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1-(Arylpiperazinylamidoalkyl)-pyrimidones: Orally Active Inhibitors of Lipoprotein-Associated Phospholipase A_2

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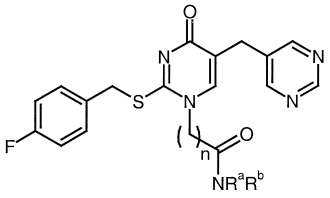
Abstract—The lipophilic 1-substituent in a series of 1-((amidolinked)-alkyl)-pyrimidones, inhibitors of recombinant lipoprotein-associated phospholipase A_2, has been modified to give inhibitors of high potency in human plasma and enhanced physicochemical properties. Phenylpiperazineacetamide derivative **23** shows very promising oral activity.

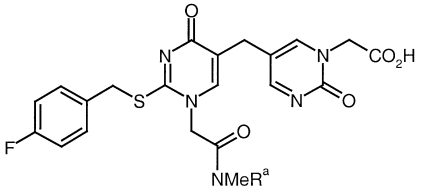
Recent evidence suggests that atherosclerosis is an inflammatory disease.[1] Treatments which influence this inflammation and as a result, atherosclerotic plaque stability,[1] represent an exciting new opportunity to treat many more of the at risk population than is possible with current therapy.[2] To this end, we have focused our attention on the inhibition of the enzyme, lipoprotein-associated phospholipase A_2 (Lp-PLA_2), which is able to release pro-inflammatory mediators, oxidised fatty acids and lysophosphatidylcholine, from oxidatively modified phosphatidylcholines.[3] Our interest in this enzyme has been reinforced by recent clinical data that indicates 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 would also be of potential value in the treatment of other inflammatory vascular diseases involving oxidative stress.

In a recent letter,[5] we reported 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 hyperlipidaemic rabbit (WHHL rabbit). Those compounds were



1 n = 1 or 3

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



2 R^a = n-C_8H_{17} , n-$C_{12}H_{25}$

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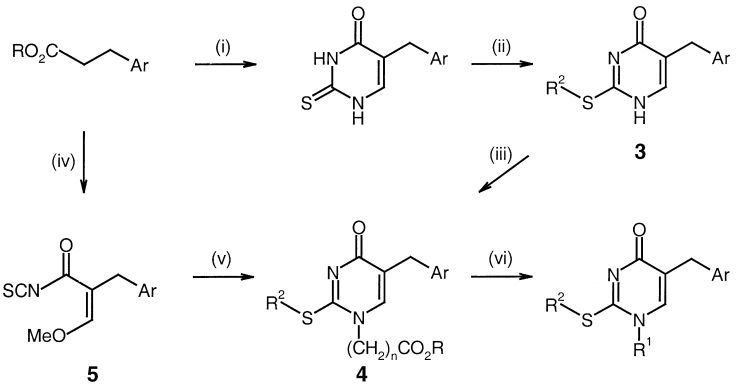
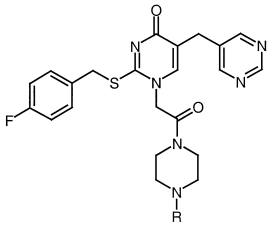


Scheme 1. Reagents: (i) HCO_2Et, KO*t*Bu, THF/Et_2O then thiourea/*i*PrOH; (ii) R^2Cl/Br, K_2CO_3, DMF; (iii) RO_2CCH_2Br/I, iPr_2NEt, CH_2Cl_2; (iv) (a) HCO_2Et, NaH, DME, (b) Me_2SO_4, K_2CO_3, DMF, (c) NaOH, H_2O, (d) $(COCl)_2$, $ClCH_2CH_2Cl$, (e) KSCN, CH_3CN; (v) (a) $RO_2C(CH_2)_3NH_2$, DMF then NaOEt, (b) R^2Cl/Br, iPr_2NEt, CH_2Cl_2; (vi) (a) NaOH, dioxan/H_2O or TFA/CH_2Cl_2 (R = *t*Bu), (b) 1-arylpiperazine, EDC, HOBT, CH_2Cl_2. For subsequent transformations within Ar (Ar = 2-oxo-pyrimidin-5-yl and derivatives), see ref 6.

