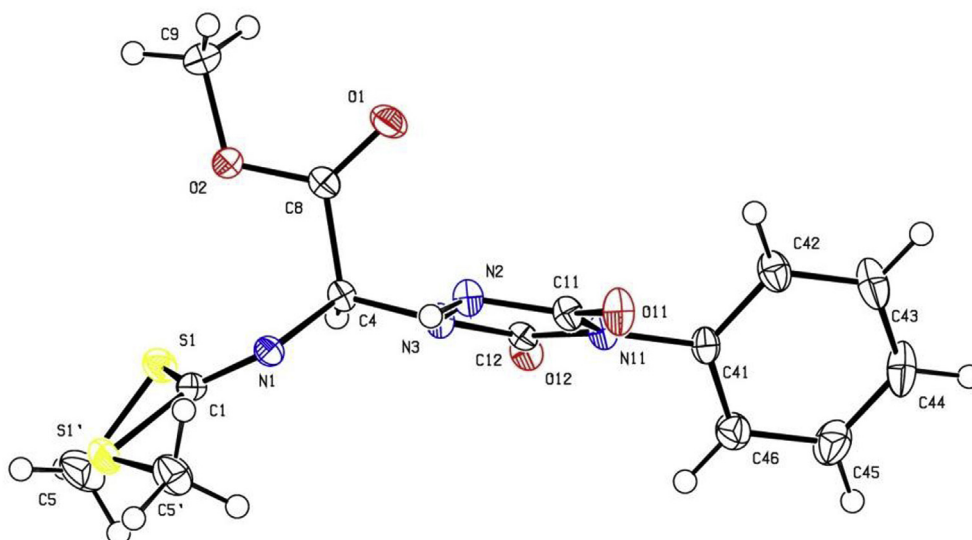


Fig. 1. X-ray structure of compound **2a**.

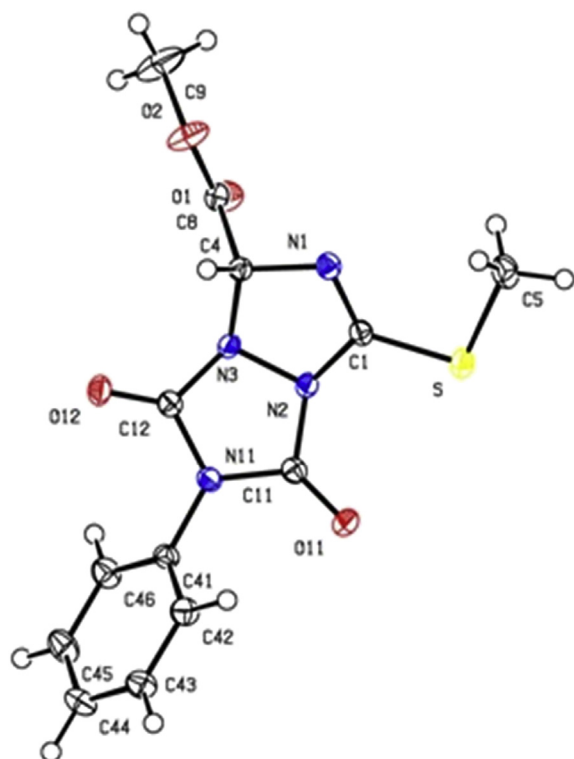
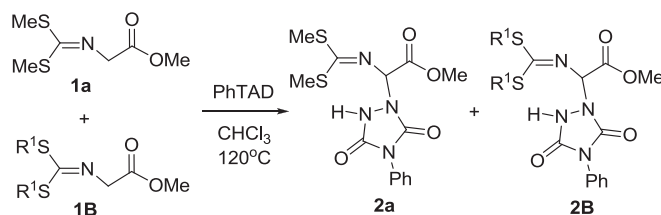


Fig. 2. X-ray structure of compound **3a**.

of the amination. The larger the size of the substituent, the faster the reaction. If a steric factor was in operation, then a bigger than unity molar ratio would be expected due to the steric hindrance during the approach of the two reactants (in benefit of the **1a** substrate).

To come up with a reliable explanation of the above observation, we turned our attention to the very first period of the 1,3-dipoles' evolution for 1,3-dipolar cycloadditions, with reports from the group of Grigg. 1,3-Azomethine dipole is an effective component for the 1,3-cycloaddition between an imine and an electrophile. A precursor 1,2-dipole needs to precede via a [1,2-*H*] prototropic shift of a H_α in the glycine imine, **1**. The more efficient formation of the two dipoles reflects to the more efficient 1,3-dipolar cycloaddition reaction. In our opinion, the differences in molar (and rate) ratios owe their existence to the different degrees of the dipoles' stabilization. Thus the larger the alkyl chain of the disulfide substituents inductively stabilizes, more the positive charge in the dipoles, Fig. 3,



Scheme 3. Intermolecular competition between imines **1** for α -amination by PhTAD.

Table 2

Calculated molar ratios of the two products [**2a**]/[**2B**], from **1a** vs **1B** intermolecular competition for their amination by PhTAD.^{a,b}

Entry	R ¹	[2a]/[2B]
1	Et	0.18
2	Pr	0.12
3	<i>i</i> -Pr	0.23
4	Bu	0.62
5	<i>i</i> -Bu	0.69
6	<i>sec</i> -Bu	0.14

^a All competitions were performed with **1a**:**1B**:PhTAD 1:1:0.5 M ratio.

^b Molar ratios were calculated from integrals of appropriate peaks from the ¹H NMR spectra of the crude reaction mixture.

together with the positive resonance effect of the sulfur atoms.

The intervention of the 1,2-dipole was further revealed by deuterium exchange of the methylenic H_α protons in **1**, heating a solution of imine **1a** (20 mg) in MeOD (0.5 mL) at 50 °C. The ¹H NMR spectra of an aliquot taken after 18 h showed ~45% di-deuterated imine **1a-d₂**, together with 55% mixture of **1a-d₀** and **1a-d₁** imines. The degree of deuteration increased after a second circle [removal of the MeOH(D) and dissolution of the partially deuterated imine **1a** in 0.5 mL of MeOD and stirring with heating at 50 °C for 16 h] to >80% in **1a-d₂** and <20% in **1a-d₁**. The deuterium exchange was almost complete (>95% in **1a-d₂**, and <5% in **1a-d₁**) after a third circle, in line with earlier observations in the exchange of a more acidic (methinic) H_α proton, of an imine of phenylglycine methyl ester [5].

Next, we made reactions between sulfur-containing Schiff bases of several different amino acid esters such as alanine, phenylalanine, leucine, tyrosine, aspartic acid, glutamic acid and N-PhTAD under the same conditions (Scheme 4, Table 3). In all cases, the main products observed were triazolone derivatives via the 5-exo-trig cyclization reaction, and α -amination products formed in very low yields (2%-trace). We have used TEMPO, DIAD, and DBAD as catalysts to get α -amination products encouraged in the literature [32] but we again observed triazolone derivatives as main products and the yields didn't change. Finally we made reactions with benzylamine derivatives's Schiff bases and N-PhTAD. In most cases, the triazolone derivatives were the only product observed. However, **6a** gave a mixture of triazolone and α -amination products with the yields 72% and 19%, respectively (Scheme 5, Table 4). Substrates **4** and **6** gave the thermal 5-exo-trig cyclization reaction which occurs due to the side-chain steric effects and this cyclization might happen via α -amination products (Scheme 6).

