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Pyrrolovesamicols—Synthesis, structure and VAChT binding of two 4-fluorobenzoyl regioisomers

Barbara Wenzel [a,*], Yan Li [b], Werner Kraus [c], Dietlind Sorger [d], Osama Sabri [d], Peter Brust [a], Jörg Steinbach [a]

[a] Institute of Radiopharmacy, Helmholtz-Zentrum Dresden-Rossendorf, Research Site Leipzig, 04318 Leipzig, Germany
[b] Key Laboratory of Radiopharmaceuticals, Ministry of Education, College of Chemistry, Beijing Normal University, Beijing 100875, PR China
[c] BAM Federal Institute for Materials Research and Testing, Richard-Willstätter-Str. 11, D-12489 Berlin, Germany
[d] Department of Nuclear Medicine, University of Leipzig, 04103 Leipzig, Germany

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ABSTRACT

This Letter describes the synthesis of two regioisomers of a new class of vesamicol analogs as possible ligands for imaging the vesicular acetylcholine transporter in future PET studies. The two pyrrolovesamicols (±)-**6a** and (±)-**6b** were synthesized by nucleophilic ring opening reaction of a tetrahydroindole epoxide precursor with 4-phenylpiperidine. The reaction mechanism of the synthesis was studied by HPLC and the molecular structures were determined by X-ray structure analysis. Unexpected low binding affinities to VAChT (K_i = 312 ± 73 nM for (±)-**6a** and K_i = 7320 ± 1840 nM for (±)-**6b**) were determined by competitive binding analysis using a cell line stably transfected with ratVAChT and (−)-[^{3}H]vesamicol.

Vesamicol (2-(4-phenylpiperidin-1-yl)cyclohexanol) binds with high affinity to an allosteric site of the vesicular acetylcholine transporter (VAChT). Exclusively located in presynaptic nerve terminals, this transporter is considered as valuable target for in vivo imaging of neurodegenerative processes using SPECT (Single Photon Emission Tomography) and PET (Positron Emission Tomography).[1] Vesamicol itself is not suitable as a radioligand due to its insufficient selectivity expressed by the additional binding to the σ_1 and σ_2 receptors, which are also present in cholinergic brain regions. Because only vesamicol-like structures are tolerated by this special transporter binding site, the development of new VAChT ligands has focused on structural modifications of vesamicol as chemical lead. Several classes of vesamicol analogs have been studied such as high affinity benzovesamicols,[2–6] trozamicols,[7,8] morpholino vesamicols,[9,10] and azaspirovesamicols[11,12] (selected ^{18}F-labeled representatives in Fig. 1).

For some of these compounds moderate or high binding affinities to VAChT were determined in vitro and in vivo. However, their selectivity in target binding was often neglected. This may be one of the reasons, that none of the known ligands has been really accepted for clinical application so far. Additionally, fast in vivo metabolism or inappropriate lipophilicity prevented further evaluation of some promising ligands. Therefore, we expanded our efforts to develop a VAChT ligand with improved binding and physicochemical properties. The annelation of a pyrrole moiety on the cyclohexanol ring of the vesamicol skeleton should mimic the benzene moiety of the high affine benzovesamicols in combination with a reduced lipophilicity and offers the possibility to introduce fluoro-containing groups for future ^{18}F-labeling. Thus, in this Letter we describe the synthesis, molecular structures and VAChT binding affinities of two regioisomeric 4-fluorobenzoyl substituted pyrrolovesamicols belonging to a novel class of structurally modified vesamicol analogs.

The synthetic pathway of the two new pyrrolovesamicols is shown in Scheme 1.[13] We have applied the very convenient method of nucleophilic ring opening of an epoxide precursor with 4-phenylpiperidine to get the two regioisomers (±)-**6a** and (±)-**6b**.

The synthesis of the two possible epoxide precursors **4** and **5** were performed in three steps starting with a Birch Reduction of indole to get the 4,7-dihydroindole **1**. As previously described[14,15] the additional formation of 15–20% of 4,5,6,7-tetrahydroindole was observed. Without separation of this byproduct, the benzoylation was performed by electrophilic aromatic substitution using basic additives. Usefulness of various bases and solvents was investigated. Among them triethylamine (TEA) and catalytic amounts of *N,N*-4-dimethylamino pyridine (DMAP) in chloroform turned out to be the most suitable method.

