RSC Medicinal Chemistry

ROYAL SOCIETY OF CHEMISTRY

RESEARCH ARTICLE

View Article Online
View Journal | View Issue

Published on 09 December 2020. Downloaded by University of Connecticut on 5/15/2021 1:42:17 PM.

Check for updates

Cite this: *RSC Med. Chem.*, 2021, **12**, 237

Synthesis and σ receptor affinity of spiro[[2]benzopyran-1,1′-cyclohexanes] with an exocyclic amino moiety in the 3′-position†

Elisabeth Kronenberg, Frauke Weber, Dirk Schepmann and Bernhard Wünsch *

Received 7th September 2020,
Accepted 15th November 2020

DOI: 10.1039/d0md00307g

rsc.li/medchem

The main functions of σ_1 receptors include the modulation of release and reuptake of neurotransmitters, the regulation of ion channels and the influence on intracellular signaling through modulation of calcium levels. Due to these properties, σ_1 receptors are interesting drug targets for the treatment of various neurological disorders, pain and cancer. In order to modify the distance between the pharmacophoric elements (the benzene ring of 2-benzopyran and an amino moiety), a set of spiro[[2]benzopyran-1,1′-cyclohexan]-3′-amines was synthesized. The key step of the synthesis was a Parham cyclization of 1-bromo-2-(2-bromoethyl)benzene (**6**) with the mono ketal **7** of cyclohexane-1,3-dione, which led in a one-pot reaction to the spirocyclic framework **8**. Reductive amination of ketone **9** stereoselectively provided secondary amines *cis*-**4**, which were methylated to afford tertiary amines *cis*-**5**. Whereas spirocyclic compounds *cis*-**4a** and *cis*-**5a** bearing a benzyl moiety at the exocyclic amino moiety showed rather low σ_1 affinity, the corresponding cyclohexylmethyl derivatives *cis*-**4b** and *cis*-**5b** exhibited low nanomolar σ_1 affinity. The secondary amine *cis*-**4b** displayed the highest σ_1 receptor affinity (K_i = 5.4 nM) in this series. Methylation of the secondary amine *cis*-**4b** led to a slightly decreased σ_1 receptor affinity of *cis*-**5b** (K_i = 15 nM).

1. Introduction

First described in 1976 by Martin *et al.*, σ receptors were initially accounted to the opioid receptor family.[1] Further investigation revealed a pharmacological profile deviating from the profile of opioid receptors.[2,3] Thus, σ receptors became their own receptor class consisting of two subtypes termed σ_1 and σ_2 receptors.[4] Both subtypes differ in their molecular weight, their distribution in different tissues and their ligand binding profile.

σ_1 receptors are localized in the central nervous system and in peripheral tissues including liver, kidney and heart.[5,6] On the cellular level, σ_1 receptors are found in the membrane of the endoplasmic reticulum and at mitochondria-associated membranes.[7] In 2016, Kruse *et al.* published the X-ray crystal structure of the σ_1 receptor revealing the receptor as a trimer in the solid state with one transmembrane domain in each protein. The carboxy and amino termini are located on opposite sides of the membrane.[8,9] σ_1 receptors play an important role in the modulation of ion channels, neurotransmitter systems and intracellular calcium homeostasis.[10,11]

Due to their modulating properties and the high expression in the central nervous system, σ_1 receptors are associated with the pathophysiology of several neurological disorders like Parkinson's and Alzheimer's disease, psychosis and depression.[12–16] σ_1 receptor antagonists can be used for the treatment of neuropathic pain.[17,18] Moreover, various human tumor cell lines show an increased σ_1 receptor expression level compared to non-tumor cells and it is reported that σ_1 receptors are involved in programmed cell death. Therefore, σ_1 receptors are promising targets for the development of innovative cancer treatment and for tumor diagnosis.[19–24]

In contrast, much less is known about the σ_2 receptor, which has a molecular weight of 21.5 kDa. Very recently, it could be isolated from calf liver preparations and was identified as endoplasmic reticulum resident transmembrane protein 97 (TMEM97).[25] σ_2 receptors are found in the central nervous system[26,27] and in several peripheral tissues, including liver and the gastrointestinal tract. In particular, high expression levels were detected in fast proliferating tumor cells. Thus, ligands addressing the σ_2 receptor subtype are useful as antitumor agents and as tumor markers allowing tumor cells with a high σ_2 receptor density to be visualized.[28–30]

Several σ_1 receptor ligands based on various scaffolds have been reported in the literature.[31] We are particularly

Institut für Pharmazeutische und Medizinische Chemie der Westfälischen Wilhelms-Universität Münster, Corrensstr. 48, D-48149 Münster, Germany.
E-mail: wuensch@uni-muenster.de; Fax: +49 251 8332144; Tel: +49 251 8333311

† Electronic supplementary information (ESI) available: Contains the procedure for the synthesis of mono ketal **7**, details on the receptor binding studies, ^{1}H and ^{13}C NMR spectra of the synthesized compounds and HPLC chromatograms of all the test compounds confirming their purity. See DOI: 10.1039/d0md00307g

Published on 09 December 2020. Downloaded by University of Connecticut on 5/15/2021 1:42:17 PM.

interested in spirocyclic systems provided with a basic amino moiety. Due to the spirocyclic structure, such types of ligands are conformationally restricted allowing the decoration of the system with various substituents in a predefined three-dimensional orientation to each other. During the last few years, we have reported several spirocyclic piperidines with a very high σ_1 receptor affinity and selectivity over the σ_2 subtype.[32–37] Pharmacophore models recommend a defined distance between the hydrophobic residues and the basic amino moiety.[38,39] High σ_1 affinity was achieved for spirocyclic piperidines **1** (K_i = 0.69 nM) and **2** (K_i = 1.3 nM) with an endocyclic amino moiety (piperidine ring, Fig. 1).[32] For these types of spirocyclic piperidines, distances of 5.6–5.7 Å and 5.1–5.2 Å between the benzene ring of the 2-benzopyran system and the basic amino moiety (piperidine N-atom) were determined for conformations with equatorially and axially oriented phenyl moieties at the piperidine ring (spiro center), respectively.[39,40]

According to the pharmacophore models, the distance between the benzene ring and the basic amino group should be longer, *i.e.* in the range of 6–10 Å. Shifting the basic functional group from inside the piperidine ring (**1** and **2**) at the 4′-position of a cyclohexane ring leads to increased distances between the benzene ring of the benzopyran system and the basic amino group. Depending on the equatorial or axial orientation of the phenyl moiety at the cyclohexane ring, the corresponding distances for *trans*-**3** are 6.0–6.3 Å and 6.5–6.6 Å, respectively, and those for *cis*-**3** are 7.2 Å and 6.3 Å, respectively. Although these distances fit exactly into the pharmacophore models, the increased distances led to a reduced σ_1 affinity: the benzyl derivatives *trans*-**3a** and *cis*-**3a** exhibit K_i-values of 256 nM and 97 nM, respectively. However, replacement of the benzyl moiety at the N-atom of **3a** with a cyclohexylmethyl moiety resulted in *trans*-**3b** and *cis*-**3b** displaying 10-fold and 5.5-fold increased σ_1 affinity, respectively[39,40] (Fig. 1 and Table 1).

