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1,2,4-Oxadiazolidin-3,5-diones and 1,3,5-triazin-2,4,6-triones as cytosolic phospholipase $A_2\alpha$ inhibitors

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Abstract—1,2,4-Oxadiazolidin-3,5-dione and 1,3,5-triazin-2,4,6-trione scaffolds were employed as templates to incorporate the pharmacophore requirements of cytosolic phospholipase $A_2\alpha$ substrate mimetics. Inhibitors that are active in both enzyme, and cell-based assays were identified from both classes. From the SAR work carried out and modeling efforts around these templates, the triazinetrione scaffold with an additional substitution point was found to be more favorable.

Leukotrienes and prostaglandins are important mediators of inflammation, generated via the 5-lipoxygenase and cyclooxygenase pathways, respectively. Prostaglandins and leukotrienes are unstable and are not stored in cells, but are instead synthesized[1] from arachidonic acid in response to stimuli. Arachidonic acid, which is fed into these two distinct inflammatory pathways, is released from the *sn-2* position of membrane phospholipids by cytosolic phospholipase $A_2\alpha$[2] ($cPLA_2\alpha$, a group IVA phospholipase). When the phospholipid substrate of PLA_2 is phosphatidylcholine with an ether linkage in the *sn-1* position, the lysophospholipid produced is the immediate precursor of platelet-activating factor (PAF), another potent mediator of inflammation.[3]

Most anti-inflammatory therapies have focused on preventing production of either prostaglandins or leukotrienes, but not both of them. It is highly desirable to identify compounds that inhibit the actions of $cPLA_2\alpha$ for use in treating or preventing inflammatory conditions, particularly where these classes of lipid mediators might have an additive or synergistic pro-inflammatory effect. Consequently, the direct inhibition of the activity of $cPLA_2\alpha$ has been suggested as a useful mechanism for a therapeutic agent with application in number of disease states including osteoarthritis, rheumatoid arthritis, and asthma.[4]

Recent reports[5] from our laboratory disclosed our efforts in this area that identified indoles **2** as $cPLA_2\alpha$ inhibitors. A substrate-based approach was taken using zafirlukast **1**[6] as a starting point since it was designed to be an arachidonic acid mimetic with a template that supports an acidic and a lipophilic group. In continuation of this project, we were evaluating alternate templates that can be utilized to package the required pharmacophore. In this report, we describe the identification of two alternate scaffolds—1,2,4-oxadiazolidin-3,5-dione **3** and 1,3,5-triazin-2,4,6-trione **4** (Figs. 1 and 2).

The compounds of interest could be readily prepared[7] as described in Schemes 1 and 2 . Benzophenone oxime **6** was reduced to the hydroxyl amine intermediate **7**, which was cyclized with chlorocarbonylisocyanate to give the scaffold oxadiazolidinedione **8** with the benzhydryl lipophilic group. Mitsunobu reaction with the required alcohol bearing the appropriate acid region precursor afforded the aldehyde **10**, which was oxidized to give the final target **11**. Triazin-2,4,6-trione analogs were synthesized starting from benzhydrylamine **12** and the isocyanate of interest to give the urea **14**. Cyclization of **14** to triazinetrione **15** followed by alkylation afforded the aldehyde, which was oxidized to give the final target acid **17**.

Keywords: 1,2,4-Oxadiazolidin-3,5-dione; 1,3,5-Triazin-2,4,6-trione; Cytosolic phospholipase $A_2\alpha$ inhibitors.
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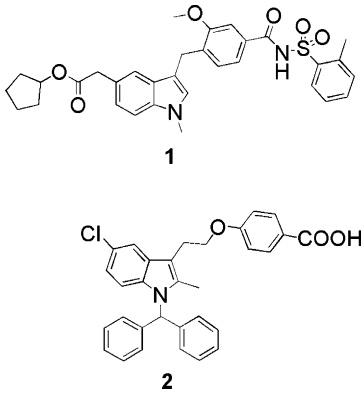


Figure 1. LTD4 receptor antagonist Zafirlukast (**1**) and cPLA$_2\alpha$ substrate mimetic inhibitor (**2**).

