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Potent inhibitors of lipoprotein-associated phospholipase A_2: Benzaldehyde *O*-heterocycle-4-carbonyloxime

Hyung Jae Jeong,[a] Yong-Dae Park,[a,b] Ho-Yong Park,[a] Il Yun Jeong,[c] Tae-Sook Jeong[a,*] and Woo Song Lee[a,b,*]

[a]*National Research Laboratory of Lipid Metabolism & Atherosclerosis, Insect Resources Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon 305-805, Republic of Korea*
[b]*University of Science and Technology, Daejeon 305-333, Republic of Korea*
[c]*Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute, Jeongeup 580-185, Republic of Korea*

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Abstract—A series of multi-substituted oximes were prepared and their potencies for inhibiting lipoprotein-associated phospholipase A_2 (Lp-PLA_2) activity were evaluated in vitro. Among them, compounds **3a**, **3b**, and **3m** were identified to display a micromolar potency for inhibiting Lp-PLA_2 in whole human plasma and isolated human LDL. Based on these results, structure–activity relationship was studied on modification of three parts of R^1, R^2, and R^3 to identify a potent pharmacophore for Lp-PLA_2. In an attempt to introduce various functional groups at R^2 and R^3, we discovered that replacement of less lipophilic groups led to an increase of inhibitory activity. Among the tested oxime derivatives, cyano- and morpholino-substituted analogue **4f** at R^2 and R^3 had the highest potency with an IC_{50} value of 0.05 μM in whole human plasma.
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Accumulation of LDL, particularly oxidized LDL, on the arterial wall is known as the most important initial step of atherosclerosis. Lp-PLA_2 is able to hydrolyze the *sn*-2 ester bond in phospholipids of which the fatty acid moiety has been shortened or altered by oxidation to yield oxidized fatty acid and lysophosphatidylcholine (lyso-PC).[1] Hydrolyzed products further accelerate chronic inflammation related to accumulation of macrophage, and a positive feedback mechanism of macrophage forming a large amount of Lp-PLA_2 further accelerates progress of vascular disorder. The biological role of Lp-PLA_2 had been controversial with seemingly contradictory anti- or pro-atherogenic functions being proposed. The anti-atherogenic properties of Lp-PLA_2 were suggested because of the enzymatic catabolism of biologically active oxidized phospholipids in LDL and degradation of the unrelated polar phospholipids, PAF.[2] In contrast, the pro-atherogenic function of Lp-PLA_2 is thought to arise from the formation of downstream inflammatory mediators derived from oxidized phospholipids. This view is supported by experimental evidence suggesting that the products of Lp-PLA_2 activity on oxidized phospholipids (oxidized fatty acid and lyso-PC) elicit potentially several pro-atherogenic effects. For example, the reported pro-atherogenic roles of lyso-PC are impairment of endothelium-dependent relaxation, inducement of vascular cell and intracellular adhesion molecules, activity as chemoattractant of monocyte and T-lymphocytes, suppressed production and release of endothelium-derived nitric oxide, inhibition of macrophage migration, toxicity at the concentration higher than 30–50 micromole, and stimulation of release of arachidonic acid from endothelial cells.[3]

Lp-PLA_2 is suggested as a pro-inflammatory agent, that is detected in macrophage of atherosclerotic lesions of hyperlipidemic human and rabbits.[4] According to Macphee's group's researches, the formation of fatty streak in Watanabe heritable hyperlipidemic rabbits is significantly decreased by dosage of Lp-PLA_2 inhibitor.[5] Therefore, inhibition of Lp-PLA_2 activity is highlighted as a target for prevention and treatment of atherosclerosis.

So far, the rational design of Lp-PLA_2 inhibitor has been somewhat difficult because the three-dimensional

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* Corresponding authors. Tel.: +82 42 860 4278; fax: +82 42 861 2675 (W.S.L.); tel.: +82 42 860 4558 (T.S.J.); e-mail addresses: tsjeong@kribb.re.kr; wslee@kribb.re.kr

