



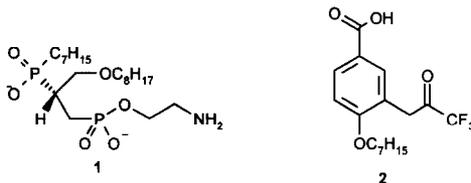
NOVEL FRAMEWORKS FOR TRIFLUOROMETHYL KETONE AND PHOSPHONATE TSA INHIBITORS OF TYPE II PLA₂

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Abstract. Design and synthesis of some TSA inhibitors on novel molecular frameworks is described. This TSA analog design culminates in the preparation of the phosphonate **18**. © 1997 Elsevier Science Ltd.

Phospholipases A₂ (PLA₂s)¹ catalyze the hydrolysis of fatty acids from the *sn*-2 position of glycerophospholipids. Arachidonic acid liberated in this manner may be metabolised to proinflammatory mediators such as prostaglandins and leukotrienes. The lysophospholipid may be acetylated to produce platelet activating factor (PAF). The low molecular weight (14 kDa), Ca²⁺-dependent, extracellular type II PLA₂² (also called *secretory* or *s*-PLA₂) has been found in high concentrations in the synovial fluid of patients with rheumatoid arthritis,³ and also in a variety of inflammatory cells, mediating arachidonic acid release and eicosanoid generation.⁴ Hence, it has been suggested that inhibitors of this enzyme may have therapeutic value. The catalytic mechanism of this enzyme has been studied in detail⁵ and the three dimensional structure has been determined both in its native form and complexed with a transition-state analogue.⁶ A more recently described high molecular weight (85 kDa) cytosolic PLA₂ (cPLA₂), selectively cleaves arachidonic acid from membrane phospholipids and is involved in the rate-limiting cellular generation of physiologically active eicosanoids.⁷ While this enzyme is much less studied, there is ample evidence to support the involvement of both the secretory and the cytosolic PLA₂s in various inflammatory conditions.⁸



We describe herein the design and synthesis of some novel nonphospholipid frameworks for type II PLA₂ inhibition. The efficacy of phosphonate transition state analogs (TSAs) as PLA₂ inhibitors has been elegantly demonstrated by Gelb et al.,⁶ using compound **1**. This compound and other substrate analogs have provided structural information on interactions with functional groups in the enzyme active site, which can be

exploited in the development of novel inhibitors. At the inception of our PLA₂ inhibitor program, it was evident that the glycerol backbone could be replaced by an appropriately substituted benzene ring.⁹ To explore the utility of TSAs based on a 1,3-disubstituted arene framework, molecular modeling was used to design the trifluoromethyl ketone (TFK) **2** as an inhibitor of the *s*-PLA₂. Figure 1 shows all the proposed polar interactions compound **2** can make with the enzyme, in comparison⁶ with the Gelb inhibitor **1**. Both the Gelb phosphonate and the TFK **2** make several similar polar interactions with PLA₂, each contributing a ligand to the active site calcium in the form of an oxygen atom. A similar strategy, in which a 1,3,5-trisubstituted indole was used as the arene framework has been recently described.¹⁰

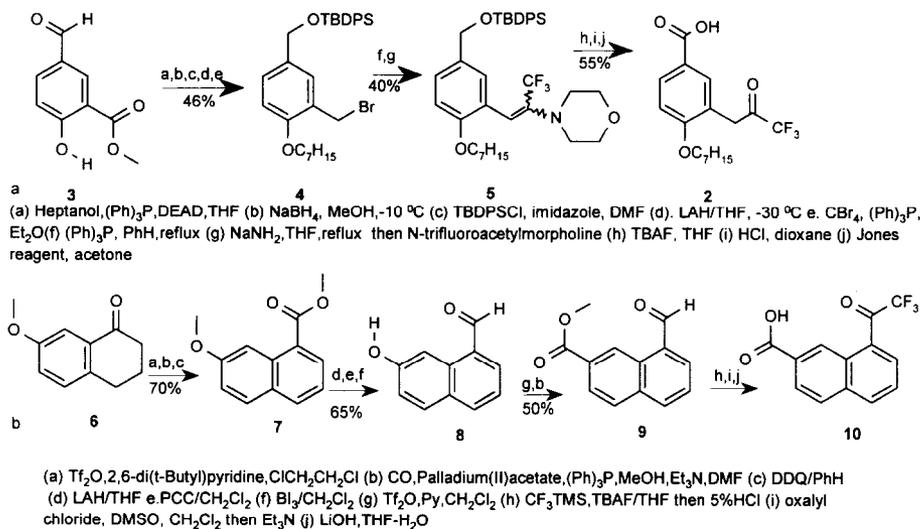
The preparation of **2** is outlined in Scheme Ia. Phenol **3** was converted to the corresponding heptyl ether under standard Mitsunobu conditions and routine functional group manipulations yielded the bromide **4**. Treatment with triphenylphosphine led to the phosphonium bromide, which was used in a Wittig reaction with 1-trifluoroacetyl morpholine to afford the alkene **5**.¹¹ Deprotection of the silyl ether and acid hydrolysis of the



