Sulfanyl 5*H*-dihydropyrrole derivatives *via* 1,3-dipolar cycloaddition, their further chemical manipulation and antioxidant activity

Kosmas Oikonomou,*[a] Dimitra Georgiou,[a] Sotirios Katsamakas,[b] Dimitra Hadjipavlou-Litina,[b] and Yiannis Elemes[a]

[a] *Department of Chemistry, University of Ioannina, 45110 Ioannina, Greece*
[b] *Department of Pharmaceutical Chemistry, School of Pharmacy, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki 54124, Greece*
E-mail: pch00958@cc.uoi.gr

Dedicated to Professor Michael Orfanopoulos for his scientific contribution in Organic Chemistry

DOI: http://dx.doi.org/10.3998/ark.5550190.0016.315

Abstract
Sulfanyl 5*H*-dihydropyrroles were synthesized through diastereoselective 1,3-dipolar cycloaddition of sulfanyl-substituted azomethine ylides from glycine esters to *N*-phenyl-maleimide under thermal or Ag-catalyzed conditions. Further manipulation with DDQ afforded novel polysubstituted pyrroles which were more potent antioxidants and lipoxygenase inhibitors than their 5*H*-dihydro analogues.

Keywords: 1,3-Dipolar cycloaddition, azomethine ylides, sulfanyl 5*H*-dihydropyrroles, polysubstituted pyrroles, antioxidant activity

Introduction

1,3-Dipolar cycloaddition (1,3-DC) with olefinic dipolarophiles is one of the most synthetically useful pericyclic reactions.[1,2] In particular, the 1,3-DC of azomethine ylides with alkenes offers a powerful method to access pyrrolidines, which frequently exist in natural alkaloids and artificial molecules with vital bioactivities.[3,4] As a result, novel developments have been described in the field of 1,3-DCs of azomethine ylides to electron-deficient alkenes by using either metal-based catalysts[5,6]or organocatalysts.[7,8] On the other hand, the 2,5-dihydropyrrole skeleton, which can be constructed by the 1,3-DC of azomethine ylides to alkenes, exists in a number of alkaloids,[9,10] serves as an important building block in organic synthesis through further functionalization of its

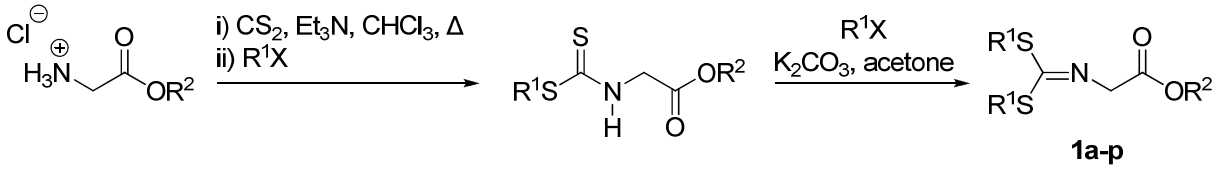
carbon-carbon double bond,[11,12] and is featured in a large family compounds that exhibit important bioactivities such as antibiotic,[13] anti-tumor[14] and anti-inflamatory[15,16] properties.

Our previous work[17] regarding the 1,3-DC of sulfur-substituted azomethine ylides of glycine esters to C_{60}, led to an investigation of the cycloaddition of these 1,3-dipoles and *N*-phenyl-maleimide (NPM) under thermal conditions.[18] Herein, we prepare new cycloadducts of the fore mentioned reaction under thermal conditions and describe how the efficiency of the cycloaddition was improved *via* the use of $AgNO_3$ as catalyst: in the presence of silver salt the reactions can be carried out at ambient conditions and in higher yields/diastereoselectivity. Furthermore, the antioxidative activity of the cycloadducts is reported: dihydropyrroles are known to exhibit interesting biological activities, among them antioxidant ability,[19] inhibition of mitotic kinesin and anticancer activity.[20] Finally, the dihydropyrroles are aromatized *via* oxidation using DDQ and the obtained polysubstituted pyrroles investigated for their antioxidative activity.

Results and Discussion

Synthesis of dihydropyrroles and pyrroles

Methyl-2-[bis(alkyl)sulfanyl or (aralkylsulfanyl)methylenamino]acetates **1a-p** used as substrates for the 1,3-dipolar cycloaddition to *N*-phenylmaleimide (NPM) were prepared by a known procedure (Figure 1).[21]



1a R^1 = Me, R^2 = Me
1b R^1 = Et, R^2 = Me
1c R^1 = Pr, R^2 = Me
1d R^1 = ^{i}Pr, R^2 = Me
1e R^1 = Bu, R^2 = Me
1f R^1 = ^{i}Bu, R^2 = Me
1g R^1 = Bn, R^2 = Me
1h R^1 = $(CH_3)_2CH{=}CHCH_2$-, R^2 = Me
1i R^1 = Me, R^2 = Et
1j R^1 = Bn, R^2 = Et
1k R^1 = Me, R^2 = ^{i}Pr
1l R^1 = Bn, R^2 = ^{i}Pr
1m R^1 = Me, R^2 = ^{t}Bu
1n R^1 = Bn, R^2 = ^{t}Bu
1o R^1 = Me, R^2 = Bn
1p R^1 = Bn, R^2 = Bn

Figure 1. General procedure for the synthesis of 2-[bis(alkyl)sulfanyl or aralkylsulfanyl)methyl-eneamino]acetates **1a-p** used in the 1,3-dipolar cycloaddition to NPM.

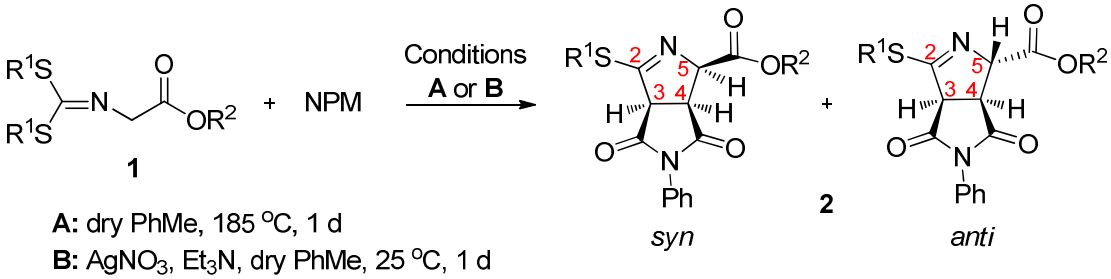
The cycloaddition reactions were performed by placing in a screw-capped test tube the specific quantity of the imine of methyl glycinate (**1a-p**) dissolved in dry toluene and flushed

with argon.[18] To this solution NPM (10% excess with respect to the imine) was added in one portion, the tube was sealed and heated to 185 ^{o}C (Table 1, Conditions **A**). When $AgNO_3$ was used as a catalyst (Table 1, Conditions **B**), dry Et_3N and the silver salt were added prior to NPM and the reaction was run at room temperature. Both routes were monitored by TLC and stopped after 24 h. The resulting *syn* and *anti* cycloaddition products **2** (Table 1) were purified by column chromatography and fully characterized. The discrimination among *syn* and *anti* diastereomers was established by ^{1}H NMR spectroscopy. In the *syn* adducts there is a large coupling constant among H_4 and H_5 (J ~9.5 Hz), whereas the corresponding coupling constant is smaller in the *anti* isomers (J ~2.0 Hz). The less polar *endo-anti* adducts elute first during chromatography and in general, the kinetically controlled *endo-syn* adduct is the predominant product. Worthy of note was that under acidic (40% AcOH in PhMe, 185 ^{o}C, 3 days) or basic conditions (40% Et_3N in PhMe, 185 ^{o}C, 3 days), the *syn* adducts are quantitatively epimerized into the thermodynamically more favorable *anti* isomers.

Upon using stoichiometric amounts of $AgNO_3$, the cycloaddition (conditions **B**) proceeds smoothly at much milder conditions (room temperature) in higher yields and diastereomeric ratios in favor of the *syn* diastereomers (Table 1). The beneficial effect of silver salts as catalysts in 1,3-DCs is well documented.[22-23] In certain cases under conditions **B**, only the *syn* diastereomer was formed. The presence of $AgNO_3$ plays a vital role in the formation of cycloadduct from substrate **1j**, which was unproductive under thermal conditions **A**. Silver nitrate activates the imine by simultaneous coordination to the carbonyl oxygen and the imine nitrogen. Thus, the activated acidic *a*-hydrogen can be deprotonated with a weak base, such as triethylamine. After elimination of mercaptan the resulting ylide adopts the reactive *E,E*-configuration. Finally, through an *endo* approach, the dipolarophile (NPM) reacts to the ylide forming the kinetically favorable *syn* cycloadduct.

