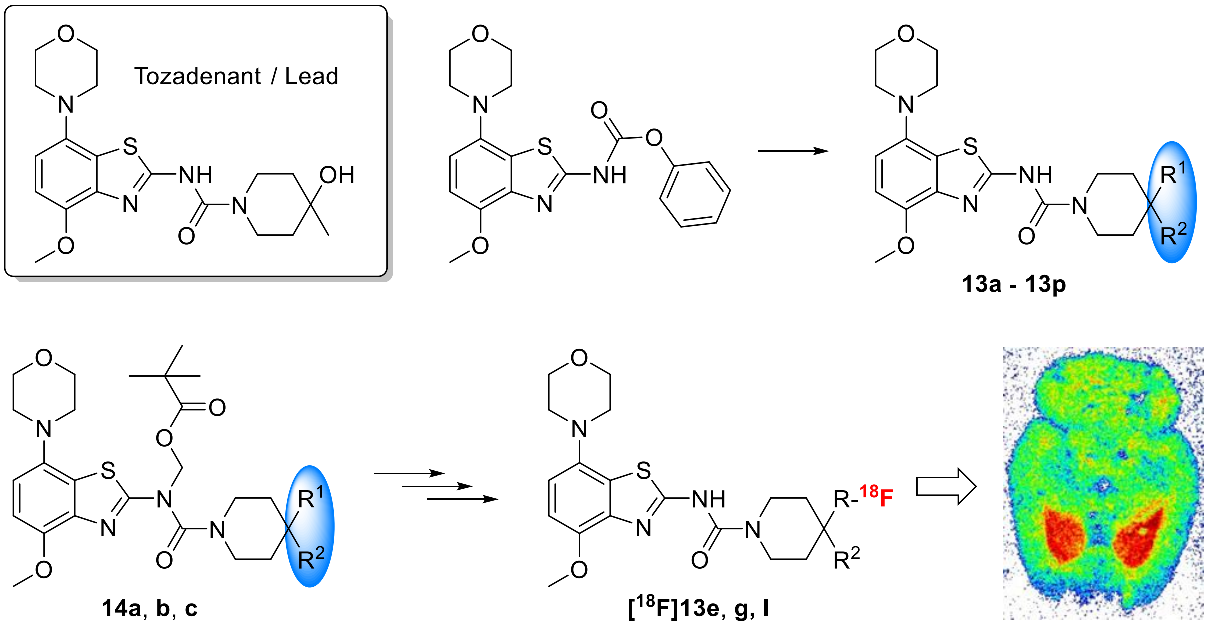
Tozadenant / Lead
13a - 13p
14a, b, c
[18F]13e, g, l

- A straightforward synthetic strategy provided tailor made tozadenant analogues.
- Compounds possess high affinity for the adenosine A_{2A} receptor ($A_{2A}R$).
- *In vitro* autoradiography of radiofluorinated derivatives showed striatal binding.
- The described compounds are candidates for the *in vivo* visualization of the $A_{2A}R$.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Design, synthesis and biological evaluation of Tozadenant analogues as adenosine A_{2A} receptor ligands.

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ABSTRACT

With the aim to obtain potent adenosine A_{2A} receptor (A_{2A}R) ligands, a series of eighteen derivatives of 4-hydroxy-*N*-(4-methoxy-7-morpholin-4-yl-1,3-benzo[*d*]thiazol-2-yl)-4-methylpiperidine-1-carboxamide (SYN-115, Tozadenant) were designed and synthesized. The target compounds were obtained by a chemical building block principle that involved reaction of the appropriate aminobenzothiazole phenyl carbamates with either commercially available or readily synthesized functionalized piperidines. Their affinity and subtype selectivity with regard to human adenosine A_1- and A_{2A} receptors were determined using radioligand binding assays. K_i values for human A_{2A}R ranged from 2.4 to 38 nM, with more than 120-fold selectivity over A_1 receptors for all evaluated compounds except **13k** which had a K_i of 361 nM and 18-fold selectivity. The most potent fluorine-containing derivatives **13e**, **13g** and **13l** exhibited K_i values of 4.9 nM, 3.6 nM and 2.8 nM for the human A_{2A}R. Interestingly, the corresponding values for rat A_{2A}R were found to be four to five times higher. Their binding to A_{2A}R was further confirmed by radiolabeling with ^{18}F and *in vitro* autoradiography in rat brain slices, which showed almost exclusive striatal binding and complete displacement by the A_{2A}R antagonist ZM 241385. We conclude that these compounds represent potential candidates for the visualization of the A_{2A} receptor and open pathways to novel therapeutic treatments of neurodegenerative disorders or cancer.

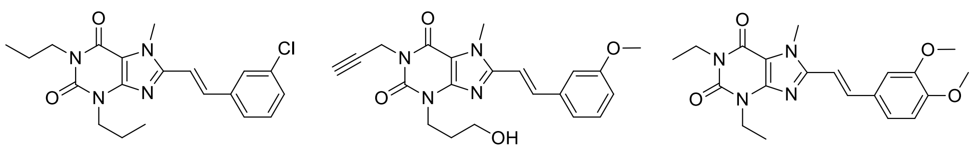
KEYWORDS

A_{2A} adenosine receptor, ligand synthesis, fluorinated analogues, fluorine-18 isotopologues, binding studies, autoradiography

INTRODUCTION

The purine nucleoside adenosine (adenine-9-β-D-ribofuranoside) is an ubiquitous but short-lived signaling molecule that exerts its effects through at least four subtypes of G-protein coupled adenosine receptors (ARs) [1]. Within the central nervous system (CNS), adenosine is a neuromodulator that participates in the regulation of sleep and arousal [2–4], is involved in cognition [5,6] and contributes to the autoregulation of cerebral blood flow [7,8]. The adenosine A_{2A} receptor ($A_{2A}R$) mediates multiple of the physiological effects of adenosine and has been implicated with a number of neurodegenerative [9,10], neuropsychiatric [11,12] and neuroinflammatory [13] disorders. In the healthy CNS, $A_{2A}R$ is most abundant in the basal ganglia [14,15], where it co-localizes with dopamine D_2 receptors on striatopallidal output neurons [16] and has been targeted for non-dopaminergic treatment of Parkinson's disease [17–19]. Receptor densities in other subcortical structures and the cerebral cortex are at least ten-fold lower [14], but many diseases appear to be associated with considerable changes in extra-striatal $A_{2A}R$ expression [10,13,14]. The relevance of $A_{2A}Rs$ in a number of specific disease states like in neurodegenerative diseases or in tumor immunotherapy [14,20,21] has stimulated the development of various selective antagonists for these receptors, which can be broadly classified into xanthine and non-xanthine derivatives [22,23].

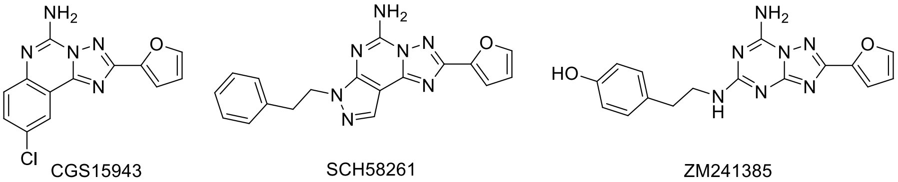
The xanthine scaffold present in naturally occurring nonselective AR antagonists like caffeine provided a first starting point for the development of selective $A_{2A}R$ antagonists, which led to the discovery of 3-chlorostyrylcaffeine (CSC), MSX-2 [24] and istradefylline [25] (Figure 1).



CSC MSX-2 istradefylline

Figure 1: Chemical structures of xanthine based $A_{2A}R$ antagonists.

However, due to their poor water solubility and metabolic instability, a search for different structural types of $A_{2A}R$ antagonists based on mono-, bi-, and triheterocycles was soon initiated. The first non-xanthine $A_{2A}R$ antagonist thus discovered was the triazoloquinazoline CGS15943 (Figure 2) [26], a very potent but unselective compound that exhibits high affinity for the other ARs as well. Nonetheless, the CGS scaffold served as a template for the development of more selective antagonists and these efforts culminated in the identification of *N*-substituted pyrazolotriazolopyrimidines as a class of heterocycles with high $A_{2A}R$ affinity and strongly reduced affinity for all other ARs [27]. One member of this class, the tricyclic SCH58261 (Figure 2), was rapidly accepted as a reference $A_{2A}R$ antagonist, although it suffered from poor water solubility and synthesizability These disadvantages have been overcome by the development of ZM241385 (Figure 2), a very potent and selective bicyclic triazolotriazine based $A_{2A}R$ antagonist [28]. By virtue of its bicyclic nature and due to the presence of two additional hydrogen donors, ZM241385 showed a much more favorable aqueous solubility profile.



CGS15943 SCH58261 ZM241385

Figure 2: Structures of non-xanthine $A_{2A}R$ antagonists CGS15943, SCH58261 and ZM241385

Since then several additional classes of non-xanthine $A_{2A}R$ antagonists with either mono-, bi-, or tricyclic core-based structures have been reported [29] and used to decipher the pharmacology and signaling pathways of these receptors. Efforts to optimize these ligands are ongoing and there is still a need for novel, easily accessible compounds with high affinity and subtype selectivity for the design of therapeutic agents.

In 2005 Hoffmann-La Roche disclosed 4-hydroxy-*N*-(4-methoxy-7-morpholin-4-yl-1,3-benzo[*d*]thiazol-2-yl)-4-methylpiperidine-1-carboxamide (SYN-115, Tozadenant), a potent and selective $A_{2A}R$ antagonist based on a benzothiazole scaffold [30] (Figure 3). Our research started with the decision to use the core structure of Tozadenant as a template to develop improved $A_{2A}R$ antagonists with high selective receptor binding and suitable physicochemical and pharmacokinetic properties. Although researchers from Hoffmann-La Roche have described a number of similar compounds in patents, the ones described here are invariably new.

After thorough analysis of the chemical structure of Tozadenant and extensive literature screening [31,32] we decided to chemically modify the substituents at the 4-position of the piperidine ring or to exchange the methoxy group at carbon-4 of the benzothiazole ring system (Figure 3).

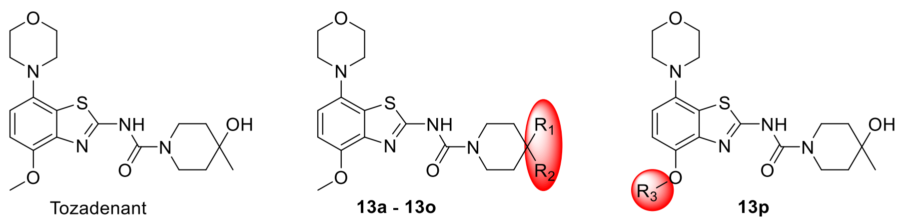


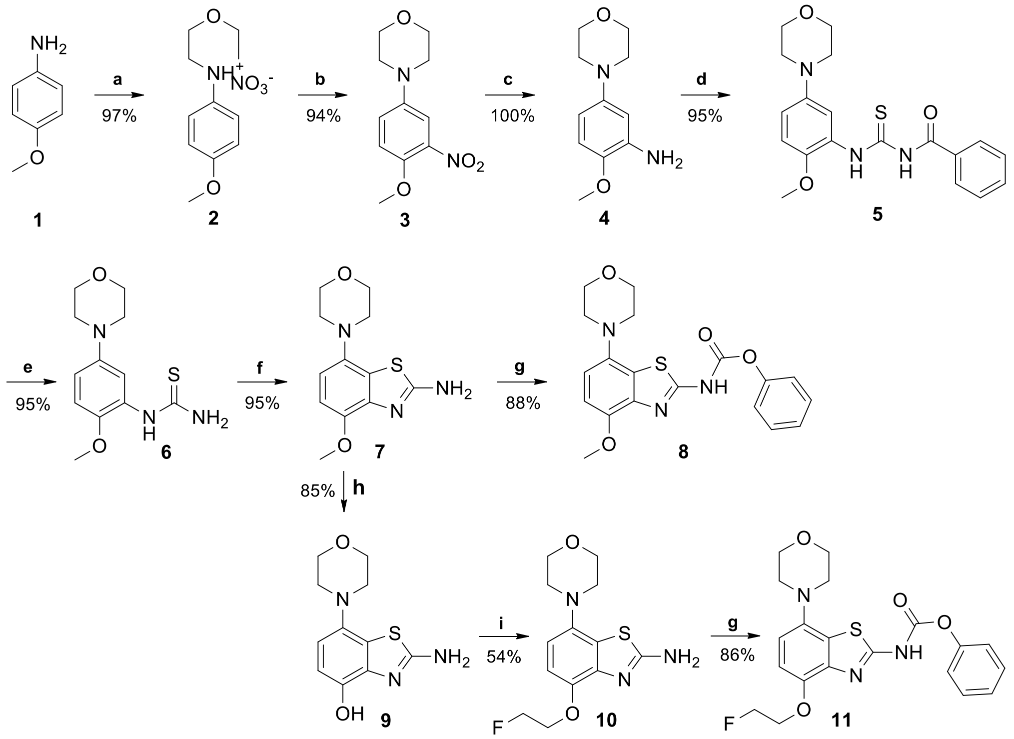
Figure 3: Structure of Tozadenant and positions for chemical modifications

As in our earlier study of antagonists for the adenosine A_1 receptor (A_1R)[33], the primary objective was to assess the impact of fluorination on binding characteristics and pharmacological activity. Herein, we describe the results of the modifications of the Tozadenant structure on binding affinity for the A_{2A} receptor and adenosine receptor selectivity. Since most of the described compounds contain a fluorine, three high-affinity candidates were selected to investigate if they are amenable to radiofluorination. The corresponding precursors were synthesized and radiofluorinated. Their potential suitability for positron emission tomography (PET) was further investigated by determination of their binding profiles by *in vitro* autoradiographic studies.

RESULTS AND DISCUSSION

CHEMISTRY

The lead heterocycle 4-methoxy-7-morpholin-4-yl-benzo[*d*]thiazol-2-ylamine **7** was prepared from *p*-anisidine in a 6-step synthesis (see Scheme 1).



Scheme 1: Synthesis of starting aminobenzothiazoles **7** and **9** and phenyl carbamates **8** and **11**.

Conditions: (**a**) i) 2-chloroethyl ether, TBAB, NaOH, 180°C, 8 h, ii) 70% HNO_3, 0-5°C, 12 h; (**b**) 95% H_2SO_4, 0-5°C, 1.5 h; (**c**) NH_4Cl, zinc powder, EtOH/ethyl acetate, r.t., 0.5 h; (**d**) benzoyl isothiocyanate, acetone, r.t., 0.5 h; (**e**) NaOMe, MeOH, r.t., 3 h; (**f**) i) 33% HBr, 80°C, 0.4 h, ii) DMSO, ethyl acetate, 80°C, 4 h; (**g**) phenyl chloroformate, pyridine, THF, r.t., 14 h; (**h**) 48% HBr, 130°C, 24 h; (**i**) 2-bromo-1-fluoroethane, Cs_2CO_3, DMF, r.t., 24 h.

In the first reaction step, *p*-anisidine **1** was reacted with 2-chloroethyl ether to form 4-(4-methoxyphenyl)morpholine. Crude morpholine was treated with nitric acid and isolated as the nitrate salt **2**. Subsequent regioselective nitration furnished 4-(4-methoxy-3-nitrophenyl)morpholine **3** [34]. Reduction of the nitro group with zinc/ammonium chloride yielded 2-methoxy-5-morpholinoaniline **4** in almost quantitative yield. Reaction with benzoyl isothiocyanate provided 1-benzoyl-3-(2-methoxy-5-morpholin-4-yl-phenyl)thiourea **5** which was deprotected with sodium methoxide to give 2-methoxy-5-morpholin-4-yl-phenylthiourea **6**. Cyclization of thiourea **6** to furnish 4-methoxy-7-morpholin-4-yl-benzothiazol-2-ylamine **7** was performed by a modified Hugershoff reaction using bromine in dimethyl sulfoxide [35]. Phenyl carbamate **8** was prepared by reaction of **7** with phenyl chloroformate and was obtained as a bench stable crystalline solid. 2-Amino-7-morpholinobenzo[*d*]thiazol-4-ol **9** was prepared in excellent yield by demethylation of **7** with aqueous 48% hydrobromic acid. Attempts to cleave the methylether **7** with boron tribromide failed. Cesium carbonate supported chemoseletive O-alkylation of aminophenol **9** with 1-bromo-2-fluoroethane furnished **10**. Reaction of aminothiazole **10** with phenyl chloroformate and pyridine as a base afforded phenyl carbamate **11** as a stable solid.

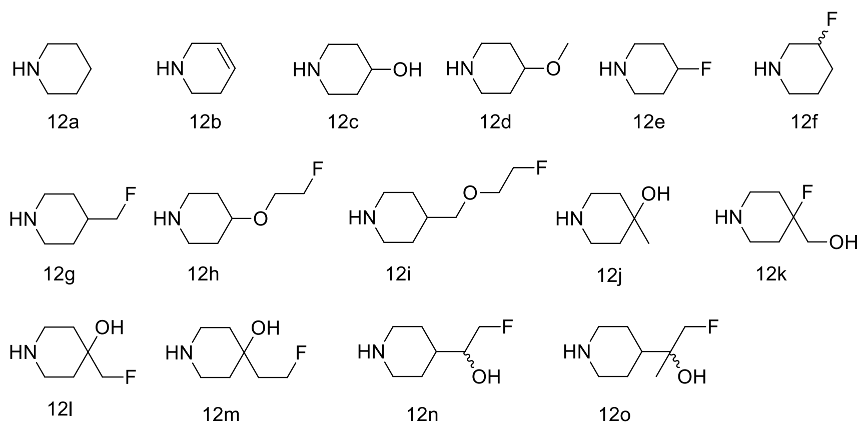
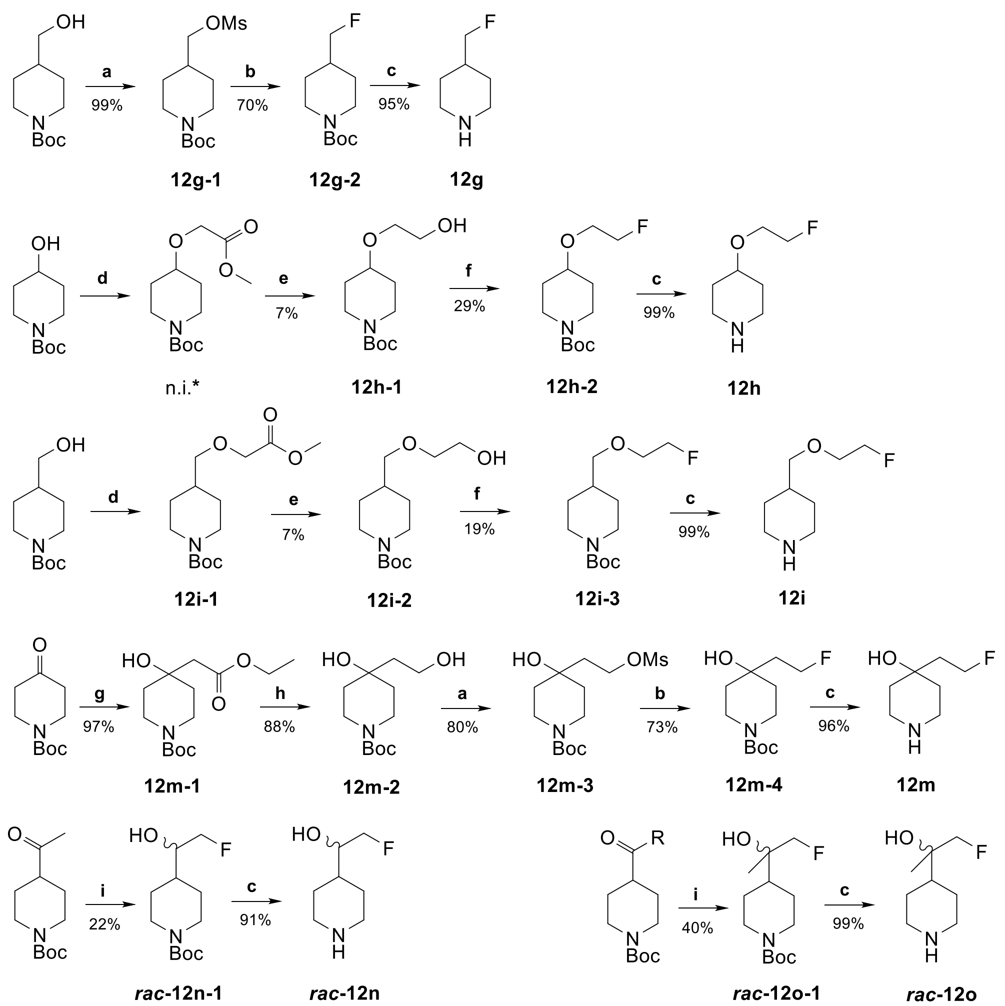


Chart 1: Structure of piperidines **12a** – **12o** applied in this work.

Piperidines used in this work are depicted in Chart 1. Most of them are commercially available but piperidines **12g**, **12h**, **12i**, **12m**, **12n**, and **12o** were unknown and had to be synthesized (Scheme 2).

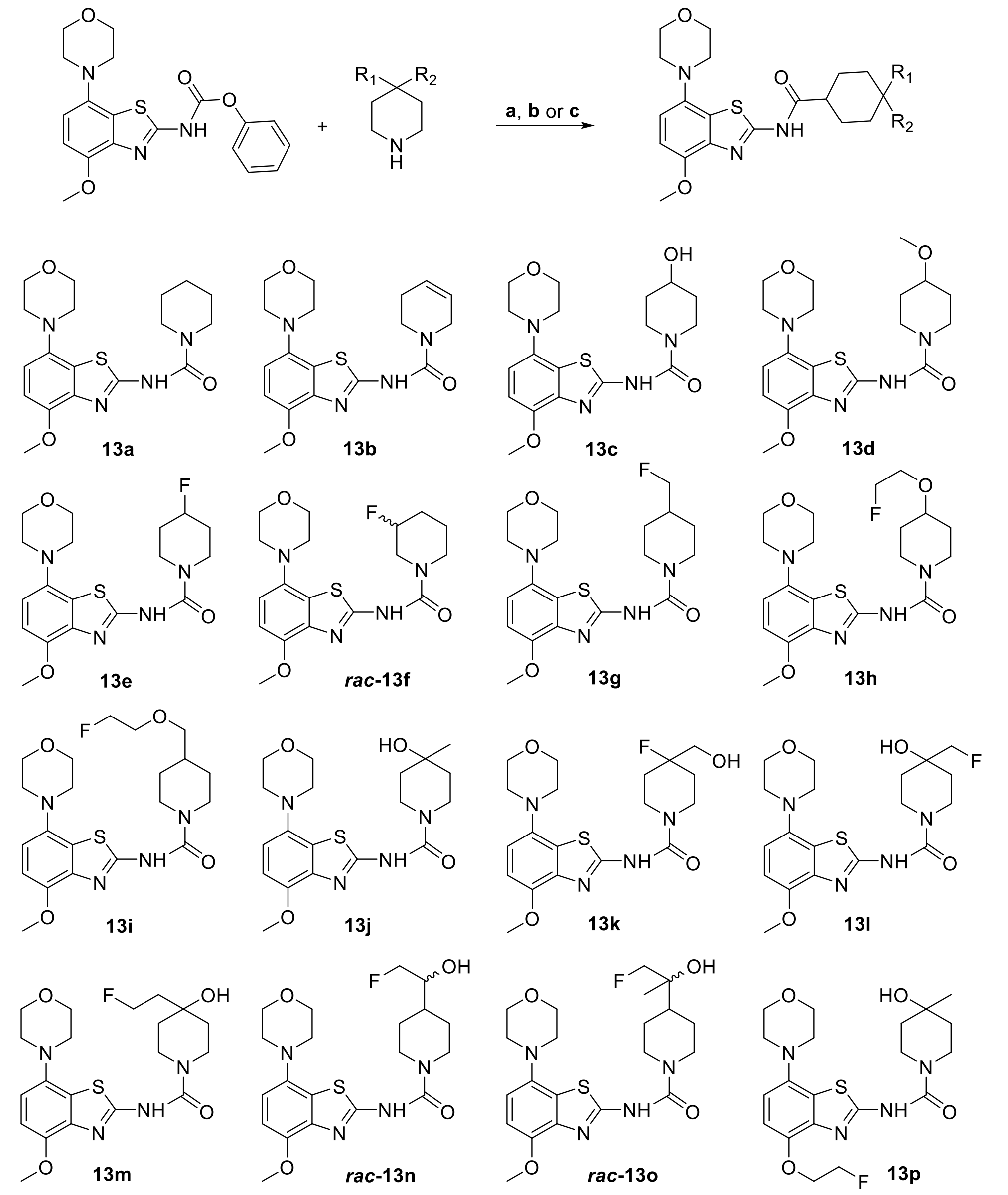


Scheme 2: Synthesis of piperidines **12g, 12h, 12i, 12m,** ***rac*****-12n** and ***rac*****-12o.**

Conditions: (**a**) MsCl, Et_3N, DCM, r.t., 1 h; (**b**) TBAF, THF, 70°C, 19-24 h; (**c**) TFA, DCM r.t., 1-4 h; (**d**) i) NaH, THF, 0°C, 20 min, ii) methyl bromoacetate, r.t., 22 h; (**e**) LAH, r.t., 3 h; (**f**) PyFluor, r.t., 48-69 h; (**g**) ethyl acetate, LiHMDS, THF, -70°C, 3.5 h; (**h**) $LiBH_4$/MeOH, THF, r.t., 2 h; (**i**) FCH_2Li, -78°C, 5 min. *n.i. not isolated.

