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Probing conformations of the glycerol backbones of triacyglycerols in the active site of lipase by 1,2-cyclopentane-carbamates: The *meso* effect for the enzyme inhibition

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Abstract

The aim of this study is to probe the glycerol backbone conformation of the substrate (or inhibitor) in the active site of *Pseudomonas* species lipase by the 1,2-cyclopentandiol analogues of the ethylene glycerol carbamate inhibitors. Cyclopentane-carbamates, *cis*-1,2-di-*N*-*n*- butylcarbamyl-cyclopentane (**1**) and *trans*-1,2-di-*N*-*n*-butylcarbamyl-cyclopentane (**2**), are the conformationally constrained analogues of 1,2-di-*N*-*n*-butylcarbamyl ethane (**3**). All carbamates are synthesized and characterized as the pseudo-substrate inhibitors of the enzyme. *Cis*-cyclopentane-di-carbamate (**1**) is a more potent inhibitor than both ethane-di-carbamate (**3**) and *trans*-cyclopentane-di-carbamate (**2**) probably because the glycerol backbone conformations of *cis*-cyclopentane-di-carbamate (**1**) are constrained by the cyclopentane ring and *cis*-cyclopentane-di-cabamate (**1**) is a *meso* compound but *trans*-cyclopentane-di-carbamate (**2**) is a racemate.

Keywords: Lipase; Enzyme inhibitor; Carbamate; Cyclopentanediol; Conformation

1. Introduction

The potential of organic syntheses catalyzed by lipases (EC 3.1.1.3) underscores the need for a comprehensive understanding of lipase structure and function and provided the impetus for many investigations [1–4].

Many X-ray structures of lipases such as *Rhizomucor miehei* lipase (RML) [5,6], *Candida rugosa* lipase (CRL) [7], human pancreatic lipase (HPL) [8], and *Pseudomonas cepacia* lipase (PCL) [9–11] (see abbreviation) and have been reported. Although different activation mechanisms, the active sites of most lipases strongly resemble one another. Most lipases have the same catalytic mechanism as serine proteases in that they have a Ser-His-Asp (or Glu) catalytic triad which is involved in nucleophilic and general acid–base catalyses and a neighboring oxyanion hole (OAH), the hydrogen bonding peptide NH functions of Gly and Ala, which stabilizes the incipient carbonyl C–O^- of the ester function during turnover [3]. The conservation of this catalytic triad suggests that most lipases share a common mechanism for substrate hydrolysis, that is, formation of discrete acyl enzyme species via the serine hydroxyl group.

Carbamates are characterized as the substrates of *Staphylococcal* lipases from the long reaction time assay [12] and as the inhibitors of *Pseudomonas* lipase from the short reaction time assay [13–18]. Therefore, carbamates are characterized as the pseudo-substrate inhibitors of lipase as those of cholesterol esterase [20].

In the presence of substrate, the kinetic scheme for the pseudo-substrate inhibition of an enzyme by a carbamate inhibitor is proposed (Scheme 1) [19–21]. Since this inhibition follows first-order kinetics over observed time period for steady-state kinetics, rate of hydrolysis of EI' (carbamyl enzyme) to E (enzyme) and Q (the second product) must be significantly slower than rate of formation of EI' ($k_2 \gg k_3$) [19]. Therefore, values of K_i and k_2 can be calculated from Eq. (1) [20].

$$K_{app} = k_2[I]/(K_i(1 + [S]/K_m) + [I]) \quad (1)$$

Abbreviations: ACS, first acyl chain binding site; CRL, *Candida rugosa* lipase; ES, esteratic site or catalytic triad; HPL, human pancreatic lipase; k_2, carbamylation constant; K_i, inhibition or dissociation constant of enzyme-inhibitor adduct; k_i, bimolecular inhibition constant; OAH, oxyanion hole; PCL, *Pseudomonas cepacia* lipase; PNPB, *p*-nitrophenyl butyrate; PSL, *Pseudomonas species* lipase; RML, *Rhizomucor miehei* lipase; SACS, second alkyl chain binding site; TACS, third alkyl chain binding site

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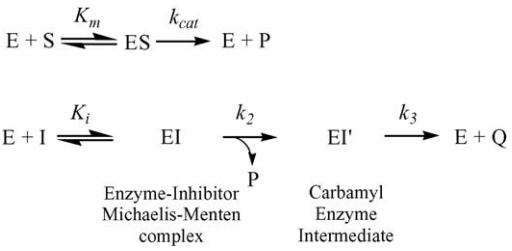

Scheme 1. Kinetic scheme for the PSL-catalyzed hydrolysis of substrate PNPB and the PSL inhibition by the pseudo-substrate carbamate inhibitor.

In Eq. (1), the k_{app} values are the first-order rate constants which are obtained by Hosie's method [20]. The bimolecular rate constant, $k_i = k_2/K_i$, is related to overall inhibitory potency.

In this report, we synthesized cyclopentane-carbamates, *cis*-1,2-di-*N*-*n*-butylcarbamyl-cyclopentane (**1**), *trans*-1,2-di-*N*-*n*-butylcarbamyl-cyclopentane (**2**), *cis*-1,2-di-*N*-*t*-butylcarbamyl-cyclopentane (**4**), *trans*-1,2-di-*N*-*t*-butylcarbamyl-cyclopentane (**5**), *cis*-2-*N*-*n*-butylcarbamyl-cyclopentanol (**7**), and *cis*-2-*N*-*t*-butylcarbamyl-cyclopentanol (**9**), as the conformationally constrained analogues of 1,2-di-*N*-*n*-butylcarbamyl-ethane (**3**), 1,2-di-*N*-*t*-butylcarbamyl-ethane (**6**), 2-*N*-*n*-butylcarbamyl-ethanol (**8**), and 2-*N*-*t*-butylcarbamyl-ethanol (**10**) (Fig. 3) and study the enzyme kinetics for the inhibition of *Pseudomonas* species lipase (PSL) by these inhibitors.

