Synthesis and evaluation of potent, highly-selective, 3-aryl-piperazinone inhibitors of protein geranylgeranyltransferase-I†

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A series of compounds based on the carboxyl-terminal CAAL sequence of PGGTase-I substrates was designed and synthesized. Using piperazin-2-one as a semi-rigid scaffold, we have introduced critical pharmacophores in a well-defined arrangement to mimic the CAAL sequence. High potency and exceptional selectivity were obtained for inhibition of PGGTase-I with structures such as **45** and **70**. Potency of this series of GGTIs was dependent on the presence of an L-leucine residue with a free carboxyl terminus, as well as an *S* configuration of the 3-aryl group. The selectivity was significantly enhanced by 5-methyl substitution on the imidazole ring and fluorine substitution on the 3-aryl group. Modification of the 6-position of the piperazinone scaffold was found to be unfavorable. Compounds **44** and **69**, the corresponding methyl esters of **45** and **70**, were found to selectively block processing of Rap1A by PGGTase-I in whole cells with IC_{50} values of 0.4 μM and 0.7 μM respectively.

Introduction

Protein prenylation is an important lipid posttranslational modification that affects about 0.5% of cellular proteins.[1] Prenylated proteins are covalently modified with either farnesyl or geranylgeranyl isoprenoid *via* thioether bonds to the C-terminal cysteine residues. Many prenylated proteins belong to the low molecular weight GTPase family, such as the Ras oncoproteins, and depend on prenylation for their proper cellular localization and biological function.

Protein geranylgeranyltransferase-I (PGGTase-I), a member of the prenyltransferase family, is responsible for the attachment of a C_{20} geranylgeranyl group to CAAX proteins, where X is usually leucine or phenylalanine.[2] PGGTase-I is a heterodimer zinc metalloenzyme, consisting of a 48 kDa α-subunit and a 43 kDa β-subunit. The X-ray crystal structure of mammalian PGGTase-I reveals that the overall secondary structure of the enzyme is mainly helical in nature and similar to protein farnesyltransferase (PFTase).[3] The α-subunit is arranged in α-helical hairpin pairs, and forms a crescent around the β-subunit. The β-subunit also contains mostly helical domains, which form a compact α–α barrel structure with a central cavity. The substrate binding pocket opens into the subunit interface and extends into the β-subunit hydrophobic funnel-shaped cavity. The catalytic zinc ion is located in the β-subunit funnel-shaped cavity.

Over the past decade, the major effort in designing prenyltransferase inhibitors focused on PFTase, with the goal of specifically blocking malignant transformation caused by mutated Ras and other farnesylated proteins. A particular emphasis was placed on developing highly selective PFTase inhibitors (FTIs). The approach has been very successful even though the antitumor activity of FTIs likely results from blocking farnesylation of one or more target proteins other than Ras.[4–6] Some FTIs have demonstrated significant antitumor activity with little toxicity in animal models, and several compounds are currently in phase II/III clinical trials.[6]

Recently, PGGTase-I has gained increased attention because many of its protein substrates, such as RhoC, RhoA, Rac-1, Cdc42, R-Ras and TC-21, have been found to be implicated in promoting tumorigenesis and/or metastasis.[7–11] In addition, K-Ras, the most commonly mutated form of Ras in human cancers, becomes geranylgeranylated when PFTase is inhibited.[5] Further reasons for targeting PGGTase-I in the development of novel anticancer agents arise from the desirable biological activities observed with early PGGTase-I inhibitors (GGTIs). These agents inhibited human tumor growth *in vitro* and *in vivo* with a mechanism that is consistent with cell cycle arrest at the G1 phase.[12–14] This includes induction of the CDK (cyclin-dependent kinase) inhibitor $p21^{waf}$, inhibition of CDK2 and CDK4 kinase activities and induction of hypophosphorylation of Rb (retinoblastoma protein).[12–14]

The complex networks of signal transduction pathways involving key GTPases have not been fully characterized. Therefore, developing highly selective GGTIs would provide valuable tools to study the related proteins in normal and cancer cell growth. Selective PGGTase-I inhibitors, in combination with

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† Electronic supplementary information (ESI) available: Experimental procedure and spectroscopy data for additional compounds. Representative ^{1}H NMR spectra for compounds **12a** and **16**, and ^{1}H–^{1}H COSY and NOESY spectra of compound **25**. Representative HPLC chromatographs for key compounds. See DOI: 10.1039/b517572k

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other anti-cancer therapies, may have significant potential as cancer chemotherapeutic agents for the treatment of malignant tumors.

Results and discussion

Inhibitor design

Relatively few studies have been published on the design of PGGTase-I inhibitors, by mimicking either the isoprenyl substrate geranylgeranyl pyrophosphate (GGPP)[15–17] or the tetrapeptide substrate sequence CAAL.[17–21] Because GGPP is the universal C_{20} source for all geranylgeranylated proteins, inhibitors that are competitive with protein substrates containing a CAAL sequence would be more selective, therefore more desirable for drug development.

As shown in Fig. 1, the tetrapeptide CVIL was found to act as an inhibitor for both PGGTase-I and PFTase with IC_{50} values of 11.3 μM and 16.7 μM, respectively.[18] Hydroxyphosphonate Merck-**8** was shown to be GGPP-competitive with an IC_{50} value against PGGTase-I of 12 nM.[17] Aryloxy substituted *N*-arylpiperazinone Merck-**2** was found to be a dual prenyl-protein transferase inhibitor (PFTase, IC_{50} = 6.8 nM, PGGTase-I, IC_{50} = 140 nM,[a] 0.7 nM,[b] Fig. 1).[17] Although this compound was designed based on the CAAX sequence, inhibition of PGGTase-I was found to be GGPP-competitive, as well as time and anion dependent. The authors have suggested that formation of an anion-inhibitor complex might mimic the transition state of PGGTase-I.[17] Using 2-aryl-4-aminobenzoic acid as a spacer, and imidazole as an oxidatively stable zinc-binding functionality, we have prepared a family of CAAX-mimetic PGGTase-I inhibitors.[12,20] For example, GGTI-2154 (PFTase, IC_{50} = 5600 nM, PGGTase-I, IC_{50} = 21 nM, Fig. 1), exhibited antitumor activity both *in vitro* and *in vivo*, and is currently undergoing preclinical evaluation.

In the hope of improving the potency, selectivity, and *in vivo* antitumor efficacy of our early PGGTase-I inhibitors, we have investigated piperazin-2-one scaffolds as alternative template CAAX mimetics. We report here a series of inhibitors that mimic the C-terminal CAAX sequence of PGGTase-I substrates, such as RhoA or Rap1A. High potency and exceptional selectivity were obtained for inhibition of PGGTase-I over PFTase with structures such as **45**, GGTI-2418 (PFTase, IC_{50} = 53 000 ± 11 000 nM, PGGTase-I, IC_{50} = 9.5 ± 2.0 nM, Fig. 1). Whole cell studies showed that **44**, the corresponding methyl ester of **45**, was able to selectively block processing of Rap1A in oncogenic H-Ras transfected NIH 3T3 cells with an IC_{50} value of 0.4 ± 0.1 μM.

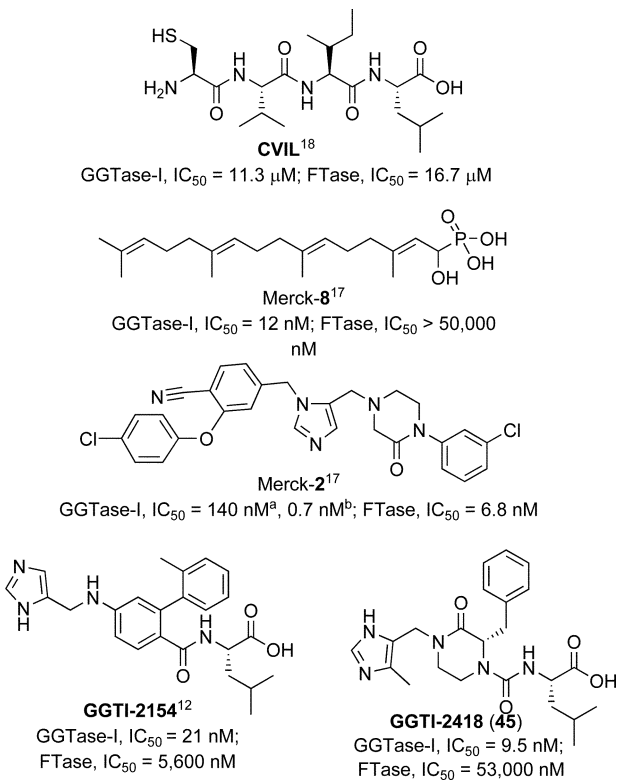


Fig. 1 Chemical structures of PGGTase-I inhibitors. [a]IC_{50} value determined in the absence of ATP and without preincubation. [b]IC_{50} value determined in the presence of 5 mM ATP and with 30 min preincubation.

Chemistry

The piperazin-2-one derivatives described in this paper were synthesized as represented in Schemes 1–6. In Scheme 1, substitution on the N-1 position of the piperazinone ring was introduced by reductive amination of *p*-fluorobenzaldehyde with aminoacetaldehyde dimethyl acetal in the presence of $NaBH(OAc)_3$. Coupling of the resulting secondary amine **1** with *N*-Cbz-L-leucine using EDCI afforded compound **2**, which cyclized in 70% TFA–H_2O[22] in good yield to produce the piperazin-2-one scaffold as a Cbz-protected enamine **3**. The crystal structure of **3** (Fig. 2)[23] obtained at −90 °C showed a single conformation corresponding to the *Z*-isomer about the Cbz-carbamate group. However, the NMR spectrum of **3** in methanol clearly showed two sets of signals representing the two distinct *Z*- and *E*-conformers.[24] Deprotection and saturation of the double bond were accomplished in one step by hydrogenation using 10% Pd/C catalyst to give the piperazin-2-one scaffold **4**.

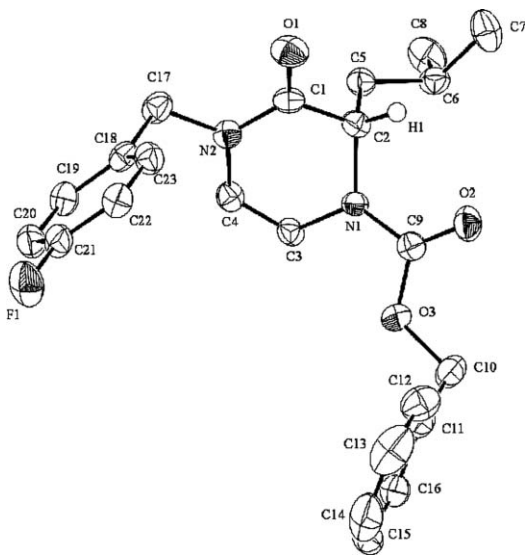
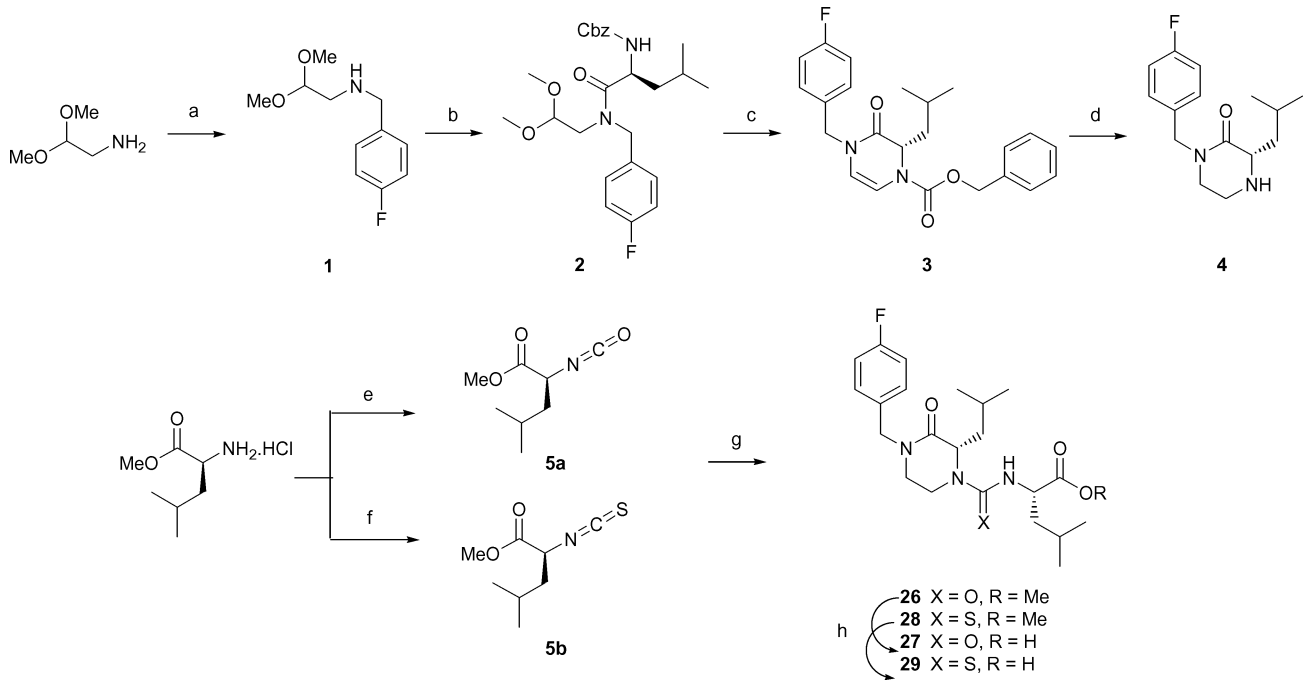


Fig. 2 An ORTEP drawing of the X-ray structure for compound **3** (30% probability ellipsoids).



Scheme 1 *Reagents and conditions*: (a) *p*-fluorobenzaldehyde, $NaBH(OAc)_3$, DCE, 24 h, 95%; (b) *N*-Cbz-L-Leu, EDCI, DIEA, CH_2Cl_2, 3 h, 98%; (c) 70% TFA–H_2O, 2 h, 90%; (d) H_2, 10% Pd/C, EtOAc–MeOH, 4 h, 98%; (e) $COCl_2$, CH_2Cl_2, pyridine, 2 h, 90%; (f) $CSCl_2$, H_2O, Na_2CO_3, 0.5 h, 65%; (g) **4**, CH_2Cl_2, 0 °C to rt, 5 h; 85–90% (h) NaOH–H_2O, MeOH, 90%.

