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New Adamantane Phenylalkylamines with σ-Receptor Binding Affinity and Anticancer Activity, Associated with Putative Antagonism of Neuropathic Pain

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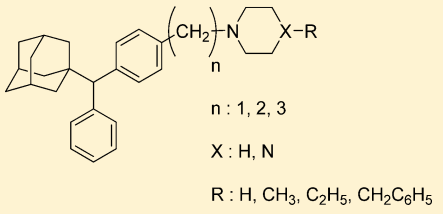
Supporting Information

ABSTRACT: The synthesis of the adamantane phenylalkylamines **2a−d**, **3a−c**, and **4a−e** is described. These compounds exhibited significant antiproliferative activity, in vitro, against eight cancer cell lines tested. The σ_1, σ_2, and sodium channel binding affinities of compounds **2a**, **3a**, **4a**, and **4c−e** were investigated. The most interesting analogue, **4a**, exhibited significant in vivo anticancer profile on pancreas, prostate, leukemia, and ovarian cancer cell line xenografts together with apoptosis and caspase-3 activation. Inhibition of the cancer cells cycle at the sub-G1 level was also obtained with **4a**. Finally, encouraging results were observed with **4a** in vivo on mice, suggesting putative antimetastatic and analgesic activities of this compound.



INTRODUCTION

Cancer and neurogenerative diseases, two of the most important areas for medical research, both implicate σ-receptors. σ-Ligands can therefore be expected to be putative anticancer drugs.[1−5] Indeed, σ-receptors are expressed to a greater degree in tumors than they are in the surrounding normal tissue.[6−13] σ-Receptors have been classified as a distinct pharmacological entity, and their function was shown to be unrelated to the function of opioid receptors.[2,5,14] On the basis of the ligand selectivity in the binding assays, two subtypes, σ_1 and σ_2 receptors, were identified.[2,5,15] Further, σ_1-receptors have been shown to be involved in programmed cell death (apoptosis), with σ_1-agonists being antiapoptotic and neuroprotective, with putative antineurodegenerative activity.[2,16−20] σ_1-Antagonists or σ_2-agonists are proapoptotic and can, because of the high expression of σ_1 and σ_2 receptors in the rapidly proliferating cancer cells,[6−13] act as putative anticancer drugs by inducing cell death via apoptosis.[21−32] There is considerable evidence of antiproliferative and cytotoxic activity for σ_1-antagonists,[21,23,24,26,29,31] σ_2-putative agonists,[22,23,25,28,30] mixed σ_1/σ_2-ligands,[21,23,26] and even one σ_1-agonist.[27] More specifically, it has been shown that σ_1-ligands (putative antagonists) induce caspase-dependent apoptosis,[24,31] which is in accord with recent observations that σ_1-agonists prevent caspase-3 activation.[2,17,19] On the other hand, σ_2-ligands (putative agonists) have been shown to activate a caspase-independent apoptotic pathway and were proposed as putative anticancer drugs,[22,25] but caspase-3 activation was also described for σ_2 anticancer ligands.[28,30,31] Recent data have also suggested the importance of the σ_1-receptor modulated ion channels (Na^+, K^+, Cl^-, Ca^{2+})[2,32−35] and σ_1-receptor binding of cholesterol in lipid rafts concerning the proliferation of cancer cells.[36,37] The Na^+ channels, in particular, are modulated by σ_1-receptors and are implicated in the adhesion, migration, and apoptosis of cancer cells.[34,35,38−40] Important advances were recently made on the mechanism of action of σ-ligands and their putative role as therapeutic anticancer agents. In particular, the σ_1-receptor was cloned[2,41] allowing more accurate pharmacological evaluation[42] of its specific role in the endoplasmic reticulum (ER), in the apoptosis of cancer cells,[1,2,4,16] and in its connection with the impairing action on the G_0/G_1 cell cycle phases.[31,32] Classical SAR studies indicated that the presence of a cycloalkyl or aryl group attached to the cationic amine center via a linker of a three- to five-membered chain (including a heteroatom) was essential for affinity at the σ-receptors.[43,44] In previous work, we reported 1-[*p*-[α-(1-adamantyl)benzyl]-phenyl]piperazines **1** as antiproliferative agents. Piperazine **1a** (R = CH_3) presented appreciable anticancer activity, which was related to its affinity for σ-receptors and binding to site 2 of the

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Na^+ channels.[31] The structures of derivatives **1** involve a scaffold of a benzene ring A linked to the second piperazine nitrogen via a chain of three atoms (N, 2C), the first piperazine nitrogen, and the following two piperazine carbons (Figure 1). The length of the above linker satisfies the structural requirement for σ-receptor binding affinity.

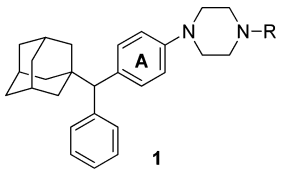


Figure 1. Adamantane phenylpiperazines **1**.

In this work, we describe the design, synthesis, and σ-receptor affinity and antiproliferative potency of 1-[*p*-[α-(1-adamantyl)benzyl]phenyl]alkylpiperazines **2a–c**, **3a,b**, and **4a–d** (Figure 2). In the new derivatives, the benzene ring A is

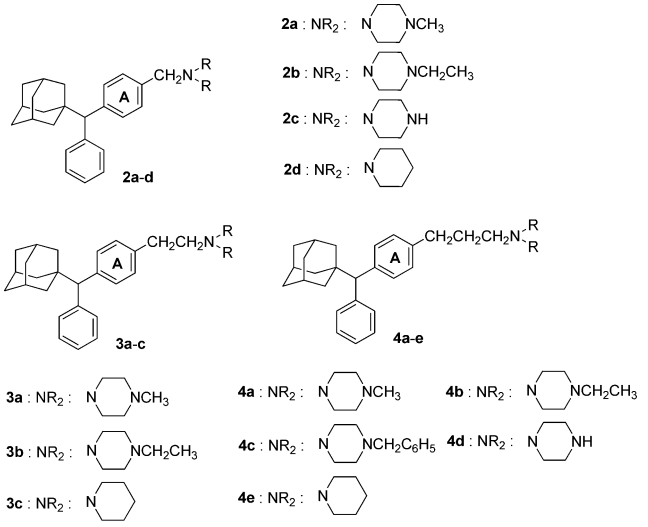


Figure 2. Adamantane phenylalkylamines **2**, **3**, and **4** with antiproliferative activity.

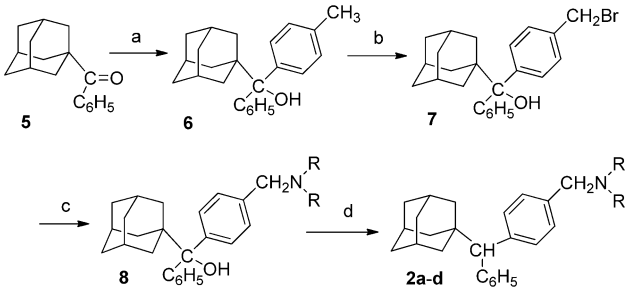
linked to the first piperazine nitrogen by one, two, and three methylene carbons. We also synthesized piperidine adducts **2d**, **3c**, and **4e** in order to evaluate the contribution of the first piperazine nitrogen to antiproliferative activity. Derivative **4a**, having shown the most interesting antiproliferative activity in vitro, was also evaluated in vivo.

CHEMISTRY

For the synthesis of benzylamines **2**, 1-adamantyl phenyl ketone (**5**) was used as starting material and was prepared either by reacting diphenylcadmium with 1-adamantanecarbonyl chloride in boiling benzene or by reacting phenyllithium with 1-adamantanecarboxylic acid in di-*n*-butyl ether at −80 °C.[45,46]

Two different synthetic pathways were followed for the preparation of benzylamines **2**, as illustrated in Schemes 1 and 2. In the first synthetic route, *p*-tolylmagnesium bromide was added to ketone **5** to give α-phenyl-α-(*p*-tolyl)-1-adamantanemethanol (**6**). Carbinol **6** was then brominated by NBS in the presence of a catalytic amount of dibenzoyl peroxide to afford bromomethylcarbinol **7**, which was coupled with the appropriate secondary amine to give amino alcohols **8**.

Scheme 1[a]



[a]Reagents and conditions: (a) (i) *p*-tolylmagnesium bromide/Et_2O, reflux, 2 h, (ii) HCl 10% at 0 °C; (b) NBS-dibenzoyl peroxide/CCl_4, reflux, 8 h; (c) R_2NH/THF, reflux, 10 h; (d) (i) TFA/DCM, Ar, rt, 15 min, (ii) Et_3SiH, Ar, rt, 1 h, (iii) H_2O at 0 °C, then sat. solution of Na_2CO_3.

Reduction of **8** with triethylsilane in trifluoroacetic acid gave the desired benzylamines **2a–d**. The second pathway was accomplished in five steps (Scheme 2). Reaction of *p*-bromophenylmagnesium bromide with ketone **5** gave bromo alcohol **9**, which was reduced by triethylsilane in trifluoroacetic acid to the aryl bromide **10**. Conversion of bromide **10** to the corresponding Grignard reagent and reaction of the latter with dry gaseous carbon dioxide gave benzoic acid **11**. Transformation of the acid **11** to the acid chloride and coupling of this with the appropriate secondary amine gave the benzamides **12a,d**, which were reduced by $LiAlH_4$ to the benzylamines **2a,d**.

Phenethylamines **3a–c** were prepared by two synthetic pathways (Schemes 4 and 5). In the first synthetic route *p*-bromophenethylamines **17** were used as starting materials, which were synthesized by the reaction sequence shown in Scheme 3.

Bromophenethyl alcohol **13** was esterified to tosylate **14**, which was converted to amines **17** by reacting with the appropriate secondary amine. Alternatively,[47] *p*-bromophenethylamines **17** can be prepared by reacting paraformaldehyde with the trifluoroacetate of 1-methyl- or 1-ethylpiperazine or piperidine hydrochloride in *N*-methyl-2-pyrrolidinone and addition of *p*-bromobenzylzinc bromide to the intermediate cations **16** (Scheme 3). The *p*-bromophenethylamines **17** were then lithiated with *tert*-butyllithium at −80 °C to give the corresponding aryllithium intermediates **18**, which were then reacted with ketone **5** to give the amino alcohols **19**. Reduction of **19** with triethylsilane in trifluoroacetic acid gave the desired phenethylamines **3a–c** (Scheme 4).

In the second route to the amines **3a–c**, aryl bromide **10** was converted to the intermediate aryllithium with *n*-butyllithium at −80 °C and then reacted with DMF to give the benzaldehyde **20**. Reduction of **20** gave the benzyl alcohol **21**, which was then treated with thionyl chloride to give the benzyl chloride **22**. The latter was converted to phenylacetonitrile **23** with sodium cyanide in DMSO. Carbonitrile **23** could also be obtained by the reaction of aldehyde **20** with tosylmethyl isocyanide (TosMIC) in the presence of potassium *tert*-butoxide, followed by addition of methanol.[48,49] Alcoholysis of nitrile **23** led to ethyl phenylacetate **24**, and this was saponified to the corresponding phenylacetic acid, which was converted to the intermediate acid chloride and then to the phenylacetamides **25**. Reduction of amides **25** with $LiAlH_4$ then gave the desired phenethylamines **3a–c** (Scheme 5).

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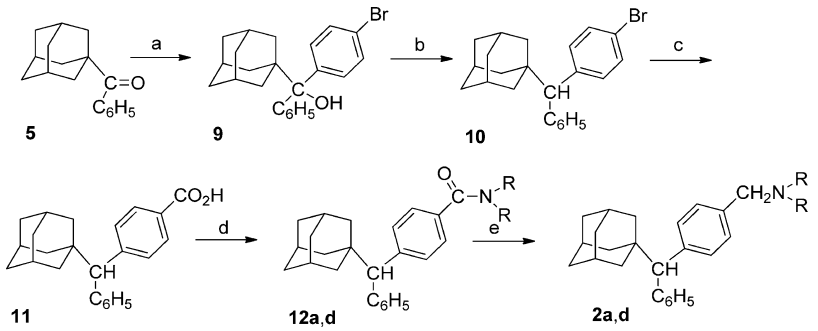
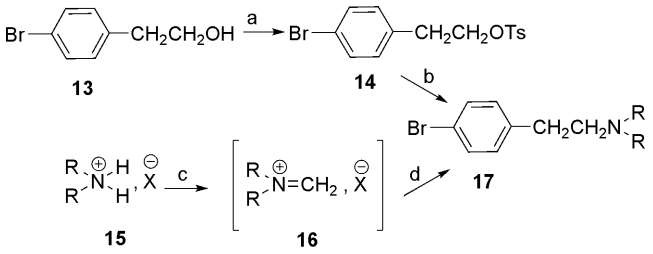
Scheme 2[a]



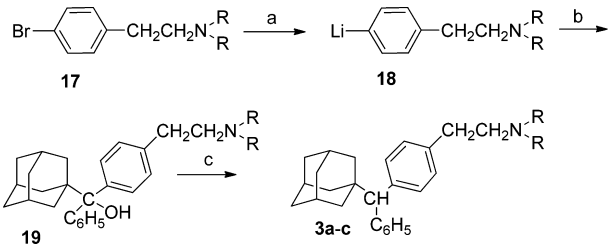
[a]Reagents and conditions: (a) *p*-bromophenylmagnesium bromide/Et_2O, reflux, 2 h, (ii) sat. solution of NH_4Cl at 0 °C; (b) (i) TFA/DCM, Ar, rt, 15 min, (ii) Et_3SiH, rt, 1 h, (iii) H_2O at 0 °C, then sat. solution of Na_2CO_3; (c) (i) Mg turnings/THF, reflux, 3 h, (ii) dry CO_2 gas, 3 h, (iii) HCl (10%) at 0 °C; (d) (i) $SOCl_2$, reflux, 1 h, (ii) R_2NH/THF, reflux, 3 h; (e) $LiAlH_4$/THF, reflux, 3 h, then NaOH 10% at 0 °C.

Scheme 3[a]



[a]Reagents and conditions: (a) TsCl/Py–DCM, at 0 °C, 30 min, then at 4 °C, 12 h; (b) R_2NH/THF, reflux, 6 h; (c) paraformaldehyde/NMP, rt, 20 min, then at 60 °C, 12 h; (d) *p*-bromobenzylzinc bromide/THF, rt, 30 min.

Scheme 4[a]



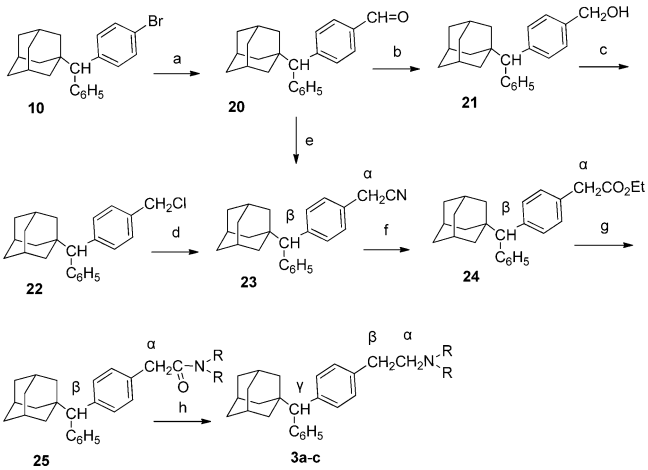
[a]Reagents and conditions: (a) *t*-BuLi/THF, 2 h at −80 °C; (b) (i) ketone **5**/THF, 30 min at −80 °C, then rt, 2 h, (ii) sat. solution of NH_4Cl at 0 °C; (c) (i) TFA/DCM, rt, 15 min, (ii) Et_3SiH, rt, 1 h, (iii) H_2O at 0 °C, then sat. solution of Na_2CO_3.

The synthesis of propylamines **4a–c,e** was accomplished by the reaction sequence shown in Scheme 6. γ-[*p*-[α-(1-Adamantyl)benzyl]phenyl]propanol (**26**) was tosylated to the intermediate ester, which was then reacted with the requisite secondary amines to give the desired amines **4a–c,e**. This substitution was accompanied by the formation of the *p*-toluenesulfonamides byproducts **27**.

Piperazine **4d** was prepared from the benzyl derivative **4c** by reductive debenzylation in the presence of palladium on charcoal, with ammonium formate as hydrogen donor (Scheme 7).

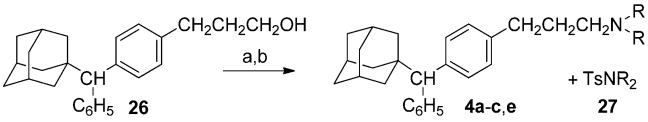
The preparation of propyl alcohol **12** is described in our previous paper as a low yield byproduct.[50] In this work, **12** was prepared by three different synthetic routes. In the first route,

Scheme 5[a]



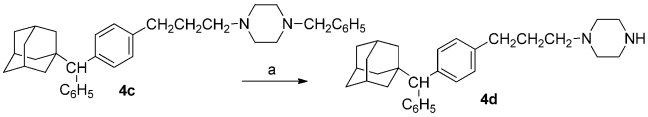
[a]Reagents and conditions: (a) (i) *n*-BuLi/THF, 2 h at −80 °C, (ii) DMF, rt, (iii) HCl 10%, at 0 °C; (b) $LiAlH_4$/THF, reflux, 1 h, then NaOH 10% at 0 °C; (c) $SOCl_2$ and $CaCO_3/Et_2O$, rt, 12 h; (d) NaCN/DMSO, 12 h; (e) (i) *t*-BuOK and TosMIC/DME, 2 h at −60 °C, (ii) MeOH, rt, then reflux, 30 min; (f) gas HCl/ethanol, ∼20%, reflux, 2 h, then water drops, reflux, 1 h; (g) (i) NaOH/EtOH–H_2O, reflux, 2 h, then HCl 10% at 0 °C, (ii) $SOCl_2$ at 60 °C, 1 h, (iii) R_2NH/THF, reflux, 12 h; (h) $LiAlH_4$/THF, reflux, 3 h, then NaOH 10% at 0 °C.

Scheme 6[a]



[a]Reagents and conditions: (a) TsCl/Py/DCM; (b) requisite amine/EtOH, Δ.