however rather lipophilic and, as a result, we sought inhibitors with improved physicochemical properties. To this end, modification of the pyrimidone 5-substituent led to the very potent, water soluble inhibitors **2**, which proved highly suitable for *intravenous* administration.[6] We next turned our attention to the identification of alternatives to the lipophilic pyrimidone N-1 substituent and now describe our studies to identify less lipophilic, orally active inhibitors through replacement of this group with a series of piperazine amides.

Acetic acid derivatives **4** ($n = 1$) were obtained via a new procedure involving direct pyrimidone N-1 alkylation of **3** using bromo- or iodoacetate in dichloromethane with diisopropylethylamine as base (Scheme 1). A wide range of other alkylation conditions gave predominantly N-3 substituted pyrimidones.[5] Introduction of the butyric acid side chain was not possible with this method, so compounds bearing this group were prepared via the acylisothiocyanate **5** in an analogous manner to that previously described (Scheme 1).[5] The synthesis of compounds bearing a 5-(2-oxo-pyrimidinylmethyl) group was completed as detailed previously.[6] Other derivatives were prepared via the corresponding 3-(2-substituted-pyrimidin-5-yl)propionic ester or 3-(1-methyl-pyrazol-4-yl)propionic ester intermediates in a similar fashion. All compounds in Tables 1–4 were evaluated using human Lp-PLA_2 (hLp-PLA_2). Assays were performed in duplicate.[5] In order to factor in any non-specific binding effects in plasma, compounds were

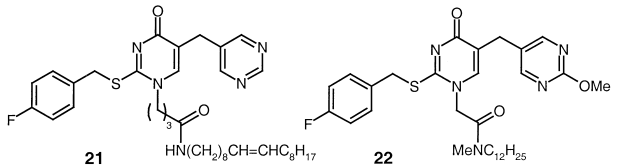
Table 1. Piperazine 4-substituent variation



No.[a]	R	IC_{50} (nM)	clogP[b]	% Inhibition in plasma @ 100 nM	
				Human	Rabbit
6	Phenyl	120	1.7	45	19
7	4-Methoxyphenyl	176	1.8	NT[c]	NT
8	4-Acetylphenyl	130	1.5	66	28
9	4-Fluorophenyl	97	2.1	54	33
10	2-Chlorophenyl	39	2.6	38	9
11	3-Chlorophenyl	66	2.6	54	19
12	4-Chlorophenyl	26	2.6	73	37
13	4-Trifluoromethylphenyl	26	2.9	72	40
14	H	460	0.8	NT	NT
15	Benzyl	85	3.1	45	13
16	4-Chlorobenzyl	140	3.8	NT	NT
17	Benzoyl	1280	2.0	NT	NT
18	Phenylsulfonyl	260	2.5	NT	NT
19	Pyridin-2-yl	350	0.8	NT	NT
20	Pyrimidin-5-yl	410	0.0	NT	NT

[a]All new compounds gave satisfactory analytical/spectral data.[8]
[b]See ref 9.
[c]Not tested.

Table 2. Profiles of previous key inhibitors



No.	IC_{50} (nM)	ClogP[a]	% Inhibition in plasma @ 100 nM	
			Human	Rabbit
21 (Z)	0.4	9.2	85	59
22	1	7.4	81	29

[a]See ref 9.

