

Synthesis and biological evaluation of chemokine receptor ligands with 2-benzazepine scaffold

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Abstract

Targeting CCR2 and CCR5 receptors is considered as promising concept for the development of novel antiinflammatory drugs. Herein, we present the development of the first probe-dependent positive allosteric modulator (PAM) of CCR5 receptors with a 2-benzazepine scaffold. Compound **14** (2-isobutyl-*N*-({[*N*-methyl-*N*-(tetrahydro-2*H*-pyran-4-yl)amino]methyl}phenyl)-1-oxo-2,3-dihydro-1*H*-2-benzazepine-4-carboxamide) activates the CCR5 receptor in a CCL4-dependent manner, but does not compete with [³H]TAK-779 binding at the CCR5. Furthermore, introduction of a *p*-tolyl moiety at 7-position of the 2-benzazepine scaffold turns the CCR5 PAM **14** into the selective CCR2 receptor antagonist **26b**. The structure affinity and activity relationships presented here offer new insights into ligand recognition by CCR2 and CCR5 receptors.

Key words

Chemokine receptors; CCR5; CCR2; positive allosteric modulator, 2-benzazepines; TAK-779, TAK-652; structure-affinity relationships; structure activity relationships.

1. Introduction

Since the first purification and description of the chemoattractant cytokine secreted platelet factor 4 (PF4/CXCL4) in 1977¹ more than 50 human chemokines have been discovered.² Their effects are mediated by 19 G-protein-coupled chemokine receptors. The chemokine receptors CCR2 and CCR5 share 72% sequence identity (82% identity in their active sites).³ Both receptors play a crucial role in trafficking of immune cells such as macrophages and monocytes, relevant for the development and progression of immunologic and cardiovascular diseases.⁴ The CCR2 receptor is abundantly expressed on blood monocytes and regulates their migration from the bone marrow into inflamed tissue, whereas the CCR5 receptor is expressed on macrophages, T-lymphocytes, and natural killer cells..⁵⁻⁷ CCR2 and

CCR5 receptors are expressed on different cells, but in a complementary manner. Therefore, dual as well as selective targeting of CCR2 and CCR5 receptors appears to have great potential in the development of novel concepts for the therapy of inflammatory diseases (e.g. atherosclerosis).⁵

The benzo[7]annulene TAK-779 (**1**) represents one of the first potent non-peptide CCR5 receptor antagonists ($IC_{50} = 1.4$ nM, Figure 1). TAK-779 does not only interact with the CCR5 receptor, but also with the CCR2 receptor, although its CCR2 affinity is about 20-fold lower ($IC_{50} = 27$ nM) compared to its CCR5 affinity.⁸ However, the quaternary ammonium group of TAK-779 leads to very low oral bioavailability. Therefore, very recently we have reported a large structure affinity relationship study with TAK-779 analogs containing a tertiary amine instead of the quaternary ammonium group. Depending on the substitution pattern, potent CCR2 selective and dual CCR2 and CCR5 targeting antagonists were found.⁹⁻¹¹

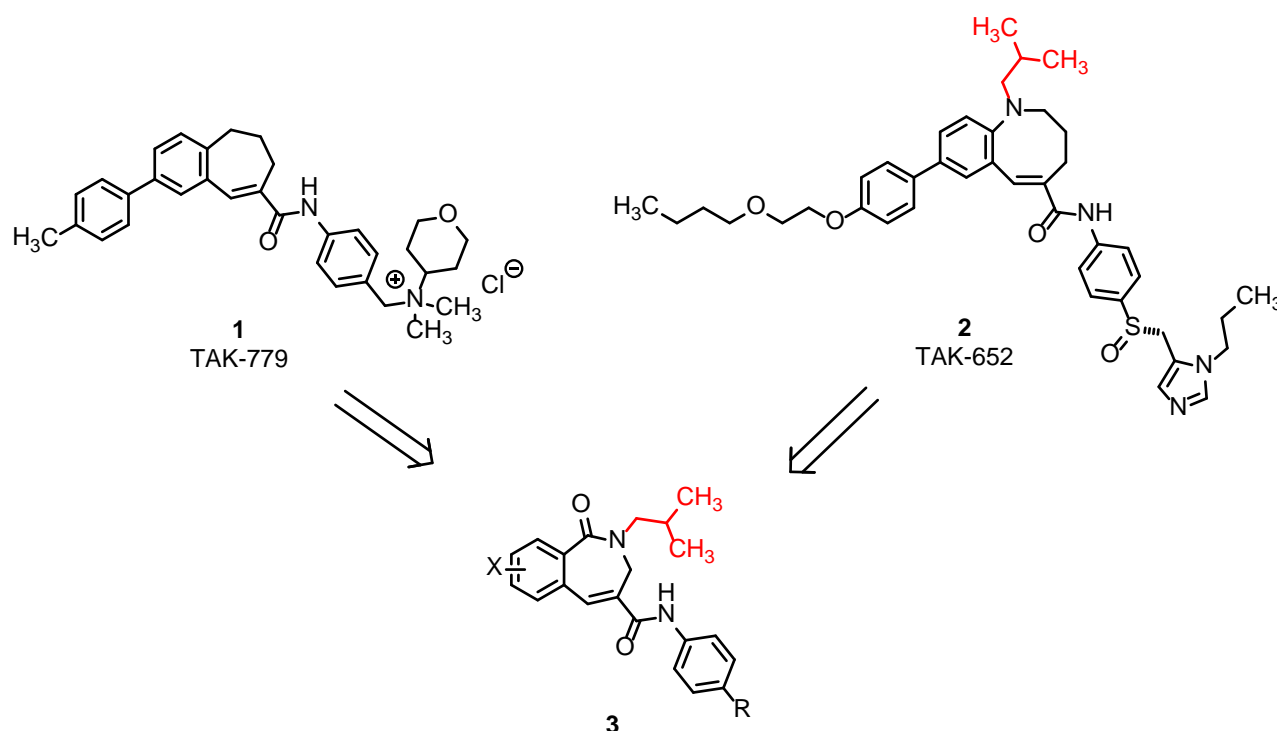


Figure 1: Design of 2-benzazepin-1-ones **3** derived from TAK-779 (**1**) and TAK-652 (**2**).

In addition to TAK-779, the benzazocine TAK-652 (**2**) served as lead compound in this project (Figure 1). TAK-652 shows high and similar affinities towards CCR5 ($IC_{50} = 3.1$ nM) and CCR2 receptors ($IC_{50} = 5.9$ nM), but does not contain a quaternary ammonium group, which had been replaced by a polar sulfoxide.¹² Structure affinity relationship (SAR) studies performed by Takeda laboratories have shown that reduction of the ring from a benzazocine to a benzazepine did not result in considerable loss of CCR5 affinity. The introduction of an isobutyl side chain onto the benzazocine ring increased CCR5 binding affinity.¹²

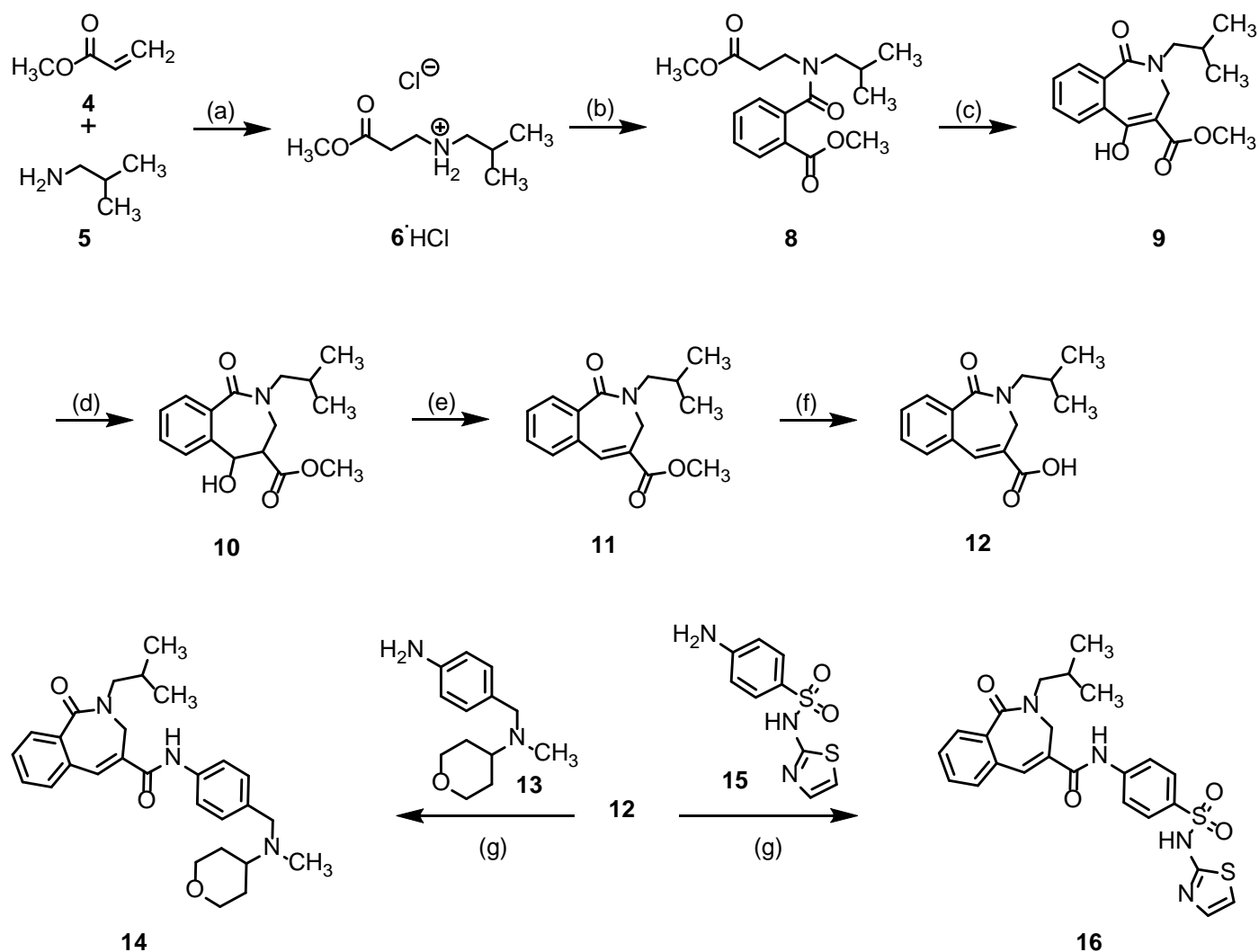
Thus, we envisaged to combine the structures of TAK-779 and TAK-652 in 2-benzazepinones **3**. The dihydro-2-benzazepin-1-one system of **3** contains a benzannulated seven-membered ring as TAK-779 and an N-heterocycle bearing an isobutyl moiety as TAK-652. The basicity of the amino group in TAK-652 (**2**) is rather low, due to its position at the phenyl ring and due to its conjugation with the amide group at 5-position (phenylogous / vinylogous urea). In the dihydro-2-benzazepin-1-one system **3** the basicity of the N-heterocycle is also negligible (lactam). The position of the lipophilic isobutyl moiety (red) is shifted from 1-position in TAK-652 (**2**) to 2-position in **3**. The selection of substituents X and R was inspired by the substituents of the lead compounds **1** and **2** and our previous SAR studies.⁹⁻¹¹ The interaction of the final compounds with CCR2 and CCR5 receptors was evaluated in various biochemical assays.

2. Results and discussion

2.1. Synthesis

The synthesis of the central building block **11** started with a *Michael* addition of isobutylamine (**5**) at methyl acrylate (**4**) yielding the aminopropanoate **6**, which was purified as HCl salt (Scheme 1). Amine **6** was acylated with commercially available 2-(methoxycarbonyl)benzoic acid (monomethyl phthalate,

7), which was first converted into its acid chloride using SOCl_2 . Treatment of the resulting diester **8** with NaH in boiling THF induced the *Dieckmann* cyclization to produce the cyclic β -ketoester **9**. According to the NMR spectra in CDCl_3 solution, **9** exists almost exclusively as enol ester as shown in Scheme 1.



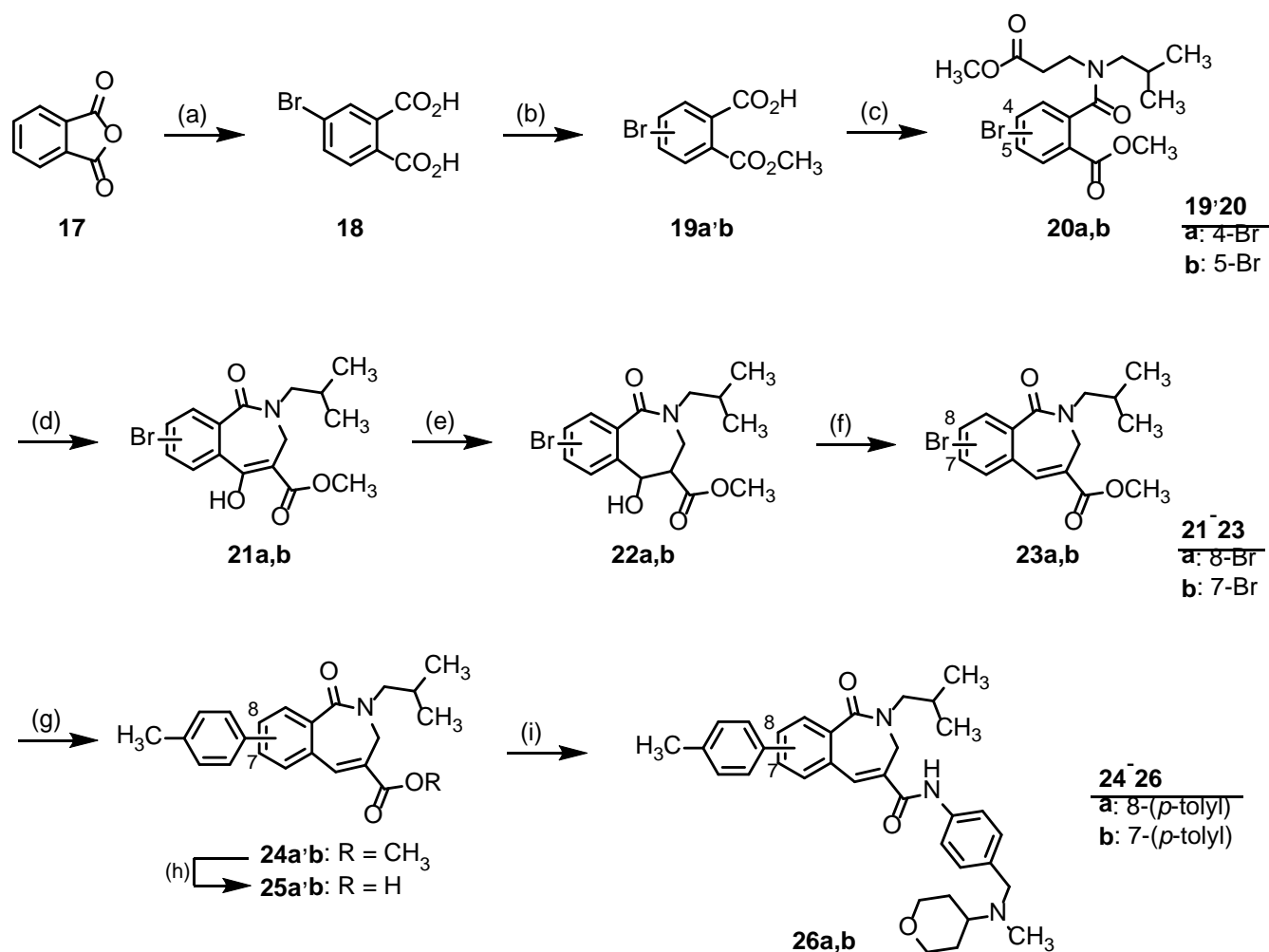
Scheme 1: Synthesis of compounds **14** and **16**. Reagents and reaction conditions: (a) 1. NaOCH_3 , H_3COH , rt, 2 h; 2. $\text{HCl}/\text{Et}_2\text{O}$, 89%. (b) Monomethyl phthalate (**7**), SOCl_2 , pyridine; then addition of **6**· HCl , pyridine, CH_2Cl_2 , rt, 4 h, 64%. (c) NaH , THF, reflux, 3 h, 58%. (d) NaBH_4 , H_3COH , 0°C , 1 h, 58%. (e) $\text{H}_3\text{CSO}_2\text{Cl}$, NEt_3 , DBU, CH_2Cl_2 , rt, 12 h, 76%. (f) 5 M NaOH , H_3COH , reflux, 40 min, 100%. (g) NEt_3 (2 equiv.), HATU (1.1 equiv.), THF, rt, 12 h, **14**: 48%, **16**: 8%.

