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Design, synthesis, and evaluation of potent and selective benzoyleneurea-based inhibitors of protein geranylgeranyltransferase-I

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Abstract—A series of novel protein geranylgeranyltransferase-I (PGGTase-I) inhibitors based on a benzoyleneurea scaffold has been synthesized. Using a benzoyleneurea scaffold as a mimetic for the central dipeptide (AA), we have developed CAAX peptidomimetic inhibitors that selectively block the activity of PGGTase-I over the closely related enzyme protein farnesyltransferase. In this new class of PGGTase-I inhibitors, compound (**6c**) with X = L-phenylalanine displayed the highest inhibition activity against PGGTase-I with an IC_{50} value of 170 nM. The inhibitors described in this study represent novel and promising leads for the development of potent and selective inhibitors of mammalian PGGTase-I for potential application as antitumor agents.

1. Introduction

Inhibition of protein prenyltransferases has received considerable interest in recent years as a promising strategy for the development of chemotherapeutic agents for a variety of diseases. The prenyltransferase family of lipid-modifying enzymes consists of protein farnesyltransferase (PFTase), geranylgeranyltransferase type I (PGGTase-I), and geranylgeranyltransferase type II (PGGTase-II). To date, most work has focused on developing PFTase inhibitors as anticancer agents because malignant Ras activity was found to be dependent on post-translational modification by PFTase. However, recent findings linking protein prenylation of PGGTase-I substrates to tumorigenesis and metastasis have highlighted PGGTase-I as a new potential target in anticancer drug research.

The protein prenyltransferase enzymes catalyze the covalent transfer of a farnesyl group (15 carbon by PFTase) or a geranylgeranyl group (20 carbon by PGGTase-I and-II) to cysteine residues near the C-terminus of certain proteins.[1] Protein prenyltransferase activity plays an important role in mediating protein–membrane and protein–protein interactions that affect a variety of cellular processes associated with, for example, cell cycle progression, cytoskeleton remodeling, apoptosis, signal transduction, and intracellular trafficking pathways.[2–6]

The protein prenyltransferases are usually classified into two categories: (1) the CAAX prenyltransferases, which include PFTase and PGGTase-I and (2) PGGTase-II. The CAAX prenyltransferases modify eukaryotic protein substrates that contain a C-terminal CAAX sequence. In the CAAX motif, C represents a cysteine amino acid residue, A represents any aliphatic amino acid and X is the determining residue that specifies PFTase or PGGTase-I activity. PFTase modifies substrates that contain a CAAX motif where X is predominantly methionine and serine, but other amino acid residues such as alanine or glutamine are common as well.[1,7] Protein substrates terminating with CAAX sequences where X is leucine, isoleucine or phenylalanine are usually modified by PGGTase-I.[1,7] The non-CAAX prenyltransferase PGGTase-II catalyzes the

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modification of protein substrates terminating in a CC or a CXC motif.

Nearly 30% of all human cancers contain oncogenic forms of Ras, with higher prevalence in pancreatic (90%), colorectal (50%), and lung (40%). Since Ras malignant activity was found to be dependent on post-translational modification by PFTase, significant efforts have been directed toward the development of PFTase inhibitors for application as antitumor agents.[8–10] However, recent studies have indicated that PGGTase-I is a new potential target for the development of anticancer therapeutics. These studies have shown that several PGGTase-I substrates such as RhoC, RhoA, Rac-1, Cdc42, and R-Ras play a critical role in tumorigenesis and metastasis.[11–15] In addition, the most frequently mutated form of Ras in human cancers, Ki-Ras4B, was found to be geranylgeranylated when PFTase is inhibited.[16,17] Thus, inhibition of both PFTase and PGGTase-I is required to inhibit prenylation of Ki-Ras4B in vivo.[18,19] Finally, the development of highly potent and selective PGGTase-I inhibitors (GGTIs) is needed to determine the effect of PGGTase-I in normal and oncogenic cell growth. Based on these recent findings, we have directed our efforts toward the development of novel selective inhibitors of PGGTase-I for further development of useful chemotherapeutic agents with in vivo antitumor activity.

Similar to PFTase, PGGTase-I is a heterodimeric zinc metalloenzyme, consisting of a 48 kDa α-subunit and a 43 kDa β-subunit.[20,21] Both PFTase and PGGTase-I share an identical α-subunit and the β-subunit is known to contain approximately 35% of amino acid sequence identity.[22–24] The recently published X-ray crystal structure of protein PGGTase-I shows that the overall structural arrangement is mainly helical in nature.[25] The α-subunit of PGGTase-I is folded in a crescent-shaped domain composed of α-helices that are arranged in α-helical hairpins. The β-subunit also contains mostly helical domains, which form a compact α–α barrel structure with a central cavity. The substrate binding site opens into the extensive subunit interface and extends into the hydrophobic funnel-shaped cavity of the β-subunit. This funnel-shaped cavity hosts a single zinc ion, which marks the location of the active site of PGGTase-I. Since an X-ray crystal structure of PGGTase-I was not available until very recently, the work described herein was based on a rational approach in which design improvements were guided by: (1) comparison between the active sites of PFTase, (from published PFTase structural information), and PGGTase-I (from sequence alignment)[26] and (2) extensive structure activity relationship studies on previous PGGTase-I inhibitors.

Our design for peptidomimetic inhibitors of PGGTase-I was based on the C-terminal CAAX motif (X = L, F) sequence found in many geranylgeranylated proteins. The first generation of PGGTase-I inhibitors were obtained by replacing the central dipeptide (AA) portion with a rigid spacer, where X was chosen to be leucine. By employing a 2-aryl-4-aminobenzoic acid scaffold as a mimetic for the dipeptide (AA), we developed a series of PGGTase-I inhibitors from which the most potent compound was found to be GGTI-2154, exhibiting an IC_{50} for PGGTase-I of 21 nM and of 5,600 nM for PFTase (Fig. 1). In an effort to improve the potency, selectivity and in vivo antitumor activity of our PGGTase-I inhibitors, we sought new inhibitors based on novel scaffolds that could be readily constructed and modified to provide useful structure activity profiles.

A variety of potentially suitable scaffolds were considered in terms of: (a) synthetic versatility and (b) structural features common to our existing two most potent PGGTase-I inhibitors based on a 2-aryl-4-aminobenzoic acid (GGTI-2154)[27] and piperazine (GGTI-2418) scaffolds.[28] Simple energy-minimizing molecular modeling studies revealed that a benzoyleneurea unit as in compound **6g**, would be an attractive scaffold for the development of a new series of GGTIs (Fig. 2). This scaffold shared structural features common to both GGTI-2154 and GGTI-2418, and suggested that inhibitors designed around it might bind effectively to the active site of

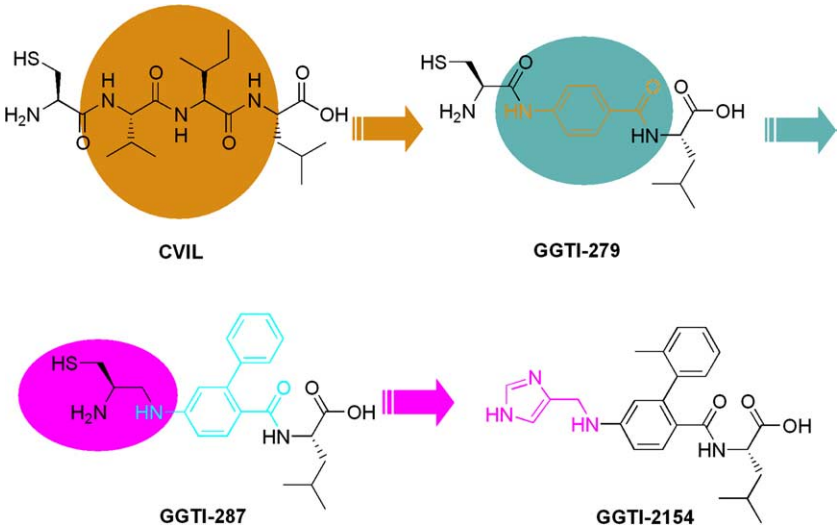


Figure 1. Evolution of CAAX peptidomimetic PGGTase-I inhibitor design.

