Journal of Medicinal Chemistry

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Article

Discovery of M_3 Antagonist-PDE4 Inhibitor Dual Pharmacology Molecules for the Treatment of Chronic Obstructive Pulmonary Disease

Elisabetta Armani,* Andrea Rizzi, Carmelida Capaldi, Renato De Fanti, Maurizio Delcanale, Gino Villetti, Gessica Marchini, Anna Rita Pisano, Vanessa Pitozzi, Maria Gloria Pittelli, Marcello Trevisani, Michela Salvadori, Valentina Cenacchi, Paola Puccini, Francesco Amadei, Alice Pappani, Maurizio Civelli, Riccardo Patacchini, Charles A.G. Baker-Glenn, Hervé Van de Poël, Wesley P. Blackaby, Kevin Nash, and Gabriele Amari

Cite This: *J. Med. Chem.* 2021, 64, 9100–9119

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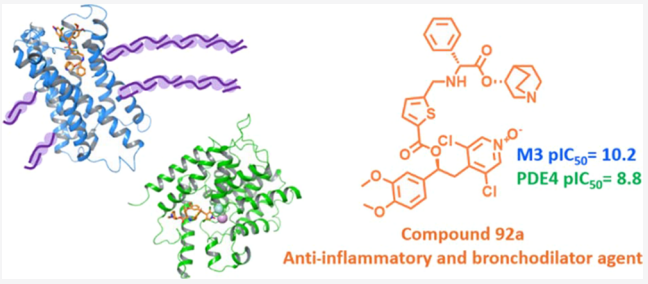
ABSTRACT: In this paper, we report the discovery of dual M_3 antagonist-PDE4 inhibitor (MAPI) compounds for the inhaled treatment of pulmonary diseases. The identification of dual compounds was enabled by the intuition that the fusion of a PDE4 scaffold derived from our **CHF-6001** series with a muscarinic scaffold through a common linking ring could generate compounds active versus both the transmembrane M_3 receptor and the intracellular PDE4 enzyme. Two chemical series characterized by two different muscarinic scaffolds were investigated. SAR optimization was aimed at obtaining M_3 nanomolar affinity coupled with nanomolar PDE4 inhibition, which translated into anti-bronchospastic efficacy *ex vivo* (inhibition of rat trachea contraction) and into anti-inflammatory efficacy *in vitro* (inhibition of TNFα release). Among the best compounds, compound **92a** achieved the goal of demonstrating *in vivo* efficacy and duration of action in both the bronchoconstriction and inflammation assays in rat after intratracheal administration.



INTRODUCTION

Chronic obstructive pulmonary disease (COPD) describes a group of lung conditions and is characterized by progressive airflow limitation. These conditions include persistent bronchitis, which is long-term inflammation of the airways, and emphysema, which damages the air sacs in the lungs, and both these conditions can occur together. COPD is a major worldwide health problem, with an estimated 3 million deaths caused globally by the disease in 2015, which is around 5% of all deaths in that year.[1]

The current treatment guidelines for COPD involve the use of bronchodilators, usually muscarinic (M_3) antagonists or β-adrenergic agonists. However, although these drugs may be effective in improving symptoms, they fail to address the underlying chronic inflammation. As such, corticosteroids are also recommended for COPD patients at high risk of exacerbations, but these drugs have shown limited efficacy as anti-inflammatory agents in COPD. However, PDE4 inhibitors, which elevate cAMP levels, have shown promise in the treatment of COPD by reducing the responses of inflammatory cells, and the latest clinically validated COPD therapy is roflumilast, a potent, long-lasting phosphodiesterase 4 (PDE4) inhibitor.

Inhalers containing a binary combination of drugs, for example, Combivent (ipratropium bromide/salbutamol), can simplify a COPD patient's drug regimen by reducing the number of inhalations per day. The concept of combining two activities in a single molecule has also been investigated in the recent years [i.e., dual-pharmacology inhaled muscarinic antagonist-β2 agonist (MABA) molecules for COPD treatment][2] and this approach may offer potential advantages over the combination of two different drugs including the potential benefit of an increase in affinity over the monofunctional and monovalent parent compounds, a high molecular weight that often translates into greater lung retention, low oral bioavailability, and reduced systemic exposure. Moreover, the development of a single compound with dual activity is simplified in terms of a unique pharmacokinetic profile,

Received: February 3, 2021
Published: June 18, 2021

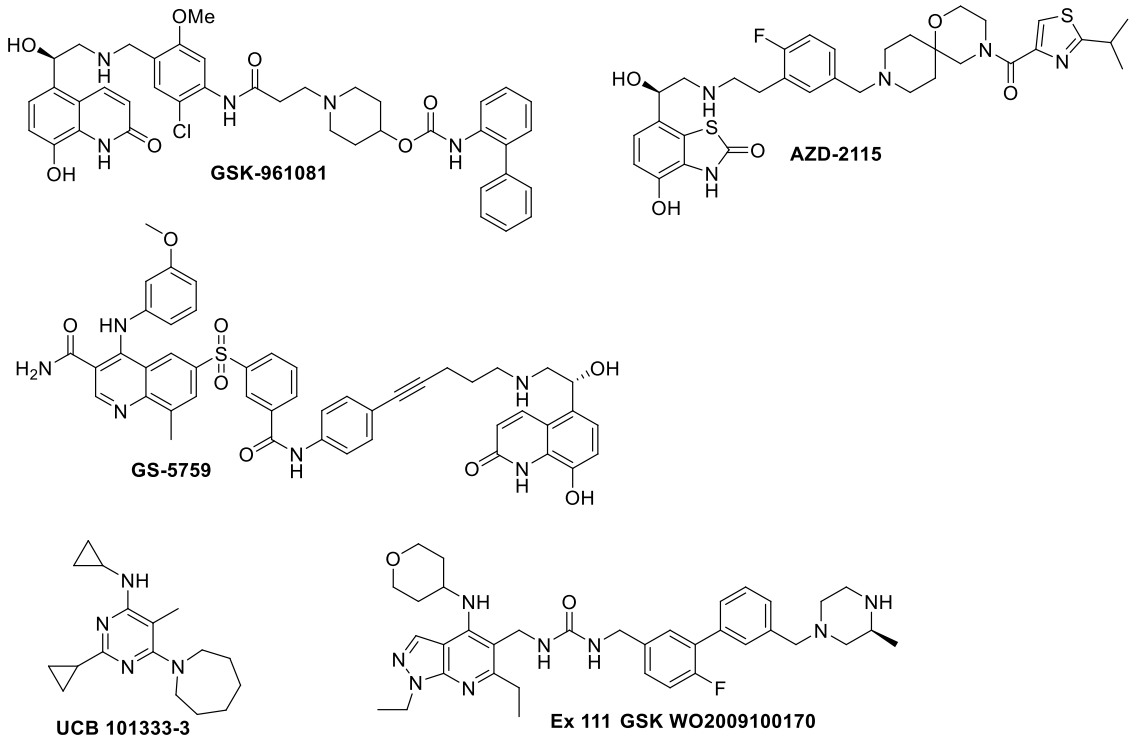


Figure 1. Known MABA (**GSK-961081** and **AZD-2115**), BAPI (**GS-5759**), and MAPI (**UCB 101333-3**, Ex 111 in WO 2009100170) compounds.

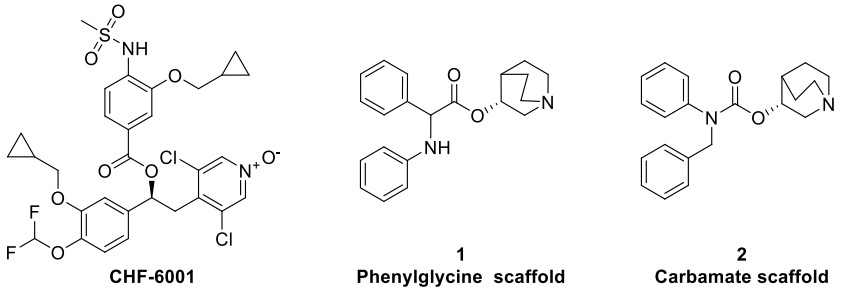


Figure 2. PDE4 inhibitor **CHF-6001** and muscarinic antagonist scaffolds.

formulation, and clinical development programs, so avoiding the associated challenges of the clinical development and co-formulation of two active pharmaceutical ingredients. On the other hand, whereas the flexible dosage of each active component is possible in the combination product, in the single dual product, the dosage is the same for the different components, meaning that the ratio of the two pharmacological activities cannot be adjusted as needed. Balancing of the pharmacological activities should be an intrinsic property of the dual molecule in order to avoid the overdosage of one component and the associated risk of unwanted side effects.

The combination in a single molecule of a muscarinic antagonist and a β-adrenergic agonist (MABA) has been widely reported,[2] with compounds from GSK (**GSK-961081**) and AZ (**AZD-2115**) being progressed into the clinic. Compounds combining either a β-adrenergic agonist with a PDE4 inhibitor (BAPI), such as **GS-5759**,[3] or combining muscarinic antagonist activity with a PDE4 inhibiting activity (MAPI), such as **UCB 101333-3**[4] or a series of pyrazolopyridines from Glaxo[5] (Figure 1), have also been reported.

In this paper, we report the discovery of a family of dual M_3 antagonist-PDE4 inhibitor molecules derived from our previously reported PDE4 inhibitor, **CHF-6001**.[6]

RESULTS AND DISCUSSION

Initial Strategy. Our aim was the identification of compounds that demonstrated activity both at the transmembrane M_3 receptor and at the intracellular PDE4 protein to deliver a MAPI for inhaled administration that displayed a balanced *in vivo* efficacy as a bronchodilator and anti-inflammatory agent with limited systemic exposure to minimize any potential side effects resulting from interaction with peripheral drug targets. Our strategy was to combine the structure of PDE4 inhibitor **CHF-6001** (Figure 2) with that of a muscarinic M_3 antagonist: a number of muscarinic antagonist scaffolds were investigated, but this paper will focus on work derived from two related scaffolds, phenylglycine scaffold **1**[7] and phenylcarbamate scaffold **2**[8] (Figure 2).

Unlike many of the MABA compounds reported in the literature, and the GSK MAPI series, which contain a linker between the two pharmacophoric fragments, we began our investigation by designing compounds where the two fragments are overlapped through a common linking ring. A series of four analogues were synthesized, all containing a *meta*-linked phenyl as the shared ring: three of these compounds contain a muscarinic head group derived from scaffold **1**, with different

https://doi.org/10.1021/acs.jmedchem.1c00204
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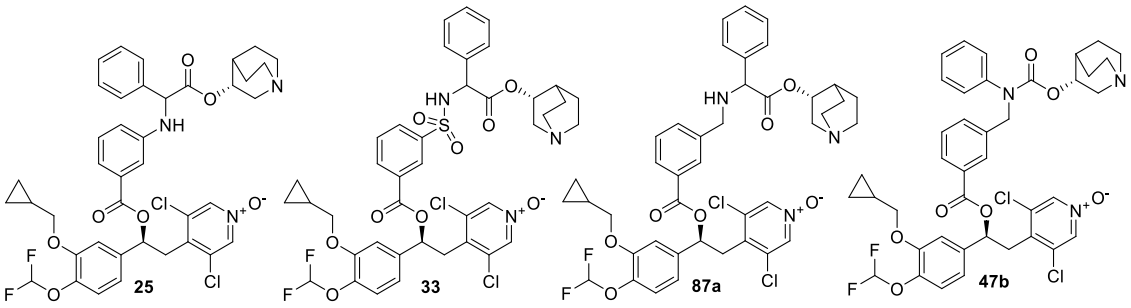
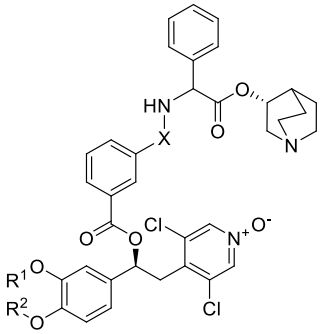
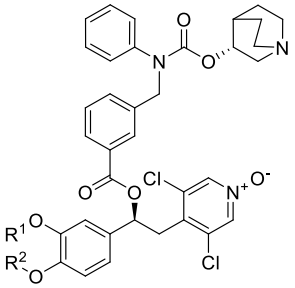


Figure 3. First MAPI compounds with muscarinic scaffold 1 (**25**, **87a**, and **33**) or scaffold 2 (**47b**).

Table 1. Biological Data and Calculated logD for Compounds 25, 33, 87a, 47b, 24, 32, 86a, and 46b





Cmp	–X–	R^1	R^2	stereochem	M_3 pK_i	GPT pIC_{50}	LARBS pIC_{50}	clogD (7.4)
25	bond	CH_2cPr	CHF_2	diast. mix	9.1	<6	9.0	4.3
33	$-SO_2-$	CH_2cPr	CHF_2	diast. mix	8.9	7.3	10.0	3.9
87a	$-CH_2-$	CH_2cPr	CHF_2	diast. mix	9.2	7.6	9.5	4.6
47b		CH_2cPr	CHF_2		8.9	6.5	9.9	4.6
24	bond	Me	Me	diast. mix	9.4	7.7	9.8	3.4
32	$-SO_2-$	Me	Me	diast. mix	9.2	8.2	9.6	2.9
86a	$-CH_2-$	Me	Me	diast. mix	9.4	8.2	9.5	3.7
46b		Me	Me		9.5	9.2	9.0	3.8

linkers between the phenylglycine amine and the shared ring, and one is derived from carbamate scaffold **2** (Figure 3).

Pleasingly, compounds **25**, **33**, **87a**, and **47b** all demonstrated good affinity for the M_3 receptor coupled with good inhibition of PDE4 in a LARBS assay. Based on this promising initial result, the compounds were assessed in the isolated guinea pig trachea (GPT) assay, measuring the inhibition of the contraction of the isolated tissue caused by carbachol (Cch) (Table 1). It was hypothesized that the poor translation from M_3 binding affinity to functional efficacy in GPT with at least a 40-fold reduction may be due to the physical properties of the molecules. The less lipophilic 3,4-dimethoxycatechol analogues of the four MAPI compounds were synthesized to investigate the impact of a reduced lipophilicity on GPT functional efficacy.

The dimethoxy catechol-containing analogues **86a**, **24**, **32**, and **46b** in Table 1 retained a good affinity for the M_3 receptor coupled with good inhibition of PDE4 and showed a reduced drop-off between the M_3 binding and GPT assays.

Optimization of Phenylcarbamate Series. Compounds containing a carbamate M_3 fragment showed a better chemical stability in comparison with their phenylglycine analogues, several of which displayed a significant reduction in chemical purity when stored as solid over a 2 month time period, mainly due to hydrolysis of the quinuclidine ester bond in the M_3 fragment. The first round of optimization was therefore undertaken using the carbamate M_3 fragment, coupled with a PDE4 fragment containing a dimethoxy catechol substituent.

At this point in the project, a technically simpler PDE4 assay was also introduced: the cell-free activity of the compounds was determined against human recombinant PDE4B2 protein where, in general, MAPI compounds showed a lower level of inhibition compared to the original LARBS assay. A PDE4 cell-based assay was added to the cell-free assay: the best compounds were progressed to the *in vitro* evaluation of activity in peripheral blood mononuclear cells (PBMCs) measuring the inhibition of (LPS)-induced tumor necrosis factor-alpha (TNF-α) release.

We focused on the optimization of the linking ring of phenylcarbamate series, synthesizing a set of derivatives that retain the (*R*)-quinuclidinyl-phenylcarbamate and dimethoxyphenyl-dichloropyridine oxide portions whilst varying the substitution pattern around the phenyl linking ring and the linking ring itself (Table 2).

Both *meta*- and *para*-substitution of the linking phenyl ring in **46b** and **46a** was tolerated at the muscarinic receptor with an improvement in PDE4 inhibitory activity for *para*-substitution. Fluorine introduction on the phenyl linking ring of **46a** and **46b** resulted in the retention of M_3 activity and the improvement of PDE4 cell-based activity for compounds **46d** and **46e**. Introduction of a methylene between the linking ring and the PDE4 ester fragment showed a good M_3 affinity

https://doi.org/10.1021/acs.jmedchem.1c00204
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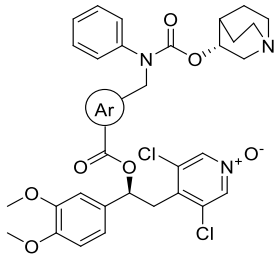
Table 2. Biological Data of Compounds 46a−e and 60



Cmp	Ar	M_3 pK_i	GPT pIC_{50}	PDE4B2 pIC_{50}	PBMC pIC_{50}
46a		9.8	8.7	8.7	8.6
46b		9.5	9.2	8.2	8.3
46c		9.5	–	8.0	–
46d		9.8	8.6	8.6	9.9
46e		9.7	9.0	8.1	8.7
60		10.0	8.4	9.0	9.1

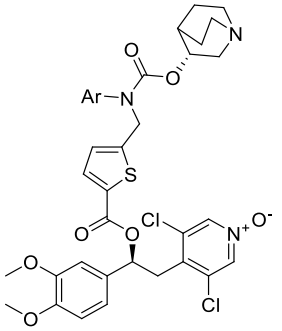
without any improvement in PDE4B2 inhibition for compound **46c**. When the phenyl ring was replaced with a series of five- and six-membered heterocycles a significant instability of the PDE4 ester fragment was observed (data not shown). However, the introduction of a 2,5-disubstituted thiophene as linking ring resulted in a stable compound: **60** showed a good *in vitro* activity and retained efficacy in the GPT assay.

Fluorophenyl **46d** and thiophene **60** showed the best overall *in vitro* profiles. Based on the observation that thiophene **60** demonstrated a better chemical stability over time than fluorophenyl derivatives, the next stage of optimization was undertaken using thiophene as the linking ring.

To investigate the importance of the non-linking M_3 ring, a series of carbamate derivatives with different M_3 rings was targeted (Table 3). Introduction of a fluorine onto the phenyl ring of **60** was tolerated (**57** and **61**), whilst introduction of larger substituents such as methoxy or cyano were not well tolerated, resulting in a drop in affinity for the M_3 receptor (data not shown). It was hypothesized that introduction of a hydroxy group at the *ortho*-position may enable a hydrogen-bonding interaction with the M_3 receptor, but both the 2-substituted phenol and the corresponding benzyl alcohol demonstrated a rapid degradation, with the hydroxyl group cyclizing onto the carbamate to release (*R*)-quinuclidin-3-ol.

In contrast, the *meta*-substituted phenol **64** was stable and demonstrated good affinity for the M_3 receptor coupled with a good inhibition of PDE4B2, which translated into a good efficacy in the GPT and PBMC assays. The bioisosteric *meta*-difluoromethyl analogue **63** showed a significant drop-off in the affinity for the M_3 receptor in comparison with **64**, although it regained efficacy in the GPT assay. Introduction of a basic 3-pyridyl group as the non-linking M_3 ring (**58**) resulted in a significant drop in affinity for the M_3 receptor. In contrast, the 2-pyridyl analogue **59** and the 2-thiazolyl

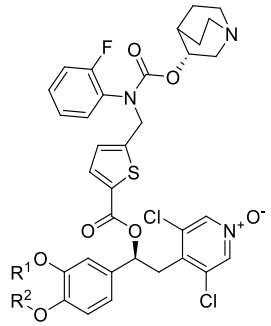
Table 3. Biological Data of Analogues of 60 with Variations in the Non-linking M_3 Ring



Cmp	Ar	M_3 pK_i	GPT pIC_{50}	PDE4B2 pIC_{50}	PBMC pIC_{50}
60	Ph	10.0	8.4	9.0	9.1
57	2-F-Ph	9.9	9.1	9.3	8.9
61	3-F-Ph	9.7	8.1	9.0	9.2
58	3-Py	8.1			
59	2-Py	9.3	8.7	9.2	9.3
62	2-thiazolyl	9.1	8.4	9.2	9.3
63	3-F_2CH-Ph	8.5	8.8	9.5	9.7
64	3-HO-Ph	9.5	9.0	9.1	9.4

analogue **62** did not show the same drop in muscarinic affinity. The analogues of **57** with alternative substitution patterns on the catechol fragment (**69−72** in Table 4) confirmed the earlier hypothesis around the impact of the physical properties on the translation from binding affinity to functional efficacy.

