European Journal of Medicinal Chemistry 44 (2009) 4306–4314

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis of σ receptor ligands with unsymmetrical spiro connection of the piperidine moiety

Annemarie Jasper [a], Dirk Schepmann [a], Kirstin Lehmkuhl [a], Jose Miguel Vela [b], Helmut Buschmann [b], Jörg Holenz [b], Bernhard Wünsch [a,*]

[a] Institut für Pharmazeutische und Medizinische Chemie, Hittorfstraße 58-62, 48145 Münster, Germany
[b] Esteve, Av. Mare de Deu de Montserrat 221, 08041 Barcelona, Spain

ARTICLE INFO

Article history:
Received 22 January 2009
Received in revised form
13 July 2009
Accepted 16 July 2009
Available online 21 July 2009

Keywords:
σ Receptor ligands
Spirocyclic piperidines
Structure–affinity relationships
Receptor binding studies
Halogen–metal exchange
Transmetallation

ABSTRACT

The symmetrically connected spiro[[2]benzopyran-1,4′-piperidines] **1** are highly potent and selective σ_1 receptor ligands. Changing the position of the spirocyclic nitrogen atom led to the unsymmetrically connected spiro[[2]benzopyran-1,3′-piperidines] **2** with a reduced distance between the aromatic system and the basic nitrogen atom. The synthesis of **2** was performed by halogen–metal exchange at the aryl bromide **3** followed by addition to the piperidone **5** and intramolecular transacetalization. The yield of **2a** was considerably improved by transmetallation of the aryllithium intermediate **4a** with $CeCl_3$ (**4c**). The *cis* and *trans* diastereomers *cis*-**2** and *trans*-**2** were separated and characterized by nuclear Overhauser effect. After removal of the benzyl group, the secondary amine **2b** was alkylated with various alkyl and arylalkyl halides. The σ_1 and σ_2 receptor affinity of the spirocyclic piperidines **2** were determined with receptor binding studies. Compared with the spirocyclic piperidines **1**, the unsymmetrically connected piperidines **2** show remarkably reduced σ_1 receptor affinities, whereas the selectivity over σ_2 and NMDA receptors was retained. A stereoselective interaction of the σ_1 receptor protein with the *cis*- or *trans*-configured spirocyclic compounds **2** was not observed. It was shown that alkyl residues at the N-atom can replace the lipophilic N-arylalkyl groups and interact with the primary hydrophobic binding site of the σ_1 receptor protein.

1. Introduction

Originally, the σ receptor has been regarded as an opioid receptor subtype [1]. However, since the first postulation it has been shown that σ receptors represent an unique class of receptors with a specific ligand binding profile and a characteristic distribution both in the central nervous system (CNS) and in many peripheral tissues (e.g. kidney, liver, heart) [2–6]. The σ receptor class can be divided into at least two subtypes: σ_1 and σ_2 receptors. The σ_1 receptor has already been cloned. It is a non-metabotropic transmembrane receptor comprising two transmembrane helices and a large extracellular loop [7,8]. The rat brain receptor protein consists of 223 amino acids, which results in a molecular weight of 23 kDa [9]. Although human and animal σ_1 receptors show a structural similarity of more than 95%, there is no resemblance to other known mammalian proteins. However, a 30% homology exists between the cloned σ_1 receptor and the yeast ergosterol-Δ^8/Δ^7-isomerase [10]. The σ_2 receptor is less characterized. So far, it has not been cloned and its molecular weight is approximately 21.5 kDa [6].

Both subtypes are involved in neuromodulatory processes and, therefore, σ receptors serve as targets for the development of novel drugs for the treatment of different neurological disorders, e.g. schizophrenia, depression or dementia [11–13]. Moreover, it has been shown that various cancer cell lines including breast, lung and prostate cancer cells overexpress σ receptors [14,15]. Thus, radiolabeled σ_1 and σ_2 receptor ligands represent attractive tools for imaging of various cancer cells, e.g. breast cancer cells [16].

Among various classes of σ_1 receptor ligands, the class of spirocyclic piperidines **1** contains potent σ_1 receptor ligands with high affinity and selectivity (Fig. 1). In particular the benzyl substituted spirocyclic compound **1a** (R = benzyl) has a K_i value of 1.29 nM for the σ_1 receptor [17]. In order to investigate the effect of the N-position in the piperidine heterocycle on the σ affinity, we planned to shift the nitrogen to the adjacent position. This shift leads to the novel class of spiro[[2]benzopyran-1,3′-piperidines] **2** with a modified distance between the aromatic benzopyran ring and the basic

* Corresponding author. Tel.: +49 251 83 33311; fax: +49 251 83 32144.
E-mail address: wuensch@uni-muenster.de (B. Wünsch).

0223-5234/$ – see front matter © 2009 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2009.07.017

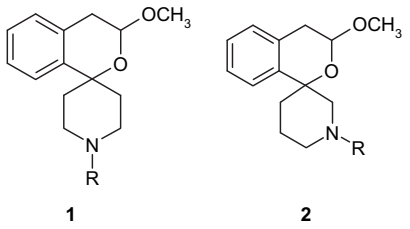


Fig. 1. Compounds with symmetrical (**1**) and unsymmetrical (**2**) spiro connection of the piperidine moiety.

nitrogen atom. Furthermore, the new center of chirality of the spiro atom leads to two diastereomeric pairs of enantiomers of the spirocyclic piperidines *cis*-**2** and *trans*-**2** (see Scheme 2).

The purpose of this work was to find a straightforward access to spiro[[2]benzopyran-1,3′-piperidines] **2** as a new class of σ receptor ligands. The focus was on the stereochemistry and the systematic variation of the residue R at the piperidine nitrogen atom in order to find an optimal nitrogen substituent (Fig. 1).

2. Chemistry

The starting compound for the synthesis of the spiro[[2]benzopyran-1,3′-piperidines] **2** was the bromo acetal **3** [18]. Upon treatment with *n*-butyllithium at −78 °C **3** was transformed by a halogen–metal exchange into the aryllithium intermediate **4a**. Subsequent trapping of **4a** with 1-benzylpiperidin-3-one (**5**) afforded the hydroxy acetal **6**. Cyclization of the hydroxy acetal **6** was catalyzed by *p*-toluenesulfonic acid [19]. In order to avoid elimination of a second molecule CH_3OH to give a cyclic enol ether, CH_3OH was the preferred solvent for the intramolecular transacetalization of isolated hydroxy acetal **6**. However, the best yield (18%) of the spirocyclic piperidine **2a** was obtained by direct cyclization of the unpurified hydroxy acetal **6** with HCl during the work-up procedure (Scheme 1).

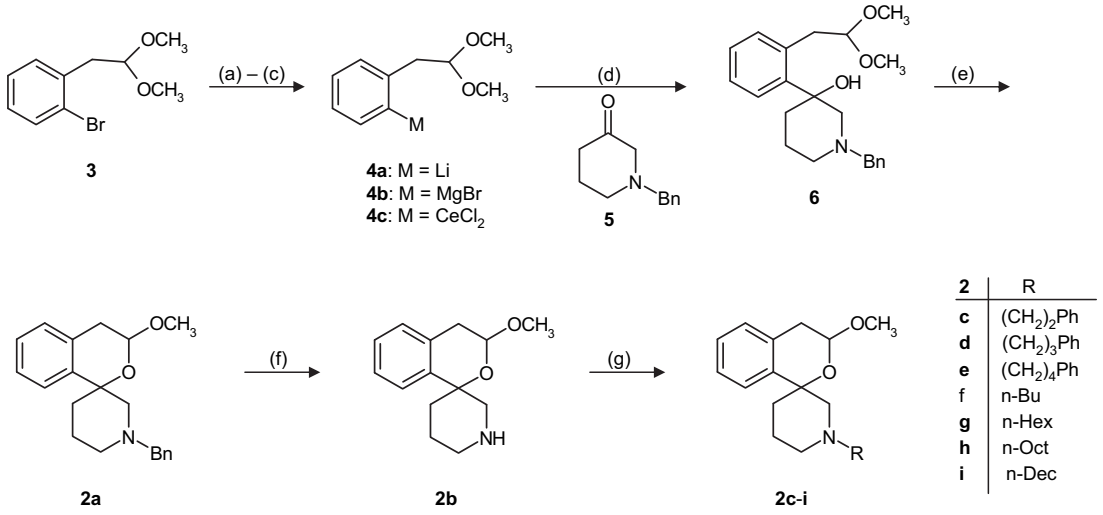
Despite several optimization attempts the yield of the spiro compound **2a** did not exceed 18%. We assume that enolate formation of **5** was competing with the nucleophilic addition. Therefore, less basic aryl metal compounds were investigated for the nucleophilic addition to the ketone **5**. At first, the Grignard reagent **4b** was generated by reaction of the aryl bromide **3** with Mg. This modification provided an increased yield of 26% of **2a** [20]. However, due to the difficult preparation of the *ortho*-substituted arylmagnesium compound **4b** by insertion of Mg metal into the aryl–C–Br bond, the yields of the spirocyclic piperidine **2a** were not reproducible.

Instead of using metallic magnesium it is sometimes more efficient to synthesize *ortho*-substituted arylmagnesium compounds by a halogen–metal exchange with neopentyl-MgBr [21]. The yield of the spirocyclic piperidine **2a** was slightly improved by this variation (29% yield).

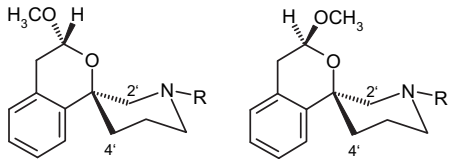
For the nucleophilic attack of substrates, susceptible to enolate formation due to the presence of acidic protons, the oxophilic organocerium reagents are the reagents of choice [22]. Therefore, the aryllithium intermediate **4a** was transmetallated with $CeCl_3$ to afford the almost non-basic arylcerium reagent **4c**. Subsequent addition of the arylcerium intermediate **4c** to the piperidone **5** gave the desired spirocyclic piperidine **2a** in 30% yield. In conclusion, the addition of the *ortho*-substituted aryl metal compounds **4** to the piperidone **5** was improved from 18% yield using the aryllithium compound **4a** to 30% using the arylcerium compound **4c**.