In this study, spirocyclic cyclohexanes of type **4** and **5** bearing an exocyclic amino moiety in the 3′-position were investigated. Attachment of the amino moiety at the 3′-position instead of the 4′-position leads to a reduced distance between the benzene moiety of the benzopyran ring and the basic amino moiety. Thus, for *trans*-**4** and **5** and *cis*-**4** and **5**, distances of 6.2–6.3 Å (*trans*) and 5.9–6.1 Å (*cis*, axial amine)/5.5–5.7 Å (*cis*, equatorial amine), respectively, were calculated, which are very similar to the distances in the spirocyclic piperidines **1** and **2**. Whereas only one substituent at the piperidine ring of spirocyclic piperidines **1** and **2** can be modified, a broader diversification of the exocyclic amino moiety by the introduction of one (secondary amines **4**) or two substituents (tertiary amines **5**) is possible.

2. Synthesis

1-Bromo-2-(2-bromoethyl)benzene (**6**) served as a starting material for the synthesis of 3′-substituted spirocyclic 2-benzopyrans *cis*-**4a** and **b** and *cis*-**5a** and **b**. The Parham cyclization[41] was performed by treatment of aryl bromide **6** with *n*-BuLi and subsequently with monoethylene ketal-protected cyclohexane-1,3-dione **7**.[42,43] Due to the high CH-acidity of cyclohexane-1,3-dione, one carbonyl moiety had to be protected before the reaction with aryllithium. In the Parham cyclization, an alcoholate was formed by the reaction of the

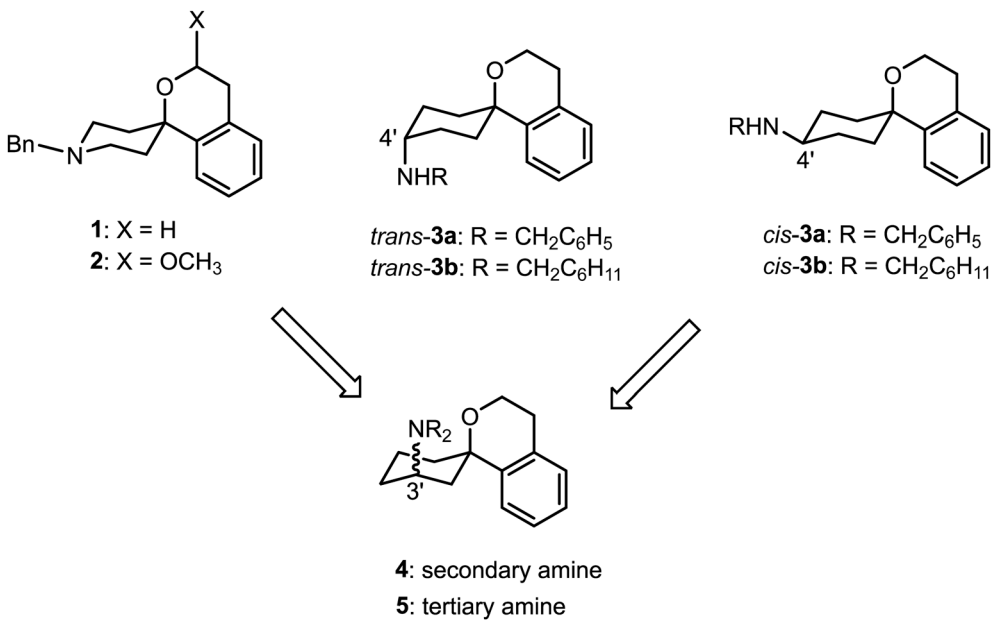
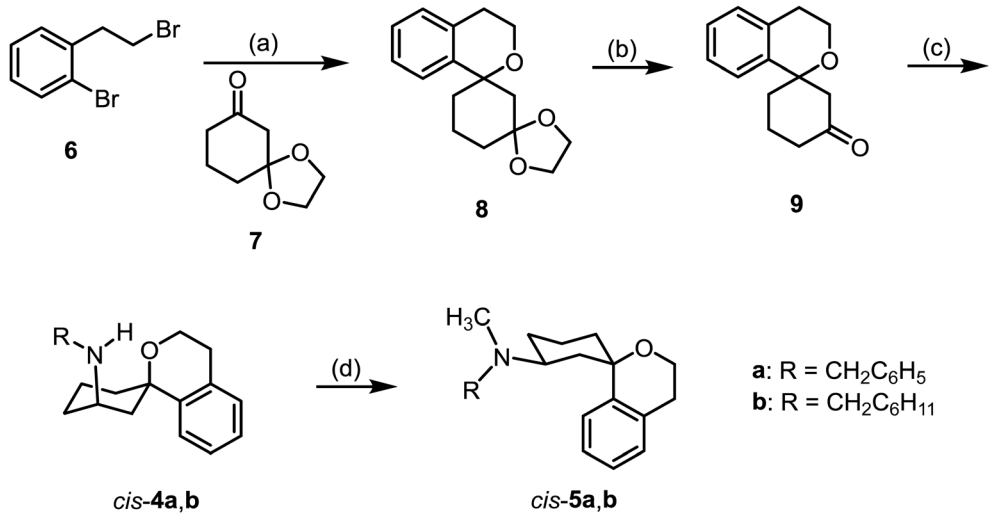


Fig. 1 Spirocyclic cyclohexanamines **4** and **5** with an exocyclic amino moiety in the 3′-position were derived from spirocyclic piperidines **1** and **2** with an endocyclic amino moiety and spirocyclic cyclohexanamine **3** with an exocyclic amino moiety in the 4′-position. The stereodescriptors *cis* and *trans* refer to the relative orientation of the O- and N-substituents at the central cyclohexane ring.

 This journal is © The Royal Society of Chemistry 2021

Published on 09 December 2020. Downloaded by University of Connecticut on 5/15/2021 1:42:17 PM.



Scheme 1 Synthesis of spirocyclic cyclohexanes *cis*-**4a** and **b** and *cis*-**5a** and **b** with an exocyclic amino function in the 3′-position. Reagents and reaction conditions: (a) THF, *n*-BuLi, cyclohexane-1,3-dione monoethylene ketal (**7**), 5 min, −88 °C, 1 h, rt, 28%. (b) Et_2O, 2 M HCl, 2 d, reflux, 85%. (c) RNH_2, CH_3CO_2H, $NaBH(OAc)_3$, THF, 24 h, rt, 90% (*cis*-**4a**), 80% (*cis*-**4b**). (d) Formalin 37%, $NaBH(OAc)_3$, CH_2Cl_2, 3 h, rt, 92% (*cis*-**5a**), 88% (*cis*-**5b**). Only one enantiomer of the racemic mixtures is shown. The stereodescriptor *cis* refers to the relative orientation of the O- and N-substituents at the central cyclohexane ring.

aryllithium intermediate with ketone **7**. A subsequent intramolecular S_N2 reaction of the alcoholate with the bromoethyl moiety led to the spirocyclic compound **8** in 28% yield. Ketone **9** was obtained in 85% yield by hydrolysis of ethylene ketal **8** with 2 M HCl in diethyl ether (Scheme 1).

