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Tetrahydro-3-benzazepines with fluorinated side chains as NMDA and σ_1 receptor antagonists: Synthesis, receptor affinity, selectivity and antiallodynic activity

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Abstract

The class of tetrahydro-1*H*-3-benzazepines was systematically modified in 1-, 3- and 7-position. In particular, a F-atom was introduced in β- or γ-position of the 4-phenylbutyl side chain in 3-position. Ligands with the F-atom in γ-position possess higher GluN2B affinity than analogs bearing the F-atom in β-position. This effect was attributed to the reduced basicity of β-fluoro amines. 3-Benzazepines with a benzylic OH moiety show moderate GluN2B affinity, but considerable selectivity over the σ_2

receptor. However, removal of the benzylic OH moiety led to increased GluN2B affinity, but reduced GluN2B/σ_2 selectivity. With respect to GluN2B affinity the phenol **17b** with a γ-fluorophenylbutyl moiety in 3-position represents the most interesting fluorinated ligand (K_i(GluN2B) = 16 nM). Most of the synthesized ligands reveal either similar GluN2B and σ_1 affinity or higher σ_1 affinity than GluN2B affinity. The methyl ether **16b** shows high σ_1 affinity (K_i(σ_1) = 6.6 nM) and high selectivity over a broad panel of receptors and transporters. The high antiallodynic activity in the mouse capsaicin assay proved the σ_1 antagonistic activity of **16b**.

Keywords

NMDA receptor, GluN2B subunit selective NMDA receptor antagonist, fluorinated side chain, tetrahydro-3-benzazepines, structure activity relationships, receptor selectivity, PET, σ_1 receptor selectivity, antiallodynic activity.

1. Introduction

N-Methyl-D-aspartate (NMDA) receptors are indispensable as a control unit for the glutamatergic network in the central nervous system (CNS), keeping the excitatory neurotransmission balanced.[1] Upon voltage-dependent activation of NMDA receptors by (*S*)-glutamate release, physiological Mg^{2+} blockade of the channel pore is unlocked and ions can pass. In contrast to the other ionotropic glutamate receptors (2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA) and kainate receptor), the NMDA receptor mediates influx of Ca^{2+} ions into neurons and therefore plays an important role in synaptic plasticity and thus in nervous system development, memory and learning.[2,3]

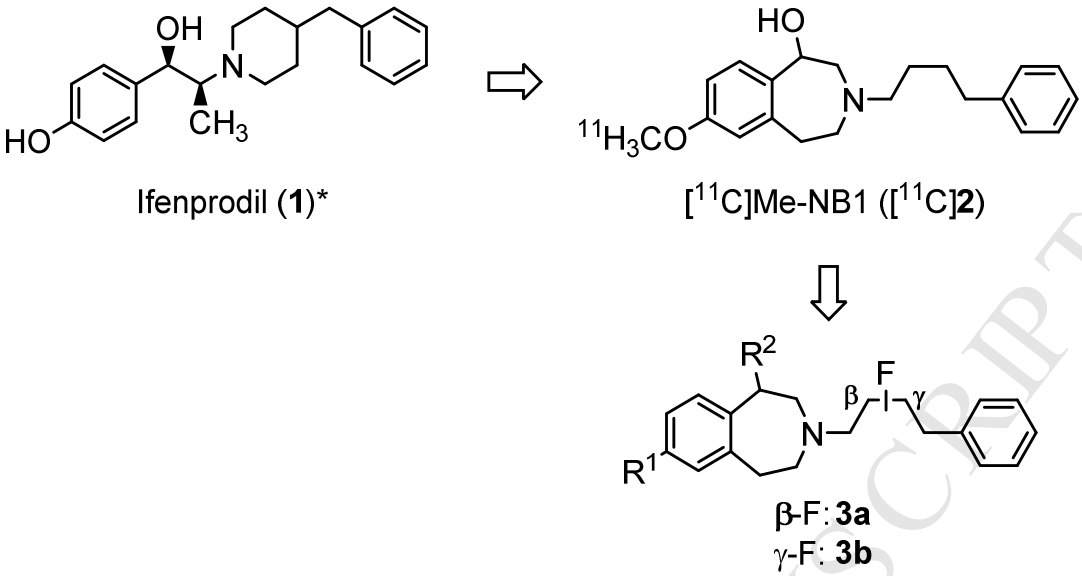
However, in addition to its positive effects on central nervous system development,

elevated glutamate concentrations in the synaptic cleft induce neurotoxic effects. Overactivation of NMDA receptors, resulting in an increased Ca^{2+} ion influx can lead finally to excitotoxicity being associated with severe cell damage and subsequent apoptosis of neurons. This process in turn is linked to the progression of neurodegenerative disorders like Alzheimer's and Parkinson's disease but also other CNS disorders such as depression, stroke/ischemia, neuropathic pain and alcohol withdrawal symptoms.[4,5,6]

Structurally, the heterotetrameric NMDA receptor is composed of two GluN1 and two GluN2 subunits. Four GluN2 subunits termed GluN2A-D subunits are known, which show a distinct expression and developmental pattern as well as a particular physiological role.[7] Therapeutically, selective addressing NMDA receptors with GluN2B subunit at the so called ifenprodil binding site, not present in subunits GluN2A, GluN2C and GluN2D, has gained increasing attention.[8] The therapeutic potential of GluN2B negative allosteric modulators with decreased cognitive side effects has promoted the development of ifenprodil-like drugs.

To verify their exclusive binding to the ifenprodil binding site, to exclude off-target binding *in vivo* and identify the required dose for receptor occupation, molecular imaging experiments with positron emission tomography (PET) are essential.[9] Therefore, high affinity GluN2B ligands, which can be radiolabeled, are of major interest. Despite considerable efforts, a radioligand for labeling the GluN2B subunit in NMDA receptors for human PET imaging studies is not yet available.[10] Very recently, carbon-11-labeled tetrahydro-3-benzazepin-1-ol [^{11}C]Me-NB1 (**2**), derived from ifenprodil (**1**), was successfully developed as GluN2B PET radiotracer without suffering from the common problems of already investigated known radioligands.

(Figure 1)[11]



Ifenprodil (**1**)*

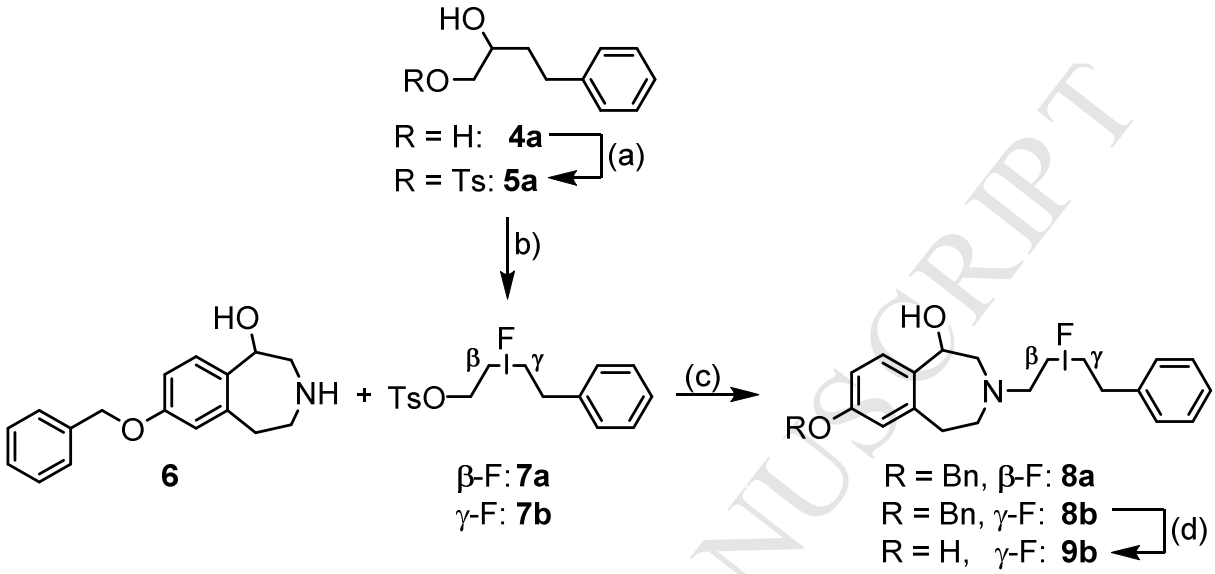
[^{11}C]Me-NB1 ([^{11}C]**2**)

β-F: **3a**
γ-F: **3b**

Figure 1. Lead structure ifenprodil (**1**), previously reported radiotracer [^{11}C]Me-NB1 ([^{11}C]**2**) and design of fluorinated 3-benzazepines **3a** and **3b**. *Ifenprodil represents the racemic *erythro* diastereomer.

To overcome the short physical half-life of carbon-11 (20.3 min), GluN2B ligands **3a**,**b** amenable for labeling with fluorine-18 (109.8 min) should be developed. In previous studies, introduction of fluorinated phenylbutyl and phenylpropyl side chains into different scaffolds was investigated to achieve high GluN2B affinity.[12,13] In these studies, tetrahydro-3-benzazepine emerged as promising scaffold for addressing the ifenprodil binding site in the GluN2B subunit. Therefore, we report herein on the systematic variation and structure-affinity relationship of 3-benzazepines bearing a F-atom in the side chain, which should be amenable for fluorine-18 labeling. To assess the impact of the position of the F-atom in the phenylbutyl side chain, the tetrahydro-3-benzazepine scaffold decorated with various substituents should be combined with fluorinated phenylbutyl side chains bearing the F-atom in β- or γ-position relative to the amino moiety. (Figure 1)

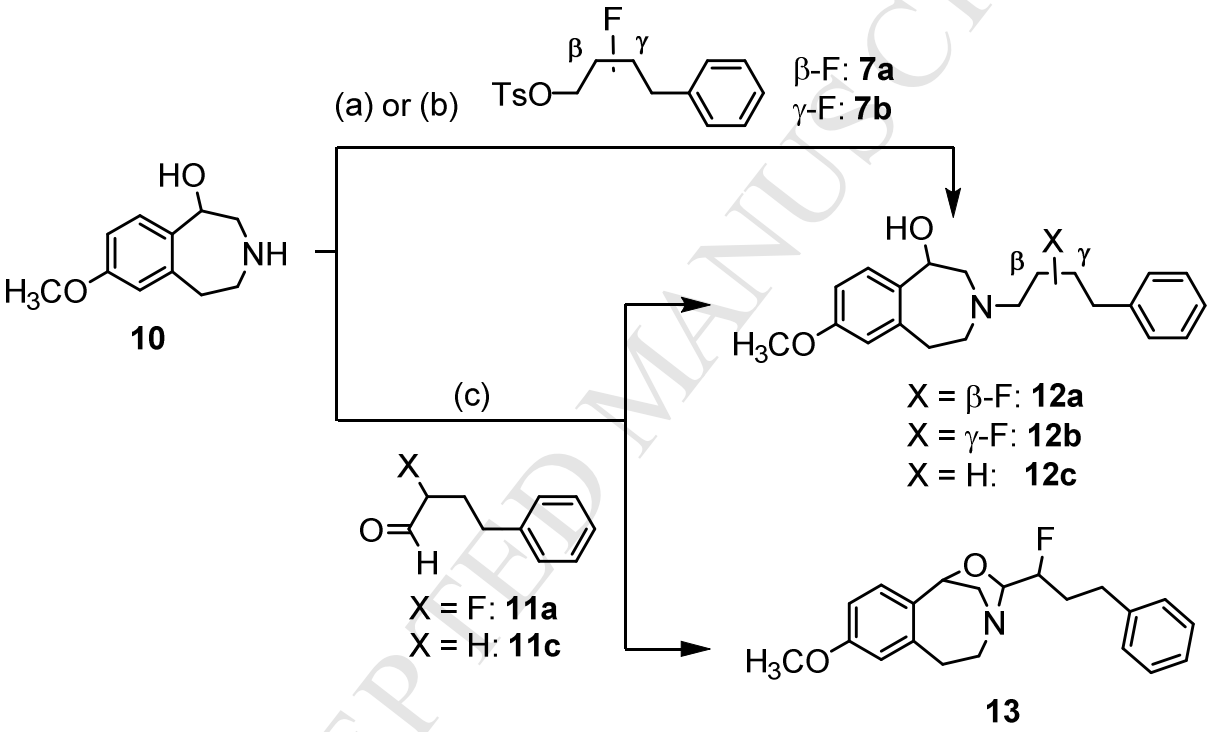
2. Synthesis



Scheme 1. Synthesis of tertiary amines **8a**, **8b** and **9b**. Reagents and reaction conditions: (a) *p*-TsCl, Et_3N, 4-DMAP, Bu_2SnO, CH_3CN:THF=1:1, 14 h, rt, 85 %.[14] (b) XtalFluor E®, Et_3N•3 HF, CH_2Cl_2, 17 h, -78 °C - rt, 72 %. (c) K_2CO_3, CH_3CN, 3 d, reflux, 14 % (**8a**), 82 % (**8b**). (d) H_2 (balloon), Pd/C, CH_3OH, rt, 3 h, 86 %.

The preparation of desired 3-benzazepines of type **3** with either a F-atom in β- or γ-position of the phenylbutyl side chain was envisaged via a nucleophilic substitution reaction. Therefore, fluorinated phenylbutyl tosylates **7a**, bearing the F-atom in β-position, and already described **7b**,[13] fluorinated in γ-position, were synthesized. (Scheme 1) Diol **4a**, obtained from 4-phenylbut-1-ene by a Sharpless dihydroxylation reaction with AD-mix, was regioselectively tosylated at the primary alcohol using Bu_2SnO to afford **5a**.[14] Subsequent deoxyfluorination of the secondary alcohol with (diethylamino)-difluorosulfonium tetrafluoroborate (XtalFluor E®) provided building block **7a**. To connect the fluorinated phenylbutyl side chains of **7a** and **7b** with benzylated 3-benzazepin-1-ol **6**[15,16], nucleophilic substitution reactions were

performed using K_2CO_3 as a base. Hence, tertiary amine **8b**, fluorinated in γ-position, was obtained in 86 % yield, whereas tertiary amine **8a**, with a F-atom in β-position was isolated in only 14 % yield, even after long reaction periods of up to three days. Thus, only benzyl ether **8b** was subjected to hydrogenolytic cleavage to provide the phenol **9b**. The 3-benzazepin-1-ols **8a**, **8b** and **9b** were obtained as 1:1-mixtures of diastereomers, which could not be separated by flash chromatography.



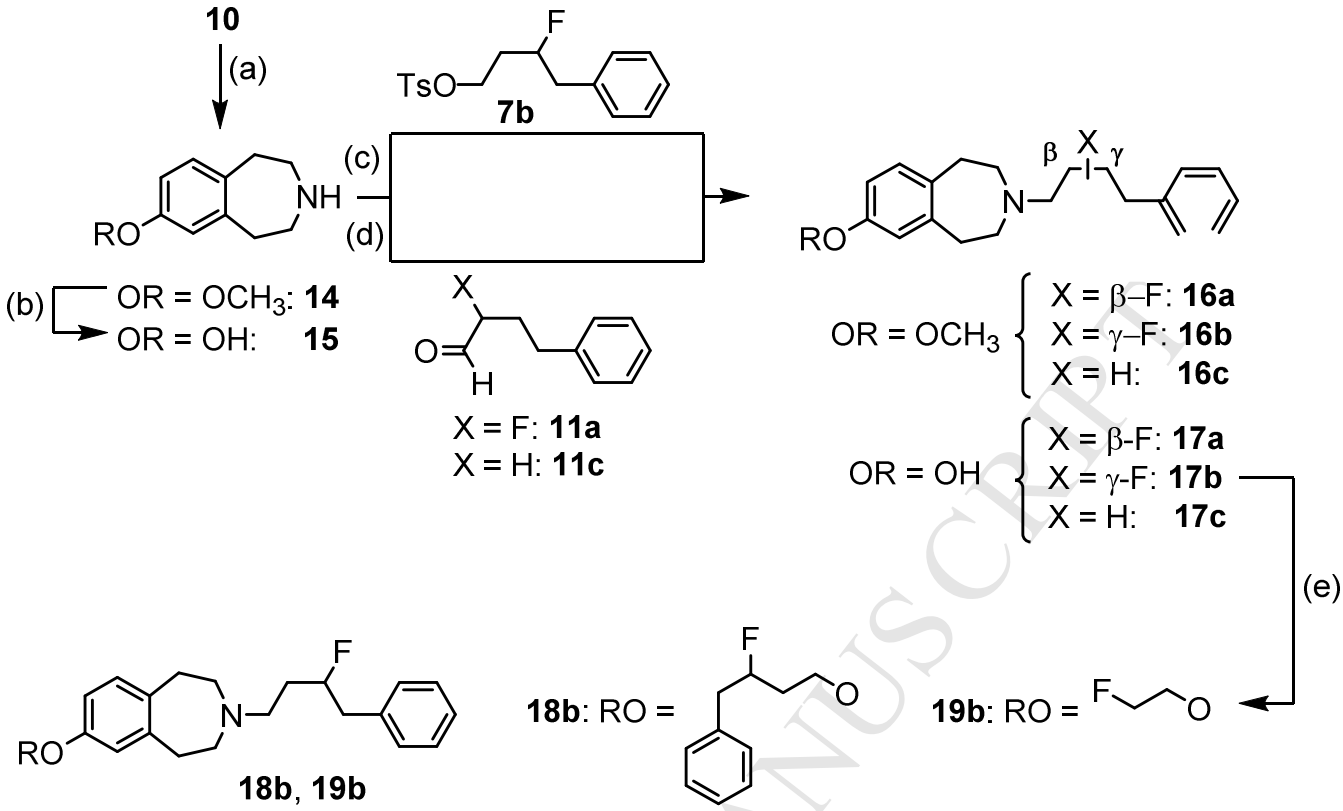
Scheme 2. Nucleophilic substitution (a,b) and reductive alkylation (c) for synthesis of tertiary amines **12a-12c**. Reagents and reaction conditions: (a) **7a**. K_2CO_3, CH_3CN, 4 d, reflux, 7 % (**12a**). (b) **7b**. K_2CO_3, CH_3CN, 20 h, reflux, 70 % (**12b**). (c) $NaBH(OAc)_3$, CH_2Cl_2, 15 h, rt, 12 % (**12a**), 62 % (**13**), 57 % (**12c**[15]).

Two reaction pathways were investigated to obtain the tertiary amine **12a** with a F-atom in β-position. (Scheme 2) As already described for secondary amine **6** (Scheme 1), substitution reaction of commercially available **10** with tosylate **7a**[13] provided the tertiary amine **12a** in only 7 % yield. This clearly indicates, that the F-

atom in β-position hinders the nucleophilic substitution reaction. This assumption is confirmed by the rather high yield (70 %) of fluorinated 3-benzazepinol **12b** prepared by nucleophilic substitution of secondary amine **10** with γ-fluorotosylate **7b**. Both **12a** and **12b** with F-atom in β- or γ-position exit as 1:1-mixtures of diastereomers.

In order to improve the yield of the β-fluoro derivative **12a** reductive alkylation of secondary amine **10** with β-fluoroaldehyd **11a**[13] and $NaBH(OAc)_3$[17] was performed. (Scheme 2) Although the β-fluoro derivative **12a** was obtained in slightly higher yield of 12 %, two pairs of diastereomeric N,O-acetals **13** were obtained as main products (62 %). The same reaction with non-fluorinated 4-phenylbutanal (**11c**) proves the participation of the F-atom in the formation of the N/O-acetal **13**, since in this case the desired tertiary amine **12c** was isolated as sole product in 57 % yield. The generation of acetalic compounds **13** is explained by intramolecular nucleophilic attack of benzylic alcohol of **10** on the intermediate iminium ion, favored by the electron withdrawing β-F-atom. The resulting N,O-acetals **13** could not be reduced by $NaBH(OAc)_3$.

