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Design, synthesis and SAR analysis of novel selective σ_1 ligands (Part 2)

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ABSTRACT

In order to investigate the molecular features involved in sigma receptors (σ-Rs) binding, new compounds based on arylalkylaminoalcoholic, arylalkenyl- and arylalkylaminic scaffolds were synthesized and their affinity towards σ_1- and σ_2-Rs subtypes was evaluated. The most promising compounds were also screened for their affinity at μ-opioid, δ-opioid and κ-opioid receptors. Biological results are herein presented and discussed.

1. Introduction

The sigma receptors (σ-Rs) system comprises two subtypes, named sigma1 (σ_1-Rs) and sigma2 (σ_2-Rs). Up to now only the σ_1-R has been purified and cloned[1] and consists of a 26 kDa protein with no homology to any other known mammalian proteins. Both σ-Rs subtypes are expressed in the central nervous system (CNS) as well as in peripheral tissues. Although their exact physiological role is not yet completely understood, their involvement in modulating complex biochemical pathways, as well as several physiopathological events is well documented.

σ_1-Rs have been studied mostly with respect to their functions within the CNS, particularly as putative targets for neuroprotection after ischemia.[2] There is strong pharmacological evidence which indicates that σ_1-Rs, at least in part, act as intracellular amplifiers creating a supersensitive state of signal transduction in biochemical pathways in the CNS.[3] It was also hypothesized that activation of σ_1-Rs can ameliorate Ca^{2+} dysregulation associated with ischemia in cortical neurons.[2] However, the mechanism of neuroprotection for some σ_1-Rs ligands has been controversially discussed because both the σ_1-Rs and the phencyclidine (PCP) binding site of the NMDA receptor channel complex have been reported to contribute to these effects.[2,4] Recently, the σ_1-Rs agonist PPBP (Fig. 1) was proved to exert a neuroprotective effect by a mechanism involving the antiapoptotic protein bcl-2 and sensitive to the σ_1-Rs antagonist rimcazole.[5] Finally, the selective σ_1-Rs agonist SA4503 (Fig. 1), was found to significantly suppress hypoxia/hypoglycaemia-induced neurotoxicity in rat primary neuronal cultures and to protect cultured rat retinal neurons from neurotoxicity induced by glutamate.[6] SA4503 is currently undergoing clinical trials for the treatment of depression and of post-stroke motor dysfunction.

Accumulating evidences suggest that both σ-Rs subtypes regulate cell proliferation and survival. Indeed, cellular transition from quiescent to proliferative status is associated with an increase in σ-Rs expression. Whereas σ_1-Rs promote cell growth and inhibit apoptosis, σ_2-Rs activation by selective as well as non-selective

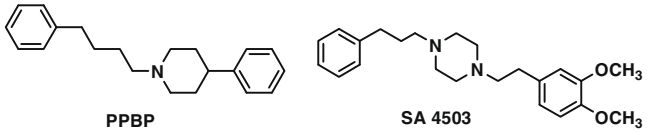

Figure 1. Structures of known σ-Rs ligands.

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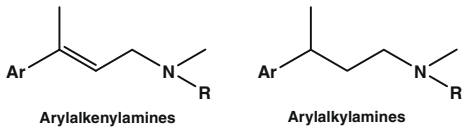


Figure 2. General structures of arylalkenyl- and arylalkylamines.

ligands induces growth arrest and cell death in various cell lines.[7] Normal cells respond to death stimuli by undergoing apoptosis, the best characterized form of programmed cell death. In contrast, cancer cells frequently escape from spontaneous or therapy-induced apoptosis due to acquired mutations in the apoptotic machinery. Therefore, development of novel anticancer drugs able to trigger alternative death pathways, not dependent on apoptosis-regulating genes, is of great interest. σ_2-Rs may represent candidate targets for such drugs.[7] Furthermore, the high densities of both σ-Rs subtypes in many tumour cell lines and tissues suggest their involvement in cancer biology. Accordingly, σ-Rs are now commonly considered to be tumour biomarkers and ligands with high affinity towards both σ-Rs subtypes could have relevant therapeutic and diagnostic applications.[8] Actually, many σ-Rs ligands are developed nowadays for the detection of tumours at early stages through imaging techniques (PET).[9]

Thus, the state of the art suggests that σ-Rs represent exciting targets for the development of novel neuroprotective agents, for the in vivo detection of tumours and their metastasis, as well as for the development of new anticancer drugs. Therapeutic applications of σ-Rs ligands depend on their receptor binding profile: potent and highly selective σ_1-Rs ligands are potential neuroprotective agents and could represent an innovative pharmacological approach for the treatment and prevention of neurodegenerative diseases, on the contrary compounds with high affinity towards both σ-Rs subtypes and high selectivity against the other receptors of the CNS are potentially useful in cancer therapy and diagnosis.

In our previous paper we presented the rational drug design, synthesis and biological evaluation of novel σ-Rs ligands based on arylalkenyl- and arylalkylaminic scaffolds (Fig. 2) and characterized by three sites of molecular diversity: the aromatic nucleus, the aminic moiety and the three carbon spacer between these two structural features.[10] In more details, we carefully evaluated the influence of both the aromatic portion and the aminic moiety on σ-Rs affinity and selectivity. Concerning the three carbon spacer, we focused on molecules characterized by either an olefinic (arylalkenylamines) or an alkylic (arylalkylamines) chain (Fig. 2), according to the σ_1-Rs model developed by us.[10] Compounds with affinity towards σ_1-Rs in the nanomolar range and appreciable selectivity over σ_2-Rs subtype were identified; moreover, moderate σ_2-Rs affinity was also observed for some compounds. Additionally, useful information concerning the structure and properties of the ligand-binding site in the σ_1-Rs was obtained and interesting structure–activity relationships were established for our novel ligands.

In the present paper we address our efforts towards a deeper evaluation of the influence of the three carbon spacer on σ-Rs affinity and selectivity, with the final aim to extend the SAR studies of these chemical classes and also to discover new σ-Rs ligands with improved affinity versus σ_2-Rs subtype. Although an electronegative site on the spacer does not seem to have relevance for conferring affinity towards σ_1-Rs to our class of compounds,[10] this molecular feature seems to be relevant for σ_2-Rs binding. Indeed a σ_2-Rs pharmacophore model recently developed suggested that a hydrogen-bond group is involved in σ_2-Rs interaction.[11] On the basis of this observation, new derivatives characterized by three different spacers (alcoholic, olefinic and alkylic spacers, Series I, II and III compounds, respectively) were designed (Fig. 3). Biphenyl-4-yl and 6-methoxynaphth-2-yl were selected as aromatic nucleus while dimethylamine, *N*-benzyl-*N*-methylamine and piperidine were chosen as aminic moieties (Fig. 3).

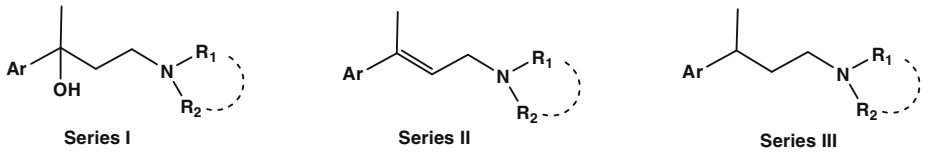
Synthetic procedures, based on Polymer Assisted Solution Phase Synthesis (PASPS) methodology, developed for preparing the designed compounds as well as SAR information on newly synthesized compounds are described in this communication.

2. Chemistry

We started our synthetic approach with the synthesis of β-aminoketone **C**, essentially prepared according to the methodology described in our previous work, with suitable modifications (Scheme 1).[10] The Michael addition of piperidine to but-3-en-2-one was firstly performed in absolute ethanol and glacial acetic acid (see Section 6.1.2.1) yielding compound **C**, for which a purification by fractional distillation was required. Afterwards, the same reaction was carried out in PEG 400 (see Section 6.1.2.2), giving the desired product in a very short time and in higher yield with respect to the procedure previously described, without any further purification.