Reduction of the enol ester **9** with NaBH₄ in H₃COH afforded two diastereomeric β-hydroxy esters **10**. Since in the next step both diastereomers of **10** form the same α,β-unsaturated ester **11**, the diastereomeric β-hydroxy esters **10** were not separated. Elimination of H₂O was performed upon treatment of β-hydroxy esters **10** with methanesulfonyl chloride in the presence of NEt₃. Subsequent addition of DBU induced the β-elimination of the intermediate methanesulfonate to give the α,β-unsaturated ester **11** in 76% yield. Saponification of methyl ester **11** with NaOH provided the acid **12** in almost quantitative yield.

The acid **12** was used to prepare secondary amides **14** and **16**, which have similar structures as the lead compounds **1** and **2**. For the amide coupling the uronium salt *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU) was used. Whereas coupling of acid **12** with the primary amine **13** provided the secondary amide **14** in 48% yield, the corresponding coupling with sulfathiazole (**15**) gave only 8% of secondary amide **16**.

In the BRET-based cAMP assay, TAK-779-derived secondary amide **14** showed a CCL4-dependent positive allosteric modulation (PAM) of CCR5 receptor (see part 3, Biological activity), whereas the sulfonamide derivative **16** was inactive, indicating the requirement of a basic benzylamine moiety for CCR5 receptor binding. This promising result stimulated further exploration of the substitution pattern of compound **14**. At first, a bromine atom should be introduced into the benzene moiety of the 2-benzazepine ring of the key compound **11**, since bromoarenes could be used for the introduction of a broad variety of diverse substituents by Pd-catalyzed cross-coupling reactions. Unfortunately, all attempts to brominate **11** using Br₂ or NBS under different reaction conditions led to loss of the double bond of the α,β unsaturated ester indicating higher reactivity of the double bond compared to the benzene ring.

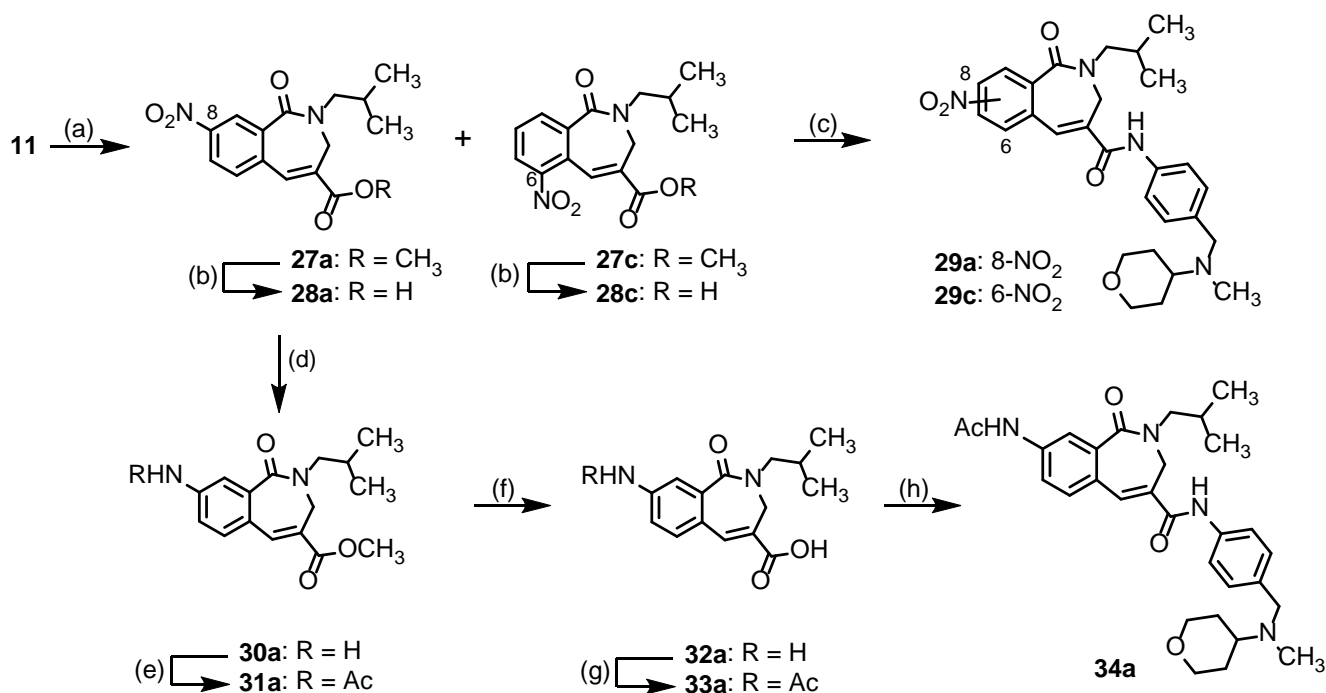
Therefore, it was planned to change the synthetic strategy and introduce the Br-atom at a very early stage of the synthesis. For this purpose, phthalic anhydride (**17**) was treated with an aqueous solution of NaOH and Br₂ which provided 4-bromophthalic acid (**18**)¹³ in 95% yield (Scheme 2). Treatment of diacid **18** with methanol in the presence of TMSCl provided a 1:1 mixture of regioisomeric monomethyl esters **19a** and **19b** in 94% yield. The further synthetic route to obtain esters **23** is very similar to the synthesis of ester **11**. Activation of the mono acids **19a,b** with SOCl₂ and subsequent reaction of the acid chlorides with aminopropanoate **6** led to the amides **20**, which underwent *Dieckmann* condensation to afford the enol esters **21a,b**. NaBH₄ reduction of **21a,b** provided the β-hydroxyesters **22a,b**, which reacted with mesyl chloride and DBU to yield the α,β-unsaturated esters **23a,b**. In the next step the *p*-tolyl moiety of the lead compound TAK-779 (**1**) should be introduced. *Suzuki-Miyaura* cross-coupling of the regioisomeric bromo derivatives **23a,b** with 4-methylbenzeneboronic acid and PdCl₂(dppf) as catalyst provided the regioisomeric *p*-tolyl derivatives **24a,b**. Saponification of the esters **24a,b** with NaOH led to acids **25a,b**, which were coupled with amine **13** to afford the final amides **26a,b** (Scheme 2).



Scheme 2: Synthesis of 7- and 8-(*p*-tolyl)-2-benzazepine-4-carboxamides **26a,b**. Reagents and reaction conditions: (a) Br₂, NaOH, H₂O, reflux, 72 h, 95%. (b) TMSCl, H₃COH, rt, 12 h, 94%. (c) 1. SOCl₂, pyridine; 2. **6**·HCl, pyridine, CH₂Cl₂, rt, 4 h, 64%. (d) NaH, THF, reflux, 3 h, **21a**: 36%, **21b**: 38%. (e) NaBH₄, H₃COH, 0 °C, 1 h, **22a**: 58%, **22b**: 45%. (f) H₃CSO₂Cl, NEt₃, DBU, CH₂Cl₂, rt, 12 h, **23a**: 74%, **23b**: 84%. (g) 4-Methylbenzeneboronic acid, PdCl₂(dppf) (5 mol%), KOAc, DME, reflux, 12 h, **24a**: 50%, **24b**: 58%. (h) 5 M NaOH, H₃COH, reflux, 30 min, 97%. (i) **13**, HATU, NEt₃, THF, rt, 12 h, **26a**: 39%, **26b**: 34%.

The separation of the regioisomers **a** and **b** was performed at various stages at the synthesis. Separation at the very early stage of enol esters **21** and performing the synthesis with pure regioisomers **21a** and **21b** gave clear spectra of all intermediates. An X-ray crystal structure of the 7-bromo derivative **21b** proved the existence of the β-ketoester in the enol tautomer and the position of the Br-atom.¹⁴

Separation of the intermediate α,β -unsaturated esters **23a** and **23b** gave higher yields than the early separation of **21a** and **21b**. However, separation of the final *p*-tolyl derivatives **26a** and **26b** turned out to be the most efficient strategy, since the corresponding transformations and purifications had to be performed only once for the mixture of regioisomers, respectively. However, preparative HPLC had to be used for the separation of **26a** and **26b** and the NMR spectra of all intermediates showed two sets of signals.

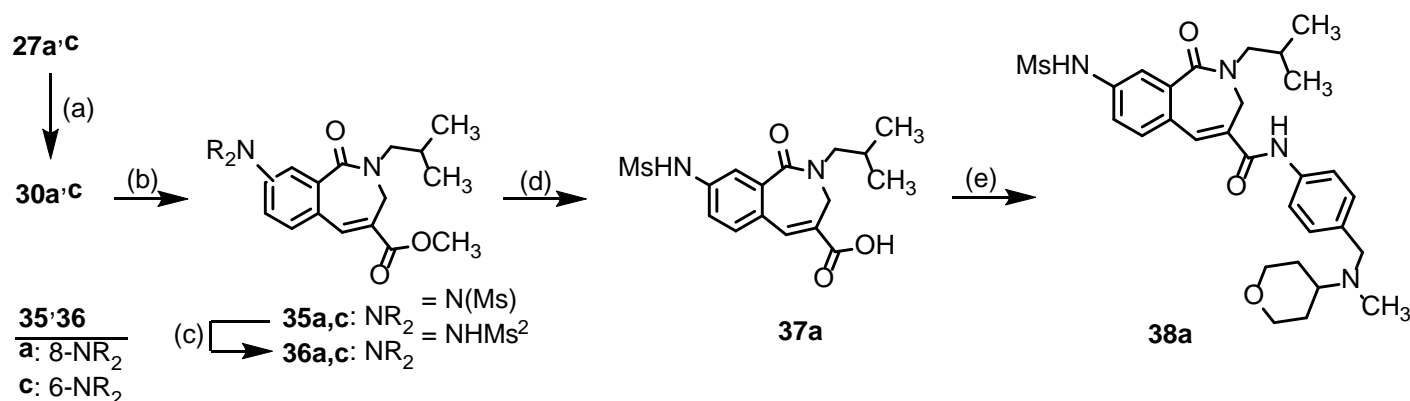


Scheme 3: Synthesis of NO₂ derivatives **29** and acetamide **34a**. Reagents and reaction conditions: (a) HNO₃ (100%), H₂SO₄ (95 – 97%), CH₃NO₂, rt, 2 h, 83%. (b) 5M NaOH, H₃COH, reflux, 97%. (c) **13**, HATU, NEt₃, THF, rt, 12 h, **29a**: 9%, **29c**: 14%. (d) Fe, conc. HCl, EtOH, reflux, 2 h, 75%. (e) Ac₂O, NEt₃, CH₂Cl₂, 6 h, rt, 59%. (f) NaOH, H₃COH, 10 min, rt. (g) Ac₂O, NEt₃, CH₂Cl₂, 6 h, rt, 69%. (h) **13**, HATU, NEt₃, THF, rt, 12 h, 47%.

In addition to the *p*-tolyl moiety, the introduction of electron withdrawing NO₂ group was envisaged, which could be converted into various other functional groups, subsequently. For this purpose, the naked α,β -unsaturated ester **11** was reacted with HNO₃/H₂SO₄ to obtain a mixture of regioisomeric 8-NO₂- and

6-NO₂-2-benzazepines **27a** and **27c** in the ratio 7:3 in 97% yield (Scheme 3). After chromatographic separation of the regioisomeric esters **27a** and **27c**, saponification with NaOH led to the acids **28a** and **28c**, which were coupled with the amine **13** to afford the final amides **29a** and **29c**.

The main regioisomer **27a** was reduced with Fe in the presence of HCl to provide the primary amine **30a**, which was acylated with Ac₂O to give the acetamide **31a** (Scheme 3). In the next step, the ester **31a** was treated with NaOH. Unexpectedly, not only the ester moiety but also the acetamide group was hydrolyzed to produce the amino acid **32a**. Therefore, the amino moiety was acetylated once more with Ac₂O, before the final HATU-coupling of the acid **33a** with amine **13** was performed to produce the desired acetamide **34a**.



Scheme 4: Synthesis of sulfonamide **38a**: Reagents and reaction conditions: (a) Fe, conc. HCl, EtOH, reflux, 2 h, 74%. (b) CH₃SO₂Cl (1 equiv.), NEt₃, CH₂Cl₂, 3 d, then 4 x 0.3 equiv. CH₃SO₂Cl and NEt₃ every 6 h, rt, 91%. (c) NaOH, H₃COH, 20 min, rt, 43%. (d) LiOH (4 equiv.), H₃COH, 3 h rt, 1 h 50 °C, 53%. (e) **13**, HATU, NEt₃, THF, rt, 12 h, 20%.

Reduction of the regioisomers NO₂-derivatives **27a,c** with Fe and conc. HCl provided a mixture of regioisomeric primary amines **30a,c**, which was treated with methanesulfonyl chloride in the presence of triethylamine. Although only one equivalent of methanesulfonyl chloride was added, considerable

amounts of disulfonamides **35a,c** were formed. Therefore, an excess of methanesulfonyl chloride was used to obtain the disulfonamides **35a,c** in 91% yield. Subsequent hydrolysis of the disulfonamides **35a,c** with NaOH in methanol produced the monosulfonamides **36a,c**. Finally, the ester moiety of **36a,c** was hydrolyzed with LiOH. Chromatographic purification of the resulting acids provided the main regioisomer **37a** in 53% yield, which was coupled with the amine **13** to afford the amide **38a** in 20% yield (Scheme 4).