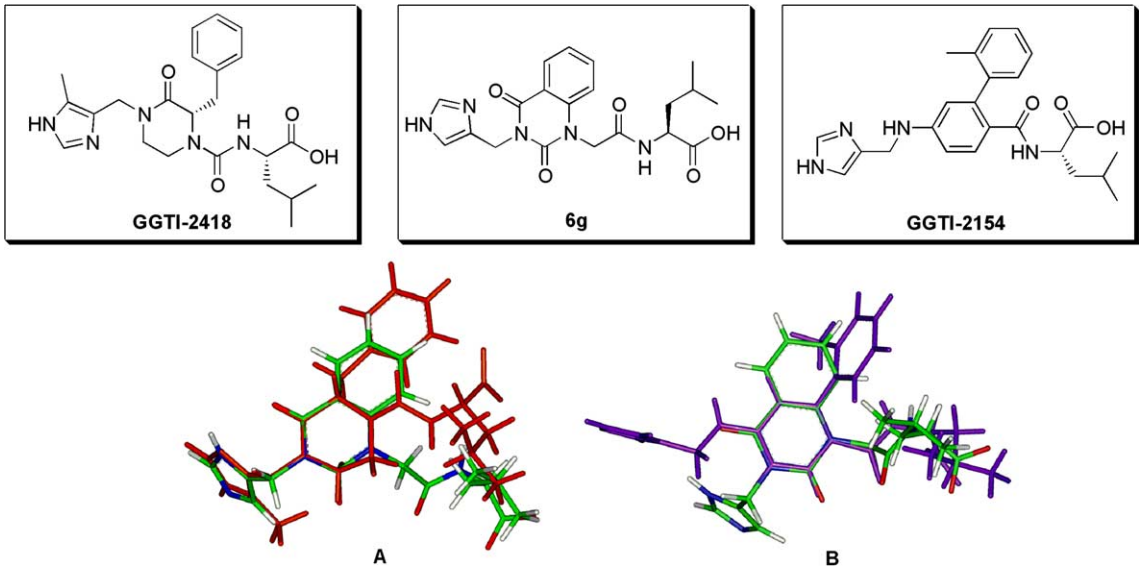


Figure 2. Comparison of proposed novel PGGTase-I inhibitor **6g** (atom color) with (a) GGTI-2418 (orange) and (b) GGTI-2154 (purple). Inhibitors were built and minimized in the Insight II (2000) suite of programs builder module.

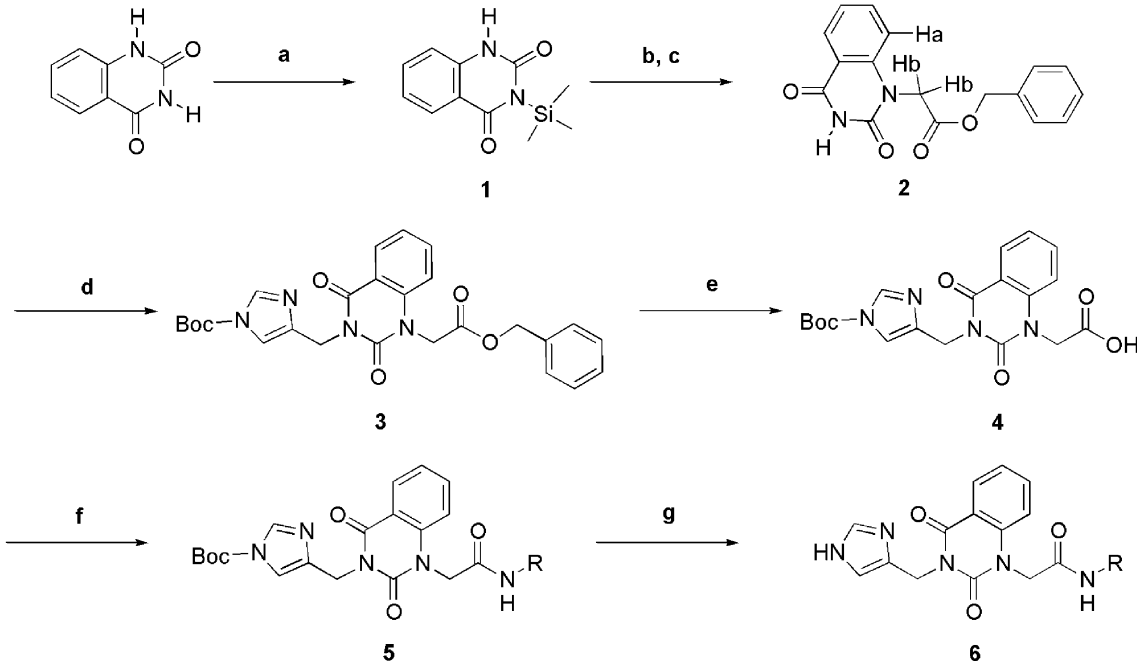
PGGTase-I. In addition, this scaffold has the advantage of being commercially available and ease of substitution would allow potential access to a wide range of novel GGTI leads.

2. Results and discussion

2.1. Chemistry

The general synthetic route utilized to prepare these compounds is outlined in Scheme 1. Silylation of commercially available benzoyleneurea with hexamethyldisilazane and ammonium sulfate gave the monosilylated product **1**, which was then alkylated with benzyl chloroacetate followed by desilylation with ethanol to afford intermediate **2**. The regiochemistry of alkylation in **2** was initially established as the N1-position by 2DNMR where an NOE signal was observed between protons H_a and H_b (Scheme 1) and later confirmed by X-ray crystallography (Fig. 3).

Treatment of compound **2** with 1-*t*-butoxycarbonyl-4-chloromethylimidazole and potassium carbonate gave the dialkylated compound **3**. Benzyl group removal was accomplished by hydrogenation with 10%



Scheme 1. Reagents and conditions: (a) $(CH_3)_3SiNHSi(CH_3)_3$, $(NH_4)_2SO_4$, toluene; (b) benzyl chloroacetate, KI, toluene; (c) EtOH, 90% for three steps; (d) 1-*t*-butoxycarbonyl-4-chloromethylimidazole, K_2CO_3, acetone, 89%; (e) H_2, Pd/C, MeOH, CH_2Cl_2, 91%; (f) H_2NR, HOBT, EDCI, Et_3N, DMF, 25–100%; (g) TFA, 82–100% or NaOH, 81–100%.

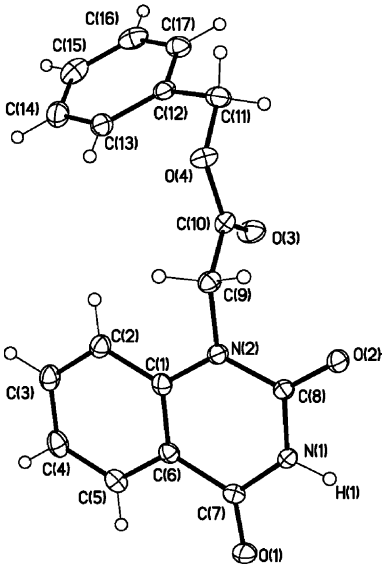


Figure 3. X-ray crystal structure of compound **2**.

palladium on activated charcoal. The resulting carboxylic acid derivative **4** was then coupled with a variety of amino acid methyl esters or amines using EDCI/HOBT coupling conditions to give the respective amides **5**. Standard Boc deprotection and, if necessary, saponification conditions using TFA and NaOH, respectively, were then employed to give the final deprotected compounds **6** (Fig. 4).

2.2. Biological activity

The enzyme inhibition activity displayed by the inhibitors shown in Figure 4 is reported in Table 1 as IC_{50} values (the concentration at which PFTase or PGGTase-I activity was inhibited by 50%). The ability of the benzoyleneurea GGTI compounds to inhibit PFTase and PGGTase-I in vitro was investigated by using partially purified PFTase and PGGTase-I from human Burkitt Lymphoma (Daudi) cells as described earlier.[29–31] Inhibition activity was determined by the ability of the compounds to inhibit the transfer of [^{3}H]farnesyl and [^{3}H]geranylgeranyl from [^{3}H]farnesyl PPi and [^{3}H]geranylgeranyl PPi to H-Ras-CVLS and H-Ras-CVLL, respectively.

A comparison between the active sites of PFTase and PGGTase-I suggested that the central dipeptide played

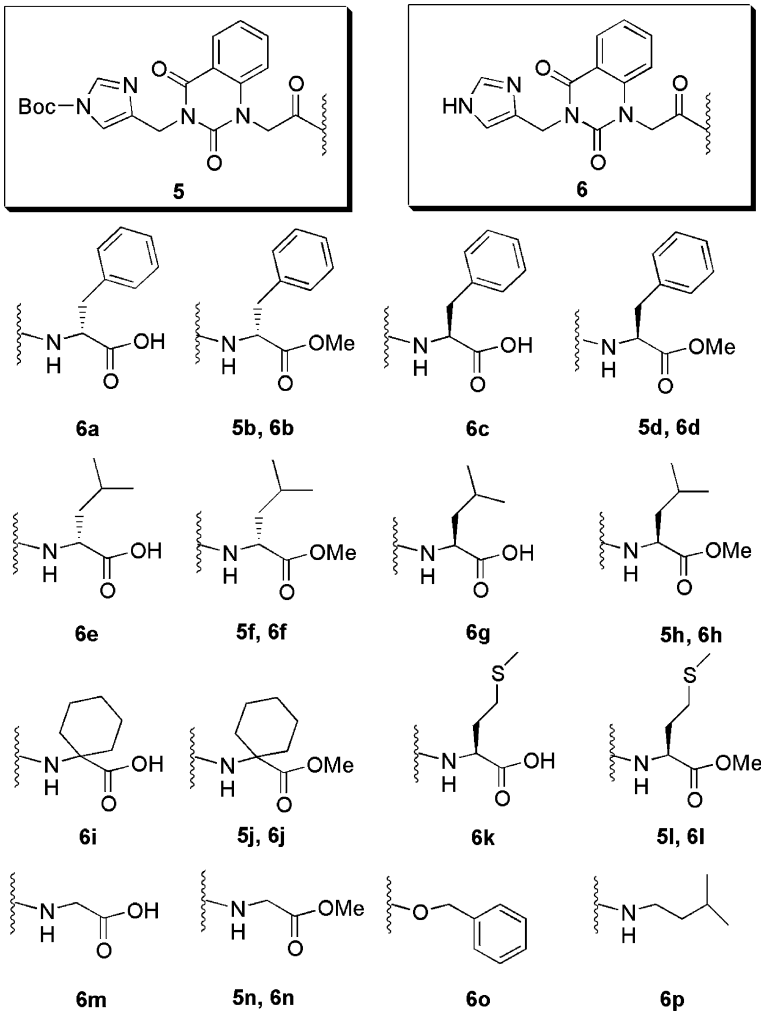


Figure 4. Benzoyleneurea-based PGGTase-I inhibitors.

