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Synthesis and evaluation of substrate-mimicking cytosolic phospholipase A_2 inhibitors—reducing the lipophilicity of the arachidonyl chain isostere

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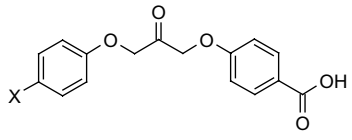
Abstract—The high lipophilicity of a series of cytosolic phospholipase A_2 inhibitors has been reduced by the modification of a decyloxyphenyl chain designed to mimic the arachidonyl group of the natural substrate. These changes have resulted in an improvement in the whole cell potency of the inhibitors.

Cytosolic phospholipase A_2 ($cPLA_2$) is the cellular enzyme believed to be responsible for cleavage of arachidonic acid from the sn-2 position of membrane phospholipids.[1–4] This process is thought to be the principal source of free arachidonic acid within activated cells.[5] Since arachidonic acid is the raw material for the cellular production of a variety of other pro-inflammatory mediators such as prostaglandins and leukotrienes,[6,7] inhibition of $cPLA_2$ activity represents an attractive approach to the control of inflammation.[8–10]

We recently described a series of potent $cPLA_2$ inhibitors such as **1a**–**c** (Fig. 1) designed to mimic the binding of the natural substrate.[11] These inhibitors comprise three core elements: (i) a terminal carboxylic acid, which functions as a phosphate isostere, (ii) an electrophilic ketone, which serves as a serine trap, (iii) an extended alkoxyphenyl chain, which mimics the arachidonyl group of the substrate. This moiety is the major contributor to the very high lipophilicity of these compounds, and the present communication describes our efforts to moderate this undesirable property and so discover more 'drug-like' inhibitors.



1a R = $CH_3(CH_2)_9O$
1b R = $Ph(CH_2)_5O$
1c R = $Ph(CH_2)_5S$

Figure 1. Structure of $cPLA_2$ inhibitors.

Since we were interested in modifying the long alkyl chain of these $cPLA_2$ inhibitors, it was desirable to employ a general synthesis which enabled this group to be introduced at a late stage. The two principal routes used to prepare these compounds are outlined in Schemes 1 and 2. The key epichlorohydrin-derived epoxide intermediate **3** may be reacted with a suitable 4-functionalised phenol as shown in Scheme 1, to generate secondary alcohols such as **4** or **5**. These may then be attached to a modified alkyl chain by either alkylation of the phenolic hydroxyl of **4**, followed by oxidation and ester hydrolysis to give **12** and **17**–**21** (Table 1), or

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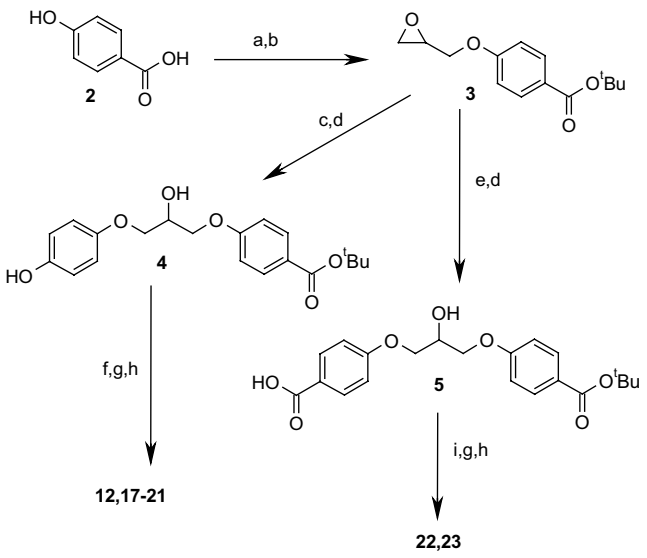
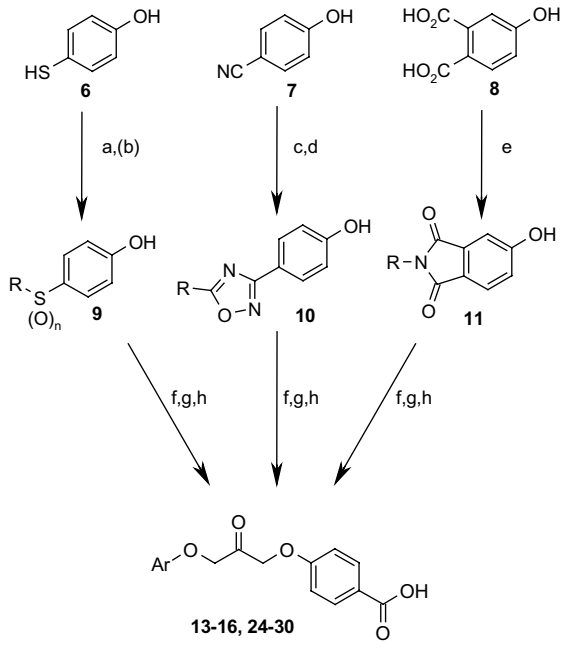


