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Chiral N^G-acylated hetarylpropylguanidine-type histamine H_2 receptor agonists do not show significant stereoselectivity

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Dedicated to Professor Dr. Dr. Dr. h.c. Walter Schunack, Berlin, on the occasion of his 75th birthday

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ABSTRACT

A set of chiral imidazolylpropylguanidines and 2-aminothiazolylpropylguanidines bearing N^G-3-phenyl- or N^G-3-cyclohexylbutanoyl residues was synthesized and investigated for histamine H_2 receptor (H_2R) agonism (guinea pig (gp) right atrium, GTPase assay on recombinant gp and human (h)H_2R) and for hH_2R selectivity compared to hH_1R, hH_3R and hH_4R. In contrast to previous studies on arpromidine derivatives, the present investigation of acylguanidine-type compounds revealed only very low eudismic ratios (1.1–3.2), indicating the stereochemistry of the acyl moiety to play only a minor role in this series of H_2R agonists.

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Imidazolylpropylguanidines such as arpromidine and related compounds (Fig. 1) are the most potent histamine H_2 receptor (H_2R) agonists reported in the literature so far.[1] Such compounds are interesting pharmacological tools and potential drugs, for example, for the treatment of severe congestive heart failure.[2] However, their applicability is limited. The strongly basic guanidino group is the main reason for very low oral bioavailability, non H_2R-mediated effects and the lack of penetration across the blood brain barrier. We previously reported on N^G-acylated guanidines as bioisosteres of alkylguanidines with strongly reduced basicity (by 4–5 orders of magnitude) and improved pharmacokinetic properties.[3,4] For instance, the *N*-(3-phenylbutanoyl)guanidine **5** proved to be up to 60 times more potent than histamine (**1**) at the spontaneously beating guinea pig right atrium. However, the first generation of these acylguanidines turned out to be insufficiently selective for H_2R, in particular regarding H_3R and H_4R.[5,6] This problem was solved by replacing the privileged structure, the imidazolylpropylguanidine moiety, with a bioisosteric 2-amino-4-methylthiazol-5-yl group resulting in highly potent and selective H_2R agonists.[4]

In the arpromidine series, the (*S*)-configured enantiomers, for example, of the 3,4-dihalogenated analogues **3** and **4**, are the eutomers with eudismic ratios of up to 40.[7] This motivated us to investigate the enantiomers of representative acylguanidines. As the oxo analogues of arpromidine-like compounds turned out to

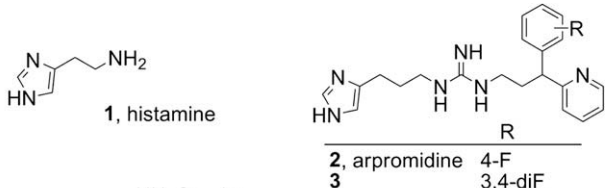

Figure 1. Structures of selected histamine H_2 receptor agonists.

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be less potent than their corresponding parent compounds,[3] branched butanoylguanidines were preferred as model compounds. The present study is focused on the synthesis and the pharmacological characterisation of the enantiomers of **5** and the corresponding cyclohexyl (**6**) and aminothiazole analogues (**7** and **8**).

The pertinent chiral building blocks were obtained via asymmetric synthesis. The building block (*R*)-3-phenylbutanoic acid ((*R*)-**11**) was synthesized from the achiral precursor, methyl (*E*)-but-2-enoate (**9**), via asymmetric addition of phenylboronic acid (**10**) as reported by Hayashi et al.,[8] using a catalytic amount of rhodium catalyst and (*S*)-binap ligand. As the enantiomeric excess (ee) of this reaction did not exceed about 80%, the methyl ester (*R*)-**11a** was recrystallized to obtain a sufficiently pure enantiomer (99% ee) which was then hydrolysed to give (*R*)-**11** (Scheme 1).

