# Discovery of Biphenyl Piperazines as Novel and Long Acting Muscarinic Acetylcholine Receptor Antagonists

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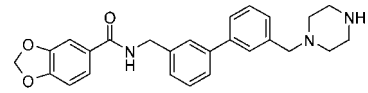
**Abstract:** A series of novel biphenyl piperazines was discovered as highly potent muscarinic acetylcholine receptor antagonists via high throughput screening and subsequent optimization. Compound **5c** with respective 500- and 20-fold subtype selectivity for $M_3$ over $M_2$ and $M_1$ exhibited excellent inhibitory activity and long duration of action in a bronchoconstriction in vivo model in mice via intranasal administration. The novel inhaled mAChR antagonists are potentially useful therapeutic agents for the treatment of chronic obstructive pulmonary disease.

Five muscarinic acetylcholine receptor (mAChR[a]) subtypes, $M_1-M_5$, are known to date.[1–3] These seven-transmembrane (7TM) receptors share a common orthosteric ligand-binding site with an extremely high sequence homology, which explains why it has been difficult historically to identify subtype selective ligands.[3] The five subtypes also exhibit a high sequence homology across species.[3] $M_1-M_5$ mAChRs are widely distributed in mammalian organs and the central and peripheral nerve system where they mediate important neuronal and autocrine functions.[4,5]

In the mammalian respiratory system, only $M_1$, $M_2$, and $M_3$ have been recognized as playing important and diverse functional roles.[6] $M_3$ is predominately expressed on airway smooth muscle and mediates smooth muscle contraction and mucus secretion.[7] Blockade of $M_3$ on airway smooth muscle reduces excess airway smooth muscle contraction. $M_2$ is primarily found on postganglionic nerve termini, where it inhibits acetylcholine release from parasympathetic nerves.[8] Blockade of the $M_2$ function is expected to enhance bronchoconstriction. $M_1$ is found in parasympathetic ganglia and facilitates neurotransmission through ganglia, thus enhancing cholinergic reflexes.[9] Blockade of $M_1$ may help to reduce bronchoconstriction.

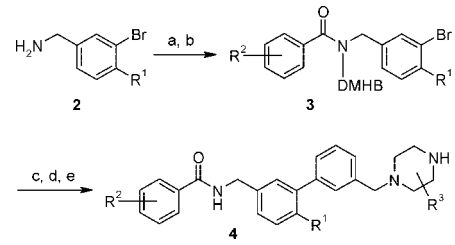
Muscarinic acetylcholine receptor dysfunction in the lungs has been noted in a variety of different pathophysiological states.[10] In particular, in chronic obstructive pulmonary disease (COPD) and asthma, inflammatory conditions lead to loss of neuronal inhibitory activity mediated by $M_2$ on parasympathetic nerves, causing excess acetylcholine reflexes,[11] which result in airway hyperreactivity and hyperresponsiveness mediated by increased stimulation of $M_3$. Therefore, potent mAChR antagonists, particularly directed toward the $M_3$ subtype, are useful as therapeutics for mAChRs-mediated disease states. Besides preventing any potential $M_2$-mediated bronchoconstriction, achieving subtype selectivity for $M_3$ over $M_2$ would be desirable as $M_2$ is found in large numbers on the myocardium and mediates negative inotropic effects and bradycardia.[12] In addition, inhaled delivery could potentially reduce side effects mediated by peripheral and/or central $M_1$, $M_2$, or $M_3$ antagonism[5] by avoiding substantial systemic exposure.



