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Structure–Activity Relationships of Indole Cytosolic Phospholipase $A_2\alpha$ Inhibitors: Substrate Mimetics☆

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Abstract—An SAR effort focused on generating $cPLA_2\alpha$ inhibitors using a substrate mimetic approach is reported. Indole inhibitors of $cPLA_2\alpha$ with promising pharmacokinetic parameters that were active in both an isolated enzyme assay and in cell-based assays were discovered. Modeling these compounds into the $cPLA_2\alpha$ structure validated the assumptions made at the start of the SAR effort.

Cytosolic phospholipase $A_2\alpha$ ($cPLA_2\alpha$, a group IVA phospholipase) selectively cleaves the *sn*-2 position of arachidonyl-glycerophospholipids to generate free arachidonic acid.[1] This arachidonic acid is in turn metabolized[2] to a variety of inflammatory mediators including leukotrienes, prostaglandins and thromboxanes. If the lysophospholipid remaining after arachidonic acid cleavage contains an alkyl ether at the *sn*-1 position, acetylation forms yet another inflammatory mediator, platelet activating factor, PAF.[3] The discovery of inhibitors of $cPLA_2\alpha$ with drug-like properties would provide a novel therapeutic with applications in many disease states including osteoarthritis, rheumatoid arthritis, and asthma.[4]

High throughput screening of the corporate database did not generate viable leads thus a substrate-based approach to inhibitors was taken. Although the substrate of $cPLA_2\alpha$ is a glycerophospholipid, there is little discrimination based on the phospholipid headgroup or fatty-acid at the *sn*-1 position. Instead, $cPLA_2\alpha$ is selective for arachidonic acid[1] and this selectivity is due to recognition by $cPLA_2\alpha$ of the first five carbons[5] of the arachidonate ester at the *sn*-2 position. The phosphate region of the substrate is also recognized as evidenced by the inability to cleave diacylglycerol substrates. Thus a successful inhibitor should incorporate the features of a lipophilic arachidonate ester and the charged phosphate. When the project began, the only published inhibitors of $cPLA_2\alpha$ were those from Merkle[6] and Merck.[7] Known inhibitors of downstream enzymes that process arachidonic acid metabolites such as Zafirlukast,[8] an LTD_4 receptor antagonist, and MK 886,[9] a Flap antagonist, are also relevant and suggested a starting point for arachidonic acid mimetics. Comparing these $cPLA_2\alpha$ inhibitors to Zafirlukast and MK 886 revealed a common motif, a template that supports an acid and a lipophilic group to mimic the arachidonate ester. The results of a study applying this approach are presented here. Zafirlukast was chosen as a starting point because it was designed to be a crude arachidonic acid mimetic[10] and it displayed desirable pharmacokinetic properties.

A monomeric or *soluble* substrate, 7-hydroxycoumarinyl 6-heptenoate, was used in the primary screening assay.[11] This 'coumarin assay' removes the possibility that the inhibitor acts by disrupting the membrane surface present in a traditional phospholipase assay. Thus the SAR is based on interactions between the inhibitor and enzyme. This substrate was chosen because it is monomeric to 80 uM, and lipophilic compounds should not be able to induce micelle formation and change the kinetics of the reaction. Cell-based assays[9,12] that demonstrated cell permeability, inhibition of arachidonic acid release as well as activity

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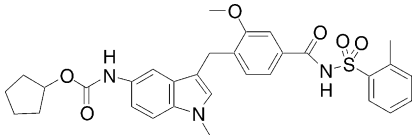
against production of the downstream metabolite LTB_4 were chosen as the secondary assays.



Zafirlukast

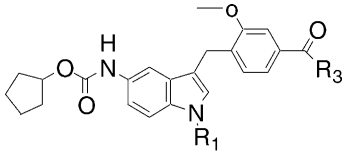
The medicinal chemistry strategy was to systematically modify the acidic region, the group at the indole N_1 position and the substituent at C_5 of the indole in Zafirlukast to increase cPLA$_2\alpha$ inhibition. The initial synthetic strategy was to follow the published route[8] however this did not provide the required synthetic flexibility. A new route[13] based on reductive alkylation at the indole C_3, with or without substitution at N_1, provided the requisite flexibility.[14]

The analogues in Table 1 quickly demonstrated that increases in the steric bulk of the group at N_1 significantly increased the affinity for cPLA$_2\alpha$, **5** is approximately 10-fold more potent than **1**. Fortuitously the increase in steric bulk at the N_1 position of Zafirlukast analogues (phenyl sulfonamides vs *o*-tolyl sulfonamides shown here) had previously been reported[8] to have a large deleterious effect on LTD_4 receptor binding. For example, the change from methyl to cyclopentyl was reported to result in an 80-fold decrease in K_i values for LTD_4 receptor antagonism. The benzoic acid is equipotent with the acylsulfonamide acid mimetic as evidenced by **6**.