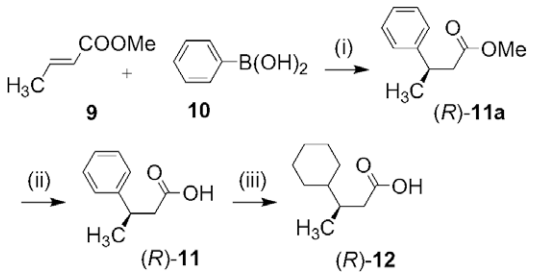
(*S*)-3-Phenylbutanoic acid was prepared according to the same procedure except for using (*R*)-binap instead of (*S*)-binap as the chiral ligand. Analytical enantioseparation of the esters (*R*)- and (*S*)-**11a** was performed by chiral HPLC, whereas the acids (*R*)- and (*S*)-**11** were analysed by means of capillary electrophoresis using (2-hydroxypropyl)-β-cyclodextrin as chiral selector. The configurations of (*R*)- and (*S*)-**11** were assigned according to the literature.[8,9] The 3-cyclohexylbutanoic acids (*R*)- and (*S*)-**12** were prepared by hydrogenation of (*R*)- and (*S*)-**11** (Scheme 1).

Coupling of the acids (*R*)-**11**, (*S*)-**11**, (*R*)-**12** and (*S*)-**12** with trityl-protected imidazolylpropylguanidine or di-Boc-protected 2-amino-4-methylthiazolylpropylguanidine, followed by cleavage of the protecting groups under acidic conditions gave the corresponding enantiomers of the N^G-acylated products **5–8** (Scheme 2).[10] For enantiomeric excess (determined by means of CE by analogy with a previously reported method[11]), HPLC purity and specific optical rotation cf. Supplementary data.

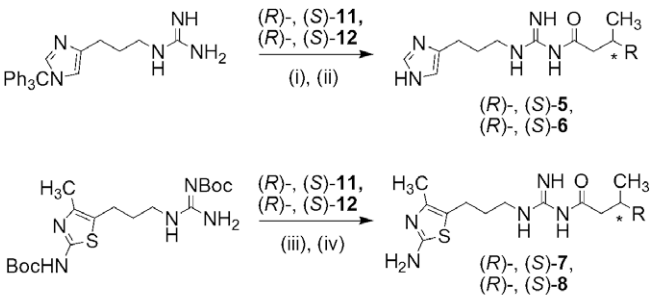
The synthesized compounds were investigated for H_2R agonistic activity on the isolated spontaneously beating guinea pig right atrium (positive chronotropic response)[3,4] (Table 1) and in a steady state GTPase assay using membrane preparations of Sf9 insect cells expressing guinea pig (gp) or human (h) H_2R-$G_{s\alpha S}$ fusion proteins[12] (Table 2). Furthermore, a selection of compounds was investigated for H_2R selectivity versus hH_1R, hH_3R and hH_4R in GTPase assays using recombinant human histamine receptors[3,4,13] (Table 3).

On the spontaneously beating guinea pig right atrium (*S*)-**5** proved to be the eutomer with a eudismic ratio of 3.7. (*S*)-**5** was a full agonist, whereas the intrinsic activity of (*R*)-**5** was slightly reduced. This is in agreement with the results for the corresponding 2-aminothiazole analogues: (*S*)-**7** was found to be more active than (*R*)-**7** by a factor of 2.6. The result is in accordance with the data from the GTPase assay on guinea pig H_2R-$G_{s\alpha S}$ fusion protein (Table 2). Here also (*S*)-**5** was found to be 3.2 times more potent than the corresponding (*R*)-enantiomer. Moreover, similar to the results from guinea pig right atrium, the 2-aminothiazole analogue (*S*)-**7** was two times more active than its optical antipode. The efficacy was also higher for the (*S*)-enantiomer. The enantiomers of the 3-cyclohexylbutanoyl- and the 3-phenylbutanoylguanidine derivatives **6** and **5** show the same preference for H_2Rs: higher potency resides in the (*S*)-configured cyclohexyl-substituted enantiomer ((*S*)-**6**) with a eudismic ratio of about three (guinea pig atrium and GTPase assay on human and guinea pig H_2R-$G_{s\alpha S}$). The aminothiazolyl enantiomers (*R*)-**8** and (*S*)-**8** apparently show an inverse preference ((*R*)-**8** > (*S*)-**8**) compared to both the corresponding imidazolylpropyl analogue ((*S*)-**6** > (*R*)-**6**) and 3-phenylbutanoyl substituted analogue ((*S*)-**7** > (*R*)-**7**), but the activity ratios of the enantiomers are not significantly different from one (guinea pig atrium and GTPase assays). The eudismic ratios of the 2-amino-4-methylthiazol-5-ylpropylguanidines were lower by trend than those of the imidazolylpropylguanidines. Taken together, the data suggest that the H_2R binding modes of the present stereoisomers are very similar.