2. Materials and methods

2.1. Materials

Pseudomonas species lipase (PSL) and *p*-nitrophenyl butyrate (PNPB) were obtained from Sigma; *cis*- and *trans*-cyclopentane-1,2-diols and other chemicals were obtained from Aldrich; silica gel used in liquid chromatography (Licorpre Silica 60, 200–400 mesh) and thin-layer chromatography plates (60 F_{254}) were obtained from Merck. All other chemicals were of the highest purity available commercially.

2.2. Instrumental methods

^{1}H and ^{13}C NMR spectra were at 400 and 100 MHz, respectively, on a Varian-GEMINI 400 spectrometer. All steady state kinetic data were obtained from an UV–vis spectrophotometer (HP 8452A or Beckman DU-650) with a cell holder circulated with a water bath.

2.3. Data reduction

Origin (version 6.0) was used for linear, nonlinear, and multiple linear least squares regression analyses.

2.4. Steady-state enzyme kinetics

The PSL inhibition was assayed by Hosie's method [20] at 25.0 °C. All inhibition reactions were preformed in sodium phosphate buffer (1 mL, 0.1 M, pH 7.0) containing NaCl (0.1 M), acetonitrile (2 vol.%), Triton X-100 (0.5 wt.%), substrate (0.2 mM), and varying concentration of inhibitors. Requisite volumes of stock solution of substrate and inhibitors in acetonitrile were injected into reaction buffer via a pipet. PSL was dissolved in sodium phosphate buffer (0.1 M, pH 7.0). First-order rate constants (k_{app}'s) for inhibition were determined as described by Hosie et al. [20]. K_i's and k_2's were obtained by fitting k_{app}'s and [I] to Eq. (1) by nonlinear least squares regression analyses [13–18,20,22–34]. Duplicate sets of data were collected for each inhibitor concentration.

2.5. Synthesis of inhibitors

The condensation of *cis*-1,2-cyclopentandiol with 1.5 equiv. of *n*-butyl isocyanate and 1.5 equiv. of NaH in tetrahydrofuran at room temperature for 48 h yielded *cis*-1,2-di-*N*-*n*-butylcarbamyl-cyclopentane (**1**) (35–40%) (Fig. 3) and *cis*-2-*N*-*n*-butylcarbamyl-cyclopentanol (**7**) (15–25%) [13–18,20,22–34]. The resulting two products were separated and purified by liquid chromatography on silica gel eluting with hexane-ethyl acetate–solvent gradients. *Cis*-1,2-di-*N*-*t*-butylcarbamylcyclopentane (**4**) (25–30% yield) and *cis*-2-*N*-*t*-butylcarbamyl-cyclopentanol (**9**) (20–30% yield) were prepared in a similar method except the use of *t*-butyl isocyanate instead. 1,2-Di-*N*-*n*- or *t*-butylcarbamyl-ethane (**3** or **6**) (20–25% yield) and 2-*N*-*n*- or *t*-butylcarbamyl ethanol (**8** or **10**) (25–35% yield) were prepared by a similar method. *Trans*-1,2-di-*N*-*n*- or *t*-butylcarbamyl-cyclopentane (**2** or **5**) (30–40% yield) was prepared from condensation of *trans*-1,2-cyclopentandiol with 1.5 equiv. of *n*- or *t*-butyl isocyanate and 1.5 equiv. of NaH in toluene at 60 °C for 48 h.

All compounds were purified by liquid chromatography on silica gel eluting with hexane–ethyl acetate solvent gradients and characterized by ^{1}H and ^{13}C NMR spectra.

2.5.1. Cis-1,2-di-N-n-butylcarbamyl-cyclopentane (*1*)

^{1}H NMR ($CDCl_3$, 400 MHz) δ/ppm 0.99 (t, $J = 7.1$ Hz, 6H, $NHCH_2CH_2CH_2CH_3$), 1.31 (sextet, $J = 7.2$ Hz, 4H, $NHCH_2CH_2CH_2CH_3$), 1.45 (quintet, $J = 7.3$ Hz, 4H, $NHCH_2CH_2CH_2CH_3$), 1.58 and 1.95 (m, 6H, cyclopentane-3,4,5-CH_2), 3.14 (t, $J = 6.8$ Hz, 4H, $NHCH_2CH_2CH_2CH_3$), 4.60 (br s, 2H, cyclopentane-1,2-C*H*), 5.01 (br s, 2H, $NHCH_2CH_2CH_2CH_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ/ppm 13.62 ($NHCH_2CH_2CH_2CH_3$), 18.99 (cyclopentane-4-CH_2), 19.80 ($NHCH_2CH_2CH_2CH_3$), 28.30 (cyclopentane-3,5-CH_2), 31.98 ($NHCH_2CH_2CH_2CH_3$), 40.65 ($NHCH_2CH_2CH_2CH_3$), 74.79 (cyclopentane-1,2-*C*H), 155.95 (carbamate *C*=O).