Table 4. Biological Data, Calculated logD, and Kinetic Solubility of Compounds 57 and 69−72



Cmp	R^1/R^2	M_3 pK_i	GPT pIC_{50}	clogD (7.4)	kinetic solubility (μM)
57	Me/Me	9.9	9.1	3.4	134
69	Me/CHF_2	9.6	8.2	3.5	<5
70	Et/CHF_2	9.5	8.3	3.9	<5
71	*i*Pr/CHF_2	9.5	7.5	4.2	<5
72	*c*PrCH_2/CHF_2	9.7	8.2	4.2	<5

Replacement of the methoxy group at the 4-position of the catechol in **57** with a difluoromethoxy group to give **69** resulted in an increase in the drop-off between the M_3 binding and the GPT assays. Increasing the lipophilicity of the substituent at the 3-position of the catechol resulted in a corresponding increase in the clogD, and the compounds (**70−72**) showed negligible kinetic solubility in phosphate-buffered saline solution (PBS) and a large drop-off in the GPT assay.

At this stage, a set of compounds were next evaluated in an *in vivo* model of bronchoconstriction (Table 5). The

https://doi.org/10.1021/acs.jmedchem.1c00204
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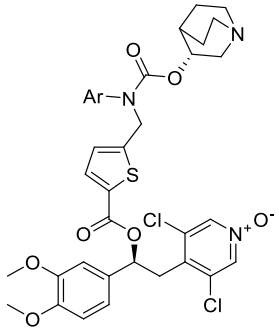
Table 5. Bronchodilator Effect of Compounds 57, 59, and 60



Cmp	Ar	M_3 pK_i	GPT pIC_{50}	bronchodilator effect *in vivo* ED_{50} (nmol/kg) @ 1 h	bronchodilator effect @ 1/16 h (%) (dose in nmol/kg)
60	Ph	10.0	8.4	1.6	82/− (3)
57	2-F-Ph	9.9	9.1	0.5	97/38 (3)
59	2-Py	9.3	8.7		78/− (10)

compounds were dosed by solution *via* intra-tracheal administration to anesthetized guinea pigs and their impact on bronchoconstriction induced by acetylcholine (ACh) was tested.

When tested at 3 nmol/kg, both **60** and **57** showed good and dose-dependent inhibition of bronchoconstriction at 1 h timepoint, giving ED_{50} values of 1.6 and 0.5 nmol/kg, respectively. The 2-pyridyl analogue **59** displayed only 78% inhibition of bronchoconstriction at 1 h timepoint when dosed at 10 nmol/kg. Based on this data, the duration of action of **57** was investigated; disappointingly, the compound showed only modest levels of inhibition 16 h after dosing.

Our focus at this point returned to MAPI compounds derived from a phenylglycine muscarinic fragment, in particular to the benzylamine-linked compounds, such as **87a** containing a second, mildly basic, amine functionality in addition to quinuclidine basic center. Our aim was to verify if the presence of the second basic center could improve the duration of action after inhalation.[9]

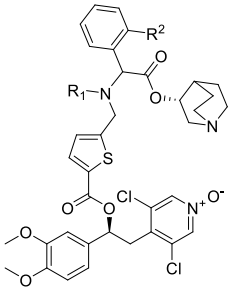
Optimization of Phenylglycine Series. The cross-over analogues of the best carbamate derivatives, **60** and **57**, were synthesized as a racemic mixture and then separated by chiral SFC into the single diastereomers, identified on the basis of the elution order as a Fast isomer or Slow isomer.

All the four compounds **91a**, **92a**, **91b**, **92b** demonstrated good affinity for the M_3 receptor coupled with good inhibition of PDE4B2, and these data translated into moderate to good efficacy in both the GPT and PBMC assays (Table 6). Capping of the phenylglycine nitrogen atom in **91a** and **92a** with a methyl group resulted in compounds **91c** and **92c** showing a conserved PDE4 activity but a clear drop in M_3 affinity. For this reason, other *N*-methyl derivatives were not investigated.

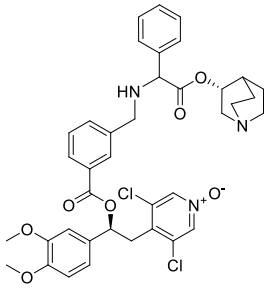
The diastereoisomers from each pair displayed similar *in vitro* profiles, as was also observed for **89** and **90**, the two single diastereoisomers of the phenyl linked analogue **86a** shown in Table 1.

The thiophene-linked phenylglycine series, showing an improved chemical stability overtime in comparison with the phenyl-linked phenylglycine series, was progressed to further investigation.

Table 6. Biological Data for Compounds 89, 90, 91a−c, and 92a−c



91a 92a 91b 92b 91c 92c



89 90

Cmp	R^1/R^2	isomer	M_3 pK_i	GPT pIC_{50}	PDE4B2 pIC_{50}	PBMC pIC_{50}
89		Fast	9.8	9.0	8.4	8.9
90		Slow	9.7	9.1	8.1	8.6
91a	H/H	Fast	9.9	8.9	8.8	8.5
92a	H/H	Slow	10.2	9.0	8.8	8.8
91b	H/F	Fast	10.0	8.6	9.2	9.0
92b	H/F	Slow	9.8	8.5	9.2	8.9
91c	Me/H	Fast	9.2		8.7	8.5
92c	Me/H	Slow	9.2		8.5	8.7

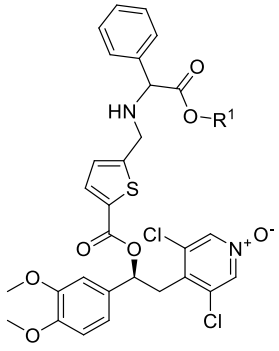
To allow for investigation of the balance of *in vivo* activity at the two pharmacological targets in the same species, it was decided to run both *in vivo* assays in rat at this stage. To support this testing paradigm, **91a** and **92a** were tested in a rat trachea (RT) assay measuring, as for the corresponding assay in guinea pig, the inhibition of the contraction of the isolated tracheal tissue caused by carbachol and a good correlation was observed between the data in the two species. Indeed **91a** showed RT pIC_{50} = 8.7 (GPT IC_{50} = 8.9) and **92a** showed RT pIC_{50} = 9.0, the same value obtained in GPT assay. Based on this good correlation, the RT assay replaced the GPT assay at this step in the project cascade.

The importance of the (*R*)-quinuclidinol head group found in all the compounds made up until this point in the program was investigated, replacing this group by a number of alternative amine-containing head groups (Table 7). The diastereomeric compounds in each case showed the same or only marginally different PDE4 profile both in cell-free and cell-based assay, but a significant difference in the affinity at the M_3 receptor was observed in contrast with the previous pairs of diastereomers, reported in Table 6, containing an (*R*)-quinuclidinol head group. For all of the non-(*R*)-quinuclidinol head groups, one diastereomer showed an M_3 affinity that was significantly higher than the other. This difference in affinity was carried through into the RT assay, except for **91f**, which regained activity in the rat trachea compared with M_3 affinity.

At this stage, compounds that demonstrated an $pIC_{50} \geq 8.5$ in both the rat trachea and hPBMC assays were evaluated in an *in vivo* model of bronchoconstriction in rat. Compounds **91a**, **92a**, **92e**, and **92f** were dosed by solution *via* intra-trachea (IT) administration to an anesthetized rat, and their impact on bronchoconstriction induced by carbachol was assessed at 1 h after dosing (Table 8). All compounds demonstrated an ED_{50} below 10 nmol/kg in this assay and were progressed to investigation of the bronchodilator effect at 16 h, each compound dosed at a concentration where at least 70% reduction of bronchoconstriction was observed at 1 h

https://doi.org/10.1021/acs.jmedchem.1c00204
J. Med. Chem. 2021, 64, 9100−9119

Table 7. Biological Data for Compounds 91a, 91d−j, 92a, and 92d−j



Cmp	R^1	Isomer	M_3 pK_i	RT pIC_{50}	PDE4B2 pIC_{50}	PBMC pIC_{50}
91a		Fast	9.9	8.7	8.8	8.5
92a		Slow	10.2	9.0	8.8	8.8
91d		Fast	9.3	8.0	9.0	8.6
92d		Slow	8.0	–	9.0	–
91e		Fast	8.0	7.7	9.0	8.8
92e		Slow	9.6	8.8	9.3	8.6
91f		Fast	8.1	9.0	8.5	8.4
92f		Slow	9.9	8.8	8.8	8.5
91g		Fast	8.2	7.7	8.7	8.5
92g		Slow	9.3	8.5	8.8	8.4
91h		Fast	8.0	7.2	9.1	8.4
92h		Slow	9.5	8.3	9.2	8.8
91i		Fast	8.6	7.7	8.6	8.1
92i		Slow	9.7	8.3	8.9	8.6
91j		Fast	7.9	7.1	9.0	8.5
92j		Slow	9.4	7.9	9.2	–

timepoint. Only (*R*)-quinuclidin-3-ol **92a** was observed to show greater than 50% inhibition at 16 h timepoint.

The sustained duration of action of **92a** in the animal model was confirmed by *in vitro* investigation on association and dissociation kinetics from the M3 receptor in comparison with the muscarinic antagonists long-acting tiotropium[10] and short-acting ipratropium.[11] Association and dissociation $t_{1/2}$ values, obtained from the association and dissociation rate constants, were very similar for compound **92a** and tiotropium with a dissociation $t_{1/2}$ much higher than ipratropium (Table 9), so suggesting that the observed long duration of action can be attributed to the off-rate at the receptor. Selectivity vs receptor M2 was also investigated at this stage: the lack of selectivity for compound **92a** was not considered a critical point, being typical of most of the M3 antagonists, tiotropium included.[10]

Compound **92a** was next tested in a rat OVA-induced airway inflammation model (Table 10), where it showed an ED_{50} of 30 nmol/kg, with about 90% reduction of eosinophils at 1 μmol/kg, and a good duration of action when dosed at 1 μmol/kg (Table 10).

Compound **92a** achieved the goal of demonstrating efficacy *in vivo* and duration of action in both the bronchoconstriction and inflammation assays, even if the 40:1 ratio of ED_{50}s between the OVA and bronchoconstriction assays raised concerns around the ability of the compound to display a clinical effect without effectively overdosing the compound at one of the target proteins. Indeed, both pharmacological activities are relevant for COPD, where maintaining airway caliber and counteracting inflammation are primary therapeutic objectives. For this reason, it is necessary that both pharmacophores of the preferred bifunctional compound are effective on their respective targets over similar concentrations *in vivo*.

The *in vivo* anti-inflammatory activity, 40-fold lower than bronchodilating activity, cannot be attributed to a difficulty of compound **92a** entering the inflammatory cells; indeed, the apical to basolateral apparent permeability (Papp A-B) value measured in Caco2 cells (Table 11) can be classified as a value of medium permeability. The ability of **92a** compound to cross the cells is also confirmed in a PBMC *in vitro* test, where the pIC_{50} data is comparable to that observed in cell-free PDE4B2. At this stage of the project, the key factors for a balanced *in vivo* activity remained unexplained.

The ADME profile of **92a** (Table 11) showing high levels of clearance in microsomes and a reduced stability in plasma proved to be in alignment with the aim of limiting the systemic exposure in order to reduce the risk of potential systemic side effects.

The emetic side effect, well known for most of the PDE4 inhibitors, was investigated for compound **92a** after intratracheal administration in ferret, considered a current gold standard to study the nausea and emetic effect of a compound,[12] at two level doses, 1 and 10 μmol/kg. The 1 μmol/kg a dose produced a sub-maximal anti-inflammatory activity in the rat ovalbumin model of lung inflammation (ED_{50} in OVA model = 30 nmol/kg) and was compared with the oral compound roflumilast at 10 μmol/kg, a dose associated with a sub-maximal efficacy in the rat ovalbumin model (data not shown) (Figure 4).

The mean of nausea-like score for the reference compound showed a significant difference in comparison with the vehicle (72.50 ± 18.55 for roflumilast vs 12.83 ± 5.10 for the vehicle), whereas compound **92a** showed at both doses a mean nausea-like score much lower than the reference compound and, importantly, without statistically significant differences from the vehicle and between the two tested doses (30.67 ± 6.13 for compound **92a** 1 μmol/kg; 19.83 ± 6.48 for compound **92a** 10 μmol/kg and 12.83 ± 5.10 for the vehicle). Notably, compound **92a** was devoid of gastrointestinal side effects at both dose levels, therefore also at a dose that exceeds the pharmacologically active doses by a factor 10, whereas roflumilast induced emesis and nausea at a pharmacological relevant dose.

The absolute configuration of compound **92a** was determined by single-crystal high-resolution X-ray diffraction. The configuration of stereocenters in the PDE4 alcohol and quinuclidinol portions were known, respectively, *S* and *R*. Based on that information, the single-crystal structure (CSD code: 2035364) allowed to attribute the configuration *R* to the stereocenter in the phenylglycine portion.

A preliminary evaluation of the developability of **92a** as a dry powder inhaler (DPI) was also started, but significant hydrolysis of the quinuclidine ester fragment was observed for all the identified solid forms when stored in accelerated conditions, suggesting a potential issue for further progression.

https://doi.org/10.1021/acs.jmedchem.1c00204
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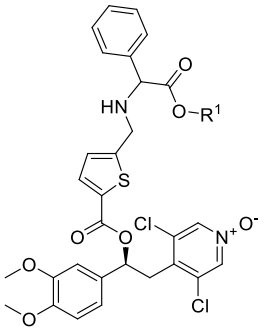
Table 8. Bronchodilator Effect of Compounds 91a, 92a, 92e, and 92f



Cmp	R^1	Isomer	M_3 pK_i	RT pIC_{50}	Bronchodilator effect *in vivo* ED_{50} (nmol/kg) @ 1 h	Bronchodilator effect @ 1 / 16 h (%) (dose)
92a		Slow	10.2	9.0	0.7	74 / 67 (3)
91a		Fast	9.9	8.7	1.6	73 / 32 (3)
92e		Slow	9.6	8.8	4.1	75 / 2 (30)
92f		Slow	9.9	8.8	1.0	96 / 41 (3)

Table 9. M3/M2 Binding and M3 Binding Kinetics of Compound 92a vs Tiotropium, Ipratropium

Cmp	M_3/M_2 pK_i	M3 association $t_{1/2}$ (min)	M3 dissociation $t_{1/2}$ (min)
92a	10.2/9.7	822	1768
tiotropium	9.9/9.8	618	1350
ipratropium	9.1/9.1	9.4	20.1

Table 10. Anti-inflammatory Effect of Compound 92a

Cmp	PDE4B2 pIC_{50}	PBMC pIC_{50}	eosinophilia reduction in OVA model ED_{50} (nmol/kg)	eosinophilia reduction in OVA model @ 24 h (%) (dose)
92a	8.8	8.8	30	54 (1 μmol/kg)

Table 11. ADME Profile of Compound 92a

Cmp	Kin. Solub. (μM)	PPB (% bound) h/r	Plasma stab. $t_{1/2}$ (min) h/r	Mics. Cl_{int} (μL/min/mg) h/r	Caco2 (nm/s) Papp A-B
92a	145	93/91.40	11/9	213/126	16

Combined with the non-optimal balance at the two pharmacological targets, this finding resulted in the progression of **92a** being halted.

CHEMISTRY

The synthetic pathway employed for the preparation of the PDE4 catechol fragment is shown in Schemes 1 and 2. Alcohol **11** was prepared following a similar synthetic approach used for the preparation of (*S*)-alcohol **12**.[6] The absolute configuration (*S*) of alcohol **11** was confirmed by the X-ray of the solid crystals of alcohol **10** (data not shown). Acidic cleavage of the cyclopropylmethyl group in **12** followed by alkylation of the catecholic hydroxyl group with K_2CO_3 and the appropriate alkyl iodide reagent led to compounds **14**, **15**, and **16** (Scheme 2).

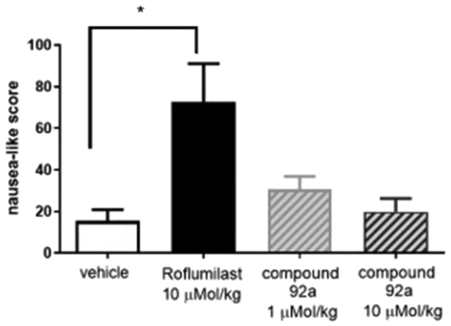


Figure 4. Evaluation of nausea-like score of **92a** compared to roflumilast. Differences in the mean of nausea-like scores were assessed with Kruskal–Wallis one-way analysis of variance on ranks followed by Dunn's test. A level of probability of 0.05 or less was accepted as significant.

The synthetic pathway for the synthesis of aniline derivatives **24** and **25** is depicted in Scheme 3. Chiral alcohols **11** and **12** were condensed with 3-nitrobenzoic acid **17** and then reduction of the nitro group with tin(II) chloride led to the amine intermediates **20** and **21**, which underwent a multicomponent Petasis reaction involving phenylboronic acid and glyoxylic acid. The obtained α-amino acids **22** and **23** were directly coupled with (*R*)-quinuclidin-3-ol using the standard DCC/HOBt procedure to afford anilines **24** and **25**.

The synthesis of sulfonamides **32** and **33** is shown in Scheme 4. Sulfonylation of *tert*-butyl 2-amino-2-phenylacetate **27** with methyl 3-(chlorosulfonyl)benzoate gave sulfonamide **28**. Sulfonamides **30** and **31** were prepared by hydrolysis of methyl ester **28** under basic conditions followed by coupling with the suitable enantiomerically pure alcohols **11** or **12** in the presence of EDC and DMAP. Acidic removal of the *tert*-butyl group followed by DCC/HOBt coupling with (*R*)-quinuclidin-3-ol afforded the final compounds **32** and **33**.

Carbamates **46a–e** and **47b** synthesized to explore the linking ring SAR were prepared following the synthetic

https://doi.org/10.1021/acs.jmedchem.1c00204
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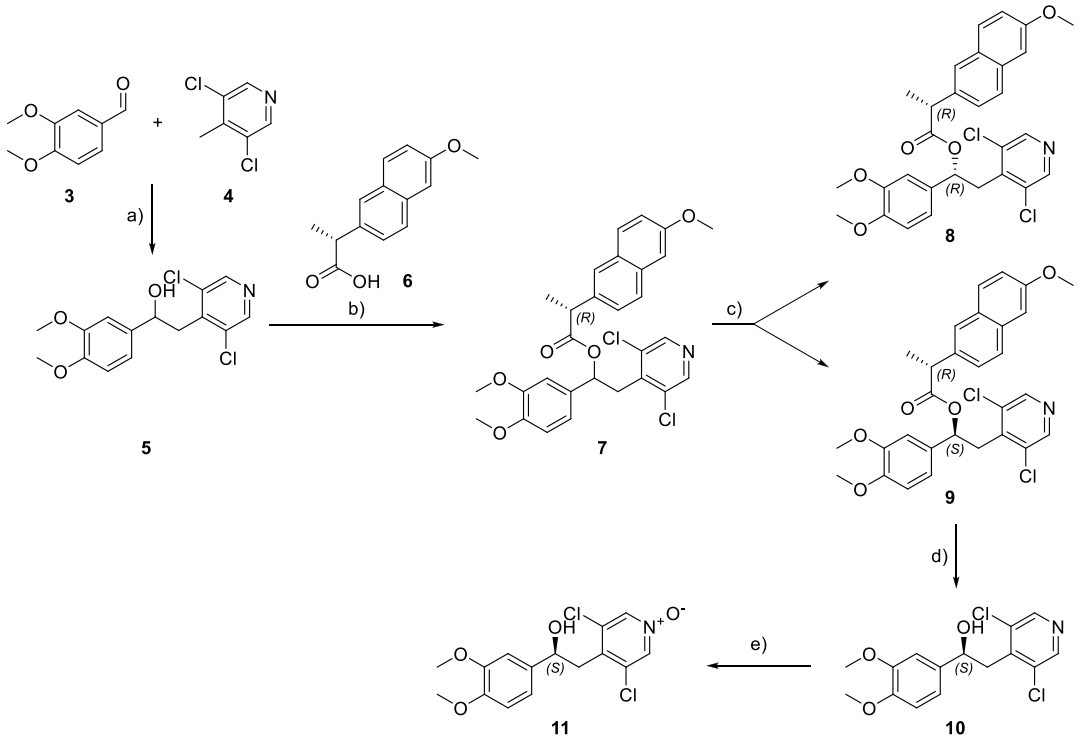
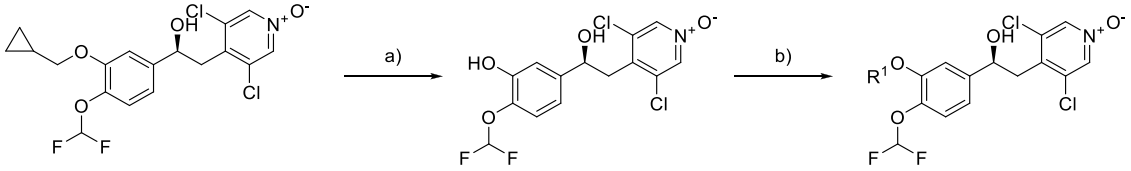
Scheme 1. Preparation of (*S*)-Alcohol 11



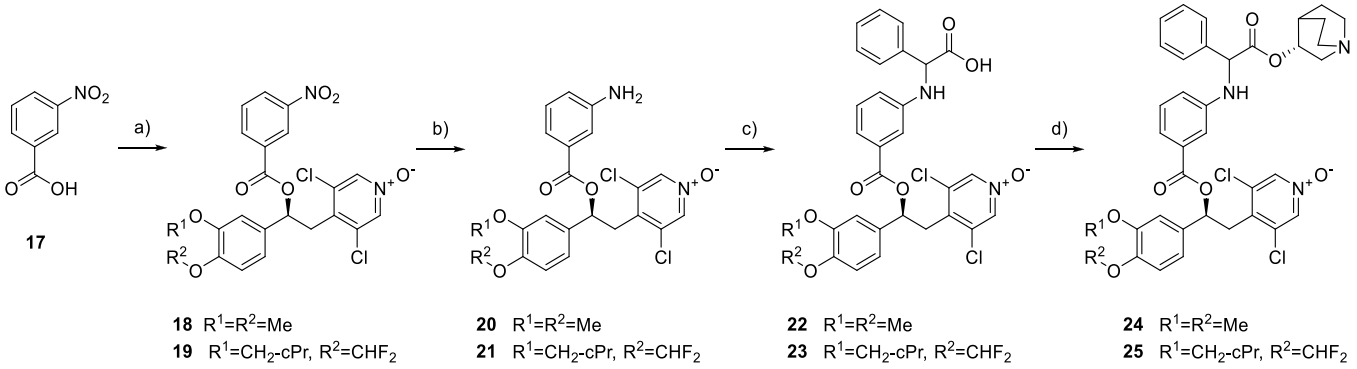
[a]Reagents and conditions: (a) 1 N LHMDS, THF, −78 °C to rt, 1 h; (b) DMAP, EDC, DMF, rt, 2 h; (c) crystallization in $CHCl_3$, MeOH, 2 h; (d) *t*-BuOK, MeOH, toluene, rt, 24 h; (e) *m*-CPBA, EtOAc, rt, 5 h.

