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New V1a receptor antagonist. Part 2. Identification and optimization of triazolobenzazepines

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ABSTRACT

Solid preclinical evidence links vasopressin to social behavior in animals, so, extensive work has been initiated to find new vasopressin V1a receptor antagonists which can improve deteriorated social behavior in humans and can treat the core symptoms of autistic behavior, as well. Our aim was to identify new chemical entities with antagonizing effects on vasopressin V1a receptors. Continuing our previous work, we found an *in vitro* and *in vivo* orally active V1a selective antagonist molecule (40) among [1,2,4]triazolo[4,3-a][1]benzazepines.

Vasopressin and oxytocin are thought to act as modulators of social information processing and also social behavior in mammals.[1] Vasopressin neuropeptide is thought to be acting its social effects *via* its vasopressin V1a receptors. It has well established yet that vasopressin V1a receptor antagonists can improve anxiety and depression[2] related behavior in animal models and it can also improve the deficit of social behavior in animal models of sociability.[3] These preclinical findings emerged the possibility to treat deteriorated social behavior in autistic patients and extensive clinical work was started to confirm these benefits of vasopressin V1a antagonist compounds on social behavior of humans.

The first selective, brain penetrating V1a antagonist was reported by the researchers of Pfizer.[4] PF-184563 **(1)** was intended for the treatment of dysmenorrhoea, however, it did not reach clinical phase. Indole derivative **2**[5] by Roche brought new therapeutic possibilities, as it was managed to prove under clinical circumstances, that the blockade of the V1a receptor may improve social communication in adults with high-functioning ASD."[6] Its follow-up molecule was the balovaptan (RG7314, **3**), which displayed significant efficacy on the Vineland-II scale (Vineland adaptive behavior scale, second edition) in adult ASD patients.[7] (Fig. 1). Up to April the molecule was being tested in Phase2 clinical studies involving children and adolescents with autism and in Phase 3 clinical trials with adult patients[8], then it was abruptly removed from Roche's pipeline.

We previously reported the identification of a tetrahydroquinolinyl urea derivative (**4**)[9] with acceptable penetrability ($Papp_{A-B}$ 10 × 10^{-6} $cm.s^{-1}$) and very potent V1a antagonist activity, which major disadvantage was its high intrinsic clearance with the microsomes of all species (h/r/m) tested[10]. We hypothesized that the replacement of the bicyclic urea with a triazole containing tricycle could improve this parameter.

Our starting point was the previously already synthesized[9], moderately active tetrahydroquinoxaline derivative **5**, which permeability ($Papp_{A-B}$ 48 × 10^{-6} $cm.s^{-1}$)[11] was high despite the presence of a very polar pyridinyl-piperidine group. By retaining this side chain, we searched for a proper tricyclic scaffold. The prepared compounds were examined on hV1a binding test[12], antagonist activity was measured on fluorimetric Ca^{2+} assay[13].

The firstly prepared [1,2,4]triazolo[4,3-*a*]benzimidazole derivative **6** was, beside its poor binding value (hV1a K_i: 5200 nM), ineffective in

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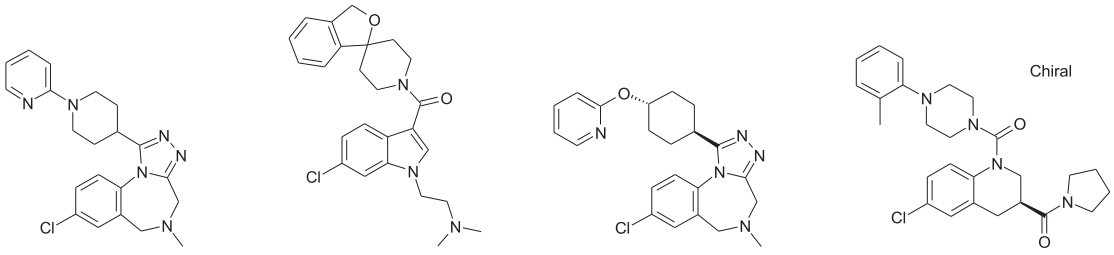


