Letters

The First Potent Inhibitor of Mammalian Group X Secreted Phospholipase A_2: Elucidation of Sites for Enhanced Binding

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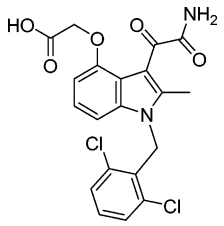
Abstract: Using the X-ray structure of human group X secreted phospholipase A_2 (hGX), we carried out structure-based design of indole-based inhibitors and prepared the compounds using a new synthetic route. The most potent compound inhibited hGX and the mouse orthologue with an IC_{50} of 75 nM. This compound is the most potent hGX inhibitor reported to date and was also found to inhibit a subset of the other mouse and human $sPLA_2$s.

Secreted phospholipases A_2 ($sPLA_2$s) are a group of enzymes with conserved active sites and calcium-binding loops that catalyze the hydrolysis of the *sn*-2 ester of glycero-phospholipids to release free fatty acids and 2-lysophospholipids.[1−4] The $sPLA_2$s are low molecular weight (∼14−20 kDa) enzymes with 5−8 disulfide bonds and require Ca^{2+} as a catalytic cofactor.[1−4] To date, there are 10 distinct mammalian $sPLA_2$s (groups IB, IIA, IIC, IID, IIE, IIF, III, V, X, XIIA), with group IIC present as a pseudogene in humans.[1,2] Biomedical interest in this class of enzymes stems from the observation that one or more plays a role in the liberation of arachidonic acid from cellular phospholipids for the biosynthesis of eicosanoids. Mammalian cells also contain a cytosolic phospholipase ($cPLA_2$-α).[5] Recent evidence shows that in macrophages and other cells, $sPLA_2$s and $cPLA_2$-α work together to liberate arachidonic acid in agonist-stimulated mammalian cells.[6−9] The molecular basis for the cross-talk between these two enzymes is not known. Potent and selective inhibitors of the $sPLA_2$ enzymes would help resolve some of the questions surrounding the mechanism of arachidonate release as well as probe other physiological functions of $sPLA_2$s.

For the past decade, the group IIA $sPLA_2$ has received the most attention among the $sPLA_2$s because it was the first nonpancreatic $sPLA_2$ to be discovered, and it is found in high levels in patients suffering from inflammatory diseases including rheumatoid arthritis[10] and acute pancreatitis.[11] The group IIA $sPLA_2$ has been the focus of medicinal chemistry efforts at several pharmaceutical companies.[12−16] Workers at Lilly Labs and Shionogi & Co. Ltd. have reported on substituted indoles, exemplified by compound **I**, as potent inhibitors of group IIA $sPLA_2$ (Figure 1).[12−15] Among the various reported inhibitors of $sPLA_2$,[17] these compounds appear to be the most potent and also appear to have the most drug-like properties. With the discovery of additional $sPLA_2$ enzymes, we have been interested in exploring these indole analogues as inhibitors of all of the members of the $sPLA_2$ enzyme class.[18] We are particularly interested in the group X $sPLA_2$ because it appears to have the highest specific activity in promoting arachidonic acid release from mammalian cells.[7,19,20] To date, very few inhibitors of the group X $sPLA_2$ have been reported, with the general $sPLA_2$ inhibitor YM-26734 being the most potent at 0.20 μM against the group X enzyme.[21] To this end, we recently determined the X-ray structure of the indole-based inhibitor Me-Indoxam bound to human group X $sPLA_2$ (hGX).[18] We now report the development of a potent indole-based inhibitor of hGX that was designed based on this structural information.



I

Figure 1. Representative structure of a substituted indole inhibitor of group IIA $sPLA_2$. Indole positions 1−7 are shown.

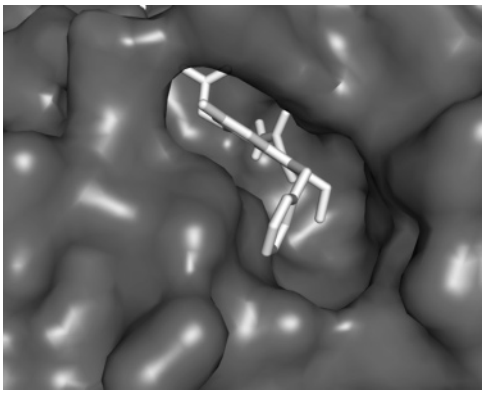


Figure 2. Compound A docked into the active site of hGX $sPLA_2$. As can be seen, the 2-ethyl substituent makes contact with the active site wall and the 6-methyl group sticks out of the active site.

In our first attempt to develop potent indole-based inhibitors of $sPLA_2$s other than the group IIA enzyme, we made a library of analogues in which the substituent attached to N1 of the indole ring was varied.[18] The range of IC_{50} values for this library was not very broad, and potent inhibitors of hGX were not obtained. Subsequent X-ray structural studies showed that this substituent largely points out of the active site of the $sPLA_2$, toward what would be the membrane plane when the enzyme is bound to the phospholipid bilayer.[18] Thus, we turned to chemical modifications of other areas of the indole ring.

