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## The Identification of Clinical Candidate SB-480848: A Potent Inhibitor of Lipoprotein-Associated Phospholipase A<sub>2</sub>

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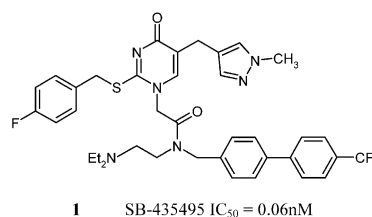
**Abstract**—Modification of the pyrimidone 5-substituent in clinical candidate SB-435495 has given a series of inhibitors of recombinant lipoprotein-associated phospholipase A<sub>2</sub> with sub-nanomolar potency. Cyclopentyl fused derivative **21**, SB-480848, showed an enhanced in vitro and in vivo profile versus SB-435495 and has been selected for progression to man.

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Atherosclerosis bears many hallmarks of a chronic inflammatory disease.<sup>1</sup> For this reason we have targeted an enzyme, lipoprotein associated phospholipase A<sub>2</sub> (Lp-PLA<sub>2</sub>), that is able to generate inflammatory products, lysophosphatidylcholine (lyso-PtdCho) and oxidised fatty acids, on hydrolysis of oxidised low density lipoprotein (LDL). Both of these hydrolysis products have been implicated in atherosclerosis.<sup>2,3</sup> Additionally, the increased levels of lyso-PtdCho in oxidised LDL can be completely accounted for by Lp-PLA<sub>2</sub>.<sup>3</sup> Furthermore, a recent study has shown a strong, positive correlation between levels of lipoprotein associated phospholipase A<sub>2</sub> (Lp-PLA<sub>2</sub>) and coronary events in asymptomatic, hypercholesterolemic men and has suggested that Lp-PLA<sub>2</sub> is a new, independent marker of coronary heart disease risk.<sup>4</sup> As a result of these data, we sought inhibitors of Lp-PLA<sub>2</sub> to assess the role of this lipase in atherosclerosis.

We have previously reported the identification of compound **1**, SB-435495, a very potent inhibitor of Lp-PLA<sub>2</sub> with a suitable profile for evaluation in man.<sup>5</sup> In view of the exceptional potency of this compound, we speculated that good activity would still be achievable in simplified (lower molecular weight) analogues. To this

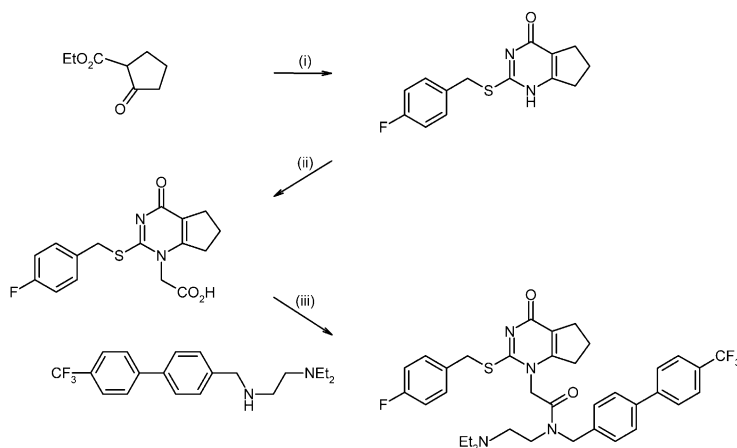
end, we decided to modify the pyrimidone 5-substituent and now report the beneficial effects of this study.



Compounds **2–7**, **10**, **11** and **14** were synthesised using methods similar to those previously described.<sup>6</sup> Halo derivatives **8** and **9** were prepared from 1,5-unsubstituted pyrimidones by reaction with the corresponding N-halosuccinimide and subsequent introduction of the 1-substituent as before.<sup>6</sup> Oxidation of **11** with meta-chloroperoxybenzoic acid gave **12**. Compound **13** was prepared via known methods.<sup>7</sup> Hydroxyethyl derivative **14** was converted into amine **15** via methanesulfonation, azide displacement and reduction (H<sub>2</sub>, Pd/C). Compounds **18–23** were prepared as exemplified for **21** in Scheme 1.

