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(*E*)-Phenyl- and -heteroaryl-substituted *O*-benzoyl- (or acyl)oximes as lipoprotein-associated phospholipase A_2 inhibitors

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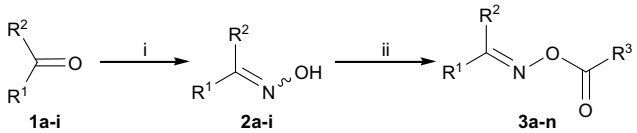
Abstract—A series of (*E*)-phenyl- and -heteroaryl-substituted *O*-benzoyl- (or acyl)oximes **3a**–**n** were synthesized for evaluating their human lipoprotein-associated phospholiphase A_2 (Lp-PLA_2) inhibitory activities. The less lipophilic derivatives **3a**–**c** showed the most potent in vitro inhibitory activity on human Lp-PLA_2.

In general, oxidized low-density lipoproteins (ox-LDLs) play a key role in the early stages of atherosclerosis.[1] Lipoprotein-associated phospholiphase A_2 (Lp-PLA_2) associates predominantly with LDL in plasma and hydrolyzes the *sn*-2 fatty acid of oxidatively modified LDL to generate lysophosphatidylcholine (lyso-PC) and oxidized free fatty acids (oxFFA).[2] In vitro studies have demonstrated that lyso-PC in ox-LDLs is a strong chemoattractant for human monocytes and promotes the chronic inflammation that is associated with macrophage accumulation. Then, macrophages secrete Lp-PLA_2 through the positive feedback mechanism driving progression of atherosclerosis.[3] Therefore, Lp-PLA_2 enzyme is a very attractive target for the treatment of atherosclerosis.

So far, the rational design of Lp-PLA_2 inhibitor is somewhat difficult because the three-dimensional structure of Lp-PLA_2 enzyme has not been elucidated. However, the enzyme is known to be a serine lipase with a catalytic triad that is formed by a histidine and aspartic or glutamic acid at the active site.[4] Thirkettle and co-workers reported that SB-253514 was isolated from *Pseudomonas fluorescens* DMS 11579 and has shown potent inhibitory activity against Lp-PLA_2.[5] Also, Smith and co-workers developed a novel series of pyrimidone derivatives through high throughput screening.[6] To explore novel Lp-PLA_2 inhibitors, we screened 4480 compounds that were deposited in Korea Chemical Bank to select (*E*)-benzaldehyde *O*-benzoyloxime (**3a**) with IC_{50} value of 3.8 μM. In this letter, we wish to describe the synthesis and in vitro Lp-PLA_2 inhibitory activity of (*E*)-benzaldehyde *O*-benzoyloxime (**3a**) and its derivatives by optimization studies.

A series of (*E*)-phenyl- and -heteroaryl-substituted *O*-benzoyl- (or acyl)oximes **3a**–**n** were synthesized according to the methods shown in Scheme 1.[7] Treatment of various aldehydes or ketones (**1a**–**i**) with hydroxylamine hydrochloride and Et_3N gave the mixture of (*E*)- and (*Z*)-oximes **2a**–**i** with a high ratio in 60–95% yields, as shown in Table 1. Reaction of the mixture of (*E*)- and (*Z*)-oximes **2a**–**i** with benzoyl or acyl chlorides gave only an (*E*)-isomer **3a**–**n** because (*Z*)-isomer could be isomerized to the (*E*)-isomer by triethyl ammonium hydrochloride,[8] as shown in Scheme 1 and Table 2.



Scheme 1. Reagents and conditions: (i) $NH_2OH{\cdot}HCl$, Et_3N, EtOH, rt; (ii) R^3COCl, Et_3N, CH_2Cl_2, 0 °C.

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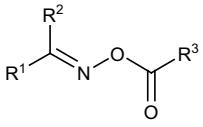
Table 1. Synthesis of (*E*)- or (*Z*)-aldoximes **2a**–**i**

Compd **2**	R^1	R^2	Yield (%)[a] (*E*/*Z*)[b]
2a	Ph	H	80 (9/1)
2b	3,4-$(F)_2$-Ph	H	95 (9:1)
2c	4-F-Ph	H	95 (8:1)
2d	(3,5-Di-*t*-Bu-4-OMe)-Ph	H	60 (6:1)
2e	PhCH=CH	H	95 (1.7:1)
2f	Furanyl	H	55 (1.2:1)
2g	Thiopheneyl	H	65 (1:2.2)
2h	Ph	Me	80 (9:1)
2i	Ph	Ph	84

[a] Isolated yields from **1a**–**i**.
[b] Isolated ratios.

