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Synthesis of (+)- and (−)-*cis*-2-[(1-adamantylamino)-methyl]-1-phenylcyclopropane derivatives as high affinity probes for σ_1 and σ_2 binding sites

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Abstract

Selective ligands for either sigma$_1$ (σ_1) or σ_2 binding sites are potentially useful for gaining a better understanding of the physiological functions of these proteins. Moreover, potent and selective homochiral σ_1 and σ_2 binding site ligands represent leads to potential radioligands for tumour imaging with positron emission tomography (PET). On the basis of their structural similarity to previous leads, new (+)- and (−)-*cis*-2-[(1-adamantylamino)-methyl]-1-phenylcyclopropane derivatives were synthesised and their binding affinities for σ_1 and σ_2 binding sites were determined. Each enantiomer showed high affinity for both σ_1 and σ_2 binding sites, but only (−)-*cis*-methyl-2-{[1-adamantyl(methyl)amino]methyl}-1-phenylcyclopropane-carboxylate, (−)-**4**, showed appreciable selectivity for binding to σ_1 versus σ_2 sites. The enantiomers of *cis*-(2-{[1-adamantyl(methyl)amino]methyl}-1-phenyl-cyclopropyl)methanol, **6**, expressed the highest affinity for σ_1 and σ_2 binding sites. Ligands (−)-4, (+)-6 and (−)-6 might be rapidly labelled in their *N*-methyl groups by methylation of the *N*-desmethyl analogues with [^{11}C]iodomethane to provide prospective radioligands for PET. The *N*-desmethyl analogues, which are also high affinity ligands, were prepared and shown to undergo satisfactory methylation with iodomethane.

Keywords: Sigma; Receptors; Ligands; Stereoselectivity; PET

1. Introduction

Since the first reports [1,2] on sigma (σ) binding sites, steady progress has been made for their biochemical, physiological and pharmacological characterisation. At least two subtypes of σ binding site have been identified through their different pharmacology, anatomical distributions and molecular weights [3–5].

The σ_1 subtype, with a specific distribution in the central nervous system (CNS) and a molecular weight of 25 kDa, shows high affinity for the dextro-isomers of *cis*-normetazocine derivatives, (+)-pentazocine, (+)-SKF-10,047 and (+)-cyclazocine. The σ_2 subtype also has a specific distribution in the CNS but at a higher concentration than the σ_1 subtype. However, it has a molecular weight of 18–21 kDa and low affinity for the aforementioned dextro *cis*-normetazocine derivatives.

σ binding sites are also present in the peripheral tissues [6]. Recently, the 223 amino acid σ_1 protein (Fig. 1) has been purified and cloned from several animal species and humans [7,8]. This shows no analogy with other mammalian protein but a homology sequence with a fungal Δ-8,7-sterol-isomerase. The high density of σ_1 sites in steroid-producing tissues and CNS provides evidence for their involvement in neuroendocrine functions, such as the synthesis and metabolism of neuroactive steroids [9]. Several other physiological functions have been reported for σ_1 sites, such as modulation of synthesis and release of the

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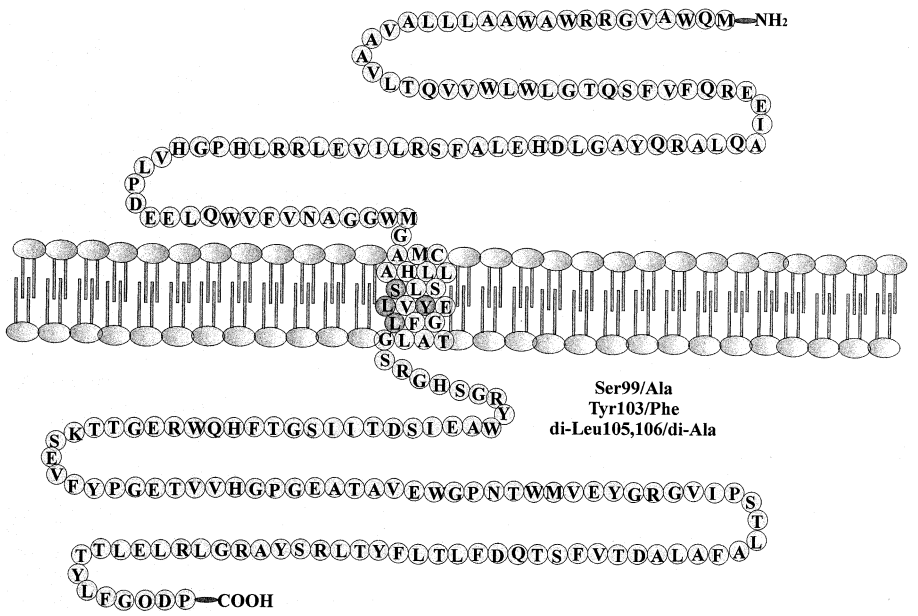


Fig. 1. Amino acid sequence of the human σ_1 receptor showing the amino acid residues (shaded) in the putative transmembrane domain critical for ligand binding [25].

neurotransmitters, acetylcholine and dopamine [10,11], neuromodulatory effects on glutamatergic NMDA-type [12] and opioid systems [13], attenuation of the cocaine-induced effect [14] and antiamnesic effects [15]. At present, the molecular mechanism involved in these effects is not clear but a recent study shows that modulation of Ca^{2+} level by σ_1 receptor–ankirin–1,4,5-trisphosphate receptor complex is a possible mode of action [16].