In the next modification, the benzylic OH-moiety of **10** should be removed by reductive deoxygenation. Thus, treatment of alcohol **10** with Et_3SiH and $BF_3{\bullet}OEt_2$ provided 3-benzazepine **14** in 54 % yield. (Scheme 3) *N*-alkylation of secondary amine **14** by nucleophilic substitution reaction with tosylate **7b** yielded in γ-position fluorinated tertiary amine **16b**. 3-Benzazepine **16a** with the F-atom in β-position and the non-fluorinated phenylbutyl derivative **16c** were prepared by reductive alkylation with α-fluorobutanal **11a** and 4-phenylbutynal **11c**, respectively.

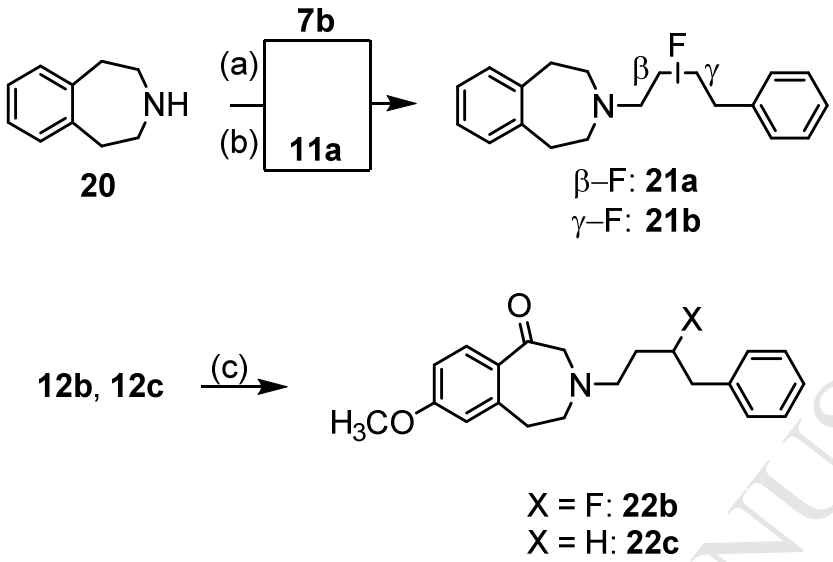


Scheme 3. Synthesis of 3-benzazepines by deoxygenation and ether cleavage. Reagents and reaction conditions: (a) Et_3SiH, BF_3•OEt_2, CH_2Cl_2, rt, 4 h, 54 %.[18] (b) 48 % HBr, 5 h, reflux, 87 %.[18] (c) **7b**, K_2CO_3, CH_3CN, 2-3 d, reflux, 80 % (**16b**); 28 % (**17b**), 14 % (**18b**). (d) $NaBH(OAc)_3$, CH_2Cl_2, 15-18 h, rt, 69 % (**16a**), 12 % (**16c**); 14 % (**17a**), 68 % (**17c**), (e) (2-Fluoroethyl) tosylate, K_2CO_3, DMF, 19 h, 80 °C, 45 %.

To obtain the corresponding phenols **17a-c**, demethylation of **14** was accomplished using 47 % HBr to afford phenol **15** in 78 % yield. (Scheme 2)[18] *N*-Alkylation of secondary amine **15** was performed by reductive alkylation with aldehydes **11a** and **11c** in the presence of $NaBH(OAc)_3$ and nucleophilic substitution with tosylate **7b**. In addition to the *N*-alkylated phenol **17b** (28 %) the double alkylated product **18b** was isolated in 14 % yield after reaction of phenol **15** with tosylate **7b**. (Scheme 3)

To introduce a further F-containing modification in 7-position, phenol **17b** was alkylated with (2-fluoroethyl) tosylate to afford the fluoroethyl ether **19b**. The late

stage fluoroethylation of phenol **17b** offers the possibility for radiofluorination at the very end of the synthesis by using ^{18}F-labeled (2-fluorethyl) tosylate. (Scheme 3)



Scheme 4. Synthesis of tertiary amines **21** and **22**. Reagents and reaction conditions: (a) K_2CO_3, CH_3CN, 3 d, reflux, 10 %. (b) $NaBH(OAc)_3$, CH_2Cl_2, 20 h, rt, 33 %.[13] (c) DMP, CH_2Cl_2, 1 h, rt, 25 % (**22b**), 32 % (**22c**).

Next, tetrahydro-3-benzazepines **21** without further substituents at the benzazepine scaffold were envisaged to investigate the impact of the different substituents in 7-position. For this purpose, commercially available tetrahydro-3-benazazepine **20** was reacted with fluorinated tosylate **7b** to provide **21b**. Reductive alkylation of secondary amine **20** with fluorinated aldehyde **11a** and $NaBH(OAc)_3$ resulted in tertiary amine **21a**.[13] Low yields of desired compounds **21a** and **21b** can be assigned to decomposition of **20** during storage and purification problems of final products. (Scheme 4)

In addition to 3-benzazepines without substituent in 1-position (**16**, **17**, **19**, **21**) and with an OH-moiety in 1-position (**8**, **9**, **12**) able to act as H-bond donor and acceptor,

an oxo group should be introduced in 1-position, which can only react as H-bond acceptor component. Thus benzylic alcohols **12b** and **12c**[15] were oxidized with Dess-Martin-Periodinane to provide the ketones **22b** and **22c**, respectively. (Scheme 4) Unexpectedly, the ketones showed a very fast decomposition, which inhibited the inclusion of the ketones into biological testing.

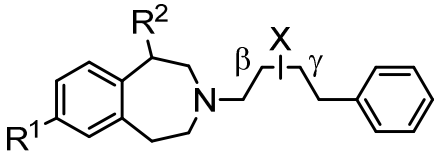
In order to investigate the effect of the configuration of the F-containing C-atom, the enantiomers of the γ-fluorophenol **17b** were separated by chiral HPLC (column Daicel Chiralpak® IA). According to this procedure, both enantiomers (-)-**17b** and (+)-17b were obtained with 98.6 and 99.8 % ee, respectively (for details see Supporting Information).

3. Receptor affinity

3.1. GluN2B affinity

The affinity towards NMDA receptors with GluN2B subunit (GluN2B affinity) of the synthesized 3-benzazepines and reference compounds was determined in a competitive radioligand binding assay, using tritium-labeled ifenprodil ([^{3}H]**1**) as radioligand. Selectivity for the GluN2B subunit was achieved by employing membrane preparations from a recombinant cell line stably expressing the GluN1a splice variant and the GluN2B subunit.[19] The results are summarized in Table 1.

Table 1. GluN2B, PCP, σ_1 and σ_2 receptor affinity of synthesized 3-benzazepines and reference compounds.



				K_i ± SEM [nM] (n=3)[a]			
compd.	R^1	R^2	X	GluN2B	PCP [%][b]	σ_1	σ_2
8a	OBn	OH	β-F	784	0	393	1400
8b	OBn	OH	γ-F	813	0	183	1800
8c[16,20]	OBn	OH	H	505 ± 170	>1 µM	293 ± 58	1050
9b	OH	OH	γ-F	422 ± 57	8	1400	130 ± 26
9c[16,20]	OH	OH	H	84 ± 18	35	194	18000
12a	OCH_3	OH	β-F	506	0	585	7900
12b	OCH_3	OH	γ-F	630	0	210	4500
12c[15,20]	OCH_3	OH	H	706 ± 100	22	182 ± 38	554 ± 27
13	OCH_3	N/O acetal	γ-F	8 %[c]	0	0 %[c]	0 %[c]
16a	OCH_3	H	β-F	602	0	213	740
16b	OCH_3	H	γ-F	120 ± 16	21	6.6 ± 2	115
16c	OCH_3	H	H	55 ± 10	0	11 ± 5	56 ± 1
17a	OH	H	β-F	86 ± 3	0	70 ± 13	153 ± 36
17b	OH	H	γ-F	16 ± 5	15	17 ± 5	39 ± 15
17c	OH	H	H	9.9 ± 3	nd	32 ± 8	12 ± 3
(-)-**17b**	OH	H	γ-F	65 ± 7	nd	25 ± 11	63 ± 8
(+)-**17b**	OH	H	γ-F	54 ± 11	nd	10 ± 2	49 ± 7
18b	$O(CH_2)_2CHFCH_2Ph$	H	γ-F	249	nd	185	643
19b	OCH_2CH_2F	H	γ-F	348	nd	25 ± 0.9	524
21a[13]	H	H	β-F	239 ± 11	0	108 ± 27	333
21b	H	H	γ-F	30 ± 9	0	8.0 ± 2.2	48 ± 9
21c[21]	H	H	H	227 ± 2	>10 µM	10 ± 8	35 ± 13
Ifenprodil (**1**)				10 ± 0.7	nd	125 ± 24	98 ± 34
Eliprodil				13 ± 2.5	nd	nd	Nd
dexoxadrol				nd	32 ± 7.4	nd	Nd
(+)-MK-801				nd	3.4 ± 0.8	nd	Nd
haloperidol				nd	nd	6.3 ± 1.6	78 ± 2.3
di-*o*-tolylguanidine				nd	nd	89 ± 29	57 ± 18

[a] K_i values of potent compounds were recorded three times (n = 3). For low-affinity compounds with K_i values at GluN2B-NMDA receptors > 150 nM, the competition curves were recorded only once (single value).

[b] Due to very low affinity towards the PCP binding site of the NMDA receptor, the replacement (in %) of the radioligand [^{3}H](+)-MK-801 from the PCP binding site at a test compound concentration of 1 µM is given.

[c] Due to very low affinity the replacement of the radioligands (in %) from the ifenprodil binding site, the σ_1 and σ_2 receptors is given.

nd indicates not determined.

Previous studies have shown that 3-benzazepines without phenolic OH moiety still interact with GluN2B containing NMDA receptors but with reduced affinity.[22] This finding is in good accordance with our results, since the highest GluN2B affinity was found for the phenolic 3-benzazepine **17c** (K_i = 9.9 nM). In general, various substituents (H, OH, OCH_3) in 7-position of the 3-benzazepine scaffold are well tolerated by the ifenprodil binding pocket, but compounds with a 7-OH moiety (**9c**, **17a**-**c**) result in the highest GluN2B affinity. Even compounds with larger substituents such as 2-fluoroethoxy (**19b**) and 4-phenylbutoxy groups (**18b**) show moderate GluN2B affinity with K_i values of 348 nM and 249 nM, respectively.

Regarding the position of the F-atom in the phenylbutyl side chain, 3-benzazepines fluorinated in γ-position relative to the N-atom reveal a remarkably increased GluN2B affinity compared to compounds bearing the F-atom in the adjacent β-position. This effect can nicely be observed for unsubstituted 3-benzazepines **21**. 3-Benzazepine **21b** (K_i = 30 nM) bearing the F-atom in γ-position is 8-fold more potent than 3-benzazepine **21a** (K_i = 239 nM) with the F-atom in β-position. Furthermore, shifting the F-atom from β- to γ-position resulted in 5-fold increased GluN2B affinity of methyl ethers **16a** and **16b**, as well as phenols **17a** and **17b**. The reduced GluN2B affinity of 3-benzazepines bearing the F-atom in β-position may be explained by a strong effect on the basicity of the cyclic amine. The basicity reducing effect is considerably lower

for γ-fluoro derivatiyes.[23] It can be concluded that the preferred position for the introduction of a F-atom in this compound class is the γ-position.

Removal of the benzylic OH moiety (1-OH group) led to increased GluN2B affinity. This effect is well demonstrated by the more active 3-benzazepines bearing the F-atom in γ-position of the side chain. As example, methyl ether **12b** with benzylic OH moiety shows a 5-fold lower GluN2B affinity (K_i = 630 nM) than methyl ether **16b** without benzylic OH moiety (K_i = 120 nM). An even stronger effect was observed for phenolic 3-benzazepines. Compound **17b** without benzylic OH moiety reveals a 26-fold higher GluN2B affinity (K_i = 16 nM) than in 1-position hydroxylated counterpart **9b** (K_i = 422 nM). Moderate to low GluN2B affinity was also observed for 3-benzazepines with benzylic OH moiety bearing the F-atom in β-position. As examples, β- and γ-fluorinated methyl ethers **12a** and **12b** (K_i = 506 and 630 nM) as well as benzyl ethers **8a** and **8b** (K_i = 784 and 813 nM) show almost the same GluN2B affinities.

Exemplarily, the enantiomers of the most promising fluorinated ligand **17b** were separated and tested. With K_i-values of 65 nM and 54 nM, both enantiomers (-)-**17b** and (+)-**17b** showed almost the same GluN2B affinity. This result led to the conclusion that the center of chirality bearing the small F-atom in γ-position of the 4-phenylbutyl side chain is not important for the interaction with the ifenprodil binding site of GluN2B-NMDA receptors.

Finally, this study demonstrated nicely that a F-atom in the phenylbutyl side chain, preferably in the γ-position, is well tolerated by GluN2B containing NMDA receptors. Therefore, the γ-CH_2 moiety of the phenylbutyl side chain represents a promising

position for radiofluorination, i.e. introduction of 18-F by nucleophilic substitution of an appropriate tosylate precursor. Introduction of a F-atom into the γ-position of the most potent 3-benzazepine **17c** (K_i = 9.9 nM) led to almost the same GluN2B affinity (K_i(**17b**) = 16 nM).

3.2. Receptor selectivity

To exclude the phencyclidine (PCP, 1-(1-phenylcyclohexyl)piperidine) binding site within the channel pore of the NMDA receptor as a potential competing binding site, PCP affinity was determined in competitive binding studies using the radioligand [^{3}H]-(+)-MK-801. Table 1 clearly reveals that the tested 3-benzazepines do not interact significantly with the phencyclidine binding site, indicating high selectivity for the ifenprodil binding site over the PCP binding site within the NMDA receptor.

Due to known cross reactivity of NMDA receptor ligands with both σ_1 and σ_2 receptors[24] and vice versa, affinity of synthesized 3-benzazepines towards σ_1 and σ_2 receptors was investigated. In the competitive binding assays [^{3}H]-(+)-pentazocine (σ_1) and [^{3}H]ditolylguanidine (σ_2) were used as radioligands.[25,26,27] The affinity data are summarized in Table 1.

With exception of phenol **9b**, 3-benzazepines bearing an OH moiety in benzyl position (1-position) reveal some selectivity against the σ_2 receptor. For the methyl ethers **12a** and **12b** the GluN2B/σ_2 selectivity factors are 15 and 7, respectively, whereas benzyl ethers **8a**-**c** show a twofold selectivity against σ_2 receptors. An opposed profile was observed for phenol **9b**, the only 3-benzazepine of this series displaying moderate σ_2 affinity (K_i = 130 nM) and selectivity over GluN2B (K_i = 422 nM) and σ_1 receptors (K_i = 1400 nM). Removal of the benzylic OH moiety

led to increased GluN2B and σ_2 affinity resulting finally in almost the same K_i values for both receptors indicating loss of GluN2B/σ_2 selectivity. Methyl ether **16c** (K_i(GluN2B) = 55 nM; $K_i(\sigma_2)$ = 56 nM) and phenol **17c** (K_i(GluN2B) = 9.9 nM; $K_i(\sigma_2)$ = 12 nM) are examples for high affinity but low selectivity GluN2B/ σ_2 ligands.

A comparable picture results from analysis of σ_1 affinity data. Only phenols **9b**, **9c** and **17c** reveal a 2-3-fold selectivity for GluN2B over σ_1 receptors. Removal of the OH moiety in 1-position increased the σ_1 affinity drastically and introduction of a F-atom in γ-position of the phenylbutyl side chain further increased the σ_1 receptor affinity. Thus, most of the synthesized compounds show either similar GluN2B and σ_1 affinities or considerably higher σ_1 affinity than GluN2B affinity. Methyl ether **16b** ($K_i(\sigma_1)$ = 6.6 nM), 2-fluoroethyl ether **19b** ($K_i(\sigma_1)$ = 5.0 nM) and unsubstituted 3-benzazepine **21b** ($K_i(\sigma_1)$ = 8.0 nM) with a F-atom in γ-position of the phenylbutyl side chain and without OH moiety in benzylic position show very high σ_1 affinity ($K_i(\sigma_1)$ < 10 nM) and selectivity over GluN2B and σ_2 receptors.

4. Pharmacological *in vitro* characterization of 16b and 21b

Due to their high σ_1 affinity and selectivity, the γ-fluorinated 3-benzazepines **16b** and **21b** were selected for further pharmacological characterization *in vitro*. The σ_1 receptor is found predominantly in the endoplasmic reticulum membrane and in mitochondria-associated membranes[28,29] Very recently, the σ_1 receptor crystal structure with various ligands has been solved by Kruse and coworkers.[30,31] It plays a central role in various neurological and neurodegenerative disorders including alcohol and drug (e.g. cocaine) dependence, depression, anxiety, Alzheimer`s, Parkinson's and Huntington's disease.[32,33,34,35,36,37] σ_1 Receptor antagonists (e.g. S1RA) are able to ameliorate neuropathic pain.[38,39,40] The high density of σ_1

receptors in various human tumor cell lines stimulates the development of σ_1 ligands for tumor therapy and diagnosis.[41,42]

Before detailed pharmacological evaluation, the chemical stability of 3-benzazepines **16b** and **21b** was determined. They were stored at pH 2 (aqueous HCl) and pH 7.4 (phosphate buffer). After 24 h, HPLC analysis did not reveal any decomposition products of **16b** and **21b** indicating high chemical stability under these conditions.

At first the selectivity of the σ_1 ligands **16b** and **21b** over a panel of further targets (receptors and transporters) was determined. As shown in Table 2, affinity towards *δ*-opioid (DOR) and *κ*-opioid (KOR) receptors was not found. However, 3-benzazepine **21b** showed a slight interaction with *μ*-opioid receptors (MOR), but could not activate MOR in the cAMP assay. At a concentration of 1 *μ*M, interactions of the 3-benzazepines **16b** and **21b** with 5-HT_{2B} and α_{1A} receptors, with serotonin (SERT), norepinephrine (NET) and dopamine transporters (DAT) could not be detected. However, **16b** and **21b** show slight affinity towards hERG channel and 5-HT_{1A} receptor. Altogether, 3-benzazepines **16b** and **21b** possess a desirable selectivity profile, especially compared to lead compound ifenprodil.

Table 2. Affinity of **16b** and **21b** towards various receptors and transporters.

	target	**16b**	**21b**
K_i [nM][a]	MOR	>1000	406
	DOR	>1000	>1000
	KOR	>1000	>1000
EC_{50} [nM][b]	MOR	>10000	>10000
IC_{50} [nM][c]	hERG	553	1183
% inhibition [1 μM][d]	SERT	<50	<50

NET	<50	<50
DAT	<50	<50
5-HT_{1A}	70	72
5-HT_{2B}	<50	<50
α_{1A}	<50	<50
H_1	<50	<50

[a] K_i values for opioid receptors.

[b] agonistic activity concerning the activation of MOR.

[c] inhibition of hERG K^+ channel determined by recording dose/response curves.

[d] Replacement of radioligands at a test compound concentration of 1 µM.