2.5.2. Trans-1,2-di-N-n-butylcarbamyl-cyclopentane (*2*)

^{1}H NMR ($CDCl_3$, 400 MHz) δ/ppm 0.88 (t, $J = 7.3$ Hz, 6H, $NHCH_2CH_2CH_2CH_3$), 1.31 (sextet, $J = 7.1$ Hz, 4H, $NHCH_2CH_2CH_2CH_3$), 1.43 (quintet, $J = 7.0$ Hz, 4H, $NHCH_2CH_2CH_2CH_3$), 1.69 and 2.04 (m, 6H, cyclopentane-3,4,5-CH_2), 3.11 (t, $J = 6.7$ Hz, 4H, $NHCH_2CH_2CH_2CH_3$), 4.72 (br s, 2H, cyclopentane-1,2-C*H*), 4.92 (br s, 2H, $NHCH_2CH_2CH_2CH_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ/ppm 13.93 ($NHCH_2CH_2CH_2CH_3$), 20.11 ($NHCH_2CH_2CH_2CH_3$),

21.29 (cyclopentane-4-*C*H_2), 30.46 (cyclopentane-3,5-*C*H_2), 32.20 (NHCH$_2$*C*H$_2$CH$_2$CH$_3$), 40.94 (NH*C*H$_2$CH$_2$CH$_2$CH$_3$), 79.44 (cyclopentane-1,2-CH), 156.18 (carbamate *C*=O).

*2.5.3. 1,2-Di-N-n-butylcarbamyl-ethane (**3**)*

^{1}H NMR ($CDCl_3$, 400 MHz) δ/ppm 0.89 (t, J = 7.2 Hz, 6H, NHCH$_2$CH$_2$CH$_2$C*H*$_3$), 1.32 (sextet, J = 7.4 Hz, 4H, NHCH$_2$CH$_2$C*H*$_2$CH$_3$), 1.45 (quintet, J = 7.3 Hz, 4H, NHCH$_2$C*H*$_2$CH$_2$CH$_3$), 3.15 (t, J = 6.8 Hz, 4H, NHC*H*$_2$CH$_2$CH$_2$CH$_3$), 4.21 (br s, 4H, ethylene glycerol-1,2-C*H*$_2$), 4.76 (br s, 1H, N*H*CH$_2$CH$_2$CH$_2$CH$_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ/ppm 13.64 (NHCH$_2$CH$_2$CH$_2$*C*H$_3$), 19.80 (NHCH$_2$CH$_2$*C*H$_2$CH$_3$), 31.91 (NHCH$_2$*C*H$_2$CH$_2$CH$_3$), 40.71 (NH*C*H$_2$CH$_2$CH$_2$CH$_3$), 63.02 (ethylene glycerol-1,2-*C*H$_2$), 156.16 (carbamate *C*=O).

*2.5.4. Cis-1,2-di-N-t-butylcarbamyl-cyclopentane (**4**)*

^{1}H NMR ($CDCl_3$, 400 MHz) δ/ppm 1.27 (s, 18H, NHC(C*H*$_3$)$_3$), 1.67 and 2.02 (m, 6H, cyclopentane-3,4,5-C*H*$_2$), 4.63 (t, 2H, J = 2 Hz, cyclopentane-1,2-C*H*), 4.88 (br s, 2H, N*H*C(CH$_3$)$_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ/ppm 19.31 (cyclopentane-4-*C*H$_2$), 28.61 (cyclopentane-3,5-*C*H$_2$), 29.12 (NHC(*C*H$_3$) $_3$), 50.35 (NH*C*(CH$_3$)$_3$), 74.39 (cyclopentane-1,2-*C*H), 157.40 (carbamate *C*=O).

*2.5.5. Trans-1,2-di-N-t-butylcarbamyl-cyclopentane (**5**)*

^{1}H NMR ($CDCl_3$, 400 MHz) δ/ppm 1.28 (s, 18H, NHC(C*H*$_3$)$_3$), 1.70 and 2.02 (m, 6H, cyclopentane-3,4,5-C*H*$_2$), 4.62 (t, 2H, J = 2 Hz, cyclopentane-1,2-C*H*), 4.90 (br s, 2H, N*H*C(CH$_3$)$_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ/ppm 21.32 (cyclopentane-4-*C*H$_2$), 29.14 (cyclopentane-3,5-*C*H$_2$), 30.50 (NHC(*C*H$_3$)$_3$), 50.53 (NH*C*(CH$_3$)$_3$), 78.86 (cyclopentane-1,2-*C*H), 154.50 (carbamate *C*=O).

*2.5.6. 1,2-Di-N-t-butylcarbamate ethane (**6**)*

^{1}H NMR ($CDCl_3$, 400 MHz) δ/ppm 1.27 (s, 18H, NHC(C*H*$_3$)$_3$), 4.13 (br s, 4H, ethylene glycerol-1,2-C*H*$_2$), 4.73 (br s, 2H, N*H*C(CH$_3$)$_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ/ppm 29.08 (NHC(*C*H$_3$)$_3$), 50.58 (NH*C*(CH$_3$)$_3$), 62.68 (ethylene glycerol-1,2-*C*H$_2$), 154.64 (carbamate *C*=O).

