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The Discovery of SB-435495: A Potent, Orally Active Inhibitor of Lipoprotein-Associated Phospholipase A_2 for Evaluation in Man

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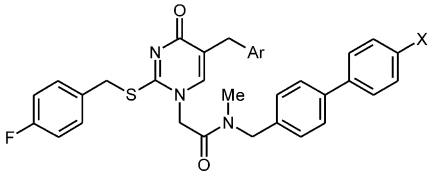
Abstract—The introduction of a functionalised amido substituent into a series of 1-(biphenylmethylacetamido)-pyrimidones has given a series of inhibitors of recombinant lipoprotein-associated phospholipase A_2 with sub-nanomolar potency and very encouraging developability properties. Diethylaminoethyl derivative **32**, SB-435495, was selected for progression to man.

A recent study has shown a strong, positive correlation between levels of lipoprotein associated phospholipase A_2 (Lp-PLA_2) and coronary events in asymptomatic, hypercholesterolemic men and has suggested that Lp-PLA_2 is a new, independent marker of coronary heart disease risk.[1] It is known that this lipase is associated predominantly with LDL and is able to hydrolyse oxidised LDL into lysophosphatidylcholine (lyso-PtdCho) and oxidised fatty acids. Both of these hydrolysis products are known to be pro-inflammatory and have been implicated in atherosclerosis.[2] Indeed, the increased levels of lyso-PtdCho in oxidised LDL can be completely accounted for by Lp-PLA_2.[3] These data highlight the need for inhibitors of Lp-PLA_2 in order to evaluate the role of this lipase in atherosclerosis.

In the preceding letter,[4] we reported the identification of a series of 1-(biphenylmethylamidoalkyl)-pyrimidones **1**, as highly potent inhibitors of Lp-PLA_2 which showed excellent activity in the Watanabe hereditable hyperlipidaemic rabbit (WHHL rabbit). These compounds however proved somewhat difficult to formulate for in vivo studies and, as a result, we sought inhibitors with further improved physicochemical properties. With this aim in mind, we investigated the effect of introducing a more polar, potentially water solubilising, group onto the amide nitrogen atom and now describe the beneficial results of this modification.



1 X = Cl, CF_3
Ar = 1-Me-pyrazol-4-yl or 2-MeO-pyrimidin-5-yl

Compounds were prepared from either methyl 3-(1-methylpyrazol-4-yl)propanoate or ethyl 3-(2-methoxypyrimidin-5-yl)propanoate using methods similar to those previously described[4] and were subsequently evaluated using recombinant human Lp-PLA_2 (rhLp-PLA_2).[5] Non-specific binding effects in plasma were assessed by evaluating compounds against the enzyme in both whole human and WHHL rabbit plasma at a single concentration of inhibitor.[5] High potency in

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human and rabbit plasma in addition to little effect on cytochrome P450 enzymes[6] (minimising potential drug–drug interactions) and reasonable levels of permeability[7] were normally required before compounds were evaluated in vivo in WHHL rabbits,[5] and for selected inhibitors, in the rat[8] and dog.[8]

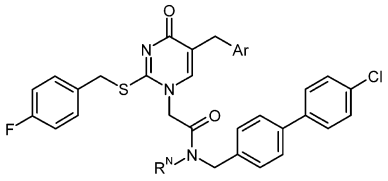
Initial SAR indicated that relative to the parent *N*-methyl derivatives, potency could be maintained or even enhanced by the introduction of a number of more polar amide substitutuents (Table 1). These included alcohols, amines and amides (c.f., **2** and **7** with **3**, **5**, **6**, **9**, **11** and **12**). The related ethers and acids were a little less potent (see **6** and **4**, also **11** and **10**). Whilst as expected, amine and acid salts proved the most water soluble, the corresponding alcohols were remarkably insoluble. Additionally, inhibitors containing the 5-(1-methylpyrazol-4-yl)methyl group showed better solubility properties than the related methoxypyrimidyl derivatives. When screened against a number of cytochrome P450 enzymes, amides such as **5** and **11** showed a strong interaction with CYP450 3A4 ($IC_{50} < 0.5$ μM) and consequently were not considered further.

With all these results to hand, we concentrated on amino substitution in the 5-(1-methylpyrazol-4-yl)methyl series. Many basic substituents were introduced, linked by either two or three methylene spacers to the amido nitrogen atom. Virtually all these amino derivatives proved to be very potent inhibitors of rhLp-PLA_2 with excellent activity in whole plasma (see **12**–**26**). Indeed, with the exception of the morpholino- and piperazino-derivatives **19**, **25** and **26** and primary amine **15** all compounds showed an $IC_{50} \leq 1$ nM versus rhLp-PLA_2. A number of compounds were also prepared in the 4-trifluoromethyl substituted biphenyl series (Table 2). Broadly similar results were observed, with diethylaminoethyl derivative **32** proving particularly potent.

