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Novel δ^2-isoxazolines as group II phospholipase A_2 inhibitors

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Abstract—The synthesized imidazolyl substituted δ^2-isoxazolines were subjected to Phospholipase A_2 (PLA_2) enzyme inhibitory activity against snake venom source and their structure–activity relationship with respect to different groups attached to this moiety is reported for the first time. The crystal structure of the compound 2-butyl-5-chloro-3*H*-imidazolyl-4-carbaldehyde oxime **2**, an intermediate for the construction of isoxazolines is reported. These compounds exerted a significant PLA_2 enzyme inhibitory activity against group II PLA_2. The in vivo activity on mice of selected compounds **3bI** and **3bIV** shows the comparable anti-inflammatory activity with the known standard ursolic acid.

1. Introduction

The PLA_2 class of enzymes catalyze hydrolysis of the 2-acyl ester of 3-Sn phosphoglycerides to yield arachidonic acid (metabolized to eicosanoids by cycloxygenase and lipoxygenase) and lysophospholipid, which is a rate limiting step of the production of pro-inflammatory lipid mediators such as prostaglandins, leukotrienes, lipoxins, and platelet activating factor.[1–4] The importance of PLA_2 enzyme in fertilization, cell proliferation, smooth muscle contraction, hyper sensitization, chronic inflammatory diseases, signal transduction, membrane homeostasis has been recognized.[5] *Vipera russelli russelli* PLA_2 enzyme has been classified as group II Asp 49 (which is critically involved in binding of Ca^{2+}) enzyme based on its structure and mechanism of catalysis. It has C-terminal tail, which forms an extra disulphide link with a cysteine residue near the active site His 48.[6]

The literature survey on δ^2-isoxazoline derivative shows that they represent an important new class of anti-inflammatory agents.[7] From this perspective, we have initiated a study on the PLA_2 enzyme inhibitors and structure–activity relationship of these type of molecules. The results from this study by in vitro and the selected compounds in vivo are presented in this paper.

2. Chemistry

As part of our drug discovery program,[8] microwave-assisted synthesis of 2-butyl-5-chloro-3*H*-imidazolyl-4-carbaldehyde **1** and its X-ray crystallographic studies have been reported.[9] The key intermediate 2-butyl-5-chloro-3*H*-imidazolyl-4-carbaldehyde oxime **2** was used to construct novel imidazolyl δ^2-isoxazoline libraries[10] (Scheme 1) through 1,3-dipolar cycloaddition reaction[11] with the dipolarophiles like mono substituted alkenes.

Recently an aldoxime **2** crystal structure has been determined by X-ray diffraction method,[12] whose crystallographic data was deposited on CCDC[17] and the probability of the molecule is as shown in Figure 1 with some selected bond lengths.

3. Biological results and discussion

The PLA_2 enzyme inhibitory activity assay were performed by using standard protocol stabilized in our

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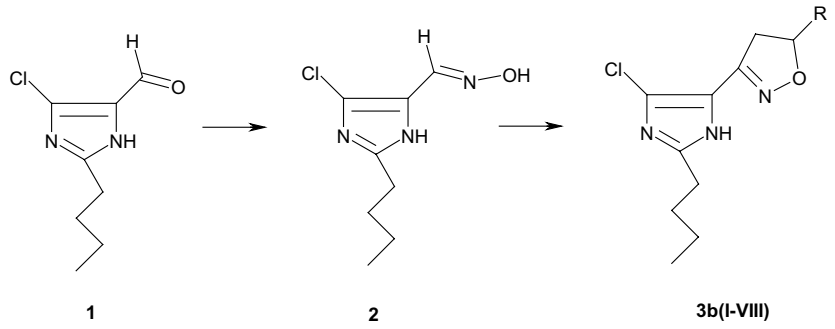

Scheme 1. **3bI**, R = –CN; **3bII**, R = $-C_6H_5$; **3bIII**, R = $-COOC_6H_5$; **3bIV**, R = $-COOC_2H_5$; **3bV**, R = $-CH_2COOCH_3$; **3bVI**, R = $-CH_2OH$; **3bVII**, R = $-COOCH_3$.

