Hydrobenzoin esters of arecaidine as potential PET tracers for muscarinic acetylcholine receptors: synthesis, characterization and docking experiments

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Background and Aim

Muscarinic acetylcholine receptors (mAChR) are widely involved in neurological but also cardiac disorders and are a particularly interesting target for investigating the heart-brain axis. Unsatisfactory imaging properties of the currently available tracers hamper clinical studies of mAChRs. We recently developed the 4,4'-difluordiphenylmethyl ester of arecaidine as mAChR ligand with high affinity and a strong preference for subtype M1 (K_i = 5 nM).[a] However, autoradiography and cell binding experiments revealed pronounced nonspecific binding. To address this issue, we aim to introduce a polar hydrogen bond donor comparable to the hydroxyl in the established mAChR ligands, *e.g.* scopolamine.

Methods

Arecaidine esters with the three possible stereoisomers of hydrobenzoin were synthesized (Figure 1) and characterized by 2D-NMR, MS and HPLC (purity, logP, stability in PBS and cell culture medium). Chiral HPLC confirmed that no racemization occurred during the reaction. Binding affinities towards the mAChR subtypes M1-M5 were determined by a competitive radioligand binding assay using [^{3}H]NMS on cell membranes isolated from stably transfected CHO cells. Molecular docking was performed on a crystal structure of human mAChR M1[b] using AutoDock 4.2 with default settings in LigandScout 4.4_RC6. **1** and **2** were docked as protonated tertiary amine to consider the expected species under physiological conditions.

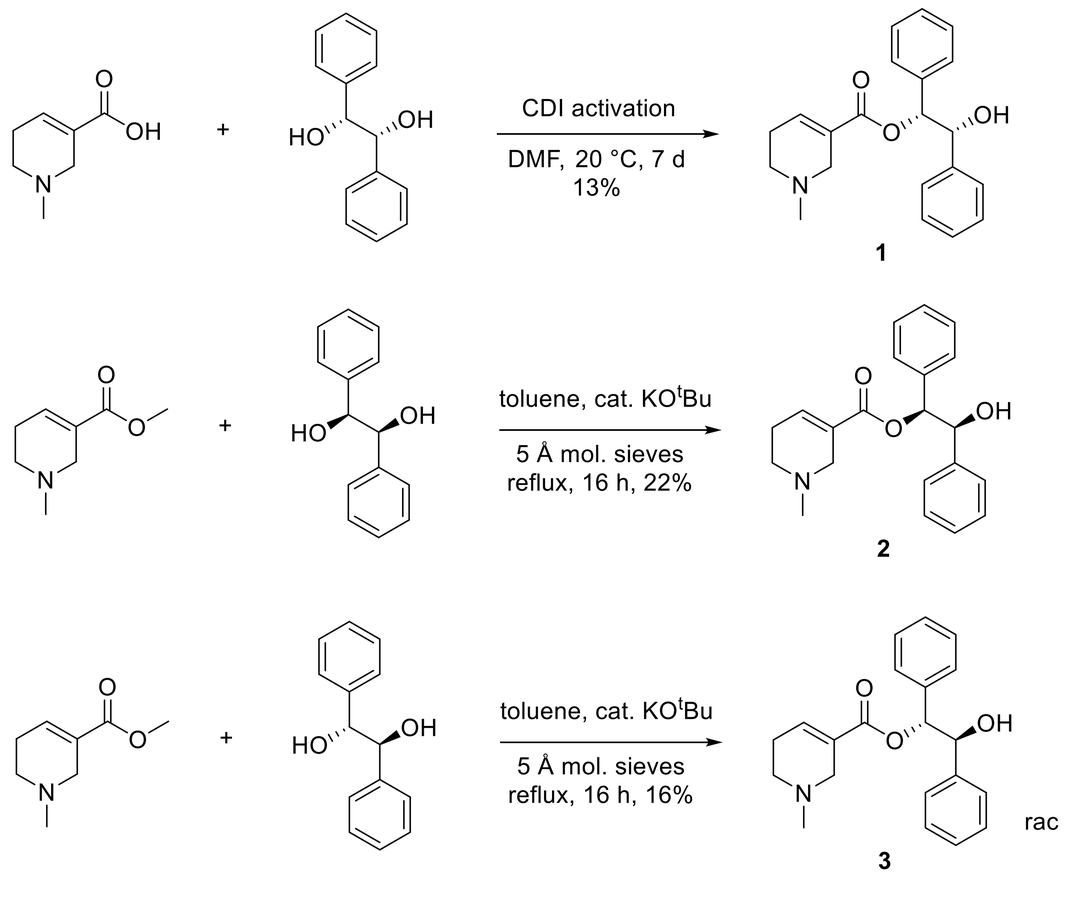

Figure 1: Stereoselective synthesis of hydrobenzoin esters of arecaidine.

Results and Discussion

HPLC-log$P_{ow}^{pH7.4}$ values were found to be 2.24±0.12 (**1**,**2**) and 2.39±0.10 (**3**), which are much lower compared to arecaidine diphenylmethyl ester (3.32±0.04)[a]. Consequently, **1**, **2** and **3** are expected to be less prone to nonspecific binding. **1**, **2** and **3** show a comparable rate of decomposition in DPBS and Ham's F12 cell culture medium at 37 °C over a time period of 60 h (Figure 2). The determined stabilities of **1**, **2** and **3** support further development as carbon-11 PET tracer, as linear interpolation between the 0 h and 10 h indicates that only 0.8% and 2.5% are decomposed after 2 h in DPBS and Ham'S F12 cell culture medium, respectively.

