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Discovery of a novel class of inhaled dual pharmacology muscarinic antagonist and β_2 agonist (MABA) for the treatment of chronic obstructive pulmonary disease (COPD)

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ABSTRACT

The targeting of both the muscarinic and β-adrenergic pathways is a well validated therapeutic approach for the treatment of chronic obstructive pulmonary disease (COPD). In this communication we report our effort to incorporate two pharmacologies into a single chemical entity, whose characteristic must be suitable for a once daily inhaled administration. Contextually, we aimed at a locally acting therapy with limited systemic absorption to minimize side effects. Our lung-tailored design of bifunctional compounds that combine the muscarinic and β-adrenergic pharmacologies by the elaboration of the muscarinic inhibitor **7**, successfully led to the potent, pharmacologically balanced muscarinic antagonist and β_2 agonist (MABA) **13**.

Chronic obstructive pulmonary disease (COPD) is a debilitating lung disease, characterised by progressive airflow limitation, and is expected to be the third most common cause of death worldwide by 2030.[1] Exacerbations and respiratory symptoms, which worsen quality of life and increase morbidity and mortality, are common manifestations. In COPD, the dysfunction in autonomic nerve regulation of airway smooth muscle tone plays a pivotal role in sustaining the airflow limitation and it is the primary reversible component.[2] Therefore, current treatment guidelines emphasize the use of bronchodilators in all stages of the disease, with inhaled β-adrenergic agonists and muscarinic (M_3) antagonists being the two mainstays of treatment.[3] Recent advances have delivered long acting muscarinic antagonists (LAMA) and long acting β_2 agonists (LABA) to enable once-daily dosing regimens. The finding that targeting both of these pharmacological targets provides enhanced clinical efficacy has resulted in the combination of LABA and LAMA into a single device, such as Anoro™ (umeclidinium and vilanterol), Ultibro™ (indacaterol and glycopyrronium) and Spiolto™ (tiotropium and olodaterol) (see Fig. 1).[4,5,6]

This approach targets the symptomatic control of bronchoconstriction but has no impact on the underlying chronic inflammation associated with COPD. To meet this end, the additional use of an inhaled corticosteroid (ICS) is recommended for COPD patients at high risk of exacerbations. Traditional inhaler design has only been able to deal with a binary combination of drugs for several years; however, clinical data from GSK (fluticasone furoate/umeclidinium/vilanterol), AstraZeneca (budesonide/glycopyrronium/formoterol fumarate) and Chiesi (beclomethasone/formoterol/glycopyrronium) have shown that triple therapy in a single device (LAMA / LABA / ICS) is now a reality.[7,8,9,10] Nevertheless, the technological complexity encountered in the development of a "triple therapy inhaler" might be reduced by incorporating two of the pharmacologies in a single molecule. In fact, the development of formulations to allow the use of more than one drug class in a single inhaler

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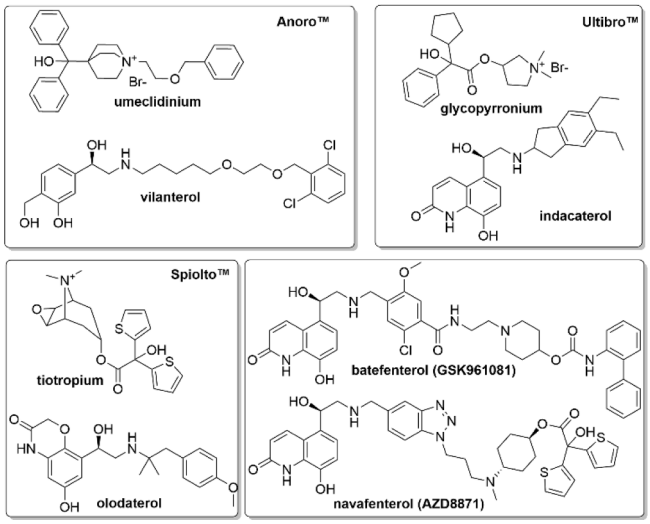


Fig. 1. Structures of reference compounds.

