Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 2129–2132

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

THE STRUCTURE–ACTIVITY RELATIONSHIPS OF A SERIES OF SUICIDE INHIBITORS OF PHOSPHOLIPASE A_2

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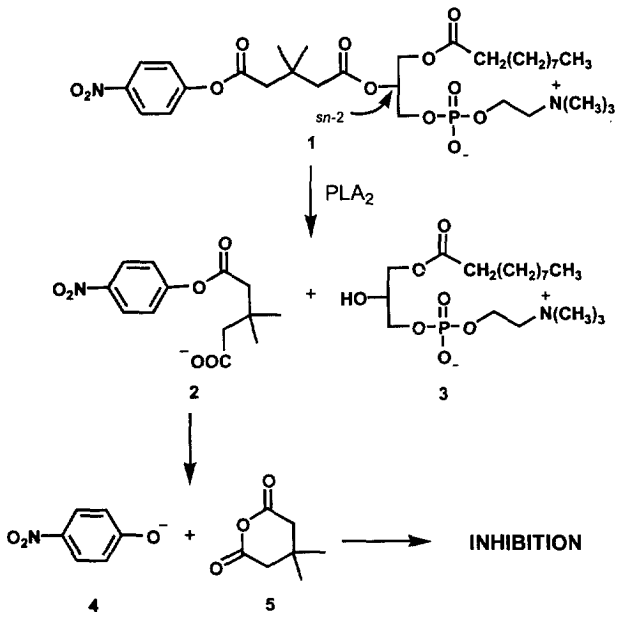
Received 30 April 1998; accepted 2 July 1998

Abstract: A series of mechanism-based inhibitors of phospholipase A_2 (SIBLINKS) were synthesized. These new SIBLINKS are phospholipid analogues that contain a *para*-substituted phenyl 3,3-dimethylglutaryl group in the place of the *sn*-2 acyl chain. The effect of the phenyl leaving group on inhibitory activity was studied by varying the electron-withdrawing ability of the *para*-substituted group. A strong correlation was observed between the leaving group potential of the suicide inhibitor and the inhibitory activity of the derivative toward cobra venom phospholipase A_2.

Phospholipase A_2 (PLA_2) is a ubiquitous enzyme that functions to hydrolyze the ester group present at the *sn*-2 position of phospholipids.[1,2] The products of this hydrolysis are known to be potent mediators of inflammation or precursors to such mediators.[3] Inhibition of the hydrolytic action of PLA_2 would be anticipated to result in a decrease in inflammation and, as such, may provide a new chemotherapeutic approach for the treatment of inflammatory disorders.[4–7]

Scheme 1.



The recent report of the discovery of a mechanism-based PLA_2 inhibitor by Washburn and Dennis[8,9] demonstrated the feasibility of suicide-inhibition of this enzyme. Its mechanism of action is illustrated in Scheme 1 as previously proposed.[9] The SIBLINKS compound **1** is recognized as a substrate by the enzyme that catalyzes hydrolysis of the ester attached to the *sn*-2 position of the phospholipid. The resulting carboxylate anion (**2**) can then undergo intramolecular anhydride formation with concomitant release of *p*-nitrophenoxide **4** (the leaving group). If the cyclization occurs rapidly relative to diffusion from the active site, enzyme inactivation via acylation by the anhydride (**5**) of a proximal nucleophilic residue can occur. We report here a structure-activity relationship study of SIBLINKS **1** by modulating the phenyl leaving

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0960-894X/98/$ - see front matter

PII: S0960-894X(98)00378-3

group to address our concerns on the stability of the original SIBLINKS and the potential toxicity associated with the *p*-nitrophenol.