*2.5.7. Cis-2-N-n-butylcarbamyl-cyclopentanol (**7**)*

^{1}H NMR ($CDCl_3$, 400 MHz) δ/ppm 0.90 (t, J = 6.8 Hz, 3H, NHCH$_2$CH$_2$CH$_2$C*H*$_3$), 1.31 (sextet, J = 7.3 Hz, 2H, NHCH$_2$CH$_2$C*H*$_2$CH$_3$), 1.46 (quintet, J = 7.3 Hz, 2H, NHCH$_2$C*H*$_2$CH$_2$CH$_3$), 1.54 and 1.71 (m, 6H, cyclopentane-3,4,5-C*H*$_2$), 3.14 (t, J = 6.8 Hz, 2H, NHC*H*$_2$CH$_2$CH$_2$CH$_3$), 4.04 (m, 2H, cyclopentane-1,2-C*H*), 4.60 (br s, 1H, O*H*), 5.01 (br s, 1H, N*H*CH$_2$CH$_2$CH$_2$CH$_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ/ppm 13.91 (NHCH$_2$CH$_2$CH$_2$*C*H$_3$), 20.09 (cyclopentane-4-*C*H$_2$), 21.49 (NHCH$_2$CH$_2$*C*H$_2$CH$_3$), 30.24 (NHCH$_2$*C*H$_2$CH$_2$CH$_3$), 32.19 and 32.50 (cyclopentane-3,5-*C*H$_2$), 40.98 (NH*C*H$_2$CH$_2$CH$_2$CH$_3$), 74.39 and 78.47 (cyclopentane-1,2-*C*H), 157.79 (carbamate *C*=O).

*2.5.8. 2-N-n-Butylcarbamyl ethanol (**8**)*

^{1}H NMR ($CDCl_3$, 400 MHz) δ/ppm 0.88 (t, J = 7.1 Hz, 3H, NHCH$_2$CH$_2$CH$_2$C*H*$_3$), 1.31 (sextet, J = 7.2 Hz, 2H, NHCH$_2$CH$_2$C*H*$_2$CH$_3$), 1.45 (quintet, J = 6.8 Hz, 2H, NHCH$_2$C*H*$_2$CH$_2$CH$_3$), 3.12 (t, J = 7.1 Hz, 2H, NHC*H*$_2$CH$_2$CH$_2$CH$_3$), 3.75 and 4.14 (m, 4H, ethylene glycerol-1,2-C*H*$_2$), 5.00 (br s, 1H, N*H*CH$_2$CH$_2$CH$_2$CH$_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ/ppm 13.62 (NHCH$_2$CH$_2$CH$_2$*C*H$_3$), 19.80 (NHCH$_2$CH$_2$*C*H$_2$CH$_3$), 31.87 (NHCH$_2$*C*H$_2$CH$_2$CH$_3$), 40.23 (NH*C*H$_2$CH$_2$CH$_2$CH$_3$), 61.69 and 66.56 (ethylene glycerol-1,2-*C*H$_2$), 157.01 (carbamate *C*=O).

*2.5.9. Cis-2-N-t-butylcarbamyl-cyclopentanol (**9**)*

^{1}H NMR ($CDCl_3$, 400 MHz) δ/ppm 1.29 (s, 9H, NHC(C*H*$_3$)$_3$), 1.60 and 2.00 (m, 6H, cyclopentane-3,4,5-C*H*$_2$), 3.62 and 4.03 (m, 2H, cyclopentane-1,2-C*H*), 4.88 (br s, 1H, N*H*C(CH$_3$)$_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ/ppm 19.28 (cyclopentane-4-*C*H$_2$), 28.22 and 28.77 (cyclopentane-3,5-*C*H$_2$), 30.56 (NHC(*C*H$_3$)$_3$), 50.26 (NH*C*(CH$_3$)$_3$), 73.24 and 76.28 (cyclopentane-1,2-*C*H), 154.79 (carbamate *C*=O).

*2.5.10. 2-N-t-Butylcarbamyl ethanol (**10**)*

^{1}H NMR ($CDCl_3$, 400 MHz) δ/ppm 1.29 (s, 9H, NHC(C*H*$_3$)$_3$), 3.83 and 4.16 (m, 4H, ethylene glycerol-1,2-C*H*$_2$), 4.70 (br s, 1H, N*H*C(CH$_3$)$_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ/ppm 32.20 (NHC(*C*H$_3$)$_3$), 50.58 (NH*C*(CH$_3$)$_3$), 62.68 and 68.65 (ethylene glycerol-1,2-*C*H$_2$), 155.95 (carbamate *C*=O).

3. Results

Carbamate inhibitors (**1**)–(**10**) (Fig. 3) are synthesized from condensation of *cis*- or *trans*-1,2-cyclopentanediol or ethylene glycerol with *n*- or *t*-butyl isocyanate in the presence of NaH at room temperature [13–18].

All inhibitors (**1**)–(**10**) (Fig. 3) are characterized as the pseudo-substrate [20] inhibitors of PSL [13–18] (Scheme 1) because the inhibition of PSL by (**1**)–(**10**) are time-dependent and follow first-order kinetics, and the enzyme activity is protected by a competitive inhibitor, trifluoroacetophenone [13–18]. The inhibition constant K_i, carbamylation constant k_2, and bimolecular rate constant k_i, for the inhibition of PSL-catalyzed hydrolysis of PNPB by inhibitors (**1**)–(**10**) are calculated from Eq. (1) [13–18,20] and summarized in Table 1. The bimolecular rate constant, $k_i = k_2/K_i$, is related to overall inhibitory potency [20]. Among carbamate inhibitors (**1**)–(**10**) (Fig. 3), *meso-cis*-1,2-di-*N*-*n*-butylcarbamyl-cyclopenatne (**1**) is the most potent PSL inhibitor with the k_i value of $3000 \pm 700\ M^{-1}\ s^{-1}$. The K_i value for *meso-cis*-1 is about one-quarter of that for *racemic-trans*-1,2-di-*N*-*n*-butylcarbamyl-cyclopenatne (**2**). The K_i value for *meso-cis*-1 is about one-third of that for conformationally free 1,2-di-*N*-*n*-butylcarbamyl-ethane (**3**). On the other hand, the K_i value for *meso-cis*-1,2-di-*N*-*t*-butylcarbamyl-cyclopentane (**4**) is about a half of that for *racemic-trans*-1,2-di-*N*-*t*-butylcarbamyl-cyclopenatne (**5**). The K_i value for *meso-cis*-4 is more than a half of that for conformationally free 1,2-di-*N*-*t*-butyl-carbamyl-ethane (**6**). *Cis*-cyclopentane-mono-carbamates (**7**) and (**9**) are more potent inhibitors than the conformationally free mono-carbamates (**8**) and (**10**). The k_2 values for *cis*-cyclopentane-carbamates (**1**, **4**, **7**, and **9**) are about the same (0.00063–$0.00066\ s^{-1}$)

