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Studies towards topical selective β_2-adrenoceptor agonists with a long duration of action

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ABSTRACT

β_2-Adrenoceptor agonists with basic and acidic groups attached via an alkyl linker to the phenyl ethanolamine core were prepared and investigated in vitro and in vivo. The compounds exhibited a high potency in a functional cellular assay and a bronchoprotective effect in a guinea pig model which lasted over the complete study period of 5 h.

β_2-Adrenoceptor agonists are well established in the treatment of both asthma and chronic obstructive pulmonary disease (COPD) due to their bronchodilatory effects. The two most prescribed representatives of this class are salmeterol **1** and formoterol **2**, which have been approved for a twice-daily (b.i.d.) regimen and are both used as monotherapy and in combination with steroids. A considerable interest has been developed to identify long-acting beta2 agonists (LABAs) with a 24 h duration of action (q.d. dosing) and an improved therapeutic window compared to the marketed b.i.d. LABAs (Fig. 1).[1]

To avoid unwanted β-adrenoceptor mediated effects like tachycardia, hypokalemia or muscle tremor it is necessary that the systemic exposure of the β-agonist be as low as possible. Low oral bioavailability would be beneficial in this respect, because a certain proportion of the inhaled drug is always swallowed and therefore available for absorption through the gut wall of the intestine. But even in such cases the agonist might become systemically available through pulmonary absorption. It has been found that drugs which show otherwise poor oral bioavailability are absorbed via the highly permeable pulmonary epithelium and it seems that only the pulmonary absorption *rate* can be influenced by the physicochemical properties of a compound.[2,3] Thus, a small pulmonary absorption rate in combination with poor oral bioavailability should contribute to low systemic exposure of a β-agonist. We hypothesized that such characteristics could be achieved through the introduction of additional basic or acidic groups to the phenyl ethanolamine structure which is common to all known β-agonists. The incorporation of such groups appeared to us most practicable at the amine residue (here, right-hand side of the molecule) since the ethanolamine backbone as well as the phenolic aryl moiety (here, left-hand side of the molecule) are important pharmacophores and highly sensitive towards minor structural modifications.

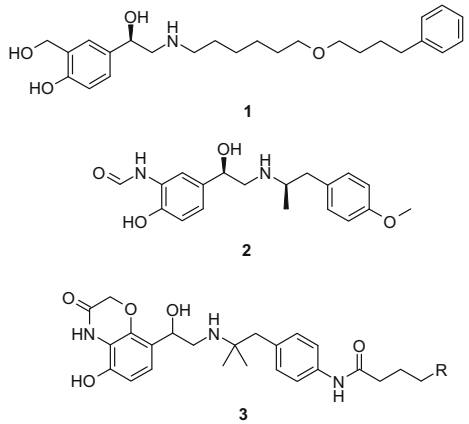

Figure 1. Structures of the bid LABAs salmeterol **1** and formoterol **2** and the 5-hydroxy-4*H*-benzo[1,4]oxazin-3-ones **3** described within this study.

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Phenyl ethanolamines like **3** with a 5-hydroxy-4*H*-benzo[1,4]oxazin-3-one moiety at the left-hand side and a phenethyl residue bound to the amine have been already suggested by our company in the past for use as bronchodilators.[4] A patent evaluation gave rise to the assumption that it should be possible to introduce substituents at the para-position of the right phenyl group under retention of a sufficient potency towards the β_2-adrenoceptor.[5,6] The structure **3** was therefore selected as template for our studies and it was envisaged that an alkyl linker connected to the phenyl group at the right-hand side of the molecule should serve as anchor for additional basic or acidic groups.