The protein corresponding to σ_2 sites has not yet been cloned and this explains a relative paucity of information. Nevertheless, its anatomical distribution in the CNS and the typical extrapyramidal side effects induced by neuroleptic drugs, such as haloperidol, provide evidence that σ_2 receptors are involved in motor control behaviour [17]. Morphology changes or apoptotic cell death upon chronic exposure to selective σ_2 ligands such as CB-64, ibogaine or treatment with neuroleptic such as haloperidol seem to be responsible for irreversible motor disorders and typical dyskinesia. Distonic effects induced by reduced haloperidol (a metabolite that has been shown to accumulate in the brain of patients taking haloperidol) might relate to citotoxicity of this compound in brain areas with high density of σ_2 receptors and controlling motor behaviour [18]. The very high concentration of σ_2 sites in different tumour cell lines, such as breast, lung, prostate and melanoma, and apoptotic-type cytotoxicicty suggest an important role in cell proliferation and viability [19].

Thus, considering the biochemical and physiological processes in which σ receptors are involved, the synthesis and discovery of selective ligands for σ_1 and σ_2 binding sites may provide potential drugs for psychosis, neuroprotection, motor control and cancer treatment, or tumour imaging agents for non-invasive diagnosis or clinical research in oncology. Besides normetazocine derivatives, many classes of compound [20] such as the neuroleptic agents, perphenazine, haloperidol analogues, U50-488 derivatives, guanidines and others, bind both σ sites.

Recently, we have investigated the design and synthesis of selective σ_1 and σ_2 binding site ligands. In particular, we have focused our attention on examining structure–affinity relationships in a new series of ($\pm$)-*cis*-2-[(1-adamantylamino)-methyl]-1-phenylcyclopropane derivatives [21] that are structurally related to (+)-MPCB (Fig. 2). Here we report that the substitution of the (+)-*cis*-*N*-normetazocine nucleus of (+)-MPCB with 1-adamantanamine or *N*-methyladamantanamine provides ligands with high affinity for σ sites and high selectivity versus binding to opioid and dopamine-2 (D_2) receptors (Table 1). These data also show that substitution of the adamantanamine moiety

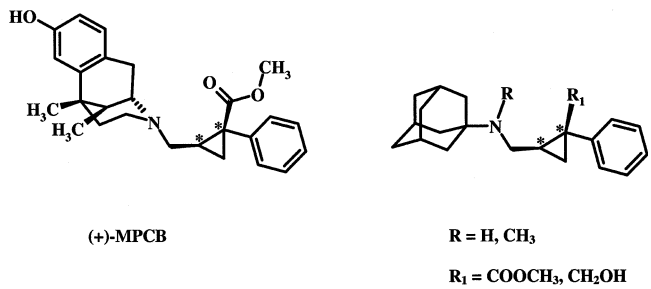
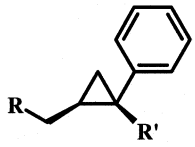
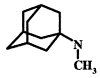


Fig. 2. Structures of the (+)-MPCB and adamantanamine derivatives.

Table 1
Binding affinities [$K_i \pm$ SEM (nM)] of (+)-MPCB and compounds **3**–**5**



Compound	R	R'	[^{3}H](+)Pentazocine (σ_1)	[^{3}H]DTG (σ_2)	[^{3}H]Naloxone (opioid)	[^{3}H]Spiperone (D_2)
(+)-**MPCB**			66.7 ± 2.2	3980 ± 42	>10,000	>10,000
(±)-**3**		$COOCH_3$	3 ± 0.4	23 ± 6.4	5000<IC_{50}<10.000	>10,000
(±)-**4**		$COOCH_3$	12 ± 1.2	11.2 ± 0.8	>10,000	>10,000
(±)-**5**		CH_2OH	5.3 ± 1.2	2.22 ± 1.0	>10,000	>10,000

(as in (±)-**3**) by an *N*-methyladamantanamine moiety (as in (±)-4) or reduction of the carboxymethyl ester (as in (±)-**3** or (±)-**4**) to a hydroxymethyl group (as in (±)-**5** and (±)-**6**, respectively) gives a substantial improvement in binding affinity to σ_2 sites. Here we also describe the synthesis of the enantiomers of each of the new *cis*-2-[(1-adamantylamino)-methyl]-1-phenylcyclopropane derivatives and report their affinities for binding to σ_1 and σ_2 sites.

Considering that σ_2 binding sites may represent potential biomarkers of proliferation in solid tumours, radioligands with high affinity and selectivity for σ_2 receptors may represent useful probes for non-invasive tumour imaging [22–24]. None of the reported compounds show high selectivity or for binding to σ_2 sites; nor do the compounds show enantioselectivity for binding to this site. However, compound (−)-**4**, is identified as having high affinity and selectivity for σ_1 sites, and is suitable for labelling with carbon-11 as a prospective homochiral radioligand for PET. Similarly, compound **6** may be a useful lead to non-subtype selective σ binding site radioligands.

2. Chemistry

The final compound in the synthetic path, racemic *cis*-**6**, was obtained from racemic lactone **1**, prepared as reported in the literature [26]. The syntheses of racemic *cis*-**3**–**5** and of the enantiomers of **3** were reported previously [21]. In order to obtain the new *cis*-enantiomers of compounds **4**–**6**, we synthesised the lactones (+)-**1** and (−)-**1** starting from phenylacetonitrile and (*S*)- or (*R*)-epichlorohydrin, respectively, as reported by Shuto et al. [27].