1 PF-184563 **2** RG7713 (RO5028442) **3** balovaptan (RG7314) **4**

Fig. 1. Structure of V1a antagonists.

the functional test (Table 1). By the relocation of the chlorine atom from position 7 to 6 (**7**), the binding activity also diminished. We then prepared [1,2,4]triazolo[4,3-*a*]quinoxaline derivatives **8–11** by expanding the middle ring to six membered one. As a result, we obtained moderately active molecule **8** by placing methoxy group to position 4. When we hydrolyzed the methyl group, the obtained oxo-compound **9** was ineffective. After these results we drew the conclusion that apolar (instead of polar) groups are required in this region. This was proven by the activity of 4,4-dimethyl derivative **10**. By the acylation of this compound (**11**), the binding potency weakened, however, the functional activity strengthened. When we replaced the acylated nitrogen with a methylene moiety, and removed geminal methyl groups, we obtained [1,2,4]triazolo[4,3-*a*]quinoline derivative **12**, which possessed sufficient activity. As its microsomal stability was promising and its brain penetration was excellent (VB-Caco-2 $Papp_{A\text{-}B}$: 51×10^{-6} $cm.s^{-1}$; PDR: 0.8, see Table 1), we considered this molecule as Lead, and carried on with its optimization.

When investigating the substituents of **12**, we retained the chlorine atom at its original place. First, we introduced an anellated cyclopropyl ring to position R^3-R^4, hoping that the receptor would tolerate a small apolar group in that direction (Table 2). Unfortunately, activity dropped (**13**), which could not be improved even by altering R^1 (**14**). When carboxymethyl group was placed next to the cyclopropyl ring (**15**), activity further decreased. Interestingly, when we replaced R^1 with methyl-oxazolyl-cyclohexyl, we obtained compound **16** having similar binding affinity, but better functional potency compared to that of **12**. We hypothesized that activity will increase after separating the enantiomers. Indeed, binding affinity of compound **18** was 10 times better than that of **17**, though functional activity did not improve. We demonstrate the synthesis of cyclopropyl derivatives through the preparation of compounds **17–19** on Scheme 1.

We protected the nitrogen of the commercially available 6-chloro-2-hydroxyquinoline **(17a)** with 4-methoxybenzyl group, and then we formed the cyclopropyl group with trimethylsulfoxonium iodide (**17c**). After the removal of the protective group, we created the thio-derivative **17e** from quinoline **17d**, from which we obtained molecule **17** by reacting with the corresponding hydrazide.[14] Its chiral chromatographical separation served us two pure enantiomers **18** and **19**.

It was not surprising that the introduction of polar NHBoc (**19**) or amine group **(20)** to R^2 resulted in the loss of activity. However, introduction of methoxycarbonylmethylene group **(21)** yielded a compound more active than the Lead (K_i: 28 nM, IC_{50}: 220 nM), albeit having poor metabolic stability.

By the investigation of the role of the R^1 position while having hydrogens on R^2, R^3, R^4 and R^5, it turned out to be evident, that polar groups, namely sulfone derivatives (**22–25**) and the piperidine ring with or without Boc (**26, 27**) resulted in significant reduction of activity. The smaller sized apolar cyclohexyl and methyl group (**28, 29**) showed comparatively weak activity. In contrast to this, when we replaced the R^1 group of Lead to methyl-oxazolyl-cyclohexyl (**30**) or pyridyloxy-cyclohexyl (**31**), we surpassed compound **12** both in terms of binding potency and antagonist activity.