Studying the scope and limitations of cycloaddition, we observed that the increasing the steric bulk of the sulfide group (R^1S) of **1**, led to a reduction in the reaction efficiency. For instance, if R^1 was the relatively bulky benzyl group, the reaction yield decreased substantially (see Table 1). The ester substituent (R^2) also influenced the reaction yields, with methyl esters being more reactive, and there was also a small effect in the *syn*/*anti* diastereoselectivity (Table 1). Apart from using glycine in the synthesis of **1**, we also synthesized *via* the same procedure several azomethine ylides, using other amino acids such as alanine, leucine, methionine, phenylalanine, tyrosine, aspartic acid, glutamic acid, serine and threonine. With these substrates the cycloaddition reaction did not take place. Only in case of alanine, the cycloadduct **2q** was formed in low yield. Tentatively, this indicated that the side-chain in the amino acid moiety of substrates **1** retarded the reaction rate, presumably owing to steric reasons.

Table 1. S*yn* (major or only) and *anti* products **2** from the 1,3-dipolar cycloaddition of imines **1** to *N*-phenylmaleimide (NPM)



Adduct	R^1	R^2	Conditions	Isolated yield (%)[a]	*syn*/*anti* (%)
2a[c]	Me	Me	**A**	76	87/13
			B	78	91/9
2b[c]	Et	Me	**A**	89	72/28
			B	69	>97/3
2c[c]	Pr	Me	**A**	68	65/35
			B	61	97/3
2d[c]	^{i}Pr	Me	**A**	52	67/33
			B	49	>97/3
2e[c]	Bu	Me	**A**	72	82/18
			B	70	96/4
2f	^{i}Bu	Me	**A**	57	93/7
			B	61	95/5
2g[c]	Bn	Me	**A**	41	83/17
			B	54	94/6
2h	Prenyl	Me	**A**	-	-
			B	33	>97/3
2i[c]	Me	Et	**A**	48	81/19
			B	58	93/7
2j	Bn	Et	**A**	42	86/14
			B	48	92/8
2k	Me	^{i}Pr	**A**	48	90/10
			B	59	93/7

Table 1 (continued)

Adduct	R^1	R^2	Conditions	Isolated yield (%)[a]	*syn*/*anti* (%)
2l	Bn	^{i}Pr	**A**	42	91/9
			B	41	>97/3
2m[c]	Me	^{t}Bu	**A**	42	86/14
			B	43	93/7
2n	Bn	^{t}Bu	**A**	21	>97/3
			B	24	>97/3
2o[c]	Me	Bn	**A**	46	83/17
			B	60	88/12
2p	Bn	Bn	**A**	37	92/8
			B	46	91/9
2q[b]	Me	Me	**A**	9	ND[d]
			B	14	>97/3

[a] Yields of isolated products after chromatography. [b] The *N*,*N*-(bismethylsulfanyl)imine of alanine ester was used as substrate. [c] Spectroscopic data for these adducts can be found in ref. 18. [d] ND: Not determined.

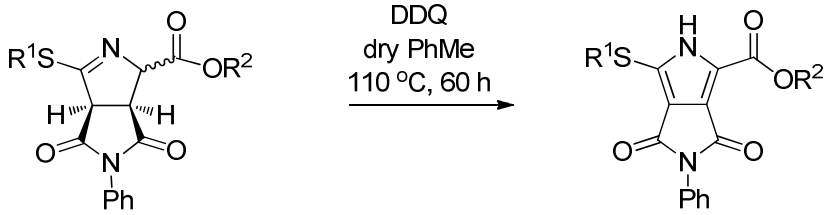
As a next step we aromatized the cycloadducts **2** into pyrrole derivatives **3** and tested their antioxidant activity; pyrroles are known antioxidants.[24] We chose 2,3-dichloro-3,4-dicyano-quinone (DDQ), a well known oxidation reagents.[25-28] The aromatization (Table 2) was performed under an inert atmosphere with the addition of cycloadducts **2** (mixture *syn* and *anti*) dissolved in dry toluene, in a screw capped test-tube and adding DDQ (100% excess with respect to **2**) in one portion. The tube was sealed and heated to 110 ^{o}C for 60 h. The pyrroles were isolated in moderate to good yields. Worthy of note was that the *syn* diastereomers reacted approximately twice as quickly as the more stable *anti* epimers.

Biological studies

Free radicals play an important role in the induction of several pathophysiological disorders *e.g.*, inflammation, cancer, arthritis. Consequently, compounds with antioxidant properties could be expected to offer protection in several diseases and to lead to potentially effective drugs. Taking into account the multifactorial character of oxidative stress and inflammation, and the role of pyrroles and dihydropyrroles in them, we evaluated the new cycloadducts and pyrroles as antioxidants and also as inhibitors of soybean LOX (Tables 3 and 4). The well-known

antioxidant agents, nordihydroguaiaretic acid (NDGA) and trolox were used as reference compounds. Several assays should be used to assess *in vitro*[29] antioxidant activity because the antioxidant ability of a compound must be evaluated in a variety of milieus. Most of them require a spectrophotometric measurement and a certain reaction time to obtain reproducible results.

Table 2. Aromatization of cycloadducts **2** with DDQ to form pyrroles **3**



2 **3**

Product	R^1	R^2	Isolated yield (%)
3a	Me	Me	61
3b	Et	Me	53
3c	Pr	Me	49
3d	^{i}Pr	Me	45
3e	Bu	Me	60
3f	^{i}Bu	Me	59
3g	Bn	Me	51
3h	Me	Et	58
3i	Bn	Et	62
3j	Me	^{i}Pr	51
3k	Bn	^{i}Pr	62
3l	Me	^{t}Bu	71
3m	Me	Bn	50
3n	Bn	Bn	63

[a]100% molar excess of DDQ.[b]Yields of isolated products after chromatographic purification.

In our studies, AAPH was used as a free radical initiator to follow oxidative changes of linoleic acid to conjugated diene hydroperoxide. Azo compounds generating free radicals through spontaneous thermal decomposition are useful for free radical production studies *in vitro*. The water-soluble azo compound AAPH has been extensively used as a clean and controllable source of thermally produced alkylperoxyl free radicals. In the AAPH assay, the highly reactive alkylperoxyl radicals are intercepted mainly by hydrogen atom transfer HAT from the antioxidant. 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.[30] All dihydropyrroles shown is Table 3 exhibit anti-lipid peroxidation activity lower than the reference compound Trolox. Only compounds **2f** and **2p** present 63%. It seems that the presence of a Bn group in position R^1 and a Me group at R^2 correlated with higher antioxidant results.

Table 3. Biological evaluation of cycloadducts **2** as antioxidants

Substrate	%LOX Inh.[a] @100 μM/IC_{50} μM	AAPH%[b] @100 μM
2f	28	63
2h	7	41
2i	14	37
2j	37	54
2k	23	38
2l	100 μM	56
2m	3	30
2n	No	28
2o	No	34
2p	83.5 μM	63
2q	9	28
NDGA	5.5 μM	
Trolox		71

[a] *In vitro* % inhibition of soybean lipoxygenase (LOX). [b] % Inhibition of lipid peroxidation (AAPH).

Considering pyrrole derivatives **3** (Table 4), the majority show lower activity than trolox. However, it should to be mentioned that there are five derivatives with significant high activity: **3e** > **3i** > **3c** > **3d** > **3f** (74-96%). The common structural characteristics are that R^1 = Pr or Bu and that R^2 = Me (for compounds **3c-f**). Considering the structural characteristics of the two groups of derivatives (**2** *vs* **3**) compounds of the group **3** seem to be more potent, owing to the pyrrolyl groups.

We evaluated compounds from both series for their ability to inhibit soybean LOX by the UV absorbance based enzyme assay.[31] The lipoxygenase (LOX) catalyzes the first two steps in the metabolism of arachidonic acid to leukotrienes. LTB4 generation is considered to be important in the pathogenesis of neutrophil-mediated inflammatory diseases[32] with a marked relation to the severity of cardiovascular diseases, asthma and cancer. For some derivatives we succeeded to calculate their IC_{50} values, otherwise their % inhibition values are given at 100 μM. Perusal of the IC_{50}'s inhibition values (Table 4) shows that the most potent, and equipotent,

inhibitors are **3a** and **3i**. Lipophilicity seems to be the major physicochemical property influencing activity. It is observed again that pyrroles of series **3** are more potent than the dihydropyrroles **2** in which inhibitory activity is not observed with the exception of **2l**.

Table 4. Biological evaluation of pyrroles **3** as antioxidants

Substrate	%LOX Inh.[a] @100 μM/IC_{50} μM	AAPH%[b] @100 μM
3a	58.5 μM	53
3b	21	50
3c	68	84
3d	89	78
3e	99	96
3f	63 μM	74
3g	75 μM	67
3h	100 μM	69
3i	58.5 μM	85
3j	100 μM	68
3k	75 μM	67
3l	31	64
3m	85 μM	68
NDGA	5.5 μM	
Trolox		71

[a] *In vitro* % inhibition of soybean lipoxygenase (LOX). [b] % Inhibition of lipid peroxidation (AAPH).

