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Synthesis of Lipophilic Aldehydes and Study of Their Inhibition Effect on Human Digestive Lipases

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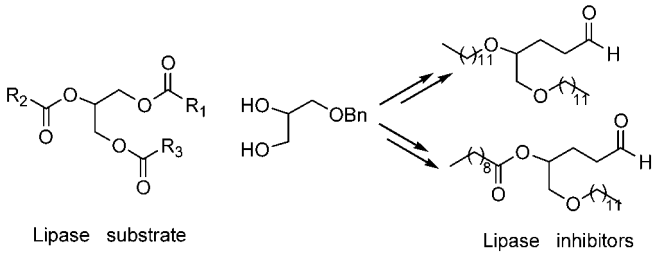
ABSTRACT



Novel inhibitors of human digestive lipases, aldehyde dialkyl and alkyl-acyl glycerol analogues, were developed. The inhibitors were prepared starting from 3-(benzyloxy)-1,2-propanediol. The inhibition of human pancreatic and gastric lipases by the aldehyde derivatives was studied using the monolayer technique. (1*R*)-1-[(Dodecyloxy)methyl]-4-oxobutyl decanoate caused a 50% decrease in HPL and HGL activities at 0.100 and 0.053 molar fractions, respectively.

In humans and most mammals the digestion of dietary triacylglycerols (TAGs) requires the successive intervention of two lipases: gastric lipase (HGL), which is secreted and is active in stomach, followed by the classical pancreatic lipase (HPL) secreted into the duodenum. The hydrolysis of TAGs by these lipases to monoacylglycerols and free fatty acids is a necessary step for efficient fat digestion and absorption by the enterocytes.[1] Therefore, inhibitors of digestive lipases are of special interest because they may find applications as anti-obesity agents.[2] A gastrointestinal lipase inhibitor of microbial origin is now a registered drug for weight reduction.[3]

Synthetic inhibitors of lipases have been used in the study of structural and mechanistic properties of lipases.[4] Among the various classes of inhibitors reported, inhibitors of the phosphonate type have been successfully used to solve the 3D structure of various lipases.[5]

The active site of pancreatic lipase contains Ser-His-Asp residues, a triad resembling the catalytic triad of serine proteases, as has been proven by site-directed mutagenesis[6] and crystallographic data.[7] HGL possesses the same catalytic triad.[8] We have recently proposed a strategy for the rational design of lipase inhibitors. In general a lipase inhibitor should consist of two components: a chemically reactive moiety,

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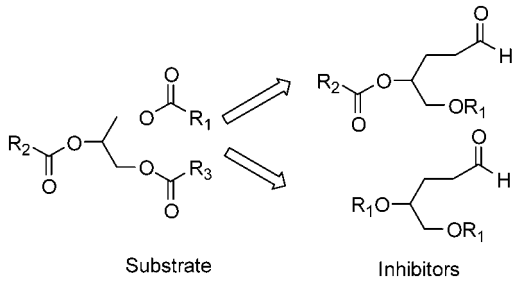
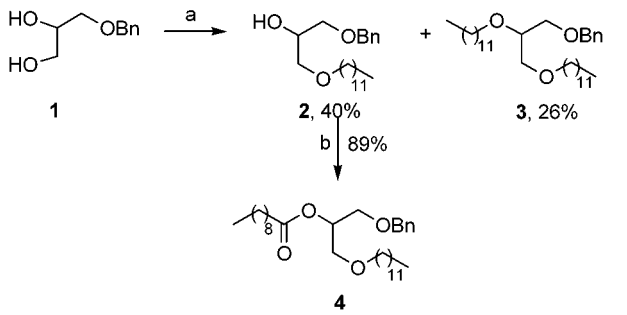
capable of reacting with the active site serine of the enzyme, and a part that contains chemical motifs, necessary for specific interactions and a proper orientation in the enzyme binding pocket. Up to now we have shown that lipophilic 2-oxo amide[9,10] and 2-oxo amide[11] and bis-2-oxo amide[12] triacylglycerol analogues are effective inhibitors of human pancreatic and gastric lipases.