Scheme 2. Preparation of (*S*)-Alcohols 14−16



[a]Reagents and conditions: (a) 37% HCl, rt, 3 min; (b) K_2CO_3, DMF, R^1I, rt, 4 h.

Scheme 3. General Route for the Preparation of MAPI Compounds with Aniline Saffold



[a]Reagents and conditions: (a) **11** or **12**, EDC, DMAP, DCM, rt, 16 h; (b) $SnCl_2{\cdot}2H_2O$, THF, 75 °C, 16 h; (c) phenylboronic acid, glyoxylic acid, DCM, rt, 16 h; (d) (*R*)-quinuclidin-3-ol, DCC, HOBt, THF, rt, 16 h.

approach outlined in Scheme 5. Reductive amination of commercially available carboxaldehydes **34a−c** with aniline led to amines **36a−c**. Amine **36d** was prepared *via* bromine displacement of **35d** with *N*-Boc-aniline in the presence of

https://doi.org/10.1021/acs.jmedchem.1c00204
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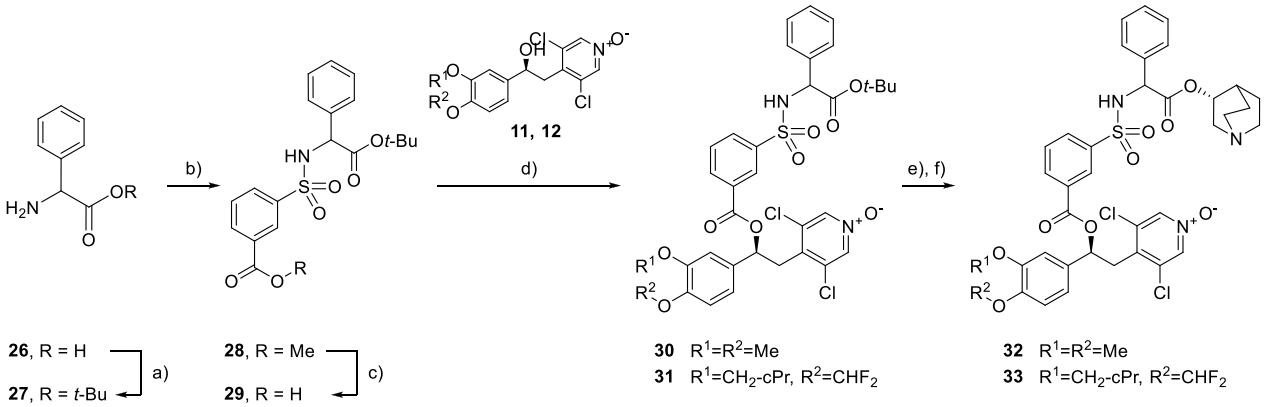
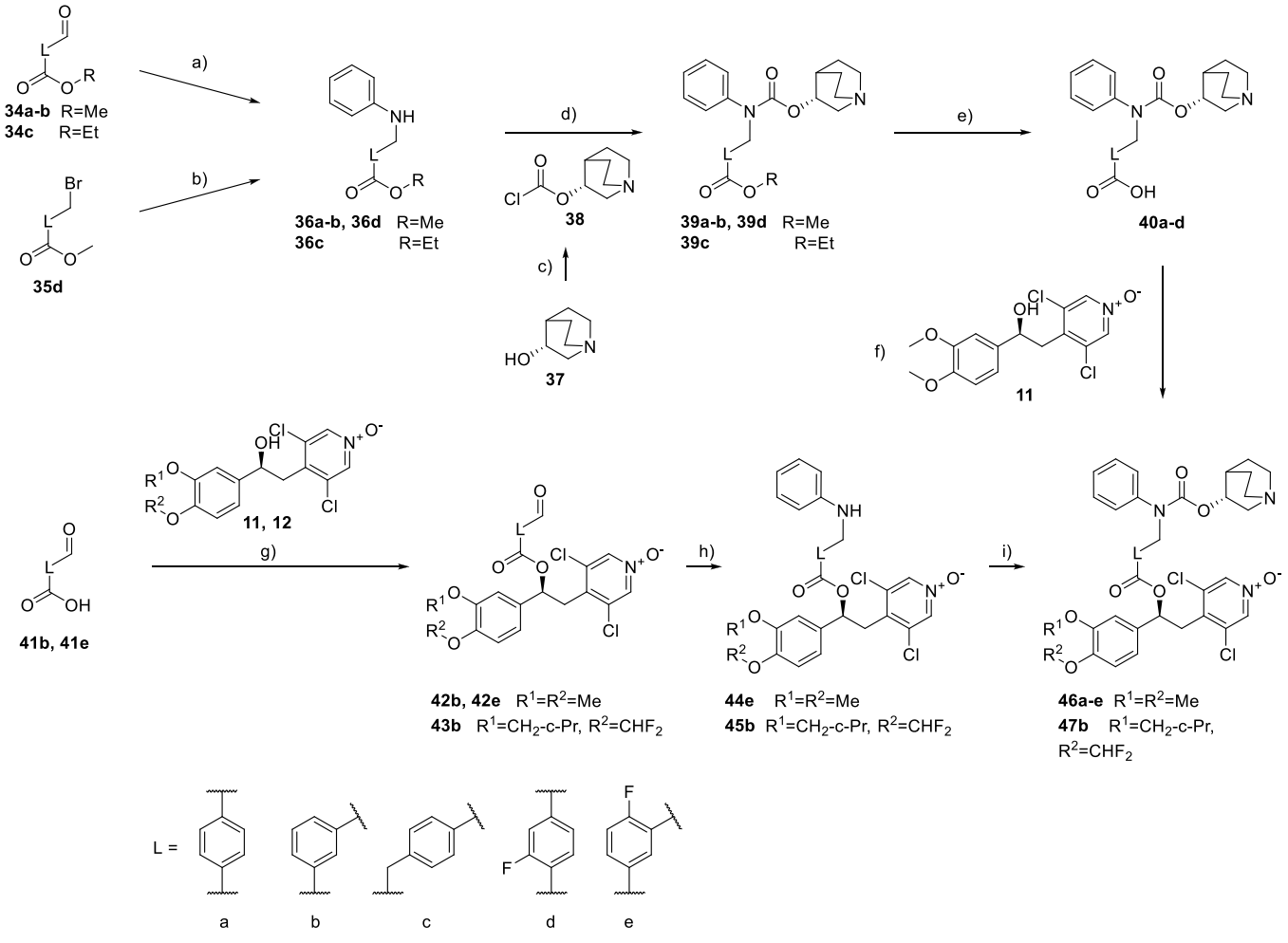
Scheme 4. General Route for the Preparation of MAPI Compounds with Sulfonamide Scaffold



[a]Reagents and conditions: (a) isobutylene, sulfuric acid, 1,4-dioxane, rt, 48 h; (b) methyl 3-(chlorosulfonyl)benzoate, Et_3N, THF, rt, 30 min; (c) 1 N LiOH, THF, MeOH, rt, 18 h; (d) **11** or **12**, EDC·HCl, DMAP, DMF, rt, 18 h; (e) TFA, DCM, 0 °C, 2.5 h to rt, 0.5 h; (f) (*R*)-quinuclidin-3-ol, DCC, HOBt, THF, rt, 18 h.

Scheme 5. General Route for the Preparation of MAPI Compounds with Carbamate Scaffold



[a]Reagents and conditions: (a) (L = a–c) Aniline, AcOH, rt, 64 h then $NaBH(OAc)_3$, rt, 2 h; (b) (L = d) Aniline, K_2CO_3, DMF, 60 °C, 18 h; (c) trichloromethyl chloroformate, CH_3CN, 0 °C, 1 h then rt, 16 h; (d) for the preparation of **39a** and **39b**: **38**, pyridine, 0 °C to rt, 48 h; for the preparation **39c** and **39d**: **38**, DMAP, pyridine, rt, 48 h; (e) LiOH·H_2O, THF, H_2O, rt, 18 h; (f) **11**, EDC·HCl, DMAP, DMF, rt, 18 h; (g) **11** or **12**, EDC·HCl, DMAP, DCM, rt, 18 h; (h) aniline, AcOH, DCM, rt, 6–18 h then $NaBH(OAc)_3$, rt, 18 h; (i) **38**, CH_3CN, microwave 80 °C, 15 min.

potassium carbonate followed by cleavage of the Boc group under acidic conditions. Final carbamates **46a–d** were prepared by reaction of amines **36a–d** with key chloroformate intermediate **38** followed by hydrolysis of the ester group and

https://doi.org/10.1021/acs.jmedchem.1c00204
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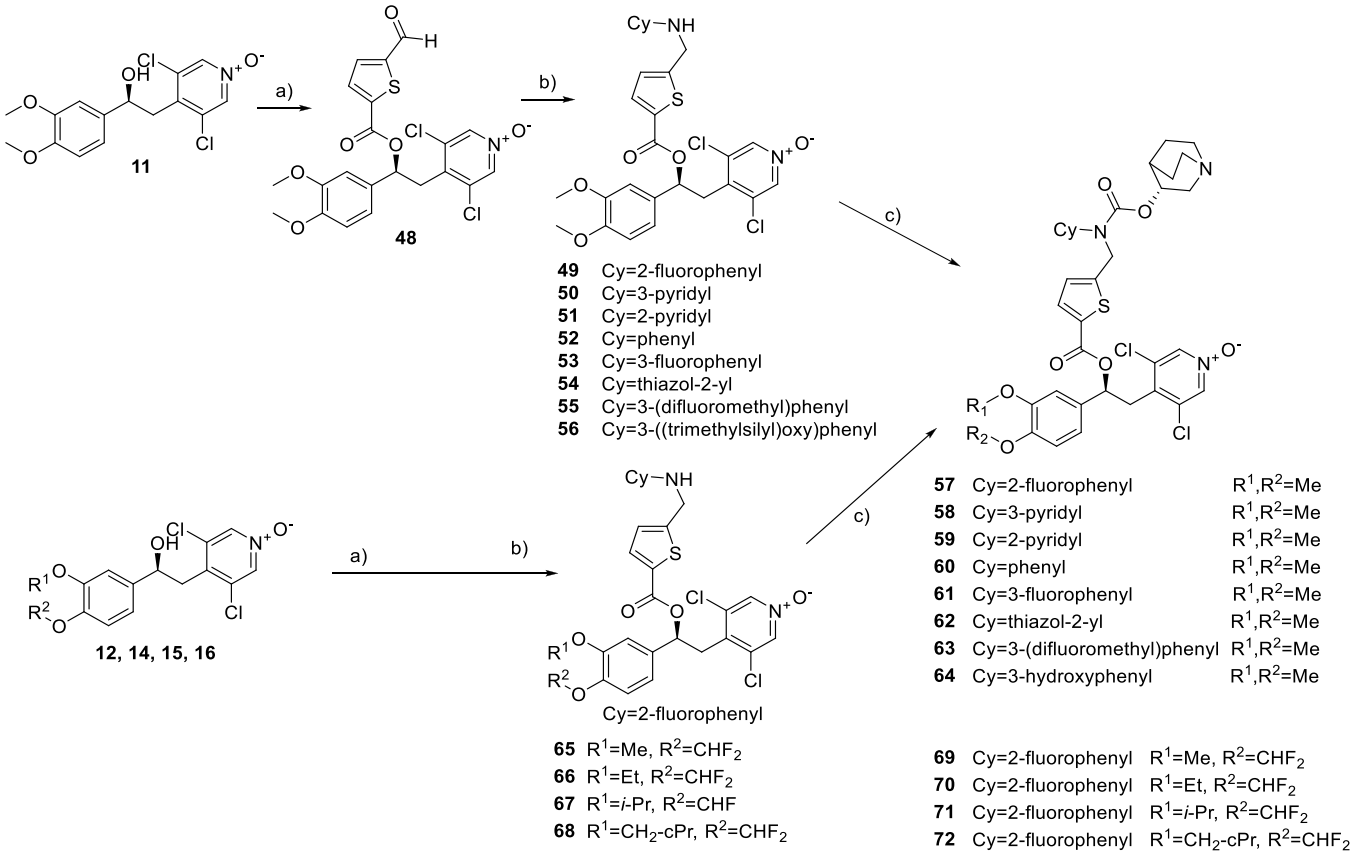
Scheme 6. General Route for the Preparation of MAPI Compounds with Carbamate Scaffold



[a]Reagents and conditions: (a) 5-formyl-2-thiophenecarboxylic acid, EDC·HCl, DMAP, DCM, rt, 18 h; (b) $CyNH_2$, AcOH, DCM, rt, 6−18 h then $NaBH(OAc)_3$, rt, 18 h; (c) **38**, CH_3CN, microwave 80 °C.

EDC/DMAP coupling with enantiomerically pure alcohol **11**. A partially modified synthetic route was followed for compounds **46e** and **47b** in which enantiomerically pure alcohols **11** or **12** were coupled with commercially available acids **41b** and **41e** followed with reductive amination with aniline and carbamate formation using chloroformate intermediate **38**. In Scheme 5, the intermediate **42b** is described to be used in Scheme 7.

Carbamate **57**, with a thiophene as the linking ring, was prepared following a similar approach from aldehyde **48** *via* reductive amination with the appropriate cyclic amine followed by carbamate formation (Scheme 6). The analogous compounds **58**−**64** and **69**−**72**, with the same linking ring, synthesized to explore the M_3 non-linking ring and the cathecolic substitution, were analogously prepared (Scheme 6).

The synthetic pathway for the preparation of MAPI compounds with a phenylglycine scaffold is outlined in Scheme 7. Boc-protected amino acids **73**−**75** were coupled with the suitable alcohol **76**−**83** in the presence of DCC and HOBt, and the subsequent Boc cleavage followed by a reductive amination reaction led to phenyl glycine analogues **86a**, **87a**, and **88a**−**j** as a mixture of two diastereoisomers. **86a** and **88a**−**j** were separated by chiral SFC to afford single diastereoisomers **89**, **90**, **91a**−**j**, and **92a**−**j** (Scheme 8).

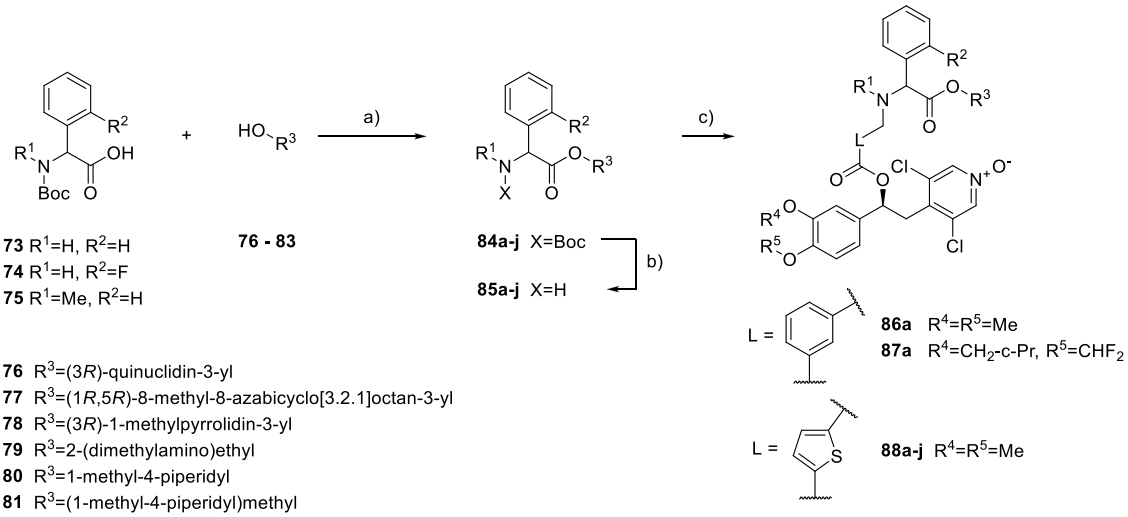
CONCLUSIONS

We have demonstrated that the PDE4 scaffold structurally related to **CHF-6001** can be combined with a muscarinic antagonist scaffold to yield compounds that display dual M_3/PDE4 activity *in vitro* and efficacy *in vivo* in both bronchoconstriction and inflammation assays. Our strategy differed from the MAPIs reported by UCB, which combined both activities in a shared scaffold, and from the GSK MAPI series, which contains a linker between M3 and PDE4 portions, in that the two pharmacophoric fragments overlapped through a common linking ring.

