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TETRAZOLE IS AN EFFECTIVE *Sn*-3 PHOSPHATE REPLACEMENT IN SUBSTRATE ANALOG INHIBITORS OF 14 kDa PHOSPHOLIPASE A_2

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Abstract: A series of substrate analog inhibitors of 14 kDa PLA_2 possessing replacements for the *sn*-3 phosphate moeity was prepared and evaluated. Tetrazole **26** possessed similar in vitro inhibition potency to phosphate-containing substrate analog inhibitors, but demonstrated superior cell permeability as monitored by LTC_4 release in monocytes. © 1997 Elsevier Science Ltd.

Introduction

Phospholipase A_2 catalyzes the *sn*-2 acylhydrolysis of phospholipids liberating free fatty acids, predominantly arachidonic acid, and lysophospholipids. These products can impart biological actions or be further metabolized to form a variety of proinflammatory lipid mediators including prostaglandins, leukotrienes, or platelet-activating factor. A human nonpancreatic Type II-14 kDa PLA_2 has been purified[1] and is found both in inflammatory cells[2] and in a variety of inflammatory exudate fluids, in soluble form,[3] Since Type II-14 kDa-PLA_2 enzyme has been associated with the initiation and/or the propagation of inflammatory episodes, its inhibition is an attractive approach toward the development of novel antiiflammatory agents.

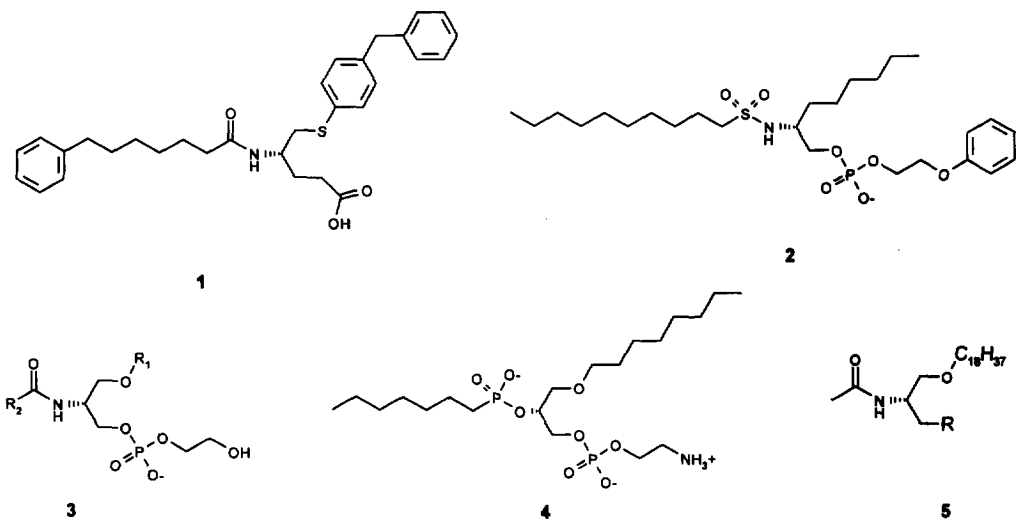


Figure 1

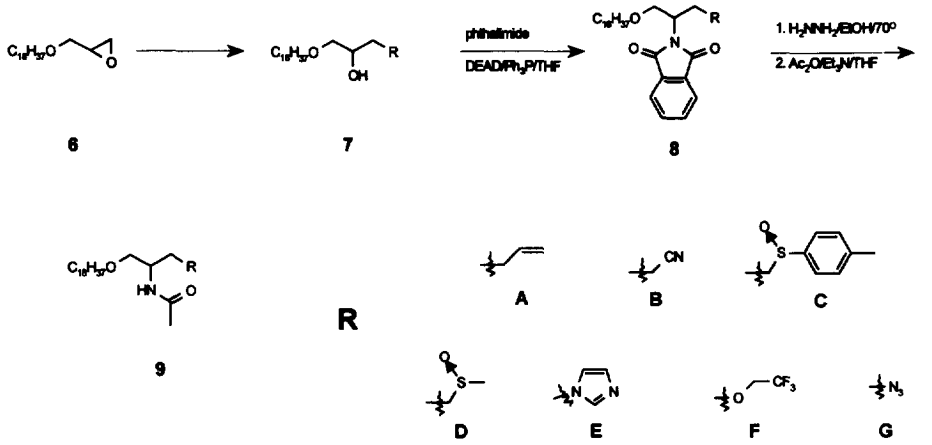
Two tight-binding substrate analogue inhibitors of 14 kDa PLA_2 (**1** and **2**, Figure 1) have been recently reported.[4,5] Earlier, de Haas had investigated a series of nonhydrolyzable *sn*-2 amides **3**,[6] and Gelb had reported on phosphonate **4**[7] as inhibitors of PLA_2. We were investigating the development of substrate analogue inhibitors for 14 kDa PLA_2 based on these studies by de Haas and Gelb. Since both **3** and **4** possess an *sn*-3 phosphate moiety, which has undesirable physical properties (membrane permeability) and metabolic instability (phosphate hydrolysis), we investigated the replacement of this functionality in order to make substrate analogs viable as potential therapeutic agents. Several *sn*-3 phosphate replacements in PLA_2 inhibitors have been reported, but their relative effects on inhibitor potency are unclear since they were prepared on varying inhibitor structures. This communication details our investigations into *sn*-3 phosphate mimics on an invariant inhibitor framework.

