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The Identification of a Potent, Water Soluble Inhibitor of Lipoprotein-Associated Phospholipase A_2

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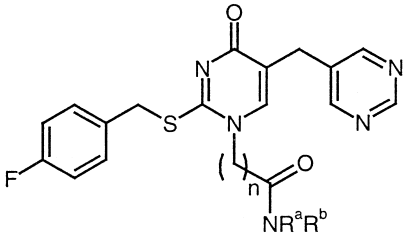
Abstract—Modification of the pyrimidone 5-substituent in a series of 1-((amidolinked)-alkyl)-pyrimidones, lipophilic inhibitors of lipoprotein-associated phospholipase A_2, has given inhibitors of nanomolar potency and improved physicochemical properties. Compound **23** was identified as a potent, highly water soluble, CNS penetrant inhibitor suitable for intravenous administration.

Current therapy for atherosclerosis is broadly based on the regulation of plasma lipid levels, particularly LDL cholesterol. The statins, although only really effective in around 30% of patients, have achieved medical and commercial success in this role.[1] Therapies which directly influence atherosclerotic plaque formation and stability are less well precedented and represent an exciting new opportunity to treat many more of the at risk population. To this end, we have focused our attention on a novel serine dependant lipase—lipoprotein-associated phospholipase A_2 (Lp-PLA_2)[2]—that is able to hydrolyse oxidatively modified phosphatidylcholines to release oxidised fatty acids and lysophosphatidylcholine (lyso-PC). Both of these hydrolysis products are known to be pro-inflammatory and have been implicated in atherosclerosis.[3] Furthermore, a recent study has shown a strong, positive correlation between Lp-PLA_2 levels and coronary events in asymptomatic, hypercholesterolemic men and suggested that Lp-PLA_2 is a new, independent marker of coronary heart disease risk.[4] The identification of inhibitors of Lp-PLA_2 would then aid our evaluation of the role of this enzyme in atherosclerosis and additionally, could also be of potential value in the treatment of other inflammatory vascular diseases involving oxidative stress (e.g., stroke).

Recently[5] we described the identification of a series of 1-((amidolinked)-alkyl)-pyrimidones **1**, as highly potent inhibitors of Lp-PLA_2 which showed activity in the Watanabe hereditable hypolipodaemic rabbit (WHHL rabbit). Those inhibitors were however rather lipophilic and, as a result, poorly water soluble. In this communication we describe our studies towards the identification of less lipophilic inhibitors via modification of the pyrimidone 5-substituent.



1 n = 1 or 3

R^a = long chain alkyl, R^b = H, Me

Compounds **3** were prepared via the acylisothiocyanate **2** in an analogous manner to that previously described (Scheme 1).[5] *O*-Demethylation of **3** was achieved with B-bromocatecholborane[6] to give compound **4** in high yield. Other dealkylation conditions proved less effective—boron tribromide for example gave a less clean product that was difficult to purify. *N*-Alkylation with ethyl bromoacetate and hydrolysis gave acid **5** (Y = OH) which was subsequently converted to the amide with EDC/HOBT. Other *N*-alkylations of compound **4** were performed similarly. All compounds in Tables 1 and 2 were evaluated using human Lp-PLA_2 (hLp-PLA_2).