It is well known, that indoles undergo electrophilic substitution at the 3-position, whereas pyrroles are commonly substituted at the 2-position.[16] 4,7-Dihydroindoles can be considered as pyrroles resulting in the preferred substitution at the 2-position compared

* Corresponding author.
E-mail address: b.wenzel@hzdr.de (B. Wenzel).

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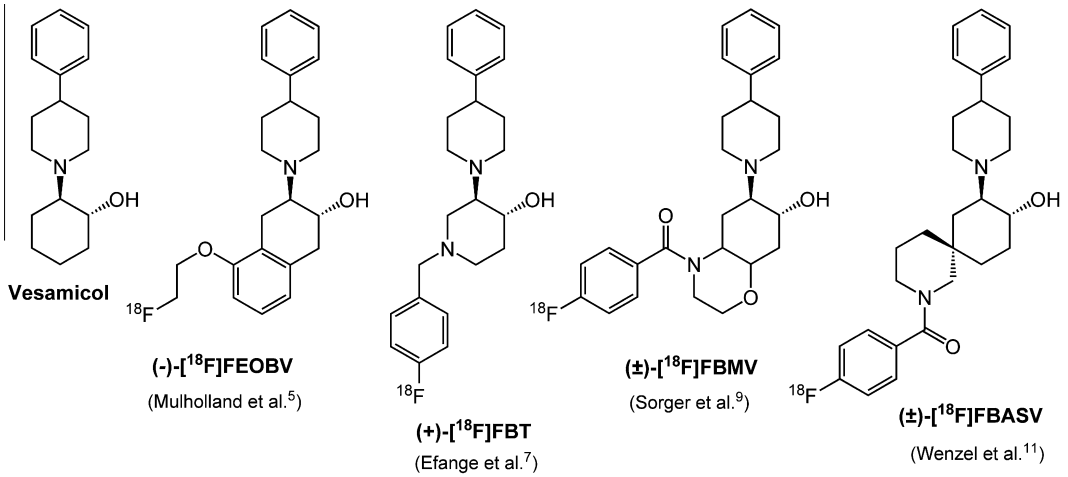
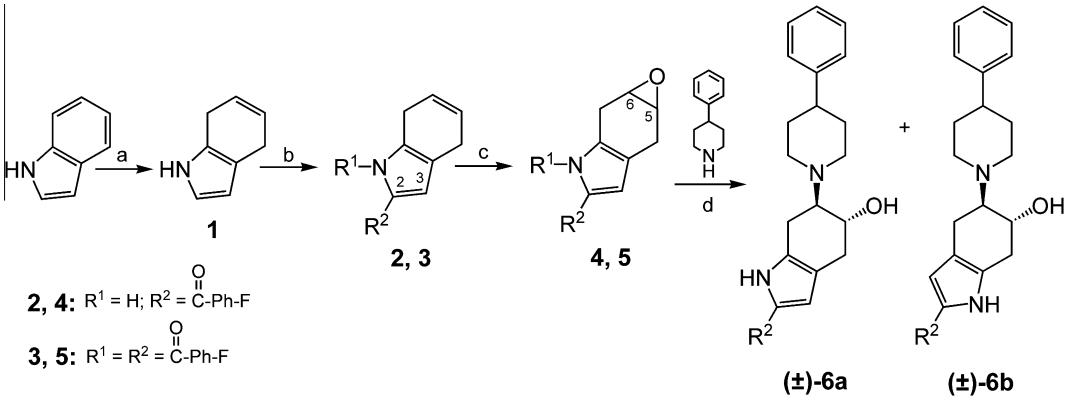


Figure 1. Vesamicol and selected ^{18}F-labeled VAChT-radioligands.