Results and Discussion

Screening of a SmithKline Beecham compound collection initially prepared as Platelet Activating Factor antagonists revealed that those possessing the general structure **5** (R = $OPO_3^-CH_2CH_2X$, X = OH, SMe, SMe_2^+, NMe_3^+) were low micromolar inhibitors of 14 kDa PLA_2. Important binding determinants within this class of inhibitors include the C_{18} tail which interacts favorably with the hydrophobic pocket of the phospholipid binding site,[8] and the amide NH which forms a hydrogen bond with Nδ of His-48. This latter interaction contributes approximately 1.5 kcal/mol of binding energy.[9] Therefore, **5** was chosen as the template onto which replacements for the *sn*-3 phosphate group were investigated.

All compounds were derived from the common racemic epoxide intermediate **6** (Scheme 1), obtained by coupling sodium octadecanoate with allyl bromide followed by MCPBA oxidation of the intermediate allyl ether. Opening of the epoxide with allyllithium (allyltriphenyltin/phenyllithium/diethyl ether/-78 °C),[10] acetonitrile anion (CH_3CN/*n*-BuLi/-42 °C),[11] *p*-toluenesulfinylmethyl anion (*p*-tol$SOCH_2Li$/1:1DME-DMF/100 °C),[12] dimsyl anion (*n*-BuLi/DMSO/-42 °C), imidazole (imidazole/K_2CO_3/DMF/70 °C),[13] trifluoroethanol (CF_3CH_2OH/KOH/90 °C), and sodium azide (NaN_3/NH_4Cl/EtOH/85 °C)[14] afforded the 2°-alcohols **7**. Conversion of the alcohols to the phthalimides under Mitsunobu conditions gave **8**,[15] and deprotection followed by acetylation furnished the acetamides **9**.

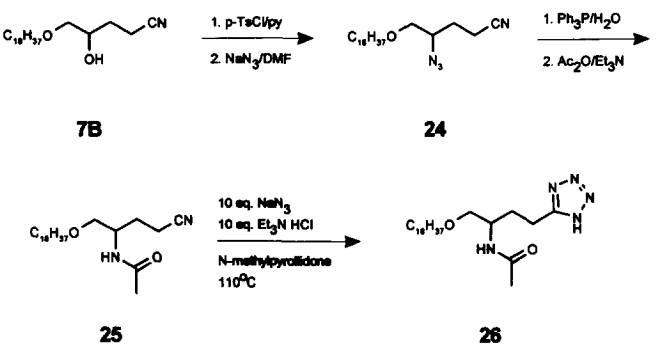
Compound **9A** served as a common intermediate for the preparations of **10** (H_2/10% Pd on C/1:1 ethyl acetate:MeOH), alcohol **11** (i. 9-BBN, ii. H_2O_2/NaOH), epoxide **12** (MCPBA/CH_2Cl_2), diol **13** (OsO_4/NMMO)[16] as an equimolar mixture of diastereomers and acid **14** ($RuCl_3$/$NaIO_4$/2:2:3 CH_3CN:CCl_4:H_2O).[17] Treatment of **14** with diazomethane in ether yielded methyl ester **15**.



Scheme 1

Ozonolysis and reductive workup of **8A** (i. O_3/-78 °C, ii. $NaBH_4$) followed by conversion of the phthalimide to the acetamide using the previously described conditions afforded **16**. Oxidation of **8A** ($MCPBA/CH_2Cl_2$), reductive opening (H_2/Pd black/5:1 EtOH:ethyl acetate) of the terminal epoxide, and conversion of the phthalimide to the acetamide gave **17**. Subsequent oxidation (TPAP/NMMO/4 A sieves)[18] of **17** yielded ketone **18**.

Oxidation of sulfoxides **9C** and **9D** ($MCPBA/CH_2Cl_2$) afforded sufones **19** and **20**, respectively. Reduction of **9C** (P_2I_4/CH_2Cl_2)[19] produced sulfide **21**. Reduction of azide **9G** (H_2/Lindlar's catalyst/2:1 ethyl acetate:MeOH) followed by acetylation and tosylation yielded **22** and **23**, respectively.