2.2. Biological activity

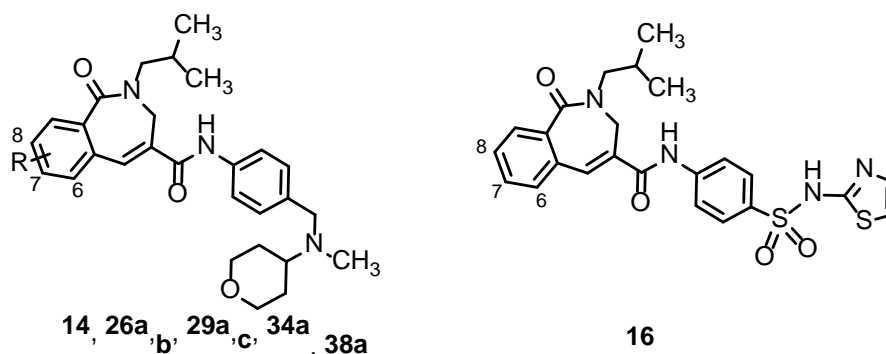
The interaction of sulfathiazole coupled amide **16** as well as 2-benzazepine-4-carboxamides **14**, **26**, **29**, **34**, and **38** bearing different substituents in 8- (**a-series**), 7- (**b-series**) and 6-position (**c-series**) with CCR2 and CCR5 receptors was tested in various CCR2 and CCR5 assays.

In the first assay, the test compounds competed with the radioligand [³H]INCB3344 for CCR2 receptor binding on membrane preparations from U2OS cells stably expressing CCR2. With exception of the 7-*p*-tolyl derivative **26b**, the test compounds did not reduce the specific binding of the radioligand [³H]INCB3344 to a large extent at a concentration of 1 μM indicating rather low CCR2 affinity (Table 1). Only the 7-*p*-tolyl derivative **26b** displayed a moderate CCR2 affinity with an IC₅₀ value of 387 nM.

In addition to binding, the antagonistic activity of the test compounds at the CCR2 receptor was determined in a Ca²⁺ flux assay employing the Chem-1 cell line stably transfected with the human CCR2b receptor. Influx of Ca²⁺ ions was induced by recombinant human CCL2 (MCP-1). The inhibition of this Ca²⁺ influx by the test compounds was recorded. Table 1 shows that only the *p*-tolyl derivatives **26** were able to inhibit the Ca²⁺ influx. Whereas the 8-*p*-tolyl derivative **26a** revealed only very low inhibition, a significant inhibition was observed for the regioisomer **26b**. This result is explained by the

structural relationship of **26b** and the lead compound TAK-779. However, the IC₅₀ value of 140 nM is considerably higher than the IC₅₀ value of TAK-779. The inhibition of the Ca²⁺ flux induced by the 7-*p*-tolyl derivative **26b** correlates nicely with its moderate CCR2 binding affinity.

Table 1: Receptor affinities and activities at CCR2 and CCR5 receptors.



Compd.	R	CCR2		CCR5	
		[³ H]INCB3344 displacement IC ₅₀ (nM) ^[a]	Ca ²⁺ flux IC ₅₀ (nM) ^[b]	[³ H]TAK-779 displacement IC ₅₀ [nM] ^[c]	cAMP-BRET (CCL4) EC ₅₀ (nM) ^[d]
14	H	18%	n.d.	0%	2470
16	H	18%	n.d.	0%	> 10
26a	8- <i>p</i> -tolyl	0%	3000	0%	> 10
26b	7- <i>p</i> -tolyl	387	140	0%	> 10
29a	8-NO ₂	9%	n.d.	0%	> 10
29c	6-NO ₂	6%	n.d.	0%	> 10
34a	8-NHAc	15%	n.d.	0%	> 10
38a	8-NHSO ₂ CH ₃	18%	n.d.	0%	> 10
TAK-779	-	50	0.95	2	7

[%] inhibition at a test compound concentration of 1 μM (n = 3). n.d. not determined.

^[a] Displacement of the radioligand [³H]INCB3344 at a concentration of 1 μM of the test compound (n = 3).

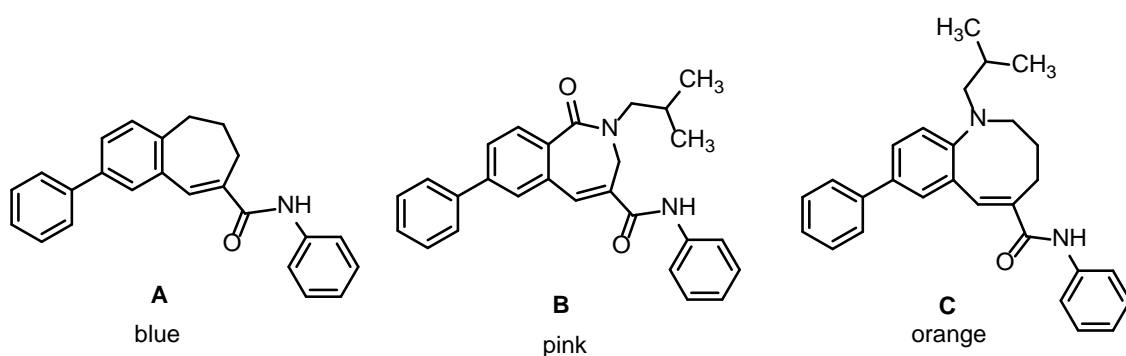
^[b] Inhibition of Ca²⁺ mobilization after activation by 5 nM MCP-1 (n = 3).

^[c] Displacement of the radioligand [³H]TAK-779 at a concentration of 1 μM of the test

compound (n = 3).

[d] Stimulation of cAMP production after inhibition with 0.2 nM CCL4 (n = 3).

For investigation of the interaction of the test compounds with CCR5 receptors, a binding assay was performed at first. In this assay the radioligand [³H]TAK-779 was used as competitor and commercially available membrane preparations containing high amounts of CCR5 receptors were employed as source of receptors. At a concentration of 1 μM the test compounds could not displace the specific radioligand binding (Table 1). In particular, the low CCR5 interaction of the *p*-tolyl derivative **26b** was unexpected, as this compound is structurally very similar to the lead compound TAK-779. To address the surprising lack of CCR5 affinity of compound **26b**, the central core structures of **26b** (B) and TAK-779 (A) were compared via flexible alignment. In Figure 2, part I the superposition of the core structures demonstrates different angles within the 7-membered rings of TAK-779 (**1**) and 2-benzazepinone **26b**. The different arrangement of the 7-membered ring of **26b** compared to TAK-779 might explain the reduced or eliminated CCR5 affinity of **26b**. Obviously, the CCR5 receptor does not tolerate the introduction of the N-isobutyl substituted amide moiety within the seven-membered part of the ring system. On the other hand, an alignment of the core structures of **26b** (B) and TAK-652 (C) (Figure 2, part II) reveals a very similar positioning of the isobutyl chain that might explain the moderate CCR2 binding affinity and activity.



I

II

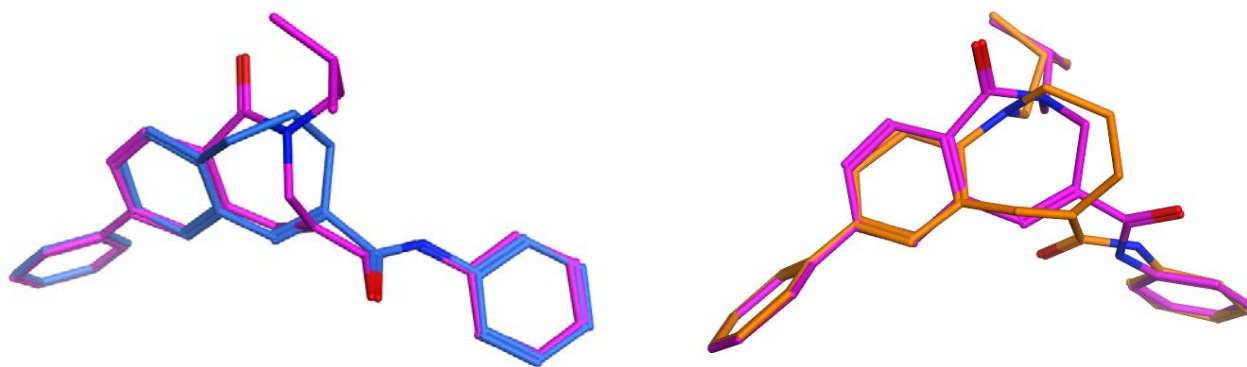


Figure 2: I) Alignment of central core structures of TAK-779 (A, blue) with 7-*p*-tolyl derivative **26b** (B, pink). II) Alignment of central core structures of TAK-652 (C, orange) with 7-*p*-tolyl derivative **26b** (B, pink).

To assess the effect of compounds on the CCL4 or CCL5-induced G_i protein-dependent signaling of CCR5, we monitored the changes in cAMP levels by use of the bioluminescence resonance energy transfer- (BRET-) based cAMP sensor CAMYEL. This biosensor is comprised of a catalytically inactive Epac1 that is fused to Citrine at its N-terminus and to *Renilla reniformis* luciferase (Rluc) at the C-terminus.¹⁵ For these experiments, HEK293T cells were transiently transfected with the human CCR5 receptor and the CAMYEL biosensor. The accumulation of cAMP was induced by forskolin. TAK-779 (**1**) was used as reference inhibitor of CCL4 and CCL5 action.

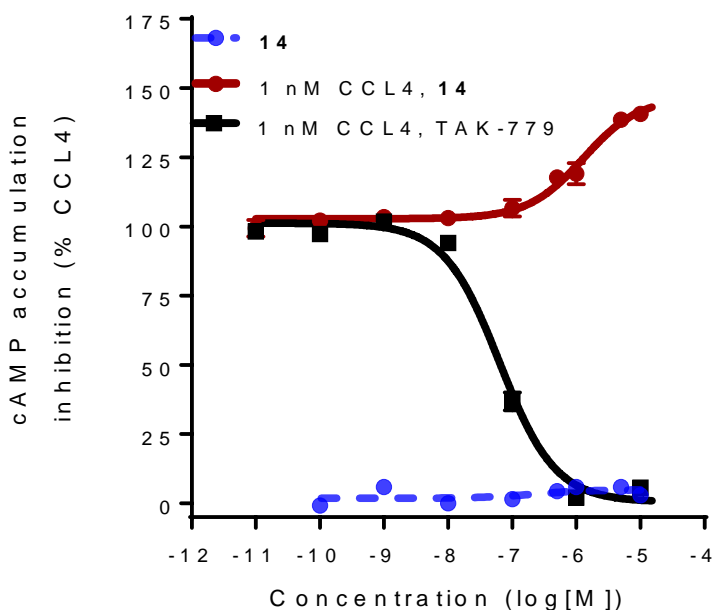


Figure 3: The BRET-based cAMP assay for compounds **14** and TAK-779 (**1**) with CCL4 at the CCR5 receptor.

In the cAMP assay only the unsubstituted 2-benzazepinone **14** displayed remarkable biological effects. This compound showed clear probe-dependence at the CCR5 receptor. Although that compound **14** itself had no intrinsic agonist activity (Figure 3), it behaved as positive allosteric modulator (PAM) when CCL4 was used to activate the CCR5 receptor ($IC_{50} = 2.47 \mu\text{M}$, Table 1, Figure 3). At the same time, the compound **14** was fully inactive in the presence of CCL5. This CCL4-dependence indicates a slightly different CCR5 receptor binding pocket for compound **26b** compared to TAK-779, since TAK-779 displays a negative allosteric modulation with both chemokines CCL4 and CCL5. Of note, probe-dependent negative allosteric modulators (NAM) of CXCR3 receptors were recently reported.^{16,17} Probe-dependent allosteric modulation provides a promising strategy for fine tuning of the chemokine response and corresponding ligands are therefore of great interest for drug development in the chemokine receptor field. If we consider that **14** binds to a different subpocket than TAK-779, the lack of CCR5 affinity of **14** in a radioligand binding assay is not surprising, as it would not compete with

[³H]TAK-779. In the benzene ring of the sulfathiazole-derived amide **16** further substituents are also missing. However, **16** does not induce similar effects as **14**, which is probably due to the lacking basic amino moiety.

3. Conclusion

The introduction of the *p*-tolyl group at 7-position of the 2-benzazepine scaffold seems to be crucial for CCR2 receptor interactions. In the CCR2 binding assay and the Ca²⁺ flux assay, the 7-*p*-tolyl derivative **26b** displayed moderate affinity (IC₅₀ = 387 nM) and activity (IC₅₀ = 140 nM). Obviously the CCR2 receptor is able to accommodate at the different structure of the 2-benzazepinone **26b** compared to the structures of the lead compounds TAK-779 and TAK-652. Despite the different conformations of the scaffolds, the isobutyl moieties of TAK-652 and **26b** adopt similar orientations, which might explain the moderate interactions of **26b** with the CCR2 receptor. The corresponding 8-*p*-tolyl regioisomer **26a** was approx. 20-fold less potent in both assays, other substituents at the 2-benzazepine framework were not tolerated by the CCR2 receptor.

The synthesized 2-benzazepinones did not show any affinity towards the CCR5 receptor in the [³H]TAK-779 competition assay. Despite the absence of TAK-779 displacement at CCR5 receptors, the naked 2-benzazepinone **14** led to an increased production of cAMP after stimulation of the CCR5 receptor with CCL4. This probe-dependent positive allosteric modulation of the CCR5 receptor, which was not observed after stimulation with CCL5, was unexpected.

4. Experimental Part

4.1. General

Unless otherwise noted, moisture sensitive reactions were conducted under dry nitrogen. THF was dried with sodium/benzophenone, CH₂Cl₂ with calcium hydride and both were freshly distilled before use. Thin layer chromatography (tlc): Silica gel 60 F₂₅₄ plates (Merck). Flash chromatography (fc): Silica gel 60, 40–64 μm (Merck); parentheses include: diameter of the column, length of column, fraction size, eluent, R_f value. Melting point: melting point system MP50 (Mettler Toledo), uncorrected. IR: IR spectrophotometer 480Plus FT-ATR-IR (Jasco). ¹H NMR (400 MHz), ¹³C NMR (100 MHz): Mercury plus 400 spectrometer (Varian); δ in ppm related to tetramethylsilane; coupling constants are given with 0.5 Hz resolution. MS: APCI = atmospheric pressure chemical ionization: MicroTOFQII (Bruker Daltonics), ESI = electro spray ionization: MicroTof (Bruker Daltonics, Bremen), calibration with sodium formate clusters before measurement. Deviations of the found exact masses from the calculated exact masses were 5 mDa or less, unless otherwise stated. The data were analyzed with DataAnalysis (Bruker).