Scheme 1. Reagents and conditions: (a) Birch reduction with Li/NH_3/MeOH; (b) 4-fluorobenzoyl chloride/TEA/DMAP in $CHCl_3$, rt, 72 h; (c) *m*-CPBA in CH_2Cl_2, rt, 5 days; (d) 4-phenylpiperidine (twofold access) in ethanol, 80 °C; 10 days.[13]

to the 3-position.[17] However, using TEA and 4-fluorobenzoyl chloride in a threefold access, the benzoylation proceeded at the C2- as well as the N-position and resulted in the disubstituted derivative **3**. With lower amounts of base and 4-fluorobenzoyl chloride, the monosubstituted C2-product **2** and the C2/N-product **3** were obtained both in low yields. Under these reaction conditions decomposition and the formation of several byproducts were observed even under argon atmosphere. Compounds **2** and **3** were epoxidized with *meta*-chloroperoxybenzoic acid (*m*-CPBA) to get the corresponding epoxides **4** and **5**. The disubstituted epoxide **5** could be obtained in much better yields than the monosubstituted epoxide **4**, which was difficult to isolate.

To study the reaction behavior of the nucleophilic epoxide ring opening reaction, we started with the disubstituted epoxide precursor **5** because of its better availability. Interestingly, at 80 °C already 15 min after adding the 4-phenylpiperidine (normally in a twofold access) to the epoxide the formation of a white precipitate was observed. This intermediate was isolated and analyzed with NMR and LRMS, providing evidence for the formation of the monosubstituted epoxide **4**. To get detailed information on this phenomenon, the conversion of **5** into **4** under these reaction conditions was studied with HPLC at different reaction times (Fig. 2). Thus, 15 min after stirring at 80 °C already 12% of the monosubstituted epoxide **4** could be observed, and 24 h later the total amount of **5** was converted into **4**. Beside the decrease of **5** and the increase of **4**, the simultaneous formation of a second product was observed, which was identified as *N*-4-fluorobenzoyl substituted 4-phenylpiperidine by LRMS. In retrospect, this exchange of the 4-fluorobenzoyl group can be explained by the higher basicity of pyrrole ($pK_a \sim 25$) compared to piperidine ($pK_a \sim 11$). In consideration of this fact it is clear that the formation of the ring opening products (±)-**6a** and (±)-**6b** could only be observed due to the access of 4-phenylpiperidine in the reaction mixture and that the monosubstituted epoxide precursor **4** was the actual reactant. Using these reaction conditions, 90% of the epoxide **4** was transformed into (±)-**6a** and (±)-**6b** in an averaged ratio of 1:1.5 after 10 days. Obviously the nucleophilic attack of the amine at the C5 position of the epoxide is slightly favored compared to the C6 positions. For analysis and in vitro binding studies, the two regioisomers were isolated with flash chromatography and the isomeric purity was verified with HPLC.[13]

To verify the molecular structures of (±)-**6a** and (±)-**6b** by X-ray structure analysis, single crystals were grown from chloroform/*n*-hexane (Fig. 3).[18] Crystal structure data and selected bond length are listed in the Supplementary data file of the online version. Both regioisomers crystallize in the triclinic space group $P\bar{1}$: each with two molecules in the unit cell. The molecules contain two asymmetric carbon atoms in the cyclohexanol ring (C12/C14 for (±)-**6a** and C13/C14 for (±)-**6b**). Due to the centrosymmetry of the space group, both compounds crystallize as racemates. In doing so, the