Interaction with CYP450 3A4 proved a particular issue with this series (target $IC_{50} \geq 10$ μM). Compounds with an $IC_{50} \geq 2$ μM against this isoform are shown in Table 3. As expected, lipophilicity plays a key role in the interaction with this CYP450 enzyme (c.f., **15**, **12** and **13**, **22** and **23** also **31** and **32**) although other factors may also be important (see above discussion on amides **5** and **11**). Trifluoromethyl derivatives generally show less interaction than their chlorobiphenyl counterparts (c.f., **31** and **12** also **32** and **13**).

As a guide to obtaining improved bioavailability, compounds of Table 3 were also evaluated in an artificial membrane permeability assay (target permeability ≥ 0.01 cm/h).[7] This assay proved a further stringent test

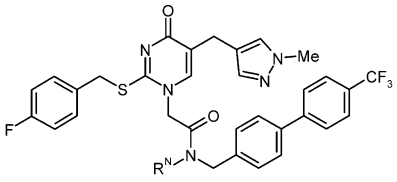
Table 1. Effect of amide substitution—4-Cl series



Compd[a]	R^N	Ar	IC_{50} (nM)	Inhibition in plasma	
				Human 10 nM	Rabbit 100 nM
2	Me	2-MeO-Pyrimidin-5-yl	0.2	51	65
3	$HOCH_2CH_2$	2-MeO-Pyrimidin-5-yl	0.2	42	55
4	HO_2CCH_2	2-MeO-Pyrimidin-5-yl	1	51	37
5	Me_2NCOCH_2	2-MeO-Pyrimidin-5-yl	0.2	80	80
6	$Me_2NCH_2CH_2$	2-MeO-Pyrimidin-5-yl	0.2	78	85
7	Me	1-Me-Pyrazol-4-yl	1	43	55
8	Et	1-Me-Pyrazol-4-yl	1.5	27	35
9	$HOCH_2CH_2$	1-Me-Pyrazol-4-yl	0.6	36	52
10	$MeOCH_2CH_2$	1-Me-Pyrazol-4-yl	4.5	30	41
11	Me_2NCOCH_2	1-Me-Pyrazol-4-yl	0.2	80	84
12	$Me_2NCH_2CH_2$	1-Me-Pyrazol-4-yl	0.6	65	72
13	$Et_2NCH_2CH_2$	1-Me-Pyrazol-4-yl	0.7	58	80
14	$EtNHCH_2CH_2$	1-Me-Pyrazol-4-yl	0.2	63	72
15	$H_2NCH_2CH_2$	1-Me-Pyrazol-4-yl	2	41	58
16	$iPr_2NCH_2CH_2$	1-Me-Pyrazol-4-yl	0.2	54	67
17	2-(Pyrrolidin-1-yl)ethyl	1-Me-Pyrazol-4-yl	0.8	53	78
18	2-(Piperidin-1yl)ethyl	1-Me-Pyrazol-4-yl	0.3	52	57
19	2-(Morpholin-4-yl)ethyl	1-Me-Pyrazol-4-yl	1.4	39	28
20	$HOCH_2CH_2(Et)NCH_2CH_2$	1-Me-Pyrazol-4-yl	0.12	71	80
21	$(HOCH_2CH_2)_2NCH_2CH_2$	1-Me-Pyrazol-4-yl	0.5	49	42
22	$Me_2NCH_2CH_2CH_2$	1-Me-Pyrazol-4-yl	0.5	63	79
23	$Et_2NCH_2CH_2CH_2$	1-Me-Pyrazol-4-yl	0.35	63	59
24	3-(Pyrrolidin-1-yl)propyl	1-Me-Pyrazol-4-yl	1	35	35
25	3-(Morpholin-4-yl)propyl	1-Me-Pyrazol-4-yl	5	10	56
26	3-(4-Me-piperazin-1-yl)propyl	1-Me-Pyrazol-4-yl	2	55	76

[a]All new compounds gave satisfactory analytical/spectral data.[9]

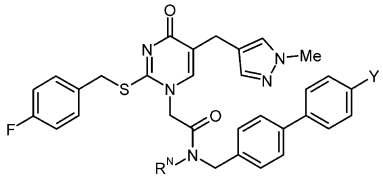
Table 2. Effect of amide substitution—4-CF_3 series



