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Design and synthesis of new bifunctional sigma-1 selective ligands with antioxidant activity.

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Abstract

Herein we report the synthesis of new bifunctional sigma-1 (σ_1)-selective ligands with antioxidant activity. To achieve this goal, we combined the structure of lipoic acid, a universal antioxidant, with an appropriate sigma aminic moiety. Ligands **14** and **26** displayed high affinity and selectivity for σ_1 receptors ($K_i\sigma_1$ = 1.8 and 5.5 nM; $K_i\sigma_2/\sigma_1$ = 354 and 414, respectively). Compound **26** exhibited in vivo antiopioid effects on kappa opioid (KOP) receptor-mediated analgesia. In rat liver and brain mitochondria (RLM, RBM), this compound significantly reduced the swelling and the oxidation of thiol groups induced by calcium ions. Our results demonstrate that the tested compound has protective effects against oxidative stress.

Introduction

The sigma receptors (σ), originally postulated by Martin et al. in the mid-1970s to be a subtype of opioid receptors,[1] are currently classified as a unique biochemical entity different from other known neurotransmitter receptors.[2] Two different subtypes, designated sigma-1 (σ_1) and sigma-2 (σ_2), have been identified;[3] however, only the σ_1 receptor has been cloned from various tissues and species.[4-6] The σ_1 receptor is involved in many cellular functions and biological processes,[7] including neuroprotection.[8-10] Many investigations have documented the involvement of σ receptors in some important pathways implicated in different neurodegenerative illnesses such as Alzheimer's,[11] Lou Gehrig's[12] and Parkinson's[13,14] diseases. It has been observed that some general pathways, such as an increase in the intracellular calcium levels, mitochondrial dysfunction, reactive species production, metal dyshomeostasis and protein misfolding, participate in the pathogenic cascades of these diseases.[15] In particular, mitochondrial damage increases the production of reactive oxygen species (ROS) and the susceptibility to cell death, playing a key role in these diseases.[16] Recently, the σ_1 protein has been characterized as a chaperone localized to the endoplasmic reticulum (ER) at the mitochondria-associated

ER membrane (MAM), where it forms a complex with the ER chaperone called Binding immunoglobulin protein (Bip).[17] The activation of σ_1, induced by the depletion of ER calcium ions or by σ ligands, leads to the dissociation of the σ_1-Bip complex, resulting in prolonged mitochondrial calcium signaling through the inositol 1,4,5-trisphosphate receptor (IP3R). These events regulate ER-mitochondrial interorganellar calcium signaling and cell survival. Some investigations have found that the σ_1 receptors are associated with oxidative stress; for example, ER stress promotes the upregulation of σ_1 receptors,[18] whereas the knockdown of σ_1 receptors potentiates ER stress-induced apoptosis.[19] Significant increases in the concentration of metabolites, considered markers of oxidative stress, have been observed in the livers of σ_1 receptor-knockout mice relative to wild-type liver. This antioxidant effect is mediated by the activation of the antioxidant response element (ARE), which upregulates the expression of NAD(P)H:quinone oxidoreductase 1 (NQO1) and superoxide dismutase 1 (SOD1).[20] Many other studies have demonstrated that σ_1 selective compounds can provide neuroprotection under cytotoxic conditions.[21-24] These data suggest that σ_1 receptors have a critical role in protection against oxidative stress and neuronal apoptosis. The use of antioxidants is considered a promising approach for slowing the progression of neurodegenerative diseases;[25] however, clinical studies have demonstrated that treatment with a single antioxidant is not able to prevent or treat these disorders.[26] A greater therapeutic benefit can be achieved with drugs that act on multiple molecular targets, which may be part of a system or may be involved in different pathways.[27] We focused our attention on α-lipoic acid **1** (ALA), a natural antioxidant. Compound **1** has amphiphilic properties, crosses the blood-brain barrier and is considered an important metabolite for energy production in mitochondria.[28,29] Compound **1** can easily quench radicals and chelate metals.[30] Furthermore, it can regenerate endogenous antioxidants and repair oxidative tissue damage. A large number of studies have documented that the therapeutic effect of **1** is based on its antioxidant properties and have found that this compound has neuroprotective effects.[31-33] Thus, our design strategy is based to the development of σ_1 agonists with improved antioxidant properties. To achieve this goal, we combined the structure of **1** with an appropriate aminic moiety

based on the previously proposed structure-activity relationship (***SAR***) for σ ligands.[34-36] In particular, Gilligan et al.[34] identified four regions in their lead compound: 1) a distal aromatic ring (Region A), 2) a nitrogen heterocycle (Region C), 3) a space between the nitrogen heterocycle and the distal hydrophobic site (Region B), and 4) a substituent on the basic nitrogen (Region D) (Figure 1). They noted that a variety of spacer groups could be inserted in Region B and that the replacement of the distal phenyl group (Region A) with other aryl or heteroaryl nuclei had no appreciable effect on the sigma binding affinity or selectivity. Furthermore, they indicated that the chemical nature of the *N*-substituent and its distance from Region C significantly affect the selectivity for σ binding over binding to other receptors such as those for dopamine (D_2) and serotonin ($5\text{-}HT_2$). Thus, we synthesized new piperazine and piperidine derivatives containing the lipoyl function group in Regions A and B and different *N*-substituents in Region D (Figure 1). Particularly, we investigated how the chemical structure of the *N*-substituent and its distance from Region C influence the σ_1/σ_2 selectivity. To determine the pharmacological profile of the most interesting compound in the series, we also performed in vivo studies on the modulation of the analgesic effect induced by the kappa opioid (KOP) agonist *trans*-(1*S*,2*S*)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]benzeneacetamide **2** [(-)-U50,488H].[37] Preliminary biological studies were also conducted to assess the antioxidant properties in rat liver and brain mitochondria (RLM, RBM).[38]

Chemistry

Commercially available secondary piperazines **3-9** acylated with lipoic acid in dry dichloromethane (DCM) using *N*,*N*′-dicyclohexylcarbodiimide (DCC) at room temperature (rt) provided the final ketones **10-16** (Scheme 1).[39] The 1-alkyl-4-hydroxypiperidines **21-23-24** were synthesized by the reaction of piperidin-4-ol (**20**) with the alkyl bromides **17-19** following the experimental procedures reported in the literature (Scheme 2).[40] 1-Benzylpiperidin-4-ol (**22**) was purchased from Sigma-Aldrich and was used without further purification. The condensation of lipoic acid with secondary alcohols **21-24** was performed in dry DCM using DCC and 4-(*N,N*-dimethylamino)pyridine (DMAP) as a nucleophilic

catalyst, furnishing the required esters **25-28** (Scheme 2).[41]

Results and Discussion

The synthesized piperazine (**10-16**) and piperidine (**25-28**) derivatives were evaluated for their affinities for both σ_1 and σ_2 receptors (Table 1). Compound **13**, in which the aromatic ring is directly bound to the basic nitrogen center, did not exhibit significant affinity for either σ subtype, whereas the corresponding *N*-methyl derivative **10** exhibited moderate affinity only for the σ_1 receptor (K_i = 115 ± 1 nM). A significant increase in the affinity for both subtypes was observed when a cyclohexyl group was present on the nitrogen heterocycle (**11**) ($K_i\sigma_1$ = 0.4 ± 0.0 nM; $K_i\sigma_2$ = 11.25 ± 0.55 nM). A comparable $\sigma_{1/2}$ binding profile was also found for the cyclohexylmethyl analog **12** ($K_i\sigma_1$ = 1.25 ± 0.25 nM; $K_i\sigma_2$ = 35.5 ± 2.5 nM). The replacement of the cyclohexylmethyl side chain with the benzyl group led to **14**, which presented approximately 354-fold selectivity for σ_1 receptors ($K_i\sigma_1$ = 1.8 ± 0.2 nM, $K_i\sigma_2$ = 637 ± 15 nM). Related compounds containing a phenylethyl (**15**) or phenylpropyl (**16**) moiety failed to display σ_1-selective binding. In fact, both showed a lower σ_1 binding affinity (K_i= 8.4 ± 1 nM and 10 ± 2 nM, respectively) compared to compound **14**, and a moderate σ_2 affinity (K_i= 209 ± 11 nM and 91.6 ± 7 nM, respectively). Comparable results were observed for the piperidine derivatives **25-28**. We found that compound **25** bound with high affinity to both subtypes ($K_i\sigma_1$ = 1.14 ± 0.8 nM; $K_i\sigma_2$ = 14 ± 2 nM). Although piperidine **26** bound with lower affinity to σ_1 (K_i = 5.56 ± 0.5 nM) than piperazine analog **14**, it exhibited a notable selectivity for σ_1 receptors ($K_i\sigma_2/K_i\sigma_1$ > 400). The elongation of the alkyl chain (**27** and **28)** led to a decreased affinity for the σ_1 sites (K_i = 13.5 ± 0.5 nM and 25.7 ± 2 nM, respectively). These compounds exhibited considerable affinity for σ_2 (K_i = 160.4 ± 8 nM; K_i = 100.8 ± 4 nM) with respect to the benzyl analog. These results suggest that the lipoyl function group is well tolerated in Regions A and B when a suitable moiety is present on the basic nitrogen center. In particular, in our compounds the *N*-benzyl substituent seems to play a pivotal role in discriminating between the sigma receptor subtypes. Further binding studies performed by Cerep, Inc (Poitiers,