The treatment of lactone **1** and its enantiomers with HBr–AcOH (33%) and the subsequent esterification of the bromoacid derivatives with $SOCl_2$ and methanol–3N HCl provided the respective intermediate **2** (Scheme 1).

Nucleophilic substitution of 1-adamantanamine with methyl 2-(bromomethyl)-1-phenylcyclopropanecarboxylate **2** gave derivatives **3** that were reduced with $EtN(Me)_2AlH_3$ to the new *cis*-enantiomers **5**. The final *N*-methylated compounds, **4** and **6**, were obtained by treatment of the respective amines **3** and **5** with iodomethane in DMF.

3. Results and discussion

Compounds with high affinity and selectivity for σ receptors are useful probes for a better comprehension of their biochemical and physiological role. PET is a uniquely sensitive and quantitative technique for imaging cancerous cells as solid tumours or metastases with molecules labelled with positron-emitting oxygen-15, carbon-11 or fluorine-18, that target metabolic

processes (e.g. glucose utilisation) or over-expressed protein (e.g. receptors, binding sites) [28]. In view of the over-expression of σ receptors in many tumour cell lines, suitably radiolabelled σ ligands may be useful for PET as tumour imaging agents or for clinical research in oncology.

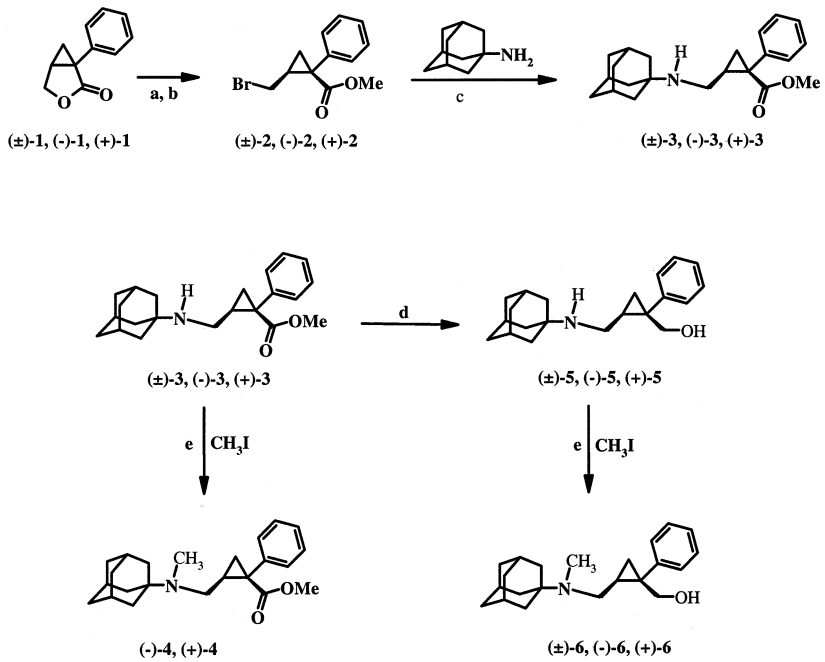
Recently, in our laboratory we have synthesised a series of *cis*-2-[(1-adamantylamino)-methyl]-1-phenylcyclopropane derivatives that show high affinity and selectivity for σ sites in respect to opioid and dopamine D_2 receptors [21]. In particular, racemic compounds **4** and **5** showed an improved affinity for σ_2 sites in respect to (±)-*cis*-methyl-2-[(1-adamantylamino)-methyl]-1-phenylcyclopropanecarboxylate **3**. A PET radioligand must have a very high affinity (Kd in nanomolar range), high selectivity, low or moderate lipophilicity (to avoid high non-specific binding) and stereoselectivity where enantiomers exist [29]. Among all the *cis*-2-[(1-adamantylamino)-methyl]-1-phenylcyclopropane derivatives reported previously, a possible candidate for development as a PET radioligand is the adamantylamino compound **5** (Table 1), for example, as either an *O*-^{11}C-methyl or *N*-^{11}C-methylated form.

In this study we report a new synthetic strategy in order to obtain the enantiomers of **4** and **5**. We also synthesised the *N*-methylated compound **6** and its enatiomers with the aim to test the binding affinity for σ receptors and verify if *N*-methylation of **5** with iodomethane occurs in acceptable yield.

Binding data obtained for these compounds (Table 2) showed that *N*-methylation generally enhanced affinity for σ_1 binding sites (as in the enantiomers of **4** and **6**), but either reduced (as in the enantiomers of **4**) or slightly increased affinity (as in the enantiomers of **6**) for σ_2 sites. Reduction of the carboxymethyl ester to a hydroxymethyl group (as in the enantiomers of **5** and **6**) improved affinity for σ_2 sites, while affinity for σ_1 sites was either increased significantly (as in the dextro enantiomers of **5** and **6**) or little affected (as in the levo enantiomers of **5** and **6**). Previously it was found that the levo-isomers generally showed a preference for σ_1 in respect to σ_2 [21]. Here, only the enantiomers of **6** lacked enantioselectivity for binding to **σ** subtypes. Compounds **4**–**6** all display improved binding affinity for σ sites in respect to the enantiomers of **3**. However, only the levo isomer of **4** showed selectivity in binding to one subtype, the σ_2 subtype ($\sigma_2/\sigma_1 = 24$).

