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Substituted Aminopyridines as Potent and Selective Phosphodiesterase-4 Inhibitors

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Abstract—The synthesis and the biological evaluation of new potent phosphodiesterase type 4 (PDE4) inhibitors are presented. This new series was elaborated by replacement of the metabolically resistant phenyl hexafluorocarbinol of L-791,943 (**1**) by a substituted aminopyridine residue. The structure–activity relationship of *N*-substitution on 3 led to the identification of (−)-**3n** which exhibited a good PDE4 inhibitor activity (HWB-TNFα = 0.12 μM) and an improved pharmacokinetic profile over L-791,943 (rat $t_{1/2}$ = 2 h). (−)-**3n** was well tolerated in ferret with an emetic threshold of 30 mg/kg (po) and was found to be active in the ovalbumin-induced bronchoconstriction model in guinea pig (54%, 0.1 mg/kg, ip) as well as the ascaris-induced bronchoconstriction model in sheep (64%/97%, early/late, 0.5 mg/kg, iv).

Over the last few years, leukotriene modulators such as leukotriene D_4-receptor antagonist[1] and 5-lipoxygenase inhibitors[2] have been one of the main antiasthmatic targets of the pharmaceutical industry. More recently, regulation of the intracellular level of cyclic nucleotides by inhibition of cAMP phosphodiesterases (PDEs) has attracted increased interest for the treatment of inflammatory diseases and asthma. It has been shown that cAMP elevation mediates airway smooth muscle relaxation[3] and also suppresses the activation of inflammatory cells.[4] It was then rapidly recognized that inhibition of the cAMP-specific isozyme phosphodiesterase type 4 (PDE4) would represent a good asthma target with dual properties of bronchodilation and anti-inflammatory activity.[5,6] However, the strong potential of the PDE4 inhibitors was found to be limited by a narrow therapeutic index mainly associated with nausea and emesis.[7] These side effects were first observed with the archetypic PDE4 inhibitor rolipram and since then, a major effort was invested to understand this phenomena and improve the so-called emetic window.

Recently, we reported the discovery of an orally active and well tolerated PDE4 inhibitor, L-791,943 (**1**) (Fig. 1).[8]

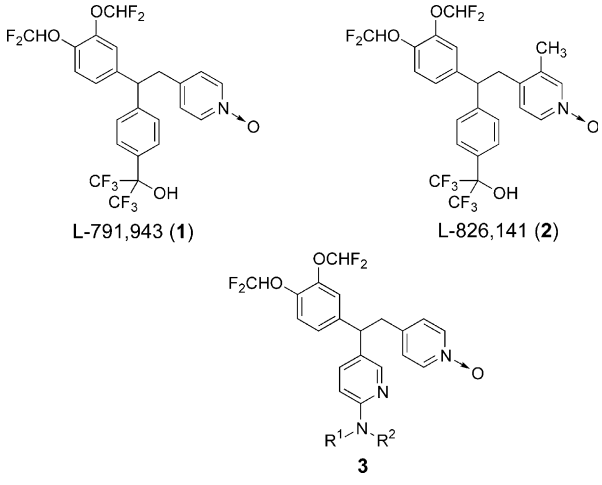

Figure 1.

An excessively long half-life (Rat: >24 h) had precluded the development of this compound but the introduction of a soft metabolic site on the structure of L-791,943 to produce L-826,141 (**2**) resulted in improved pharmacokinetics.[9] To overcome this long half-life problem we also considered replacing the metabolically resistant phenyl hexafluorocarbinol by a substituted aminopyridine residue. Here we wish to report the synthesis

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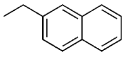
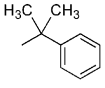
and the biological properties of the PDE4 inhibitor aminopyridines **3**.