According to the above strategy we decided to use the aldehyde group as the reactive functionality. Peptide aldehydes have been reported to inhibit serine and cysteine proteases.[13] It should be noted that two tripeptide aldehyde inhibitors of thrombin have entered clinical trials.[14] The novel lipase inhibitors were designed taking into consideration the structure of triacylgycerols, which are the natural substrate of lipases. The carbonyl of the ester bond at the *sn*-1 position of the substrate was replaced by the carbonyl of the aldehyde functionality. The ester bond at the *sn*-3 position was replaced by an ether bond to avoid hydrolysis at this position. The ester bond at the *sn*-2 position was either maintained or replaced by a non-hydrolyzable ether bond. Given the preference of HPL and HGL to hydrolyze ester bonds at the *sn*-1 and *sn*-3 positions of triacylglycerol, the ester bond of the inhibitor corresponding to that of the *sn*-2 position is not anticipated to undergo enzymatic hydrolysis.

Etherification of 3-(benzyloxy)-1,2-propanediol (**1**) with 1-bromododecane took place under phase transfer conditions and produced a mixture of compounds **2** (40%) and **3** (26%), which were separated (Scheme 1). Compound **2** was then coupled with decanoic acid using 1,3-dicyclohexylcarbodiimide (DCC) as a condensing agent in the presence of 4-(dimethylamino)pyridine (DMAP)[15] to produce compound **4**.

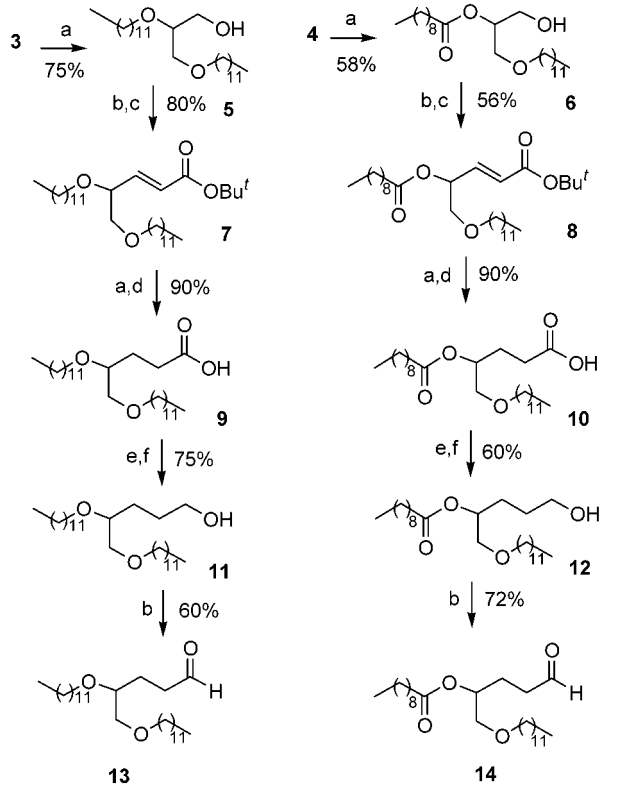
Scheme 1[a]



[a] (a) $C_{12}H_{25}Br$, Bu_4NHSO_4, 50% NaOH/C_6H_6, 45 °C; (b) $C_9H_{19}COOH$, DCC, DMAP, CH_2Cl_2.

The removal of the benzyl group from compounds **3** and **4** was carried out by catalytic hydrogenation (Scheme 2).

Scheme 2[a]



[a] (a) H_2, 10% Pd/C; (b) AcNH-TEMPO, NaOCl, NaBr, $PhCH_3$/EtOAc/H_2O, −10 °C; (c) $Ph_3P{=}CHCOOBu^t$, THF, 65 °C; (d) 50% CF_3COOH/CH_2Cl_2; (e) NMM, ClCOOEt, THF, −10 °C; (f) $NaBH_4$, MeOH.

Compounds **5**, **6** were then oxidized to the corresponding aldehydes by NaOCl in the presence of 4-acetamido-2,2,6,6-tetramethyl-1-piperidinyloxy free radical (AcNH-TEMPO).[16]