Reductive amination of ketone **9** with benzylamine and cyclohexylmethylamine in the presence of reducing agent $NaBH(OAc)_3$ [44] and one equivalent of HOAc provided the secondary amines *cis*-**4a** (90%) and *cis*-**4b** (80%). This reaction proceeded with high diastereoselectivity, since only the *cis*-configured secondary amines *cis*-**4a** and *cis*-**4b** were formed. The high diastereoselectivity is explained by an H-bond between the intermediate iminium ion and the axially oriented O-atom of the benzopyran ring. This H-bond stabilizes the transition state leading to an axially oriented amino moiety in the 3′-position by equatorial transfer of hydride from $NaBH(OAc)_3$. The ddd at 2.32 ppm for the equatorially oriented 2′-H_{eq}, the dd at 1.79 ppm for the axially oriented 2′-H_{ax} and the rather narrow signal for 3′-H_{eq} at 2.98–3.04 ppm (width of 20 Hz) exclude an axial orientation of 3′-H and thus proves unequivocally the axial position of the benzylamino moiety of *cis*-**4a**.

The secondary amines *cis*-**4a** and *cis*-**4b** were finally methylated with formalin and $NaBH(OAc)_3$ [44] to afford the tertiary amines *cis*-**5a** (92%) and *cis*-**5b** (88%), respectively. Unexpectedly, an inversion of the chair conformation of the cyclohexane ring was observed for the tertiary amines *cis*-**5**. The tt for 3′-H at 3.01 ppm (J = 9.9/4.4 Hz) with two large coupling constants of 9.9 Hz towards the axially oriented neighbor protons in the 2′- and 4′-positions confirms the axial orientation of 3′-H_{ax}. Thus, the NR_2-moiety has to adopt the equatorial position. The conformational change occurring during methylation is explained by the stabilizing H-bond between the NH moiety and the axially oriented O-atom of the benzopyran ring of secondary amine **4**. After methylation, this H-bond is no longer possible leading to a conformational change with two substituents NR_2 and OR in the energetically favored equatorial position.

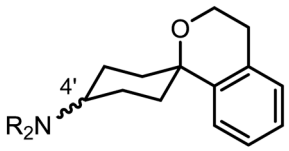
3. σ_1 and σ_2 receptor affinity

The σ_1 and σ_2 receptor affinity of the synthesized spirocyclic 2-benzopyrans was determined in competition experiments with radioligands.[45–47] Briefly, homogenates of guinea pig brain and rat liver served as sources of σ_1 and σ_2 receptors, respectively. [^{3}H]-(+)-pentazocine was used as a radioligand in the σ_1 assay. The nonspecific binding was determined with an excess of nonlabeled (+)-pentazocine. Due to the missing availability of a selective σ_2 receptor radioligand, [^{3}H]-di-*o*-tolylguanidine (DTG) was used as an unselective radioligand in the presence of an excess of (+)-pentazocine to mask the σ_1 receptors. Compounds with high affinity were tested three times (n = 3).[45–47] For compounds with low σ affinity, only the inhibition of the radioligand binding at a concentration of 1.0 μM is given.

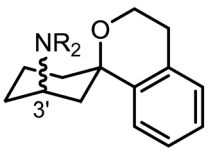
The spirocyclic 2-benzopyrans *cis*-**4a** and *cis*-**5a** bearing a benzylamino moiety in the 3′-position show a similar low σ_1 affinity as the 2-benzopyrans *trans*-**3a** and *cis*-**3a** with the benzylamino moiety located in the 4′-position. (Table 1) The crucial distance between the center of the benzene ring and the N-atom of *cis*-**4a** (5.9–6.1 Å) and *cis*-**5a** (5.5–5.7 Å) is shorter than the distance in *trans*-**3a** and *cis*-**3a** (6.0–7.2 Å), but close to the distance in the high-affinity spirocyclic piperidine derivative **1** (5.1 Å/5.6 Å). It is assumed that the low affinity of *cis*-**4a** and *cis*-**5a** compared to the high affinity of **1** is due to the different orientation of the pharmacophoric

Published on 09 December 2020. Downloaded by University of Connecticut on 5/15/2021 1:42:17 PM.

Table 1 σ_1 and σ_2 receptor affinity of the spirocyclic cyclohexanes with an amino moiety in the 3′-position and reference compounds



3



4,5

Compd.	NR_2	Distance[a] (Å)	K_i ± SEM [nM] σ_1	K_i ± SEM [nM] σ_2
1 (ref. 32)	—	5.6 - 5.7 Å 5.1–5.2 Å	0.69 ± 0.17	99.7 ± 19.8
trans-**3a** (ref. 40)	$NHCH_2C_6H_5$	6.0–6.3 Å 6.5–6.6 Å,	256 ± 72	13%[c]
cis-**3a** (ref. 40)	$NHCH_2C_6H_5$	7.2 Å 6.3 Å	97 ± 4.8	503[b]
trans-**3b** (ref. 40)	$NHCH_2C_6H_{11}$	6.0–6.3 Å 6.5–6.6	15 ± 2.6	99 ± 17
cis-**3b** (ref. 40)	$NHCH_2C_6H_{11}$	7.2 Å 6.3 Å	18 ± 3.8	84 ± 19
cis-**4a**	$NHCH_2C_6H_5$	5.9–6.1 Å	95 ± 35	340[b]
cis-**4b**	$NHCH_2C_6H_{11}$	5.9–6.1 Å	5.4 ± 2.8	60 ± 40
cis-**5a**	$N(CH_3)CH_2C_6H_5$	5.5–5,7 Å	278 ± 2	24%[c]
cis-**5b**	$N(CH_3)CH_2C_6H_{11}$	5.5–5.7 Å	15 ± 4.6	34%[c]
(+)-pentazocine			5.4 ± 0.5	—
di-*o*-Tolylguanidine			71 ± 8	54 ± 8

[a] Distance refers to the distance between the center of the benzene ring of the 2-benzopyran and the N-atom; the value in the first line defines the distance, when the benzene ring of the 2-benzopyran adopts the equatorial orientation at the cyclohexane ring and the second value describes the distance for the axial orientation of the benzene ring. [b] Result from one measurement. [c] Inhibition of radioligand binding (%) at a test compound concentration of 1 μM.

structural elements, in particular, the benzylamino moiety relative to the benzopyran system.

As observed for the spirocyclic cyclohexanes *trans*-**3b** and *cis*-**3b**, replacement of the benzyl moiety with a cyclohexylmethyl moiety at the amino group led to considerably increased σ_1 affinity of *cis*-**4b** and *cis*-**5b**. At 5.4 nM and 15 nM, their K_i values are in the low nanomolar range and comparable with the K_i values of the cyclohexylmethylamines *trans*-**3b** and *cis*-**3b**. However, both compounds do not reach the subnanomolar affinity of the spirocyclic piperidine **1** (K_i = 0.69 nM).

In contrast to the result of recent studies,[40] transformation of the secondary amines *cis*-**4a** and *cis*-**4b** into methylated tertiary amines *cis*-**5a** and *cis*-**5b** led to 3-fold decreased σ_1 receptor affinity.

Spirocyclic cyclohexanes **4** and **5** with an amino moiety in the 3′-position display a very low σ_2 affinity indicating the high selectivity for σ_1 over σ_2 receptors. *N*-Methylation of secondary amines *cis*-**4** leading to tertiary amines *cis*-**5** resulted in a particularly low σ_2 affinity and thus high σ_1:σ_2 selectivity.

Since the σ receptor was originally classified as an opioid receptor, the affinity of the cyclohexylmethylamines *cis*-**4b** and *cis*-**5b** towards the opioid receptors MOR, KOR and DOR was determined in receptor binding studies. Up to a concentration of 1 μM, the tertiary methylamine *cis*-**5b** did not interact with all three opioid receptors. However, the most potent σ_1 ligand *cis*-**4b** showed a moderate MOR (K_i = 696 ± 180 nM), DOR (K_i = 150 ± 108 nM), and KOR affinity (K_i = 407 ± 165 nM). The selectivity for the σ_1 receptor over the three opioid receptors is at least 25-fold (over DOR). Also, the low-σ_1-affinity benzylamines *cis*-**4a** and *cis*-**5a** did not display MOR affinity up to a concentration of 1 μM.