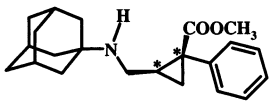
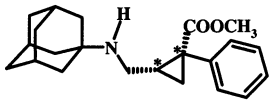
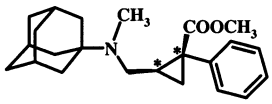
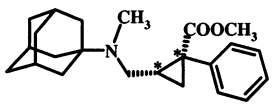
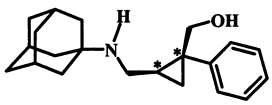
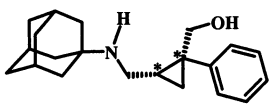
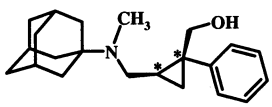
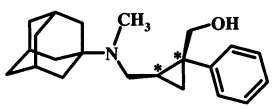
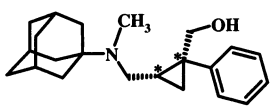
Thus, considering the binding data obtained, we further supported the importance of the substitution of (+)-*cis*-*N*-normetazocine nucleus with 1-adamantanamine or *N*-methyl-1-adamantanamine in order to obtain compounds with very high affinity for σ_1 and σ_2 receptors. Moreover, it seems that control of stereochemistry of substituents on the cyclopropyl ring might improve binding selectivity for either σ_1 or σ_2 receptors, but to obtain highly selective σ_1 and σ_2 ligands additional structural modifications will be necessary.

In conclusion, considering the potential PET radioligand **6**, both enantiomers showed a very high affinity for σ_1 and σ_2 receptors. Hence, each enantiomer of **6**



Scheme 1. (a) HBr–AcOH (33%), 80 °C, 2 h; (b) benzene, $SOCl_2$, MeOH–3 N HCl, 5 h; (c) DMF, $NaHCO_3$, 70 °C, 6 h; (d) $EtN(Me)_2AlH_3$, THF an., 2.5 h; (e) MeI, DMF, $NaHCO_3$, 80 °C, 2 h.

Table 2
σ_1 and σ_2 binding affinities [$K_i \pm$ SEM (nM)]

Compd.		[^{3}H](+)Pentazocine (σ_1)	[^{3}H]-DTG (σ_2)
(+)-**3**		234 ± 7	39.4 ± 0.6
(−)-**3**		4 ± 0.3	35 ± 2
(+)-**4**		38.9 ± 3	83 ± 5
(−)-**4**		2.1 ± 0.3	48 ± 2
(+)-**5**		11.6 ± 0.5	8.8 ± 0.3
(−)-**5**		6.5 ± 0.2	8.8 ± 0.6
(±)-**6**		1.20 ± 0.1	6.6 ± 0.3
(+)-**6**		1.26 ± 0.2	2.75 ± 0.3
(−)-**6**		1.39 ± 0.2	2.69 ± 0.4

will be labelled by *N*-^{11}C-methylation of the corresponding enantiomer of **5**. ^{11}C-Methylations of amines with [^{11}C]iodomethane are very common reactions for preparing PET radioligands [29], and we have shown here that compounds **3** and **5** are readily *N*-methylated by iodomethane. Each labelled enantiomer of **6** will be evaluated as a radioligand for PET with assessment of any possible differences in kinetics, biodistribution, metabolism and resultant brain distribution. Likewise, (−)-**4** will be labelled with carbon-11 and evaluated as prospective σ_1-selective radioligand.

4. Experimental

Reagents were purchased from Aldrich Chemicals Co. Ltd. unless otherwise specified. Melting points were determined on a Buchi 530 capillary apparatus and were uncorrected. Analytical thin-layer chromatography (TLC) was performed on pre-coated silica gel 60 F_{254} aluminium sheets (Merck). Visualization was with accomplished under UV light or in an iodine chamber. Merck silica gel 60 (230–400 mesh) was used for flash column chromatography. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity INOVA (200 MHz)

spectrometer with TMS as an internal standard. Optical rotations were determined in methanol ($c = 1$) with a Perkin–Elmer 241 polarimeter. Infrared spectra were recorded on a 1600 FT-IR Perkin–Elmer instrument and are consistent with the assigned structures. Elemental analyses were measured with an elemental analyzer (model 1106, Carlo Erba). Analyses are indicated by symbols of the elements. Analytical results obtained for those elements were within $\pm 0.4\%$ of the theoretical values. Molecular weights of the obtained products were determined by EI MS (70 eV) on a Kratos 2S RFA spectrometer using a Tektronix 4205 computer system.

5. Radioligand binding assays

5.1. σ_1-Site binding assays

σ_1-Site binding assays were carried out on guinea pig brain membranes according to Matsumoto et al. [30]. The protein concentration of the suspension was measured by the method of Lowry et al. [31] and generally ranged from 6.5 to 8.5 mg of protein/ml. Binding assays were performed as described by DeHaven et al. [32]. Each tube contained 500 μg of membrane protein and was incubated with 3 nM [^{3}H]-(+)-pentazocine (45 Ci/mmol); the value of the apparent dissociation constant (K_d) was 1.2 ± 0.3 nM ($n = 3$) in 50 mM Tris–HCl (pH 7.4). Test compounds were dissolved in dimethyl sulfoxide, and then diluted in buffer to a total volume of 1 ml. Test compounds were added to give a concentration in the range 10^{-5}–10^{-11} M. Non-specific binding was assessed in the presence of 10 μM haloperidol. The reaction was performed for 150 min at 37 °C and terminated by filtering the solution through a Whatman GF/B glass fiber filter which had been pre-soaked for 1 h in a 0.5% poly(ethylenimine) solution. Filters were washed twice with 4 ml of ice-cold buffer.

