

Porcine Liver Carboxylesterase Requires Polyisoprenylation for High Affinity Binding to Cysteinyl Substrates

Nazarius S. Lamango^{1,*}, Randolph Duverna¹, Wang Zhang¹ and Seth Y. Ablordeppey¹

¹College of Pharmacy and Pharmaceutical Sciences, Florida A&M University, Tallahassee, FL 32307, USA

Abstract: The polyisoprenylation pathway enzymes have been the focus of numerous studies to better understand the roles of polyisoprenylated proteins in eukaryotic cells and to identify novel targets against diseases such as cancer. The final step of the pathway is a reversible reaction catalyzed by isoprenyl carboxymethyl transferase (icmt) whose products are then hydrolyzed by polyisoprenylated methylated protein methyl esterase (PMPMEase). Unlike the other pathway enzymes, the esterase has received little attention. We recently purified PMPMEase from porcine liver using an S-polyisoprenylated cysteine methyl ester substrate-dependent screening assay. However, no data is available showing its relative interaction with structurally diverse substrates. As such, its role as the putative endogenous PMPMEase has not been demonstrated. A series of substrates with S-alkyl substituents ranging from 2 to 20 carbons, including the two moieties found in polyisoprenylated proteins, were synthesized. Enzyme kinetics analysis revealed a 33-fold increase in affinity (K_M values) from ethyl- (C-2, $505 \pm 63 \mu\text{M}$), prenyl- (C-5, $294 \pm 25 \mu\text{M}$), *trans*-geranyl- (C-10, $87 \pm 12 \mu\text{M}$), *trans*, *trans*-farnesyl- (C-15, $29 \pm 2.2 \mu\text{M}$) to *all trans*-geranylgeranyl- (C-20-, $15 \pm 2.7 \mu\text{M}$) based analogs. Comparative molecular field analysis of the data yielded a cross-validated q^2 of 0.863 ± 0.365 and a final R^2 of 0.995. Since the substrates with the *S-trans*, *trans*-farnesyl and *S-all trans*-geranylgeranyl moieties that occur in proteins show the highest affinity towards PMPMEase and are not hydrolyzed by the cholinesterases, the results suggest that polyisoprenylated proteins are the endogenous substrates of this esterase. The results suggest design strategies for high affinity and selective inhibitors of PMPMEase.

Keywords: Polyisoprenylation, Isoprenylation, Esterase, Carboxylesterase, Farnesyl, Geranylgeranyl, Farnesylation, Geranylgeranylation.

INTRODUCTION

Polyisoprenylation is an essential secondary modification on a wide range of proteins in eukaryotic cells [1, 2]. Their contributions to disease due to mutations or improper metabolism have been widely reported [1]. For example, an estimated 15 to 30% of cancers involve overexpression [3] or mutations of monomeric G-proteins that render them constitutively active [4]. On the other extreme of the cell viability spectrum is the degeneration observed in choroideramia caused by a defective polyisoprenylation enzyme [5, 6]. Heart-specific knockout mice devoid of the polyisoprenylated-dependent endoprotease eventually develop cardiomyopathy [7] while knockout mice lacking isoprenylated carboxymethyltransferase (icmt, also known as prenylated protein methyl transferase, PPMTase) activity do not survive through mid gestation [8].

While the pathophysiological effects of deficient polyisoprenyl transferase and PPMTase activities yield critical insights into the importance of the polyisoprenylation pathway, the role of the esterase that counterbalances the effects of PPMTase and makes possible the only reversible step of the pathway is less understood. Polyisoprenyl binding sites

have been identified in some proteins, thus underlying the likelihood that polyisoprenylation promotes protein-protein interactions [9, 10]. Given that PPMTase removes and PMPMEase introduces a negative charge in the vicinity of the polyisoprenyl moiety, it has been suggested that these enzymes switch the polyisoprenylated proteins between the active and inactive conformations [11].

Although the existence of PMPMEase has been predicted from biochemical studies [12, 13], few studies have been conducted to better understand its substrate specificity and tissue variants. PMPMEase was recently purified and identified from porcine liver [14]. It is over 70% identical and 80% similar to various ubiquitously distributed carboxylesterases [14]. The crystal structures of human carboxylesterase 1 have been solved [15-18]. The data reveal an overarching feature of human carboxylesterase 1; that of a large, hydrophobic and flexible active site believed to accommodate a wide variety of substrates [15]. This may be an adaptation to accommodate the large and structurally diverse polypeptide portions bearing the hydrophobic polyisoprenylated methylated cysteines at their carboxyl terminals [14]. The present study is aimed at determining whether the enzyme is selective for polyisoprenylated methylated protein methyl esterase (PMPMEase), in which case the farnesylated and geranylgeranylated substrates would have the highest affinities for the enzyme. A series of S-alkyl,N-acyl cysteine methyl esters were thus synthesized, purified and characterized by proton and ¹³C NMR and elemental analysis. Substrate kinetics analysis with the enzyme revealed a strong

*Address correspondence to this author at the College of Pharmacy and Pharmaceutical Sciences, Florida A&M University, Tallahassee, FL 32307; USA; Tel: 850 412 7377; Lab: 850 599 8443; Fax: 850 599 3347; E-mail: nazarius.lamango@famu.edu

dependence on the polyisoprenyl moiety for high affinity binding to the esterase as shown by the significantly lower Michaelis-Menten constants for the *S-trans,trans*-farnesyl (C15) and *S-all trans*-geranylgeranyl (C20) compared to the *S*-ethyl cysteine methyl and other ester substrates. The requirement of polyisoprenylation for high affinity binding to PMPMEase and selectivity against cholinesterase hydrolysis is indicative of an endogenous role in the metabolism of polyisoprenylated proteins and important for the rational design of selective high-affinity inhibitors of the enzyme.

MATERIALS AND METHODS

Materials

Esterase from porcine liver (EPL), acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) were obtained from Sigma-Aldrich, St. Louis, MO. SDS-PAGE electrophoresis gels were obtained from BioRad, Hercules, CA. SDS-PAGE molecular weight markers were from Fisher Scientific (Suwanee, GA).

Synthesis of Substrates

Experiments were conducted under argon in flame-dried glassware and anhydrous solvents, when necessary (Scheme 1). Flash chromatography was performed on Fisher 40-63 μ silica gel. Unless otherwise stated, NMR spectra (300 MHz) were obtained in CDCl₃ on Varian Mercury 300. Chemical shifts (δ) are given in ppm relative to the signal of the deuterated solvent. IR spectra were taken on Shimadzu 840S in KBr pellets. Optical rotations were measured on JASCO P-1020 polarimeter in a 10 cm cell. All substrates were >99% pure by HPLC. Elemental analyses were conducted by Atlantic Microlab, Inc, Norcross, GA, and the accepted values are within 0.4% of the theoretical values except otherwise noted.

trans-Geranyl bromide. A solution of PBr₃ (6 ml, 63.2 mmol) in anhydrous ether (10 mL) was added drop-wise to *trans*-geraniol (21.7 g, 141 mmol) in anhydrous ether (250 mL) at -15 to -10°C. At the end of the addition, the mixture was kept at 0°C for 10 min and then at 20°C for 30 min [19]. The organic phase was decanted, washed with cold 5% NaHCO₃, brine, dried over MgSO₄ and the solvent removed at 40°C/3 mm Hg, giving a colorless liquid (22.2 g, 73%). ¹H NMR: 1.60 (s, CH₃), 1.69 (s, CH₃), 1.74 (s, CH₃),

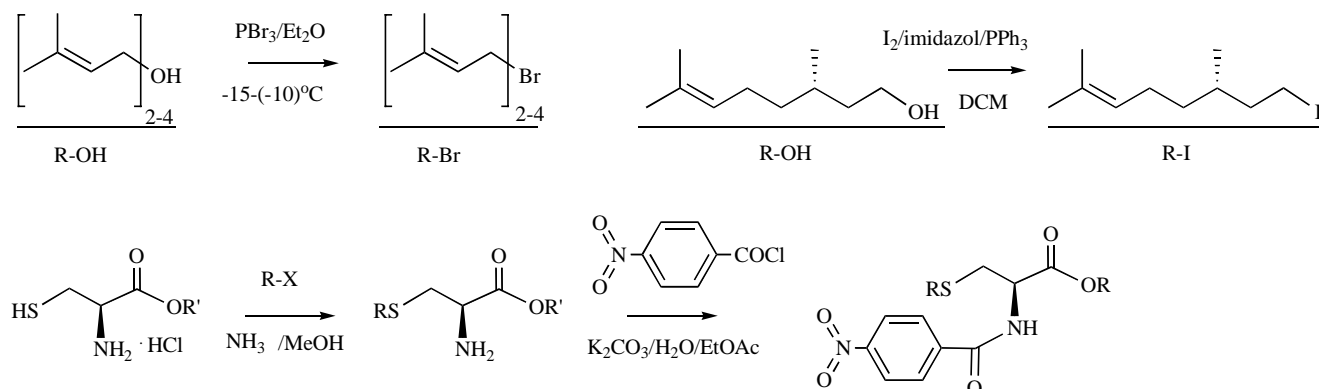
1.95-2.20 (m, 2 CH₂), 4.03 (d, CH₂Br, *J*=8.4 Hz), 5.09 (m, C=CH), 5.54 (m, C=CHCBr); ¹³C NMR: 16.2, 17.9, 25.9, 26.4, 29.9, 39.8, 120.8, 123.8, 132.2, 143.8.

trans, trans-Farnesyl bromide. A solution of PBr₃ (6 mL, 63.2 mmol) in anhydrous ether (10mL) was added drop-wise to *trans, trans*-farnesol (20.5 g, 92 mmol) in anhydrous ether (250 mL) at -15 to -10°C and treated as for the synthesis of *trans*-geranyl bromide. A pale-yellow liquid (24.43 g, 93%) was obtained. ¹H NMR: 1.60 (s, 2 CH₃), 1.67 (s, CH₃), 1.72 (s, CH₃), 1.95-2.15 (m, 4 CH₂), 4.16 (d, CH₂Br, *J*=8.4 Hz), 5.09 (m, 2 x C=CH), 5.54 (m, C=CHCBr); ¹³C NMR: 16.2, 16.3, 17.9, 25.9, 26.3, 26.9, 29.7, 39.7, 39.9, 120.8, 123.6, 124.5, 131.5, 135.8, 143.7.

trans, trans, trans-Geranylgeranyl bromide: As for *trans, trans*-farnesyl bromide from PBr₃ (1 mL, 1.1 mmol) and *trans, trans, trans*-geranylgeraniol (1 g, 3.4 mmol) in anhydrous ether. A pale-yellow liquid (1.2 g) was obtained. ¹H NMR: 1.60 (s, 3 CH₃), 1.68 (s, CH₃), 1.73 (s, CH₃), 1.95-2.15 (m, 6 CH₂), 4.02 (d, CH₂Br, *J*=8.1 Hz), 5.09 (m, 3 C=CH), 5.53 (m, C=CHCBr); ¹³C NMR: 16.19, 16.23, 16.28, 17.91, 25.92, 26.35, 26.83, 27.01, 29.80, 39.76, 39.90, 39.96, 120.80, 123.63, 124.41, 124.62, 131.44, 135.17, 135.86, 143.78.

(*S*)-(+)-Citronellyl iodide. PPh₃ (6.3 g, 24 mmol) was added to dry DCM (80 ml) followed by imidazole (1.64 g, 24 mmol), iodine (6.1 g, 24 mmol) and a solution of (*S*)-(-)-citronellol (3.6 ml, 20 mmol) in dry DCM (20 ml). The mixture was stirred at RT until the alcohol disappeared (TLC in DCM, R_f 0.1 alcohol, 0.9 iodide). The precipitate was filtered off, the solvent removed and the semi-solid residue was treated with hexane. The hexane extract was purified by column chromatography (in hexane) [20]. The solvent was removed under vacuum, yielding 5.1g (96%) of a colorless liquid, [α]_D²⁵ = +1.02° (pure, 1 cm). ¹H-NMR: δ 0.88 (d, 6.6 Hz, CHCH₃), 1.19 (m, H₄), 1.33 (m, H₄'), 1.5-1.7 (m, H₂+CHCH₃, 2H), 1.60 (s, CH₃), 1.68 (s, CH₃), 1.8-2.1 (m, H_{5,5'}+H₂, 3H), 3.20 (m, CH₂I), 5.08 (m, C=CH). ¹³C-NMR: δ 5.28, 17.93, 18.90, 25.55, 25.96, 33.80, 36.54, 41.15, 124.70, 131.62.