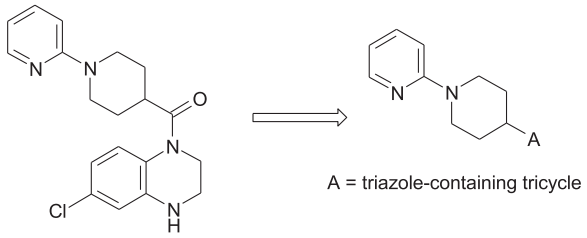
Both compounds (**30, 31**) had high microsomal stability for all species (Table 2) and high penetrability in VB-Caco-2 cultures (Compound **30** $Papp_{A\text{-}B}$: 33×10^{-6} $cm.s^{-1}$, PDR 0.8, tested at 1 μM; Compound **31** $Papp_{A\text{-}B}$: $42 \times 10^{-6} cm.s^{-1}$, PDR: 0.8, tested at 10 μM). Moreover, they turned out to be selective over the hV2 receptor (K_i: 9% and 13% at 1 μM) [12] and their hERG data[15] were also acceptable (IC_{50}: 7.6 μM and 7.2 μM), so they proceeded to *in vivo* phase. The compounds showed 45% and 66% inhibition in the oxytocin-induced scratching test[16] at the dose of 10 mg/kg in mice given intraperitoneally, respectively.

In contrary to i.p. administration, after *per os* treatment we did not observe any activity with these compounds, thus we had to improve efficacy while retaining the good physicochemical parameters. To achieve this, we modified the scaffold again: the middle ring of the tricycle was replaced to azepine (Fig. 2). The *in vitro* activity of the so obtained 5,6-dihydro-4*H*-[1,2,4]triazolo [4,3-*a*][1]benzazepine **32** reached that of **4** (K_i: 1.4 nM, IC_{50}: 3.2 nM), and its human metabolism also changed in a favorable way (see Table 3).

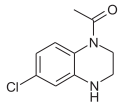
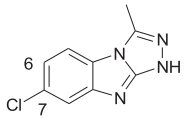
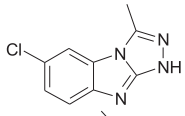
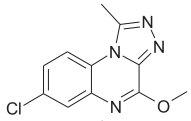
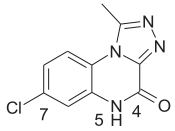
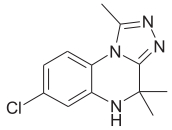
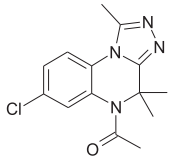
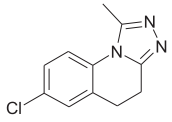
Later, we only changed R^1 and R^6 in position 8 on this highly active scaffold in order to achieve improved human metabolic stability. Keeping the pyridyl-piperidine group in R^1, substitution of R^6 with hydrogen (**34**) or ethyl (**36**) resulted in one magnitude drop of hV1a binding affinity and two orders of magnitude of functional potency. In case of bromo-substitution (**33**) binding affinity increased together with metabolic stability, though antagonist activity slightly decreased. In case of ethynyl group (**35**) *in vitro* and metabolism data were similar to those of **32** (Table 3).

When putting methyl-oxazolyl-cyclohexyl group to R^1, chloro and bromo derivatives **37** and **38** reached the activity of the balovaptan in *in vitro* assays ($K_i < 1$ nM, $IC_{50} \leq 3$ nM), while the unsubstituted **39** failed to do so. Lastly, we placed pyridyloxy-cyclohexyl group to R^1 position.

Table 1
Exploration of the triazole-containing tricyclics in position A.

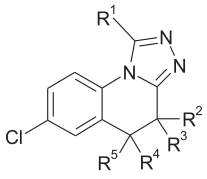


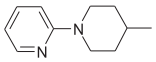
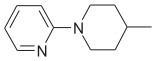
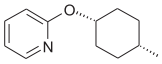
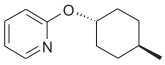
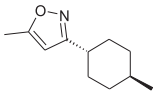
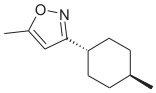
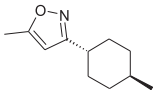
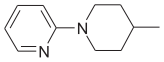
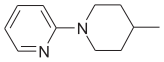
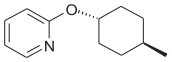
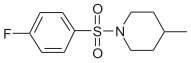
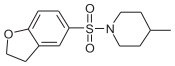
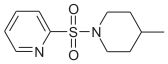
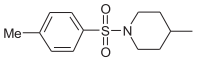
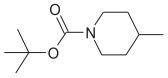
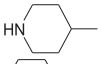
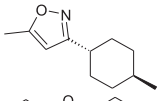
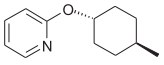
5