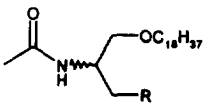
Scheme 2

Tetrazole **26** was prepared via a modified route (Scheme 2). Tosylation of **7B** followed by displacement with sodium azide gave azide **24**. Reduction (Ph_3P/H_2O)[20] and acetylation yielded acetamide **25**. Finally, treatment of the nitrile with an excess of sodium azide and triethylamine hydrochloride in N-methylpyrollidone at 110 °C afforded **26**.

All compounds were tested for the inhibition of recombinant human 14 kDa PLA_2 acyl hydrolysis using a membrane assay (0.1 ng enzyme and ^{3}H-arachidonic acid *E. coli*) as previously described[2] and their IC_{50}'s are listed in Table 1. IC_{50}s > 200 uM are considered inactive. A report from Jain and Gelb[21] that the C_{16} analog of **9F** was a potent inhibitor of 14 kDa PLA_2 encouraged us to prepare **9F**, **9A**, and **10**. Surprisingly, **9F** was relatively inactive in our hands. However, olefin **9A** exhibited 8 uM activity while the saturated derivative **10** was relatively inactive. The activity of **9A** was surprising but was not further investigated because the lipophilic nature of this class of compounds rendered them difficult to handle in our assays. Instead, more polar derivatives were prepared.

X-ray crystal structures have revealed specific ionic interactions between the two *sn*-3 phosphate oxygens and the catalytic calcium ion and Lys69 sidechain.[22] With this in mind, several neutral oxygenated derivatives were prepared to take advantage of these interactions. Diol **13** gave good inhibition while the monools **11**, **16**, and **17** were inactive. Ketone **18**, epoxide **12**, and ester **15** also lacked activity.

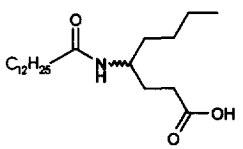
Table 1



R	IC_{50} (mM)	R	IC_{50} (mM)	R	IC_{50} (mM)
9F	100	**10**	>200		100
9A	8	**12**	>200		>200
10	60	**18**	>200		>200
13	8.9	**9C**	>200		>200
11	>200	**19**	50	**14**	9
17	>200	**21**	>200	**26**	0.57
16	>200	**9E**	60		

Sulfoxides and sulfones were examined next as surrogates for the phosphate center. Examination of the crystal structure reveals that aryl groups can be accomodated within the *sn*-3 pocket. 4-Toluenethio derivatives **9C**, **19**, and **21** were tested, and of these, only the sulfone **19** exhibited moderate activity. The methylsulfinyl (**9D**) and methylsulfonyl (**20**) analogs also possessed intermediate activites. The remaining neutral, polar derivatives (**9E**, **22**, and **23**) were inactive.

The phosphate/calcium ionic interaction may contribute significantly to binding, and if so, effective replacement of the phosphate group would require a negatively-charged moiety. Thus, two additional phosphate substitutions were examined, a carboxylic acid and a tetrazole. Carboxylic acid **14** was a good inhibitor (IC_{50} of 8 uM), but tetrazole **26** proved superior with an IC_{50} of 0.57 uM. Both groups retain the potential to simultaneously coordinate to the catalytic calcium ion[23] and engage in a hydrogen bond with Lys69. These two functionalities were also evaluated on another inhibitor framework previously studied by others (Figure 2).[6] Both maintained good inhibitory activity, though in this case the carboxylic acid **27** (0.4 uM) was better than the tetrazole **28** (6.6 uM).[24]



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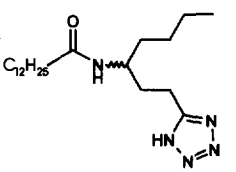

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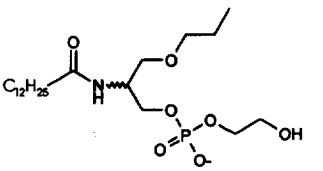

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Figure 2

The activity of **26** in whole cells was evaluated in the human monocyte LTC_4 release assay.[25] For comparison, *sn*-3 phosphate-containing **29** and **4** were also assayed. Both **29** and **4** are approximately 1 uM inhibitors against the isolated enzyme, but neither demonstrated inhibition of LTC_4 release in the human monocytic cell line. On the other hand, 75% inhibition of LTC_4 release was effected in the presence of 30 uM **26**. No inhibition of 5-lipoxygenase by 50 uM **26** was detected indicating that the inhibition of LTC_4 was not via this pathway. No activity in the *E. coli* assay with 50 uM of **26** was observed in the presence of 10% human whole blood. This is most likely a result of high protein binding due to the lipophilicity of the C_{18} tail, and replacements for this group are under investigation.

Summary

The tetrazole moiety is an effective replacement for the *sn*-3 phosphate in substrate analog inhibitors of PLA_2. It imparts greatly enhanced cell permeability while maintaining in vitro potency, and should also be superior in terms of chemical stability (i.e., resistance to hydrolysis).

Acknowledgement

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