We showed that the introduction of a second basic center into our MAPI molecules could improve the duration of action in the bronchoconstriction assay, and optimization of the benzylamine-linked series led to **92a**. The *in vitro* pharmacological characterization of **92a** demonstrated that this compound has low nanomolar potency for both M_3 muscarinic receptor and PDE4B2 isoenzyme. Functional potency of **92a** evaluated in rat isolated tracheal strips and in human PBMCs was in the low nanomolar range in both assays with no drop-off in potency compared with cell-free IC_{50} values. A key factor for bifunctional compounds such as MAPI molecules is that a balanced pharmacological profile at the two targets *in vitro* translates into a bronchodilator and anti-inflammatory activity that can be achieved in a similar dose range in relevant *in vivo* models. Experiments performed in the rat looked at bronchodilator and anti-inflammatory activity in two separate models to investigate potency and duration of action of **92a** using the same formulation and methodology of inhaled administration. The compound displayed full efficacy and a sustained duration of action in both the bronchoconstriction and OVA assays; however, the ED_{50} for anti-inflammatory activity was 40-fold higher than the one for the bronchodilator

https://doi.org/10.1021/acs.jmedchem.1c00204
J. Med. Chem. 2021, 64, 9100−9119

Scheme 7. General Route for the Preparation of MAPI Compounds with Phenylglycine Scaffold



73 R^1=H, R^2=H
74 R^1=H, R^2=F
75 R^1=Me, R^2=H

76 - 83

84a-j X=Boc
85a-j X=H

86a R^4=R^5=Me
87a R^4=CH_2-c-Pr, R^5=CHF_2

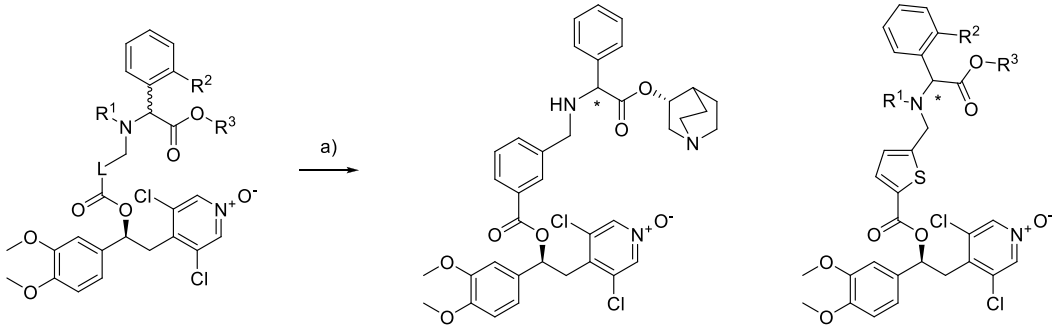
88a-j R^4=R^5=Me

76 R^3=(3*R*)-quinuclidin-3-yl
77 R^3=(1*R*,5*R*)-8-methyl-8-azabicyclo[3.2.1]octan-3-yl
78 R^3=(3*R*)-1-methylpyrrolidin-3-yl
79 R^3=2-(dimethylamino)ethyl
80 R^3=1-methyl-4-piperidyl
81 R^3=(1-methyl-4-piperidyl)methyl
82 R^3=[(2*R*)-1-methylazetidin-2-yl]methyl
83 R^3=[(3*R*)-quinuclidin-3-yl]methyl

a R^1=H, R^2=H, R^3=(3*R*)-quinuclidin-3-yl
b R^1=H, R^2=F, R^3=(3*R*)-quinuclidin-3-yl
c R^1 = Me, R^2=H, R^3=(3*R*)-quinuclidin-3-yl
d R^1=H, R^2=H, R^3=(1*R*,5*R*)-8-methyl-8-azabicyclo[3.2.1]octan-3-yl
e R^1=H, R^2=H, R^3=1-methyl-4-piperidyl
f R^1=H, R^2=H, R^3=(3*R*)-1-methylpyrrolidin-3-yl
g R^1=H, R^2=H, R^3=[(3*R*)-quinuclidin-3-yl]methy
h R^1=H, R^2=H, R^3=(1-methyl-4-piperidyl)methyl
i R^1=H, R^2=H, R^3=[(2*R*)-1-methylazetidin-2-yl]methyl
j R^1=H, R^2=H, R^3=2-(dimethylamino)ethyl

[a]Reagents and conditions: (a) DCC, HOBt, THF, rt, 18 h; (b) for the preparation of **85a,b**: 2 N HCl in diethylether, 1,4-dioxane, rt, 20 h; for the preparation of **85c−j**: 4 N HCl in 1,4-dioxane, 1,4-dioxane, rt, 20 h; (c) **42b** or **43b** or **48**, CH_3CN, AcOH, rt, 20 h then $NaBH(OAc)_3$, rt, 18 h.

Scheme 8. Preparation of Single Diastereoisomers 89, 90, 91a−j and 92a−j



86a (L=phenyl), 88a-j (L=thiophene)

89 fast diastereoisomer
90 slow diastereoisomer

91a-j fast diastereoisomer
92a-j slow diastereoisomer

a R^1=H, R^2=H, R^3=(3*R*)-quinuclidin-3-yl
b R^1=H, R^2=F, R^3=(3*R*)-quinuclidin-3-yl
c R^1 = Me, R^2=H, R^3=(3*R*)-quinuclidin-3-yl
d R^1=H, R^2=H, R^3=(1*R*,5*R*)-8-methyl-8-azabicyclo[3.2.1]octan-3-yl
e R^1=H, R^2=H, R^3=1-methyl-4-piperidyl
f R^1=H, R^2=H, R^3=(3*R*)-1-methylpyrrolidin-3-yl
g R^1=H, R^2=H, R^3=[(3*R*)-quinuclidin-3-yl]methy
h R^1=H, R^2=H, R^3=(1-methyl-4-piperidyl)methyl
i R^1=H, R^2=H, R^3=[(2*R*)-1-methylazetidin-2-yl]methyl
j R^1=H, R^2=H, R^3=2-(dimethylamino)ethyl

[a]Reagents and conditions: (a) Chiral SFC or chiral HPLC.

effect, meaning that a significant and sustained anti-inflammatory activity may not be achieved within the window of doses for bronchodilation. These data raised concerns not only for the effectiveness of the compound but also for its safety profile since the need of overdosing to reach a significant local PDE4 inhibition may determine the appearance of

https://doi.org/10.1021/acs.jmedchem.1c00204
J. Med. Chem. 2021, 64, 9100−9119

unwanted systemic side effects due to muscarinic antagonism. These considerations, coupled with the developability evaluation, which introduced questions about the chemical stability of the compound, caused that **92a** was not progressed any further, and the MAPI program was continued to address the key issues of compound **92a**; among them, the need of balancing the bronchodilator/anti-inflammatory profile, while minimizing side effects, proved to be particularly challenging to govern and quite unpredictable because a number of factors, including *in vitro* affinities on M3 and PDE4 targets and pharmacokinetic and physicochemical properties, contribute to *in vivo* efficacy. The knowledge gained during the investigation described in the present paper was the basis for the identification of MAPI compounds chemically stable and pharmacologically balanced, which will be subject of forthcoming publication.

EXPERIMENTAL SECTION

Animals. Male albino Dunkin-Hartley guinea pigs (450−550 g), male CD Sprague Dawley rats (220−250 g), and male Brown-Norway rats (150−200 g) were obtained from Charles River Laboratories Italia (Calco, Italy), while male Putorious Furo ferrets (0.9−1.5 kg) were obtained from Marshall Ferrets France. Animals housed in plastic cages (Tecniplast Gazzada, Varese, Italy) in air-conditioned rooms at 22 °C in a 12 h light/dark cycle. Food and water were available at libitum. All animals were acclimatized for at least 5 days before any experimental work began. All the experiments were carried out in accordance with the national and European legislation and approved by local ethical committees.

Chemistry. All reagents and starting materials were obtained from commercial suppliers and used without further purification unless otherwise stated. ^{1}H nuclear magnetic resonance (NMR) spectroscopy was carried out using a Bruker instrument operating at 400 MHz using the stated solvent at around room temperature unless otherwise stated. In all cases, NMR data were consistent with the proposed structures. Characteristic chemical shifts (δ) are given in parts per million using conventional abbreviations for designation of major peaks: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublets; dt, doublet of triplets; m, multiplet; br, broad. UV purity and [MH+] of compounds were assessed by LCMS analysis performed on a Waters 2795 Alliance HT HPLC with Waters 2996 Diode Array Detector coupled to a Micromass ZQ, single quadrupole mass spectrometer using a Phenomenex Luna C18 (2) column (5 μm, 100 × 4.6 mm plus guard cartridge) with a linear gradient of 5−95% acetonitrile/water (with 0.1% formic acid in each mobile phase) within 3.5 min and held at 95% for 2.0 min or using a Waters Xterra MS C18 column (5 μm, 100 × 4.6 mm plus guard cartridge) initially held at 5% acetonitrile/water (with 10 mM ammonium bicarbonate) for 0.5 min followed by a linear gradient of 5−95% within 3.5 min and then held at 95% for 1.5 min. Alternatively, UV purity and [MH+] of compounds were assessed by UPLC-MS analysis performed on a Waters UPLC H Class with a Diode Array Detector coupled to a Waters XEVO-TQS, triple quadrupole mass spectrometer with an electrospray ionization (ESI) source, using a Acquity UPLC CSH C18 1.7 μm 50 × 2.10 with mobile phase A consisting of ammonium formate buffer (25 mM at pH 3) and mobile phase B consisting of 0.1% formic acid in acetonitrile. The flow rate was 0.35 mL/min, and the gradient started from 20% to 80% of mobile phase B in 5.5 min followed by an isocratic step at 80% for 2 min. Preparative HPLC purification was performed by reversed-phase HPLC using a Waters Fraction Lynx preparative HPLC system (2525 pump, 2996/2998 UV/VIS detector, 2767 liquid handler) or an equivalent HPLC system such as a Gilson Trilution UV directed system. The Waters 2767 liquid handler acted as both an auto-sampler and fraction collector. The columns used for the preparative purification of the compounds were a Waters Sunfire OBD Phenomenex Luna Phenyl Hexyl or Waters Xbridge Phenyl at 10 μm 19 × 150 mm or Waters CSH Phenyl Hexyl, 19 × 150, 5 μm column. Appropriate focused gradients were selected based on acetonitrile and methanol solvent systems under either acidic or basic conditions. The modifiers used under acidic/basic conditions were formic acid or trifluoroacetic acid (0.1% v/v) and ammonium bicarbonate (10 mM), respectively. The purification was controlled by Waters Fractionlynx software through monitoring at 210−400 nm and triggered a threshold collection value at 260 nm and, when using the Fractionlynx, the presence of target molecular ion as observed under APi conditions. Collected fractions were analyzed by LCMS (Waters Acquity systems with Waters SQD). The diastereomeric separation of compounds was achieved either by chiral high-performance liquid chromatography (HPLC) using a Gilson Trilution preparative HPLC system (322 pump, 155 UV/VIS, GX281 liquid handler and fraction collector) or by supercritical luid chromatography (SFC) using a Waters Thar Prep100 preparative SFC system (P200 CO2 pump, 2545 modifier pump, 2998 UV/VIS detector, 2767 liquid handler with stacked injection module). The Waters 2767 liquid handler acted as both an auto-sampler and fraction collector. The column used for the preparative purification of the compounds was a Diacel Chiralpak IA/IB or an YMC Amylose-C at 5 μm 250 × 20−21.2 mm ID. Appropriate isocratic methods were selected based on methanol, ethanol, or isopropanol solvent systems under un-modified or basic/acidic conditions. The standard SFC method used was modifier, CO_2, 100 mL/min, 120 bar backpressure, and 40 °C column temperature. The standard HPLC method used was modifier, heptane, 5 mL/min, and room temperature. The modifier used under basic conditions was diethylamine (0.1% v/v). The modifier used under acidic conditions was either formic acid (0.1% v/v) or trifluoroacetic acid (0.1% v/v). Chiral analysis methods 1−10 are described in the Supporting Information.

All compounds submitted for biological screening had a purity >95%.

The preparation of intermediates **11**, **14**, **15**, **16**, **29**, **39a−d**, and **85a−j**, the analytical characterization of intermediates **19**, **21**, **23**, **31**, **42b**, **42e**, **43b**, **44e**, **45b**, **65−68**, and **50−55**, LCMS and SFC-MS traces for compounds **92e**, **92f**, **91a**, and **92a**, and the crystal structure determination of **92a** are reported in the Supporting Information.

Preparation of Final Compounds 24 and 25. *[(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)-ethyl] 3-Nitrobenzoate (**18**).* To a stirred solution of (1*S*)-2-(3,5-dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)-ethanol **11** (0.500 g, 1.45 mmol) and 3-nitrobenzoic acid (0.242 g, 1.45 mmol) in DCM (15.0 mL) was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (0.560 g, 2.91 mmol) and *N*,*N*-dimethylpyridin-4-amine (0.089 g, 0.73 mmol). The reaction was stirred at room temperature for 16 h. The reaction was quenched by addition of saturated aqueous sodium bicarbonate solution (25.0 mL) and extracted with DCM (2 × 60 mL). The combined organic extracts were dried on magnesium sulfate and filtered, and the solvent was removed *in vacuo* to afford a yellow oil. The crude material was purified by chromatography on silica gel, eluting sequentially with isohexane, ethyl acetate, and 10% methanol in ethyl acetate to afford the title compound as a yellow solid (0.642 g, 90% yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.89 (d, J = 2.0 Hz, 1 H), 8.44−8.41 (m, 1 H), 8.33−8.30 (m, 1 H), 8.14 (s, 2 H), 7.63 (t, J = 8.0 Hz, 1 H), 7.06−7.03 (m, 2 H), 6.88 (d, J = 8.4 Hz, 1 H), 6.30 (dd, J = 9.6, 4.4 Hz, 1 H), 3.92 (s, 3 H), 3.90 (s, 3 H), 3.78 (dd, J = 9.6, 14.0 Hz, 1 H), 3.38 (dd, J = 14.0, 4.4 Hz, 1 H). LCMS: [MH+] = 493.

*[(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 3-Aminobenzoate (**20**).* To a stirred solution of [(1*S*)-2-(3,5-dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 3-nitrobenzoate **18** (0.640 g, 1.30 mmol) in anhydrous THF (30.0 mL) was added tin chloride dihydrate (1.18 g, 5.2 mmol). After heating at 75 °C for 16 h, the reaction was allowed to cool to room temperature. The reaction was partitioned between ethyl acetate and saturated aqueous sodium bicarbonate solution and extracted with ethyl acetate (2 × 60 mL). The combined organic extracts were dried on magnesium sulfate and filtered, and the solvent was removed *in vacuo* to afford the title compound as a brown solid (0.520 g, 87% yield).

https://doi.org/10.1021/acs.jmedchem.1c00204
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^{1}H NMR (400 MHz, DMSO-d_6): δ 8.58 (s, 2 H), 7.20−7.15 (m, 3 H), 7.04−6.98 (m, 3 H), 6.83−6.81 (m, 1 H), 6.22−6.19 (dd, J = 4.4, 9.6 Hz, 1 H), 5.39 (s, 2 H), 3.82 (s, 3 H), 3.78 (s, 3 H), 3.63−3.59 (dd, J = 9.6, 14.0 Hz, 1 H), 3.33−3.29 (m, 1 H). LCMS: [MH+] = 463.

*2-[3-[(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethoxy]carbonylanilino]-2-phenylacetic Acid (**22**).* To a stirred solution of [(1*S*)-2-(3,5-dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 3-aminobenzoate **20** (0.850 g, 1.83 mmol) in DCM (20 mL) was added phenylboronic acid (0.225 g, 1.83 mmol) and glyoxylic acid (205 μL, 1.83 mmol). The reaction was stirred at room temperature for 16 h. The reaction was quenched by addition of water and extracted with DCM (2 × 60 mL). The combined organic extracts were dried on magnesium sulfate and filtered, and the solvent was removed *in vacuo*. A small sample of crude residue (90 mg) was purified by preparative HPLC to provide the title compound as a white solid (33.6 mg). The remaining amount of crude residue (0.940 g) was taken on to the next step without further purification.

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.54*^{or†} (s, 2 H), 8.53*^{or†} (s, 2H), 7.51−7.49 (m, 2 H), 7.39−7.35 (m, 2 H), 7.32−7.30 (m, 1 H), 7.26−7.24 (m, 1 H), 7.18−7.16 (m, 2 H), 7.01−6.92 (m, 4 H), 6.68−6.51 (brs, 1 H), 6.18−6.14 (m, 1 H), 5.08 (s, 1H), 3.59 (s, 3 H), 3.58 (s, 3 H), 3.56−3.51 (m, 1 H), 3.32−3.27 (m , 1 H). OH not observed. † and * refer to different isomers. LCMS: [MH+] = 597.

*[(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 3-[[2-Oxo-1-phenyl-2-[(3R)-quinuclidin-3-yl]oxyethyl]amino]benzoate (**24**).* To a stirred solution of previously obtained crude **22** (0.940 g, 1.57 mmol) in anhydrous THF (30.0 mL) was added (*R*)-quinuclidin-3-ol (0.300 g, 2.35 mmol), *N*,*N*′-dicyclohexylcarbodiimide (0.375 g, 1.80 mmol), and 1-hydroxybenzotriazole (0.245 g, 1.80 mmol). The reaction was stirred at room temperature under nitrogen for 16 h. The reaction was filtered through fine sintered glass, and the filtrate was removed *in vacuo*. The residue was partitioned between ethyl acetate and saturated aqueous sodium bicarbonate solution and extracted with ethyl acetate (2 × 50 mL). The combined organic layers were dried on magnesium sulfate and filtered, and the solvent was removed *in vacuo*. The residue was purified by preparative HPLC to afford the title compound **24** as a white solid (118 mg, 9% yield over two steps).

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.61*^{or†} (s, 2 H), 8.57*^{or†} (s, 2 H), 7.57 (d, J = 7.5 Hz, 2 H), 7.47−7.31 (m, 4 H), 7.28−7.20 (m, 2 H), 7.06−6.97 (m, 4 H), 6.78−6.71 (m, 1 H), 6.21 (dd, J = 9.4, 4.7 Hz, 1 H), 5.37−5.29 (m, 1 H), 4.80−4.71 (m, 1 H), 3.83−3.76 (m, 6 H), 3.65−3.56 (m, 1 H), 3.18−3.10 (m, 1 H), 3.07−2.96 (m, 1 H), 2.68−2.57 (m, 3 H), 2.32−2.22 (m, 1 H), 2.11 (d, J = 14.5 Hz, 1 H), 1.93 (s, 1 H), 1.68−1.60 (m, 1 H), 1.60−1.54 (m, 1 H), 1.53−1.43 (m, 1 H), 1.34−1.17 (m, 1 H). † and * refer to different isomers. LCMS: [MH+] = 706.

*[(1S)-1-[3-(Cyclopropylmethoxy)-4-(difluoromethoxy)phenyl]-2-(3,5-dichloro-1-oxido-pyridin-1-ium-4-yl)ethyl] 3-[[2-Oxo-1-phenyl-2-[(3R)-quinuclidin-3-yl]oxyethyl]amino]benzoate Trifluoroacetate Salt (**25**).* Compound **25** was synthesized following the same procedure described for the preparation of **24** using enantiomerically pure alcohol **12** instead of **11** via intermediates **19**, **21**, and **23**.

^{1}H NMR (300 MHz, DMSO-d_6): δ 9.36 and 9.50 (br. s, 1 H), 8.54 (s, 2 H), 7.49−7.68 (m, 3 H), 7.12−7.49 (m, 6 H), 6.90−7.12 (m, 3 H), 7.07 (t, 1 H), 6.75 (m, 1 H), 6.19 (dd, 1 H), 5.35 and 5.40 (d, 1 H), 4.95−5.13 (m, 1 H), 3.92 and 3.93 (d, 2H), 3.48−3.77 (m, 2 H), 3.01−3.29 (m, 4 H), 2.67−2.80 (m, 1 H), 2.54−2.61 (m, 1 H), 1.94−2.08 and 2.20−2.26 (m, 1 H), 1.61−1.93 (m, 3 H), 1.36−1.57 (m, 1 H), 1.08−1.36 (m, 1 H), 0.45−0.70 (m, 2 H), 0.25.0.45 (m, 2 H). LCMS [MH+] = 782.

Preparation of Final Compounds 32 and 33. *[(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)-ethyl] 3-[(2-tert-Butoxy-2-oxo-1-phenyl-ethyl)sulfamoyl]benzoate (**30**).* 3-[(2-*tert*-Butoxy-2-oxo-1-phenyl-ethyl)sulfamoyl]benzoic acid **29** (1.216 g, 3.11 mmol) was added to a stirred suspension of (1*S*)-2-(3,5-dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)-ethanol **11** (1.07 g, 3.11 mmol) in DMF (12 mL). To the resultant solution was added DMAP (0.189 g, 1.55 mmol) followed by EDC hydrochloride (1.192 g, 6.22 mmol), and the reaction was stirred at room temperature for 18 h. After this time, the reaction mixture was concentrated under reduced pressure and water (30 mL) was added; the resulting off-white precipitate was filtered and dried in air. The crude material was purified by chromatography on silica gel, eluting with 0−100% EtOAc in isohexane to give the title compound as a white solid (1.34 g, 60%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.33 (dt, J = 7.9, 1.6 Hz, 1 H), 8.18 (s, 1 H), 8.16 (s, 1 H), 8.19−8.05 (m, 1 H), 7.90−7.82 (m, 1 H), 7.43 (q, J = 7.6 Hz, 1 H), 7.21−7.08 (m, 5 H), 7.07−6.98 (m, 2 H), 6.88 (d, J = 8.3 Hz, 1 H), 6.26 (dd, J = 9.5, 4.8 Hz, 1 H), 6.07 (dd, J = 33.2, 7.9 Hz, 1 H), 4.96 (t, J = 7.8 Hz, 1 H), 3.93 (s, 2H), 3.92 (s, 1 H), 3.89 (s, 3 H), 3.8−3.69 (m, 1 H), 3.39 (dt, J = 14.0, 4.2 Hz, 1 H), 1.22 (s, 6 H), 1.21 (s, 3 H). LCMS: [MH+] = 717.