4-(Fluoromethyl)piperidine **12g** was prepared by mesylation of the *N*-*tert*-butoxycarbonyl (Boc) protected alcohol followed by nucleophilic fluorination with excess tetrabutylammonium fluoride and subsequent acid induced Boc deprotection. Piperidines **12h** and **12i** were obtained by O-alkylation of the sodium salts of *N*-Boc-4-hydroxypiperidine (**12h**) or *N-Boc*-4-hydroxymethylpiperidine (**12i**) with methyl bromoacetate followed by ester reduction with lithium aluminium hydride. Fluorination of the respective alcohols with PyFluor [36] and Boc deprotection with trifluoroacetic acid provided 4-(2-fluoroethoxy)piperidine **12h** and 4-((2-fluoroethoxy)methyl)piperidine **12i** in moderate yields. 2-Fluoro-1-(piperidin-4-yl)ethanol **12n** and 1-fluoro-2-(piperidin-4-yl)propan-2-ol **12o** were synthesized by fluoromethylation of either *N*-Boc-4-formylpiperidine or *N*-Boc-4-acetylpiperidine with *in situ* prepared fluoromethyllithium [37]. Acidic Boc deprotection furnished fluorohydrins **12n** and **12o** in acceptable yields (Scheme 3). 4-(2-Fluoroethyl)piperidin-4-ol **12m** was obtained starting from *N*-Boc-4-piperidone. Low temperature reaction with lithio ethyl acetate, prepared *in situ* from ethyl acetate and lithium hexamethyldisilazide [38], provided *N*-Boc-4-ethoxycarbonylmethyl-4-hydroxypiperidine which was reduced with lithium borohydride in methanol to furnish *N*-Boc-4-hydroxy-4-(2-hydroxyethyl)piperidine. Fluorination with excess tetrabutylammonium fluoride followed by acidic Boc deprotection gave 4-(2-fluoroethyl)piperidin-4-ol **12m** in good yield.

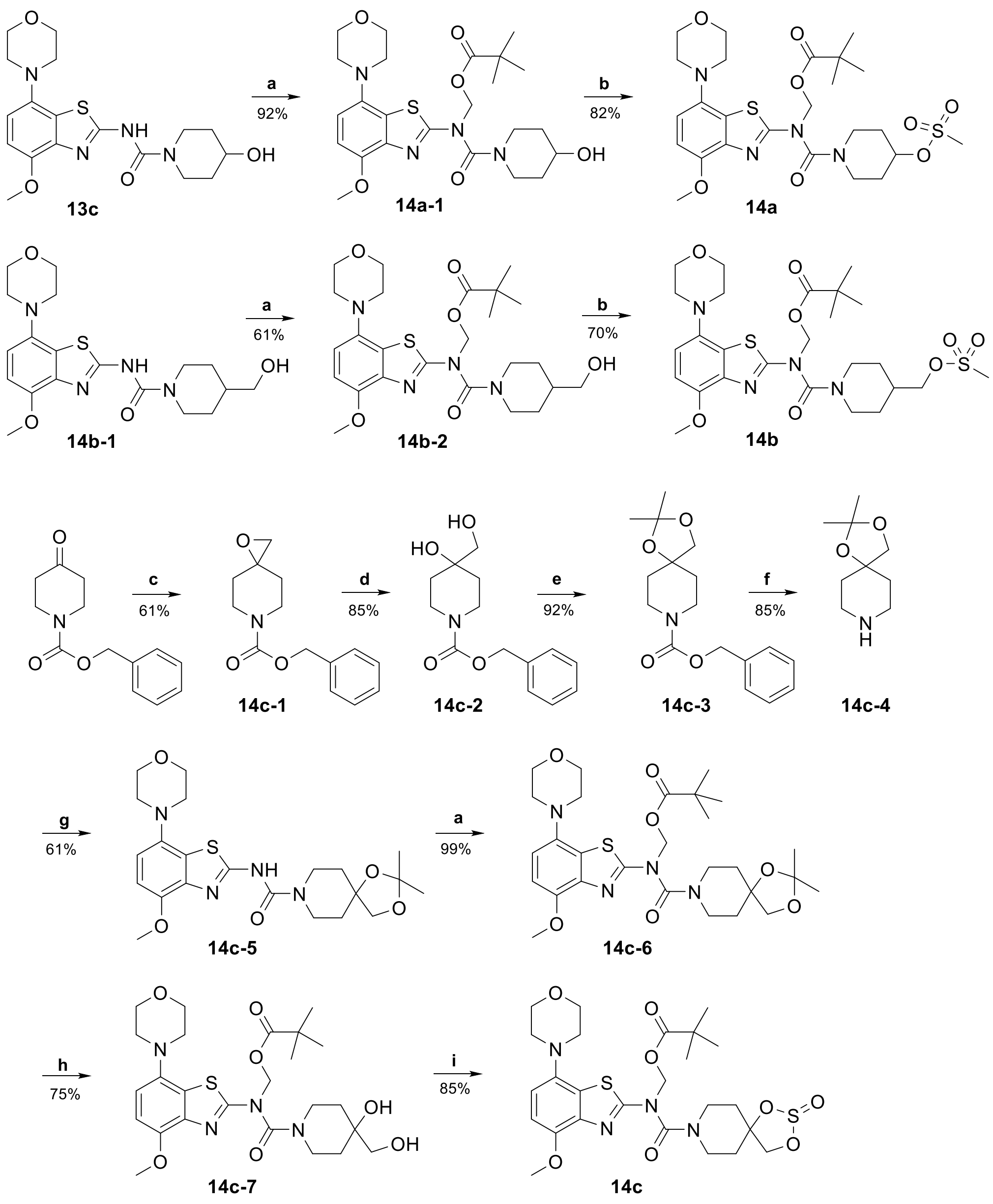
The asymmetric *N*,*N*'-disubstituted target ureas **13a** – **13g** and **13p** were synthesized via aminolysis of phenyl carbamate **8** under neutral conditions (Scheme 3) [39]. Thus, using dimethyl sulfoxide as the solvent, reaction of **8** or **10** with a stoichiometric amount of the respective piperidine at ambient or slightly elevated temperature rapidly generated the asymmetric target ureas in high yield. Ureas **13h** to **13o** were obtained by base catalyzed reaction of **8** with the respective piperidine at slightly elevated temperature using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as a base.



Scheme 3: Synthesis and chemical structures of asymmetric *N*,*N*'-disubstituted target ureas **13a** – **13p**.

Conditions: (**a**) 1.08 eq. substituted piperidine, DMSO, ambient temperature or 60°C (for **13a** - **13g**, **13p**); (**b**) 1 – 1.5 eq. substituted piperidine, DMSO, DBU, 60°C (for **13h, 13i** and **13k** - **13o**); (**c**) 2 eq. substituted piperidine, DCE, 40°C (for **13j**).

For nucleophilic radiofluorination of the three pharmacologically most promising candidates **13e**, **13g** and **13l** with fluorine-18, the appropriate radiolabeling precursors **14a** – **14c** were prepared as follows (Scheme 4). The synthesis of mesylates **14a** and **14b** was straightforward. It started with the *N*-pivaloyloxymethyl (*N*-Pom) protection of 4-hydroxy-*N*-(4-methoxy-7-morpholinobenzo[*d*]thiazol-2-yl)piperidine-1-carboxamide **13c** to give **14a-1**. *N*-Pom protection of 4-(hydroxymethyl)-*N*-(4-methoxy-7-morpholinobenzo[*d*]thiazol-2-yl)piperidine-1-carboxamide **14b-1**, prepared from phenyl carbamate **8** and 4-piperidinemethanol, provided **14b-2**. Mesylation with methanesulfonyl chloride and triethylamine as base furnished the protected precursor sulfonates **14a** and **14b** as crystalline solids with high yields (Scheme 4). Sulfite **14c** was obtained through a multistep synthesis starting from *N*-benzyloxycarbonyl-4-piperidone (*N*-Cbz-4-piperidone). Corey-Chaykowsky epoxidation of *N*-Cbz-4-piperidone with the methylene-transfer reagent dimethyloxosulfonium methylide, which was prepared by the action of sodium hydride on trimethylsulfonium iodide in tetrahydrofuran [40] provided benzyl 1-oxa-6-azaspiro[2.5]octane-6-carboxylate **14c-1** in 61% yield. Stirring the epoxide in 0.02 N aqueous hydrochloric acid led to ring opening to give the diol, benzyl 4-hydroxy-4-(hydroxymethyl)piperidine-1-carboxylate **14c-2**, in almost quantitative yield. Diol protection as the acetonide using dimethoxypropane and camphersulfonic acid (CSA) as a catalyst followed by hydrogenolytic removal of the Cbz group provided piperidine **14c-4**. Treatment of phenyl carbamate **8** with a stoichiometric amount of **14c-4** in DMSO at 60°C overnight generated urea **14c-5**. *N*-Pom protection and subsequent hydrolysis of the cyclic ketal **14c-6** with CSA in methanol liberated the free diol **14c-7**, which was treated in the cold with thionyl chloride/triethylamine to furnish the crystalline cyclic sulfite **14c** [41].



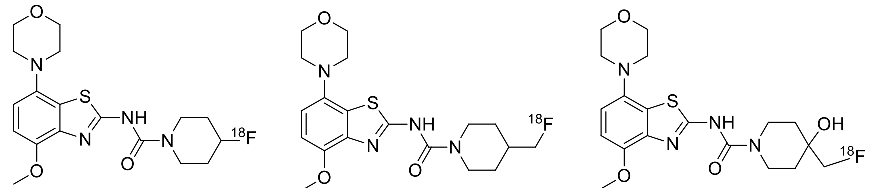
Scheme 4: Synthesis of the radiolabeling precursors **14a** - **14c**.

Conditions: (**a**) POM-Cl, K_2CO_3, DMF, 60°C, 3.5 h; (**b**) Ms-Cl, Et_3N, DCM, 0°C, 3 h; (**c**) NaH, $(CH_3)_3S(I)$, DMSO, 55°C, 2 h; (**d**) 0.02 N HCl, 50°C, 1 h; (**e**) 2,2-dimethoxypropane, CSA, r.t., 24 h; (**f**) H_2, Pd/C, MeOH, r.t., 12 h; (**g**) **8**, DMSO, 60°C, 24 h; (**h**) CSA, MeOH, 50°C, 12 h; (**i**) $SOCl_2$, Et_3N, DCM, 0°C, 15 min.

RADIOCHEMISTRY

Radiolabeling of the mesylates **14a** and **14b** and the cyclic sulfite **14c** with no carrier added (n.c.a.) fluorine-18 and subsequent Pom deprotection afforded fluorine-18 isotopologues **$[^{18}F]$13e**, **$[^{18}F]$13g** and **$[^{18}F]$13l** (Chart 2). Thus, **$[^{18}F]$13e** and **$[^{18}F]$13g** were prepared by nucleophilic substitution of the mesyl leaving group by $[^{18}F]$fluoride under phase transfer conditions (PTC) and subsequent Pom deprotection. Following standard labeling and deprotection procedures ($[^{18}F]$KF in the presence of Kryptofix 2.2.2., anhydrous DMSO, 85°C, 15 min, sodium methylate, r.t., 3 min) radiotracers **$[^{18}F]$13e** and **$[^{18}F]$13g** (1.6-2 GBq) were obtained in 25%–30% radiochemical yield (RCY) with a molar activity (MA) of 249-300 GBq/µmol and a radiochemical purity (RCP) of > 99%.

A similar procedure was used for the radiofluorination of the cyclic sulfite **14c** using $[^{18}F]$*n*-Et_4NF as radiolabeling reagent. Standard labeling conditions ($[^{18}F]$*n*-Et_4NF, dimethyl sulfoxide, 140°C, 15 min, sodium methylate, r.t., 3 min) yielded radiotracer **$[^{18}F]$13l** (1.2 GBq) in 10%–15% RCY with a MA of 600 GBq/µmol and a RCP of > 99%.



$[^{18}F]$13e **$[^{18}F]$13g** **$[^{18}F]$13l**

Chart 2: Structure of fluorine-18 isotopologues **$[^{18}F]$13e**, **$[^{18}F]$13g** and **$[^{18}F]$13l**.

RADIOLIGAND BINDING ASSAYS

The different ligands described above and the lead compound Tozadenant were subjected to receptor binding studies to determine their ability to displace $[^{3}H]$ZM241385 from the cloned human $A_{2A}R$ stably expressed in Chinese hamster ovary (CHO) cells. The resulting K_I values, calculated with the approximation formula of Cheng and Prusoff, are summarized in

Table 1. All compounds showed a high to excellent affinity for the human $A_{2A}R$, with K_i values ranging from 2.4 to 361 nM, while the K_i value for the lead compound tozadenant **13j** was determined to be 3.9 ± 0.7 nM (Table 1).

	Human $A_{2A}R$		Rat $A_{2A}R$		Human A_1R		
Compound	**K_i [nM]**	**n**	**K_i [nM]**	**n**	**K_i [nM] (95% CI)**	**hA_{2A}/hA_1**	**clogP**
13a	6.0 ± 1.0	3			1559 (836-2114)	260	3.02
13b	2.4 ± 0.24	3			577 (417-799)	240	2.74
13c	3.8 ± 0.48	3			1719 (858-3442)	452	0.94
13d	33 ± 6.8	3			7563 (4936-11588)	229	1.66
13e	4.9 ± 1.3	3	19.2 ± 3.9	3	591 (205-1700)	120	2.72
***rac*-13f**	6.4 ± 0.68	3			1173 (853-1612)	183	2.87
13g	3.6 ± 0.18	3	20.4 ± 13.3	5	1198 (971-1478)	332	2.82
13h	23.9 ± 7.2	3			>20000[a]	>830	1.77
13i	35.1 ± 1.18	3			7975 (5245-12126)	227	2.39
13j (Toz)	3.9 ± 0.7	7	18.7 ± 5.4	10	3380 (1875-6093)	866	1.46
13k	3.6 ± 0.54	3			2887 (1985-4201)	801	1.70
13l	2.8 ± 0.9	7	14.9 ± 7.9	14	497 (342-721)	177	1.18
13m	9.4 ± 3.7	3			4802 (2940-7841)	510	1.41
***rac*-13n**	6.4 ± 2.3	3	195 ± 65.3	4	16680 (9370-29689)	2606	1.59
***rac*-13o**	37.9 ± 15.1	3			>20000[a]	>520	1.99
13p	361 ± 112	3			6663 (2051-21658)	18	1.71

Table 1: Results of the receptor binding experiments. K_i values for human and rat $A_{2A}R$ were determined in competition experiments with 0.5 µM [^{3}H]ZM 241385 and are expressed as mean ± standard deviation based on n, the number of experiments. K_i values for human A_1R were determined in competition experiments with 0.5 nM [^{3}H]DPCPX and are shown with the corresponding 95% confidence interval. clogP values were calculated using ChemDraw Ultra 12.0.
[a] no displacement of the radioligand could be observed even at the highest concentrations of the compound used (5µM).

Comparison of the binding data among different test compounds (for structures see Scheme 2) shows that modifications of the piperidine ring with small polar electron withdrawing substituents were well tolerated and resulted in analogues with K_i values of 3 - 8 nM (compounds **13c**, **e**, **f**, **l**, **n**, **k** and **g**). Introduction of an ether moiety on the other hand reduced the affinity for the $A_{2A}R$ and resulted in analogues with K_i values of 20 to 33 nM (compounds **13d**, **h** and **i**). Replacement of the methoxy group at the 4-position of the benzothiazole scaffold by a fluoroethoxy moiety (**13p**) led to a more significant loss of potency (K_i 361 nM for **13p**). Sterically more demanding functionalities as in **13n** and **13o** led to analogues with good to medium $A_{2A}R$ affinity (K_i 6.4 for **13n** and K_i 38 nM for **13o**). Surprisingly, unsaturated analogue **13b** showed the highest potency (K_i 2.4 nM) of all evaluated compounds. For three of the compounds which were chosen for radiolabeling with fluorine-18 and further evaluation by *in vitro* autoradiography (**13e**, **13g** and **13l,** see below), the affinity for the murine $A_{2A}R$ was determined using homogenates from rat brain corpora striata, which revealed K_i values roughly 4-5 times higher than the values obtained for the human receptor but still within the two-digit nanomolar range (Table 1).

IN VITRO AUTORADIOGRAPHY

Three of the most potent structures containing a fluorine atom (**13e**, **13g** and **13l)** were radiolabeled with ^{18}F and used for *in vitro* autoradiography. Figure 1 shows the results of autoradiographic experiments of rat brain slices obtained by incubation with the ^{18}F-labeled isotopologues **[^{18}F]13e**, **[^{18}F]13g** and **[^{18}F]13l**. Due to the n.c.a. radiolabeling, extremely low concentrations (< 10 pM) of the radioligands were sufficient for the autoradiograms shown in Fig. 4. Total binding in all three autoradiographic experiments was very similar and showed the striatal accumulation pattern expected for $A_{2A}R$-selective ligands, with low uptake in all extra-striatal brain regions. In addition, striatal binding was consistently and completely displaced by 1 µM of the potent and selective $A_{2A}R$ antagonist ZM 241385, suggesting specific binding of all three radioligands in this region. Thus, in summary,

[^{18}F]13e, **[^{18}F]13g** and **[^{18}F]13l** are promising $A_{2A}R$ radioligand candidates for PET imaging with excellent molar activity, high *in vitro* affinity (especially for human receptors), and an autoradiographic distribution pattern corresponding to the *in vivo* distribution of the $A_{2A}R$ in rat brain. Thus, the high accumulation of radioligands **[^{18}F]13e**, **[^{18}F]13g** and **[^{18}F]13l** in $A_{2A}R$ rich striatal regions can be selectively blocked by the application of the selective $A_{2A}R$ antagonist ZM 241385. These findings should encourage further studies to determine their metabolic stability, blood-brain-barrier permeability and *in vivo* receptor affinity and specificity.

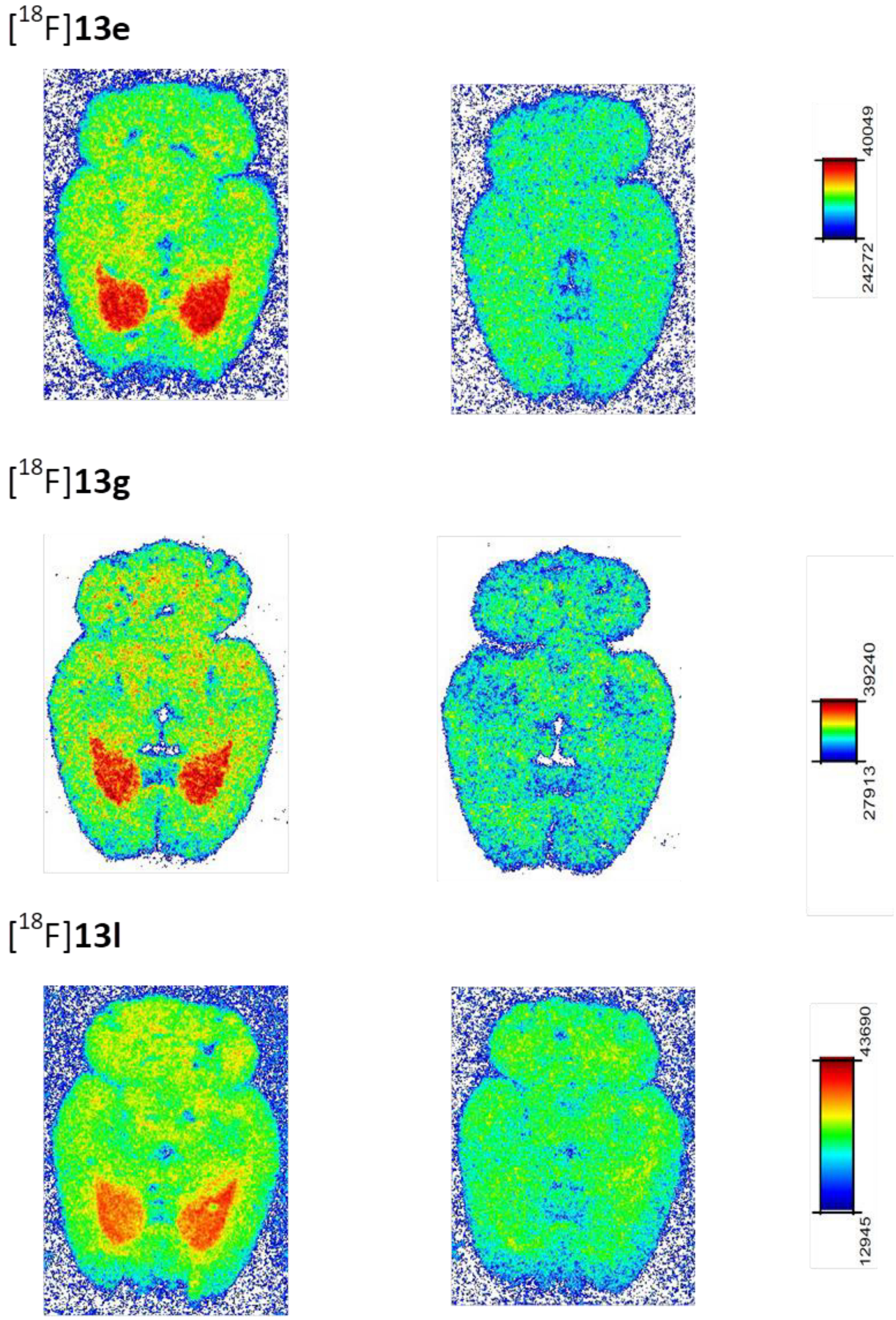


Figure 4: *In vitro* autoradiography with **[^{18}F]13e** (top), **[^{18}F]13g** (middle), and **[^{18}F]13l** (bottom) in horizontal rat brain slices (20 µm). The slices were incubated in a solution of 0.01 M TRIS buffer (pH 7.4), 1 µM EDTA and the indicated radioligands with an activity concentration of 2.3 kBq/mL. The exposure time was 12 hours. Autoradiograms on the left show total binding of the respective ligands, while those on the right show unspecific

binding obtained if specific binding was inhibited by 1 µM of the $A_{2A}R$ antagonist ZM 241385. The numbers next to the color scales represent the relative activity units associated with the color shades.

CONCLUSION

In the present study, a simple chemical building block principle has been used to prepare a series of Tozadenant analogues. The described approach for the late stage piperidine ring functionalization of the A_{2A} receptor antagonist Tozadenant provided derivatives with high affinity to the A_{2A} receptor and good selectivity over the A_1 receptor. Among these compounds are a number of fluorine-containing analogues, which bind reversibly and with high affinity to the adenosine A_{2A} receptor while maintaining a very high selectivity over the adenosine A_1 receptor. Three compounds with the most promising binding properties were radiofluorinated and examined by *in vitro* autoradiography in rat brain sections. All three derivatives showed almost exclusive striatal binding. As such, the described compounds represent an interesting starting point for the development of new PET ligands. The strategy of performing structural modifications at the 4-position of the piperidine ring of Tozadenant while maintaining high affinity and selectivity for the target receptor offers the possibility to synthesize a variety of analogues that differ in their physicochemical properties (e.g. lipophilicity, non-specific binding, metabolic stability) and can be designed tailor-made for specific applications.

EXPERIMENTAL

General information

Unless noted otherwise, all reactions were carried out under a nitrogen or argon atmosphere at ambient temperature (22 ± 2°C) in oven-dried glassware. Standard inert atmosphere techniques were used in handling all air and moisture sensitive reagents.

Melting points were measured on a Melting Point B-540 instrument (Büchi Labortechnik, Flawil, Switzerland).

No-carrier-added [^{18}F]fluoride was produced via the $^{18}O(p,n)^{18}F$ nuclear reaction by bombardment of isotopically enriched [^{18}O]water in a Ti-target with 16.5 MeV protons at the JSW cyclotron BC1710 (INM-5, Forschungszentrum Jülich*)*.

Solvents and reagents

Solvents were either purchased commercially in sufficient purity (MerckKGaA, Darmstadt, Germany) or purified and dried by standard methods [42]. All chemicals used for the syntheses were commercially available (Merck, Taufkirchen, Germany; Activate Scientific, Prien, Germany; ABCR, Karlsruhe, Germany) or were prepared as described in the text.

Spectroscopy

^{1}H, ^{13}C, and ^{19}F NMR spectra were recorded at 400.13, 100.61, and 376.49 MHz by means of a Bruker Avance Neo 400 instrument (Bruker Bio Spin GmbH, Rheinstetten, Germany), in 5% solution at 299 K. Chemical shifts (δ) are given in parts per million (ppm). Internal reference was set to the residual solvent signals (for $CDCl_3$, δ_H = 7.26, δ_C = 77.16; for DMSO-*d$_6$*, δ_H = 2.50, δ_C = 50.32). The ^{1}H NMR spectra are reported as follows: δ / ppm (number of protons, multiplicity, coupling constant J / Hz (where appropriate), assignment). The following abbreviations and their combinations are used when reporting NMR data: s = singlet, d

=doublet, t = triplet, q = quartet, m = multiplet, br s = broad singlet, obsc = obscured, v = very. NMR signals were assigned based on information from additional two-dimensional experiments (COSY, gHSQC, gHMBC, NOESY). Coupling constants *J* (in Hertz) for protons are given in the form $^{n}J(^{1}H, X)$, those for carbons as $^{n}J(^{13}C,^{19}F)$. All ^{13}C- and ^{19}F NMR spectra were recorded under ^{1}H-broadband decoupling (CPD). Compound names are those generated by ChemBioDraw™ (CambridgeSoft) following IUPAC nomenclature. However, the NMR assignment numbering used is arbitrary and does not follow any particular convention. Numbering of compounds is illustrated on the structures themselves; *vide infra*.