Table 1. PFTase and PGGTase-I activity for compounds **6a–p**

Inhibitor	PGGTase-I IC_{50} (nM)	PFTase IC_{50} (nM)	PFTase/ PGGTase-I
6a	6300	>10,000	>1.5
6b	>10,000	>10,000	
6c	170	>10,000	>58
6d	4500	>10,000	>2
6e	2700	>10,000	>3.5
6f	>10,000	>10,000	
6g	580	>10,000	>17
6h	>10,000	>10,000	
6i	6425	>10,000	>1.5
6j	>10,000	>10,000	
6k	3350	>10,000	>2.5
6l	>10,000	>10,000	
6m	>10,000	>10,000	
6n	>10,000	>10,000	
6o	>10,000	>10,000	
6p	>10,000	>10,000	

a simple hydrophobic role in binding to PGGTase-I, and could thus be substituted by other scaffolds that would allow for better occupation of the PGGTase-I active site and consequently lead to an improvement in binding affinity. Previous studies have indicated that the cysteine sulfur of CAAL substrates coordinates to the zinc ion of PGGTase-I and constitutes an important step in PGGTase-I catalytic activity.[32] Replacement of the cysteine thiol by an imidazole metal-coordinating group has been previously explored and shown to increase the metabolic stability and also the selectivity for PGGTase-I.[33] Based on the CAAL tetrapeptide motif, we replaced the central dipeptide AA portion by a benzoyleneurea scaffold and substituted the cysteine residue with an imidazole and the X residue with several groups.

Though these compounds were designed before a crystal structure of PGGTase-I was available, the obtained structure-activity relationships can be rationalized by docking studies based on the recently published X-ray crystal structure of mammalian protein PGGTase-I (PDB code: 1N4Q).[25] Docking studies were performed with the genetic algorithm included in Gold v. 2.1 with 10 independent runs per ligand.[34] Crystallographic water molecules were removed and the GGPP analog used in the crystal structure was retained for the molecular modeling studies. The parent compound of this series, **6g**, was docked into mammalian PGGTase-I, and the single low energy enzyme-bound conformation is shown in Figure 5.

Docking studies show that compound **6g** binds to PGGTase-I in the CVIL binding site, suggesting that its activity may arise from a peptide-competitive mechanism. As with CVIL, compound **6g** adopts an extended conformation, with the imidazole nitrogen coordinating to the catalytic zinc ion. The benzoyleneurea scaffold makes extensive hydrophobic contacts with the hydrophobic pocket formed by the geranylgeranylpyrophosphate analog. The C-terminus carboxylate group of **6g** interacts strongly with Arg 173β and the leucine side chain fits tightly to the hydrophobic cleft formed by the GGPP analog, Phe 53β and Leu 320β.

In this series, compounds **3** (Scheme 1) and **5** (Fig. 4), containing a Boc-substituted imidazole, showed no activity against PGGTase-I. Interestingly, previous studies with our PFTase inhibitors, have shown that substitution on the imidazole ring leads to an increase in activity and selectivity against PFTase. The lack of activity exhibited by **3** and **5** was presumed to be due to an unfavorable conformational change or steric interaction between the inhibitor and the enzyme imposed by

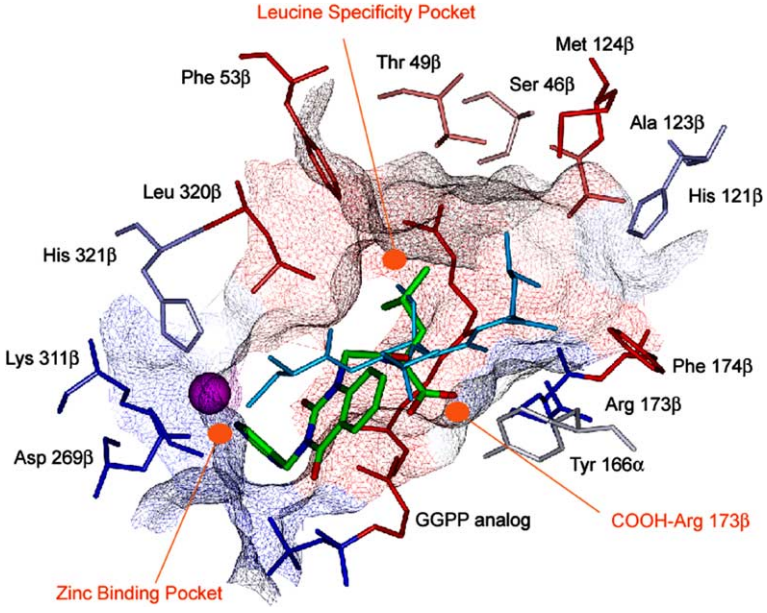


Figure 5. Comparison of the GOLD-predicted docked conformation of **6g** (atom color) in the active site of PGGTase-I (pdb 1N4Q, colored by hydrophobicity, red hydrophobic to blue hydrophilic) with the enzyme bound conformation of the native substrate peptide CVIL (cyan blue).

the presence of the *t*-butyl group on the imidazole. This possible explanation was further supported by X-ray crystal structures, which show that the zinc ion binding pocket in PGGTase-I is more congested than in PFTase, suggesting a strict size limitation for the presence of any substitution on either imidazole nitrogen. Thus, increased size of the imidazole substitution cannot be tolerated by the tight PGGTase-I zinc binding pocket, which likely leads to the observed loss in activity. Furthermore, the electron-withdrawing Boc-group may decrease the basicity of the imidazole and weaken its coordination to zinc in PGGTase-I, which may also contribute to a decrease in binding of the inhibitor to the enzyme.

In order to probe the binding environment of the specificity pocket of PGGTase-I, compounds containing a variety of side chains at the X-position of the CAAX motif were prepared and evaluated. The glycine-containing compounds, **6m** and **6n** showed no activity against PGGTase-I, which suggested that hydrophobic interactions between the side chain group and the enzyme are important for activity. This observation was further confirmed by the high activity exhibited by L-leucine derivative **6g** and L-phenylalanine derivative **6c**, with IC_{50} values of 580 and 170 nM, respectively. These results suggest that a favorable interaction is gained through the hydrophobic side-chain of the phenylalanine and leucine residues. The potency of **6g** and **6c** is also consistent with the known preference of PGGTase-I for proteins that contain leucine or phenylalanine on their X terminal residue. Docking studies with **6g** show that the hydrophobic leucine side chain can indeed occupy the hydrophobic specificity pocket of PGGTase-I. The docking studies with **6g** also point to a strict size limitation for substitution on the C-terminal position and suggest that only small aliphatic groups will be tolerated in the PGGTase specificity pocket.

In order to determine if the L-configuration is required to observe PGGTase-I inhibition in this series, we prepared inhibitors containing both D-phenylalanine (**6a**, **6b**), and D-leucine (**6e**, **6f**) residues as well as an achiral cyclohexyl side chain (**6i**, **6j**). Compounds **6b** and **6f** showed no inhibition, and **6a** and **6e** showed negligible activity against PGGTase-I. Cyclohexyl achiral peptidomimetics **6i** and **6j** also showed no or poor activity against the enzyme. The importance of the L-configuration of the side chain for PGGTase-I inhibition in this series is most probably due to the strict size limitation of the specificity pocket in the PGGTase-I active site as shown in Figure 5. A change in configuration of the side chain is not well tolerated by the tight specificity pocket of PGGTase-I, forcing the inhibitor to bind in an alternative unfavorable orientation leading to the observed loss in activity.

Finally, peptidomimetics **6o** and **6p** lacking a free carboxyl group did not show any activity against PGGTase-I, which suggested the presence of a critical interaction between the carboxyl group and a positively charged residue in the PGGTase-I active site. This was further supported by the fact that only the free acid compounds **6c** and **6g** showed potent activities against the enzyme and that most of the methyl ester compounds, with the exception of **6d**, showed no inhibition against PGGTase-I. These results are clearly explained by docking studies, which show that compounds lacking the carboxylate can no longer form a hydrogen bond with Arg 173β.

2.3. Conclusions

In conclusion, a series of novel benzoyleneurea-based inhibitors for PGGTase-I were successfully synthesized. These derivatives exploit a benzoyleneurea scaffold as a replacement for the AA portion of the CAAX tetrapeptide, and led to a highly potent and selective inhibitor series for protein PGGTase-I. Only compounds containing a free carboxyl group exhibited potent activity against PGGTase-I and phenylalanine seems to be the preferred amino acid residue to bind in the hydrophobic X specificity pocket of the enzyme. In addition, the L-configuration of the amino acid side chain is of great importance for PGGTase-I inhibition activity, as significant loss of activity was observed for compounds containing D-configured residues.