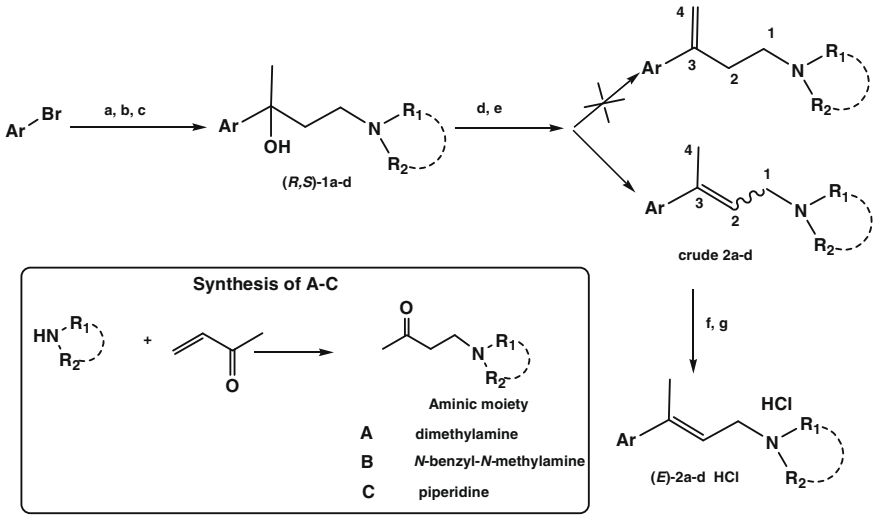
The syntheses of racemic **1a–d** were then accomplished via nucleophilic addition of the appropriate arylic anion to the suitable β-aminoketone, according to the procedure already described by us (Scheme 1).[12] Desired products were obtained in good yields.

Concerning the synthesis of arylalkenylamines **2a–d**, preliminary dehydration experiments of the corresponding arylalkylaminoalcohols under standard acidic conditions (37% HCl or 98% H_2SO_4) led to desired olefinic compounds with unsatisfactory yields and purities. Therefore we considered to take advantage of the triphenylphosphine–iodine system, recently described for the regioselective dehydration of tertiary alcohols.[13] Moreover, to simplify the reaction work-up, polymer-bound triphenylphosphine



Compound			Ar	R_1	R_2
Series I	Series II	Series III			
1a	**2a**	**3a**	6-methoxy-naphth-2-yl	CH_3	CH_3
1b	**2b**	**3b**	6-methoxy-naphth-2-yl	CH_3	$CH_2C_6H_5$
1c	**2c**	**3c**	6-methoxy-naphth-2-yl	$(CH_2)_5$	
1d	**2d**	**3d**	biphenyl-4-yl	CH_3	$CH_2C_6H_5$

Figure 3. Structures of designed arylalkylaminoalcohols (Series I), arylalkenyl (Series II) and arylalkylamines (Series III).



Scheme 1. Synthesis of arylalkylaminoalcohols (*R*/*S*)-**1a–d** and arylalkenylamines (*E*)-**2a–d**. Reagents and conditions: (a) *t*-BuLi, anhydrous Et_2O, −78 °C to rt; (b) ketones A–C, −78 °C to 0 °C; (c) H_2O rt; (d) I_2, TPPP rt; (e) aq 5% $NaHSO_3$ rt; (f) 37% HCl rt; (g) crystallization from acetone.

(TPPP) was employed instead of the conventional reagent (Scheme 1). To the best of our knowledge, the efficacy of the TPPP–iodine system in the dehydration of tertiary aminoalcohols as well as the effects of this system on both regioselectivity and stereoselectivity of the reaction have never been studied before.

Regarding the regioselective behaviour of the reaction, the treatment of (*R*/*S*)-**1a–d** with the TPPP–iodine system afforded exclusively the trisubstituted regioisomers. The ^1H NMR analysis of crude **2a–d** revealed only the formation of a C2–C3 double bond; no signals related to C3–C4 olefinic regioisomers were detectable. Regarding the configuration of the C2–C3 double bond, ^1H NMR analysis of crude **2a–d** showed signals related to both (*E*) and (*Z*) stereoisomers. The main reaction product was the (*E*) stereoisomer for all prepared compounds, as confirmed by NOESY experiments. Indeed, a significant NOE effect corresponding to the interaction correlating the methyl group on the double bond and the olefinic hydrogen was evidenced only for the minor stereoisomer, to which the (*Z*) configuration was assigned (see Fig. 4). As regards the stereoselectivity of the reaction, the (*E*):(*Z*) isomeric ratio was about 75:25 for all prepared compounds, suggesting that it was not significantly affected by the structural properties of both the aromatic nucleus and the aminic portion of the molecule.

Crude **2a–d** were then converted into their respective hydrochlorides and successively crystallized from acetone, yielding pure (*E*)-**2a–d** HCl in satisfactory yields.

Finally, racemic **3a–d** (Fig. 3) were synthesized by catalytic reduction of the corresponding arylalkenylamines in hydrogen atmosphere, applying our already developed protocol with convenient modifications.[10] In these experiments Pd(0) EnCat™ 30NP was used instead of Pd/C (Scheme 2, see Section 6.1.5.1). The reduction protocol was developed and optimized on compound (*E*)-**2a** and then applied to (*E*)-**2b–d**. Among the newly synthesized compounds, the arylalkylamines **3b** and **3d**, characterized by an *N*-benzyl group, could not be prepared in satisfactory yields using the

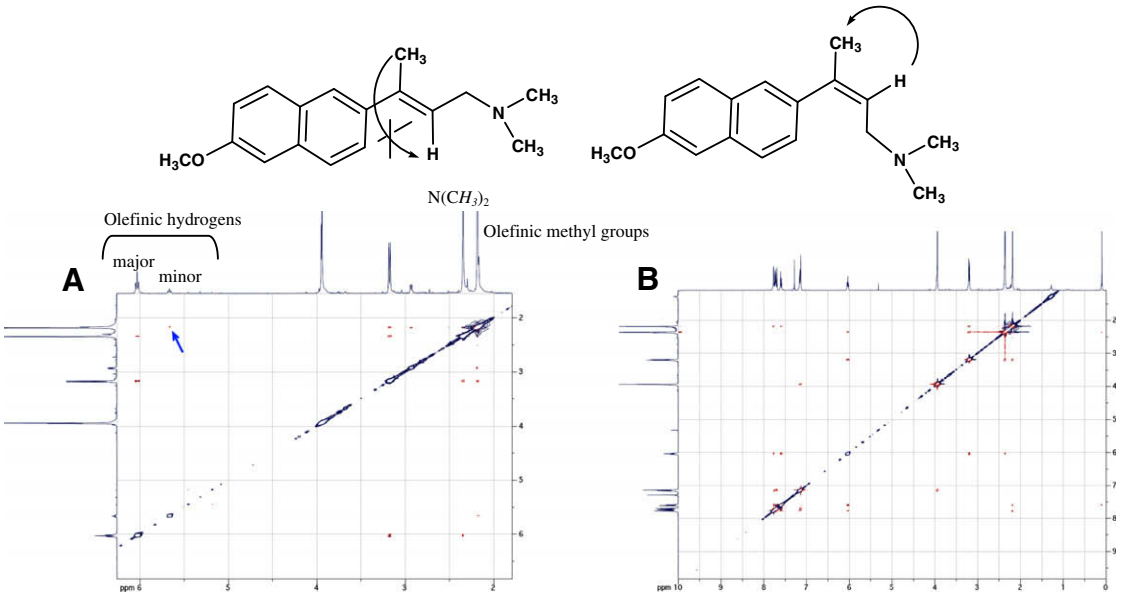
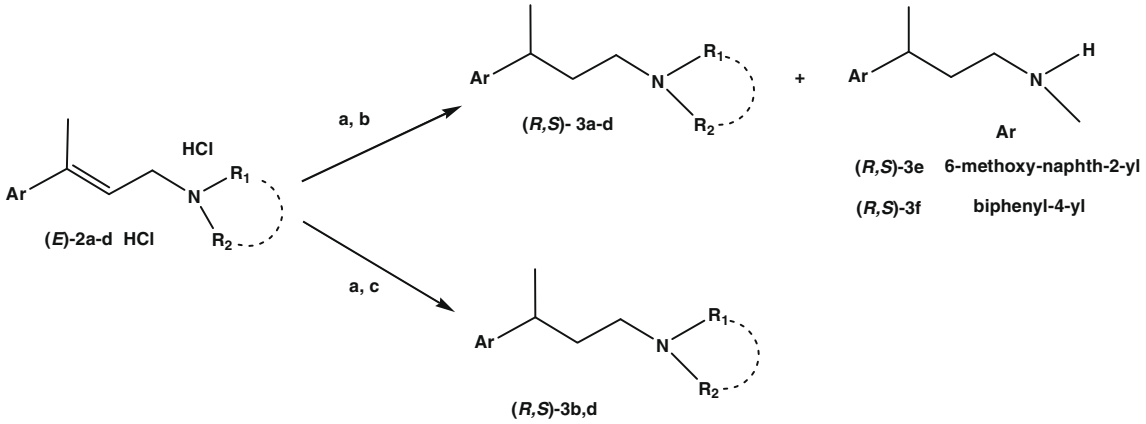