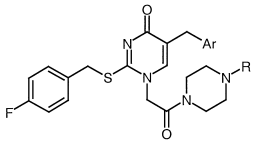
also assessed against the plasma enzyme in both whole human and WHHL rabbit plasma at a single concentration of inhibitor.[5] Good activity in rabbit and human plasma was required before compounds were evaluated for metabolic stability in pooled human and rat liver microsomes[7] and subsequently in vivo in WHHL rabbits.[5]

We initially investigated the effect of substitution of the aryl ring, modification of this ring and the introduction of a linker between the piperazine and aryl rings (Table 1). Within the phenyl piperazine series, the most potent compounds against the isolated enzyme contained a lipophilic electron withdrawing substituent at the 4-position (see **6**–**13**). Activity in whole plasma appeared a balance of potency versus isolated Lp-PLA_2 and lipophilicity (cf. **8** and **12**, see also below). From these initial results, it also appeared that phenyl was preferred over some more polar heterocycles (cf. **6** with **19** and **20**) and that direct substitution of this phenyl ring onto the piperazine was optimal (cf. **6** and **12** with **16**–**18**). Although compound **12** was less potent against isolated Lp-PLA_2 than our previous lead inhibitors (Table 2), we were very encouraged that activity in whole plasma was only marginally lower for this much more 'drug-like' molecule (cf. clogP of **12** versus **21** and **22**) and as a result we decided to evaluate this series more thoroughly.

The effect of modifying the pyrimidinone 5-substituent (particularly the introduction of a pyrimidine 2-substituent) alongside some further changes to the piperazine substituent is detailed in Table 3. Once again, results from the inhibition of Lp-PLA_2 in whole plasma indicated that a balance exists between potency against the isolated enzyme and lipophilicity, suggesting a reduced level of non-specific binding in plasma for the less lipophilic compounds (cf. **23** with **33**, also **32** and **34**). Of particular note in this respect were the two 2-oxo-pyrimidine derivatives **30** and **31** which, although considerably less potent than, for example **23** and **28**, show similar profiles in whole plasma. Overall, the most interesting compounds contained a 2-methoxy, 2-methyl-, 2-cyclopropyl-, 2-(4-morpholino)- or a 2-oxo-pyrimidine substituent (**23**–**25**, **28** and **30**) or a 1-methyl-pyrazol-4-yl group **32**.

Further indications that a 4-chlorophenylpiperazinyl group gave the best profile came from compounds **35**–**37** (Table 3), **38** and **39**. A 4-bromophenylpiperazinyl group gave no advantage over the corresponding 4-chloro derivative (cf. **35** and **32**). Replacement of the halophenyl ring with an aliphatic/alicyclic group was not advantageous—the cyclohexyl analogue **36** proved weakly active against the isolated enzyme and although, as expected, potency could be regained by the introduction of a suitably long aliphatic group, this was not

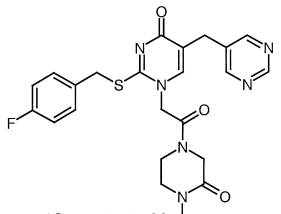
Table 3. Effect of varying pyrimidone 5-substituent



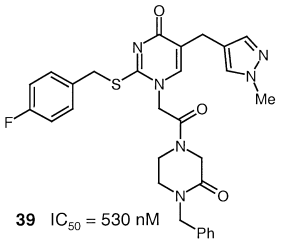
No.[a]	R	Ar	IC_{50} (nM)	ClogP[b]	% Inhibition in plasma @ 100 nM	
					Human	Rabbit
23	4-Chlorophenyl	2-Methoxy-pyrimidin-5-yl	20	3.6	77	33
24	4-Chlorophenyl	2-Methyl-pyrimidin-5-yl	18	3.1	75	35
25	4-Chlorophenyl	2-*c*-Propyl-pyrimidin-5-yl	21	3.6	70	22
26	4-Chlorophenyl	2-*t*-Butyl-pyrimidin-5-yl	34	4.5	44	4
27	4-Chlorophenyl	2-Trifluoromethyl-pyrimidin-5-yl	69	3.6	25	6
28	4-Chlorophenyl	2-(4-Morpholino)-pyrimidin-5-yl	10	3.2	70	26
29	4-Chlorophenyl	2-Dimethylamino-pyrimidin-5-yl	29	3.5	65	13
30	4-Chlorophenyl	1-Methyl-2-oxo-pyrimidin-5-yl	70	1.8	75	34
31	4-Chlorophenyl	1-Carboxymethyl-2-oxo-pyrimidin-5-yl	89	1.9	73	32
32	4-Chlorophenyl	1-Methyl-pyrazol-4yl	44	3.0	68	23
33	3,4-Chlorophenyl	2-Methoxy-pyrimidin-5-yl	8	4.2	67	22
34	3,4-Chlorophenyl	1-Methyl-pyrazol-4yl	12	3.6	51	16
35	4-Bromophenyl	1-Methyl-pyrazol-4yl	51	3.1	59	26
36	*c*-Hexyl	1-Methyl-pyrazol-4yl	720	3.5	NT[c]	NT
37	*n*-Octyl	1-Methyl-pyrazol-4yl	24	5.6	10	17

