Journal of
Medicinal Chemistry

Article

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J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.0c00297 • Publication Date (Web): 23 Mar 2020

Just Accepted

is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Regiospecific introduction of halogens on the 2-aminobiphenyl subunit leads to highly potent and selective M3 muscarinic acetylcholine receptor antagonists and weak inverse agonists

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Abstract

Muscarinic M_3 receptor antagonists and inverse agonists displaying high affinity and subtype selectivity over the anti-target M_2 are valuable pharmacological tools and may enable improved treatment of chronic obstructive pulmonary disease (COPD), asthma or urinary incontinence. Based on known M_3 antagonists comprising a piperidine or quinuclidine unit attached to a biphenyl carbamate, 5-fluoro substitution was responsible for M_3 subtype selectivity over M_2, while 3'-chloro substitution substantially increased affinity through a σ hole interaction. Resultantly, two piperidinyl- and two quinuclidinium-substituted biphenyl carbamates OFH243 (**13n**), OFH244

(**13m**), OFH3911 (**14n**) and OFH3912 (**14m**) were discovered, which display two-digit picomolar affinities with K_i values from 0.069 nM to 0.084 nM, as well as high selectivity over the M_2 subtype (46- to 68-fold). While weak inverse agonistic properties were determined for the biphenyl carbamates **13m** and **13n**, neutral antagonism was observed for **14m** and **14n** and tiotropium under identical assay conditions.

Introduction

Around thirty percent of the drugs marketed today exhibit their effects via G protein-coupled receptors. Among this family of membrane proteins, muscarinic acetylcholine receptors (mAChRs) play an important role in various physiological processes.[1] The group of mAChRs is known to comprise five receptor subtypes M_1 to M_5, which signal either via the G_q (for M_1, M_3, M_5) or G_i pathway (for M_2 and M_4). While the M_1 subtype has been shown to be involved in CNS functions including cognition, the M_2 receptor is mainly located at the heart and is further associated with the regulation of gastrointestinal motility. For the M_3 subtype, a predominant occurrence has been found at the lung and the bladder. The M_4 and M_5 receptor subtypes mainly occur in the central nervous system.[2] While the M_4 receptor has been investigated with regard to the treatment of Alzheimer[3], schizophrenia[4] and Parkinson's disease[5], little is so far known about the M_5 subtype and its biological relevance.[6]

Against this background and the multitude of diverse pharmacological functions mediated by mAChRs, high subtype selectivity is undoubtedly an important aspect for therapeutic efficacy and limitation of side effects.[7] Nevertheless, compounds with low subtype selectivity may also serve as useful pharmaceuticals for two reasons: At first, the low selectivity among the subtypes may be, to a certain extent, counterbalanced by kinetic selectivity, meaning that the drug's residence time in the desired receptor subtype is much longer than in the competing subtypes.[8] Secondly, selective

activity can be also a result of local application taking advantage of tissue-specific expression. A prominent example, in which the drug benefits from both effects, is tiotropium bromide (**1**) a non-selective muscarinic M_3 receptor antagonist to treat chronic obstructive pulmonary disease (COPD) (Figure 1).[9-10]

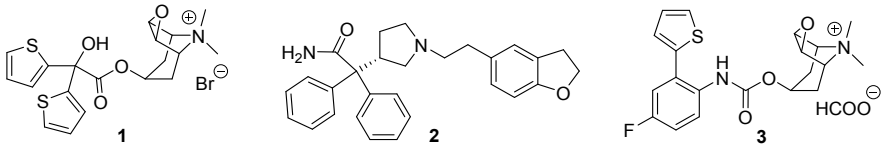


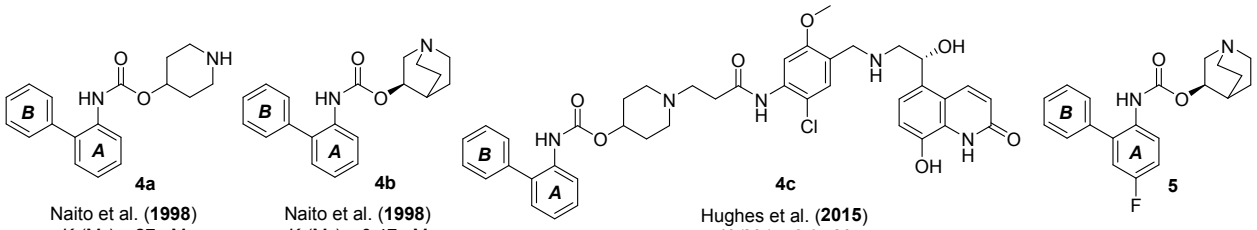
Figure 1. Tiotropium bromide (**1**), darifenacin (**2**), and BS46 (**3**).

While a number of recent studies strongly support the cardiovascular safety of tiotropium bromide,[11-12] other clinical studies still give hints to M_2 receptor mediated side effects,[13-15] thereby indicating that kinetic selectivity and local application might not fully be able to overcome a lack of subtype selectivity. An orally applied M_3 receptor antagonist showing much higher subtype selectivity is darifenacin (**2**) (Figure 1), which is less potent in respiratory disease but widely used for the treatment of urinary incontinence.[16-17] Despite its selectivity towards the M_3 subtype (6.9-fold over M_1, 56-fold over M_2), darifenacin (**2**) may still lead to adverse effects, including dry mouth, constipation and abnormal vision.[18] In addition, its *in-vivo* activity suffers from problematic metabolism and fast degradation.[19] Against this background, and despite the existence of broadly established and well-studied M_3 antagonistic drugs, such as **1** and **2**, more detailed insight about the activity, pharmacology and structural recognition of the related pharmaceutically active ligands would be highly desirable.

Today, it is possible to obtain valuable information from crystal structures leading to an increased number of GPCR ligands being designed and optimized by structure-based drug design, thereby demonstrating the vast potential of this rationale.[20] Recent crystal structures of the human M_2 and

M_3 receptor binding the antagonists *R*-(-)-3-quinuclidinyl benzilate and tiotropium bromide, respectively, showed that both ligands are located in a deeply buried binding pocket within the highly conserved transmembrane receptor core adopting a similar binding pose.[21] One significant difference between the M_2 and the M_3 subtype derives from the exchange of Phe181 (M_2) for a sterically less demanding Leu225 (M_3) in the extracellular loop ECL2. This modification offers additional space for ligands binding to the M_3 receptor, which is not available in the M_2 receptor, and it can thus be exploited to achieve high affinity and good subtype selectivity over the M_2 subtype, as we have shown in our very recent development of the M_3 receptor antagonist BS46 (**3**) (Figure 1). [21-22]

Having shown that the Phe181/Leu225 distinction between the M_2 and the M_3 receptor subtype represents an eligible starting point for the optimization of M_3 subtype-selective ligands, we continued our research on the lipophilic aromatic moieties pointing at the critical amino acids in ECL2 thereby particularly focusing on non-heterocyclic biphenyl carbamates. The biphenyl motif already was the focus of previous studies[23-26] and patents,[27-29] however none of those focused on a more detailed exploration of substituent effects on the ring systems ***A*** and ***B*** of the biphenyl moiety (Figure 2). Biphenyl carbamates such as **4a** and **4b** were for example studied by Naito,[23] and the pharmacophoric moiety was also incorporated into bivalent ligands[25-26] addressing muscarinic and adrenergic receptors. For the most prominent example of this approach, batefenterol (**4c**),[30] a dosage for phase III clinical trials was selected in 2019.[31]



4a
Naito et al. (**1998**)
K_i (M_3) = 27 nM
M_2/M_3 = 10

4b
Naito et al. (**1998**)
K_i (M_3) = 0.47 nM
M_2/M_3 = 16

4c
Hughes et al. (**2015**)
K_i (M_3) = 2.0 nM
EC_{50} (β_2) = 0.25 nM

5
K_i (M_3) = 0.34 nM
M_2/M_3 = 91

Figure 2. Examples of M_3 receptor antagonists **4a**, **4b**, batefenterol (**4c**) and ABH423 (**5**) with a biphenyl carbamate unit as common structural motif.

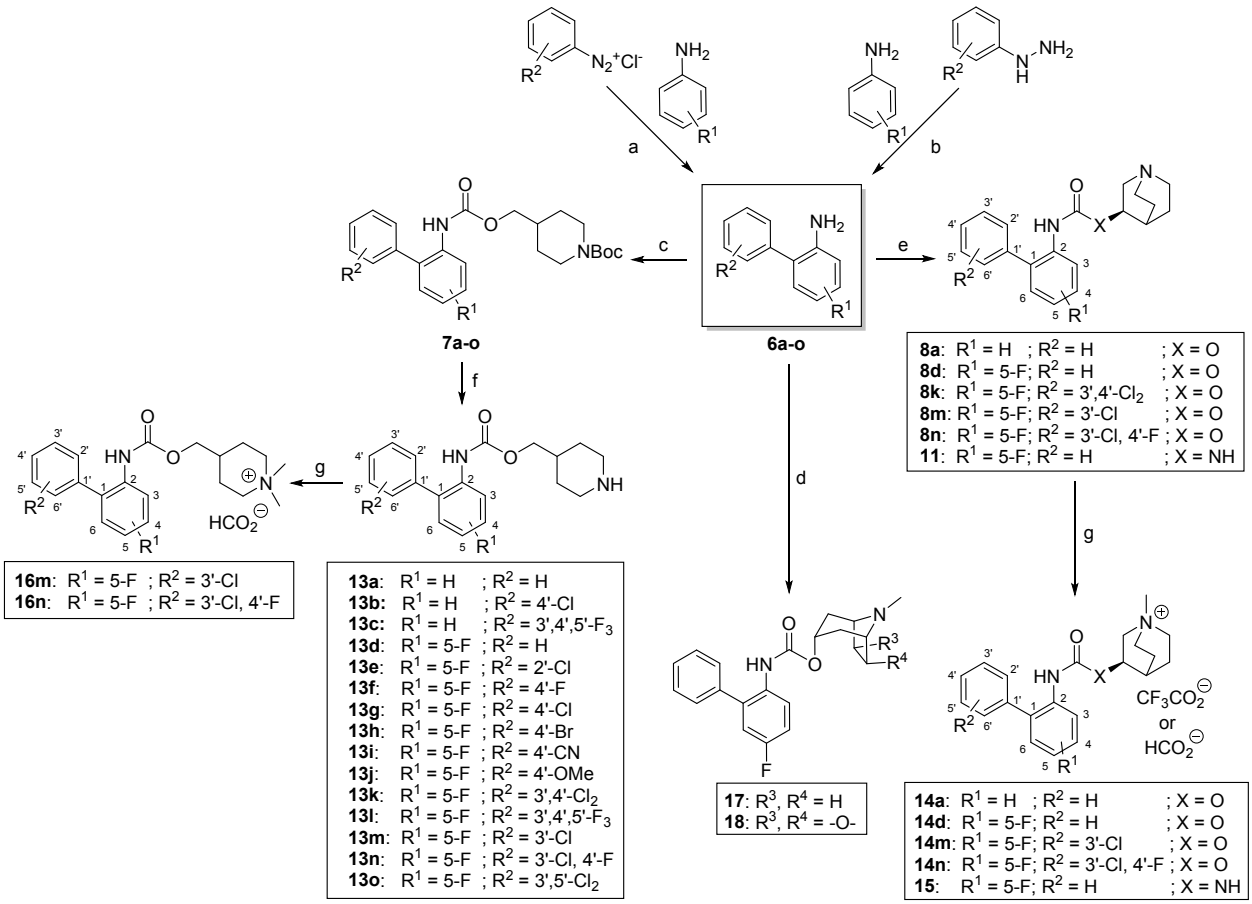
In this work, we provide insights into effects of substitution on the biphenyl unit combined with structural changes in the basic alkyl amine moiety using our previously developed, highly subtype selective compound ABH423 (**5**)[22] as a lead structure. Besides its M_3 receptor affinity and subtype selectivity over M_2, this compound was chosen due to its substantial reduction of airway resistance in mice while leaving the heart rate unaffected.[22] Important aspects addressed in this study are whether selectivity without 5-fluorine substitution on ring ***A*** can be achieved, and whether further substituents on ring ***B*** may increase affinity and selectivity. Generally, it was our goal to not only develop a ligand with high affinity for the M_3 receptor and substantial selectivity for the M_3 over the M_2 subtype, but one which would also be synthetically accessible in larger quantities to enable broad pharmacological investigations in the future. In a chemistry-driven approach using regioselective radical arylation, these multiple requirements could be met by carefully optimizing the biphenyl carbamate lead structure and by exploiting an uncommon interaction by mediated by regioselective halogen substitution.

Results

Chemistry

The synthesis of the 30 ligands **8a,d,k,m,n 11, 13a-o, 14a,d,m,n, 15, 16m,n, 17** and **18** was based on three novel radical chemistry methods for the preparation of 2-aminobiphenyls (Scheme 1).[32-34] These reactions exploit the comparably high regioselectivity that can be achieved in radical arylations of anilines under neutral or basic conditions.[35] Since the methods start from simple precursors such as aryldiazonium salts or arylhydrazines, whereat the aniline can be employed

without additional functionalization or protecting group, these reactions represent an interesting alternative to well-established Suzuki-type cross couplings. Using triphosgene, all 2-aminobiphenyls **6a-o** were then further converted to the corresponding isocyanates, which provided the carbamates **7a-o** and **8a,d,k,m,n** upon reaction with either *N*-Boc 4-piperidinemethanol (**9**) or (*R*)-quinuclidin-3-ol (**10**). The carbonyl diamide (urea) derivative **11** was obtained from **6d** and (*R*)-quinuclidin-3-amine (**12**). Trifluoroacetic acid mediated deprotection of **7a-o** gave the ligands **13a-o**, and methylation of **8a,d,m,n**, **11** and **13m,n** using methyl iodide furnished the quaternary ammonium salts **14a,d,m,n**, **15** and **16m,n** after exchange of the counterion to trifluoroacetate. A slightly modified strategy turned out as beneficial for the tropane and scopine derivatives **17** and **18**, whereat 3α-tropanol (**19**) or scopine (**20**) were at first converted by triphosgene to their corresponding chloroformates, which were finally coupled to 2-amino-5-fluorobiphenyl (**6d**).



Scheme 1. Synthetic routes to ligands **8a,d,k**,**m,n 11**, **13a-o**, **14a,d**,**m,n**, **15, 16m,n, 17** and **18** based on the radical arylation of anilines. [a]Reagents and conditions: a) NaOH, 75-95 °C; b) O_2 (air), 60 °C or MnO_2, air, rt; c) triphosgene, CH_2Cl_2, 0 °C, then *N*-boc-4-piperidinemethanol, DIPEA, CH_2Cl_2, 0 °C; d) triphosgene, CH_3CN, 0 °C, 3α-tropanol (**19)** or scopine (**20**); then **6d**, pyridine, 80 °C or 60 °C; e) triphosgene, CH_2Cl_2, 0 °C, then (*R*)-quinuclidin-3-ol (**10**) or (*R*)-quinuclidin-3-amine (**12**), toluene, 110 °C f) 10% TFA in CH_2Cl_2, rt; g) MeI, K_2CO_3, CH_2Cl_2, rt.

The identity of all prepared ligands was verified by ^{1}H NMR spectroscopy and their purity was established by analytical HPLC.

Ligand binding and functional properties

Ligand binding. Radioligand competition binding assays were performed to determine binding affinities and the selectivity profiles of the new muscarinic receptor ligands **8a,d,k**,**m,n, 11**, **13a-o**, **14a,d**,**m,n**, **15, 16m,n, 17** and **18**, in comparison with the reference drug darifenacin (**2**). The binding data were generated by measuring the ligands' ability to compete with [^{3}H]*N*-methylscopolamine using membranes of HEK293T cells transiently transfected with the cDNA of the human M_1, M_2 or M_3 receptor.

In the initial stage, the lead structure ABH423 (**5**)[22] was modified at diverse positions to explore starting points for further variations (Figure 3, variations shown in blue). Replacement of the basic quinuclidine moiety by a tropane and or a scopine unit, such as in **17** and **18**, partially reduced M_3 receptor affinity and selectivity. Variation of carbamate to give the urea derivative **11** led to a significant loss in affinity, which became even stronger after quaternization for the ammonium salt **15**. In contrast, quaternization of ABH423 (**5**) increased affinity but slightly reduced selectivity,

as determined for ligand **14d**. Further substituents at the non-fluorinated phenyl ring ***B***, as they were introduced in the 3',4'-dichloro derivative **8k**, resulted in a minor loss of affinity and selectivity, thus showing that there could be possible room for improvements associated with the optimization of the substitution pattern on ring ***B***. Among the diverse variations shown in Figure 3, the piperdin-4-ylmethyl derivative **13d** retained the highest M_3 subtype selectivity relative to the M_2 receptor at an acceptable loss of M_3 receptor affinity. Against this background, we decided to continue our studies with further variations on the biphenyl core and in combination with the (*R*)-quinuclidin-3-yl and the piperidin-4-ylmethyl units as basic side chains.

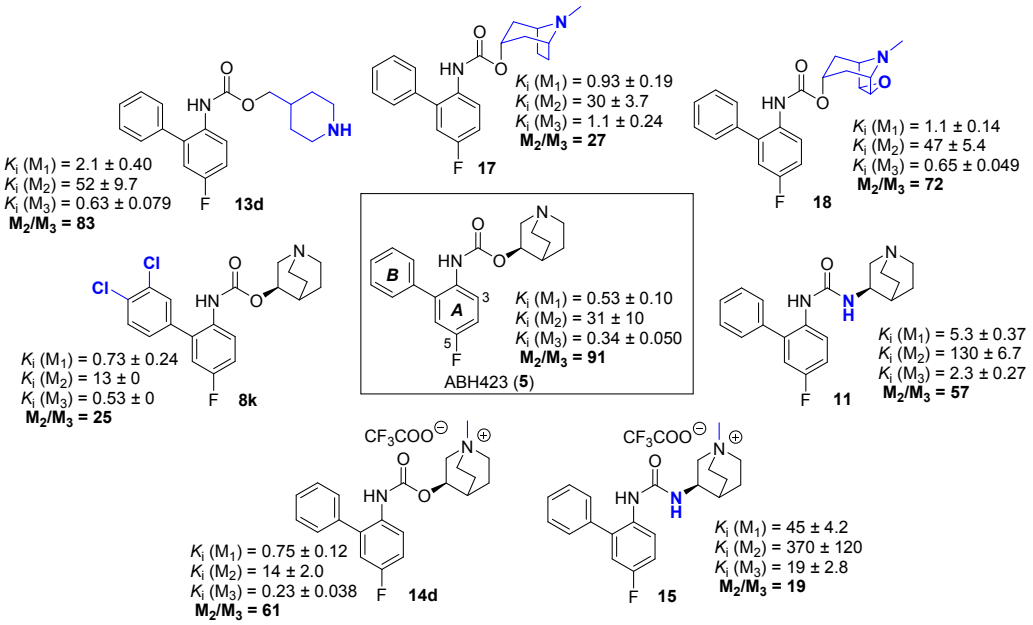


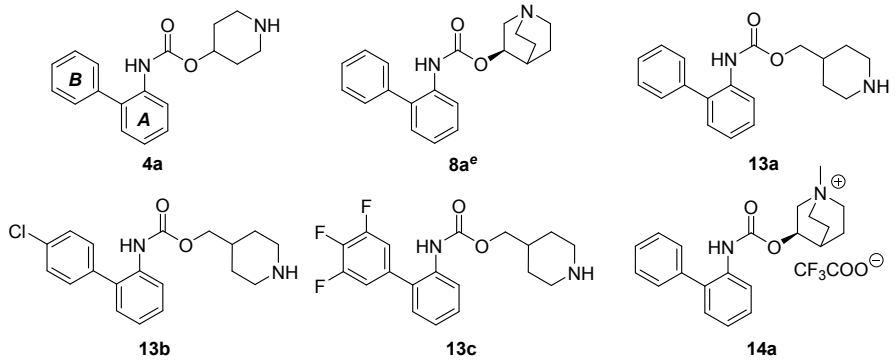
Figure 3. Initial structural variations of the lead structure ABH423 (**5**). K_i values are the means from 3-7 individual experiments each done in triplicate [nM±SEM]. For compound **8k**, **13d** and **15** binding affinity is displayed in K_i±SD derived from two experiments.

Having observed that the piperidin-4-ylmethyl unit might be a useful replacement for the (*R*)-quinuclidin-3-yl moiety, the question arose whether a certain subtype selectivity could even be achieved without 5-fluorine substitution on the ring ***A***. Table 1 gives an overview over the binding

and selectivity data determined for darifenacin (**2**) and six ligands bearing unsubstituted 2-aminobiphenyl moieties, whereat the biphenyl carbamates **4a** and **8a** (**8a** identical to **4b**, see Figure 2) were introduced to enable a comparison to the previous studies by Naito.[23] A comparison of the data obtained for darifenacin (**2**) with those previously reported,[36] shows that our assay conditions lead to comparable results for binding to the M_1 and M_3 subtypes, but our biological evaluation tends to be more sensitive for the M_2 subtype. This deviation for the M_2 subtype, which roughly corresponds to a factor of 4, was also found for the carbamate **8a** that was studied by us and previously by Naito et al.[23] As a result, the M_2/M_3 subtype selectivities reported in this work may to a certain extent be underestimated.

Notably, the best M_3 over M_2 subtype selectivities among our biphenyl carbamates were found for the piperidin-4-ylmethyl derivatives **13a** and **13c**, and **13a** also displayed a high affinity of K_i = 0.47 nM towards the M_3 receptor. The highest binding affinity to the M_3 subtype was determined for the quaternary ammonium salt **14a**, which is in agreement with the data of the closely related analogue **14d** (Figure 3), albeit **14a** showed a significantly lower M_3 over M_2 subtype selectivity than **14d**.