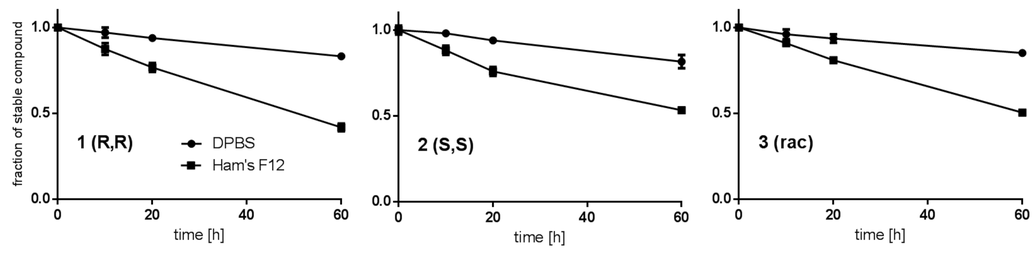

Figure 2: Stability of **1**, **2** and **3** in DPBS and Ham's F12 cell culture medium at 37 °C.

Based on the *in silico* docking experiments, the enantiomers **1** and **2** feature substantially different binding poses (Figure 3). In addition to the hydrophobic interactions and the ionic Asp105 interaction, both enantiomers show hydrogen bonds to two amino acid side chains. However, in the predicted binding pose of **1** the position of the hydroxyl group enables it to act as hydrogen bond donor and acceptor to Asn382, an amino acid that is known to be strongly involved in the binding of high affinity antagonists. Conclusively, **1** exhibits an additional hydrogen bond interaction compared to **2**, which can be seen as an indicator for higher affinity.

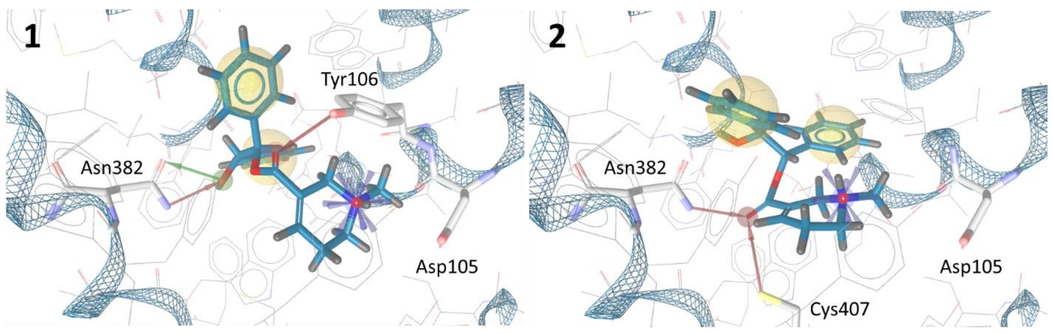

Figure 3: Calculated binding poses of protonated **1** and **2** in the orthosteric binding pocket of human mAChR M1. Amino acid residues participating in non-hydrophobic interactions are highlighted. **1** shows hydrophobic interactions to Val113, Trp157, Leu183, Ala193, Ala196, Trp378 and **2** to Tyr106, Trp157, Ala193, Ala196, Trp378, Tyr381, Val385 (not highlighted).

Comparing the *in vitro* data, all tested compounds show the highest affinity toward M1 followed by M5 and the lowest affinity to M2 (Table 1). **1** is considered most promising because it displays the highest affinity to the preferred M1 subtype. Furthermore, **1** shows the strongest subtype selectivity against all subtypes (M1 over M2, M3, M4 and M5) and the highest overall selectivity between M1 and M2 (19-fold) within the tested compounds. Especially the 7-fold M1 over M4 subtype selectivity has to be mentioned. Although it is the second lowest selectivity compared to the other receptor subtypes, it is even higher than found in pirenzepine (3.5-fold) and trihexyphenidyl (1.6-fold).[c] Using a calcium efflux assay all stereoisomers where shown to act as antagonists towards mAChR M1.

Table 1: Equilibrium dissociation constants (Ki) given in nM, n≥3, determined by [^{3}H]NMS competition binding.

	M1	M2	M3	M4	M5
1 (*R,R*)	99±19	1900±300	1300±600	700±300	600±75
2 (*S,S*)	800±200	8000±2000	1600±300	2700±600	1300±300
3 (*rac*)	380±90	3700±1000	3200±500	1600±200	970±90

The K_i value of the (*R*,*R*)-isomer **1** toward mAChR M1 is slightly above the recommended range of 3-50 nM.[d] However, the majority of previous mAChR PET tracer candidates failed because of too high affinity resulting in blood flow-dependent, instead of target-dependent, tracer distribution. Consequently, we nevertheless strive to carbon-11 radiolabel and further evaluate **1** as potential mAChR M1 PET tracer.

Conclusion

Within this work we synthesized chiral hydrobenzoin esters of arecaidine and investigated their stability and binding properties toward mAChRs. Stereochemistry was shown to have a strong impact on *in vitro* target affinity, which was also anticipated by *in silico* molecular docking. The antagonistic (*R*,*R*)-isomer **1** shows most promising characteristics to act as suitable mAChR M1 PET tracer and especially stands out because of its broad subtype selectivity compared to clinically established mAChR M1 antagonists. These properties motivate us to radiolabel **1** with carbon-11 in the future to further evaluate its applicability as brain PET tracer.

References

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