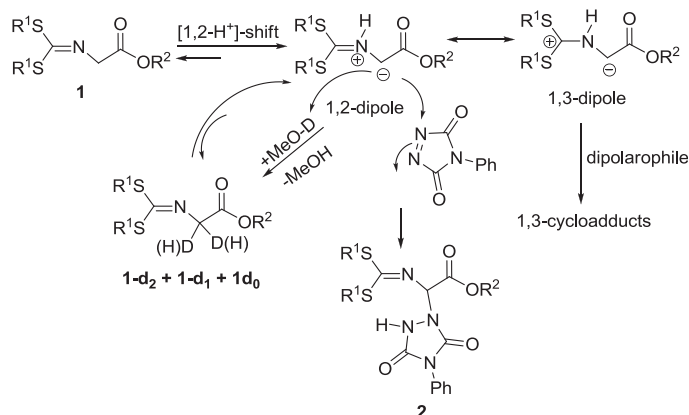
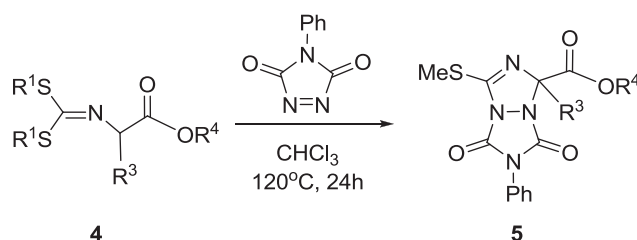


Fig. 3. Plausible mechanistic interpretation for the α -amination sequence through the quench of the 1,2-dipole by electrophiles such as PhTAD and D^+ .



Scheme 4. General reaction scheme for the synthesis of the triazolines **5**.

Table 3
Isolated yields of products **5**.^a

Entry	Adduct	R ³	R ⁴	Yield % ^b
1	5a	Methyl	Methyl	72
2	5b	2-Methoxy-2-oxoethyl	Methyl	68
3	5c	3-Methoxy-3-oxopropyl	Methyl	64
4	5d	1-Isobutyl	Methyl	69
5	5e	2-Methylthioethyl	Methyl	32
6	5f	Benzyl	Methyl	61
7	5g	4-Methoxybenzyl	Methyl	60
8	5h	Ethoxycarbonyl	Ethyl	61

^a All the reactions were performed with 0.290 mmol of imine **4**, 0.725 mmol of PhTAD, in 5 mL of dry $CHCl_3$, at 120 °C for 24 h.

^b Isolated yields of products **5** after chromatographic purification.

Products **5** and **7** were characterized by 1H and ^{13}C NMR, and FTIR spectroscopies and by HRMS spectrometry. Characteristic resonances to be mentioned from their 1H NMR spectra are: a) the OCH_3 as a single peak at ~ 3.85 – 3.60 ppm (for **5**), b) the sulfide substituent as a single peak at ~ 2.50 – 2.65 ppm (for **5** and **7**).

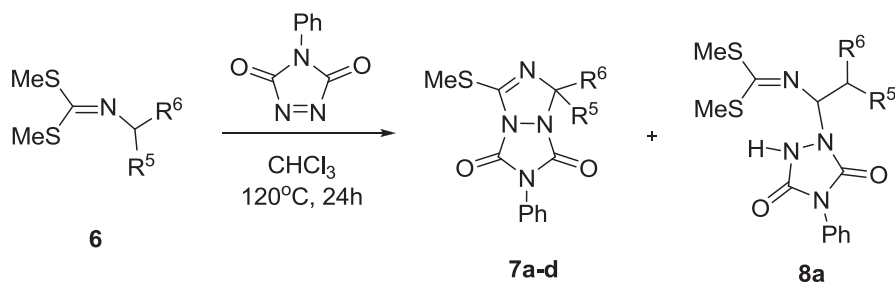
3. Biological studies

We tested the new cycloadducts with regard to their anti-lipid peroxidation ability and as soybean lipoxygenase inhibitors (LOX) in comparison to well known reference compounds, for example, nordihydroguaiaretic acid (NDGA), and trolox (Fig. 4).

The use of the free radical reaction initiator 2,2-azobis(2-aminopropane) dihydrochloride (AAPH) is recommended as more appropriate for measuring radical-

scavenging activity *in vitro*, and anti-lipid peroxidation ability. This is supported by the fact that the activity of the peroxy radicals produced by the action of AAPH shows a greater similarity to cellular activities such as lipid peroxidation [33]. Oxidation of sodium linoleate by a thermal free radical initiator (AAPH) is followed by UV spectrophotometry in a highly diluted sample [34]. In the AAPH assay, the highly reactive alkylperoxy radicals are intercepted mainly by a hydrogen atom transfer (HAT) from the antioxidant [35]. Therefore, particularly effective HAT agents are compounds with high hydrogen atom donating ability, that is, compounds with low heteroatom-H bond dissociation energies and/or compounds from which hydrogen abstraction leads to sterically hindered radicals as well as compounds from which abstraction of hydrogen leads to C-centered radicals stabilized by resonance. Compounds **2c**, **2h**, **7b**, **7c** and **7d** caused strong inhibition of lipid peroxidation (70–93%) and higher than trolox (65%). Compound **7d** with the highest lipophilicity ($\log P = 4.769$) is the most potent. The presence of at least one phenyl group (R^6) is correlated with higher antioxidant activity. Since these compounds lack the easily oxidizable phenol unit present in trolox, their inhibitory potency should be attributed to other factors. Perusal of Table 5 points to lower activity (29–54%) for the rest adducts whereas one (**5g**) was almost inactive (5%). This could be attributed to stereochemical reasons, due to the presence of bulky substituents in R^3 R^4 R^5 R^6 , especially for the products of series **2** and **5**.

Antioxidants are defined as substances that, even at low concentration, significantly delay or prevent oxidation of



Scheme 5. General reaction scheme for the synthesis of the triazolines **7a–d** and α -aminated Schiff base **8a**.

Table 4

Isolated yields of products **7a–d** and **8a**.^a

Entry	Adduct	R ⁵	R ⁶	Yield % ^b
1	7a	H	Ph	57
2	7b	H	4-F Ph	49
3	7c	Me	Ph	53
4	7d	Ph	Ph	53
5	8a	H	Ph	19

^a All the reactions were performed with 0.290 mmol of imine **6**, 0.725 mmol of PhTAD, in 5 mL of dry CHCl₃, at 120 °C for 24 h.

