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Discovery of olodaterol, a novel inhaled β_2-adrenoceptor agonist with a 24 h bronchodilatory efficacy

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ABSTRACT

Compound **4p** was identified from a series of 6-hydroxy-4*H*-benzo[1,4]oxazin-3-ones as potent agonist of the human β_2-adrenoceptor with a high β_1/β_2-selectivity. A complete reversal of acetylcholine-induced bronchoconstriction which lasted over the whole study period of 5 h was demonstrated for **4p** in a guinea pig in vivo model without any signs of cardiovascular effects up to 10-fold above the first dose reaching 100% bronchoprotection. The enantiomerically pure (*R*)-form of **4p** exerted a bronchodilatory efficacy over 24 h in dogs and guinea pigs in the absence of systemic pharmacodynamic effects. Formoterol which was tested as comparator in the same in vivo models of acetylcholine-induced bronchoconstriction did not retain efficacy after 24 h. In summary, the preclinical profile of compound (*R*)-**4p** (olodaterol, also known as BI 1744 CL) suggests a potential for once-daily dosing in man accompanied with an improved safety profile.

COPD and asthma are characterized by a reduced airflow on expiration and difficulty in breathing. β_2-Adrenoceptor agonists are used for the treatment of both conditions due to their bronchodilatory effect mediated by a relaxation of the airway smooth muscles through an increase of cAMP. The availability of once-daily corticosteroids and the once-daily anticholinergic tiotropium, a bronchodilator with a different mechanism of action, suggested the development of β_2-agonists suitable for a q.d. dosing regiment[1] as potential combination partner. This requirement is not fulfilled by the two marketed long-acting β_2-agonists (LABAs) salmeterol and formoterol **1** (Fig. 1) which are both exerting a duration of action over 12 h only. Consequently, programs targeting towards the identification of q.d. LABAs are ongoing in different laboratories[2] and we herewith continue the disclosure of our efforts in this area.[3,4]

Recently, we described a series of potent β_2-adrenoceptor agonists consisting of a 5-hydroxy-4*H*-benzo[1,4]oxazin-3-one head group and a phenethylamine residue.[4] Unfortunately, the moderate sub-type selectivity of these compounds could not be significantly improved through the introduction of lipophilic substituents at the phenyl group of their amine moiety. The only compound identified during this study displaying a high β_1/β_2-selectivity was the carboxylic acid bearing example **2** (Fig. 1), which, however, demonstrated an inferior safety margin compared to formoterol **1** (Fig. 1) in an in vivo model of bronchodilation. In our present work, we now show how a minor structural modification of these 5-hydroxy-4*H*-benzo[1,4]oxazin-3-ones led to a series of compounds with a considerably optimized profile.

It is documented in the literature that the β_2-selectivity improves if the phenolic hydroxyl group of the β_2-agonist is shifted from the *para*- to the *meta*-position with respect to the ethanolamine substituent.[5,6] A prominent selective β_2-agonist with a phenolic hydroxyl group in the *meta*-position is for example terbutaline **3** (Fig. 1). Driven by this hypothesis, a series of 6-hydroxy-4*H*-benzo[1,4]oxazin-3-ones **4** with different substituents bound to the right hand side phenyl group was investigated. As in our previous work, mainly lipophilic substituents were selected due to reports stating that the duration of action increases with lipophilicity.[7,8]

The synthetic route towards the 6-hydroxy-4*H*-benzo[1,4]oxazin-3-ones **4** described in this study is outlined in Scheme 1. Selective alkylation of commercially available 2,5-dihydroxy acetophenone **5** followed by nitration of the resulting benzyl ether

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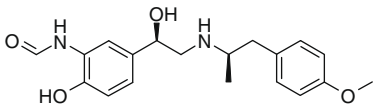

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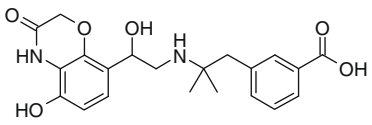
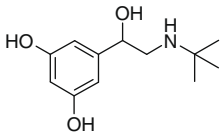


