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Discovery of L-791,943: A Potent, Selective, Non Emetic and Orally Active Phosphodiesterase-4 Inhibitor

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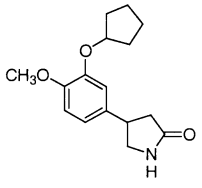
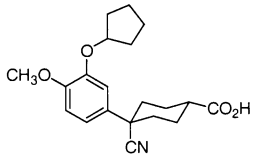
Abstract—Structure–activity relationship studies directed toward improving the potency and metabolic stability of CDP-840 (**3**) resulted in the discovery of L-791,943 (**11n**) as a potent (HWB TNF-$\alpha = 0.67\,\mu M$) and orally active phosphodiesterase type 4 (PDE4) inhibitor. This compound, which bears a stable bis-difluoromethoxy catechol and a pendant hexafluorocarbinol, exhibited a long half-life in rat and in squirrel monkey. It is well tolerated in ferret with an emetic threshold greater than 30 mg/kg (po) and was found to be active in the ovalbumin-induced bronchoconstriction model in guinea pig and in the ascaris-induced bronchoconstriction models in sheep and squirrel monkey.

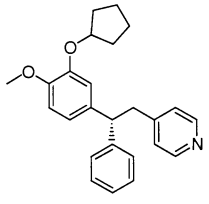
The broad family of cyclic nucleotide phosphodiesterases (PDE) represents a number of hydrolases responsible for the degradation of the second messengers cAMP and cGMP.[1] Cyclic nucleotides are involved in a wide range of biological responses. Strong evidence suggests that cAMP plays a central role in regulating the function of airway smooth muscle,[2] inflammatory cells,[3] and immune cells and the cAMP specific PDE4 is the predominant isoenzyme found in pro-inflammatory cells associated with a number of airway disorders.[4] It is believed that selective PDE4 inhibitors could be good therapeutic agents for the treatment of asthma, chronic obstructive pulmonary disease (COPD), and other inflammatory conditions and a number of them have already entered clinical trials.[5] However, severe limiting side effects precluded the development of the first generation inhibitors such as (*R*)-rolipram (**1**) and many promising candidates have been discontinued.[6] The most advanced PDE4 inhibitor in clinical trials is Cilomilast (**2**, Ariflo™). This compound has a much improved tolerability profile relative to **1** and is being primarily developed for the oral treatment of COPD.[7]



Rolipram (**1**)



Ariflo™ (**2**, SB207499)



CDP-840 (**3**)

When we began this program, CDP-840 (**3**) was one of our lead structures. This compound has been reported to significantly reduce antigen-induced bronchoconstriction in animal models[8] and in asthmatic patients.[9] Although this compound is a potent PDE4 inhibitor, it suffers from extensive metabolism in vitro[10] and this translates into a short half-life in vivo. In the rat, the major metabolic pathway of CDP-840 is the *para*-hydroxylation on the pendant phenyl group whereas in human hepatocytes, the major metabolite is the pyridinium

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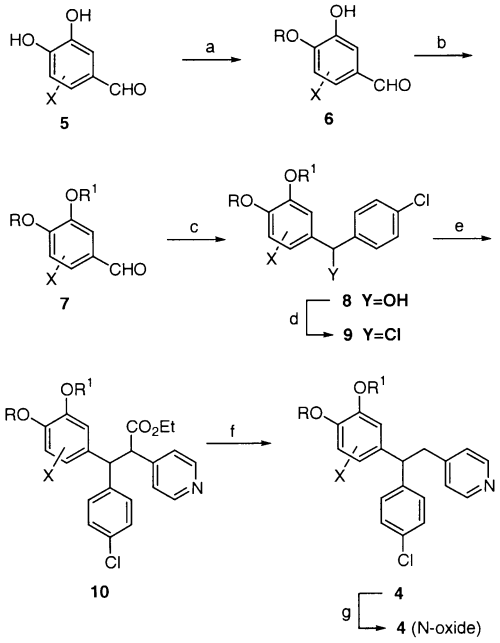
glucuronide, which is not detected in rat hepatocytes. Other major sites of metabolism are centered on the catechol portion of the molecule with hydroxylation, dealkylation and glucuronide and sulfate conjugation. We thus performed SAR studies on CDP-840. Our goal was to modify the structure in order to not only increase its potency but also to improve its metabolic stability and pharmacokinetic profile. Herein, we report the results of this study that lead to the identification of the highly potent and metabolically stable PDE4 inhibitor **11n** (L-791,943).