Due to the additional center of chirality in the spirocyclic system **2a**, two diastereomers were formed almost in the ratio 1:1 (Scheme 2). In the *cis* isomer *cis*-**2a** the methoxy group and the 2′-CH_2 group of the piperidine ring are on the same side and in the *trans* isomer *trans*-**2a** these groups are on opposite sides of the benzopyran ring plane. The diastereomers *cis*-**2a** and *trans*-**2a** were separated by flash chromatography, the corresponding structures were determined by ^1H NMR spectroscopy and the relative configuration was assigned by NOE difference spectroscopy, respectively.

Cleavage of the benzyl group of **2a** was performed with ammonium formate [23] in the presence of the catalyst Pd/C and led to the secondary amine **2b**. The homologous series of phenylalkyl (**2c**–**2e**) and alkyl substituted spirocyclic piperidines (**2f**–**2i**) were synthesized by S_N2 reaction of the secondary amine **2b** with various arylalkyl and alkyl halides. Reaction of the secondary amine **2b** with the primary halides R–X was performed in refluxing THF, acetone or acetonitrile in the presence of K_2CO_3 to afford the (aryl)alkylated spirocyclic piperidines **2c**–**2i**. In order to improve



Scheme 1. Synthesis of compounds with an unsymmetrical spiro connection of the piperidine moiety. Reagents and reaction conditions: (a) *n*-BuLi, −78 °C, THF, formation of **4a**. (b) Mg, THF, 60 °C, then −30 °C or neopentyl-MgBr, THF, −40 °C, formation of **4b**. (c) *n*-BuLi, −78 °C, THF, 1 h, then addition of $CeCl_3$ in THF, 1 h, −78 °C, formation of **4c**. (d) reaction of **4a** with **5**, 2 h, −78 °C, 16 h, rt, 28%. (e) from **6**: *p*-TosOH, CH_3OH, 4 d, rt; from **3**: conditions as described under (c) via **4c** and work-up with 5% HCl, 30%. (f) NH_4HCO_2, CH_3OH, Pd/C, 2 h, 65 °C, 91%. (g) R-X, THF or CH_3CN or acetone, K_2CO_3.



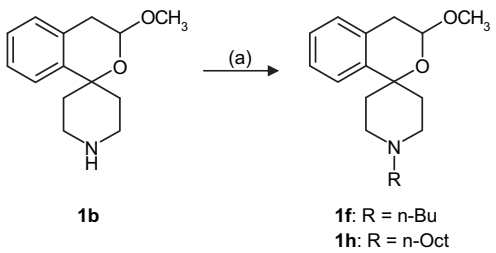
Scheme 2. Structures for *cis*- and *trans*-configured diastereomers of **2**.

the yields and reduce the very long reaction times, most of the nucleophilic substitution reactions were performed using microwave irradiation [24] instead of heating to reflux. The *n*-hexyl residue was introduced with 1-iodohexane which had been synthesized from 1-chlorohexane by a Finkelstein reaction with NaI under microwave irradiation.

The diastereomeric spirocyclic piperidines *cis*-**2a** and *trans*-**2a** were formed in a ratio of about 1:1. Since the diastereomeric composition did not change during the hydrogenolytic removal of the benzyl group and the alkylation step the *cis*- and *trans*-configured spirocyclic piperidines **2c**–**2i** were also obtained in the ratio of about 1:1. The diastereomers were separated by flash chromatography, respectively. However, due to very similar R_f values and strong tailing of the diastereomers the isolation of pure stereoisomers required more than one (usually 3–5) chromatographic run with considerable loss of yields. The *cis* and *trans* isomers of the phenylbutyl derivative **2e** could not be separated by fc. Therefore the pure diastereomers *cis*-**2b** and *trans*-**2b**, which were prepared by hydrogenolytic removal of the benzyl group from the diastereomerically pure benzyl derivatives *cis*-**2a** and *trans*-**2a**, were alkylated with 1-chloro-4-phenylbutane under microwave irradiation to yield the pure diastereomers *cis*-**2e** and *trans*-**2e**, respectively.

In order to prove the configurational stability of the isolated diastereomers solutions of the pure diastereomers *cis*-**2a** and *trans*-**2a** were stored in different aqueous TRIS/HCl-buffers (pH 4.0, pH = 7.4) over a period of 1 week. Careful tlc analysis showed that both diastereomers did not isomerize under these conditions (Scheme 2).

The receptor binding studies revealed that an aromatic residue within the N-substituent is not crucial for high σ_1 receptor affinity since ligands with linear alkyl substituents showed comparable σ_1 receptor affinities as compounds with arylalkyl moieties (see Receptor binding studies). In order to confirm this observation, spirocyclic 1,4′-piperidines **1** with alkyl substituents were envisaged. The spirocyclic 1,4′-piperidine derivative **1b** was prepared according to Ref. [17]. Alkylation of the secondary amine **1b** with butyl bromide and *n*-octyl bromide provided the spiro[[2]benzopyran-1,4′-piperidines] **1f** with a short (C_4H_9) and **1h** with a long (C_8H_{17}) alkyl residue, respectively (Scheme 3).



Scheme 3. Synthesis of alkyl substituted compounds with a symmetrical spiro connection. Reagents and reaction conditions: (a) *n*-BuBr or *n*-Oct-Br, CH_3CN, K_2CO_3, 30 h, 81 °C, 37% (**1f**) and 68% (**1h**).

3. Receptor binding studies

The σ_1 and σ_2 receptor affinities of the novel spirocyclic compounds **1** and **2** were determined by means of competition experiments with radioligands. Homogenates of guinea pig brains were used as receptor material in the σ_1 receptor assay. The σ_1 selective ligand [^{3}H]-(+)-pentazocine served as radioligand, and the non-specific binding was determined in the presence of a large excess of non-labeled (+)-pentazocine [17,25,26]. Homogenates of rat livers served as source for σ_2 receptors in the σ_2 assay. Since a σ_2 selective radioligand is not commercially available, the non-selective σ ligand [^{3}H]-di(*o*-tolyl)guanidine was employed in the presence of an excess of the non-radiolabeled σ_1 selective ligand (+)-pentazocine for selective masking of σ_1 receptors. Performing of the σ_2 assay in the presence of an excess of non-tritiated 1,3-di(*o*-tolyl)guanidine led to the non-specific binding of the radioligand [6,26,27].

4. Results and discussion

The σ_1 and σ_2 receptor affinities of the *cis*- and *trans*-configured spiro[benzopyran-1,3′-piperidines] **2a**–**2i** are listed in Table 1 together with the σ affinities of the spiro[benzopyran-1,4′-piperidines] **1a**, **1f** and **1h**. The spiro[benzopyran-1,3′-piperidines] **2a** and **2c**–**2e** substituted with arylalkyl residues possess σ_1 receptor affinities in the low micromolar to high nanomolar range. The benzyl compounds *cis*-**2a** and *trans*-**2a** show the lowest σ_1 receptor affinity (K_i = 1.72 μM and 1.44 μM, respectively), whereas the phenylpropyl derivative *trans*-**2d** is the most potent σ_1 ligand within this series of compounds (K_i = 376 nM). Generally, the *trans*-configured derivatives reveal slightly higher σ_1 affinities than the corresponding *cis*-diastereomers, e.g. *trans*-**2d** has about threefold higher σ_1 receptor affinity than the *cis*-diastereomer *cis*-**2d**. The σ_1 affinity increases with increasing length of the side chain (**2a** < **2c** < **2d** ≈ **2e**) with the *trans*-configured phenylpropyl and phenylbutyl derivatives *trans*-**2d** and *trans*-**2e** being the most active compounds. Certainly three to four methylene moieties represent the optimal N-aryl distance in the *trans*-configured derivatives.

The σ_1 receptor affinity is retained after removing of the phenyl moiety in the nitrogen side chain. The alkyl substituted compounds **2f**–**2i** and the analogous arylalkyl substituted compounds **2a**, **2c**–**2e** reveal comparable σ_1 affinities. The *n*-hexyl compounds *cis*-**2g** and *trans*-**2g** with a medium chain length show the highest σ_1 receptor affinities, which even exceeds the σ_1 affinities of the phenylpropyl (*trans*-**2d**) and phenylbutyl derivatives (*trans*-**2e**). Ligands with a shorter (**2f**) or a longer (**2h**, **2i**) side chain are less active. In the spirocyclic benzopyran-1,3′-piperidine series of compounds the *cis*-configured hexyl derivative *cis*-**2g** is the most active σ_1 ligand with a K_i value of 159 nM. Thus, aliphatic alkyl chains, in particular alkyl chains with six C-atoms, are able to replace arylalkyl residues at the N-atom of potent σ_1 ligands.

The σ_2 receptor affinities of the spiro[benzopyran-1,3′-piperidines] **2** are considerably lower than their σ_1 receptor affinities. Compounds **2** show σ_2 affinities in the low micromolar range. The phenylpropyl compound *trans*-**2d** possesses the highest σ_2 affinity (K_i = 1.11 μM) within this series. The determined K_i values indicate that the σ_2 receptor protein does not differentiate between compounds with arylalkyl and alkyl substituents and between *cis* and *trans* isomers.

Generally, most of the new spirocyclic ligands show a slight preference for the σ_1 receptor. Compounds with the highest σ_1 affinity reveal the highest σ_1/σ_2 selectivity. For example the phenylpropyl derivative *trans*-**2d** and the *n*-hexyl derivative *cis*-**2g** have selectivity factors of 3.0 and 7.7, respectively.

