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QSAR for phospholipase A_2 inhibitions by 1-acyloxy-3-*N*-*n*-octylcarbamyl-benzenes

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Abstract—1-Acyloxy-3-*N*-*n*-octylcarbamyl-benzenes are potent reversible competitive inhibitors of *Naja mocambique mocambique* phospholipase A_2 with the K_i values from 9.6 to 119 μM. The pK_i values are correlated to both Taft substituent constant σ^* and Hansch hydrophobicity constant π. The pre-steady state inhibition studies indicate that the pK_S values for the first inhibition step are linearly correlated to σ^* alone with the ρ^* of −0.09 for this correlation. Thus, the first inhibition step may involve the insertion of the inhibitor to hepta-coordinated Ca^{2+} ion of the enzyme to form the octa-coordinated Ca^{2+} ion of the enzyme. The log(k_2/k_{-2}) values for the second inhibition step are linearly correlated to π alone, and the ψ value for this correlation is 0.13. Therefore, the second step inhibition step may involve the van der Waals' interaction between the acyl group of the inhibitor and Tyr 69 of the enzyme.
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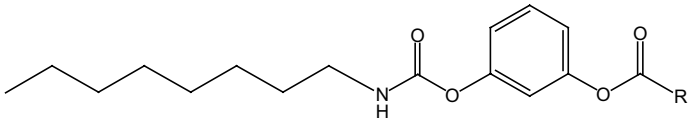
Phospholipase A_2 (PLA2, EC 3.1.1.4) catalyzes hydrolysis of the *sn*-2 ester bonds of phospholipids, liberating lysophospholipid, and free fatty acid products. Apart from being the products of phospholipid digestion, fatty acids and lysophopholipids may be transformed into inflammatory lipid mediators, thereby implicating PLA2s in the pathogenesis of many inflammatory disease states. In fact, PLA2s are found in a variety of extracellular locations and are involved in a range of physicological processes including phospholipid digestion, signal transduction, and host defense.[1] PLA2 has served as an useful prototype for elucidating the mechanism for an enzyme that functions at a lipid-aqueous interface.[2]

The X-ray crystal structure of a complex between a transition state analogue, 1-*O*-octyl-2-heptylphosphonyl-*sn*-glycero-3-phosphoethanolamine and *Naja naja atra* PLA2 has shown that the active site Ca^{2+} binds to both the *sn*-2 phosphonate oxygen and the *sn*-3 phosphodiester oxygen of the inhibitor and that the dihedral angle between the C(*sn*-2)-O and C(*sn*-3)-O bonds is about 180° (*anti* conformation).[3] However, the X-ray structure of the phosphonate inhibitor-porcine PLA2 complex has revealed the same active site Ca^{2+} binding to those oxygens but the dihedral angle is about 60° (*gauche* conformation).[4] Thus, the glycerol *sn*-2-*sn*-3 backbond conformation plays an important role in the PLA2 catalysis. We have found that 1,3-benzene-di-*N*-*n*-octylcarbamate



Inhibitors: **1** **2** **3** **4** **5** **6** **7** **8** **9**

R = CH_3, n-C_4H_9, n-C_7H_{15}, n-$C_{15}H_{31}$, Ph, CH_2Ph, CPh_3, t-Bu, CCl_3

Figure 1. Structures of inhibitors **1**–**9**.

Keywords: Phospholipase A_2; QSAR; Enzyme mechanism; Carbamate inhibitor.
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Table 1. Inhibition constants for the PLA2 inhibitions by 1-acyloxy-3-*N*-*n*-octylcarbamyl-benzenes (**1**–**9**) and Taft substituent and Hansch hydrophobicity constants

Inh.	R	σ^*	π	K_i (μM)	K_S (μM)[a]	k_2 (s^{-1})[a]	k_{-2} (s^{-1})[b]
1	CH_3	0	0.5	98 ± 8	6.7 ± 0.7	0.27 ± 0.03	3.9 ± 0.7
2	*n*-C_4H_9	−0.13	2	75 ± 6	6.67 ± 0.09	0.22 ± 0.02	2.5 ± 0.3
3	*n*-C_7H_{15}	−0.13	3.5	27 ± 4	5.7 ± 0.7	0.16 ± 0.01	0.7 ± 0.1
4	*n*-$C_{15}H_{31}$	−0.13	7.5	9.6 ± 0.9	6.55 ± 0.09	0.16 ± 0.02	0.27 ± 0.04
5	Ph	0.6	2.13	95 ± 9	6.45 ± 0.08	0.16 ± 0.02	2.4 ± 0.4
6	CH_2Ph	0.215	2.26	73 ± 8	6.5 ± 0.7	0.16 ± 0.01	1.8 ± 0.3
7	CPh_3	0.89	5.4[c]	38 ± 3	7.1 ± 0.1	0.18 ± 0.02	1.0 ± 0.1
8	$C(CH_3)_3$	−0.3	1.98	55 ± 6	5.13 ± 0.07	0.18 ± 0.02	1.9 ± 0.3
9	CCl_3	3.15[d]	1.67	120 ± 10	12.1 ± 0.3	0.46 ± 0.04	4.5 ± 0.6