Analoging at the indole C_5 position while maintaining the benzhydryl and acid functionality led to the data shown in Table 2. Amino, carbamate, urea and sulfonamide substitution, **7**–**10**, were less potent than **6**. As electron-withdrawing groups were substituted at this position a significant increase in activity was seen. Halo, nitro and cyano indoles, **12**–**16**, all showed inhibition <1 μM against cPLA$_2\alpha$. In addition, both methyl and methoxy substitution showed low micromolar IC_{50}s.

The most potent of the analogues from Table 2, compounds **12** and **14**–**17**, were more closely examined in cell-based assays. The results of these assays and pharmacokinetic analysis are shown in Table 3. These data indicate that all of these compounds are cell permeable, and that they are able to inhibit production of arachidonic acid, which gives a direct measurement of cPLA$_2\alpha$ inhibition in a cell-based system. They are also able to

Table 1. Modification of N_1 and the acidic functionality of Zafirlukast



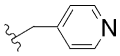
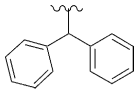
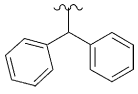
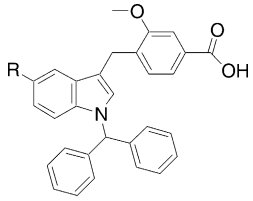
Compd	R_1	R_2	Coumarin IC_{50} uM
1[8]	CH_3	$NHSO_2$-*o*-tolyl	85
2		$NHSO_2$-*o*-tolyl	20
3		$NHSO_2$-*o*-tolyl	17
4		$NHSO_2$-*o*-tolyl	38
5		$NHSO_2$-*o*-tolyl	6
6		OH	5

Table 2. Modification of the indole C_5 position



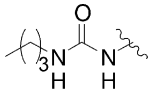
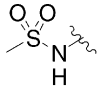
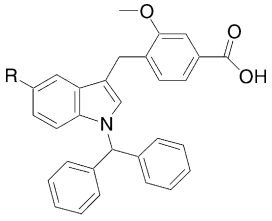
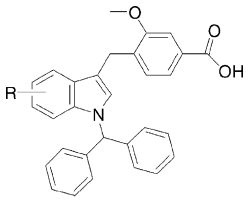
Compd	R	Coumarin IC_{50} uM
7	NH_2	41
8	$N(CH_3)_2$	24
9		15
10		20
11	CH_3SO_2	2.0
12	NO_2	0.4
13	F	1.0
14	Cl	0.4
15	Br	0.6
16	CN	0.8
17	CH_3	0.9
18	OCH_3	1.9
19	H	3.6

Table 3. Secondary assays and pharmacokinetic data



Compd	R	PMN AA release IC_{50} uM	PMN LTB4 IC_{50} uM	MC-9 LTB4 IC_{50} uM	% F Rat 10 mg/kg
12	NO_2	1.8	0.5	2.8	NT
14	Cl	1.8	0.4	1.6	22
15	Br	1.2	0.4	1.1	16
16	CN	1.3	0.8	2.1	19
17	CH_3	NT	0.4	0.9	38

Table 4. Substituion of indole carbocycle



Compd	R	Coumarin IC_{50} uM	% F Rat 10 mg/kg
20	4-Cl	4.5	NT
14	5-Cl	0.5	22
21	6-Cl	0.8	63
22	7-Cl	2.1	NT

inhibit metabolites of arachidonic acid, LTB_4 for example, in both human neutrophils, PMN assay, and in a mast cell line, MC-9 assay. Early application of discovery pharmacokinetics (PK) was considered essential because $cPLA_2\alpha$ has an affinity for large, lipophilic inhibitors and substrates. Discovery PK[15] was used in a screening mode to quickly remove compounds with poor bioavailability in rats (% F). The results in Table 3 show that all of the selected compounds have reasonable oral absorption. The chloro analogue, **14**, was chosen for further analoging.

The effect of moving the substitution around the indole carbocycle is presented in Table 4. The results demonstrate that C_4 and C_7 substitution, **20** and **22**, had a significantly negative impact on potency in the isolated enzyme assay. Both C_5 and C_6 substituted analogues were potent inhibitors in both assay systems (**21** has an IC_{50} in the MC-9 LTB_4 assay of 0.8 μM) however, **21** presents a significant advantage due to the high bioavailability seen in the pharmacokinetics studies. Thus, **21** represents a viable lead in that it is a potent inhibitor in an isolated enzyme assay, it inhibits in cell-based assay systems, and it possesses useful pharmacokinetic properties.