Since animal experiments were planned, metabolism of 3-benzazepines **16b** and **21b** was investigated. After incubation of 3-benzazepines **16b** and **21b** with human, mouse and rat liver microsomes for 1 h, both test compounds disappeared almost completely. Obviously, both 3-benzazepines underwent fast metabolism. Additionally, interactions of **16b** and **21b** with CYP1A2, 2C9, 2C19, 3A4 and 2D6 were evaluated. Only CYP2D6 was inhibited by both tertiary amines **16b** (55%) and **21b** (85 %). Cytotoxic effects of **16b** and **21b** were detected in the MTT and NR assays.

5. *In vivo* activity of 16b in mechanical allodynia assay

Since methyl ether **16b** possesses high σ_1 affinity (K_i = 6.6 nM) as well as a promising selectivity profile (Tables 1 and 2), its antiallodynic activity should be investigated in an *in vivo* experiment. It has been shown that σ_1 receptor antagonists are useful for the treatment of neuropathic pain such as allodynia.[40] In addition to preclinical evidence, σ_1 receptor knockout mice did not develop mechanical allodynia after intraplantar (ipl) capsaicin injection.[43]

Intraplantar administration of capsaicin provokes protective and defense reflexes up to 3 min after injection. After this period, hypersensitivity follows to thermal as well as

mechanical stimuli.[44] 30 min before capsaicin administration, 3-benzazepine **16b**, the σ_1 receptor antagonist BD-1063 (1-[2-(3,4-dichlorophenyl)ethyl]-4-methylpiperazine, **23**)[45] as positive control as well as the solvent hydroxypropylmethylcellulose (HPMC) as negative control were applied subcutaneously (sc) to mice. Subsequently, capsaicin-induced mechanical allodynia was evaluated by stimulation with electronic von-Frey device.[46] As depicted in Figure 2, 3-benzazepine **16b** shows an antiallodynic effect, which is stronger than the positive control BD-1063 (**23**). A statistically significant antiallodynic effect was found at a low dose of 8 mg/kg of **16b**, whereas 32 mg/kg were necessary in case of positive control **23**.

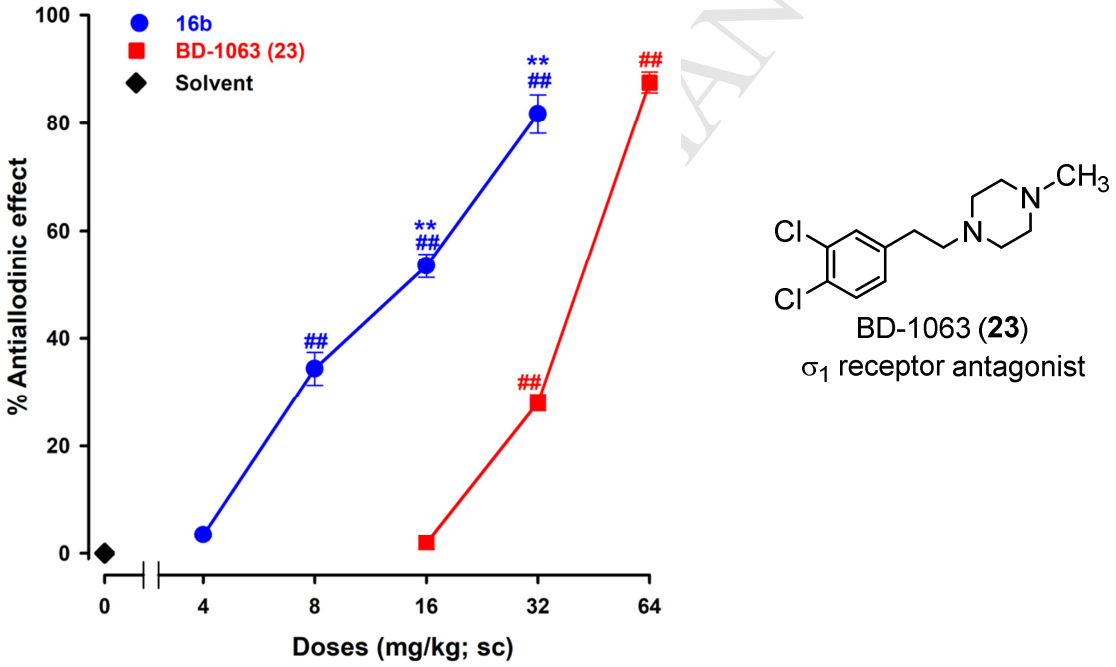


Figure 2. Effect of fluorinated 3-benzazepine **16b** and reference compound BD-1063 (**23**) on mechanical allodynia induced by ipl administration of capsaicin in mice. Animals were treated sc with **16b**, **23** or the solvent (HPMC) 30 min prior to capsaicin injection. Each bar and vertical line represents the mean ± 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 **16b** or **23**, in comparison with control animals (HPMC-treated, dose 0) value ($^{\#\#}P < 0.01$) and between the values obtained in mice treated with the same

dose of **16b** and **23** (**P < 0.01).

In order to show that the antiallodynic effect of **16b** results from inhibition of σ_1 receptors, mice were pretreated with the σ_1 receptor agonist PRE-084 (2-(morpholin-4-yl)ethyl 1-phenylcyclohexane-1-carboxylate, **24**, 32 mg/kg)[47] and the experiment was conducted once more as described above. As shown in Figure 3, the antiallodynic effect of 3-benzazepine **16b** up to a dose of 16 mg/kg was antagonized completely by PRE-084 (**24**). However, PRE-084 (**24**) was not able to antagonize completely the antiallodynic effect of 3-benzazepine **16b** at the highest dose of 32 mg/kg. The observed shift to the right of the dose-response curve of **16b** proves unequivocally the correlation between σ_1 receptor affinity and the identified antiallodynic effect.

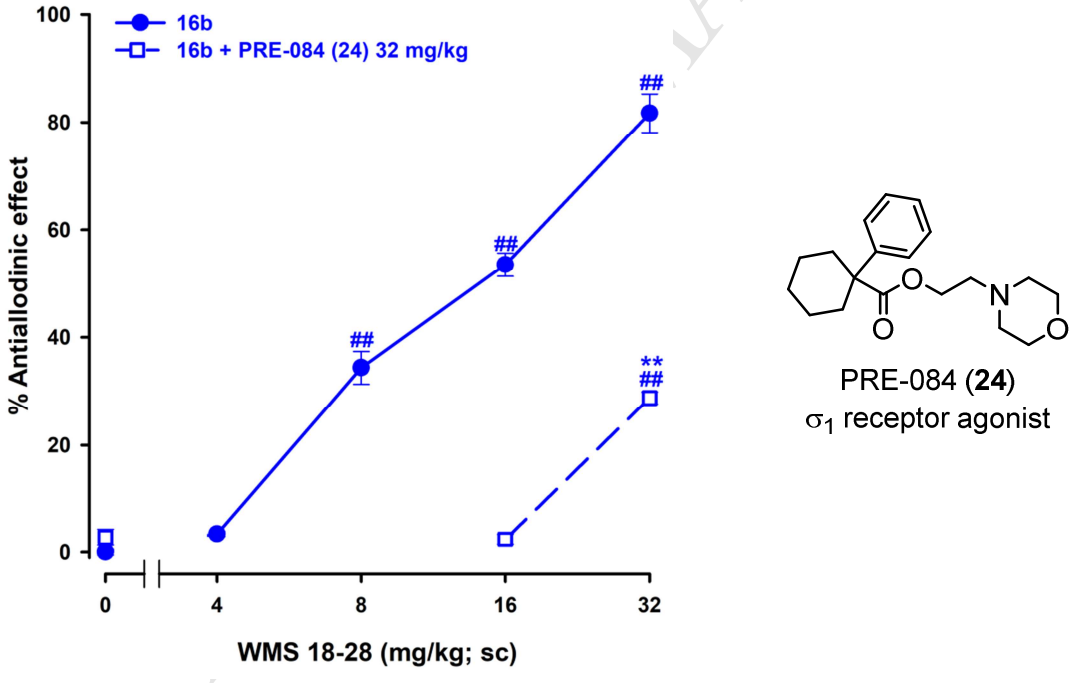


Figure 3. Effect of fluorinated 3-benzazepine **16b** alone and in combination with σ_1 receptor agonist PRE-084 (**24**) on mechanical allodynia induced by ipl administration of capsaicin to mice. Animals were treated sc with **24** 5 min before administration of **16b**, which was administered sc 30 min before capsaicin. Each bar and vertical line represents the mean ± 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 **16b** or its combination with **24**, in comparison with control animals (dose 0) value ($^{\#\#}P < 0.01$) and between the values obtained in mice treated with 16b and 16b + 23 (**P < 0.01).

6. Conclusion

A series of 3-benzazepines with 4-phenylbutyl substituents in 3-position bearing a F-atom in either β- or γ-position was synthesized. The impact of the F-atom and the substitution pattern on affinity and selectivity was evaluated in various pharmacological assays. Phenolic 3-benzazepine **17b** bearing the F-atom in γ-position reveals the most promising GluN2B affinity (K_i = 16 nM). In general, 3-benzazepines with benzylic OH moiety show reduced GluN2B affinity compared to 3-benzazepines without OH-moiety in 1-position. Removal of benzylic OH moiety resulted in increased GluN2B affinity, but loss of selectivity over the σ_2 receptor. Unfortunately, the 3-benzazepines do not exhibit selectivity over σ_1 receptors.

On the other side, some of the fluorinated 3-benzazepines prefer σ_1 receptor over GluN2B receptors with a selectivity factor of up to 18-fold for **16b**. The pharmacological properties and selectivity profile of **16b** were further evaluated in *in vitro* studies. In a mouse model, 3-benzazeine **16b** showed high antiallodynic activity, which was attributed unequivocally to σ_1 receptor antagonism.

7. Experimental

7.1. Chemistry, General

Unless otherwise noted, moisture sensitive reactions were conducted under dry nitrogen. Acetonitrile was dried over molecular sieves (3 Å). CH_2Cl_2 was distilled over CaH_2. THF was distilled over sodium/benzophenone. Thin layer chromatography (tlc): Silica gel 60 F_{254} plates (Merck). Flash chromatography (fc): Silica gel 60, 40–64

µm (Merck); parentheses include: diameter of the column (d), fraction size (v), eluent, R_f value. Melting point: Melting point apparatus Mettler Toledo MP50 Melting Point System, uncorrected. MS: microTOF-Q II (Bruker Daltonics); APCI, atmospheric pressure chemical ionization. IR: FT-IR spectrophotometer MIRacle 10 (Shimadzu) equipped with ATR technique. Nuclear magnetic resonance (NMR) spectra were recorded on Agilent 600-MR (600 MHz for ^{1}H, 151 MHz for ^{13}C) or Agilent 400-MR spectrometer (400 MHz for ^{1}H, 101 MHz for ^{13}C); δ in ppm related to tetramethylsilane and measured referring to $CHCl_3$ (δ = 7.26 ppm (^{1}H NMR) and δ = 77.2 ppm (^{13}C NMR)) and CHD_2OD (δ = 3.31 ppm (^{1}H NMR) and δ = 49.0 ppm (^{13}C NMR)); trichlorofluoromethane (CCl_3F) was used as reference compound in ^{19}F NMR spectroscopy; coupling constants are given with 0.5 Hz resolution; the assignments of ^{13}C and ^{1}H NMR signals were supported by 2-D NMR techniques where necessary. HPLC: Merck Hitachi Equipment; UV detector: L-7400; autosampler: L-7200; pump: L-7100; degasser: L-7614; column: LiChrospher® 60 RP-select B (5 µm); LiChroCART® 250-4 mm cartridge; flow rate: 1.0 mL/min; injection volume: 5.0 µL; detection at λ = 210 nm; solvents: A: water with 0.05% (v/v) trifluoroacetic acid; B: acetonitrile with 0.05% (v/v) trifluoroacetic acid: gradient elution: (A%): 0-4 min: 90%, 4-29 min: 90 → 0%, 29-31 min: 0%, 31-31.5 min: 0 → 90%, 31.5-40 min: 90%. The purity of all compounds was determined by this method. The purity of all test compounds is higher than 95%, unless otherwise noted.

7.3. Synthetic procedures

7.3.1. (2-Fluoro-4-phenylbutyl) 4-methylbenzenesulfonate (7a)

(Diethylamino)difluorosulfonium tetrafluoroborate (XtalFluor E®, 180 mg, 0.79 mmol, 1.5 eq) and triethylamine trihydrofluoride (427 µL, 2.62 mmol, 5.0 eq) were dissolved in CH_2Cl_2 (6 mL). The solution was cooled to -78 °C. Alcohol **5a** (168 mg, 0.52 mmol,

1.0 eq) was added and the mixture was stirred for 1 h at -78 °C and overnight at rt. A saturated solution of $NaHCO_3$ (25 mL) was added and the mixture was stirred for 20 min. The aqueous layer was extracted with CH_2Cl_2 (3 x 10 mL), then the combined organic layers were dried (Na_2SO_4), filtered and the solvent was removed *in vacuo*. The crude product was purified by fc (d = 2 cm, l = 20 cm, V = 7 mL, cyclohexane:ethyl acetate = 9:1). Colorless oil, yield 122 mg (0.38 mmol, 72 %). $C_{17}H_{19}O_3S$ (322.4). R_f = 0.37 (cyclohexane:ethyl acetate = 8:2). ^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] = 1.73-2.07 (m, 2H, $PhCH_2C\mathit{H}_2CHF$), 2.45 (s, 3H, $C\mathit{H}_3$), 2.62-2.82 (m, 2H, $PhC\mathit{H}_2CH_2CHF$), 4.04-4.18 (m, 2H, $CHFC\mathit{H}_2OTs$), 4.53-4.70 (dm, *J* = 48.5 Hz, 1H, $C\mathit{H}F$), 7.12-7.23 (m, 3H, 2-H_{phenyl}, 4-H_{phenyl}, 6-H_{phenyl}), 7.25-7.31 (m, 2H, 3-H_{phenyl}, 5-H_{phenyl}), 7.33-7.37 (m, 2H, 3-H_{tosyl}, 5-H_{tosyl}), 7.77-7.81 (m, 2H, 2-H_{tosyl}, 6-H_{tosyl}). ^{13}C NMR (101 MHz, $CDCl_3$): δ [ppm] = 21.8 (1C, $C\mathit{H}_3$), 30.8 (d, *J* = 4.4 Hz, 1C, $PhC\mathit{H}_2CH_2CHF$), 32.7 (d, *J* = 20.7 Hz, 1C, $PhCH_2C\mathit{H}_2CHF$), 70.7 (d, *J* = 23.6 Hz, 1C, $CHFC\mathit{H}_2OTs$), 89.6 (d, *J* = 175.5 Hz, 1C, $C\mathit{H}F$), 126.4 (1C, C-4_{phenyl}), 128.1 (2C, C-2_{tosyl}, C-6_{tosyl}), 128.5 (2C, C-2_{phenyl}, C-6_{phenyl}), 128.7 (2C, C-3_{phenyl}, C-5_{phenyl}), 130.1 (2C, C-3_{tosyl}, C-5_{tosyl}), 132.8 (1C, C-1_{tosyl}), 140.4 (1C, C-1_{phenyl}), 145.2 (1C, C-4_{tosyl}). ^{19}F NMR (376 MHz, CD_3OD): δ [ppm] = -189.0 (m, 1F). HR-MS (APCI): m/z = 323.1114 (calcd. 323.1112 for $C_{17}H_{20}FO_3S$ $[M+H]^+$). Purity (HPLC): 98.5 %, t_R = 24.3 min. IR: $\tilde{\nu}$ [cm^{-1}] = 2951 (C-$H_{aliph.}$), 1597, 1493 (C=$C_{arom.}$), 1173, 1362 (O-SO_2), 814 (disubst. arom.), 748, 698 (monosubst. arom.).

7.3.2. 7-(Benzyloxy)-3-(2-fluoro-4-phenylbutyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepin-1-ol (8a)

Secondary amine **6** (104 mg, 0.39 mmol, 1.0 eq) was dissolved in CH_3CN (8 mL). After addition of tosylate **7a** (150 mg, 0.47 mmol, 1.2 eq) in CH_3CN (3 mL) and K_2CO_3 (427 mg, 3.09 mmol, 8.0 eq), the suspension was heated to reflux for 3 d. At

rt, K_2CO_3 was filtered off, washed with CH_3CN and the solvent was removed *in vacuo*. The residue was purified by fc three times (1. d = 2 cm, l = 20 cm, V = 7 mL, cyclohexane:ethyl acetate = 8:2 + 1 % *N*,*N*-dimethylethanamine; 2. d = 1 cm, l = 22 cm, V = 4 mL, CH_2Cl_2:CH_3OH = 99:1 + 1 % *N*,*N*-dimethylethanamine; 3. d = 1 cm, l = 15 cm, V = 4 mL, CH_2Cl_2:ethyl acetate = 97:3 + 1 % NH_3). Colorless oil, yield 22.0 mg (0.05 mmol, 14 %). $C_{27}H_{30}FNO_2$ (419.5). R_f = 0.24 (cyclohexane:ethyl acetate = 7:3+ 1 % *N*,*N*-dimethylethanamine). ^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] = 1.78-2.08 (m, 2H, $NCH_2CHFC\mathit{H_2}CH_2Ph$), 2.58-3.09 (m, 8H, 2-H, 2 x 4-H, 5-H, $NC\mathit{H_2}CHFCH_2C\mathit{H_2}Ph$), 3.16-3.32 (m, 2H, 2-H, 5-H), 4.57-4.82 (m, 2H, 1-H, C*H*F), 5.03 (s, 2H, $OC\mathit{H_2}Ph$), 6.71-6.77 (m, 2H, 6-H, 8-H), 7.11 (d, *J* = 8.9 Hz, 1H, 9-H), 7.18-7.24 (m, 3H, 2-H_{phenyl}, 4-H_{phenyl}, 6-H_{phenyl}), 7.28-7.44 (m, 7H, 3-H_{phenyl}, 5-H_{phenyl}, 2-H_{benzyl}, 3-H_{benzyl}, 4-H_{benzyl}, 5-H_{benzyl}, 6-H_{benzyl}). A signal for the OH proton is not seen in the spectrum. ^{13}C NMR (101 MHz, $CDCl_3$): δ [ppm] = 31.3, 31.4 (d, *J* = 4.6 Hz, 1C, $NCH_2CHFCH_2\mathit{C}H_2Ph$), 35.27, 35.32 (d, *J* = 20.8 Hz, 1C, $NCH_2CHF\mathit{C}H_2CH_2Ph$), 36.95, 37.04 (1C, C-5), 56.6, 56.9 (1C, C-4), 61.5, 61.7 (1C, C-2), 63.77, 63.79 (d, *J* = 20.8 Hz, 1C, $N\mathit{C}H_2CHFCH_2CH_2Ph$), 70.1 (1C, $O\mathit{C}H_2Ph$), 72.6, 72.7 (1C, C-1), 91.6, 91.9 (d, *J* = 171.3 Hz, 1C, *C*HF), 111.4 (1C, C-8), 117.62, 117.63 (1C, C-6), 126.3 (1C, C-4_{phenyl}), 127.6 (2C, C-2_{benzyl}, C-6_{benzyl}), 128.1 (1C, C-4_{benzyl}), 128.6 (2C, C-2_{phenyl}, C-6_{phenyl}), 128.68 (2C, C-3_{phenyl}, C-5_{phenyl}), 128.70 (2C, (C-3_{benzyl}, C-5_{benzyl}), 129.8, 129.9 (1C, C-9), 135.6, 135.7 (1C, C-9a), 137.2 (1C, C-1_{benzyl}), 141.14, 141.18 (1C, C-5a), 141.20 (1C, C-1_{phenyl}), 158.346, 158.354 (1C, C-7). Ratio of diastereomers is approx. 1:1. ^{19}F NMR (376 MHz, $CDCl_3$): δ [ppm] = -183.5, -182.7 (m, 1F). HR-MS (APCI): m/z = 420.2321 (calcd. 420.2333 for $C_{27}H_{31}FNO_2$ $[M+H]^+$). Purity (HPLC): 91.6 %, t_R = 20.8 min. IR: ṽ [cm^{-1}] = 3422 (OH), 3028 (C-H_{aryl}), 2940 (C-$H_{aliph.}$), 1605, 1578, 1497 (C=$C_{arom.}$), 1454 (C-$H_{aliph.}$), 1234 (C-O).

