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2-(Alkylthio)pyrimidin-4-ones as Novel, Reversible Inhibitors of Lipoprotein-Associated Phospholipase A_2

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Abstract—Starting from two weakly active hits from high throughput screening, a novel series of 2-(alkylthio)-pyrimidin-4-ones with high potency and selectivity for lipoprotein-associated phospholipase A_2 has been designed. In contrast to previously known inhibitors, these have been shown to act by a non-covalent and substrate competitive mechanism. © 2000 Elsevier Science Ltd. All rights reserved.

Lipoprotein-associated phospholipase A_2 (Lp-PLA_2)[1] is a novel serine dependent lipase that is able to hydrolyse oxidatively modified phosphatidylcholines to release both oxidised fatty acids and lysophosphatidylcholine (lyso-PC). Both of these hydrolysis products have been shown to be pro-inflammatory and have been implicated in atherosclerosis. Lyso-PC for example is both chemoattractant for circulating monocytes and induces endothelial dysfunction.[2] Furthermore, the increased lyso-PC content of oxidised low density lipoprotein can be completely accounted for by Lp-PLA_2. Inhibition of Lp-PLA_2, therefore, represents an attractive alternative approach to current antiatherosclerotic therapy.

We have previously described one class of selective inhibitors (exemplified by SB-222657) which acted by covalent modification of the active site.[2,3] In a parallel strategy, we sought mechanistically distinct inhibitors which lacked the covalent reactivity of the initial series. To this end, high throughput screening of our compound bank led to the identification of pyrimidones **1** and **2** as weak (IC_{50} values of 12 and 55 μM respectively) but fully reversible inhibitors of Lp-PLA_2. In this Letter we describe our initial optimisation studies with these leads.

We focussed our attention on the sulfide series as robust preparations were available.[4,5] This allowed the ready optimisation of R^1, R^2 and R^3 (Scheme 1). As the corresponding ethers suffered from capricious and low yielding syntheses only selected compounds were prepared in that series. All compounds in Tables 1–3 were evaluated using recombinant human Lp-PLA_2. Assays were performed in duplicate.[6]

We initially turned our attention to the pyrimidone 2-substituent (Table 1). It quickly became apparent that, as had previously been found for the azetidinone series,[7] activity proved sensitive to the length of the arylalkyl chain R^1. The highest level of activity was seen in those compounds with either a single methylene spacer (e.g compound **3a**) or a linker $n \geq 6$ with no advantage beyond $n = 8$ (compounds **3g** and **4d**) suggesting two different binding modes for these inhibitors and thus, two series for further development.

Within the C_8 series (Table 2), active compounds were obtained with either a 3- or 4-pyridyl group as the heterocyclic substituent at C-5. Activity was reduced if either a heteroatom or a substituent was present *ortho* to the methylene linkage (cf. compounds **3g**, **4d**, **5** and **8** with **6** and **7**. Also compound **18** with **26**). Based on these results and the poor activity of the corresponding phenyl and furyl derivatives **9** and **10**, we propose that a hydrogen bond acceptor is required at this site of the molecule. This view was further supported by the preparation of

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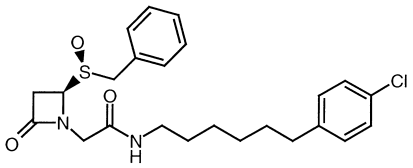
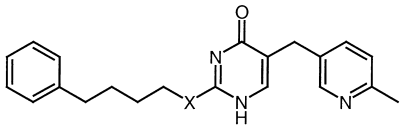
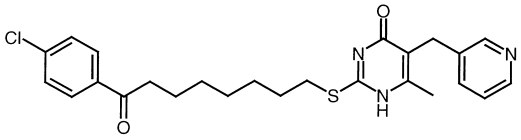


