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Substituted 4-(2,2-Diphenylethyl)pyridine-*N*-oxides as Phosphodiesterase-4 Inhibitors: SAR Study Directed Toward the Improvement of Pharmacokinetic Parameters

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Abstract—A detailed SAR study directed toward the optimization of pharmacokinetic parameters for analogues of L-791,943 is reported. The introduction of a soft metabolic site on this structure permitted the identification of L-826,141 as a potent phosphodiesterase type 4 (PDE4) inhibitor that is well absorbed and that presents a shorter half-life than L-791,943 in a variety of animal species. The efficacy of L-826,141 is also demonstrated in different in vivo models.

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] Among this superfamily of 11 PDE isoenzymes, the PDE type 4 (PDE4) enzymes, encoded by four genes (A–D),[2] have received particular attention due to the fact that they are particularly abundant in inflammatory, immune, and airway smooth muscle cells.[3,4] Since elevation of cAMP levels is known to inhibit the activation of these cells, PDE4 inhibitors may have potential as anti-inflammatory drugs.[3c] Selective PDE4 inhibitors could become promising therapeutic agents for the treatment of asthma and a wide range of other inflammatory diseases, by virtue of their ability to block both inflammation and bronchoconstriction.[3b] In fact, a number of PDE4 inhibitors are being evaluated in the clinic and they have shown promising clinical efficacy for asthma,[5] chronic obstructive pulmonary disease (COPD)[6] and atopic dermatitis.[7]

We already reported that the potent PDE4 inhibitor CDP-840, known to significantly reduce the bronchoconstriction induced by antigen in animal models[8] and in asthmatic patients,[9] was found to be extensively metabolized in vitro with a concomitant short half-life in vivo.[10] In a previous publication, we reported how the replacement of the alkoxy substituents of the catechol by the difluoromethoxy group and the substitution of the pendant phenyl ring by the bis(trifluoromethyl) carbinol group, transformed CDP-840 into L-791,943, a highly potent and metabolically more stable inhibitor.[11] Unfortunately, despite a good biological profile, L-791,943 shows an excessively long half-life (>48 h) in a variety of animal species.

We report here the results of a SAR study directed toward the optimization of pharmacokinetic parameters for analogues of L-791,943. Our strategy to reduce the half-life in this series consisted in introducing soft metabolic sites to the structure of L-791,943 as shown in Figure 1.

In Vitro Studies

Intrinsic potency of compounds were assayed against human PDE4A^{248} isoform using construct representing the common region of spliced variants expressed as GST-fusion proteins in Sf9 cells.[12] IC_{50} values represent a mean of $n=3$ or more. The cellular potency of the compounds was evaluated by the inhibition of the LPS-induced TNF-α formation in human whole blood (HWB).[13]

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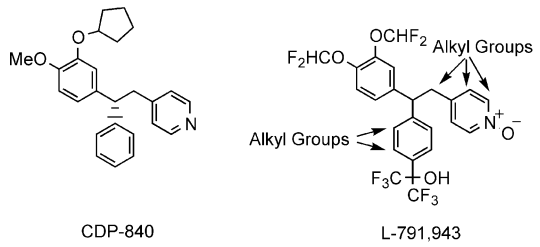
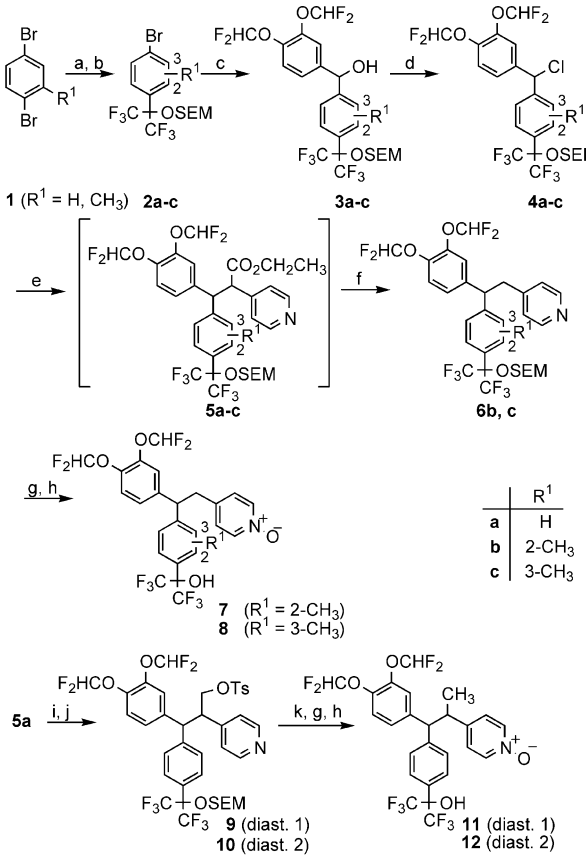