Ex	A	hV1a[12,b] (K_i, nM or inh.% at 10 μM)	hV1a[13,b] (IC_{50}, nM orinh.% at 10 Mm)	CL_{int} [h/r/m][10,a](μL/min/mg protein)	$Papp_{A-B}$ × 10^{-6}cm.s^{-1}/PDR[11]
4	–	0.6	2.4	72/170/235	10/1.3 at1μM
5		14	260	80/404/132	48/0.7 at10μM
6		5200	11%	–	–
7		27%	8%	–	–
8		700	40%	8/39/115	28/0.8 at1μM
9		3%	0%	–	–
10		800	28%	–	–
11		2400	43%	–	–
12 (Lead)		71	1400	0.3/32/36	51/0.8 at10μM

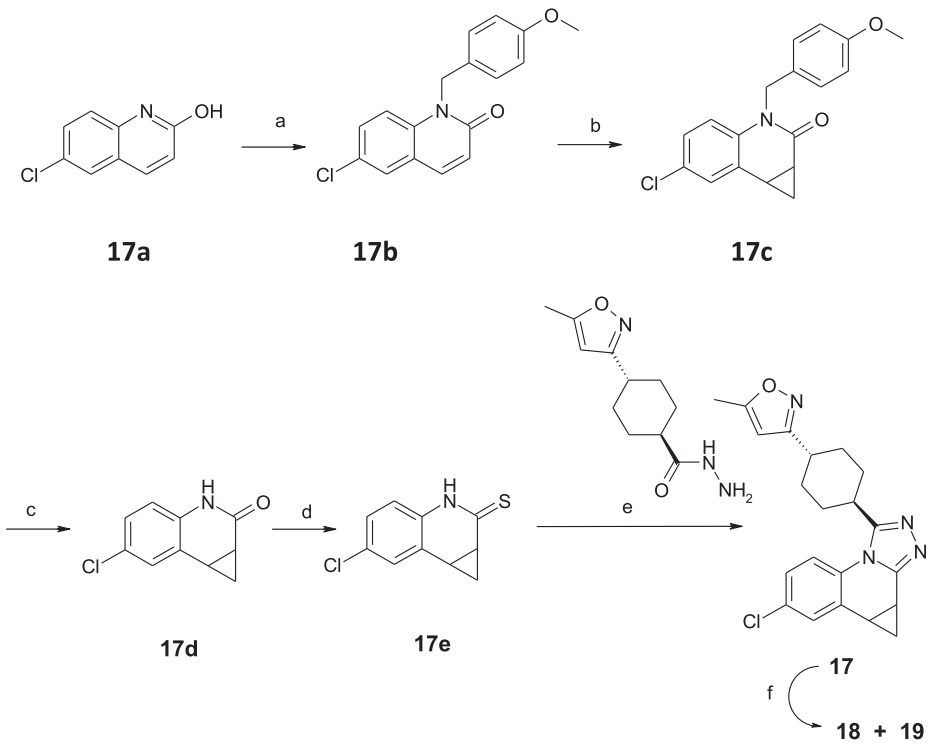
[a] CL_{int} [h/r/m] intrinsic clearance measured in h (human), r (rat) and m (mouse) liver microsomes
[b]Ki or IC_{50} was determined only if inhibition surpassed 50% at 10 μM concentration

Table 2
Exploration of substituents of [1,2,4]triazolo[4,3-*a*]quinolone.



