Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 2557–2561

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

N-1 Substituted Pyrimidin-4-ones: Novel, Orally Active Inhibitors of Lipoprotein-Associated Phospholipase A_2

Helen F. Boyd, Stephen C. M. Fell, Sean T. Flynn, Deirdre M. B. Hickey, Robert J. Ife, Colin A. Leach, Colin H. Macphee, Kevin J. Milliner, Kitty E. Moores, Ivan L. Pinto, Rod A. Porter, D. Anthony Rawlings, Stephen A. Smith,* Ian G. Stansfield, David G. Tew, Colin J. Theobald and Caroline M. Whittaker

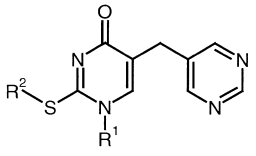
SmithKline Beecham Pharmaceuticals, New Frontiers Science Park, Third Avenue, Harlow, Essex, CM19 5AW, UK

Received 24 July 2000; revised 28 August 2000; accepted 6 September 2000

Abstract—From two related series of 2-(alkylthio)-pyrimidones, a novel series of 1-((amidolinked)-alkyl)-pyrimidones has been designed as nanomolar inhibitors of human lipoprotein-associated phopholipase A_2. These compounds show greatly enhanced activity in isolated plasma. Selected derivatives such as compounds **51** and **52** are orally active with a good duration of action.

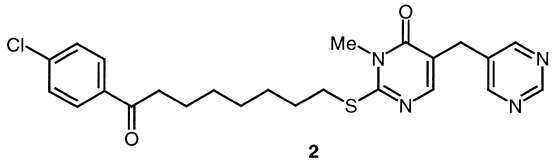
The oxidative modification of low density lipoprotein (LDL) has been suggested to play a key role in atherosclerosis through the release of a pro-inflammatory oxidative signal.[1] Whilst the exact mechanism for the release of such a message has not been fully characterised, it has been shown that a phospholipase associated predominantly with LDL, termed lipoprotein-associated phospholipase A_2 (Lp-PLA_2),[2,3] 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.[1] Indeed, the increased levels of lyso-PtdCho in oxidised LDL can be completely accounted for by Lp-PLA_2.[1] With this evidence in hand, we decided that there was a clear need for inhibitors of Lp-PLA_2 to more fully understand the role of this lipase in the atherosclerotic process.

In a recent report[4] we described the identification of a series of 2-(alkylthio)-pyrimidones, such as **1a** and **1b**, as fully reversible inhibitors of Lp-PLA_2. Further evaluation of these compounds however, indicated a low level of inhibition in whole plasma and as a result, little activity in vivo. In this communication we describe our follow-up studies to investigate the effect of pyrimidone nitrogen substitution (e.g. **1** $R^1 \neq H$) on potency, the potential for improved in vivo properties and, for selected compounds, activity in the Watanabe hereditable hyperlipidaemic rabbit (WHHL rabbit).



1a R^1 = H, R^2 = $(CH_2)_7COPh(4\text{-}Cl)$

1b R^1 = H, R^2 = CH_2Ph



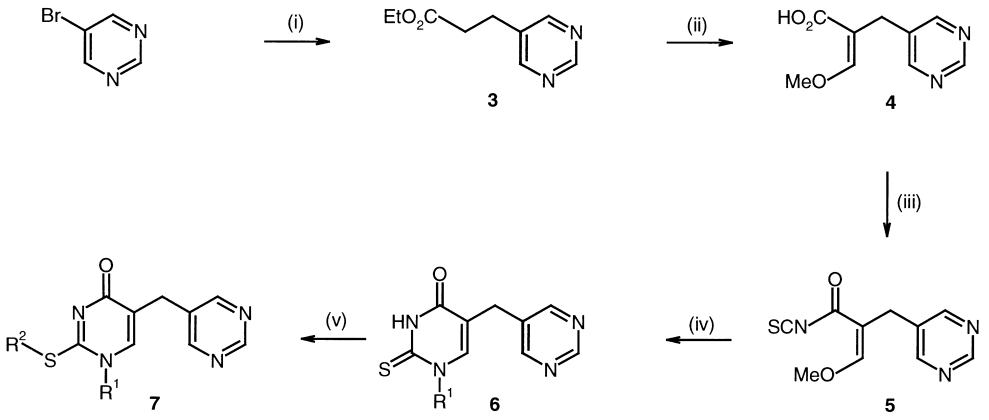
2

28% inhibition Lp-PLA_2 @ 2.5μM

Attempts to N-alkylate compound **1a** under a range of conditions (e.g., sodium ethoxide or Hunig's base in ethanol or dimethylformamide, potassium hydroxide/tetrabutylammonium bromide in tetrahydrofuran), indicated that N-3 alkylation predominated (compound **2** was made in this way). As a result, N-1 alkylated adducts were made via acylisothiocyanate **5** (Scheme 1).[5,6] This key intermediate is prepared from propionic ester **3** which is itself made by a Heck reaction with 5-bromopyrimidine followed by reduction. Ester **3** is formylated, O-alkylated and carefully hydrolysed to acid **4** which is

