Design, Synthesis, and Biological Evaluation of 4,4'-Difluorobenzhydrol Carbamates as Selective M_1 Antagonists

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Background

Muscarinic acetylcholine receptors (mAChRs) have been found to regulate a multitude of physiological processes and are involved in many pathologies like Alzheimer's disease or multiple sclerosis. Despite ongoing research efforts, clinicians' portfolios are characterized by a lack of truly subtype-selective mAChR ligands. Our group recently made tangible progress in this direction with the discovery of highly M_1 selective benzhydrol esters of arecaidine with K_i values in the single-digit nanomolar range.[1] However, excessive nondisplaceable binding limits the usability of these ligands for molecular imaging purposes. Thus, we envisioned a structural modification of the 1,2,3,6-tetrahydropyridine moiety, which will be accompanied by replacement of the ester linkage with a carbamate motif.

Methods

A library of 52,857 commercially available amines was filtered for cyclic aliphatic primary and secondary at least mono *N*-methyl diamines. After further filtering steps, this focused selection of 331 diamine fragments was linked with 4,4'-difluorobenzhydrol via a carbamate bridge.[2] These molecules were loaded into the binding site of M_1 (5CXV) and docked using AutoDock Vina.[3] The highest ranked pose of each docked compound exhibiting an ionic interaction with Asp105$^{3.32}$ was selected as a representative resulting in a final dataset of 129 potential ligands. Manual selection from this dataset resulted in 12 primary and secondary carbamates which were synthesized via CDI-mediated alkoxycarbonylation of diamines (Figure 1 and Table 1).

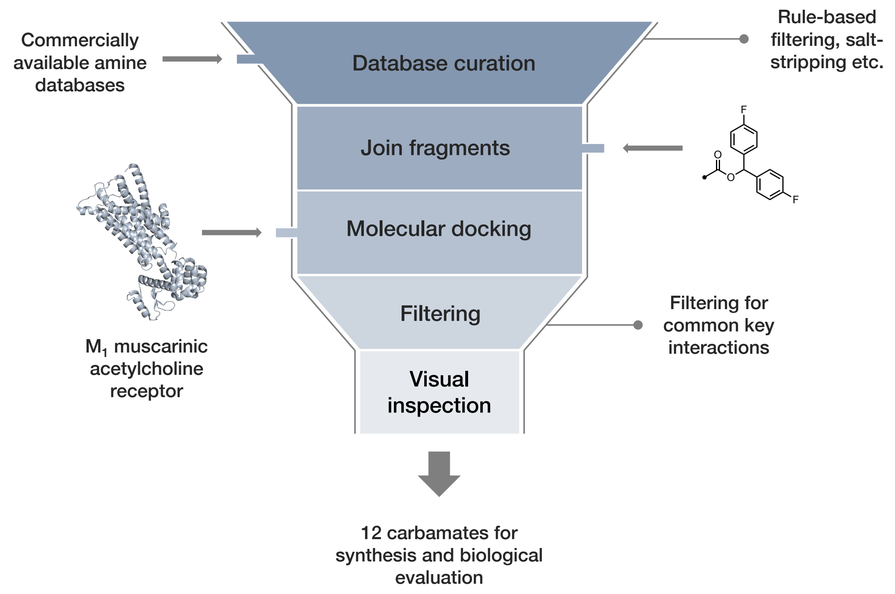
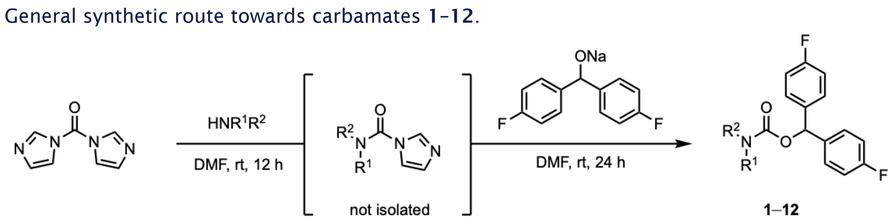
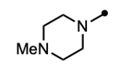
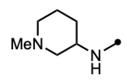
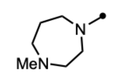
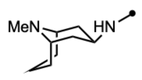
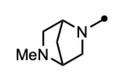
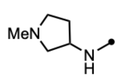
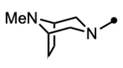
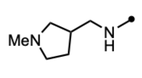
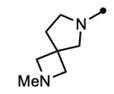
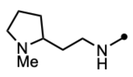
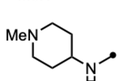
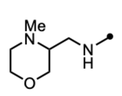


Figure 1: Schematic depiction of the utilized in silico workflow in this study.

The compounds were further evaluated for their physico-chemical parameters, especially the lipophicity in a HPLC-based assay. The compounds' biological affintiy was assessed in a competitive radiolabeling assay, and their functionality was assessed by means of Fluo-4 Direct™ Calcium Assay Kit on stably transfected CHO-*h*M_1 cells. Carbachol and scopolamine were used as positive control for evaluating the agonistic and antagonistic response.

Table 1: General synthetic route towards carbamates **1**–**12**.



Cmpd.	R^2, N, R^1	Yield (%)	Cmpd.	R^2, N, R^1	Yield (%)
1		34	**7**		34
2		38	**8**		25
3		10	**9**		19
4		25	**10**		29
5		26	**11**		28
6		22	**12**		29

Results and Discussion

A structurally diverse set of 12 carbamates was synthesized in moderate yields of up to 38% using CDI as coupling agent (Table 1). Importantly, the diamine building blocks contained an *N*-methyl tertiary amine functionality, which due to protonation under physiological conditions enables an important salt bridge interaction. This hypothesis is supported by in silico docking experiments, which show an ionic interaction between the *N*-methyl tertiary amine and Asp105$^{3.32}$ for compounds **1**–**12**. Figure 2 shows in an exemplary way, the 2D and 3D pharmacophore of **2** in the orthosteric binding pocket of M_1.

