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Synthesis of sulfur containing dihydro-pyrrolo derivatives and their biological evaluation as antioxidants

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ABSTRACT

The 1,3-dipolar cycloaddition to *N*-phenylmaleimide of azomethine ylides, generated in situ from sulfanyl-substituted imines of glycine esters, yields 5*H*-dihydro-pyrrolo products with *syn* diastereoselectivity. The *syn* (major) and *anti* (minor) products were isolated chromatographically and fully characterized by spectroscopic methods and in two cases also by X-ray analysis. The diastereomeric cycloadducts were tested for their antioxidant activity with good results.

1. Introduction

Cycloaddition reactions are among the most important and powerful tools for ring construction in organic synthesis.[1] In particular, 1,3-dipolar cycloadditions have been a fertile source of ring systems with extended applications in medicine, industry and the laboratory.[2] Azomethine ylides are reactive 1,3-dipoles and, with electrophiles, give rise to a variety of five-membered ring nitrogen heterocyclic compounds which have synthetic applications in heterocyclic and natural product chemistry.[3] Heterocyclic compounds are significant because of their biological activity and their applications in diverse fields. Heterocyclic moieties are important in naturally occurring compounds, as in alkaloids, vitamins, hormones, and antibiotics. They are also widely present in pharmaceutical products, herbicides, and dyes.

A major route to azomethine ylides involves imines of amino acids or their derivatives (either previously synthesized or formed in situ); numerous reviews testify to the importance of this topic.[4] Sulfur-containing imines are important intermediate substrates for amino acid synthesis: with a chiral auxiliary they produce excellent precursors for the highly diastereoselective synthesis of optically active amino acid derivatives,[5] which has found applications on the synthesis of labeled amino acid derivatives.[5e,f]

Our recent interest in 1,3-dipolar cycloadditions of sulfur-substituted azomethine ylides to C_{60},[6] led us to a systematic investigation of the fore mentioned reaction between sulfur-containing imines of glycine esters with electrophile such as *N*-phenylmaleimide (NPM). Sulfur-containing nitrogen heterocycles might be of biological interest and may play an antioxidative or a biological role in general due to the sulfur functionality.[7] We decided to explore the applicability of this reaction in terms of different sulfide- and alkoxy-substituents in the imines. We report here on the isolation of *syn* and *anti* 5*H*-dihydro-pyrrolo derivatives, from the cycloaddition of a series of *N*,*N*-[bis-methylsulfanyl]-imines of glycine esters to NPM, in good yields under neutral conditions at elevated temperatures, and their full spectroscopic characterization. In addition we tested most of the cycloadducts for their possible antioxidant activity. In the literature there was only one example of such a reaction (with imine **1a** and NPM) in strongly acidic solution (stoichiometric, to the reactants, quantity of AcOH)

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reported to give only the **2a**-*syn* adduct,[9] which was isolated and briefly characterized by ^{1}H NMR.

2. Results and discussion

The following methyl 2-[bis (alkylsulfanyl or aralkylsulfanyl) methyleneamino] acetates, **1a–f**, were prepared by known procedures,[5a] Figure 1.

In general, the cycloaddition reaction was performed by placing in a screw-capped test-tube flushed with Ar the desired quantity of the imine of methyl glycinate, **1**, dissolved in dry toluene. To this solution solid NPM (10% excess with respect to the imine) was added in one portion. The tube was closed and heated to ~185–190 °C (below this temperature little or no reaction took place as detected by TLC). The reaction was followed by TLC and stopped after 24 h. Volatile compounds were removed with a rotary evaporator and high vacuum pump. Column chromatography on SiO_2, EtOAc/*n*-hexanes, ~1/2 v/v) afforded *syn* and *anti* cycloaddition products **2**, Scheme 1.

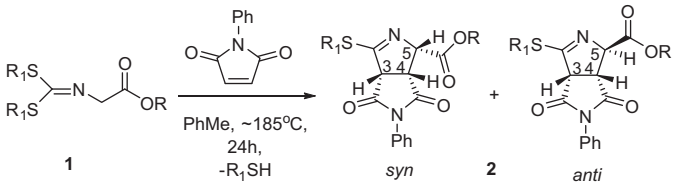
These were the only products detected by TLC during the reaction. An aliquot taken periodically from the reaction mixture showed the formation of the *syn* product already within 20 minutes after the beginning of the reaction. Throughout of this work the expected cycloaddition products (with two –SR substituents) never detected nor isolated. In addition the elimination of a mercaptane molecule during the reaction course was indirectly detected with the isolation and characterization of the Thia Michael addition of MeSH to NPM (see footnote b in Table 2).

The two diastereomers isolated from the reaction of each imine, **1**, were fully characterized by spectrometric means and by elemental analysis. In general, ^{13}C NMR reveals four resonances for the three carbonyls and the imine carbon nuclei. In the ^{1}H NMR the stereogenic carbon center imposes multiplicity to the diastereotopic methylene hydrogens of the sulfide substituents. Furthermore in the *syn* adducts, there is a large coupling constant between H_4 and H_5 ($J_{H4,H5}$ ~9.50 Hz), whereas the corresponding coupling constant is smaller in the *anti* isomers ($J_{H4,H5}$ ~2.20 Hz), testifying to their relative stereochemistry (i.e., *cis* and *trans*, respectively). Smaller differences were observed for the $J_{H3,H4}$ coupling constants (i.e., ~9.20 Hz for the *syn* and ~8.40 Hz for the *anti* isomer. The above coupling constants are in close relation with the reported values for **2a** stereoisomers in Ref. 9: $J_{H4,H5}$ = 9.9 Hz and $J_{H3,H4}$ = 8.8 Hz for the *syn* and $J_{H4,H5}$ = 2.5 Hz and $J_{H3,H4}$ = 8.45 Hz for the *anti* isomer.

It is interesting to note that in all cases the *anti* adduct invariably eluted first from the column, revealing its smaller polarity with regard to the *syn* diastereomer. Yields and molar ratios of the isolated products, together with diastereomeric ratios (d.r.) are shown in Table 1.

In the case of adducts **2a**, crystals of adequate quality for XRD were obtained from CH_2Cl_2/*n*-hexanes, 2/1, v/v. From the resulting diffraction analyses, Figure 2, the *anti*- or *syn*-relation of the ester group and the maleimide ring and also between the ring hydrogens is evident.

Finally, the cycloaddition was performed under the same, as above, experimental conditions with the ethyl-, benzyl-, and



Scheme 1. Mixture of *syn* and *anti* products **2**, from the 1,3-dipolar cycloaddition of imines **1**, to *N*-phenylmaleimide.

Table 1
Yields of *syn* and *anti* diastereomers isolated from the reaction of imines **1** to NPM[a,b]

Adduct	R	*syn* (%)	*anti* (%)	Total (%)	d.r.
2a	Me	66	10	76	6.6
2b	Et	69 (60)[c]	25 (0)[c]	94	2.8
2c	*n*-Pr	44	34	78	1.3
2d	*i*-Pr	35	27	62	1.3
2e	*n*-Bu	59	3	62	19.7
2f	Bn	30	9	39	3.3

[a] 10% excess of NPM was used in every run, unless otherwise specified.
[b] Yields % of isolated adducts after chromatographic separation.
[c] Under strong acidic conditions, see Ref. 9.