Reaction of L-leucine methyl ester with phosgene or thiophosgene gave the corresponding isocyanate **5a** or isothiocyanate **5b**, which could then be coupled with **4** to give **26** and **28**, respectively. The methyl esters were hydrolyzed under basic conditions to give acids **27** and **29**.

Protected imidazole chloride derivatives (**7–10**) were prepared using previously reported procedures[25–27] as outlined in Scheme 2. Compounds with the imidazole group substituted on the N-1 position of the piperazinone ring were prepared by alkylation of the amide nitrogen in compounds **12a–12d** (Scheme 3). Protected scaffolds **12a–12d** were synthesized using procedures similar to that of scaffold **3**, except that the reductive amination step was omitted to leave the N-1 position open for further substitution. The crystal structure[28] of compound **12a** is shown in Fig. 3. The acid-catalyzed cyclization went smoothly for most of the scaffolds in 85–88% yield, except for **12b** (30% yield) which has a bulky naphthyl group. Alkylation of **12** with Boc-protected chloromethylimidazole **7** went to completion within 1 h at rt. However, the yield of the N-1 alkylation was only about 10%, while the major products resulted from C-5 alkylation. Compounds **13a2–13a4** were synthesized by reacting scaffold **12a** with NaH and trityl-protected chloromethylimidazoles **8–10** in THF at 60 °C for 2 h in 35–70% yield. The temperature and reaction time were monitored carefully to prevent racemization at the C-3 chiral center. Compounds **13b–13d** were synthesized from scaffolds **12b–12d**, respectively, under similar conditions using 4-chloromethyl-5-methyl-1-tritylimidazole **9**. Hydrogenation at atmospheric pressure using 10% Pd/C removed the Cbz protective group and the double bond, while leaving the trityl group intact. Coupling of the piperazinone scaffold **14** with isocyanates or

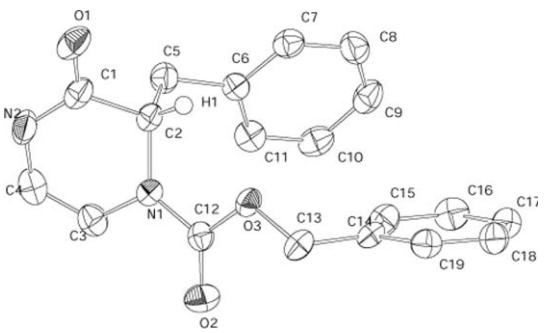
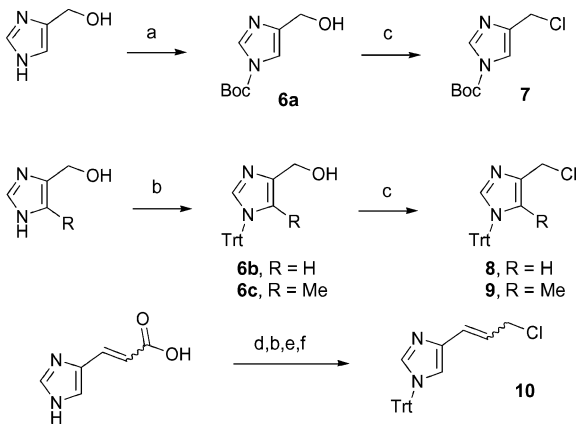


Fig. 3 An ORTEP drawing of the X-ray structure for compound **12a** (30% probability ellipsoids).



Scheme 2 *Reagents and conditions*: (a) $(Boc)_2O$, DMF, overnight, 80%; (b) TrtCl, Et_3N, DMF, overnight; 85–95%; (c) $SOCl_2$, DMF, CH_2Cl_2, 0 °C, 15 min, 80%; (d) $SOCl_2$, MeOH, 98%; (e) $LiAlH_4$, THF, 75%; (f) $SOCl_2$, THF, 1 h, 75%.

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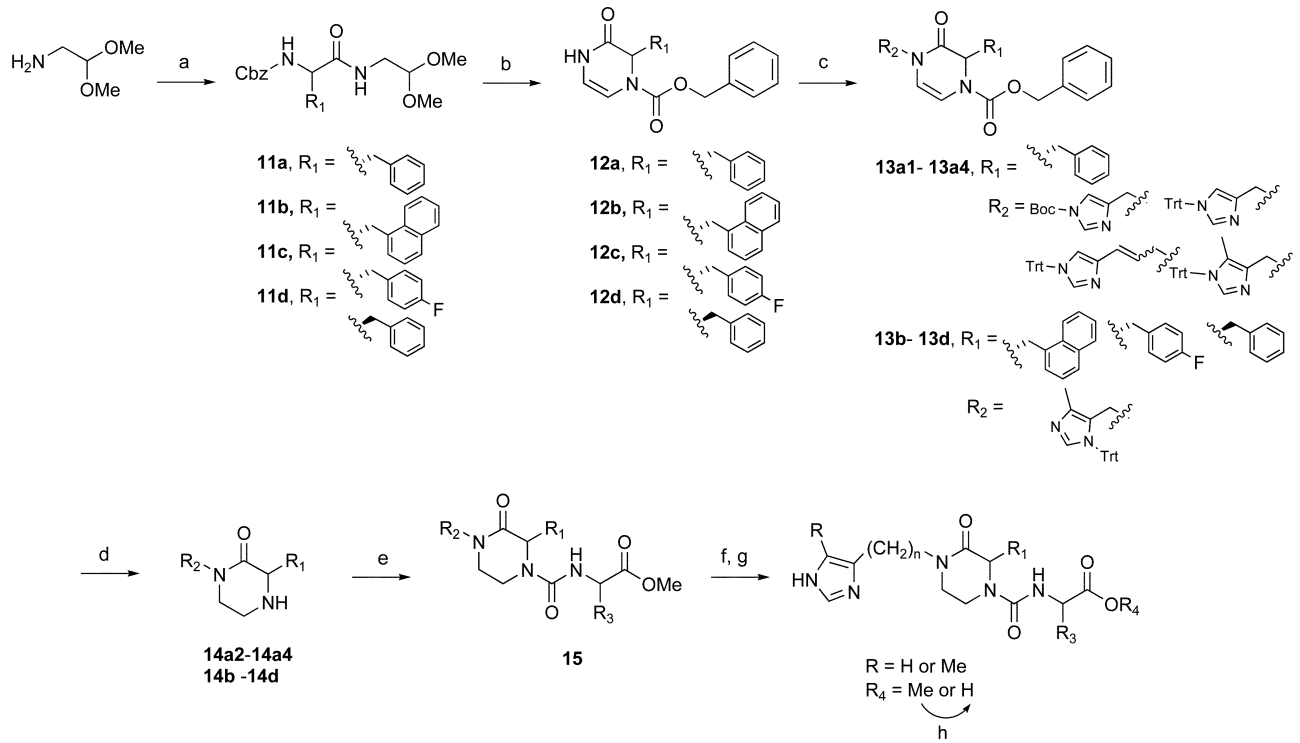


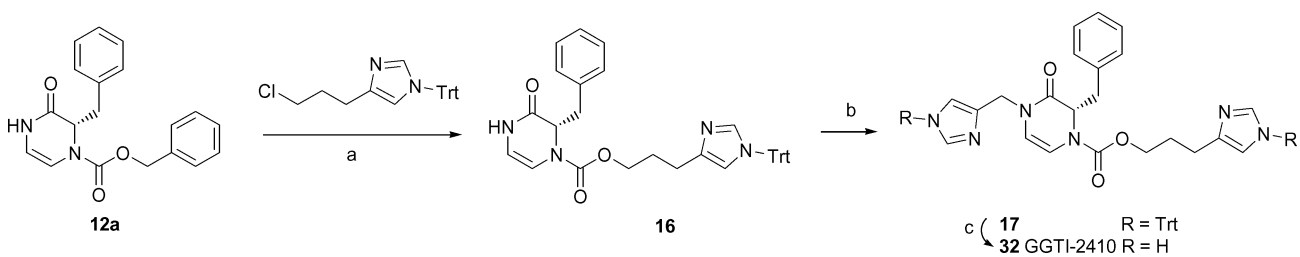
Scheme 3 *Reagents and conditions*: (a) *N*-Cbz-amino acid, EDCI, DIEA, CH_2Cl_2, 90–95%; (b) 70% TFA–H_2O, 30–88%; (c) NaH, **7–10**, THF, 60 °C, 2 h, 15–70%; (d) H_2, 10% Pd/C, EtOAc–MeOH, 98%; (f) amino acid methyl ester isocyanates, CH_2Cl_2, 0 °C to rt, 4 h, 85–88%; (g) 40% TFA–CH_2Cl_2, triethylsilane, 90–95%; (h) 1 N NaOH–H_2O, MeOH, 90%.

isothiocyanates generated from the corresponding commercially available amino acid methyl esters afforded trityl-protected inhibitors **15**. Deprotection of the trityl group using 40% TFA–CH_2Cl_2 and triethylsilane gave the methyl esters, which were then saponified to give the corresponding acids.

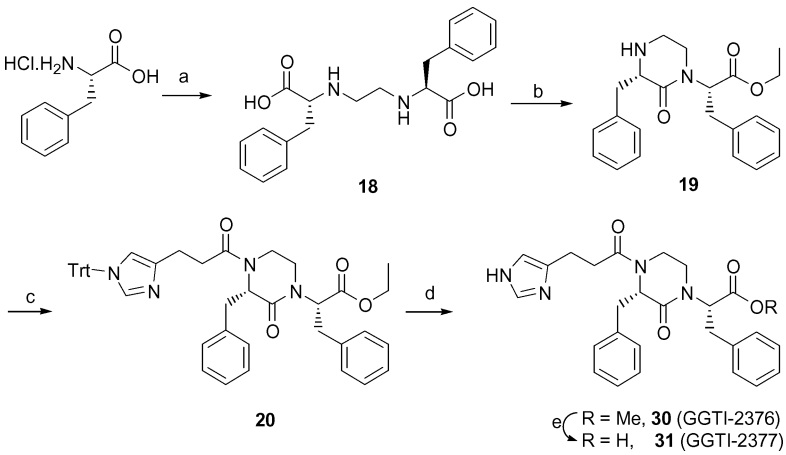
As shown in Scheme 4, initial attempts to synthesize compound **13a2** using 4-(3-chloro-propyl)-1-tritylimidazole and NaH in THF were unsuccessful. Instead, compound **16** was obtained using catalytic amounts of Bu_4NI under reflux in THF. Reaction of compound **16** with NaH and **8** gave **17**, which, after deprotection, generated **32** with two imidazole substituents.

As shown in Scheme 5, **30** and **31** were synthesized using Yamashita's method[29] which is useful in synthesizing constrained dipeptide mimics composed of two identical amino acids. Compound **19** was synthesized in two steps (75% and 85% yields, respectively) from L-phenylalanine *via* ethylene-bridged compound **18**. Coupling of scaffold **19** with *N*-1-trityl-deaminohistidine gave compound **20**, which after removal of the trityl group and saponification gave the desired products.

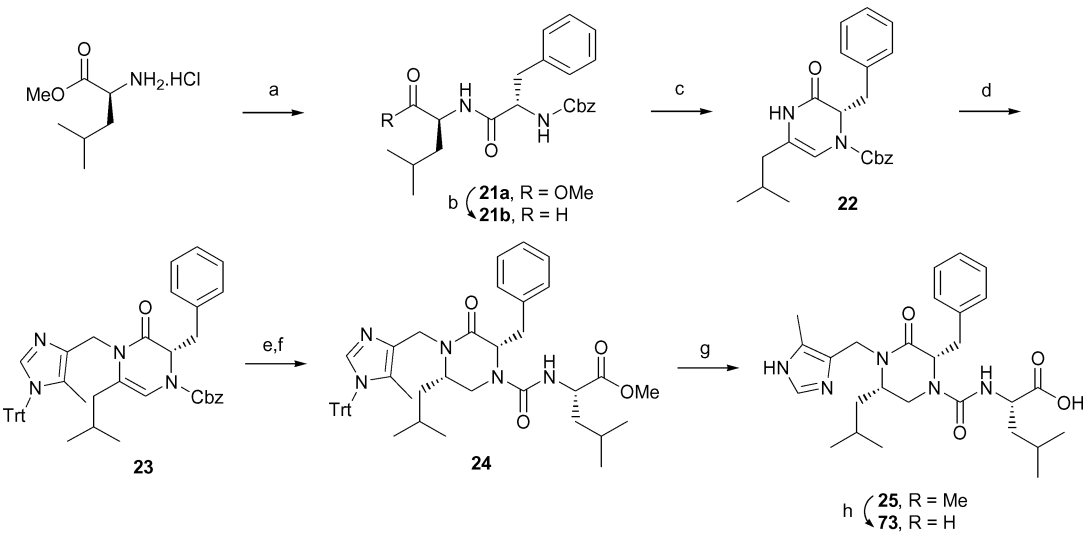
As shown in Scheme 6, compound **21** was synthesized in 40% yield by coupling of L-leucine methyl ester with *N*-Cbz-L-phenylalanine using EDCI, followed by DIBAL-H reduction in CH_2Cl_2 at −78 °C. Cyclization of **21** in 70% TFA–H_2O generated compound **22** in 87% yield. Reaction of compound **22** with NaH and trityl-protected imidazole chloride **9** gave compound **23** in poor yield (15%), presumably due to the steric hindrance between the isobutyl group at the 6-position and the bulky trityl substitution on the imidazole ring. Hydrogenation of compound **23** removed the Cbz group and saturated the double bond, resulting in predominantly one isomer with a de of 80% based on NMR analysis. The newly generated stereocenter was predicted to be in a 6*S* configuration, due to the approach of the catalyst-bound hydrogen from the top face to avoid a steric clash with the 3*S* benzyl group. The crude deprotected scaffold was coupled to L-leucine methyl ester isocyanate to give compound **24**, which after purification, deprotection of the trityl group, and saponification gave methyl ester **25** and acid **73**, respectively.