Table 2. Lp-PLA_2 inhibitory activities of **3a**–**n**



Compd **3**	R^1	R^2	R^3	Yield (%)[a]	IC_{50} (μM)[b]
3a	Ph	H	Ph	65	3.8
3b	3,4-$(F)_2$-Ph	H	Ph	80	2.0
3c	4-F-Ph	H	Ph	80	4.4
3d	(3,5-Di-*t*-Bu-4-OMe)-Ph	H	Ph	50	11%[c]
3e	PhCH=CH	H	Ph	85	25
3f	Furanyl	H	Ph	72	26.0
3g	Thiopheneyl	H	Ph	75	11.2
3h	Ph	Me	Ph	80	29%[c]
3i	Ph	Ph	Ph	77	NA[d]
3j	3,4-$(F)_2$-Ph	H	9(*Z*)-$C_{17}H_{33}$	46	16%[c]
3k	3,4-$(F)_2$-Ph	H	9(*Z*), 12(*Z*)-$C_{17}H_{31}$	24	20%[c]
3l	3,4-$(F)_2$-Ph	H	C_9H_{19}	50	12%[c]
3m	3,4-$(F)_2$-Ph	H	Ph (4-NO_2)	65	17%[c]
3n	3,4-$(F)_2$-Ph	H	Ph (3,4-F_2)	85	38

[a] Isolated yields from **2**.
[b] Using isolated LDL. Data are shown as mean values of two independent experiments performed in duplicate.
[c] Percentage at 25 μM.
[d] NA = not active.

The potential of **3a**–**n** was evaluated as an inhibitor of Lp-PLA_2 (LDL-PLA_2). Because the plasma isoform of Lp-PLA_2 is 85–90% bound to LDL,[9] the LDL isolated from the plasma of normalipidemic volunteers[10] was used as the source of enzyme. Then, the amount of [^{3}H]acetate produced from [^{3}H]PAH (1-*O*-hexadecyl-acetyl-^{3}H(N)-phosphatidylcholine) was determined by scintillation counting to reveal the Lp-PLA_2 inhibitory activity.[11] The Lp-PLA_2 inhibitory activities of **3a**–**n** were confirmed by the positive control with SB381320 supplied by GlaxoSmithKline. Then, SB381320 inhibited Lp-PLA_2 (LDL-PLA_2) with IC_{50} value of 8.8 nM (IC_{50} value of 8.0 nM in recombinant Lp-PLA_2 and 67% inhibition in whole human plasma at 100 nM).[12] The data for all compounds **3a**–**n** has been shown in Table 2. Compound **3a**–**c** showed an encouraging inhibitory activity against Lp-PLA_2 with IC_{50} values of 3.8, 2.0, and 4.4 μM, respectively. The styryl and heterocycle derivatives **3e**–**g** at R^1 proved a little less potent than **3a**. On the other hand, a heavily substituted phenyl derivative **3d** was almost devoid of activity. Compounds **3h** and **3i**, which were substituted with methyl and phenyl groups at R^2, had little effect on potency against Lp-PLA_2. The highly lipophilic C_9 or C_{18} derivatives **3j**–**l** at R^3 showed a weak inhibitory activity, even though increasing the length of the alkyl chain increased activity.[13] Substitution at R^3 gave compound **3m** with little activity, however, 3,4-difluorophenyl derivative **3n** at R^3 showed a somewhat increased inhibitory activity. These results may be rationalized that Lp-PLA_2 inhibitory activity depends on lipophilicity of the functional groups at R^1 and R^3.

In conclusion, we have discovered a novel series of Lp-PLA_2 enzyme inhibitors, (*E*)-phenyl- and -heteroaryl-substituted *O*-benzoyl- (or acyl)oximes **3a**–**n**. Furthermore, the efficacy test of anti-atherogenic activity will be the subject of future publications.

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brine, dried over anhydrous $MgSO_4$, filtered, and evaporated to give the crude product. Purification by flash column chromatography on silica gel *n*-hexane–EtOAc (1:1) gave the pure (*E*)-**2a** (3.0 g, 50%) and (*Z*)-**2a** (0.32 g, 5%). To a solution of (*E*)-**2a** (0.22 g, 1.8 mmol) in CH_2Cl_2 (10 mL) was added benzoyl chloride (0.3 mL, 2.3 mmol) in the presence of Et_3N (0.32 mL, 2.3 mmol) at 0 °C. After being stirred for 1 h, the reaction mixture was quenched with 1 N HCl and extracted with CH_2Cl_2 to give the residue, which was washed with saturated $NaHCO_3$ aqueous solution and brine and dried over anhydrous $MgSO_4$. Purification by flash column chromatography on silica gel [*n*-hexane–EtOAc (1:1)] gave the pure (*E*)-**3a** (0.26, 65%) as colorless prisms, mp 98–105 °C (*n*-hexane–CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48 (m, 5H), 7.62 (m, 1H), 7.82 (dd, J = 1.8, 7.8 Hz, 2H), 8.14 (dd, J = 2.1, 8.4 Hz, 2H), 8.57 (s, 1H).

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