Inspection of the X-ray structure of Me-Indoxam bound to hGX $sPLA_2$[18] reveals a large hydrophobic pocket near the 2-substituent that makes little interaction with the inhibitor. We hypothesized that a larger 2-alkyl substituent would bind to the hydrophobic pocket and increase the binding affinity of the inhibitor. Also, the 6-position of the indole points out of the enzyme active site and does not contribute to binding affinity. However, modification of the 6-position may be useful for modifying the physiochemical properties of the indole for

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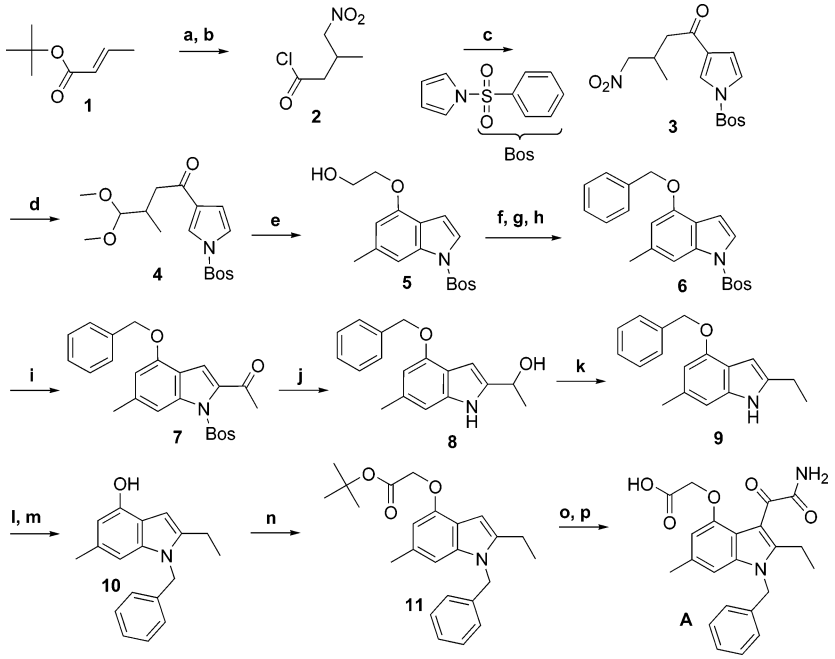
Scheme 1. Synthesis of $SPLA_2$ Inhibitor **A**[a]



[a] Reagents: (a) CH_3NO_2, KF, 18-Crown-6, ACN, reflux; (b) (i) H_2SO_4, $CHCl_3$, reflux; (ii) $SOCl_2$, reflux; (c) $AlCl_3$, $ClCH_2CH_2Cl$; (d) (i) NaOH, −20 °C, MeOH; (ii) H_2SO_4, −20 °C, MeOH; (e) p-TsOH, toluene, $HOCH_2CH_2OH$, reflux; (f) CCl_4, PPh_3; (g) 12 equiv of n-BuLi, THF; (h) NaH, BnBr, DMF; (i) n-BuLi, THF, −78 °C, Ac_2O; (j) LAH, THF, reflux; (k) $NaBH_4$, TFA, THF; (l) NaH, BnBr, DMF; (m) H_2, Pd/C, MeOH; (n) NaH, $BrCH_2CO_2t$-Bu, DMF; (o) (i) $(ClCO)_2$, CH_2Cl_2; (ii) NH_3; (p) TFA, CH_2Cl_2.

subsequent use in whole animal studies to affect pharmacokinetics. Docking studies were performed on the hGX enzyme in which the indole inhibitor was modified to include a 2-ethyl and 6-methyl, and the N1 substituent replaced with a benzyl (Figure 2). As suspected, the larger 2-ethyl group contacts the inner wall of the enzyme much better than a 2-methyl group, and a 6-methyl group sticks out of the enzyme active site and should not affect binding. As the reported synthesis[12] ofsubstituted 2-ethyl indoles was unsuccessful in our laboratory and the starting material for introduction of the 6-methyl substituent is not commercially available, a novel synthesis for 2-ethyl-6-methyl indoles was developed (Scheme 1). As there are few known literature reactions to functionalize the 6-position of an indole, the indole core had to be built up from pyrrole. Michael addition of nitromethane to *tert*-butyl crotonate followed by deprotection of this ester and subsequent treatment with thionyl chloride produced the acyl chloride **2.** This was then added to benzenesulfonyl protected pyrrole in the presence of aluminum trichloride to give ketone **3**. Treatment of **3** with NaOH in MeOH at low temperature followed by concentrated H_2SO_4 yielded dimethyl acetal **4**. Ring closure to form the 4-oxyethanol indole **5** was accomplished by addition of a catalytic amount of acid with refluxing in toluene/ethylene glycol solvent. Conversion to the chloride followed by addition of excess *n*-butyllithium and benzyl protection yielded indole **6**. Addition of *n*-butyllithium and acetic anhydride produced the desired 2-acetyl compound **7** due to the ortho-lithiating director used to protect the N1-position of the indole. Removal of the benzenesulfonyl protecting group and reduction of the ketone was accomplished in one step by refluxing in excess lithium aluminum hydride. Deoxygenation at the 2-position was accomplished using $NaBH_4$ and trifluoroacetic acid to produce the 2-ethyl indole **9**. N1-benzylation and 4-hydroxy deprotection followed by addition of *tert*-butyl bromoacetate yielded the *tert*-butyl oxyethanoate **11**. Treatment of compound **11** with dilute oxalyl chloride followed by ammonia gas and deprotection of the *tert*-butyl ester with trifluoroacetic acid yielded the desired