France), on compounds **14**, **15** and **26** using a different experimental protocol procedure (see the experimental section) furnished better results in terms of σ_1 affinity and selectivity. Compounds **14** and **15** had σ_1 affinities similar to our values (K_i = 2.5 and 6.5 nM, respectively) but lower σ_2 affinities (K_i = 2500 and 3000 nM, respectively). A considerable increase in the σ_1 affinity (K_i = 0.4 nM) and a reduction in the σ_2 affinity (K_i = 8300 nM) were found for piperidine **26**. The different K_i values obtained by Cerep are attributed to the different experimental conditions. However, all together, these data confirm the affinity and selectivity for σ_1 of our compounds. Given the implication of σ_1 receptors in KOP-mediated analgesia,[42] we analyzed the ability of compound **26** to modulate the analgesic effect of the systemically injected kappa agonist, **2**. Our results demonstrate that the systemic administration of **26** (1 mg/kg sc) does not modify basal tail-flick latency. Pre-treatment with compound **26**, at the same dose, significantly decreased the antinociceptive effect of **2** (5 mg/kg sc) from 30 min after opioid administration up to the entire observation period (Figure 2). This antiopioid effect was prevented by the σ_1 antagonist 4-[4-(4-chlorophenyl)-4-hydroxy-1-piperidyl]-1-(4-fluorophenyl)-butan-1-one (haloperidol).[43] To assess the antioxidant properties of compound **26**, we performed in vitro studies in RLM and RBM.[38] In particular, we tested this compound's effects on calcium ion-induced swelling and on the percentage of reduced thiol groups. Figure 3 shows that the RLM suspension energized by the oxidation of succinate in the presence of rotenone, when treated with Ca^{2+} and phosphate, exhibited a strong decrease in the apparent absorbance at 540 nm. This reduction, indicative of large-amplitude swelling, was strongly inhibited by compound **26** at 25 μM and abolished at 50 μM. The absorbance decrease was also completely prevented in the presence of cyclosporin A (CsA), adenosine 5□-diphosphate (ADP) or dithioerythritol (DTE) (Figure 3). The colloid osmotic swelling of the matrix, related to the absorbance decrease, is induced by the mitochondrial permeability transition (MPT), which is associated with the opening of a proteinaceous pore on the membrane of this organelle.[44,45] The observation that two typical inhibitors of the MPT, CsA and ADP, are able to abolish matrix swelling confirmed the above statement. Furthermore, the decrease in absorbance prevented by the reductant

DTE indicated that MPT is also related to mitochondrial oxidative stress. To study the protective mechanism of compound **26**, we analyzed the mitochondrial redox state by evaluating the percentage of the sulfhydryl groups in their reduced state under different experimental conditions. The RLM, in the presence of Ca^{2+} plus phosphate, exhibited a 30% reduction in SH groups versus the control (50 nmol/mg prot.) (Figure 4). This percentage of reduced sulfhydryl groups was restored to the control value when RLM were treated with compound **26** at 25 μM or 50 μM or with the antioxidant DTE (Figure 4). Although the protective mechanism of compound **26** is not completely understood, our results suggest that it has antioxidant effects. Nevertheless, some differences have been found between assays performed with RBM and those performed with RL mitochondria. As shown in Figure 5, the RBM suspension exhibited a spontaneous drop off in absorbance at 540 nm, which gradually decreased over time. In the presence of Ca^{2+} and phosphate, this reduction was further enhanced without reaching the extent observed in the RLM. CsA completely abolished this decrease, revealing that this effect is a consequence of MPT-induced mitochondrial swelling. ADP had only a slight protective effect on RBM, whereas the reductant DTE was almost completely ineffective (Figure 5). Under these experimental conditions, compound **26** strongly (25 μM) or completely inhibited (50 μM) MPT induction, confirming that this compound is much more effective than the tested MPT inhibitors. As shown in Figure 6, the calcium ion in the presence of phosphate induced oxidation and a decrease in sulfhydryl groups (approximately 10%) with respect to the untreated RBM. This effect was completely prevented by compound **26** and DTE, suggesting an antioxidant-like effect. Consistent with these findings, compound **26** had a significant protective effect against MPT induction and oxidative stress in both RLM and RBM. However, it must be noted that in RLM, MPT induction requires the establishment of oxidative stress; in fact, some typical MPT inhibitors are antioxidant agents (e.g., DTE). According to all of the above results, compound **26** behaves like these inhibitors. However, the results obtained in the RBM assays support the hypothesis, proposed in a previous paper,[38] that the mechanism of MPT induction is not correlated only with oxidative stress. Thus, the strong protective effect exhibited by **26** against the

MPT is due not only to its antioxidant activity but, most likely, also another mechanism involving pore opening in RBM. This mechanism could involve the interaction of Ca^{2+} with its critical binding site(s)[46] on adenine nucleotide translocase (AdNT), which is considered the main event leading to the MPT. Therefore, our results indicate that **26** is an MPT inhibitor with antioxidant properties in both RLM and RBM. Further studies are in progress to better characterize its possible role in some important process such as protection against intrinsic apoptosis and the maintenance of mitochondrial redox homeostasis.

Conclusions

In conclusion, we designed a set of novel bifunctional compounds that have both σ_1 agonist and antioxidant activities that act synergistically. To reach our goal, we combined a natural antioxidant moiety, α-lipoic acid, and an appropriate aminic moiety based on the piperidine or piperazine scaffold following the Gilligan pharmacophore hypothesis of σ ligands. We also investigated how the chemical structure of the *N*-substituent and its distance from Region C influence σ_1/σ_2 selectivity. Compounds with the *N*-benzyl moiety, **14** and **26**, displayed the highest affinity and selectivity for the σ_1 receptors ($K_i\sigma_1$ = 1.8 and 5.5 nM; $K_i\sigma_2/\sigma_1$ = 354 and 414, respectively). Among the newly tested ligands, we selected compound **26** on the basis of its excellent σ_1 selectivity and agonist activity for evaluation of its possible antioxidant property. Our results indicate that this compound possesses not only antioxidant activity but also MPT inhibitor activity. Taking into account that the cell death due to intrinsic apoptosis is mediated by the opening of the transition pore (MPT induction) as the result of an oxidative stress-dependent (RLM) or oxidative stress-independent (RBM) mechanism, the observation that the σ_1 receptor agonist **26** acts as an MPT inhibitor supports its potential protective role against diseases related to intrinsic apoptosis and makes it a very promising tool for the treatment of neurodegenerative diseases.

Experimental Section.

Chemical Materials and Methods. Reagents and reactants used for synthesis were purchased from Sigma & Aldrich (Milan, Italy) with the exception of the 1-(3-phenylpropyl)piperazine that was furnished by Apollo Scientific Ltd (UK). They were utilized as received without further purification. Reaction courses were monitored by thin-layer chromatography (TLC) on precoated silica gel 60 F_{254} aluminium sheets (Merck, Darmstadt, Germany) and the spots were visualized under UV light or in an iodine chamber. Merck silica gel 60, 230-400 mesh was used for flash column chromatography. Melting points were determined in open capillary tubes on digital Electrothermal apparatus 9100 (Rochford, UK) and are reported uncorrected. ^{1}H NMR and ^{13}C-NMR spectra were recorded with a Varian Inova 200 and 500 MHz or Varian Mercury 300 MHz spectrometer. Proton chemical shifts were reported in ppm (δ) relative to the internal standard tetramethylsilane (TMS, δ = 0.00 ppm). Carbon chemical shifts were reported in ppm (δ) relative to the residual solvent signal ($CDCl_3$, δ = 77.0 ppm). Gas chromatography-mass spectroscopy (GC-MS) analysis was recorded using a Shimadzu QP500 EI 171 (70 eV). Elemental analyses (C, H, N), performed on an elemental analyser Carlo Erba Model 1106 (Carlo Erba, Milan, Italy), confirm that the samples had a purity equal to or greater than 95%; the analytical results were within ± 0.4% of the theoretical values (Table S1, see Supporting Information). The radioactive materials [^{3}H]-(+)-Pentazocine (sa 29 Ci/mmol) and [^{3}H]-DTG [1,3-di-(2-tolyl)-guanidine] (sa 53.3 Ci/mmol) were obtained from Perkin Elmer.

Radioligand Binding Assays. *In vitro* σ-Binding experiments were carried out as previously reported.[47] σ_1 binding assays were performed on guinea pig brain membranes according to experimental protocol described by DeHaven et al.[48] Briefly, 500 μg of membrane protein were incubated with 3 nM [^{3}H]-(+)-pentazocine (29 Ci/mM; the value of the apparent dissociation constant (K_d) was 14 ± 0.3 nM, n = 3) in 50 mM Tris-HCl (pH 7.4). Test compounds were added in concentration ranging from 10^{-5} to 10^{-11} M. Nonspecific binding was assessed in the presence of 10 μM of unlabeled haloperidol. The reaction was performed for 150 min at 37 °C and terminated by filtering the solution through Whatman GF/B glass

fiber filters which were presoaked for 1 h in a 0.5% poly(ethylenimine) solution. Filters were washed with ice cold buffer (2 x 4 mL). In the matter of σ_2 binding assays[49] the membranes were incubated with 3 nM [^{3}H]DTG (53.3 Ci/mM; K_d = 11 ± 0.8 nM; n = 3) in the presence of 400 nM (+)-SKF10,047 so as to mask σ_1 sites. Nonspecific binding was evaluated with DTG (5 μM). Incubation was carried out in 50 mM Tris-HCl (pH 8.0) for 120 min at room temperature and assays were terminated by the addition of ice-cold 10 mM Tris-HCl (pH 8.0). Each sample was filtered through Whatman GF/B glass fibers filters, which were presoaked for 1 h in a 0.5% poly(ethylenimine) solution, using a millipore filter apparatus. Filters were washed twice with 4 mL of ice cold buffer. Radioactivity was counted in 4 mL of "Ultima Gold MV" in a 1414 Winspectral PerkinElmer Wallac liquid scintillation counter. Inhibition constants (K_i values) were calculated using the EBDA/LIGAND program purchased from Elsevier/Biosoft. Further σ receptor binding studies were performed by Cerep on compounds **12**, **13** and **24** as described by Ganapathy et al.[50] and Bowen et al.[51] Briefly, the σ_1 binding assays were conducted by incubating Jurkat cell membranes with 15 nM [^{3}H]-(+)-pentazocine (K_d = 37 nM) in 5 nM Tris-HCl buffer (pH 7.4). After addition of test compounds in concentration ranging from 10^{-5} to 10^{-11} M, the reaction was conducted for 2 h at 22 °C. Nonspecific binding was evaluated in the presence of unlabeled haloperidol (10 μM). σ_2 Receptors binding assays were performed on rat cerebral cortex membranes that were incubated with 5 nM [^{3}H](+)-DTG (K_d = 32 nM) in the presence of (+)pentazocine (300 nM) for masking σ_1 sites binding. Test compounds were added in concentration ranging from 10^{-5} to 10^{-11} M in 5 nM Tris-HCl buffer (pH 7.4). The reaction was performed at 22 °C for 120 min. Nonspecific binding was defined by the addition of haloperidol (10 μM). The data were obtained using a software developed at Cerep (Hill software) and validated by comparison with data generated by the commercial software SigmaPlot-4.0 for Windows.