2

EC_{50} (hβ_2-AR) = 2.9 nM, β_1/β_2 = 87



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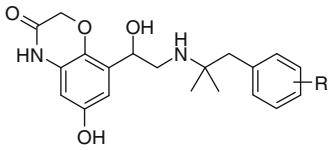


4

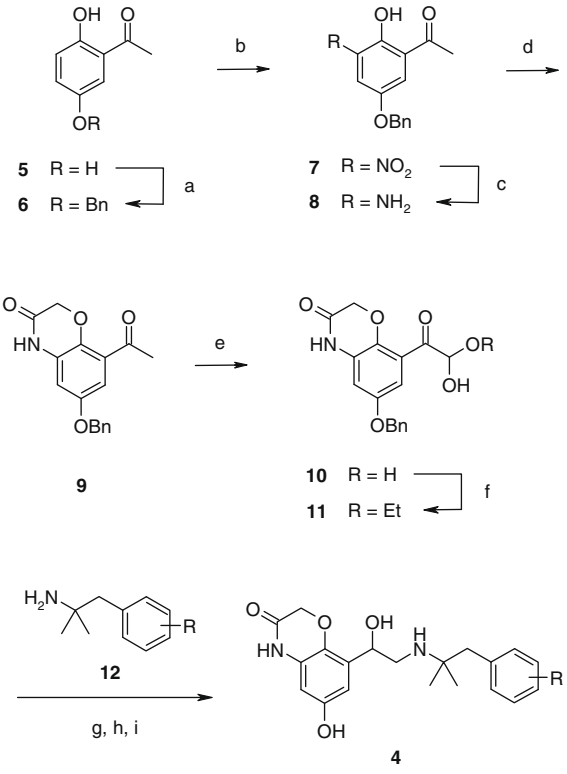
Figure 1. Structure of the b.i.d. LABA formoterol **1**, the 5-hydroxy-4*H*-benzo[1,4]oxazin-3-one **2**, terbutaline **3**, and the 6-hydroxy-4*H*-benzo[1,4]oxazin-3-ones **4** from this study.

afforded intermediate **7**. Hydrogenation of the nitro group of **7** and treatment with chloro-acetyl chloride yielded the 4*H*-benzo[1,4]oxazin-3-one **9**. The acetyl group of **9** was oxidized with selenium dioxide to the corresponding glyoxal aldehyde which was further converted into a hemi-acetal through heating in ethanol. The hemi-acetal **11** was then condensed with the phenethylamines **12** in the presence of a reducing agent. A subsequent debenzylation delivered the target compounds **4** as racemates. Alternatively, the target compounds were obtained from the reaction of the glyoxal aldehyde **10** with the phenethylamines **12**.

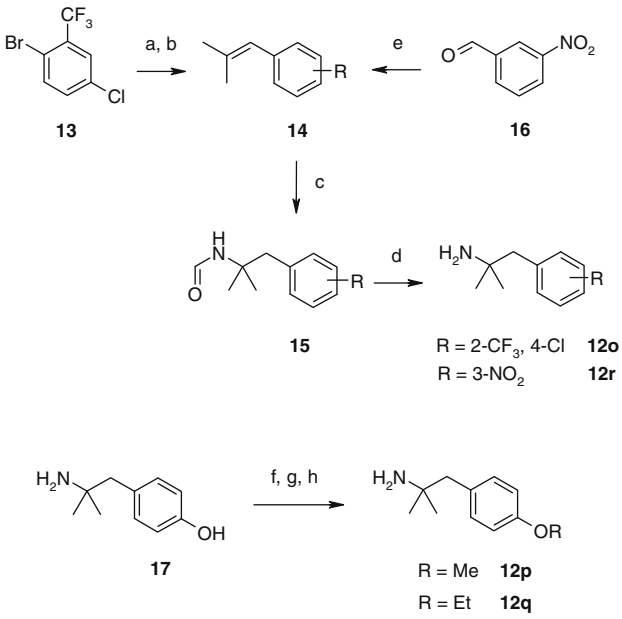
The phenethylamines **12a–k** were synthesized as previously described.[4] Compounds **12l–n** were derived from commercial sources and the preparation of the remaining amines **12o–r** is detailed in Scheme 2.