Conclusions

The 1,3-dipolar cycloaddition of *N*,*N*-bissulfanyl-substituted imines of glycinates onto *N*-phenyl-maleimide under thermal and Ag-catalyzed conditions provides mainly or exclusively the kinetically favorable *syn* diastereomers. Yields and selectivity are higher under the mild $AgNO_3$ catalysis conditions. The dihydropyrrolo cycloadducts were further oxidized to polysubstituted pyrroles in moderate to good yields upon reaction with DDQ. Pyrroles are more potent antioxidants and lipoxygenase inhibitors than their dihydro derivatives.

Experimental Section

General. All NMR spectra were taken in $CDCl_3$, on a Bruker-Spectrospin, Avance spectrometer. NMR peak assignments were achieved by 2D NMR spectroscopy (COSY, HSQC and HMBC experiments). HRMS were taken on an Orbitrap LTQ/XL instrument. IR spectra were taken on a Perkin Elmer Spectrum BX. All reagents and solvents were obtained from commercial suppliers and used without further purification. Dry quality solvents were obtained according to literature procedures[33] and stored over MS 4Å under inert atmosphere. Compounds **1a-p** were prepared according to literature procedures.[21]

General procedure for 1,3-dipolar cycloadditions

Thermal route A: In a screw capped tube(10 mL) flushed with Ar were introduced glycine ester imines **1** (0.5 mmol), *N*-phenylmaleimide (0.55 mmol) and dry toluene (3.5 mL). The solution was stirred at *ca*. 185 ^oC for 24 h. Volatiles were removed using a rotary evaporator. The remaining material was chromatographed on a silica gel column using EtOAc/hexane (1:3), as eluant.

$AgNO_3$-catalyzed route B: In a screw capped tube (10 mL) flushed with Ar were introduced glycine ester imine **1** (0.5 mmol), *N*-phenylmaleimide (0.55 mmol), $AgNO_3$ (0.5 mmol), dry Et_3N (0.5 mmol) and dry toluene (3.5 mL). The solution was stirred at 25 ^oC for 24 h. Volatiles were removed using a rotary evaporator and the remaining material was chromatographed as above.

Methyl 3-(isobutylthio)-4,6-dioxo-5-phenyl-1,3a,4,5,6,6a-hexahydropyrrolo[3,4-*c*]pyrrole-1-carboxylate (2f).

2f-*syn*. Yellow powder. mp 107-108 ^oC (hexane/$CHCl_3$). R_f 0.39 (EtOAc/hexane, 1:2). IR (ν_{max} cm^{-1}): 1722 and 1744 (C=O), 1582 (C=N). ^1H NMR (250 MHz, $CDCl_3$): 7.47-7.21 (m, 5H, aromatic), 5.13 (dd, $J_{H5,H4}$ 9.5 Hz, $J_{H5,H3}$ 1.0 Hz, $1H_5$), 4.20 (dd, $J_{H3,H4}$ 9.0 Hz, $J_{H3,H5}$ 1.0 Hz, $1H_3$), 3.92 (dd, $J_{H4,H5}$ 9.5 Hz, $J_{H4,H3}$ 9.0 Hz, $1H_4$,), 3.73 (s, 3H, OMe), 3.12-2.93 (m, 2H, SCH_2), 1.91-1.96 (m, 1H, CH), 0.99 (d, *J* 6.5 Hz, 6H, 2 × Me). ^{13}C NMR (62.5 MHz, $CDCl_3$): 174.3 (C=O), 170.6 (C=N), 170.5 (C=O), 169.9 (CO_2R), 131.3, 129.2, 129.1, 126.5 (aromatic), 75.9 (C_5), 59.7 (C_3), 52.7 (OMe), 47.0 (C_4), 40.1 (SCH_2), 27.9 (CH), 21.9 (Me), 21.8 (Me). HRMS (ESI-Orbit trap) *m/z* $[M+H]^+$calcd for $C_{18}H_{20}N_2O_2S$+H, 361.1217; found 361.1204.

2f-*anti*. Yellow powder. mp 111-112 ^oC (hexane/EtOAc). R_f 0.52 (EtOAc/hexane, 1:2). IR (ν_{max} cm^{-1}): 1716 and 1733 (C=O), 1568 (C=N). ^1H NMR (250 MHz, $CDCl_3$): 7.54-7.21 (m, 5H, aromatic), 5.20 (dd, $J_{H5,H4}$ 9.5 Hz, $J_{H5,H3}$ 2.0 Hz, $1H_5$), 4.33 (dd, $J_{H3,H4}$ 5.5 Hz , $J_{H3,H5}$ 2.0 Hz, $1H_3$), 4.10 (dd, $J_{H4,H5}$ 9.5 Hz, $J_{H4,H3}$ 5.5 Hz, $1H_4$), 3.73 (s, 3H, OMe), 3.12-2.93 (m, 2H, SCH_2), 1.91-1.95 (m, 1H, CH), 0.99 (d, *J* 6.5 Hz, 6H, 2 × Me). ^{13}C NMR (62.5 MHz, $CDCl_3$): 175.1 (C=O), 170.9 (C=N), 170.7 (C=O), 170.2 (CO_2R), 131.1, 129.1, 128.9, 126.2 (aromatic), 76.5 (C_5), 59.1 (C_3), 52.9 (OMe), 47.9 (C_4), 40.0 (SCH_2), 27.9 (CH), 21.9 (Me). HRMS (ESI-Orbit trap) *m/z* $[M+H]^+$calcd for $C_{18}H_{20}N_2O_2S$+H, 361.1217; found 361.1216.

Methyl 3-(3-methylbut-2-enylthio)-4,6-dioxo-5-phenyl-1,3a,4,5,6,6a-hexahydropyrrolo[3,4-*c*]pyrrole-1-carboxylate (2h).

2h-*syn*. Colorless powder. mp 126-127 ^{o}C (hexane/$CHCl_3$). R_f 0.29 (EtOAc/hexane, 1:2). IR (ν_{max} cm^{-1}): 1722 and 1744 (C=O), 1578 (C=N). ^{1}H NMR (250 MHz, $CDCl_3$): 7.49-7.23 (m, 5H, aromatic), 5.34-5.27 (m, 1H, vinyl), 5.19 (d, $J_{H5,H4}$ 9.5 Hz, $1H_5$), 5.04 (dd, $J_{H3,H4}$ 9.0 Hz, $J_{H3,H5}$ 1.0 Hz, $1H_3$), 3.96 (dd, $J_{H4,H5}$ 9.5 Hz, $J_{H4,H3}$ 9.0 Hz, $1H_4$), 3.77 (s, 3H, OMe), 3.80-3.74 (m, 2H, SCH_2), 1.72 (s, 3H, Me), 1.69 (s, 3H, Me). ^{13}C NMR (400 MHz, $CDCl_3$): 174.2 (C=O), 170.7 (C=N), 170.6 (C=O), 169.9 (CO_2R), 131.4, 129.2, 128.8, 126.5 (aromatic), 117.3 (C=<u>C</u><), 76.1 (C_5), 59.7 (C_3), 52.7 (OMe), 47.0 (C_4), 30.2 (Me), 25.7 (CH, vinyl), 17.9 (Me), 17.8 (Me). HRMS (ESI-Orbit trap) *m/z* $[M+H]^+$calcd for $C_{19}H_{20}N_2O_2S$+H, 373.1217; found 373.1212.

Ethyl 3-(benzylthio)-4,6-dioxo-5-phenyl-1,3a,4,5,6,6a-hexahydropyrrolo[3,4-*c*]pyrrole-1-carboxylate (2j).

2j-*syn*. Colorless powder. mp 105-106 ^{o}C (hexane/EtOAc). R_f 0.37 (EtOAc/hexane, 1:2). IR (ν_{max} cm^{-1}): 1738 and 1708 (C=O), 1578 (C=N). ^{1}H NMR (250 MHz, $CDCl_3$): 7.51-7.24 (m, 10H, aromatic), 5.15 (dd, $J_{H5,H4}$ 9.0 Hz, $J_{H5,H3}$ 1.0 Hz, $1H_5$), 4.39 (d, *J* 14.0 Hz, 1H, $PhC\underline{H}_aH_bS$,), 4.29 (d, *J* 14.0 Hz, 1H, $PhCH_a\underline{H}_bS$), 4.25-4.15 (m, 3H, $1H_3$+2H, OCH_2), 3.91 (dd, $J_{H4,H5}$ 9.0 Hz, $J_{H4,H3}$ 8.5 Hz, $1H_4$), 1.27 (t, *J* 7.5 Hz, $OCH_2C\underline{H}_3$). ^{13}C NMR (62.5 MHz, $CDCl_3$): 174.1 (C=O), 170.5 (C=N), 169.9 (C=O), 169.5 (CO_2R), 136.1, 131.4, 129.1, 128.9, 128.6, 127.6, 126.5 (aromatic), 76.1 (C_5), 62.1 (C_3), 59.6 (OCH_2), 47.1 (C_4), 36.1 (SCH_2), 14.0 (Me). HRMS (ESI-Orbit trap) *m/z* $[M+H]^+$calcd for $C_{22}H_{20}N_2O_2S$+H, 409.1217; found 409.1211.