4. Conclusions

A small series of spirocyclic cyclohexanes with an amino moiety in the 3′-position was synthesized and pharmacologically evaluated. Unexpectedly, reductive amination of the spirocyclic cyclohexanone **9** stereoselectively provided only *cis*-configured amines *cis*-**4** and *cis*-**5**. Therefore, only *cis*-configured amines were investigated.

Whereas the σ_1 affinity of the benzylamines *cis*-**4a** and *cis*-**5a** is rather low, the σ_1 affinity of the corresponding cyclohexylmethylamines *cis*-**4b** and *cis*-**5b** is in the low nanomolar range. The secondary amine *cis*-**4b** represents the most active σ_1 ligand (K_i = 5.4 nM) of this series of ligands. However, the σ_1 affinity of *cis*-**4a** does not reach the σ_1 affinity of the spirocyclic piperidines **1** (K_i = 0.69 nM) and **2** (K_i = 1.3 nM). Since the distance of the benzene moiety of the 2-benzopyran and the N-atom is very similar for the spirocyclic piperidines **1** and **2** (5.1 Å/5.6 Å) and the cyclohexanes *cis*-**4** (5.9–6.1 Å) and *cis*-**5** (5.5–5.7 Å), it was concluded that in addition to the correct distance, the orientation of the pharmacophoric elements is crucial for high σ_1

 This journal is © The Royal Society of Chemistry 2021

Published on 09 December 2020. Downloaded by University of Connecticut on 5/15/2021 1:42:17 PM.

affinity. In particular, the H-bond donor or basic functional group of the spirocyclic cyclohexanes *cis*-**4** and *cis*-**5** adopts an orientation different from the spirocyclic piperidines **1** and **2**. Although an equatorial orientation of the tertiary amines *cis*-**5a** and *cis*-**5b** was shown by ^{1}H NMR spectroscopy ($CDCl_3$), in a buffer system at pH 7.4, the amino moiety will be protonated and able to form the same H-bond as the secondary amines *cis*-**4a** and *cis*-**4b**. Altogether, further pharmacological properties of these types of spirocyclic σ_1 ligands were not further investigated.

5. Experimental part

5.1 Chemistry and general methods

Oxygen and moisture sensitive reactions were carried out under nitrogen, dried with silica gel with a moisture indicator (orange gel, VWR, Darmstadt, Germany) and in dry glassware (Schlenk flask or Schlenk tube). Temperature was controlled with dry ice/acetone (−78 °C), ice/water (0 °C), a cryostat (Julabo TC100E-F, Seelbach, Germany), a magnetic stirrer MR 3001 K (Heidolph, Schwalbach, Germany) or an RCT CL (IKA, Staufen, Germany), together with a temperature controller EKT HeiCon (Heidolph) or a VT-5 (VWR) and PEG or silicone bath. All solvents were of analytical or technical grade quality. Demineralized water was used. CH_2Cl_2 was distilled from CaH_2; THF was distilled from sodium/benzophenone; MeOH was distilled from magnesium methanolate. Thin layer chromatography (TLC): TLC silica gel 60 F_{254} on aluminum sheets (VWR). Flash chromatography (FC): silica gel 60, 40–63 μm (VWR); parentheses include: the diameter of the column (ϕ), the length of the stationary phase (l), and the eluent and fraction size (v). Melting point: melting point system MP50 (Mettler Toledo, Gießen, Germany), open capillary, uncorrected. MS: MAT GCQ (Thermo-Finnigan); EI = electron impact; Thermo Finnigan LCQ® ion trap mass spectrometer with an ESI = electrospray ionization interface. 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.

5.2 HPLC method for the determination of the purity

Equipment 1: pump: L-7100, degasser: L-7614, autosampler: L-7200, UV detector: L-7400, interface: D-7000, data transfer: D-line, data acquisition: HSM-software (all from Merck Hitachi, Darmstadt, Germany); equipment 2: pump: LPG-3400SD, degasser: DG-1210, autosampler: ACC-3000T, UV-detector: VWD-3400RS, interface: DIONEX UltiMate 3000, data acquisition: Chromeleon 7 (equipment and software from Thermo Fisher Scientific, Lauenstadt, Germany); column: LiChrospher® 60 RP-select B (5 μm), LiChroCART® 250–4 mm cartridge; flow rate: 1.0 mL min^{-1}; injection volume: 5.0 μL; detection at λ = 210 nm; solvents: A: demineralized water with 0.05% (V/V) trifluoroacetic acid, B: CH_3CN with 0.05% (V/V) trifluoroacetic acid; gradient elution (% A): 0–4 min: 90%; 4–29 min: gradient from 90% to 0%; 29–31 min: 0%; 31–31.5 min: gradient from 0% to 90%; 31.5–40 min: 90%. Unless otherwise mentioned, the purity of all the test compounds is greater than 95%.

5.3 Synthetic procedures

The synthesis of cyclohexane-1,3-dione monoethylene ketal (**7**)[42,43] is described in the ESI.†

3,4-Dihydrospiro[[2]benzopyran-1,1′-cyclohexan]-3′-one ethylene ketal (8). 1-Bromo-2-(2-bromoethyl)benzene (**6**, 118 mg, 0.45 mmol) was dissolved in THF (5 mL) under a N_2 atmosphere and the solution was cooled to −88 °C. Subsequently, *n*-BuLi (1.6 M in *n*-hexane. 0.33 mL, 0.93 mmol) was added dropwise. After 5 min at −88 °C, a solution of cyclohexane-1,3-dione monoethylene ketal (**7**, 77 mg, 0.49 mmol) in THF (2 mL)) was added rapidly and the mixture was stirred at −88 °C for 5 min and 2 h at rt. H_2O (10 mL) was added and the mixture was extracted with Et_2O (3 × 30 mL). The combined organic layers were dried (K_2CO_3), concentrated *in vacuo* and the residue was purified by FC (ϕ 3 cm, 20 cm, cyclohexane : ethyl acetate 4 : 1, 20 mL). R_f (cyclohexane : ethyl acetate 4 : 1 = 0.14). Pale yellow oil, yield 33 mg (28%). $C_{16}H_{20}O_3$ (260.4). MS (EI): *m*/*z* (%) = 260 [M, 12], 232 [M–CH_2═CH_2, 34], 217 [M–$CH_3CH_2CH_2$*, 100], 159 [M–CH_3CketalCH_2*, 60], 146 [M–CH_2Cketal, –CH_2═CH_2, 61], 86 [C*H_2C(═O + CH_2CH_2O), 31]. FT-IR: $\tilde{\nu}$ (cm^{-1}) = 2945, 2921, 2862 (s, v, C–H, alkyl), 1492 (w, v, C═C, arom), 1087 [s, v, C–O), 765 (s, δ, C–H, *o*-disubst. arom). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.54–1.69 (m, 3H, CH_2-CH_2CH_2C═O), 1.89–2.07 (m, 4H, CH_2CH_2CH_2C═O (3H), CCH_2C(OCH_2CH_2O) (1H)), 2.19 (dt, J = 14.7/2.5 Hz, 1H, CCH_2-C(OCH_2CH_2O), 2.77 ("dt", J = 16.2/5.1 Hz, 1H, ArC$H_2$$CH_2$O), 2.89 (ddd, J = 16.2/6.9/4.9 Hz, 1H, ArC$H_2$$CH_2$O), 3.81–4.15 (m, 6H, OC$H_2CH_2$O (4H), Ar$CH_2CH_2$O (2H)), 7.07–7.20 (m, 4H, Ar–H). Purity (HPLC): 96.2%, t_R = 17.3 min.