Table 1. Muscarinic acetylcholine M_3 receptor ligands bearing an unsubstituted ring ***A*** in the 2-aminobiphenyl moiety.



4a **8a**[e] **13a** **13b** **13c** **14a**

compound	K_i (nM ± SEM)[a]			selectivity[b]	
	M_1	M_2	M_3	M_1/M_3	M_2/M_3
darifenacin (**2**)	8.6 ± 3.3	12 ± 1.2	0.74 ± 0.14	12	16
	5.5[c]	47[c]	0.8[c]	6.9	56
4a	32[d]	270[d]	27[d]	1.2	10
8a[e]	0.56 ± 0.11	2.0 ± 0.33	0.36 ± 0.047	1.6	5.6
	0.65[d]	7.6[d]	0.47[d]	1.4	16
13a	1.8 ±0.33	17 ±3.5	0.47 ± 0.090	3.8	36
13b	34 ± 4.4	120 ± 5.8	11 ± 3.5	3.0	11
13c	3.5 ± 0.75	28 ±1.7	0.90 ± 0.080	3.9	31
14a	0.40 ± 0.090	1.7 ± 0.42	0.17 ± 0.040	2.4	10

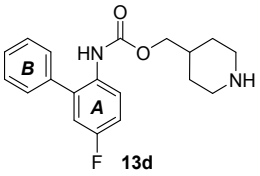
[a]K_i values in nM±SEM are the means of 3-5 individual experiments each done in triplicate. [b]Selectivity was determined by calculating the ratio of $K_i(M_1)/K_i(M_3)$ or $K_i(M_2)/K_i(M_3)$, respectively. [c]Reported affinities according to Hirose et al. (**2**, Figure 1).[36] [d]Reported affinities according to Naito et al. (**4a**, Figure 2).[23] [e]Ligand **8a** is identical to reference compound **4b** described by Naito et al. (Figure 2)[23].

The fact that a remarkable selectivity for the M_3 over the M_2 receptor can be achieved with piperidin-4-ylmethyl ligands could be due to an increased distance between the basic amine, acting as anchor unit, and the biphenyl carbamate. In this way, ring ***A*** might be pushed closer to the differentiating amino acids Phe181 (M_2) and Leu225 (M_3) in the extracellular loop ECL2, which would explain the increased subtype selectivity. Related results from computational studies are presented below and support this assumption.

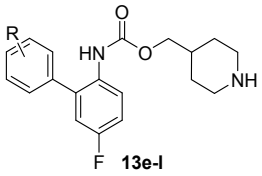
Having observed that substitution on ring ***B*** can be tolerated (Figure 3), we turned to investigate the substituent effects in more detail (Table 2). At first, it appeared that, compared to the unsubstituted biphenyl **13d**, only the small fluorine atom of **13f** in position 4' of ring ***B*** would be beneficial, albeit a slight loss of affinity. Larger atoms or groups, or poly-substitution, as they are present in the biphenyl carbamates **13e**, **13g-j** and **13l** were not well tolerated. Then, however, the high binding affinity and subtype selectivity of the 3',4'-dichloro derivative **13k** (K_i = 0.25 nM, M_2/M_3 = 68) attracted our attention, which is even more remarkable since the 4'-chloro analogue **13g** (K_i = 14 nM, M_2/M_3 = 23) showed a significantly worse profile. The increase in M_3 receptor affinity (and similarly to the other subtypes M_1 and M_2) should thus be due to a favorable ligand-

receptor interaction mediated by the chlorine atom in 3'-position. Notably, 3',4'-dichloro substitution on ring ***B*** had previously led to a reduction of M_3 receptor affinity and M_3 over M_2 subtype selectivity (c.f. ligands **8d** and **8k**, Figure 3), thereby pointing out the highly sensitive interplay of the basic amine and the substitution on the biphenyl.

Table 2. Substitution at ring ***B*** combined with 5-fluorine substitution on ring ***A***



13d



13e-l

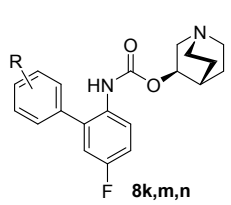
compound	R =	K_i (nM ± SEM)[a]			selectivity[b]	
		M_1	M_2	M_3	M_1/M_3	M_2/M_3
13d	--	2.1 ± 0.40	52 ± 9.7	0.63 ± 0.080	3.3	83
13e	2′-Cl	53 ± 14	980 ± 210	30 ± 6.4	1.8	33
13f	4′-F	4.8 ± 0.95	98 ± 29	1.0 ± 0.24	4.8	98
13g	4′-Cl	34 ± 1.6	320 ± 91	14 ± 2.3	2.4	23
13h	4′-Br	15 ± 1.8	340 ± 40	7.0 ± 0.41	2.1	49
13i	4`-CN	15000 ± 710[c]	28000 ± 6400[c]	2000 ± 0[c]	7.5	14
13j	4`-OMe	250 ± 21[c]	2400 ± 1400[c]	84 ± 7.1[c]	3.0	29
13k	3′,4′-Cl_2	0.66 ± 0.25	17 ± 3.5	0.25 ± 0.020	2.6	68
13l	3′,4′,5′-F_3	24 ± 2.7	520 ± 43	6.6 ± 0.42	3.6	79

[a]K_i values in nM±SEM are the means of 3-7 individual experiments each done in triplicate. [b]Selectivity was determined by calculating the ratio of $K_i(M_1)/K_i(M_3)$ or $K_i(M_2)/K_i(M_3)$, respectively. [c]*K*i values in nM±SD are the means of two individual experiments each done in triplicate.

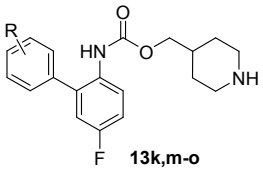
The results from the more detailed investigation of the 3'-chloro substitution effect are summarized in Table 3. Taking the 3',4'-dichloro derivatives **8k** and **13k** as reference compounds, whereat the direct comparison reveals an advantage of the basic piperidin-4-ylmethyl over the quinuclidin-3-yl moiety, the single chlorine atom in 3'-position in biphenyl carbamate **13m** was able to increase the binding affinity towards the M_3 receptor by a factor of 3 compared to **13k** while maintaining subtype selectivity on a high level. As a σ hole interaction of the 3'-chloro substituent with a closely located donor group in the receptor appeared to be a reasonable explanation for the stronger binding

of **13m**, we tried to amplify this effect by adding a further fluorine atom in 4' position of ring ***B***. Carbamate **13n**, possessing a larger σ hole at the 3'-chlorine atom than **13m** (see also molecular electrostatic potential (MEP) calculations below), then indeed displayed a further increased binding affinity (K_i = 0.069 nM), albeit at a slightly decreased M_2/M_3 subtype selectivity of 49. The effect of a second *meta* substituent on ring ***B***, which was investigated with the 3',5'-dichloro derivative **13o**, turned out to be a significantly decreased binding to the M_3 subtype, thus supporting the assumption that the binding affinity is very sensitive to changes on the ***B*** ring.

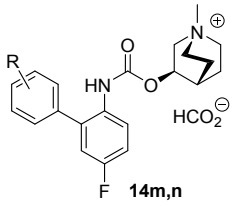
Table 3. Ligands bearing a 3'-chloro substituent on ring ***B***.



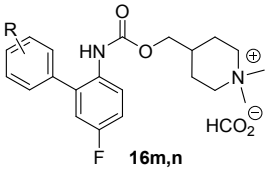
8k,m,n



13k,m-o



14m,n



16m,n

compound	R =	K_i (nM ± SEM)[a]			selectivity[b]	
		M_1	M_2	M_3	M_1/M_3	M_2/M_3
8k	3′,4′-Cl_2	0.73 ± 0.24[c]	13 ± 0[c]	0.53 ± 0[c]	1.4	25
8m	3′-Cl	0.53 ± 0.11	31 ± 18	0.39 ± 0.081	1.4	79
8n	3′-Cl, 4′-F	0.36 ± 0.079	14 ± 2.7	0.35 ± 0.083	1.0	40
13k	3′,4′-Cl_2	0.66 ± 0.25	17 ± 3.5	0.25 ± 0.020	2.6	68
13m	3′-Cl	0.34 ± 0.14	5.7 ± 1.4	0.084 ± 0.010	4.0	68
13n	3′-Cl, 4′-F	0.27 ± 0.11	3.4 ± 0.64	0.069 ± 0.010	3.9	49
13o	3′,5′-Cl_2	3.3 ± 1.2	70 ± 12	1.5 ± 0.18	2.2	47
14m	3′-Cl	0.11 ± 0.022	3.3 ± 1.2	0.072 ± 0.004	1.5	46
14n	3′-Cl, 4′-F	0.12 ± 0.009	3.7 ± 0.71	0.070 ± 0.011	1.7	53
16m	3′-Cl	1.8 ± 0.14	82 ± 10	2.7 ± 0.44	0.67	30
16n	3′-Cl, 4′-F	1.1 ± 0.096	39 ± 2.3	1.7 ± 0.13	0.65	23

[a]*K*i values in nM±SEM are the means of 3-4 individual experiments each done in triplicate. [b]Selectivity was determined by calculating the ratio of $K_i(M_1)/K_i(M_3)$ or $K_i(M_2)/K_i(M_3)$, respectively. [c]*K*i values in nM±SD are the means of two individual experiments each done in triplicate.

The favorable 3'-chloro and 3'-chloro,4'-fluoro substitution patterns on the biphenyl cores of **13m** and **13n** were further evaluated in combination with the basic quinuclidine moiety to give ligands **8m** and **8n**. Through this modification, the M_3 over M_2 subtype selectivity was largely retained, or

even improved for **8m**, but the affinities of **8m** and **8n** towards the M_3 receptor dropped significantly. Finally, quaternization of **8m,n** and **13m,n** was carried out to yield the corresponding ammonium salts **14m,n** and **16m,n**, which should show improved pharmacokinetic profiles as they cannot pass the blood-brain barrier and are thus unable to interact with M_1 receptors located in the CNS. Again, the strong interplay of the amine moiety – now being present as ammonium ion – and the biphenyl subunit became apparent. While the dimethylpiperidinium derivatives **16m,n** displayed relatively weak binding to the M_3 subtype and below average M_3 over M_2 subtype selectivities, the quinuclidinium salts **14m** and **14n** reached highly favorable binding profiles with affinities of K_i = 0.072 nM and K_i = 0.070 nM, and M_3 over M_2 subtype selectivities of 46 and 53, respectively.

In terms of binding affinity towards the M_3 subtype and selectivity for the M_3 over the M_2 receptor the biphenyl carbamates OFH244 (**13m**), OFH243 (**13n**), OFH 3912 (**14m**) and OFH3911 (**14n**) range among the best ligands ever developed for this particular muscarinic receptor subtype. In this context, it is interesting to note that 3'-chloro substitution on the ***B*** ring already appeared in earlier studies, but this effect was so far mainly exploited for the development of highly potent M_1 receptor agonists and non-selective muscarinic antagonists.[25,37-39] As briefly mentioned above, the comparably high affinities of OFH 3912 (**14m**) and OFH3911 (**14n**) to the M_1 subtype, which is predominantly located in the CNS, postganglionic nerves and exocrine glands,[40-42] are less problematic for the quaternary ammonium salts **14m** and **14n**, as these compounds are unable to pass the blood-brain barrier. For the amines OFH243 (**13n**) and OFH244 (**13m**), in contrast, brain penetration can basically not be excluded. In this context, it was interesting to see that the mild CNS related side effects of darifenacin (**2**)[43] are not related to the presence of a protonatable, aliphatic amine in the structure, but due to the fact that darifenacin (**2**) is a substrate for P-glycoprotein (P-gp) and, therefore, is actively transported away from the brain.[44] In agreement with

that, it has been reported that darifenacin (**2**) at pharmacological doses does not decrease the binding potential of a PET radioligand which can label muscarinic receptors in the rat cerebral cortex and corpus striatum.[45] Against this background, and to evaluate whether the amines OFH243 (**13n**) and OFH244 (**13m**) might also benefit from an interaction with P-gp, transcellular transport studies have been carried out (see below).

Functional profile of target compounds. Functional data at the M_3 receptor were obtained applying an HTFR based IP_1 accumulation assay (IP-One®) to measure the $G\alpha_q$ coupled activation. Arrestin recruitment was measured with an enzyme fragment complementation assay (PathHunter®) (Figure 4). Relative to the reference agonist carbachol the test compounds OFH244 (**13m**) and OFH243 (**13n**) revealed weak inverse agonist profiles with efficacies of 23% under basal activity, while OFH3912 (**14m**) and OFH3911 (**14n**) were found to be neutral antagonists for G-protein coupled signaling (Figure 4A, Table 4). For the reference compound darifenacin (**2**) a very weak inverse agonist effect (efficacy of -10%) was determined, while tiotropium bromide (**1**) was found to act as a neutral antagonist (Figure S3A). Looking at arrestin recruitment all test compounds show neutral antagonism at the M_3 subtype (Figure 4C, Figure S3E,G, Table 4). This overall observation was confirmed when looking at the inhibitory effect of the test compounds on the EC_{80} concentration of carbachol. While the quinuclidinium ligands **14m** and **14n** showed inhibitory potencies of 6.3 and 6.8 nM (Figure 4B), respectively, which is in the same range as the inhibitory power of tiotropium (IC_{50} = 5.8 nM) (Figure S3B, Table 4), **13m** and **13n** revealed IC_{50} values of 14 nM and 9.3 nM with an inverse efficacy of -14% and -13%, respectively. Together with the piperidine derivatives **8m** and **8n**, which showed IC_{50} values of 17 nM and 19 nM, all tested ligands revealed a 2.5- to 7-fold better M_3 receptor inhibition when compared to the reference

darifenacin (IC_{50} = 45 nM) (Figure S3B,C,D, Table 4). Similar results could be observed for arrestin recruitment (Figure 4D, Figure S3E-H, Table 4).

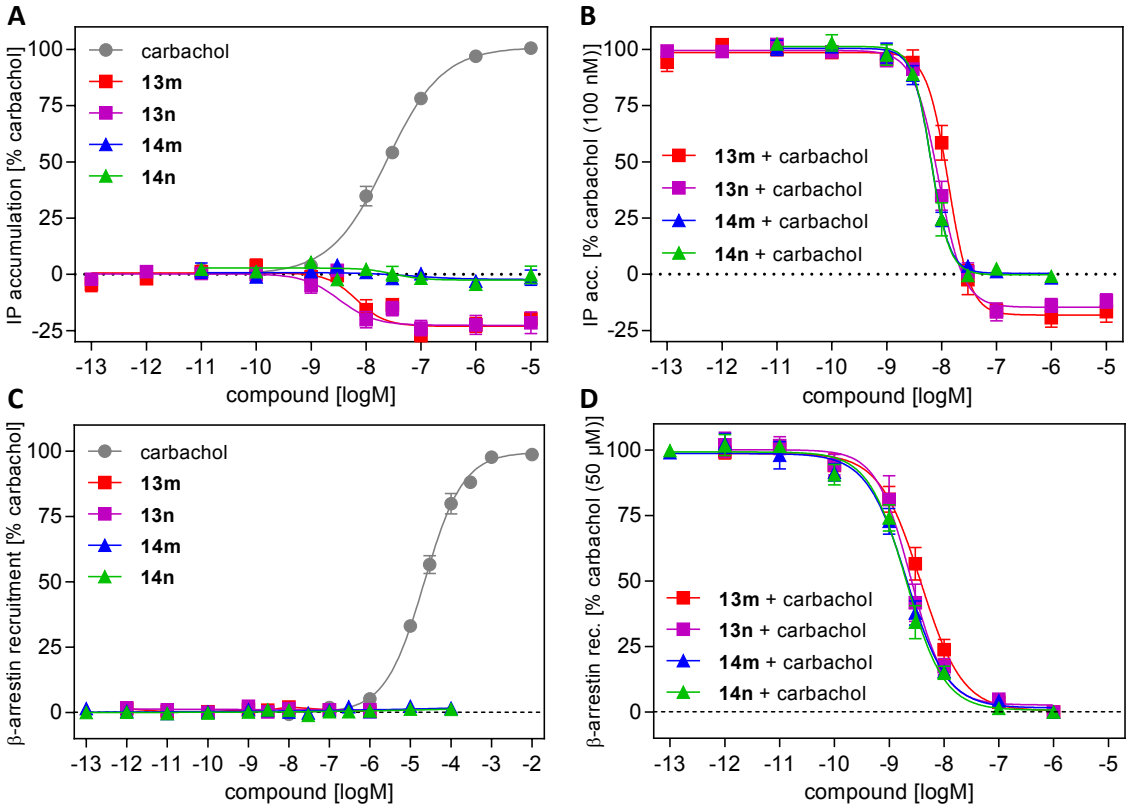


Figure 4. Functional properties of OFH243 (**13n**), OFH244 (**13m**), OFH3911 (**14n**) and OFH3912 (**14m**) applying an IP_1 accumulation assay (IP-One®) as readout for G-protein-mediated signaling (**A**, **B**) and a β-arrestin recruitment (PathHunter®) assay for arrestin signaling (**C**, **D**). **A**) The piperidines **13m** and **13n** show inverse agonist activity, while neutral antagonism was determined for the quaternized quinuclidines **14m** and **14n** when looking at G-protein signaling. **B**) Inhibition of the agonist effect of 100 nM carbachol was measured for **13m**, **13n**, **14m**, and **14n** with inverse agonist properties for **13m** and **13n**. **C**) Neutral antagonism of arrestin signaling was observed for all test compounds. **D**) Inhibition of arrestin recruitment induced by 50 µM of carbachol show

inhibitory properties for **13m**, **13n**, **14m**, and **14n**, which are similar to that of the G-protein pathway. (A-D) Pooled curves from 3-10 experiments each done in duplicates).

Table 4. Functional data for the test compounds **8m**, **8n**, **13m**, **13n**, **14m**, **14n** and the references tiotropium (**1**), darifenacin (**2**) and carbachol derived from an IP_1 accumulation test and an arrestin recruitment assay with M_3R.

	G-protein signaling[a]				arrestin recruitment[b]			
compound	agonist mode[c]		antagonist mode[d]		agonist mode[c]		antagonist mode[d]	
	EC_{50} [nM]	eff [%][e]	EC_{50} [nM]	eff [%][e]	EC_{50} [µM]	eff [%][e]	EC_{50} [nM]	eff [%][e]
carbachol	25±3.1	100	-	-	22±2.2	100	-	-
8m	-	<3	17±3.2	0.4±1.2	-	<3	27±4.9	-2.2±0.6
8n	-	<3	19±2.7	-0.2±0.9	-	<3	25±4.7	-1.8±1.2
13m	6.9±1.6	-24±2.5	14±1.6	-14±5.8	-	<3	26±4.8	-1.8±1.2
13n	4.1±1.5	-23±3.2	9.3±1.3	-13±3.8	-	<3	4.1±1.4	2.4±0.8
14m	-	<3	6.3±0.2	1.6±2.3	-	<3	2.2±0.3	1.4±0.6
14n	-	<3	6.8±0.9	-0.8±0.6	-	<3	2.2±0.3	1.0±0.5
tiotropium	-	<3	5.8±0.7	0.8±1.1	-	<3	0.4±0.1	-1.2±0.7
darifenacin	32±13	-9.9±2.2	45±14	-6.2±3.4	-	<3	11±3.2	2.0±1.9

[a]IP-One® assay (Cisbio) measuring the receptor stimulated accumulation of IP_1 in HEK293T cells transiently transfected with M_3 receptors. Values are the means±SEM derived from 4-10 individual experiments each done in duplicate. [b]Test on arrestin-2 recruitment (DiscoverX) applying an arrestin complementation assay. Values are the means±SEM derived from 3-11 single experiments each done in duplicate. [c]Agonist mode displaying the stimulating effect of a test compound. [d]Antagonist mode expressing the inhibitory effect of an antagonist on the EC_{80} concentration of carbachol. [e]Efficacy determined relative to the full effect of quinpirole (100%) and the basal effect of buffer (0%).

Regarding the functional data of established M_3 receptor ligands, it is worth to note that tiotropium (**1**) is typically referred to as an antagonist although inverse agonistic properties have also been reported.[46] Since tiotropium (**1**) appears as a neutral antagonist under our assay conditions, while OFH243 (**13n**) and OFH244 (**13m**) show a weak inverse agonistic profile, the actual decrease of activation attainable by **13m** and **13n** may even be underestimated in our present study. For the biphenyl carbamates **4a** and **4b** (Figure 2), no functional data has been reported that would give a hint towards inverse antagonism.[23] The same is true for batefenterol (**4c**) (Figure 2), which currently enters clinical phase III trials.[30]

Transcellular transport studies

The data obtained from the transcellular transport studies clearly indicate that both OFH243 (**13n**) and OFH244 (**13m**) are substrates of P-glycoprotein. OFH243 (**13n**) basal-to-apical translocation at 1 μM was considerably higher compared to and apical-to-basal translocation (9.2 ± 1.7 vs 3.5 ± 1.0 a.u., $P < 0.001$) yielding an efflux ratio (i.e. ratio of basal-to-apical and apical-to-basal translocation) of 2.7. Similarly, OFH243 (**13n**) at 10 μM showed a pronounced polarized transport in the basal to apical direction (99.5 ± 13.7 vs 46.2 ± 23.2 a.u., efflux ratio 2.2, $P < 0.05$).

OFH244 (**13m**) basal-to-apical translocation at 1 μM was also considerably higher compared to apical-to-basal translocation (9.0 ± 3.4 vs 3.7 ± 1.1 a.u., $P < 0.001$) yielding an efflux ratio of 2.4. Moreover, OFH244 (**13m**) at 10 μM showed a pronounced polarized transport in the basal to apical direction (79.3 ± 7.0 vs 46.5 ± 17.2 a.u., efflux ratio 1.7, $P < 0.01$). Polarized, basal to apical net transport was abolished by adding the P-gp inhibitor valspodar to the apical compartment for both OFH243 (**13n**) and OFH244 (**13m**) (basal to apical vs apical to basal, n.s., for both compounds and both concentrations).