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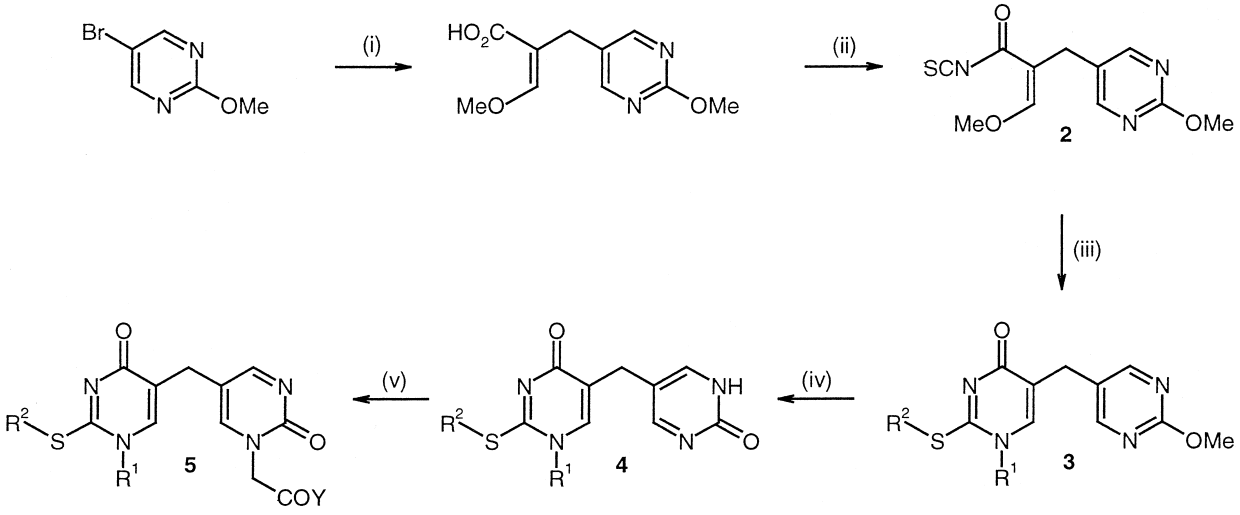
Assays were performed in duplicate.[7] In order to factor in any non-specific binding effects in plasma, compounds were also assessed against the plasma enzyme in both whole human and WHHL rabbit plasma at a single concentration of inhibitor.[5] Good activity in rabbit and human plasma was required before compounds were evaluated in vivo in either WHHL rabbits[5] or in the rat.[8,9]

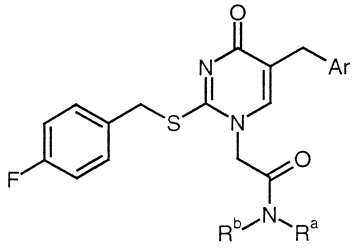
We quickly focused our attention (Table 1) on tertiary acetamides, as their secondary counterparts (e.g., **6** and **8**) proved very insoluble and as a result, difficult to evaluate fully. Two *N*-1 substituents were chosen: (*N*-methyl-*N*-octylcarbonyl)methyl and the more lipophilic, but often more potent, (*N*-dodecyl-*N*-methylcarbonyl)methyl group. Activity versus hLp-PLA_2 was retained or enhanced on substitution of the (pyrimidin-5-yl)methyl group at the pyrimidine 2-position. It is interesting to note that both lipophilic and more hydrophilic substituents are well tolerated (cf. **7** vs **10**–**15** and **9** vs **16**–**18**).

As a result of the encouraging activity in whole plasma for the more polar 2-oxopyrimidines **13**, **15**, **17** and **18**, we decided to investigate substitution of the 2-oxopyrimidine ring with more polar substituents (Table 2). Very encouragingly, high potency versus hLp-PLA_2 was



Scheme 1. Reagents: (i) (a) $CH_2{=}CHCO_2Et$, $Pd(OAc)_2$, P(*o*-Tol)$_3$, Et_3N, (b) H_2, Pd/C, EtOH/Et_3N, (c) HCO_2Et, NaH, DME, (d) Me_2SO_4, K_2CO_3, DMF, (e) NaOH, H_2O; (ii) (a) $(COCl)_2$, $ClCH_2CH_2Cl$, (b) KSCN, CH_3CN; (iii) (a) R^1NH_2, DMF then NaOEt, (b) R^2Cl/Br, iPr_2NEt, CH_2Cl_2; (iv) B-bromocatecholborane, CH_2Cl_2; (v) (a) $BrCH_2CO_2Et$, K_2CO_3, DMF, (b) NaOH, H_2O/dioxan, (c) R^3R^4NH, EDC, HOBT, CH_2Cl_2.

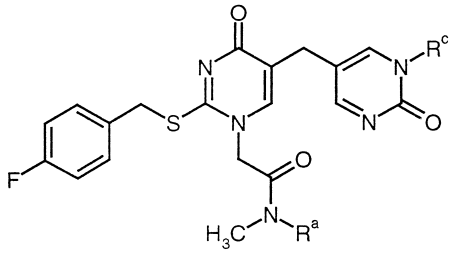
Table 1. Pyrimidone 5-substituent variation