*[(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 3-[[2-Oxo-1-phenyl-2-[(3R)-quinuclidin-3-yl]oxyethyl]sulfamoyl]benzoate (**32**).* Trifluoroacetic acid (5.0 mL) was added to a stirred solution of [(1*S*)-2-(3,5-dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl) ethyl] 3-[(2-*tert*-butoxy-2-oxo-1-phenyl-ethyl)sulfamoyl]benzoate **30** (1.0 g, 1.39 mmol) in DCM (5.0 mL) at 0 °C, and stirring was maintained at 0 °C for 2.5 h. The reaction mixture was then stirred at ambient temperature for 30 min, after which time toluene (50 mL) was added and the solvent removed *in vacuo* to yield a yellow solid. The crude intermediate 2-[[3-[(1*S*)-2-(3,5-dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethoxy]-carbonylphenyl]sulfonylamino]-2-phenylacetic acid was dissolved in THF (20 mL), and *N*,*N*′-dicyclohexylcarbodiimide (431 mg, 2.09 mmol), 1-hydroxybenzotriazole hydrate (282 mg, 2.09 mmol), and (*R*)-quinuclidin-3-ol (355 mg, 2.79 mmol) were sequentially added. The resulting reaction mixture was stirred at ambient temperature for 18 h, after which time the reaction mixture was filtered through a pad of Celite, washing with THF (10 mL). The filtrate was concentrated *in vacuo*, the resulting crude was partitioned between ethyl acetate (20 mL) and water (10 mL), the organic phases were dried over magnesium sulfate, filtered, and concentrated *in vacuo*. The resulting crude was triturated in diethyl ether and purified by preparative HPLC to afford the title compound as a pale yellow solid (17.9 mg, 2%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.30 (s, 1 H), 8.16 (s, 2 H), 8.06 (d, J = 7.4 Hz, 1 H), 7.82 (d, J = 8.0 Hz, 1 H), 7.42 (t, J = 8.0 Hz, 1 H), 7.20−7.08 (m, 5 H), 7.07−6.98 (m, 2 H), 6.88 (d, J = 8.5 Hz, 1 H), 6.26 (dd, J = 9.6, 4.8 Hz, 1 H), 5.10 (s, 1 H), 4.72−4.62 (m, 1 H), 3.94 (s, 3H), 3.89 (s, 3 H), 3.73 (dd, J = 14.0, 9.6 Hz, 1 H), 3.39 (dd, J = 14.0, 4.7 Hz, 1 H), 2.99 (ddd, J = 13.0, 8.1, 2.0 Hz, 1 H), 2.74−2.53 (m, 3 H), 2.48−2.35 (m, 1 H), 2.23 (d, J = 14.8 Hz, 1 H), 1.90−1.81 (m, 1 H) 1.76−1.2 (m, 4 H). LCMS: [MH+] = 770.

*[(1S)-1-[3-(Cyclopropylmethoxy)-4-(difluoromethoxy)phenyl]-2-(3,5-dichloro-1-oxido-pyridin-1-ium-4-yl)ethyl] 3-[[2-Oxo-1-phenyl-2-[(3R)-quinuclidin-3-yl]oxyethyl]sulfamoyl]benzoate (**33**).* Compound **33** was synthesized following the same procedure described for the preparation of **32** using enantiomerically pure alcohol **12** instead of **11** via intermediate **31**.

^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 9.52 and 9.59 (br. s., 1 H), 9.14 (d, 1 H), 8.55 and 8.57 (s, 2 H), 8.23 (t,1 H), 8.07 and 8.14 (dt, 1 H), 7.93 and 8.01 (dt, 1 H), 7.58 and 7.66 (t, 1 H), 7.09−7.32 (m, 8 H), 7.08 (t, 1 H), 6.10−6.30 (m, 1 H), 5.15 and 5.19 (d, 1 H), 4.81−4.99 (m, 1 H), 3.95 (d, 2 H), 3.46−3.75 (m, 2 H), 3.30−3.45 (m, 1 H), 2.74−3.27 (m, 5 H), 1.89−1.98 and 1.98−2.10 (m, 1 H), 0.99−1.87 (m, 5 H), 0.47−0.66 (m, 2 H), 0.30−0.42 (m, 2 H). MS/ESI+ [MH+] = 846.

Preparation of Final Compounds 46a−d. *[(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)-ethyl] 4-[(N-[(3R)-Quinuclidin-3-yl]oxycarbonylanilino)methyl]-benzoate (**46a**).* To a stirred solution of methyl 4-[(*N*-[(3*R*)-quinuclidin-3-yl]oxycarbonylanilino)methyl]benzoate **39a** (0.451 g, 1.144 mmol) in THF (5.8 mL) and methanol (5.8 mL) was added a solution of lithium hydroxide monohydrate (0.096 g, 2.29 mmol) in water (2.3 mL). The reaction was stirred rapidly at room temperature for 18 h. The mixture was cooled using an ice bath and acidified by dropwise addition of concentrated hydrochloric acid (0.46 mL, 5.52

https://doi.org/10.1021/acs.jmedchem.1c00204
J. Med. Chem. 2021, 64, 9100−9119

mmol). The mixture was allowed to warm to room temperature, and then the solvent was removed *in vacuo* (3 × toluene azeotrope followed by 2 × acetonitrile azeotrope), and the residue was dried in the vacuum oven at 40 °C to afford a pale yellow solid. The crude 4-[(*N*-[(3*R*)-quinuclidin-3-yl]oxycarbonylanilino)methyl]benzoic acid **40a** was dissolved in DMF (5 mL) and added to a stirred suspension of (1*S*)-2-(3,5-dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethanol **11** (0.394 g, 1.144 mmol) in DMF (12 mL). To the resultant solution was added DMAP (0.070 g, 0.572 mmol) followed by EDC hydrochloride (0.439 g, 2.288 mmol), and the reaction was stirred at room temperature for 18 h. The majority of DMF was removed *in vacuo*, and the residue was partitioned between ethyl acetate and saturated aqueous sodium bicarbonate solution. The organic layer was washed with brine, and the solvent was removed *in vacuo* to afford an off-white solid. The crude material was partially purified using an SCX-2 cartridge eluting sequentially with 1:1:1 methanol:acetonitrile:water and then methanol and 2.3 M methanolic ammonia. Final purification was achieved by preparative HPLC to afford the title compound as a pale yellow solid (0.240 g, 30%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.13 (s, 2 H), 7.97 (d, J = 8.0 Hz, 2 H), 7.35−7.21 (m, 5 H), 7.10 (br s, 2 H), 7.04−6.95 (m, 2 H), 6.85 (d, J = 8.2 Hz, 1 H), 6.30 (dd, J = 9.7, 4.6 Hz, 1 H), 4.95−4.89 (m, 1 H), 4.89 (s, 2 H), 3.90 (s, 3 H), 3.88 (s, 3 H), 3.71 (dd, J = 14.0, 9.7 Hz, 1 H), 3.40−3.28 (m, 2 H), 2.90 (t, J = 9.1 Hz, 2 H), 2.81 (d, J = 18.1 Hz, 2 H), 2.73−2.59 (m, 1 H), 2.16−2.10 (m, 1 H), 1.84−1.73 (m, 1 H), 1.72−1.60 (m, 1 H), 1.55−1.45 (m, 1 H), 1.44−1.33 (m, 1 H). LCMS: [MH+] = 706.

Compounds **46b−d** were synthesized following the same procedure described for the preparation of compound **46a**, using intermediates **39b−d**.

*[(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 3-[(N-[(3R)-Quinuclidin-3-yl]-oxycarbonylanilino)methyl]benzoate (**46b**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11 (s, 2 H), 7.93−7.91 (m, 2 H), 7.48−7.46 (m, 1 H), 7.40−7.36 (m, 1 H), 7.32−7.22 (m, 3 H), 7.14−7.12 (m, 2 H), 7.00−6.96 (m, 2 H), 6.86−6.84 (m, 1 H), 6.27 (dd, J = 9.6, 4.4 Hz, 1 H), 4.89 (s, 2 H), 4.82−4.81 (m, 1 H), 3.89 (s, 3 H), 3.87 (s, 3 H), 3.68 (dd, J = 14.0, 9.6 Hz, 1 H), 3.33 (dd, J = 14.0, 4.4 Hz, 1 H), 3.22−3.18 (m, 1H), 2.76−2.62 (m, 5 H), 1.97−1.96 (m, 1 H), 1.65−1.62 (m, 1 H), 1.55−1.54 (m, 1 H), 1.40−1.39 (m, 1 H), 1.25−1.21 (m, 1 H). LCMS: [MH+] = 706

*[(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 2-[4-[(N-[(3R)-Quinuclidin-3-yl]-oxycarbonylanilino)methyl]phenyl]acetate (**46c**).* ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.54 (s, 2 H), 8.31 (s, 1 H), 7.34−7.30 (m, 2 H), 7.26−7.21 (m, 2 H), 7.20−7.12 (m, 4 H), 6.93−6.90 (m, 1 H), 6.83−6.81 (m, 2 H), 5.95 (dd, J = 9.6, 4.8 Hz, 1 H), 4.87 (s, 2 H), 4.67−4.64 (m, 1 H), 3.74 (s, 3 H), 3.70 (s, 3 H), 3.64−3.54 (m, 2 H),3.49−3.35 (m, 2 H), 3.11−3.05 (m, 1 H), 2.67−2.47 (m, 5 H), 1.88−1.83 (m, 1 H), 1.58−1.52 (m, 1 H), 1.50−1.39 (m, 1 H), 1.37−1.22 (m, 1 H), 1.19−1.12 (m, 1 H). LCMS: [MH+] = 720

*[(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 2-Fluoro-4-[(N-[(3R)-quinuclidin-3-yl]-oxycarbonylanilino)methyl]benzoate (**46d**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.13 (s, 2 H), 7.85 (t, J = 7.7 Hz, 1 H), 7.33 (t, J = 7.6 Hz, 2 H), 7.28−6.93 (m, 6 H), 6.84 (d, J = 8.3 Hz, 1 H), 6.33 (dd, J = 9.1, 5.1 Hz, 1 H), 4.94−4.88 (m, 1 H), 4.86 (d, J = 6.8 Hz, 2 H), 3.91 (s, 3 H), 3.88 (s, 3 H), 3.67 (dd, J = 13.9, 9.1 Hz, 1 H), 3.35 (dd, J = 13.9, 5.0 Hz, 1 H), 3.30 (ddd, J = 14.8, 8.2, 2.2 Hz, 1 H), 2.87 (t, J = 7.8 Hz, 3 H), 2.78 (d, J = 14.8 Hz, 1 H), 2.71−2.62 (m, 1 H), 2.13−2.08 (m, 1 H), 2.05−1.71 (m, 2 H), 1.69−1.58 (m, 1 H), 1.54−1.44 (m, 1 H), 1.44−1.33 (m, 1 H). LCMS: [MH+] = 724.

Preparation of Intermediates 42b, 42e, 43b, and 48. *[(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 5-Formylthiophene-2-carboxylate (**48**).* To a stirred solution of 5-formyl-2-thiophenecarboxylic acid (400 mg, 2.56 mmol) in DCM (20 mL) was added (1*S*)-2-(3,5-dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethanol **11** (881 mg, 2.56 mmol) followed by DMAP (156 mg, 1.28 mmol) and EDC hydrochloride (983 mg, 5.12 mmol). The resulting mixture was stirred at room temperature for 18 h. The reaction was partitioned between DCM and saturated aqueous sodium bicarbonate solution. The organic layer was washed with brine and passed through a hydrophobic frit, and the solvent was removed *in vacuo*. The crude material was purified by chromatography on silica gel, eluting with 0−100% EtOAc in DCM, to afford the title compound (488 mg, 39%) as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ 9.97 (s, 1 H), 8.15 (s, 2 H), 7.81 (d, J = 3.6 Hz, 1 H), 7.72 (d, J = 3.6 Hz, 1 H), 7.03−6.99 (m, 2 H), 6.87 (d, J = 8.7 Hz, 1 H), 6.26 (dd, J = 4.4, 10.0 Hz, 1 H), 3.91 (s, 3 H), 3.88 (s, 3 H), 3.72 (dd, J = 10.0, 14.0 Hz, 1 H), 3.33 (dd, J = 4.4, 14.0 Hz, 1 H). LCMS: [MH+] = 482.

Intermediates **42b**, **42e**, and **43b** were synthesized following the same procedure of **48** using the suitable formyl acid.

Preparation of Intermediates 44e, 45b, 49, 50−55, and 65−68. *[(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 5-[(2-Fluoroanilino)methyl]thiophene-2-carboxylate (**49**).* To a stirred solution of [(1*S*)-2-(3,5-dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 5-formylthiophene-2-carboxylate **48** (128 mg, 0.265 mmol) in DCM (5 mL) was added 2-fluoroaniline (29 mg, 0.265 mmol) followed by glacial acetic acid (0.015 mL, 0.265 mmol). The reaction was stirred at room temperature for 6 h. Sodium triacetoxyborohydride (140 mg, 0.662 mmol) was added, and the reaction was stirred at room temperature for 18 h. Water was added to quench the reaction, and the organic layer was washed with brine and passed through a hydrophobic frit, and the solvent was removed *in vacuo*. The crude material was purified by chromatography on silica gel, eluting with 0−100% EtOAc in DCM, to afford the title compound (50 mg, 32%) as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11 (s, 2 H), 7.65 (d, J = 4.0 Hz, 1 H), 7.02−6.95 (m, 5 H), 6.84 (d, J = 8.0 Hz, 1 H), 6.71−6.65 (m, 2 H), 6.18 (dd, J = 4.4, 9.6 Hz, 1 H), 4.57−4.56 (m, 2 H), 4.51−4.48 (m, 1 H), 3.87 (s, 3 H), 3.86 (s, 3 H), 3.64 (dd, J = 9.6, 14.0 Hz, 1 H), 3.29 (dd, J = 4.4, 14.0 Hz, 1 H). LCMS: [MH+] = 577.

Intermediates **44e**, **45b**, and **50−55** were synthesized following the same procedure of intermediate **49** from intermediate **48** using suitable anilines.

Intermediates **65−68** were synthesized in two steps reacting alcohols **12**, **14**, **16**, **18**, and 5-formyl-2-thiophenecarboxylic acid, as described for intermediate **48** and then the formyl derivatives obtained with 2-fluoroaniline, as described for intermediate **49**.

Preparation of Final Compounds 46e, 47b, 57, 69−72, 58−63, and 64. *[(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 5-[(2-Fluoro-N-[(3R)-quinuclidin-3-yl]-oxycarbonylanilino)methyl]thiophene-2-carboxylate Formate Salt (**57**).* A microwave tube was charged with [(1*S*)-2-(3,5-dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 5-[(2-fluoroanilino)methyl]thiophene-2-carboxylate **49** (50 mg, 0.09 mmol), [(3*R*)-quinuclidin-3-yl] carbonochloridate hydrochloride (81 mg, 0.36 mmol), and anhydrous acetonitrile (0.8 mL). The mixture was heated at 80 °C for 3 min under microwave irradiation. The reaction was evaporated to dryness. The residue was dissolved in DMSO (1.5 mL) and purified by preparative HPLC to afford the tittle compound as a white solid (26 mg, 41%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.40 (s, 1 H), 8.15 (s, 2 H), 7.59 (d, J = 3.6 Hz, 1 H), 7.32−7.28 (m, 1 H), 7.15−7.11 (m, 3 H), 6.99−6.96 (m, 2 H), 6.86−6.84 (m, 2 H), 6.20 (dd, J = 4.8, 10.0 Hz, 1 H), 4.95−4.93 (m, 3 H), 3.90 (s, 3 H), 3.88 (s, 3 H), 3.66 (dd, J = 10.0, 14.0 Hz, 1 H), 3.38−3.28 (m, 2 H), 2.97−2.91 (m, 3 H), 2.83−2.61 (m, 2 H), 2.18−2.16 (m, 1 H), 1.84−1.79 (m, 1 H), 1.78−1.70 (m, 1 H), 1.50−1.37 (m, 2 H). LCMS: [MH+] = 730.

Compounds **46e**, **47b**, **69−72**, and **58−63** were synthesized following the same procedure using the intermediates **44e**, **45b**, **65−68**, and **50−55**.

*[(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 4-Fluoro-3-[(N-[(3R)-quinuclidin-3-yl]-oxycarbonylanilino)methyl]benzoate (**46e**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.10−8.06 (m, 3 H), 7.95−7.91 (m, 1 H), 7.33−7.29 (m, 2 H), 7.24−7.18 (m, 3 H), 7.05 (t, J = 8.9 Hz, 1 H), 6.99−6.95 (m, 2 H), 6.85 (d, J = 8.0 Hz, 1 H), 6.25 (dd, J = 4.4, 9.6 Hz, 1 H), 4.97 (s, 2 H), 4.79−4.77 (m, 1 H), 3.88 (s, 3 H), 3.87 (s, 3 H), 3.66 (dd, J = 9.6, 14.0 Hz, 1 H), 3.33 (dd, J = 4.4, 14.0 Hz, 1 H), 3.17−3.15 (m, 1

https://doi.org/10.1021/acs.jmedchem.1c00204
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H), 2.72−2.59 (m, 5 H), 2.00−1.92 (m, 1 H), 1.60−1.57 (m, 1 H), 1.52−1.47 (m, 1 H), 1.42−1.31 (m, 1 H), 1.24−1.17 (m, 1 H). LCMS: [MH+] = 724

*[(1S)-1-[3-(Cyclopropylmethoxy)-4-(difluoromethoxy)phenyl]-2-(3,5-dichloro-1-oxido-pyridin-1-ium-4-yl)ethyl] 3-[(N-[(3R)-Quinuclidin-3-yl]oxycarbonylanilino)methyl]benzoate (**47b**).* ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.51 (s, 2 H), 7.81−7.94 (m, 2 H), 7.54 (dt, 1 H), 7.48 (t, 1 H), 7.30−7.44 (m, 2 H), 7.17−7.30 (m, 5 H), 7.05 (dd, 1 H), 7.06 (t, 1 H), 6.21 (dd, 1 H), 4.88−5.04 (m, 2 H), 4.63−4.82 (m, 1 H), 3.92 (d, 2 H), 3.59 (dd, 1 H), 3.31−3.40 (m, 1 H), 3.08−3.26 (m, 1 H), 2.56−2.85 (m, 5 H), 1.77−1.99 (m, 1 H), 1.42−1.75 (m, 2 H), 1.10−1.42 (m, 3 H), 0.44−0.68 (m, 2 H), 0.22−0.44 (m, 2 H). MS/ESI+ [MH+] = 782.

*[(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-[4-(difluoromethoxy)-3-methoxyphenyl]ethyl] 5-[(2-Fluoro-N-[(3R)-quinuclidin-3-yl]oxycarbonylanilino)methyl]thiophene-2-carboxylate Formate Salt (**69**).* ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.55 (s, 2 H), 8.20 (s, 1 H), 7.66 (d, J = 3.6 Hz, 1 H), 7.39−7.30 (m, 2 H), 7.28−7.20 (m, 4 H), 7.07−6.88 (m, 3 H), 6.15 (dd, J = 4.0, 9.2 Hz, 1 H), 5.05−4.96 (m, 2 H), 4.71−4.67 (m, 1 H), 3.85 (s, 3 H), 3.60−3.54 (m, 1 H), 3.35−3.30 (m, 1 H), 3.16−3.13 (m, 1 H), 2.81−2.61 (m, 4 H), 2.50−2.35 (m, 1 H), 1.98−1.86 (m, 1 H), 1.67−1.40 (m, 2 H), 1.24−1.15 (m, 2 H). LCMS: [MH+] = 766.