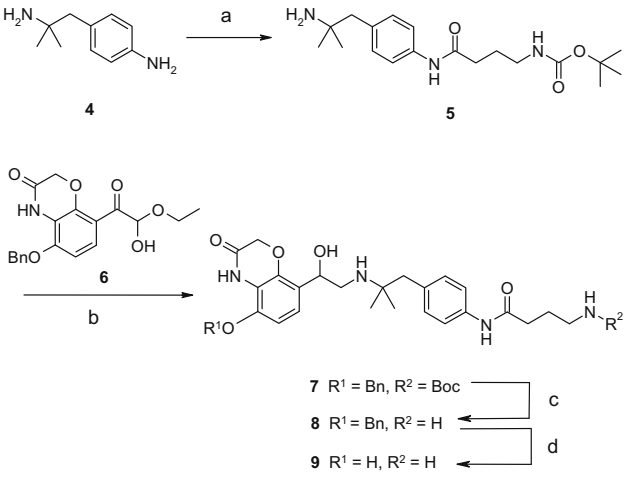
The synthesis of the 5-hydroxy-4*H*-benzo[1,4]oxazin-3-ones described in this study is outlined in Schemes 1 and 2. Acylation of the phenethylamine **4** with Boc protected 4-amino-butyric acid and subsequent coupling with the literature known hemi-acetal **6** yielded the ethanolamine **7**.[4] Boc removal afforded intermediate **8** which was further converted into compound **9** through hydrogenation of the benzyl group.

The introduction of basic and acidic groups to the alkyl amine side chain of intermediate **8** delivered the target compounds **10**–**18**. Hence, the primary amine of **8** was converted into a guanidine through treatment with 1-amidinopyrazole. A TBTU mediated acylation and removal of the benzyl and Cbz groups through hydrogenolysis afforded the compounds **11**–**14**. Reaction of the chloroacetamide obtained from **8** with different amines and deprotection yielded the 2-aminoacetamides **15** and **16** as well as the trimethyl ammonium compound **17**. The sulfonic acid **18** was obtained from **8** through treatment with 1,3-propane sultone and subsequent debenzylation.[7,8]

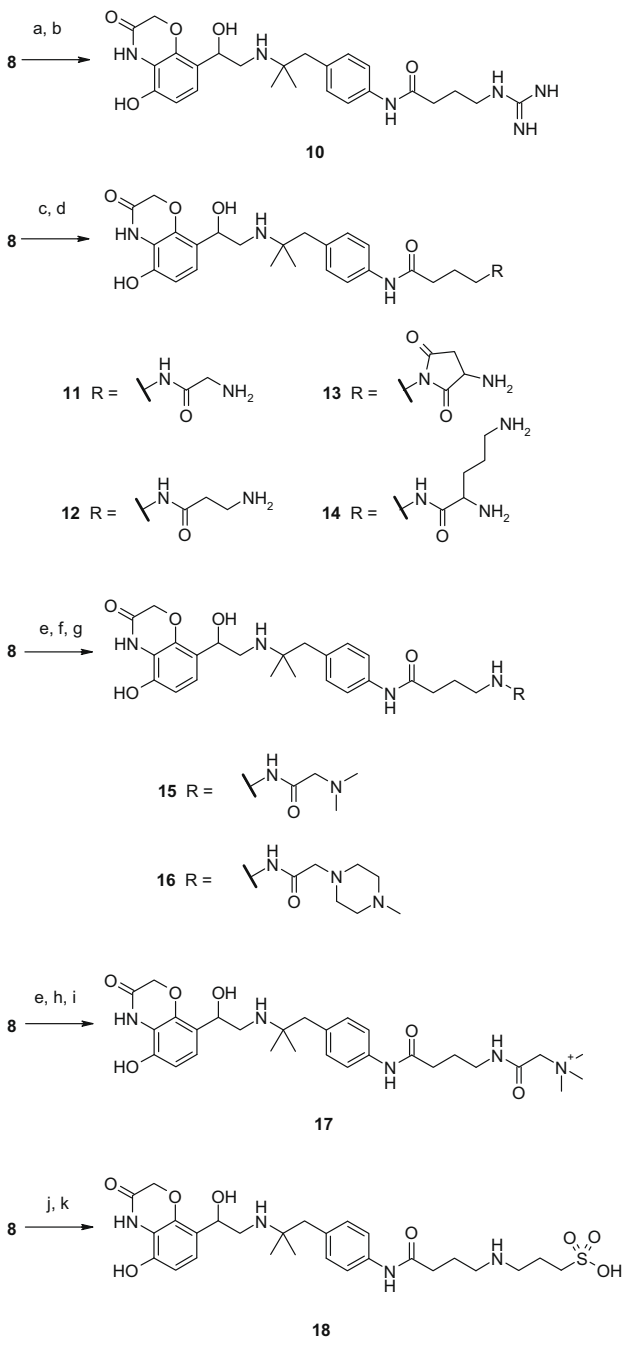
The in vitro characteristics of the synthesized compounds were assessed in CHO-K1 cell lines stably expressing the human β_1- and β_2-adrenoceptors. The agonist-induced rise of intracellular cAMP was measured as a functional read out in these assays. The potencies (EC_{50}) of the compounds were calculated and the intrinsic activity as a marker for efficacy was reported as percentage of the maximal effect observed for isoprenaline. Marketed formoterol fumarate salt was measured in these assays for comparison (Table 1).