**1**, $M_3$ FLIPR $pIC_{50}$ = 7.5
$M_2$ FLIPR $pIC_{50}$ = < 5.5
$M_1$ FLIPR $pIC_{50}$ = 6.8

**Figure 1.** In vitro profile of HTS hit **1**.

**Scheme 1.** General Synthesis of Biphenyl Piperazines[a]



[a] (a) 2,6-Dimethoxy-4-polystyrenebenzyloxybenzaldehyde (DMHB-resin), $Na(OAc)_3BH$, DIEA, 10% of HOAc in NMP, rt; (b) various benzoic acids, DIC, DCE/DMF (1:1), rt; (c) 3-formylphenyl boronic acid, $Pd(PPh_3)_4$, $K_2CO_3$ or $Cs_2CO_3$, DME, 80 °C; (d) various piperazines, $Na(OAc)_3BH$, $Na_2SO_4$, DCE, rt; (e) TFA, DCE, rt.

High throughput screening (HTS) of our in-house compound collection using a fluorometric imaging plate reader (FLIPR) assay[13] resulted in the identification of biphenyl piperazine **1**, as a $M_3$ antagonist with a $pIC_{50}$ of 7.5 (Figure 1).[14–16] In subsequent evaluation in subtype selectivity assays, compound **1** was found to be more than 100-fold selective for $M_3$ over $M_2$ and about 5-fold selective for $M_3$ over $M_1$. On the basis of its good potency and subtype selectivity for $M_3$ over $M_2$, **1** was considered an acceptable starting point for our lead optimization program aimed at identifying long acting mAChR antagonists.

An efficient and robust solid-phase synthesis was developed to explore this novel series (Scheme 1). Commercially available 3-bromo benzylamines (**2**) were loaded onto 2,6-dimethoxy-4-polystyrenebenzyloxybenzaldehyde resin (DMHB resin)[17] via reductive amination, then coupled with benzoic acids to afford resin-bound aryl bromides **3**. Suzuki coupling of aryl bromides **3** with 3-formylphenyl boronic acid and subsequent reduction amination of the resulting benzaldehydes with substituted piperazines, followed by resin cleavage, produced the targeted biphenyl piperazines **4** in excellent yields and purity.

During the course of the lead optimization, potent $M_3$ antagonists such as **4a**, **4b**, and **4c**, which were single enantiomers possessing a (3*S*)-3-methylpiperazin-1-yl moiety at the right-hand side (RHS), were identified (Table 1). Methyl ketone

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[a] Abbreviations: mAChRs, muscarinic acetylcholine receptors; 7TM, seven-transmembrane; COPD, chronic obstructive pulmonary disease; HTS, high throughput screening; CYP450, cytochrome P450; PK, pharmacokinetic; Penh, enhanced pause.

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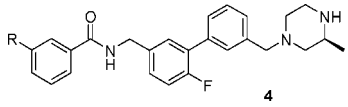
**Table 1.** Potency of Compounds **4a**, **4b**, and **4c**



**4**

| Compound | R | FLIPR p$A_2$ | | |
|---|---|---|---|---|
| | | $M_3$ | $M_2$ | $M_1$ |
| **4a** |  | 10.3 | 7.0 | 8.6 |
| **4b** |  | 10.3 | 7.6 | 9.0 |
| **4c** | CN | 9.6 | 6.5 | 8.1 |

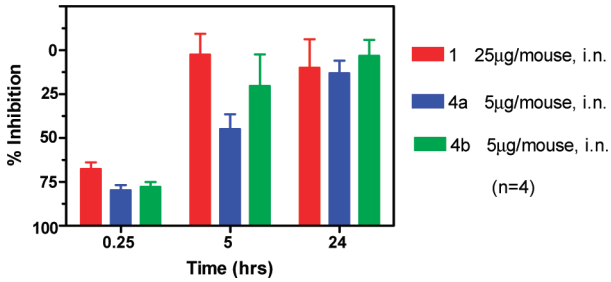


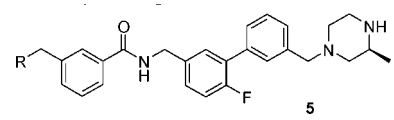

**Figure 2.** Effect of intranasal administration of **1**, **4a**, and **4b** on methacholine-induced bronchoconstriction in conscious mice.