Table 2
Isolated yields of *syn* and *anti* cycloadducts and diastereomeric ratios after chromatographic purification, for the cycloaddition of imines **1g–i** with NMP[a]

Ester	*syn* (%)	*anti* (%)	Total (%)	d.r.
–OMe	66.0	10.0	76.0	6.6
–OEt	37.8	10.2	48.0	3.7
–OBn	38.0	8.2[b]	46.2	4.6
–O^{t}Bu	32.2	5.7	37.9	5.6

[a] 10% excess of NPM was used in every run, unless otherwise specified.
[b] Characterized as a mixture with the MeSH Michael addition product to NPM.[8]

t-butyl- esters of the –SMe substituted glycine imines **1g–i**, Scheme 2. Isolated yields of the two diastereomers and the obtained d.r. are listed in Table 2.

At this point we should comment on the absence of any clear correlation between the d.r. and the size of either the sulfide or the alkoxy substituents used in this work.

According to an earlier report, that only closed adducts were isolated in the reaction of a cyclic thioiminoether (cyclic monosubstituted imine) with NPM,[9] and the results of the present work we have to conclude that the reaction follows the azomethine ylide path. More work though is needed to a definite answer for a more detailed mechanism of this process.

3. Biological studies

We tested the new compounds, from Table 1, with regard to their antioxidant ability and in comparison to well known antioxidant agents, for example, nordihydroguaiaretic acid (NDGA) and trolox.

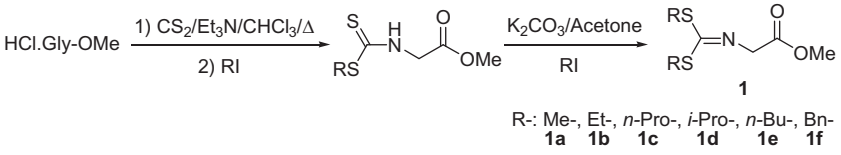


Figure 1. General synthetic procedure for methyl 2-[bis (alkylsulfanyl or aralkylsulfanyl) methyleneamino] acetate **1**, used as substrates for the 1,3-dipolar cycloaddition to NPM.

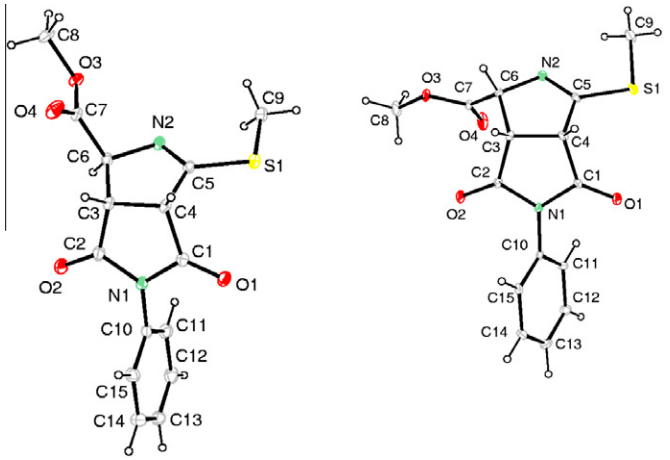
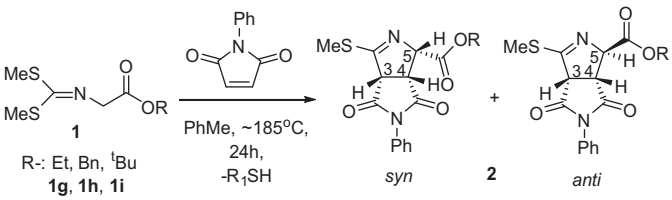


Figure 2. Views of the molecules of the **2a**-*anti* (left) and **2a**-*syn* (right) isomers. The samples were racemic. In the **2a**-*anti* molecule shown all three chiral centers have *S* configurations whereas in the **2a**-*syn* molecule the configurations of the chiral carbon atoms are 3-*S*, 4-*S*, 6-*R*.



Scheme 2. 1,3-Dipolar cycloadditions of imines **1g–i** with NPM.

Antioxidants are defined as substances that, even at low concentration, significantly delay or prevent oxidation of easily oxidizable substrates. The formation of Reactive Oxygen Species (ROS) is characteristic of aerobic organisms, as an unavoidable consequence of cell metabolism. Their involvement in inflammation, cancer, myocardial and central nervous system (CNS) ischemia is under intensive study. For the estimation of the antioxidative potential of chemical components, different experimental approaches were used. The most popular screening assays have been developed to be fast and easy. Most of them require a spectrophotometric measurement and a certain reaction time in order to obtain reproducible results.[10] However, all the used assays do not measure the same chemistry. In this way, factors such as solubility or steric hindrance, which may be of overriding importance in one environment but not in another, can be varied and the antioxidant ability of a compound in a variety of milieus may be evaluated.

The interaction/reducing activity (RA) of the examined compounds with the stable free radical DPPH is shown in Table 3, first row. This interaction, which indicates their radical scavenging ability in an iron-free system, was measured at 50 μM (equimolar to the DPPH concentration) for 20/60 min). In the DPPH assay, the dominant chemical reaction involved is the reduction of the DPPH radical by an ET from the antioxidant. Particularly effective such antioxidants are the phenoxide anions from phenolic compounds like catechol and derivatives, such as NDGA. Under our experimental conditions very low interaction values were observed.

The use of the free radical reactions initiator 2,2-azobis(2-amidinopropane) dihydrochloride (AAPH) is recommended as more appropriate for measuring radical-scavenging activity in vitro, because the activity of the peroxyl radicals produced by the action of AAPH shows a greater similarity to cellular activities such as lipid peroxidation.[11] Oxidation of sodium linoleate by a thermal free radical initiator (AAPH) is followed by UV spectrophotometry in a highly diluted sample.[12] In the AAPH assay, the highly reactive alkylperoxyl radicals are intercepted mainly by a hydrogen atom transfer (HAT) from the antioxidant.[13] 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. All the present compounds caused strong inhibition of lipid peroxidation (72–98%) and higher than trolox (63%) (Table 3, third row). Not much difference is observed between *syn* and *anti* isomers. Since these compounds lack the easily oxidizable phenol unit present in trolox, their inhibitory potency should be attributed to other factors. The antioxidant effect of 5*H*-dihydro-pyrrolo products is possible to be supported by the following mechanism of C centered radical stabilization by resonance, Figure 3.

