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Discovery of substituted phenyl urea derivatives as novel long-acting β_2-adrenoreceptor agonists

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ABSTRACT

The synthesis of diverse functionalized ureas in a semi-parallel fashion is described, as well as their β_1/β_2-adrenergic activities and the corresponding structure–activity relationship (SAR). We have focused on lipophilicity and duration of action, and we have discovered a strong correlation in this series of molecules. A quantitative structure–activity relationship (QSAR) analysis will be presented that quantifies this relationship.

The search for new ultra long-acting β_2-adrenoreceptor agonists (LABA's), for the treatment of asthma and COPD, has become a very active area of drug discovery in recent years. Once-a-day compounds are entering the market (indacaterol[1], **3**) or in advanced clinical phase (vilanterol[2], **4**) and will potentially become the therapy of choice over salmeterol **1** or formoterol **2**, the two currently marketed (twice-daily) long acting β_2-adrenoreceptor agonists (Fig. 1). Duration of action is one of the most challenging issues in the field, and there are many publications[3–6] related to this topic. Two distinct hypotheses have been put forward to explain this issue: the exo-site hypothesis[7] and the diffusion microkinetic hypothesis.[8]

The exosite theory requires the presence of a specific binding site for the drug, apart from the orthosteric site, which contributes to retain the agonist for receptor activation. Such a mechanism would result in longer receptor residence time.

On the other hand the microkinetic theory is based on the partition of the drug into the membrane. Thus, in theory, lipophilic compounds will remain longer in the cellular membrane resulting in drugs with extended duration of action. The more soluble a molecule is in aqueous media, the more prone to be diffused away from the tissue.

We focused our attention on the microkinetic theory, and the effect of lipophilicity on duration of action was investigated in a series of compounds developed in our LABA research program.

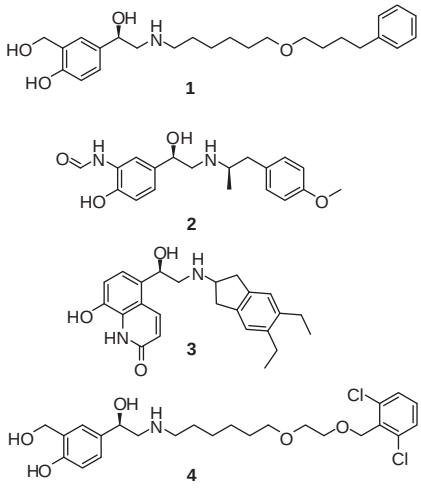

Figure 1. Structures of salmeterol **1**, formoterol **2**, indacaterol **3**, and vilanterol **4**.

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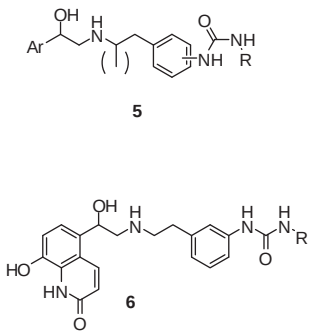


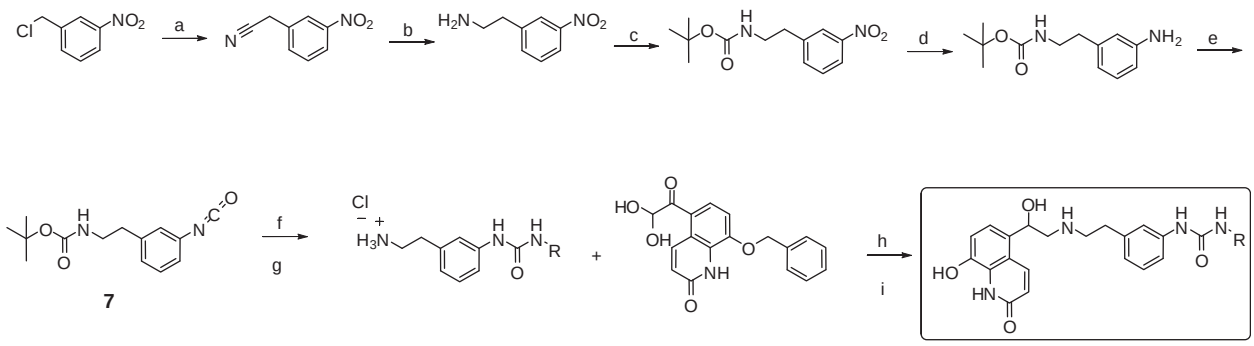
Figure 2. Structures of general scaffold **5** and refined scaffold **6**.

