SUPPORTING INFORMATION FOR

The effect of *N*-methylation on transition state mimetic inhibitors of the *Plasmodium* protease, plasmepsin V

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1. Experimental

1.1 Chemistry

Analytical thin-layer chromatography was performed on Merck silica gel $60F^{254}$ aluminum-backed plates and were visualized by fluorescence quenching under UV light or by $KMnO_4$ staining. Flash chromatography was performed with silica gel 60 (particle size 0.040-0.063 μm). NMR spectra were recorded on a Bruker Avance DRX 300 or a Varian 600 MHz at 298K unless specified with the solvents indicated. Chemical shifts are reported in ppm on the δ scale and referenced to the appropriate solvent peak. MeOD contains H_2O. Infrared spectra were obtained on a Bruker Tensor 27 FT-IR spectrometer at a resolution of 4 cm^{-1} and absorptions are given in wavenumbers (cm^{-1}). HRMS were acquired by Jason Dang at the Monash Institute of Pharmaceutical Sciences Spectrometry Facility using an Agilent 1290 infinity 6224 TOF LCMS. Column used was RRHT 2.1 x 50 mm 1.8 μm C18. Gradient was applied over the 5 min with the flow rate of 0.5 mL/min. For MS: Gas temperature was 325^oC; drying gas 11 L/min; nebulizer 45 psig and the fragmentor 125V. LCMS were recorded on a Waters ZQ 3100 using a 2996 Diode Array Detector. LCMS conditions used to assess purity of compounds were as follows, column: XBridge TM C18 5 μm 4.6 x 100 mm, injection volume 10 μL, gradient: 10-100% B over 10 min (solvent A: water 0.1% formic acid; solvent B: AcCN 0.1% formic acid), flow rate: 1.5 mL/min, detection: 100-600 nm. All final compounds were analyzed using a Agilent HP1100 high performance liquid chromatograph. HPLC conditions used to assess purity of final compounds were as follows, column: Phenomenex Gemini C18, 2.0 x 50 mm; injection volume 10 μL; gradient: 0-100% Buffer B over 6 min (buffer A: 0.1% formic acid in autoclaved MilliQ water; buffer B: 0.1% formic acid in 100% acetonitrile), flow rate: 1.0 mL/min, detection: 214 nm. Unless otherwise noted, all compounds were found to be >95% pure by this method.

The following compounds were purchased commercially and used without further purification, 2-(4-chlorophenyl)-ethylamine, Cbz-Orn(*N*-Boc)-OH, HCl.NH_2-Ala-OMe, Cbz-Arg(*N*,*N*-diBoc)-OH, HCl.NH_2-Ala-OEt, Cbz-Orn(Phth)-OH, Boc-Ala-OH, Boc-Sta(3*S*,4*S*)-OH, *N,N′*-bis-Boc-1-guanylpyrazole, benzyl bromide, 2-(4-chlorophenyl)-*N*-methylethanamine, phenylethylamine.

General Procedure A

Boc-Sta-NH(CH_2)$_2$Ph(4'-Cl) 11

To a stirred solution of Boc-Sta(3*S*,4*S*)-OH (500 mg, 1.82 mmol) in DMF (10 mL) was added HBTU (826 mg, 2.18 mmol) and DIPEA (1.58 mL, 9.08 mmol). The reaction mixture was stirred for 10 min at 20°C. An excess of 2-(4-chlorophenyl)-ethylamine (505 µL, 3.63 mmol) was added and the resulting suspension was allowed to stir for 18 h at 20°C. The reaction mixture was quenched with 10% citric acid solution and extracted with EtOAc (2 x 15 mL). The combined organic layers were then washed with saturated $NaHCO_3$ solution (1 x 20 mL). The organic layer was washed with brine (20 mL), dried with $MgSO_4$ and the solvent was concentrated *in vacuo* to obtain **11** as an oil (745 mg, 99%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers) δ 7.31 – 7.26 (m, 2H), 7.19 – 7.11 (m, 2H), 6.48 (s, 1H), 4.83 – 4.77 (m, 1H), 3.96 – 3.88 (m, 1H), 3.60 – 3.44 (m, 4H), 2.84 – 2.76 (m, 2H), 2.43 – 2.23 (m, 2H), 1.71 – 1.50 (m, 2H), 1.44 (s, 9H), 1.38 – 1.26 (m, 1H), 0.91 (dd, *J* = 6.5, 2.5 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$, rotamers) δ 172.87, 156.87, 137.23, 132.50, 130.27, 130.18, 129.25, 128.94, 128.85, 79.75, 70.76, 52.31, 41.79, 41.56, 40.74, 40.50, 34.95, 32.89, 28.48, 24.93, 23.17, 22.19. IR (cm^{-1}) *v* 3369 (NH), 2958-2874 (CH), 1681 (C=O). MS, *m/z* = 413.3 $[M + H]^+$.