3. Experimental

3.1. General methods

Melting points were determined with an electrothermal capillary melting point apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on a Bruker AM-500 and 400 spectrometer. Chemical shifts were reported in δ (ppm) relative to tetramethylsilane. All coupling constants were described in Hz. Flash column chromatography was performed on silica gel (40–63 μm) under a pressure of about 4 psi. Solvents were obtained from commercial suppliers and purified as follows: tetrahydrofuran and ether were distilled from sodium benzophenone ketyl; dichloromethane was distilled over calcium hydride. Synthesized final compounds were checked for purity by analytical HPLC, which was performed using a Rainin HP controller and a Rainin UV-C detector with a Rainin 250 mm × 4.6 mm, 5 μm Microsorb C-18 column, eluted with gradient 10–90% of CH_3CN in 0.1% TFA in H_2O in 30 min. High-resolution mass spectra (HRMS) and low-resolution mass spectra (LRMS) were performed on a Varian MAT-CH-5 (HRMS) or VG 707 (LRMS) mass spectrometer.

3.1.1. 3-Trimethylsilanyl-1H-quinazoline-2,4-dione (1). A suspension of 1H-quinazoline-2,4-dione (1.62 g, 10 mmol), 1,1,1,3,3,3-hexamethyldisilazane (5.05 mL, 24 mmol), ammonium sulfate (0.20 g, 1.5 mmol) in anhydrous toluene was refluxed under N_2 overnight. The reaction mixture was allowed to reach room temperature and the solvent was removed under reduced pressure to afford a light yellow residue that was used for the next reaction without further purifications.

3.1.2. (2,4-Dioxo-3,4-dihydro-2H-quinazolin-1-yl)-acetic acid benzyl ester (2). A mixture of **1**, potassium iodide (1.66 g, 10 mmol), and benzyl chloroacetate (6 mL, 39.5 mmol) was stirred at 85 °C under N_2 overnight. To the reaction mixture, ethanol (20 mL) was added and the reaction was refluxed for 2 h. The reaction mixture was allowed to reach room temperature and the solvent was removed under reduced pressure. The resulting residue was rinsed first with acetone (2 × 25 mL), H_2O (2 × 20 mL), and with additional acetone (2 × 20 mL). The solid was dried in a vacuum oven overnight to afford the product as a white powder (2.81 g, 90%). Mp 236–238 °C; ^{1}H NMR (DMSO-d_6) δ 4.99 (s, 2H), 5.19 (s, 2H), 7.29–7.38 (m, 7H), 7.71 (t, J = 7.0 Hz, 1H), 8.02 (dd, J = 1.5 and 7.8 Hz, 1H), 11.78 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 168.56, 162.04, 150.67, 141.22, 135.92, 135.75, 128.80, 128.55, 128.27, 127.99, 123.34, 115.77, 114.97, 66.89, 44.12; LRMS (FAB) m/z calcd for $C_{17}H_{14}N_2O_4H^+$ 311, found 311; HRMS (FAB) m/z calcd for $C_{17}H_{14}N_2O_4H^+$ 311.1032, found 311.1033.

3.1.3. 4-(1-Benzyloxycarbonylmethyl-2,4-dioxo-1,4-dihydro-2H-quinazolin-3-ylmethyl)-imidazole-1-carboxylic acid *tert*-butyl ester (3). A solution of **2** (116 mg, 0.37 mmol), 4-chloromethyl-imidazole-1-carboxylic acid *tert*-butyl ester[35] (105 mg, 0.48 mmol) and K_2CO_3 (62 mg, 0.45 mmol) in dry acetone (2 mL) was heated at 60 °C under N_2 overnight. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The resulting white residue was diluted with EtOAc (50 mL) and washed with H_2O (2 × 25 mL). Combined organic phases were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Flash chromatography (2:1:0.01 $CHCl_3$/ethyl acetate/NH_4OH eluent) furnished dialkylated product **3** (161 mg, 89%) as a clear, colorless oil. HPLC R_t 19.21; ^{1}H NMR ($CDCl_3$) δ 8.26 (dd, J = 1.5, 7.9 Hz, 1H), 7.97 (d, J = 1.2 Hz, 1H), 7.58 (ddd, J = 1.6, 7.4, 8.7 Hz, 1H), 7.24–7.36 (m, 7H), 6.89 (d, J = 8.4 Hz, 1H), 5.25 (s, 2H), 5.21 (s, 2H), 4.97 (s, 2H), 1.58 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 168.05, 161.67, 151.25, 147.28, 140.01, 139.14, 137.20, 135.63, 135.32, 129.79, 129.01, 128.95, 128.69, 123.73, 116.01, 115.74, 113.42, 85.91, 67.93, 45.30, 39.36, 28.24; LRMS (FAB) m/z calcd for $C_{26}H_{26}N_4O_6H^+$ 491, found 491; HRMS (FAB) m/z calcd for $C_{26}H_{26}N_4O_6H^+$ 491.1931, found 491.1932.

3.1.4. 4-(1-Carboxymethyl-2,4-dioxo-1,4-dihydro-2H-quinazolin-3-ylmethyl)-imidazole-1-carboxylic acid *tert*-butyl ester (4). A flask containing **3** (2.07 g, 4.21 mmol) in MeOH (15 mL) and CH_2Cl_2 (10 mL) was evacuated 3 times and filled with N_2. To the flask 10% Pd–C (0.18 g) was added and the reaction mixture was hydrogenated at atmospheric pressure for 2.5 h. The catalyst was filtered and the filtrate concentrated under reduced pressure to afford the carboxylic acid **4** (1.54 g, 91%) as a clear, colorless oil. ^{1}H NMR (DMSO-d_6) δ 8.08–8.11 (m, 2H), 7.58 (t, J = 7.0 Hz, 1H), 7.30–7.43 (m, 3H), 5.06 (s, 2H), 4.89 (s, 2H), 1.55 (s, 9H); ^{13}C NMR (DMSO-d_6) δ 169.78, 161.07, 150.61, 146.91, 140.23, 139.14, 137.02, 135.88, 128.41, 123.48, 114.99, 114.92, 114.63, 85.75, 45.15, 38.95, 27.72; LRMS (FAB) m/z calcd for $C_{19}H_{20}N_4O_6H^+$ 401, found 401; HRMS (FAB) m/z calcd for $C_{19}H_{20}N_4O_6H^+$ 401.1461, found 401.1460.

3.1.5. 4-{1-[(1-Methoxycarbonyl-3-methyl-butylcarbamoyl)-methyl]-2,4-dioxo-1,4-dihydro-2H-quinazolin-3-ylmethyl}-imidazole-1-carboxylic acid *tert*-butyl ester (5h). A stirred solution containing acid **4** (68 mg, 0.17 mmol), L-leucine methyl ester hydrochloride (34 mg, 0.19 mmol), HOBt (25 mg, 0.19 mmol), Et_3N (26 μL, 0.19 mmol) in anhydrous DMF (4 mL) was cooled to −10 °C. To the cooled reaction mixture was added EDCI (33 mg, 0.17 mmol) and the reaction was stirred at room temperature overnight. The solvent was removed under reduced pressure and the white residue was diluted with EtOAc (60 mL) and washed with saturated K_2CO_3 (10 mL). The aqueous phase was washed with EtOAc (10 mL) and the organic phases were combined. The organic phase was extracted with saturated K_2CO_3 (10 mL) and saturated NaCl (2 × 10 mL). Combined organic phases were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Flash chromatography (2:1:0.01 $CHCl_3$/ethyl acetate/Et_3N eluent) furnished L-leucine derivative product **5h** (38 mg, 42%) as a clear, colorless oil. ^{1}H NMR (CD_3OD) δ 8.06 (dd, J = 1.3, 7.9 Hz, 1H), 7.98 (d, J = 1.0 Hz, 1H), 7.63 (t, J = 7.1 Hz, 1H), 7.34 (s, 1H), 7.21 (t, J = 7.5 Hz, 1H), 7.09 (d, J = 8.5 Hz, 1H), 5.10 (s, 2H), 4.88 (d, J = 17.3 Hz, 1H), 4.77 (d, J = 17.1 Hz, 1H), 4.38–4.42 (m, 1H), 3.58 (s, 3H), 1.48–1.57 (m, 12H), 0.79–0.86 (m, 6H); ^{13}C NMR (CD_3OD) δ 174.70, 170.33, 163.54, 152.88, 148.46, 141.98, 140.23, 138.49, 136.93, 129.98, 124.83, 117.24, 116.99, 115.73, 87.65, 53.14, 52.67, 47.53, 41.54, 40.12, 28.37, 26.35, 23.75, 22.05; LRMS (ESI) m/z calcd for $C_{26}H_{33}N_5O_7H^+$ 528, found 528; HRMS (ESI) m/z calcd for $C_{26}H_{33}N_5O_7H^+$ 528.2458, found 528.2463.