Our aim was to find potent, selective and long-acting β_2 agonists suitable for inhaled dry powder formulation. The starting points were β_2-adrenoreceptor agonists with the general formula **5** where Ar = saligenin or 8-hydroxycarbostyril moieties (Fig. 2).

As was the case for other compounds in the literature, the carbostyril moiety provided higher potency compared to saligenin and additionally *meta* substitution of phenyl urea showed improved potency over *para* substitution.

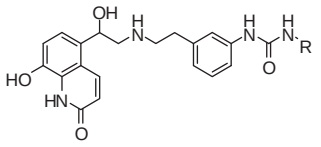
In general, the presence of an α-methyl adjacent to amine afforded less selective compounds versus the β1 receptor and this feature was removed early from our strategy.

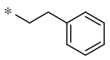
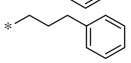
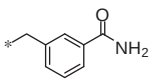
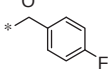
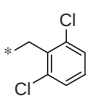
Thus **6** became our refined scaffold for further modifications (Fig. 2).

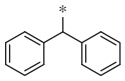
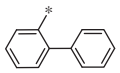
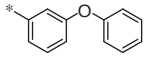
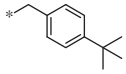


Scheme 1. Reagents and conditions: (a) KCN, MeOH/H_2O, 95 °C, 4 h, 62%; (b) $BH_3{\cdot}SMe_2$, THF, rt, 24 h, 43%; (c) $(BOC)_2O$, THF, rt, 2 h, 85%; (d) Pd/C 10%, H_2(30 psi), rt, 3 h, MeOH, 44%; (e) triphosgene, Et_3N, CH_2Cl_2, rt, 2.5 h, no isolation; (f) isocyanate split into aliquots, NH_2-R (1.1 equiv), rt, on; (g) HCl concd, dioxane, rt, 2 h, 51–82% (yields over three last steps); (h) Et_3N, $NaBH_4$, DMSO/MeOH, rt, 6 h, 43–77%; (i) Pd/C 10%, H_2, MeOH, rt, 4 h, 25–87%.

Table 1
Biological data and calculated log *P* values for urea analogues



Compound number	R	*c* log *P*[a]	In vitro duration of action (% of trachea tone recovery in 1 h)[b]	β_2 potency in G-P trachea (EC_{50}; nM)[c]	β_1 potency in rat left atria (EC_{50}; nM)[d]	In vivo potency[e] (μg/ml)	
						(IC_{50} at 4 h)	(IC_{50} at 24 h)
8		2.28	12	0.07	>10,000	0.2	3
9		2.31	15	0.2	900	0.3	>100
10		2.72	9	0.09	>10,000	2	>>10
11		3.15	2	0.05	>10,000	1	3
12		0.83	52	0.13	—	—	—
13		2.22	23	0.2	550	—	—
14		2.36	16	0.2	6300	—	—
15		3.51	4	0.2	1600	~4	>>100

16		4.04	2	0.2	8890	1	1.5
17		4.04	4	0.7	5770	3	7
18		3.94	8	3.2	296	—	—
19		4.62	0	1	≈10,000	—	—
20		3.99	5	0.9	—	—	—
3	Indacaterol	3.88	0	1	2310	5	12

[a] Calculated log *P* using ACD/ChemSketch.
[b] In vitro duration of action; expressed as the percentage of tone recovery reached after 1 h of washout of the test compound.
[c] β_2 adrenoreceptor agonist activity in isolated guinea-pig tracheal rings expressed as the concentration required to produce a 50% of relaxation (EC_{50}) versus the 100% relaxation (E_{max}) produced by 0.1 μM of isoprenaline.
[d] β1 adrenoreceptor agonist activity in rat left atria expressed as the concentration required to induce a 50% of an ionotropic effect.[10]
[e] Bronchocoprotective potency and duration of action was measured in guinea-pigs after 4 and 24 h of compounds' administration by aerosol. Data are expressed as the concentration of the compound required to produce a 50% inhibition of the bronchoconstriction induced by acetylcholine (iv).

