

Bodilisant—A Novel Fluorescent, Highly Affine Histamine H₃ Receptor Ligand

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Supporting Information

ABSTRACT: A piperidine-based lead structure for the human histamine H_3 receptor (hH_3R) was coupled with the BODIPY fluorophore and resulted in a strong green fluorescent (quantum yield, 0.92) hH_3R ligand with affinity in the nanomolar concentration range (K_i $hH_3R = 6.51 \pm 3.31$ nM), named Bodilisant. Screening for affinities at histamine and dopamine receptor subtypes showed high hH_3R preference. Bodilisant was used for visualization of hH_3R in hH_3R overexpressing HEK-293 cells with fluorescence confocal laser scanning microscopy. In addition, in native human brain tissues, Bodilisant showed clear and displaceable images of labeled hH_3R .



KEYWORDS: histamine, H_3 receptor, nonimidazole derivative, GPCR, BODIPY, HEK-293 cells, tissue labeling, fluorescence confocal laser scanning microscopy, displacement, pharmacological tool

 \square he human histamine H_3 receptor is one of the four human histamine receptor subtypes $(hH_{1-4}R)$. It is a membranebound class A family G-protein-coupled receptor (GPCR) (G_{i/o} coupled) mainly expressed in the central nervous system (CNS) acting as an auto- as well as a heteroreceptor. The human histamine H₃ receptor (hH₂R) modulates the release of several neuronal neurotransmitters.^{2,3} Because of different central effects of several hH3R antagonists in preclinical and clinical trials, we have seen a need for labeled hH₃R ligands to be taken as diagnostic tools to further investigate neurological disorders in the CNS based on receptor distribution, occupation, and regulation. In histochemistry, fluorescentlabeled antibodies are of common use. 4 For the manufacturing process of antibodies, mostly animal experiments are required.^{5,6} These antibodies have to be labeled in a followup work step with a second labeled antibody for immunofluorescence; merely a few primary antibodies are directly labeled. Most antibodies are sensitive to temperature and have to be stored in freezers. The main disadvantage as compared to small fluorescent GPCR ligands is their undisplacement properties without target destruction. Small fluorescent GPCR ligands can be displaced by other ligands and consequently enable the development of fluorescence displacement assays. There are many efforts to design fluorescent GPCR ligands on the strength of their prominence as the largest and most versatile group of cell surface receptors, therefore responsible for various pharmacological functions.^{8,9} Recent research in our working group resulted in chalconebased fluorescent hH3R ligands able to label hH3R in cells and

human tissue¹⁰ based on earlier results (Mirisant-405, Mirisant-470, and Benz-Mirisant-405).^{11–13} Because these early ligands were not usable under all conditions, we wanted to design fluorescent hH₃R ligands with more properties in common in one molecule, that is, higher hH₃R affinity, versatile fluorescent wavelength, optimized fluorescence intensity, and thus a better signal-to-noise ratio. The decision has been made for 4,4difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY) dve as a fluorophore due to its high extinction coefficient, its sharp fluorescence emission peaks with high quantum yield, 14 its insensitivity to pH and solvent polarity, and its greater chemical and photochemical stability. Therefore, BODIPY is often used as a sensor for analytical detection of transmitters, like metal ions, reducing agents, nitrogen-monoxide, 15 or hydroperoxides, 16 or as labeling reagent for peptides. 17 Recently, there are efforts to design BODIPY-labeled GPCR ligands, such as purinergic receptor ligands, $^{18-21}$ β_2 -adrenoceptor agonists, 22 M_1 muscarinic receptor ligands, 23 and dopamine D_1 and D_2 receptor ligands.24

Different histamine receptor ligands were linked with BODIPY derivatives. In 2012, the human histamine H_1 receptor (h H_1R) ligand mepyramine-BODIPY 630–650 was published. Aminopotentidine was fluorescence-tagged but with moderate H_2R affinity, and a red fluorescent clobenpropit-based H_3R ligand is available: H_3/H_4 -633-AN

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269

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Scheme 1. Synthesis of Bodilisant^a

^aReagents and conditions: (i) SOCl₂, 0 °C → 60 °C, toluene, 3 h. (ii) Methyl 4-hydroxybenzoate, K_2CO_3 , KI, acetone, reflux, 72 h. (iii) LiOH, THF; H_2O , 60 °C, 1 h, precipitation with HCl. (iv) SOCl₂, 70 °C, 5 h. (v) 2,4-Dimethyl-1*H*-pyrrol, CH_2Cl_2 , $RT \rightarrow 40$ °C, 1 h. (vi) NEt₃, BF₃ × Et₂O, toluene, 80 °C, 15 min.