Surprisingly, the *trans*-configured *n*-butyl derivative *trans*-**2f** was chemically unstable. Over a period of 2 weeks the σ_1 receptor

Table 1
Affinities of the spirocyclic piperidines **1** and **2** towards σ_1 and σ_2 receptors.

Compound	R	$K_i \pm$ SEM (nM)[a]		σ_1/σ_2 Selectivity
		σ_1 ([^{3}H]-(+)-pentazocine)	σ_2 ([^{3}H]-di-*o*-tolylguanidine)	
1a [17]	Bn	1.3 ± 0.2	3500 ± 350	2708
1f	*n*-Bu	2.3	1020	443
1h	*n*-Oct	9.8	534	55
cis-**2a**	Bn	1720	0%[b]	n.d.
trans-**2a**	Bn	1440	1900	1.3
cis-**2c**	$-(CH_2)_2Ph$	470 ± 87	13%[b]	n.d.
trans-**2c**	$-(CH_2)_2Ph$	725 ± 94	1450	2.0
cis-**2d**	$-(CH_2)_3Ph$	1220	2940	2.4
trans-**2d**	$-(CH_2)_3Ph$	376 ± 62	1110	3.0
cis-**2e**	$-(CH_2)_4Ph$	1170	1340	1.1
trans-**2e**	$-(CH_4)_2Ph$	392 ± 106	1300	3.3
cis-**2f**	*n*-Bu	984	0%[b]	n.d.
trans-**2f**	*n*-Bu	(535)	0%[b]	n.d.
cis-**2g**	*n*-Hex	159 ± 27	1230	7.7
trans-**2g**	*n*-Hex	288 ± 256	1480	5.1
cis-**2h**	*n*-Oct	1070	7230	6.8
trans-**2h**	*n*-Oct	2980	1390	0.5
cis-**2i**	*n*-Dec	2080	5280	2.5
trans-**2i**	*n*-Dec	4360	4110	0.9
Haloperidol		3.9 ± 1.5	78 ± 2.3	20
(+)-Pentazocine		4.2 ± 1.1	–	n.d.
Ditolylguanidine		61 ± 18	42 ± 17	0.7

n.d. = not determined due to the non-specified σ_2 affinity.
The K_i value of *trans*-**2f** is given in brackets, since the compound was not stable.
[a] The standard error of the mean (SEM) is given, when three independent experiments have been performed. For compounds with low affinity ($K_i > 1000$ nM) the K_i value was recorded only once and, then, a SEM is not given.
[b] Inhibition of radioligand binding at a test compound concentration of 1 μM.

affinity was determined three times. The K_i values changed from 535 nM to 2.0 μM, to 3.4 μM. This variation was due to compound decomposition in the test solution (DMSO, TRIS buffer). Thus, the σ_1 affinity of the pure compound is in the nanomolar range, a standard error of the mean (SEM) could however not be determined.

The promising σ_1 affinity of the N-alkyl derivatives, in particular the N-butyl (**2f**) and N-hexyl (**2g**) derivatives, prompted us to synthesize and evaluate pharmacologically the corresponding N-alkyl derivatives **1** derived from the symmetrical spiro piperidine ring connection. The N-butyl (**1f**) and N-octyl (**1h**) derivatives interact with high affinity with σ_1 receptors (Table 1) indicating that the σ_1 receptor protein accepts an aliphatic residue at the piperidine N-atom instead of an arylalkyl chain. With a K_i value of 2.3 nM the butyl derivative **1f** is almost as potent as the benzyl derivative **1a**. Compared with **1a** (2708) the σ_1/σ_2 selectivity of **1f** (443) and **1g** (55) is reduced due to their slightly increased σ_2 receptor affinities.

A comparison of the σ_1 receptor affinities of the N-benzyl (**1a**/**2a**), N-butyl (**1f**/**2f**) and N-octyl (**1h**/**2h**) pairs clearly shows that compounds **2** with an unsymmetrical spiro piperidine ring connection are considerably less potent than the corresponding symmetrical spirocyclic piperidines **1**. The reduced σ_1 affinity of the unsymmetrical spirocyclic piperidines is explained by the pharmacophore model of Glennon et al. [28,29]. According to this model the distance between the basic N-atom and the primary hydrophobic region should be 6–10 Å to achieve high σ_1 receptor affinity.

The piperidine ring of the spirocyclic piperidines **1** and **2** can adopt two chair conformations with the phenyl moiety of the benzopyran ring in the equatorial or axial orientation. In both types of compounds the conformation with equatorial orientation of the phenyl ring is energetically favored. In the favored conformations the distance between the basic N-atom and the center of the benzopyran phenyl moiety is 5.68 Å (**1**) and 4.95 Å (**2**), respectively. The obtained K_i values support the pharmacophore model of Glennon [28,29], which requires a distance of 6–10 Å between the basic N-atom and the primary hydrophobic region. The shorter distance of compounds **2** with an unsymmetrical spiro connection might explain the reduced σ_1 receptor affinity.

5. Conclusion

A series of spirocyclic σ_1 receptor ligands with an unsymmetrical spiro connection of the piperidine moiety has been synthesized. Since the molecules contain two centers of chirality two diastereomeric pairs of enantiomers are possible. The σ_1 affinity of most of the *cis*- and *trans* isomers is very similar, although the σ_1 receptor protein has a small preference for some *trans*-configured N-arylalkyl derivatives (e.g. *trans*-**2d**, *trans*-**2e**). Compared with the symmetrically connected spiro compounds **1** the unsymmetrically connected spiro derivatives **2** are considerably less active. This may be due to the reduced distance between the basic N-atom and the benzene moiety of the benzofuran system (4.95 Å). According to the pharmacophore model of Glennon, a distance of 6–10 Å between the basic N-atom and the primary hydrophobic region is required for high σ_1 receptor affinity. It was shown that N-alkyl derivatives (e.g. **2f**, **2g**) are also potent σ_1 receptor ligands with even higher σ_1 affinity than the corresponding N-arylalkyl derivatives. This result was transferred to the symmetrically connected spirocyclic piperidines **1** and it was shown that N-alkyl derivatives (e.g. **1f**, **1h**) are very potent σ_1 receptor ligands.

6. Experimental

6.1. Chemistry, general methods

Unless otherwise noted, moisture sensitive reactions were conducted under dry nitrogen. THF was distilled from sodium/benzophenone ketyl prior to use. CH_3OH was distilled from magnesium/iodine prior to use. Microwave irradiation: Discover synthesis microwave apparatus (CEM); the following parameters are given in parenthesis: program; max. power; max. pressure; temperature; the reaction time is divided into ramp time, hold time

and cool off time. Thin layer chromatography (tlc): Silica gel 60 F_{254} plates (Merck). Flash chromatography (fc): Silica gel 60, 40–64 μm (Merck); parentheses include: diameter of the column, eluent, fraction size, R_f value. Melting point: Melting point apparatus SMP 3 (Stuart Scientific), uncorrected. Elemental analyses: Vario EL (Elementaranalysesysteme GmbH). MS: MAT GCQ (Thermo-Finnigan); mode of ionization: electron impact (EI). IR: IR spectrophotometer 480Plus FT-ATR-IR (Jasco). ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz): Mercury-400BB spectrometer (Varian); δ in ppm related to tetramethylsilane; coupling constants are given with 0.5 Hz resolution and the assignments of ^{1}H and ^{13}C NMR signals were supported by 2D NMR techniques (COSY, HETCOR). Compound **3** was prepared according to Ref. [17].

6.2. 2-(1-Benzyl-3-hydroxypiperidin-3-yl)phenylacetaldehyde dimethyl Acetal (**6**)

Under N_2 the aryl bromide **3** (1.24 g, 5.06 mmol) was dissolved in THF (10 mL) and cooled to −78 °C. A 1.6 M solution of *n*-BuLi in hexane (3.75 mL, 6.0 mmol) was slowly added. After 20 min at −78 °C, a solution of 1-benzylpiperidin-3-one (**5**, 1.24 g, 5.08 mmol) in THF (4 mL) was added dropwise and the mixture was stirred at −78 °C for 2 h. After stirring at rt for another 16 h a saturated NH_4Cl solution was added, and the mixture was extracted with CH_2C1_2. The organic layer was washed with a $NaHSO_3$ solution (10%), dried (Na_2SO_4) and filtered. The solvent was removed in vacuo, and the crude product was purified by fc (6 cm, petroleum ether/ethyl acetate 1:2, 30 mL, $R_f = 0.43$). Pale yellow oil, yield 515 mg (28%). $C_{22}H_{29}NO_3$ (355.5). MS: $m/z = 340$ [$M^+ - CH_3$], 263 [$M^+ - OH - CH(OCH_3)_2$]. IR (neat). ν (cm^{-1}) = 2922 (C–H). ^{1}H NMR ($CDC1_3$): δ (ppm) = 1.58–1.70 (m, 3H, $CH_2CH_2CH_2N$), 1.96–2.12 (in, 3H, $CH_2CH_2CH_2N$), 2.42–2.49 (m, 1H, $(CH_2)_3NCH_2$), 2.84–2.99 (m, 2H, $(CH_2)_3NCH_2$, $ArCH_2CH$), 3.11–3.19 (in, 1 H. $ArCH_2CH$), 3.28 (s, 3H, OCH_3), 3.32 (s, 3H, OCH_3), 3.57–3.65 (m, 2H, NCH_2Ph), 4.79 (t, $J = 5.2$ Hz, 1H, $ArCH_2CH$), 7.14–7.34 (m, 9H, arom).