Throughout this study, compounds have been synthesized as racemates. Our initial synthetic efforts were directed toward reducing the metabolism at the catechol portion. It had been demonstrated that introducing a substituent such as a chlorine atom on the pendant phenyl group greatly reduces the metabolism of this moiety[10] so we performed modifications on the 4-chlorophenyl analogue of CDP-840 (**4a**). The general route to these compounds is described in Scheme 1.[11] Sequential alkylation of an appropriately substituted 3,4-dihydroxybenzaldehyde **5** with alkyl halides in DMF gave the corresponding derivative **7**. Alkylation using methyl chlorodifluoroacetate at 90 °C provided the corresponding difluoromethyl ethers. Treatment of benzaldehydes **7** with 4-chlorophenyl magnesium bromide gave the corresponding alcohols **8**. Conversion to the chloride and alkylation with the potassium enolate of ethyl 4-pyridyl acetate in the presence of HMPA produced a diastereomeric mixture of esters **10**. Saponification followed by acidification led to spontaneous decarboxylation to give derivatives **4**.



Scheme 1. Reagents and conditions: (a) RX or ClF_2COOMe, Cs_2CO_3, DMF; (b) R^1X, Cs_2CO_3, DMF, 54–80% (2 steps); (c) 4-Chlorophenyl magnesium bromide, THF, −30 to 0 °C, 80–95%; (d) $SOCl_2$, Hunig's base, C_6H_6; (e) KHMDS, HMPA, ethyl 4-pyridylacetate, THF, −30 to 25 °C; (f) NaOH, THF, EtOH, 60 °C; HCl; (g) MMPP, CH_2Cl_2, MeOH, 62–86% (4 steps).

Many structural modifications of the catechol ring including joining the two alkoxy groups in a cyclic fashion and additional ring substitution (cf. **4b**, **4c**, Table 1) led to a very significant loss of potency. Steric constraints limit the size of the R group to one carbon ethers. A major improvement came with the bis-difluoromethoxy analogue **4e**, this led to an increased potency for the inhibition of LPS-induced TNF-α in human whole blood (HWB) (Table 1).[13] In addition, these alkoxy groups were now very stable towards metabolic degradation in vitro.[14] It was also found that in many cases, the pyridine *N*-oxide derivatives were equipotent in our HWB assay (cf. **4e** *N*-oxide).

Having discovered the metabolically stable catechol, we then modified the substituents on the phenyl group to further improve the potency. A few hundred analogues have been synthesized in this series and in general, substitution in the *para* position was more tolerant with respect to the overall activity. These analogues (**11**) were prepared using a strategy similar to the one described in Scheme 1 and involve the addition of substituted phenyl lithium or Grignard reagents to 3,4-bis(difluoromethoxy)benzaldehyde **7e** derived from 3,4-dihydroxybenzaldehyde.[11]

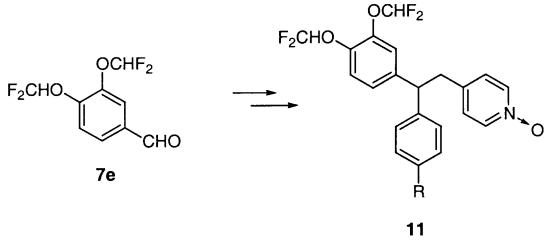
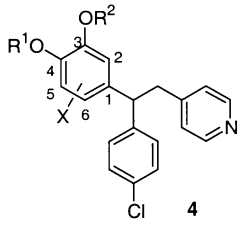