3.1.6. 2-{2-[3-(1H-Imidazol-4-ylmethyl)-2,4-dioxo-3,4-dihydro-2H-quinazolin-1-yl]-acetylamino}-4-methyl-pentanoic acid (6g). Compound **5h** (8.2 mg, 0.015 mmol) was dissolved in MeOH (1.0 mL) and 1 N NaOH (0.1 mL) was added. The reaction mixture was stirred at room temperature for 1 h and 1 N HCl (0.15 mL) was then added to the solution. The solvent was removed under reduced pressure and the resulting white residue was further purified by gel permeation on Sephadex LH-20 with MeOH to afford to the final compound (6.5 mg, 96%) as a white foam. HPLC R_t 10.79; ^{1}H NMR (CD_3OD) δ 8.72 (s, 1H), 8.10 (dd, J = 1.2, 7.8 Hz, 1H), 7.64 (t, J = 7.2 Hz, 1H), 7.45 (s, 1H), 7.24 (t, J = 7.5 Hz, 1H), 7.15 (d, J = 8.2 Hz, 1H), 5.24 (s, 2H), 4.96 (d, J = 17.2 Hz, 1H), 4.81 (d, J = 17.5 Hz, 1H), 4.37 (t, J = 7.4 Hz, 1H), 1.54–1.67 (m, 3H), 0.86 (dd, J = 6.1, 22.5 Hz, 6H); ^{13}C NMR (CD_3OD) δ 174.76, 170.37, 163.57, 153.05, 141.90, 137.01, 134.93, 132.84, 129.95, 124.88, 117.07, 115.89, 115.74, 52.80, 47.46, 41.52, 26.38, 23.73, 22.12; LRMS (FAB) m/z calcd for $C_{20}H_{23}N_5O_5H^+$ 414, found 414; HRMS (FAB) m/z calcd for $C_{20}H_{23}N_5O_5H^+$ 414.1777, found 414.1778.

3.1.7. 2-{2-[3-(1H-Imidazol-4-ylmethyl)-2,4-dioxo-3,4-dihydro-2H-quinazolin-1-yl] acetylamino}-4-methyl-pentanoic acid methyl ester (6h). A solution of **5h** (18 mg, 0.034 mmol) in CH_2Cl_2 (1 mL) was cooled to 0 °C and TFA was added (0.17 mL). The reaction mixture was allowed to reach room temperature and was stirred for an additional 35 min. The mixture was concentrated and the residue was rinsed with Et_2O (2 × 5 mL) to give **6h** as a light yellow beige foamy residue (19 mg, 100%). HPLC R_t 11.75; ^{1}H NMR (CD_3OD) δ 8.70 (s, 1H), 8.09 (dd, J = 1.4, 7.9 Hz, 1H), 7.65 (t, J = 7.1 Hz, 1H), 7.47 (s, 1H), 7.25 (t, J = 7.3 Hz, 1H), 7.12 (d, J = 8.4 Hz, 1H), 5.24 (s, 2H), 4.94 (d, J = 17.3 Hz, 1H), 4.80 (d, J = 17.0 Hz, 1H), 4.39 (t, J = 6.7 Hz, 1H), 3.60 (s, 3H), 1.52–1.60 (m, 3H), 0.84 (dd, J = 6.2, 21.3 Hz, 6H); ^{13}C NMR (CD_3OD) δ 173.08, 168.68, 161.81, 151.27, 140.21, 135.20, 134.93, 132.84, 128.29, 123.13, 118.80, 115.53, 114.01, 51.57, 51.11, 45.86, 39.95, 37.55, 24.74, 22.13, 20.52; LRMS (FAB) *m/z* calcd for $C_{21}H_{25}N_5O_5H^+$ 428, found 428; HRMS (FAB) *m/z* calcd for $C_{21}H_{25}N_5O_5H^+$ 428.1934, found 428.1932.

3.1.8. 4-{1-[(1-Methoxycarbonyl-2-phenyl-ethylcarbamoyl)-methyl]-2,4-dioxo-1,4-dihydro-2H-quinazolin-3-ylmethyl}-imidazole-1-carboxylic acid *tert*-butyl ester (5b). This compound was prepared in a similar manner to that described for compound **5h** with **4** and D-phenylalanine methyl ester hydrochloride to afford a white oily residue (84%). HPLC R_t 16.54; ^{1}H NMR (CD_3OD) δ 8.09 (dd, J = 1.5, 7.9 Hz, 1H), 8.01 (d, J = 1.3 Hz, 1H), 7.57 (t, J = 7.4 Hz, 1H), 7.43 (d, J = 1.0 Hz, 1H), 7.12–7.27 (m, 6H), 6.89 (d, J = 8.5 Hz, 1H), 5.15 (s, 2H), 4.86 (d, J = 17.2 Hz, 1H), 4.73–4.77 (m, 2H), 3.68 (s, 3H), 3.19 (dd, J = 5.2, 13.9 Hz, 1H), 2.96 (dd, J = 9.3, 13.8 Hz, 1H), 1.57 (s, 9H); ^{13}C NMR (CD_3OD) δ 173.51, 169.76, 163.32, 152.71, 148.45, 141.78, 140.29, 138.54, 138.50, 136.99, 130.74, 130.01, 129.94, 128.37, 124.75, 117.09, 117.04, 115.65, 87.62, 55.61, 53.39, 47.49, 40.25, 38.77, 28.52; LRMS (ESI) *m/z* calcd for $C_{29}H_{31}N_5O_7H^+$ 562, found 562; HRMS (ESI) *m/z* calcd for $C_{29}H_{31}N_5O_7H^+$ 562.2302, found 562.2292.

3.1.9. 2-{2-[3-(1H-Imidazol-4-ylmethyl)-2,4-dioxo-3,4-dihydro-2H-quinazolin-1-yl]-acetylamino}-3-phenyl-propionic acid (6a). This compound was prepared in a similar manner to that described for compound **6g** with **5b** to afford the final compound as a clear colorless oil (100%). HPLC R_t 11.25; ^{1}H NMR (CD_3OD) δ 8.81 (s, 1H), 8.15 (d, J = 7.5 Hz, 1H), 7.64 (t, J = 7.9 Hz, 1H), 7.17–7.62 (m, 7H), 6.91 (d, J = 8.4 Hz, 1H), 5.31 (br s, 2H), 4.70–5.22 (m, 3H), 3.29 (dd, J = 4.5, 13.9 Hz, 1H), 3.00 (dd, J = 9.7, 13.8 Hz, 1H); ^{13}C NMR (CD_3OD) δ 173.05, 168.14, 161.66, 151.10, 140.07, 137.16, 135.69, 133.81, 129.32, 129.06, 128.31, 128.24, 126.64, 123.31, 118.41, 115.19, 114.10, 53.93, 45.55, 37.08, 34.88; LRMS (ESI) *m/z* calcd for $C_{23}H_{21}N_5O_5H^+$ 448, found 448; HRMS (ESI) *m/z* calcd for $C_{23}H_{21}N_5O_5H^+$ 448.1621, found 448.1621.

3.1.10. 2-{2-[3-(1H-Imidazol-4-ylmethyl)-2,4-dioxo-3,4-dihydro-2H-quinazolin-1-yl]-acetylamino}-3-phenyl-propionic acid methyl ester (6b). This compound was prepared in a similar manner to that described for compound **6h** with **5b** to afford the final compound as a clear colorless oil (96%). HPLC R_t 11.10; ^{1}H NMR (CD_3OD) δ 8.69 (d, J = 0.9 Hz, 1H), 8.04 (dd, J = 1.3, 7.9 Hz, 1H), 7.54 (t, J = 7.2 Hz, 1H), 7.44 (s, 1H), 7.05–7.24 (m, 6H), 6.79 (d, J = 8.4 Hz, 1H), 5.20 (s, 2H), 4.71–4.90 (m, 2H), 4.62–4.66 (m, 1H), 3.61 (s, 3H), 3.11 (dd, J = 5.11, 14.0 Hz, 1H), 2.87 (dd, J = 9.6, 13.9 Hz, 1H); ^{13}C NMR (CD_3OD) δ 173.50, 169.88, 163.33, 152.76, 141.74, 138.51, 137.34, 135.41, 131.01, 130.68, 130.03, 129.96, 128.42, 125.00, 120.05, 116.88, 115.68, 55.69, 53.31, 47.14, 38.69, 36.46; LRMS (ESI) *m/z* calcd for $C_{24}H_{23}N_5O_5H^+$ 462, found 462; HRMS (ESI) *m/z* calcd for $C_{24}H_{23}N_5O_5H^+$ 462.1777, found 462.1776.

3.1.11. {1-[(1-Methoxycarbonyl-2-phenyl-ethylcarbamoyl)-methyl]-2,4-dioxo-1,4-dihydro-2H-quinazolin-3-yl} methyl-imidazole-1-carboxylic acid *tert*-butyl ester (5d). This compound was prepared in a similar manner to that described for compound **5h** with **4** and L-phenylalanine methyl ester hydrochloride to afford a white oily residue (100%). HPLC R_t 17.63; $[\alpha]_D^{25} + 0.7$ (*c* 0.01, MeOH); ^{1}H NMR (CD_3OD) δ 8.19 (dd, J = 1.5, 7.8 Hz, 1H), 8.11 (d, J = 1.3 Hz, 1H), 7.68 (t, J = 7.3 Hz, 1H), 7.47 (d, J = 1.2 Hz, 1H), 7.18–7.36 (m, 6H), 6.97 (d, J = 8.6 Hz, 1H), 5.22 (s, 2H), 4.93 (d, J = 17.2 Hz, 1H), 4.85 (d, J = 17.0 Hz, 1H), 4.74–4.80 (m, 1H), 3.73 (s, 3H), 3.23 (dd, J = 5.4, 14.1 Hz, 1H), 3.00 (dd, J = 9.3, 14.0 Hz, 1H), 1.63 (s, 9H); ^{13}C NMR (CD_3OD) δ 173.51, 169.86, 163.41, 152.77, 148.44, 141.78, 140.22, 138.54, 138.50, 137.02, 130.70, 129.99, 129.92, 128.35, 124.76, 117.15, 117.05, 115.63, 87.65, 55.62, 53.31, 47.41, 40.14, 38.66, 28.43; LRMS (FAB) *m/z* calcd for $C_{29}H_{31}N_5O_7H^+$ 562, found 562; HRMS (FAB) *m/z* calcd for $C_{29}H_{31}N_5O_7H^+$ 562.2302, found 562.2302.

