INHIBITION OF Ca^{2+}- INDEPENDENT PHOSPHOLIPASE A_2 BY 2-OXOAMIDES BASED ON DIPEPTIDES AND ETHER PSEUDODIPEPTIDES

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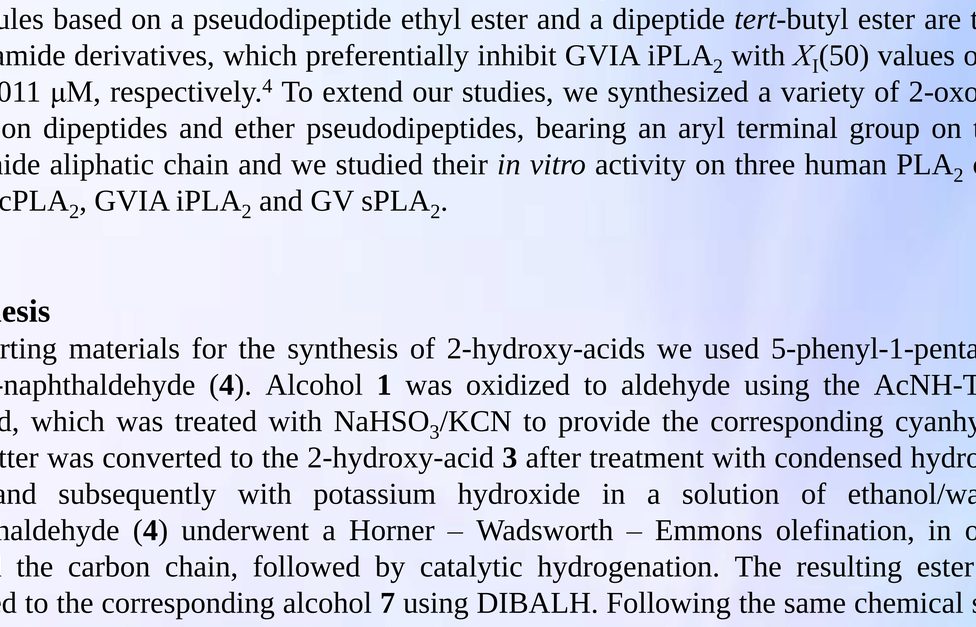
Introduction

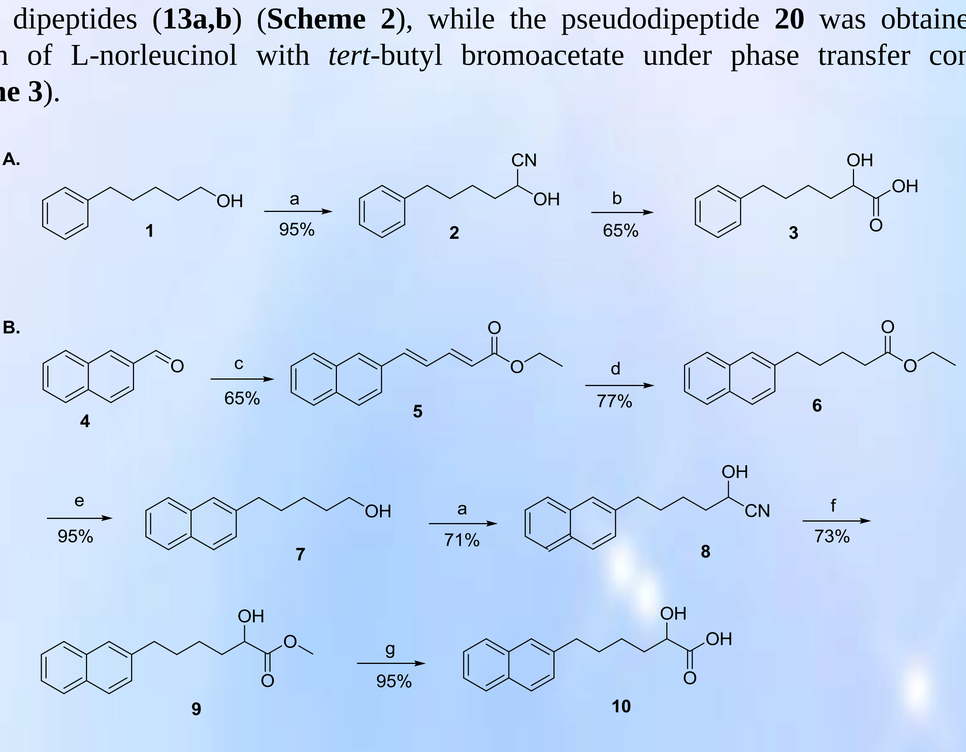
Phospholipases A_2 (PLA_2) catalyze the hydrolysis of the *sn-2* ester bond of glycerophospholipids producing free fatty acids and lysophospholipids. The free arachidonic acid (AA) that is released may be converted to a variety of proinflammatory eicosanoids. Therefore, inhibiting AA release is of great therapeutic relevance for the development of new anti-inflammatory drugs. Moreover, selective inhibition of the less studied enzyme of the three main categories, GVIA $iPLA_2$, may offer valuable information about the enzyme's physiological role. A new class of PLA_2 inhibitors has been developed by our group: long chain 2-oxoamides based on amino acids.[1-3] Among others, we have shown that two molecules based on a pseudodipeptide ethyl ester and a dipeptide *tert*-butyl ester are the first 2-oxoamide derivatives, which preferentially inhibit GVIA $iPLA_2$ with $X_I(50)$ values of 0.017 and 0.011 μM, respectively.[4] To extend our studies, we synthesized a variety of 2-oxoamides based on dipeptides and ether pseudodipeptides, bearing an aryl terminal group on their 2-oxoamide aliphatic chain and we studied their *in vitro* activity on three human PLA_2 classes: GIVA $cPLA_2$, GVIA $iPLA_2$ and GV $sPLA_2$.