is sometimes challenging, as there are often differences in duration of action of the monocomponents and issues concerning chemical compatibility and stability, as well as galenic challenges relating to the different physiochemical properties of the different drug classes.[11] Therefore, the advent of a bifunctional molecule could provide several advantages relative to fixed combination products, which include simplified clinical development and more straightforward pharmacokinetic profile, facilitated development of combinations with a third therapeutic modality[12] and the possibility to harness the synergy between the mechanisms.[13] These ideas have given rise to the MABA dual pharmacology molecule concept of combining the LAMA and LABA moieties into a single molecule.[14] The conjugation of previously reported muscarinic fragments with the quinolinone β_2 pharmacophore present in indacaterol has furnished a series of MABAs, resulting in the discovery of the Theravance/GSK clinical candidate batefenterol (GSK961081)[15] and navafenterol (AZD8871).[16]

Both batefenterol and navafenterol reached Phase II clinical studies, the first both as a standalone therapy and in "triple therapy" mode in conjunction with the ICS fluticasone furoate while the latter was administered as a standalone therapy. The clinical investigation of both compounds appears to be discontinued with the inference, based upon available data, that neither compound provided a good balance of the antimuscarinic and the adrenergic properties, exerting one of the two as the predominant clinical mode of action. Batefenterol behaved as a potent β_2 agonist but was less potent than Tiotropium in its anti-M_3 efficacy, whereas navafenterol showed a more marked antimuscarinic activity.[17]

Herein we wish to report our efforts in the conversion of previously described M_3 antagonists into MABAs. Our aim was to deliver a MABA compatible with once daily dosing that displayed a balanced efficacy profile at each pharmacological target over the 24 h period. Such an efficacy profile would ensure that any clinical effect was achieved without effectively overdosing the compound at either receptor. In order to minimise interactions with cardiac β receptors, thus mitigating any potential cardiac liabilities, and peripheral muscarinic receptors, curbing the xerostomia associated with the clinical use of M_3 selective anticholinergic agents, we wished to limit the systemic exposure of our compounds. To meet the requirement for low systemic exposure we initially targeted compounds with high tPSA, (topological polar surface area), since membrane permeability is typically reduced when tPSA exceeds 140 Å[18], thus limiting the potential absorption of the swallowed component of any inhaled dose, and high clogP to maximize plasma protein binding, given that in general the more lipophilic compounds display lower free fraction in plasma.[19] Achieving both these goals

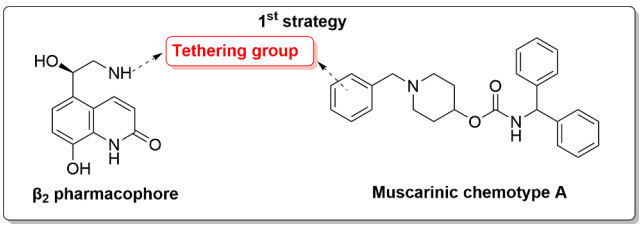


Fig. 2. MABA design - 1st Strategy.

would have reduced the interaction of the MABA with peripheral muscarinic and β-receptors, preventing undesired side-effects.[20]

Investigation of the congested intellectual property space surrounding MABAs had identified carbamate chemotype **A** (Fig. 2) as a potential muscarinic fragment on which to build our new series of MABAs.[21] Chemotype **A** had not been previously incorporated into any bifunctional compounds and offered the attractive possibility to take advantage of its benzylic moiety as a chemically accessible anchoring point to grow the linker towards the β_2 fragment. In common with the approach described by Theravance, we decided to retain the same quinolinone β_2 pharmacophore.

Unfortunately, applying the strategy of linking the individual muscarinic and β-adrenergic moieties through the piperidine nitrogen (Fig. 2), as previously described by Theravance in the discovery of GSK961081, with a variety of linkers failed to deliver a MABA with measureable affinity for the M_3 muscarinic receptor. As for every bifunctional molecule, indeed, the choice of the exit vector attachment point has a high impact on the activity of the final compound. A new strategy in which the linker was appended to one of the phenyl rings in the muscarinic chemotype, surprisingly lead to recovery of affinity for the M_3 muscarinic receptor (Fig. 3). This strategy allowed for the introduction of a wide range of possible amines in the muscarinic "headgroup". Examination of the previously reported work by our[22] and other groups[23] on this scaffold led us to concentrate our efforts on compounds containing the (*R*)-3-hydroxyquinuclidine as the amine of choice for incorporation in our new series of MABAs, since its adoption into similar chemotypes led to selective (displaying fewer M_2-related side-effects) and potent inhibitors.[24]

To aid in the synthesis of our newly designed MABAs, the linker was attached to one of the phenyl groups via an ether linkage and the initial findings from this exploration are shown in MABAs **1** to **6** in Table 1 (assay used to determine binding to the individual receptors are detailed in the table footnotes).