The sub-type selectivity and agonistic activity of the target compounds were assessed in functional cellular assays. In these assays, the rise of cAMP is measured in CHO cells expressing either the human β_1- or β_2-adrenergic receptor. cAMP is generated from ATP upon stimulation of the adenylyl cyclase by the $G_{\alpha s}$ subunit which translocates from the β-adrenergic receptor on binding of an agonist. The results from these tests including those for formoterol which was measured as comparator are summarized in Table 1.

The majority of the racemic 6-hydroxy-4*H*-benzo[1,4]oxazin-3-ones **4** display a considerable potency at the β_2-adrenoceptor with EC_{50} values ranging between 1 and 15 nM. The most potent example **4b** (R = 2-Me) has an EC_{50} of 0.7 nM which is comparable to formoterol in this assay. Modest EC_{50} values and intrinsic activities (means efficacy; expressed in percentage of the maximal efficacy of the full agonist isoprenaline) were only obtained for examples **4d**, **e** and **g** with sterically more demanding groups in the *para*-position at the phenyl residue. Interestingly, the introduction of 4-*tert*-butyl (example **4f**) resulted in an inactive compound, whereas a phenyl group (example **4g**) was still tolerated at this position.



Scheme 1. Reagents and conditions: (a) 1 equiv BnBr, K_2CO_3, acetone, rt, 4 d, 64%; (b) fuming HNO_3, acetic acid, rt, 74%; (c) Rh/C, H_2, MeOH, 89%; (d) chloro-acetyl chloride, K_2CO_3, acetonitrile, 0 °C to rt, then 6 h reflux, 66%; (e) selenium dioxide, dioxane, H_2O, reflux; (f) EtOH, 2 h reflux, 63% over two steps; (g) 1 equiv of amine **12**, THF, then $LiBH_4$, 0 °C to rt; for **4d**, **e**, **h**, **j**, **q**: 1 equiv of amine **12**, EtOH, 50–80 °C, then $NaBH_4$, rt; (h) Pd/C, H_2, MeOH; only for **4r**: (i) concd H_2SO_4, *tert*-butyl nitrite, MeOH; examples **4l**, **m**, **n** and **p** were prepared from glyoxal hydrate **10** in an analogous fashion.



Scheme 2. Reagents and conditions: (a) Mg-Grignard preparation in Et_2O, then isobutyraldehyde; (b) *p*-toluenesulfonic acid monohydrate, toluene, reflux; (c) NaCN, AcOH, concd H_2SO_4; (d) HCl, Et_2O; (e) isopropyl triphenylphosphonium iodide, n-BuLi, THF, Et_2O; (f) 1 equiv ClCbz, DCM, H_2O, Na_2CO_2; (g) alkyliodide, K_2CO_3, DMF; (h) Pd/C, H_2, MeOH.

Table 1
Potency and intrinsic activity at the human β1- and β2-adrenoceptors for formoterol fumerate **1** and the 6-hydroxy-4*H*-benzo[1,4]oxazin-3-ones **4** from this study