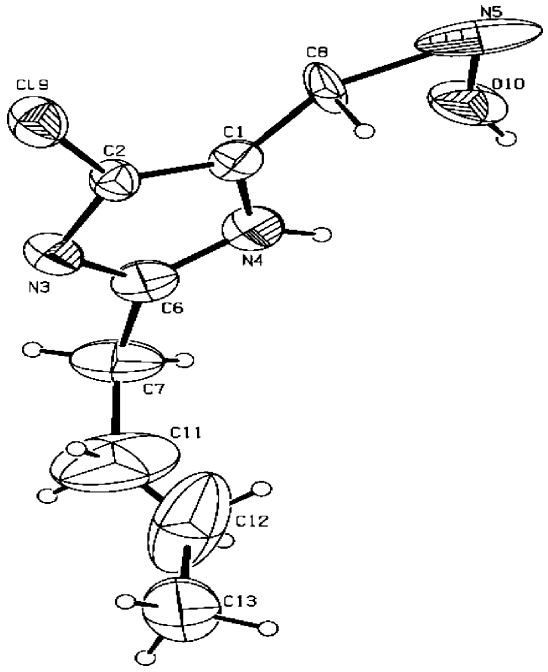

Figure 1. Oak ridge thermal ellipsoids (ORTEP) diagram[16] of the molecule **2** at 40% probability with crystallographic numbering scheme. The selected bond lengths are: N_5–O_{10}: 1.354(13) Å, C_8–N_5: 1.33(2) Å, C_1–N_4: 1.38(1) Å, N_4–C_6: 1.327(13) Å, C_6–N_3: 1.349(13) Å, N_3–C_2: 1.378(10) Å, C_1–C_2: 1.34(1) Å, C_2–Cl_9: 1.78(8) Å.

lab.[13a,b] An extended conformation of the δ^2-isoxazoline derivatives of R groups appears to be required for optimal filling of the hydrophobic active site of the enzyme with the common butyl substituent, since alteration of this chain by addition of ethyl group increases inhibitory potency (Table 1). This optimal length of R groups for the series is critical as it has a characteristic feature to displace His 48 of the enzyme and could probably lie at the base of the hydrophobic cavity and is unique. Also the mainly electron withdrawing groups of δ^2-isoxazoline and the substituted imidazole rings at the fifth position enhances the inhibitory activity while the electron releasing groups do not, which is clearly confirmed by the experimental results. The benzene ring can also form a nonbonded intermolecular interaction with the tyrosine derived aromatic ring, by being angled to one another. Such nonbonded interactions may contribute significantly toward the binding affinity of **3bVII** and **3bII** to the PLA_2 enzyme. These results suggest that the nonpolar functional groups are well tolerated within the hydrophobic cavity and contribute much to inhibitor binding and also easily hydrolyzable R group (ethoxy) shown to be good inhibitors.

The relative potencies of the compounds as inhibitors of the PLA_2 are shown in Table 1. Compounds **3bI** and **3bIV** demonstrate very strong in vitro inhibition of PLA_2 enzyme. Edema inducing activity[13c] of group II PLA_2 was inhibited by **3bI** and **3bIV**, which positively correlated with in vitro inhibition.

Amount of *Vipera russelli russelli* venom (Gift from Prof. T. Veerabasappa Gowda) taken for each in vitro

Table 1. Inhibitory effect of compounds **3b(I–VII)** on *Vipera russelli russelli* venom PLA_2 enzyme activity