The control experiments using the P-glycoprotein substrate digoxin showed the expected results. In the absence of valspodar, basal-to-apical translocation of digoxin was considerably higher compared to apical-to-basal translocation (19.9 ± 2.4% vs 6.7 ± 0.3% of the administered dose, $P < 0.001$). This pronounced polarized digoxin transport was completely abolished with apical administration of valspodar: basal to apical and apical to basal digoxin translocation were now nearly identical (10.2 ± 0.6 vs 10.5 ± 0.5, n.s.).

Metabolic stability

The metabolic stability of the secondary amines OFH243 (**13n**) and OFH244 (**13m**), as well as the quaternary ammonium salts OFH3911 (**14n**) and OFH3912 (**14m**) was studied using male rat liver microsomes and imipramine as a positive control under established conditions.[47,48] The results for OFH243 (**13n**), OFH244 (**13m**), OFH3911 (**14n**), OFH3912 (**14m**), and the reference compound imipramine are shown in Figure 5.

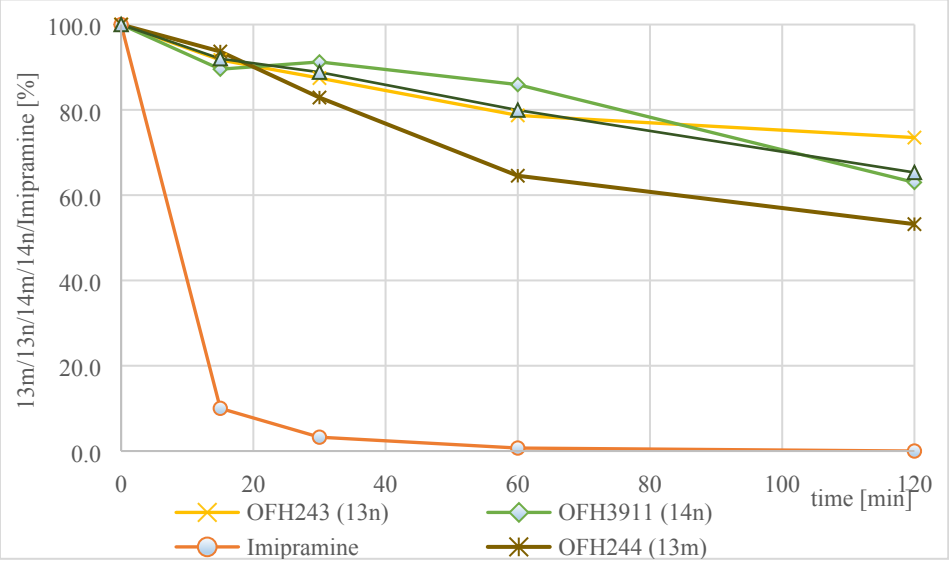


Figure 5. Oxidative metabolism of OFH243 (**13n**), OFH244 (**13m**), OFH3911 (**14n**), OFH3912 (**14m**), and imipramine after exposure to male rat liver microsomes. Results from LC−MS analysis are depicted in percentage of non-metabolized **13m**, **13n**, **14m**, **14n** and imipramine.

In comparison to imipramine, which is typically classified as a moderately stable positive control, all tested substances **13m**, **13n**, **14m**, and **14n** show substantially greater metabolic stability. After 120 min of incubation time, LC/MS analysis showed ca. 74% of unchanged amine **13n**, 53% of unchanged amine **13m**, 63% of unchanged ammonium salt **14n**, and 65% of unchanged ammonium salt **14m**, respectively, whereas imipramine was fully converted after 60 min.

Docking and molecular electrostatic potential calculations

Docking studies[49] and calculations of the molecular electrostatic potential (MEP)[50-51] were performed to provide possible explanations for the effects observed in the ligand binding studies. In the first stage of our investigations, we evaluated whether subtype selectivity towards the M_3 over the M_2 subtype could also be achieved without the 5-fluoro substitution on the ***A*** ring of the biphenyl. Taking into account the results summarized in Table 1, it became obvious that small structural changes in the basic aliphatic side chain can lead to a remarkable selectivity as observed for ligand **13a** (K_i = 0.47 nM, M_2/M_3 = 36). Since the underlying effect on subtype selectivity is probably the chain length between the hydrogen-bond accepting carbamate, which undergoes hydrogen bonding with Asn507 (M_3) and the positively charged, protonated amine, which forms an ionic interaction with Asp147 (M_3), three representative ligands **4a**, **8a**, **13a** were docked into the M_3 receptor (PDB entry 4U15)[52] (see Figure 6, A-C). Indeed, changing the chain from piperidin-4-yl to 3-quinuclidinyl and piperidin-4-ylmethyl for **4a**, **8a** and **13a**, respectively, leads to slight changes in relative orientation and distance of the biphenyl unit with respect to the critical side chains of Leu225 (M_3) or Phe181 (M_2) in ECL2, which is likely to be the reason for the experimentally observed differentiation between the M_2 and M_3 subtypes. Comparing the small effects for ligands **4a**, **8a**, **13a** (Figure 6, A-C), the 5-fluorine substitution in ligand **13d** (Figure 6, D) undoubtedly is an optimal structural requirement to achieve subtype selectivity. Regarding the recently published crystal structure of BS46 (**3**) in the M_3 receptor subtype, the docking poses shown in Figure 6 are in good agreement.[22]

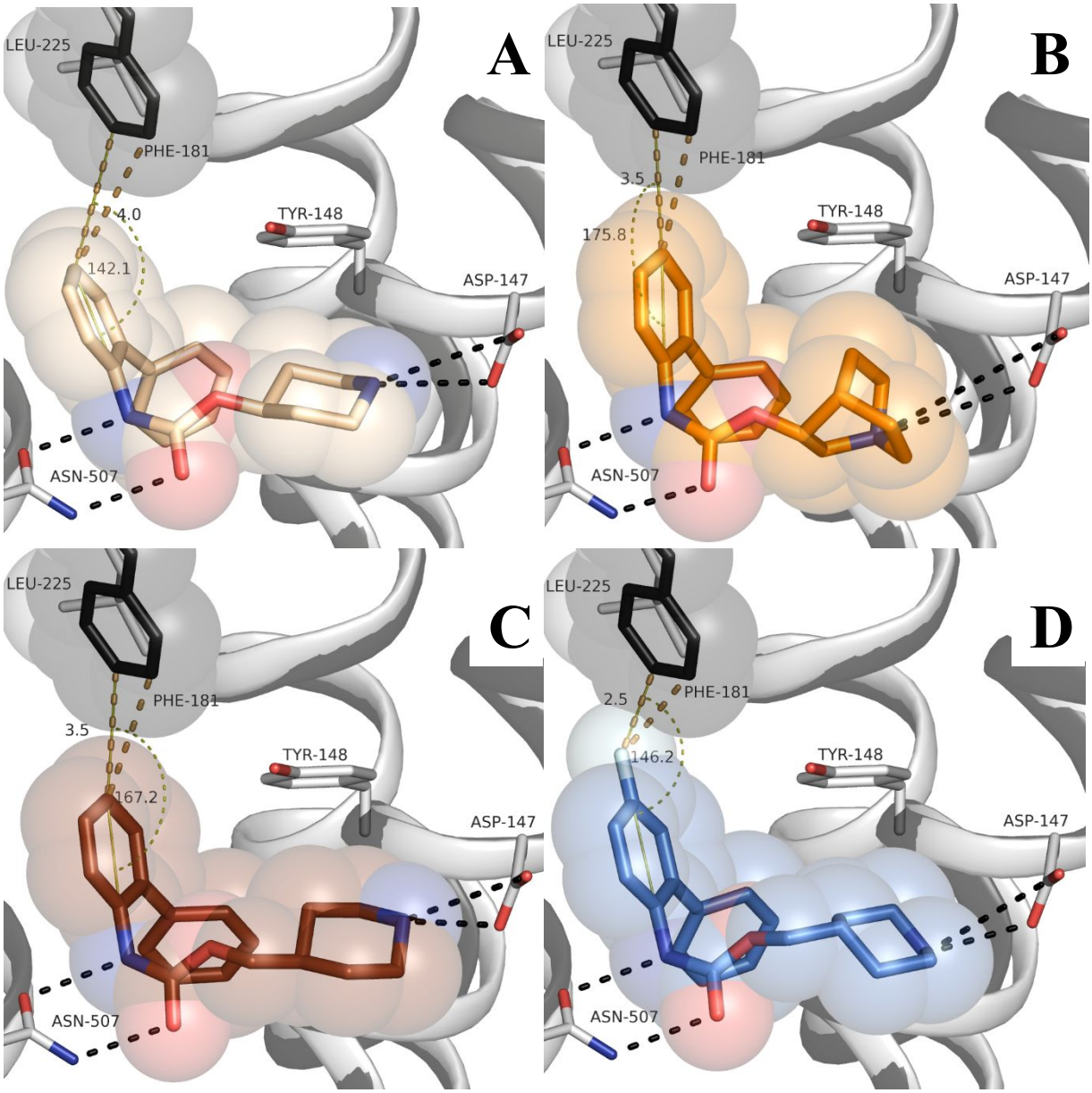


Figure 6. Docking results of **4a** (light brown, **A**), **8a** (orange, **B**), **13a** (brown, **C**) and **13d** (blue, **D**) at the M_3 receptor crystal structure (PDB entry 4U15, white)[52] superimposed with the M_2 receptor crystal structure (PDB entry 3UON, black)[53]. Shortest distance of the 5-substituent as well as the relative orientation angle of the biphenyl towards Phe181 (M_2) are shown.

Further docking experiments showed a possible interaction between a chloro substituent in 3'-position of ring ***B*** and the backbone carbonyl unit of Tyr148 from TM3 in the M_3 subtype (Figure 7). This so called halogen bonding is an important interaction seen in several receptor-ligand complexes.[54] Basically, halogens bond may be formed between the positively polarized σ hole of a halogen atom (F << Cl < Br < I) and a proximate hydrogen-bond acceptor. The halogen bond interaction typically follows strict geometric rules, whereas the halogen atom should ideally point at the acceptor at an angle of 180°. Deviations of 25-30° already lead to an approximately 50% decrease of bond strength. The distance should be smaller than the sum of the individual van der Waals radii of the respective atoms (< 3.27 Å for a chlorine-oxygen interaction), and in case that a carbonyl unit is involved, the C-O-X angle should be close to 120° so that the halogen atom points directly towards one of the lone pairs of the carbonyl oxygen.[55]

The favorable effect of a 3'-chloro substitution on ring ***B***, first observed for ligand **13k** in comparison with **13g** (Table 2), provided the rationale of synthesizing further 3'-chloro substituted derivatives (Table 3) to test the hypothesis. In this context, it should be noted that the used docking software (*AutoDock Vina*)[49] does not include halogen bonding in its scoring function. Docked poses that would allow halogen bond interactions could only be observed for **13k** and OFH243 (**13n**). The high binding affinity for OFH244 (**13m**) suggests that similar contacts are very likely to be present, even so the σ hole is less pronounced than for **13k** and **13n**. Interestingly, performing the docking with *AutoDock VinaXB*[56], a newer version including halogen interactions in its scoring function, did not show any relevant differences for the binding poses or their scoring.

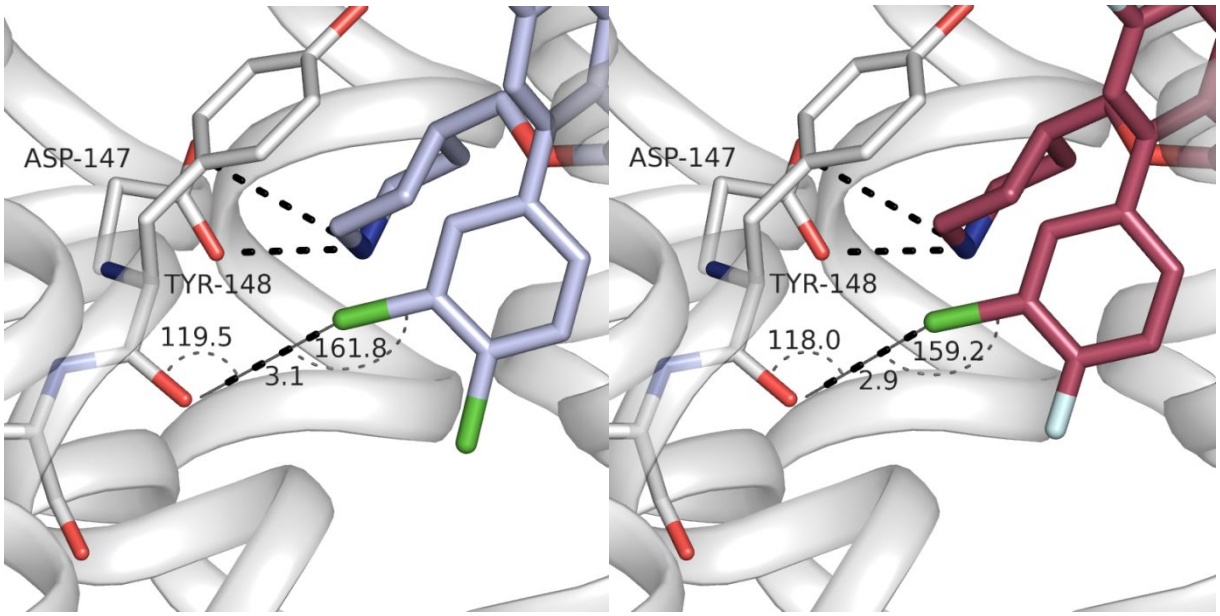


Figure 7. Docking poses of **13k** (left) and OFH243 (**13n**, right) in the M_3 receptor subtype (PDB entry 4U15, white)[52] suggesting halogen bonding between 3'-chloro substituent and the carbonyl unit of Tyr148.

Additional support for the halogen bond hypothesis was obtained by molecular electrostatic potential (MEP) calculations[50-51] of the 3'-chlorinated ligands **13g**,**k**,**m**,**n**, and **13o** (Figure 8). These show a comparatively large σ hole for all 3'-chloro substituted ligands due to the electronic properties of the aromatic biphenyl moiety. As predicted, this σ hole can be increased by the introduction of further inductively electron-withdrawing substituents on the ***B*** ring such as 4'-fluoro (as in ligand OFH243 (**13n**)), 4'-chloro (as in ligand **13k**) or 5'-chloro (as in ligand **13o**).

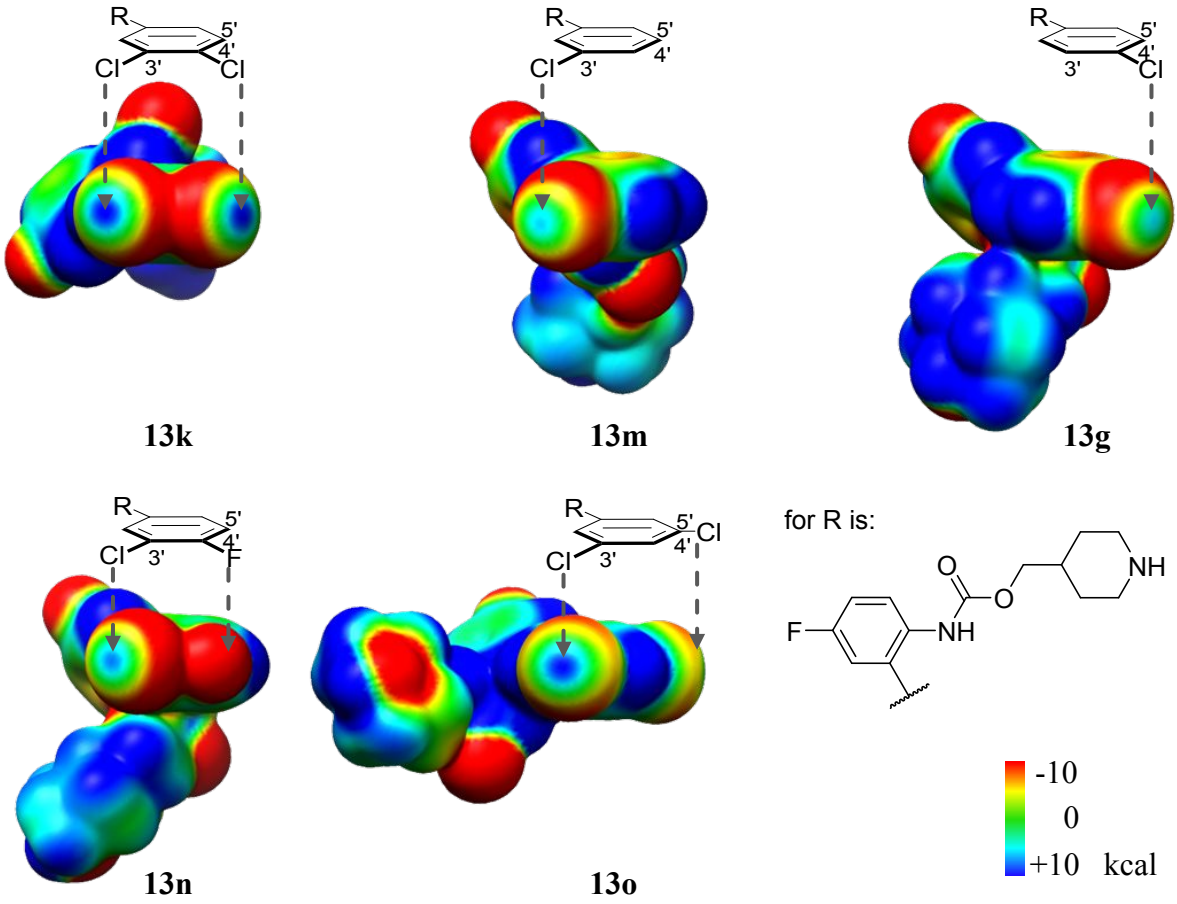


Figure 8. The molecular electrostatic potential (MEP) of ligands **13k**, **13m**, **13g**, **13n** and **13o** bearing at least one chloro substituent on ring ***B*** is mapped onto isodensity surface (0.001 au) calculated at the HF/6-31G* level.

Discussion and Conclusions

In summary, it has been shown that biphenyl carbamates can be developed towards highly potent and subtype selective ligands for the muscarinic M_3 receptor. Besides 5-fluoro substitution, which enables subtype selectivity for the M_3 over the M_2 receptor, the introduction of a chlorine atom in 3' position leads to an increased binding affinity to all three subtypes. This effect, which can be

rationalized by a halogen bond interaction between the σ hole on the chlorine atom and the carbonyl of Tyr148, may however only be exploited if the amine or ammonium moiety of the ligand is correctly chosen. Whereas 3',4'-dichloro substitution on the lead compound ABH423 (**5**) led to a decrease of both M_3 receptor affinity and M_2/M_3 subtype selectivity in ligand **8k**, the identical modification of **13d**, now bearing a basic 4-piperidinylmethyl instead of a quinuclidinyl moiety, results in an increased M_3 receptor affinity and a more or less unchanged M_2/M_3 selectivity observed for ligand **13k**. Further optimization of **13k** revealed that a σ hole on the 3'-chlorine atom, as it is also present in OFH243 (**13n**), OFH244 (**13m**), OFH3911 (**14n**) and OFH3912 (**14m**) can translate into even stronger binding to the M_3 subtype, with at the same time good selectivities towards the M_3 over the M_2 subtype ranging from 46 to 68. The comparably high affinities of **14n** and **14m** to the M_1 subtype can be considered as less problematic due to the inability of permanently charged ammonium compounds to pass the blood-brain barrier. For the secondary amines **13n** and **13m**, which also display high affinities to the M_1 receptor, it has been shown that these compounds are substrates of P-gp, so that they are likely to be actively transported away from the brain as it has been reported for darifenacin (**2**). Since the efflux ratios calculated from monolayer experiments with P-gp expressing cell lines are higher than 2 for the reference compound darifenacin (**2**) as well as for OFH243 (**13n**) and OFH244 (**13m**) at low micromolar concentrations, we expect a qualitatively similar effect of P-gp expressed in the blood-brain barrier on CNS accumulation of these compounds.[57]

Related to the σ hole effect, which can explain the high affinities of 3'-chloro substituted biphenyl carbamates to the M_3 subtype, the size of the σ hole was estimated by molecular electrostatic potential (MEP) calculations. Docking studies using recently published crystal structures further showed that the subtype selectivity for the M_3 over the M_2 receptor can to a certain extent be achieved without 5-fluoro substitution, but that 5-fluorination remains the optimal structural

requirement. All in all, OFH243 (**13n**), OFH244 (**13m**), OFH 3911 (**14n**) and OFH 3912 (**14m**) range among the top ligands developed in this field so far, which are moreover accessible via a straightforward and scalable synthetic route.

Given the favorable properties of **13m,n** and **14m,n**, whereat the amines **13m,n** were found to be weak inverse agonists and the ammonium salts **14m,n** to be neutral antagonists in the G_q pathway, these compounds represent valuable starting points for future developments towards highly potent and selective blockers of M_3 receptor signaling with potential applications in the fields of COPD and overactive bladder treatment. Studies on the incorporation of **13m,n** and **14m,n** into bivalent ligands, such as the clinical candidate batefenterol (**4c**), are currently underway in our laboratory. For research groups interested in a deeper investigation of the pharmacology of OFH243 (**13n**), OFH244 (**13m**), OFH3911 (**14n**) and OFH3912 (**14m**), we would be happy to provide samples – also on a larger scale – within a future collaboration.

Experimental Section

Synthesis.