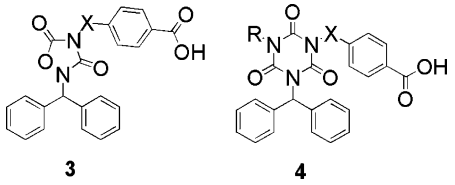
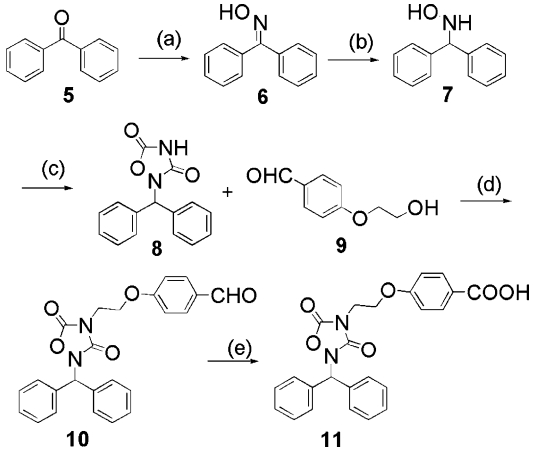
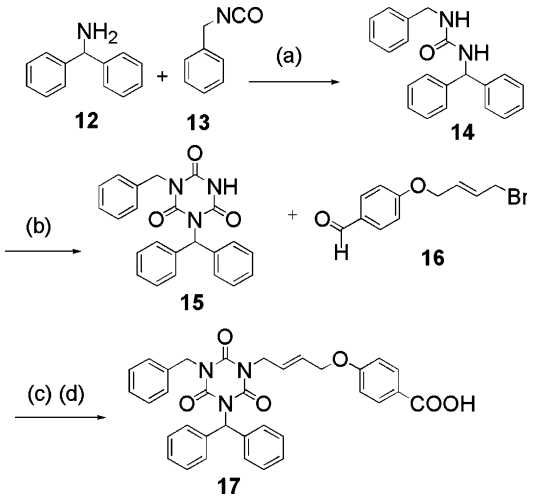


Figure 2. Template modification for cPLA$_2\alpha$ substrate mimetic **2**.

To explore the feasibility of scaffold exchange in **2**, oxadiazolidin-3,5-dione analog **11** was synthesized retaining the benzhydryl moiety for the lipophilic region and the benzoic acid portion of the molecule. While this direct analog was not potent, a small change in the lipophilic group by introducing a methylene spacer between the ring and the benzhydryl group afforded **18** with a significant boost in enzyme activity. However, analog **19** with a more flexible lipophilic moiety was inactive. At this stage we decided to maintain the lipophilic region as diphenylethyl group and systematically vary the acid region. Moving the acid to a 3-benzoic acid derivative as in analog **20** was unfavorable. Adding an additional 3-hydroxyl substitution to the 4-benzoic acid (analog **21**) led to a 4-fold loss in activity. Chain extension between the scaffold and benzoic acid by one



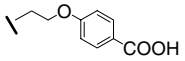
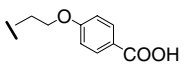
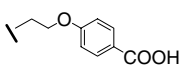
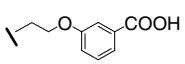
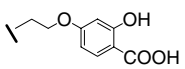
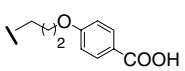
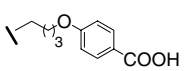
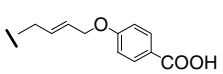
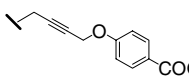
Scheme 1. Reagents and conditions: (a) NH_2OH, pyridine, ethanol, 60 °C, 3 h, 89%; (b) BH_3-Py, MeOH, 10% HCl (pH 2), rt, 18 h, 68%; (c) ClCONCO, THF, 0 °C to rt, 3 h, 80%; (d) **9**, Ph_3P, DEAD, THF, rt, 8 h, 68%; (e) sulfamic acid, $NaClO_2$, THF–H_2O, rt, 3 h, 86%.



Scheme 2. Reagents and conditions: (a) CH_2Cl_2, rt, 12 h, 90%; (b) ClCONCO, THF, 0 °C to rt, 3 h, 78%; (c) NaH, THF, 16, rt, 18 h, 67%; (d) sulfamic acid, $NaClO_2$, THF–H_2O, rt, 3 h, 81%.

methylene unit as in compound **22** led to a slight loss in activity, but further extension (compounds **23**) was not desirable. Introduction of unsaturation (compounds **24**–**25**) diminished the activity as well (see Table 1).