Compound	Name	Venom PLA_2 IC_{50} (μM)	Edema[a] ratio
3bI	3-(2-Butyl-5-chloro-3*H*-imidazolyl-4-yl)-4,5-dihydro-isoxazole-carbonitrile	96.4	120 ± 2
3bII	3-(2-Butyl-5-chloro-3*H*-imidazolyl-4-yl)-5-phenyl-4,5-dihydro-isoxazole	142	ND[b]
3bIII	3-(2-Butyl-5-chloro-3*H*-imidazolyl-4-yl)-4,5-dihydro-isoxazole-5-carboxylic acid phenyl ester	195	ND[b]
3bIV	3-(2-Butyl-5-chloro-3*H*-imidazolyl-4-yl)-4,5-dihydro-isoxazole-5-carboxylic acid ethyl ester	86.2	116 ± 4
3bV	[3-(2-Butyl-5-chloro-3*H*-imidazolyl-4-yl)-4,5-dihydro-isoxazole]-5-acetic acid methyl ester	208.7	ND[b]
3bVI	[3-(2-Butyl-5-chloro-3*H*-imidazolyl-4-yl)-4,5-dihydro-isoxazole]-methanol	151.5	ND[b]
3bVII	3-(2-Butyl-5-chloro-3*H*-imidazolyl-4-yl)-4,5-dihydro-isoxazole-5-carboxylic acid methyl ester	250	ND[b]
Standard	Ursolic acid	2.5	102 ± 1

[a] The PLA_2 enzyme for in vivo (1 μg): δ^2-isoxazolines/ursolic acid (2.5 μM) mixture was preincubated at 37 °C for 1 h prior to injection into the mice footpads. Values of edema ratio are expressed as mean ± SD ($n = 4$), P values <0.05 were considered significant when compared to the control by Student's t-test.

[b] ND = Not determined.

assay was 1.4 μg. Ursolic acid 2.5 μM (Sigma) was taken as known PLA_2 inhibitor. Compounds **3bI** and **3bIV** alone did not cause edema when injected into mice footpads.