Table 1. Substituent Constant of *p*-Substituted Phenol

X	σ_p^-
H	0.00
Br	0.25
CO_2CH_3	0.64
CF_3	0.74
CN	0.88
$SOCF_3$	0.95
SO_2CH_3	0.98
CHO	1.04
NO_2	1.28
SO_2CF_3	1.47

Structure–Activity Studies: Our approach to selecting compounds for synthesis was based upon the premise that a more reactive leaving group (related to the phenoxide **4** in Scheme 1) would allow for more rapid anhydride (**5**) formation. It was hoped that an increase in the rate of anhydride formation (relative to the rate of diffusion from the active site) would allow for more efficient enzyme inactivation. To test this hypothesis, we chose to modulate the leaving group ability of **4** by changing the nature of the *para* substituent. It was anticipated that this leaving group ability would be directly related to the ability of the *para* substituent to stabilize the negative charge as estimated by σ_p^- values of *para*-substituted phenols.[10] Thus, a more potent electron withdrawing group should translate into a more reactive leaving group and hopefully a more efficient PLA_2 inhibitor. Our initial studies were directed toward the derivatives bearing substituents as listed in Table 1 which would provide a series of compounds with a wide range of σ_p^- values.

Chemistry: The new SIBLINKS molecules were prepared in a manner similar to that reported by Washburn and Dennis.[11] A lysophospholipid 1-decanoyl-*sn*-glycerol-3-phosphorylcholine was acylated with 3,3-dimethylglutaric anhydride. The resultant half-acid was converted into the acid chloride with an excess of oxalylchloride and subsequently reacted with a substituted phenol to yield a SIBLINKS molecule.

Biology: All SIBLINKS derivatives were tested for inhibitory activity against cobra venom PLA_2 (*Naja naja atra*). Typically, the enzyme (5 μg mL^{-1}) was preincubated at 40 °C with 100 μM sonicated SIBLINKS vesicles in 50 mM Tris-HCl, pH 8.0, 100 mM KCl, and 10 mM $CaCl_2$. Aliquots were removed at various times and tested for residual enzymatic activity using the Dole assay.[12] The residual activity for each time point is expressed as a percentage relative to the activity of each incubation mixture at time zero. Of the ten derivatives studied, five (including the original SIBLINKS) inactivated PLA_2 whereas the other five did not under the experimental conditions. The time-dependent, irreversible inactivation of PLA_2 for the five active SIBLINKS is shown in Figure 1. Apparent rate constants for enzyme inactivation were calculated using pseudo first-order kinetics (Table 2). These data show that the inhibitory activity of a SIBLINKS is dramatically affected by the σ_p^- value of its phenyl leaving group. The inactivation rate constant increases as the σ_p^- value increases. As shown in Figure 1, those SIBLINKS that displayed significant inhibitory activity have σ_p^- values of greater than 0.88. The only exception is the formyl derivative, which has an σ_p^- value of 1.04, but does not inhibit the enzyme. The most potent SIBLINKS is the trifluoromethylsulfonylphenyl derivative that

Table 2. Kinetic Parameters for the Inhibition of PLA_2

X	k_{app} x 10^3 (min^{-1})	Activity (nmol min^{-1} mg^{-1})	P
SO_2CF_3	18.4	11.9 ± 1.2	6
NO_2	8.1	2.5 ± 0.4	16
SO_2CH_3	2.9	11.1 ± 0.2	36
SO CF_3	3.2	2.0 ± 0.3	49
CN	1.1		

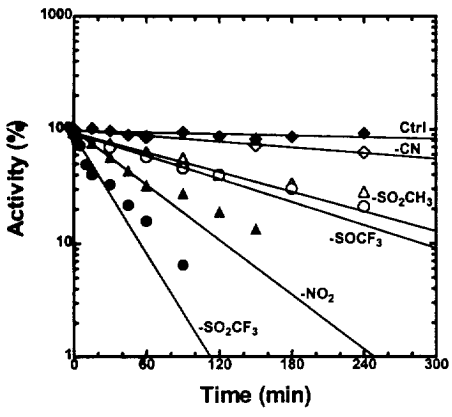

Figure 1. Inhibition of PLA_2 with SIBLINKS.