Low-resolution mass spectra were obtained in electrospray ionization (ESI positive) mode with a Thermo Finnigan Surveyor mass spectrometer (Thermo Fisher Scientific GmbH, Dreieich, Germany). The analytes were dissolved in methanol (about 1 mg / mL) and injected directly through a valve on the ionisation interface. The flow rate of the eluent (methanol/water/acetic acid, 50/50/0.2, v/v/v) was 200 µL/min. Reported are the m/z-values of the pseudo-ion $[M + H]^{+}$.

High resolution mass spectra and elemental analyses were performed by the Central Division of Analytical Chemistry at the Forschungszentrum Jülich. Analyses indicated by the symbols of the elements are within ± 0.4 % of the theoretical values.

Chromatography

Thin layer chromatographic analyses were performed in all reactions to monitor the reaction progress and served as a purity check of the obtained products. The respective eluent was selected so that the R_f values of the individual compounds ranged from 0.2 to 0.8. Visualization was done by detection under UV light using silica coated TLC aluminium sheets with fluorescent indicator (SIL ALUGRAM G/UV254 Macherey-Nagel GmbH, Düren, Germany) and/or by iodine staining.

For flash chromatography a Grace Reveleris® iES flash chromatography system equipped with RevealX™ detection, allowing for multisignal (UV/ELSD) collection, and Reveleris® flash silica cartridges (size 40 µm) as stationary phase were employed.

Chemical Syntheses

4-(4-Methoxyhenyl)morpholine nitrate salt **2**

A 1 L 3-neck round bottom flask, equipped with a mechanical stirrer, was charged with *p*-anisidine **1** (20 g, 0.162 mol), 2-chloroethyl ether (48 g, 0.336 mol), tetrabutylammonium bromide (1.04 g, 0.003 mol), and 42% sodium hydroxide solution (77 g, 0.8 mol). The mixture was stirred at 120 °C for 8 h. After completion of the reaction the mixture was cooled to 20 °C and extracted with TBME (80 mL) and ethyl acetate (80 mL). The combined organic phases were washed with water (80 mL), the dark organic solution was cooled to 0 - 5 °C, and 70% HNO_3 (14.6 g, 0.162 mol) was slowly added. Scratching with a glass rod induced crystallization of the nitrate salt. After cooling to 5°C for 12 h the solid was filtered, washed with TBME (40 mL), and dried under vacuum at 45 °C overnight to give the title compound (40.2 g, 97%) as a tan solid, mp 112 - 113°C (dec.). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 3.52 (m, 4H, 2 x N-CH_2), 3.79 (s, 3H, O-CH_3), 3.94 (m, 4H, 2 x O-CH_2), 7.08 (d, 2H, J = 8.5 Hz, aryl-H^2, aryl-H^6), 7.53 (d, 2H, J = 9.1 Hz, aryl-H^3, aryl-H^5). ^{13}C-NMR (101 MHz, DMSO-d_6) δ 54.2 (N-CH$_2$), 56.0 (O-CH$_3$), 64.9 (O-CH$_2$), 115.5 (aryl-C^3 + aryl-C^5), 122.1 (aryl-C^2 + aryl-C^6), 136.4 (aryl-C^1), 160.0 (aryl-C^4).

4-(4-Methoxy-3-nitrophenyl)morpholine **3**

A 250 mL 3-neck round bottom flask, equipped with a magnetic stirrer, was charged with 95% sulfuric acid (80 g, 0.815 mol). The acid was cooled to 0°C (ice/salt bath) and a solution of the nitrate salt **2** (20 g, 0.078 mol) of in dichloromethane (125 mL) was added dropwise from a dropping funnel over 1 h while the reaction temperature was maintained at 0 - 5°C. After complete addition the mixture was stirred for 30 min, the bottom acid layer was separated and slowly added to ice / water (200 mL , 1 L beaker) while maintaining the temperature at <10°C. To this diluted acid solution was then slowly added 28% NH_4OH solution (190 mL) while the temperature was maintained at <10°C by the addition of ice. At the end of the addition the pH of the mixture should be higher than 10 (TLC: ethyl acetate/methanol, 98/2). The batch was cooled at 5°C for 1 h, the solid was filtered, washed with 28% NH_4OH solution (50 mL) and water (50 mL), and dried under vacuum at 45°C overnight to give the title compound (17.5 g, 94% yield) as an orange solid, mp 93°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 3.09 (m, 4H, 2 x N-CH_2), 3.74 (m, 4H, 2 x O-CH_2), 3.85 (s, 3H, O-CH_3), 7.28 (m, 2H, aryl-H^5, aryl-H^6), 7.33 (m, 1H, aryl-H^2). ^{13}C-NMR (101 MHz, DMSO-d_6) δ 49.2 (N-CH$_2$), 57.3 (O-CH$_3$), 66.4 (O-CH$_2$), 111.3 (aryl-C^2), 115.8 (aryl-C^5), 122.8 (aryl-C^6), 140.2 (aryl-C^3), 145.3 (aryl-C^4), 145.3 (aryl-C^1). $C_{11}H_{14}N_2O_4$ MS (ESI+) m/z: $[M+H]^+$ Calcd 239.10; Found 239.19.

2-Methoxy-5-morpholinoaniline **4**

To a stirred solution of nitroarene **3** (11.9 g, 50 mmol) and ammonium chloride (26.7 g, 500 mmol) in a mixture of ethanol (300 mL) and ethyl acetate (300 mL) was added zinc powder (32.7 g, 500 mmol). The reaction was stirred at room temperature for 0.5 h, then diluted with EtOAc, filtered through CELITE®, and the filtrate evaporated *in vacuo* to give aniline **4** (10.4 g, 99%) as dark brown crystals. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 2.91 (m, 4H, 2 x N-CH_2), 3.68 (s, 3H, O-CH_3), 3.70 (m, 4H, 2 x O-CH_2), 4.60 (s_{br}, 2H, NH_2), 6.09 (dd, 1H, J = 8.7 Hz, 2.8

Hz, aryl-H^6), 6.31 (d, 1H, J = 2.8 Hz, aryl-H^2), 6.31 (d, 1H, J = 8.7 Hz, aryl-H^5). ^{13}C-NMR (101 MHz, DMSO-d_6) δ 50.4 (N-CH$_2$), 56.2 (O-CH$_3$), 66.7 (O-CH$_2$), 1103.4 (aryl-C^2), 103.9 (aryl-C^5), 112.0 (aryl-C^6), 138.5 (aryl-C^3), 141.3 (aryl-C^4), 146.5 (aryl-C^1). $C_{11}H_{16}N_2O_2$ MS (ESI+) m/z: $[M+H]^+$ Calcd 209.12; Found 209.17.

1-Benzoyl-3-(2-methoxy-5-morpholin-4-yl-phenyl)-thiourea **5**

To a solution of 2-methoxy-5-morpholinoaniline **4** (4.6 g, 22 mmol) in acetone (140 mL) was added dropwise a solution of benzoyl isothiocyanate (3.4 mL, 25 mmol) in acetone (80 mL) and after complete addition the mixture was stirred at ambient temperature for another 0.5 h (TLC: ethyl acetate/hexane/AcOH, 50/50/0.2). Acetone was distilled off at atmospheric pressure with the continuous dropwise addition of water (total volume 150 mL). After cooling the suspension to ambient temperature and to 5°C overnight the product was collected by filtration, washed with water, dried, and recrystallized from MeOH to give the benzoyl thiourea as yellow crystals, mp 139-140°C (95% yield). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 3.02 (m, 4H, 2 x N-CH_2), 3.76 (m, 4H, 2 x O-CH_2), 3.85 (s, 3H, O-CH_3), 6.82 (dd, 1H, J = 9 Hz, 3 Hz, aryl-H^6), 7.04 (d, 1H, J = 9 Hz, aryl-H^5), 7.55 (m, 2H, Bz-$H^{3,5}$), 7.67 (m, 1H, Bz-H^4) 7.98 (m, 2H, Bz-$H^{2,6}$), 8.5 (d, 1H, J = 3 Hz, aryl-H^2), 11.55 (s, 1H, SCNHCO), 13.10 (s, 1H, arylNHCS). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 50.1 (N-CH$_2$), 56.9 (O-CH$_3$), 66.6 (O-CH$_2$), 111.4 (aryl-C^2), 112.4 (aryl-C^5), 113.8 (aryl-C^6), 127.8 (aryl-C^3), 128.9 (Bz-$C^{2,6}$), 129.2 (Bz-$C^{3,5}$), 132.5 (Bz-C^1), 133.6 (Bz-C^4), 144.7 (aryl-C^1), 145.1 (aryl-C^4), 168.9 (CO), 177.9 (CS). $C_{19}H_{21}N_3O_3S$ MS (ESI+) m/z: $[M+H]^+$ Calcd 372.13; Found 372.09.

(2-Methoxy-5-morpholin-4-yl-phenyl)-thiourea **6**

At ambient temperature the benzoylthiourea **5** (10.6 g, 28 mmol) was suspended in MeOH (65 mL). Under magnetic stirring sodium methoxide (35% solution in methanol, 9.3 mL, 43 mmol) was added and the clear brown solution was stirred for 3 h. During this time a grey suspension formed. The mixture was cooled to 0 – 5°C and was kept at that temperature for 1 h while stirring was continued. Filtration, washing with ice cold MeOH (10 mL) and hexane (10 mL) and air drying gave 7.1 g (95% yield) of the title compound as brown crystals, mp 184-185°C (TLC: ethyl acetate/hexane/AcOH, 80/20/0.2, v/v/v) ^{1}H-NMR (400 MHz, DMSO-d_6) δ 2.98 (m, 4H, 2 x N-CH_2), 3.73 (m, 4H, 2 x O-CH_2), 3.76 (s, 3H, O-CH_3), 6.71 (m, 1H, J = 9 Hz, 3 Hz, aryl-H^6), 6.92 (m, 1H, J = 9 Hz, aryl-H^4), 7.50 (s_{br}, 2H, NH_2), 7.64 (m, 1H, aryl-H^5), 9.02 (s_{br}, 1H, NH). ^{13}C-NMR (101 MHz, DMSO-d_6) δ 50.2 (N-CH$_2$), 56.5 (O-CH$_3$), 66.6 (O-CH$_2$), 112. 6 (aryl-C^2), 112.9 (aryl-C^5), 113.9 (aryl-C^6), 128. 5 (aryl-C^3), 145.2 (aryl-C^1), 145.7 (aryl-C^4), 181.6 (CS). $C_{12}H_{17}N_3O_2S$ MS (ESI+) m/z: $[M+H]^+$ Calcd 268.10; Found 268.04.

4-Methoxy-7-morpholin-4-yl-benzothiazol-2-ylamine **7**

Under efficient mechanical stirring the thiourea **6** (6.05 g, 22.6 mmol) was suspended in ethyl acetate (67 mL) and heated to 80°C. At that temperature 33% HBr in acetic acid (8.18 mL, 45.2 mmol) was added dropwise over 0.2 h. After complete addition the thick suspension was refluxed (oil bath 100°C) for 0.2 h and DMSO (1.93 mL, 27.12 mmol) was added at that temperature in one portion. Ten minutes after the addition ethyl acetate (20 mL) was added and stirring and heating was continued for 4 h. The mixture was cooled to ambient temperature, the solid collected by filtration and washed with ethyl acetate (50 mL). The wet material was suspended under stirring in EtOH (55 mL), water (72 mL) was added, and the red solution thus formed was heated to 50°C. Aqueous ammonia (28%, 5.4 mL) was added (pH 9 – 10), the mixture was cooled in an ice bath, and stirred overnight (during this time the ice bath was allowed to thaw). The light brown precipitate formed was collected by filtration and washed with 50% aqueous ethanol (TLC: ethyl acetate/methanol, 90/10). The solid was extracted with boiling THF (100 mL), cooled, collected by filtration, and oven-dried at 100°C to give the aminobenzothiazole as grey crystals, 5.7 g (95% yield), mp >275°C (dec) (TLC: ethyl acetate/methanol/AcOH, 90/10/0.2, v/v/v). If further purification was needed the solid was dissolved in aqueous 0.5 N HCl (50 mL, 25 mmol) and stirred for 0.2 h at ambient temperature. Insoluble material was removed by filtration, aqueous NH_4OH (28%, 14.47 M, 2.07 mL, 30 mmol) was added, and the formed suspension was stirred for 0.2 h. EtOH (75 mL) was added and the mixture was chilled overnight. The solid was collected by filtration, washed with 50% aqueous ethanol and vacuum-dried in a desiccator over P_4O_{10}. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 2.94 (m, 4H, 2 x N-CH_2), 3.74 (m, 4H, 2 x O-CH_2), 3.81 (s, 3H, O-CH_3), 6.64 (d, 1H, J = 8.7 Hz, H^5), 6.78 (d, 1H, J = 8.7 Hz, H^6), 7.42 (s_{br}, 2H, NH_2). ^{13}C-NMR (101 MHz, DMSO-d_6) δ 51.7 (N-CH$_2$), 56.6 (O-CH$_3$), 67. (O-CH$_2$), 109.4 (C^6), 110.7 (C^5), 126.1 (C^{7a}), 140.4 (C^4), 143.4 (C^7), 146.9 (C^{4a}) 165.8 (C^2). $C_{12}H_{15}N_3O_2S$ MS (ESI+) m/z: $[M+H]^+$ Calcd 266.09; Found 266.17.

Phenyl (4-Methoxy-7-morpholin-4-yl-benzo[*d*]thiazol-2-yl)carbamate **8**

To a well stirred suspension of 4-methoxy-7-morpholin-4-yl-benzo[d]thiazol-2-ylamine (2.65 g, 10 mmol) in dry THF (50 mL) was added dry pyridine (2.43 mL, 30 mmol) at 0 – 5°C. After stirring for 5 min, a solution of phenyl chloroformate (1.44 mL, 11.5 mmol) in THF (5 mL) was added very slowly. The reaction mixture was allowed to stir at ambient temperature for 14 h (TLC: ethyl acetate/methanol/AcOH, 98/2/0.2, $R_{fCarbamate}$ 0.85; ethyl acetate/hexane/acetic acid, 80/20/0.2, $R_{fCarbamate}$ 0.82) and was then diluted with ethyl acetate (250 mL) and 50% brine (75 mL). The organic layer was separated, washed with aqueous HCl (0.5N, 50 mL) and brine (75 mL), dried, filtered and rotoevaporated *in vacuo* to furnish a fawn solid residue (3.4 g, 88%). An analytical sample was recrystallized from acetonitrile. (400 MHz, DMSO-d_6) δ 3.01 (m, 4H, 2 x N-CH_2), 3.76 (m, 4H, 2 x O-CH_2), 3.91 (s, 3H, O-CH_3), 6.91 (d, 1H, J = 8.6 Hz, H^5), 6.95 (d, 1H, J = 8.6 Hz, H^6), 7.31 (m, 3H, phenyl), 7.47 (m, 2H, phenyl), 12.67 (s_{br}, 1H, NH).

^{13}C-NMR (101 MHz, DMSO-d_6) δ 51.9 (N-CH_2), 56.4 (O-CH_3), 67. (O-CH_2), 108.8 (C^6), 113.4 (C^5), 122.2 (phenyl $C^{2,6}$), 126.6 (phenyl C^4), 127.3 (C^{7a}), 130.1 (phenyl $C^{3,5}$), 140.0 (C^7), 140.4 (C^4), 148.5 (C^{4a}), 150.5 (phenyl C^1), 153.2 (C=O), 158.6 (C^2). $C_{19}H_{19}N_3O_4S$ MS (ESI+) m/z: $[M+H]^+$ Calcd 386.44; Found 386.57.

2-Amino-7-morpholinobenzo[*d*]thiazol-4-ol **9**

Under efficient stirring a solution of 2-amino-4-methoxy-7-morpholinobenzothiazole (1.3 g, 5 mmol) in 48% HBr (8.9M, 50 mL, 445 mmol, good quality: colorless to slightly yellowish) was stirred at 130°C for 24 h. The formed suspension was cooled to ambient temperature and then set aside overnight at 5°C, The grey brown solid was collected by filtration and was taken up in water (30-40 mL). Under efficient stirring the pH of the mixture was brought to 8–9, first by the addition of 10N aqueous sodium hydroxide until a precipitate started to form, then by the addition of saturated aqueous sodium bicarbonate. The formed suspension was diluted with water (50 mL), stirred for 10 min at ambient temperature and was kept at 5°C for one hour. The product was collected by filtration, washed with water and dried in air. The aminophenol was obtained as a tan solid in 85% yield (1150 mg, TLC: ethyl acetate/methanol/AcOH, 98/2/0.2, R_f 0.69; ethyl acetate/hexane/AcOH, 80/20/0.2, R_f 0.57). The phenol is soluble in 0.5N NaOH. ^1H-NMR (400 MHz, DMSO-d_6) δ 2.90 (t, 4H, *J* = 9.1 Hz, 2 x N-CH_2). 3.72 (t, 4H, *J* = 9.1 Hz, 2 x O-CH_2), 6.55 (d, 1H, *J* = 8.4 Hz, H^6), 6.61 (d, 1H, *J* = 8.4 Hz, H^5), 7.23 (s_{br}, 2H, NH_2), 8.89 (s, 1H, O*H*). ^{13}C-NMR (101 MHz, DMSO-d_6) δ 51.8 (*C*N*C*). 67.1 (*C*O*C*), 111.3 (C^6), 112.1 (C^5), 126.2 (C^{7a}), 138.8 (C^4), 142.3 (C^7), 144.5 (C^{4a}), 165.1 (C^2). $C_{11}H_{13}N_3O_2S$ MS (ESI+) m/z: $[M+H]^+$ Calcd 252.30; Found 252.21.

4-(2-Fluoroethoxy)-7-morpholinobenzo[*d*]thiazol-2-amine **10**

Under argon Cs_2CO_3 (2.65 mg, 7.5 mmol) was added to a stirred solution of 2-amino-7-morpholinobenzo[d]thiazol-4-ol (1.25 mg, 5 mmol) in dry DMF (50 mL). Immediately after the carbonate addition 2-bromo-1-fluoroethane (450 µL, 6 mmol) was added and the mixture was stirred at ambient temperature for 24 h (TLC: ethyl acetate/methanol, 98/2, R_f 0.79; ethyl acetate/hexane, 80/20 R_f 0.54). Upon the addition of water (100 mL) the product precipitated as a fine granular solid that was collected by filtration, washed with methanol, and air dried. Yield 800 mg (54%), tan solid. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 2.94 (m, 4H, 2 x N-CH_2), 3.73 (m, 4H, 2 x O-CH_2), 4.29 (dt, $^3J_{H\text{-}F}$ = 31 Hz, ^3J = 3.9 Hz, 2H, CH_3CH$_2$F), 4.73 (dt, $^2J_{H\text{-}F}$ = 48 Hz, ^3J = 3.9 Hz, 2H, CH$_3$CH_2F), 6.64 (d, 1H, J = 8.6 Hz, H^6), 6.78 (d, 1H, J = 8.6 Hz, H^5), 7.47 (s_{br}, 2H, NH_2). ^{19}F-NMR (377 MHz, DMSO-d_6) δ -221.76. ^{13}C-NMR (101 MHz, DMSO-d_6) δ 51.7 (*C*-N-*C*), 67. (*C*-O-*C*), 68.9(d, $^2J_{C\text{-}F}$ = 19 Hz, *C*H$_2$CH$_2$F), 82.9(d, $^1J_{C\text{-}F}$ = 167 Hz, CH$_2$*C*H$_2$F), 110.7 (C^6), 111.4 (C^5), 126.2 (C^{7a}), 140.9 (C^4), 143.8 (C^7), 145.5 (C^{4a}) 166 (C^2). $C_{13}H_{16}FN_3O_2S$ HRMS (ESI+) m/z: $[M+H]^+$ Calcd 298.1020; Found 298.1016.

Phenyl (4-(2-fluoroethoxy)-7-morpholinobenzo[*d*]thiazol-2-yl)carbamate **11**

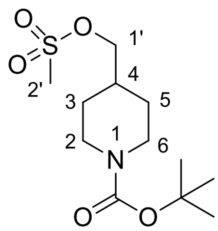
At 0 – 5°C pyridine (243 µL, 3 mmol) was added to a well stirred suspension (prepared in ultrasound bath) of 4-(2-fluorethoxy)-7-morpholin-4-yl-benzo[d]thiazol-2-ylamine (297 mg, 1 mmol) in dry THF (7.5 mL). After stirring for 5 min, phenyl chloroformate (144 µL, 1.15 mmol) was added slowly. The reaction mixture was allowed to stir at ambient temperature for 5 h (TLC (sample in acetone not methanol): ethyl acetate/hexane, 80/20, R_f 0.90; if reaction was not quant. after 5 h 10 µL of chloroformate was added and reaction time was 1 h longer) and was then diluted with ethyl acetate (25 mL) and water (7.5 mL). The organic layer was separated, washed two times with aqueous HCl (0.5N, 5 mL) and brine (10 mL), dried, filtered and rota-evaporated *in vacuo* to furnish a fawn solid residue. Recrystallisation from ethyl acetate gave the carbamate as an off-white solid (360 mg, 86%). ^{1}H-NMR (400

MHz, DMSO-d_6) δ 3.01 (m, 4H, 2 x N-CH_2), 3.76 (m, 4H, 2 x O-CH_2), 4.29 (dt, $^3J_{H-F}$ = 31 Hz, ^3J = 3.9 Hz, 2H, CH_2CH$_2$F), 4.73 (dt, $^2J_{H-F}$ = 48 Hz, ^3J = 3.9 Hz, 2H, CH$_2$CH_2F), 6.91 (d, 1H, J = 8.6 Hz, H^6), 6.95 (d, 1H, J = 8.6 Hz, H^5), 7.31 (m, 3H, phenyl), 7.47 (m, 2H, phenyl), 12.67 (s_{br}, 1H, NH). ^{19}F-NMR (377 MHz, DMSO-d_6) δ -223.21. ^{13}C-NMR (101 MHz, DMSO-d_6) δ 51.9 (C-N-C), 67. (C-O-C), 68.8(d, $^2J_{C-F}$ = 19 Hz, CH$_2$CH$_2$F), 82.9(d, $^1J_{C-F}$ = 167 Hz, CH$_2$$CH_2$F), 108.8 ($C^6$), 113.4 ($C^5$), 122.2 (phenyl $C^{2,6}$), 126.6 (phenyl C^4), 127.3 (C^{7a}), 130.1 (phenyl $C^{3,5}$), 140.0 (C^7), 140.4 (C^4), 148.5 (C^{4a}), 150.5 (phenyl C^1), 153.2 (C=O), 158.6 (C^2). $C_{20}H_{20}FN_3O_4S$ HRMS (ESI+) m/z: $[M+H]^+$ Calcd 418.1231; Found 418.1229.

Preparation of piperidines **12g** - **12i** and **12m – 12o**

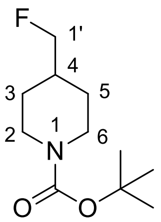
4-(Fluoromethyl)piperidine **12g**

a) 1-(*tert*-Butoxycarbonyl)-4-(methylsulfonyloxy)methylpiperidine **12g-1**



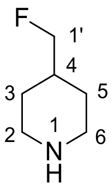
To an approximately 0.2M solution of the alcohol (1.076 g, 5 mmol) in ethanol free DCM (25 mL) containing a 50% molar excess of triethylamine (1050 µL, 7.5 mmol) and kept between 0°C and -10°C, was added a 10% excess of methanesulfonyl chloride (465 µL, 6 mmol) over a period of 2-5 minutes. Ten minutes after the addition the cooling bath was removed and the mixture was stirred for 60 min at ambient temperature to complete the reaction (TLC: ethyl acetate/hexane, 50/50, $R_{fproduct}$ 0.06 – 0.19 (Λ), iodine staining). The reaction mixture was transferred to a separatory funnel with the aid of more DCM. The mix was first extracted with ice water, followed by cold 10% HCl acid, sat. sodium bicarbonate, and sat. brine. Drying the DCM solution over Na_2SO_4 followed by solvent removal gave the product (1.46 g, 99%) as a clear colorless oil, that solidified upon standing in the refrigerator overnight to furnish a colorless waxy solid, mp 76-77°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.14-1.28 (m, 2H, 0.5 C^3H_2, 0.5 C^5H_2), 1.44 (9H, s, Boc CH_3), 1.72 (d_{br}, J = 13.4 Hz, 2H, 0.5 C^3H_2, 0.5 C^5H_2), 1.84-1.96 (m, 1H, C^4H), 2.70 (td, J = 13.4, 2.6 Hz, 2H, 0.5 C^2H_2, 0.5 C^6H_2), 3.02 (s, 3H,Mes CH_3), 4.05 (d, J = 6.4 Hz, 2H, $C^{1'}H_2$), 4.13 (d_{br} J = 13.4 Hz, 2H, 0.5 C^2H_2, 0.5 C^6H_2,). ^{13}C NMR (101 MHz, $CDCl_3$) δ 28.2 (C^3, C^5), 28.4 (Boc CH3), 35.9 (C^4), 37.3 (Ms CH3), 39.6 ($C^{1'}$), 43.2 (C^2, C^6), 79.6 (Boc C), 154.7 (Boc C=O). $C_{12}H_{23}NO_5S$ HRMS (ESI+) m/z: $[M+H]^+$ Calcd 294.1369; Found 294.1369

b) 1-(*tert*-Butoxycarbonyl)-4-fluoromethylpiperidine **12g-2**



Under an atmosphere of argon TBAF (1M solution in THF, 25 mL, 25 mmol, 5 eq) was added to 1-(*tert*-butoxycarbonyl)-4-(methylsulfonyloxy)methyl-1,2,3,6-tetrahydropyridine **12g-1** (1.46 g, 5 mmol). The mixture was stirred and heated to 70°C and kept at that temperature for 24 h. (TLC (spots of reaction mixture, no dilution): ethyl acetate/hexane, 50/50, R_{fTBAF} 0.00 $R_{fproduct}$ 0.94, R_{feduct} 0.06 – 0.19, iodine staining). After cooling to ambient temperature the mixture was partitioned with diethyl ether and sat. aqueous NH_4Cl solution. The organic fraction was washed with sat NH_4Cl, water, and brine, dried over Na_2SO_4, filtered, and concentrated to afford an almost colorless solid residue (760 mg, 3.5 mmol, 70%), mp 51-52°C. . ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.15-1.28 (m, 2H, 0.5 C^3H_2, 0.5 C^5H_2), 1.46 (9H, s, Boc CH_3), 1.69 (d_{br}, J = 13.4 Hz, 2H, 0.5 C^3H_2, 0.5 C^5H_2), 1.77-1.94 (m, 1H, C^4H), 2.71 (td, J = 13.4, 2.7 Hz, 2H, 0.5 C^2H_2, 0.5 C^6H_2), 4.14 (d_{br}, J = 6.4 Hz, 2H, 0.5 C^2H_2, 0.5 C^6H_2), 4.27 (dd, , $^2J_{H-F}$ = 47.6, $^3J_{H-H}$ = 6,1 Hz, 2H, $C^{1'}H_2$). ^{19}F NMR (377 MHz, $CDCl_3$) δ -224.10. ^{13}C NMR (101 MHz, $CDCl_3$) δ 27.6 (d, $^3J_{C-F}$ = 5.9 Hz C^3, C^5), 28.4 (Boc CH_3), 36.9 (d, $^2J_{C-F}$ = 18 Hz, C^4), 43.4 (C^2, C^6), 79.6 (Boc C), 87.5 (d, $^1J_{C-F}$ = 169.3 Hz, $C^{1'}$), 154.8 (Boc C=O). $C_{11}H_{20}FNO_2$ HRMS (ESI+) m/z: $[M+H]^+$ Calcd 218.1551; Found 218.1550.