Ex	R^1	R^2	R^3	R^4	R^5	hV1a[12,b] (Ki, nM or inh.% at10 Mm)	hV1a[13] (IC_{50}, nM)	CL_{int} [h/r/m][10,a] (μL/min/mg protein)
12 (lead)		H	H	H	H	71	1400	0.3/32/36
13		H	–CH_2-		H	260	–	–
14		H	–CH_2-		H	180	–	–
15		COOMe	–CH_2-		H	400	–	–
16 (rac)		H-	–CH_2-		H	100	500	0.3/1/3
17 (8b*R*,9a*S*)			–CH_2-			570	–	–
18 (8b*S*,9a*R*)			–CH_2-			55	635	–
19		$NHBoc$	H	H	H	25%	–	–
20		NH_2	H	H	H	34%	–	–
21		CH_2COOMe	H	H	H	28	220	81/72/373
22		H	H	H	H	36%	–	–
23		H	H	H	H	43%	–	–
24		H	H	H	H	19%	–	–
25		H	H	H	H	42%	–	–
26		H	H	H	H	37%	–	–
27		H	H	H	H	14%	–	–
28		H	H	H	H	35%	–	–
29	Me	H	H	H	H	9%	–	–
30		H	H	H	H	13	100	0.3/13/13
31		H	H	H	H	9	70	3/12/15

[a] CL_{int} [h/r/m] intrinsic clearance measured in h (human), r (rat) and m (mouse) liver microsomes.
[b] Ki was determined only if inhibition surpassed 50% at 10 μM concentration.



Scheme 1. Synthesis of compound **17–19.** Reagents and conditions: (a) 4-MeO-benzyl chloride, NaH, DMF, rt, 24 h, 38% yield; (b) $(CH_3)_3S(I)O$, NaH, DMSO, 0–25 °C, 24 h, 40% yield; (c) cerium ammonium nitrate, acetonitrile/H_2O = 9:1, 0–25 °C, 12 h, 50% yield; (d) Lawesson, pyridine, 140 °C, 3 h, quant. yield; (e) hydrazide derivative,[14] xylenes (isomer mixture), reflux, 72 h, 61% yield; (f) chiral separation, 45–46% yield.

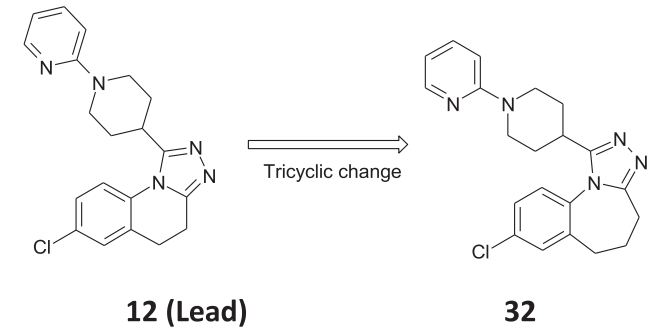


Fig. 2. Changing to 5,6-dihydro-4*H*-[1,2,4]triazolo[4,3-*a*][1]benzazepine scaffold *(for this scaffold we used the term "triazolobenzazepine" in this article)*.

As it was expected, chloro and bromo derivative **40** and **41** were more active than the unsubstituted **42**. We were glad to see that compound **40** was potent *p.o. in vivo* (94%at

30 mg/kg) and approached the *p.o.* activity of balovaptan (80%at30 mg/kg) in the mouse scratching test. The compound was turned out to be not only effective but also selective over the hV2 receptor (hV2 K_i: 560 nM) and no hERG activity (IC_{50}: 14 μM). Although compound **40** differs only by two atoms from balovaptan (CH_2 group instead of *N*-Me), methylene groups of triazolobenzazepine core can be substituted at least three different positions to enable further optimization of this core.

