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Discovery of muscarinic acetylcholine receptor antagonist and beta 2 adrenoceptor agonist (MABA) dual pharmacology molecules

Adam D. Hughes *, Kay H. Chin, Sarah L. Dunham, Jeffrey R. Jasper, Kristin E. King, Tae Weon Lee, Mathai Mammen, Jerri Martin, Tod Steinfeld

Theravance, Inc., 901 Gateway Blvd, South San Francisco, CA 94080, USA

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ABSTRACT

We sought to design dual pharmacology bronchodilators targeting both the M_3 muscarinic acetylcholine and beta-2 adrenergic (β_2) receptors by applying our multivalent approach to drug discovery. Herein, we describe our initial discovery and the SAR of the first such compounds with matched potencies at both receptors.

Chronic obstructive pulmonary disease (COPD) is the fifth leading cause of death worldwide and its prevalence is thought to be greatly underestimated.[1] COPD is most commonly associated with cigarette smoking; however other risk factors include air pollutants and occupational dust, vapors and fumes. This debilitating disease is characterized by a progressive airflow limitation that is not fully reversible. Treatment guidelines emphasize the use of bronchodilators at all stages of the disease with a combination of long-acting bronchodilators recommended for patients with moderate to severe COPD.[2] The addition of an inhaled corticosteroid (ICS) is recommended for severe and very severe COPD, but recent studies indicate that a broader patient population may benefit from treatment with an ICS in addition to a long-acting bronchodilator.[3]

The most frequently used bronchodilators for COPD are inhaled beta-2 adrenergic receptor (β_2) agonists and inhaled muscarinic acetylcholine receptor antagonists. Short acting or 'rescue' compounds with a 2–4 h duration of action, are recommended for the relief of symptoms in patients with mild COPD. Clinical studies have shown that drugs from these two classes may be used effectively in combination to provide enhanced efficacy.[4] Combivent® consists of a short acting muscarinic antagonist (SAMA) ipratropium[5] and a short acting beta agonist (SABA) albuterol[6] formulated in a single inhalation device.

Longer acting agents for both mechanisms are also prescribed such as formoterol (**1**),[7] salmeterol (**2**)[8] (*bid* LABAs) and tiotropium (**3**)[9] (*qd* LAMA). New once daily LABAs such as indacaterol (**4**)[10] and GW642444[11] and a twice daily LAMA, aclidinium (**5**),[12] are in late stage clinical trials. Combination studies with tiotropium and formoterol have confirmed that the complementary effects of the two mechanisms can provide greater improvements in lung function compared to single agent bronchodilators.[13]

Due to the treatment successes of combination products such as Combivent®, Advair®[3] (salmeterol and fluticasone propionate) and Symbicort®[14] (formoterol and budesonide), it is anticipated that a coformulation of a LABA with a LAMA will soon be available.[15]

In this Letter, we describe an alternative approach to obtain dual pharmacology through the design of compounds possessing dual activity as muscarinic antagonists and β_2 agonists (MABAs).

By providing both activities in a single molecule, there is the potential to provide efficacy comparable to a combination product

* Corresponding author.
E-mail address: ahughes@theravance.com (A.D. Hughes).

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without the challenges of coformulation and clinical development of a combination of two active agents. The MABA approach also offers the potential for simplified pharmacokinetics and the opportunity for a binary combination with an ICS to achieve 'triple therapy' in a single inhalation device.

Using our multivalent approach to drug design we investigated the tolerability of a secondary binding site motif with an additional pharmacology on both the muscarinic (M_3) and β_2 receptors. Our prior experience with lead optimization for both muscarinic antagonists[16] and β_2 agonists[17] indicated that distal secondary binding sites are accessible on both receptors for single pharmacology compounds.