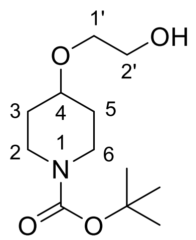
c) 4-Fluoromethylpiperidine **12g**



Trifluoroacetic acid (7 mL) was added to a solution of 1-(*tert*-Butoxycarbonyl)-4-fluoromethyl-piperidine **12g-2** (760 mg, 3.5 mmol) in CH_2Cl_2 (7 mL) and the mixture was stirred at ambient temperature for two hours. The solution was concentrated *in vacuo* and co-evaporated with methanol (4 x 5 mL) to furnish the trifuoroacetate salt of the deprotected compound as a yellow oil (800 mg, 99%). $C_8H_{13}F_4NO_2$ MS (ESI+) m/z: $[M+H]^+$ Calcd 231.19; Found 118.18 (free base MW 117.16). The oil from above was taken up in CH_2Cl_2 (10 mL) and the trifluoroacetate salt was freebased with a saturated $NaHCO_3$ (aq) solution (10 mL). The organic layer was separated and the aqueous phase was extracted with CH_2Cl_2 (20 mL). The organic layers were combined, dried over Na_2SO_4, and concentrated under reduced pressure to afford the amine (390 mg, 95% yield) as an oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.64 (m, 2H, 0.5 C^3H_2, 0.5 C^5H_2), 1.97 (m, 2H, 0.5 C^3H_2, 0.5 C^5H_2), 2.07 (m, 1H, C^4H), 2.92 (m, 2H, 0.5 C^2H_2, 0.5 C^6H_2), 3.49 (m, 2H, 0.5 C^2H_2, 0.5 C^6H_2), 4.33 (dd, , $^2J_{H-F}$ = 47.6, $^3J_{H-H}$ = 6,1 Hz, 2H, $C^{1'}H_2$). ^{19}F NMR (377 MHz, $CDCl_3$) δ -224.43. $C_6H_{12}FN$ HRMS (ESI+) m/z: $[M+H]^+$ Calcd 118.1027; Found 118.1021.

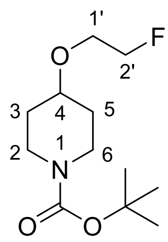
4-(2-Fluoroethoxy)piperidine hydrochloride **12h HCl**

a) *tert*-Butyl-4-(2-hydroxyethoxy)piperidine-1-carboxylate **12h-1** (according to [43])



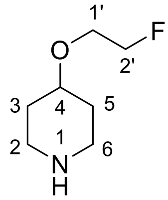
Sodium hydride (60 % dispersion in mineral oil, 650 mg, 16.3 mmol, 1.01 eq.) was suspended in dry THF under an argon atmosphere and cooled to 0 °C. *N*-Boc-4-hydroxypiperidine (3.25 g, 16.3 mmol, 1.01 eq.) was added and the suspension was stirred for twenty minutes at 0°C. After the addition of methyl bromoacetate (1.5 mL, 16.2 mmol, 1.00 eq.) the reaction was stirred for 22 hours at room temperature. The reaction was quenched by addition of water and extracted with ethyl acetate. The combined organic phases were washed with brine and dried over sodium sulfate and the solvent was removed *in vacuo*. A colorless oil (1.262 g, impure) was obtained by column chromatography (eluent: petrol ether/ethyl acetate, 4/1) and was dissolved in dry THF under an argon atmosphere. The solution was cooled to 0 °C and lithium aluminium hydride (205 mg, 5.40 mmol) was added in portions. The reaction was allowed to warm to room temperature and stirred for three hours. The reaction was quenched by addition of an aqueous solution of sodium hydroxide (10 %). The mixture was extracted with ethyl acetate. The combined organic phases were washed with brine and dried over sodium sulfate. After removing the solvent *in vacuo* the residue was purified by column chromatography to afford a colorless oil (256 mg, 1.04 mmol, 6.4 %). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.44 (s, 9H, Boc-CH_3), 1.51 (ddd, *J* = 13.4, 8.5, 4.5 Hz, 2H, 0.5 C^3H_2, 0.5 C^5H_2), 1.79 – 1.88 (m, 2H, 0.5 C^3H_2, 0.5 C^5H_2), 2.01 (br, 1H, O*H*), 3.06 (ddd, *J* = 13.6, 8.5, 3.9 Hz, 2H, 0.5 C^2H_2, 0.5 C^6H_2), 3.45 – 3.52 (m, 1H, C^4H), 3.54 – 3.59 (m, 2H, $C^{1'}H_2$), 3.69 – 3.80 (m, 4H, $C^{2'}H_2$, 0.5 C^2H_2, 0.5 C^6H_2).

b) *tert*-Butyl-4-(2-fluoroethoxy)piperidine-1-carboxylate **12h-2**



tert-Butyl-4-(2-hydroxyethoxy)piperidine-1-carboxylate **12h-1** (256 mg, 1.04 mmol, 1.00 eq.) and PyFluor (184 mg, 1.14 mmol, 1.10 eq.) were dissolved in toluene (1.0 mL). DBU (310 µL, 2.08 mmol, 2.00 eq.) was added and the reaction was stirred for 48 hours. The reaction was quenched by the addition of water und extracted with ethyl acetate. The combined organic phases were washed with brine and dried over sodium sulfate. The solvent was removed *in vacuo* and the residue was purified by column chromatography (eluent: petrol ether/ethyl acetate, 4/1) to afford a colorless oil (74 mg, 0.299 mmol, 29 %). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.44 (s, 9H, Boc-C*H*$_3$), 1.47 – 1.58 (m, 2H, 0.5 C^3*H*$_2$, 0.5 C^5*H*$_2$), 1.77 – 1.88 (m, 2H, 0.5 C^3*H*$_2$, 0.5 C^5*H*$_2$), 3.07 (ddd, *J* = 15.8, 9.6, 5.1 Hz, 2H, 0.5 C^2*H*$_2$, 0.5 C^6*H*$_2$), 3.45 – 3.55 (m, 1H, C^4*H*), 3.65 – 3.80 (m, 4H, $C^{1'}$*H*$_2$, 0.5 C^2*H*$_2$, 0.5 C^6*H*$_2$), 4.46 – 4.61 (m, 2H, $C^{2'}$*H*$_2$). ^{19}F-NMR (377 MHz, $CDCl_3$) δ -223.16. ^{13}C-NMR (101 MHz, $CDCl_3$): δ 28.55 (Boc-*C*H$_3$), 31.06 (*C*^{3}, *C*^{5}), 43.28 (*C*^{2}, *C*^{6}), 67.25 (d, *J* = 20.1 Hz, *C*$^{1'}$), 68.35, 75.36 (*C*^{4}), 79.59 (Boc-*C*), 83.38 (d, *J* = 169.3 Hz, *C*$^{2'}$), 154.96 (Boc-CO). $C_{12}H_{23}NO_4$ HRMS m/z: $[M+Na]^+$ Calcd 270.1476; Found 270.1470.

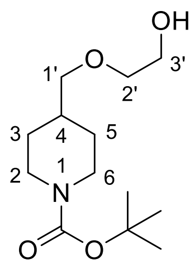
c) 4-(2-Fluoroethoxy)piperidine hydrochloride **12h**



tert-Butyl-4-(2-fluoroethoxy)piperidine-1-carboxylate **12h-2** (74 mg, 0.299 mmol) was dissolved in dichloromethane (2.0 mL) and treated with trifluoroacetic acid (2.0 mL). The reaction was stirred at room temperature for two hours. The solvent was removed *in vacuo* and the residue was treated with 3 M methanolic HCl. After removal of the solvent *in vacuo* a yellow oil (55 mg, 0.299 mmol, >99 %) was obtained that was used without further purification. ^{1}H-NMR (400 MHz, DMSO-d$_6$) δ 1.65 – 1.76 (m, 2H, 0.5 C^3*H$_2$*, 0.5 C^5*H$_2$*), 1.92 – 2.01 (m, 2H, 0.5 C^3*H$_2$*, 0.5 C^5*H$_2$*), 2.86 – 2.96 (m, 2H, 0.5 C^2*H$_2$*, 0.5 C^6*H$_2$*), 3.03 – 3.15 (m, 2H, 0.5 C^2*H$_2$*, 0.5 C^6*H$_2$*), 3.57 – 3.73 (m, 2H, C$^{1'}$*H$_2$*, C^4*H*), 4.27 (s, 2H), 4.43 – 4.59 (m, 2H, C$^{2'}$*H$_2$*), 9.20 (s, 2H). ^{19}F-NMR (377 MHz, DMSO-d$_6$) δ -221.69. ^{13}C-NMR (101 MHz, DMSO-d$_6$) δ 27.20 (*C*^{3}, *C*^{5}), 40.29 (*C*^{2}, *C*^{6}), 66.78 (d, *J* = 18.9 Hz, *C*$^{1'}$), 70.94 (*C*^{4}), 83.15 (d, *J* = 165.8 Hz, *C*$^{2'}$). $C_7H_{14}FNO$ HRMS m/z: [M+H]$^+$ Calcd 148.1132; Found 148.1130.

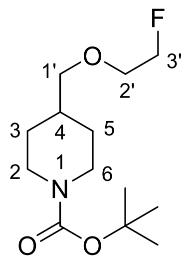
4-((2-Fluoroethoxy)methyl)piperidine hydrochloride **12i HCl**

a) *tert*-Butyl-4-((2-hydroxyethoxy)methyl)piperidine-1-carboxylate **12i-1**



Sodium hydride (60 % dispersion in mineral oil, 1.00 g, 25.0 mmol, 1.01 eq.) was suspended in dry THF under an argon atmosphere and cooled to 0 °C. N-Boc-4-piperidinemethanol (5.35 g, 24.8 mmol, 1.00 eq.) was added and the suspension was stirred for twenty minutes at 0°C. After the addition of methyl bromoacetate (2.4 mL, 25.9 mmol, 1.04 eq.) the reaction was stirred for 24 hours at room temperature. The reaction was quenched by addition of water and extracted with ethyl acetate. The combined organic phases were washed with brine and dried over sodium sulfate and the solvent was removed *in vacuo*. A colorless oil (4.11 g, impure) was obtained by column chromatography (eluent: petrol ether/ethyl acetate, 4/1) and was dissolved in dry THF. Under an argon atmosphere the solution was added dropwise to a suspension of lithium aluminium hydride (568 mg, 15.0 mmol) in dry THF cooled to 0°C. The reaction was allowed to warm to room temperature and stirred for three hours. The reaction was quenched by addition of an aqueous solution of sodium hydroxide (15 %) and the mixture was extracted with ethyl acetate. The combined organic phases were washed with brine and dried over sodium sulfate. After removing the solvent *in vacuo* the residue was purified by column chromatography to afford a colorless oil (454 mg, 1.75 mmol, 7.0 %). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.13 (qd, J = 12.6, 4.3 Hz, 2H, 0.5 C^3H_2, 0.5 C^5H_2), 1.44 (s, 9H, Boc-CH_3), 1.65 – 1.81 (m, 3H, 0.5 C^3H_2, 0.5 C^5H_2, C^4H), 2.03 (br, 1H, O*H*), 2.68 (t, J = 12.4 Hz, 2H, 0.5 C^2H_2, 0.5 C^6H_2), 3.31 (d, J = 6.3 Hz, 2H, $C^{1'}H_2$), 3.49 – 3.53 (m, 2H, $C^{2'}H_2$), 3.69 – 3.73 (m, 2H, $C^{3'}H_2$), 4.02 – 4.16 (m, 2H, 0.5 C^2H_2, 0.5 C^6H_2). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 28.58 (Boc-CH_3), 29.13 (C^3, C^5), 36.61 (C^4), 61.94 (C^2, C^6), 67.67 ($C^{3'}$), 72.20 ($C^{2'}$), 76.13 ($C^{1'}$), 79.43 (Boc-*C*), 155.0 (Boc-*C*O). $C_{13}H_{25}NO_4$ HRMS m/z: $[M+Na]^+$ Calcd 182.1676; Found 182.1675.

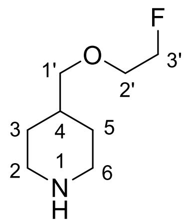
b) *tert*-Butyl-4-((2-fluoroethoxy)methyl)piperidine-1-carboxylate **12i-2**



tert-Butyl-4-(2-hydroxyethoxy)piperidine-1-carboxylate **12i-1** (417 mg, 1.61 mmol, 1.00 eq.) and PyFluor (285 mg, 1.77 mmol, 1.10 eq.) were dissolved in toluene (1.6 mL). DBU (480 µL, 3.22 mmol, 2.00 eq.) was added and the reaction was stirred for 69 hours. The solvent was removed *in vacuo* and the residue was dissolved in dichloromethane. The organic layer was washed with water and dried over sodium sulfate. The solvent was removed *in vacuo* and the residue was purified by column chromatography (eluent: petrol ether/ethyl acetate, 5/1) to afford a colorless oil (80 mg, 0.306 mmol, 19 %). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.06 – 1.19

(m, 2H, 0.5 C^3*H₂*, 0.5 C^5*H₂*), 1.44 (s, 9H, Boc-C*H₃*), 1.67 – 1.80 (m, 3H, 0.5 C^3*H₂*, 0.5 C^5*H₂*, C^4*H*), 2.68 (td, J = 13.2, 2.6 Hz, 2H, 0.5 C^2*H₂*, 0.5 C^6*H₂*), 3.33 (d, J = 6.3 Hz, 2H, $C^{1'}$*H₂*), 3.59 – 3.71 (m, 2H, $C^{2'}$*H₂*), 4.05 – 4.12 (m, 2H, 0.5 C^2*H₂*, 0.5 C^6*H₂*), 4.45 – 4.61 (m, 2H, $C^{3'}$*H₂*). ^{19}F-NMR (377 MHz, $CDCl_3$) δ -222.92. ^{13}C-NMR (101 MHz, $CDCl_3$): δ 28.58 (Boc-*C*H₃), 29.10 (*C³, C⁵*), 36.62 (*C⁴*), 43.74 (*C², C⁶*), 70.33 (d, J = 19.6 Hz, $C^{2'}$), 76.41 ($C^{1'}$), 79.39 (Boc-*C*), 83.23 (d, J = 169.0 Hz, $C^{3'}$), 155.00 (Boc-*C*O). $C_{13}H_{24}FNO_3$ HRMS m/z: $[M+H]^+$ Calcd 284.1632; Found 284.1630.

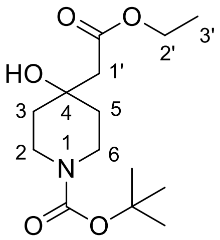
c) 4-((2-Fluoroethoxy)methyl)piperidine hydrochloride **12i HCl**



tert- Butyl-4-((2-fluoroethoxy)methyl)piperidine-1-carboxylate **12i-2** (75 mg, 0.287 mmol) was dissolved in dichloromethane (2.0 mL) and treated with trifluoroacetic acid (2.0 mL). The reaction was stirred at room temperature for four hours. The solvent was removed *in vacuo* and the residue was treated with 3 M methanolic HCl. After removal of the solvent *in vacuo* a colorless oil (67 mg, 0.287 mmol, >99 %) was obtained that was used without further purification. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.59 – 1.76 (m, 2H, 0.5 C^3*H₂*, 0.5 C^5*H₂*), 1.82 – 2.04 (m, 3H, 0.5 C^3*H₂*, 0.5 C^5*H₂*, C^4*H*), 2.24 (br, 1H), 2.77 – 2.98 (m, 2H, 0.5 C^2*H₂*, 0.5 C^6*H₂*), 3.38 (d, J = 5.1 Hz, 2H, $C^{1'}$*H₂*), 3.44 – 3.55 (m, 2H, 0.5 C^2*H₂*, 0.5 C^6*H₂*), 3.59 – 3.72 (m, 2H, $C^{2'}$*H₂*), 4.44 – 4.61 (m, 2H, $C^{3'}$*H₂*), 9.26 (s, 1H), 9.56 (s, 1H). ^{19}F-NMR (377 MHz, $CDCl_3$) δ -222.92. ^{13}C-NMR (101 MHz, $CDCl_3$): δ 25.90 (*C³, C⁵*), 34.76 (*C⁴*), 44.00 (*C², C⁶*), 70.49 (d, J = 19.6 Hz, $C^{2'}$), 75.18 ($C^{1'}$), 83.12 (d, J = 169.3 Hz, $C^{3'}$). $C_8H_{16}FNO$ HRMS m/z: $[M+H]^+$ Calcd 162.1289; Found 162.1285.

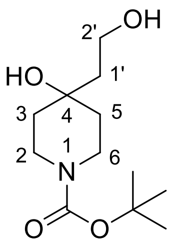
4-(2-Fluoroethyl)-4-hydroxypiperidine **12m**

a) 4-Ethoxycarbonylmethyl-4-hydroxypiperidine-1-carboxylic acid *tert*-butyl ester **12m-1**



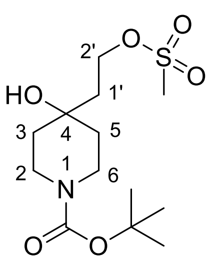
A 1.0 M solution of lithium hexamethyldisilazide (LiHMDS) in THF (40 mL, 40 mmol) was cooled to -70° C under an argon atmosphere. Ethyl acetate (3.91 mL, 40 mmol) was slowly added dropwise over the course of 5 min. The reaction mixture was stirred for 10 min and a solution of 1-(*tert*-butyloxycarbonyl)-4-piperidone (7.34 g, 36.84 mmol) in THF (16 mL) was slowly added dropwise over the course of 20 min. After 3 hours at -70°C the reaction solution was warmed to 0°C, water (50 mL) was added and the reaction mixture extracted with ether (3 x 75 mL). The combined organic phases were washed with sat. aq. NaCl solution (150 mL), dried over Na_2SO_4 and the solvent was removed under a vacuum. The desired product 4-ethoxycarbonylmethyl-4-hydroxypiperidine-1-carboxylic acid *tert*-butyl ester (10.31 g, 97%) was obtained as a clear yellow oil and reacted further without purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.23 (t, *J* = 7.2 Hz, 3H, $C^{3'}H_3$), 1.46 (s, 9H, Boc C*H*$_3$), 1.47-1.55 (m, 2H, 0.5 C^3H_2, 0.5 C^5H_2), 1.61-1.71 (m, 2H, 0.5 C^3H_2, 0.5 C^5H_2), 2.47 (s, 2H, $C^{1'}H_2$), 3.20 (t, *J* = 11.7 Hz, 2H, 0.5 C^2H_2, 0.5 C^6H_2), 3.61 (s, 1H, O*H*), 3.82 (m, 2H, 0.5 C^2H_2, 0.5 C^6H_2), 4.18 (q, *J* = 7.2 Hz, 2H, $C^{2'}H_2$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 14.1 ($C^{3'}$), 28.4 (Boc *C*H$_3$) 36.6 (C^3, C^5), 39.5 (weak, C^2, C^6), 45.5 ($C^{1'}$), 60.8 ($C^{2'}$), 68.1 (C^4), 80.4 (Boc *C*), 154.8 (Boc *C*=O), 172.6 (*C*=O). Calcd. for $C_{14}H_{25}NO_5$: C, 58.52; H, 8.77; N, 4.87; O, 27.84%; Found: C, 58.60; H, 8.69; N, 4.93. $C_{14}H_{25}NO_5$ HRMS (ESI+) m/z: $[M+H]^+$ Calcd 288.1805; Found 288.1803.

b) *tert*-Butyl 4-hydroxy-4-(2-hydroxyethyl)piperidine-1-carboxylate **12m-2**



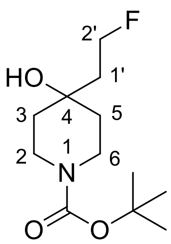
tert-Butyl-4-(2-ethoxy-2-oxoethyl)-4-hydroxypiperidine-1-carboxylate **12m-1** (2.87 g, 10 mmol) was dissolved in THF (40 mL). After adding $LiBH_4$ (2M in THF, 15 mL, 30 mmol, slightly exothermic) methanol (3 mL) was added and the resulting solution stirred at ambient temperature for 2 hours. (TLC: ethyl acetate/hexane, 80/20, $R_{fproduct}$ 0.75, R_{feduct} 0.88, iodine staining, compound is not UV-active). The mixture was externally cooled with a water bath and saturated ammonium chloride solution was carefully added in portions (5 x 1 mL over 25 min, hydrogen gas evolution). After stirring for another 20 min, the mixture was concentrated under reduced pressure and the residue diluted with ethyl acetate and water. The organic layer was separated and washed successively with water and brine and dried over Na_2SO_4. After filtration, the solvent was removed *in vacuo* to obtain a clear colorless oil (2.16 g, 88%) that was reacted further without purification. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.96 (t, 2H), 3.78 (m, 2H), 3.23 (m, 2H), 1.76 (t, 2H) 1.70 (d, 2H), 1.49 (m, 2H), 1.47 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 28.5 (Boc CH_3) 36.8 (C^3, C^5), 39.6 ($C^{1'}$), 41.3 (weak, C^2, C^6), 66.0 ($C^{2'}$), 68.7 (C^4), 80.1 (Boc *C*), 154.7 (Boc *C*=O). $C_{12}H_{23}NO_4$ HRMS (ESI+) m/z: $[M+H]^+$ Calcd 246.1699; Found 246.1691.

c) *tert*-Butyl 4-hydroxy-4-(2-((methylsulfonyl)oxy)ethyl)piperidine-1-carboxylate **12m-3**



To an approximately 0.2M solution of the diol **12m-2** (1.39 g, 5.67 mmol) in ethanol free DCM (28 mL) containing a 50% molar excess of trimethylamine (1190 µL, 8.5 mmol) and kept between 0°C and -10°C, was added a 10% excess of methanesulfonyl chloride (488 µL, 6.3 mmol) over a period of 5-10 minutes. Ten minutes after the addition the cooling bath was removed and the mixture was stirred for one hour at ambient temperature to complete the reaction (TLC: ethyl acetate/hexane, 70/30, $R_{fproduct}$ 0.43, R_{feduct} 0.31, iodine staining, compound is not UV-active). The reaction mixture was transferred to a separatory funnel with the aid of more DCM. The mix was first extracted with ice water, followed by cold 10% HCl acid, sat. sodium bicarbonate, sat. brine. Drying the DCM solution over Na_2SO_4 followed by solvent removal gave the product as a colorless oil (1.45 g, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.46 (s, 9H, Boc CH_3), 1.54-1.71 (m, *4*H, C^3H_2, C^5H_2), 1.95 (t, *J* = 6.6 Hz, 2H, $C^{1'}H_2$), 2.27 (s_{br}, 1H, O*H*), 3.03 (s, 3H,Mes CH_3), 3.13-3.24 (m, 2H, 0.5 C^2H_2, 0.5 C^6H_2), 3.76 (t, *J* = 6.6 Hz, 2H, $C^{2'}H_2$), 3.81 (dt, *J* = 13.3, 3.4 Hz, 2H, 0.5 C^2H_2, 0.5 C^6H_2). ^{13}C NMR (101 MHz, $CDCl_3$) δ 28.4 (Boc *C*H_3) 36.9 (C^3, C^5), 37.5 (Ms *C*H_3), 39.6 ($C^{1'}$), 41.4 (weak, C^2, C^6), 66.0 ($C^{2'}$), 68.8 (C^4), 79.6 (Boc *C*), 154.7 (Boc *C*=O). $C_{13}H_{25}NO_6S$ HRMS (ESI+) m/z: $[M+H]^+$ Calcd 324.1475; Found 324.1474.