In vivo studies

Animals. Male Sprague-Dawley rats (Morini, S. Polo d'Enza, Reggio Emilia, Italy), weighing 180–200

g, were used. Animals were kept at a constant room temperature (25±1 °C) under a 12:12 h light and dark cycle with free access to food and water. Each rat was used for only one experiment. Experimental procedures were approved by the Local Ethical Committee (IACUC) and conducted in accordance with international guidelines as well as European Communities Council Directive and National Regulations (CEE Council 86/609 and DL 116/92).

Nociceptive test. Nociception was evaluated by the radiant heat tail-flick test[52]. Briefly, it consisted of irradiation of the lower third of the tail with an infrared source (Ugo Basile, Comerio, Italy). The day before the experiment, rats were habituated to the procedure for measuring nociception threshold. Experiments were performed at room temperature (25±1 °C). The basal pre-drug latency was established between 3 and 4 s, and was calculated as the average of the first three measurements, which were performed at 5 min intervals. A cut-off latency of 10 s was established to minimize damage to the tail. Post-treatment tail flick latencies (TFLs) were determined 30, 45, 60, 75 and 90 min after subcutaneous (sc) injection.

Statistical analysis. All values are presented as means ± SD. Intergroup comparisons were assessed using an initial two-way analysis of variance (ANOVA) followed by Duncan's multiple range post-hoc test. Differences were considered significant when $*p<0.05$.

In vitro biological studies

Isolation of RLM and RBM. Rat liver homogenized in isolation medium (pH 7.4) composed of 250 mM sucrose, 5 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and 0.5 mM ethylene glycol tetraacetic acid (EGTA) was centrifuged at 900 x g for 5 min. The resulting supernatant was re-centrifuged at 12,000 x g for 10 min to separate the crude mitochondrial pellets; the obtained pellets were suspended in isolation medium lacking EGTA.[53] RBM were isolated using a conventional differential centrifugation method and purified by the Ficoll gradient method essentially according to Nicholls[54] but with some modifications. Briefly, rat brain (cerebral cortex) was homogenized in

isolation medium (320 mM sucrose, 5 mM HEPES, 0.5 mM EDTA, pH 7.4) supplemented with 0.3% bovine serum albumin (BSA) and centrifuged (900 x g) for 5 min. The supernatant was then centrifuged at 17,000 x g for 10 min to precipitate the crude mitochondrial pellets. These pellets were resuspended in isolation medium supplemented with 1 mM ATP and layered on top of a discontinuous gradient composed of 2 ml of isolation medium containing 12% (w/v) Ficoll and 3 ml each of isolation medium containing 9% and 6% (w/v) Ficoll. The mitochondrial suspension and gradient were centrifuged for 30 min at 75,000 x g. The mitochondrial pellets were suspended in isolation medium and centrifuged for 10 min at 17,000 x g. The resulting pellets were then resuspended in isolation medium without ethylenediaminetetraacetic acid (EDTA). The protein content was measured by the biuret method using BSA as the standard.[55] These studies were performed in accordance with the guiding principles for the care and use of animals and were approved by the Italian Ministry of Health.

Standard incubation conditions for RLM and RBM. RBM and RLM (1 mg protein/ml) were incubated in a water-jacketed cell at 20 °C. The standard medium contained 200 mM sucrose, 10 mM HEPES (pH 7.4), 5 mM sodium succinate, 1 mM sodium phosphate, and 1.25 μM rotenone. Ca^{2+} at 50 or 100 μM was added for the assays using RLM or RBM, respectively. Variations and/or other additions are described in the sections for the specific experiments.

Evaluation of mitochondrial swelling. The extent of mitochondrial swelling was determined by measuring the apparent absorbance change of the mitochondrial suspensions at a wavelength of 540 nm using a Kontron Uvikon model 922 spectrophotometer equipped with a thermostatic control.

Analysis of mitochondrial function. The analysis of protein sulfhydryl groups was performed with aliquots of mitochondrial suspensions taken from the incubations used to evaluate mitochondrial swelling. In brief, at the end of the incubation period (15 min), the total suspension (1 mg/ml) was placed in an Eppendorf 4515c tube and centrifuged for 1 min at 12,000 x g. Then, the supernatant was discarded, and the pellet used for both measurements. A mitochondrial thiol oxidation assay was

performed after solubilization of the pellet with 1 mg of solubilization medium (pH = 8.3) composed of 10 mM EDTA, 0.2 M Tris-HCl and 1% sodium dodecyl sulfate (SDS) and supplemented with Ellman's reagent [5,5'-dithiobis-(2-nitrobenzoic acid) or DTNB]. The analysis was performed at 412 nm using a Kontron Uvikon Model 922 spectrophotometer according to the method of Santos et al.[56] Variations between samples were analyzed by ANOVA, and significance was determined using Student's t-test.

Compound Syntheses.

General procedure for the preparation of ketones 10-16

5-(1,2-Dithiolan-3-yl)-1-(4-methylpiperazin-1-yl)pentan-1-one (10)

A solution of lipoic acid (1 g, 5 mmol) in DCM (20 mL) was added dropwise to a solution of DCC (1.031 g, 5 mmol) in anhydrous DCM (20 mL) under stirring and nitrogen atmosphere. After 10 minutes a solution of 1-methylpiperazine (**3**) (0.5 g, 5 mmol) in DCM (10 mL) was added dropwise and the resulting mixture was stirred for 3 h. The *N,N'*-dicyclohexylurea (DCU) that has precipitated is removed by filtration through a fritted-glass Büchner funnel, and the filtrate is washed with sodium bicarbonate solution (5%) (2 x 10 mL) and brine (2 x 10 mL). After dried over Na_2SO_4, the solvent was removed under reduced pressure. During this procedure the additional precipitated DCU was removed by several filtrations. The organic phase was concentrated and the obtained oil was purified by flash chromatography using CH_2Cl_2, CH_2Cl_2/CH_3OH 9,75:0,25 and 9.5/0.5 as mobile phase. The free base was converted into the corresponding hydrochloride salt with 2 N HCl in diethyl ether and recrystallized from methanol to give **10** as yellow solid. Yield = 20%; R_f = 0.52 [CH_2Cl_2/CH_3OH, 8:2]; mp = 141.7 °C dec; MS *m/z* $[M]^+$ = 288.1; IR (NaCl) 2924, 2852, 2792, 1642, 1440; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 1.43-1.52 (m, 2H), 1.60-1.74 (m, 4H), 1.87-1.94 (m, 1H), 2.30-2.34 (m, 5H), 2.40-2.48 (m, 5H), 3.08-3.10 (m, 1H), 3.11-3.20 (m, 1H), 3.44-3.47 (m, 2H), 3.49-3.50 (m, 1H), 3.54-3.65 (broad t, 2H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 171.1, 56.4, 55.0, 54.6, 45.8, 45.2, 41.2, 40.2, 38.5, 34.7, 32.9, 29.1, 24.9. Anal. ($C_{13}H_{24}N_2OS_2$ HCl) C, H, N, S.

1-(4-Cyclohexylpiperazin-1-yl)-5-(1,2-dithiolan-3-yl)pentan-1-one (11)

This compound was synthesized with the same procedure reported above using the 1-cyclohexylpiperazine (**4**) (0.787 g, 4.84 mmol). The crude product was purified by flash chromatography on silica gel (EtOAc/cyclohexane 8:2, EtOAc 10, EtOAc/EtOH 9.5:0.5). The free base was converted into the corresponding hydrochloride salt with 2 N HCl in diethyl ether and recrystallized from methanol. Yield = 44.1%; R_f = 0.55 [CH_2Cl_2/MeOH, 9:1]; mp = 173.1 °C dec; MS *m*/*z* $[M]^+$ = 356; IR (KBr) 2933, 2850, 2809, 1634, 1433. ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 1.11-1.30 (m, 4H), 1.42-1.97 (m, 11H), 2.29-2.72 (m, 9H), 3.05-3.26 (m, 2H), 3.46-3.78 (m, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.0, 63.6, 56.4, 49.3, 48.7, 46.1, 42.0, 40.2, 38.5, 34.8, 32.9, 29.1, 28.9, 26.2, 25.8, 25.0. Anal. ($C_{18}H_{32}N_2OS_2$ HCl) C, H, N, S.

1-(4-Cyclohexylmethylpiperazin-1-yl)-5-(1,2-dithiolan-3-yl)pentan-1-one (12)

This compound was prepared by using the 1-(cyclohexylmethyl)piperazine (**5**) (0.91 g, 5 mmol) according to the synthetic method described above. The crude product was purified by flash chromatography on silica gel using ethyl acetate/ cyclohexane 8:2 as eluting system, converted into the corresponding hydrochloride salt with 2 N HCl in diethyl ether and recrystallized from methanol. Yield = 31.2%; R_f = 0.47 [AcOEt/EtOH, 9:1]; mp = 202 °C dec; MS *m*/*z* $[M]^+$ = 370.3; IR (NaCl) 2922, 2850, 1643, 1444, 1004. ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 0.76-0.83 (m, 2H), 1.17-1.20 (m, 2H), 1.37-1.45 (m, 3H), 1.54-1.70 (m, 9H), 1.81-1.88 (m, 1H), 2.06-2.10 (d, 2H), 2.23-2.30 (m, 6H), 2.36-2.43 (m, 1H), 3.02-3.14 (m, 2H), 3.36-3.43 (m, 3H), 3.49-3.54 (m, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 171.1, 65.4, 56.4, 53.9, 53.2, 45.6, 41.5, 40.2, 38.4, 34.9, 34.7, 32.9, 31.8, 29.1, 26.7, 26.0, 25.0. Anal. ($C_{19}H_{34}N_2OS_2$ HCl) C, H, N, S.