**4a** was more than 100-fold more potent compared to HTS hit **1** in the $M_3$ FLIPR assay with a p$A_2$ of 10.3. The compound had excellent $M_2$ subtype selectivity, about 2000-fold selective for $M_3$ over $M_2$, and was also 50-fold selective for $M_3$ over $M_1$. Ethyl ketone **4b** also exhibited excellent $M_3$ potency (p$A_2$ = 10.3) and good subtype selectivity (500-fold selective for $M_3$ over $M_2$ and 20-fold selective for $M_3$ over $M_1$). In addition to ketones **4a** and **4b**, compounds such as 3-cyanobenzamide **4c** also had high $M_3$ potency (p$A_2$ = 9.6) and good subtype selectivity (1300-fold selective for $M_3$ over $M_2$ and 30-fold selective for $M_3$ over $M_1$).

Compounds **1**, **4a**, and **4b** were evaluated in a methacholine-induced bronchoconstriction model in conscious mice, measuring enhanced pause (Penh), an indicator of bronchoconstriction,[18] using barometric plethysmography (Figure 2). Intranasal administration[19] of **4a** and **4b** at a single dose (5 $\mu$g/animal) significantly inhibited methacholine-induced bronchoconstriction at 15 min, while HTS hit **1**, a more than 100-fold less potent antagonist, exhibited less inhibition at a higher dose (25 $\mu$g/animal) at 15 min post dosing. However, the excellent inhibitory activity exhibited by **4a** and **4b** was not maintained over a 24 h period. Compounds **4a** and **4b** showed respective 45% and 20% of bronchoprotection at 5 h and little bronchoprotection at 24 h post the single dose.

Further optimization aimed at identifying long acting mAChR antagonists from the series led to discovery of compounds **5a**, **5b**, and **5c**, which possess an amino or quaternary ammonium moiety at the left-hand side (LHS), as potent $M_3$ antagonists (Table 2).[20] Similar to **4a**, **4b**, and **4c**, piperazine **5a** had excellent potency with a p$A_2$ of 10.6 in the $M_3$ FLIPR assay and showed good subtype selectivity (400-fold selective for $M_3$ over $M_2$ and 6-fold selective for $M_3$ over $M_1$). Converting the secondary piperazine (**5a**) to the quaternary piperazinium moiety (**5b**) maintained the excellent $M_3$ potency (p$A_2$ = 9.8) but had lower $M_2$ subtype selectivity (80-fold selective for $M_3$ over $M_2$). Piperazine **5a** and quaternary piperazinium salt **5b** were less

**Table 2.** Potency of Compounds **5a**, **5b**, and **5c**



**5**

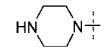
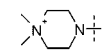
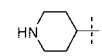
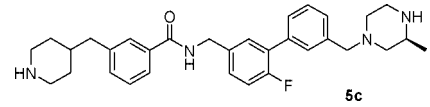
| Compound | R | FLIPR p$A_2$ | | |
|---|---|---|---|---|
| | | $M_3$ | $M_2$ | $M_1$ |
| **5a** |  | 9.8 | 7.2 | 9.0 |
| **5b** |  | 9.8 | 7.9 | 8.7 |
| **5c** |  | 11.0 | 8.3 | 9.7 |

