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Improving Metabolic Stability of Phosphodiesterase-4 Inhibitors Containing a Substituted Catechol: Prevention of Reactive Intermediate Formation and Covalent Binding

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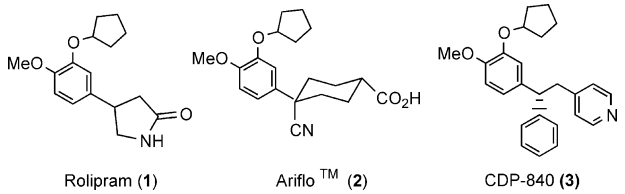
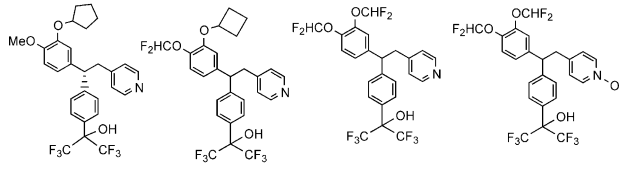
Abstract—A detailed study directed towards metabolic stability optimization of the alkoxy substituents on the catechol moiety of CDP-840 is reported. Replacement of the methoxy and cyclopentyloxy substituents by cyclobutyloxy and/or difluromethoxy groups resulted in the discovery of potent and selective PDE4 inhibitors where the formation of reactive metabolites that could covalently bind to microsomal protein was significantly reduced or eliminated.

Cyclic nucleotide phosphodiesterases (PDEs) constitute a broad family of enzymes responsible for the hydrolysis and consequent deactivation of the second messengers cAMP and cGMP.[1] There is strong evidence that cAMP plays a central role in regulating the function of inflammatory,[2] immune and airway smooth muscle cells.[3] The PDE4 enzymes have received particular attention due to the fact that they are particularly abundant in these cells.[4] Selective PDE4 inhibitors therefore could become promising therapeutic agents for the treatment of asthma and a wide range of other inflammatory diseases.[5] A number of them have entered clinical trials, the first generation being exemplified by Rolipram[6] (**1**) and the most advanced one in clinical trials being Ariflo™ [7] (**2**).



We already reported that the PDE4 inhibitor CDP-840 (**3**), known to significantly reduce the bronchoconstriction induced by antigen in asthmatic patients[8] was found to be extensively metabolized in vitro with a concomitant short half-life in vivo.[9] Several sites of metabolism were identified which included *p*-hydroxylation of the phenyl ring, glucuronidation of the pyridine moiety and oxidation at the methoxy and cyclopentyloxy catechol moieties. We had shown that simple modification of the phenyl ring, such as the introduction of a *para* substituent, improved the metabolism profile of CDP-840 by reducing metabolism and species differences in metabolic profiles.[9] We report here the results of a study directed towards the optimization of the catechol moiety using the *p*-hexafluorocarbinol analogue **4** as a model compound. Our strategy was to replace the alkoxy substituents to avoid dealkylation knowing that phenol-type metabolites can generate quinones such as those reported with α-methyl-dopa[10] and troglitazone.[11]



Reactive intermediates, including quinones, have been shown to covalently bind to proteins and this phenomenon has been associated to toxic events such as acute toxicity[12] or idiosyncratic reactions.[13] As we modified

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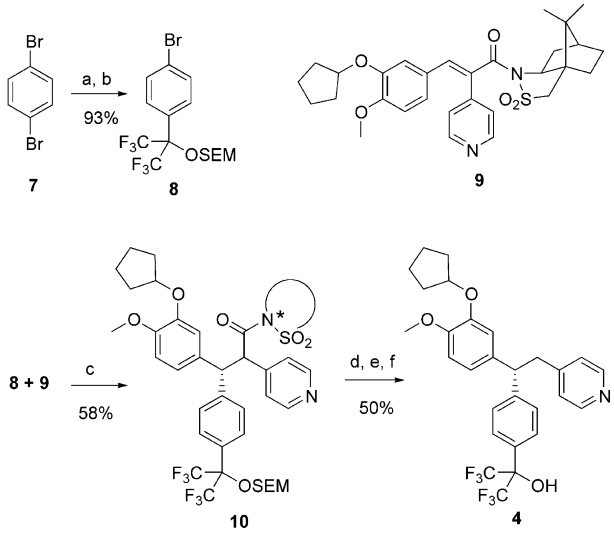
the catechol moiety to prevent covalent binding to proteins, we also wanted to preserve the potency of the PDE4 inhibitors. The SAR for compounds with different substituents on the catechol moiety has been reported in a previous publication.[14]

Compound **4** was synthesized as shown in Scheme 1. The fluorinated carbinol portion was introduced from hexafluoroacetone and 4-bromophenyl lithium. The use of the chiral sultam auxiliary allowed excellent control of the absolute stereochemistry during the conjugate 1,4-addition of the Grignard reagent derived from **8** onto intermediate **9**[15] to give a mixture to two diastereoisomers **10**. Removal of the chiral auxiliary and final deprotection afforded compound **4**.