2j-*anti*. Yellow oil. R_f 0.42 (EtOAc/hexane, 1:2). IR (ν_{max} cm^{-1}): 1720 (C=O), 1578 (C=N). ^{1}H NMR (250 MHz, $CDCl_3$): 7.51-7.19 (m, 10H, aromatics), 5.23 (dd, $J_{H5,H4}$ 2.5 Hz, $J_{H5,H3}$ 2.0 Hz, $1H_5$), 4.46 (d, *J* 14.0 Hz, 1H, $PhC\underline{H}_aH_bS$), 4.34 (dd, $J_{H3,H4}$ 8.5 Hz, $J_{H3,H5}$ 2.0 Hz, $1H_3$) 4.26 (d, *J* 14.0 Hz, 1H, $PhCH_a\underline{H}_bS$), 4.33-4.23 (m, 2H, OCH_2), 4.12 (dd, $J_{H4,H3}$ 8.5 Hz, $J_{H4,H5}$ 2.5 Hz, $1H_4$), 1.36 (t, *J* 7.5 Hz, 3H, $OCH_2C\underline{H}_3$,). ^{13}C NMR (62.5 MHz, $CDCl_3$): 175.2 (C=O), 170.9 (C=N), 169.9 (C=O), 169.7 (CO_2R), 136.0, 131.1, 129.3, 129.2, 129.1, 128.9, 127.6, 126.4 (aromatic), 76.2 (C_5), 62.3 (C_3), 59.1 (OCH_2), 48.0 (C_4), 36.1 (SCH_2), 14.2 (Me). HRMS (ESI-Orbit trap) *m/z* $[M+H]^+$calcd for $C_{22}H_{20}N_2O_4S$+H, 409.1217; found 409.1208.

Isopropyl 3-(methylthio)-4,6-dioxo-5-phenyl-1,3a,4,5,6,6a-hexahydropyrrolo[3,4-*c*]pyrrole-1-carboxylate (2k).

2k-*syn*. Colorless powder. mp 121-122 ^{o}C (hexane/$CHCl_3$). R_f 0.32 (EtOAc/hexane, 1:2). ^{1}H NMR (250 MHz, $CDCl_3$): 7.47-7.23 (m, 5H, aromatic), 5.10 (dd, $J_{H5,H4}$ 9.5 Hz, $J_{H5,H3}$ 1.0 Hz, $1H_5$), 5.03 (heptet, *J* 6.5 Hz, 1H, $OC\underline{H}Me_2$), 4.22 (dd, $J_{H3,H4}$ 9.0 Hz, $J_{H3,H5}$ 1.0 Hz, $1H_3$), 3.94 (dd, $J_{H4,H5}$ 9.5 Hz, $J_{H4,H3}$ 9.0 Hz, $1H_4$), 2.52 (s, 3H, SMe), 1.27 (d, *J* 7.0 Hz, 3H, $OCH\underline{Me}_2$), 1.25 (d, *J* 7.0 Hz, 3H, $OCH\underline{Me}_2$). ^{13}C NMR (62.5 MHz, $CDCl_3$): 173.9 (C=O), 170.7 (C=N), 170.5 (C=O), 169.1 (CO_2R), 131.3, 128.9, 128.7, 126.4 (aromatics), 75.9 (C_5), 69.7 (C_3), 59.4 (OCH), 47.1 (C_4), 21.5 (Me), 21.4 (Me), 14.2 (SMe). HRMS (ESI-Orbit trap) *m/z* $[M+H]^+$calcd for $C_{17}H_{20}N_2O_4S$+H, 347.1060; found 347.1055.

2k-*anti*. Yellow oil. R_f 0.35 (EtOAc/hexane, 1:2). ^{1}H NMR (250 MHz, $CDCl_3$): 7.51-7.26 (m, 5H, aromatic), 5.10 (dd, $J_{H5,H4}$ 9.5 Hz, $J_{H5,H3}$ 1.0 Hz, $1H_5$), 5.03 (heptet, *J* 6.5 Hz, 1H, $OC\underline{H}Me_2$),

4.22 (dd, $J_{H3,H4}$ 9.0 Hz, $J_{H3,H5}$ 1.0 Hz, $1H_3$), 3.94 (dd, $J_{H4,H5}$ 9.5 Hz, $J_{H4,H3}$ 9.0 Hz, $1H_4$), 2.54 (s, 3H, SMe), 1.33 (d, *J* 6.0 Hz, 3H OCH$\underline{Me}_2$), 1.32 (d, *J* 6.0 Hz, 3H, CH$\underline{Me}_2$). ^{13}C NMR (62.5 MHz, $CDCl_3$): 175.3 (C=O), 171.1 (C=N), 170.9 (C=O), 169.5 (CO_2R), 134.2, 131.1, 129.1, 126.1 (aromatic), 75.9 (C_5), 70.1 (C_3), 59.0 (OCH), 48.3 (C_4), 21.7 (Me), 21.6 (Me), 14.4 (SMe). HRMS (ESI-Orbit trap) *m/z* $[M+H]^+$calcd for $C_{17}H_{18}N_2O_4S$+H, 347.1060; found 347.1054.

Isopropyl 3-(benzylthio)-4,6-dioxo-5-phenyl-1,3a,4,5,6,6a-hexahydropyrrolo[3,4-*c*]pyrrole-1-carboxylate (2l).

2l-*syn*. Colorless powder. mp 127-128 ^{o}C (hexane/$CHCl_3$). R_f 0.27 (EtOAc/hexane, 1:2). IR (ν_{max} cm^{-1}): 1720 (C=O), 1558 (C=N). ^{1}H NMR (250 MHz, $CDCl_3$): 7.48-7.26 (m, 10H, aromatic), 5.17 (dd, $J_{H5,H4}$ 9.5 Hz, $J_{H5,H3}$ 1.0 Hz, $1H_5$), 5.07 (heptet, *J* 6.5 Hz, 1H, OC$\underline{H}Me_2$), 4.41 (d, *J* 13.5 Hz, 1H, SC$\underline{H}_aH_b$Ph), 4.33 (d, *J* 13.5 Hz, 1H, $CH_a\underline{H}_b$Ph), 4.25 (dd, $J_{H3,H4}$ 9.5 Hz, $J_{H3,H5}$ 1.0 Hz, $1H_3$), 3.97 (dd, $J_{H4,H5}$ 9.5 Hz, $J_{H4,H3}$ 9.5 Hz, $1H_4$), 1.30 (d, *J* 6.5 Hz, 3H OCH$\underline{Me}_2$), 1.28 (d, *J* 6.5 Hz, 3H, OCH$\underline{Me}_2$). ^{13}C NMR (62.5 MHz, $CDCl_3$): 174.1 (C=O), 170.8 (C=N), 170.7 (C=O), 169.3 (CO_2R), 136.1, 131.5, 129.2, 129.1, 128.9, 128.6, 126.6, 126.1 (aromatic), 76.1 (C_5), 69.9 (C_3), 59.6 (OCH), 47.1 (C_4), 35.8 (SCH_2), 21.8 (Me), 21.7 (Me). HRMS (ESI-Orbit trap) *m/z* $[M+H]^+$calcd for $C_{23}H_{22}N_2O_4S$+H, 423.1373; found 423.1361.

2l-*anti*. Yellow oil. R_f 0.31. ^{1}H NMR (250 MHz, $CDCl_3$): 7.48-7.26 (m, 10H, aromatic), 5.19 (dd, $J_{H5,H4}$ 2.0 Hz, $J_{H5,H3}$ 2.0 Hz, $1H_5$), 5.08 (heptet, *J* 6.5 Hz, 1H, OC$\underline{H}Me_2$), 4.48 (d, *J* 13.5 Hz, 1H, SC$\underline{H}_aH_b$Ph), 4.33 (d, *J* 13.5 Hz, 1H, $CH_a\underline{H}_b$Ph,), 4.34 (dd, $J_{H3,H4}$ 8.5 Hz, $J_{H3,H5}$ 2.0 Hz, $1H_3$), 4.11 (dd, $J_{H4,H3}$ 8.5 Hz, $J_{H4,H5}$ 2.0 Hz, $1H_4$), 1.34 (d, *J* 6.5 Hz, 3H, OCH$\underline{Me}_2$), 1.33 (d, *J* 6.5 Hz, 3H, OCH$\underline{Me}_2$). ^{13}C NMR (62.5 MHz, $CDCl_3$): 175.3 (C=O), 170.9 (C=N), 169.8 (C=O), 169.2 (CO_2R), 136.2, 131.1, 129.2, 129.0, 128.9, 128.6, 127.6, 126.3 (aromatic), 76.5 (C_5), 70.1 (C_3), 59.1 (OCH), 48.1 (C_4), 36.0 (SCH_2), 21.7 (Me), 14.4 (SMe). HRMS (ESI-Orbit trap) *m/z* $[M+H]^+$calcd for $C_{23}H_{22}N_2O_4S$+H, 423.1373; found 423.1350.