Compd[a]	R^N	IC_{50} (nM)	Inhibition in plasma	
			Human 10 nM	Rabbit 100 nM
27	$HOCH_2CH_2$	0.6	59	56
28	HO_2CCH_2	1	78	64
29	Me_2NCOCH_2	0.3	92	92
30	(Morpholin-4-yl)$COCH_2$	0.1	78	65
31	$Me_2NCH_2CH_2$	0.7	82	87
32	$Et_2NCH_2CH_2$	0.06	87	95
33	$EtNHCH_2CH_2$	0.12	66	46
34	2-(Piperidin-1-yl)ethyl	0.17	64	82
35	$Me_2NCH_2CH_2CH_2$	1	18	8

[a]All new compounds gave satisfactory analytical/spectral data.[9]

Table 3. Interaction of selected compounds with CYP450 3A4 and black membrane permeability



Compd	R^N	Y	CYP450 3A4 IC_{50} (μM)[a]	Permeability (cm/h[b])
9	$HOCH_2CH_2$	Cl	19	0.002
12	$Me_2NCH_2CH_2$	Cl	8	0.002
13	$Et_2NCH_2CH_2$	Cl	5	0.029
15	$H_2NCH_2CH_2$	Cl	15	0.002
17	2-(Pyrrolidin-1-yl)ethyl	Cl	6	0.004
22	$Me_2NCH_2CH_2CH_2$	Cl	17	0.001
23	$Et_2NCH_2CH_2CH_2$	Cl	2	<0.001
26	2-(4-Me-piperazin-1-yl)propyl	Cl	10	<0.001
28	HO_2CCH_2	CF_3	31	<0.001
31	$Me_2NCH_2CH_2$	CF_3	30	<0.001
32	$Et_2NCH_2CH_2$	CF_3	10	0.017
34	2-(Piperidin-1-yl)ethyl	CF_3	10	0.068

[a]See ref 6.
[b]See ref 7. Permeability >0.01 cm/h considered acceptable.

for these inhibitors — many of the compounds that passed the CYP450 criteria proved particularly impermeable. This included not only carboxylic acid **28** but also many of the basic derivatives. Differences were seen between the series bearing a two- or three-methylene spacer with only the former proving permeable. This may be due in part to differences in pK_a of these two groups (c.f., **13**, $pK_a = 8.3$ and **23** $pK_a = 9.0$). Overall, a balance of lipophilic properties appeared necessary in order that good membrane permeability could be achieved without strong interaction with CYP450 3A4. Compounds **32** and **34** possessed this balance and were progressed to the WHHL rabbit. Unlike our previous inhibitors which showed low aqueous solubility, we were able to dose these compounds, as their salts, in pure water (compound **32**, for example was soluble at 5 mg/mL at pH 4.7). Based on the impressive results following gavage dosing (Fig. 1), **32** was subsequently chosen for a more detailed evaluation.

Mechanistic studies using steady state and transient kinetics indicated compound **32** to be a freely reversible, non-covalently bound, inhibitor of rhLp-PLA_2 with a K_i of 30 pM and an off-rate of 2.4 h.[10] Moreover, in agreement with the single point data in Table 2, **32** inhibited the enzyme in whole human plasma with an IC_{50} of 3 ± 0.4 nM. Impressively, and consistent with the proposed mechanism of action for an inhibitor of Lp-PLA_2, the presence of compound **32** during the copper catalysed oxidation of human LDL prevented the production of lyso-PtdCho ($IC_{50} = 23 \pm 4$ nM) and subsequent monocyte chemotaxis ($IC_{50} = 10 \pm 1$ nM).[11]

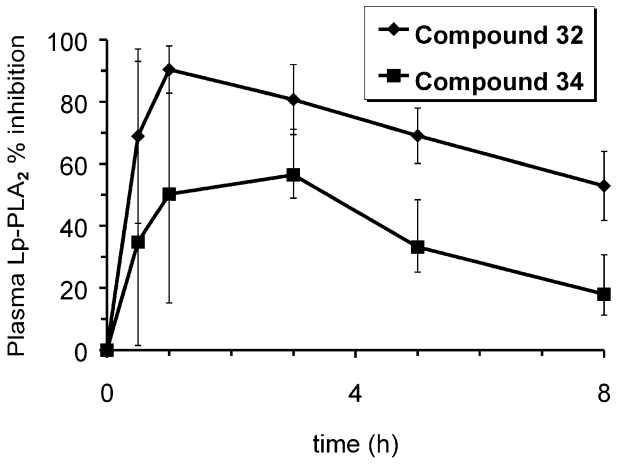


Figure 1. Inhibition of plasma Lp-PLA_2 in the WHHL rabbit @ 10 mg/kg po.

Furthermore, **32** showed little interaction with other CYP450 enzymes (CYP450 IC_{50}: 1A2 > 100 μM, 2C9 > 100 μM, 2C19 > 40 μM, 2D6 = 37 μM).