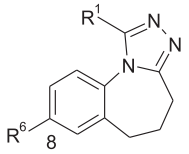
The synthesis of compounds involved in Table 3 is demonstrated on Scheme 2. The starting material was the commercially available benzazepinone **33a**, which we converted to **34** through its thio derivative in two steps. We obtained bromo-benzazepinone **33b** by bromination with good yield, and through the thio derivative **33c**, compound **33** was also obtained. The bromo compound could be converted to 8-ethynyl derivative **35** via 2 steps, and then to 8-ethyl derivative **36** by hydrogenation. The rest of the compounds **(32, 37–42)** were synthesized with the corresponding hydrazides and benzazepinones in similar manner.

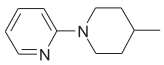
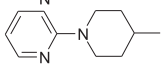
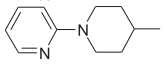
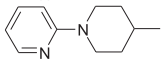
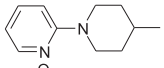
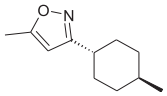
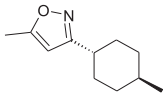
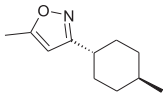
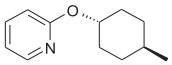
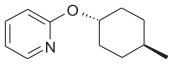
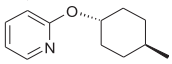
In summary, we found an *in vitro* and *in vivo orally* active V1a selective antagonist molecule (**40**) among triazolobenzazepines. The physicochemical properties and the potency of the molecule encouraged us to continue the research of selective, brain-penetrant V1a antagonists[17], which we will report later on.

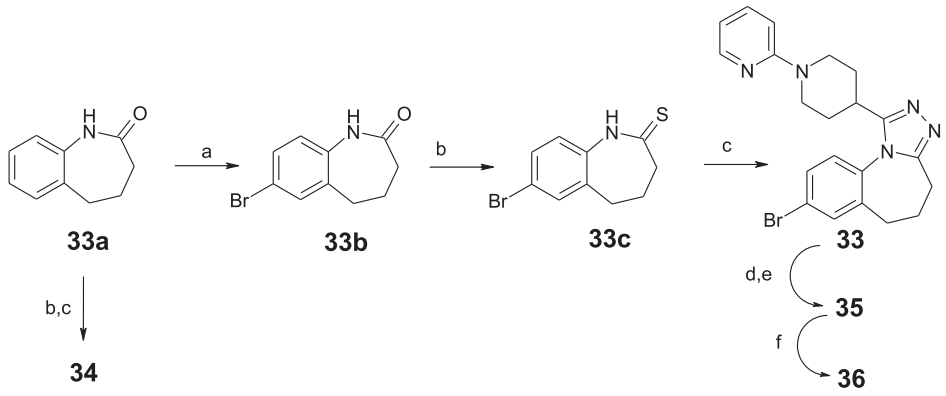
Declaration of Competing Interest

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.

Table 3
Exploration of R^1 and R^6group of triazolobenzazepine core and their ADME parameters.



Ex	R^1	R^6	hV1a [12] (Ki, nM)	hV1a [13] (IC_{50}, nM)	CL_{int} (h/r/m) [10] (µL/min/mg protein)	$Papp_{A-B}$ $\times 10^{-6}$ cm.s^{-1}/PDR[11]
32		Cl	1.4	3.2	19/196/116	37/1.1 at 1 µM
33		Br	0.7	13	8/111/50	46/0.8 at 10 µM
34		H	15	117	12/130/163	–
35		HC≡C—	0.45	2.7	27/121/115	–
36		Et	15	252	31/163/170	–
37		Cl	0.7	2.2	1/83/38	–
38		Br	0.6	1.7	3/76/36	–
39		H	1.6	25	0.4/125/114	–
40		Cl	0.8	1.0	7/124/94	29/1.3 at 10 µM
41		Br	0.6	1.3	9/110/90	29/0.9 at 10 µM
42		H	2.3	20	8/231/188	–
3	**balovaptan**	–	0.7	3	0.5/22/17	43/1.1 at 1 µM



Scheme 2. Synthesis of compound **33–36**. Reagents and conditions: (a) bromine, AcOH, H2O, rt, 3 h quant. yield; (b) Lawesson, pyridine, reflux, 3 h, 90% yield; (c) 1-(piperidin-2-yl)piperidine-4-carbohydrazide, xylene, reflux, 4 days, 70% yield; (d) trimethylsilyl acetylene, bis(triphenylphosphino) palladium(II)-diacetate, TEA, CH_3CN, reflux. 6 h, 71% yield; (e) Tetrabutylammonium fluoride, THF, rt, 1 h, 85% yield; (f) H-cube, fill H2, 0.5 mL/min, 60% yield.