Scheme 7[a]



[a]Reagents and conditions: (a) HCO_2NH_4/10% Pd–C/MeOH, Δ.

addition of allylmagnesium chloride to aryl bromide **10** in presence of a catalytic amount of copper(I) iodide gave a

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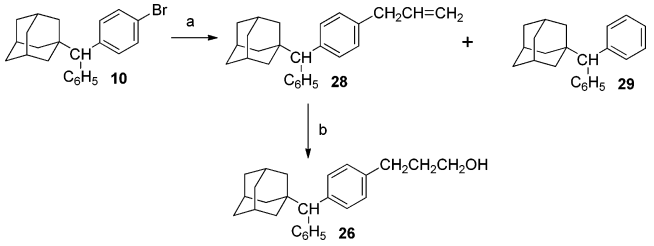
mixture of olefin **28** and 1-(α-benzhydryl)adamantane (**29**) via a free radical mechanism. Hydroboration of the above mixture led to propyl alcohol **26**, which was separated from hydrocarbon **29** by column chromatography (Scheme 8).

Scheme 8[a]



[a]Reagents and conditions: (a) allylmagnesium chloride/THF, CuI; (b) (i) BH_3·THF; (ii) $NaOH/H_2O_2$.

In the second route, the application of Heck reaction conditions,[51,52] treatment of aryl bromide **10** with ethyl acrylate in presence of palladium(II) acetate and triphenylphosphine, gave the ethyl cinnamate **30**. The corresponding cinnamic acid is a mixture of diastereomers (*R*,*E*) and (*S*,*E*) (Figure 3)

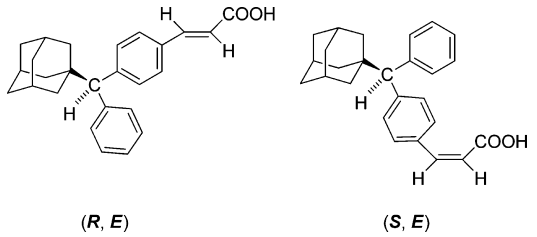
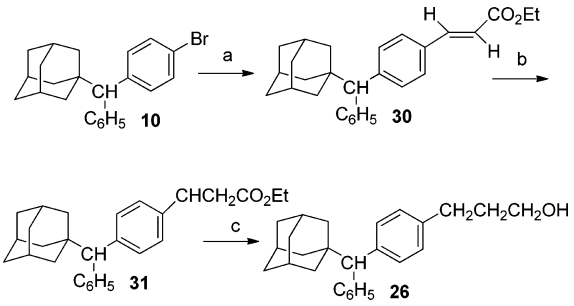


Figure 3. Mixture of corresponding cinnamic acid diastereomers.

according to the ^1H NMR spectra for this compound. Catalytic hydrogenation of the unsaturated ester **30** and sequential reduction of the intermediate propionic ester **31** with $LiAlH_4$ gave the desired alcohol **26** (Scheme 9).

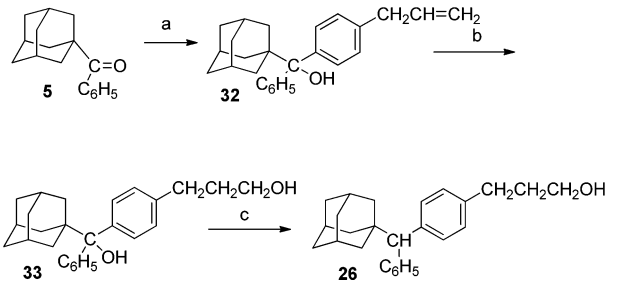
Scheme 9[a]



[a]Reagents and conditions: (a) ethyl acrylate, palladium(II) acetate, triphenylphosphine, triethylamine at 95−100 °C; (b) H_2/PtO_2, EtOH at 50−60 psi; (c) (i) $LiAlH_4$/THF; (ii) H_2O/OH^- at 0 °C.

In the third synthetic route (Scheme 10), addition of *p*-allylphenylmagnesium bromide to ketone **5** gave the unsaturated alcohol **32**, which was transformed to diol **33** by hydroboration. The latter was reduced to propyl alcohol **26** with triethylsilane in trifluoroacetic acid. Of the three synthetic pathways, the third route gave the best yield of **26**.

Scheme 10[a]



[a]Reagents and conditions: (a) *p*-allylphenylmagnesium bromide/THF, NH_4Cl/H_2O at 0 °C; (b) BH_3 (>2 equiv)/THF and then $NaOH/H_2O_2$ at 0 °C; (c) (i) TFA/DCM and then Et_3SiH; (ii) H_2O at 0 °C; (iii) $NaOH/H_2O$/THF at rt.

BIOLOGY

The results for the affinities of the derivatives for both the σ_1 and σ_2 receptors and site 2 of Na^+ channels are summarized in Table 1. The results for the in vitro antiproliferative activity of the derivatives **2**, **3**, and **4** are shown in Table 2.

Table 1. Affinities of Some Adamantane Phenylalkylamines for the σ_1 and σ_2 Receptors

	IC_{50} ± SEM (nM) (n = 3)			
compd	σ_1	σ_2	σ_1/σ_2	IC_{50} ± SEM (nM) (n = 3) Na^+ channels
2a	5.2 ± 1.3	110.4 ± 13.1	21.2	nd[a]
3a	2.9 ± 0.7	80.1 ± 9.4	27.6	nd[a]
4a	48.1 ± 17.7	85.0 ± 18.3	1.77	>1000
4c	36.1 ± 8.3	28.3 ± 7.2	0.78	11.5 ± 7.0
4d	42.0 ± 21.0	461.4 ± 141.7	11.0	>1000
4e	12.3 ± 4.2	13.0 ± 3.9	1.06	>100

[a]nd: not determined.

It is clear from Table 1 that adamantane analogues **4a−e** have a significant binding affinity for the σ_1 and σ_2 receptors and, except for the **4c**, a much lower or insignificant affinity for site 2 of the Na^+ channels. Analogue **4d** seems to have moderate binding affinity at the σ_2-receptors, while derivatives **2a** and **3a** seem to act as selective σ_1-ligands.

These results, in conjunction with the in vitro antiproliferative action of the new adamantane derivatives (Table 2), imply that the new molecules act as mixed σ_1/σ_2 ligands. Since binding data do not classify the ligands as agonists or antagonists, further work is required to define the characteristics of adamantane ligands that are needed for receptor activation, subtype selectivity, and particularly the nature of the action (agonistic or antagonistic). No validated in vitro isolated organ test exists for the functional characterization of σ-ligands as agonists or antagonists.[53] However, the antiproliferative activity of the new compounds is possibly linked with their affinity for the σ-receptors, while other mechanisms of action cannot be ruled out. Indeed, we argue that in contrast to analogues **1**,[31] where the antiproliferative activity and the σ affinities are clearly related to the second piperazine nitrogen (benzyl and morpholino derivatives exhibited diminution of or no σ affinities and antiproliferative activities), the antiproliferative action and σ binding affinities of analogues **2a**, **3a**, and **4a−d** are due to both piperazine atoms. The relative

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Table 2. Summary Results from the in Vitro Screening of Antiproliferative Activity of Tested Derivatives on Cancer Cell Lines (Colon, Prostate, Breast, Ovarian, Central Nervous System, Leukemia, Pancreas, Liver) and on Normal Cell Lines

		cancer cell lines												normal cell lines		
		colon		prostate			ovarian				pancreas					
compd	parameter[a]	HCT-116	HCT-15	DU145	PC3	breast MCF7	IGROV-1	OVCAR-5	CNS U251	leukemia HL-60 (TB)	MiaPaca2	BX-PC3	liver SKHep1	HUVEC	hMSC	NHDF
2a	LC_{50}		9.1	9.08	68.51	8.33		57.70	9.47	100	9.06	9.13	8.59	100	26.39	100
	TGI		6.3	6.41	6.25	5.29		19.67	6.55	9.14	6.34	6.98	5.97	7.3	4.44	10.44
	GI_{50}		3.5	3.01	3.55	2.25		5.31	3.62	4.63	3.61	4.83	3.35	4.12	1	5.12
2b	LC_{50}		9.4	100	62.35	9.58		70.03	68.54	100	88.62	9.35	90.21	9.54	100	100
	TGI		6.36	46.1	7.9	5.92		43.78	7.8	7.75	43.67	6.84	24.34	5.34	4.61	66.83
	GI_{50}		3.36	5.73	7	2.26		18.05	4.33	4.33	8.24	4.33	5.9	1.14	1	6.59
2c	LC_{50}		8.24	39.2	69.21	8.57			8.43	100		9.51	8.84	17.43	8.27	9.73
	TGI		5.76	17.84	7.52	5.51			5.92	7.59		6.9	6.17	5.98	3.14	6.69
	GI_{50}		3.28	4.23	3.89	2.45			3.41	4.48		4.3	3.51	2.91	1	3.66
2d	LC_{50}		100	88.97	81.31	80.6		91.53	75.83	100	81.26	87.45	82.24	72.47	78.84	81.19
	TGI		79	47.32	26.18	43		60.8	40.17	72.87	43.17	51.64	38.39	21.73	20.06	52.46
	GI_{50}		8.8	8.98	6.07	8.89		31.12	8.6	5.91	7.98	15.84	7.46	5.11	1	23.74
3a	LC_{50}		100		13.48	8.60	13.58	62.37	8.81		76.2	100	9.02	9.6	22.53	100
	TGI		8.26		9.00	5.70	8.93	29.28	6.09		36	19.9	6.36	6.46	6.39	8.07
	GI_{50}		4.64		4.57	2.77	4.29	17.35	3.37		7.5	5.76	3.69	3.32	2.35	4.18
3b	LC_{50}		100		86.73	97.10	96.77	68.54	8.47		77.5	89.77	100	9.62	100	100
	TGI		17.2		9.53	8.50	9.83	53.78	6.19		49.2	45.16	7.66	7.36	2.93	83.58
	GI_{50}		5.64		4.83	3.90	4.49	28.11	3.54		20.9	8.35	4.14	5.1	1	7.31
3c	LC_{50}		82		91.99	98.54	68.66	97.69	86.69		91.14	100	100	100	62.77	99.34
	TGI		43.7		41.23	42.38	43.67	67.80	55.76		63.32	100	58.25	9.33	18.32	58.95
	GI_{50}		9.12		11.57	6.93	17.70	37.33	24.83		35.51	64.7	8.61	5.31	2.95	18.56
4a	LC_{50}	8.73	9.2	100	10.01	9.00	74.77	10.20	8.24	100	72.63	8.8	9.24	8.62	80.01	100
	TGI	6.05	6.35	7.11	6.72	6.10	11.91	6.90	5.93	7.88	40.11	6.1	6,37	5.8	30.94	78.2
	GI_{50}	3.38	3.5	3.77	3.43	3.20	4.54	3.60	3.62	4.02	9.39	3.41	3.49	2.98	5.44	9.68
4b	LC_{50}	8.52	47.3	11.66	10.89	8.61			9.09			100	8.61	9.4	53.96	100
	TGI	5.9	7.64	8.5	7.84	5.87			6.69			10.57	6.01	6.73	8.69	36.59
	GI_{50}	3.29	4.09	5.33	4.79	3.05			4.29			5.3	3.41	4.07	5.36	5.04
4c	LC_{50}	82.91				100			100			100				100
	TGI	39.66				61.7			79.12			100				100
	GI_{50}	7.72				6.76			36.41			36.81				39.45
4d	LC_{50}	8.74	9.11	8.46	8.37	8.84	9.39		8.44	100		9.44	8.7	100	9.65	9.58
	TGI	5.85	6.13	5.65	5.71	5.97	6.34		5.87	8.22		6.41	6.19	7.17	7.28	6.82
	GI_{50}	2.96	3.15	2.83	3.06	3.1	3.29		3.3	3.01		3.38	3.67	3.97	4.91	4.07
4e	LC_{50}	8.61	9.68	11.15	10.72	9.63			9.77			9.17	8.68	9.18	9.68	9.47
	TGI	5.99	6.78	8.17	7.32	6.07			7.19			6.47	5.97	6.23	1.93	6.15
	GI_{50}	3.37	3.89	5.18	3.92	2.41			4.61			3.78	3.25	3.27	1	2.83
gemcitabine	LC_{50}										100	8.71				
	TGI										100	9.05				
	GI_{50}										35.83	3.64				

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Table 2. continued

		cancer cell lines													normal cell lines		
		colon		prostate		breast	ovarian		CNS	leukemia	pancreas		liver				
compd	parameter[a]	HCT-116	HCT-15	DU145	PC3	MCF7	IGROV-1	OVCAR-5	U251	HL-60 (TB)	MiaPaca2	BX-PC3	SKHep1	HUVEC	hMSC	NHDF
5FU	LC_{50}	100	100	100	100	100	100	100	100	100	100	100	100	100	1	100
	TGI	100	100	100	100	100	100	100	100	100	100	8.4	100	100	1	100
	GI_{50}	8.8	21.1	20	10.45	14.5	8.12	26.4	69.6	100	30.6	2.9	7.6	3.6	1	62.02

[a]For each experimental agent: LC_{50} is the concentration in nM of drug resulting in 50% reduction in measured protein, TGI the concentration in nM resulting in total growth inhibition, GI_{50} the concentration in nM resulting in growth inhibition of 50% (see also pharmacological protocol).

conservation of the antiproliferative activity of the piperidino analogue **4e** reinforces this argument. However, the high σ binding affinity of the benzyl-substituted compound **4c** is not accompanied by any significant antiproliferative activity. This, which has also been observed in analogous benzyl group bearing adamantanes,[50] is possibly due to the σ_2-receptor antagonistic profile of **4c** induced by the benzyl substitution.

Considering the in vitro results of antiproliferative activity of derivatives **4** on cancer cell lines (colon, prostate, breast, ovarian, central nervous system, leukemia, pancreas, liver) and on normal cell lines (HUVEC, human umbilical vein endothelial cell; hMSC, human mesenchymal stem cell; NHDF, normal human dermal fibroblast) (Table 2), it appears that **4a** exhibited the most selective activity against cancer cells, given that the cytotoxic effect of **4a** on the HUVECs could, in vivo, also be an antiangiogenic factor against the tumors. Interesting results were also obtained with **4a** in the BxPC-3 (pancreas), OVCAR-5 (ovarian), DU-145 and PC3 (prostate), and HL-60 (leukemia) cancer xenografts (described in Experimental Section[38,39]). Antitumor activity of **4a** was, in most of these xenografts, superior to those of 5-fluorouracil (5FU) and aracytin and similar to those of gemcitabine, paclitaxel (Taxol), and cis-Pt, with **4a** exerting interesting synergies with both the reference drugs 5FU and gemcitabine (Figures 4−8). Finally, some encouraging results were obtained with **4a** on the metastasis of PC3 and OVCAR-5 cancer cells xenografts (Figures 6 and 8)

The pharmacological profile of compound **4a** was further extended by toxicological experiments. These studies were performed on CD-1 male and female mice and, for xenografts, on mice with severe combined immune deficiency (SCID). Compound **4a** was well tolerated at 40−55 mg/kg (ip) in chronic 2−3 weeks of treatments on CD-1 and SCID mice. There were no more dead animals than in Tween-80 (5%) or Cremophor or reference drugs used in the above protocols and no important loss of weight in SCID mice during the xenograft experiments. Concerning the mechanistic effects induced by **4a** on the BxPC-3, IGROV-1, and PC-3 cell cycle and apoptosis (Table 3), significant increases of sub-G1 for BxPC-3, IGROV-1, and PC-3 at 15 μM (for PC3 even from 5 μM) and also of G1 populations of BxPC3 at 5 μM were observed in cells treated with **4a**, compared to the vehicle treated group. Combined with a decrease of populations engaged in the S phase, these alterations of the cell cycle are indicative of the apoptotic activity of **4a**. Indeed, **4a** exhibited apoptotic plasmatic membrane modifications at 15 and 50 μM (for BxPC-3 at 15 μM and for IGROV-1 at 15 and 50 μM) with caspase-3 activation. It is noteworthy that the antiproliferative activity of **4a** on BxPC-3 (pancreas), IGROV-1 (ovarian), and PC3 (prostate) cancer cells is conjugated with apoptotic activity (in the order IGROV-1 > BxPC-3 > PC3), an inhibition of these cancer cells cycle at the sub-G1 level (in the order PC3 > IGROV-1 ≥ BxPC-3), and caspase-3 activation only for IGROV-1 and BxPC-3. The above results could indicate that the mixed σ_1/σ_2 ligand **4a**, in agreement with previous data,[28,30−33,54] exhibited cell cycle inhibition and caspase-3 activation in BxPC-3 and IGROV-1 but also cell cycle inhibition and caspase-3 independent apoptosis in PC3 cancer cells, in agreement with results concerning σ_2-ligands.[22,25,54] Results of in vitro assays concerning the effect of **4a** on the cell cycle and apoptosis of BxPC3, IGROV-1, and PC3 cancer cells are summarized in Table 3.