As reported recently,[4] the N^G-acylated aminothiazolylpropylguanidines are devoid of any relevant agonistic or antagonistic activity on hH_1R, hH_3R and hH_4R. This is also true for the racemates and the enantiomers of compounds **7** and **8** (Table 3). In contrast,



Scheme 1. Synthesis of (*R*)-3-phenylbutanoic acid ((*R*)-**11**) and (*R*)-3-cyclohexylbutanoic acid ((*R*)-**12**). Reagents and conditions: (i) [Rh(acac)(C_2H_4)$_2$]/(*S*)-binap, dioxane/H_2O (10/1), 100 °C, 16 h; (ii) LiOH, THF, rt; (iii) H_2, Rh/C or Rh/Al_2O_3 (cat.), AcOH, 8 bar, 48 h, rt.



compd.	R	compd.	R
(*R*)- and (*S*)-**5**	Ph	(*R*)- and (*S*)-**7**	Ph
(*R*)- and (*S*)-**6**	cHex	(*R*)- and (*S*)-**8**	cHex

Scheme 2. Coupling of chiral acids with guanidine building blocks. Reagents and conditions: (i) CDI (1.1 equiv), NaH (60% dispersion in mineral oil) (2 equiv), THF, 5 h, rt; (ii) TFA (20%), DCM, 5 h, rt; (iii) EDC···HCl (1 equiv), HOBt···H_2O (1 equiv), DIPEA (1 equiv), DCM, 15 h, rt; (iv) TFA (20%), DCM, 3–5 h, rt.[10]

Table 1
Histamine H_2 receptor agonism at the spontaneously beating guinea pig right atrium

Compd	pEC_{50}[a]	Relative potency[b]	E_{max}[c] (%)	(*R*):(*S*)[d]
Histamine	6.00 ± 0.10	1	100 ± 2	
(±)-**5**[3]	7.80 ± 0.07	63.50 ± 17.85	99 ± 2	1:3.7 ± 1.2
(*R*)-(+)-**5**	7.03 ± 0.12	10.71 ± 3.85	93 ± 1	
(*S*)-(−)-**5**	7.60 ± 0.08	39.81 ± 11.74	102 ± 2	
(±)-**6**[3]	7.17 ± 0.07	14.70 ± 4.13	101 ± 3	1:3.1 ± 1.0
(*R*)-(+)-**6**	6.99 ± 0.07	9.77 ± 2.75	82 ± 4	
(*S*)-(−)-**6**	7.48 ± 0.12	30.20 ± 10.86	103 ± 2	
(±)-**7**[4]	7.31 ± 0.17	20.42 ± 9.27	89 ± 3	1:2.6 ± 0.9
(*R*)-(−)-**7**	6.99 ± 0.10	9.77 ± 3.18	73 ± 4	
(*S*)-(+)-**7**	7.40 ± 0.11	25.12 ± 8.60	87 ± 3	
(±)-**8**[4]	7.16 ± 0.06	14.45 ± 3.88	74 ± 8	1.6:1 ± 0.7
(*R*)-(+)-**8**	7.24 ± 0.14	17.38 ± 6.89	80 ± 4	
(*S*)-(−)-**8**	7.04 ± 0.11	10.96 ± 3.75	78 ± 3	

[a] pEC_{50} was calculated from the mean shift ΔpEC_{50} of the agonist curve relative to the histamine reference curve by equation: $pEC_{50} = 6.00 + 0.13 + \Delta pEC_{50}$; summand 0.13 represents the mean desensitization observed for control organs when two successive curves for histamine were performed (0.13 ± 0.02, $N = 16$). The SEM given for pEC_{50} is the SEM calculated for ΔpEC_{50} for 3–6 experiments.
[b] Potency, relative to histamine = 1%.
[c] Efficacy, maximal response (%) relative to the maximal increase in heart rate induced by the reference compound histamine.
[d] SEM of the eutomer/distomer ratio due to error propagation.