*Corresponding author. Fax +44-1279-627841; e-mail: Stephen_1_Smith@sbphrd.com

0960-894X/00/$ - see front matter
PII: S0960-894X(00)00510-2



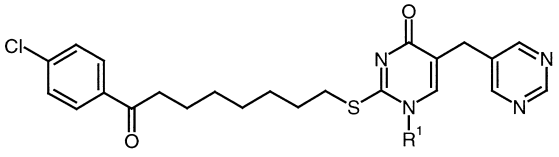
Scheme 1. Reagents: (i) (a) $CH_2{=}CHCO_2Et$, $Pd(OAc)_2$, P(*o*-Tol)$_3$, Et_3N, (b) H_2, Pd/C, EtOH/Et_3N; (ii) (a) HCO_2Et, NaH, DME, (b) Me_2SO_4, K_2CO_3, DMF, (c) NaOH, H_2O; (iii) (a) $(COCl)_2$, $ClCH_2CH_2Cl$, (b) KSCN, CH_3CN; (iv) R^1NH_2, DMF then NaOEt; (v) R^2Cl/Br, iPr_2NEt, CH_2Cl_2.

converted to the required acylisothiocyanate. Addition of an amine to **5** followed by ring closure gives thiouracils **6** regiospecifically. These were S-alkylated to give the required pyrimidones **7**. For those compounds bearing additional functionality in R^1 (Table 3), further chemistry, e.g., hydrolysis of an ester ($R^1 = (CH_2)_n$-CO_2Et) followed by amide coupling (carbodiimide/HOBT), was performed after S-alkylation. Whenever possible (e.g., optimisation of R^2, amide formation within R^1) compounds were prepared using array techniques.

All structures in Tables 1–3 were evaluated using human Lp-PLA_2. Assays were performed in duplicate.[4] In order to factor in any non-specific binding effects in plasma, the majority of compounds were also assessed against the plasma enzyme in both whole human and WHHL rabbit plasma at a single concentration of inhibitor.[7] Good activity in rabbit and human plasma was required before compounds were evaluated in vivo in WHHL rabbits.[8]

We initially focused our attention on those compounds bearing a long arylalkyl substituent on the C-2 sulfur atom. This work showed N-1 substitution (Table 1) to be preferred over N-3 (cf. compounds **8** and **2**). Furthermore, although the introduction of an N-1 substituent gave some useful enhancements in potency against the isolated enzyme (cf. compound **1a** with **8**, **14** and **15**), the most marked effect was the much improved activity of these compounds in whole plasma. Potency in rabbit plasma was generally lower than in human plasma suggesting that compounds may be more potent in man than in the rabbit.

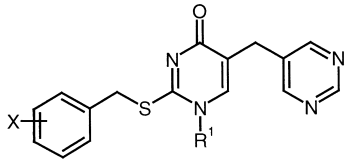
Table 1. Effect of N-1 substitution



No.[a]	R^1	IC_{50} (nM)	Inhibition in plasma: Human % @ 300 nM	Inhibition in plasma: Rabbit % @ 1 μM
1a	H	54	2	0
8	Me	19	53	23
9	Et	39	35	38
10	CH_2CH_2OMe	110	41	20
11	CH_2Ph	36	29	14
12	CH_2CH_2Ph	34	28	45
13	$CH_2CH_2CH_2Ph$	90	10	9
14	CH_2(2-furyl)	16	36	30
15	CH_2(2-thienyl)	11	27	31

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

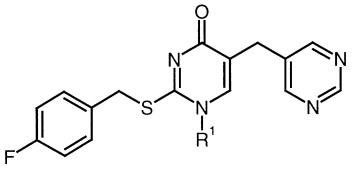
Table 2. Effect of N-1 substitution in the S-benzyl series