Compound **5** was synthesized following the same route as already described for the preparation of **6**.[14] The radioactive analogues (**18**–**20**) of racemic **4** and of **5**–**6** were prepared by tritiation of the corresponding 3,5-dichloropyridine derivatives **15**–**17**. These were in turn synthesized by applying the chemistry, as shown in Scheme 2, to different starting benzaldehydes. Radiolabeled L-791943[14] was prepared from the oxidation of **20** at the pyridine ring to yield the pyridine-*N*-oxide.

Another radiolabeled version of compound **4** was prepared where the tritium was placed on the catechol ring (**22**). This compound was prepared by electrophilic iodination of **4** to give the iodo derivative **21**. Tritiation of the latter provided the desired analogue **22** (Scheme 3).

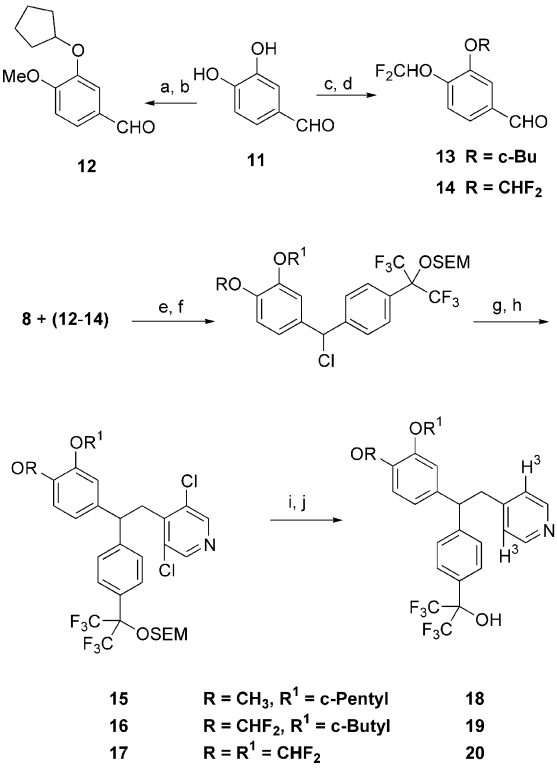
Initial in vitro metabolism studies were carried out in rat hepatocytes using standard procedures.[9] Briefly, compounds were incubated at a concentration of 50 μM with 10^6 cells for 3 h at 37 °C. HPLC analysis of the incubations of **4** revealed the presence of eight metabolites, as shown in Figure 1.



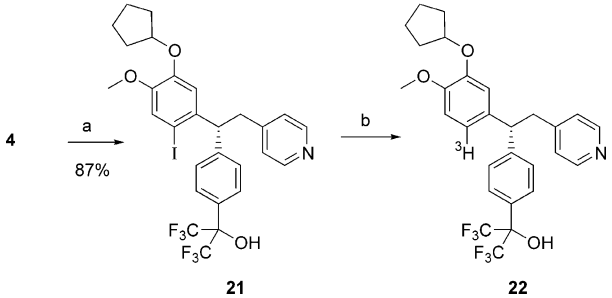
Scheme 1. Reagents and conditions: (a) *n*-BuLi, Et_2O, −78 °C; $(CF_3)_2CO$, (b) *i*-Pr_2NEt, SEMCl, CH_2Cl_2; (c) Mg, **8**, THF, then **9**, 0 °C; (d) *n*-PrSLi, THF, 0 °C to rt; (e) LiOH, THF, H_2O; (f) TBAF,

Based on HPLC/MS,[9] five metabolites of **4** were shown to be associated with dealkylation on the catechol moiety (desmethylation with or without subsequent glucuronidation and descyclopentylation with or without subsequent sulfation or glucuronidation). The presence of these metabolites were previously reported for CDP-840[9] and Rolipram,[16] two compounds containing methoxy and cyclopentyloxy subtituents on the catechol moiety. The other metabolites were identified as hydroxy on the cyclopentyl, *O*-glucuronide at the carbinol moiety and *N*-oxide pyridine, as indicated in Figure 2.