Figure 4. NOESY spectra of crude **2a** (A) and pure (*E*)-**2a** (B). The arrow indicates the NOE effect between the methyl group on the double bond and the olefinic hydrogen of the minor (*Z*) stereoisomer.



Scheme 2. Synthesis of arylalkylamines (*R/S*)-**3a**–**f**. Reagents and conditions: (a) SCX cartridge, (b) H_2, Pd(0) EnCat™ 30NP, abs EtOH, rt (Method I); (c) H_2, Pd(0) EnCat™ 30NP(en), abs EtOH, rt (Method II).

above described procedure. Complete reduction was achieved for both compounds, but the concurrent cleavage of the *N*-benzyl group was observed. Although both target products **3b** and **3d** were isolated from the reaction mixtures by flash chromatography, the corresponding *N*-debenzyl-derivatives **3e** and **3f** (Scheme 2) resulted the main products.

In order to avoid the cleavage of the *N*-benzyl group and to develop a protocol suitable for the synthesis of **3b** and **3d**, our strategy consisted in developing a new poisoned catalyst based on Pd(0) EnCat™ 30NP. Ethylenediamine was used as catalytic poison, according to the procedure already described by Sajiki et al. for the Pd/C catalyst.[14] The new catalyst will be called by now on Pd(0) EnCat™ 30NP(en).

Pd(0) EnCat™ 30NP(en) was firstly experimented on compound (*E*)-**2a** to verify its efficacy in reducing double bonds. ^{1}H NMR analysis of crude **3a** clearly evidenced the effectiveness of Pd(0) EnCat™ 30NP(en) in the hydrogenation of the olefinic precursor. The catalyst was then used for the catalytic hydrogenation of (*E*)-**2b** in hydrogen atmosphere (Scheme 2, see Section 6.1.5.2). Interestingly, applying our optimized protocol compound **3b** was obtained with good crude purity and satisfactory yield (>45%). ^{1}H NMR analysis of crude **3b** revealed the complete hydrogenation of the double bond (absence of the signal related to the olefinic hydrogen) and the presence of one main product in which the *N*-benzyl group is still present (Fig. 5). The identity of this product was then confirmed by both ^{1}H NMR analysis and mass spectroscopy of pure compound, isolated by solid phase extraction (SPE, SCX cartridge) followed by flash chromatography.

The hydrogenation protocol was then successfully applied to (*E*)-**2d**, providing pure **3d** in good yields.

Compounds belonging to both Series I and III, which present one stereogenic centre, have not been resolved prior to perform binding studies, therefore they were assayed as racemates.

3. Receptor binding studies

Sigma receptor binding assays were performed on rat liver membranes according to the methods of Hellewell et al.,[15] slightly modified as previously described.[16] [^{3}H]-(+)-Pentazocine was employed for σ_1-Rs binding assays, whereas the non-selective radioligand [^{3}H]-DTG (1,3-di-2-tolylguanidine) was employed for σ_2-Rs studies in the presence of an excess of non-tritiated (+)-pentazocine (100 nM) to mask σ_1-Rs. In both assays, the non-specific binding was defined in the presence of haloperidol (10 μM).

In order to determine receptor selectivity the most promising compounds were screened for their affinity at μ-opioid, δ-opioid and κ-opioid receptors.[17]

4. Results and discussion

All novel compounds showed interesting binding affinities for σ_1-Rs subtype (K_i values ranging from 1.29 to 38.8 nM, Table 1) and poor σ_2-Rs affinity (K_i values >136 nM, Table 1), with the only exception of compound **1a** which exhibits a negligible affinity for σ_2-Rs subtype (K_i value >10,000 nM, Table 1). All new molecules were mapped onto our 5-points pharmacophore model, which consisted of four hydrophobic and one positive ionizable features.[10] Good fit values were obtained for all compounds belonging to both Series I and Series II. Figure 6 shows compounds (*E*)-**2c** and (*S*)-**3d** mapped to pharmacophore model, exemplifying how it maps the different residues present among our most active compounds. Calculated fit values for the displayed compounds were 3.38 [(*E*)-**2c**] and 4.64 [(*S*)-**3d**], out of a maximum score of 5.0 (perfect fit). The lowest fit values were observed for compounds belonging to Series I. There the matching algorithm in the software appears to penalize the proximity of the polar hydroxyl group to the hydrophobic region, despite the hydroxyl group being well tolerated.

Contrary to our initial aim, in the present study we identified novel potent σ_1-Rs ligands with interesting selectivity against σ_2-Rs subtypes, suggesting that the structural properties of the three carbon spacer between the aromatic nucleus and the aminic moiety do not exert any relevant influence on σ_1-Rs affinity. On the contrary, we found that the structural properties of the three carbon spacer noticeably affect the selectivity over the σ_2-Rs subtype. Indeed Series I and III compounds generally showed selectivity against σ_2-Rs subtype higher than compounds belonging to Series II. Therefore it can be postulated that the alcoholic group on the spacer is not essential for σ_1-Rs binding affinity, in accordance with our σ_1-Rs pharmacophore model, while it appears to be unfavourable for σ_2-Rs interaction—at least for certain substitution patterns—in contrast with the σ_2-Rs pharmacophore recently developed for α-tropanyl derivatives.[11] Among the most interesting compounds in terms of both σ_1-Rs affinity and selectivity over σ_2-Rs subtype, racemic **1b**, **1d**, **3b** and **3d** were selected for a deeper investigation of their binding profiles. In Table 2 their μ-opioid, δ-opioid and κ-opioid receptor affinities are reported. The residual binding of the radioligand is given at a concentration of 1 μM of tested compounds. When a significant inhibition of the radioligand was observed, the K_i value was determined.