***tert*-Butyl 3-(benzylthio)-4,6-dioxo-5-phenyl-1,3a,4,5,6,6a-hexahydropyrrolo[3,4-*c*]pyrrole-1-carboxylate (2n).**

2n-*syn*. Yellow powder. mp 147-148 ^{o}C (hexane/EtOAc). R_f 0.22 (EtOAc/hexane, 1:2). IR (ν_{max} cm^{-1}): 1718 (C=O), 1702 (C=O), 1560 (C=N). ^{1}H NMR (250 MHz, $CDCl_3$): 7.48-7.21 (m, 10H, aromatic), 5.11 (d, $J_{H5,H4}$ 9.5 Hz, $1H_5$,), 4.40 (d, *J* 13.0 Hz, 1H, SC$\underline{H}_aH_b$Ph), 4.33 (d, *J* 13.0 Hz, 1H, $CH_a\underline{H}_b$Ph), 4.24 (d, $J_{H3,H4}$ 9.5 Hz, $1H_3$), 3.95 (dd, $J_{H4,H5}$ 9.5 Hz, $J_{H4,H3}$ 9.5 Hz, $1H_4$), 1.49 (s, 9H, *t*-BuO). ^{13}C NMR (62.5 MHz, $CDCl_3$): 174.0 (C=O), 170.7 (C=N), 169.4 (C=O), 168.7 (CO_2R), 136.2, 131.5, 129.1, 128.8, 128.6, 127.5, 126.6 (aromatic), 83.1 ($Me_3\underline{C}$), 76.9 (C_5), 59.7 (C_3), 47.2 (C_4), 36.0 (SCH_2), 28.0 ($\underline{Me}_3CO$), 14.4 (SMe). HRMS (ESI-Orbit trap) *m/z* $[M+H]^+$calcd for $C_{24}H_{24}N_2O_4S$+H, 437.1530; found 437.1537.

Benzyl 3-(benzylthio)-4,6-dioxo-5-phenyl-1,3a,4,5,6,6a-hexahydropyrrolo[3,4-*c*]pyrrole-1-carboxylate (2p).

2p-*syn*. Yellow oil. R_f 0.26 (EtOAc/hexane, 1:2). IR (ν_{max} cm^{-1}): 1734 (C=O), 1718 (C=O), 1560 (C=N). ^{1}H NMR (250 MHz, $CDCl_3$): 7.48-7.24 (m, 15H, aromatic), 5.25 (dd, $J_{H5,H4}$ 9.5 Hz, $J_{H5,H3}$ 1.0 Hz, $1H_5$), 5.20 (s, 2H, OCH_2), 4.42 (d, *J* 13.5 Hz, 1H, SC$\underline{H}_aH_b$Ph), 4.31 (d, *J* 13.5 Hz, 1H, $CH_a\underline{H}_b$Ph,), 4.26 (dd, $J_{H3,H5}$ 1.0 Hz, $J_{H3,H4}$ 9.0 Hz, $1H_3$,), 4.01 (dd, $J_{H4,H5}$ 9.5 Hz, $J_{H4,H3}$ 9.0

Hz, 1H$_4$). ^{13}C NMR (62.5 MHz, $CDCl_3$): 174.1 (C=O), 170.5 (C=N), 170.0 (C=O), 169.5 (CO_2R), 136.1, 134.9, 131.3, 129.1, 128.8, 128.6, 128.5, 127.5,126.5 (aromatic), 76.0 (C_5), 67.8 (C_3), 59.6 (OCH_2), 47.1 (C_4), 36.0 (SCH_2). HRMS (ESI-Orbit trap) *m/z* $[M+H]^+$calcd for $C_{27}H_{22}N_2O_4S$+H, 471.1373; found 471.1363.

2p-*anti*. Yellow oil. R_f 0.32 (EtOAc/hexane, 1:2). IR (ν_{max} cm^{-1}): 1732 (C=O), 1718 (C=O), 1556 (C=N). ^{1}H NMR (250 MHz, $CDCl_3$): 7.49-7.21 (m, 15H, aromatic), 5.29 (dd, $J_{H5,H4}$ 2.5 Hz, $J_{H5,H3}$ 2.0 Hz, 1H$_5$), 5.26 (s, 2H, OCH_2), 4.43 (d, *J* 13.0 Hz, 1H, $SC\underline{H}_aH_bPh$), 4.27 (d, *J* 13.0 Hz, 1H, $CH_a\underline{H}_bPh$,), 4.33 (dd, $J_{H3,H4}$ 8.0 Hz, $J_{H3,H5}$ 2.0 Hz, 1H$_3$), 4.11 (dd, $J_{H4,H3}$ 8.0 Hz, $J_{H4,H5}$ 2.5 Hz, 1H$_4$). ^{13}C NMR (62.5 MHz, $CDCl_3$): 175.1 (C=O), 170.9 (C=N), 170.2 (C=O), 169.5 (CO_2R), 136.0, 135.1, 131.1, 129.2, 129.1, 128.9, 128.7, 128.3, 127.6, 126.2 (aromatic), 76.0 (C_5), 67.8 (C_3), 59.1 (OCH_2), 48.0 (C_4), 36.2 (SCH_2). HRMS (ESI-Orbit trap) *m/z* $[M+H]^+$calcd for $C_{27}H_{22}N_2O_2S$+H, 471.1373; found 471.1375.

Methyl 3-(methylthio)-4,6-dioxo-5-phenyl-1,3a,4,5,6,6a-hexahydropyrrolo[3,4-*c*]pyrrole-1-carboxylate (2q).

2q-*syn*. Yellow oil. R_f 0.23 (EtOAc/hexane, 1:2). IR (ν_{max} cm^{-1}): 1744 (C=O), 1718 (C=O), 1582 (C=N). ^{1}H NMR (250 MHz, $CDCl_3$): 7.53-7.26 (m, 5H, aromatic), 4.33 (d, $J_{H3,H4}$ 9.0 Hz, 1H$_3$), 3.68 (s, 3H, OMe), 3.54 (d, $J_{H4,H3}$ 9.0 Hz, 1H$_4$), 2.51 (s, 3H, SMe), 1.70 (s, 3H, Me). ^{13}C NMR (62.5 MHz, $CDCl_3$): 174.1 (C=O), 170.9 (C=N), 170.7 (C=O), 168.3 (CO_2R), 131.4, 129.3, 129.2, 129.1, 128.9, 128.8, 126.4, 126.3 (aromatic), 82.6 (C_5), 59.6 (C_3), 54.8 (C_4), 52.9 (OMe), 26.6 (Me), 14.4 (SMe). HRMS (ESI-Orbit trap) *m/z* $[M+H]^+$calcd for $C_{16}H_{16}N_2O_4S$+H, 333.0904; found 333.0907.

General procedure for aromatization of the cycloadducts 2 with DDQ to give pyrroles 3

In a screw capped tube (10 mL) flushed with Ar were placed cycloadduct **2** (0.3 mmol) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (0.6 mmol) in dry toluene (3.5 mL). The mixture was stirred at *ca*. 110 ^{o}C for 60 h. Volatiles were removed using a rotary evaporator and the remaining material was chromatographed (silica gel) with a gradient mixture of EtOAc/hexaneas eluant.

Methyl 3-(methylthio)-4,6-dioxo-5-phenyl-2,4,5,6-tetrahydropyrrolo[3,4-*c*]pyrrole-1-carboxylate (3a). Colorless powder. mp 171-172 ^{o}C (hexane/$CHCl_3$). R_f 0.23 (EtOAc/hexane, 1:2). IR (ν_{max} cm^{-1}): 3444 (NH), 1722 (C=O), 1703 (C=O). ^{1}H NMR (250 MHz, $CDCl_3$): 9.91 (br s, 1H, NH), 7.51-7.32 (m, 5H, phenyl group), 3.99 (s, 3H, OMe), 2.85 (s, 3H, SMe). ^{13}C NMR (62.5 MHz, $CDCl_3$): 162.0 (C=O), 160.9 (C=O), 158.2 (CO_2R), 132.2, 129.2, 126.2, 118.9 (phenyl group), 131.2 (C_2), 129.2 (C_4 or C_3), 126.2 (C_4 orC_3), 118.9 (C_5), 52.9 (OMe), 17.0 (SMe). HRMS (ESI-Orbit trap) *m/z* $[M+H]^+$ calcd for $C_{15}H_{12}N_2O_4S$+H, 317.0591; found 317.0593.