SB-222657



1 X = O, **2** X = S



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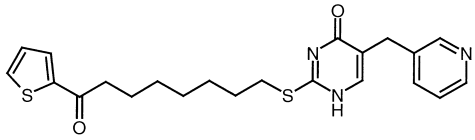

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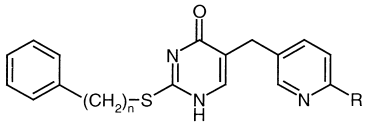
potent pyridones **12** and **13**. Disruption of this putative H-bond due to orientation effects on the 5-substituent may also explain the loss of potency seen on the introduction of a 6-substituent into the pyrimidone nucleus—compound **29** for example gave only 40% inhibition at 10 μM.

4-Chloro substitution of the phenyl group within the *S*-arylalkyl moiety proved to be well tolerated (compound **14)** whereas other substitution or heterocyclic replacement of the phenyl ring was not advantageous (cf. compound **30**, $IC_{50} = 1.3$ μM, with **4d** and **18**). The introduction of polar groups such as sulfoxide and amide into the C_8 chain proved detrimental (compounds **19**, **22** and **23**) whereas less polar groups such as ethers and thioethers—compounds **20**, **21** and **24**—were well tolerated. The introduction of a keto group adjacent to the phenyl ring as in compound **18** proved the most effective substitution. The optimised *S*-(4-chlorobenzoylheptyl) group was combined with a variety of optimised C-5 substituents to give highly potent inhibitors of the Lp-PLA_2 enzyme. Indeed, compounds **27** and **28** represent a 1000-fold improvement on our original lead **2**.

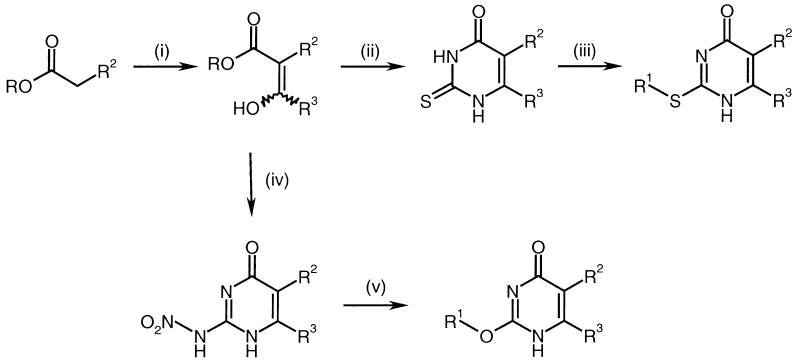
Within the *S*-benzyl series based on compounds **3a** and **4a** it quickly became apparent that a number of the structural modifications described for the C_8 series in Table 2 did not give useful potency increases (Table 3). In these shorter chain derivatives there appears a greater size constraint on the sulfur substituent with benzyl substituents H, F giving greater potency than Cl (cf. compounds **33** with **4a** and **34** and **32)**. For stability

Table 1. Effect of *S*-arylalkyl chain length on potency



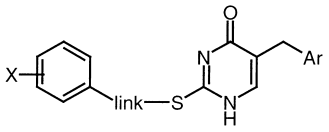
	R = Me		R = H	
n	Compound[a]	IC_{50} (μM)	Compound	IC_{50} (μM)
1	**3a**	1.4	**4a**	1.1
2	**3b**	5.7		
3	**3c**	10% @ 30μM		
4	**2**	55		
5	**3d**	14		
6	**3e**	1.1	**4b**	2.3
7	**3f**	1.0	**4c**	1.1
8	**3g**	0.78	**4d**	0.33
9	**3h**	0.99	**4e**	0.42

[a]All new compounds gave satisfactory analytical/spectral data.[8]



Scheme 1. Preparation of 2-substituted pyrimidones. Methods: (i) NaH, THF, R^3COOEt; (ii) thiourea, NaOEt, EtOH; (iii) R^1Br/R^1I, NaOEt, EtOH; (iv) nitroguanidine, NaOEt, EtOH; (v) R^1OH, 10% dimethylaminopyridine in pyridine.