^b Isolated yields of products **7a–d** and **8a** after chromatographic purification.

easily oxidizable substrates. The formation of Reactive Oxygen Species (ROS) is a characteristic of aerobic organisms, as an unavoidable consequence of cell metabolism. lipoxygenase (LOX) catalyzes the first two steps in the metabolism of arachidonic acid, (which is cleaved from membrane phospholipids) to leukotrienes (LTB₄). LTB₄ generation is considered to be important in the pathogenesis of neutrophil-mediated inflammatory diseases [35] with a marked relation to the severity of cardiovascular diseases, asthma and cancer. In this context, we decided to further evaluate the synthesized cycloadducts as soybean LOX inhibitors by the UV absorbance based enzyme assay [36]. NDGA, a known inhibitor of soybean LOX, has been used as a reference compound (83% or 45 μM) and as a positive control. The compounds exhibited low or no inhibition under the reported conditions. Most potent was found to be compound **7b** followed by **2h**, **7d** and **2c**. Since the differences among **7b** and **2h** (as well as **7d**) adducts in terms of IC₅₀ are very small, their structural characteristics are quite different. Comparing **2c** and **2h**, it is obvious that higher lipophilicity is correlated with higher inhibition (clog *P* 4.98/IC₅₀ = 60 μM , clog *P* 4.27/IC₅₀ = 85.5 μM). It should be mentioned that most of the LOX inhibitors are antioxidants or free radical scavengers. Since these compounds present the highest antioxidant abilities, their lipoxygenase inhibition is proceeded in parallel through this mechanism.

4. Conclusion

In summary we presented here a simple procedure for the α -amination of glycine Schiff bases with PhTAD as the aminating component, under neutral conditions without the use of a catalyst, and 5-exo-trig cyclization reactions of several sulfur-containing imines with PhTAD under the same conditions. These products present interesting biological activities as inhibitors of lipid peroxidation and of lipoxygenase. Especially the **7b**, **7d** and **2h** derivatives highly inhibit lipid peroxidation and soybean LOX and they might serve as lead compounds.

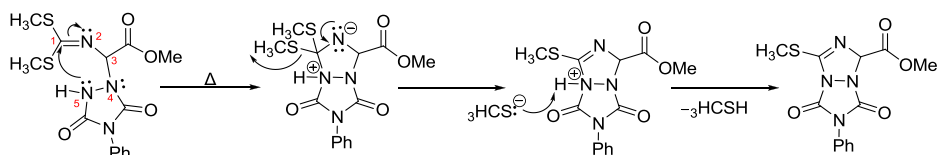
5. Experimental

5.1. General

All NMR spectra were taken in CDCl₃ 98% D, on a Bruker-Spectrospin, Avance spectrometer. ESI MS spectra were taken on an Agilent Technologies 1100 Series LC/MSD-Trap-SL spectrometer. HRMS were taken on an Orbitrap LTQ/XL instrument. FTIR spectra were taken on a Perkin Elmer Spectrum GX, FTIR System spectrometer. All reagents and solvents were obtained from commercial suppliers and used without further purification. Dry quality solvent was obtained according to literature procedures and stored under an Ar atmosphere. Compound **1** was prepared by known literature methods [37].

5.2. Typical procedure for preparation of the α -aminated Schiff bases and triazoline derivatives

A screw-capped 10-mL tube flashed with Ar. In it were placed 0.290 mmol sulfur substituted amino acid esters **1** or **4**, or sulfur substituted imines **6**, and 0.725 mmol N-Phenyltriazolinedione (N-PhTAD) in 5 mL of dry CHCl₃. This solution was stirred at 120 °C for 24 h. The tube was left to reach room temperature. Volatiles were removed in a rotary evaporator and the remaining material was then



Scheme 6. Plausible mechanism of 5-exo-trig reaction.

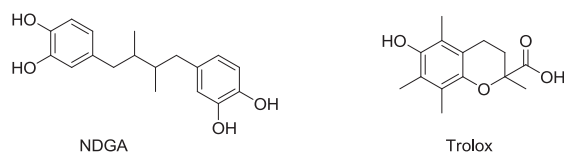


Fig. 4. The structure of the antioxidant compounds nordihydroguaiaretic acid (NDGA) and trolox.

chromatographed on a silica gel column with a mixture of EtOAc/*n*-hexane, 1/3, v/v, as eluant in order to separate pure products.

5.2.1. Methyl-2-(bis(methylthio)methyleneamino)-2-(3,5-dioxo-4-phenyl-1,2,4-triazolidin-1-yl)acetate (2a) $C_{14}H_{16}N_4O_4S_2$

Colorless powder, yield 61%, mp: 134–136 °C. FTIR (KBr), ν_{\max} : 3477, 3151, 3070, 3004, 2950, 2922, 1779, 1749, 1697, 1564, 1503, 148, 1285, 1252, 1138, 1075, 1048, 1024, 1005, 818, 767, 727, and 713 cm^{-1} . 1H NMR (250 MHz, $CDCl_3$) δ : 7.86 (br s, 1H), 7.50–7.44 (m, 5H), 6.42 (s, 1H), 3.81 (s, 3H), 2.62 (s, 3H), and 2.38 (s, 3H) ppm. ^{13}C NMR (62.5 MHz, $CDCl_3$) δ : 171.03, 166.59, 153.71, 153.19, 130.94, 129.13, 128.37, 125.64, 73.03, 53.40, 15.81, and 15.04 ppm. HRMS (ESI-Orbit trap) m/z : $[M+H]^+$ calcd for $C_{14}H_{16}N_4O_4S_2+H$, 369.1265; found 369.1265.

5.2.2. Methyl-2-(bis(ethylthio)methyleneamino)-2-(3,5-dioxo-4-phenyl-1,2,4-triazolidin-1-yl)acetate (2b) $C_{16}H_{20}N_4O_4S_2$

Colorless powder, yield 55%, mp: 122–124 °C. FTIR (KBr), ν_{\max} : 3464, 3151, 3055, 2972, 2935, 2870, 1781, 1734, 1709, 1600, 1562, 1504, 1431, 1340, 1277, 1233, 1138, 1077, 1026, 821, 768, 725, 709 cm^{-1} . 1H NMR (250 MHz, $CDCl_3$) δ : 8.32 (br s, 1H), 7.47–7.49 (m, 5H), 6.46 (s, 1H), 3.80 (s, 3H),

3.16 (qd, 2H, $J_1 = 7.25$ Hz, $J_2 = 5.50$ Hz), 2.98 (qd, 2H, $J_1 = 7.25$ Hz, $J_2 = 6.00$ Hz), 1.37 (t, 3H, $J = 7.38$ Hz), 1.19 (t, 3H, $J = 7.38$ Hz) ppm. ^{13}C NMR (62.5 MHz, $CDCl_3$) δ : 169.70, 166.60, 153.83, 153.32, 131.03, 129.13, 128.37, 125.68, 73.38, 53.37, 27.89, 26.28, 14.99, 13.61 ppm. HRMS (ESI-Orbit trap) m/z : $[M+H]^+$ calcd for $C_{16}H_{20}N_4O_4S_2+H$, 397.0999; found 397.0996.

