Journal of Medicinal Chemistry Article

J. Med. Chem. **2010,** *53,* 3675–3684 **3675**
DOI: 10.1021/jm100068m

The Identification of Indacaterol as an Ultralong-Acting Inhaled β_2-Adrenoceptor Agonist

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Received January 16, 2010

Following a lipophilicity-based hypothesis, an 8-hydroxyquinolinone 2-aminoindan derived series of β_2-adrenoceptor agonists have been prepared and evaluated for their potential as inhaled ultralong-acting bronchodilators. Determination of their activities at the human β_2-adrenoceptor receptor showed symmetrical substitution of the 2-aminoindan moiety at the 5- and 6-positions delivered the targeted intermediate potency and intrinsic-efficacy profiles relative to a series of clinical reference β_2-adrenoceptor agonists. Further assessment with an in vitro superfused electrically stimulated guinea-pig tracheal-strip assay established the onset and duration of action time courses, which could be rationalized by considering the lipophilicity, potency, and intrinsic efficacy of the compounds. From these studies the 5,6-diethylindan analogue indacaterol **1c** was shown to possess a unique profile of combining a rapid onset of action with a long duration of action. Further in vivo profiling of **1c** supported the long duration of action and a wide therapeutic index following administration to the lung, which led to the compound being selected as a development candidate.

Introduction

Inhaled β_2-adrenoceptor agonists play a central role in the symptomatic management of reversible obstructive airways diseases. As a compound class, they represent the most widely used, safe, and effective bronchodilators currently in clinical use. Additionally, in combination with either an inhaled corticosteroid, or a muscarinic antagonist, β_2-adrenoceptor agonists form the current gold-standard treatments for both asthma and COPD[a].[1] The evolution of this class of compounds has continued for over a century, and many groups have contributed to making improvements to the endogenous β_2-adrenoceptor ligand adrenalin, with the current state of the art being: salbutamol, a rapid onset-of-action and short acting compound, used PRN as a rescue medication for symptomatic relief; formoterol, a high intrinsic-efficacy, rapid onset-of-action, and long-acting compound with a 12 h duration of effect, used both for maintenance and as a rescue medication; salmeterol, a lower intrinsic efficacy agonist, with a slow onset-of-action, and also with a 12 h duration of effect, and used as a maintenance therapy.[2] Although the current long-acting inhaled β_2-adrenoceptor agonists in clinical use are very effective, poor compliance and poor control of nocturnal asthma have been highlighted as issues limiting their effectiveness in certain patient populations. To address these limitations, the next generation of compounds from this therapeutic class are anticipated to be inhaled β_2-adrenoceptor agonists capable of delivering sustained 24 h bronchodilation and thus suitable for once-daily administration.[3] Such once-daily agents, in combination with an emerging number of similarly dosed combination partners, are anticipated to deliver improved patient compliance and superior disease control.[4] In this article, we describe the medicinal chemistry approach leading to the identification of the ultralong-acting β_2-adrenoceptor agonist indacaterol, which has been shown in both asthma and COPD patient populations to be a fast onset-of-action 24 h bronchodilator that is well tolerated.[5] Figure 1 shows the structure of indacaterol and other β_2-adrenoceptor agonists of interest.

Several strategies have been proposed for the design and for the rationalization of agents that exhibit a sustained duration of effect when applied topically to the lung. These hypotheses are commonly used to account for differences in the duration of activity observed in both in vitro and in vivo preclinical models. In the case of β_2-adrenoceptor agonists, the compounds are typically compared at equieffective submaximal bronchodilating doses. These types of studies have proven useful for ranking compounds with respect to their intrinsic duration of action potential and for selecting examples for further study. Attempts to rationalize the data from these

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[a]Abbreviations: COPD, chronic obstructive pulmonary disease; PRN, pro re nata; NBS, *N*-bromosuccinimide; DME, 1,2-dimethoxyethane; ORTEP, Oak Ridge Thermal-Ellipsoid Plot; CHI_{IAM}, chromatographic hydrophobicity index; %HSA, percentage binding to human serum albumin.

Published on Web 04/19/2010

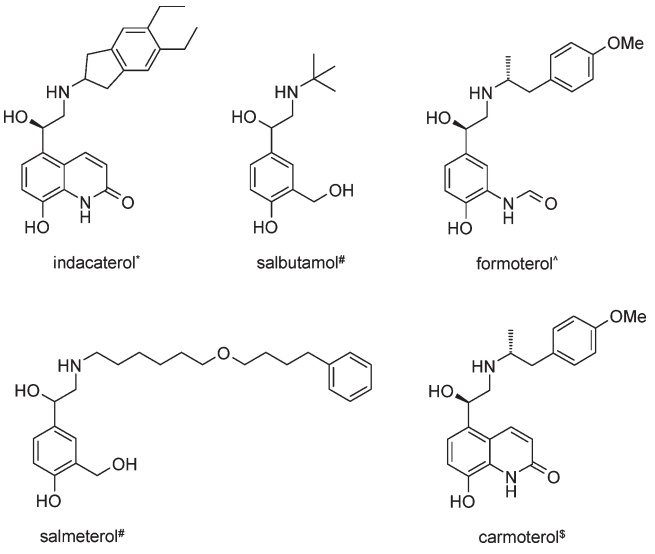


Figure 1. Structure of indacaterol **1c** and other important β_2-adrenoceptor agonists. *Indacaterol is a single enantiomers of the (*R*)-configuration, #salbutamol and salmeterol are racemic mixtures, ^formoterol is a racemic mixture of the L-diastereoisomer, $carmoterol is a single enantiomer with the (*R*)-configuration at the carbons bearing the hydroxyl and methyl groups.

studies has resulted in the development of several hypotheses to account for the duration of effect differences observed between inhaled β_2-adrenoceptor agonists. These include: the diffusion microkinetic theory, in which the high partitioning of lipophilic bases into phospholipids is used to account for the long duration of action of formoterol and salmeterol through the uptake of the compound into airway smooth muscle following administration;[6] exosite binding, in which the 4-phenylbutoxyhexyl amino-substituent of salmeterol is hypothesized to interact with a region of the β_2-adrenoceptor, remote from the catechol binding site, resulting in retention of the compound in close proximity to the receptor;[7] receptor kinetics, in which slow receptor off-rates have been proposed to account for differences observed within a series of 8-hydroxyquinolinone derived β_2-adrenoceptor agonists;[8] tight ligand-binding, in which the formation of a stable binary drug−receptor complex, which can repeatedly stimulate G-proteins, has been proposed to account for the duration of action of carmoterol.[9] Additionally, other factors can be anticipated to make a contribution to the duration of action of inhaled β_2-adrenoceptor agonists: intrinsic efficacy, where the level of agonist receptor occupancy required for delivering efficacy may influence the ease of maintaining the pharmacological effect;[10] limiting solubility and permeability, where a delayed passage from airway lumen to blood, due to slowed dissolution and permeation, has the potential to further extend lung residency.[11]