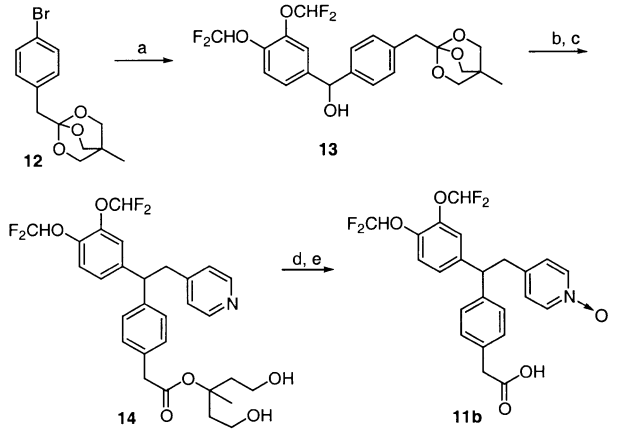
Table 1. SAR for R and R^1 substituents on **4**



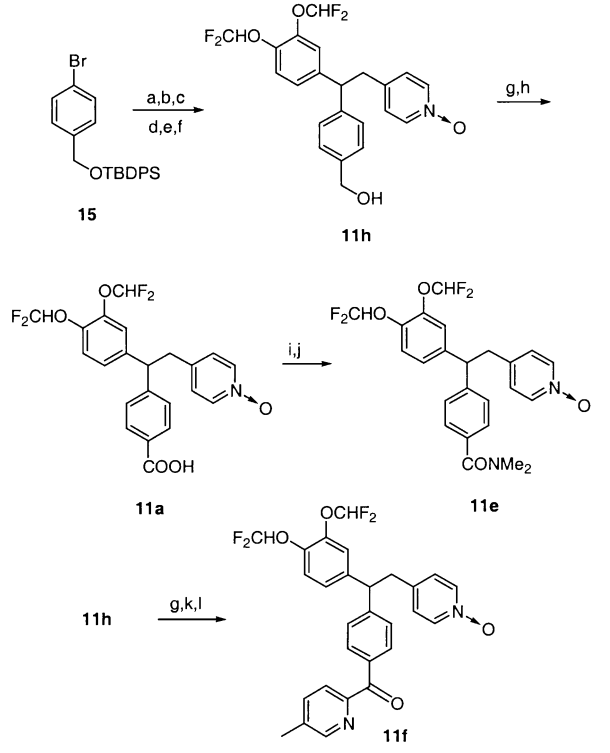
Compd	X	R^1	R^2	GST-PDE4A[248a] IC_{50} (nM)	HWB (TNF-α)[b] IC_{50} (μM)
3	—	Me	c-Pentyl	4.3	16
4a	—	Me	c-Pentyl	2.8	12
4b	6-F	Me	c-Pentyl	13	>25
4c	2-F	Me	c-Pentyl	110	98
4d	—	CHF_2	c-Pentyl	13	8.7
4e	—	CHF_2	CHF_2	11	4.5
4e	(N-oxide)	CHF_2	CHF_2	32	3.8
4f	—	$-CH_2-$		>1000	n.d.
4g		$-CH_2CH_2-$		772	n.d.

[a]Assayed against human PDE4A isoform using a construct representing the common region of spliced variants expressed as GST-fusion protein in Sf9 cells.[12] IC_{50} represent a mean of $n=3$ or more.
[b]Inhibition of LPS-induced TNF-α in human whole blood.[13] IC_{50} represent a mean of $n=3$ or more.

Metallation of bromobenzene derivative **12** with *n*-butyl lithium followed by treatment with benzaldehyde **7e** provided secondary alcohol **13** (Scheme 2). Alkylation of the corresponding chloride with the potassium enolate of ethyl 4-pyridylacetate followed by ester hydrolysis and decarboxylation upon acidification gave dihydroxyester **14**. Final oxidation and saponification afforded the analogue **11b**.