The first linker selected for introduction into our series of MABAs was the simple nonyl linker previously described by Theravance.[10] The affinity difference in M_3 binding in MABAs **1** and **2** suggested that optimal M_3 binding was associated with the *meta*-substitution pattern in the linking ring. In contrast, the substitution pattern of the linking ring did not impact on the affinity for the β_2 receptor. Satisfyingly, the addition of the linking chain and the β-moiety did not have a negative impact on the affinity of **2** for the M_3 receptor compared to the unsubstituted pure M_3 antagonist **7**. Compounds **1** and **2** were next assessed in

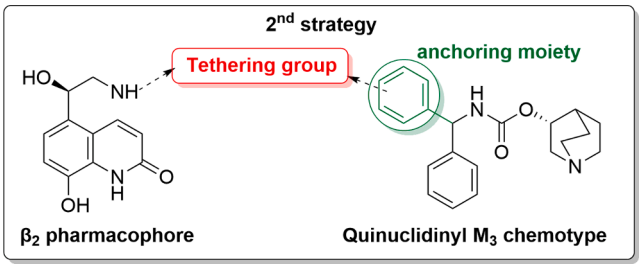
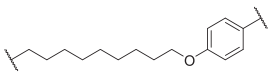
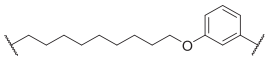
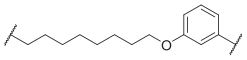
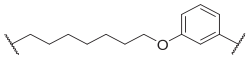
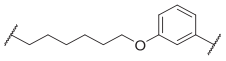
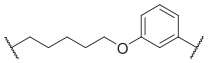
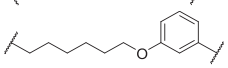
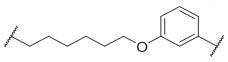
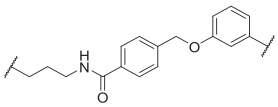
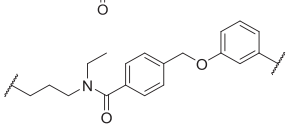
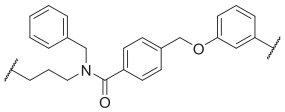
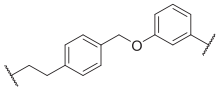
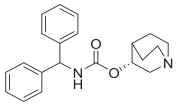


Fig. 3. MABA design - 2nd Strategy.

Table 1
SAR (Structure Activity Relationships) of the new MABAs designed applying the 2nd Strategy (Fig. 3).

MABA	Tethering group	R/S	pK_i		GPT pIC_{50}[c]			Duration of action MABA (mins)	clogP	tPSA[d]	PPB[e]	
			M_3[a]	β_2[b]	M_3	β_2	MABA				h	r
1		*R/S*	8.0	9.6	8.3	7.3	7.9	>270	5.5	132	nd	nd
2		*R/S*	9.6	9.7	7.5	7.3	7.9	>270	5.5	132	nd	nd
3		*R/S*	9.8	9.7	8.7	7.8	9.2	<90	4.9	132	nd	nd
4		*R/S*	9.4	10.0	9.3	8.1	8.7	<90	4.5	132	nd	nd
5		*R/S*	9.5	10.8	9.9	9.8	9.7	<135	4.3	132	nd	nd
6		*R/S*	9.4	10.6	8.5	9.0	9.1	<45	3.8	132	nd	nd
8		*R*	8.4	10.4	nd	nd	nd	nd	4.3	132	nd	nd
9		*S*	9.9	9.3	nd	nd	nd	nd	4.3	132	nd	nd
10		*S*	10.0	10.9	9.7	9.8	9.8	>270	3.5	162	95	95
11		*S*	9.6	10.3	9.5	9.5	10.1	>225	4.0	153	96	94
12		*S*	9.9	10.0	8.7	9.0	8.7	>180	5.4	153	>99	97
13		*S*	9.9	9.6	9.3	9.1	9.2	>270	4.5	132	98	98
GSK961081			8.4 8.9	9.8	9.3	9.1	9.4	90	4.4	162	99	ns

[a] Binding assay, human cloned M_3 receptor using [^{3}H]-*N*-methylscopolamine.
[b] Binding assay, human cloned β_2 receptor using ^{125}I-cyanopindolol.
[c] GPT (Guinea Pig Trachea) assay see text and SI for experimental details.
[d] Topological Polar Surface Area determined within ACDLabs Percepta Portal.
[e] Plasma protein Binding. h = human; r = rat. nd not determined. ns not stable.

the isolated guinea pig trachea (GPT) assay.[25] In this assay, the compounds were tested for their ability to inhibit the contraction of the isolated tissue caused by either the muscarinic agonist carbachol, to determine the MABA potency and efficacy, or histamine (bronchoconstriction H1-mediated, counteracted by the β_2 component but not by the M_3 component), to estimate the β_2 component of the MABA effect. The M_3 component of the MABA effect was then estimated on carbachol contraction by pharmacologically blocking the β_2 element of the MABA effect with the pan β-antagonist propranolol.