Figure 1.

Sites of Substitution

We evaluated the potential sites of attachment of the soft metabolic moieties by adding first a methyl group at various positions of L-791,943. As outlined in Scheme 1, the first sites of attachment studied were on the bis(trifluoromethyl)carbinol phenyl ring and on the carbon adjacent to the pyridine ring.

Starting from the appropriate dibromobenzene derivative (**1**), the bis(trifluoromethyl)carbinol group was introduced and protected as its SEM ether (**2a**–**c**). When 2,5-dibromotoluene was used (**1**, $R^1 = CH_3$), both regioisomers **2b** and **2c** were available from the separation, by chromatography in pure hexane, of the mixture of alcohols produced. The corresponding aryl Grignard reagents of **2a**–**c** were reacted with 3,4-(difluoromethoxy)benzaldehyde[11] to produce the diaryl alcohols **3a**–**c**. Treatment of the alcohols with thionyl chloride permitted the efficient isolation, after quick chromatography in toluene, of the diaryl chlorides **4a**–**c**.

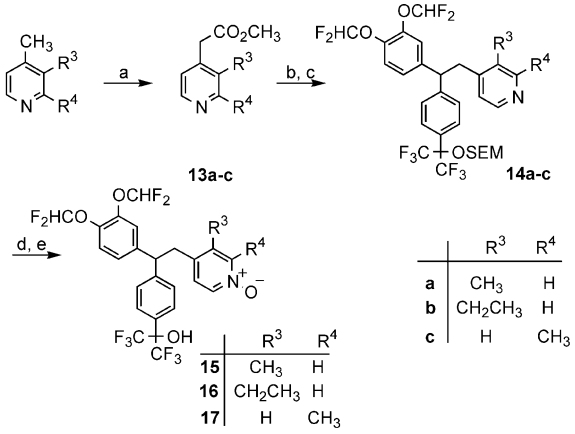


Scheme 1. Reagents and conditions: (a) BuLi, Et_2O, −78 °C, 30 min; then $(CF_3)_2CO$, −50 °C, 2 h, 70–75%; (b) SEMCl, *i*-Pr_2NEt, CH_2Cl_2, rt, 18 h, 95%; (c) Mg, THF, reflux, 1.5 h; then 3,4-(difluoromethoxy) benzaldehyde,[11] THF, −78 °C, 1.5 h, 80–85%; (d) $SOCl_2$, pyridine, toluene, rt, 30 min, 75–85%; (e) ethyl 4-pyridylacetate, KHMDS, HMPA, THF, rt, 3 h; then **4a**–**c** in THF, added to previous mixture, rt, 3 h; (f) 2 N LiOH, THF:MeOH (3:1), 65 °C, 1 h; then 4 N HCl followed by evaporation of volatiles, 50–75% (2 steps); (g) TBAF, THF, 65 °C, 6 h; (h) MMPP, CH_2Cl_2: MeOH (10:1), rt, 3 h, 85% (2 steps). (i) $LiAlH_4$, THF, rt, 1 h, 90%; (j) TsCl, pyridine, rt, 7 h, 70%; followed by flash chromatography in ethyl acetate: hexane (1:1); (k) $LiAlH_4$, THF, rt, 1 h, 50%.