Solvents and reagents are obtained from commercial sources and used as received. ^{1}H-NMR and ^{13}C-NMR spectra are recorded on Bruker Avance 600 (^{1}H: 600 MHz; ^{13}C: 151 MHz), Bruker Avance 360 (^{1}H: 360 MHz; ^{13}C: 91 MHz), and Bruker Avance 400 (^{1}H: 400 MHz; ^{13}C: 101 MHz) spectrometers. For ^{1}H-NMR spectra $CDCl_3$ is used as solvent referenced to TMS (0 ppm): $CHCl_3$ (7.26 ppm), $(CD_3)_2SO$ (2.50 ppm), CD_3OD (3.31 ppm). Chemical shifts are reported in parts per million (ppm). Coupling constants are in Hertz (Hz). The following abbreviations are used for the description of signals: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), bs (broad singlet). ^{13}C-NMR (DEPTQ) spectra are recorded in $CDCl_3$ using $CDCl_3$ (77.16 ppm) and in

$(CD_3)_2SO$ using $(CD_3)_2SO$ (39.52 ppm) as standard, respectively. Chemical shifts are given in parts per million (ppm). Mass spectra are recorded using electron spray ionization (ESI) and Atomospheric Pressure Photoionization (APPI) and a sector field mass analyzer for HRMS measurements. Analytical TLC is carried out on Merck silica gel plates using short wave (254 nm) UV light to visualize components. Silica gel (Kieselgel 60, 40-63mm, Merck) is used for flash column chromatography. Solvent ratios of the liquid phase are given in v/v. The purity of the substances was determined using an analytical HPLC: System A: Phenomenex Gemini NX-C18 analytical column, 4.6 mm x 250 mm, 5 µm, flow rate: 0.75 mL/min, Eluent: CH_3CN in H_2O + 0.1% HCO_2H (0-15 min 5%-90%, 15-20 min 90%). System B: Waters XBridge C18 analytical column, 4.6 mm x 50 mm, 3.5 µm, flow rate: 0.5 mL/min, Eluent: CH_3CN in H_2O + 0.1% HCO_2H (0-15 min 10%-90%, 15-20 min 90%). The purity of all target compounds **8a-n**, **11**, **13a-o**, **14a-n**, **15**, **16m-n**, **17**, and **18** was determined as >95%.

General Procedure A: Synthesis of aminobiphenyls from arylhydrazine derivatives with MnO_2

To a stirred suspension of the aniline derivative (20.0 mmol, 20 eq.) and MnO_2 (435 mg, 5 mmol, 5 eq.) in acetonitrile (5.0 mL), a solution of the arylhydrazine derivative (1.00 mmol, 1.0 eq.) in acetonitrile (2.0 mL) was added over a course of 1 h. After termination of the reaction, the reaction mixture was filtered over Celite® and washed with ethyl acetate. Afterwards, the solvent was removed under reduced pressure and further purification of the biphenyl-2-amines was achieved using Kugelrohr distillation and subsequent flash column chromatography.

General Procedure B: Synthesis of aminobiphenyls from arylhydrazine hydrochlorides with NaOH

To the neat aniline derivate (20.0 mmol) an aqueous solution of 1 M sodium hydroxide (1.0 ml) was added. After heating to 60 °C - 90 °C the arylhydrazine hydrochloride was added portionwise over a period of 9 hours. The reaction is completed after 24 hours at the given temperature, monitored by TLC. The remaining aniline was recovered by Kugelrohr distillation, and the products were purified by column chromatography on silica gel as described in the respective reaction procedure.

Biphenyl-2-amine (6a) was prepared from phenyl hydrazine (0.098 mL, 1.00 mmol), MnO_2 (435 mg, 5.00 mmol) and aniline (20.0 mmol, 1.83 mL) according to General Procedure A. The excess of aniline was removed by Kugelrohr distillation *in vacuo* at 85 °C. The desired product **6a** was obtained by flash column chromatography (hexane / EtOAc = 8 : 1) as a brown oil (105 mg, 0.62 mmol, 62% yield). ^{1}H-NMR (600 MHz, $CDCl_3$) δ 6.70 (d, J = 8.0 Hz, 1H), 6.82 (dt, J = 1.2, 7.5 Hz, 1H), 7.08 - 7.15 (m, 2H), 7.28 - 7.32 (m, 1H), 7.37 - 7.43 (m, 4H). Nitrogen-bound proton signals missing.

4'-Chlorobiphenyl-2-amine (6b) was prepared from aniline (20.0 mmol, 1.82 mL) and 4'-chlorophenylhydrazine hydrochloride (1.00 mmol, 179 mg) according to General Procedure B at 60 °C. The excess of aniline was removed by Kugelrohr distillation *in vacuo* at 75 °C. The crude products were purified by column chromatography (hexane / EtOAc = 8:1) to give **6b** as a brown oil (0.48 mmol, 99.0 mg, 48% yield). ^{1}H-NMR (600 MHz, $CDCl_3$) δ 6.76 (dd, J = 1.2, J = 8.6 Hz, 1H), 6.86 (dt, J = 1.2, 7.5 Hz, 1H), 7.08 (dd, J = 1.6, 7.6 Hz, 1H), 7.16 (ddd, J = 1.6, 7.4, 8.0 Hz, 1H), 7.38 - 7.42 (m, 4H). Nitrogen-bound proton signals missing.

3',4',5'-Trifluorobiphenyl-2-amine (**6c**) was prepared from 3,4,5-trifluorophenyl hydrazine (0.90 mmol, 146 mg), MnO_2 (392 mg, 4.50 mmol) and aniline (18.0 mmol, 1.64 mL) according to General Procedure A. The excess of aniline was removed by Kugelrohr distillation *in vacuo* at

75 °C. The desired product **6c** was obtained by flash column chromatography (hexane / EtOAc = 10:1 → 4:1) as a brown oil (0.53 mmol, 118 mg, 59% yield). ^{1}H-NMR (360 MHz, $CDCl_3$) δ 3.79 (bs, 2H), 6.77 (dd, *J* = 0.8, 8.0 Hz, 1H), 6.83 (dt, *J* = 1.0, 7.5 Hz, 1H), 7.06 (dd, *J* = 1.6, 7.6 Hz, 1H), 7.07-7.13 (m, 2H), 7.19 (ddd, *J* = 1.6, 7.4, 8.0 Hz, 1H).

5-Fluorobiphenyl-2-amine (6d) was prepared from phenyl hydrazine (0.10 mL, 1.00 mmol), MnO_2 (435 mg, 5.00 mmol) and 4-fluoroaniline (20.0 mmol, 1.92 mL) according to General Procedure A. The excess of 4-fluoroaniline was removed by Kugelrohr distillation *in vacuo* at 85 °C. The desired product **6d** was obtained by flash column chromatography (hexane / EtOAc = 10 : 1) as a brown oil (559 µmol, 138 mg, 56% yield). ^{1}H-NMR (600 MHz, $CDCl_3$) δ 3.54 (bs, 2H), 6.73 - 6.75 (m, 1H), 6.86 - 6.90 (m, 1H), 7.34 - 7.47 (m, 1H), 7.42 - 7.47 (m, 5H).

2'-Chloro-5-fluorobiphenyl-2-amine (6e) was prepared from 2-chlorophenyl hydrazine (1.00 mmol, 143 mg), MnO_2 (435 mg, 5.00 mmol) and 4-fluoroaniline (20.0 mmol, 1.92 mL) according to General Procedure A. The excess of 4-fluoroaniline was removed by Kugelrohr distillation *in vacuo* at 75 °C. The desired product **6e** was obtained by flash column chromatography (hexane/EtOAc = 10:1) as a brown oil (0.56 mmol, 124 mg, 56% yield). ^{1}H-NMR (600 MHz, $CDCl_3$) δ 3.47 (bs, 2H), 6.73 (dd, *J* = 4.8, 8.8 Hz, 1H), 6.81 (dd, *J* = 3.0, 8.9 Hz, 1H), 6.93 (dt, *J* = 3.0, 8.4, 8.5 Hz, 1H), 7.30-7.36 (m, 3H), 7.48-7.52 (m, 1H).

4',5-Difluorobiphenyl-2-amine (6f) prepared from 4-fluoroaniline (20.0 mmol, 1.92 mL) and 4-fluorophenylhydrazine hydrochloride (1.00 mmol, 163 mg) according to General Procedure B at 60 °C. The excess of 4-fluoroaniline was removed by Kugelrohr distillation *in vacuo* at 80 °C. The crude product was purified by column chromatography (hexane / EtOAc = 8:1) to give **6f** as black crystals (0.53 mmol, 108 mg, 53% yield). ^{1}H-NMR (600 MHz, $CDCl_3$) δ 6.69 (dd, *J* = 4.8, 8.7 Hz,

1H), 6.83 (dd, *J* = 3.0, 9.2 Hz, 1H), 6.84 - 6.89 (m, 1H), 7.13 (t, *J* = 8.7 Hz, 2H), 7.40 (dd, *J* = 5.4, 8.8 Hz, 2H). Nitrogen-bound proton signals missing.

4'-Chloro-5-fluorobiphenyl-2-amine (6g) was prepared from 4-fluoroaniline (20.0 mmol, 1.92 mL) and 4-chlorophenylhydrazine hydrochloride (1.00 mmol, 179 mg) according to General Procedure B at 60 °C. The excess of 4-fluoroaniline was removed by Kugelrohr distillation *in vacuo* at 80 °C. The crude product was purified by column chromatography (hexane / EtOAc = 8:1) to give **6g** as a dark brown oil (0.55 mmol, 121 mg, 55% yield). ^{1}H-NMR (360 MHz, $CDCl_3$) δ 6.70 (dd, *J* = 4.8, 8.7 Hz, 1H), 6.83 (dd, *J* = 3.0, 9.2 Hz, 1H), 6.85 (ddd, *J* = 3.0, 8.2, 8.3 Hz, 1H), 7.38 (d, *J* = 8.8 Hz, 2H), 7.43 (d, *J* = 8.8 Hz, 2H). Nitrogen-bound proton signals missing.

4'-Bromo-5-fluorobiphenyl-2-amine (6h) was prepared from 4-Bromophenyl hydrazine (2.00 mmol, 374 mg), MnO_2 (870 mg, 10.00 mmol) and 4-fluoroaniline (40.0 mmol, 3.84 mL) according to General Procedure A. The excess of aniline was removed by Kugelrohr distillation *in vacuo* at 90 °C. The desired product **6h** was obtained by flash column chromatography (hexane/EtOAc = 20:1) as a brown oil (0.50 mmol, 134 mg, 25% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.8 (s, 2H), 6.7 – 6.8 (m, 1H), 6.8 – 6.9 (m, 1H), 6.9 (ddd, *J* = 3.0, 8.1, 8.7 Hz, 1H), 7.3 – 7.4 (m, 2H), 7.6 – 7.7 (m, 2H).

4'-Cyano-5-fluorobiphenyl-2-amine (6i) was prepared from 4-cyanophenyl hydrazine (1.00 mmol, 133 mg), MnO_2 (435 mg, 5.00 mmol) and 4-fluoroaniline (20.0 mmol, 1.92 mL) according to General Procedure A. The excess of aniline was removed by Kugelrohr distillation *in vacuo* at 75 °C. The desired product **6i** was obtained by flash column chromatography (hexane/EtOAc = 10:1) as a brown oil (0.58 mmol, 123 mg, 58% yield). ^{1}H-NMR (600 MHz, $CDCl_3$) δ 3.68 (bs, 2H), 6.73 (dd, *J* = 4.7, 8.7 Hz, 1H), 6.84 (dd, *J* = 2.9, 9.0 Hz, 1H), 6.93 (dt, *J* = 2.9, 8.4, 8.4 Hz, 1H), 7.59 (d, *J* = 8.2 Hz, 2H), 7.75 (d, *J* = 8.3 Hz, 2H).

5-Fluoro-4'-methoxybiphenyl-2-amine (6j) was prepared from 4-methoxyphenyl hydrazine (1.00 mmol, 138 mg), MnO_2 (435 mg, 5.00 mmol) and 4-fluoroaniline (20.0 mmol, 1.92 mL) according to General Procedure A. The excess of aniline was removed by Kugelrohr distillation *in vacuo* at 75 °C. The desired product **6j** was obtained by flash column chromatography (hexane/EtOAc = 10:1) as a brown oil (0.32 mmol, 69.2 mg, 32% yield). ^{1}H-NMR (360 MHz, $CDCl_3$) δ 3.85 (bs, 3H), 6.68 (dd, J = 4.9, 9.2 Hz, 1H), 6.80-6.88 (m, 2H), 6.98 (d, J = 8.8 Hz, 2H), 7.36 (d, J = 8.9 Hz, 2H).

3',4'-Dichloro-5-fluorobiphenyl-2-amine (6k) was prepared from 3,4-dichlorophenyl hydrazine (1.00 mmol, 177 mg), MnO_2 (435 mg, 5.00 mmol) and 4-fluoroaniline (20.0 mmol, 1.92 mL) according to General Procedure A. The excess of aniline was removed by Kugelrohr distillation *in vacuo* at 75 °C. The desired product **6k** was obtained by flash column chromatography (hexane/EtOAc = 10:1) as a brown oil (0.62 mmol, 158 mg, 62% yield). ^{1}H-NMR (360 MHz, $CDCl_3$) δ 3.65 (bs, 2H), 6.70 (dd, J = 4.8, 8.8 Hz, 1H), 6.82 (dd, J = 2.9, 9.1 Hz, 1H), 6.89 (ddd, J = 3.0, 8.1, 8.7 Hz, 1H), 7.29 (dd, J = 2.1, 8.3 Hz, 1H), 7.52 (d, J = 8.2 Hz, 1H), 7.55 (d, J = 2.1 Hz, 1H).

3',4',5,5'-Tetrafluorobiphenyl-2-amine (6l) was prepared from 4-fluoroaniline (20.0 mmol, 1.92 mL) and 3,4,5-trifluorophenylhydrazine hydrochloride (1.00 mmol, 199 mg) according to General Procedure B at 60 °C. The excess of aniline was removed by Kugelrohr distillation *in vacuo* at 80 °C. The desired product **6l** was obtained by flash column chromatography (hexane/EtOAc = 8:1) as a brown oil (0.24 mmol, 57.0 mg, 24% yield). ^{1}H-NMR (600 MHz, $CDCl_3$) δ 6.71 (dd, J = 4.8, 8.8 Hz, 1H), 6.80 (dd, J = 3.0, 9.0 Hz, 1H), 6.90 (ddd, J = 3.0, 8.1, 8.7 Hz, 1H), 7.06 - 7.13 (m, 2H). Nitrogen-bound proton signals missing.

3'-Chloro-5-fluorobiphenyl-2-amine (6m) was prepared from 3-Chlorophenyl hydrazine (2.00 mmol, 285 mg), MnO_2 (870 mg, 10.00 mmol) and 4-fluoroaniline (40.0 mmol, 3.84 mL) according to General Procedure A. The excess of aniline was removed by Kugelrohr distillation *in vacuo* at 90 °C. The desired product **6m** was obtained by flash column chromatography (hexane/EtOAc = 15:1) as a brown oil (0.82 mmol, 182 mg, 41% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.39 (s, 2H), 6.79 (dd, *J* = 4.8, 8.7 Hz, 1H), 6.86 – 6.98 (m, 2H), 7.32 – 7.50 (m, 4H).

3'-Chloro-4',5-difluorobiphenyl-2-amine (6n) was prepared from 3-Chloro-4-fluorophenyl hydrazine (2.00 mmol, 321 mg), MnO_2 (870 mg, 10.00 mmol) and 4-fluoroaniline (40.0 mmol, 3.84 mL) according to General Procedure A. The excess of aniline was removed by Kugelrohr distillation *in vacuo* at 90 °C. The desired product **6n** was obtained by flash column chromatography (hexane/EtOAc = 15:1) as a brown oil (0.86 mmol, 207 mg, 43% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.81 (s, 2H), 6.74 (dd, *J* = 4.8, 8.7 Hz, 1H), 6.84 (dd, *J* = 2.9, 9.1 Hz, 1H), 6.92 (ddd, *J* = 3.0, 8.1, 8.7 Hz, 1H), 7.19 – 7.29 (m, 1H), 7.34 (ddd, *J* = 2.2, 4.6, 8.5 Hz, 1H), 7.53 (dd, *J* = 2.2, 7.1 Hz, 1H).

3',5'-Dichloro-5-fluorobiphenyl-2-amine (6o) was prepared from 3,5-Dichlorophenyl hydrazine (2.00 mmol, 354 mg), MnO_2 (870 mg, 10.00 mmol) and 4-fluoroaniline (40.0 mmol, 3.84 mL) according to General Procedure A. The excess of aniline was removed by Kugelrohr distillation *in vacuo* at 90 °C. The desired product **6o** was obtained by flash column chromatography (hexane/EtOAc = 15:1) as a brown oil (1.1 mmol, 280 mg, 55% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.49 (s, 2H), 6.81 (dd, *J* = 4.8, 8.8 Hz, 1H), 6.87 (dd, *J* = 2.9, 8.9 Hz, 1H), 6.95 (ddd, *J* = 3.0, 8.0, 8.8 Hz, 1H), 7.38 (q, *J* = 1.3 Hz, 3H).

General Procedure C: Synthesis of isocyanates followed by the formation of the carbamate

To a solution of triphosgene (119 mg, 400 µmol, 0.4 eq.) in dry CH_2Cl_2 (5.0 mL), a solution of the aminobiphenyl (1 mmol, 1.0 eq.) in dry CH_2Cl_2 (2.5 mL) was added under argon atmosphere over a period of 20 minutes at 0 °C. Subsequently a solution of *N*,*N*-diisopropylethylamine (680 µL, 4 mmol, 4.0 eq.) in dry CH_2Cl_2 (2.5 mL) was added over 5 minutes and the mixture was stirred for additional 30 minutes at 0 °C. A solution of the alcohol (1.2 mmol, 1.2 eq.) in dry CH_2Cl_2 (2.5 mL) was added and the solution was stirred overnight at room temperature. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and the organic phase was washed both, with a saturated aqueous solution of sodium carbonate, and a saturated aqueous solution of sodium chloride and dried over sodium sulfate. The solvent was removed under reduced pressure and the crude products were purified by column chromatography on silica gel or used directly for the subsequent step (**7m, 7o**).

***tert*-Butyl ((biphen-2-ylcarbamoyl)oxymethyl)piperidine-1-carboxylate (7a)** was prepared from biphenyl-2-amine (**6a**) (400 µmol, 68.0 mg) according to General Procedure C. The crude product was purified by column chromatography (CH_2Cl_2 / EtOAc = 50:1) to give the title compound **7a** as a brown oil (276 µmol, 113 mg, 69% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.1 – 1.2 (m, 2H), 1.4 (s, 9H), 1.6 – 1.7 (m, 2H), 1.7 – 1.8 (m, 1H), 2.7 (s, 2H), 4.0 (d, *J* = 6.5 Hz, 2H), 4.0 – 4.3 (m, 2H), 6.6 (s, 1H), 7.1 (td, *J* = 1.2, 7.5 Hz, 1H), 7.2 – 7.2 (m, 1H), 7.3 – 7.4 (m, 3H), 7.4 – 7.4 (m, 1H), 7.5 – 7.5 (m, 2H), 8.1 (s, 1H).

***tert*-Butyl 4-(((4'-chlorobiphen-2-yl)carbamoyl)oxymethyl)piperidine-1-carboxylate (7b)** was prepared from 4'-chloro-biphenyl-2-amine (**6b**) (200 µmol, 46.7 mg) according to General Procedure C. The crude product was purified by column chromatography (CH_2Cl_2 / EtOAc = 50:1) to give **7b** as a brown oil (60.0 µmol, 27.0 mg, 30% yield). ^{1}H-NMR (600 MHz, $CDCl_3$) δ 1.16 (ddd, *J* = 4.4, 12.5, 24.9 Hz, 2H), 1.45 (s, 9H), 1.66 (d, *J* = 14.1 Hz, 2H), 1.73 - 1.87 (m, 1H), 2.69 (t, *J* = 12.1 Hz, 2H), 3.98 (d, *J* = 6.6 Hz, 2H), 4.04 - 4.20 (m, 2H), 6.48 (s, 1H), 7.10 - 7.20 (m,

2H), 7.30 (d, *J* = 8.6 Hz, 2H), 7.34 - 7.40 (m, 1H), 7.46 (d, *J* = 8.5 Hz, 2H), 8.06 (d, *J* = 8.1 Hz, 1H).

***tert*-Butyl 4-(((3',4',5'-trifluorobiphen-2-yl)carbamoyl)oxymethyl)piperidine-1-carboxylate (7c)** was prepared from 3',4',5'-trifluoro-biphenyl-2-amine (**6c**) (224 µmol, 50 mg) according to General Procedure C. The crude product was purified by column chromatography (hexane / EtOAc = 6:1) to give **7c** as a brown oil (195 µmol, 90.6 mg, 87% yield). ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.1 – 1.2 (m, 2H), 1.5 (s, 10H), 1.7 (d, *J* = 12.9 Hz, 2H), 1.8 (th, *J* = 3.4, 13.7 Hz, 1H), 2.7 (t, *J* = 12.8 Hz, 2H), 4.0 (d, *J* = 6.6 Hz, 2H), 4.0 – 4.2 (m, 2H), 6.4 (s, 1H), 7.0 – 7.1 (m, 2H), 7.1 – 7.2 (m, 2H), 7.4 – 7.4 (m, 1H), 8.0 (d, *J* = 8.3 Hz, 1H).