Scheme 4 *Reagents and conditions*: (a) NaH, Bu_4NI, THF, reflux, 4 h, 40%; (b) NaH, **8**, THF, 60 °C, 2 h, 70%; (c) 40% TFA–CH_2Cl_2, triethylsilane, 90%.



Scheme 5 *Reagents and conditions*: (a) 1,2-dibromoethane, K_2CO_3, NaOH, H_2O, 95 °C, 5 h, 75%; (b) H_2SO_4, EtOH, reflux, 85%; (c) *N*-1-trityl-deaminohistidine, EDCI, DIEA, CH_2Cl_2, 90%; (d) 40% TFA–CH_2Cl_2, triethylsilane, 90%; (e) NaOH–H_2O, MeOH, 90%.



Scheme 6 *Reagents and conditions*: (a) N-Cbz-L-Phe, EDCI, DIEA, CH_2Cl_2, 90%; (b) DIBAL–CH_2Cl_2, 40%; (c) 70% TFA–H_2O, 87%; (d) NaH, **9**, THF, 60 °C, 2 h; 15% (e) H_2, 10% Pd/C, EtOAc–MeOH, 98%; (f) **5a**, CH_2Cl_2, 0 °C to rt, 4 h, 88%; (g) 40% TFA–CH_2Cl_2, triethylsilane; 90%; (h) 1 N NaOH–H_2O, MeOH, 90%.

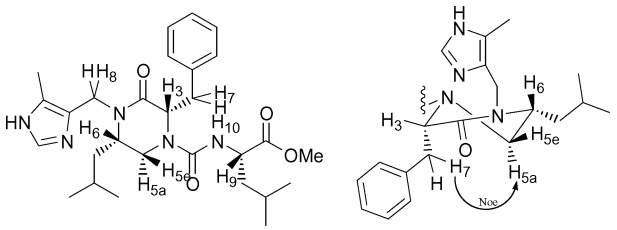


Fig. 4 NOE observed in **25**.

The 6*S* stereochemistry was confirmed by 2D NMR experiments, including ^{1}H–^{1}H COSY and NOESY, of compound **25**.[30] As shown in Fig. 4, an NOE was observed between axial-H-5 and one of the H-7 protons confirming the pseudoaxial orientation of the 3*S* benzyl group (as seen in the crystal structures of compounds **3** and **12a**, Figs. 2 and 3), and the axial, β-orientation of H-6 (6*S* configuration). This is consistent with earlier studies which showed that acylation of an amino group induces an allylic (1,3)-strain-enforced pseudoaxial position of the C_α side chain substituent.[31]

Structure–activity relationships

The piperazinone derivatives were evaluated for their inhibitory activity against PGGTase-I and PFTase by measuring their ability to inhibit incorporation of [^{3}H]GGPP and [^{3}H]FPP into H-Ras-CVLL and H-Ras-CVLS, respectively, as previously described.[32] Furthermore, inhibition of Rap1A and H-Ras processing was used as a measure of the ability of GGTIs to prevent prenylation in whole cells.[13] An example of this Western blot analysis for several key compounds is shown in Fig. 5.

Compounds **26–32** were prepared to test the synthetic feasibility and scope of biological activities of the designed scaffolds and inhibitors. As shown in Table 1, urea derivative **27** without a zinc-binding functionality exhibited reasonable inhibitory activity (IC_{50} = 6.4 μM) and some selectivity for PGGTase-I. Its corresponding methyl ester showed whole cell activity (**26**, IC_{50} = 20 μM) in inhibiting Rap1A processing. Changing the urea into a thiourea linkage as in **29** led to reduced activity. Therefore, the thiourea derivatives were not pursued in the later design.

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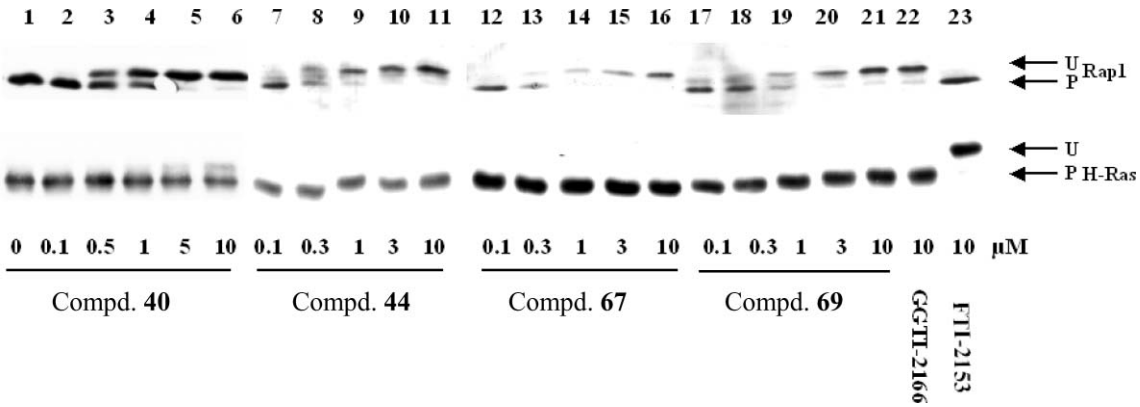
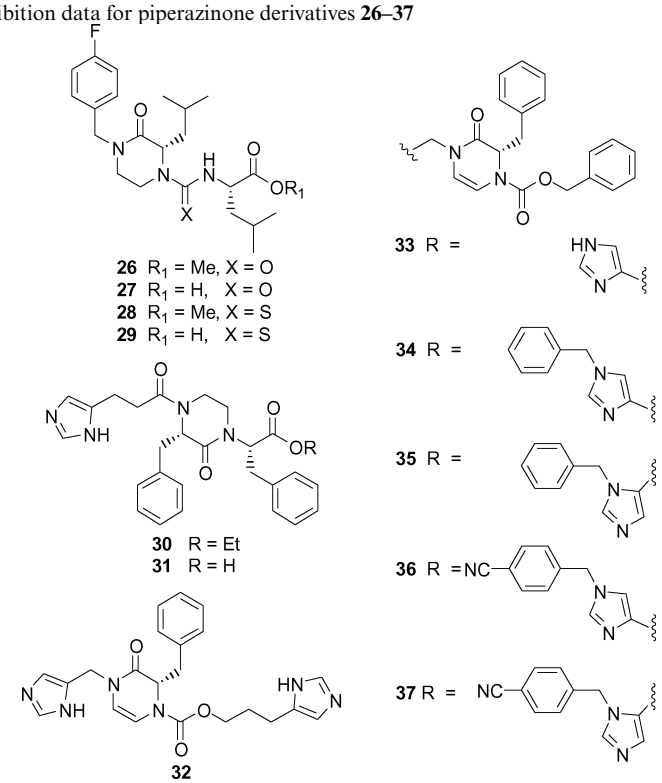


Fig. 5 Effect of piperazinone-derived PGGTase-I inhibitors on processing of H-Ras and Rap1A in NIH 3T3 cells (H-Ras 61L). Cells were treated on each of two consecutive days with inhibitors, then harvested, and subjected to Western blot analysis to demonstrate the inhibition of geranylgeranylated Rap1A or farnesylated H-Ras as seen in the band shift from processed (P) protein to unprocessed (U) protein.

Table 1 PGGTase-I and PFTase inhibition data for piperazinone derivatives **26–37**



Compd no.	IC_{50}/nM GGTase	PFTase/GGTase	PFTase	IC_{50}/μM[a] Rap1A	H-Ras
26	>10 000	>10 000		20	>30
27	6400	>10 000	>1.5	>10	>10
28	> 100 000	>100 000		>10	>10
29	24 000	>100 000	>4.2	>10	>10
30	18 000	8850	0.5	>10	>10
31	760 ± 96	8150	10.7	>10	>10
32	16 000 ± 14 000	20 000 ± 14 000	1.25	>10	>10
33	8000	>10 000	>1.25	>10	>10
34	>10 000	>10 000		ND	ND
35	>10 000	20 000	<2	ND	ND
36	>10 000	>10 000		ND	ND
37	>10 000	1300 ± 870	<0.13	ND	ND

[a] ND: Not determined.

Yamashita's method was chosen to generate compounds **30** and **31**. When **30** and **31** are aligned to overlap the CAAX sequence, the piperazinone adopts an inverted orientation as compared to other inhibitors, such as **45** (Fig. 1). Even in the presence of the zinc binding imidazole functionality, inhibitory potency and selectivity (PGGTase-I, $IC_{50} = 0.76\ \mu M$; PFTase, $IC_{50} = 8.15\ \mu M$) for **31** was moderate. Therefore, this direction was not further pursued. As shown in Table 1, incorporation of two imidazole groups as in **32** or an imidazole and a benzyl as in **33** gave no significant observable activity. Imidazole substitution at the N-4 (**34**, **36**) position did not lead to an increase in activity, however N-5 imidazole substitution reversed the selectivity for compound **37**. This observation is consistent with the fact that the zinc binding pocket of PGGTase-I is smaller than that of PFTase, and does not accommodate large groups in the imidazole ring.

It was not surprising that the L-methionine-derived **39** was slightly more selective for PFTase (PGGTase-I, $IC_{50} = 450 \pm 95$ nM; PFTase, $IC_{50} = 300 \pm 220$ nM; Table 2); while the L-leucine-derived **41** reversed the selectivity (PGGTase-I, $IC_{50} = 62 \pm 14$ nM; PFTase, $IC_{50} = 4400 \pm 1970$ nM; Table 2). A longer spacer chain between the imidazole group and the piperazinone scaffold increased PGGTase-I inhibitory potency and selectivity (PGGTase-I, $IC_{50} = 25 \pm 13$ nM; PFTase, $IC_{50} > 10\,000$ nM; Table 2), as seen in **43**, while a small methyl substitution at the 5-position of the imidazole ring also significantly reduced PFTase affinity for **45** (PGGTase-I, $IC_{50} = 9.5 \pm 2.0$ nM; PFTase, $IC_{50} = 53\,000 \pm 11\,000$ nM; Table 2).

Compound **45** is one of the most potent PGGTase-I inhibitors discovered in this series, exhibiting more than 5500-fold selectivity *in vitro* for PGGTase-I over PFTase. The relatively low cellular PGGTase-I inhibitory potency of **45** may be caused by poor penetration of this free acid through the plasma membrane. However, the PGGTase-I inhibitory potency and selectivity are well-retained by the corresponding methyl ester **44** in the cell based assay (Rap1A, $IC_{50} = 0.4 \pm 0.1\ \mu M$; H-Ras, $IC_{50} > 50\ \mu M$; Table 2, Fig. 5). The cellular PGGTase-I inhibition of **44** is reduced by approximately 30-fold as compared to the *in vitro* IC_{50} value. This discrepancy between *in vitro* and cell-based potencies has been seen by others. For example, Merck-**2**[17] in Fig. 1 is a subnanomolar inhibitor *in vitro*, but showed an IC_{50} value of 0.3 μM in cell based assays.

Docking studies based on the recently published X-ray crystal structure of mammalian protein PGGTase-I (PDB code: 1N4Q) with compound **45** were performed using the flexible ligand docking approach (GOLD).[33] Fig. 6 shows the lowest energy conformation of compound **45**, using MACROMODEL,[34] docked in the active site of PGGTase-I. The imidazole group coordinates to the catalytic zinc ion, and the methyl group occupies a very small pocket formed by Lys 311β and the terminal phosphate of the GGPP analog. The 3-aryl pharmacophore is located at an open hydrophobic cleft formed by Leu 320β, Phe 53β and Leu 43β. The urea spacer of compound **45** makes no significant binding interactions, and the C-terminal carboxylate moiety interacts strongly with Arg 173β. The leucine side chain fits tightly in the hydrophobic pocket lined with the GGPP analog, Phe 53β and Leu 320β.

While the introduction of a small 5-methyl group on the imidazole ring is well tolerated and leads to an increase in selectivity for PGGTase-I, as in **45** and **51**, the existence of a

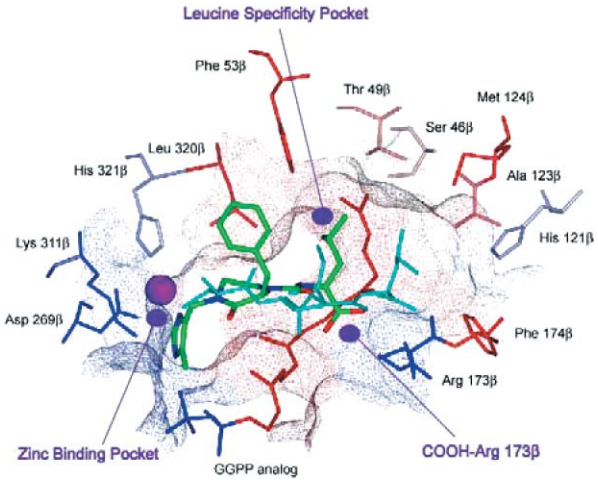


Fig. 6 Comparison of the GOLD-predicted docked conformation of **45** (GGTI-2418) (atom color) in the active site of PGGTase (PDB 1N4Q, colored by hydrophobicity, red hydrophobic to blue hydrophilic) with the enzyme bound conformation of the native substrate peptide CVIL (cyan blue).

strict size limitation for imidazole substitution in this series is confirmed by compounds **46–49**. These results show that as the size of the substituent increases, the selectivity towards PGGTase-I decreases. Docking studies with **45** clearly show that the methyl group is accommodated tightly in the small zinc binding pocket of PGGTase-I and that larger substitutions are therefore disfavored. Compound **53**, in which the R_2 group is an isobutyl group with an *R* configuration introduced from D-leucine, is essentially inactive toward both PFTase and PGGTase-I (IC_{50} values > 10 000 nM, Table 2). Compounds **55**, **57** and **59** containing isopropyl, benzyl and cyclohexylmethyl substituents in an *S* configuration exhibited lower PGGTase-I inhibitory potency and selectivity as compared to **45**.