3,4-Dihydrospiro[[2]benzopyran-1,1′-cyclohexan]-3′-one (9). Ketal **8** (85 mg, 0.33 mmol) was dissolved in Et_2O (10 mL) and HCl (2 M, 10 ml) was added. The mixture was heated to reflux for 48 h. The mixture was extracted with Et_2O (3 × 40 mL) and the combined organic layers were dried (K_2CO_3), concentrated *in vacuo* and the residue was purified by FC (ϕ 2 cm, 20 cm, cyclohexane : ethyl acetate 9 : 1, 10 mL). R_f (cyclohexane : ethyl acetate 9 : 1 = 0.12, cyclohexane : ethyl acetate 4 : 1 = 0.20). Colorless solid, mp 91 °C, yield 60 mg (85%). $C_{14}H_{16}O_2$ (216.3). MS (EI): *m*/*z* (%) = 216 [M, 25], 188 [M–CH_2═CH_2, 11], 173 [M–$CH_3CH_2CH_2$*, 47], 159 [M–CH_3-C═OCH_2*, 100]. FT-IR: $\tilde{\nu}$ (cm^{-1}) = 3062, 3025 (w, v, C–H, arom), 2939, 2869 (s, v, C–H, Alkyl), 1712 (s, v, C═O), 1491 (w, v, C═C, arom), 1085 (s, v, C–O), 754 (s, δ, C-H, *o*-disubst. arom). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.93–2.01 (m, 1H, CH_2-CH_2CH_2C═O), 2.03–2.22 (m, 3H, CH_2CH_2CH_2C═O), 2.34–2.42 (m, 1H, CH_2CH_2CH_2C═O), 2.44–2.52 (m, 1H, CH_2CH_2-CH_2C═O), 2.72 (s, 2H, CH_2C═O), 2.79 ("dt", J = 16.2/5.5 Hz, 1H, ArC$H_2$$CH_2$O), 2.85 ("dt", J = 16.3/5.5 Hz, ArC$H_2$$CH_2$O), 3.86 (ddd, J = 11.5/6.1/5.1 Hz, 1H ArCH_2CH_2O), 3.9 (ddd, J = 11.5/6.3/5.0 Hz, 1H, ArCH_2CH_2O), 7.11–7.25 (m, 4H, Ar–H). Purity (HPLC): 97.4%, t_R = 15.49 min.

Published on 09 December 2020. Downloaded by University of Connecticut on 5/15/2021 1:42:17 PM.

***cis*-*N*-Benzyl-3,4-dihydrospiro[[2]benzopyran-1,1′-cyclohexan]-3′-amine (*cis*-4a).** Ketone **9** (100 mg, 0.46 mmol) was dissolved in THF (5 mL). Benzylamine (79 μL, 0.70 mmol), acetic acid (27 μL, 0.46 mmol) and $NaBH(OAc)_3$ (95%, 186 mg, 0.83 mmol) were added and the mixture was stirred for 24 h under a N_2 atmosphere at rt. NaOH (1 M, 10 mL) was added and the mixture was extracted with CH_2Cl_2 (3 × 20 mL) and with Et_2O (3 × 20 mL). The combined organic layers were dried (K_2CO_3), concentrated *in vacuo* and the residue was purified by FC (ϕ 2 cm, 20 cm, cyclohexane + 2% *N*,*N*-dimethylethanamine, 10 mL). R_f (cyclohexane + 2% *N*,*N*-dimethylethanamine = 0.17). Colorless oil, yield 128 mg (90%). $C_{21}H_{25}NO$ (307.5). MS (ESI): *m*/*z* (%) = 308 [MH,100]. FT-IR: $\tilde{\nu}$ (cm^{-1}) = 3341 (w, v, N–H), 3060, 3025 (w, C–H, arom), 2929, 2865 (s, v, C–H, alkyl), 1604, 1490 (w, C═C, arom), 1451 (m, δ, C–H, alkyl), 1089 (s, v, C–O), 751 (s, δ, C–H, *o*-disubst. arom), 729, 696 (s, δ, C–H, mono-subst. arom). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.45–1.54 (m, 2H, 4′-H, 5′-H or 6′-H), 1.70 ("td", J = 13.2/3.6 Hz, 1H, 6′-H or 5′-H), 1.79 (dd, J = 14.6/4.6 Hz, 1H, 2′-H), 1.85–1.94 (m, 1H, 5′-H or 6′-H), 1.97 – 2.03 (m, 1H, 6′-H or 5′-H), 2.06 – 2.13 (m, 1H, 4′-H), 2.32 (ddd, *J* = 14.6/4.4/2.2 Hz, 1H, 2′-H), 2.76 – 2.88 (m, 2H, $ArCH_2CH_2O$), 2.98–3.04 (m, 1H, 3′-H), 3.79 (d, *J* = 13.2 Hz, 1H, $ArCH_2NH$), 3.83 (d, *J* = 13.2 Hz, 1H, $ArCH_2NH$), 3.88 (ddd, *J* = 11.2/5.6/5.1 Hz, 1H, $ArCH_2CH_2O$), 3.97 (ddd, *J* = 11.5/6.2/5.1 Hz, 1H, $ArCH_2CH_2O$), 7.06–7.12 (m, 2H, Ar–H), 7.13–7.18 (m, 2H, Ar–H), 7.20–7.25 (m, 1H, Ar–H), 7.28–7.34 (m, 2H, Ar–H), 7.35–7.39 (m, 2H, Ar–H). A signal for the NH-proton is not seen in the spectrum. ^{13}C NMR ($CDCl_3$): δ (ppm) = 16.6 (1C, C-5′), 29.3 (1C, 4′-C), 29.9 (1C, $ArCH_2CH_2O$), 37.9 (1C, 6′-C), 38.8 (1C, 2′-C), 51.3 (1C, $NHCH_2Ar$), 52.0 (1C, 3′-C), 59.3 (1C, $ArCH_2CH_2O$), 77.1 (1C, 1′-C), 125.8 (1C, arom), 126.3 (1C, arom), 126.4 (1C, arom), 126.9 (1C, arom), 128.4 (2C, arom), 128.6 (2C, arom), 129.2 (1C, arom), 133.9 (1C, arom), 141.5 (1C, arom), 143.1 (1C, arom). Purity (HPLC): 99.1%, t_R = 15.9 min.