Scheme 1. Reagents and conditions: (a) ^{t}BuOH, DCC, DMAP, rt, 69%; (b) epichlorohydrin, Cs_2CO_3, MeCN, reflux, 76%; (c) 4-benzyloxyphenol, DABCO, DMF, 120 °C, 61%; (d) H_2 (4 bar), Pd/C, EtOAc, rt, 95%; (e) benzyl 4-hydroxybenzoate, DABCO, DMF, 120 °C, 50%; (f) alkyl halide, Cs_2CO_3, MeCN, rt; (g) Ac_2O, DMSO, rt; (h) TFA, DCM, rt; (i) 5-phenyl-1-pentanol or 4-phenylbutylamine, DCC, DCM, rt.



Scheme 2. Reagents and conditions: (a) Alkyl halide, Cs_2CO_3, MeCN, rt; (b) *m*CPBA, DCM, rt (for **26** and **27**); (c) $NH_2OH{\cdot}HCl$, Na_2CO_3, EtOH, H_2O, 70 °C, 33%; (d) acid chloride, pyridine, reflux; (e) amine, DCC, THF, reflux; (f) **3**, DABCO, DMF, 120 °C; (g) Ac_2O, DMSO, rt; (h) TFA, DCM, rt.

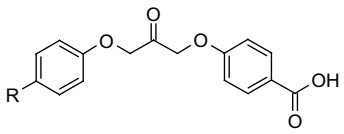
condensation of an alcohol or amine with the carboxyl group of **5**, giving rise to **22** and **23** after oxidation and hydrolysis (Table 2). Alternatively, the modified alkyl chain can be attached first to a suitably functionalised phenol such as **6**, **7** or **8** by alkylation or condensation with appropriate electrophiles or nucleophiles as shown in Scheme 2. The resultant phenols **9**, **10** and **11** can be subsequently combined with epoxide **3** to furnish the inhibitor products **13**–**16** (Table 1) and **24**–**30** (Table 2) after oxidation and ester hydrolysis.

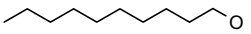
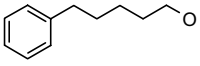
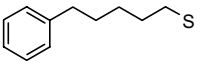
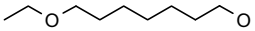
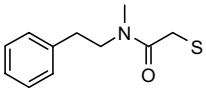
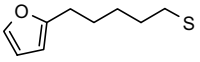
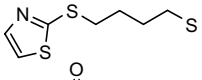
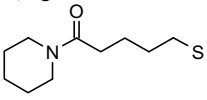
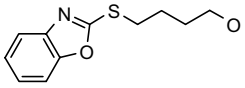
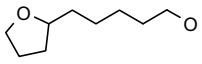
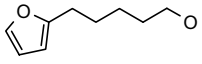
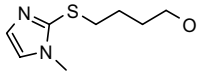
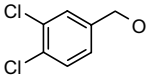
The activities of the compounds described herein were compared in two biological screens, a 'monomeric' assay based on the $cPLA_2$-mediated hydrolysis of a simple, soluble synthetic substrate,[12] and a cellular screen based on the stimulated production of arachidonic acid by HL60 cells.[11] Our initial attempts sought to reduce the log*P* of these inhibitors by introducing a polar functionality such as an ether oxygen as in compound **12** or an amide linkage (**13**) within the body of the long alkyl chain. The poor enzyme inhibition shown by these two compounds (Table 1) was shared by many other similar analogues, and so we turned our attention instead to varying the end of the lipophilic chain. Previous SAR[11] had shown that a phenylpentyl group as in **1b** or **1c** maintained most of the potency of the decyl chain of **1a**, so a number of analogues containing a terminal heterocycle were prepared (**14**–**20**). Three of these analogues (**14**, **15** and **17**) showed some promise with near micromolar whole cell potencies and reduced lipophilicities (Table 1). Another attractive approach entailed replacing the decyloxy chain with a substituted benzyloxy group, and the best of these, the 3,4-dichlorobenzyl derivative **21**, was equipotent with its decyloxy analogue **1a** in the whole cell assay despite its markedly lower *c*log*P* (Table 1).

