

Synthesis, Receptor Affinity, and Antiallodynic Activity of Spirocyclic σ Receptor Ligands with Exocyclic Amino Moiety

Bergkemper, M.; Kronenberg, E.; Thum, S.; Börgel, F.; Daniliuc, C.; Schepmann, D.; Nieto, F. R.; Brust, P.; Reinoso, R. F.; Alvarez, I.; Wünsch, B.;

Originally published:

October 2018

Journal of Medicinal Chemistry 61(2018), 9666-9690

DOI: <https://doi.org/10.1021/acs.jmedchem.8b01183>

Perma-Link to Publication Repository of HZDR:

<https://www.hzdr.de/publications/Publ-28319>

Release of the secondary publication
on the basis of the German Copyright Law § 38 Section 4.

1
2
3 **Synthesis, receptor affinity and antiallodynic activity of spirocyclic σ receptor**
4
5 **ligands with exocyclic amino moiety**
6
7
8

9 Melanie Bergkemper,^a Elisabeth Kronenberg,^a Simone Thum,^a Frederik Börgel,^a
10 Constantin Daniliuc,^b Dirk Schepmann,^a Francisco Rafael Nieto,^c Peter Brust,^d
11 Raquel F. Reinoso,^e Inés Alvarez,^e Bernhard Wünsch*^{a,f}
12
13
14
15

16
17
18 ^a Institut für Pharmazeutische und Medizinische Chemie der Universität Münster,
19 Corrensstraße 48, D-48149 Münster, Germany
20

21
22 Tel.: +49-251-8333311; Fax: +49-251-8332144; E-mail: wuensch@uni-muenster.de
23

24 ^b Organisch-Chemisches Institut der Westfälischen Wilhelms-Universität Münster,
25 Corrensstraße 40, D-48149 Münster, Germany
26
27

28 ^c Department of Pharmacology and Institute of Neuroscience, School of Medicine and
29 Biomedical Research Center, University of Granada, Avenida de la Investigación 11,
30 18016 Granada, Spain
31
32
33

34 ^d Helmholtz-Zentrum Dresden-Rossendorf, Institut für Radiopharmazeutische
35 Krebsforschung, Forschungsstelle Leipzig, Permoserstr. 15, 04318 Leipzig, Germany
36
37
38

39 ^e Esteve Pharmaceuticals S.A., Baldiri Reixach 4-8, 08028 Barcelona, Spain.
40

41 ^f Cells-in-Motion Cluster of Excellence (EXC 1003 – CiM), Westfälische Wilhelms-
42 Universität Münster, Germany
43
44
45

46
47
48 **Abstract**
49

50 In order to detect novel σ receptor ligands the rigid spiro[[2]benzopyran-1,1'-
51 cyclohexan]-4'-one was connected with amino moieties derived from σ_2 receptor
52 preferring lead compounds resulting in mixtures of *trans*- and *cis*-configured amines
53
54
55
56
57
58
59
60

1
2
3 **6, 18** and **27**. In a four step synthesis the methyl acetals **6** were converted into
4
5 fluoroethyl derivatives **13** and **30**. The most promising σ_2 receptor ligand is the methyl
6
7 acetal **6a** bearing a 2,4-dimethylbenzylamino moiety. The fluoroethyl derivatives **13c**
8
9 and **13d** reveal high σ_1 affinity, but moderate selectivity over the σ_2 subtype. In mice
10
11 **13c** and **13d** showed antiallodynic activity, which is stronger than those of the
12
13 reference σ_1 antagonist BD-1063 (**34**). Since the antiallodynic activity of **13c** could
14
15 only be partially reversed by the σ_1 agonist PRE-084 (**35**), it is postulated that a
16
17 second mechanism contributes to its overall antiallodynic effect. In contrast, the
18
19 antiallodynic effect of its diastereomer **13d** can be totally explained by a σ_1
20
21 antiallodynic effect of its diastereomer **13d** can be totally explained by a σ_1
22
23 antagonism.
24
25
26

27 **Keywords**

28
29 σ receptors; spirocyclic ligands; X-ray crystal structure; Domino reaction; reductive
30
31 amination; *cis-trans*-configuration; structure-affinity relationship; receptor selectivity;
32
33 stability; antiallodynic activity; σ_1 antagonist.
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1. Introduction

Although a lot of research was performed in the field of σ receptors, their biological function is not totally understood. The σ receptor was first described as opioid receptor subtype, since some of the pharmacological effects of the racemic benzomorphan SKF-10,047 (N-allylnormetazocine) could be antagonized by the opioid antagonist naltrexone.¹ Later it was observed that (+)-SKF-10,047 acts as a σ agonist, whereas (-)-SKF-10,047 shows affinity towards MOR (μ opioid receptor) and KOR (κ opioid receptor).² Moreover, the effects of (+)-SKF-10,047 could not be blocked by the opioid antagonist naloxone.³ This led to the classification of σ receptors as an own class of receptors with two subtypes, σ_1 and σ_2 receptors.^{4; 5} Their endogenous ligands are still unknown, although different endogenous compounds with affinity towards σ_1 receptors including neuroactive steroids⁶⁻⁸, *N,N*-dimethyltryptamine⁹ and sphingosine derivatives¹⁰ were identified.

The σ_1 receptor has been cloned from different tissues like guinea pig liver,¹¹ mouse kidney¹² and human placental choriocarcinoma cells. The human σ_1 receptor consists of 223 amino acids¹³ and has a molecular weight of 25 kDa.¹⁴ Its sequence shows a similarity of 66 % with the yeast Δ^8/Δ^7 sterol isomerase but no similarity to any other mammalian protein. Indeed, sterol isomerase activity could not be observed for the σ_1 receptor.¹¹ In 2016, the σ_1 receptor was crystallized by Kruse *et al.* showing a trimeric organization of the receptor with one transmembrane domain for each monomer.¹⁵ σ_1 receptors are particularly enriched in mitochondrion-associated endoplasmic reticulum (ER) membranes (MAM)¹⁶ of neuronal¹⁷ and peripheral cells like liver cells¹⁸ or cardiac myocytes.¹⁹ Under stress situations σ_1 receptors can also translocate to the plasma membrane²⁰ or ER-associated

1
2
3 membranes,²¹ where they can regulate other proteins. The cellular mechanisms
4
5 connected to the σ_1 receptor are not elucidated in detail. The σ_1 receptor seems to
6
7 act as a chaperone²² interacting with IP₃ receptor and modulating Ca²⁺, K⁺, Na⁺ and
8
9 Cl⁻ channels.²³⁻²⁷ It is involved in pathomechanisms of neurological diseases like
10
11 addiction,²⁸ Alzheimer's disease,²⁹ depression³⁰ and schizophrenia.³¹ Moreover, it
12
13 has an influence on pain³² and allodynia, which makes it an interesting target for the
14
15 therapy of these diseases.
16
17
18
19

20 Allodynia belongs to the symptoms of neuropathic pain, which is induced by several
21
22 CNS diseases like postherpetic neuralgia, multiple sclerosis or stroke. It is
23
24 characterized by the evocation of pain by stimuli, which normally do not provoke pain.
25
26 Therapeutic options for the treatment of allodynia are tricyclic antidepressants,
27
28 serotonin-norepinephrine reuptake inhibitors, gabapentinoids, opioids, cannabinoids,
29
30 lamotrigine, mexiletine, lidocaine gel, and botulinum toxin-A. Besides Na⁺ channels,
31
32 protein kinases, glutamate and tyrosine kinase receptors,³³ the σ_1 receptor is
33
34 involved in the molecular mechanisms, which cause allodynia. The knockout of the σ_1
35
36 receptor in mice led to the inhibition of capsaicin-induced mechanical allodynia. The
37
38 same effect was observed dose-dependently by treatment of wild-type mice with σ_1
39
40 receptor antagonists. Moreover, this antiallodynic effect could be blocked by σ_1
41
42 receptor agonists.³⁴ Therefore, σ_1 receptor antagonists offer a new possibility for the
43
44 treatment of allodynia. The most developed candidate is the σ_1 receptor antagonist
45
46 S1RA, which shows high activity in different models of neuropathic pain. After
47
48 successful completion of phase I, the phase II clinical trial for neuropathic pain of
49
50 S1RA is currently ongoing.³⁵
51
52
53
54
55
56
57
58
59
60

1
2
3 Recently, the identity of the σ_2 receptor and TMEM97, an endoplasmic reticulum-
4 resident transmembrane protein, was demonstrated, which also led to the
5 identification of the gene coding for the σ_2 receptor.³⁶ The σ_2 receptor has a
6 molecular weight of 21.5 kDa¹⁴ and is located in the central nervous system³⁷ and in
7 peripheral tissue like liver and kidney.¹⁴ On the cellular level it is located in
8 mitochondria and ER membranes.³⁸ Although the signal transduction of the σ_2
9 receptor is not yet fully understood, its influence on cell differentiation and survival is
10 well-known.³⁸ It seems to have an influence on Ca^{2+} and K^+ channels^{39; 40} and
11 interacts with caspase-3 and the mTOR signaling pathway.⁴¹ These findings can
12 explain the apoptotic effect of σ_2 receptor ligands.⁴²
13
14
15
16
17
18
19
20
21
22
23
24
25

26 Both, the σ_1 and σ_2 receptor, are expressed in fast proliferating cells like prostate
27 cancer, breast carcinoma or leukemia cells. For the σ_2 receptor it was observed that
28 the expression in proliferating cancer cells is 10-fold higher than in quiescent cancer
29 cells, which still show higher expression than the surrounding tissue. Additionally, in
30 stem cells as a type of proliferating cells the σ_2 receptor density is higher than in
31 differentiated cells.⁴³ Therefore, the σ_2 receptor can be considered as a biomarker for
32 the proliferative status of cells, offering new opportunities for cancer diagnosis and
33 therapy.
34
35
36
37
38
39
40
41
42
43
44
45

46 Various compound classes with affinity towards σ_1 and σ_2 receptors are described in
47 the literature. Compounds bearing a cyclohexylpiperazine moiety such as PB28 (**1**)
48 show high σ_1 and σ_2 affinity ($K_i(\sigma_1) = 0.38$ nM; $K_i(\sigma_2) = 0.68$ nM).⁴⁴ The 1-(4-
49 fluorophenyl)-substituted indole derivative siramesine (**2**) displays a preference for
50 the σ_2 receptor ($K_i(\sigma_1) = 17$ nM; $K_i(\sigma_2) = 0.12$ nM).⁴⁵ A high selectivity towards the σ_2
51
52
53
54
55
56
57
58
59
60

receptor was achieved by compounds of type **3** ($K_i(\sigma_1) = 12.9 \mu\text{M}$; $K_i(\sigma_2) = 8.2 \text{ nM}$) containing a benzamide and an isoquinoline moiety connected by an aliphatic spacer. (Figure 1).⁴⁶ A common feature of these compounds is the conformationally flexible linker between the basic amino moiety and the aromatic system.

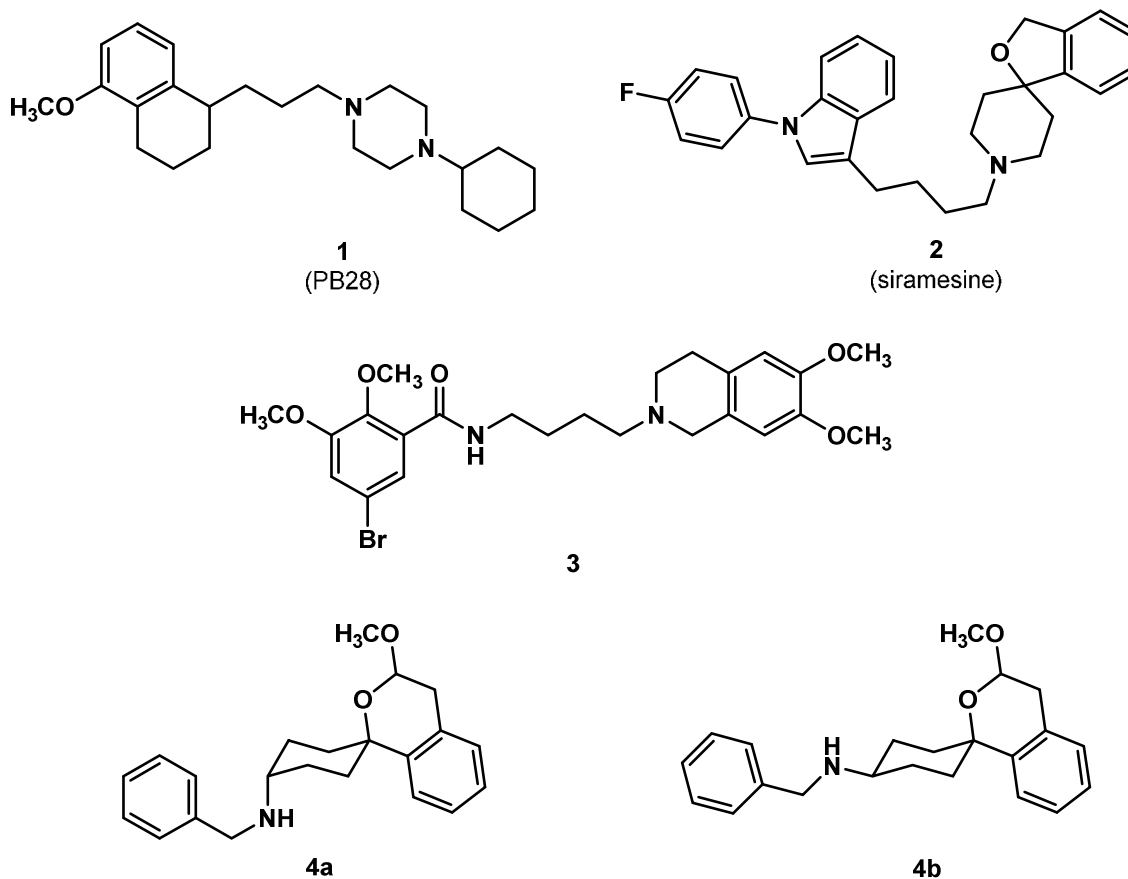
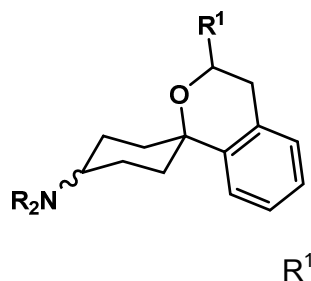


Figure 1. Chemical structures of σ receptor ligands.

In our group different σ ligands with a spirocyclic ring system as conformationally restricted scaffold were synthesized.⁴⁷⁻⁵² The spirocyclic 2-benzopyrans **4a** and **4b** with exocyclic amino moiety show moderate affinity towards both σ receptors (**4a**: $K_i(\sigma_1) = 538 \text{ nM}$; $K_i(\sigma_2) > 1000 \text{ nM}$; **4b**: $K_i(\sigma_1) = 158 \text{ nM}$; $K_i(\sigma_2) > 1000 \text{ nM}$).⁴⁹ Based on these affinity data, combination of the spirocyclic 2-benzopyran with pharmacophoric moieties of the reference compounds **1** - **3** was envisaged to obtain

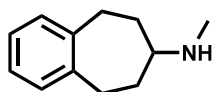
compounds with high σ_1 and/or σ_2 affinity. Furthermore, exchanging the methoxy group in 3-position of the 2-benzopyrans **4a** and **4b** by a fluoroethyl side chain should increase the metabolic stability of the compounds. An overview of all synthesized compounds is shown in Table 1. The influence of the introduction of a fluoroethyl side chain at 3-position of the 2-benzopyran on σ_1 and σ_2 receptor affinity will be investigated. Promising candidates will be selected for further pharmacological characterization in *in vitro* and *in vivo* experiments to determine the antiallodynic activity of these σ receptor ligands.

Table 1: Synthesized spirocyclic compounds with modification of the basic moiety and the residue in 3-position of the 2-benzopyran scaffold.



	NR ₂	OCH ₃	OH	CH ₂ CO ₂ Et	CH ₂ CH ₂ OH	CH ₂ CH ₂ F
		6a,b	10a,b	11a,b	12a,b	13a,b
		6c,d	10c,d	11c,d	12c,d	13c,d
		6e,f	10e,f	11e,f	12e,f	13e,f
		18a,b	-	-	-	-

8



27a,b

-

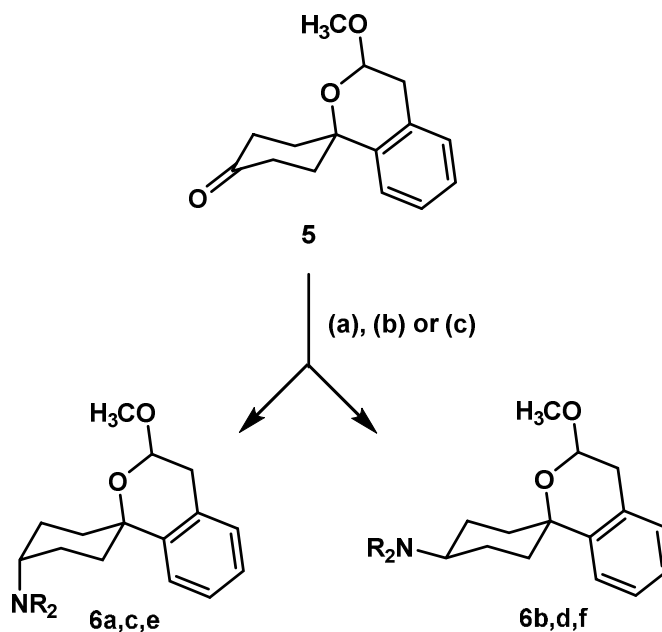
-

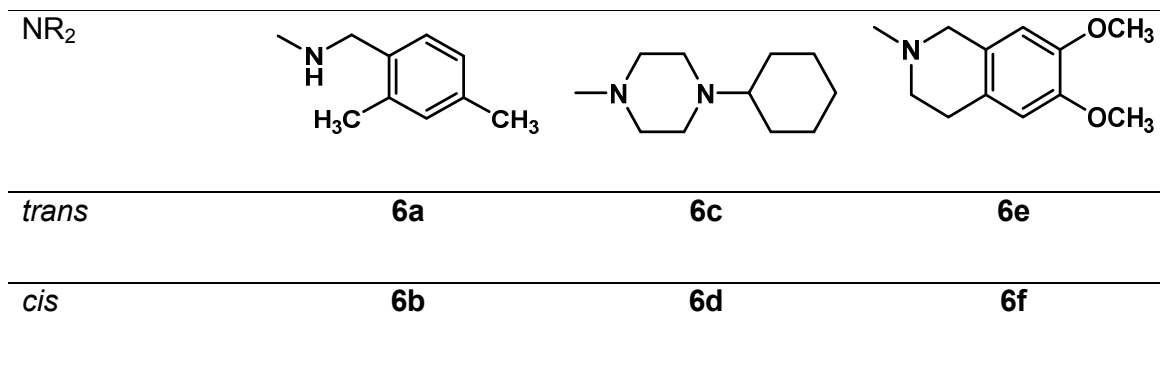
-

30a,b

2. Synthesis

For the synthesis of the designed σ ligands the spirocyclic ketone **5**, which was obtained in four steps starting from 2-bromobenzaldehyde,⁴⁹ served as important intermediate. The reductive amination of ketone **5** with 2,4-dimethylbenzylamine (**7**) and NaBH(OAc)₃ in THF and HOAc led to the diastereomeric amines **6a** and **6b**, which were separated by fc. Performing this reaction under the same conditions with 1-cyclohexylpiperazine (**8**) led to amines **6c** and **6d**. Because of low solubility of 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (**9**·HCl) in THF, the synthesis of amines **6e** and **6f** was conducted in CH₂Cl₂ and without HOAc (Scheme 1).





Scheme 1. Synthesis of spirocyclic σ receptor ligands with exocyclic amino moiety.

Reagents and reaction conditions: (a) 2,4-dimethylbenzylamine, NaBH(OAc)₃, HOAc, THF, 20 h, rt; **6a**, 30 %; **6b**, 44 %. (b) 1-cyclohexylpiperazine, NaBH(OAc)₃, HOAc, THF, 23 h, rt; **6c**, 9 %; **6d**, 38 %. (c) 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride, NaBH(OAc)₃, CH₂Cl₂, 43 h, rt; **6e**, 19 %; **6f**, 37 %.

According to the isolated yields the formation of the diastereomers **6b**, **6d** and **6f** with the amino moiety in equatorial orientation is preferred. This can be explained by stereoelectronic effects during the hydride transfer to intermediate iminium ions. The attack of the hydride in axial orientation at the cyclohexane ring can be stabilized by an overlap of the doubly occupied σ orbital of the newly formed C-H bond and the unoccupied, axial oriented σ^* orbitals of the C-H bonds in α position.⁵³ Therefore, the axial hydride attack is more favorable than the equatorial approach, which leads to an excess of the diastereomer with the amino moiety in equatorial orientation. Using secondary amines instead of primary amines for the reductive amination, the difference between the yields of the diastereomers is higher.

To determine the configuration of the spirocyclic amines in 1-position of the 2-benzopyran, X-ray crystal structures of the isoquinoline derivatives **6e** and **6f** were recorded exemplarily. As shown in Figure 2, the oxygen atom of the 2-benzopyran of

both diastereomers is in axial orientation and the aromatic moiety is equatorially oriented regarding the cyclohexane chair. Therefore, in compounds **6a**, **6c**, and **6e** with the amino moiety in axial orientation, the O- and N-atoms are *trans*-oriented and therefore the compounds are termed *trans*-configured in this manuscript. On the other side, in compounds **6b**, **6d**, and **6f** O- and N-atoms are *cis*-oriented with respect to the cyclohexane chair and are termed *cis*-diastereomers in this manuscript.

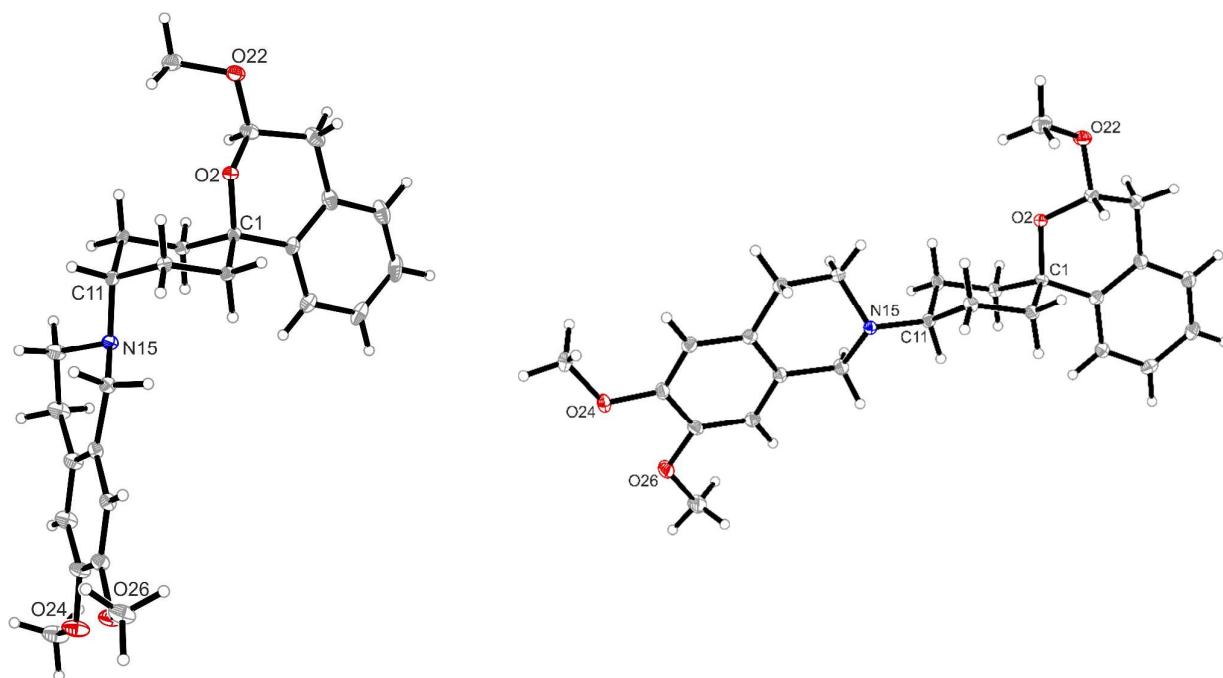
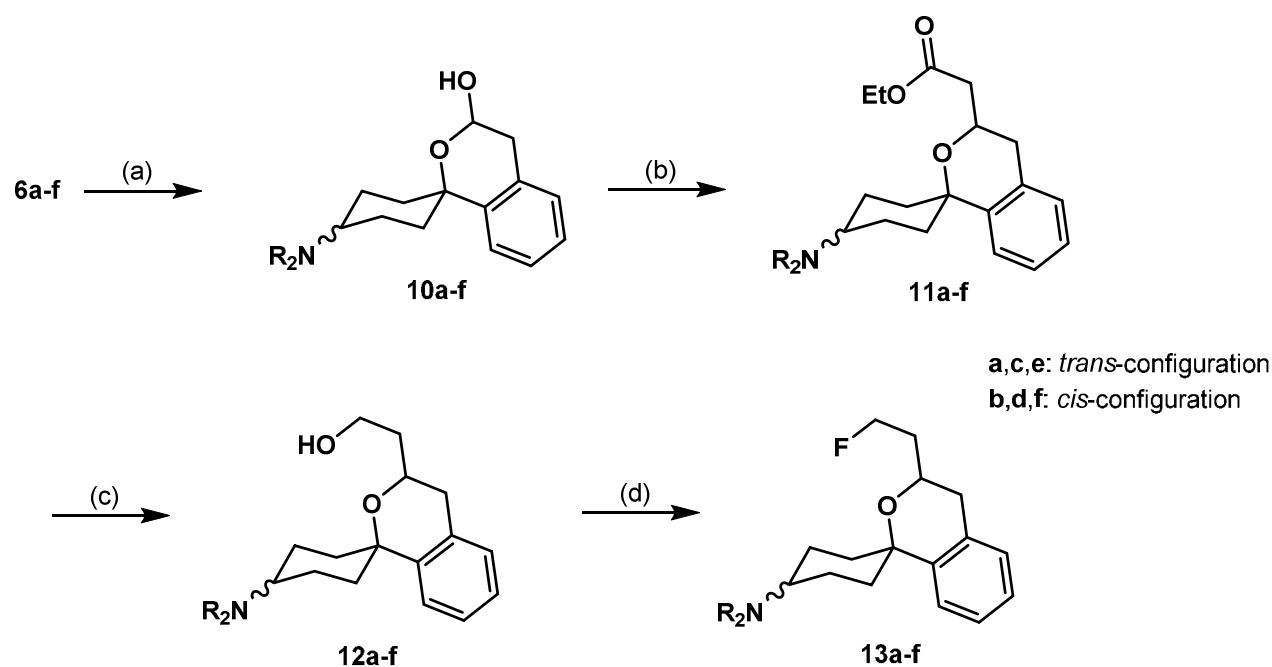


Figure 2. X-ray crystal structures of the *trans*- and *cis*- configured acetalic isoquinoline derivatives **6e** and **f**. Thermal ellipsoids are shown at 30 % and 50 %, respectively. CCDC number: **6e**: 1855388; **6f**: 1855389.

Hydrolysis of the acetals **6a-f** resulted in the formation of lactols **10a-f**. Afterwards, a tandem reaction with (ethoxycarbonylmethylene)triphenylphosphorane in toluene was performed. The first step in this one pot reaction is the opening of the lactol to afford

an hydroxy aldehyde, followed by a Wittig reaction and an intramolecular Michael addition at the formed α,β -unsaturated ester.⁴⁸ The esters **11a-f** were obtained in yields of 39 - 94 %. The following reduction of the ester with LiAlH_4 in Et_2O gave the alcohols **12a-f** in high yields. Fluorination of the alcohols **12a-f** with DAST (diethylaminosulfur trifluoride) led to compounds **13a-f** in yields of 13 - 67 % (Scheme 2).

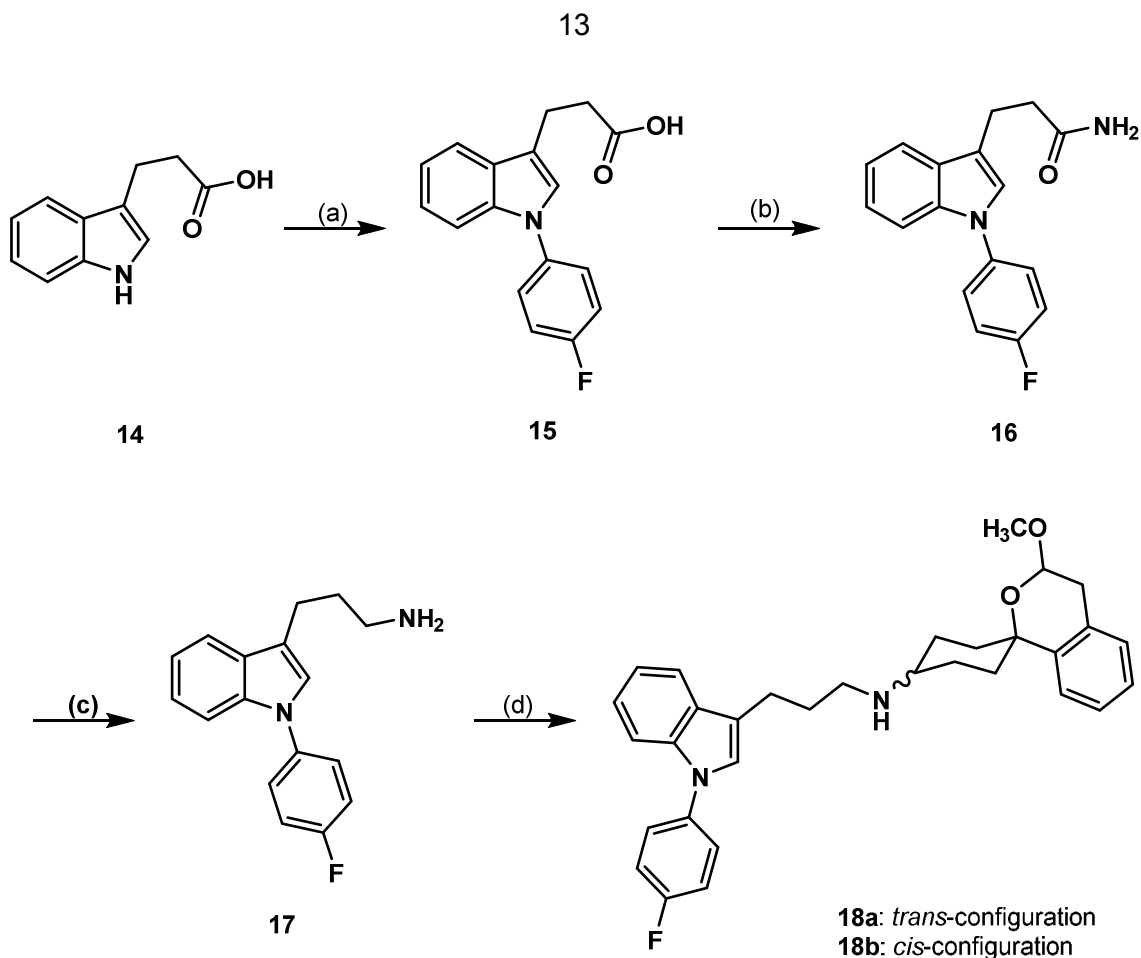


Scheme 2. Synthesis of spirocyclic σ receptor ligands with fluoroethyl side chain.

Reagents and reaction conditions: (a) HCl , THF , 3 - 4 d, rt; **10a**, 71 %; **10b**, 80 %; **10c**, 86 %; **10d**, 81 %; **10e**, 86 %; **10f**, 74 %. (b) $\text{Ph}_3\text{P}=\text{CHCO}_2\text{Et}$, toluene, 3 - 5 d, reflux; **11a**, 39 %; **11b**, 79 %; **11c**, 52 %; **11d**, 89 %; **11e**, 94 %; **11f**, 77 %. (c) LiAlH_4 , Et_2O , 2 - 4 h, $-20\text{ }^\circ\text{C}$; **12a**, 79 %; **12b**, 82 %; **12c**, 85 %; **12d**, 82 %; **12e**, 71 %; **12f**, 69 %. (d) DAST, CH_2Cl_2 , 1 h $-78\text{ }^\circ\text{C}$, then 20 h rt; **13a**, 67 %; **13b**, 22 %; **13c**, 67 %; **13d**, 13 %; **13e**, 21 %; **13f**, 27 %.

10-13a,b: $\text{NR}_2 = 2,4\text{-dimethylbenzylamino}$; **10-13c,d**: $\text{NR}_2 = 4\text{-cyclohexylpiperazin-1-yl}$; **10-13e,f**: $\text{NR}_2 = 6,7\text{-dimethoxyisoquinolin-2-yl}$. Structures see Table 1.

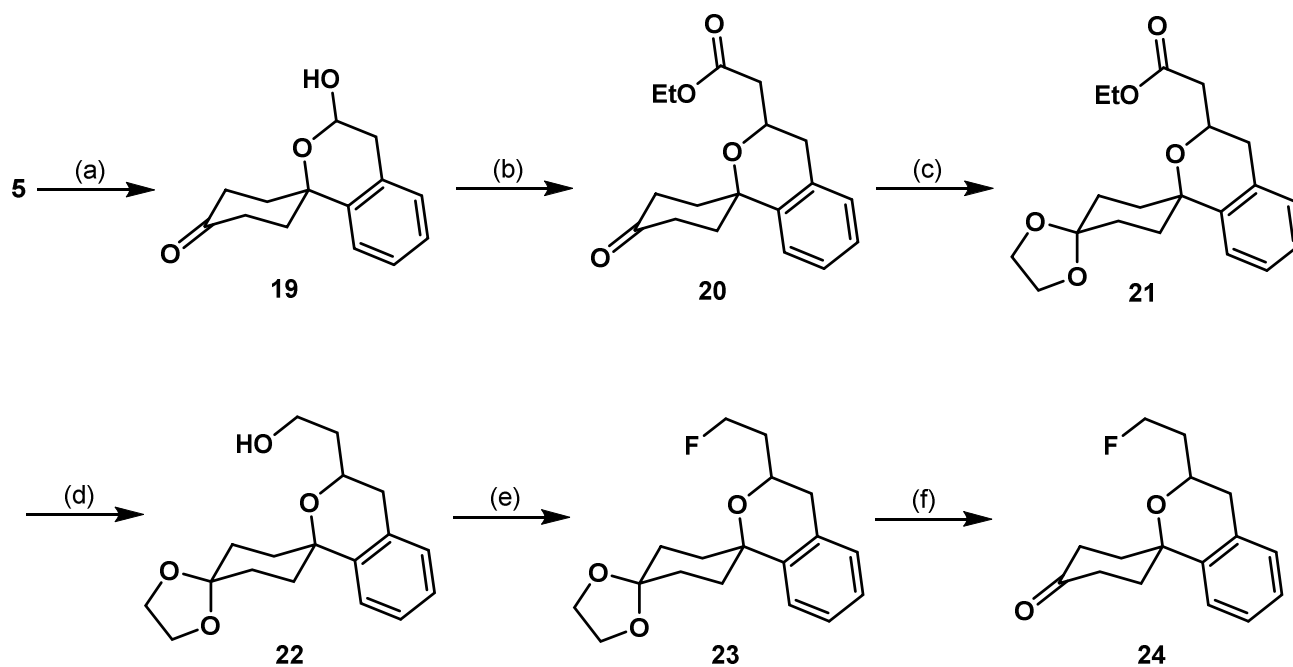
1
2
3
4
5
6
7
8
9 Since compounds with a 1-(4-fluorophenyl)indole moiety like **2** show high affinity
10 towards σ receptors with a preference for the σ_2 subtype,⁴⁵ spirocyclic
11 cyclohexylamine derivatives with this residue were envisaged. In the first step of the
12 synthesis 3-(indol-3-yl)propionic acid (**14**) was arylated with 1-bromo-4-fluorobenzene
13 by an Ullmann reaction to obtain the arylated propionic acid **15** in 78 % yield.
14
15 Activation of acid **14** with ethyl chloroformate and subsequent treatment with NH_3
16 provided the primary amide **16** in 97 % yield. Reduction of the amide **16** with LiAlH_4
17 in THF led to the primary amine **17**, which was used for the aforementioned reductive
18 amination of ketone **5**. The diastereomeric amines **18a** and **18b** were separated by fc
19
20
21
22
23
24
25
26
27
28
29 (Scheme 3).
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



Scheme 3. Synthesis of spirocyclic σ receptor ligands with [1-(4-fluorophenyl)-(indol-3-yl)]propyl moiety. Reagents and reaction conditions: (a) 1-bromo-4-fluorobenzene, CuI, Cs₂CO₃, DMF, 2 d, reflux; 78 %. (b) ClCO₂Et, NH₃, Et₃N, THF, 3 h, 0 °C; 97 %. (c) LiAlH₄, THF, 2 h, reflux; 64 %. (d) ketone **5**, NaBH(OAc)₃, HOAc, THF, 20 h, rt; **18a**, 30 %; **18b**, 42 %.

In order to get fast access to diverse spirocyclic amines with fluoroethyl side chain, a spirocyclic ketone with fluoroethyl side chain (**24**) was envisaged. The synthesis of **24** was conducted according to the aforementioned synthesis of amines **13a-f**. Hydrolysis of the acetalic moiety of ketone **5** led to the lactol **19** in high yield, which was transformed to the ester **20** by (ethoxycarbonylmethylene)triphenylphosphorane and Cs₂CO₃. In order to avoid reduction, the ketone **20** was protected with ethylene

glycol to afford the cyclic ketal **21**. Reduction of the ester **21** was performed with LiAlH_4 and gave alcohol **22** in 81 % yield. Compound **23** was obtained after fluorination of the alcohol **22** with DAST. In the last step, the ketal was hydrolyzed with diluted HCl to provide the ketone **24** in a yield of 95 % (Scheme 4).

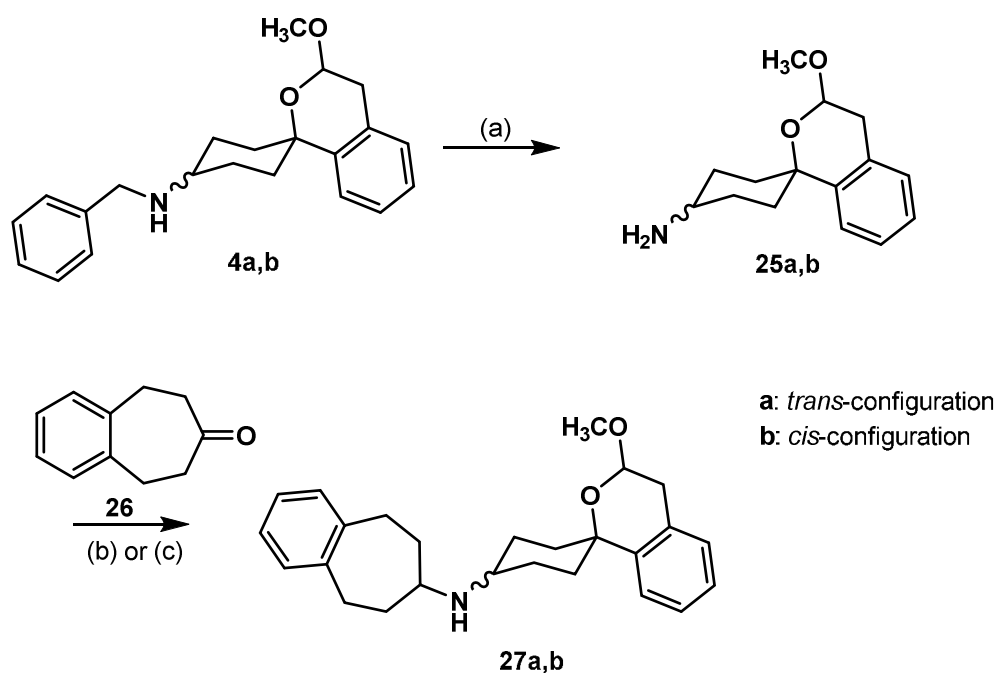


Scheme 4. Synthesis of the spirocyclic building block **24** with fluoroethyl side chain.