Eicosanoids are oxygenated metabolites of arachidonic acid with a broad implication in a diversity of diseases. Upon appropriate stimulation of neutrophils, arachidonic acid (AA) is cleaved from membrane phospholipids and can be converted into leukotrienes (LTs). 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[14] with a marked relation to the severity of cardiovascular diseases, asthma and cancer. In this context, we decided to further evaluate the synthesized compounds for their ability to inhibit soybean LOX by the UV absorbance based enzyme assay.[15] Most of the LOX inhibitors are antioxidants or free radical scavengers. NDGA, a known inhibitor of soybean LOX, has been used as a reference compound (64%) and as a positive control. All the compounds inhibit soybean LOX, with the exception of Me-*syn* isomer (Table 3, first column, second row). No big differences are observed between the % inhibition values of Pr-*syn*/*anti* isomers and *i*Pr-*syn*/*anti* isomers. In two cases, the *anti*-isomers of Bu- and Bn-substituted adducts, are more potent than their respective *syn*. This might be attributed to a better fit of the former isomer in the active space of the enzyme due to steric reasons compared to the latter.

Table 3
Biological evaluation of cycloadducts **2a–f** as antioxidants

Method/adduct	Me *syn*	Me *anti*	Et *syn*	Et *anti*	Pr *syn*	Pr *anti*	*i*-Pr *syn*	*i*-Pr *anti*	Bu *syn*	Bu *anti*	Bn *syn*	Bn *anti*	NDGA	Trolox
DPPH%@50 μM 20min[a]	8	26	na[d]	11	9	7	13	12	7	11	11	15	81	
%LOX Inh. @100μM[b]	dec[e]	51	26	81	39	43	44	49	56	91	73	83	64	
AAPH%@100μM[c]	84	93	89	91	86	86	72	89	89	82	76	98		63

[a] % Interaction with DPPH (RA%).
[b] In vitro % inhibition of soybean lipoxygenase (LOX).
[c] % Inhibition of lipid peroxidation (AAPH).
[d] No activity under the experimental conditions.
[e] Not stable under the experimental conditions.

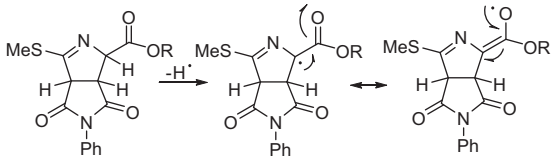


Figure 3. Radical stabilization by resonance.

4. Conclusions

In summary, we show here that *syn* diastereoselectivity prevails when a series of *N*,*N*-bis[sulfanyl]-substituted imines of glycinates undergo 1,3-dipolar cycloaddition to *N*-phenylmaleimide, and the two diastereomeric dihydro-pyrrolo adducts bearing a vinylic sulfide substituent were isolated and characterized in each reaction. Furthermore, it was found that the size of the sulfanyl and alkoxy substituents in the starting imine plays no definite role on the rate or the diastereoselectivity of the reaction. Qualitatively, and also in the light of a previous study the observed behavior is consonant with a cycloaddition through an azomethine ylide dipole, although more mechanistic studies are needed to settle more accurate the mechanism. These cycloadducts present interesting biological activities as antioxidant agents. Especially the *anti*- Bu and Bn substituted adducts with the higher lipophilicity values[16] (*c*log*P* 3.31 and 3.38, respectively) highly inhibit lipid peroxidation and soybean LOX and they might serve as lead compounds. Finally the isolated diastereomeric cycloadducts showed a hyperoxidation activity with three major standard methods.

5. Experimental

5.1. General

All NMR spectra were taken in $CDCl_3$ 98% D, on a Bruker-Spectrospin, Avance spectrometer. ESI MS spectra were taken on a Agilent Technologies 1100 Series LC/MSD-Trap-SL spectrometer. FAB MS were taken on a MICROMASS ZABSPEC spectrometer (FAB modus, with 3-nitrobenzylalcohol as matrix). HRMS were taken on an Orbitrap LTQ/XL instrument. FT IR spectra were taken on a Perkin Elmer Spectrum GX, FT IR System, spectrometer. UV–vis spectra were recorded on a Hitachi U-2001 spectrophotometer. All reagents and solvents were obtained from commercial suppliers and used without further purification. Dry quality solvents were obtained according to literature procedures,[17] and stored over MS 4Å under Ar atmosphere. Thus, PhMe distilled from Na with benzophenone as an indicator. Compounds **1**, were prepared by known literature methods.[5a]

5.2. General procedure for 1,3-dipolar cycloaddditions; isolation of adducts 2

A screw-capped 10 mL tube flashed with Ar. In it were placed 5.17×10^{-4} mol of glycine ester imine **1**, and 5.69×10^{-4} mol of *N*-Phenylmaleimide (NMP) in 3.5 mL of dry toluene. This solution was stirred at ~185 °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 chromatographed on a silica gel column (SiO_2, 12 cm height), starting with a mixture of EtOAc/*n*-hexanes, 1/8, v/v, as eluant, in order to remove safely the unreactive starting materials without contaminating the desired products. The solvent polarity was increased to elute the products **2**. A mixture of EtOAc/*n*-hexanes, 1/3, v/v, was normally used as eluant for efficient separation. Yields are listed in the text. Products usually solidified by treatment with mixtures of $CHCl_3$/*n*-hexanes. NMR peak assignments were made by 2D NMR spectroscopy (COSY, HSQC, and HMBC experiments).

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

5.2.1.1. Compound 2a-*syn*. White needles. Mp: 163.5–164 °C. R_f (EtOAc/*n*-hexanes 1:2, v/v) 0.15. ^{1}H NMR (250 MHz, $CDCl_3$): δ_H 7.49–7.17 (m, 5H, aromatics), 5.20 (dd, 1H$_5$, $J_{H5,H4}$ = 9.51 Hz, $J_{H5,H3}$ = 0.93 Hz), 4.27 (dd, 1H$_3$, $J_{H3,H4}$ = 9.19 Hz, $J_{H3,H5}$ = 0.93 Hz), 4.00 (dd, 1H$_4$, $J_{H4,H3}$ = 9.19 Hz, $J_{H4,H5}$ = 9.51 Hz), 3.77 (s, 3H, OMe), 2.53 (s, 3H, –SCH_3). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ_C 174.2 (C=O), 170.9 (C=N), 170.5 (C=O), 169.9 [C(=O)OR], 131.3, 129.0, 128.8, 126.4 (aromatics), 75.7 (C_5), 59.4 (C_3), 52.6 (OMe), 47.1 (C_4), 14.3 (–SMe). FT-IR (KBr pellet) 3484, 3066, 3045, 3002, 2950, 2936, 2847, 1785(C=O), 1715 (C=O),1575 (C=N), 1494, 1454, 1436, 1379, 1350, 1290, 1200, 1092, 1041, 993, 942, 916, 888, 871, 844, 823, 796, 770, 757, 736, 691, 656, 635, 618, 573, 544, 500, 475, 434, 413 cm^{-1}. UV–vis ($CHCl_3$): λ_{max} 244 nm. Anal. Calcd for $C_{15}H_{14}N_2SO_4$: N, 8.80; C, 56.59; H, 4.43; S, 10.07. Found: N, 8.84; C, 56.67; H, 4.45; S, 10.03. MS (FAB$^+$) *m/z*: 319, [M+H]$^+$. MS (ESI$^+$) *m/z*: 319.21 [M+H]$^+$, [M]: 318.35. HRMS Calcd for $C_{15}H_{15}N_2SO_4$, *m/z*: 319.0747, [M+H]$^+$. Found: 319.0740.