**Table 3.** Profile of Piperidine **5c**



**5c**

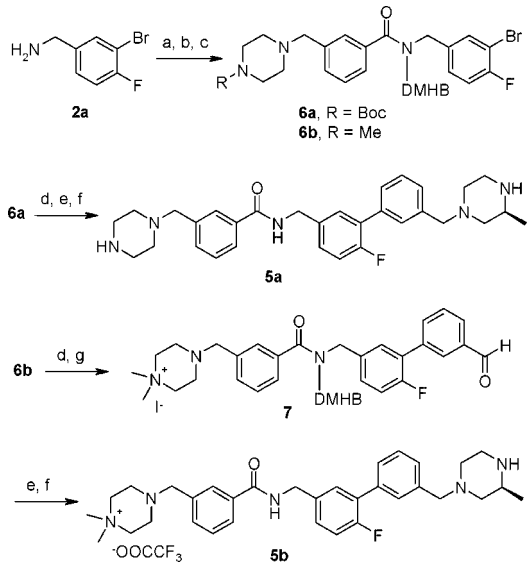
| | | | |
|---|---|---|---|
| in vitro potency | $M_3$ | $M_2$ | $M_1$ |
| FLIPR p$A_2$ | 11.0 | 8.3 | 9.7 |
| $M_3$ binding | | p$K_i$ = 10.0 | |
| $M_3$ kinetics | | competitive, p$K_B$ = 10.5 | |
| intrinsic clearance | | human: $Cl_{int}$ = 4 mL/min/g liver<br>rat: $Cl_{int}$ >50 mL/min/g liver | |
| permeability | | <3 nm/s | |
| solubility | | 200 $\mu$M | |
| CYP450 | | $pIC_{50}$ < 5.0 vs 1A2, 2C19, 2C9, 2D6, and 3A4 | |
| hERG binding | | $pIC_{50}$ = 5.0 | |

subtype selective compared to ketone **4a** in general. Most notably, piperidine **5c** showed outstanding $M_3$ potency with a p$A_2$ of 11.0 − more than 10-fold more potent than piperazine **5a** and piperazinium salt **5b**. **5c** also had good subtype selectivity (500-fold selective for $M_3$ over $M_2$ and 20-fold selective for $M_3$ over $M_1$).

In addition to high potency in the $M_3$ FLIPR assay and good subtype selectivity over $M_2$ and $M_1$, piperidine **5c** had excellent binding affinity to $M_3$ with a p$K_i$ of 10.0 (Table 3). In kinetics studies using the $M_3$ FLIPR assay, **5c** was found to be a competitive $M_3$ antagonist with a p$K_B$ of 10.5, consistent with its FLIPR p$A_2$ and binding p$K_i$. Although **5c** showed 70 to 100% inhibition at 10 $\mu$M against α adrenergic, opioid, and serotonin receptors in the Cerep selectivity screen, the compound exhibited less than 30% inhibition at 10 $\mu$M against enzymes, ion channels, and transporters in the panel. Most of the potential 7TM liabilities could be mitigated by avoiding substantial systemic exposure via low membrane permeability and the relatively low dose administrated by inhaled delivery (vide infra). In the in vitro human and rat liver microsome stability studies, **5c** showed low to moderate intrinsic clearance vs human liver microsome ($Cl_{int}$ = 4.3 mL/min/g) but high intrinsic clearance vs rat enzyme ($Cl_{int}$ >50 mL/min/g). **5c** had extremely low artificial membrane permeability[21] (less than 3 nm/s), suitable for inhaled delivery and targeting membrane-bound receptors such as mAChRs. In addition, **5c** had good developability properties. For example, **5c** had high aqueous solubility, was clean against five common cytochrome P450 (CYP450) isozymes ($pIC_{50}$ < 5.0), and was more than 100000-fold selective for $M_3$ over hERG (binding, $pIC_{50}$ = 5.0).

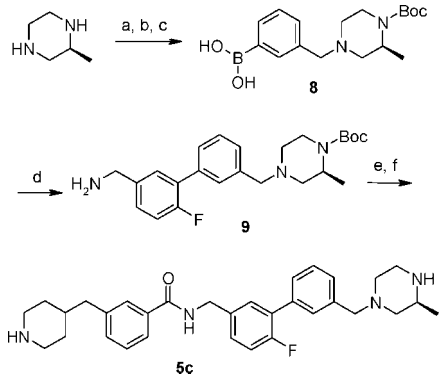
Piperazine **5a** and piperazinium salt **5b** were synthesized according to the route outlined in Scheme 2. Resin-bound