7.3.3. 7-(Benzyloxy)-3-(3-fluoro-4-phenylbutyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepin-1-ol (8b)

Secondary amine **6**(125 mg, 0.46 mmol, 1.0 eq) was dissolved in CH_3CN (5 mL). After addition of tosylate **7b** (165 mg, 0.51 mmol, 1.2 eq) in CH_3CN (3 mL) and K_2CO_3 (427 mg, 3.09 mmol, 8.0 eq), the suspension was heated to reflux for 3 d. At rt, K_2CO_3 was filtered off, washed with CH_3CN and the solvent was removed *in vacuo*. The residue was purified by fc (d = 2 cm, l = 20 cm, V = 7 mL, cyclohexane:ethyl acetate = 7:3 + 1.5 % *N*,*N*-dimethylethanamine). Pale yellow resin, yield 160 mg (0.38 mmol, 82 %). $C_{27}H_{30}FNO_2$ (419.5). R_f = 0.22 (cyclohexane:ethyl acetate = 6:4 + 1 % *N*,*N*-dimethylethanamine). ^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] = 1.73-1.93 (m, 2H, $NCH_2CH_2CHFCH_2Ph$), 2.39-3.30 (m, 10H, 2 x 2-H, 2 x 4-H, 2 x 5-H, $NCH_2CH_2CHFCH_2Ph$), 4.58, 4.59 (d, *J* = 6.7 Hz, 1H, 1-H), 4.70-4.90 (dm, *J* = 49.1 Hz, 1H, C*H*F), 5.03 (s, 2H, OCH_2Ph), 6.71-6.75 (m, 2H, 6-H, 8-H), 7.10 (d, *J* = 9.0 Hz, 1H, 9-H), 7.21-7.43 (m, 10H, 2-H_{phenyl}, 3-H_{phenyl}, 4-H_{phenyl}, 5-H_{phenyl}, 6-H_{phenyl}, 2-H_{benzyl}, 3-H_{benzyl}, 4-H_{benzyl}, 5-H_{benzyl}, 6-H_{benzyl}). A signal for the OH proton is not seen in the spectrum. ^{13}C NMR (101 MHz, $CDCl_3$): δ [ppm] = 32.40, 32.42 (d, *J* = 20.6 Hz, 1C, $NCH_2CH_2CHFCH_2Ph$), 36.8, 36.9 (1C, C-5), 41.90, 41.94 (d, *J* = 21.5 Hz, 1C, $NCH_2CH_2CHFCH_2Ph$), 55.76, 55.78 (d, *J* = 3.8 Hz, 1C, $NCH_2CH_2CHFCH_2Ph$), 56.0, 56.5 (1C, C-4), 60.7, 61.1 (1C, C-2), 70.1 (1C, OCH_2Ph), 72.4, 72.5 (1C, C-1), 92.96, 92.98 (d, *J* = 171.4 Hz, 1C, *C*HF), 111.37, 111.38 (1C, C-8), 117.60, 117.62 (1C, C-6), 126.9 (1C, C-4_{phenyl}), 127.6 (2C, C-2_{benzyl}, C-6_{benzyl}), 128.1 (2C, C-3_{phenyl}, C-5_{phenyl}), 128.66, 128.68 (1C, C-4_{benzyl}), 128.7 (2C, C-3_{phenyl}, C-5_{phenyl}), 129.5 (2C, C-2_{phenyl}, C-6_{phenyl}), 129.80, 129.82 (1C, C-9), 135.72, 135.74 (1C, C-9a), 137.00, 137.02 (d, *J* = 5.4 Hz, 1C, C-1_{phenyl}), 137.2 (1C, C-1_{benzyl}), 141.18, 141.20 (1C, C-5a), 158.33, 158.34 (1C, C-7). Ratio of diastereomers is 1:1.

^{19}F NMR (376 MHz, $CDCl_3$): δ [ppm] = -179.9 (m, 0.5F), -179.3 (m, 0.5F). HR-MS (APCI): m/z = 420.2362 (calcd. 420.2333 for $C_{27}H_{31}FNO_2$ $[M+H]^+$). Purity (HPLC): 98.7 %, t_R = 21.2 min. IR: ṽ [cm^{-1}] = 3395 (OH), 3028 (C-H_{aryl}), 2940 (C-$H_{aliph.}$), 1609, 1582, 1497 (C=$C_{arom.}$), 1454 (C-$H_{aliph.}$), 1246 (C-O).

7.3.4. 3-(3-Fluoro-4-phenylbutyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepine-1,7-diol (9b)

8b (100 mg, 0.24 mmol, 1.0 eq) was dissolved in CH_3OH (4 mL) and the solution was added to a suspension of Pd/C (10 %, 20.0 mg) in CH_3OH (8 mL). The reaction mixture was stirred for 3 h at rt under H_2 atmosphere (1 bar, balloon). The catalyst was removed by filtration over Celite® and the solvent was removed *in vacuo*. The residue was purified by fc (d = 2 cm, l = 18 cm, V = 7 mL, CH_2Cl_2:CH_3OH = 97:3 + 1.5 % *N*,*N*-dimethylethanamine). Yellow oil, yield 67.5 mg (0.21 mmol, 86 %). $C_{20}H_{24}FNO_2$ (329.4). R_f = 0.30 (CH_2Cl_2:CH_3OH = 96:4 + 1 % *N*,*N*-dimethyl-ethanamine). ^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] = 1.70-1.91 (m, 2H, $NCH_2CH_2CHFCH_2Ph$), 2.34-2.62 (m, 3H, 2-H, 4-H, 5-H), 2.65-2.81 (m, 2H, $NCH_2CH_2CHFCH_2Ph$), 2.83-3.24 (m, 5H, 2-H, 4-H, 5-H, $NCH_2CH_2CHFCH_2Ph$), 4.54, 4.55 (d, *J* = 6.6 Hz, 1H, 1-H), 4.68-4.89 (dm, *J* = 48.8 Hz, 1H, C*H*F), 6.55-6.59 (m, 2H, 6-H, 8-H), 7.00 (d, *J* = 8.6 Hz, 1H, C-9), 7.20-7.25 (m, 3H, 2-H_{phenyl}, 4-H_{phenyl}, 6-H_{phenyl}), 7.29-7.34 (m, 2H, 3-H_{phenyl}, 5-H_{phenyl}). Signals for the OH protons are not seen in the spectrum. ^{13}C NMR (101 MHz, $CDCl_3$): δ [ppm] = 32.45, 32.48 (d, *J* = 20.6 Hz, 1C, $NCH_2CH_2CHFCH_2Ph$), 36.8, 36.9 (1C, C-5), 41.91, 41.95 (d, *J* = 21.5 Hz, 1C, $NCH_2CH_2CHFCH_2Ph$), 55.75, 55.78 (d, *J* = 3.6 Hz, 1C, $NCH_2CH_2CHFCH_2Ph$), 56.1, 56.6 (1C, C-4), 60.7, 61.2 (1C, C-2), 72.6, 72.7 (1C, C-1), 93.00, 93.04 (d, *J* = 171.4 Hz, 1C, *C*HF), 112.6 (1C, C-8), 117.71, 117.74 (1C, C-6), 126.8 (1C, C-4_{phenyl}), 128.66, 128.67 (2C, C-3_{phenyl}, C-5_{phenyl}), 129.5 (2C, C-

2_{phenyl}, C-6_{phenyl}), 130.02, 130.04 (1C, C-9), 134.94, 134.95 (1C, C-9a), 137.03, 137.04 (d, *J* = 5.4 Hz, 1C, C-1_{phenyl}), 141.35, 141.37 (1C, C-5a), 155.75, 155.77 (1C, C-7). Ratio of diastereomers is approx. 1:1. ^{19}F NMR (376 MHz, $CDCl_3$): δ [ppm] = -179.6 (m, 0.5F), 179.3 (m, 0.5F). HR-MS (APCI): m/z = 330.1865 (calcd. 330.1864 for $C_{20}H_{25}FNO_2$ $[M+H]^+$). Purity (HPLC): 88.5 %, t_R = 15.8 min. IR: ṽ [cm^{-1}] = 3244 (OH), 3028 (C-H_{aryl}), 2924 (C-$H_{aliph.}$), 1609, 1586, 1497 (C=$C_{arom.}$), 1454 (C-$H_{aliph.}$), 1227 (C-O).

7.3.5. 3-(2-Fluoro-4-phenylbutyl)-7-methoxy-2,3,4,5-tetrahydro-1*H*-3-benzazepin-1-ol (12a) and 3-(1-Fluoro-3-phenylpropyl)-8-methoxy-5,6-dihydro-1*H*,3*H*-1,4-methanobenzo-1,3-oxazocine (13)

Procedure 1: Secondary amine **10** (39.0 mg, 0.20 mmol, 1.0 eq) was dissolved in CH_3CN (5 mL). After addition of tosylate **7a** (72.0 mg, 0.22 mmol, 1.1 eq) in CH_3CN (5 mL) and K_2CO_3 (223 mg, 1.61 mmol, 8.0 eq), the suspension was heated to reflux for 4 d. At rt, K_2CO_3 was filtered off, washed with CH_3CN and the solvent was removed *in vacuo*. The residue was purified by fc (d = 1 cm, l = 18 cm, V = 4 mL, cyclohexane:ethyl acetate = 7:3) to give tertiary amine **12a**. Yellow oil, yield 4.5 mg (0.01 mmol, 7 %). $C_{21}H_{26}FNO_2$ (343.4). R_f = 0.30 (cyclohexane:ethyl acetate = 1:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] = 1.78-2.04 (m, 2H, $NCH_2CHFC\mathit{H}_2CH_2Ph$), 2.57-2.94 (m, 7H, 2-H, 4-H, 5-H, $NC\mathit{H}_2CHFCH_2C\mathit{H}_2Ph$), 3.00-3.09 (m, 1H, 4-H), 3.15-3.32 (m, 2H, 2-H, 5-H), 3.78 (s, 3H, $OC\mathit{H}_3$), 4.58, 4.60 (d, *J* = 6.4 Hz, 1H, 1-H), 4.61-4.81 (dm, *J* = 49.9 Hz, 1H, C*H*F), 6.63 (d, *J* = 2.6 Hz, 1H, 6-H), 6.66 (dd, *J* = 8.1/2.6 Hz, 1H, 8-H), 7.11 (d, *J* = 8.1 Hz, 1H, 9-H), 7.18-7.24 (m, 3H, 2-H_{phenyl}, 4-H_{phenyl}, 6-H_{phenyl}), 7.28-7.33 (m, 2H, 3-H_{phenyl}, 5-H_{phenyl}). A signal for the OH proton is not seen in the spectrum. ^{13}C NMR (101 MHz, $CDCl_3$): δ [ppm] = 31.34, 31.36 (d, *J* = 4.4 Hz, 1C, $NCH_2CHFCH_2\mathit{C}H_2Ph$), 35.27, 35.33 (d, *J* = 20.8 Hz, 1C, $NCH_2CHF\mathit{C}H_2CH_2Ph$),

37.1, 37.2 (1C, C-5), 55.4 (1C, O*C*H_3), 56.7, 56.9 (d, *J* = 1.4 Hz, 1C, C-4), 61.6, 61.7 (d, *J* = 1.3 Hz, 1C, C-2), 63.80, 63.81 (d, *J* = 20.7 Hz, 1C, N*C*H_2CHFCH_2CH_2Ph), 72.7, 72.8 (1C, C-1), 91.7, 92.0 (d, *J* = 171.0 Hz, 1C, *C*HF), 110.4 (1C, C-8), 116.74, 116.76 (1C, C-6), 126.3 (1C, C-4_{phenyl}), 128.6 (2C, C-2_{phenyl}, C-6_{phenyl}), 128.7 (2C, C-3_{phenyl}, C-5_{phenyl}), 129.8, 129.9 (1C, C-9), 135.4, 135.5 (1C, C-9a), 141.1, 141.2 (1C, C-5a), 141.2 (d, *J* = 0.8 Hz, 1C, C-1_{phenyl}), 159.10, 159.11 (1C, C-7). ^{19}F NMR (376 MHz, $CDCl_3$): δ [ppm] = -183.6 (m, 0.5F), -182.7 (m, 0.5F). HR-MS (APCI): m/z = 344.2007 (calcd. 344.2020 for $C_{21}H_{27}FNO_2$ $[M+H]^+$). Purity (HPLC): 90.3 %, t_R = 17.6 min. IR: ṽ [cm^{-1}] = 3399 (OH), 3024 (C-H_{aryl}), 2936 (C-$H_{aliph.}$), 1609, 1582, 1497 (C=$C_{arom.}$), 1454 (C-$H_{aliph.}$), 1258 (C-O). Procedure 2: Secondary amine **10** (300 mg, 1.55 mmol, 1.0 eq) was added to a stirred solution of freshly prepared aldehyde **11a** (excess) in CH_2Cl_2 (10 mL). After addition of $NaBH(OAc)_3$ (658 mg, 3.11 mmol, 2.0 eq), the reaction mixture was stirred for 15 h at rt. A saturated solution of $NaHCO_3$ (10 mL), water (5 mL) and CH_2Cl_2 (5 mL) were added, the organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (2 x 5 mL) and ethyl acetate (2 x 5 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated *in vacuo*. The crude product was purified by fc (d = 3 cm, l = 22 cm, V = 20 mL, cyclohexane:ethyl acetate = 8:2 + 1 % *N*,*N*-dimethylethanamine) to give **12a** and two pairs of diastereomers of **13** in separate fractions. **12a**: Yellow oil, yield 62.0 mg (0.18 mmol, 12 %). $C_{21}H_{26}FNO_2$ (343.4). R_f = 0.30 (cyclohexane:ethyl acetate = 1:1). The spectroscopic data are identical with those of **12a** from procedure 1. HR-MS (APCI): m/z = 344.1981 (calcd. 344.2020 for $C_{21}H_{27}FNO_2$ $[M+H]^+$). Purity (HPLC): 90.3 %, t_R = 17.6 min. **13** (diastereomer 1): Yellow oil, yield 182 mg (0.53 mmol, 34 %). $C_{21}H_{24}FNO_2$ (341.4). R_f = 0.62 (cyclohexane:ethyl acetate = 8:2+ 1 % *N*,*N*-dimethylethanamine). ^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] = 1.93-2.20 (m, 2H, CHFC*H_2*CH_2Ph), 2.38-2.49 (m, 1H,

6-H), 2.70-2.96 (m, 2H, $CHFCH_2C\mathit{H}_2Ph$), 2.99-3.63 (m, 5H, 2 x 5-H, 6-H, 2 x 11-H), 3.76 (s, 3H, $OC\mathit{H}_3$), 4.22-4.40 (dm, J = 49.4 Hz, 0.7H, C*H*F), 4.28-4.46 (dm, J = 49.2 Hz, 0.3H, C*H*F), 4.76 (d, J = 6.9 Hz, 0.7H, 1-H), 4.89 (d, J = 7.1 Hz, 0.3H, 1-H), 4.91-4.98 (m, 1H, 3-H), 6.60 (dd, J = 8.2/2.6 Hz, 1H, 8-H), 6.64-6.67 (m, 1H, 6-H), 6.97 (d, J = 8.2 Hz, 0.7H, 9-H), 6.98 (d, J = 8.2, 0.3H, 9-H), 7.17-7.25 (m, 3H, 2-H_{phenyl}, 4-H_{phenyl}, 6-H_{phenyl}), 7.27-7.32 (m, 2H, 3-H_{phenyl}, 5-H_{phenyl}). Ratio of diastereomers is 7:3. ^{13}C NMR (101 MHz, $CDCl_3$): δ [ppm] = 31.15 (d, J = 4.0 Hz, 0.3C, $CHFCH_2\mathit{C}H_2Ph$), 31.19 (d, J = 4.0 Hz, 0.7C, $CHFCH_2\mathit{C}H_2Ph$), 33.4 (d, J = 20.5 Hz, 0.3C, $CHF\mathit{C}H_2CH_2Ph$), 33.8 (d, J = 20.6 Hz, 0.7C, $CHF\mathit{C}H_2CH_2Ph$), 34.9 (0.3C, C-6), 35.2 (0.7C, C-6), 55.35 (0.7C, OCH_3), 55.37 (0.3C, OCH_3), 55.6 (0.7C, C-5), 55.8 (0.3C, C-5), 60.1 (d, J = 1.3 Hz, 0.7C, C-11), 61.0 (d, J = 2.7 Hz, 0.3C, C-11), 80.0 (0.7C, C-1), 80.5 (0.3C, C-1), 91.0 (d, J = 175.5 Hz, 0.7C, *C*HF), 93.6 (d, J = 175.5 Hz, 0.3C, *C*HF), 96.8 (d, J = 23.0 Hz 0.7C, C-3), 96.9 (d, J = 21.9 Hz, 0.3C, C-3), 110.1 (0.3C, C-9), 110.2 (0.7C, C-9), 117.53 (0.3C, C-7), 117.54 (0.7C, C-7), 126.12 (0.7C, C-4$_{phenyl}$), 126.14 (0.3C, C-4$_{phenyl}$), 128.57 (0.7C, C-10), 128.60 (0.3C, C-10), 128.60 (2 x 0.7C, C-3$_{phenyl}$, C-5$_{phenyl}$), 128.57 (2 x 0.3C, C-3$_{phenyl}$, C-5$_{phenyl}$), 128.67 (2 x 0.7C, C-2$_{phenyl}$, C-6$_{phenyl}$), 128.69 (2 x 0.3C, C-2$_{phenyl}$, C-6$_{phenyl}$), 135.1 (0.7C, C-10a), 135.4 (0.3C, C-10a), 140.3 (0.3C, C-6a), 140.4 (0.7C, C-6a), 141.4 (0.3C, C-1$_{phenyl}$), 141.5 (0.7C, C-1$_{phenyl}$), 158.8 (0.3C, C-8), 158.9 (0.7C, C-8). ^{19}F NMR (376 MHz, $CDCl_3$): δ [ppm] = -196.3 (m, 0.3F), -188.9 (m, 0.7F). HR-MS (APCI): m/z = 342.1843 (calcd. 342.1864 for $C_{21}H_{25}FNO_2$ $[M+H]^+$). Purity (HPLC): 46.1 % t_R = 9.2 min; 52.1 %, t_R = 15.1 min. IR: ṽ [cm^{-1}] = 3024 (C-H_{aryl}), 2932 (C-$H_{aliph.}$), 1605, 1578, 1497 (C=$C_{arom.}$), 1454 (C-$H_{aliph.}$), 1254 (C-O). **13** (diastereomer 2): Yellow oil, yield 148 mg (0.43 mmol, 28 %). $C_{21}H_{24}FNO_2$ (341.4). R_f = 0.50 (cyclohexane:ethyl acetate = 8:2+ 1 % *N*,*N*-dimethylethanamine). ^{1}H NMR (400 MHz, $CDCl_3$)**:** δ [ppm] = 1.91-2.20 (m, 2H, $CHFC\mathit{H}_2CH_2Ph$), 2.39-2.49 (m, 1H, 6-H), 2.70-

