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Synthesis of benzophenone oxime analogues as inhibitor of secretory phospholipase A_2 with anti-inflammatory activity

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Abstract—The title compound have been synthesized and tested for structure activity relationship for Phospholipase A_2 (PLA_2) [E.C. 3.1.1.4] enzyme inhibition. The in vitro PLA_2 enzyme inhibitory activity of benzophenone oxime analogue and in vivo anti-inflammatory activity studies using mice are highlighted.

1. Introduction

PLA_2 is a growing family of distinct enzymes that exhibits different substrate specificities, cofactor requirements, subcellular localization and cellular functions.[1] 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.[2–5] In many inflammatory diseases, high levels of PLA_2 enzymes are detected and are believed to be responsible for part of the inflammatory reactions. Injection of purified PLA_2 enzyme from synovial fluid and from snake venom into animal joints confirmed the development of an acute inflammatory response with edema, swelling of synovial cells and hyperplasia.[6,7] Benzophenone oxime analogues have shown anti-inflammatory activity. Benzophenone oximes have structural resemblance with fenoprofen, which belong to the class of aryl acetic acids. Alkylation of the benzophenone oxime potentiates the binding to the PLA_2 enzyme and enhances the anti-inflammatory activity. Presence of electron withdrawing groups such as Cl, Br, I, etc. at *meta*-position potentiates the activity.[8] Clinical results with cycloxygenase and lipoxygenase inhibitions demonstrate that inhibition of PLA_2 enzyme results in reduction of both lipid mediators, as a result these PLA_2 inhibitors can be used as anti-inflammatory drugs.

2. 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 benzophenone analogue 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 removed 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 ratio of 120%. Injecting a fixed dose of protein into mice footpads and sacrificing them at regular period of time obtained time course curve of edema inducing activity. Edema ratio was calculated as defined.[9]

3. PLA_2 activity

Assayed with [^{14}C]oleate-labelled autoclaved *E. coli* as the substrate.[10] 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

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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 benzophenone analogue. Adding labelled *E. coli* substrate started the reaction. The reaction was terminated by adding 100 μL 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 and counted in a Hewlett Packard liquid Scintillation Analyzer TRI CARB 2100 TR.

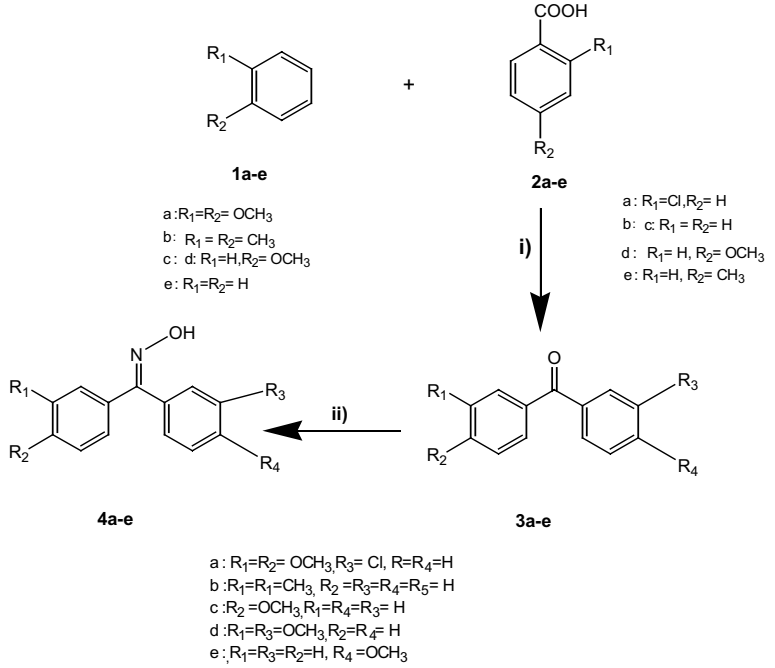
Benzophenone oxime alone did not cause edema when injected into mice footpads. As shown in Table 1, **4a** and **4b** demonstrated very strong in vitro PLA_2 enzyme inhibition. The compounds **4a** and **4b** inhibited the edema inducing activity of four different PLA_2 isoenzymes. The edema ratio at 1 μg PLA_2 enzyme concentration dropped below 120% in the presence of **4c** and **4d**

Table 1. Effect of compounds **4a**–**e** on PLA_2 enzyme activity and edema inducing activity of PLA_2

Phospholipase A_2	Compounds (inhibitors)[b]									
	4a		**4b**		**4c**		**4d**		Luffariellin B	
	Inhibitory rate (%)	Edema ratio	Inhibitory rate (%)	Edema ratio	Inhibitory rate (%)	Edema ratio	Inhibitory rate (%)	Edema ratio	Inhibitory rate (%)	Edema ratio
From *V. russelli* venom[a]	85.3	101 ± 6	79.2	102 ± 1	29.9	180 ± 5	58.7	136 ± 4	99.6	101 ± 6
From *N. naja* venom	90.2	109 ± 2	82.9	110 ± 5	42.4	142 ± 4	36.9	142 ± 2	100	103 ± 2
From *T. malabaricus* venom	72.4	105 ± 2	81.6	101 ± 3	NI	183 ± 2	NI	181 ± 4	99.8	101 ± 3
From pleural fluid	93.5	106 ± 3	91.0	103 ± 4	36.7	147 ± 4	16.9	147 ± 6	100	102 ± 5
From ascites fluid	76.8	107 ± 4	87.8	109 ± 3	11.7	151 ± 1	NI	149 ± 2	100	102 ± 1

Amount of venom taken for each assay was 1 μg ([a]1.4 μg). NI = No inhibition.