**Scheme 2.** Synthesis of Piperazine **5a** and Piperazinium Salt **5b**[a]




[a] (a) 2,6-Dimethoxy-4-polystyrenebenzyloxybenzaldehyde (DMHB-resin), $Na(OAc)_3BH$, DIEA, 10% of HOAc in NMP, rt; (b) 3-formyl benzoic acid, DIC, DCE/DMF (1:1), rt; (c) *N*-Boc piperazine or *N*-methyl piperazine, $Na(OAc)_3BH$, $Na_2SO_4$, DCE, rt; (d) 3-formylphenyl boronic acid, $Pd(PPh_3)_4$, $Cs_2CO_3$, DME, 80 °C; (e) (2*S*)-2-methylpiperazine, $Na(OAc)_3BH$, $Na_2SO_4$, DCE, rt; (f) TFA, DCE, rt; (g) MeI, $CH_3CN$, rt.

**Scheme 3.** Synthesis of Piperidine **5c**[a]




[a] (a) *n*-BuLi, TBDMSCl, $(Boc)_2O$, THF, rt; (b) 3-bromobenzaldehyde, $Na(OAc)_3BH$, DCM, rt; (c) *n*-BuLi, $B(OMe)_3$, THF, 78 °C−rt; (d) **2a**, $Pd(PPh_3)_4$, $K_2CO_3$, dioxane, $H_2O$, 150 °C; (e) 3-[(1-Boc-piperidin-4-yl)methyl]benzoic acid, EDC, HOBT, DIEA, $CHCl_3$, rt; (f) TFA, DCM, 0 °C − rt.

*N*-Boc-piperazine **6a** and *N*-methylpiperazine **6b** were prepared from commercially available 3-bromo-4-fluorobenzylamine (**2a**) via loading onto DMHB resin, coupling with 3-formyl benzoic acid and subsequent reductive amination. Suzuki coupling of **6a** with 3-formylphenyl boronic acid and subsequent reductive amination of the resulting benzaldehyde with optically pure (2*S*)-2-methylpiperazine, followed by resin cleavage, produced the desired compound **5a** in excellent yield. *N*-methylpiperazine **6b** was converted to quaternary ammonium salt **7** via Suzuki coupling and quaternization of the methyl piperazine. Reductive amination of **7** with optically pure (2*S*)-2-methylpiperazine and subsequent resin cleavage produced the desired piperazinium salt **5b** again in good yield. Synthesis of piperidine **5c** is outlined in Scheme 3. Optically pure (2*S*)-2-methylpiperazine was selectively protected via a one-pot two-step procedure. Reductive amination of the resulting amine with 3-bromobenzaldehyde and subsequent conversion of the corresponding aryl bromide to boronic acid afforded compound **8**. Suzuki coupling of **8** with

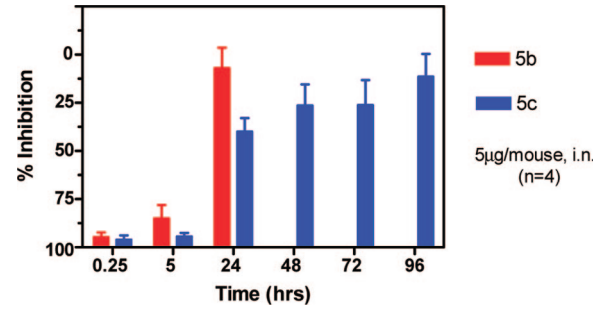



**Figure 3.** Effect of intranasal administration of **5b** and **5c** on methacholine-induced bronchoconstriction in conscious mice.

3-bromo-4-fluorobenzylamine (**2a**) produced benzyl amine **9**, which was then converted to the desired piperidine **5c** via coupling with commercially available 3-[(1-Boc-piperidin-4-yl)methyl] benzoic acid, followed by the removal of the Boc group.