(Abcam, Cambridge, MA). This purchasable compound possesses moderate affinities at H₃R and does not act specifically selective toward the other histamine receptors.

In brief, the initializing precursor 3-(piperidin-1-yl)propan-1-ol hydrochloride (I) was synthesized by alkylation of piperidine with 3-chloropropan-1-ol as described in the literature²⁷ and chlorinated.²⁸ The intermediate II was used for alkylation of methyl 4-hydroxybenzoate by Williamson ether reaction.²⁹ Methyl ether III²⁸ was cleaved in basic milieu. Resulting acid IV²⁸ was chlorinated with thionyl chloride to the appropriate acid chloride V, which was reacted via condensation with 2,4-dimethyl-1H-pyrrole to VI. Final ring closure to Bodilisant was achieved with triethyl amine and boron trifluoride diethyl etherate.¹⁶ Bodilisant was purified via column chromatography (Scheme 1). For detailed synthesis procedures and analytical data, see the Supporting Information.

For the design of our novel BODIPY-labeled hH3R ligands, we chose ciproxifan and related nonimidazoles as lead structures. The imidazole moiety of ciproxifan was replaced by the piperidine group (see Pitolisant). Nonimidazole ciproxifan analogues have been claimed to possess a highly reduced interaction with the cytochrome P450 system. The basic piperidine element was labeled with a propyl group (spacer A). An ether as a polar group links the phenyl group (spacer B). Previously, we have coupled spacer B with cyanoisoindole¹³ as a fluorophore. In Mirisant-405, the phenyl group of spacer B was extended by a tetralone group. 10 Via aldol condensation, the tetralone group was converted to the fluorescent hH₃R ligand, which is part of the fluorophore. In Bodilisant, the phenyl group of spacer B is part of the fluorophore moiety. The BODIPY group represents the lipophilic residue as an element in the pharmacophore (cf. Figure 1).

The affinity of the newly designed Bodilisant at the hH₃R in a displacement assay³² is seven times higher than that of the reference compound, ciproxifan (hH₃R $K_i = 46 \pm 4$ nM).³⁷ Bodilisant is 10 times more potent at the hH₃R (hH₃R $K_i = 6.51 \pm 3.31$ nM) than recently described fluorescent chalcones,¹⁰ indicating the BODIPY-core as a tolerated lipophilic residue in the binding pocket.

For selectivity validation, Bodilisant has been screened on affinities at related hH₁R and human histamine H₄ receptor (hH₄R).^{33–35} Bodilisant possesses the highest affinity at the hH₃R with selectivity ratios higher than 250 and 1100 for hH₁R and hH₄R, respectively (hH₁R K_i = 1662 \pm 88 nM; hH₄R = K_i 7476 \pm 3853 nM) (Supporting Information).

Affinities at human dopamine receptor subtypes for Bodilisant have been determined due to colocalization of these aminergic GPCRs in different brain tissues. 3,36,38 Affinities of Bodilisant at the human dopamine D_2 and D_3

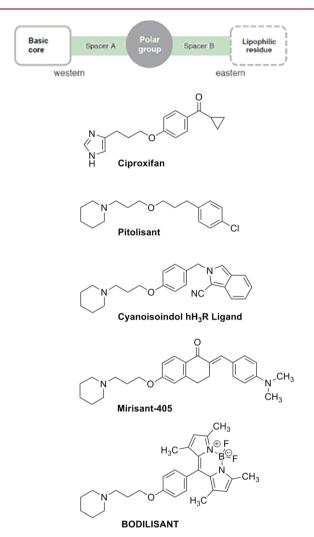


Figure 1. Design strategy for the novel fluorescent human histamine H_3 receptor ligand.

receptors are about one log unit and at the human dopamine D₁ and D₅ receptors about two log units lower than that at hH₃R (hD₁R K_i = 12513 \pm 9937 nM; hD₂R K_i = 1971 \pm 216 nM; hD₃R K_i = 1492 \pm 537 nM; and hD₅R K_i = 5210 \pm 1739 nM). Bodilisant has clearly only marginal affinity at human dopamine receptor subtypes investigated as compared to histamine receptor subtypes, especially to hH₃R (Supporting Information).