5.2. σ_2-Site binding assays

σ_2-Site binding assays were carried out on guinea pig brain membranes, prepared as described by Mach et al. [33]. The membranes were incubated with 3 nM [^{3}H]DTG [1,3-di-(2-tolyl)-guanidine] (31 Ci/mM; $K_d = 9.9 \pm 0.8$ nM; $n = 3$) in the presence of 400 nM (+)-SKF10,047 to block σ_1 sites. Incubation was carried out in 50 mM Tris–HCl (pH 8.0) for 120 min at room temperature (r.t.). Each assay was terminated by the addition of ice-cold 10 mM Tris–HCl pH 8.0, followed by filtration through a Whatman GF/B glass fiber filter which had been pre-soaked for 1 h in a 0.5% poly(ethylenimine) solution. Filters were washed twice with 4 ml of ice-cold buffer. Non-specific binding was evaluated in the presence of 5 mM DTG. Inhibition constants (K_i values) for test compounds were calculated using the EBDA–LIGAND program [34] purchased from Elsevier/Biosoft.

5.3. (±)-Methyl-cis-2-(bromomethyl)-1-phenylcyclopropanecarboxylate ((±)-**2**)

Compound **1** (1.24 g, 7.11 mmol) was added to a solution of hydrogen bromide in 33% acetic acid (12 ml) and the resultant mixture was stirred at 80 °C for 2 h. After cooling to 40 °C, the reaction mixture was poured into an ice-water bath to obtain the (1*S*,2*R*) (1*R*,2*S*) - 2 - (bromomethyl) - 1 - phenylcyclopropanecarboxylic acid as a white precipitate, which was filtered and dried in vacuo. [1.66 g, 91%; m.p. 148 °C; IR (KBr): ν(C=O) 1665; ^{1}H NMR ($CDCl_3$) δ 1.61 (dd, 1H, $J = 4.8$, 9.0 Hz), 1.88 (dd, 1H, $J = 4.8$, 7.0 Hz), 2.12–2.22 (m, 1H), 3.72 (dd, 1H, $J = 10.2$, 11.4 Hz), 3.94 (dd, 1H, $J = 5.6$, 11.4 Hz), 5.28 (br s, 1H), 7.29–7.50 (m, 5H)]. Subsequently, $SOCl_2$ (1.24 ml, 16.9 mmol) was added to a solution of the (1*S*,2*R*) (1*R*,2*S*)-2-(bromomethyl)-1-phenylcyclopropanecarboxylic acid (1.66 g, 6.5 mmol) in anhydrous benzene (8 ml) at 0 °C. After heating to reflux for 5 h, the reaction was cooled to 0 °C. A solution of 3N MeOH–HCl (2.76 ml) was added dropwise and the reaction mixture allowed to stir overnight at room temperature. The mixture was evaporated in vacuo and the residue dissolved in Et_2O, washed with a solution of 4% $NaHCO_3$ and dried over anhydrous Na_2SO_4. The solvent was then evaporated in vacuo to give the desired ester (+)-**2** (1.67 g, 88%); m.p. 53–55 °C; R_f 0.57 (toluene–chloroform, 8:2 v/v); IR (KBr): ν(C=O) 1716 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.52 (dd, 1H, $J = 4.6$, 8.5 Hz), 1.78 (dd, 1H, $J = 4.6$, 7.5 Hz), 2.05–2.12 (m, 1H), 3.72 (dd, 1H, $J = 10.0$, 11.2 Hz), 3.80 (s, 3H), 3.89 (dd, 1H, $J = 5.6$, 11.2 Hz), 7.29–7.50 (m, 5H)]. ^{13}C NMR ($CDCl_3$) δ 22.32, 27.54, 38.36, 38.91, 50.51, 118.30, 125.32, 128.65, 1 38.41, 173.61; MS: m/z 269 $[M]^+$; *Anal.* $C_{12}H_{13}BrO_2$ (C, H).

The following compounds were prepared using the above procedure.

5.4. (−)-(1R,2S)-Methyl 2-(bromomethyl)-1-phenylcyclopropanecarboxylate ((−)-**2**)

(1.61 g, 85%); m.p. 53–55 °C; $[\alpha]_{20}^{D}$ −36°; R_f 0.57 (toluene–chloroform, 4:1, v/v); IR (KBr): ν(C=O) 1716 cm^{-1}; MS: m/z 269 $[M]^+$. *Anal.* $C_{12}H_{13}BrO_2$ (C, H).

5.5. (+)-(1R,2S)-Methyl-2-(bromomethyl)-1-phenylcyclopropanecarboxylate ((+)-**2**)

(1.65 g, 87%); m.p. 53–55 °C; $[\alpha]_{20}^{D}$ +37,6°; R_f 0.57 (toluene–chloroform, 8:2 v/v); IR (KBr): ν(C=O) 1716 cm^{-1}; MS: m/z 269 $[M]^+$; *Anal.* $C_{12}H_{13}BrO_2$ (C, H).