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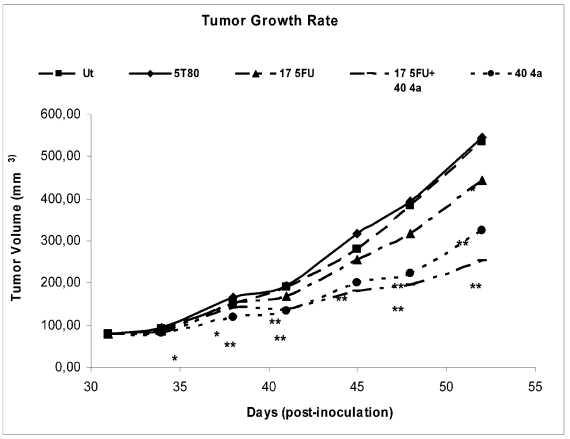
A[a]



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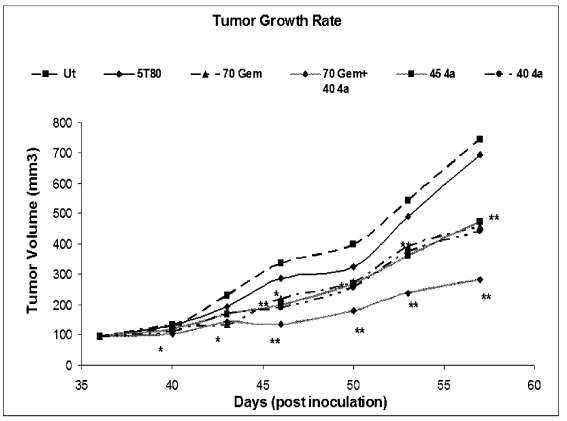

B[b]

	Post mortem weight	
	Average weight (mg)±SE	% reduction
Ut	744 ± 254	
5T80	730±156	
17 5FU	568±192	23.66 *
17 5FU+ 40 4a	377±95	48.36 **
40 4a	492±148	32.67 **

D[d]

	Post mortem weight	
	Average weight (mg)±SE	% reduction
Ut	819 ± 295	
5T80	862±198	
70 Gem	507±199	38.14**
45 4a	541±247	37.18**
40 4a	674±211	21.81**
70 Gem+ 40 4a	335±165	61.10**

Figure 4. Growth of BXPC3 (pancreas) tumors in SCID mice treated with fluorouracil (5FU) (reference drug for pancreatic cancer) and **4a**. (A[a]) Tumor size (in mm^3) of each mouse group (10 mice per group, that is, 20 tumors per group). (B[b]) Table showing reduction of tumors on day of termination of experiment for BXPC3 xenograft: Ut, untreated animals; 5T80, animals treated with 5% Tween 80; 17 5FU, animals treated with 17 mg/kg 5FU (dissolved in water for injection) administered twice a week (three cycles of treatment); 40 4a, animals treated with 40 mg/kg **4a** (dissolved in 5% Tween 80) administered for 3 consecutive days per week (three cycles of treatment)l 17 5FU + 40 4a, coadministration of 17 mg/kg 5FU and 40 mg/kg **4a**. Statistical evaluation was carried out using a two-tailed Student's *t* test. Points with $p < 0.05$ are indicated by one asterisk (*), and points with $p < 0.001$ are indicated by two asterisks (**). Shown is the growth of BXPC3 (pancreas) tumors in SCID mice treated with gemcitabine (Gem) (reference drug for pancreatic cancer) and **4a**. (C[c]) Tumor size (in mm^3) of each mouse group (10 mice per group, that is, 20 tumors per group). (D[d]) Table showing reduction of tumors on day of termination of experiment for BXPC3 xenograft: Ut, untreated animals; 5T80, animals treated with 5% Tween 80; 70Gem, animals treated with 70 mg/kg Gem (dissolved in water for injection) administered twice a week (three cycles of treatment); 45 4a, animals treated with 45 mg/kg **4a** (dissolved in 5% Tween 80) administered for 3 consecutive days per week (three cycles of treatment); 40 4a, animals treated with 40 mg/kg **4a** (dissolved in 5%Tween 80) administered for 3 consecutive days per week (three cycles of treatment; 70Gem + 40 4a, coadministration of 70 mg/kg Gem and 40 mg/kg **4a**. Statistical evaluation was carried out using a two-tailed Student's *t* test. Points with $p < 0.05$ are indicated by one asterisk (*) and those with $p < 0.001$ by two asterisks (**).

Finally, in relation to the role of σ_1-receptors in the central sensitization processes of neuropathic pain,[2,55−58] **4a** was tested in a neuropathic pain model (described in Experimental Section[31,59]). **4a** exhibited a notable analgesic effect in the formalin test[60,61] operated on mice on which pain sensitization was obtained by previous (2 weeks) administration of paclitaxel,[31,59] as shown in Figure 9.

CONCLUSION

The in vitro and in vivo toxicological and xenograft screening studies showed that piperazine **4a** exhibited an acceptable toxicological profile associated with a notable antitumor activity that, in good agreement with its in vitro cell line results, was particularly prominent in pancreas, prostate, ovarian, and leukemia xenografts on SCID mice (Figures 4−8). It is noteworthy that this antitumor activity of **4a** is associated with a putative antagonism of the neuropathic pain induced by the clinically used anticancer drugs (particularly, taxanes). Encouraging results were also obtained with **4a** on the metastasis of prostate and ovarian xenografts (Figures 7 and 8). Consequently, compound **4a** is currently under investigation with particular interest in its putative antimetastatic activity.

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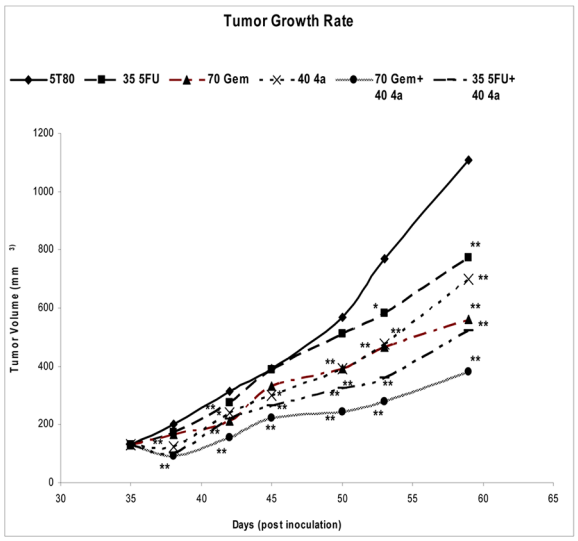
A[a]



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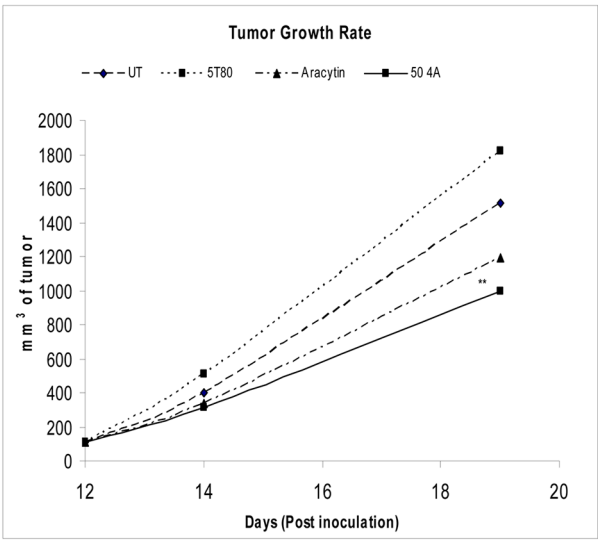

B[b] Post mortem weight

	Average weight (mg)±SE	% reduction
5T80	1226 ± 478	
35 5FU	777±187	36.66**
70 Gem	669±207	45.40**
40 4a	732±199	40.34**
70 Gem+ 40 4a	494±161	59.71**
35 5FU+ 40 4a	542±164	55.84**

D[d] Post mortem weight

	Average weight (mg)±SE	% reduction
Ut	1824 ± 582	
5T80	2397±588	
10 Ara	1390±611	23.80
50 4a	1400±706	41.60**

Figure 5. Growth of BXPC3 (pancreas) tumors in SCID mice treated with fluorouracil (5FU), gemcitabine (Gem) (reference drugs for pancreatic cancer) and **4a**. (A[a]) Tumor size (in mm^3) of each mouse group (10 mice per group, that is, 20 tumors per group). (B[b]) Table showing reduction of tumors on day of termination of experiment for BXPC3 xenograft: 5T80, animals treated with 5% Tween 80; 35 5FU, animals treated with 35 mg/kg 5FU (dissolved in water for injection) administered twice a week (three cycles of treatment); 70Gem, animals treated with 70 mg/kg gemcitabine (dissolved in water for injection) administered twice a week (three cycles of treatment); 40 4a, animals treated with 40 mg/kg **4a** (dissolved in 5% Tween 80) administered for 3 consecutive days per week (three cycles of treatment); 35 5FU + 40 4a, coadministration of 35 mg/kg 5FU and 40 mg/kg **4a**; 70 Gem + 40 4a, coadministration of 70 mg/kg Gem and 40 mg/kg **4a**. Shown is the growth of HL-60(TB) (leucaemias) tumors in SCID mice treated with aracytin (Ara) (reference drug for leucaemias) and **4a**. (C[c]) Tumor size (in mm^3) of each mouse group (9 mice per group, that is, 20 tumors per group). (D[d]) Table showing reduction of tumors on day of termination of experiment for HL-60 (TB) xenograft: 5T80, animals treated with 5% Tween 80; 10Aracytin, animals treated with 10 mg/kg aracytin (dissolved in water for injection) administered 5 times a week (one cycle of treatment); 50 4a, animals treated with 50 mg/kg **4a** (dissolved in 5% Tween 80) administered for 3 consecutive days per week (one cycle of treatment). Statistical evaluation was carried out using a two-tailed Student's *t* test. Points with $p < 0.05$ are indicated by one asterisk (*) and those with $p < 0.001$ by two asterisks (**).

EXPERIMENTAL SECTION

Binding Studies. Binding studies were carried out by CEREP (France) with the σ_1 binding assay performed in triplicate. Affinities of the new adamantane alkylamines for the σ_1 and σ_2 receptors were measured by displacement of [^{3}H](+)-pentazocine and [^{3}H]1,3-di-*o*-tolylguanidine, respectively. The σ_1 binding assay[62] was performed by incubating Jurkat cell membranes (10−20 mg of protein per tube) with [^{3}H](+)-pentazocine (8 nM) and a range of concentrations of the compounds at 22 °C for 2 h in 5 nM Tris-HCl buffer, pH 7.4. The σ_2 binding assay[63] was performed by incubating rat cerebral cortex membranes (10−20 mg of protein per tube) with [^{3}H](+)-1-DTG (3-di-*o*-tolylguanidine, 5 nM) in the presence of (+)-pentazocine (300 nM) to saturate σ_1 site binding and a range of concentrations of the compounds at 22 °C for 2 h in 5 nM Tris-HCl buffer, pH 7.4. The final assay volume was 0.5 mL. Nonspecific binding was defined, in both assays, as that remaining in the presence of 10 μM haloperidol. Affinities for site 2 of Na^+ channel were measured by displacement of [^{3}H]batrachotoxin benzoate (^{3}H-BTX-B).[64] Binding reactions were initiated by addition of 150 μL of vesicular preparation containing 150−500 μg of protein to a solution in standard incubation buffer of ^{3}H-BTX-B, 50 μg of *Leiurius quinquestriatus* scorpion venom, and various unlabeled effectors as indicated. The concentration of ^{3}H-BTX-B was generally 20−25 nM, and the total assay volume was 335 μL. Standard incubation buffer contained 130 mM choline chloride, 50 mM HEPES buffer adjusted to pH 7.4 with Tris base, 5.5 mM glucose, 0.8 mM $MgSO_4$, 5.4 mM KCl, and 1 mg/mL BSA. Incubations were carried out for 60 min at the indicated temperature and were then terminated by addition of 3 mL of ice-cold wash buffer. The tissue was immediately collected on Whatman GF/C glass fiber filters and washed 3 more times with 3 mL of cold wash buffer. The wash buffer

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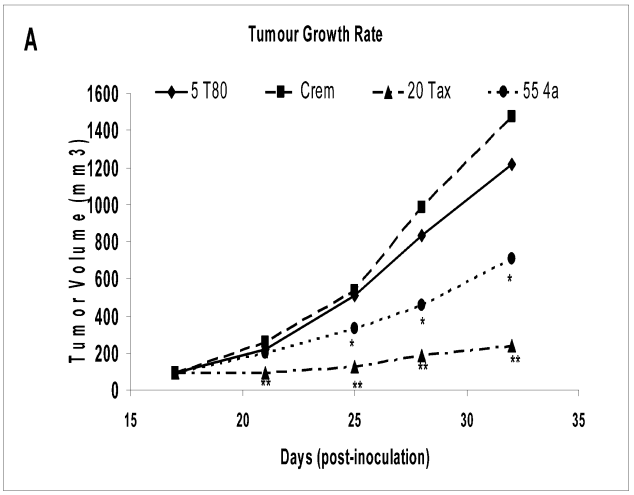
A[a]



B[b]

	Post mortem weight	
	Average weight (mg±SE)	% reduction
5T80	869±64,77	
Crem	882.14±180,44	
20 Tax	184.29±182,0	79.1**
55 4a	451.67±40,20	48.0*

C[c]

Treatment Groups	Primary Tumour Occurrence	Secondary Tumour Occurrence	
		Axillary Metastasis (AM)	Bone Metastasis (BM)
5 T80	7/7 (100%)	4/7 right; 5/7 left (64%)	7/7 (100%)
Crem	7/7 (100%)	3/7 right; 4/7 left (50%)	6/7 (86%)
20 Tax	7/7 (100%)	1/7 (14%)	6/7 (86%)
55 4a	7/7 (100%)	0/7 (0%)	3/7 (43%)

Figure 6. Growth of PC3 (prostate) tumors in SCID mice treated with paclitaxel (reference drug for prostate cancer) and **4a**. (A[a]) Tumor size (in mm^3) of each mouse group (14 mice per group, that is, 28 tumors per group). (B[b]) Table showing reduction of tumors on day of termination of experiment for PC3 xenograft. (C[c]) Occurrence of primary (n = 7) and secondary metastatic tumors (n = 7) at the axillary (AM) and bone (BM) sites in the control and drug-treated groups: 5 T80, animals treated with 5% Tween 80; Crem, animals treated with a (1:1 ethanol and Cremophor stock solution diluted 1:3 in saline; 20 Tax, animals treated with 20 mg/kg paclitaxel (dissolved in Cremophor and diluted in saline) administered once a week (two cycles of treatment); 55 4a, animals treated with 55 mg/kg **4a** (dissolved in 5% Tween 80) administered for 5 consecutive days (two cycles of treatment). Statistical evaluation was carried out using a two-tailed Student's t test. Points with $p < 0.05$ are indicated by one asterisk (*) and those with $p < 0.001$ by two asterisks (**).

contained 163 mM choline chloride, 5 mM HEPES (pH 7.4), 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, and 1 mg/mL BSA. Radioactivity associated with the tissue was determined by liquid scintillation spectroscopy of the filters suspended in 10 mL of scintillation cocktail (3a70B;RPI). Nonspecific binding was determined from parallel incubations containing 250 μM veratridine and has been subtracted from the data. Specific binding measured in this way was nominally 75% of the total binding.

In Vitro Antiproliferative and Cytotoxic Activity. All human cancer cell lines were obtained from the National Cancer Institute, NIH (Bethesda, MD, U.S.) with the exception of BX-PC3 and the normal cells Hs888Lu and CCD18Co that were obtained from ATCC and the hMSCs, NHF, and HUVECs that were purchased from Lonza. All cell lines were adapted to propagate in RPMI 1640 medium supplemented with 5% heat-inactivated fetal calf serum, 2 mM L-glutamine, and antibiotics. The cultures were grown in a humidified 37 °C incubator with 5% CO_2 atmosphere. Cell viability was assessed at the beginning of each experiment by the trypan blue dye exclusion method and was always greater than 95%. Cells were seeded into 96-well microtiter plates in 100 μL of medium at the corresponding density (3500−30000 cells per well), and subsequently, the plates were incubated at standard conditions for 24 h to allow the cells to resume exponential growth prior to addition of the agents to be tested. Then in order to measure the cell population, cells in one plate were fixed in situ with trichloroacetic acid (TCA) followed by sulforhodamine B solution (SRB) staining, as described elsewhere.[65−67] To determine the activity, each compound was dissolved in dimethylsulfoxide (DMSO) and then was added at 10-fold dilutions (from 100 to 0.01 μM), and incubation continued for an additional period of 48 h. The assay was terminated by addition of cold TCA followed by SRB staining and absorbance measurement at 540 nm in an DAS plate reader to determine the GI_{50}, that is, the concentration required in the cell culture to inhibit cell growth by 50%; TGI, the concentration that is required to completely inhibit cell growth; and the LC_{50}, the concentration that is needed in culture to kill 50% of the cellular population as described.[65−67]

In Vivo Antitumor Activity. SCID (NOD.CB17 Prkdcscid) mice were purchased from Jackson Laboratories/Charles River Laboratories (L'Arbresle, France). The mouse colony was maintained under restricted flora conditions in a pathogen-free environment in type

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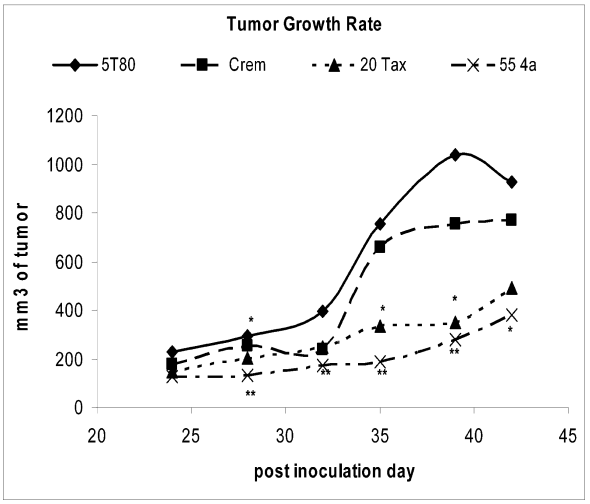
A[a]



B[b]

	Post mortem weight	
	Average weight (mg)±SE	% reduction
5T80	790±98,57	
Crem	617.14±103,19	
20 Tax	452.86±104,64	26.6
55 4a	225.83±86,57	71.4**

Figure 7. Growth of DU145 (prostate) tumors in SCID mice treated with paclitaxel (reference drug for prostate cancer) and **4a**. (A[a]) Tumor size (in mm^3) of each mouse group (minimum number of animals used $n = 30$). (B[b]) Table showing reduction of tumors on day of termination of experiment for DU145 xenograft: 5 T80, animals treated with 5% Tween 80; Crem, animals treated with a 1:1 ethanol and Cremophor stock solution diluted 1:3 in saline; 20 Tax, animals treated with 20 mg/kg paclitaxel (dissolved in Cremophor and diluted in saline) administered once a week (two cycles of treatment); 55 4a, animals treated with 55 mg/kg **4a** (dissolved in 5% Tween 80) administered for 5 consecutive days (two cycles of treatment). Statistical evaluation was carried out using a two-tailed Student's t test. Points with $p < 0.05$ are indicated by one asterisk (*) and those with $p < 0.001$ by two asterisks (**).