Reagents and reaction conditions: (a) HCl, THF, 3 d, rt; 95 %. (b) $\text{Ph}_3\text{P}=\text{CHCO}_2\text{Et}$, Cs_2CO_3 , toluene, 2 d, reflux; 63 %. (c) ethylene glycol, $\text{CH}(\text{OCH}_3)_3$, *p*-toluenesulfonic acid (*p*-TsOH), CH_2Cl_2 , 17 h, RT; 88 %. (d) LiAlH_4 , Et_2O , 2 h, 81 %. (e) DAST, CH_2Cl_2 , 1 h $-78\text{ }^\circ\text{C}$, then 20 h rt; 49 %. (f) 2 M HCl, Et_2O , 3 d, $40\text{ }^\circ\text{C}$; 95 %.

Benzo[7]annulenamines **27a,b** and tetrahydroisoquinolines **6e,f** have comparable sizes, but show different hydrogen bonding properties. To investigate the influence of these different properties on the σ affinity, benzo[7]annulene derivatives **27** with

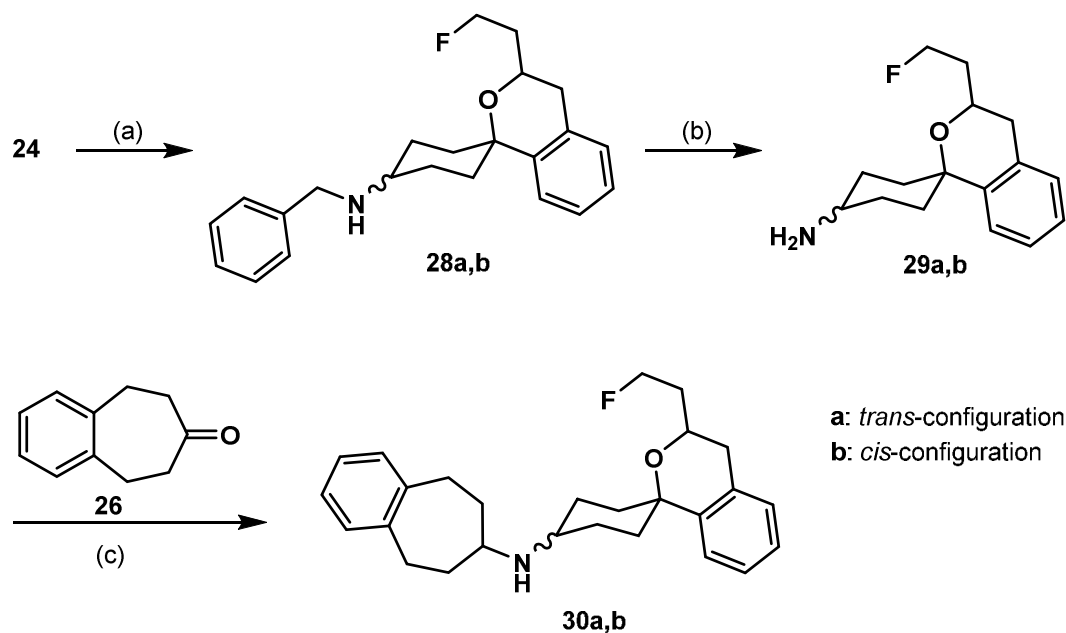
1
2
3 acetalic moiety and **30** with fluoroethyl side chain were synthesized. As shown in
4
5 Scheme 5, debenzoylation of benzylamines **4a** and **4b** with H₂ and Pd/C as catalyst
6
7 led to the primary amines **25a** and **25b** in 72 % and 89 % yield, respectively.
8
9 Reductive alkylation of the primary amines **25a** and **25b** with benzo[7]annulene **26**
10
11 and NaBH(OAc)₃ provided the secondary amines **27a** and **27b** in yields of 48 % and
12
13 28 %, respectively.
14



Scheme 5. Synthesis of spirocyclic σ receptor ligands with benzo[7]annulenyli moiety.
Reagents and reaction conditions: (a) H₂, 10 % Pd/C, CH₃OH, 20 h, rt; **25a**, 72 %;
25b, 89 %. (b) NaBH(OAc)₃, HOAc, CH₂Cl₂, 3 h, rt; **27a**, 48 %. (c) NaBH(OAc)₃,
CH₂Cl₂, 12 h, rt; **27b**, 28 %.

For the synthesis of benzo[7]annulene derivatives **30a** and **30b** with fluoroethyl side
chain, ketone **24** was reductively aminated with benzylamine and NaBH(OAc)₃. The
obtained amines **28a** and **28b** were separated and afterwards debenzoylated using

ammonium formate as hydrogen source and Pd/C as catalyst. Reductive alkylation of the primary amines **29a** and **29b** with benzo[7]annulenone **26** and NaBH(OAc)₃ led to the secondary amines **30a** and **30b** in yields of 76 % and 81 %, respectively (Scheme 6).



Scheme 6. Synthesis of spirocyclic σ receptor ligands with benzo[7]annulenyl moiety and fluoroethyl side chain. Reagents and reaction conditions: (a) Benzylamine, NaBH(OAc)₃, HOAc, THF, 3 h, rt; **28a**, 45 %; **28b**, 45 %. (b) NH₄HCO₂, 10 % Pd/C, CH₃OH, 2 – 4.5 h, 65 °C; **29a**, 96 %; **29b**, 88 %. (c) NaBH(OAc)₃, HOAc, CH₂Cl₂, 1 d, rt; **30a**, 76 %; **30b**, 81 %.

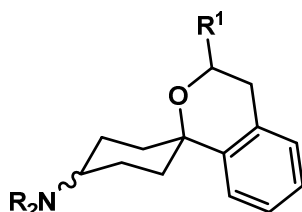
3. σ_1 and σ_2 receptor affinity

The affinity of all synthesized spirocyclic amines towards σ_1 and σ_2 receptors was determined by radioligand binding assays. The σ_1 assay was performed with homogenates of guinea pig brains as receptor material and [³H]-(+)-pentazocine as σ_1 selective radioligand. Homogenates of rat liver were used as receptor material for

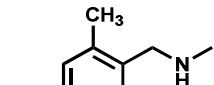
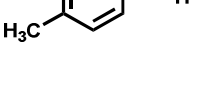
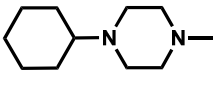
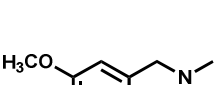
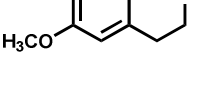
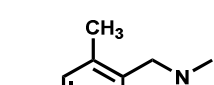
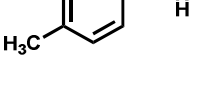
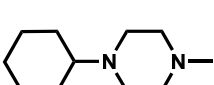
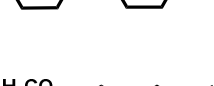
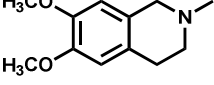
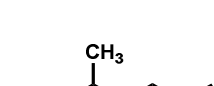
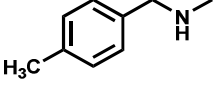
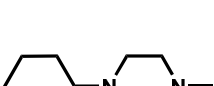
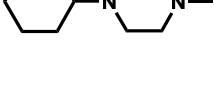
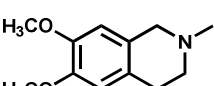
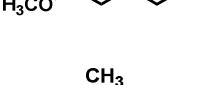
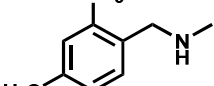
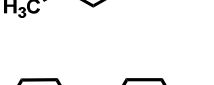
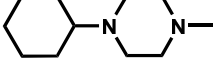



the σ_2 assay. Due to the lack of a σ_2 selective radioligand, the assay was performed with the non-selective radioligand [^3H]-1,3-di(o-tolyl)guanidine (DTG) and an excess of non-tritiated (+)-pentazocine to selectively occupy σ_1 receptors.^{47; 48} Since the amino acid sequence of the human σ_1 receptor shows 93 % identity with the guinea pig σ_1 receptor, affinity data for the guinea pig and human σ_1 receptor are comparable.¹³ Until now, the sequence of the human σ_2 receptor is unknown, but binding studies with σ_2 selective ligands reveal comparable affinity data for rat and human σ_2 receptors.^{54; 55} The determined affinities of the synthesized compounds and some reference and lead compounds towards both receptors are summarized in Table 2.

Table 2: σ_1 and σ_2 receptor affinity of spirocyclic amines and reference substances.

n = 3, if SEM is given, otherwise n = 1.



compd.	config.	R ¹	NR ₂	K _i ± SEM [nM]	
				σ_1	σ_2
(+)-pentazocine	-	-	-	5.4 ± 0.5	-
1 (PB28) ⁴⁴	-	-	-	0.38 ± 0.10	0.68 ± 0.20
2 (siramesine) ⁴⁵	-	-	-	17	0.12
3 ⁴⁶	-	-	-	12900 ± 111	8.2 ± 1.4
4a	<i>trans</i>	OCH ₃		538 ± 56	>1000

1						
2						
3	4b	<i>cis</i>	OCH ₃		158 ± 5	>1000
4						
5	6a	<i>trans</i>	OCH ₃		736	7.6 ± 4.1
6						
7	6b	<i>cis</i>	OCH ₃		> 1000*	54 ± 15
8						
9						
10	6c	<i>trans</i>	OCH ₃		6.3 ± 0.44	62 ± 17
11						
12	6d	<i>cis</i>	OCH ₃		4.4 ± 0.71	213 ± 25
13						
14	6e	<i>trans</i>	OCH ₃		639	58 ± 27
15						
16	6f	<i>cis</i>	OCH ₃		> 1000	105 ± 8
17						
18						
19	10a	<i>trans</i>	OH		349	181
20						
21	10b	<i>cis</i>	OH		> 1000	66 ± 10
22						
23						
24	10c	<i>trans</i>	OH		77 ± 25	302
25						
26	10d	<i>cis</i>	OH		47 ± 10	27 ± 16
27						
28	10e	<i>trans</i>	OH		583	99 ± 24
29						
30	10f	<i>cis</i>	OH		> 1000	> 1000
31						
32						
33	11a	<i>trans</i>	CH ₂ CO ₂ Et		>1000	231
34						
35	11b	<i>cis</i>	CH ₂ CO ₂ Et		547	159
36						
37						
38	11c	<i>trans</i>	CH ₂ CO ₂ Et		12 ± 2	355
39						
40	11d	<i>cis</i>	CH ₂ CO ₂ Et		12 ± 4	51 ± 5
41						
42	11e	<i>trans</i>	CH ₂ CO ₂ Et		> 1000	>1000
43						
44	11f	<i>cis</i>	CH ₂ CO ₂ Et		> 1000	343
45						
46						
47	12a	<i>trans</i>	CH ₂ CH ₂ OH		> 1000	381 ± 113
48						
49	12b	<i>cis</i>	CH ₂ CH ₂ OH		548	179
50						
51						
52	12c	<i>trans</i>	CH ₂ CH ₂ OH		61 ± 12	172
53						
54	12d	<i>cis</i>	CH ₂ CH ₂ OH		28 ± 5	505
55						
56						
57						
58						
59						
60						

12e	<i>trans</i>	CH ₂ CH ₂ OH		> 1000	>1000
12f	<i>cis</i>	CH ₂ CH ₂ OH		>1000	>1000
13a	<i>trans</i>	CH ₂ CH ₂ F		> 1000	302 ± 23
13b	<i>cis</i>	CH ₂ CH ₂ F		966	> 1000
13c	<i>trans</i>	CH ₂ CH ₂ F		12 ± 2	45 ± 14
13d	<i>cis</i>	CH ₂ CH ₂ F		19 ± 2	432 ± 168
13e	<i>trans</i>	CH ₂ CH ₂ F		807	> 1000
13f	<i>cis</i>	CH ₂ CH ₂ F		> 1000	505
18a	<i>trans</i>	OCH ₃		>1000	>1000
18b	<i>cis</i>	OCH ₃		635	>1000
27a	<i>trans</i>	OCH ₃		>1000	49 ± 7
27b	<i>cis</i>	OCH ₃		265	77 ± 5
30a	<i>trans</i>	CH ₂ CH ₂ F		667	305
30b	<i>cis</i>	CH ₂ CH ₂ F		198	527

* No correlation between concentration and receptor affinity.

The introduction of two methyl groups in *o*- and *p*-position of the benzyl moiety of lead compounds **4a** and **4b** led to considerably increased σ_2 affinity of amines **6a** ($K_i(\sigma_2) = 7.6$ nM) and **6b** ($K_i(\sigma_2) = 54$ nM). Amine **6a** shows very high σ_2 affinity and selectivity over the σ_1 subtype with a K_i -values of 7.6 nM for the σ_2 receptor and 736 nM for the σ_1 receptor. Also, amines **6e** and **6f** with the isoquinolinyl moiety and amines **27a** and **27b** with benzannulenyl moiety display a preference for the σ_2 receptor. In contrast, amines **6c** and **6d** bearing the cyclohexylpiperazinyl moiety show high σ_1 affinity with 10- to 50-fold selectivity over the σ_2 receptor. Unexpectedly,

1
2
3 siramesine derived amines **18a** and **18b** with indolylpropyl moiety show only very low
4
5 affinity towards both σ receptor subtypes. Due to the very low σ affinity of the
6
7 methoxy derivatives, **18a** and **18b** were not transformed into the corresponding
8
9 fluoroethyl derivatives.
10

11
12
13 The affinity of the lactols **10a-e** towards both σ receptors is slightly lower than the
14
15 affinity of the corresponding methyl acetals. Up to a test compound concentration of
16
17 1 μ M, lactol **10f** did not compete with the radioligands for the σ receptors. With
18
19 elongation of the side chain the affinity towards both σ receptor subtypes decreased
20
21 for esters **11a** and **11b** with dimethylbenzylamino moiety and **11e** and **11f** with
22
23 isoquinolinyl residue. The reduction of the esters to the alcohols **12a**, **12b**, **12e** and
24
25 **12f** did not change the affinity towards the σ receptors. Also, the fluorination did not
26
27 lead to increased σ affinity of compounds **13a**, **13b**, **13e** and **13f**. In comparison to
28
29 the acetals **27a** and **27b** the introduction of the fluoroethyl side chain led to a
30
31 decrease in σ_2 affinity for the compounds with benzannulenyl moiety **30a** and **30b**.
32
33
34
35
36

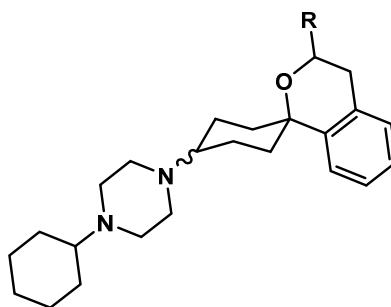
37
38 Esters **11c** and **11d** with cyclohexylpiperazinyl moiety show comparable σ_1 affinity as
39
40 the acetals **6c** and **6d**. In comparison to ester **11c**, alcohol **12c** shows a small
41
42 decrease in σ_1 affinity and increase in σ_2 affinity resulting in lower σ_1/σ_2 selectivity.
43
44 The diastereomeric alcohol **12d** displays a higher σ_1/σ_2 selectivity due to the 10-fold
45
46 reduced σ_2 affinity of **12d** compared to the σ_2 affinity of the ester **11d**. The fluorinated
47
48 compound **13c** shows high affinity towards both σ receptors with K_i -values of 12 nM
49
50 for the σ_1 receptor and 45 nM for the σ_2 receptor. A preference for one of the σ
51
52 receptors was not observed. However, the diastereomeric fluoroethyl derivative **13d**
53
54 reveals high σ_1 affinity and selectivity ($K_i(\sigma_1) = 19$ nM; $K_i(\sigma_2) = 432$ nM).
55
56
57
58
59
60

4. Pharmacological *in vitro* characterization of selected compounds **10d**, **11d**, **13c** and **13d**

Due to their high σ_1 and σ_2 affinity, lactol **10d**, ester **11d** and fluoroethyl compounds **13c** and **13d** bearing the cyclohexylpiperazinyll moiety were selected for further characterization *in vitro*. At first, the selectivity over a panel of receptors and transporters was investigated. All four compounds show low affinity towards μ -opioid receptors (MOR), κ -opioid receptors (KOR), δ -opioid receptors (DOR) and the phencyclidine (PCP) binding site of the *N*-methyl-*D*-aspartate (NMDA) receptor (Table 3). However, they display high affinity to the ifenprodil binding site of GluN2B subunit containing NMDA receptors. At a concentration of 1 μ M, the compounds **10d**, **11d** and **13c** did not interact with 5-HT_{2B} receptor, norepinephrine transporter (NET), dopamine transporter (DAT) and serotonin transporter (SERT). However, they displayed low affinity towards hERG channel, 5-HT_{1A}, α_{1A} and H₁ receptor (Table 4). Measuring the functional activity at MOR by determination of the intracellular cAMP concentration, only fluoroethyl derivative **13c** showed a very weak effect (EC_{50} = 1757 nM). Nevertheless, **13c** displayed the best selectivity profile for σ receptors.

Table 3: Affinity towards σ receptors, opioid receptors, the PCP binding site and the ifenprodil binding site of GluN2B subunit containing NMDA receptors of amines **10d**, **11d**, **13c** and **13d**. n = 3, if SEM is given, otherwise n = 1.

22

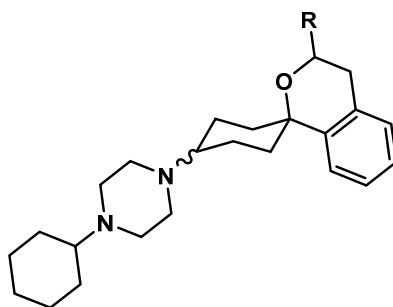


compd.		10d	11d	13c	13d
config.		<i>cis</i>	<i>cis</i>	<i>trans</i>	<i>cis</i>
R		OH	CH ₂ CO ₂ Et	CH ₂ CH ₂ F	CH ₂ CH ₂ F
K_i [nM] ⁺	σ_1	47 ± 10	12 ± 4	12 ± 2	19 ± 2
	σ_2	27 ± 16	51 ± 5	45 ± 14	432 ± 168
	MOR	> 1000	458	> 1000	434
	KOR	781	> 1000	> 1000	> 1000
	DOR	> 1000	> 1000	> 1000	647
	PCP	> 1000	> 1000	> 1000	> 1000
	GluN2B	34 ± 7	54 ± 6	65 ± 9	31 ± 9

⁺ Results are the mean of at least two replicates

Table 4: Inhibition of σ , α_1 , H₁, 5-HT_{1A} and 5-HT_{2B} receptor, hERG channel, NET, DAT and SERT and functional activity at MOR of amines **10d**, **11d** and **13c**. n = 3, if SEM is given, otherwise n = 1.

23



compd.		10d	11d	13c
config.		<i>cis</i>	<i>cis</i>	<i>trans</i>
R		OH	CH ₂ CO ₂ Et	CH ₂ CH ₂ F
<i>K_i</i> [nM]	σ ₁	47 ± 10	12 ± 4	12 ± 2
	σ ₂	27 ± 16	51 ± 5	45 ± 14
<i>EC</i> ₅₀ [nM]	MOR	> 10000	> 10000	1757
<i>IC</i> ₅₀ [nM]	hERG	3207	1753	2620
% Inhibition [1 μM]	α _{1A}	< 50	78	< 50
	H ₁	76	98	< 50
	5-HT _{1A}	55	68	62
	5-HT _{2B}	< 50	< 50	< 50
	NET	< 50	< 50	< 50
	DAT	< 50	< 50	< 50
	SERT	< 50	< 50	< 50

In order to determine the chemical stability of compounds **10d**, **11d**, and **13d** they were dissolved in aqueous solution at pH 2 (aqueous HCl) and pH 7.4 (phosphate buffer). The remaining amount of parent compound was determined by HPLC. After a

1
2
3 period of 24 h the parent compounds remained completely intact indicating high
4
5 chemical stability under these conditions.
6
7

8
9 Since amines **13c** and **13d** were chosen for *in vivo* tests in mice, the metabolic
10 stability of these compounds during incubation with mouse liver microsomes over a
11 period of 1 h was investigated (Table 4). Imipramine served as reference compound.
12
13 Using a concentration of 25 μM , both **13c** as well as **13d** showed high metabolic
14
15 stability. After a period of 60 min 72 % and 70 % of the parent compound remained
16
17 intact. These findings allow the *in vivo* application of **13c** and **13d** in experiments with
18
19 a duration of 1 h.
20
21
22
23
24
25

26 Table 5: Metabolic stability of Imipramine (reference compound) and amines **13c** and
27
28 **13d** after incubation with mouse liver microsomes for 1 h (n = 3).
29

30 Compd.	31 remaining parent compound \pm SEM [%]
32 Imipramine	33 17 \pm 1.1
34 13c	35 71.8 \pm 2.4
36 13d	37 69.5 \pm 1.6

38 39 40 41 42 43 **5. *In vivo* activity of amine 13c and 13d in mechanical allodynia assay**

44 For the *in vivo* investigations the fluoroethyl derivatives **13c** and **13d** were selected.
45
46 **13c** shows promising affinity towards both σ receptor subtypes, a very good
47
48 selectivity profile over other receptors and transporters and, moreover, does not
49
50 contain an acid labile acetal or hemiacetal moiety. Its diastereomer **13d** indeed
51
52 displays high σ_1 receptor affinity and selectivity. Since it has been reported that σ_1
53
54
55
56
57
58
59
60

receptors are involved in the development of pain,³² the effect of amines **13c** and **13d** on mechanical allodynia was analyzed. By using the σ_1 selective **13d** and the dual σ_1/σ_2 ligand **13c** the influence of the different σ receptor binding profile on the antiallodynic activity was determined.

Fluoroethyl derivatives **13c** and **13d**, the σ_1 receptor antagonist BD-1063 (1-[2-(3,4-dichlorophenyl)ethyl]-4-methylpiperazine, **34**)⁵⁶ as positive control and their solvent hydroxypropyl-methyl-cellulose (HPMC) as negative control were administered subcutaneously (s.c.) to mice and capsaicin-induced mechanical allodynia was evaluated.³⁴ As shown in Figure 4, **13c**, **13d** and **34** have a strong effect on allodynia. In both assays **13c** and **13d** were more potent than **34**, since lower doses of **13c** and **13d** than **34** were necessary to achieve the same effects.

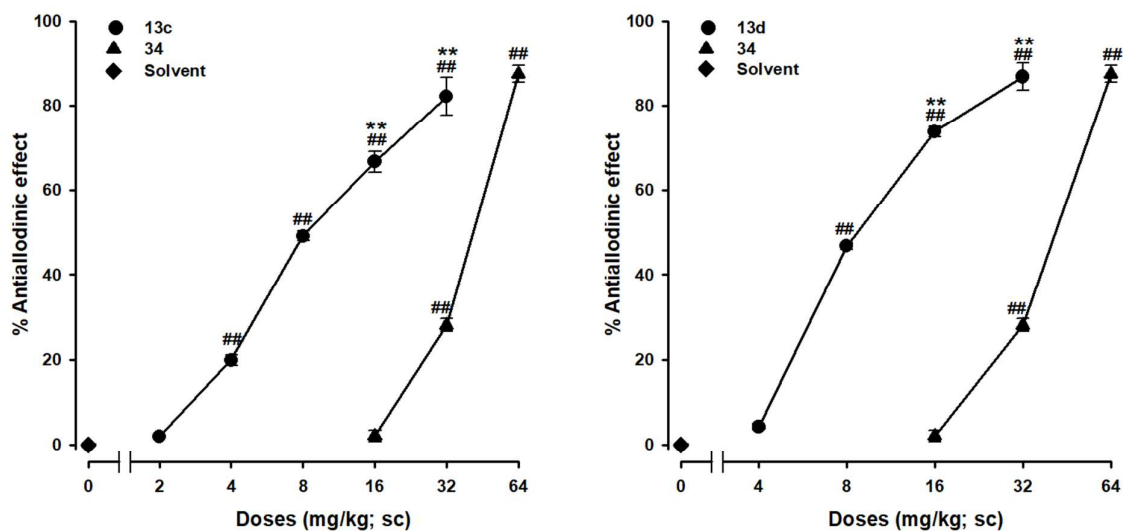


Figure 4. Effects of fluoroethyl derivatives **13c** (left) and **13d** (right) and the reference compound **34** (left and right) on mechanical allodynia induced by intraplantar (i.pl) administration of capsaicin in mice. Animals were treated s.c. with **13c**, **13d**, **34** or their solvent (HPMC, dose 0) 30 min before capsaicin. Each bar and vertical line

1
2
3 represents the mean \pm SEM of values obtained in 6–8 animals. One-way analysis of
4
5 variance followed by the Bonferroni test was used to determine statistically significant
6
7 differences between values obtained in mice treated with compounds **13c**, **13d** or **34**
8
9 in comparison with control animals (HPMC-treated, dose 0) value ($^{##}P < 0.01$); and
10
11 between the values obtained in mice treated with the same dose of **13c** and **34** (left)
12
13 or the same dose of **13d** and **34** (right) ($^{**}P < 0.01$).
14
15

16
17
18 In order to evaluate the influence of σ_1 affinity on the antiallodynic effects of **13c** and
19
20 **13d**, the experiment was repeated with pretreatment of the mice with σ_1 agonist
21
22 PRE-084 (2-morpholin-4-ylethyl 1-phenylcyclohexane-1-carboxylate, **35**, 32 mg/kg).⁵⁷
23
24 It was observed that for low doses of **13c** (8 – 16 mg/kg), **35** was able to antagonize
25
26 the antiallodynic effect. At higher doses of **13c** (32 mg/kg), reversion of the
27
28 antiallodynic effect could not be observed (Figure 5). This indicates an involvement of
29
30 the σ_1 receptor in the antiallodynic effect of **13c**. However, since the σ_1 agonist **35**
31
32 was not able to antagonize the effect of **13c** completely at higher doses, the
33
34 antiallodynic effect of **13c** at least at high doses will be mediated by a second
35
36 unknown mechanism. In contrast, when compound **13d** was associated to **35** a
37
38 parallel shift to the right of the dose-response line of **13d** was observed (Figure 5),
39
40 indicating that the antiallodynic effect of **13d** is probably due only to its interaction
41
42 with σ_1 receptor.
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

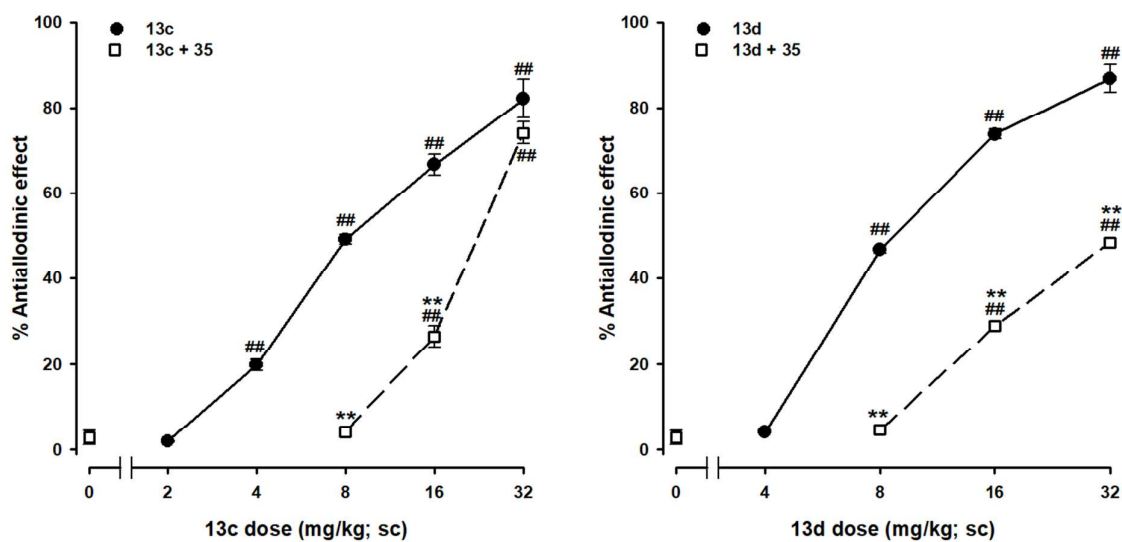


Figure 5. Effects of fluoroethyl derivatives **13c** (left) or **13d** (right) alone and in combination with the σ_1 receptor agonist **35** on mechanical allodynia induced by intraplantar (i.pl) administration of capsaicin in mice. Animals were treated s.c. with **35** 5 min before administration of **13c** or **13d**, which were s.c. administered 30 min before capsaicin. Each bar and vertical line represents the mean \pm SEM of values obtained in 6–8 animals. One-way analysis of variance followed by the Bonferroni test was used to determine statistically significant differences between values obtained in mice treated with compounds **13c**, **13d** or their combination with **35** in comparison with the value obtained in control animals (HPMC-treated, dose 0) (##P < 0.01); and between the values obtained in mice treated with **13c** and **13c** + **35** (left) or **13d** and **13d** + **35** (right) (**P < 0.01).

6. Conclusion

A series of spirocyclic 2-benzopyrans bearing exocyclic amino moieties derived from ligands preferring the σ_2 receptor subtype was designed, synthesized and evaluated pharmacologically. Whereas compounds with the 4-cyclohexylpiperazinyl moiety

1
2
3 show high σ_1 affinity, ligands bearing the dimethylbenzylamino, 6,7-
4 dimethoxyisoquinolinyl or benzo[7]annulenyl residue display a preference for the σ_2
5 receptor. The affinity towards both σ receptors decreases with elongation of the side
6 chain in position 3 of the 2-benzopyran system: $\text{OCH}_3 = \text{OH} > \text{CH}_2\text{CO}_2\text{Et} =$
7 $\text{CH}_2\text{CH}_2\text{OH} = \text{CH}_2\text{CH}_2\text{F}$. Introduction of the indolylpropyl moiety derived from the
8 high-affinity σ_2 ligand siramesine led to considerably reduced affinity towards both σ
9 receptors.
10
11
12
13
14
15
16
17
18
19

20 Further pharmacological properties of the most promising 4-cyclohexylpiperazinyl
21 derivatives **10d**, **11d** and **13c** were investigated in *in vitro* experiments. In the mouse
22 model of mechanical allodynia the most selective, fluorinated compound **13c** and its
23 diastereomer **13d** showed high antiallodynic activity that can be partially explained by
24 σ_1 receptor antagonism. A second mechanism was postulated to explain the
25 complete antiallodynic effect of **13c**. In contrast, the antiallodynic effect of **13d** can be
26 totally explained by σ_1 receptor antagonism.
27
28
29
30
31
32
33
34
35
36

37 7. Experimental

38 7.1. Chemistry, General

39
40 Unless otherwise noted, moisture sensitive reactions were conducted under dry
41 nitrogen. CH_2Cl_2 was distilled over CaH_2 . THF was distilled over
42 sodium/benzophenone. Et_2O and toluene were dried over molecular sieve 0.4 Å. Thin
43 layer chromatography (tlc): Silica gel 60 F254 plates (Merck). Flash chromatography
44 (fc): Silica gel 60, 40–64 μm (Merck); parentheses include: diameter of the column
45 (d), length of the stationary phase (l), fraction size (V), eluent. Melting point: Melting
46 point apparatus Mettler Toledo MP50 Melting Point System, uncorrected. MS:
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 microTOF-Q II (Bruker Daltonics); APCI, atmospheric pressure chemical ionization.
4
5 IR: FT-IR spectrophotometer MIRacle 10 (Shimadzu) equipped with ATR technique.
6
7 Nuclear magnetic resonance (NMR) spectra were recorded on Agilent 600-MR (600
8
9 MHz for ^1H , 151 MHz for ^{13}C) or Agilent 400-MR spectrometer (400 MHz for ^1H , 101
10
11 MHz for ^{13}C); δ in ppm related to tetramethylsilane and measured referring to CHCl_3
12
13 ($\delta = 7.26$ ppm (^1H NMR) and $\delta = 77.2$ ppm (^{13}C NMR)), CHD_2OD ($\delta = 3.31$ ppm (^1H
14
15 NMR) and $\delta = 49.0$ ppm (^{13}C NMR)) and $\text{DMSO-}d_6$ ($\delta = 2.54$ ppm (^1H NMR) and $\delta =$
16
17 39.5 ppm (^{13}C NMR)); coupling constants are given with 0.5 Hz resolution; the
18
19 assignments of ^{13}C and ^1H NMR signals were supported by 2-D NMR techniques
20
21 where necessary. HPLC: Pump: LPG-3400SD, degasser: DG-1210, autosampler:
22
23 ACC-3000T, UV-detector: VWD-3400RS, interface: DIONEX UltiMate 3000, data
24
25 acquisition: Chromeleon 7 (Thermo Fisher Scientific); column: LiChrospher[®] 60 RP-
26
27 select B (5 μm), LiChroCART[®] 250-4 mm cartridge; guard column: LiChrospher[®] 60
28
29 RP-select B (5 μm), LiChroCART[®] 4-4 mm cartridge (No.: 1.50963.0001), manu-
30
31 CART[®] NT cartridge holder; flow rate: 1.0 mL/min; injection volume: 5.0 μL ; detection
32
33 at $\lambda = 210$ nm; solvents: A: method 1: water with 0.05 % (v/v) trifluoroacetic acid;
34
35 method 2: water; B: method 1: acetonitrile with 0.05 % (v/v) trifluoroacetic acid;
36
37 method 2: acetonitrile: gradient elution: (A %): 0-4 min: 90 %, 4-29 min: 90 \rightarrow 0 %,
38
39 29-31 min: 0 %, 31-31.5 min: 0 \rightarrow 90 %, 31.5-40 min: 90 %. The purity of all
40
41 compounds was determined by this method. Unless otherwise mentioned, the purity
42
43 of all test compounds is higher than 95 %.
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

7.2. Synthetic procedures

7.2.1. *trans*-N-(2,4-Dimethylbenzyl)-3-methoxy-3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-4'-amine (**6a**) and *cis*-N-(2,4-Dimethylbenzyl)-3-methoxy-3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexane]-4'-amine (**6b**)

A solution of ketone **5** (201 mg, 0.82 mmol), 2,4-dimethylbenzylamine (161 mg, 1.19 mmol, 1.5 eq) and acetic acid (50 μ L, 0.88 mmol, 1.1 eq) in THF (30 mL) was stirred under N₂ atmosphere at rt. After 2.5 h, NaBH(OAc)₃ (310 mg, 1.46 mmol, 1.9 eq) was added and the mixture was stirred for 20 h at rt. 1 M NaOH (20 mL) was added and the aqueous layer was extracted with Et₂O (4 x 30 mL). The combined organic layers were dried (Na₂SO₄), filtered, concentrated in vacuo and the residue was purified by fc (d = 3 cm, l = 26 cm, V = 20 mL, cyclohexane/ethyl acetate 90:10 + 1 % *N,N*-dimethylethanamine). **6a** was eluted first and **6b** afterwards. **6b** was purified again by fc (d = 2 cm, l = 16 cm, V = 10 mL, cyclohexane/ethyl acetate 80:20 + 1 % *N,N*-dimethylethanamine).

trans-**6a**: Pale yellow solid, mp 74 °C, yield 91 mg (30 %). C₂₄H₃₁NO₂ (365.6 g/mol). R_f = 0.30 (cyclohexane/ethyl acetate 90:10 + 1 % *N,N*-dimethylethanamine). HR-MS (APCI): m/z = 366.2437 (calcd. 366.2428 for C₂₄H₃₂NO₂ [MH⁺]). ¹H NMR (600 MHz, CD₃OD): δ (ppm) = 1.57 (dq, *J* = 13.5/3.1 Hz, 1H, 2'-H_{equ}), 1.74 – 1.79 (m, 2H, 3'-H, 5'-H), 1.82 (dq, *J* = 13.3/2.8 Hz, 1H, 6'-H_{equ}), 1.98 – 2.16 (m, 3H, 3'-H, 5'-H, 6'-H_{ax}), 2.29 – 2.34 (m, 1H, 2'-H_{ax}), 2.30 (s, 1H, 4-CH₃), 2.39 (s, 3H, 2-CH₃), 2.79 (dd, *J* = 15.6/7.5 Hz, 1H, 4-H), 2.91 (dd, *J* = 15.6/3.1 Hz, 1H, 4-H), 3.01 (quint, *J* = 3.1 Hz, 1H, 4'-H_{equ}), 3.54 (s, 3H, OCH₃), 3.77 (s, 2H, ArCH₂NH), 4.90 (dd, *J* = 7.7/3.1 Hz, 1H, 3-H), 6.96 – 7.04 (m, 2H, 3-H_{benzyl}, 5-H_{benzyl}), 7.06 (dd, *J* = 7.6/1.2 Hz, 1H, 5-H), 7.13 (td, *J* = 7.4/1.4 Hz, 1H, 6-H), 7.16 – 7.20 (m, 1H, 7-H), 7.23 (d, *J* = 7.6 Hz, 1H, 6-

1
2
3 H_{benzyl}), 7.33 (dd, $J = 7.8/1.3$ Hz, 1H, 8- H). A signal for the NH proton is not observed
4
5 in the spectrum. ^{13}C NMR (151 MHz, CD_3OD): δ (ppm) = 19.2 (1C, 2- CH_3), 21.1 (1C,
6
7 4- CH_3), 26.6 (1C, C-3'), 26.8 (1C, C-5'), 31.6 (1C, C-6'), 34.3 (1C, C-2'), 36.3 (1C, C-
8
9 4), 50.2 (1C, ArCH_2NH), 52.4 (1C, C-4'), 56.3 (1C, OCH_3), 78.3 (1C, C-1), 97.8 (1C,
10
11 C-3), 126.1 (1C, C-8), 127.47 (1C, C-7), 127.52 (1C, C-6), 127.6 (1C, C-5 $_{\text{benzyl}}$),
12
13 130.0 (1C, C-5), 130.2 (1C, C-6 $_{\text{benzyl}}$), 132.0 (1C, C-3 $_{\text{benzyl}}$), 132.4 (1C, C-4a), 136.2
14
15 (1C, C-1 $_{\text{benzyl}}$), 137.4 (1C, C-2 $_{\text{benzyl}}$), 137.8 (1C, C-4 $_{\text{benzyl}}$), 143.5 (1C, C-8a). FT-IR
16
17 (neat): ν [cm^{-1}] = 3310 (N-H), 2948, 2930, 2851 (C- H_{alkyl}), 1615, 1476 (C=C $_{\text{arom}}$).
18
19
20 Purity (HPLC): 96.7 %, $t_{\text{R}} = 19.8$ min.

