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Discovery of TD-4306, a long-acting β_2-agonist for the treatment of asthma and COPD

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ABSTRACT

A multivalent approach focused on amine-based secondary binding groups was applied to the discovery of long-acting inhaled β_2-agonists. Addition of amine moieties to the neutral secondary binding group of an existing β_2-agonist series was found to provide improved in vivo efficacy, but also led to the formation of biologically active aldehyde metabolites which were viewed as a risk for the development of these compounds. Structural simplification of the scaffold and blocking the site of metabolism to prevent aldehyde formation afforded a potent series of dibasic β_2-agonists with improved duration of action relative to their monobasic analogs. Additional optimization led to the discovery of **29** (TD-4306), a potent and selective β_2-agonist with potential for once-daily dosing.

The β_2-adrenoreceptor belongs to the superfamily of seven transmembrane G protein-coupled receptors (GPCRs) and is widely expressed in the respiratory tract. β_2-agonists exert a bronchodilatory effect through relaxation of airway smooth muscle, and as such play a central role in the treatment of respiratory disorders such as asthma and chronic obstructive pulmonary disease (COPD).[1] First generation β_2-agonists such as albuterol have a short duration of action (4–6 h) and are normally used as-needed to relieve acute exacerbations (rescue therapy). Second generation, long-acting β_2-agonists (LABAs) such as salmeterol and formoterol (Fig. 1) are typically used as maintenance therapy due to their longer duration of action (approximately 12 h) and are dosed twice-daily, usually in combination with an inhaled corticosteroid. In an effort to further improve patient compliance and disease control, significant drug development activity has recently focused on third generation β_2-agonists with a duration of action suitable for once-daily dosing.[2,3] Olodaterol (**3**), indacaterol (**4**) and vilanterol (**5**) (in combination with the glucocorticoid fluticasone furoate) have been approved or are in late stage development for the treatment of COPD.

In previous publications we described the application of a multivalent approach towards the discovery of novel, selective, β_2-agonists with improved duration of action, leading to the identification of milveterol[4] (**6**, phase 2) and TD-5471[5] (**7**, phase 1). Two distinct hypotheses concerning the mechanism of increased duration have been proposed. The exosite theory postulates that the duration of action is driven by the LABA molecule binding to an exosite adjacent to the agonist binding site, leading to prolonged and repeated stimulation of the receptor.[6] The diffusion microkinetic theory postulates that the duration of action is driven by partitioning of highly lipophilic β_2-agonists into the lipid bilayer membranes of bronchial smooth muscle cells, which in turn act as a depot providing effective concentrations of the agonist over an extended timeframe.[7] Irrespective of the precise mechanism by which duration is achieved, the optimized linkers and secondary binding groups (Fig. 2) of **6** and **7** are the key to their enhanced duration of action relative to first generation agonists such as salmeterol. In the case of **7**, the linker consists of a 4-phenethylamine moiety whilst the secondary binding group consists of a highly lipophilic biarylamine fragment, which was shown to be particularly beneficial in terms of receptor potency and duration of action in the guinea pig model of bronchoprotection. Herein, we describe an extension of this work in which we explored the introduction of amines to the secondary binding group as a strategy to provide LABAs with differentiated structural and physicochemical properties relative to milveterol and TD-5471. Amines are known to have a high affinity for lung tissue (possibly through lysosomal partitioning), and it was postulated that introduction of a second basic group (to form a dibasic LABA) might enhance this effect and have a beneficial impact on efficacy and duration of action.[8,9] Furthermore, it was envisaged that introduction of an amine to this region of the

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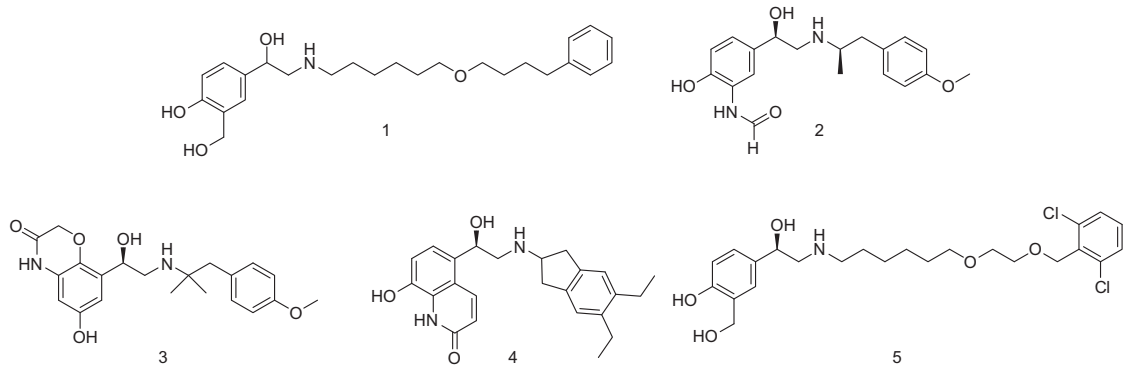


Figure 1. Structures of the β_2-agonists salmeterol (**1**), formoterol (**2**), olodaterol (**3**), indacaterol (**4**) and vilanterol (**5**).

molecule would enhance solubility and therefore facilitate formulation development.