IIL cages. Male or female mice, 7–9 weeks old, were subjected to subcutaneous injections according to the British practice of bilateral trocar implants at the axillary region. Each inoculum contained 106 cells exponentially growing at the time of harvesting. The mice were subsequently randomly divided into groups of 6–20 animals per group when the average tumor volume had reached about 100 mm^3. Treatments started at that point. Tumor volume was calculated as described elsewhere.[67,68] All administrations were intraperitoneal. Treated animals received a single injection daily for 5 days per week throughout the experiments. Tumor volume was measured with a caliper twice per week. In addition to tumor volume, we calculated the parameter, % $\Delta T/\Delta C$, where $\Delta T = T - D_o$ and $\Delta C = C - D_o$ (D_o is the average tumor volume at the beginning of the treatment; T and C are the volumes of treated and untreated tumors, respectively, at a specified day). Concurrently, we scored the number of tumor-free animals, number of drug-related deaths, and average number of days required to reach a defined tumor volume. Optimal $\Delta T/\Delta C$ was used as a measure of drug activity. Losses of weight, neurological disorders, and behavioral and dietary changes were also recorded as indicators of toxicity (side effects). The experiment was terminated when tumor size in untreated animals reached a volume of about 10–11% of the animals' weight.

Evaluation of Metastasis in the OVCAR5 Xenograft. The OVCAR5 ovarian cancer xenograft was observed for the presence of metastasis. On day 54 (days postinoculation), animals from all drug groups were sacrificed and observed for the occurrence of metastasis. Metastatic tumors were observed at two main sites: the auxiliary region (named "auxillary metastasis", AM) and the abdominal region attached to the bone (named "bone metastasis", BM). Figure 8, Table C represents the occurrence of the AM and BM tumors for the different drug groups as observed on day 54 of the experiment. Isolation, subculture and in vitro analysis of the metastatic-derived tissue indicated that the three types of cultures (primary, AM-derived, and BM-derived) were indeed of similar morphological arigin, confirming that the metastatic tissue was OVCAR5 ovarian-cancer-cell derived.

In Vitro Cell Cycle Modifications and Apoptotic Activity. Supplying PC-3 (human prostatic adenocarcinoma), BxPC-3 (human pancreatic adenocarcinoma), and IGROV-1 (human ovarian carcinoma) cancer cells and carrying out cell cycle and apoptosis assays were done by Oncodesign S.A. (France). Cells were plated 24 h before treatment at the appropriate seeding density in six-well plates or 25 cm^2 flasks according to the assay. Compound **4a** was dissolved at 10 mM in 100% DMSO and then diluted in cascade to obtain concentrations of 0.1, 1, and 3 mM in 100% DMSO. These dilutions were further diluted at 1:10 with RPMI 1640 medium. The last dilution was performed on cells at 1:20 to reach the appropriate concentrations of 0.5, 5, 15, and 50 μM. The final concentration of DMSO was 0.5% in cell culture medium. The treatment period was 24 h. The effect of compound **4a** on cell cycle was evaluated by quantification of propidium iodide (PI) incorporation into genomic DNA. After incubation, cells were detached from the well by trypsinisation, transferred into tubes, washed, and resuspended in 500 μL of ice cold PBS before being fixed with 1.5 mL dropwise of 100% cold ethanol for 3 h at 4 °C . Then the cell suspensions were centrifuged at 1500 rpm for 5 min, and pellets were resuspended in a mix of 100 μL of 200 μg/mL RNase and 100 μL of 1 mg/mL PI. Cells were incubated for 45 min at room temperature in the dark. The preparation was centrifuged 5 min at 1500 rpm. Then the pellets were resuspended in PBS for FACS analysis.

The apoptotic membrane modifications induced by compound **4a** were evaluated by annexin V binding at the end of the treatment period. 7-AAD, a fluorescent agent incorporated into DNA, was also used to differentiate early (no membrane disruption) and late (membrane disruption) apoptosis. After incubation, cells were detached from the well by gentle scraping, transferred to FACS tubes, and labeled according to the annexin V-FITC/7-AAD kit (Beckman Coulter). Briefly, after being washed, cells were incubated in ice-cold binding buffer. Then 10 μL of annexin V-FITC solution and 20 μL of 7-AAD viability dye were added to 100 μL of cell suspension and incubated 15 min on ice in the dark. After incubation, an amount of 800 μL of binding buffer was added. Cell preparations were analyzed by FACS within 1 h.

The activation of caspase pathway by compound **4a** was evaluated by measuring the level of activated caspase-3 by FACS. After incubation, cells were detached from the culture flask by trypsinization, transferred to FACS tubes, and labeled according to PE active caspase-3 apoptosis kit (Pharmingen). Briefly, after being washed, 106 cells were fixed and permeabilized in ice-cold BD Cytofix/Cytoperm buffer

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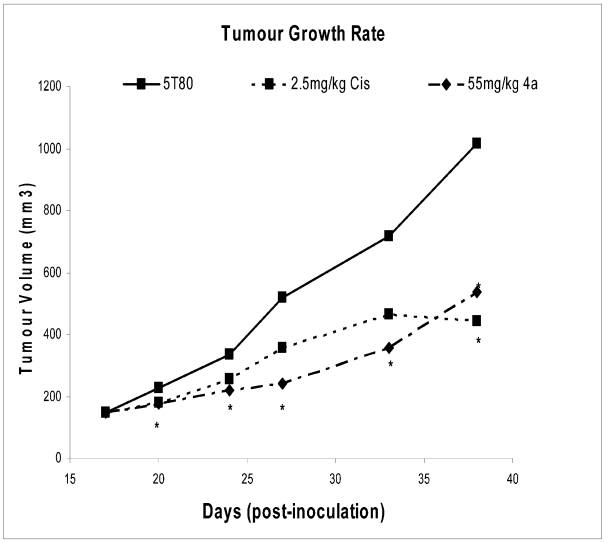
A[a]



B[b]

	Post mortem weight	
	Average weight (mg±SE)	% reduction
5T80	852.5±253.7	
2.5 Cis	392.9±154.6	53.9*
55 4a	472.9±37.7	44.5*

C[c]

Treatment Groups	Average weight (mg) of Primary tumours	% Reduction	Secondary Tumour Occurrence	
			Axillary Metastasis (AM)	Bone Metastasis (GM)
5 T80	1087.5+372.14	-	44%	56%
2.5mg/kg Cis	372+213.97	65.8*	40%	60%
55 4a	577.5+127.11	46.9*	25%	25%

Figure 8. Growth of OVCAR5 (ovarian) tumors in SCID mice treated with cisplatin (reference drug for ovarian cancer) and **4a**. (A[a]) Tumor size (in mm^3) of each mouse group (16 mice per group, that is, 32 tumors per group). (B[b]) Table showing reduction of tumors on day of termination of experiment for OVCAR5 xenograft. (C[c]) Average weight of primary tumors (n = 7−8) with % reduction and occurrence of secondary metastatic tumors (n = 7−8) at the axillary (AM) and bone (BM) sites in the control and drug-treated groups: 5 T80, animals treated with 5% Tween 80; 2.5 Cis, animals treated with 2.5 mg/kg cisplatin administered twice a week (three cycles of treatment); 55 4a, animals treated with 55 mg/kg **4a** (dissolved in 5% Tween 80) administered for 5 consecutive days (two cycles of treatment). Statistical evaluation was carried out using a two-tailed Student's t test. Points with $p < 0.05$ are indicated by one asterisk (∗) and those with $p < 0.001$ by two asterisks (∗∗).

for 20 min. Cells were then pelleted and washed with BD Perm/Wash buffer. Incubation with antibody included in the kit was performed in 100 μL of the same buffer complete with 20 μL of antibody for 30 min. Cell preparations were analyzed by FACS within 1 h.

For all assays staining cells were analyzed with a CyFlow space flow cytometer (Partec) using a 488 nm wavelength laser excitation. The acquisition was stopped after a total of 10 000 FSC/SSC gated cells were collected for each sample.

Formalin Test. CD1 male mice weighing 34−40 g were used. They were kept in a room maintained at 21−22 °C with free access to standard laboratory diet and tap water. Paclitaxel (Brystol Myers Squibb Company) was diluted in saline and administered at one ip injection[59] (6 mg/kg) on day 0. On day 14, 1 h after oral administration (po) of compound **4a** (100 mg/kg) the formalin test, as a tonic and persistent pain model of nociception, was performed. Injection of formalin into the hind paw is followed by two phases of behavior.[60,61] The first phase consists of intense licking and biting of the injected paw for the first 5 min followed by a period of little activity. The second phase spans from 15 to 40 min after the formalin injection and involves periods of licking and biting of the injected paw. The first phase is thought to be a model of acute chemical pain, whereas the second phase reflects a state of central sensitization driven by the presumed first phase.[60,61] The amount of time spent licking and biting the injected paw and leg was recorded at 5 min intervals for 0−5 and 35−40 min after the formalin injection.

Statistical Analysis. Significant difference in tumor volume was determined by the Student's t test using the SPSS for Windows (release 11.0.0, SPSS Inc., U.S.) software package. A difference was considered significant if $p < 0.05$.

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Table 3. Summary Results of the Cell Cycle and Apoptotic in Vitro Assays Performed with Compound 4a at 0.5, 5, 15, and 50 μM on BxPC-3, PC-3, and IGROV-1 Cancer Cell Lines[a]

		cell cycle analysis (%)						annexin V binding/7-AAD incorporation (%)			
cancer cell line	treatment (μM)	<G1	G_1	S	G_2	>G2	cleaved caspase-3 containing cells (%)	healthy cells annexin V^-/7-AAD^-	early apoptosis annexin V^+/7-AAD^-	late apoptosis annexin V^+/7-AAD^+	total apoptotic death (%)
BxPC-3 (pancreas)	0	9	41	33	12	6	8.2	79	9	9	18
	0.5	11	41	31	11	7	4	82	10	7	17
	5	8	53	23	10	5	4	77	11	10	21
	15	24	32	27	12	5	17.5	35	19	26	44
	50	23	31	33	9	4	4.8	12	2	44	46
IGR-OV-1 (ovary)	0	4	27	29	19	21	3.9	86	9	5	13
	0.5	4	25	26	23	20	4.2	89	8	3	11
	5	4	29	27	21	20	12.6	83	10	5	15
	15	12	23	25	27	17	61.4	35	37	20	57
	50	18	30	28	10	15	15.8	26	40	25	64
PC-3 (prostate)	0	4	36	42	12	6	3.2	91	5	3	8
	0.5	4	34	38	15	7	1.7	95	3	1	5
	5	11	36	36	10	6	2	92	5	2	7
	15	25	25	31	11	7	2.5	71	9	7	16
	50	12	29	31	11	17	4	43	15	9	23

[a]In cell cycle analysis, the percentage of cells was estimated for each phase of cell cycle (G0/G1, S, and G2/M) according to their DNA content. Cells having less than *n* chromosomes (<G0/G1) or more than 2*n* chromosomes (>G2/M) were also reported. The high toxicity of compound **4a** at 50 μM in the IGROV-1 cell line prevented correct cell cycle analysis. In the caspase-3 assay, the percentage of cells showing cleaved/activated caspase-3 is indicated. In the annexin V/7-AAD assay, the percentage of healthy, early apoptotic, and late apoptotic cells was estimated according to their affinity to annexin V-FITC and 7-AAD intercalating agent.

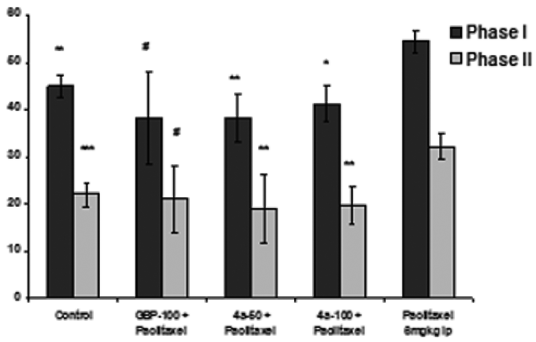

Figure 9. Effects of orally administered (po) **4a** at doses of 50 and 100 mg/kg po on paclitaxel treated mice in the formalin test compared to the reference drug gabapentin (GBP) at 100 mg/kg (ip). Administration of **4a** exerted a significant analgesic effect in paclitaxel treated animals. **4a**, 50 and 100 mg/kg (po), in paclitaxel treated animals resulted in lower licking times compared to paclitaxel treated animals at 0−5 min ((**) $p = 0.0013$ and (*) $p = 0.041$, respectively) and 35−40 min ((**) $p = 0.012$ and (*) $p = 0.015$) after formalin injection.

Methods and Materials. Melting points were determined using a Büchi capillary apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 833 spectrometer. ^1H and ^{13}C NMR spectra were recorded on a Bruker MSL 400 spectrometer using $CDCl_3$ as solvent and TMS as internal standard. Carbon multiplicities were established by DEPT experiments. The 2D NMR experiments (HMQC, COSY, and NOESY) were performed for the elucidation of the structures of the new compounds. Microanalyses were carried out by the Service Central de Microanalyse (CNRS), France, and the results obtained had a maximum deviation of ±0.4% from the theoretical value.

α-(4-Methylphenyl)-α-phenyl-1-tricyclo[3.3.1.1^{3,7}]-decanemethanol (6). To a stirred solution of *p*-tolylmagnesium bromide, which was prepared from magnesium turnings (0.8 g, 0.032 g at) and 4-bromotoluene (6 g, 0.035 mol) in anhydrous diethyl ether (50 mL), was added dropwise a solution of 1-adamantyl phenyl ketone (**5**) (3.4 g, 0.014 mol) in anhydrous diethyl ether (50 mL) under an argon atmosphere. The mixture was heated to mild reflux for 1 h and then quenched by adding water and aqueous HCl (10%) in an ice bath. The aqueous layer was separated and extracted with diethyl ether. The combined organics were washed with water, saturated solution of Na_2CO_3 (10%), dried over Na_2SO_4, and concentrated in vacuo. The residue obtained was crystallized and triturated with *n*-pentane to give 2.8 g of a solid product. Yield 60%. Mp 146−148 °C (ether−*n*-pentane).

α-(4-Bromomethylphenyl)-α-phenyl-1-tricyclo-[3.3.1.1^{3,7}]decanemethanol (7). To a stirred solution of carbinol **6** (1.35 g, 4 mmol) in dry CCl_4 (20 mL) were added NBS (0.9 g, 5 mmol) and a catalytic amount of dibenzoylperoxide (50 mg). The reaction mixture was gently refluxed for 8 h, then cooled to ambient temperature and filtered. The insoluble material was washed with CCl_4 and the combined filtrates were evaporared in vacuo to give a residue, which was used as such in the next step.

α-[4-(4-Methyl-1-piperazinylmethyl)phenyl]-α-phenyl-1-tricyclo[3.3.1.1^{3,7}]decanemethanol (8a). To a solution of the crude benzyl bromide **7** in anhydrous THF (10 mL) was added 1-methylpiperazine (1.8 g, 18 mmol), and the reaction mixture was heated to reflux for 10 h. The solvent was then removed in vacuo. Water was poured into the residue, and the resulting mixture was extracted with DCM. The organic layer was thoroughly washed with water, dried over Na_2SO_4, and evaporated to give a residue, which was purified by flash column chromatography, using as eluent a mixture of $CHCl_3$/

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MeOH (9:1) to afford 1.02 g of foamy product (yield 59% from alcohol **6**).

α-[4-(4-Ethyl-1-piperazinylmethyl)phenyl]-α-phenyl-1-tricyclo[3.3.1.1^{3,7}]decanemethanol (8b). Amino alcohol **8b** was prepared from carbinol **6** in a similar way to amino alcohol **8a**. The product was purified by flash column chromatography, using as eluent a mixture of DCM/MeOH (9:1) to afford amino alcohol **8b** as a foamy product (yield 57.5% from alcohol **6**).

α-[4-(1-Piperazinylmethyl)phenyl]-α-phenyl-1-tricyclo[3.3.1.1^{3,7}]decanemethanol (8c). Amino alcohol **8c** was prepared from carbinol **6** in a similar way to amino alcohol **8a**. The product was purified by flash column chromatography, using as eluent a mixture of DCM/MeOH (7:3) to afford amino alcohol **8c** as a foamy product (yield 38% from alcohol **6**).

α-[4-(1-Piperidinylmethyl)phenyl]-α-phenyl-1-tricyclo[3.3.1.1^{3,7}]decanemethanol (8d). Amino alcohol **8d** was prepared from carbinol **6** in a similar way to amino alcohol **8a**. The product was purified by flash column chromatography, using as eluent a mixture of DCM/MeOH (9:1) to afford amino alcohol **8d** as a sticky product (yield 67.5% from alcohol **6**).

α-(4-Bromophenyl)-α-phenyl-1-tricyclo[3.3.1.1^{3,7}]-decanemethanol (9). Carbinol **9** was synthesized by adding *p*-bromophenylmagnesium bromide to 1-adamantyl phenyl ketone (**5**) in a similar way as for carbinol **6**. Yield 81%. Mp 128−129 °C (ether−*n*-pentane).

4-Bromo-α-phenyl-α-(1-tricyclo[3.3.1.1^{3,7}]decyl)-methylbenzene (10). To a solution of carbinol **9** (4 g, 10 mmol) in anhydrous DCM (10 mL) was added trifluoroacetic acid (7.6 g, 67 mmol) under an argon atmosphere, and the mixture stirred at room temperature for 15 min. Triethylsilane (1.28 g, 1.8 mL, 11 mmol) was added dropwise to the reaction mixture under mild external cooling, and the resulting mixture was stirred at room temperature for 1 h. Then the reaction was quenched with chilled water and the mixture extracted with DCM. The combined organic layers were washed with water, saturated solution of Na_2CO_3 (10%), dried over Na_2SO_4, and concentrated in vacuo. To a solution of the residue obtained in THF, KOH (2 g) in a minimum amount of water was added and the resulting mixture stirred for 2 h. Then the solvent was evaporated in vacuo. Water was added to the residue, and the mixture was extracted with ether. The combined ethereal phases were washed with water, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash column chromatography, using hexane as eluent to afford 2.1 g of a crystalline product (yield 54%). Mp 144−145 °C (Et_2O).