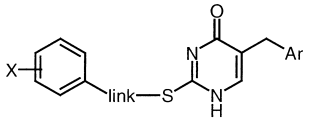
Table 2. Optimisation of C_8 series



Compound[a]	X	Link	Ar[b]	IC_{50} (μM)
3g	H	$(CH_2)_8$	6-Me-3-pyridyl	0.78
4d	H	$(CH_2)_8$	3-Pyridyl	0.33
5	H	$(CH_2)_8$	2-MeO-4-pyridyl	1.0
6	H	$(CH_2)_8$	4,6-diMe-3-pyridyl	3.3
7	H	$(CH_2)_8$	2-Pyridyl	38% @10
8	H	$(CH_2)_8$	4-Pyridyl	0.63
9	H	$(CH_2)_8$	Ph	0% @10
10	H	$(CH_2)_8$	3-Furyl	63% @10
11	H	$(CH_2)_8$	2-Oxo-4-pyridyl	1.0
12	H	$(CH_2)_8$	1-Me-2-oxo-4-pyridyl	0.25
13	H	$(CH_2)_8$	1-Bu-2-oxo-4-pyridyl	0.78
14	4-Cl	$(CH_2)_8$	3-Pyridyl	0.20
15	3-Cl	$(CH_2)_8$	3-Pyridyl	0.85
16	4-Acetyl	$(CH_2)_8$	3-Pyridyl	2.9
17	4-OMe	$(CH_2)_8$	6-Me-3-pyridyl	1.1
18	4-Cl	$CO(CH_2)_7$	3-Pyridyl	0.11
19	4-Cl	$CONH(CH_2)_6$	3-Pyridyl	41% @10
20	4-Cl	$O(CH_2)_7$	3-Pyridyl	0.23
21	4-Cl	$S(CH_2)_7$	3-Pyridyl	0.25
22	4-Cl	$SO(CH_2)_7$	3-Pyridyl	17
23	4-Cl	$SO_2(CH_2)_7$	3-Pyridyl	12.6
24	4-Cl	$CH_2O(CH_2)_6$	6-Me-3-pyridyl	1.2
25	4-Cl	$CO(CH_2)_8$	3-Pyridyl	0.65
26	4-Cl	$CO(CH_2)_7$	2-Pyrazinyl	0.64
27	4-Cl	$CO(CH_2)_7$	1-Me-2-oxo-4-pyridyl	0.072
28	4-Cl	$CO(CH_2)_7$	5-Pyrimidyl	0.054

[a]All new compounds gave satisfactory analytical/spectral data.[8]
[b]2-Oxo-4-pyridyl derivatives are 1,2-dihydro isomers.

Table 3. Optimisation of *S*-benzyl series

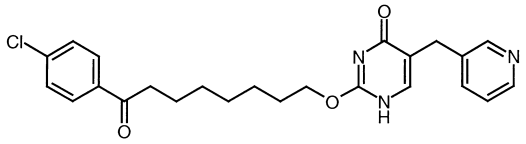


Compound[a]	X	Link	Ar	IC_{50} (μM)
3a	H	CH_2	6-Me-3-pyridyl	1.4
4a	H	CH_2	3-Pyridyl	1.1
31	H	CH_2	4-Pyridyl	4.4
32	H	CH_2	5-Pyrimidyl	1.1
33	4-Cl	CH_2	3-Pyridyl	4.4
34	4-F	CH_2	5-Pyrimidyl	0.85
35	H	$COCH_2$	3-Pyridyl	0% @ 10

[a]All new compounds gave satisfactory analytical/spectral data.[8]

reasons, the introduction of a carbonyl into the methylene linker required an additional carbon in the linker. The resultant compound **35**, proved considerably less active than either the benzyl or the related phenethyl analogues, **4a** and **3b.**

Following SAR development in the sulfide series, ether **36** was prepared. As expected, this compound proved very active ($IC_{50} = 0.064$ μM), being almost 200-fold more potent than initial lead **1**. This result is consistent with similar SAR patterns in the ether and sulfide series.