To explore the impact of amine-substituted secondary binding groups on β_2 activity, we initially prepared a series of derivatives of **7** in which a primary aminomethyl moiety was appended to the *ortho*-, *meta*- and *para*-positions of the distal phenyl ring of the secondary binding group (Scheme 1). Synthesis of these secondary binding groups was accomplished by Suzuki coupling of bromide **8** with the appropriate benzonitrile boronic acids. Reduction of the nitrile substituent using borane in THF followed by hydrogenation of the nitro group furnished intermediates **9**–**11**. The headgroup-linker fragment **13** was constructed by alkylation of 4-bromophenethylamine with protected headgroup **12**.[10] Buchwald coupling of intermediates **9**–**11** to bromide **13** followed by deprotection of the headgroup afforded the desired compounds **14**–**16**.

These compounds were profiled in our in vitro screens and compared to standards (Table 1). Binding affinities for the β_1- and β_2-adrenoceptors and β_2/β_1 selectivity were measured using competition binding assays with [^{3}H]dihydroalprenolol as tracer and plasma membranes from cells expressing the human recombinant β_1- and β_2-adrenoceptors. Agonist potencies were measured in cAMP accumulation assays using recombinant cell lines heterologously expressing the human β_1-, β_2-, and β_3-adrenoceptors, and the BEAS-2B lung epithelial cell line with endogenous expression of the β_2-adrenoceptor. BEAS-2B cells were also used to measure intrinsic agonist activity (IA) relative to isoproterenol. Compounds **14**–**16** illustrate that, relative to **7**, the addition of a basic moiety was well tolerated in terms of β_2-receptor affinity and functional potency. However, β_2/β_1 selectivity of **14** was too low for progression, since selectivity similar to or greater than that of formoterol (**2**) was targeted. Based on their attractive in vitro profile, compounds **15** and **16** were advanced to the guinea pig in vivo bronchoprotection model.[11]

At this stage of our β_2-agonist program, our criteria for advancing compounds required significant bronchoprotective efficacy at the 72 h time point in the guinea pig model. Following nebulized dosing, both **15** and **16** were shown to possess excellent bronchoprotective activity over this extended time frame. Although neither compound showed a statistically significant improvement over **7**, a promising trend towards improved efficacy was apparent (Fig. 3). All three compounds were superior to salmeterol in this model.

During additional preclinical evaluation of compounds **15**–**16**, we observed significant oxidation (potentially catalyzed by monoamine oxidase[12]) of the primary amine functionality to the respective aldehyde in the plasma and/or hepatocytes of various species (Scheme 2). These aldehyde metabolites were subsequently found to have in vitro activity similar to parent molecules at the β_2-receptor (data not shown). The presence of circulating active metabolites bearing a potentially reactive functional group was seen as a risk for the development of these compounds, and further evaluation was suspended.

Based on the encouraging efficacy of compounds **15**–**16**, we sought an alternative strategy by which to incorporate metabolically stable amines into this series. The large number of synthetic steps required to produce **15**–**16** also led us to consider structural simplification of the scaffold. The terminal alkyl ether group of **7** was identified as a convenient synthetic handle with which to rapidly explore a variety of basic amines in the same general region of the molecule. In order to simplify the synthesis and reduce structural similarity to **7**, the distal phenyl ring of **7** was excised. Synthesis of the parent compound **19** was accomplished by coupling BOC-protected 4-bromophenethylamine with 4-ethoxyaniline followed by deprotection to provided intermediate **17**. Treatment of **17** with epoxide **18**[13] followed by debenzylation afforded **19** (Scheme 3). Synthesis of the amine-based compounds was accomplished by nucleophilic aromatic substitution of *p*-fluoronitrobenzene with the alkoxide of the requisite aminoalcohol. Hydrogenation of the nitro group afforded aniline intermediates which were then coupled to intermediate **13** using Buchwald chemistry. Deprotection of the headgroup afforded compounds **20**–**30** (Scheme 4).