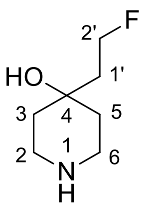
d) *tert*-Butyl 4-(2-fluoroethyl)-4-hydroxypiperidine-1-carboxylate **12m-4**



Under an atmosphere of argon TBAF (1M solution in THF, 15 mL, 15 mmol, 5 eq) was added to *tert*-butyl 4-hydroxy-4-(2-((methylsulfonyl)oxy)ethyl)piperidine-1-carboxylate **12m-3** (960 mg, 3 mmol). The mixture was stirred, heated to 70°C and kept at that temperature for 19 h.

(TLC: ethyl acetate/hexane, 50/50, $R_{fproduct}$ 0.96, R_{feduct} 0.27, iodine staining, compound is not UV-active). After cooling to ambient temperature the mixture was partitioned with diethyl ether and sat. aqueous NH_4Cl solution. The organic fraction was washed with sat NH_4Cl (x 2), water, and brine, dried over Na_2SO_4, filtered, and concentrated to afford the fluoride as a clear orange oil (540 mg, 2.19 mmol, 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.46 (9H, s, Boc CH_3), 1.52-1.67 (m, 4H, C^3H_2, C^5H_2), 1.89 (dt, $^3J_{H-F}$ = 28.4, $^3J_{H-H}$ = 5.7 Hz, 2H, $C^{1'}H_2$), 2.51 (s_{br}, 1H, OH), 3.13 – 3.26 (m, 2H, 0.5 C^2H_2, 0.5 C^6H_2), 3.73 – 3.85 (m, 2H, 0.5 C^2H_2, 0.5 C^6H_2), 4.70 (dt, , $^2J_{H-F}$ = 47.3, $^3J_{H-H}$ = 5,7 Hz, 2H, $C^{2'}H_2$). ^{19}F NMR (377 MHz, $CDCl_3$) δ -218.05. ^{13}C NMR (101 MHz, $CDCl_3$) δ 28.4 (Boc CH$_3$), 36.9 (C^3, C^5), 39.6 (C^2, C^6), 42.4 (d, $^2J_{C-F}$ = 18.4 Hz, $C^{1'}$), 69.0 (d, $^3J_{C-F}$ = 2.5 Hz, C^4), 79.6 (Boc C), 80.8 (d, $^1J_{C-F}$ = 163.5 Hz, $C^{2'}$), 154.8 (Boc C=O). $C_{12}H_{22}FNO_3$ HRMS (ESI+) m/z: $[M+H]^+$ Calcd 248.1656; Found 248.1653.

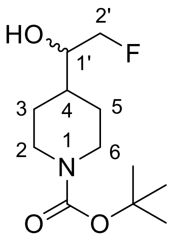
e) 4-(2-Fluoroethyl)-4-hydroxypiperidine trifluoroacetate **12m**



Trifluoroacetic acid (4 mL) was added to a solution of *tert*-butyl 4-(2-fluoroethyl)-4-hydroxypiperidine-1-carboxylate **12m-4** (540 mg, 2.19 mmol) in DCM (5 mL) and the mixture was stirred at ambient temperature for one hour. The solution was concentrated *in vacuo* and co-evaporated with methanol (4 x 5 mL) to furnish the trifuoroacetate salt (570 mg, 96 %) of the deprotected compound as an amber oil. $C_7H_{15}F_4NO_3$ MS (ESI+) m/z: $[M+H]^+$ Calcd 262.21; Found 148.17 (free base $[M+H]^+$ 148.19).

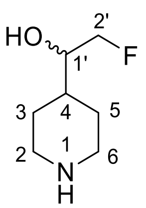
rac-2-Fluoro-1-(piperidine-4-yl)ethan-1-ol hydrochloride **12 n**

a) *rac-tert*-Butyl-4-(2-fluoro-1-hydroxyethyl)piperidine-1-carboxylate **12n-1**



Under an argon atmosphere *N*-boc-piperidine-4-carboxaldehyde (960 mg, 4.50 mmol, 1.50 eq.) and fluoroiodomethane (201 µL, 474 mg, 3.00 mmol, 1.00 eq.) were dissolved in THF (10 mL) and diethyl ether (10 mL). A 1.5 M solution of methyllithium lithiumbromide complex (4.00 mL, 6.00 mmol, 2.00 eq.) in diethyl ether was added at -78 °C. The reaction mixture was stirred for five minutes at -78 °C before a saturated aqueous NH_4Cl solution (3.0 mL) was added. The mixture was extracted three times with diethyl ether. The combined organic phases were dried over sodium sulfate and the solvent was removed *in vacuo*. The residue was purified by column chromatography (eluent: petrol ether/ethyl acetate, 3/1) to obtain a yellow oil (165 mg, 0.667 mmol, 22 %). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.23 – 1.31 (m, 2H, 0.5 C^3H_2, 0.5 C^5H_2), 1.44 (s, 9H, Boc-CH_3), 1.54 – 1.65 (m, 2H, 0.5 C^3H_2, 0.5 C^5H_2), 1.80 – 1.88 (m, 1H, C^4H), 2.18 (br, 1H, O*H*), 2.56 – 2.75 (m, 2H, 0.5 C^2H_2, 0.5 C^6H_2), 3.54 – 3.69 (m, 1H, $C^{1'}H$), 4.02 – 4.25 (m, 2H, 0.5 C^2H_2, 0.5 C^6H_2), 4.29 – 4.60 (m, 2H, $C^{2'}H_2$). ^{19}F-NMR (377 MHz, $CDCl_3$) δ -234.69. $C_{12}H_{22}FNO_3$ HRMS m/z: $[M+Na]^+$ Calcd 270.1476; Found 270.1477.

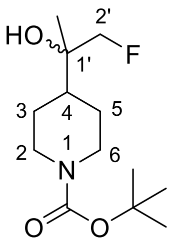
b) *rac*-2-Fluoro-1-(piperidine-4-yl)ethan-1-ol hydrochloride **12n**



rac-*tert*-Butyl-4-(2-fluoro-1-hydroxyethyl)piperidine-1-carboxylate **12n-1** (165 mg, 0.667 mmol) was dissolved in dichloromethane (5 mL) and treated with trifluoroacetic acid (5 mL). The reaction mixture was stirred at room temperature for one hour. The solvent was removed *in vacuo* and the residue was treated with 3 M methanolic HCl. After removal of the solvent *in vacuo* a yellow oil (112 mg, 0.610 mmol, 91 %) was obtained that was used without further purification. ^{1}H-NMR (400 MHz, DMSO-d$_6$) δ 1.40 – 1.60 (m, 2H, 0.5 C^3*H*$_2$, 0.5 C^5*H*$_2$), 1.60 – 1.72 (m, 2H, 0.5 C^3*H*$_2$, 0.5 C^5*H*$_2$), 1,78 – 1.87 (m, 1H, C^4*H*), 2.70 – 2.85 (m, 2H, 0.5 C^2*H*$_2$, 0.5 C^6*H*$_2$), 3.17 – 3.27 (m, 2H, 0.5 C^2*H*$_2$, 0.5 C^6*H*$_2$), 3.36 – 3.53 (m, 1H, C$^{1'}$*H*), 4.22 – 4.47 (m, 2H, C$^{2'}$*H*$_2$), 5.14 (br, 1H), 8.81 (s, 1H), 9.05 (s, 1H). ^{19}F-NMR (377 MHz, DMSO-d$_6$) δ -227.48. ^{13}C-NMR (101 MHz, DMSO-d$_6$) δ = 24.28 (d, J = 113.4 Hz, *C*^{3}, *C*^{5}), 35.53 (d, J = 5.8 Hz, *C*^{4}), 42.85 (d, J = 14.4 Hz, *C*^{2}, *C*^{6}), 71.36 (d, J = 18.4 Hz, *C*$^{1'}$), 85.08 (d, J = 168.1 Hz, *C*$^{2'}$). $C_7H_{14}FNO$ HRMS m/z: $[M+H]^+$ Calcd 148.1132; Found 148.1131.

rac-1-Fluoro-2-(piperidine-4-yl)propan-2-ol hydrochloride **12o**

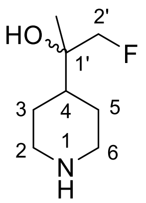
a) *rac*-*tert*-Butyl-4-(1-fluoro-2-hydroxypropan-2-yl)piperidine-1-carboxylate **12o-1**



Under an argon atmosphere *N*-boc-4-acetylpiperidine (681 mg, 3.00 mmol, 1.50 eq.) and fluoroiodomethane (134 µL, 316 mg, 2.00 mmol, 1.00 eq.) were dissolved in THF (10 mL) and diethyl ether (10 mL). A 1.5 M solution of methyllithium-lithium bromide complex (2.66 mL, 4.00 mmol, 2.00 eq.) in diethyl ether was added at -78 °C. The reaction mixture was stirred for five minutes at -78 °C before a saturated aqueous NH_4Cl solution (2.0 mL) was added. The mixture was extracted three times with diethyl ether. The combined organic phases were dried over sodium sulfate and the solvent was removed *in vacuo*. The residue was purified by column chromatography (eluent: petrol ether/ethyl acetate, 3/1) to obtain a yellow oil (207 mg, 0.792 mmol, 40 %). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.10 (d, J = 2.4 Hz, 3H,

CH_3), 1.15 – 1.33 (m, 2H, 0.5 C^3H_2, 0.5 C^5H_2), 1.43 (s, 9H, Boc-CH_3), 1.57 – 1.68 (m, 2H, 0.5 C^3H_2, 0.5 C^5H_2), 1.73 – 1.81 (m, 1H, C^4H), 2.08 (s, 1H, O*H*), 2.54 – 2.70 (m, 2H, 0.5 C^2H_2, 0.5 C^6H_2), 4.05 – 4.39 (m, 4H, 0.5 C^2H_2, 0.5 C^6H_2, $C^{2'}H_2F$). ^{19}F-NMR (377 MHz, $CDCl_3$) δ -230.14. ^{13}C-NMR (101 MHz, $CDCl_3$) δ 19.73 (CH_3), 25.87 (C^3, C^5), 26.90 (C^2, C^6), 28.55 (Boc-CH_3), 42.86 (C^4), 73.33 (d, J = 17.1 Hz, $C^{1'}$), 79.55 (Boc-*C*), 88.14 (d, J = 172.9 Hz, $C^{2'}$), 154.84 (Boc-*C*O). $C_{13}H_{24}FNO_3$ HRMS m/z: $[M+Na]^+$ Calcd 284.1632; Found 284.1631.

b) 1-Fluoro-2-(piperidine-4-yl)propan-2-ol hydrochloride **12o**

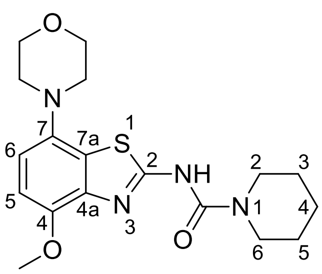


rac-tert-Butyl-4-(1-fluoro-2-hydroxypropan-2-yl)piperidine-1-carboxylate **12o-1** (291 mg, 1.11 mmol) was dissolved in dichloromethane (5 mL) and treated with trifluoroacetic acid (5 mL). The reaction mixture was stirred at room temperature for one hour. The solvent was removed *in vacuo* and the residue was treated with 3 M methanolic HCl. After removal of the solvent *in vacuo* a yellow oil (217 mg, 1.10 mmol, 99 %) was obtained that was used without further purification. ^1H-NMR (400 MHz, DMSO-d_6) δ 1.02 (d, J = 2.2 Hz, 3H, CH_3), 1.41 – 1.69 (m, 3H, 0.5 C^3H_2, 0.5 C^5H_2, NCH_2CH_2CH), 1.70 – 1.84 (m, 2H, 0.5 C^3H_2, 0.5 C^5H_2), 2.62 – 2.89 (m, 2H, 0.5 C^2H_2, 0.5 C^6H_2), 3.18 – 3.30 (m, 2H, 0.5 C^2H_2, 0.5 C^6H_2), 4.18 (dq, J = 47.8, J = 9.3 Hz, $C^{2''}H_2$), 8.77 (br), 9.13 (br). ^{19}F-NMR (377 MHz, DMSO-d_6) δ -225.45. ^{13}C-NMR (101 MHz, DMSO-d_6) δ 20.29 (d, J = 4.8 Hz, CH_3), 22.92 (d, J = 63.3 Hz, C^3, C^5), 40.05 (s, C^4), 43.59 (d, J = 12.0 Hz, C^2, C^6), 71.43 (d, J = 17.2 Hz, $C^{1'}$), 87.89 (d, J = 173.3 Hz, $C^{2'}$). $C_8H_{16}FNO$ HRMS m/z: $[M+H]^+$ Calcd 162.1289; Found 162.1287.

Preparation of ureas **13a** to **13p**, general procedure

Under argon at ambient temperature (4-methoxy-7-morpholin-4-yl-benzo[*d*]thiazol-2-yl)-carbamic acid phenyl ester (385 mg, 1 mmol) was dissolved in dry DMSO (3-5 mL). Under stirring the respective amine (or its hydrochloride,1.08 mmol) was added (for amine salts aqueous 10 N NaOH (108 µL, 1.08 mmol) was additionally added). The light brown solution was stirred for a given time at a given temperature (TLC: ethyl acetate/hexane, 90/10, $R_{fCarbamate}$ 0.85 or ethyl acetate/hexane/acetic acid, 80/20/0.2, $R_{fCarbamate}$ 0.82) after which ice cold 50% brine (15 mL) was added. To the stirred turbid mixture ethyl acetate (50 mL) was added, the organic layer was separated and the aqueous phase extracted with ethyl acetate (50 mL). The combined organic layers were washed successively with 10% citric acid (50 mL), sat. aqueous Na_2CO_3 (50 mL) and 50% brine (50 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure to give a solid or an oil that was crystallized by dissolving the oil in boiling aqueous 50-70% MeOH (5-10 mL / mmol) and slow cooling. The urea was collected by filtration and dried in air.

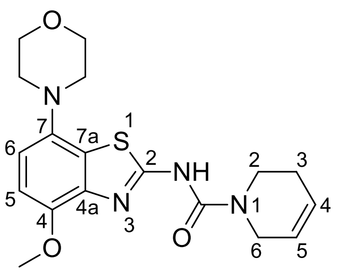
N-(4-methoxy-7-morpholin-4-yl-benzo[*d*]thiazol-2-yl)piperidine-1-carboxamide **13a**



Carbamate **8** (385 mg, 1 mmol) was reacted with piperidine (107 µL, 1.08 mmol) in DMSO (5 mL) for 8 h at ambient temperature (TLC: ethyl acetate/hexane, 90/10, $R_{fCarbamate}$ 0.85, R_{fUrea} 0.54). The organic (EA) layer was washed with 10% citric acid, sat. aqueous Na_2CO_3 and brine. After drying, filtration and evaporation of the solvent the oily residue was treated with

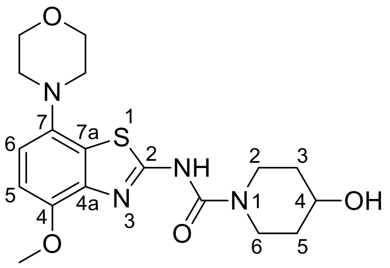
TBME (20 mL), concentrated *in vacuo*, and the solid residue was recrystallized from 70% MeOH. Yield 326 mg (80%), colorless crystals, mp 191°C (70% MeOH). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.43 - 1.53 (m, 4H, Pip-C^3H_2 + Pip-C^5H_2), 1.53 – 1.62 (m, 2H, Pip-C^4H_2), 2.99 (t, 4H, J = 8.5 Hz, 2 x N-CH_2), 3.59 - 3.28 (m, 4H, Pip-C^2H_2 + Pip-C^6H_2), 3.77 (t, 4H, J = 8.5 Hz, 2 x OCH_2), 3.84 (s, 3H, OCH_3), 6.78 (d, 1H, J = 8.5 Hz, H^6), 6.86 (d, 1H, J = 8.5 Hz, H^5) 11.33 (s_{br}, 1H, NH). ^{13}C-NMR (101 MHz, DMSO-d_6) δ 24.3 (Pip-C^4), 25.9 (Pip-$C^{3,5}$), 45 (Pip-$C^{2,6}$), 51.9 (CNC), 56.3 (OCH$_3$), 67.0 (COC), 108.4 (C^5), 112.2 (C^6), 140.4 (C^4). Anal. Calcd. for $C_{18}H_{24}N_4O_3S$: C, 57.43; H, 6.43; N, 14.88; Found C, 57.41; H, 6.39; N, 14.81. HRMS m/z: $[M+H]^+$ Calcd 377.1642, Found 377.1640.

N-(4-methoxy-7-morpholinobenzo[*d*]thiazol-2-yl)-5,6-dihydropyridine-1(*2H*)-carboxamide **13b**



Carbamate **8** (192 mg, 0.5 mmol) was reacted with 1,2,3,6-tetrahydropyridine (47.5 µL, 0.505 mmol) in DMSO (3 mL) for 15 h at ambient temperature (TLC: ethyl acetate/hexane, 90/10, $R_{fCarbamate}$ 0.85, R_{fUrea} 0.51). Yield 287 mg (73%), off-white felted needles, mp 212°C (70% MeOH). $C_{18}H_{22}FN_4O_3S$ HRMS m/z: $[M+H]^+$ ^{1}H-NMR (400 MHz, CDCl$_3$) δ 2.20 – 2.28 (m, 2H, Pip-C^3H_2), 3.12 (t, 4H, J = 9.2 Hz, 2 x N-CH_2), 3.68 (t, 2H, J = 11.2 Hz, Pip-C^2H_2), 3.89 (t, 4H, J = 9.2 Hz, 2 x O-CH_2), 3.94 (s, 3H, OCH_3), 4.03 (m, 2H, Pip-C^6H), 5.66 – 5.73 (m, 1H, Pip-C^4H), 5.88 – 5.96 (m, 1H, Pip-C^5H), 6.80 (s, 2H, C^5H, C^6H). ^{13}C-NMR (101 MHz, CDCl$_3$) δ 24.9 (Pip-C^3), 43.5 (Pip-C^2), 51.8 (CNC), 55.9 (OCH$_3$), 67.4 (COC + Pip-C^6), 107 (C^6), 112.2 (C^5), 123.2 (Pip-C^4), 127.2 (C^{7a}), 135 (Pip-C^5), 137.9 (C^7), 140.5 (C^4), 147.3 (C^2), 154 (C^{4a}), 160.9 (*C*O). Anal. Calcd. for $C_{18}H_{22}N_4O_3S$: C, 57.73; H, 5.92; N, 14.96; Found C, 57.70; H, 5.89; N, 14.94. HRMS m/z: $[M+H]^+$ Calcd 394.1469, Found 394.1468.

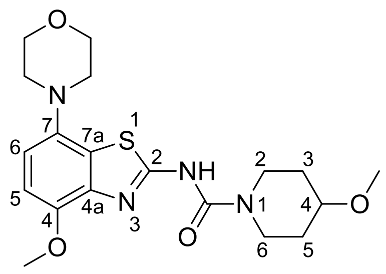
4-Hydroxy-*N*-(4-methoxy-7-morpholin-4-yl-benzo[*d*]thiazol-2-yl)piperidine-1-carboxamide **13c**



Carbamate **8** (1155 mg, 3 mmol) was reacted with 4-hydroxypiperidine (319 mg, 3.24 mmol, 1.08 eq) in DMSO (10 mL) for 16 h at ambient temperature (TLC: ethyl acetate/hexane, 80/20, $R_{fCarbamate}$ 0.82, R_{fUrea} 0.02-0.2). For the extraction 2 x 75 mL DCM / mmol is needed (not ethyl acetate, **13c** shows a tendency to precipitate from ethyl acetate in the separation funnel during work-up!) After extraction the combined organic layers were washed with 50% brine. Concentration furnished a light brown oil that was crystallized from hot ethyl acetate (15 mL). Yield 970 mg (82%), off-white crystals, mp 188°C (EA). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.81 - 1.97 (m, 2H, 0.5 C^3*H*$_2$, 0.5 Pip C^5*H*$_2$), 1.98 - 2.12 (m, 2H, 0.5 Pip C^3H_2, Pip-0.5 C^5*H*$_2$), 3.05 (t, ^3J = 9 Hz, 4H, 2 x N-C*H*$_2$), 3.53 - 3.64 (m, 1H, 0.25 Pip $C^{2/6}$*H*$_2$), 3.66 - 3.77 (m, 1H, 0.25 Pip $C^{2/6}$*H*$_2$), 3.88 (t, ^3J = 9 Hz, 4H, 2 x O-C*H*$_2$), 3.89 (s, 3H, O-C*H*$_3$), 3.96-4.07 (m, 1H, 0.25 Pip $C^{2/6}$*H*$_2$), 4.09-4.20 (m, 1H, 0.25 Pip $C^{2/6}$*H*$_2$), 4.97 (sept., 1H, Pip C^4*H*), 6.86 (d,^3J = 5.2 Hz, 2H, aryl), 11.32 (s_{br}, 1H, N*H*). ^{13}C NMR (101 MHz, $CDCl_3$) δ 33.9 (Pip C^3, C^5), 41.5 (Pip C^2, C^6), 51.6 (*C*N*C*), 55.8 (O*C*H$_3$), 66.2 (Pip C^4), 67.2 (*C*O*C*), 107 (C^6), 111.7 (C^5), 126.9 (C^{7a}), 138.2 (C^7), 140.3 (C^4), 147.3 (C^{4a}), 154.1 (*C*=O), 161.1 (C^2). Anal. Calcd. for $C_{18}H_{24}N_4O_4S$: C, 55.08; H, 6.16;

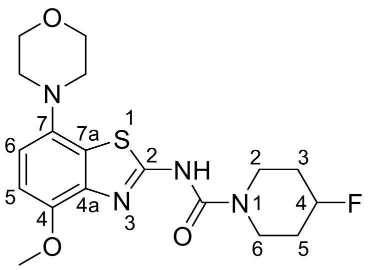
N, 14.28; Found C, 55.11; H, 6.11; N, 14.22. HRMS m/z: $[M+H]^+$ Calcd 393.1591, Found 393.1588.

4-Methoxy-*N*-(4-methoxy-7-morpholin-4-yl-benzo[*d*]thiazol-2-yl)piperidine-1-carboxamide **13d**



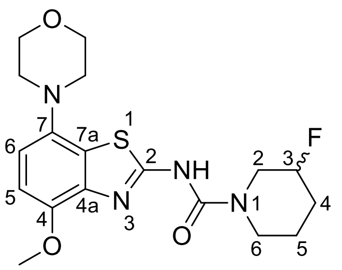
Carbamate **8** (385 mg, 1 mmol) was reacted with 4-methoxypiperidine (125 mg, 135 µL, 1.08 mmol) in DMSO (5 mL) for 5 h at ambient temperature (TLC: ethyl acetate/hexane, 80/20, $R_{fCarbamate}$ 0.80, R_{fUrea} 0.15-0.2). For the extraction DCM (2 x 75 mL) was used. The organic layer was washed with 10% citric acid (75 mL) and brine (75 mL). After drying, filtration and evaporation of the solvent the oily residue was treated with tert-butyl methyl ether (20 mL) and concentrated *in vacuo*. The obtained cream colored crystals were dissolved in MeOH (3 mL) and the product was precipitated by the addition of water (9 mL, cloudy white mixture). After standing at 5°C for 48 h a white solid had formed, which was collected by filtration. Yield 326 mg (80%), mp 172°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.33 - 1.48 (m, 2H, 0.5 C^3H_2, 0.5 Pip C^5H_2), 1.77 – 1.94 (m, 2H, 0.5 Pip C^3H_2, Pip-0.5 C^5H_2), 3.0 (t, ^3J = 9.1 Hz, 4H, 2 x N-CH_2), 3.20 - 3.31 (m, 5H, 0.5 Pip $C^{2/6}H_2$ + Pip O-CH_3), 3.36 - 3.45 (m, 1H, Pip C^4H), 3.77 (t, ^3J = 9.1 Hz, 4H, 2 x O-CH_2), 3.86 (s, 3H, OCH_3), 3.80-3.91 (m, 2H, 0.5 Pip $C^{2/6}H_2$), 6.80 (d,^3J = 8.3 Hz, 1H, C^5H), 6.90 (d,^3J = 8.3 Hz, 1H, C^6H), 11.34 (s_{br}, 1H, NH).^{13}C NMR (101 MHz, $CDCl_3$) δ 30.8 (Pip C^3, C^5), 41.5 (Pip C^2, C^6), 51.9 (*C*N*C*), 55.5 (Pip O*C*H$_3$), 56.4 (O*C*H$_3$), 67 (*C*O*C*), 75.4 (Pip C^4), 108.5 (C^6), 112.3 (C^5), 140.45 (C^4). Anal. Calcd. for $C_{19}H_{26}N_4O_4S$: C, 56.14; H, 6.45; N, 13.78; Found C, 56.08; H, 6.43; N, 13.83. HRMS m/z: $[M+H]^+$ Calcd 407.17475, Found 407.17472.