Figure 1. Relative binding of hydrated TFK2 (Yellow, only three carbons of the heptyloxy group are shown for clarity) and the Gelb Phosphonate⁶ **1** (purple) in *s*-PLA₂ (cyan).²⁰ Dashed lines show the relevant polar interactions.

enamine followed by Jones oxidation yielded TFK 2.^{12,13} As shown in table 1, this compound had an IC₅₀ of 1.5 μM for type II PLA₂ inhibition. Unfortunately, this compound proved too unstable to allow further evaluation and we sought another framework.¹⁴ Based on the binding hypothesis depicted in Figure 1, we reasoned that replacement of the benzene ring of TFK 2 with a 1,7-substituted naphthalene scaffold would eliminate the reactive benzylic protons as well as rigidify the molecule. In addition, it would add some lipophilic bulk in a favorable location and afford the possibility of forming aromatic-aromatic interactions with Phe⁵.

The preparation of (1-trifluoroacetyl)-7-naphthoic acid (**10**) is detailed in Scheme Ib. The commercially available 7-methoxytetralone (**6**) was converted to methyl 7-methoxy-1-naphthoate (**7**) via enoltriflate carbonylation and DDQ dehydrogenation. Conversion of the methyl ester to the corresponding aldehyde and deprotection of the methyl ether led to the phenolic naphthaldehyde **8**. Triflation of the phenol and carbonylation led to compound **9**, which was converted to the corresponding trifluoromethylketone¹² by reacting with trifluoromethyl trimethylsilane and a catalytic amount of TBAF followed by oxidation of the carbinol. Saponification of the methyl ester led to the naphthoic acid **10**.¹³



Scheme I

Although the trifluoromethylketone **10** was a *s*-PLA₂ inhibitor¹⁵ at micromolar concentrations, the inhibitor dose-response was minimal. The pH profile of *s*-PLA₂ inhibition for TFK **10**, and TFK **2**, had very little pH dependence,¹⁶ suggesting that these compounds were not functioning as TSA inhibitors. Studies with compound **1** and non-TSA inhibitors have demonstrated significant pH dependence, consistent with either the

TSA hypothesis or a close interaction with hydrogen bonding or ionizable groups at the active site.¹⁶ In contrast, either the trifluoromethyl ketones are not acting as TSAs,¹⁶ or the hydroxyl interacting with the catalytic histidine is able to function as a hydrogen bond donor at high pH and as an acceptor at low pH. This poor inhibition profile can also be due to the fact that these compounds can not be enzymatically hydrated.¹³ To further test the viability of the naphthyl framework, we prepared the corresponding phosphonate(s) as transition state mimic.

Tetralone **6** was once again used as the starting material and was demethylated and benzylated to afford 7-benzyloxy tetralone. This compound was converted to the corresponding enolphosphonate,¹⁸ which was dehydrogenated to afford the naphthylphosphonate **11**. Phosphonate **11** was monodemethylated and alkylated with either octyl bromide or octadecyl bromide to afford the corresponding mixed phosphonates, which were debenzylated to afford the phenols **12a** and **12b**. Phenols **12a** and **12b** were transformed to the esters **13a** and **13b** by standard carbonylation protocol. The methyl ester and the methyl phosphonate could not be saponified in one step, and hence the methyl phosphonate was selectively dealkylated with LiBr, followed by basic hydrolysis to yield the phosphonates **14a** and **14b**. Phosphonate **14b** was a much better inhibitor of *s*-PLA₂, emphasizing the importance of appropriate lipophilic functional groups. Phosphonate **14b** still did not demonstrate the strong pH dependence of inhibition expected by comparison with compound **1**, although a modest improvement in inhibition was observed below pH 7.