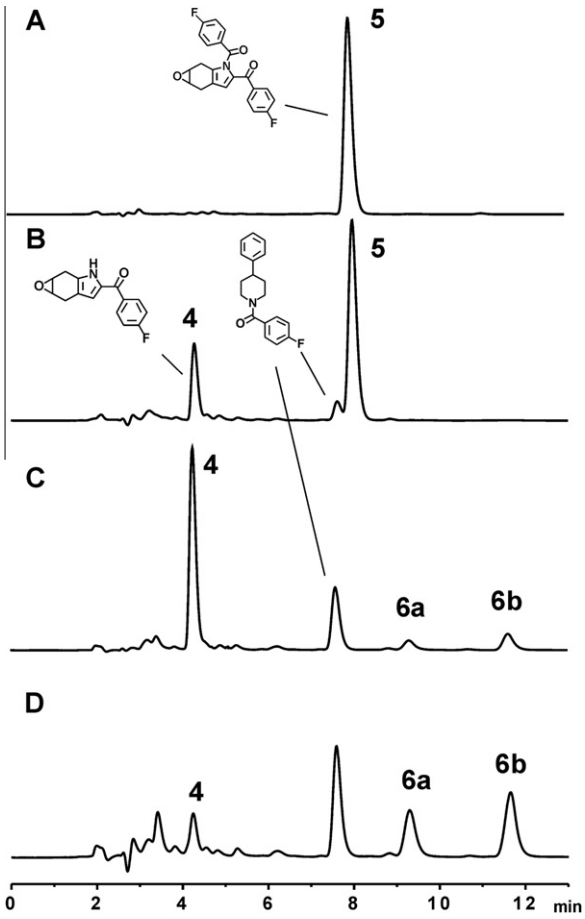


Figure 2. Chromatograms of the ring opening reaction at different reaction times. (A) epoxide precursor **5** in EtOH before reaction, (B) reaction mixture after 15 min at 80 °C, (C) reaction mixture after 24 h at 80 °C, and (D) reaction mixture after 10 days at 80 °C. *Column*: Reprosil-Pur Basic HD (250 × 4.6 mm); 70% ACN/20 mM NH_4OAc aq; 1.0 mL/min; 254 nm.

enantiomers of (±)-**6a** and (±)-**6b** are dimeric in the crystal structure because of the formation of hydrogen bonds between N1-H of the pyrrole ring and O1 of the benzoyl group as shown in part B of Figure 3. The piperidine rings are typically in chair conformation, however due to the annelation of the pyrrole moiety the cyclohexanol rings show almost half chair conformation with the hydroxyl and piperidine substituents in diequatorial position. Looking perpendicular to the imaginary plane formed by the cyclohexanol and pyrrole rings, the two oxygen atoms O1 and O2 are *trans*-oriented in (±)-**6a** and *cis*-oriented in (±)-**6b**. As illustrated in part A of Figure 3, the phenyl- and piperidine rings from molecule (±)-**6b** are disordered over two positions.

In contrast to (±)-**6b**, which contains an additional chloroform molecule in the asymmetric unit (not depicted), crystals of (±)-**6a** do not contain any solvent molecules or voids in the structure.

The binding affinities of (±)-**6a** and (±)-**6b** to VAChT were determined by competitive binding analysis using rat PC12 cells stably transfected with cDNA of rat VAChT. Commercially available (−)-[^{3}H]vesamicol ((−)-[^{3}H]AH5183) was used as radioligand to address VAChT binding sites. Its K_D value was determined as 25.6 nM (n = 30). The affinity of regioisomer (±)-**6a** is with a K_i value of 312 ± 73 nM (n = 5) slightly better than of (±)-**6b** with a value of K_i = 7320 ± 1840 nM (n = 5). Compared to (±)-vesamicol which was used as reference compound with a K_i value of 46.1 ± 4.1 nM (n = 9), the two pyrrolovesamicol derivatives demonstrated unexpected low affinities. Several causes for this considerably loss of binding affinity are possible: (i) The annelation of a five-membered aromatic heterocycle on the cyclohexanol ring of the vesamicol skeleton may be not favorable for binding. So far, only benzene or six-membered saturated heterocyclic ring systems were substituted at this position and the resulting derivatives showed good or moderate binding affinities. (ii) The secondary NH-group of the pyrrole ring may decrease the affinity as it was observed for trozamicol[19] and the decahydroquinoline[20] derivatives. This NH-group could also be responsible for the more than 20-fold lower affinity of (±)-**6b** in comparison to (±)-**6a**. A similar influence of the spatial position of a NH_2-group was observed for the regioisomers of