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Studies on representative compounds confirmed that the pyrimidones, in contrast to the previous azetidinone series, are simple reversible, substrate-competitive inhibitors of Lp-PLA_2 with no time dependency. For example, Lineweaver–Burk analysis with compound **27**, indicated a competitive mechanism of action and a K_i of 41 nM.

Selected compounds were also screened against the most closely related phospholipase A_2 (human serine dependent-PLA_2).[9] Pleasingly, compounds showed >>100-fold selectivity for Lp-PLA_2 over this lipase.

In conclusion, starting from weakly active HTS leads **1** and **2**, we have identified a number of potent, fully reversible, non-time dependent inhibitors of Lp-PLA_2. Consistent with the natural substrates of this lipase, inhibitors bearing a long lipophilic substituent proved the most effective. Potent inhibitors such as **28** and **36** should prove of considerable value as tools for the evaluation of the role of Lp-PLA_2 in atherosclerosis.

References and Notes

1. The enzyme is also known as PAF acetyl hydrolase, though it is clear that the enzyme has much broader substrate specificity than this name implies. 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* (4), 591. 3-Dimensional structural information on this enzyme is not available. Recently, the structure of a potentially related bacterial enzyme has been published: Wei, Y.; Swenson, L.; Castro, C.; Derewenda, U.; Minor, W.; Arai, H.; Aoki, J.; Inoue, K.; Servin-Gonzalez, L.; Derewenda, Z. S. *Structure* **1998**, *6* (4), 511
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6. IC_{50} values were determined by measuring the rate of turnover of the artificial substrate 1-decanoyl-2-(4-nitrophenylglutaryl)phosphatidylcholine (DNPG) (Washburn, W. N.; Dennis E. A. *J. Am. Chem. Soc.* **1990**, *112*, 2040). Assays were performed in 96 well microtitre plates using recombinant human Lp-PLA_2. Duplicate assay plates of compound or vehicle plus buffer (50 mM Hepes, 150 mM NaCl, pH 7.4) were set up to a volume of 0.17 ml. This was followed by the addition of 20 μL of 200 μM DNPG and reaction was initiated with 10 μl of diluted enzyme to a final concentration of 0.1 nM. The reaction was followed for 20 min at 37 °C using a Molecular Devices Tmax plate reader at 405 nM. Data were fitted to a 4

parameter logistic IC_{50} equation using Grafit (*Erithacus Software*).
7. Boyd, H. F.; Hickey, D. M. B.; Ife, R. J.; Leach, C. A.; Macphee, C. H.; Tew, D. G. unpublished results.
8. Representative examples: Compound **3g** (250 MHz) ^{1}H NMR (d_6 DMSO) δ 1.3 (8H, m), 1.6 (4H, m), 2.40 (3H, s), 2.55 (2H, t), 3.02 (2H, t), 3.54 (2H, s), 7.11 (1H, d), 7.16 (3H, m), 7.25 (2H, m), 7.50 (1H, m), 7.69 (1H, s), 8.33 (1H, d). Compound **28** (250 MHz) ^{1}H NMR (d_6 DMSO) δ 1.2–1.5 (6H, m), 1.5–1.8 (4H, m), 2.93 (2H, t), 3.15 (2H, t), 3.71 (2H, s), 7.43 (2H, d), 7.78 (1H, s), 7.89 (2H, d), 8.72 (2H, s) and 9.07 (1H, s); Mass spectrum (EI) M = 456; $C_{23}H_{25}ClN_4O_2S$ requires 456.
9. Rice, S. Q. J.; Southan, C.; Boyd, H. F.; Terrett, J. A.; Macphee, C. H.; Moores, K.; Tew, D. G. *Biochem. J.* **1998**, *330*, 1309.