Synthesis

As starting materials for the synthesis of 2-hydroxy-acids we used 5-phenyl-1-pentanol (**1**) and 2-naphthaldehyde (**4**). Alcohol **1** was oxidized to aldehyde using the AcNH-TEMPO method, which was treated with $NaHSO_3$/KCN to provide the corresponding cyanhydrin **2**. The latter was converted to the 2-hydroxy-acid **3** after treatment with condensed hydrochloric acid and subsequently with potassium hydroxide in a solution of ethanol/water. 2-Naphthaldehyde (**4**) underwent a Horner – Wadsworth – Emmons olefination, in order to extend the carbon chain, followed by catalytic hydrogenation. The resulting ester **6** was reduced to the corresponding alcohol **7** using DIBALH. Following the same chemical steps as previously, cyanhydrin **8** was obtained, which was converted to the 2-hydroxy-methylester **9** after treatment with methanolic hydrochloride. The desired 2-hydroxy-acid **10** was produced after saponification (Scheme 1). *N*-Protected L-norleucines **11a,b** and glycine esters **12a,b** were coupled using WSCI.HCl as a condensing agent in the presence of HOBt to provide the desired dipeptides (**13a,b**) (**Scheme 2**), while the pseudodipeptide **20** was obtained after reaction of L-norleucinol with *tert*-butyl bromoacetate under phase transfer conditions (**Scheme 3**).



Scheme 1. *Reagents and conditions:* a) i. NaOCl, NaBr, AcNH-TEMPO, $NaHCO_3$, AcOEt/toluene/H_2O 3:3:0.5, -5 °C; ii. $NaHSO_3$, KCN, CH_2Cl_2, H_2O; b) i. conc. HCl, ii. KOH, EtOH/H_2O; c) $C_2H_5OOCCH{=}CHCH_2P(=O)(OC_2H_5)_2$, LiOH, THF, reflux; d) H_2, 10% Pd/C, EtOH; e) DIBALH, anhydrous Et_2O; f) 3N HCl/ MeOH; g) NaOH 1N, MeOH.