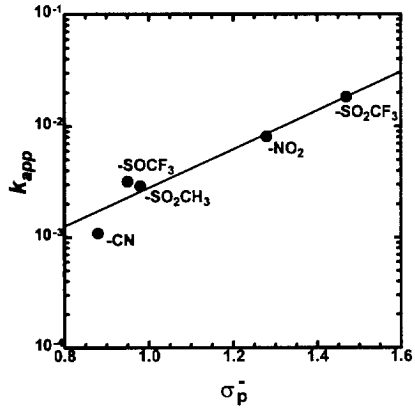

Figure 2. Correlation of PLA_2 inactivation with the activity of leaving group

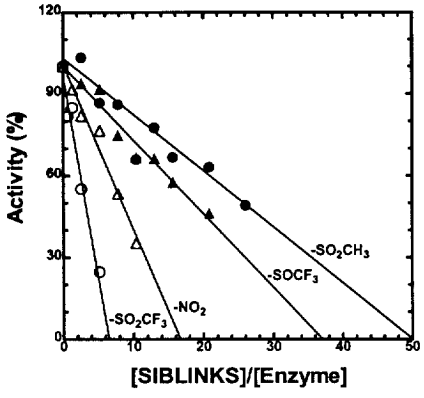

Figure 3. Determination of partition ratio.

inhibits the enzyme twice as rapidly as the original, *para*-nitro SIBLINKS.

To quantify the effect of phenyl leaving groups on inhibitory activities, the apparent rate constants of enzyme inactivation for the five active inhibitors are also expressed as a function of σ_p^- values of their *para*-substituted phenyl groups. As shown in Figure 2, the rate constant of enzyme inactivation increases linearly with the σ_p^- value. From this linear correlation, a reaction constant (ρ) is calculated for the SIBLINKS inactivation of cobra venom PLA_2 using the Hammett equation $\log(k/k_o) = \sigma_p^-\rho$, where k is the apparent rate constant for a specific SIBLINKS molecule and k_o the apparent rate constant for that SIBLINKS molecule that has a proton as its *para*-substituent. The ρ value reflects the sensitivity of this enzyme inactivation reaction to *para*-substitution effects. For this suicide inhibition, a value of 2.4 for ρ was obtained, indicating that the inhibitory activity of SIBLINKS is extremely sensitive to the relative ability of the substituted phenyl group to leave. This implies that only a limited number of phenols can be used to replace *para*-nitrophenol without compromising or losing inhibitory activity.

The initial velocities for PLA_2 hydrolysis of the five active SIBLINKS were determined spectrophotometrically by following the release of *para*-substituted phenol (Table 2). No correlation was observed between the hydrolysis of the SIBLINKS and their σ_p^- values. Interestingly, the enzymatic hydrolysis was significantly affected by the structure of the *para*-substituent. Trifluoromethylsulfonyl and methylsulfonyl derivatives are hydrolyzed at 11 nmol/min/mg whereas nitro and trifluoromethylsulfoxide derivatives are hydrolyzed at 2 nmol/min/mg. The initial velocity for the nitro derivative determined here is in consistent with the literature.[8]

The efficiencies of enzymatic inactivation as characterized by partition ratio were determined by titrating the enzyme with a SIBLINKS inactivator.[13,14] To ensure the maximal inhibition, the enzyme was

incubated for two days with different inhibitor concentrations.[15] The residual enzyme activity is plotted as a function of the ratio of the moles of inhibitor per mole of the enzyme (Figure 3). The intercept at the x axis gives the turnover number that equals the partition ratio plus one. The partition ratio measures how many inactivator molecules have to be hydrolyzed by each enzyme molecule before becoming inactivated. As the leaving potential of the phenyl substituent increases, the efficiency of enzyme inactivation is also improved. The most potent inhibitor, the trifluoromethylsulfonyl derivative, has a partition ratio of 6. This derivative inhibits the enzyme about twice as efficient as the original SIBLINKS.

Conclusions: We have prepared an improved version of the original suicide-based PLA_2 inhibitor described by Washburn and Dennis.[8] The compound does not contain the *para*-nitro aromatic moiety and exhibits more rapid enzyme inactivation with a more favorable partition number. In addition, a strong correlation has been established between the rate of PLA_2 inactivation and the σ_p^- value for the aromatic substituent of the inhibitor.

References and Notes

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15. The enzyme (5 mg/mL) was preincubated at 40 °C for 2 days in 50 mM Tris-HCl, pH 8.0, 100 mM KCl, and 10 mM $CaCl_2$. Enzymatic activity was measured using the Dole assay.