2.96 (m, 2H, CHFCH$_2$C*H$_2$*Ph), 3.00-3.63 (m, 5H, 2 x 5-H, 6-H, 2 x 11-H), 3.76 (s, 3H, OC*H$_3$*), 4.22-4.40 (dm, *J* = 49.4 Hz, 0.5H, C*H*F), 4.28-4.46 (dm, *J* = 49.2 Hz, 0.5H, C*H*F), 4.76 (d, *J* = 6.9 Hz, 0.5H, 1-H), 4.89 (d, *J* = 7.1 Hz, 0.5H, 1-H), 4.91-4.98 (m, 1H, 3-H), 6.60 (dd, *J* = 8.2/2.6 Hz, 1H, 9-H), 6.64-6.67 (m, 1H, 7-H), 6.97 (d, *J* = 8.2 Hz, 0.5H, 10-H), 6.98 (d, *J* = 8.2, 0.5H, 10-H), 7.17-7.25 (m, 3H, 2-H$_{phenyl}$, 4-H$_{phenyl}$, 6-H$_{phenyl}$), 7.27-7.32 (m, 2H, 3-H$_{phenyl}$, 5-H$_{phenyl}$). Ratio of diastereomers is 1:1. ^{13}C NMR (101 MHz, $CDCl_3$): δ [ppm] = 31.15 (d, *J* = 4.0 Hz, 0.5C, CHFCH$_2$*C*H$_2$Ph), 31.19 (d, *J* = 4.0 Hz, 0.5C, CHFCH$_2$*C*H$_2$Ph), 33.4 (d, *J* = 20.5 Hz, 0.5C, CHF*C*H$_2$CH$_2$Ph), 33.8 (d, *J* = 20.6 Hz, 0.5C, CHF*C*H$_2$CH$_2$Ph), 34.9 (0.5C, C-6), 35.2 (0.5C, C-6), 55.35 (0.5C, O*C*H$_3$), 55.37 (0.5C, O*C*H$_3$), 55.6 (0.5C, C-5), 55.8 (0.5C, C-5), 60.1 (0.5C, C-11), 61.0 (0.5C, C-11), 80.0 (0.5C, C-1), 80.5 (0.5C, C-1), 91.0 (d, *J* = 175.5 Hz, 0.5C, *C*HF), 93.6 (d, *J* = 175.5 Hz, 0.5C, *C*HF), 96.7 (d, *J* = 23.0 Hz, 0.5C, C-3), 96.9 (d, *J* = 21.9 Hz, 0.5C, C-3), 110.1 (0.5C, C-9), 110.2 (0.5C, C-9), 117.53 (0.5C, C-7), 117.54 (0.5C, C-7), 126.12 (0.5C, C-4$_{phenyl}$), 126.14 (0.5C, C-4$_{phenyl}$), 128.57 (0.5C, C-10), 128.60 (0.5C, C-10), 128.61 (2 x 0.7C, C-3$_{phenyl}$, C-5$_{phenyl}$), 128.62 (2 x 0.3C, C-3$_{phenyl}$, C-5$_{phenyl}$), 128.67 (2 x 0.5C, C-2$_{phenyl}$, C-6$_{phenyl}$), 128.69 (2 x 0.5C, C-2$_{phenyl}$, C-6$_{phenyl}$), 135.1 (0.5C, C-10a), 135.4 (0.5C, C-10a), 140.3 (0.5C, C-6a), 140.4 (0.5C, C-6a), 141.4 (0.5C, C-1$_{phenyl}$), 141.5 (0.5C, C-1$_{phenyl}$), 158.8 (0.5C, C-8), 158.9 (0.5C, C-8). ^{19}F NMR (376 MHz, $CDCl_3$): δ [ppm] = -196.3 (m, 0.5F), -188.9 (m, 0.5F). HR-MS (APCI): m/z = 342.1843 (calcd. 342.1864 for $C_{21}H_{25}FNO_2$ $[M+H]^+$). Purity (HPLC): 46.5 %, t_R = 9.1 min; 52.4 %, t_R = 15.1 min. IR: ṽ [cm^{-1}] = 3024 (C-H$_{aryl}$), 2940 (C-H$_{aliph.}$), 1613, 1578, 1497 (C=C$_{arom.}$), 1454 (C-H$_{aliph.}$), 1254 (C-O).

7.3.6. 3-(3-Fluoro-4-phenylbutyl)-7-methoxy-2,3,4,5-tetrahydro-1*H*-3-benzazepin-1-ol (12b)

Secondary amine **10** (35.0 mg, 0.18 mmol, 1.0 eq) was dissolved in CH_3CN (8 mL). After addition of tosylate **7b** (64.5 mg, 0.20 mmol, 1.1 eq) in CH_3CN (2 mL) and K_2CO_3 (200 mg, 1.45 mmol, 8.0 eq), the suspension was heated to reflux for 20 h. At rt, K_2CO_3 was filtered off, washed with CH_3CN and the solvent was removed *in vacuo*. The residue was purified by fc (d = 2 cm, l = 20 cm, V = 7 mL, cyclohexane:ethyl acetate = 7:3). Colorless oil, yield 43.2 mg (0.13 mmol, 70 %). $C_{21}H_{26}FNO_2$ (343.4). R_f = 0.26 (cyclohexane:ethyl acetate = 1:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] = 1.72-1.91 (m, 2H, $NCH_2CH_2CHFCH_2Ph$), 2.38-3.31 (m, 10H, 2 x 2-H, 2 x 4-H, 2 x 5-H, $NCH_2CH_2CHFCH_2Ph$), 3.77 (s, 3H, OCH_3), 4.58, 4.59 (d, *J* = 6.7 Hz, 1H, 1-H), 4.70-4.90 (dm, *J* = 47.4 Hz, 1H, C*H*F), 6.62-6.68 (m, 2H, 6-H, 8-H), 7.10 (d, *J* = 8.1 Hz, 1H, 9-H), 7.21-7.28 (m, 3H, 2-H_{phenyl}, 4-H_{phenyl}, 6-H_{phenyl}), 7.29-7.35 (m, 2H, 3-H_{phenyl}, 5-H_{phenyl}). A signal for the OH proton is not seen in the spectrum.´^{13}C NMR (101 MHz, $CDCl_3$): δ [ppm] = 32.4, 32.5 (d, *J* = 20.6 Hz, 1C, $NCH_2CH_2CHFCH_2Ph$), 36.9, 37.0 (1C, C-5), 41.91, 42.0 (d, *J* = 21.5 Hz, 1C, $NCH_2CH_2CHFCH_2Ph$), 55.4 (1C, OCH_3), 55.75, 55.79 (d, *J* = 3.5 Hz, 1C, $NCH_2CH_2CHFCH_2Ph$), 56.1, 56.6 (1C, C-4), 60.7, 61.2 (1C, C-2), 72.5, 72.6 (1C, C-1), 92.97, 93.00 (d, *J* = 171.4 Hz, 1C, *C*HF), 110.39, 110.40 (1C, C-8), 116.72, 116.74 (1C, C-6), 126.9 (1C, C-4_{phenyl}), 128.66, 128.67 (2C, C-3_{phenyl}, C-5_{phenyl}), 129.5 (2C, C-2_{phenyl}, C-6_{phenyl}), 129.81, 129.84 (1C, C-9), 135.48, 135.50 (1C, C-9a), 137.01, 137.03 (d, *J* = 5.3 Hz, 1C, C-1_{phenyl}), 141.16, 141.18 (1C, C-5a), 159.09, 159.10 (1C, C-7). Ratio of diastereomers is approx. 1:1. ^{19}F NMR (376 MHz, $CDCl_3$): δ [ppm] = -179.4 (m, 0.5F), -179.9 (m, 0.5F). HR-MS (APCI): m/z = 344.2003 (calcd. 344.2020 for $C_{21}H_{27}FNO_2$ $[M+H]^+$). Purity (HPLC): 97.0 %, t_R = 17.9 min. IR: ṽ [cm^{-1}] = 3383 (OH), 3028 (C-H_{aryl}), 2940 (C-$H_{aliph.}$), 1609, 1582, 1497 (C=$C_{arom.}$), 1454 (C-$H_{aliph.}$), 1254 (C-O).

7.3.7. 7-Methoxy-2,3,4,5-tetrahydro-1*H*-3-benzazepine (14)[48]

Triethylsilane (3.3 mL, 20.7 mmol, 8.0 eq) and $BF_3 \cdot OEt_2$ (48 %, 2.7 mL, 10.4 mmol, 4.0 eq) were added to a solution of alcohol **10** (500 mg, 2.59 mmol, 1.0 eq) in CH_2Cl_2 (20 mL). The mixture was stirred for 4 h at rt. Then, 1 M NaOH (30 mL) was added, the organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (3 x 15 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated *in vacuo*. The crude product was purified by fc (d = 3 cm, l = 20 cm, V = 12 mL, CH_2Cl_2:CH_3OH = 93:7 + 1 % *N*,*N*-dimethylethanamine). Pale yellow oil, yield 249 mg (1.40 mmol, 54 %). $C_{11}H_{15}NO$ (177.3). R_f = 0.32 (CH_2Cl_2:CH_3OH = 95:5 + 1 % *N*,*N*-dimethylethanamine). ^{1}H NMR (600 MHz, $CDCl_3$): δ [ppm] = 2.91-2.97 (m, 4H, 2 x 1-H, 2 x 5-H), 3.00-3.05 (m, 4H, 2 x 2-H, 2 x 4-H), 3.78 (s, 3H, OC*H*$_3$), 6.65 (dd, *J* = 8.2/2.7 Hz, 1H, 8-H), 6.68 (d, *J* = 2.6 Hz, 1H, 6-H), 7.02 (d, *J* = 8.2 Hz, 1H, 9-H). A signal for the NH proton is not seen in the spectrum. ^{13}C NMR (151 MHz, $CDCl_3$): δ [ppm] = 37.8 (1C, C-1), 38.9 (1C, C-5), 48.2 (1C, C-4), 48.6 (1C, C-2), 55.4 (1C, O*C*H$_3$), 110.9 (1C, C-8), 115.6 (1C, C-6), 130.4 (C-9), 133.8 (1C, C-9a), 142.9 (1C, C-5a), 158.2 (1C, C-7). HR-MS (APCI): m/z = 178.1215 (calcd. 178.1226 for $C_{11}H_{16}NO$ $[M+H]^+$). Purity (HPLC): 95.5 %, t_R = 11.0 min. IR: ṽ [cm^{-1}] = 3059 (C-H_{aryl}), 2947 (C-$H_{aliph.}$), 1605, 1582, 1501 (C=$C_{arom.}$), 1262 (C-O).

7.3.8. 3-(2-Fluoro-4-phenylbutyl)-7-methoxy-2,3,4,5-tetrahydro-1*H*-3-benzazepine (16a)

A mixture of secondary amine **14** (70.0 mg, 0.40 mmol, 1.0 eq), an excess of freshly prepared crude aldehyde **11a** and $NaBH(OAc)_3$ (168 mg, 0.79 mmol, 2.0 eq) in CH_2Cl_2 (4 mL) was stirred for 13 h at rt. A saturated solution of $NaHCO_3$ (10 mL) was added, the organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (3 x 10 mL). The combined organic layers were dried (Na_2SO_4), filtered and

concentrated *in vacuo*. The crude product was purified by fc twice (1. d = 2 cm, l = 23 cm, V = 7 mL, CH_2Cl_2:CH_3OH = 99:1; 2. d = 2 cm, l = 21 cm, V = 7 mL, cyclohexane:ethyl acetate = 8:2). Pale yellow oil, yield 89.4 mg (0.27 mmol, 69 %). $C_{21}H_{26}FNO$ (327.4). R_f = 0.30 (cyclohexane:ethyl acetate = 8:2). ^{1}H NMR (400 MHz, CD_3OD): δ [ppm] = 1.78-2.09 (m, 2H, $NCH_2CHFC\mathit{H}_2CH_2Ph$), 2.58-2.87 (m, 12H, 2 x 1-H, 2 x 2-H, 2 x 4-H, 2 x 5-H, $NC\mathit{H}_2CHFCH_2C\mathit{H}_2Ph$), 3.73 (s, 3H, $OC\mathit{H}_3$), 4.61-4.80 (dm, *J* = 50.4 Hz, 1H, C*H*F), 6.62 (dd, *J* = 8.1/2.7 Hz, 1H, 8-H), 6.65 (dd, *J* = 2.6 Hz, 1H, 6-H), 6.97 (d, *J* = 8.1 Hz, 1H, 9-H), 7.14-7.22 (m, 3H, 2-H_{phenyl}, 4-H_{phenyl}, 6-H_{phenyl}), 7.24-7.30 (m, 2H, 3-H_{phenyl}, 5-H_{phenyl}). ^{13}C NMR (101 MHz, $CDCl_3$): δ [ppm] = 32.2 (d, *J* = 4.7 Hz, 1C, $NCH_2CHFCH_2\mathit{C}H_2Ph$), 35.8 (1C, C-1), 36.6 (d, *J* = 20.9 Hz, 1C, $NCH_2CHF\mathit{C}H_2CH_2Ph$), 36.9 (1C, C-5), 55.6 (1C, OCH_3), 57.2 (1C, C-2 or C-4), 57.6 (1C, C-2 or C-4), 63.7 (d, *J* = 21.2 Hz, 1C, $NCH_2CHFCH_2CH_2Ph$), 92.4 (d, *J* = 169.9 Hz, 1C, *C*HF), 111.8 (1C, C-8), 115.9 (1C, C-6), 127.0 (1C, C-4$_{phenyl}$), 129.5 (4C, C-2$_{phenyl}$, C-3$_{phenyl}$, C-5$_{phenyl}$, C-6$_{phenyl}$), 130.8 (1C, C-9), 135.1 (1C, C-9a), 142.7 (1C, C-1$_{phenyl}$), 144.3 (1C, C-5a), 159.6 (1C, C-7). ^{19}F NMR (376 MHz, CD_3OD): δ [ppm] = -183.0 (m, 1F). HR-MS (APCI): m/z = 328.2080 (calcd. 328.2071 for $C_{21}H_{27}FNO$ $[M+H]^+$). Purity (HPLC): 98.9 %, t_R = 18.4 min. IR: ṽ [cm^{-1}] = 3024 (C-H_{aryl}), 2932 (C-$H_{aliph.}$), 1609, 1582, 1501 (C=$C_{arom.}$), 1454 (C-$H_{aliph.}$), 1262 (C-O).

7.3.9 3-(3-Fluoro-4-phenylbutyl)-7-methoxy-2,3,4,5-tetrahydro-1*H*-3-benzazepine (16b)

Secondary amine **14** (60.0 mg, 0.34 mmol, 1.0 eq) was dissolved in CH_3CN (3 mL). After addition of tosylate **7b** (109 mg, 0.34 mmol, 1.0 eq) in CH_3CN (2 mL) and K_2CO_3 (374 mg, 2.71 mmol, 8.0 eq), the suspension was heated to reflux for 70 h. At rt, K_2CO_3 was filtered off, washed with CH_3CN and the solvent was removed *in*

vacuo. The residue was purified by fc (d = 2 cm, l = 18 cm, V = 7 mL, cyclohexane:ethyl acetate = 7:3 + 0.5 % *N*,*N*-dimethylethanamine). Colorless oil, yield 88.7 mg (0.27 mmol, 80 %). $C_{21}H_{26}FNO$ (327.4). R_f = 0.28 (cyclohexane:ethyl acetate = 7:3). ^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] = 1.85-2.10 (m, 2H, $NCH_2CH_2CHFCH_2Ph$), 2.59-3.11 (m, 12H, 2 x 1-H, 2 x 2-H, 2 x 4-H, 2 x 5-H, $NCH_2CH_2CHFCH_2Ph$), 3.78 (s, 3H, OCH_3), 4.70-4.90 (dm, *J* = 48.9 Hz, 1H, *C*HF), 6.64-6.68 (m, 2H, 6-H, 8-H), 7.00 (d, *J* = 9.0 Hz, 1H, 9-H), 7.20-7.26 (m, 3H, 2-H_{phenyl}, 4-H_{phenyl}, 6-H_{phenyl}), 7.29-7.33 (m, 2H, 3-H_{phenyl}, 5-H_{phenyl}). ^{13}C NMR (101 MHz, $CDCl_3$): δ [ppm] = 31.5 (d, *J* = 23.5 Hz, 1C, $NCH_2CH_2CHFCH_2Ph$), 34.4 (1C, C-1), 35.6 (1C, C-5), 41.8 (d, *J* = 21.2 Hz, 1C, $NCH_2CH_2CHFCH_2Ph$), 55.0 (d, *J* = 3.7 Hz, 1C, $NCH_2CH_2CHFCH_2Ph$), 55.38 (1C, C-2 or C-4), 55.39 (1C, OCH_3), 55.8 (1C, C-2 or C-4), 93.0 (d, *J* = 171.1 Hz, 1C, CHF), 111.1 (1C, C-8), 115.2 (1C, C-6), 126.9 (1C, C-4$_{phenyl}$), 128.6 (2C, C-3$_{phenyl}$, C-5$_{phenyl}$), 129.5 (2C, C-2$_{phenyl}$, C-6$_{phenyl}$), 130.0 (1C, C-9), 133.5 (1C, C-9a), 136.9 (1C, C-1$_{phenyl}$), 142.6 (1C, C-5a), 158.4 (1C, C-7). ^{19}F NMR (376 MHz, $CDCl_3$): δ [ppm] = -180.7 (m, 1F). HR-MS (APCI): m/z = 328.2080 (calcd. 328.2071 for $C_{21}H_{27}FNO$ $[M+H]^+$). Purity (HPLC): 96.0 %, t_R = 17.9 min. IR: $\tilde{\nu}$ [cm^{-1}] = 3024 (C-H_{aryl}), 2928 (C-$H_{aliph.}$), 1609, 1582, 1505 (C=$C_{arom.}$), 1454 (C-$H_{aliph.}$), 1265 (C-O).