No.[a]	X	R^1	IC_{50} (nM)	Inhibition in plasma: Human % @ 300 nM	Inhibition in plasma: Rabbit % @ 1 μM
1b	H	H	1110	4	4
16	H	Me	660	54	33
17	H	CH_2CH_2OMe	1200	27	25
18	H	CH_2Ph	450	30	42
19	H	CH_2CH_2Ph	210	35	73
20	H	$CH_2CH_2CH_2Ph$	300	18	28
21	H	CH_2(2-furyl)	150	46	37
22	H	CH_2(2-thienyl)	210	34	40
23	H	CH_2CH_2(2-thienyl)	350	59	75
24	4-F	Me	285	55	32
25	4-Cl	CH_2(2-furyl)	1400	NT[b]	NT
26	4-Me	CH_2(2-furyl)	2850	NT	NT
27	3-Cl	CH_2(2-furyl)	960	NT	NT
28	2-F	CH_2(2-furyl)	450	41	40
29	3-F	CH_2(2-furyl)	250	44	33
30	4-F	CH_2(2-furyl)	300	49	40
31	3,4-diF	CH_2(2-furyl)	235	53	47
32	4-F	CH_2CH_2(2-thienyl)	250	42	65
33	4-F	CH_2CH_2Ph	200	60	82
34	3,4-diF	CH_2CH_2Ph	110	75	87

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

Table 3. The introduction of a lipophilic substituent at N-1



No.[a]	R^1	IC_{50} nM	Plasma inhib. (%) @ 100 nM	
			Human	Rabbit
35	$(CH_2)_9Ph$	25	10	10
36	*n*-$C_{11}H_{23}$	19	11	10
37	$(CH_2)_2Ph(4\text{-}C_6H_{13})$	12	5	4
24	Me	285	28	6
30	CH_2(2-furyl)	300	26	7
33	$(CH_2)_2Ph$	200	27	29
38	$CH_2CONH(CH_2)_6Ph(4\text{-}F)$	2	67	33
39	$CH_2CONHC_7H_{15}$	19	37	29
40	$CH_2CONHC_8H_{17}$	7	43	38
41	$CH_2CONHC_{12}H_{25}$	0.3	86	54
42	$CH_2CONMeC_8H_{17}$	15	40	30
43	$CH_2CONMeC_{12}H_{25}$	1	79	39
44	$(CH_2)_3CONHC_6H_{13}$	7	63	59
45	$(CH_2)_3CONHC_7H_{15}$	2	78	62
46	$(CH_2)_3CONHC_8H_{17}$	0.4	85	55
47	$(CH_2)_3CONHC_{10}H_{21}$	0.1	94	77
48	$(CH_2)_3CONMeC_7H_{15}$	3	18	45
49	(*E*) $CH_2CONH(CH_2)_8CH{=}CHC_8H_{17}$	2	55	32
50	(*Z*) $CH_2CONH(CH_2)_8CH{=}CHC_8H_{17}$	1	66	38
51	(*E*) $(CH_2)_3CONH(CH_2)_8CH{=}CHC_8H_{17}$	0.7	71	47
52	(*Z*) $(CH_2)_3CONH(CH_2)_8CH{=}CHC_8H_{17}$	0.4	85	59
53	$(CH_2)_3CONHC_{18}H_{37}$	4	29	21
54	$(CH_2)_5CONHC_4H_9$	260	4	0
55	$(CH_2)_2NHCOC_7H_{15}$	140	8	9
56	$(CH_2)_2NHCONHC_7H_{15}$	70	18	10

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

Although potency against Lp-PLA_2 was lower in the S-benzyl series (Table 2) than for the longer chain compounds of Table 1, activity in human plasma was broadly similar for compounds with similar N-1 substituents (cf. compounds **16** and **8**, **21** and **14**), suggesting a reduced level of non-specific binding in plasma for the S-benzyl series. The effect of substitution on the S-benzyl group was striking, with groups larger than fluoro causing a marked reduction in potency (cf. compound **21** with **25**–**31** and **19** with **33** and **34**). For subsequent work the 4-fluorobenzyl group was chosen on the grounds of potential metabolic advantage (i.e., a deactivated aromatic ring) with minimal increase in molecular weight.

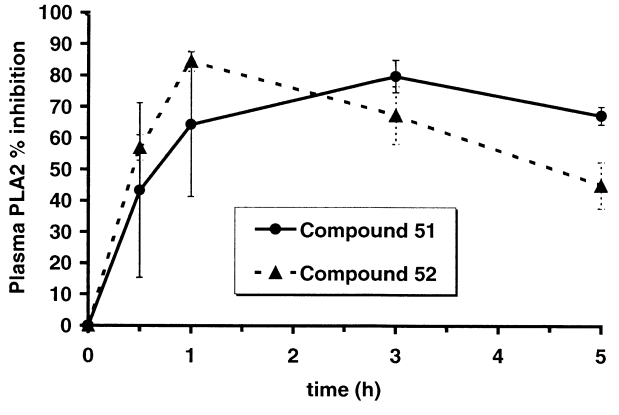


Figure 1. Inhibition of plasma Lp-PLA_2 in the WHHL rabbit @ 10 mg/kg ($n = 2$).