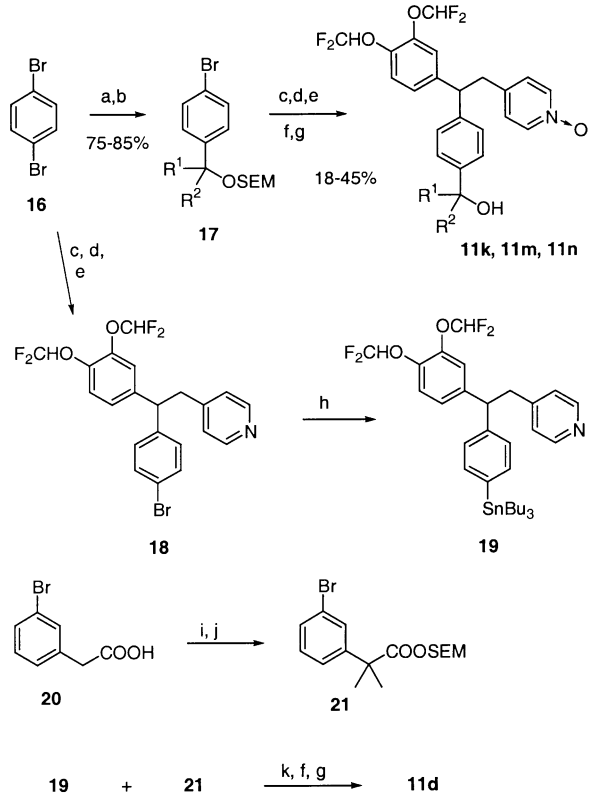
Scheme 2. Reagents and conditions: (a) *n*-BuLi, THF, −78 °C, then **7e**, 50%; (b) $SOCl_2$, pyridine, toluene, 0 °C; KHMDS, HMPA, ethyl 4-pyridylacetate, THF, 0 °C; (c) LiOH, THF, MeOH, 65 °C; HCl, 60% (2 steps); (d) MMPP, CH_2Cl_2, MeOH; (e) NaOH, THF, EtOH.



Scheme 3. Reagents and conditions: (a) *n*-BuLi, THF, −78 °C, then **7e**, 65%; (b) $SOCl_2$, toluene, $(i\text{-Pr})_2NEt$, 60%; (c) KHMDS, HMPA, ethyl 4-pyridylacetate, THF, 0 °C; (d) LiOH, THF, MeOH, 65 °C; HCl, 76% (2 steps); (e) AcOH, 92%; (f) MMPP, CH_2Cl_2, MeOH, 93%; (g) MnO_2, EtOAc, 87%; (h) $NaClO_2$, NaH_2PO_4, isobutylene, 83%; (i) $SOCl_2$, CH_2Cl_2; (j) Me_2NH, $(i\text{-Pr})_2NEt$; (k) *n*-BuLi, 2-bromo-5 methyl pyridine, THF, −78 °C, $CeCl_3$, 48%; (l) MnO_2, 91%.

Starting with silyl ether **15**, a similar sequence of events provided analogue **11h** (Scheme 3). Oxidation of the latter to the corresponding aldehyde allowed for the preparation of benzoic acid analogue **11a**, whereas amide formation gave **11e**. Ketone **11f** was obtained from the same aldehyde after treatment with the cerium reagent derived from 5-methyl-2-bromopyridine and MnO_2 oxidation of the resulting secondary alcohol. Finally, compound **11c** was prepared starting from 4-bromo-*N*-(*tert*-butyl)benzenesulfonamide using a similar synthetic strategy.

A number of carbinol derivatives were synthesized starting from 1,4-dibromobenzene **16** as shown in Scheme 4. Metal halogen exchange followed by condensation with a ketone and protection of the resulting alcohol afforded a number of bromophenyl fragments **17**. Further elaboration as described above gave alcohol derivatives **11k**, **11m**, and **11n**. Also available from **16** was the bromophenyl intermediate **18**. The latter was subjected to tin–halogen exchange under palladium catalysis to give the tributyltin derivative **19**. Esterification of 3-bromophenyl acetic acid followed by double alkylation with methyl iodide led to fragment **21**. Stille type coupling of bromide **21** with stannane **19** afforded the corresponding biaryl derivative **11d** after final depro-

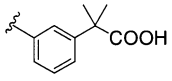
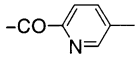
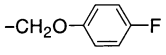


Scheme 4. Reagents and conditions: (a) *n*-BuLi, THF, −78 °C, R^1COR^2; (b) SEMCl, CH_2Cl_2, $i\text{-Pr}_2NEt$; (c) Mg or *n*-BuLi, THF, **7e**; (d) $SOCl_2$, pyridine, toluene, 0 °C; KHMDS, HMPA, ethyl 4-pyridylacetate, THF, 0 °C; (e) LiOH, THF, MeOH, 65 °C; HCl; (f) TBAF, THF or TFA, CH_2Cl_2, then NaOH, MeOH; (g) MMPP, CH_2Cl_2, MeOH; (h) $(Bu_3Sn)_2$, $Pd(PPh_3)_4$, LiCl, dioxane 65 °C; (i) $TMSCH_2CH_2OH$, EDC, DMAP, CH_2Cl_2; (j) LDA, CH_3I, THF, −78–0 °C; repeat; (k) PPh_3, $PdCl_2(PPh_3)$, dioxane, 65 °C.

tection and pyridine oxidation. Mitsunobu reaction of compound **11h** with 4-fluorophenol afforded analogue **11g** whereas treatment with ethyl isocyanate gave the carbamate **11i**.