***tert*-Butyl 4-(((5-fluorobiphen-2-yl)carbamoyl)oxymethyl)piperidine-1-carboxylate (7d)** was prepared from 5-fluoro-biphenyl-2-amine (**6d**) (200 µmol, 37.4 mg) according to General Procedure C. The crude product was purified by column chromatography (CH_2Cl_2 / EtOAc = 50:1) to give **7d** as a brown oil (28.0 µmol, 12.0 mg, 49% yield). ^{1}H-NMR (600 MHz, $CDCl_3$) δ 1.15 (dq, *J* = 4.4, 12.5 Hz, 2H), 1.45 (s, 9H), 1.64 (d, *J* = 12.6 Hz, 2H), 1.74 - 1.84 (m, 1H), 2.68 (t, *J* = 12.3 Hz, 2H), 3.97 (d, *J* = 6.6 Hz, 2H), 4.05 - 4.15 (m, 2H), 6.47 (s, 1H), 6.95 (dd, *J* = 3.0, 8.9 Hz, 1H), 7.06 (ddd, *J* = 3.0, 8.1, 9.0 Hz, 1H), 7.35 (dd, *J* = 1.3, 8.2 Hz, 2H), 7.41 - 7.45 (m, 1H), 7.49 (tt, *J* = 1.5, 8.3 Hz, 2H), 8.00 (s, 1H).

***tert*-Butyl 4-(((2'-chloro-5-fluorobiphen-2-yl)carbamoyl)oxymethyl)piperidine-1-carboxylate (7e)** was prepared from 2'-chloro-5-fluoro-biphenyl-2-amine (**6e**) (200 µmol, 44.3 mg) according to General Procedure C. The crude product was purified by column chromatography (CH_2Cl_2 / EtOAc = 100:1) to give **7e** as a brown oil (60.1 µmol, 27.0 mg, 30% yield). ^{1}H-NMR (600 MHz, $CDCl_3$) δ 1.13 (dq, *J* = 4.5, 12.5 Hz, 2H), 1.45 (s, 9H), 1.62 (d, *J* = 12.0 Hz, 2H), 1.76 - 1.84 (m, 1H), 2.67 (t, *J* = 12.3 Hz, 2H), 4.00 - 4.18 (m, 2H), 4.10 (d, *J* = 11.9 Hz, 2H), 6.15 (s,

1H), 6.91 (dd, *J* = 3.0, 8.6 Hz, 1H), 7.09 - 7.14 (m, 1H), 7.28 (dd, *J* = 2.1, 7.2 Hz, 1H), 7.36 - 7.40 (m, 2H), 7.53 (dd, *J* = 1.7, 7.6 Hz, 1H), 7.95 (s, 1H).

***tert*-Butyl 4-(((4',5-difluorobiphen-2-yl)carbamoyl)oxymethyl)piperidine-1-carboxylate (7f)** was prepared from 4',5-difluoro-biphenyl-2-amine (**6f**) (244 µmol, 50 mg) according to General Procedure C. The crude product was purified by column chromatography (hexane / EtOAc = 6:1) to give **7f** as a brown oil (56.2 µmol, 25.1 mg, 23% yield). ^{1}H-NMR (600 MHz, $CDCl_3$) δ 1.16 (dq, *J* = 4.4 Hz, 12.7 Hz, 2H), 1.46 (s, 9H), 1.65 (d, *J* = 15.0 Hz, 2H), 1.75 - 1.85 (m, 1H), 2.69 (t, *J* = 12.3 Hz, 2H), 3.98 (d, *J* = 6.5 Hz, 2H), 4.05 - 4.17 (m, 2H), 6.45 (s, 1H), 7.17 - 7.20 (m, 3H), 7.30 - 7.35 (m, 4H).

***tert*-Butyl 4-(((4'-chloro,5-fluorobiphen-2-yl)carbamoyl)oxymethyl)piperidine-1-carboxylate (7g)** was prepared from 4'-chloro-5-fluoro-biphenyl-2-amine (**6g**) (226 µmol, 50 mg) according to General Procedure C. The crude product was purified by column chromatography (hexane / EtOAc = 10:1) to give **7g** as brown oil (87.7 µmol, 40.6 mg, 39% yield). ^{1}H-NMR (360 MHz, $CDCl_3$) δ 1.16 (ddd, *J* = 4.5 Hz, 12.5 Hz, 25.1 Hz, 2H), 1.46 (s, 9H), 1.66 (d, *J* = 12.7 Hz, 2H), 1.76 - 1.85 (m, 1H), 2.69 (t, *J* = 11.8 Hz, 2H), 3.98 (d, *J* = 6.6 Hz, 2H), 4.12 (d, *J* = 12.0 Hz, 2H), 6.45 (s, 1H), 7.18 (d, *J* = 2.5 Hz, 1H), 7.29 (d, *J* = 8.6 Hz, 2H), 7.34 (dd, *J* = 2.5 Hz, *J* = 8.9 Hz, 1H), 7.48 (d, *J* = 8.6 Hz, 2H), 8.05 (bs, 1H).

***tert*-Butyl 4-(((4'-bromo-5-fluorobiphen-2-yl)carbamoyl)oxymethyl)piperidine-1-carboxylate (7h)** was prepared from 4'-bromo-5-fluoro-biphenyl-2-amine (**6h**) (200 µmol, 53.2 mg) according to General Procedure C. The crude product was purified by column chromatography (CH_2Cl_2 / EtOAc = 50:1) to give **7h** as a brown oil (13.8 µmol, 7.00 mg, 7% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.1 – 1.2 (m, 2H), 1.5 (s, 9H), 1.6 – 1.7 (m, 2H), 1.8 (dddd, *J* = 14.7, 11.7, 6.7, 3.1 Hz, 1H), 2.7 (td, *J* = 13.1, 12.9, 2.7 Hz, 2H), 4.0 (d, *J* = 6.6 Hz, 2H), 4.1 – 4.1 (m, 2H), 6.4 (s, 1H),

6.9 (dd, *J* = 8.8, 3.1 Hz, 1H), 7.0 – 7.1 (m, 2H), 7.2 – 7.3 (m, 2H), 7.6 – 7.7 (m, 2H), 8.0 (dd, *J* = 8.9, 5.2 Hz, 1H).

***tert*-Butyl 4-(((4'-cyano-5-fluorobiphen-2-yl)carbamoyl)oxymethyl)piperidine-1-carboxylate (7i)** was prepared from 4'cyano-5-fluorobiphenyl-2-amine (**6i**) (400 µmol, 84.0 mg) according to General Procedure C. The crude product was purified by column chromatography (CH_2Cl_2 / EtOAc = 50:1) to give **7i** as a brown oil (225 µmol, 102 mg, 56% yield). ^{1}H-NMR (600 MHz, $CDCl_3$) δ 1.15 (dq, *J* = 4.4, 12.6 Hz, 2H), 1.45 (s, 9H), 1.64 (d, *J* = 12.7 Hz, 2H), 1.74 - 1.84 (m, 1H), 2.68 (t, *J* = 12.3 Hz, 2H), 4.00 (d, *J* = 6.6 Hz, 2H), 4.13 (d, *J* = 13.3 Hz, 2H), 6.33 (s, 1H), 6.95 (dd, *J* = 3.0, 8.6 Hz, 1H), 7.12 (ddd, *J* = 3.0, 7.9, 9.0 Hz, 1H), 7.50 (d, *J* = 8.5 Hz, 2H), 7.79 (d, *J* = 8.5 Hz, 2H), 7.91 (s, 1H).

***tert*-Butyl 4-(((5-fluoro-4'-methoxybiphen-2-yl)carbamoyl)oxymethyl)piperidine-1-carboxylate (7j)** was prepared from 5-fluoro-4'-methoxy-biphenyl-2-amine (**6j**) (250 µmol, 55.0 mg) according to General Procedure C. The crude product was purified by column chromatography (CH_2Cl_2 / EtOAc = 50:1) to give **7j** as a brown oil (24.0 µmol, 11.0 mg, 9% yield). ^{1}H-NMR (600 MHz, $CDCl_3$) δ 1.15 (ddd, *J* = 4.4, 12.5, 25.1 Hz, 2H), 1.45 (s, 9H), 1.65 (d, *J* = 12.5 Hz, 2H), 1.75 - 1.85 (m, 1H), 2.68 (dt, *J* = 2.4, 13.2 Hz, 2H), 3.87 (s, 3H), 3.97 (d, *J* = 6.6 Hz, 2H), 4.11 (d, *J* = 12.9 Hz, 2H), 6.49 (bs, 1H), 6.90 - 6.96 (m, 2H), 7.01 (d, *J* = 8.8 Hz, 2H), 7.27 (d, *J* = 8.7 Hz, 2H), 7.81 (s, 1H).

***tert*-Butyl 4-(((3',4'-dichloro-5-fluorobiphen-2-yl)carbamoyl)oxymethyl)piperidine-1-carboxylate (7k)** was prepared from 3',4'-dichloro-5-fluoro-biphenyl-2-amine (**6k**) (195 µmol, 50 mg) according to General Procedure C. The crude product was purified by column chromatography (hexane / EtOAc = 10:1) to give **7k** as a brown oil (31.5 µmol, 15,7 mg, 16% yield). ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.2 (qd, *J* = 4.2, 12.2 Hz, 2H), 1.5 (s, 9H), 1.6 – 1.7 (m, 2H),

1.7 – 1.9 (m, 1H), 2.7 (t, *J* = 12.6 Hz, 2H), 4.0 (d, *J* = 6.6 Hz, 2H), 4.1 (d, *J* = 11.2 Hz, 2H), 6.1 – 6.4 (m, 1H), 6.9 (ddd, *J* = 0.4, 3.0, 8.7 Hz, 1H), 7.1 (dddd, *J* = 0.4, 3.0, 7.9, 9.0 Hz, 1H), 7.2 (dd, *J* = 2.1, 8.2 Hz, 1H), 7.5 (dd, *J* = 0.3, 2.1 Hz, 1H), 7.6 (dd, *J* = 0.3, 8.3 Hz, 1H), 7.8 – 8.0 (m, 1H).

***tert*-Butyl 4-(((3',4',5,5'-tetrafluorobiphen-2-yl)carbamoyl)oxymethyl)piperidine-1-carboxylate (7l)** was prepared from 3',4',5,5'-tetrafluoro-biphenyl-2-amine (**6l**) (85.0 µmol, 20.0 mg) according to General Procedure C. The crude product was purified by column chromatography (hexane / CH_2Cl_2 = 5:1) to give **7l** as a yellow oil (17.0 µmol, 8.00 mg, 20% yield). ^{1}H-NMR (600 MHz, $CDCl_3$) δ 1.18 (ddd, *J* = 4.4, 12.5, 25.1 Hz, 2H), 1.46 (s, 9H), 1.67 (d, *J* = 12.7 Hz, 2H), 1.76 - 1.86 (m, 1H), 2.70 (dt, *J* = 2.7, 13.3 Hz, 2H), 4.00 (d, *J* = 6.6 Hz, 2H), 4.13 (d, *J* = 13.3 Hz, 2H), 6.26 (bs, 1H), 6.93 (dd, *J* = 3.0, 8.6 Hz, 1H), 7.01 (ddd, *J* = 3.0, 6.4, 7.8 Hz, 2H), 7.12 (ddd, *J* = 3.0, 7.9, 9.0 Hz, 1H), 7.91 (s, 1H).

***tert*-Butyl 4-(((3'-chloro-5-fluorobiphen-2-yl)carbamoyl)oxymethyl)piperidine-1-carboxylate (7m)** was prepared from 3'-chloro-5-fluoro-biphenyl-2-amine (**6m**) (383 µmol, 85.0 mg) according to General Procedure C. The crude mixture was obtained as a brown oil and directly converted to **13m** according to General Procedure D.

***tert*-Butyl 4-(((3'-chloro-4',5-difluorobiphen-2-yl)carbamoyl)oxymethyl)piperidine-1-carboxylate (7n)** was prepared from 3'-chloro-4',5-difluoro-biphenyl-2-amine (**6n**) (1040 µmol, 250 mg) according to General Procedure C. The crude product was purified by column chromatography (hexane / EtOAc = 5:1) to give **7n** as a yellow oil (510 µmol, 245 mg, 49% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.08 – 1.22 (m, 2H), 1.45 (s, 9H), 1.59 – 1.72 (m, 2H), 1.80 (dddt, *J* = 3.6, 6.7, 11.7, 15.4 Hz, 1H), 2.53 – 2.76 (m, 2H), 3.98 (d, *J* = 6.5 Hz, 2H), 4.05 – 4.18 (m, 2H), 6.38 (s, 1H), 6.93 (dd, *J* = 3.0, 8.7 Hz, 1H), 7.08 (ddd, *J* = 3.0, 7.9, 9.0 Hz, 1H), 7.20 – 7.31 (m, 2H), 7.42 (ddd, *J* = 0.6, 2.0, 6.9 Hz, 1H), 7.90 (s, 1H).

***tert*-Butyl 4-(((3',5'-dichloro-5-fluorobiphen-2-yl)carbamoyl)oxymethyl)piperidine-1-carboxylate (7o)** was prepared from 3',5'-dichloro-5-fluoro-biphenyl-2-amine (**6o**) (310 µmol, 79.5 mg) according to General Procedure C. The crude mixture was obtained as a brown oil and directly converted to **13o** according to General Procedure D.

General Procedure D: Synthesis of the biaryl carbamates/urea using (*R*)-quinuclidin-3-ol or (*R*)-quinuclidin-3-amine

A solution of the biphenyl-2-amine (1.0 mmol, 1.0 eq.) in dry CH_2Cl_2 (1.5 mL) under argon was cooled to 0 °C. Over a course of 20 min, a solution of triphosgene (118 mg, 0.40 mmol, 0.40 eq.) in dry CH_2Cl_2 (3.0 mL) was added slowly. The reaction mixture was stirred for another 15 min at 0 °C, the reaction progress was monitored using TLC. Afterwards, the solvent was removed under reduced pressure and the residue was redissolved in toluene (3.0 mL). A solution of the (*R*)-quinuclidin-3-ol (153 mg, 1.2 mmol, 1.2 eq.) or (*R*)-quinuclidin-3-ol (153 mg, 1.2 mmol, 1.2 eq.) (in the case of **11**) in toluene (1.5 mL) was added and the reaction mixture was stirred at 110 °C for 4 h. After cooling to room temperature, the reaction mixture was diluted with ethyl acetate (60 mL) and the organic phase was extracted with 1 N HCl (3 × 45 mL). The combined aqueous phases were adjusted to a pH of 9, using K_2CO_3 and subsequently extracted with chloroform (3 x 60 mL). The combined organic phases were then dried over Na_2SO_4. The crude product was purified by flash column chromatography.

(1*S*,3*R*,4*S*)-Quinuclidin-3-yl-biphen-2-ylcarbamate (8a) was prepared from triphosgene (35.6 mg, 120 µmol, 0.4 eq.), biphenyl-2-amine (**6a**) (50.0 mg, 300 µmol) and (*R*)-quinuclidin-3-ol (45.7 mg, 360 µmol, 1.2 eq.) according to General Procedure D. The carbamate **8a** was obtained by flash

column chromatography (CH_2Cl_2 / methanol = 15 : 1) as a beige oil (29 mg, 90.0 µmol, 30% yield). ^{1}H-NMR (600 MHz, $CDCl_3$) δ 1.38 – 1.44 (m, 1H), 1.57 – 1.64 (m, 1H), 1.69 – 1.81 (m, 2H), 2.07 – 2.11 (m, 1H), 2.72 – 2.92 (m, 5 H), 3.27 (dd, *J* = 8.5, 14.6 Hz, 1H), 4.79 – 4.83 (m, 1H), 6.68 (s, 1H), 7.15 (dt, *J* = 1.2, 7.5 Hz, 1H), 7.23 (dd, *J* = 1.6, 7.6 Hz, 1H), 7.34 – 7.39 (m, 3H), 7.42 (t, *J* =7.4 Hz, 1H), 7.49 (t, *J* = 7.4 Hz, 2H), 8.05 (bs, 1H). ^{13}C-NMR (91 MHz, $CDCl_3$) δ 19.1, 24.0, 25.2, 46.3, 47.1, 55.0, 71.5, 123.7 (d, J_{CF} = 22.1 Hz), 125.6 (d, J_{CF} = 23.1 Hz), 128.4, 129.1, 129.1, 129.2, 130.2, 134.6, 138.1, 153.2. HPLC (254 nm, system A): t_R= 9.3 min, purity: >95% yield. HRMS (EI) calcd. for $C_{20}H_{22}N_2O_2$ [M+H$^+$]: 322.1681, found: 322.1682. $[\alpha]^{24.1}_D$ 13 (c 0.15, CH_3CN). The analytical data obtained is in agreement with those reported in literature.[23]

(1*S*,3*R*,4*S*)-Quinuclidin-3-yl-(5-fluorobiphen-2-yl)carbamate (8d) was prepared from triphosgene (120 mg, 400 µmol, 0.4 eq.), 5-fluoro-biphenyl-2-amine (**6d**) (189 mg, 1.00 mmol) and (*R*)-quinuclidin-3-ol (155 mg, 1.20 mmol, 1.2 eq.) according to General Procedure D. The carbamate **8d** was obtained by flash column chromatography (CH_2Cl_2 / methanol = 15 : 1) as a beige oil (206 mg, 600 µmol, 60% yield). ^{1}H NMR (600 MHz,) δ 1.8 (s, 1H), 1.9 (s, 1H), 2.1 (s, 2H), 2.5 (s, 1H), 3.3 (d, *J* = 43.1 Hz, 5H), 3.7 (s, 1H), 5.1 (s, 1H), 6.6 (s, 1H), 6.9 – 7.0 (m, 1H), 7.1 (ddd, *J* = 9.0, 7.9, 3.0 Hz, 1H), 7.3 – 7.4 (m, 2H), 7.4 – 7.5 (m, 1H), 7.5 (t, *J* = 7.6, 7.6 Hz, 2H), 7.9 (s, 1H). ^{13}C-NMR (91 MHz, $CDCl_3$) δ 114.8 (d, J_{CF} = 22.3 Hz), 116.5 (d, J_{CF} = 3.6 Hz), 116.6 (d, J_{CF} = 26.7 Hz), 127.6, 128.8 (d, J_{CF} = 7.2 Hz), 128.9, 129.0, 133.6, 139.4 (d, J_{CF} = 2.2 Hz), 156.4 (d, J_{CF} = 236.9 Hz). HPLC (254 nm, system B): t_R= 8.2 min, purity: >95%. HRMS (EI) calcd. for $C_{20}H_{22}FN_2O_2$ [M+H$^+$]: 341.1660, found: 341.1656. $[\alpha]^{24.3}_D$ 3 (c 0.32, CH_3CN). The analytical data obtained is in agreement with those reported in literature.[22]

(1*S*,3*R*,4*S*)-Quinuclidin-3-yl (3',4'-dichloro-5-fluorobiphen-2-yl)carbamate (8k) was prepared from triphosgene (23 mg, 75 µmol, 0.33 eq.), 3',4'-dichloro-5-fluoro-biphenyl-2-amine (**6k**) (57.9

mg, 0.23 mmol) and (*R*)-quinuclidin-3-ol (29.3 mg, 0.23 mmol, 1.0 eq.) according to General Procedure D. The carbamate **8k** was obtained by flash column chromatography (CH_2Cl_2 / methanol = 15 : 1) as a beige oil (43.3 mg, 0.11 mmol, 48% yield) in a reasonable purity (>90%). The crude product was further purified by preparative HPLC and obtained as a beige oil (23.7 mg, 0.052 mmol, 23% yield). ^{1}H-NMR (600 MHz, $CDCl_3$) δ 1.35 - 1.48 (m, 1H), 1.53 - 1.64 (m, 1H), 1.66 - 1.83 (m, 2H), 2.04 - 2.10 (m, 1H), 2.66 - 2.94 (m, 5H), 3.28 (dd, *J* = 8.4, 14.6 Hz, 1H), 4.80 (bs, 1H), 6.35 (bs, 1H), 6.94 (dd, *J* = 2.9, 8.7 Hz, 1H), 7.09 (ddd, *J* = 3.0, 7.9, 9.0 Hz, 1H), 7.21 (dd, *J* = 2.1, 8.2 Hz, 1H), 7.48 (d, *J* = 2.0 Hz, 1H), 7.56 (d, *J* = 8.2 Hz, 1H) 7.85-7.94 (bs, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 17.0, 20.7, 24.3, 45.3, 46.2, 53.0, 67.9, 116.0 (d, J_{CF} = 22.1 Hz), 116.9 (d, J_{CF} = 23.0 Hz), 128.2, 130.9, 131.1, 133.1, 133.4, 136.9, 152.5 (one signal missing). HPLC (254 nm, system A): t_R= 10.8 min, purity: >95%. HRMS (EI) calcd. for $C_{20}H_{20}Cl_2FN_2O_2$ [M^+]: 409.0880, found: 409.0878. $[\alpha]^{24.4}_D$ 7 (c 0.01, CH_3CN).

(1*S*,3*R*,4*S*)-Quinuclidin-3-yl (3',4'-dichloro-5-fluorobiphen-2-yl)carbamate (8m) was prepared from triphosgene (24.8 mg, 24.8 µmol, 0.5 eq.), 3'-Chloro-5-fluoro-biphenyl-2-amine (**6m**) (11.0 mg, 50 µmol) and (*R*)-quinuclidin-3-ol (9.5 mg, 74 µmol, 1.5 eq.) according to General Procedure D. The carbamate **8m** was purified by preperative HPLC and obtained as a beige oil (2.0 mg, 5.5 µmol, 11% yield). ^{1}H NMR (400 MHz, MeOD) δ 1.8 (d, *J* = 22.3 Hz, 2H), 1.9 – 2.0 (m, 1H), 2.1 (d, *J* = 13.2 Hz, 1H), 2.2 (s, 1H), 2.8 – 3.3 (m, 2H), 3.6 (t, *J* = 11.5 Hz, 1H), 7.1 – 7.2 (m, 2H), 7.4 (d, *J* = 7.5 Hz, 1H), 7.4 – 7.5 (m, 4H), 8.5 (s, 1H). ^{13}C NMR (101 MHz, MeOD) δ 16.8, 20.3, 24.1, 45.4, 46.3, 53.4, 68.1, 114.9 (d, J_{CF} = 22.5 Hz), 116.5 (d, J_{CF} = 22.5 Hz), 127.1, 127.5, 128.5, 129.9, 130.2, 130.2, 133.8, 133.8, 136.8, 140.3, 140.3, 160.1 (d, J_{CF} = 241.1 Hz), 168.5. HPLC (254 nm, system A): t_R= 10.1 min, purity: >95%. HRMS (EI) calcd. for $C_{20}H_{20}ClFN_2O_2$ [M^+]: 375.1270, found: 375-1178. $[\alpha]^{25.06}_D$ 4 (c 0.08, CH_3CN).