Novel compounds generally showed low affinity for μ-opioid and κ-opioid receptors, with the only exception of **3d** (K_i value at μ-opioid receptor = 248 nM) and **3b** (K_i value at κ-opioid receptor = 841 nM). Concerning δ-opioid receptor binding assays, only compounds **1b** and **3b** showed affinities remarkable high, being

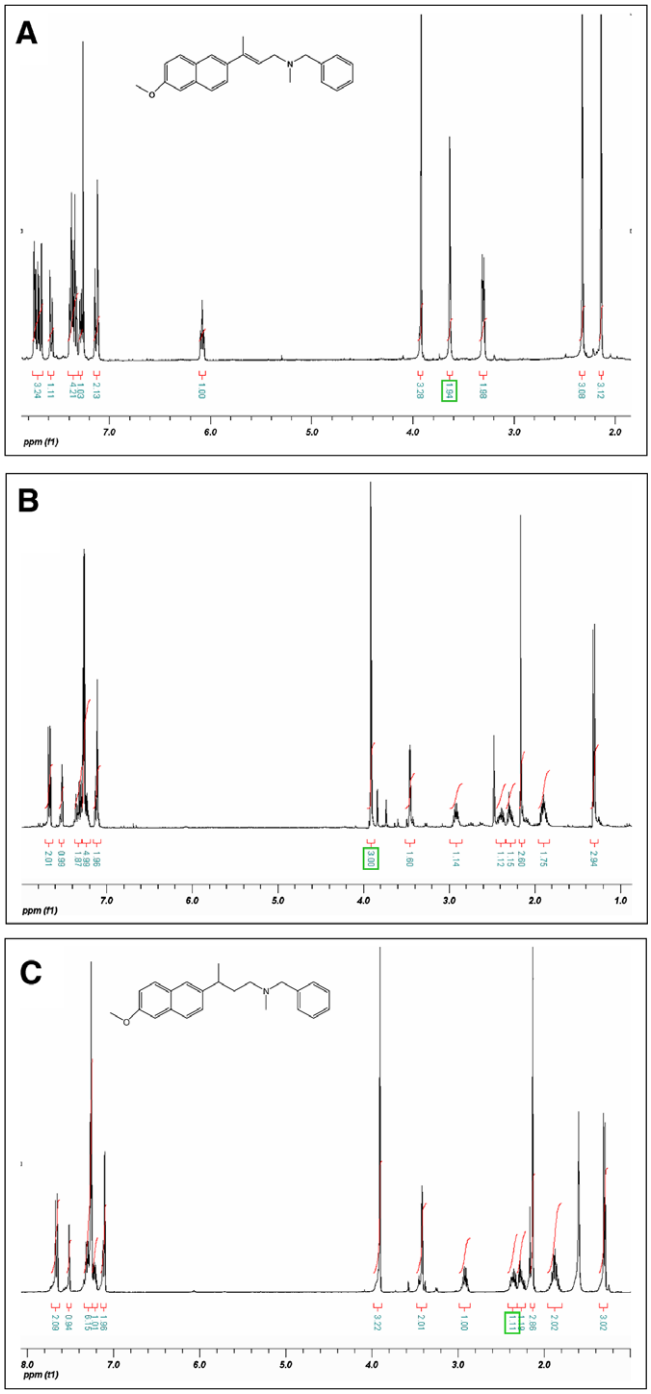


Figure 5. ^{1}H NMR spectra of (*E*)-**2b** (A), crude and pure **3b** (B and C, respectively).

the K_i values 41 and 171 nM, respectively. Interestingly, binding results clearly evidenced that compound **1d** (K_i σ_1 = 2.38 nM, K_i σ_2 = 215 nM) display high selectivity towards μ-opioid, δ-opioid and κ-opioid receptors.

5. Conclusion

Three different series of σ-Rs ligands were synthesized applying PASPS methodology whenever it was possible. In this context a new catalyst suitable for the selective reduction of olefinic bonds in the presence of an *N*-benzyl group was developed using Pd(0) EnCat™ 30NP and ethylenediamine as catalytic poison. Although the use of poisoned Pd/C to reduce olefins in the presence of groups sensitive to hydrogenolytic conditions has been already reported, this is the first time that Pd(0) EnCat™ 30NP(en) is described and its efficacy in the selective reduction of double bonds is demonstrated.

All novel compounds generally showed interesting affinities for σ_1-Rs (K_i values ranging from 1.29 to 40 nM) and poor or negligible σ_2-Rs affinity (K_i values >136 nM). Among the most σ_1-active compounds, racemic **1d** showed a noticeable selectivity towards σ_2-Rs (factor 90) and also an excellent selectivity with respect to μ-opioid, δ-opioid and κ-opioid receptors. Therefore, our current efforts are directed towards a deeper understanding of the role of chirality in affecting σ-Rs ligand binding by resolving the racemic mixtures of **1d** and at evaluating the σ-Rs affinities of pure enantiomers. Finally, we intend to address our attention to clarify the influence of a hydrogen-bond donor group on σ_2-Rs interaction applying a molecular modelling approach.

Table 1
Binding affinities of the prepared compounds towards σ_1- and σ_2-Rs subtypes

Compound	K_i (nM)		
	σ_1[a]	σ_2[b]	σ_2/σ_1
Series I			
1a	38.8 ± 4.5	>10,000	>258
1b	6.04 ± 1.3	940 ± 148	156
1c	10.4 ± 2.4	154 ± 54	15
1d	2.38 ± 0.92	215 ± 105	90
Series II			
(*E*)-**2a**	27.3 ± 2.4	707 ± 111	26
(*E*)-**2b**	13.7 ± 5.52	568 ± 222	41
(*E*)-**2c**	4.41 ± 0.60	136 ± 114	31
(*E*)-**2d**	4.17 ± 1.18	309 ± 116	74
Series III			
3a	14.4 ± 1.1	1548 ± 288	108
3b	2.31 ± 0.82	169 ± 80	73
3c	1.29 ± 0.23	169 ± 105	131
3d	3.59 ± 0.57	214 ± 136	60

Values are means ± SEM of three experiments performed in duplicate.
Haloperidol (10 μM) was used to define non-specific binding.
Series I and Series III compounds were assayed as racemates.
[a] Displacement of 1 nM [^{3}H]-(+)-pentazocine.
[b] Displacement of 3 nM [^{3}H]-DTG in the presence of (+)-pentazocine (100 nM).

6. Experimental

6.1. Chemistry

6.1.1. General

All reagents and solvents were purchased from commercial suppliers and used without any further purification. Anhydrous solvents were obtained according to standard procedures. Pd(0) EnCat™ 30NP (loading = 0.4 mmol/g) was purchased from Sigma–Aldrich. Melting points were determined in open capillaries on SMP3 Stuart Scientific apparatus and are uncorrected. NOESY NMR spectra were recorded on Bruker AVANCE 400 MHz spectrometer using tetramethylsilane as internal standard (δ = 0) and the chemical shift values are expressed in ppm (δ). IR spectra were recorded on a Jasco FT/IR-4100 spectrophotometer; only noteworthy absorptions are given. Reaction courses and the purity of compounds were checked by thin layer chromatography (TLC) on silica gel (Kieselgel 60 F_{254}, Merck) pre-coated glass-backed plates purchased from Fluka and the chromatograms were detected by UV radiations, potassium permanganate and acidic ammonium molybdate(IV). Flash chromatography was performed with Silica Gel 60 (particle size 230–400 mesh) purchased from Nova Chimica. Bond Elut SCX cartridges were purchased from Varian. MS spectra

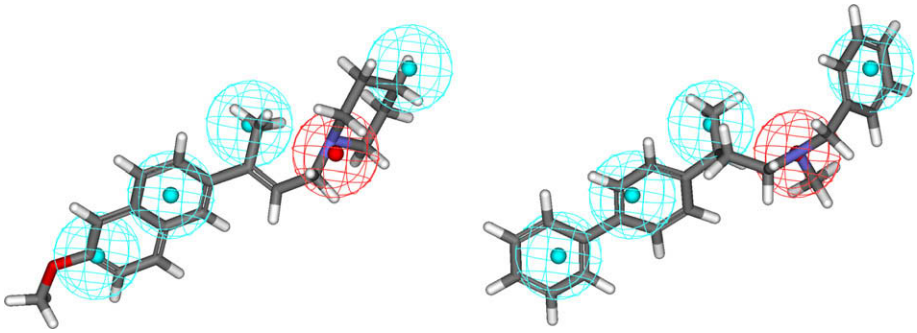


Figure 6. Compounds (*E*)-**2c** (left) and (*S*)-**3d** (right) mapped to our σ_1-Rs pharmacophore model. Cyan spheres: hydrophobic features, red sphere: positive ionizable feature.

