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ENANTIODIFFERENTIATION OF DIHYDROPYRIDINE PAF ANTAGONISTS

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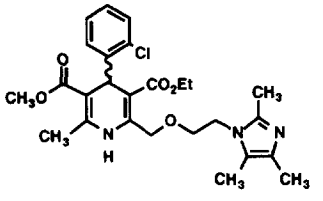
Abstract: The PAF antagonist activity of a series of enantiomeric dihydropyridines is described. In the first example, **1**, the PAF antagonist activity and calcium channel blocking activity reside in opposite enantiomers. Subsequent examples also display enantioselectivity and the SAR of the series is described.

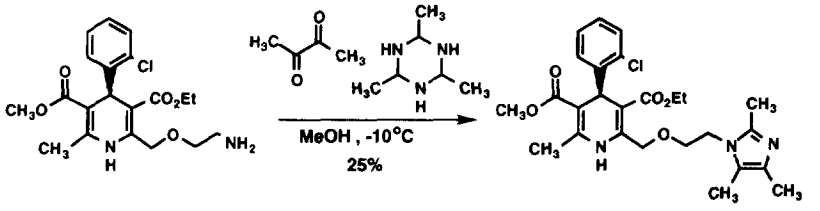
Platelet activating factor (PAF, 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) is an ether phospholipid which exhibits, in addition to potent platelet aggregating activity, a wide spectrum of biological activities elicited directly or *via* the release of other mediators. Extensive research studies in many laboratories have implicated PAF as a potential mediator in a number of pathophysiological conditions. In particular, great interest has focused on the role of PAF in bronchial hyperreactivity and the late phase bronchospasm following allergen challenge to the lung, and thus the role of PAF in asthma. In addition, PAF has been linked to several other disease states, including rhinitis, psoriasis, endotoxic shock, and ischemia and it has also been credited with a role in immunological mechanisms principally *via* a priming mechanism.[2] To evaluate fully and validate the role of PAF in *in vitro* and *in vivo* settings, as well as in clinical endpoints, it has been of primary importance to develop potent and specific PAF antagonists.[3] In the Pfizer laboratories, we initiated a program to identify potent and selective PAF antagonists based on the early reports of the activity of calcium channel blockers as inhibitors of PAF-induced platelet aggregation.[4] We extended literature reports with the testing of some calcium channel blockers from our sample files and found that the dihydropyridine **1** was a potent inhibitor of PAF-induced platelet aggregation.



1

The full profile of **1** showed balanced PAF antagonist and calcium channel blocker activity. However, PAF antagonist activity could not be demonstrated *in vivo* because the compound causes profound hypotension on dosing to animals. Thus, it was difficult to unequivocally establish whether the PAF antagonist activity was a distinct and separate property of the molecule. Enantioselective activity is a well

established phenomenon within the calcium channel blocker SAR, and it was of significant interest to separate the two enantiomers of **1** and assay them in the individual pharmacological test systems. The enantiomers of **1**, which were synthesized by modification of the amlodipine enantiomers (see **Scheme 1**),[5] led to complete separation of the PAF antagonist and calcium channel blocking activities.



R-amlodipine **2**

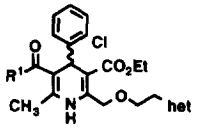
Scheme 1[6]

Thus, the R enantiomer **2** possessed the majority of the PAF antagonist activity, whereas the S enantiomer **3** possessed all of the calcium channel blocking activity (see **Table 1**).

Compound	PAF IC_{50} nM[7]	CCB IC_{50} nM[8]	DHP binding IC_{50} nM[9]
1	21	14	96
2	15	>1000	>10000
3	183	12	41

Table 1[10]

Thus, the two activities were demonstrated to be pharmacologically distinct and supported a full chemistry follow-up program to pursue selective PAF antagonist activity in dihydropyridines. During previous work we had explored the SAR of calcium channel blocking activity in dihydropyridines and were able to pursue a number of different approaches to reduce or abolish the calcium channel activity, such as replacement of the 4-aryl group with an alkyl group, or substitution at the 4' position of the 4-aryl group. This work rapidly led to the identification of the 5-amide derivatives (see **Table 2**, examples **4-6**), the first racemic series of compounds which possessed PAF antagonist activity without calcium channel blocking activity.[11] These compounds were also the first to demonstrate significant *in vivo* activity and the best compound was the 5-[6-methylpyrid-2-yl]-carbamoyl derivative **6**. The compound was submitted to pharmacokinetic evaluation in the dog but its profile was disappointing, with a moderate plasma half life ($t^1/_2$) (4.4 h) and only 9% oral bioavailability. However, the 5-amide series provided the encouragement to proceed with the program, having achieved the first key objective of designing **out** the calcium channel blocking activity.