Methyl 3-(ethylthio)-4,6-dioxo-5-phenyl-2,4,5,6-tetrahydropyrrolo[3,4-*c*]pyrrole-1-carboxylate (3b). Colorless powder. mp 172-173 ^{o}C (hexane/$CHCl_3$). R_f 0.24 (EtOAc/hexane, 1:2). IR (ν_{max} cm^{-1}): 3418 (NH), 1719 (C=O), ^{1}H NMR (250 MHz, $CDCl_3$): 9.92 (br s, 1H, NH), 7.50-7.29 (m, 5H, phenyl group), 4.00 (s, 3H, OMe), 3.38 (q, *J* 7.5 Hz, 2H, SCH_2), 1.35 (t, *J* 7.5 Hz, 3H, Me). ^{13}C NMR (62.5 MHz, $CDCl_3$): 162.0 (C=O), 160.9 (C=O), 159.3 (CO_2R), 132.2, 128.9,

127.9, 126.9 (phenyl group), 130.6 (C_2), 129.2 (C_4 or C_3), 126.2 (C_4 or C_3), 119.1 (C_5), 53.0 (OMe), 28.8 (SCH_2), 15.1 (Me). HRMS (ESI-Orbit trap) *m/z* $[M+H]^+$ calcd for $C_{16}H_{14}N_2O_4S+H$, 331.0747; found 331.0741.

Methyl 4,6-dioxo-5-phenyl-3-(propylthio)-2,4,5,6-tetrahydropyrrolo[3,4-*c*]pyrrole-1-carboxylate (3c). Colorless powder. mp 175-176 °C (hexane/$CHCl_3$). R_f 0.27 (EtOAc/hexane, 1:2). IR (ν_{max} cm^{-1}): 3404 (NH), 1713 (C=O). ^1H NMR (250 MHz, $CDCl_3$): 9.97 (br s, 1H, NH), 7.50-7.28 (m, 5H, phenyl group), 3.99 (s, 3H, OMe), 3.34 (t, *J* 7.0 Hz, 2H, SCH_2), 1.71-1.68 (m, 2H, CH_2), 1.02 (t, *J* 7.5 Hz, 3H, Me). ^{13}C NMR (62.5 MHz, $CDCl_3$): 162.0 (C=O), 161.0 (C=O), 159.4 (CO_2R), 131.2, 128.9, 127.9, 126.9 (phenyl group), 131.1 (C_2), 129.2 (C_4 or C_3), 126.2 (C_4 orC_3), 119.0 (C_5), 53.0 (OMe), 36.3 (SCH_2), 23.1 (CH_2), 12.9 (Me). HRMS (ESI-Orbit trap) *m/z* $[M+H]^+$ calcd for $C_{17}H_{16}N_2O_4S+H$, 345.0904; found 345.0896.

Methyl 3-(isopropylthio)-4,6-dioxo-5-phenyl-2,4,5,6-tetrahydropyrrolo[3,4-*c*]pyrrole-1-carboxylate (3d). Colorless powder. mp 174-175 °C (hexane/$CHCl_3$). R_f 0.26 (EtOAc/hexane, 1:2). IR (ν_{max} cm^{-1}): 3232 (NH), 1715 (C=O), ^1H NMR (250 MHz, $CDCl_3$): 9.91 (br s, 1H, NH), 7.50-7.29 (m, 5H, phenyl group), 4.13 (heptet, *J* 6.5 Hz, 1H, SCH), 4.00 (s, 3H, OMe), 1.35 (d, *J* 6.5 Hz, 6H, 2 × Me). ^{13}C NMR (62.5 MHz, $CDCl_3$): 162.0 (C=O), 160.9 (C=O), 159.3 (CO_2R), 132.2, 128.9, 127.9, 126.8 (phenyl group), 129.4 (C_2), 129.2 (C_4 or C_3), 126.9 (C_4 or C_3), 119.3 (C_5), 53.0 (OMe), 40.1 (SCH), 23.4 (Me). HRMS (ESI-Orbit trap) *m/z* $[M+H]^+$ calcd for $C_{17}H_{16}N_2O_4S+H$, 345.0904; found 345.090.

Methyl 3-(butylthio)-4,6-dioxo-5-phenyl-2,4,5,6-tetrahydropyrrolo[3,4-*c*]pyrrole-1-carboxylate (3e). Colorless powder. mp 151-152 °C (hexane/$CHCl_3$). R_f 0.28 (EtOAc/hexane, 1:2). IR (ν_{max} cm^{-1}): 3548 (NH), 1715 (C=O). ^1H NMR (250 MHz, $CDCl_3$): 9.95 (br s, 1H, NH), 7.50-7.29 (m, 5H, phenyl group), 4.00 (s, 3H, OMe), 3.37 (t, *J* 7.5 Hz, 2H, SCH_2,), 1.67-1.63 (m, 2H, CH_2), 1.48-1.41 (m, 2H, CH_2), 0.91 (t, *J* 7.5 Hz, 3H, Me). ^{13}C NMR (62.5 MHz, $CDCl_3$): 162.0 (C=O), 161.0 (C=O), 159.4 (CO_2R), 132.2, 128.9, 127.9, 126.9 (phenyl group), 131.1 (C_2), 129.1 (C_4 or C_3), 126.2 (C_4 or C_3), 119.0 (C_5), 53.0 (OMe), 34.1 (SCH_2), 31.7 (CH_2), 21.5 (CH_2), 13.5 (Me). HRMS (ESI-Orbit trap) *m/z* $[M+H]^+$ calcd for $C_{17}H_{16}N_2O_4S+H$, 359.1060; found 359.1047.

Methyl 3-(isobutylthio)-4,6-dioxo-5-phenyl-2,4,5,6-tetrahydropyrrolo[3,4-*c*]pyrrole-1-carboxylate (3f). Colorless powder. mp 174-175 °C (hexane/$CHCl_3$). R_f 0.30 (EtOAc/hexane, 1:2). IR (ν_{max} cm^{-1}): 3228 (NH), 1718 (C=O). ^1H NMR (250 MHz, $CDCl_3$): 9.99 (br s, 1H, NH), 7.50-7.27 (m, 5H, phenyl group), 3.99 (s, 3H, OMe), 3.28 (d, *J* 7.0 Hz, 2H, SCH_2,), 1.92-1.87 (m, 1H, CH), 1.53 (d, *J* 6.5 Hz 6H, 2 × Me). ^{13}C NMR (62.5 MHz, $CDCl_3$): 162.0 (C=O), 161.0 (C=O), 159.5 (CO_2R), 132.2, 128.9, 127.9, 126.9 (phenyl group), 131.6 (C_2), 124.5 (C_4 or C_3), 121.3 (C_4 or C_3), 118.9 (C_5), 53.0 (OMe), 42.8 (SCH_2), 28.9 (CH), 21.5 (Me). HRMS (ESI-Orbit trap) *m/z* $[M+H]^+$ calcd for $C_{18}H_{18}N_2O_4S+H$, 381.0879; found 381.0872.

Methyl 3-(benzylthio)-4,6-dioxo-5-phenyl-2,4,5,6-tetrahydropyrrolo[3,4-*c*]pyrrole-1-carboxylate (3g). Colorless needles. mp 185-186 °C (hexane/EtOAcR_f 0.18 (EtOAc/hexane, 1:2). IR (ν_{max} cm^{-1}): 3480 (NH), 1716 (C=O). ^1H NMR (250 MHz, $CDCl_3$): 9.42 (br s, 1H, NH), 7.50-7.27 (m, 10H, phenyl groups), 4.50 (s, 2H, SCH_2), 3.96 (s, 3H, OMe). ^{13}C NMR (62.5 MHz,

$CDCl_3$): 162.1 (C=O), 161.0 (C=O), 159.1 (CO_2R), 136.3, 129.0, 128.9, 128.8, 126.9, 126.9 (phenyl groups), 132.2 (C_2), 124.1 (C_4 or C_3), 122.8 (C_4 or C_3), 119.4 (C_5), 52.9 (OMe), 32.9 (SCH_2). HRMS (ESI-Orbit trap) *m/z* $[M+H]^+$ calcd for $C_{21}H_{16}N_2O_4S$+H, 415.0723; found 415.0717.

Ethyl 3-(methylthio)-4,6-dioxo-5-phenyl-2,4,5,6-tetrahydropyrrolo[3,4-*c*]pyrrole-1-carboxylate (3h). Colorless powder. mp 161-162 °C (hexane/$CHCl_3$). R_f 0.27 (EtOAc/hexane, 1:2). IR (ν_{max} cm^{-1}): 3230 (NH), 1718 (C=O). ^{1}H NMR (250 MHz, $CDCl_3$): 10.11 (br s, 1H, NH), 7.51-7.29 (m, 5H, phenyl group), 4.45 (q, *J* 7.0 Hz, 2H, OCH_2), 2.85 (s, 3H, SMe), 1.44 (t, *J* 7.0 Hz, 3H, Me). ^{13}C NMR (62.5 MHz, $CDCl_3$): 162.1 (C=O), 161.1 (C=O), 160.0 (CO_2R), 132.3, 129.0, 128.0, 127.0 (phenyl group), 132.2 (C_2), 124.5 (C_4 or C_3), 121.0 (C_4 or C_3), 119.4 (C_5), 62.3 (OCH_2), 17.0 (Me), 14.2 (SMe). HRMS (ESI-Orbit trap) *m/z* $[M+H]^+$ calcd for $C_{16}H_{14}N_2O_4S$+H, 331.0747; found 331.0754.