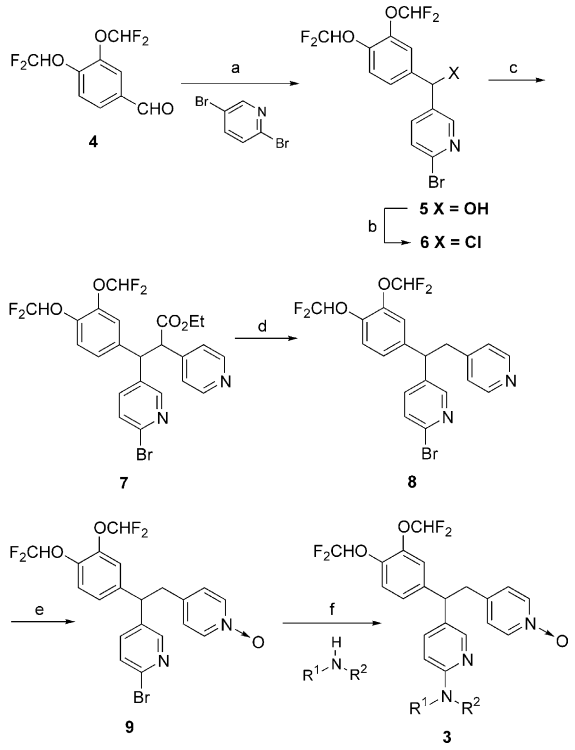
Over 100 analogues of type **3** were prepared to investigate the SAR of *N*-substitution with R^1 and R^2.[10] Three different strategies were used to synthesize compounds **3a** to **3p** (Table 1). In general, the steric hindrance of the amine involved in the Cu (I) promoted aryl amination dictated the appropriate route. The first general synthesis, presented in Scheme 1, was applied to the preparation of non-hindered secondary and tertiary amines **3**.[11] Secondary alcohol **5** was obtained by regioselective metalation of 2,5-dibromopyridine and nucleophilic addition on bis(difluoromethoxy)benzaldehyde **4**.[12] The potassium enolate of ethyl 4-pyridylacetate was then alkylated with the chloride **6**. After hydrolysis of ester **7**, the corresponding acid spontaneously decarboxylated to afford the pyridine **8** which could then be oxidized to the 4-pyridyl-*N*-oxide with either MMPP or MCPBA.

Table 1. SAR for R^1 and R^2 substituents on **3**

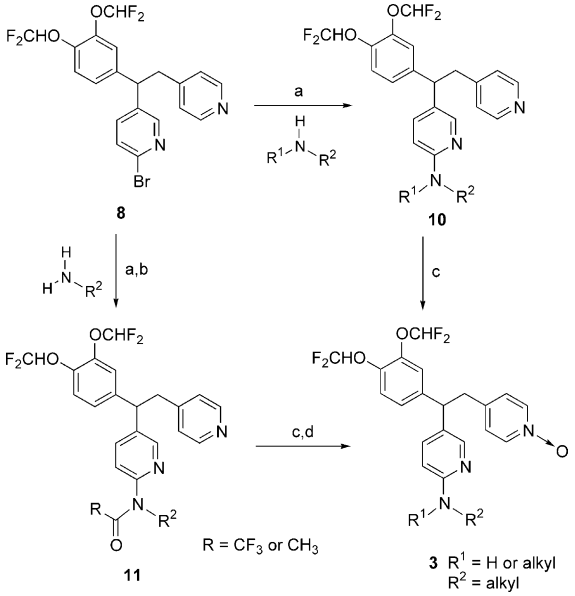
3	R^1	R^2	GST-PDE4A^{248} IC_{50} (nM)[a]	HWB (TNF-α) IC_{50} (μM)[b]
3a	Me	CH_2Ph	1.2	0.3
3b	Et	CH_2Ph	2.3	0.4
3c	*i*-Pr	CH_2Ph	9.3	1.0
3d	*t*-Bu	CH_2Ph	18	17
3e	Me	CH_2CH_2Ph	5.8	0.8
3f	Me	Ph	0.8	2.1
3g		—$(CH_2)_4$—	31	—
3h	Me		1.5	0.3
3i	Me		0.3	0.7
3j	Et		0.6	0.2
3k	H	CH_2Ph	1.2	0.4
3l	H	$CH_2CH_2PhCH_3$	2.3	0.8
3m	H		1.7	0.9
3n	H		1.6	0.2
3o[c]	H	H	31	1.6
3p	H	*i*-Pr	12	0.8
3q[d]	H	SO_2Ph	16	34
3r[d]	H	CONHEt	8.3	1.7
3s[d]	H	$COCH_3$	33	1.8
3t[e]	H	CO_2CH_2Ph	7.8	3.8
L-791,943 (**1**)			4.2	0.7
L-826-141 (**2**)			1.3	0.3
SB207499 (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.[15] IC_{50} represent a mean of N = 3.
[b]Inhibition of LPS-induced TNF-α in human whole blood.[16] IC_{50} represent a mean of $N = 3$.
[c]Prepared from **3n** with TFA/CH_2Cl_2.
[d]For the synthesis of these analogues see ref 17.
[e]Prepared by treating **16** with TFA/CH_2Cl_2.