Table 1
Kinetic constants for the inhibition of PSL by inhibitors (**1**–**10**)[a]

Inhibitors	K_i (μM)	k_2 (10^{-4} s^{-1})	k_i (M^{-1} s^{-1})	k_i ratio[b]
1	2.2 ± 0.5	6.6 ± 0.4	300 ± 70	1.8 ± 0.5
2	8 ± 1	10.2 ± 0.9	120 ± 20	0.7 ± 0.1
3	6.0 ± 0.7	10.1 ± 0.6	170 ± 20	1.0 ± 0.2[c]
4	5 ± 1	6.4 ± 0.5	130 ± 30	0.8 ± 0.2
5	12 ± 2	10 ± 1	80 ± 20	0.5 ± 0.1
6	8 ± 1	9.5 ± 0.8	120 ± 20	0.7 ± 0.1
7	4.4 ± 0.9	6.3 ± 0.5	140 ± 30	0.8 ± 0.2
8	7 ± 1	8.3 ± 0.7	120 ± 20	0.7 ± 0.1
9	8 ± 2	6.6 ± 0.7	90 ± 20	0.5 ± 0.1
10	10 ± 2	7.6 ± 0.9	80 ± 20	0.5 ± 0.1

[a] K_i and k_2 values were calculated from Eq. (1); $k_i = k_2/K_i$ [20,21].
[b] k_i ratio = k_i of an inhibitor/k_i of inhibitor (**3**).
[c] Defined as unity.

and are less than those for *trans*-cyclopentane-carbamates (**2** and **5**) and conformationally free mono-carbamates (**3**, **6**, **8** and **10**).

4. Discussion

4.1. Active site of PSL

According to the X-ray structure of PCL [11], the active site of PSL may be consist of at least six major binding sites since both enzymes are from the same *Pseudomonas* family (Fig. 1): (a) the first acyl chain binding site (ACS) which binds to one of the acyl chain of the triacylglycerol substrate and then hydrolyzes the acyl chain, (b) an oxyanion hole (OAH) which stabilizes the tetrahedral intermediates, (c) an esteratic site or catalytic triad (ES), comprised of the active site Ser87[1] which attacks the ester carbonyl group of substrate, His286, and Asp264, (d) the second acyl chain binding site (SACS) which binds to the cholesterol part of cholesterol ester or the second acyl chain of triacylglycerol, which is relatively larger than ACS, (e) a leaving group hydrophilic binding site which binds to the hydrophilic part of the leaving group and is located at the opposite direction of ACS, and (f) the third acyl chain binding site (TACS) which binds to the third acyl chain of triacylglycerol, is located at the opposite direction of ACS, has exposures to the solvent, and has room to adopt many different conformations. There are at least three stable conformers for the *sn*-1-carbon-*sn*-2-carbon (or *sn*-2-carbon-*sn*-3-carbon) backbone, e.g. *anti*-, (+)-*gauche*-, and (−)-*gauche*-conformers (Fig. 2A). Only (+)-*gauche*-conformers for the phosphonate analogues of triacylglycerols are likely present in the X-ray crystal structures of the phosphonate–lipase complexes [4,7,8,11]. Moreover, these inhibitors bind to both ACS and SACS of the enzyme, and the active Ser87 of the enzyme nucleophilically attacks the phosphorus atom (corresponding to the carbonyl carbon of triacylglycerol) of ACS. On the other hand, cyclopentanoid analogues of the ethylene glycerol moieties (Fig. 2B and C) only have

[1] The amino residue numbers are related to PCL [11]. Therefore, PCL is the reference lipase for PSL.

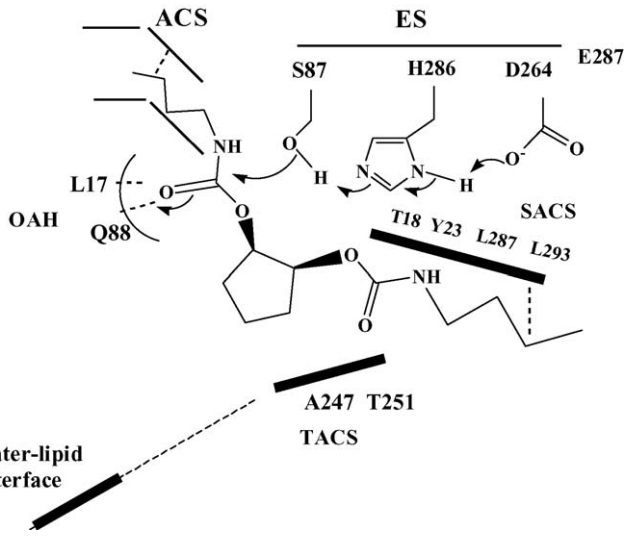

Fig. 1. Interactions between the pseudo-substrate inhibitor [20], *meso-cis*-1,2-cyclopentane-di-*N*-*n*-butylcarbamate (**1**) and PSL.

two stable conformations for the ethylene glycerol backbones and have been characterized as the conformationally restricted analogues of phospholipid substrates of phospholipase A2 [22].