***cis*-*N*-(Cyclohexylmethyl)-3,4-dihydrospiro[[2]benzopyran-1,1′-cyclohexan]-3′-amine (*cis*-4b).** Ketone **9** (37 mg, 0.17 mmol) was dissolved in THF (5 mL). Cyclohexylmethylamine (98%, 30 mg, 0.26 mmol in THF (2 mL)), acetic acid (10 μL, 0.17 mmol), and $NaBH(OAc)_3$ (95%, 69 mg, 0.31 mmol) were added and the mixture was stirred for 24 h under a N_2 atmosphere at rt. Subsequently, NaOH (1 M, 10 mL) was added and the mixture was extracted with Et_2O (3 × 20 mL) and once with CH_2Cl_2 (3 × 20 mL). The combined organic layers were dried (K_2CO_3), concentrated *in vacuo* and the residue was purified by FC (ϕ 2 cm, 0 cm, cyclohexane: ethyl acetate + 1% *N*,*N*-dimethylethanamine, 10 mL). R_f (cyclohexane : ethyl acetate 9 : 1 + 1% *N*,*N*-dimethylethanamine = 0.11). Colorless oil, yield 43 mg (80%). $C_{21}H_{31}NO$ (313.5). MS (ESI): *m*/*z* (%) = 314 [MH, 100]. FT-IR: $\tilde{\nu}$ (cm^{-1}) = 3357 (w, v, N–H), 3059, 3019 (w, C–H, arom), 2919, 2848 (s, v, C–H, alkyl), 1489 (w, C═C, arom), 1448 (m, δ, C–H, alkyl), 1090 (s, v, C–O), 751 (s, δ, C–H, *o*-disubst. arom), 731, 668 (m, δ, monosubst. arom). ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.86–0.97 (m, 2H, NCH_2(cyclohexyl-*H*)), 1.10–1.32 (m, 3H, NCH_2(cyclohexyl-*H*)), 1.42–1.52 (m, 3H, NCH_2(cyclohexyl-*H*) (1H), 4′-H, 5′-H or 6′-H (2H)), 1.62 – 1.90 (m, 8H, NCH_2(cyclohexyl-*H*) (5H), 2′-H, 5′-H, 6′-H, (3H)), 1.92–1.99 (m, 1H, 6′-H or 5′-H), 1.99–2.08 (m, 1H, 4′-H), 2.26 (ddd, *J* = 14.8/4.4/2.1 Hz, 1H, 2′-H), 2.36 (dd, *J* = 11.1/6.5 Hz, 1H, NCH_2(cyclohexyl-H), 2.40 (dd, *J* = 11.1/7.0 Hz, 1H, NCH_2(cyclohexyl-H), 2.75–2.88 (m, 2H, $ArCH_2CH_2O$), 2.92–2.97 (m, 1H, 3′-H), 3.88 ("dt", *J* = 11.6/5.2 Hz, 1H, $ArCH_2CH_2O$), 3.94 (ddd, *J* = 11.5/7.0/4.7 Hz, 1H, $ArCH_2CH_2O$), 7.06–7.19 (m, 4H, Ar–H). A signal for the NH-proton is not seen in the spectrum. Purity (HPLC): 99.5%, t_R = 17.4 min.

***cis*-*N*-Benzyl-*N*-methyl-3,4-dihydrospiro[[2]benzopyran-1,1′-cyclohexan]-3′-amine (*cis*-5a).** Benzylamine *cis*-**4a** (56.6 mg, 0.18 mmol) was dissolved in CH_2Cl_2 (3.5 mL) and formalin (37%, stab. with 10–15% MeOH, 274 μL, 3.68 mmol) and $NaBH(OAc)_3$ (95%, 66 mg, 0.29 mmol) were added. The reaction mixture was stirred under a N_2 atmosphere at rt for 3 h. Subsequently, H_2O (10 mL) was added and the mixture was extracted with CH_2Cl_2 (4 × 20 mL) and once with Et_2O (20 mL). The combined organic layers were dried (K_2CO_3), concentrated *in vacuo* and the residue was purified by FC (ϕ 2 cm, 30 cm, cyclohexane + 2% *N*,*N*-dimethylethanamine, 10 mL). R_f (cyclohexane + 2% *N*,*N*-dimethylethanamine = 0.31, cyclohexane + 1% *N*,*N*-dimethylethanamine = 0.13). Colorless oil, yield 55 mg (92%). $C_{22}H_{27}NO$ (321.5). MS (ESI): *m*/*z* (%) = 322 [MH, 100]. FT-IR: $\tilde{\nu}$ (cm^{-1}) = 3060, 3023 (w, v, C–H, arom), 2932, 2858 (s, v, C–H, alkyl), 2782 (m, v, N–CH_3), 1603, 1490 (w, v, C═C, arom), 1450 (m, δ, C–H, alkyl), 1092 (s, v, C–O), 754 (s, δ, C–H, *o*-disubst. arom), 733, 698 (s, δ, C–H, mono-subst. arom). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.61–1.81 (m, 3H, 4′-H, 5′-H or 6′-H), 1.83–2.02 (m, 4H, 2′-H (1H), 4′-H, 5′-H or 6′-H (3H)), 2.17–2.21 (m, 1H, 2′-H), 2.21 (s, 3H, NCH_3), 2.86 (t, *J* = 5.7 Hz, 2H, $ArCH_2CH_2O$), 3.01 ("tt", *J* = 9.9/4.4 Hz, 1H, 3′-H), 3.54 (d, *J* = 13.5 Hz, 1H, NCH_2Ar), 3.65 (d, *J* = 13.5 Hz, 1H, NCH_2Ar), 3.94–4.03 (m, 2H, $ArCH_2CH_2O$), 7.08–7.18 (m, 3H, Ar–H), 7.20–7.33 (m, 6H, Ar–H). Purity (HPLC): 98.5%, t_R = 15.9 min.

***cis*-*N*-(Cyclohexylmethyl)-*N*-methyl-3,4-dihydrospiro[[2]benzopyran-1,1′-cyclohexan]-3′-amine (*cis*-5b).** Cyclohexylmethylamine *cis*-**4b** (19 mg, 0.06 mmol) was dissolved in CH_2Cl_2 (3.5 mL) and formalin (37%, stab. with 10–15% MeOH, 90 μL, 1.20 mmol) and $NaBH(OAc)_3$ (95%, 22 mg, 0.10 mmol) were added. The reaction mixture was stirred for 3 h under a N_2 atmosphere at rt. H_2O (10 mL) was added and the mixture was extracted with CH_2Cl_2 (4 × 20 mL). The combined organic layers were dried (K_2CO_3), concentrated *in vacuo* and the residue was purified by FC (ϕ 1.5 cm, cyclohexane + 1% *N*,*N*-dimethylethanamine, 20 cm, 10 mL). R_f (cyclohexane + 2% *N*,*N*-dimethylethanamine = 0.31, (cyclohexane + 1% *N*,*N*-dimethylethanamine = 0.15). Colorless oil, yield 18 mg (88%). $C_{22}H_{33}NO$ (327.6). MS (ESI): *m*/*z* (%) = 328 [MH, 100]. IR: $\tilde{\nu}$ (cm^{-1}) = 3067, 3021 (w, C–H, arom), 2920, 2848 (s, v, C–H, alkyl), 2786 (m, v, N–CH_3), 1488 (w, C═C, arom), 1448 (m, δ, C–H, alkyl), 755 (s, δ, C–H, *o*-disubst. arom), 733 (m, δ, C–H, mono-subst. arom). ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.75–0.89 (m, 2H, NCH_2(cyclohexyl-*H*)), 1.08–1.28 (m, 3H, NCH_2(cyclohexyl-*H*)), 1.30–1.40 (m, 1H, NCH_2(cyclohexyl-*H*)), 1.46–1.56 (m, 1H, 4′-H), 1.60–1.76 (m, 8H,(cyclohexyl-H) (4H),

 This journal is © The Royal Society of Chemistry 2021

Published on 09 December 2020. Downloaded by University of Connecticut on 5/15/2021 1:42:17 PM.