The inhaled route of administration is selected in the majority of cases for β_2-adrenoceptor agonists to maximize bronchodilator activity while minimizing their exposure to the systemic circulation. In the clinical setting, the ability to deliver efficacy with an acceptable separation from doses that are associated with significant β_2-adrenoceptor mediated systemic side-effects is one of the key factors in defining the human dose.[12] Additionally, for inhaled compounds, increasing the delivered dose typically leads to increases in the duration of effect. Following on from the above observations: the separation of bronchodilating doses from the doses that produce unacceptable levels of systemic β_2-adrenoceptor activation additionally provides a measure of the duration of action potential for a compound. Agents with a larger separation offer the potential to increase the duration of action beyond the intrinsic duration of action with relatively higher doses, if required, or to deliver a greater safety margin in addition to a long duration of effect. Applying this rationale, potentially allows an understanding for the large preclinical differences seen in the intrinsic duration of action measurements between the clinically similar agents salmeterol and formoterol.[13] The principal systemic β_2-adrenoceptor agonist effects observed in man being: tremor, tachycardia, hyperglycemia, and hypokalemia.[14] Several factors can be anticipated to contribute to a more favorable separation of the bronchodilating properties from the systemic β_2-adrenoceptor mediated side effects including: protein binding, where higher levels will lead to a reduced circulating free-fraction of compound being available for receptor activation;[15] rapid clearance from the systemic circulation, where rapid elimination of parent, or through the formation of metabolites of reduced activity, would minimize the impact of the compound redistributing from the lung, such as the formation of the phenolic glucuronide of formoterol;[16] interorgan distribution, where preferential distribution into lung tissue would lead to sustained concentrations of compound at the targeted site of action and delayed redistribution via the systemic circulation, such preferential distribution, as assessed by whole-lung levels, having been observed for a number of lipophilic bases in lower species;[17] intraorgan and intracellular distribution, where increased distribution into specific organelles, or intracellular regions, favoring lung retention and/or bronchodilation could assist in increasing the separation from doses at which undesired systemic side effects are observed.[18] Each of the above factors not being exclusive to a particular property, and in particular the latter two can also be considered as positive attributes for increasing the intrinsic duration of action of a molecule when topically applied to the lung.

The goal of the project from the outset was to identify an inhaled β_2-adrenoceptor agonist that was: well tolerated, with a rapid onset of action, comparable to formoterol and salbutamol, and most importantly, which could provide 24 h bronchodilation following once-daily administration. At the initiation of the project an analysis of the published information, and previous in-house experience with formoterol, had highlighted the important role of lipophilicity in regulating the duration of action of inhaled β_2-adrenoceptor agonists.[6,19] Thus, regulating the lipophilicity of the compounds targeted for synthesis and relating this parameter to their biological activities formed a key part of the early medicinal chemistry strategy. In particular, one question that remained unanswered at the start of the project was if a profile consistent with a rapid onset of action could be retained when the intrinsic duration of action was extended through increasing lipophilicity. This was based upon the observation that salmeterol, as the most lipophilic of the clinically well characterized β_2-adrenoceptor agonists, exhibits a slower onset of action in man. However, the lower intrinsic efficacy of salmeterol and subsequent requirement for a higher level of receptor occupancy to deliver bronchodilation, was also considered as a potential contributing factor to the delayed onset of action observed with this compound. For the optimal profile, a rapid onset of bronchodilation following administration was considered to be a favorable attribute to further improve upon patient compliance.[14] Thus, identifying series

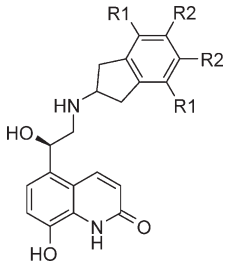


Figure 2. 2-Aminoindan analogues **1a**−**g**.

of compounds that could provide the desired β_2-adrenoceptor agonist properties and also allow homologation to sequentially increase lipophilicity were sought. During the course of the project, the disclosures of others,[20] in addition to some of our own experiences,[21] further supported lipophilicity based approaches as a means to identify inhaled long-acting β_2-adrenoceptor agonists. However, these disclosures also highlighted that increased levels of lipophilicity can lead to preclinical profiles consistent with the potential for a delayed onset of action. Thus, following on from an initial flowchart focus on controlling lipophilicity to regulate in vitro measurements of duration and onset of action, selected compounds were further evaluated to characterize their in vivo duration of action and separation of bronchodilator activity from systemic β_2-adrenoceptor mediated side effects. The goal being to ultimately select candidates with a long intrinsic duration of action, rapid onset of action, and as wide a therapeutic margin as possible with respect to the separation of systemic β_2-adrenoceptor mediated side-effects from the doses delivering bronchodilation.

Results and Discussion

One series which provided an interesting starting point was the combination of an 8-hydroxyquinolinone catechol mimetic with the 2-indanyl amino-substituent, as outlined by the general structure **1**, shown in Figure 2. The reasoning being that by selecting the highly efficacious 8-hydroxyquinolinone moiety a wide range of amino substituents were anticipated to provide derivatives within the desired β_2-adrenoceptor agonist potency and efficacy range.[22] Additionally, the 8-hydroxyquinolinone containing β_2-adrenoceptor agonist carmoterol had been reported to deliver >24 h bronchodilation, albeit with a narrow therapeutic margin with respect to systemic β_2-adrenoceptor mediated side-effects, when dosed in asthma patients as a nebulized solution.[23] In contrast, the less efficacious saligenin based variants, as exemplified by salbutamol and salmeterol, were anticipated to be more limited in the range of modifications that could yield the targeted potency and efficacy range. In addition, as part of the initial assessment, targeting an intrinsic efficacy closer to that of formoterol, and higher than for the above saligenin analogues, was considered as having a greater possibility for identifying a lipophilic compound with a rapid onset of action. Compounds with higher intrinsic efficacy were also favored to avoid the potential complication of antagonising the PRN salbutamol response, as previously reported preclinically for salmeterol.[24] However, saligenin based agonists have recently been reported as having provided the basis for designing ultralong-acting β_2-adrenoceptor agonist development candidates.[25] Additionally, a series of ultralong-acting β_2-adrenoceptor agonists based upon an alternative 6-hydroxybenzo[1,4]oxazin-3-one catechol mimetic have recently been reported to provide the clinical candidate olodaterol with an intrinsic efficacy significantly lower than formoterol, and the clinical evaluation of these agents is awaited with interest.[26] The selection of the 2-indanyl amino-moiety in **1** was based upon the ability to readily introduce substituents into the phenyl portion of this structural element to regulate the lipophilicity at positions remote from the key adrenalin-mimicking pharmacophore. Moreover, employing a symmetrical substitution pattern in the indan moiety results in the opportunity to maintain a plane of symmetry with a secondary α-carbon in the amino residue and thus provides a stereochemically simpler cyclic version of the α-methyl-β-phenethyl amino residues present in formoterol and carmoterol. Additionally, alkyl groups were an attractive option for the indan substituent as an achiral moiety could be retained when incremental homologations of two methylene units were made between analogues. The selection of alkyl groups was also anticipated to enable a wide range of lipophicities to be rapidly explored in a systematic fashion. Thus, the symmetrical 4,7- and 5,6-disubstituted 2-aminoindan analogues **1** became the targets for synthesis.