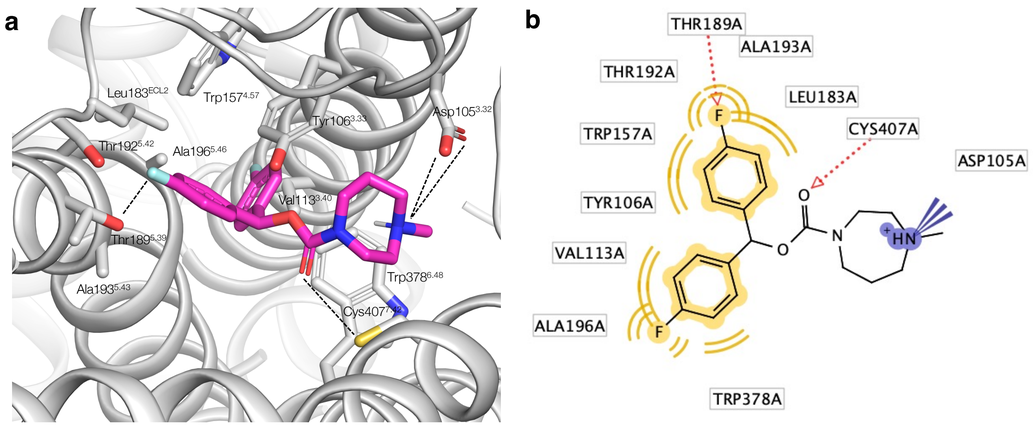


Figure 2: (a) Docking pose of **2** (carbons in magenta) in the orthosteric binding site of M_1 (PDB 5CXV) with interacting amino acid residues and key polar interactions highlighted (dashed lines); (**b**) corresponding 2D pharmacophore.

The synthetized carbamates' HPLC-logD values were found to be in a range of 2.2-3.25. The lipophilicity of this set of compounds lies below the one of the recently published highly M_1 selective benzhydrol esters,[1] enabling the assumption of lower non-specific binding and blood-brain barrier (BBB) permeability of the herein presented compounds. This is supported by calculated BBB transport parameters (log BB and log PS), which, for all of the compounds, with the exception of **12**, predict BBB permeability.[4]

Table 2: Inhibition of [^{3}H]NMS binding in CHO-*h*$M_{1\text{-}5}$ cell membrane preparations and subtype selectivity profiles for selected compounds.

	Affinity: K_i ± SD (nM)					x-fold Selectivity for *h*M_1 vs. *h*M_x			
Cmpd.	***h*M$_1$**	***h*M$_2$**	***h*M$_3$**	***h*M$_4$**	***h*M$_5$**	***h*M$_2$**	***h*M$_3$**	***h*M$_4$**	***h*M$_5$**
1	15.2 ± 3.6	>1000	225.6 ± 85.2	54.8 ± 20.5	50.6 ± 3.9	>66	14.8	3.6	3.3
2	1.2 ± 0.4	227.2 ± 85.9	28.4 ± 10.7	14.4 ± 5.5	4.8 ± 1.6	189.3	23.7	12.0	4.0
3	33.1 ± 8.1	>1000	357.8 ± 83.0	115.1 ± 51.0	68.0 ± 22.1	>30	10.8	3.5	2.1
5	24.9 ± 6.2	>1000	164.5 ± 37.5	150.3 ± 52.9	230.8 ± 25.7	>40	6.6	6.0	9.3
7	1.22 ± 0.06	32.8 ± 11.4	16.1 ± 4.5	6.2 ± 2.1	3.7 ± 1.3	27.3	13.4	5.2	3.1

While none of the tested compounds was devoid of any affinity for mAChRs, significant differences were observed among them. Table 2 highlights the affinity and subtype selectivity profiles for selected examples. Of all tested compounds, tertiary carbamate **2** and secondary carbamate **7** displayed the highest affinity towards *h*M_1 with almost equal K_i values of 1.2 and 1.22 nM. Interestingly, both compounds follow the same selectivity trend, i.e. decreasing affinities in the order *h*M_1R > *h*M_5R > *h*M_4R > *h*M_3R > *h*M_2R; however, while **7** shows moderate *h*M_1 selectivity over the *h*$M_{2\text{-}5}$ subtypes, **2** exhibits good-to-excellent selectivity versus the *h*$M_{2\text{-}4}$R (up to 189-fold) with a slightly lower 4-fold selectivity versus the *h*M_5R. All tested substances act as antagonists toward stably transfected CHO-*h*M_1 cells.

Conclusion

We present the design, synthesis, and biological evaluation of twelve 4,4'-difluorobenzhydrol carbamates available for both, radiofluorination or -methylation. The compounds show promising binding features, assessed by docking experiments as well as in binding and functional assays, for the application as antagonistic PET imaging agents.

References

[1] M. Ozenil, K. Pacher, T. Balber, C. Vraka, A. Roller, W. Holzer, H. Spreitzer, M. Mitterhauser, W. Wadsak, M. Hacker, V. Pichler, *European Journal of Medicinal Chemistry* **2020**, *204*, 112623.
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