The compounds prepared in this first series are potent, full agonists of the human β_2-adrenoceptor. The most potent compound, example **15**, exhibited a similar potency and intrinsic activity as formoterol, while EC_{50} values around 1 nM were measured for the majority of the other examples. The least potent β_2-agonist from this set of compounds, example **14**, still has an acceptable EC_{50} value of 5.4 nM. In contrast to formoterol, the compounds do not show any reasonable selectivity over the β_1-adrenoceptor.

The in vivo efficacy, potency, and duration of action in comparison to formoterol were determined in guinea pigs according to the method of Konzett–Roessler.[9] In this model, the test compound is applied intra-tracheally to anaesthetized guinea pigs and bronchoconstriction is induced by intravenous application of acetylcholine at various time points after administration of the compound.



Scheme 1. Reagents and conditions: (a) isobutyl chloroformate, *N*-BOC-aminobutyric acid, *N*-methyl-morpholine, −25 °C, then **4**, 64%; (b) EtOH, 50–80 °C, then $NaBH_4$, rt, 84%; (c) TFA, CH_2Cl_2, 42%; (d) Pd/C, H_2, MeOH, 68%.



Scheme 2. Reagents and conditions: (a) 1-amidinopyrazole hydrochloride, DMF, 67%; (b) Pd/C, H_2, MeOH, 80%; (c) carboxylic acid (benzyloxycarbonylamino-acetic acid for **11**, 3-benzyloxycarbonylamino-propionic acid for **12**, 2-benzyloxycarbonylamino-succinic acid 4-benzyl ester for **13**, 2,5-bis-benzyloxycarbonylamino-pentanoic acid for **14**), TBTU, DIPEA, THF; (d) Pd/C, H_2, MeOH; (e) chloroacetyl chloride, DIPEA, CH_2Cl_2, 48%; (f) amine (2 M dimethylamine in THF for **15**, *N*-methyl-piperazine for **16**), DIPEA, THF; (g) Pd/C, H_2, MeOH; (h) 4 M trimethylamine in EtOH, THF, 60 °C, 52%; (i) Pd/C, H_2, MeOH, 72%; (j) 1,3-propane sultone, ACN, EtOH, reflux, 18%; (k) Pd/C, H_2, MeOH, 32%.