To better understand the binding of these inhibitors to the enzyme a connection between the previously published[5] crystal structure for full length $cPLA_2\alpha$ was sought. To this end a docking experiment was run for a group of inhibitors using the program FLO[16] for Monte Carlo simulation. Figure 1[17] shows **21** docked into the active site of $cPLA_2\alpha$. The protein is depicted as a Connolly surface colored by electrostatic potential and the docked ligand **21** is shown as van der Waals radii spheres to visualize inhibitor fit. As postulated earlier, the acid interacts with the phosphate-binding region, Arg 200, Asn 555 and the backbone amide nitrogen of Gly 197 (Fig. 2). The catalytic Ser 228 is more than 5 Å away from the acid. The substituent at C_5 or C_6 must be small to avoid severe van der Waals clashes with the enzyme. One of the phenyl groups of the benzhydryl fits nicely into a lipophilic groove and the other interacts with the lipophilic underbelly of the lid, an α helical region that covers the active site. The modeling results confirmed the initial hypothesis of inhibitor binding and shed light on the allowed groups on the indole carbocycle.

Indole inhibitors of $cPLA_2\alpha$ have been developed using a substrate mimetic approach. Optimization of the acid functionality, the N_1 and the C_5 substituents generated potent inhibitors of $cPLA_2\alpha$ in an isolated enzyme assay. Specifically, an acid or acid mimetic had equal potency, and a large lipophilic group at the N_1 was preferred as was a small electron withdrawing group on the indole carbocycle at the C_5 or C_6 position. The most

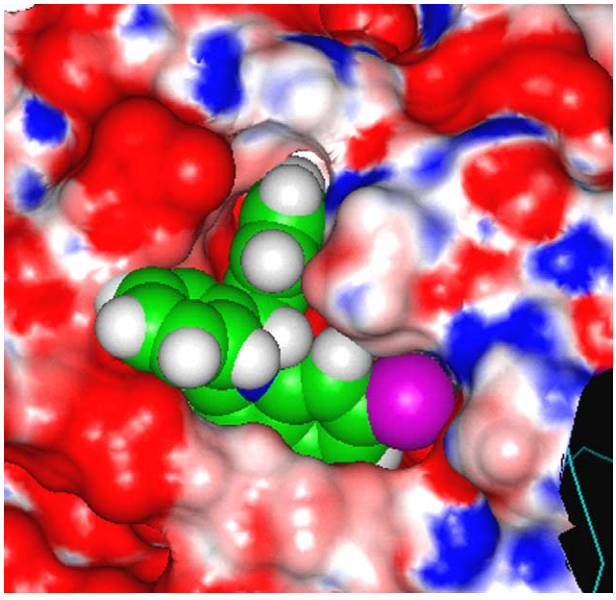


Figure 1. 21 depicted as van der Waals radii spheres shown in the protein with a Connolly surface colored by electrostatic potential.

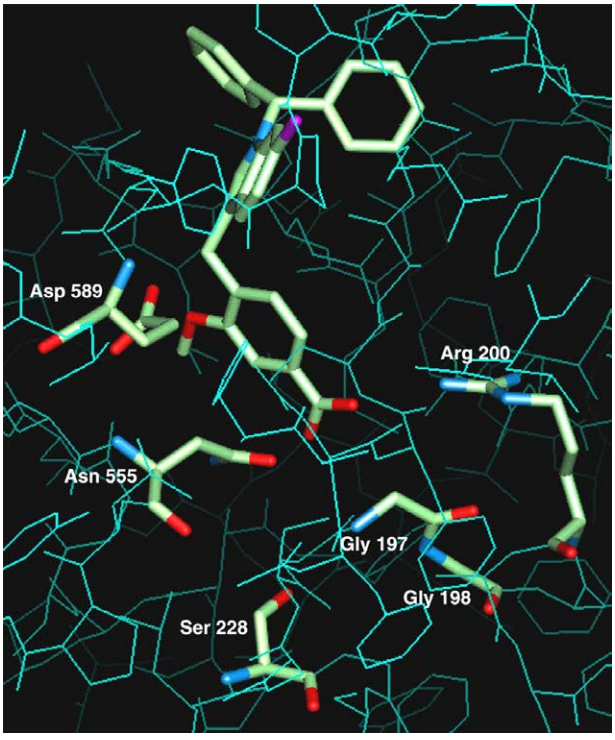


Figure 2. Interactions of the docked inhibitor (**21**) with the $cPLA_2\alpha$ protein structure.