In vivo studies with **32** indicated a promising oral bioavailability profile of 13 ± 1% in the rat and 24 ± 7% in the dog.[8] Furthermore, following a dose of 30 mg/kg of this compound to the WHHL rabbit, excellent inhibition of Lp-PLA_2 within the atherosclerotic plaque was achieved with 74 ± 9% inhibition observed 2 h after dosing.[12]

Based on this excellent package of in vitro and in vivo data, compound **32**, SB-435495 was selected for evaluation in man. Details of these very encouraging studies will be presented in due course.

In conclusion, we have shown that modification of the amido substituent in our previously described biarylamide derivatives[4] gives highly potent inhibitors of Lp-PLA_2. Using in vitro developability assays we identified compounds with suitable characteristics for further evaluation and subsequently showed that one of these, compound **32**, SB-435495, was worthy of progression to man. Further studies with SB-435495 will clearly enhance our assessment of Lp-PLA_2 as a target for therapeutic intervention.

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6. See Bambal, R.; Bloomer, J.C. WO Patent 0144495, 2001; *Chem. Abstr.* **2001**, 453279.
7. Egg phosphatidyl choline (2%) and cholesterol (1%) were dissolved in *n*-decane. A small amount of the mixture was applied to the bottom of microfiltration filter inserts (Transwell, Corning-Costar, Cambridge, MA, USA). Phosphate buffer (0.05 M, pH 7.05, containing 1% hydroxypropyl-β-cyclodextrin) was added to the donor and receiver sides, and the lipids were allowed to form self-assembled bilayers across small holes in the filter. 10-mM solutions of compound in DMSO were spiked into the donor side to a final concentration of 100 μM. Samples from both donor and receiver sides were withdrawn at predetermined elapse times (typically, 2 and 3 h) for HPLC analysis. The permeability parameters, expressed in cm/h, were calculated from the experimentally determined receiver/donor ratios.
8. Oral bioavailability and clearance parameters were determined for the hydrochloride or bitartrate salt of **32** by non-compartmental pharmacokinetic analysis following iv infusion over 1 h [4 mg/kg (rat) or 2 mg/kg (dog) in 5% w/v glucose] and oral gavage administration (10 mg/kg in 1% aq methylcellulose) in the conscious, cannulated male rat or dog. Serial blood samples were collected up to 24 h post dose and analysed by LC/MS/MS.
9. Representative example: Compound **32** (250 MHz) ^{1}H NMR ($CDCl_3$, rotamer mixture) δ 0.9–1.0 (6H, m) δ 2.4–2.6 (6H, m), 3.24/3.4–3.6 (4H, 2×m), 3.85 (3H, s), 4.46/4.53/4.66/4.83 (6H, 4×s), 6.75/6.8 (1H, 2×s), 6.9–7.0 (2H, m), 7.3–7.7 (12H, m); Elemental Analysis C 63.21, H 5.57, N 11.56%; $C_{38}H_{40}F_4N_6O_2S$ requires C 63.32, H 5.59, N 11.66%. MS (APCI+) found (M + 1) = 721; $C_{38}H_{40}F_4N_6O_2S$ requires 720.
10. For representative methods, see: Pope, A. J.; Moore, K. J.; McVey, M.; Mensah, L.; Benson, N.; Osbourne, N.; Broom, N.; Brown, M. J. B.; O'Hanlon, P. *J. Biol. Chem.* **1998**, *273*, 31691. Full details will be published in due course.
11. For methods, see: Tew, D. G.; Southan, C.; Rice, S. Q. J.; Lawrence, M. P.; Haodong, L.; Boyd, H. F.; Moores, K.; Gloger, I. S.; Macphee, C. H. *Atheroscler. Thromb. Vasc. Biol.* **1996**, *16*, 591.
12. WHHL rabbits (n = 4 per group) were gavage dosed with either 30 mg/kg **32** or vehicle and euthanased after 2 h. 0.5-cm sections of aorta were removed from just after the aortic arch, washed, frozen in liquid nitrogen and stored at −80 °C to await analysis. Each slice of aorta was homogenised in buffer (mmol/L: Tris 50[pH8], CHAPS 10, EGTA 2, and EDTA 2) containing 1 μg/mL each of leupeptin, antipain, and pepstatin-A and microfuged for 20 min at 4 °C. 50-uL aliquots of supernatant were incubated (in the presence or absence of 100 nM SB-435495 to define the contribution of Lp-PLA_2 to the total PLA_2 activity) with 130 μL of Lp-PLA_2 assay buffer (50-mM Hepes, 150 mM NaCl, pH 7.4) and 20 μL of 500 μM [^{3}H]PAF. Activity was measured as in ref 5.