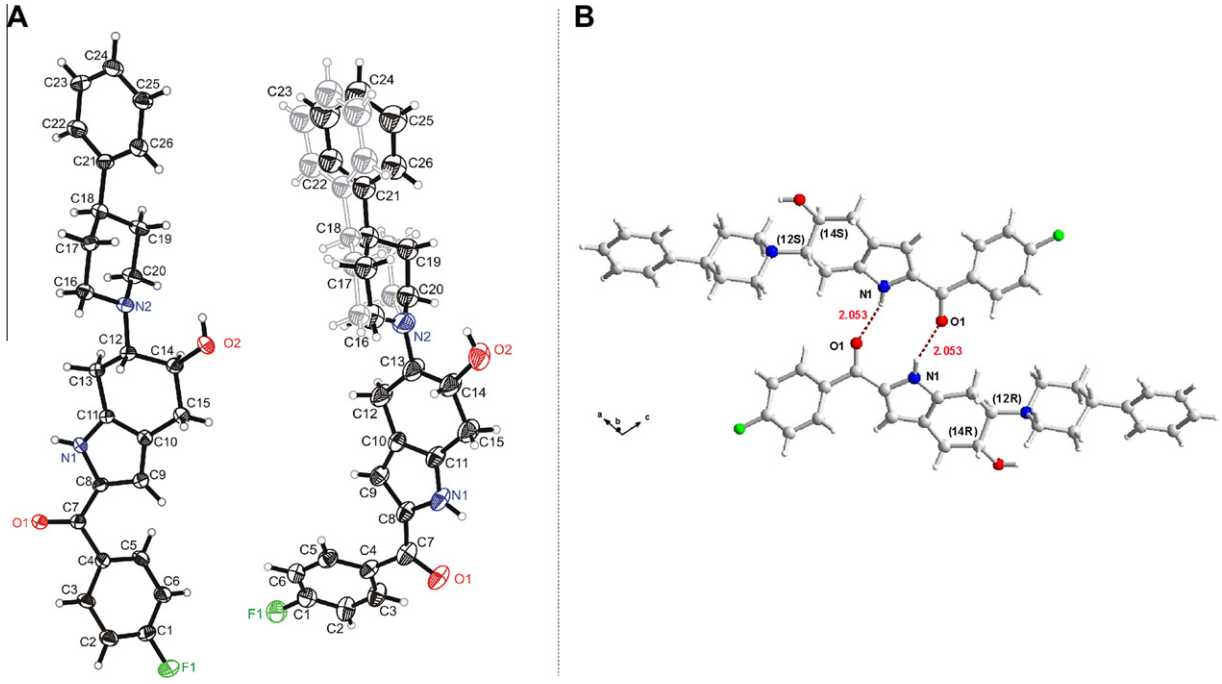


Figure 3. (A) ORTEP representation of (±)-**6a** (left) and (±)-**6b** (right) shown with 30% probability displacement ellipsoids. (B) Dimer formation of *S*,*S*-enantiomer and *R*,*R*-enantiomer in the crystal structure of (±)-**6a** with hydrogen bonds indicated by dotted lines ($d_{O1\text{-}N1}$ = 2.843(2) Å).

aminobenzovesamicol.[21] (iii) At last, the sterically demanding 4-fluorobenzoyl group might reduce the binding affinity because it is placed within the molecule at a region which is obviously sensitive for steric bulk. This was also observed for prezamicol derivatives[19] with substituents bound on the nitrogen of the cyclohexanol ring in opposite to the piperidinyl moiety. As we have recently shown in a QSAR study,[22] this region is not favorable for steric interactions. More promising would be an incorporation of the 4-fluorobenzoyl group on the pyrrole nitrogen (in opposite to the OH-group of the cyclohexanol ring) of regioisomer (±)-**6a**. This region is favorable for bulky substituents as shown for FBT (K_i = 0.44 ± 0.11 nM),[23] FBMV (K_i = 27.5 ± 8.8 nM)[9] or the aminobenzovesamicol derivative NEFA (K_i = 0.32 ± 0.04 nM).[24] However, as described in this Letter, the N-substitution of the pyrrole ring was not stable during the nucleophilic epoxide ring opening reaction. Therefore, further synthetic work and structural modifications within this new class of pyrrolovesamicols are needed to verify their suitability as VAChT ligands and to get detailed information on structure-activity relationship.

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Supplementary data

Supplementary data (detailed descriptions of syntheses and the binding assay as well as analytical data (NMR, HRMS, HPLC) and crystal structure data) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2012.01.127.