Based on the above results and the knowledge that further simple modification of the structures of Tables 1 and 2 had not given further enhancements in activity, we speculated that potent inhibitors would be obtained if the long arylalkyl chain on sulfur as in Table 1 was moved to N-1 whilst maintaining the S-benzyl group of the compounds of Table 2 (see Table 3). Pleasingly, compounds **35** and **12** showed similar profiles, confirming our hypothesis (note the lower concentration used for the single point determination of plasma inhibition).[10] We also showed that high potency against Lp-PLA_2 could be achieved by either extending the phenethyl group of compound **33** or, perhaps more usefully, by the replacement of the arylalkyl group by a long alkyl chain (lower MWt, cf. **36** with **35** and **37**). Our real breakthrough however came when, in an effort to make these inhibitors less lipophilic, we introduced an amide group into the C_{11} chain of compound **36**. Both acetamide and butyramide derivatives[11] proved

not only more potent against Lp-PLA_2 but were considerably more active in whole plasma (cf. **40** and **44** with **36**). The corresponding hexanamides (e.g. **54**) proved much less active and were not considered further, nor were the reversed amides (cf. **55** and **44**) or ureas (cf. **56** and **45**).

Increasing the length of the alkyl chain increased activity, giving sub-nanomolar inhibitors in both the acetamide and the butyramide series (cf. **39**–**41**, also **44**–**47**), although there appeared a maximum for this effect with the highly lipophilic C_{18} derivatives **49**–**53** proving to be no more potent than, for example, compounds **41** and **47**. Amide *N*-methylation had little effect on potency against Lp-PLA_2. Although a similar effect was seen in whole plasma for the acetamides (cf. **42** and **40**, **43** and **41**), tertiary butyramides proved significantly less efficacious in plasma than their secondary counterparts (e.g. **48** versus **45**).

Selected compounds from Table 3 were evaluated in the WHHL rabbit (at 10 mg/kg po).[8] Butyramides **51** and **52** from the C_{18} amide series proved the most efficacious, demonstrating good levels of inhibition alongside a promising duration of action ($\geq$5 h, Fig. 1).

In conclusion, we have shown that orally active, sub-nanomolar inhibitors of Lp-PLA_2 may be obtained by the introduction of a suitable amidic N-1 substituent into our initially optimised series of pyrimidone inhibitors.[4] Furthermore, our evaluation of the role of Lp-PLA_2 in the atherosclerotic disease process and as a target for therapeutic intervention should be markedly enhanced by the development of potent, orally active inhibitors such as compounds **51** and **52**.

References and Notes

1. Macphee, C. H.; Moores, K. E.; Boyd, H. F.; Dhanak, D.; Ife, R. J.; Leach, C. A.; Leakes, D. S.; Milliner, K. J.; Patterson, R. A.; Suckling, K. E.; Tew, D. G.; Hickey, D. M. B. *Biochem. J.* **1999**, *338*, 479.
2. Tew, D. G.; Boyd, H. F.; Ashman, S.; Theobald, C. J.; Leach, C. A. *Biochemistry* **1998**, *37*, 10087.
3. 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.
4. Boyd, H. F.; Flynn, S. F.; Hickey, D. M. B.; Ife, R. J.; Jones, M.; Leach, C. A.; Macphee, C. H.; Milliner, K. J.; Rawlings, D. A.; Slingsby, B. P.; Smith, S. A.; Stansfield, I. G.; Tew, D. G.; Theobald, C. J. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 395.
5. See Agathocleous, D. C.; Shaw, G. J. *J. Chem. Soc., Perkin Trans. 1* **1993**, 2555.
6. See Shaw, G.; Warrener, R. N. *J. Chem. Soc.* **1959**, 50.
7. Activity in plasma was assessed following measurement of PAF-AH activity. 10 μL [^{3}H]PAF (50 μM) and whole plasma were incubated at 37 °C in a final volume of 200 μL for either 15 s (rabbit plasma) or 60 s (human plasma). The reaction was stopped by vortexing with 600 μL of $CHCl_3$/MeOH (2:1) and the $CHCl_3$ and aqueous layers were separated by centrifugation. The aqueous layer was removed (250 μL) and vortexed with 250 μL of $CHCl_3$. The aqueous layer was again removed and the [^{3}H]acetate determined by scintillation counting. The inhibitory effect of various compounds was determined by mixing whole plasma (190 μL) and 2 μL of a DMSO solution of compound and preincubating at 37 °C for 10 min before running the enzyme assay as above. Results at a single concentration proved very reproducible and were used for assessing SAR trends. A full IC_{50} curve was generated ($n=3$) for compounds of most interest (Figs. 2 and 3) and confirmed assay reproducibility.