5.2.3. Methyl-2-(bis(propylthio)methyleneamino)-2-(3,5-dioxo-4-phenyl-1,2,4-triazolidin-1-yl)acetate (2c)

$C_{18}H_{24}N_4O_4S_2$

Colorless powder, yield 56%, mp: 111–113 °C. FTIR (KBr), ν_{\max} : 3483, 3114, 2963, 2863, 1744, 1690, 1559, 1502, 1465, 1398, 1336, 1221, 1137, 1072, 1023, 1003, 815, 779, 707 cm^{-1} . 1H NMR (250 MHz, $CDCl_3$) δ : 7.87 (br s, 1H), 7.49–7.47 (m, 5H), 6.46 (s, 1H), 3.78 (s, 3H), 3.12 (td, 2H, $J_1 = 7.25$ Hz, $J_2 = 5.75$ Hz), 2.93 (td, 2H, $J_1 = 7.25$ Hz, $J_2 = 6.25$ Hz), 1.73 (sextet, 2H, $J = 7.25$ Hz), 1.53 (sextet, 2H, $J = 7.25$ Hz), 1.01 (t, 3H, $J = 7.25$ Hz), 0.88 (t, 3H, $J = 7.38$). ^{13}C NMR (62.5 MHz, $CDCl_3$) δ : 169.91, 166.54, 153.89, 153.35, 131.02, 129.06, 128.30, 125.65, 73.40, 53.30, 50.73, 35.38, 33.67, 23.27, 21.87, 13.23 ppm. HRMS (ESI-Orbit trap) m/z : $[M+H]^+$ calcd for $C_{18}H_{24}N_4O_4S_2+H$, 425.1312; found 425.1313.

5.2.4. Methyl-2-(bis(isopropylthio)methyleneamino)-2-(3,5-dioxo-4-phenyl-1,2,4-triazolidin-1-yl)acetate (2d)

$C_{18}H_{24}N_4O_4S_2$

Colorless powder, yield = 40%, mp: 108–110 °C. FTIR (KBr), ν_{\max} : 3467, 3165, 3078, 2969, 2927, 2866, 1781, 1738, 1713, 1600, 1556, 1504, 1434, 1385, 1367, 1236, 1139, 1078, 1056, 1025, 818, 773, 713 cm^{-1} . 1H NMR (250 MHz, $CDCl_3$) δ : 8.11 (br s, 1H), 7.48–7.38 (m, 5H), 6.53 (s, 1H), 3.85 (septet, 2H, $J = 6.75$ Hz), 3.80 (s, 3H), 1.38 (d, 6H, $J = 7.00$ Hz), 1.26 (d, 6H, $J = 7.00$ Hz). ^{13}C NMR (62.5 MHz, $CDCl_3$) δ : 169.43, 166.66, 153.83, 153.50, 131.01, 129.19, 128.40, 125.70, 73.83, 53.37, 39.62, 37.05, 24.58, 23.12, 22.32, 21.74 ppm. HRMS (ESI-Orbit trap) m/z : $[M+H]^+$ calcd for $C_{18}H_{24}N_4O_4S_2+H$, 425.1312; found 425.1318.

5.2.5. Methyl-2-(bis(butylthio)methyleneamino)-2-(3,5-dioxo-4-phenyl-1,2,4-triazolidin-1-yl)acetate (2e) $C_{20}H_{28}N_4O_4S_2$

Colorless powder, yield = 49%, mp: 100–102 °C. FTIR (KBr), ν_{\max} : 3468, 3151, 3076, 2957, 2928, 2871, 1781, 1760, 1708, 1569, 1495, 1427, 1276, 1234, 1181, 1138, 1080, 1069, 1027, 821, 778, 740, 716 cm^{-1} . 1H NMR (250 MHz, $CDCl_3$) δ : 8.30 (br s, 1H), 7.49–7.44 (m, 5H), 6.46 (s, 1H), 3.79 (s, 3H), 3.13 (td, 2H, $J_1 = 7.25$ Hz, $J_2 = 5.25$ Hz), 2.97 (td, 2H, $J_1 = 7.25$ Hz, $J_2 = 5.25$ Hz), 1.67 (quintet, 2H, $J = 7.25$ Hz), 1.49 (sextet, 2H, $J = 7.25$ Hz), 1.34 (sextet, 2H, $J = 7.25$ Hz), 0.93 (t, 3H, $J = 7.25$ Hz), 0.85 (t, 3H, $J = 7.25$ Hz). ^{13}C NMR (62.5 MHz, $CDCl_3$) δ : 170.07, 166.62, 153.79, 153.31, 131.05, 129.09, 128.30, 125.98, 125.61, 73.31, 53.31, 33.18, 31.75, 31.55, 30.47, 21.83, 21.78, 13.63, 13.48 ppm. HRMS (ESI-Orbit trap) m/z : $[M+H]^+$ calcd for $C_{20}H_{28}N_4O_4S_2+H$, 453.1625; found 453.1626.

5.2.6. Methyl-2-(bis(isobutylthio)methyleneamino)-2-(3,5-dioxo-4-phenyl-1,2,4-triazolidin-1-yl)acetate (2f) $C_{20}H_{28}N_4O_4S_2$

Colorless powder, yield = 42%, mp: 95–97 °C. FTIR (KBr), ν_{\max} : 3472, 3153, 3065, 2967, 2926, 2870, 1784, 1746, 1713, 1602, 1569, 1504, 1434, 1385, 1368, 1331, 1250, 1192, 1138,

Table 5
Biological evaluation of novel compounds as antioxidants.

Entry	Adduct	%LOX Inh. @100 μM^a / IC ₅₀ μM	AAPH% @100 μM^b	Clog P ^c
1	2a	No	37	2.15
2	2b	No	37	3.21
3	2c	85.5 μM (59%)	70	4.27
4	2d	No	31	3.83
5	2e	13%	29	5.33
6	2f	No	21	5.07
7	2g	4%	33	4.89
8	2h	60 μM (92%)	93	4.98
9	2i	8%	46	2.68
10	2j	16%	44	3.39
11	2k	20%	47	3.38
12	5a	7%	52	2.60
13	5b	24%	46	2.37
14	5c	No	41	2.089
15	5d	No	43	4.11
16	5f	33%	48	4.02
17	5g	10%	5	3.94
18	5h	26%	54	2.57
19	7a	88 μM	49	3.21
20	7b	57.5 μM (94%)	90	3.35
21	7c	17%	79	3.87
22	7d	65 μM (81%)	92	4.767
23	NDGA Trolox	45 μM (83%)	65	

^a *In vitro* % inhibition of soybean lipoxygenase (LOX).

^b % Inhibition of Lipid Peroxidation (AAPH).

^c Ref. 36.

1082, 1026, 822, 778, 717 cm^{-1} . ^1H NMR (250 MHz, CDCl_3) δ : 7.73 (br s, 1H), 7.49–7.47 (m, 5H), 6.51 (s, 1H), 3.81 (s, 3H), 3.10 (dd, 2H, $J_1 = 7.75$ Hz, $J_2 = 5.50$ Hz), 2.81 (dd, 2H, $J_1 = 7.00$ Hz, $J_2 = 6.25$ Hz), 1.90 (dt, 2H, $J_1 = 6.75$ Hz, $J_2 = 6.50$ Hz), 1.03 (dd, 6H, $J_1 = 4.75$ Hz, $J_2 = 1.75$ Hz), 0.96 (dd, 6H, $J_1 = 6.00$ Hz, $J_2 = 0.75$ Hz) ppm. ^{13}C NMR (62.5 MHz, CDCl_3) δ : 170.39, 166.77, 153.61, 153.41, 131.07, 129.17, 128.37, 125.57, 73.31, 53.36, 42.01, 40.12, 29.24, 28.18, 22.00, 21.86, 21.68 ppm. HRMS (ESI-Orbit trap) m/z : $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{20}\text{H}_{28}\text{N}_4\text{O}_4\text{S}_2+\text{H}$, 453.1625; found 453.1627.