*[(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-[4-(difluoromethoxy)-3-ethoxyphenyl]ethyl] 5-[(2-Fluoro-N-[(3R)-quinuclidin-3-yl]oxycarbonylanilino)methyl]thiophene-2-carboxylate Formate Salt (**70**).* ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.53 (s, 2 H), 8.20 (s, 1 H), 7.66 (d, J = 3.6 Hz, 1 H), 7.38−7.33 (m, 2 H), 7.27−7.18 (m, 4 H), 7.05−6.98 (m, 3 H), 6.13 (dd, J = 4.0, 9.2 Hz, 1 H), 5.04−4.97 (m, 2 H), 4.72−4.68 (m, 1 H), 4.15−4.06 (m, 2 H), 3.59−3.52 (m, 1 H), 3.32 (dd, J = 4.0, 14.2 Hz, 1 H), 3.15−3.12 (m, 1 H), 2.76−2.63 (m, 4 H), 2.49−2.42 (m, 1 H), 1.89−1.73 (m, 1 H), 1.65−1.42 (m, 2 H), 1.32 (t, J = 7.0 Hz, 3 H), 1.24−1.09 (m, 2 H). LCMS: [MH+] = 780

*[(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-[4-(difluoromethoxy)-3-isopropoxyphenyl]ethyl] 5-[(2-Fluoro-N-[(3R)-quinuclidin-3-yl]oxycarbonylanilino)methyl]thiophene-2-carboxylate Trifluoroacetate Salt (**71**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.22 (s, 2 H), 7.60 (d, J = 3.8 Hz, 1 H), 7.38−7.30 (m, 1 H), 7.19−7.10 (m, 4 H), 7.05−6.98 (m, 2 H), 6.88 (d, J = 3.8 Hz, 1 H), 6.56 (t, J = 75.3 Hz, 1 H), 6.19 (dd, J = 9.9, 4.4 Hz, 1 H), 5.14−5.06 (m, 1 H), 4.97−4.89 (m, 2 H), 4.57 (h, J = 5.96 Hz, 1 H), 3.66 (dd, J = 14.1, 10.0 Hz, 1 H), 3.62−3.54 (m, 1 H), 3.32 (dd, J = 14.5, 4.6 Hz, 1 H), 3.28−3.14 (m, 3 H), 3.10 (d, J = 15.2 Hz, 1 H), 2.96−2.89 (m, 1 H), 2.39−2.31 (m, 1 H), 2.02−1.93 (m, 1 H), 1.91−1.81 (m, 1 H), 1.71−1.58 (m, 2 H), 1.36 (dd, J = 11.4, 6.0 Hz, 6 H). LCMS: [MH+] = 794

*[(1S)-1-[3-(Cyclopropylmethoxy)-4-(difluoromethoxy)phenyl]-2-(3,5-dichloro-1-oxido-pyridin-1-ium-4-yl)ethyl] 5-[(2-Fluoro-N-[(3R)-quinuclidin-3-yl]oxycarbonylanilino)methyl]thiophene-2-carboxylate Formate Salt (**72**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.43 (s, 1 H), 8.16 (s, 2 H), 7.60 (d, J = 3.8 Hz, 1 H), 7.34−7.27 (m, 1 H), 7.21−7.09 (m, 4 H), 7.06−7.00 (m, 2 H), 6.87 (d, J = 3.8 Hz, 1 H), 6.62 (t, J = 75.3 Hz, 1 H), 6.18 (dd, J = 10.0, 4.2 Hz, 1 H), 4.94 (m, 3 H), 3.89 (d, J = 6.9 Hz, 2 H), 3.64 (dd, J = 14.1, 10.1 Hz, 1 H), 3.36−3.23 (m, 3 H), 2.87 (m, 3 H), 2.76 (m, 1 H), 2.09 (s, 1 H), 1.82−1.55 (m, 2 H) 1.32−1.24 (m, 3 H), 0.68−0.62 (m, 2 H), 0.40−0.35 (m, 2 H). LCMS: [MH+] = 806

*[(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 5-[[3-Pyridyl-[(3R)-quinuclidin-3-yl]-oxycarbonylamino]methyl]thiophene-2-carboxylate (**58**).* ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.56 (s, 2 H), 8.50 (brs, 1 H), 8.44 (d, J = 4.4 Hz, 1 H), 7.72−7.70 (m, 1 H), 7.66−7.65 (m, 1 H), 7.44−7.41 (m, 1 H), 7.04−6.97 (m, 4 H), 6.12 (dd, J = 4.0, 9.6 Hz, 1 H), 5.11 (brs, 2 H), 4.707−4.688 (m, 1 H), 3.77 (s, 3 H), 3.75 (s, 3 H), 3.60−3.54 (dd, J = 9.6, 14.0 Hz, 1 H), 3.31−3.26 (m, 1 H), 3.13−3.07 (m, 1 H), 2.67−2.54 (m, 6 H), 1.81−1.79 (m, 1 H), 1.58−1.56 (m, 1 H), 1.55−1.50 (m, 1 H), 1.24−1.22 (m, 1 H). LCMS: [MH+] = 713

*[(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 5-[[2-Pyridyl-[(3R)-quinuclidin-3-yl]-oxycarbonylamino]methyl]thiophene-2-carboxylate Formate Salt (**59**).* ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.54 (s, 2 H), 8.45−8.44 (m, 1 H), 7.86−7.82 (m, 1 H), 7.75−7.73 (m, 1 H), 7.66 (d, J = 3.7 Hz, 1 H), 7.23−7.20 (m, 1 H), 7.10 (d, J = 3.7 Hz, 1 H), 6.99−6.96 (m, 3 H), 6.11 (dd, J = 4.1, 9.7 Hz, 1 H), 5.37−5.28 (m, 2 H), 4.78−4.76 (m, 1 H), 3.75 (s, 3 H), 3.74 (s, 3 H), 3.56 (dd, J = 9.7, 14.2 Hz, 1 H), 3.33−3.28 (m, 1 H), 3.18−3.13 (m, 1 H), 2.80−2.63 (m, 5 H), 1.96−1.95 (m, 1 H), 1.67−1.61 (m, 1 H), 1.59−1.51 (m, 2 H), 1.30−1.29 (m, 1 H). LCMS: [MH+] = 713.

*[(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 5-[(N-[(3R)-Quinuclidin-3-yl]-oxycarbonylanilino)methyl]thiophene-2-carboxylate Formate Salt (**60**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.42 (s, 1 H), 8.15 (s, 2 H), 7.60 (d, J = 3.8 Hz, 1 H), 7.39−7.32 (m, 2 H), 7.29 (d, J = 7.2 Hz, 1 H), 7.17−7.06 (m, 2 H), 7.00−6.95 (m, 2 H), 6.85 (dd, J = 5.9, 2.2 Hz, 2 H), 6.21 (dd, J = 9.8, 4.5 Hz, 1 H), 5.02−4.93 (m, 2 H), 4.94−4.87 (m, 1 H), 3.90 (s, 3 H), 3.88 (s, 3 H), 3.67 (dd, J = 14.0, 9.8 Hz, 1 H), 3.37−3.27 (m, 1 H), 3.31 (dd, J = 13.9, 4.9 Hz, 1 H), 2.92−2.84 (m, 3 H), 2.79 (d, J = 15.5 Hz, 1 H), 2.74−2.62 (m, 1H), 2.17−2.07 (m, 1 H), 1.82−1.72 (m, 1 H), 1.71−1.58 (m, 1 H), 1.56−1.31 (m, 2 H). LCMS: [MH+] = 712.

*[(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 5-[(3-Fluoro-N-[(3R)-quinuclidin-3-yl]-oxycarbonylanilino)methyl]thiophene-2-carboxylate (**61**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.15 (s, 2 H), 7.61 (d, J = 3.8 Hz, 1 H), 7.34 (td, J = 8.2, 6.2 Hz, 1 H), 7.05−6.94 (m, 3 H), 6.95−6.88 (m, 1 H), 6.91−6.80 (m, 3 H), 6.21 (dd, J = 9.8, 4.5 Hz, 1 H), 5.04−4.97 (m, 1 H), 4.98−4.91 (m, 2 H), 3.91 (s, 3 H), 3.88 (s, 3 H), 3.68 (dd, J = 14.0, 9.8 Hz, 1 H), 3.45−3.38 (m, 1 H), 3.32 (dd, J = 14.0, 4.6 Hz, 1 H), 3.08−2.88 (m, 3 H), 2.90−2.76 (m, 2 H), 2.29−2.21 (m, 1 H), 1.91−1.86 (m, 1 H), 1.82−1.69 (m, 1 H), 1.68−1.46 (m, 2 H). LCMS: [MH+] = 730.

*[(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 5-[[[(3R)-Quinuclidin-3-yl]oxycarbonyl-thiazol-2-yl-amino]methyl]thiophene-2-carboxylate (**62**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.13 (s, 2 H), 7.61 (d, J = 3.8 Hz, 1 H), 7.49 (d, J = 3.6 Hz, 1 H), 7.09 (d, J = 3.7 Hz, 1 H), 7.01 (d, J = 3.6 Hz, 1 H), 6.98−6.93 (m, 2 H), 6.84 (d, J = 8.1 Hz, 1 H), 6.18 (dd, J = 9.8, 4.5 Hz, 1 H), 5.52 (s, 2 H), 4.97 (s, 1 H), 3.88 (s, 3 H), 3.87 (s, 3 H), 3.64 (dd, J = 14.0, 9.8 Hz, 1 H), 3.33 (dd, J = 14.9, 8.6 Hz, 1 H), 3.29 (dd, J = 14.0, 4.3 Hz, 1 H), 2.92−2.73 (m, 5 H), 2.19−2.14 (m, 1 H), 1.80−1.69 (m, 1 H), 1.66−1.53 (m, 1 H), 1.52−1.40 (m, 1 H), 1.31−1.23 (m, 1 H). LCMS: [MH+] = 719.

*[(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 5-[[3-(Difluoromethyl)-N-[(3R)-quinuclidin-3-yl]oxycarbonylanilino]methyl]thiophene-2-carboxylate Formate Salt (**63**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.41 (s, 1 H), 8.15 (s, 2 H), 7.61 (d, J = 3.8 Hz, 1 H), 7.48−7.39 (m, 2 H), 7.33 (s, 1 H), 7.26 (s, 1 H), 7.01−6.95 (m, 2 H), 6.88−6.81 (m, 2 H), 6.64 (t, J = 56.3 Hz, 1 H), 6.21 (dd, J = 9.8, 4.5 Hz, 1 H), 5.02−4.90 (m, 3 H), 3.90 (s, 3 H), 3.88 (s, 3 H), 3.67 (dd, J = 14.5, 9.7 Hz, 1 H), 3.39−3.26 (m, 2 H), 2.99−2.71 (m, 5 H), 2.14 (s, 1 H), 1.82−1.76 (m, 1 H), 1.72−1.60 (m, 1 H), 1.63−1.34 (m, 2 H). LCMS: [MH+] = 762

*[(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 5-[(3-Hydroxy-N-[(3R)-quinuclidin-3-yl]-oxycarbonylanilino)methyl]thiophene-2-carboxylate (**64**).* To a solution of 3-aminophenol (0.67 g, 6.15 mmol) in hexamethyldisilazane (10 mL) was added a catalytic amount of concentrated sulfuric acid (0.05 mL), and the mixture was heated at reflux for 18 h. The mixture was cooled to room temperature, and the excess solvent was removed *in vacuo*. Trituration with diethyl ether gave a precipitate, which was filtered, and the filtrate was evaporated *in vacuo*. The crude was purified by chromatography on silica gel, eluting with 0−100% EtOAc in isohexane to give 3-((trimethylsilyl)oxy)aniline as a red-brown oil (782 mg, 70%, LCMS: [MH+] = 182). A mixture of 3-((trimethylsilyl)oxy)aniline (0.14 g, 0.77 mmol) and [(1*S*)-2-(3,5-dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 5-formylthiophene-2-carboxylate **48** (0.186 g, 0.39 mmol) in DCM (5 mL) was stirred at room temperature for 18 h. $NaBH(OAc)_3$ (0.206 g, 0.97 mmol) and acetic acid (0.043 mL, 0.75 mmol) were added, and the reaction mixture was stirred at room temperature for a further 3 h. The reaction mixture was diluted with DCM (10 mL) and

https://doi.org/10.1021/acs.jmedchem.1c00204
J. Med. Chem. 2021, 64, 9100−9119

washed with saturated aqueous $NaHCO_3$ solution (25 mL). The aqueous phase was further extracted with DCM (10 mL), and the combined organic phases were washed with brine (20 mL) and filtered through a phase separator cartridge, and the solvent was removed *in vacuo*, co-evaporated with MeCN to give the crude [(1*S*)-2-(3,5-dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 5-[(3-trimethylsilyloxyanilino)methyl]-thiophene-2-carboxylate (**56**) as a light brown gum. A mixture of the crude **56** (0.185 g, 0.29 mmol) and [(3*R*)-quinuclidin-3-yl] carbonochloridate (0.162 g, 0.72 mmol) in MeCN (5 mL) was heated in a microwave at 80° C for 6 min. Additional [(3*R*)-quinuclidin-3-yl] carbonochloridate (0.08 g, 0.35 mmol) was added and heated in the microwave at 80° C for a further 6 min. The solvent was removed *in vacuo*, and the residue was partitioned between EtOAc (20 mL) and water (20 mL). The separated aqueous phase was basified by the addition of saturated aqueous $NaHCO_3$ solution and extracted with EtOAc (2 × 20 mL). The organic extracts were combined and filtered through a phase separator, and the solvent was removed *in vacuo*. Purification by preparative HPLC gave the title compound as a white solid (0.02 g, 9.6%).

^{1}H NMR (400 MHz, DMSO-d_6): δ 9.53 (s, 1 H), 8.55 (s, 2 H), 7.66 (d, J = 3.78 Hz, 1 H), 7.15 (t, J = 8.00 Hz, 1 H), 7.03−6.96 (m, 4 H), 6.70−6.61 (m, 3 H), 6.14 (dd, J = 9.71, 4.26 Hz, 1 H), 5.00 (s, 2 H), 4.68−4.63 (m, 1 H), 3.76 (d, J = 6.84 Hz, 6 H), 3.58 (dd, J = 14.23, 9.79 Hz, 1 H), 3.54 (dd, J = 14.19, 9.83 Hz, 1 H), 3.25 (dd, J = 14.20, 4.22 Hz, 1 H), 3.10 (dd, J = 14.57, 7.97 Hz, 1 H), 2.69−2.55 (m, 4 H), 1.87 (s, 1 H), 1.62−1.42 (m, 3 H), 1.21 (s, 1 H). LCMS: [MH+] = 728.

Preparation of Final Compounds 86a, 87a, and 88a−j. *[(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)-ethyl] 3-[[[2-Oxo-1-phenyl-2-[(3R)-quinuclidin-3-yl]-oxyethyl]amino]methyl]benzoate (**86a**).* To a suspension of (3*R*)-quinuclidin-3-yl 2-amino-2-phenylacetate hydrochloride **85a** (0.200 g, 0.600 mmol) in EtOAc (5 mL) was added Et_3N (0.176 mL, 1.26 mmol). The reaction mixture was stirred at room temperature for 2 h. The precipitate obtained was filtered and washed with EtOAc (∼5 mL), and the solvent was removed *in vacuo*. This residue was dissolved in CH_3CN (4 mL), and to the solution was added [(1*S*)-2-(3,5-dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)-ethyl] 3-formylbenzoate **42b** (0.286 g, 0.6 mmol) followed by acetic acid (0.034 mL, 0.6 mmol). The reaction mixture was stirred at room temperature for 20 h. $NaBH(OAc)_3$ (0.318 g, 1.5 mmol) was added, and the reaction mixture was stirred at room temperature for a further 24 h. The excess solvent was removed *in vacuo*, and the residue was partitioned between EtOAc (70 mL) and saturated aqueous $NaHCO_3$ solution (15 mL). The organic layer was washed with saturated brine (2 × 15 mL), dried on magnesium sulfate, and filtered, and the solvent was removed *in vacuo*. Purification by preparative HPLC gave a yellow gum (0.158 g), which was dissolved in EtOAc (25 mL), washed with saturated aqueous $NaHCO_3$ solution (5 mL), and saturated brine (5 mL), dried on magnesium sulfate, and filtered. The solvent was removed *in vacuo*, and trituration with diethyl ether gave the title compound (1:1 mixture of diastereoisomers) as a yellow solid (126.9 mg, 29%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.12*^{or†} (s, 2 H), 8.11*^{or†} (s, 2 H), 8.00−7.97 (m, 1 H), 7.92 (d, J = 7.8 Hz, 1 H), 7.54−7.52 (m, 1 H), 7.42−7.30 (m, 6 H), 7.03−6.98 (m, 2 H), 6.85 (d, J = 8.2 Hz, 1 H), 6.30−6.28 (m, 1 H), 4.88−4.78 (m, 1 H), 4.40*^{or†} (s, 1 H), 4.38*^{or†} (s, 1 H), 3.89 (s, 3 H), 3.88 (s, 3 H), 3.78−3.71 (m, 3 H), 3.38−3.34 (m, 1 H), 3.25−3.10 (m, 1 H), 2.81−2.65 (m, 4 H), 2.60−2.35 (m, 1 H), 2.00−1.88 (m, 1 H), 1.75−1.45 (m, 3 H), 1.40−1.10 (m, 2 H). * and † refer to different isomers. LCMS: [MH+] =720.

Compounds **87a** and **88a−j** were synthesized following the same procedure described for the preparation of **86a** using intermediates **85a−j** and **43b** or **48.**

*[(1S)-1-[3-(Cyclopropylmethoxy)-4-(difluoromethoxy)phenyl]-2-(3,5-dichloro-1-oxido-pyridin-1-ium-4-yl)ethyl] 3-[[[2-Oxo-1-phenyl-2-[(3R)-quinuclidin-3-yl]oxyethyl]amino]methyl]benzoate Hydrobromide Salt (**87a**).* ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 9.44 and 9.55 (brs, 1 H), 8.54 (s, 2 H), 7.89−8.11 (m, 2 H), 7.37−7.73 (m, 8 H), 7.19−7.27 (m, 2 H), 7.10 (dd, 1 H), 7.07 (t, 1 H), 6.16−6.31 (m, 1 H), 4.98−5.19 (m, 1 H), 4.03 (br. s., 2 H), 3.93 (d, 2 H), 3.57−3.77 (m, 1 H), 3.63 (dd, 1 H), 2.78−3.30 (m, 6 H), 2.00−2.14 and 2.32−2.45 (m, 1 H), 1.42−1.95 (m, 4 H), 1.06−1.28 (m, 1 H), 0.48−0.64 (m, 2 H), 0.23−0.45 (m, 2 H). LCMS: [MH+] = 796

*[(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 5-[[[2-Oxo-1-phenyl-2-[(3R)-quinuclidin-3-yl]oxyethyl]amino]methyl]thiophene-2-carboxylate (**88a**).* ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.56 (s, 2 H), 7.69−7.68 (d, J = 3.8, 1 H), 7.45−7.28 (m, 5 H), 7.04−6.94 (m, 4 H), 6.14 (dd, J = 9.7, 4.3 Hz, 1 H), 4.73−4.65 (m, 1 H), 4.43 (d, J = 9.3, Hz, 1 H), 3.92−3.82 (m, 2 H), 3.80−3.74 (m, 6 H), 3.66−3.54 (m, 2 H), 3.28−3.26 (m, 1 H), 3.10−3.03*^{or†} (m, 1 H), 3.02−2.95*^{or†} (m, 1 H), 2.63−2.50 (m, 3 H), 2.46−2.29 (m, 1 H), 2.16 (m, 1 H), 1.89−1.84*^{or†} (m, 1 H), 1.75−1.71*^{or†} (m, 1 H), 1.61−1.38 (m, 3 H), 1.24−1.18 (m,1 H). † and * refer to different isomers (arbitrarily assigned). LCMS: [MH+] = 726.