Table 2
Affinities of racemic **1b**, **1d**, **3b** and **3d** towards opioid receptors

Compound	μ ([^{3}H]-DAMGO)	δ ([^{3}H]-DPDPE)	κ ([^{3}H]-U 69593)
1b	32%[a]	41 nM	30%[a]
1d	45%[a]	31%[a]	30%[a]
3b	32%[a]	171 nM	841 nM
3d	248 nM	0%[a]	15%[a]

[a] Percent inhibition of the radioligand at a concentration of 1 μM of the test compound.

were recorded on a Waters Micromass ZQ using an APCI ionization source operating in positive ion mode. Elemental analyses (C, H, N) were performed on a Carlo Erba 1106 analyzer and the analysis results were within ±0.4% of the theoretical values.

6.1.2. 4-Piperidin-1-yl-butan-2-one (C)

6.1.2.1. Method a. But-3-en-2-one (30 mL, 370 mmol) was added dropwise to a stirred solution of piperidine (40.6 mL, 410 mmol) and glacial acetic acid (1 mL) in absolute ethanol (63 mL). Reaction mixture was stirred at room temperature for 3 h; the solvent was then removed under reduced pressure, affording crude β-aminoketone **C** (brown oil), which was purified by fractional distillation, yielding the desired product as a yellow oil (yield 46%).

6.1.2.2. Method b. A mixture of piperidine (0.1 mL, 1 mmol), but-3-en-2-one (0.12 mL, 1.5 mmol) and PEG 400 (2.5 g) was stirred at room temperature for 35 min and then 10% HCl was added until pH 2 was reached. The aqueous phase was washed with CH_2Cl_2, made alkaline with 1 N NaOH solution (pH 10) and extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure affording the desired product as yellow oil (yield 62%).

$bp_{6\ mmHg}$ 103 °C. IR (cm^{-1}): 2932, 2852, 2763–2797, 1711, 1442, 1376, 1354, 1301, 1226, 1165, 1152, 1117, 1038, 862, 773–752. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.39 [m, 2H, $N(CH_2CH_2)_2CH_2$], 1.54 [m, 4H, $N(CH_2CH_2)_2CH_2$], 2.12 (s, 3H, CH_3CO), 2.33 [br s, 4H, $N(CH_2CH_2)_2CH_2$], 2.58 (m, 4H, $COCH_2CH_2N$). MS: *m/z* 156.01 [MH^+].

6.1.3. General procedure for the preparation of arylalkylaminoalcohols (*R/S*)-1a–d

t-BuLi (25 mmol, 1.7 M in pentane) was added dropwise to a solution of the appropriate aromatic precursor (12.5 mmol) in anhydrous diethyl ether (50 mL) cooled to −78 °C, under nitrogen atmosphere, keeping the temperature for 20 min. The reaction mixture was then slowly allowed to warm to room temperature. Stirring was continued for 1 h and a solution of the appropriate β-aminoketone (10 mmol) in anhydrous diethyl ether (15 mL) was then added dropwise at −78 °C. The reaction mixture was slowly allowed to warm to 0 °C, stirred for 3 h and then quenched with water (30 mL); after an acid–base work-up, the combined organic phases were evaporated under vacuum. Crude products were purified by flash chromatography, affording the desired compounds.

6.1.3.1. (*R/S*)-4-(Dimethylamino)-2-(6-methoxynaphth-2-yl)butan-2-ol [(*R/S*)-1a]. Flash chromatography mobile phase: hexane–ethyl acetate–7 N NH_3 in methanol (80:20:1.5). Yield: 69%; white solid; mp 98–100 °C. IR (cm^{-1}): 3183, 2969, 2948, 2821, 2779, 1604, 1461, 1261, 1201, 1030, 849, 750. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.48 (s, 3H, CH_3–C), 1.88–1.96 (m, 2H, C–CH_2), 1.98–2.10 (m, 7H, HCH–N + $N(CH_3)_2$), 2.14–2.25 (m, 1H, HCH–N), 3.86 (s, 3H, OCH_3), 5.93 (br s, 1H, O*H*, exchanges with D_2O), 7.13 (dd, 1H, *J* = 2.4 Hz, *J* = 8.9 Hz, aromatic), 7.28 (d, 1H, *J* = 2.2 Hz, aromatic), 7.52 (dd, 1H, *J* = 1.1 Hz, *J* = 8.5 Hz, aromatic), 7.75 (d, 1H, *J* = 8.6 Hz, aromatic), 7.80 (d, 1H, *J* = 8.9 Hz, aromatic), 7.87 (s, 1H, aromatic). MS: *m/z* 274.18 [MH^+].

6.1.3.2. (*R/S*)-4-(Benzyl(methyl)amino)-2-(6-methoxynaphth-2-yl)butan-2-ol [(*R/S*)-1b]. Flash chromatography mobile phase: hexane–ethyl acetate–7 N NH_3 in methanol (90:10:1.5). Yield: 81%; yellow oil. IR (cm^{-1}): 3190, 3026, 2965, 2930, 2836, 2799, 1632, 1604, 1262, 1202, 734, 697. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.48 (s, 3H, CH_3–C), 1.98–2.06 (m, 5H, C–CH_2 + N–CH_3), 2.18–2.28 (m, 1H, HCH–N), 2.33–2.44 (m, 1H, HCH–N), 3.29 (d, 1H, *J* = 13.1 Hz, HCH-Ph), 3.42 (d, 1H, *J* = 13.1 Hz, HCH-Ph), 3.86 (s, 3H, OCH_3), 5.84 (br s, 1H, O*H*, exchanges with D_2O), 7.13 (dd, 1H, *J* = 2.5 Hz, *J* = 8.9 Hz, aromatic), 7.18–7.31 (m, 6H, aromatic), 7.52 (dd, 1H, *J* = 1.8 Hz, *J* = 8.6 Hz, aromatic), 7.73 (d, 1H, *J* = 8.6 Hz, aromatic), 7.78 (d, 1H, *J* = 8.9 Hz, aromatic), 7.86 (d, 1H, *J* = 1.9 Hz, aromatic). MS: *m/z* 350.21 [MH^+].

6.1.3.3. (*R/S*)-2-(6-Methoxynaphth-2-yl)-4-(piperidin-1-yl)butan-2-ol [(*R/S*)-1c]. Flash chromatography mobile phase: hexane–ethyl acetate–7 N NH_3 in methanol (98:2:2). Yield: 46%; white solid; mp 79–86.5 °C. IR (cm^{-1}): 3126, 2965, 2932, 2828, 2789, 1632, 1604, 1481, 1262, 1204, 1121, 851, 808. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.28–1.39 (m, 2H, $N(CH_2CH_2)_2CH_2$), 1.40–1.54 (m, 7H, CH_3–C + $N(CH_2CH_2)_2CH_2$), 1.87–1.98 (m, 2H, C–CH_2), 2.05–2.20 (m, 4H, CH_2–N + $N(CHHCH_2)_2CH_2$), 2.24–2.40 (br, 2H, $N(CHHCH_2)_2CH_2$), 3.86 (s, 3H, OCH_3), 6.20 (br s, 1H, O*H*, exchanges with D_2O), 7.12 (dd, 1H, *J* = 2.5 Hz, *J* = 8.9 Hz, aromatic), 7.27 (d, 1H, *J* = 2.4 Hz, aromatic), 7.51 (dd, 1H, *J* = 1.7 Hz, *J* = 8.5 Hz, aromatic), 7.74 (d, 1H, *J* = 8.6 Hz, aromatic), 7.79 (d, 1H, *J* = 8.9 Hz, aromatic), 7.87 (s, 1H, *J* = 1.8 Hz, aromatic). MS: *m/z* 314.23 [MH^+].