Fluorescence absorption and emission measurements were carried out in buffer (12.5 mM MgCl₂, 100 mM NaCl, and 75 mM Tris/HCl, pH 7.4) at a concentration of 10 mM to imitate fluorescence microscopy conditions. An absorption maximum

at 468 nm has been found in aqueous media. Two emission maxima could be detected: 494 and 563 nm (Figure 2). The

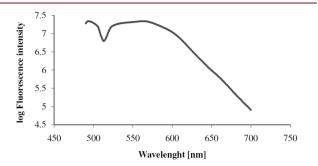


Figure 2. Fluorescence emission spectrum of Bodilisant.

Stokes shift between the absorption and the first emission maximum is very low (26 nm). To avoid additionally recording the excitation energy, the second emission maximum was only used for fluorescence microscopy.

Measurement of Bodilisant's quantum yield resulted in a value of 0.92 for which it is similar to sodium fluorescein (quantum yield, 0.91). Consequentially, Bodilisant's illuminating power is as high as sodium fluorescein, a standard for strong fluorescence substances. An advantage over sodium fluorescein is the stability of luminescence. Fluorescein compounds are easily quenched, which has led to their application in MELC technology (multiepitope ligand cartography). Bleaching experiments for 1 h with Bodilisant were not successful (results not shown).

To minimize unspecific binding, cells were incubated with 1–3% BSA solution for 30 min. Bodilisant was incubated in a concentration range between 1 and 10 nM. The best signal-to-noise ratio was achieved with a concentration of 8 nM. Bodilisant labeled hH₃R with strong green fluorescence signals in the outer cell membrane of hH₃R overexpressing human embryonergic kidney cell line (HEK-293 cells), where these membrane-bound GPCRs are localized (Figure 3 and Supporting Information). No overlay of blue fluorescent 4′,6-diamidino-2-phenylindole (DAPI), staining cell nuclei, and

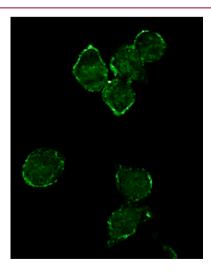


Figure 3. Labeling of hH_3R with Bodilisant on hH_3R -HEK-293 cells in the outer cell membrane. Several hH_3R -HEK-293 cells were visualized. In all cells, Bodilisant enriches in the outer cell membrane, where hH_3R are mainly expressed.

green fluorescent BODIPY could be detected. Consequently, Bodilisant was not internalized. HEK-293 cells that do not overexpress hH $_3$ R were used as a control. On the basis of Bodilisant's illuminating power, fluorescence microscopy conditions could be adjusted that negligible autofluorescence occurs. In contrast to antibodies, small fluorescent GPCR ligands are displaceable. To confirm this thesis, displacement experiments with 10 μ M pitolisant were successfully carried out. Marginal remaining signal could be detected.

To confirm localization of hH_3R on hH_3R overexpressing HEK-293 cells, an anti- hH_3R rabbit monoclonal IgG1 antibody and a red fluorescent Texas Red dye conjugated goat antirabbit IgG (H+L) secondary antibody were used for immunostaining (Supporting Information).

After successful staining of hH $_3$ R on hH $_3$ R overexpressing HEK-293 cells, we tested Bodilisant on human brain tissue. As reported from Martinz-Mir et al., hH $_3$ R are expressed in the cerebral cortex, nucleus caudatus, and globus pallidus. Nuclei of synapses were stained with DAPI. After staining with 1 μ M Bodilisant, hH $_3$ R next to synapses nuclei and distant hetero hH $_3$ R were labeled (Supporting Information). These signals were deleted after treatment with 500 μ M pitolisant or 1 mM ciproxifan. Also, Bodilisant shows high displaceable properties in brain tissue with structurally different unlabeled hH $_3$ R ligands. In paraffin tissue of globus pallidus, hH $_3$ R were detectable and structures of histaminergic nerve tracts (Figure 4). These findings confirm Bodilisant as a labeling tool in stored tissues.