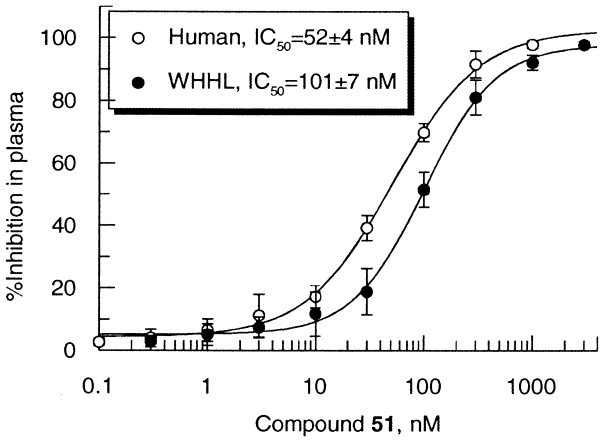


Figure 2.

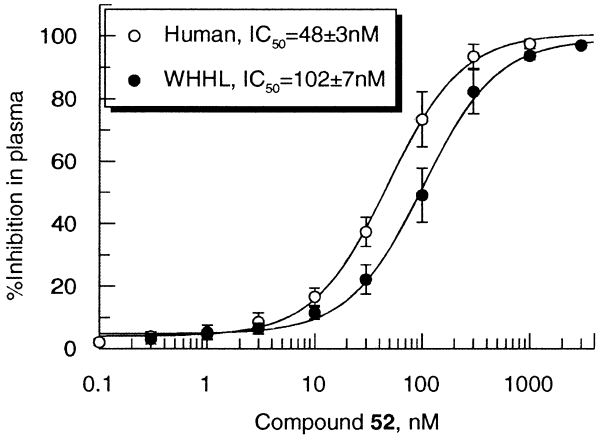


Figure 3.

8. Watanabe heritable hyperlipidaemic rabbits were fasted overnight prior to dosing and were allowed to feed ad lib immediately following dosing. All dosing was by gavage using 3 mL of PEG300 containing compound. Prior to dosing and at 0.5, 1, 3 and 5 h post dosing, 1.5 mL blood samples were taken via the ear vein and transferred to Eppendorf tubes containing 30 μL 250 mM EDTA solution. Plasma was immediately prepared by microfuging for 30 s and frozen on dry ice to await analysis for plasma PLA_2 activity using the method of ref 7. Percentage inhibitions were determined relative to the pre dose value.
9. Representative examples: Compound **45** (250 MHz) ^{1}H NMR ($CDCl_3$) δ 0.88 (3H, t), 1.27 (8H, m), 1.47 (2H, m), 1.98–2.25 (4H, m), 3.20 (2H, m), 3.69 (2H, s), 3.92 (2H, t), 4.52 (2H, s), 5.42 (1H, bm), 6.98 (2H, m), 7.25 (1H, s), 7.37 (2H, m), 8.68 (2H, s), 9.08 (1H, s); Mass Spectrum (APCI+)

M + 1 = 512, $C_{27}H_{34}FN_5O_2S$ requires 511. Compound **51** (250 MHz) ^{1}H NMR ($CDCl_3$) δ 0.88 (3H, t), 1.27 (22H, m), 1.47 (2H, m), 1.99–2.20 (8H, m), 3.20 (2H, m), 3.69 (2H, s), 3.92 (2H, m), 4.46 (2H, s), 5.37 (2H, m), 5.44 (1H, m), 6.99 (2H, m), 7.26 (1H, s), 7.37 (2H, m), 8.71 (2H, s), 9.08 (1H, s); Mass Spectrum (APCI+) M + 1 = 664, $C_{38}H_{54}FN_5O_2S$ requires 663.

10. Compounds **24**, **30** and **33** were screened at both concentrations and are included in Table 3 for comparison with the data in Table 2.

11. The corresponding propionamides proved susceptible to retro-Michael elimination of the N-1 substituent and as a result were not evaluated.