The pyridine moiety was introduced when a chloride solution (**4a**–**c**) in THF was treated with a solution of the anion of ethyl 4-pyridylacetate, prepared previously from treatment with KHMDS in the presence of HMPA in THF. The adduct **5** was obtained as a mixture of diastereomers. This mixture was usually hydrolyzed and acidified without separation of the diastereomers. The decarboxylation occured during the work-up. The pyridine derivatives **6b**,**c** obtained were subjected to cleavage of the SEM group with TBAF and oxidized with MMPP to the corresponding pyridine-*N*-oxides **7** and **8**, existing as mixtures of enantiomers.

The introduction of a methyl group on the carbon adjacent to the pyridine ring was accomplished by transforming the diastereomeric mixture of acetates **5a** to the corresponding tosylates. At this point, the two diastereomers were separated by chromatography to give **9** (diastereomer 1, fast eluting compound) and **10** (diastereomer 2, slow eluting compound). Both tosylates were reduced to the methyl derivatives and transformed as described above to give **11** and **12**.

The compounds prepared to study the effect of the substitution of the pyridine ring were accessible by using the appropriately substituted 4-methylpyridine analogues (Scheme 2). Alkylation of 3,4-dimethylpyridine and 3-ethyl-4-methylpyridine with dimethyl carbonate



Scheme 2. Reagents and conditions: (a) LDA, THF, −78 °C, 30 min; then dimethyl carbonate, THF, −78 to 0 °C, over 1 h, 75%; (b) KHMDS, HMPA, THF, rt, 30 min; then **4a** in THF, added to previous mixture, 55 °C, 18 h; (c) 2 N LiOH, THF:MeOH (3:1), 65 °C, 1 h; then 4 N HCl followed by evaporation of volatiles, 60–75% (2 steps); (d) TBAF, THF, 65 °C, 6 h; (e) MMPP, CH_2Cl_2: MeOH (10:1), rt, 3 h, 80–90% (2 steps).

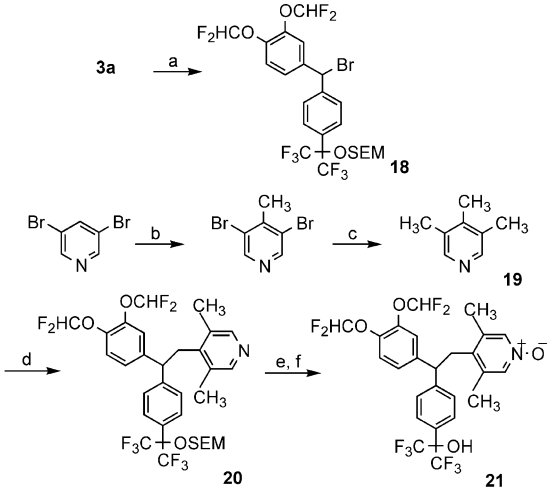
produced, respectively, the 4-pyridylacetates **13a** and **13b**. Methyl 2-methyl-4-pyridylacetate (**13c**) was prepared as reported in the literature.[14] Using the same methodology as described above, the substituted pyridines **14a–c** were obtained from **4a** and functionalized to give the substituted pyridine-*N*-oxides **15**, **16**, and **17**.

Because of the steric factors associated with the 3,5-dimethylpyridine moiety, the preparation of this analogue was not accessible by the above methodology. The preparation of the more reactive diarylbromide **18**, prepared from the alcohol **3a** with thionyl bromide, was necessary in combination with a more reactive nucleophile, the lithium anion of 3,4,5-trimethylpyridine **19** (Scheme 3). The latter compound was prepared from 3,5-dibromopyridine, which is methylated successively with methyl iodide, followed by a double Stille-coupling with tetramethyltin. Coupling of the lithium anion of **19** with the diarylbromide **18** provided the dimethylpyridine adduct **20** that was transformed to its pyridine-*N*-oxide analogue **21** as previously described.