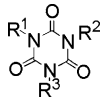
Table 1. 1,2,4-Oxadiazolidin-3,5-diones

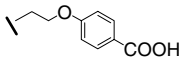
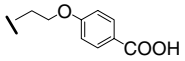
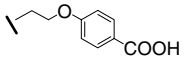
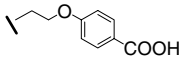
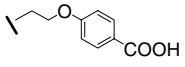
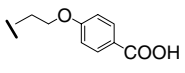
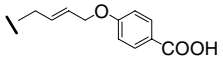
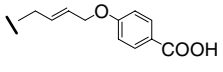
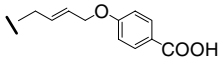
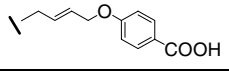
Compd	R^1	R^2	Glu-Mic IC_{50} (μM)[8]
2	Reference compound		3
11			>50
18			6.7
19			>50
20			>50
21			27
22			12.4
23			>50
24			>50
25			23

A similar approach was undertaken to evaluate the second scaffold of interest—triazine-2,4,6-trione. However, this scaffold has the potential to carry three different substituents. For direct comparison to **2**, analogs were prepared retaining the benzhydryl group for the lipophilic region and the ethoxy-4-benzoic acid for the acid mimetic. The third substituent was varied from a small ethyl group to a bulky benzyl group (compounds **26**–**29**). It was interesting to notice that the compounds were active and the activity range was within a 2-fold variation, indicating that while the third substituent gives a boost in activity, it does not show any significant preference for another bulky group. However, a rigid phenyl group directly attached to the scaffold, **30** was inactive.

Unlike the oxadiazolidin-3,5-dione scaffold in the case of triazin-2,4,6-trione, installing diphenylethyl group (compound **31**) for the lipophilic region did not improve the enzyme potency rather rendered the molecule 10-fold less active. Probably the rigid benzhydryl group is well accommodated in the binding pocket since this scaffold has a larger six-membered ring. Also, with triazin-2,4,6-triones, chain extension was well tolerated and the butenyloxy benzoic acid chain (compound **32**) was found to be slightly more potent than the ethoxy benzoic acid group. As we observed before, with this acid chain, the size of the third substituent did not play a major role since compounds **32**–**35** showed similar potency (see Table 2).

Table 2. 1,3,5-Triazin-2,4,6-triones



Compd	R^1	R^2	R^3	Glu-Mic IC_{50} (μM)[8]
2	Reference compound			3
26			Ethyl	7.5
27			Allyl	5
28			*n*-Butyl	5.6
29			Benzyl	3.5
30			Phenyl	>50
31			Benzyl	30
32			Ethyl	1.6
33			Allyl	1.3
34			*n*-Butyl	2.4
35			Benzyl	1.3

One of the most potent analogs **35** from this effort was further characterized in secondary cell-based assays.[9] The compound was found to be cell permeable and was able to inhibit both LTB_4 (IC_{50} = 0.5 μM) and $PGF_{2\text{-}\alpha}$ (IC_{50} = 0.8 μM) synthesis in a mast cell line (MC-9 assay). In addition, the compound no longer inhibited $PGF_{2\text{-}\alpha}$ synthesis when exogenous arachidonic acid was added to bypass $cPLA_2\alpha$ indicating that the compound is also selective for the target in this cell-based assay. The compound was also active (IC_{50} TXB_2: 5.2 μM) in the ionophore stimulated rat whole blood assay (COX-1-dependent assay).

Modeling efforts were undertaken to validate the scaffold tolerance. Towards this end, compound **35** and a series of triazine analogs were docked into the X-ray structure of $cPLA_2\alpha$[10] via Monte Carlo simulation[11]. Figure 3 shows a model of compound **35** bound to $cPLA_2\alpha$ with a partial molecular surface (colored by element) of the active site region. The acid interacts with the backbone amide nitrogens of Gly 197 and Gly 198, which are in an approximate *cis* conformation thought to stabilize the developing oxyanion during ester hydrolysis, and also makes a weak interaction with Ser 228 (4.0 Å to the hydroxyl oxygen), the nucleophile that attacks the *sn*-2 ester[12]. The linker oxygen makes a hydrogen bond with the backbone amide nitrogen of Ala 578 closer to the opening of the pocket.