3.1.12. 2-{2-[3-(1H-Imidazol-4-ylmethyl)-2,4-dioxo-3,4-dihydro-2H-quinazolin-1-yl]-acetylamino}-3-phenyl-propionic acid (6c). This compound was prepared in a similar manner to that described for compound **6g** with **5d** to afford the final compound as a clear colorless oil (87%). HPLC R_t 11.80; $[\alpha]_D^{25} + 14.7$ (*c* 0.01, MeOH); ^{1}H NMR (CD_3OD) δ 8.64 (br s, 1H), 8.06 (s, 1H), 7.53 (t, J = 7.1 Hz, 1H), 7.08–7.26 (m, 7H), 6.81 (d, J = 7.6 Hz, 1H), 5.21 (br s, 2H), 4.59–4.91 (m, 3H), 3.17 (t, J = 10.9 Hz, 1H), 2.89 (t, J = 10.9 Hz, 1H); ^{13}C NMR (CD_3OD) δ 175.92, 169.77, 163.33, 152.78, 141.77, 138.93, 137.34, 130.74, 129.94, 128.27, 124.97, 116.91, 115.77, 60.17, 47.25, 38.78, 36.59; LRMS (FAB) *m/z* calcd for $C_{23}H_{21}N_5O_5H^+$ 448, found 448; HRMS (FAB) *m/z* calcd for $C_{23}H_{21}N_5O_5H^+$ 448.1621, found 448.1622.

3.1.13. 2-{2-[3-(1H-Imidazol-4-ylmethyl)-2,4-dioxo-3,4-dihydro-2H-quinazolin-1-yl]-acetylamino}-3-phenyl-propionic acid methyl ester (6d). This compound was prepared in a similar manner to that described for compound **6h** with **5d** to afford the final compound as a clear colorless oil (82%). HPLC R_t 13.17; $[\alpha]_D^{25} + 0.9$ (*c* 0.012, MeOH); ^{1}H NMR (CD_3OD) δ 8.68 (d, J = 1.3 Hz, 1H), 8.05 (dd, J = 1.5, 7.9 Hz, 1H), 7.54 (t, J = 7.4 Hz, 1H), 7.44 (s, 1H), 7.08–7.21 (m, 6H), 6.80 (d, J = 8.4 Hz, 1H), 5.20 (s, 2H), 4.82 (d, J = 17.3 Hz, 1H), 4.69 (d,

J = 17.3 Hz, 1H), 4.62–4.67 (m, 1H), 3.60 (s, 3H), 3.12 (dd, J = 5.2, 13.9 Hz, 1H), 2.87 (dd, J = 9.6, 13.9 Hz, 1H); ^{13}C NMR (CD_3OD) δ 173.49, 169.85, 163.34, 152.77, 141.75, 138.48, 137.33, 135.38, 131.04, 130.65, 130.02, 129.96, 128.40, 124.99, 120.04, 116.90, 115.67, 55.67, 53.28, 47.14, 38.69, 36.45; LRMS (FAB) *m/z* calcd for $C_{24}H_{23}N_5O_5H^+$ 462, found 462; HRMS (FAB) *m/z* calcd for $C_{24}H_{23}N_5O_5H^+$ 462.1777, found 462.1776.

3.1.14. 4-{1-[(1-Methoxycarbonyl-3-methyl-butylcarbamoyl)-methyl]-2,4-dioxo-1,4-dihydro-2H-quinazolin-3-ylmethyl}-imidazole-1-carboxylic acid *tert*-butyl ester (5f). This compound was prepared in a similar manner to that described for compound **5h** with **4** and D-leucine methyl ester hydrochloride to afford a white oily residue (84%). HPLC R_t 16.29; ^{1}H NMR (CD_3OD) δ 8.01 (dd, J = 1.5, 7.9 Hz, 1H), 7.96 (d, J = 1.3 Hz, 1H), 7.58 (t, J = 7.3 Hz, 1H), 7.32 (d, J = 1.2 Hz, 1H), 7.17 (t, J = 7.3 Hz, 1H), 7.07 (d, J = 8.4 Hz, 1H), 5.07 (s, 2H), 4.86 (d, J = 17.3 Hz, 1H), 4.74 (d, J = 17.2 Hz, 1H), 4.37–4.41 (m, 1H), 3.57 (s, 3H), 1.45–1.55 (m, 12H), 0.80 (dd, J = 6.2, 14.1 Hz, 6H); ^{13}C NMR (CD_3OD) δ 174.70, 170.26, 163.48, 152.86, 148.48, 141.94, 140.27, 138.51, 136.93, 129.99, 124.83, 117.21, 117.05, 115.74, 87.67, 53.21, 52.72, 47.58, 41.62, 40.18, 28.46, 26.39, 23.81, 22.18; LRMS (ESI) *m/z* calcd for $C_{26}H_{33}N_5O_7H^+$ 528, found 528; HRMS (ESI) *m/z* calcd for $C_{26}H_{33}N_5O_7H^+$ 528.2458, found 528.2448.

3.1.15. 2-{2-[3-(1H-Imidazol-4-ylmethyl)-2,4-dioxo-3,4-dihydro-2H-quinazolin-1-yl]-acetylamino}-4-methyl-pentanoic acid (6e). This compound was prepared in a similar manner to that described for compound **6g** with **5f** to afford the final compound as a clear colorless oil (100%). HPLC R_t 10.46; ^{1}H NMR (CD_3OD) δ 8.70 (s, 1H), 8.07 (d, J = 7.7 Hz, 1H), 7.64 (t, J = 8.0 Hz, 1H), 7.46 (s, 1H), 7.23 (t, J = 7.5 Hz, 1H), 7.14 (d, J = 8.4 Hz, 1H), 5.23 (s, 2H), 4.96 (d, J = 17.3 Hz, 1H), 4.81 (d, J = 17.3 Hz, 1H), 4.36 (t, J = 6.2 Hz, 1H), 1.56–1.68 (m, 3H), 0.84 (dd, J = 6.1, 22.9 Hz, 6H); ^{13}C NMR (CD_3OD) δ 176.08, 170.23, 163.42, 152.85, 141.89, 137.28, 135.48, 131.11, 129.97, 125.05, 120.02, 116.97, 115.94, 52.73, 47.32, 41.83, 36.78, 26.49, 23.85, 22.08; LRMS (ESI) *m/z* calcd for C_{20} $H_{23}N_5O_5H^+$ 414, found 414; HRMS (ESI) *m/z* calcd for $C_{20}H_{23}N_5O_5H^+$ 414.1777, found 414.1778.

3.1.16. 2-{2-[3-(1H-Imidazol-4-ylmethyl)-2,4-dioxo-3,4-dihydro-2H-quinazolin-1-yl]-acetylamino}-4-methyl-pentanoic acid methyl ester (6f). This compound was prepared in a similar manner to that described for compound **6h** with **5f** to afford the final compound as a clear colorless oil (98%). HPLC R_t 11.90; ^{1}H NMR (CD_3OD) δ 8.63 (s, 1H), 8.07 (dd, J = 1.4, 7.8 Hz, 1H), 7.64 (t, J = 7.1 Hz, 1H), 7.44 (s, 1H), 7.23 (t, J = 7.5 Hz, 1H), 7.11 (d, J = 8.5 Hz, 1H), 5.22 (s, 2H), 4.93 (d, J = 17.3 Hz, 1H), 4.79 (d, J = 17.3 Hz, 1H), 4.36–4.40 (m, 1H), 3.59 (s, 3H), 1.51–1.62 (m, 3H), 0.83 (dd, J = 6.2, 26.1 Hz, 6H); ^{13}C NMR (CD_3OD) δ 174.68, 170.24, 163.42, 152.85, 141.89, 137.24, 135.47, 131.29, 130.02, 125.06, 120.07, 117.01, 115.80, 53.18, 52.81, 47.26, 41.67, 36.65, 26.43, 23.68, 22.11; LRMS (ESI) *m/z* calcd for $C_{21}H_{25}N_5O_5H^+$ 428, found 428; HRMS (ESI) *m/z* calcd for $C_{21}H_{25}N_5O_5H^+$ 428.1934, found 428.1935.