5.2.7. *Methyl-2-(bis(sec-butylthio)methyleneamino)-2-(3,5-dioxo-4-phenyl-1,2,4-triazolidin-1-yl)acetate (2g)*

$\text{C}_{20}\text{H}_{28}\text{N}_4\text{O}_4\text{S}_2$

Colorless powder, yield = 46%, mp: 97–99 °C. FTIR (KBr), ν_{max} : 3441, 3100, 2965, 2915, 2878, 1782, 1749, 1713, 1598, 1560, 1503, 1428, 1380, 1332, 1222, 1170, 1133, 1056, 1026, 820, 772, 690 cm^{-1} . ^1H NMR (250 MHz, CDCl_3) δ : 7.75 (br s, 1H), 7.49–7.38 (m, 5H), 6.56 (4s, 1H, $J = 2.42$ Hz), 3.81 (s, 3H), 3.75–3.65 (m, 2H), 1.74–1.50 (m, 4H), 1.38 (dd, 3H, $J_1 = 4.00$ Hz, $J_2 = 2.75$ Hz), 1.29 (dd, 3H, $J_1 = 6.00$ Hz, $J_2 = 1.00$ Hz), 1.06–0.88 (m, 6H) ppm. ^{13}C NMR (62.5 MHz, CDCl_3) δ : 170.00, 166.82, 153.69, 153.67, 131.03, 129.20, 128.40, 125.65, 125.58, 73.75, 53.38, 46.35, 45.87, 43.32, 43.19, 29.77, 29.10, 20.59, 11.60, 11.41 ppm. HRMS (ESI-Orbit trap) m/z : $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{20}\text{H}_{28}\text{N}_4\text{O}_4\text{S}_2+\text{H}$, 453.1625; found 453.1629.

5.2.8. *Methyl-2-(bis(benzylthio)methyleneamino)-2-(3,5-dioxo-4-phenyl-1,2,4-triazolidin-1-yl)acetate (2h)*

$\text{C}_{26}\text{H}_{24}\text{N}_4\text{O}_4\text{S}_2$

Colorless powder, yield = 15%, mp: 159–161 °C. FTIR (KBr), ν_{max} : 3468, 3166, 3062, 3033, 2953, 2922, 2848, 1778, 1745, 1709, 1599, 1561, 1495, 1450, 1235, 1174, 1136, 1071, 1026, 813, 770, 699 cm^{-1} . ^1H NMR (250 MHz, CDCl_3) δ : 8.21 (br s, 1H), 7.48–7.30 (m, 15H), 6.43 (s, 1H), 4.32 (d, 2H, $J = 14.50$ Hz), 4.22 (d, 2H, $J = 13.75$ Hz), 3.75 (s, 3H) ppm. ^{13}C NMR (62.5 MHz, CDCl_3) δ : 168.50, 166.41, 153.81, 153.12, 136.44, 135.18, 130.92, 129.04, 128.94, 128.74, 128.53, 128.28, 127.83, 127.38, 125.58, 73.13, 53.37, 50.81, 38.03, 36.32 ppm. HRMS (ESI-Orbit trap) m/z : $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{26}\text{H}_{24}\text{N}_4\text{O}_4\text{S}_2+\text{H}$, 521.1312; found 521.1316.

5.2.9. *Ethyl-2-(bis(methylthio)methyleneamino)-2-(3,5-dioxo-4-phenyl-1,2,4-triazolidin-1-yl)acetate (2i)* $\text{C}_{15}\text{H}_{18}\text{N}_4\text{O}_4\text{S}_2$

Colorless powder, yield = 35%, mp: 118–120 °C. FTIR (KBr), ν_{max} : 3460, 3165, 3070, 2987, 2930, 1778, 1749, 1694, 1574, 1504, 1434, 1277, 1136, 1078, 1068, 1016, 832, 773, 711 cm^{-1} . ^1H NMR (250 MHz, CDCl_3) δ : 8.60 (br s, 1H), 7.49–7.37 (m, 5H), 6.37 (s, 1H), 4.33 (qd, 1H, $J_1 = 7.00$ Hz, $J_2 = 3.50$ Hz), 4.15 (qd, 1H, $J_1 = 7.25$ Hz, $J_2 = 3.50$ Hz), 2.60 (s, 3H), 2.34 (s, 3H), 1.28 (t, 3H, $J = 7.25$ Hz) ppm. ^{13}C NMR (62.5 MHz, CDCl_3) δ : 170.68, 165.88, 153.86, 153.21, 131.05, 129.07, 128.29, 125.60, 73.30, 62.74, 15.83, 14.99, 14.06 ppm. HRMS (ESI-Orbit trap) m/z : $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{15}\text{H}_{18}\text{N}_4\text{O}_4\text{S}_2+\text{H}$, 383.0842; found 383.0839.

5.2.10. *tert-Butyl-2-(bis(methylthio)methyleneamino)-2-(3,5-dioxo-4-phenyl-1,2,4-triazolidin-1-yl)acetate (2j)* $\text{C}_{17}\text{H}_{22}\text{N}_4\text{O}_4\text{S}_2$

Colorless powder, yield = 30%, mp: 160–162 °C. FTIR (KBr), ν_{max} : 3474, 3195, 3062, 2976, 2924, 1778, 1745, 1699,

1574, 1493, 1435, 1369, 1262, 1225, 1151, 1084, 1024, 1004, 844, 775, 721, 695 cm^{-1} . ^1H NMR (250 MHz, CDCl_3) δ : 8.45 (br s, 1H), 7.53–7.39 (m, 5H), 6.28 (s, 1H), 2.59 (s, 3H), 2.35 (s, 3H), 1.45 (s, 9H) ppm. ^{13}C NMR (62.5 MHz, CDCl_3) δ : 169.87, 164.56, 153.78, 153.07, 131.12, 129.05, 128.17, 125.44, 84.20, 73.83, 27.91, 15.85, 14.98 ppm. HRMS (ESI-Orbit trap) m/z : $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{17}\text{H}_{22}\text{N}_4\text{O}_4\text{S}_2+\text{H}$, 411.1155; found 411.1157.

5.2.11. *Benzyl-2-(bis(methylthio)methyleneamino)-2-(3,5-dioxo-4-phenyl-1,2,4-triazolidin-1-yl)acetate (2k)*

$\text{C}_{20}\text{H}_{20}\text{N}_4\text{O}_4\text{S}_2$

Colorless powder, yield = 58%, mp: 154–156 °C. FTIR (KBr), ν_{max} : 3468, 3151, 3066, 3025, 2944, 2922, 2885, 1785, 1730, 1705, 1570, 1501, 1455, 1424, 1336, 1248, 1181, 1134, 1075, 1064, 1023, 1006, 852, 770, 750, 708 cm^{-1} . ^1H NMR (250 MHz, CDCl_3) δ : 8.16 (br s, 1H), 7.41–7.31 (m, 10H), 6.44 (s, 1H), 5.29 (d, 1H, $J = 12.25$ Hz), 5.15 (d, 1H, $J = 12.25$ Hz), 2.60 (s, 3H), 2.35 (s, 3H) ppm. ^{13}C NMR (62.5 MHz, CDCl_3) δ : 171.03, 165.84, 153.71, 153.21, 134.58, 130.86, 129.03, 128.67, 128.19, 125.67, 73.23, 68.25, 15.82, 15.04 ppm. HRMS (ESI-Orbit trap) m/z : $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{20}\text{H}_{20}\text{N}_4\text{O}_4\text{S}_2+\text{H}$, 445.0999; found 445.0999.