Table 1. Summary of Physicochemical Properties of the Aminoindan Analogues **1a**−**g** and Reference β_2-Adrenoceptor Agonists

compd	R1	R2	ClogP[a]	CHI_{IAM} pH 7.4	%HSA
1a	H	H	0.96	38.2	63.8
1b	H	Me	1.91	54.9	89.8
1c indacaterol	H	Et	2.97	59.7	95.7
1d	H	*n*-Pr	4.02	>70	98.6
1e	H	*n*-Bu	5.08	>70	99.5
1f	H	OMe	0.62	32.4	59.5
1g	Et	H	3.02	62.9	94.0
salbutamol			0.06	21.0	29.9
salmeterol			3.06	56.7	91.1
formoterol			1.26	40.4	31.7
carmoterol			1.31	37.7	57.1

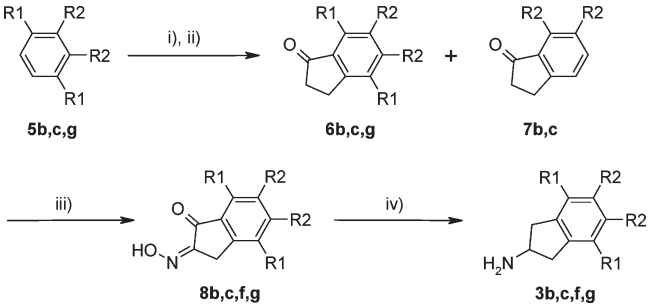
[a]clogP were calculated using Biobyte CLOGP version 4.71.

Chemistry

To prepare the targeted analogues **1a**−**g** in Table 1, the regioselective opening of the previously described (*R*)-8-benzyloxyquinolinone-5-epoxide **2** with a series of appropriately substituted 2-aminoindanes **3a**−**g** was anticipated to give the corresponding ethanolamine derivatives **4a**−**g** in line with previously reported routes to this class of compound.[27] Two approaches were taken to prepare the substituted aminoindan coupling partners **3a**−**g** required for the above epoxide-opening reaction, either by construction of the 5-membered indan ring by annulation of the corresponding disubstituted benzene derivatives **5** or by the sequential introduction of the two alkyl groups into 2-aminoindan.

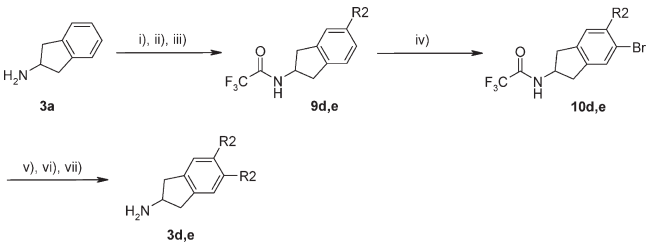
The first annulation route was used for the preparation of the 5,6-dimethyl, 5,6-diethyl, and 4,7-dimethyl 2-aminoindan intermediates **3b**,**c**,**g** and followed a literature procedure, as outlined in Scheme 1.[28] In the case of **3f**, 5,6-dimethoxyindan-1-one was sourced commercially and processed through the latter steps of the sequence in an analogous manner. Thus, a Friedel−Crafts acylation of the appropriate dialkyl-substituted benzene derivative with 3-chloropropionyl chloride was followed by a further intramolecular Friedel−Crafts alkylation to give the 1-indanones **6**. The regioselectivity in the cyclization for the sequences starting from the 1,2-dialkyl-sustituted benzenes **5b**,**c** was found to be modest, only favoring the 5,6-disubstituted indanone intermediates **6** over the 4,5-regioisomers **7** by a factor of ca. 2:1. However, for these

Scheme 1. Annulation Route to the Aminoindan Intermediates **3b**,**c**,**f**,**g**[a]



[a] (i) 1.0 equiv 3-chloropropionylchloride, 2.5 equiv $AlCl_3$, $MeNO_2$, 3 h, rt (94−99%); (ii) conc H_2SO_4, 3 h, rt (72−83%); (iii) 1.1 equiv *n*-BuONO, 25:1 MeOH: conc HCl, 2 h, 40 °C (54−93%); (iv) 1 atm H_2, catalytic 10% Pd on C, 15:1 AcOH:conc H_2SO_4, 48 h, rt (11−88%).

Scheme 2. Alkylation Route to the Aminoindan Intermediates **3d**,**e**[a]



[a] (i) 1.2 equiv $CF_3C(O)OEt$, *i*-PrOAc, 0 °C to rt, 3 h (71%); (ii) 6 equiv RC(O)Cl, 2.6 equiv $AlCl_3$, 0 °C, 2 h (82−90%); (iii) 1.4 atm H_2, catalytic 10% Pd on C, EtOH, 2 weeks, rt (79−96%); (iv) 1.0 equiv NBS, 1.1 equiv Br_2, 0 °C, 30 min (97%); (v) 3 equiv 1-alkenyl boronic acid, 4 equiv CsF, 0.03 equiv $Pd(PPh_3)_4$, DME, reflux, 18 h (45−86%); (vi) 1 atm H_2, H-Cube flow reactor with 10% Pd on C cartridge, EtOAc, rt (90−95%); (vii) 1:1 EtOH:6 M $NaOH_{(aq)}$, reflux, 1 h (82−90%).

examples, the direct reaction of the isolated mixtures of the indanones **6** and **7** with *n*-butylnitrite resulted in the precipitation of the desired 5,6-disubstituted keto-oximes **8** directly from the reaction mixture. This approach made for an efficient process that could be readily performed on a multigram scale. Subsequent reduction of the keto-oximes **8** gave the targeted 2-aminoindan intermediates **3b**,**c**,**f**,**g**.

For the 5,6-*n*-propyl and 5,6-*n*-butyl intermediates **3d**,**e**, an alternative route to that described above involving the sequential introduction of the two alkyl groups was found to be more efficient, as shown in Scheme 2. Starting from 2-aminoindan, protection as the trifluoroacetamide was followed by a Friedel−Crafts acylation reaction, which proceeded with a high level of regioselectivity for the 5-position. Hydrogenation of the ketone completed the installation of the first alkyl substituent to give the intermediates **9**. In contrast to the procedure reported for the diethyl analogue, preparation of the homologues **3d**,**e** via a second Friedel−Crafts acylation of **9** proved not to be an efficient process and an alternative sequence was developed.[29] Thus, bromination of the intermediates **9** was found to proceed at the 6-position with a high level of regioselectivity to give the bromoindans **10**. Installation of the second alkyl group into **10** was achieved with a palladium-catalyzed cross-coupling reaction with the appropriate 1-alkenyl boronic acid, followed by hydrogenation of the resulting olefin. Subsequent deprotection of the trifluoroacetamide under basic conditions gave the targeted 2-aminoindan intermediates **3d**,**e** in good overall yield.