(*R*)-(-)-Citronellyl iodide. (*R*)-(+)-citronellal (9.06 mL, 50 mmol) was reduced with 1N solution of LiAlH₄ in ether (55 ml, 55 mmol). TLC control in hexane-EtOAc 4:1, R_f of



Scheme 1. General procedures for the synthesis of the *S*-alkylated *N*-acylated cysteine ester substrates.

alcohol 0.2, of aldehyde 0.5. Vacuum distillation of the crude product (6.1 g, 78%) resulted in 4.1 g of (*R*)-(+)-citronellol, b.p. 85-87°C/4 mm Hg, $[\alpha]_D^{23.5} = +3.44^\circ$ (pure, 1 cm). (*R*)-(+)-citronellol was converted to the iodide as described for the synthesis of (*S*)-(+)-Citronellyl iodide [21]. Instead of filtration, the solvent was removed under reduced pressure and the residue treated with 2 portions of hexane. Column chromatography of the soluble fraction afforded 3.37 g (63%) of a colorless liquid, $[\alpha]_D^{23.5} = -1.26^\circ$ (pure, 1 cm). $[\alpha]_D^{20.5} = -14.34^\circ$ (pure, 10 cm). The NMR spectrum was identical to that for the (*S*)-(+)-isomer.

D-Cysteine methyl ester hydrochloride. D-Cys·HCl·H₂O (1.02 g, 6.7 mmol) was dehydrated at 50 °C, 3mm Hg for 2 h and reacted with MeOH saturated with anhydrous HCl for 20 h at RT. Evaporation of the solvent in vacuum resulted in 1.14 g (quantitative yield) of a crystalline solid with $[\alpha]_D^{24} +2.22$ (c 0.095, MeOH). [Aldrich $[\alpha]_D^{20} -1.8$ (c 10, MeOH) for L-CysOMe·HCl].

N-(4-Nitrobenzoyl)-glycine methyl ester [methyl 2-(4-nitrobenzamido)acetate, **L-102**]. N-(4-Nitrobenzoyl)-glycine (2.24 g, 10 mmol) in MeOH (20 ml) and TMS-Cl (3 mL, 23.5 mmol) were left overnight at RT and the precipitated yellowish solid filtered off. Yield 1.45 g (61%), *R_f* 0.25 (hexane-EtOAc 1:1), m.p. 157-158°C [[22] m.p. 150-153 °C]. Anal. Calcd for C₁₀H₁₀N₂O₅: C, 50.42; H, 4.23; N, 11.76. Found: C, 50.35; H, 4.22; N, 11.77.

L-Cysteine *i*-propyl ester hydrochloride. A suspension of L-Cys (0.3 g, 2.5 mmol) in anhydrous *i*-PrOH (30 mL) and TMS-Cl (0.85 g, 92 mmol) was heated at 70°C for 2 days in a pressure tube. The CysOPr-*i*-HCl (0.5 g, quantitative yield) obtained after solvent removal was used for the next step without purification. ¹H NMR (D₂O): 1.17 [dd, 1.2, 6.3 Hz, OCH(CH₃)₂], 2.98 (dd, 15.3, 4.2 Hz, H_β), 3.06 (dd, 15.3, 5.4 Hz, H_{β'}), 4.24 (m, H_α), 5.00 [sept, OCH(CH₃)₂]; ¹³C NMR (D₂O): 21.39, 24.31, 55.15, 73.46, 168.29.

L-S-(*trans, trans*-farnesyl)cysteine methyl ester. Farnesyl bromide (4.56 g, 16 mmol) was added to a solution of L-cysteine methyl ester hydrochloride (2.75 g, 16 mmol) in anhydrous methanol (28 mL) and 7N anhydrous NH₃ solution in methanol (92 ml) at -5°C and the mixture stirred at this temperature for 2 h [23]. The solvent was evaporated under vacuum and the residue purified by flash chromatography, eluting with hexane-EtOAc 1:1 (1.69 g, 31%). *R_f* 0.2 (hexane-EtOAc 1:1). ¹H-NMR: δ 1.48 (s, CH₃), 1.51 (s, CH₃), 1.59 (s, CH₃), 1.66 (s, CH₃), 1.59 (s, CH₃), 1.65 (s, NH₂), 1.80-2.10 (m, 4 CH₂), 2.61 and 2.77 (dd, S-C-H *J*=7.5Hz), 3.09 (d, CH₂S, *J*=7.2Hz), 3.55 (dd, α-H, *J*=3.0Hz), 3.65 (s, OCH₃), 5.04 (broad, 2 x C=CH), 5.14 (t, C=CHCS, *J*=1.2Hz); ¹³C-NMR: δ 16.17, 16.26, 17.84, 25.85, 25.48, 26.55, 29.94, 36.48, 39.77, 39.85, 52.27, 54.39, 120.2, 123.9 124.5, 131.3, 135.4, 139.6, 174.6.

D-S-(*trans, trans*-farnesyl)cysteine methyl ester. Farnesyl chloride (626 mg, 2.7 mmol) was added to a solution of D-CysOMe·HCl (446 mg, 2.6 mmol) in anhydrous methanol (1 ml) and 7N anhydrous NH₃ solution in methanol (2 mL) at -5°C. The reaction mixture was stirred at -5°C for 10 min, at 0°C for 30 min and at RT for 45 min. After an identical treatment, the product (635 mg, 72%) with $[\alpha]_D^{20} -6.35$

(c 0.1, CHCl₃), $[\alpha]_D^{21} -12.3$ (c 0.1, PhMe) displayed the same NMR spectra and *R_f* as the L-isomer.

L-S-(*trans*-geranyl)cysteine methyl ester. Geranyl bromide (1.3 mL, 6.6 mmol) was added to a stirred solution of L-cysteine methyl ester hydrochloride (1.02 g, 6 mmol) in anhydrous methanol (8 ml) and 7N anhydrous NH₃ solution in anhydrous methanol (15 mL) at -10°C. The mixture was kept at -10°C for 10 min, at 0°C for 50 min and at RT for 1h. The solvent was removed under vacuum and the residue suspended in EtOAc. The solid was removed by centrifugation and the organic phase evaporated. The product was applied onto a column and eluted with a gradient of DCM - DCM-EtOAc 30:1 (699 mg, 43%). *R_f* 0.2 (hexane-EtOAc 1:2), $[\alpha]_D^{22} +17.0$ (c 0.22, PhMe). ¹H-NMR: δ 1.53 (s, CH₃), 1.59 (s, CH₃), 1.61 (s, CH₃), 1.65 (s, NH₂), 1.80-2.20 (m, 4 CH₂), 2.61 (dd, 13.5, 7.5 Hz, H_β), 2.80 (dd, 13.5, 4.8 Hz, H_{β'}), 3.11 (m, CH₂S), 3.55 (dd, 4.8, 7.5 Hz, H_α), 3.66 (s, OCH₃), 5.02 (m, C=CH), 5.17 (m, C=CHCS); ¹³C-NMR: δ 16.2, 17.7, 25.6, 26.7, 30.2, 36.8, 39.7, 52.0, 54.7, 120.4, 124.1, 131.6, 139.4, 174.5.

D-N-Acetyl-S-(*trans, trans*-farnesyl)cysteine methyl ester [(*S*)-methyl 3-((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trienylthio)-2-acetamidopropanoate, **L-69**]. D-(*S-trans,trans*-farnesyl)cysteine methyl ester (106 mg, 0.3 mmol) was reacted with acetic anhydride (77 mg, 0.75 mmol) and K₂CO₃ (189 mg, 1.36 mmol) in PhMe-water (1.5+1 mL). The organic phase was dried over K₂CO₃, the solvent removed and the residue purified by column chromatography eluting with hexane-EtOAc 1:1.5 to afford the product (114 mg, 96%). *R_f* 0.3 (hexane-EtOAc 1:1), $[\alpha]_D^{22} +25.4$ (c 0.025, acetone). ¹H-NMR: δ 1.57 (s, CH₃), 1.64 (s, CH₃), 1.64 (s, CH₃), 1.65 (d, 0.9 Hz, CH₃), 1.87-2.10 (m, 8 CH₂), 2.02 (s, COCH₃), 2.84 (dd, 13.8, 5.7 Hz, H_β), 2.93 (dd, 13.8, 5.1 Hz, H_{β'}), 3.13 (m, CH₂S), 3.73 (s, OCH₃), 4.78 (m, H_α), 5.06 (m, 2 C=CH), 5.17 (m, C=CHCS), 6.31 (d, CONH); ¹³C-NMR: δ 16.2, 16.3, 17.9, 23.3, 25.9, 26.6, 26.9, 30.2, 33.6, 39.8, 39.9, 52.0, 52.7, 119.8, 123.9, 124.5, 131.5, 135.6, 140.2, 170.0, 171.7. Anal. Calcd for C₂₁H₃₅NO₃S·0.6H₂O: C, 64.28; H, 9.30; N, 3.57. Found: C, 64.02; H, 9.03; N, 3.52.

L-N-(4-Nitrobenzoyl)-S-(*trans, trans*-farnesyl)cysteine methyl ester [(*R*)-methyl 3-((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trienylthio)-2-(4-nitrobenzamido)propanoate, **RD-PNB**]. Triethylamine (181 μl, 1.32 mmol) was added to a stirred suspension of *p*-nitrobenzoyl chloride (245 mg, 1.32 mmol) and L-S-(*trans,trans*-farnesyl)cysteine methyl ester (412 mg, 1.2 mmol) in anhydrous DCM (10 ml) on ice. After 10 min the mixture was left at RT for 4 h. The solvent was removed and the solid residue was dissolved in ethyl acetate and washed with 5% NaHCO₃, 10% HCl and brine. It was then dried over MgSO₄ and the solvent evaporated. The resulting oil was purified by column chromatography eluting with DCM to give a yellowish solid (180 mg, 30%) with a m.p. of 52-54.5 °C; $[\alpha]_D^{20} = -66.0^\circ$ (c = 0.015, acetone). IR (KBr) NH (3300), C=O (1750), O=CN (1620), NO₂ (1355), and OMe (1250) cm⁻¹; *R_f* (hexane-EtOAc 1:1) 0.65 amide, 0.25 amine. ¹H-NMR: δ 1.58 (s, CH₃), 1.42 (s, CH₃), 1.66 (s, CH₃), 1.95-2.10 (m, 4 x CH₂), 3.00-3.07 (β' and β) (dd, S-C-H *J*=7.5Hz), 3.09-3.21 (t, CH₂S, *J*=7.2Hz), 3.81 (s, OCH₃), 4.97-5.04 (d, HC-NH₂), 5.04-5.06 (broad, 2

C=CH), 5.09-5.15 (broad, C=CHCS, $J=1.2$ H), 7.12 (d, HN, $J=10.2$ Hz), 7.98 (m, Ar, 2H), 8.28 (m, Ar, 2H). 13 C-NMR: δ 16.27, 16.39, 17.95, 25.97, 26.64, 26.93, 30.07, 33.28, 39.87, 39.27, 43.71, 52.25, 53.08, 119.6, 123.8, 124.0, 124.5, 128.7, 131.6, 135.7, 139.2, 140.5, 149.9, 165.8, 168.9, 171.3. Anal. Calcd for $C_{26}H_{36}N_2O_5S$: C, 63.91; H, 7.43; N, 5.73. Found: C, 63.92; H, 7.44; N, 5.67.

D-N-(4-Nitrobenzoyl)-S-(*trans, trans*-farnesyl)cysteine methyl ester [(*S*)-methyl 3-((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trienylthio)-2-(4-nitrobenzamido)propanoate, **L-70**]. Triethylamine (0.2 mL, 1.4 mmol) was added to a stirred suspension of *p*-nitrobenzoyl chloride (238 mg, 1.28 mmol) and D-S-(*trans,trans*-farnesyl)-cysteine methyl ester (396 mg, 1.17 mmol) in dry $CHCl_3$ (3 mL) at $-10^\circ C$. The mixture was left in a fridge overnight and treated as described for the L-isomer, giving a quantitative yield of a yellowish solid with m.p. 57-58 $^\circ C$; $[\alpha]_D^{22} = +79.3^\circ$ ($c = 0.015$, acetone) and the same NMR spectrum and R_f as the L-isomer. Anal. Calcd for $C_{26}H_{36}N_2O_5S$: C, 63.91; H, 7.43; N, 5.73. Found: C, 63.99; H, 7.43; N, 5.71.