4.2. HPLC methods

4.2.1. Method 1: Purity of compounds

Merck Hitachi equipment; UV detector: L-7400; autosampler: L-7200; pump: L-7100; degasser: L-7614; Method A: column: LiChrospher® 60 RP-select B (5 μm), 250-4 mm cartridge; flow rate: 1.00 mL/min; injection volume: 5.0 μL; detection λ = 210 nm; solvents: A: water with 0.05% (v/v) trifluoroacetic acid; B: acetonitrile with 0.05% (v/v) trifluoroacetic acid: gradient elution: (A %): 0-4 min: 90% , 4-29 min: gradient from 90% to 0%, 29-31 min: 0%, 31-31.5 min: gradient from 0% to 90%, 31.5-40 min: 90%.

4.2.2. Method 2: Preparative HPLC

Merck Hitachi equipment; UV detector: L-7400; autosampler: L-7200; pump: L-7100; interface: D-7000; data acquisition: HSM Software (LaChrom, Merck-Hitachi); solvent: acetonitrile : H₂O 70:30; column: Phenomenex[®] Gemini 5 μ m C18 110A, 250 – 21.2 mm; flow rate: 10.00 mL/min; injection volume 500.0 μ L; detection: wavelength: 254 nm, stop time: 60.0 min.

4.3. Synthetic procedures

4.3.1. Methyl 3-(isobutylamino)propanoate (6•HCl)

Methyl 4-acrylate (9.81 g, 114 mmol, 15.0 mL) was added slowly to a vigorously stirred mixture of isobutylamine (10 g, 137 mmol, 13.6 mL) and sodium methanolate (123 mg, 2.28 mmol) in methanol (70 mL). The mixture was stirred for 2 h at rt, concentrated *in vacuo* and the residue was purified by fc ($\emptyset = 4$ cm, h = 16 cm, cyclohexane / ethyl acetate = 1 : 2 + 1% triethylamine, $R_f = 0.71$) to give a colorless oil. The oil was dissolved in Et₂O and 2 M HCl in Et₂O was added to the solution. A colorless precipitate was formed, which was separated by filtration with Et₂O to give **6** as a HCl-salt. Colorless solid, mp 187 °C, yield 23.8 g (89%). C₈H₁₈ClNO₂, $M_r = 195.7$. Exact MS (APCI): $m/z = 160.1380$ (calcd. 160.1332 for C₈H₁₈NO₂ [MH⁺]). ¹H NMR (DMSO-*d*₆): δ [ppm] = 0.94 (d, $J = 6.7$ Hz, 6H, NCH₂CH(CH₃)₂), 1.90 - 2.05 (m, 1H, NCH₂CH(CH₃)₂), 2.75 (d, $J = 7.1$ Hz, 2H, NCH₂CH(CH₃)₂), 2.83 (t, $J = 7.5$ Hz, 2H, CH₂CH₂CO₂CH₃), 3.12 (t, $J = 7.4$ Hz, 2H, CH₂CH₂CO₂CH₃), 3.64 (s, 3H, CO₂CH₃), 8.88 (s, 2H, NH₂). ¹³C NMR (DMSO-*d*₆): δ [ppm] = 19.9 (2C, NCH₂CH(CH₃)₂), 25.2 (1C, NCH₂CH(CH₃)₂), 29.8 (1C, CH₂CH₂CO₂CH₃), 42.6 (1C, CH₂CH₂CO₂CH₃), 51.7 (1C, CO₂CH₃), 53.7 (1C, NCH₂CH(CH₃)₂), 170.4 (1C, CO₂CH₃). IR (neat): ν [cm⁻¹] = 1736 (C=O), 1593 (C-N).

4.3.2. Methyl 2-{N-isobutyl-N-[2-(methoxycarbonyl)ethyl]-carbamoyl}-benzoate (8)

Monomethyl phthalate (**7**, 6.24 g, 34.6 mmol) was added to a vigorously stirred solution of SOCl₂ (6.18

g, 52 mmol, 3.77 mL) in CH₂Cl₂ (20 mL) and the mixture was heated to reflux for 1.5 h. The reflux condenser was replaced by a Liebig micro distillation apparatus to remove CH₂Cl₂ and SOCl₂. The residue was dissolved in CH₂Cl₂ (20 mL). Then pyridine (8.21 g, 104 mmol, 8.4 mL, 3 equiv.) and **6**•HCl (8.13 g, 41.5 mmol, 1.2 equiv.) were added under ice cooling. After 4 h, the mixture was concentrated in vacuo, the residue was suspended in sat. NaHCO₃ and the mixture was extracted with CH₂Cl₂ (3 x 50 mL). The combined organic layers were dried (Na₂SO₄), concentrated in vacuo and the residue was purified by fc (Ø = 8 cm, h = 16 cm, cyclohexane / ethyl acetate = 2 : 1, R_f = 0.45) to give **8** as a colorless solid, mp 62 °C, yield 7.12 g (64%). C₁₇H₂₃NO₅, M_r = 321.4. Exact MS (APCI): *m/z* = 322.1659 (calcd. 322.1649 for C₁₇H₂₄NO₅ [MH⁺]). ¹H NMR (CDCl₃): δ [ppm] = 0.76 (d, *J* = 6.7 Hz, 6H, NCH₂CH(CH₃)₂), 1.81 – 1.92 (m, 1H, NCH₂CH(CH₃)₂), 2.79 – 2.91 (m, 4H, CH₂CH₂CO₂CH₃), 3.72 (s, 3H, CH₂CH₂CO₂CH₃), 3.73 – 3.86 (m, 2H, NCH₂CH(CH₃)₂), 3.86 (s, 3H, ArCO₂CH₃), 7.28 (dd, *J* = 7.9/1.0 Hz, 1H, Ar-*H*), 7.40 – 7.45 (m, 1H, Ar-*H*), 7.53 – 7.56 (m, 1H, Ar-*H*), 7.97 – 8.05 (m, 1H, Ar-*H*). ¹³C NMR (CDCl₃): δ [ppm] = 20.1 (2C, NCH₂CH(CH₃)₂), 27.1 (1C, NCH₂CH(CH₃)₂), 31.4 (1C, CH₂CH₂CO₂CH₃), 41.2 (1C, NCH₂CH(CH₃)₂), 51.7 (1C, CH₂CH₂CO₂CH₃), 52.3 (1C, ArCO₂CH₃), 56.2 (1C, CH₂CH₂CO₂CH₃), 127.7 (1C, Ar-*C*), 128.5 (1C, Ar-*C*), 128.7 (1C, Ar-*C*), 130.4 (1C, Ar-*C*), 132.6 (1C, Ar-*C*), 132.7 (1C, Ar-*C*), 165.8 (1C, ArCO₂CH₃), 171.4 (1C, CONR₂), 172.8 (1C, CO₂CH₃). IR (neat): ν [cm⁻¹] = 1725 (C=O), 1625 (NC=O).

4.3.3. Methyl 2-isobutyl-5-hydroxy-1-oxo-2,3-dihydro-1*H*-2-benzazepine-4-carboxylate (**9**)

Under ice cooling a dispersion of NaH (60% w/w in mineral oil 540 mg, 13.5 mmol) was added slowly to a solution of the amide **8** (1.41 g, 4.5 mmol) in THF (20 mL) and left to stir for 0.5 h, then mixture was heated to reflux for 1.5 h. After cooling to rt the mixture was concentrated *in vacuo* and the residue was suspended in 1 M HCl. The aqueous layer was extracted with CH₂Cl₂ (3 x 50 mL). The combined organic layers were dried (Na₂SO₄), concentrated in vacuo and the residue was purified by automatic fc

(Biotage[®], Cartridge SNAP 100 g, cyclohexane / ethyl acetate, $R_f = 0.35$ (cyclohexane / ethyl acetate = 2 : 1)) to give the 2-benzazepine **9** as a pale yellow solid, mp 84 °C, yield 750 mg (58%). $C_{16}H_{19}NO_4$, $M_r = 289.3$. Exact MS (APCI): $m/z = 290.1387$ (calcd. 290.1392 for $C_{16}H_{20}NO_4$ [MH^+]). 1H NMR ($CDCl_3$): δ [ppm] = 0.95 (d, $J = 6.7$ Hz, 6H, $NCH_2CH(CH_3)_2$), 2.00 – 2.13 (m, 1H, $NCH_2CH(CH_3)_2$), 3.23 - 3.38 (m, 1H, $NCH_2CH(CH_3)_2$), 3.45 - 3.61 (m, 1H, $NCH_2CH(CH_3)_2$), 3.68 - 3.82 (m, 1H, 3- CH_2), 3.90 (s, 3H, CO_2CH_3), 4.01 - 4.17 (m, 1H, 3- CH_2), 7.53 - 7.61 (m, 2H, Ar- H), 7.84 - 7.90 (m, 1H, Ar- H), 7.98 - 8.03 (m, 1H, Ar- H), 12.56 (s, 1H, OH). ^{13}C NMR ($CDCl_3$): δ [ppm] = 20.3 (2C, $NCH_2CH(CH_3)_2$), 27.7 (1C, $NCH_2CH(CH_3)_2$), 43.6 (1C, C-3), 52.4 (1C, CO_2CH_3), 55.8 (1C, $NCH_2CH(CH_3)_2$), 102.3 (1C, C-4), 127 (1C, Ar-C), 130.6 (1C, Ar-C), 130.9 (1C, Ar-C), 131.2 (1C, Ar-C), 131.3 (1C, Ar-C), 136.3 (1C, Ar-C), 167.8 (1C, $CONR_2$), 170.4 (1C, CO_2CH_3), 171.2 (1C, C-5). IR (neat): ν [cm^{-1}] = 1728 (C=O), 1632 (C=O), 1611 (NC=O).

4.3.4. Methyl 2-isobutyl-5-hydroxy-1-oxo-2,3,4,5-tetrahydro-1H-2-benzazepine-4-carboxylate (**10**)

$NaBH_4$ (523 mg, 13.8 mmol) was added slowly to a solution of the enol ester **9** (2.0 g, 6.91 mmol) in abs. H_3COH (50 mL) under ice cooling. After 1 h, 1 M HCl (50 mL) was added and the mixture was extracted with CH_2Cl_2 (3 x 50 mL). The combined organic layers were dried (Na_2SO_4), concentrated *in vacuo* and the residue was purified by fc ($\emptyset = 3$ cm, $h = 16$ cm, cyclohexane / ethyl acetate = 4 : 1, $R_f = 0.18$) to give **10** as a colorless solid, mp 154 °C, yield 1.16 g (58%). $C_{16}H_{21}NO_4$, $M_r = 291.4$. Exact MS (APCI): $m/z = 292.1590$ (calcd. 292.1543 for $C_{16}H_{22}NO_4$ [MH^+]). 1H NMR ($CDCl_3$): δ [ppm] = 0.92 (d, $J = 6.7$ Hz, 6H, $NCH_2CH(CH_3)_2$), 1.98 – 2.06 (m, 1H, $NCH_2CH(CH_3)_2$), 3.19 – 3.36 (m, 5H, $NCH_2CH(CH_3)_2$, 3- CH_2 , 4- CH), 3.68 (s, 3H, CO_2CH_3), 4.60 (d, $J = 6.7$ Hz, 1H, 5- CH), 7.30 – 7.40 (m, 1H, Ar- H), 7.41 – 7.48 (m, 2H, Ar- H), 7.63 (d, $J = 7.3$ Hz, 1H, Ar- H). A signal for OH proton is not seen in the spectrum. ^{13}C NMR ($CDCl_3$): δ [ppm] = 20.5 (2C, $NCH_2CH(CH_3)_2$), 27.8 (1C, $NCH_2CH(CH_3)_2$), 48.1 (1C, $NCH_2CHCO_2CH_3$), 51.6 (1C, C-3), 52.1 (1C, CO_2CH_3), 55.1 (1C, C-4),

70.2 (1C, C-5), 125.1 (1C, Ar-C), 128.2 (1C, Ar-C), 128.5 (1C, Ar-C), 130.9 (1C, Ar-C), 133.2 (1C, Ar-C), 138.1 (1C, Ar-C), 171.1 (1C, C-1), 171.5 (1C, CO₂CH₃). IR (neat): ν [cm⁻¹] = 3221 (-OH), 1725 (C=O), 1620 (NC=O).

4.3.5. Methyl 2-isobutyl-1-oxo-2,3-dihydro-1*H*-2-benzazepine-4-carboxylate (**11**)

Under ice cooling CH₃SO₂Cl (1117 mg, 9.75 mmol) was added to a solution of β -hydroxy ester **10** (943.5 mg, 3.25 mmol) and triethylamine (986 mg, 9.75 mmol) in CH₂Cl₂ (20 mL). The reaction mixture was stirred overnight at rt. Then DBU (2.47 g, 16.3 mmol) was added under ice cooling. The reaction mixture was stirred for 1 h at rt. Then 1 M HCl was added and the mixture was extracted with CH₂Cl₂ (3 x 50 mL). The combined organic layers were dried (Na₂SO₄), concentrated in vacuo and the residue was purified by fc (\emptyset = 3 cm, h = 16 cm, cyclohexane / ethyl acetate = 4 : 1, R_f = 0.6) to give **11** as a colorless solid, mp 69 °C, yield 679 mg (76%). C₁₆H₂₀NO₃, M_r = 273.3. Exact MS (APCI): m/z = 274.1500 (calcd. 274.1432 for C₁₆H₂₁NO₃ [MH⁺]). ¹H NMR (CDCl₃): δ [ppm] = 0.93 (d, J = 6.7 Hz, 6H, NCH₂CH(CH₃)₂), 2.01 – 2.13 (m, 1H, NCH₂CH(CH₃)₂), 3.44 (d, J = 7.6 Hz, 2H, NCH₂CH(CH₃)₂), 3.88 (s, 3H, CO₂CH₃), 3.97 (s, 2H, 3-CH₂), 7.34 (dd, J = 5.9/3.7 Hz, 1H, Ar-*H*), 7.50 (dd, J = 6.0/3.7 Hz, 2H, Ar-*H*), 7.82 (s, 1H, 5-*CH*), 8.09 (dd, J = 5.8/3.5 Hz, 1H, Ar-*H*). ¹³C NMR (CDCl₃): δ [ppm] = 20.2 (2C, NCH₂CH(CH₃)₂), 27.7 (1C, NCH₂CH(CH₃)₂), 44.4 (1C, C-3), 52.6 (1C, NCH₂CH(CH₃)₂), 56.3 (1C, CO₂CH₃), 129.7 (1C, Ar-C), 129.9 (1C, Ar-C), 130.5 (1C, Ar-C), 131.8 (1C, Ar-C), 132.9 (1C, Ar-C), 133.2 (1C, Ar-C), 136.8 (1C, C-4), 141.7 (1C, C-5), 166.1 (1C, CO₂CH₃), 168.1 (1C, C-1). IR (neat): ν [cm⁻¹] = 2924 (C-H_{aliph.}), 1725 (C=O), 1597 (C=C).