In the methacholine-induced bronchoconstriction model in conscious mice, piperazinium salt **5b** had excellent inhibitory activity at 15 min (95% inhibition) and 5 h (85% inhibition) and showed significant improvement at 5 h compared to ketones **4a** and **4b** following intranasal administration of a single dose (5 $\mu$g/animal) (Figure 3). However, the bronchoprotective activity exhibited by **5b** was not maintained at 24 h. On the other hand, intranasal administration of piperidine **5c** (5 $\mu$g/animal), which was 15-fold more potent against $M_3$ compared to piperazinium salt **5b**, significantly inhibited methacholine-induced bronchoconstriction not only at 15 min (96% inhibition) and 5 h (94% inhibition) but also at 24 h (40% inhibition). Even at 48 and 72 h post the single low dose, **5c** still exhibited over 25% of bronchoprotection, thus demonstrating excellent in vivo efficacy and long duration of action.

In conclusion, a series of highly potent and competitive biphenyl piperazines was discovered as novel mAChR antagonists. Piperidine **5c** with respective 500- and 20-fold subtype selectivity for $M_3$ over $M_2$ and $M_1$ exhibited excellent inhibitory activity and long duration of action in a bronchoconstriction in vivo model, demonstrating that the novel inhaled mAChR antagonists are potentially useful therapeutic agents for the treatment of COPD and other bronchoconstriction disorders.

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**Supporting Information Available:** Synthetic procedures, characterization data, and LC-MS spectra for all compounds. Procedures for $M_3$, $M_2$, and $M_1$ FLIPR and $M_3$ binding assays and in vivo bronchoconstriction mouse model. This material is available free of charge via the Internet at http://pubs.acs.org.

## References

(1) Caulfield, M. P.; Birdsall, N. J. M. International Union of Pharmacology. XVII. Classification of Muscarinic Acetylcholine Receptors. *Pharmacol. Rev.* **1998**, *50*, 279–290.
(2) Eglen, R. M. Muscarinic Receptor Subtype Pharmacology and Physiology. *Prog. Med. Chem.* **2005**, *43*, 105–136.
(3) Hulme, E. C.; Birdsall, N. J. M.; Buckley, N. J. Muscarinic Receptor Subtypes. *Annu. Rev. Pharmacol. Toxicol.* **1990**, *30*, 633–673.
(4) Caulfield, M. P. Muscarinic Receptors—Characterization, coupling and function. *Pharmacol. Ther.* **1993**, *58*, 319–379.
(5) Eglen, R. M. Muscarinic receptor subtypes in neuronal and non-neuronal cholinergic function. *Auto. Autacoid Pharmacol.* **2006**, *26*, 219–233.