4-[α-(1-Tricyclo[3.3.1.1^{3,7}]decyl)phenylmethyl]-benzene Carboxylic Acid (11). A solution of aryl bromide **10** (3.2 g, 8.4 mmol) in anhydrous THF (16 mL) containing 1,2-dibromoethane (10 drops) was added dropwise to magnesium turnings (0.44 g, 18.4 mmol) activated with iodine, under an argon atmosphere. The reaction mixture was heated to reflux for 3 h and then cooled to room temperature. Dry carbon dioxide gas bubbled into the mixture for 3 h. The reaction was quenched by adding an aqueous solution of HCl (10%) at 0 °C. The mixture was extracted with DCM, and the combined organic layers were washed with water, dried over Na_2SO_4, and concentrated in vacuo to give 2.24 g of a sticky product, which was used as such in the next step without further purification.

1-Methyl-4-{4-[α-(1-tricyclo[3.3.1.1^{3,7}]decyl)-phenylmethyl]phenylcarbonyl}pirerazine (12a). Thionyl chloride (12 mL) was added to the carboxylic acid **11**, and the mixture was gently refluxed for 1 h. The excess of thionyl chloride was evaporated in vacuo, and the last traces were removed azeotropically with dry benzene. The residue was dissolved in anhydrous THF (20 mL), and the resulting solution was added dropwise to a solution of 1-methylpiperazine (2.6 g, 25.6 mmol) in anhydrous THF (20 mL) at 0 °C. The reaction mixture was refluxed for 3 h, and then the solvent was evaporated in vacuo. Water was added to the residue, and the mixture was extracted with DCM. The combined organic phases were washed with water and dried over Na_2SO_4. The residue was purified by flash column chromatography, using as eluent a mixture of DCM/MeOH (9:1) to give 1.32 g of a viscous oil, which was crystallized after cooling (yield 37%). Mp 137−138 °C (Et_2O).

1-{4-[α-(1-Tricyclo[3.3.1.1^{3,7}]decyl)phenylmethyl]-phenylcarbonyl}pirerazine (12d). Carboxamide **12d** was prepared from carboxylic acid **11** in a similar way to benzamide **12a**. Flash column chromatography gave a solid product, using as eluent a mixture of DCM/MeOH (9:1). Yield 42% from aryl bromide **12**. Mp 196−198 °C (Et_2O).

1-Methyl-4-{4-[α-(1-tricyclo[3.3.1.1^{3,7}]decyl)-phenylmethyl]phenylmethyl}pirerazine (2a). *Method A.* To a solution of amino alcohol **8a** (1 g, 2.3 mmol) in anhydrous DCM (10 mL) was added trifluoroacetic acid (3.6 g, 31.2 mmol) under an argon atmosphere, and the mixture was stirred at room temperature for 15 min. Triethylsilane (350 mg, 3 mmol) was added dropwise to the reaction mixture under mild external cooling, and the resulting mixture was stirred at room temperature for 1 h. The reaction mixture was quenched with chilled water, made alkaline on treatment with solid Na_2CO_3, and then extracted with DCM. The combined organic layers were washed with water, dried over Na_2SO_4, and concentrated in vacuo. To a solution of the residue obtained in THF was added KOH (1 g) in a minimum amount of water, and the resulting mixture was stirred for 2 h. Then the solvent was evaporated in vacuo. Water was added to the residue, and the mixture was extracted with DCM. The combined organic phases were washed with water and dried over Na_2SO_4. The residue was purified by flash column chromatography, using as eluent a mixture of $CHCl_3$/MeOH (9:1) to afford 630 mg of a solid product (yield 60%). Mp 196−198 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.45−1.55 (complex m, 12H, 2,4,6, 8,9,10-H), 1.85 (br s, 3H, 3,5,7-H), 2.18−2.62 (very br s, 8H, 2,3,5,6-Hp), 2.20 (s, 3H, CH_3), 3.38 (br s, 2H, α-H), 3.39 (s, 1H, β-H), 7.10−7.13 (m, 3H, 2,6,4′-Har), 7.16−7.20 (m, 2H, 3′, 5′-Har), 7.28−7.30 (m, 2H, 3,5-Har), 7.33−7.35 (m, 2H, 2, 6-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 28.8 (3,5,7-C), 36.8 (2,8,9-C), 41.0 (1,4,6,10-C), 46.0 (CH_3), 53.0 (2,6-Cp), 55.1 (3,5-Cp), 62.7 (α-C), 66.1 (β-C), 125.9 (4′-Car), 127.8 (2,6-Car), 128.7 (3′,5′-Car), 129.8 (3,5-Car), 130.0 (2′,6′-Car), 135.6 (1-Car), 140.9 (4-Car), 142.2 (1′-Car). Dihydrochloride, mp > 260 °C.

Method B. To a stirred suspension of $LiAlH_4$ (1.0 g, 26 mmol) in anhydrous THF (20 mL) was added dropwise a solution of benzamide **12a** (800 mL, 1.92 mmol) in anhydrous THF (10 mL). The reaction mixture was refluxed for 3 h, then hydrolyzed by adding ethanol, water, and a solution of NaOH (10%) at 0 °C. The inorganic material was filtered, and the filtrate was evaporated. Water was added to the residue, and the resulting mixture was extracted with DCM. The combined

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organic phases were washed with water, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash column chromatography, using as eluent a mixture of $CHCl_3$/MeOH (9:1) to afford 677 mg of a solid product (yield 85%).

1-Ethyl-4-{4-[α-(1-tricyclo[3.3.1.1^{3,7}]decyl)-phenylmethyl]phenylmethyl}pirerazine (2b). Benzylamine **2b** was prepared from amino alcohol **8b** in a similar way to benzylamine **2a**. The product was purified by flash column chromatography, using as eluent a mixture of DCM/MeOH (9:1) to afford amino alcohol **2b** as a solid product (yield 60%). Mp 45−47 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.98−1.02 (t, 3H, A_3X_2, $J_{AX} \approx 7.2$ Hz, CH_3), 1.45−1.57 (m, 12H, 2,4,6,8,9,10-H), 1.86 (br s, 3H, 3,5,7-H), 2.22−2.62 (very br s, 8H, 2,3,5,6-Hp), 2.32−2.37 (q, 2H, A_3X_2, $J_{AX} \approx 7.2$ Hz, CH_2CH_3), 3.39 (s, 3H, α, β-H), 7.08−7.13 (m, 3H, 2,6,4′-Har), 7.28−7.30 (m, 2H, 3′,5′-H), 7.33−7.35 (m, 2H, 2′,6′-Har). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 11.9 (CH_3), 28.8 (3,5,7-C), 36.8 (2,8,9-C), 41.1 (1,4,6,10-C), 52.3 (CH_2CH_3), 52.7 (2,6-Cp), 52.9 (3,5-Cp), 62.7 (α-C), 66.1 (β-C), 125.9 (4′-Car), 127.8 (2′,6′-Car), 128.7 (3′,5′-Car), 130.0 (3,5,2′,6′-Car), 135.5 (1-Car), 141.0 (4-Car), 142.2 (1′-Car). Dihydrochloride, mp > 260 °C (dec) (EtOH−Et_2O).

1-{4-[α-(1-Tricyclo[3.3.1.1^{3,7}]decyl)phenylmethyl]-phenylmethyl}pirerazine (2c). Benzylamine **2c** was prepared from amino alcohol **8c** in a similar way to benzylamine **2a**. The product was purified by flash column chromatography, using as eluent a mixture of DCM/MeOH (7:3) to afford amino alcohol **2c** as a solid product (yield 63%). Mp 63−65 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.45−1.57 (m, 12H, 2,4,6,8,9,10-H), 1.86 (br s, 4H, 3,5,7-H, 4-Hp), 2.32 (br s, 4H, 2,6-Hp), 2.78−2.80 (t, 4H, $J \approx 4.8$ Hz, 3,5-H), 3.36 (s, 2H, α-H), 3.39 (s, 1H, β-H), 7.10−7.13 (m, 3H, 2, 6, 4′-Har), 7.17−7.20 (m, 2H, 3′,5′-Har), 7.28−7.30 (m, 2H, 3,5-Har), 7.34−7.35 (m, 2H, 2′,6′-Har). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 28.8 (3,5,7-C), 36.8 (2,8,9-C), 41.1 (1,4,6,10-C), 48.0 (3,5-Cp), 54.3 (2,6-Cp), 63.3 (α-C), 66.1 (β-C), 125.9 (4′-Car), 127.8 (2,6-Car), 128.7 (3′,5′-Car), 129.8 (3,5-Car), 130.0 (2′,6′-Car), 135.5 (1-Car), 140.9 (4-Car), 142.2 (1′-Car). Dihydrochloride, mp 240 °C (dec) (EtOH−Et_2O).

1-{4-[α-(1-Tricyclo[3.3.1.1^{3,7}]decyl)phenylmethyl]-phenylmethyl}piperidine (2d). *Method A.* Benzylpiperidine **2d** was prepared from amino alcohol **8d** in a similar way to benzylpiperazine **2a**. The product was purified by flash column chromatography, using as eluent a mixture of DCM/MeOH (9:1) to afford amine **2d** as a solid product (yield 87%). Mp 56−57 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.35 (br s, 2H, 4-Hp), 1.45−1.56 (complex m, 16H, 2,4,6,8,9,10-H, 3,5-Hp), 1.86 (br s, 4H, 3,5,7-H, 4-Hp), 2.30 (br s, 4H, 2,6-Hp), 3.36 (s, 2H, β-H), 3.41 (s, 1H, α-H), 7.09−7.14 (m, 3H, 2,6,4′-Har), 7.17−7.21 (m, 2H, 3′,5′-H), 7.29−7.31 (m, 2H, 3,5-Har), 7.34−7.37 (2,6-Har). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 24.3 (4-Cp), 25.8 (3,5-Cp), 28.8 (3,5,7-C), 36.8 (2,8,9-C), 41.1 (1,4,6,10-C), 54.4 (2,6-Cp), 63.4 (β-C), 66.1 (α-C), 125.9 (4′-Car), 127.8 (2,6-Car), 128.8 (3′,5′-Car), 129.8 (3,5-Car), 130.1 (2′,6′-Car), 131.9 (1-Car), 140.8 (4-Car), 142.3 (1′-Car), Hydrochloride, mp 244−246 °C (dec) (EtOH−Et_2O).

Method B. Benzylpiperidine **2d** was also prepared from benzamide **12d** in a similar way to benzylpiperazine **2a**. Yield 87%.

4-Bromobenzenethanol 4-Methylbenzenesulfonate (14). To a stirred solution of *p*-bromophenethyl alcohol (**13**) in a mixture of anhydrous DCM (5 mL) and pyridine (4.7 mL) was added dropwise a solution of tosyl chloride (1.9 g, 10 mmol) in anhydrous DCM (3 mL) at 0 °C. The reaction mixture was stirred at the same temperature for 30 min and then at 4 °C for 12 h. Water was added to the mixture, then acidified with a solution of aqueous HCl (10%) and extracted with DCM. The combined organic phases were washed with water, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash column chromatography, using DCM as eluent to afford 1.65 g of a solid product (yield 90%). Mp 86−88 °C.

1-Methyl-4-(4-bromophenylethyl)piperazine (17a). *Method A.* To a solution of tosylate **14** (1.5 g, 4.2 mmol) in anhydrous THF (15 mL) was added 1-methylpiperazine (2.1 g, 2.1 mmol), and the reaction mixture was refluxed for 8 h. Then the solvent was removed in vacuo, water was added into the residue, and the mixture was extracted with DCM. The combined organic phases were washed with water, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash column chromatography, using as eluent a mixture of DCM/MeOH (9:1) to afford 750 mg of a solid product (yield 63%). Mp 52−53 °C.

Method B. To a stirred solution of 1-methylpiperazine (1g, 10 mmol) in NMP (4 mL) was added dropwise a solution of trifluoroacetic acid (1.14 g, 10 mmol) in NMP (4 mL) at 0 °C. Paraformaldehyde (445 mg, 15 mmol) was added to the reaction mixture, and the resulting suspension was stirred at room temperature for 10 min, then heated to 60 °C for 12 h under an argon atmosphere. After the mixture was cooled to room temperature, a solution of *p*-bromobenzylzinc bromide (8 mL, 0.5M in THF, 4 mmol) was added into the reaction mixture in one portion. The reaction mixture was stirred at room temperature for 20 min. Then ethyl acetate (40 mL) and a saturated solution of Na_2CO_3 were added. The resulting suspension was stirred at room temperature for 30 min. Then the white solid was filtered through a pad of Celite and washed with ethyl acetate. The combined filtrates were washed with a saturated solution of Na_2CO_3 and extracted with 1 N HCl. The acidic layer was washed with ethyl acetate and basified to pH > 10 with a solution of NaOH (50%) in the presence of ethyl acetate. The organic layer was washed with a saturated solution of $NaHCO_3$, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash column chromatography, using as eluent a mixture of DCM/MeOH (9:1) to afford 510 mg of amine **17a** (yield 45% based on *p*-bromobenzylzinc bromide).

1-Ethyl-4-(4-bromophenylethyl)piperazine (17b). *Method A.* Phenethylamine **17b** was prepared from tosylate **14** in a similar way to amine **17a**. Yield 77%. Mp 51−53 °C.

Method B. Phenethylamine **17b** was also prepared from 1-ethylpiperazine trifluoroacetate in a similar way to amine **17a**. Yield 47%.

1-(4-Bromophenylethyl)piperidine (17c). *Method A.* Phenylethylpiperidine **17c** was prepared from tosylate **14** in a similar way to piperazine **17a**. Oily product, yield 79%.

Method B. Phenylethylpiperidine **17c** was also prepared from piperidine hydrochloride in a similar way to amine **17a**. Yield 70%.

α-{4-[2-(4-Methyl-1-piperazinyl)ethyl]phenyl}-α-phenyl-1-tricyclo[3.3.1.1^{3,7}]decanemethanol (19a). To a stirred solution of aryl bromide **17a** (1.2 g, 4.2 mmol) in anhydrous THF (10 mL) was added *tert*-butyllithium (3 mL, 1.7 M solution in hexane, 5 mmol) at −80 °C under an argon atmosphere. The reaction mixture was stirred at the same temperature for 2 h. Then a solution of ketone **5** (1.2 g, 5

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mmol) in anhydrous THF (10 mL) was added dropwise into the mixture, which was stirred for 1 more hour at the same temperature. The reaction mixture was allowed to gradually reach ambient temperature and was stirred at the same temperature for 2 h. The reaction was quenched by adding a saturated solution of NH_4Cl at 0 °C. The organic solvents were removed in vacuo, and water was added to the residue. The mixture was extracted with DCM. The combined organic phases were washed with water, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash column chromatography, using as eluent a mixture of DCM/MeOH (9:1) to give 870 mg of a foamy solid (yield 47%).

α-{4-[2-(4-Ethyl-1-piperazinyl)ethyl]phenyl}-α-phenyl-1-tricyclo[3.3.1.1^{3,7}]decanemethanol (19b). Amino alcohol **19b** was prepared from ketone **5** and aryl bromide **17b** in a similar way as for amino alcohol **19a**. Viscous oil. Yield 45%.

α-{4-[2-(1-Piperidinyl)ethyl]phenyl}-α-phenyl-1-tricyclo[3.3.1.1^{3,7}]decanemethanol (19c). Amino alcohol **19c** was prepared from ketone **5** and aryl bromide **17c** in a similar way as for amino alcohol **19a**. Viscous oil. Yield 48%.

4-[α-(1-Tricyclo[3.3.1.1^{3,7}]decyl)phenylmethyl]-benzenecarboxaldehyde (20). To a stirred solution of aryl bromide **10** (1.6 g, 4.23 mmol) in anhydrous THF (15 mL) was added *n*-butyllithium (2.1 mL, 2.5 M solution in hexane, 5.21 mmol) at −80 °C, under an argon atmosphere. The reaction mixture was stirred at the same temperature for 2 h, and then DMF (1.7 mL) was added to the mixture, which was allowed to gradually reach ambient temperature. The reaction was quenched by adding a solution of HCl (10%) at 0 °C. The organic solvents were removed in vacuo, and water was added to the residue. The mixture was extracted with DCM. The combined organic phases were washed with water, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash column chromatography, using as eluent a mixture of *n*-hexane/Et_2O (5:1) to give 1.07 g of a crystalline product (yield 76.5%). Mp 141−143 °C (ether−*n*-pentane).

4-[α-(1-Tricyclo[3.3.1.1^{3,7}]decyl)phenylmethyl]-benzenemethanol (21). To a stirred suspension of $LiAlH_4$ (700 mg, 18.5 mmol) in anhydrous THF (10 mL) was added dropwise a solution of benzaldehyde **20** (970 mg, 2.94 mmol) in anhydrous THF (10 mL). The reaction mixture was gently refluxed for 2 h, then hydrolyzed by adding ethanol, water, and a solution of NaOH (10%) at 0 °C. The inorganic material was filtered, and the filtrate was evaporated. Water was added to the residue, and the resulting mixture was extracted with ether. The combined organic phases were washed with water, dried over Na_2SO_4, and concentrated in vacuo to give 880 mg of a solid product (yield 90%). Mp 150−151 °C (ether).

4-[α-(1-Tricyclo[3.3.1.1^{3,7}]decyl)phenylmethyl]-phenylmethyl Chloride (22). To a stirred solution of benzyl alcohol **21** (2 g, 6 mmol) in dry ether (10 mL) were added a small amount of anhydrous calcium chloride and then a solution of thionyl chloride (0.8 g, 6.7 mmol) in dry ether (3 mL). Then, calcium carbonate (0.6 g) was added into the reaction mixture, which was stirred at room temperature for 12 h. The inorganic material was filtered out and the combined filtrates were evaporated in vacuo to give a residue (yield almost quantitative), which was used as such in the next step without any further purification. TLC (ether−*n*-pentane) shows one spot.

4-[α-(1-Tricyclo[3.3.1.1^{3,7}]decyl)phenylmethyl]-benzeneacetonitrile (23). *Method A.* To a stirred solution of crude benzyl chloride **22** (1 g, 2.2 mmol) in DMSO (10 mL) was added sodium cyanide (430 mg, 8.8 mmol). The reaction mixture was stirred at room temperature for 12 h under an argon atmosphere. The reaction was quenched with chilled water, and the mixture was extracted with DCM. The combined organic phases were washed with water, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash column chromatography, using as eluent a mixture of *n*-hexane/Et_2O (4:1) to give 650 mg of a crystalline product (yield 86.5%). Mp 168−169 °C (ether).