[a] The K_S, k_2 values were determined from nonlinear least squares curve fitting of the pre-steady state inhibition rate constant (k_{obs}) on inhibitor concentration [I] according to the following equation: $k_{obs} = k_{-2} + k_2[I]/(K_S + [I])$.[25–31] The k_{obs} value was obtained from the initial burst phase of the pH-stat titration.

[b] Calculated from $1/K_i = (1/K_S)(k_2/k_{-2})$.[25–31]

[c] This value is calculated from 2.26 + 2(1.57), where 2.26 is the π value for benzyl and 1.57 is the π value difference between phenyl (2.13) and methyl (0.5).

[d] Calculated from $3\sigma^*(CH_2Cl)$.

Table 2. Correlation results for the PLA2 inhibitions by 1-acyloxy-3-*N*-*n*-octylcarbamyl-benzenes (**1**–**9**)[a]

Parameters	pK_i	pK_S	log(k_2/k_{-2})
ρ^*	−0.08 ± 0.03	−0.09 ± 0.01	—
ψ	0.15 ± 0.02	—	0.13 ± 0.02
h[b]	3.88 ± 0.06	5.21 ± 0.01	−1.29 ± 0.07
R	0.94095	0.94078	0.92606

[a] Correlations of pK_i, pK_S, and log(k_2/k_{-2}) against Eq. 1.

[b] Calculated pK_i, pK_S, and log(k_2/k_{-2}) values for $\sigma = \pi = 0$.

is a potent inhibitor of PLA2 due to the constrained conformation between two C (benzene)-O bonds.[5] In order to study quantitative structure–activity relationship (QSAR) for PLA2, 1-acyloxy-3-*N*-*n*-octylcarbamyl-benzenes (**1**–**9**) (Fig. 1) are synthesized for this purpose.

Inhibitors **1**–**6**, **8**, and **9** were synthesized from condensation of resorcinol (1,3-benzene-diol) with 1 equiv of *n*-octyl isocyanate in the presence of 1.2 equiv of triethylamine in THF at 25 °C for 1 day to yield 1-hydroxy-3-*N*-*n*-octylcarbamyl-benzene (35–45% yield) then followed by the condensation of the latter with 1.2 equiv of the corresponding acyl chloride in the presence of 1 equiv of triethylamine in THF at 25 °C for 1 day to yield inhibitors **1**–**6**, **8**, and **9** (70–90% yield). 1-*N*-*n*-Octyl-carbamyl-3-triphenyl-acetoxy-benzene (**7**) was synthesized from condensation of resorcinol with 1 equiv of triphenylacetic acid in the presence of 1 equiv of 1,3-dicyclohexylcarbodiimide (DCC), 1 equiv of triethylamine, and a catalytic amount of 4-(dimethylamino) pyridine (DMAP) in CH_2Cl_2 at 25 °C for 4 h to yield 1-hydroxy-3-triphenyl-acetoxy-benzene (20–25% yield) then followed by the condensation of the latter with 1 equiv of *n*-octyl isocyanate in presence of 1 equiv triethylamine in THF at 25 °C for 2 h to yield inhibitor **7** (50–60% yield). All products were characterized by ^1H and ^{13}C NMR spectra, mass spectra, and elemental analyses.

The K_i values were obtained from the Lineweaver–Burk plots. Steady-state initial rates of *Naja mocambique* PLA2 (Sigma)-catalyzed hydrolysis of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (Sigma, 0.1 mM) in the presence of inhibitors **1**–**9** were followed by pH-stat titration (Radiometer PHM 290) under a

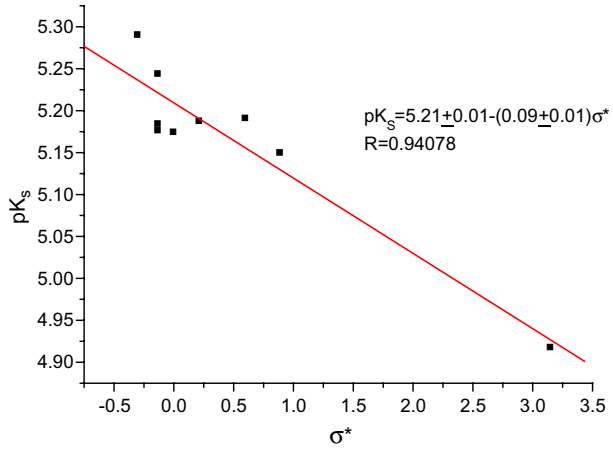

Figure 2. Plot of the pK_S values against σ^*.