*[(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 5-[[[1-(2-Fluorophenyl)-2-oxo-2-quinuclidin-3-yloxyethyl]amino]methyl]thiophene-2-carboxylate (**88b**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.14 (s, 2 H), 7.64 (dd, J = 3.8, 1.5 Hz, 1 H), 7.42−7.35 (m, 1 H), 7.35−7.29 (m, 1 H), 7.21−7.13 (m, 1 H), 7.13−7.05 (m, 1 H), 7.02−6.96 (m, 2 H), 6.92−6.88 (m, 1 H), 6.85 (d, J = 8.1 Hz, 1 H), 6.22 (dd, J = 9.7, 4.5 Hz, 1 H), 4.90−4.79 (m, 1 H), 4.76 (d, J = 4.2 Hz, 1 H), 3.97 (s, 2 H), 3.90 (s, 3 H), 3.88 (d, J = 1.3 Hz, 3 H), 3.70−3.62 (m, 1 H), 3.35−3.28 (m, 1 H), 3.24−3.09 (m, 1 H), 2.80−2.62 (m, 5 H), 2.54−2.34 (m, 1 H), 2.01−1.85 (m, 1 H), 1.57−1.43 (m, 2 H), 1.36−1.13 (m, 2 H). LCMS: [MH+] = 744

*[(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 5-[[Methyl-[2-oxo-1-phenyl-2-[(3R)-quinuclidin-3-yl]oxyethyl]amino]methyl]thiophene-2-carboxylate (**88c**).* ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.56 (s, 2 H), 7.68 (d, J = 3.8 Hz, 1 H), 7.45−7.36 (m, 5 H), 7.04−7.03 (m, 2 H), 7.01−6.96 (m, 2 H), 6.14 (dd, J = 4.4, 9.7 Hz, 1 H), 4.83−4.76 (m, 1 H), 4.51 (d, J = 6.8 Hz, 1 H), 3.89−3.83 (m, 1 H), 3.78 (s, 3 H), 3.76 (s, 3 H), 3.75−3.72 (m, 1 H), 3.60 (dd, J = 9.9, 14.1 Hz, 1 H), 3.32−3.27 (m, 1 H), 3.16−3.03 (m, 1 H), 2.68−2.58 (m, 4 H), 2.37−2.31 (m, 1 H), 2.22 (s, 3 H), 1.92−1.84 (m, 1 H), 1.62−1.39 (m, 3 H), 1.29−1.21 (m, 1 H). LCMS: [MH+] =740

*[(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 5-[[[2-[[(1R,5R)-8-Methyl-8-azabicyclo-[3.2.1]octan-3-yl]oxy]-2-oxo-1-phenylethyl]amino]methyl]-thiophene-2-carboxylate (**88d**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.13 (s, 2 H), 7.64 (d, J = 3.8 Hz, 1 H), 7.37−7.32 (m, 5 H), 7.01−6.96 (m, 2 H), 6.88−6.83 (m, 2 H), 6.26−6.20 (m, 1 H), 5.01 (s, 1 H), 4.37 (s, 1 H), 3.95−3.85 (m, 8 H), 3.70−3.63 (m, 1 H), 3.34−3.28 (m, 1 H), 3.01 (s, 1 H), 2.88 (s, 1 H), 2.70 (s, 1 H), 2.19 (s, 3 H), 2.10 (d, J = 15.7 Hz, 1 H), 2.01 (s, 1 H), 1.90−1.80 (m, 1 H), 1.70−1.57 (m, 3 H), 1.41 (d, J = 15.2 Hz, 1 H), 1.04 (d, J = 9.7 Hz, 1 H). LCMS: [MH+] = 740

*[(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 5-[[[2-[(1-Methyl-4-piperidyl)oxy]-2-oxo-1-phenylethyl]amino]methyl]thiophene-2-carboxylate (**88e**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.14*^{or†} (s, 2 H), 8.13*^{or†} (s, 2 H), 7.64 (d, J = 3.8 Hz, 1 H), 7.37−7.30 (m, 5 H), 7.02−6.96 (m, 2 H), 6.91−6.83 (m, 2 H), 6.25−6.19 (m, 1 H), 4.88−4.77 (m, 1 H), 4.42 (d, J = 3.7 Hz, 1 H), 3.97−3.85 (m, 8 H), 3.67 (dd, J = 14.1, 9.7 Hz, 1 H), 3.34−3.27 (m, 1 H), 2.60−2.40 (m, 2 H), 2.30−2.15 (m, 2 H), 2.21 (s, 3 H), 1.92−1.80 (m, 2 H), 1.75−1.45 (m, 3 H), * and † refer to different isomers. LCMS: [MH+] = 714

*[(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 5-[[[2-[(3R)-1-Methylpyrrolidin-3-yl]oxy-2-oxo-1-phenylethyl]amino]methyl]thiophene-2-carboxylate (**88f**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.14*^{or†} (s, 2 H), 8.13*^{or†} (s, 2 H), 7.63 (d, J = 3.5 Hz, 1 H), 7.38−7.33 (m, 5 H), 7.01−6.96 (m, 2 H), 6.89−6.83 (m, 2 H), 6.25−6.19 (m, 1 H), 5.26−5.17 (m, 1 H), 4.43 (s, 1 H), 3.95−3.91 (m, 2 H), 3.91*^{or†} (s, 3 H), 3.90*^{or†} (s, 3 H), 3.88 (s, 3 H) 3.66 (dd, J = 9.9, 13.9 Hz, 1 H), 3.35−3.27 (m, 1 H), 2.73−2.58 (m, 3 H), 2.50−2.43 (m, 1 H), 2.29−2.19 (m, 5 H), 1.88−

https://doi.org/10.1021/acs.jmedchem.1c00204
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1.79 (m, 1 H). * and † refer to different isomers. NH not visible. LCMS: [MH+] = 700.

*[(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 5-[[[2-Oxo-1-phenyl-2-[[(3R)-quinuclidin-3-yl]methoxy]ethyl]amino]methyl]thiophene-2-carboxylate (**88g**).* ^{1}H NMR (400 MHz, CD_3CN): δ 8.19*^{or†} (s, 2 H), 8.18*^{or†} (s, 2 H), 7.69 (d, J = 3.8 Hz, 1 H), 7.40−7.38 (m, 5 H), 7.07−7.01 (m, 2 H), 6.97−6.93 (m, 2 H), 6.18 (dd, J = 4.5, 9.6 Hz, 1 H), 4.43 (s, 1 H), 4.15−4.02 (m, 2 H), 3.99−3.89 (m, 2 H), 3.84*^{or†} (s, 3 H), 3.83*^{or†} (s, 3 H), 3.82 (s, 3 H), 3.67 (dd, J = 9.5, 14.0 Hz, 1 H), 3.34 (dd, J = 4.5, 14.1 Hz, 1 H), 2.86−2.80 (m, 2 H), 2.75−2.63 (m, 4 H), 2.29−2.16 (m, 1 H), 1.89−1.86 (m, 1 H), 1.58−1.49 (m, 3 H), 1.44−1.39 (m, 1 H), 1.34−1.23 (m, 1 H). † and * refer to different isomers (arbitrarily assigned). LCMS: [MH+] = 740

*[(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 5-[[[2-[(1-Methyl-4-piperidyl)methoxy]-2-oxo-1-phenylethyl]amino]methyl]thiophene-2-carboxylate (**88h**).* ^{1}H NMR (400 MHz, $CDCl_3$ + D_2O): δ 8.15*^{or†} (s, 2 H), 8.14*^{or†} (s, 2 H), 7.64 (d, J = 3.8 Hz, 1 H), 7.39−7.35 (m, 5 H), 7.01−6.96 (m, 2 H), 6.89−6.84 (m, 2 H), 6.24−6.20 (m, 1 H), 4.42 (d, J = 3.0 Hz, 1 H), 3.93−3.87 (m, 10 H), 3.67 (dd, J = 9.5, 13.3 Hz, 1 H), 3.34−3.28 (m, 1 H), 2.84−2.81 (m, 2 H), 2.26 (s, 3 H), 1.91−1.83 (m, 2 H), 1.54−1.54 (m, 3 H), 1.31−1.24 (m, 2 H). † and * refer to different isomers (arbitrarily assigned) LCMS: [MH+] = 728

*[(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 5-[[[2-[[(2R)-1-Methylazetidin-2-yl]-methoxy]-2-oxo-1-phenylethyl]amino]methyl]thiophene-2-carboxylate (**88i**).* ^{1}H NMR (400 MHz, CD_3CN): δ 8.19*^{or†} (s, 2 H), 8.18*^{or†} (s, 2 H), 7.69 (d, J = 3.8 Hz, 1 H), 7.43−7.39 (m, 5 H), 7.07−7.01 (m, 2 H), 6.97−6.94 (m, 2 H), 6.18 (dd, J = 4.5, 9.6 Hz, 1 H), 4.45 (s, 1 H), 4.22−4.12 (m, 1 H), 4.00−3.93 (m, 3 H), 3.84*^{or†} (s, 3 H), 3.83*^{or†} (s, 3 H), 3.82 (s, 3 H), 3.71−3.64 (m, 1 H), 3.34 (dd, J = 4.5, 14.1 Hz, 1 H), 3.24−3.16 (m, 1 H), 3.11−3.02 (m, 1 H), 2.87 (s, 1 H), 2.73−2.64 (m, 1 H), 2.09*^{or†} (s, 3 H), 2.09*^{or†} (s, 3 H), 1.92−1.76 (m, 2 H). † and * refer to different isomers (arbitrarily assigned). LCMS: [MH+] = 700.

*[(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 5-[[[2-[2-(Dimethylamino)ethoxy]-2-oxo-1-phenylethyl]amino]methyl]thiophene-2-carboxylate (**88j**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.14*^{or†} (s, 2 H), 8.13*^{or†} (s, 2 H), 7.64 (d, J = 3.8 Hz, 1 H), 7.38−7.35 (m, 5 H), 7.00−6.96 (m, 2 H), 6.89−6.83 (m, 2 H), 6.24−6.20 (m, 1 H), 4.42 (d, J = 2.8 Hz, 1 H), 4.23−4.09 (m, 2 H), 3.93 (d, J = 5.4 Hz, 2 H), 3.90*^{or†} (s, 3 H), 3.90*^{or†} (s, 3 H), 3.88 (s, 3 H), 3.66 (dd, J = 10.0, 13.8 Hz, 1 H), 3.31 (ddd, J = 2.7, 4.4, 13.9 Hz, 1 H), 2.18−2.15 (m, 2 H), 2.14 (s, 6 H), 1.75−1.69 (m, 2 H). † and * refer to different isomers (arbitrarily assigned), NH not observed. LCMS: [MH+] = 688.

Purification of the 1:1 mixture of diastereoisomers **86a** and **88a−j** by chiral preparative SFC or chiral preparative HPLC afforded the single diastereoisomers **89**, **90**, **91a−j**, and **92a−j**.

*Single Diastereoisomers of [(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 3-[[[2-Oxo-1-phenyl-2-[(3R)-quinuclidin-3-yl]oxyethyl]amino]methyl]benzoate (**89**, Fast Diastereoisomer).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.12 (s, 2 H), 7.99 (s, 1 H), 7.92 (d, J = 7.8 Hz, 1 H), 7.55 (d, J = 7.7 Hz, 1 H), 7.42−7.29 (m, 6 H), 7.03−6.97 (m, 2 H), 6.85 (d, J = 8.2 Hz, 1 H), 6.29 (dd, J = 9.6, 4.7 Hz, 1 H), 4.82−4.76 (m, 1 H), 4.40 (s, 1 H), 3.89 (s, 3 H), 3.87 (s, 3 H), 3.78 (s, 2 H), 3.71 (dd, J = 13.9, 9.7 Hz, 1 H), 3.35 (dd, J = 13.9, 4.7 Hz, 1 H), 3.10 (ddd, J = 14.8, 8.2, 2.3 Hz, 1 H), 2.78−2.61 (m, 2 H), 2.56−2.47 (m, 1 H), 2.34 (d, J = 15.0 Hz, 1 H), 2.04−1.95 (m, 2 H), 1.69−1.59 (m, 2 H), 1.55−1.45 (m, 1 H), 1.35−1.25 (m, 2 H). LCMS: [MH+] = 720. Chiral analysis (Method 3) at 5.41 min.

*Single Diastereoisomers of [(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 3-[[[2-Oxo-1-phenyl-2-[(3R)-quinuclidin-3-yl]oxyethyl]amino]methyl]benzoate (**90**, Slow Diastereoisomer).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.12 (s, 2 H), 8.00 (s, 1 H), 7.92 (d, J = 7.8 Hz, 1 H), 7.53 (d, J = 7.7 Hz, 1 H), 7.42−7.27 (m, 6 H), 7.04−6.97 (m, 2 H), 6.85 (d, J = 8.2 Hz, 1 H), 6.28 (dd, J = 9.7, 4.6 Hz, 1 H), 4.87−4.82 (m, 1 H), 4.38 (s, 1 H), 3.89 (s, 3 H), 3.88 (s, 3 H), 3.78 (d, J = 3.6 Hz, 2 H), 3.71 (dd, J = 13.8, 9.6 Hz, 1 H), 3.35 (dd, J = 14.0, 4.6 Hz, 1 H), 3.19 (dd, J = 14.7, 8.4 Hz, 1 H), 2.80−2.65 (m, 4 H), 2.27−2.05 (m, 2 H), 1.89−1.83 (m, 1 H), 1.66−1.55 (m, 1 H), 1.54−1.44 (m, 1 H), 1.30−1.21 (m, 1 H), 1.21−1.10 (m, 1 H). LCMS: [MH+] = 720. Chiral analysis (Method 3) at 6.59 min.

*Single Diastereoisomer of [(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 5-[[(2-Oxo-1-phenyl-2-[(3R)-quinuclidin-3-yl]oxyethyl)amino]methyl]thiophene-2-carboxylate (**91a**, Fast Diastereoisomer).* ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.56 (s, 2 H), 7.69 (d, J = 3.78 Hz, 1 H), 7.44−7.27 (m, 5 H), 7.04−6.96 (m, 4 H), 6.14 (dd, J = 9.62, 4.40 Hz, 1 H), 4.72−4.67 (m, 1 H), 4.43 (d, J = 9.25 Hz, 1 H), 3.88 (d, J = 5.70 Hz, 2 H), 3.76 (d, J = 5.87 Hz, 6 H), 3.63−3.53 (m, 2 H), 3.06 (dd, J = 14.61, 8.26 Hz, 1 H), 2.59 (t, J = 10.90 Hz, 5 H), 2.45 (s, 1 H), 1.73 (s, 1 H), 1.61−1.13 (m, 4 H). LCMS: [MH+] = 726. Chiral analysis (Method 4) at 12.97 min and (Method 10) at 2.57 min.

*Single Diastereoisomer of [(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 5-[[(2-Oxo-1-phenyl-2-quinuclidin-3-yloxyethyl)amino]methyl]thiophene-2-carboxylate (**92a**, Slow Diastereoisomer).* ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.55 (s, 2 H), 7.68 (d, J = 3.78 Hz, 1 H), 7.45−7.29 (m, 5 H), 7.05−6.95 (m, 4 H), 6.14 (dd, J = 9.59, 4.29 Hz, 1 H), 4.67 (d, J = 7.13 Hz, 1 H), 4.43 (d, J = 9.21 Hz, 1 H), 3.89 (t, J = 5.31 Hz, 2 H), 3.77 (d, J = 8.16 Hz, 6 H), 3.59 (t, J = 11.05 Hz, 2 H), 3.00−2.93 (m, 1 H), 2.57 (s, 4 H), 2.36 (d, J = 20.05 Hz, 1 H), 2.15 (d, J = 14.66 Hz, 1 H), 1.86 (s, 1 H), 1.60−1.50 (m, 2 H), 1.44 (s, 1 H), 1.24 (s, 1 H). LCMS: [MH+] = 726. Chiral analysis (Method 4) at 15.02 min and (Method 10) at 3.29 min.

*Single Diastereoisomer of [(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 5-[[[1-(2-Fluorophenyl)-2-oxo-2-[(3R)-quinuclidin-3-yl]oxyethyl]amino]methyl]thiophene-2-carboxylate (**91b**, Fast Diastereoisomer).* ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.55 (s, 2 H), 7.68 (d, J = 3.8 Hz, 1 H), 7.57−7.51 (m, 1 H), 7.42−7.35 (m, 1 H), 7.28−7.19 (m, 2 H), 7.04−6.96 (m, 4 H), 6.14 (dd, J = 9.6, 4.3 Hz, 1 H), 4.72−4.66 (m, 2 H), 3.94 (d, J = 5.6 Hz, 2 H), 3.77 (s, 3 H), 3.76 (s, 3 H), 3.67−3.53 (m, 2 H), 3.30 (dd, J = 14.3, 4.5 Hz, 1 H), 3.01 (dd, J = 14.8, 8.1 Hz, 1 H), 2.69−2.44 (m, 3 H), 2.38−2.27 (m, 1 H), 2.20−2.12 (m, 1 H), 1.89−1.82 (m, 1 H), 1.60−1.39 (m, 3 H), 1.28−1.14 (m, 1 H). LCMS: [MH+] = 744. Chiral analysis (Method 9) at 15.61 min.

*Single Diastereoisomer of [(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 5-[[[1-(2-Fluorophenyl)-2-oxo-2-[(3R)-quinuclidin-3-yl]oxyethyl]amino]methyl]thiophene-2-carboxylate (**92b**, Slow Diastereoisomer).* ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.55 (s, 2 H), 7.69 (d, J = 3.8 Hz, 1 H), 7.56−7.50 (m, 1 H), 7.41−7.34 (m, 1 H), 7.26−7.18 (m, 2 H), 7.04−6.96 (m, 4 H), 6.14 (dd, J = 9.6, 4.4 Hz, 1 H), 4.77−4.67 (m, 2 H), 3.94 (d, J = 5.1 Hz, 2 H), 3.77 (s, 3 H), 3.76 (s, 3 H), 3.67−3.53 (m, 2 H), 3.30 (dd, J = 14.1, 4.4 Hz, 1 H), 3.09 (dd, J = 14.8, 8.4 Hz, 1 H), 2.70−2.44 (m, 5 H), 1.77−1.71 (m, 1 H), 1.59−1.38 (m, 2 H), 1.31−1.09 (m, 2 H). LCMS: [MH+] = 744. Chiral analysis (Method 9) at 17.65 min.

*Single Diastereoisomer of [(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 5-[[Methyl-[2-oxo-1-phenyl-2-[(3R)-quinuclidin-3-yl]oxyethyl]amino]methyl]thiophene-2-carboxylate (**91c**, Fast Diastereoisomer).* ^{1}H NMR (400 MHz,CD_3CN): δ 8.19 (s, 2 H), 7.67 (d, J = 3.8 Hz, 1 H), 7.51 (d, J = 7.8 Hz, 2 H), 7.46−7.38 (m, 3 H), 7.08−7.01 (m, 2 H), 6.97−6.93 (m, 2 H), 6.18 (dd, J = 4.5, 9.6 Hz, 1 H), 4.86−4.81 (m, 1 H), 4.44 (s, 1 H), 3.90−3.85 (m, 4 H), 3.83−3.77 (m, 4 H), 3.68 (dd, J = 9.9, 14.1 Hz, 1 H), 3.35 (dd, J = 4.5, 13.9 Hz, 1 H), 3.12 (ddd, J = 2.1, 8.2, 14.5 Hz, 1 H), 2.75−2.60 (m, 4 H), 2.46 (d, J = 14.4 Hz, 1 H), 2.30 (s, 3 H), 1.94−1.88 (m, 1 H), 1.76−1.50 (m, 3 H), 1.41−1.29 (m, 1 H). LCMS: [MH+] = 740. Chiral analysis (Method 7) at 2.27 min.

*Single Diastereoisomer of [(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 5-[[Methyl-[2-oxo-1-phenyl-2-[(3R)-quinuclidin-3-yl]oxyethyl]amino]methyl]thiophene-2-carboxylate (**92c**, Slow Diastereoisomer).* ^{1}H NMR (400 MHz, CD_3CN): δ 8.19 (s, 2 H), 7.68 (d, J = 3.8 Hz, 1 H), 7.50 (d, J = 6.8 Hz, 2 H), 7.46−7.37 (m, 3 H), 7.08−7.02 (m, 2 H), 6.97−6.94 (m, 2 H), 6.18 (dd, J = 4.5, 9.6 Hz, 1 H), 4.88−4.83 (m, 1 H), 4.46 (s, 1 H), 3.91 (d, J = 15.2 Hz, 1 H), 3.84 (s, 3 H), 3.84−3.78 (m, 4 H),

https://doi.org/10.1021/acs.jmedchem.1c00204
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3.68 (dd, J = 9.7, 14.0 Hz, 1 H), 3.35 (dd, J = 4.5, 14.1 Hz, 1 H), 3.19 (dd, J = 8.3, 14.7 Hz, 1 H), 2.79−2.64 (m, 4 H), 2.64−2.55 (m, 1 H), 2.30 (s, 3 H), 1.94−1.88 (m, 1 H), 1.70−1.50 (m, 3 H), 1.37−1.29 (m, 1 H). LCMS: [MH+] = 740. Chiral analysis (Method 7) at 3.08 min.

*Single Diastereoisomer of [(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 5-[[[2-[[(1R,5R)-8-Methyl-8-azabicyclo[3.2.1]octan-3-yl]oxy]-2-oxo-1-phenylethyl]amino]-methyl]thiophene-2-carboxylate (**91d**, Fast Diastereoisomer).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.13 (s, 2 H), 7.64 (d, J = 3.8 Hz, 1 H), 7.38−7.36 (m, 5 H), 7.02−6.96 (m, 2 H), 6.88−6.84 (m, 2 H), 6.22 (dd, J = 4.3, 9.9 Hz, 1 H), 5.00 (dd, J = 5.4, 5.4 Hz, 1 H), 4.36 (s, 1 H), 3.93−3.87 (m, 8 H), 3.67 (dd, J = 10.0, 14.0 Hz, 1 H), 3.31 (dd, J = 4.5, 13.9 Hz, 1 H), 3.03−3.00 (m, 1 H), 2.90−2.88 (m, 1 H), 2.62 (d, J = 70.9 Hz, 1 H), 2.19 (s, 3 H), 2.14−1.98 (m, 2 H), 1.86−1.81 (m, 1 H), 1.60−1.70 (m, 3 H), 1.42 (d, J = 15.2 Hz, 1 H), 1.1−1.0 (m, 1 H). LCMS: [MH+] = 740. Chiral analysis (Method 2) at 9.50 min.

*Single Diastereoisomer of [(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 5-[[[2-[[(1R,5R)-8-Methyl-8-azabicyclo[3.2.1]octan-3-yl]oxy]-2-oxo-1-phenylethyl]amino]-methyl]thiophene-2-carboxylate (**92d**, Slow Diastereoisomer).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.14 (s, 2 H), 7.65 (d, J = 3.8 Hz, 1 H), 7.39−7.35 (m, 5 H), 6.99 (d, J = 8.3 Hz, 2 H), 6.88−6.83 (m, 2 H), 6.23 (dd, J = 4.7, 9.7 Hz, 1 H), 5.01 (dd, J = 5.2, 5.2 Hz, 1 H), 4.38 (s, 1 H), 3.93−3.88 (m, 8 H), 3.67 (dd, J = 9.9, 13.9 Hz, 1 H), 3.31 (dd, J = 4.7, 14.0 Hz, 1 H), 3.07−3.05 (m, 1 H), 2.92 (d, J = 3.0 Hz, 1 H), 2.21 (s, 3 H), 2.20−2.00 (m, 2 H), 1.86−1.70 (m, 4 H), 1.44 (d, J = 14.9 Hz, 1 H), 1.06−1.04 (m, 1H), NH not visible. LCMS: [MH+] = 740. Chiral analysis (Method 2) at 11.81 min.