Summarized in Table 2 are the intrinsic PDE4A potency and their cellular efficacy in HWB using the inhibition of TNF-α production as an index. General trends concerning the effect of substituents in the para position of the pendant phenyl group can be observed. First, the benzoic acid **11a** and the phenyl acetic acid **11b** both exhibit poor activity on the enzyme when compared to **4a**. Also, a very significant loss of potency for the inhibition of LPS-induced TNF-α formation in HWB was observed. This shift in the activity appeared to be linked to the acid nature and the polarity of the substituent. As the size of the group was increased, the intrinsic activity also increased (**11c**–**d**). In addition, steric crowding around the acid functionality (**11d**) did improve the HWB potency. However, it was found that neutral substituents with moderate polarity and lipophilicity such as amide **11e** and ketone **11f** were even more potent in HWB with IC_{50}s around 1 μM. Most interestingly, the simple hydroxymethyl derivative **11h** was also found to be potent with an IC_{50} of 0.5 μM in HWB. However, low plasma concentration was observed after oral administration of this compound in rat and the parent drug was rapidly metabolized to the corresponding aldehyde and acid derivatives. Attempts to improve on the pharmacokinetic profile by ether (**11g**) or carbamate (**11i**) formation were not very successful. A more encouraging trend was noticed when the bulkiness and the nature of the carbinol moiety were modified. Going from secondary alcohol **11j** to tertiary derivatives **11k** and **11m** increased the in vitro activity, maintained good potency in HWB and led to significantly improved pharmacokinetics for these analogues. For example, the bioavailability in rat of the dimethyl tertiary alcohol (**11k**) was 55% whereas that of spirocyclobutyl analogue (**11m**) was 100%. Unfortunately, the half-lives for these compounds remained short (i.e., in the order of 1 or 2 h). Finally, we found that blocking the metabolism of the alcohol moiety by introduction of a bis-trifluoromethyl carbinol resulted in **11n** (L-791,943) a compound with good in vitro activity and much longer half-life (Table 3).

Table 2. SAR for R substituents on **11**

11	R	GST-PDE4A[248a] IC_{50} (nM)	HWB (TNF-α)[b] IC_{50} (μM)
11a	–COOH	24	77
11b	$–CH_2COOH$	53	58
11c[c]	$–SO_2NHCO(o\text{-Tol})$	3.1	75
11d		2.2	6.5
11e	$–CONMe_2$	8.6	1.0
11f		7.3	0.89
11g		0.5	1.9
11h	$–CH_2OH$	44	0.47
11i	$–CH_2OCONHEt$	4.6	0.41
11j[d]	$–CH(CH_3)OH$	45	0.51
11k	$–C(CH_3)_2OH$	9.6	0.36
11l[e]		16	1.7
11m		7.5	0.38
11n (L-791,943)	$–C(CF_3)_2OH$	4.2	0.67
3 (CDP-840)		4.3	16
Ariflo™		38	18

[a]Assayed against human PDE4A isoform using construct representing the common region of spliced variants expressed as GST-fusion protein in Sf9 cells.[12] IC_{50} represent a mean of $n=3$ or more.
[b]Inhibition of LPS-induced TNF-α in human whole blood.[13] IC_{50} represent a mean of $n=3$ or more.
[c]For the synthesis of this analogue, see ref 15.
[d]For the synthesis of this analogue see ref 16.
[e]For the synthesis of this analogue see ref 17.

The extent of metabolism of L-791,943 (**11n**) was evaluated in vitro in rat hepatocytes and compared to the data obtained with CDP-840 (**3**). In our standard incubation conditions, >98% of the parent drug remained in the case of L-791,943 whereas only 11% of CDP-840 was left intact. These results are in good agreement with the respective half-lives (>24 h vs less than 30 min) of these compounds following iv dosing in rats.