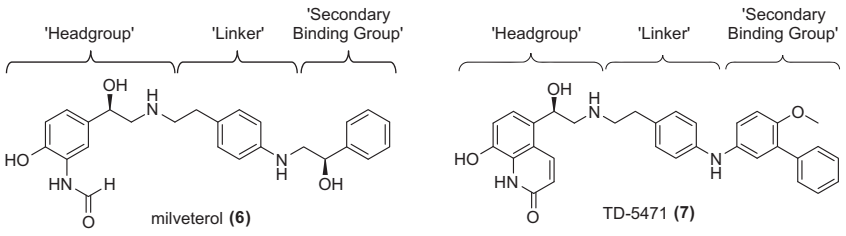
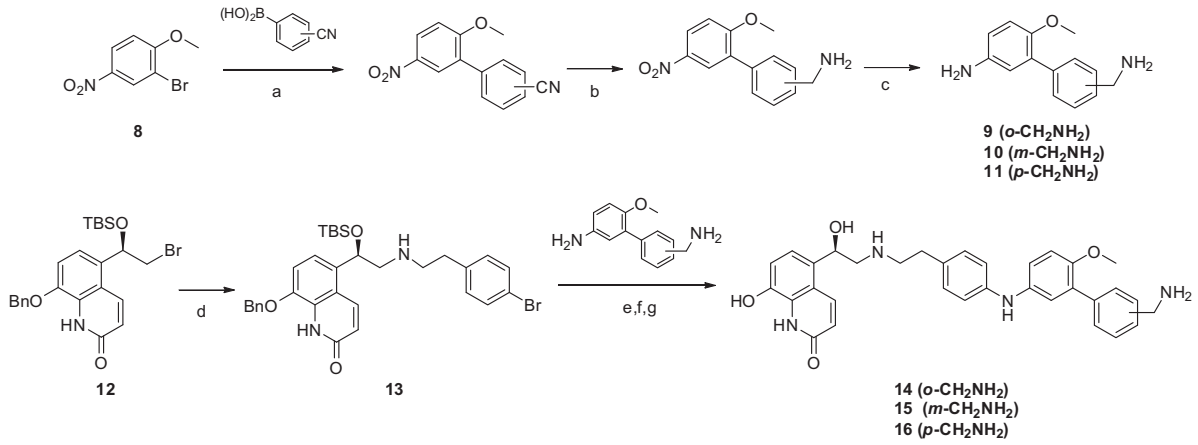


Figure 2. Structure of milveterol (**6**) and TD-5471 (**7**) indicating the β_2- agonist 'headgroup' as well as the regions we have termed the 'linker' and the 'secondary binding group'.



Scheme 1. Reagents and conditions: (a) $PdCl_2(dppf).CH_2Cl_2$, Cs_2CO_3, DME; (b) BH_3.THF, THF; (c) H_2, 10% Pd/C, MeOH; (d) 4-bromophenethylamine, DMSO; (e) **9**–**11**, $Pd_2(dba)_3$, *rac*-BINAP, NaO*t*-Bu, toluene; (f) $Et_3N.3HF$, THF; (g) H_2, 10% Pd/C, MeOH, CH_2Cl_2.

Table 1
In vitro activity of compounds **1**, **2**, **7**, **14**–**16**

Compound	*c*Log*D*$_{7.4}$	Competition binding assay		Recombinant cell lines			Endogenous cell line (BEAS-2B)	
		β_2 pK_i	β_2/β_1 Selectivity	β_1 pEC$_{50}$	β_2 pEC$_{50}$	β_3 pEC$_{50}$	β_2 pEC$_{50}$	IA (%)
1	3.0	8.6	1940	6.6	9.5	6.0	9.3	34
2	0.4	7.5	114	7.9	9.7	7.6	8.6	93
7	3.8	8.2	115	8.1	9.9	7.9	9.4	83
14	1.9	8.5	20	8.6	9.9	8.2	9.1	87
15	2.8	8.8	146	8.4	10.1	7.6	9.5	87
16	2.7	8.9	101	8.6	10.0	8.0	9.3	74

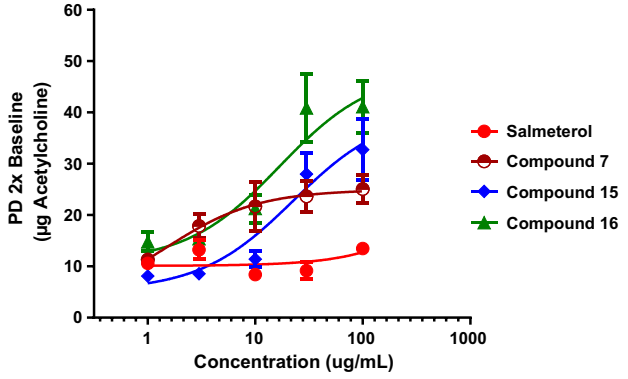
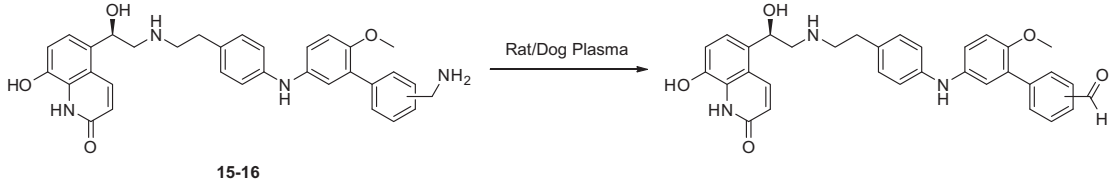