6.3. cis-1′-Benzyl-3-methoxy-3,4-dihydrospiro[[2]benzopyran-1,3′-piperidine] (cis-**2a**) and trans-1′-benzyl-3-methoxy-3,4-dihydrospiro[[2]benzopyran-1,3′-piperidine] (trans-**2a**)

Under N_2 $CeCl_3$ (138 mg, 0.56 mmol) was suspended in THF (3 mL) and stirred at -78 °C for 2 h. Every 30 min the suspension was treated with ultrasonic waves for *5* min. In a second flask the aryl bromide **3** (130 mg, 0.53 mmol) was dissolved in THF (3 mL) and cooled to −78 °C. A 1.6 M solution of *n*-BuLi in hexane (0.34 mL, 0.55 mmol) was slowly added, and the mixture was stirred at −78 °C for 1 h. With a transfer needle and N_2 pressure the solution of the formed aryllithium intermediate **4a** was transferred to the $CeCl_3$ suspension and the needle was washed with THF (2 mL). After stirring for 1 h at −78 °C, a solution of the piperidone **5** (94.6 mg, 0.50 mmol) in THF (2 mL) was added dropwise to the solution of the arylcerium intermediate **4c**, and the mixture was stirred for another 1 h at −78 °C. A saturated NH_4Cl solution was added, the mixture was extracted with Et_2O (6x), and the Et_2O layer was washed with H_2O (2x). Afterwards, the organic layer was extracted with aqueous HCl (5%, 6x) and the acidic aqueous layer was washed with Et_2O (2x). Solid KOH was added to the aqueous layer (pH > 9) which was extracted with Et_2O (6x). The organic layer was dried (Na_2SO_4), filtered, the solvent was removed in vacuo and the residue was purified by fc (2 cm, cyclohexane/ethyl acetate 7:3, 5 mL). Due to tailing only small amounts of the pure diastereomers were obtained after several chromatographic runs. Total yield 48 mg (30%).

cis-**2a** ($R_f = 0.38$): Pale yellow oil. $C_{21}H_{25}NO_2$ (323.4). MS: $m/z = 323$ [M^+]. 292 [$M^+ - OCH_3$], 263 [$M^+ - OCHOCH_3$]. IR (neat): ν (cm^{-1}) = 2923 (C–H), 1076, 1034 (C–O). ^{1}H NMR ($CDC1_3$): δ (ppm) = 1.60–1.66 (m, 2H, $CH_2CH_2CH_2N$), 1.81–1.87 (m, 1H, $CH_2CH_2CH_2N$), 1.92–1.98 (m, 1H, $CH_2CH_2CH_2N$), 2.00–2.04 (m, 1H, $CH_2CH_2CH_2N$), 2.10–2.15 (m, 1H, $(CH_2)_3NCH_2$), 2.85–3.06 (m, 4H, $CH_2CH_2CH_2NCH_2$), $ArCH_2CH$). 3.40 (d. $J = 11.2$ Hz, 1H, NCH_2Ph), 3.70 (d, $J = 13.3$ Hz, 1H, NCH_2Ph), 3.58 (s, 3 H. $CHOCH_3$), 4.79 (t, $J = 3.9$ Hz, $ArCH_2CH$), 7.05–7.33 (m, 9H, arom). Anal. calcd. for $C_{21}H_{25}NO_2$: C 77.99 H 7.79 N 4.33 found C 77.89 H 8.06 N 4.04.

trans-**2a** ($R_f = 0.20$): Pale yellow oil. $C_{21}H_{25}NO_2$ (323.4). MS: $m/z = 323$ [M^+], 292 [$M^+ - OCH_3$], 263 [$M^+ - OCHOCH_3$]. IR (neat): ν (cm^{-1}) = 2921 (C–H), 1076 (C–O). ^{1}H NMR $CDC1_3$): δ (ppm) = 1.55–1.67 (m, 2H, $CH_2CH_2CH_2N$), 1.91–2.18 (m, 3H, $CH_2CH_2CH_2N$), 2.44–2.48 (m, 1H, $(CH_2)_3NCH_2$), 2.83–3.04 (m, 4H, CH_2NCH_2, $ArCH_2CH$ (2 H)). 3.44 (s, 3 H. $CHOCH_3$), 3.50 (d, $J = 13.2$ Hz, 1H, NCH_2Ph), 3.63 (d, $J = 13.2$ Hz, 1H, NCH_2Ph), 4.98 (t, $J = 3.5$ Hz, 1H, $ArCH_2CH$), 7.07–7.39 (m, 9H, arom). Anal. calcd. for $C_{21}H_{25}NO_2$: C 77.99 H 7.79 N 4.33 found C 77.73 H 8.04 N 4.10.

6.4. cis- and trans-3-Methoxy-3,4-dihydrospiro[[2]benzopyran-1,3′-piperidine] (cis-**2b** and trans-**2b**)

Under N_2 the benzylamine **2a** (734 mg, 2.27 mmol, mixture of *cis*-**2a** and *trans*-**2a**) was dissolved in dry methanol (12 mL). Ammonium formate (716 mg, 11.3 mmol) and 10% Pd/C (145 mg) were added. The mixture was heated to reflux for 2 h. Then it was filtered through Celite, the solvent was removed in vacuo and the residue was purified by fc (3 cm, methanol/ammonia 98:2, 20 mL, $R_f = 0.69$). Colorless needles (Et_2O), mp 54–58 °C, yield 480 mg (91%). $C_{14}H_{19}NO_2$ (233.3). MS: $m/z = 233$ [M^+], 202 [$M^+ - OCH_3$], 190 [$M^+ - NH(CH_2)_2$]. IR (neat): ν (cm^{-1}) = 2933 (C–H), 1055 (C–O). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.54–1.58 (m, 1H, $CH_2CH_2CH_2N$), 1.79–2.04 (m, 4H, $CH_2CH_2CH_2N$), 2.15–2.19 (m, 1H, $(CH_2)_2CH_2N$), 2.66–2.78 (m, 1H, $(CH_2)_3NCH_2$), 2.86–2.98 (m, 2 H $(CH_2)_3NCH_2$, $ArCH_2CH$), 3.05–3.16 (m, 1H, $ArCH_2CH$), 3,55/3.60 (2 s, together 3H, $CHOCH_3$ (*cis*-**2b**, *trans*-**2b**)), 4.87–4.96 (2 t, $J = 3.3$ Hz each, together 1H, $ArCH_2CH$), 7.09–7.26 (m, 4H, arom).

6.5. cis-3-Methoxy-1′-(2-phenylethyl)-3,4-dihydrospiro[[2]benzopyran-1,3′-piperidine] (cis-**2c**) and trans-3-methoxy-1′-(2-phenylethyl)-3,4-dihydrospiro[[2]benzopyran-1,3′-piperidine] (trans-**2c**)

Method 1: 1-Chloro-2-phenylethane (93.2 mg, 0.66 mmol) and K_2CO_3 (334 mg, 2.42 mmol) were added to a mixture of the secondary amine **2b** (96.2 mg, 0.41 mmol) in THF (5 mL) and the mixture was heated to reflux for 19 h. Then, the mixture was filtered through Celite, the solvent was removed in vacuo and the residue was purified by fc (1 cm, cyclohexane/ethyl acetate 7:3, 5 mL). Pale yellow oil, total yield 49 mg (35%).

Method 2: A mixture of the secondary amine **2b** (187 mg, 0.80 mmol), 1-chloro-2-phenylethane (141 mg, 1.00 mmol), K_2CO_3 (663 g. 4.80 mmol) and acetonitrile (3 mL) was filled into a 10 mL-microwave pressure vial. The mixture was irradiated with microwaves (program: standard; max. power 220 W; max. pressure 4 bar; temperature 100 °C; time program: 5 min ramp time; 40 min hold time; 5 min cool off time). Afterwards, the mixture was filtered through Celite, the solvent was removed under reduced pressure and the residue was purified by fc (2 cm, cyclohexane/ethyl acetate 7:3, 10 mL). Due to strong tailing only small amounts of the pure diastereomers were obtained after several chromatographic runs. Total yield 125 mg (46%).

cis-**2c** ($R_f = 0.36$): Pale yellow oil. $C_{72}H_{27}NO_2$ (337.4). MS (EI): $m/z = 306$ [$M^+ - OCH_3$], 246 [$M^+ - CH_2Ph$]. IR (neat): ν (crn^{-1}) = 2924 (C–H), 1076, 1034 (C–O). ^{1}H NMR ($CDC1_3$): δ (ppm) = 1.68–1.75 (m, 1H, $CH_2CH_2CH_2N$), 1.80–1.89 (m, 1H,

$CH_2CH_2CH_2N$), 1.97–2.06 (m, 1H, $CH_2CH_2CH_2N$), 2.22 (m, 3H, $CH_2CH_2CH_2N$), 2.52–2.63 (m, 1H, NCH_2CH_2Ph), 2.70–2.79 (m, 1H, NCH_2CH_2Ph), 2.80–2.87 (m, 2H, NCH_2CH_2Ph), 2.86–2.93 (m, 1H, $ArCH_2CH$), 3.05–3.10 (m. 3H, $ArCH_2CH$, $(CH_2)_3NCH_2$), 3.46 (s, 3H, OCH_3), 5.12 (t, $J = 3.5$ Hz, 1H, $ArCH_2CH$), 7.13–7.29 (m, 9H, arom). Anal. calcd. for $C_{22}H_{27}NO_2$: C 78.30 H 8.06 N 4.15 found C 78.18 H 8.37 N 4.08.

trans-**2c** ($R_f = 0.30$): Pale yellow oil. $C_{22}H_{27}NO_2$ (337.4). MS (EI): $m/z = 306$ [$M^+ - OCH_3$], 246 [$M^+ - CH_2Ph$]. IR (neat): ν (cm^{-1}) = 2923 (C–H), 1077 (C–O). ^1H NMR ($CDC1_3$): δ (ppm) = 1.53–1.74 (m, 4H, $CH_2CH_2CH_2N$), 1.90–2.17 (m, 2H, $CH_2CH_2CH_2N$), 2.21–2.26 (m, 1H, NCH_2CH_2Ph), 2.43–2.52 (m, 1H, NCH_2CH_2Ph), 2.60–2.69 (m, 1H, NCH_2CH_2Ph), 2.77–2.99 (m, 3H, $ArCH_2CH$, NCH_2CH_2Ph), 3.00–3.13 (m, 2H, $(CH_2)_3NCH_2$), 3.50 (s, 3H, OCH_3), 4.93 (t, $J = 3.5$ Hz, 1H, $ArCH_2CH$), 7.01–7.27 (m, 9H, arom). Anal. calcd. for $C_{22}H_{27}NO_2$: C 78.30 H 8.06 N 4.15 found C 78.55 H 8.00 N 3.84.