5-(1,2-Dithiolan-3-yl)-1-(4-phenylpiperazin-1-yl)pentan-1-one (13)

This compound was synthesized by the same procedure reported above using the 1-phenylpiperazine (**6**) (0.811 g, 5.0 mmol). The crude product was purified by flash chromatography on silica gel using ethyl acetate, converted into the corresponding hydrochloride salt with 2 N HCl in diethyl ether and recrystallized from methanol. Yield = 49.2%; R_f = 0.64 [AcOEt/C_2H_5OH), 9:1]; mp = 136.2 °C dec; MS

m/z $[M]^+$ = 350.1; IR (KBr) 3042, 2932, 2853, 1642, 1599, 1578. ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 1.47-155 (m, 2H), 1.64-1.78 (m, 4H), 1.89-1.95 (m, 1H), 2.38 (t, *J* = 7.5 Hz, 2H), 2.44-2.50 (m, 1H), 3.10-3.22 (m, 6H), 3.58-3.64 (m, 3H), 3.78 (t, *J* = 5 Hz, 2H), 6.89-6.94 (m, 3H), 7.29 (t, *J* = 7.5 Hz, 2H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 171.2, 150.9, 129.2, 120.6, 116.6, 56.4, 49.8, 49.4, 45.5, 41.5, 40.2, 38.5, 34.8, 33.0, 29.1, 25.0. Anal. ($C_{18}H_{26}N_2OS_2$ HCl) C, H, N, S.

1-(4-Benzylpiperazin-1-yl)-5-(1,2-dithiolan-3-yl)pentan-1-one (14)

Compound **12** was prepared by use of the 1-benzylpiperazine (**7**) (0.33 g, 1.34 mmol) in agreement with the procedure reported above. Purification of the crude product was carried out by flash chromatography on silica gel eluting with ethyl acetate. The free base was converted to the corresponding hydrochloride salt using 4 M HCl in dioxane and recrystallized from diethyl ether and methanol. Yield = 93%; R_f = 0.3 [CH_2Cl_2/CH_3OH 9:1]; mp = 200-201.3 °C; MS *m/z* $[M]^+$ = 364.2; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 1.39-1.55 (m, 2H), 1.61-1.76 (m, 4H), 1.82-1.94 (m, 1H), 2.23-2.50 (m, 7H), 3.03-3.20 (m, 2H), 3.42-3.62 (m, 7H), 7.20-7.35 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.3, 138.0, 129.4, 128.6, 127.5, 63.1, 56.7, 53.4, 53.0, 45.7, 41.7, 40.5, 38.7, 35.0, 33.2, 29.4, 25.2. Anal. ($C_{19}H_{28}N_2OS_2$ HCl) C, H, N, S.

5-(1,2-Dithiolan-3-yl)-1-(4-phenethylpiperazin-1-yl)pentan-1-one (15)

This compound was synthesized via the same procedure detailed above using the 1-(2-phenethyl)piperazine (**8**) (0.19 g; 1mmol). The crude product was purified by flash chromatography on silica gel using CH_2Cl_2/CH_3OH 9.5:0.5 as mobile phase. The free base was converted to its hydrochloride salt with 4 M HCl in dioxane and recrystallized from diethyl ether and methanol. Yield = 45%; R_f = 0.3 [CH_2Cl_2/CH_3OH 9.5:0.5]; mp = 160-161.5 °C; MS *m/z* $[M]^+$ = 378.2; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 1.40-1.53 (m, 2H), 1.58-1.77 (m, 4H), 1.80-1.92 (m, 1H), 2.23-2.64 (m, 7H), 2.72-2.81 (m, 2H), 3.01-3.19 (m, 2H), 3.43-3.65 (m, 7H), 7.15-7.29 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.4, 140.0, 129.0, 128.7, 126.4, 60.3, 56.7, 53.5, 53.0, 45.5, 41.5, 40.5, 38.7, 35.0, 33.4, 33.2, 29.3, 25.2. Anal. ($C_{20}H_{30}N_2OS_2$ HCl) C, H, N, S.

5-(1,2-Dithiolan-3-yl)-1-(4-(3-phenylpropyl)piperazin-1-yl)pentan-1-one (16)

This compound was obtained from the 1-(3-phenylpropyl)piperazine (**9**) (1.02 g, 5 mmol) in the same manner as described above and was purified using the procedure described for compound **12**. The oil obtained was converted into the corresponding hydrochloride salt with 2 N diethyl ether and recrystallized from methanol. Yield = 31.2%; R_f = 0.36 [AcOEt/EtOH, 8:2], mp = 142.3 °C dec; MS *m*/*z* $[M]^+$ = 392.2; IR (NaCl) 3024, 2931, 1643, 1440, 748, 702. ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 1.44-1.54 (m, 2H), 1.66-1.76 (m, 3H), 1.80-1.86 (m, 2H), 1.89-1.94 (m, 1H), 2.60 (t, *J* = 7.5 Hz, 2H), 2.36-2.50 (m, 8H), 2.66 (t, *J* = 7.5 Hz, 2H), 3.10-3.21 (m, 2H), 3.46-3.48 (m, 2H), 3.56-3.64 (m, 3H), 7.18-7.21 (m, 3H), 7.29 (t, *J* = 7.5 Hz, 2H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 171.1, 142.0, 128.3, 125.8, 57.7, 56.4, 53.4, 52.8, 45.6, 41.5, 40.2, 38.5, 34.8, 33.5, 32.9, 29.1, 28.4, 25.0. Anal. ($C_{21}H_{32}N_2OS_2$ HCl) C, H, N, S.

The synthesis of alcohols **21**, **23** and **24** has already been reported.[40]

General procedure for the preparation of esters 25-28.

1-(Cyclohexylmethyl)piperidin-4-yl 5(1,2-dithiolan-3-yl)pentanoate (25)

To a solution of 1-(Cyclohexylmethyl)piperidin-4-ol (**21**) (0.43 g, 2.20 mmol), α-lipoic acid (0.45 g, 42.20 mmol) and DMAP (0.22 mmol, 0.026 mg) in 5.4 mL of dry CH_2Cl_2 at 0 °C was added 0.45 g of DCC (2.20 mmol) under stirring and nitrogen atmosphere. After 10 minutes the reaction temperature was slowly raised to room temperature and the reaction was stirred for over 3 h. The dicyclohexylurea (DCU) that has precipitated is removed by filtration through a fritted Büchner funnel. The filtrate was washed twice with 10-mL of sodium bicarbonate solution (5%) and twice with 10-mL of brine solution. After dried (Na_2SO_4) the solvent was removed under reduced pressure. During this procedure the additional precipitated DCU was removed by several filtrations. The organic phase was concentrated and purified by flash chromatography using ethyl acetate/cyclohexane (7:3). The free base was converted into the corresponding hydrochloride salt with 4 N HCl in dioxane and recrystallized from methanol. Yield = 39.6%; R_f = 0.55 (AcOEt); mp = 174.1 °C dec; MS *m*/*z* $[M]^+$ = 385.2; IR (NaCl) 2921, 2850, 2803, 1730, 1449, 1179, 1034; ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 0.83-0.93 (m, 2H), 1.11-

1.99 (m, 19H), 2.05-2.21 (m, 4H), 2.31 (t, J = 7.4 Hz, 2H), 2.39-2.54 (m, 1H), 2.61-2.79 (m, 3H), 3.05-3.25 (m, 2H), 3.50-3.64 (m, 1H), 4.70-4.87 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 172.9, 70.5, 65.5, 56.3, 51.3, 40.2, 38.4, 35.3, 34.5, 34.4, 31.9, 30.8, 28.7, 27.7, 26.1, 24.7. Anal. ($C_{20}H_{35}NO_2S_2$ HCl) C, H, N, S.

1-Benzylpiperidin-4-yl 5-(1,2-dithiolan-3-yl)pentanoate (26)

This compound was prepared from commercially available 1-benzylpiperidin-4-ol (**22**) (0.19 g, 1mmol) according to previously reported procedure for the compound **25**. The crude product was purified by flash chromatography on silica gel using cyclohexane/ethyl acetate (7:3) as eluting system. The oil obtained was converted to its hydrochloride salt with 4 M HCl in dioxane and recrystallized from isopropanol. Yield = 78%; R_f = 0.6 (AcOEt/cyclohexane 7:3); mp = 191.8–193.1 °C; MS m/z $[M]^+$ = 379.58; ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 1.44-1.99 (m, 11H), 2.21-2.68 (m, 7H), 3.05-3.25 (m, 2H), 3.50-3.63 (m, 3H), 4.75-4.85 (m, 1H), 7.26-7.32 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.2, 138.6, 129.3, 128.4, 127.3, 70.5, 63.2, 56.6, 51.0, 40.4, 38.7, 34.8, 34.6, 31.1, 29.0, 25.0. Anal. ($C_{20}H_{29}NO_2S_2$ HCl) C, H, N, S.

1-Phenethylpiperidin-4-yl 5-(1,2-dithiolan-3-yl)pentanoate (27)

This compound was synthesized from the 1-phenethyl-piperidin-4-ol (**23**) (0.995 g, 4.85 mmol) by the general via reported above. The crude product was purified using the procedure described for **25** and the obtained oil was converted to its hydrochloride salt with 4 N HCl in dioxane and recrystallized from methanol. Yield = 69.5%; R_f = 0.5 [AcOEt/CH_3OH, 8:2]; mp = 155.8 °C dec; MS m/z $[M]^+$ = 394.3; IR (NaCl) 3026, 2932, 2808, 1728, 1603, 1548; ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 1.46-1.57 (m, 2H), 1.60-2.0 (m, 8H), 2.29-2.61 (m, 8H), 2.72-2.85 (m, 4H), 3.05-3.26 (m, 2H), 3.51-3.64 (m, 1H), 4.75-4.87 (m, 1H), 7.16-7.34 (m, 5H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 172.9, 140.3, 128.6, 128.3, 125.9, 70.1, 60.4, 56.3, 50.8, 40.2, 38.4, 34.6, 34.4, 33.8, 30.8, 28.7, 24.7. Anal. ($C_{21}H_{31}NO_2S_2$ HCl) C, H, N, S.