Eliminating the free carboxyl group as in compounds **60–66** rendered most compounds inactive, suggesting a critical interaction of the carboxyl group with positively charged residues in the PGGTase-I active site (Table 3). This is consistent with the previous observation from X-ray crystallographic studies, that the C-terminal carboxyl group of the CVIM peptide forms a hydrogen bond to the side chain amide group of Gln-167 in the PFTase α-subunit.[35] According to docking studies with **45**, the loss of activity observed by compounds in this series lacking a C-terminal carboxyl group is due to the fact that these inhibitors can no longer interact with Arg 173β in the PGGTase-I active site. The importance of the carboxyl group also explains the drastic change imposed by inverting the stereochemistry of the R_2 substitution, since optimal binding requires satisfaction of both the hydrophobic and hydrogen bonding interactions introduced by R_2 substitution and the carboxyl group, respectively.

As shown in Table 4, modification of the R_1 substitution to an *R* configuration, as introduced from D-phenylalanine in **72**, leads to significantly reduced PGGTase-I inhibitory potency ($IC_{50} = 680 \pm 120$ nM, Table 4) as compared to its counterpart with an *S* configuration in this position (**45**, Table 4). The difference likely arises from the opposite pseudoaxial orientations of the phenylalanine side chains of inverted stereochemistry. Unexpectedly, when all the four important pharmacophores are assembled

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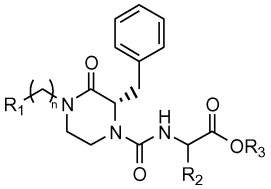
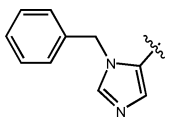
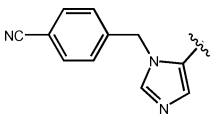
Table 2 PGGTase-I and PFTase inhibition data for piperazinone derivatives **38–59**

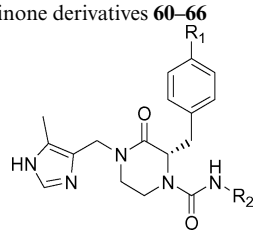


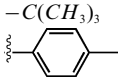
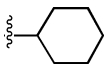
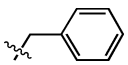
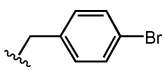
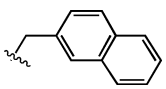
Compd no.	n	R_1	R_2	R_3	IC_{50}/nM GGTase	IC_{50}/nM PFTase	PFTase/GGTase	IC_{50}/μM[a] **Rap1A**	IC_{50}/μM[a] **H-Ras**
38	1			Me	4450	2350		10	10
39				H	450 ± 95	300 ± 220	0.7	>10	>10
40	1			Me	2400 ± 1950	>10 000		0.85	>10
41				H	62 ± 14	4400 ± 1970	71	>10	>10
42	3			Me	8150	>10 000		4	>10
43				H	25 ± 13	>10 000	>400	>10	>10
44	1			Me	1800 ± 1200	>10 000		0.4 ± 0.1	>50
45				H	9.5 ± 2.0	53 000 ± 11 000	5580	>10	>10
46	1			Me	40 000	>10 000		ND	ND
47				H	8550	7900	0.9	ND	ND
48	1			Me	6100	800 ± 150		ND	ND
49				H	3700	680 ± 150	0.2	ND	ND
50	1			Me	8000 ± 1200	>10 000		>10	>10
51				H	230 ± 140	800 ± 310	3.5	>10	>10
52	1			Me	>10 000	>10 000		>10	>10
53				H	>10 000	>10 000		>10	>10
54	1			Me	>10 000	>10 000		>10	>10
55				H	520 ± 130	22 000 ± 11 000	42	>10	>10
56	1			Me	>10 000	>10 000		>10	>10
57				H	5500 ± 150	29 500	5.4	>10	>10
58	1			Me	6500	>10 000		>15	>10
59				H	440 ± 180	>10 000	>23	>10	>10

[a] ND: Not determined.

onto the piperazinone scaffold to mimic the tetrapeptide CAAL sequence as in **73**, poor activity was observed (PGGTase-I, IC_{50} = 6100 nM, Table 4), suggesting unfavorable interaction with the enzyme in the presence of an isobutyl residue at the R_2 position. When R_1 is changed from an L-phenylalanine side chain as in **44** (IC_{50} = 1800 nM, Table 4) to a β-1-naphthyl-alanine residue as in **67** (IC_{50} = 24 ± 13 nM, Table 4), the latter methyl ester exhibited significantly enhanced inhibitory potency for PGGTase-I, suggesting that a bulkier side chain is favored. However, the inhibitory activity of the corresponding free acid **68** (IC_{50} = 14 ± 6.4 nM, Table 4), was similar to that of **45** (Table 4). This may be caused by the inability of the bulky naphthyl group to adopt an optimal orientation while satisfying the hydrogen bonding capacity of the free carboxyl group. In **70**, introduction of a *p*-fluoro-phenylalanine side chain led to a highly potent and selective PGGTase-I inhibitor (PGGTase-I, IC_{50} = 7.1 ± 4.3 nM; PFTase, IC_{50} = 130 000 ± 58 000 nM, Table 4). We also evaluated the ability of the inhibitors to disrupt Rap1A and H-Ras processing

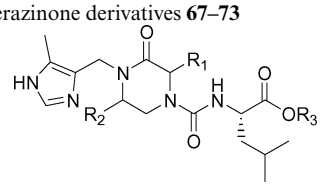
Table 3 PGGTase-I and PFTase inhibition data for piperazinone derivatives **60–66**

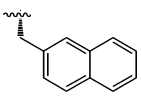
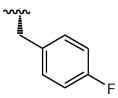


Compd no.	R_1	R_2	IC_{50}/nM		IC_{50}/μM[a]	
			GGTase	PFTase	Rap1A	H-Ras
60	H	$-C(CH_3)_3$	>10 000	>10 000	>10	>10
61	H		>10 000	>10 000	>10	>10
62	F		>10 000	>10 000	>10	>10
63	F		20 000	>10 000	>10	>10
64	F		6100	>10 000	>10	>10
65	F		69 000	>10 000	>10	>10
66	F		>10 000	>10 000	ND	ND

[a] ND: Not determined.

Table 4 PGGTase-I and PFTase inhibition data for piperazinone derivatives **67–73**



Compd no.	R_1	R_2	R_3	IC_{50}/nM		PFTase/GGTase	IC_{50}/μM	
				GGTase	PFTase	Rap1A	H-Ras	
44		H	Me	1800 ± 1200	>10 000		0.4 ± 0.1	>50
45			H	9.5 ± 2.0	53 000 ± 11 000	5580	>10	>10
67		H	Me	24 ± 13	24 000		0.6	50
68			H	14 ± 6.4	4800 ± 1100	343	>10	>10
69		H	Me	8000	>10 000		0.7	>50
70			H	7.1 ± 4.3	130 000 ± 58 000	18 300	>10	>10
71		H	Me	12 000	>10 000		>10	>10
72			H	680 ± 120	480 000	706	>10	>10
73			H	6100	>10 000	>1.6	>10	>10

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in a cellular assay. Table 2 shows that **40** was able to inhibit Rap1A geranylgeranylation but not H-Ras farnesylation, and that 5-methyl substitution in the imidazole, as in **44**, led to an increase in potency to inhibit Rap1A processing ($IC_{50} = 0.4 \pm 0.1$ μM). Substituting the phenylalanine by a naphthyl or a *p*-fluorophenylalanine, as in **67** and **69**, had little effect on the ability of the derivatives to inhibit Rap1A processing ($IC_{50} = 0.6$ and 0.7 μM).

Conclusions

A series of derivatives based on the C-terminal CAAX (X = L, F) sequence of PGGTase-I substrates, such as Rho, was designed and synthesized. Using piperazin-2-one as a relatively rigid scaffold, we have introduced critical recognition groups in a well-defined arrangement to mimic the peptide sequence. High potency and exceptional selectivity were obtained for inhibition of PGGTase-I with structures such as **45** (Fig. 1) and **70** (Table 2). The potency of this series of GGTIs is dependent on the presence of an L-leucine residue with a free carboxyl terminus, as well as an *S* configuration of the 3-aryl group. The selectivity is significantly promoted by 5-methyl substitution on the imidazole ring and fluorine substitution on the 3-aryl group. Modification of the 6-position of the piperazinone scaffold was found to be unfavorable. PGGTase-I inhibitor **44**, the corresponding methyl ester of **45**, was found to selectively block processing of Rap1A by PGGTase-I with an IC_{50} of 0.4 μM in NIH 3T3 cells.

Experimental

Nuclear magnetic resonance spectra (^{1}H, 400 or 500 MHz), (^{13}C, 100 or 125 MHz) were acquired using Bruker-500 or Bruker-400 spectrometers, and are reported in δ (ppm) with TMS as the internal reference. The homogeneity of all the compounds was routinely checked by TLC on silica gel plates, and new compounds were checked for purity by analytical HPLC using a Rainin 250 × 4.6 mm, 5 μm Microsorb C18 column with a gradient of 0–100% acetonitrile in water buffered with 0.1% TFA. High-resolution mass spectra (EI or FAB) were recorded on Micro-mass VSE and Micro-mass 70-4F mass spectrometers, respectively. Melting points were obtained on an Electrochem melting point apparatus and are uncorrected.

General procedure for the syntheses of amino acid ester isocyanates and subsequent urea formation

Amino acid methyl ester hydrochloride (0.6 mmol) was suspended in 2.0 mL of CH_2Cl_2, and to the solution was added 0.2 mL of pyridine (2.4 mmol). The resulting suspension was cooled at 0 °C for 15 min. Then a solution of phosgene (20% in toluene, 0.4 mL, 0.72 mmol) (**Caution**: use fume hood) was added by syringe. The resulting mixture was stirred at 0 °C under N_2 for 2 h. The solution was then diluted to a volume of 8 mL with CH_2Cl_2 and extracted with 10 mL of cold 0.1 N HCl, and *ca.* 7 mL of crushed ice. Each aqueous phase was re-extracted with 4 mL of CH_2Cl_2. The combined organic phases were extracted with cold brine and dried over Na_2SO_4. The resulting isocyanate solution was used for the subsequent urea formation reaction without further purification.

To a 25 mL round flask charged with piperazinone scaffold (0.25 mmol) was added a fraction of the above solution (*ca.* 0.30 mmol, assuming 90% yield according to the literature[36]). The mixture was stirred under N_2 at 0 °C for 1 h, and at rt for 4 h. Then the solvent was removed under reduced pressure and the resulting residue was subjected to silica gel column chromatography using 1–5% MeOH–CH_2Cl_2 as eluant to afford the urea. The same procedure was employed for attaching different amino acid methyl esters to the piperazinone scaffolds through a urea linkage with 85–88% yields.

Syntheses of compounds 26–29

To a solution of aminoacetaldehyde dimethyl acetal (1.1 mL, 10 mmol) in dichloroethane was added 4-fluorobenzaldehyde (1.07 mL, 10 mmol) and glacial acetic acid 0.5 mL. The reaction mixture was stirred at rt for 0.5 h, then sodium triacetoxyboron hydride (2.6 g, 13 mmol) was added at one time. The reaction mixture was stirred under N_2 for 3 h, then an additional 400 mg of sodium triacetoxyboron hydride was added and the mixture was stirred at rt for another 5–7 h. The reaction was stopped by quenching with 1 N NaOH in an ice bath and the mixture was extracted with methylene chloride. The combined organic phases were dried over sodium carbonate, filtered and the solvent was removed under vacuum to give compound **1** as a colorless oil (2.1 g, 92%), which was used without further purification.

A mixture of crude **1** (1.2 g, 5.6 mmol), Cbz-L-leucine (1.2 g, 0.55 mmol), EDCI (1.07 g, 5.6 mmol), DIEA (0.9 mL, 5.6 mmol) in 20 mL anhydrous methylene chloride was stirred at rt for 5 h. The reaction mixture was diluted with 80 mL methylene chloride, and the solution was washed with 1 N HCl (20 mL), sat. sodium bicarbonate solution (20 mL), and brine (20 mL). The organic phase was dried over sodium sulfate and the solvent was removed under reduced pressure to give a crude oil, which was purified by silica gel column chromatography with hexanes–EtOAc (5 : 1) as eluant to afford compound **2** as a colorless oil (2.2 g, 95%): δ_H (500 MHz, d_4-methanol) 0.73 (1.3 H, d, *J* 6.5), 0.84 (1.5 H, d, *J* 7.0), 0.95 (3.3H, d, *J* 7.0), 1.17–1.77 (3H, m), 3.07–3.27 (1H, m), 3.48 (0.5H, dd, *J* 14.0 and 5.5), 3.73 (0.5H, dd, *J* 15.5 and 6.5), 4.52 (1H, dd, *J* 11.0 and 5.5), 4.57–4.82 (3H, m), 5.06 (1H, d, *J* 12.5), 5.11 (1H, d, *J* 12.5), 7.00 (1H, t, *J* 8.5), 7.07 (1H, t, *J* 9.0), 7.21 (1H, dd, *J* 8.5 and 5.5), 7.27 (1H, dd, *J* 8.5 and 5.5), 7.32 (5H, m).

Compound **2** (2.0 g, 4.33 mmol) was dissolved in 20 mL 70% TFA–H_2O and the solution was stirred at rt for 2 h. The solvent was removed on a rotary evaporator to give a yellowish oil, which was dissolved in 100 mL ethyl acetate and washed with saturated aqueous $NaHCO_3$ solution and brine. The organic phase was dried over anhydrous Na_2SO_4, and the solvent was removed to give compound **3** as a white solid (1.55 g, 91%). A diffraction quality single crystal was obtained by slow evaporation of a chloroform solution of compound **3**: mp 91–92 °C; δ_H (500 MHz, d_4-methanol) 0.78 (1H, d, *J* 6.0), 0.83 (1H, d, *J* 6.0), 0.91 (2H, d, *J* 6.0), 0.94 (2H, d, *J* 6.0), 1.40–1.53 (3H, m), 4.65 (2H, d, *J* 7.0), 4.72 (0.5H, m), 4.83 (0.5H, m), 5.09–5.26 (2H, m), 5.80 (0.5H, d, *J* 6.0), 5.90 (0.5H, d, *J* 6.0), 6.32 (0.5H, d, *J* 5.5), 6.29 (0.5H, d, *J* 5.5), 7.02 (1H, d, *J* 8.5), 7.04 (1H, d, *J* 8.5), 7.26 (2H, d, *J* 8.5 and 6.0), 7.32

(5H, m); *m/z* (FAB) 397.1926 ($M^+ + 1$, $C_{23}H_{26}FN_2O_3$ requires 397.1927).