Table 1
Potency and intrinsic activity at human β1- and β2-adrenoceptors for compounds **2**, **9–12** and **14–21**

Compounds	hβ1 EC_{50} (nM)	hβ1 IA[a] (%)	hβ2 EC_{50} (nM)	hβ2 IA[a] (%)	β1/β2
2	86 ± 23	122 ± 7	0.4 ± 0.2	133 ± 48	215
9	3.4 ± 1.1	93 ± 33	1.1 ± 0.1	128 ± 28	3.1
10	1.8 ± 0.4	98 ± 24	1.0 ± 0.1	115 ± 2	1.8
11	4.5 ± 0.2	125 ± 74	1.2 ± 0.3	142 ± 30	3.8
12	4.6 ± 1.5	135 ± 50	0.9 ± 0.6	161 ± 16	5.1
14	24.0 ± 11.3	117 ± 36	5.4 ± 0.7	130 ± 16	4.4
15	5.3 ± 2.7	76 ± 18	0.5 ± 0.1	125 ± 47	10.6
16	5.7	76	1.2	126	4.8
17	3.5 ± 0.3	72 ± 9	1.0 ± 0.1	162 ± 92	3.5
18	9.9 ± 1.6	95 ± 60	1.8 ± 0.4	121 ± 4	5.5
19	~11 ± 4	14 ± 1	9.0 ± 4.2	78 ± 15	1.2
20	~28 ± 8	12 ± 1	11.6 ± 7.6	37 ± 0	2.4
21	~18 ± 9	13 ± 2	4.1 ± 3.4	54 ± 6	4.4

[a] Cloned 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%). Values are means of two independent experiments (exceptions: compound **2** *N* = 3, compound **16** *N* = 1). The standard deviation is given.

In addition to measuring bronchoprotection, the heart rate of the guinea pigs is also recorded in this experiment. As an increase in heart rate indicates a systemic exposure of the test compound, the pharmacokinetic properties of the agonists are assessed indirectly in this model via the systemic pharmacodynamic effects.

Four examples from the first series were selected based on the structurally diversity of their side chains and tested in vivo (**10** = guanidine; **11** = mono-amino; **14** = di-amino; **18** = mono-amino and sulfonic acid).[10] All examples showed a dose dependent bronchoprotection in this model. The lowest dose causing a complete reversal (=100% bronchoprotection) of the induced bronchospasm was 10 μg/kg for the three compounds (examples **10**, **11**, **14**) with purely basic side chains. Further, a duration of action over the complete study period of 5 h was determined for the three examples at doses of 3 and 10 μg/kg (Fig. 2B). Unfortunately, a physiologically significant (>10%) increase in heart rate was already evident at 10 μ/kg. A heart rate increase was also observed for the sulfonic acid bearing example **18** at a sub-maximal dose of 3 μg/kg. All four examples showed a similar time course of the heart rate inasmuch as the maximum was reached between 10 and 20 min after administration of the test compound indicating a similar pulmonary absorption rate. Furthermore, a sustained elevation of the heart rate was determined at the highest tested dose for all four examples although the increase was only marginal after 90 min compared to the heart rate before drug administration.[11]

The comparator formoterol also exhibited a dose dependent bronchoprotection in this model with a complete reversal of the induced bronchospasm at a dose of 1 μg/kg. The lower doses of 0.3 and 1 μg/kg showed a decline in bronchoprotection after 30 and 150 min, respectively. A significant increase in heart rate was detected for formoterol only at a dose of 10 μg/kg, which is 10-fold higher then the first dose reaching 100% bronchoprotection (Fig. 2A).

Due to a therapeutic window inferior to the marketed formoterol, the compounds of the first series where not profiled any further. The small therapeutic window was attributed to the modest selectivity of the compounds over the β_1-adrenoceptor and in the further course of this study it was attempted to prepare agonists without this liability.