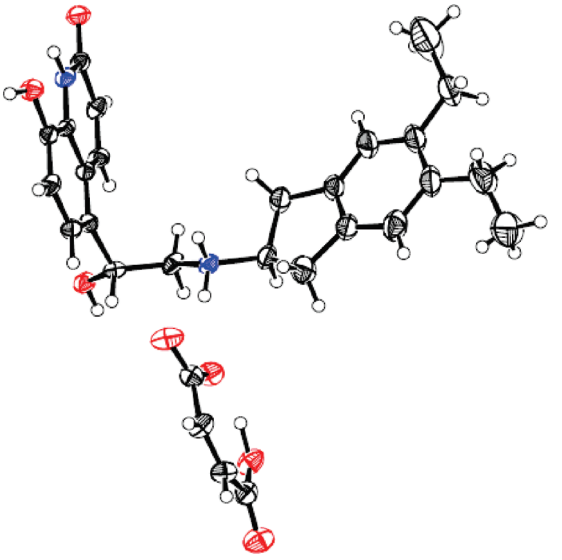
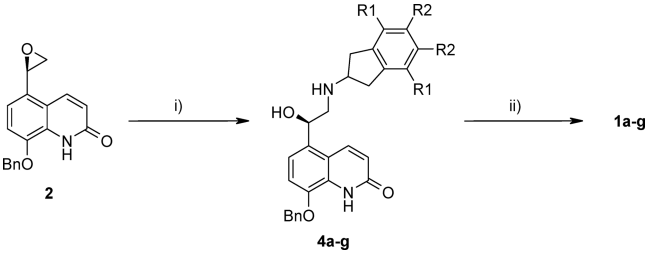


Figure 3. X-ray structure of the maleate salt of **1c**: ORTEP showing 30% displacement ellipsoids.

Scheme 3. Synthesis of the Indan Derivatives **1a**−**g**[a]



[a] (i) 1.1 equiv 2-aminoindan derivative **3**, 1-BuOH, 110 °C, Emrys Optimizer microwave, 1 h (21−36%); (ii) 1 atm H_2, catalytic 10% Pd on C, MeOH, rt, 1 h (20−93%).

With the 2-aminoindan intermediates **3a**−**g** in hand regioselective opening of the epoxide **2** at the sterically least demanding position could be achieved under a variety of conditions, of which heating a slight excess of the 2-aminoindan with respect to the epoxide in 1-butanol at 110 °C proved to be the most reliable. The targeted compounds **1a**−**g** were then obtained following hydrogenolysis of the benzyl residue of the intermediates **4a**−**g**, as shown in Scheme 3. Conformation of the regiochemical outcomes in the above sequence were obtained following a single-crystal X-ray structural analysis with the maleate salt of the 5,6-diethyl analogue **1c**, as shown in Figure 3.

Physical Chemistry Evaluation

With the analogues **1a**−**g** in hand, the lipophilicities and affinities for biological matrices were assessed in silico by calculating log *P* values and also experimentally with HPLC methods being used to measure affinity for immobilized artificial membranes (data expressed as CHI_{IAM}) and for binding to human serum albumin (%HSA).[30] These physicochemical data for the compounds **1a**−**g** and the reference β_2-adrenoceptor agonists are shown in Table 1.

Evaluating the physicochemical properties in Table 1, passing down the homologous series of 5,6-disubstituted indan analogues **1a**−**e**, the clogP values predict an approximately 10-fold increase in the partitioning into a 1-octanol phase relative to an aqueous phase for the addition of each pair of

methylene groups. These calculated increases in lipophilicity correlate well with the increase in affinity for phospholipids measured by CHI_{IAM}. The magnitude increasing as the homologous series is ascended before reaching the dynamic range of the HPLC method used to measure CHI_{IAM} for the dipropyl and dibutyl analogues **1d**,**e**. Comparison of the regioisomeric diethyl analogues **1c** and **1g** indicates little difference in clogP and CHI_{IAM} values between the alternative arrangement of the indan alkyl residues. The 5,6-dimethoxy analogue **1f** has the lowest calculated clogP value of the series and also the lowest measured affinity for the phospholipid phase from the CHI_{IAM} values. Similarly, the affinity for human serum albumin follows the same trend with free fractions calculated from the retention times ranging from 40.5% and 36.3% for the 5,6-dimethoxy and unsubstituted analogues **1g** and **1a** to 1.4% and 0.5% for the dipropyl and dibutyl analogues **1d**,**e**. In the case of **1c**, the estimated free fraction derived from the %HSA HPLC method of 4.3% correlated well with the measured human protein binding figure of 95.1−96.2%.

Comparison of the physicochemical properties of the 8-hydroxyquinolinone 2-aminoindan analogues **1a**−**g** with the reference β_2-adrenoceptor agonists: all show an increased lipophilicity and phospholipid affinity compared to the short acting salbutamol; both formoterol and carmoterol exhibit similar levels of lipophilicity which fall between the unsubstituted and 5,6-dimethylindan analogues **1a** and **1b**; salmeterol, the most lipophilic of the reference compounds, is comparable to the diethyl analogues **1c** and **1g**. To better understand the amphiphilic nature of the interaction with phospholipids the pK_a values for **1c**, salmeterol, formoterol, and carmoterol were measured as representative members of the three different catechol mimetics from the compounds in Table 1, and these pK_a data, as determined by two independent methods, are shown in Table 2. Analysis of these pK_a data indicates an increased acidity for the 8-hydroxyquinolinone phenol moiety of **1c** and carmoterol relative to the equivalent functionality present in salmeterol and formoterol. A consequence of this difference is that, at physiological pH, the 8-hydroxyquinolinone containing examples are anticipated to exist in solution predominantly as the zwitterionic species (protonated amine/phenoxide). In contrast, for formoterol and salmeterol, the neutral uncharged-species will be the predominate one in solution. However, these differences resulted in no substantial deviation in the bulk amphiphilic interaction, as measured by CHI_{IAM}, from that which would be predicted by extrapolation from the clogP values for the 8-hydroxyquinolinone containing examples compared to the other β_2-adrenoceptor agonist series. In contrast, a more detailed study of membrane kinetics and the impact upon membrane fluidity, in membranes of varying composition, has revealed some differences between the interactions with **1c** and with salmeterol.[31]

Table 2. pK_a Values Measured by Potentiometric Titration and UV Titration for **1c**, Salmeterol, Formoterol and Carmoterol

	potentiometric titration		UV titration	
compd	pK_a phenol	pK_a amine	pK_a phenol	pK_a amine
1c	6.7	8.3	6.7	8.3
salmeterol	10.2	8.5	9.9	9.0
formoterol	8.8	8.2	9.1	8.1
carmoterol	7.3	8.6	7.3	8.6

Comparison of the free fractions derived from the human serum-albumin elution rates: the highest values, of ca. 40%, were determined for the unsubstituted **1a** and 5,6-dimethoxy **1f** aminoindan analogues which were lower than salbutamol and comparable to formoterol and carmoterol. The 5,6-dimethyl analogue **1b** exhibited a comparable ca. 10% free fraction to salmeterol, with the higher homologues exhibiting progressively lower unbound fractions to the level of 0.5% for the 5,6-dibutyl analogue **1e**.

Biological Evaluation

To evaluate the activities at the human β_2-adrenoceptor studies were performed on Chinese hamster ovarian cells expressing stable recombinant human β_2-adrenoceptors.[32] Filtration binding assays were performed with membranes from these cells using [^{125}I]cyanopindolol as the radioligand. Functional cyclic adenosine monophosphate studies were performed on whole cells. Additionally, an electrically stimulated superfused guinea pig tracheal-strip assay provided a further measure of potency.[33] However, the principal role of this assay was as an initial measure of the onset of action and intrinsic duration of action profiles of the compounds. The data from the in vitro β_2-adrenoceptor assays are shown in Table 3.