The pharmacological profile of L-791,943 (**11n**) was evaluated in comparison to CPD-840 (**3**) and is summarized in Table 3. One of our initial goals to improve the metabolic stability of CDP-840 was met with L-791,943 and this is reflected in the longer half-lives measured in rat and in squirrel monkey. Moreover, an increase in potency was also achieved with this compound. L-791,943 was active in blocking the ovalbumin-induced bronchoconstriction in conscious guinea pig by 58% at a dose of 1.0 mg/kg (ip 4 h pretreatment). It also showed good in vivo activity in the anesthetized squirrel monkey[18] and in the conscious sheep[19] models of ascaris-induced bronchoconstriction. An oral dose of 3 mg/kg, 4 h before ascaris challenge, blocked the early phase response in the monkey by 96%. In the sheep model, 85% of the early phase and 95% of the late phase response were inhibited following an iv dose of 2 mg/kg, 2 h pre-treatment. Finally, the potential for causing emesis, one of the major drawbacks of typical PDE4 inhibitors, was also assessed. Ferrets could be dosed orally with up to 30 mg/kg of L-791,943 with plasma concentrations reaching 14 μM without causing emesis.

Table 3. In vivo profile of CDP-840 and L-791,943

	CPD-840	L-791,943
Rat $t_{1/2}$	<0.5 h	>24 h
Squirrel monkey $t_{1/2}$	<1 h	48 h
Efficacy in squirrel monkey[18] (dose)	64%[a] (5 mg/kg)	96%[b] (3 mg/kg)
Efficacy in sheep[19] -early/late (dose iv)	—	85%/95% (2 mg/kg)
Emesis in ferret	30 mg/kg	>3 mg/kg

[a]Compound administered iv (30 min pretreatment).
[b]Compound administered po (4 h pretreatment).

In conclusion, the structure activity relationship study performed in the CDP-840 series with the aim to reduce metabolism has led to the discovery of L-791,943 (**11n**), a highly potent and metabolically stable PDE4 inhibitor. This was achieved by replacement of the alkoxy groups of the catechol with bis-difluoromethoxy moieties, substitution of the phenyl ring with a bis-trifluoromethyl carbinol and oxidation of the pyridine to the corresponding pyridine *N*-oxide. L-791,943 is potent in vitro and in vivo as demonstrated by the inhibition of antigen-induced bronchoconstriction in a variety of models. The compound is well absorbed and is well tolerated in the ferret.