7.3.10. 7-Methoxy-3-(4-phenylbutyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepine (16c)

A mixture of secondary amine **14** (69.0 mg, 0.39 mmol, 1.0 eq), an excess of 4-phenylbutanal (**11c**) and $NaBH(OAc)_3$ (165 mg, 0.78 mmol, 2.0 eq) in CH_2Cl_2 (6 mL) was stirred for 24 h at rt. A saturated solution of $NaHCO_3$ (10 mL) was added, the organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (3 x 10 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated *in vacuo*. The crude product was purified by fc twice (1. d = 2 cm,

l = 17 cm, V = 7 mL, cyclohexane:ethyl acetate = 8:2 + 1 % *N*,*N*-dimethylethanamine; 2. d = 2 cm, l = 19 cm, V = 7 mL, cyclohexane:ethyl acetate = 9:1 + 1 % *N*,*N*-dimethylethanamine). Colorless oil, yield 14.8 mg (0.05 mmol, 12 %). $C_{21}H_{27}NO$ (309.4). R_f = 0.36 (cyclohexane:ethyl acetate = 8:2 + 1 % *N*,*N*-dimethylethanamine). ^{1}H NMR (600 MHz, $CDCl_3$): δ [ppm] = 1.53-1.59 (m, 2H, $NCH_2CH_2CH_2CH_2Ph$), 1.61-1.67 (m, 2H, $NCH_2CH_2CH_2CH_2Ph$), 2.47-2.51 (m, 2H, $NCH_2CH_2CH_2CH_2Ph$), 2.56-2.66 (m, 6H, 2 x 2-H, 2 x 4-H, $NCH_2CH_2CH_2CH_2Ph$), 2.85-2.89 (m, 4H, 2 x 1-H, 2 x 5-H), 3.78 (s, 3H, OCH_3), 6.64 (dd, *J* = 8.1/2.7 Hz, 1H, 8-H), 6.66 (d, *J* = 2.6 Hz, 1H, 6-H), 7.00 (d, *J* = 8.1 Hz, 1H, 9-H), 7.16-7.19 (m, 3H, 2-H_{phenyl}, 4-H_{phenyl}, 6-H_{phenyl}), 7.26-7.29 (m, 2H, 3-H_{phenyl}, 5-H_{phenyl}). ^{13}C NMR (151 MHz, $CDCl_3$): δ [ppm] = 26.7 (1C, $NCH_2CH_2CH_2CH_2Ph$), 29.6 (1C, $NCH_2CH_2CH_2CH_2Ph$), 35.8 (1C, C-1), 36.0 (1C, $NCH_2CH_2CH_2CH_2Ph$), 37.0 (1C, C-5), 55.4 (1C, OCH_3), 55.5 (1C, C-2 or C-4), 55.9 (1C, C-2 or C-4), 59.2 (1C, $NCH_2CH_2CH_2CH_2Ph$), 110.7 (1C, C-8), 115.1 (1C, C-6), 125.8 (1C, C-4_{phenyl}), 128.4 (2C, C-3_{phenyl}, C-5_{phenyl}), 128.5 (2C, C-2_{phenyl}, C-6_{phenyl}), 129.9 (1C, C-9), 134.6 (1C, C-9a), 142.6 (1C, C-1_{phenyl}), 143.6 (1C, C-5a), 158.1 (1C, C-7). HR-MS (APCI): m/z = 310.2161 (calcd. 310.2165 for $C_{21}H_{28}NO$ $[M+H]^+$). Purity (HPLC): 98.6 %, t_R = 18.5 min. IR: ṽ [cm^{-1}] = 3024 (C-H_{aryl}), 2932 (C-$H_{aliph.}$), 1609, 1582, 1497 (C=$C_{arom.}$), 1454 (C-$H_{aliph.}$), 1262 (C-O).

7.3.11. 3-(2-Fluoro-4-phenylbutyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepin-7-ol (17a)

A mixture of secondary amine **15** (90.0 mg, 0.55 mmol, 1.0 eq), an excess of freshly prepared crude aldehyde **11a** and $NaBH(OAc)_3$ (234 mg, 1.10 mmol, 2.0 eq) in CH_2Cl_2 (6 mL) was stirred for 16 h at rt. A saturated solution of $NaHCO_3$ (20 mL) was added, the organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (3 x 10 mL). The combined organic layers were dried (Na_2SO_4), filtered and

concentrated *in vacuo*. The crude product was purified by fc twice (1. d = 2 cm, l = 30 cm, V = 7 mL, CH_2Cl_2 + 1 % *N*,*N*-dimethylethanamine; 2. d = 2 cm, l = 20 cm, V = 7 mL, cyclohexane:ethyl acetate = 8:2 + 1 % *N*,*N*-dimethylethanamine). Colorless oil, yield 24.6 mg (0.08 mmol, 14 %). $C_{20}H_{24}FNO$ (313.4). R_f = 0.24 (cyclohexane:ethyl acetate = 7:3). ^{1}H NMR (600 MHz, CD_3OD): δ [ppm] = 1.81-1.98 (m, 2H, $NCH_2CHFC\mathit{H}_2CH_2Ph$), 2.60-2.87 (m, 12H, 2 x 1-H, 2 x 2-H, 2 x 4-H, 2 x 5-H, $NC\mathit{H}_2CHFCH_2C\mathit{H}_2Ph$), 4.63-4.78 (dm, *J* = 50.4 Hz, 1H, C*H*F), 6.50 (dd, *J* = 8.0/2.6 Hz, 1H, 8-H), 6.53 (d, *J* = 2.5 Hz, 1H, 6-H), 6.87 (d, *J* = 8.1 Hz, 1H, 9-H), 7.15-7.23 (m, 3H, 2-H_{phenyl}, 4-H_{phenyl}, 6-H_{phenyl}), 7.25-7.29 (m, 2H, 3-H_{phenyl}, 5-H_{phenyl}). A signal for the OH proton is not seen in the spectrum. ^{13}C NMR (151 MHz, CD_3OD): δ [ppm] = 32.2 (d, *J* = 4.7 Hz, 1C, $NCH_2CHFCH_2\mathit{C}H_2Ph$), 35.7 (1C, C-1), 36.7 (d, *J* = 20.9 Hz, 1C, $NCH_2CHF\mathit{C}H_2CH_2Ph$), 36.8 (1C, C-5), 57.2 (1C, C-2 or C-4), 57.7 (1C, C-2 or C-4), 63.7 (d, *J* = 20.8 Hz, 1C, $NCH_2CHFCH_2CH_2Ph$), 92.3 (d, *J* = 169.9 Hz, 1C, *C*HF), 113.5 (1C, C-8), 116.9 (1C, C-6), 127.0 (1C, C-4$_{phenyl}$), 129.476 (2C, C-2$_{phenyl}$, C-6$_{phenyl}$ or C-3$_{phenyl}$, C-5$_{phenyl}$), 129.483 (2C, C-2$_{phenyl}$, C-6$_{phenyl}$ or C-3$_{phenyl}$, C-5$_{phenyl}$), 130.8 (1C, C-9), 133.9 (1C, C-9a), 142.7 (1C, C-1$_{phenyl}$), 144.2 (1C, C-5a), 156.7 (1C, C-7). ^{19}F NMR (376 MHz, $CDCl_3$): δ [ppm] = -189.8 (m, 1F). HR-MS (APCI): m/z = 314.1896 (calcd. 314.1915 for $C_{20}H_{25}FNO$ $[M+H]^+$). Purity (HPLC): 87.6 %, t_R = 16.8 min. IR: ṽ [cm^{-1}] = 3210 (OH), 2947 (C-$H_{aliph.}$), 1605, 1582, 1501 (C=$C_{arom.}$), 1454 (C-$H_{aliph.}$), 1262 (C-O).

7.3.12. 3-(3-Fluoro-4-phenylbutyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepin-7-ol (17b) and 7-(3-Fluoro-4-phenylbutoxy)-3-(3-fluoro-4-phenylbutyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepine (18b)

Secondary amine **15** (90.0 mg, 0.55 mmol, 1.0 eq) was dissolved in CH_3CN (3 mL). After addition of tosylate **7b** (178 mg, 0.55 mmol, 1.0 eq) in CH_3CN (4 mL) and

K_2CO_3 (609 mg, 4.41 mmol, 8.0 eq), the suspension was heated to reflux for 50 h. At rt, K_2CO_3 was filtered off, washed with CH_3CN and the solvent was removed *in vacuo*. The crude product was purified by fc twice (1. d = 2 cm, l = 19 cm, V = 7 mL, CH_2Cl_2:CH_3OH = 99:1 + 1 % *N*,*N*-dimethylethanamine; 2. d = 2 cm, l = 24 cm, V = 7 mL, cyclohexane:ethyl acetate = 8:2 + 1 % *N*,*N*-dimethylethanamine).

18b: Pale yellow oil, yield 36.3 mg (0.08 mmol, 14 %). $C_{30}H_{35}F_2NO$ (463.6). R_f = 0.60 (cyclohexane:ethyl acetate = 1:1 + 1 % *N*,*N*-dimethylethanamine). ^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] = 1.87-2.15 (m, 4H, $NCH_2C\mathit{H}_2CHFCH_2Ph$, $OCH_2C\mathit{H}_2CHFCH_2Ph$'), 2.64-3.10 (m, 14H, 2 x 1-H, 2 x 2-H, 2 x 4-H, 2 x 5-H, $NC\mathit{H}_2CH_2CHFC\mathit{H}_2Ph$, $OCH_2CH_2CHFC\mathit{H}_2Ph$'), 4.03-4.12 (m, 2H, $OC\mathit{H}_2CH_2CHFCH_2Ph$'), 4.70-4.89 (dm, *J* = 50.4 Hz, 1H, $NCH_2CH_2C\mathit{H}FCH_2Ph$), 4.85-5.06 (dm, *J* = 48.7 Hz, 1H, $OCH_2CH_2C\mathit{H}FCH_2Ph$'), 6.63-6.68 (m, 2H, 6-H, 8-H), 6.99 (d, *J* = 8.6 Hz, 1H, 9-H), 7.20-7.27 (m, 6H, 2-H_{phenyl}, 4-H_{phenyl}, 6-H_{phenyl}, 2-$H_{phenyl'}$, 4-$H_{phenyl'}$, 6-$H_{phenyl'}$), 7.29-7.35 (m, 4H, 3-H_{phenyl}, 5-H_{phenyl}, 3-$H_{phenyl'}$, 5-$H_{phenyl'}$). ^{13}C NMR (101 MHz, $CDCl_3$): δ [ppm] = 31.4 (d, *J* = 21.4 Hz, 1C, $NCH_2\mathit{C}H_2CHFCH_2Ph$), 34.3 (1C, C-1), 34.7 (d, *J* = 21.0 Hz, 1C, $OCH_2\mathit{C}H_2CHFCH_2Ph$'), 35.4 (1C, C-5), 41.78 (d, *J* = 21.2 Hz, 1C, $NCH_2CH_2CHF\mathit{C}H_2Ph$), 41.80 (d, *J* = 21.2 Hz, 1C, $OCH_2CH_2CHF\mathit{C}H_2Ph$'), 55.0 (d, *J* = 3.9 Hz, 1C, $N\mathit{C}H_2CH_2CHFCH_2Ph$), 55.3 (1C, C-2 or C-4), 55.7 (1C, C-2 or C-4), 63.9 (d, *J* = 4.6 Hz, 1C, $O\mathit{C}H_2CH_2CHFCH_2Ph$'), 91.4 (d, *J* = 171.1 Hz, 1C, $OCH_2CH_2\mathit{C}HFCH_2Ph$'), 92.6 (d, *J* = 171.7 Hz, 1C, $NCH_2CH_2\mathit{C}HFCH_2Ph$), 112.0 (1C, C-8), 115.8 (1C, C-6), 126.8 (1C, C-4_{phenyl} or C-$4_{phenyl'}$), 126.9 (1C, C-4_{phenyl} or C-$4_{phenyl'}$), 128.61 (2C, C-2_{phenyl}, C-6_{phenyl} or C-$2_{phenyl'}$, C-$6_{phenyl'}$), 128.64 (2C, C-2_{phenyl}, C-6_{phenyl} or C-$2_{phenyl'}$, C-$6_{phenyl'}$), 129.5 (2C, C-3_{phenyl}, C-5_{phenyl} or C-$3_{phenyl'}$, C-$5_{phenyl'}$), 129.6 (2C, C-3_{phenyl}, C-5_{phenyl} or C-$3_{phenyl'}$, C-$5_{phenyl'}$), 130.0 (1C, C-9), 133.6 (1C, C-9a), 136.9 (d, *J* = 4.4 Hz, 1C, C-1_{phenyl} or C-$1_{phenyl'}$), 137.0 (d, *J* = 4.5 Hz, 1C, C-1_{phenyl} or C-$1_{phenyl'}$), 142.7 (1C, C-5a), 157.5

(1C, C-7). ^{19}F NMR (376 MHz, $CDCl_3$): δ [ppm] = -182.2 (m, 1F), -180.7 (m, 1F). HR-MS (APCI): m/z = 464.2787 (calcd. 464.2759 for $C_{30}H_{36}F_2NO$ $[M+H]^+$). Purity (HPLC): 94.5 %, t_R = 22.2 min. IR: ṽ [cm^{-1}] = 3028 (C-H_{aryl}), 2924 (C-$H_{aliph.}$), 1605, 1582, 1497 (C=$C_{arom.}$), 1454 (C-$H_{aliph.}$), 1258 (C-O).

17b: Colorless solid, mp 128 °C, yield 49.1 mg (0.16 mmol, 28 %). $C_{20}H_{24}FNO$ (313.4). R_f = 0.20 (cyclohexane:ethyl acetate = 1:1 + 1 % *N*,*N*-dimethylethanamine). ^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] = 1.85-2.05 (m, 2H, $NCH_2C\mathit{H}_2CHFCH_2Ph$), 2.57-3.05 (m, 12H, 2 x 1-H, 2 x 2-H, 2 x 4-H, 2 x 5-H, $NC\mathit{H}_2CH_2CHFC\mathit{H}_2Ph$), 4.66-4.87 (dm, *J* = 49.3 Hz, 1H, C*H*F), 6.58-6.64 (m, 2H, 6-H, 8-H), 6.91 (d, *J* = 8.7 Hz, 1H, 9-H), 7.18-7.26 (m, 3H, 2-H_{phenyl}, 4-H_{phenyl}, 6-H_{phenyl}), 7.27-7.33 (m, 2H, 3-H_{phenyl}, 5-H_{phenyl}). A signal for the OH proton is not seen in the spectrum. ^{13}C NMR (101 MHz, $CDCl_3$): δ [ppm] = 31.3 (d, *J* = 21.6 Hz, 1C, $NCH_2\mathit{C}H_2CHFCH_2Ph$), 34.2 (2C, C-1, C-5), 41.7 (d, *J* = 22.9 Hz, 1C, $NCH_2CH_2CHF\mathit{C}H_2Ph$), 54.9 (d, *J* = 4.7 Hz, 1C, $N\mathit{C}H_2CH_2CHFCH_2Ph$), 55.3 (1C, C-3 or C-4), 55.8 (1C, C-3 or C-4), 92.9 (d, *J* = 170.8 Hz, 1C, *C*HF), 113.3 (1C, C-8), 116.4 (1C, C-6), 126.9 (1C, C-4$_{phenyl}$), 128.7 (2C, C-3$_{phenyl}$, C-5$_{phenyl}$), 129.5 (2C, C-2$_{phenyl}$, C-6$_{phenyl}$), 130.2 (1C, C-9), 132.9 (1C, C-9a), 136.9 (1C, C-1$_{phenyl}$), 142.0 (1C, C-5a), 154.7 (1C, C-7). ^{19}F NMR (376 MHz, $CDCl_3$): δ [ppm] = -180.8 (m, 1F). HR-MS (APCI): m/z = 314.1902 (calcd. 314.1915 for $C_{20}H_{25}FNO$ $[M+H]^+$). Purity (HPLC): 95.5 %, t_R = 16.6 min. IR: ṽ [cm^{-1}] = 3175 (OH), 3024 (C-H_{aryl}), 2924 (C-$H_{aliph.}$), 1609, 1586, 1497 (C=$C_{arom.}$), 1451 (C-$H_{aliph.}$).

7.3.13. 3-(4-Phenylbutyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepin-7-ol (17c)

A mixture of secondary amine **15** (80.0 mg, 0.49 mmol, 1.1 eq), 4-phenylbutanal (**11c**, 68.5 mg, 0.46 mmol, 1.0 eq) and $NaBH(OAc)_3$ (148 mg, 0.70 mmol, 1.5 eq) in CH_2Cl_2 (5 mL) was stirred for 18 h at rt. A saturated solution of $NaHCO_3$ (10 mL) was added, the organic layer was separated and the aqueous layer was extracted with

CH_2Cl_2 (3 x 10 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated *in vacuo*. The crude product was purified by fc (d = 2 cm, l = 16 cm, V = 7 mL, cyclohexane:ethyl acetate = 6:4 + 1 % *N*,*N*-dimethylethanamine). Colorless solid, mp 123 °C, yield 92.3 mg (0.31 mmol, 68 %). $C_{20}H_{25}NO$ (295.4). R_f = 0.28 (cyclohexane:ethyl acetate = 1:1 + 1 % *N*,*N*-dimethylethanamine). ^{1}H NMR (400 MHz, CD_3OD): δ [ppm] = 1.51-1.66 (m, 4H, $NCH_2CH_2CH_2CH_2Ph$), 2.45-2.50 (m, 2H, $NCH_2CH_2CH_2CH_2Ph$), 2.53-2.65 (m, 6H, 2 x 2-H, 2 x 4-H, $NCH_2CH_2CH_2CH_2Ph$), 2.78-2.81 (m, 4H, 2 x 1-H, 2 x 5-H), 6.50 (dd, *J* = 8.0/2.6 Hz, 1H, 8-H), 6.54 (d, *J* = 2.5 Hz, 1H, 6-H), 6.87 (d, *J* = 8.0 Hz, 1H, 9-H), 7.12-7.19 (m, 3H, 2-H_{phenyl}, 4-H_{phenyl}, 6-H_{phenyl}), 7.22-7.27 (m, 2H, 3-H_{phenyl}, 5-H_{phenyl}). A signal for the OH proton is not seen in the spectrum. ^{13}C NMR (101 MHz, CD_3OD): δ [ppm] = 26.9 (1C, $NCH_2CH_2CH_2CH_2Ph$), 30.7 (1C, $NCH_2CH_2CH_2CH_2Ph$), 35.5 (1C, C-1), 36.5 (1C, C-5), 36.7 (1C, $NCH_2CH_2CH_2CH_2Ph$), 56.6 (1C, C-2 or C-4), 57.1 (1C, C-2 or C-4), 60.0 (1C, $NCH_2CH_2CH_2CH_2Ph$), 113.6 (1C, C-8), 116.9 (1C, C-6), 126.8 (1C, C-4_{phenyl}), 129.3 (2C, C-3_{phenyl}, C-5_{phenyl}), 129.4 (2C, C-2_{phenyl}, C-6_{phenyl}), 130.8 (1C, C-9), 133.8 (1C, C-9a), 143.6 (1C, C-1_{phenyl}), 144.1 (1C, C-5a), 156.8 (1C, C-7). HR-MS (APCI): m/z = 296.1989 (calcd. 296.2009 for $C_{20}H_{26}NO$ $[M+H]^+$). Purity (HPLC): 97.9 %, t_R = 16.7 min. IR: $\tilde{\nu}$ [cm^{-1}] = 3028 (C-H_{aryl}), 2924 (C-$H_{aliph.}$), 1605, 1493 (C=$C_{arom.}$), 1447 (C-$H_{aliph.}$), 1238 (C-O).