(6) Lee, A. M.; Jacoby, D. B.; Fryer, A. D. Selective muscarinic receptor antagonists for airway diseases. *Curr. Opin. Pharmacol.* **2001**, *1*, 223–229.
(7) (a) Barnes, P. J. Muscarinic receptor subtypes: implications for lung disease. *Thorax* **1989**, *44*, 161–167. (b) Gater, P. J.; Alabastar, V. J.; Piper, I. A study of the muscarinic receptor subtype mediating mucus secretion in the cat trachea in vitro. *Pulm. Pharmacol.* **1989**, *2*, 87–92.
(8) Faulkner, D.; Fryer, A. D.; MacLagan, J. Postganglionic muscarinic inhibitory receptors in pulmonary parasympathetic nerves in the guinea pig. *Br. J. Pharmacol.* **1986**, *88*, 181–187.
(9) Lammers, J. W. J.; Minnette, P. A.; McCuster, M.; Barnes, P. J. The role of pirenzepine sensitive muscarinic receptors in vagally mediated bronchoconstriction in humans. *Am. Rev. Respir. Dis.* **1989**, *139*, 446–449.
(10) Hay, D. W. P. Chronic obstructive pulmonary disease: emerging therapies. *Curr. Opin. Chem. Biol.* **2000**, *4*, 412–419.
(11) Fryer, A. D.; Adamko, D. J.; Yost, B. L.; Jacoby, D. B. Effects of inflammatory cells on neuronal M2 muscarinic receptor function in the lung. *Life Sci.* **1999**, *64*, 449–455.
(12) Harvey, R. D.; Belevych, A. E. Muscarinic regulation of cardiac ion channels. *Br. J. Pharmacol.* **2003**, *139*, 1074–1084.
(13) Measuring inhibition of acetylcholine-mediated $[Ca^{2+}]_i$-mobilization in Chinese hamster ovary (CHO) cells stably expressing human recombinant $M_3$ receptor. See supporting information for FLIPR assay details.
(14) (a) Budzik, B. W.; Cooper, A. W. J.; Corbett, D. F.; Jin, J.; Laine, D. I.; Wang, Y.; Moore, M. L.; Rivero, R. A.; Shi, D.; Wang, F.; Xie, H.; Zhu, C. Preparation of biaryl amines as M3 muscarinic acetylcholine receptor antagonists. Patent WO 087236, 2005. (b) Jin, J.; Wang, Y.; Moore, M. L.; Rivero, R. A. Preparation of biaryl quaternary ammonium salts as M3 muscarinic acetylcholine receptor antagonists. Patent WO 086873, 2005. (c) Budzik, B. W.; Jin, J.; Laine, D. I.; Palovich, M. R.; Rivero, R. A.; Wang, Y.; Xie, H. Preparation of *N*-[(piperazinylmethyl)biphenyl] benzamides as M3 muscarinic acetylcholine receptor antagonists. Patent WO 055503, 2006. (d) Budzik, B. W; Jin, J.; Laine, D. I.; McCleland, B.; Palovich, M. R.; Rivero, R. A.; Wang, Y.; Xie, H.; Zhu, C.; Cooper, A. W. J. Preparation of *N*-[(piperazinylmethyl)biphenyl]benzamide derivatives as M3 muscarinic acetylcholine receptor antagonists. Patent WO 055553, 2006.
(15) The biological assay results in the paper are a mean of at least 2 determinations with standard deviation of $<\pm 0.3$ unless otherwise noted.
(16) The final compounds in the paper were also tested under the agonist mode in the $M_3$, $M_2$, and $M_1$ FLIPR assays and showed no agonism in the three assays.
(17) (a) Jin, J.; Graybill, T. L.; Wang, M. A.; Davis, L. D.; Moore, M. L. Convenient preparation of 4-formyl-3,5-dimethoxyphenol and its incorporation into linkers and resins for solid-phase synthesis. *J. Comb. Chem.* **2001**, *3*, 97–101. (b) Available from Polymer Laboratories, part number 1466-6689, 150−300 $\mu$M, 1.5 mmol/g loading.
(18) Hamelmann, E.; Schwarze, J.; Takeda, K.; Oshiba, A.; Larsen, G. L.; Irvin, C. G.; Gelfand, E. W. Noninvasive Measurement of Airway Responsiveness in Allergic Mice Using Barometric Plethysmography. *Am. J. Respir. Crit. Care Med.* **1997**, *156*, 766–775.
(19) Foster, W. M.; Walters, D. M.; Longphre, M.; Macri, K.; Miller, L. M. Methodology for the measurement of mucociliary function in the mouse by scintigraphy. *J. Appl. Physiol.* **2001**, *90*, 1111–1118.
(20) **5a**, **5b**, and **5c** were single enantiomers with a (3*S*)-3-methylpiperazin-1-yl moiety at the right-hand side (RHS) similar to **4a**, **4b**, and **4c**.
(21) Measuring the permeation ability of compounds of interest across artificial phospholipid membranes. The technique is very similar to the widely used Caco-2 monolayer permeation technique.

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