4.2. Pseudo-substrate inhibition

All carbamate inhibitors (**1**–**10**) (Fig. 3) are characterized as the pseudo-substrate [19–21] inhibitors of PSL [13–18] (Scheme 1) (Table 1). Therefore, the first carbamate moiety of inhibitors (**1**–**10**) binds to the ACS of the enzyme and the active site Ser87 of the enzyme reacts with the carbonyl carbon of this carbamate moiety (Fig. 1). On the other hand, the second carbamate moiety of inhibitors (**1**–**6**) binds to the SACS of the enzyme due to the U shape of the ACS–SACS of the enzyme [4,7,8,11] (Fig. 1).

The inhibition constant K_i, carbamylation constant k_2, and bimolecular rate constant k_i, for the inhibition of PSL-catalyzed hydrolysis of PNPB by carbamates (**1**–**10**) are calculated from Eq. (1) [13–21] and summarized in Table 1. The bimolecular rate constant, $k_i = k_2/K_i$, is related to overall inhibitory potency [20].

4.3. K_i values

The fact that the K_i value for *cis*-1,2-di-*N*-*n*-butylcarbamyl-cyclopentane (**1**) (Fig. 3) (Table 1) is about one-third of the value for the conformationally free analogue, 1,2-di-*N*-*n*-butylcarbamyl-ethane (**3**), indicates that all four identical conformers of 1 (Fig. 2C) bind to the enzyme while about one third of the conformers, likely the (+)-*gauche* one (Fig. 2A), of (**3**) binds to the enzyme. The fact that the K_i value for *cis*-1,2-di-*N*-*n*-butylcarbamyl-cyclopenatne (**1**) is about one-quarter of the value for *trans*-1,2-di-*N*-*n*-butylcarbamyl-cyclopenatne (**2**) (Table 1) suggests that all four identical conformers of *meso*-1 (Fig. 2C) bind to the enzyme while only half of *racemic*-(**2**) (composed of both more reactive and less reactive enantiomers) effectively