References and Notes

1. (a) Yuasa, K.; Kanoh, Y.; Okumura, K.; Omori, K. *Eur. J. Biochem.* **2001**, *268*, 168. (b) Soderling, S. H.; Beavo, J. A. *Curr. Opin. Cell. Biol.* **2000**, *12*, 174.
2. Torphy, T. J. *Agents Actions* **1998**, *23*, S37.
3. (a) Kuehl, F. A.; Zanetti, M. E.; Soderman, D. D.; Miller, D. K.; Ham, E. A. *Am. Rev. Respir. Dis.* **1987**, *136*, 210. (b) Souness, J. E.; Aldous, D.; Sargent, C. *Immunopharmacol.* **2000**, *47*, 127.
4. (a) Torphy, T. *J. Am. J. Respir. Crit. Care Med.* **1998**, *157*, 351. (b) Barnes, P. J. *Nature* **1999**, *402*, B31.
5. (a) Huang, Z.; Ducharme, Y.; MacDonald, D.; Robichaud, A. *Curr. Opin. Chem. Biol.* **2001**, *5*, 432. (b) Martin, T. J. *Idrugs* **2001**, *4*, 312. (c) Nell, H.; Louw, C.; Leichtl, S.; Rathgeb, F.; Neuhauser, M.; Bardin, P. G. *Am. J. Respir. Crit. Care Med.* **2000**, *161*, A200. (d) Timmer, W.; Leclerc, V.; Birraux, G.; Neuhauser, M.; Hatzelmann, A.; Bethke, T.; Wurst, W. *Am. J. Respir. Crit. Care Med.* **2000**, *161*, A505.
6. (a) Horowski, R.; Sastre-y-hernandez, M. *Curr. Therapeutic Res.* **1985**, *38*, 23. (b) Robichaud, A.; Tattersall, F. D.; Choudhury, I.; Rodger, I. W. *Neuropharmacology* **1999**, *38*, 289. (c) Robichaud, A.; Savoie, C.; Stamaciou, P. B.; Tattersall, F. D.; Chan, C. C. *Neuropharmacology* **2001**, *40*, 262 and 465 (erratum). (d) Barnette, M. S. *Prog. Drug Res.* **1999**, *53*, 193.
7. (a) Torhpy, T. J.; Barnette, M. S.; Underwood, D. C.; Griswold, D. E.; Christensen, S. B.; Murdoch, R. D.; Nieman, R. B.; Compton, C. H. *Pulm. Pharmacol. Ther.* **1999**, *12*, 131. (b) Compton, C. H.; Gubb, J.; Cedar, E.; Nieman, R. B.; Amit, O.; Brambilla, C.; Ayres, J. *Am. J. Respir. Crit. Care Med.* **1999**, *159*, A806.
8. Hughes, B.; Howat, D.; Lisle, H.; Holbrook, M.; James, T.; Gozzard, N.; Blease, K.; Hughes, P.; Kingaby, R.; Warrellow, G.; Alexander, R.; Eaton, M.; Perry, M.; Wales, M.; Smith, B.; Owens, R.; Catterall, C.; Lumb, S.; Russel, A.; Allen, R.; Merriman, M.; Bloxham, D.; Higgs, G. *Br. J. Pharmacol.* **1996**, *118*, 1192.
9. Harbinson, P. L.; MacLeod, D.; Hawksworth, R.; O'Toole, S.; Sullivan, P. J.; Health, P.; Kilfeather, S.; Page, C. P.; Costello, J.; Holgate, S. T.; Lee, T. H. *Eur. Respir. J.* **1997**, *10*, 1008.
10. Li, C.; Chauret, N.; Trimble, L. A.; Nicoll-Griffith, D. A.; Silva, J. M.; Macdonald, D.; Perrier, H.; Yergey, J. A.; Parton, T.; Alexander, R. P.; Warrellow, G. J. *Drug Metab. Disp.* **2001**, *29*, 232.
11. Guay, D.; Girard, Y.; Ducharme, Y.; Blouin, M.; Hamel, P.; Girard, M. 1998, US 5,710,170. *Chem. Abstr.* **1998**, *128*, 140611.
12. Laliberté, F.; Han, Y.; Govindarajan, A.; Giroux, A.; Liu, S.; Bobechko, B.; Lario, P.; Bartlett, A.; Gorseth, E.; Gresser, M.; Huang, Z. *Biochemistry* **2000**, *39*, 6449.
13. Brideau, C.; Van Staden, C.; Sthyler, A.; Rodger, I. W.; Chan, C.-C. *Br. J. Pharmacol.* **1999**, *126*, 979.
14. For example, in our standard in vitro hepatocyte incubations,[10] compounds **4a** and **4e** were 45% and 15% metabolized respectively. Only **4e** *N*-oxide was formed with **4e**.
15. Compound **11c** was synthesized starting from 4-bromo-*N*-(*tert*-butyl)benzenesulfonamide and **7e**. Final cleavage of the *N*-*tert*-butyl group was effected with TFA in CH_2Cl_2. Acylation with *o*-toluic acid was done using DMAP and EDC in CH_2Cl_2.
16. Compound **11j** was prepared from analogue **11h** by oxidation (MnO_2) followed by Grignard addition (MeMgCl).
17. Synthesis of **11l** began with cyclopropanation (LDA, THF; SmI_2, CH_2I_2) and protection (SEMCl, Hunig's base) of 4- bromo acetophenone. Remaining steps as for **11k**.
18. Jones, T. R.; McAuliffe, M.; McFarlane, C. S.; Piechuta, H.; Macdonald, D.; Rodger, I. W. *Can. J. Phys. Pharmacol.* **1998**, *76*, 210.
19. Abraham, W. M.; Ahmed, A.; Cortes, A.; Sielczak, M. W.; Hinz, W.; Bouska, J.; Lanni, C.; Bell, R. L. *Eur. J. Pharmacol.* **1992**, *217*, 119.