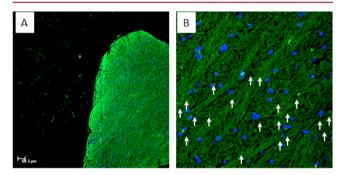


Figure 4. Staining of hH_3R with Bodilisant on human globus pallidus slices. (A) Detail of a Bodilisant-labeled globus pallidus slice. Blue areas mark Bodilisant-labeled hH_3R and histaminergic nerves. (B) Zoom of panel A. Structures of the globus pallidus and hH_3R are visible. White arrows show some histaminergic nerves in the lower part of picture.

In our work, we achieved the optimization of physicochemical and pharmacological properties via introduction of a BODIPY group as compared to our previously reported chalcone-based fluorescent hH $_3$ R ligands. The novel BODIPY dye is a high-affinity hH $_3$ R ligand with excellent fluorescence properties. Bodilisant is a strong illuminating green fluorescent compound with high affinity and good selectivity ratios. The investigation on hH $_3$ R overexpressing HEK-293 cells and in hH $_3$ R rich human brain tissue demonstrated Bodilisant's application as an useful pharmacological tool for receptor imaging. Bodilisant can be used in low concentrations (1–10 nM) for the detection of hH $_3$ R in hH $_3$ R overexpressing cells and in a concentration of approximately 1 μ M in hH $_3$ R-rich tissue. Bodilisant is applicable in aqueous neutral buffer.

ASSOCIATED CONTENT

S Supporting Information

Synthesis and analytical data of compounds V, VI, and Bodilisant and pharmacological and imaging procedures with additional material. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

 hH_1R , human histamine H_1 receptor; hH_3R , human histamine H_3 receptor; hH_4R , human histamine H_4 receptor; GPCR, G-protein-coupled receptor; CNS, central nervous system; BODIPY, 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene; HEK-293 cell, human embryonergic kidney cell line; DAPI, 4',6-diamidino-2-phenylindole

REFERENCES

- (1) Walter, M.; Stark, H. Histamine receptor subtypes: S century of rational drug design. Front. Biosci. (Schol. Ed.) 2012, 4, 461–488.
- (2) Martinez-Mir, M. I.; Pollard, H.; Moreau, J.; Arrang, J. M.; Ruat, M.; Traiffort, E.; Schwartz, J.-C.; Palacios, J. M. Three histamine receptors (H₁, H₂ and H₃) visualized in the brain of human and nonhuman primates. *Brain Res.* **1990**, *526*, 322–327.
- (3) Sander, K.; Kottke, T.; Stark, H. Histamine H₃ Receptor Antagonists Go to Clinics. *Biol. Pharm. Bull.* **2008**, *31*, 2163–2181.
- (4) Grandi, D.; Shenton, F. C.; Chazot, P. L.; Morini, G. Immunolocalization of histamine H₃ receptors on endocrine cells in the rat gastrointestinal tract. *Histol. Histopathol.* **2008**, 23, 789–798.
- (5) Chazot, P. L.; Hann, V.; Wilson, C.; Lees, G.; Thompson, C. L. Immunological identification of the mammalian H3 histamine receptor in the mouse brain. *NeuroReport* **2001**, *12*, 259–262.