The phenolic hydroxyl group is important for the potency and selectivity of a β-adrenoceptor agonist. Precedents from the literature showed that a shift of this hydroxyl group from the para- to the meta-position in respect to the ethanolamine substituent increases the β_2-selectivity of the agonist.[12,13] These findings inspired us to prepare the examples **19**–**21** which differ only in the position of the phenolic hydroxyl group from the previously investigated 5-hydroxy-4*H*-benzo[1,4]oxazin-3-ones and which were

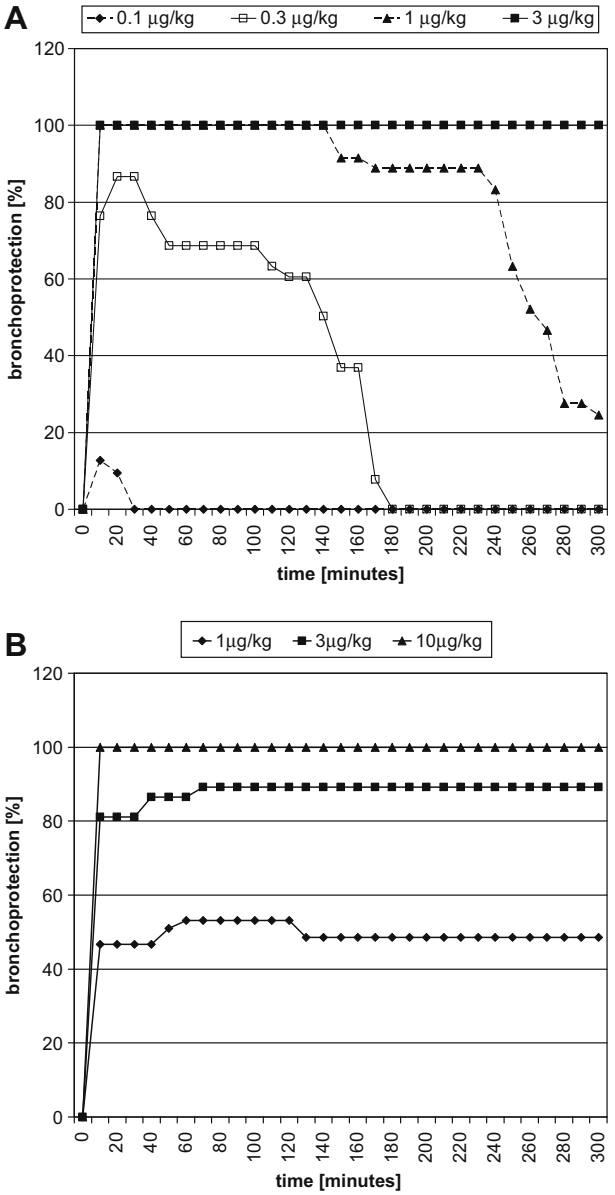


Figure 2. Bronchoprotective effect of formoterol (A) and example **14** (B) in the Konzett–Roessler model. Bronchospams were induced in guinea pigs by iv injections of acetylcholine every 10 min. Bronchoprotection is expressed as the percentage of inhibition of the increase in pulmonary resistance induced by acetylcholine (*N* = 2 animals per dose).

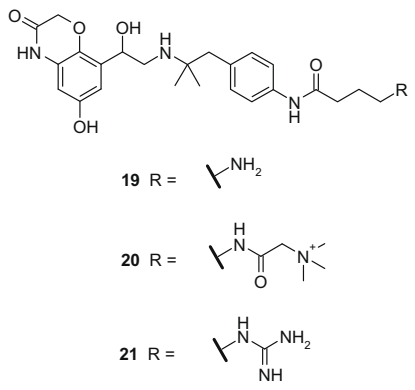
obtained in an analogous fashion (Scheme 3). The potency of these compounds towards the β_2-adrenoceptor was lower compared to their 5-hydroxy analogs but still in an acceptable range. Advantageously, the examples **19–21** exhibited only a negligible intrinsic activity at the β_1-adrenoceptor (Table 1).

These three examples were then tested in the Konzett–Roessler model at doses of 3 and 10 μg/kg. Again, an increase in heart rate was observed at 10 μg/kg which was the first dose reaching a full reversal of the induced bronchospasm. Consequently, the further profiling of these compounds was stopped.

Consecutively, 6-hydroxy-4*H*-benzo[1,4]oxazin-3-ones were prepared with acidic (carboxylic and sulfonic acids) instead of basic groups but these compounds displayed an insufficient potency in the cellular cAMP assay and were not submitted to any further experiments (data not shown).