21
22 **cis-6b**: Pale yellow solid, mp 93 °C, yield 131 mg (44 %). $\text{C}_{24}\text{H}_{31}\text{NO}_2$ (365.6 g/mol).
23
24 $R_{\text{f}} = 0.09$ (cyclohexane/ethyl acetate 90:10 + 1 % N,N -dimethylethanamine). HR-MS
25
26 (APCI): $m/z = 366.2390$ (calcd. 366.2428 for $\text{C}_{24}\text{H}_{32}\text{NO}_2$ [MH^+]). ^1H NMR (400 MHz,
27
28 CD_3OD): δ (ppm) = 1.70 (td, $J = 13.4/3.5$ Hz, 1H, 2'- H_{ax}), 1.74 – 1.83 (m, 1H, 3'- H),
29
30 1.83 – 1.96 (m, 4H, 3'- H , 5'- H , 6'- H), 1.96 – 2.05 (m, 1H, 6'- H), 2.13 (dq, $J = 13.6/2.7$
31
32 Hz, 1H, 2'- H_{equ}), 2.31 (s, 3H, 4- CH_3), 2.36 (s, 3H, 2- CH_3), 2.77 (tt, $J = 10.8/3.9$ Hz,
33
34 1H, 4'- H_{ax}), 2.83 (dd, $J = 16.1/7.4$ Hz, 1H, 4- H), 2.94 (dd, $J = 15.7/3.0$ Hz, 1H, 4- H),
35
36 3.59 (s, 3H, OCH_3), 3.83 (s, 2H, ArCH_2NH), 4.93 (dd, $J = 7.4/3.2$ Hz, 1H, 3- H), 6.99 –
37
38 7.05 (m, 2H, 3- H_{benzyl} , 5- H_{benzyl}), 7.10 (d, $J = 7.6$ Hz, 1H, 5- H), 7.14 – 7.21 (m, 3H, 6-
39
40 H , 7- H , 8- H), 7.22 (d, $J = 7.5$ Hz, 1H, 6- H_{benzyl}). A signal for the NH proton is not
41
42 observed in the spectrum. ^{13}C NMR (101 MHz, CD_3OD): δ (ppm) = 19.1 (1C, 2- CH_3),
43
44 21.1 (1C, 4- CH_3), 29.1 (1C, C-5'), 29.1 (1C, C-3'), 36.2 (1C, C-4), 36.6 (1C, C-2'),
45
46 39.1 (1C, C-6'), 48.7 (1C, ArCH_2NH), 56.5 (1C, OCH_3), 57.2 (1C, C-4'), 77.5 (1C, C-
47
48 1), 97.8 (1C, C-3), 125.7 (1C, C-8), 127.5 (1C, C-7), 127.6 (1C, C-5 $_{\text{benzyl}}$), 127.7 (1C,
49
50 C-6), 129.9 (1C, C-6 $_{\text{benzyl}}$), 130.1 (1C, C-5), 132.1 (1C, C-3 $_{\text{benzyl}}$), 132.6 (1C, C-4a),
51
52 135.8 (1C, C-1 $_{\text{benzyl}}$), 137.2 (1C, C-2 $_{\text{benzyl}}$), 137.9 (1C, C-4 $_{\text{benzyl}}$), 142.7 (1C, C-8a). FT-
53
54
55
56
57
58
59
60

1
2
3 IR (neat): ν [cm⁻¹] = 3290 (N-H), 2941, 2918 (C-H_{alkyl}), 1614, 1488 (C=C_{arom}). Purity
4
5 (HPLC): 95.6 %, t_R = 19.53 min.
6
7

9
10 **7.2.2. *trans*-1-Cyclohexyl-4-(3-methoxy-3,4-dihydrospiro[[2]benzopyran-1,1'-**
11 **cyclohexan]-4'-yl)piperazine (6c) and *cis*-1-Cyclohexyl-4-(3-methoxy-3,4-**
12 **dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-4'-yl)piperazine (6d)**
13
14
15

16 A solution of ketone **5** (80 mg, 0.32 mmol), 1-cyclohexylpiperazine (76 mg,
17 0.45 mmol, 1.4 eq) and acetic acid (22 μ L, 0.39 mmol, 1.2 eq) in THF (10 mL) was
18 stirred under N₂ atmosphere at rt. After 3 h, NaBH(OAc)₃ (129 mg, 0.61 mmol, 1.9
19 eq) was added and the mixture was stirred for 23 h at rt. 1 M NaOH (20 mL) was
20 added and the aqueous layer was extracted with Et₂O (4 x 30 mL). The combined
21 organic layers were dried (Na₂SO₄), filtered, concentrated in vacuo and the residue
22 was purified by fc (d = 2 cm, l = 26 cm, V = 5 mL, cyclohexane/ethyl acetate 66:33 +
23 2 % *N,N*-dimethylethanamine). **6c** was eluted first and **6d** afterwards.
24
25
26
27
28
29
30
31
32

33 *trans*-**6c**: Colorless solid, mp 146 °C, yield 13 mg (9 %). C₂₅H₃₈N₂O₂ (398.6 g/mol). R_f
34 = 0.34 (cyclohexane/ethyl acetate 90:10 + 2 % *N,N*-dimethylethanamine). HR-MS
35 (APCI): m/z = 399.3013 (calcd. 399.3006 for C₂₅H₃₉N₂O₂ [MH⁺]). ¹H NMR (600 MHz,
36 CD₃OD): δ (ppm) = 1.13 – 1.21 (m, 1H, 4-*H*_{cyclohexyl}), 1.13 – 1.37 (m, 4H, 2-*H*_{cyclohexyl},
37 3-*H*_{cyclohexyl}, 5-*H*_{cyclohexyl}, 6-*H*_{cyclohexyl}), 1.51 (dq, *J* = 13.3/3.1 Hz, 1H, 2'-*H*_{equ}), 1.64 –
38 1.71 (m, 1H, 4-*H*_{cyclohexyl}), 1.76 (dq, *J* = 12.6/3.3/2.8/2.3 Hz, 1H, 6'-*H*_{equ}), 1.82 – 1.88
39 (m, 2H, 3-*H*_{cyclohexyl}, 5-*H*_{cyclohexyl}), 1.90 – 2.05 (m, 7H, 3'-*H*, 5'-*H*, 6'-*H*_{ax}, 2-*H*_{cyclohexyl}, 6-
40 *H*_{cyclohexyl}), 2.22 – 2.30 (m, 3H, 2'-*H*_{ax}, 4'-*H*_{equ}, 1-*H*_{cyclohexyl}), 2.45 – 2.66 (broad signal,
41 4H, 2-*H*_{piperazine} and 6-*H*_{piperazine} or 3-*H*_{piperazine} and 5-*H*_{piperazine}), 2.66 – 2.76 (broad
42 signal, 4H, 2-*H*_{piperazine} and 6-*H*_{piperazine} or 3-*H*_{piperazine} and 5-*H*_{piperazine}), 2.78 (dd, *J* =
43 15.4/7.7 Hz, 1H, 4-*H*), 2.90 (dd, *J* = 15.6/3.2 Hz, 1H, 4-*H*), 3.54 (s, 3H, OCH₃), 4.89
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

(dd, $J = 7.5/3.1$ Hz, 1H, 3-*H*), 7.04 – 7.08 (m, 1H, 5-*H*), 7.11 – 7.15 (m, 1H, 6-*H*), 7.16 – 7.22 (m, 2H, 7-*H*, 8-*H*). ^{13}C NMR (151 MHz, CD_3OD): δ (ppm) = 24.9 (1C, C-3'), 25.0 (1C, C-5'), 26.9 (2C, C-2_{cyclohexyl}, C-6_{cyclohexyl}), 27.3 (1C, C-4_{cyclohexyl}), 29.8 (2C, C-3_{cyclohexyl}, C-5_{cyclohexyl}), 32.1 (1C, C-6'), 34.8 (1C, C-2'), 36.2 (1C, C-4), 50.6 (2C, C-2_{piperazine} and C-6_{piperazine} or C-3_{piperazine} and C-5_{piperazine}), 51.4 (2C, C-2_{piperazine} and C-6_{piperazine} or C-3_{piperazine} and C-5_{piperazine}), 56.3 (1C, OCH₃), 59.4 (1C, C-4'), 65.0 (1C, C-1_{cyclohexyl}), 78.4 (1C, C-1), 97.9 (1C, C-3), 125.9 (1C, C-8), 127.4 (1C, C-7), 127.5 (1C, C-6), 130.0 (1C, C-5), 132.5 (1C, C-4a), 143.6 (1C, C-8a). FT-IR (neat): ν [cm^{-1}] = 2947, 2914 (C-H_{alkyl}), 2800, 2758 (N-CH₂), 1486 (C=C_{arom}). Purity (HPLC): 98.1 %, $t_{\text{R}} = 14.42$ min.

cis-6d: Colorless solid, mp 146 °C, yield 48 mg (38 %). $\text{C}_{25}\text{H}_{38}\text{N}_2\text{O}_2$ (398.6 g/mol). $R_{\text{f}} = 0.17$ (cyclohexane/ethyl acetate 90:10 + 2 % *N,N*-dimethylethanamine). HR-MS (APCI): $m/z = 399.2996$ (calcd. 399.3006 for $\text{C}_{25}\text{H}_{39}\text{N}_2\text{O}_2$ [MH^+]). ^1H NMR (600 MHz, CD_3OD): δ (ppm) = 1.10 – 1.20 (m, 1H, 4-*H*_{cyclohexyl}), 1.20 – 1.35 (m, 4H, 2-*H*_{cyclohexyl}, 3-*H*_{cyclohexyl}, 5-*H*_{cyclohexyl}, 6-*H*_{cyclohexyl}), 1.63 – 1.68 (m, 1H, 4-*H*_{cyclohexyl}), 1.68 – 1.74 (m, 1H, 2'-*H*_{ax}), 1.78 – 1.92 (m, 7H, 3'-*H*, 5'-*H*, 6'-*H*, 2-*H*_{cyclohexyl}, 6-*H*_{cyclohexyl}), 1.92 – 2.03 (m, 3H, 6'-*H*, 3-*H*_{cyclohexyl}, 5-*H*_{cyclohexyl}), 2.14 (dq, $J = 14.2/3.2$ Hz, 1H, 2'-*H*_{equ}), 2.25 (tt, $J = 11.0/3.4$ Hz, 1H, 1-*H*_{cyclohexyl}), 2.47 (tt, $J = 10.6/4.3$ Hz, 1H, 4'-*H*_{ax}), 2.61 – 2.76 (broad signal, 8H, *H*_{piperazine}), 2.79 (dd, $J = 15.6/7.5$ Hz, 1H, 4-*H*), 2.91 (dd, $J = 15.7/3.1$ Hz, 1H, 4-*H*), 3.55 (s, 3H, OCH₃), 4.90 (dd, $J = 7.5/3.1$ Hz, 1H, 3-*H*), 7.07 (d, $J = 7.3$ Hz, 1H, 5-*H*), 7.11 – 7.16 (m, 1H, 6-*H*), 7.16 – 7.19 (m, 2H, 7-*H*, 8-*H*). ^{13}C NMR (151 MHz, CD_3OD): δ (ppm) = 24.86 (1C, C-3' or C-5'), 24.89 (1C, C-3' or C-5'), 26.9 (2C, C-2_{cyclohexyl}, C-6_{cyclohexyl}), 27.3 (1C, C-4_{cyclohexyl}), 29.7 (2C, C-3_{cyclohexyl}, C-5_{cyclohexyl}), 36.2 (1C, C-4), 36.9 (1C, C-2'), 39.4 (1C, C-6'), 49.9 (2C, C-3_{piperazine}, C-5_{piperazine}), 50.1 (2C, C-2_{piperazine}, C-6_{piperazine}), 56.4 (1C, OCH₃), 63.9 (1C, C-4'), 64.9

(1C, C-1_{cyclohexyl}), 77.3 (1C, C-1), 97.8 (1C, C-3), 125.7 (1C, C-8), 127.5 (1C, C-7), 127.7 (1C, C-6), 130.1 (1C, C-5), 132.6 (1C, C-4a), 142.6 (1C, C-8a). FT-IR (neat): ν [cm⁻¹] = 2926 (C-H_{alkyl}), 2827, 2798 (N-CH₂), 1489 (C=C_{arom}). Purity (HPLC): 99.7 %, t_R = 13.9 min.

7.2.3. *trans*-6,7-Dimethoxy-2-(3-methoxy-3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-4'-yl)-1,2,3,4-tetrahydroisoquinoline (6e) and *cis*-6,7-Dimethoxy-2-(3-methoxy-3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-4'-yl)-1,2,3,4-tetrahydroisoquinoline (6f)

A solution of ketone **5** (205 mg, 0.83 mmol) and 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (262 mg, 1.14 mmol, 1.4 eq) in CH₂Cl₂ (30 mL) was stirred under N₂ atmosphere at rt. After 4 h, NaBH(OAc)₃ (395 mg, 1.87 mmol, 2.3 eq) was added and the mixture was stirred for 43 h at rt. 1 M NaOH (20 mL) was added and the aqueous layer was extracted with CH₂Cl₂ (4 x 30 mL). The combined organic layers were dried (Na₂SO₄), filtered, concentrated in vacuo and the residue was purified by fc (d = 2 cm, l = 20 cm, V = 10 mL, cyclohexane/ethyl acetate 80:20 + 1 % *N,N*-dimethylethanamine → 66:33 + 1 % *N,N*-dimethylethanamine). **6e** was eluted first and **6f** afterwards. **6f** was purified twice by fc (d = 2 cm, l = 16 cm, V = 10 mL, cyclohexane/ethyl acetate 50:50 + 1 % HCOOH → 66:33 + 1 % *N,N*-dimethylethanamine; d = 2 cm, l = 20 cm, V = 10 mL, cyclohexane/ethyl acetate 80:20 + 1 % *N,N*-dimethylethanamine → 60:40 + 1 % *N,N*-dimethylethanamine).

***trans*-6e**: Yellow solid, mp 158 °C, yield 66 mg (19 %). C₂₆H₃₃NO₄ (423.6 g/mol). R_f = 0.67 (cyclohexane/ethyl acetate 50:50 + 1 % *N,N*-dimethylethanamine). HR-MS (APCI): m/z = 424.2454 (calcd. 424.2482 for C₂₆H₃₄NO₄ [MH⁺]). ¹H NMR (400 MHz, DMSO-*d*₆): δ (ppm) = 1.48 (dq, *J* = 12.3/2.7 Hz, 1H, 2'-*H*), 1.70 – 1.78 (m, 1H, 6'-*H*),

1
2
3 1.86 – 2.07 (m, 5H, 3'-H, 5'-H, 6'-H), 2.21 (td, $J = 12.8/3.9$ Hz, 1H, 2'-H), 2.36 – 2.44
4 (m, 1H, 4'-H_{equ}), 2.69 – 2.77 (m, 3H, 4-H, 3-H_{isoquinoline}), 2.77 – 2.82 (m, 2H, 4-
5 H_{isoquinoline}), 2.90 (dd, $J = 15.8/3.1$ Hz, 1H, 4-H), 3.46 (s, 3H, 3-OCH₃), 3.63 (s, 2H, 1-
6 H_{isoquinoline}), 3.72 (s, 3H, 6-OCH₃), 3.74 (s, 3H, 7-OCH₃), 4.90 (dd, $J = 7.2/3.1$ Hz, 1H,
7 3-H), 6.71 (s, 2H, 5-H_{isoquinoline}, 8-H_{isoquinoline}), 7.03 (dd, $J = 7.0/2.0$ Hz, 1H, 8-H), 7.08
8 (dd, $J = 7.3/1.8$ Hz, 1H, 5-H), 7.11 – 7.19 (m, 2H, 6-H, 7-H). ¹³C NMR (101 MHz,
9 DMSO-*d*₆): δ (ppm) = 23.9 (1C, C-3' or C-5'), 24.1 (1C, C-3' or C-5'), 28.7 (1C, C-
10 4_{isoquinoline}), 30.9 (1C, C-6'), 33.3 (1C, C-2'), 34.7 (1C, C-4), 47.0 (1C, C-3_{isoquinoline}),
11 52.9 (1C, C-1_{isoquinoline}), 55.0 (1C, 3-OCH₃), 55.45 (1C, 6-OCH₃ or 7-OCH₃), 55.50
12 (1C, 6-OCH₃ or 7-OCH₃), 56.5 (1C, C-4'), 76.0 (1C, C-1), 95.8 (1C, C-3), 110.3 (1C,
13 C-8_{isoquinoline}), 111.6 (1C, C-5_{isoquinoline}), 124.4 (1C, C-8), 126.2 (1C, C-4a_{isoquinoline}),
14 126.2 (1C, C-6 or C-7), 126.3 (1C, C-6 or C-7), 127.0 (1C, C-8a_{isoquinoline}), 128.8 (1C,
15 C-5), 131.1 (1C, C-4a), 142.1 (1C, C-8a), 146.9 (1C, C-6_{isoquinoline} or C-7_{isoquinoline}),
16 147.1 (1C, C-6_{isoquinoline} or C-7_{isoquinoline}). FT-IR (neat): ν [cm⁻¹] = 2943, 2920, 2832 (C-
17 H_{alkyl}), 1516, 1447 (C=C_{arom}). Purity (HPLC): 98.8 %, $t_R = 17.7$ min. A sample was
18 recrystallized from CH₂Cl₂ to obtain crystals, which were suitable to for X-ray crystal
19 structure analysis.

20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40 *cis*-**6f**: Yellow solid, mp 180 °C, yield 133 mg (37 %). C₂₆H₃₃NO₄ (423.6 g/mol). R_f =
41 0.22 (cyclohexane/ethyl acetate 50:50 + 1 % *N,N*-dimethylethanamine). HR-MS
42 (APCI): $m/z = 424.2439$ (calcd. 424.2482 for C₂₆H₃₄NO₄ [MH⁺]). ¹H NMR (400 MHz,
43 DMSO-*d*₆): δ (ppm) = 1.61 – 1.71 (m, 1H, 2'-H), 1.71 – 1.85 (m, 5H, 3'-H, 5'-H, 6'-H),
44 1.85 – 2.02 (m, 1H, 6'-H), 2.02 – 2.11 (m, 1H, 2'-H), 2.64 – 2.76 (m, 5H, 4-H, 4'-H_{ax},
45 4-H_{isoquinoline}), 2.80 (t, $J = 5.7$ Hz, 2H, 3-H_{isoquinoline}), 2.90 (dd, $J = 15.9/3.2$ Hz, 1H, 4-
46 H), 3.47 (s, 3H, 3-OCH₃), 3.70 (s, 8H, 1-H_{isoquinoline}, 6-OCH₃, 7-OCH₃), 4.89 (dd, $J =$
47 7.2/3.1 Hz, 1H, 3-H), 6.65 (s, 1H, 5-H_{isoquinoline}), 6.66 (s, 1H, 8-H_{isoquinoline}), 7.09 (dd, J
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 = 7.3/1.8 Hz, 1H, 5-*H*), 7.12 – 7.21 (m, 2H, 6-*H*, 7-*H*), 7.25 (dd, $J = 7.4/1.8$ Hz, 1H, 8-
4 *H*). ^{13}C NMR (101 MHz, DMSO- d_6): δ (ppm) = 23.3 (2C, C-3', C-5'), 28.7 (1C, C-
5 4_{isoquinoline}), 34.6 (1C, C-4), 35.6 (1C, C-2'), 37.9 (1C, C-6'), 46.7 (1C, C-3_{isoquinoline}),
6 50.5 (1C, C-1_{isoquinoline}), 55.2 (1C, 3-OCH₃), 55.46 (1C, 6-OCH₃ or 7-OCH₃), 55.52
7 (1C, 6-OCH₃ or 7-OCH₃), 61.4 (1C, C-4'), 75.4 (1C, C-1), 95.7 (1C, C-3), 110.2 (1C,
8 C-8_{isoquinoline}), 111.7 (1C, C-5_{isoquinoline}), 124.5 (1C, C-8), 126.0 (1C, C-8a_{isoquinoline}),
9 126.2 (1C, C-6 or C-7), 126.3 (1C, C-6 or C-7), 126.6 (1C, C-4a_{isoquinoline}), 128.9 (1C,
10 C-5), 131.3 (1C, C-4a), 141.5 (1C, C-8a), 146.8 (1C, C-6_{isoquinoline} or C-7_{isoquinoline}),
11 147.1 (1C, C-6_{isoquinoline} or C-7_{isoquinoline}). FT-IR (neat): ν [cm⁻¹] = 2978, 2940, (C-H_{alkyl}),
12 1516, 1466, 1450 (C=C_{arom}). Purity (HPLC): 95.3 %, $t_R = 17.9$ min. A sample was
13 recrystallized from CH₂Cl₂ to obtain crystals, which were suitable to for X-ray crystal
14 structure analysis.
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30

31 **7.2.4. *trans*-4'-[(2,4-Dimethylbenzyl)amino]-3,4-dihydrospiro[[2]benzopyran-** 32 **1,1'-cyclohexan]-3-ol (10a)**

33
34
35
36 A solution of acetal **6a** (254 mg, 0.69 mmol) and 0.2 M HCl (17 mL, 3.5 mmol, 5.0 eq)
37 in THF (20 mL) was stirred at rt for 3 d. 1 M NaOH (10 mL) was added and the
38 aqueous layer was extracted with Et₂O (4 x 10 mL). The combined organic layers
39 were dried (Na₂SO₄), filtered, concentrated in vacuo and the residue was purified by
40 fc (d = 2 cm, l = 20 cm, V = 10 mL, cyclohexane/ethyl acetate 80:20 + 1 % *N,N*-
41 dimethylethanamine). Pale yellow solid, mp 142 °C, yield 173 mg (71 %). C₂₃H₂₉NO₂
42 (351.5 g/mol). $R_f = 0.11$ (cyclohexane/ethyl acetate 80:20 + 1 % *N,N*-
43 dimethylethanamine). HR-MS (APCI): $m/z = 352.2277$ (calcd. 352.2271 for
44 C₂₃H₃₀NO₂ [MH⁺]). ^1H NMR (400 MHz, CDCl₃): δ (ppm) = 1.60 – 1.74 (m, 3H, 2'-*H*,
45 3'-*H*, 5'-*H*), 1.74 – 1.81 (m, 1H, 6'-*H*), 1.94 – 2.15 (m, 3H, 3'-*H*, 5'-*H*, 6'-*H*), 2.25 –
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

2.40 (m, 1H, 2'-H), 2.32 (s, 1H, 4-CH₃), 2.41 (s, 3H, 2-CH₃), 2.85 (dd, *J* = 15.5/8.0 Hz, 1H, 4-H), 3.01 (dd, *J* = 15.5/3.0 Hz, 1H, 4-H), 3.03 – 3.07 (m, 1H, 4'-H_{equ}), 3.75 (s, 2H, ArCH₂NH), 5.28 (dd, *J* = 7.8/2.9 Hz, 1H, 3-H), 6.98 – 7.04 (m, 2H, 3-H_{benzyl}, 5-H_{benzyl}), 7.06 – 7.11 (m, 1H, 5-H), 7.13 – 7.26 (m, 4H, 6-H, 7-H, 8-H, 6-H_{benzyl}). Signals for the NH and OH protons are not observed in the spectrum. ¹³C NMR (101 MHz, CDCl₃): δ (ppm) = 19.2 (1C, 2-CH₃), 21.2 (1C, 4-CH₃), 26.1 (1C, C-3' or C-5'), 26.3 (1C, C-3' or C-5'), 30.6 (1C, C-6'), 33.5 (1C, C-2'), 37.3 (1C, C-4), 49.9 (1C, ArCH₂NH), 51.4 (1C, C-4'), 77.9 (1C, C-1), 89.7 (1C, C-3), 125.2 (1C, C-8), 126.6 (1C, C-6 or C-7), 126.7 (2C, C-6 or C-7, C-5_{benzyl}), 129.1 (1C, C-6_{benzyl}), 129.2 (1C, C-5), 131.2 (1C, C-4a), 131.3 (1C, C-3_{benzyl}), 136.1 (1C, C-1_{benzyl}), 136.7 (1C, C-2_{benzyl}), 136.8 (1C, C-4_{benzyl}), 142.5 (1C, C-8a). FT-IR (neat): ν [cm⁻¹] = 3337 (N-H/O-H), 2924 (C-H_{alkyl}), 1454, 1435 (C=C_{arom}). Purity (HPLC): 98.6 %, *t*_R = 19.5 min.

7.2.5. *cis*-4'-[(2,4-Dimethylbenzyl)amino]-3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-3-ol (10b)

A solution of acetal **6b** (201 mg, 0.55 mmol) and 2 M HCl (3 mL, 6.0 mmol, 11 eq) in THF (10 mL) was stirred at rt for 3 d. 1 M NaOH (10 mL) was added and the aqueous layer was extracted with CH₂Cl₂ (4 x 10 mL). The combined organic layers were dried (Na₂SO₄), filtered, concentrated in vacuo and the residue was purified by fc (*d* = 2 cm, *l* = 16 cm, *V* = 10 mL, cyclohexane/ethyl acetate 50:50 + 1 % *N,N*-dimethylethanamine). Colorless solid, mp 177 °C, yield 156 mg (80 %). C₂₃H₂₉NO₂ (351.5 g/mol). *R*_f = 0.03 (cyclohexane/ethyl acetate 50:50 + 1 % *N,N*-dimethylethanamine). HR-MS (APCI): *m/z* = 352.2254 (calcd. 352.2271 for C₂₃H₃₀NO₂ [MH⁺]). ¹H NMR (400 MHz, CDCl₃): δ (ppm) = 1.62 (td, *J* = 13.6/3.6 Hz, 1H, 2'-H_{ax}), 1.68 – 1.81 (m, 1H, 3'-H), 1.81 – 1.93 (m, 4H, 3'-H, 5'-H, 6'-H), 1.93 –

2.01 (m, 1H, 6'-H), 2.07 (dq, $J = 13.9/3.0$ Hz, 1H, 2'-H_{equ}), 2.30 (s, 3H, 4-CH₃), 2.34 (s, 3H, 2-CH₃), 2.69 (m, 1H, 4'-H_{ax}), 2.83 (dd, $J = 15.6/7.7$ Hz, 1H, 4-H), 2.97 (dd, $J = 15.7/3.1$ Hz, 1H, 4-H), 3.82 (s, 2H, ArCH₂NH), 5.26 (dd, $J = 7.7/3.0$ Hz, 1H, 3-H), 6.96 – 7.02 (m, 2H, 3-H_{benzyl}, 5-H_{benzyl}), 7.07 – 7.13 (m, 2H, 5-H, 8-H), 7.14 – 7.23 (m, 3H, 6-H, 7-H, 6-H_{benzyl}). Signals for the NH and OH protons are not observed in the spectrum. ¹³C NMR (101 MHz, CDCl₃): δ (ppm) = 19.1 (1C, 2-CH₃), 21.1 (1C, 4-CH₃), 28.7 (1C, C-3'), 28.8 (1C, C-5'), 35.6 (1C, C-6'), 36.9 (1C, C-4), 38.3 (1C, C-2'), 48.5 (1C, ArCH₂NH), 56.2 (1C, C-4'), 77.4 (1C, C-1), 89.5 (1C, C-3), 124.6 (1C, C-8), 126.6 (1C, C-6 or C-7), 126.7 (1C, C-5_{benzyl}), 126.8 (1C, C-6 or C-7), 128.8 (1C, C-6_{benzyl}), 129.4 (1C, C-5), 131.3 (1C, C-3_{benzyl}), 131.7 (1C, C-4a), 135.3 (1C, C-1_{benzyl}), 136.2 (1C, C-2_{benzyl}), 136.8 (1C, C-4_{benzyl}), 141.7 (1C, C-8a). FT-IR (neat): ν [cm⁻¹] = 3256 (N-H), 2932 (C-H_{alkyl}), 1454, 1366 (C=C_{arom}). Purity (HPLC): 99.5 %, $t_R = 17.5$ min.

7.2.6. *trans*-4'-(4-Cyclohexylpiperazin-1-yl)-3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-3-ol (10c)

A solution of acetal **6c** (174 mg, 0.44 mmol) and 0.2 M HCl (11 mL, 2.2 mmol, 5 eq) in THF (10 mL) was stirred at rt for 3 d. 1 M NaOH (5 mL) was added and the aqueous layer was extracted with Et₂O (4 x 10 mL). The combined organic layers were dried (Na₂SO₄), filtered, concentrated in vacuo and the residue was purified by fc (d = 2 cm, l = 15 cm, V = 10 mL, cyclohexane/ethyl acetate 50:50 + 2 % *N,N*-dimethylethanamine). Pale yellow solid, mp 214 °C, yield 145 mg (86 %). C₂₄H₃₆N₂O₂ (384.6 g/mol). $R_f = 0.10$ (cyclohexane/ethyl acetate 50:50 + 2 % *N,N*-dimethylethanamine). HR-MS (APCI): $m/z = 385.2885$ (calcd. 385.2850 for C₂₄H₃₇N₂O₂ [MH⁺]). ¹H NMR (600 MHz, CDCl₃): δ (ppm) = 1.07 – 1.19 (m, 1H, 4-

1
2
3 $H_{\text{cyclohexyl}}$), 1.19 – 1.34 (m, 4H, 2- $H_{\text{cyclohexyl}}$, 3- $H_{\text{cyclohexyl}}$, 5- $H_{\text{cyclohexyl}}$, 6- $H_{\text{cyclohexyl}}$), 1.58
4 (dq, $J = 13.7/3.5/3.0$ Hz, 1H, 2'- H_{equ}), 1.62 – 1.67 (m, 1H, 4- $H_{\text{cyclohexyl}}$), 1.70 (dq, $J =$
5 13.4/3.0 Hz, 1H, 6'- H_{equ}), 1.78 – 1.91 (m, 5H, 3'- H , 5'- H , 2- $H_{\text{cyclohexyl}}$, 6- $H_{\text{cyclohexyl}}$), 1.91
6 – 2.04 (m, 4H, 3'- H or 5'- H , 6'- H_{ax} , 3- $H_{\text{cyclohexyl}}$, 5- $H_{\text{cyclohexyl}}$), 2.22 (td, $J = 13.2/3.6$ Hz,
7 1H, 2'- H_{ax}), 2.25 – 2.36 (m, 2H, 4'- H_{equ} , 1- $H_{\text{cyclohexyl}}$), 2.69 (broad signal, 7H,
8 $H_{\text{piperazine}}$), 2.85 (dd, $J = 15.5/7.8$ Hz, 2H, 4- H , $H_{\text{piperazine}}$), 3.00 (dd, $J = 15.5/2.9$ Hz,
9 1H, 4- H), 5.28 (dd, $J = 7.8/3.5$ Hz, 1H, 3- H), 7.08 (d, $J = 7.5$ Hz, 1H, 5- H), 7.15 – 7.24
10 (m, 3H, 6- H , 7- H , 8- H). A signal for the OH proton is not observed in the spectrum.
11
12
13 ^{13}C NMR (151 MHz, CDCl_3): δ (ppm) = 24.0 (1C, C-3' or C-5'), 24.2 (1C, C-3' or C-
14 5'), 26.0 (2C, C-2 $_{\text{cyclohexyl}}$, C-6 $_{\text{cyclohexyl}}$), 26.4 (1C, C-4 $_{\text{cyclohexyl}}$), 29.2 (2C, C-3 $_{\text{cyclohexyl}}$, C-
15 5 $_{\text{cyclohexyl}}$), 31.0 (1C, C-6'), 34.1 (1C, C-2'), 37.3 (1C, C-4), 49.9 (2C, C-2 $_{\text{piperazine}}$ and
16 C-6 $_{\text{piperazine}}$ or C-3 $_{\text{piperazine}}$ and C-5 $_{\text{piperazine}}$), 50.5 (2C, C-2 $_{\text{piperazine}}$ and C-6 $_{\text{piperazine}}$ or C-
17 3 $_{\text{piperazine}}$ and C-5 $_{\text{piperazine}}$), 57.5 (1C, C-4'), 63.8 (1C, C-1 $_{\text{cyclohexyl}}$), 78.0 (1C, C-1), 89.7
18 (1C, C-3), 125.2 (1C, C-8), 126.6 (2C, C-6, C-7), 129.2 (1C, C-5), 131.3 (1C, C-4a),
19 142.6 (1C, C-8a). FT-IR (neat): ν [cm^{-1}] = 3325 (O-H), 2959, 2932, 2805 (C-H $_{\text{alkyl}}$),
20 1454 (C=C $_{\text{arom}}$). Purity (HPLC): 97.4 %, $t_{\text{R}} = 12.7$ min.
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39

40 **7.2.7. *cis*-4'-(4-Cyclohexylpiperazin-1-yl)-3,4-dihydrospiro[[2]benzopyran-1,1'-** 41 **cyclohexan]-3-ol (10d)**

42
43
44 A solution of acetal **6d** (273 mg, 0.68 mmol) and 0.5 M HCl (7 mL, 3.5 mmol, 5 eq) in
45 THF (10 mL) was stirred at rt for 4 d. 1 M NaOH (8 mL) was added and the aqueous
46 layer was extracted with Et₂O (4 x 8 mL). The combined organic layers were dried
47 (Na₂SO₄), filtered, concentrated in vacuo and the residue was purified by fc (d =
48 3 cm, l = 18 cm, V = 20 mL, cyclohexane/ethyl acetate 50:50 + 2 % *N,N*-
49 dimethylethanamine). Colorless solid, mp 204 °C, yield 211 mg (81 %). C₂₄H₃₆N₂O₂
50
51
52
53
54
55
56
57
58
59
60

(384.6 g/mol). $R_f = 0.03$ (cyclohexane/ethyl acetate 50:50 + 2 % *N,N*-dimethylethanamine). HR-MS (APCI): $m/z = 385.2874$ (calcd. 385.2850 for $C_{24}H_{37}N_2O_2$ [MH^+]). 1H NMR (600 MHz, $CDCl_3$): δ (ppm) = 1.05 – 1.16 (m, 1H, 4- $H_{cyclohexyl}$), 1.17 – 1.34 (m, 4H, 2- $H_{cyclohexyl}$, 3- $H_{cyclohexyl}$, 5- $H_{cyclohexyl}$, 6- $H_{cyclohexyl}$), 1.58 – 1.67 (m, 2H, 2'- H_{ax} , 4- $H_{cyclohexyl}$), 1.75 – 1.86 (m, 5H, 3'- H , 5'- H , 2- $H_{cyclohexyl}$, 6- $H_{cyclohexyl}$), 1.86 – 1.97 (m, 4H, 5'- H , 6'- H_{ax} , 3- $H_{cyclohexyl}$, 5- $H_{cyclohexyl}$), 1.99 (dq, $J = 9.6/3.0/2.6$ Hz, 1H, 6'- H_{equ}), 2.11 (dq, $J = 14.3/3.3$ Hz, 1H, 2'- H_{equ}), 2.27 (broad signal, 1H, 1- $H_{cyclohexyl}$), 2.40 – 2.48 (m, 1H, 4'- H_{ax}), 2.58 – 2.81 (broad signal, 8H, $H_{piperazine}$), 2.85 (dd, $J = 15.6/7.7$ Hz, 1H, 4- H), 2.99 (dd, $J = 15.6/2.9$ Hz, 1H, 4- H), 5.29 (dd, $J = 7.9/3.1$ Hz, 1H, 3- H), 7.07 – 7.14 (m, 2H, 5- H , 8- H), 7.14 – 7.22 (m, 2H, 6- H , 7- H). A signal for the OH proton is not observed in the spectrum. ^{13}C NMR (151 MHz, $CDCl_3$): δ (ppm) = 24.0 (1C, C-5'), 24.2 (1C, C-3'), 26.0 (2C, C-2 $_{cyclohexyl}$, C-6 $_{cyclohexyl}$), 26.4 (1C, C-4 $_{cyclohexyl}$), 29.01 (1C, C-3 $_{cyclohexyl}$ or C-5 $_{cyclohexyl}$), 29.03 (1C, C-3 $_{cyclohexyl}$ or C-5 $_{cyclohexyl}$), 36.2 (1C, C-2'), 37.0 (1C, C-4), 38.9 (1C, C-6'), 49.4 (2C, C-2 $_{piperazine}$ and C-6 $_{piperazine}$ or C-3 $_{piperazine}$ and C-5 $_{piperazine}$), 49.5 (2C, C-2 $_{piperazine}$ and C-6 $_{piperazine}$ or C-3 $_{piperazine}$ and C-5 $_{piperazine}$), 63.1 (1C, C-4'), 63.8 (1C, C-1 $_{cyclohexyl}$), 76.8 (1C, C-1), 89.5 (1C, C-3), 124.7 (1C, C-8), 126.6 (1C, C-7), 126.8 (1C, C-6), 129.4 (1C, C-5), 131.6 (1C, C-4a), 141.5 (1C, C-8a). FT-IR (neat): ν [cm^{-1}] = 3352 (O-H), 2928 (C-H $_{alkyl}$), 1454 (C=C $_{arom}$). Purity (HPLC): 97.7 %, $t_R = 12.7$ min.

7.2.8. *trans*-4'-(6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl)-3,4-dihydro-spiro[[2]benzopyran-1,1'-cyclohexan]-3-ol (10e)

A solution of acetal **6e** (154 mg, 0.36 mmol) and 0.2 M HCl (9 mL, 1.8 mmol, 5 eq) in THF (10 mL) was stirred at rt for 3 d. 1 M NaOH (5 mL) was added and the aqueous layer was extracted with CH_2Cl_2 (4 x 10 mL). The combined organic layers were dried

(Na₂SO₄), filtered, concentrated in vacuo and the residue was purified by fc (d = 2 cm, l = 19 cm, V = 10 mL, cyclohexane/ethyl acetate 80:20 + 1 % *N,N*-dimethylethanamine → cyclohexane/ethyl acetate 50:50 + 1 % *N,N*-dimethylethanamine). Colorless solid, mp 174 °C, yield 127 mg (86 %). C₂₅H₃₁NO₄ (409.5 g/mol). R_f = 0.31 (cyclohexane/ethyl acetate 50:50 + 1 % *N,N*-dimethylethanamine). HR-MS (APCI): m/z = 410.2294 (calcd. 410.2326 for C₂₅H₃₂NO₄ [MH⁺]). ¹H NMR (600 MHz, DMSO-*d*₆): δ (ppm) = 1.46 (dq, *J* = 12.9/2.9 Hz, 1H, 2'-*H*_{equ}), 1.72 (dq, *J* = 13.2/2.8 Hz, 1H, 6'-*H*_{equ}), 1.84 – 1.91 (m, 1H, 6'-*H*_{ax}), 1.91 – 1.99 (m, 4H, 3'-*H*, 5'-*H*), 2.17 (ddd, *J* = 12.9/10.6/6.4 Hz, 1H, 2'-*H*_{ax}), 2.36 (quint, *J* = 3.0 Hz, 1H, 4'-*H*_{equ}), 2.68 (dd, *J* = 15.7/8.2 Hz, 1H, 4-*H*), 2.71 – 2.76 (m, 2H, 3-*H*_{isoquinoline}), 2.76 – 2.80 (m, 2H, 4-*H*_{isoquinoline}), 2.82 (dd, *J* = 15.7/2.8 Hz, 1H, 4-*H*), 3.61 (s, 2H, 1-*H*_{isoquinoline}), 3.70 (s, 3H, 7-OCH₃), 3.73 (s, 3H, 6-OCH₃), 5.11 (ddd, *J* = 8.6/5.9/2.9 Hz, 1H, 3-*H*), 6.41 (d, *J* = 5.9 Hz, 1H, OH), 6.70 (s, 1H, 5-*H*_{isoquinoline}), 6.70 (s, 1H, 8-*H*_{isoquinoline}), 7.00 (dd, *J* = 7.5/1.6 Hz, 1H, 8-*H*), 7.06 (dd, *J* = 6.1/1.6 Hz, 1H, 5-*H*), 7.09 – 7.15 (m, 2H, 6-*H*, 7-*H*). ¹³C NMR (151 MHz, DMSO-*d*₆): δ (ppm) = 23.8 (1C, C-3'), 23.9 (1C, C-5'), 28.7 (1C, C-4_{isoquinoline}), 30.3 (1C, C-6'), 33.7 (1C, C-2'), 36.8 (1C, C-4), 47.0 (1C, C-3_{isoquinoline}), 52.9 (1C, C-1_{isoquinoline}), 55.4 (1C, 7-OCH₃), 55.5 (1C, 6-OCH₃), 56.6 (1C, C-4'), 76.3 (1C, C-1), 88.3 (1C, C-3), 110.3 (1C, C-8_{isoquinoline}), 111.6 (1C, C-5_{isoquinoline}), 124.5 (1C, C-8), 126.11 (1C, C-6), 126.14 (1C, C-7), 126.2 (1C, C-4a_{isoquinoline}), 127.1 (1C, C-8a_{isoquinoline}), 128.9 (1C, C-5), 132.1 (1C, C-4a), 142.5 (1C, C-8a), 146.9 (1C, C-7_{isoquinoline}), 147.1 (1C, C-6_{isoquinoline}). FT-IR (neat): ν [cm⁻¹] = 3360 (O-H), 2928, 2882, 2808 (C-H_{alkyl}), 1520, 1454 (C=C_{arom}). Purity (HPLC): 95.4 %, *t*_R = 15.2 min.