5.2.12. *Methyl-3-(methylthio)-5,7-dioxo-6-phenyl-1,5,6,7-tetrahydro[1,2,4]triazolo[1,2-a][1,2,4]triazole-1-carboxylate (3a)* $\text{C}_{13}\text{H}_{12}\text{N}_4\text{O}_4\text{S}$

Colorless powder, yield = 8%, mp: 142–144 °C. FTIR (KBr), ν_{max} : 3084, 3025, 2952, 2925, 2848, 1798, 1755, 1594, 1561, 1500, 1428, 1404, 1362, 1233, 1213, 1170, 1141, 1115, 1070, 1019, 834, 782, 759 cm^{-1} . ^1H NMR (250 MHz, CDCl_3) δ : 7.49–7.45 (m, 5H), 6.13 (s, 1H), 3.89 (s, 3H), 2.60 (s, 3H) ppm. ^{13}C NMR (62.5 MHz, CDCl_3) δ : 165.93, 158.51, 154.41, 149.91, 130.29, 129.39, 129.07, 125.70, 82.63, 53.57, 14.36 ppm. HRMS (ESI-Orbit trap) m/z : $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{13}\text{H}_{12}\text{N}_4\text{O}_4\text{S}+\text{H}$, 321.0652; found 321.0647.

5.2.13. *Methyl-3-(ethylthio)-5,7-dioxo-6-phenyl-1,5,6,7-tetrahydro[1,2,4]triazolo[1,2-a][1,2,4]triazole-1-carboxylate (3b)* $\text{C}_{14}\text{H}_{14}\text{N}_4\text{O}_4\text{S}$

Colorless powder, yield = 4%, mp: 148–150 °C. FTIR (KBr), ν_{max} : 3062, 2952, 2926, 2848, 1797, 1740, 1575, 1500, 1454, 1432, 1258, 1174, 1141, 1104, 1067, 1019, 835, 762, 690 cm^{-1} . ^1H NMR (250 MHz, CDCl_3) δ : 7.47–7.45 (m, 5H), 6.12 (s, 1H), 3.89 (s, 3H), 3.19 (q, 2H, $J = 7.37$ Hz), 1.43 (t, 3H, $J = 7.37$ Hz) ppm. ^{13}C NMR (62.5 MHz, CDCl_3) δ : 166.00, 158.50, 153.00, 148.00, 129.47, 129.44, 129.37, 129.12, 129.04, 125.70, 82.56, 53.55, 30.93, 13.79 ppm. HRMS (ESI-Orbit trap) m/z : $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{14}\text{H}_{14}\text{N}_4\text{O}_4\text{S}+\text{H}$, 335.0809; found 335.0806.

5.2.14. *Methyl-1-methyl-3-(methylthio)-5,7-dioxo-6-phenyl-1,5,6,7-tetrahydro[1,2,4]triazolo[1,2-a][1,2,4]triazole-1-carboxylate (5a)* $\text{C}_{14}\text{H}_{14}\text{N}_4\text{O}_4\text{S}$

Colorless crystals, yield = 72%, mp: 148–150 °C. FTIR (KBr), ν_{max} : 3110, 3066, 3004, 2944, 1728, 1568, 1404, 1272, 1144, 984, 910, 750 cm^{-1} . ^1H NMR (250 MHz, CDCl_3) δ : 7.38–7.53 (m, 5H), 3.81 (s, 3H), 2.56 (s, 3H), 1.96 (s, 3H) ppm. ^{13}C NMR (62.5 MHz, CDCl_3) δ : 167.20, 155.80, 153.10, 148.20, 130.60, 129.30, 128.90, 125.80, 91.60, 53.60, 23.30, 14.10 ppm. HRMS (ESI-Orbit trap) m/z : $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{14}\text{H}_{14}\text{N}_4\text{O}_4\text{S}+\text{H}$, 335.0809; found 335.0808.

5.2.15. *Methyl-1-(2-methoxy-2-oxoethyl)-3-(methylthio)-5,7-dioxo-6-phenyl-1,5,6,7-tetrahydro[1,2,4]triazolo[1,2-a][1,2,4]triazole-1-carboxylate (5b)* C₁₆H₁₆N₄O₄S

White powder, yield = 68%, mp: 152–154 °C. FTIR (KBr), ν_{\max} : 3064, 2978, 2956, 2936, 1794, 1702, 1599, 1583, 1554, 1499, 1448, 1279, 1255, 1170, 1074, 1027, 883, 747 cm⁻¹. ¹H NMR (250 MHz, CDCl₃) δ : 7.28–7.53 (m, 5H), 3.87 (s, 3H), 3.87 (d, 1H, *J* = 17.76 Hz), 3.66 (s, 3H), 3.37 (d, 1H, *J* = 17.76 Hz), 2.54 (s, 3H) ppm. ¹³C NMR (62.5 MHz, CDCl₃) δ : 167.20, 155.80, 153.10, 148.20, 130.60, 129.30, 128.90, 125.80, 91.60, 53.60, 23.30, 14.10 ppm. HRMS (ESI-Orbit trap) *m/z*: [M+H]⁺ calcd for C₁₆H₁₆N₄O₆S+H, 393.0863; found 393.0858.

5.2.16. *Methyl-1-(3-methoxy-3-oxopropyl)-3-(methylthio)-5,7-dioxo-6-phenyl-1,5,6,7-tetrahydro[1,2,4]triazolo[1,2-a][1,2,4]triazole-1-carboxylate (5c)* C₁₇H₁₈N₄O₄S

Yellow powder, yield = 64%, mp: 155–157 °C. FTIR (KBr), ν_{\max} : 3128, 3070, 3018, 2956, 2856, 1800, 1734, 1574, 1406, 1266, 1130, 936, 758 cm⁻¹. ¹H NMR (250 MHz, CDCl₃) δ : 7.37–7.52 (m, 5H), 3.81 (s, 3H), 3.69 (s, 3H), 2.50–2.70 (m, 4H), 2.55 (s, 3H) ppm. ¹³C NMR (62.5 MHz, CDCl₃) δ : 172.80, 166.40, 156.60, 153.60, 148.40, 130.50, 129.30, 128.90, 125.90, 93.90, 53.70, 51.80, 31.00, 28.30, 14.10 ppm. HRMS (ESI-Orbit trap) *m/z*: [M+H]⁺ calcd for C₁₇H₁₈N₄O₆S+H, 407.1020; found 407.1012.