Further success was achieved by altering the functionality tethering the lipophilic chain to the phenoxy group believed to mimic the Δ-5,6 double bond of arachidonic acid.[11] Several different polar functional groups were investigated as alternatives to ether and sulfide linkages (Table 2), with phthalimide **24** and oxadiazole **25** both giving rise to submicromolar whole cell potencies. Finally, the best features of these different strategies were combined to give the dichlorophenyl analogues **28**–**30** (Table 2). The two oxadiazoles **28** and **29** showed pleasing activities in the monomeric assay, and the dichlorobenzylphthalimide **30** was micromolar in the whole cell assay.

The 'intrinsic' SAR of the compounds described herein in the soluble assay is largely lipophilicity-driven with a component of ketone electrophilicity also playing a part. Thus the most polar compounds (**13**, **18**, **20**, **23**) all show very poor activity in the soluble assay whereas the most lipophilic (**14**, **17**, **22**, **28**) are all highly active. The influence of ketone electrophilicity may be seen in the 10-fold higher activity of **22** over **1b**: although these compounds share the phenylpentyl group and have similar *c*log*P*'s, the linking ester group of **22** is likely to render its ketone serine trap more electrophilic than the corresponding ether-linked ketone of **1b**.[14] This soluble assay SAR is consistent with an inhibition mechanism which involves the lipophilic groups binding in the arachidonyl-binding pocket of $cPLA_2$, presumably a highly lipophilic domain, and the ketone functionality binding the serine nucleophile of the enzyme as a hemiacetal. The SAR of the whole cell assay is more

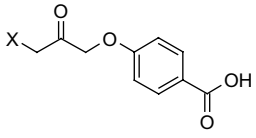
Table 1. cPLA$_2$ inhibition and *c* log *P* of compounds **1a–c**, **12–21**

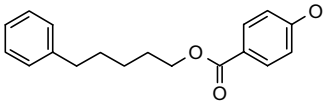


Compound	R	Soluble assay IC_{50} (μM[a])	HL60 cell assay IC_{50} (μM[b])	*c*Log*P*[c]
1a		0.03	2.8	7.8
1b		0.46	2.6	6.6
1c		0.08	5.8	7.2
12		1.7	NT[d]	5.5
13		34	NT[d]	4.6
14		0.08	1.0	6.3
15		0.76	1.1	5.9
16		12	NT[d]	6.1
17		0.14	1.1	6.5
18		5.2	NT[d]	4.8
19		0.59	4.8	5.8
20		63	NT[d]	4.7
21		0.25	2.0	5.8

[a] Inhibition of recombinant cPLA$_2$ measured against a soluble substrate. IC_{50} values are the means of at least two independent observations. Errors are within ±20%.
[b] Inhibition of ^{3}H-arachidonic acid production by stimulated HL60 cells.
[c] Lipophilicity as calculated by Daylight.[13]
[d] Not tested.