References and notes

1. Giboureau, N.; Som, I. M.; Boucher-Arnold, A.; Guilloteau, D.; Kassiou, M. *Curr. Top. Med. Chem.* **2010**, *10*, 1569.
2. Efange, S. M. N.; Khare, A. B.; von Hohenberg, K.; Mach, R. H.; Parsons, S. M.; Tu, Z. *J. Med. Chem.* **2010**, *53*, 2825.
3. Kilbourn, M. R.; Hockley, B.; Lee, L.; Sherman, P.; Quesada, C.; Frey, K. A.; Koeppe, R. A. *Nucl. Med. Biol.* **2009**, *36*, 489.
4. Mavel, S.; Kovac, M.; Deuther-Conrad, W.; Meheux, N.; Glockner, J.; Wenzel, B.; Anderluh, M.; Brust, P.; Guilloteau, D.; Emond, P. *Bioorg. Med. Chem.* **2010**, *18*, 7659.
5. Jung, Y. W.; Frey, K. A.; Mulholland, G. K.; del Rosario, R.; Sherman, P. S.; Raffel, D. M.; Van Dort, M. E.; Kuhl, D. E.; Gildersleeve, D. L.; Wieland, D. M. *J. Med. Chem.* **1996**, *39*, 3331.
6. Mulholland, G. K.; Wieland, D. M.; Kilbourn, M. R.; Frey, K. A.; Sherman, P. S.; Carey, J. E.; Kuhl, D. E. *Synapse* **1998**, *30*, 263.
7. Efange, S. M.; Mach, R. H.; Khare, A.; Michelson, R. H.; Nowak, P. A.; Evora, P. H. *Appl. Radiat. Isot.* **1994**, *45*, 465.
8. Efange, S. M.; Michelson, R. H.; Khare, A. B.; Thomas, J. R. *J. Med. Chem.* **1993**, *36*, 1754.
9. Sorger, D.; Scheunemann, M.; Vercouillie, J.; Grossmann, U.; Fischer, S.; Hiller, A.; Wenzel, B.; Roghani, A.; Schliebs, R.; Steinbach, J.; Brust, P.; Sabri, O. *Nucl. Med. Biol.* **2009**, *36*, 17.
10. Sorger, D.; Scheunemann, M.; Grossmann, U.; Fischer, S.; Vercouille, J.; Hiller, A.; Wenzel, B.; Roghani, A.; Schliebs, R.; Brust, P.; Sabri, O.; Steinbach, J. *Nucl. Med. Biol.* **2008**, *35*, 185.
11. Wenzel, B.; Hiller, A.; Fischer, S.; Sorger, D.; Deuther-Conrad, W.; Scheunemann, M.; Brust, P.; Sabri, O.; Steinbach, J. *J. Labelled Compd. Radiopharm.* **2011**, *54*, 426.
12. Wenzel, B.; Fischer, S.; Brust, P.; Steinbach, J. *J. Chromatogr., A* **2010**, *1217*, 7884.
13. Details in Supplementary data.
14. Remers, W. A.; Gibs, G. J.; Pidacks, C.; Weiss, M. J. *J. Org. Chem.* **1971**, *36*, 279.
15. Saracoglu, N.; Cavdar, H. *Tetrahedron* **2005**, *61*, 2401.
16. Scopes, D. I. C.; Allen, M. S.; Hignett, G. J.; Wilson, N. D. V.; Harris, M.; Joule, J. A. *J. Chem. Soc., Perkin Trans. 1* **1977**, 2376.
17. Wang, R.; Hong, L.; Liu, C. X.; Sun, W. S.; Wang, L.; Wong, K. *Org. Lett.* **2009**, *11*, 2177.
18. Crystallographic data (excluding structure factors) for the structures have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 853323 for (±)-**6a** and CCDC 853324 for (±)-**6b**. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, fax: +44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk.
19. Efange, S. M.; Khare, A.; Parsons, S. M.; Bau, R.; Metzenthin, T. *J. Med. Chem.* **1993**, *36*, 985.
20. Efange, S. M.; Khare, A. B.; Mach, R. H.; Parsons, S. M. *J. Med. Chem.* **1999**, *42*, 2862.
21. Rogers, G. A.; Parsons, S. M.; Anderson, D. C.; Nilsson, L. M.; Bahr, B. A.; Kornreich, W. D.; Kaufman, R.; Jacobs, R. S.; Kirtman, B. *J. Med. Chem.* **1989**, *32*, 1217.
22. Szymoszek, A.; Wenzel, B.; Scheunemann, M.; Steinbach, J.; Schüürmann, G. *J. Med. Chem.* **2008**, *51*, 2128.
23. Efange, S. M.; Mach, R. H.; Smith, C. R.; Khare, A. B.; Foulon, C.; Akella, S. K.; Childers, S. R.; Parsons, S. M. *Biochem. Pharmacol.* **1995**, *49*, 791.
24. Rogers, G. A.; Kornreich, W. D.; Hand, K.; Parsons, S. M. *Mol. Pharmacol.* **1993**, *44*, 633.