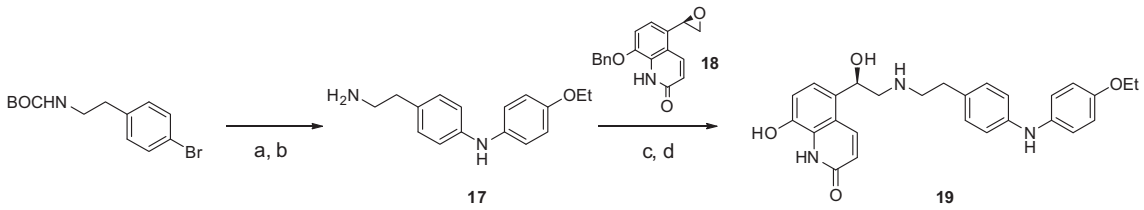
Figure 3. Activity of compounds **1**, **7**, **15** and **16** at 72 h in the guinea pig bronchoprotection model.

The initial series of analogs is shown in Table 2 and are compared to salmeterol (**1**), formoterol (**2**), milveterol (**6**) and TD-5471 (**7**). The parent molecule **19** had moderate binding affinity for the β_2-receptor, but was equipotent with salmeterol in the recombinant functional assay, and was a full agonist. Addition of a primary amine to this secondary binding group (compound **20**) was beneficial in terms of both β_2 binding affinity and functional potency, without detriment to selectivity. Introduction of the amine group results in a two log unit reduction in *c*log*D*, suggesting that reducing the lipophilicity had a positive influence on the in vitro agonist potency of this series. Having demonstrated that the desired in vitro profile could be obtained with this novel series of amine-substituted, linear secondary binding groups, we then began a systematic exploration of the preferred position and nature of the amine group.

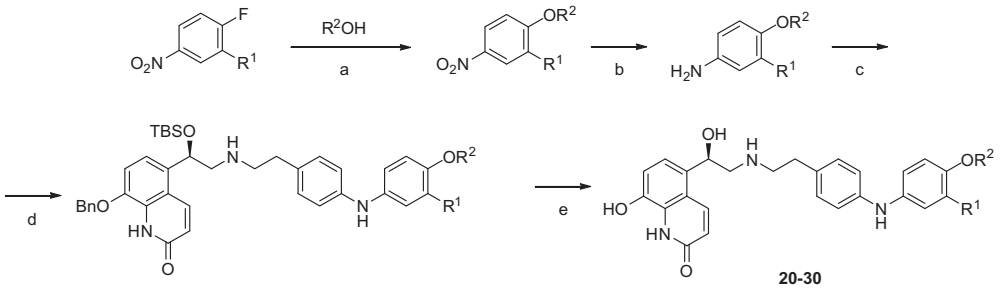
Extension of the alkylamine chain by 1–3 atoms (compounds **21**–**23**) had no significant impact on the measured in vitro properties, suggesting general tolerance for a cationic moiety in this region of the binding site. Masking the amine as a bulky *t*-butyl carbamate (**24**) or benzamide (**25**) reduced the β_2/β_1-selectivity to unacceptably low levels and was also deleterious to functional potency and intrinsic activity in the endogenous cell line, relative to the free amine analog **20**. This data suggests that the amine moiety is not simply a spectator fragment but makes a beneficial contribution to the in vitro profile of this series. In order to understand the role of the pK_a of the amine moiety, morpholine (**26**) and piperazine-sulfonamide (**27**) analogs were synthesized. The calculated pK_a of these compounds were 6.6 and 5.9 compared to 8.2 for **20**.[14] This resulted in a significant drop in β_2 binding affinity, β_2/β_1



Scheme 2. Metabolism of compounds **15**–**16**.