Ethyl 3-(benzylthio)-4,6-dioxo-5-phenyl-2,4,5,6-tetrahydropyrrolo[3,4-*c*]pyrrole-1-carboxylate (3i). Colorless powder. mp 181-182 °C (hexane/EtOAc). R_f 0.33 (EtOAc/hexane, 1:2). IR (ν_{max} cm^{-1}): 3230 (NH), 1720 (C=O). ^{1}H NMR (250 MHz, $CDCl_3$): 9.78 (br s, 1H, NH), 7.59-7.26 (m, 10H, phenyl groups), 4.50 (s, 2H, SCH_2), 4.41 (q, *J* 7.0 Hz, 2H, -OCH_2,), 1.41 (t, *J* 7.0 Hz, 3H, Me). ^{13}C NMR (62.5 MHz, $CDCl_3$): 162.2 (C=O), 161.0 (C=O), 160.9 (CO_2R), 136.3, 129.0, 128.9, 128.8, 128.7, 127.9, 127.0 (phenyl groups), 132.2 (C_2), 124.1 (C_4 or C_3), 122.7 (C_4 or C_3), 119.8 (C_5), 62.3 (OCH_2), 39.0 (SCH_2), 14.1 (Me). HRMS (ESI-Orbit trap) *m/z* $[M+H]^+$ calcd for $C_{22}H_{18}N_2O_4S$+H, 407.1060; found 407.1065.

Isopropyl 3-(methylthio)-4,6-dioxo-5-phenyl-2,4,5,6-tetrahydropyrrolo[3,4-*c*]pyrrole-1-carboxylate (3j). Colorless powder. mp 174-175 °C (hexane/EtOAc). R_f 0.36 (EtOAc/hexane, 1:2). IR (ν_{max} cm^{-1}): 3234 (NH), 1760 (C=O), 1708 (C=O). ^{1}H NMR (250 MHz, $CDCl_3$): 9.99 (br s, 1H, NH), 7.56-7.28 (m, 5H, phenyl group), 5.62 (heptet, *J* 7.0 Hz, 1H, CH), 2.84 (s, 3H, SMe), 1.42 (d, *J* 7.0 Hz, 6H, OCH$\underline{Me}_2$). ^{13}C NMR (62.5 MHz, $CDCl_3$): 162.1 (C=O), 161.0 (C=O), 158.6 (CO_2R), 132.3, 129.0, 128.0, 127.1 (phenyl group), 131.9 (C_2), 124.4 (C_4 or C_3), 121.0 (C_4 or C_3), 119.9 (C_5), 70.5 (OCH), 21.8 (CH$\underline{Me}_2$), 17.1 (SMe). HRMS (ESI-Orbit trap) *m/z* $[M+H]^+$ calcd for $C_{17}H_{16}N_2O_4S$+H, 345.0904; found 345.0912.

Isopropyl 3-(benzylthio)-4,6-dioxo-5-phenyl-2,4,5,6-tetrahydropyrrolo[3,4-*c*]pyrrole-1-carboxylate (3k). Colorless powder. mp 181-182 °C (hexane/$CHCl_3$). R_f 0.41 (EtOAc/hexane, 1:2). IR (ν_{max} cm^{-1}): 3245 (NH), 1718 (C=O). ^{1}H NMR (250 MHz, $CDCl_3$): 9.69 (br s, 1H, NH), 7.51-7.23 (m, 10H, phenyl groups), 5.20 (heptet, *J* 6.0 Hz, 1H, CH,), 4.50 (s, 2H, SCH_2), 1.39 (d, *J* 6.0 Hz, 6H, CH$\underline{Me}_2$). ^{13}C NMR (62.5 MHz, $CDCl_3$): 162.3 (C=O), 161.0 (C=O), 158.4 (CO_2R), 136.4, 129.0, 128.9, 128.8, 128.7, 127.8, 127.0, 126.2 (phenyl groups), 132.3 (C_2), 124.1 (C_4 or C_3), 122.6 (C_4 or C_3), 120.3 (C_5), 70.5 (OCH), 38.9 (CH$\underline{Me}_2$), 21.8 (SCH_2). HRMS (ESI-Orbit trap) *m/z* $[M+H]^+$ calcd for $C_{23}H_{20}N_2O_4S$+H, 421.1217; found 421.1220.

***tert*-Butyl 3-(methylthio)-4,6-dioxo-5-phenyl-2,4,5,6-tetrahydropyrrolo[3,4-*c*]pyrrole-1-carboxylate (3l).** Colorless powder. mp 173-174 °C (hexane/$CHCl_3$). R_f 0.48 (EtOAc/hexane, 1:2). IR (ν_{max} cm^{-1}): 3211 (NH), 1760 (C=O), 1718 (C=O). ^{1}H NMR (250 MHz, $CDCl_3$): 10.18 (br s, 1H, NH), 7.62-7.28 (m, 5H, phenyl group), 2.82 (s, 3H, SMe), 1.64 (s, 9H, *t*-BuO).^{13}C NMR

(62.5 MHz, $CDCl_3$): 162.2 (C=O), 161.1 (C=O), 158.3 (CO_2R), 132.4, 129.0, 128.0, 127.2 (phenyl group), 131.5 (C_2), 124.0 (C_4 or C_3), 121.1 (C_4 or C_3), 121.0 (C_5), 84.0 (OC), 28.2 ($C\underline{Me}_3$), 17.1 (SMe). HRMS (ESI-Orbit trap) *m/z* $[M+Na]^+$ calcd for $C_{18}H_{18}N_2O_4S$+H, 381.0879; found 381.0872.

Benzyl 3-(methylthio)-4,6-dioxo-5-phenyl-2,4,5,6-tetrahydropyrrolo[3,4-*c*]pyrrole-1-carboxylate (3m). Colorless powder. mp 186-187 ^oC (hexane/$CHCl_3$). R_f 0.42 (EtOAc/hexane, 1:2). IR (ν_{max} cm^{-1}): 3232 (NH), 1762 (C=O), 1718 (C=O). ^1H NMR (250 MHz, $CDCl_3$): 9.57 (br s, 1H, NH), 7.58-7.26 (m, 10H, phenyl groups), 5.43 (s, 2H, OCH_2), 2.84 (s, 3H, SMe). ^{13}C NMR (62.5 MHz, $CDCl_3$): 162.2 (C=O), 160.9 (C=O), 158.5 (CO_2R), 135.0, 129.3, 129.2, 128.9, 128.6, 128.5, 128.4, 128.1, 128.0, 127.9, 127.1 (phenyl groups), 132.3 (C_2), 124.8 (C_4 or C_3), 121.2 (C_4 or C_3), 119.0 (C_5), 67.5 (OCH_2), 17.0 (SMe). HRMS (ESI-Orbit trap) *m/z* $[M+Na]^+$ calcd for $C_{21}H_{16}N_2O_4S$+H, 415.0723; found 415.0710.

Benzyl 3-(benzylthio)-4,6-dioxo-5-phenyl-2,4,5,6-tetrahydropyrrolo[3,4-*c*]pyrrole-1-carboxylate (3n). Colorless powder. mp 189-190 ^oC (hexane/$CHCl_3$). R_f 0.38 (EtOAc/hexane, 1:2). IR (ν_{max} cm^{-1}): 3230 (NH), 1724 (C=O).^1H NMR (250 MHz, $CDCl_3$): 9.74 (br s, 1H, NH), 7.52-7.26 (m, 15H, phenyl groups), 5.40 (s, 2H, OCH_2), 4.49 (s, 2H, SCH_2). ^{13}C NMR (62.5 MHz, $CDCl_3$): 162.2 (C=O), 160.9 (C=O), 158.5 (CO_2R), 136.3, 134.9, 129.0, 128.9, 128.8, 128.6, 128.4, 128.1, 128.0, 127.9, 127.1 (phenyl groups), 132.2 (C_2), 124.5 (C_4 or C_3), 119.4.2 (C_4 or C_3), 112.6 (C_5), 67.5 (OCH_2), 39.0 (SCH_2). HRMS (ESI-Orbit trap) *m/z* $[M+Na]^+$ calcd for $C_{27}H_{20}N_2O_4S$+H, 491.1036; found 491.1032.

Biological evaluation

All reagents used were commercially available by Aldrich Chemical Co. Milwaukee, WI, (USA): nordihydroguaiaretic acid (NDGA), trolox, 2,2-azobis(2-amidinopropane) dihydrochloride (AAPH), soybean lipoxygenase and linoleic acid sodium salt.