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References and notes

1. Fox, N.; Song, M.; Schermenti, J.; Sharp, J. D.; White, D. L.; Snyder, D. W.; Hartley, L. W.; Carlson, D. G.; Bach, N. J.; Dillard, R. D.; Draheim, S. E.; Bobbit, J. L.; Fisher, L.; Mihelich, E. D. *Eur. J. Pharmacol.* **1996**, *308*, 195.
2. Glaser, K. B. *Adv. Pharmacol.* **1995**, *32*, 31.
3. Mayer, R. J.; Marshall, L. A. *FASEB J.* **1993**, *7*, 339.
4. (a) Vishwanath, B. S.; Fawzy, A. A.; Franson, R. C. *Inflammation* **1988**, *12*, 549; (b) Sekar, K.; Mala, S. V.; Yogavel, M.; Velmurugan, D.; Ming-Jye, P.; Viswanath, B. S.; Gowda, T. V.; Jeyaprakash, A. A.; Tsai, M.-D. *J. Mol. Biol.* **2003**, *333*, 367.
5. Manjunath Kini, R. Venom Phospholipase A_2 enzymes. Structure, Function and Mechanism; John Wiley and Sons: England, 1997.
6. Fujii, S.; Tahara, Y.; Toyomoto, M. *Biochem. J.* **1995**, *7*(308), 297.
7. Amgad, G.; Habeeb; Praveen Rao, P. N.; Edward, E. K. *J. Med. Chem.* **2001**, *44*, 2921.
8. (a) Ravikumar, K. R.; Mallesha, H.; Basappa; Rangappa, K. S. *Eur. J. Med. Chem.* **2003**, *38*, 163; (b) Ravikumar, K. R.; Mallesha, H.; Rangappa, K. S. *Arch. Pharm.* **2003**, *336*, 159.
9. Beeranahally Doreswamy, H.; Basappa; Madegowda, M.; Mantelingu, K.; Sridhar Anandalwar, M.; Javaregowda Prasad, S.; Rangappa, K. S. *Anal. Sci.* **2003**, 31–32.
10. Basappa; Sadashiva, M. P.; Mantelingu, K.; Nanjunda Swamy, S.; Rangappa, K. S. *Bioorg. Med. Chem.* **2003**, *11*, 4539.
11. (a) Caramella, P.; Grunanger, P. In *1,3-Dipolar Cycloaddition Chemistry*; Padwa, A., Ed.; John Wiley and Sons: New York, 1984; Vol. 1, p 337; (b) Huisgen, R. *Angew. Chem., Int. Ed. Engl.* **1963**, *2*, 565.
12. Good quality single crystals of **2**, were grown by slow evaporation method (methanol as a solvent) at room temperature, and chosen after examination under a polarizing microscope and coated with glue before mounting. X-ray diffraction intensities were measured by ω-scans using a DIPlabo Kappa Imaging Plate (IP) Diffractometer equipped with IP area detector and a graphite monochromator for the Mo Kα radiation (50 kV, 36 mA). All frames of reflection data were processed by using DENZO[14] oscillation data processing program and data sets were scaled and merged by using the SCALEPACK[14] program. The phase problem was solved by direct methods, and the nonhydrogen atoms were refined anisotropically, by the means of full matrix least-squares methods using the SHELXL[15] program. Some of the hydrogen atoms were located using the difference Fourier map and the remaining were placed at geometrically idealized positions. The crystallographic details are deposited in CCDC database.[17]
13. (a) PLA_2 activity: Assayed with [^{14}C] oleate-labeled autoclaved *E. coli* as the substrate. The reaction mixture, 350 μL contained 100 mM Tris/HCl, pH 8.0, 5 mM Ca^{2+}, and 3.15×10^9 autoclaved *E. coli* cells (corresponding to 10,000 cpm and 60 nmol of lipid phosphorous). The amount of enzyme protein was chosen such that 10–15% hydrolysis of substrate was obtained when incubated at 37 °C for 60 min. The reaction components were mixed in the following order: buffer, calcium, water, and δ^2-isoxazoline derivatives. Adding labeled *E. coli* substrate started the reaction. The reaction was terminated by adding 100 μL of 2.0 M HCl, and 100 μL of fatty acid free BSA (100 mg/mL). The tubes were vortex mixed and centrifuged at 20,000*g* for 5 min. Aliquot (140 μL) of the supernatant containing released [^{14}C] oleic acid was mixed with scintillation cocktail 13 and counted in a Hewlett Packard liquid Scintillation Analyzer TRI CARB 2100 TR (b) Vishwanath, B. S.; Frey, F. J.; Bradbury, M. J.; Dallman, M. F.; Frey, B. M. *J. Clin. Invest.* **1993**, *92*, 1974; (c) Determination of edema inducing activity: Groups of six mice (22–24 g) were injected in the right footpads of hind limbs with 3 mM dose of δ^2-isoxazolines in 20 μL saline. The left footpads received 20 μL of saline, which served as control. After 45 min, mice were sacrificed by cervical dislocation and both legs were cut at the ankle joint and weighed individually. The increase in weight due to edema was calculated as the edema ratio, which equals the weight of edematous leg × 100/weight of the normal leg minimum edema dose, is defined as the microgram of protein causing an edema ration of 120%. Edema ratio = weight of edematous leg × 100/weight of normal leg.
14. Otwinowski, Z.; Minor, W. Processing of X-ray Diffraction Data Collected in Oscillation mode. In *Methods in Enzymology, Macromolecular Crystallography*; Carter, C. W., Jr., Sweet, R. M., Eds.; Academic: Dallas, TX, 1997; p 307.
15. Sheldrick, G.M. SHELX-97; University of Göttingen, Göttingen, Germany, 1997.
16. Johnson, C.K. ORTEP-II. A Fortran Thermal-Ellipsoid Plot Program. Report ORNL-5138; Oak Ridge National Laboratory, Oak Ridge, TN, USA, 1976.
17. Crystallographic data (excluding structure factors) for the structures have been deposited with the Cambridge Crystallographic center as supplementary publication numbers CCDC No 235618 for compound **2**. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union road, Cambridge, CB2 IEZ, UK (fax: +44(0) 1223336033 or e-mail: deposit@ccdc.cam.ac.uk).