General Procedure B

HCl.NH_2- Sta-NH(CH_2)$_2$Ph(4'-Cl) 14

A mixture of Boc-Sta-NH(CH_2)$_2$Ph(4'-Cl) **11** (884 mg, 2.14 mmol), in 4N HCl in dioxane (4 mL) was allowed to stir for 1 h at 20°C. The reaction mixture was concentrated to dryness *in vacuo*. The oil was triturated with Et_2O and the supernatant decanted to obtain **14** as a solid (740 mg, 99% yield). ^{1}H NMR (600 MHz, MeOD) δ 7.26 (d, *J* = 8.3 Hz, 2H), 7.22 (d, *J* = 8.4 Hz, 2H), 4.05 – 3.98 (m, 1H), 3.41 (t, *J* = 7.2 Hz, 2H), 3.25 – 3.20 (m, 1H), 2.80 (t, *J* = 7.3 Hz, 2H), 2.56 (dd, *J* = 14.8, 5.2 Hz, 1H), 2.47 (dd, *J* = 14.8, 7.0 Hz, 1H), 1.79 – 1.70 (m, 1H), 1.60 – 1.46 (m, 2H), 0.96 (d, *J* = 6.5 Hz, 6H). ^{13}C NMR (151 MHz, MeOD, rotamers) δ 172.88, 139.20, 133.03, 131.55, 131.31, 129.54, 129.33, 68.20, 68.07, 54.97, 54.60, 41.77, 41.47, 40.25, 35.62, 25.42, 25.30, 22.97, 22.92, 22.49, 22.43. IR (cm^{-1}) *v* 3272 (NH), 2958 (CH), 1636 (C=O). MS, *m/z* = 313.3 $[M + H]^+$.

Cbz-Arg(*N,N*-diBoc)-Ala-OEt 9

General Procedure A was followed using Cbz-Arg(*N,N*-diBoc)-OH **8** (200 mg, 0.393 mmol) and HCl.NH_2-Ala-OEt (121 mg, 0.787 mmol) to obtain a crude residue. The crude residue was subjected to

silica chromatography gradient eluting with 100% DCM to 10% MeOH/DCM to obtain **9** as an oil (239 mg). Chemical characterisation data was identical to that previously described.[1]

Cbz-Arg(*N,N*-diBoc)-Ala-OH 10

A mixture of Cbz-Arg(*N,N*-diBoc)-Ala-OEt **9** (200 mg, 0.329 mmol), and LiOH hydrate (41 mg, 0.987 mmol) in a mixture of water (1.3 mL) and THF (4 mL) was allowed to stir for 5 min at 20°C. 10% Citric acid solution was added to the reaction mixture. The solution was then extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (10 mL) and dried with $MgSO_4$. The solvent was concentrated *in vacuo* to obtain **10** as a solid (189 mg, 99%). Chemical characterisation data was identical to that previously described.[1]

Cbz-Arg(*N,N*-diBoc)-Ala-Sta-NH$(CH_2)_2$Ph(4'-Cl) 17

General Procedure A was followed using Cbz-Arg(*N,N*-diBoc)-Ala-OH **10** (60 mg, 0.103 mmol) and HCl.NH_2-Sta-NH$(CH_2)_2$Ph(4'-Cl) **14** (72 mg, 0.207 mmol) to obtain a crude residue. The oil was subjected to silica chromatography gradient eluting with 100% DCM to 10% MeOH/DCM to obtain **17** as an oil (58 mg, 64%). Chemical characterisation data was identical to that previously described.[1]