N-(4-Nitrobenzoyl)-S-(*trans, trans, trans*-geranylgeranyl)cysteine methyl ester [(*R*)-methyl 3-((2*E*,6*E*,10*E*)-3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraenylthio)-2-(4-nitrobenzamido)propanoate, **L-80**]. *trans, trans, trans*-Geranylgeranyl bromide (1.29 g, 3.7 mmol) dissolved in anhydrous ether (3 mL) was added to a solution of L-cysteine methyl ester (0.68 g, 3.7 mmol) in methanol (2 mL) and 7N solution of NH_3 in methanol (3.5 mL, 24.5 mmol) at -5 to $-10^\circ C$. The reaction mixture was stirred at $-5^\circ C$ for 10 min, at $0^\circ C$ for 30 min and at RT 45 min. The solvent was evaporated under vacuum and the residue taken into EtOAc. The organic phase was rotovaped and the product purified on a column eluting with hexane-EtOAc 1:1 (1.14g, 75%). R_f 0.15 (hexane-EtOAc 1:1). A solution of L-S-(*trans, trans, trans*-geranylgeranyl)cysteine methyl ester (1.14 g, 2.8 mmol) in EtOAc (50 mL) was stirred with *p*-nitrobenzoyl chloride (0.64 g, 3.4 mmol) and a solution of K_2CO_3 (0.77 g, 5.6 mmol) in water (5 mL). After completion of the reaction (TLC, R_f hexane-EtOAc 1:1 0.2 amine, 0.8 amide), the organic phase was separated, washed with diluted NaOH, brine, dried over K_2CO_3 and the solvent removed under vacuum. Column chromatography (gradient from DCM to DCM-EtOAc 30:1) afforded 1.12 g (72%) of a light yellow solid, R_f (hexane-EtOAc 1:1) m.p. 45-46 $^\circ C$, $[\alpha]_D^{23} -39.4^\circ$ ($c = 0.08$, acetone). 1H -NMR: δ 1.57 (s, 3 CH_3), 1.63 (s, CH_3), 1.65 (s, CH_3), 1.90-2.05 (m, 6 CH_2), 2.95-3.15 (m, $H_{\beta, \beta'} + CH_2S$), 3.79 (s, OCH_3), 4.96 (m, H_a , 1H), 5.06 (m, 3 C=CH), 5.18 (m, C=CHCS, 1H), 7.10 (d, HN, $J=6.6$ Hz), 7.96 (m, Ar, 2H), 8.25 (m, Ar, 2H). ^{13}C -NMR: δ 16.21, 16.24, 16.37, 17.95, 25.97, 26.63, 26.83, 26.97, 30.19, 33.45, 39.84, 39.89, 39.92, 52.53, 53.06, 119.55, 123.85, 124.04, 124.36, 124.59, 128.62, 131.42, 135.17, 135.67, 139.46, 140.54, 150.03, 165.27, 171.3. Anal. Calcd for $C_{31}H_{44}N_2O_5S$: C, 66.88; H, 7.97; N, 5.03. Found: C, 66.85; H, 7.95; N, 5.07.

L-N-(4-Nitrobenzoyl)-S-(*trans*-geranyl)cysteine methyl ester [(*R*)-methyl 3-((*E*)-3,7-dimethylocta-2,6-dienylthio)-2-(4-nitrobenzamido)propanoate, **L-72**]. This was synthesized from L-(*S*-*trans*-geranyl)cysteine methyl ester (699 mg, 2.6 mmol), *p*-nitrobenzoyl chloride (530 mg, 2.9 mmol) and K_2CO_3 (790 mg, 5.7 mmol) in water - EtOAc. A light yellow

solid was obtained, m.p. 60-65 $^\circ C$, $[\alpha]_D^{25} -78.0^\circ$ ($c = 0.1$, acetone). 1H -NMR: δ 1.55 (s, CH_3), 1.61 (s, CH_3), 1.63 (s, CH_3), 1.90-2.10 (m, 2 CH_2), 2.29-3.22 (m, $H_{\beta, \beta'} + CH_2S$), 3.77 (s, OCH_3), 4.94 (m, H_a), 5.12 (m, C=CH), 5.15 (m, C=CHCS), 7.15 (d, HN), 7.96 (m, Ar, 2H), 8.23 (m, Ar, 2H). ^{13}C -NMR: δ 16.3, 17.9, 25.9, 26.6, 30.1, 33.3, 39.8, 52.5, 53.1, 119.6, 123.9, 124.0, 128.6, 132.0, 139.4, 140.4, 150.0, 165.3, 171.4. Anal. Calcd for $C_{21}H_{28}N_2O_5S$: C, 59.98; H, 6.71; N, 6.66. Found: C, 59.80; H, 6.66; N, 6.60.

L-N-(4-Nitrobenzoyl)-[*S*-(*S*)-citronellyl]cysteine methyl ester [(*R*)-methyl 3-((*S*)-3,7-dimethyloct-6-enylthio)-2-(4-nitrobenzamido)propanoate, **L-83**]. (*S*)-(+)-Citronellyl iodide (1.33 g, 5 mmol) was added to a stirred solution of L-cysteine methyl ester hydrochloride (0.858 g, 5 mmol) in anhydrous methanol (2 mL) and 7N anhydrous NH_3 solution in methanol (4.8 mL) at $-10^\circ C$. The mixture was kept for 10 min at $-10^\circ C$ followed by continuous stirring without cooling for 1 h. The solvent was evaporated in vacuum, the residue suspended in EtOAc, the solid filtered off and the organic phase was evaporated. The resulting residue was applied onto a column and eluted with hexane-EtOAc 1:2 followed by EtOAc. Fractions containing L-S-(*S*)-citronellyl]cysteine methyl ester (R_f 0.25 hexane-EtOAc 1:2) were combined, shaken with *p*-nitrobenzoyl chloride (1.04 g, 5.4 mmol) and a solution of K_2CO_3 (1.38 g, 10 mmol) in water (10 mL) and treated as above. Column chromatography (gradient from DCM to DCM-EtOAc 50:1) afforded 0.979 g (46% yield) of a light yellow solid, R_f 0.6, hexane-EtOAc 1:1, m.p. 47.5-48.5 $^\circ C$, $[\alpha]_D^{25} -45.8^\circ$ ($c = 0.08$, acetone). 1H -NMR: δ 0.84 (d, 6.3 Hz, $CHCH_3$), 1.12 (m, H_4 , 1H), 1.20-1.60 (m, $H_4 + H_{5,5'} + CHCH_3$, 4H), 1.56 (s, CH_3), 1.65 (s, CH_3), 1.92 (m, $H_{5,5'}$), 2.29 (m, CH_2S), 3.11 (m, $H_{\beta, \beta'}$), 3.80 (s, OCH_3), 5.00 (m, $H_a + C=CH$), 7.11 (d, HN), 7.97 (m, Ar, 2H), 8.28 (m, Ar, 2H). ^{13}C -NMR: δ 17.85, 19.37, 25.59, 25.89, 30.79, 32.03, 34.32, 36.89, 36.92, 52.60, 53.09, 124.07, 124.67, 128.62, 131.59, 139.44, 150.06, 165.21, 171.30. Anal. Calcd for $C_{21}H_{30}N_2O_5S$: C, 59.69; H, 7.16; N, 6.63. Found: C, 59.94; H, 7.05; N, 6.55.

L-N-(4-Nitrobenzoyl)-[*S*-(*R*)-citronellyl]cysteine methyl ester [(*R*)-methyl 3-((*R*)-3,7-dimethyloct-6-enylthio)-2-(4-nitrobenzamido)propanoate, **L-86**]. (*R*)-(-)-citronellyl iodide was used for the synthesis in essentially the same procedure as for L-N-(4-Nitrobenzoyl)-[*S*-(*S*)-citronellyl]cysteine methyl ester. Yield 1.478 g (70%), m.p. 57 $^\circ C$, $[\alpha]_D^{23} -57.4^\circ$ ($c = 0.09$, acetone). 1H -NMR: δ 0.81 (d, 6.3 Hz, $CHCH_3$), 1.00-1.60 (m, $H_{4,4'} + H_{5,5'} + CHCH_3$, 5H), 1.54 (s, CH_3), 1.62 (s, CH_3), 1.90-2.10 (m, $H_{5,5'}$), 2.49 (m, CH_2S), 3.04 (dd, 5.4, 13.8 Hz, H_{β}), 3.12 (dd, 4.8, 13.8 Hz, $H_{\beta'}$), 3.77 (s, OCH_3), 4.95 (m, H_a), 5.00 (m, C=CH), 7.19 (d, HN), 7.95 (m, Ar, 2H), 8.24 (m, Ar, 2H). ^{13}C -NMR: δ 17.83, 19.34, 25.59, 25.87, 30.71, 32.01, 34.25, 36.83, 36.91, 52.61, 53.06, 124.01, 124.69, 128.64, 131.53, 139.42, 150.01, 165.28, 171.33. Anal. Calcd for $C_{21}H_{30}N_2O_5S$: C, 59.69; H, 7.16; N, 6.63. Found: C, 59.93; H, 7.12; N, 6.55.

L-N-(4-Nitrobenzoyl)-S-(ethyl)cysteine methyl ester [(*R*)-methyl 2-(4-nitrobenzamido)-3-(ethylthio)propanoate, **L-74**]. L-(*S*-ethyl)cysteine (1.49 g, 10 mmol) was reacted overnight with TMS-Cl (3 mL, 23.5 mmol) in MeOH (13 mL) at RT, giving L-(*S*-ethyl)cysteine methyl ester. After removal of solvent, the solid was reacted with *p*-nitrobenzoyl chloride (2.33 g, 12 mmol) and K_2CO_3 (2.76 g, 20 mmol) in

water – EtOAc (10+20 ml) and treated as above. Column chromatography (gradient from DCM to DCM-EtOAc 40:1) afforded 1.36 g (43%) of a yellowish solid, m.p. 95-96°C, $[\alpha]_D^{21}$ -56.1° (c 0.065, acetone). R_f 0.45 (hexane-EtOAc 1:1). $^1\text{H-NMR}$: δ 1.22 (t, CH₃), 2.53 (q, CH₂), 3.22 (m, H _{β} , β'), 3.80 (s, OCH₃), 4.98 (m, H _{α}), 7.11 (d, HN), 7.97 (m, Ar, 2H), 8.28 (m, Ar, 2H). $^{13}\text{C-NMR}$: δ 14.9, 26.8, 33.9, 52.5, 53.1, 124.1, 128.6, 139.4, 150.0, 165.2, 171.3. Anal. Calcd for C₁₃H₁₆N₂O₅S: C, 49.99; H, 5.16; N, 8.97. Found: C, 50.18; H, 5.04; N, 8.81.

L-N-(4-Nitrobenzoyl)-S-(*i*-amyl)cysteine methyl ester [(*R*)-methyl 2-(4-nitrobenzamido)-3-(isopentylthio)propanoate, **L-81**]. *i*-Amyl bromide (0.615 g, 4.1 mmol) was added to a solution of L-cysteine methyl ester (0.68 g, 3.7 mmol) in methanol (2 mL) and 7N solution of NH₃ in methanol (3.5 mL, 24.5 mmol) at -5 - -10°C. The reaction mixture was stirred for 30 min at -5°C, 1h at 0°C and 1 h at RT. The solvent was evaporated in vacuum, the residue taken into EtOAc, the organic phase rotovaped and the residue purified on a column in EtOAc (1.14 g, 75%). Fractions containing L-(*S*-*i*-amyl)cysteine methyl ester (R_f 0.15, hexane-EtOAc 1:1) were combined and shaken with *p*-nitrobenzoyl chloride (0.64 g, 3.4 mmol) and a solution of K₂CO₃ (0.77 g, 5.6 mmol) in water (5 mL) in a separatory funnel for 5 min. After completion of the reaction (TLC in EtOAc 1:1, R_f of amide 0.65) the organic phase was separated, washed with diluted NaOH, brine, dried over K₂CO₃ and the solvent removed in vacuum. Column chromatography (gradient from DCM to DCM-EtOAc 20:1) afforded 0.49 g (38%) of a light yellow solid, m.p. 93-93.5°C, $[\alpha]_D^{25}$ -53.3° (c 0.05, acetone). $^1\text{H-NMR}$: δ 0.85 (dd, 6.6, 1.5 Hz, CHCH₃), 1.42 (m, CH₂CH₂S, 2H), 1.67 (m, CHMe₂), 2.51 (m, CH₂CH₂S), 3.11 (H _{β} , β' , m), 3.80 (s, OCH₃), 4.98 (H _{α} , m), 7.11 (d, HN, broad), 7.97 (m, Ar, 2H), 8.28 (m, Ar, 2H). $^{13}\text{C-NMR}$: δ 22.40, 27.55, 30.98, 34.29, 38.68, 52.59, 53.10, 124.07, 128.62, 139.45, 150.06, 165.23, 171.31. Anal. Calcd for C₁₆H₂₂N₂O₅S: C, 54.22; H, 6.26; N, 7.90. Found: C, 54.23; H, 6.30; N, 7.85.