[a]All new compounds gave satisfactory analytical/spectral data.[8]
[b]See ref 9.
[c]Not tested.

maintained in whole plasma (see **37**). Inhibitors bearing an oxopiperazinyl group showed little activity (**38** and **39**).



38 IC_{50} = 6700 nM



39 IC_{50} = 530 nM

In addition to the above pyrimidone acetamide derivatives, we also prepared a number of pyrimidone butyramides (Table 4). Whilst compounds were often considerably more potent against isolated Lp-PLA_2, inhibition in whole plasma was rather disappointing (cf. **40** and **23**, **41** and **33**, **43** and **32**), suggesting a higher degree of non-specific binding in the butyramide series.

With these results to hand, selected compounds were then assessed for their metabolic stability in rat and human liver microsomes (Table 5). Most encouragingly, methoxypyrimidine derivative **23** showed a low rate of intrinsic clearance in both species. In stark contrast was the high rate of clearance in human liver microsomes for the morpholinopyrimidine **28** and butyramide **40** which were not considered further. The most promising compounds from Table 5 were then evaluated in WHHL rabbits at 10 mg/kg po.[5] Compound **23** proved the most interesting with inhibition seen for 5 h (Fig. 1). This encouraging profile alongside a much greater activity in human rather than in rabbit plasma would suggest that compound **23** has the potential for potent inhibition of Lp-PLA_2 in man.

In conclusion, we have shown that the long lipophilic chain present in our previously described orally active inhibitors may be replaced by a chlorophenylpiperazine moiety. Whilst potency against isolated Lp-PLA_2 was reduced to some extent with these new inhibitors, inhibition in whole human plasma could be maintained (cf. **23** vs **21**). Compound **23** shows much improved developability properties over our previously described orally active leads (e.g., **21**) and as such will be of great value in our evaluation of Lp-PLA_2 as a target for therapeutic intervention in both atherosclerosis and other inflammatory vascular diseases involving oxidative stress.

Table 5. Intrinsic clearance of selected derivatives in liver microsomes

No.	CLi (mL/min/g liver)	
	Rat	Human
23	2	3
24	3	7
28	13	> 50
30	3	4
33	2	7
34	6	11
40	19	> 50

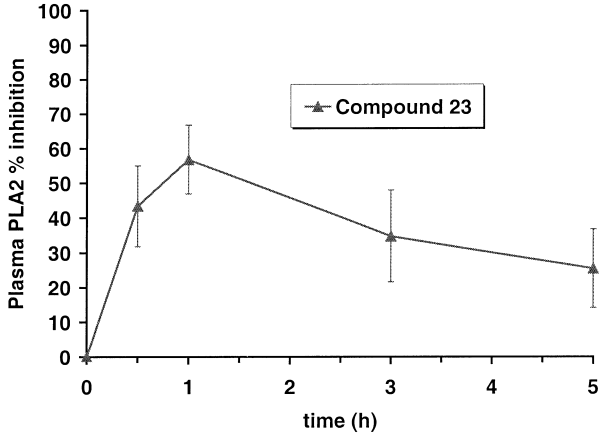
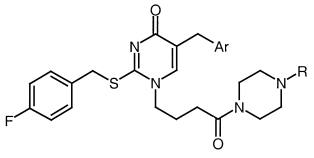


Figure 1. Inhibition of plasma Lp-PLA_2 in the WHHL rabbit @ 10 mg/kg ($n = 2$).