Compounds	R	hβ1 EC_{50} (nM)	hβ1 IA[a] (%)	hβ1 *n*	hβ2 EC_{50} (nM)	hβ2 IA[a] (%)	hβ2 *n*	β1/β2
1		86 ± 23	22 ± 7	2	0.4 ± 0.2	133 ± 48	3	215
4a	H	35.0 ± 22.6	15 ± 4	2	2.4 ± 0.7	57 ± 2	2	15
4b	2-Me	4.5 ± 5.1	28 ± 8	2	0.7 ± 0.1	64 ± 6	2	6
4c	3-Me	42.0	8	1	4.1 ± 1.8	48 ± 8	2	10
4d	4-Et	215.0	36	1	49.7	29	1	4
4e	4-*i*-Pr	207.0	13	1	58.0	23	1	4
4f	4-*t*-Bu	989.5 ± 29.0	14 ± 6	2	5167.0 ± 6835.6	2 ± 1	2	<1
4g	4-Ph	207.0	41	1	68.0	52	1	3
4h	3,5-di-Fluor	>1000.0	8 ± 1	2	12.1 ± 11.2	50 ± 12	2	>83
4i	4-CF_3	711.5 ± 60.1	17 ± 5.7	2	12.4 ± 3.4	40 ± 0	2	57
4j	3-CF_3	436.5 ± 217.1	13 ± 3	2	3.4 ± 1.6	44 ± 2	3	128
4k	4-OCF_3	240.0 ± 22.6	24 ± 3	2	11.9 ± 2.7	50 ± 17	2	20
4l	4-F	101.0 ± 13.9	18 ± 7	3	3.4 ± 0.9	65 ± 10	3	30
4m	4-Cl	171.0 ± 103.4	30 ± 11	2	1.3 ± 0.7	55 ± 35	2	132
4n	4-Br	103.4 ± 91.9	20 ± 12	3	10.7 ± 7.8	64 ± 19	3	10
4o	2-CF_3	27.0 ± 12.7	19 ± 7	2	1.4 ± 0.4	25 ± 3	2	19
4p	4-OMe	358.0 ± 69.3	7 ± 7	2	1.4 ± 0.8	53 ± 6	2	256
(*S*)-**4p**	4-OMe	2725.0 ± 620.8	17 ± 4	3	97.7 ± 1.4	50 ± 8	3	28
4q	4-OEt	200.0 ± 112.7	15 ± 38	3	15.4 ± 13.5	43 ± 48	3	13
4r	3-OMe	278.4 ± 288.6	8 ± 5	7	14.3 ± 9.5	44 ± 11	5	19

[a] Recombinant human β1- and β2-adrenoceptors are expressed in CHO-K1 cell lines and the intracellular accumulation of cAMP after addition of various test compounds is measured. The intrinsic activity (IA) is reported as percentage of the maximal effect of isoprenaline (=100%). The standard deviation is given.

Table 2
Results from the 5 h guinea pig model of acetylcholine-induced bronchoconstriction (Konzett–Roessler model)

Compounds	Doses[a] (μg/kg)	Dur >80%[b] (min)	FED[c] (μg/kg)	TI[d]
1	0.1, 0.3, 1, 3, 10	300	1	10
4a	1, 3	300	3	1
4c	3, 10, 30, 100	300	30	3
4h	3, 10, 30	300	10	3
4i	3, 10	150	None	<1
4j	3, 10, 30, 100	300	None	<1
4l	3, 10	300	10	1
4m	3, 10	220	None	<1
4p	1, 3, 10, 30	300	3	10
4q	1, 3, 10, 30	300	3	10

[a] Doses administered intratracheally.
[b] Time period with >80% bronchoprotecton at the highest tested dose.
[c] The fully effective dose (FED) corresponds to the first dose reaching a full inhibition of the acetylcholine-induced bronchospasm (=100% bronchoprotection).
[d] Therapeutic index (TI), ratio of the first dose producing an increase in heart rate of >10% and the FED.