Compounds were evaluated using recombinant human Lp-PLA<sub>2</sub> (rhLp-PLA<sub>2</sub>).<sup>8</sup> Non-specific binding effects in plasma were assessed by evaluating compounds against the enzyme in both whole human and Watanabe hereditary

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**Scheme 1.** Reagents: (i) (a) thiourea, NaOEt, EtOH; (b) 4-F-PhCH<sub>2</sub>Cl, K<sub>2</sub>CO<sub>3</sub>, DMF; (ii) (a) *t*BuO<sub>2</sub>CCH<sub>2</sub>I, *i*Pr<sub>2</sub>NEt, CH<sub>2</sub>Cl<sub>2</sub>; (b) TFA, CH<sub>2</sub>Cl<sub>2</sub>; (iii) EDC, HOBT, DMF.

hyperlipidaemic (WHHL) rabbit plasma at a single concentration of inhibitor.<sup>8</sup> High potency in rabbit and human plasma, alongside little interaction with a battery of cytochrome P450 enzymes<sup>5</sup> and reasonable levels of permeability<sup>5</sup> were normally required before compounds were evaluated in vivo in WHHL rabbits,<sup>8</sup> and for selected inhibitors, in the rat and dog.<sup>9</sup>

Although removal of the entire 5-substituent in **1** resulted in a marked reduction in potency (Table 1, cf. **1** and **2**), sub-nanomolar inhibition could be regained on the introduction of a simple methyl, or more preferably ethyl, group (see compounds **1–6**). Increasing the chain length further (see **7** vs **6**) was not advantageous either in terms of potency or CYP450 interaction (see below).

Compounds bearing a number of other non-polar substituents typically proved highly potent inhibitors with only the 5-methoxy derivative **10** less active (cf. **8**, **9** and **11** with **10**). Of the more polar derivatives prepared (compounds **12–17**) none matched the potency of the 5-ethyl derivative **6** and, in contrast to the less polar analogues (**3–10**), none proved sufficiently permeable across artificial membranes.<sup>5</sup>

A simple methyl substituent when placed at the 6-position gave a compound of lower potency (Table 2, cf. **18** and **4**). However in contrast to our original work with compounds bearing a 5-heterocyclylmethyl substituent,<sup>11</sup> the introduction of a 6-methyl group into the 5-methyl series was not detrimental (see **19** and **4**), likely

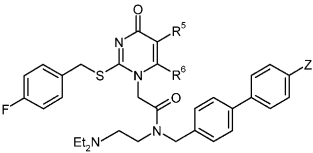
**Table 1.** 5-Substituent modification

No. <sup>a</sup>	Z	R <sup>5</sup>	% Inhibition in plasma			
			IC <sub>50</sub> nM	Human 10 nM	Rabbit 100 nM	Permeability cm/h <sup>b</sup>
1	CF <sub>3</sub>	CH <sub>2</sub> (1-Me-pyrazol-4-yl)	0.06	87	95	0.017
2	Cl	H	50	1	10	NT <sup>c</sup>
3	Cl	Me	5	10	41	0.10
4	CF <sub>3</sub>	Me	1	33	63	0.08
5	Cl	Et	1	24	48	0.12
6	CF <sub>3</sub>	Et	0.4	48	69	0.10
7	CF <sub>3</sub>	Pr	0.8	18	43	0.13
8	CF <sub>3</sub>	Cl	3	12	47	0.11
9	CF <sub>3</sub>	Br	1	29	60	0.09
10	CF <sub>3</sub>	OCH <sub>3</sub>	19	NT	NT	0.025
11	CF <sub>3</sub>	SCH <sub>3</sub>	0.8	22	45	0.03
12	CF <sub>3</sub>	SOCH <sub>3</sub>	0.8	NT	NT	0.006
13	CF <sub>3</sub>	CH <sub>2</sub> OH	1.5	18	56	0.002
14	CF <sub>3</sub>	CH <sub>2</sub> CH <sub>2</sub> OH	2	18	29	0.003
15	CF <sub>3</sub>	CH <sub>2</sub> CH <sub>2</sub> NH <sub>2</sub>	38	NT	NT	NT
16	CF <sub>3</sub>	CH <sub>2</sub> CH <sub>2</sub> NHAc	6	17	33	0.003
17	CF <sub>3</sub>	CH <sub>2</sub> CH <sub>2</sub> NHSO <sub>2</sub> CH <sub>3</sub>	1	NT	NT	0.005

<sup>a</sup>All new compounds gave satisfactory analytical/spectral data.<sup>10</sup>

<sup>b</sup>See ref 5. Permeability > 0.01 cm/h considered acceptable.