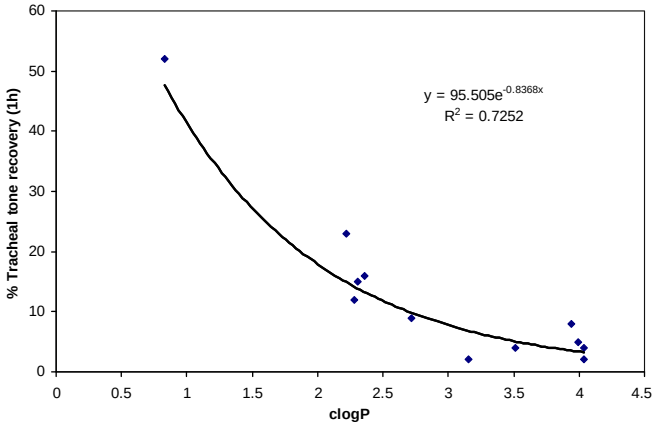


Figure 3. Correlation between % of tone recovery at 1 h versus *c* log *P* of the compounds selected.

The synthesis of these compounds was achieved starting from 1-(chloromethyl)-3-nitrobenzene as described in Scheme 1. Final compounds **6** were initially prepared in racemic form. The key intermediate was the isocyanate **7**, which was formed using triphosgene in the presence of triethylamine. This intermediate was not isolated and allowed us to incorporate a variety of different amines following a parallel strategy.

The resulting crude isocyanate was split into aliquots and added simultaneously the corresponding amines to form their ureas. Once the urea was formed the amine was deprotected. The coupling step with the quinolone moiety was done via reductive amination. The yield of this step was dependent upon the amine used.

Finally a deprotection of the hydroxyl via debenzylation gave the desired compounds with good yields and purities.

In the case of the most interesting molecules we developed an enantioselective synthesis[9] to obtain the more active R-enantiomers.

Following the above strategy several ureas were synthesized. To assess in vitro duration of action, we measured the percent recovery of tracheal tone at 1 h (the lower the recovery the longer the duration of action). Compound **8** gave high β_2 functional potency, excellent β_1/β_2 selectivity and 12% recovery of tracheal tone at 1 h (Table 1). For more lipophilic residues (entries 9–11), a decrease in the % recovery of tracheal tone at 1 h was observed, with phenpropyl analog **11** showing an exceptional one (2% recovery), while maintaining high potency and selectivity.

The use of a more polar residue (compound **12**) gave a faster recovery of tracheal tone (52%), suggesting a relationship between lipophilicity of the fragment 'R' and the corresponding in vitro duration of action.

As a result, we chose to further investigate 'R' residues with high log *P*. Given the distance between the secondary amine and the residue 'R', we considered the pK_a of the amine would be unaffected by the nature of 'R'. So, we used *c* log *P* instead of *c* log *D* to assess lipophilicity.

As shown in Table 1, all compounds showed high potency in the isolated guinea-pig trachea assay, most in the subnanomolar range, as well as good selectivity versus the left atria assay (low β_1 activity). The remarkably high tolerance of our scaffold to maintain high potency and selectivity over a wide diversity of substituents 'R' is noteworthy.

Many of the ureas described also demonstrated good duration of action values in the in vitro model and for some of them this translated into long duration of action in vivo.

In an attempt to quantify the relationship between lipophilicity and in vitro duration of action, we plotted both variables in a graph, and an interesting correlation was found as shown in Figure 3. (Compound **19** was excluded from the graph for mathematical reasons, as curve was below the abscissa).

Therefore, we could establish a clear link between lipophilicity and duration of action, and we can conclude that lipophilicity is one of the main aspects that drive duration of action, both in vitro and in vivo in our chemical series—a novel example of how to achieve a desired pharmacological profile in a series of LABA's by modulation of physicochemical properties.

These findings support the microkinetic theory as the more plausible explanation of the extended duration of action observed in the series, since it is the lipophilicity of the 'R' substituent, and not its individual chemical features that appears to be driving duration of action.

Furthermore, three of our ureas (**11, 16, 17**) showed a sustained in vivo duration of action in guinea-pig comparable to that of reference compound **3** (indacaterol).

Due to their excellent in vitro profile and in vivo duration of action, we synthesized selected ureas in enantiomerically pure R-form for further profiling, to fully assess their potential to produce a long lasting and safer inhaled bronchodilator.

The results of these studies will be presented in due course.

Acknowledgments

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