4-Fluoro-*N*-(4-methoxy-7-morpholin-4-yl-benzo[*d*]thiazol-2-yl)piperidine-1-carboxamide **13e**



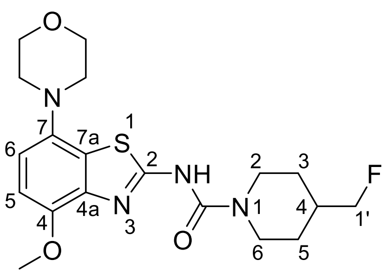
Carbamate **8** (385 mg, 1 mmol) was reacted with 4-fluoropiperidine hydrochloride (150 mg, 1.08 mmol) and aqueous 10 N NaOH (108 µL, 1.08 mmol) in DMSO (10 mL) for 24 h at ambient temperature (TLC: ethyl acetate/hexane, 90/10, $R_{fCarbamate}$ 0.85, R_{fUrea} 0.68; ethyl acetate/hexane/AcOH, 80/20/0.2, $R_{fCarbamate}$ 0.82, R_{fUrea} 0.58). Yield 260 mg (66%), colorless crystals, mp 194 °C (50% MeOH). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.76 – 1.91 (m, 4H, Pip-C^3H_2, Pip-C^5H_2), 3.05 – 3.11 (m, 4H, 2 x NCH_2), 3.53 – 3.73 (m, 4H, Pip-C^2H_2, Pip-C^6H_2), 3.84 – 3.88 (m, 7H, 2 x OCH_2, O-CH_3), 4.74 – 4.92 (m, 1H, Pip-C^4H), 6.76 (s, 2H, C^5H, C^6H), 10.25 (br, 1H, N*H*). ^{19}F-NMR (377 MHz, $CDCl_3$) δ -183.71. ^{13}C-NMR (101 MHz, $CDCl_3$) δ 31 (d, $^2J_{C-F}$ = 20.1 Hz, Pip-C^3, Pip-C^5), 40.2 (d, J = 3.7 Hz, Pip-C^2, Pip-C^6), 51.8 (*C*N*C*), 55.9 (CH_3), 67.4 (*C*O*C*), 87.5 (d, $^1J_{C-F}$ = 171.5 Hz, Pip-C^4), 107.3 (C^6), 112.3 (C^5), 126.7 (C^{7a}), 136.8 (C^7), 140.6 (C^4), 146.8 (C^2), 154.9 (C^{4a}), 162.3 (*C*O). Anal. Calcd. for $C_{18}H_{23}FN_4O_3S$: C, 54.81; H, 5.88; N, 14.20; Found C, 54.82; H, 5.89; N, 14.14. HRMS m/z: $[M+H]^+$ Calcd 395.1548, Found 395.1545.

rac-3-Fluoro-*N*-(4-methoxy-7-morpholin-4-yl-benzo[*d*]thiazol-2-yl)piperidine-1-carboxamide **13f**



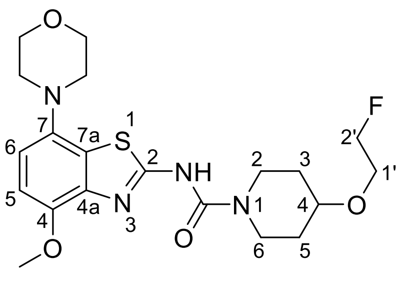
Carbamate **8** (385 mg, 1 mmol) was reacted with 3-fluoropiperidine hydrochloride (150 mg, 1.08 mmol) and aqueous 10 N NaOH (108 µL, 1.08 mmol) in DMSO (10 mL) for 24 h at ambient temperature (TLC: ethyl acetate/hexane, 90/10, $R_{fCarbamate}$ 0.85, R_{fUrea} 0.44). Yield 287 mg (73%), colorless crystals, mp 196°C (50% MeOH). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.41 - 1.59 (m, 1H), 1.62 – 1.76 (m, 1H), 1.79 – 1.96 (m, 2H), 3.01 (t, 4H, J = 9 Hz, 2 x N-CH_2), 3.20 - 3.33 (m, 1H), 3.77 (t, 4H, J = 9 Hz, 2 x O-CH_2), 3.86 (s, 3H, OCH_3), 3.92 - 4.07 (m, 1H), 4.75 (d_{br}, J = 47.8 Hz, 1H), 6.80 (d, 1H, J = 8.5 Hz, H^6), 6.88 (d, 1H, J = 8.5 Hz, H^5) 11.44 (s_{br}, 1H, NH). ^{19}F-NMR (377 MHz, $CDCl_3$) δ -183.67. ^{13}C-NMR (101 MHz, DMSO-d_6) δ 21.1 (s_{br}, Pip C^5), 29.4 (d, $^2J_{C-F}$ = 20.3 Hz, Pip C^4), 43.9 (s_{br}, Pip C^6), 47.8 (d, $^2J_{C-F}$ = 24 Hz, Pip C^2), 87.1 (d, $^1J_{C-F}$ = 174.1 Hz, Pip C^3), 51.9 (*C*N*C*), 56.4 (OCH_3), 67.0 (*C*O*C*), 108.5 (C^5), 112.3 (C^6), 140.5 (C^4). Anal. Calcd. for $C_{18}H_{23}FN_4O_3S$: C, 54.81; H, 5.88; N, 14.20; Found C, 54.77; H, 5.79; N, 14.29. HRMS m/z: $[M+H]^+$ Calcd 395.1548, Found 395.1543.

4-(Fluoromethyl)-*N*-(4-methoxy-7-morpholinobenzo[*d*]thiazol-2-yl)piperidine-1-carboxamide **13g**



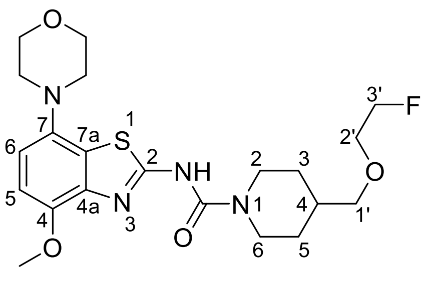
Phenyl (4-methoxy-7-morpholinobenzo[*d*]thiazol-2-yl)carbamate (385 mg, 1.00 mmol, 1.00 eq.) and 4-(fluoromethyl)piperidine hydrochloride (196 mg, 1.28 mmol, 1.28 eq.) were dissolved in dry DMSO (10 mL) under an argon atmosphere. DBU (420 µL, 2.81 mmol, 2.81 eq.) was added and the reaction was heated to 60°C. After four hours of stirring the reaction was allowed to cool to room temperature and stirred for another 66 hours. The solution was diluted with ethyl acetate and subsequently washed with a saturated aqueous solution of sodium bicarbonate, twice with water and brine. The organic layer was dried over sodium sulfate and the solvent was removed *in vacuo*. The residue was purified by column chromatography (eluent: petrol ether/ethyl acetate, 45/55 to 55/45) to obtain a white solid (277 mg, 0.678 mmol, 68 %), mp 110°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.22 – 1.36 (m, 2H, 0.5 Pip-C^3H_2, 0.5 Pip-C^5H_2), 1.70 – 1.78 (m, 2H, 0.5 Pip-C^3H_2, 0.5 Pip-C^5H_2), 1.82 – 1.96 (m, 1H, Pip-C^4H), 2.84 – 2.94 (m, 2H, 0.5 Pip-C^2H_2, 0.5 Pip-C^6H_2), 3.06 – 3.11 (m, 4H, 2 x N-CH_2), 3.83 – 3.90 (m, 7H, 2 x O-CH_2, O-CH_3), 4.16 – 4.34 (m, 4H, 0.5 Pip-C^2H_2, 0.5 Pip-C^6H_2, Pip-$C^{1'}H_2$), 6.76 (s, 2H, C^5H, C^6H), 9.82 (br, 1H, N*H*). ^{19}F-NMR (377 MHz, $CDCl_3$) δ -223.96. ^{13}C-NMR (101 MHz, $CDCl_3$): δ 27.5 (d, J = 5.7 Hz, Pip-C^3, Pip-C^5), 36.8 (d, $^2J_{C-F}$ = 19.0 Hz, Pip-C^4), 44 (Pip-C^2, Pip-C^6), 51.8 (*C*N*C*), 55.9 (CH_3), 67.5 (*C*O*C*), 87.1 (d, $^1J_{C-F}$ = 169.5 Hz, Pip-$C^{1'}$), 107.1 (C^6), 112.2 (C^5), 126.99 (C^{7a}), 137.5 (C^7), 140.6 (C^4), 147.2 (C^2), 154.3 (C^{4a}), 161.8 (*C*O). Anal. Calcd. for $C_{19}H_{25}FN_4O_3S$: C, 55.87; H, 6.17; N, 13.72; Found C, 55.81; H, 6.09; N, 13.80. HRMS m/z: $[M+H]^+$ Calcd 409.1704; Found 409.1702.

4-(2-Fluoroethoxy)-*N*-(4-methoxy-7-morpholinobenzo[*d*]thiazol-2-yl)piperidine-1-carboxamide **13h**



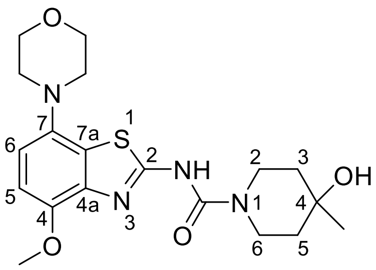
Phenyl (4-methoxy-7-morpholinobenzo[*d*]thiazol-2-yl)carbamate (55 mg, 0.143 mmol, 1.00 eq.) and 1-4-(2-fluoroethoxy)piperidine hydrochloride (60 mg, 0.327 mmol, 2.28 eq.) were dissolved in dry DMSO (4 mL) under an argon atmosphere. DBU (60.1 µL, 0.402 mmol, 2.82 eq.) was added and the reaction was heated to 60°C. After two hours of stirring the reaction was allowed to cool to room temperature and stirred overnight. The solution was diluted with ethyl acetate and subsequently washed with a saturated aqueous solution of sodium bicarbonate, twice with water and brine. The organic layer was dried over sodium sulfate and the solvent was removed *in vacuo*. The residue was purified by column chromatography (eluent: dichloromethane/methanol, 99/1 to 97/3) to obtain a white solid (32 mg, 73.0 µmol, 51 %), mp 95°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.61 – 1.70 (m, 2H, 0.5 Pip-C^3H_2, 0.5 Pip-C^5H_2), 1.81 – 1.90 (m, 2H, 0.5 Pip-C^3H_2, 0.5 Pip-C^5H_2), 3.06 – 3.11 (m, 4H, 2 x N-CH_2), 3.32 – 3.41 (m, 2H, 0.5 Pip-C^2H_2, 0.5 Pip-C^6H_2), 3.56 – 3.90 (m, 11H, 0.5 Pip-C^2H_2, 0.5 Pip-C^6H_2, Pip-$C^{1'}H_2$, 2 x O-CH_2, *O*-CH_3), 4.55 (dt, $^3J_{H-F}$ = 47.6 Hz, ^3J = 4.2 Hz, 2H, $C^{2'}H_2$), 6.76 (s, 2H, C^5H, C^6H), 9.86 (br, 1H, N*H*. ^{19}F-NMR (377 MHz, $CDCl_3$) δ -223.11. ^{13}C-NMR (101 MHz, $CDCl_3$): δ 30.66 (Pip-C^3, Pip-C^5), 41.3 (Pip-C^2, Pip-C^6), 51.9 (*C*N*C*), 56 (*C*H$_3$), 67.30 ($C^{1'}$), 67.5 (*C*O*C*), 74.1 (Pip-C^4), 83.3 (d, $^1J_{C-F}$ = 169.7 Hz, $C^{2'}$), 107.2 (C^6), 112.2 (C^5), 127 (C^{7a}), 137.6 (C^7), 140.6 (C^4), 147.2 (C^2), 154.3 (C^{4a}), 161.7 (*C*O). Anal. Calcd. for $C_{20}H_{27}FN_4O_4S$: C, 54.78; H, 6.21; N, 12.78; Found C, 54.69; H, 6.19; N, 12.81. HRMS m/z: $[M+H]^+$ Calcd 439.1810; Found 439.1806.

4-((2-Fluoroethoxy)methyl)-*N*-(4-methoxy-7-morpholinobenzo[*d*]thiazol-2-yl)piperidine-1-carboxamide **13i**



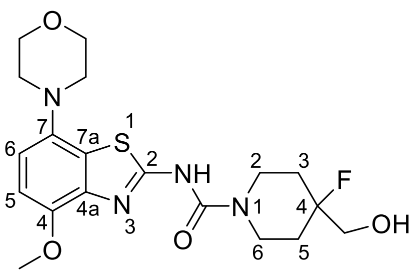
Phenyl (4-methoxy-7-morpholinobenzo[*d*]thiazol-2-yl)carbamate (96 mg, 0.250 mmol, 1.00 eq.) and 1-4-((2-fluoroethoxy)methyl)piperidine hydrochloride (70 mg, 0.354 mmol, 1.42 eq.) were dissolved in dry DMSO (4 mL) under an argon atmosphere. DBU (104 µL, 0.697 mmol, 2.79 eq.) was added and the reaction was heated to 60 °C. After three hours of stirring the reaction was allowed to cool to room temperature and stirred for another 69 hours. The solution was diluted with ethyl acetate and subsequently washed with a saturated aqueous solution of sodium bicarbonate, twice with water and brine. The organic layer was dried over sodium sulfate and the solvent was removed *in vacuo*. The residue was purified by column chromatography (eluent: dichloromethane/methanol, 100/0 to 98/2) to obtain a colorless solid (64 mg, 141 µmol, 40 %), mp 112°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.18 – 1.32 (m, 2H, 0.5 Pip-C^3H_2, 0.5 Pip-C^5H_2), 1.76 – 1.91 (m, 2H, 0.5 Pip-C^3H_2, 0.5 Pip-C^5H_2, Pip-C^4H), 2.85 – 2.96 (m, 2H, 0.5 Pip-C^2H_2, 0.5 Pip-C^6H_2), 3.06 – 3.13 (m, 4H, 2 x N-CH_2), 3.34 (d, J = 6.1 Hz, 2H, Pip-$C^{1'}H_2$), 3.59 – 3.72 (m, 2H, Pip-$C^{2'}H_2$), 3.82 – 3.93 (m, 7H, 2 x O-CH_2, O-CH_3), 4.11 – 4.24 (m, 2H, , 0.5 Pip-C^2H_2, 0.5 Pip-C^6H_2), 4.44 – 4.61 (m, 2H, Pip-$C^{3'}H_2$), 6.77 (s, 2H, C^5H, C^6H), 9.42 (br, 1H, N*H*). ^{19}F-NMR (377 MHz, $CDCl_3$) δ -222.84. ^{13}C-NMR (101 MHz, $CDCl_3$): δ 28.8 (Pip-C^3, Pip-C^5), 36.4 (Pip-C^4), 44.3 (Pip-C^2, Pip-C^6), 51.9 (*C*N*C*), 56 (*C*H$_3$), 67.5 (*C*O*C*), 70.3 (d, $^2J_{C\text{-}F}$ = 19.6 Hz, $C^{2'}$), 76 ($C^{1'}$), 83.2 (d, $^1J_{C\text{-}F}$ = 169.1 Hz, $C^{3'}$), 107.1 (C^6), 112.2 (C^5), 127.2 (C^{7a}), 138 (C^7), 141 (C^4), 147.4 (C^2), 154 (C^{4a}), 161.4 (*C*O). Anal. Calcd. for $C_{21}H_{29}FN_4O_4S$: C, 55.73; H, 6.46; N, 12.38; Found C, 55.72; H, 6.39; N, 12.42. HRMS m/z: $[M+H]^+$ Calcd 453.1966; Found 453.1960.

4-Hydroxy-4-methyl-*N*-(4-methoxy-7- morpholinobenzo[*d*]thiazol-2-yl) piperidine-1-carboxamide (Tozadenant) **13j**



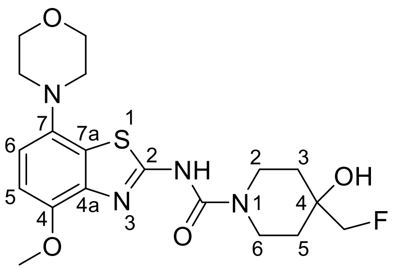
A solution of phenyl carbamate **8** (1.07 g, 2.8 mmol) and 4-methylpiperidin-4-ol (650 mg, 5,6 mmol) in 1,2-dichloroethane (30 mL) was stirred for 2.5 h at 40°C (TLC: ethyl acetate/methanol/AcOH, 98/2/0.2, R_f 0.37; hexane/ethyl acetate/AcOH 60/40/0.2, Rf = 0.67). After removal of the solvent under reduced pressure the residue was taken up in a small volume of methanol. After addition of ethyl acetate, the product (680 mg, 61%) precipitated as a slightly beige solid, mp. 215°C (dec.). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.17 (s, 3H), 1.50 (d, *J* = 11.2 Hz, 4H), 3.01 (d, *J* = 4.7 Hz, 4H), 3.32 (t, *J* = 11.1 Hz, 2H), 3.71 - 3.99 (m, 9H), 6.64 - 6.84 (m, 2H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 18.7, , 30.2, 38.5, 40.7, 51.8, 55.9, 57, 66.6, 67.1, 107.5, 111.9, 126.7, 138.6, 140.1, 147.5, 154.2, 161.2. Anal. Calcd. for $C_{19}H_{26}N_4O_4S$: C, 56.14; H, 6.45; N, 13.78; Found C, 56.12; H, 6.39; N, 13.79. HRMS m/z: $[M+H]^+$ Calcd 407.1743; Found 407.1744.

4-Fluoro-4-(hydroxymethyl)-*N*-(4-methoxy-7-morpholinobenzo[*d*]thiazol-2-yl)piperidine-1-carboxamide **13k**



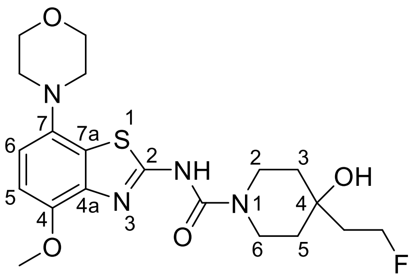
A solution of phenyl carbamate **8** (501 mg, 1.3 mmol) and (4-fluoropiperidin-4-yl)-methanol (186.42 mg, 1.4 mmol) in DMSO (12 mL) was stirred for 2 h at ambient temperature followed by 5 h at 80°C (TLC: ethyl acetate/methanol/AcOH, 98/2/0.2, R_f 0.46). After cooling to ambient temperature 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (153 µL, 1 mmol) was added and stirring was continued for 30 min. The mixture was diluted with ethyl acetate (60 mL) and washed twice with water, 1 N HCl, water, 1N NaOH and brine. Drying over Na_2SO_4 and removal of the solvent under reduced pressure left a solid residue that was recrystallized from ethanol to furnish the target urea (310 mg, 56%) as a cream colored solid, mp. 226°C (dec.). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.56 (t, *J* = 11.8 Hz, 4H), 3.28 – 2.80 (m, 6H), 3.28 - 3.62 (m, 3H), 3.61 - 4.00 (m, 7H), 4.12 (d, *J* = 13.,6 Hz, 2H), 5.04 (t, *J* = 5,9 Hz, 1H), 6.70 - 6.95 (m, 2H), 11.50 (s, 1H). ^{19}F-NMR (377 MHz, $CDCl_3$) δ -164.84. ^{13}C-NMR (101 MHz, $CDCl_3$) δ 31.3, 31.7, 40.1, 51.9, 56.3, 66.3, 66.8, 67.01, 93.4, 98, 108.4, 112.3, 115.7, 129.8, 140.4, 157.8. Anal. Calcd. for $C_{19}H_{25}FN_4O_4S$: C, 53.76; H, 5.94; N, 13.20; Found C, 53.72; H, 5.99; N, 13.23. HRMS m/z: $[M+H]^+$ Calcd 425,1659; Found 425,1652.

4-Fluoromethyl-4-hydroxy-*N*-(4-methoxy-7-morpholinobenzo[*d*]thiazol-2-yl)piperidine-1-carboxamide **13l**



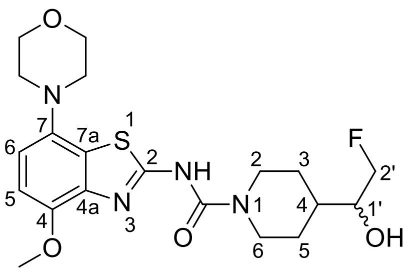
A solution of phenyl carbamate **8** (771 mg, 2 mmol), 4-fluormethyl-4-hydroxypiperidine hydrochloride (475 mg, 2.8 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (826 µL, 5.4 mmol) in DMSO (25 mL) was stirred for 4 h at ambient temperature (TLC: hexane/ethyl acetate, 30/70, R_f 0.19). The mixture was diluted with ethyl acetate (50 mL) and basified with saturated aqueous $NaHCO_3$ solution. Washing with water, drying over Na_2SO_4 and evaporation of the solvents left a residue that was purified by flash-chromatography (hexane/ethyl acetate, 30/70). The product (848 mg, 99%) was obtained as a colorless solid, mp. 211°C (dec.). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.51 (s, 4H), 3.11 (d, *J* = 43,8 Hz, 7H), 3.82 (d, *J* = 15.6 Hz, 7H), 4.08 (s, 3H), 4.32 (s, 1H), 4.91 (s, 1H), 6.83 (dd, *J* = 17.8, 8.3 Hz, 2H), 11.42 (s, 1H). ^{19}F-NMR (377 MHz, $CDCl_3$) δ -227.60. ^{13}C-NMR (101 MHz, $CDCl_3$) δ 14.5, 21.2, 32.5, 39.8, 51.9, 56.3, 67, 67.9, 68.3, 88, 91.4, 108.4, 112.2, 115.7, 126.5, 129.8, 140.4, 147.7, 154.3, 160.8. Anal. Calcd. for $C_{19}H_{25}FN_4O_4S$:C, 53.76; H, 5.94; N, 13.20; Found C, 53.72; H, 5.99; N, 13.19. HRMS m/z: $[M+H]^+$ Calcd 425,1659; Found 425,1654

4-(2-Fluoroethyl)-4-hydroxy-N-(4-methoxy-7-morpholinobenzo[d]thiazol-2-yl)piperidine-1-carboxamide **13m**



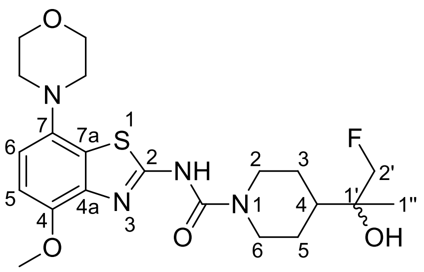
To a solution of (4-methoxy-7-morpholin-4-yl-benzothiazol-2-yl)-carbamic acid phenyl ester (385 mg, 1 mmol) and N-ethyl-diisopropyl-amine (680 µL, 4 mmol, 4 equiv) in trichloromethane (10 mL) was added a solution of 4-(2-fluoroethyl)-4-hydroxypiperidine trifluoroacetate (392 mg, 1.5 mmol, 1.5 equiv) in trichloromethane (1 mL) and tetrahydrofurane (1 mL) and the resulting mixture heated to reflux (80°C) for 1.5 h (TLC: EA/Hex, 80/20, $R_{fproduct}$ 0.02, R_{feduct} 0.72). The reaction mixture was then cooled to ambient temperature, diluted with dichloromethane (40 mL) and extracted with saturated aqueous sodium carbonate (15 mL) and water (2 x 5 ml). Final drying with sodium sulphate followed by evaporation of the solvent and recrystallization from 2-propanol afforded the title compound as white crystals (78percent yield), mp 191°C (dec.). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.45-1.58 (m, 4H, Pip-C^3H_2, Pip-C^5H_2), 1.81 (dt, $^3J_{H-F}$ = 25.8, $^3J_{H-H}$ = 6.3 Hz, 2H, F-CH$_2$-CH_2), 2.99 (t, $^3J_{H-H}$ = 4.5 Hz, 4H, CH_2NCH_2), 3.18 – 3.31 (m, 2H, 0.5 Pip-C^2H_2, 0.5 Pip-C^6H_2), 3.78 (t, $^3J_{H-H}$ = 4.5 Hz, 4H, CH_2OCH_2), 3.85 (s, 3H, OCH_3), 3.88 – 3.98 (m, 2H, 0.5 Pip-C^2H_2, 0.5 Pip-C^6H_2), 4.54 (s_{br}, 1H, OH), 4.63 (dt, , $^2J_{H-F}$ = 47.5, $^3J_{H-H}$ = 6.2 Hz, 2H, F-CH_2), 6.79 (d, $^3J_{H-H}$ = 8.5 Hz, C^6H), 6.79 (d, $^3J_{H-H}$ = 8.5 Hz, C^5H),.11.29 (s_{br}, 1H, NH). ^{19}F-NMR (376 MHz, DMSO-d_6) δ -216.48. ^{13}C-NMR (100 MHz, DMSO-d_6) δ 37.0 (Pip-C^3, Pip-C^5), 40.3 (Pip-C^2, Pip-C^6), 42.7 (d, $^2J_{C-F}$ = 18.2 Hz, F-CH$_2$-CH$_2$),51.9 (C-N-C), 56.3 (OCH$_3$), 67.0 (C-O-C), 67.5 (d, $^3J_{C-F}$ = 4.7 Hz, C^4), 81.1 (d, $^1J_{C-F}$ = 159 Hz, F-CH$_2$), 108.4 (C^6), 112.2 (C^5), 115.7 ($C^{7'}$), 119.2 ($C^{4'}$), 129.8 (C^7), 140.5 (C^4), 153.0 (C=O), 173.9 (C^2). Anal. Calcd. for $C_{20}H_{27}FN_4O_4S$: C, 54.78; H, 6.21; N, 12.78; Found C, 54.72; H, 6.19; N, 12.69. HRMS m/z: [M+H]$^+$ Calcd 438.1737; Found 438.1733

rac-4-(2-Fluoro-1-hydroxyethyl)-*N*-(4-methoxy-7-morpholinobenzo[*d*]thiazol-2-yl)piperidine-1-carboxamide **13n**