L-N-(4-Nitrobenzoyl)-S-(prenyl)cysteine methyl ester [(*R*)-methyl 3-(3-methylbut-2-enylthio)-2-(4-nitrobenzamido)propanoate, **L-77**]. A solution of 7N ammonia in MeOH (70 mmol, 10 mL) was added to a solution of L-CysOMeHCl (1.03 g, 6 mmol) in anhydrous MeOH (2 mL) that had been cooled to -5°C. Prenyl chloride (0.69 g, 6.6 mmol) was then added in one portion. After 15 min at -5°C, the mixture was put on ice for 30 min, the solvent removed in vacuum, the residue taken into EtOAc, precipitated ammonium chloride extracted with water and the organic phase vigorously shaken with *p*-nitrobenzoyl chloride (1.22 g, 6.6 mmol) and a solution of K₂CO₃ (1.66 g, 12 mmol) in water (7.7 mL). After completion of the reaction, the organic phase was separated, dried over K₂CO₃ and the solvent evaporated in vacuum. Column chromatography (gradient from DCM to DCM-EtOAc 10:7) afforded 1.44 g (68% for 2 steps) of a yellowish solid with m.p. 89-90°C, $[\alpha]_D^{24}$ -57.4° (c 0.07, acetone), R_f 0.7 (hexane-EtOAc 1:2). $^1\text{H-NMR}$: δ 1.63 (s, CH₃), 1.70 (s, CH₃), 2.95-3.15 (m, H _{β} , β' , + CH₂S) 3.81 (s, OCH₃), 4.97 (m, H _{α}), 5.17 (m, C=CH), 7.06 (d, NH), 7.98 (m, Ar, 2H), 8.28 (m, Ar, 2H); $^{13}\text{C-NMR}$: δ 18.0, 25.9, 26.55, 30.4, 33.6, 52.5, 53.1, 119.8, 124.1, 128.6, 136.9, 139.5, 165.2, 171.3. Anal. Calcd for C₁₆H₂₀N₂O₅S: C, 54.53; H, 5.72; N, 7.95. Found: C, 54.54; H, 5.68; N, 7.85.

L-N-(4-Nitrobenzoyl)-S-(benzyl)cysteine methyl ester [(*R*)-methyl 2-(4-nitrobenzamido)-3-(benzylthio)propanoate, **L-93**]. A suspension of L-Cys(Bn)OMeHCl (1.36 g, 5 mmol) in EtOAc (50 mL) was shaken with *p*-nitrobenzoyl chloride (1.37 g, 6 mmol) and a solution of K₂CO₃ (2.07 g, 15 mmol) in water (15 mL). After completion of the reaction, concentrated NH₄OH (1 mL) was added, the mixture was kept at RT for 15 min and the organic phase was treated as above. Column chromatography (gradient from DCM to DCM-EtOAc 30:1) afforded 1.27 g (68%) of a yellowish solid with m.p. 114-115°C, $[\alpha]_D^{26}$ -77.4° (c 0.05, acetone), R_f 0.6 (hexane-EtOAc 1:1). $^1\text{H-NMR}$: δ 2.9-3.1 (m, H _{β} , β'), 3.71 (s, CH₂S) 3.77 (s, OCH₃), 4.97 (m, H _{α}), 7.04 (d, NH), 7.2-7.3 (m, Ph), 7.91 (m, Ar, 2H), 8.25 (m, Ar, 2H); $^{13}\text{C-NMR}$: δ 35.6, 36.9, 52.4, 53.1, 124.0, 127.6, 128.6, 128.9, 129.1, 137.7, 139.3, 150.0, 165.3, 171.2. Anal. Calcd for C₁₈H₁₈N₂O₅S: C, 57.74; H, 4.85; N, 7.48. Found: C, 57.71; H, 4.83; N, 7.51.

L-N-(4-Nitrobenzoyl)-S-(*trans*, *trans*-farnesyl)cysteine ethyl ester [(*R*)-ethyl 3-((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trienylthio)-2-(4-nitrobenzamido)propanoate, **L-76**]. A solution of 7N ammonia in MeOH (19 mmol, 2.7 mL) was added to a solution of L-CysOEtHCl (0.56 g, 3 mmol) in anhydrous MeOH (1.5 mL), followed by the addition of farnesyl bromide (0.94 g, 3.3 mmol) in one portion at 0°C. After 30 min at 0°C, the mixture was stirred at RT for 30 min, the solvent was removed in vacuum and the residue taken into EtOAc. The precipitated ammonium bromide was centrifuged and the organic phase stirred with *p*-nitrobenzoyl chloride (0.67 g, 3.6 mmol) and a solution of K₂CO₃ (0.83 g, 6 mmol) in water (3 mL) with ice cooling for 10 min and then for 15 min at RT. The organic phase was separated, dried over K₂CO₃ and the solvent evaporated. Column chromatography in DCM afforded 0.51 g (33% for 2 steps) of a yellowish solid with m.p. 53-54°C, $[\alpha]_D^{24}$ -60.0° (c 0.06, acetone), R_f 0.75 (hexane-EtOAc 1:1). $^1\text{H-NMR}$: δ 1.28 (t, CH₂CH₃), 1.55 (s, 2 CH₃), 1.62 (s, 2 CH₃), 1.95-2.05 (m, 4 x CH₂), 2.9-3.3 (m, H _{β} , β' + CH₂S), 4.22 (s, OCH₂CH₃), 4.92 (m, H _{α}), 5.04 (m, 2 C=CH), 5.17 (m, C=CHCS), 7.21 (d, HN), 7.95 (m, Ar, 2H), 8.22 (m, Ar, 2H). $^{13}\text{C-NMR}$: δ 14.36, 16.20, 16.35, 17.87, 25.89, 26.61, 26.91, 30.13, 33.42, 39.82, 39.88, 52.63, 62.31, 119.65, 123.85, 123.99, 124.51, 128.65, 131.45, 135.59, 139.51, 140.40, 149.95, 165.33, 170.99. Anal. Calcd for C₂₇H₃₈N₂O₅S: C, 64.51; H, 7.62; N, 5.57. Found: C, 64.54; H, 7.58; N, 5.64.

L-N-(4-Nitrobenzoyl)-S-(*trans*, *trans*-farnesyl)cysteine *i*-propyl ester [(*R*)-isopropyl 3-((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trienylthio)-2-(4-nitrobenzamido)propanoate, **L-75**]. Similar to the synthesis of L-N-(4-nitrobenzoyl)-S-(*trans*,*trans*-farnesyl)cysteine ethyl ester from CysOPr-*i*HCl (0.44 g, 2.2 mmol), 7N ammonia in MeOH (14 mmol, 2 mL), farnesyl bromide (0.69 g, 2.4 mmol), *p*-nitrobenzoyl chloride (0.52 g, 2.8 mmol) and a solution of K₂CO₃ (0.61 g, 4.4 mmol) in water (3 mL). A yield of 0.33 g (29% for 2 steps) of a yellowish solid with m.p. 60-61°C, $[\alpha]_D^{24}$ -71.7° (c 0.05, acetone), R_f 0.8 (hexane-EtOAc 1:1). $^1\text{H-NMR}$: 1.26 [dd, 1.2, 6.3 Hz, OCH(CH₃)₂], 1.54 (s, 2 CH₃), 1.62 (s, 2 CH₃), 1.9-2.1 (m, 4 x CH₂), 2.93 (dd, 6.0, 13.8 Hz, H _{β}), 3.05-3.30 (m, H _{β} + CH₂S), 4.89 (m, H _{α}), 5.0-5.2 [m, 2 C=CH + C=CHCS + OCH(CH₃)₂], 7.18 (d, HN), 7.95 (m, Ar, 2H), 8.22 (m, Ar, 2H). $^{13}\text{C-NMR}$: δ 16.20, 16.36, 21.92, 21.95, 25.88, 26.63, 26.90, 30.18, 33.46, 39.82, 39.87, 52.75, 70.28,

119.68, 123.84, 123.98, 124.51, 128.61, 131.44, 135.58, 139.57, 140.31, 149.95, 165.29, 170.49. Anal. Calcd for $C_{28}H_{40}N_2O_5S$: C, 65.09; H, 7.80; N, 5.42. Found: C, 65.31; H, 7.77; N, 5.51.

N-(4-Nitrobenzoylglycyl)-S-(*trans, trans*-farnesyl)cysteine methyl ester [**RD-18**]. 1N solution of N,N'-dicyclohexylcarbodiimide in DCM (1.5 mL, 1.5 mmol) was added to a stirred suspension of 4-nitrohippuric acid (336 mg, 1.5 mmol) and FarCysOMe (488 mg, 1.44 mmol) in anhydrous DCM (10 mL) and the mixture was kept at RT for one day. The solvent was evaporated in vacuum and the solid residue crystallized from hot methanol (18-1, 418 mg, 53%); mp 119-120°C; R_f 0.4 (hexane-EtOAc). The mother liquor was evaporated in vacuum, the residue dissolved in DCM and separated on a column using a gradient DCM-EtOAc 15:1 to 7:1. Crystallization from methanol with activated carbon gave (18-2, 210 g, 27%); mp 118-120°C; $[\alpha]_D^{20} = -20.9^\circ$ (c = 0.03, acetone); IR (KBr) NH (3300), C=O (1750), O=CN (1650), NO₂ (1340) cm^{-1} . ¹H-NMR: δ 1.55-1.60 (s, CH₃), 1.61-1.66 (s, CH₃), 1.59 (s, CH₃), 1.66 (s, CH₃), 1.93-2.10 (m, 4 x CH₂), 2.82-2.90 (H_β) and 2.95-3.01 (H_β) (dd, S-C-H $J=7.5$ Hz), 3.01-3.24 (t, CH₂S, $J=7.2$ Hz), 3.77 (s, OCH₃), 4.276 (d, 2 x), 4.78-4.84 (t, HC-NH₂), 5.05-5.09 (m, 2 x C=CH), 5.15-5.20 (m, C=CHCS, $J=1.2$ H), 7.21-7.27 (d, HN, $J=10.2$ Hz), 7.58 (s, NH), 7.98 (m, Ar, 2H, 2H), 8.27 (m, Ar, 2H, 2H). ¹³C-NMR: δ 16.27, 16.39, 17.95, 25.97, 26.64, 26.93, 30.07, 33.28, 39.87, 39.27, 43.71, 52.25, 53.08, 119.6, 123.8, 124.0, 124.5, 128.7, 131.6, 135.7, 139.2, 140.5, 149.9, 165.8, 168.9, 171.3. Anal. Calcd for $C_{28}H_{39}N_3O_6S$: C, 61.63; H, 7.20; N, 7.70. Found: C, 61.48; H, 7.02; N, 7.59.

L-N-(4-Nitrobenzoyl)-alanine methyl ester [(*S*)-methyl 2-(4-nitrobenzamido)propanoate, **L-111**]. TMS-Cl (3 mL, 23.5 mmol) was added to a suspension of L-Ala (891 mg, 10 mmol) in MeOH (17 mL) at RT and left overnight. After removal of the solvent, solid L-Ala methyl ester hydrochloride was stirred with *p*-nitrobenzoyl chloride (2.04 g, 11 mmol), K₂CO₃ (4.15 g, 30 mmol), water (15 mL) and EtOAc (35 mL) for 1h. The organic layer was dried over Na₂SO₄, evaporated and crystallized from hot MeOH (10 mL). A pale yellow solid (1.67 g, 66%) with R_f 0.8 (hexane-EtOAc 1:2) and m.p. 134-135°C, $[\alpha]_D^{25} +42.1$ (c 0.04, CHCl₃) was obtained. Anal. Calcd for $C_{11}H_{12}N_2O_5$: C, 52.28; H, 4.80; N, 11.11. Found: C, 52.65; H, 4.76; N, 11.02.

Sequencing and Identification of Esterase from Porcine Liver

We previously reported the purification of a single esterase of about 57 kDa (SDS-PAGE) to 62 kDa (calculated) from porcine liver that hydrolyzes *trans, trans*-farnesylated cysteine methyl ester substrates [14]. EPL obtained from Sigma-Aldrich (St. Louis, MO) was found to hydrolyze polyisoprenylated substrates while AChE and BChE did not. With limited information regarding the purity, molecular weight and amino acid sequence identity of the Sigma-Aldrich EPL, we sought to analyze it for three reasons; (1) assess its purity, (2) compare its molecular weight with the PMPMEase we previously purified from porcine liver and (3) if identical, switch to the more cost-effective commercially available enzyme. To achieve this, an aliquot of the enzyme (1 μ g) was analyzed by SDS-PAGE on an 8% gel followed by EZ-Blue colloidal coomas-

sie blue staining. The protein band was excised and submitted to ProtTech (Eagleville, PA) for trypsin digestion and LC-MS/MS sequencing and identification. An aliquot of EPL was washed on C-4 Q-tips and mixed with sinapinic acid matrix followed by analyses on the Applied Biosystems 4800 Mass Spectrometer in linear mode (Applied Biosystems, Framingham, MA).