<sup>c</sup>Not tested.

**Table 2.** Pyrimidone-5,6-substitution


No. <sup>a</sup>	R <sup>5</sup>	R <sup>6</sup>	Z	Inhibition in plasma			
				IC <sub>50</sub> nM	Human 10 nM	Rabbit 100 nM	Permeability cm/h <sup>b</sup>
4	Me	H	CF <sub>3</sub>	1	33	63	0.08
18	H	Me	CF <sub>3</sub>	3	6	39	0.05
19	Me	Me	CF <sub>3</sub>	0.5	39	71	0.03
20	(CH <sub>2</sub> ) <sub>3</sub>	Cl		0.4	36	67	0.14
21	(CH <sub>2</sub> ) <sub>3</sub>	CF <sub>3</sub>		0.25	60	79	0.10
22	(CH <sub>2</sub> ) <sub>4</sub>	Cl		0.25	NT <sup>c</sup>	NT	NT
23	(CH <sub>2</sub> ) <sub>4</sub>	CF <sub>3</sub>		0.1	NT	NT	0.06

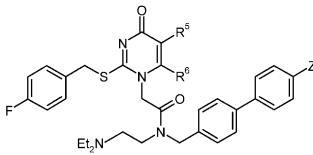
<sup>a</sup>All new compounds gave satisfactory analytical/spectral data.<sup>10</sup><sup>b</sup>See ref 5. Permeability > 0.01 cm/h considered acceptable.<sup>c</sup>Not tested.

due to the absence of any significant conformational effect in this series. Following this result, and in view of the good activity of compound **6**, we prepared cyclic analogues **20–23**. All of these compounds proved very potent inhibitors of rhLp-PLA<sub>2</sub> with the trifluoromethylbiphenyl derivatives again more potent than their 4-chloro analogues.<sup>5</sup> Furthermore, whilst compounds of Table 2 passed the permeability guidelines, cyclopentyl fused derivatives **20** and **21** proved particularly permeable.

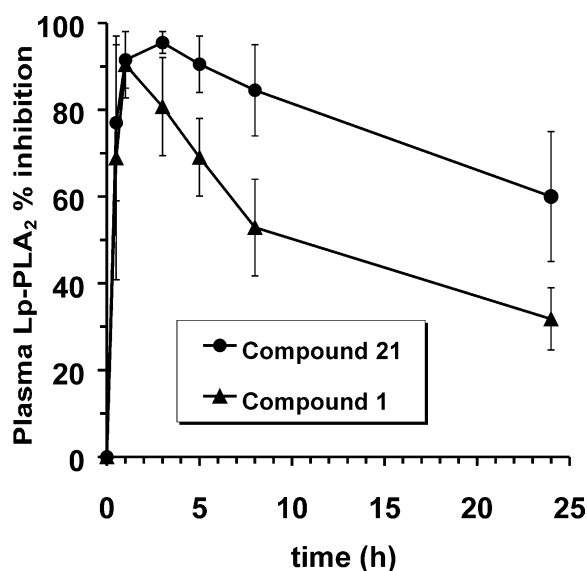
In contrast to our previous series,<sup>5</sup> the majority of compounds passed our criteria for CYP450 interaction (target IC<sub>50</sub> ≥ 10 μM) although the potent cyclohexyl fused pyrimidones **22** and **23** proved important exceptions to this finding (results against key isozymes 2D6 and 3A4 are shown for compounds of interest in Table 3).