1
2
3 **7.2.9. *cis*-4'-(6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl)-3,4-dihydro-**
4 **spiro[[2]benzopyran-1,1'-cyclohexan]-3-ol (10f)**
5
6

7
8 A solution of acetal **6f** (97 mg, 0.23 mmol) and 0.2 M HCl (6 mL, 1.2 mmol, 5.2 eq) in
9 THF (10 mL) was stirred at rt for 3 d. 1 M NaOH (5 mL) was added and the aqueous
10 layer was extracted with CH₂Cl₂ (4 x 10 mL). The combined organic layers were dried
11 (Na₂SO₄), filtered, concentrated in vacuo and the residue was purified by fc (d =
12 2 cm, l = 19 cm, V = 10 mL, cyclohexane/ethyl acetate 50:50 + 1 % *N,N*-
13 dimethylethanamine → ethyl acetate + 1 % *N,N*-dimethylethanamine). Pale yellow
14 solid, mp 202 °C, yield 69 mg (74 %). C₂₅H₃₁NO₄ (409.5 g/mol). R_f = 0.06
15 (cyclohexane/ethyl acetate 50:50 + 1 % *N,N*-dimethylethanamine). HR-MS (APCI):
16 m/z = 410.2278 (calcd. 410.2326 for C₂₅H₃₂NO₄ [MH⁺]). ¹H NMR (600 MHz, DMSO-
17 d₆): δ (ppm) = 1.59 (td, *J* = 13.6/3.8 Hz, 1H, 2'-H_{ax}), 1.61 – 1.68 (m, 2H, 3'-H, 5'-H),
18 1.74 – 1.78 (m, 1H, 6'-H), 1.80 – 1.94 (m, 3H, 3'-H, 5'-H, 6'-H), 2.02 (dq, *J* = 13.7/3.2
19 Hz, 1H, 2'-H_{equ}), 2.58 – 2.64 (m, 1H, 4'-H_{ax}), 2.64 – 2.70 (m, 3H, 4-H, 4-H_{isoquinoline}),
20 2.75 (t, *J* = 5.8 Hz, 2H, 3-H_{isoquinoline}), 2.81 (dd, *J* = 15.8/2.9 Hz, 1H, 4-H), 3.64 (s, 2H,
21 1-H_{isoquinoline}), 3.68 (s, 6H, 6-OCH₃, 7-OCH₃), 5.09 (ddd, *J* = 8.6/5.9/2.9 Hz, 1H, 3-H),
22 6.41 (d, *J* = 5.9 Hz, 1H, OH), 6.62 (s, 2H, 5-H_{isoquinoline}, 8-H_{isoquinoline}), 7.05 (dd, *J* =
23 7.5/1.5 Hz, 1H, 5-H), 7.11 (td, *J* = 7.3/1.5 Hz, 1H, 6-H), 7.14 (td, *J* = 7.5/1.7 Hz, 1H,
24 7-H), 7.20 (dd, *J* = 7.7/1.5 Hz, 1H, 8-H). ¹³C NMR (151 MHz, DMSO-d₆): δ (ppm) =
25 23.2 (1C, C-3' or C-5'), 23.3 (1C, C-3' or C-5'), 29.1 (1C, C-4_{isoquinoline}), 35.2 (1C, C-
26 2'), 36.7 (1C, C-4), 38.3 (1C, C-6'), 46.9 (1C, C-3_{isoquinoline}), 50.7 (1C, C-1_{isoquinoline}),
27 55.48 (1C, 6-OCH₃ or 7-OCH₃), 55.52 (1C, 6-OCH₃ or 7-OCH₃), 61.6 (1C, C-4'), 75.6
28 (1C, C-1), 88.2 (1C, C-3), 110.2 (1C, C-8_{isoquinoline}), 111.8 (1C, C-5_{isoquinoline}), 124.6
29 (1C, C-8), 126.1 (1C, C-7), 126.2 (1C, C-6), 126.3 (1C, C-4a_{isoquinoline}), 127.4 (1C, C-
30 8a_{isoquinoline}), 129.0 (1C, C-5), 132.2 (1C, C-4a), 142.0 (1C, C-8a), 146.8 (1C, C-
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 6_{isoquinoline} or C-7_{isoquinoline}), 147.0 (1C, C-6_{isoquinoline} or C-7_{isoquinoline}). FT-IR (neat): ν [cm⁻¹]
4 = 3102 (O-H), 2931, 2939 (C-H_{alkyl}), 1516, 1466 (C=C_{arom}). Purity (HPLC): 97.9 %,
5
6 t_R = 15.7 min.
7
8
9

10
11
12
13
14
15
16 **7.2.10. Ethyl *trans*-2-{4'-[(2,4-dimethylbenzyl)amino]-3,4-**
17
18 **dihydrospiro[[2]benzo-pyran-1,1'-cyclohexan]-3-yl}acetate (11a)**
19

20 A solution of lactol **10a** (154 mg, 0.44 mmol) and (ethoxycarbonylmethylene)-
21 triphenylphosphorane (308 mg, 0.89 mmol, 2.0 eq) in toluene (25 mL) was heated to
22 reflux under N₂ atmosphere for 5 d. After cooling to rt, the mixture was concentrated
23 in vacuo and the residue was purified by fc (d = 3 cm, l = 20 cm, V = 20 mL,
24 cyclohexane/ethyl acetate 95:5 + 2 % *N,N*-dimethylethanamine). Yellow oil, yield
25 72 mg (39 %). C₂₇H₃₅NO₃ (421.6 g/mol). R_f = 0.14 (cyclohexane/ethyl acetate 90:10
26 + 1 % *N,N*-dimethylethanamine). HR-MS (APCI): m/z = 422.2688 (calcd. 422.2690
27 for C₂₇H₃₆NO₃ [MH⁺]). ¹H NMR (400 MHz, CDCl₃): δ (ppm) = 1.30 (t, J = 7.1 Hz, 3H,
28 OCH₂CH₃), 1.45 – 1.52 (m, 1H, 2'-*H*), 1.55 – 1.70 (m, 2H, 3'-*H*, 5'-*H*), 1.81 – 1.92 (m,
29 2H, 5'-*H*, 6'-*H*), 1.94 – 2.05 (m, 2H, 3'-*H*, 6'-*H*), 2.28 – 2.38 (m, 1H, 2'-*H*), 2.32 (s, 3H,
30 4-CH₃), 2.41 (s, 3H, 2-CH₃), 2.56 – 2.68 (m, 2H, CH₂CO₂Et), 2.68 – 2.80 (m, 2H, 4-
31 *H*), 2.96 – 3.00 (m, 1H, 4'-*H*_{equ}), 3.74 (s, 2H, ArCH₂NH), 4.11 – 4.29 (m, 3H, 3-*H*,
32 OCH₂CH₃), 6.98 – 7.06 (m, 3H, 5-*H*, 3-*H*_{benzyl}, 5-*H*_{benzyl}), 7.09 – 7.15 (m, 1H, 6-*H*), 7.15
33 – 7.21 (m, 2H, 7-*H*, 8-*H*), 7.21 – 7.26 (m, 1H, 6-*CH*_{benzyl}). A signal for the NH proton is
34 not observed in the spectrum. ¹³C NMR (101 MHz, CDCl₃): δ (ppm) = 14.4 (1C,
35 OCH₂CH₃), 19.2 (1C, 2-CH₃), 21.1 (1C, 4-CH₃), 26.0 (2C, C-3', C-5'), 29.3 (1C, C-6'),
36 33.5 (1C, C-2'), 35.3 (1C, C-4), 41.8 (1C, CH₂CO₂Et), 49.9 (1C, ArCH₂NH), 51.5 (1C,
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

C-4'), 60.7 (1C, OCH₂CH₃), 65.3 (1C, C-3), 76.4 (1C, C-1), 125.6 (1C, C-8), 126.1 (1C, C-6), 126.4 (1C, C-7), 126.7 (1C, C-5_{benzyl}), 128.7 (1C, C-5), 129.1 (1C, C-6_{benzyl}), 131.3 (1C, C-3_{benzyl}), 132.9 (1C, C-4a), 136.3 (1C, C-1_{benzyl}) 136.7 (1C, C-2_{benzyl}), 136.8 (1C, C-4_{benzyl}), 143.1 (1C, C-8a), 171.7 (1C, C=O). FT-IR (neat): ν [cm⁻¹] = 3063 (N-H), 2955, 2924 (C-H_{alkyl}), 1732 (C=O), 1450, 1369 (C=C_{arom}). Purity (HPLC): 98.1 %, t_R = 21.8 min.

7.2.11. Ethyl *cis*-2-{4'-[(2,4-dimethylbenzyl)amino]-3,4-dihydrospiro[[2]benzo-pyran-1,1'-cyclohexan]-3-yl}acetate (11b)

A solution of lactol **10b** (164 mg, 0.47 mmol) and (ethoxycarbonylmethylene)-triphenylphosphorane (330 mg, 0.95 mmol, 2.0 eq) in toluene (30 mL) was heated to reflux under N₂ atmosphere for 3 d. After cooling to rt, the mixture was concentrated in vacuo and the residue was purified by fc (d = 3 cm, l = 20 cm, V = 20 mL, cyclohexane/ethyl acetate 80:20 + 1 % *N,N*-dimethylethanamine). Yellow oil, yield 156 mg (79 %). C₂₇H₃₅NO₃ (421.6 g/mol). R_f = 0.18 (cyclohexane/ethyl acetate 80:20 + 1 % *N,N*-dimethylethanamine). HR-MS (APCI): m/z = 422.2690 (calcd. 422.2690 for C₂₇H₃₆NO₃ [MH⁺]). ¹H NMR (600 MHz, CDCl₃): δ (ppm) = 1.29 (t, J = 7.1 Hz, 3H, OCH₂CH₃), 1.54 – 1.60 (m, 2H, 2'-H, 3'-H), 1.66 – 1.75 (m, 1H, 5'-H), 1.77 – 1.93 (m, 4H, 3'-H, 5'-H, 6'-H), 2.21 (dt, J = 10.7/2.9 Hz, 1H, 2'-H), 2.30 (s, 3H, 4-CH₃), 2.34 (s, 3H, 2-CH₃), 2.58 (dd, J = 15.0/5.0 Hz, 1H, CH₂CO₂Et), 2.63 – 2.80 (m, 4H, 4-H, 4'-H_{ax}, CH₂CO₂Et), 3.80 (s, 2H, ArCH₂NH), 4.14 – 4.27 (m, 3H, 3-H, OCH₂CH₃), 6.97 – 7.00 (m, 2H, 3-H_{benzyl}, 5-H_{benzyl}), 7.06 (dd, J = 7.5/1.3 Hz, 1H, 5-H), 7.09 (dd, J = 7.7/1.4 Hz, 1H, 8-H), 7.13 (td, J = 7.4/1.4 Hz, 1H, 6-H), 7.16 – 7.20 (m, 1H, 7-H), 7.21 (d, J = 7.9 Hz, 1H, 6-H_{benzyl}). A signal for the NH proton is not observed in the spectrum. ¹³C NMR (151 MHz, CDCl₃): δ (ppm) = 14.4 (1C, OCH₂CH₃), 19.1 (1C, 2-

1
2
3 CH₃), 21.1 (1C, 4-CH₃), 28.6 (2C, C-3', C-5'), 34.3 (1C, C-2'), 35.2 (1C, C-4), 38.5
4 (1C, C-6'), 41.6 (1C, CH₂CO₂Et), 48.4 (1C, ArCH₂NH), 56.4 (1C, C-4'), 60.8 (1C,
5 OCH₂CH₃), 65.3 (1C, C-3), 75.7 (1C, C-1), 125.1 (1C, C-8), 126.2 (1C, C-6), 126.3
6 (1C, C-7), 126.7 (1C, C-5_{benzyl}), 128.7 (1C, C-6_{benzyl}), 128.9 (1C, C-5), 131.3 (1C, C-
7 3_{benzyl}), 133.3 (1C, C-4a), 135.6 (1C, C-1_{benzyl}), 136.3 (1C, C-2_{benzyl}), 136.6 (1C, C-
8 4_{benzyl}), 142.3 (1C, C-8a), 171.6 (1C, C=O). FT-IR (neat): ν [cm⁻¹] = 3063 (N-H), 2924
9 (C-H_{alkyl}), 1732 (C=O), 1447, 1373 (C=C_{arom}). Purity (HPLC): 95.2 %, t_R = 21.8 min.

10
11
12
13
14
15
16
17
18
19
20 **7.2.12. Ethyl *trans*-2-{4'-(4-cyclohexylpiperazin-1-yl)-3,4-**
21 **dihydrospiro[[2]benzo-pyran-1,1'-cyclohexan]-3-yl}acetate (11c)**

22
23
24
25 A solution of lactol **10c** (120 mg, 0.31 mmol) and (ethoxycarbonylmethylene)-
26 triphenylphosphorane (216 mg, 0.62 mmol, 2.0 eq) in toluene (10 mL) was heated to
27 reflux under N₂ atmosphere for 5 d. After cooling to rt, the mixture was concentrated
28 in vacuo and the residue was purified by fc (d = 2.5 cm, l = 20 cm, V = 10 mL,
29 cyclohexane/ethyl acetate 80:20 + 2 % *N,N*-dimethylethanamine). Yellow solid, mp
30 135 °C, yield 73 mg (52 %). C₂₈H₄₂N₂O₃ (454.7 g/mol). R_f = 0.18 (cyclohexane/ethyl
31 acetate 80:20 + 2 % *N,N*-dimethylethanamine). HR-MS (APCI): m/z = 455.3303
32 (calcd. 455.3268 for C₂₈H₄₃N₂O₃ [MH⁺]). ¹H NMR (400 MHz, CDCl₃): δ (ppm) = 1.07 –
33 1.19 (m, 1H, 4-*H*_{cyclohexyl}), 1.19 – 1.34 (m, 7H, OCH₂CH₃, 2-*H*_{cyclohexyl}, 3-*H*_{cyclohexyl}, 5-
34 *H*_{cyclohexyl}, 6-*H*_{cyclohexyl}), 1.38 – 1.45 (m, 1H, 2'-*H*), 1.60 – 1.69 (m, 1H, 4-*H*_{cyclohexyl}),
35 1.70 – 1.93 (m, 8H, 3'-*H*, 5'-*H*, 6'-*H*, 2-*H*_{cyclohexyl}, 6-*H*_{cyclohexyl}), 1.93 – 2.04 (m, 2H, 3-
36 *H*_{cyclohexyl}, 5-*H*_{cyclohexyl}), 2.16 – 2.23 (m, 2H, 2'-*H*, 4'-*H*_{eq}), 2.23 – 2.33 (m, 1H, 1-
37 *H*_{cyclohexyl}), 2.47 – 2.79 (m, 12H, 4-*H*, CH₂CO₂Et, H_{piperazine}), 4.09 – 4.28 (m, 3H, 3-*H*,
38 OCH₂CH₃), 7.04 (d, *J* = 7.3 Hz, 1H, 5-*H*), 7.09 – 7.15 (m, 1H, 6-*H*), 7.15 – 7.23 (m,
39 2H, 7-*H*, 8-*H*). ¹³C NMR (101 MHz, CDCl₃): δ (ppm) = 14.4 (1C, OCH₂CH₃), 23.86
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

(1C, C-3'), 23.91 (1C, C-5'), 26.0 (2C, C-2_{cyclohexyl}, C-6_{cyclohexyl}), 26.4 (1C, C-4_{cyclohexyl}), 29.2 (2C, C-3_{cyclohexyl}, C-5_{cyclohexyl}), 29.6 (1C, C-6'), 34.0 (1C, C-2'), 35.3 (1C, CH₂CO₂Et), 41.7 (1C, C-4), 49.9 (2C, C-2_{piperazine} and C-6_{piperazine} or C-3_{piperazine} and C-5_{piperazine}), 50.5 (2C, C-2_{piperazine} and C-6_{piperazine} or C-3_{piperazine} and C-5_{piperazine}), 57.5 (1C, C-4'), 60.7 (1C, OCH₂CH₃), 63.8 (1C, C-1_{cyclohexyl}), 65.4 (1C, C-3), 76.5 (1C, C-1), 125.6 (1C, C-8), 126.1 (1C, C-6), 126.3 (1C, C-7), 128.7 (1C, C-5), 133.0 (1C, C-4a), 143.2 (1C, C-8a), 171.7 (1C, C=O). FT-IR (neat): ν [cm⁻¹] = 2928, 2805 (C-H_{alkyl}), 1736 (C=O), 1450 (C=C_{arom}). Purity (HPLC): 97.5 %, t_R = 16.8 min.

7.2.13. Ethyl *cis*-2-{4'-(4-cyclohexylpiperazin-1-yl)-3,4-dihydrospiro[[2]benzo-pyran-1,1'-cyclohexan]-3-yl}acetate (**11d**)

A solution of lactol **10d** (174 mg, 0.45 mmol) and (ethoxycarbonylmethylene)-triphenylphosphorane (326 mg, 0.94 mmol, 2.1 eq) in toluene (20 mL) was heated to reflux under N₂ atmosphere for 4 d. After cooling to rt, the mixture was concentrated in vacuo and the residue was purified twice by fc (d = 3 cm, l = 20 cm, V = 10 mL, cyclohexane/ethyl acetate 66:33 + 2 % *N,N*-dimethylethanamine; d = 2 cm, l = 21 cm, V = 10 mL, cyclohexane/ethyl acetate 80:20 + 2 % *N,N*-dimethylethanamine). Yellow solid, mp 117 °C, yield 182 mg (89 %). C₂₈H₄₂N₂O₃ (454.7 g/mol). R_f = 0.17 (cyclohexane/ethyl acetate 66:33 + 2 % *N,N*-dimethylethanamine). HR-MS (APCI): m/z = 455.3256 (calcd. 455.3268 for C₂₈H₄₃N₂O₃ [MH⁺]). ¹H NMR (600 MHz, CDCl₃): δ (ppm) = 1.05 – 1.15 (m, 1H, 4-*H*_{cyclohexyl}), 1.16 – 1.26 (m, 4H, 2-*H*_{cyclohexyl}, 3-*H*_{cyclohexyl}, 5-*H*_{cyclohexyl}, 6-*H*_{cyclohexyl}), 1.28 (t, *J* = 7.2 Hz, 3H, OCH₂CH₃), 1.51 – 1.58 (m, 1H, 2'-*H*), 1.59 – 1.65 (m, 1H, 4-*H*_{cyclohexyl}), 1.65 – 1.75 (m, 3H, 3'-*H*, 5'-*H*), 1.75 – 1.88 (m, 5H, 5'-*H*, 6'-*H*, 2-*H*_{cyclohexyl}, 6-*H*_{cyclohexyl}), 1.88 – 1.95 (m, 2H, 3-*H*_{cyclohexyl}, 5-*H*_{cyclohexyl}), 2.20 – 2.28 (m, 2H, 2'-*H*, 1-*H*_{cyclohexyl}), 2.38 – 2.47 (m, 1H, 4'-*H*_{ax}), 2.57 (dd,

1
2
3 $J = 15.0/4.7$ Hz, 1H, $\text{CH}_2\text{CO}_2\text{Et}$), 2.60 – 2.70 (m, 9H, $\text{CH}_2\text{CO}_2\text{Et}$, $H_{\text{piperazine}}$), 2.70 –
4 2.77 (m, 2H, 4- H), 4.12 – 4.25 (m, 3H, 3- H , OCH_2CH_3), 7.05 (d, $J = 7.5$ Hz, 1H, 5- H),
5 7.09 (d, $J = 7.7$ Hz, 1H, 8- H), 7.12 (td, $J = 7.4/1.4$ Hz, 1H, 6- H), 7.17 (t, $J = 7.7$ Hz,
6 1H, 7- H). ^{13}C NMR (151 MHz, CDCl_3): δ (ppm) = 14.4 (1C, OCH_2CH_3), 23.7 (1C, C-
7 5'), 23.8 (1C, C-3'), 26.1 (2C, C-2_{cyclohexyl}, C-6_{cyclohexyl}), 26.4 (1C, C-4_{cyclohexyl}), 29.1
8 (2C, C-3_{cyclohexyl}, C-5_{cyclohexyl}), 34.9 (1C, C-2'), 35.2 (1C, C-4), 39.1 (1C, C-6'), 41.6
9 (1C, $\text{CH}_2\text{CO}_2\text{Et}$), 49.4 (2C, C-2_{piperazine} and C-6_{piperazine} or C-3_{piperazine} and C-5_{piperazine}),
10 49.5 (2C, C-2_{piperazine} and C-6_{piperazine} or C-3_{piperazine} and C-5_{piperazine}), 60.7 (1C,
11 OCH_2CH_3), 63.2 (1C, C-4'), 63.8 (1C, C-1_{cyclohexyl}), 65.3 (1C, C-3), 75.6 (1C, C-1),
12 125.1 (1C, C-8), 126.2 (1C, C-6), 126.3 (1C, C-7), 128.9 (1C, C-5), 133.2 (1C, C-4a),
13 142.2 (1C, C-8a), 171.5 (1C, C=O). FT-IR (neat): ν [cm^{-1}] = 2978, 2928, 2855 (C-
14 H_{alkyl}), 1728 (C=O), 1450 (C=C_{arom}). Purity (HPLC): 97.2 %, $t_R = 16.4$ min.

30
31 **7.2.14. Ethyl *trans*-2-{4'-(6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl)-**
32 **3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-3-yl}acetate (11e)**

33
34
35
36 A solution of lactol **10e** (160 mg, 0.39 mmol) and (ethoxycarbonylmethylene)-
37 triphenylphosphorane (272 mg, 0.78 mmol, 2.0 eq) in toluene (12 mL) was heated to
38 reflux under N_2 atmosphere for 4 d. After cooling to rt, the mixture was concentrated
39 in vacuo and the residue was purified by fc (d = 3 cm, l = 19 cm, V = 20 mL,
40 cyclohexane/ethyl acetate 90:10 + 1 % *N,N*-dimethylethanamine). Yellow oil, yield
41 175 mg (94 %). $\text{C}_{29}\text{H}_{37}\text{NO}_5$ (479.6 g/mol). $R_f = 0.31$ (cyclohexane/ethyl acetate 80:20
42 + 1 % *N,N*-dimethylethanamine). HR-MS (APCI): $m/z = 480.2755$ (calcd. 480.2744
43 for $\text{C}_{29}\text{H}_{38}\text{NO}_5$ [MH^+]). ^1H NMR (400 MHz, CDCl_3): δ (ppm) = 1.31 (t, $J = 7.2$ Hz, 3H,
44 OCH_2CH_3), 1.43 – 1.51 (m, 1H, 2'- H), 1.76 – 2.04 (m, 6H, 3'- H , 5'- H , 6'- H), 2.30 (dt, J
45 = 14.0/8.7 Hz, 1H, 2'- H), 2.36 – 2.41 (m, 1H, 4'- H_{equ}), 2.56 – 2.68 (m, 2H,
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 $\text{CH}_2\text{CO}_2\text{Et}$), 2.69 – 2.76 (m, 2H, 4-*H*), 2.76 – 2.81 (m, 2H, 3-*H*_{isoquinoline}), 2.82 – 2.90
4 (m, 2H, 4-*H*_{isoquinoline}), 3.66 (s, 2H, 1-*H*_{isoquinoline}), 3.86 (s, 3H, 7- OCH_3), 3.88 (s, 3H, 6-
5 OCH_3), 4.11 – 4.23 (m, 2H, OCH_2CH_3), 4.23 – 4.31 (m, 1H, 3-*H*), 6.59 (s, 1H, 8-
6 *H*_{isoquinoline}), 6.65 (s, 1H, 5-*H*_{isoquinoline}), 7.03 (d, $J = 6.7$ Hz, 1H, 5-*H*), 7.07 – 7.17 (m,
7 3H, 6-*H*, 7-*H*, 8-*H*). ^{13}C NMR (101 MHz, CDCl_3): δ (ppm) = 14.5 (1C, OCH_2CH_3), 24.3
8 (2C, C-3', C-5'), 29.4 (1C, C-4_{isoquinoline}), 29.6 (1C, C-6'), 34.0 (1C, C-2'), 35.3 (1C, C-
9 4), 41.8 (1C, $\text{CH}_2\text{CO}_2\text{Et}$), 47.5 (1C, C-3_{isoquinoline}), 53.5 (1C, C-1_{isoquinoline}), 56.1 (2C, 6-
10 OCH_3 , 7- OCH_3), 57.2 (1C, C-4'), 60.7 (1C, OCH_2CH_3), 65.4 (1C, C-3), 76.6 (1C, C-
11 1), 109.9 (1C, C-8_{isoquinoline}), 111.3 (1C, C-5_{isoquinoline}), 125.8 (1C, C-6 or C-8), 126.1
12 (1C, C-6 or C-8), 126.3 (1C, C-7), 127.1 (1C, C-4_{isoquinoline}), 127.8 (1C, C-
13 8_{isoquinoline}), 128.6 (1C, C-5), 132.9 (1C, C-4a), 143.0 (1C, C-8a), 147.4 (1C, C-
14 7_{isoquinoline}), 147.5 (1C, C-6_{isoquinoline}), 171.7 (1C, C=O). FT-IR (neat): ν [cm^{-1}] = 2967,
15 2920 (C- H_{alkyl}), 1736 (C=O), 1520, 1454 (C=C_{arom}). Purity (HPLC): 98.4 %, $t_{\text{R}} = 20.0$ min.

32
33
34
35 **7.2.15. Ethyl *cis*-2-{4'-(6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl)-**
36 **3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-3-yl}acetate (11f)**

37
38
39
40 A solution of lactol **10f** (175 mg, 0.43 mmol) and (ethoxycarbonylmethylene)-
41 triphenylphosphorane (305 mg, 0.88 mmol, 2.1 eq) in toluene (12 mL) was heated to
42 reflux under N_2 atmosphere for 5 d. After cooling to rt, the mixture was concentrated
43 in vacuo and the residue was purified by fc (d = 3 cm, l = 19 cm, V = 20 mL,
44 cyclohexane/ethyl acetate 80:20 + 1 % *N,N*-dimethylethanamine). Yellow oil, yield
45 157 mg (77 %). $\text{C}_{29}\text{H}_{37}\text{NO}_5$ (479.6 g/mol). $R_{\text{f}} = 0.09$ (cyclohexane/ethyl acetate 80:20
46 + 1 % *N,N*-dimethylethanamine). HR-MS (APCI): $m/z = 480.2752$ (calcd. 480.2744
47 for $\text{C}_{29}\text{H}_{38}\text{NO}_5$ [MH^+]). ^1H NMR (400 MHz, CDCl_3): δ (ppm) = 1.31 (t, $J = 7.1$ Hz, 3H,
48
49
50
51
52
53
54
55
56
57
58
59
60

OCH₂CH₃), 1.55 – 1.65 (m, 1H, 2'-H_{ax}), 1.73 – 1.99 (m, 6H, 3'-H, 5'-H, 6'-H), 2.28 (dq, *J* = 14.2/3.1 Hz, 1H, 2'-H_{equ}), 2.61 (dd, *J* = 15.1/4.6 Hz, 1H, CH₂CO₂Et), 2.65 – 2.79 (m, 4H, 4-H, 4'-H_{ax}, CH₂CO₂Et), 2.79 – 2.92 (m, 4H, 3-H_{isoquinoline}, 4-H_{isoquinoline}), 3.77 (s, 2H, 1-H_{isoquinoline}), 3.84 (s, 3H, 6-OCH₃ or 7-OCH₃), 3.84 (s, 3H, 6-OCH₃ or 7-OCH₃), 4.16 – 4.23 (m, 2H, OCH₂CH₃), 4.23 – 4.31 (m, 1H, 3-H), 6.53 (s, 1H, 8-H_{isoquinoline}), 6.59 (s, 1H, 5-H_{isoquinoline}), 7.07 (d, *J* = 7.1 Hz, 1H, 5-H), 7.10 – 7.13 (m, 1H, 8-H), 7.13 – 7.16 (m, 1H, 6-H), 7.16 – 7.22 (m, 1H, 7-H). ¹³C NMR (101 MHz, CDCl₃): δ (ppm) = 14.4 (1C, OCH₂CH₃), 23.3 (1C, C-3' or C-5'), 23.6 (1C, C-3' or C-5'), 29.4 (1C, C-4_{isoquinoline}), 34.9 (1C, C-2'), 35.2 (1C, C-4), 39.1 (1C, C-6'), 41.7 (1C, CH₂CO₂Et), 46.9 (1C, C-3_{isoquinoline}), 51.4 (1C, C-1_{isoquinoline}), 56.06 (1C, 6-OCH₃ or 7-OCH₃), 56.08 (1C, 6-OCH₃ or 7-OCH₃), 60.7 (1C, OCH₂CH₃), 62.7 (1C, C-4'), 65.3 (1C, C-3), 75.6 (1C, C-1), 109.8 (1C, C-8_{isoquinoline}), 111.6 (1C, C-5_{isoquinoline}), 125.1 (1C, C-8), 126.3 (1C, C-6), 126.4 (1C, C-7), 126.7 (1C, C-4a_{isoquinoline}), 127.3 (1C, C-8a_{isoquinoline}), 128.9 (1C, C-5), 133.2 (1C, C-4a), 142.1 (1C, C-8a), 147.3 (1C, C-7_{isoquinoline}), 147.6 (1C, C-6_{isoquinoline}), 171.6 (1C, C=O). FT-IR (neat): ν [cm⁻¹] = 2928 (C-H_{alkyl}), 1732 (C=O), 1516, 1450 (C=C_{arom}). Purity (HPLC): 96.0 %, *t*_R = 20.1 min.

7.2.16. trans-2-{4'-[(2,4-Dimethylbenzyl)amino]-3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-3-yl}ethanol (12a)

A solution of LiAlH₄ (22 mg, 0.59 mmol, 3.1 eq) in THF (5 mL) was added slowly to a solution of ester **11a** (82 mg, 0.19 mmol) in Et₂O (10 mL) at -20 °C under N₂ atmosphere. After stirring for 4 h, H₂O (10 mL) was added, the precipitate was filtered off and the aqueous layer was extracted with ethyl acetate (3 x 10 mL). The combined organic layers were dried (Na₂SO₄), filtered, concentrated in vacuo and the residue was purified by fc (d = 1.5 cm, l = 18 cm, V = 5 mL, cyclohexane/ethyl

1
2
3 acetate 90:10 + 1 % *N,N*-dimethylethanamine → 70:30 + 1 % *N,N*-
4 dimethylethanamine). Yellow oil, yield 58 mg (79 %). $C_{25}H_{33}NO_2$ (379.5 g/mol). $R_f =$
5 0.11 (cyclohexane/ethyl acetate 80:20 + 1 % *N,N*-dimethylethanamine). HR-MS
6 (APCI): $m/z = 380.2568$ (calcd. 380.2584 for $C_{25}H_{34}NO_2$ [MH^+]). 1H NMR (600 MHz,
7 $CDCl_3$): δ (ppm) = 1.49 – 1.56 (m, 1H, 2'-*H*), 1.64 – 1.78 (m, 2H, 3'-*H*, 5'-*H*), 1.85 –
8 1.98 (m, 5H, 3'-*H*, 5'-*H*, 6'-*H*, CH_2CH_2OH), 2.00 – 2.10 (m, 1H, 6'-*H*), 2.32 (s, 3H, 4-
9 CH_3), 2.34 – 2.44 (m, 1H, 2'-*H*), 2.41 (s, 3H, 4- CH_3), 2.61 (dd, $J = 15.8/2.7$ Hz, 1H, 4-
10 *H*), 2.84 (dd, $J = 15.7/11.4$ Hz, 1H, 4-*H*), 3.01 – 3.10 (m, 1H, 4'- H_{eq}), 3.75 (s, 2H,
11 $ArCH_2NH$), 3.86 – 3.93 (m, 2H, CH_2CH_2OH), 4.00 – 4.06 (m, 1H, 3-*H*), 6.99 – 7.03
12 (m, 2H, 3- H_{benzyl} , 5- H_{benzyl}), 7.05 (d, $J = 7.6$ Hz, 1H, 5-*H*), 7.11 – 7.15 (m, 1H, 6-*H*),
13 7.15 – 7.20 (m, 2H, 7-*H*, 8-*H*), 7.22 – 7.26 (m, 1H, 6- H_{benzyl}). Signals for the NH and
14 OH protons are not observed in the spectrum. ^{13}C NMR (151 MHz, $CDCl_3$): δ (ppm) =
15 19.2 (1C, 2- CH_3), 21.2 (1C, 4- CH_3), 26.3 (2C, C-3', C-5'), 29.3 (1C, C-6'), 33.4 (1C,
16 C-2'), 35.5 (1C, C-4), 38.0 (1C, CH_2CH_2OH), 49.9 (1C, $ArCH_2NH$), 51.2 (1C, C-4'),
17 61.5 (1C, CH_2CH_2OH), 68.5 (1C, C-3), 76.7 (1C, C-1), 125.7 (1C, C-8), 126.2 (1C, C-
18 6), 126.3 (1C, C-7), 126.7 (1C, C-5 $_{benzyl}$), 128.7 (1C, C-5), 129.1 (1C, C-6 $_{benzyl}$), 131.3
19 (1C, C-3 $_{benzyl}$), 133.0 (1C, C-4a), 136.1 (1C, C-1 $_{benzyl}$), 136.7 (1C, C-2 $_{benzyl}$), 136.9
20 (1C, C-4 $_{benzyl}$), 142.8 (1C, C-8a). FT-IR (neat): ν [cm^{-1}] = 3426 (O-H/N-H), 2920 (C-
21 H_{alkyl}), 1435, 1373 (C=C $_{arom}$), 1061 (C-O). Purity (HPLC): 98.2 %, $t_R = 18.3$ min.
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45

46 **7.2.17. cis-2-{4'-(2,4-Dimethylbenzyl)amino}-3,4-**
47 **dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-3-yl}ethanol (12b)**
48
49

50
51 $LiAlH_4$ (55 mg, 1.44 mmol, 2.9 eq) was added slowly to a solution of ester **11b**
52 (211 mg, 0.50 mmol) in Et_2O (10 mL) at -20 °C under N_2 atmosphere. After stirring for
53 2 h, H_2O (10 mL) was added, the precipitate was filtered off and the aqueous layer
54
55
56
57
58
59
60

1
2
3 was extracted with ethyl acetate (3 x 10 mL). The combined organic layers were
4
5 dried (Na₂SO₄), filtered, concentrated in vacuo and the residue was purified by fc (d =
6
7 3 cm, l = 16 cm, V = 20 mL, cyclohexane/ethyl acetate 10:20 + 1 % *N,N*-
8
9 dimethylethanamine → ethyl acetate + 1 % *N,N*-dimethylethanamine). Pale yellow
10
11 solid, mp 89 °C, yield 157 mg (82 %). C₂₅H₃₃NO₂ (379.5 g/mol). R_f = 0.06
12
13 (cyclohexane/ethyl acetate 50:50 + 1 % *N,N*-dimethylethanamine). HR-MS (APCI):
14
15 *m/z* = 380.2568 (calcd. 380.2568 for C₂₅H₃₄NO₂ [MH⁺]). ¹H NMR (600 MHz, CDCl₃):
16
17 δ (ppm) = 1.53 – 1.66 (m, 3H, 2'-*H*, 3'-*H*, 5'-*H*), 1.80 – 1.85 (m, 1H, 6'-*H*), 1.85 – 1.99
18
19 (m, 5H, 3'-*H*, 5'-*H*, 6'-*H*, CH₂CH₂OH), 2.20 (dt, *J* = 10.8/3.0 Hz, 1H, 2'-*H*), 2.30 (s, 3H,
20
21 4-CH₃), 2.34 (s, 3H, 2-CH₃), 2.63 (dd, *J* = 15.9/2.7 Hz, 1H, 4-*H*), 2.65 – 2.71 (m, 1H,
22
23 4'-*H*_{ax}), 2.82 (dd, *J* = 15.9/11.1 Hz, 1H, 4-*H*), 3.81 (s, 2H, ArCH₂NH), 3.86 – 3.95 (m,
24
25 2H, CH₂CH₂OH), 4.01 (ddt, *J* = 11.4/8.6/3.1 Hz, 1H, 3-*H*), 6.97 – 7.01 (m, 2H, 3-
26
27 *H*_{benzyl}, 5-*H*_{benzyl}), 7.05 – 7.11 (m, 2H, 5-*H*, 8-*H*), 7.14 (td, *J* = 7.4/1.5 Hz, 1H, 6-*H*),
28
29 7.17 (t, *J* = 7.5 Hz, 1H, 7-*H*), 7.21 (d, *J* = 8.2 Hz, 1H, 6-*H*_{benzyl}). Signals for the NH
30
31 and OH protons are not observed in the spectrum. ¹³C NMR (151 MHz, CDCl₃): δ
32
33 (ppm) = 19.1 (1C, 2-CH₃), 21.1 (1C, 4-CH₃), 29.0 (1C, C-3' or C-5'), 29.1 (1C, C-3' or
34
35 C-5'), 34.4 (1C, C-2'), 35.5 (1C, C-4), 38.1 (1C, CH₂CH₂OH), 38.4 (1C, C-6'), 48.4
36
37 (1C, ArCH₂NH), 56.0 (1C, C-4'), 60.8 (1C, CH₂CH₂OH), 67.6 (1C, C-3), 75.8 (1C, C-
38
39 1), 125.1 (1C, C-8), 126.26 (1C, C-6 or C-7), 126.27 (1C, C-6 or C-7), 126.7 (1C, C-
40
41 5_{benzyl}), 128.6 (1C, C-6_{benzyl}), 128.9 (1C; C-5), 131.3 (1C, C-3_{benzyl}), 133.5 (1C, C-4a),
42
43 135.6 (1C, C-1_{benzyl}), 136.3 (1C, C-2_{benzyl}), 136.6 (1C, C-4_{benzyl}), 142.1 (1C, C-8a). FT-
44
45 IR (neat): ν [cm⁻¹] = 3364 (O-H/N-H), 2924 (C-H_{alkyl}), 1454 (C=C_{arom}), 1061 (C-O).
46
47
48
49 Purity (HPLC): 99.8 %, *t*_R = 18.7 min.
50
51
52
53
54
55
56
57
58
59
60

1
2
3 **7.2.18. trans-2-{4'-(4-Cyclohexylpiperazin-1-yl)-3,4-**
4 **dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-3-yl}ethanol (12c)**
5
6

7
8 A solution of LiAlH₄ (15 mg, 0.38 mmol, 2.9 eq) in THF (2 mL) was added slowly to a
9
10 solution of ester **11c** (60 mg, 0.13 mmol) in Et₂O (5 mL) at -20 °C under N₂
11
12 atmosphere. After stirring for 5 h, H₂O (8 mL) was added, the precipitate was filtered
13
14 off and the aqueous layer was extracted with ethyl acetate (3 x 10 mL). The
15
16 combined organic layers were dried (Na₂SO₄), filtered, concentrated in vacuo and the
17
18 residue was purified by fc (d = 1.5 cm, l = 18 cm, V = 10 mL, cyclohexane/ethyl
19
20 acetate 50:50 + 2 % *N,N*-dimethylethanamine). Colorless solid, mp 185 °C, yield
21
22 45 mg (85 %). C₂₆H₄₀N₂O₂ (412.6 g/mol). R_f = 0.11 (cyclohexane/ethyl acetate 50:50
23
24 + 2 % *N,N*-dimethylethanamine). HR-MS (APCI): *m/z* = 413.3154 (calcd. 413.3163
25
26 for C₂₆H₄₁N₂O₂ [MH⁺]). ¹H NMR (600 MHz, CDCl₃): δ (ppm) = 1.08 – 1.18 (m, 1H, 4-
27
28 *H*_{cyclohexyl}), 1.19 – 1.31 (m, 4H, 2-*H*_{cyclohexyl}, 3-*H*_{cyclohexyl}, 5-*H*_{cyclohexyl}, 6-*H*_{cyclohexyl}), 1.45
29
30 (dq, *J* = 13.3/3.0 Hz, 1H, 2'-*H*_{equ}), 1.61 – 1.68 (m, 1H, 4-*H*_{cyclohexyl}), 1.70 – 1.78 (m,
31
32 2H, 3'-*H*, 5'-*H*), 1.78 – 1.86 (m, 3H, 6'-*H*, 2-*H*_{cyclohexyl}, 6-*H*_{cyclohexyl}), 1.86 – 2.02 (m, 7H,
33
34 CH₂CH₂OH, 3'-*H*, 5'-*H*, 6'-*H*, 3-*H*_{cyclohexyl}, 5-*H*_{cyclohexyl}), 2.20 – 2.31 (m, 3H, 2'-*H*_{ax}, 4'-
35
36 *H*_{equ}, 1-*H*_{cyclohexyl}), 2.40 – 2.77 (broad signal, 8H, 4-*H*, *H*_{piperazine}), 2.83 (dd, *J* =
37
38 15.8/11.1 Hz, 1H, 4-*H*), 2.86 – 2.93 (broad signal, 1H, *H*_{piperazine}) 3.86 – 3.93 (m, 2H,
39
40 CH₂CH₂OH), 4.02 (ddt, *J* = 11.6/8.6/3.1 Hz, 1H, 3-*H*), 7.04 (d, *J* = 7.5 Hz, 1H, 5-*H*),
41
42 7.13 (td, *J* = 7.2/1.7 Hz, 1H, 6-*H*), 7.15 – 7.22 (m, 2H, 7-*H*, 8-*H*). A signal for the OH
43
44 proton is not observed in the spectrum. ¹³C NMR (151 MHz, CDCl₃): δ (ppm) = 24.2
45
46 (1C, C-3' or C-5'), 24.3 (1C, C-3' or C-5'), 26.0 (2C, C-2_{cyclohexyl}, C-6_{cyclohexyl}), 26.4
47
48 (1C, C-4_{cyclohexyl}), 29.2 (2C, C-3_{cyclohexyl}, C-5_{cyclohexyl}), 29.7 (1C, C-6'), 34.0 (1C, C-2'),
49
50 35.6 (1C, C-4), 38.0 (1C, CH₂CH₂OH), 49.9 (2C, C-2_{piperazine} and C-6_{piperazine} or C-
51
52 3_{piperazine} and C-5_{piperazine}), 50.5 (2C, C-2_{piperazine} and C-6_{piperazine} or C-3_{piperazine} and C-
53
54 5_{piperazine}).