Table 2
Agonistic activities on hH_2R-$G_{s\alpha S}$ and gpH_2R-$G_{s\alpha S}$ (GTPase assay)

Compd	hH_2R-$G_{s\alpha S}$[a]				gpH_2R-$G_{s\alpha S}$[a]				EC_{50} hH_2R-$G_{s\alpha S}$/ EC_{50} gpH_2R-$G_{s\alpha S}$
	E_{max}	pEC_{50}	Rel. pot.	(*R*):(*S*)[b]	E_{max}	pEC_{50}	Rel. pot.	(*R*):(*S*)[b]	
His[c]	1.00	5.90 ± 0.09	1	—	1.00	5.92 ± 0.09	1	—	1.05
(±)-**5**[3,13]	0.87 ± 0.01	7.17 ± 0.01	18.81 ± 3.77		1.03 ± 0.06	7.92 ± 0.04	100.00 ± 21.61		5.58
(*R*)-**5**	1.01 ± 0.16	6.92 ± 0.01	10.48 ± 2.08	1:1.5	1.01 ± 0.10	7.12 ± 0.12	15.83 ± 5.24	1:3.2	1.58
(*S*)-**5**	1.07 ± 0.17	7.10 ± 0.03	15.87 ± 3.36	±0.1	1.19 ± 0.17	7.62 ± 0.09	50.21 ± 14.53	±1.1	3.32
(±)-**6**[3,13]	0.87 ± 0.05	7.64 ± 0.06	54.78 ± 13.01		1.11 ± 0.16	8.05 ± 0.05	133.33 ± 30.50		2.55
(*R*)-**6**	0.85 ± 0.02	7.73 ± 0.05	67.02 ± 15.44	1:2.9	0.90 ± 0.02	7.72 ± 0.13	62.83 ± 22.30	1:2.8	0.98
(*S*)-**6**	0.99 ± 0.01	8.19 ± 0.03	193.85 ± 40.73	±0.4	0.93 ± 0.00	8.11 ± 0.10	155.84 ± 46.42	±1.1	0.84
(±)-**7**[4]	0.82 ± 0.01	7.30 ± 0.13	25.15 ± 8.83		0.98 ± 0.00	7.65 ± 0.01	53.10 ± 10.68		2.22
(*R*)-**7**	0.65 ± 0.04	7.27 ± 0.03	23.59 ± 4.99	1:1.9	0.86 ± 0.00	7.77 ± 0.18	70.59 ± 31.94	1:2.2	3.14
(*S*)-**7**	0.87 ± 0.01	7.55 ± 0.12	44.84 ± 15.56	±0.5	0.99 ± 0.02	8.11 ± 0.12	153.85 ± 51.60	±1.1	3.60
(±)-**8**[4]	0.56 ± 0.05	8.03 ± 0.13	134.04 ± 47.97		0.82 ± 0.03	8.12 ± 0.17	157.89 ± 69.87		1.24
(*R*)-**8**	0.62 ± 0.04	7.69 ± 0.14	62.07 ± 23.38	1.4:1	0.80 ± 0.04	7.88 ± 0.22	90.22 ± 48.27	1.1:1	1.53
(*S*)-**8**	0.71 ± 0.05	7.54 ± 0.09	43.60 ± 12.63	±0.2	0.90 ± 0.14	7.85 ± 0.19	85.11 ± 40.56	±0.7	2.05

[a] Steady state GTPase activity in Sf9 membranes expressing hH_2R-$G_{s\alpha S}$ and gpH_2R-$G_{s\alpha S}$ was determined as described.[12] Reaction mixtures contained ligands at concentrations from 1 nM to 10 μM as appropriate to generate saturated concentration–response curves. Data were analysed by nonlinear regression and were best fit to sigmoidal concentration–response curves. Typical basal GTPase activities ranged between ~0.5 and 2.5 pmol/mg/min, and activities stimulated by histamine (His) (100 μM) ranged between ~2 and 13 pmol/mg/min. The efficacy (E_{max}) of histamine was determined by nonlinear regression and was set to 1.0. The E_{max} values of other agonists were referred to this value. Data shown are the mean ± SEM of two to three experiments or one experiment* performed in duplicate each. The relative potency of histamine was set to 1, and the potencies of other agonists were referred to this value. The ratio of the EC_{50} values of H_2R agonists for hH_2R-$G_{s\alpha S}$ and gpH_2R-$G_{s\alpha S}$ were also calculated.
[b] SEM of the eutomer/distomer ratio due to error propagation.
[c] Histamine.