6.6. cis-*3-Methoxy-1′-(3-phenylpropyl)-3,4-dihydrospiro[[2]benzopyran-1,3′-piperidine]* (cis-***2d***) *and* trans-*3-methoxy-1′-(3-phenylpropyl)-3,4-dihydrospiro[[2]benzopyran-1,3′-piperidine]* (trans-***2d***)

1-Bromo-3-phenylpropane (126 mg, 0.63 mmol) and K_2CO_3 (554 mg, 4.01 mmol) were added to a mixture of the secondary amine **2b** (135 mg, 0.58 mmol) in acetonitrile (12 mL). The mixture was heated to reflux for 5 d. Then it was filtered through Celite, concentrated under reduced pressure and the residue was purified by fc (2 cm, petroleum ether/ethyl acetate 8:2, 10 mL). Due to strong tailing only small amounts of the pure diastereomers were obtained after several chromatographic runs. Total yield 166 mg (82%).

cis-**2d** ($R_f = 0.30$): Pale yellow oil. $C_{23}H_{29}NO_2$ (351.3). MS (EI): $m/z = 351$ [M^+], 320 [$M^+ - OCH_3$], 246 [$M^+ - (CH_2)_2Ph$]. IR (neat): ν (cm^{-1}) = 2943 (C–H), 1077, 1033 (C–O). ^1H NMR ($CDC1_3$): δ (ppm) = 1.52–1.63 (m, 1H, $CH_2CH_2CH_2N$), 1.72–1.76 (m, 3H, $CH_2CH_2CH_2N$ (1 H), CH_2CH_2Ph (2 H)), 1.85–2.20 (m, 3H, $CH_2CH_2CH_2N$), 2.08–2.17 (m, 1H, $CH_2CH_2CH_2N$), 2.21–2.28 (m, 1H, $NCH_2(CH_2)_2Ph$), 2.37–2.45 (m, 1H, $NCH_2(CH_2)_2Ph$), 2.53 (t, $J = 7.8$ Hz, 2H, CH_2CH_2Ph), 2.78–2.98 (m, 4H, $ArCH_2CH$, $(CH_2)_3NCH_2$), 3.42 (s, 3H, OCH_3), 5.00 (t, $J = 4.1$ Hz, 1H, $ArCH_2CH$), 7.02–7.19 (m, 9H, arom). Anal. calcd. for $C_{23}H_{29}NO_2$: C 78.59 H 8.32 N 3.99 found C 78.10 H 8.39 N 3.75.

trans-**2d** ($R_f = 0.23$): Pale yellow oil. $C_{23}H_{29}NO_2$ (351.3). MS (EI): $m/z = 351$ [M^+], 320 [$M^+ - OCH_3$], 246 [$M^+ - (CH_2)_2Ph$]. IR (neat): ν (cm^{-1}) = 2923 (C–H), 1077. (C–O). ^1H NMR ($CDCl_3$): δ (ppm) = 1.53–1.58 (m, 2H, $CH_2CH_2CH_2Ph$), 1.66–1.70 (m, 2H, $CH_2CH_2CH_2N$), 1.78–1.83 (m, 2H, $CH_2CH_2CH_2N$), 1.90–2.03 (m, 2H, $CH_2CH_2CH_2N$), 2.27–2.80 (m, 2H, $NCH_2(CH_2)_2Ph$), 2.52 (t, $J = 7.2$ Hz, 2H, $(CH_2)_2CH_2Ph$), 2.80–2.96 (m, 4H, $ArCH_2CH$, $(CH_2)_3NCH_2$), 3.43 (s, 3H, OCH_3), 4.88 (t, $J = 3.9$ Hz, 1H, $ArCH_2CH$), 6.98–7.21 (m, 9H, arom). Anal. calcd. for $C_{23}H_{29}NO_2$: C 78.59 H 8.32 N 3.99 found C 78.31 H 8.27 N 3.81.

6.7. cis-*3-Methoxy-1′-(4-phenylbutyl)-3,4-dihydrospiro[[2]benzopyran-1,3′-piperidine]* (cis-***2e***) *and* trans-*3-methoxy-1′-(4-phenylbutyl)-3,4-dihydrospiro[[2]benzopyran-1,3′-piperidine]* (trans-***2e***)

Method 1: 1-Chloro-4-phenylbutane (53.8 mg, 0.32 mmol) and K_2CO_3 (243 mg, 1.76 mmol) were added to a solution of the secondary amine **2b** (59.3 mg, 0.25 mmol) in acetonitrile (8 mL) and the mixture was heated to reflux for 40 h. It was filtered through Celite, concentrated in vacuo and the residue was purified by fc (2 cm, petroleum ether/ethyl acetate 7:3, 10 mL). Due to tailing, a fc separation of the diastereomers *cis*-**2e** and *trans*-**2e** was not possible. Pale yellow oil, total yield 33 mg (36%).

Method 2 (*cis*-**2e**): A solution of the pure diastereomer *cis*-**2b** (51.3 mg, 0.22 mmol) and 1-chloro-4-phenylbutane (50.4 mg, 0.30 mmol) in acetonitrile (3 mL) was filled into a 10 mL-microwave pressure vial. K_2CO_3 (240 mg, 1.74 mmol) was added, and the mixture was irradiated with microwaves (program: standard; max. power 220 W; max. pressure 4 bar; temperature 100 °C; time program: 5 min ramp time; 40 min hold time; 5 min cool off time). The mixture was filtered through Celite, concentrated in vacuo and the residue was purified by fc (1 cm, petroleum ether/ethyl acetate 7:3, 5 mL, $R_f = 0.18$). Pale yellow oil (*cis*-**2e**), yield 20 mg (25%). $C_{24}H_{31}NO_2$ (365.4). MS (EI): $m/z = 365$ [M^+], 334 [$M^+ - OCH_3$], 246 [$M^+ - (CH_2)_3Ph$]. IR (neat): ν (cm^{-1}) = 2927 (C–H), 1076 (C–O). ^1H NMR ($CDC1_3$): δ (ppm) = 1.45–1.63 (m, 6H, $CH_2(CH_2)_2CH_2Ph$, $CH_2CH_2CH_2N$), 1.69–1.78 (m, 1H, $CH_2CH_2CH_2N$), 1.89–1.97 (m, 3H, $CH_2CH_2CH_2N$), 2.22–2.40 (m, 2H, $CH_2(CH_2)_3Ph$), 2.50–2.58 (m, 2H, $(CH_2)_3CH_2Ph$), 2.78–2.98 (m, 4H, $ArCH_2CH$, $(CH_2)_3NCH_2$), 3.40 (s, 3H, OCH_3), 5.00–5.06 (m, 1H, $ArCH_2CH$), 7.04–7.20 (m, 9H, arom). Anal. calcd. for $C_{24}H_{31}NO_2$: C 78.86 H 8.55 N 3.83 found C 78.54 H 8.94 N 3.64.

Method 3 (*trans*-**2e**): As described in Method 2 the diastereomer *trans*-**2b** (70.0 mg, 0.30 mmol) was reacted with 1-chloro-4-phenylbutane (69.0 mg, 0.41 mmol), the mixture was worked up and the product was isolated by fc (1 cm, petroleum ether/ethyl acetate 7:3, 5 mL, $R_f = 0.13$). Pale yellow oil (*trans*-**2e**), yield 28 mg (26%). $C_{24}H_{31}NO_2$ (365.4). MS (EI): $m/z = 365$ [M^+], 334 [$M^+ - OCH_3$], 246 [$M^+ - (CH_2)_3Ph$]. IR (neat): ν (cm^{-1}) = 2925 (C–H), 1077 (C–O). ^1H NMR ($CDC1_3$): δ (ppm) = 1.64–1.70 (m, 6H, $CH_2(CH_2)_2CH_2Ph$, $CH_2CH_2CH_2N$). 1.88–1.91 (m, 1H, $CH_2CH_2CH_2N$), 2.07–2,19 (m, 3H, $CH_2CH_2CH_2N$), 2.42–2.58 (m, 2H, $CH_2(CH_2)_3Ph$), 2.71–2.75 (m, 2H, $(CH_2)_3CH_2Ph$), 2.99–3.15 (m, 4H, $ArCH_2CH$, $(CH_2)_3NCH_2$), 3.64 (s, 3H, OCH_3), 5.08–5.11 (m, 1H, $ArCH_2CH$), 7.23–7.44 (m, 9H, arom). Anal. calcd. for $C_{24}H_{31}NO_2$: C 78.86 H 8.55 N 3.83 found C 78.20 H 8.72 N 3.86.