With these data in hand, the most potent compounds in whole plasma (**6**, **19** and **21**) were progressed to the WHHL rabbit for evaluation and comparison with compound **1**. In this model, compounds **6** and **19** gave very similar profiles to **1** (data not shown), whilst cyclopentyl fused derivative **21** proved considerably more effective, showing prolonged inhibition of plasma Lp-PLA<sub>2</sub> over 24 h (Fig. 1) and a good correlation of pharmacodynamic and pharmacokinetic effects (Fig. 2). As a consequence of these findings, **21** was chosen for a more detailed evaluation.

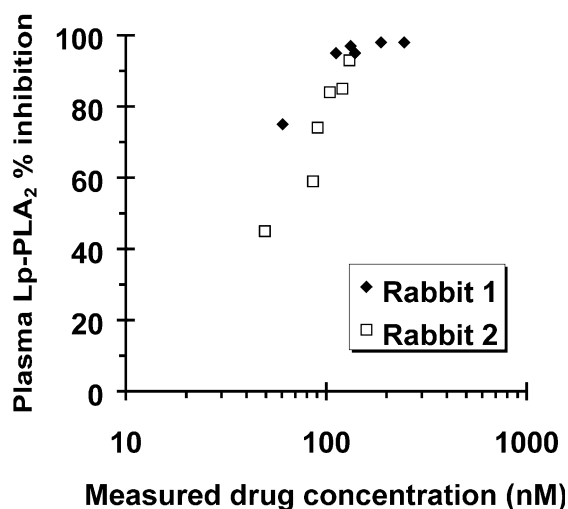
Mechanistic studies using steady state and transient kinetics indicated compound **21** to be a freely reversible, non-covalently bound, inhibitor of rhLp-PLA<sub>2</sub> with a K<sub>i</sub> of 110 pM and an off-rate of 27 min.<sup>5</sup> Potent inhibition of the enzyme in whole human plasma was confirmed (IC<sub>50</sub> = 5 ± 2 nM). Furthermore, the presence of compound **21** during the copper catalysed oxidation of human LDL prevented the production of lyso-PtdCho (IC<sub>50</sub> = 4 ± 3 nM) and subsequent monocyte chemotaxis (IC<sub>50</sub> = 4 ± 1 nM).<sup>5</sup>

**Table 3.** Interaction of selected compounds with CYP450 2D6 and 3A4


No.	R <sup>5</sup>	R <sup>6</sup>	Z	Lp-PLA <sub>2</sub> IC <sub>50</sub> nM	CYP450 2D6	IC <sub>50</sub> (μM) <sup>a</sup>
						3A4
1	<b>b</b>	H	CF <sub>3</sub>	0.06	37	10
3	Me	H	Cl	5	15	13
4	Me	H	CF <sub>3</sub>	1	39	17
5	Et	H	Cl	1	14	7
6	Et	H	CF <sub>3</sub>	0.4	24	11
7	Pr	H	CF <sub>3</sub>	0.8	22	5
8	Cl	H	CF <sub>3</sub>	3	83	29
9	Br	H	CF <sub>3</sub>	1	56	39
11	SMe	H	CF <sub>3</sub>	0.8	39	15
18	H	Me	CF <sub>3</sub>	3	47	22
19	Me	Me	CF <sub>3</sub>	0.5	24	22
20	(CH <sub>2</sub> ) <sub>3</sub>	Cl		0.4	25	18
21	(CH <sub>2</sub> ) <sub>3</sub>	CF <sub>3</sub>		0.25	26	27
22	(CH <sub>2</sub> ) <sub>4</sub>	Cl		0.25	6	5
23	(CH <sub>2</sub> ) <sub>4</sub>	CF <sub>3</sub>		0.1	9	10

<sup>a</sup>See ref 5, target IC<sub>50</sub> ≥ 10 μM.<sup>b</sup>CH<sub>2</sub>(1-Me-pyrazol-4-yl).**Figure 1.** Inhibition of plasma Lp-PLA<sub>2</sub> in the WHHL rabbit @ 10 mg/kg (*n* = 2) po.