3.1.17. 4-{1-[(1-Methoxycarbonyl-cyclohexylcarbamoyl)-methyl]-2,4-dioxo-1,4-dihydro-2H-quinazolin-3-ylmethyl}-imidazole-1-carboxylic acid *tert*-butyl ester (5j). Preparation of 1-amino-cyclohexanecarboxylic acid methyl ester: A suspension of 1-amino-cyclohexanecarboxylic acid (2.0 g, 14 mmol) in anhydrous MeOH (10 mL) was cooled to −10 °C. To the suspension was added cold $SOCl_2$ (2.81 mL, 35.9 mmol) dropwise. The reaction was allowed to reach room temperature and was refluxed for 4 h. The solvent was removed under reduced pressure and the residue was diluted with AcOEt (50 mL) and extracted with $NaHCO_3$ solution (3 × 25 mL). The combined organic layers were washed with saturated NaCl solution (25 mL) and dried with Na_2SO_4. The dried organic layer was filtered and concentrated to afford 1-amino-cyclohexanecarboxylic acid methyl ester as a light yellow oil, that was used in the synthesis of **5j** without further purification (460 mg, 21%). ^{1}H NMR (CD_3OD) δ 3.61 (s, 3H), 1.87 (t, J = 8.9 Hz, 2H), 1.51–1.58 (m, 2H), 1.34–1.36 (m, 6H); ^{13}C NMR (CD_3OD) δ 178.58, 58.84, 52.99, 36.78, 26.85, 23.55.

Compound **5j** was prepared in a similar manner to that described for compound **5h** with **4** and 1-amino-cyclohexanecarboxylic acid methyl ester to afford the desired final compound as a white clear oil (100%). HPLC R_t 17.14; ^{1}H NMR (CD_3OD) δ 8.01 (dd, J = 1.3, 7.9 Hz, 1H), 7.97 (s, 1H), 7.59 (t, J = 7.0 Hz, 1H), 7.31 (s, 1H), 7.16 (t, J = 7.4 Hz, 1H), 7.06 (d, J = 8.4 Hz, 1H), 5.06 (s, 2H), 4.81 (s, 2H), 3.47 (s, 3H), 1.90–1.98 (m, 2H), 1.64–1.73 (m, 2H), 1.44–1.48 (m, 15H); ^{13}C NMR (CD_3OD) δ 176.44, 169.62, 163.47, 152.86, 148.48, 142.01, 140.33, 138.47, 136.87, 129.96, 124.77, 117.13, 117.06, 115.78, 87.65, 61.05, 53.11, 47.22, 40.19, 33.76, 28.40, 26.75, 22.90; LRMS (FAB) *m/z* calcd for $C_{27}H_{33}N_5O_7H^+$ 540, found 540; HRMS (FAB) *m/z* calcd for $C_{27}H_{33}N_5O_7H^+$ 540.2458, found 540.2460.

3.1.18. 1-{2-[3-(1H-Imidazol-4-ylmethyl)-2,4-dioxo-3,4-dihydro-2H-quinazolin-1-yl]-acetylamino}-cyclohexanecarboxylic acid (6i). This compound was prepared in a similar manner to that described for compound **6g** with **5j** to afford the final compound as a clear colorless oil (81%). HPLC R_t 10.87; ^{1}H NMR (CD_3OD) δ 8.77 (s, 1H), 8.12 (d, J = 6.9 Hz, 1H), 7.70 (t, J = 6.8 Hz, 1H), 7.53 (s, 1H), 7.16–7.37 (m, 2H), 5.29 (s, 2H), 4.97 (s, 2H), 1.96–2.21 (m, 2H), 1.72–1.91 (m, 2H), 1.22–1.71 (m, 6H); ^{13}C NMR (CD_3OD) δ 177.78, 169.70, 163.35, 152.80, 141.84, 137.22, 135.57, 131.04, 129.83, 124.98, 120.01, 116.88, 116.13, 61.04, 47.19, 36.57, 33.62, 26.79, 22.94; LRMS (FAB) *m/z* calcd for $C_{21}H_{23}N_5O_5H^+$ 426, found 426; HRMS (FAB) *m/z* calcd for $C_{21}H_{23}N_5O_5H^+$ 426.1777, found 426.1778.

3.1.19. 1-{2-[3-(1H-Imidazol-4-ylmethyl)-2,4-dioxo-3,4-dihydro-2H-quinazolin-1-yl]-acetylamino}-cyclohexanecarboxylic acid methyl ester (6j). This compound was prepared in a similar manner to that described for compound **6h** with **5j** to afford the final compound as a clear colorless oil (96%). HPLC R_t 12.34; ^{1}H NMR (CD_3OD) δ 8.69 (d, J = 1.4 Hz, 1H), 8.08 (dd, J = 1.5, 7.9 Hz, 1H), 7.67 (t, J = 7.3 Hz, 1H), 7.46 (d,

J = 1.2 Hz, 1H), 7.23 (t, J = 7.3 Hz, 1H), 7.12 (d, J = 8.4 Hz, 1H), 5.23 (s, 2H), 4.88 (s, 2H), 3.47 (s, 3H), 1.89–2.00 (m, 2H), 1.71–1.79 (m, 2H), 1.17–1.61 (m, 6H); ^{13}C NMR (CD_3OD) δ 176.38, 169.76, 163.39, 152.84, 141.89, 137.22, 135.39, 131.11, 129.99, 125.05, 119.99, 116.97, 115.83, 61.14, 53.08, 46.98, 36.49, 33.74, 26.71, 22.89; LRMS (FAB) *m/z* calcd for $C_{22}H_{25}N_5O_5H^+$ 440, found 440; HRMS (FAB) *m/z* calcd for $C_{22}H_{25}N_5O_5H^+$ 440.1934, found 440.1936.

3.1.20. 4-{1-[(1-Methoxycarbonyl-3-methylsulfanyl-propyl-carbamoyl)-methyl]-2,4-dioxo-1,4-dihydro-2H-quinazolin-3-ylmethyl}-imidazole-1-carboxylic acid *tert*-butyl ester (5l). This compound was prepared in a similar manner to that described for compound **5h** with **4** and L-methionine methyl ester hydrochloride to afford the desired final compound as a white clear oil (38%). ^{1}H NMR (CD_3OD) δ 8.04 (dd, J = 1.6, 7.9 Hz, 1H), 7.98 (d, J = 1.4 Hz, 1H), 7.61 (t, J = 7.0 Hz, 1H), 7.33 (d, J = 1.2 Hz, 1H), 7.19 (t, J = 7.3 Hz, 1H), 7.11 (d, J = 8.5 Hz, 1H), 5.08 (s, 2H), 4.86 (d, J = 17.3 Hz, 1H), 4.77 (d, J = 17.2 Hz, 1H), 4.50–4.54 (m, 1H), 3.59 (s, 3H), 2.31–2.48 (m, 2H), 1.97–2.06 (m, 1H), 1.93 (s, 3H), 1.82–1.92 (m, 1H), 1.49 (s, 9H); ^{13}C NMR (CD_3OD) δ 173.90, 170.34, 163.50, 152.87, 148.48, 141.97, 140.24, 138.51, 136.97, 129.99, 124.84, 117.23, 116.96, 115.77, 87.67, 54.47, 53.29, 47.70, 40.15, 32.11, 31.53, 28.42, 15.67; LRMS (FAB) *m/z* calcd for $C_{25}H_{31}N_5O_7SH^+$ 546, found 546; HRMS (FAB) *m/z* calcd for $C_{25}H_{31}N_5O_7SH^+$ 546.2022, found 546.2020.

3.1.21. 2-{2-[3-(1H-Imidazol-4-ylmethyl)-2,4-dioxo-3,4-dihydro-2H-quinazolin-1-yl]-acetylamino}-4-methylsulfanyl-butyric acid (6k). This compound was prepared in a similar manner to that described for compound **6g** with **5l** to afford the final compound as a clear colorless oil (97%). HPLC R_t 10.49; ^{1}H NMR (CD_3OD) δ 8.73 (d, J = 1.5 Hz, 1H), 8.08 (dd, J = 1.5, 7.9 Hz, 1H), 7.64 (t, J = 7.0 Hz, 1H), 7.48 (d, J = 1.2 Hz, 1H), 7.24 (t, J = 7.2 Hz, 1H), 7.18 (d, J = 8.4 Hz, 1H), 5.24 (s, 2H), 4.97 (d, J = 17.3 Hz, 1H), 4.83 (d, J = 17.3 Hz, 1H), 4.49–4.52 (m, 1H), 2.39–2.54 (m, 2H), 2.03–2.11 (m, 1H), 1.98 (s, 3H), 1.86–1.96 (m, 1H); LRMS (FAB) *m/z* calcd for $C_{19}H_{21}N_5O_5SH^+$ 432, found 432; HRMS (FAB) *m/z* calcd for $C_{19}H_{21}N_5O_5SH^+$ 432.1342, found 432.1344.

3.1.22. 2-{2-[3-(1H-Imidazol-4-ylmethyl)-2,4-dioxo-3,4-dihydro-2H-quinazolin-1-yl]-acetylamino}-4-methylsulfanyl-butyric acid methyl ester (6l). This compound was prepared in a similar manner to that described for compound **6h** with **5l** to afford the final compound as a clear colorless oil (82%). HPLC R_t 10.72; ^{1}H NMR (CD_3OD) δ 8.70 (d, J = 1.4 Hz, 1H), 8.09 (dd, J = 1.5, 7.9 Hz, 1H), 7.66 (t, J = 7.0 Hz, 1H), 7.47 (d, J = 1.1 Hz, 1H), 7.24 (t, J = 7.2 Hz, 1H), 7.15 (d, J = 8.5 Hz, 1H), 5.24 (s, 2H), 4.94 (d, J = 17.3 Hz, 1H), 4.81 (d, J = 17.3 Hz, 1H), 4.52–4.55 (m, 1H), 3.62 (s, 3H), 2.37–2.57 (m, 2H), 2.02–2.09 (m, 1H), 1.97 (s, 3H), 1.85–1.93 (m, 1H); ^{13}C NMR (CD_3OD) δ 175.64, 172.09, 165.15, 154.54, 143.63, 138.99, 131.76, 126.80, 122.32, 118.77, 117.57, 55.03, 54.92, 49.07, 33.77, 33.20, 32.09, 17.27; LRMS (FAB) *m/z* calcd for $C_{20}H_{23}N_5O_5SH^+$ 446, found 446; HRMS (FAB) *m/z* calcd for $C_{20}H_{23}N_5O_5SH^+$ 446.1498, found 446.1497.