General Procedure C

Cbz-Arg(NH_2)-Ala-Sta-NH$(CH_2)_2$Ph(4'-Cl).TFA 1

A mixture of Cbz-Arg(*N,N*-diBoc)-Ala-Sta-NH$(CH_2)_2$Ph(4'-Cl) **17** (44 mg, 0.050 mmol), in TFA (0.4 mL) and DCM (0.4 mL) was allowed to stir for 18 h at 20°C. The reaction mixture was concentrated to dryness *in vacuo*. The oil was triturated with Et_2O and the supernatant decanted to obtain **1** as a solid (30 mg, 76%). ^{1}H NMR and HRMS were identical to that previously described.[1] ^{13}C NMR (75 MHz, MeOD, 325K, rotamers) δ 174.93, 174.75, 174.22, 174.07, 158.78, 139.37, 137.99, 133.17, 131.38, 129.50, 129.10, 128.85, 71.66, 71.41, 67.97, 56.28, 56.01, 52.75, 51.13, 50.84, 42.06, 41.71, 41.62, 41.33, 35.80, 35.60, 30.38, 30.22, 26.25, 26.11, 26.01, 25.91, 23.64, 23.58 , 22.44, 22.28, 18.12, 17.99. IR (cm^{-1}) *v* 3339 (NH), 2957 (CH), 1637 (C=O).

Cbz-NCH_3-Orn(NPhth)-OH 38

Compound **38** was synthesised according to previously described procedure.[2]

Boc-Ala-Sta-NH(CH_2)$_2$Ph(4'-Cl) 39

General Procedure A was followed using Boc-L-Ala-OH (150 mg, 0.793 mmol) and HCl.NH_2-Sta-NH(CH_2)$_2$Ph(4'-Cl) **14** (346 mg, 0.991 mmol) to obtain a crude residue. The crude residue was subjected to silica chromatography gradient eluting with 100% DCM to 10% MeOH/DCM to obtain **39** as a solid (242 mg, 63%). ^{1}H NMR (300 MHz, MeOD, 325K) δ 7.27 (d, *J* = 8.7 Hz, 2H), 7.20 (d, *J* = 8.5 Hz, 2H), 4.07 – 3.85 (m, 3H), 3.40 (t, *J* = 7.2 Hz, 2H), 2.79 (t, *J* = 7.3 Hz, 2H), 2.28 – 2.21 (m, 2H), 1.67 – 1.47 (m, 3H), 1.44 (s, 9H), 1.32 (d, *J* = 7.2 Hz, 3H), 0.91 (dd, *J* = 6.5, 4.6 Hz, 6H). ^{13}C NMR (75 MHz, MeOD, 325K) δ 175.89, 173.91, 157.76, 139.37, 133.18, 131.39, 129.50, 80.97, 71.18, 52.38, 52.15, 42.07, 41.76, 41.67, 35.75, 28.77, 25.91, 23.54, 22.48, 18.17. IR (cm^{-1}) *v* 3321 (NH), 2953 (CH), 1691 (C=O). MS, *m/z* = 484.3 $[M + H]^+$.

Cbz-NCH_3-Orn(Phth)-Ala-Sta-NH(CH_2)$_2$Ph(4'Cl) 41

General Procedure B was followed using Boc-Ala-Sta-NH(CH_2)$_2$Ph(4'-Cl) **39** (273 mg, 0.564 mmol), and 4N HCl in dioxane (1.5 mL) to obtain HCl.NH_2-Ala-Sta-NH(CH_2)$_2$Ph(4'-Cl) **40** as a crude oil (235 mg, 99%). General Procedure A was then followed using Cbz-NCH_3-Orn(NPhth)-OH **38** (175 mg, 0.426 mmol), HCl.NH_2-Ala-Sta-NH(CH_2)$_2$Ph(4'-Cl) **40** (222 mg, 0.529 mmol) and TFFH (146 mg, 0.554 mmol) (in place of HBTU), to obtain **41** as a an oil (328 mg, 99%). ^{1}H NMR (300 MHz, MeOD, 325K) δ 7.87 – 7.70 (m, 4H), 7.38 – 7.11 (m, 9H), 5.14 (s, 2H), 4.75 – 4.65 (m, 1H), 4.27 (q, *J* = 7.2 Hz, 1H), 3.97 – 3.90 (m, 1H), 3.90 – 3.81 (m, 1H), 3.72 – 3.60 (m, 2H), 3.38 (t, *J* = 7.0 Hz, 2H), 2.89 (s, 3H), 2.77 (t, *J* = 7.2 Hz, 2H), 2.26 – 2.16 (m, 2H), 1.97 – 1.43 (m, 6H), 1.41 – 1.27 (m, 4H), 0.88 (t, *J* = 6.6 Hz, 6H). ^{13}C NMR (75 MHz, MeOD, 325K) δ 174.76, 173.86, 172.97, 169.89, 139.38, 137.97, 135.34, 133.40, 133.15, 131.39, 129.51, 129.49, 129.10, 128.91, 124.14, 71.19, 68.75, 59.89, 52.38, 51.19, 41.91, 41.76, 41.68, 38.25, 35.77, 31.11, 27.04 26.22, 25.93, 23.54, 22.47, 17.99. IR (cm^{-1}) *v* 3307 (NH), 2953 (CH), 1708-1648 (C=O). MS, *m/z* = 776.3 $[M + H]^+$.