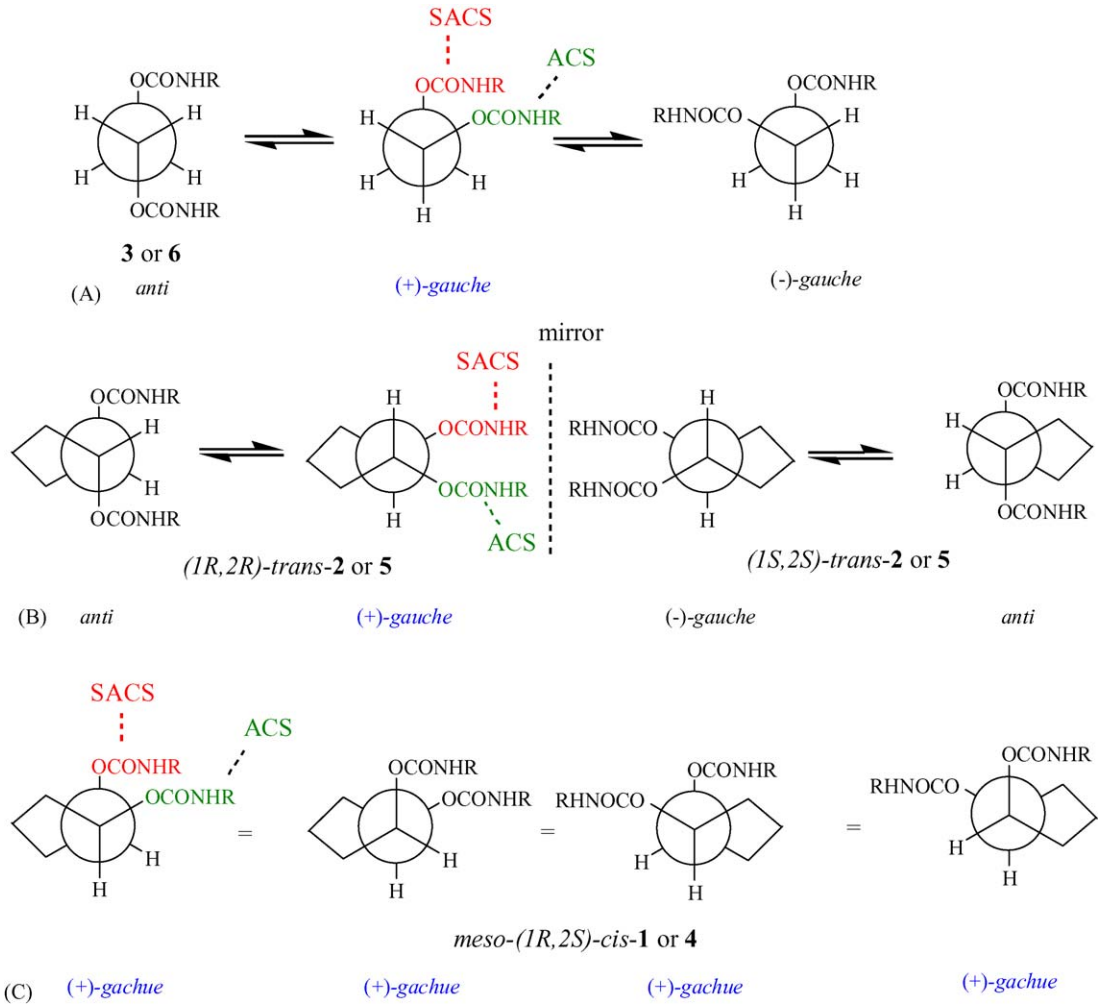

Fig. 2. Possible conformations for: (A) 1,2-di-*N*-butylcarbamyl-ethane (**3** or **6**), (B) *racemic-trans*-1,2-cyclopentane-di-*N*-butylcarbamate (**2** or **5**), and (C) *meso-cis*-1,2-cyclopentane-di-*N*-butylcarbamate (**1** or **4**).

binds to the enzyme and half of conformers of the more reactive enantiomer, likely the (+)-*gauche* one (Fig. 2B), of (**2**) bind to the enzyme. On the other hand, the fact that the K_i value for *meso-cis*-1,2-di-*N*-*t*-butylcarbamyl-cyclopenatne (**4**) is about a half of the value for *racemic-trans*-1,2-di-*N*-*t*-butylcarbamyl-cyclopenatne (**5**) (Table 1) implies that the *t*-butyl groups of the inhibitors disfavor for both *anti* conformers of (**5**) (Fig. 2B) and the (+)- or (−)-*gauche* conformer of (**5**) is the only possible con-

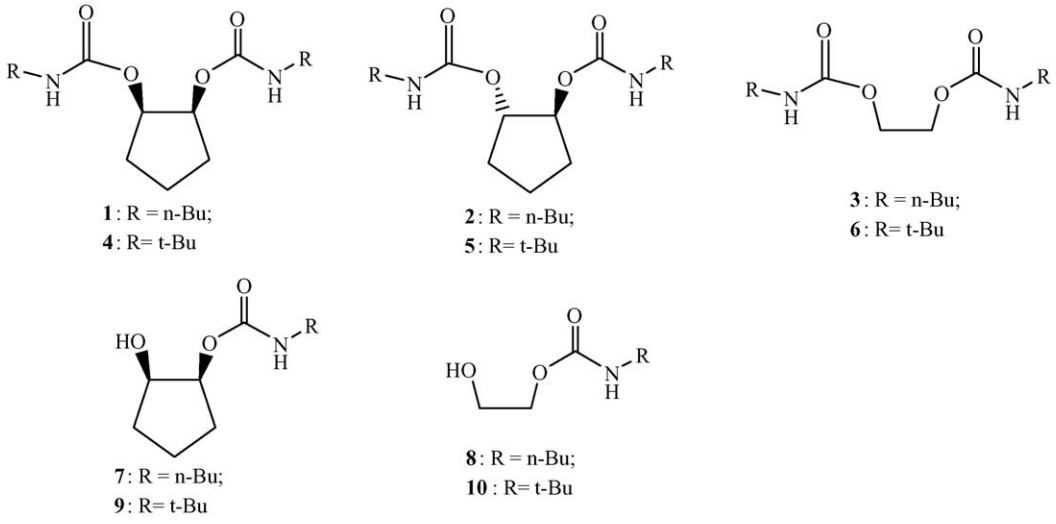

Fig. 3. Structures of inhibitors (**1**–**10**).

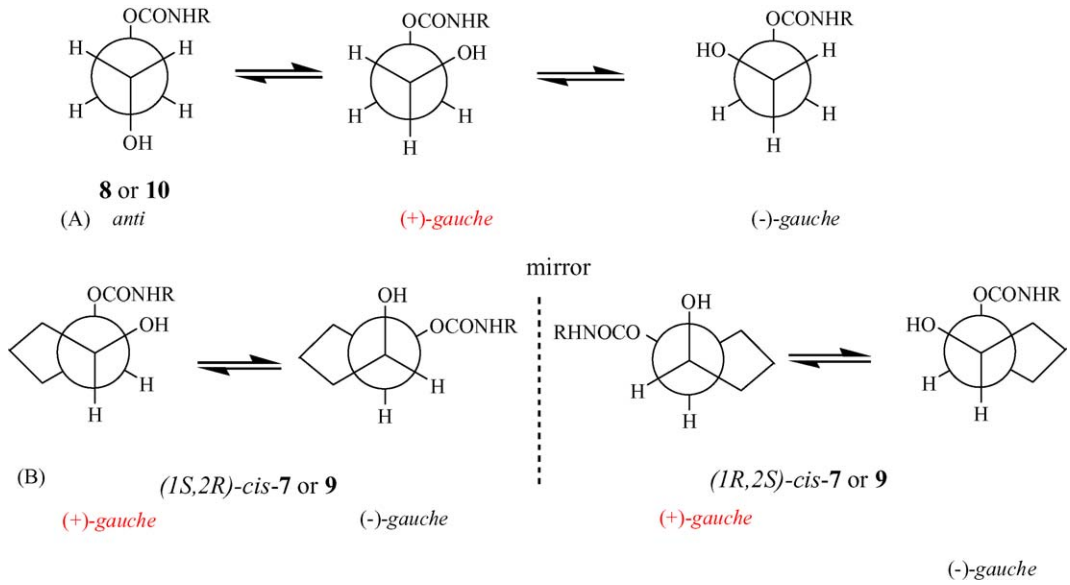

Fig. 4. Possible conformations for: (A) 2-*N*-butylcarbamyl-ethanol (**8** or **10**) and (B) *racemic-cis*-2-*N*-butylcarbamyl-cyclopentanol (**7** or **9**).

formation. Therefore, the effective inhibition concentration for *meso*-(**4**) is about twice of that for *racemic*-(**5**). Likewise, the fact that the K_i value for *meso-cis*-1,2-di-*N*-*t*-butylcarbamyl-cyclopenatne (**4**) (Table 1) is about a half of the value for the conformationally free analogue, 1,2-di-*N*-*t*-butylcarbamyl-ethane (**6**), implies that the bulky *t*-butyl group of (**6**) instabilizes both *gauche* conformers and that the effective conformer of (**6**) is likely the *anti* one (Fig. 2A).