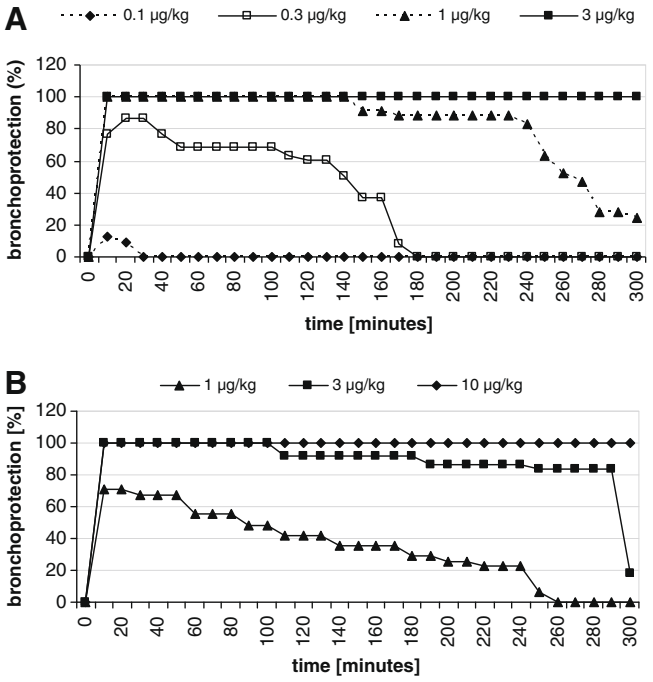


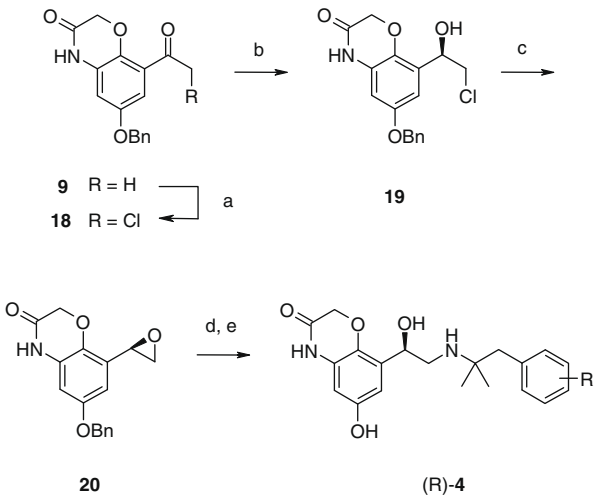
Figure 2. Bronchoprotective effect of formoterol **1** (A) and example **4p** (B) in the Konzett-Roessler model. Bronchospams were induced in guinea pigs by iv injections of acetylcholine every 10 min. Bronchoprotection is expressed as the percentage of inhibition of the increase in pulmonary resistance induced by acetylcholine ($N = 2$ animals per dose). The 10 μg/kg dose for formoterol and the 30 μg/kg dose for **4p** were omitted for clarity.

Fortunately, several compounds exert an excellent sub-type selectivity based on the ratio of the EC_{50} values for the β_1- and β_2-receptor which is for compound **4p**[9] even higher than for formoterol. In this respect, it has to be considered that a decreased intrinsic activity at the β_1-adrenoceptor is observed for all compounds from this study which is in some cases even negligible, adding to the favorable overall profile of this class.

Only a weak β-adrenergic activity was determined for the distomer (*S*)-**4p** ((*S*)-enantiomer of compound **4p**). These data are strong evidence that the measured EC_{50}s of the racemates are mainly influenced by the eutomers and thereby justify the use of racemates in this early stage of the test cascade. If the measured residual activity of (*S*)-**4p** stems from the distomer itself or from a contamination with its eutomer is unknown.

Nine compounds from this study (examples **4a**, **c**, **h**–**j**, **l**, **m**, **p**, **q**) were selected for further profiling and tested in an in vivo model of bronchodilation (Table 2).[10] In this model, bronchospasms were induced in anaesthetized guinea pigs by iv injections of acetylcholine.[11] The test compound was administered via a tracheal cannula using a Respimat® soft mist inhaler and bronchodilation was quantified over the entire study period of 5 h. In addition, the heart rate was recorded and used as an indicator for the systemic availability of a test compound.[12]

Cardiovascular effects which are observed for β_2-agonists with high sub-type selectivity can be attributed both to the residual β_1-acitivity and to the dominant β_2-component of the compound. One potential cause for an accelerated heart rate results from the β_2-adrenoceptor mediated vasodilation and concomitant reduced blood flow which is counterbalanced through an increased cardiac output. The β_2-adrenoceptor can also directly act on the heart,