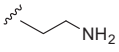
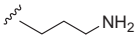
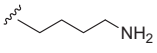
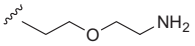
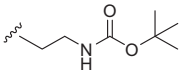
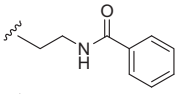
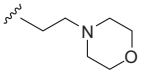
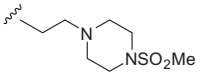
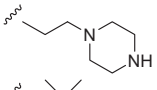


Scheme 3. Reagents and conditions: (a) 4-ethoxyaniline, $Pd_2(dba)_3$, *rac*-BINAP, NaO*t*-Bu, toluene; (b) TFA/CH_2Cl_2 (1:1); (c) **18**, *i*-PrOH; (d) H_2, 10% Pd/C, MeOH.



Scheme 4. Reagents and conditions: (a) NaH, DMSO; (b) H_2, 10% Pd/C, MeOH; (c) **13**, $Pd_2(dba)_3$, *rac*-BINAP, NaO*t*-Bu, toluene; (d) $Et_3N.3HF$, *i*-PrOH, EtOH; (e) H_2, 20% $Pd(OH)_2$, EtOH.

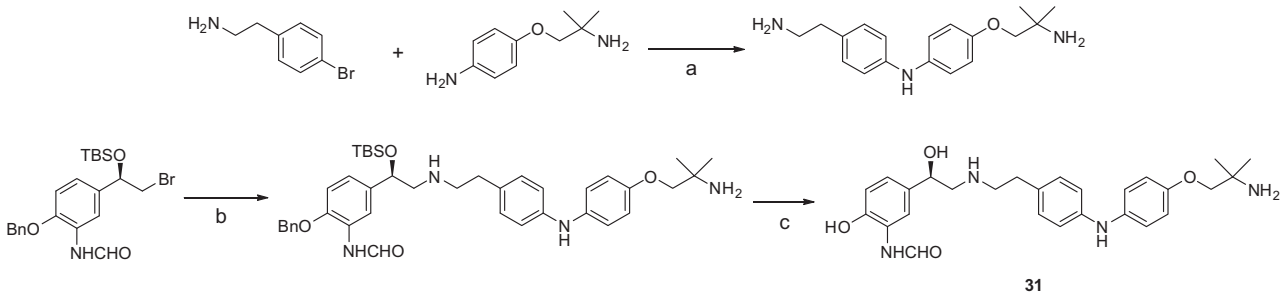
Table 2
In vitro potency and binding selectivity of standards and compounds **19–31**

Compound	R^1	R^2	*c*Log$D_{7.4}$	β_2 pK_i	β_2/β_1 Selectivity	β_1 pEC_{50}	β_2 pEC_{50}	β_3 pEC_{50}	BEAS pEC_{50}	BEAS IA (%)
1	n/a	n/a	3	8.6	1940	6.6	9.5	6	9.3	34
2	n/a	n/a	0.4	7.5	114	7.9	9.7	7.6	8.6	93
6	n/a	n/a	0.9	8.5	2910	7.6	10.2	7.4	9.3	87
7	n/a	n/a	3.8	8.2	115	8.1	9.9	7.9	9.4	83
19	n/a	n/a	2.6	7.9	72	ND	9.6	ND	8.7	104
20	H		0.4	8.2	64	8.1	10	7.4	9.3	82
21	H		0.5	8.3	95	8.1	9.8	7.4	9.2	80
22	H		1.1	8.3	85	8.1	9.9	7.4	9.2	74
23	H		0.3	8.2	95	8.1	9.8	7.4	9.2	80
24	H		3	7.7	26	7.9	9.3	7.9	8.6	67
25	H		3.1	8	13	8.7	9.3	7.9	8.8	67
26	H		2	7.7	41	7.9	9.5	7.3	8.5	49
27	H		−0.2	7.5	30	8.1	9.2	7.5	8.2	53
28	H		0.2	8	116	8	9.9	7.4	8.8	71
29	H		1	8.2	64	8.2	10.1	7.2	9	70
30	Ph		2.5	9	140	8.8	10.2	8.1	9.2	77
31	n/a	n/a	0.3	7.6	94	8.2	9.6	7.4	8.5	68

β_2/β_1 Selectivity is expressed as the ratio of K_i's (β_1 K_i/β_2 K_i).

selectivity, agonist intrinsic activity, and functional potency in both recombinant and endogenous β_2 cell lines. The piperizine analog **28**, sterically similar to the morpholine but with greater basicity (pK_a = 9.1) restored these in vitro properties to levels similar to **20**. Thus, the pK_a of the amine was important for optimal potency.

As previously noted for compounds **15** and **16**, the inclusion of a primary amine in these agonists was associated with formation of



Scheme 5. Reagents and conditions: (a) $Pd_2(dba)_3$, *rac*-BINAP, NaO*t*-Bu, toluene; (b) K_2CO_3, NaI, DMSO; (c) (i) $Et_3N{\cdot}3HF$, THF; (ii) H_2, 20% $Pd(OH)_2$, EtOH.