5.2.1.2. Compound 2a-*anti*. White needles. Mp: 170.5–171 °C. R_f (EtOAc/*n*-hexanes 1:2, v/v) 0.28. ^{1}H NMR (250 MHz, $CDCl_3$): δ_H 7.50–7.25 (m, 5H, aromatics), 5.21 (dd, 1H$_5$, $J_{H5,H4}$ = 2.27 Hz, $J_{H5,H3}$ = 2.04 Hz), 4.34 (dd, 1H$_3$, $J_{H3,H4}$ = 8.40 Hz, $J_{H3,H5}$ = 2.04 Hz), 4.13 (dd, 1H$_4$, $J_{H4,H3}$ = 8.40 Hz, $J_{H4,H5}$ = 2.27 Hz), 3.84 (s, 3H, OMe), 2.53 (s, 3H, –SCH_3). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ_C 175.1 (C=O), 171.0 (C=N), 170.9 (C=O), 170.1 [C(=O)OR], 131.1, 129.1, 128.9, 126.2 (aromatics), 76.7 (C_5), 58.9 (C_3), 53.0 (OMe), 48.1 (C_4), 14.3 (–SMe). FT-IR (KBr pellet): 3478, 3006, 2976, 2955, 2853, 1782 (C=O), 1742 (C=O), 1713 (C=O), 1575 (C=N), 1498, 1442, 1387, 1351, 1321, 1278,,1209, 1188, 1186, 1097, 1032, 1013, 975, 956, 945, 915, 804, 788, 757, 733, 693, 662, 622, 571, 526, 494, 459, 381 cm^{-1}. UV–vis ($CHCl_3$): λ_{max} 243 nm. Anal. Calcd for $C_{15}H_{14}N_2SO_4$: N, 8.80; C, 56.59; H, 4.43; S, 10.07. Found: N, 8.75; C, 56.70; H, 4.45; S, 10.11. MS (FAB$^+$) *m/z*: 319, [M+H]$^+$. MS (ESI$^+$) *m/z*: 319.27 [M+H]$^+$, [M]: 318.35. HRMS Calcd for $C_{15}H_{15}N_2SO_4$, *m/z*: 319.0747, [M+H]$^+$. Found: 319.0745.

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

5.2.2.1. Compound 2b-*syn*. White powder. Mp: 169.5–170 °C. R_f (EtOAc/*n*-hexanes 1:2, v/v) 0.25. ^{1}H NMR (250 MHz, $CDCl_3$): δ_H 7.46–7.24 (m, 5H, aromatics), 5.21 (dd, 1H$_5$, $J_{H5,H4}$ = 9.48 Hz, $J_{H5,H3}$ = 0.89 Hz), 4.26 (dd, 1H$_3$, $J_{H3,H4}$ = 9.20 Hz, $J_{H3,H5}$ = 0.89 Hz), 3.98 (dd, 1H$_4$, $J_{H4,H3}$ = 9.20 Hz, $J_{H4,H5}$ = 9.48 Hz), 3.77 (s, 3H, OMe), 3.20–3.06 (m, 2H, –SCH_2–, *not well resolved*), 1.36 (t, 3H, –CH_3, *J* = 7.39 Hz); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ_C 174.2 (C=O), 170.6 (C=N), 170.3 (C=O), 169.9 [C(=O)OR], 131.4, 129.2, 128.9, 126.5 (aromatics), 76.1 (C_5), 59.8 (C_3), 52.7 (-OMe), 47.0 (C_4), 26.2 (–SCH_2–), 13.7 (Me); FT-IR (KBr pellet): 3488, 3063, 2945, 1720 (C=O),1573 (C=N), 1495, 1454, 1437, 1383, 1348, 1291, 1248, 1200, 1176, 1090, 1044, 943, 870, 826, 797, 732, 692, 661, 623, 545, 476, 425 cm^{-1}. UV–vis ($CHCl_3$): λ_{max} 240 nm. Anal. Calcd for $C_{16}H_{16}N_2SO_4$: N, 8.43; C, 57.82; H, 4.85; S, 9.65. Found: N, 8.40; C, 57.94; H, 4.83; S, 9.62. MS (FAB$^+$) *m/z*: 333, [M+H]$^+$. MS (ESI$^+$) *m/z*: 333.34 [M+H]$^+$, [M]: 332.37. HRMS calcd for $C_{16}H_{17}N_2SO_4$, *m/z*: 333.0904, [M+H]$^+$. Found: 333.0898.

5.2.2.2. Compound 2b-*anti*. Oil. R_f (EtOAc/*n*-hexanes 1:2, v/v) 0.47. ^{1}H NMR (250 MHz, $CDCl_3$): δ_H 7.46–7.17 (m, 5H, aromatics), 5.21 (dd, 1H$_5$, $J_{H5,H4}$ = 2.14 Hz, $J_{H5,H3}$ = 2.02 Hz), 4.32 (dd, 1H$_3$, $J_{H3,H4}$ = 8.39 Hz, $J_{H3,H5}$ = 2.27 Hz), 4.02 (dd, 1H$_4$, $J_{H4,H3}$ = 8.39 Hz, $J_{H4,H5}$ = 2.27 Hz), 3.83 (s, 3H, OMe), 3.24 (qd, –SCH–, J_1=13.02 Hz,

J_2 = 7.47 Hz), 3.13 (qd, –SCH–, J_1 = 13.02 Hz, J_2 = 7.47 Hz), 1.40 (t, 3H, –CH_3, J_1 = 7.47 Hz). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ_C 175.2 (C=O), 170.9 (C=N), 170.4 (C=O), 170.3 [C(=O)OR], 131.2, 129.2, 128.9, 126.4, 126.2 (aromatics), 76.8 (C_5), 59.1 (C_3), 53.1 (OMe), 47.9 (C_4), 26.1 (–SCH_2–), 13.7 (Me). FT-IR (KBr pellet): 3488, 3063, 2945, 1720 (C=O), 1573 (C=N), 1495, 1454, 1437, 1383, 1348, 1291, 1248, 1200, 1176, 1090, 1044, 943, 870, 826, 797, 732, 692, 661, 623, 545, 476, 425 cm^{-1}. UV–vis ($CHCl_3$): λ_{max} 238 nm. Anal. Calcd for $C_{16}H_{16}N_2SO_4$: N, 8.43; C, 57.82; H, 4.85; S, 9.65. Found: N, 8.36; C, 58.07; H, 4.81; S, 9.60. MS (FAB^+) *m/z*: 333, $[M+H]^+$. MS (ESI^+) *m/z*: 333.34 $[M+H]^+$, [M]: 332.37. HRMS calcd for $C_{16}H_{17}N_2SO_4$, *m/z*: 333.0904, $[M+H]^+$. Found: 333.0902.