A poor translation from binding affinity to functional efficacy was observed at both pharmacological targets for **2**, with a 100-fold reduction in the individual M_3 and β_2 responses in the isolated GPT compared to their binding affinity estimates. Curiously, no such reduction was observed with **1** in its M_3 profile. Gratifyingly the response of **2** in the MABA arm of the experiment was maintained throughout the duration of the experiment (>4h). Our initial investigation targeted the impact that linker length had on the affinities at both the M_3 and β_2 receptor, and the subsequent translation into efficacy in the GPT. No significant impact on affinity for the M_3 receptor was observed on reducing the chain length from nine (**2**) to five (**6**). A ten-fold improvement in binding affinity to the β_2 receptor was seen upon the truncation of the linking chain, suggesting that the muscarinic "head-group" may have a positive interaction with the β_2 receptor and thus on β_2 affinity. This finding is in line with previous work that showed how a synergistic effect enhancing binding affinity for the multivalent ligand relative to monovalent fragments can be appreciated for such bifunctional compounds, due to crosstalk mechanisms.[26,27] Furthermore, a possible allosteric effect should be taken into account since MABA can be considered a bitopic ligand with an orthosteric site-binding moiety and an allosteric site-binding moiety. It has been suggested that its orientation relative to orthosteric and allosteric sites present in the cavity of both receptors, is bimodal and they are able to interact at the same time with both sites[28], shedding light on a possible structural reason behind their synergistic behaviour. The other impact that this linker shortening had was on the translation from binding affinity to functional GPT efficacy; MABA **5** showed an excellent translation. This property may be partially

Table 2
In vivo profile of **12** and **13**. See text for definition of terms.

MABA	Dose (nmol / kg)	% inhibition of bronchoconstriction					
		at 1 h post dose			at 24 h post dose		
		M_3 (n)	β_2 (n)	MABA (n)	M_3 (n)	β_2 (n)	MABA (n)
12	1.0	73 ± 7 (6)	27 ± 8 (8)	73 ± 11 (6)			
12	3.0	73 ± 7 (4)	63 ± 6 (6)	86 ± 6 (4)	51 ± 7 (6)	20 ± 5 (6)	64 ± 7 (6)
13	1.0	33 ± 6 (10)	20 ± 8 (7)	64 ± 6 (10)	44 ± 10 (5)	42 ± 2 (6)	71 ± 11 (5)
13	3.0	78 ± 6 (7)	67 ± 9 (6)	91 ± 4 (8)	54 ± 7 (6)	35 ± 7 (6)	77 ± 8 (7)

n = number of replicates.

governed by the reduction in clogP associated with **2**, and the expected improved solubility and reduced non-specific tissue binding associated with **5**, forasmuch as permeability through cell membranes is strongly dependent on the molecule physicochemical properties, among which lipophilicity plays a key role. **5** reaches an adequate balance between hydrophobicity, needed to effectively cross the hydrophobic phospholipid bilayer, and hydrophilicity, necessary to achieve solubility in aqueous body fluids before being absorbed. Unfortunately, the duration of action previously observed with the nonyl **2** was lost upon truncating the linker to hexyl (**5**).

MABAs **1** to **6** were initially prepared as a mixture of diastereoisomers at the benzhydryl carbon; however, the identification of **5** allowed for investigation into the effect that chirality at this position had on the pharmacological profile of our MABAs.

The individual isomers of MABA **5** were prepared as **8** and **9** and their profiles shown in Table 1. As expected from the proximity of the chiral centre to the muscarinic "head-group," a significant difference in affinity for the M_3 receptor was observed, with the (*S*)-isomer **9** showing a 30-fold increase over the corresponding (*R*)-isomer. Less expected, due to the distance separating the resolved stereogenic center and the β_2 pharmacophore was the finding that there was a 10-fold difference in the β_2 affinity for the two isomers, with the (*R*)-isomer unfortunately displaying the higher affinity. In light of this finding, further exploration of the linker was undertaken, whilst maintaining the optimal (*S*)-benzhydrylamine isomer, since changes proximal to the β-head group would be expected to heavily influence the β_2 affinity of the newly prepared MABAs.