1-(3-Phenylpropyl)piperidin-4-yl 5-(1,2-dithiolan-3-yl)pentanoate (28)

This compound was prepared by using the 1-(3-phenylpropyl)-piperidin-4-ol (**24**) (1.06 g, 4.85 mmol)

by the same procedure previously employed for **25-27**. The crude product was purified by flash chromatography and eluted with ethyl acetate/cyclohexane ethyl (7:3). The free base was converted to its hydrochloride salt with 4 N HCl in dioxane and recrystallized from methanol. Yield = 76.2%; R_f = 0.53 [AcOEt/CH_3OH, 8:2]; mp = 121.4 °C dec; MS *m*/*z* $[M]^+$ = 407.15; IR (NaCl) 3024, 2929, 2856, 1729, 1453; ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 1.43-1.99 (m, 13H), 2.16-2.59 (m, 7H), 2.63-2.74 (m, 4H), 3.08-3.25 (m, 2H), 3.46-3.64 (m, 1H), 4.72-4.84 (m, 1H), 7.13-7.33 (m, 5H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 172.9, 142.1, 128.3, 128.2, 125.7, 70.2, 57.9, 56.3, 50.8, 40.2, 38.4, 34.6, 34.3, 33.7, 30.8, 28.8, 28.7, 24.7. Anal. ($C_{22}H_{33}NO_2S_2$ HCl) C, H, N, S.

Supporting Information Available: Elemental analysis results. This material is available free of charge via the Internet at http://pubs.acs.org.

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Abbreviations Used

AdNT, adenine nucleotide translocase; ADP, adenosine 5□-diphosphate; ALA, alpha lipoic acid; ARE, antioxidant response element; ATP, adenosine 5□-triphosphate; Bip, Binding immunoglobulin protein; BSA, bovine serum albumin; CsA, cyclosporin A; DCC, *N*,*N*′-dicyclohexylcarbodiimide; DCM, dichloromethane; DCU, *N*,*N*′-dicyclohexylurea; DMAP, 4-(*N*,*N*-dimethylamino)pyridine; DTE, dithioerythriol; DTG, 1,3-di-(2-tolyl)-guanidine; DTNB, 5,5'-dithiobis-(2-nitrobenzoic acid); EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol tetraacetic acid; ER, endoplasmic reticulum; GC-MS, gas chromatography-mass spectrometry; haloperidol, 4-[4-(4-chlorophenyl)-4-hydroxy-1-piperidyl]-1-(4-fluorophenyl)-butan-1-one; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; IACUC, Institutional Animal Care and Use Committee; IP3R, inositol 1,4,5-trisphosphate receptor; IR, infrared; K_i, inhibition constant; KOP, kappa opioid; M^+, parent molecular ion; MAM, mitochondria-associated ER membrane; mp, melting point; MPT, mitochondrial permeability transition;

MS, mass spectrometry; *m*/*z*, mass-to-charge ratio (not *m*/*e*); NMR, nuclear magnetic resonance; NQO1, NAD(P)H:quinone oxidoreductase 1; (+)-pentazocine, (2*S*,6*S*,11*S*)-6,11-dimethyl-3-(3-methylbut-2-en-1-yl)-1,2,3,4,5,6-hexahydro-2,6-methano-3-benzazocin-8-ol; RBM, rat brain mitochondria; R_f, retention factor (in chromatography); RLM, rat liver mitochondria; ROS, reactive oxygen species; Rotenone; (2*R*,6a*S*,12a*S*)-1,2,6,6a,12,12a- hexahydro-2-isopropenyl-8,9- dimethoxychromeno[3,4-b] furo(2,3-h)chromen-6-one; rt, room temperature; SAR, structure-activity relationship; sc, subcutaneous; SD, standard deviation; SDS, sodium dodecyl sulphate;σ, sigma receptor; SOD1, superoxide dismutase 1; TFLs, tail flick latencies; TLC, thin-layer chromatography; Tris-HCl, Tris(hydroxymethyl)aminomethane hydrochloride; UV, ultraviolet.

References

(1) Martin, W. R.; Eades, C. G.; Thompson, J. A.; Huppler, R. E.; Gilbert, P. E. The effects of morphine-and nalorphine- like drugs in the nondependent and morphine-dependent chronic spinal dog. *J. Pharmacol. Exp. Ther*. **1976**, *197*, 517-532.

(2) Quirion, R.; Bowen, W. D.; Itzhak, Y.; Junien, J. L.; Musacchio, J. M.; Rothman, R. B.; Su, T. P.; Tam, S. W.; Taylor, D. P. A proposal for the classification of sigma binding sites. *Trends Pharmacol. Sci*. **1992**, *13*, 85-86.

(3) Hellewell, S. B., Bruce, A., Feinstein, G., Orringer, J., Williams, W., Bowen, W. D. Rat liver and kidney contain high densities of sigma-1 and sigma-2 receptors: Characterization by ligand binding and photoaffinity labeling. *Eur. J. Pharmacol*. **1994**, *268*, 9-18.

(4) Hanner, M.; Moebius, F. F.; Flandorfer, A.; Knaus, H. G.; Striessnig, J.; Kempner, E.; Glossmann, H. Purification, molecular cloning, and expression of the mammalian sigma1-binding site. *Proc. Natl. Acad. Sci. U S A*. **1996**, *93*, 8072-8077.

(5) Pan, Y. X.; Mei, J.; Xu, J.; Wan, B. L.; Zuckerman, A.; Pasternak, G. W. Cloning and

characterization of a mouse sigma1 receptor. *J. Neurochem*. **1998**, *70*, 2279-2285.

(6) Mei, J.; Pasternak, G.W. Molecular cloning and pharmacological characterization of the rat sigma1 receptor. *Biochem Pharmacol*. **2001**, *62*, 349-355.

(7) Maurice, T.; Su, T. P. The pharmacology of sigma-1 receptors. *Pharmacol Ther*. **2009**, *124*, 195-206.

(8) DeCoster, M. A.; Klette, K. L.; Knight, E. S.; Tortella, F. C. Sigma receptor-mediated neuroprotection against glutamate toxicity in primary rat neuronal cultures. *Brain Res*. **1995**, *671*, 45-53.

(9) Nakazawa, M.; Matsuno, K.; Mita, S. Activation of sigma1 receptor subtytpe leads to neuroprotection in the rat primary neuronal cultures. *Neurochem Int*. **1998**, *32*, 337-343.

(10) Tchedre, K. T.; Yorio, T. Sigma-1 receptors protect RGC-5 cells from apoptosis by regulating intracellular calcium, Bax levels, and caspase-3 activation. *Invest. Ophthalmol. Vis. Sci*. **2008**, *49*, 2577-2588.

(11) Huang, Y.; Zheng, L.; Halliday, G.; Dobson-Stone, C.; Wang, Y.; Tang, H. D.; Cao, L.; Deng, Y. L.; Wang, G.; Zhang, Y. M.; Wang, J. H.; Hallupp, M.; Kwok, J.; Chen, S. D. Genetic polymorphisms in sigma-1 receptor and apolipoprotein E interact to influence the severity of Alzheimer's disease. *Curr. Alzheimer Res*. **2011**, *8*, 765-770.

(12) Al-Saif, A.; Al-Mohanna, F.; Bohlega, S. A mutation in sigma-1 receptors causes juvenile amyotrophic lateral sclerosis. *Ann. Neurol*. **2011**, *70*, 913-919.

(13) Mishina, M.; Ishiwata, K.; Ishii, K.; Kitamura, S.; Kimura, Y.; Kawamura, K.; Oda, K.; Sasaki, T.; Sakayori, O.; Hamamoto, M.; Kobayashi, S.; Katayama, Y. Function of sigma1 receptors in Parkinson's disease. *Acta Neurol. Scand*. **2005**, *112*, 103-107.

(14) Mori, T.; Hayashi, T.; Su, T. P. Compromising sigma-1 receptors at the ER renders cytotoxicity to

physiologically relevant concentations of dopamine in a NF-κB/Bcl-2-dependent mechanism: Potential relevance to Parkinson's disease. *J. Pharmacol. Exp. Ther*. **2012**, *341*, 663-671.

(15) Jellinger, K. A. Basic mechanisms of neurodgeneration: a critical update. *J. Cell. Mol. Med*. **2010**, *14*, 457-487.

(16) Facecchia, K.; Fochesato, L. A.; Ray, S.D.; Stohs, S. J.; Pandey, S. Oxidative toxicity in neurodegenerative diseases: role of mitochondrial dysfunction and therapeutic strategies. *J. Toxicol*. [Online early access]. DOI:10.1155/2011/683728. Published Online: July 14, 2011.

(17) Hayashi, T.; Rizzuto, R.; Hajnoczky, G.; Su, T. P. MAM: more than just a housekeeper. *Trends Cell. Biol*. **2009**, *19*, 2, 81-88.

(18) Hayashi, T.; Su, T. P. Sigma-1 receptor chaperones at the ER-mitochondrion interface regulate Ca^{2+} signaling and cell survival. *Cell*. **2007**, 131, 596-610.

(19) Meunier, J.; Hayashi, T. Sigma-1 receptors regulate Bcl-2 expression by reactive oxygen species-dependent transcriptional regulation of nuclear factor kappaB. *J. Pharmacol. Exp. Ther*. **2010**, *332*, 388-397.

(20) Pal, A.; Fontanilla, D.; Gopalakrishnan, A.; Chae, Y. K.; Markley, J. L.; Ruoho, A. E. The sigma-1 receptor protects against cellular oxidative stress and activates response elements. *Eur. J. Pharmacol*. **2012**, *682*, 12-20.

(21) Marrazzo, A.; Caraci, F.; Salinaro, E. T.; Su, T. P.; Copani, A.; Ronsisvalle, G. Neuroprotective effects of sigma-1 receptor agonists against beta-amyloid-induced toxicity. *Neuroreport*. **2005**, *16*, 1223-1226.

(22) Smith, S. B.; Duplantier, J.; Dun, Y.; Mysona, B.; Roon, P.; Martin, P. M.; Ganapathy, V. In vivo protection against retinal neurodegeneration by sigma receptor 1 ligand (+)-pentazocine. *Invest.*

Ophthalmol. Vis. Sci. **2008**, *49*, 4154-4161.

(23) Prezzavento, O.; Campisi, A.; Parenti, C.; Ronsisvalle, S.; Aricò, G.; Arena, E.; Pistolozzi, M.; Scoto, G. M., Bertucci, C., Vanella, A., Ronsisvalle, G. Synthesis and resolution of cis-(+/-)-methyl (1R,2S/1S,2R)-2-[(4-hydroxy-4-phenylpiperidin-1-yl)methyl]-1-(4-methylphenyl)cyclopropanecarboxylate[(+/-)-PPCC)]: new sigma receptor ligands with neuroprotective effect. *J. Med. Chem*. **2010**, *53*, 5881-5885.