Cbz-NCH_3-Arg(*N,N*-diBoc)-Ala-Sta-NH(CH_2)$_2$Ph(4'Cl) 42

A mixture of Cbz-NCH_3-Orn(NPhth)-Ala-Sta-NH(CH_2)$_2$Ph(4'Cl) **41** (116 mg, 0.149 mmol) and hydrazine monohydrate (15 μL, 0.299 mmol) in EtOH (4.5 mL) was allowed to stir for 18 h at 20°C. The reaction mixture was concentrated to dryness *in vacuo* to obtain the unprotected residue as a crude oil (96 mg, 99%). The crude oil was dissolved in DCM (3 mL), THF (1mL) and Et_3N (42 μL, 0.302 mmol) was added. The solution was stirred vigorously for 5 min. *N,N'*-Bis-Boc-1-guanylpyrazole (94 mg, 0.302 mmol) was added and the solution was allowed to stir for 18 h at 20°C. The reaction mixture

was concentrated to dryness *in vacuo* to obtain a crude residue. The crude residue was subjected to silica chromatography gradient eluting with 100% DCM to 10% MeOH/DCM to obtain **42** as an oil (40 mg, 20%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.46 (s, 1H), 8.36 – 8.27 (m, 1H), 7.38 – 7.28 (m, 5H), 7.23 (d, *J* = 8.4 Hz, 2H), 7.11 (d, *J* = 8.4 Hz, 2H), 6.77 (s, 1H), 6.65 (s, 1H), 6.48 (d, *J* = 9.5 Hz, 1H), 5.18 – 5.13 (m, 2H), 4.61 – 4.51 (m, 1H), 4.32 – 4.23 (m, 1H), 3.99 – 3.92 (m, 1H), 3.90 – 3.82 (m, 1H), 3.55 – 3.28 (m, 4H), 2.86 (s, 3H), 2.77 (t, *J* = 7.3 Hz, 2H), 2.33 – 2.14 (m, 2H), 1.97 – 1.83 (m, 2H), 1.77 – 1.42 (m, 22H), 1.37 – 1.27 (m, 4H), 0.87 (dd, *J* = 6.1, 3.4 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$, 325K) δ 172.42, 172.15, 170.76, 163.37, 156.24, 153.47, 137.54, 136.49, 132.47, 130.20, 128.82, 128.73, 128.36, 128.05, 83.44, 79.59, 70.55, 68.00, 59.04, 51.04, 50.13, 41.38, 40.77, 40.62, 35.14, 30.47, 28.47, 28.23, 25.97, 25.55, 25.05, 23.13, 22.31, 18.23. IR (cm^{-1}) *v* 3325 (NH), 2956 (CH), 1640 (C=O). MS, *m/z* = 888.4 $[M + H]^+$.

Cbz-NCH$_3$-Arg(NH$_2$)-Ala-Sta-NH(CH$_2$)$_2$Ph(4'Cl).TFA 2

General Procedure C was followed using Cbz-NCH$_3$-Arg(*N,N*-diBoc)-Ala-Sta-NH(CH$_2$)$_2$Ph(4'Cl) **42** (30 mg, 0.034 mmol), to obtain **2** as an oil (25 mg, 92%). ^{1}H NMR (600 MHz, MeOD, rotamers) δ 7.42 – 7.15 (m, 9H), 5.24 – 5.09 (m, 2H), 4.74 – 4.57 (m, 1H), 4.28 (br s, 1H), 3.99 – 3.84 (m, 2H), 3.46 – 3.35 (m, 2H), 3.25 – 3.10 (m, 2H), 2.90 (s, 3H), 2.78 (t, *J* = 7.2 Hz, 2H), 2.22 (d, *J* = 6.2 Hz, 2H), 1.95 (br s, 1H), 1.82 – 1.69 (m, 1H), 1.67 – 1.45 (m, 4H), 1.45 – 1.27 (m, 4H), 1.01 – 0.83 (m, 6H). IR (cm^{-1}) *v* 3292 (NH), 2957 (CH), 1651 (C=O). MS, *m/z* = 688.3 $[M + H]^+$. HRMS found: (M+H) 688.3593; $C_{34}H_{50}ClN_7O_6$ requires (M+H), 688.3589.