Table 4. Pyrimidone 1-butyramides



No.[a]	R	Ar	IC_{50} (nM)	ClogP[b]	% Inhibition in plasma @ 100 nM	
					Human	Rabbit
40	4-Chlorophenyl	2-Methoxy-pyrimidin-5-yl	8	3.8	43	34
41	3,4-Chlorophenyl	2-Methoxy-pyrimidin-5-yl	2	4.5	22	20
42	4-Trifluoromethylphenyl	2-Methoxy-pyrimidin-5-yl	2	4.1	29	42
43	4-Chlorophenyl	1-Methyl-pyrazol-4yl	3	3.2	21	24
44	4-Trifluoromethylphenyl	1-Methyl-pyrazol-4yl	5	3.5	10	41
45	3,4-Chlorophenyl	1-Methyl-2-oxo-pyrimidin-5-yl	9	2.7	17	30
46	3,4-Chlorophenyl	1-Carboxymethyl-2-oxo-pyrimidin-5-yl	9	2.8	38	39

[a]All new compounds gave satisfactory analytical/spectral data.[8]
[b]See ref 9.

References and Notes

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7. Intrinsic clearance values (CLi) were determined by quantifying the disappearance of compound following incubation with hepatic microsomes. Compound (final concentration 0.5 μM in 2% v/v DMSO) was incubated with rat or human hepatic microsomes (final concentration 0.5 mg protein/mL) and 50 mM phosphate buffer, pH 7.4 at 37 °C. Oxidative metabolism was initiated by addition of NADPH regenerating system [final concentration 0.5 mM NADP, 5 mM glucose-6-phosphate and 1 U/mL glucose-6-phosphate dehydrogenase in 2% (w/v) sodium hydrogen carbonate]. Aliquots were removed from the incubation mix at intervals of 3 min over a 30 min time period and added to internal standard in acetonitrile. Samples were analysed for parent compound using LC/MS/MS and the data fitted to a single exponential decay equation using Grafit (Erithacus Software). Intrinsic clearance values (mL/min/g liver) were calculated assuming a microsomal yield of 52.5 mg protein/g liver.
8. Representative examples: Compound **23** (250 MHz) ^{1}H NMR ($CDCl_3$) δ 3.07–3.28 (4H, br.t), 3.51–3.62 (2H, br.t), 3.65 (2H, s), 3.70–3.85 (2H, br.t), 3.98 (3H, s), 4.48 (2H, s), 4.61 (2H, s), 6.75–6.90 (3H, m), 6.91–7.05 (2H, t), 7.17–7.42 (4H, m), 8.42 (2H, s); MS (APCI−) found (M−1) = 593, $C_{29}H_{28}ClFN_6O_3S$ requires 594. Compound **40** (250 MHz) ^{1}H NMR ($CDCl_3$) δ 1.95–2.15 (2H, m), 2.30–2.45 (2H, t), 3.00–3.20 (4H, m), 3.47–3.59 (2H, t), 3.67 (2H, s), 3.69–3.8 (2H, t), 3.84–3.96 (2H, t), 3.99 (3H, s), 4.47 (2H, s), 6.75–6.90 (2H, d), 6.91–7.05 (2H, t), 7.10 (1H, s), 7.19–7.43 (4H, m), 8.48 (2H, s); MS (APCI+) found (M+1) = 623; $C_{31}H_{32}ClFN_6O_3S$ requires 622.
9. Daylight CIS, Mission Viejo CA, USA version 4.71.