5.2.17. *Methyl-1-isobutyl-3-(methylthio)-5,7-dioxo-1,5,6,7-tetrahydro-1,2,4-triazolo[1,2-a][1,2,4]triazole-1-carboxylate (5d)* C₁₇H₂₀N₄O₄S

Yellow crystals, yield = 69%, mp: 158–160 °C. FTIR (KBr), ν_{\max} : 3032, 2958, 2870, 1786, 1726, 1562, 1496, 1398, 1230, 1018, 768 cm⁻¹. ¹H NMR (250 MHz, CDCl₃) δ : 7.37–7.52 (m, 5H), 3.80 (s, 3H), 2.56 (s, 3H), 2.26 (dd, 2H, *J*₁ = 6.37 Hz, *J*₂ = 14.95 Hz), 1.93 (nonet, 1H, *J* = 6.37 Hz), 1.02 (d, *J* = 6.37 Hz), 1.01 (d, *J* = 6.37 Hz) ppm. ¹³C NMR (62.5 MHz, CDCl₃) δ : 167.30, 155.10, 153.10, 148.20, 130.70, 129.30, 128.80, 125.90, 94.80, 53.60, 43.10, 23.90, 23.70, 14.10 ppm. HRMS (ESI-Orbit trap) *m/z*: [M+H]⁺ calcd for C₁₇H₂₀N₄O₄S+H, 377.1278; found 377.1263.

5.2.18. *Methyl-(3-methylthio)-1-(2-methylthio)ethyl)-5,7-dioxo-6-phenyl-1,5,6,7-tetrahydro-1,2,4-triazolo[1,2-a][1,2,4]triazole-1-carboxylate (5e)* C₁₆H₁₈N₄O₄S₂

Yellow powder, yield = 32%, mp: 154–156 °C. FTIR (KBr), ν_{\max} : 3022, 2978, 2956, 2916, 1783, 1725, 1597, 1559, 1494, 1459, 1392, 1263, 1236, 1191, 1142, 1064, 1014, 965, 843, 795 cm⁻¹. ¹H NMR (250 MHz, CDCl₃) δ : 7.39–7.54 (m, 5H), 3.83 (s, 3H), 2.61–2.68 (m, 4H), 2.58 (s, 3H), 2.15 (s, 3H) ppm. ¹³C NMR (62.5 MHz, CDCl₃) δ : 166.50, 156.30, 153.40, 148.30, 130.60, 129.40, 129.00, 125.90, 94.10, 53.80, 35.50, 27.80, 15.70, 14.20 ppm. HRMS (ESI-Orbit trap) *m/z*: [M+H]⁺ calcd for C₁₆H₁₈N₄O₄S₂+H, 395.0842; found 395.0843.

5.2.19. *Methyl-1-benzyl-(3-methylthio)-5,7-dioxo-6-phenyl-1,5,6,7-tetrahydro-1,2,4-triazolo[1,2-a][1,2,4]triazole-1-carboxylate (5f)* C₂₀H₁₈N₄O₄S

Colorless powder, yield = 61%, mp: 152–154 °C. FTIR (KBr), ν_{\max} : 3110, 3068, 3032, 2960, 2944, 1788, 1732, 1570, 1496, 1402, 1282, 1212, 1144, 760 cm⁻¹. ¹H NMR (250 MHz,

CDCl₃) δ : 7.39–7.23 (m, 10H), 3.86 (s, 3H), 3.67 (d, 1H, *J* = 14.39 Hz), 3.57 (d, 1H, *J* = 14.39 Hz), 2.53 (s, 3H) ppm. ¹³C NMR (62.5 MHz, CDCl₃) δ : 166.80, 155.30, 152.40, 147.50, 132.90, 131.20, 130.50, 129.20, 128.80, 127.40, 125.80, 93.90, 53.70, 40.50, 14.10 ppm. HRMS (ESI-Orbit trap) *m/z*: [M+H]⁺ calcd for C₂₀H₁₈N₄O₄S+H, 411.1122; found 411.1120.

5.2.20. *Methyl-1-(4-methoxybenzyl)-3-(methylthio)-5,7-dioxo-6-phenyl-1,5,6,7-tetrahydro[1,2,4]triazolo[1,2-a][1,2,4]triazole-1-carboxylate (5g)* C₂₁H₂₀N₄O₅S

Yellow oil, yield = 60%. FTIR (KBr), ν_{\max} : 3049, 2978, 2957, 2933, 2903, 2843, 1787, 1730, 1704, 1613, 1572, 1513, 1491, 1452, 1396, 1324, 1295, 1249, 1204, 1179, 1139, 1054, 1033, 1001, 953, 841, 753 cm⁻¹. ¹H NMR (250 MHz, CDCl₃) δ : 7.17–7.47 (m, 5H), 7.17–7.36 (m, 2H), 6.80–6.85 (m, 2H), 3.88 (s, 3H), 3.64 (d, 1H, *J* = 14.51 Hz), 3.54 (d, 1H, *J* = 14.51 Hz), 2.57 (s, 3H) ppm. ¹³C NMR (62.5 MHz, CDCl₃) δ : 166.90, 158.80, 155.20, 152.30, 147.50, 132.20, 130.50, 129.20, 128.80, 125.80, 124.70, 113.50, 94.0, 55.1, 53.7, 14.1 ppm. HRMS (ESI-Orbit trap) *m/z*: [M+H]⁺ calcd for C₂₁H₂₀N₄O₅S+H, 441.1227; found 441.1231.

5.2.21. *Diethyl-3-(methylthio)-5,7-dioxo-6-phenyl-6,7-dihydro-1,2,4-triazolo[1,2-a][1,2,4]triazole-1,1(5H)-dicarboxylate (5h)* C₁₇H₁₈N₄O₆S

Yellow oil, yield = 61%. FTIR (KBr), ν_{\max} : 3069, 2982, 2965, 2928, 2872, 1799, 1739, 1578, 1496, 1476, 1402, 1370, 1295, 1264, 1226, 1184, 1126, 1102, 1049, 1012, 980, 862, 789 cm⁻¹. ¹H NMR (250 MHz, CDCl₃) δ : 7.39–7.48 (m, 5H), 4.40–4.28 (m, 4H), 2.62 (s, 3H), 1.32 (t, 6H, *J* = 4.50 Hz) ppm. ¹³C NMR (62.5 MHz, CDCl₃) δ : 163.60, 158.40, 152.50, 148.10, 130.30, 129.20, 129.00, 125.80, 92.10, 63.70, 14.30, 13.80 ppm. HRMS (ESI-Orbit trap) *m/z*: [M+H]⁺ calcd for C₁₇H₁₈N₄O₆S+H, 407.1020; found 407.1015.

5.2.22. *7-(Methylthio)-2,5-diphenyl-1,2,4-triazolo[1,2-a][1,2,4]triazole-1,3(2H,5H)-dione (7a)* C₁₇H₁₄N₄O₂S

Colorless powder, yield = 57%, mp: 140–142 °C. FTIR (KBr), ν_{\max} : 3062, 3049, 3036, 2932, 2850, 1785, 1733, 1594, 1579, 1499, 1453, 1398, 1301, 1247, 1222, 1166, 1138, 1097, 1041, 1012, 852, 793, 741 cm⁻¹. ¹H NMR (250 MHz, CDCl₃) δ : 7.26–7.53 (m, 10H), 6.82 (s, 1H), 2.63 (s, 3H). ¹³C NMR (62.5 MHz, CDCl₃) δ : 169.50, 166.70, 157.70, 153.50, 149.20, 130.60, 129.30, 129.20, 128.90, 126.30, 120.00, 90.10, 53.80, 52.10, 34.70, 14.20 ppm. HRMS (ESI-Orbit trap) *m/z*: [M+H]⁺ calcd for C₁₇H₁₄N₄O₂S+H, 330.0910; found 330.0909.