Table 1. Biological Activity¹⁵ of *s*-PLA₂ inhibitors

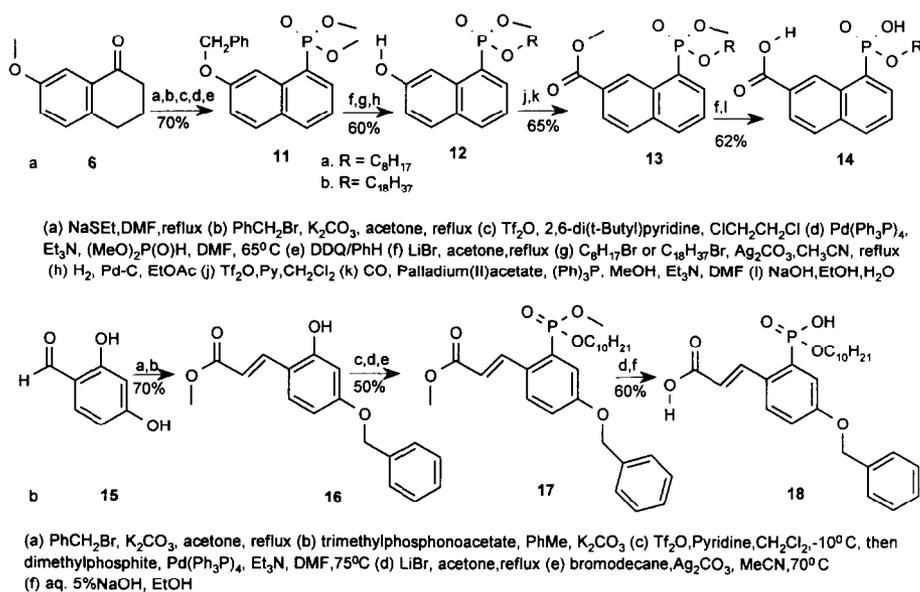
Compound	IC ₅₀ (μ M)	pH dependence	Human whole blood (LTB ₄)	Human Monocyte (LTC ₄)	c-PLA ₂ activity
1	1.5	none	inactive	not cell permeable ¹⁹	166 μ M
2	1.7	pKa 7.1	ND	not cell permeable ¹⁹	-40% at 30 μ M
10	2.4	none	ND	-76% at 30 μ M	-36% at 100 μ M
14a	>200	ND	ND	ND	ND
14b	3.9	weak	ND	not cell permeable ¹⁹	ND
18	4.5	pKa 7.1	-90% at 30 μ M	-44% at 30 μ M	ND

ND = not determined

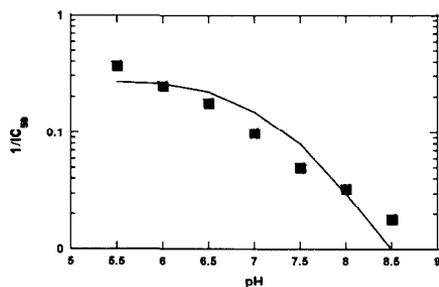
A further improvement in inhibitory activity was sought by introduction of a lipophilic substituent to mimic the *sn*-1 chain. Based on our postulated binding mode for the 1,7-naphthalene scaffold, we felt that the 3-position offered the best position for the *sn*-1 chain mimic. Because the preparation of such a trisubstituted naphthalene could be laborious, we chose compound **18** instead. The preparation of this phosphonate was initiated from 2,4-dihydroxy benzaldehyde (**15**), which was selectively benzylated and converted to the

cinnamate **16**. The phenol **16** was activated with triflic anhydride and converted to the corresponding dimethyl phosphonate. Nucleophilic cleavage and alkylation with bromodecane afforded the mixed phosphonate **17**. Selective cleavage and saponification of the methyl ester yielded the phosphonate **18**.

Phosphonate **18** was a potent inhibitor of PLA₂ (Table 1) and showed the expected pH profile for a phosphonate TSA (Figure 2), indicating the successful design of a TSA inhibitor. Compound **18** demonstrated good activity in whole cells and was active in whole human blood (Table 1). In conclusion, we have successfully employed the tools of molecular modeling and TSA analog design in conjunction with the X-ray crystallographic structure of *s*-PLA₂, to demonstrate the feasibility of designing non-glycerol substrate analog inhibitors of *s*-PLA₂, with improved activity in cellular assays.



Scheme II

Figure 2. pH profile of **18** (pK_a = 7.1)

References and Notes

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