Table 3
Agonist/antagonist activities on recombinant human histamine H_1, H_3 and H_4 receptors in GTPase assays[a]

Compd	hH_1R + RGS4		hH_3R + $G_{\alpha i2}$ + $\beta1\gamma2$ + RGS4		hH_4R-GAIP + $G_{\alpha i2}$ + $\beta1\gamma2$	
	pEC_{50} (pK_B)	E_{max}	pEC_{50} (pK_B)	E_{max}	pEC_{50} (pK_B)	E_{max}
Histamine	6.72 ± 0.02	1.00	7.89 ± 0.07	1.00	8.04 ± 0.18	1.00
(±)-**5**	4.88[b] ± 0.17	0.35 ± 0.05	(7.71)	—	7.82 ± 0.20	0.89 ± 0.02
(*R*)-**5**	5.21 ± 0.09	0.34 ± 0.03	(7.34 ± 0.34)	—	7.64 ± 0.24	0.85 ± 0.08
(*S*)-**5**	6.08 ± 0.20	0.53 ± 0.05	(8.73 ± 0.02)	—	8.58 ± 0.23	0.82 ± 0.04
(±)-**6**	6.82 ± 0.16	0.54 ± 0.05	(8.44)	—	8.03 ± 0.11	0.51 ± 0.09
(*R*)-**6**	7.05 ± 0.33	0.41 ± 0.04	(8.54 ± 0.17)	—	8.08 ± 0.14	0.57 ± 0.03
(*S*)-**6**	7.18 ± 0.16	0.51 ± 0.05	(8.23)	—	8.34 ± 0.05	0.59 ± 0.01
(±)-**7**	(<5)	—	(<5)	—	(<5)	—
(*R*)-**7**	(<5)	—	(<5)	—	(<5)	—
(*S*)-**7**	(<5)	—	(<5)	—	(<5)	—
(±)-**8**	(<5)	—	(5.35 ± 0.04)	—	(5.22 ± 0.04)	—
(*R*)-**8**	(<5)	—	(<5)	—	(<5)	—
(*S*)-**8**	(<5)	—	(5.56 ± 0.01)	—	(5.86 ± 0.07)	—

[a] Membrane preparations of Sf9 insect cells expressing hH_1R (co-expressed with RGS4),[14] hH_3R (co-expressed with $G_{\alpha i2}$, $G_{\beta1\gamma2}$ and RGS4),[15] or hH_4R-GAIP fusion protein co-expressed with $G_{\alpha i2}$ and $G_{\beta1\gamma2}$ were used.[16] Data shown are means ± SEM of two to three experiments in duplicates each.
[b] Data from Ref. 3.

the investigated imidazolylpropylguanidines turned out to be weak partial hH_1R agonists, potent hH_3R antagonists as well as potent partial hH_4R agonists (Table 3). As for the results at the H_2Rs, there is a distinct preference of (*S*)-**5** versus (*R*)-**5** at the hH_1R, hH_3R and hH_4R, respectively, whereas the stereoselectivity of the cyclohexyl-substituted analogues is weak and subtype-dependent.

In summary, there was no significant stereoisomeric preference at histamine receptors detectable in this set of acylguanidine-type H_2R agonists. In contrast to the arpromidine series, where eudismic ratios up to 40 (guinea pig atrium) were determined in favour of the (*S*)-enantiomers, the present acylguanidines show very low eudismic ratios in the range of 1.1–3.2 (gpH_2R-$G_{s\alpha S}$). Thus, the stereochemistry of the acyl moiety plays only a minor role in this series of H_2R agonists.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.03.082.

References and notes

1. Buschauer, A. *J. Med. Chem.* **1989**, *32*, 1963.
2. Felix, S. B.; Buschauer, A.; Baumann, G. *Eur. J. Clin. Invest.* **1995**, *25*, 42.
3. Ghorai, P.; Kraus, A.; Keller, M.; Götte, C.; Igel, P.; Schneider, E.; Schnell, D.; Bernhardt, G.; Dove, S.; Zabel, M.; Elz, S.; Seifert, R.; Buschauer, A. *J. Med. Chem.* **2008**, *51*, 7193.
4. Kraus, A.; Ghorai, P.; Birnkammer, T.; Schnell, D.; Elz, S.; Seifert, R.; Dove, S.; Bernhardt, G.; Buschauer, A. *ChemMedChem* **2009**, *4*, 232.
5. Igel, P.; Schneider, E.; Schnell, D.; Elz, S.; Seifert, R.; Buschauer, A. *J. Med. Chem.* **2009**, *52*, 2623.
6. Igel, P.; Schnell, D.; Bernhardt, G.; Seifert, R.; Buschauer, A. *ChemMedChem* **2009**, *4*, 225.