7.3.14. 3-(3-Fluoro-4-phenylbutyl)-7-(2-fluoroethoxy)-2,3,4,5-tetrahydro-1*H*-3-benzazepine (19b)

A mixture of phenol **17b** (18.0 mg, 0.06 mmol, 1.0 eq), K_2CO_3 (47.6 mg, 0.35 mmol, 6.0 eq) and (2-fluoroethyl) tosylate (13.8 mg, 0.06 mmol, 1.1 eq) in DMF (2 mL) was stirred for 19 h at 80 °C. Then, water (100 mL) was added and the mixture was extracted with CH_2Cl_2 (3 x 10 mL). The combined organic layers were dried (Na_2SO_4)

and concentrated *in vacuo*. The crude product was purified by fc (d = 1 cm, l = 19 cm, V = 4 mL, cyclohexane:ethyl acetate = 7:3 + 1 % *N*,*N*-dimethylethanamine). Pale yellow oil, yield 9.3 mg (0.03 mmol, 45 %). $C_{22}H_{27}F_2NO$ (359.5). R_f = 0.44 (cyclohexane:ethyl acetate = 6:4 + 1 % *N*,*N*-dimethylethanamine). ^{1}H NMR (600 MHz, $CDCl_3$): δ [ppm] = 1.90-2.09 (m, 2H, $NCH_2CH_2CHFCH_2Ph$), 2.61-3.16 (m, 12H, 2 x 1-H, 2 x 2-H, 2 x 4-H, 2 x 5-H, $NCH_2CH_2CHFCH_2Ph$), 4.18 (dt, *J* = 27.9/4.2 Hz, 2H, OCH_2CH_2F), 4.73 (dt, *J* = 47.6/4.2 Hz, 2H, OCH_2CH_2F), 4.73-4.86 (dm, *J* = 48.7 Hz, 1H, C*H*F), 6.68 (dd, *J* = 8.2/2.1 Hz, 1H, 8-H), 6.70 (d, *J* = 2.5 Hz, 1H, 6-H), 7.01 (d, *J* = 8.1 Hz, 1H, 9-H), 7.21-7.25 (m, 3H, 2-H_{phenyl}, 4-H_{phenyl}, 6-H_{phenyl}), 7.30-7.33 (m, 2H, 3-H_{phenyl}, 5-H_{phenyl}). ^{13}C NMR (151 MHz, $CDCl_3$): δ [ppm] = 31.2 (d, *J* = 20.9 Hz, 1C, $NCH_2CH_2CHFCH_2Ph$), 34.2 (1C, C-1), 35.3 (1C, C-5), 41.8 (d, *J* = 23.9 Hz, 1C, $NCH_2CH_2CHFCH_2Ph$), 55.0 (d, *J* = 4.9 Hz, 1C, $NCH_2CH_2CHFCH_2Ph$), 55.3 (1C, C-2 or C-4), 55.7 (1C, C-2 or C-4), 67.3 (d, *J* = 20.6 Hz, 1C, OCH_2CH_2F), 82.1 (d, *J* = 170.7 Hz, 1C, OCH_2CH_2F), 92.9 (d, *J* = 171.3 Hz, 1C, *C*HF), 112.0 (1C, C-8), 116.0 (1C, C-6), 126.9 (1C, C-4$_{phenyl}$), 128.7 (2C, C-3$_{phenyl}$, C-5$_{phenyl}$), 129.5 (2C, C-2$_{phenyl}$, C-6$_{phenyl}$), 130.1 (1C, C-9), 133.8 (1C, C-9a), 136.8 (1C, C-1$_{phenyl}$), 142.5 (1C, C-5a), 157.2 (d, *J* = 6.5 Hz, 1C, C-7). ^{19}F NMR (376 MHz, $CDCl_3$): δ [ppm] = -224.4 (m, 1F, CH_2F), -180.8 (m, 1F, CHF). HR-MS (APCI): m/z = 360.2114 (calcd. 360.2133 for $C_{22}H_{28}F_2NO$ $[M+H]^+$). Purity (HPLC): 85.9 %, t_R = 18.9 min. IR: ṽ [cm^{-1}] = 3028 (C-H_{aryl}), 2924 (C-$H_{aliph.}$), 1609, 1582 (C=$C_{arom.}$), 1454 (C-$H_{aliph.}$), 1262 (C-O), 745, 702 (monosubst. arom.).

7.3.15. 3-(3-Fluoro-4-phenylbutyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepine (21b)

2,3,4,5-Tetrahydro-1*H*-3-benzazepine (**20**, 100 mg, 0.68 mmol, 1.0 eq) was dissolved in CH_3CN (6 mL). Then K_2CO_3 (751 mg, 5.43 mmol, 8.0 eq) and tosylate **7b** (219 mg, 0.68 mmol, 1.0 eq) were added and the mixture was heated to reflux for 70 h. The

suspension was filtered and the solvent was removed *in vacuo*. The crude product was purified three times by fc (1. d = 2 cm, l = 18 cm, V = 7 mL, cyclohexane:ethyl acetate = 8:2 + 1 % *N*,*N*-dimethylethanamine; 2. d = 1 cm, l = 20 cm, V = 4 mL, CH_2Cl_2:CH_3OH = 99:1 + 1 % *N*,*N*-dimethylethanamine; 3. d = 1 cm, l = 18 cm, V = 4 mL, cyclohexane:ethyl acetate = 9:1 + 1 % *N*,*N*-dimethylethanamine). Yellow oil, yield 20.1 mg (0.07 mmol, 10 %). $C_{20}H_{24}FN$ (297.4). R_f = 0.40 (cyclohexane:ethyl acetate = 8:2 + 1 % *N*,*N*-dimethylethanamine). ^{1}H NMR (600 MHz, $CDCl_3$): δ [ppm] = 1.84-1.94 (m, 2H, $NCH_2C\mathit{H}_2CHFCH_2Ph$), 2.63-2.80 (m, 6H, 2 x 2-H, 2 x 4-H, $NC\mathit{H}_2CH_2CHFCH_2Ph$), 2.88-3.04 (m, 6H, 2 x 1-H, 2 x 5-H, $NCH_2CH_2CHFC\mathit{H}_2Ph$), 4.74-4.88 (dm, *J* = 48.6 Hz, 1H, C*H*F), 7.09-7.11 (m, 2H, 6-H, 9-H), 7.12-7.14 (m, 2H, 7-H, 8-H), 7.22-7.26 (m, 3H, 2-H_{phenyl}, 4-H_{phenyl}, 6-H_{phenyl}), 7.30-7.33 (m, 2H, 3-H_{phenyl}, 5-H_{phenyl}). ^{13}C NMR (151 MHz, $CDCl_3$): δ [ppm] = 31.9 (d, *J* = 16.1 Hz, 1C, $NCH_2\mathit{C}H_2CHFCH_2Ph$), 36.1 (2C, C-1, C-5), 41.9 (d, *J* = 21.4 Hz, 1C, $NCH_2CH_2CHF\mathit{C}H_2Ph$), 54.9 (d, *J* = 3.7 Hz, 1C, $N\mathit{C}H_2CH_2CHFCH_2Ph$), 55.4 (2C, C-2, C-4), 93.2 (d, *J* = 172.7 Hz, 1C, *C*HF), 126.5 (2C, C-7, C-8), 126.8 (1C, C-4_{phenyl}), 128.6 (2C, C-3_{phenyl}, C-5_{phenyl}), 129.0 (2C, C-6, C-9), 129.5 (2C, C-2_{phenyl}, C-6_{phenyl}), 137.1 (1C, C-1_{phenyl}), 141.7 (2C, C-5a, C-9a). ^{19}F NMR (376 MHz, $CDCl_3$): δ [ppm] = -180.5 (m, 1F). HR-MS (APCI): m/z = 298.1957 (calcd. 298.1966 for $C_{20}H_{25}FN$ $[M+H]^+$). Purity (HPLC): 98.6 %, t_R = 18.7 min. IR: ṽ [cm^{-1}] = 3024 (C-H_{aryl}), 2947, 2916 (C-$H_{aliph.}$), 1605, 1586, 1493 (C=$C_{arom.}$), 1454 (C-$H_{aliph.}$).

7.3.16. 3-(3-Fluoro-4-phenylbutyl)-7-methoxy-2,3,4,5-tetrahydro-3-benzazepin-1-one (22b)

A solution of alcohol **12b** (90.0 mg, 0.26 mmol, 1.0 eq) in CH_2Cl_2 (2 mL) was added dropwise to a solution of Dess-Martin Periodinane (122 mg, 0.29 mmol, 1.1 eq) in CH_2Cl_2 (1 mL). The reaction mixture was stirred for 1.5 h at rt. Then, 10 % $Na_2S_2O_3$

(5 mL) and a saturated solution of $NaHCO_3$ (5 mL) were added and the mixture was stirred for 15 min. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (2 x 5 mL) and ethyl acetate (2 x 5 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated *in vacuo*. The crude product was purified by fc (d = 2 cm, l = 16 cm, V = 10 mL, cyclohexane:ethyl acetate = 9:1 + 1 % *N*,*N*-dimethylethanamine). Yellow oil, yield 20.4 mg (0.07 mmol, 25 %). $C_{21}H_{24}FNO_2$ (341.4). R_f = 0.50 (cyclohexane:ethyl acetate = 7:3 + 1 % *N*,*N*-dimethylethanamine). ^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] = 1.61-1.83 (m, 2H, $NCH_2CH_2CHFCH_2Ph$), 2.64-2.96 (m, 8H, 2 x 4-H, 2 x 5-H, $NCH_2CH_2CHFCH_2Ph$), 3.48 (s, 2H, 2 x 2-H), 3.85 (s, 3H, OCH_3), 4.53-4.71 (dm, *J* = 48.6 Hz, 1H, C*H*F), 6.70 (d, *J* = 2.5 Hz, 1H, 6-H), 6.84 (dd, *J* = 8.6/2.5 Hz, 1H, 8-H), 7.07-7.11 (m, 2H, 2-H_{phenyl}, 6-H_{phenyl}), 7.19-7.29 (m, 3H, 3-H_{phenyl}, 4-H_{phenyl}, 5-H_{phenyl}), 7.73 (d, *J* = 8.6 Hz, 1H, 9-H).

^{13}C NMR (101 MHz, $CDCl_3$): δ [ppm] = 32.7 (d, *J* = 21.0 Hz, 1C, $NCH_2CH_2CHFCH_2Ph$), 33.8 (1C, C-5), 41.7 (d, *J* = 21.5 Hz, 1C, $NCH_2CH_2CHFCH_2Ph$), 52.6 (1C, C-4), 53.0 (d, *J* = 4.5 Hz, 1C, $NCH_2CH_2CHFCH_2Ph$), 55.5 (1C, OCH_3), 62.2 (1C, C-2), 92.8 (d, *J* = 170.7 Hz, 1C, C*H*F), 112.1 (1C, C-8), 114.9 (1C, C-6), 126.7 (1C, C-4$_{phenyl}$), 128.5 (2C, C-3$_{phenyl}$, C-5$_{phenyl}$), 129.4 (2C, C-2$_{phenyl}$, C-6$_{phenyl}$), 130.8 (1C, C-9), 131.3 (1C, C-9a), 137.2 (d, *J* = 4.9 Hz, 1C, C-1$_{phenyl}$), 142.1 (1C, C-5a), 163.2 (1C, C-7), 203.9 (1C, C-1). ^{19}F NMR (376 MHz, $CDCl_3$): δ [ppm] = -180.0 (m, 1F). HR-MS (APCI): m/z = 342.1838 (calcd. 342.1864 for $C_{21}H_{25}FNO_2$ $[M+H]^+$). IR: ṽ [cm^{-1}] = 3028 (C-H_{aryl}), 2940 (C-$H_{aliph.}$), 1655 (C=O), 1601, 1582, 1497 (C=$C_{arom.}$), 1454 (C-$H_{aliph.}$), 1238 (C-O).

7.3.17. 7-Methoxy-3-(4-phenylbutyl)-2,3,4,5-tetrahydro-3-benzazepin-1-one (22c)

A solution of alcohol **12c** (95 mg, 0.29 mmol, 1.0 eq) in CH_2Cl_2 (3 mL) was added

dropwise to a solution of Dess-Martin Periodinane (149 mg, 0.35 mmol, 1.2 eq) in CH_2Cl_2 (1 mL). The reaction mixture was stirred for 1 h at rt. Then, 10 % $Na_2S_2O_3$ (5 mL) and a saturated solution of $NaHCO_3$ (5 mL) were added and the mixture was stirred for 5 min. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (3 x 10 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated *in vacuo*. The crude product was purified by fc (d = 2 cm, l = 16 cm, V = 7 mL, cyclohexane:ethyl acetate = 9:1 + 1 % *N*,*N*-dimethylethanamine). Yellow resin, yield 30.1 mg (0.09 mmol, 32 %). $C_{21}H_{25}NO_2$ (323.4). R_f = 0.36 (cyclohexane:ethyl acetate = 7:3 + 1 % *N*,*N*-dimethylethanamine). ^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] = 1.47-1.65 (m, 4H, $NCH_2CH_2CH_2CH_2Ph$), 2.53-2.65 (m, 4H, $NCH_2CH_2CH_2CH_2Ph$), 2.87-2.96 (m, 4H, 2 x 4-H, 2 x 5-H), 3.49 (s, 2H, 2-CH_2), 3.85 (s, 3H, OCH_3), 6.70 (d, *J* = 2.5 Hz, 1H, 6-H), 6.83 (dd, *J* = 8.6/2.5 Hz, 1H, 8-H), 7.07-7.10 (m, 2H, 2-H_{phenyl}, 6-H_{phenyl}), 7.13-7.25 (m, 3H, 3-H_{phenyl}, 4-H_{phenyl}, 5-H_{phenyl}), 7.74 (d, *J* = 8.6 Hz, 1H, 9-H). ^{13}C NMR (101 MHz, $CDCl_3$): δ [ppm] = 27.0 (1C, $NCH_2CH_2CH_2CH_2Ph$), 29.1 (1C, $NCH_2CH_2CH_2CH_2Ph$), 33.9 (1C, C-5), 35.8 (1C, $NCH_2CH_2CH_2CH_2Ph$), 52.6 (1C, C-4), 55.5 (1C, OCH_3), 57.1 (1C, $NCH_2CH_2CH_2CH_2Ph$), 62.3 (1C, C-2), 112.1 (1C, C-8), 114.8 (1C, C-6), 125.8 (1C, C-4_{phenyl}), 128.4 (2C, C-3_{phenyl}, C-5_{phenyl}), 128.5 (2C, C-2_{phenyl}, C-6_{phenyl}), 130.8 (1C, C-9), 131.4 (1C, C-9a), 142.3 (1C, C-4a), 142.6 (1C, C-1_{phenyl}), 163.2 (1C, C-7), 204.3 (1C, C-1). HR-MS (APCI): m/z = 324.1954 (calcd. 324.1958 for $C_{21}H_{26}NO_2$ $[M+H]^+$). IR: ṽ [cm^{-1}] = 3024 (C-H_{aryl}), 2932 (C-$H_{aliph.}$), 1663 (C=O), 1601, 1566, 1497 (C=$C_{arom.}$), 1454 (C-$H_{aliph.}$), 1234 (C-O).

7.4. Receptor binding studies

7.4.1 Materials

Guinea pig brains, rat brains and rat livers were commercially available (Harlan-

Winkelmann, Borchen, Germany). Pig brains were a donation of the local slaughterhouse (Coesfeld, Germany). The recombinant L(tk-) cells stably expressing the GluN2B receptor were obtained from Prof. Dr. Dieter Steinhilber (Frankfurt, Germany). Homogenizers: Elvehjem Potter (B. Braun Biotech International, Melsungen, Germany) and Soniprep® 150 (MSE, London, UK). Centrifuges: Cooling centrifuge Eppendorf 5424R (Eppendorf, Hamburg, Germany) and High-speed cooling centrifuge model Sorvall® RC-5C plus (Thermo Fisher Scientific, Langenselbold, Germany). Multiplates: standard 96 well multiplates (Diagonal, Muenster, Germany). Shaker: self-made device with adjustable temperature and tumbling speed (scientific workshop of the institute). Harvester: MicroBeta® FilterMate 96 Harvester. Filter: Printed Filtermat Typ A and B. Scintillator: Meltilex® (Typ A or B) solid state scintillator. Scintillation analyzer: MicroBeta® Trilux (all Perkin Elmer LAS, Rodgau-Jügesheim, Germany).

7.4.2. Cell culture and preparation of membrane homogenates for the GluN2B.[19]

Mouse L(tk-) cells stably transfected with the dexamethasone-inducible eukaryotic expression vectors pMSG GluN1a, pMSG GluN2B (1:5 ratio) were grown in Modified Earl's Medium (MEM) containing 10 % of standardized FCS (Biochrom AG, Berlin, Germany). The expression of the NMDA receptor at the cell surface was induced after the cell density of the adherent growing cells had reached approximately 90 % of confluency. For the induction, the original growth medium was replaced by growth medium containing 4 µM dexamethasone and 4 µM ketamine (final concentration). After 24 h, the cells were rinsed with phosphate buffered saline solution (PBS, Biochrom AG, Berlin, Germany), harvested by mechanical detachment and pelleted (10 min, 5.000 x g).

For the binding assay, the cell pellet was resuspended in PBS solution and the

number of cells was determined using a Scepter® cell counter (MERCK Millipore, Darmstadt, Germany). Subsequently, the cells were lysed by sonication (4 °C, 6 x 10 s cycles with breaks of 10 s). The resulting cell fragments were centrifuged with a high performance cool centrifuge (23.500 x g, 4 °C). The supernatant was discarded and the pellet was resuspended in a defined volume of PBS yielding cell fragments of approximately 500.000 cells/mL. The suspension of membrane homogenates was sonicated again (4 °C, 2 x 10 s cycles with a break of 10 s) and stored at -80 °C.

7.4.3. Preparation of membrane homogenates from pig brain cortex

Fresh pig brain cortex was homogenized with the potter (500-800 rpm, 10 up and down strokes) in 6 volumes of cold 0.32 M sucrose. The suspension was centrifuged at 1,200 x g for 10 min at 4 °C. The supernatant was separated and centrifuged at 31,000 x g for 20 min at 4 °C. The pellet was resuspended in 5-6 volumes of TRIS/EDTA buffer (5 mM TRIS/1 mM EDTA, pH 7.5) and centrifuged again at 31,000 x g (20 min, 4 °C). The final pellet was resuspended in 5-6 volumes of buffer and frozen (-80 °C) in 1.5 mL portions containing about 0.8 mg protein/mL.

7.4.4. Preparation of membrane homogenates from rat liver

Two rat livers were cut into small pieces and homogenized with the potter (500-800 rpm, 10 up and down strokes) in 6 volumes of cold 0.32 M sucrose. The suspension was centrifuged at 1,200 x g for 10 min at 4 °C. The supernatant was separated and centrifuged at 31,000 x g for 20 min at 4 °C. The pellet was resuspended in 5-6 volumes of buffer (50 mM TRIS, pH 8.0) and incubated at rt for 30 min. After the incubation, the suspension was centrifuged again at 31,000 x g for 20 min at 4 °C. The final pellet was resuspended in 5-6 volumes of buffer and stored

at -80 °C in 1.5 mL portions containing about 2 mg protein/mL.

7.4.5. Protein determination

The protein concentration was determined by the method of Bradford,[35] modified by Stoscheck.[36] The Bradford solution was prepared by dissolving 5 mg of Coomassie Brilliant Blue G 250 in 2.5 mL of EtOH (95 %, v/v). 10 mL deionized H_2O and 5 mL phosphoric acid (85 %, m/v) were added to this solution, the mixture was stirred and filled to a total volume of 50 mL with deionized water. The calibration was carried out using bovine serum albumin as a standard in 9 concentrations (0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.5, 2.0 and 4.0 mg/mL). In a 96 well standard multiplate, 10 µL of the calibration solution or 10 µL of the membrane receptor preparation were mixed with 190 µL of the Bradford solution, respectively. After 5 min, the UV absorption of the protein-dye complex at λ = 595 nm was measured with a plate reader (Tecan Genios®, Tecan, Crailsheim, Germany).