1
2
3 5_{piperazine}), 57.3 (1C, C-4'), 61.4 (1C, CH₂CH₂OH), 63.7 (1C, C-1_{cyclohexyl}), 68.4 (1C, C-
4 3), 76.8 (1C, C-1), 125.6 (1C, C-8), 126.1 (1C, C-6), 126.3 (1C, C-7), 128.6 (1C, C-
5 5), 133.2 (1C, C-4a), 143.0 (1C, C-8a). FT-IR (neat): ν [cm⁻¹] = 3198 (O-H), 2978,
6 2932, 2816 (C-H_{alkyl}), 1450 (C=C_{arom}). Purity (HPLC, method 1): 99.5 %,
7 t_R = 14.0 min.
8
9
10
11
12
13
14
15
16
17
18
19

20 **7.2.19. cis-2-{4'-(4-Cyclohexylpiperazin-1-yl)-3,4-
21 dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-3-yl}ethanol (12d)**
22
23

24
25 A solution of LiAlH₄ (30 mg, 0.79 mmol, 2.8 eq) in THF (2 mL) was added slowly to a
26 solution of ester **11d** (125 mg, 0.28 mmol) in Et₂O (6 mL) at -20 °C under N₂
27 atmosphere. After stirring for 4 h, H₂O (15 mL) was added, the precipitate was filtered
28 off and the aqueous layer was extracted with ethyl acetate (3 x 15 mL). The
29 combined organic layers were dried (Na₂SO₄), filtered, concentrated in vacuo and the
30 residue was purified twice by fc (d = 2 cm, l = 17 cm, V = 10 mL, cyclohexane/ethyl
31 acetate 33:66 + 2 % *N,N*-dimethylethanamine → ethyl acetate + 2 % *N,N*-
32 dimethylethanamine; d = 1.5 cm, l = 10 cm, V = 3 mL, cyclohexane/ethyl acetate
33 66:33 + 2 % *N,N*-dimethylethanamine → 33:66 + 2 % *N,N*-dimethylethanamine).
34 Colorless solid, mp 128 °C, yield 94 mg (82 %). C₂₆H₄₀N₂O₂ (412.6 g/mol). R_f = 0.08
35 (cyclohexane/ethyl acetate 33:66 + 2 % *N,N*-dimethylethanamine). HR-MS (APCI):
36 m/z = 413.3137 (calcd. 413.3163 for C₂₆H₄₁N₂O₂ [MH⁺]). ¹H NMR (400 MHz, CDCl₃):
37 δ (ppm) = 1.04 – 1.16 (m, 1H, 4-*H*_{cyclohexyl}), 1.17 – 1.31 (m, 4H, 2-*H*_{cyclohexyl}, 3-
38 *H*_{cyclohexyl}, 5-*H*_{cyclohexyl}, 6-*H*_{cyclohexyl}), 1.53 – 1.68 (m, 2H, 2'-*H*, 4-*H*_{cyclohexyl}), 1.67 – 1.85
39 (m, 6H, 3'-*H*, 5'-*H*, 2-*H*_{cyclohexyl}, 6-*H*_{cyclohexyl}), 1.85 – 1.98 (m, 6H, CH₂CH₂OH, 6'-*H*, 3-
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

$H_{\text{cyclohexyl}}$, $5\text{-}H_{\text{cyclohexyl}}$, 2.19 – 2.30 (m, 2H, $2'\text{-}H$, $1\text{-}H_{\text{cyclohexyl}}$), 2.48 (tt, $J = 11.3/3.8$ Hz, 1H, $4'\text{-}H_{\text{ax}}$), 2.62 (dd, $J = 16.0/2.8$ Hz, 1H, $4\text{-}H$), 2.65 – 2.76 (broad signal, 8H, $H_{\text{piperazine}}$), 2.81 (dd, $J = 15.9/11.1$ Hz, 1H, $4\text{-}H$), 3.87 – 3.92 (m, 2H, $\text{CH}_2\text{CH}_2\text{OH}$), 3.96 – 4.05 (m, 1H, $3\text{-}H$), 7.05 (d, $J = 6.8$ Hz, 1H, $5\text{-}H$), 7.09 (dd, $J = 7.3/1.8$ Hz, 1H, $8\text{-}H$), 7.11 – 7.20 (m, 2H, $6\text{-}H$, $7\text{-}H$). A signal for the OH proton is not observed in the spectrum. ^{13}C NMR (101 MHz, CDCl_3): δ (ppm) = 23.8 (1C, $\text{C-}3'$ or $\text{C-}5'$), 23.8 (1C, $\text{C-}3'$ or $\text{C-}5'$), 26.0 (2C, $\text{C-}2_{\text{cyclohexyl}}$, $\text{C-}6_{\text{cyclohexyl}}$), 26.4 (1C, $\text{C-}4_{\text{cyclohexyl}}$), 29.06 (1C, $\text{C-}3_{\text{cyclohexyl}}$ or $\text{C-}5_{\text{cyclohexyl}}$), 29.07 (1C, $\text{C-}3_{\text{cyclohexyl}}$ or $\text{C-}5_{\text{cyclohexyl}}$), 34.9 (1C, $\text{C-}2'$), 35.5 (1C, $\text{C-}4$), 38.1 (1C, $\text{CH}_2\text{CH}_2\text{OH}$), 38.8 (1C, $\text{C-}6'$), 49.3 (2C, $\text{C-}2_{\text{piperazine}}$ and $\text{C-}6_{\text{piperazine}}$ or $\text{C-}3_{\text{piperazine}}$ and $\text{C-}5_{\text{piperazine}}$), 49.4 (2C, $\text{C-}2_{\text{piperazine}}$ and $\text{C-}6_{\text{piperazine}}$ or $\text{C-}3_{\text{piperazine}}$ and $\text{C-}5_{\text{piperazine}}$), 61.1 (1C, $\text{CH}_2\text{CH}_2\text{OH}$), 63.0 (1C, $\text{C-}4'$), 63.8 (1C, $\text{C-}1_{\text{cyclohexyl}}$), 68.1 (1C, $\text{C-}3$), 75.8 (1C, $\text{C-}1$), 125.1 (1C, $\text{C-}8$), 126.30 (1C, $\text{C-}6$ or $\text{C-}7$), 126.32 (1C, $\text{C-}6$ or $\text{C-}7$), 128.9 (1C, $\text{C-}5$), 133.4 (1C, $\text{C-}4\text{a}$), 141.9 (1C, $\text{C-}8\text{a}$). FT-IR (neat): ν [cm^{-1}] = 3225 (O-H), 2970, 2928, 2851 (C-H_{alkyl}), 1447 (C=C_{arom}). Purity (HPLC, method 1): 99.2 %, $t_R = 13.5$ min.

7.2.20. *trans*-2-{4'-(6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl)-3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-3-yl}ethanol (12e)

LiAlH_4 (36 mg, 0.93 mmol, 2.7 eq) was added slowly to a solution of ester **11e** (163 mg, 0.34 mmol) in Et_2O (9 mL) at -20 °C under N_2 atmosphere. After stirring for 3 h, H_2O (10 mL) was added, the precipitate was filtered off and the aqueous layer was extracted with ethyl acetate (3 x 10 mL). The combined organic layers were dried (Na_2SO_4), filtered, concentrated in vacuo and the residue was purified by fc ($d = 3$ cm, $l = 17$ cm, $V = 20$ mL, cyclohexane/ethyl acetate 66:33 + 1 % *N,N*-dimethylethanamine). Pale yellow solid, mp 160 °C, yield 105 mg (71 %). $\text{C}_{27}\text{H}_{35}\text{NO}_4$

(437.6 g/mol). $R_f = 0.23$ (cyclohexane/ethyl acetate 66:33 + 1% *N,N*-dimethylethanamine). HR-MS (APCI): $m/z = 438.2686$ (calcd. 438.2639 for $C_{27}H_{36}NO_4$ [MH^+]). 1H NMR (600 MHz, $CDCl_3$): δ (ppm) = 1.48 – 1.55 (m, 1H, 2'-H), 1.80 – 2.09 (m, 8H, 3'-H, 5'-H, 6'-H, CH_2CH_2OH), 2.28 – 2.38 (m, 1H, 2'-H), 2.40 – 2.52 (m, 1H, 4'- H_{equ}), 2.62 (dd, $J = 15.9/2.6$ Hz, 1H, 4-H), 2.75 – 2.93 (m, 5H, 4-H, 3- $H_{isoquinoline}$, 4- $H_{isoquinoline}$), 3.62 – 3.72 (m, 2H, 1- $H_{isoquinoline}$), 3.86 (s, 3H, 7- OCH_3), 3.88 (s, 3H, 6- OCH_3), 3.89 – 3.95 (m, 2H, CH_2CH_2OH), 4.04 (ddt, $J = 11.0/8.2/2.9$ Hz, 1H, 3-H), 6.59 (s, 1H, 8- $H_{isoquinoline}$), 6.65 (s, 1H, 5- $H_{isoquinoline}$), 7.03 (d, $J = 7.7$ Hz, 1H, 5-H), 7.09 – 7.17 (m, 3H, 6-H, 7-H, 8-H). A signal for the OH proton is not observed in the spectrum. ^{13}C NMR (151 MHz, $CDCl_3$): δ (ppm) = 24.6 (2C, C-3', C-5'), 29.4 (1C, C-4 $_{isoquinoline}$), 29.7 (1C, C-6'), 33.9 (1C, C-2'), 35.6 (1C, C-4), 38.0 (1C, CH_2CH_2OH), 47.4 (1C, C-3 $_{isoquinoline}$), 53.5 (1C, C-1 $_{isoquinoline}$), 56.1 (2C, 6- OCH_3 , 7- OCH_3), 57.0 (1C, C-4'), 61.4 (1C, CH_2CH_2OH), 68.5 (1C, C-3), 76.9 (1C, C-1), 109.9 (1C, C-8 $_{isoquinoline}$), 111.3 (1C, C-5 $_{isoquinoline}$), 125.9 (1C, C-6 or C-8), 126.2 (1C, C-6 or C-8), 126.3 (1C, C-7), 127.0 (1C, C-4a $_{isoquinoline}$), 127.6 (1C, C-8a $_{isoquinoline}$), 128.5 (1C, C-5), 133.0 (1C, C-4a), 142.7 (1C, C-8a), 147.4 (1C, C-7 $_{isoquinoline}$), 147.6 (1C, C-6 $_{isoquinoline}$). FT-IR (neat): ν [cm^{-1}] = 3437 (O-H), 2920 (C- H_{alkyl}), 1516, 1450 (C=C $_{arom}$). Purity (HPLC): 96.1 %, $t_R = 16.4$ min.

7.2.21. *cis*-2-{4'-(6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl)-3,4-dihydro-spiro[[2]benzopyran-1,1'-cyclohexan]-3-yl}ethanol (12f)

A solution of $LiAlH_4$ (32 mg, 0.85 mmol, 2.9 eq) in THF (5 mL) was added slowly to a solution of ester **11f** (137 mg, 0.29 mmol) in Et_2O (9 mL) at -20 °C under N_2 atmosphere. After stirring for 3.5 h, H_2O (10 mL) was added, the precipitate was filtered off and the aqueous layer was extracted with ethyl acetate (4 x 10 mL). The

combined organic layers were dried (Na_2SO_4), filtered, concentrated in vacuo and the residue was purified by fc ($d = 2 \text{ cm}$, $l = 18 \text{ cm}$, $V = 10 \text{ mL}$, cyclohexane/ethyl acetate 50:50 + 1 % *N,N*-dimethylethanamine \rightarrow ethyl acetate + 1 % *N,N*-dimethylethanamine). Yellow solid, mp $148 \text{ }^\circ\text{C}$, yield 88 mg (69 %). $\text{C}_{27}\text{H}_{35}\text{NO}_4$ (437.6 g/mol). $R_f = 0.06$ (cyclohexane/ethyl acetate 50:50 + 1 % *N,N*-dimethylethanamine). HR-MS (APCI): $m/z = 438.2673$ (calcd. 438.2639 for $\text{C}_{27}\text{H}_{36}\text{NO}_4$ [MH^+]). ^1H NMR (400 MHz, CDCl_3): δ (ppm) = 1.60 – 1.70 (m, 1H, 2'-*H*), 1.81 – 1.90 (m, 4H, 3'-*H*, 5'-*H*), 1.90 – 2.03 (m, 4H, 6'-*H*, $\text{CH}_2\text{CH}_2\text{OH}$), 2.25 – 2.33 (m, 1H, 2'-*H*), 2.64 (dd, $J = 15.9/2.6 \text{ Hz}$, 1H, 4-*H*), 2.70 – 2.79 (m, 2H, 4'- H_{ax}), 2.80 – 2.97 (m, 5H, 4-*H*, 3- $H_{\text{isoquinoline}}$, 4- $H_{\text{isoquinoline}}$), 3.81 (s, 2H, 1- $H_{\text{isoquinoline}}$), 3.84 (s, 6H, 6- OCH_3 , 7- OCH_3), 3.90 – 4.00 (m, 2H, $\text{CH}_2\text{CH}_2\text{OH}$), 4.00 – 4.09 (m, 1H, 3-*H*), 6.55 (s, 1H, 8- $H_{\text{isoquinoline}}$), 6.60 (s, 1H, 5- $H_{\text{isoquinoline}}$), 7.07 (d, $J = 7.3 \text{ Hz}$, 1H 5-*H*), 7.10 – 7.22 (m, 3H, 6-*H*, 7-*H*, 8-*H*). A signal for the OH proton is not observed in the spectrum. ^{13}C NMR (101 MHz, CDCl_3): δ (ppm) = 23.7 (1C, C-5'), 23.8 (1C, C-3'), 29.4 (1C, C-4 $_{\text{isoquinoline}}$), 35.0 (1C, C-2'), 35.5 (1C, C-4), 38.1 (1C, $\text{CH}_2\text{CH}_2\text{OH}$), 39.0 (1C, C-6'), 46.9 (1C, C-3 $_{\text{isoquinoline}}$), 51.5 (1C, C-1 $_{\text{isoquinoline}}$), 56.06 (1C, 6- OCH_3 or 7- OCH_3), 56.14 (1C, 6- OCH_3 or 7- OCH_3), 61.2 (1C, $\text{CH}_2\text{CH}_2\text{OH}$), 62.7 (1C, C-4'), 68.3 (1C, C-3), 75.9 (1C, C-1), 109.8 (1C, C-8 $_{\text{isoquinoline}}$), 111.6 (1C, C-5 $_{\text{isoquinoline}}$), 125.1 (1C, C-8), 126.3 (1C, C-6 or C-7), 126.4 (1C, C-6 or C-7), 126.6 (1C, C-4a $_{\text{isoquinoline}}$), 127.0 (1C, C-8a $_{\text{isoquinoline}}$), 128.9 (1C, C-5), 133.4 (1C, C-4a), 141.8 (1C; C-8a), 147.4 (1C, C-7 $_{\text{isoquinoline}}$), 147.6 (1C, C-6 $_{\text{isoquinoline}}$). FT-IR (neat): ν [cm^{-1}] = 3179 (O-H), 2943, 2920, 2851 (C- H_{alkyl}), 1516, 1443 (C=C $_{\text{arom}}$). Purity (HPLC): 96.3 %, $t_R = 16.7 \text{ min}$.

7.2.22. *trans*-N-(2,4-Dimethylbenzyl)-3-(2-fluoroethyl)-3,4-dihydrospiro[[2]benzo-pyran-1,1'-cyclohexan]-4'-amine (13a)

A solution of alcohol **12a** (36 mg, 0.09 mmol) in CH₂Cl₂ (4 mL) was added dropwise to a solution of DAST (0.03 mL, 0.23 mmol, 2.6 eq) in CH₂Cl₂ (5 mL) under N₂ atmosphere at -78 °C. After 90 min, the mixture was warmed to rt and stirred for 20 h. 1 M NaOH (3 mL) was added and the aqueous layer was extracted with CH₂Cl₂ (4 x 5 mL). The combined organic layers were dried (Na₂SO₄), filtered, concentrated in vacuo and the residue was purified by fc (d = 1 cm, l = 18 cm, V = 5 mL, cyclohexane/ethyl acetate 90:10 + 1 % *N,N*-dimethylethanamine → 80:20 + 1 % *N,N*-dimethylethanamine). Pale yellow solid, mp 67 °C, yield 24 mg (67 %). C₂₅H₃₂FNO (381.5 g/mol). R_f = 0.56 (cyclohexane/ethyl acetate 80:20 + 1 % *N,N*-dimethylethanamine). HR-MS (APCI): *m/z* = 382.2541 (calcd. 382.2541 for C₂₅H₃₃FNO₂ [MH⁺]). ¹H NMR (400 MHz, CDCl₃): δ (ppm) = 1.50 (dq, *J* = 13.4/3.0 Hz, 1H, 2'-*H*), 1.60 – 1.72 (m, 2H, 3'-*H*, 5'-*H*), 1.81 – 1.94 (m, 3H, 5'-*H*, 6'-*H*, CH₂CH₂F), 1.94 – 2.18 (m, 3H, 3'-*H*, 6'-*H*, CH₂CH₂F), 2.31 – 2.41 (m, 1H, 2'-*H*), 2.33 (s, 3H, 4-CH₃), 2.43 (s, 3H, 2-CH₃), 2.66 (dd, *J* = 15.8/3.1 Hz, 1H, 4-*H*), 2.75 (dd, *J* = 15.8/10.7 Hz, 1H, 4-*H*), 3.02 (quint, *J* = 3.0 Hz, 1H, 4'-*H*_{equ}), 3.75 (s, 2H, ArCH₂NH), 3.97 (ddt, *J* = 10.6/9.3/3.3 Hz, 1H, 3-*H*), 4.60 (ddd, *J* = 9.0/5.7/4.3 Hz, 0.5H, CH₂CH₂F), 4.72 (m, 2 x 0.5H, CH₂CH₂F), 4.84 (td, *J* = 9.0/4.5 Hz, 0.5H, CH₂CH₂F), 6.99 – 7.04 (m, 2H, 3-*H*_{benzyl}, 5-*H*_{benzyl}), 7.06 (m, 1H, 5-*H*), 7.10 – 7.16 (m, 1H, 6-*H*), 7.16 – 7.20 (m, 2H, 7-*H*, 8-*H*), 7.24 (d, *J* = 7.5 Hz, 1H, 6-*H*_{benzyl}). A signal for the NH proton is not observed in the spectrum. ¹³C NMR (101 MHz, CDCl₃): δ (ppm) = 19.1 (1C, 2-CH₃), 21.2 (1C, 4-CH₃), 26.1 (1C, C-3'), 26.3 (1C, C-5'), 29.3 (1C, C-6'), 33.6 (1C, C-2'), 35.8 (1C, C-4), 37.1 (d, *J* = 19.4 Hz, 1C, CH₂CH₂F), 49.9 (1C, ArCH₂NH), 51.4 (1C, C-4'), 63.7 (d, *J* = 5.0 Hz, 1C, C-3), 76.1 (1C, C-1), 81.1 (d, *J* = 163.7 Hz, 1C, CH₂CH₂F), 125.6 (1C, C-8), 126.0 (1C, C-6), 126.3 (1C, C-7), 126.7 (1C, C-5_{benzyl}), 128.7 (1C, C-5), 129.0 (1C, C-6_{benzyl}), 131.3 (1C, C-3_{benzyl}), 133.3 (1C, C-4a), 136.2 (1C, C-1_{benzyl}),

1
2
3 136.69 (1C, C-2_{benzyl}), 136.73 (1C, C-4_{benzyl}), 143.3 (1C, C-8a). FT-IR (neat): ν [cm⁻¹]
4 = 3306 (N-H), 2947, 2924 (C-H_{alkyl}), 1447 (C=C_{arom}). Purity (HPLC): 98.1 %,
5 t_R = 21.4 min.
6
7
8
9

10
11 **7.2.23. cis-N-(2,4-Dimethylbenzyl)-3-(2-fluoroethyl)-3,4-**
12 **dihydrospiro[[2]benzo-pyran-1,1'-cyclohexan]-4'-amine (13b)**
13
14

15
16 A solution of alcohol **12b** (70 mg, 0.18 mmol) in CH₂Cl₂ (6 mL) was added dropwise
17 to a solution of DAST (0.07 mL, 0.53 mmol, 2.9 eq) in CH₂Cl₂ (5 mL) under N₂
18 atmosphere at -78 °C. After 90 min, the mixture was warmed to rt and stirred for
19 20 h. 1 M NaOH (5 mL) was added and the aqueous layer was extracted with CH₂Cl₂
20 (4 x 8 mL). The combined organic layers were dried (Na₂SO₄), filtered, concentrated
21 in vacuo and the residue was purified twice by fc (d = 2 cm, l = 18 cm, V = 10 mL,
22 cyclohexane/ethyl acetate 90:10 + 1 % *N,N*-dimethylethanamine; d = 1.5 cm, l =
23 23 cm, V = 5 mL, cyclohexane/ethyl acetate 80:20 + 1 % *N,N*-dimethylethanamine).
24
25

26 Colorless oil, yield 15 mg (22 %). C₂₅H₃₂FNO (381.5 g/mol). R_f = 0.40
27 (cyclohexane/ethyl acetate 50:50 + 1 % *N,N*-dimethylethanamine). HR-MS (APCI):
28 m/z = 382.2526 (calcd. 382.2541 for C₂₅H₃₃FNO₂ [MH⁺]). ¹H NMR (400 MHz, CDCl₃):
29 δ (ppm) = 1.51 – 1.63 (m, 2H, 2'-H, 3'-H), 1.66 – 1.94 (m, 5H, 3'-H, 5'-H, 6'-H), 1.94 –
30 2.15 (m, 2H, CH₂CH₂F), 2.15 – 2.22 (m, 1H, 2'-H), 2.31 (s, 3H, 4-CH₃), 2.35 (s, 3H,
31 2-CH₃), 2.63 – 2.71 (m, 2H, 4'-H_{ax}, 4-H), 2.76 (dd, J = 15.8/10.8 Hz, 1H, 4-H), 3.82 (s,
32 2H, ArCH₂NH), 3.95 (ddt, J = 10.7/9.0/3.4 Hz, 1H, 3-H), 4.65 (dddd, J =
33 46.8/9.4/5.4/4.4 Hz, 1H, CH₂CH₂F), 4.76 (dtd, J = 47.5/9.0/4.4 Hz, 1H, CH₂CH₂F),
34 6.97 – 7.03 (m, 2H, 3-H_{benzyl}, 5-H_{benzyl}), 7.05 – 7.11 (m, 2H, 5-H, 8-H), 7.11 – 7.18 (m,
35 2H, 6-H, 7-H), 7.18 – 7.24 (m, 1H, 6-H_{benzyl}). A signal for the NH proton is not
36 observed in the spectrum. ¹³C NMR (101 MHz, CDCl₃): δ (ppm) = 19.1 (1C, 2-CH₃),
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 21.1 (1C, 4-CH₃), 28.89 (1C, C-3'), 28.94 (1C, C-5'), 34.2 (1C, C-2'), 35.7 (1C, C-4),
4
5 37.0 (d, *J* = 19.4 Hz, 1C, CH₂CH₂F), 38.6 (1C, C-6'), 48.5 (1C, ArCH₂NH), 56.3 (1C,
6
7 C-4'), 64.0 (d, *J* = 4.9 Hz, 1C, C-3), 75.4 (1C, C-1), 81.1 (d, *J* = 163.6 Hz, 1C,
8
9 CH₂CH₂F), 125.1 (1C, C-8), 126.2 (1C, C-6 or C-7), 126.3 (1C, C-6 or C-7), 126.7
10
11 (1C, C-5_{benzyl}), 128.7 (1C, C-6_{benzyl}), 128.9 (1C, C-5), 131.3 (1C, C-3_{benzyl}), 133.7 (1C,
12
13 C-4a), 135.6 (1C, C-1_{benzyl}), 136.3 (1C, C-2_{benzyl}), 136.7 (1C, C-4_{benzyl}), 142.5 (1C, C-
14
15 8a). FT-IR (neat): ν [cm⁻¹] = 3017 (N-H), 2924, 2855 (C-H_{alkyl}), 1447 (C=C_{arom}). Purity
16
17 (HPLC): 97.4 %, *t*_R = 21.4 min.
18
19
20
21
22
23

24 7.2.24. *trans*-1-Cyclohexyl-4-[3-(2-fluoroethyl)-3,4-

25 dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-4'-yl]piperazine (13c)

26
27
28
29 A solution of alcohol **12c** (35 mg, 0.09 mmol) in CH₂Cl₂ (4 mL) was added dropwise
30
31 to a solution of DAST (0.03 mL, 0.23 mmol, 2.6 eq) in CH₂Cl₂ (3 mL) under N₂
32
33 atmosphere at -78 °C. After 1 h, the mixture was warmed to rt and stirred for 23 h.
34
35 1 M NaOH (3 mL) was added and the aqueous layer was extracted with CH₂Cl₂
36
37 (4 x 5 mL). The combined organic layers were dried (Na₂SO₄), filtered, concentrated
38
39 in vacuo and the residue was purified by fc (*d* = 1 cm, *l* = 18 cm, *V* = 5 mL,
40
41 cyclohexane/ethyl acetate 66:33 + 2 % *N,N*-dimethylethanamine). Colorless solid, mp
42
43 167 °C, yield 24 mg (67 %). C₂₆H₃₉FN₂O (414.6 g/mol). *R*_f = 0.35 (cyclohexane/ethyl
44
45 acetate 66:33 + 2 % *N,N*-dimethylethanamine). HR-MS (APCI): *m/z* = 415.3103
46
47 (calcd. 415.3119 for C₂₆H₄₀FN₂O₂ [MH⁺]). ¹H NMR (400 MHz, CDCl₃): δ (ppm) = 1.07
48
49 – 1.20 (m, 1H, 4-*H*_{cyclohexyl}), 1.20 – 1.34 (m, 4H, 2-*H*_{cyclohexyl}, 3-*H*_{cyclohexyl}, 5-*H*_{cyclohexyl}, 6-
50
51 *H*_{cyclohexyl}), 1.38 – 1.46 (m, 1H, 2'-*H*), 1.61 – 1.68 (m, 1H, 4-*H*_{cyclohexyl}), 1.69 – 1.92 (m,
52
53 8H, 3'-*H*, 5'-*H*, 6'-*H*, 2-*H*_{cyclohexyl}, 6-*H*_{cyclohexyl}), 1.92 – 2.15 (m, 4H, CH₂CH₂F, 3-
54
55
56
57
58
59
60

$H_{\text{cyclohexyl}}$, $5\text{-}H_{\text{cyclohexyl}}$, 2.20 – 2.34 (m, 3H, $2'\text{-}H$, $4'\text{-}H_{\text{equ}}$, $1\text{-}H_{\text{cyclohexyl}}$), 2.48 – 2.80 (m, 10H, $4\text{-}H$, $H_{\text{piperazine}}$), 3.95 (ddt, $J = 10.6/9.3/3.3$ Hz, 1H, $3\text{-}H$), 4.59 (ddd, $J = 9.4/5.6/4.3$ Hz, 0.5H, $\text{CH}_2\text{CH}_2\text{F}$), 4.67 – 4.74 (m, $2 \times 0.5\text{H}$, $\text{CH}_2\text{CH}_2\text{F}$), 4.83 (td, $J = 9.0/4.4$ Hz, 0.5H, $\text{CH}_2\text{CH}_2\text{F}$), 7.05 (d, $J = 7.5$ Hz, 1H, $5\text{-}H$), 7.12 (td, $J = 6.9/6.1/2.3$ Hz, 1H, $6\text{-}H$), 7.15 – 7.22 (m, 2H, $7\text{-}H$, $8\text{-}H$). ^{13}C NMR (101 MHz, CDCl_3): δ (ppm) = 24.0 (1C, $\text{C-}3'$ or $\text{C-}5'$), 24.1 (1C, $\text{C-}3'$ or $\text{C-}5'$), 26.0 (2C, $\text{C-}2_{\text{cyclohexyl}}$, $\text{C-}6_{\text{cyclohexyl}}$), 26.4 (1C, $\text{C-}4_{\text{cyclohexyl}}$), 29.1 (2C, $\text{C-}3_{\text{cyclohexyl}}$, $\text{C-}5_{\text{cyclohexyl}}$), 29.6 (1C, $\text{C-}6'$), 34.1 (1C, $\text{C-}2'$), 35.9 (1C, $\text{C-}4$), 37.1 (d, $J = 19.4$ Hz, 1C, $\text{CH}_2\text{CH}_2\text{F}$), 49.9 (2C, $\text{C-}2_{\text{piperazine}}$ and $\text{C-}6_{\text{piperazine}}$ or $\text{C-}3_{\text{piperazine}}$ and $\text{C-}5_{\text{piperazine}}$), 50.5 (2C, $\text{C-}2_{\text{piperazine}}$ and $\text{C-}6_{\text{piperazine}}$ or $\text{C-}3_{\text{piperazine}}$ and $\text{C-}5_{\text{piperazine}}$), 57.5 (1C, $\text{C-}4'$), 63.8 (1C, $\text{C-}1_{\text{cyclohexyl}}$), 63.9 (d, $J = 5.0$ Hz, 1C, $\text{C-}3$), 76.2 (1C, $\text{C-}1$), 81.1 (d, $J = 163.6$ Hz, 1C, $\text{CH}_2\text{CH}_2\text{F}$), 125.6 (1C, $\text{C-}8$), 126.0 (1C, $\text{C-}6$), 126.2 (1C, $\text{C-}7$), 128.7 (1C, $\text{C-}5$), 133.4 (1C, $\text{C-}4\text{a}$), 143.4 (1C, $\text{C-}8\text{a}$). FT-IR (neat): $\nu[\text{cm}^{-1}] = 2916, 2851, 2801$ ($\text{C-H}_{\text{alkyl}}$), 1451 (C=C_{arom}). Purity (HPLC): 93.6 %, $t_{\text{R}} = 16.6$ min.

7.2.25. *cis*-1-Cyclohexyl-4-[3-(2-fluoroethyl)-3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-4'-yl]piperazine (13d)

A solution of alcohol **12d** (64 mg, 0.15 mmol) in CH_2Cl_2 (5 mL) was added dropwise to a solution of DAST (0.05 mL, 0.38 mmol, 2.5 eq) in CH_2Cl_2 (5 mL) under N_2 atmosphere at -78 °C. After 1 h, the mixture was warmed to rt and stirred for 20 h. 1 M NaOH (4 mL) was added and the aqueous layer was extracted with CH_2Cl_2 (4 x 5 mL). The combined organic layers were dried (Na_2SO_4), filtered, concentrated in vacuo and the residue was purified by fc (d = 2 cm, l = 15 cm, V = 10 mL, cyclohexane/ethyl acetate 66:33 + 2 % *N,N*-dimethylethanamine; d = 1.5 cm, l = 20 cm, V = 5 mL, cyclohexane/ethyl acetate 95:5 + 1 % *N,N*-dimethylethanamine →

80:20 + 1 % *N,N*-dimethylethanamine; $d = 2$ cm, $l = 16$ cm, $V = 5$ mL, cyclohexane/ethyl acetate 90:10 + 1 % *N,N*-dimethylethanamine). Yellow solid, mp 152 °C, yield 9 mg (13 %). $C_{26}H_{39}FN_2O$ (414.6 g/mol). $R_f = 0.17$ (cyclohexane/ethyl acetate 66:33 + 2 % *N,N*-dimethylethanamine). HR-MS (APCI): $m/z = 415.3155$ (calcd. 415.3119 for $C_{26}H_{40}FN_2O_2$ [MH^+]). 1H NMR (400 MHz, $CDCl_3$): δ (ppm) = 1.04 – 1.17 (m, 1H, 4- $H_{cyclohexyl}$), 1.17 – 1.32 (m, 4H, 2- $H_{cyclohexyl}$, 3- $H_{cyclohexyl}$, 5- $H_{cyclohexyl}$, 6- $H_{cyclohexyl}$), 1.51 – 1.60 (m, 1H, 2'- H), 1.60 – 1.71 (m, 1H, 4- $H_{cyclohexyl}$), 1.71 – 1.90 (m, 8H, 3'- H , 5'- H , 6'- H , 2- $H_{cyclohexyl}$, 6- $H_{cyclohexyl}$), 1.90 – 2.14 (m, 4H, CH_2CH_2F , 3- $H_{cyclohexyl}$, 5- $H_{cyclohexyl}$), 2.16 – 2.25 (m, 1H, 2'- H), 2.25 – 2.34 (m, 1H, 1- $H_{cyclohexyl}$), 2.42 – 2.52 (m, 1H, 4'- H_{ax}), 2.60 – 2.85 (m, 10H, 4- H , $H_{piperazine}$), 3.94 (ddt, $J = 10.3/8.9/3.4$ Hz, 1H, 3- H), 4.58 (ddd, $J = 8.9/5.6/4.5$ Hz, 0.5H, CH_2CH_2F), 4.66 – 4.73 (m, 2 x 0.5H, CH_2CH_2F), 4.82 (td, $J = 9.1/4.4$ Hz, 0.5H CH_2CH_2F), 7.06 (d, $J = 7.4$ Hz, 1H, 5- H), 7.08 – 7.11 (m, 1H, 8- H), 7.11 – 7.15 (m, 1H, 6- H), 7.15 – 7.20 (m, 1H, 7- H). ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) = 23.7 (1C, C-5'), 23.9 (1C, C-3'), 26.0 (2C, C-2 $_{cyclohexyl}$, C-6 $_{cyclohexyl}$), 26.4 (1C, C-4 $_{cyclohexyl}$), 29.0 (2C, C-3 $_{cyclohexyl}$, C-5 $_{cyclohexyl}$), 34.8 (1C, C-2'), 35.7 (1C, C-4), 37.0 (d, $J = 19.3$ Hz, 1C, CH_2CH_2F), 39.0 (1C, C-6'), 49.3 (4C, $C_{piperazine}$), 63.2 (1C, C-4'), 63.8 (1C, C-1 $_{cyclohexyl}$), 64.0 (d, $J = 4.9$ Hz, 1C, C-3), 75.3 (1C, C-1), 81.1 (d, $J = 163.8$ Hz, 1C, CH_2CH_2F), 125.1 (1C, C-8), 126.2 (1C, C-6 or C-7), 126.3 (1C, C-6 or C-7), 128.9 (1C, C-5), 133.6 (1C, C-4a), 142.3 (1C, C-8a). FT-IR (neat): ν [cm^{-1}] = 2924, 2851, 2824 (C- H_{alkyl}), 1450 (C=C $_{arom}$). Purity (HPLC): 94.2 %, $t_R = 15.5$ min.