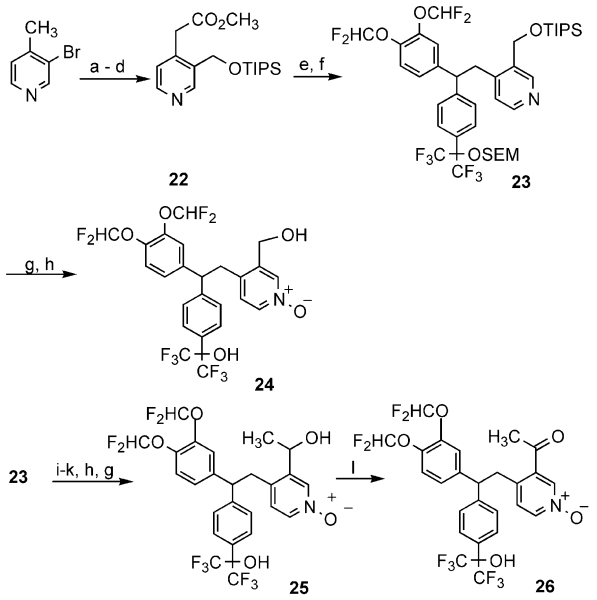
All compounds were tested as enantiomeric mixtures and compared to L-791,943 and Ariflo™, the most advanced inhibitor in clinical development.[15] Table 1 shows the effect of the substitution at various positions by a methyl group on L-791,943. The potency to inhibit PDE4 was preserved for compounds **7**, **15**, and **17**. Among these three compounds, compound **15** that bears a methyl group at the position 3 on the pyridine-*N*-oxide ring shows the best overall profile. As a mixture of enantiomers, it showed improved pharmacokinetics relative to L-791,943 in rats.

Substitution at Position 3 on the Pyridine-*N*-oxide

Since position 3 on the pyridine-*N*-oxide ring was identified as a good site for substitution, we studied in more detail the effect of various functional groups. The 3-ethyl analogue **16** was prepared as reported in Scheme 2. Some polar substituents were also introduced. A primary alcohol substituent was attached to position 3 by preparing the pyridylacetate **22** (Scheme 4). 3-Bromo-4-methylpyridine was formylated at position 3 and, after reduction of the aldehyde and protection of the primary alcohol, the methyl group at carbon 4 was transformed to the corresponding acetate. The pyridylacetate **22** was subjected to the same sequence of reactions as described above in

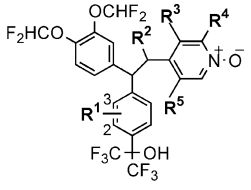


Scheme 3. Reagents and conditions: (a) $SOBr_2$, pyridine, toluene, rt, 30 min, 57%; (b) LDA, THF, −78 °C, 30 min; then methyl iodide, −78 °C to rt, 18 h, 66%; (c) Me_4Sn, $PdCl_2(PPh_3)_2$, toluene, reflux, 18 h, 33%; (d) LDA, HMPA, THF, −78 °C, 30 min; then **18**, THF, −78 °C, 3 h, 28%; (e) TBAF, THF, 65 °C, 6 h; (f) MMPP, CH_2Cl_2:MeOH (10:1), rt, 3 h, 77% (2 steps).