5.2.23. *5-(4-Fluorophenyl)-7-(methylthio)-2-phenyl-1,2,4-triazolo[1,2-a][1,2,4]triazole-1,3(2H,5H)-dione (7b)* C₁₇H₁₃FN₄O₂S

Colorless powder, yield = 49%, mp: 156–158 °C. FTIR (KBr), ν_{\max} : 3076, 3013, 2970, 2925, 2851, 1787, 1735, 1606, 1578, 1510, 1459, 1402, 1310, 1256, 1225, 1173, 1141, 1107, 1048, 1016, 873, 781, 748 cm⁻¹. ¹H NMR (250 MHz, CDCl₃) δ : 7.53–6.80 (m, 9H), 6.79 (s, 1H), 2.63 (s, 3H) ppm. ¹³C NMR (62.5 MHz, CDCl₃) δ : 155.70, 155.00, 150.40, 165.10, 161.10, 133.50, 130.50, 129.40, 129.30, 128.90, 128.40, 128.30, 125.50, 125.10, 116.00, 115.60, 86.00, 14.40 ppm. HRMS (ESI-Orbit trap) *m/z*: [M+H]⁺ calcd for C₁₇H₁₃FN₄O₄S+H, 357.0816; found 357.0811.

5.2.24. 5-Methyl-7-(methylthio)-2,5-diphenyl-[1,2,4]triazolo[1,2-a][1,2,4]triazole-1,3(2H,5H)-dione (**7c**) $C_{18}H_{16}N_4O_2S$

Yellow powder, yield = 53%, mp: 163–165 °C. FTIR (KBr), ν_{\max} : 3063, 2987, 2957, 2930, 2851, 1784, 1722, 1590, 1577, 1493, 1447, 1390, 1316, 1253, 1137, 1072, 1022, 833, 754 cm^{-1} . ^1H NMR (250 MHz, CDCl_3) δ : 7.36–7.67 (m, 10H), 2.65 (s, 3H), 2.10 (s, 3H) ppm. ^{13}C NMR (62.5 MHz, CDCl_3) δ : 151.90, 151.40, 148.00, 142.10, 130.80, 129.20, 128.60, 128.50, 128.40, 125.60, 125.40, 94.20, 27.60, 14.10 ppm. HRMS (ESI-Orbit trap) m/z : $[\text{M}+\text{H}]^+$ calcd for $C_{17}H_{16}N_4O_2S+\text{H}$, 353.1067; found 353.1061.

5.2.25. 7-(Methylthio)-2,5,5-triphenyl-[1,2,4]triazolo[1,2-a][1,2,4]triazole-1,3(2H,5H)-dione (**7d**) $C_{23}H_{18}N_4O_2S$

Yellow powder, yield = 53%, mp: 166–168 °C. FTIR (KBr), ν_{\max} : 3070, 3050, 2977, 2959, 2920, 2850, 1792, 1732, 1706 ($\text{C}=\text{O}$), 1595, 1580, 1490, 1451, 1428, 1393, 1315, 1242, 1181, 1164, 1141, 1116, 1087, 1045, 1009, 832, 778, 741 cm^{-1} . ^1H NMR (250 MHz, CDCl_3) δ : 7.33–7.51 (m, 15H), 2.71 (s, 3H) ppm. ^{13}C NMR (62.5 MHz, CDCl_3) δ : 152.50, 151.10, 148.00, 139.40, 130.70, 129.10, 129.00, 128.60, 128.30, 127.50, 125.50, 97.90, 14.20 ppm. HRMS (ESI-Orbit trap) m/z : $[\text{M}+\text{H}]^+$ calcd for $C_{23}H_{18}N_4O_2S+\text{H}$, 415.1223; found 415.1210.

5.2.26. Dimethyl(3,5-dioxo-4-phenyl-1,2,4-triazolidin-1-yl)(phenyl) methylcarbonimidodithioate (**8a**) $C_{18}H_{18}N_4O_2S_2$

White powder yield = 19%, mp: 123–125 °C. FTIR (KBr), ν_{\max} : 3132, 3070, 3054, 2916, 2850, 1764, 1686, 1450, 1268, 1140, 980, 728. ^1H NMR (250 MHz, CDCl_3) δ : 7.36–7.51 (m, 10H), 6.42 (s, 1H), 2.67 (s, 3H), 2.28 (s, 3H). ^{13}C NMR (62.5 MHz, CDCl_3) δ : 159.2, 153.1, 152.3, 134.9, 130.9, 130.1, 129.3, 129.2, 129.0, 128.8, 128.4, 127.4, 126.4, 125.5, 65.7, 15.1, 15.0. HRMS (ESI-Orbit trap) m/z : $[\text{M}+\text{H}]^+$ calcd for $C_{17}H_{13}FN_4O_4S+\text{H}$, 387.0944; found 387.0944.

5.3. Biological evaluation

All the reagents used were commercially available by Merck, 1,1-diphenyl-2-picrylhydrazyl (DPPH), nordihydroguaiaretic acid (NDGA) were purchased from the Aldrich Chemical Co. Milwaukee, WI, (USA). Soybean lipooxygenase, linoleic acid sodium salt, indomethacin were obtained from Sigma Chemical, Co. (St. Louis, MO, USA).

5.3.1. Soybean lipooxygenase inhibition study in vitro

(Lit. [36]) The *in vitro* study was evaluated as reported previously. The test compounds dissolved in ethanol were incubated at room temperature with sodium linoleate (100 μM) and 0.2 mL of enzyme solution ($1/9 \times 10^{-4}$ w/v in saline). The conversion of sodium linoleate to 13-hydroperoxylinoleic acid at 234 nm was recorded and compared with the appropriate standard inhibitor (nordihydroguaiaretic acid 45 μM) (Table 5).

5.3.2. Inhibition of linoleic acid peroxidation

(Lit. [36]) Production of conjugated diene hydroperoxide by oxidation of linoleic acid in an aqueous dispersion was monitored at 234 nm. 2, 2'-Azobis(2-amidinopropane) dihydrochloride (AAPH) is used as a free radical initiator. This assay can be used to follow oxidative changes and to

determine the inhibition of linoleic acid peroxidation induced by each tested compound. Ten microliters of the 16 mM linoleic acid dispersion were added to the UV cuvette containing 0.93 mL of 0.05 M phosphate buffer, pH 7.4, thermostatted at 37 °C. The oxidation reaction was initiated at 37 °C under air by the addition of 50 μL of 40 mM AAPH solution. Oxidation was carried out in the presence of aliquots (10 μL) of the tested compounds. In the assay without antioxidant, lipid oxidation was measured in the presence of the same level of DMSO. The rate of oxidation at 37 °C was monitored by recording the increase in absorption at 234 nm caused by conjugated diene hydroperoxide formation (Table 5).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.crci.2016.05.024>.

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