[b] Percent inhibition of benzophenone oxime analogue concentration of 2.67 mM edema ratio = weight of edematous leg × 100/weight of normal leg. The PLA_2 enzyme (1 μg): benzophenone analogues/Luffariellin B (3 mM) mixture was preincubated at 37 °C for 1 h prior to injection into the mice footpads. Prolonged preincubation time up to 12 h, at 37 °C did not have any additional effect on edema ratio. Values of edema ratio are expressed as mean ± SD ($n = 6$), P values <0.05 were considered significant when compared to the control by Student's t-test.
Inhibitory rate (%) = nanomoles of labelled substrate released as compared to the control.



Scheme 1. Synthesis of compounds **4a**–**e**. Reagents and conditions: (i) polyphosphoric acid (PPA) 100 °C, 2 h; (ii) hydroxylaminehydrochloride, reflux.

at 3 mM concentration. Compounds **4c** and **4d** were not much effective compared to **4a** and **4b** in both in vivo anti-inflammatory activity and in vitro PLA_2 enzyme inhibition.

The synthetic route of compounds **3a**–**e** and **4a**–**e** is as depicted in Scheme 1. Intermolecular Fridel Crafts reaction of substituted hydrocarbons with substituted aromatic acids in the presence of PPA (polyphosphoric acid) as catalyst afforded compounds **3a**–**e**.[11] Condensation of **3a**–**e** with hydroxylamine hydrochloride gave corresponding oximes **4a**–**e** in excellent yield.[12] The IR and ^1H NMR data of compounds **3a**–**e** and **4a**–**e** are in complete agreement.[13,14]

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13. **3a**: mp 149–150 °C (lit. mp 150–151 °C); IR (Nujol): 1600 cm^{-1} (aromatic C=C), 1640 (C=O), 1660 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.73 (s, 6H, $2OCH_3$), 6.9–7.9 (m, 8H, Ar-H). Anal. Calcd for $C_{17}H_{17}NO_3$ (283): C, 72.08; H, 6.01; N, 4.95. Found: C, 72.06; H, 6.04; N, 4.93.
3b: mp 165–166 °C; (lit. mp 150–151 °C); IR (Nujol): 1608 cm^{-1} (aromatic C=C), 1652 (C=O), 1662 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.35 (s, 3H, $2CH_3$), 7.0–7.9 (m, 8H, Ar-H). Anal. Calcd for $C_{17}H_{17}NO_3$ (283): C, 72.08; H, 6.01; N, 4.95. Found: C, 72.06; H, 6.04; N, 4.93.
3c: mp 155–160 °C (lit. mp 150–151 °C); IR (Nujol): 1600 cm^{-1} (aromatic C=C), 1640 (C=O), 1663 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.73 (s, 6H, $2OCH_3$), 6.9–7.65 (m, 8H, Ar-H). Anal. Calcd for $C_{17}H_{17}NO_3$ (283): C, 72.08; H, 6.01; N, 4.95. Found: C, 72.06; H, 6.04; N, 4.93.
3d: mp 145–150 °C (lit. mp 150–151 °C); IR (Nujol): 1600 cm^{-1} (aromatic C=C), 1642 (C=O), 1660 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.73 (s, 6H, $2OCH_3$), 6.9–7.9 (m, 8H, Ar-H). Anal. Calcd for $C_{17}H_{17}NO_3$ (283): C, 72.08; H, 6.01; N, 4.95. Found: C, 72.06; H, 6.04; N, 4.93.
3e: mp 165–170 °C (lit. mp 150–151 °C); IR (Nujol): 1600 cm^{-1} (aromatic C=C), 1640 (C=O), 1660 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.73 (s, H, $2OCH_3$), 6.9–7.9 (m, 8H, Ar-H). Anal. Calcd for $C_{17}H_{17}NO_3$ (283): C, 72.08; H, 6.01; N, 4.95. Found: C, 72.06; H, 6.04; N, 4.93.
14. **4a**: mp 149–150 °C (lit. mp 150–151 °C); IR (Nujol): 1600 cm^{-1} (aromatic C=C), 1650 (–N–OH), 1660 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.73 (s, 6H, $2OCH_3$), 6.9–7.9 (m, 8H, Ar-H). Anal. Calcd for $C_{17}H_{17}NO_3$ (283): C, 72.08; H, 6.01; N, 4.95. Found: C, 72.06; H, 6.04; N, 4.93.
4b: mp 165–166 °C; (lit. mp 150–151 °C); IR (Nujol): 1608 cm^{-1} (aromatic C=C), 1652 (–N–OH), 1662 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.35 (s, 3H, $2CH_3$), 7.0–7.9 (m, 8H, Ar-H). Anal. Calcd for $C_{17}H_{17}NO_3$ (283): C, 72.08; H, 6.01; N, 4.95. Found: C, 72.06; H, 6.04; N, 4.93.
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