Compound **3** (1.5 g, 3.78 mmol) was dissolved in 40 mL MeOH–EtOAc (1 : 1), and to the solution was added 10% Pd/C. The solution was hydrogenated at atmospheric pressure for 4 h. The solution was filtered and the solvent removed to give compound **4** as a colorless oil (0.98 g, 99%): δ_H (500 MHz, $CDCl_3$) 0.88 (3H, d, *J* 6.5), 0.91 (3H, d, *J* 6.5), 1.51 (1H, ddd, *J* 14.0, 10.5 and 4.5), 1.72 (1H, m), 1.86 (1H, ddd, *J* 14.0, 10.5 and 4.0), 2.89 (1H, ddd, *J* 13.5, 10.5 and 4.5), 3.08 (2H, m), 3.23 (1H, m), 3.42 (1H, dd, *J* 10.0 and 3.5), 4.40 (1H, d, *J* 15.0), 4.55 (1H, d, *J* 15.0), 6.93 (1H, d, *J* 8.5), 6.95 (1H, d, *J* 8.5), 7.17 (2H, dd, *J* 8.5 and 5.5); *m/z* (FAB) 265.1716 ($M^+ + 1$, $C_{15}H_{22}FN_2O$, 265.1716).

Reaction of **4** with the isocyanate generated from L-leucine methyl ester (general procedure) afforded **26** as a colorless oil in 85% yield: δ_H (500 MHz, d_4-methanol) 0.87 (3H, d, *J* 6.5), 0.93 (3H, d, *J* 6.5), 0.95 (3H, d, *J* 6.5), 0.96 (3H, d, *J* 6.6), 1.53–1.80 (6H, m), 3.18 (1H, m), 3.39 (2H, m), 3.67 (3H, s), 3.98 (1H, m), 4.27 (1H, dd, *J* 10.0 and 4.5), 4.57 (2H, d, *J* 4.5), 4.83 (1H, m), 7.04 (1H, d, *J* 9.0), 7.06 (1H, d, *J* 9.0), 7.27 (2H, dd, *J* 9.0 and 5.5); *m/z* (FAB) 436.2612 ($M^+ + 1$, $C_{23}H_{35}N_3O_4F$ requires 436.2612).

To a solution of **26** (100 mg, 0.23 mmol) in 0.5 mL methanol was added 1 mL 1 N NaOH solution. The resulting mixture was stirred at rt for 1 h, then the solvent was removed under reduced pressure. The residue was suspended in 2 mL of 30% MeOH–CH_2Cl_2, and the suspension was passed through a pad of silica gel (500 mg). The solid phase was further eluted with 30–50% MeOH–CH_2Cl_2 solution. The fractions containing the pure product were combined and the solvent was removed to afford **27** as a colorless oil in 80% yield: δ_H (500 MHz, d_4-methanol) 0.78 (3H, d, J = 6.0), 0.82 (3H, d, *J* 6.0), 0.85 (3H, d, *J* 6.0), 0.88 (3H, d, *J* 6.0), 1.50–1.60 (6H, m), 3.08 (1H, m), 3.33 (2H, m), 3.90 (1H, br d, *J* 4.5), 4.18 (1H, dd, *J* 10.5 and 5.0), 4.47 (2H, br s), 4.75 (1H, dd, *J* 9.5 and 2.5), 6.94 (1H, d, *J* 8.5), 6.96 (1H, d, *J* 8.5), 7.17 (2H, dd, *J* 8.5 and 5.0); *m/z* (FAB) 422.2455 ($M^+ + 1$, $C_{22}H_{33}N_3O_4F$ requires 422.2455).

Syntheses of L-leucine methyl ester isothiocyanate

L-Leucine methyl ester hydrochloride (110 mg, 0.6 mmol) was dissolved in 0.3 mL of water and stirred with 1 mL of chloroform at 0 °C. The pH was adjusted to 9.0 with aqueous sodium carbonate solution. Then a solution of thiophosgene 70 μL (1.0 mmol) in 150 μL $CHCl_3$ was added dropwise with stirring while the pH was kept at 9.0 with sodium carbonate solution. After 30 min stirring at 0 °C, the organic phase was separated, and diluted to a volume of 8 mL with $CHCl_3$. The solution was extracted with 10 mL of cold 0.1 N HCl, and *ca.* 7 mL of crushed ice. Each aqueous phase was re-extracted with 4 mL of $CHCl_3$. The combined organic phases were extracted with cold brine, and dried over Na_2SO_4. The resulting isothiocyanate solution was used for the subsequent urea formation reaction without further purification.

To a 25 mL round flask charged with piperazinone scaffold **4** (100 mg, 0.38 mmol) was added a fraction (1.2 equiv.) of the above solution. The mixture was stirred under N_2 at 0 °C for 1 h, and at rt for 4 h. Then the solvent was removed under reduced pressure and the resulting residue was subjected to silica gel column chromatography using 0.5–2.5% MeOH–CH_2Cl_2 as eluant to afford the thiourea **28** (140 mg, 83% yield) as a colorless oil: δ_H (500 MHz, $CDCl_3$) 0.94 (6H, d, *J* 6.2), 1.01 (6H, d, *J* 6.7), 1.65 (2H, m), 1.72 (2H, m), 1.81 (1H, m), 1.90 (1H, m), 3.15 (1H, m), 3.45 (2H, m), 3.73 (3H, s), 4.30 (1H, d, *J* 14.5), 4.73 (1H, d, *J* 14.5), 4.79 (1H, m), 4.93 (1H, m), 5.18 (1H, dd, *J* 13.2 and 7.0), 5.91 (1H, d, *J* 7.5), 7.00 (2H, t, *J* 8.5), 7.20 (2H, dd, *J* 8.5 and 5.5); *m/z* (FAB) 452 ($M^+ + 1\text{-}SH_2$).

Saponification of **28** in a manner similar to that described for the synthesis of **27**, afforded **29** as colorless oil in 80% yield: δ_H (400 MHz, $CDCl_3$) 0.93 (3H, d, *J* 6.5), 0.98 (12H, m), 1.61–1.83 (6H, m), 3.12 (1H, br d, *J* 12.5), 3.40 (2H, m), 4.16 (1H, d, *J* 14.5), 4.83 (1H, d, *J* 14.5), 5.37 (2H, m), 5.47 (1 h, br d, *J* 14.0), 6.63 (1H, d, *J* 7.0), 7.01 (2H, t, *J* 8.5), 7.18 (2H, dd, *J* 8.5 and 5.5); *m/z* (FAB) 438 ($M^+ + 1\text{-}SH_2$).

Syntheses of compound 33

A mixture of aminoacetaldehyde dimethyl acetal (1.1 mL, 10 mmol), Cbz-L-leucine (2.99 g, 10 mmol), EDCI (1.92 g, 10 mmol), in 20 mL anhydrous methylene chloride was stirred at rt for 5 h. The reaction mixture was diluted with 80 mL methylene chloride, and the solution was washed with 1 N HCl (20 mL), saturated sodium bicarbonate solution (20 mL), and brine (20 mL). The organic phase was dried over sodium sulfate, and passed through a pad of silica gel, and the solid phase was washed with 1–2.5% MeOH–CH_2Cl_2. Fractions were combined and the solvent was removed to afford compound **11a** as a white solid (3.3 g, 86%): mp 123–124 °C; δ_H (500 MHz, d_4-methanol) 2.72 (1H, dd, *J* 14.0 and 9.0), 2.95 (1H, dd, *J* 14.0 and 6.0), 3.13 (2H, m), 3.18 (6H, s), 4.17 (1H, t, *J* 6.0), 4.23 (1H, dd, *J* 9.0 and 6.0), 4.87 (1H, d, *J* 13.0), 4.91 (1H, d, *J* 13.0), 7.06–7.20 (10H, m); *m/z* (FAB) 387.1917 ($M^+ + 1$, requires 387.1920).

Compound **11a** (3.0 g, 7.8 mmol) was dissolved in 30 mL 70% TFA–H_2O and the solution was stirred at rt for 2 h. The solvent was removed on a rotary evaporator to give a yellow oil, which was dissolved in 150 mL ethyl acetate and washed with saturated $NaHCO_3$ and brine. The organic phase was dried over anhydrous Na_2SO_4, and the solvent removed to give compound **12a** as a white solid (2.1 g, 84%). A single crystal for analysis was obtained by slow evaporation of a hexanes–EtOAc solution of **12a**: mp 141–142 °C; ^1H δ_H (500 MHz, d_4-methanol) 2.77–2.85 (2H, m), 4.41 (0.5H, d, *J* 12.5), 4.66 (0.5H, ddd, *J* 9.0, 5.0 and 1.5), 4.77 (0.5H, m), 4.80 (0.5H, d, *J* 12.0), 4.88 (0.5H, d, *J* 12.5), 4.99 (0.5H, d, *J* 12.5), 5.44 (0.5H, d, *J* 6.0), 5.67 (0.5H, d, *J* 6.0), 6.08 (0.5H, dd, *J* 6.0 and 1.5), 6.19 (0.5H, dd, *J* 6.0 and 1.5), 6.95–7.24 (10H, m); *m/z* (FAB) 323.1396 ($M^+ + 1$, $C_{19}H_{19}N_2O_3$ requires 323.1396).

To a stirred solution of compound **12a** (966 mg, 3.0 mmol) in 12 mL anhydrous THF was added 60% NaH (120 mg, 3.0 mmol) at 0 °C. The solution was stirred at rt for 0.5 h. Then 4-chloromethyl-1-Boc-imidazole (**7**, 700 mg, 3.2 mmol) was added, and the solution was stirred at rt for 0.5 h. The reaction mixture was then cooled to room temperature and the solvent was removed on a rotary evaporator. The resulting residue was dissolved in EtOAc, washed with aqueous NH_4Cl solution and brine. The organic phase was dried over Na_2SO_4 and concentrated to give a yellow oil, which was subjected to silica gel column chromatography

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using hexanes–EtOAc (3 : 1–1 : 1) to afford **13a1** as a colorless oil (150 mg, 10%): δ_H (500 MHz, $CDCl_3$) 1.53 (9H, s), 2.86 (2H, m), 4.35–4.60 (2.5H, m), 4.84–5.04 (2.5H, m), 5.58 (0.5H, d, *J* 6.0), 5.79 (0.5H, d, *J* 6.0), 6.10 (0.5H, d, *J* 6.0), 6.31 (0.5H, d, *J* 6.0), 6.96–7.30 (11H, m), 7.95 (1H, s); *m/z* (FAB) 503.2294 ($M^+ + 1$, requires 503.2294).

Compound **13a1** (100 mg, 0.2 mmol) was treated with 2 mL 20% TFA–CH_2Cl_2 at rt for 1 h. After removal of the solvent, **33** was obtained as a colorless oil (78 mg, 97%): δ_H (500 MHz, $CDCl_3$) 2.82 (2H, m), 4.48 (1.5H, m), 4.73 (1H, m), 4.83–5.05 (2.5H, m), 5.49 (0.5H, d, *J* 6.0), 5.70 (0.5H, d, *J* 6.0), 6.19 (0.5H, d, *J* 6.0), 6.34 (0.5H, d, *J* 6.0), 6.88–7.28 (11H, m), 8.38 (1H, s); *m/z* (FAB) 403.1770 ($M^+ + 1$, $C_{23}H_{23}N_4O_3$ requires 403.1770).

Syntheses of compounds 34 and 35

To a stirred solution of compound **12a** (1 g, 3.1 mmol) in 14 mL anhydrous THF was added 60% NaH (124 mg, 3.1 mmol) at 0 °C. The solution was stirred at rt for 0.5 h. Then 4-chloromethyl-1-tritylimidazole[25] (**8**, 850 mg, 3.1 mmol) was added, and the solution was stirred at 60 °C for 2 h. The reaction mixture was then cooled to room temperature and the solvent was removed on a rotary evaporator. The residue obtained was subjected to silica gel column chromatography using hexanes–EtOAc (3 : 1–1 : 1) to afford compound **13a2** as a colorless oil (1.2 g, 60%): δ_H (500 MHz, d_4-methanol) 2.71 (2H, m), 4.43 (1.5H, m), 4.54 (0.5H, d, *J* 15.0), 4.57 (0.5H, d, *J* 15.0), 4.70 (0.5H, m), 4.75 (0.5H, m), 4.80 (0.5H, d, *J* 12.0), 4.87 (0.5H, d, *J* 12.0), 4.96 (0.5H,d, *J* 12.5), 5.55 (0.5H, d, *J* 6.0), 5.76 (0.5H, d, *J* 6.0), 6.10 (0.5H, dd, *J* 6.0 and 1.5), 6.22 (0.5H, dd, *J* 6.0 and 1.5), 6.76 (1H, s), 6.85–7.28 (20H, m), 7.30 (1H, s); *m/z* (FAB) 645.2865 ($M^+ + 1$, $C_{42}H_{37}N_4O_3$ requires 645.2866).

Reaction of scaffold **12a** with 1-benzyl-4-chloromethyl-1*H*-imidazole following previously described procedure for **13a2** gave **34** as a colorless oil (315 mg, 44%): δ_H (500 MHz, d_4-methanol) 2.79–2.94 (2H, m), 4.47–4.63 (2.5H, m), 4.84–5.01 (2.5H, m), 5.10 (2H, s), 5.66 (0.5H, d, *J* 5.9), 5.87 (0.5H, d, *J* 5.9), 6.17 (0.5H, d, *J* 5.9), 6.32 (0.5H, d, *J* 5.9), 6.98–7.34 (16H, m), 7.67 (1H, s); δ_C (125 MHz, d_4-methanol) 37.37, 44.12, 52.19, 59.87, 69.42, 109.96, 110.12, 115.03, 120.29, 128.29, 129.19, 129.58, 129.71, 129.82, 129.98, 130.44, 131.15, 138.40, 138.50, 139.16, 154.84, 166.30; *m/z* (FAB) 493.2226 ($M^+ + 1$, $C_{30}H_{28}N_4O_3$ requires 493.2240).