Enzyme Assays

Each substrate (1 mM final concentration) was incubated with either PMPMEase (1-10 μ g depending on substrate), acetylcholinesterase (10 μ g) or butyrylcholinesterase (10 μ g) in 100 mM Tris-HCl, pH 7.4 containing 5% DMSO at 37°C (100 μ l total volume). Michaelis-Menten kinetics analysis was conducted under identical conditions except with varying concentrations of the different substrates and EPL (0.1 to 5 μ g/per assay depending on substrate). The reactions were stopped and analyzed as previously described [14, 24] with UV-detection at 260 nm (for the para-nitrobenzoyl substrates) or 214 nm for BzGFCM, HCFCM and L-69 (D-AFCM). Aliquots of the substrates were dissolved in a 50% (v/v) mixture of methanol in water containing 0.05 M NaOH and incubated at 37°C for 1 h to hydrolyze the esters to the corresponding acid. These were then used as HPLC retention time markers as well as to generate calibration plots for data analysis. The K_M and V_{max} values were obtained by applying Michaelis-Menten kinetics analysis on the data using the GraphPad Prism software.

Modeling of S-Alkylated Cysteiny-Ester Substrates

The pK_M for twelve of the substrates (Table 1) were used in Comparative Molecular Field Analysis (CoMFA). The initial geometries were generated using SKETCH/Sybyl 8.0 (Tripos Associates Inc., St. Louis, MO) and the side-chains were set to the extended conformations. The Sybyl software was run under the LINUX operating system (Fedora 8.0) and the lowest energy (minimized) conformations were obtained under vacuum using the conjugate gradient method with 0.001 kcal/(mol \AA) gradient termination option and 5000 maximum iterations, using MMFF94s force field and MMFF94 charges.

Several alignment hypotheses were investigated in the absence of a known pharmacophore for this group of compounds. The alignment that led to the highest cross-validated (q^2) value was selected for the development of the final model. The minimized structure of one of the most active compounds (L-75) was used as the template. The rest of the compounds were aligned using the SYBYL "database alignment" function and a common set of 6 atoms from the ester carbonyl carbon to the first double-bond carbon of the side chain (C11-C10-C15-S16-C17-C18 shown in L-75 in Table 1).

RESULTS

Synthesis

The general procedures for the synthesis of the substrates are shown in Scheme 1. Proton and ¹³C NMR, elemental analysis and reversed-phase high performance liquid chromatography confirmed the identities of the synthesized compounds and the over 95% purity of the compounds.

Table 1. Michaelis-Menten Kinetic Data for the Hydrolysis of Carboxyl Esters by PMPMEase. The pK_M Values for the Training Dataset used in the Molecular Modeling are Shown in Square Brackets

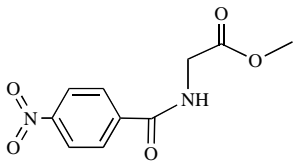
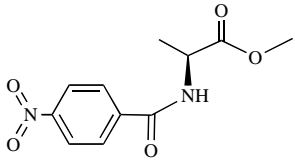
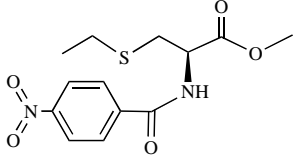
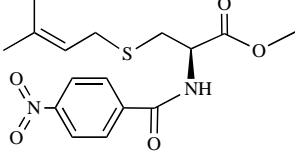
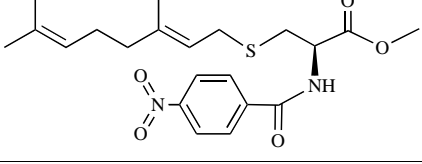
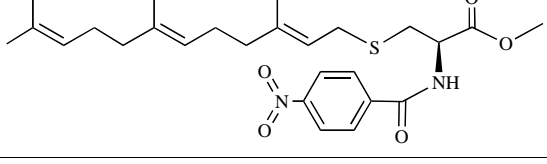
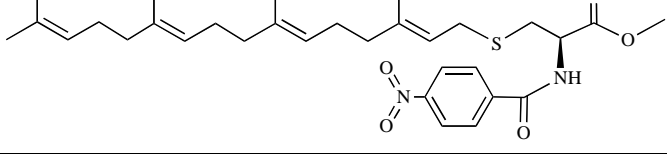
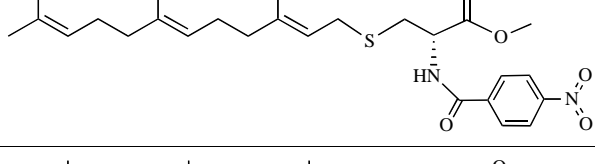
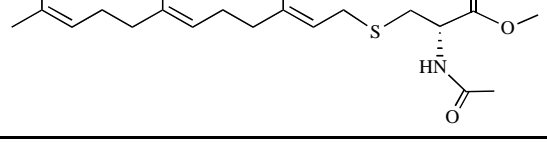
Compound	Structure	K_M (μM) [pK_M]	V_{max} (nmol/s/mg)	$^a k_{\text{cat}}$ (s^{-1})	$^a k_{\text{cat}}/K_M$ ($\text{M}^{-1}\text{s}^{-1}$)
L-102		400 \pm 115	4.5 \pm 0.6	0.28 \pm 0.04	700
L-111		300 \pm 38	13.3 \pm 0.7	0.82 \pm 0.04	2700
L-74		505 \pm 63 [3.30]	95.5 \pm 5.4	5.92 \pm 0.34	11700
L-77		294 \pm 25 [3.53]	39.8 \pm 1.6	2.47 \pm 0.10	8400
L-72		87 \pm 12 [4.06]	12.6 \pm 0.5	0.78 \pm 0.03	9000
RD-PNB		29 \pm 2.2 [4.54]	1.06 \pm 0.03	0.07 \pm 0.002	2400
L-80		15 \pm 2.7	0.15 \pm 0.007	0.009 \pm 0.0004	600
L-70		11 \pm 0.9 [4.96]	0.19 \pm 0.003	0.01 \pm 0.0002	900
L-69		21 \pm 4.2 [4.68]	0.41 \pm 0.03	0.03 \pm 0.002	1400

Table 1. contd....

Compound	Structure	K_M (μM) [PK_M]	V_{max} (nmol/s/mg)	$^a k_{\text{cat}}$ (s^{-1})	$^a k_{\text{cat}}/K_M$ ($\text{M}^{-1}\text{s}^{-1}$)
L-81		960 ± 180 [3.02]	75 ± 10	4.65 ± 0.62	4800
L-93		40.5 ± 3.7	29 ± 1	1.80 ± 0.06	44000
L-83		56 ± 9.6 [4.25]	9.5 ± 0.4	0.59 ± 0.03	11000
L-86		53 ± 7.0 [4.28]	9.5 ± 0.3	0.59 ± 0.02	11000
L-75		5.9 ± 0.7 [5.23]	0.53 ± 0.01	0.03 ± 0.001	5100
L-76		11 ± 0.9 [4.96]	1.5 ± 0.03	0.09 ± 0.002	8200
RD-18		8.4 ± 0.89 [5.08]	0.30 ± 0.007	0.02 ± 0.0004	2400
BzGFCM		3.0 ± 0.4	0.46 ± 0.01	0.03 ± 0.001	10000
HCFCM		3.2 ± 0.5	0.74 ± 0.02	0.05 ± 0.001	15600

^a k_{cat} assumes a molecular weight for PMPMEase (EPL) of 62,000 for the translated cDNA sequence.

EPL is the Same Protein as PMPMEase

SDS-PAGE analysis of EPL revealed a single major protein band of about 60.5 kDa (Fig. 1A). Linear mode MS analysis showed two peaks corresponding to the $[M+H]^+$ and $[M+2H]^{2+}$ molecular ions with M/Z values of 61,200 and 30,600, respectively (Fig. 1B). LC-MS/MS analysis of the coomassie band yielded the sequences of over twenty-five tryptic peptides which were used to identify a single protein with greater than 98% certainty. EPL was identified as EST1_PIG Liver carboxylesterase precursor, Proline-beta-naphthylamidase, Retinyl ester hydrolase or carboxylesterase precursor (*Sus scrofa*) with a calculated molecular weight of 62621.95. EPL is thus the same protein as PMPMEase that was previously purified and identified as carboxylesterase precursor (*Sus scrofa*) [14].

S-Alkylated Substrates are Hydrolyzed by PMPMEase but not AChE and BChE

When the substrates were analyzed for hydrolysis by PMPMEase, AChE and BChE, all of the substrates were significantly hydrolyzed by PMPMEase. Little or no hydrolysis was observed with AChE and BChE (Fig. 2). The S-substitutions had a strong effect on the degree of hydrolysis by PMPMEase and the cholinesterase enzymes. The *all trans*-geranylgeranylated and the *trans,trans*-farnesylated substrates (L-69, L-70, L-75, L-76, L-80 and RD-PNB) were all very resistant to cholinesterase hydrolysis (Fig. 2). Substrates with significantly reduced side-chains (L-74, L-102 and L-111) were more susceptible to hydrolysis by acetyl- and butyrylcholinesterases (Fig. 2). A time-dependent study further revealed more rapid hydrolysis of the S-prenylated (L-77) and S-geranylated (L-72) substrates over the *trans,trans*-farnesylated and S-*all trans*-geranylgeranylated substrates by PMPMEase (Fig. 3).

Michaelis-Menten Kinetics Analysis

We hypothesized that if polyisoprenylated proteins are the substrates for EPL/PMPMEase, then substrates with *trans,trans*-farnesyl or *all trans*-geranylgeranyl moieties will display the lowest K_M values. The analysis revealed the typi-

cal concentration-dependent effects (Fig. 4). As expected, the substrates with small S-substituents (e.g. ethyl and prenyl) displayed the least affinity while the *trans,trans*-farnesyl and *all trans*-geranylgeranyl substrates had the highest affinities (Fig. 4). The K_M values ranged from $960 \pm 180 \mu\text{M}$ (L-81, L-alanine) to $3.0 \pm 0.4 \mu\text{M}$ (BzGFCM), a 320-fold increase in affinity (Table 1).

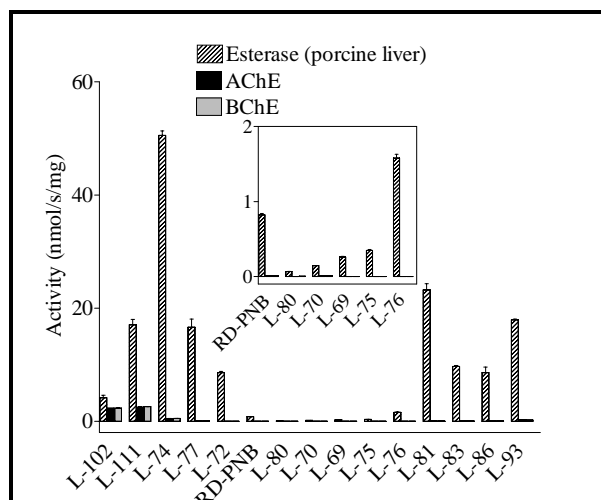


Fig. (2). Relative substrate hydrolysis by PMPMEase, acetyl- and butyrylcholinesterases. Each substrate was incubated with the different enzymes as described in the procedures section. These were then analyzed for the amounts of products formed. The specific activities were then computed based on the amounts of enzyme used and the duration of the incubation to determine the specific activity. The results are the means \pm SEM (n=3).