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

5.2.3.1. Compound 2c-*syn*. White powder. Mp: 145–146 °C. R_f (EtOAc/*n*-hexanes 1:2, v/v) 0.35. ^{1}H NMR (250 MHz, $CDCl_3$): δ_H 7.49–7.23 (m, 5H, aromatics), 5.17 (dd, 1H_5, $J_{H5,H4}$ = 9.50 Hz, $J_{H5,H3}$ = 0.89 Hz), 4.23 (dd, 1H_3, $J_{H3,H4}$ = 9.16 Hz, $J_{H3,H5}$ = 0.89 Hz), 3.95 (dd, 1H_4, $J_{H4,H3}$ = 9.16 Hz, $J_{H4,H5}$ = 9.50 Hz), 3.76 (s, 3H, OMe), 3.13 (td, 1H, –SCH–, J_1 = 12.76 Hz, J_2=7.31 Hz), 3.06 (td, 1H, –SCH– J_1 = 12.76 Hz, J_2=7.31 Hz), 1.73 (sextet, 2H, –CH_2–, J = 7.31 Hz), 1.01 (t, 3H, Me, J = 7.34 Hz). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ_C 174.2 (C=O), 170.6 (C=N), 170.4 (C=O), 169.9 [C(=O)OR], 131.4, 129.1, 128.8, 126.5 (aromatics), 76.0 (C_5), 59.7 (C_3), 52.7 (OMe), 47.0 (C_4), 33.6 (–S–CH_2–), 21.9 (–CH_2–), 13.4 (Me). FT-IR (KBr pellet): 3487, 2946, 2875, 1721 (C=O), 1575 (C=N), 1496, 1439, 1381, 1352, 1291, 1199, 1088, 1040, 944, 827, 768, 734, 691, 636, 544, 478 cm^{-1}. UV–vis ($CHCl_3$): λ_{max} 241 nm. Anal. Calcd for $C_{17}H_{18}N_2SO_4$: N, 8.09; C, 58.94; H, 5.24; S, 9.26. Found: N, 7.99; C, 58.75; H, 5.26; S, 9.22. MS (FAB^+) *m/z*: 347 $[M+H]^+$. HRMS calcd for $C_{17}H_{19}N_2SO_4$, *m/z*: 347.1060, $[M+H]^+$. Found: 347.1056.

5.2.3.2. Compound 2c-*anti*. White powder. Mp: 151–152 °C. R_f (EtOAc/*n*-hexanes 1:2, v/v) 0.47. ^{1}H NMR (250 MHz, $CDCl_3$): δ_H 7.50–7.25 (m, 5H, aromatics), 5.20 (dd, 1H_5, $J_{H5,H4}$=2.21 Hz, $J_{H5,H3}$ = 2.02 Hz), 4.32 (dd, 1H_3, $J_{H3,H4}$ = 8.38 Hz, $J_{H3,H5}$ = 2.02 Hz), 4.08 (dd, 1H_4, $J_{H4,H3}$ = 8.38 Hz, $J_{H4,H5}$ = 2.21 Hz), 3.83 (s, 3H, OMe), 3.17 (td, 1H, –SCH-, J_1 = 12.80 Hz, J_2 = 7.27 Hz), 3.05 (td, 1H, –SCH–, J_1=12.80 Hz, J_2 = 7.27 Hz), 1.72 (sextet, 2H, –CH_2–, J = 7.27 Hz) 1.01 (t, 3H, Me, J = 7.27 Hz). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ_C 175.2 (C=O), 171.0 (C=N), 170.6 (C=O), 170.4 [C(=O)OR], 147.1, 129.2, 128.9, 126.2 (aromatics), 76.8 (C_5), 59.2 (C_3), 53.1 (OMe), 48.0 (C_4), 33.7 (–S–CH_2–), 21.9 (–CH_2–), 13.4 (Me). FT-IR (KBr pellet): 3478, 3059, 2963, 2934, 2873, 1968, 1748, 1712 (C=O), 1578 (C=N), 1502, 1455, 1436, 1390, 1342, 1291, 1264, 1241, 1196, 1087, 1044, 1007, 990, 957, 941, 914, 807, 784, 756, 733, 694, 665, 618, 577, 541, 506, 480, 432 cm^{-1}. UV–vis ($CHCl_3$): λ_{max} 243 nm. Anal. Calcd for $C_{17}H_{18}N_2SO_4$: N, 8.09; C, 58.94; H, 5.24; S, 9.26. Found: N, 7.97; C, 58.846; H, 5.26; S, 9.15. MS (FAB^+) *m/z*: 347 $[M+H]^+$. HRMS calcd for $C_{17}H_{19}N_2SO_4$, *m/z*: 347.1060, $[M+H]^+$. Found: 347.1059.

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

5.2.4.1. Compound 2d-*syn*. Oil. R_f (EtOAc/*n*-hexanes 1:2, v/v) 0.34. ^{1}H NMR (250 MHz, $CDCl_3$): δ_H 7.44–7.24 (m, 5H, aromatics), 5.21 (dd, 1H_5, $J_{H5,H4}$ = 9.53 Hz, $J_{H5,H3}$ = 0.98 Hz), 4.23 (dd, 1H_3, $J_{H3,H4}$ = 9.20 Hz, $J_{H3,H5}$ = 0.98 Hz), 3.94 (dd, 1H_4, $J_{H4,H3}$ = 9.20 Hz, $J_{H4,H5}$ = 9.53 Hz), 3.86 (septet, 1H, –SCH<, J = 6.81 Hz), 3.77 (s, 3H, OMe), 1.39 (d, 6H, 2-CH_3, J = 6.81 Hz). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ_C 174.2 (C=O), 170.6 (C=N), 170.0 (C=O), 169.9 [C(=O)OR], 131.4, 129.1, 128.9, 126.5 (aromatics), 76.3 (C_5), 60.0 (C_3), 52.7 (OMe), 46.7 (C_4), 37.3 (–SCH<), 22.7 (Me), 22.6 (Me). FT-IR (KBr pellet): 3340, 2959, 1718 (C=O), 1581 (C=N), 1498, 1383, 1187, 740, 692 cm^{-1}. UV–vis ($CHCl_3$): λ_{max} 242 nm. Anal. Calcd for $C_{17}H_{18}N_2SO_4$: N, 8.09; C, 58.94; H, 5.24; S, 9.26. Found: N, 7.92; C, 58.64; H, 5.27; S, 9.13. MS (FAB^+) *m/z*: 347 $[M+H]^+$.