6.1.3.4. (*R/S*)-4-(Benzyl(methyl)amino)-2-(biphenyl-4-yl)butan-2-ol [(*R/S*)-1d]. Flash chromatography mobile phase: hexane–ethyl acetate–7 N NH_3 in methanol (80:20:1.5). Yield: 84%; yellow oil. IR (cm^{-1}): 3186, 3026, 2967, 2923, 2841, 2798, 1736, 1599,

1484, 1452, 1075, 1005, 837, 765, 732. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.42 (s, 3H, CH_3–C), 1.94–2.00 (m, 2H, C–CH_2), 2.06 (s, 3H, N–CH_3), 2.22–2.31 (m, 1H, HC*H*–N), 2.36–2.46 (m, 1H, *H*CH–N), 3.33 (d, 1H, *J* = 12.7 Hz, *H*CH-Ph), 3.45 (d, 1H, *J* = 13.0 Hz, HC*H*-Ph), 5.83 (br s, 1H, O*H*, exchanges with D_2O), 7.20–7.27 (m, 3H, aromatic), 7.28–7.39 (m, 3H, aromatic), 7.43–7.52 (m, 4H, aromatic), 7.54–7.61 (m, 2H, aromatic), 7.63–7.69 (m, 2H, aromatic). MS: *m/z* 346.17 [MH$^+$].

6.1.4. General procedure for the preparation of arylalkenylamines (*E*)-2a–d

Triphenylphosphine polymer-bound (TPPP) (1.5 mmol) was added to a solution of iodine (1.5 mmol) in CH_2Cl_2 (12 mL) and the mixture was stirred at room temperature for 10 min. A solution of the appropriate alcohol (1.0 mmol) in CH_2Cl_2 (5 mL) was then added and the mixture was further stirred at room temperature. Aqueous 5% $NaHSO_3$ was added and the mixture was stirred for 10 min and then filtered through Celite. The organic phase was washed with 1 M NaOH, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure yielding crude **2a–d**, which were converted into the corresponding hydrochlorides and crystallized from acetone, providing pure (*E*)-**2a–d** HCl as white solids. All compounds were characterized as free bases.

6.1.4.1. (*E*)-3-(6-Methoxynaphth-2-yl)-*N*,*N*-dimethylbut-2-en-1-amine [(*E*)-2a]. Yield: 53%; white solid; mp 87.4–88.4 °C. IR (cm^{-1}): 3008, 2956, 2631, 2590, 2487, 1625, 1598, 1482, 1247, 1210, 1030, 851. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.18 (s, 3H, CH_3–C=CH), 2.35 (s, 6H, N(CH_3)$_2$), 3.18 (d, 2H, *J* = 6.7 Hz, CH_2–N), 3.94 (s, 3H, OCH_3), 6.00–6.07 (m, 1H, CH$_3$–C=C*H*), 7.10–7.19 (m, 2H, aromatic), 7.60 (dd, 1H, *J* = 1.7 Hz, *J* = 8.6 Hz, aromatic), 7.70 (d, 1H, *J* = 8.7 Hz, aromatic), 7.74 (d, 1H, *J* = 8.7 Hz, aromatic), 7.77 (d, 1H, *J* = 1.8 Hz, aromatic). MS: *m/z* 256.11 [MH$^+$].

6.1.4.2. (*E*)-*N*-Benzyl-3-(6-methoxynaphth-2-yl)-*N*-methylbut-2-en-1-amine [(*E*)-2b]. Yield: 30%; yellow solid; mp 61.2–63.4 °C. IR (cm^{-1}): 3059, 2955, 2561, 2501, 2409, 1912, 1624, 1599, 1483, 1458, 1206, 1024, 854, 749, 700. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.16 (s, 3H, CH_3–C=CH), 2.32 (s, 3H, N–CH_3), 3.29 (d, 2H, *J* = 6.7 Hz, CH_2–N), 3.62 (s, 2H, CH_2-Ph), 3.95 (s, 3H, OCH_3), 6.07–6.13 (m, 1H, CH$_3$–C=C*H*), 7.11–7.18 (m, 2H, aromatic), 7.26–7.32 (m, 1H, aromatic), 7.33–7.41 (m, 4H, aromatic), 7.60 (dd, 1H, *J* = 1.7 Hz, *J* = 8.6 Hz, aromatic), 7.70 (d, 1H, *J* = 8.7 Hz, aromatic), 7.74 (d, 1H, *J* = 8.7 Hz, aromatic), 7.76 (d, 1H, *J* = 1.8 Hz, aromatic). MS: *m/z* 332.24 [MH$^+$].

6.1.4.3. (*E*)-1-(3-(6-Methoxynaphth-2-yl)but-2-enyl)piperidine [(*E*)-2c]. Yield: 50%; pale yellow solid; mp 91.2–93.1 °C. IR (cm^{-1}): 2935, 2507, 1628, 1600, 1482, 1455, 1203, 1028, 844, 810. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.42–1.55 (m, 2H, N(CH$_2$CH$_2$)$_2$-CH$_2$), 1.61–1.71 (m, 4H, N(CH$_2$CH$_2$)$_2$CH$_2$), 2.17 (s, 3H, CH_3–C=CH), 2.42–2.62 (m, 4H, N(CH$_2$CH$_2$)$_2$CH$_2$), 3.23 (d, 2H, *J* = 6.7 Hz, CH_2–N), 3.94 (s, 3H, OCH_3), 6.04–6.11 (m, 1H, CH$_3$–C=C*H*), 7.10–7.18 (m, 2H, aromatic), 7.58–7.64 (m, 1H, aromatic), 7.70 (d, 1H, *J* = 8.7 Hz, aromatic), 7.73 (d, 1H, *J* = 8.7 Hz, aromatic), 7.77 (d, 1H, *J* = 1.2 Hz, aromatic). MS: *m/z* 296.21 [MH$^+$].

6.1.4.4. (*E*)-*N*-Benzyl-3-(biphenyl-4-yl)-*N*-methylbut-2-en-1-amine [(*E*)-2d]. Yield: 49%; pale yellow solid; mp 67.2–68.3 °C. IR (cm^{-1}): 3032, 2978, 2537, 2488, 2388, 1644, 1486, 1469, 1420, 765, 696. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.11 (s, 3H, CH_3–C=CH), 2.32 (s, 3H, N–CH_3), 3.27 (d, 2H, *J* = 6.7 Hz, CH_2–N), 3.61 (s, 2H, CH_2-Ph), 6.02–6.10 (m, 1H, CH$_3$–C=C*H*), 7.25–7.32 (m, 1H, aromatic), 7.33–7.42 (m, 5H, aromatic), 7.43–7.50 (m, 2H, aromatic), 7.51–7.56 (m, 2H, aromatic), 7.57–7.67 (m, 4H, aromatic). MS: *m/z* 328.17 [MH$^+$].

6.1.5. General procedure for the preparation of arylalkylamines (*R/S*)-3a–f

6.1.5.1. Method I. Before use, Pd(0) EnCat™ 30NP (supplied as a water wet solid with water content 45% w/w) was washed thoroughly with absolute ethanol to remove water. Pre-washed Pd(0) EnCat™ 30NP (0.20 equiv) was added to a stirred solution of the appropriate arylalkenylamine as free base (0.14 mmol) in absolute ethanol (11 mL) and the reaction mixture was left at room temperature in hydrogen atmosphere (balloon) for 30 h. The catalyst was then filtered off and washed with absolute ethanol; the filtrates were loaded on SCX cartridge and eluted with 1 M NH_3 in methanol, the organic phases were finally dried in vacuo. In this way, pure **3a** and **3c** were obtained as yellow oils. Compounds **3b** and **3d**–**f** were isolated from crude residue by flash chromatography.