In evaluating the activities at the human β_2-adrenoceptor, all the 5,6-disubstituted indan analogues **1b**−**f** exhibited similar levels of affinity to the unsubstituted parent compound **1a** with only a 6-fold spread between the highest affinity

Table 3. Summary of the in Vitro β_2-Adrenoceptor Agonist Activities of the Aminoindan Analogues **1a**−**g** and Reference Compounds[a]

	binding[b]	functional activity[c]		guinea pig tracheal strip[d]		
compd	K_i (nM)	EC_{50} (nM)	IE %	IC_{50} (nM)	onset (min)	duration (h)
1a	218 ± 26	5.0 ± 0.56	88	0.83 ± 0.3	28 ± 2	1.2 ± 0.5
1b	522 ± 115	39 ± 3.4	74	48 ± 1	42 ± 9	1.4 ± 0.3
1c indacaterol	76 ± 7.2	11 ± 3.1	75	7.9 ± 0.1	35 ± 2	> 12
1d	119 ± 15	14 ± 1.6	74	20 ± 1	55 ± 10	> 12
1e	112 ± 17	25 ± 6.0	79	> 1000	> 180	> 12
1f	342 ± 30	18 ± 3.0	72	2.3 ± 0.9	28 ± 3	1.2 ± 0.3
1g	692 ± 87	115 ± 14	51	75 ± 1	51 ± 6	2.1 ± 0.5
salbutamol	1828 ± 312	68 ± 8.3	45	17 ± 0.8	28 ± 3	0.9 ± 0.1
salmeterol	0.39 ± 0.09	0.32 ± 0.04	30	3.5 ± 0.9	120 ± 34	> 12
formoterol	23 ± 2.0	1.3 ± 0.28	100	0.4 ± 0.1	28 ± 1	1.2 ± 0.2
carmoterol	3.19 ± 0.52	0.76 ± 0.17	95	0.30 ± 0.01	28 ± 2	1.6 ± 0.1

[a]All methods for biological measurements are as previously reported.[32] [b]Human β_2-adrenoceptor binding ± SEM (n = 3−4). [c]Human β_2-adrenoceptor functional activity ± SEM (n = 3). [d]Mean IC_{50} ± SEM (n = 3−5), onset of action, and duration of action were measured at compound concentrations nearest to their IC_{50} values.

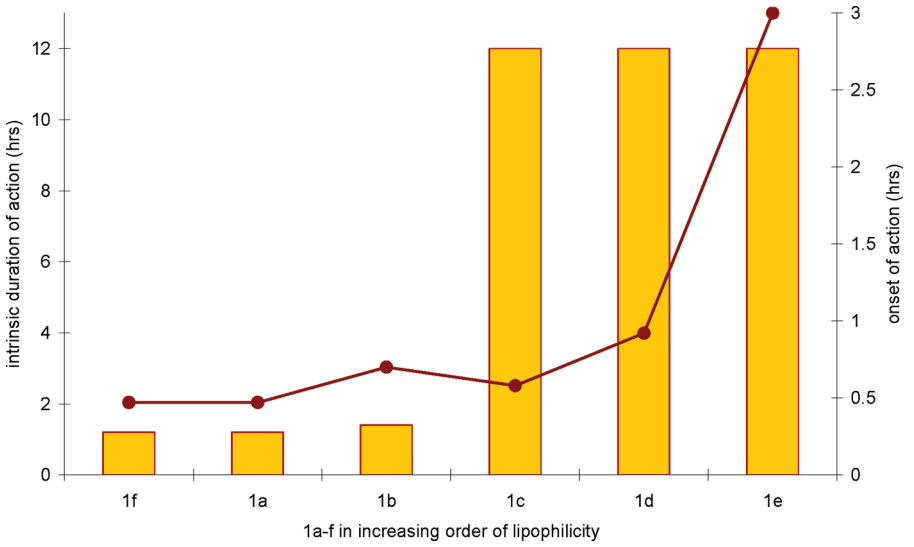


Figure 4. Relationship between lipophilicity and onset (linear plot) and duration (bar graph) of action for the 5,6-disubstituted indan derivatives **1a**−**f**.

observed for the diethyl analogue **1c** to the lowest affinity of the dimethyl analogue **1b**. The isomeric 4,7-diethyl analogue **1g** had the lowest affinity, which was 10-fold lower than the isomeric **1c**. A similar pattern was also observed in the functional assay in which the unsubstituted example **1a** was found to be the most potent and efficacious agonist, achieving an intrinsic efficacy of 88% compared to formoterol. The 5,6-disubstituted analogues **1b**−**f** all exhibited similar 2- to 8-fold lower potencies compared to **1a** and slightly lower intrinsic efficacies in the 72−79% range. The 4,7-diethyl analogue **1g** again had the lowest potency, which was 10-fold lower than the isomeric **1c** and also the lowest intrinsic efficacy of 51%, indicating the 4,7-substitution pattern to be less effective for activating the β_2-adrenoceptor. All the human β_2-adrenoceptor data are shown in Table 3.

Comparison of the human β_2-adrenoceptor agonist data with the reference compounds indicates the 5,6-disubstituted aminoindan analogues **1a**−**f** to be intermediate in potency between the more potent salmeterol, formoterol, and carmoterol and the less potent salbutamol. Similarly, the intrinsic efficacies of the 5,6-disubstituted aminoindan analogues **1a**−**f** fall within an intermediate range relative to the reference compounds but closer to the high efficacy agonist formoterol. Even the least potent, and least efficacious, isomeric 4,7-diethylindan analogue **1g** exhibited an intrinsic efficacy greater than the saligenin analogues salbutamol and salmeterol.

To assess the onset and intrinsic duration of action profiles the 8-hydroxyquinolinone 2-aminoindan derivatives **1a**−**g** were tested using a superfused guinea pig tracheal-strip assay.[33] Onset of action was assessed by the time to reach maximal inhibition of contraction following the initial 30 min drug administration phase and intrinsic duration of action from the time for the response to decay to 50% of the maximal response during the washout phase. Concentrations close to their IC_{50} values were used from dose−response studies to compare the parameters between compounds. For the 5,6-disubstituted indan examples **1a**−**f**, a good correlation between lipohilicity and increasing onset and intrinsic duration of action was observed, as shown by the representation in Figure 4. The plots show that with increasing lipophilicity, between the dimethyl analogue **1b** and the diethyl analogue **1c**, a transition from a short duration of action (< 2 h) to a long duration of action (> 12 h) was observed. In contrast, rapid onset of action profiles, with maximal responses being observed close to the end of the 30 min drug administration phase, were determined with increasing lipophilicity through the series to the diethyl analogue **1c**. A further increase in lipophilicity to the dipropyl analogue **1d** resulted in a trend for an increasing onset of action, extending for 25 min beyond the end of the drug administration phase, which became even further protracted for the dibutyl analogue **1e**. The dibutyl analogue **1e** also being of much lower potency in the tissue preparation, in contrast to the membrane and cellular assays, indicating that such high levels of affinity for biological matrices (lipophilicity and serum binding) may be sufficient to significantly limit the free drug concentration available for receptor activation in intact tissue as compared to measurements in less complex assay systems.