5.2.4.2. Compound 2d-*anti*. White powder. Mp: 136–137 °C. R_f (EtOAc/*n*-hexanes 1:2, v/v) 0.46. ^{1}H NMR (250 MHz, $CDCl_3$): δ_H 7.46–7.26 (m, 5H, aromatics), 5.23 (dd, 1H_5, $J_{H5,H4}$ = 2.21 Hz, $J_{H5,H3}$ = 2.02 Hz), 4.29 (dd, 1H_3, $J_{H3,H4}$ = 8.32 Hz, $J_{H3,H5}$ = 2.02 Hz), 4.05 (dd, 1H_4, $J_{H4,H3}$ = 8.32 Hz, $J_{H4,H5}$ = 2.21 Hz), 3.90 (septet, partially overlapped, –SCH<, J = 6.85 Hz), 3.83 (s, 3H, OMe), 1.41 (d, 3H, CH_3, J = 6.85 Hz), 1.38 (d, 3H, CH_3, J = 6.85 Hz). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ_C 175.2 (C=O), 171.0 (C=N), 170.4 (C=O), 170.1 [C(=O)OR], 131.2, 129.1, 128.8, 126.2 (aromatics), 76.9 (C_5), 59.3 (C_3), 53.0 (OMe), 47.7 (C_4), 37.2 (–SCH<), 22.7 (Me), 22.5 (Me). FT-IR (KBr pellet): 3479, 2954, 2929, 2868, 1742 (C=O), 1718 (C=O), 1595, 1564 (C=N), 1496, 1439, 1387, 1320, 1276, 1239, 1205, 1185, 1086, 1031, 1012, 980, 957, 911, 806, 787, 738, 696, 665, 647, 625, 540, 494, 459 cm^{-1}. UV–vis ($CHCl_3$): λ_{max} 244 nm. Anal. Calcd for $C_{17}H_{18}N_2SO_4$: N, 8.09; C, 58.94; H, 5.24; S, 9.26. Found: N, 7.92; C, 58.84; H, 5.27; S, 9.13. MS (FAB^+) *m/z*: 347 $[M+H]^+$. HRMS calcd for $C_{17}H_{19}N_2SO_4$, *m/z*: 347.1060, $[M+H]^+$. Found: 347.1056.

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

5.2.5.1. Compound 2e-*syn*. White powder. Mp: 102–103 °C. R_f (EtOAc/*n*-hexanes 1:2, v/v) 0.41. ^{1}H NMR (250 MHz, $CDCl_3$): δ_H 7.50–7.23 (m, 5H, aromatics), 5.17 (dd, 1H_5, $J_{H5,H4}$=9.51 Hz, $J_{H5,H3}$ = 0.86 Hz), 4.22 (dd, 1H_3, $J_{H3,H4}$ = 9.20 Hz, $J_{H3,H5}$ = 0.86 Hz), 3.95 (dd, 1H_4, $J_{H4,H3}$ = 9.20 Hz, $J_{H4,H5}$ = 9.51 Hz), 3.76 (s, 3H, OMe), 3.16 (td, 1H, –SCH–, J_1 = 12.54 Hz, J_2 = 7.35 Hz), 3.09 (td, 1H, –SCH–, J_1 = 12.54 Hz, J_2=7.35 Hz), 1.68 (quintet, 2H, –CH_2–, J = 7.35 Hz), 1.43 (sextet, 2H, –CH_2–, J = 7.31 Hz), 0.93 (t, 3H, Me, J = 7.31 Hz). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ_C 174.2 (C=O), 170.6 (C=N), 170.4 (C=O), 169.9 [C(=O)OR], 131.3, 129.1, 128.8, 126.4 (aromatics), 75.9 (C_5), 59.7 (C_3), 52.6 (OMe), 46.9 (C_4), 31.4 (–S–CH_2–), 30.3 (–CH_2–), 21.8 (–CH_2–), 13.5 (Me). FT-IR (KBr pellet): 3490, 2937, 2841, 1784, 1722 (C=O), 1578 (C=N), 1438, 1384, 1352, 1291, 1244, 1198, 1085, 1038, 945, 889, 825, 797, 748, 734, 691, 638, 619, 545, 481, 442, 414 cm^{-1}. UV–vis ($CHCl_3$): λ_{max} 242 nm. Anal. Calcd for $C_{18}H_{20}N_2SO_4$: N, 7.77; C, 59.98; H, 5.59; S, 8.90. Found: N, 7.81; C, 59.73; H, 5.57; S, 8.93. MS (FAB^+) *m/z*: 361, $[M+H]^+$. MS (ESI^+) *m/z*: 361.41 $[M+H]^+$, [M]: 360.43.

5.2.5.2. Compound 2e-*anti*. Oil. R_f (EtOAc/*n*-hexanes 1:2, v/v) 0.53. ^{1}H NMR (250 MHz, $CDCl_3$): δ_H 7.50–7.24 (m, 5H, aromatics), 5.20 (dd, 1H_5, $J_{H5,H4}$ = 2.14 Hz, $J_{H5,H3}$ = 1.99 Hz), 4.32 (dd, 1H_3, $J_{H3,H4}$ = 8.34 Hz, $J_{H3,H5}$ = 1.99 Hz), 4.09 (dd, 1H_4, $J_{H4,H3}$ = 8.34 Hz, $J_{H4,H5}$ = 2.14 Hz), 3.83 (s, 3H, OMe), 3.18 (td, 1H, –SCH–, J_1 = 12.72 Hz, J_2 = 7.28 Hz), 3.08 (td, 1H, –SCH–, J_1 = 12.72 Hz, J_2 = 7.28 Hz), 1.67 (quintent, 2H, –CH_2–, J = 7.28 Hz), 1.42 (sextet, 2H, –CH_2–, J = 7.30 Hz), 0.92 (t, 3H, Me, J = 7.30 Hz). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ_C 175.2 (C=O), 171.0 (C=N), 170.7 (C=O), 170.4 [C(=O)OR], 131.2, 129.2, 128.9, 126.3 (aromatics), 76.8 (C_5), 59.2 (C_3), 53.1 (OMe), 48.0 (C_4), 31.5 (–S–CH_2–), 30.4 (–CH_2–), 21.9 (–CH_2–), 13.6 (Me). FT-IR (KBr pellet): 3437, 2954, 2915, 2856, 1742, 1721 (C=O), 1571 (C=N), 1491, 1386, 1207, 1191, 1086, 794, 735, 694, 464 cm^{-1}. UV–vis ($CHCl_3$): λ_{max} 244 nm. Anal. Calcd for $C_{18}H_{20}N_2SO_4$: N, 7.77; C, 59.98; H, 5.59; S, 8.90. Found: N, 7.80; C, 59.81; H, 5.57; S, 8.96. MS (FAB^+) *m/z*: 361, $[M+H]^+$. MS (ESI^+) *m/z*: 361.40 $[M+H]^+$, [M]: 360.43.