(24) Luedtke, R. R.; Perez, E.; Yang, S. H.; Liu, R.; Vangveravong, S.; Tu, Z.; Mach, R. H.; Simpkins, J. W. Neuroprotective effects of high affinity sigma 1 receptor selective compounds. *Brain Res*. **2012**, *1441*, 17-26.

(25) Moosmann, B.; Behl, C. Antioxidants as treatment for neurodegenerative disorders. *Expert Opin. Investig. Drugs*. **2002**, *11*, 1407-1435.

(26) Casetta, I.; Govoni, V.; Granieri, E. Oxidative stress, antioxidants and neurodegenerative diseases. *Curr. Pharm. Des*. **2005**, *11*, 2033-2052.

(27) Morphy, R.; Rankovic, Z. Designing multiple ligands – medicinal chemistry strategies and challenges. *Curr. Pharm. Des*. **2009**, *15*, 587-600.

(28) Packer, L.; Witt, E. H.; Tritschler, H. J. Alpha-Lipoic acid as a biological antioxidant. *Free Radic. Biol. Med*. **1995**, *19*, 227-250.

(29) Liu, J. The effects and mechanisms of mitochondrial nutrient alpha-lipoic acid on improving age-associated mitochondrial and cognitive dysfunction: an overview. *Neurochem. Res*. **2008**, *33*, 194-203.

(30) Biewenga, G.P.; Haenen, G.R.; Bast, A. The pharmacology of the antioxidant lipoic acid. *Gen. Pharmacol*. **1997**, *29*, 315-331.

(31) Zhang, L.; Xing, G. Q.; Barker, J. L.; Chang, Y.; Maric, D.; Ma, W.; Li, B. S.; Rubinow, D. R.

Alpha-lipoic acid protects rat cortical neurons against cell death induced by amyloid and hydrogen peroxide through the Akt signalling pathway. *Neurosci. Lett*. **2001**, *312*, 125-128.

(32) Ono, K.; Hirohata, M.; Yamada, M. Alpha-lipoic acid exhibits anti-amyloidogenicity for beta-amyloid fibrils in vitro. *Biochem. Biophys. Res. Commun*. **2006**, *341*, 1046-1052.

(33) Toklu, H. Z.; Hakan, T.; Celik, H.; Biber, N.; Erzik, C.; Ogunc, A. V.; Akakin, D.; Cikler, E.; Cetinel, S.; Ersahin, M.; Sener, G. Neuroprotective effects of alpha-lipoic acid in experimental spinal cord injury in rats. *J. Spinal Cord Med*. **2010**, *33*, 401-409.

(34) Gilligan, P. J.; Cain, G. A.; Christos, T. E.; Cook, L.; Drummond, S.; Johnson, A. L.; Kergaye, A. A.; McElroy, J. F.; Rohrbach, K. W.; Schmidt, W. K.; et al. Novel piperidine sigma receptor ligands as potential antipsychotic drugs. *J. Med. Chem*. **1992**, *35*, 4344-4361.

(35) Largent, B. L.; Wikström, H., Gundlach, A. L.; Snyder, S. H. Structural determinants of sigma receptor affinity. *Mol. Pharmacol*. **1987**, *32*, 772-784.

(36) Manallack, D. T.; Wong, M. G.; Costa, M.; Andrews, P. R.; Beart, P. M. Receptor site topographies for phencyclidine-like and sigma drugs: predictions from quantitative conformational, electrostatic potential, and radioreceptor analyses. *Mol. Pharmacol*. **1988**, *34*, 863-879.

(37) Prezzavento, O.; Parenti, C.; Marrazzo, A.; Ronsisvalle, S.; Vittorio, F.; Arico, G.; Scoto, G. M.; Ronsisvalle, G. A new sigma ligand, (±)-PPCC, antagonizes kappa opioid receptor-mediated antinociceptive effect. *Life Sci*. **2008**, *82*, 549-553.

(38) Grancara, S.; Battaglia, V.; Martinis, P.; Viceconte, N.; Agostinelli, E.; Toninello, A.; Deana, R. Mitochondrial oxidative stress induced by Ca^{2+} and monoamines: different behaviour of liver and brain mitochondria in undergoing permeability transition. *Amino Acids*. **2012**, *42*, 751-759.

(39) Zhang, Y.; Williams, W.; Torrence-Campbell, C.; Bowen, W. D.; Rice, K. C. Characterization of

novel *N*,*N*'-disubstituted piperazines as sigma receptor ligands. *J. Med. Chem*. **1998**, *41*, 4950-4957.

(40) Weis, R.; Faist, J.; di Vora, U.; Schweiger, K.; Brandner, B.; Kungl, A. J.; Seebacher, W. Antimycobacterial activity of diphenylpyraline derivatives. *Eur. J. Med. Chem*. **2008**, *43*, 872-879.

(41) Neises, B.; Steglich, W. Simple Method for the Esterification of Carboxylic Acids. *Angew. Chem. Int. Ed. Engl*. **1978**, *17*, 522-524.

(42) Pasternack, G. W. σ_1 Receptors and the Modulation of Opiate Analgesics. In *Sigma Receptors: Chemistry, Cell Biology and Clinical Implications*; Kluwer Academic Publishers: New York, 2006; pp 337-350.

(43) King, M.; Pan, Y. X.; Mei, J.; Chang, A.; Xu, J.; Pasternak, G. W. Enhanced kappa-opioid receptor-mediated analgesia by antisense targeting the sigma1 receptor. *Eur. J. Pharmacol*. **1997**, *331*, R5-6.

(44) Zoratti, M.; Szabò, I. The mitochondrial permeability transition. *Biochim. Biophys. Acta*. **1995**, *1241*, 139-176.

(45) Kroemer, G.; Galluzzi, L.; Brenner, C. Mitochondrial membrane permeabilization in cell death. *Physiol. Rev*. **2007**, *87*, 99-163.

(46) Toninello, A.; Salvi, M.; Mondovì, B. Interaction of biologically active amines with mitochondria and their role in the mitochondrial-mediated pathway of apoptosis. *Curr. Med. Chem*. **2004**, *11*, 2349-2374.

(47) Matsumoto, R. R.; Bowen, W. D.; Tom, M. A.; Vo, V. N.; Truong, D. D.; De Costa, B. R. Characterization of two novel sigma receptor ligands: Antidystonic effects in rats suggest sigma receptor antagonism. *Eur. J. Pharmacol.* **1995**, *280*, 301-310.

(48) DeHaven-Hudkins, D. L.; Ford-Rice, Y. Characterization of the binding of [3H]-(+)-pentazocine to

sigma recognition site in guinea pig brain. *Eur. J. Pharmacol.* **1992**, *227*, 371-378.

(49) Macth, R. H.; Smith, C. R.; Childers, S. R. Ibogaine possesses a selective affinity for sigma-2 receptors. *Life Sci*. **1995**, *57*, 57-62.

(50) Ganapathy, M. E.; Prasad, P. D.; Huang, W.; Seth, P.; Leibach, F. H.; Ganapathy, V. J. Molecular and ligand-binding characterization of the sigma-receptor in the Jurkat human T lymphocyte cell line. *J. Pharmacol. Exp. Ther*. **1999**, *289*, 251-260.

(51) Bowen, W. D.; De Costa, B. R.; Hellewell, S. B.; Walker, M.; Rice, K. C. Pentazocine: A potent and highly selective benzomorphan-based probe for sigma-1 receptors. *Mol. Neuropharmacol*. **1993**, *3*, 117-126.

(52) Scoto, G. M.; Aricò, G.; Ronsisvalle, S.; Parenti, C. Blockade of the nociceptin/orphanin FQ/NOP receptor system in the rat ventrolateral periaqueductal gray potentiates DAMGO analgesia. *Peptides*. **2007**, *28*, 1441-1446.

(53) Schneider, W. C.; Hogeboon, H. G. Intracellular distribution of enzymes: XI. Glutamic dehydrogenase. *J. Biol. Chem*. **1953**, *204*, 233-238.

(54) Nicholls, D. G. Calcium transport and proton electrochemical potential gradient in mitochondria from guinea-pig cerebral cortex and rat heart. *Biochem. J*. **1978**, *170*, 511-522.

(55) Gornall, A. G.; Bardawill, C. J.; David, M. M. Determination of serum proteins by means of the biuret method. *J. Biol. Chem*. **1949**, *177*, 751-766.

(56) Santos, A. C.; Uyemura, S. A.; Lopes, J. L.; Bazon, J. N.; Mingatto, F. E.; Curti, C. Effect of naturally occurring flavonoids on lipid peroxidation and membrane permeability transition in mitochondria. *Free Radic. Biol. Med*. **1998**, *24*, 1455-1461.

Table 1. σ Binding affinities of piperidines 10-16 and piperazines 25-28

σ Binding affinities (K_i, nM ± SD)[a]			
Cmpd	**σ_1**	**σ_2**	**$K_i\sigma_2/K_i\sigma_1$**
	[^{3}H]-Pentazocine	**[^{3}H]-DTG**	
10	115 ± 1	21000 ± 400	182.6
11	0.4 ± 0.0	11.25 ± 0.55	28
12	1.25 ± 0.25	35.5 ± 2.5	28.4
13	1700 ± 100	10600 ± 300	6.23
14	1.8 ± 0.2 2.5*	637 ± 15 2500*	353.8 1000
15	8.4 ± 1 6.5*	209 ±11 3000*	24.8 461
16	10 ± 2	91.6 ± 7	9.16
25	1.14 ± 0.8	14 ± 2	12.3
26	5.56 ± 0.5 0.4*	2302 ± 45 8300*	414 >20000
27	13.5 ± 0.5	160.4 ± 8	11.8
28	25.7 ± 2	100.8 ± 4	3.9

[a] Each value is the means ± SD of three determinations. *Data obtained from Cerep laboratories were determined by single experiments.

Figure Legends

Figure 1. The four pharmacophore regions proposed by Gilligan et al., matched with our piperazine and piperidine derivatives containing the lipoyl function, are represented by a distal aromatic ring (Region A), a space between the nitrogen heterocycle and the distal hydrophobic site (Region B), a nitrogen heterocycle (Region C), and a substituent on the basic nitrogen (Region D).