No.[a]	R^a	R^b	Ar	IC_{50} nM	Inhibition in plasma (%) @ 100 nM	
					Human	Rabbit
6	*n*-C_8H_{17}	H	Pyrimidin-5-yl	7	43	38
7	*n*-C_8H_{17}	Me	Pyrimidin-5-yl	15	40	30
8	*n*-$C_{12}H_{25}$	H	Pyrimidin-5-yl	0.3	86	54
9	*n*-$C_{12}H_{25}$	Me	Pyrimidin-5-yl	1	79	39
10	*n*-C_8H_{17}	Me	2-MeO-pyrimidin-5-yl	4	39	13
11	*n*-C_8H_{17}	Me	2-EtO-pyrimidin-5-yl	3	23	7
12	*n*-C_8H_{17}	Me	2-$PhCH_2O$-pyrimidin-5-yl	7	10	8
13	*n*-C_8H_{17}	Me	2-Oxo-pyrimidin-5-yl	4	66	34
14	*n*-C_8H_{17}	Me	1-Me-2-oxo-pyrimidin-5-yl	27	55	19
15	*n*-C_8H_{17}	Me	1-Et-2-oxo-pyrimidin-5-yl	20	48	5
16	*n*-$C_{12}H_{25}$	Me	2-MeO-pyrimidin-5-yl	1	81	29
17	*n*-$C_{12}H_{25}$	Me	2-Oxo-pyrimidin-5-yl	0.9	83	39
18	*n*-$C_{12}H_{25}$	Me	1-Me-2-oxo-pyrimidin-5-yl	5	75	32

[a]All new compounds gave satisfactory analytical/spectral data.[10]

Table 2. Effect of substitution in C-5 pyrimidone ring



No.[a]	R^a	R^c	IC_{50} (nM)	Inhibition in plasma (%) @ 100 nM	
				Human	Rabbit
13	*n*-C_8H_{17}	H	4	66	34
19	*n*-C_8H_{17}	CH_2CO_2Et	75	NT	NT
20	*n*-C_8H_{17}	CH_2CO_2H	14	56	27
21	*n*-C_8H_{17}	CH_2CH_2OH	5	59	36
17	*n*-$C_{12}H_{25}$	H	0.9	83	39
22	*n*-$C_{12}H_{25}$	CH_2CO_2Et	4	55	15
23	*n*-$C_{12}H_{25}$	CH_2CO_2H	1	74	28
24	*n*-$C_{12}H_{25}$	CH_2CH_2OH	1	77	36
25	*n*-C_8H_{17}	$CH_2CONHMe$	6	63	42
26	*n*-C_8H_{17}	$CH_2CONHPr$	11	42	16
27	*n*-C_8H_{17}	$CH_2CONHCH_2CH_2OH$	8	68	40
28	*n*-C_8H_{17}	CH_2CO(morpholin-4-yl)	10	64	36
29	*n*-C_8H_{17}	CH_2CO(1-Me-piperazin-4-yl)	3	68	40
30	*n*-C_8H_{17}	CH_2CO(2-oxo-piperazin-4-yl)	2	80	55
31	*n*-C_8H_{17}	CH_2CO(1-acetyl-piperazin-4-yl)	5	80	49
32	*n*-$C_{12}H_{25}$	$CH_2CONHMe$	0.4	80	28
33	*n*-$C_{12}H_{25}$	$CH_2CONHCH_2CH_2OH$	0.6	79	37
34	*n*-$C_{12}H_{25}$	CH_2CO(morpholin-4-yl)	0.7	80	30
35	*n*-$C_{12}H_{25}$	CH_2CO(2-oxo-piperazin-4-yl)	0.4	86	49

[a]All new compounds gave satisfactory analytical/spectral data.[10]

maintained on the introduction of a wide range of substituents. Indeed, acidic, polar neutral and basic groups were all well tolerated, suggesting that the substituent may be directed away from the enzyme and towards the surrounding aqueous environment. As has been previously observed, potency is higher in human than rabbit plasma suggesting that compounds may be more potent in man than in the rabbit.

Selected compounds from Tables 1 and 2 (**16**, **20**, **23** and **34**) were also screened against the most closely related phospholipase A_2 (human serine dependent-PLA_2).[11] Encouragingly, all compounds tested showed >1000-fold selectivity for Lp-PLA_2 over this lipase.

Whilst a number of compounds in Table 2 showed some improvement in solubility over the parent pyrimidines (**7** and **9**), our attention quickly focused on the acetic acid derivatives **20** and **23** whose potency was matched by high solubility (>15 mg/mL) in normal saline at pH 7.4. Based on these data, it was decided to evaluate compounds **20** and **23** in vivo in order to assess oral availability, their use as intravenous agents and, with a view to assessing the role of Lp-PLA_2 in centrally mediated inflammatory vascular disease (e.g., stroke), CNS penetration.