4.3.6. 2-Isobutyl-1-oxo-2,3-dihydro-1*H*-2-benzazepine-4-carboxylic acid (**12**)

The ester **11** (120 mg, 0.44 mmol) was dissolved in H₃COH (10 mL) and 5 M NaOH (10 mL) was added. The mixture was heated to reflux for 40 min. After cooling down to 0 °C, the mixture was

acidified with conc. HCl (2.5 mL) and extracted with ethyl acetate (3 x 50 mL). The combined organic layers were dried (Na₂SO₄) and concentrated in vacuo to give the acid **12**. Colorless solid, mp 192 °C, yield 114 mg (100%). C₁₅H₁₇NO₃, M_r = 259.3. R_f = 0.12 (cyclohexane / ethyl acetate = 2 : 1). Exact MS (APCI): *m/z* = 260.1285 (calcd. 260.1281, for C₁₅H₁₈NO₃ [MH⁺]). ¹H NMR (methanol-*d*₄): δ [ppm] = 0.94 (d, *J* = 6.7 Hz, 6H, NCH₂CH(CH₃)₂), 2.05 - 2.18 (m, 1H, NCH₂CH(CH₃)₂), 3.46 (d, *J* = 7.6 Hz, 2H, NCH₂CH(CH₃)₂), 4.01 (s, 2H, 3-CH₂), 7.50 (dd, *J* = 7./1.5 Hz, 1H, Ar-*H*), 7.53 - 7.63 (m, 2H, Ar-*H*), 7.91 (s, 1H, 5-*H*), 7.99 (dd, *J* = 7.7/1.6 Hz, 1H, Ar-*H*). A signal for COOH proton is not seen in the spectrum. ¹³C NMR (methanol-*d*₄): δ [ppm] = 20.4 (2C, N-CH₂-CH-(CH₃)₂), 28.8 (1C, N-CH₂-CH-(CH₃)₂), 45.4 (1C, C-3), 57.2 (1C, N-CH₂-CH-(CH₃)₂), 130.7 (1C, Ar-C), 131.1 (1C, Ar-C), 132.0 (1C, Ar-C), 132.1 (1C, Ar-C), 135.0 (1C, C-4), 135.30 (1C, Ar-C), 137.11 (1C, Ar-C), 142.00 (1C, C-5), 168.2 (1C, CO₂CH₃), 170.2 (1C, C-1). IR (neat): ν [cm⁻¹] = 2937 (C-H_{aliph.}), 1715 (C=O), 1600 (C=C). HPLC (method 1): t_R = 17.21 min, purity 98.8%.

4.3.7. 2-Isobutyl-*N*-({[*N*-methyl-*N*-(tetrahydro-2*H*-pyran-4-yl)amino]methyl}phenyl)-1-oxo-2,3-dihydro-1*H*-2-benzazepine-4-carboxamide (**14**)

Amine **13** (113 mg, 0.44 mmol) was added to a vigorously stirred mixture of acid **12** (113.8 mg, 0.44 mmol), NEt₃ (89.1 mg, 0.88 mmol) and HATU (182.5 mg, 0.48 mmol) in abs. THF (5 mL). The mixture was stirred overnight at rt. Then the mixture was concentrated in vacuo. Saturated NaHCO₃-solution (20 mL) was added to the residue and the mixture was extracted with CH₂Cl₂ (3 x 20 mL). The combined organic layers were concentrated in vacuo to give a yellow solid, which was purified by fc (Ø = 1.5 cm, h = 20 cm, CH₂Cl₂ : H₃COH = 9 : 1 + 1% NH₃, V = 30 mL, R_f = 0.66) and then by automatic fc (Biotage[®], SNAP Cartridge KP-C18-HS 30 g, water/acetonitrile) to give **14**. Colorless solid, yield 97 mg (48%). C₂₈H₃₅N₃O₃, M_r = 461.6. Exact MS (APCI): *m/z* = 462.2709 (calcd. 462.2678 for C₂₈H₃₆N₃O₃ [MH⁺]). ¹H NMR (CDCl₃): δ [ppm] = 0.84 (d, *J* = 6.6 Hz, 6H, NCH₂CH(CH₃)₂), 1.64 – 1.77 (m, 2H, 3-

CH_{pyran} , 5- CH_{pyran}), 1.77 – 1.86 (m, 2H, 3- CH_{pyran} , 5- CH_{pyran}), 1.91 – 2.04 (m, 1H, $NCH_2CH(CH_3)_2$), 2.26 (s, 3H, CH_3), 2.70 – 2.83 (m, 1H, 4- CH_{pyran}), 3.34 (d, $J = 6.8$ Hz, 2H, $NCH_2CH(CH_3)_2$), 3.39 (d, $J = 11.4$ Hz, 2H, 2- CH_{pyran} , 6- CH_{pyran}), 3.64 (s, 2H, CH_2N), 3.91 (s, 2H, CH_2NCO), 4.04 (dd, $J = 11.2/3.3$ Hz, 2H, 2- CH_{pyran} , 6- CH_{pyran}), 6.88 (d, $J = 7.1$ Hz, 1H, 6- CH), 7.13 (s, 1H, 5- CH), 7.32 (t, $J = 8.3$ Hz, 3H, 3- CH_{aniline} , 5- CH_{aniline} , 7- CH), 7.41 (t, $J = 7.5$ Hz, 1H, 8- CH), 7.76 (d, $J = 8.4$ Hz, 2H, 2- CH_{aniline} , 6- CH_{aniline}), 7.98 (d, $J = 7.8$ Hz, 1H, 9- CH), 9.22 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ [ppm] = 20.1 (2C, $NCH_2CH(CH_3)_2$), 27.7 (1C, $NCH_2CH(CH_3)_2$), 29.1 (2C, 3- C_{pyran} , 5- C_{pyran}), 37.4 (1C, CH_3), 44.6 (1C, 3-C), 56.2 (1C, $NCH_2CH(CH_3)_2$), 57.4 (1C, CH_2N), 59.9 (1C, 4- C_{pyran}), 67.6 (2C, 2- C_{pyran} , 6- C_{pyran}), 120.3 (2C, 2- C_{aniline} , 6- C_{aniline}), 129.0 (1C, 8-C), 129.4 (1C, 6-C), 129.7 (2C, 3- C_{aniline} , 5- C_{aniline}), 130.7 (1C, C-8), 130.7 (1C, C-9a), 130.9 (1C, C-9), 135.1 (1C, C-7), 134.4 (1C, C-4 $_{\text{aniline}}$), 135.7 (1C, C-5a), 137.8 (1C, C-1 $_{\text{aniline}}$), 164.9 (1C, CONH), 168.6 (1C, C-1). The signal for C-4 is not seen in the spectrum. IR (neat): ν [cm^{-1}] = 3300 (NH), 2939 ($CH_{\text{aliph.}}$), 1650 (C=O), 1022 (C-O). HPLC (method 1): $t_R = 20.62$ min, purity 98.4%.

4,3,8, 2-Isobutyl-1-oxo-N-{4-[N-(thiazol-2-yl)sulfoamoyl]phenyl}-2,3-dihydro-1H-2-benzazepine-4-carboxamide (16)

Sulfathiazole (**15**, 47 mg, 0.19 mmol, 1 equiv.) was added to a vigorously stirred mixture of **12** (48 mg, 0.19 mmol, 1 equiv.), NEt_3 (51 μ L, 38 mg, 0.37 mmol, 2 equiv.) and HATU (77 mg, 0.20 mmol, 1.1 equiv.) in abs. THF (3 mL). The mixture was stirred overnight at rt. The solvent was removed in vacuo and a saturated solution of $NaHCO_3$ (10 mL) was added to the residue. The mixture was extracted with CH_2Cl_2 (2 x 10 mL) and ethyl acetate (2 x 10 mL). The combined organic layers were dried (Na_2SO_4) and concentrated in vacuo to give the crude product as yellow oil, which was purified by fc ($\varnothing = 2$ cm, $h = 16$ cm, $CH_2Cl_2 : H_3COH = 97 : 3 + 1\% NH_3$, $V = 10$ mL, $R_f = 0.34$ ($CH_2Cl_2 : H_3COH = 95 : 5 + 1\% NH_3$)). Colorless oil, yield 7 mg (8%). $C_{24}H_{24}N_4O_4S_2$, $M_r = 496.6$. Exact mass

(APCI): $m/z = 497.1296$ (calcd. 497.1312 for $C_{24}H_{25}N_4O_4S_2 [MH^+]$). 1H NMR (DMSO- d_6): δ [ppm] = 0.85 (d, $J = 6.7$ Hz, 6H, $NCH_2CH(CH_3)_2$), 1.90 – 1.96 (m, 1H, $NCH_2CH(CH_3)_2$), 3.36 (d, $J = 7.5$ Hz, 2H, $NCH_2CH(CH_3)_2$), 3.93 (s, 2H, 3- CH_2), 6.83 (d, $J = 4.6$ Hz, 1H, 5- $CH_{thiazole}$), 7.26 (d, $J = 4.6$ Hz, 1H, 4- $CH_{thiazole}$), 7.55 (m, 2H, 6- CH , 8- CH), 7.62 (td, $J = 7.7/1.4$ Hz, 1H, 7- CH), 7.77 (s, 1H, 5- CH), 7.79 (d, $J = 8.8$ Hz, 2H, 2- CH_{phenyl} , 6- CH_{phenyl}), 7.88 (d, $J = 8.8$ Hz, 2H, 3- CH_{phenyl} , 5- CH_{phenyl}), 7.93 (m, 1H, 9- CH), 10.55 (s, 1H, NH), 12.70 (s, 1H, SO_2NH). ^{13}C NMR (DMSO- d_6): δ [ppm] = 19.9 (2C, $NCH_2CH(CH_3)_2$), 27.2 (1C, $NCH_2CH(CH_3)_2$), 44.1 (1C, C-3), 55.0 (1C, $NCH_2CH(CH_3)_2$), 108.1 (1C, C-5 $_{thiazole}$), 119.6 (2C, C-2 $_{phenyl}$, C-6 $_{phenyl}$), 124.4 (1C, C-4 $_{thiazole}$), 126.8 (2C, C-3 $_{phenyl}$, C-5 $_{phenyl}$), 129.1 (1C, C-8), 129.6 (1C, C-6), 130.5 (1C, C-7), 131.0 (1C, C-9), 133.1 (1C, C-5a), 135.9 (1C, C-9a), 136.2 (1C, C-5), 136.8 (1C, C-4 $_{phenyl}$), 137.0 (1C, C-4), 142.2 (1C, C-1 $_{phenyl}$), 165.0 (1C, CONH), 167.0 (1C, C-1), 168.7 (1C, C-2 $_{thiazole}$). IR (neat): ν [cm^{-1}] = 3433 (CONH), 1053 (SO_2), 1662 (C=O). HPLC (method 1): $t_R = 18.68$ min, purity 96.8%.

4.3.9. Methyl 2-isobutyl-1-oxo-8-(*p*-tolyl)-2,3-dihydro-1*H*-2-benzazepine-4-carboxylate (**24a**)

Under a permanent flow of N_2 , ester **23a** (160 mg, 0.45 mmol), $PdCl_2(dppf)$ (22 mg, 5 mol %), KOAc (104 mg, 1 mmol) and 4-methylbenzeneboronic acid (79 mg, 0.5 mmol) were suspended in dry dimethoxyethane (10 mL). The Schlenk tube was sealed and heated to 100 °C for 12 h. After cooling down to rt, the mixture was filtered through a short silica pad (ethyl acetate). The filtrate was concentrated in vacuo to give the crude product as a brown oil, which was purified by automatic fc (Biotage[®], SNAP 50 g, cyclohexane / ethyl acetate) to give **24a**. Colorless solid, mp 124 °C, yield 82 mg (50%). $C_{23}H_{25}NO_3$, $M_r = 363.5$. 1H NMR ($CDCl_3$): δ [ppm] = 0.92 (d, $J = 6.7$ Hz, 6H, $NCH_2CH(CH_3)_2$), 2.08 – 2.17 (m, 1H, $NCH_2CH(CH_3)_2$), 2.40 (s, 3H, CH_3), 3.51 (d, $J = 7.6$ Hz, 2H, $NCH_2CH(CH_3)_2$), 3.95 (s, 3H, CO_2CH_3), 4.14 (s, 2H, 3- CH_2), 7.28 – 7.36 (m, 2H, 3- CH_{tolyl} , 5- CH_{tolyl}), 7.47 – 7.65 (m, 3H, 2- CH_{tolyl} , 6- CH_{tolyl}), 7.64 (dd, $J = 8.2/2.0$ Hz, 1H, 7- CH), 7.71 (dd, $J = 8.2/2.1$ Hz, 1H, 6- CH), 7.89 (s, 1H,

5-*CH*), 8.20 (s, 1H, 9-*CH*). ^{13}C NMR (CDCl_3): δ [ppm] = 20.2 (2C, $\text{NCH}_2\text{CH}(\text{CH}_3)_2$), 21.3 (1C, CH_3), 27.8 (1C, $\text{NCH}_2\text{CH}(\text{CH}_3)_2$), 44.5 (1C, C-3), 52.6 (1C, CO_2CH_3), 56.4 (1C, $\text{NCH}_2\text{CH}(\text{CH}_3)_2$), 124.3 (1C, C-7), 127.1 (2C, C-2_{tolyl}, C-6_{tolyl}), 128.1 (1C, C-4), 130.3 (2C, C-3_{tolyl}, C-5_{tolyl}), 132.5 (1C, C-9), 132.9 (1C, C-9a), 134.6 (1C, C-4_{tolyl}), 138.0 (1C, C-5a), 138.8 (1C, C-1_{tolyl}), 141.9 (1C, C-6), 149.2 (1C, C-8), 168.1 (1C, CO_2CH_3), 170.0 (1C, C-1). IR (neat): ν [cm^{-1}] = 2937 (C-H_{aliph.}), 1728 (C=O_{amide}), 1600 (C=C).

4.3.10. Methyl 2-isobutyl-1-oxo-7-(*p*-tolyl)-2,3-dihydro-1*H*-2-benzazepine-4-carboxylate (**24b**)

Under a permanent flow of N_2 , ester **23b** (160 mg, 0.45 mmol), $\text{PdCl}_2(\text{dppf})$ (22 mg, 5 mol%), KOAc (104 mg, 1 mmol) and 4-methylbenzeneboronic acid (79 mg, 0.5 mmol) were suspended in dry dimethoxyethane (10 mL). The Schlenk tube was sealed and heated to 100 °C for 12 h. After cooling down to rt, the mixture was filtered through a short silica pad (ethyl acetate). The filtrate was concentrated in vacuo and purified by automatic fc (Biotage[®], SNAP 50 g, cyclohexane / ethyl acetate) to give **24b**. Colorless solid, mp 128 °C, yield 95 mg (58%). $\text{C}_{23}\text{H}_{25}\text{NO}_3$, $M_r = 363.5$. ^1H NMR (CDCl_3): δ [ppm] = 0.94 (d, $J = 6.7$ Hz, 6H, $\text{NCH}_2\text{CH}(\text{CH}_3)_2$), 2.02 – 2.15 (m, 1H, $\text{NCH}_2\text{CH}(\text{CH}_3)_2$), 2.41 (s, 3H, CH_3), 3.46 (d, $J = 7.6$ Hz, 2H, $\text{NCH}_2\text{CH}(\text{CH}_3)_2$), 3.89 (s, 3H, CO_2CH_3), 4.02 (s, 2H, 3-*CH}_2*), 7.26 – 7.30 (m, 2H, 3-*CH*_{tolyl}, 5-*CH*_{tolyl}), 7.47 – 7.61 (m, 3H, 2-*CH*_{tolyl}, 6-*CH*_{tolyl}, 6-*CH*), 7.71 (dd, $J = 8.2/1.8$ Hz, 1H, 8-*CH*), 7.88 (s, 1H, 5-*CH*), 8.15 (d, $J = 8.2$ Hz, 1H, 9-*CH*). ^{13}C NMR (CDCl_3): δ [ppm] = 20.2 (2C, $\text{NCH}_2\text{CH}(\text{CH}_3)_2$), 21.3 (1C, CH_3), 27.8 (1C, $\text{NCH}_2\text{CH}(\text{CH}_3)_2$), 44.5 (1C, C-3), 52.6 (1C, CO_2CH_3), 56.4 (1C, $\text{NCH}_2\text{CH}(\text{CH}_3)_2$), 127.1 (2C, C-2_{tolyl}, C-6_{tolyl}), 128.1 (1C, C-8), 128.4 (1C, C-6), 129.9 (2C, C-3_{tolyl}, C-5_{tolyl}), 132.4 (1C, C-9), 133.6 (1C, C-9a), 135.1 (1C, C-5a), 136.6 (1C, C-4_{tolyl}), 138.3 (1C, C-1_{tolyl}), 141.9 (1C, C-5), 143.3 (1C, C-7), 166.1 (1C, CO_2CH_3), 168.0 (1C, C-1). IR (neat): ν [cm^{-1}] = 2937 (C-H_{aliph.}), 1727 (C=O_{amide}), 1597 (C=C).