potent of these inhibitors were also shown to have activity in cell-based assays inhibiting generation of both arachidonic acid and its metabolites. Additionally these compounds were profiled in discovery pharmacokinetic experiments and **21** proved to have the best combination of potency and pharmacokinetic properties. Finally, a rationalization of the SAR was observed upon docking **21** into the crystal structure of full length $cPLA_2\alpha$. This exercise validated the theory that the carboxylate was acting as a phosphate mimetic and defined a small binding pocket for the C_5 or C_6 substituent which agreed with the observed SAR data. Further optimization of **21** will be presented in due course.

Acknowledgements

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References and Notes

1. (a) Clark, J. D.; Schievella, A. R.; Nalefski, E. A.; Lin, L.-L. *J. Lipid Mediators Cell Signal.* **1995**, *12*, 83. (b) Six, D. A.; Dennis, E. A. *BioChim. BioPhys. Acta* **2000**, *1488*, 1.
2. Smith, W. L. *Biochem. J.* **1989**, *259*, 315.
3. Wasserman, S. I. *Hosp. Pract.* **1988**, *15*, 49.
4. (a) Sapirstein, A.; Bonventre, J. V. *Biochim. Biophys. Acta* **2000**, *1488*, 139. (b) Nagase, T.; Uozumi, N.; Ishii, S.; Kume, K.; Izumi, T.; Ouchi, Y.; Shimizu, T. *Nat. Immunol.* **2000**, *1*, 42. (c) Lehr, M. *Drugs Future* **2000**, *25*, 823.
5. Dessen, A.; Tang, J.; Schmidt, H.; Stahl, M.; Clark, J. D.; Seehra, J.; Somers, W. *Cell* **1999**, *97*, 349.
6. Lehr, M. *J. Med. Chem.* **1997**, *40*, 2694.
7. Abdullah, K. H.; Perrier, H.; Leger, S. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 519.
8. Matassa, V. G.; Maduskuie, T. P.; Shapiro, H. S.; Heep, B.; Snyder, D. W.; Aharony, D.; Krell, R. D.; Keith, R. A. *J. Med. Chem.* **1990**, *33*, 1781.
9. Gilliard, J. W.; Ford-Hutchinson, A. W.; Chan, C.; Charleson, S.; Denis, D.; Foster, A.; Fortin, R.; Legere, S.; McFarlane, C. S.; Morton, H.; Piechuta, H.; Riendeau, D.; Rouzer, C. A.; Rokach, J.; Young, R. N.; MacIntyre, D. E.; Peterson, L.; Bach, T.; Eiermann, G.; Hopple, S.; Humes, J.; Hupe, D.; Luell, S.; Metzger, J.; Meurer, R.; Miller, D. K.; Opas, E.; Pachalok, S. *Can. J. Physiol. Pharmacol.* **1989**, *67*, 456.
10. (a) For a more rigorous approach to arachidonic acid mimetics, see: Bonzom, P. M.; Toth, G.; Gibbons, W. A. *Biochemical Society Transactions* **1998**, *26*, S32. (b) Bonzom, P. M.; Zloh, M.; Reid, R. E.; Gibbons, W. A. *Biochem. Soc. Trans.* **1997**, *25*, 26 S.
11. Biological data are the mean of multiple experiments performed in duplicate: Huang, Z.; Laliberte, F.; Tremblay, N. M.; Weech, P. K.; Street, I. P. *Anal. Biochem.* **1994**, *222*, 110.
12. Hagmann, W. *Biochem. J.* **1994**, *299* (Pt 2), 467.
13. (a) Mahadevan, A.; Sard, H.; Gonzalez, M.; McKew, J. C. *Tetrahedron Lett.* **2003**, *44*, 4589. (b) Appleton, J. E.; Dack, K. N.; Green, A. D.; Steele, J. *Tetrahedron Lett.* **1993**, *34*, 1529.
14. All final compounds were characterized by ^1H NMR and either HRMS, LC/MS or CHN.
15. Formulation for PO PK studies was EtOH/PEG 400. Analysis was performed with HPLC (UV detection).
16. McMartin, C.; Bohacek, R. S. *J. Comput.-Aided Mol. Des.* **1997**, *11*, 333.
17. The figure was generated using InsightII (Accelyrs, 2002, San Diego, CA, USA).