*5.6. (±)-cis-Methyl-2-[(1-adamantylamino)methyl]-1-phenylcyclopropanecarboxylate ((±)-**3**)*

$NaHCO_3$ (168 mg, 2 mmol) and compound **2** (150 mg, 0.66 mmol) were added to a solution of 1-adamantanamine (184 mg, 1,22 mmol) in DMF (7 ml). The reaction mixture was stirred at 70 °C for 8 h. The solvent was evaporated under reduced pressure and the residue was dissolved in $CHCl_3$ and washed with a solution of 4% $NaHCO_3$. The mixture was dried over anhydrous Na_2SO_4, evaporated in vacuo and the crude product was purified by flash chromatography eluting with $CHCl_3$–cyclohexane–EtOH (6:4.5:0.5, by vol.). Compound **3** was then dissolved in THF and treated with a solution of oxalic acid in THF to give the salt as a white solid. The analytically pure sample was obtained by recrystallization from methanol–diethyl ether.

(Yield: 15%); m.p. 248–250 °C; ^{1}H NMR (DMSO-d_6) δ 1.48 (dd, 1H, $J = 4.0$, 9.2 Hz), 1.51–198 (m, 14H), 2.05–2.20 (m, 4H), 3.12 (dd, 1H, $J = 7.5$, 12.6 Hz), 3.50 (dd, 1H, $J = 5.5$, 12.6 Hz), 3.60 (s, 3H), 4.80 (br s, 2H), 7.20–7.45 (m, 5H); ^{13}C NMR (DMSO-d_6) δ 19.85, 23.31, 28.35, 34.22, 35.88, 38.08, 46.25, 52.98, 61.01, 127.41, 128.61, 130.80, 139.35, 165.50, 172.19; MS: m/z 339 $[M]^+$; *Anal.* $C_{22}H_{29}NO_2 \cdot 0.5H_2C_2O_4 \cdot 1.5H_2O$ (C, H, N).

Enantiomers (−)-**3** and (+)-**3** were prepared using the above procedure.

*5.7. (−)-(1S,2R)-Methyl-2-[(1-adamantylamino)-methyl]-1-phenylcyclopropanecarboxylate ((−)-**3**)*

(Yield: 22%); m.p. 248–250 °C; $[\alpha]_{20}^{D}$ −50°; ^{1}H NMR (DMSO-d_6) δ 1.45 (dd, 1H, $J = 4.0$, 9.1 Hz), 1.50–2.00 (m, 14H), 2.08–2.25 (m, 4H), 3.14 (dd, 1H, $J = 7.2$, 12.1 Hz), 3.53 (dd, 1H, $J = 5.4$, 12.1 Hz), 3.61 (s, 3H), 5.5 (br s, 2H), 7.27–7.45 (m, 5H); ^{13}C NMR (DMSO-d_6) δ 20.58, 23.31, 27.98, 34.50, 35.66, 38.40, 45.91, 51.02, 60.92, 126.33, 128.03, 131.00, 139.02, 166.48, 172.20; MS: m/z 339 $[M]^+$; *Anal.* $C_{22}H_{29}NO_2 \cdot 0.6H_2C_2O_4$ (C, H, N).

*5.8. (+)-(1R,2S)-Methyl-2-[(1-adamantylamino)-methyl]-1-phenylcyclopropanecarboxylate ((+)-**3**)*

(Yield: 18%); m.p. 105–112 °C; $[\alpha]_{20}^{D}$ +49°; ^{1}H NMR (DMSO-d_6) δ 1.47 (dd, 1H, $J = 4.1$, 9.1 Hz), 1.50–2.00 (m, 14H), 2.08–2.25 (m, 4H), 3.14 (dd, 1H, $J = 7.2$, 12.1 Hz), 3.50 (dd, 1H, $J = 5.4$, 12.1 Hz), 3.59 (s, 3H), 6.2 (br s, 2H), 7.28–7.50 (m, 5H); ^{13}C NMR (DMSO-d_6) δ 19.98, 23.10, 28.01, 33.98, 35.00, 39.65, 46.00, 51.32, 61.22, 126.15, 128.32, 131.50, 139.10, 165.98, 172.50; MS: m/z 339 $[M]^+$. *Anal.* $C_{22}H_{29}NO_2 \cdot 0.7H_2C_2O_4 \cdot 2H_2O$ (C, H, N).

*5.9. (−)-cis-Methyl-2-{[1-adamantyl(methyl)amino]-methyl}-1-phenylcyclopropanecarboxylate ((−)-**4**)*