Mixture of **24a** and **24b**: As described above, a mixture of the regioisomers **23a** and **23b** (120 mg, 0.34 mmol) was reacted with 4-methylbenzeneboronic acid (51 mg, 0.37 mmol). Pale yellow oil, yield 74 mg (60%). Ratio **24a:24b** = 1:1.

4.3.11. 2-Isobutyl-N-{4-[N-methyl-N-(tetrahydro-2H-pyran-4-yl)aminomethyl]phenyl}-1-oxo-8-(*p*-tolyl)-2,3-dihydro-1H-2-benzazepine-4-carboxamide (**26a**)

The ester **24a** (90 mg, 0.25 mmol) was dissolved in H₃COH (10 mL) and 5 M NaOH (10 mL) was added. The mixture was heated to reflux for 30 min. After cooling down to 0 °C, the mixture was acidified with conc. HCl to give a precipitate. The aqueous layer was extracted with ethyl acetate (3 x 25 mL), the combined organic layers were dried (Na₂SO₄), added to the precipitate and concentrated in vacuo to give the acid **25a**. Colorless solid, yield 87 mg (97%). C₂₂H₂₃NO₃, M_r = 349.4. Amine **13** (64 mg, 0.25 mmol, 1 equiv.) was added to a vigorously stirred mixture of acid **25a** (87 mg, 0.25 mmol, 1 equiv.), trimethylamine (51 mg, 0.5 mmol, 2 equiv.) and HATU (105 mg, 0.28 mmol, 1.1 equiv.) in abs. THF (10 mL). The mixture was stirred overnight at rt. Then the mixture was concentrated in vacuo. Sat. NaHCO₃-solution (20 mL) was added to the residue and the mixture was extracted with CH₂Cl₂ (3 x 20 mL). The combined organic layers were concentrated in vacuo to give a yellow oil, which was first purified by automatic fc (Biotage[®], SNAP Cartridge KP-C18-HS 30g, water/acetonitrile) to give **26a**. Pale yellow solid, yield 54 mg (39%). Exact MS (APCI): $m/z = 552.3236$ (calcd. 552.3221 for C₃₅H₄₁N₃O₃ [MH⁺]). ¹H NMR (CDCl₃): δ [ppm] = 0.86 (d, $J = 6.6$ Hz, 6H, NCH₂CH(CH₃)₂), 1.70 – 1.84 (m, 2H, 3-CH₂pyran, 5-CH₂pyran), 1.91 (d, $J = 11.6$ Hz, 2H, 3-CH₂pyran, 5-CH₂pyran), 1.95 – 2.07 (m, 1H, NCH₂CH(CH₃)₂), 2.36 (s, 3H, CH₃tolyl), 2.40 (s, 3H, NCH₃), 3.09 (m, 1H, 4-CH_{pyran}), 3.31 (m, 2H, 2-CH₂pyran, 6-CH₂pyran) 3.35 (d, 2H, $J = 7.6$ Hz, NCH₂CH(CH₃)₂), 3.90 (s, 2H, 3-CH₂), 3.95 (s, 2H, NCH₂), 4.03 (dd, $J = 11.2/4.1$ Hz, 2H, 2-CH₂pyran, 6-CH₂pyran), 7.13 (s, 1H, 5-CH), 7.16 (d, $J = 8.1$ Hz, 2H, 3-CH_{tolyl}, 5-CH_{tolyl}), 7.17 – 7.20 (m, 1H, 6-CH), 7.35 (d, $J = 6.2$ Hz, 2H, 2-CH_{tolyl}, 6-CH_{tolyl}), 7.40 (d,

$J = 8.3$ Hz, 2H, 3- CH_{phenyl} , 5- CH_{phenyl}), 7.62 (dd, $J = 8.2/1.8$ Hz, 1H, 7- CH), 7.82 (d, $J = 8.2$ Hz, 2H, 2- CH_{phenyl} , 6- CH_{phenyl}), 8.08 (s, 1H, 9- CH), 9.62 (s, 1H, NH). ^{13}C NMR (CDCl_3): δ [ppm] = 20.2 (2C, $\text{NCH}_2\text{CH}(\text{CH}_3)_2$), 21.3 (1C, $\text{CH}_{3\text{tolyl}}$), 27.7 (1C, $\text{NCH}_2\text{CH}(\text{CH}_3)_2$), 27.9 (2C, C-3 $_{\text{pyran}}$, C-5 $_{\text{pyran}}$), 35.9 (1C, NCH_3), 44.6 (1C, PhCH_2N), 56.23 (1C $\text{NCH}_2\text{CH}(\text{CH}_3)_2$), 56.5 (1C, C-3), 59.5 (1C, C-4 $_{\text{pyran}}$), 67.0 (2C, C-2 $_{\text{pyran}}$, C-6 $_{\text{pyran}}$), 120.6 (2C, C-2 $_{\text{phenyl}}$, C-6 $_{\text{phenyl}}$), 126.9 (2C, C-2 $_{\text{tolyl}}$, C-6 $_{\text{tolyl}}$), 127.0 (1C, C-7), 127.1 (1C, C-5), 127.2 (1C, C-5a), 127.4 (1C, C-6), 129.2 (1C, C-4 $_{\text{phenyl}}$), 129.7 (1C, C-1 $_{\text{tolyl}}$), 129.8 (2C, C-3 $_{\text{tolyl}}$, C-5 $_{\text{tolyl}}$), 130.8 (2C, C-3 $_{\text{phenyl}}$, C-5 $_{\text{phenyl}}$), 131.8 (1C, C-9), 134.2 (1C, C-8), 138.3 (1C, C-4 $_{\text{tolyl}}$), 139.2 (1C, C-1 $_{\text{phenyl}}$), 141.4 (1C, C-4), 143.0 (1C, C-9a), 165.1 (1C, CONH), 168.5 (1C, C-1). IR (neat): ν [cm^{-1}] = 2937 (C- H_{aliph}), 1727 (C=O $_{\text{amide}}$), 1600 (C=C). HPLC (method 1): $t_{\text{R}} = 21.51$ min, purity 98.3%.

4.3.12. 2-Isobutyl-*N*-{4-[*N*-methyl-*N*-(tetrahydro-2*H*-pyran-4-yl)aminomethyl]phenyl}-1-oxo-7-(*p*-tolyl)-2,3-dihydro-1*H*-2-benzazepine-4-carboxamide (**26b**)

The ester **24b** (82 mg, 0.23 mmol) was dissolved in H_3COH (10 mL) and 5 M NaOH (10 mL) was added. The mixture was heated to reflux for 30 min. After cooling down to 0 °C, the mixture was acidified with conc. HCl to give a precipitate. The aqueous layer was extracted with EtOAc (3 x 25 mL), the combined organic layers were dried (Na_2SO_4), added to the precipitate and concentrated in vacuo to give the acid **25b**. Colorless solid, yield 79 mg (97%). $\text{C}_{22}\text{H}_{23}\text{NO}_3$ $M_{\text{r}} = 349.4$ g/mol. Amine **13** (58 mg, 0.23 mmol) was added to a vigorously stirred mixture of acid **25b** (79 mg, 0.23 mmol), triethylamine (46 mg, 0.45 mmol) and HATU (95 mg, 0.25 mmol) in THF (10 mL). The mixture was stirred overnight at rt. Then the mixture was concentrated in vacuo. Saturated NaHCO_3 solution (20 mL) was added to the residue and the mixture was extracted with CH_2Cl_2 (3 x 20 mL). The combined organic layers were concentrated in vacuo to give a yellow oil, which was purified by automatic fc (Biotage®, SNAP KP-C18-HS 30 g, water/acetonitrile) to give **26b**. Pale yellow solid, yield 42 mg (34%). $\text{C}_{35}\text{H}_{41}\text{N}_3\text{O}_3$,

$M_r = 551.7$. Exact MS (APCI): $m/z = 552.3236$ (calcd. 552.3221 for $C_{35}H_{41}N_3O_3$ [MH^+]). 1H NMR ($CDCl_3$): δ [ppm] = 0.88 (d, $J = 6.7$ Hz, 6H, $NCH_2CH(CH_3)_2$), 1.72 – 1.79 (m, 2H, 3- CH_{2pyran} , 5- CH_{2pyran}), 1.86-1.92 (d, $J = 11.6$ Hz, 2H, 3- CH_{2pyran} , 5- CH_{2pyran}), 2.03 (non, $J = 6.9$ Hz, 1H, $NCH_2CH(CH_3)_2$), 2.36 (s, 3H, NCH_3), 2.40 (s, 3H, CH_{3tolyl}), 2.89 – 3.02 (m, 1H, 4- CH_{pyran}), 3.30 – 3.37 (m, 2H, 2- CH_{2pyran} , 6- CH_{2pyran}), 3.40 (d, $J = 7.6$ Hz, 2H, $NCH_2CH(CH_3)_2$), 3.81 (s, 2H, NCH_2), 3.99 (s, 2H, 3- CH_2), 4.04 (dd, $J = 11.3/3.8$ Hz, 2H, 2- CH_{2pyran} , 6- CH_{2pyran}), 7.18 (d, $J = 7.9$ Hz, 2H, 3- CH_{tolyl} , 5- CH_{tolyl}), 7.25 (m, 1H, 6- CH), 7.38 (m, 3H, 5- CH , 2- CH_{phenyl} , 6- CH_{phenyl}), 7.42 (d, $J = 8.1$ Hz, 2H, 3- CH_{phenyl} , 5- CH_{phenyl}), 7.64 (dd, $J = 8.2/1.8$ Hz, 1H, 8- CH), 7.78 (d, $J = 8.3$ Hz, 2H, 2- CH_{phenyl} , 6- CH_{phenyl}), 8.10 (d, $J = 8.2$ Hz, 1H, 9- CH), 9.23 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ [ppm] = 20.2 (2C, $NCH_2CH(CH_3)_2$), 21.3 (1C, CH_{3tolyl}), 27.7 (1C, $NCH_2CH(CH_3)_2$), 28.3 (2C, C-3 $_{pyran}$, C-5 $_{pyran}$), 36.5 (1C, NCH_3), 44.7 (1C, C-3), 56.2 (1C $NCH_2CH(CH_3)_2$), 56.9 (1C, $PhCH_2N$), 59.7 (1C, C-4 $_{pyran}$), 67.3 (2C, C-2 $_{pyran}$, C-6 $_{pyran}$), 120.5 (2C, C-2 $_{phenyl}$, C-6 $_{phenyl}$), 127.0 (2C, C-2 $_{tolyl}$, C-6 $_{tolyl}$), 127.3 (1C, C-6), 127.5 (1C, C-8), 129.8 (2C, C-3 $_{tolyl}$, C-5 $_{tolyl}$), 130.5 (2C, C-3 $_{phenyl}$, C-5 $_{phenyl}$), 131.9 (1C, C-9), 133.8 (1C, C-5a), 134.4 (1C, C-9a), 135.6 (1C, C-5), 136.2 (1C, C-1 $_{tolyl}$), 137.6 (1C, C-1 $_{phenyl}$), 138.3 (1C, C-4 $_{tolyl}$), 138.6 (1C, C-4 $_{phenyl}$), 143.1 (1C, C-7), 165.0 (1C, $CONH$), 168.4 (1C, C-1). IR (neat): ν [cm^{-1}] = 2937 (C-H $_{aliph.}$), 1728 (C=O $_{amide}$), 1600 (C=C). HPLC (method 1): $t_R = 23.08$ min, purity 97.1%.

Separation of regioisomeric **26a** and **26b**: As described above, a mixture of the regioisomers **24a** and **24b** (74 mg, 0.2 mmol) was reacted with 5 M NaOH (5 mL) and the resulting mixture of acids **25a** and **25b** was subsequently coupled with primary amine **13** (51 mg, 0.2 mmol) and HATU (83 mg, 0.22 mmol). Pale yellow oil, yield 51 mg (46%). The 1:1-mixture of regioisomers **26a** and **26b** was separated by preparative HPLC (method 2, acetonitrile : $H_2O = 70:30$, 60 min per run). **26a**: Colorless solid, yield 12 mg (24%). **26b**: Colorless solid, yield 16 mg (31%).

4.3.13. Methyl 2-isobutyl-8-nitro-1-oxo-2,3-dihydro-1*H*-2-benzazepine-4-carboxylate (27a) and Methyl 2-isobutyl-6-nitro-1-oxo-2,3-dihydro-1*H*-2-benzazepine-4-carboxylate (27c)

Concentrated H₂SO₄ (1.04 mL) was added slowly, with ice-water cooling and stirring, to 100% HNO₃ (1.04 mL). **11** (280 mg, 1.02 mmol, 1 equiv.) was dissolved in nitromethane (0.5 mL) and cooled to 0 °C. After nitrating acid was added slowly at this temperature, the solution was warmed to rt and stirred for 2 h. Then ice-water was poured into the reaction mixture, neutralized with 3 M NaOH (10 mL) and extracted with CH₂Cl₂ (2 x 15 mL) and ethyl acetate (2 x 15 mL). The combined organic layers were dried (Na₂SO₄) and concentrated in vacuo. The residue was purified by fc (Ø = 3 cm, h = 17 cm, cyclohexane / ethyl acetate = 7 : 2, V = 20 mL, R_f = 0.02 (cyclohexane / ethyl acetate = 7 : 2)) to give a mixture of regioisomers **27a** and **27c**. Pale yellow oil, yield 273 mg (83%). C₁₆H₁₈N₂O₅, M_r = 318.3. Ratio of **27a**:**27c** = 7:3. Exact mass (ESI): *m/z* = 341.1109 (calcd. 341.1108 for C₁₆H₁₈N₂NaO₅ [MNa⁺]). IR (neat): ν [cm⁻¹] = 2959 (C-H_{alkyl}), 1713 (C=O), 1636 (NC=O), 1524 (N=O), 1346 (NO₂ arom.). HPLC (method 1): t_R = 20.43 min, purity 97.7%. The two regioisomers were separated by fc (Ø = 2.5 cm, h = 24 cm, CH₂Cl₂ : ethyl acetate = 95 : 5, V = 20 mL) to give **27a** and **27c**.