Schemes 1–9 illustrate the preparation of the compounds described in this Letter. We had previously investigated muscarinic receptor antagonists for the treatment of overactive bladder and had identified the biphenyl carbamate (**8a**) as a potent M_3 receptor antagonist with tolerance for piperidine N-substitution.[18] Our search for a secondary binding site began by evaluating different alkyl chain lengths to probe for a lipophilic pocket while concomitantly assessing the tolerance of the piperidine nitrogen to steric bulk, Table 1, Scheme 1.[19]

For alkyl chains of 6–9 atoms in length, a potency gain of 5–10-fold was observed at the M_3 receptor relative to the unsubstituted piperidine **8a**. Since both shorter and longer alkyl chains led to reduced potency we concluded that compounds **9f–i** were more suited to accessing a secondary binding site on the muscarinic receptor.

To quickly assess the tolerance of a basic moiety at the secondary binding site, a primary amine was used as a probe, Table 2, Scheme 2. Since all known efficacious β_2 agonists contain a secondary amine as part of the epinephrine ligand mimic, this would be vital for our MABA design.

Table 1
M_3 potencies of *n*-alkyl chain derivatives of **8a**

Compound	*n*	hM_3 K_i[20] (nM)
8a	NA	46
9a	0	22
9b	1	55
9c	2	77
9d	3	17
9e	4	14
9f	5	4.8
9g	6	5.1
9h	7	7
9i	8	9.5
9j	9	25
9k	10	48

Table 2
M_3 potencies of *n*-alkyl primary amine derivatives of **8a**

Compound	*n*	hM_3 K_i (nM)
10a	2	50
10b	3	67
10c	4	84
10d	5	98
10e	6	19
10f	7	0.53
10g	8	1.8
10h	9	0.75
10i	10	1.6
10j	11	2.5
10k	12	2.6

Evaluation of a series of aminoalkyl substituents showed that linkers of 7–12 carbon atoms between the two basic nitrogens afforded low nanomolar potency at M_3 (**10f–k**). Based on the combined results from the alkyl and primary amine series, we focused on 7- to 9-carbon linkers to elaborate the primary amine further to build in β_2 agonist activity.

Substituting the primary amine with selected β_2 agonist fragments (shown in Fig. 1) gratifyingly provided a further enhancement of M_3 receptor binding potency for all entries in Table 3,

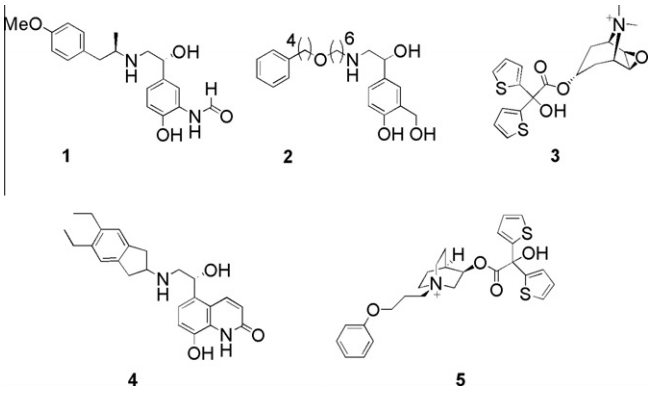
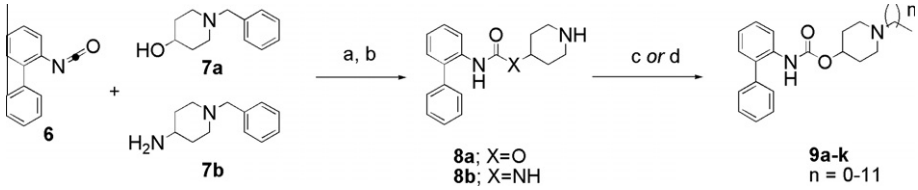
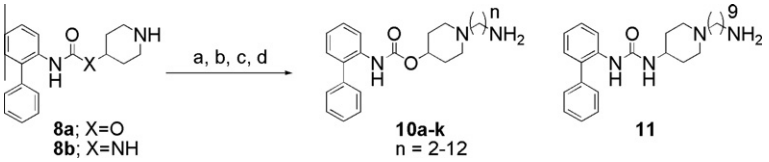


Figure 1. Marketed respiratory medicines and those in late stage clinical development.