(1*S*,3*R*,4*S*)-Quinuclidin-3-yl (3'-chloro-4',5-difluorobiphen-2-yl)carbamate (8n) was prepared from triphosgene (37.2 mg, 125 µmol, 0.5 eq.), 3'-Chloro-4',5-difluoro-biphenyl-2-amine (**6n**) (60.0 mg, 250 µmol) and (*R*)-quinuclidin-3-ol (47.8 mg, 376 µmol, 1.5 eq.) according to General Procedure D. The carbamate **8n** was purified by preperative HPLC and obtained as a beige oil (17.8 mg, 45.3 µmol, 18% yield). ^{1}H NMR (400 MHz, MeOD) δ 1.7 (d, *J* = 37.1 Hz, 2H), 1.9 – 2.1 (m, 2H), 2.1 – 2.4 (m, 1H), 2.8 – 3.3 (m, 5H), 3.5 – 3.6 (m, 1H), 7.2 (dd, *J* = 6.9, 9.6 Hz, 2H), 7.2 – 7.4 (m, 2H), 7.5 (dd, *J* = 5.4, 8.3 Hz, 1H), 7.5 – 7.6 (m, 4H), 8.6 (s, 1H). ^{13}C NMR (101 MHz, MeOD) δ 17.3, 21.2, 24.4, 45.4, 46.4, 53.7, 69.0, 114.8, 115.1, 116.3 (d, J_{CF} = 6.6 Hz), 116.6 (d, J_{CF} = 8.7 Hz), 120.2 (d, J_{CF} = 17.7 Hz), 129.1, 130.4, 130.4, 130.7, 135.8, 154.9, 157.6 (d, J_{CF} = 248.7 Hz), 160.9 (d, J_{CF} = 244.7 Hz), 168.9. HPLC (254 nm, system A): t_R= 10.3 min, purity: >95%. HRMS (EI) calcd. for $C_{20}H_{19}ClF_2N_2O_2$ [M^+]: 393.1176, found: 393.1178. $[\alpha]^{25.06}_D$ -10 (c 0.61, CH_3CN).

(1*S*,3*R*,4*S*)-Quinuclidin-3-yl-(5-fluorobiphen-2-yl)urea (11) was prepared from triphosgene (61.5 mg, 205 µmol), 5-fluoro-biphenyl-2-amine (**6d**) (95.9 mg, 513 µmol) and (*R*)-quinuclidin-3-amine (77.6 mg, 616 µmol) according to General Procedure D. The urea **11** was obtained by flash column chromatography (CH_2Cl_2 / methanol = 15 : 1) as a beige oil (80 mg, 236 µmol, 46% yield). ^{1}H-NMR (600 MHz, $CDCl_3$, trifluoroacetate salt) δ 1.81-1.89 (m, 1H), 1.93 - 2.04 (m, 3H), 2.06-2.10 (m, 1H), 2.93 (ddd, *J* = 2.5, 5.1, 13.4 Hz, 1H), 3.21 - 3.33 (m, 4H), 3.66 (ddd, *J* = 2.5, 9.6, 13.3 Hz, 1H), 4.03 - 4.08 (m, 1H), 7.01 (dd, *J* = 2.9, 9.2 Hz, 1H), 7.07 (ddd, *J* = 3.0 Hz, 8.2 Hz, 8.9 Hz, 1H), 7.35 - 7.42 (m, 3H), 7.44 - 7.48 (m, 2H), 7.64 (dd, *J* = 5.3, 8.9 Hz, 1H). Nitrogen-bound proton signals missing. ^{13}C-NMR (91 MHz, $CDCl_3$) δ 18.3, 22.9, 25.9, 46.1, 47.0, 47.6, 54.9, 115.5 (d, J_{CF} = 22.3 Hz), 117.7 (d, J_{CF} = 23.0 Hz), 128.0 (d, J_{CF} = 8.4 Hz), 129.1, 129.9, 130.0, 132.7 (d, J_{CF} = 2.9 Hz), 139.1 (d, *J* = 7.8 Hz), 139.6 (d, J_{CF} = 1.6 Hz), 158.3, 161.2 (d, J_{CF} = 243.2 Hz). HPLC (254 nm, system A): t_R= 9.0 min, purity: >95%. HRMS (EI) calcd. for $C_{20}H_{23}FN_3O$ [M^+]: 340.1820, found: 340.1822. $[\alpha]^{24.6}_D$ -3 (c 0.87, CH_3CN).

General Procedure E: Elimination of the *tert*-butoxycarbonylgroup

The corresponding Boc-protected compound (1.0 mmol, 1.0 eq.) was stirred in trifluoroacetic acid in CH_2Cl_2 (10% yield, 5.0 mL) for 10 hours at room temperature, monitored by TLC. The solvent was removed under reduced pressure and the crude product was dried *in vacuo*.

((Biphen-2-yl-carbamoyl)oxymethyl)piperidin-1-ium trifluoroacetate (13a) was prepared from *tert*-butyl ((biphen-2-yl-carbamoyl)oxymethyl)piperidine-1-carboxylate (**7a)** (276 µmol, 113 mg) according to General Procedure E. Without any further purification **13a** was identified as a brown oil (268 µmol, 114 mg, 97%).^{1}H NMR (600 MHz, $CDCl_3$) δ 1.17 – 1.44 (m, 3H), 1.50 – 1.78 (m, 2H), 1.84 – 2.11 (m, 2H), 2.67 – 3.09 (m, 2H), 3.37 – 3.63 (m, 2H), 3.85 – 4.17 (m, 2H), 6.65 (s, 1H), 7.15 (t, J = 7.3 Hz, 1H), 7.23 (dd, J = 1.6, 7.6 Hz, 1H), 7.33 – 7.39 (m, 2H), 7.42 (t, J = 7.3 Hz, 1H), 7.48 (t, J = 7.4 Hz, 1H), 8.07 (s, 1H). Nitrogen-bound proton signals missing. ^{13}C NMR (151 MHz, $CDCl_3$) δ 25.5, 29.7, 33.8, 55.7, 128.0, 128.5, 129.1, 129.2, 130.3, 138.0. HPLC (254 nm, system A): t_R= 9.4 min, purity: >95%. HRMS (EI) calcd. for $C_{19}H_{23}N_2O_2$ [M^+ - CF_3COO]: 311.1754, found: 311.1754.

4-(((4'-Chlorobiphen-2-yl)carbamoyl)oxymethyl)piperidin-1-ium trifluoroacetate (13b) was prepared from *tert*-butyl 4-(((4'-chloro-biphen-2-yl)carbamoyl)oxymethyl)piperidine-1-carboxylate (**7b**) (60.0 µmol, 27.0 mg) according to General Procedure E. Without any further **13b** was identified as a red oil (58.0 µmol, 26.0 mg, 96% yield). ^{1}H-NMR (600 MHz, CD_3OD) δ 0.98 - 1.02 (m, 2H), 1.38 - 1.52 (m, 3H), 2.54 (t, J = 12.1 Hz, 2H), 2.97 (d, J = 12.7 Hz, 2H), 3.50 (d, J = 5.6 Hz, 2H), 6.85 - 6.92 (m, 2H), 6.94 - 6.98 (m, 3H), 7.02 (d, J = 8.6 Hz, 2H), 7.04 - 7.12 (m, 1H). ^{13}C-NMR (151 MHz, CD_3OD) δ 26.5, 34.8, 44.7, 69.0, 114.4, 117.6, 127.3, 129.5, 129.6, 131.4, 131.7, 134.4, 135.7, 139.4, 156.0. HPLC (254 nm, system A): t_R= 9.8 min, purity: >95%. HRMS (EI) calcd. for $C_{19}H_{22}ClN_2O_2$ [M^+ - CF_3COO]: 345.1364, found: 345.1366.

4-(((3',4',5'-Trifluorobiphen-2-yl)carbamoyl)oxymethyl)piperidin-1-ium trifluoroacetate (13c) was prepared from *tert*-butyl-4-(((3',4',5'-trifluoro-biphen-2-yl)carbamoyl)oxymethyl)piperidine-1-carboxylate (**7c**) (195 µmol, 90.6 mg,) according to General Procedure E. Without any further purification **13c** was identified as a dark brown oil (195 µmol, 93.7 mg, 99% yield). ^{1}H-NMR (600 MHz, CD_3OD) δ 7.47 (d, J = 7.91 Hz, 1H), 7.40 (ddd, J = 2.05, 6.93, 8.04 Hz, 1H), 7.35 – 7.29 (m, 2H), 7.18 – 7.12 (m, 2H), 3.95 (d, J = 6.43 Hz, 2H), 3.40 (d, J = 12.74 Hz, 2H), 2.97 (td, J = 2.41, 12.82 Hz, 2H), 1.99 – 1.87 (m, 3H), 1.43 (td, J = 3.96, 14.35 Hz, 2H). ^{13}C-NMR (91 MHz, CD_3OD) δ 26.6, 34.9, 44.8, 69.2, 114.3, 144.8, 117.7, 127.6, 130.2, 131.4, 135.8, 137.5 (td, J_{CF} = 4.97, 8.33 Hz), 140.3 (dt, J_{CF} = 15.38, 249.66 Hz), 152.3 (ddd, J_{CF} = 4.12, 9.88, 247.96 Hz), 156.8. HPLC (254 nm, system A): t_R= 9.8 min, purity: >95%. HRMS (EI) calcd. for $C_{19}H_{20}F_3N_2O_2$ [M^+ - CF_3COO]: 365.1471, found: 365.1469.

4-((((5-Fluorobiphen-2-yl)carbamoyl)oxy)methyl)piperidin-1-ium trifluoroacetate (13d) was prepared from *tert*-butyl 4-(((5-fluoro-biphenyl-2-yl)carbamoyl)oxymethyl)piperidine-1-carboxylate **(7d)** (32.6 µmol, 13.9 mg) according to General Procedure E. Without any further **13d** was identified as a red oil (32.6 µmol, 12.0 mg, quant. yield). ^{1}H-NMR (600 MHz, CD_3OD) δ 1.38 (dt, J = 3.9 Hz, J = 14.9 Hz, 2H), 1.76 - 1.92 (m, 3H), 2.94 (t, J = 12.1 Hz, 2H), 3.37 (d, J = 12.7 Hz, 2H), 3.87 - 3.96 (m, 2H), 7.05 - 7.13 (m, 2H), 7.36 - 7.40 (m, 3H), 7.42 - 7.45 (m, 2H), 7.46 - 7.55 (m, 1H). ^{13}C-NMR (151 MHz, CD_3OD) δ 26.5, 34.7, 44.7, 69.0, 115.6 (d, J_{CF} = 22.5 Hz), 117.9 (d, J_{CF} = 23.1 Hz), 128.9 (d, J_{CF} = 8.1 Hz), 129.7, 129.9 (d, J_{CF} = 8.3 Hz), 130.0, 131.9 (d, J_{CF} = 2.9 Hz), 132.0, 139.6 (d, J_{CF} = 245.3 Hz), 156.9. HPLC (254 nm, system A): t_R= 9.6 min, purity: >95%. HRMS (EI) calcd. for $C_{19}H_{22}FN_2O_2$ [M^+ - CF_3COO]: 329.1665, found: 329.1664.

4-(((2'-Chloro-5-fluorobiphen-2-yl)carbamoyl)oxymethyl)piperidin-1-ium trifluoroacetate (13e) was prepared from *tert*-butyl 4-(((2'-chloro-5-fluoro-biphen-2-yl)carbamoyl)oxymethyl)piperidine-1-carboxylate (**7e**) (60.1 µmol, 27.0 mg) according to General Procedure E. Without any further purification **13e** was identified as a dark brown oil (55.3 µmol, 25.0 mg, 92% yield). ^{1}H-NMR (600 MHz, CD_3OD) δ 1.27 (dt, J = 4.0 Hz, J = 15.6 Hz, 2H), 1.73 (d, J = 14.2 Hz, 2H), 1.75 - 1.85 (m, 1H), 2.83 (dt, J = 2.8 Hz, J = 12.9 Hz, 2H), 3.26 (d, J = 13.0 Hz, 2H), 3.77 - 3.83 (m, 2H), 6.83 (dd, $_J$ = 2.9 Hz, J = 8.9 Hz, 1H), 7.05 (ddd, $_J$ = 3.0 Hz, J = 8.2 Hz, J = 8.9 Hz, 1H), 7.18 - 7.31 (m, 3H), 7.37 - 7.40 (m, 1H), 7.43 (s, 1H). ^{13}C-NMR (151 MHz, DEPTQ, CD_3OD) δ 26.5, 34.8, 44.7, 69.1, 116.4 (d, J_{CF} = 22.5 Hz), 118.4, 118.2 (d, J_{CF} = 22.5 Hz), 128.2, 131.6, 130.8, 132.9 (d, J_{CF} = 8.5 Hz), 133.0, 134.4, 137.9, 138.0 (d, J_{CF} = 250.3 Hz), 156.6. HPLC (254 nm, system A): t_R= 9.8 min, purity: >95%. HRMS (EI) calcd. for $C_{19}H_{21}ClFN_2O_2$ [M^+ - CF_3COOH]: 363.1276, found: 363.1275.

4-(((4',5-Difluorobiphen-2-yl)carbamoyl)oxymethyl)piperidin-1-ium trifluoroacetate (13f) was prepared from *tert*-butyl 4-(((4',5-difluoro-biphenyl-2-yl)carbamoyl)oxymethyl)piperidine-1-carboxylate **(7f)** (56.4 µmol, 25.2 mg) according to General Procedure E. Without any further purification **13f** was identified as a dark brown oil (54.7 µmol, 25.2 mg, 97% yield). ^{1}H NMR (360 MHz, MeOD) δ 1.3 – 1.5 (m, 2H), 1.9 (d, J = 14.9 Hz, 3H), 2.9 – 3.0 (m, 2H), 3.4 (dt, J = 13.0, 3.3, 3.3 Hz, 2H), 3.9 (d, J = 6.2 Hz, 2H), 7.0 – 7.1 (m, 2H), 7.1 – 7.2 (m, 2H), 7.4 – 7.5 (m, 2H), 7.4 – 7.5 (m, 1H). ^{13}C-NMR (91 MHz, $CDCl_3$) δ 115.0 (d, J_{CF} = 22.3 Hz), 115.8 (d, J_{CF} = 21.4 Hz), 116.6 (d, J_{CF} = 7.7 Hz), 116.7 (d, J_{CF} = 22.5 Hz), 127.7 (d, J_{CF} = 7.2 Hz), 130.6 (d, J_{CF} = 8.0 Hz), 134.4 (dd, J_{CF} = 1.6 Hz, J_{CF} = 3.3 Hz), 139.4 (d, J_{CF} = 2.3 Hz), 156.3 (d, J_{CF} = 237.5 Hz), 162.3 (d, J_{CF} = 247.2 Hz). HPLC (254 nm, system A): t_R= 9.5 min, purity: >95%. HRMS (EI) calcd. for $C_{19}H_{21}F_2N_2O_2$ [M^+ - CF_3COO]: 347.1566, found: 347.1566.

4-(((4'-Chloro-5-fluorobiphen-2-yl)carbamoyl)oxymethyl)piperidin-1-ium trifluoroacetate (13g) was prepared from *tert*-butyl 4-(((4'-chloro-5-fluoro-biphenyl-2-yl)carbamoyl)oxymethyl)piperidine-1-carboxylate **(7g)** (87.7 µmol, 40.6 mg) according to General Procedure E. Without any further purification **13g** was identified as a dark brown oil (68.9 µmol, 32.9 mg, 79% yield). ^{1}H NMR (600 MHz, MeOD) δ 1.3 – 1.4 (m, 2H), 1.8 (d, J = 16.5 Hz, 3H), 2.8 – 3.1 (m, 2H), 3.4 (dt, J = 13.0, 3.2, 3.2 Hz, 2H), 3.9 (s, 2H), 7.1 – 7.2 (m, 2H), 7.4 – 7.4 (m, 2H), 7.4 – 7.5 (m, 2H). ^{13}C-NMR (91 MHz, $CDCl_3$) δ 115.2 (d, J_{CF} = 22.2 Hz), 116.5 (d, J_{CF} = 22.6 Hz), 116.8 (d, J_{CF} = 7.8 Hz), 127.5 (d, J_{CF} = 7.3 Hz), 129.1, 130.3, 133.6, 136.9 (d, J_{CF} = 1.7 Hz), 139.2 (d, J_{CF} = 2.2 Hz), 155.1 (d, J_{CF} = 243.9 Hz). HPLC (254 nm, system A): t_R= 9.9 min, purity: >95%. HRMS (EI) calcd. for $C_{19}H_{21}ClFN_2O_2$ [M^+ - CF_3COO]: 363.1268, found: 363.1270.

4-(((4'-Bromo-5-fluorobiphen-2-yl)carbamoyl)oxymethyl)piperidin-1-ium trifluoroacetate (13h) was prepared from *tert*-butyl 4-(((4'-bromo-5-fluoro-biphenyl-2-yl)carbamoyl)oxymethyl)piperidine-1-carboxylate **(7h)** (13.5 µmol, 6.9 mg) according to General Procedure E. Without any further purification **13h** was identified as a dark brown oil (13.4 µmol, 7 mg, 99% yield). ^{1}H-NMR (600 MHz, CD_3OD) δ 1.32-1.43 (m, 2H), 1.76 - 1.94 (m, 3H), 2.96 (t, J = 12.7 Hz, 2H), 3.39 (d, J = 12.7 Hz, 2H), 3.86 - 3.98 (m, 2H), 7.07 - 7.15 (m, 3H), 7.28-7.35 (m, 2H), 7.57-7.61 (m, 2H). One signal missing. ^{13}C-NMR (151 MHz, CD_3OD) δ 26.5, 34.8, 44.8, 69.0, 116.1 (d, J_{CF} = 22.5 Hz), 117.6 (d, J_{CF} = 23.3 Hz), 122.8, 132.0, 132.7, 139.0, 156.9. HPLC (254 nm, system A): t_R= 10.2 min, purity: >95%. HRMS (EI) calcd. for $C_{19}H_{21}BrFN_2O_2$ [M^+ - CF_3COO]: 407.0765, found: 407.0768.

4-(((4'-Cyano-5-fluorobiphen-2-yl)carbamoyl)oxymethyl)piperidin-1-ium trifluoroacetate (13i) was prepared from *tert*-butyl 4-(((4'-cyano-5-fluoro-biphenyl-2-

yl)carbamoyl)oxymethyl)piperidine-1-carboxylate **(7i)** (225 µmol, 102 mg) according to General Procedure E. Without any further purification **13i** was identified as a dark brown oil (218 µmol, 99.0 mg, 97% yield). ^{1}H-NMR (600 MHz, CD_3OD) δ 1.37 (dt, J = 11.9 Hz, J = 23.6 Hz, 2H), 1.78 - 1.92 (m, 3H), 2.96 (dt, J = 2.0 Hz, J = 12.9 Hz, 2H), 3.39 (d, J = 12.7 Hz, 2H), 3.86 (d, J = 6.2 Hz, 2H), 7.13 - 7.21 (m, 2H), 7.45 (dd, J = 5.3 Hz, J = 8.6 Hz, 1H), 7.58 (d, J = 8.6 Hz, 2H), 7.80 (d, J = 8.6 Hz, 2H). One signal missing. ^{13}C-NMR (151 MHz, CD_3OD) δ 26.6, 34.8, 44.8, 69.2, 112.5, 116.8 (d, J_{CF} = 22.5 Hz) 117.8 (d, J_{CF} = 23.6 Hz), 119.7, 131.1 (d, J_{CF} = 245.1 Hz) 131.3 (d, J_{CF} = 8.3 Hz), 133.4, 133.6 (d, J_{CF} = 8.3 Hz,), 144.9 (d, J_{CF} = 3.1 Hz), 148.2, 156.9. HPLC (254 nm, system A): t_R= 9.3 min, purity: >95%. HRMS (EI) calcd. for $C_{20}H_{21}FN_3O_2$ [M^+ - CF_3COO]: 354.1618, found: 354.1618.

4-(((5-Fluoro-4'-methoxybiphen-2-yl)carbamoyl)oxymethyl)piperidin-1-ium trifluoroacetate (13j) was prepared from *tert*-butyl 4-(((5-fluoro-4'-methoxy-biphen-2-yl)carbamoyl)oxymethyl)piperidine-1-carboxylate (**7j**) (24.0 µmol, 11.0 mg) according to General Procedure E. Without any further purification **13j** was identified as a dark brown oil (22.0 µmol, 10.0 mg, 92% yield). ^{1}H-NMR (600 MHz, CD_3OD) δ 1.37 (d, J = 13.8 Hz, 2H), 1.76 – 1.94 (m, 3H) 2.94 (t, J = 12.7 Hz, 2H), 3.34 – 3.40 (m, 2H), 3.84 (s, 3H), 3.87 – 4.00 (m, 2H), 6.97 – 7.10 (m, 2H), 7.04 – 7.09 (m, 2H), 7.31 (d, J = 8.6 Hz, 2H), 7.47 (bs, 1H). One signal missing. ^{13}C-NMR (151 MHz, $CDCl_3$) δ 55.3, 114.3, 114.5 (d, J_{CF} = 22.2 Hz), 116.4 (d, J_{CF} = 7.8 Hz), 116.7 (d, J_{CF} = 22.2 Hz), 128.5 (d, J_{CF} = 7.3 Hz), 130.0, 130.8, 139.6 (d, J_{CF} = 2.3 Hz), 156.4 (d, J_{CF} = 236.2 Hz), 159.1. HPLC (254 nm, system A): t_R= 9.2 min, purity: >95%. HRMS (EI) calcd. for $C_{20}H_{24}FN_2O_2$ [M^+ - CF_3COO]: 359.1766, found. 359.1764.