A potential explanation for the failure of this approach could be that β_2-agonists are already protonated at the basic center of their ethanolamine moiety under physiological conditions (pH 7.4) implying that an additional charged group will have not much influence on the pulmonary absorption rate. Furthermore, to control the absorption rate seems to be insufficient since a rapid clearance of the absorbed fraction of the agonist is another important contributor to a low systemic exposure and a beneficial therapeutic window of the drug.

In conclusion, a number of highly potent β_2-adrenoceptor agonists have been identified. Selected examples from this series were tested in a guinea pig in vivo model and exhibited a dose dependent bronchoprotective effect which lasted over the complete study period. Further results from our continued efforts towards the identification of long-acting β_2-adrenoceptor agonists with an improved therapeutic window will be reported in due course.



Scheme 3. 6-Hydroxy analogs of examples **8**, **10** and **17**.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.06.104.

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7. The purity and identity of the synthesized compounds detailed in this publication were determined by ^{1}H NMR and mass spectroscopy.
8. *Synthesis protocol of a representative example* (*compound* **12**): 190 mg (0.86 mmol) benzyloxycarbonylamino-acetic acid, 290 mg (0.90 mmol) TBTU and 160 μL (0.92 mmol) ethyl-diisopropyl-amine in 30 mL anhydrous THF were stirred for 30 min at ambient temperature. 500 mg (0.92 mmol) of compound **8** were added and stirring was continued overnight. The solvent was removed in vacuo and the residue was dissolved in dichloromethane and washed with aqueous potassium carbonate solution. The organic phase was dried over sodium sulfate, concentrated and purified by chromatography on silica gel eluting with dichloromethane/methanol/ammonia (95:4.5:0.5→60:35:5 by volume). The product containing fractions were combined and the solvents evaporated. The Cbz-protected compound **12** was obtained as pale solid after triturating with diisopropyl-ether. Yield: 240 mg (38%). 210 mg (0.28 mmol) Cbz-protected compound **12** were hydrogenated in the presence of 80 mg palladium on carbon (10 wt %) in 20 mL methanol at ambient temperatures and a hydrogen pressure of 50 psi. The catalyst was filtered off and the solvent was removed in vacuo. Trituration of the residue with diisopropyl-ether yielded compound **12** as white solid. Yield: 95 mg (65%). Analytical data of compound **12**: mass spectroscopy: $[M+H]^+ = 528$. ^{1}H NMR (400 MHz, methanol-d_4) $\delta = 7.45$ (d, 2H, $J = 8.4$ Hz), 7.10 (d, 2H, $J = 8.4$ Hz), 6.94 (d, 1H, $J = 8.5$ Hz), 6.51 (d, 1H, $J = 8.5$ Hz), 4.98 (m, 1H), 4.52 (d, 1H, $J = 15.3$ Hz), 4.48 (d, 1H, $J = 15.3$ Hz), 3.28 (t, 2H, $J = 6.7$ Hz), 2.96 (t, 2H, $J = 6.8$ Hz), 2.84 (m, 2H), 2.70 (m, 2H), 2.40 (m, 4H), 1.89 (m, 2H), 1.09 (s, 3H), 1.08 (s, 3H). ^{13}C NMR (100 MHz, methanol-d_4) $\delta = 174.1$, 173.8, 166.9, 147.0, 143.0, 138.3, 134.5, 131.9 (2C), 122.4, 122.1, 121.0 (2C), 116.3, 110.5, 68.1, 68.0, 55.1, 46.9, 39.8, 38.6, 38.0, 35.2, 26.6, 26.4, 26.1.
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10. The results from the anaesthetized guinea pig experiments are available in the Supplementary data for all compounds from this publication tested in the model.
11. Similar time courses of the heart rate were observed for the examples **19–21**. See also Supplementary data.
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