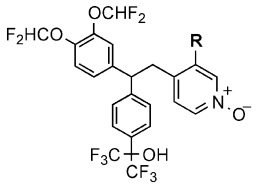
Scheme 4. Reagents and conditions: (a) BuLi, THF, −100 °C, 10 min; then methyl formate, −96 °C to −78 °C, 1 h, 95%; (b) $NaBH_4$, THF:MeOH (2:1), 0 °C to rt, 1 h, 47%; (c) TIPSOTf, 2,6-lutidine, CH_2Cl_2, −78 °C to rt, 2 h; (d) LDA, HMPA, THF, −78 °C, 20 min; then dimethyl carbonate, THF, −78 °C to 0 °C, 30 min, 76% (2 steps); (e) KHMDS, HMPA, THF, rt, 30 min; then **4a** in THF, added to previous mixture, 55 °C, 18 h; (f) 2 N LiOH, THF:MeOH (3:1), 50 °C, 18 h; then acetic acid followed by evaporation of volatiles, 80% (2 steps); (g) MMPP, CH_2Cl_2:MeOH (10:1), rt, 3 h, 92%; (h) TBAF, THF, 65 °C, 6 h, 85%; (i) TBAF, THF, 0 °C, 30 min, 89%; (j) MnO_2, CH_2Cl_2, rt, 17 h, 98%; (k) MeMgCl, CH_2Cl_2, 0 °C to rt, 15 min, 97%; (l) CrO_3, pyridine, celite, CH_2Cl_2, rt, 1 h, 40%.

Table 1. Biological data for methyl substituted L-791,943 analogues



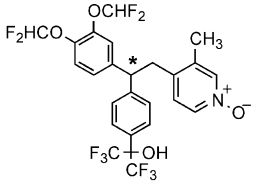
Compd	R^1	R^2	R^3	R^4	R^5	GST-PDE4A^{248} IC_{50} (nM)	HWB (TNF-α) IC_{50} (μM)
L-791, 943	H	H	H	H	H	4.2	0.7
7	2-CH_3	H	H	H	H	4.4	1.0
8	3-CH_3	H	H	H	H	32	7.8
11	H	CH_3	H	H	H	39	23
12	H	CH_3	H	H	H	39	7.8
15	H	H	CH_3	H	H	2.1	0.4
17	H	H	H	CH_3	H	18	0.3
21	H	H	CH_3	H	CH_3	57	5.8
CDP-840						4.2	15
Ariflo™						38	18

Table 2. Substitution on carbon 3 of the pyridine-*N*-oxide



Compd	R	GST-PDE4A^{248} IC_{50} (nM)	HWB (TNF-α) IC_{50} (μM)
15	CH_3	2.1	0.4
16	CH_2CH_3	27	3.9
24	CH_2OH	38	7.9
25	$CH(OH)CH_3$	42	13
26	$COCH_3$	2.7	2.9

Table 3. Enantiomers of **15**



Compd		GST-PDE4A^{248} IC_{50} (nM)	HWB (TNF-α) IC_{50} (μM)
15	rac. mixt.	2.1	0.4
L-826,141	enantiomer 1	1.3	0.3
27	enantiomer 2	23	3.7

Table 4. Comparative in vivo profile of L-791,943 and L-826,141

	L-791,943	L-826,141
Rat $t_{1/2}$	>48 h	10 h
Squirrel monkey $t_{1/2}$	>48 h	36 h
Dog $t_{1/2}$	>48 h	19 h
Efficacy in guinea pig (dose ip, pre-treatment)	58% (1 mg/kg, 4h)	–
	0% (1 mg/kg, 0.5h)	82% (1 mg/kg, 0.5h)
Efficacy in squirrel monkey early/late (dose po)	96%[a] (3 mg/kg)	59%/100% (3 mg/kg)
	–	33%/63% (1 mg/kg)
	–	26%/38% (0.5 mg/kg)
Efficacy in sheep early/late (dose iv)	85%/95% (2 mg/kg)	62%/90% (2 mg/kg)
Emesis in ferret (C_{max})	>30 mg/kg (14 μM)	>30 mg/kg (10 μM)

[a]Anaesthetized squirrel monkey model (early-phase reported).

the presence of the diarylchloride **4a** to give the coupled product **23**. Oxidation of the pyridine followed by the simultaneous cleavage of the SEM and the TIPS groups with TBAF at 65 °C afforded the primary alcohol **24**.