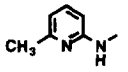
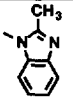
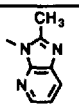
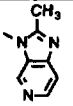
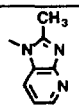
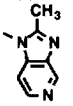
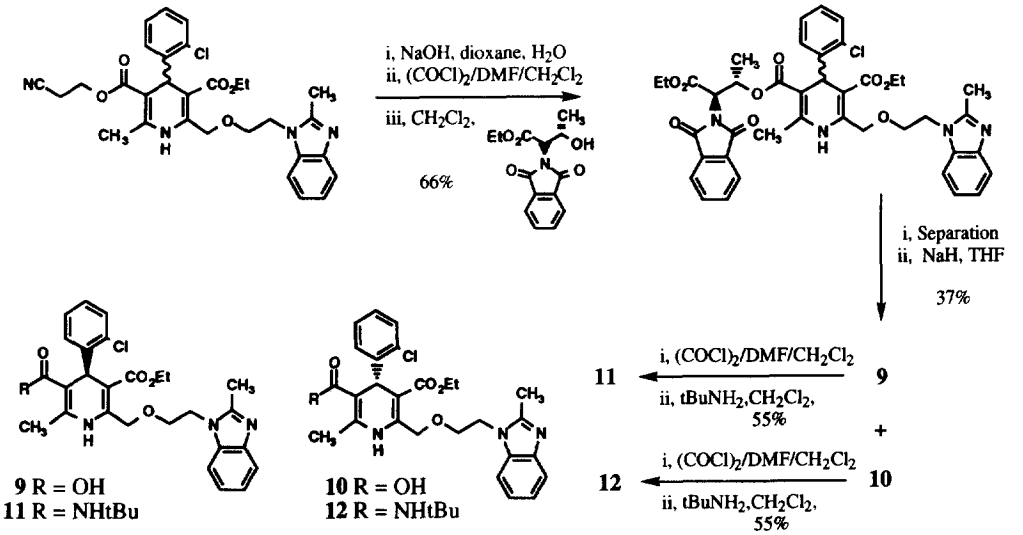
Compound	R^1	het	PAF IC_{50} nM[7]	ED_{50} mg/kg[12]
4			17	18
5	tBuNH		22	10.1
6			11	4.5
7			3	49
8	tBuNH		7.5	34
13			8	25
14			49	29
15			6.5	31
16			8	2

Table 2[10]

We next turned our attention to modification of the heterocycle on the 2-substituent of the dihydropyridine. From this series of modifications, the 2-methylbenzimidazol-1-yl derivative **8**, was selected for additional profiling. The compound was a potent PAF antagonist *in vitro* and showed moderate *in vivo* activity in the

mouse model. In dog pharmacokinetics it possessed a rather short plasma $t^1/_2$ (1.2 h), but improved 30% oral bioavailability. The profile of **8** was of sufficient interest for us to proceed to a resolution, which was achieved *via* chromatographic separation of diastereoisomeric esters and conversion of the acids **9** and **10** to the enantiomeric amides **11** and **12** as outlined in **Scheme 2**.[13]



Scheme 2

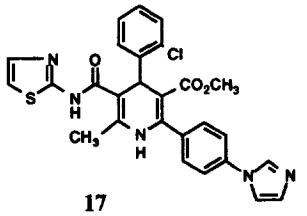
As with **1**, the R enantiomer **11** was the most potent PAF antagonist with the S enantiomer **12** being the weaker PAF antagonist (see **Table 3**).[14]

Compound	PAF IC_{50} nM[7]	ED_{50} mg/kg[12]	Compound	PAF IC_{50} nM[7]	ED_{50} mg/kg[12]
8	7.5	40	**18**	4.9±0.24 (3)	0.26±0.03 (6)
11	2.3	-	(+) **18**	2±0.37 (3)	0.15±0.01 (3)
12	60	-	(-) **18**	31.3±5.2 (3)	3.8±0.28 (4)

Table 3[10]

However, the *in vivo* potency of **8** was only moderate and the program continued with the goal of identifying better *in vivo* activity. The next major breakthrough came with the introduction of a nitrogen

atom into the benzimidazole ring to give the four imidazopyridines **13** to **16**. Of the four derivatives, **16** was the most potent antagonist, both *in vitro* and *in vivo*, and the compound was evaluated in the dog for its pharmacokinetic profile. Disappointingly, it possessed a short plasma $t^1/_2$ (0.75 h) and had poor oral bioavailability (4%). Clearly the pharmacokinetics of this series was a problem and we began to focus on compounds which had potential for more metabolic stability. We re-examined an earlier lead **17** which placed a phenyl ring between the dihydropyridine ring and the heterocycle (PAF IC_{50} = 415 nM, n=2).