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

5.2.6.1. Compound 2f-*syn*. Oil. R_f (EtOAc/*n*-hexanes 1:2, v/v) 0.33. ^{1}H NMR (250 MHz, $CDCl_3$): δ_H 7.46–7.24 (m, 10H, aromatics), 5.21 (dd, 1H_5, $J_{H5,H4}$ = 9.51 Hz, $J_{H5,H3}$ = 0.98 Hz), 4.42 (d, 1H,

benzylic, J = 13.14 Hz), 4.30 (d, 1H, benzylic, J = 13.14 Hz), 4.24 (dd, $1H_3$, $J_{H3,H4}$ = 9.20 Hz, $J_{H3,H5}$ = 0.98 Hz), 3.97 (dd, $1H_4$, $J_{H4,H3}$ = 9.20 Hz, $J_{H4,H5}$ = 9.51 Hz), 3.78 (s, 3H, OMe). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ_C 174.1 (C=O), 170.4 (C=N), 170.0 (C=O), 169.9 [C(=O)OR], 136.1, 131.4, 129.2, 129.1, 128.9, 128.6, 127.6, 126.5 (aromatics), 76.1 (C_5), 59.6 (C_3), 52.7 (OMe), 47.1 (C_4), 36.1 (benzylic). FT-IR (KBr pellet): 3480, 3063, 3029, 2952, 1716 (C=O), 1581 (C=N), 1497, 1454, 1435, 1383, 1347, 1286, 1177, 1086, 1030, 944, 913, 873, 819, 766, 734, 693, 641, 618 cm^{-1}. UV–vis ($CHCl_3$): λ_{max} 242 nm. Anal. Calcd for $C_{21}H_{18}N_2SO_4$: N, 7.10; C, 63.94; H, 4.60; S, 8.13. Found: N, 7.12; C, 63.74; H, 4.57; S, 8.15. MS (FAB^+) m/z: 395 $[M+H]^+$, [M]: 394.44.

5.2.6.2. Compound 2f-*anti*. White powder, m.p. 129.5–130 °C; R_f (EtOAc/*n*-hexanes 1:2, v/v) 0.39; ^{1}H NMR (250 MHz, $CDCl_3$): δ_H 7.49–7.23 (m, 10H, aromatics), 5.24 (dd, $1H_5$, $J_{H5,H4}$ = 2.23 Hz, $J_{H5,H3}$ = 2.04 Hz), 4.44 (d, 1H, benzylic, J = 13.08 Hz), 4.33 (dd, $1H_3$, $J_{H3,H4}$ = 8.37 Hz, $J_{H3,H5}$ = 2.04 Hz), 4.28 (d, 1H, benzylic, J = 13.08 Hz), 4.12 (dd, $1H_4$, $J_{H4,H3}$ = 8.37 Hz, $J_{H4,H5}$ = 2.23 Hz), 3.84 (s, 3H, OMe). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ_C 175.1 (C=O), 170.8 (C=N), 170.2 (C=O), 170.1 [C(=O)OR], 135.9, 131.1, 129.2, 129.1, 128.9, 128.6, 127.6, 126.2 (aromatics), 76.9 (C_5), 59.1 (C_3), 53.1 (OMe), 48.0 (C_4), 36.1 (benzylic). FT-IR (KBr pellet): 3447, 3030, 2958, 2927, 1716 (C=O), 1574 (C=N), 1497, 1454, 1382, 1260, 1181, 1089, 1028, 911, 803, 747, 693, 618 cm^{-1}. UV–vis ($CHCl_3$): λ_{max} 244 nm. Anal. Calcd for $C_{21}H_{18}N_2SO_4$: N, 7.10; C, 63.94; H, 4.60; S, 8.13. Found: N, 7.13; C, 63.78; H, 4.58; S, 8.16. MS (FAB^+) m/z: 395 $[M+H]^+$, [M]: 394.44. HRMS calcd for $C_{21}H_{19}N_2SO_4$, m/z: 395.1060, $[M+H]^+$. Found: 395.1052.

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

5.2.7.1. Compound 2g-*syn*. Oil. R_f (EtOAc/*n*-hexanes 1:2, v/v) 0.11. ^{1}H NMR (250 MHz, $CDCl_3$): δ_H 7.50–7.24 (m, 5H, aromatics), 5.18 (dd, $1H_5$, $J_{H5,H4}$ = 9.53 Hz, $J_{H5,H3}$ = 0.74 Hz), 4.26 (dd, $1H_3$, $J_{H3,H4}$ = 9.39 Hz, $J_{H3,H5}$ = 0.74 Hz), 4.28–4.16 (m, 3H, $-OCH_2-$,$+H_3$), 3.99 (dd, $1H_4$, $J_{H4,H3}$ = 9.39 Hz, $J_{H4,H5}$ = 9.53 Hz), 2.52 (s, 3H, –SMe), 1.28 (t, 3H, Me, J = 7.22 Hz). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ_C 174.1 (C=O), 170.9 (C=N), 170.6 (C=O), 169.6 [C(=O)OR], 131.4, 129.2, 128.9, 126.5 (aromatics), 76.1 (C_5), 62.1 ($-OCH_2-$), 59.6 (C_3), 47.3 (C_4), 14.5 (SMe), 14.0 (Me). Anal. Calcd for $C_{16}H_{16}N_2SO_4$: N, 8.43; C, 57.82; H, 4.85; S, 9.65. Found: N, 8.46; C, 57.59; H, 4.88; S, 9.63. MS (FAB^+) m/z: 355.0 $[M+Na]^+$, [M]: 332.57.

5.2.7.2. Compound 2g-*anti*. Oil. R_f (EtOAc/*n*-hexanes 1:2, v/v) 0.25. ^{1}H NMR (250 MHz, $CDCl_3$): δ_H 7.50–7.25 (m, 5H, aromatics), 5.19 (dd, $1H_5$, $J_{H5,H4}$ = 2.19 Hz, $J_{H5,H3}$ = 2.00 Hz), 4.34 (dd, $1H_3$, $J_{H3,H4}$ = 8.32 Hz, $J_{H3,H5}$ = 2.00 Hz), 4.30 (dq, 1H, -OCH-, J_1 = 7.13 Hz, J_2 = 3.22 Hz), 4.28 (dq, 1H, -OCH-, J_1 = 7.13 Hz, J_2 = 3.22 Hz), 4.11 (dd, $1H_4$, $J_{H4,H3}$ = 8.32 Hz, $J_{H4,H5}$ = 2.19 Hz), 2.53 (s, 3H, –SMe), 1.35 (t, 3H, Me, J = 7.13 Hz). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ_C 175.2 (C=O), 171.0 (C=N), 169.8 (C=O), 131.1, 129.2, 128.9, 126.2 (aromatics), 76.9 (C_5), 62.3 ($-OCH_2-$), 58.9 (C_3), 48.2 (C_4), 14.4 (SMe), 14.1 (Me). Anal. Calcd for $C_{16}H_{16}N_2SO_4$: N, 8.43; C, 57.82; H, 4.85; S, 9.65. Found: N, 8.47; C, 58.07; H, 4.90; S, 9.58. MS (FAB^+) m/z: 355.0 $[M+Na]^+$, [M]: 332.57.