7.2.26. *trans*-6,7-Dimethoxy-2-[3-(2-fluoroethyl)-3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-4'-yl]-1,2,3,4-tetrahydroisoquinoline (13e)

A solution of alcohol **12e** (62 mg, 0.14 mmol) in CH₂Cl₂ (6 mL) was added dropwise to a solution of DAST (0.06 mL, 0.45 mmol, 3.2 eq) in CH₂Cl₂ (8 mL) under N₂ atmosphere at -78 °C. After 1.5 h, the mixture was warmed to rt and stirred for 21 h. 1 M NaOH (5 mL) was added and the aqueous layer was extracted with CH₂Cl₂ (4 x 8 mL). The combined organic layers were dried (Na₂SO₄), filtered, concentrated in vacuo and the residue was purified twice by fc (d = 1.5 cm, l = 22 cm, V = 5 mL, cyclohexane/ethyl acetate 80:20 + 1 % *N,N*-dimethylethanamine; d = 1.5 cm, l = 23 cm, V = 5 mL, cyclohexane/ethyl acetate 90:10 + 2 % *N,N*-dimethylethanamine). Colorless solid, mp 125 °C, yield 15 mg (21 %). C₂₇H₃₄FNO₃ (439.6 g/mol). R_f = 0.23 (cyclohexane/ethyl acetate 80:20 + 1 % *N,N*-dimethylethanamine). HR-MS (APCI): *m/z* = 440.2627 (calcd. 440.2595 for C₂₇H₃₅FNO₃ [MH⁺]). ¹H NMR (400 MHz, CDCl₃): δ (ppm) = 1.44 – 1.52 (m, 1H, 2'-H), 1.77 – 2.19 (m, 8H, 3'-H, 5'-H, 6'-H, CH₂CH₂F), 2.26 – 2.40 (m, 1H, 2'-H), 2.40 – 2.47 (m, 1H, 4'-H_{equ}), 2.65 (dd, *J* = 15.9/3.0 Hz, 1H, 4-H), 2.73 (dd, *J* = 15.9/5.1 Hz, 1H, 4-H), 2.77 – 2.83 (m, 2H, 3-H_{isoquinoline}), 2.83 – 2.91 (m, 2H, 4-H_{isoquinoline}), 3.68 (s, 2H, 1-H_{isoquinoline}), 3.86 (s, 3H, 7-OCH₃), 3.88 (s, 3H, 6-OCH₃), 3.91 – 4.06 (m, 1H, 3-H), 4.61 (dt, *J* = 9.3/5.0 Hz, 0.5H, CH₂CH₂F), 4.69 – 4.78 (m, 2 x 0.5H, CH₂CH₂F), 4.85 (td, *J* = 8.8/4.3 Hz, 0.5H, CH₂CH₂F), 6.60 (s, 1H, 8-H_{isoquinoline}), 6.66 (s, 1H, 5-H_{isoquinoline}), 7.04 (d, *J* = 6.6 Hz, 1H, 5-H), 7.07 – 7.17 (m, 3H, 6-H, 7-H, 8-H). ¹³C NMR (101 MHz, CDCl₃): δ (ppm) = 24.4 (1C, C-3'), 24.6 (1C, C-5'), 29.4 (1C, C-4_{isoquinoline}), 29.6 (1C, C-6'), 34.1 (1C, C-2'), 35.9 (1C, C-4), 37.1 (d, *J* = 19.3 Hz, 1C, CH₂CH₂F), 47.5 (1C, C-3_{isoquinoline}), 53.5 (1C, C-1_{isoquinoline}), 56.1 (2C, 6-OCH₃, 7-OCH₃), 57.2 (1C, C-4'), 63.9 (d, *J* = 4.7 Hz, 1C, C-3), 76.3 (1C, C-1), 81.1 (d, *J* = 163.7 Hz, 1C, CH₂CH₂F), 109.9 (1C, C-8_{isoquinoline}), 111.3 (1C, C-5_{isoquinoline}), 125.8 (1C, C-6 or C-8), 126.0 (1C, C-6 or C-8), 126.3 (1C, C-7), 127.0 (1C, C-8a_{isoquinoline}), 127.7 (1C, C-4a_{isoquinoline}), 128.6 (1C, C-5), 133.3 (1C, C-

1
2
3 4a), 143.2 (1C, C-8a), 147.4 (1C, C-7_{isoquinoline}), 147.5 (1C, C-6_{isoquinoline}). FT-IR (neat):
4
5 ν [cm⁻¹] = 2951, 2932, 2781 (C-H_{alkyl}), 1520, 1462, 1447 (C=C_{arom}). Purity (HPLC,
6
7 method 1): 93.2 %, t_R = 19.7 min.
8
9

10
11
12 **7.2.27. cis-6,7-Dimethoxy-2-[3-(2-fluoroethyl)-3,4-**
13
14 **dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-4'-yl]-1,2,3,4-**
15
16 **tetrahydroisoquinoline (13f)**
17

18 A solution of alcohol **12f** (67 mg, 0.15 mmol) in CH₂Cl₂ (8 mL) was added dropwise to
19 a solution of DAST (0.06 mL, 0.45 mmol, 3.0 eq) in CH₂Cl₂ (6 mL) under N₂
20 atmosphere at -78 °C. After 1 h, the mixture was warmed to rt and stirred for 20 h.
21
22 1 M NaOH (5 mL) was added and the aqueous layer was extracted with CH₂Cl₂
23 (4 x 6 mL). The combined organic layers were dried (Na₂SO₄), filtered, concentrated
24 in vacuo and the residue was purified twice by fc (d = 1.5 cm, l = 23 cm, V = 5 mL,
25 cyclohexane/ethyl acetate 66:33 + 1 % *N,N*-dimethylethanamine; d = 1.5 cm, l =
26 23 cm, V = 5 mL, cyclohexane/ethyl acetate 80:20 + 2 % *N,N*-dimethylethanamine).
27 Colorless solid, mp 136 °C, yield 18 mg (27 %). C₂₇H₃₄FNO₃ (439.6 g/mol). R_f = 0.31
28 (cyclohexane/ethyl acetate 50:50 + 1 % *N,N*-dimethylethanamine). HR-MS (APCI):
29 m/z = 440.2613 (calcd. 440.2595 for C₂₇H₃₅FNO₃ [MH⁺]). ¹H NMR (400 MHz, CDCl₃):
30 δ (ppm) = 1.56 – 1.68 (m, 1H, 2'-H), 1.74 – 2.15 (m, 8H, 3'-H, 5'-H, 6'-H, CH₂CH₂F),
31 2.22 – 2.29 (m, 1H, 2'-H), 2.63 – 2.82 (m, 3H, 4-H, 4'-H_{ax}), 2.82 – 2.99 (m, 4H, 3-
32 *H*_{isoquinoline}, 4-*H*_{isoquinoline}), 3.82 (s, 2H, 1-*H*_{isoquinoline}), 3.85 (s, 6H, 6-OCH₃, 7-OCH₃),
33 3.91 – 4.04 (m, 1H, 3-H), 4.69 (dddd, J = 46.7/9.2/5.3/4.4 Hz, 1H, CH₂CH₂F), 4.82
34 (dtd, J = 47.4/9.1/4.3 Hz, 1H, CH₂CH₂F), 6.55 (s, 1H, 8-*H*_{isoquinoline}), 6.60 (s, 1H, 5-
35 *H*_{isoquinoline}), 7.06 – 7.10 (m, 1H, 5-H), 7.10 – 7.22 (m, 3H, 6-H, 7-H, 8-H). ¹³C NMR
36 (101 MHz, CDCl₃): δ (ppm) = 23.8 (2C, C-3', C-5'), 29.1 (1C, C-4_{isoquinoline}), 34.8 (1C,
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

C-2'), 35.7 (1C, C-4), 37.1 (d, $J = 19.3$ Hz, 1C, CH₂CH₂F), 39.1 (1C, C-6'), 47.0 (1C, C-3_{isoquinoline}), 51.5 (1C, C-1_{isoquinoline}), 56.07 (1C, 6-OCH₃ or 7-OCH₃), 56.12 (1C, 6-OCH₃ or 7-OCH₃), 62.7 (1C, C-4'), 64.0 (d, $J = 4.7$ Hz, 1C, C-3), 75.2 (1C, C-1), 81.2 (d, $J = 164.0$ Hz, 1C, CH₂CH₂F), 109.8 (1C, C-8_{isoquinoline}), 111.6 (1C, C-5_{isoquinoline}), 125.1 (1C, C-8), 126.29 (1C, C-6 or C-7), 126.32 (1C, C-6 or C-7), 126.5 (1C, C-4_{isoquinoline}), 126.8 (1C, C-8_{a isoquinoline}), 128.9 (1C, C-5), 133.6 (1C, C-4a), 142.2 (1C, C-8a), 147.4 (1C, C-7_{isoquinoline}), 147.7 (1C, C-6_{isoquinoline}). FT-IR (neat): ν [cm⁻¹] = 2978, 2920 (C-H_{alkyl}), 1512, 1462, 1443 (C=C_{arom}). Purity (HPLC): 97.8 %, $t_R = 19.7$ min.

7.2.28. 3-[1-(4-Fluorophenyl)-indol-3-yl]propanoic acid (15)

A solution of 4-bromofluorobenzene (0.26 mL, 2.38 mmol), 3-(indol-3-yl)propionic acid (**14**, 500 mg, 2.64 mmol, 1.1 eq), CuI (320 mg, 1.68 mmol, 0.7 eq) and Cs₂CO₃ (2.58 g, 7.93 mmol, 3.3 eq) in DMF (20 mL) was heated to reflux for 2 d under N₂ atmosphere. After cooling to rt, ethyl acetate (100 mL) was added and the mixture was washed with 0.1 M HCl (2 x 80 mL). The aqueous layer was extracted with ethyl acetate (3 x 60 mL). The combined organic layers were dried (Na₂SO₄), filtered, concentrated in vacuo and the residue was purified by fc (d = 4 cm, l = 18 cm, V = 30 mL, cyclohexane/ethyl acetate 80:20 + 1 % HCOOH). Brownish solid, mp 140 °C, yield 586 mg (78 %). C₁₇H₁₄FNO₂ (283.3 g/mol). R_f = 0.69 (cyclohexane/ethyl acetate 50:50 + 1 % HCOOH). HR-MS (APCI): $m/z = 283.1077$ (calcd. 283.1081 for C₁₇H₁₅FNO₂ [MH⁺]). ¹H NMR (600 MHz, CDCl₃): δ (ppm) = 2.83 (t, $J = 7.6$ Hz, 2H, CH₂CH₂CO), 3.17 (t, $J = 7.6$ Hz, 2H, CH₂CH₂CO), 7.13 (s, 1H, 2-H), 7.17 – 7.22 (m, 3H, 5-H, 3-H_{phenyl}, 5-H_{phenyl}), 7.22 – 7.25 (m, 1H, 6-H), 7.41 – 7.44 (m, 2H, 2-H_{phenyl}, 6-H_{phenyl}), 7.44 – 7.47 (m, 1H, 7-H), 7.64 – 7.66 (m, 1H, 4-H). A signal for the COOH

1
2
3 proton is not observed in the spectrum. ^{13}C NMR (151 MHz, CDCl_3): δ (ppm) = 20.4
4 (1C, $\text{CH}_2\text{CH}_2\text{CO}$), 34.5 (1C, $\text{CH}_2\text{CH}_2\text{CO}$), 110.4 (1C, C-7), 115.8 (1C, C-3), 116.6 (d,
5
6 $J = 22.8$ Hz, 2C, C-3_{phenyl}, C-5_{phenyl}), 119.2 (1C, C-4), 120.2 (1C, C-5), 122.9 (1C, C-
7
8 6), 125.5 (1C, C-2), 126.1 (d, $J = 8.1$ Hz, 2C, C-2_{phenyl}, C-6_{phenyl}), 128.5 (1C, C-3a),
9
10 135.9 (d, $J = 3.0$ Hz, 1C, C-1_{phenyl}), 136.5 (1C, C-7a), 161.1 (d, $J = 246.2$ Hz, 1C, C-
11
12 4_{phenyl}), 178.8 (1C, C=O). FT-IR (neat): ν [cm^{-1}] = 3051 (COOH), 2978, 2909 (C-
13
14 H_{alkyl}), 1717 (C=O), 1512, 1458, 1450 (C=C_{arom}). Purity (HPLC): 99.4 %,
15
16 $t_{\text{R}} = 21.2$ min.
17
18
19
20
21

22 **7.2.29. 3-[1-(4-Fluorophenyl)-indol-3-yl]propanamide (16)**

23
24
25 Et_3N (1.0 mL, 7.2 mmol, 3.7 eq) and ethyl chloroformate (0.28 mL, 2.94 mmol, 1.5
26
27 eq) were added to a solution of **15** (548 mg, 1.93 mmol) in THF (10 mL) at 0 °C
28
29 under N_2 atmosphere. After 1 h, NH_3 (0.5 M in THF, 20.0 mL, 10.0 mmol, 5.2 eq) was
30
31 added and the mixture was stirred for 2 h. The precipitate was filtered off and the
32
33 filtrate was concentrated in vacuo. H_2O (50 mL) was added and the aqueous phase
34
35 was extracted with EtOAc (4 x 50 mL). The combined organic layers were dried
36
37 (Na_2SO_4), filtered and concentrated in vacuo. Brownish solid, mp 123 °C, yield
38
39 532 mg (97 %). $\text{C}_{17}\text{H}_{15}\text{FN}_2\text{O}$ (282.3 g/mol). $R_f = 0.22$ (cyclohexane/ethyl acetate
40
41 50:50 + 1 % HCOOH). HR-MS (APCI): $m/z = 189.1012$ (calcd. 189.1022 for
42
43 $\text{C}_{17}\text{H}_{16}\text{FN}_2\text{O}$ [MH^+]). ^1H NMR (400 MHz, CDCl_3): δ (ppm) = 2.68 (t, $J = 7.4$ Hz, 2H,
44
45 $\text{CH}_2\text{CH}_2\text{CO}$), 3.17 (t, $J = 7.2$ Hz, 2H, $\text{CH}_2\text{CH}_2\text{CO}$), 5.49 (s, 1H, CONH_2), 5.66 (s, 1H,
46
47 CONH_2), 7.14 (s, 1H, 2-*H*), 7.15 – 7.21 (m, 3H, 5-*H*, 3-*H*_{phenyl}, 5-*H*_{phenyl}), 7.21 – 7.26
48
49 (m, 1H, 6-*H*), 7.39 – 7.44 (m, 2H, 2-*H*_{phenyl}, 6-*H*_{phenyl}), 7.44 – 7.48 (m, 1H, 7-*H*), 7.63 –
50
51 7.68 (m, 1H, 4-*H*). ^{13}C NMR (101 MHz, CDCl_3): δ (ppm) = 21.0 (1C, $\text{CH}_2\text{CH}_2\text{CO}$),
52
53 36.4 (1C, $\text{CH}_2\text{CH}_2\text{CO}$), 110.5 (1C, C-7), 116.0 (1C, C-3), 116.6 (d, $J = 22.9$ Hz, 2C,
54
55
56
57
58
59
60

C-3_{phenyl}, C-5_{phenyl}), 119.2 (1C, C-4), 120.2 (1C, C-5), 122.8 (1C, C-6), 125.7 (1C, C-2), 126.0 (d, $J = 8.4$ Hz, 2C, C-2_{phenyl}, C-6_{phenyl}), 128.5 (1C, C-3a), 135.9 (d, $J = 3.0$ Hz, 1C, C-1_{phenyl}), 136.5 (1C, C-7a), 161.1 (d, $J = 246.1$ Hz, 1C, C-4_{phenyl}), 175.3 (1C, C=O). FT-IR (neat): ν [cm⁻¹] = 3395, 3206 (N-H), 2963, 2920 (C-H_{alkyl}), 1654 (C=O), 1508, 1454 (C=C_{arom}). Purity (HPLC): 93.2 %, $t_R = 19.8$ min.

7.2.30. 3-[1-(4-Fluorophenyl)-indol-3-yl]propan-1-amine (17)

LiAlH₄ (150 mg, 3.94 mmol, 3.4 eq) was added to a solution of amide **16** (329 mg, 1.17 mmol) in THF (25 mL) at 0 °C under N₂ atmosphere. The mixture was heated to reflux for 2 h. After cooling to rt, H₂O (15 mL) was added, the precipitate was filtered off and the aqueous phase was extracted with CH₂Cl₂ (4 x 20 mL). The combined organic layers were dried (Na₂SO₄), filtered, concentrated in vacuo and the residue was purified by fc (d = 4 cm, l = 18 cm, V = 30 mL, CH₂Cl₂: CH₃OH 95:5 + 3 % NH₃ (25 %)). Yellow oil, yield 200 mg (64 %). C₁₇H₁₇FN₂ (268.3 g/mol). R_f = 0.13 (CH₂Cl₂/CH₃OH 95:5 + 1 % *N,N*-dimethylethanamine). HR-MS (APCI): $m/z = 269.1464$ (calcd. 269.1449 for C₁₇H₁₈FN₂ [MH⁺]). ¹H NMR (400 MHz, CDCl₃): δ (ppm) = 1.93 (quint, $J = 7.5$ Hz, 2H, CH₂CH₂CH₂NH₂), 2.80 – 2.90 (m, 4H, CH₂CH₂CH₂NH₂), 7.09 (s, 1H, 2-*H*), 7.14 – 7.25 (m, 4H, 5-*H*, 6-*H*, 3-*H*_{phenyl}, 5-*H*_{phenyl}), 7.39 – 7.47 (m, 3H, 7-*H*, 2-*H*_{phenyl}, 6-*H*_{phenyl}), 7.64 – 7.67 (m, 1H, 4-*H*). A signal for the NH₂ protons is not observed in the spectrum. ¹³C NMR (101 MHz, CDCl₃): δ (ppm) = 22.5 (1C, CH₂CH₂CH₂NH₂), 33.9 (1C, CH₂CH₂CH₂NH₂), 42.1 (1C, CH₂CH₂CH₂NH₂), 110.3 (1C, C-7), 116.5 (d, $J = 22.8$ Hz, 2C, C-3_{phenyl}, C-5_{phenyl}), 117.5 (1C, C-3), 119.4 (1C, C-4), 120.0 (1C, C-5), 122.7 (1C, C-6), 125.2 (1C, C-2), 126.0 (d, $J = 8.4$ Hz, 2C, C-2_{phenyl}, C-6_{phenyl}), 129.0 (1C, C-3a), 136.1 (d, $J = 3.0$ Hz, 1C, C-1_{phenyl}), 136.5 (1C, C-7a), 161.0 (d, $J = 246.0$ Hz, 1C, C-4_{phenyl}). FT-IR (neat): ν [cm⁻¹] = 3051 (N-H),

2924, 2851 (C-H_{alkyl}), 1508, 1458 (C=C_{arom}). Purity (HPLC): 94.7 %, t_R = 17.9 min.

7.2.31. *trans*-N-{3-[1-(4-Fluorophenyl)-(Indol-3-yl)]propyl}-3-methoxy-3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-4'-amine (18a) and *cis*-N-{3-[1-(4-Fluorophenyl)-(Indol-3-yl)]propyl}-3-methoxy-3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-4'-amine (18b)

A solution of ketone **5** (164 mg, 0.67 mmol), amine **17** (265 mg, 0.67 mmol, 1.5 eq) and acetic acid (45 μ L, 0.79 mmol, 1.2 eq) in THF (20 mL) was stirred under N₂ atmosphere at rt. After 2.5 h, NaBH(OAc)₃ (252 mg, 1.19 mmol, 1.8 eq) was added and the mixture was stirred for 17 h at rt. 1 M NaOH (10 mL) was added and the aqueous layer was extracted with CH₂Cl₂ (4 x 20 mL). The combined organic layers were dried (Na₂SO₄), filtered, concentrated in vacuo and the residue was purified by fc (d = 3 cm, l = 19 cm, V = 20 mL, CH₂Cl₂/CH₃OH 98:2 + 1 % *N,N*-dimethylethanamine). **18a** was eluted first and **18b** afterwards.

trans-**18a**: Yellow oil, yield 99 mg (30 %). C₃₂H₃₅FN₂O₂ (498.6 g/mol). R_f = 0.51 (CH₂Cl₂/CH₃OH 95:5 + 1 % *N,N*-dimethylethanamine). HR-MS (APCI): m/z = 499.2770 (calcd. 499.2755 for C₃₂H₃₆FN₂O₂ [MH⁺]). ¹H NMR (600 MHz, CD₃OD): δ (ppm) = 1.55 (dq, J = 12.5/2.7 Hz, 1H, 2'- H_{equ}), 1.68 – 1.73 (m, 2H, 3'- H , 5'- H), 1.80 (ddt, J = 14.3/4.6/2.8 Hz, 1H, 6'- H_{equ}), 1.87 (td, J = 13.9/3.7 Hz, 1H, 6'- H_{ax}), 2.00 – 2.04 (m, 1H, 5'- H), 2.04 – 2.13 (m, 3H, 3'- H , CH₂CH₂CH₂NH), 2.13 – 2.19 (m, 1H, 2'- H_{ax}), 2.76 – 2.82 (m, 3H, 4- H , CH₂CH₂CH₂NH), 2.90 (dd, J = 15.7/2.9 Hz, 1H, 4- H), 2.91 – 2.96 (m, 3H, 4'- H_{equ} , CH₂CH₂CH₂NH), 3.53 (s, 3H, OCH₃), 4.88 (dd, J = 7.6/3.1 Hz, 1H, 3- H), 7.06 (d, J = 7.0 Hz, 1H, 5- H), 7.11 – 7.17 (m, 3H, 6- H , 7- H , 5- H_{indole}), 7.20 (ddd, J = 8.2/6.9/1.2 Hz, 1H, 6- H_{indole}), 7.26 – 7.30 (m, 3H, 2- H_{indole} , 3- H_{phenyl} , 5- H_{phenyl}), 7.32 (dd, J = 7.3/1.7 Hz, 1H, 8- H), 7.46 (d, J = 8.2 Hz, 1H,

1
2
3 7-*H*_{indole}), 7.50 – 7.54 (m, 2H, 2-*H*_{phenyl}, 6-*H*_{phenyl}), 7.69 (d, *J* = 7.7 Hz, 1H, 4-*H*_{indole}). A
4 signal for the NH proton is not observed in the spectrum. ¹³C NMR (151 MHz,
5 CD₃OD): δ (ppm) = 24.0 (1C, CH₂CH₂CH₂NH), 26.1 (1C, C-3'), 26.3 (1C, C-5'), 30.7
6 (1C, CH₂CH₂CH₂NH), 31.5 (1C, C-6'), 34.1 (1C, C-2'), 36.2 (1C, C-4), 48.6 (1C,
7 CH₂CH₂CH₂NH), 52.5 (1C, C-4'), 56.3 (1C, OCH₃), 78.0 (1C, C-1), 97.8 (1C, C-3),
8 111.1 (1C, C-7_{indole}), 117.4 (d, *J* = 23.1 Hz, 2C, C-3_{phenyl}, C-5_{phenyl}), 118.5 (1C, C-
9 3_{indole}), 120.2 (1C, C-4_{indole}), 120.9 (1C, C-5_{indole}), 123.6 (1C, C-6_{indole}), 126.1 (1C, C-
10 8), 126.4 (1C, C-2_{indole}), 127.0 (d, *J* = 8.3 Hz, 2C, C-2_{phenyl}, C-6_{phenyl}), 127.5 (1C, C-7),
11 127.6 (1C, C-6), 130.0 (1C, C-5), 130.3 (1C, C-3a_{indole}), 132.4 (1C, C-4a), 137.5 (d, *J*
12 = 3.1 Hz, 1C, C-1_{phenyl}), 137.8 (1C, C-7a_{indole}), 143.4 (1C, C-8a), 162.2 (d, *J* = 244.0
13 Hz, 1C, C-4_{phenyl}). FT-IR (neat): ν [cm⁻¹] = 3318 (N-H), 2928, 2808 (C-H_{alkyl}), 1508,
14 1458 (C=C_{arom}). Purity (HPLC): 97.4 %, *t*_R = 23.0 min.

15
16
17
18
19
20
21
22
23
24
25
26
27
28
29 **cis-18b**: Yellow solid, mp 118 °C, yield 138 mg (42 %). C₃₂H₃₅FN₂O₂ (498.6 g/mol).
30 *R*_f = 0.49 (CH₂Cl₂/CH₃OH 95:5 + 1 % *N,N*-dimethylethanamine). HR-MS (APCI):
31 *m/z* = 499.2769 (calcd. 499.2755 for C₃₂H₃₆FN₂O₂ [MH⁺]). ¹H NMR (600 MHz,
32 CD₃OD): δ (ppm) = 1.62 – 1.72 (m, 2H, 2'-*H*, 3'-*H*), 1.72 – 1.86 (m, 4H, 3'-*H*, 5'-*H*, 6'-
33 *H*), 1.94 (td, *J* = 13.7/4.1 Hz, 1H, 6'-*H*), 1.99 – 2.10 (m, 3H, 2'-*H*, CH₂CH₂CH₂NH),
34 2.71 (tt, *J* = 11.0/3.9 Hz, 1H, 4'-*H*_{ax}), 2.78 (dd, *J* = 15.6/7.5 Hz, 1H, 4-*H*), 2.81 – 2.85
35 (m, 2H, CH₂CH₂CH₂NH), 2.88 – 2.93 (m, 3H, 4-*H*, CH₂CH₂CH₂NH), 3.53 (s, 3H,
36 OCH₃), 4.86 – 4.89 (m, 1H, 3-*H*), 7.05 – 7.08 (m, 1H, 5-*H*), 7.12 – 7.17 (m, 4H, 6-*H*,
37 7-*H*, 8-*H*, 5-*H*_{indole}), 7.17 – 7.21 (m, 1H, 6-*H*_{indole}), 7.25 – 7.30 (m, 3H, 2-*H*_{indole}, 3-
38 *H*_{phenyl}, 5-*H*_{phenyl}), 7.46 (dt, *J* = 8.2/1.0 Hz, 1H, 7-*H*_{indole}), 7.49 – 7.55 (m, 2H, 2-*H*_{phenyl},
39 6-*H*_{phenyl}), 7.67 (dt, *J* = 8.0/1.0 Hz, 1H, 4-*H*_{indole}). A signal for the NH proton is not
40 observed in the spectrum. ¹³C NMR (151 MHz, CD₃OD): δ (ppm) = 23.8 (1C,
41 CH₂CH₂CH₂NH), 28.6 (1C, C-3' or C-5'), 28.7 (1C, C-3' or C-5'), 30.6 (1C,
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 CH₂CH₂CH₂NH), 36.1 (1C, C-4), 36.4 (1C, C-2'), 38.9 (1C, C-6'), 47.3 (1C,
4 CH₂CH₂CH₂NH), 56.4 (1C, OCH₃), 57.3 (1C, C-4'), 77.3 (1C, C-1), 97.8 (1C, C-3),
5
6 111.1 (1C, C-7_{indole}), 117.4 (d, *J* = 23.0 Hz, 2C, C-3_{phenyl}, C-5_{phenyl}), 118.3 (1C, C-
7
8 3_{indole}), 120.2 (1C, C-4_{indole}), 120.9 (1C, C-5_{indole}), 123.6 (1C, C-6_{indole}), 125.6 (1C, C-6
9
10 or C-8), 126.4 (1C, C-2_{indole}), 126.9 (d, *J* = 8.6 Hz, 2C, C-2_{phenyl}, C-6_{phenyl}), 127.5 (1C,
11
12 C-7), 127.7 (1C, C-6 or C-8), 130.1 (1C, C-5), 130.3 (1C, C-3a_{indole}), 132.6 (1C, C-
13
14 4a), 137.5 (d, *J* = 3.2 Hz, 1C, C-1_{phenyl}), 137.7 (1C, C-7a_{indole}), 142.5 (1C, C-8a),
15
16 162.2 (d, *J* = 244.4 Hz, 1C, C-4_{phenyl}). FT-IR (neat): ν [cm⁻¹] = 3318 (N-H), 2928, 2851
17
18 (C-H_{alkyl}), 1508, 1458 (C=C_{arom}). Purity (HPLC): 98.3 %, *t*_R = 23.0 min.
19
20
21
22
23

24 **7.2.32. 3-Hydroxy-3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-4'-one**
25
26 **(19)**

27
28 A solution of acetal **5** (502 mg, 2.04 mmol) and 0.2 M HCl (50 mL, 10.0 mmol, 5 eq)
29
30 in THF (50 mL) was stirred at rt for 3 d. 1 M NaOH (25 mL) was added and the
31
32 aqueous layer was extracted with Et₂O (3 x 30 mL) and CH₂Cl₂ (1 x 30 mL). The
33
34 combined organic layers were dried (Na₂SO₄), filtered, concentrated in vacuo and the
35
36 residue was purified by fc (d = 3 cm, l = 18 cm, V = 20 mL, cyclohexane/ethyl acetate
37
38 67:33 → cyclohexane/ethyl acetate 1:1). Colorless solid, mp 169 °C, yield 448 mg
39
40 (95 %). C₁₄H₁₆O₃ (232.3 g/mol). R_f = 0.14 (cyclohexane/ethyl acetate 67:33). HR-MS
41
42 (APCI): *m/z* = 215.1067 (calcd. 215.1067 for C₁₄H₁₅O₂ [M-H₂O+H⁺]). ¹H NMR (400
43
44 MHz, DMSO-*d*₆): δ (ppm) = 1.94 – 2.03 (m, 1H, 2'-H), 2.07 – 2.14 (m, 3H, 3'-H, 5'-H,
45
46 6'-H), 2.14 – 2.25 (m, 1H, 6'-H), 2.37 (td, *J* = 13.4/4.9 Hz, 1H, 2'-H), 2.69 – 2.82 (m,
47
48 3H, 4-H, 3'-H, 5'-H), 2.89 (dd, *J* = 15.8/3.0 Hz, 1H, 4-H), 5.24 (ddd, *J* = 7.3/5.3/3.0
49
50 Hz, 1H, 3-H), 6.56 (d, *J* = 5.3 Hz, 1H, 3-OH), 7.06 – 7.12 (m, 1H, 5-H), 7.12 – 7.18
51
52 (m, 2H, 6-H, 7-H), 7.22 – 7.28 (m, 1H, 8-H). ¹³C NMR (101 MHz, DMSO-*d*₆): δ (ppm)
53
54
55
56
57
58
59
60

= 35.3 (1C, C-6'), 36.3 (1C, C-4), 36.9 (1C, C-3' or C-5'), 37.0 (1C, C-3' or C-5'), 37.9 (1C, C-2'), 74.7 (1C, C-1), 88.7 (1C, C-3), 124.4 (1C, C-8), 126.2 (1C, C-7), 126.6 (1C, C-6), 129.1 (1C; C-5), 132.1 (1C, C-4a), 140.4 (1C, C-8a), 210.3 (1C, C-4'). FT-IR (neat): ν [cm⁻¹] = 3283 (O-H), 2916, 2851 (C-H_{alkyl}), 1686 (C=O), 1450, 1439, 1412 (C=C_{arom}). Purity (HPLC): 96.4 %, t_R = 14.8 min.

7.2.33. Ethyl 2-(4'-oxo-3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-3-yl)acetate (20)

A solution of lactol **19** (100 mg, 0.43 mmol), (ethoxycarbonylmethylene)-triphenylphosphorane (211 mg, 0.60 mmol, 1.4 eq) and Cs₂CO₃ (141 mg, 0.43 mmol, 1.0 eq) in toluene (15 mL) was heated to reflux under N₂ atmosphere for 2 d. After cooling to rt, H₂O (10 mL) was added and the aqueous layer was extracted with Et₂O (3 x 30 mL). The combined organic layers were washed with brine, dried (Na₂SO₄), filtered, concentrated in vacuo and the residue was purified by fc (d = 2 cm, l = 20 cm, V = 10 mL, cyclohexane/ethyl acetate 90:10 → 80:20). Colorless solid, mp 83 °C, yield 81 mg (63 %). C₁₈H₂₂O₄ (302.4 g/mol). R_f = 0.50 (cyclohexane/ethyl acetate 67:33). HR-MS (APCI): m/z = 303.1563 (calcd. 303.1591 for C₁₈H₂₃O₄ [MH⁺]). ¹H NMR (600 MHz, CDCl₃): δ (ppm) = 1.27 (t, J = 7.2 Hz, 3H, OCH₂CH₃), 2.03 (td, J = 14.1/4.8 Hz, 1H, 2'-H), 2.06 – 2.12 (m, 1H, 6'-H), 2.22 – 2.36 (m, 3H, 3'-H, 5'-H, 6'-H), 2.48 – 2.54 (m, 1H, 2'-H), 2.63 – 2.73 (m, 2H, CH₂CO₂Et), 2.76 (dd, J = 15.8/3.0 Hz, 1H, 4-H), 2.79 – 2.93 (m, 3H, 4-H, 3'-H, 5'-H), 4.11 – 4.24 (m, 2H, OCH₂CH₃), 4.35 – 4.41 (m, 1H, 3-H), 7.07 (dd, J = 7.4/1.7 Hz, 1H, 8-H), 7.10 – 7.12 (m, 1H, 5-H), 7.16 – 7.22 (m, 2H, 6-H, 7-H). ¹³C NMR (151 MHz, CDCl₃): δ (ppm) = 14.4 (1C, OCH₂CH₃), 35.0 (1C, C-4), 35.3 (1C, C-2'), 37.3 (1C, C-3'), 37.4 (1C, C-5'), 39.3 (1C, C-6'), 41.4 (1C, CH₂CO₂Et), 60.9 (1C, OCH₂CH₃), 65.8 (1C, C-3), 75.0 (1C,

C-1), 124.8 (1C, C-8), 126.7 (1C, C-7), 126.8 (1C, C-6), 129.1 (1C, C-5), 133.0 (1C, C-4a), 140.2 (1C, C-8a), 171.5 (1C, C=O), 212.2 (1C, C-4'). FT-IR (neat): ν [cm⁻¹] = 2978, 2905 (C-H_{alkyl}), 1724 (C=O_{ester}), 1697 (C=O_{ketone}), 1435, 1408 (C=C_{arom}). Purity (HPLC): 96.0 %, t_R = 21.0 min.

7.2.34. Ethyl 2-(3,4-dihydrodispiro[[2]benzopyran-1,1'-cyclohexan-4',2''-[1,3]dioxolan]-3-yl)acetate (21)

A solution of ester **20** (1.37 g, 4.52 mmol), ethylene glycol (6 mL, 107 mmol, 24 eq), trimethyl orthoformate (3 mL, 27.4 mmol, 6 eq) and *p*-toluenesulfonic acid (174 mg, 0.91 mmol, 0.2 eq) in CH₂Cl₂ (100 mL) was stirred at rt for 24 h. The mixture was washed with saturated Na₂CO₃ solution (2 x 60 mL) and brine (1 x 60 mL). The organic layer was dried (Na₂SO₄), filtered and concentrated in vacuo. Yellow oil, yield 1.55 g (99 %). C₂₀H₂₆O₅ (346.4 g/mol). R_f = 0.28 (CH₂Cl₂/CH₃OH 99:1). HR-MS (APCI): m/z = 347.1873 (calcd. 347.1853 for C₂₀H₂₇O₅ [MH⁺]). ¹H NMR (600 MHz, CDCl₃): δ (ppm) = 1.30 (t, J = 7.2 Hz, 3H, OCH₂CH₃), 1.55 – 1.64 (m, 2H, 3'-H, 5'-H), 1.71 – 1.76 (m, 1H, 6'-H), 1.86 (td, J = 13.9/3.7 Hz, 1H, 2'-H), 1.94 (td, J = 13.9/3.4 Hz, 1H, 3'-H), 2.04 – 2.12 (m, 1H, 5'-H), 2.14 – 2.21 (m, 2H, 2'-H, 6'-H), 2.58 (dd, J = 15.1/4.5 Hz, 1H, CH₂CO₂Et), 2.66 (dd, J = 14.8/8.3 Hz, 1H, CH₂CO₂Et), 2.71 – 2.80 (m, 2H, 4-H), 3.98 (s, 4H, OCH₂CH₂O), 4.13 – 4.27 (m, 3H, 3-H, OCH₂CH₃), 7.06 (d, J = 7.3 Hz, 1H, 5-H), 7.12 – 7.15 (m, 1H, 6-H), 7.16 – 7.20 (m, 2H, 7-H, 8-H). ¹³C NMR (151 MHz, CDCl₃): δ (ppm) = 14.4 (1C, OCH₂CH₃), 30.46 (1C, C-3'), 30.50 (1C, C-5'), 32.9 (1C, C-2'), 35.2 (1C, C-4), 37.2 (1C, C-6'), 41.6 (1C, CH₂CO₂Et), 60.8 (1C, OCH₂CH₃), 64.3 (1C, OCH₂CH₂O), 64.4 (1C, OCH₂CH₂O), 65.5 (1C, C-3), 75.4 (1C, C-1), 108.8 (1C, C-4'), 125.3 (1C, C-8), 126.3 (1C, C-6), 126.4 (1C, C-7), 128.8

(1C, C-5), 133.1 (1C, C-4a), 141.8 (1C, C-8a), 171.6 (1C, C=O). FT-IR (neat): ν [cm⁻¹] = 2978, 2928, 2889 (C-H_{alkyl}), 1728 (C=O), 1435, 1369 (C=C_{arom}). Purity (HPLC): 90.0 %, t_R = 22.4 min.

7.2.35. 2-(3,4-Dihydrodispiro[[2]benzopyran-1,1'-cyclohexan-4',2''-[1,3]dioxolan]-3-yl)ethanol (22)

LiAlH₄ (494 mg, 12.99 mmol, 2.9 eq) was added slowly to a solution of ester **21** (1.54 g, 4.46 mmol) in Et₂O (30 mL) at -20 °C under N₂ atmosphere. After stirring for 2 h, H₂O (50 mL) was added, the precipitate was filtered off and the aqueous layer was extracted with ethyl acetate (3 x 50 mL). The combined organic layers were dried (Na₂SO₄), filtered, concentrated in vacuo and the residue was purified by fc (d = 5 cm, l = 18 cm, V = 50 mL, cyclohexane/ethyl acetate 50:50). Colorless oil, yield 1.10 g (81 %). C₁₈H₂₄O₄ (304.4 g/mol). R_f = 0.19 (cyclohexane/ethyl acetate 50:50). HR-MS (APCI): m/z = 305.1736 (calcd. 305.1747 for C₁₈H₂₅O₄ [MH⁺]). ¹H NMR (600 MHz, CDCl₃): δ (ppm) = 1.61 – 1.71 (m, 2H, 3'-H, 5'-H), 1.76 – 1.81 (m, 1H, 2'-H), 1.85 – 1.99 (m, 4H, 5'-H, 6'-H, CH₂CH₂OH), 2.00 – 2.06 (m, 1H, 3'-H), 2.14 – 2.19 (m, 1H, 6'-H), 2.22 (td, J = 13.8/4.3 Hz, 1H, 2'-H), 2.64 (dd, J = 15.9/2.7 Hz, 1H, 4-H), 2.82 (dd, J = 15.6/11.2 Hz, 1H, 4-H), 3.85 – 3.95 (m, 2H, CH₂CH₂OH), 4.00 (s, 4H, OCH₂CH₂O), 4.00 – 4.04 (m, 1H, 3-H), 7.06 (d, J = 7.6 Hz, 1H, 5-H), 7.10 – 7.16 (m, 1H, 6-H), 7.17 – 7.19 (m, 2H, 7-H, 8-H). A signal for the OH proton is not observed in the spectrum. ¹³C NMR (151 MHz, CDCl₃): δ (ppm) = 30.7 (1C, C-3' or C-5'), 30.8 (1C, C-3' or C-5'), 33.0 (1C, C-6'), 35.4 (1C, C-4), 37.1 (1C, C-2'), 38.2 (1C, CH₂CH₂OH), 60.6 (1C, CH₂CH₂OH), 64.4 (1C, OCH₂CH₂O), 64.5 (1C, OCH₂CH₂O), 67.3 (1C, C-3), 75.4 (1C, C-1), 108.5 (1C, C-4'), 125.3 (1C, C-8), 126.30 (1C, C-7),

1
2
3 126.33 (1C, C-6), 128.9 (1C, C-5), 133.5 (1C, C-4a), 141.7 (1C, C-8a). FT-IR (neat):
4
5 ν [cm⁻¹] = 3421 (O-H), 2928, 2886 (C-H_{alkyl}), 1508, 1435 (C=C_{arom}). Purity (HPLC):
6
7 95.4 %, t_R = 18.0 min.
8
9

10
11
12
13 **7.2.36. 3-(2-Fluoroethyl)-3,4-dihydrodispiro[[2]benzopyran-1,1'-**
14
15 **cyclohexan-4',2''-[1,3]dioxolan] (23)**
16

17
18 A solution of alcohol **22** (55 mg, 0.18 mmol) in CH₂Cl₂ (10 mL) was added dropwise
19
20 to a solution of DAST (0.05 mL, 0.38 mmol, 2.1 eq) in CH₂Cl₂ (10 mL) under N₂
21
22 atmosphere at -78 °C. After 1 h, the mixture was warmed to rt and stirred for 18 h.
23
24 H₂O (10 mL) was added and the aqueous layer was extracted with CH₂Cl₂
25
26 (4 x 10 mL). The combined organic layers were dried (Na₂SO₄), filtered, concentrated
27
28 in vacuo and the residue was purified by fc (d = 1.5 cm, l = 19 cm, V = 5 mL,
29
30 cyclohexane/ethyl acetate 90:10). Yellow oil, yield 27 mg (49 %). C₁₈H₂₃FO₃
31
32 (306.4 g/mol). R_f = 0.66 (cyclohexane/ethyl acetate 67:33). HR-MS (APCI):
33
34 m/z = 307.1690 (calcd. 307.1704 for C₁₈H₂₄FO₃ [MH⁺]). ¹H NMR (600 MHz, CDCl₃): δ
35
36 (ppm) = 1.61 – 1.69 (m, 2H, 3'-H, 5'-H), 1.77 (ddt, J = 13.2/3.8/2.8 Hz, 1H, 2'-H_{equ}),
37
38 1.84 – 1.99 (m, 3H, 5'-H, 6'-H, CH₂CH₂F), 2.04 – 2.18 (m, 3H, 3'-H, 6'-H, CH₂CH₂F),
39
40 2.22 (td, J = 13.6/4.0 Hz, 1H, 2'-H_{ax}), 2.67 (dd, J = 15.8/2.8 Hz, 1H, 4-H), 2.76 (dd, J
41
42 = 15.8/10.7 Hz, 1H, 4-H), 3.96 (ddt, J = 11.0/9.1/3.2 Hz, 1H, 3-H), 4.00 (s, 4H,
43
44 OCH₂CH₂O), 4.63 (dddd, J = 46.8/9.5/5.5/4.3 Hz, 1H, CH₂CH₂F), 4.76 (dtd, J =
45
46 47.6/9.4/4.1 Hz, 1H, CH₂CH₂F), 7.05 – 7.09 (m, 1H, 5-H), 7.11 – 7.17 (m, 1H, 6-H),
47
48 7.17 – 7.22 (m, 2H, 7-H, 8-H). ¹³C NMR (151 MHz, CDCl₃): δ (ppm) = 30.6 (1C, C-3'),
49
50 30.7 (1C, C-5'), 32.9 (1C, C-6'), 35.7 (1C, C-4), 37.0 (d, J = 19.3 Hz, 1C, CH₂CH₂F),
51
52 37.3 (1C, C-2'), 64.0 (d, J = 4.7 Hz, 1C, C-3), 64.4 (1C, OCH₂CH₂O), 64.5 (1C,
53
54
55
56
57
58
59
60

OCH₂CH₂O), 75.1 (1C, C-1), 81.0 (d, *J* = 163.4 Hz, 1C, CH₂CH₂F), 108.7 (1C, C-4'), 125.3 (1C, C-8), 126.29 (1C, C-7), 126.32 (1C, C-6), 128.9 (1C, C-5), 133.5 (1C, C-4a) 142.0 (1C, C-8a). FT-IR (neat): ν [cm⁻¹] = 2967, 2928, 2855 (C-H_{alkyl}), 1489, 1439 (C=C_{arom}). Purity (HPLC): 95.8 %, *t*_R = 22.4 min.