Iodomethane (0.05 ml, 0.792 mmol) was added to a mixture of $NaHCO_3$ (84 mg, 1 mmol) and compound (−)-**3** (150 mg, 0.66 mmol) in DMF (6 ml). The reaction vessel was sealed and heated at 80 °C for 2 h. After recovering of reaction mixture, the solvent was evaporated under reduced pressure and the residue was dissolved in $CHCl_3$ and washed with brine. The mixture was dried over anhydrous Na_2SO_4, evaporated in vacuo and the crude product was purified by flash chromatography eluting with $CHCl_3$–cyclohexane–EtOH (6:4.5:0.2, by vol.). Compound **4** was then dissolved in THF and treated with a solution of HCl in $(C_2H_5)_2O$ to give the respective salt. The pure sample was obtained by recrystallization from methanol–diethyl ether. (Yield: 62%); m.p. 180–182 °C; $[\alpha]_{20}^{D}$ −54°; ^{1}H NMR (DMSO-d_6) δ 1.51 (dd, 1H, $J = 4.1$, 8.8 Hz), 1.60–1.78 (m, 7H), 1.82–2.23 (m, 10H), 2.75 (s, 3H), 3.25–3.50 (m, 2H), 3.58 (s, 3H), 5.40 (br s, 2H), 7.22–7.45 (m, 5H); ^{13}C NMR (DMSO-d_6) δ 20.65, 23.75, 28.92, 32.38, 34.10, 35.07, 35.52, 46.81, 52.62, 62.97, 127.31, 128.19, 130.07, 139.31, 164.63, 172.04; MS: m/z 353 $[M]^+$; *Anal.* $C_{23}H_{31}NO_2 \cdot HCl$ (C, H, N).

Enantiomer (+)-4 was prepared using the above procedure.

*5.10. (+)-cis-Methyl-2-{[1-adamantyl(methyl)amino]-methyl}-1-phenylcyclopropanecarboxylate ((+)-**4**)*

(Yield: 61%); m.p. 183–186 °C; $[\alpha]_{20}^{D}$ +56°; ^{1}H NMR (DMSO-d_6) δ 1.53 (dd, 1H, $J = 4.1$, 8.8 Hz), 1.62–1.80 (m, 7H), 1.83–2.25 (m, 10H), 2.78 (s, 3H), 3.27–3.53 (m, 2H), 3.60 (s, 3H), 6.50 (br s, 2H), 7.24–7.47 (m, 5H); ^{13}C NMR (DMSO-d_6) δ 20.66, 23.77, 28.94, 32.39, 34.14, 35.10, 35.54, 46.83, 52.64, 62.99, 127.34, 128.21, 130.10, 139.33, 164.65, 172.05; MS: m/z 353 $[M]^+$; *Anal.* $C_{23}H_{31}NO_2 \cdot HCl$ (C, H, N).

*5.11. (±)-cis-{2-[(1-Adamantylamino)methyl]-1-phenylcyclopropyl}methanol ((±)-**5**)*

A solution of compound (±)-**3** (1 g, 2.95 mmol) in anhydrous THF (10 ml) was added dropwise to a 0.5 M solution of alane-*N*,*N*-dimethylethylamine complex (6 ml, 3 mmol) in anhydrous THF (10 ml) at 0 °C under a nitrogen atmosphere. The reaction mixture was stirred for 2.5 h and quenched with a water–THF (1:1 v/v) solution to give a white precipitate. The mixture was dissolved in 1N NaOH and extracted with Et_2O (3 × 30 ml); the organic extracts were dried with anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash chromatography eluting with cyclohexane–ethyl acetate (1:1 v/v) to afford compound (±)-**5**.

(Yield: 76%); m.p. 280–283 °C; ^{1}H NMR (DMSO-d_6) δ 1.05 (dd, 1H, $J = 5.0$, 8.6 Hz), 1.11 (dd, 1H, $J = 5.5$, 8.6 Hz), 1.46 (m, 1H) 1.58 (m, 6H), 1.87 (m, 6H), 2.14 (m, 3H), 3.11 (dd, 1H, $J = 6.4$, 12.2 Hz), 3.29 (dd, 1H, $J = 5.1$, 12.2 Hz), 3.53 (d, 1H, $J = 11.6$ Hz), 4.10 (d, 1H, $J = 11.6$ Hz) 5.60 (br s, 3H), 7.15–7.45 (m, 5H); ^{13}C NMR (DMSO-d_6) δ 17.51, 20.28, 28.42, 31.72, 35.21, 37.79, 40.12, 55.94, 64.74, 126.29, 128.01, 128.72, 1 43.93; MS: m/z 311 $[M]^+$; *Anal.* $C_{21}H_{29}NO_2 \cdot HCl$ (C, H, N).

Enantiomers (−)-**5** and (+)-**5** were prepared using the above procedure.

*5.12. (−)-cis-{2-[(1-Adamantylamino)methyl]-1-phenylcyclopropyl}methanol ((−)-**5**)*

(Yield: 76%); m.p. 282–283 °C; $[\alpha]_{20}^{D}$ −48°; ^{1}H NMR (DMSO-d_6) δ 1.05 (dd, 1H, $J = 5.0$, 8.6 Hz), 1.11 (dd, 1H, $J = 5.5$, 8.6 Hz), 1.46 (m, 1H) 1.58 (m, 6H), 1.87 (m, 6H), 2.14 (m, 3H), 3.11 (dd, 1H, $J = 6.4$, 12.2 Hz), 3.29 (dd, 1H, $J = 5.1$, 12.2 Hz), 3.53 (d, 1H, $J = 11.6$ Hz), 4.10 (d, 1H, $J = 11.6$ Hz) 5.60 (br s, 3H), 7.15–7.45 (m, 5H); ^{13}C NMR (DMSO-d_6) δ 17.51, 20.28, 28.42, 31.72, 35.21, 37.79, 40.12, 55.94, 64.74, 126.29, 128.01, 128.72, 143.93; MS: m/z 311 $[M]^+$; *Anal.* $C_{21}H_{29}NO_2 \cdot HCl \cdot 0.2H_2O$ (C, H, N).