Finally, the amine was introduced by a Cu (I) promoted aryl amination.[13] For this transformation only small amines could be used. In fact, more sterically hindered amines slow down the coupling reaction and competitive reduction of the *N*-oxide is observed. It is then recommended to do the Cu coupling before the *N*-oxidation of the 4-pyridyl residue as shown in Scheme 2. In this sequence, secondary amines are coupled in the presence



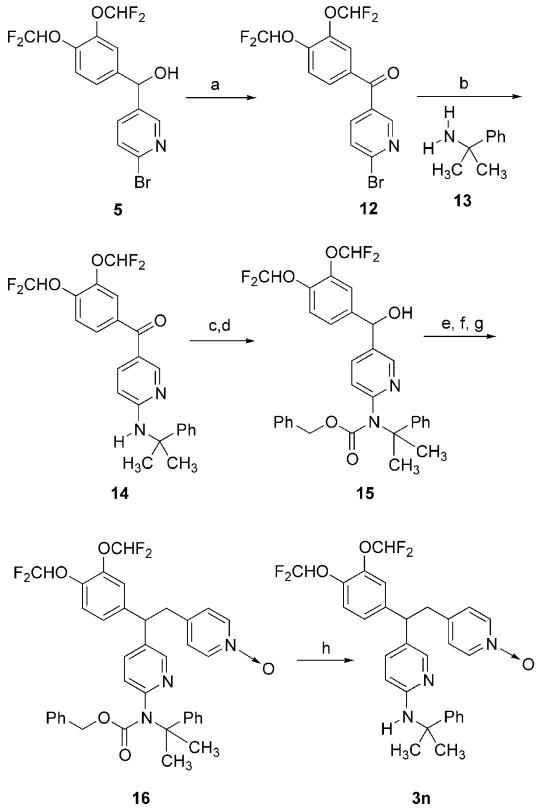
Scheme 1. (a) BuLi, Et_2O, −78 °C, 53%; (b) $SOCl_2$, CH_2Cl_2; (c) KHMDS, HMPA, ethyl 4-pyridylacetate, THF; (d) LiOH, THF, MeOH, 65 °C; HCl, 97% (three steps); (e) MMPP, CH_2Cl_2, MeOH, 93%; (f) CuI, 100–160 °C.



Scheme 2. (a) CuI, >100 °C; (b) $(RCO)_2O$, pyridine, CH_2Cl_2; (c) MMPP, CH_2Cl_2, MeOH; (d) LiOH, THF, MeOH.

of CuI followed by MMPP oxidation, but primary amines required an additional protection step to avoid oxidation of the aminopyridine functionality. Oxidation of the 4-pyridine **11** and amide hydrolysis afforded the aminopyridine **3**.[14]

The synthesis of aminopyridine **3n** is presented in Scheme 3. Compounds **3f** and **3i** were also prepared following this sequence which was found to be more appropriate for bulky amines. In a first attempt to synthesize **3n** by coupling cumylamine **13** with the bromopyridine **8**, no reaction was observed and we had to increase the electrophilic character of the bromopyridine functionality. This was achieved by oxidizing the alcohol **5** to the ketone **12** in the presence of MnO_2. Cumylamine **13** was then successfully coupled to this activated bromopyridine with copper iodide in 89% yield. The resulting secondary amine **14** was then protected to avoid competitive hydroxylamine formation during the subsequent oxidation of the 4-pyridyl residue. Ketone reduction followed by treatment of the alcohol **15** with thionyl chloride led to the corresponding chloride which was used to alkylate the potassium enolate of ethyl 4-pyridylacetate. Aminopyridine **16** was then obtained by ester hydrolysis and decarboxylation of the corresponding carboxylic acid followed by oxidation of the 4-pyridyl residue. Final deprotection of the benzyl carbamate under hydrogenolysis conditions afforded the analogue **3n**.



Scheme 3. (a) MnO_2, CH_2Cl_2, 88%; (b) CuI, 140 °C, 89%; (c) benzyl chloroformate, *i*-Pr_2NEt, dioxane; (d) $NaBH_4$, THF, MeOH, 95% (two steps); (e) $SOCl_2$, pyridine, toluene, 0 °C; KHMDS, HMPA, ethyl 4-pyridylacetate, THF, 0 °C; (f) LiOH, THF, MeOH, 65 °C; HCl, 80% (two steps); (g) MMPP, CH_2Cl_2, MeOH; (h) H_2, 10% Pd/C, EtOH, 55% (two steps).