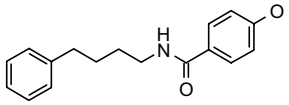
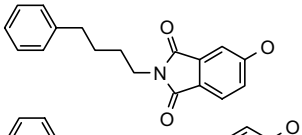
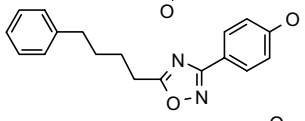
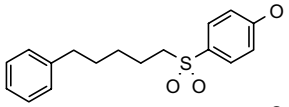
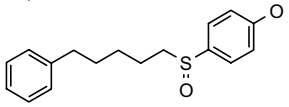
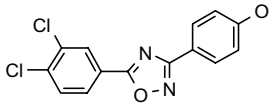
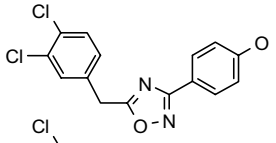
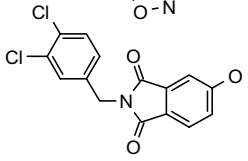
Table 2. cPLA$_2$ inhibition and *c* log *P* of compounds **22–30**



Compound	X	Soluble assay IC_{50} (μM[a])	HL60 cell assay IC_{50} (μM[b])	*c*Log*P*[c]
22		0.05	1.3	6.8

(*continued on next page*)

Table 2 (*continued*)

Compound	X	Soluble assay IC_{50} (μM[a])	HL60 cell assay IC_{50} (μM[b])	*c*Log*P*[c]
23		20	NT[d]	5.1
24		0.18	0.76	5.8
25		0.27	0.76	6.2
26		1.0	5.2	5.5
27		1.8	18	5.2
28		0.04	4.7	6.7
29		0.09	2.7	5.9
30		0.56	1.0	5.5

[a] Inhibition of recombinant $cPLA_2$ measured against a soluble substrate. IC_{50} values are the means of at least two independent observations. Errors are within ±20%.
[b] Inhibition of ^{3}H-arachidonic acid production by stimulated HL60 cells.
[c] Lipophilicity as calculated by Daylight.[13]
[d] Not tested.

complex: superimposed on the intrinsic lipophilicity requirement of the enzyme is a tendency for the more lipophilic inhibitors to partition into the cell membranes of the assay, thus lowering their effective concentration and therefore their apparent activity in the cellular assay. Consequently, the ratio of whole cell IC_{50} to soluble assay IC_{50} tends to decrease as the inhibitor $c\log P$ is lowered (Fig. 2), a trend which accounts for the improvement in cellular potency on going from **28** to **29** to **30** in counterpoint to the accompanying decrease in soluble assay activity. Clearly an understanding of these complex, overlaying SAR's, driven by both true enzyme binding and lipophilic partitioning, is necessary to design potent $cPLA_2$ inhibitors of lower lipophilicity.

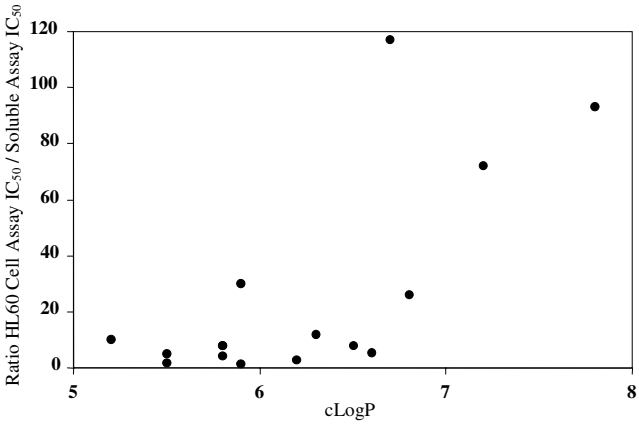


Figure 2. Relationship of ratio of HL60 cell assay IC_{50}/soluble assay IC_{50} with inhibitor $c\log P$.

In conclusion, an investigation into the SAR of the lipophilic alkyl chain of the $cPLA_2$ inhibitors **1** has been carried out, with the identification of several more 'drug-like' analogues such as **30** which display improved whole cell potencies against $cPLA_2$ along with lower $c\log P$'s. Further exploration of the electrophilic ketone serine trap and the carboxylic acid phosphate mimic will be the subject of future communications.

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