Figure 2. Effect of **26** on the antinociception induced by **2**. Haloperidol reversed the antiopioid effect of **26**. The results are expressed as the mean ± SD. **p*<0.05 *vs* **2**-treated rats (n= 8−10). $^{\#}p$<0.05 *vs* **26** + **2**-treated rats (n= 8−10).

Figure 3. Liver mitochondria swelling induced by Ca^{2+}. Prevention of swelling by compound **26**, CsA, ADP and DTE. Where indicated, 1 μM CsA, 0.5 mM ADP, or 3 mM DTE was present. **26** was present at the indicated concentrations (μM). A downward deflection indicates a decrease in absorbance. The assays were performed five times with comparable results.

Figure 4. Effect of **26** or DTE on sulfhydryl group oxidation induced by Ca^{2+} in RLM. **26** was present at the indicated concentrations (μM). DTE was present at a concentration of 3 mM. Data are expressed as the percentage of reduced thiol groups and represent average ± mean SD from five independent experiments.

Figure 5. Brain mitochondria swelling induced by Ca^{2+}. Prevention of swelling by compound **26**, CsA, ADP and DTE. The RBM incubation conditions, compound concentrations, meaning of the downward deflection, and statistics were as described for Figure 3.

Figure 6. Effect of **26** and DTE on the sulfhydryl group oxidation induced by Ca^{2+} in RBM. The RBM incubation conditions compound concentrations, representation of the data, and statistics were as described in Figure 4. Where indicated, 50 μM **26** or 3 mM DTE was present.

Scheme Legends

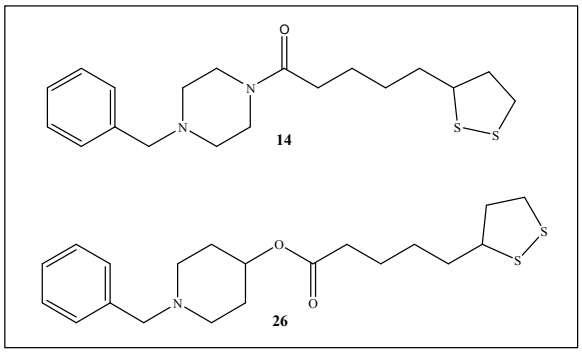
Scheme 1.[a]

[a] **Reagents and conditions:** a) lipoic acid, DCC, DCM, 3 h rt.

Scheme 2.[a]

[a] **Reagents and conditions: a)** Toluene, K_2CO_3, reflux 24 h; **b**) lipoic acid, DCC, DMAP, DCM, 3 h rt.

Table of Contents graphic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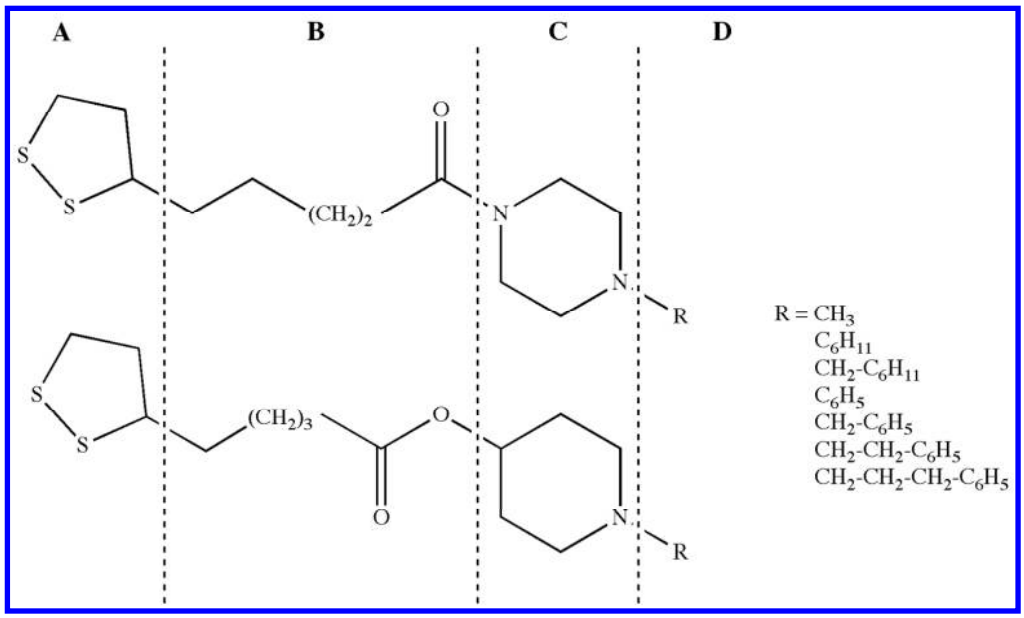

Figure 1. The four pharmacophore regions proposed by Gilligan et al., matched with our piperazine and piperidine derivatives containing the lipoyl function, are represented by a distal aromatic ring (Region A), a space between the nitrogen heterocycle and the distal hydrophobic site (Region B), a nitrogen heterocycle (Region C), and a substituent on the basic nitrogen (Region D).
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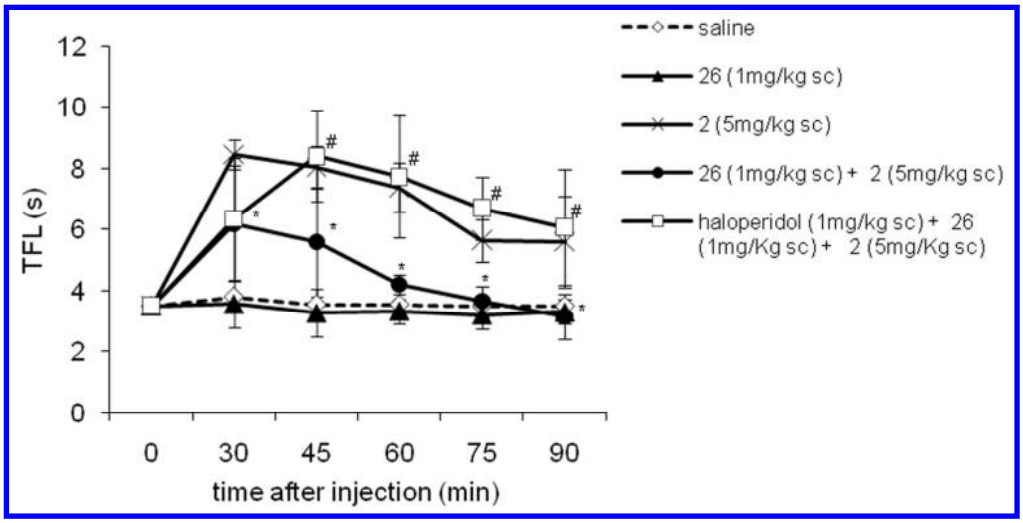

Figure 2. Effect of 26 on the antinociception induced by 2. Haloperidol reversed the antiopioid effect of 26. The results are expressed as the mean ± SD. *p<0.05 vs 2-treated rats (n= 8−10). #p<0.05 vs 26 + 2-treated rats (n= 8−10).
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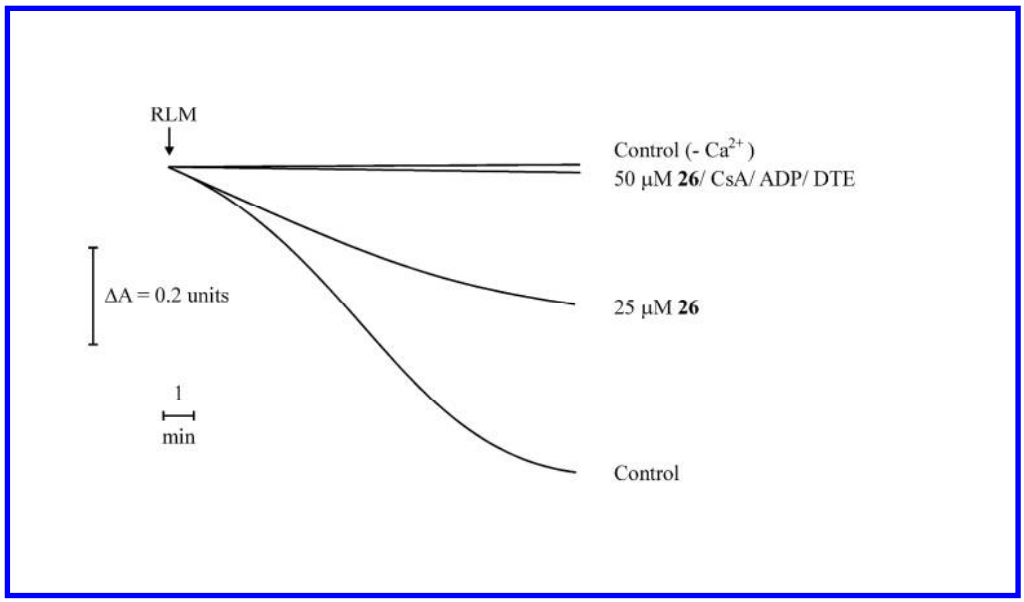

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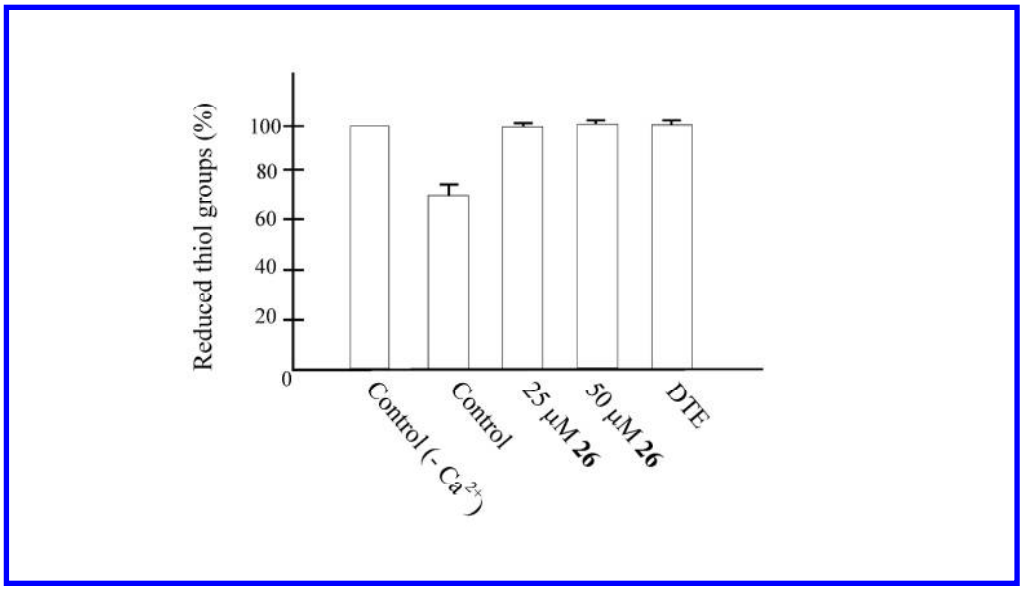


Figure 4. Effect of 26 or DTE on sulfhydryl group oxidation induced by Ca2+ in RLM. 26 was present at the indicated μM concentrations. DTE was present at 3 mM concentration. Data are expressed as percentage of thiol reduction and represent average ± mean SD from five independent experiments.