The TIPS group of **23** was removed selectively without cleaving the SEM group when treated with TBAF at 0 °C. The alcohol produced was oxidized with MnO_2 to give an aldehyde, which, when subjected to methyl Grignard addition followed by the cleavage of the SEM ether and oxidation of the pyridine, gave the secondary alcohol **25**. The corresponding methyl ketone **26** was obtained by oxidation with chronium (III) oxide.

From Table 2, it is clear that increasing the size or the polarity of the substituent at position 3, rapidly reduces the potency on the PDE4 enzymes and in the HWB assay. For example, the ethyl derivative **16** is about 10-fold less potent than the methyl analogue **15**. The substitution with a methyl group seems to be optimal.

Since **15** is a mixture of enantiomers, we decided to resolve it. Individual pure enantiomers were obtained, after an efficient HPLC resolution on chiral column of the pyridine precursor prior to the final oxidation step.[16] The fast eluting compound was identified as enantiomer 1 and the slow eluting compound as enantiomer 2. Separated enantiomers 1 and 2 were oxidized with MMPP to give respectively L-826,141 and **27**. Table 3 shows that the PDE4 inhibitory activity resides predominantly in enantiomer 1, with activities at least 10-fold superior to enantiomer 2 and comparable to L-791,943 (see Table 1).

In Vivo Studies

The pharmacokinetic profile of L-826,141 was then evaluated, in comparison with L-791,943 in various species. Table 4 shows that L-826,141 presents a shorter half-life than L-791,943 in various species. The introduction of a methyl group at position 3 on the pyridine-*N*-oxide resulted in a diminished half-life while maintaining the PDE4 inhibitory activity. For example, the half-life observed in rats were >48 h and 10 h for L-791,943 and L-826,141, respectively.

Compound L-826,141 is well absorbed in rat with a bioavailability of 60% and a plasma level of 0.4 μM at 6 h when administered po at 3 mg/kg in 60% PEG200. Its in vitro metabolism profile was investigated in human and rat liver microsomal incubations performed under previously described oxidative conditions.[10] In the case of L-791,943, the rate of metabolism was less than 20 pmol/mg protein*h with no detectable metabolites. For L-826,141, the metabolic rates were 75 and 250 pmol/mg protein*h in rat and human incubations, respectively, with one metabolite being observed. The metabolite was identified as the hydroxymethyl pyridinium *N*-oxide by LC/MS analysis according to previously described techniques.[10] In rats, the metabolic rates observed in vitro correlate with the half-lives, indicating that the oxidative metabolism plays an important role in the elimination of these compounds.

Its in vivo efficacy was also demonstrated in the following models. It inhibited ovalbumin-induced bronchoconstriction in conscious guinea pigs by 82% when administered ip at 1 mg/kg (0.5 h pre-treatment).[17] It was also orally effective for the inhibition of bronchoconstriction induced by ascaris in conscious squirrel monkey with 26–59% and 38–100% inhibition of the early-phase and late-phase responses at doses varying from 0.5 to 3 mg/kg, 4 h pre-treatment.[18] Efficacy was also demonstrated in the ascaris-induced bronchoconstriction sheep model when administered iv at 2 mg/kg, 2 h pre-treatment.[19] Furthermore, it does not induce emesis in ferrets at 30 mg/kg po.

In conclusion, the introduction of a methyl group at position 3 on the pyridine-*N*-oxide ring of L-791,943 led to the identification of the PDE4 inhibitor L-826,141 which exhibits excellent in vitro activity. Furthermore, it is well absorbed and presents a shorter half-life than L-791,943 in a variety of animal species. Also, it is orally active in the squirrel monkey model of antigen induced bronchoconstriction (0.5–3 mg/kg) and is not emetic in ferrets at a dose of 30 mg/kg.

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