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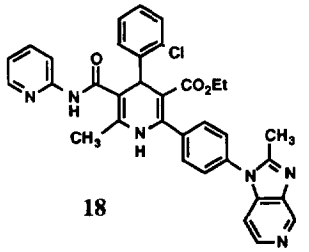
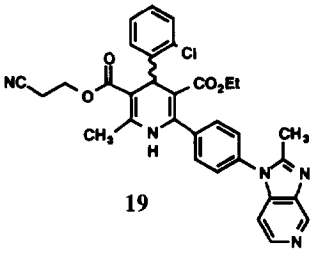


18

Development of the SAR around **17** led rapidly to the discovery of **18** which in all respects surpassed all our previous antagonists and it was profiled extensively.[15] In dog pharmacokinetics **18** showed complete (100%) oral bioavailability, although it had a short plasma $t^1/_2$ (1.2 h). Resolution of **18** was achieved *via* fractional crystallization of a salt[16] of the key intermediate **19**, and then conversion to the two enantiomeric amides (see **Scheme 3**).



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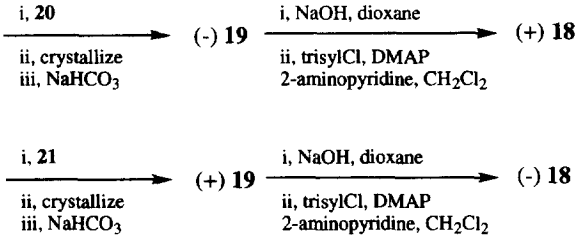

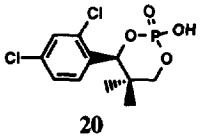

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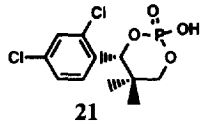

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Scheme 3

The separation of the enantiomers of **18** led to a separation of the PAF antagonist activity which was similar to those obtained with the two previous resolutions (see **Table 3**). Thus, (+) **18** was 15 fold more potent than (-) **18** *in vitro*, a difference also reflected *in vivo* where (+) **18** was 25 fold more potent than(-) **18**. Unfortunately, we have not been able to determine the absolute stereochemistry of (+) **18** by X-ray crystallography due to disorder in the crystals of (+) **18**, and thus it is not known whether (+)**18** has the same chirality as **2** and **11**.

Conclusions

In our work on the dihydropyridine PAF antagonist program, we demonstrated clear enantioselectivities for the PAF receptor with compounds **2**, **11**, and **18**. The first resolution showed that the calcium channel blocking activity and PAF antagonist activities were separate and this discovery was enough to support a continued program. Subsequent resolutions allowed us to focus on the follow up profiling and evaluation of the enantiomer with the required pharmacological activity and thereby discard any effects of the weaker enantiomer. The program successfully identified **18** as a potent and specific PAF antagonist and the more active enantiomer, **(+)18, (UK-80,067, modipafant)**, was progressed for clinical evaluation in asthma.[17]

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References and Notes

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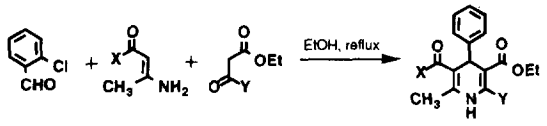
6. The S-enantiomer was made using identical reaction conditions starting with S-amlodipine
7. PAF functional activity was estimated in a PAF-induced rabbit, washed-platelet aggregation assay. Full details of the assay can be found in Cooper, K.; Fray, M. J.; Parry, M. J.; Richardson, K.; Steele, J. *J. Med. Chem.* **1992**, *35*, 3115.
8. The activity of the compounds as calcium channel blockers was measured in a K^+-stimulated rat aorta assay. For details see reference 5.
9. For details of the DHP binding assay, measuring displacement of [^{3}H]-nitrendipine from bovine frontal cortex membranes see reference 15.
10. All values are single determinations unless otherwise stated.
11. The dihydropyridines in **Table 2** were synthesized using a three component Hantszch reaction outlined as follows:



12. *In vivo* activity was estimated after oral administration using PAF-induced lethality in mice. Full details are given in reference 7.
13. The starting material for the resolution of **8** was obtained *via* a three component Hantszch reaction between 2-chlorobenzaldehyde, 2'-cyanoethyl 3-aminobut-2-enoate, and ethyl (2-methylbenzimidazol-1-yl)ethoxy-3-oxobutanoate.
14. The absolute stereochemistry of **10** was determined by single crystal X-ray crystallography.
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