Additional in vivo studies with **21** indicated an oral bioavailability of 11 ± 2% in the fed rat. This was a marked improvement over compound **1** for which further studies had shown that rat bioavailability fell from 13 ± 1% in the fasted state to 2 ± 1% in the fed animal. The fed:fasted ratio for compound **21** was 1:1.9 ± 0.4, again a considerable advance over **1** and consistent with an oral bioavailability of ~21% in the fasted rat.<sup>9</sup> The oral bioavailability of **21** was 28 ± 4% in the dog (cf. 24 ± 7% for **1**).<sup>9</sup> Furthermore excellent inhibition of Lp-PLA<sub>2</sub> within the atherosclerotic plaque was achieved for **21**, with 95 ± 1% inhibition observed 2 h after an oral dose of 30 mg/kg to the WHHL rabbit.<sup>12</sup>



**Figure 2.** Pharmacodynamic/pharmacokinetic correlation in the WHHL rabbit: Compound 21 @ 10 mg/kg ( $n=2$ ) po.

As a result of the excellent in vitro effects of **21** alongside both an enhanced in vivo profile and shorter route of synthesis versus SB-435495, compound **21**, SB-480848, was selected to replace SB-435495 for our evaluation of the effects of Lp-PLA<sub>2</sub> inhibition in man. The encouraging initial human studies with SB-480848 will be published shortly.

In conclusion, we have shown that highly potent inhibitors of Lp-PLA<sub>2</sub> may be obtained on simplification of the pyrimidone 5-substituent in compound **1**. One of these simplified inhibitors, compound **21**, SB-480848, demonstrated excellent in vitro and in vivo profiles and has been chosen for progression to man. Our assessment of Lp-PLA<sub>2</sub> as a therapeutic target will undoubtedly be enhanced by further studies with SB-480848.

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- Oral bioavailability was determined for the free base of **21** as described in ref 5, except that the iv infusion was in normal saline containing 10% (w/v) Encapsin and 2% (v/v) DMSO and the oral dose by gavage administration in 1% aq methylcellulose (rat) or by loose filled gelatine capsule (dog). The fed:fasted ratio in the rat was measured by oral gavage administration (10 mg/kg in 1% Tween 80 and 1% aq methylcellulose) to the conscious, cannulated fed or fasted male rat. Serial blood samples were collected up to 14 h post dose and analysed by LC/MS/MS. The fed:fasted ratio was calculated as AUC (fed)/AUC (fasted).
- Representative examples: compound **6** bitartrate salt <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, rotamer mixture)  $\delta$  0.96 (3H, m), 1.07 (6H, m), 2.27 (2H, m), 2.59 (2H, m), 2.84 (2H, m), 3.37/3.50 (4H, m), 4.26 (2H, s), 4.39/4.43 (2H, 2 $\times$ s), 4.64/4.72 (2H, 2 $\times$ s), 4.94/5.09 (2H, 2 $\times$ s), 7.11/7.14 (2H, 2 $\times$ m), 7.36–7.49 (5H, m), 7.63/7.72 (2H, 2 $\times$ d), 7.84 (4H, m); MS (APCI+) found (M+1)=655; C<sub>35</sub>H<sub>38</sub>F<sub>4</sub>N<sub>4</sub>O<sub>2</sub>S requires 654. Compound **21** free base <sup>1</sup>H NMR (CDCl<sub>3</sub>, rotamer mixture)  $\delta$  0.99 (6H, t), 2.10 (2H, m), 2.50 (4H, q), 2.58/2.62 (2H, 2 $\times$ t), 2.70/2.82 (2H, 2 $\times$ t), 2.86 (2H, t), 3.28/3.58 (2H, 2 $\times$ t), 4.45/4.52 (2H, 2 $\times$ s), 4.68/4.70 (2H, 2 $\times$ s), 4.93 (2H, s), 6.95 (2H, m), 7.31 (2H, d), 7.31/7.37 (2H, 2 $\times$ m), 7.48/7.52 (2H, d), 7.65 (2H, m), 7.72 (2H, m); MS (APCI+) found (M+1)=667; C<sub>36</sub>H<sub>38</sub>F<sub>4</sub>N<sub>4</sub>O<sub>2</sub>S requires 666.
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- Determined as in ref 5, data from 15 month old female WHHL rabbits. Compound **1** gave an inhibition of 74 $\pm$ 9% in sex and age (14 months) matched WHHL rabbits.