Use of the D- in place of L-cysteine did not prevent hydrolysis by PMPMEase. Instead, the affinity was increased by almost 3-fold as shown by the K_M values for the L-cysteine analog, RD-PNB ($29 \pm 2.2 \mu\text{M}$) compared to the D-cysteine analog, L-70 ($11 \pm 0.9 \mu\text{M}$). Larger O-alkyl

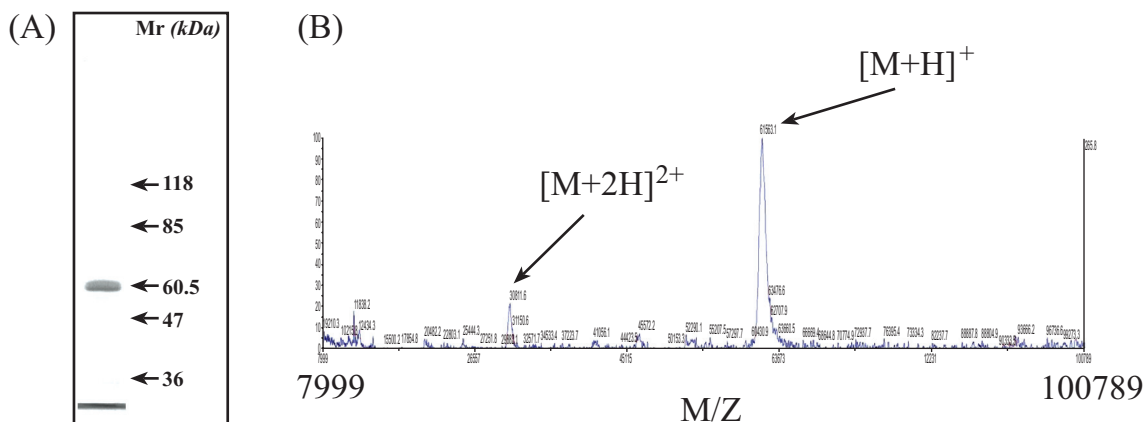


Fig. (1). SDS-PAGE and mass spectrometry analyses reveal EPL is the same protein as PMPMEase. (A) A $1 \mu\text{g}$ aliquot of EPL was boiled in SDS-PAGE sample buffer and subjected to electrophoresis on an 8% polyacrylamide gel followed by EZ-Blue colloidal coomassie blue staining. The molecular weight of the protein band was determined based on its migration relative to the Fisher's EZ-Run pre-stained molecular weight protein markers shown in kilodaltons (β -galactosidase, 118.0; bovine serum albumin 85.0; ovalbumin, 47.0 and lactate dehydrogenase, 36.0). (B) MALDI-TOF analysis of an aliquot of EPL using the Applied Biosystems 4800 mass spectrometer. The protein peaks represent the $[M+H]^+$ ($M/Z = 61,200$) and $[M+2H]^{2+}$ ($M/Z = 30,600$) molecular ions of EPL.

substituents also increased the affinity towards the esterase as O-ethyl (L-76) and O-isopropyl (L-75) had lower K_M values (11 ± 0.9 and $5.9 \pm 0.66 \mu\text{M}$, respectively) when compared to RD-PNB ($29 \pm 2.2 \mu\text{M}$).

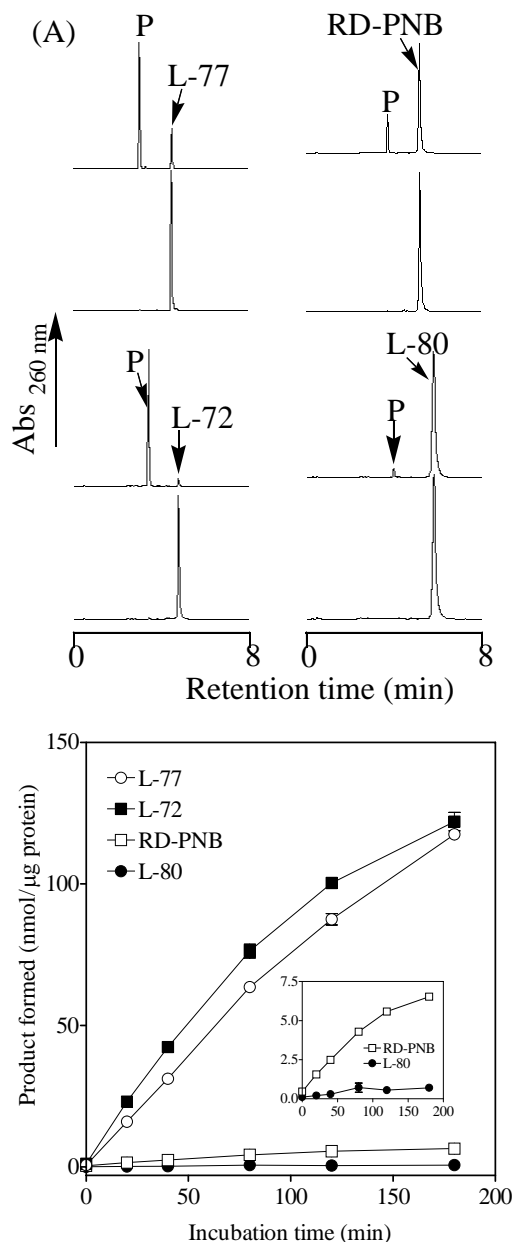


Fig. (3). Time-dependent hydrolysis of selected S-alkylated substrates. An S-prenylated (L-77), S-*trans* geranylated (L-72), S-*trans, trans*-farnesylated (RD-PNB) and S-*all trans*-geranylgeranylated (L-80) substrates at 1 mM concentrations were incubated with either 0.25 or 1 μg of EPL. At the various time intervals, the reactions were stopped and analyzed as described in the procedures section. The amounts of enzyme used and the amounts of products formed were used to compute the relative amounts of products formed. Panel A: Reversed-phase HPLC chromatograms showing the substrate hydrolysis to products (P) by 5 μg PMPMEase following 160 min incubation (upper chromatograms) compared to the respective substrates before incubation (lower chromatograms). Panel B: Time-dependent hydrolysis of the selected substrates. The results are the means \pm SEM ($n=3$).

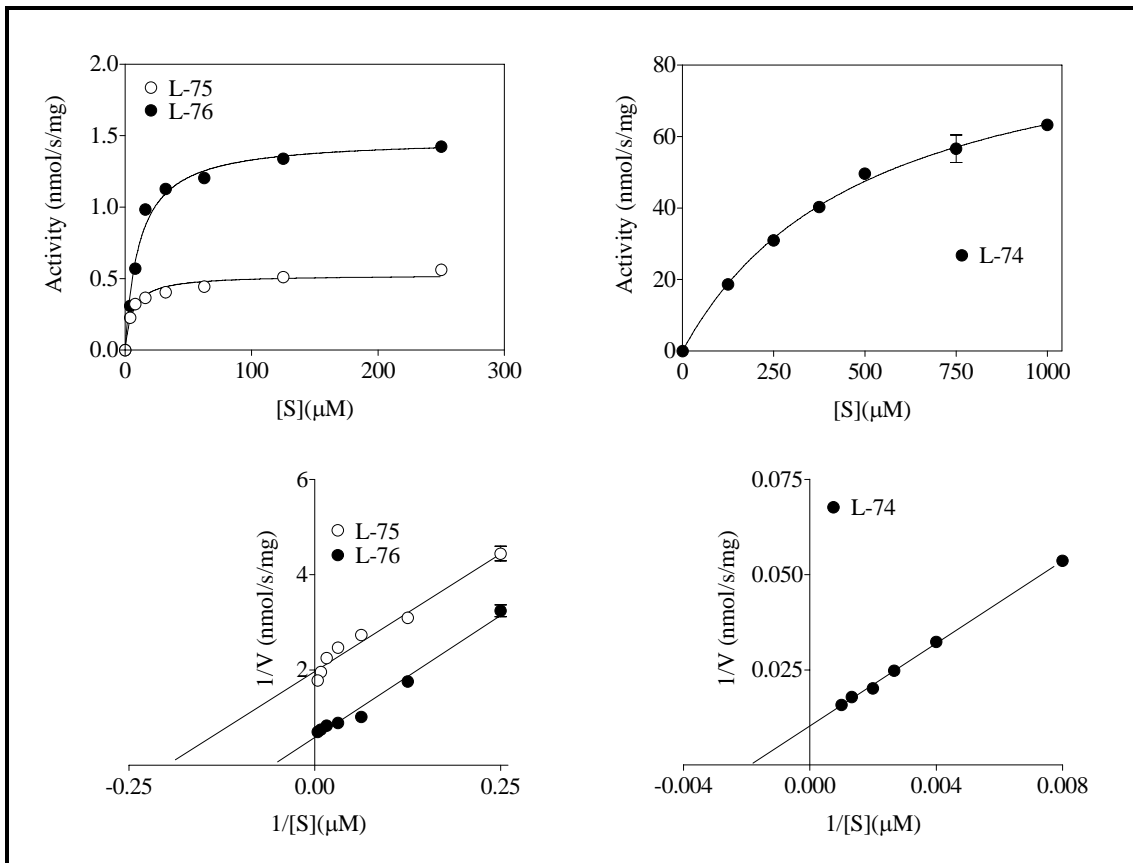
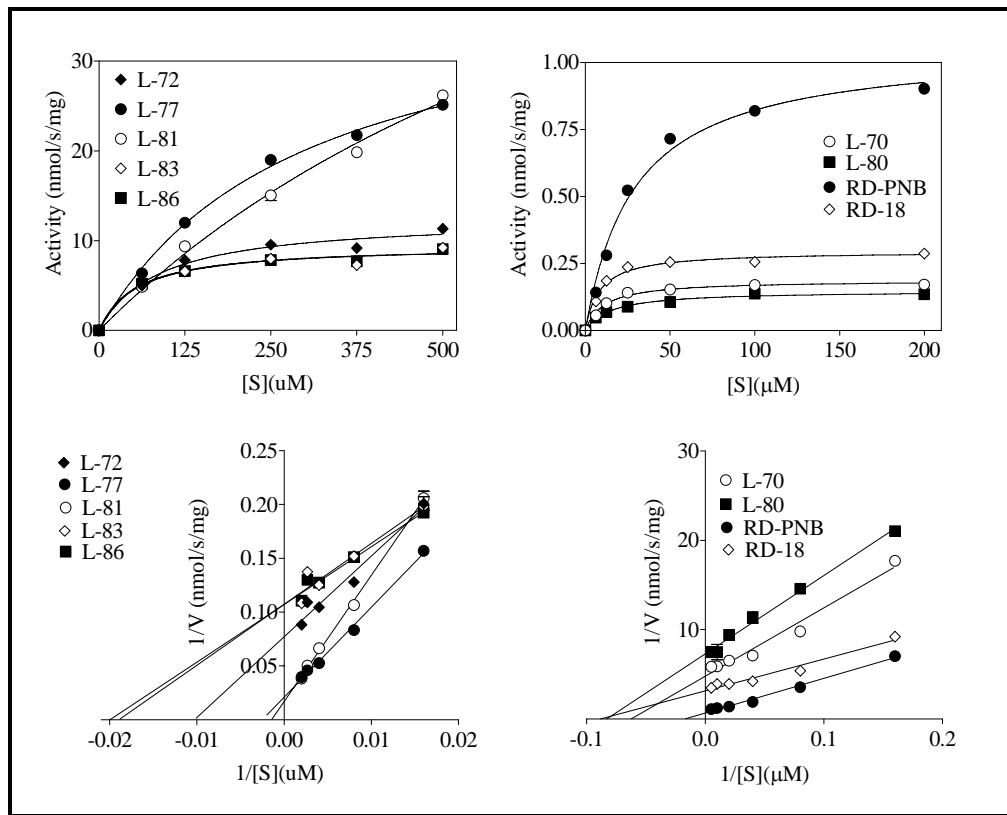
Bond saturation on the S-substituents yielded ambiguous results as shown by the K_M values for L-77 ($290 \pm 25 \mu\text{M}$) versus L-81 ($960 \pm 180 \mu\text{M}$) as well as L-72 ($87 \pm 12 \mu\text{M}$) compared to L-83 ($56 \pm 9.6 \mu\text{M}$) and L-86 ($53 \pm 7.0 \mu\text{M}$). The S-benzyl analog (L-93) displayed a biphasic character in which the typical Michaelis-Menten type saturation kinetics was observed at concentrations lower than 125 μM but an inhibitory effect was observed at higher concentrations. A limited concentration range was then used to obtain the K_M of 40.5 ± 3.7 . Because it is difficult to determine the substrate concentration at which the inhibitory effects begin, the true K_M value for L-93 may be significantly higher than the obtained value. This may have resulted in the much larger k_{cat}/K_M value for L-93 of 44,000.

For the S-alkylated *p*-nitrobenzoyl-L-cysteine methyl-ester substrates, the V_{max} values increased inversely with the K_M values and decreased S-alkyl substituent sizes. The V_{max} values for the substrates lacking the sulfur atom (L-102 and L-111) did not fit this general pattern as might have been predicted from the large K_M values. Decreasing the alkyl chain from the 20-carbon *all trans*-geranylgeranyl through *trans,trans*-farnesyl, *trans*-geranyl, prenyl to ethyl resulted in at least 7-, 80-, 260- and 600-fold increases in the relative hydrolysis rates (Table 1).

Modeling of S-alkyl,N-acyl Cysteiny-Ester Substrates

CoMFA is widely used to study the correlation of biological activities with the structural properties of ligands. It samples the steric and electrostatic fields around a set of ligands and correlates them to the experimental binding affinities. Evaluation of the alignment maps (Fig. (5), top panels) shows that the spatial location of the *p*-nitrobenzoyl moiety in the S-isomers compared to the R-isomers (Fig. (5) bottom left panel) is in the opposite direction. Also, in the extended side chains of compounds L-83 and L-86, the C-18 saturated analogs, were placed away from those of the template compound as depicted with L-86 and L-75 (Fig. (5) bottom right panel). The initial Leave-One-Out cross-validated partial least squares (PLS) analysis of the aligned dataset yielded a cross-validated q^2 of 0.863 with a standard error of 0.365 at component 5. The final quantitative structure-activity relationship (QSAR) model yielded an R^2 of 0.995 with a standard error of 0.071. The statistical data of the PLS analysis are summarized in Table 2 and a plot of the predicted against experimental binding affinities ($\text{p}K_M$) is shown in Fig. (6). A predicted $\text{p}K_M$ of 4.72 was obtained for L-80 that was not included in the training set, compared to its experimental value of 4.82.