2′-H, 4′-H, 5′-H, 6′-H (4H)), 1.80–1.92 (m, 2H, (cyclohexyl-H) (1H), 5′-H (1H)), 1.96–2.05 (m, 1H, 6′-H), 2.12–2.22 (m, 3H, 2′-H (1H), NCH_2cyclohexyl (2H)) 2.24 (s, 3H, NCH_3), 2.86 (t, J = 5.9 Hz, 2H, ArCH_2CH_2O), 2.95 (tt, J = 11.4/3.9 Hz, 1H, 3′-H), 3.93–4.03 (m, 2H, ArCH_2CH_2O), 7.08–7.19 (m, 3H, Ar–H), 7.33–7.39 (m, 1H, Ar–H). ^{13}C NMR ($CDCl_3$): δ (ppm) = 21.1 (1C, 5′-C), 26.6 (2C, cyclohexyl-C), 26.7 (1C, 4′-C), 27.2 (1C, cyclohexyl-C), 29.7 (1C, ArCH$_2$CH$_2$O), 32.3 (2C, cyclohexyl-C), 36.7 (1C, cyclohexyl-C), 37.2 (1C, 6′-C), 39.3 (1C, NCH_3), 39.7 (1C, 2′-C), 59.3 (1C, ArCH$_2$$CH_2$O), 59.4 (1C, 3′-C), 60.6 (1C, NHCH$_2$Ar), 76.5 (1C, 1′-C), 125.6 (1C, arom), 126.0 (1C, arom), 126.4 (1C, arom), 129.5 (1C, arom), 133.9 (1C, arom), 144.0 (1C, arom). Purity (HPLC): 99.0%, t_R = 16.9 min.

5.4 Receptor binding studies

The σ_1 and σ_2 affinities were recorded as described in references.[45–47] Details of the assays are given in the ESI.†

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft, which is gratefully acknowledged.

References

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

2 S. W. Tam, Naloxone-inaccessible in rat central nervous system, *Proc. Natl. Acad. Sci. U. S. A.*, 1983, **80**, 6703–6707.

3 D. B. Vaupel, Naltrexone fails to antagonize the σ effects of PCP and SKF 10,047 in the dog, *Eur. J. Pharmacol.*, 1983, **92**, 269–274.

4 W. D. Bowen, S. B. Hellewell and K. A. McGarry, Evidence for a multi-site model of the rat brain sigma receptor, *Eur. J. Pharmacol.*, 1989, **163**, 309–318.

5 S. B. Hellewell, A. Bruce, G. Feinstein, J. Orringer, W. Williams and W. D. Bowen, Rat liver and kidney contain high densities of sigma 1 and sigma 2 receptors: characterization by ligand binding and photoaffinity labelling, *Eur. J. Pharmacol.*, 1994, **268**, 9–18.

6 C. Ela, J. Barg, Z. Vogel, Y. Hasin and Y. Eilam, Sigma receptor ligands modulate contractility, Ca++ influx and beating rate in cultured cardiac myocytes, *J. Pharmacol. Exp. Ther.*, 1994, **269**, 1300–1309.

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

8 H. R. Schmidt, S. Zheng, E. Gurpinar, A. Koehl, A. Manglik and A. C. Kruse, Crystal structure of the human σ_1 receptor, *Nature*, 2016, **532**, 527–530.

9 H. R. Schmidt, R. M. Betz, R. O. Dror and A. C. Kruse, Structural basis for σ_1 receptor ligand recognition, *Nat. Struct. Mol. Biol.*, 2018, **25**, 981–987.

10 P. D. Lupardus, R. A. Wilke, E. Aydar, C. P. Palmer, Y. Chen, A. E. Ruoho and M. B. Jackson, Membrane-delimited coupling between sigma receptors and K^+ channels in rat neurohypophysial terminals requires neither G-Protein nor ATP, *J. Physiol.*, 2000, **526**, 527–539.

11 W. C. Hong, H. Yano, T. Hiranita, F. T. Chin, C. R. McCurdy, T. P. Su, S. G. Amara and J. L. Katz, The sigma-1 receptor modulates dopamine transporter conformation and cocaine binding and may thereby potentiate cocaine self-administration in rats, *J. Biol. Chem.*, 2017, **292**, 11250–11261.

12 E. J. Cobos, J. M. Entrena, F. R. Nieto, C. M. Cendan and E. Del Pozo, Pharmacology and Therapeutic Potential of Sigma(1) Receptor Ligands, *Curr. Neuropharmacol.*, 2008, **6**(4), 344–366.

13 T. Hayashi, S. Y. Tsai, T. Mori, M. Fujimoto and T. P. Su, Targeting ligand-operated chaperone sigma-1 receptors in the treatment of neuropsychiatric disorders, *Expert Opin. Ther. Targets*, 2011, **15**, 557–577.

14 T. Hayashi and T. P. Su, Sigma-1 receptor ligands – Potential in the treatment of neuropsychiatric disorders, *CNS Drugs*, 2004, **18**(5), 269–284.

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

16 A. Urani, F. J. Roman, V. L. Pham, T. P. Su and T. Maurice, The Antidepressant-Like Effect Induced by σ_1-Receptor Agonists and Neuroactive Steroids in Mice Submitted to the Forced Swimming Test, *J. Pharmacol. Exp. Ther.*, 2001, **298**, 1269–1279.

17 J. L. Diaz, R. Cuberes, J. Berrocal, M. Contijoch, U. Christmann, A. Fernandez, A. Port, J. Holenz, H. Buschmann, C. Laggner, M. T. Serafini, J. Burgueno, D. Zamanillo, M. Merlos, J. M. Vela and C. Almansa, Synthesis and biological evaluation of the 1-arylpyrazole class of σ(1) receptor antagonists: identification of 4-{2-[5-methyl-1-(naphthalen-2-yl)-1H-pyrazol-3-yloxy]ethyl}morpholine (S1RA, E-52862), *J. Med. Chem.*, 2012, **55**, 8211–8224.

18 B. Wünsch, The σ_1 receptor antagonist S1RA is a promising candidate for the treatment of neurogenic pain, *J. Med. Chem.*, 2012, **55**, 8209–8210.

19 E. Aydar, C. P. Palmer and M. B. Djamgoz, Sigma receptors and cancer: possible involvement of ion channels, *Cancer Res.*, 2004, **64**, 5029–5035.

20 A. van Waarde, A. A. Rybczynska, N. K. Ramakrishnan, K. Ishiwata, P. H. Elsinga and R. A. J. O. Dierckx, Potential applications for sigma receptor ligands in cancer diagnosis and therapy, *Biochim. Biophys. Acta*, 2015, **1848**(10), 2703–2714.

21 F. J. Kim and C. M. Maher, Sigma1 pharmacology in the context of cancer, *Handb. Exp. Pharmacol.*, 2017, **244**, 237–308.

22 B. A. Spruce, L. A. Campbell, N. McTavish, M. A. Cooper, M. V. Appleyard, M. O'Neill, J. Howie, J. Samson, S. Watt, K.

Published on 09 December 2020. Downloaded by University of Connecticut on 5/15/2021 1:42:17 PM.