Summarized in Table 1 are the intrinsic PDE4A potency and their cellular efficacy in human whole blood (HWB) using the inhibition of TNF-α production as an index. Although these compounds represent only a small fraction of all the aminopyridines tested, they illustrate very well the SAR that was observed while replacing R^1 and R^2. In the tertiary amine series (**3a** to **3j**), the data suggested that R^1 has to be kept small since the inhibitory activity declined progressively going from **3a** ($R^1 = CH_3$) to **3d** ($R^1 = t$-butyl). With a small R^1 in place ($R^1 = Me$), several R^2 replacements were compared with the benzyl aminopyridine **3a**. Selected examples **3e** to **3h** led to the conclusion that the benzylic motif was a superior pharmacophore at this position since phenethyl **3e**, aniline **3f** and pyrrolidine **3g** were found to be less potent. As we had a good hand on potency with prototypic aminopyridine **3a**, the next series of analogues were then designed based on pharmacokinetic data. Oral and iv administration of **3a** to rats and squirrel monkeys, indicated that the drug was rapidly metabolized ($t_{1/2} < 1$ h) by dealkylation of the methyl substituent to afford the secondary amine **3k**. This in vivo metabolic pathway was also observed with analogue **3e** leading to the formation of **3l** as the major metabolite. Interestingly, **3k** and **3l** were found to maintain a good PDE4 activity but unfortunately, when dosed iv in rats, no pharmacokinetic improvement was observed and these two compounds were cleared rapidly with a half-life of less than one h. Another interesting observation was made when the most potent compound of the series, **3j** (HWB 0.15 μM), was dosed iv in rats. In this case, the ethyl residue remains untouched but the amine was rapidly debenzylated resulting in a $t_{1/2}$ comparable to **3a**. Its was postulated that in vivo oxidation of the benzylic position followed by hemiacetal fragmentation could be responsible for the observed debenzylation. To prevent this metabolic pathway the benzylic position was blocked with a gem dimethyl while keeping $R^1 = H$. This new potent analogue **3n** displayed a half-life of 2–3 h in rats and squirrel monkeys which represented a major improvement over the previous aminopyridines. Several benzyl replacements were also investigated but in general, as shown with analogue **3o** to **3t**, a reduced PDE4 activity was observed.

The enantiomers of **3n** were then resolved using a Chiralpack AD HPLC column (Table 2). (−)-**3n** was found to be the most potent enantiomer exhibiting, in the human whole blood assay, improved PDE4 inhibitory potency over L-791,943 (5.6-fold), L-826,141 (3-fold) and SB207499 (Ariflo™)[18] (150-fold). (−)-**3n** was also found to be very selective against all the other PDEs.[19]

Table 3 highlights some of the in vivo characteristics of (−)-**3n** in comparison with L-791,943 (**1**). One of our

Table 2. Resolution of aminopyridine **3n**

Compd	GST-PDE4A IC_{50} (nM)	HWB (TNF-α) IC_{50} (μM)
3n	1.6	0.2
(+)-**3n**	23	10
(−)-**3n**	0.8	0.1

Table 3. Comparative in vivo profile of (−)-**3n** with L-791,943

	L-791,943	(−)-**3n**
Rat: $t_{1/2}$	>24 h	2 h
F, C_{max} (P.O. 20 mpk)	70%, 3.5 μM	100%, 2.4 μM
Efficiacy — Guinea Pig	58%[a] (1.0 mpk)	54%[b] (0.1 mpk)
Efficiacy — Sheep – early/late.[c]	85%/95% (2.0 mpk)	64%/97% (0.5 (mpk)
Emesis in ferret (C_{Max})	>30 mpk (14 μM)	30 mpk (16 μM)

[a]Dosed ip (4 h of pre-treatment).
[b]Dosed ip (30 min of pre-treatment).
[c]Dosed iv for 4 days (2 h of pre-treatment).

initial objectives was to reduce the long $t_{1/2}$ observed with L-791,943. With a half-life of 2 h (rat, iv 5 mg/kg), while maintaining the PDE4 activity, the aminopyridine pharmacophore proved to be a good replacement for the phenyl hexafluorocarbinol. Ferrets receiving (−)-**3n** showed signs of emesis only at a po dose of 30 mg/kg. Considering its potency, this emetic threshold compares very well with L-791,943 (**1**). Finally, inhibitor (−)-**3n** demonstrated good in vivo activity by blocking ovalbumin-induced bronchoconstriction in conscious guinea pig[20] by 54% at a dose of 0.1 mg/kg (ip). (−)-**3n** was also found to be efficacious in the sheep model[21] with 64%(early phase) and 97% (late phase) of inhibition of ascaris-induced bronchoconstriction.

In conclusion, we have developed a new series of potent PDE4 inhibitors by replacement of the metabolically resistant phenyl hexafluorocarbinol of L-791,943 (**1**) by a substituted aminopyridine residue. The structure–activity relationship of *N*-substitution on **3** led to the identification of (−)-**3n** which exhibited a good PDE4 inhibitor activity (HWB-TNF-$\alpha = 0.12$ μM) and an improved pharmacokinetic over L-791,943 (Rat $t_{1/2} = 2$ h). (−)-**3n** was well tolerated in ferret with an emetic threshold of 30 mg/kg (po) and was found to be active in the ovalbumin-induced bronchoconstriction model in guinea pig (54%, 0.1 mg/kg, ip) as well as the ascaris-induced bronchoconstriction model in sheep (64%/97%, early/late, 0.5 mg/kg, iv).

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