4-(((3',4'-Dichloro-5-fluorobiphen-2-yl)carbamoyl)oxymethyl)piperidin-1-ium trifluoroacetate (13k) was prepared from *tert*-butyl 4-(((3',4'-dichloro-5-fluoro-biphenyl-2-

yl)carbamoyl)oxymethyl)piperidine-1-carboxylate **(7k)** (31.5 µmol, 15.7 mg) according to General Procedure E. Without any further purification **13k** was identified as a dark brown oil (29.0 µmol, 14.4 mg, 92% yield). ^{1}H-NMR (600 MHz, CD_3OD) δ 1.39 (dt, J = 4.5 Hz, J = 11.5 Hz, 2H), 1.80 – 1.92 (m, 3H), 2.95 (t, J = 11.9 Hz, 2H), 3.39 (d, J = 12.8 Hz, 2H), 3.89 – 3.93 (m, 2H), 7.12 (m, 1H), 7.15 (ddd, J = 3.0 Hz, J = 8.1 Hz, J = 8.7 Hz, 1H), 7.32 (m, 1H), 7.39 - 7.44 (m, 1H), 7.57 (d, J = 2.0 Hz, 1H), 7.59 (d, J = 8.3 Hz, 1H). One signal missing. ^{13}C-NMR (151 MHz, CD_3OD) δ 26.5, 34.9, 44.8, 69.1, 116.5 (d, J_{CF} = 22.5 Hz), 117.8 (d, J_{CF} = 23.6 Hz), 130.0, 131.7, 131.9, 132.7, 133.2, 139.2, 140.2, 156.3 (d, J_{CF} = 241.0 Hz), 161.5. HPLC (254 nm, system A): t_R= 10.3 min, purity: >95%. HRMS (EI) calcd. for $C_{19}H_{20}Cl_2FN_2O_2$ [M^+ - CF_3COO]: 397.0880, found. 397.0877.

4-(((3',4',5,5'-Tetrafluorobiphen-2-yl)carbamoyl)oxymethyl)piperidin-1-ium trifluoroacetate (13l) was prepared from *tert*-butyl 4-(((3',4',5,5'-tetrafluoro-biphen-2-yl)carbamoyl)oxymethyl)piperidine-1-carboxylate (**7l**) (18.0 µmol, 8.0 mg) according to General Procedure E. Without any further purification **13l** was identified as a black oil (18.0 µmol, 8.9 mg, quant. yield). ^{1}H-NMR (600 MHz, CD_3OD) δ 1.42 (dd, J = 11.0 Hz, J = 23.8 Hz, 2H), 1.86 - 1.98 (m, 3H), 2.97 (t, J = 11.9 Hz, 2H), 3.40 (d, J = 12.8 Hz, 2H), 3.95 (d, J = 5.9 Hz, 2H), 713 - 7.20 (m, 3H), 7.27 - 7.31 (m, 1H), 7.42 (dd, J = 4.5 Hz, J = 9.0 Hz, 1H). One signal missing. ^{13}C-NMR (151 MHz, DEPTQ, CD_3OD) δ 26.6, 34.9, 44.8, 69.2, 114.6 (d, J_{CF} = 4.6 Hz), 114.7 (d, J_{CF} = 22.5 Hz), 116.1 (d, J_{CF} = 264.7 Hz), 116.8 (d, J_{CF} = 22.5 Hz), 117.9 (d, J_{CF} = 23.8 Hz), 118.1 (d, J_{CF} = 23.7 Hz), 126.0 (d, J_{CF} = 9.3 Hz), 132.0 (d, J_{CF} = 3.2 Hz), 152.2 (d, J_{CF} = 247.7 Hz), 152.2 (d, J_{CF} = 247.4 Hz), 152.3 (d, J_{CF} = 247.9 Hz), 157.0. HPLC (254 nm, system A): t_R= 10.1 min, purity: >95%. HRMS (EI) calcd. for $C_{19}H_{19}F_4N_2O_2$ [M^+ - CF_3COO]:383.1377, found: 383.1379.

4-(((3'-Chloro-5-fluorobiphen-2-yl)carbamoyl)oxymethyl)piperidin-1-ium trifluoroacetate (13m) was prepared from crude *tert*-butyl 4-(((3'-chloro-5-fluoro-biphenyl-2-yl)carbamoyl)oxymethyl)piperidine-1-carboxylate **(7m)** (383 µmol, 85.0 mg) according to General Procedure E. The crude product was purified via preparative HPLC ((H_2O / CH_3CN = 70 : 30 → 20 : 80, 0.1% FA as modifier) to obtain **13m** as a white solid (62.5 mg, 172 µmol, 45% yield over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.63 (q, *J* = 13.4, 14.9 Hz, 2H), 1.91 (d, *J* = 13.7 Hz, 2H), 2.36 (bs, 1H), 2.88 (q, *J* = 12.0 Hz, 2H), 3.44 (d, *J* = 12.8 Hz, 2H), 4.04 (d, *J* = 6.0 Hz, 2H), 6.45 (s, 1H), 6.97 (dd, *J* = 3.0, 8.7 Hz, 1H), 7.11 (ddd, *J* = 3.0, 7.9, 9.0 Hz, 1H), 7.26 (ddd, *J* = 1.7, 2.5, 6.4 Hz, 1H), 7.36 (td, *J* = 0.8, 1.7 Hz, 1H), 7.41 – 7.48 (m, 2H), 7.94 (bs, 1H), 9.10 (bs, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 25.4 , 33.8 , 43.5 , 68.1 , 115.6 (d, J_{CF} = 20.1 Hz), 116.7 (d, J_{CF} = 24.1 Hz), 127.1 , 128.5, 129.2, 130.3, 130.4, 135.0, 138.9, 153.5, 159.2 (d, J_{CF} = 244.8 Hz), 162.4, 162.7. HPLC (254 nm, system A): t_R= 10.0 min, purity: >95%. HRMS (EI) calcd. for $C_{19}H_{20}ClFN_2O_2$ [M^+ - CF_3COO]: 363.1270, found: 363.1270.

4-(((3'-Chloro-4',5-difluorobiphen-2-yl)carbamoyl)oxymethyl)piperidin-1-ium trifluoroacetate (13n) was prepared from *tert*-butyl 4-(((3'-chloro-4',5-difluoro-biphenyl-2-yl)carbamoyl)oxymethyl)piperidine-1-carboxylate **(7n)** (250 µmol, 120 mg) according to General Procedure E. . The crude product was purified via preparative HPLC ((H_2O / CH_3CN = 70 : 30 → 20 : 80, 0.1% FA as modifier) to obtain **13n** as a white solid (120 mg, 243 µmol, 97% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.55 – 1.74 (m, 2H), 1.90 (d, *J* = 14.4 Hz, 2H), 2.87 (td, *J* = 3.0, 12.9 Hz, 2H), 3.44 (d, *J* = 12.8 Hz, 2H), 4.05 (d, *J* = 5.9 Hz, 2H), 6.44 (s, 1H), 6.96 (dd, *J* = 3.0, 8.7 Hz, 1H), 7.11 (ddd, *J* = 3.0, 7.9, 9.1 Hz, 1H), 7.23 – 7.27 (m, 1H), 7.40 – 7.56 (m, 1H), 7.91 (bs, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 25.3 , 33.8 , 43.5 , 72.8 (d, J_{CF} = 924.0 Hz), 115.71 (d, J_{CF} = 21.4 Hz), 116.82 (d, J_{CF} = 23.4 Hz), 117.3 (d, J_{CF} = 20.8 Hz), 121.8 (d, J_{CF} = 18.1 Hz), 128.8 , 130.4 , 131.3 , 134.3 , 153.6, 158.0 (d, J_{CF} = 251.2 Hz), 159.2 (d, J_{CF} = 245.1 Hz), 162.6 (d, J_{CF} = 35.4

Hz), 166.5. HPLC (254 nm, system A): t_R= 10.1 min, purity: >95%. HRMS (EI) calcd. for $C_{19}H_{19}ClF_2N_2O_2$ [M^+ - CF_3COO]: 381.1176, found: 381.1174.

4-(((3',5'-Dichloro-5-fluorobiphen-2-yl)carbamoyl)oxymethyl)piperidin-1-ium trifluoroacetate (13o) was prepared from crude *tert*-butyl 4-(((3',5'-dichloro-5-fluoro-biphenyl-2-yl)carbamoyl)oxymethyl)piperidine-1-carboxylate **(7o)** (310 µmol, 79.5 mg) according to General Procedure E. . The crude product was purified via preparative HPLC ((H_2O / CH_3CN = 70 : 30 → 20 : 80, 0.1% FA as modifier) to obtain **13o** as a violet solid (64.0 mg, 161 µmol, 52% yield over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.65 (q, *J* = 12.2 Hz, 2H), 1.90 (d, *J* = 14.2 Hz, 2H), 2.87 (td, *J* = 2.9, 13.0 Hz, 2H), 3.43 (d, *J* = 12.7 Hz, 2H), 4.05 (d, *J* = 5.9 Hz, 2H), 6.47 (s, 1H), 6.97 (dd, *J* = 3.0, 8.6 Hz, 1H), 7.13 (ddd, *J* = 3.0, 7.9, 9.0 Hz, 1H), 7.27 (d, *J* = 1.9 Hz, 2H), 7.44 (t, J_{CF} = 1.9 Hz, 1H), 7.86 (bs, 1H). ^{13}C NMR (151 MHz, DEPTQ, $CDCl_3$) δ 25.3, 33.9, 43.4, 68.3, 116.1 (d, J_{CF} = 22.1 Hz), 116.7 (d, J_{CF} = 23.4 Hz), 127.5, 128.4, 130.3, 135.6, 140.2, 153.7, 159.5 (d, J_{CF} = 247.2 Hz), 162.5 (d, J_{CF} = 35.1 Hz), 167.85. HPLC (254 nm, system A): t_R= 10.5 min, purity: >95%. HRMS (EI) calcd. for $C_{19}H_{19}Cl_2FN_2O_2$ [M^+ - CF_3COO]: 397.0880, found: 397.0880.

General procedure F: Quarternization using methyl iodide

The respective quinuclidinyl carbamate **8**, quinuclidinyl urea **11** or methylpiperidine carbamate **13** (1 mmol, 1.0 eq.) was taken up in dry CH_2Cl_2 (4.0 mL) at room temperature. Methyl iodide (710 mg, 0.31 mL, 5 mmol, 5.0 eq.) and K_2CO_3 (415 mg, 3 mmol, 3.0 eq.) were added and the reaction mixture was stirred for 6 h. Afterwards, the reaction mixture was filtered and the solvent was removed under reduced pressure. The quaternary ammonium compound was transformed into the trifluoroacetate salt using trifluoroacetic acid.

(1*S*,3*R*,4*S*)-3-(((Biphen-2-yl)carbamoyl)oxy)-1-methylquinuclidin-1-ium trifluoroacetate (14a) was prepared from **8a** (43.0 mg, 125 µmol, 1.0 eq.), methyl iodide (39 µl, 625 mmol, 5.0 eq.) and K_2CO_3 (52 mg, 375 µmol, 3.0 eq.) according to General Procedure F. The quaternary ammonium trifluoroacetate **14a** (quant. conversion assumed) was purified by preparative HPLC and obtained as a beige oil. ^{1}H-NMR (600 MHz, $CDCl_3$) δ 1.83 – 1.98 (m, 2H), 2.02 – 2.10 (m, 1H), 2.40 (m, 1H), 2.95 (s, 3H), 3.10 (m, 1H), 3.31 – 3.49 (m, 5 H), 3.75 (m, 1H), 4.93 (m, 1H), 7.05 – 7.12 (m, 2H), 7.34 – 7.43 (m, 3H), 7.45 – 7.49 (m, 3H), 7.60 (bs, 1H). ^{13}C-NMR (151 MHz, $CDCl_3$) δ 19.3, 22.1, 25.1, 52.2, 57.1, 58.0, 64.2, 69.2, 127.39, 128.4, 128.6, 129.3, 129.7, 130.2, 131.7, 135.4, 140.7, 155.5. HPLC (254 nm, system A): t_R= 9.2 min, purity: >95%. HRMS (EI) calcd. for $C_{21}H_{25}FN_2O_2$ [M^+]: 337.1914, found: 337.1911. $[\alpha]^{24.3}{}_D$ -16 (c 2.48, CH_3CN).

(1*S*,3*R*,4*S*)-3-(((5-Fluorobiphen-2-yl)carbamoyl)oxy)-1-methylquinuclidin-1-ium trifluoroacetate (14d) was prepared from **8d** (43.0 mg, 125 µmol, 1.0 eq.), methyl iodide (39 µl, 625 mmol, 5.0 eq.) and K_2CO_3 (52 mg, 375 µmol, 3.0 eq.) according to General Procedure F. The quaternary ammonium trifluoroacetate **14d** (quant. conversion assumed) was purified by preparative HPLC and obtained as a beige oil. ^{1}H-NMR (600 MHz, $CDCl_3$) δ 1.83 – 1.98 (m, 2H), 2.02 – 2.10 (m, 1H), 2.12 – 2.21 (m, 1H), 2.30 (m, 1H), 2.95 (s, 3H), 3.12 – 3.25 (m, 1H), 3.30 – 3.36 (m, 1H), 3.41 – 3.51 (m, 2H), 3.71 – 3.82 (m, 1H), 4.88 – 5.00 (m, 1H), 7.05 – 7.12 (m, 2H), 7.34 – 7.43 (m, 3H), 7.45 – 7.48 (m, 2H), 7.50 (bs, 1H). One signal missing. ^{13}C-NMR (151 MHz, $CDCl_3$) δ 19.3, 22.1, 25.2, 52.3, 57.1, 58.0, 64.2, 69.3, 115.5 (d, J_{CF} = 22.0 Hz), 117.9 (d, J_{CF} = 22.8 Hz), 129.0, 129.7, 130.0, 131.6 (d, J_{CF} = 2.9 Hz), 139.7. HPLC (254 nm, system A): t_R= 9.4 min, purity: >95%. HRMS (EI) calcd. for $C_{21}H_{24}FN_2O_2$ [M^+]: 355.1809, found: 355.1805. $[\alpha]^{24.5}{}_D$ -8 (c 0.29, CH_3CN).

3-(((3'-Chloro-5-fluorobiphen-2-yl)carbamoyl)oxy)-1-methylquinuclidin-1-ium formate (14m) was prepared from **8m** (2.0 mg, 5 µmol, 1.0 eq.), methyl iodide (3.3 µl, 54 µmol, 10.0 eq.) and K_2CO_3 (3.7 mg, 27 µmol, 5.0 eq.) according to General Procedure F. The quaternary ammonium salt **14m** (quant. conversion assumed) was purified by preparative HPLC and obtained as a beige oil. ^{1}H NMR (400 MHz, MeOD) δ 1.8 – 2.6 (m, 5H), 3.0 (s, 3H), 3.2 (d, J = 13.4 Hz, 1H), 3.4 – 3.6 (m, 4H), 3.8 (t, J = 11.1 Hz, 1H), 4.6 (s, 1H), 7.2 (ddt, J = 3.0, 7.0, 12.2 Hz, 2H), 7.4 (d, J = 7.3 Hz, 1H), 7.4 – 7.6 (m, 4H), 8.6 (s, 1H). ^{13}C NMR (101 MHz, MeOD) δ 17.9, 20.7, 50.8, 55.7, 56.6, 62.8, 67.9, 114.9 (d, J_{CF} = 22.6 Hz), 116.5 (d, J_{CF} = 23.4 Hz), 127.2, 127.5, 128.5, 129.9, 130.1 (d, J_{CF} = 3.1 Hz), 133.8, 140.3, 140.3, 154.4, 162.1, 168.8. (one signal missing). HPLC (254 nm, system A): t_R= 10.1 min, purity: >95%. HRMS (EI) calcd. for $C_{21}H_{23}ClFN_2O_2$ [M^+]: 389.1427, found: 389.1427. $[\alpha]^{25.31}_D$ -21 (c 0.28, CH_3CN).

3-(((3'-Chloro-4',5-difluorobiphen-2-yl)carbamoyl)oxy)-1-methylquinuclidin-1-ium formate (14n) was prepared from **8n** (23.0 mg, 61 µmol, 1.0 eq.), methyl iodide (38.2 µl,614 µmol, 10.0 eq.) and K_2CO_3 (42 mg, 307 µmol, 5.0 eq.) according to General Procedure F. The quaternary ammonium salt **14n** (quant. conversion assumed) was purified by preparative HPLC and obtained as a beige oil. ^{1}H NMR (400 MHz, MeOD) δ 1.9 – 2.1 (m, 2H), 2.1 – 2.3 (m, 2H), 2.4 (s, 1H), 3.0 (s, 3H), 3.3 – 3.6 (m, 5H), 3.9 (ddd, J = 2.9, 8.4, 13.9 Hz, 1H), 5.0 (s, 1H), 7.0 – 7.3 (m, 2H), 7.3 – 7.5 (m, 2H), 7.5 – 7.7 (m, 2H), 8.6 (s, 1H). One signal missing. ^{13}C NMR (101 MHz, MeOD) δ 17.9, 20.7, 23.8, 50.9, 55.7, 56.6, 62.7, 67.9, 114.9, 115.1, 116.4 (d, J_{CF} = 3.1 Hz), 116.6 (d, J_{CF} = 5.3 Hz), 120.3 (d, J_{CF} = 18.0 Hz), 129.2 (d, J_{CF} = 7.4 Hz), 130.2, 130.2, 130.7, 135.3 – 136.3 (m), 154.4, 157.6 (d, J_{CF} = 248.7 Hz), 160.8 (d, J_{CF} = 245.7 Hz), 168.7. HPLC (254 nm, system A): t_R= 10.3 min, purity: >95%. HRMS (EI) calcd. for $C_{21}H_{23}ClFN_2O_2$ [M^+]: 407.1332, found: 407.1334. $[\alpha]^{25.19}_D$ -16 (c 1.11, CH_3CN).

(1*S*,3*R*,4*S*)-3-(((5-Fluorobiphen-2-yl)ureido)-1-methylquinuclidin-1-ium trifluoroacetate (15) was prepared from **11** (20.3 mg, 60 µmol, 1.0 eq.), methyl iodide (11 µl, 180 mmol, 3.0 eq.) and K_2CO_3 (9 mg, 60 µmol, 1.0 eq.) according to General Procedure F. The quaternary ammonium trifluoroacetate **15** (quant. conversion assumed) was purified by preparative HPLC and obtained as a beige oil. ^{1}H-NMR (600 MHz, $CDCl_3$, trifluoroacetate salt) δ 1.88-1.98 (m, 1H), 2.00 - 2.15 (m, 4H), 2.98 (s, 3H), 3.12 (ddd, J = 2.9, 4.9, 13.1 Hz, 1H), 3.35 - 3.50 (m, 4H), 3.78 (ddd, J = 2.5, 9.6, 13.3 Hz, 1H), 4.07 - 4.13 (m, 1H), 7.01 (dd, J = 2.9, 9.2 Hz, 1H), 7.07 (ddd, J = 3.0, 8.2, 8.9 Hz, 1H), 7.36 - 7.42 (m, 3H), 7.44 - 7.48 (m, 2H), 7.65 (dd, J = 5.3, 8.9 Hz, 1H). Nitrogen-bound signals missing. ^{13}C-NMR (91 MHz, $CDCl_3$) δ 19.8, 23.9, 25.8, 47.1, 52.4, 57.3, 57.9, 65.0, 115.5 (d, J_{CF} = 22.3 Hz), 117.7 (d, J_{CF} = 23.0 Hz), 127.9 (d, J_{CF} = 8.4 Hz), 129.1, 129.9, 130.2, 132.6 (d, J_{CF} = 2.9 Hz), 139.1 (d, J_{CF} = 7.8 Hz), 139.6 (d, J_{CF} = 1.6 Hz), 158.3, 161.1 (d, J_{CF} = 243.1 Hz). HPLC (254 nm, system A): t_R= 9.0 min, purity: >95%. HRMS (EI) calcd. for $C_{21}H_{25}FN_3O$ [M^+]: 354.1974, found: 354.1976. $[\alpha]^{25.0}_D$ 7 (c 0.95, CH_3CN).

4-((((3'-Chloro-5-fluorobiphen-2-yl)carbamoyl)oxy)methyl)-1,1-dimethylpiperidin-1-ium formate (16m) was prepared from **13n** (31.0 mg, 65 µmol, 1.0 eq.), methyl iodide (39 µl, 626 µmol, 10.0 eq.) and K_2CO_3 (52.0 mg, 376 µmol, 6.0 eq.) according to General Procedure F. The quaternary ammonium salt **16m** (quant. conversion assumed) was purified by preparative HPLC and obtained as a beige oil. ^{1}H NMR (400 MHz, MeOD) δ 8.5 (s, 1H), 7.5 – 7.4 (m, 5H), 7.3 (dt, J = 1.7, 6.5 Hz, 1H), 7.2 – 7.0 (m, 2H), 4.0 (s, 2H), 3.5 – 3.4 (m, 2H), 3.4 (d, J = 3.4 Hz, 2H), 3.3 (d, J = 3.9 Hz, 1H), 3.2 (s, 3H), 3.1 (s, 3H), 2.1 – 1.6 (m, 5H). ^{13}C NMR (101 MHz, MeOD) δ 16.8, 20.3, 24.1, 45.4, 46.3, 53.4, 68.1, 114.9 (d, J_{CF} = 22.5 Hz), 116.5 (d, J_{CF} = 23.5 Hz), 127.1, 127.5, 128.5, 129.9, 130.2, 130.2, 133.8, 136.7, 140.3, 161.0 (d, J_{CF} = 241.1 Hz), 168.5. HPLC (254 nm,

system A): t_R= 10.1 min, purity: >95%. HRMS (EI) calcd. for $C_{21}H_{25}FN_2O_2$ [M^+]: 391.1583, found: 391.1583.