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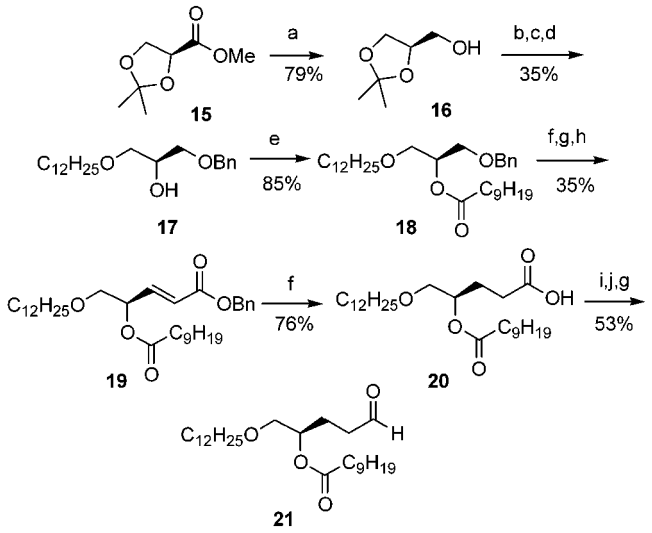
The aldehydes, without further purification, reacted with $Ph_3P{=}CHCOOBu^t$ to afford compounds **7** and **8**. The corresponding carboxylic acids **9** and **10** were obtained after catalytic hydrogenation and acidic removal of the Bu^t group. Reduction of the mixed carbonic anhydrides of **9** and **10** by $NaBH_4$[17] and oxidation of the products produced the target compounds **13** and **14**.

To study the stereoselectivity of the inhibition, the *R*-enantiomer of compound **14**, (1*R*)-1-(dodecyloxymethyl)-4-oxobutyl decanoate (**21**), was prepared by similar reactions starting from compound **15**, as outlined in Scheme 3. All the intermediates and final products gave satisfactory analytical and spectroscopic data.[18]

Scheme 3[a]



[a] (a) DIBALH, Et_2O, 0 °C; (b) $C_6H_5CH_2Br$, Bu_4NHSO_4, 50% NaOH/C_6H_6, 45 °C; (c) 4 N HCl/MeOH; (d) $C_{12}H_{25}Br$, Bu_4NHSO_4, 50% NaOH/C_6H_6, 45 °C; (e) $C_9H_{19}COOH$, DCC, DMAP, CH_2Cl_2; (f) H_2, 10% Pd/C; (g) AcNH-TEMPO, NaOCl, NaBr, $PhCH_3$/AcOEt/H_2O, −10 °C; (h) $Ph_3P{=}CHCOOBn$, THF, 65 °C; (i) NMM, ClCOOEt, THF, −10 °C; (j) $NaBH_4$, MeOH.

The study of inhibition of lipolytic enzymes is a difficult task, because of nonmutually exclusive processes such as interfacial denaturation, changes in interfacial quality[19] and surface dilution phenomena.[20] The use of the monolayer technique, which is based upon surface pressure decrease owing to lipid-film hydrolysis, is advantageous for the study of lipases inhibitors since with conventional emulsified systems it is not possible to control the interfacial quality. The kinetic studies of the lipase hydrolysis reactions require that the lipids used form a stable monomolecular film at the air/water interface.[21]

To determine the film stability and the interfacial properties at the air/water interface of the aldehyde derivatives synthesized, we recorded their force/area curves. Figure 1 shows

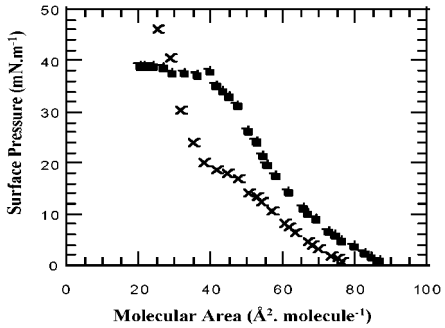


Figure 1. Force/area curves for compounds **13** (×) and **14** (■). The aqueous subphase was composed of Tris/HCl 10 mM, pH 8, NaCl 150 mM, $CaCl_2$ 21 mM, EDTA 1 mM. The continuous compression experiment was performed in the rectangular reservoir of the "zero order" trough.[22]

the molecular area dependency as a function of the surface pressure for compounds **13** and **14** spread over a buffered subphase at pH 8.0. The inhibition of HPL and HGL was studied by means of the monomolecular film technique[22,23] with mixed films of 1,2-dicaprin containing variable proportions of each inhibitor. The inhibition studies were performed at a constant surface pressure of 25 mN m^{-1} for HPL and 27 mN m^{-1} for HGL.