Scaffold **13a2** (213 mg, 0.33 mmol) was dissolved in AcOEt (2 mL). To the solution it was added bromomethyl-benzene (44 μL, 0.37 mmol) and the mixture was stirred under N_2 at 60 °C for 24 h. The solvent was removed, MeOH (2.5 mL) were added, and the solution was stirred under N_2 at 60 °C for 24 h. The solution was concentrated under reduced pressure and the residue was washed with hexanes (2 × 5 mL). The resulting residue was subjected to silica gel column chromatography using 1–5% MeOH–CH_2Cl_2 as eluant to afford **35** as a colorless oil (20 mg, 12%): δ_H (500 MHz, d_4-methanol) 2.51 (1H, d, *J* 6.6), 2.55–2.72 (1H, m), 3.94–4.26 (1.5H, m), 4.39 (0.5H, t, *J* 5.9), 4.50 (0.5H, t, *J* 5.7), 4.68–4.90 (2.5H, m), 4.95 (2.5H, s), 5.23 (0.5H, d, *J* 6.1), 5.78 (0.5H, d, *J* 6.0), 6.03 (0.5H, dd, *J* 1.4 and 6.0), 6.63–7.29 (16H, m), 7.42 (1H, s); δ_C (125 MHz, d_4-methanol) 36.7, 48.8, 58.3, 59.0, 68.2, 109.5, 109.9, 111.0, 126.2, 127.3, 128.2, 128.5, 128.6, 128.7, 128.8, 129.0, 129.0, 129.1, 129.9, 129.9, 135.9, 136.3, 153.0, 164.4; *m/z* (FAB) 493.2242 ($M^+ + 1$, $C_{30}H_{28}N_4O_3$ requires 493.2240).

Syntheses of compounds 40 and 41

Compound **13a2** (1.2 g, 1.86 mmol) was dissolved in 30 mL MeOH–EtOAc (2 : 1) and to the solution was added 10% Pd/C. The mixture was hydrogenated at atmospheric pressure overnight. Then the solution was filtered, and the solvent was removed to give compound **14a2** as a colorless oil (0.92 g, 96%): δ_H (500 MHz, d_4-methanol) 2.50 (2H, m), 3.00 (2H, m), 3.22 (1H, t, *J* 5.0), 2.26 (1H, dd, *J* 8.0 and 5.0), 3.60 (1H, dd, *J* 11.5 and 5.0), 4.39–4.57 (3H, m), 6.90 (1H, s), 7.07–7.39 (20H, m), 7.42 (1H, s); *m/z* (FAB) 513.2653 ($M^+ + 1$, $C_{34}H_{33}N_4O$ requires 513.2654).

Reaction of scaffold **14a2** with L-leucine methyl ester isocyanate following the previously described general procedures gave trityl-protected **40** as a colorless oil in 80% yield: δ_H (500 MHz, d_4-methanol) 0.81 (3H, d, *J* 6.5), 0.82 (3H, d, *J* 6.5), 1.02 (1H, m), 1.26 (2H, m), 2.83 (1H, ddd, *J* 14.0, 11.0 and 4.0), 3.01 (1H, dd, *J* 13.5 and 9.0), 3.12 (1H, dt, *J* 12.2 and 3.0), 3.31 (1H, dd, *J* 13.5 and 3.8), 3.40 (1H, ddd, *J* 11.7, 11.7 and 4.0), 3.64 (3H, s), 4.03 (2H, m), 4.21 (1H, dt, *J* 8.3 and 5.2), 4.31 (1H, d, *J* 14.5), 4.41 (1H, br s), 4.65 (1H, d, *J* 14.5), 6.78 (1H, s), 7.05–7.34 (20H, m), 7.36 (1H, s); *m/z* (FAB) 684.3552 ($M^+ + 1$, $C_{42}H_{46}N_5O_4$ requires 684.3550).

General procedure for deprotection and hydrolysis

Trityl-protected compound **15** (0.2 mmol), was dissolved in 2 mL of 40% TFA–CH_2Cl_2. Triethylsilane was added dropwise until the deep yellow color disappeared. The mixture was stirred at rt for 1 h. The solvent was removed and the resulting residue was dried under reduced pressure to give a yellow solid. After washing with hexanes, the residue was subjected to silica gel column chromatography using CH_2Cl_2 followed by 5–10% MeOH–CH_2Cl_2 as eluant. The fractions were combined and concentrated to afford a colorless oil. The deprotected product (0.2 mmol) was then dissolved in a 0.5 mL of MeOH, and then 1 mL of 1 N NaOH. The mixture was stirred at rt for 1 h. The solvent was removed under reduced pressure, and the resulting residue was suspended in 2 mL of 30% MeOH–CH_2Cl_2, and the suspension was passed through a pad of silica gel. The solid phase was further eluted with 30–50% MeOH–CH_2Cl_2 solution. The fractions containing the product were combined and the solvent was removed to afford the target molecules in 80–85% yields.

Deprotection of trityl-protected **40** following the general procedure described previously afforded compound **40** as a colorless oil in 88% yield: δ_H (500 MHz, d_4-methanol) 0.83 (3H, d, *J* 6.5), 0.84 (3H, d, *J* 6.5), 1.20 (1H, m), 1.36 (2H, m), 2.97 (1H, m), 3.10 (2H, m), 3.25 (1H, dt, *J* 13.5 and 3.5), 3.45 (1H, m), 3.64 (3H, s), 4.10 (1H, br d, *J* 12.0), 4.21 (1H, m), 4.46 (1H, d, *J* 15.5), 4.73 (2H, m), 4.90 (1H, br s), 7.10–7.34 (6H, m), 8.67 (1H, s); δ_C (125 MHz, d_4-methanol) 22.1, 23.0, 24.9, 37.7, 37.8, 41.4, 41.5, 47.0, 52.4, 52.6, 60.2, 118.5, 127.6, 129.2, 129.2, 129.2, 129.9, 129.9, 134.6, 137.4, 156.7, 168.8, 174.8; *m/z* (FAB) 442.2455 ($M^+ + 1$, $C_{23}H_{32}N_5O_4$ requires 442.2454).

Saponification of **40** following the general procedure described previously afforded **41** as a colorless oil in 85% yield: δ_H (500 MHz,

d_4-methanol) 0.68 (3H, d, *J* 6.0), 0.69 (3H, d, *J* 6.0), 1.23 (1H, m), 1.31 (2H, m), 2.61 (1H, ddd, *J* 14.0, 10.5 and 3.8), 2.76 (1H, dt, *J* 12.3 and 3.2), 3.03–3.13 (3H, m), 3.66 (1H, br d, *J* 13.5), 3.96 (1H, dd, *J* 9.8 and 4.4), 4.25 (1H, d, *J* 15.0), 4.43 (1H, d, *J* 15.0), 4.61 (1H, t, *J* 5.5), 6.84 (1H, s), 6.95–7.03 (5H, m), 7.51 (1H, s); δ_C (500 MHz, d_4-methanol) 22.5, 24.1, 26.3, 38.8, 39.6, 43.3, 44.5, 47.1, 55.8, 60.3, 119.1, 128.3, 129.9, 129.9, 131.3, 131.3, 135.0, 137.1, 139.1, 158.8, 170.1, 180.6; *m/z* (FAB) 428.2297 ($M^+ + 1$, $C_{22}H_{30}N_5O_4$ requires 428.2298).

Syntheses of compounds 42 and 43

To a stirred solution of compound **12a** (450 mg, 1.4 mmol) in 5 mL anhydrous THF was added 60% NaH (56 mg, 1.4 mmol) at 0 °C. The solution was stirred at rt for 0.5 h. Then 4-chloroallyl-1-tritylimidazole[26] (**10**, 540 mg, 1.4 mmol) was added, and the solution was stirred at 60 °C for 2 h. Then the reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The residue obtained was subjected to silica gel column chromatography using hexanes–EtOAc (3 : 1–1 : 1) to afford compound **13a3** as a colorless oil (200 mg, 21%): δ_H (500 MHz, d_4-methanol) 2.80 (2H, m), 4.08 (2H, m), 4.48 (0.5H, d, *J* 12.0), 4.78 (0.5H, m), 4.84 (0.5H, m), 4.85 (0.5H, d, *J* 12.0), 4.93 (0.5H, d, *J* 12.0), 5.04 (0.5H, d, *J* 12.0), 5.49 (0.5H, d, *J* 6.0), 5.73 (0.5H, d, *J* 6.0), 6.05 (1H, m), 6.16 (0.5H, d, *J* 6.0), 6.27 (1.5H, m), 6.86 (0.5H, s), 6.87 (0.5H, s), 6.90–7.32 (25H, m), 7.41 (1H, s); *m/z* (FAB) 670.3024 ($M^+ + 1$, $C_{44}H_{39}N_4O_3$ requires 671.3022).

Compound **13a3** (200 mg, 0.3 mmol) was dissolved in 10 mL MeOH–EtOAc (2 : 1) and to the solution was added 10% Pd/C. The mixture was hydrogenated at atmospheric pressure overnight. Then the solution was filtered, and the solvent was removed to give compound **14a3** as a colorless oil (160 mg, 99%): δ_H (500 MHz, d_4-methanol) 1.81 (2H, m), 2.47 (2H, t, *J* 8.0), 2.78 (2H, m), 2.98 (1H, dt, *J* 12.3 and 3.5), 3.06 (1H, dt, *J* 11.6 and 3.5 Hz), 3.29 (2H, m), 3.35 (1H, m), 3.51 (1H, dd, *J* 10.0 and 3.5), 6.48 (1H, s), 7.00–7.28 (20H, m), 7.29 (1H, s); *m/z* (FAB) 541.2966 ($M^+ + 1$, $C_{36}H_{37}N_4O$ requires 541.2967).

Scaffold **14a3** was coupled to the L-leucine methyl ester isocyanate following the previously described general procedures to give trityl-protected **42** as a colorless oil in 85% yield: δ_H (500 MHz, d_4-methanol) 0.76 (3H, d, *J* 6.0), 0.77 (3H, d, *J* 6.0), 1.00 (1H, m), 1.23 (2H, m), 1.80 (2H, m), 2.46 (2H, t, *J* 7.5), 2.85 (2H, m), 3.01 (1H, dd, *J* 13.5 and 8.5), 3.16 (1H, ddd, *J* 13.5, 8.8 and 6.0), 3.30 (2H, m), 3.45 (1H, m), 3.59 (3H, s), 4.02 (1H, br d, *J* 13.5), 4.07 (1H, d, *J* 8.0), 4.18 (1H, m), 4.36 (1H, br s), 6.48 (1H, s), 7.03–7.28 (20H, m), 7.29 (1H, s); *m/z* (FAB) 712.3861 ($M^+ + 1$, $C_{44}H_{50}N_5O_4$ requires 712.3863).

Deprotection of the above compound following the general procedure described previously afforded **42** as colorless oil in 90% yield: δ_H (500 MHz, d_4-methanol) 0.77 (3H, d, *J* 5.0), 0.78 (3H, d, *J* 5.0), 1.12 (1H, m), 1.29 (2H, m), 1.82 (2H, m), 2.61 (2H, m), 2.89 (2H, m), 3.03 (1H, dd, *J* 13.5 and 8.0), 3.26 (3H, m), 3.40 (1H, m), 3.59 (3H, s), 4.04 (1H, br d, *J* 13.5), 4.15 (1H, m), 4.55 (1H, m), 4.66 (1H, d, *J* 7.5), 7.04 (1H, s), 7.10–7.23 (5H, m), 8.46 (1H, s); δ_C (125 MHz, d_4-methanol) 21.9, 22.1, 23.0, 24.9, 26.0, 37.8, 37.9, 41.6, 46.5, 46.6, 52.5, 52.5, 60.3, 116.0, 127.5, 129.2, 129.2, 130.0, 130.0, 133.4, 133.5, 137.7, 156.7, 168.5, 174.9; *m/z* (FAB) 470.2766 ($M^+ + 1$, $C_{25}H_{36}N_5O_4$ requires 470.2767).

Saponification of **42** following the general procedure described previously afforded **43** as a colorless oil in 85% yield: δ_H (500 MHz, d_4-methanol) 0.76 (3H, d, *J* 6.5), 0.77 (3H, d, *J* 6.5), 1.27 (1H, m), 1.38 (2H, m), 1.76 (2H, m), 2.49 (2H, t, *J* 7.5), 2.70 (1H, ddd, *J* 14.0, 11.0 and 4.0), 2.80 (1H, dt, *J* 12.5 and 3.2), 3.10 (2H, d, *J* 6.0), 3.18 (1H, m), 3.31 (2H, m), 3.78 (1H, br d, *J* 13.2), 4.03 (1H, dd, *J* 10.0 and 4.5), 4.60 (1H, t, *J* 5.5), 6.80 (1H, s), 7.04–7.16 (5H, m), 7.70 (1H, s); δ_C (125 MHz, d_4-methanol) 22.5, 24.1, 24.8, 26.4, 27.7, 38.7, 39.5, 43.5, 47.6, 48.3, 56.1, 60.4, 117.8, 128.4, 130.0, 130.0, 131.3, 131.3, 136.0, 137.4, 139.2, 158.8, 170.2, 181.1; *m/z* (FAB) 456.2612 ($M^+ + 1$, $C_{24}H_{34}N_5O_4$ requires 456.2611).

Syntheses of compound 32

To a stirred solution of compound **12a** (400 mg, 1.2 mmol) in 6 mL anhydrous THF was added 60% NaH (50 mg, 1.2 mmol) at 0 °C. The solution was stirred at rt for 0.5 h. Then 4-(3-chloro-propyl)-1-tritylimidazole (480 mg, 1.2 mmol) in 4 mL anhydrous THF and catalytic amounts of Bu_4NI were added. The mixture was stirred at reflux for 4 h, cooled and quenched with sat. NH_4Cl aqueous solution. The mixture was extracted with dichloromethane. The organic layer was washed with brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue obtained was a mixture of unreacted starting materials and compound **16**. The mixture was subjected to silica gel column chromatography using MeOH–CH_2Cl_2 (0.5–5%) to afford compound **16** as a colorless oil (210 mg, 30%): δ_H (500 MHz, d_4-methanol) 1.60 (1H, m), 1.92 (1H, m), 2.42 (1H, t, *J* 7.6), 2.58 (1H, t, *J* 7.6), 2.92–3.06 (2H, m), 3.58 (0.5H, m), 3.89 (0.5H, m), 4.03 (0.5H, m), 4.10 (0.5H, m), 4.85 (0.5H. t, *J* 7.2), 5.03 (0.5H, t, *J* 7.2), 5.43 (0.5H, dd, *J* 6.0 and 2.8), 5.68 (0.5H, dd, *J* 5.6 and 3.2), 6.16 (0.5H, d, *J* 6.0), 6.38 (0.5H, d, *J* 6.0), 6.50 (0.5H, s), 6.53 (0.5H, s), 7.10–7.35 (21H, m), 8.30 (0.5H, d, *J* 4.0), 8.36 (0.5H, d, *J* 4.0); *m/z* (FAB) 583.2710 ($M^+ + 1$, $C_{37}H_{35}N_4O_3$ requires 583.2709).