*Single Diastereomer of [(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 5-[[[2-[(1-Methyl-4-piperidyl)oxy]-2-oxo-1-phenylethyl]amino]methyl]thiophene-2-carboxylate (**91e**, Fast Diastereoisomer).* ^{1}H NMR (400 MHz, CD_3CN): δ 8.19 (s, 2 H), 7.69 (d, J = 3.8 Hz, 1 H), 7.43−7.32 (m, 5 H), 7.08−7.01 (m, 2 H), 6.97−6.92 (m, 2 H), 6.18 (dd, J = 4.5, 9.6 Hz, 1 H), 4.81−4.73 (m, 1 H), 4.42 (s, 1 H), 3.95 (dd, J = 15.0, 22.4 Hz, 2 H), 3.83 (s, 3 H), 3.82 (s, 3 H), 3.67 (dd, J = 9.6, 14.1 Hz, 1 H), 3.34 (dd, J = 4.5, 14.1 Hz, 1 H), 2.53−2.08 (m, 4 H), 2.15 (s, 3 H), 1.90−1.81 (m, 1 H), 1.77−1.59 (m, 2 H), 1.55−1.44 (m, 1 H), NH not observed. LCMS: [MH+] = 714. Chiral analysis (Method 7) at 1.68 min.

*Single Diastereomer of [(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 5-[[[2-[(1-Methyl-4-piperidyl)oxy]-2-oxo-1-phenylethyl]amino]methyl]thiophene-2-carboxylate (**92e**, Slow Diastereoisomer).* ^{1}H NMR (400 MHz, CD_3CN): δ 8.18 (s, 2 H), 7.69 (d, J = 4.3 Hz, 1 H), 7.44−7.32 (m, 5 H), 7.07−7.01 (m, 2 H), 6.97−6.91 (m, 2 H), 6.18 (dd, J = 4.4, 9.7 Hz, 1 H), 4.81−4.73 (m, 1 H), 4.41 (s, 1 H), 3.95 (dd, J = 14.9, 25.1 Hz, 2 H), 3.83 (s, 3 H), 3.82 (s, 3 H), 3.67 (dd, J = 9.6, 14.1 Hz, 1 H), 3.33 (dd, J = 4.5, 14.1 Hz, 1 H), 2.56−2.11 (m, 4 H), 2.16 (s, 3 H), 1.89−1.81 (m, 1 H), 1.77−1.59 (m, 2 H), 1.55−1.45 (m, 1 H), NH not observed. LCMS: [MH+] = 714. Chiral analysis (Method 7) at 2.50 min.

*Single Diastereoisomer of [(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 5-[[[2-[(3R)-1-Methylpyrrolidin-3-yl]oxy-2-oxo-1-phenylethyl]amino]methyl]thiophene-2-carboxylate (**91f**, Fast Diastereoisomer).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.14 (s, 2 H), 7.64 (d, J = 3.8 Hz, 1 H), 7.37−7.29 (m, 5 H), 7.00−6.97 (m, 2 H), 6.87−6.84 (m, 2 H), 6.22 (dd, J = 4.4, 10.0 Hz, 1 H), 5.24−5.21 (m, 1 H), 4.43 (s, 1 H), 3.91−3.90 (m, 2 H), 3.89 (s, 3 H), 3.87 (s, 3 H), 3.66 (dd, J = 10.0, 14.0 Hz, 1 H), 3.31 (dd, J = 4.4, 14.0 Hz, 1 H), 2.69−2.67 (m, 3 H), 2.29 (s, 3 H), 2.27−2.25 (m, 1 H), 2.19−2.14 (m, 1 H), 1.69−1.58 (m, 2 H). LCMS: [MH+] =700. Chiral analysis (Method 1) at 11.66 min.

*Single Diastereoisomer of [(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 5-[[[2-[(3R)-1-Methylpyrrolidin-3-yl]oxy-2-oxo-1-phenylethyl]amino]methyl]thiophene-2-carboxylate (**92f**, Slow Diastereoisomer).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.13 (s, 2 H), 7.64 (d, J = 3.8 Hz, 1 H), 7.37−7.29 (m, 5 H), 7.01−6.96 (m, 2 H), 6.86−6.84 (m, 2 H), 6.22 (dd, J = 4.4, 9.7 Hz, 1 H), 5.23−5.17 (m, 1 H), 4.43 (s, 1 H), 3.91−3.90 (m, 2 H), 3.89 (s, 3 H), 3.87 (s, 3 H), 3.67 (dd, J = 9.9, 13.9 Hz, 1 H), 3.31 (dd, J = 4.5, 13.9 Hz, 1 H), 2.77−2.70 (m, 1 H), 2.64−2.62 (m, 1 H), 2.48 (dd, J = 2.1, 11.0 Hz, 1 H), 2.34−2.29 (m, 2 H), 2.28 (s, 3 H), 1.86−1.80 (m, 2 H). LCMS: [MH+] =700. Chiral analysis (Method 1) at 13.61 min.

*Single Diastereomer of [(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 5-[[[2-Oxo-1-phenyl-2-[[(3R)-quinuclidin-3-yl]methoxy]ethyl]amino]methyl]thiophene-2-carboxylate (**91g**, Fast Diastereoisomer).* ^{1}H NMR (400 MHz, CD_3CN): δ 8.19 (s, 2 H), 7.69 (d, J = 3.8 Hz, 1 H), 7.41−7.32 (m, 5 H), 7.08−7.02 (m, 2 H), 6.97−6.94 (m, 2 H), 6.19 (dd, J = 4.5, 9.6 Hz, 1 H), 4.44 (s, 1 H), 4.11−4.08 (m, 2 H), 4.00−3.90 (m, 2 H), 3.84 (s, 3 H), 3.83 (s, 3 H), 3.71−3.64 (m, 1 H), 3.35 (dd, J = 4.5, 14.1 Hz, 1 H), 2.84 (dd, J = 9.9, 13.6 Hz, 2 H), 2.76−2.64 (m, 4 H), 2.26−2.19 (m, 1 H), 1.90−1.81 (m, 1 H), 1.61−1.50 (m, 3 H), 1.44−1.25 (m, 2 H). LCMS: [MH+] = 740. Chiral analysis (Method 6) at 6.31 min.

*Single Diastereomer of [(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 5-[[[2-Oxo-1-phenyl-2-[[(3R)-quinuclidin-3-yl]methoxy]ethyl]amino]methyl]thiophene-2-carboxylate (**92g**, Slow Diastereoisomer).* ^{1}H NMR (400 MHz, CD_3CN): δ 8.18 (s, 2 H), 7.69 (d, J = 3.8 Hz, 1 H), 7.43−7.34 (m, 5 H), 7.09−7.01 (m, 2 H), 6.98−6.94 (m, 2 H), 6.19 (dd, J = 4.4, 9.7 Hz, 1 H), 4.45 (s, 1 H), 4.15−4.04 (m, 2 H), 4.00−3.89 (m, 2 H), 3.85 (s, 3 H), 3.82 (s, 3 H), 3.71−3.64 (m, 1 H), 3.35 (dd, J = 4.4, 14.0 Hz, 1 H), 2.89−2.65 (m, 5 H), 2.31−2.23 (m, 1 H), 1.90 (dd, J = 8.4, 20.4 Hz, 1 H), 1.61−1.44 (m, 3 H), 1.34−1.26 (m, 2 H), NH not observed. LCMS: [MH+] = 740. Chiral analysis (Method 6) at 7.51 min.

*Single Diastereomer of [(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 5-[[[2-[(1-Methyl-4-piperidyl)methoxy]-2-oxo-1-phenylethyl]amino]methyl]thiophene-2-carboxylate (**91h**, Fast Diastereoisomer).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.14 (s, 2 H), 7.64 (d, J = 3.8 Hz, 1 H), 7.37−7.35 (m, 5 H), 7.01−6.97 (m, 2 H), 6.89−6.84 (m, 2 H), 6.22 (dd, J = 4.5, 9.9 Hz, 1 H), 4.43 (s, 1 H), 3.98−3.91 (m, 4 H), 3.90 (s, 3 H), 3.88 (s, 3 H), 3.67 (dd, J = 9.7, 14.0 Hz, 1 H), 3.31 (dd, J = 4.5, 13.9 Hz, 1 H), 2.81 (d, J = 10.6 Hz, 2 H), 2.25 (s, 3 H), 1.89−1.82 (m, 2 H), 1.53−1.52 (m, 3 H), 1.29−1.20 (m, 2 H). LCMS: [MH+] = 728. Chiral analysis (Method 5) at 6.14 min.

*Single Diastereomer of [(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 5-[[[2-[(1-Methyl-4-piperidyl)methoxy]-2-oxo-1-phenylethyl]amino]methyl]thiophene-2-carboxylate (**92h**, Slow Diastereoisomer).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.13 (s, 2 H), 7.64 (d, J = 3.8 Hz, 1 H), 7.38−7.36 (m, 5 H), 7.02−6.96 (m, 2 H), 6.88−6.84 (m, 2 H), 6.22 (dd, J = 4.5, 9.9 Hz, 1 H), 4.42 (s, 1 H), 3.99−3.92 (m, 4 H), 3.91 (s, 3 H), 3.88 (s, 3 H), 3.67 (dd, J = 9.9, 13.9 Hz, 1 H), 3.31 (dd, J = 4.4, 14.0 Hz, 1 H), 2.86 (d, J = 8.8 Hz, 2 H), 2.29 (s, 3 H), 1.92−1.87 (m, 2 H), 1.54−1.54 (m, 3 H), 1.35−1.20 (m, 2 H). LCMS: [MH+] = 728. Chiral analysis (Method 5) at 7.21 min.

*Single Diastereomer of [(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 5-[[[2-[[(2R)-1-Methylazetidin-2-yl]methoxy]-2-oxo-1-phenylethyl]amino]methyl]-thiophene-2-carboxylate (**91i**, Fast Diastereoisomer).* ^{1}H NMR (400 MHz, CD_3CN): δ 8.19 (s, 2 H), 7.69 (d, J = 3.8 Hz, 1 H), 7.44−7.34 (m, 5 H), 7.07−7.01 (m, 2 H), 6.97−6.93 (m, 2 H), 6.19 (dd, J = 4.5, 9.6 Hz, 1 H), 4.46 (s, 1 H), 4.20 (dd, J = 3.8, 11.4 Hz, 1 H), 4.02−3.90 (m, 3 H), 3.83 (s, 3 H), 3.82 (s, 3 H), 3.67 (dd, J = 9.6, 14.1 Hz, 1 H), 3.35 (dd, J = 4.5, 14.1 Hz, 1 H), 3.22−3.16 (m, 1 H), 3.09−3.01 (m, 1 H), 2.69 (ddd, J = 6.6, 8.0, 9.3 Hz, 1 H), 2.09 (s, 3 H), 1.91−1.76 (m, 2 H). Note: NH not visible LCMS: [MH+] = 700. Chiral analysis (Method 6) at 3.44 min.

*Single Diastereomer of [(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 5-[[[2-[[(2R)-1-Methylazetidin-2-yl]methoxy]-2-oxo-1-phenylethyl]amino]methyl]-thiophene-2-carboxylate (**92i**, Slow Diastereoisomer).* ^{1}H NMR (400 MHz, CD_3CN): δ 8.18 (s, 2 H), 7.69 (d, J = 3.8 Hz, 1 H), 7.43−7.40 (m, 5 H), 7.07−7.02 (m, 2 H), 6.97−6.94 (m, 2 H), 6.19 (dd, J = 4.5, 9.9 Hz, 1 H), 4.46 (s, 1 H), 4.19−4.12 (m, 1 H), 4.01−3.89 (m, 3 H), 3.83 (s, 3 H), 3.82 (s, 3 H), 3.71−3.63 (m, 1 H), 3.34 (dd, J = 4.5, 14.1 Hz, 1 H), 3.25−3.20 (m, 1 H), 3.15−3.07 (m, 1 H), 2.74−

https://doi.org/10.1021/acs.jmedchem.1c00204
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2.67 (m, 1 H), 2.10 (s, 3 H), 1.91−1.76 (m, 2 H).NH not visible LCMS: [MH+] = 700. Chiral analysis (Method 6) at 4.30 min.

*Single Diastereoisomer of [(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 5-[[[2-[2-(Dimethylamino)ethoxy]-2-oxo-1-phenylethyl]amino]methyl]-thiophene-2-carboxylate (**91j**, Fast Diastereoisomer).* ^{1}H NMR (400 MHz, CD_3CN): δ 8.19 (s, 2 H), 7.70 (d, J = 3.5 Hz, 1 H), 7.42−7.36 (m, 5 H), 7.08−7.01 (m, 2 H), 6.98−6.93 (m, 2 H), 6.18 (dd, J = 4.5, 9.6 Hz, 1 H), 4.44 (s, 1 H), 4.23−4.14 (m, 2 H), 4.01−3.90 (m, 2 H), 3.84 (s, 3 H), 3.82 (s, 3 H), 3.67 (dd, J = 9.7, 14.0 Hz, 1 H), 3.35 (dd, J = 4.5, 14.1 Hz, 1 H), 2.47 (t, J = 5.7 Hz, 2 H), 2.15 (s, 6 H), NH not visible. LCMS: [MH+] = 688. Chiral analysis (Method 8) at 10.27 min.

*Single Diastereoisomer of [(1S)-2-(3,5-Dichloro-1-oxido-pyridin-1-ium-4-yl)-1-(3,4-dimethoxyphenyl)ethyl] 5-[[[2-[2-(Dimethylamino)ethoxy]-2-oxo-1-phenylethyl]amino]methyl]-thiophene-2-carboxylate (**92j**, Slow Diastereoisomer).* ^{1}H NMR (400 MHz, CD_3CN): δ 8.18 (s, 2 H), 7.69 (d, J = 3.7 Hz, 1 H), 7.42−7.39 (m, 5 H), 7.07−7.01 (m, 2 H), 6.97−6.93 (m, 2 H), 6.21−6.15 (m, 1 H), 4.43 (s, 1 H), 4.23−4.14 (m, 2 H), 3.96 (dd, J = 15.2, 29.6 Hz, 2 H), 3.83 (s, 3 H), 3.82 (s, 3 H), 3.72−3.63 (m, 1 H), 3.34 (d, J = 26.1 Hz, 1 H), 2.47 (t, J = 5.6 Hz, 2 H), 2.15 (s, 6 H), NH not observed. LCMS: [MH+] = 688. Chiral analysis (Method 8) at 12.21 min.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.1c00204.

Preparation of intermediates **11**, **14**, **15**, **16**, **29**, **39a−d**, and **85a-j**; analytical characterization of intermediates **19**, **21**, **23**, **31**, **42b**, **42e**, **43b**, **44e**, **45b**, **65−68**, and **50−55**; chiral analytical methods, LCMS and SFC-MS traces for compounds **92e**, **92f**, **91a**, and **92a**; crystal structure determination of **92a**; and methods for clogD determination, kinetic solubility measure, assays: PDE4B2 HTRF, PDE4 LARBS, M3 receptor radioligand binding, THFa release in hPBMC, guinea pig and rat isolated trachea contraction, bronchoconstriction in anaesthetized guinea pigs and rats, M3 binding kinetics, ovalbumin-induced eosinophilia in the sensitized Brown Norway rat, nausea and emetic effect in ferret, *in vitro* microsomial Stability, *in vitro* plasma protein binding, *in vitro* plasma stability, and cellular permeability (PDF)

Molecular formula strings of final compounds with biological data (CSV)

■ AUTHOR INFORMATION

Corresponding Author

Elisabetta Armani − *Nuovo Centro Ricerche, Chiesi Farmaceutici S.p.A., 43122 Parma, Italy;* orcid.org/0000-0002-2835-2652; Phone: +39 0521 279477; Email: e.armani@chiesi.com; Fax: +39 0521 279880

Authors

Andrea Rizzi − *Nuovo Centro Ricerche, Chiesi Farmaceutici S.p.A., 43122 Parma, Italy*

Carmelida Capaldi − *Nuovo Centro Ricerche, Chiesi Farmaceutici S.p.A., 43122 Parma, Italy*

Renato De Fanti − *Nuovo Centro Ricerche, Chiesi Farmaceutici S.p.A., 43122 Parma, Italy;* Present Address: rdf0510noah@gmail.com, actually in retirement.

Maurizio Delcanale − *Nuovo Centro Ricerche, Chiesi Farmaceutici S.p.A., 43122 Parma, Italy*

Gino Villetti − *Nuovo Centro Ricerche, Chiesi Farmaceutici S.p.A., 43122 Parma, Italy*

Gessica Marchini − *Nuovo Centro Ricerche, Chiesi Farmaceutici S.p.A., 43122 Parma, Italy*

Anna Rita Pisano − *Nuovo Centro Ricerche, Chiesi Farmaceutici S.p.A., 43122 Parma, Italy*

Vanessa Pitozzi − *Nuovo Centro Ricerche, Chiesi Farmaceutici S.p.A., 43122 Parma, Italy*

Maria Gloria Pittelli − *Nuovo Centro Ricerche, Chiesi Farmaceutici S.p.A., 43122 Parma, Italy*

Marcello Trevisani − *Nuovo Centro Ricerche, Chiesi Farmaceutici S.p.A., 43122 Parma, Italy*

Michela Salvadori − *Nuovo Centro Ricerche, Chiesi Farmaceutici S.p.A., 43122 Parma, Italy*

Valentina Cenacchi − *Nuovo Centro Ricerche, Chiesi Farmaceutici S.p.A., 43122 Parma, Italy*

Paola Puccini − *Nuovo Centro Ricerche, Chiesi Farmaceutici S.p.A., 43122 Parma, Italy*

Francesco Amadei − *Nuovo Centro Ricerche, Chiesi Farmaceutici S.p.A., 43122 Parma, Italy*

Alice Pappani − *Nuovo Centro Ricerche, Chiesi Farmaceutici S.p.A., 43122 Parma, Italy*

Maurizio Civelli − *Nuovo Centro Ricerche, Chiesi Farmaceutici S.p.A., 43122 Parma, Italy*

Riccardo Patacchini − *Nuovo Centro Ricerche, Chiesi Farmaceutici S.p.A., 43122 Parma, Italy*

Charles A.G. Baker-Glenn − *Chesterford Research Park, Charles River Discovery Research Services UK Ltd, Saffron Walden CB10 1XL, United Kingdom*

Hervé Van de Poël − *Chesterford Research Park, Charles River Discovery Research Services UK Ltd, Saffron Walden CB10 1XL, United Kingdom*

Wesley P. Blackaby − *Chesterford Research Park, Charles River Discovery Research Services UK Ltd, Saffron Walden CB10 1XL, United Kingdom;* Present Address: Servier, 125 Chemin de Ronde, 78290 Croissy sur Seine, France.

Kevin Nash − *Chesterford Research Park, Charles River Discovery Research Services UK Ltd, Saffron Walden CB10 1XL, United Kingdom*

Gabriele Amari − *Nuovo Centro Ricerche, Chiesi Farmaceutici S.p.A., 43122 Parma, Italy*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jmedchem.1c00204

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

All the experiments with animals were carried out in accordance with national and European legislation and approved by local ethical committees.

■ ABBREVIATIONS

Ach, acetylcholine; ADME, absorption, distribution, metabolism, excretion; Cch, carbachol; Cl_{int}, intrinsic clearance; COPD, chronic obstructive pulmonary disease; CSD, crystal structure databank; ED, effective dose; GPT, guinea pig trachea; IC, inhibitory concentration; Ki, inhibition constant; LARBS, low affinity rolipram binding site; LogD, distribution coefficient logarithm; LPS, lipopolysaccharides; MABA,

https://doi.org/10.1021/acs.jmedchem.1c00204
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muscarinic antagonist beta adrenergic agonist; MAPI, muscarinic antagonist, phosphodiesterase 4 inhibitor; M3, muscarinic receptor 3; Mics, microsomes; OVA, ovalbumin; PBMC, peripheral blood mononuclear cells; PDE4, phosphodiesterase 4; PDE4B2, isoform B2 of phosphodiesterase 4; PPB, plasma protein binding; RT, rat trachea; TNFα, tumor necrosis factor alpha

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https://doi.org/10.1021/acs.jmedchem.1c00204
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