CoMFA contour maps are useful in providing visual displays of areas where the steric and electrostatic fields significantly affect binding affinities. Fig. (7) (top left panel) shows the contour map of the model with the polyisoprenyl group of the template compound, L-75, interacting with the green region of the final CoMFA fields. On the other hand, L-81, (Fig. (7) top right panel), does not extend to interact with this region. The insufficient interactions (Fig. (7) bottom left panel) are observed with other substrates with smaller S-substituents than L-75. The S-alkyl side chains of L-83 and L-86 have C-18-saturated carbons which result in them being placed away from the equivalent side chain of the template molecule (Fig. (7) bottom right panel), preventing them from



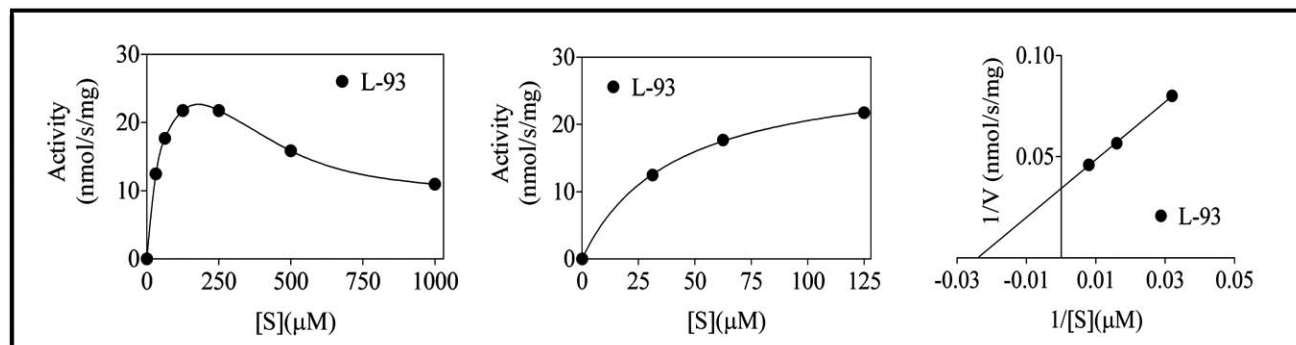


Fig. (4). Michaelis-Menten and Double-reciprocal analysis of ester substrates hydrolysis by PMPMEase. PMPMEase (0.1 to 5 μg depending substrate) was incubated with varying concentrations of each substrate. The reactions were stopped and analyzed by reversed phase-HPLC as described in the procedures section. The specific activities were calculated and used to determine the K_M and V_{max} values using the Graph-Pad Prism software. The results are the means \pm SEM ($n=3$).

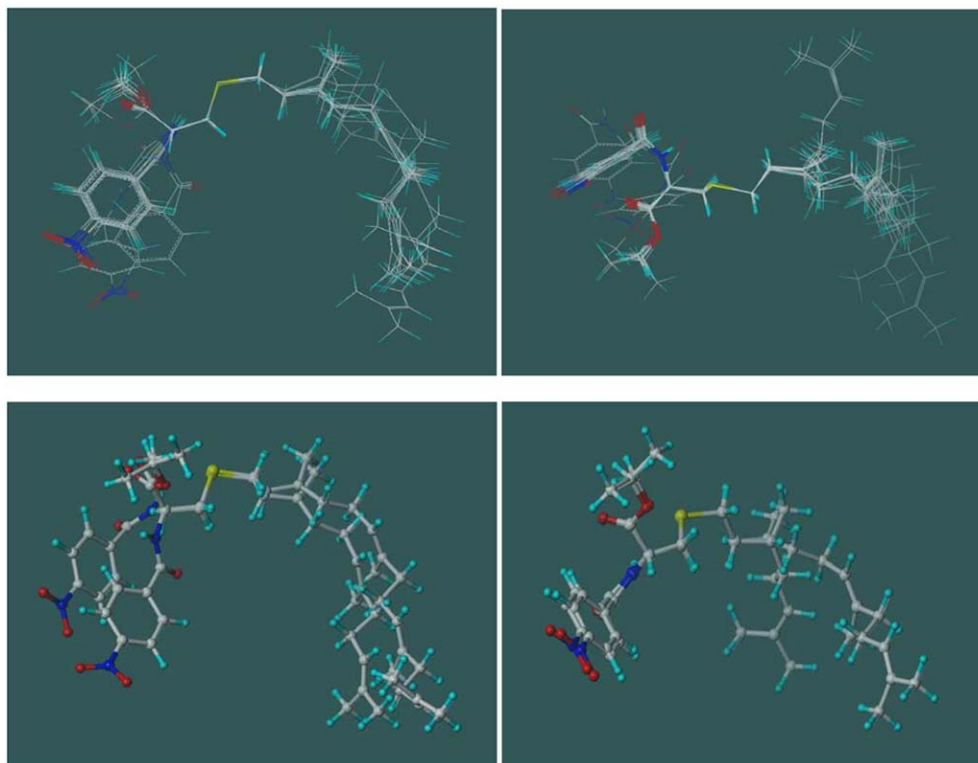


Fig. (5). Two views of the alignment of all molecules in the training set (top panels). The alignment of L-70 and L-75 (bottom left panel) and L-86 and L-75 (bottom right panel).

interacting with the green contour region. The steric contour map also shows a smaller green region adjacent to the ester carbonyl carbon (C11). The contribution of the electrostatic field is low (28%) and does not provide an interpretive result.

DISCUSSION

Although a single polyisoprenylated substrate-metabolizing esterase was previously purified, sequenced and identified from porcine liver [14], its selectivity towards small molecule *trans,trans*-farnesylated and *S-all trans*-

geranylgeranylated cysteine methyl esters that would be indicative of its status as the polyisoprenylation-dependent esterase has not been demonstrated. Such an esterase would have higher affinities (lower K_M values) for *S-trans,trans*-farnesylated and *S-all trans*-geranylgeranylated cysteine methyl esters than for other S-alkyl substituents. The significantly higher affinity of the enzyme towards the *S-trans,trans*-farnesylated and *S-all trans*-geranylgeranylated cysteine methyl esters indicates that polyisoprenylated proteins are most likely the endogenous substrates of the enzyme. Since the substrates with progressively shorter S-alkyl groups displayed increasingly lower affinity towards the enzyme, it can be inferred, as shown in the modeling contour maps, that the polyisoprenyl moiety is pivotal in the active site binding interactions. Studies with synthetic mimics of the carboxyl terminals of polyisoprenylated proteins revealed similar affinities towards PPMTase [25-28]. The strong correlation between the experimental and predicted binding affinities affirms the principal finding that *S-trans, trans*-farnesylation and *S-all trans*-geranylgeranylation constitute high affinity elements of a substrate towards the enzyme.

Table 2. Summary of the CoMFA Results

PLS Statistics	
Cross-validated q^2	0.863
Standard error of prediction (Cross-validated)	0.365 (at comp 5)
% Variance	0.994 (at comp 5)
R^2	0.995
Standard error of estimate (Non-cross validated)	0.071
F values (n1=5, n2=6)	227
PLS components	5
Field contributions	
Steric	0.722
Electrostatic	0.278

The low turnover rates for the *S-trans,trans*-farnesylated and *S-all trans*-geranylgeranylated cysteine methyl ester substrates compared to the short S-alkyl substrates is not unexpected. As the modeling contour maps reveal, the strong binding site is beyond the reach of short S-alkyl chains compared to the *S-tran,trans*-farnesyl and *S-all trans*-geranylgeranyl moieties. The products from substrates bearing the latter two therefore interact more strongly with the enzyme resulting in slower dissociations than the short chain S-alkyls, thereby prolonging enzyme recovery times resulting in lower turnover rates. Bond saturation around C-18 as in compounds L-83 and L-86 compared to L-72 and L-81 compared to L-77 had opposing effects on the affinity towards the enzyme. This suggests that a trigonal planar structure may be preferred at this position. The tetrahedral structure arising from the saturation, though negatively impacting the affinity (L-81), resulted in a different spatial orientation of the additional isoprene in L-83 and L-86 as the modeling depicts. This might have resulted in affinity contacts for L-83 and L-86 with the enzyme molecule that more than com-

pensates for the diminished affinity displayed in L-81. This is corroborated by the aligned structures which reveal the profiles of the S-alkyl portions of L-83 and L-86 slightly turning at a different trajectory at the saturation point from those of L-72 and the other molecules. The alignment from the susceptible carbonyl carbon through to the first two carbon atoms of the S-alkyl substituents explains why the D-cysteine analogs were surprisingly hydrolyzed by the enzyme. It appears from these that the spatial orientation of the N-substituents is the most altered while the S-alkyl groups that are important for affinity and the ester group that is the target for hydrolysis are not affected by the switch from L- to D-cysteine. The much higher k_{cat}/K_M value for the S-benzyl substrate (L-93) is most likely a result of the inhibitory effect (on the K_M) that is more obvious at high concentrations. Although the lower concentrations were used in the Michaelis-Menten kinetics computations, the inhibitory effects may have been large enough to significantly suppress the K_M . The K_M as shown is at least 2 to 24-fold smaller than the K_M values for the comparably sized L-72, L-81 and L-77. A lesser pronounced inhibitory effect at concentrations over 200 μ M was also observed for HCFCM and BzGFCM. Unlike the other substrates, the latter two compounds and L-93 are characterized by a planar unsubstituted benzene ring in the molecule which may fit into a fissure-like allosteric inhibitory site on the enzyme molecule.

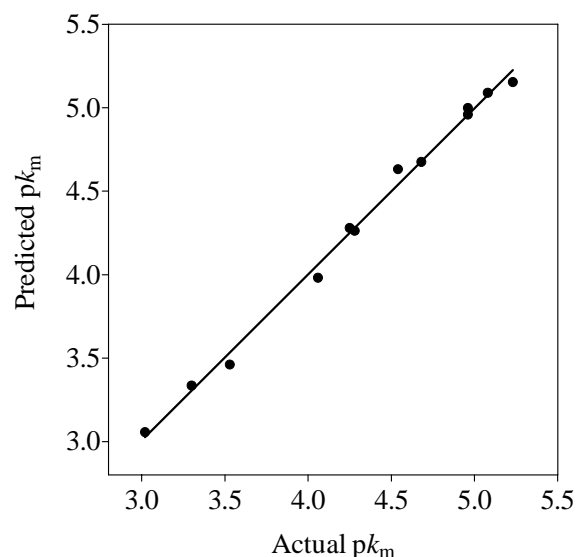


Fig. (6). Predicted (y-axis) versus experimental logs of the binding affinities (pK_M , X-axis). The experimental pK_M for one compound was removed from the model and the model was then used to obtain the predicted pK_M for that substrate. This process was repeated for the other substrates to obtain the respective pK_M values. The results of the predicted pK_M values were then plotted against the experimentally-derived binding affinities.

The wide distribution of PMPMEase in various organs [24] is in line with the highly ubiquitous nature of their putative polyisoprenylated proteins. Although these enzymes are yet to be characterized, various esterases with sequence identities to PMPMEase in excess of 70% have been reported from the intestines [29], human brain [30], human alveolar macrophages [31], human monocytes and macrophages [31, 32].

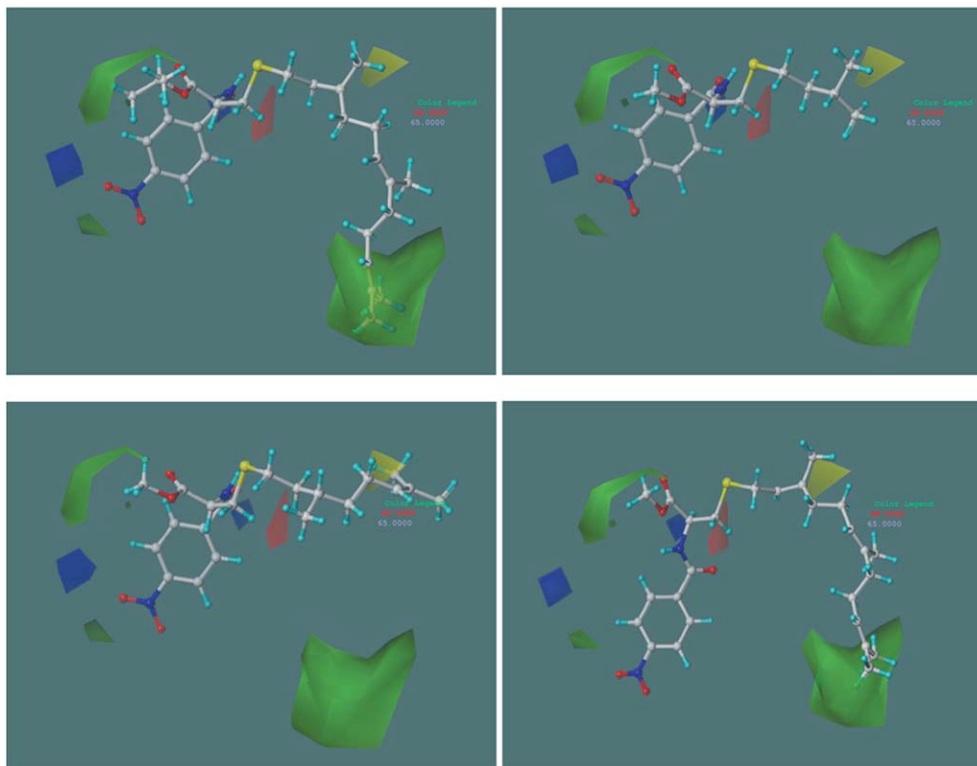


Fig. (7). The contour map with L-75 (top left panel), L-81 (top right panel), L-86 (bottom left panel) and L-70 (bottom right panel) embedded in it.