Radiolabeled **4**, made by tritiation at the catechol moiety (**22**) or at the pyridine ring (**18**), was used to assess



Scheme 2. Reagents and conditions: (a) CH_3I, K_2CO_3, DMF; (b) Cs_2CO_3, C_5H_9Br, DMF, heat; (c) $CClF_2COOMe$, Cs_2CO_3, DMF; (d) C_4H_7Br, Cs_2CO_3, DMF; or repeat (c); (e) **8**, Mg, THF, then benzaldehyde **12**, **13** or **14**, 0 °C; (f) $SOCl_2$, *i*-Pr_2NEt, toluene; (g) KHMDS, HMPA, ethyl 3,5-dichloro-4-pyridylacetate, THF, −30 to 25 °C; (h) NaOH, THF, EtOH, 60 °C; HCl aq; (i) TBAF, THF; (j) 10% Pd/C, 0.2 atm $^3H_2$, EtOAc, MeOH.



Scheme 3. Reagents and conditions: (a) NIS, $CHCl_3$, TFA; (b) 10% Pd/C, 0.2 atm $^3H_2$, EtOAc, Et_3N, MeOH.

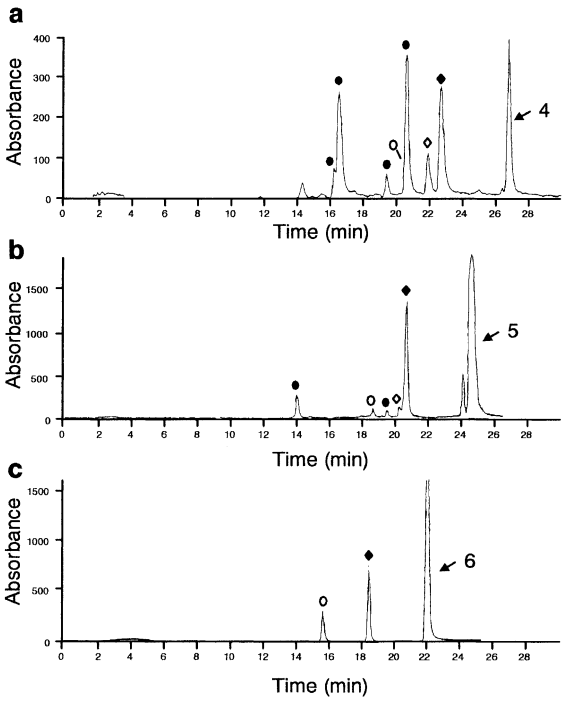


Figure 1. HPLC chromatogram of rat hepatocyte incubates of (a) **4** (b) **5** and (c) **6** (Peaks identified with ● are related to dealkylation of the catechol moiety, with ◆ are pyridine *N*-oxide, with ○ are carbinol *O*-glucoronide with ◇ are hydroxylation on the cyclohexyl or cyclo-

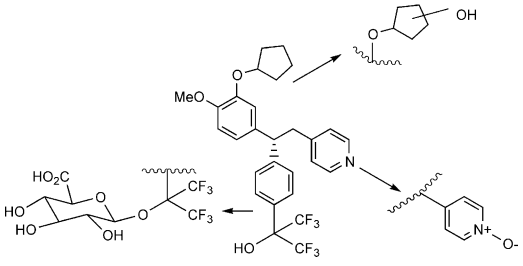


Figure 2. Metabolic pathways not involving dealkylation.

the potential to cause covalent protein adducts upon metabolism. The in vitro metabolism studies with ^{3}H **4** were performed in rat microsomes according to a published procedure.[17] Briefly, ^{3}H compounds (10 μM) were incubated with 2.5 mg of microsomal proteins containing a NADPH-regenerating system for 1 h at 37 °C. Reactions were stopped by adding acetonitrile, mixtures were centrifuged. Supernatants obtained were analyzed by HPLC with radiometric detection. Protein pellets were extracted until negligible radioactivity counts were found in the washes. The pellets were solubilized before counting. The difference between the amounts of radioactivity found in the protein pellet obtained from the incubations under oxidative conditions versus control incubations (no NADPH or heat deactivated microsomes) are indicated in Table 1. From these data, it was clear that, upon metabolism, **18** and **22** formed intermediates that were covalently binding to microsomal proteins.