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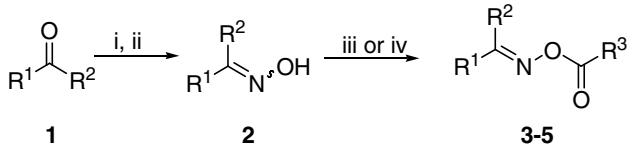
structure of Lp-PLA_2 was not elucidateed. However, the enzyme is known as a serine protease with a catalytic triad that was formed by a histidine and aspartic or glutamic acid at the active site.[6] Thirkettle et al. reported that SB-253514 was isolated from *Pseudomonas fluorescens* DMS 11579 and has shown potent inhibitory activity against Lp-PLA_2.[7–9] Subsequently, Smith and his coworker developed a novel series of pyrimidone derivatives through the high-throughput screening.[10] A potent, orally active Lp-PLA_2 inhibitor, SB-480848, has been developed by the modification of pyrimidone derivatives, which is currently in a phase II clinical study.[11] Recently, we have identified a new class of Lp-PLA_2 inhibitors, (*E*)-benzaldehyde *O*-benzoyloxime series, using isolated human LDL as Lp-PLA_2 enzyme sources.[12] The Lp-PLA_2 inhibitory effects of (*E*)-benzaldehyde *O*-benzoyloxime series were not quite different between each isolated human LDL and whole human plasma. In this article, we describe our initial optimization studies with a series of multi-substituted oximes in whole human plasma to afford a lead compound with the highest inhibitory potency of Lp-PLA_2.

A series of (*E*)- or (*Z*)-phenyl- and -heteroaryl-substituted *O*-benzoyl- (or acyl)oximes **3a–n**, **4a–j**, and **5a–s** were synthesized according to the methods shown in Scheme 1. Treatment of various aldehydes or ketones with hydroxylamine in the presence of Na_2CO_3 gave the mixture of (*E*)- and (*Z*)-oximes **2a–r** with a high ratio in high yields (Table 1). Among them, (*E*)-*N*-4-fluorobenzylpyrrole-2-carboaldehyde oxime (**2j**) was obtained by the reaction of *N*-fluorobenzyl-pyrrole-2-carbonylaldehyde with hydroxylamine. The reaction of benzonitrile with hydroxylamine in the presence of Et_3N gave (*Z*)-1-amino-benzaldehyde oxime (**2o**). Treatment of (*E*)-benzaldehyde oxime (**2a**) with *N*-chlorosuceinimide in dichloromethane gave (*Z*)-1-chloro-benzaldehyde oxime (**2p**). Then, pure (*Z*)-compounds **2o–q** are obtained by silica gel column chromatography. In order to introduce various functional groups, multi-substituted aryl or heteroaryl oximes **2a–r** were treated with acyl chlorides, morpholine-4-carbonyl chloride, and heteroaryl chlorides to give (*E*)-isomers **3a–n**, **4a**, **5a–s**, and (*Z*)-isomers **4b–j**. Then, (*Z*)-isomers **3a–n**, **5a–s** could be isomerized to the (*E*)-isomers **3a–n** and **5a–s** by triethyl ammonium hydrochloride[13] (Scheme 1 and Table 2).

The potential of (*E*)- or (*Z*)-oxime derivatives was evaluated as an inhibitor of Lp-PLA_2. Because the Lp-PLA_2 inhibitory effects of oxime derivatives on the assay system with [^{3}H]PAH (1-*O*-hexadecyl-acetyl-^{3}H(N)-phosphatidylcholine) as a substrate[12] were almost same between each isolated human LDL and whole human



Scheme 1. Reagents and condition: (i) NH_2OH, Et_3N, EtOH, rt; (ii) NBS, CH_2Cl_2; (iii) R^3COCl, Et_3N, CH_2Cl_2; (iv) R^3COCl, NaH, THF.

Table 1. Synthesis of (*E*)- or (*Z*)-oximes **2a–r**

Compound	R^1	R^2	Yield[a] (%) (*E*/*Z*)[b]
2a	Ph	H	90 (9/1)
2b	4-F-Ph	H	95 (8/1)
2c	(3,5-Di-*t*-Bu-4-OMe)-Ph	H	60 (6/1)
2d	PhCH=CH	H	95 (1.7/1)
2e	Furanyl	H	55 (1.2/1)
2f	Thiophenyl	H	65 (1/2.2)
2g	*N*-4-F-Benzylpyrrole	H	65
2h	4-CF_3-Ph	H	87
2i	4-F-Ph-Ph	H	78
2j	2,4-$(F)_2$-Ph	H	92
2k	2,5-$(F)_2$-Ph	H	76 (4/1)
2l	2,6-$(F)_2$-Ph	H	71
2m	3,4-$(F)_2$-Ph	H	73
2n	3,5-$(F)_2$-Ph	H	76
2o	Ph	NH_2	92
2p	Ph	Cl	85
2q	Ph	CN	—[c]
2r	Ph	Me	80 (9/1)