27a (R_f = 0.38, CH₂Cl₂ : ethyl acetate = 95 : 5): Pale yellow solid, mp 109 °C, yield 178 mg (54%). C₁₆H₁₈N₂O₅, M_r = 318.3. Exact mass (ESI): *m/z* = 341.1106 (calcd. 341.1108 for C₁₆H₁₈N₂NaO₅ [MNa⁺]). ¹H NMR (CDCl₃): δ [ppm] = 0.94 (d, *J* = 6.7 Hz, 6H, NCH₂CH(CH₃)₂), 2.14 – 2.21 (m, 1H, NCH₂CH(CH₃)₂), 3.45 (d, *J* = 7.6 Hz, 2H, NCH₂CH(CH₃)₂), 3.91 (s, 3H, CO₂CH₃), 4.02 (s, 2H, 3-CH₂), 7.53 (d, *J* = 8.5 Hz, 1H, 6-CH), 7.84 (s, 1H, 5-CH), 8.30 (dd, *J* = 8.5/2.4 Hz, 1H, 7-CH), 8.92 (d, *J* = 2.0 Hz, 1H, 9-CH). ¹³C NMR (CDCl₃): δ [ppm] = 20.1 (2C, NCH₂CH(CH₃)₂), 27.7 (1C, NCH₂CH(CH₃)₂), 44.2 (1C, C-3), 53.0 (1C, CO₂CH₃), 56.6 (1C, NCH₂CH(CH₃)₂), 124.8 (1C, C-7), 127.3 (1C, C-9), 130.9 (1C, C-6), 136.0 (1C, C-4), 138.2 (1C, C-9a), 138.4 (1C, C-5a), 139.2 (1C, C-5), 148.3 (1C, C-8), 165.4 (1C, CO₂CH₃), 166.2 (1C, C-1). FT-IR (neat): ν [cm⁻¹] = 2963 (C-H_{alkyl}), 1709 (C=O), 1632 (NC=O), 1520 (N=O), 1354 (NO₂ arom.). HPLC (method 1): t_R = 20.81 min, purity 96.5%.

27c ($R_f = 0.24$, CH_2Cl_2 : ethyl acetate = 95 : 5): Pale yellow oil, yield 29 mg (9%). $\text{C}_{16}\text{H}_{18}\text{N}_2\text{O}_5$, $M_r = 318.3$. Exact mass (ESI): $m/z = 341.1116$ (calcd. 341.1108 for $\text{C}_{16}\text{H}_{18}\text{N}_2\text{NaO}_5$ [MNa^+]). ^1H NMR (CDCl_3): δ [ppm] = 0.94 (d, $J = 6.7$ Hz, 6H, $\text{NCH}_2\text{CH}(\text{CH}_3)_2$), 2.02 – 2.12 (m, 1H, $\text{NCH}_2\text{CH}(\text{CH}_3)_2$), 3.44 (d, $J = 6.2$ Hz, 2H, $\text{NCH}_2\text{CH}(\text{CH}_3)_2$), 3.89 (s, 3H, CO_2CH_3), 4.00 (s, 2H, 3- CH_2), 7.63 (t, $J = 8.0$ Hz, 1H, 8- CH), 7.96 (s, 1H, 5- CH), 8.10 (dd, $J = 8.1/1.4$ Hz, 1H, 7- CH), 8.31 (dd, $J = 7.9/1.3$ Hz, 1H, 9- CH). ^{13}C NMR (CDCl_3): δ [ppm] = 20.1 (2C, $\text{NCH}_2\text{CH}(\text{CH}_3)_2$), 27.6 (1C, $\text{NCH}_2\text{CH}(\text{CH}_3)_2$), 43.8 (1C, C-3), 53.0 (1C, CO_2CH_3), 56.2 (1C, $\text{NCH}_2\text{CH}(\text{CH}_3)_2$), 126.7 (1C, C-7), 127.2 (1C, C-5a), 129.9 (1C, C-8), 134.6 (1C, C-4), 134.9 (1C, C-5), 136.2 (1C, C-9), 139.2 (1C, C-9a), 148.3 (1C, C-6), 165.6 (1C, CO_2CH_3), 166.3 (1C, C-1). IR (neat): ν [cm^{-1}] = 2959 (C- H_{alkyl}), 1712 (C=O), 1636 (NC=O), 1528 (N=O), 1343 ($\text{NO}_2_{\text{arom.}}$). HPLC (method 1): $t_R = 20.81$ min, purity 92.9%.

4.3.14. 2-Isobutyl-*N*-{4-[*N*-methyl-*N*-(tetrahydro-2*H*-pyran-4-yl)aminomethyl]phenyl}-8-nitro-1-oxo-2,3-dihydro-1*H*-2-benzazepine-4-carboxamide (29a) and 2-Isobutyl-*N*-{4-[*N*-methyl-*N*-(tetrahydro-2*H*-pyran-4-yl)aminomethyl]phenyl}-6-nitro-1-oxo-2,3-dihydro-1*H*-2-benzazepine-4-carboxamide (29c)}

Amine **13** (193 mg, 0.66 mmol, 1 equiv.) was added to a vigorously stirred mixture of the regioisomeric acids **28a** and **28c** (200 mg, 0.66 mmol, 1 equiv.), NEt_3 (182 μL , 133 mg, 1.32 mmol, 2 equiv.) and HATU (275 mg, 0.72 mmol, 1.1 equiv.) in abs. THF (8 mL). The mixture was stirred overnight at rt. After further addition of NEt_3 (133 mg, 1.32 mmol, 2 equiv.) and HATU (274.9 mg, 0.72 mmol, 1.1 equiv.) the mixture was stirred for 3 h. The solvent was removed in vacuo and saturated NaHCO_3 -solution (25 mL) was added to the residue. The mixture was extracted with ethyl acetate (3 x 30 mL). The combined organic layers were dried (Na_2SO_4) and concentrated in vacuo to give the amides **29a** and **29c** as a light brown oil as mixture of regioisomers. This was purified and separated by fc ($\varnothing = 2.5$ cm, $h = 17$ cm, CH_2Cl_2 : $\text{H}_3\text{COH} = 96 : 4 + 1\%$ NH_3 , $V = 20$ mL) to give the pure amides **29a** and **29c**, and a

fraction containing a mixture, which was further separated by another fc ($\emptyset = 1$ cm, $h = 17$ cm, $\text{CH}_2\text{Cl}_2 : \text{H}_3\text{COH} = 97 : 3 + 1\% \text{NH}_3$, $V = 5$ mL).

29a ($R_f = 0.28$, $\text{CH}_2\text{Cl}_2 : \text{H}_3\text{COH} = 95 : 5 + 1\% \text{NH}_3$). Yellow solid, mp 200 °C, yield 32 mg (9%).

$\text{C}_{28}\text{H}_{34}\text{N}_4\text{O}_5$, $M_r = 506.6$. Exact mass (ESI): $m/z = 507.2595$ (calcd. 507.2602 for $\text{C}_{28}\text{H}_{35}\text{N}_4\text{O}_5$ [MH^+]).

^1H NMR (CDCl_3): δ [ppm] = 0.89 (d, $J = 6.6$ Hz, 6H, $\text{NCH}_2\text{CH}(\text{CH}_3)_2$), 1.67 – 1.84 (m, 4H, 3- CH_{pyran} , 5- CH_{pyran}), 1.98 – 2.06 (m, 1H, $\text{NCH}_2\text{CH}(\text{CH}_3)_2$), 2.29 (s, 3H, CH_3), 2.81 – 2.88 (m, 1H, 4- CH_{pyran}), 3.31 – 3.36 (m, 2H, 2- CH_{pyran} , 6- CH_{pyran}), 3.40 (d, $J = 7.6$ Hz, 2H, $\text{NCH}_2\text{CH}(\text{CH}_3)_2$), 3.72 (s, 2H, CH_2N), 3.99 (s, 2H, 3- CH_2), 4.02 – 4.06 (m, 2H, 2- CH_{pyran} , 6- CH_{pyran}), 7.28 (d, $J = 8.5$ Hz, 1H, 6- CH), 7.36 (d, $J = 8.4$ Hz, 2H, 3- $\text{CH}_{\text{aniline}}$, 5- $\text{CH}_{\text{aniline}}$), 7.38 (s, 1H, 5- CH), 7.68 (d, $J = 8.4$ Hz, 2H, 2- $\text{CH}_{\text{aniline}}$, 6- $\text{CH}_{\text{aniline}}$), 8.18 (dd, $J = 8.5/2.4$ Hz, 1H, 7- CH), 8.86 (d, $J = 2.4$ Hz, 1H, 9- CH), 9.01 (s, 1H, NH). ^{13}C NMR (CDCl_3): δ [ppm] = 20.1 (2C, $\text{NCH}_2\text{CH}(\text{CH}_3)_2$), 27.7 (1C, $\text{NCH}_2\text{CH}(\text{CH}_3)_2$), 28.6 (2C, C-3 $_{\text{pyran}}$, C-5 $_{\text{pyran}}$), 36.8 (1C, CH_3), 44.5 (1C, C-3), 56.4 (1C, $\text{NCH}_2\text{CH}(\text{CH}_3)_2$), 56.9 (1C, CH_2N), 59.5 (1C, C-4 $_{\text{pyran}}$), 67.5 (2C, C-2 $_{\text{pyran}}$, C-6 $_{\text{pyran}}$), 120.4 (2C, C-2 $_{\text{aniline}}$, C-6 $_{\text{aniline}}$), 124.8 (1C, C-7), 126.8 (1C, C-9), 130.3 (2C, 3- $\text{C}_{\text{aniline}}$, 5- $\text{C}_{\text{aniline}}$), 130.4 (1C, C-6), 132.8 (1C, C-5), 133.6 (1C, C-4 $_{\text{aniline}}$), 137.4 (1C, C-1 $_{\text{aniline}}$), 137.5 (1C, C-9a), 138.6 (1C, C-5a), 140.8 (1C, C-4), 147.8 (1C, C-8), 164.0 (1C, CONH), 166.5 (1C, C-1). IR (neat): ν [cm^{-1}] = 3267 (N-H), 2920 (C- H_{alkyl}), 1632 (C=O), 1520 (N=O), 1343 (NO_2 arom.). HPLC (method 1): $t_R = 17.43$ min, purity 97.3%.

29c ($R_f = 0.32$, $\text{CH}_2\text{Cl}_2 : \text{H}_3\text{COH} = 95 : 5 + 1\% \text{NH}_3$). Pale yellow solid, mp 197 °C, yield 45.6 mg

(14%). $\text{C}_{28}\text{H}_{34}\text{N}_4\text{O}_5$, $M_r = 506.6$. Exact mass (ESI): $m/z = 507.2595$ (calcd. 507.2602 for $\text{C}_{28}\text{H}_{35}\text{N}_4\text{O}_5$

[MH^+]). ^1H NMR (CDCl_3): δ [ppm] = 0.87 (d, $J = 6.6$ Hz, 6H, $\text{NCH}_2\text{CH}(\text{CH}_3)_2$), 1.64 – 1.79 (m, 4H, 3- CH_{pyran} , 5- CH_{pyran}), 1.94 – 2.05 (m, 1H, $\text{NCH}_2\text{CH}(\text{CH}_3)_2$), 2.22 (s, 3H, CH_3), 2.62 – 2.70 (m, 1H, 4- CH_{pyran}), 3.33 – 3.40 (m, 4H, $\text{NCH}_2\text{CH}(\text{CH}_3)_2$, 2- CH_{pyran} , 6- CH_{pyran}), 3.60 (s, 2H, CH_2N), 3.89 – 4.06 (m, 4H, 3- CH_2 , 2- CH_{pyran} , 6- CH_{pyran}), 7.33 (d, $J = 8.4$ Hz, 2H, 3- $\text{CH}_{\text{aniline}}$, 5- $\text{CH}_{\text{aniline}}$), 7.40 (s, 1H, 5- CH), 7.56 (t, $J = 8.0$ Hz, 1H, 8- CH), 7.69 (d, $J = 8.5$ Hz, 2H, 2- $\text{CH}_{\text{aniline}}$, 6- $\text{CH}_{\text{aniline}}$), 8.04 (dd, $J = 8.1/1.4$ Hz,

¹H, 7-CH), 8.25 (dd, $J = 7.9/1.3$ Hz, 1H, 9-CH). A signal for the NH proton is not seen in the spectrum. ¹³C NMR (CDCl₃): δ [ppm] = 19.9 (2C, NCH₂CH(CH₃)₂), 27.5 (1C, NCH₂CH(CH₃)₂), 29.1 (2C, C-₃pyran, C-₅pyran), 37.5 (1C, CH₃), 44.0 (1C, C-3), 55.9 (1C, NCH₂CH(CH₃)₂), 57.3 (CH₂N), 59.5 (C-₄pyran), 67.6 (2C, C-₂pyran, C-₆pyran), 120.2 (2C, C-₂aniline, C-₆aniline), 126.9 (1C, C-7), 127.3 (1C, C-5a), 128.2 (1C, C-5), 129.4 (2C, C-₃aniline, C-₅aniline), 129.5 (1C, C-8), 135.8 (1C, C-9), 136.1 (1C, C-₁aniline), 136.8 (1C, C-₄aniline), 138.5 (1C, C-9a), 139.3 (1C, C-4), 147.2 (1C, C-6), 164.0 (1C, CONH), 166.3 (1C, C-1). IR (neat): ν [cm⁻¹] = 3291 (N-H), 2955 (C-H_{alkyl}), 1628 (C=O), 1524 (N=O), 1343 (NO₂arom.). HPLC (method 1): $t_R = 17.13$ min, purity 98.4%.