6.1.5.2. Method II: procedure for the preparation of the arylalkylamines (*R/S*)-3b, 3d. 6.1.5.2.1. *Preparation of Pd(0) EnCat™ 30NP(en)*: Pd(0) EnCat™ 30NP (240 mg, 0.096 mmol of Pd) was stirred with ethylenediamine (67.2 mL of 0.1 M solution in methanol, 6.72 mmol) at room temperature for 48 h and then the catalyst was filtered off, washed with methanol and diethyl ether, dried under a vacuum pump for 48 h and stored under nitrogen.

6.1.5.2.2. *Preparation of compounds (R/S)*-**3b**, 3d: Pd(0) EnCat™ 30NP(en) (0.3 equiv) was added to a stirred solution of the appropriate arylalkenylamine as free base (0.14 mmol) in absolute ethanol (11 mL). The reaction mixture was stirred at room temperature in hydrogen atmosphere (balloon) for 24 h. The catalyst was then filtered off and washed with absolute ethanol; the filtrate was loaded on SCX cartridge and eluted with 1 M NH_3 in methanol and the organic phase was finally dried in vacuo. Desired compounds were finally isolated by flash chromatography.

6.1.5.3. (*R/S*)-3-(6-Methoxynaphth-2-yl)-*N*,*N*-dimethylbutan-1-amine [(*R/S*)-3a]. Yield: 68%; yellow oil. IR (cm^{-1}): 3053, 2954, 2856, 2813, 2762, 1604, 1459, 1262, 1030, 849, 805. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.35 (d, 3H, *J* = 6.9 Hz, CH_3–C), 1.86–2.00 (m, 2H, CH–CH_2), 2.19–2.48 (m, 2H, CH_2–N), 2.30 (s, 6H, N–(CH_3)$_2$), 2.83–2.97 (m, 1H, CH$_3$–C*H*), 3.93 (s, 3H, OCH_3), 7.10–7.20 (m, 2H, aromatic), 7.34 (dd, 1H, *J* = 1.6 Hz, *J* = 8.4 Hz, aromatic), 7.56 (s, 1H, aromatic), 7.68–7.73 (m, 2H, aromatic). MS: *m/z* 258.05 [MH$^+$].

6.1.5.4. (*R/S*)-*N*-Benzyl-3-(6-methoxynaphth-2-yl)-*N*-methylbutan-1-amine [(*R/S*)-3b]. Flash chromatography mobile phase: hexane–ethyl acetate–7 N NH_3 in methanol (80:20:1.5). Yield: 46%; yellow oil. IR (cm^{-1}): 2952, 2925, 2836, 2784, 1633, 1604, 1452, 1263, 1030, 849, 734, 697. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.33 (d, 3H, *J* = 6.9 Hz, CH_3–C), 1.82–1.99 (m, 2H, CH–CH_2), 2.16 (s, 3H, N–CH_3), 2.24–2.34 (m, 1H, HC*H*–N), 2.35–2.45 (m, 1H, *H*CH–N), 2.89–3.02 (m, 1H, CH$_3$–C*H*), 3.45 (AB system, 2H, *J* = 13.0 Hz, CH_2-Ph), 3.94 (s, 3H, OCH_3), 7.11–7.18 (m, 2H, aromatic), 7.22–7.39 (m, 6H, aromatic), 7.55 (s, 1H, aromatic), 7.66–7.74 (m, 2H, aromatic). MS: *m/z* 334.28 [MH$^+$].

6.1.5.5. (*R/S*)-1-(3-(6-Methoxynaphth-2-yl)butyl)piperidine [(*R/S*)-3c]. Yield: 63%; yellow oil. IR (cm^{-1}): 3053, 2928, 2850, 2761, 2058, 1904, 1633, 1604, 1263, 1158, 1032, 848. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.34 (d, 3H, *J* = 6.9 Hz, CH_3–CH), 1.38–1.48 (m, 2H, N(CH$_2$CH$_2$)$_2$CH_2), 1.54–1.64 (m, 4H, N(CH$_2$CH_2)$_2$CH$_2$), 1.84–1.98 (m, 2H, CH–CH_2), 2.13–2.24 (m, 1H, *H*CH–N), 2.25–2.45 (m, 5H, HC*H*–N + N(CH_2CH$_2$)$_2$CH$_2$), 2.80–2.94 (m, 1H, CH$_3$–C*H*), 3.94 (s, 3H, OCH_3), 7.11–7.18 (m, 2H, aromatic), 7.34 (dd, 1H, *J* = 1.6 Hz, *J* = 8.4 Hz, aromatic), 7.56 (s, 1H, aromatic), 7.67–7.73 (m, 2H, aromatic). MS: *m/z* 298.27 [MH$^+$].

6.1.5.6. (*R/S*)-*N*-Benzyl-3-(biphenyl-4-yl)-*N*-methylbutan-1-amine [(*R/S*)-3d]. Flash chromatography mobile phase: hexane–ethyl acetate–7 N NH_3 in methanol (80:20:1.0). Yield: 49%; yellow oil. IR (cm^{-1}): 3059, 3025, 2922, 2851, 2783, 1484, 1451, 1026, 1006, 835, 731, 695. ^1H NMR (400 MHz, $CDCl_3$) δ: 1.30 (d, 3H, J = 6.9 Hz, CH_3–C), 1.79–1.94 (m, 2H, CH–CH_2), 2.19 (s, 3H, N–CH_3), 2.27–2.46 (m, 2H, CH_2–N), 2.82–2.93 (m, 1H, CH_3–CH), 3.48 (s, 2H, CH_2-Ph), 7.23–7.39 (m, 8H, aromatic), 7.42–7.49 (m, 2H, aromatic), 7.51–7.56 (m, 2H, aromatic), 7.59–7.64 (m, 2H, aromatic). MS: m/z 330.24 [MH^+].

6.1.5.7. (*R/S*)-3-(6-Methoxynaphth-2-yl)-*N*-methylbutan-1-amine [(*R/S*)-3e]. Flash chromatography mobile phase: ethyl acetate–methanol–7 N NH_3 in methanol (70:30:2). Yield: 35%; yellow oil. IR (cm^{-1}): 2953, 2922, 2851, 2789, 1604, 1262, 1029, 849, 807. ^1H NMR (400 MHz, DMSO-d_6) δ: 1.26 (d, 3H, J = 6.9 Hz, CH_3–C), 1.68–1.79 (m, 2H, CH–CH_2), 2.20 (s, 3H, N–CH_3), 2.25–2.43 (m, 2H, CH_2–N), 2.84–2.96 (m, 1H, CH_3–CH), 3.27–3.32 (br s, 1H, NH, exchanges with D_2O), 3.86 (s, 3H, OCH_3), 7.10–7.15 (m, 1H, aromatic), 7.25–7.29 (m, 1H, aromatic), 7.34–7.39 (m, 1H, aromatic), 7.61 (s, 1H, aromatic), 7.72–7.78 (m, 2H, aromatic). MS: m/z 244.08 [MH^+].