For mono-carbamates (**7**–**10**), the fact that the K_i value for *cis*-2-*N*-*n*-butylcarbamyl-cyclopentanol (**7**) is about half of that for 2-*N*-*n*-butylcarbamyl-ethanol (**8**) (Table 1) indicates that (**7**) is no longer a *meso* but *racemic* compound (Fig. 4B) and that the conformations of these inhibitors play a major role in these inhibitions. The fact that the K_i value for (**7**) is about twice of the value for (**1**) (Table 1) implies that (**7**) is a racemate (Fig. 4B) but (**1**) is a *meso* compound (Fig. 2C) and that the effective inhibition concentration for (**1**) is about twice of that for (**7**). The fact that the K_i value for (**7**) is about half of that for (**8**) (Table 1) implies that (**7**) has two active conformers, likely two (+)-*gauche* ones (Fig. 4B), but (**8**) has only one (Fig. 4A). The fact that the K_i values for compounds (**2**, **6**, **9**, and **10**) are about the same suggests that all these inhibitors bind to the enzyme with a similar conformation, likely the (+)-*gauche* conformer (Fig. 2B).

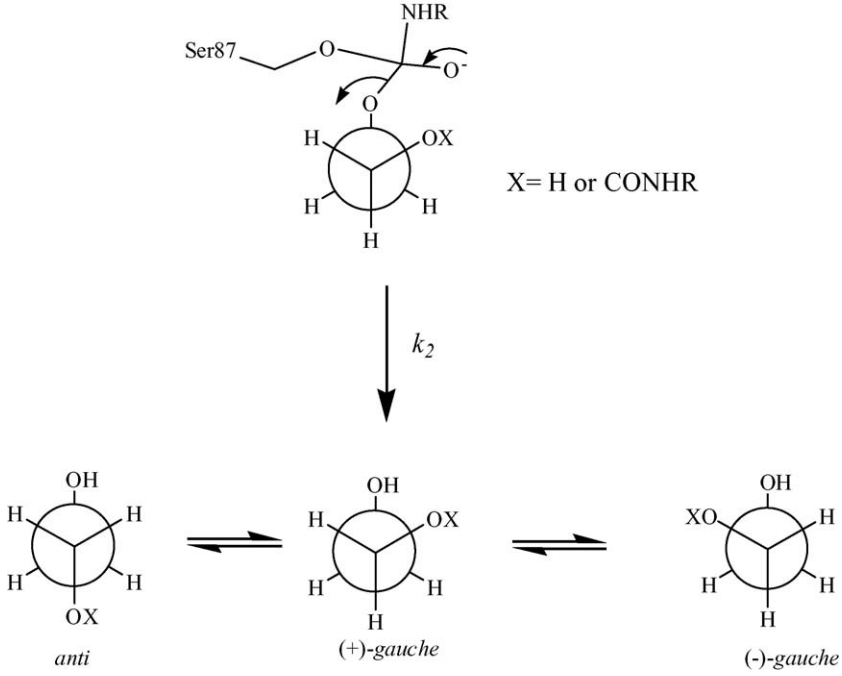

Fig. 5. Proposed mechanism for the post-equilibrium of the k_2 step (Scheme 1) for the PSL inhibition by inhibitors (**3**, **6**, **8**, and **10**).

4.4. k_2 values

The fact that the k_2 values for *cis*-cyclopentane-carbamates (**1**, **4**, **7**, and **9**) are about the same (0.00063–0.00066 s^{-1}) (Table 1) and are less than the values for *trans*-cyclopentane-carbamates (**2** and **5**) and the values for the conformationally free mono-carbamates (**3**, **6**, **8** and **10**) indicates that the *cis*-cyclopentandiol moieties are poor leaving groups when compared to both *trans*-cyclopentandiol and ethylene glycerol moieties. Likely, the post-equilibrium (equilibrium after the k_2 step) between the *gauche* and *anti* conformers (Fig. 5) may stabilize of both *trans*-cyclopentandiol and ethylene glycerol leaving groups but *cis*-cyclopentandiol leaving groups remain the *gauche* conformers without the extra stabilization from the post-equilibrium.

5. Conclusion

The substitution of the ethylene glycerol moiety by the analogous 1,2-cyclopentandiol moiety for carbamate inhibitors shows the increase of the inhibitory potency of PSL. This is probably due to the fact that conformations of the cyclopentane-inhibitors are constrained. Besides the character for constrained conformations, *cis*-cyclopentane-di-carbamates are more potent inhibitors of the enzyme than *trans*-cyclopentane-di-carbamates and *cis*-cyclopentane-mono-carbamates due to the *cis*-dicarbamates are *meso* compounds but both *trans*-carbamates and *cis*-monocarbamates are racemates.

Acknowledgement

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