7.2.37. 3-(2-Fluoroethyl)-3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-4'-one (24)

A solution of ketal **23** (694 mg, 2.27 mmol) and 2 M HCl (20 mL, 40.0 mmol, 17.6 eq) in Et₂O (50 mL) was heated to reflux for 3 d. After cooling to rt, H₂O (20 mL) was added and the aqueous layer was extracted with CH₂Cl₂ (3 x 30 mL). The combined organic layers were dried (Na₂SO₄), filtered, concentrated in vacuo and the residue was purified by fc (d = 4 cm, l = 18 cm, V = 30 mL, cyclohexane/ethyl acetate 90:10). Colorless solid, mp 105 °C, yield 566 mg (95 %). C₁₆H₁₉FO₂ (262.3 g/mol). R_f = 0.39 (cyclohexane/ethyl acetate 80:20). HR-MS (APCI): *m/z* = 263.1459 (calcd. 263.1442 for C₁₆H₂₀FO₂ [MH⁺]). ¹H NMR (400 MHz, CDCl₃): δ (ppm) = 1.89 – 1.98 (m, 1H, CH₂CH₂F), 1.98 – 2.07 (m, 1H, 2'-H), 2.07 – 2.23 (m, 2H, 6'-H, CH₂CH₂F), 2.27 – 2.40 (m, 3H, 3'-H, 5'-H, 6'-H), 2.43 – 2.51 (m, 1H, 2'-H), 2.67 – 2.79 (m, 2H, 4-H, 3'-H), 2.83 (dd, *J* = 16.0/10.9 Hz, 1H, 4-H), 2.88 – 2.99 (m, 1H, 5'-H), 4.09 (ddt, *J* = 10.6/9.1/3.4 Hz, 1H, 3-H), 4.59 (ddd, *J* = 9.3/5.2/4.3 Hz, 0.5H, CH₂CH₂F), 4.70 (td, *J* = 9.6/4.4 Hz, 2 x 0.5H, CH₂CH₂F), 4.82 (td, *J* = 9.3/3.9 Hz, 0.5H, CH₂CH₂F), 7.05 – 7.10 (m, 1H, 8-H), 7.10 – 7.15 (m, 1H, 5-H), 7.15 – 7.23 (m, 2H, 6-H, 7-H). ¹³C NMR (101 MHz, CDCl₃): δ (ppm) = 35.3 (1C, C-2'), 35.6 (1C, C-4), 36.9 (d, *J* = 19.2 Hz, 1C, CH₂CH₂F), 37.3 (1C, C-3'), 37.5 (1C, C-5'), 39.3 (1C, C-6'), 64.6 (d, *J* = 4.3 Hz, 1C, C-3), 74.7 (1C, C-1), 80.6 (d, *J* = 164.5 Hz, 1C, CH₂CH₂F), 124.7 (1C, C-8),

1
2
3 126.6 (1C, C-7), 126.8 (1C, C-6), 129.2 (1C, C-5), 133.4 (1C, C-4a), 140.4 (1C, C-
4 8a), 211.9 (1C, C=O). FT-IR (neat): ν [cm⁻¹] = 2932, 2897 (C-H_{alkyl}), 1705 (C=O),
5
6 1493, 1439 (C=C_{arom}). Purity (HPLC): 97.0 %, t_R = 20.9 min.
7
8
9

10
11
12 **7.2.38. trans-3-Methoxy-3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-**
13 **4'-amine (25a)**
14
15

16
17 A solution of amine **4a** (274 mg, 0.81 mmol) and 10 % Pd/C (36 mg, 0.03 mmol,
18 4 mol-%) in CH₃OH (20 mL) was stirred at rt for 20 h under H₂ atmosphere. The
19 mixture was filtered through Celite, washed with CH₂Cl₂ (150 mL), concentrated in
20 vacuo and the residue was purified by fc (d = 2 cm, l = 20 cm, V = 10 mL,
21 CH₂Cl₂/CH₃OH 95:5 + 1 % *N,N*-dimethylethanamine). Pale yellow solid, mp 94 °C,
22 yield 143 mg (72 %). C₁₅H₂₁NO₂ (247.3 g/mol). R_f = 0.09 (cyclohexane/ethyl acetate
23 67:33 + 1 % *N,N*-dimethylethanamine). HR-MS (APCI): m/z = 248.1648 (calcd.
24 248.1645 for C₁₅H₂₂NO₂ [MH⁺]). ¹H NMR (600 MHz, CD₃OD): δ (ppm) = 1.58 – 1.63
25 (m, 3H, 3'-H, 5'-H, 6'-H), 1.84 – 1.89 (m, 1H, 2'-H), 1.98 (td, J = 14.1/3.8 Hz, 1H, 2'-
26 H), 2.11 – 2.20 (m, 1H, 3'-H), 2.20 – 2.30 (m, 2H, 5'-H, 6'-H), 2.80 (dd, J = 15.6/7.6
27 Hz, 1H, 4-H), 2.92 (dd, J = 15.6/3.1 Hz, 1H, 4-H), 3.26 (quint, J = 3.2 Hz, 1H, 4'-H_{equ}),
28 3.55 (s, 3H, OCH₃), 4.91 (dd, J = 7.6/3.1 Hz, 1H, 3-H), 7.08 (d, J = 7.5 Hz, 1H, 5-H),
29 7.15 (td, J = 7.4/1.3 Hz, 1H, 6-H), 7.18 – 7.22 (m, 1H, 7-H), 7.38 (dd, J = 7.7/1.3 Hz,
30 1H, 8-H). A signal for the NH₂ protons is not observed in the spectrum. ¹³C NMR (151
31 MHz, CD₃OD): δ (ppm) = 28.8 (1C, C-5'), 29.0 (1C, C-3'), 31.0 (1C, C-2'), 33.6 (1C,
32 C-6'), 36.2 (1C, C-4), 45.6 (1C, C-4'), 56.3 (1C, OCH₃), 78.0 (1C, C-1), 97.8 (1C, C-
33 3), 126.1 (1C, C-8), 127.5 (1C, C-7), 127.6 (1C, C-6), 130.0 (1C, C-5) 132.4 (1C, C-
34 4a), 143.4 (1C, C-8a). FT-IR (neat): ν [cm⁻¹] = 3360 (N-H), 2947, 2870 (C-H_{alkyl}),
35 1562, 1447, 1431 (C=C_{arom}). Purity (HPLC): 96.4 %, t_R = 13.3 min.
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5 **7.2.39. cis-3-Methoxy-3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-4'-**
6 **amine (25b)**
7

8
9
10 A solution of amine **4b** (326 mg, 0.97 mmol) and 10 % Pd/C (41 mg, 0.04 mmol,
11 4 mol-%) in CH₃OH (20 mL) was stirred at rt for 19 h under H₂ atmosphere. The
12 mixture was filtered through Celite, washed with CH₂Cl₂ (150 mL), concentrated in
13 vacuo and the residue was purified by fc (d = 2 cm, l = 20 cm, V = 10 mL,
14 CH₂Cl₂/CH₃OH 97:3 + 1 % *N,N*-dimethylethanamine). Pale yellow solid, mp 114 °C,
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
yield 202 mg (89 %). C₁₅H₂₁NO₂ (247.3 g/mol). R_f = 0.17 (CH₂Cl₂/CH₃OH 95:5 + 1 %
N,N-dimethylethanamine). HR-MS (APCI): *m/z* = 248.1626 (calcd. 248.1645 for
C₁₅H₂₂NO₂ [MH⁺]). ¹H NMR (600 MHz, CD₃OD): δ (ppm) = 1.72 – 1.94 (m, 6H, 2'-*H*_{ax},
3'-*H*, 5'-*H*, 6'-*H*), 1.99 – 2.07 (m, 1H, 6'-*H*), 2.11 (dq, *J* = 14.0/3.0 Hz, 1H, 2'-*H*_{equ}),
2.80 (dd, *J* = 15.7/7.3 Hz, 1H, 4'-*H*), 2.92 (dd, *J* = 15.8/3.1 Hz, 1H, 4'-*H*), 3.01 – 3.08
(m, 1H, 4'-*H*_{ax}), 3.56 (s, 3H, OCH₃), 4.91 (dd, *J* = 7.4/3.1 Hz, 1H, 3'-*H*), 7.08 (d, *J* =
7.6 Hz, 1H, 5'-*H*), 7.13 – 7.20 (m, 3H, 6'-*H*, 7'-*H*, 8'-*H*). A signal for the NH₂ protons is
not observed in the spectrum. ¹³C NMR (151 MHz, CD₃OD): δ (ppm) = 29.9 (1C, C-3'
or C-5'), 30.0 (1C, C-3' or C-5'), 36.1 (1C, C-4), 36.3 (1C, C-2'), 38.7 (1C, C-6'), 50.7
(1C, C-4'), 56.4 (1C, OCH₃), 76.7 (1C, C-1), 97.9 (1C, C-3), 125.6 (1C, C-8), 127.6
(1C, C-7), 127.8 (1C, C-6), 130.2 (1C, C-5), 132.6 (1C, C-4a), 142.2 (1C, C-8a). FT-
IR (neat): ν [cm⁻¹] = 3445, 3352 (N-H), 2928, 2859 (C-H_{alkyl}), 1574, 1443, 1385
(C=C_{arom}). Purity (HPLC, method 1): 98.6 %, *t*_R = 13.4 min.

51 **7.2.40. trans-3-Methoxy-N-(6,7,8,9-tetrahydro-5H-benzo[7]annulen-7-yl)-**
52 **3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-4'-amine (27a)**
53
54
55
56
57
58
59
60

A solution of ketone **26** (52 mg, 0.32 mmol), amine **25a** (77 mg, 0.31 mmol, 1.0 eq) and acetic acid (18 μ L, 0.32 mmol, 1.0 eq) in CH_2Cl_2 (5 mL) was stirred under N_2 atmosphere at rt. After 30 min, $\text{NaBH}(\text{OAc})_3$ (119 mg, 0.56 mmol, 1.8 eq) was added and the mixture was stirred for 3 h at rt. 1 M NaOH (15 mL) was added and the aqueous layer was extracted with CH_2Cl_2 (3 x 20 mL). The combined organic layers were dried (Na_2SO_4), filtered, concentrated in vacuo and the residue was purified by fc (d = 2.5 cm, l = 20 cm, V = 10 mL, cyclohexane/ethyl acetate 80:20 + 1 % *N,N*-dimethylethanamine). Colorless oil, yield 58 mg (48 %). $\text{C}_{26}\text{H}_{33}\text{NO}_2$ (391.6 g/mol). R_f = 0.32 (cyclohexane/ethyl acetate 50:50 + 1 % *N,N*-dimethylethanamine). HR-MS (APCI): m/z = 392.2599 (calcd. 392.2584 for $\text{C}_{26}\text{H}_{34}\text{NO}_2$ [MH^+]). ^1H NMR (400 MHz, CD_3OD): δ (ppm) = 1.25 – 1.37 (m, 2H, 6- $H_{\text{benzannulene}}$, 8- $H_{\text{benzannulene}}$), 1.64 (dt, J = 12.6/3.0 Hz, 1H, 2'- H), 1.71 – 1.78 (m, 2H, 3'- H , 5'- H), 1.85 – 1.92 (m, 1H, 6'- H), 1.96 (td, J = 13.5/3.6 Hz, 1H, 6'- H), 2.06 – 2.29 (m, 5H, 2'- H , 3'- H , 5'- H , 6- $H_{\text{benzannulene}}$, 8- $H_{\text{benzannulene}}$), 2.78 – 2.87 (m, 5H, 4- H , 5- $H_{\text{benzannulene}}$, 9- $H_{\text{benzannulene}}$), 2.89 – 2.98 (m, 2H, 4- H , 7- $H_{\text{benzannulene}}$), 3.18 (quint, J = 3.1 Hz, 1H, 4'- H_{equ}), 3.58 (s, 3H, OCH_3), 4.93 (dd, J = 7.5/3.2 Hz, 1H, 3- H), 7.08 – 7.14 (m, 5H, 5- H , 1- $H_{\text{benzannulene}}$, 2- $H_{\text{benzannulene}}$, 3- $H_{\text{benzannulene}}$, 4- $H_{\text{benzannulene}}$), 7.17 (td, J = 7.4/1.4 Hz, 1H, 6- H), 7.22 (td, J = 7.5/1.6 Hz, 1H, 7- H), 7.35 (dd, J = 7.7/1.4 Hz, 1H, 8- H). A signal for the NH proton is not observed in the spectrum. ^{13}C NMR (101 MHz, CD_3OD): δ (ppm) = 26.6 (1C, C-3'), 26.8 (1C, C-5'), 31.6 (1C, C-6'), 33.5 (2C, C-5 $_{\text{benzannulene}}$, C-9 $_{\text{benzannulene}}$), 34.2 (1C, C-2'), 35.7 (2C, C-6 $_{\text{benzannulene}}$, C-8 $_{\text{benzannulene}}$), 36.2 (1C, C-4), 48.5 (1C, C-4'), 56.3 (1C, OCH_3), 60.0 (1C, C-7 $_{\text{benzannulene}}$), 78.0 (1C, C-1), 97.8 (1C, C-3), 126.0 (1C, C-8), 127.3 (2C, C-2 $_{\text{benzannulene}}$, C-3 $_{\text{benzannulene}}$), 127.5 (1C, C-7), 127.6 (1C, C-6), 129.8 (2C, C-1 $_{\text{benzannulene}}$, C-4 $_{\text{benzannulene}}$), 130.0 (1C, C-5), 132.4 (1C, C-4a), 143.4 (1C, C-8a), 143.7 (2C, C-4a $_{\text{benzannulene}}$, C-9a $_{\text{benzannulene}}$). FT-IR (neat): ν [cm^{-1}] = 3314 (N-H), 2924,

1
2
3 2843 (C-H_{alkyl}), 1489, 1443 (C=C_{arom}). Purity (HPLC, method 1): 99.1 %,
4
5 t_R = 20.0 min.
6
7
8
9

10 **7.2.41. cis-3-Methoxy-N-(6,7,8,9-tetrahydro-5H-benzo[7]annulen-7-yl)-3,4-**
11 **dihydrospiro([2]benzopyran-1,1'-cyclohexan)-4'-amine (27b)**
12
13

14 A solution of ketone **26** (50.0 mg, 0.31 mmol, 1.0 eq), amine **25b** (77.0 mg,
15 0.31 mmol, 1.0 eq) and NaBH(OAc)₃ (132 mg, 0.62 mmol, 2.0 eq) in CH₂Cl₂ (4 mL)
16 was stirred for 12 h at rt. A saturated solution of NaHCO₃ (10 mL) and CH₂Cl₂ (5 mL)
17 were added and the aqueous layer was extracted with CH₂Cl₂ (3 x 10 mL). The
18 combined organic layers were dried (Na₂SO₄), filtered, concentrated in vacuo and the
19 residue was purified by fc (d = 2 cm, l = 25 cm, V = 7 mL, cyclohexane:ethyl
20 acetate = 20:80 + 1 % *N,N*-dimethylethanamine). Colorless solid, mp 132 °C, yield
21 34 mg (28 %). C₂₆H₃₃NO₂ (391.6). R_f = 0.36 (cyclohexane:ethyl acetate = 1:9 +
22 1 % *N,N*-dimethylethanamine). HR-MS (APCI): m/z = 392.2572 (calcd. 392.2584 for
23 C₂₆H₃₄NO₂ [M+H]⁺). ¹H NMR (600 MHz, CD₃OD): δ [ppm] = 1.18 - 1.26 (m, 2H,
24 6-*H*_{benzannulene}, 8-*H*_{benzannulene}), 1.66 - 1.80 (m, 3H, 3'-*H*, 5'-*H*, 6'-*H*), 1.82 - 1.89 (m, 3H,
25 2'-*H*, 3'-*H*, 5'-*H*), 2.01 (dt, *J* = 13.6/4.2 Hz, 1H, 2'-*H*), 2.10 (dt, *J* = 10.5/3.0 Hz, 1H,
26 6'-*H*), 2.16 - 2.23 (m, 2H, 6-*H*_{benzannulene}, 8-*H*_{benzannulene}), 2.77 - 2.89 (m, 6H,
27 5-*H*_{benzannulene}, 9-*H*_{benzannulene}, 4'-*H*_{ax}, 4-*H*), 2.91 (dd, *J* = 15.6/3.0 Hz, 1H, 4-*H*), 3.01 (tt,
28 *J* = 10.6/3.5 Hz, 1H, 7-*H*_{benzannulene}), 3.57 (s, 3H, OCH₃), 4.90 (dd, *J* = 7.5/3.1 Hz, 1H,
29 3-*H*), 7.05 - 7.10 (m, 5H, 5-*H*, 1-*H*_{benzannulene}, 2-*H*_{benzannulene}, 3-*H*_{benzannulene},
30 4-*H*_{benzannulene}), 7.13 - 7.20 (m, 3H, 6-*H*, 7-*H*, 8-*H*). A signal for the NH proton is not
31 observed in the spectrum. ¹³C NMR (151 MHz, CD₃OD): δ [ppm] = 29.5 (1C, C-3'),
32 29.7 (1C, C-5'), 33.4 (2C, C-5_{benzannulene}, C-9_{benzannulene}), 35.51 (1C, C-6_{benzannulene} or
33 C-8_{benzannulene}), 35.53 (1C, C-6_{benzannulene} or C-8_{benzannulene}), 36.2 (1C, C-4), 36.8 (1C,
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 C-6'), 39.3 (1C, C-2'), 53.5 (1C, C-4'). 56.5 (1C, OCH₃), 59.0 (1C, C-7_{benzannulene}),
4
5 77.6 (1C, C-1), 97.8 (1C, C-3), 125.7 (1C, C-8), 127.3 (2C, C-2_{benzannulene},
6
7 C-3_{benzannulene}), 127.5 (1C, C-6), 127.6 (1C, C-7), 129.8 (2C, C-1_{benzannulene},
8
9 C-4_{benzannulene}), 130.1 (1C, C-5), 132.6 (1C, C-4a), 142.8 (1C, C-8a), 143.6 (2C,
10
11 C-4a_{benzannulene}, C-9a_{benzannulene}). FT-IR (neat): $\tilde{\nu}$ [cm⁻¹] = 3024, 2924 (C-H), 1489,
12
13 1443 (C=C). Purity (HPLC): 95.3 %, t_R = 19.9 min.

14
15
16
17
18 **7.2.42. trans-N-Benzyl-3-(2-fluoroethyl)-3,4-dihydrospiro[[2]benzopyran-**
19
20 **1,1'-cyclohexan]-4'-amine (28a) and cis-N-Benzyl-3-(2-fluoroethyl)-3,4-**
21
22 **dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-4'-amine (28b)**

23
24
25 A solution of ketone **24** (248 mg, 0.94 mmol), benzylamine (distilled, 0.16 mL,
26
27 1.47 mmol, 1.6 eq), acetic acid (59 μ L, 1.04 mmol, 1.1 eq) and NaBH(OAc)₃ (366 mg,
28
29 1.73 mmol, 1.8 eq) in THF (20 mL) was stirred under N₂ atmosphere at rt. After 3 h,
30
31 1 M NaOH (15 mL) was added and the aqueous layer was extracted with Et₂O
32
33 (3 x 25 mL). The combined organic layers were dried (Na₂SO₄), filtered, concentrated
34
35 in vacuo and the residue was purified by fc (d = 4 cm, l = 16 cm, V = 20 mL,
36
37 cyclohexane/ethyl acetate 80:20 + 1 % *N,N*-dimethylethanamine → 67:33 + 1 % *N,N*-
38
39 dimethylethanamine). **28a** was eluted first and **28b** afterwards.

40
41
42 **trans-28a**: Colorless oil, yield 148 mg (45 %). C₂₃H₂₈FNO (353.8 g/mol). R_f = 0.57
43
44 (cyclohexane/ethyl acetate 67:33 + 1 % *N,N*-dimethylethanamine). HR-MS (APCI):
45
46 m/z = 354.2224 (calcd. 354.2228 for C₂₃H₂₉FNO [MH⁺]). ¹H NMR (600 MHz, CD₃OD):
47
48 δ (ppm) = 1.45 (dq, J = 13.6/3.2 Hz, 1H, 2'-H_{equ}), 1.64 – 1.73 (m, 2H, 3'-H, 5'-H), 1.82
49
50 – 1.96 (m, 4H, 5'-H, 6'-H, CH₂CH₂F), 2.00 – 2.11 (m, 2H, 3'-H, CH₂CH₂F), 2.29 (td, J
51
52 = 13.7/4.0 Hz, 1H, 2'-H_{ax}), 2.65 – 2.69 (m, 2H, 4-H), 2.92 (quint, J = 3.2 Hz, 1H, 4'-
53
54 H_{equ}), 3.82 (s, 2H, ArCH₂NH), 3.94 (tdd, J = 8.8/5.3/3.5 Hz, 1H, 3-H), 4.61 (dddd, J =
55
56
57
58
59
60

1
2
3 47.0/9.2/5.5/4.8 Hz, 1H, CH₂CH₂F), 4.72 (dtd, $J = 47.7/9.0/4.4$ Hz, 1H CH₂CH₂F),
4
5 7.05 (d, $J = 7.5$ Hz, 1H, 5-*H*), 7.10 (td, $J = 7.4/1.3$ Hz, 1H, 6-*H*), 7.16 (t, $J = 7.7$ Hz,
6
7 1H, 7-*H*), 7.24 – 7.29 (m, 1H, 4-*H*_{benzyl}), 7.33 – 7.38 (m, 3H, 8-*H*, 3-*H*_{benzyl}, 5-*H*_{benzyl}),
8
9 7.39 – 7.43 (m, 2H, 2-*H*_{benzyl}, 6-*H*_{benzyl}). A signal for the NH proton is not observed in
10
11 the spectrum. ¹³C NMR (151 MHz, CD₃OD): δ (ppm) = 26.1 (1C, C-3'), 26.2 (1C, C-
12
13 5'), 30.0 (1C, C-6'), 34.4 (1C, C-2'), 36.6 (1C, C-4), 38.1 (d, $J = 19.6$ Hz, 1C,
14
15 CH₂CH₂F), 51.3 (1C, C-4'), 52.3 (1C, ArCH₂NH), 65.1 (d, $J = 5.0$ Hz, 1C, C-3), 76.9
16
17 (1C, C-1), 81.7 (d, $J = 163.2$ Hz, 1C, CH₂CH₂F), 126.5 (1C, C-8), 127.0 (1C, C-6),
18
19 127.2 (1C, C-7), 128.0 (1C, C-4_{benzyl}), 129.4 (2C, C-3_{benzyl}, C-5_{benzyl}), 129.6 (3C, C-5,
20
21 C-2_{benzyl}, C-6_{benzyl}), 134.3 (1C, C-4a), 141.3 (1C, C-1_{benzyl}), 144.2 (1C, C-8a). FT-IR
22
23 (neat): ν [cm⁻¹] = 3317 (N-H), 2978, 2924, 2893 (C-H_{alkyl}), 1489, 1450 (C=C_{arom}).
24
25
26 Purity (HPLC, method 1): 98.8 %, $t_R = 19.2$ min.

27
28
29 **cis-28b**: Colorless oil, yield 150 mg (45 %). C₂₃H₂₈FNO (353.8 g/mol). $R_f = 0.22$
30
31 (cyclohexane/ethyl acetate 67:33 + 1 % *N,N*-dimethylethanamine). HR-MS (APCI):
32
33 $m/z = 354.2221$ (calcd. 354.2228 for C₂₃H₂₉FNO [MH⁺]). ¹H NMR (400 MHz, CD₃OD):
34
35 δ (ppm) = 1.56 – 1.69 (m, 2H, 2'-*H*, 3'-*H*), 1.70 – 1.79 (m, 1H, 6'-*H*), 1.80 – 2.02 (m,
36
37 5H, 3'-*H*, 5'-*H*, 6'-*H*, CH₂CH₂F), 2.03 – 2.13 (m, 1H, CH₂CH₂F), 2.13 – 2.20 (m, 1H,
38
39 2'-*H*), 2.64 – 2.77 (m, 3H, 4-*H*, 4'-*H*_{ax}), 3.86 (s, 2H, ArCH₂NH), 3.96 (tdd, $J =$
40
41 8.9/5.6/3.5 Hz, 1H, 3-*H*), 4.67 (dddd, $J = 46.9/10.1/6.0/4.5$ Hz, 1H, CH₂CH₂F), 4.83
42
43 (dtd, $J = 47.8/9.2/4.7$ Hz, 1H, CH₂CH₂F), 7.06 – 7.10 (m, 1H, 5-*H*), 7.10 – 7.19 (m,
44
45 3H, 6-*H*, 7-*H*, 8-*H*), 7.26 – 7.32 (m, 1H, 4-*H*_{benzyl}), 7.34 – 7.43 (m, 4H, 2-*H*_{benzyl}, 3-
46
47 *H*_{benzyl}, 5-*H*_{benzyl}, 6-*H*_{benzyl}). A signal for the NH proton is not observed in the spectrum.
48
49 ¹³C NMR (101 MHz, CD₃OD): δ (ppm) = 28.7 (1C, C-5'), 28.8 (1C, C-3'), 34.8 (1C, C-
50
51 2'), 36.6 (1C, C-4), 38.1 (d, $J = 19.5$ Hz, 1C, CH₂CH₂F), 39.3 (1C, C-6'), 51.5 (1C,
52
53 ArCH₂NH), 56.4 (1C, C-4'), 65.2 (d, $J = 5.3$ Hz, 1C, C-3), 76.4 (1C, C-1), 81.8 (d, $J =$
54
55
56
57
58
59
60

1
2
3 162.6 Hz, 1C, CH₂CH₂F), 126.1 (1C, C-8), 127.1 (1C, C-6 or C-7), 127.2 (1C, C-6 or
4
5 C-7), 128.1 (1C, C-4_{benzyl}), 129.52 (2C, C-2_{benzyl} and C-6_{benzyl} or C-3_{benzyl} and C-
6
7 5_{benzyl}), 129.53 (2C, C-2_{benzyl} and C-6_{benzyl} or C-3_{benzyl} and C-5_{benzyl}), 129.7 (1C, C-5),
8
9 134.7 (1C, C-4a), 140.9 (1C, C-1_{benzyl}), 143.4 (1C, C-8a). FT-IR (neat): ν [cm⁻¹] =
10
11 3059 (N-H), 2924, 2855 (C-H_{alkyl}), 1489, 1450 (C=C_{arom}). Purity (HPLC): 98.2 %,
12
13 t_R = 19.2 min.
14
15
16
17

18
19 **7.2.43. trans-3-(2-Fluoroethyl)-3,4-dihydrospiro[[2]benzopyran-1,1'-**
20
21 **cyclohexan]-4'-amine (29a)**
22

23 A solution of amine **28a** (118 mg, 0.33 mmol), ammonium formate (102 mg,
24 1.61 mmol, 4.9 eq) and 10 % Pd/C (15 mg, 0.01 mmol, 4 mol-%) in CH₃OH (15 mL)
25 was heated to reflux for 2 h under N₂ atmosphere. After cooling to rt, the mixture was
26 filtered through Celite, washed with CH₂Cl₂ (100 mL) and concentrated in vacuo.
27 0.1 M NaOH (60 mL) was added and the aqueous phase was extracted with CH₂Cl₂
28 (3 x 50 mL). The combined organic layers were dried (Na₂SO₄), filtered and
29 concentrated in vacuo. Yellow oil, yield 83 mg (96 %). C₁₆H₂₂FNO (263.4 g/mol). R_f =
30 0.06 (cyclohexane/ethyl acetate 67:33 + 1 % *N,N*-dimethylethanamine). HR-MS
31 (APCI): m/z = 264.1753 (calcd. 264.1758 for C₁₆H₂₃FNO [MH⁺]). ¹H NMR (400 MHz,
32 CDCl₃): δ (ppm) = 1.43 – 1.57 (m, 3H, 2'-H, 3'-H, 5'-H), 1.83 – 2.01 (m, 4H, 5'-H, 6'-
33
34 H, CH₂CH₂F), 2.02 – 2.16 (m, 2H, 3'-H, CH₂CH₂F), 2.31 (td, J = 13.5/4.2 Hz, 1H, 2'-
35
36 H), 2.66 (dd, J = 15.8/3.2 Hz, 1H, 4-H), 2.74 (dd, J = 15.9/10.6 Hz, 1H, 4-H), 3.32 –
37
38 3.37 (m, 1H, 4'-H_{equ}), 3.95 (ddt, J = 10.6/9.3/3.4 Hz, 1H, 3-H), 4.58 (ddd, J =
39
40 9.1/5.7/4.3 Hz, 0.5H, CH₂CH₂F), 4.67 – 4.73 (m, 2 x 0.5H, CH₂CH₂F), 4.82 (td, J =
41
42 9.1/4.4 Hz, 0.5H, CH₂CH₂F), 7.04 – 7.08 (m, 1H, 5-H), 7.13 (td, J = 7.3/1.6 Hz, 1H, 6-
43
44 H), 7.16 – 7.21 (m, 1H, 7-H), 7.23 (dd, J = 7.7/1.7 Hz, 1H, 8-H). A signal for the NH₂
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

protons is not observed in the spectrum. ^{13}C NMR (101 MHz, CDCl_3): δ (ppm) = 28.4 (1C, C-3'), 28.5 (1C, C-5'), 28.6 (1C, C-6'), 32.8 (1C, C-2'), 35.8 (1C, C-4), 37.1 (d, J = 19.4 Hz, 1C, $\text{CH}_2\text{CH}_2\text{F}$), 44.5 (1C, C-4'), 63.8 (d, J = 5.0 Hz, 1C, C-3), 75.8 (1C, C-1), 81.0 (d, J = 163.7 Hz, 1C, $\text{CH}_2\text{CH}_2\text{F}$), 125.5 (1C, C-8), 126.2 (1C, C-6), 126.3 (1C, C-7), 128.8 (1C, C-5), 133.3 (1C, C-4a), 143.1 (1C, C-8a). FT-IR (neat): ν [cm^{-1}] = 3372 (N-H), 2920, 2851 (C- H_{alkyl}), 1489, 1439 (C=C $_{\text{arom}}$). Purity (HPLC): 94.4 %, t_{R} = 15.6 min.

7.2.44. *cis*-3-(2-Fluoroethyl)-3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-4'-amine (29b)

A solution of amine **28b** (137 mg, 0.39 mmol), ammonium formate (125 mg, 1.99 mmol, 5.1 eq) and 10 % Pd/C (17 mg, 0.02 mmol, 4 mol-%) in CH_3OH (15 mL) was heated to reflux for 4.5 h under N_2 atmosphere. After cooling to rt, the mixture was filtered through Celite, washed with CH_2Cl_2 (100 mL) and concentrated in vacuo. 0.1 M NaOH (60 mL) was added and the aqueous phase was extracted with CH_2Cl_2 (3 x 50 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated in vacuo. Yellow oil, yield 90 mg (88 %). $\text{C}_{16}\text{H}_{22}\text{FNO}$ (263.4 g/mol). R_{f} = 0.06 (cyclohexane/ethyl acetate 67:33 + 1 % *N,N*-dimethylethanamine). HR-MS (APCI): m/z = 264.1748 (calcd. 264.1758 for $\text{C}_{16}\text{H}_{23}\text{FNO}$ [MH^+]). ^1H NMR (400 MHz, CDCl_3): δ (ppm) = 1.45 – 1.60 (m, 2H, 2'-H, 3'-H), 1.60 – 1.81 (m, 4H, 3'-H, 5'-H, 6'-H), 1.85 – 2.12 (m, 3H, 6'-H, $\text{CH}_2\text{CH}_2\text{F}$), 2.12 – 2.19 (m, 1H, 2'-H), 2.66 (dd, J = 15.8/3.1 Hz, 1H, 4-H), 2.75 (dd, J = 15.8/10.7 Hz, 1H, 4-H), 2.82 (tt, J = 11.3/10.6/4.6 Hz, 1H, 4'- H_{ax}), 3.94 (ddt, J = 10.6/9.1/3.4 Hz, 1H, 3-H), 4.59 (ddd, J = 9.0/5.6/4.4 Hz, 0.5H, $\text{CH}_2\text{CH}_2\text{F}$), 4.68 – 4.76 (m, 2 x 0.5H, $\text{CH}_2\text{CH}_2\text{F}$), 4.84 (td, J = 9.0/4.4 Hz, 0.5H, $\text{CH}_2\text{CH}_2\text{F}$), 7.04 – 7.12 (m, 2H, 5-H, 8-H), 7.12 – 7.20 (m, 2H, 6-H, 7-H). A signal for

the NH₂ protons is not observed in the spectrum. ¹³C NMR (101 MHz, CDCl₃): δ (ppm) = 32.1 (1C, C-5'), 32.2 (1C, C-3'), 34.4 (1C, C-2'), 35.7 (1C, C-4), 37.1 (d, *J* = 19.4 Hz, 1C, CH₂CH₂F), 38.7 (1C, C-6'), 50.4 (1C, C-4'), 64.0 (d, *J* = 4.9 Hz, 1C, C-3), 74.9 (1C, C-1), 81.1 (d, *J* = 163.7 Hz, 1C, CH₂CH₂F), 125.1 (1C, C-8), 126.2 (1C, C-6 or C-7), 126.3 (1C, C-6 or C-7), 128.9 (1C, C-5), 133.6 (1C, C-4a), 142.4 (1C, C-8a). FT-IR (neat): ν [cm⁻¹] = 3356 (N-H), 2924, 2855 (C-H_{alkyl}), 1516, 1447 (C=C_{arom}). Purity (HPLC): 96.0 %, *t*_R = 15.2 min.

7.2.45. trans-3-(2-Fluoroethyl)-N-(6,7,8,9-tetrahydro-5H-benzo[7]annulen-7-yl)-3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-4'-amine (30a)

A solution of ketone **26** (25 mg, 0.16 mmol), amine **29a** (42 mg, 0.16 mmol, 1.0 eq), acetic acid (10 μL, 0.18 mmol, 1.1 eq) and NaBH(OAc)₃ (62 mg, 0.29 mmol, 1.8 eq) in CH₂Cl₂ (3 mL) was stirred under N₂ atmosphere at rt. After 21 h, 1 M NaOH (3 mL) was added and the aqueous layer was extracted with CH₂Cl₂ (3 x 10 mL). The combined organic layers were dried (Na₂SO₄), filtered, concentrated in vacuo and the residue was purified by fc (d = 2 cm, l = 19 cm, V = 10 mL, cyclohexane/ethyl acetate 90:10 + 1 % *N,N*-dimethylethanamine). Colorless oil, yield 50 mg (76 %). C₂₇H₃₄FNO (407.6 g/mol). R_f = 0.34 (cyclohexane/ethyl acetate 67:33 + 1 % *N,N*-dimethylethanamine). HR-MS (APCI): *m/z* = 408.2668 (calcd. 408.2697 for C₂₇H₃₅FNO [MH⁺]). ¹H NMR (400 MHz, CDCl₃): δ (ppm) = 1.22 – 1.39 (m, 2H, 6-*H*_{benzannulene}, 8-*H*_{benzannulene}), 1.46 – 1.53 (m, 1H, 2'-*H*), 1.53 – 1.65 (m, 2H, 3'-*H*, 5'-*H*), 1.81 – 2.14 (m, 6H, 3'-*H*, 5'-*H*, 6'-*H*, CH₂CH₂F), 2.14 – 2.24 (m, 2H, 6-*H*_{benzannulene}, 8-*H*_{benzannulene}), 2.30 (td, *J* = 13.3/3.6 Hz, 1H, 2'-*H*), 2.65 (dd, *J* = 16.2/3.1 Hz, 1H, 4-*H*), 2.69 – 2.80 (m, 3H, 4-*H*, 5-*H*_{benzannulene}, 9-*H*_{benzannulene}), 2.80 – 2.89 (m, 3H, 5-*H*_{benzannulene}, 7-*H*_{benzannulene}, 9-*H*_{benzannulene}), 3.13 – 3.19 (m, 1H, 4'-*H*_{equ}), 3.96 (ddt, *J* =

1
2
3 10.6/9.2/3.3 Hz, 1H, 3-*H*), 4.60 (dddd, $J = 49.9/9.0/5.7/4.3$ Hz, 1H, CH₂CH₂F), 4.78
4 (dtd, $J = 47.4/9.1/4.4$ Hz, 1H, CH₂CH₂F), 7.06 (d, $J = 7.3$ Hz, 1H, 5-*H*), 7.09 – 7.16
5 (m, 5H, 6-*H*, 1-*H*_{benzannulene}, 2-*H*_{benzannulene}, 3-*H*_{benzannulene}, 4-*H*_{benzannulene}), 7.16 – 7.22
6 (m, 2H, 7-*H*, 8-*H*). A signal for the NH proton is not observed in the spectrum. ¹³C
7 NMR (101 MHz, CDCl₃): δ (ppm) = 26.4 (1C, C-3'), 26.6 (1C, C-5'), 29.2 (1C, C-6'),
8 32.7 (2C, C-5_{benzannulene}, C-9_{benzannulene}), 33.5 (1C, C-2'), 35.4 (2C, C-6_{benzannulene}, C-
9 8_{benzannulene}), 35.8 (1C, C-4), 37.1 (d, $J = 19.3$ Hz, 1C, CH₂CH₂F), 47.4 (1C, C-4'),
10 58.2 (1C, C-7_{benzannulene}), 63.8 (d, $J = 5.1$ Hz, 1C, C-3), 76.0 (1C, C-1), 81.1 (d, $J =$
11 163.7 Hz, 1C, CH₂CH₂F), 125.5 (1C, C-8), 126.1 (1C, C-6), 126.3 (3C, C-7, C-
12 2_{benzannulene}, C-3_{benzannulene}), 128.7 (1C, C-5), 129.0 (2C, C-1_{benzannulene}, C-4_{benzannulene}),
13 133.3 (1C, C-4a), 142.8 (2C, C-4a_{benzannulene}, C-9a_{benzannulene}), 143.3 (1C, C-8a). FT-IR
14 (neat): ν [cm⁻¹] = 3318 (N-H), 2924, 2843 (C-H_{alkyl}), 1489, 1450 (C=C_{arom}). Purity
15 (HPLC): 96.8 %, $t_R = 21.3$ min.
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32

33
34 **7.2.46. *trans*-3-(2-Fluoroethyl)-N-(6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-7-
35 yl)-3,4-dihydrospiro[[2]benzopyran-1,1'-cyclohexan]-4'-amine (30b)**
36