Murray, D. McLean, N. R. Leslie, S. T. Safrany, M. J. Ferguson, J. A. Peters, A. R. Prescott, G. Box, A. Hayes, B. Nutley, F. Raynaud, C. P. Downes, J. J. Lambert, A. M. Thompson and S. Eccles, Small molecule antagonists of the sigma-1 receptor cause selective release of the death program in tumor and self-reliant cells and inhibit tumor growth in vitro and in vivo, *Cancer Res.*, 2004, **64**, 4875–4886.

23 B. J. Vilner, C. S. John and W. D. Bowen, Sigma-1 and Sigma-2 Receptors Are Expressed in a Wide Variety of Human and Rodent Tumor Cell Lines, *Cancer Res.*, 1995, **55**, 408–413.

24 K. Hashimoto and K. Ishiwata, Sigma receptor ligands: possible application as therapeutic drugs and radiopharmaceuticals, *Curr. Pharm. Des.*, 2006, **12**, 3857–3876.

25 A. Alon, H. R. Schmidt, M. D. Wood, J. J. Sahn, S. F. Martin and A. C. Kruse, Identification of the gene that codes for the σ2 receptor, *Proc. Natl. Acad. Sci. U. S. A.*, 2017, **114**, 7160–7165.

26 K. Terada, K. Migita, Y. Matsushima and C. Kamei, Sigma-2 receptor as a potential therapeutic target for treating central nervous system disorders, *Neural Regener. Res.*, 2019, **14**, 1893–1894.

27 C. Katnik, A. Garcia, A. A. Behensky, I. E. Yasny, A. M. Shuster, S. B. Seredenin, A. V. Petrov and J. Cuevas, Activation of σ_1 and σ_2 receptors by afobazole increases glial cell survival and prevents glial cell activation and nitrosative stress after ischemic stroke, *J. Neurochem.*, 2016, **139**, 497–509.

28 V. Megalizzi, M. Le Mercier and C. Decaestecker, Sigma receptors and their ligands in cancer biology: overview and new perspectives for cancer therapy, *Med. Res. Rev.*, 2012, **32**, 410–427.

29 R. H. Mach, C. Zeng and W. G. Hawkins, The σ_2 receptor: a novel protein for the imaging and treatment of cancer, *J. Med. Chem.*, 2013, **56**, 7137–7160.

30 C. Zeng, J. Rothfuss, J. Zhang, W. Chu, S. Vangveravong, Z. Tu, F. Pan, K. C. Chang, R. Hotchkiss and R. H. Mach, Sigma-2 ligands induce tumor cell death by multiple signaling pathways, *Br. J. Cancer*, 2012, **106**, 693–701.

31 S. Collina, R. Gaggeri, A. Marra, A. Bassi, S. Negrinotti, F. Negri and D. Rossi, Sigma receptor modulators: A patent review, *Expert Opin. Ther. Pat.*, 2013, **23**, 597–613.

32 C. A. Maier and B. Wünsch, Novel Spiropiperidines as Highly Potent and Subtype Selective σ-Receptor Ligands. Part 1, *J. Med. Chem.*, 2002, **45**, 438–448.

33 C. A. Maier and B. Wünsch, Novel σ Receptor Ligands. Part 2. SAR of Spiro[[2]benzopyran-1,4`-piperidines] and Spiro[[2]-benzofuran-1,4`-piperidines] with Carbon Substituents in Position 3, *J. Med. Chem.*, 2002, **45**, 4923–4930.

34 T. Schläger, D. Schepmann, K. Lehmkuhl, J. Holenz, J. M. Vela, H. Buschmann and B. Wünsch, Combination of two pharmacophoric systems: synthesis and pharmacological evaluation of spirocyclic pyranopyrazoles with high σ_1 receptor affinity, *J. Med. Chem.*, 2011, **54**, 6704–6713.

35 C. Oberdorf, D. Schepmann, J. M. Vela, H. Buschmann, J. Holenz and B. Wünsch, Thiophene bioisosteres of spirocyclic σ receptor ligands: Relationships between substitution pattern and σ receptor affinity, *J. Med. Chem.*, 2012, **55**, 5350–5360.

36 C. Meyer, D. Schepmann, S. Yanagisawa, J. Yamaguchi, V. Dal Col, E. Laurini, K. Itami, S. Pricl and B. Wünsch, Thiopehene Pd-catalyzed direct C-H bond functionalization of spirocyclic σ_1 ligands: Generation of a pharmacophore model and analysis of the reverse binding mode by docking into a 3D homology model of the σ_1 receptor, *J. Med. Chem.*, 2012, **55**, 8047–8065.

37 K. Miyata, G. Möller, D. Schepmann and B. Wünsch, Pyridine analogues of spirocyclic σ_1 receptor ligands, *Bioorg. Med. Chem.*, 2014, **22**, 4277–4284.

38 S. Y. Ablordeppey, J. B. Fischer, H. Law and R. A. Glennon, Probing the proposed phenyl-A region of the sigma-1 receptor, *Bioorg. Med. Chem.*, 2002, **10**, 2759–2765.

39 E. Rack, R. Fröhlich, D. Schepmann and B. Wünsch, Design, synthesis and pharmacological evaluation of spirocyclic σ_1 receptor ligands with exocyclic amino moiety (increased distance 1), *Bioorg. Med. Chem.*, 2011, **19**, 3141–3151.

40 E. Kronenberg, F. Weber, S. Brune, D. Schepmann, C. Almansa, K. Friedland, E. Laurini, S. Pricl and B. Wünsch, Synthesis and structure-affinity relationships of spirocyclic benzopyrans with exocyclic amino moiety, *J. Med. Chem.*, 2019, **62**, 4204–4217.

41 W. E. Parham, L. D. Jones and Y. A. Sayed, Selective halogen-lithium exchange in bromophenylalkyl halides, *J. Org. Chem.*, 1976, **41**, 1184–1186.

42 T. Hayashi, H. Takagi, H. Masuda and H. Ogoshi, Synthesis and structure of new cis-1,3-dihydroxycyclohexane derivative having four convergent hydroxy groups, *J. Chem. Soc., Chem. Commun.*, 1993, 346–365.

43 H. Takagi, T. Hayashi, H. Mizutani and H. Ogoshi, Synthesis and structure of tetraols with convergent and divergent arrays of hydroxy groups, *J. Chem. Soc., Perkin Trans. 1*, 1999, 1885–1892.

44 A. F. Abdel-Magid and S. J. Mehrman, A Review on the Use of Sodium Triacetoxyborohydride in the Reductive Amination of Ketones and Aldehydes, *Org. Process Res. Dev.*, 2006, **10**, 971–1031.

45 P. Hasebein, B. Frehland, K. Lehmkuhl, R. Fröhlich, D. Schepmann and B. Wünsch, Synthesis and pharmacological evaluation of like- and unlike-configured tetrahydro-2-benzazepines with the α-substituted benzyl moiety in the 5-position, *Org. Biomol. Chem.*, 2014, **12**, 5407–5426.

46 C. Meyer, B. Neue, D. Schepmann, S. Yanagisawa, J. Yamaguchi, E.-U. Würthwein, K. Itami and B. Wünsch, Improvement of σ1 receptor affinity by late-stage C-H-bond arylation of spirocyclic lactones, *Bioorg. Med. Chem.*, 2013, **21**, 1844–1856.

47 K. Miyata, D. Schepmann and B. Wünsch, Synthesis and σ receptor affinity of regioisomeric spirocyclic furopyridines, *Eur. J. Med. Chem.*, 2014, **83**, 709–716.

 This journal is © The Royal Society of Chemistry 2021