4.4. Biological evaluation

4.4.1. Affinity towards CCR2 receptors

Chemicals and reagents

[³H]INCB3344 (32 Ci/mmol) was purchased from ViTrax (Placentia, USA - custom synthesis). BMS22 was purchased from Tocris Bioscience (Bristol, UK)

Cell culture and membrane preparation

U2OS cells stably expressing the human CCR2 receptor (Invitrogen, Carlsbad, CA) were cultured in McCoy5a medium supplemented with 10% fetal calf serum, 2 mM glutamine, 0.1 mM non-essential amino acids (NEAAs), 25 mM 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES), 1 mM sodium pyruvate, 100 IU/mL penicillin, 100 μ g/mL streptomycin, 100 μ g/mL G418, 50 μ g/mL hygromycin, and 125 μ g/mL zeocin in a humidified atmosphere at 37 °C and 5% CO₂. Cell culture and membrane preparation were performed as described previously.¹⁸

[³H]INCB3344 binding assays

Binding assays were performed in a 100 μ L reaction volume containing 50 mM Tris-HCl buffer (pH 7.4), 5 mM MgCl₂, 0.1% 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonic acid (CHAPS)

and 20 μg of membrane protein at 25 °C. Nonspecific binding was determined with 10 μM BMS22. Displacement assays were performed with 7 nM [^3H]INCB3344 using at least 6 concentrations of competing ligand for 120 minutes of incubation. The HP D300 digital dispenser from Tecan (Männedorf, Switzerland) was used to dispense the compounds in DMSO directly into the assay plate. Incubations were terminated by dilution with ice-cold 50 mM Tris-HCl buffer supplemented with 0.05% CHAPS. Separation of bound from free radioligand was performed by rapid filtration through a 96-well GF/B filter plate using a PerkinElmer Filtermate-harvester (PerkinElmer, Groningen, The Netherlands). Filters were washed 10 times with ice-cold wash buffer, and 25 μL of Microscint scintillation cocktail (PerkinElmer) was added to each well; the filter-bound radioactivity was determined by scintillation spectrometry using the P-E 1450 Microbeta Wallac Trilux scintillation counter (PerkinElmer).

Data analysis

All experiments were analysed using the nonlinear regression curve fitting program Prism 5 (GraphPad, San Diego, CA).

4.4.2. Ca^{2+} flux assay at CCR2 receptors

Cell culture

Chem-1 cell line transfected with human CCR2 (ChemiSCREEN™ CCR2B Calcium-Optimized FLIPR Cell Line, Merck Millipore) was used for the intracellular calcium flux assay. U2OS β -arrestin cell line transfected with murine CCR2 (93-0543C3, DiscoverX Corporation, Ltd.) was used for the β -arrestin recruitment assay.

Measurement of intracellular calcium flux (G_q signaling pathway)

Chem-1 cells transfected with human CCR2 were cultured in DMEM high glucose medium (supplemented by 10% FCS, 1 mM pyruvate, 15 mM HEPES, 500 $\mu\text{g}/\text{mL}$ geniticine and non-essential

amino acids (NEAA)). The cells were transferred into Optimem (supplemented by 5% FCS, 50 U/mL penicillin and 50 µg/mL streptomycin and NEAA) and seeded into 384-well plates (µCLEAR/black Greiner Bio One) at a density of 5000 cells/25 µL. Cells were incubated for approximately 24 h at 37 °C, 5% CO₂. Before the assay medium was removed and the cells were incubated with Fluo-4 solution (25 µL Tyrode's solution containing 3 µM Fluo-4 AM (1 mM DMSO stock solution), 0.4 mg/mL brilliant black, 2.5 mM probenecide, 0.03% pluronic F-127) for 60 min at 37 °C, 5% CO₂. The compounds were dissolved in DMSO with 10 mM stock concentration followed by further dilution with DMSO in 1/3.16 steps. Required test solutions for the assay were obtained by dilution with Tyrode's solution containing 2 mM CaCl₂ and 0.05% BSA. Compounds (10 µL per well) were added and cells were incubated for 10 min at 37 °C, 5% CO₂. Then 20 µL of agonist solution (recombinant human CCL2 (PeproTech, 300-04) in Tyrode's solution with 0.05% BSA) were added. CCL2 was applied at EC₅₀, which was determined in an experiment prior to compound testing (approximately 5 nM). Fluorescence intensity (excitation: 485 nm, emission: 520 nm) was measured for 120 s in 1.0 s intervals by a proprietary fluorescence measuring device. IC₅₀ values were fitted using a 4 parameter logistic function (Hill function).

4.4.3. Affinity toward the CCR5

Chemicals and reagents

[³H]TAK-779 (79.1 Ci/mmol) was purchased from PerkinElmer (custom synthesis).

Cell culture and membrane preparation and [³H]TAK-779 binding assay

The CCR5 receptor containing membrane fragments were used according to the instructions of the manufacturer and incubated with various concentrations of the test compound, 2 nM [³H]TAK-779 and binding buffer (50 mM HEPES pH 7.4, 5 mM MgCl₂, 1 mM CaCl₂ and 0.2% BSA) at room temperature. The filter mats were washed with a buffer solution (50 mM HEPES pH = 7.4, 500 mM

NaCl-solution and 0.1% BSA). The test compound solutions were prepared by dissolving approximately 10 μmol (usually 2-4 mg) of the test compound in DMSO so that a 10 mM stock solution was obtained. To obtain the required test solutions for the assay, the DMSO stock solution was diluted with the respective assay buffer. The filter mats were presoaked in 0.5% aqueous polyethylenimine solution for 2 h at room temperature before use. All binding experiments were carried out in duplicates in 96-well multiplates. The concentrations given are the final concentrations in the assay. Generally, the assays were performed by addition of 50 μL of the respective assay buffer, 50 μL test compound solution in various concentrations (10^{-5} , 10^{-6} , 10^{-7} , 10^{-8} , 10^{-9} and 10^{-10} mol/L), 50 μL of corresponding radioligand solution and 50 μL of the respective receptor preparation into each well of the multiplate (total volume 200 μL). The receptor preparation was always added last. During the incubation, the multiplates were shaken at a speed of 500-600 rpm at the specified temperature. The assays were terminated after 120 min by rapid filtration using the harvester. During the filtration, each well was washed five times with 300 μL of water. Subsequently, the filter mats were dried at 95 °C. The solid scintillator was melted on the dried filter mats at a temperature of 95 °C for 5 min. After solidifying of the scintillator at room temperature, the trapped radioactivity in the filter mats was measured with the scintillation analyser. Each position on the filter mat corresponding to one well of the multiplate was measured for 5 min with the [^3H]-counting protocol. The overall counting efficiency was 20%.

Data analysis

All experiments were analysed using the nonlinear regression curve fitting program Prism 3 (GraphPad, San Diego, CA).

4.4.4. BRET-based cAMP assay at the CCR5 receptor

Cell culture

The human embryonic kidney (HEK293 T) were cultured in 10 cm cell culture plates in DMEM/F-12

medium, supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 1% penicillin-streptomycin and incubated at 37°C in a humid atmosphere with 5% CO₂.

cAMP BRET assay

HEK293T cells were transiently transfected with 1µg of the human receptor CCR5 and 2µg of the biosensor CAMYEL, using the TransIT-293 transfection reagent from Mirus 293 (Mirus Bio LLC, Madison, USA). 24 hours after transfection, the cells were seeded in a half-area white 96-wells plate (Grainer-Bio One) containing 20.000 cells/well in 100 µL of complete DMEM/F-12 phenol red-free medium. After incubation at 37°C in a humid atmosphere with 5% CO₂ overnight, the medium was removed and 30 µL of dPBS supplemented with CaCl₂ and MgCl₂ (Gibco, Life Technologies) were added to each well. After additional hour of incubation at 37°C the substrate coelenterazine h (Promega, Madison, USA) was added in each well to a final concentration of 5 µM and incubated for 5 min in darkness. Various concentrations of test compounds (prepared in dPBS, pH 7.4, containing 0.2% BSA, 1% DMSO, 10 µM forskoline, and 0.2 nM of the chemokine CCL4 or 1 nM of the chemokine CCL5) were added to a final volume of 50 µL per well and incubated for additional 10 min at room temperature. Bioluminescence was measured with a microplate reader Clariostar (BMG labtech, Ortenberg, Germany). Each concentration was measured in triplicate.

Data analysis

To characterize the allosteric profile of novel ligands, we applied the ternary complex model of allosterism to analyze the data obtained from the functional assays. The assumptions were that the allosteric modulators do not cause the depression of maximal response or the suppression of the basal activity. The depression of maximal response or the suppression of the basal activity are not accounted for in an ATCM model. Importantly, even if these assumptions do not hold entirely true for all the novel allosteric modulators, this analysis enables a first approximation and a semi-empirical estimate of

cooperativity.^{19,20} The data from functional studies, where discrete concentrations of agonist CCL4 was used, were fitted to following equations using Prism 7.0:

$$K_{app} = \frac{K_A \left(1 + \frac{[B]}{K_B}\right)}{\left(1 + \frac{\alpha\beta[B]}{K_B}\right)} \quad (1)$$

$$Y = \frac{Y_0 (1 + K_A)}{([c] + K_{app})} \quad (2)$$

Where K_{app} described the occupancy of the orthosteric site, K_A was the EC_{50} value of CCL4 or CCL5 for the investigated receptor, $[c]$ the concentration of CCL4 or CCL5 used, $[B]$ the concentration of novel allosteric modulator, K_B the equilibrium dissociation constant of modulator binding, and $\alpha\beta$ the ternary complex constant, which denotes cooperativity factor.²¹ In the cAMP assay the K_A value for CCL4 was set to 0.2 nM. The concentration of the chemokine CCL5 was set to 1 nM.

4.5 Molecular modelling

All molecular modelling studies were carried out on an Intel Core™ i7 processor, 8 GB memory with Windows 10 operating system using MOE (Molecular Operating Environment, 2016.08; Chemical Computing Group Inc., 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, 2012.) as the computational software.

For the calculations performed using AM1 of the MOE software package (*Molecular Operating Environment (MOE)*, 2012.10; Chemical Computing Group Inc., 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, 2012) the 3D-structures were drawn using Module Builder. The stochastic conformational search was performed under standard conditions. (Method: Stochastic, Rejection Limit 100, Iteration Limit 10000, RMS gradient: 0.005, MM iteration limit 500, RMSD limit: 0.25, Strain Cutoff: 7 kcal/mol, Conformation Limit 10000).

Acknowledgement

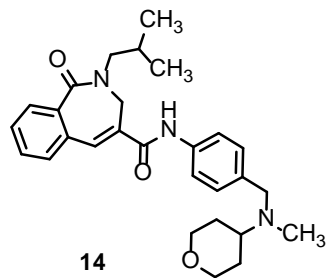
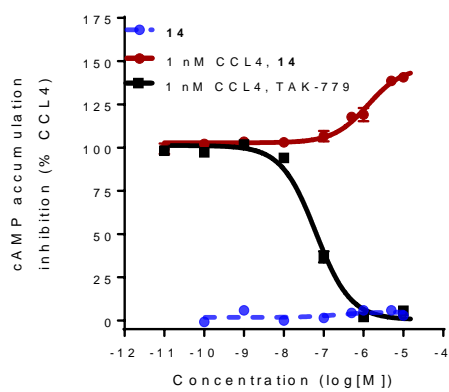
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Graphical Abstract



positive allosteric modulator
at CCR5 receptor

Captions of all Figures, Schemes and Tables

Figure 1: Design of 2-benzazepin-1-ones **3** derived from TAK-779 (**1**) and TAK-652 (**2**).

Figure 2: I) Alignment of central core structures of TAK-779 (**A**, blue) with 7-*p*-tolyl derivative **26b** (**B**, pink). II) Alignment of central core structures of TAK-652 (**C**, orange) with 7-*p*-tolyl derivative **26b** (**B**, pink).

Figure 3: The BRET-based cAMP assay for compounds **14** and TAK-779 (**1**) with CCL4 at the CCR5 receptor.

Scheme 1: Synthesis of compounds **14** and **16**. Reagents and reaction conditions: (a) 1. NaOCH₃, H₃COH, rt, 2 h; 2. HCl/Et₂O, 89%. (b) Monomethyl phthalate (**7**), SOCl₂, pyridine; then addition of **6**·HCl, pyridine, CH₂Cl₂, rt, 4 h, 64%. (c) NaH, THF, reflux, 3 h, 58%. (d) NaBH₄, H₃COH, 0 °C, 1 h, 58%. (e) H₃CSO₂Cl, NEt₃, DBU, CH₂Cl₂, rt, 12 h, 76%. (f) 5 M NaOH, H₃COH, reflux, 40 min, 100%. (g) NEt₃ (2 equiv.), HATU (1.1 equiv.), THF, rt, 12 h, **14**: 48%, **16**: 8%.

Scheme 2: Synthesis of 7- and 8-(*p*-tolyl)-2-benzazepine-4-carboxamides **26a,b**. Reagents and reaction conditions: (a) Br₂, NaOH, H₂O, reflux, 72 h, 95%. (b) TMSCl, H₃COH, rt, 12 h, 94%. (c) 1. SOCl₂, pyridine; 2. **6**·HCl, pyridine, CH₂Cl₂, rt, 4 h, 64%. (d) NaH, THF, reflux, 3 h, **21a**: 36%, **21b**: 38%. (e) NaBH₄, H₃COH, 0 °C, 1 h, **22a**: 58%, **22b**: 45%. (f) H₃CSO₂Cl, NEt₃, DBU, CH₂Cl₂, rt, 12 h, **23a**: 74%, **23b**: 84%. (g) 4-Methylbenzeneboronic acid, PdCl₂(dppf) (5 mol%), KOAc, DME, reflux, 12 h, **24a**: 50%, **24b**: 58%. (h) 5 M NaOH, H₃COH, reflux, 30 min, 97%. (i) **13**, HATU, NEt₃, THF, rt, 12 h, **26a**: 39%, **26b**: 34%.

Scheme 3: Synthesis of NO₂ derivatives **29** and acetamide **34a**. Reagents and reaction conditions: (a) HNO₃ (100%), H₂SO₄ (95 – 97%), CH₃NO₂, rt, 2 h, 83%. (b) 5M NaOH, H₃COH, reflux, 97%. (c) **13**, HATU, NEt₃, THF, rt, 12 h, **29a**: 9%, **29c**: 14%. (d) Fe, conc. HCl, EtOH, reflux, 2 h, 75%. (e) Ac₂O, NEt₃, CH₂Cl₂, 6 h, rt, 59%. (f) NaOH, H₃COH, 10 min, rt. (g) Ac₂O, NEt₃, CH₂Cl₂, 6 h, rt, 69%. (h) **13**, HATU, NEt₃, THF, rt, 12 h, 47%.

Scheme 4: Synthesis of sulfonamide **38a**: Reagents and reaction conditions: (a) Fe, conc. HCl, EtOH, reflux, 2 h, 74%. (b) CH₃SO₂Cl (1 equiv.), NEt₃, CH₂Cl₂, 3 d, then 4 x 0.3 equiv. CH₃SO₂Cl and NEt₃ every 6 h, rt, 91%. (c) NaOH, H₃COH, 20 min, rt, 43%. (d) LiOH (4 equiv.), H₃COH, 3 h rt, 1 h 50 °C, 53%. (e) **13**, HATU, NEt₃, THF, rt, 12 h, 20%.

Table 1: Receptor affinities and activities at CCR2 and CCR5 receptors.

Under the Table

[%] inhibition at a test compound concentration of 1 μM (n = 3). n.d. not determined.

- [a] Displacement of the radioligand [³H]INCB33344 at a concentration of 1 μM of the test compound (n = 3).
- [b] Inhibition of Ca²⁺ mobilization after activation by 5 nM MCP-1 (n = 3).
- [c] Displacement of the radioligand [³H]TAK-779 at a concentration of 1 μM of the test compound (n = 3).
- [d] Stimulation of cAMP production after inhibition with 0.2 nM CCL4 (n = 3).