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

5.2.8.1. Compound 2h-*syn*. Oil. R_f (EtOAc/*n*-hexanes 1:2, v/v) 0.14. ^{1}H NMR (250 MHz, $CDCl_3$): δ_H 7.47–7.20 (m, 5H, aromatics), 5.18–5.20 (d+s, partially overlaped, 3H, 2H benzylic+H_5), 5.18 (s, 2H, benzylic), 4.22 (d, $1H_3$, $J_{H3,H4}$ = 8.94 Hz), 3.97 (dd, $1H_4$, $J_{H4,H3}$ = 8.94 Hz, $J_{H4,H5}$ = 9.38 Hz), 2.52 (s, 3H, SMe). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ_C 174.1 (C=O), 171.0 (C=N), 170.6 (C=O), 169.5 (C=O), 134.9, 131.3, 129.1, 128.8, 128.6, 128.5, 128.4, 128,1, 126.5 (aromatics), 75.9 (C_5), 67.8 (benzylic), 59.5 (C_3), 47.2 (C_4), 14.4 (SMe). Anal. Calcd for $C_{21}H_{18}N_2SO_4$: N, 7.10; C, 63.94; H, 4.60; S, 8.13. Found: N, 7.06; C, 64.21; H, 4.63; S, 8.10. MS (FAB^+) m/z: 417.0 $[M+Na]^+$, 393.1 $[M-H]^-$, [M]: 394.44.

5.2.8.2. Compound 2h-*anti*. Oil. R_f (EtOAc/*n*-hexanes 1:2, v/v) 0.28. ^{1}H NMR (250 MHz, $CDCl_3$): δ_H 7.47–7.23 (m, 5H, aromatics), 5.24 (br s, 3H, 2H benzylic+$1H_5$), 4.30 (dd, $1H_3$, $J_{H3,H4}$ = 9.19 Hz, $J_{H3,H5}$ = 1.87 Hz), 4.07 (dd, $1H_4$, $J_{H4,H3}$ = 9.19 Hz, $J_{H4,H5}$ = 2.25 Hz), 2.50 (s, 3H, SMe). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ_C 175.0 (C=O), 171.1 (C=N), 170.9 (C=O), 169.5 (C=O), 135.0, 131.0, 129.1, 128.8, 128.5, 128.4, 128,1, 126.3, 126.1 (aromatics), 76.7 (C_5), 67.6 (benzylic), 58.9 (C_3), 48.1 (C_4), 14.3 (SMe). MS (FAB^+) m/z: 417.0 $[M+Na]^+$, [M]: 394.44.

5.2.9. Michael adduct of MeSH to NPM

Oil. R_f (EtOAc/*n*-hexanes 1:2, v/v) 0.33. ^{1}H NMR (250 MHz) δ_H (ppm): 7.47–7.23 (m, 5H, aromatics), 3.77 (dd, $1H_3$, J_1 = 9.00 Hz, J_2 = 3.57 Hz), 3.28 (dd, $1H_{4a}$, J_1 = 18.82 Hz, J_2 = 9.00 Hz), 2.66 (dd, $1H_{4b}$, J_1 = 18.82 Hz, J_2 = 3.57 Hz), 2.36 (s, 3H, SMe). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ_C 175.2 (C=O), 173.6 (C=O), 134.1, 131.5, 128.7, 127.9, 126.0 (aromatics), 40.6 (C_3), 35.8 (C_4), 14.8 (SMe). MS (FAB^+) m/z: 244.0 $[M+Na]^+$, [M]: 221.28.

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

5.2.10.1. Compound 2i-*syn*. Oil. R_f (EtOAc/*n*-hexanes 1:2, v/v) 0.17. ^{1}H NMR (250 MHz, $CDCl_3$): δ_H 7.49–7.26 (m, 5H, aromatics), 5.01 (d, $1H_5$, $J_{H5,H4}$ = 9.61 Hz), 4.23 (d, $1H_3$, $J_{H3,H4}$ = 9.36 Hz), 3.95 (dd, $1H_4$, $J_{H4,H5}$ = 9.61 Hz, $J_{H4,H3}$ = 9.36 Hz), 2.52 (s, 3H, SMe), 1.48 (s, 9H, –OBu-*t*). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ_C 174.0 (C=O), 170.8 (C=N), 170.4 (C=O), 168.8 (C=O), 131.5, 129.1, 128.8, 126.6 (aromatics), 83.1 (OBu-*t*), 76.9 (C_5), 59.6 (C_3), 47.3 (C_4), 28.0 (CH_3), 14.4 (SMe). Anal. Calcd for $C_{18}H_{20}N_2SO_4$: N, 7.77; C, 59.98; H, 5.59; S, 8.90. Found: N, 7.80; C, 59.71; H, 5.58; S, 8.94. MS (FAB^+) m/z: 383.0 $[M+Na]^+$, 359.3 $[M-H]^-$, [M]: 360.42.

5.2.10.2. Compound 2i-*anti*. Oil. R_f (EtOAc/*n*-hexanes 1:2, v/v) 0.39. ^{1}H NMR (250 MHz, $CDCl_3$): δ_H 7.50–7.24 (m, 5H, aromatics), 5.09 (dd, $1H_5$, $J_{H5,H4}$ = 2.17 Hz, $J_{H5,H3}$ = 1.99 Hz), 4.32 (dd, $1H_3$, $J_{H3,H4}$ = 9.00 Hz, $J_{H3,H5}$ = 1.99 Hz), 4.01 (dd, $1H_4$, $J_{H4,H3}$ = 9.00 Hz, $J_{H4,H5}$ = 2.17 Hz), 2.52 (s, 3H, SMe) 1.51 (s, 9H, –OBu-*t*). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ_C 175.3 (C=O), 171.2 (C=N), 170.7 (C=O), 168.9 (C=O), 131.2, 129.2, 128.9, 126.3 (aromatics), 82.9 (OBu-*t*), 77.7 (C_5), 58.9 (C_3), 48.4 (C_4), 27.9 (CH_3), 14.6 (SMe). Anal. Calcd for $C_{18}H_{20}N_2SO_4$: N, 7.77; C, 59.98; H, 5.59; S, 8.90. Found: N, 7.79; C, 59.79; H, 5.61; S, 8.94. MS (FAB^+) m/z: 361.0 $[M+H]^+$, 383.0 $[M+Na]^+$, 359.3 $[M-H]^-$, [M]: 360.42.

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 Lipoxygenase, linoleic acid sodium salt, indomethacin were obtained from Sigma Chemical, Co. (St. Louis, MO, USA).

5.3.1. Determination of the reducing activity of the stable radical 1,1-diphenyl-picrylhydrazyl (DPPH)[15]

To a solution of DPPH (final concentration 50 μM) in absolute ethanol was added an equal volume of the compounds dissolved in dimethylsulfoxide. As control solution ethanol was used. The final concentration of the test compounds was 50 μM. After 20 min at room temperature, the absorbance was recorded at 517 nm (Table 3)

5.3.2. Soybean lipoxygenase inhibition study in vitro[15]

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 (caffeic acid 600 μM) (Table 3)

5.3.3. Inhibition of linoleic acid peroxidation[15]

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 3).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.07.014.

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