6.1.5.8. (*R/S*)-3-(Biphenyl-4-yl)-*N*-methylbutan-1-amine [(*R/S*)-3f]. Flash chromatography mobile phase: ethyl acetate–methanol–7 N NH_3 in methanol (70:30:2). Yield: 38%; white solid; mp 178–180 °C. IR (cm^{-1}): 3295, 3025, 2956, 2923, 2868, 2776, 2461, 1597, 1484, 1349, 1006, 763, 696. ^1H NMR (400 MHz, DMSO-d_6) δ: 1.25 (d, 3H, J = 6.9 Hz, CH_3–C), 1.84–1.98 (m, 2H, CH–CH_2), 2.48 (s, 3H, N–CH_3), 2.59–2.70 (m, 1H, HCH–N), 2.75–2.92 (m, 2H, CH_3–CH + HCH–N), 7.32–7.39 (m, 3H, aromatic), 7.42–7.50 (m, 2H, aromatic), 7.59–7.69 (m, 4H, aromatic), 8.46–8.90 (br s, 1H, NH, exchanges with D_2O). MS: m/z 240.10 [MH^+].

6.2. Pharmacology

6.2.1. Sigma radioligand binding assays

Binding assays were performed on rat liver membranes as previously described.[16] For σ_1-Rs assay 250 μg of rat liver homogenate was incubated for 120 min at 37 °C with 1 nM [^3H]-(+)-pentazocine (Perkin–Elmer, specific activity 34.9 Ci/mmol) in 50 mM Tris–HCl, pH 8.0, 0.5 mL final volume. Non-specific binding was defined in the presence of 10 μM haloperidol. The reaction was stopped by vacuum filtration through GF/B glass-fibre filters presoaked with 0.5% polyethylenimine, followed by rapid washing with 2 mL ice-cold buffer. Filters were placed in 3 mL scintillation cocktail and the radioactivity determined by liquid scintillation counting.

For σ_2-Rs assay, 150 μg of rat liver homogenate were incubated for 120 min at room temperature with 3 nM [^3H]-DTG (Perkin–Elmer, specific activity 58.1 Ci/mmol) in 50 mM Tris–HCl, pH 8.0, 0.5 mL final volume. (+)-pentazocine (100 nM) and haloperidol (10 μM) were used to mask σ_1-Rs and to define non-specific binding, respectively.

Competition studies were done using at least 11 different concentrations of the compound under investigation prepared as 10 mM stock solutions in 100% DMSO and diluted with buffer on the day of the experiment. The maximal DMSO final concentration in the incubation tubes was 0.1%.

IC_{50} values and Hill's coefficients n_H were calculated by non-linear regression using a four parameters curve-fitting algorithm of the SigmaPlot software, and are the means ± SEM of three separate determinations performed in duplicate. The corresponding K_i values were obtained by means of the Cheng–Prusoff equation.

6.2.2. Opioid receptor radioligand binding assays

6.2.2.1. Materials and general procedures. The guinea pig brains and rat brains were commercially available (Harlan-Winkelmann, Borchen, Germany). Homogenizer: Elvehjem Potter (B. Braun Biotech International, Melsungen, Germany). Centrifuge: High-speed cooling centrifuge model Sorvall RC-5C plus (Thermo Fisher Scientific, Langenselbold, Germany). Filter: Printed Filtermat Typ A and B (Perkin–Elmer LAS, Rodgau-Jügesheim, Germany), presoaked in 0.5% aqueous polyethylenimine for 2 h at room temperature before use. The filtration was carried out with a MicroBeta FilterMate-96 Harvester (Perkin–Elmer). The scintillation analysis was performed using Meltilex (Typ A or B) solid scintillator (Perkin–Elmer). The solid scintillator was melted on the filtermat at a temperature of 95 °C for 5 min. After solidifying of the scintillator at room temperature, the scintillation was measured using a MicroBeta Trilux scintillation analyzer (Perkin–Elmer). The overall counting efficiency was 20%. All experiments were carried out in triplicates using standard 96-well-multiplates (Diagonal, Muenster, Germany). The IC_{50} values were determined in competition experiments with at least six concentrations of the test compounds and were calculated with the program GRAPHPAD PRISM® 3.0 (GraphPad Software, San Diego, CA, USA) by non-linear regression analysis. The K_i values were calculated according to the formula of Cheng and Prusoff.

6.2.2.2. Preparation of the tissue. Five guinea pig brains (μ- and κ-opioid receptor assay) or six rat brains (δ opioid receptor assay) were homogenized with the potter (500–800 rpm, 10 up-and-down strokes) in six volumes of cold 0.32 M sucrose. The suspension was centrifuged at 1200*g* for 10 min at 4 °C. The supernatant was separated and centrifuged at 23,500*g* for 20 min at 4 °C. The pellet was resuspended in 5-6 volumes of buffer (50 mM Tris, pH 7.4) and centrifuged again at 23,500*g* (20 min, 4 °C). This procedure was repeated twice. The final pellet was resuspended in 5-6 volumes of buffer, the protein concentration was determined according to the method of Bradford using bovine serum albumin as standard, and subsequently the preparation was frozen (−80 °C) in 1.5 mL portions containing about 1.5 mg protein/mL.

6.2.2.3. κ-Opioid receptor binding assay. The test was performed with the radioligand [^3H]-U-69593 (55 Ci/mmol, Amersham, Little Chalfont, UK). The thawed membrane preparation (about 75 μg of the protein) was incubated with various concentrations of test compounds, 1 nM [^3H]-U-69593, and Tris–$MgCl_2$-buffer (50 mM, 8 mM $MgCl_2$, pH 7.4) in a total volume of 200 μL for 120 min at 37 °C. The incubation was terminated by rapid filtration through the presoaked filtermats using a cell harvester. After washing each well five times with 300 μL of water, the filtermats were dried at 95 °C. The bound radioactivity trapped on the filters was counted as described above. The non-specific binding was determined with 10 μM unlabelled U-69593. The K_d-value of U-69593 is 0.69 nM.

6.2.2.4. μ-Opioid receptor binding assay. The test was performed with the radioligand [^3H]-DAMGO (51 Ci/mmol, Perkin–Elmer LAS). The thawed membrane preparation (about 75 μg of the protein) was incubated with various concentrations of test compounds, 3 nM [^3H]-DAMGO, and Tris–$MgCl_2$–PMSF-buffer (50 mM, 8 mM $MgCl_2$, 400 μM PMSF, pH 7.4) in a total volume of 200 μL for 150 min at 37 °C. The incubation was terminated by rapid filtration through the presoaked filtermats using a cell harvester. After washing each well five times with 300 μL of water, the filtermats were dried at 95 °C. The bound radioactivity trapped on the filters was counted as described above. The non-specific binding was determined with 10 μM unlabelled naloxone. The K_d-value of DAMGO is 0.57 nM.

6.2.2.5. δ-Opioid receptor binding assay. The test was performed with the radioligand [^{3}H]-DPDPE (40 Ci/mmol, Perkin–Elmer LAS). The thawed membrane preparation (about 75 μg of the protein) was incubated with various concentrations of test compounds, 1 nM [^{3}H]-DPDPE, and Tris–$MgCl_2$–PMSF-buffer (50 mM, 8 mM $MgCl_2$, 400 μM PMSF, pH 7.4) in a total volume of 200 μL for 150 min at 37 °C. The incubation was terminated by rapid filtration through the presoaked filtermats using a cell harvester. After washing each well five times with 300 μL of water, the filtermats were dried at 95 °C. The bound radioactivity trapped on the filters was counted as described above. The non-specific binding was determined with 10 μM unlabelled morphine. The K_d-value of DPDPE is 0.65 nM.

6.3. Molecular modelling

Computations were performed in Discovery Studio 2.0 (Accelrys Inc., San Diego, CA, USA) on a PC equipped with an Intel Core2Duo 2 × 2.13 GHz processor and 2 GB RAM running Fedora 8 Linux. A set of low-energy conformations for the investigated compounds was computed using the 'best' algorithm with a maximum of 255 conformations per molecule and an energy maximum of 20 kcal/mol above the calculated minimum. The compounds were then mapped to our previously reported σ_1-R pharmacophore model using the 'best' mapping algorithm.[10]

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.12.039.

References and notes

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