To a stirred solution of compound **16** (200 mg, 0.36 mmol) in 5 mL anhydrous THF was added 60% NaH (16 mg, 0.4 mmol) at 0 °C. The solution was stirred at rt for 0.5 h. Then 4-chloromethyl-tritylimidazole[25] (**8**, 133 mg, 0.37 mmol) was added, and the solution was stirred at 60 °C for 2 h. The reaction mixture was then cooled to room temperature and the solvent was removed under reduced pressure. The residue obtained was subjected to silica gel column chromatography using hexanes–EtOAc (3 : 1–1 : 1) to afford compound **17** as a colorless oil (270 mg, 80%): δ_H (500 MHz, d_4-methanol) 1.39 (1H, m), 1.67 (1H, m), 2.18 (1H, t, *J* 7.5), 2.35 (1H, m), 2.50–2.68 (2H, m), 3.28 (0.5H, dt, *J* 10.5 and 6.5), 3.60 (0.5H, dt, *J* 10.5 and 6.5), 3.73 (0.5H, dt, *J* 10.5 and 6.5), 3.84 (0.5H, dt, *J* 10.5 and 6.5), 4.22 (0.5H, d, *J* 15.0), 4.25 (0.5H, d, *J* 15.0), 4.46 (0.5H, d, *J* 15.0), 4.51 (0.5H, d, *J* 15.0), 4.60 (0.5H, t, *J* 7.0), 4.77 (1H, t, *J* 7.0), 5.45 (0.5H, d, *J* 6.0), 5.65 (0.5H, d, *J* 6.0), 5.89 (0.5H, d, *J* 6.0), 6.12 (0.5H, d, *J* 6.0), 6.28 (0.5H, s), 6.32 (0.5H, s), 6.56 (0.5H, s), 6.57 (0.5H, s), 6.78–7.20 (37H, m), 7.28 (1H, d, *J* 7.0); *m/z* (FAB) 905.4183 ($M^+ + 1$, $C_{60}H_{53}N_6O_3$ requires 905.4179).

Deprotection of compound **17** following the general procedure described previously, using 40% TFA–triethylsilane, afforded **32** as

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a colorless oil in 85% yield: δ_H (500 MHz, d_4-methanol) 1.68 (1H, m), 1.90 (1H, m), 2.53 (1H, t, *J* 7.5), 2.68 (1H, t, *J* 7.5), 2.79–2.95 (2H, m), 3.55 (0.5H, dt, *J* 10.0 and 6.0), 3.86 (0.5H, dt, *J* 10.5 and 6.5), 4.05 (1H, m), 4.64–4.85 (3H, m), 5.73 (0.5H, d, *J* 6.0), 5.95 (0.5H, d, *J* 6.0), 6.25 (0.5H, d, *J* 6.0), 6.36 (0.5H, d, *J* 6.0), 6.98–7.20 (5H, m), 7.24 (1H, s), 7.42 (0.5H, s), 7.45 (1H, s), 8.74 (1H, s), 8.76 (1H, s); *m/z* (FAB) 421.1987 ($M^+ + 1$, $C_{22}H_{25}N_6O_3$ requires 421.1988).

Syntheses of compounds 44 and 45

Alkylation of compound **12a** with 4-chloromethyl-5-methyl-1-tritylimidazole[25] (**9**) using conditions similar to that described for the synthesis of compound **13a2**, afforded compound **13a4** as a colorless oil in 70% yield: δ_H (500 MHz, d_4-methanol) 1.40 (3H, s), 2.76 (2H, m), 4.44 (1.5H, m), 4.55 (0.5H, d, *J* 15.0), 4.59 (0.5H, d, *J* 15.0), 4.77 (0.5H, m), 4.84 (0.5H, d, *J* 12.0), 4.87 (0.5H, m), 4.93 (0.5H, d, *J* 15.0), 5.02 (0.5H, d, *J* 15.0), 5.63 (0.5H, d, *J* 5.8), 5.83 (0.5H, d, *J* 5.8), 6.19 (0.5H, d, *J* 5.8), 6.31 (0.5H, d, *J* 5.8), 6.76 (1H, s), 6.96–7.40 (21H, m); *m/z* (FAB) 659.3025 ($M^+ + 1$, $C_{43}H_{39}N_4O_3$ requires 659.3022).

Compound **14a4** was obtained as a colorless oil in 98% yield by hydrogenation of compound **14a3**, using similar conditions described previously: δ_H (500 MHz, d_4-methanol) 1.42 (3H, s), 2.10 (1H, br), 2.77 (2H, m), 3.00 (1H, dt, *J* 12.5 and 4.0), 3.32 (2H, m), 3.38 (1H, dd, *J* 13.5 and 3.2), 3.53 (1H, dd, *J* 10.0 and 3.3), 4.35 (1H, d, *J* 14.5), 4.60 (1H, d, *J* 14.5), 7.00–7.34 (21H, m); *m/z* (FAB) 527.2812 ($M^+ + 1$, $C_{35}H_{35}N_4O$ requires 527.2811).

Scaffold **14a4** was coupled to the L-leucine methyl ester isocyanate following he previously described general procedures to give trityl-protected **44** as a colorless oil in 85% yield: δ_H (500 MHz, d_4-methanol) 0.81 (3H, d, *J* 6.0), 0.82 (3H, d, *J* 6.0), 1.02 (1H, m), 1.27 (2H, m), 1.46 (3H, s), 2.91 (1H, ddd, *J* 13.5, 10.5 and 3.5), 3.03 (1H, dd, *J* 14.0 and 8.8), 3.15 (1H, dt, *J* 12.0 and 3.0), 3.33 (1H, dd, *J* 13.5 and 3.5), 3.39 (1H, ddd, *J* 11.7, 11.7 and 4.0), 3.64 (3H, s), 4.00 (1H, br d, *J* 8.0), 4.04 (1H, br d, *J* 13.5), 4.22 (1H, dt, *J* 8.3 and 5.0), 4.41 (1H, d, *J* 14.5), 4.42 (1H, br s), 4.58 (1H, d, *J* 14.5), 7.06–7.35 (21H, m); *m/z* (FAB) 698.3706 ($M^+ + 1$, $C_{43}H_{48}N_5O_4$ requires 698.3706).

Deprotection of the above mentioned compound following the general procedure described previously afforded **44** as a colorless oil in 88% yield: δ_H (500 MHz, d_4-methanol) 0.81 (3H, d, *J* 6.0), 0.82 (3H, d, *J* 6.0), 1.10 (1H, m), 1.30 (2H, m), 2.36 (3H, s), 3.06 (2H, m), 3.28 (1H, *J* 13.5 and 3.8), 3.45 (1H, ddd, *J* 12.0, 12.0 and 4.5), 3.63 (3H, s), 4.08 (1H, br d, *J* 13.5), 4.20 (1H, m), 4.54 (4H, m), 7.13–7.25 (5H, m), 8.43 (1H, s); δ_C (100 MHz, d_4-methanol) 9.5, 22.1, 23.0, 24.8, 37.7, 37.9, 40.4, 41.7, 47.0, 52.4, 52.5, 60.6, 124.6, 127.6, 128.6, 129.2, 129.2, 129.9, 129.9,132.8, 137.6, 156.7, 168.5, 174.6; *m/z* (FAB) 456.2612 ($M^+ + 1$, $C_{24}H_{34}N_5O_4$ requires 456.2611).

Saponification of **44** following the general procedure described previously afforded **45** as a colorless oil in 85% yield: δ_H (500 MHz, d_4-methanol) 0.68 (3H, d, *J* 6.0), 0.70 (3H, d, *J* 6.0), 1.21 (1H, m), 1.31 (2H, m), 2.08 (3H, s), 2.66 (1H, ddd, *J* 13.5, 10.0 and 3.7), 2.75 (1H, dd, *J* 12.3 and 3.2), 3.00–3.15 (3H, m), 3.66 (1H, br d, *J* 13.5), 3.95 (1H, dd, *J* 10.0 and 4.5), 4.28 (1H, d, *J* 14.8), 4.38 (1H, d, *J* 14.8), 4.60 (1H, t, *J* 5.5), 6.95–7.04 (5H, m), 7.42 (1H, s); δ_C (125 MHz, d_4-methanol) 10.4, 22.6, 24.1, 26.3, 38.8, 39.5, 42.9, 43.6, 46.8, 56.1, 60.3, 128.3, 129.1, 129.6, 129.9, 129.9, 131.2, 131.3, 135.4, 139.1, 158.7, 169.9, 181.1; *m/z* (FAB) 442.2455 ($M^+ + 1$, $C_{23}H_{32}N_5O_4$ requires 442.2454).

Syntheses of compounds 69 and 70

Compounds **11c** were synthesized using conditions similar to that described for the synthesis of compound **11a**, and were purified using the same chromatographic condition. Using Cbz-*p*-fluoro-L-phenylalanine, compound **11c** was obtained as a white solid in 95% yield: mp 118–119 °C; δ_H (500 MHz, $CDCl_3$) 3.01 (2H, m), 3.28 (1H, s), 3.29 (1H, s), 4.19 (1H, t, *J* 5.0), 4.33 (1H, m), 5.07 (2H, br s), 5.29 (1H, m), 5.78 (1H, m), 6.95 (2H, t, *J* 8.7), 7.13 (2H, m), 7.28–7.36 (5H, m); *m/z* (FAB) 405.1825 ($M^+ + 1$, $C_{21}H_{26}N_2O_5F$ requires 405.1826).

Compound **12c** was synthesized using conditions similar to that described for the synthesis of compound **12a**. Compound **12c** was obtained in 85% yield as a colorless solid: δ_H (500 MHz, $CDCl_3$) 2.85–3.06 (2H, m), 4.65 (0.5H, d, *J* 12.0), 4.87 (0.5H, t, *J* 6.5), 4.96 (0.5H, d, *J* 12.0), 5.03 (1.0H, m), 5.14 (0.5H, d, *J* 12.0), 5.40 (0.25H, d, *J* 5.5), 5.41 (0.25H, d, *J* 5.5), 5.64 (0.25H, d, *J* 5.5), 5.65 (0.25H, d, *J* 5.5), 6.16 (0.5H, d, *J* 6.0), 6.37 (0.5H, d, *J* 6.0), 6.83–7.40 (10H, m); *m/z* (FAB) 341.1302 ($M^+ + 1$, $C_{19}H_{18}N_2O_3F$ requires 341.1301).

Alkylation of compound **12c** with 4-chloromethyl-5-methyl-1-tritylimidazole[27] (**9**) using conditions similar to that described for the synthesis of compound **13a2**, afforded compounds **13c** as colorless oils in 65% yield: δ_H (500 MHz, $CDCl_3$) 1.44 (1.5H, s), 1.45 (1.5H, s), 2.75–2.92 (2H, m), 4.46 (0.5H, d, *J* 12.0), 4.48 (0.5H, d, *J* 12.0), 4.58 (0.5H, d, *J* 12.0), 4.64 (0.5H, d, $J = 14.5$), 4.73 (0.5H, d, *J* 14.5), 4.85 (0.5H, t, *J* 6.5), 4.91 (0.5H, d, *J* 12.0), 4.98 (0.5H, d, *J* 12.0), 5.00 (0.5H, t, *J* 6.5), 5.10 (0.5H, d, *J* 2.0), 5.76 (0.5H, d, *J* 6.0), 5.91 (0.5H, d, *J* 6.0), 6.14 (0.5H, d, *J* 6.0), 6.34 (0.5H, d, *J* 6.0), 7.04–7.35 (25H, m); *m/z* (FAB) 677.2928 ($M^+ + 1$, $C_{43}H_{38}N_4O_3F$ requires 677.2928).

Compounds **14c** were obtained in 95% yields by hydrogenation of compound **13c**, using similar conditions described previously, as a colorless oil: δ_H (500 MHz, $CDCl_3$) 1.41 (3H, s), 2.80 (2H, m), 3.00 (1H, dt, *J* 12.5 and 4.0), 3.28–3.33 (3H, m), 3.50 (1H, dd, *J* 10.0 and 3.5), 4.36 (1H, d, *J* 14.5), 4.58 (1H, d, *J* 14.5), 6.86–7.40 (20H, m); *m/z* (FAB) 545.2717 ($M^+ + 1$, $C_{35}H_{34}N_4OF$ requires 545.2717).

Scaffold **14c** was coupled to L-leucine methyl ester isocyanate following the previously described general procedures to give trityl-protected **69** as colorless oil in 87% yield: δ_H (500 MHz, d_4-methanol) 0.80 (6H, d, *J* 6.0), 1.09 (1H, m), 1.30 (2H, m), 1.49 (3H, s), 2.78 (1H, ddd, *J* 13.5, 10.5 and 3.5), 3.01 (1H, dd, *J* 14.0 and 8.5), 3.08 (1H, dt, *J* 12.0 and 3.0), 3.22 (1H, dd, *J* 14.0 and 4.0), 3.33 (1H, ddd, *J* 12.5, 11.0 and 4.5), 3.61 (3H, s), 3.94 (1H, br d, *J* 14.0), 4.11 (1H, br d, *J* 8.0), 4.24 (1H, m), 4.34 (1H, d, *J* 14.5), 4.39 (1H, m), 4.55 (1H, d, *J* 14.5), 6.85–7.34 (20H, m); *m/z* (FAB) 716.3609 ($M^+ + 1$, $C_{43}H_{47}N_5O_4F$ requires 716.3612).