6.8. cis-*1′-Butyl-3-methoxy-3,4-dihydrospiro[[2]benzopyran-1,3′-piperidinej* (cis-***2f***) *and* trans-*1′-butyl-3-methoxy-3,4-dihydrospiro[[2]benzopyran-1,3′-piperidinej* (trans-***2f***)

1-Bromobutane (108 mg, 0.79 mmol) and K_2CO_3 (482 mg, 3.49 mmol) were added to a mixture of the secondary amine **2b** (119 mg, 0.51 mmol) in acetonitrile (13 mL). The mixture was heated to reflux for 30 h. After filtration through Celite the solvent was removed in vacuo and the residue was purified by fc (2 cm, petroleum ether/ethyl acetate 7:3, 10 mL). Due to strong tailing only small amounts of the pure diastereomers were obtained after several chromatographic runs. Total yield 129 mg (77%).

cis-**2f** ($R_f = 0.40$): Pale yellow oil. $C_{18}H_{27}NO_2$ (289.3). MS (EI): $m/z = 289$ [M^+], 258 [$M^+ - OCH_3$], 246 [$M^+ - (CH_2)_2CH_3$]. IR (neat): ν (cm^{-1}) = 2928 (C–H), 1077 (C–O). ^1H NMR ($CDCl_3$): δ (ppm) = 0.88 (t, $J = 7.4$ Hz, 3H, $(CH_2)_3CH_3$), 1.30 (sext, $J = 7.4$ Hz, 2H, $(CH_2)_2CH_2CH_3$), 1.42–1.50 (m, 2H, $CH_2CH_2CH_2CH_3$), 1.61–1.68 (m, 1H, $CH_2CH_2CH_2N$), 1.77–1.83 (m, 1H, $CH_2CH_2CH_2N$), 1.95–2.08 (m, 3H, $CH_2CH_2CH_2N$), 2.12–2.30 (m, 2H, $CH_2CH_2CH_2N$, $NCH_2(CH_2)_2CH_3$), 2.38–2.46 (m, 1H, $NCH_2(CH_2)_2CH_3$), 2.85–3.06 (m, 4H, $(CH_2)_3NCH_2$, $ArCH_2CH$), 3.50 (s, 3H, OCH_3), 5.08 (t. $J = 3.9$ Hz, 1H, $ArCH_2CH$), 7.07–7.19 (m, 4H, arom). Anal. calcd. for $C_{18}H_{27}NO_2$: C 74.70 H 9.40 N 4.84 found C 74.48 H 9.47 N 4.71.

trans-**2f**: $R_f = 0.30$): Pale yellow oil. $C_{18}H_{27}NO_2$ (289.3). MS (EI): $m/z = 289$ [M^+], 258 [$M^+ - OCH_3$], 246 [$M^+ - (CH_2)_2CH_3$]. IR (neat): ν (cm^{-1}) = 2930 (C–H), 1078 (C–O). ^1H NMR ($CDCl_3$): δ (ppm) = 0.89 (t, $J = 7.4$ Hz, 3H, $(CH_2)_3CH_3$), 1.29 (sext, $J = 7.4$ Hz, 2H, $(CH_7)_2CH_2CH_3$), 1.50 (quint, $J = 7.4$ Hz, 2H, $CH_2CH_2CH_2CH_3$), 1.57–1.66 (m, 2H, $CH_2CH_7CH_2N$), 1.94–1.97 (m, 1 H. $CH_2CH_2CH_2N$), 1.97–2.10 (m, 2H, $CH_2CH_2CH_2N$), 2.31–2.45 (m, 3H, CH_2CH,CH_2N, $NCH_2(CH_2)_2CH_3$), 2.91–3.01 (m, 4H, $(CH_2)_3NCH_2$, $ArCH_2CH$), 3.54 (s, 3H, OCH_3), 4.98 (t,

J = 3.9 Hz, 1H, $ArCH_2CH$), 7.09–7.31 (m, 4H, arom). Anal. calcd. for $C_{18}H_{27}NO_2$: C 74.70 H 9.40 N 4.84 found C 75.10 H 9.60 N 4.60.

6.9. cis-*1′-Hexyl-3-methoxy-3,4-dihydrospiro[[2]benzopyran-1,3′-piperidine]* (cis-**2g**) *and* trans-*1′-hexyl-3-methoxy-3,4-dihydrospiro[[2]benzopyran-1,3′-piperidine]* (trans-**2g**)

Method 1: The secondary amine **2b** (91.3 mg, 0.39 mmol) and 1-chlorohexane (63.7 mg, 0.53 mmol) were dissolved in acetonitrile (9 mL), K_2CO_3 (334 mg, 2.42 mmol) was added and the mixture was heated to reflux for 30 h. After filtration through Celite the solvent was removed in vacuo and the residue was purified by fc (2 cm, petroleum ether/ethyl acetate 8:2, 10 mL). Pale yellow oil, total yield 28 mg (23%).

Method 2: 1-Chlorohexane (183 mg, 1.52 mmol) and NaI (271 mg, 1.81 mmol) were dissolved in acetone (3 mL),the solution was filled into a 10 mL-microwave pressure vial and irradiated with microwaves (program: standard; max. power 180 W; max. pressure 2 bar; temperature 80 °C; time program: 5 min ramp time; 10 min hold time; 5 min cool off time). The precipitated NaCl was removed by filtration and the filtrate (1-iodohexane in acetone) was used for further reaction. The secondary amine **2b** (230 mg, 0.99 mmol) and K_2CO_3 (1.10 g. 8.0 mmol) along with acetone (1 mL) were added to the solution of the produced 1-iodohexane. The mixture was irradiated with microwaves (program: standard; max. power 180 W; max. pressure 5 bar; temperature 100 °C; time program: 5 min ramp time; 40 min hold time; 5 min cool off time). After filtration through Celite and washing with CH_2C1_2 the solvent was removed under reduced pressure and the residue was purified by fc (2 cm, petroleum ether/ethyl acetate 8:2, 10 mL). Due to tailing only small amounts of the pure diastereomers were isolated after several chromatographic runs. Total yield 276 mg (88%).

cis-**2g** (R_f = 0.28): Pale yellow oil. $C_{20}H_{31}NO_2$ (317.5). MS (EI): m/z = 317 [M^+], 286 [$M^+ - OCH_3$], 246 [$M^+ - (CH_2)_4CH_3$}. IR (neat): ν (cm^{-1}) = 2925 (C–H), 1077 (C–O). ^1H NMR ($CDC1_3$): δ (ppm) = 0.84–0.87 (m. 3H, $(CH_2)_5CH_3$), 1.20–1.32 (m, 6H, $CH_2CH_2(CH_2)_3CH_3$). 1.42–1.51 (m, 2H, $CH_2CH_2(CH_2)_3CH_3$), 1.63–1.66 (m, 1H, $CH_2CH_2CH_2N$), 1.76–1.84 (m, 1H, $CH_2CH_2CH_2N$), 1.95 – 2.10 (m, 3H, $CH_2CH_2CH_2N$), 2.15–2.29 (m, 2H, $CH_2CH_2CH_2N$, $NCH_2(CH_2)_4CH_3$), 2.39–2.47 (m, 1H, $NCH_2(CH_2)_4CH_3$), 2.85–3.06 (m, 4H, $(CH_2)_3NCH_2$, $ArCH_2CH$), 3.49 (s, 3H, OCH_3), 5.08 (t, J = 3.9 Hz. 1H, $ArCH_2CH$), 7.09–7.21 (m, 4H, arom). Anal. calcd. for $C_{20}H_{31}NO_2$: C 75.67 H 9.84 N 4.41 found C 75.71 H 9.93 N 4.28.

trans-**2g** (R_f = 0.23): Pale yellow oil. $C_{20}H_{31}NO_2$ (317.5). MS (EI): m/z = 317 [M^+], 286 [$M^+ - OCH_3$], 246 [$M^+ - (CH_2)_4CH_3$]. IR (neat): ν (cm^{-1}) = 2926 (C–H), 1078 (C–O). ^1H NMR ($CDC1_3$): δ (ppm) = 0.78–0.84 (m, 3H, $(CH_2)_5CH_3$), 1.15–1.24 (m, 6H, $CH_2CH_2(CH_2)_3CH_3$), 1.39–1.47 (m, 2H, $CH_2CH_2(CH_2)_3CH_3$), 1.50–1.59 (m, 2H, $CH_2CH_2CH_2N$), 1.84–2.05 (m, 3H, $CH_2CH_2CH_2N$), 2.21–2.35 (m, 3H, $CH_2CH_2CH_2N$, $NCH_2(CH_2)_4CH_3$), 2.84–2.92 (m. 4H, $(CH_2)_3NCH_2$, $ArCH_2CH$), 3.46 (s, 3H, OCH_3), 493 (t, J = 3.5 Hz, 1H, $ArCH_2CH$), 7.02–7.23 (m, 4H, arom). Anal. calcd. for $C_{20}H_{31}NO_2$: C 75.67 H 9.84 N 4.41 found C 75.41 H 10.00 N 4.41.