- (6) Lethbridge, N. L.; Shenton, F. C.; Chazot, P. L. Generation and characterization of the first anti-human H₃R445/453 isoform specific antibody probe. *Inflammation Res.* **2009**, *58* (Suppl. 1), S43–S44.
- (7) Ramos-Vara, J. A. Technical Aspects of Immunohistochemistry. *Vet. Pathol.* **2005**, 42, 405–426.
- (8) Middleton, R. J.; Kellam, B. Fluorophore-tagged GPCR ligands. Curr. Opin. Chem. Biol. 2005, 9, 517–525.
- (9) Kuder, K. J.; Kiec-Kononowicz, K. Fluorescent GPCR ligands as new tools in pharmacology. *Curr. Med. Chem.* **2008**, *21*, 2132–2143.
- (10) Tomasch, M.; Schwed, J. S.; Weizel, L.; Stark, H. Novel chalcone-based fluorescent human histamine H₃ receptor ligands as pharmacological tools. *Front. Syst. Neurosci.* **2012**, *6* (14), DOI: 10.3389/fnsys.2012.00014.
- (11) Kuder, K.; Kottke, T.; Stark, H.; Ligneau, X.; Camelin, J.-C. Search for novel, high affinity histamine H₃ receptor ligands with fluorescent properties. *Inflammation Res.* **2010**, *59*, S247–S248.
- (12) Amon, M.; Ligneau, X.; Schwartz, J.-C.; Stark, H. Fluorescent non-imidazole histamine H₃ receptor ligands with nanomolar affinities. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1938–1940.
- (13) Amon, M.; Ligneau, X.; Camelin, J.-C.; Berrebi-Bertrand, I.; Schwartz, J.-C.; Stark, H. Highly potent fluorescence-tagged non-imidazole histamine H₃ receptor ligands. *Chem. Med. Chem.* **2007**, *2*, 708–716
- (14) Loudet, A.; Burgess, K. BODIPY Dyes and Their Derivatives: Syntheses and Spectroscopic Properties. *Chem. Rev.* **2007**, *107*, 4891–4932.
- (15) Kálai, T.; Hideg, K. Synthesis of new, BODIPY-based sensors and labels. *Tetrahedron* **2006**, *62*, 10352–10360.
- (16) Inoue, N.; Suzuki, Y.; Yokoyama, K.; Karube, I. Novel Fluorescent Probe for Analysis of Hydroperoxides Based on Boron Dipyrromethane Fluorophore. *Biosci., Biotechnol., Biochem.* **2009**, 73, 1215–1217.
- (17) Dumont, Y.; Gaudreau, P.; Mazzuferi, M.; Langlois, D.; Chabot, J.-G.; Fournier, A.; Simonato, M.; Quirion, R. BODIPYs-conjugated neuropeptide Y ligands: New fluorescent tools to tag Y1, Y2, Y4 and Y5 receptor subtypes. *Br. J. Pharmacol.* 2005, 146, 1069–1081.
- (18) Freudenthal, S. J. Fluorophordesign und Fluoreszenzmarkierung: Synthese funktionalisierter BODIPY-Derivate und Markierung von Purinrezeptor-Liganden. Ph.D. Thesis; Rheinische Friedrich-Wilhelms-Universität Bonn: Bonn, Germany, 2002.
- (19) Briddon, S. J.; Middleton, R. J.; Yates, A. S.; George, M. W.; Kellamb, B.; Hill, S. J. Application of fluorescence correlation spectroscopy to the measurement of agonist binding to a G-protein coupled receptor at the single cell level. *Faraday Discuss.* **2004**, *126*, 197–207.
- (20) Briddon, S. J.; Middleton, R. J.; Cordeaux, Y.; Flavin, F. M.; Weinstein, J. A.; George, M. W.; Kellam, B.; Hill, S. J. Quantitative analysis of the formation and diffusion of A1-adenosine receptor—antagonist complexes in single living cells. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 4673–4678.
- (21) Dale, C. L.; Hill, S. J.; Kellam, B. New potent, short-linker BODIPY-630/650 labelled fluorescent adenosine receptor agonists. *Med. Chem. Comm.* **2012**, *3*, 333–338.
- (22) Baker, J. G.; Hall, I. P.; Hill, S. J. Pharmacology and direct visualisation of BODIPY-TMR-CGP: A long-acting fluorescent β_2 -adrenoceptor agonist. *Br. J. Pharmacol.* **2003**, *139*, 232–242.
- (23) Tahtaoui, C.; Parrot, I.; Klotz, P.; Guillier, F.; Galzi, J.-L.; Hibert, M.; Ilien, B. Fluorescent Pirenzepine Derivatives as Potential Bitopic Ligands of the Human M1Muscarinic Receptor. *J. Med. Chem.* **2004**, 47, 4300–4315.
- (24) Monsma, F. J.; Barton, A. C.; Kang, H. C.; Brassard, D. L.; Haugland, R. P.; Sibley, D. R. Characterization of Novel Fluorescent Ligands with High Affinity for D1 and D2 Dopaminergic Receptors. *J. Neurochem.* 1989, 52, 1641–1644.
- (25) Rose, R. H.; Briddon, S. J.; Hill, S. J. A novel fluorescent histamine H1 receptor antagonist demonstrates the advantage of using fluorescence correlation spectroscopy to study the binding of lipophilic ligands. *Br. J. Pharmacol.* **2012**, *165*, 1789–1800.