Method B. To a stirred suspension of potassium *tert*-butoxide (943 mg, 8.4 mmol) in dry DME (5 mL) was added a solution of tosylmethyl isocyanide (TosMIC) (860 mg, 4.4 mmol) in dry DME (5 mL) at −30 °C under an argon atmosphere. The reaction mixture was cooled at −60 °C, and then a solution of aldehyde **20** (1.32 g, 4 mmol) in dry DME (10 mL) was added dropwise into the mixture, which was stirred at the same temperature for 1 more hour. The reaction was controlled with TLC (*n*-pentane/ether, 4:1), and an excess of TosMIC (100 mg) was added in one portion into the mixture, which stirred for 30 more minutes. Absolute methanol (12 mL) was added, and the reaction mixture was allowed to reach room temperature and then heated at 75−80 °C for 30 min. The solvents were removed in vacuo. Water and acetic acid (1 mL) were added into the residue, and the resulting mixture was extracted with DCM. The combined organic phases were washed with water and a saturated solution of $NaHCO_3$, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash column chromatography, using as eluent a mixture of *n*-hexane/Et_2O, 4:1, to give 755 mg of a crystalline product (yield 55%).

Ethyl 4-[α-(1-Tricyclo[3.3.1.1^{3,7}]decyl)phenylmethyl]-benzeneacetate (24). Phenylacetonitrile **23** (1.4 g, 4.1 mmol) was added to a mixture of saturated ethanolic solution of hydrogen chloride (12 mL) and absolute ethanol (8 mL). The reaction mixture was refluxed for 2 h. Then water (8 drops) was added to the mixture, which was heated for 1 more hour. Ethanol was removed in vacuo. Water was added to the residue, and the mixture was extracted with ether. The combined organic phases were washed with water and a saturated solution of Na_2CO_3, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash column chromatography, using as eluent a mixture of *n*-hexane/Et_2O (2:1) to give 1.31 g of a solid product (yield 82%). Mp 81−82 °C.

1-Methyl-4{4-[α-(1-tricyclo[3.3.1.1^{3,7}]decyl)-phenylmethyl]phenylacetyl}pirerazine (25a). To a stirred solution of ethyl ester **24** (670 mg, 1.72 mmol) in ethanol (10 mL), a solution of NaOH (2 g) in a minimum amount of water was added, and the reaction mixture was refluxed for 2 h. Ethanol was removed in vacuo. Water was added to the residue, and the resulting mixture was acidified with an aqueous solution of HCl (10%) at 0 °C. The mixture was extracted with ether, and the organic phase was washed with water, dried over Na_2SO_4, and concentrated in vacuo. Then thionyl chloride (3 mL) was added and the reaction mixture was gently refluxed for 1 h. The excess of thionyl chloride was removed in vacuo with the aid of dry benzene. The residue was dissolved in anhydrous THF (10 mL), and the resulting solution was added dropwise to a solution of 1-methylpiperazine (2 mL) in anhydrous THF (10 mL) at 0 °C. The reaction mixture was stirred at room temperature for 12 h. Then the solvent was removed in vacuo and water was added to the residue. The mixture was extracted with DCM. The combined organic phases were washed with

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water, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash column chromatography, using as eluent a mixture of DCM/MeOH (97:3) to give 480 mg of a sticky product (yield 63%).

1-Ethyl-4{4-[α-(1-tricyclo[3.3.1.1^{3,7}]decyl)-phenylmethyl]phenylacetyl}pirerazine (25b). Amide **25b** was prepared in a similar way as for amide **25a** using ester **24** as starting material. Flash column chromatography, using as eluent a mixture of DCM/MeOH (97:3), gave a sticky solid (yield 60%).

1-{4-[α-(1-Tricyclo[3.3.1.1^{3,7}]decyl)phenylmethyl]-benzeneacetyl}pireridine (25c). Phenylacetylpiperidine **25c** was prepared in a similar way as for amide **25a**, using ester **24** as starting material. Flash column chromatography, using as eluent a mixture of DCM/MeOH (97:3), gave a sticky solid (yield 71%).

1-Methyl-4-{4-[α-(1-tricyclo[3.3.1.1^{3,7}]decyl)-phenylmethyl]phenylethyl}pirerazine (3a). *Method A.* Phenethylamine **3a** was synthesized by reduction of amino alcohol **19a** with triethylsilane/trifluoracetic acid in a similar way to benzylamine **2a** from amino alcohol **8a**. Flash column chromatography, using as eluent a mixture of DCM/MeOH (9:1), gave a glassy solid (yield 63%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.46−1.57 (m, 6H, 2,8,9-H), 1.54 (s, 6H, 4,6,10-H), 1.85 (br s, 3H, 3,5, 7-H), 2.23 (s, 3H, CH_3), 2.30−2.75 (very br s, 8H, 2,3,5,6-Hp), 2.49−2.51 (m, 2H, α-H), 2.66−2.68 (m, 2H, β-H), 3.37 (s, 1H, γ-H), 7.01−7.03 (∼d, 2H, 3,5-Har), 7.07−7.11 (m, 1H, 4′-Har), 7.16−7.19 (m, 2H, 3′,5′-Har), 7.25−7.27 (∼d, 2H, 3,5-Har), 7.32−7.34 (∼d, 2H, 2′,6′-Har). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 28.8 (3,5,7-C), 33.0 (β-C), 36.8 (2,8,9-C), 41.1 (1,4,6,10-C), 45.9 (CH_3), 53.0 (3,5-Cp), 55.0 (2,6-Cp), 60.4 (α-C), 66.0 (γ-C), 126.0 (4′-Car), 127.8 (2,6-Car), 128.2 (3′,5′-Car), 130.0 (3,5-Car), 130.1 (2′, 6′Car), 137.6 (1-Car), 139.9 (4-Car), 142.3 (1′-Car). Dihydrochloride, mp > 260 °C (EtOH−Et_2O).

Method B. Phenethylamine **3a** was also prepared by reduction of phenylacetamide **25a** with the aid of $LiAlH_4$ in a similar way to benzylamine **2a** from benzamide **12a**. Yield almost quantitative.

1-Ethyl-4-{4-[α-(1-tricyclo[3.3.1.1^{3,7}]decyl)-phenylmethyl]phenylethyl}pirerazine (3b). *Method A.* Phenethylamine **3b** was prepared from amino alcohol **19b** in a similar way to phenethylamine **3a**. Flash column chromatography, using as eluent a mixture of DCM/MeOH (8:2), gave a viscous oil (yield 48%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.00−1.04 (t, 3H, A_3X_2, $J_{AX} \approx 7.2$ Hz, CH_3), 1.44−1.55 (m, 6H, 2,8,9-H), 1.54 (br s, 6H, 4,6,10-H), 1.85 (br s, 3H, 3,5,7-H), 2.30−2.72 (very br s, 8H, 2, 3,5,6-Hp), 2.33−2.39 (q, 2H, A_3X_2, $J_{AX} \approx 7.2$ Hz, CH_2CH_3), 2.48−2.52 (m, 2H, α-H), 2.67−2.71 (m, 2H, β-H), 3.37 (s, 1H, γ-H), 7.01−7.03 (∼d, 2H, 2,6-Har), 7.07−7.11 (m, 1H, 4′-Har), 7.15−7.19 (m, 2H, 3′,5′-Har), 7.25−7.27 (∼d, 2H, 3,5-Har), 7.32−7.34 (∼d, 2H, 2′,6′-Har). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 11.9(CH_3), 28.8 (3,5,7-C), 33.1 (β-C), 36.8 (2,8,9-C), 41.1 (1,4,6,10-C), 52.3 (CH_2CH_3), 52.8 (3,5-Cp), 53.1 (2,6-Cp), 60.5 (α-C), 66.0 (γ-C), 125.9 (4′-Car), 127.8 (2,6-Car), 128.2(3′,5′-Car), 130.0 (3,5,2′,6′-Car), 137.8 (1-Car), 139.9 (4-Car), 142.3 (1′-Car). Dihydrochloride, mp > 250 °C (EtOH−Et_2O).

Method B. Phenethylamine **3b** was also prepared by reduction amide **25b** with the aid of LiAlH4 in a similar way to benzylamine **2a**. Yield almost quantitative.

1-{4-[α-(1-Tricyclo[3.3.1.1^{3,7}]decyl)phenylmethyl]-phenylethyl}pireridine (3c). *Method A.* Phenethylpiperidine **3c** was prepared from amino alcohol **19c** in a similar way to phenethylamine **3a**. Flash column chromatography, using as eluent a mixture of DCM/MeOH (9:1), gave a solid product (yield 58%). Mp 127−129 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.35−1.30 (m, 2H, 4-Hp), 1.46−1.56 (complex m, 10H, 2,8,9-H, 3,5-Hp), 1.55 (br s, 6H, 4,6,10-H), 1.86 (br s, 3H, 3,5,7-H), 2.35−2.47 (complex m, 4H, α-H, 2,6-Hp), 2.67−2.71 (m, 2H, β-H), 3.37 (s, 1H, γ-H), 7.01−7.03 (d, 2H, 3, 5-Har), 7.07−7.12 (m, 1H, 4′-Har), 7.14−7.21 (m, 2H, 3′, 5′-Har), 7.25−7.27 (∼d, 2H, 3,5-Har), 7.33−7.34 (∼d, 2H, 2′,6′-Har). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 24.4 (4-Cp), 25.9 (3,5-Cp), 28.8 (3,5,7-C), 33.1 (β-C), 36.5 (2,8,9-C), 41.1 (1,4,6,10-C), 54.5 (2,6-Cp), 61.3 (α-C), 66.0 (γ-C), 125.9 (4′-Car), 127.8 (2,6-Car), 128.2 (3′,5′-Car), 130.0 (3,5,2′,6′-Car), 138.0 (1-Car), 139.8 (4-Car), 142.3 (1′-Car). Hydrochloride, mp > 250 °C (EtOH−Et_2O).

Method B. Phenethylamine **3c** was also prepared by reduction amide **25c** with the aid of $LiAlH_4$ in a similar way to benzylamine **2a**. Yield 97%.

4-[α-(1-Tricyclo[3.3.1.1^{3,7}]decyl)phenylmethyl]-benzenepropanol (26). *Method A.* To a stirred solution of allylmagnesium chloride (2 M) in THF (8 mL, 16 mmol) was added dropwise, under an argon atmosphere, a solution of aryl bromide **10** (1.9 g, 5 mmol) in anhydrous THF (20 mL) in the presence of catalytic amount of copper(I) iodide. The reaction mixture was heated to reflux for 4 h, then quenched by adding a saturated solution of NH_4Cl at 0 °C. The mixture was filtered, and the filtrate was evaporated to remove the solvent. Water was added to the residue, and the mixture was extracted with ether. The combined organic layers were washed with water, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash column chromatography, using cyclohexane as an eluent, to give 1.1 g of a viscous product. ^{1}H NMR analysis showed a mixture of alkene **28** and 1-(α-benzhydryl)-adamantane (**29**) (45:55). To a solution of the above mixture in THF (15 mL) was added a solution of borane (7 mL, 1 M in THF, 7 mmol) slowly at 0 °C under an argon atmosphere. The reaction mixture was stirred at room temperature for 3 h, then quenched by adding chilled water until no foaming was further formed. A solution of NaOH, 10% (1.5 mL), and H_2O_2, 30% (1.5 mL), was added dropwise into the previous mixture. The resulting mixture was heated to 50−60 °C for 1 h. Then the solvent was evaporated and water was added to the residue. The resulting mixture was extracted with ether. The combined organic layers were washed with water, dried over Na_2SO_4, and concentrated in vacuo to give a residue, which was further purified by flash column chromatography, using cyclohexane as an eluent, to give 550 mg of 1-(α-benzhydryl)adamantane (**29**) (yield 36%). Mp 81−83 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.48−1.56 (complex m, 12H, 2,4,6, 8,9,10-H), 2.20 (br s, 3H, 3,5,7-H), 3.40 (s, 1H, α-H), 7.08−7.11 (m, 2H, 4-Har), 7.16−7.20 (m, 4H, 3, 5-Har), 7.34−7.36 (∼d, 4H, 2,6-Har). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 28.8 (3,5,7-C), 30.3 (1-C), 36.8 (2,8,9-C), 41.1 (4,6,10-C), 66.4 (α-C), 126.0 (4-Car), 127.8 (3,5-Car), 130.1 (2,6-Car), 142.2 (1-Car). An amount of 640 mg of crystalline alcohol **26** was obtained (yield 35%) by using a mixture of cyclohexane/ethyl acetate (8:2) as an eluent. Mp 71−73 °C (*n*-pentane).

Ethyl 4-[α-(1-Tricyclo[3.3.1.1^{3,7}]decyl)phenylmethyl]-benzenepropanoate (31). To a stirred solution of aryl bromide **10** (1.92 g, 5 mmol) in triethylamine (10 mL) were added triphenylphosphine (2.62 g, 10 mmol), palladium(II) acetate (112 mg, 0.5 mmol), and ethyl acrylate (1.2 g, 12

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mmol). The reaction mixture was heated to 95−100 °C for 15 h under an argon atmosphere. After cooling, the mixture was filtered through a Celite pad and the precipitate was washed well with water and ethyl acetate. The organic layer was separated, and the inorganic phase was extracted with ethyl acetate. The combined organic layers were washed with water, dried over Na_2SO_4, decolorized with norit, and evaporated. The residue was purified by flash column chromatography to give the unreacted aryl bromide **10** (less polar extract), using *n*-hexane as eluent, and ethyl cinnamate **30**, using a mixture of *n*-hexane/Et_2O (1:1) as eluent. Ethyl cinnamate **30** was obtained in 1.54 g yield (77%) as crystalline product. Mp 109−111 °C. ^{1}H NMR analysis of the corresponding cinnamic acid from ester saponification showed a mixture of (*R*,*E*) and (*S*,*E*) diastereomers. ^{1}H NMR δ (ppm): 1.45−1.55 (very br m, 12H, 2,4,6,8,9,10-H), 1.87 (br s, 3H, 3H, 3,5,7-H), 3.44 (s, 1H, γ-H), 6.30−6.34 and 6.36−6.40 (dd, 2H, $J \simeq 12$ Hz, α-H), 7.12−7.48 (complex dm, 9H, Har), 7.65−7.69 and 7.70−7.74 (dd, 1H, $J \simeq 12$ Hz, β-H). To a solution of ethyl cinnamate **30** in a small portion of ethyl acetate and ethanol (30 mL) was added platinum oxide (200 mg), and the reaction mixture was hydrogenated for 7−8 h under a pressure of 55−60 psi. Then the catalyst was filtered out and the filtrate evaporated in vacuo. The residue was purified by flash column chromatograph, using a mixture of *n*-hexane/Et_2O (4:1) as eluent, to afford 1.4 g of ethyl propionate **31** as a viscous oil, which was crystallized on standing (total yield from aryl bromide **10** 70%). Mp 43−45 °C.

4-[α-(1-Tricyclo[3.3.1.1^{3,7}]decyl)phenylmethyl]-benzenepropanol (26). *Method B.* To a stirred suspension of $LiAlH_4$ (1.08 g, 28.4 mmol) in anhydrous THF (30 mL) was added dropwise a solution of ethyl propanoate **31** (1.14 g, 2.84 mmol) in anhydrous THF (20 mL). The reaction mixture was stirred at room temperature overnight, then hydrolyzed by adding ethanol, water, and a solution of NaOH (10%) at 0 °C. The inorganic material was filtered, and the filtrate was evaporated. Water was added to the residue, and the resulting mixture was extracted with ether. The combined organic phases were washed with water, dried over Na_2SO_4, and concentrated in vacuo to give 1.02 g of alcohol **26** (yield almost quantitative).

α-Phenyl-α-[4-(2-propenyl)pheny]-1-tricyclo-[3.3.1.1^{3,7}]decanemethanol (32). To a stirred solution of *p*-allylphenylmagnesium bromide, which was prepared from magnesium turnings (0.8 g, 0.032 g at) and *p*-allylbromobenzene (5.9 g, 0.03 mol) in anhydrous THF (40 mL) was added dropwise a solution of 1-adamantyl phenyl ketone (**5**) (2.4 g, 0.01 mol) in anhydrous THF (15 mL) under an argon atmosphere. The mixture was heated at 40 °C for 3 h and then quenched by adding a saturated solution of NH_4Cl in an ice bath. The inorganic material was filtered off and washed with THF. The filtrate was evaporated in vacuo, and water was added to the residue. The resulting mixture was extracted with ether. The ethereal extracts were washed with water, dried over Na_2SO_4, and concentrated. The residue was heated at 70 °C under high vacuum to remove the volatiles and then further purified by flash column chromatography using a gradient eluent from *n*-hexane to a mixture of *n*-hexane/Et_2O (1:1) and gave 3.28 g of a waxy solid (yield 91%).

4-[α-Hydroxy-α-(1-tricyclo[3.3.1.1^{3,7}]decyl)-phenylmethyl]benzenepropanol (33). Diol **33** was synthesized by hydroboration of unsaturated alcohol **32** (4.66 g, 0.013 mol) in anhydrous THF (30 mL) with a solution of borane (30 mL, 1 M in THF, 0.030 mol) in a similar way as for alcohol **26** (method A). Flash column chromatography, using as eluent a mixture of cyclohexane/ethyl acetate (3:2), gave 3.18 g of diol **33** as a viscous oil (yield 65%).

4-[α-(1-Tricyclo[3.3.1.1^{3,7}]decyl)phenylmethyl]-benzenepropanol (26). *Method C.* Reduction of diol **33** with triethylsilane in a similar way as for aryl bromide **10** gave propanol **26**. Flash column chromatography, using as eluent a mixture of cyclohexane/ethyl acetate (2:1), afforded propanol **26**. Yield 71%.