37
38 A solution of ketone **26** (25 mg, 0.15 mmol), amine **29b** (43 mg, 0.16 mmol, 1.1 eq),
39 acetic acid (10 μ L, 0.18 mmol, 1.1 eq) and NaBH(OAc)₃ (59 mg, 0.28 mmol, 1.9 eq)
40 in CH₂Cl₂ (3 mL) was stirred under N₂ atmosphere at rt. After 22 h, 1 M NaOH (5 mL)
41 was added and the aqueous layer was extracted with CH₂Cl₂ (3 x 10 mL). The
42 combined organic layers were dried (Na₂SO₄), filtered, concentrated in vacuo and the
43 residue was purified by fc (d = 2 cm, l = 20 cm, V = 10 mL, cyclohexane/ethyl acetate
44 80:20 + 1 % *N,N*-dimethylethanamine \rightarrow 67:33 + 1 % *N,N*-dimethylethanamine).
45 Colorless oil, yield 49 mg (81 %). C₂₇H₃₄FNO (407.6 g/mol). $R_f = 0.47$
46 (cyclohexane/ethyl acetate 50:50 + 1 % *N,N*-dimethylethanamine). HR-MS (APCI):
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 $m/z = 408.2656$ (calcd. 408.2697 for $C_{27}H_{35}FNO$ [MH^+]). 1H NMR (400 MHz, $CDCl_3$):
4
5 δ (ppm) = 1.28 – 1.39 (m, 2H, 6- $H_{benzannulene}$, 8- $H_{benzannulene}$), 1.44 – 1.55 (m, 1H, 3'- H),
6
7 1.60 (td, $J = 13.7/3.2$ Hz, 1H, 2'- H), 1.65 – 1.75 (m, 1H, 5'- H), 1.75 – 1.85 (m, 3H, 3'-
8
9 H , 5'- H , 6'- H), 1.85 – 2.10 (m, 3H, 6'- H , CH_2CH_2F), 2.10 – 2.23 (m, 3H, 2'- H , 6-
10
11 $H_{benzannulene}$, 8- $H_{benzannulene}$), 2.66 (dd, $J = 15.8/3.0$ Hz, 1H, 4- H), 2.70 – 2.88 (m, 6H, 4-
12
13 H , 4'- H_{ax} , 5- $H_{benzannulene}$, 9- $H_{benzannulene}$), 2.92 – 3.02 (m, 1H, 7- $H_{benzannulene}$), 3.95 (ddt,
14
15 $J = 11.7/8.8/3.4$ Hz, 1H, 3- H), 4.65 (ddt, $J = 46.7/9.0/5.5/4.5$ Hz, 1H, CH_2CH_2F), 4.85
16
17 (dtd, $J = 47.5/9.0/4.4$ Hz, 1H, CH_2CH_2F), 7.07 (d, $J = 7.3$ Hz, 1H, 5- H), 7.09 – 7.16
18
19 (m, 6H, 6- H , 8- H , 1- $H_{benzannulene}$, 2- $H_{benzannulene}$, 3- $H_{benzannulene}$, 4- $H_{benzannulene}$), 7.16 –
20
21 7.22 (m, 1H, 7- H). A signal for the NH proton is not observed in the spectrum. ^{13}C
22
23 NMR (101 MHz, $CDCl_3$): δ (ppm) = 29.6 (1C, C-3' or C-5'), 29.7 (1C, C-3' or C-5'),
24
25 32.6 (2C, C-5 $_{benzannulene}$, C-9 $_{benzannulene}$), 34.5 (1C, C-2'), 35.2 (2C, C-6 $_{benzannulene}$, C-
26
27 8 $_{benzannulene}$), 35.7 (1C, C-4), 37.0 (d, $J = 19.3$ Hz, 1C, CH_2CH_2F), 38.9 (1C, C-6'), 53.3
28
29 (1C, C-4'), 58.0 (1C, C-7 $_{benzannulene}$), 64.1 (d, $J = 4.8$ Hz, 1C, C-3), 75.4 (1C, C-1), 81.1
30
31 (d, $J = 163.6$ Hz, 1C, CH_2CH_2F), 125.1 (1C, C-8), 126.2 (1C, C-6 or C-7), 126.27 (1C,
32
33 C-6 or C-7), 126.32 (2C, C-2 $_{benzannulene}$, C-3 $_{benzannulene}$), 128.9 (1C, C-5), 129.0 (2C, C-
34
35 1 $_{benzannulene}$, C-4 $_{benzannulene}$), 133.7 (1C, C-4a), 142.5 (1C, C-8a), 142.7 (2C, C-
36
37 4a $_{benzannulene}$, C-9a $_{benzannulene}$). FT-IR (neat): ν [cm^{-1}] = 3059 (N-H), 2924, 2847 (C- H_{alkyl}),
38
39 1489, 1447 (C=C $_{arom}$). Purity (HPLC): 98.8 %, $t_R = 21.4$ min.
40
41
42
43
44
45
46
47
48
49

50 7.3. X-Ray diffraction

51 For compound *trans*-**6e** data sets were collected with an APEX II CCD diffractometer.

52 For compound *cis*-**6f** data sets were collected with a D8 Venture Dual Source 100

1
2
3 CMOS diffractometer. Programs used: data collection: *APEX3* V2016.1-0 (Bruker
4 AXS Inc., **2016**);⁵⁸ cell refinement: *SAINT* V8.37A (Bruker AXS Inc., **2015**);⁵⁹ data
5 reduction: *SAINT* V8.37A (Bruker AXS Inc., **2015**);⁵⁹ absorption correction, *SADABS*
6 V2014/7 (Bruker AXS Inc., **2014**);⁶⁰ structure solution *SHELXT*-2015 (Sheldrick,
7 **2015**);⁶¹ structure refinement *SHELXL*-2015 (Sheldrick, **2015**)⁶¹ and graphics, *XP*
8 (Bruker AXS, **1998**).⁶² *R*-values are given for observed reflections, and *wR*² values
9 are given for all reflections.
10
11
12
13
14
15
16
17
18
19

20 **7.3.1. X-ray crystal structure analysis of *trans*-6e (dan8992)**

21
22 A colorless prism-like specimen of C₂₆H₃₃NO₄, approximate dimensions 0.200 mm x
23 0.200 mm x 0.230 mm, was used for the X-ray crystallographic analysis. The X-ray
24 intensity data were measured. A total of 1635 frames were collected. The total
25 exposure time was 24.64 hours. The frames were integrated with the Bruker SAINT
26 software package using a wide-frame algorithm. The integration of the data using a
27 monoclinic unit cell yielded a total of 34283 reflections to a maximum θ angle of
28 66.67° (0.84 Å resolution), of which 3931 were independent (average redundancy
29 8.721, completeness = 99.9%, *R*_{int} = 4.32%, *R*_{sig} = 2.24%) and 3521 (89.57%) were
30 greater than 2 σ (*F*²). The final cell constants of *a* = 13.7232(4) Å, *b* = 6.5580(2) Å, *c* =
31 24.7015(7) Å, β = 93.5540(10)°, volume = 2218.78(11) Å³, are based upon the
32 refinement of the XYZ-centroids of 9967 reflections above 20 σ (*I*) with 7.186° < 2 θ <
33 133.1°. Data were corrected for absorption effects using the multi-scan method
34 (SADABS). The ratio of minimum to maximum apparent transmission was 0.889. The
35 calculated minimum and maximum transmission coefficients (based on crystal size)
36 are 0.8600 and 0.8770. The structure was solved and refined using the Bruker
37 SHELXTL Software Package, using the space group *P2*_{1/n}, with *Z* = 4 for the formula
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

unit, $C_{26}H_{33}NO_4$. The final anisotropic full-matrix least-squares refinement on F^2 with 283 variables converged at $R1 = 3.88\%$, for the observed data and $wR2 = 9.74\%$ for all data. The goodness-of-fit was 1.043. The largest peak in the final difference electron density synthesis was $0.342 \text{ e}/\text{\AA}^3$ and the largest hole was $-0.203 \text{ e}/\text{\AA}^3$ with an RMS deviation of $0.040 \text{ e}/\text{\AA}^3$. On the basis of the final model, the calculated density was $1.268 \text{ g}/\text{cm}^3$ and $F(000)$, 912 e^- . CCDC number: 1855388.

7.3.2. X-ray crystal structure analysis of *cis*-6f (dan8995)

A colorless plate-like specimen of $C_{26}H_{33}NO_4$, approximate dimensions 0.094 mm x 0.279 mm x 0.327 mm, was used for the X-ray crystallographic analysis. The X-ray intensity data were measured. A total of 1121 frames were collected. The total exposure time was 16.57 hours. The frames were integrated with the Bruker SAINT software package using a wide-frame algorithm. The integration of the data using a monoclinic unit cell yielded a total of 24421 reflections to a maximum θ angle of 68.34° (0.83 \AA resolution), of which 4026 were independent (average redundancy 6.066, completeness = 99.5%, $R_{\text{int}} = 3.12\%$, $R_{\text{sig}} = 2.26\%$) and 3798 (94.34%) were greater than $2\sigma(F^2)$. The final cell constants of $a = 21.9660(9) \text{ \AA}$, $b = 6.2458(3) \text{ \AA}$, $c = 32.4538(13) \text{ \AA}$, $\beta = 99.1270(10)^\circ$, volume = $4396.1(3) \text{ \AA}^3$, are based upon the refinement of the XYZ-centroids of 9316 reflections above $20 \sigma(I)$ with $5.516^\circ < 2\theta < 136.6^\circ$. Data were corrected for absorption effects using the multi-scan method (SADABS). The ratio of minimum to maximum apparent transmission was 0.893. The calculated minimum and maximum transmission coefficients (based on crystal size) are 0.8080 and 0.9390. The structure was solved and refined using the Bruker SHELXTL Software Package, using the space group $C2/c$, with $Z = 8$ for the formula unit, $C_{26}H_{33}NO_4$. The final anisotropic full-matrix least-squares refinement on F^2 with

1
2
3 283 variables converged at $R1 = 3.56\%$, for the observed data and $wR2 = 8.99\%$ for
4
5 all data. The goodness-of-fit was 1.055. The largest peak in the final difference
6
7 electron density synthesis was $0.267 \text{ e}^-/\text{\AA}^3$ and the largest hole was $-0.215 \text{ e}^-/\text{\AA}^3$ with
8
9 an RMS deviation of $0.046 \text{ e}^-/\text{\AA}^3$. On the basis of the final model, the calculated
10
11 density was 1.280 g/cm^3 and $F(000)$, 1824 e^- . CCDC number: 1855389.
12
13
14
15
16

17 **7.4. *In Vitro* studies**

18 **7.4.1. Receptor binding studies**

19 **7.4.1.1. Materials**

20
21
22
23 Guinea pig brains and rat livers were commercially available (Harlan-Winkelmann,
24
25 Borchon, Germany). Homogenizers: Elvehjem Potter (B. Braun Biotech International,
26
27 Melsungen, Germany) and Soniprep[®] 150, MSE, London, UK). Centrifuges: Cooling
28
29 centrifuge Eppendorf 5424R (Eppendorf, Hamburg, Germany) and High-speed
30
31 cooling centrifuge model Sorvall[®] RC-5C plus (Thermo Fisher Scientific,
32
33 Langenselbold, Germany). Multiplates: standard 96 well multiplates (Diagonal,
34
35 Muenster, Germany). Shaker: self-made device with adjustable temperature and
36
37 tumbling speed (scientific workshop of the institute). Harvester: MicroBeta[®]
38
39 FilterMate 96 Harvester. Filter: Printed Filtermat Typ A and B. Scintillator: Meltilex[®]
40
41 (Typ A or B) solid state scintillator. Scintillation analyzer: MicroBeta[®] Trilux (all Perkin
42
43 Elmer LAS, Rodgau-Jügesheim, Germany).
44
45
46
47
48
49
50

51 **7.4.1.1.1. Preparation of membrane homogenates from guinea pig brain**

52
53
54 5 guinea pig brains were homogenized with the potter (500-800 rpm, 10 up and down
55
56 strokes) in 6 volumes of cold 0.32 M sucrose. The suspension was centrifuged at
57
58
59
60

1
2
3 1,200 x g for 10 min at 4 °C. The supernatant was separated and centrifuged at
4
5 23,500 x g for 20 min at 4 °C. The pellet was resuspended in 5-6 volumes of buffer
6
7 (50 mM TRIS, pH 7.4) and centrifuged again at 23,500 x g (20 min, 4 °C). This
8
9 procedure was repeated twice. The final pellet was resuspended in 5-6 volumes of
10
11 buffer and frozen (-80 °C) in 1.5 mL portions containing about 1.5 mg protein/mL.
12
13
14
15

16 **7.4.1.1.2. Preparation of membrane homogenates from rat liver**

17
18
19 Two rat livers were cut into small pieces and homogenized with the potter (500-
20
21 800 rpm, 10 up and down strokes) in 6 volumes of cold 0.32 M sucrose. The
22
23 suspension was centrifuged at 1,200 x g for 10 min at 4 °C. The supernatant was
24
25 separated and centrifuged at 31,000 x g for 20 min at 4 °C. The pellet was
26
27 resuspended in 5-6 volumes of buffer (50 mM TRIS, pH 8.0) and incubated at rt for
28
29 30 min. After the incubation, the suspension was centrifuged again at 31,000 x g for
30
31 20 min at 4 °C. The final pellet was resuspended in 5-6 volumes of buffer and stored
32
33 at -80 °C in 1.5 mL portions containing about 2 mg protein/mL.
34
35
36
37
38

39 **7.4.1.2. Protein determination**

40
41
42 The protein concentration was determined by the method of Bradford,⁶³ modified by
43
44 Stoscheck.⁶⁴ The Bradford solution was prepared by dissolving 5 mg of Coomassie
45
46 Brilliant Blue G 250 in 2.5 mL of EtOH (95 %, v/v). 10 mL deionized H₂O and 5 mL
47
48 phosphoric acid (85 %, m/v) were added to this solution, the mixture was stirred and
49
50 filled to a total volume of 50 mL with deionized water. The calibration was carried out
51
52 using bovine serum albumin as a standard in 9 concentrations (0.1, 0.2, 0.4, 0.6, 0.8,
53
54 1.0, 1.5, 2.0 and 4.0 mg /mL). In a 96 well standard multiplate, 10 µL of the
55
56
57
58
59
60

1
2
3 calibration solution or 10 μL of the membrane receptor preparation were mixed with
4
5 190 μL of the Bradford solution, respectively. After 5 min, the UV absorption of the
6
7 protein-dye complex at $\lambda = 595 \text{ nm}$ was measured with a plate reader (Tecan
8
9 Genios[®], Tecan, Crailsheim, Germany).
10

11 12 13 14 **7.4.1.3. General procedures for binding assays**

15
16 The test compound solutions were prepared by dissolving approximately 10 μmol
17
18 (usually 2-4 mg) of test compound in DMSO so that a 10 mM stock solution was
19
20 obtained. To obtain the required test solutions for the assay, the DMSO stock
21
22 solution was diluted with the respective assay buffer. The filtermats were presoaked
23
24 in 0.5 % aqueous polyethylenimine solution for 2 h at rt before use. All binding
25
26 experiments were carried out in duplicates in the 96 well multiplates. The
27
28 concentrations given are the final concentration in the assay. Generally, the assays
29
30 were performed by addition of 50 μL of the respective assay buffer, 50 μL of test
31
32 compound solution in various concentrations (10^{-5} , 10^{-6} , 10^{-7} , 10^{-8} , 10^{-9} and 10^{-10}
33
34 mol/L), 50 μL of the corresponding radioligand solution and 50 μL of the respective
35
36 receptor preparation into each well of the multiplate (total volume 200 μL). The
37
38 receptor preparation was always added last. During the incubation, the multiplates
39
40 were shaken at a speed of 500-600 rpm at the specified temperature. Unless
41
42 otherwise noted, the assays were terminated after 120 min by rapid filtration using
43
44 the harvester. During the filtration, each well was washed five times with 300 μL of
45
46 water. Subsequently, the filtermats were dried at 95 $^{\circ}\text{C}$. The solid scintillator was
47
48 melted on the dried filtermats at a temperature of 95 $^{\circ}\text{C}$ for 5 min. After solidifying of
49
50 the scintillator at rt, the trapped radioactivity in the filtermats was measured with the
51
52 scintillation analyzer. Each position on the filtermat corresponding to one well of the
53
54
55
56
57
58
59
60

1
2
3 multiplate was measured for 5 min with the [³H]-counting protocol. The overall
4
5 counting efficiency was 20 %. The *IC*₅₀ values were calculated with the program
6
7 GraphPad Prism[®] 3.0 (GraphPad Software, San Diego, CA, USA) by non-linear
8
9 regression analysis. Subsequently, the *IC*₅₀ values were transformed into *K*_i values
10
11 using the equation of Cheng and Prusoff.⁶⁵ The *K*_i values are given as mean value ±
12
13 SEM from three independent experiments.
14
15

16 17 18 **7.4.1.4. σ_1 receptor affinity**

19
20 The assay was performed with the radioligand [³H]-(+)-pentazocine (22.0 Ci/mmol;
21
22 Perkin Elmer). The thawed membrane preparation of guinea pig brain cortex (about
23
24 100 µg of the protein) was incubated with various concentrations of test compounds,
25
26 2 nM [³H]-(+)-pentazocine, and TRIS buffer (50 mM, pH 7.4) at 37 °C. The non-
27
28 specific binding was determined with 10 µM unlabeled (+)-pentazocine. The *K*_d value
29
30 of (+)-pentazocine is 2.9 nM.⁶⁶
31
32
33
34
35

36 37 38 **7.4.1.5. σ_2 receptor affinity**

39
40 The assay was performed with the radioligand [³H]di-*o*-tolyguanidine (specific activity
41
42 50 Ci/mmol; ARC, St. Louis, MO, USA). The thawed rat liver membrane preparation
43
44 (about 100 µg protein) was incubated with various concentrations of the test
45
46 compound, 3 nM [³H]di-*o*-tolyguanidine and buffer containing (+)-pentazocine
47
48 (500 nM (+)-pentazocine in TRIS buffer (50 mM TRIS, pH 8.0)) at rt. The non-specific
49
50 binding was determined with 10 µM non-labeled di-*o*-tolyguanidine. The *K*_d value of
51
52 di-*o*-tolyguanidine is 17.9 nM.⁶⁷
53
54
55

56 57 58 **7.5. Pain behavioral studies**

1
2
3 To evaluate the effect of drugs on mechanical allodynia induced by capsaicin, a
4 previously described experimental procedure was used.³⁴ The compound under
5 study or its solvent (HPMC) was administered s.c. to female CD-1 mice (Charles
6 River, Barcelona, Spain) 30 min before the intraplantar (i.pl.) administration of 20 μ L
7 capsaicin (1 μ g in 1% DMSO). 15 min after the i.pl. administration of capsaicin, a
8 mechanical punctate stimulation (0.5 g force) was applied with an electronic von Frey
9 device (Dynamic Plantar Aesthesiometer, Ugo Basile, Comerio, Italy) at least 5 mm
10 from the site of injection toward the toes (area of secondary mechanical
11 hypersensitivity), and the paw withdrawal latency time was automatically recorded.
12 Each mouse was tested in three trials at 30 s intervals and the mean of the 3
13 measurements was calculated. A cutoff time of 50 s was used in each trial.
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28

29 In the experiments to elucidate the influence of σ_1 receptors on the antiallodynic
30 effect of compound tested, the σ_1 receptor agonist PRE-084 (compound **35**) was
31 administered s.c. 5 minutes before the s.c. administration of the compound tested
32 and 30 min later (i.e.. 35 min after compound **35** administration) capsaicin was ipl
33 injected and the above-mentioned procedures were performed to measure
34 mechanical allodynia.
35
36
37
38
39
40
41
42
43

44 Animal care was provided in accordance with institutional (Research Ethics
45 Committee of the University of Granada, Granada, Spain), regional (Junta de
46 Andalucía, Spain), and international standards (European Communities Council
47 Directive 2010/63). The protocol of the experiments was approved by Junta de
48 Andalucía (Licence 04/09/2017/113). The degree of effect on capsaicin-induced
49 mechanical allodynia was calculated as: % Antiallodynic effect = [(LTD-LTS)/(CT-
50
51
52
53
54
55
56
57
58
59
60

1
2
3 LTS)] x 100 where LTD is the latency time for paw withdrawal in drug-treated
4 animals, LTS is the latency time in solvent-treated animals (mean value 12.03 s), and
5 CT is the cutoff time (50 s). The statistical significance of differences between values
6
7 obtained in the different experimental groups were analyzed with one-way analysis of
8 variance (ANOVA) followed by the Bonferroni test. The differences between means
9
10 were considered statistically significant when the value of P was below 0.05.
11
12
13
14
15
16
17

18 All animal experiments performed in the manuscript were conducted in compliance
19 with institutional guidelines. Licence number: 04/09/2017/113.
20
21
22
23
24
25

26 **Supporting Information**

27
28 Supporting Information contains purity data of all compounds. Moreover, ¹H and ¹³C
29 NMR spectra and HPLC chromatograms (purity) are included. Experimental details
30 of *in vitro* assays and Molecular Formula Strings are given.. This material is available
31 free of charge via the Internet at <http://pubs.acs.org>.
32
33
34
35
36
37
38
39
40

41 **Author Information**

42 Corresponding author

43
44 *Tel: +49-251-8333311. Fax: +49-8332144. Email: wuensch@uni-muenster.de
45
46
47
48
49
50
51

52 **Acknowledgments**

53
54
55
56
57
58
59
60

1
2
3 This work was supported by the Deutsche Forschungsgemeinschaft (DFG) and the
4 Cells-in-motion Cluster of Excellence (EXC, 1300-CiM), University of Münster,
5 Germany.
6
7
8
9

10 11 **Conflict of interests**

12
13 The authors declare no conflict of interest.
14
15
16

17 **Abbreviations Used**

18
19

20 APCI	atmospheric pressure chemical ionization
21	
22 d	diameter of the column
23	
24 DAST	diethylaminosulfur trifluoride
25	
26 DOR	δ opioid receptor
27	
28 DTG	1,3-di(o-tolyl)guanidine
29	
30 ER	endoplasmic reticulum
31	
32 fc	flash column chromatography
33	
34 HPMC	hydroxypropyl-methyl-cellulose
35	
36 IP ₃	Inositol trisphosphate
37	
38 i. pl.	intraplantar
39	
40 KOR	κ opioid receptor
41	
42 l	length of the stationary phase
43	
44 MAM	mitochondrion-associated endoplasmic reticulum membranes
45	
46 MOR	μ opioid receptor
47	
48 mTOR	mammalian target of Rapamycine
49	
50 NET	norepinephrine transporter
51	
52 NMDA	N-methyl-D-aspartate
53	
54	
55	
56	
57	
58	
59	
60	

1		
2		
3	PCP	phencyclidine
4		
5	p-TsOH	<i>p</i> -toluenesulfonic acid
6		
7	SEM	standard error of the mean
8		
9	TRIS	tris(hydroxymethyl)aminomethane
10		
11	V	fraction size
12		
13		
14		
15		
16		
17		
18		
19		
20		
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		
31		
32		
33		
34		
35		
36		
37		
38		
39		
40		
41		
42		
43		
44		
45		
46		
47		
48		
49		
50		
51		
52		
53		
54		
55		
56		
57		
58		
59		
60		

References

- (1) Martin, W. R.; Eades, C. G.; Thompson, J. A.; Huppler, R. E.; Gilbert, P. E. 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) Tam, S. W.; Cook, L. Sigma opiates and certain antipsychotic drugs mutually inhibit (+)-[3H] SKF 10,047 and [3H]haloperidol binding in guinea pig brain membranes, *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 5618–5621.
- (3) Tam, S. W. Naloxone-inaccessible sigma receptor in rat central nervous system, *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 6703–6707.
- (4) Bowen, W. D.; Hellewell, S. B.; McGarry, K. A. Evidence for a multi-site model of the rat brain sigma receptor, *Eur. J. Pharmacol.* **1989**, *163*, 309–318.
- (5) Quirion, R.; Bowen, W. D.; Itzhak, Y.; Junien, J. L.; Musacchio, J.; Rothman, R. B.; Tsung-Ping, S.; Tam, S.W.; Taylor, D. P. A proposal for the classification of sigma binding sites, *Trends Pharmacol. Sci.* **1992**, *13*, 85–86.
- (6) Maurice, T.; Roman, F. J.; Privat, A. Modulation by neurosteroids of the in vivo (+)-[3H]SKF-10,047 binding to sigma 1 receptors in the mouse forebrain, *J. Neurosci. Res.* **1996**, *46*, 734–743.
- (7) Maurice, T.; Junien, J. L.; Privat, A. Dehydroepiandrosterone sulfate attenuates dizocilpine-induced learning impairment in mice via sigma 1-receptors, *Behav. Brain Res.* **1997**, *83*, 159–164.
- (8) Romieu, P.; Martin-Fardon, R.; Bowen, W. D.; Maurice, T. Sigma 1 receptor-related neuroactive steroids modulate cocaine-induced reward, *J. Neurosci.* **2003**, *23*, 3572–3576.

- 1
2
3 (9) Fontanilla, D.; Johannessen, M.; Hajipour, A. R.; Cozzi, N. V.; Jackson, M. B.;
4
5 Ruoho, A. E. The hallucinogen N,N-dimethyltryptamine (DMT) is an endogenous
6
7 sigma-1 receptor regulator, *Science* **2009**, *323*, 934–937.
8
9 (10) Ruoho, A. E.; Chu, U. B.; Ramachandran, S.; Fontanilla, D.; Mavlyutov, T.;
10
11 Hajipour, A. R. The ligand binding region of the sigma-1 receptor: studies utilizing
12
13 photoaffinity probes, sphingosine and N-alkylamines, *Curr. Pharm. Des.* **2012**, *18*,
14
15 920–929.
16
17 (11) Hanner, M.; Moebius, F. F.; Flandorfer, A.; Knaus, H. G.; Striessnig, J.;
18
19 Kempner, E.; Glossmann, H. Purification, molecular cloning, and expression of the
20
21 mammalian sigma1-binding site, *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 8072–8077.
22
23 (12) Seth, P.; Leibach, F. H.; Ganapathy, V. Cloning and structural analysis of the
24
25 cDNA and the gene encoding the murine type 1 sigma receptor, *Biochem. Biophys.*
26
27 *Res. Commun.* **1997**, *241*, 535–540.
28
29 (13) Kekuda, R.; Prasad, P. D.; Fei, Y. J.; Leibach, F. H.; Ganapathy, V. Cloning
30
31 and functional expression of the human type 1 sigma receptor (hSigmaR1), *Biochem.*
32
33 *Biophys. Res. Com.* **1996**, *229*, 553–558.
34
35 (14) Hellewell, S. B.; Bruce, A.; Feinstein, G.; Orringer, J.; Williams, W.; Bowen, W.
36
37 D. Rat liver and kidney contain high densities of σ_1 and σ_2 receptors:
38
39 characterization by ligand binding and photoaffinity labeling, *Eur. J. Pharmacol. Mol.*
40
41 *Pharmacol.* **1994**, *268*, 9–18.
42
43 (15) Schmidt, H. R.; Zheng, S.; Gurpinar, E.; Koehl, A.; Manglik, A.; Kruse, A. C.
44
45 Crystal structure of the human σ_1 receptor, *Nature* **2016**, *532*, 527–530.
46
47 (16) Hayashi, T.; Su, T.-P. Sigma-1 receptor chaperones at the ER-mitochondrion
48
49 interface regulate Ca(2+) signaling and cell survival, *Cell* **2007**, *131*, 596–610.
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 (17) Weissman, A. D.; Su, T. P.; Hedreen, J. C.; London, E. D. Sigma receptors in
4 post-mortem human brains, *J. Pharmacol. Exp. Ther.* **1988**, *247*, 29–33.
5
6
7 (18) Samovilova, N. N.; Nagornaya, L. V.; Vinogradov, V. A. (+)-[3H]SKF 10,047
8 sites in rat liver, *Eur. J. Pharmacol.* **1988**, *147*, 259–264.
9
10
11 (19) Ela, C.; Barg, J.; Vogel, Z.; Hasin, Y.; Eilam, Y. Sigma receptor ligands
12 modulate contractility, Ca⁺⁺ influx and beating rate in cultured cardiac myocytes, *J.*
13 *Pharmacol. Exp. Ther.* **1994**, *269*, 1300–1309.
14
15
16 (20) Morin-Surun, M. P.; Collin, T.; Denavit-Saubié, M.; Baulieu, E.-E.; Monnet, F.
17 P. Intracellular σ_1 receptor modulates phospholipase C and protein kinase C
18 activities in the brainstem, *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 8196–8199.
19
20
21 (21) Hayashi, T.; Su, T.-P. Intracellular dynamics of sigma-1 receptors (sigma(1)
22 binding sites) in NG108-15 cells, *J. Pharmacol. Exp. Ther.* **2003**, *306*, 726–733.
23
24
25 (22) Tsai, S.-Y.; Hayashi, T.; Mori, T.; Su, T.-P. Sigma-1 Receptor Chaperones and
26 Diseases, *Cent. Nerv. Syst Agents Med. Chem.* **2009**, *9*, 184–189.
27
28
29 (23) Zhang, H.; Cuevas, J. Sigma receptors inhibit high-voltage-activated calcium
30 channels in rat sympathetic and parasympathetic neurons, *J. Neurophysiol.* **2002**, *87*,
31 2867–2879.
32
33
34 (24) Soriani, O.; Foll, F. L.; Roman, F.; Monnet, F. P.; Vaudry, H.; Cazin, L. A-
35 Current down-modulated by sigma receptor in frog pituitary melanotrope cells
36 through a G protein-dependent pathway, *J. Pharmacol. Exp. Ther.* **1999**, *289*, 321–
37 328.
38
39
40 (25) Soriani, O.; Le Foll, F.; Galas, L.; Roman, F.; Vaudry, H.; Cazin, L. The sigma-
41 ligand (+)-pentazocine depresses M current and enhances calcium conductances in
42 frog melanotrophs, *Am. J. Physiol.* **1999**, *277*, E73-80.
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 (26) Cheng, Z.-X.; Lan, D.-M.; Wu, P.-Y.; Zhu, Y.-H.; Dong, Y.; Ma, L.; Zheng, P.
4
5 Neurosteroid dehydroepiandrosterone sulphate inhibits persistent sodium currents in
6
7 rat medial prefrontal cortex via activation of sigma-1 receptors, *Exp. Neurol.* **2008**,
8
9 *210*, 128–136.
10
11 (27) Renaudo, A.; L'Hoste, S.; Guizouarn, H.; Borgèse, F.; Soriani, O. Cancer cell
12
13 cycle modulated by a functional coupling between sigma-1 receptors and Cl-
14
15 channels, *J. Biol. Chem.* **2007**, *282*, 2259–2267.
16
17 (28) Maurice, T.; Romieu, P. Involvement of the sigma1 receptor in the appetitive
18
19 effects of cocaine, *Pharmacopsychiatry* **2004**, *37 Suppl 3*, S198-207.
20
21 (29) Jin, J.-L.; Fang, M.; Zhao, Y.-X.; Liu, X.-Y. Roles of sigma-1 receptors in
22
23 Alzheimer's disease, *Int. J. Clin. Exp. Med.* **2015**, *8*, 4808–4820.
24
25 (30) Kulkarni, S. K.; Dhir, A. sigma-1 receptors in major depression and anxiety,
26
27 *Expert Rev. Neurother.* **2009**, *9*, 1021–1034.
28
29 (31) Niitsu, T.; Iyo, M.; Hashimoto, K. Sigma-1 receptor agonists as therapeutic
30
31 drugs for cognitive impairment in neuropsychiatric diseases, *Curr. Pharm. Des.* **2012**,
32
33 *18*, 875–883.
34
35 (32) Zamanillo, D.; Romero, L.; Merlos, M.; Vela, J. M. Sigma 1 receptor: a new
36
37 therapeutic target for pain, *Eur. J. Pharmacol.* **2013**, *716*, 78–93.
38
39 (33) Jensen, T. S.; Finnerup, N. B. Allodynia and hyperalgesia in neuropathic pain:
40
41 clinical manifestations and mechanisms, *Lancet Neurol.* **2014**, *13*, 924–935.
42
43 (34) Entrena, J. M.; Cobos, E. J.; Nieto, F. R.; Cendán, C. M.; Gris, G.; del Pozo,
44
45 E.; Zamanillo, D.; Baeyens, J. M. Sigma-1 receptors are essential for capsaicin-
46
47 induced mechanical hypersensitivity: studies with selective sigma-1 ligands and
48
49 sigma-1 knockout mice, *Pain* **2009**, *143*, 252–261.
50
51
52
53
54
55
56
57
58
59
60

1
2
3 (35) Díaz, J. L.; Cuberes, R.; Berrocal, J.; Contijoch, M.; Christmann, U.;
4
5 Fernández, A.; Port, A.; Holenz, J.; Buschmann, H.; Laggner, C.; Serafini, M. T.;
6
7 Burgueño, J.; Zamanillo, D.; Merlos, M.; Vela, J. M.; Almansa, C. Synthesis and
8
9 biological evaluation of the 1-arylpyrazole class of $\sigma(1)$ receptor antagonists:
10
11 identification of 4-{2-5-methyl-1-(naphthalen-2-yl)-1H-pyrazol-3-yloxyethyl}morpholine
12
13 (S1RA, E-52862), *J. Med. Chem.* **2012**, *55*, 8211–8224.

14
15 (36) Alon, A.; Schmidt, H. R.; Wood, M. D.; Sahn, J. J.; Martin, S. F.; Kruse, A. C.
16
17 Identification of the gene that codes for the σ_2 receptor, *Proc. Natl. Acad. Sci. U.S.A.*
18
19 **2017**, *114*, 7160–7165.

20
21 (37) Walker, J. M.; Bowen, W. D.; Walker, F. O.; Matsumoto, R. R.; Costa, B. de;
22
23 Rice, K. C. Sigma receptors: biology and function, *Pharmacol. Rev.* **1990**, *42*, 355–
24
25 402.

26
27 (38) Bowen, W. D. Sigma receptors: recent advances and new clinical potentials,
28
29 *Pharm. Acta. Helv.* **2000**, *74*, 211–218.

30
31 (39) Vilner, B. J.; Bowen, W. D. Modulation of cellular calcium by sigma-2
32
33 receptors: release from intracellular stores in human SK-N-SH neuroblastoma cells,
34
35 *J. Pharmacol. Exp. Ther.* **2000**, *292*, 900–911.

36
37 (40) Wilke, R. A.; Mehta, R. P.; Lupardus, P. J.; Chen, Y.; Ruoho, A. E.; Jackson,
38
39 M. B. Sigma receptor photolabeling and sigma receptor-mediated modulation of
40
41 potassium channels in tumor cells, *J. Biol. Chem.* **1999**, *274*, 18387–18392.

42
43 (41) Huang, Y.-S.; Lu, H.-L.; Zhang, L.-J.; Wu, Z. Sigma-2 receptor ligands and
44
45 their perspectives in cancer diagnosis and therapy, *Med. Res. Rev.* **2014**, *34*, 532–
46
47 566.

48
49 (42) Guitart, X.; Codony, X.; Monroy, X. Sigma receptors: biology and therapeutic
50
51 potential, *Psychopharmacology* **2004**, *174*, 301–319.

- 1
2
3 (43) van Waarde, A.; Rybczynska, A.; K. Ramakrishnan, N.; Ishiwata, K.; H.
4
5 Elsinga, P.; A.J.O. Dierckx, R. Sigma receptors in oncology: therapeutic and
6
7 diagnostic applications of sigma ligands, *Curr. Pharm. Des.* **2010**, *16*, 3519–3537.
8
9 (44) Berardi, F.; Abate, C.; Ferorelli, S.; Colabufo, N.; Perrone, R. 1-
10
11 Cyclohexylpiperazine and 3,3-dimethylpiperidine derivatives as sigma-1 (σ_1) and
12
13 sigma-2 (σ_2) receptor ligands: a review, *Cent. Nerv. Syst. Agents Med. Chem.* **2009**,
14
15 *9*, 205–219.
16
17 (45) Perregaard, J.; Moltzen, E. K.; Meier, E.; Sanchez, C. sigma. Ligands with
18
19 Subnanomolar Affinity and Preference for the .sigma.2 Binding Site. 1. 3-(.omega.-
20
21 Aminoalkyl)-1H-indoles, *J. Med. Chem.* **1995**, *38*, 1998–2008.
22
23 (46) Mach, R. H.; Huang, Y.; Freeman, R. A.; Wu, L.; Vangveravong, S.; Luedtke,
24
25 R. R. Conformationally-flexible benzamide analogues as dopamine D3 and σ_2
26
27 receptor ligands, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 195–202.
28
29 (47) Maier, C. A.; Wünsch, B. Novel spiropiperidines as highly potent and subtype
30
31 selective σ -receptor ligands. Part 1., *J. Med. Chem.* **2002**, *45*, 438–448.
32
33 (48) Maier, C. A.; Wünsch, B. Novel σ receptor ligands. Part 2. SAR of
34
35 spiro[[2]benzopyran-1,4'-piperidines] and spiro[[2]benzofuran-1,4'-piperidines] with
36
37 carbon substituents in position 3, *J. Med. Chem.* **2002**, *45*, 4923–4930.
38
39 (49) Rack, E.; Fröhlich, R.; Schepmann, D.; Wünsch, B. Design, synthesis and
40
41 pharmacological evaluation of spirocyclic $\sigma(1)$ receptor ligands with exocyclic amino
42
43 moiety (increased distance 1), *Bioorg. Med. Chem.* **2011**, *19*, 3141–3151.
44
45 (50) Grosse Maestrup, E.; Wiese, C.; Schepmann, D.; Hiller, A.; Fischer, S.;
46
47 Scheunemann, M.; Brust, P.; Wünsch, B. Synthesis of spirocyclic sigma1 receptor
48
49 ligands as potential PET radiotracers, structure-affinity relationships and in vitro
50
51 metabolic stability, *Bioorg. Med. Chem.* **2009**, *17*, 3630–3641.
52
53
54
55
56
57
58
59
60

- 1
2
3 (51) Oberdorf, C.; Schepmann, D.; Vela, J. M.; Diaz, J. L.; Holenz, J.; Wünsch, B.
4 Thiophene bioisosteres of spirocyclic sigma receptor ligands. 1. N-substituted
5 spiro[piperidine-4,4'-thieno[3,2-c]pyrans], *J. Med. Chem.* **2008**, *51*, 6531–6537.
6
7
8 (52) Schläger, T.; Schepmann, D.; Würthwein, E.-U.; Wünsch, B. Synthesis and
9 structure-affinity relationships of novel spirocyclic sigma receptor ligands with
10 furopyrazole structure, *Bioorg. Med. Chem.* **2008**, *16*, 2992–3001.
11
12
13 (53) Bruckner, R.; Wender, P. A.; Beifuss, K.; Zettlmeier, W. *Organic mechanisms*;
14 Springer-Verlag: Berlin, Heidelberg, **2010**.
15
16 (54) Rack, E. *Struktur-Affinitäts- und Selektivitätsuntersuchungen spirocyclischer*
17 *Sigma-Rezeptorliganden mit exocyclischer Aminfunktion (doctoral dissertation)*:
18 Münster, **2010**.
19
20 (55) Søyby, K. K.; Mikkelsen, J. D.; Meier, E.; Thomsen, C. Lu 28-179 labels a σ_2 -
21 site in rat and human brain, *Neuropharmacology* **2002**, *43*, 95–100.
22
23 (56) Matsumoto, R. R.; McCracken, K. A.; Friedman, M. J.; Pouw, B.; Costa, B. R.
24 de; Bowen, W. D. Conformationally restricted analogs of BD1008 and an antisense
25 oligodeoxynucleotide targeting σ_1 receptors produce anti-cocaine effects in mice,
26 *Eur. J. Pharmacol.* **2001**, *419*, 163–174.
27
28 (57) Maurice, T.; Su, T.-P.; Parish, D. W.; Nabeshima, T.; Privat, A. PRE-084, a σ
29 selective PCP derivative, attenuates MK-801-induced impairment of learning in mice,
30 *Pharmacol. Biochem. Behav.* **1994**, *49*, 859–869.
31
32 (58) Bruker AXS Inc. APEX3, *Madison, Wisconsin, USA* **2016**.
33
34 (59) Bruker AXS Inc. SAINT, *Madison, Wisconsin, USA* **2015**.
35
36 (60) Bruker AXS Inc. SADABS, *Madison, Wisconsin, USA* **2014**.
37
38 (61) Sheldrick, G. M. SHELXT - integrated space-group and crystal-structure
39 determination, *Acta Crystallogr. A Found. Adv.* **2015**, *71*, 3–8.
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 (62) Bruker AXS Inc. XP-Interactive molecular graphics, Version 5.1, *Madison,*
4 *Wisconsin, USA 1998.*
5
6
7 (63) Bradford, M. M. A rapid and sensitive method for the quantitation of microgram
8 quantities of protein utilizing the principle of protein-dye binding, *Anal. Biochem.*
9 **1976**, 72, 248–254.
10
11
12
13 (64) Stoscheck, C. M. Quantitation of protein, *Methods Enzymol.* **1990**, 182, 50–68.
14
15 (65) Yung-Chi, C.; Prusoff, W. H. Relationship between the inhibition constant (KI)
16 and the concentration of inhibitor which causes 50 per cent inhibition (I50) of an
17 enzymatic reaction, *Biochem. Pharmacol.* **1973**, 22, 3099–3108.
18
19
20
21
22 (66) DeHaven-Hudkins, D. L.; Fleissner, L. C.; Ford-Rice, F. Y. Characterization of
23 the binding of [3H](+)-pentazocine to σ recognition sites in guinea pig brain, *Eur. J.*
24 *Pharmacol. Mol. Pharmacol.* **1992**, 227, 371–378.
25
26
27
28 (67) Mach, R. H.; Smith, C. R.; Childers, S. R. Ibogaine possesses a selective
29 affinity for σ_2 receptors, *Life Sci.* **1995**, 57, PL57-PL62.
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Table of Contents Graphic