[a] Isolated yields.
[b] Isolated ratios or the ratios were not determined.
[c] Commercial reagent.

plasma (Table 2) as described above, the whole human plasma was used as Lp-PLA_2 enzyme sources in this study. Then, the amount of [^{3}H]acetate produced from [^{3}H] was determined to measure the Lp-PLA_2 inhibitory activity.[12] The Lp-PLA_2 inhibitory activities of **3a–n**, **4a–j**, and **5a–s** were confirmed by the positive control with SB480848 which was synthesized by us. Then, SB480848 inhibited Lp-PLA_2 with IC_{50} value of 0.17 μM. The strategy to explore the structure–activity relationship (SAR) of **3a** (IC_{50} = 5.1 μM), which was selected as a hit, focused on examining the optimal substituents at R^1, R^2, and R^3. The results from (*E*)- or (*Z*)-oxime derivatives are presented in Table 2. Modification of R^1 gave the corresponding compounds **3b–n**. Among them, 4-fluoro-substituted **3b** showed an enhanced activity, whereas 3,4-difluoro-substituted **3m** was equipotent to **3a**. The furan-, thiophene-, and pyrrole-substituted analogues **3e–g** at R^1 showed significantly lower activity. Analogues **3c** and **3d** involving *tert*-butyl and styrene groups resulted in a significant loss in potency. These analogues having more lipophilic groups generally showed more decreased potency compared with the corresponding analogues having less lipophilic groups. The position of the fluorine was examined according to the result of 4-fluoro-substituted analogue **3b**. 3,4-Difluoro-substituted derivative **3m** showed an encouraging inhibitory activity, whereas 2,3-, 2,4-, 2,5-, 2,6-, and 3,5-difluoro-substituted analogues **3i–l** and **3n** showed an attenuated potency. The effects of substitution and position on the phenyl ring were striking to show the potency.

Based on the above results that further simple modification of the structure had not given further enhancements in activity, we envisioned that more potent inhibitors would be obtained by the substitution at R^2 or R^3. So, various functional groups such as methyl, amine, chlorine, *N*,*N*-diacetyl, cyanide, and ethoxy groups were introduced at R^2 to give compounds **4a–e** and **4j**. Then,

Table 2. Lp-PLA_2 inhibitory activities of multi-substituted oxime derivatives **3a–n**, **4a–j**, and **5a–s**

Compound	R^1	R^2	R^3	Yield[a] (%)	IC_{50}[b] (μM)	% Inhibition[b]	
						At 25 μM	At 10 μM
3a	Ph	H	Ph	65	5.1 (3.8)[c]		74
3b	4-F-Ph	H	Ph	80	3.2 (4.4)[c]		85
3c	(3,5-Di-*t*-Bu-4-OMe)-Ph	H	Ph	50		4	
3d	PhCH=CH	H	Ph	85		35	
3e	Furanyl	H	Ph	72		16	
3f	Thiophenyl	H	Ph	75		35	
3g	*N*-F-Benzylpyrrole	H	Ph	82			16
3h	4-CF_3-Ph	H	Ph	86			0
3i	2,3-$(F)_2$-Ph	H	Ph	78			20
3j	2,4-$(F)_2$-Ph	H	Ph	85			50
3k	2,5-$(F)_2$-Ph	H	Ph	74			6
3l	2,6-$(F)_2$-Ph	H	Ph	72			0
3m	3,4-$(F)_2$-Ph	H	Ph	80	5.0 (2.0)[c]		91
3n	3,5-$(F)_2$-Ph	H	Ph	82			33
4a	Ph	Me	Ph	80			38
4b	Ph	NH_2	Ph	82			13
4c	Ph	Cl	Ph	90		75	31
4d	Ph	$N(Ac)_2$	Ph	73			0
4e	Ph	CN	Ph	78			0
4f	Ph	CN	Morpholine	85	0.051 (0.12)[c]		100
4g	Ph	CN	Thiophenyl	75			0
4h	Ph	CN	Furanyl	88			15
4i	Ph	CN	3,4-F-Ph	73			0
4j	Ph	OEt	Morpholine	75			48
5a	Ph	H	Morpholine	90			43
5b	Ph	H	Cyclohexyl	79			20
5c	Ph	H	Thiophenyl	83			47
5d	Ph	H	Furanyl	81			37
5e	4-F-Ph	H	Morpholine	88	3.5		94
5f	4-F-Ph	H	4-F-Ph	76	6.1		73
5g	2,3-$(F)_2$-Ph	H	Morpholine	79	2.2		100
5h	2,4-$(F)_2$-Ph	H	4-F-Ph	83			49
5i	2,4-$(F)_2$-Ph	H	Morpholine	85	2.4		97
5j	2,5-$(F)_2$-Ph	H	Morpholine	97			61
5k	2,6-$(F)_2$-Ph	H	Morpholine	81			0
5l	3,4-$(F)_2$-Ph	H	Morpholine	87	4.2		98
5m	3,4-$(F)_2$-Ph	H	4-F-Ph	72			66
5n	3,4-$(F)_2$-Ph	H	9(*Z*)-$C_{17}H_{33}$	46		16	
5o	3,4-$(F)_2$-Ph	H	9(*Z*),12(*Z*)-$C_{17}H_{31}$	24		20	
5p	3,4-$(F)_2$-Ph	H	C_9H_{19}	50		1	
5q	3,4-$(F)_2$-Ph	H	4-NO_2-Ph	65		9	
5r	3,4-$(F)_2$-Ph	H	3,4-$(F)_2$-Ph	85		24	
5s	3,5-$(F)_2$-Ph	H	Morpholine	84			58