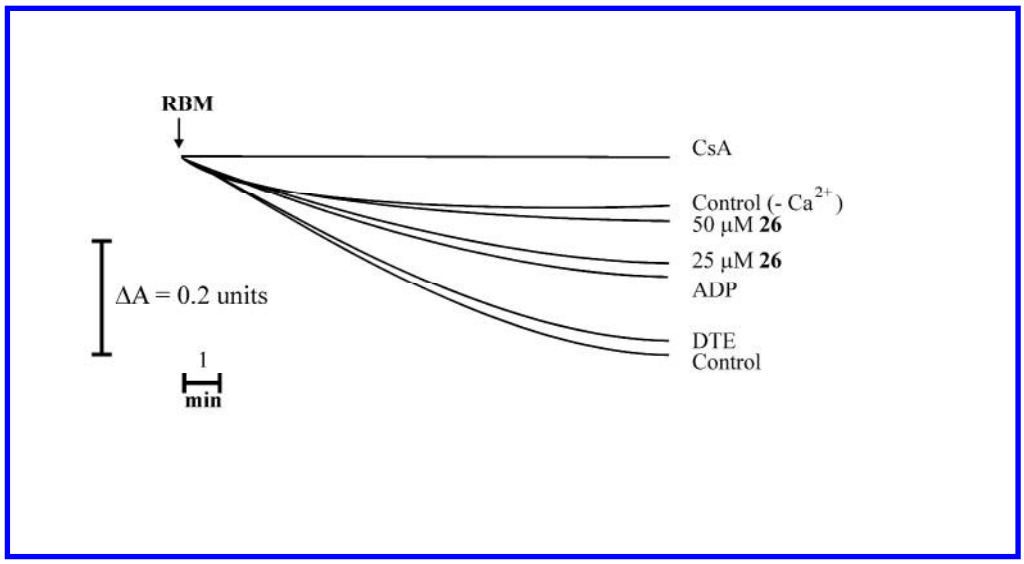


Figure 5. Brain mitochondria swelling, induced by Ca2+. Prevention by compound 26, CsA, ADP and DTE. RBM incubation, compound concentrations, meaning of downward deflection, and statistics as in Figure 3.

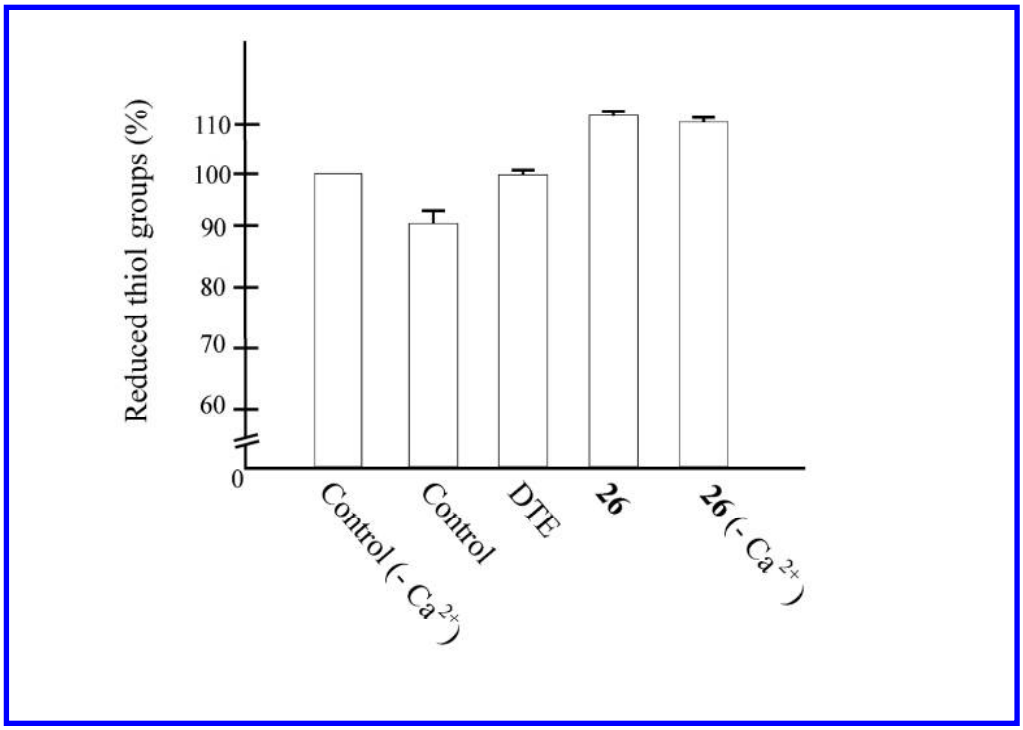
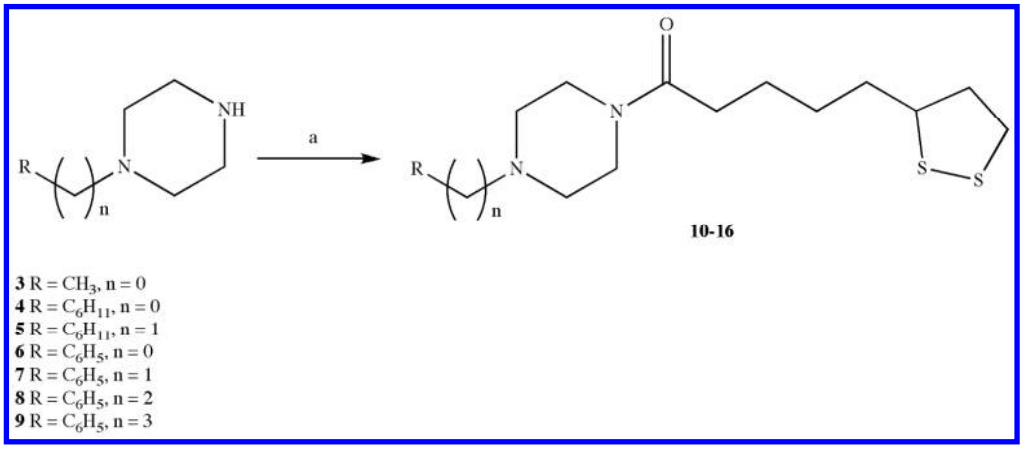


Figure 6. Effect of 26 and DTE on sulfhydryl group oxidation induced by Ca2+ in RBM. RBM incubation, compound concentrations, data expression, and statistics as in Figure 4. Where indicated 50 μM 26, and 3 mM DTE were present.



Scheme 1.a
a Reagents and conditions: a) lipoic acid, DCC, DCM, 3 h rt.
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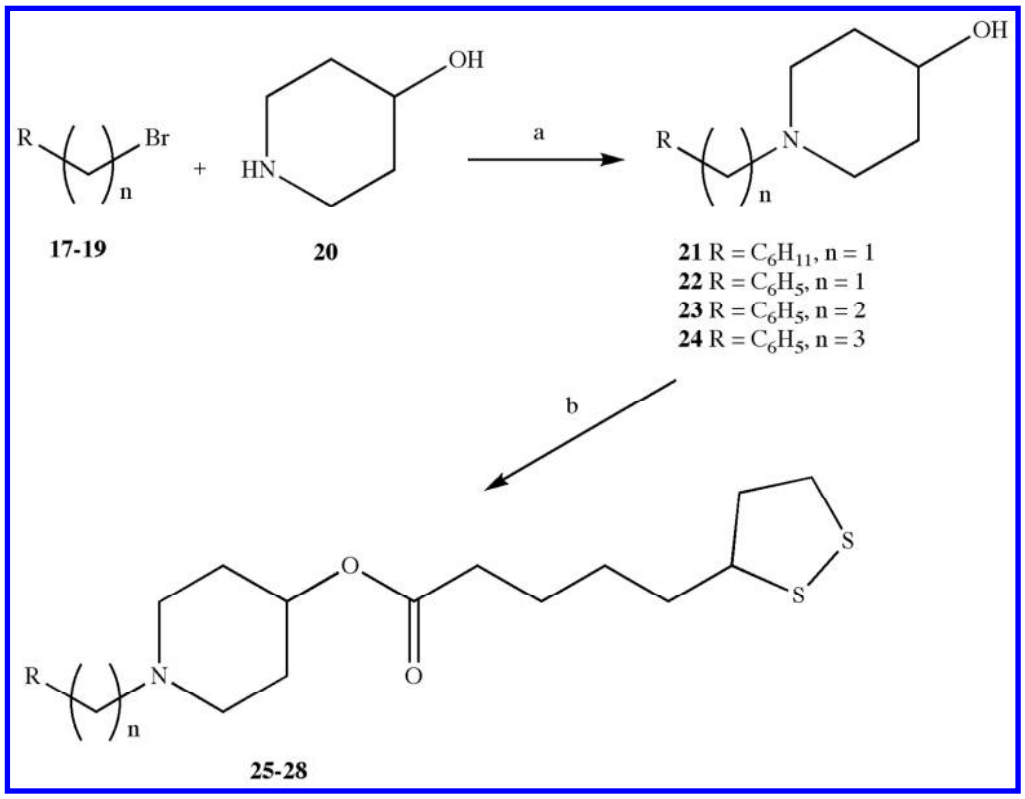

Scheme 2.a
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