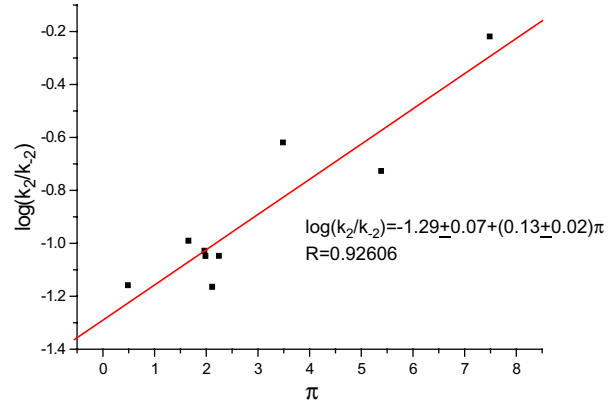

Figure 3. Plot of log(k_2/k_{-2}) values against π.

stream of nitrogen at pH 8.0 and 25 °C in a 5 mL reaction mixture containing NaCl (10 mM).[5]

Quantitative structure–activity relationships (QSARs) represent an attempt to correlate structural properties of compounds with biological activities and chemical reactivities.[6–9] These chemical descriptors, which include parameters to account for hydrophobicity, electronic, inductive, or polar properties, and steric effects, are determined empirically or by calculations. Many drug activities and chemical reactivities are linearly correlated to three substituent parameters such as the Taft–Ingold–Hanscdh–Järv equation (Eq. 1).[9]

$$\log k = h + \rho^*\sigma^* + \delta E_S + \psi\pi \quad (1)$$

In Eq. 1, the parameters h, ρ^*, σ^*, E_S, δ, ψ, and π are $\log k_o$ for the standard reaction, reaction constant to substituent inductive effect, Taft substituent constant, Taft steric constant, reaction intensity factor to substituent steric effect, reaction intensity factor to substituent hydrophobicity, and Hansch hydrophobicity constant,[10] respectively. Many successful QSAR studies for cholesterol esterase,[11–18] acetylcholinesterase,[19–22] and PSL[11,23] inhibitions by aryl carbamates have been reported. In this paper, we further report QSARs for PLA2 inhibitions by carbamate inhibitors **1–9** (Fig. 1).

All carbamate inhibitors **1–9** are reversible, competitive inhibitors of *Naja mocambique mocambique* PLA2 with