Inhibition of linoleic acid peroxidation[34]

Production of conjugated diene hydroperoxide by oxidation of linoleic acid in an aqueous dispersion was monitored at 234 nm; azobis(2-amidinopropane) dihydrochloride (AAPH) was used as a free radical initiator. 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, thermostated at 37 ^oC 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 ^oC was monitored by recording the increase in absorption at 234 nm caused by conjugated diene hydroperoxide formation (Tables 3-4).

In vitro soybean lipoxygenase inhibition study

The *in vitro* study was evaluated as reported previously.[34] The test compounds dissolved in ethanol were incubated at room temperature with sodium linoleate (100 μL) and 0.2 mL of

enzyme solution (1/9 × 10^{-4} w/v in saline). The conversion of sodium linoleate to 13-hydro-peroxylinoleic acid at 234 nm was recorded and compared with the appropriate standard inhibitor (NDGA) (Tables 3-4).

Acknowledgements

K.O. thanks IKY for a fellowship. We thank the MS and NMR facility centers of the University of Ioannina and especially Mr. Konstantinos Tsiafoulis for obtaining 2D NMR experiments. K. O. also thanks Professors L. P. Hadjiarapoglou and M. Stratakis and Dr. Z. Syrgiannis for encouragement, helpful comments, discussion and guidance and most of all to the late Professor Yiannis Elemes, who recently passed away in July 2014, that came up with this work, guided with all his heart and passion for Organic Chemistry and always was there as a great tutor and friend.

References

1. Gothelf, K. V.; Jorgensen, K. A. *Chem. Rev.* **1998**, *98*, 863. http://dx.doi.org/10.1021/cr970324e
2. Coldham, I.; Hufton, R. *Chem. Rev.* **2005**, *105*, 2765. http://dx.doi.org/10.1021/cr040004c
3. Michael, J. P. *Nat. Prod. Rep*. **2008**, *25*, 139. http://dx.doi.org/10.1039/b612166g
4. Nair, V.; Suja, T. D. *Tetrahedron* **2007**, 63, 12247. http://dx.doi.org/10.1016/j.tet.2007.09.06
5. Husinec, S.; Savic, V. *Tetrahedron:Asymmetry* **2005**, *16*, 2047. http://dx.doi.org/10.1016/j.tetasy.2005.05.020
6. Mancebo-Aracil, J.; Marti-Rodriguez, M.; Najera, C.; Sansano, J. M.; Costa P. R. R.; Crizanto de Lima E.; Dias A. G. *Tetrahedron:Asymmetry* **2012**, *23*, 1596. http://dx.doi.org/10.1016/j.tetasy.2012.10.015
7. Puglisi, A.; Benaglia, M.; Cinquini, M.; Cozzi, F.; Celentano, G. *Eur. J. Org. Chem*. **2004**, 567. http://dx.doi.org/10.1002/ejoc.200300571
8. Jiang, K.; Chen, Y. C. *Tetrahedron Lett*. **2014**, *55*, 2049. http://dx.doi.org/10.1016/j.tetlet.2014.02.036
9. Shi, F.; Xing, G. J.; Tan, W.; Zhu, R. Y.; Tu, S. *Org. Biomol. Chem.* **2013**, *11*, 1482. http://dx.doi.org/10.1039/c2ob26566d
10. Pearson, W. H.; Stoy, P.; Mi, Y. *J. Org. Chem*. **2004**, *69*, 1919. http://dx.doi.org/10.1021/jo030334h

11. Chen, Y.; Zeng, D. X.; Xie, N.; Dang, Y. Z. *J. Org. Chem.* **2005**, *70*, 5001. http://dx.doi.org/10.1021/jo050236r
12. Sonesson, C.; Larhed, M.; Nyqvist, C.; Hallberg, A. *J. Org. Chem.* **1996**, *61*, 4756. http://dx.doi.org/10.1021/jo952112s
13. Shi, W.; Duan, Y.; Qian, Y.; Li, M.; Yang, L.; Hu, W. *Bioorg. Med. Chem. Lett*. **2010**, *20*, 3592. http://dx.doi.org/10.1016/j.bmcl.2010.04.123
14. Anderson, W. K.; Milowsky, A. S. *J. Med. Chem*. **1987**, *30*, 2144. http://dx.doi.org/10.1021/jm00394a036
15. Kalai, T.; Petrlova, J.; Balog, M.; Aung, H. H.; Voss, J. C.; Hideg, K. *Eur. J. Med. Chem.* **2011**, *46*, 1348. http://dx.doi.org/10.1016/j.ejmech.2011.01.059
16. Shi, F.; Tao, Z. L.; Yu, J.; Tu, S. J. *Tetrahedron:Asymmetry* **2011**, *22*, 2056. http://dx.doi.org/10.1016/j.tetasy.2011.11.020
17. Ioannou, E.; Hirsch, A.; Elemes, Y. *Tetrahedron* **2007**, *63*, 7070. http://dx.doi.org/10.1016/j.tet.2007.05.011
18. Georgiou, D.; Toutountgoglou, V.; Kenneth, W. M.; Hadjipavlou-Litina, D.; Elemes, Y. *Bioorg. Med. Chem.* **2012**, *20*, 5103. http://dx.doi.org/10.1016/j.bmc.2012.07.014
19. Garbaccio, R. M.; Fraley, M. E.; Tasber, E. S.; Olson, C. M.; Hoffman, W. F.; Arrington, K. L.; Torrent, M.; Buser, C. A.; Walsh, E. S.; Hamilton, K.; Schaber, M. D.; Fernandes, C.; Lobell, R. B.; Tao, W.; South, V. J.; Yan, Y.; Kuo, L. C.; Prueksaritanont, T.; Slaughter, D. E.; Shu, C.; Heimbrook, D. C.; Kohl, N. E.; Huber, H. E.; Hartman, G. E. *Bioorg. Med. Chem. Lett*. **2006**, *16*, 1775. http://dx.doi.org/10.1016/j.bmcl.2005.12.094
20. Denisov, E. T.; Denisova, T.; In *Handbook of Antioxidants: Bond Dissociation Energies, Rate Constants, Activation Energies, and Enthalpies of Reactions*, Taylor Francis Inc, United States, Second Edition, 1999.
21. Hoppe, D.; Beckmann, L. *Liebigs Ann. Chem*. **1979**, 2066. http://dx.doi.org/10.1002/jlac.197919791217
22. Llompart, D. F.; Sarroti, A. M.; Corne, V.; Suarez, A. G.; Spanevello, R. A.; Echeverria, G. A.; Piro, O. E.; Castellano, E. E. *Tetrahedron Lett*. **2014**, *55*, 2394. http://dx.doi.org/10.1016/j.tetlet.2014.02.113
23. Barr, D. A.; Dorrity, M. J.; Grigg, R.; Hargreaves, S.; Malone, J. F.; Montgomery, J.; Redpath, J.; Stevenson, P.; Thornton-Pett, M. *Tetrahedron* **1995**, *51*, 273. http://dx.doi.org/10.1016/0040-4020(94)00940-V
24. Lehuede, J.; Fauconneau, B.; Barrier, L.; Ourakow, M.; Piriou, A.; Vierfonf, J. M. *Eur. J. Med. Chem.* **1999**, *34*, 991. http://dx.doi.org/10.1016/S0223-5234(99)00111-7

25. Kumagai, T.; Tanaka, S.; Mukai, T. *Tetrahedron Lett*. **1984**, *25*, 5669. http://dx.doi.org/10.1016/S0040-4039(01)91408-X
26. Wang, J.-L.; Ueng, C.-H.; Yeh, M.-C. P. *Tetrahedron Lett*. **1995**, *36*, 2823. http://dx.doi.org/10.1016/0040-4039(95)00407-4
27. Padwa, A.; Haffmanns, G.; Tomas, M. *J. Org. Chem.* **1984**, *49*, 3314. http://dx.doi.org/10.1021/jo00192a013
28. Martin, M. C.; Patil, D. V.; France, S. *J. Org. Chem.* **2014**, *79*, 3030. http://dx.doi.org/10.1021/jo5001059
29. Liegois, C.; Lermusieau, G.; Colin, S. *J. Agric. Food Chem*. **2000**, *48*, 1129. http://dx.doi.org/10.1021/jf9911242
30. Huang, D.; Ou, B.; Prior, R. L. *J. Agric. Food Chem*. **2005**, *53*, 1841. http://dx.doi.org/10.1021/jf030723c
31. Taraborewala, R. B.; Kauffman, J. M. *J. Pharm. Sci*. **1990**, *79*, 173. http://dx.doi.org/10.1002/jps.2600790219
32. Crooks, S. W.; Stockley, R. A. *Int. J. Biochem. Cell Biol*. **1998**, *30*, 173.
33. Armarego, W. L. F.; Perrin, D. D. In *Purification of Laboratory Chemicals*, Redd Educational and Profesional Publishing: Oxford, 1996.
34. Hadjipavlou-Litina, D.; Garneli, T.; Athanassopoulos, C. M.; Papaioannou, D. *J. Enzyme Inhib. Med. Chem*. **2009**, *24*, 1188.