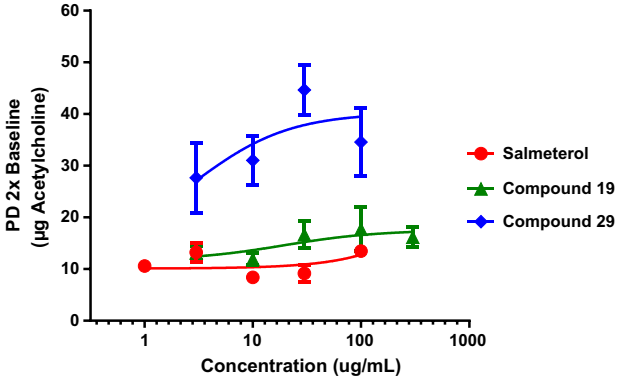


Figure 4. Activity of compound **29** relative to compounds **19** and **1** in the 72 h guinea pig bronchoprotection model.

biologically active aldehyde metabolites in plasma of various species. This liability was also found to present to varying degrees in compounds **20**–**23** (12–100% aldehyde formation in dog plasma). In order to block this metabolic process, *gem*-dimethyl analog **29** was synthesized using commercially available β-amino-isobutanol. Introduction of the two methyl groups did not have a detrimental impact on the in vitro profile and negated the possibility of aldehyde formation. Reintroduction of the phenyl ring *ortho*- to the alkoxy amine chain (**30**) was explored. Whilst this structural change boosted the β_2 binding affinity and selectivity, it had no impact on the functional potency and also introduced greater structural similarity to the previous clinical candidate **7**. Finally, the carbostyril headgroup was replaced with the formanilide headgroup (**31**). The increased chemical sensitivity of this headgroup necessitated an alternative synthesis, in which the complete tail section was synthesized first, followed by alkylation with the protected headgroup[15] and deprotection (Scheme 5). β_2 binding affinity and agonist potency of this compound were inferior to **29**.

Compound **29** was prioritized for evaluation in the guinea pig model of in vivo bronchoprotection. Bronchoprotection at 72 h was dose-dependent and was significantly greater than salmeterol at nebulizer concentrations of $\geqslant$30 µg/mL (Fig. 4). The beneficial impact of basic versus neutral secondary binding groups with respect to prolonged duration of action is readily apparent from comparing the efficacy of **29** relative to the neutral analog **19**, which did not show evidence of bronchoprotection at the 72 h time point.

Based on its long duration in this model, compound **29** was selected for further evaluation. Since a significant fraction of an inhaled therapeutic is swallowed, it is important for novel β_2 agonists to have low oral bioavailability and therefore minimize the potential for toxicity mediated by systemic exposure. In rats **29** exhibited low clearance (0.4 L/h/kg), but its oral bioavailability was only 1% presumably due to poor absorption from the GI tract. In dogs, **29** exhibited high clearance (2.4 L/h/kg) as well as low oral bioavailability (5%). This compound also had negligible inhibition of the hERG potassium ion channel at physiologically relevant concentrations (7% at a concentration of 40 nM). In a broad panel off-target screen (CEREP), compound **29** tested at 1 µM showed binding activity only at muscarinic receptors. Follow-up studies demonstrated low binding affinity towards the five human muscarinic acetylcholine receptor subtypes, with highest binding affinity observed for the M_2 subtype ($pK_i = 6.2$).

In an effort to discover novel and selective β_2 agonists with extended duration of action relative to salmeterol, we explored the introduction of basic secondary binding groups to the LABA scaffold. The dibasic nature of the resultant compounds was also expected to provide differentiated solid form, ADME and toxicological profiles relative to monobasic compounds **6** and **7**. Compared to their neutral analogs, basic secondary binding groups appear to extend the duration of bronchoprotective activity in the guinea pig model. The origin of this extended duration may be higher affinity for lung tissue commonly associated with basic molecules. These basic secondary binding groups also maintained suitable in vitro selectivity over closely related β-adrenoceptor subtypes. Optimization of the linker and secondary binding groups afforded TD-4306 **(29)**, a β_2-agonist with superior duration of action relative to salmeterol. The second basic center is also conducive to crystalline salt form identification, as crystalline napadisylate, sulfate, and 4-methyl cinnamate salts were readily identified. Compound **29** was subsequently selected for progression into toxicology studies.