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8. This is a Phase II multi-center, randomized, double-blind, 24-week, 3-arm, parallel group, placebo-controlled study to investigate the efficacy, safety, and pharmacokinetics of balovaptan in children and adolescents aged 5-17 years with ASD

(NCT02901431; AV1ATION) and a Phase III Randomized, Double-Blind, Placebo-Controlled, Efficacy, and Safety Study of Balovaptan in Adults With Autism Spectrum Disorder With a Two-Year, Open-Label Extension (NCT03504917; V1aduct).
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10. 5 mg/mL human, rat (Wistar) and mouse (NMRI) liver microsomes (n = 3) were incubated with the test compound (at 1 μM) in the presence of NADPH at 37°C. Screen Clearance classification (μL/min/mg protein): Human: Low: < 11; Medium: between 11 and 47; High: > 47; Rat: Low: < 27, Medium: between 27 and 72; High: > 72; Mouse: Low: < 25, Medium: between 25 and 48; High: > 48.
11. Bi-directional transport of test compounds at 1 or 10 μM was performed with VB-Caco-2 monolyers using the method published by Hellinger, É.; Bakk, M. L., Pócza, P. ; Tihanyi, K.; Vastag, M. Eur J Pharm Sci 2010, 41, 96.
12. hV1a and hV2 binding method: membrane prepared from 1321N1 cells (hV1a, hV2) expressing human vasopressin V1a or V2 receptors were incubated with radioligand ([3H]AVP) and increasing concentrations of test compounds (1-3 reference compounds were synthesized in house on the basis of the literature). Nonspecific binding was measured in the presence of 2 μM arginine-vasopressin in case of both targets. Radioligand displacement ability of a test compound (specific binding data) was plotted against concentration of each compound. IC50 values (affinity) were calculated from concentration-displacement curves with the help of the model 'sigmoidal fit' [y = (A1-A2)/(1 + (x/x0)p) + A2]. Finally, Ki values were calculated using the Cheng-Prusoff equation.
13. Functional hV1a in vitro assay protocol: Inhibition of vasopressin receptor activation caused by (Arg8)-vasopressin was determined in 1321N1 cells expressing human V1a receptors. Changes in intracellular Ca2+ concentrations were measured. Three average IC50 values were calculated from at least three independent measurements in all cases.
14. Corresponding hydrazide was synthesized based on the US 2014/0221350 A1 patent.
15. hERG channel measurements were performed on QPatch HTX automated patch clamp system (Sophion).
16. Functional V1a in vivo test protocol: During the experiment, mice were pretreated with the test substance or vehicle, and after the pretreatment period scratching-inducing compound (s.c. 0.3 mg/kg oxytocin) was administered, and then the animals were individually placed into measuring cages. Their behavior was observed for 1 hour. To reduce the exploratory activity, the animals were measured after a 1-hour habituation to the cage. The behavioral parameters were compared to the parallel measured parameters of the control animals (by LABORASTM). The behavioral inhibitory effect of the substances was calculated with average values of parallel measured vehicle treated groups and presented as the percentage of inhibition: 0% was expressed as average value of scratching behavior of vehicle pretreated animals (and phys. saline s.c. pretreated with vehicle), while 100% was expressed as average value of scratching of vehicle pretreated animals that received oxytocin subcutaneously. For statistical analysis one-way analysis of variance (ANOVA) with Tukey post hoc test were used.
17. Gedeon Richter's patent: Triazolobenzazepines as vasopressin V1a receptor antagonists (WO2019/116324A1).