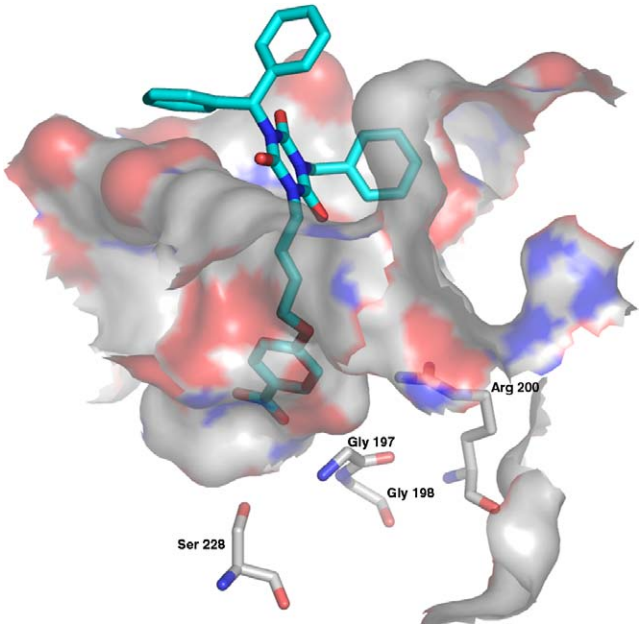


Figure 3. Interaction of 1,3,5-triazin-2,4,6-trione inhibitor **35** with $cPLA_2\alpha$ protein structure. The figure was generated using PyMOL (DeLano Scientific, 2002, San Carlos, CA, USA).

In conclusion, we have explored the feasibility of pharmacophore repackaging with two different novel scaffolds—1,2,4-oxadiazolidin-3,5-dione and 1,3,5-triazin-2,4,6-trione. While 1,2,4-oxadiazolidin-3,5-dione provided modest inhibitors, 1,3,5-triazin-2,4,6-trione with an additional substituent to pick up further interaction was more favorable. Good SAR trend for the enzyme activity was observed for this novel scaffold along with activity in the cell-based systems.

Acknowledgment

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References and notes

1. Smith, W. L. *Biochem. J.* **1989**, *259*, 315.
2. (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.
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. (a) McKew, J. C.; Lovering, F.; Clark, J. D.; Bemis, J.; Xiang, Y.; Shen, M.; Zhang, W.; Alvarez, J. C.; Joseph-McCarthy, D. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4501; (b) McKew, J. C.; Foley, M. A.; Thakker, P.; Behnke, M. L.; Lovering, F. E.; Sum, F.-W.; Tam, S.; Wu, K.; Shen, M. W. H.; Zhang, W.; Gonzalez, M.; Liu, S.; Mahadevan, A.; Sard, H.; Khor, S. P.; Clark, J. D. *J. Med. Chem.* **2005**, *49*, 135.
6. 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.
7. (a) Parallel solution-phase synthesis was carried out in 8 mL vials in 0.1 mmol scale using an orbital shaker. Reaction completion was monitored by LC–MS and the solvent was removed in Savant evaporator and compounds were purified by HPLC and the purity was >90%. LC conditions: HP 1100, 23 °C, 10 μL injected; column: YMC-ODS-A 4.6 × 50 mm 5μ; gradient A: 0.05% TFA/water, gradient B: 0.05% TFA/acetonitrile; time: 0 and 1 min: 98% A and 2% B: 7 min: 10% A and 90% B; 8 min: 10% A and 90% B; 8.9 min: 98% A and 2% B; post-time 1 min; flow rate 2.5 mL/min; detection: 215 and 254 nm, DAD. Semi-Prep HPLC: Gilson with Unipoint software; Column: Phenomenex C18 Luna 21.6 mm × 60 mm, 5 μ; solvent A: water (0.02% TFA buffer); solvent B: acetonitrile (0.02% TFA buffer); solvent gradient: time 0: 5% B; 2.5 min: 5% B; 12 min: 95% B; hold 95% B 3 min; flow rate: 22.5 mL/min; detection: 215 and 254 nm. (b) ^{1}H NMR data for compound 35: (DMSO) δ 7.85 (d, 2H), 7.26–7.36 (m, 15H), 7.25 (s, 1H), 7.0 (d, 2H), 5.88 (br s, 2H), 4.92 (s, 2H), 4.6 (s, 2H), 4.39 (s, 2H).
8. For Glu-Mic assay details, see Ref. 5b.
9. (a) 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; (b) Hagmann, W. *Biochem. J.* **1994**, *299*(Pt. 2), 467.
10. Dessen, A.; Tang, J.; Schmidt, H.; Stahl, M.; Clark, J. D.; Seehra, J.; Somers, W. *Cell* **1999**, *97*, 349.
11. McMartin, C.; Bohacek, R. S. *J. Comput. Aided Mol. Des.* **1997**, *11*, 333.
12. While Arg 200 is proposed to stabilize the binding of the negatively charged phosphate group of the phospholipid substrate, Ser 228 acts as the nucleophile and attacks the *sn*-2 ester to form the acyl enzyme intermediate. The oxyanion hole, putatively formed by Gly 197 and Gly 198, polarizes the *sn*-2 ester and stabilizes the tetrahedral intermediate (see Ref. 10). In the model of 35, the acid group most closely mimics the ester. Models of less potent analogs, with shorter linkers, showed the acid group interacting with Arg 200. However, compounds predicted to bind deeper in the active site cleft showed enhanced affinity.