An overall analysis of the guinea pig tracheal-strip data for the 8-hydroxyquinolinone 2-aminoindan analogues **1a**−**g** in comparison with the reference compounds indicated a good correlation between lipophilicity and duration of action: salbutamol as the least lipophilic example exhibited the shortest duration of effect; formoterol and carmoterol with similar lipophilicity, falling between that of **1a** and **1b**, gave rise in all four cases to relatively short intrinsic duration of action in the range of 1.2−1.6 h; salmeterol, as the most lipophilic reference β_2-adrenoceptor agonist, with a comparable lipophilicity to **1c**, gave rise to long intrinsic durations of action for both compounds of > 12 h. Interestingly, the 4,7-diethyl regioisomer **1g**, with comparable lipophilicity to salmeterol and **1c**, showed a much shorter intrinsic duration of action and slower onset of action. The shorter duration of effect determined for **1g** is presumably due to the 10- to 20-fold lower potency compared to salmeterol and **1c** in the guinea pig preparation. However, the intermediate intrinsic efficacy of **1g** also has the possibility to contribute to these differences in the intrinsic duration of action as well as to the differences observed in onset of action. An assessment of the guinea pig tracheal-strip data for the 8-hydroxyquinolinone 2-aminoindan derived series indicated that the 5,6-diethyl analogue **1c** was able to fulfill the projects targeted in vitro profile by combining a rapid onset of action, comparable with salbutamol, formoterol, and carmoterol, with a long duration of action comparable with salmeterol.

After evaluating all the above in vitro data, the 5,6-diethyl indan analogue **1c** was selected for further profiling based upon: the unique rapid onset and long duration of action profile observed in the guinea pig tracheal-strip preparation, which could also be reproduced in human isolated bronchi and precision-cut lung slices;[24,34] the intermediate intrinsic efficacy at the human β_2-adrenoceptor, which was considered ideal to avoid both antagonism of PRN bronchodilator use, and to be confident of avoiding tachyphylaxis to ensure maintaining the bronchodilating properties following chronic administration; and the intermediate potency, which was seen as a desirable attribute when extrapolating this observation to a likely human dose. A clinical dose greater than that required for formoterol was considered advantageous for producing a robust drug-product with the available Novartis in-house inhalation devices. Pharmacokinetic studies with **1c** showed high clearance values in the rat, with the phenolic glucuronide identified as the primary circulating metabolite. The phenolic glucuronide of **1c** was shown to possess no appreciable affinity for the β_2-adrenoceptor. This metabolic profile in combination with the relatively low plasma free-fraction were considered as positive attributes for providing the widest separation of bronchodilating doses from doses producing significant β_2-adrenoceptor agonist mediated side effects. A counter screen with **1c** against a panel of 82 enzymes and receptors revealed the closely related β_1- and β_3-adrenoceptor as the most potent off-target activities. The quantification of the relatively weak functional effects of **1c** against the β_1- and β_3-adrenoceptor have been previously reported.[32] To further profile **1c**, the duration of action and the separation of the bronchodilating doses from doses producing systemic β_2-adrenoceptor mediated side effects were assessed in two in vivo models: a serotonin-induced bronchoconstriction model in the guinea pig and a methacholine-induced bronchoconstriction model in the rhesus monkey. The results from these two in vivo models have been previously reported, with the key findings being: when dosed at an ED_{80} dose level in the guinea pig, **1c** produced an intrinsic duration of action of 24 h compared to 4 and 12 h for formoterol and salmeterol, respectively, and when dosed at an ED_{80} dose level in the rhesus monkey, **1c** was associated with a lower level of systemic β_2-adrenoceptor mediated side effects when compared to formoterol and salmeterol.[32] On the basis of the above data, the 5,6-diethyl-amino indan analogue **1c** was selected as a candidate for clinical development.

Conclusion

In summary, within a series of 8-hydroxyquinoline 2-aminoindan derived β_2-adrenoceptor agonists, lipophilicity was used as the basis for the design and rationalization of their onset and duration of action profiles, as assessed by a guinea pig tracheal-strip assay. In addition to lipophilicity, potency and intrinsic efficacy have also been shown to be contributing factors in regulating these in vitro time course profiles. Selected from these studies was the 5,6-diethyl substituted indan analogue **1c**, which was found to have a unique rapid onset of action and long intrinsic duration of action profile when compared to a series of clinically used β_2-adrenoceptor agonists. Following further profiling, compound **1c** was selected as a development candidate and has now been shown in extensive clinical trials when administered once-daily via a dry-powder inhalation device to be a rapid onset-of-action bronchodilator that is well tolerated and capable of providing sustained 24 h improvements in lung function in both asthma and COPD patient populations. Compound **1c** now bears the International Nonproprietary Name indacaterol and has recently been approved for the treatment of COPD in Europe, with New Drug applications currently undergoing regulatory review in other regions.

Experimental Section

General Methods. All reactions were carried out at normal atmospheric pressure, under argon, unless otherwise stated. Solvents and reagents were purchased from commercial suppliers (Aldrich, Fluka, Maybridge and Fisher) and used without further purification. For purification, normal phase silica gel prepacked columns were used (Isolute Flash Si II); for TLC, plates precoated with silica gel 60 F 254 on aluminum (Merck KGaA) were used, detection was by UV (254 nm); pH of solutions were determined using pHix 0−14 paper (FisherBrand). Purity was determined by HPLC chromatography with both UV (diode array detection) and MS detection with reverse phase Xterra C_{18} columns (50 mm × 4.6 mm, 5 μm particle size) and mobile phases consisting of 0.1% trifluoroacetic acid in acetonitrile/water, with gradients running from 5% to 95% acetonitrile in water. The minimum purities indicated are based upon uncorrected UV peak-areas at 220 and 245 nm. Mass spectrometry was performed using a Micromass time-of-flight LCT instrument. Proton, fluorine, and carbon (standard and dept) NMR were performed on Bruker Advance (400 and 500 MHz) and Varian Mercury Plus Oxford AS400 (400 MHz) instruments. Chemical shifts (δ) are quoted in ppm relative to tetramethylsilane (TMS) as an internal standard, where (δ) TMS = 0.00 ppm. The multiplicity of the signal is indicated as s, singlet; d, doublet; t, triplet; q, quartet; dd, double of doublets; dt, doublet of triplets; dq, doublet of quartets; td, triplet of doublet; tt, triplet of triplets; m, multiplet, defined as all multipeak signals where overlap or complex coupling of signals makes definitive descriptions of peaks difficult; br, broad. All peaks should be taken as sharp unless otherwise described. Peaks not described have not been observed due to extensive coupling broadening the signal. For example, the carbon spectra of most of the trifluoroacetamide species are missing the ^{13}C signal from the $-CF_3$ carbon; this is due to significant coupling from the three adjacent ^{19}F atoms, broadening the ^{13}C signal beyond detection at low concentrations. In certain solvents, the ^{13}C spectra of the aminoindan moieties in **1** and **4** exhibit diastereotopicity and are observed as double resonances. Coupling constants are quoted in Hz to one decimal place. Room temperature (RT) is within the range 19−22 °C. The term in vacuo is used to describe solvent removal by Büchi rotary evaporation between 17 and 40 °C at 25−250 mbar unless otherwise stated. Pd/C refers to palladium on amorphous carbon catalyst, with w/w referring to weight of catalyst used as a percentage of weight of substrate. HRMS is high resolution mass spectrometry. For NMR experiments, $CDCl_3$ is fully deuterated (*d*) chloroform, DMSO is fully deuterated (d_6) dimethylsulfoxide, and MeOD is fully deuterated (d_4) methanol. Solvents were chosen according to position of solvent peak in spectra and solubility. The purity of all compounds screened in biological assays was determined to be >95% by HPLC/MS analysis, and for selected examples, purity was additionally assessed by elemental analysis. The purity of the synthetic intermediates was determined to be >95% by HPLC/MS unless otherwise indicated, and for selected examples purity was additionally assessed by elemental analysis.