*5.13. (+)-cis-{2-[(1-Adamantylamino)methyl]-1-phenylcyclopropyl}methanol ((+)-**5**)*

(Yield: 76%); m.p. 282–283 °C; $[\alpha]_{20}^{D}$ +46°; ^{1}H NMR (DMSO-d_6) δ 1.05 (dd, 1H, $J = 5.0$, 8.6 Hz), 1.11 (dd, 1H, $J = 5.5$, 8.6 Hz), 1.46 (m, 1H) 1.58 (m, 6H), 1.87 (m, 6H), 2.14 (m, 3H), 3.11 (dd, 1H, $J = 6.4$, 12.2 Hz), 3.29 (dd, 1H, $J = 5.1$, 12.2 Hz), 3.53 (d, 1H, $J = 11.6$ Hz), 4.10 (d, 1H, $J = 11.6$ Hz) 5.60 (br s, 3H), 7.15–7.45 (m, 5H); ^{13}C NMR (DMSO-d_6) δ 17.51, 20.28, 28.42, 31.72, 35.21, 37.79, 40.12, 55.94, 64.74, 126.29, 128.01, 128.72, 143.93; MS: m/z 311 $[M]^+$; *Anal.* $C_{21}H_{29}NO_2 \cdot HCl$ (C, H, N).

Compounds (±)-**6**, (−)-**6** and (+)-**6** were prepared using the same procedure of compound (−)-**4**.

*5.14. (±)-cis-(2-{[1-Adamantyl(methyl)amino]-methyl}-1-phenylcyclopropyl)methanol ((±)-**6**)*

(Yield: 75%); m.p. 282–283 °C; ^{1}H NMR (DMSO-d_6) δ 1.10 (dd, 1H, $J = 5.0$, 8.3 Hz), 1.14 (dd, 1H, $J = 5.5$, 8.3 Hz), 1.48 (m, 1H) 1.61 (m, 6H), 1.92 (m, 6H), 2.15 (m, 3H), 2.78 (s, 3H), 3.42 (dd, 1H, $J = 4.4$, 12.8 Hz), 3.48 (dd, 1H, $J = 7.1$, 12.8 Hz), 3.72 (d, 1H, $J = 11.5$ Hz), 4.10 (d, 1H, $J = 11.5$ Hz) 5.60 (br s, 3H), 7.13–7.43 (m, 5H); ^{13}C NMR (DMSO-d_6) δ 18.33, 19.57, 28.88, 32.09, 35.03, 35.41, 48.63, 63.21, 64.90, 126.17, 127.98, 128.42, 143.91; MS: m/z 325 $[M]^+$; *Anal.* $C_{21}H_{29}NO_2 \cdot HCl \cdot 0.1H_2O$ (C, H, N).

*5.15. (−)-cis-(2-{[1-Adamantyl(methyl)amino]-methyl}-1-phenylcyclopropyl)methanol ((−)-**6**)*

(Yield: 75%); m.p. 282–283 °C; $[\alpha]_{20}^{D}$ −70°; ^{1}H NMR (DMSO-d_6) δ 1.10 (dd, 1H, $J = 5.0$, 8.3 Hz), 1.14 (dd, 1H, $J = 5.5$, 8.3 Hz), 1.48 (m, 1H) 1.61 (m, 6H), 1.92 (m, 6H), 2.15 (m, 3H), 2.78 (s, 3H), 3.42 (dd, 1H, $J = 4.4$, 12.8 Hz), 3.48 (dd, 1H, $J = 7.1$, 12.8 Hz), 3.72 (d, 1H, $J = 11.5$ Hz), 4.10 (d, 1H, $J = 11.5$ Hz), 5.60 (br s, 3H), 7.13–7.43 (m, 5H), 7.60 (br s, 3H); ^{13}C NMR (DMSO-d_6) δ 18.36, 19.57, 28.87, 32.09, 35.04, 35.43, 48.63, 63.21, 64.90, 126.17, 127.99, 128.42, 143.91; MS: m/z 325 $[M]^+$; *Anal.* $C_{21}H_{29}NO_2 \cdot HCl$ (C, H, N).

*5.16. (+)-cis-(2-{[1-Adamantyl(methyl)amino]-methyl}-1-phenylcyclopropyl)methanol ((+)-**6**)*

(Yield: 75%); m.p. 282–283 °C; $[\alpha]_{20}^{D}$ +70°; ^{1}H NMR (DMSO-d_6) δ 1.10 (dd, 1H, $J = 5.0$, 8.3 Hz), 1.14 (dd, 1H, $J = 5.5$, 8.3 Hz), 1.48 (m, 1H) 1.61 (m, 6H), 1.92 (m, 6H), 2.15 (m, 3H), 2.78 (s, 3H), 3.42 (dd, 1H, $J = 4.4$, 12.8 Hz), 3.48 (dd, 1H, $J = 7.1$, 12.8 Hz), 3.72 (d, 1H, $J = 11.5$ Hz), 4.10 (d, 1H, $J = 11.5$ Hz) 6.60 (br s, 3H), 7.13–7.43 (m, 5H); ^{13}C NMR (DMSO-d_6) δ 18.34, 19.57, 28.87, 32.09, 35.03, 35.41, 48.63, 63.21, 64.90, 126.17, 127.99, 128.42, 143.91; MS: m/z 325 $[M]^+$; *Anal.* $C_{21}H_{29}NO_2 \cdot HCl \cdot 0.2H_2O$ (C, H, N).

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