Inhibitors of lipolytic enzymes are best reported in terms of molar fraction of the inhibitor in the interface. Remaining lipase activity was plotted as a function of the inhibitor molar fraction (α). The data obtained for HPL and HGL are presented in Figures 2 and 3, respectively. Lipase hydrolysis rates of 1,2-dicaprin decreased sharply as the molar fraction of inhibitors increased. The dotted line corresponds to the

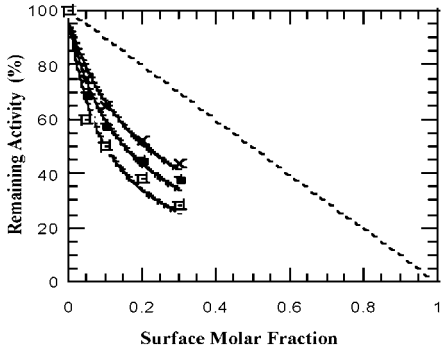


Figure 2. Effect of increasing concentrations of **13** (×), **14** (■), and **21** (□) on the remaining activity of HPL on 1,2-dicaprin monolayer maintained at a constant surface pressure of 25 mN m^{-1}. The aqueous subphase was composed of Tris/HCl 10 mM, pH 8, NaCl 100 mM, $CaCl_2$ 21 mM, EDTA 1 mM. The kinetics of hydrolysis were recorded during 15−20 min.

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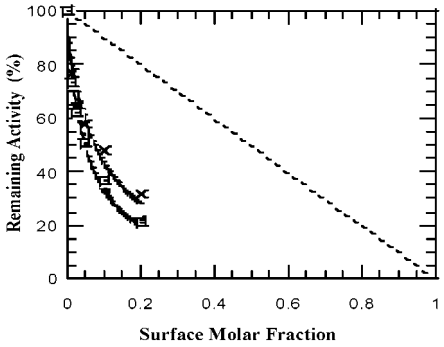

Figure 3. Effect of increasing concentrations of **13** (×), **14** (■), and **21** (□) on the remaining activity of HGL on 1,2-dicaprin monolayer maintained at a constant surface pressure 27 mN m^{-1}. The aqueous subphase was composed of CH_3COONa 10 mM, pH 5, NaCl 150 mM, $CaCl_2$ 21 mM, EDTA 1 mM. The kinetics of hydrolysis were recorded during 15−20 min.

surface dilution phenomena, which reflects the decrease in lipase activity that would be observed if a nonsubstrate, noninhibitor compound were present in the monomolecular film. The molar fractions, α_{50}, obtained for all the inhibitors tested are summarized in Table 1. The α_{50} is defined as the molar fraction of inhibitor that reduces by 50% the initial rate of lipolysis.

Table 1. Inhibition Constants (α_{50}) of Inhibitors Tested on HPL and HGL with the Monolayer Technique

	α_{50}	
compound	HPL[a]	HGL[b]
13	0.180 ± 0.019	0.086 ± 0.009
14	0.143 ± 0.012	0.052 ± 0.003
21	0.100 ± 0.007	0.053 ± 0.004

[a] Surface pressure 25 mN m^{-1}. [b] Surface pressure 27 mN m^{-1}.

As shown from these data, all inhibitors tested were 2−3 times more powerful on HGL as compared to HPL. The ester derivatives **14** and **21** were better inhibitors than the ether derivative **13** both for HPL and HGL. No stereoselective discrimination was observed during the inhibition of HPL or HGL. The racemic compound **14** and its *R*-enantiomer **21** caused almost the same inhibitory effect on both digestive lipases. These findings may be explained on the basis of the three-dimensional model proposed for the inhibited forms of HPL[5a] and HGL.[5b] As shown by crystallographic data, both enantiomers of alkyl phosphonate inhibitors may be accommodated into the catalytic crevice. Alcohol **12** was also tested, but no inhibition of either HPL or HGL was observed, indicating that the presence of the aldehyde group is critical for the inhibitory activity.

The α_{50} values reported for a series of chiral organophosphorus acylglycerol analogues,[24] in which one carbonyl was replaced by a phosphonate group, varied from 0.13 to 0.20 for HPL and 0.05 to 0.22 for HGL. Up to now the best synthetic inhibitor of HPL reported is *O*-hexadecyl-*O*-(*p*-nitrophenyl) *n*-undecyl phosphonate, with an α_{50} value of 0.003. For HGL the highest inhibition was obtained with *O*-undecyl-*O*-(*p*-nitrophenyl) *n*-decyl phosphonate, which exhibits an α_{50} value of 0.008.[25] The most active 1,3-bis-2-oxo amide triacylglycerol analogue[12] exhibited α_{50} values of 0.076 and 0.020 for HPL and HGL, respectively.

In conclusion, we have demonstrated that aldehyde dialkyl and alkyl-acyl glycerol analogues inhibit both digestive lipases. To the best of our knowledge this is the first time that the inhibition of a lipolytic enzyme by a synthetic aldehyde derivative was observed.

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Supporting Information Available: Experimental procedures and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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