Knowing that **4** underwent extensive metabolism at the catechol moiety, it was speculated that the reactive

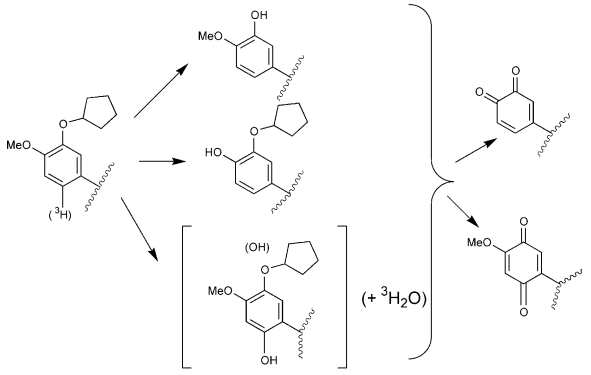


Figure 3. Potential mechanisms leading to reactive *p* and *o* quinones.

Table 1. Covalent binding of ^{3}H compounds to microsomal protein

Compd	% Covalent binding[a]
22	3.1±0.8 ($n=4$)
18	2.9±0.7 ($n=4$)
19	0.45±0.05 ($n=2$)
20	<0.05 ($n=2$)
L-791943	<0.05 ($n=2$)

[a]Expressed as a percentage of the difference in radioactivity in the protein pellet obtained under oxidative versus control incubations over the total radioactivity in incubations (average of *n* experiments performed in triplicate).

intermediates were *para* or *ortho* quinone-type metabolites as shown in Figure 3.

The formation of the phenol leading to *para*-quinone was not detected but its formation was suggested based on the HPLC analysis of the supernatant obtained from the oxidative incubations of **22** (tritiated at the catechol moiety). A radioactive peak was detected at the solvent front. Injection of tritiated water also resulted in a peak eluting at the solvent front. This observation supported that, upon metabolism, the tritium was lost to form tritiated water, likely through an oxidation at the carbon para to the cyclopentoxyl. This early peak was not detected in the supernatants obtained from the incubations of **18** (tritiated at the pyridine position). There was also evidence that tritiated water was formed in vivo. The analysis of plasma samples obtained from rats dosed with **22** radiolabeled at the catechol moiety (5 mg/kg of **22,** 50 μCi per rat) showed that up to 60% of the total radioactivity detected in plasma samples (24 h time point) could be distilled. No radioactivity could be distilled in control plasma samples spiked with **22**. These in vivo findings supported the role of metabolism in the release of tritiated water and in the formation of phenol that could lead to the *para*-quinone.

The chemistry efforts were directed toward the synthesis of analogues designed to reduce metabolism at the catechol moiety while maintaining the PDE4 potency. Difluoromethyl group as a replacement of the catechol diethylether had been reported by other groups[18] and used in our SAR.[14] In addition, we had found that the replacement of the cyclopentyl by a cyclobutyl significantly improved the potency of the PDE4 inhibitors

[40-fold more potent on the human whole blood assay[14] (222 nM for the *O*-cyclobutyl vs 8211 nM for the *O*-cyclopentyl analogue)]. Therefore, the metabolism profile of the promising analogues **5** and **6** in rat hepatocytes was compared to **4**. From the HPLC analysis of the incubates (Fig. 1), **5** and **6** were found to be metabolically more stable than **4** (10% metabolism as compared to 30%). HPLC/MS analysis indicated that two minor peaks in the incubates of **5** originated from the dealkylation of the cyclobutyloxy moiety. The other metabolites were related to the hydroxylation on the cyclobutyl, the glucuronidation of the carbinol moiety and oxidation of the pyridine, analogous to what was observed for **4**. Regarding compound **6**, the *O*-glucuronide of the carbinol and *N*-oxidation of the pyridine were observed but no metabolites derived from the catechol moiety were found. The *N*-oxide metabolite was identified as L-791,943.[14] Compounds **5**, **6**, and L-791943 were tritiated at the pyridine moiety and incubated in rat microsomes to address covalent binding to proteins. As indicated in Table 1, the levels of covalent binding due to metabolism were significantly reduced with **19** while there was none observed with **20** or ^{3}H L-791943. These results support the hypothesis that metabolic stability of the alkoxy substituents of the catechol prevents the formation of reactive quinone intermediates responsible for covalent binding.

In conclusion, formation of reactive metabolites that can covalently bind to microsomal protein were identified with the *p*-hexafluorocarbinol analogue of CDP-840. Metabolic liability of the alkoxy substituents makes the catechol moiety susceptible for further oxidation and thus potential source of reactive intermediates. Stable catechol moieties were introduced and these significantly reduced or eliminated the covalent binding. In addition, these new analogues exhibited excellent PDE4 potency and improved pharmacokinetics.[14] These findings had a significant impact on the synthetic efforts as many PDE4 inhibitors synthesized in our laboratory now bear the stable difluoromethoxy moiety.

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