Scheme 1. Reagents and conditions: (a) 70 °C, neat; (b) NH_4HCO_2, cat Pd/C, 40 °C, EtOH, 95% over 2 steps; (c) aldehyde, $Na(OAc)_3BH$, 1:1 DCM–MeOH, 50–80%; (d) alkyl halide, DIPEA, DMF, 45–75%.



Scheme 2. Reagents and conditions: (a) bromoalcohol, DIPEA, DMF, 50 °C, 49–82%; (b) DMSO, Py·SO_3, DIPEA, DCM, 0 °C, 60–90%; (c) dibenzylamine, $Na(OAc)_3BH$, 1:1 DCM–MeOH, 70–85%; (d) H_2, cat $Pd(OH)_2$, EtOH, 70–90%.

Table 3
M_3 potency, β_2 potency and functional data (EC_{50}) for biphenyl carbamate MABAs containing various β_2 pharmacophores

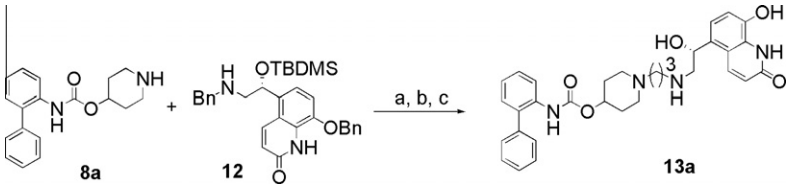
Compound	*n*	hM$_3$ K_I (nM)	HEK hβ2 K_I (nM)	HEK hβ2 EC_{50}[22] (nM)
13a	3	28	19	1.3
13b	7	0.74	10	1.1
13c	8	0.32	3.4	0.32
13d	9	0.01	3.5	0.36
17	9	0.14	21	2.6
19	9	0.14	61	14
1	NA	NA	33	0.19
2	NA	NA	2.4	0.33
3	NA	0.034	NA	NA
4	NA	NA	61	0.82

Schemes 3–5. The carbostyril and hydroxy formanilide β_2 moieties present in **13a–d** and **17**, respectively, offered superior β_2 potencies relative to the saligenin containing compound **19**. Additionally two compounds demonstrated matched, subnanomolar potencies[21] at both the M_3 and β_2 receptors (**13c** and **13d**).

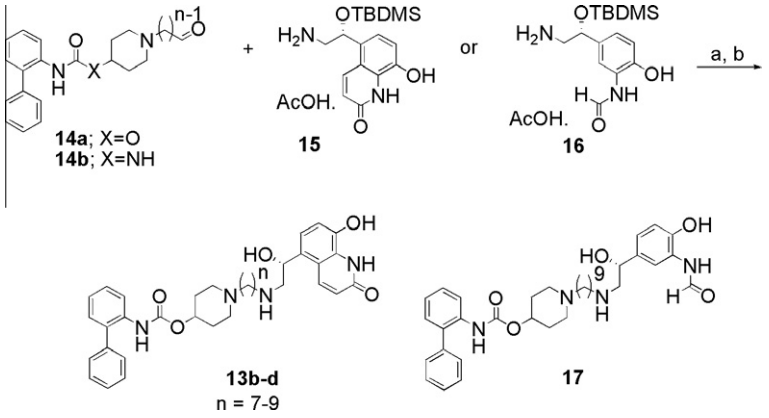
Maintaining the carbostyril β_2 moiety, several other muscarinic receptor binding motifs were investigated, Schemes 6–9. Table 4 illustrates that all of these compounds retained activity, but were inferior to the biphenyl carbamate compounds described in Table 3. Intriguingly, not only was potency reduced at the M_3 receptor, but also at the β_2 receptor, thus indicating the considerable influence of the secondary binding muscarinic motif on binding to the β_2 receptor.