Deprotection of the above mentioned compound following the general procedure described previously afforded **69** as a colorless oil in 85% yield: δ_H ($CDCl_3$, 500 MHz) 0.79 (d, $J = 6.2$, 3H), 0.82 (d, $J = 6.2$, 3H), 1.17 (m, 1H), 1.28 (m, 1H), 1.34 (m, 1H), 2.31 (s, 3H), 3.03 (m, 2H), 3.20 (br d, $J = 10.0$, 1H), 3.42 (m,1H), 3.61 (s, 3H), 4.06 (br d, $J = 11.5$, 1H), 4.18 (m, 1H), 4.47 (d, $J = 15.0$,

1H), 4.55 (d, $J = 15.0$, 1H), 4,63 (br s, 1H), 4.74 (br s, 1H), 6.89 (t, $J = 8.0$, 2H), 7.08 (dd, $J = 7.5$, 5.5, 2H), 8.44 (s, 1H); δ_C ($CDCl_3$, 125 MHz) 9.3, 22.0, 22.8, 24.9, 36.8, 37.8, 40.4, 41.6, 47.0, 52.5, 52.6, 60.0, 115.9, 116.1, 124.4, 128.6, 131.4, 131.5, 133.0, 133.1, 156.7, 161.4, 163.4, 168.7, 174.7; *m/z* (FAB) 474.2517 ($M^+ + 1$, $C_{24}H_{33}N_5O_4F$ requires 474.2517).

Saponification of **69** following the general procedure described previously afforded **70** as a colorless oil in 85% yield: δ_H (500 MHz, d_4-methanol) 0.76 (3H, d, *J* 6.0), 0.78 (3H, d, *J* 6.0), 1.28–1.44 (3H, m), 2.17 (3H, s), 2.82 (1H, ddd, *J* 14.0, 10.0 and 3.5), 2.90 (1H, dt, *J* 12.0 and 3.5), 3.10 (2H, m), 3.21 (2H, m), 3.78 (1H, br d, *J* 13.0), 4.03 (1H, dd, *J* 10.0 and 4.5), 4.36 (1H, d, *J* 14.8), 4.48 (1H, d, *J* 14.8), 4.65 (1H, t, *J* 5.5), 6.82 (2H, t, *J* 8.5), 7.06 (2H, dd, *J* 8.5 and 5.5), 7.57 (1H, s); δ_C (125 MHz, d_4-methanol) 10.2, 22.4, 24.0, 26.3, 37.8, 39.5, 42.8, 43.2, 47.0, 55.7, 60.2, 116.4, 116.6, 129.1, 129.5, 132.9, 133.0, 133.0, 135.0, 135.3, 135.3, 158.8, 169.9, 180.5; *m/z* (FAB) 460.2359 ($M^+ + 1$ requires 460.2360).

Synthesis of compound 73

A mixture of L-leucine methyl ester hydrochloride (1.83 g, 10 mmol), Cbz-L-leucine (2.99 g, 10 mmol), DIEA (1.8 mL, 10 mmol), EDCI (1.92 g, 10 mmol), in 20 mL anhydrous methylene chloride was stirred at rt for 5 h. The reaction mixture was diluted with 80 mL methylene chloride, and the solution was washed with 1 N HCl, saturated sodium bicarbonate solution, and brine. The organic phase was dried over sodium sulfate, and passed through a pad of silica gel, and the solid phase was washed with 1–2.5% MeOH–CH_2Cl_2. Fractions were combined and the solvent was removed to afford compound **21a** (3.7 g, 87%) as a colorless oil: δ_H (500 MHz, d_4-methanol) 0.80 (3H, d, *J* 6.5), 0.81 (3H, d, *J* 6.5), 1.38 (1H, m), 1.43 (1H, m), 1.49 (1H, m), 2.95–3.08 (2H, m), 3.62 (3H, s), 4.36 (1H, m), 4.48 (1H, m), 5.01 (1H, d, *J* 14.8), 5.03 (1H, d, *J* 14.8), 5.22 (1H, br s), 6.04 (1H, m), 7.11–7.32 (10H, m).

To a solution of compound **21a** (1 g, 2.35 mmol) in 15 mL anhydrous dichloromethane was added DIBAL-H (1.5 M in toluene) (3.2 mL, 4.8 mmol) at −78 °C. The reaction was stirred at this temperature for 1 h before being quenched by adding 1 mL of methanol and 7 mL of water. After warming to rt, the reaction mixture was extracted with dichloromethane. The organic layer was separated and dried over Na_2SO_4 and concentrated to give a yellow solid. The mixture was subjected to silica gel column chromatography using hexanes–EtOAc (2 : 1) as eluant to afford aldehyde **21b** (380 mg, 40%) as a colorless oil: δ_H (500 MHz, d_4-methanol) 0.88 (6H, m), 1.24 (1H, m), 1.31 (1H, m), 1.42 (1H, m), 3.06 (1H, m), 3.14 (1H, m), 4.43 (2H, m), 5.11 (2H, br s), 5.30 (1H, m), 6.11 (1H, m), 7.10–7.40 (10H, m), 9.40 (0.5H, s), 9.47 (0.5H, s); *m/z* (FAB) 397.2127 ($M^+ + 1$, $C_{23}H_{29}N_2O_4$ 397.2127).

Compound **21b** (300 mg, 0.76 mmol) was dissolved in 5 mL 70% TFA–H_2O, and the solution was stirred at rt for 2 h. The solvent was removed *in vacuo* to give a yellowish oil, which was dissolved in ethyl acetate and washed with saturated $NaHCO_3$ aqueous solution and brine. The organic phase was dried over anhydrous Na_2SO_4, and the solvent removed to give scaffold **22** (250 mg, 87%) as a colorless oil: δ_H (500 MHz, d_4-methanol) 0.86–1.00 (6H, m), 1.68–2.08 (3H, m), 2.89–3.10 (2H. m), 4.51 (0.5H, d, *J* 12.0), 4.90 (0.5H, dd, *J* 9.0 and 5.0), 4.97 (0.5H, d, *J* 12.0), 5.05 (0.5H, d, *J* 12.5), 5.07 (0.5H, m), 5.15 (0.5H, d, *J* 12.5), 5.97 (0.5H, s), 6.15 (1H, s), 7.10–7.50 (10H, m), 7.69 (1H, br s); *m/z* (FAB) 379.2023 ($M^+ + 1$, $C_{23}H_{27}N_2O_3$ requires 379.2022).

Alkylation of scaffold **22** (250 mg, 0.78 mmol) with 4-chloromethyl-5-methyl-1-tritylimidazole[27] (**9**), using conditions similar to that described for the synthesis of compound **13a2**, afforded compound **23** in 15% yield after chromatography on a silica gel column using hexanes–EtOAc (3 : 1–1 : 1) as eluant. Unreacted starting materials were recovered. Compound **23** was obtained as a colorless oil (80 mg, 15%): δ_H (500 MHz, d_4-methanol) 0.89–1.01 (6H, m), 1.36 and 1.39 (3H, s), 1.66 (2H, m), 1.80 and 1.88 (1H, dd, *J* 15.0 and 10.0), 2.75–2.94 (2H, m), 4.15–4.25 (1.5H, m), 4.77–4.84 (1.5H, m), 4.94–5.00 (1H, m), 5.10–5.16 (1H, m), 6.00 and 6.20 (1H, s), 6.93–7.27 (21H, m); *m/z* (FAB) 715.3651 ($M^+ + 1$, $C_{47}H_{47}N_4O_3$ requires 715.3648).

Compound **23** was hydrogenated, using conditions similar to those described previously, to generate predominantly the 6*S* isomer in 90% yield: δ_H (500 MHz, d_4-methanol) 0.79 (3H, d, *J* 6.5), 0.80 (3H, d, *J* 6.5), 1.14 (1H, m), 1.48 (3H, s), 1.52 (2H, m), 2.83 (2H, m), 3.08 (1H, dd, *J* 13.5 and 8.0), 3.22 (1H, dd, *J* 13.5 and 4.0), 3.38 (1H, m), 3.66 (1H, dd, *J* 7.5 and 4.0), 3.87 (1H, d, *J* 15.0), 5.19 (1H, d, *J* 15.0), 7.07–7.35 (21H, m); *m/z* (FAB) 583.3437 ($M^+ + 1$). Without further purification, the crude product (60 mg) was coupled to L-leucine methyl ester isocyanate following previously described general procedures. The product was purified by silica gel column chromatography using MeOH–CH_2Cl_2 (0.5–5%) as eluant to afford compound **24** (63 mg, 80%) as a colorless oil: δ_H (500 MHz, d_4-methanol) 0.79 (3H, d, *J* 6.5), 0.80 (3H, d, *J* 6.5 Hz), 0.82 (3H, d, *J* 6.5), 0.86 (1H, m), 0.92 (3H, d, *J* 6.5), 0.93 (1H, m), 1.16 (2H, m), 1.33 (3H, s), 1.63 (1H, m), 1.84 (1H, m), 2.82 (1H, dd, *J* 3.0 and 10.0), 3.04 (1H, dd, *J* 14.0 and 10.0), 3.42 (1H, dd, *J* 14.0 and 3.0), 3.46 (1H, m), 3.49 (3H, s), 3.88 (1H, d, *J* 12.5), 4.08 (1H, d, *J* 15.5), 4.19 (1H, m), 4.28 (1H, dd, *J* 14.0 and 3.0), 4.37 (1H, dd, *J* 10.0 and 2.5), 5.37 (1H, d, *J* 15.5), 7.06–7.35 (21H, m); *m/z* (FAB) 754.4335 ($M^+ + 1$, requires 754.4332).

Deprotection of compound **24** following the general procedure described previously afforded compound **25** as a colorless oil (35 mg, 85% yield): δ_H (500 MHz, d_4-methanol) 0.78 (3H, d, *J* 6.5), 0.79 (3H, d, *J* 6.5), 0.84 (3H, d, *J* 6.5), 0.87 (3H, d, *J* 6.5), 1.06 (1H, m), 1.15 (2H, m), 1.28 (1H, m), 1.39 (1H, m), 1.60 (1H, m), 2.30 (1H, s), 2.82 (1H, dd, *J* 14.0 and 10.0), 3.08 (1H, dd, *J* 13.0 and 10.0), 3.33 (1H, br d, *J* 13.0), 3.52 (1H, m), 3.63 (3H, s), 4.17 (1H, dd, *J* 14.0 and 7.5), 4.34 (1H, dd, *J* 14.0 and 3.5), 4.46 (1H, br d, *J* 7.5 Hz), 4.54 (2H, br s), 4.68 (1H, br s), 7.15–7.30 (5H, m), 8.51 (1H, s); δ_C (125 MHz, d_4-methanol) 9.5, 21.4, 22.0, 23.0, 24.3, 24.7, 24.9, 37.2, 38.2, 41.1, 41.5, 41.9, 52.3, 52.5, 55.7, 61.2, 125.2, 126.8, 127.6, 129.3, 129.3, 129.9, 129.9, 133.4, 137.6, 156.9, 168.9, 174.8; *m/z* (FAB) 512.3238 ($M^+ + 1$, $C_{28}H_{42}N_5O_4$ requires 512.3237).

Saponification of compound **25** following the general procedure described previously afforded **73** as a colorless oil (27 mg, 85% yield): δ_H (500 MHz, d_4-methanol) 0.59 (3H, d, *J* 6.0), 0.73 (3H, d, *J* 6.0), 0.76 (3H, d, *J* 6.0), 0.77 (3H, d, *J* 6.0), 1.20–1.50 (6H, m), 2.09 (3H, s), 2.72 (1H, dd, *J* 14.0, 10.0), 3.15–3.19 (2H, m), 3.24 (1H, m), 3.96 (1H, dd, *J* 14.0, 4.5), 4.00 (1H, dd, *J* 11.0, 4.5), 4.06 (1H, d, *J* 15.5), 4.72 (1H, t, *J* 5.5), 5.18 (1H, d, *J* 15.5), 7.12–7.20 (5H, m), 7.62 (1H, s); δ_C (125 MHz, d_4-methanol) 10.7, 21.5, 22.3, 24.1, 24.8, 25.9, 26.2, 38.2, 39.2, 41.3, 43.1, 43.8, 54.4, 55.5, 61.1,

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128.3, 129.2, 129.8, 130.0, 130.0, 131.2, 131.2, 135.3, 139.4, 159.3, 171.7, 180.5; *m/z* (FAB) 498.3079 ($M^+ + 1$, $C_{27}H_{40}N_5O_4$ requires 498.3080).

Biological assays

The *in vitro* inhibition assays of PGGTase-I and PFTase were carried out by measuring the [^{3}H]GGPP and [^{3}H]FPP incorporated into H-Ras-CVLL and H-Ras-CVLS, respectively, as previously described.[32] The *in vivo* inhibition of geranylgeranylation and farnesylation was determined based on the level of inhibition of Rap1A and H-Ras processing, respectively.[13] Briefly, oncogenic H-Ras-transformed NIH 3T3 cells were treated with various concentrations of inhibitors, and the cell lysates were separated on 12.5% SDS-PAGE. The separated proteins were transferred to nitrocellulose and immunoblotted using an anti-Ras antibody (Y13-258) or an anti-Rap1A antibody (SC-65, Santa Cruz Biotechnology, Santa Cruz, CA). Antibody reactions were visualized using either peroxidase-conjugated goat anti-rat IgG or goat anti-rabbit IgG and an enhanced chemiluminescence detection system.

Molecular modeling

Docking studies were done using GOLD v. 2.1 on a Dell i686 running RedHat Linux 7.2. The GOLD program[33] set the atom types for both the protein and the ligands. The structure of mammalian protein PGGTase-I was obtained from its complexed X-ray crystal structure with GGPP and a peptide substrate (PDB code: 1N4Q). The active site was defined with a radius of 10 Å from the zinc ion and the GOLD Program detected the active-site pocket with a radius of 10 Å. The imidazole nitrogen of the inhibitors and the protein zinc atom interaction was increased by a factor of 10 as a desired constraint. The ten best docked conformations were collected for each inhibitor. The default parameters were used for the GOLD Scoring fitness function. For generating conformations, the default genetic algorithm and population parameters were also used: 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 used were: van der Waals = 2.5 and hydrogen bonding = 4.0.

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