[a] Isolated yields from **2**.
[b] Using whole human plasma (1–2 mg protein).
[c] Using isolated human LDL (13–18 μg protein). Data are shown as mean values of two independent experiments performed in duplicate.

compounds **4a** and **4b** had a little effect on potency, whereas compound **4c** was proved more potent in Lp-PLA_2 inhibitory activity. More exciting was that compound **4e** was not active, but less lipophilic morpholine-substituted analogue **4f** at R^3 had dramatically improved inhibitory activity against Lp-PLA_2 with sub-micromolar inhibition in this series (IC_{50} = 0.05 μM). Therefore, we focused on variations to R^3. The inhibitory activities enhanced on substitution of the morpholine group at R^3 (**4j**, **5a**, **5e**, **5g**, **5i**, **5j**, **5l**, **5s**), whereas 2,6-difluoro-substituted **5k** was not active, as shown in analogue **3l**. Also, mono- or difluorophenyl-substituted derivatives **5f**, **5h**, and **5m** at R^1 and R^3 had somewhat increased inhibitory activity. Subsequently, the highly lipophilic C_9- or C_{18}-substituted derivatives **5n–p** at R^3 were much less potent, even though increasing the length of the alkyl chain increased inhibitory activity.[14] Inhibitors of lipolytic enzymes are best reported in terms of mole fraction of inhibitor in the interface (number of moles of inhibitor to the total number of moles of lipid, detergent, and inhibitor in the micelle).[15] One common critique of phospholipase inhibition assays is that they can be subject to numerous false positives, due to disruption of the membrane/water interface by lipophilic compounds. It is clear from data (Table 2) that there is no proportional correlation between the lipophilicity of the compound and the inhibitory activity in this assay.

In conclusion, we have discovered a novel series of human Lp-PLA_2 inhibitors, (*E*)- or (*Z*)-phenyl- and

-heteroaryl-substituted *O*-benzoyl- (or acyl)oximes **3a**–**n**, **4a**–**j**, and **5a**–**s**, by SAR study of **3a**. 3-Fluoro- and 3,4-difluoro-substituted analogues **3b** and **3m** by modification at R^1 showed equipotent Lp-PLA_2 inhibitory activity in whole human plasma. More exciting was that compound **4e** was not active, but cyanide- and morpholine-substituted analogue **4f** at R^2 and R^3 had dramatically improved Lp-PLA_2 inhibitory activity with sub-micromolar inhibition in this series (IC_{50} = 0.05 μM). These analogues having more lipophilic groups generally showed more decreased potency compared with the corresponding analogues. This would indicate that the increases in potency of Lp-PLA_2 inhibitory activity are related to more substantial interaction with the enzyme rather than through change in lipophilicity. Furthermore, oral efficacy test in rabbits will be the subject of future publications to develop an anti-atherogenic agent.

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