7.4.6. General protocol for the binding assay[19]

The test compound solutions were prepared by dissolving approximately 10 µmol (usually 2-4 mg) of test compound in DMSO so that a 10 mM stock solution was obtained. To obtain the required test solutions for the assay, the DMSO stock solution was diluted with the respective assay buffer. The filtermats were presoaked in 0.5% aqueous polyethylenimine solution for 2 h at room temperature before use. All binding experiments were carried out in duplicates in 96 well multiplates. The concentrations given are the final concentrations in the assay. Generally, the assays were performed by addition of 50 µL of the respective assay buffer, 50 µL test compound solution in various concentrations (10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9} and 10^{-10} mol/L), 50 µL of corresponding radioligand solution and 50 µL of the respective

receptor preparation into each well of the multiplate (total volume 200 µL). The receptor preparation was always added last. During the incubation, the multiplates were shaken at a speed of 500-600 rpm at the specified temperature. Unless otherwise noted, the assays were terminated after 120 min by rapid filtration using the harvester. During the filtration each well was washed five times with 300 µL of water. Subsequently, the filtermats were dried at 95 °C. The solid scintillator was melted on the dried filtermats at a temperature of 95 °C for 5 min. After solidifying of the scintillator at room temperature, the trapped radioactivity in the filtermats was measured with the scintillation analyzer. Each position on the filtermat corresponding to one well of the multiplate was measured for 5 min with the [^{3}H]-counting protocol. The overall counting efficiency was 20 %. The IC_{50}-values were calculated with the program GraphPad Prism® 3.0 (GraphPad Software, San Diego, CA, USA) by non-linear regression analysis. Subsequently, the IC_{50} values were transformed into K_i-values using the equation of Cheng and Prusoff.[37] The K_i-values are given as mean value ± SEM from three independent experiments.

Performance of the binding assays

7.4.7. GluN2B binding site of the NMDA receptor[19]

The competitive binding assay was performed with the radioligand [^{3}H]ifenprodil (60 Ci/mmol; BIOTREND, Cologne, Germany). The thawed cell membrane preparation from the transfected L(tk-) cells (about 20 µg protein) was incubated with various concentrations of test compounds, 5 nM [^{3}H]ifenprodil, and TRIS/EDTA-buffer (5 mM TRIS/1 mM EDTA, pH 7.5) at 37 °C. The non-specific binding was determined with 10 µM unlabeled ifenprodil. The K_d value of ifenprodil is 7.6 nM.

7.4.8. PCP binding site of the NMDA receptor[49,50]

The assay was performed with the radioligand [^{3}H]-(+)-MK-801 (22.0 Ci/mmol; Perkin Elmer). The thawed membrane preparation of pig brain cortex (about 100 µg of the protein) was incubated with various concentrations of test compounds, 2 nM [^{3}H]-(+)-MK-801, and TRIS/EDTA buffer (5 mM TRIS/1 mM EDTA, pH 7.5) at room temperature. The non-specific binding was determined with 10 µM unlabeled (+)-MK-801. The K_d value of (+)-MK-801 is 2.26 nM.

7.4.9. σ_1 receptor[51]

The assay was performed with the radioligand [^{3}H]-(+)-pentazocine (22.0 Ci/mmol; Perkin Elmer). The thawed membrane preparation of guinea pig brain cortex (about 100 µg of the protein) was incubated with various concentrations of test compounds, 2 nM [^{3}H]-(+)-pentazocine, and TRIS buffer (50 mM, pH 7.4) at 37 °C. The non-specific binding was determined with 10 µM unlabeled (+)-pentazocine. The K_d value of (+)-pentazocine is 2.9 nM.

7.4.10. σ_2 receptor[52]

The assays were performed with the radioligand [^{3}H]di-*o*-tolylguanidine (specific activity 50 Ci/mmol; ARC, St. Louis, MO, USA). The thawed rat liver membrane preparation (about 100 µg protein) was incubated with various concentrations of the test compound, 3 nM [^{3}H]di-*o*-tolylguanidine and buffer containing (+)-pentazocine (500 nM (+)-pentazocine in TRIS buffer (50 mM TRIS, pH 8.0)) at rt. The non-specific binding was determined with 10 µM non-labeled di-*o*-tolylguanidine. The K_d value of di-*o*-tolylguanidine is 17.9 nM.

7.4.11. Performance of the binding assay for the human MOR

Transfected CHO-K1 cell membranes (20 µg) were incubated with [^{3}H]DAMGO (1

nM) in assay buffer containing 50 mM Tris-HCl and 5 mM $MgCl_2$ at pH 7.4. NSB (non-specific binding) was measured by adding 10 µM naloxone. The binding of the test compound was measured at five different concentrations. Plates were incubated at 27 °C for 60 min. After the incubation period, the reaction mixture was then transferred to MultiScreen HTS, FC plates (Millipore), filtered and plates were washed 3 times with ice-cold 10 mM Tris-HCl (pH 7.4). Filters were dried and counted at approximately 40% efficiency in a MicroBeta scintillation counter (Perkin-Elmer) using EcoScint liquid scintillation cocktail.

7.4.12. Performance of the binding assay for the human 5-HT_{1A}

Human 5-HT_{1A} enriched membranes (10 µg) were incubated with 2 nM radiolabeled [^{3}H]-8-hydroxy-DPAT in 250 µL of assay buffer containing 50mM Tris-HCl, 5 mM $MgCl_2$, pH 7.4. NSB (non-specific binding) was measured by adding 10 µM 5-HT in 25 µl volume. Final DMSO concentration was 0.1% (v/v). After 2 h incubation at 37 ºC, binding reaction was terminated by filtering 200 µl through Multiscreen GF/C (Millipore) presoaked in 150 µL of 0.5 % polyethylenimine in Vacuum Manifold Station, followed by 3 washes with 200 µl/well of ice-cold filtration buffer containing 50 mM Tris-HCl, pH 7.4.

7.4.13. Performance of the binding assay for the human 5-HT_{2B}

Human 5-HT_{2B} enriched membranes (5µg) were incubated with 1 nM radiolabeled [^{3}H]LSD in 250 µL of assay buffer containing 50 mM Tris-HCl, 4 mM $CaCl_2$, 1% ascorbic acid, pH 7.4. NSB (non-specific binding) was measured by adding 50 µM 5-HT in 2.5 µL volume. Final DMSO concentration was 0.1% (v/v). After 30 min incubation at 37 ºC, binding reaction was terminated by filtering 200 µL through Multiscreen GF/C (Millipore) presoaked in 150 µL of 0.5 % polyethylenimine in

Vacuum Manifold Station, followed by 3 washes with 200 µL/well of ice-cold filtration buffer containing 50 mM Tris-HCl, pH 7.4.

7.4.14. Performance of the binding assay for the human α_{1A} adrenoreceptor

Human α_{1A} adrenoreceptor enriched membranes (10 µg) were incubated with 0.2 nM of radiolabeled [^{3}H]prazosin in 250 µL of assay buffer containing 50 mM HEPES, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 0.2% BSA, pH 7.4. NSB (non-specific binding) was measured by adding 1 µM prazosin in 25 µL volume. Final DMSO concentration was 0.1% (v/v). After 90 min incubation at 25 ºC, binding reaction was terminated by filtering 200 µL through Multiscreen GF/C (Millipore) presoaked in 150 µL of 0.5 % polyethylenimine in Vacuum Manifold Station, followed by 3 washes with 200µl/well of ice-cold filtration buffer containing 50mM HEPES, 500mM NaCl, BSA 0.1%, pH 7.4.

7.4.15. Performance of the binding assay for the human H_1 receptor

Human histamine H_1 receptor enriched membranes (25 µg) were incubated with 2.4 nM of radiolabeled [^{3}H]pyrilamine in 250 µL of 8.6 mM KH_2PO_4, 41.4 mM Na_2HPO_4 Na^+/K^+ phosphate buffer, pH 7.4. NSB (non-specific binding) was measured by adding 10 µM triprolidine in 25 µL volume. Final DMSO concentration was 0.1% (v/v). After 90 min incubation at 25 ºC, binding reaction was terminated by filtering 200 µL through Multiscreen GF/C (Millipore) presoaked in 150 µL of 0.5 % polyethylenimine in Vacuum Manifold Station, followed by 3 washes with 200 µL/well of ice-cold 8.6 mM KH_2PO4, 41.4 mM Na_2HPO_4 Na^+/K^+ phosphate buffer, pH 7.4.

7.4.16. Performance of the binding assay for the human DAT

Human Dopamine transporter (DAT) enriched membranes (5 µg) were incubated

with 2 nM of radiolabeled [^{3}H]GBR12935 in 250 µL of assay buffer containing 50 mM Tris-HCl, 100 mM NaCl, pH 7.4. NSB (non-specific binding) was measured by adding 10 µM GBR12909 in 25 µL volume. Final DMSO concentration was 0.1% (v/v). After 120 min incubation at 4 ºC, binding reaction was terminated by filtering 200 µL through Multiscreen GF/C (Millipore) presoaked in 150 µL of 0.5 % polyethylenimine in Vacuum Manifold Station, followed by 3 washes with 200 µL/well of ice-cold filtration buffer containing 50 mM Tris-HCl, 100 mM NaCl, pH 7.4.

7.4.17. Performance of the binding assay for the human NET

Human norepinephrine transporter (NET) enriched membranes (1 µg) were incubated with 5 nM of radiolabeled [^{3}H]nisoxetine in 250 µL of assay buffer containing 50 mM Tris-HCl, 120 mM NaCl, 5mM KCl, pH 7.4. NSB (non-specific binding) was measured by adding 10 µM desipramine in 2.5 µL volume. Final DMSO concentration was 1 % (v/v). After 60 min incubation at 4 ºC, binding reaction was terminated by filtering 200 µL through Multiscreen GF/C (Millipore) presoaked in 150 µL of 0.5 % polyethylenimine in Vacuum Manifold Station, followed by 3 washes with 200 µL/well of ice-cold filtration buffer containing 50 mM Tris-HCl, 0.9% NaCl, pH 7.4.

7.4.18. Performance of the binding assay for the human SERT

Human serotonin transporter (SERT) enriched membranes (3.5 µg) were incubated with 3 nM of radiolabeled [^{3}H]imipramine in 250 µL of assay buffer containing 5 0 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, pH 7.4. NSB (non-specific binding) was measured by adding 200 µM imipramine in 25 µL volume. Final DMSO concentration was 0.1% (v/v). After 30 min incubation at 27 ºC, binding reaction was terminated by filtering 200 µL through Multiscreen GF/C (Millipore) presoaked in 150 µL of 0.5 %

polyethylenimine in Vacuum Manifold Station, followed by 3 washes with 200 µL/well of ice-cold filtration buffer containing 50 mM Tris-HCl, 0.9% NaCl, pH 7.4.

7.4.19. Patch clamp study for determination of hERG-Affinity

Automated patch clamp electrophysiology studies using QPatch. CHO cells stably expressing hERG channels (Millipore) were cultured in F12 HAM medium supplemented with 10% FBS and 400µg/L Geneticin. The extracellular Ringer's solution consisted of (in mM): 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 4 KCl, 145 NaCl, 10 glucose, pH 7.4, 305 mOsm. The intracellular Ringer's solution consisted of (in mM): 5.37 $CaCl_2$, 1.75 $MgCl_2$, 31.25/10 KOH/EGTA, 10 HEPES, 210 KCl, pH 7.2, 295 mOsm. 4 mM Na_2-ATP was added to intracellular Ringer's solution shortly before use. Whole-cell currents were measured with a QPatch system (Sophion) in response to continuously executed voltage protocols as per manufacturer's recommendations. Upon onset of the voltage protocol, cells were maintained at a holding potential (Vh) of -80 mV, then clamped briefly to -50 mV (20 ms), subsequently depolarized to 20 mV for 4800 ms, and finally repolarized to -50 mV for 5000 ms, at which potential the peak outward tail current was measured. Finally, the voltage returned to Vh for 3100 ms. Thus, voltage protocols were repeated each 15 seconds. For each cell, extracellular solution was applied previous to increasing concentrations of the tested compound.[53]

7.5. *In vitro* characterization of

7.5.1. Chemical stability

A solution of the test compound (25 µL, 10 mM in DMSO) was treated with aqueous buffered solution (1 mL, pH 2 or pH 7.4) and stirred at room temperature for 24 h. Then the solution was analyzed by HPLC-MS and the compound considered stable if

the peak area was maintained between 95 and 105 %.

7.5.2. Stability in liver microsomes

The assay was carried out in a robotic liquid handling system (Freedom Evo, Tecan). All incubations were performed individually for each test compound. Compounds (1 µM) were incubated in 96-well plates at 37 ºC during 1 h under standard incubation conditions: sodium-potassium phosphate buffer (50 mM, pH 7.4), $MgCl_2$ (3 mM), the NADPH-regenerating system and CYP content (0.3 nmol/mL). At preset times (0, 10, 20, 40 and 60 min) aliquots of the reaction mixture were stopped with an equal volume of cold acetonitrile. Upon centrifugation of the resultant mixture, supernatants were analyzed by a generic UPLC-MS/MS method. Metabolic stability was determined by the disappearance of compound over time. Ln-linear plots of the % of compound remaining based on chromatographic peak area versus time were plotted, and the slope was calculated by linear fitting of the curve. Results were expressed as percentage of test compound remaining at the end of the incubation. Terfenadine was used as positive control in all assays.[54]

7.5.3. Determination of CYP inhibition

CYP inhibition assay was carried out in a robotic liquid handling system (Packard Multiprobe II, Perkin-Elmer). All incubations were performed individually for each test compound. Compounds were incubated in 96-well plates at 37 ºC at final concentration of 1 µM. The inhibition potential of test compounds was evaluated using fluorescent probe substrates and recombinant human cytochrome P450 isoenzymes (rhCYP1A2, 2C9, 2C19, 2D6 and 3A4). Selective known inhibitors were screened alongside the test compounds as positive controls for the assay. Method including composition of incubation media, time of incubation and substrates used

per each isoform are described in detail elsewhere (Stresser, 2004). Fluorescence per well was measured using a fluorescence plate reader. Results were expressed as percentage of inhibition.[55]

7.5.4. Cytotoxicity

Compound-induced cytotoxicity was analyzed in human HepG2 cells using two different colorimetric methodologies: MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and NR (Neutral Red Uptake). Briefly, 3 x 10^4 cells/well were seeded in 96 well plates containing culture media: MEM (GIBCO) supplemented with 10 % FBS (Sigma), 2 mM L-Glutamine (Sigma), 1 % non-essential amino acids (Sigma), 1 % Pyruvate (Sigma) and 100 U/µg/mL of Penicillin/Streptomycin (Sigma). Plates were incubated at 37 ºC and 5 % CO_2 for approximately 24 h. At this time, cells were treated with 1, 10 and 100 µM DMSO solutions of the test compounds during 20 h in serum-free culture media, using the same incubation conditions mentioned above. Afterwards, compounds were washed out with PBS (Sigma). For MTT assay, MTT solution (Sigma, 100 µL) at a final concentration of 0.5 mg/ml were added to each well and cells were incubated at 37 ºC and 5 % CO_2. After 4 h, MTT solution was eliminated and DMSO (100 µL) was added to dissolve formazan crystals. Optical density was then measured at 550 nm (SpectraMax® 340PC384, Molecular Devices). For Red Neutral Uptake, NR solution (Sigma, 100 µL) at a final concentration of 50 µg/mL were added to the well and cells were incubated at 37 ºC and 5 % CO_2 for 3 h. Then, NR solution was eliminated washing with PBS and 100 µL of a solution of 50 % ethanol and 1 % of acetic acid in water was added to each well to extract the Neutral Red. Likewise, optical density was then measured at 550 nm. The lowest compound concentration (1, 10 or 100 µM) producing ≥50% of cytotoxicity, compared to non-treated cells, was taken as IC_{50}

value. Each compound was analyzed by duplicate in two different assays (n = 4).[56]

7.6. Pain behavioral studies

To evaluate the effect of drugs on mechanical allodynia induced by capsaicin, a previously described experimental procedure was used.[46] The compound under study or its solvent (HPMC) was administered sc to female CD-1 mice (Charles River, Barcelona, Spain) 30 min before the intraplantar (ipl) administration of 20 µL capsaicin (1 µg in 1% DMSO). 15 min after the ipl administration of capsaicin, a mechanical punctate stimulation (0.5 g force) was applied with an electronic von Frey device (Dynamic Plantar Aesthesiometer, Ugo Basile, Comerio, Italy) at least 5 mm from the site of injection toward the toes (area of secondary mechanical hypersensitivity), and the paw withdrawal latency time was automatically recorded. Each mouse was tested in three trials at 30 s intervals, and the mean of the three measurements was calculated. A cutoff time of 50 s was used in each trial. In the experiments to elucidate the influence of σ1 receptors on the antiallodynic effect of compound tested, the σ_1 receptor agonist PRE-084 (**24**) was administered sc. 5 min before the sc administration of the compound tested and 30 min later (i.e. 35 min after compound **24** administration) capsaicin was ipl injected and the above-mentioned procedures were performed to measure mechanical allodynia. Animal care was provided in accordance with institutional (Research Ethics Committee of the University of Granada, Granada, Spain), regional (Junta de Andaluciá, Spain), and international standards (European Communities Council Directive 2010/63). The protocol of the experiments was approved by Junta de Andalucia (License 04/09/2017/113). The degree of effect on capsaicin-induced mechanical allodynia was calculated as % antiallodynic effect = [(LTD − LTS)/(CT − LTS)] × 100 where LTD is the latency time for paw withdrawal in drug-treated animals, LTS is the latency

time in solvent-treated animals (mean value 12.03 s), and CT is the cutoff time (50 s).The statistical significance of differences between values obtained in the different experimental groups was analyzed with one-way analysis of variance (ANOVA) followed by the Bonferroni test. The differences between mean values were considered statistically significant when the value of P was below 0.05. All animal experiments performed in the manuscript were conducted in compliance with institutional guidelines. License number: 04/09/2017/113.

Supporting Information

The Supporting Information contains purity data and ^{1}H, ^{13}C and ^{19}F NMR spectra of prepared compounds.

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Conflict of interests

The authors declare no conflict of interest.

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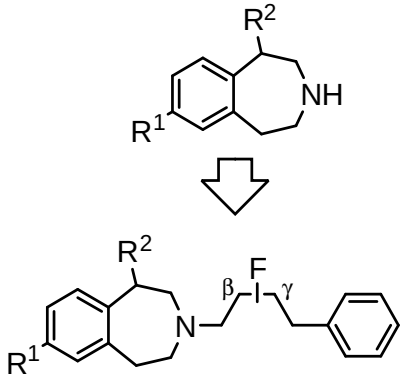
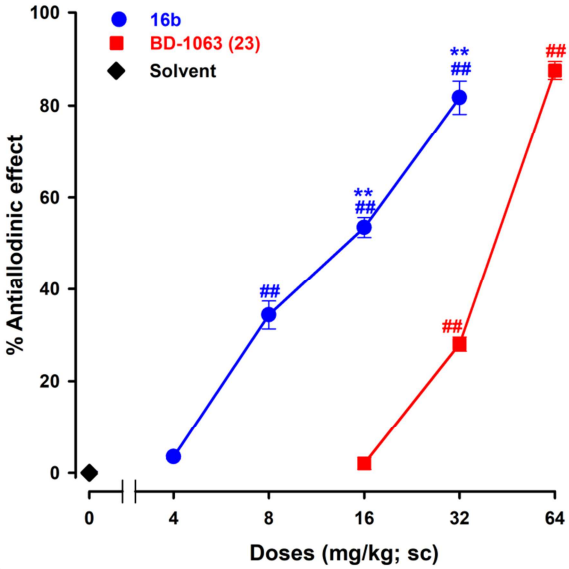
Graphical abstract



17b
R^1 = OH, R^2 = H, γ-F
GluN2B affinity: 16 nM

16b
R^1 = OCH_3, R^2 = H, γ-F
σ_1 affinity: 6.6 nM
antiallodynic activity



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Research Highlights

> 3-Benzazepines were modified systematically with fluorinated side chains.

> γ-Fluorobutyl derivatives show higher GluN2B affinity than β-fluoro analogs

> Removal of benzylic OH moiety led to higher GluN2B affinity but reduced selectivity.

> A high-affinity and selective σ_1 ligand was found.

> This σ_1 ligand showed higher antiallodynic activity than the reference ligand.