3.1.23. 4-{1-[(Methoxycarbonylmethyl-carbamoyl)-methyl]-2,4-dioxo-1,4-dihydro-2H-quinazolin-3-ylmethyl}-imidazole-1-carboxylic acid *tert*-butyl ester (5n). This compound was prepared in a similar manner to that described for compound **5h** with **4** and glycine methyl ester hydrochloride to afford the desired final compound as a white clear oil (100%). HPLC R_t 12.31; ^{1}H NMR (CD_3OD) δ 8.01 (dd, J = 1.5, 7.9 Hz, 1H), 7.96 (d, J = 1.4 Hz, 1H), 7.59 (t, J = 7.3 Hz, 1H), 7.32 (d, J = 1.2 Hz, 1H), 7.16 (t, J = 7.3 Hz, 1H), 7.12 (d, J = 8.5 Hz, 1H), 5.05 (s, 2H), 4.80 (s, 2H), 3.85 (s, 2H), 3.57 (s, 3H), 1.47 (s, 9H); ^{13}C NMR (CD_3OD) δ 169.41, 168.35, 161.08, 150.45, 146.02, 139.47, 137.75, 136.07, 134.56, 130.38, 127.47, 122.42, 114.77, 113.47, 85.21, 50.61, 45.29, 39.83, 37.69, 25.92; LRMS (FAB) *m/z* calcd for $C_{22}H_{25}N_5O_7H^+$ 472, found 472; HRMS (FAB) *m/z* calcd for $C_{22}H_{25}N_5O_7H^+$ 472.1832, found 472.1834.

3.1.24. {2-[3-(1H-Imidazol-4-ylmethyl)-2,4-dioxo-3,4-dihydro-2H-quinazolin-1-yl]-acetylamino}-acetic acid (6m). This compound was prepared in a similar manner to that described for compound **6g** with **5n** to afford the final compound as a clear colorless oil (100%). ^{1}H NMR (CD_3OD) δ 8.44 (s, 1H), 8.01 (d, J = 6.3 Hz, 1H), 7.65 (t, J = 7.6 Hz, 1H), 7.37 (s, 1H), 7.18–7.27 (m, 2H), 5.22 (s, 2H), 4.88 (s, 2H), 3.84 (s, 2H); ^{13}C NMR (CD_3OD) δ 172.66, 170.61, 163.45, 152.96, 141.93, 137.35, 137.19, 130.28, 129.95, 125.08, 117.02, 116.06, 47.42, 42.29, 31.19; LRMS (FAB) *m/z* calcd for $C_{16}H_{15}N_5O_5H^+$ 358, found 358; HRMS (FAB) *m/z* calcd for $C_{16}H_{15}N_5O_5H^+$ 358.1151, found 358.1151.

3.1.25. {2-[3-(1H-Imidazol-4-ylmethyl)-2,4-dioxo-3,4-dihydro-2H-quinazolin-1-yl]-acetylamino}-acetic acid methyl ester (6n). This compound was prepared in a similar manner to that described for compound **6h** with **5n** to afford the final compound as a clear colorless oil (89%). HPLC R_t 8.00; ^{1}H NMR (CD_3OD) δ 8.69 (s, 1H), 8.07 (dd, J = 1.3, 7.8 Hz, 1H), 7.65 (t, J = 7.2 Hz, 1H), 7.47 (s, 1H), 7.23 (t, J = 7.5 Hz, 1H), 7.17 (d, J = 8.5 Hz, 1H), 5.23 (s, 2H), 4.87 (s, 2H), 3.89 (s, 2H), 3.60 (s, 3H); ^{13}C NMR (CD_3OD) δ 171.89, 170.70, 163.42, 152.85, 141.87, 137.33, 135.42, 131.08, 129.99, 125.09, 120.10, 117.00, 115.99, 53.09, 47.45, 42.31, 36.49; LRMS (FAB) *m/z* calcd for $C_{17}H_{17}N_5O_5H^+$ 372, found 372; HRMS (FAB) *m/z* calcd for $C_{17}H_{17}N_5O_5H^+$ 372.1308, found 372.1308.

3.1.26. [3-(1H-Imidazol-4-ylmethyl)-2,4-dioxo-3,4-dihydro-2H-quinazolin-1-yl]-acetic acid benzyl ester (6o). This compound was prepared in a similar manner to that described for compound **6h** with **3** to afford the final compound as a clear colorless oil (90%). HPLC R_t 12.97; ^{1}H NMR ($CDCl_3$) δ 8.70 (d, J = 1.3 Hz, 1H), 8.12 (dd, J = 1.5, 7.9 Hz, 1H), 7.63 (t, J = 7.3 Hz, 1H), 7.44 (s, 1H), 7.21–7.28 (m, 6H), 7.17 (d, J = 8.5 Hz, 1H), 5.23 (s, 2H), 5.13 (s, 2H), 4.98 (s, 2H); ^{13}C NMR ($CDCl_3$)

δ 170.05, 163.25, 152.65, 141.67, 137.42, 137.23, 135.49, 131.08, 130.14, 130.02, 129.93, 129.71, 125.17, 120.18, 116.84, 115.71, 68.94, 46.41, 36.39; LRMS (FAB) *m/z* calcd for $C_{21}H_{18}N_4O_4H^+$ 391, found 391; HRMS (FAB) *m/z* calcd for $C_{21}H_{18}N_4O_4H^+$ 391.1406, found 391.1406.

3.1.27. 2-[3-(1H-Imidazol-4-ylmethyl)-2,4-dioxo-3,4-dihydro-2H-quinazolin-1-yl]-N-(3-methyl-butyl)-acetamide (6p). This compound was prepared in a similar manner to that described for compound **5h** with **4** and isoamylamine to afford the desired final compound as a white clear oil (25%). HPLC R_t 12.39; ^{1}H NMR (CD_3OD) δ 8.05 (dd, J = 1.4, 7.9 Hz, 1H), 7.59 (t, J = 7.0 Hz, 1H), 7.49 (s, 1H), 7.18 (t, J = 7.7 Hz, 1H), 7.05 (d, J = 8.5 Hz, 1H), 6.95 (s, 1H), 5.12 (s, 2H), 4.74 (s, 2H), 3.13 (t, J = 7.1 Hz, 2H), 1.44–1.51 (m, 1H), 1.24–1.34 (m, 2H), 0.79 (d, J = 6.7 Hz, 6H); ^{13}C NMR (CD_3OD) δ 169.85, 163.56, 152.97, 141.98, 136.90, 136.45, 134.36, 129.99, 124.80, 120.16, 117.30, 115.51, 47.82, 39.66, 39.24, 39.03, 27.20, 23.18; LRMS (FAB) *m/z* calcd for $C_{19}H_{23}N_5O_3H^+$ 370, found 370; HRMS (FAB) *m/z* calcd for $C_{19}H_{23}N_5O_3H^+$ 370.1879, found 370.1880.

3.2. In vitro mammalian PFTase and PGGTase-I activity assay

In vitro inhibition assays of mammalian PFTase and PGGTase-I were determined by measuring the incorporation of ^{3}H-FPP (Amersham Biosciences, Piscataway, NJ) and ^{3}H-GGPP (Perkin Elmer Life and Analytical Sciences, Boston, MA) into wild type H-Ras (PFTase) and H-Ras-CVLL (PGGTase-I), respectively, as previously described.[36] Briefly, 75 μg of 60,000 × *g* post-microsomal supernatant from Daudi cells was incubated in the presence of increasing concentration of compound, 10 μM H-Ras or H-Ras-CVLL substrate, and 0.5 μCi/sample of either ^{3}H-FPP or ^{3}H-GGPP. Samples were TCA-precipitated and then filtered onto glass fiber filters; unbound ^{3}H-FPP or ^{3}H-GGPP was washed through the filters. Samples were counted on a scintillation counter and activity compared to vehicle controls to obtain IC_{50} values.

3.3. Docking studies

Docking studies were done using Gold v. 2.1 on a Dell i686 running RedHat Linux 7.2. The protein and ligands were used as MOL2 files. Gold program set the atom types for both the protein and the ligands. The active site was defined with a radius of 10 Å from the zinc ion and Gold Program detected the active-site pocket with a radius of 10 Å. Ten docked conformations were found for each inhibitor. The Gold Scoring fitness function was used with all default parameters. Genetic algorithm and population parameters were used with all default parameters: population size = 100, selection pressure = 1.10, islands = 5, maximum operations = 100,000, niche size = 2, crossover weight = 95, mutate weight = 95, and migrate weight = 10. The default annealing parameters van der Waals = 2.5 and hydrogen bonding = 4.0 were used.

4. Supplementary data

Crystallographic data (excluding structure factors) for structure **2** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 250739. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK, (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

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