Using MABA **9** as a starting point, a wide range of linkers were prepared, with a selection shown in Table 3. No significant impact on the affinity of the newly prepared MABAs at the M_3 receptor was observed; however, as predicted, the nature of the linker had a large effect on the affinity profile at the β_2 receptor. This finding was highlighted by the high affinity observed with **10**, which displayed a 30-fold increase in affinity over **9**. The aim of generating MABAs with high plasma protein binding was realized with **12** and **13**; unfortunately, this high binding was at the expense of a good translation from binding affinity to functional efficacy in the GPT assay.

MABAs **12** and **13** were next evaluated in an *in vivo* model of bronchoconstriction.[29] The compounds were dosed as liquid formulation (solution) *via* intra-tracheal (IT) administration to an anesthetized guinea-pig and their impact on bronchoconstriction induced by either acetylcholine (M_3 and β_2 effect) or histamine (β_2 effect only) assessed using a modified Konzett-Roessler methodology.[30] In analogy with the GPT assay, the antimuscarinic effect was assessed by challenging the animals with acetylcholine, silencing the β_2-mediated response with a selective β_2-antagonist, ICI118,55118. The data from these experiment is shown in Table 2.

Compound **12** showed a dose dependent inhibition of bronchoconstriction in the β-arm of the experiment. When tested at the higher dose (3 nmol/kg), **12** showed a balanced efficacy profile across both M_3 and β_2 receptors at one hour post dosing. However, when the efficacy was determined 24 h after dosing, the inhibition against acetylcholine-induced bronchoconstriction was heavily biased towards the antimuscarinic effect.

In contrast, a clear dose response effect was observed for **13** in all three arms of the experiment when examined one hour after dosing. A balance between the two pharmacologies, in agreement with that previously observed in the GPT assay, was observed at this time point.

When the 24 h efficacy of **13** was determined, no significant difference in efficacy was observed between the two doses, with the individual M_3 and β_2 efficacies balanced across both doses. This finding fulfils one of our original objectives of delivering a MABA that showed a balanced efficacy against both pharmacological targets over 24 h.

We have shown that compound **13** already displayed high plasma protein binding, which should minimize the free levels of **13** available to interact with either receptor. To further understand the potential systemic fate of **13**, the compound was profiled in a panel of *in vitro* ADME assays (Table 3). Compound **13** displayed low permeability, consistent with the high tPSA, suggesting that systemic exposure from the swallowed portion of the dose would be minimal. As a surrogate for lung stability, **13** was assessed in an isolated lung S9 assay, with **13** displaying a long half-life in all three species tested. Low to modest clearance was determined for **13** in both isolated microsomes and hepatocytes.

In conclusion, we have shown that muscarinic chemotype A can be converted, following the switch to (*R*)-3-quinuclinyl amine in the muscarinic "head-group", into MABAs that show a desired balance of efficacy across the interactions with the muscarinic and β-adrenergic receptors in pre-clinical *in vivo* models over a 24 h period, making them suitable for a one-daily inhaled administration. This remarkably involved, at the time, a novel means of linking the individual muscarinic and β-adrenergic moieties through the lipophilic portion of the muscarinic antagonist differently from previous state-of-the-art, leading to a groundbreaking and unexpected structural finding.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Table 3
In vitro ADME profile of **13**.

MABA	Hepatocyte clearance*			Microsome clearance†		Lung S9 stability $t_{½}$ (min)			PPB#			Caco-2 permeability‡		
	H	GP	R	H	R	H	R	GP	H	GP	R	A-B	B-A	ER
13	6	17	25	16	10	216	200	70	98	99	98	2.0	3.1	1.5

* μL/min/10^6 cells. † μL/min/mg prot. #Plasma Protein Binding %. ‡ x 10^{-6} cm */sec*; A-B = apical to basal; B-A = basal to apical; ER = efflux ratio. H = human; GP = guinea pig; R = rat.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2021.127975.

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25 All experimental procedures involving animal research performed in this work were approved by Italian Ministry of Health (Prot.n 162/2011) and complied with the European and Italian regulations for animal care.
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