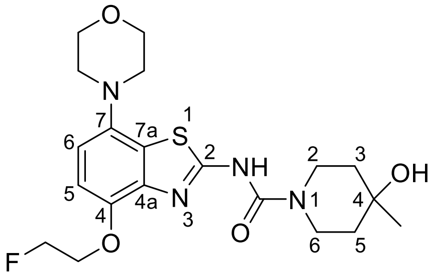
Phenyl (4-methoxy-7-morpholinobenzo[*d*]thiazol-2-yl)carbamate (150 mg, 0.389 mmol, 1.00 eq.) and 2-fluoro-1-(piperidine-4-yl)ethan-1-ol hydrochloride (100 mg, 0.545 mmol, 1.40 eq.) were dissolved in dry DMSO (10 mL) under an argon atmosphere. DBU (83.4 µL, 0.559 mmol, 1.43 eq.) was added and the reaction was heated to 60°C. After four hours of stirring the reaction was allowed to cool to room temperature and stirred for another 46 hours. The solution was diluted with ethyl acetate and subsequently washed with a saturated aqueous solution of sodium bicarbonate, twice with water and brine. The organic layer was dried over sodium sulfate and the solvent was removed *in vacuo*. The residue was purified by column chromatography (eluent: dichloromethane/methanol, 99/1 to 97/3) to obtain a white solid (124 mg, 0.283 mmol, 73 %), mp 219°C. ^{1}H-NMR (400 MHz, DMSO-d$_6$) δ 1.18 – 1.37 (m, 2H, 0.5 Pip-C^3*H$_2$*, 0.5 Pip-C^5*H$_2$*), 1.55 – 1.68 (m, 2H, 0.5 Pip-C^3*H$_2$*, 0.5 Pip-C^5*H$_2$*), 1.74 – 1.81 (m, 1H, C^4*H*), 2.70 – 2.83 (m, 2H, 0.5 Pip-C^2*H$_2$*, 0.5 Pip-C^6*H$_2$*), 2.97 – 3.02 (m, 4H, 2 x N-C*H$_2$*), 3.40 – 3.51 (m, 1H, Pip-C$^{1'}$*H*), 3.72 – 3.86 (m, 7H, 2 x O-C*H$_2$*,O-C*H$_3$*), 4.22 – 4.45 (m, 4H, 0.5 Pip-C^2*H$_2$*, 0.5 Pip-C^6*H$_2$*, Pip-C$^{2'}$*H$_2$*), 4.88 – 4.94 (m, 1H), 6.69 – 6.79 (m, 2H, C^5*H*, C^6*H*), 11.19 (s, 1H, N*H*). ^{19}F-NMR (377 MHz, DMSO-d$_6$) δ -227.90 ppm. ^{13}C-NMR (101 MHz, DMSO-d$_6$) δ 27.3 (d, J = 104.4 Hz, Pip-*C*^{3}, Pip-*C*^{5}), 37.8 (d, J = 5.8 Hz, Pip-*C*^{4}), 43.7 (d, J = 8.8 Hz, Pip-*C*^{2}, Pip-*C*^{6}), 51.23 (*C*N*C*), 55.4 (*C*H$_3$), 66.5 (*C*O*C*), 71.8 (d, $^2J_{C\text{-}F}$ = 18.4 Hz, Pip-*C*$^{1'}$), 84.9 (d, $^1J_{C\text{-}F}$ = 169.1 Hz, Pip-*C*$^{2'}$), 107.17 (*C*^{6}), 111.3 (*C*^{5}), 139.8 (CO). Anal. Calcd. for $C_{20}H_{27}FN_4O_4S$: C, 54.78; H, 6.21; N, 12.78; Found C, 54.82; H, 6.27; N, 12.69. HRMS m/z: [M+H]$^+$ Calcd 439.1810; Found 439.1806.

rac-4-(1-Fluoro-2-hydroxypropan-2-yl)-*N*-(4-methoxy-7-morpholinobenzo[*d*]thiazol-2-yl)piperidine-1-carboxamide **13o**



Phenyl (4-methoxy-7-morpholinobenzo[*d*]thiazol-2-yl)carbamate (316 mg, 0.821 mmol, 1.00 eq.) and 1-fluoro-2-(piperidine-4-yl)propan-2-ol hydrochloride (228 mg, 1.15 mmol, 1.40 eq.) were dissolved in dry DMSO (10 mL) under an argon atmosphere. DBU (175 µL, 1.17 mmol, 1.43 eq.) was added and the reaction was heated to 60°C. After four hours of stirring the reaction was allowed to cool to room temperature and stirred for another 46 hours. The solution was diluted with ethyl acetate and subsequently washed with a saturated aqueous solution of sodium bicarbonate, twice with water and brine. The organic layer was dried over sodium sulfate and the solvent was removed *in vacuo*. The residue was purified by column chromatography (eluent: dichloromethane/methanol, 99/1 to 97/3) to obtain a white solid (241 mg, 0.533 mmol, 65 %), mp 218 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.10 (d, J = 2.2 Hz, 3H, Pip-$C^{1''}H_3$), 1.28 – 1.44 (m, 1H, C^4H), 1.66 – 1.76 (m, 2H, 0.5 Pip-C^3H_2, 0.5 Pip-C^5H_2), 1.81 – 1.88 (m, 2H, 0.5 Pip-C^3H_2, 0.5 Pip-C^5H_2), 2.76 – 2.89 (m, 2H, 0.5 Pip-C^2H_2, 0.5 Pip-C^6H_2), 3.04 – 3.13 (m, 4H, 2 x N-CH_2), 3.81 – 3.90 (m, 7H, 2 x O-CH_2, O-CH_3), 4.14 – 4.38 (m, 4H, 0.5 Pip-C^2H_2, 0.5 Pip-C^6H_2, Pip-$C^{2'}H_2$), 6.75 (s, 2H, C^5H, C^6H), 9.99 (br, 1H, N*H*). ^{19}F-NMR (377 MHz, $CDCl_3$) δ -229.57. ^{13}C-NMR (101 MHz, $CDCl_3$) δ 19.8 (d, J = 5.1 Hz, Pip-$C^{1''}$), 26.3 (d, J = 93.4 Hz, Pip-C^3, Pip-C^5), 42.6 (d, J = 2.8 Hz, Pip-C^4), 44.6 (d, J = 20.6 Hz, Pip-C^2, Pip-C^6), 51.8 (NCH_2), 55.9 (CH_3), 67.5 (OCH_2), 73.2 (d, $^2J_{C-F}$ = 17.3 Hz, Pip-$C^{1'}$), 88 (d, $^1J_{C-F}$ = 173.5 Hz, Pip-$C^{2'}$), 107.2 (C^6), 112.2 (C^5), 127 (C^{7a}), 137.6 (C^7), 140.6 (C^4), 147.2 (C^2), 154.1 (C^{4a}), 161.7 (*C*O). Anal. Calcd. for $C_{21}H_{29}FN_4O_4S$: C, 55.73; H, 6.46; N, 12.38; Found C, 55.68; H, 6.49; N, 12.29. HRMS m/z: $[M+H]^+$ Calcd 453.1966; Found 453.1965.

4-Hydroxy-4-methyl-*N*-(4-(2-fluoroethoxy)-7-morpholinobenzo[*d*]thiazol-2-yl)piperidine-1-carboxamide **13p**

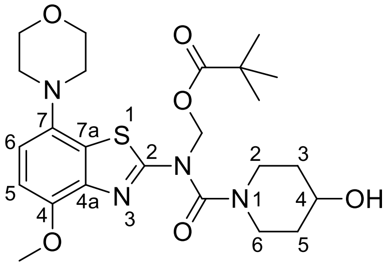


Under argon at ambient temperature phenyl (4-(2-fluoroethoxy)-7-morpholinobenzo[*d*]thiazol-2-yl)carbamate **11** (417 mg, 1 mmol) was dissolved in dry DMSO (8 mL). Under stirring 4-hydroxy-4-methylpiperidine hydrochloride (166.7 mg, 1.1 mmol) was added followed by triethylamine (556 µL, 4 mmol) and the dark yellow solution was stirred for 2.5 h at 50°C (TLC: sample in acetone, ethyl acetate/hexane/acetic acid, 80/20/0.2, $R_{fCarbamate}$ 0.90, R_{fUrea} 0.35). After cooling to ambient temperature, ethyl acetate (50 mL) was added and the mixture was washed successively with 50% brine (2 x 25 mL), dried over Na_2SO_4, and concentrated under reduced pressure to give an oily residue that was purified by flash chromatography (ethyl acetate/hexane, 80/20). Evaporation of the product fractions gave a clear colorless oil that was treated with *tert*-butyl methyl ether. Rota-evaporation of TBME furnished the product (410 mg, 93%) as a fawn solid, mp 197°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.30 (s, 3H, CH_3), 1.60 – 1.69 (m, 4H, Pip-C$^3H_2$ + Pip-C$^5H_2$), 1.81 (s_{br}, 1H, OH), 3.12 (t, J = 4.5 Hz, 4H, 2 x N-CH_2), 3.35 – 3.49 (m, 2H, 0.5 Pip C$^2H_2$ + 0.5 Pip-C$^6H_2$), 3.89 (m, t, J = 4.5 Hz, 6H, 2 x O-CH_2 + 0.5 Pip C$^2H_2$ + 0.5 Pip-C$^6H_2$), 4.36 (dt, $^3J_{H\text{-}F}$ = 28 Hz, ^3J = 4.2 Hz, 2H, CH_3CH_2F), 4.79 (dt, $^2J_{H\text{-}F}$ = 47 Hz, ^3J = 4.2 Hz, 2H, CH_3CH_2F), 6.77 (d, 1H, J = 8.5 Hz, H^6), 6.83 (d, 1H, J = 8.5 Hz, H^5), 9.47 (s_{br}, 1H, NH). ^{19}F-NMR (377 MHz, $CDCl_3$) δ -223.11. ^{13}C-NMR (101 MHz, $CDCl_3$) δ 30.3 (CH$_3$),38.3 (Pip-$C^{3/5}$), 40.6 (Pip-$C^{2/6}$), 51.7 (NCH$_2$), 67.4 (OCH$_2$), 67.8 (Pip-C^4), 68.4(d, $^2J_{C\text{-}F}$ = 21 Hz, CH$_2$CH$_2$F), 81.8(d, $^1J_{C\text{-}F}$ = 171 Hz, CH$_2$$CH_2$F), 109.3 ($C^6$), 112 ($C^5$), 141.2 ($C^7$), 140.4 ($C^4$), 145.6 ($C^{4a}$), 154.2 (C=O). Anal. Calcd. for $C_{20}H_{27}FN_4O_4S$: C, 54.78; H, 6.21; N, 12.78; Found C, 54.82; H, 6.19; N, 12.72. HRMS (ESI+) m/z: $[M+H]^+$ Calcd 439.1810; Found 439.1806.

Synthesis of labeling precursors **14a** – **14c**

(*N*-(4-methoxy-7-morpholinobenzo[*d*]thiazol-2-yl)-4-((methylsulfonyl)oxy)-piperidine-1-carboxamido)methyl pivalate **14a**

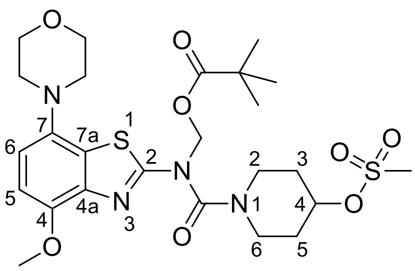
a) 4-Hydroxy-*N*-(4-methoxy-7-morpholinobenzo[*d*]thiazol-2-yl)piperidine-1-carboxamido)methyl pivalate **14a-1**



Under argon dry potassium carbonate (207 mg, 1.5 mmol) was added to a stirred solution of 4-hydroxypiperidine-1-carboxylic acid (4-methoxy-7-morpholin-4-yl-benzo[*d*]thiazol-2-yl)-amide **13c** (392 mg, 1 mmol) in dry DMF (20 mL). The mixture was heated to 60°C and stirred at that temperature for 0.5 h. A solution of chloromethyl pivalate (290 µL, 1.5 mmol) in dry DMF (500 µL) was slowly added and the mixture was stirred for 1 h at 60°C (TLC: ethyl acetate/methanol, 95/5, R_{fUrea} 0.25, $R_{fPom\text{-}urea}$ 0.65). After cooling to ambient temperature the mixture was poured into ice/water (150 mL), the aqueous layer was extracted with ethyl acetate (2 x 75 mL) and the organic phase was washed successively with 10% aqueous citric acid solution (100 mL) and water (100 mL). Drying over sodium sulfate, filtration and rota evaporation of the solvent *in vacuo* left an oily residue that was crystallized by treating with TBME followed by concentration. Recrystallization from aqueous 50% MeOH (10 mL) furnished the POM protected urea as a solid. Yield 465 mg (92%), off-white crystals, mp 121°C. $C_{24}H_{34}N_4O_6S$ HRMS (ESI+) m/z: $[M+H]^+$ Calcd 507.22718; Found 507.22715. (400 MHz, DMSO-d_6) δ 1.11 (s, 9H,Pom C*H*$_3$), 1.21 – 1.36 (m, 2H, 0.5 Pip C^3H_2, 0.5 Pip C^5H_2), 1.66-1.82 (m, 2H, 0.5 Pip C^3H_2, 0.5 Pip C^5H_2), 2.94 (t, ^3J = 4.5 Hz, 4H, 2 x N-C*H*$_2$), 3.02 – 3.17 (m, 1H, 0.25 Pip $C^{2/6}H_2$), 3.19-3.29 (m, 1H, 0.25 Pip $C^{2/6}H_2$), 3.62-3.72 (m, 1H, 0.25 Pip $C^{2/6}H_2$), 3.72 (t, ^3J = 4.5 Hz, 4H, 2 x O-C*H*$_2$), 3.84 (s, 3H, O-C*H*$_3$), 3.90-4.01 (m, 1H, 0.25 Pip $C^{2/6}H_2$), 4.11-4.25 (m, 1H, 0.25 Pip $C^{2/6}H_2$), 4.72 (s_{br}, 1H, Pip C^4H), 6.40 (s_{br}, 2H, Pom C*H*$_2$), 6.94 (d, *J* = 8.8 Hz, 2H, H^6), 7.06 (d, *J* = 8.8 Hz, 2H, H^5). ^{13}C NMR (100 MHz, DMSO-d_6) δ 27.1 (Pom *C*H$_3$), 34.6 (Pip C^3, C^5), 38.8 (Pom C^q), 42.1 (Pip C^2, C^6), 52.0 (*C*N*C*), 57.2 (O*C*H$_3$), 66.4 (Pip C^4), 66.9 (*C*O*C*), 70.1

(Pom CH_2), 111.6 (C^6), 114.3 (C^5), 121.8 (C^{7a}), 125.4 (C^7), 140.9 (C^4), 143.4 (C^{4a}), 160.2 (urea C=O), 166.4 (C^2), 177.1 (Pom C=O).

b) (*N*-(4-methoxy-7-morpholinobenzo[*d*]thiazol-2-yl)-4-((methylsulfonyloxy)-piperidine-1-carboxamido)methyl pivalate **14a**

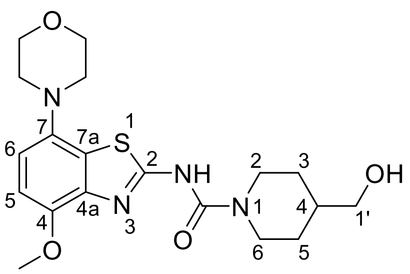


To an approximately 0.1M solution of the alcohol **14a-1** (253 mg, 0.5 mmol) in ethanol free DCM (5 mL) containing a 200% molar excess of triethylamine (210 µL, 1.5 mmol) and kept between 0°C and -10°C, was added a 100% excess of methanesulfonyl chloride (77 µL, 1 mmol) over a period of 2-5 minutes. Ten minutes after the addition (the color of the solution had changed from colorless to deep red) the cooling bath was removed and the solution was stirred for 90 min at ambient temperature to complete the reaction (TLC: ethyl acetate/hexane, 80/20, $R_{fPom\text{-}urea}$ 0.24, $R_{fMesylate}$ 0.56 or ethyl acetate/methanol, 95/5, $R_{fPom\text{-}urea}$ 0.52, $R_{fMesylate}$ 0.77). The reaction mixture was transferred to a separatory funnel with the aid of more DCM. The mix was first extracted with ice water, followed by cold sat. sodium bicarbonate and water. Drying the DCM solution over Na_2SO_4 followed by solvent removal gave the product as red-brown crystals. They were taken up in TBME (15 mL), ultrasonicated for one minute and the turbid solution was filtered through a layer (± 1 cm) of Celite. Evaporation of the solvent yielded the mesylate (240 mg, 82%) as colorless crystals, mp 97°C. $C_{25}H_{36}N_4O_8S_2$ HRMS (ESI+) m/z: $[M+H]^+$ Calcd 585.20473; Found 585.20470. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.19 (s, 9H, Pom CH_3), 1.81-1.97 (m, 2H, 0.5 Pip C^3H_2, 0.5 Pip C^5H_2), 1.98-2.12

(m, 2H, 0.5 Pip C^3H_2, 0.5 Pip C^5H_2), 3.05 (t, ^3J = 4.5 Hz, 4H, 2 x N-CH_2), 3.07 (s, 3H, Mes CH_3), 3.53-3.64 (m, 1H, 0.25 Pip $C^{2/6}H_2$), 3.66-3.77 (m, 1H, 0.25 Pip $C^{2/6}H_2$), 3.88 (t, ^3J = 4.5 Hz, 4H, 2 x O-CH_2), 3.89 (s, 3H, O-CH_3), 3.96-4.07 (m, 1H, 0.25 Pip $C^{2/6}H_2$), 4.09-4.20 (m, 1H, 0.25 Pip $C^{2/6}H_2$), 4.97 (sept., 1H, Pip C^4H), 6.55 (s_{br}, 2H, Pom CH_2), 6.86 (d, *J* = 5.2 Hz, 2H, aryl-*H*). ^{13}C NMR (100 MHz, $CDCl_3$) δ 27 (Pom *C*H_3), 31.8 (Pip C^3, C^5), 38.9 (Mes *C*H_3), 39.6 (Pom C^q), 43.2 (Pip C^2, C^6), 52.0 (*C*N*C*), 57.3 (O*C*H_3), 66.7 (*C*O*C*), 70.2 (Pom *C*H_2), 78 (Pip C^4), 110.1 (C^6), 113.4 (C^5), 123 (C^{7a}), 125.6 (C^7), 140.9 (C^4), 143.3 (C^{4a}), 161 (urea *C*=O), 167.8 (C^2), 177.7 (Pom *C*=O).

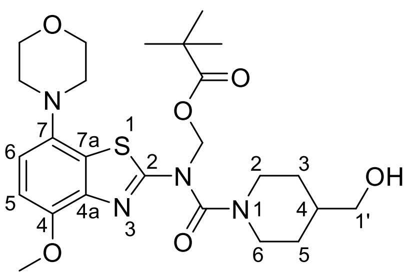
(*N*-(4-methoxy-7-morpholinobenzo[*d*]thiazol-2-yl)-4-(methylsulfonyloxy)methyl)piperidine-1-carboxamido)methyl pivalate **14b**

a) 4-(Hydroxymethyl)-*N*-(4-methoxy-7-morpholinobenzo[*d*]thiazol-2-yl)piperidine-1-carboxamide **14b-1**



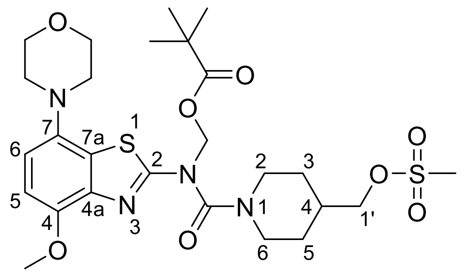
N-Boc-4-piperidinemethanol (775 mg, 3.60 mmol, 1.20 eq.) was dissolved in dichloromethane (2.0 mL) and treated with trifluoroacetic acid (2.0 mL). After stirring for two hours at room temperature, the solution was evaporated to dryness. The residue was dissolved in dry DMSO (5.0 mL) under an argon atmosphere and phenyl (4-methoxy-7-morpholinobenzo[*d*]thiazol-2-yl)carbamate **8** (1.16 g, 3.00 mmol, 1.00 eq.) and DBU (1.30 mL, 1.33 g, 8.74 mmol, 2.91 eq.) were added. After stirring for five hours at 80°C, the reaction was cooled to room temperature and stirred overnight. The solution was diluted with ethyl acetate and diethyl ether and subsequently washed with a saturated aqueous solution of sodium bicarbonate, twice with water and brine. The organic layer was dried over sodium sulfate and the solvent was removed *in vacuo*. The residue was recrystallised from methanol and water (70/30). The desired product was obtained as white solid (365 mg, 30 %), mp 206°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 0.99 – 1.13 (m, 2H, 0.5 Pip-C^3H_2, 0.5 Pip-C^5H_2), 1.54 – 1.72 (m, 3H, Pip-C^4H, 0.5 Pip-C^3H_2, 0.5 Pip-C^5H_2), 2.76 – 2.86 (m, 2H, 0.5 Pip-C^2H_2, 0.5 Pip-C^6H_2), 2.95 – 3.02 (m, 4H, 2 x NCH_2), 3.26 (d, *J* = 6.1 Hz, 2H, Pip-$C^{1'}H_2$), 3.74 – 3.79 (m, 4H, 2 x OCH_2), 3.84 (s, 3H, OCH_3), 4.20 – 4.28 (m, 4H, 0.5 Pip-C^2H_2, 0.5 Pip-C^6H_2), 4.48 (br, 1H, O*H*), 6.79 (d, *J* = 8.5 Hz, 1H, C^6H), 6.87 (d, *J* = 8.6 Hz, 1H, C^5H), 11.25 (br, 1H, N*H*). ^{13}C-NMR (101 MHz, DMSO-d_6) δ 28.55 (Pip-C^3, Pip-C^5), 33.30 (Pip-C^4), 43.70 (Pip-C^2, Pip-C^6), 51.43 (*C*N*C*), 55.86 (CH_3), 65.53 (*C*O*C*), 66.57 (Pip-$C^{1'}$), 107.94 (C^6), 111.76 (C^5), 139.99 (*C*O). $C_{19}H_{26}N_4O_4S$ HRMS m/z: $[M+H]^+$ Calcd 407.1748; Found 407.1746.

b) (4-(Hydroxymethyl)-*N*-(4-methoxy-7-morpholinobenzo[*d*]thiazol-2-yl)piperidine-1-carboxamido)methyl pivalate **14b-2**



4-(Hydroxymethyl)-*N*-(4-methoxy-7-morpholinobenzo[*d*]thiazol-2-yl)piperidine-1-carboxamide **14b-1** (335 mg, 0.824 mmol, 1.00 eq.) and potassium carbonate (185 mg, 1.34 mmol, 1.62 eq.) were suspended in DMF (15 mL) and stirred at 60 °C for 0.5 hours. A solution of chloromethyl pivalate (378 µL, 2.62 mmol, 3.18 eq.) in DMF (1.0 mL) was added and the reaction was stirred at 60 °C for three hours. The reaction was then cooled to room temperature. After stirring for 20 hours the reaction mixture was poured over ice and extracted with ethyl acetate. The combined organic phases were washed successively with an aqueous solution of citric acid (10 %), water and brine. The organic layer was dried over sodium sulfate and the solvent was removed *in vacuo*. The residue was purified by column chromatography (eluent: dichloromethane/methanol, 99/1 to 95/5) to obtain a brown solid (260 mg, 61 %), mp 100°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.09 – 1.26 (m, 11H, 3 POM-C*H₃*, 0.5 Pip-C^3H_2, 0.5 Pip-C^5H_2), 1.69 – 1.83 (m, 3H, 0.5 Pip-C^3H_2, 0.5 Pip-C^5H_2, Pip-C^4H), 2.72 – 3.08 (m, 6H, 0.5 Pip-C^2H_2, 0.5 Pip-C^6H_2, 2 x NCH_2), 3.50 (d, *J* = 6.1 Hz, 2H, Pip-$C^{1'}H_2$), 3.80 – 3.86 (m, 7H, 2 x OCH_2, CH_3), 4.52 – 4.75 (m, 2H, 0.5 Pip-C^2H_2, 0.5 Pip-C^6H_2), 6.51 (s, 2H, POM-CH_2), 6.78 (d, *J* = 8.7 Hz, 1H, C^6H), 6.83 (d, *J* = 8.7 Hz, 1H, C^5H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 27.14 (s, POM-C(CH_3)$_3$), 28.79 (0.5 Pip-C^3, 0.5 Pip-C^5), 29.19 (0.5 Pip-C^3, 0.5 Pip-C^5), 39.20 (s, Pip-C^4), 42.94 (0.5 Pip-C^2, 0.5 Pip-C^6), 44.63 (0.5 Pip-C^2, 0.5 Pip-C^6), 51.98 (*C*N*C*), 56.45 (OCH_3), 67.42 (*C*O*C*), 67.74 (s, Pip-$C^{1'}$), 69.97 (s, POM-CH_2), 110.03 (C^6), 113.29 (C^5), 123.23 (C^{7a}), 125.77 (C^7), 141.03 (C^{4a}), 143.31 (C^4), 161.05 (*C*O), 167.30 (C^2), 177.96 (POM-*C*O). $C_{25}H_{36}N_4O_6S$ HRMS m/z: $[M+H]^+$ Calcd 521.24283; Found 521.24262.

c) (*N*-(4-methoxy-7-morpholinobenzo[*d*]thiazol-2-yl)-4-(methylsulfonyloxy)methyl)piperidine-1-carboxamido)methyl pivalate **14b**

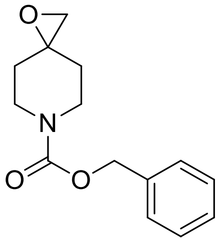


(4-(Hydroxymethyl)-*N*-(4-methoxy-7-morpholinobenzo[*d*]thiazol-2-yl)piperidine-1-carboxamido)methyl pivalate **14b-2** (230 mg, 0.442 mmol, 1.00 eq.) was dissolved in dichloromethane (3 mL) and cooled to 0 °C. The solution was treated with triethylamine

(91.7 µL, 0.657 mmol, 1.49 eq.) and methanesulfonyl chloride (44.4 µL, 0.574 mmol, 1.30 eq.) and stirred at 0 °C. After 1.5 hours another 1.49 equivalents of triethylamine and 1.30 equivalents of methanesulfonyl chloride were added. The reaction was stirred at 0 °C for another 1.5 hours before being diluted with dichloromethane. The organic solution was washed successively with saturated aqueous solutions of ammonium chloride, sodium bicarbonate and brine. After drying over sodium sulfate the solvent was removed *in vacuo* and the residue was purified by column chromatography (eluent: petrol ether/ethyl acetate, 30/70) to obtain a white solid (184 mg, 70 %), mp 163°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.17 (s, 9H, 3 POM-C*H_3*), 1.19 – 1.37 (m, 2H, 0.5 Pip-C^3*H_2*, 0.5 Pip-C^5*H_2*), 1.74 – 1.86 (m, 2H, 0.5 Pip-C^3*H_2*, 0.5 Pip-C^5*H_2*), 1.93 – 2.06 (m, 1H, Pip-C^4*H*), 2.74 – 3.09 (m, 9H, 0.5 Pip-C^2*H_2*, 0.5 Pip-C^6*H_2*, 2 x NC*H_2*, Ms-C*H_3*), 3.82 – 3.89 (m, 7H, 2 x OC*H_2*, OC*H_3*), 4.08 (d, *J* = 6.5 Hz, 2H, Pip-$C^{1'}$*H_2*), 4.56 – 4.80 (m, 2H, 0.5 Pip-C^2*H_2*, 0.5 Pip-C^6*H_2*), 6.52 (s, 2H, POM-C*H_2*), 6.80 (d, *J* = 8.7 Hz, 1H, C^6*H*), 6.84 (d, *J* = 8.7 Hz, 1H, C^5*H*). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 27.17 (s, POM-C(*C*H_3)$_3$), 28.42 (0.5 Pip-*C^3*, 0.5 Pip-*C^5*), 28.82 (0.5 Pip-*C^3*, 0.5 Pip-*C^5*), 36.40 (s, Pip-*C^4*), 37.49 (s, Ms-*C*H_3), 42.52 (0.5 Pip-*C^2*, 0.5 Pip-*C^6*), 44.22 (0.5 Pip-*C^2*, 0.5 Pip-*C^6*), 52.03 (*C*N*C*), 56.49 (O*C*H_3), 67.45 (*C*O*C*), 69.91 (s, POM-*C*H_2), 73.51 (s, Pip-*$C^{1'}$*), 110.11 (*C^6*), 113.43 (*C^5*), 123.23 (*C^{7a}*), 125.77 (*C^7*), 141.09 (*C^{4a}*), 143.39 (*C^4*), 161.10 (*C*O), 167.59 (*C^2*), 177.93 (POM-*C*O). $C_{26}H_{38}N_4O_8S_2$ HRMS m/z: $[M+H]^+$ Calcd 599.2204; Found 599.2201.