Initial results in both the rat and WHHL rabbit indicated a rather rapid clearance following intravenous administration (e.g., **23**: rat CLb = 76 mL/min/kg, ~85% liver blood flow)[8] and as a result little oral activity/systemic exposure. We postulated that this profile, alongside the ease of formulating **20** and **23** for intravenous dosing, would give these acids the correct characteristics as drugs to be administered by infusion dosing—good control of enzyme inhibition should be achieved as levels of inhibitor would fall rapidly after infusion is terminated. As a result, we proceeded to an infusion study that also included measurement of CNS penetration in the rat.[9] Compounds **9**, **13**, **25** and **34**, although more difficult to formulate, were included in this study for comparison. We were very pleased to show that, in contrast to the less soluble analogues, both **20** and particularly **23** proved to be CNS penetrant (Table 3). Furthermore, sampling during the 6–8 h time period of the infusion, indicated that good blood levels of **23** had been achieved (steady-state attained) alongside a high level of inhibition of rat Lp-PLA_2—a dose of 2 μmol/kg/h gave a blood concentration of 533 ± 50 nM and 83% inhibition of rat Lp-PLA_2.

Table 3. CNS penetration results

No.	CNS penetration (%) (*n*)
9	< 2 (3)
13	7 (1)
20	10 ± 1 (3)
23	37 ± 8 (3)
25	< 7 (1)
34	< 7 (1)

In conclusion, we have shown that high potency is retained on modification of the (pyrimidin-5-yl)methyl group present in our previously described inhibitors. Two of these new inhibitors, acetic acid derivatives **20** and **23**, exhibit excellent water solubility at physiological pH. Compound **23** shows an admirable profile following infusion dosing, including CNS penetration, and will be of great value in evaluating the role of Lp-PLA_2 in atherosclerosis and other inflammatory vascular diseases involving oxidative stress (e.g., stroke) in situations that require administration by this route.

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8. Oral bioavailability and clearance parameters were determined (e.g., for the sodium salt of **23**) by non-compartmental pharmacokinetic analysis following iv infusion over 1 h (in saline 0.9% w/v) at a target dose of 1.6 μmol/kg/h and oral gavage administration (3 μmol/kg in distilled water) in the conscious, cannulated male rat. Serial blood samples were collected over 10 h post dose and analysed by LC/MS/MS.
9. CNS penetration at steady-state was investigated in the rat. Compounds were dissolved in 2% (v/v) DMSO, 2% ethanol and 10% Encapsin™ in saline and administered at a constant rate infusion over 8 h at a target dose of 2 μmol/kg/h. Blood samples were removed over the last 2 h of the infusion to confirm steady-state concentrations. Blood and brain samples were analysed by LC/MS/MS. For compound **23**, inhibition of Lp-PLA_2 was determined by a method similar to that described in ref 5.
10. Representative examples: Compound **10** ^{1}H NMR ($CDCl_3$) δ 0.8–0.95 (3H, m), 1.1–1.7 (12H, m), 2.95 and 2.99 (3H, 2×s), 3.21 and 3.36 (2H, 2×t), 3.66 (2H, s), 3.99 (3H, s), 4.48 (2H, s), 4.51 and 4.55 (2H, d), 6.80 (1H, s), 6.9–7.1 (2H, m), 7.3–7.45 (2H, m), 8.45 (2H, s); MS (APCI+) found (M+1) = 542; $C_{28}H_{36}FN_5O_3S$ requires 541. Compound **23** (250 MHz) ^{1}H NMR (DMSO-d_6) δ 0.85 (3H, t), 1.22 (18H, m), 1.35–1.61 (2H, m), 2.78, 2.95 (3H, 2×s), 3.20–3.35 (2H, m), 3.58 (2H, s), 4.40 (2H, s), 4.55 (2H, s), 4.83 (2H, m), 7.10 (2H, m), 7.44 (2H, m), 7.54 and 7.57 (1H, 2×s), 8.03 (1H, m), 8.54 (1H, m) 13.10 (1H, br. s); MS (APCI+) found (M+1) = 642, $C_{33}H_{44}FN_5O_5S$ requires 641.
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