Scheme 3. Reagents and conditions: (a) Benzyltrimethylammonium-chloroiodate, 1,2-dichloroethane, acetic acid, H_2O, 65 °C, 86%; (b) 1 mol % Cp* RhCl[(*S*,*S*)-TsDPEN], formic acid/triethylamine (5:2), DMF, −15 °C, 76%, 95.4% ee; (c) 2 M aqueous NaOH, DMF, 0 °C, 96%; (d) amine **12**, *i*-PrOH, 140 °C, microwave; (e) Pd/C, H_2, MeOH.

although its occurence in this organ is less prevalent than that of the β_1-receptor.

All 6-hydroxy-4*H*-benzo[1,4]oxazin-3-ones **4** tested in the guinea pig model showed a dose-dependent bronchodilation. A complete reversal of the induced bronchospasms (=100% bronchoprotection) was always observed with the exception of **4i**, **j** and **m**, which already caused a significant increase in heart rate (>10%) at a sub-maximal dose. Cardiovascular effects were also accounted for compounds **4a** and **4l** at the first dose reaching 100% bronchoprotection resulting in a therapeutic index of 1, only. In contrast, a therapeutic index of 10 was determined for the examples **4p** and **4q** which is as good as for formoterol in this setting (Fig. 2). All compounds producing a maximal bronchoprotection of 100% and with a therapeutic index >1 were moved to the next stage in our screening cascade, a dog model of acetylcholine-induced bronchodilation.

In this model, only the eutomers ((*R*)-enantiomers) were studied to exclude pharmacodynamic effects associated with the distomer (including off-target effects). For this purpose, an enantioselective route towards the 6-hydroxy-4*H*-benzo[1,4]oxazin-3-ones **4** was developed (Scheme 3). The key step of the synthesis is a transfer hydrogenation of the 2-chlororacetophenone **18**. The reaction is catalyzed by a recently described rhodium complex and provided the chlorohydrine **19** in good yield and excellent enantiomeric excess.[13] Conversion of the chlorohydrine **19** into the styrene oxide **20**, followed by coupling with the phenethylamines **12** and subsequent removal of the benzyl group delivered the enantiomerically pure (*R*)-form of **4**. This method gave ready access towards the eutomers and was used to generate the amounts required for the profiling of the compounds from this study.[14]

The bronchoprotective effect of the selected examples ((*R*)-**4c**, (*R*)-**4h**, (*R*)-**4p**, (*R*)-**4q**) was studied in anaesthetized beagle dogs over a time period of 3 h. Again, the test compound was administered by inhalation using the Respimat® soft mist inhaler and bronchoconstriction was induced by iv injections of acetylcholine. Since beagle dogs are very sensitive to cardiovascular and metabolic effects (decrease in serum potassium, increase in serum glucose and lactate) mediated by the stimulation of β-adrenoceptors,[15] these parameters were assessed during the experiment to monitor the systemic availability of the test compound. Example (*R*)-**4p** demonstrated a dose-dependent bronchoprotection in this model in the absence of any changes in serum levels of potassium, glucose or lactate at all doses tested. In contrast, formoterol showed at an equally effective dose a decrease in serum potassium level and a tachycardia (heart rate increase) was more pronounced and longer lasting than for (*R*)-**4p**. None of the other 6-hydroxy-4*H*-benzo[1,4]oxazin-3-ones from this study exhibited a comparable profile in these tests (data not shown).

In further studies, bronchodilatory efficacy over 24 h was demonstrated in two species (dog and guinea pig) and similar experimental settings for (*R*)-**4p** but not for formoterol.[16] The results from these experiments in connection with a measured log *D* value of 1.2 (at pH 7.4) for compound (*R*)-**4p** are a clear disprove of the hypothesis that lipophilicity is a prerequisite for an inhalatively applied LABA exerting a long duration of action.