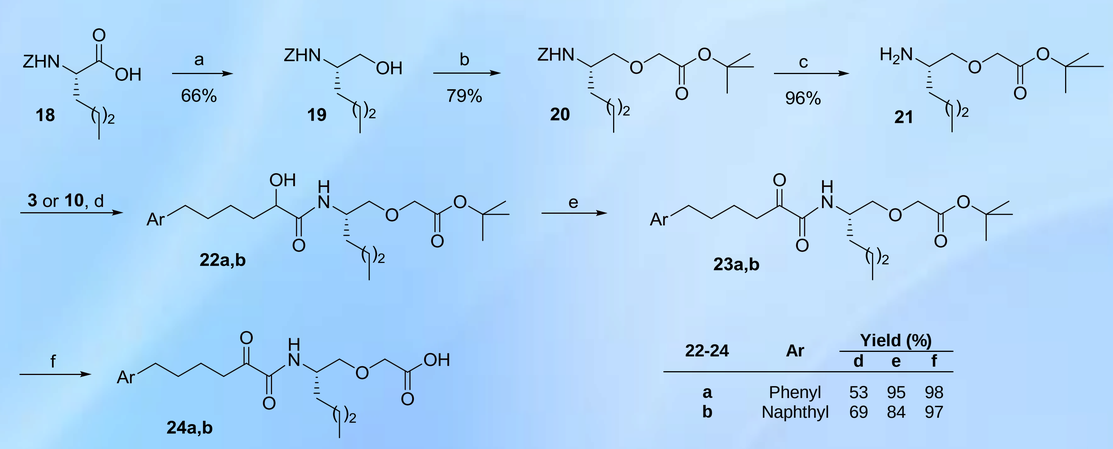


13, 14	P	R	Yield (%) a	b	c
a	Z	Bu^t	81	97	
b	Boc	Et	72		98

15-17	Ar	R	Yield (%) a	d	e
a	Phenyl	Bu^t	66	79	88
b	Phenyl	Et	58	80	
c	Naphthyl	Bu^t	47	99	93
d	Naphthyl	Et	42	80	

Scheme 2. *Reagents and conditions:* a) WSCI, HOBt, Et_3N, CH_2Cl_2; b) H_2, 10% Pd/C, THF; c) 4N HCl/Et_2O; d) Dess-Martin periodinane, CH_2Cl_2; e) 50% TFA/CH_2Cl_2.

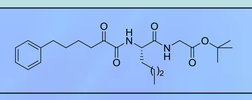
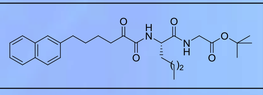
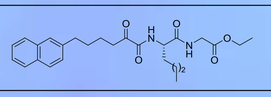
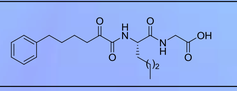
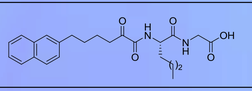
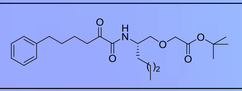
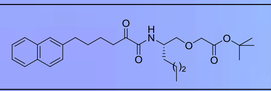
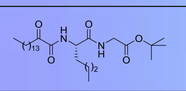
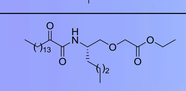
Coupling reaction between the free amino group of the dipeptides or the ether pseudodipeptide and the 2-hydroxy acid took place in the presence of WSCI/HOBt and the resulting 2-hydroxy amides **15a-d** and **22a,b** were oxidized to 2-oxoamides **16a-d** and **23a,b** using the Dess-Martin method. Oxoamides **17a,c** and **24a,b** containing a free carboxyl group, were obtained by treatment of **16a,c** and **23a,b** with trifluoroacetic acid (**Schemes 2 and 3**).



22-24	Ar	Yield (%) d	e	f
a	Phenyl	53	95	98
b	Naphthyl	69	84	97

Scheme 3. *Reagents and conditions:* a) i. NMM, ClCOOEt, THF; ii. $NaBH_4$, CH_3OH; b) $BrCH_2COOBu^t$, 50% NaOH, Bu_4NHSO_4, benzene; c) H_2, 10% Pd/C; d) WSCI, HOBt, Et_3N, CH_2Cl_2; e) Dess-Martin periodinane, CH_2Cl_2; f) 50% TFA/CH_2Cl_2.

Table 1. *In vitro* results of the human phospholipases A_2 inhibition by 2-oxoamides.

Compound	Structure	% Inhibition $cPLA_2$	$iPLA_2$	$sPLA_2$
16a		46.9	91.1	43.4
16c		57.5	89.1	44.8
16d		70.5	77.1	54.4
17a		11.2	-	-
17c		43.1	-	-
23a		82.3	76.0	57.5
23b		85.5	88.4	63.9
24[a]		72	**$X_I(50)$** 0.011	59
25[a]		52	**$X_I(50)$** 0.017	81

[a] Data taken from Ref. 4

Results and discussion

Preliminary PLA_2 inhibition results are summarized in Table 1. Derivatives **24** and **25** have been also included for comparison reasons. Among the seven compounds tested, **16a** and **16c**, based on Nle-Gly *tert*-butyl ester, bearing phenyl and naphthyl side chain terminal moieties respectively, appear to inhibit preferentially GVIA $iPLA_2$. This observation is in full agreement with our previous report, that the 2-oxoamide derivative based on Nle-Gly *tert*-butyl ester (**24**) is potent and selective inhibitor of GVIA $iPLA_2$.[4] The other two *tert*-butyl esters of this series, namely **23a** and **23b**, based on ether pseudodipeptides, exhibited considerable but lower activity towards GVIA $iPLA_2$, though they inhibit also GIVA $cPLA_2$ to a similar level. 2-Oxoamide derivatives containing free carboxylic acids (**17a** and **17c**) do not inhibit GVIA $iPLA_2$.

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