Preparation of the 2-Aminoindan Derivatives 1a−g. 8-Hydroxy-5-[(*R*)-1-hydroxy-2-(indan-2-ylamino)-ethyl]-1*H*-quinolin-2-one (1a). A solution of 8-benzyloxy-5-[(*R*)-1-hydroxy-2-(indan-2-ylamino)-ethyl]-1*H*-quinolin-2-one **4a** (1.64 g, 0.004 mol) in methanol (200 mL) was purged with nitrogen prior to the addition of 10% Pd on carbon (153 mg, 10% w/w). The reaction vessel was

purged successively with nitrogen and hydrogen and then finally pressurized to 0.05 bar above atmospheric pressure with hydrogen. After stirring at this hydrogen pressure for 1 h, the reaction vessel was purged with nitrogen and the reaction mixture filtered through celite and washed with methanol. The solvent was removed in vacuo, and the resulting beige solid was triturated with isohexane and compound **1a** was collected by filtration and dried under vacuum to give 245 mg yellow powder (20% yield). HPLC/MS: > 95% purity; *m*/*z* 337 [(M + H)$^+$ 100%]. ^{1}H NMR (400 MHz, $CDCl_3$): 2.72 (2H, br, dt, J = 15.3 CH_2), 2.83 (2H, d, J = 6.6, CH_2), 3.15 (2H, m, CH_2), 3.61 (1H, t, J = 6.7, CH), 5.11 (1H, m, CH), 6.61 (1H, d, J = 9.9, CH), 6.88 (1H, d, J = 8.2, Ar-H), 7.11 (5H, m, 5 × Ar-H), 8.14 (1H, d, J = 9.9, CH). ^{13}C NMR (100 MHz, $CDCl_3$): 39.83, 55.34, 59.50, 69.48, 114.38, 118.00, 120.90, 121.32, 125.00, 126.95, 128.43, 130.34, 137.89, 141.35, 143.45, 162.63. Analysis: Calcd for $C_{20}H_{20}N_2O_3 \cdot 0.8H_2O$ (0.8 equiv of water observed by ^{1}H NMR): C 68.48; H, 6.21; N, 7.99. Found: C, 68.56; H, 6.34; N, 8.04.

8-Hydroxy-5-[(*R*)-1-hydroxy-2-(5,6-dimethylindan-2-ylamino)-ethyl]-1*H*-quinolin-2-one (1b). Prepared in an analogous fashion to compound **1a**. A reaction on a 0.33 mmol scale yielded 95 mg of compound **1b** as a yellow powder (80% yield). HPLC/MS: > 95% purity; *m*/*z* 366 [(M + H)$^+$, 100%]. ^{1}H NMR (500 MHz, DMSO): 2.15 (6H, s, 2 × CH_3), 2.60 (2H, m, 2 × CH), 2.75 (2H, m, CH_2), 2.97 (2H, m, 2 × CH), 3.52 (1H, m, CH), 5.02 (1H, m, CH), 6.51 (1H, d, J = 9.9, Ar-H), 6.93 (1H, s, Ar-H), 6.93 (1H, s, Ar-H), 7.09 (1H, d, J = 8.1, Ar-H) 8.18 (1H, d, J = 9.9, CH) 10.6−9.7 (1H, s, br). ^{13}C NMR (125 MHz, DMSO): 19.31, 38.84, 55.56, 59.18, 69.10, 113.85, 117.00, 119.64, 121.48, 125.42, 125.46, 128.36, 131.32, 133.59, 136.92, 139.04, 139.09, 142.56, 160.68. Analysis: Calcd for $C_{22}H_{24}N_2O_3 \cdot 1.5H_2O$ (1.5 equiv of water observed by ^{1}H NMR): C, 67.50, H, 6.95, N, 7.16. Found: C, 67.36, H, 6.79, N, 7.07.

8-Hydroxy-5-[(*R*)-1-hydroxy-2-(5,6-diethylindan-2-ylamino)-ethyl]-1*H*-quinolin-2-one (1c). Prepared in an analogous fashion to compound **1a**. A reaction on a 0.75 mmol scale yielded 265 mg of compound **1c** as a white solid (83% yield). ^{1}H NMR (400 MHz, DMSO): 1.10 (t, 6H, J = 7.5, 2 × CH_3), 2.51 (q, 4H, J = 7.5, 2 × CH_2), 2.53−2.62 (m, 2H, indanyl CH_2), 2.68−2.76 (m, 2H, CH_2N), 2.90−3.03 (m, 2H, indanyl CH_2), 3.48−3.55 (m, 1H, indanyl CH), 4.97−5.04 (m, 1H, CHOH), 6.49 (d, 1H, J = 11.0, Ar-H), 6.90 (d, 1H, J = 9.1, Ar-H), 6.91 (s, 2H, indanyl Ar-H), 7.07 (d, 1H, J = 9.1, Ar-H), 8.17 (d, 1H, J = 11.0, Ar-H). ^{13}C NMR (100 MHz, DMSO): 16.34, 25.54, 39.32, 39.50, 56.30, 59.88, 69.71, 114.56, 120.38, 122.13, 124.83, 124.88, 129.15, 131.71, 137.64, 139.65, 139.88, 139.94, 143.61, 161.39. HRMS (*m*/*z*): [M + H]$^+$ (100%) calcd for $C_{24}H_{29}N_2O_3$, 393.2178; found, 393.2174 (error 1.0 ppm).