1-Methyl-4-{3-[4-[α-(1-tricyclo[3.3.1.1^{3,7}]decyl)-phenylmethyl]phenyl]propyl}piperazine (4a). A solution of propanol **26** (1.19 g, 3.29 mmol) in dry DCM (5 mL) was added dropwise to a stirring mixture of *p*-toluenesulfonyl chloride (1.26 g, 5.29 mmol) in DCM/Py (6 mL, 1:1) at 0 °C under an argon atmosphere. The reaction mixture was stirred at 0 °C for 4 h and then at 10 °C overnight. The above mixture was acidified with a solution of aqueous HCl (1:4) and extracted with DCM. The combined organic phases were washed with water and an aqueous solution of Na_2CO_3, dried over Na_2SO_4, and concentrated in vacuo to afford 1.69 g of a viscous tosyl ester (yield 97%). TLC analysis with a mixture of *n*-hexane/ether (2:1) showed a single spot. To a solution of the intermediate tosyl ester (1.03 g, 2 mmol) in EtOH absolute (10 mL) was added 1-methylpiperazine (2 mL), and the reaction mixture was refluxed for 30 min. Then the solvent was evaporated, water was added to the residue, and the resulting mixture was extracted with DCM. The combined organic phases were washed with water and dried over Na_2SO_4. The residue was purified by flash column chromatography using a mixture of DCM/MeOH (98:2) to afford 204 mg of sulfonamide **27a** as a less polar product. Mp 155−157 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.19 (s, 3H, CH_3N), 2.35 (s, 3H, 4-$CH_3C_6H_4$), 2.39−2.42 (t, 2H, $J \simeq 5$ Hz, 2,6-Hp), 2.95 (br. s, 2H, 3, 5-Hp), 7.24−7.26 (d, 2H, AA′BB′, $J_{AB} = J_{A'B'} \simeq 8.2$ Hz, $J_{AA'} = J_{BB'} \simeq 0$ Hz, 3,5-Har), 7.55−7.57 (d, 2H, AA′BB′, $J_{AB} = J_{A'B'} \simeq 8.2$ Hz, $J_{AA'} = J_{BB'} \simeq 0$ Hz, 2, 6-Har). Piperazine **4a** was eluted as the more polar extract. Yield 490 mg (55%) of a viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.45−1.48 (m, 3H, 2,8,9-Hax), 1.54−1.57 (complex m, 9H, 2,8,9-Heq, 4,6,10-H), 1.66−1.75 (m, 2H, β-H), 1.85 (br s, 3H, 3, 5, 7-H), 2.21 (s, 3H, CH_3), 2.27−2.31 (t, 2H, $J \simeq 7.6$ Hz, α-H), 2.48−2.52 (t, 2H, $J \simeq 7.8$ Hz, γ-H), 2.25−2.65 (very br s, 8H, 2, 3, 5, 6-Hp), 3.37 (s, 1H, δ-H), 6.99−7.01 (d, 2H, $J \simeq 8.1$ Hz, 2, 6-Har), 7.08−7.11 (m, 1H, 4′-Har), 7.16−7.20 (m, 2H, 3′, 5′-Har), 7.24−7.26 (d, 2H, $J \simeq 8.1$ Hz, 3,5-Har), 7.33−7.35 (m, 2H, 2′,6′-Har). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 28.5 (β-C), 28.7 (3,5,7-C), 33.2 (γ-C), 36.8 (2,8,9-C), 41.1 (1,4,6,10-C), 46.0 (CH_3), 53.1 (3,5-Cp), 55.1 (2,6-Cp), 58.1 (α-C), 67.0 (δ-C), 125.9 (4′-Car), 127.8 (2,6,3′,5′-Car), 129.9 (3, 5-Car), 130.0 (2′, 6′-Car), 139.5 (1-Car), 139.6 (4-Car), 142.3 (1′-Car). Dihydrochloride, mp 276 °C (EtOH−Et_2O).

1-Ethyl-4-{3-[4-[α-(1-tricyclo[3.3.1.1^{3,7}]decyl)-phenylmethyl]phenyl]propyl}piperazine (4b). Ethylpiperazine **4b** was prepared from alcohol **26** in a similar way as for piperazine **4a**. The product was purified by flash column chromatography using a mixture of DCM/MeOH (98:2) to afford sulfonamide **27b** (yield 50%) as a less polar product. Mp 43−44 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.94−0.97 (t, 3H, A_3X_2, $J_{AX} \simeq 7.2$ Hz, CH_3CH_2), 2.31−2.35 (q, 2H, A_3X_2, $J_{AX} \simeq 7.2$ Hz, CH_3CH_2), 2.35 (s, 3H, 4-$CH_3C_6H_4$), 2.44 (br s, 2H, 2, 6-Hp), 2.96 (br s, 2H, 3,5-Hp), 7.23−7.24 (d, 2H, AA′BB′, $J_{AB} = J_{A'B'} \simeq 7.8$ Hz, $J_{AA'} = J_{BB'} \simeq 0$ Hz, 3,5-Har), 7.55−

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7.57 (d, 2H, AA′BB′, $J_{AB} = J_{A'B'} \simeq 7.8$ Hz, $J_{AA'} = J_{BB'} \simeq 0$ Hz, 2,6-Har).

Piperazine **4b** was eluted as an oil (yield 45%). ^{1}H NMR δ (ppm): 0.99−1.02 (t, 3H, A_3X_2, $J_{AX} \simeq 7.2$ Hz, CH_3CH_2), 1.46−1.48 (m, 3H, 2,8,9-Hax), 1.55−1.57 (complex m, 9H, 2,8,9-Heq, 4,6,10-H), 1.69−1.74 (m, 2H, β-H), 1.85 (br s, 3H, 3, 5, 7-H), 2.28−2.30 (t, 2H, $J \simeq 8$ Hz, α-H), 2.32−2.35 (q, 2H, A_3X_2, $J_{AX} \simeq 7.2$ Hz, CH_3CH_2), 2.49−2.51 (t, 2H, $J \simeq 7.7$ Hz, γ-H), 2.23−2.55 (very br s, 8H, 2,3,5,6-Hp), 3.37 (s, 1H, δ-H), 6.98−7.00 (d, 2H, $J \simeq 8.1$ Hz, 2,6-Har), 7.07−7.10 (m, 1H, 4′-Har), 7.16−7.18 (m, 2H, 3′,5′-Har), 7.23−7.25 (d, 2H, $J \simeq 8.1$ Hz, 3,5-Har), 7.32−7.34 (m, 2H, 2′, 6′-Har). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 11.9 (CH_3CH_2), 28.5 (β-C), 28.8 (3,5,7-C), 33.2 (γ-C), 36.8 (2,8,9-C), 41.1 (1,4,6,10-C), 52.3 (CH_3CH_2), 52.7 (3,5-Cp), 53.1 (2,6-Cp), 58.1 (α-C), 66.0 (δ-C), 125.8 (4′-Car), 127.7 (2,6-Car), 127.8 (3′,5′-Car), 129.9 (3,5-Car), 130.0 (2′,6′-Car), 139.5 (1-Car), 139.7 (4-Car), 142.3 (1′-Car). Dihydrochloride, mp 250 °C (EtOH−Et_2O).

1-Phenylmethyl-4-{3-[4-[α-(1-tricyclo[3.3.1.1^{3,7}]decyl)-phenylmethyl]phenyl]propyl}piperazine (4c). Ethylpiperazine **4c** was prepared from alcohol **26** in a similar way as for piperazine **4a**. The product was purified by flash column chromatography using a mixture of *n*-hexane/ether (2:1) to afford sulfonamide **27c** (yield 40%) as a less polar product. Mp 48−50 °C. ^{1}H NMR, δ (ppm): 2.35 (s, 3H, CH_3), 2.43−2.45 (t, 4H, $J \simeq 4.7$ Hz, 2,6-Hp), 2.93 (br s, 4H, 3,5-Hp), 3.40 (s, 2H, α-H),7.15−7.18 (m, 5H, 2′,3′,4′,5′,6′-Har), 7.22−7.23 (d, 2H, AA′BB′, $J_{AB} = J_{A'B'} \simeq 8.1$ Hz, $J_{AA'} = J_{BB'} \simeq 0$ Hz, 3, 5-Har), 7.54−7.56 (d, 2H, AA′BB′, $J_{AB} = J_{A'B'} \simeq 8.1$ Hz, $J_{AA'} = J_{BB'} \simeq 0$ Hz, 2,6-Har). Benzylpiperazine **4c** as a semisolid product (yield 55%), ^{1}H NMR, (400 MHz, $CDCl_3$) δ (ppm): 1.45−1.48 (m, 3H, 2,8,9-Hax), 1.53−1.55 (complex m, 9H, 2,8,9-Heq, 4,6,10-H), 1.67−1.75 (m, 2H, β-H), 1.85 (br s, 3H, 3,5,7-H), 2.27−2.31 (t, 2H, $J \simeq 7.7$ Hz, α-H), 2.47−2.51 (t, 2H, $J \simeq 7.8$ Hz, γ-H), 2.22−2.55 (very br s, 8H, 2,3,5,6-Hp), 3.37 (s, 1H, δ-H), 3.43 (s, 2H, ε-H), 6.98−7.00 (d, 2H, $J \simeq 8.1$ Hz, 2,6-Har), 7.07−7.11 (m, 1H, 4′-Har), 7.16−7.19 (m, 3H, 3′,5′,4″-Har), 7.23−7.26 (m, 6H, 3,5,2″,3″,5″,6″-Har), 7.33−7.35 (m, 2H, 2′,6′-Har). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 28.4 (β-C), 28.8 (3,5,7-C), 33.2 (γ-C), 36.8, 36.8 (2,8,9-C), 41.1 (1,4,6,10-C), 52.5 (2,6-Cp), 53.1 (3,5-Cp), 58.1 (α-C), 63.0 (ε-C), 66.0 (δ-C), 125.9 (4′-Car), 127.0 (4″-Car), 127.8 (2,6,3′,5′-Car), 128.2 (3″,5″-Car), 129.2 (2″,6″-Car), 129.9 (3,5-Car), 130.0 (2′,6′-Car), 138.0 (1″-Car),139.5 (1-Car), 139.6 (4-Car), 142.3 (1′-Car). Dihydrochloride·H_2O, mp > 250 °C (EtOH−Et_2O). Dipicrate, mp 237−238 °C (dec) (acetone).

1-{3-[4-[α-(1-Tricyclo[3.3.1.1^{3,7}]decyl)phenylmethyl]-phenyl]propyl}piperidine (4e). Piperidine **4e** was prepared from alcohol **26** in a similar way as for piperazine **4a**. The product was purified by flash column chromatography using DCM as an eluent to afford sulfonamide **27e** (yield 40%) as a less polar product. Mp 51−53 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.31−1.35 (m, 2H, 4-Hp), 1.53−1.57(m, 4H, 3, 5-Hp), 2.35 (s, 3H, CH_3), 2.87−2.89 (t, 4H, $J \simeq 5.5$ Hz, 2, 6-Hp), 7.23−7.25 (d, 2H, AA′BB′, $J_{AB} = J_{A'B'} \simeq 8.3$ Hz, $J_{AA'} = J_{BB'} \simeq 0$ Hz, 3,5-Har), 7.54−7.56 (d, 2H, AA′BB′, $J_{AB} = J_{A'B'} \simeq 8.3$ Hz, $J_{AA'} = J_{BB'} \simeq 0$ Hz, 2,6-Har). Piperidine **4e** was eluted as an oil (yield 55%) using a mixture of DCM/MeOH (98:2) as eluent. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.33−1.34 (m, 2H, 4-Hp), 1.43−1.62 (very br m, 16H, 2, 4, 6, 8,9, 10-H, 3, 5-Hp), 1.69−1.74 (m, 2H, β-H), 1.84 (br s, 3H, 3, 5, 7-H), 2.23−2.35 (m, 6H, α-H, 2,6-Hp), 2.45−2.49 (t, 2H, $J \simeq 7.7$ Hz, γ-H), 3.36 (s, 1H, δ-H), 6.97−6.99 (d, 2H, $J \simeq 8.1$ Hz, 2, 6-Har), 7.07−7.10 (m, 1H, 4′-Har), 7.14−7.18 (m, 2H, 3′,5′-Har), 7.22−7.24 (d, 2H, $J \simeq 8.1$ Hz, 3,5-Har), 7.32−7.33 (∼d, 2H, $J \simeq 7.6$ Hz, 2′,6′-Har). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 24.4 (4-Cp), 25.8 (3, 5-Cp), 28.3 (β-C), 28.8 (3,5,7-C), 33.3 (γ-C), 36.8 (2, 8,9-C), 41.1 (1,4,6,10-C), 54.5 (2, 6-Cp), 58.9 (α-C), 66.0 (δ-C), 125.9 (4′-Car), 127.8 (2,6,3′,5′-Car), 129.9 (3,5-Car), 130.0 (2′,6′-Car), 139.5 (1-Car), 139.7 (4-Car), 142.4 (1′-Car). Hydrochloride, mp 230−232 °C (dec) (EtOH−Et_2O).

1-{3-[4-[α-(1-Tricyclo[3.3.1.1^{3,7}]decyl)phenylmethyl]-phenyl]propyl}piperazine (4d). To a stirred suspension of *N*-benzyl derivative **4c** (800 mg, 1.5 mmol) and 10% palladium on charcoal (800 mg) in methanol (20 mL) was added ammonium formate (480 mg, 7.5 mmol) all at once. The reaction mixture was refluxed under argon for 1.5 h. The reaction was monitored by TLC analysis. After consumption of the starting material, the mixture was cooled to room temperature and the catalyst was removed by filtration and washed with chloroform (20 mL). The filtrate was concentrated in vacuo and the residue was purified by flash column chromatography using a mixture of DCM/MeOH (5:1) as eluent to afford 420 mg of piperazine derivative **4d** in 64% yield as a viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.44−1.47 (m, 3H, 2,8,9-Hax), 1.53−1.56 (complex m, 9H, 2,8,9-Heq, 4,6,10-H), 1.65−1.73 (m, 2H, β-H), 1.84 (br s, 3H, 3,5,7-H), 2.25−2.28 (t, 2H, $J \simeq 7.7$ Hz, α-H), 2.35 (br s, 4H, 2,6-Hp), 2.47−2.51 (t, 2H, $J \simeq 7.8$ Hz, γ-H), 2.82−2.84 (t, 4H, $J \simeq 5$ Hz, 3,5-Hp), 2.88 (br s, 1H, NH), 3.37 (s, 1H, δ-H), 6.97−7.00 (d, 2H, $J \simeq 8.1$ Hz, 2,6-Har), 7.06−7.10 (m, 1H, 4′-Har), 7.15−7.19 (m, 2H, 3′,5′-Har), 7.23−7.25 (d, 2H, $J \simeq 8.1$ Hz, 3,5-Har), 7.32−7.34 (m, 2H, 2′,6′-Har). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 28.2 (β-C), 28.8 (3,5,7-C), 33.2 (γ-C), 36.8 (2,8,9-C), 41.1 (1,4,6,10-C), 45.7 (3,5-Cp), 53.9 (2,6-Cp), 58.5 (α-C), 66.0 (δ-C), 125.9 (4′-Car), 127.8 (2,6,3′,5′-Car), 129.9 (3,5-Car), 130.0 (2′,6′-Car), 139.5 (1-Car), 139.6 (4-Car), 142.3 (1′-Car). Monofumarate, mp 225−227 °C (dec) (EtOH−Et_2O). Dipicrate, mp 230−233 °C (dec) (acetone).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

IR and NMR characterization data of all compounds but finals and elemental analysis data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

DCM, dichloromethane; EtOH, ethanol; MeOH, methanol; Et_2O, diethyl ether; DMSO, dimethylsulfoxide; Py, pyridine; THF, tetrahydrofuran; TFA, trifluoroacetic acid; TosMIC, tosylmethyl isocyanide; NMR, nuclear magnetic resonance;

NMP, *N*-methyl-2-pyrrolidone; NBS, *N*-bromosuccinimide; Et_3SiH, triethylsilane; TCA, trichloroacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; BCA, bicinchoninic acid; SRB, sulforhodamine B; HUVEC, human umbilical vein endothelial cell; hMSC, human mesenchymal stem cell; NHDF, normal human dermal fibroblast; SCID, severe combined immune deficiency; 5FU, 5-fluorouracil; GBP, gabapentin; Gem, gemcitabine; Ara, aracytin

REFERENCES

(1) Megalizzi, V.; Le Mercier, M.; Decaetsecker, C. Sigma receptors and their ligands in cancer biology: overview and new perspectives for cancer therapy. *Med. Res. Rev.* **2012**, *32* (2), 410−427.

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

(3) Van Waarde, A.; Rybczynska, A. A.; Ramakrishnan, N.; Ishiwata, K.; Elsinga, P. H.; Dierckx, R. A. Sigma receptors in oncology: therapeutic and diagnostic applications of sigma ligands. *Curr. Pharm. Des.* **2010**, *16* (31), 3519−3537.

(4) Tsai, S. Y.; Hayashi, T.; Mori, T.; Su, T. P. Sigma-1 receptor charepones and diseases. *Cent. Nerv. Syst. Agents Med. Chem.* **2009**, *9* (3), 184−189.

(5) Bowen, D. W. Sigma receptors: recent advances and new clinical potentials. *Pharm. Acta Helv.* **2000**, *74*, 211−218.

(6) Bem, T. W.; Thomas, E. G.; Mamone, Y. J.; Homan, M. S.; Levy, K. B.; Johnson, E. F.; Coscia, J. C. Overexpression of sigma receptors in nonneural human tumors. *Cancer Res.* **1991**, *51*, 6558−6562.

(7) Vilner, B. J.; John, C. S.; Bowen, W. D. Sigma-1 and sigma-2 receptors are expressed in a wide variety of human and rodent tumor cell lines. *Cancer Res.* **1995**, *55*, 408−413.

(8) John, C. S.; Vilner, B. J.; Schwartz, A. M.; Bowen, W. D. Characterization of sigma receptor binding sites in human biopsied solid breast tumors. *J. Nucl. Med.* **1996**, *37*, 267P.

(9) Zamora, P. O.; Moody, T. W.; John, C. S. Increased binding to sigma sites of *N*-[1′(2-piperidinyl)ethyl-4-[I-125]-iodobenzamide (I-125-PAB) with onset of tumor cell proliferation. *Life Sci.* **1998**, *63*, 1611−1618.

(10) Wang, B.; Rouzier, R.; Albarracin, C. T.; Sahin, A.; Wagner, P.; Yang, Y.; Smith, T. L.; Meric-Bernstam, F.; Aldaz, C. M.; Hortobagyi, G. N.; Puszta, L. Expression of sigma-1 receptors in human breast cancer. *Breast Cancer Res. Treat.* **2004**, *87* (3), 205−214.