It is interesting to note that although the marketed inhaled muscarinic antagonists ipratropium and tiotropium both contain quaternary ammonium groups, we found the incorporation of a quaternary ammonium pharmacophore into our bifunctional MABA constructs resulted in lower affinities for the M_3 receptor (**29** and **31**).

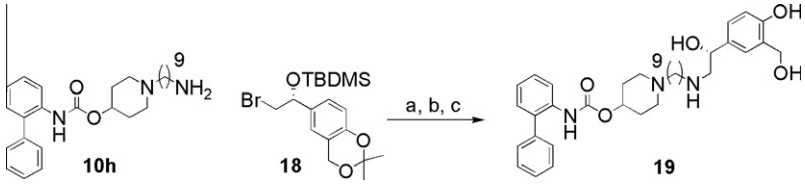
We have discovered several compounds with matched in vitro M_3 and β_2 potencies using a linked multivalent discovery approach.



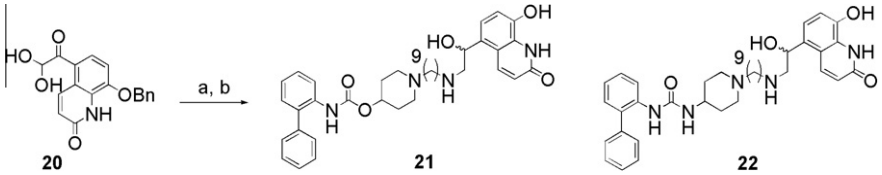
Scheme 3. Reagents and conditions: (a) **12**,[23] 1,3-dibromopropane, $NaHCO_3$, DMF, 50 °C, 55%; (b) H_2, cat $Pd(OH)_2$, EtOH; (c) $Et_3N{\cdot}3HF$, 1:9 DMF–DCM, 20% over 2 steps.



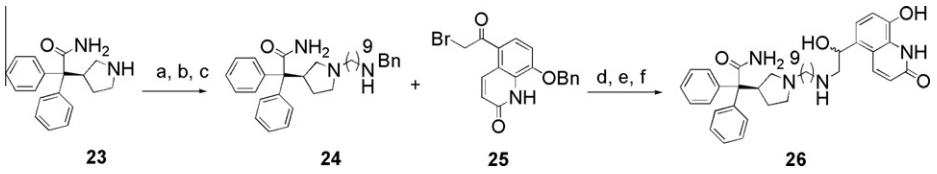
Scheme 4. Reagents and conditions: (a) Amine **15**[21] or **16**[24], $Na(OAc)_3BH$, 1:1 DCM–MeOH, 60–75%; (b) $Et_3N{\cdot}3HF$, 1:1 DMF–DCM, 60–70%.



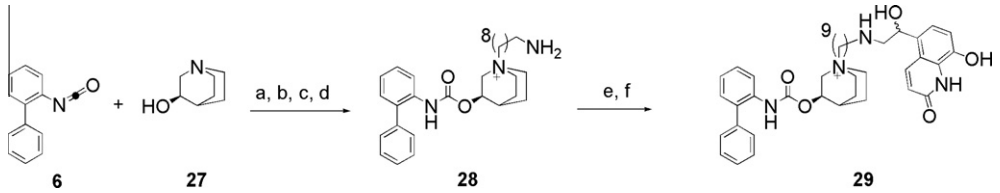
Scheme 5. Reagents and conditions: (a) **18**,[25] $NaHCO_3$, NaI, THF, 80 °C, 60%; (b) $Et_3N{\cdot}3HF$, 1:9 DMF–DCM; (c) TFA, 1:1 THF–H_2O, 30% over 2 steps.