4-((((3'-Chloro-4',5-difluorobiphen-2-yl)carbamoyl)oxy)methyl)-1,1-dimethylpiperidin-1-ium formate (16n) was prepared from **13n** (70.0 mg, 141 µmol, 1.0 eq.), methyl iodide (88 µl, 1.41 mmol, 10.0 eq.) and K_2CO_3 (117 mg, 849 µmol, 6.0 eq.) according to General Procedure F. The quaternary ammonium salt **16n** (quant. conversion assumed) was purified by preparative HPLC and obtained as a beige oil. ^{1}H NMR (400 MHz, MeOD) δ 1.8 (t, *J* = 13.2 Hz, 2H), 1.8 (bs, 1H), 1.9 – 2.1 (m, 2H), 3.1 (s, 3H), 3.2 (s, 3H), 3.4 (dd, *J* = 3.4, 12.8 Hz, 2H), 3.5 (d, *J* = 12.6 Hz, 2H), 4.0 (d, *J* = 5.3 Hz, 2H), 7.1 – 7.2 (m, 2H), 7.3 – 7.4 (m, 2H), 7.5 (dd, *J* = 5.3, 8.7 Hz, 1H), 7.5 – 7.6 (m, 1H), 8.3 (s, 1H). One signal missing. ^{13}C NMR (101 MHz, MeOD) δ 17.3 , 21.2 , 24.4 , 45.4 , 46.4 , 53.7 , 69.0 , 114.8 , 115.1 , 116.3 (d, J_{CF} = 6.6 Hz), 116.6 (d, J_{CF} = 8.7 Hz), 120.2 (d, J_{CF} = 17.7 Hz), 129.1 , 130.4 , 130.4 , 130.7 , 135.8 , 154.9 , 157.6 (d, J_{CF} = 248.7 Hz), 160.9 (d, J_{CF} = 244.7 Hz), 168.9. HPLC (254 nm, system A): t_R= 10.3 min, purity: >95%. HRMS (EI) calcd. for $C_{21}H_{24}F_2N_2O_2$ [M^+]: 409.1489, found: 409.1492.

(1*R*,3*r*,5*S*,8*s*)-3-(((5-Fluorobiphen-2-yl)carbamoyl)oxy)-8-methyl-8-azabicyclo[3.2.1]octan-8-ium chloride (17): In a flame dried flask, to a stirred suspension of tropine (141 mg, 1.00 mmol, 2.3 eq.) in dry CH_3CN (0.8 mL), triphosgene (258 mg, 0.87 mmol, 2.0 eq.) was added portion wise at 0 °C. The reaction mixture was stirred at this temperature for 5 min and, then, at rt for 36 h more. The solvent was removed *in vacuo* and the residue was triturated with dry Et_2O and carefully dried under a stream of nitrogen. Pushing forward, the partial reaction mixture (128 mg, 530 µmol, 1.2 eq., 100% purity assumed) was added portion wise to a solution of 5-fluoro-biphenyl-2-amine (**6d**) (83.0 mg, 440 µmol, 1.0 eq.) in dry pyridine (2 mL) in a dry flask at rt. The mixture was then heated to 80 °C and stirred for 20 h. Then, the mixture was allowed to cool to rt and the solvent was

removed *in vacuo*. The crude product was purified via flash column chromatography (CH_2Cl_2 / methanol (+ 2% NH_4OH (25% in H_2O) = 95 : 5 → 88 : 12) to obtain **17** as a brown residue (60 mg, 170 µmol, 38% yield over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.87 (d, *J* = 15.5 Hz, 4H), 2.01 – 2.13 (m, 2H), 2.45 (s, 5H), 3.33 (s, 2H), 4.99 (t, *J* = 5.1 Hz, 1H), 6.40 (s, 1H), 7.01 (dd, *J* = 3.0, 8.9 Hz, 1H), 7.09 (ddd, *J* = 3.0, 8.0, 9.0 Hz, 1H), 7.36 – 7.42 (m, 2H), 7.42 – 7.57 (m, 3H), 7.93 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 25.1, 35.5, 39.7, 60.5, 67.7, 115.0 (d, *J* = 22.4 Hz), 116.7 (d, J_{CF} = 23.7 Hz), 128.4, 129.0, 129.1, 130.4, 137.1, 153.2, 160.4. HPLC (254 nm, system A): t_R= 9.5 min, purity: >95%. HRMS (EI): calcd. for $C_{21}H_{24}FN_2O_2$ [M^+]: 355.1816, found: 355.1821.

(1*R*,2*R*,4*S*,5*S*,7*s*,9*r*)-7-(((5-Fluorobiphen-2-yl)carbamoyl)oxy)-9-methyl-3-oxa-9-azatricyclo[3.3.1.02,4]nonan-9-ium chloride (18): In a flame dried flask, to a stirred suspension of scopine (46 mg, 240 µmol, 1.1 eq.) in dry CH_3CN (0.8 mL), triphosgene (71.2 mg, 240 µmol, 1.1 eq.) was added portion wise at 0 °C. The reaction mixture was stirred at this temperature for 5 min and, then, at rt for 36 h more. The solvent was removed *in* vacuo and the residue was triturated with dry Et_2O and carefully dried under a stream of nitrogen. Pushing forward, the full reaction mixture (61.2 mg, 240 µmol, 1.1 eq., 100% purity assumed) was added portion wise to a solution of 5-fluoro-biphenyl-2-amine (**6d**) (41.0 mg, 220 µmol, 1.0 eq.) in dry pyridine (2 mL) in a dry flask at rt. The mixture was then heated to 60 °C and stirred for 24 h. Then, the mixture was allowed to cool to rt and the solvent was removed *in vacuo*. The crude product was purified via preparative HPLC (H_2O / CH_3CN = 95 : 5 → 20 : 80, 0.1% FA as modifier) to obtain **18** as a white solid (6.8 mg, 18 µmol, 8% yield over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.82 (d, *J* = 14.9 Hz, 2H), 2.74 (s, 5H), 3.49 (s, 2H), 3.56 (s, 2H), 5.02 (s, 1H), 6.37 (s, 1H), 7.01 (dd, *J* = 3.0, 8.9 Hz, 1H), 7.09 (ddd, *J* = 3.0, 8.0, 9.0 Hz, 1H), 7.32 – 7.39 (m, 2H), 7.44 – 7.58 (m, 3H), 7.91 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 29.6, 29.7, 30.7, 41.6, 55.2, 115.1 (d, *J* = 22.3 Hz), 116.9 (d, *J* = 24.4

Hz), 128.6, 128.9, 129.2, 130.1, 137.1, 152.5. HPLC (254 nm, system B): t_R= 8.5 min, purity: >95%. HRMS (EI): calcd. for $C_{21}H_{22}FN_2O_3$ [M^+]: 369,1609, found: 369.1609.

Receptor binding studies.

Radioligand binding studies with the M_1, M_2 or M_3 subtypes were performed as described previously.[22] In brief, competition binding experiments were done using membranes of HEK293T cells transiently transfected with the cDNA of the human M_1, M_2 or M_3 receptor (cDNA Resource Center, Bloomsberg, PA), respectively. Radioligand displacement assays were performed in binding buffer (25 mM HEPES, 5 mM $MgCl_2$, 1 mM EDTA, 0.006% BSA at a pH of 7.4) with [^{3}H]N-methylscopolamine (specific activity = 70 Ci/mmol, PerkinElmer, Rodgau, Germany) at final concentrations of 0.10-0.30 nM. The assays were carried out at protein concentrations of 2-6 µg/assay tube, a B_{max} value of 2200±460 fmol/µg, a K_D value of 0.15±0.001 nM for M_1, of 5-10 µg protein/assay tube, B_{max} of 1100±220 fmol/µg, K_D of 0.23±0.01 nM for M_2, and of 2-4 µg protein/assay tube, B_{max} of 3300±590 fmol/µg, K_D of 0.12±0.02 nM for M_3, respectively. Unspecific binding was determined in the presence of 10 µM atropine, protein concentration was established by the method of Lowry using bovine serum albumin as standard.[58] The resulting competition curves of the receptor binding experiments were analyzed by nonlinear regression using the algorithms in PRISM 6.0 (GraphPad Software, San Diego, CA). The data were initially fit using a sigmoid model to provide an IC_{50} value, representing the concentration corresponding to 50% of maximal inhibition. IC_{50} values were transformed to K_i values according to the equation of *Cheng* and *Prusoff*.[59]

Accumulation of inositol mono phosphate (IP-One Assay).

Determination of the activation of the muscarinic M_3 receptor was measured applying the IP-One HTRF® assay (Cisbio, Codolet, France) according to the manufacturer's protocol and as described

previously.[22] In brief, HEK-293T cells were grown to confluence of approximately 70% and transiently transfected with the cDNA of the human M_3 receptor (cDNA Resource Center, Bloomsberg, PA) applying the TransIT-293 Mirus transfection reagent (Peqlab, Erlangen, Germany). After one day cells were detached from the culture dish with Versene (Life Technologies GmbH, Darmstadt, Germany), seeded into black 384-well plates (10000 cells/well) (Greiner Bio-One, Frickenhausen, Germany) and maintained for 24 h at 37 °C. In general, after incubation with the test compounds dissolved in stimulation buffer (final range of concentration from 10 pM up to 10 µM) for 90 min at 37 °C the detection reagents were added (IP1-d2 conjugate and Anti-IP1cryptate TB conjugate each dissolved in lysis buffer) and incubation was continued for further 1 hr at room temperature. Time resolved fluorescence resonance energy transfer (HTRF) was determined using the Clariostar plate reader (BMG, Ortenberg, Germany). For measuring in the agonist mode each compound was tested in duplicates in 4-9 individual experiments in comparison to the reference compound carbachol (n=9). Antagonist properties were determined after preincubation of the test compound for 30 min, subsequent addition of the EC_{80} concentration of the reference agonist carbachol (100 nM) and continued incubation for 90 min at 37°C (5-10 experiments each). The resulting dose response curves were analyzed by nonlinear regression using the algorithms in PRISM 6.0 (GraphPad software, San Diego, CA), fitted with a sigmoid model and normalized to basal activity (0%) and the maximal effect caused by the reference full agonist quinpirole (100%) (agonist mode). For analysis of the results of the antagonist mode the effect of 100 nM carbachol was set as 100% and the basal activity as 0%.

Determination of arrestin recruitment (PathHunter Assay)

Muscarin M_3 receptor mediated arrestin recruitment was determined applying the PathHunter® assay (DiscoverX, Birmingham, U.K.) as described in the manufacturer's protocol. In brief, HEK-293 cells stably expressing the enzyme acceptor (EA) tagged β-arrestin-2 fusion protein were

transiently transfected with the ProLink tagged M_3-PK1 construct employing Mirus TransIT-293 transfection reagent. After 24 h cells were transferred into white clear bottom 384-well plates (5000 cells/well) (Greiner Bio-One) and maintained for further 24 h at 37 °C, 5% CO_2. For the agonist mode test compounds dissolved in PBS were incubated for 90 min at 37°C and tested in duplicates in 3-6 individual experiments in comparison to the reference compound carbachol (n=7). For the antagonist mode the test compounds were preincubated for 30 min at 37°C, and subsequently carbachol (50 μM) was added and incubation was continued for 90 min at 37°C (5-11 experiments each). Stimulation stopped by addition of detection mix and reaction continued for further 60 min at room temperature. Formed chemiluminescence was determined using a Clariostar plate reader. Data analysis was done by nonlinear regression using the algorithms for log(agonist) vs. response of PRISM 6.0 (GraphPad, San Diego, CA) and normalization of the raw data to basal (0%) and the maximum effect of carbachol (100%).

Metabolism Studies.

Metabolism experiments were performed as described previously in the publication by *Hiller et al.*[48] Pooled microsomes of male rat liver (Sprague-Dawley) were purchased from Sigma Aldrich and stored at -84 °C. NADPH was purchased from Carl and Roth and stored at 5 °C. The incubation reactions were performed in polyethylene caps (Eppendorf 1.5 mL) at 37 °C. The incubation mixture contained OFH243 (**13n)** (20 μM), OFH244 (**13m**) (20 μM), OFH3911 (**14n**) (20 μM), OFH3912 (**14m**) (20 μM) or imipramine (20 μM), used as positive control, pooled rat liver microsomes (0.5 mg of microsomal protein/mL of incubation mixture) and Tris-$MgCl_2$ buffer (50 mM Tris and 5.0 mM $MgCl_2$, pH 7.4). The final incubation volume was 0.5 mL. Microsomal reactions were started adding 50 μL of enzyme cofactor solution NADPH (final concentration of 1 mM). After predetermined times of 0, 15, 30, 60 and 120 min 100 μL samples were drawn from the incubation mixture and the reactions were terminated by adding 100 μL of ice-cold acetonitrile

(containing internal standard); final concentration of 25 µM). After removing precipitated proteins by centrifugation (10.000 rpm, 3 min) the supernatant was analyzed by HPLC/MS (binary solvent system, eluent methanol in 0.1% aqueous formic acid, 10-100% methanol in 20 min, 100% methanol for 6 min, 100%-10% in 4 min). All incubations were performed in duplicate. Parallel control experiments were performed in the absence of cofactor NADPH solution to determine unspecific binding to matrix and in the absence of OFH243 (**13n)** (20µM) or OFH3911 (**14n**) (20 µM) or imipramine, respectively.

Investigation of OFH243 and OFH244 as substrates of P-glycoprotein

Transcellular transport studies using monolayers of Caco-2 cells expressing P-glycoprotein. Monolayers of Caco-2 cells, which express P-glycoprotein in the apical membrane, were cultured as described[61] using DMEM low glucose medium. For transcellular transport studies [i.e. investigation of transport from the basal to the apical compartment and from the apical to the basal compartment], 5 x 10^5 cells were plated on Transwell Filters (Sarstedt, Germany). Transport experiments were performed on day 4 after plating. For transport experiments, the medium in each compartment was replaced with 800 µL uptake buffer (142 mM NaCl, 5 mM KCl, 1 mM K_2PO_4, 1.2 mM $MgSO_4$, 1.5 mM $CaCl_2$, 5 mM glucose and 12.5 mM HEPES, pH 7.3) with the addition of OFH243 (**13n**) (1 µM or 10 µM) or OFH244 (**13m**) (1 µM or 10 µM) into the basal or the apical compartment of the monolayer. In order to study the inhibition of potential P-glycoprotein-mediated OFH243 (**13n**) or OFH244 (**13m**) transport, the P-glycoprotein inhibitor valspodar (2 µM, Merck KGaA, Germany) was added together with OFH243 (**13n**) or OFH244 (**13m**) to the apical compartment of the monolayer in additional experiments.

As positive control P-glycoprotein-mediated transport of the P-glycoprotein substrate digoxin was studied using the same experimental setup. Digoxin [5 µM, sum of trace amounts of [^{3}H]digoxin

(ARC, USA) and unlabeled digoxin (Merck KGaA, Germany)] was administered to either the basal or the apical side of the monolayer without or with valspodar (2 μM) added to the apical compartment.

During the transport experiments monolayers were incubated at 37 °C, and the amount of the OFH243 (**13n**) and OFH244 (**13m**) appearing in the opposite compartment after 3 hours was measured in aliquots (200 μL) using liquid chromatography (LC) coupled to a mass spectrometer (MS, see below). For the control experiments using digoxin, aliquots (80 μL) were taken after 3 hours. The radioactivity of digoxin was measured by liquid scintillation counting (Tricarb 2 800, Perkin Elmer Life Science GmbH, Germany). All experiments were performed on two separate days with n = 3 per day (i.e. n = 6).

Mass spectrometric measurement of OFH243 and OFH244. The relative concentrations of OFH243 (**13n**) and OFH244 (**13m**) in the basal and apical compartments were measured using an Orbitrap QExactive Plus MS (Thermo Fisher Scientific, Germany). The MS was hyphenated to an UltiMate 3000 liquid chromatograph (Thermo Fisher Scientific, USA) equipped with a binary pump, thermostated column compartment and autosampler. An Acquity UPLC BEH Amide column (1.7 μm, 2.1 mm, 100 mm, Waters Corporation, USA) was installed and maintained at 25 °C. The eluent consisted of 10 mM ammonium formate (VWR, USA) with 0.1% formic acid (eluent A) and 10 mM ammonium formate in 95% HPLC grade acetonitrile (CH_3CN; VWR, USA) and 5% ultrahigh purity water (18.2 $M\Omega cm^{-1}$; VWR, USA) (eluent B).

10 μL of the 200 μL aliquots were diluted (1:50) using the internal standard imipramine (10 μg/mL in 50% CH_3CN; Merck KGaA, Germany) in order to minimize ion suppression and monitor the instrument performance. The injection volume was 5 μL. The chromatographic separation was achieved under isocratic conditions with 5% A and 95% B and a flow rate of 0.2 mL/ min. The run time was 5 min.

The MS was operated in positive mode with a scan range of 50-750 amu. Imipramine was detected as $[M+H]^+$, the two target compounds OFH243 (**13n**) and OFH244 (**13m**) were detected as M^+. The retention times and m/z values of OFH243 (**13n**), OFH244 (**13m**) and imipramine were 2.27 min/ 381.11759 amu, 2.32 min/ 363.12701 amu and 2.01 min/ 281.20110 amu respectively. The relative abundancies were reported as ratios between the OFH compound areas and the areas of imipramine multiplied by 100 (for data handling purposes) and data are reported in arbitrary units (a.u.).

S**tatistics.** Data are shown as mean ± SD. Groupwise comparisons were calculated by ANOVA with Tukey-Kramer post hoc tests. A P value below 0.05 was considered as statistically significant. Calculations were performed using GraphPad InStat (version 3.10, GraphPad Software Inc., CA, USA).

Molecular Docking studies.

All ligands for molecular modelling were built using the programs Avogadro (version 1.2.0)[61] and AutoDockTools (version 1.5.6)[49]. The ligands were protonated at the secondary amine group, to receive the protonation state under the physiological pH value. The geometry of each ligand was optimized by energy minimization. For the docking studies the crystal structures of the human muscarinic M_2 and M_3 receptors were used in complex with antagonists 3-quinuclidinyl-benzilate (PDB: 4U15), and tiotropium (PDB: 3UON), respectively. The related PDB files were downloaded from the RCSB PDB webpage.[52-53] Docking was performed using AutoDock Vina[49] while a search space of 18×18×18 Å was applied to cover the whole binding pocket, which was defined by the appropriate crystal ligand for each receptor sub type. In order to validate our methods, we reproduced the binding poses of 3-quinuclidinyl-benzilate and tiotropium in the M_2 and the M_3 receptor, respectively. An exhaustiveness value of 16 was used with randomly selected

starting positions. For each ligand, twenty conformations were received and analyzed manually. Visualization of the receptor ligand complexes was accomplished with the PyMOL Molecular Graphics System, Version 1.3 (Schrödinger, LLC).

Calculations of the molecular electrostatic potential (MEP)

Structures of the ligands were optimized by means of Gaussian 09 at the B3LYP/6-31G* level,[50] subsequently the MEPs was calculated at the HF/6-31G* level. Visualization was performed using UCSF Chimera Version 1.13.1, developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco.[51]

PAINS screening. The target compounds (converted into SMILES codes using ChemDraw 18.0, PerkinElmer) were screened for pan assay interference and aggregation liability using the tools and standard filters[62] implemented in the ZINC 15 database.[63] Results from the screening (Supporting Information Table S1) indicate no liabilities for the target compounds.

Ancillary Information

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.xxxxx.

HPLC purity analysis data; ^{1}H and ^{13}C NMR spectra of all target compounds, additional docking poses, additional biological data, molecular formula string list, and PAINS screening results.

PDB ID Codes

M_2 receptor crystal structure (PDB entry 3UON)[53] and M_3 receptor crystal structure (PDB entry 4U15)[52] were used for docking experiments.

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Author contributions

O. F. performed the research, metabolism and modeling studies, designed, synthesized, analyzed the data, and wrote the manuscript. J. H., H. R., G. P. and A. B performed the research, designed, and synthesized. J. K. performed the MEP calculations and analyzed the data. R. V. T. and M. F. F. performed and analysed transport studies. H. H. conducted the biological assays, analyzed the data, wrote the manuscript. P. G. analyzed the data, wrote the manuscript. M. R. H. designed the research, analyzed the data, and wrote the manuscript.

Acknowledgements

The authors are very grateful for the financial support of this work by the Deutsche Forschungsgemeinschaft (DFG) (GRK1910/B3). O. F. would like to thank Anja Hubert for experimental assistance.

Conflict of interest statement

Parts of this work have been recently published as a patent (WO2019110521).

List of abbreviations

APPI, atomospheric pressure photoionization; calcd., calculated; COPD, chronic obstructive pulmonary disease; DIPEA, *N,N*-diisopropylethylamin; DFG, Deutsche Forschungsgemeinschaft; ECL2, extracellular loop 2; FA, formic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid); HTRF, homogeneous time resolved fluorescence; mAChR, muscarinic acetylcholine receptor; MEP, molecular electronic potential; SD, standard deviation; SEM, standard error of the mean; SMILES, simplified molecular-input line-entry system; TM3, transmembrane domain 3; t_r, retention time

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