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References and notes

1. Cazzola, M.; Page, C. P.; Rogliani, P.; Matera, M. *Am. J. Respir. Crit. Care* **2013**, *187*, 690.
2. Toy, E. L.; Beaulieu, N. U.; McHale, J. M.; Welland, T. R.; Plauschinat, C. A.; Swensen, A., et al *Respir. Med.* **2011**, *105*, 435.
3. (a) Baur, F.; Beattie, D.; Beer, D.; Bentley, D.; Bradley, M.; Bruce, I.; Charlton, S. J.; Cuenoud, B.; Ernst, R.; Fairhurst, R. A.; Faller, B.; Farr, D.; Keller, T.; Fozard, J. R.; Fullerton, J.; Garman, S.; Hatto, J.; Hayden, C.; He, H.; Howes, C.; Janus, D.; Jiang, Z.; Lewis, C.; Loeuillet-Ritzler, F.; Moser, H.; Reilly, J.; Steward, A.; Sykes, D.; Tedaldi, L.; Trifilieff, A.; Tweed, M.; Watson, S.; Wissler, E.; Wyss, D. *J. Med. Chem.* **2010**, *53*, 3675; (b) Procopiou, P. A.; Barrett, V. J.; Bevan, N. J.; Biggadike, K.; Box, P. C.; Butchers, P. R.; Coe, D. M.; Conroy, R.; Emmons, A.; Ford, A. J.; Holmes, D. S.; Horsley, H.; Kerr, F.; Li-Kwai-Cheung, A.-M.; Looker, B. E.; Mann, I. S.; McLay, I. M.; Morrison, V. S.; Mutch, P. J.; Smith, C. E.; Tomlin, P. *J. Med. Chem.* **2010**, *53*, 4522; (c) Procopiou, P. A.; Barrett, V. J.; Bevan, N. J.; Biggadike, K.; Butchers, P. R.; Coe, D. M.; Conroy, R.; Edney, D. D.; Field, R. N.; Ford, A. J.; Guntrip, S. B.; Looker, B. E.; McLay, I. M.; Monteith, M. J.; Morrison, V. S.; Mutch, P. J.; Richards, S.; Sasse, R.; Smith, C. E. *J. Med. Chem.* **2009**, *52*, 2280; (d) Bouyssou, T.; Rudolf, K.; Hoenke, C.; Lustenberger, P.; Schnapp, A.; Konetzki, I. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5237; (e) Bouyssou, T.; Hoenke, C.; Rudolf, K.;

Lustenberger, P.; Pestel, S.; Sieger, P.; Lotz, R.; Heine, C.; Buttner, F. H.; Schnapp, A.; Konetzki, I. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1410; (f) Hoenke, C.; Bouyssou, T.; Tautermann, C. S.; Rudolf, K.; Schnapp, A.; Konetzki, I. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6640; (g) Brown, A. D.; Bunnage, M. E.; Glossop, P. A.; James, K.; Jones, R.; Lane, C. A. L.; Lewthwaite, R. A.; Mantell, S.; Perros-Huguet, C.; Price, D. A.; Trevethick, M.; Webster, R. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1280; (h) Brown, A. D.; Bunnage, M. E.; Glossop, P. A.; Holbrook, M.; Jones, R. D.; Lane, C. A. L.; Lewthwaite, R. A.; Mantell, S.; Perros-Huguet, C.; Price, D. A.; Webster, R. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6188; (i) Brown, A. D.; Bunnage, M. E.; Glossop, P. A.; James, K.; Jones, R.; Lane, C. A. L.; Lewthwaite, R. A.; Mantell, S.; Perros-Huguet, C.; Price, D. A.; Trevethick, M.; Webster, R. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4012; (j) Glossop, P. A.; Lane, C. A. L.; Price, D. A.; Bunnage, M. E.; Lewthwaite, R. A.; James, K.; Brown, A. D.; Yeadon, M.; Perros-Huguet, C.; Trevethick, M. A.; Clarke, N. P.; Webster, R.; Jones, R. M.; Burrows, J. L.; Feeder, N.; Taylor, S. C. J.; Spence, F. J. *J. Med. Chem.* **2010**, *53*, 6640; (k) Stocks, M. J.; Alcaraz, L.; Bailey, A.; Bonnert, R.; Cadogan, E.; Christie, J.; Connolly, S.; Cook, A.; Fisher, A.; Flaherty, A.; Hill, S.; Humphries, A.; Ingall, A.; Jordan, S.; Lawson, M.; Mullen, A.; Nicholls, D.; Paine, S.; Pairaudeau, G.; St-Gallay, S.; Young, A. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 4027; (l) Perez, D.; Crespo, M.; Sole, L.; Prat, M.; Carcasona, C.; Calama, E.; Otal, R.; Gavalda, A.; Gomez-Angelats, M.; Miralpeix, M.; Puig, C. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 1545; (m) Connolly, S.; Alcaraz, L.; Bailey, A.; Cadogan, E.; Christie, J.; Cook, A. R.; Fisher, A. J.; Hill, S.; Humphries, A.; Ingall, A. H.; Kane, Z.; Paine, S.; Pairaudeau, G.; Stocks, M. J.; Young, A. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 4612; (n) Procopiou, P. A.; Barrett, V. J.; Bevan, N. J.; Butchers, P. R.; Conroy, R.; Emmons, A.; Ford, A. J.; Jeulin, S.; Looker, B. E.; Lunniss, G. E.; Morrison, V. S.; Mutch, P. J.; Perciaccante, R.; Ruston, M.; Smith, C. E.; Somers, G. *Bioorg. Med. Chem.* **2011**, *19*, 4192; (o) Alcaraz, L.; Bailey, A.; Cadogan, E.; Connolly, S.; Jewell, R.; Jordan, S.; Kindon, N.; Lister, A.; Lawson, M.; Mullen, A.; Dainty, I.; Nicholls, D.; Paine, S.; Pairaudeau, G.; Stocks, M. J.; Thorne, P.; Young, A. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 689; (p) Stocks, M. J.; Alcaraz, L.; Bailey, A.; Bonnert, R.; Cadogan, E.; Christie, J.; Dixon, J.; Connolly, S.; Cook, A.; Fisher, A.; Flaherty, A.; Humphries, A.; Ingall, A.; Jordan, S.; Lawson, M.; Mullen, A.; Nicholls, D.; Paine, P.; Pairaudeau, G.; Young, A. *ACS Med. Chem. Lett.* **2014**, *5*, 416; (q) Procopiou, P. A.; Barrett, V. J.; Biggadike, K.; Butchers, P. R.; Craven, A.; Ford, A. J.; Guntrip, S. B.; Holmes, D. S.; Hughes, S. C.; Jones, A. E.; Looker, B. E.; Mutch, P. J.; Ruston, M.; Needham, D.; Smith, C. E. *J. Med. Chem.* **2014**, *57*, 159.