- (26) Xie, S.-X.; Petrache, G.; Schneider, E.; Ye, Q.-Z.; Bernhardt, G.; Seifert, R.; Buschauer, A. Synthesis and pharmacological characterization of novel fluorescent histamine H2-receptor ligands derived from aminopotentidine. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3886–3890.
- (27) Apelt, J.; Grassmann, S.; Ligneau, X.; Pertzt, H. H.; Ganellin, C. R.; Arrang, J.-M.; Schwartz, J.-C.; Schunack, W.; Stark, H. Search for histamine H_3 receptor antagonists with combined inhibitory potency at N^{r} -methyltransferase: ether derivatives. *Pharmazie* **2005**, 60, 97–106.
- (28) Sander, K.; Kottke, T.; Weizel, L.; Stark., H. Kojic acid derivatives as histamine H₃ receptor ligands. *Chem. Pharm. Bull.* **2010**, *58*, 1353–1361.
- (29) Williamson, A. About the theory of the formation of ethers. *Ann. Chem.* **1851**, *77*, 37–49.
- (30) Fery-Forgues, S.; Lavabre, D. Are Fluorescence Quantum Yields So Tricky to Measure? A Demonstration Using Familiar Stationary Products. *J. Chem. Educ.* **1999**, *76*, 1260–1264.
- (31) Madge, D.; Wong, R.; Seybold, P. G. Fluorescence Quantum Yields and Their Relation to Lifetime of Rhodamine 6G and Fluorescein in Nine Solvents: Improved Absolute Standards for Quantum Yields. *Photochem. Photobiol.* **2002**, *75*, 327–334.
- (32) Kottke, T.; Sander, K.; Weizel, L.; Schneider, E. H.; Seifert, R.; Stark, H. Receptor-specific functional efficacies of alkyl imidazoles as dual histamine H₃/H₄ receptor ligands. *Eur. J. Pharmacol.* **2011**, 654, 200–208
- (33) Bradford, M. M. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248–254.
- (34) Rossbach, K.; Nassenstein, C.; Gschwandtner, M.; Schnell, D.; Sander, K.; Seifert, R.; Stark, H.; Kietzmann, M.; Bäumer, W. Histamine H_1 , H_3 and H_4 receptors are involved in pruritus. *Neuroscience* **2011**, *190*, 89–102.
- (35) Von Coburg, Y.; Kottke, T.; Weizel, L.; Ligneau, X.; Stark., H. Potential utility of histamine H3 receptor antagonist pharmacophore in antipsychotics. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 538–542.
- (36) Boecker, A.; Sasse, B. C.; Nietert, M.; Stark, H.; Schneider, G. GPCR Targeted Library Design: Novel Dopamine D3 ReceptorLigands. *Chem. Med. Chem.* 2007, 2, 1000–1005.
- (37) Ligneau, X.; Morisset, S.; Tardivel-Lacombe, J.; Gbahou, F.; Ganellin, C. R.; Stark, H.; Schunack, W.; Schwartz, J.-C.; Arrang, J. M. Distinct pharmacology of rat and human histamine H₃ receptors: Role of two amino acids in the third transmembrane domain. *Br. J. Pharmacol.* **2000**, *131*, 1247–1250.
- (38) Haas, H. L.; Sergeeva, O.; Selbach, O. Histamine in the Nervous System. *Physiol. Rev.* **2008**, *88*, 1183–1241.
- (39) Bonnekoh, B.; Böckelmann, R.; Pommer, A. J.; Malykh, Y.; Phillipsen, L. The CD11a Binding Site of Efalizumab in Psoriatic Skin Tissue as Analyzed by Multi-Epitope Ligand Cartography Robot Technology. Skin Pharmacol. Physiol. 2007, 20, 96–111.