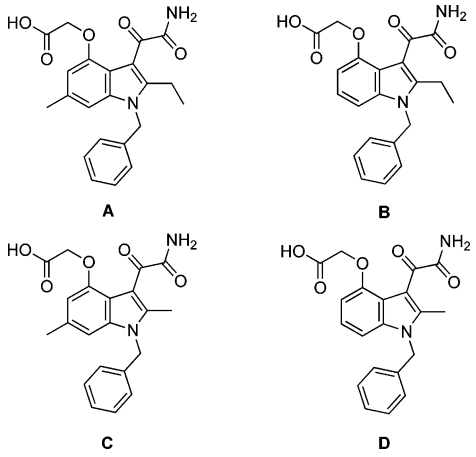


Figure 3. Structures of $sPLA_2$ inhibitors with varying alkyl groups at the 2- and 6-positions.

substituted 2-ethyl-6-methyl indole (compound **A)**. Compounds **B**−**D** (Figure 3) were synthesized similarly (Supporting Information).

To test the indole analogues as $sPLA_2$ inhibitors, we used a fluorometric assay consisting of unilamellar vesicles of 1-hexadecanoyl-2-(10-pyrenedecanoyl)-*sn*-glycero-3-phosphoglycerol.[22] The $sPLA_2$-catalyzed liberation of 10-pyrenedecanoic acid allows the fluorophore to dislodge from the vesicles and bind to albumin in the buffer phase where it now undergoes monomer fluorescent emission rather than excimer emission. The assay results (Table 1) show the 2-ethyl substituent to have a dramatic affect on binding to the hGX, with IC_{50} values of 75 nM for compounds **A** and **B**. The 2-ethyl compounds (**A** and **B**) are 26-fold more potent than the analogous 2-methyl compounds (**C** and **D**) against hGX, which have IC_{50} values of 2 μM. The 6-methyl substituent has no effect on hGX binding; compounds **A** and **B** have identical IC_{50} values. The inhibitors were then screened against a panel of recombinant human and mouse $sPLA_2$s (hGIB, mGIB, hGIIA, mGIIA, hGIIE, mGIIE, hGV,

Table 1. Inhibition Data against Mammalian $sPLA_2$s for Compounds **A**−**D**[a]

	compound IC_{50} (μM)			
$sPLA_2$	**A**	**B**	**C**	**D**
hGIB	0.80 ± 0.10	0.75 ± 0.15	2.00 ± 0.20	2.50 ± 0.25
mGIB	0.20 ± 0.05	0.14 ± 0.075	2.00 ± 0.10	2.20 ± 0.15
hGIIA	0.125 ± 0.03	0.125 ± 0.02	0.30 ± 0.05	0.275 ± 0.05
mGIIA	0.05 ± 0.01	0.07 ± 0.02	0.125 ± 0.02	0.125 ± 0.02
hGIIE	0.05 ± 0.01	0.05 ± 0.02	0.125 ± 0.03	0.075 ± 0.01
mGIIE	0.075 ± 0.02	0.075 ± 0.02	0.40 ± 0.05	0.40 ± 0.04
hGV	0.50 ± 0.1	0.50 ± 0.05	0.80 ± 0.05	0.80 ± 0.05
mGV	0.75 ± 0.15	0.75 ± 0.10	0.85 ± 0.05	1.00 ± 0.075
hGX	0.075 ± 0.01	0.075 ± 0.01	2.20 ± 0.10	2.00 ± 0.15
mGX	0.075 ± 0.01	0.075 ± 0.01	2.50 ± 0.15	2.50 ± 0.20

[a] IC_{50}s are based on duplicate or triplicate analyses.

mGV, hGX, and mGX). In all cases the 2-ethyl compounds are more potent than the 2-methyl derivatives, and the 6-methyl group is tolerated (Table 1). Compounds **A** and **B** should be useful in distinguishing the groups X and V $sPLA_2$s based on the ∼10- fold increased potency for the former. This is significant because current evidence favors a role of these two $sPLA_2$s in arachidonate liberation in mammalian cells. Although these compounds are also potent inhibitors of the group IIA $sPLA_2$s, the original lead compound Me-Indoxam is 50-fold more potent on hGIIA and mGIIA versus hGX and mGX.[18] Thus, by carrying out studies with a combination of inhibitors, it should be possible to probe for the role of specific $sPLA_2$s in cellular processes.

In conclusion, the first potent inhibitor against hGX and mGX $sPLA_2$s has been discovered. A new chemical route to these indole-based $sPLA_2$ inhibitors has been developed.

Supporting Information Available: Experimental details including the synthesis of all compounds and assay procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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