The low systemic availability of (*R*)-**4p** suggested by the absence of undesired systemic pharmacodynamic effects was supported by data from pharmacokinetic studies. A low permeability was determined for (*R*)-**4p** in an in vitro assay measuring the apical to basolateral flux across a monolayer of CaCo-2 cells (P_{app} ap > bas = 3.5×10^{-7} cm/s, P_{app} bas > ap = 9.0×10^{-6} cm/s). Further, a medium metabolism was observed in hepatocytes from humans (CL = 13 mL/min/kg) and rats (CL = 45 mL/min/kg). Consequently, a low oral bioavailability of the swallowed fraction was concluded from these in vitro data. This prediction was confirmed in vivo where an oral bioavailability of 3 ± 2% was found for (*R*)-**4p** after po administration in rats.

A key requirement for the development of a q. d. LABA was the identification of a salt form suitable for an inhalative application. Fortunately, a crystalline hydrochloride salt of (*R*)-**4p** was identified with physico-chemical properties consistent with a formulation in a dry power inhalation (DPI) device and as an aqueous solution in the Respimat® soft mist inhaler. Based on these characteristics, (*R*)-**4p** was selected to be the first clinical candidate from our program aiming to identify a q. d. LABA. This compound designated BI 1744 CL or olodaterol is currently undergoing clinical studies in man. The manuscript represents the first disclosure of the name and structure of this compound (olodaterol).

In conclusion, several potent and selective β_2-agonists have been identified from a series of 6-hydroxy-4*H*-benzo[1,4]oxazin-3-ones. One compound, (*R*)-**4p** demonstrated a duration of action over 24 h in two preclinical in vivo models of bronchoprotection and a superior safety margin compared to the marketed formoterol. The encouraging pharmacodynamic profile and CMC characteristics are supporting further clinical development of this compound.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.12.087.

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14. *Synthesis protocol of a representative example (compound* (R)-**4p**): 750 mg (2.52 mmol) 6-benzyloxy-(8*R*)-oxiranyl-4*H*-benzo[1,4]oxazin-3-one **20** and 900 mg (5.02 mmol) 2-(4-methoxy-phenyl)-1,1-dimethyl-ethylamine **12p** in 3 mL isopropanol were stirred for 30 min at 140 °C under irradiation of microwaves (Emrys Optimizer from Personal Chemistry). The mixture was concentrated, filtered twice through a column of alumina (dichloromethane/methanol = 50:1, then ethylacetate/methanol = 100:1 to 20:1) and then crystallized from diethylether/diisopropylether to yield 1360 mg (68%) of the benzyl-protected intermediate. 1340 mg (2.81 mmol) of this intermediate in 28 mL methanol were hydrogenated in the presence of 200 mg palladium on carbon (10% wt) at 50 °C and a hydrogen pressure of 50 psi. The catalyst was filtered off and the solvent removed in vacuo. The residue was dissolved in methanol and 0.6 mL 5 M hydrogen chloride in isopropanol and then precipitated through addition of ether. Yield: 1120 mg (94%; hydrogen chloride; 94% ee by chiral HPLC).
Analytical data of compound (*R*)-**4p**: Mass spectroscopy: $[M+H]^+$ = 387. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 10.62 (s, 1H), 9.28 (s, 1H), 8.76 (m, 1H), 8.54 (m, 1H), 7.13 (d, 2H, J = 8.6 Hz), 6.90 (d, 2H, J = 8.6 Hz), 6.58 (d, 1H, J = 2.8 Hz), 6.37 (d, 1H, J = 2.8 Hz), 6.06 (d, 1H, J = 3.8 Hz), 5.12 (m, 1H), 4.58 (d, 1H, J = 15.0 Hz), 4.49 (d, 1H, J = 15.0 Hz), 3.74 (s, 3H), 3.17 (m, 1H), 2.90 (m, 3H), 1.19 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ = 165.3, 158.2, 152.5, 132.3, 131.6, 130.1, 127.8, 127.0, 113.6, 106.6, 102.2, 66.9, 63.5, 59.5, 55.0, 46.5, 41.5, 22.2, 20.2.
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