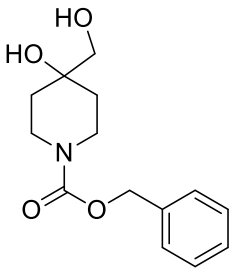
Pivaloxymethyl-(4-methoxy-7-morpholinobenzo[*d*]thiazol-2-yl)(1,3-dioxa-2-thia-8-azaspiro[4.5]decan-2-oxide)-8-carboxamide **14c**

a) Benzyl 1-Oxa-6-azaspiro[2,5]octane-6-carboxylate **14c-1** (according to [44])



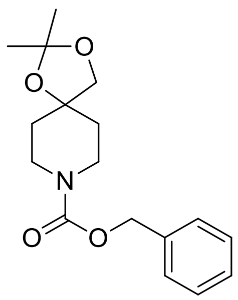
NaH (60 % in mineral oil, 2 g, 50 mmol) was added in portions to a solution of trimethylsulfonium iodide (6.6 g, 30 mmol) in DMSO (100 mL) cooled to 0 °C. After complete addition the ice bath was removed and the solution was stirred for another 40 min at ambient temperature. After addition of 1-(benzyloxycarbonyl)-4-piperidone (4.66 g, 20 mmol), the mixture was heated to 55°C for 2 h (TLC: hexane/ethyl acetate, 70/30, R_f 0.38), cooled to ambient temperature and poured onto ice. The product was extracted with ethyl acetate, washed with water and dried over Na_2SO_4. After removing the solvent under reduced pressure, further purification was carried out by flash chromatography (hexane/ethyl acetate, 70/30). The product **14c-1** (3 g, 61%) was obtained as a colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.50 (dd, *J* = 11.6, 6.7 Hz, 2H), 1.74 - 1.98 (m, 2H), 2,64 (d, *J* = 9.1 Hz, 1H), 3.52 (ddd, *J* = 13.3, 9.6, 3.7 Hz, 2H), 3.76 - 3.98 (m, 2H), 5.18 (d, *J* = 0.6 Hz, 2H), 7.27 - 7.48 (m, 5H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 32.9, 39.5, 42.7, 49.5, 53.7, 56.9, 65.2, 67.2, 73.2, 126.9, 127.5, 127.6, 127.8, 127.9, 128, 128.1, 128.4, 128.5, 136.7, 138.1,. 141.2, 155.2. $C_{14}H_{17}NO_3$ HRMS m/z: $[M+H]^+$ Calcd 248.1281; Found 248.1280.

b) Benzyl-4-hydroxy-4-(hydroxymethyl)piperidine-1-carboxylate **14c-2**



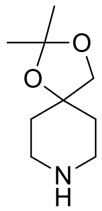
Benzyl 1-oxa-6-azaspiro[2,5]octane-6-carboxylate **14c-1** (2.9 g, 12 mmol) was heated to 50 °C in 0.02 N HCl (54 mL) for 1 h (TLC: hexane/ethyl acetate, 60/40, R_f 0.1; hexane/ethyl acetate/AcOH, 30/70/0.2, R_f 0.41). After cooling to ambient temperature, the pH was adjusted to neutral with 2 N NaOH solution. The aqueous phase was extracted three times with dichloromethane and the combined organic phases were washed with brine and dried over Na_2SO_4. After removal of the solvent under reduced pressure, the product (2.7 g, 85%) was obtained as a colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1,13 - 2.02 (m, 4H), 2.80 (s, 2H), 3.28 (t, *J* = 10.8 Hz, 2H),), 3.45 (s, 2H), 3.74 - 4.16 (m, 2H), 5.15 (s, 2H), 7.08 - 7.53 (m, 5H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 33.4, 39.8,. 42.7, 53.5, 57.0, 67.2, 69.9, 70.3, 127.8, 127.9, 128.1, 128.5, 128.6, 136.7, 155.4. $C_{14}H_{19}NO_4$ HRMS m/z: $[M+H]^+$ Calcd 266,1387; Found 266,1387.

c) Benzyl-2,2-dimethyl-1,3-dioxa-8-azaspiro[4.5]decan-8-carboxylate **14c-3** (according to [45])



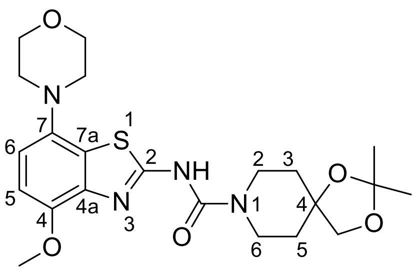
Benzyl 4-hydroxy-4-(hydroxymethyl)piperidine-1-carboxylate **14c-2** (2.65 g, 10 mmol) and CSA (27 mg, 0.11 mmol) were dissolved in 2,2-dimethoxypropane (100 mL) and stirred at ambient temperature for 24 h (TLC: hexane/ethyl acetate/AcOH, 60/40/0,2, R_f 0,72). The reaction was stopped by the addition of triethylamine (2.5 mL), the solvent was removed and the residue was taken up in water and extracted three times with dichloromethane (3 x 15 mL). The combined organic phases were washed with saturated $NaHCO_3$ solution, water, brine and water, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The product (2.8 g, 92%) was obtained as a colorless oil. ^{1}-H-NMR (400 MHz, $CDCl_3$) δ 1.38 (s, 6H), 1.45 - 1.81 (m, 4H), 3.51 – 3.14 (m, 2H), 3.61 - 3.82 (m, 4H), 5.12 (s, 2H), 7.18 - 7.45 (m, 5H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 27.3, 35.6, 41.2, 67.1, 73.6, 78.4, 109.5, 126.9, 127.8, 128, 128.5, 136.8, 155.3. $C_{17}H_{23}NO_4$ HRMS m/z: $[M+H]^+$ Calcd 306.1700; Found 306.1698.

d) 2,2-Dimethyl-1,3-dioxa-8-azaspiro[4.5]decane **14c-4**



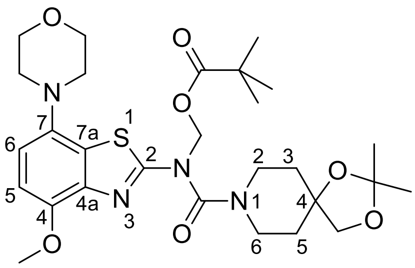
A suspension of benzyl 2,2-dimethyl-1,3-dioxa-8-azaspiro[4.5]decane-8-carboxylate **14c-3** (2,8 g, 9,2 mmol) and Pd/C (10%, 333 mg, 1 mmol) in methanol (40 mL) was stirred overnight in a hydrogen atmosphere (TLC: hexane/ethyl acetate/AcOH, 60/40/0,2, R_f 0,38). After filtration through Celite and washing of the filter cake with MeOH, the filtrates were combined and the solvent was removed under reduced pressure. The product (1.3 g, 85%) was obtained as a grey waxy solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.36 (s, 6H), 1.49 – 1.88 - (m, 4H), 2.17 - 2.67 (m, 1H), 2.68 - 3.17 (m, 3H), 3.75 (d, *J* = 4.6 Hz, 2H), 4.30 (s_{br}, 1H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 27.3, 36, 43.1, 49.3, 73.21, 73.6, 78.4, 79.5, 80.8, 108.8, 109.2. $C_9H_{17}NO_2$ HRMS m/z: $[M+H]^+$ Calcd 172.1332; Found 172.1330.

e) *N*-(4-Methoxy-7-morpholinobenzothiazol-2-yl)-2,2-dimethyl-1,3-dioxa-8-azaspiro[4.5]-decan-8-carboxamide **14c-5**



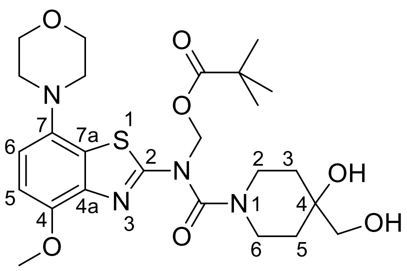
A solution of phenyl-4-methoxy-7-morpholinobenzothiazol-2-ylcarbamate **8** (1.9 mg, 5 mmol) and 2,2-dimethyl-1,3-dioxa-8-azaspiro[4.5]decane **14c-4** (1.02 g, 6 mmol) in DMSO (25 mL) was stirred for 24 hours at 60°C (TLC: hexane/ethyl acetate, 30/70, R_f 0,46). After cooling to ambient temperature, ethyl acetate (150 mL) was added and the organic solution was washed successively with water, saturated aqueous $NaHCO_3$ solution and brine. After drying over Na_2SO_4 the solvent was removed under reduced pressure to furnish a brown residue that was purified by flash chromatography (hexane/ethyl acetate, 50/50). The product was obtained as a colorless solid (1.41 g, 61%), mp. 198°C (dec.). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1,37 (s, 6H), 1.47 – 1.88 (m, 4H), 2.89 – 3.19 (m, 4H), 3.44 (t, *J* = 9,8 Hz, 2H), 3.58 – 4.09 (m, 11H), 6.76 (s, 2H), 7.69 (s_{br}, 1H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 27.2, 35.5, 41.6, 51.8, 55.9, 67.4, 73.7, 78.1, 107.3, 109.7, 112.4, 140.5, 146.85, 154.1. $C_{22}H_{30}N_4O_5S$ HRMS m/z: $[M+H]^+$ Calcd 463.2009; Found 463.2007.

f) (*N*-(4-methoxy-7-morpholinobenzothiazol-2-yl)-2,2-dimethyl-1,3-dioxa-8-azaspiro-[4.5]decan-8-carboxamido)methyl pivalate **14c-6**



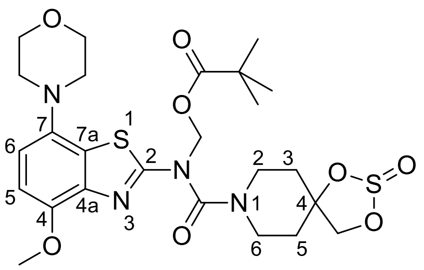
To a solution of *N*-(4-methoxy-7-morpholinobenzothiazol-2-yl)-2,2-dimethyl-1,3-dioxa-8-azaspiro[4.5]decan-8-carboxamide **14c-5** (926 mg, 2 mmol) in DMF (40 mL), was added K_2CO_3 (414 mg, 3 mmol). The suspension was heated to 60°C and stirred for 30 min. A solution of POM-Cl (580 µL, 3 mmol) in DMF (1000 µL) was added and after stirring for 2 h at 60°C (TLC: hexane/ethyl acetate, 30/70, R_f 0,69) the mixture was cooled to ambient temperature and poured into ice-water. The product was extracted into ethyl acetate and the organic phase was washed with 10 % citric acid and twice with water. Drying over Na_2SO_4 and removal of the solvent under reduced pressure afforded a solid residue that was recrystallized from methanol to give the product as a colorless solid (1.15 g, 99%), mp. 207°C (dec.). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.16 (s, 9H), 1.40 (s, 6H), 1.51 – 1.86 (m, 2H), 2.81 – 3.19 (m, 4H), 3.35 – 4.27 (m, 13H), 6.81 (s, 2H), 6.52 (s, 2H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 27.1, 27.3, 39, 51.9, 56.4, 67.3, 69.8, 73.6, 78.9, 109.4, 110, 113.3, 123.1, 125.6, 140.8, 143.4, 160.9, 167.3, 177.8. $C_{28}H_{40}N_4O_7S$ HRMS m/z: $[M+H]^+$ Calcd 577.2690; Found 577.2688.

g) 4-Hydroxy-4-(hydroxymethyl)-*N*-(4-methoxy-7-morpholinobenzo[*d*]thiazol-2-yl)-N-pivaloyloxymethylpiperidine-1-carboxamide **14c-7**



To a solution of *N*-(4-methoxy-7-morpholinobenzothiazol-2-yl)-2,2-dimethyl-1,3-dioxa-8-azaspiro[4.5]decan-8-carboxamido)methyl pivalate **14c-6** (1.62 g, 2.8 mmol) in MeOH (10 mL) was added camphorsulfonic acid (98 mg, 0.42 mmol, 15 mol %) and the mixture was stirred at 50°C overnight (TLC: ethyl acetate/methanol/AcOH, 98/2/0.2, R_f 0.21). After cooling to ambient temperature triethylamine (700 µL) was added and the solvent was removed under reduced pressure. Further purification was done by flash chromatography (ethyl acetate/methanol, 98/2). The product (967 mg, 75%) was obtained as a colorless solid, mp.: 107.9°C (dec.). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.10 (s, 9H), 1.28 - 1.76 (m, 2H), 2.75 – 3.59 (m, 11H), 3,80 (s, 7H), 4.25 (dd, *J* = 34.0, 11.8 Hz, 2H), 6.45 (s, 2H), 6.76 (s, 2H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 27, 33.9, 39, 40.5, 51.8, 56.3, 67.3, 69.8, 70.2, 110, 113.3, 122.9, 125.6, 140.8, 143.2, 160.9, 167.2, 177.8. $C_{25}H_{36}N_4O_7S$ HRMS m/z: $[M+H]^+$ Calcd 537.2377; Found 537.2376.

h) Pivaloxymethyl-(4-methoxy-7-morpholinobenzo[*d*]thiazol-2-yl)(1,3-dioxa-2-thia-8-azaspiro[4.5]decan-2-oxide)-8-carboxamide **14c** (according to [46])



To a stirred solution of 4-hydroxy-4-(hydroxymethyl)-*N*-(4-methoxy-7-morpholinobenzo[*d*]thiazol-2-yl)-*N*-pivaloyloxymethylpiperidine-1-carboxamide **14c-7** (1.07 g, 2 mmol) in dichloromethane (10 mL) was added triethylamine (1.12 mL, 8 mmol). After cooling to 0°C, a solution of thionyl chloride (220 µL, 3 mmol) in dichloromethane (500 µL) was added over 5 min (TLC: hexane/ethyl acetate, 50/50, R_f 0.25). Ten minutes after the addition, the solution was diluted with ethyl acetate (10 mL), washed with cold water (25 mL), diluted again with ethyl acetate (10 mL), and washed twice with cold brine. After removal of the solvent under reduced pressure, the product (1 g, 85%) was obtained as a light grey solid, mp. 213°C (dec.). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.16 (s, 9H), 1.31 – 2.35 (m, 4H), 2.81 – 3.21 (m, 4H), 3.21 – 3.65 (m, 2H), 3.72 – 4.01 (m, 7H), 4.12 – 4.61 (m, 4H), 6.52 (s, 2H), 6.72 – 6.97 (m, 2H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 27.1, 29.7, 35.3, 35.5, 35.6, 35.7, 35.9, 39, 39.3, 39.9, 40.9, 41.6, 51.9, 56.4, 67.3, 69.7, 74.8, 88.8, 110.1, 113.6, 123, 125.6, 140.8, 143.4, 161, 167.8. $C_{25}H_{34}N_4O_8S_2$ HRMS m/z: $[M+H]^+$ Calcd 583.1890; Found 583.1886.

Radiochemistry

Cyclotron produced [^{18}F]fluoride (80–100 GBq) was trapped on a Sep-Pak Accell Plus QMA Carbonate Plus Light Cartridge (46 mg, Waters) preconditioned with sterile water (3 mL) and flushed with air (3 mL). For the radiofluorination of **14a** and **14b** the cartridge was eluted with a solution of Kryptofix 2.2.2 (4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]-hexacosane) (20 mg) in acetonitrile (700 µL) and potassium carbonate (3.5 mg) in water (200 µL), for the radiofluorination of **14c** the trapped radiofluoride was eluted with Et_4NHCO_3 (2 mg) in water (1 mL). The effluent was collected in a tightly closed 10 mL vial and the solvent was evaporated under a stream of nitrogen and reduced pressure (50–80 mbar) at 100°C. The residue was azeotropically dried by the successive addition of 3 x 1 mL of dry acetonitrile. Precursor **14a**, **14b** or **14c** (5 mg, 8.3–8.5 µmol) predissolved in DMSO (500 µL) was added to the dry [^{18}F]F-Kryptofix complex and the reaction mixture was heated to 85°C (**14a** and **14b**) or 140°C (**14c**) for 15 min. After cooling to ambient temperature 1M NaOH solution (100 µL) in methanol (100 µL) was added to the vial and the hydrolysis reaction was allowed to proceed for 3 minutes. Acidified eluent (100 mL eluent + 200 µL H_3PO_4, 3 mL) was added and the mixture was injected into semi-preparative HPLC (column: Prontosil 120-5-C18 ace-EPS, 250 x 20 mm; eluent: sterile water/ phosphate buffer Braun MiniPlasco, 35/65/2, v/v/v; flow: 15 mL/min; detection: UV254, radioactivity). The respective radioactive fractions eluting at tR = 11–12 min (**[^{18}F]13e**), tR = 16–17 min (**[^{18}F]13g**) or tR = 11–12 min (**[^{18}F]13l**) were collected and the radiofluorinated product was concentrated using solid phase extraction. For this, the collected solution was diluted with water for injection (60 mL) and passed through a Strata X 33 C18 cartridge (30 mg), preconditioned in advance with ethanol (5 mL) followed by sterile water (10 mL). The cartridge was washed with sterile water (5 mL), the product was eluted either with 80% ethanol (0.8 mL, (**[^{18}F]13e** and **[^{18}F]13g**) or 70% ethanol (0.6 mL, **[^{18}F]13l**) and the ethanol fraction was diluted with isotonic NaCl (5.2 mL for **[^{18}F]13e** and **[^{18}F]13g** or 3.6 mL for **[^{18}F]13l**). Filtration through a sterile filter into a sterile vial gave injectable sterile solutions of **[^{18}F]13e**, **[^{18}F]13g** and **[^{18}F]13l** containing 10% ethanol by volume which were used for biological experiments. Radiotracers **[^{18}F]13e** and **[^{18}F]13g** were obtained in 25%–30% radiochemical yield (RCY) with a molar activity (MA) of 249 - 300 GBq/µmol and a radiochemical purity (RCP) of > 99%. **[^{18}F]13l** was prepared in 10% – 15% RCY with a MA of 600 GBq/µmol and a RCP of > 99%.

In vitro studies

Stable transfection of cells

Human adenosine receptors ADORA1 and ADORA2A from human whole brain cDNA (Clontech) cloned into pcDNA3.1+ (Invitrogen) at EcoRI (5'), XhoI (3') for ADORA1 and BamHI (5'), XhoI (3') for ADORA2A were commercially obtained from The Missouri S&T cDNA Resource Center, USA. Plasmid DNA was amplified and purified using standard molecular biological techniques. For transfection, we used a modified version of the calcium phosphate precipitation method [47,48].

Briefly, 4 x 10^5 CHO-K1 cells were seeded in 6 cm Petri dishes and transfected with 8 µg of hA_1AR-encoding plasmid DNA for 20 h at 37°C. Selection of stably transfected cells was initiated with 1 mg/mL geneticin (G418) until cell colonies had formed. From these colonies, single clonal lines were isolated by limiting dilution. Propagation of receptor expressing cells was performed in medium containing 450 µg/mL G418. Expression of hA_1ARs or $hA_{2A}ARs$ was verified by ligand binding ([^{3}H]DPCPX or [^{3}H]ZM 241385) and Western blotting.

Cell culture

The cells were grown adherently and kept in Ham's F12 Nutrient Mixture, containing 10% fetal bovine serum, penicillin (100 U/mL), streptomycin (100 µg/mL), L-glutamine (2 mM) and geneticin (G418, 0.2 mg/mL) at 37 °C in 5% CO_2/95% air. Cells were split two or three times a week at a ratio between 1:5 and 1:20. For binding assays, the culture medium was removed, cells were washed with PBS buffer (pH 7.4), scraped off, suspended in 1 ml PBS per dish and stored at −80°C.

Membrane preparation

Membrane preparations for ligand binding experiments followed a modified established protocol: Frozen cell samples were thawed and homogenized on ice (Ultra-Turrax, 1 x 30 sec at full speed) [49]. The homogenate was centrifuged at 600 x g for 10 min at 4°C. To collect the membrane fraction, the supernatant was centrifuged at 50,000 x g for 60 min at 4°C. The resulting membrane pellet was re-suspended in 50 mM Tris/HCl buffer (pH 7.4), frozen in liquid N_2 at a protein concentration of 6 mg/mL and stored at -80°C. Protein content was determined with a naphthol blue black photometric assay after solubilization in 15% NH_4OH containing 2% SDS (w/v) using human serum albumin as a standard [50].

Binding affinity

Binding experiments were performed with membranes from CHO K1 cells stably transfected with either the human A_1 or A_{2A} adenosine receptor and homogenates of pig striata. Dissociation constants (K_Ds) of [^{3}H]DPCPX and [^{3}H]ZM241385 were determined to be 3.0±0.7

nM (n=3) , 1.3±0.4 nM (n=6) and 2.7±1.7 (n=3) for the adenosine A_1 and A_{2A} receptor in cell membranes and pig striata homogenates, respectively. Membrane homogenates with a protein content of 7.5 µg immobilized in a gel matrix [51] were incubated with the radioligands in a total volume of 1.5 mL 50 mM Tris/HCl buffer (pH 7.4). After an incubation time of 70 min the immobilized membrane homogenates were washed with water and transferred into scintillation cocktail (5 mL each, Ultima Gold, Perkin Elmer). The radioactivity of the samples (bound radioactivity) was measured with a liquid scintillation counter (Beckman, USA). All binding data were calculated by non-linear curve fitting with a computer aided curve fitting program (Prism version 4.0, GraphPad Software, Inc., La Jolla, USA).

Autoradiography

For *in vitro* autoradiography, frozen horizontal sections (20 µm) of rat brains were used. After preincubation for five minutes in 50 mM Tris–HCl buffer solution (pH 7.4) the sections were incubated in the same buffer containing 2.3 kBq/mL of either **[^{18}F]13e**, **[^{18}F]13g** or **[^{18}F]13l** for 60 min. For detection of unspecific binding DPCPX or ZM 241385 (1 µmol/L) were added. The sections were washed in the buffer solution twice, immersed in deionized water, and dried at 37°C for 45 min. They were placed on a phosphor imaging plate for 30 min, scanned with a phosphor imager BAS 500 (Fujifilm, Tokyo, Japan) and analyzed with appropriate software.

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