6.10. cis-*3-Methoxy-1′-octyl-3,4-dihydrospiro[[2]benzopyran-1,3′-piperidine]* (cis-**2h**) *and* trans-*3-methoxy-1′-octyl-3,4-dihydrospiro[[2]benzopyran-1,3′-piperidine]* (trans-**2h**)

1-Bromooctane (137 mg, 0.71 mmol) and K_2CO_3 (481 mg, 3.48 mmol) were added to a solution of the secondary amine **2b** (136 mg, 0.56 mmol) in acetonitrile (14 mL). The mixture was heated to reflux for 26 h, then filtered through Celite and concentrated in vacuo. The residue was purified by fc (3 cm, petroleum ether/ethyl acetate 8:2, 20 mL). Due to tailing only small amounts of the pure diastereomers were obtained after several chromatographic runs. Total yield 183 mg (95%).

cis-**2h** (R_f = 0.24): Pale yellow oil. $C_{22}H_{35}NO_2$ (345.5). MS (EI): m/z = 345 [M^+], 314 [$M^+ - OCH_3$], 246 [$M^+ - (CH_2)_6CH_3$]. IR (neat): ν (cm^{-1}) = 2924 (C–H), 1078 (C–O). ^1H NMR ($CDC1_3$): δ (ppm) = 0.84–0.88 (m, 3H, $(CH_2)_7CH_3$), 1.21–1.30 (m, 10H, $CH_2CH_2(CH_2)_5CH_3$), 1.42–1.53 (m, 2H, $CH_2CH_2(dH_2)_5CH_3$), 1.62–1.68 (m, 1H, $CH_2CH_2CH_2N$), 1.76–1.84 (m, 1H, $CH_2CH_2CH_2N$), 1.95–2.10 (m, 3H, $CH_2CH_2CH_2N$), 2.16–2.30 (m, 2H, $CH_2CH_2CH_2N$, $NCH_2(CH_2)_6CH_3$), 2.39–2.48 (m, 1H, $NCH_2(CH_2)_6CH_3$), 2.85–3.06 (m, 4H, $(CH_2)_3NCH_2$, $ArCH_2CH$), 3.49 (s, 3H, OCH_3), 5.08 (t, J = 3.9 Hz, 1H, $ArCH_2CH$), 7.10–7.26 (m, 4H, arom). Anal. calcd. for $C_{22}H_{35}NO_2$: C 76.48 H 10.21 N 4.05 found C 76.55 H 10.17 N 3.88.

trans-**2h** (R_f = 0.14): Pale yellow oil. $C_{22}H_{35}NO_2$ (345.5). MS (EI): m/z = 345 [M^+], 314 [$M^+ - OCH_3$], 246 [$M^+ - (CH_2)_6CH_3$]. IR (neat): ν (cm^{-1}) = 2923 (C–H), 1079 (C–O). ^1H NMR ($CDC1_3$): δ (ppm) = 0.85–0.89 (m, 3H, $(CH_2)_7CH_3$), 1.21–1.30 (m, 10H, $CH_2CH_2(CH_2)_5CH_3$), 1.45–1.53 (m, 2H, $CH_2CH_2(CH_2)_5CH_3$), 1.57–1.63 (m, 2H, $CH_2CH_2CH_2N$), 1.92–2.12 (m, 3H, $CH_2CH_2CH_2N$), 2.32–2.42 (m, 3H, $CH_2CH_2CH_2N$, $NCH_2(CH_2)_6CH_3$), 2.87–3.02 (m, 4H, $(CH_2)_3NCH_2$, $ArCH_2CH$), 3.54 (s, 3H, OCH_3), 4.98 (t, J = 3.5 Hz, 1H, $ArCH_2CH$), 7.08–7.31 (m, 4H, arom). Anal. calcd. for $C_{22}H_{35}NO_2$: C 76.48 H 10.21 N 4.05 found C 76.52 H 10.48 N 4.24.

6.11. cis-*1′-Decyl-3-methoxy-3,4-dihydrospiro[[2]benzopyran-1,3′-piperidine]* (cis-**2i**) *and* trans-*1′-decyl-3-methoxy-3,4-dihydrospiro[[2]benzopyran-1,3′-piperidine]* (trans-**2i**)

1-Bromodecane (156 mg, 0.71 mmol) and K_2CO_3 (486 mg, 3.52 mmol) were added to a solution of the secondary amine **2b** (127 mg. 0.55 mmol) in acetonitrile (14 mL). The reaction mixture was heated to reflux for 26 h. Then it was filtered through Celite, the solvent was removed in vacuo and the residue was purified by fc (3 cm, petroleum ether/ethyl acetate 9:1, 20 mL). Due to tailing only small amounts of the pure diastereomers were obtained after several chromatographic runs. Total yield 173 mg (85%).

cis-**2i** (R_f = 0.21): Pale yellow oil. $C_{24}H_{39}NO_2$ (373.6). MS (EI): m/z = 373 [M^+], 342 [$M^+ - OCH_3$], 246 [$M^+ - (CH_2)_8CH_3$]. IR (neat): ν (cm^{-1}) = 2923 (C–H), 1078 (C–O). ^1H NMR ($CDCl_3$): δ (ppm) = 0.85–0.89 (t, J = 7.0 Hz, 3H, $(CH_2)_9CH_3$), 1.19–1.25 (m, 14H, $CH_2CH_2(CH_2)_7CH_3$), 1.42–1.51 (m, 2H, $CH_2CH_2(CH_2)_7CH_3$), 1.76–1.84 (m, 1H, $CH_2CH_2CH_2N$), 1.76–1.84 (m, 1H, $CH_2CH_2CH_2N$), 1.94–2.07 (m, 3H, $CH_2CH_2CH_2N$), 2.15–2.28 (m, 2H, $CH_2CH_2CH_2N$, $NCH_2(CH_2)_8CH_3$), 2.37–2.45 (m, 1H, $NCH_2(CH_2)_8CH_3$), 2.85–3.06 (m, 4 H. $(CH_2)_3NCH_2$, $ArCH_2CH$), 3.49 (s, 3H, OCH_3), 5.08 (t, J = 3.9 Hz, 1H, $ArCH_2CH$), 7.09–7.22 (m. 4H, arom). Anal. calcd. for $C_{24}H_{39}NO_2$: C 77.16 H 10.52 N 3.75 found C 76.70 H 10.63 N 3.50.

trans-**2i** (R_f = 0.14): Pale yellow oil. $C_{24}H_{39}NO_2$ (373.6). MS (EI): m/z = 373 [M^+], 342 [$M^+ - OCH_3$], 246 [$M^+ - (CH_2)_8CH_3$]. IR (neat): ν (cm^{-1}) = 2922 (C–H), 1079 (C–O). ^1H NMR ($CDC1_3$): δ (ppm) = 0.85–0.89 (t, J = 7.0 Hz, 3H, $(CH_2)_9CH_3$), 1.20–1.33 (m, 14H, $CH_2CH_2(CH_2)_7CH_3$), 1.46–1.55 (m, 2H, $CH_2CH_2(CH_2)_7CH_3$), 1.55–1.65 (m, 2H, $CH_2CH_2CH_2N$), 1.94–2.12 (m, 3H, $CH_2CH_2CH_2N$), 2.30–2.42 (m. 3H, $CH_2CH_2CH_2N$, $NCH_2(CH_2)_8CH_3$), 2.87–3.02 (m, 4H, $(CH_2)_3NCH_2$, $ArCH_2CH$), 3.54 (s, 3H, OCH_3), 4.98 (t, J = 3.5 Hz, 1H, $ArCH_2CH$), 7.09–7.31 (m, 4H, arom). Anal. calcd. for $C_{24}H_{39}NO_2$: C 77.16 H 10.52 N 3.75 found C 76.75 H 10.80 N 3.83.

6.12. *1′-Butyl-3-methoxy-3,4-dihydrospiro [[2]benzopyran-1,4′-piperidine]* (**1f**)

1-Bromobutane (70.1 mg, 0.51 mmol) and K_2CO_3 (599 mg, 2.57 mmol) were added to a solution of the secondary amine **1b** [17] (100 mg, 0.43 mmol) in acetonitrile (12 mL) and the mixture was heated to reflux for 30 h. It was filtered through Celite, the

solvent was removed in vacuo and the residue was purified by fc (2 cm, petroleum ether/ethyl acetate 7:3, 5 mL, $R_f = 0.31$). Pale yellow oil, yield 46 mg (37%). $C_{18}H_{27}NO_2$ (289.2). MS (EI): $m/z = 289$ [M^+], 258 [$M^+ - OCH_3$], 246 [$M^+ - (CH_2)_2CH_3$]. IR (neat): ν (cm^{-1}) = 2935 (C–H), 1076 (C–O). ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.94 (t, $J = 7.0$ Hz, 3H, $(CH_2)_3CH_3$), 1.38 (sext, $J = 7.0$ Hz, 2H, $(CH_2)_2CH_2CH_3$), 1.51–1.58 (m, 2H, $CH_2CH_2CH_3$), 1.83–2.04 (m, 3H, $N(CH_2CH_2)_2$), 2.21 (td, $J = 13.3/4.3$ Hz, 1H, $N(CH_2CH_2)_2$), 2.39–2.54 (m, 4H, $NCH_2(CH_2)_2CH_3$, $N(CH_2CH_2)_2$), 2.83–2.88 (m, 2H, $N(CH_2CH_2)_2$), 2.90–2.93 (m, 2H, $ArCH_2CH$), 3.57 (s, 3H, OCH_3), 2.87 (dd, $J = 3.9/2.7$ Hz, 1H, $ArCH_2CHOCH_3$), 7.15–7.22 (m, 4H, arom). Anal. calcd. for $C_{18}H_{27}NO_2$: C 74.70 H 9.40 N 4.84 found C 74.62 H 9.28 N 4.93.

6.13. 3-Methoxy-1′-octyl-3,4-dihydrospiro [[2]benzopyran-1,4′-piperidine] (**1h**)

1-Bromooctane (121 mg. 0.51 mmol) and K_2CO_3 (599 mg, 2.57 mmol) were added to a solution of the secondary amine **1b** [17] (100 mg, 0.43 mmol) in acetonitrile (12 mL) and the mixture was heated to reflux for 30 h. Then the mixture was filtered through Celite, concentrated in vacuo and the residue was purified by fc (2 cm, petroleum ether/ethyl acetate 7:3, 5 mL, $R_f = 0.22$). Pale yellow oil, yield 101 mg (68%). $C_{22}H_{35}NO_2$ (345.2). MS (EI): $m/z = 345$ [M^+], 314 [$M^+ - OCH_3$], 246 [$M^+ - (CH_2)_6CH_3$]. IR (neat): ν (cm^{-1}) = 2935 (C–H). 1075 (C–O). ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.89 (t, $J = 6.1$ Hz, 3H, $(CH_2)_7CH_3$), 1.23–1.32 (m, 10H, $(CH_2)_5CH_3$), 1.53–1.57 (m, 2H, $CH_2CH_2(CH_2)_5CH_3$), 1.84–2.00 (m, 3H, $N(CH_2CH_2)_2$), 2.21 (td, $J = 12.9/5.7$ Hz, 1H, $N(CH_2CH_2)_2$), 2.39–2.54 (m. 4 H. $NCH_2(CH_2)_6CH_3$, $N(CH_2CH_2)_2$), 2.82–2.85 (m, 2H, $N(CH_2CH_2)_2$), 2.90–2.93 (m, 2H, $ArCH_2CH$), 3.57 (s, 3H, OCH_3), 2.87 (dd, $J = 3.9/2.7$ Hz, 1H, $ArCH_2CHOCH_3$), 7.15–7.22 (m, 4H, arom). Anal. calcd. for $C_{22}H_{35}NO_2$: C 76.47 H 10.21 N 4.05 found C 76.59 H 9.97 N 4.22.