The resistance of the substrates to hydrolysis by the cholinesterase enzymes is further testimony that the endogenous polyisoprenylated methylated proteins are indeed most likely specifically metabolized by PMPMEase. An unidentified rod outer segment hydrolase which also shows a preference for polyisoprenylated substrates has been reported [12]. The reliance on polyisoprenylation for high affinity is in agreement with previous work in which no other esterase was identified following the enzyme purification with polyisoprenylated substrate-dependent assay screening of the collected fractions [14]. Polyisoprenyl transferases are highly specific towards the *trans,trans*-farnesyl and *all trans*-geranylgeranyl groups [33]. Likewise, the endoprotease and the methyl transferase are dependent on the polyisoprenylation for effective catalysis [26, 28, 34, 35]. The modifications probably serve a protein-protein interaction function [9] as well as recognition elements for the pathway enzymes.

Previous work in which *S*-adenosyl-L-methionine-dependent methylation precipitated a Parkinson's disease-like effect [36] that was abrogated by *trans,trans*-farnesyl-L-cysteine analogs [37, 38] indicates that aberrations in the pathway may contribute to more clinical conditions than has been reported. The current study validates the enzyme as the likely liver polyisoprenylated protein-metabolizing esterase and provides a platform for the synthesis of specific high affinity inhibitors to further explore the role of the enzyme in physiology and disease as well as lead compounds for pharmaceutical drug development.

CONCLUSION

With all other parameters constant and varying only the *S*-substituents, the Michaelis-Menten kinetics results demon-

strate that substrates with the *all trans*-geranylgeranyl and *trans,trans*-farnesyl have the highest affinities towards PMPMEase. These are the moieties used in the secondary modification of proteins in eukaryotic cells. Furthermore, PMPMEase has the endoplasmic reticulum-retention signal that would co-localize it with PPMTase. These are strong indications that polyisoprenylated proteins are the putative endogenous substrates of the enzyme. The results further demonstrate that inhibitors synthesized with *trans,trans*-farnesyl and *all trans*-geranylgeranyl groups are more likely to bind to PMPMEase with higher affinity than to such enzymes as the cholinesterases.

ABBREVIATIONS

AChE	=	Acetylcholinesterase
BChE	=	Butyrylcholinesterase
CoMFA	=	Comparative molecular field analysis
DCM	=	Dichloromethane
EPL	=	Esterase from porcine liver, also known as
PMPMEase	=	Polyisoprenylated methylated protein methyl esterase
HPLC	=	High performance liquid chromatography
icmt	=	Isoprenylated carboxymethyl transferase
k_{cat}	=	Steady state enzyme activity
kDa	=	Kilodalton

K_M	=	Michaelis-Menten constant
NMR	=	Nuclear magnetic resonance
pK_M	=	$-\log$ Michaelis-Menten constant (in molar concentration units)
PNB	=	p-nitrobenzoyl
PPMTase	=	Polysisoprenylated protein methyl transferase
QSAR	=	Quantitative structure-activity relationship
TLC	=	Thin layer chromatography

ACKNOWLEDGEMENTS

This work was supported by NIH/NIGMS/SCORE Grant number GM 08111-35 and by the Pharmaceutical Research Center NIH/NICRR Grant number G12 RR0 3020.

We thank Dr. Leonid Koikov for most of the synthesis, NMR and optical rotation analysis of the substrates.

REFERENCES

- Perez-Sala D. Protein isoprenylation in biology and disease: general overview and perspectives from studies with genetically engineered animals. *Front Biosci* 2007; 12: 4456-72.
- Roskoski R, Jr. Protein prenylation: a pivotal posttranslational process. *Biochem Biophys Res Commun* 2003; 303:1-7.
- Konstantinopoulos PA, Karamouzis MV, Papavassiliou AG. Post-translational modifications and regulation of the RAS superfamily of GTPases as anticancer targets. *Nat Rev Drug Discov* 2007; 6: 541-55.
- Ghobrial IM, Adjei AA. Inhibitors of the ras oncogene as therapeutic targets. *Hematol Oncol Clin North Am* 2002; 16: 1065-88.
- Tolmachova T, Anders R, Abrink M, *et al.* Independent degeneration of photoreceptors and retinal pigment epithelium in conditional knockout mouse models of choroideremia. *J Clin Invest* 2006; 116: 386-94.
- Seabra MC, Ho YK, Anant JS. Deficient geranylgeranylation of Ram/Rab27 in choroideremia. *J Biol Chem* 1995; 270: 24420-27.
- Bergo MO, Lieu HD, Gavino BJ, *et al.* On the physiological importance of endoproteolysis of CAAX proteins: heart-specific RCE1 knockout mice develop a lethal cardiomyopathy. *J Biol Chem* 2004; 279: 4729-36.
- Bergo MO, Leung GK, Ambroziak P, *et al.* Isoprenylcysteine carboxyl methyltransferase deficiency in mice. *J Biol Chem* 2001, 276: 5841-45.
- Gosser YQ, Nomanbhoj TK, Aghazadeh B, *et al.* C-terminal binding domain of Rho GDP-dissociation inhibitor directs N-terminal inhibitory peptide to GTPases. *Nature* 1997; 387: 814-19.
- Kloog Y, Cox AD. Prenyl-binding domains: potential targets for Ras inhibitors and anti-cancer drugs. *Semin Cancer Biol* 2004; 14: 253-61.
- Philips MR, Pillinger MH, Staud R, *et al.* Carboxyl methylation of Ras-related proteins during signal transduction in neutrophils. *Science* 1993; 259: 977-80.
- Tan EW, Rando RR. Identification of an isoprenylated cysteine methyl ester hydrolase activity in bovine rod outer segment membranes. *Biochemistry* 1992; 31: 5572-78.
- Perez-Sala D, Tan EW, Canada FJ, Rando RR. Methylation and demethylation reactions of guanine nucleotide-binding proteins of retinal rod outer segments. *Proc Natl Acad Sci USA* 1991; 88: 3043-46.
- Obboh OT, Lamango NS. Liver prenylated methylated protein methyl esterase is the same enzyme as sus scrofa carboxylesterase. *J Biochem Mol Toxicol* 2008; 22: 51-62.
- Bencharit S, Edwards CC, Morton CL, *et al.* Multisite promiscuity in the processing of endogenous substrates by human carboxylesterase 1. *J Mol Biol* 2006; 363: 201-14.
- Fleming CD, Edwards CC, Kirby SD, *et al.* Crystal structures of human carboxylesterase 1 in covalent complexes with the chemical warfare agents soman and tabun. *Biochemistry* 2007; 46: 5063-71.
- Bencharit S, Morton CL, Xue Y, Potter PM, Redinbo MR. Structural basis of heroin and cocaine metabolism by a promiscuous human drug-processing enzyme. *Nat Struct Biol* 2003; 10: 349-56.
- Bencharit S, Morton CL, Hyatt JL, *et al.* Crystal structure of human carboxylesterase 1 complexed with the Alzheimer's drug tacrine: from binding promiscuity to selective inhibition. *Chem Biol* 2003; 10: 341-49.
- Gosselin P, Maignan, C, Rouessac F. Stereospecific synthesis of homogeranic and homomeric acids. *Synthesis*, 1984; 1984: 876-81.
- Lange GL, Gottardo, C. Facile conversion of primary and secondary alcohols to alkyl iodides. *Synth Comm* 1990; 20: 1473-79.
- Mori K, Suguro, T, Masuda, S. Stereocontrolled synthesis of all of the possible stereoisomers of 3,11-dimethylnonacosan-2-one and 29-hydroxy-3,11-dimethylnonacosan-2-one: The female sex pheromone of the german cockroach. *Tetrahedron* 1981; 37: 1329-40.
- Gemperli M, Hofmann W, Rottenberg M. On the Mechanism of Trypsin-Catalyzed Ester-Hydrolysis: Model Experiments on Substrates. *Helv Chim Acta* 1965; 48: 939-45.
- Brown MJ, Milano PD, Lever DC, Epstein WW, Poulter CD. Prenylated Proteins. A convenient synthesis of farnesyl cysteinyl thioethers. *J Am Chem Soc* 1991; 113: 3176-77.
- Lamango NS. Liver prenylated methylated protein methyl esterase is an organophosphate-sensitive enzyme. *J Biochem Mol Toxicol* 2005; 19: 347-57.
- Tan EW, Perez-Sala D, Canada FJ, Rando RR. Identifying the recognition unit for G protein methylation. *J Biol Chem* 1991; 266: 10719-22.
- Perez-Sala D, Gilbert BA, Tan EW, Rando RR. Prenylated protein methyltransferases do not distinguish between farnesylated and geranylgeranylated substrates. *Biochem J* 1992; 284: 835-40.
- De Busser HM, Van Dessel GA, Lagrou AR. Identification of prenylcysteine carboxymethyltransferase in bovine adrenal chromaffin cells. *Int J Biochem Cell Biol* 2000; 32: 1007-16.
- Stephenson RC, Clarke S. Identification of a C-terminal protein carboxyl methyltransferase in rat liver membranes utilizing a synthetic farnesyl cysteine-containing peptide substrate. *J Biol Chem* 1990; 265: 16248-54.
- David L, Guo XJ, Villard C, Moulin A, Puigserver A. Purification and molecular cloning of porcine intestinal glycerol-ester hydrolase--evidence for its identity with carboxylesterase. *Eur J Biochem* 1998; 257: 142-48.
- Mori M, Hosokawa M, Ogasawara Y, Tsukada E, Chiba K. cDNA cloning, characterization and stable expression of novel human brain carboxylesterase. *FEBS Lett* 1999; 458: 17-22.
- Munger JS, Shi GP, Mark EA, Chin DT, Gerard C, Chapman HA. A serine esterase released by human alveolar macrophages is closely related to liver microsomal carboxylesterases. *J Biol Chem* 1991; 266: 18832-38.
- Ghosh S. Cholesteryl ester hydrolase in human monocyte/macrophage: cloning, sequencing, and expression of full-length cDNA. *Physiol Genomics* 2000; 2: 1-8.
- Yokoyama K, Goodwin GW, Ghomashchi F, Glomset JA, Gelb MH. A protein geranylgeranyltransferase from bovine brain: implications for protein prenylation specificity. *Proc Natl Acad Sci USA* 1991; 88: 5302-06.
- Ma YT, Chaudhuri A, Rando RR. Substrate specificity of the isoprenylated protein endoprotease. *Biochemistry* 1992; 31:11772-77.
- Jang GF, Yokoyama K, Gelb MH. A prenylated protein-specific endoprotease in rat liver microsomes that produces a carboxyl-terminal tripeptide. *Biochemistry* 1993; 32: 9500-07.

- [36] Lamango NS, Nesby RA, Charlton CG. Quantification of S-adenosylmethionine-induced tremors: a possible tremor model for Parkinson's disease. *Pharmacol Biochem Behav* 2000; 65: 523-29.
- [37] Lamango NS, Ayuk-Takem LT, Nesby R, Zhao WQ, Charlton CG. Inhibition mechanism of S-adenosylmethionine-induced movement deficits by prenylcysteine analogs. *Pharmacol Biochem Behav* 2003; 76: 433-42.
- [38] Lamango NS, Charlton CG. Farnesyl-L-cysteine analogs block SAM-induced Parkinson's disease-like symptoms in rats. *Pharmacol Biochem Behav* 2000; 66: 841-49.

Received: April 4, 2009

Revised: May 7, 2009

Accepted: May 7, 2009

© Nazarius S. Lamango; Licensee *Bentham Open*.

This is an open access article licensed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted, non-commercial use, distribution and reproduction in any medium, provided the work is properly cited.