4. Jacobsen, J. R.; Choi, S.-K.; Combs, J.; Fournier, E. J. L.; Klein, U.; Pfeiffer, J. W.; Thomas, G. R.; Yu, C.; Moran, E. J. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 1213.
5. Jacobsen, J. R.; Aggen, J. B.; Church, T. J.; Klein, U.; Pfeiffer, J. W.; Pulido-Rios, M. T.; Thomas, G. R.; Yu, C.; Moran, E. J. *Bioorg. Med. Chem. Lett.*, in press.
6. (a) Nials, A. T.; Sumner, M. J.; Johnson, M.; Coleman, R. A. *Br. J. Pharmacol.* **1993**, *108*, 507; (b) Coleman, R. A.; Johnson, M.; Nials, A. T.; Vardey, C. J. *Trends Pharmacol. Sci.* **1996**, *17*, 234; (c) Johnson, M.; Butchers, P. R.; Coleman, R. A.; Nials, A. T.; Strong, P.; Sumner, M. J.; Vardey, C. J.; Whelan, C. *J. Life Sci.* **1993**, *52*, 2131.
7. (a) Anderson, G. P. *Life Sci.* **1993**, *52*, 2145; (b) Anderson, G. P.; Lindén, A.; Rabe, K. F. *Eur. Respir. J.* **1994**, *7*, 569.
8. (a) Macintyre, A. C.; Cutler, D. J. *Biopharm. Drug Dispos.* **1988**, *9*, 513; (b) Boer, F. *Br. J. Anaesth.* **2003**, *91*, 50.
9. A potential complication of this strategy is the increased risk of phospholipidosis associated with cationic amphiphilic molecules. See Reasor, M. J.; Hastings, K. L.; Ulrich, R. G. *Expert Opin. Drug Saf.* **2006**, *5*, 567.
10. Synthesis described in US Patent Number US6670376.
11. Male guinea pigs were exposed for 10 min in a whole body exposure chamber to nebulized aqueous solutions of test compound (0.30–100 g/mL) delivered in a mixture of gases (5% CO_2, 21% O_2, 74% N_2). Post-treatment, pulmonary resistance was measured in anesthetized, spontaneously-breathing animals using a head-out plethysmograph. Bronchoconstriction was induced by increasing doses of intravenous acetylcholine (ACh) and the dose of ACh needed to double the baseline resistance (PD2) was calculated.
12. Strolin Benedetti, M.; Dostert, P.; Tipton, K. F. *Prog. Drug Metab.* **1988**, *11*, 149.
13. Synthesis described in US Patent Number US5750701.
14. Calculated using Advanced Chemistry Development (ACD/Labs) Software V11.02.
15. Synthesis described in US Patent Number US6268533.