6.14. Receptor binding studies, materials and general procedures

Guinea pig brains and rat livers were commercially available (Harlan–Winkelmann, Germany). Homogenizer: Elvehjem Potter (B. Braun Biotech International). Centrifuge: High-speed cooling centrifuge model Sorvall RC-5C plus (Thermo-Finnigan). Filter: Printed Filtermat Type A (Perkin Elmer), presoaked in 0.5% aqueous polyethylenimine for 2 h at rt before use. The filtration was carried out with a MicroBeta FilterMate-96 Harvester (Perkin Elmer). The scintillation analysis was performed using Meltilex (Type A) solid scintillator (Perkin Elmer). The solid scintillator was melted on the filtermat at a temperature of 95 °C for 5 min. After solidification of the scintillator at rt, the scintillation was measured using a MicroBeta Trilux scintillation analyzer (Perkin Elmer). The counting efficiency was 20%.

6.15. Membrane preparation for the σ_1 assay [17,26]

Five guinea pig brains were homogenized with the potter (500–800 rpm, 10 up-and-down strokes) in 6 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 [30] 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.

6.16. Performing of the σ_1 assay [17,26]

The test was performed with the radioligand [^{3}H]-(+)-pentazocine (42.5 Ci/mmol; Perkin Elmer). The thawed membrane preparation (about 75 μg of the protein) was incubated with various concentrations of test compounds, 2 nM [^{3}H]-(+)-pentazocine, and buffer (50 mM TRIS, pH 7.4) in a total volume of 200 μL for 180 min at 37 °C. The incubation was terminated by rapid filtration through the presoaked filtermats by using the cell harvester. After washing each well five times with 300 μL of water, the filtermats were dried at 95 °C. Subsequently, the solid scintillator was put on the filtermat and melted at 95 °C. After 5 min, the solid scintillator was allowed to solidify at rt. The bound radioactivity trapped on the filters was counted in the scintillation analyzer. The non-specific binding was determined with 10 μM unlabeled (+)-pentazocine. The K_d-value of the radioligand [^{3}H]-(+)-pentazocine is 2.9 nM [25].

6.17. Membrane preparation for the σ_2 assay [17,26]

Two rat livers were cut into small pieces and homogenized with a potter (500–800 rpm, 10 up-and-down strokes) in 6 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 31,000× *g* for 20 min at 4 °C. The pellet was resuspended in buffer (50 mM TRIS, pH 8.0) and incubated at rt for 30 min. After the incubation, the suspension was centrifuged again at 31,000× *g* for 20 min at 4 °C. The final pellet was resuspended in buffer, the protein concentration was determined according to the method of Bradford [30] using bovine serum albumin as standard, and subsequently the preparation was frozen (−80 °C) in 1.5 mL portions containing about 2 mg protein/mL.

6.18. Performing of the σ_2 assay [17,26]

The test was performed with the radioligand [^{3}H]-di-*o*-tolylguanidine (50 Ci/mmol; ARC). The thawed membrane preparation (about 100 μg of the protein) was incubated with various concentrations of test compounds, 3 nM [^{3}H]-di-*o*-tolylguanidine, 500 nM (+)-pentazocine and buffer (50 mM TRIS, pH 8.0) in a total volume of 200 μL for 180 min at r.t. 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. Subsequently, the solid scintillator was put on the filtermat and melted at 95 °C. After 5 min, the solid scintillator was allowed to solidify at rt. The bound radioactivity trapped on the filters was counted in the scintillation analyzer. The non-specific binding was determined with 10 μM unlabeled ditolylguanidine. The K_d-value of the radioligand [^{3}H]-ditolylguanidine is 17.9 nM [27].

6.19. Data analysis

Usually, all experiments were carried out in triplicates using standard 96-well-multiplates (Diagonal). The IC_{50}-values were determined in competition experiments with six concentrations of the test compounds and were calculated with the program GraphPad Prism® 3.0 (GraphPad Software) by non-linear regression analysis. The K_i values were calculated according to Cheng and Prusoff [31]. For potent compounds the K_i values are given as mean values + SEM from three independent experiments. For compounds with low affinity (K_i value > 1 μM) the K_i value is recorded only once. When the competition curve does not provide a clear correlation between the test compound concentration and the inhibition of radioligand binding only the inhibition at a test compound concentration of 1 μM is given.

Acknowledgement

Financial support of this project by the Deutsche Forschungsgemeinschaft is gratefully acknowledged. Thanks are also due to the Degussa AG, Janssen-Cilag GmbH, and Bristol-Myers Squibb for donation of chemicals and reference compounds.

References

[1] W.R. Martin, C.G. Eades, W.O. Thornpson, R.E. Huppler, P.E. Glibert, J. Pharmacol. Exp. Ther. 197 (1976) 517–532.
[2] C. Kaiser, M. Pontecorvo, R.E. Mewshaw, Neurotransinissions 7 (1991) 1–5.
[3] S.R. Zukin, A.L. Tempel, R.S. Zukein, J. Neurosci. 46 (1986) 1032–1041.
[4] A.L. Gundlach, B.L. Largent, S.H. Snyder, Eur. J. Pharmacol. 113 (1985) 465–466.
[5] T.P. Su, Sigma Receptors in the Central Nervous System and the Periphery, in: Y. Itzhak (Ed.), The Sigma Receptors, Academic Press, London, 1994 pp. 21–44.
[6] S.B. Hellewell, A. Bruce, G. Feinstein, J. Orringer, W. Williams, W.D. Bowen, Eur. J. Pharmacol. 268 (1994) 9–18.
[7] R. Kekuda, P.D. Prasad, Y.J. Fei, F.H. Leibach, V. Ganapathy, Biochem. Biophys. Res. Commun. 229 (1996) 553–558.
[8] E. Aydar, C.P. Palmer, V.A. Klachko, M.B. Jackson, Neuron 31 (2002) 399–410.
[9] P. Seth, F.H. Leibach, V. Ganapathy, Biophys. Res. Commun. 241 (1997) 535–540.
[10] F.F. Moebius, I. Striessnig, H. Glossmann, Trends Pharmacol. Sci. 18 (1997) 67–70.
[11] D.P. Taylor, U.A. Shukla, S.A. Wolfe, P.Q. Owen, R. Pyke, et al., Schizophrenia Res. 4 (1991) 327.
[12] A. Urani, P. Romieu, F. Roman, K. Yamada, Y. Noda, et al., Eur. J. Pharmacol. 486 (2004) 15–16.
[13] A. Marrazzo, F. Caraci, E.T. Salinaro, T.P. Su, A. Copani, et al., Neuroreport 16 (2005) 1223–1226.
[14] C.S. John, B.B. Lim, B.C. Geyer, B.J. Vilner, W.D. Bowen, Bioconjug. Chem. 8 (1997) 304–309.
[15] B.J. Vilner, C.S. John, W.D. Bowen, Cancer Res. 55 (1995) 408–413.
[16] V. Caveliers, H. Everaert, C.S. John, T. Lahoutte, A. Bossuyt, J. Nucl. Med. 43 (2002) 1647–1649.
[17] C.A. Maier, B. Wünsch, J. Med. Chem. 45 (2002) 438–448.
[18] B. Wünsch, Arch. Pharm. 323 (1990) 493–499.
[19] B. Wünsch, G. Höfner, G. Bauschke, Arch. Pharm. 326 (1993) 513–518.
[20] B. Macchia, M. Macchia, C. Manera, E. Martinotti, S. Nencetti, et al., Eur. J. Med. Chem. 30 (1995) 869–880.
[21] G. Varchi, C. Kofink, D.M. Lindsav, A. Ricci, P. Knochel, Chem. Commun. 3 (2003) 396–397.
[22] F. Kaiser, L. Schwink, J. Velder, H.G. Schmalz, J. Org. Chem. 67 (2002) 9248–9256.
[23] S. Ram, L.D. Spicer, Tetrahedron Lett. 28 (1987) 515–516.
[24] B.L. Hayes, Microwave Synthesis, CEM Publishing, 2002.
[25] D.L. DeHaven-Hudkins, L.C. Fleissner, F.Y. Ford-Rice, Eur. J. Pharmacol. Mol. 227 (1992) 371–378.
[26] U. Wirt, D. Schepmann, B. Wünsch, Eur. J. Org. Chem. (2007) 462–475.
[27] R.H. Mach, C.R. Smits, S.R. Childers, Life Sci. 57 (1995) 57–62.
[28] R.A. Glennon, S.Y. Ablordeppey, A.M. Ismaiel, M.B. El-Ashmawy, J.B. Fischer, K.B. Howie, J. Med. Chem. 37 (1994) 1214–1219.
[29] R.A. Glennon, Minirev. Med. Chem. 5 (2005) 927–940.
[30] M.M. Bradford, Anal. Biochem. 72 (1976) 248–254.
[31] Y.C. Cheng, W.H. Prusoff, Biochem. Pharmacol. 22 (1973) 3099–3108.