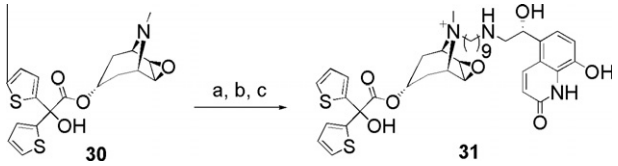
Scheme 6. Reagents and conditions: (a) **10h** or **11**, $Na(OAc)_3BH$, **20**,[24] DCE, 40–50%; (b) H_2, cat Pd/C, MeOH, 10–28%.



Scheme 7. Reagents and conditions: (a) **23**,[26] 1-bromononan-9-ol, Et_3N, MeCN, 50 °C, 62%; (b) DMSO, Py·SO_3, DIPEA, DCM, 0 °C, 90%; (c) benzylamine, Na(CN)BH_3, MeOH, 46%; (d) **25**,[23] K_2CO_3, DMF, 45 °C; (e) $NaBH_4$, EtOH, 50% over 2 steps; (f) H_2, cat Pd/C, AcOH, 20%.



Scheme 8. Reagents and conditions: (a) 70 °C, neat, 95%; (b) 1,9-dibromononane, Et_3N, MeCN, 50 °C, 52%; (c) NaH, di-*tert*-butyl iminodicarboxylate, DMF; (d) 1:3 TFA–DCM, 23% over 2 steps; (e) **20**, Na$(OAc)_3$BH, DCE, 38%; (f) H_2, cat Pd/C, MeOH, 10%.



Scheme 9. Reagents and conditions: (a) **30**,[27] 1,9-dibromononane, DIPEA, DMF, 50%; (b) **15**, DMF, 50 °C; (c) Et_3N·3HF, 1:9 DMF–DCM, 5% over 2 steps.

Table 4
M_3 potency, β_2 potency and functional data (EC_{50}) for carbostyril MABAs containing various M_3 pharmacophores

Compound	hM$_3$ K_I (nM)	HEK hβ2 K_I (nM)	HEK hβ2 EC_{50} (nM)
21[a]	0.18	13	1.1
26	32	60	3.3
22	7.5	115	8.4
29	140	123	8.9
31	4.6	36	0.9

[a] This is the racemic derivative of **13d**. All marketed β_2 agonists now possess the (*R*) enantiomer, however for our early investigations the racemic mixtures served as useful research tools and offered comparable in vitro potencies.

A biphenyl carbamate was identified as the preferred muscarinic moiety while a carbostyril was favored for β_2 potency. Interestingly potency at both receptors was affected when either group was modified or changed, suggesting a secondary binding site is being accessed in both cases.

Furthermore, the MABA compounds **13c** and **13d**, had subnanomolar M_3 potencies and additionally possessed subnanomolar β_2 activity equivalent to LABAs such as formoterol (**1**), salmeterol (**2**) and indacaterol (**4**).

The compounds in this article follow on from our first disclosure of the MABA concept[25] with publications from other organizations now appearing in the literature.[28] A related publication from Theravance is now available suggesting that **13d** exhibits a multivalent bimodal orientation in the orthosteric and allosteric binding pockets of the M_3 and β_2 receptors.[29] Future publications will disclose the in vivo activity of the lead compound **13d**.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.01.043. These data include MOL files and InChiKeys of the most important compounds described in this article.

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19. All yields are unoptimized. Final step yields in each scheme refer to isolated compounds after purification by reverse phase preparative HPLC.
20. Muscarinic receptor inhibition binding constants (K_I) were measured using a radioligand binding assay. Briefly, cell membranes expressing the human M_3 muscarinic receptor were incubated for up to 6 h at 37 °C with 1 nM [^{3}H] *N*-methyl-scopolamine and various dilutions of test compounds in a buffer consisting of 10 mM Hepes, 100 mM NaCl, 10 mM $MgCl_2$, (pH 7.4 at 37 °C). K_I values were calculated from IC_{50} values according to Cheng, Y.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099. Muscarinic acetylcholine receptor antagonist behavior has previously been reported in our patent publications and confirmed in our in vivo models.
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