8-Hydroxy-5-[(*R*)-1-hydroxy-2-(5,6-di-*n*-propylindan-2-ylamino)-ethyl]-1*H*-quinolin-2-one (1d). Prepared in an analogous fashion to compound **1a**. A reaction on a 5 mmol scale yielded 460 mg of compound **1d** as a yellow powder (21% yield). HPLC/MS: > 95% purity; *m*/*z* 422 [(M + H)$^+$, 100%]. ^{1}H NMR (400 MHz, DMSO): 0.94 (6H, t, J = 7.3, 2 × CH_3), 1.50 (4H, q, J = 7.5, 2 × CH_2), 2.48 (4H, m, 2 × CH_2), 2.58 (2H, dt, J = 15.6 and 5.4, CH_2), 2.74 (2H, m, CH_2), 2.99 (2H, m, CH_2), 3.53 (1H, m, CH), 5.01 (1H, t, J = 5.4, CH), 6.50 (1H, d, J = 9.9, CH), 6.92 (3H, m, 3 × Ar-H), 7.08 (1H, d, J = 8.2, Ar-H) 8.19 (1H, d, J = 9.9, Ar-H). ^{13}C NMR (100 MHz, DMSO): 14.43, 24.59, 34.47, 42.18, 55.71, 59.41, 69.07, 114.24, 117.39, 120.07, 121.94, 125.33, 128.73, 137.26, 138.07, 139.27, 139.32, 143.02, 161.13. Analysis: Calcd for $C_{26}H_{32}N_2O_3 \cdot 1.2H_2O$ (1.2 equiv of water observed by ^{1}H NMR): C, 70.63; H, 7.84; N, 6.34. Found: C, 70.48; H, 7.89; 6.46.

8-Hydroxy-5-[(*R*)-1-hydroxy-2-(5,6-di-*n*-butylindan-2-ylamino)-ethyl]-1*H*-quinolin-2-one (1e). Prepared in an analogous fashion to compound **1a**. A reaction on a 0.22 mmol scale yielded 56 mg of compound **1e** as a yellow solid (58% yield). ^{1}H NMR (400 MHz, DMSO): 0.90 (6H, t, J = 7.3, 2 × CH_3), 1.34 (4H, m, 2 × CH_2), 1.47 (4H, m, 2 × CH_2), 2.48 (4H, m, 2 × CH_2), 2.58 (2H, m, CH_2), 2.74 (2H, m, CH_2), 2.97 (2H, m, CH_2), 3.51 (1H, m, CH), 5.02 (1H, m, CH), 6.50 (1H, d, J = 10.0, CH), 6.91 (1H, s, Ar-H), 6.92 (1H, s, Ar-H), 6.93 (1H, d, J = 8.2, Ar-H), 7.08 (1H, d, J = 8.2, Ar-H), 8.18 (1H, d, J = 10.0, Ar-H). ^{13}C NMR (100 MHz, DMSO): 13.86, 22.21, 31.68, 33.43, 38.98, 39.13, 55.63, 59.18, 68.98, 113.86, 117.01, 119.68, 121.54, 124.95, 125.00, 128.39, 131.28, 136.94, 137.75, 139.10, 139.15, 142.64, 160.74. HRMS: calculated for (M + H)$^+$, $C_{28}H_{37}N_2O_3$: 449.2804; found: 449.2806, tolerance of 5 ppm. Analysis: Calcd for $C_{28}H_{37}N_2O_3 \cdot 0.7H_2O$ (0.7 equiv of water observed by ^{1}H NMR): C, 72.92; H, 8.17; N, 6.07. Found: C, 72.61; H, 8.02; N, 6.01.

8-Hydroxy-5-[(*R*)-1-hydroxy-2-(5,6-dimethoxyindan-2-ylamino)-ethyl]-1*H*-quinolin-2-one (1f). Prepared in an analogous fashion to compound **1a**. A reaction on a 1.4 mmol scale yielded 525 mg of compound **1f** as a yellow powder (93% yield). HPLC/MS: > 95% purity; *m*/*z* 397 [(M + H)$^+$, 100%]. ^{1}H NMR (400 MHz, $CDCl_3$): 2.52 (2H, br, dd, J = 15.35 and 5.5, CH_2), 2.78 (2H, m, CH_2), 3.50 (2H, m, CH_2), 3.51 (1H, t, J = 6.7, CH), 3.77 (6H, s, 2 × CH_3) 5.50 (1H, dd, J = 3.64 and 3.82, CH), 6.50 (1H, d, J = 9.8, CH), 6.67 (2H, s, 2 × Ar-H), 6.81 (1H, d, J = 8.1, Ar-H), 7.56 (1H, d, J = 8.1, Ar-H), 8.03 (1H, d, J = 9.8, CH). ^{13}C NMR (100 MHz, $CDCl_3$): 44.96, 61.23, 64.99, 74.64, 114.19, 114.24, 119.49, 122.63, 125.31, 127.13, 134.03, 138.71, 137.78, 142.51, 148.39, 153.35, 166.36. Analysis: Calcd for $C_{22}H_{24}N_2O_5 \cdot 1.2H_2O$ (1.2 equiv of water observed by ^{1}H NMR): C, 63.21; H, 6.37; N, 6.70. Found: C, 62.92; H, 6.52; N, 6.63.

8-Hydroxy-5-[(*R*)-1-hydroxy-2-(4,7-diethylindan-2-ylamino)-ethyl]-1*H*-quinolin-2-one (1g). Prepared in an analogous fashion to compound **1a**. A reaction on a 0.21 mmol scale yielded 70 mg of compound **1g** as a yellow powder (86% yield). HPLC/MS: > 95% purity; *m*/*z* 393 (M$^+$, 100%). ^{1}H NMR (400 MHz, DMSO): 1.10 (6H, t, J = 7.6, 2 × CH_3), 2.49 (4H, m, 2 × CH_3), 2.58 (2H, dt, J = 5.3, CH_2), 2.82 (2H, d, J = 6.1, CH_2), 3.07 (2H, m, CH_2), 3.55 (1H, t, J = 6.4, CH), 5.03 (1H, t, J = 5.4, CH), 6.51 (1H, d, J = 9.9, CH), 6.89 (2H, s, 2 × Ar-H), 6.92 (1H, d, J = 8.1, Ar-H), 7.10 (1H, d, J = 8.1, Ar-H), 8.19 (d, J = 9.9, CH), 10.31 (1H, s, br, OH). ^{13}C NMR (100 MHz, DMSO): 14.86, 25.95, 37.35, 58.43, 94.89, 114.25, 117.35, 120.11, 122.03, 126.11, 128.78, 136.95, 137.17, 139.55, 143.09, 161.07. Analysis: Calcd for $C_{24}H_{28}N_2O_3 \cdot 1.2H_2O$ (1.2 equiv of water observed by ^{1}H NMR): C, 69.61; H, 7.40; N, 6.76. Found: C, 69.77; H, 7.46; N, 6.70.

Acknowledgment. We thank Brian Everatt for analytical support, Grety Rihs and Hansrudolf Walter for performing the X-ray structural analysis, and Reiner Aichholz, Wolfgang Blum, Matthias Kittelmann, and Kurt Laumen for performing metabolite identification studies.

Supporting Information Available: Experimental procedures for the synthesis of the aminoindans **3b**−**g** and the ethanolamine derivatives **4a**−**g**, characterizing data for the whole sequence leading to indacaterol **1c**, HPLC methods for CHI_{IAM} and % HSA binding determinations, methods for pK_a and plasma protein binding determinations, details of the single-crystal X-ray structure determination for **1c**, pharmacokinetic data for **1c** compared to formoterol, procedures for the isolation and determination of the β_2-adrenoceptor activity of the phenolic glucuronide of **1c**, and time versus inhibition of contraction plots from the electrically stimulated superfused guinea-pig tracheal-strip assays with aminoindan derivatives **1a**−**g**, and the reference β_2-adrenoceptor agonists. This material is available free of charge via the Internet at http://pubs.acs.org.

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