7. Götte, C.; Kracht, J.; A, M.; Schuster, A.; Bollwein, S.; Schalkhaußer, F.; Bernhardt, G.; Buschauer, A. *Arch. Pharm. Med. Chem.* **2000**, *333*, 1.21.
8. (a) Takaya, Y.; Ogasawara, M.; Hayashi, T. *Tetrahedron Lett.* **1999**, *40*, 6957; Takaya, Y.; Senda, T.; Kurushima, H.; Ogasawara, M.; Hayashi, T. *Tetrahedron: Asymmetry* **1999**, *4047*, 10.
9. (a) Defieber, C.; Paquin, J.-F.; Serna, S.; Carreira, E. M. *Org. Lett.* **2004**, *6*, 3873; (b) Suzuki, I.; Kin, H.; Yamamoto, Y. *J. Am. Chem. Soc.* **1993**, *115*, 10139; (c) Kanemasa, S.; Suenaga, H.; Onimura, K. *J. Org. Chem.* **1994**, *59*, 6949.
10. For general experimental protocols and analytical procedures cf. Supplementary data and Ref. 3,4. Experimental and analytical data of (*S*)-N^1-[3-(1*H*-imidazol-4-yl)propyl)-N^2-(3-phenylbutanoyl)guanidine, (*S*)-**5**. The enantiomer (*S*)-**5** was obtained as a colourless oil (yield 61% over two steps). ^{1}H NMR (CD_3OD), δ (ppm): 8.76 (s, 1H, Im-2-*H*), 7.34 (s, 1H, Im-5-*H*), 7.30–7.10 (m, 5H, Ph-*H*), 3.32 (m, 3H, Ph-C*H*CH_3 and NHCH_2), 2.78 (m, 4H, CH_2CO and Im-4-CH_2CH_2), 2.00 (m, 2H, Im-CH_2), 1.30 (d, J = 7.1 Hz, 3H, Ph-CHCH_3); ^{13}C NMR (CD_3OD), δ (ppm): 176.1 (quat, CO), 155.2 (quat, C=N), 146.4 (quat, Im-C-4), 134.8 (+, Im-C-2), 134.2 (quat, Ph-C-1), 129.6, 128.1, 127.6 (+, Ph-C), 117.1 (+, Im-C-5), 46.1 (−, *C*H_2CO), 41.4 (−, *C*H_2NH), 37.6 (+, Ph-*C*H), 28.1 (−, Im-4-CH_2CH_2), 22.5 (+, Im-4-CH_2CH_2), 22.3 (+, CH_3); IR (neat): 3207, 2877, 1667, 1570, 1365, 1277, 1119, 728 cm^{-1}; HREIMS: m/z for ($C_{17}H_{23}N_5O$) calcd: 313.1903, found 313.1906; 95.5% ee (CE); $[\alpha]_D^{20}$ +15.03 (c 1.1, MeCN/H_2O (1:1)); anal. HPLC: t_R 11.05 min, k' 2.84 (Luna C18, 150 × 4.6, 3 μm; 0 min: 0.05% TFA/CH_3CN 97:3, 24 min: 85:15), purity: 99%. $C_{17}H_{23}N_5O$·2TFA (541.8).
11. Schuster, A.; Bernhardt, G.; Eibler, E.; Buschauer, A.; Hesselink, W. *J. Chromatogr., A* **1998**, *793*, 77.
12. Kelley, M. T.; Bürckstümmer, T.; Wenzel-Seifert, K.; Dove, S.; Buschauer, A.; Seifert, R. *Mol. Pharmacol.* **2001**, *60*, 1210.
13. Xie, S.-X.; Kraus, A.; Ghorai, P.; Ye, Q.-Z.; Elz, S.; Buschauer, A.; Seifert, R. *J. Pharmacol. Exp. Ther.* **2006**, *317*, 1262.
14. Seifert, R.; Wenzel-Seifert, K.; Bürckstümmer, T.; Pertz, H. H.; Schunack, W.; Dove, S.; Buschauer, A.; Elz, S. *J. Pharmacol. Exp. Ther.* **2003**, *305*, 1104.
15. Schnell, D.; Burleigh, K.; Trick, J.; Seifert, R. *J. Pharmacol. Exp. Ther.* **2010**, *332*, 996.
16. Schneider, E. H.; Schnell, D.; Papa, D.; Seifert, R. *Biochemistry* **2009**, *48*, 1424.