(11) Aydar, E.; Onganer, P.; Perrett, R.; Djamgoz, M. B.; Palmer, C. P. The expression and functional characterization of sigma (sigma) 1 receptors in breast cancer cell lines. *Cancer Lett.* **2006**, *242*, 245−257.

(12) Colabufo, N. A.; Berardi, F.; Contino, M.; Ferorelli, S.; Niso, M.; Perrone, R; Paqliarulo, A.; Saponaro, P.; Paqliarulo, V. Correlation between σ2 receptor protein expression and histopathologic grade in human bladder cancer. *Cancer Lett.* **2006**, *237*, 83−88.

(13) Tucci, M.; Quatraro, C.; Dammacco, F.; Silvestris, F. Role of active drug transporters in refractory multiple myeloma. *Curr. Top. Med. Chem.* **2009**, *9*, 218−224.

(14) Martin, R. W.; Eades, G. C.; Thompson, E. R.; Huppler, E. P.; Gilbert, E. P. 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.

(15) Hellewell, S. B.; Bruce, A.; Feinstein, J.; 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.

(16) 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.

(17) Yang, S.; Bhardwaj, A.; Cheng, J.; Alkayed, N. J.; Hurn, P. D.; Kirsch, J. R. Sigma receptor agonists provide neuroprotection in vitro by preserving bcl-2. *Anesth. Analg.* **2007**, *104*, 1179−1184.

(18) Smith, S. B.; Duplantier, J.; Dun, Y.; Mysona, B.; Roon, P.; Martin, P. M.; Ganapathy, V. In vivo protection against retinal neyrodegeneration by sigma receptor 1 ligand (+)-pentazocine. *Invest. Ophthalmol. Visual Sci.* **2008**, *49* (9), 4154−4161.

(19) Villard, V.; Espallergues, J.; Keller, E.; Alkam, T.; Nitta, A.; Yamada, K.; Nabeshima, T.; Vamvakides, A.; Maurice, T. Antiamnesic and neuroprotective effects of the aminotetrahydrofuran derivative ANAVEX1-41 against amyloid beta(25-35)-induced toxicity in ice. *Neuropsychopharmacology* **2009**, *34*, 1552−1566.

(20) Villard, V.; Espallergues, J.; Keller, E.; Vamvakides, A.; Maurice, T. Anti-amnesic and neuroprotective potentials of the mixed muscarinic receptor/sigma 1 (σ1) ligand ANAVEX2-73, a novel aminotetrahydrofuran derivative. *J. Psychopharmacol.* **2011**, *25* (8), 1101−1117.

(21) Vilner, B. J.; De Costa, B. R.; Bowen, W. D. Cytotoxic effects of sigma ligands: sigma receptor-mediated alterations in cellular morphology and viability. *J. Neurosci.* **1995**, *15*, 117−134.

(22) Crawford, K. W.; Bowen, W. D. Sigma-2 receptor agonists activate a novel apoptotic pathway and potentiate antineoplastic drugs in breast tumor cell lines. *Cancer Res.* **2002**, *62*, 313−322.

(23) Colabufo, N. A.; Berardi, F.; Contino, M.; Niso, M.; Abate, C.; Perrone, R.; Tortorella, V. Antiproliferative and cytotoxic effects of some σ2 agonists and σ1 antagonists in tumour cell lines. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2004**, *370*, 106−113.

(24) Spruce, B. A.; Campbell, L. A.; McTavish, N.; Cooper, A.; Appleyard, M. V. L; O'Neil, M.; Howie, J.; Samson, J.; Watt, S.; Murray, K.; McLean., D.; Leslie, N. R.; Safrany, S. T.; Ferguson, M. J.; Peters, J. A.; Prescott, A. R.; Box, G.; Hayes, A.; Nutley, B.; Raynand, F.; Downes, C. P.; Lambert, J. J.; Thompson, A. M.; Eccles, S. Small molecule antagonists of the σ-1 receptor cause selective release of the death program in tumor and self-reliant cells and inhibit tumor growth in vitro and in vivo. *Cancer Res.* **2004**, No. 64, 4875−4886.

(25) Ostenfeld, M. S.; Fehrenbacher, N.; Høyer-Hansen, M.; Thomsen, C.; Farkas, T.; Jäätelä, M. Effective tumor cell death by sigma-2 receptor ligand siramesine involves lysosomal leakage and oxidative stress. *Cancer Res.* **2005**, *65*, 8975−8983.

(26) Azzariti, A.; Colabufo, N. A.; Berardi, F.; Porcelli, L.; Niso, M.; Simone, G. M.; Perrone, R.; Paradiso, A. Cyclohexylpiperazine derivative PB28, a sigma2 agonist and sigma1 antagonist receptor, inhibits cell growth, modulates P-glycoprotein, and synergizes with anthracyclines in breast cancer. *Mol. Cancer Ther.* **2006**, *5*, 1807−1816.

(27) Megalizzi, V.; Mathieu, V.; Mijatovic, T.; Gailly, P.; Debeir, O.; De Neve, N.; Van Damme, M.; Bontempi, G.; Haibe-Kains, B.; Decaestecker, C.; Kondo, Y.; Kiss, R.; Lefranc, F. 4-IBP, a sigma1 receptor agonist, decreases the migration of human cancer cells, including glioblastoma cells, in vitro and sensitises them in vitro and in vivo to cytotoxic insults of proapoptotic and proautophagic drugs. *Neoplasia* **2007**, *9*, 358−369.

(28) Kashiwagi, H.; McDunn, J. E.; Simon, P. O., Jr; Goedegebuure, P. S.; Xu, J.; Jones, L.; Chang, K.; Johnston, F.; Trinkaus, K.; Hotchkiss, R. S.; Mach, R. H.; Hawkins, W. G. Selective sigma-2 ligands preferentially bind to pancreatic adenocarcinomas: applications in diagnostic imaging and therapy. *Mol. Cancer* **2007**, *6*, 48−60.

(29) Piergentili, A.; Amantini, C.; Del Bello, F.; Giannella, M.; Mattioli, L.; Palmery, M.; Perfurmi, M.; Pigini, M.; Santoni, G.; Tucci, P.; Zotti, M.; Quaglia, W. Novel highly potent and selective sigma 1 receptor antagonists related to spipethiane. *J. Med. Chem.* **2010**, *53* (3), 1261−1269.

(30) Hornick, J. R.; Xu, J.; Vangveravong, S.; Tu, Z.; Mitchem, J. B.; Spitzer, D.; Goedegebuure, P.; Mach, R. H.; Hawkins, W. G. The novel sigma-2 receptor ligand SW43 stabilizes pancreas cancer progression in combination with gemcitabine. *Mol. Cancer* **2010**, *22* (9), 298.

(31) Riganas, S.; Papanastasiou, I.; Foscolos, G. B.; Tsotinis, A.; Bourguignon, J. J.; Serin, G.; Mirjolet, J. F.; Dimas, K.; Kourafalos, V. N.; Eleutheriades, A.; Moutsos, V. I.; Khan, H.; Georgakopoulou, S.; Zaniou, A.; Prassa, M.; Theodoropoulou, M.; Pondiki, S.; Vamvakides, A. Synthesis, σ(1), σ(2)-receptors binding affinity and antiproliferative action of new C1-substituted adamantanes. *Bioorg. Med. Chem.* **2012**, *20* (10), 3323−3331.

(32) Renaudo, A.; Watry, V.; Chassot, A. A.; Ponzio, G.; Ehrenfeld, J.; Soriani, O. Inhibition of tumor cell proliferation by σ ligands is

associated with K^+ channel inhibition and p27kip1 accumulation. *J. Pharmacol. Exp. Ther.* **2004**, *311*, 1105−1114.
(33) Renaudo, A.; L' Hoste, S.; Guizouarn, H.; Borgese, F.; Soriani, O. Cancer cell cycle modulated by a functional coupling between sigma-1 receptors and Cl^- channels. *J. Biol. Chem.* **2007**, *282*, 2259−2267.
(34) Johannessen, M.; Ramachandran, S.; Riemer, L.; Ramos-Serrano, A.; Ruoho, A. E.; Jackson, M. B. Voltage-gated sodium channel modulation by sigma-receptors in cardiac myocytes and heterologous systems. *Am. J. Physiol.: Cell Physiol.* **2009**, *296*, 1049−1057.
(35) Johannessen, M.; Fontanilla, D.; Mavlyutov, T.; Ruoho, A. E.; Jackson, M. B. Antagonist action of progesterone at σ-receptors in the modulation of voltage-gated sodium channels. *Am. J. Physiol.: Cell Physiol.* **2011**, *300* (2), C328−37.
(36) Palmer, C. P.; Mahen, R.; Schnell, E.; Djamgoz, M. B. A.; Aydar, E. Sigma-1 receptors bind cholesterol and remodel lipid rafts in breast cancer cell lines. *Cancer Res.* **2007**, *67*, 11166−11175.
(37) Hayashi, T.; Su, T. P. Cholesterol at the endoplasmic reticulum: roles of the sigma-1 receptor charepone and implications thereof in human diseases. *Subcell. Biochem.* **2010**, *51*, 381−398.
(38) Ding, Y.; Brackenbury, W. J.; Onganer, P. U.; Montano, X.; Porter, L. M.; Bates, L. F.; Djangoz, M. B. Epidermal growth factor upregulates motility of Mat-LyLu rat prostate cancer cells partially via voltage-gated Na^+ channel activity. *J. Cell. Physiol.* **2008**, *215*, 77−81.
(39) Onkal, R.; Djamgoz, M. B. Molecular pharmacology of voltage-gated sodium channel expression in metastatic disease: clinical potential of neonatal Nav1.5 in breast cancer. *Eur. J. Pharmacol.* **2009**, *625*, 206−219.
(40) Jansson, K. H.; Lynch, J. E.; Lepori-Bui, N.; Czymmek, K. J.; Duncan, R. L.; Sikes, R. A. Overexpression of the Vssc-associated CAM, beta-2, enhances LNCaP cell metastasis associated behavior. *Prostate* **2012**, *72* (10), 1080−1092.
(41) 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* (15), 8072−8077.
(42) Cobos, E. J.; Entrena, J. M.; Nieto, F. R.; Cendan, C. M.; Del Pozo, E. Pharmacology and therapeutic potential of sigma(1) receptor ligands. *Curr. Neuropharmacol.* **2008**, *6* (4), 344−366.
(43) Ablordeppey, S. Y.; Fischer, J. B.; Law, H.; Glennon, R. A. Probing the proposed phenyl-A region of the sigma-1 receptor. *Bioorg. Med. Chem.* **2002**, *10*, 2759−2765.
(44) Ganapathy, V.; Ganapathy, M. E.; Inoue, K. In *Sigma Receptors: Chemistry in Cell Biology and Clinical Implications*; Matsumoto, R. R., Bowen, W. D., Su, T.-P., Eds.; Springer: New York, 2007; pp 99−112.
(45) Papaconstantinou-Garoufalias, S.; Foscolos, G. B.; Costakis, E. Adamantane analogs of diphenhydramine. *Chim. Chron., New Ser.* **1984**, *13*, 225−237.
(46) Stetter, H.; Rauscher, E. Uber verbindungen mit urotropin-struktur XVII. Zur kenntnkder adamantan-carbosaure-(I). *Chem. Ber.* **1960**, *93*, 1161−1166.
(47) Ku, Y.-Y.; Grieme, T.; Pu, Y. M.; Bhatia, A. V.; King, S. A. A simple one-pot procedure for the imminium salt formation: an efficient route to [beta]-arylethylamines. *Tetrahedron Lett.* **2005**, *46*, 1471−1474.
(48) Oldenziel, O. H.; Van Leusen, D.; Van Leusen, A. M. Chemistry of sulfonylmethyl isocyanides. A general one-step synthesis of nitriles from ketones using tosylmethyl isocyanide. Introduction of a one carbon unit. *J. Org. Chem.* **1977**, *42*, 3114−3118.
(49) Van Leusen, A. M.; Oomkes, P. G. One-step conversion of aldehydes to nitriles. Introduction of a one-carbon unit. *Synth. Commun.* **1980**, *10*, 399−403.
(50) Riganas, S.; Papanastasiou, I.; Foscolos, G. B.; Tsotinis, A.; Dimas, K.; Kourafalos, V. N.; Eleutheriades, A.; Moutsos, V. I.; Khan, H.; Prassa, M.; Georgakopoulou, S.; Zaniou, A.; Theodoropoulou, M.; Mantelas, A.; Pondiki, S.; Vamvakides, A. New adamantane derivatives with sigma affinity and antiproliferative activity. *Med. Chem.* **2012**, *8*, 569−586.
(51) Heck, R. F.; Nolley, J. P., Jr. Palladium-catalyzed vinylic hydrogen substitution reactions with aryl, benzyl and styryl halides. *J. Org. Chem.* **1972**, *37*, 2320−2322.
(52) Mizoroki, T.; Mori, K.; Bulll, O. A. Arylation of olefin with aryliodide catalyzed by palladium. *Chem. Soc. Jpn.* **1971**, *44*, 581−583.
(53) Colabufo, N. A.; Contino, M.; Inglese, C.; Niso, M.; Perrone, R.; Roperto, S.; Roperto, F. In vitro and ex vivo characterization of sigma-1 and sigma-2 receptors: agonists and antagonists in biological assays. *Cent. Nerv. Syst. Agents Med. Chem.* **2009**, *9*, 161−171.
(54) Zeng, C.; Rothfuss, J.; Zhang, J.; Chu, W.; Vangveravong, S.; Tu, Z.; Pan, F.; Chang, K. C.; Hotchkiss, R.; Mach, R. H. Sigma-2 ligands induce tumour cell death by miltiple signalling pathways. *Br. J. Cancer* **2012**, *106* (4), 693−701.
(55) De la Puente, B.; Nadal, X.; Portillo-Salido, E.; Sánchez-Arroyos, R.; Ovalle, S.; Palacios, G.; Muro, A.; Romero, L.; Entrena, J. M.; Baeyens, J. M.; López-García, J. A.; Maldonado, R.; Zamanillo, D.; Vela, J. M. Sigma-1 receptors regulate activity-induced spinal sensitization and neuropathic pain after peripheral nerve injury. *Pain* **2009**, *145* (3), 294−303.
(56) Díaz, J. L.; Zamanillo, D.; Corbera, J.; Baeyens, J. M.; Aldonado, R.; Pericàs, M. A.; Vela, J. M.; Torrens, A Selective sigma-1 (sigma1) receptor antagonists: emerging target for the treatment of neuropathic pain. *Cent. Nerv. Syst. Agents Med. Chem.* **2009**, *9* (3), 172−183.
(57) Cendán, C. M.; Pujalte, J. M.; Portillo-Salido, E.; Montoliu, L.; Baeyens, J. M. Formalin-induced pain is reduced in sigma(1) receptor knockout mice. *Eur. J. Pharmacol.* **2005**, *511* (1), 73−74.
(58) Roh, D. H.; Kim, H. W.; Yoon, S. Y.; Seo, H. S.; Kwon, Y. B.; Kim, K. W.; Han, H. J.; Beitz, A. J.; Na, H. S.; Lee, J. H. Intrathecal injection of the sigma(1) receptor antagonist BD1047 blocks both mechanical allodynia and increases in spinal NR1 expression during the induction phase of rodent neuropathic pain. *Anesthesiology* **2008**, *109* (5), 879−889.
(59) Matsumoto, M.; Inoue, M.; Hald, A.; Xie, W.; Ueda, H. Inhibition of paclitaxel-induced A-fiber hypersensitization by gabapentin. *J. Pharmacol. Exp. Ther.* **2006**, *318* (2), 735−740.
(60) Coderre, T. J.; Vaccarino, A. L.; Melzack, R. Central nervous system plasticity in the tonic pain response to subcutaneous formalin injection. *Brain Res.* **1990**, *535* (1), 155−158.
(61) Laughlin, T. M.; Tram, K. V.; Wilcox, G. L.; Birnbaum, A. K. Comparison of antiepileptic drugs tiagabine, lamotrigine and gabapentin in mouse models of acute, prolonged and chronic nociception. *J. Pharmacol. Exp. Ther.* **2002**, *302* (3), 1168−1175.
(62) Ganapathy, M. E.; Prasad, P. D.; Huang, W.; Seth, P.; Leibach, F. H.; Ganapathy, V. 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.
(63) Bowen, W. D.; De Costa, B. R.; Hellewell, S. B.; Walker, M.; Rice, K. C. [^{3}H](+)-Pentazocine: a potent and highly selective benzomorphan-based probe for sigma-1 receptors. *Mol. Neuropharmacol.* **1993**, *3*, 117−126.
(64) Brown, G. B. ^{3}H-Batrachotoxinin-A benzoate binding to voltage-sensitive sodium channels: inhibition by the channel blockers tetrodotoxin and saxitoxin. *J. Neurosci.* **1986**, *6*, 2064−2070.
(65) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. New coloremetric cytotoxicity assay for anticancer-drug screening. *J. Natl. Cancer Inst.* **1990**, *82* (13), 1107−1112.
(66) Keepers, Y. P.; Pizao, P. E.; Peters, G. J.; Van Ark-Otte, J.; Winograd, B.; Pinedo, H. M. Comparison of the sulforhodamine B protein and tetrazolium (MMT) assays for in vitro chemosensitivity testing. *Eur. J. Cancer* **1991**, *27* (7), 897−900.
(67) Dimas, K.; Hatziantoniou, S.; Tseleni, S.; Khan, H.; Georgopoulos, A.; Alevizopoulos, K.; Wyche, J. H.; Pantazis, P.; Demetzos, C. Sclareol induces apoptosis in human HCT116 colon cancer cells in vitro and suppression of HCT116 tumor growth in immunodeficient mice. *Apoptosis* **2007**, *12* (4), 685−694.
(68) Overholser, J. P.; Prewett, M. C.; Hooper, A. T.; Waksal, H. W.; Hicklin, D. J. Epidermal growth factor receptor blockade by antibody

IMC-C225 inhibits growth of a human pancreatic carcinoma xenograft in nude mice. *Cancer* **2000**, *89* (1), 74−82.