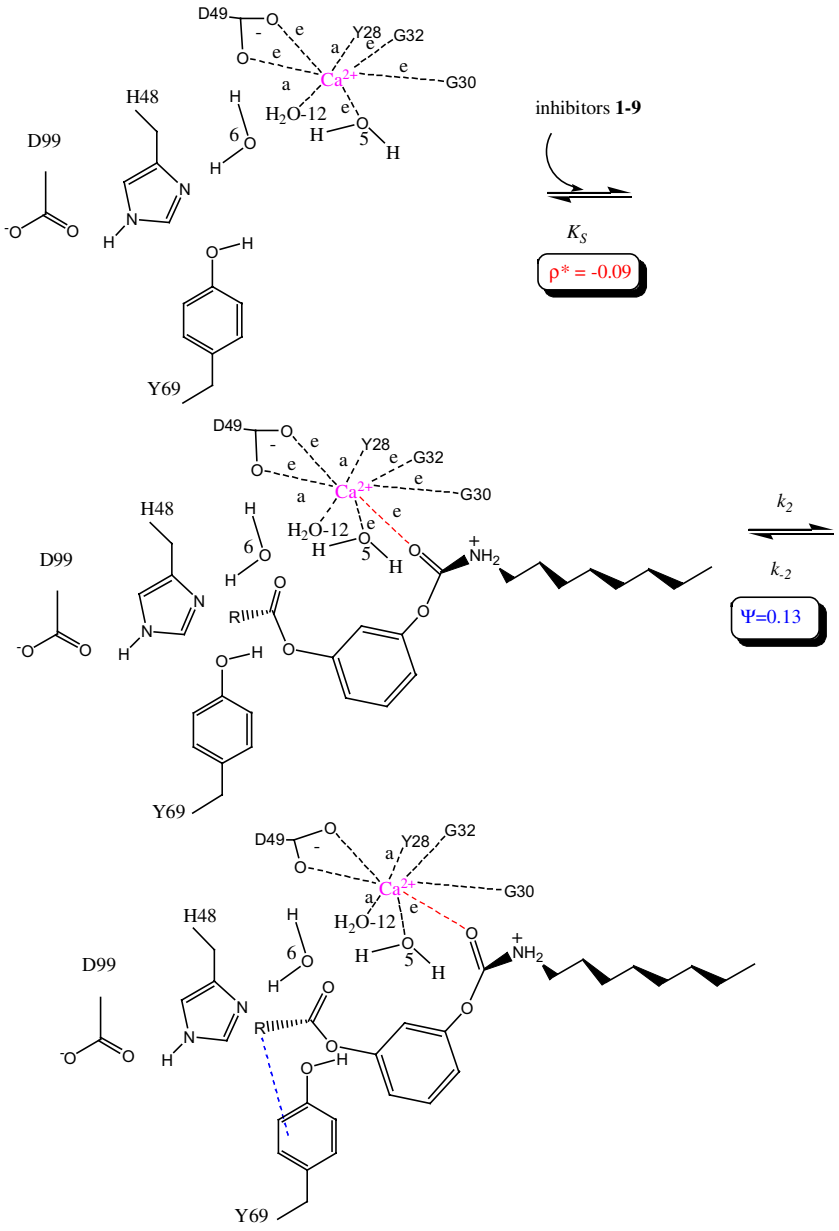

Figure 4. The proposed PLA2 inhibition mechanism by inhibitors **1–9**. The first step (K_S) is an insertion of the carbamyl carbonyl oxygen of the inhibitor to the hepta-coordinated Ca^{2+} ion of the enzyme and then the formation of the octa-coordinated Ca^{2+} ion of the enzyme-inhibitor complex. The second step (k_2/k_{-2}) may involve the van der Waals' interaction between the acyl R moiety and Tyr69 of the enzyme and therefore this interaction stabilizes the enzyme-inhibitor complex.

the K_i values from 9.6 to 119 μM (Table 1). Moreover, the pK_i values are correlated to both σ^* and π values against Eq. 2 (Table 2).

$$pK_i = h + \rho^*\sigma^* + \psi\pi \quad (2)$$

The pre-steady inhibitions indicate that each reversible K_i step is further divided into two steps:[25–31] the K_S ($= k_{-1}/k_1$) step and the k_2/k_{-2} step ($1/K_i = (1/K_S)k_2/k_{-2}$) (Table 1). Moreover, the pK_S values are linearly correlated to the σ^* values alone with the ρ^* value of −0.09 (Fig. 2 and Table 2), and the $\log(k_2/k_{-2})$ values are linearly correlated to the π values alone with the ψ value of 0.13 (Fig. 3 and Table 2).

According to these QSAR results, a two-step inhibition mechanism is proposed (Fig. 4). Thus, an insertion of the carbamyl carbonyl oxygen of the inhibitor to the hepta-coordinated Ca^{2+} ion of PLA2[2,24] (Fig. 4) may occur in the K_S step due to a small negative value of ρ^* ($= -0.09$) for the pK_S–σ^* correlation (Fig. 2 and Table 2). This value also suggests that reaction centers are far away from the varied substituents (R moieties in Fig. 1) of the inhibitors and that the products (octa-coordinated Ca^{2+} ion of PLA2-inhibitor complexes)[2,24] (Fig. 4) are more positively charged than the inhibitors themselves. The second inhibition step (k_2/k_{-2}) may involve the van der Waals' interaction between the acyl R moiety (Fig. 1) of the inhibitor and the Tyr69 residue of PLA2[2] due to a small value of ψ ($= 0.13$) for the $\log(k_2/k_{-2})$–π correlation (Fig. 3 and Table 2). Thus, the more the inhibitor is hydrophobic at the acyl R moiety the more the inhibitor interacts with the Tyr69 residue of PLA2. Since the $\log(k_2/k_{-2})$–σ^* correlation is poor (data not shown), the electronic characters of the enzyme-inhibitor complexes do not alter at all in the k_2/k_{-2} step.

Carbamates **3** and **4** are potential candidates for nonsteroidal anti-inflammatory drugs (NSAIDs) due to the fact that both carbamates are potent inhibitors of the snake venom PLA2. Further investigations for the inhibitions of pancreatic and bee venom PLA2s by carbamates **1**–**9** will be communicated in due course.

Acknowledgements

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