Cbz-Orn(*N*-Boc)-NCH$_3$-Ala-OMe 23

General Procedure A was followed using Cbz-Orn(*N*-Boc)-OH **21** (200 mg, 0.546 mmol), and HCl.NH(CH$_3$)-Ala-OMe **19** (168 mg, 1.09 mmol) to obtain a crude residue. The crude residue was subjected to silica chromatography gradient eluting with 100% DCM to 10% MeOH/DCM to obtain **23** as an oil (102 mg, 40 %). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers) δ 7.39 – 7.27 (m, 5H), 5.72 (s, 1H), 5.23 (q, *J* = 7.3 Hz, 1H), 5.09 (s, 2H), 4.77 – 4.55 (m, 2H), 3.70 (s, 3H), 3.13 (s, 2H), 2.99 & 2.83 (2s, 3H), 1.87 – 1.48 (m, 4H), 1.47 – 1.31 (m, 12H). ^{13}C NMR (75 MHz, $CDCl_3$, 325K, rotamers) δ 172.38, 171.92, 156.12, 136.60, 128.58, 128.18, 128.09, 108.00, 79.25, 67.71, 67.02, 52.50, 52.28, 50.90, 40.44, 31.21, 30.15, 29.30, 28.53, 25.60, 24.01, 14.16. IR (cm^{-1}) *v* 3330 (NH), 2975 (CH), 1709 (C=O). MS, *m/z* = 466.4 $[M + H]^+$.

Cbz-Orn(*N*-Boc)-NCH_3-Ala-Sta-$NH(CH_2)_2$Ph(4'-Cl) 30

A mixture of Cbz-Orn(*N*-Boc)-NCH_3-Ala-OMe **23** (80 mg, 0.172 mmol), and LiOH hydrate (18 mg, 0.430 mmol) in a mixture of water (0.8 mL) and THF (2.4 mL) was allowed to stir for 3 h at 20°C. 10% Citric acid solution was added to the reaction mixture. The solution was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (20 mL) and dried with $MgSO_4$. The solvent was concentrated *in vacuo* to obtain Cbz-Orn(*N*-Boc)-NCH_3-Ala-OH **27** as a light yellow oil (77 mg, 99%). General Procedure A was then followed using Cbz-Orn(*N*-Boc)-NCH_3-Ala-OH **27** (77 mg, 0.171 mmol), and HCl.NH_2-Sta-$NH(CH_2)_2$Ph(4'-Cl) **14** (101 mg, 0.290 mmol) to obtain a crude residue. The crude residue was subjected to silica chromatography gradient eluting with 100% DCM to 10% MeOH/DCM to obtain **30** as an oil (74 mg, 58%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers) δ 7.38 – 7.27 (m, 5H), 7.25 – 7.18 (m, 2H), 7.15 – 7.02 (m, 2H), 6.95 – 6.50 (m, 1H), 6.42 – 6.21 (m, 1H), 6.04 – 5.77 (m, 1H), 5.13 – 5.00 (m, 2H), 5.00 – 4.88 (m, 1H), 4.82 – 4.43 (m, 1H), 4.01 – 3.77 (m, 2H), 3.56 – 3.27 (m, 3H), 3.19 – 3.04 (m, 2H), 3.03 – 2.69 (m, 7H), 2.38 – 2.12 (m, 2H), 1.78 – 1.27 (m, 19H), 0.93 – 0.77 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$, 325K, rotamers) δ 173.50, 172.99, 172.73, 172.36, 172.02, 171.28, 171.12, 170.22, 161.86, 157.35, 156.92, 156.30, 156.24, 137.63, 137.45, 136.50, 136.20, 132.46, 132.32, 130.25, 130.19, 130.16, 129.11, 128.79, 128.70, 128.67, 128.63, 128.35, 128.27, 128.13, 127.99, 79.49, 70.99, 70.75, 70.31, 67.58, 67.30, 67.11, 55.81, 53.38, 53.23, 52.05, 51.46, 51.40, 51.34, 51.10, 50.45, 46.46, 41.44, 40.96, 40.60, 39.96, 35.05, 31.19, 30.98, 30.07, 29.75, 29.40, 29.20, 28.55, 27.04, 26.28, 25.79, 25.03, 23.14, 22.23, 22.01, 15.25, 13.85, 13.72. IR (cm^{-1}) *v* 3306 (NH), 2956 (CH), 1647 (C=O). MS, *m/z* = 746.5 $[M + H]^+$.

Cbz-Arg(*N,N*-diBoc)-NCH_3-Ala-Sta-$NH(CH_2)_2$Ph(4'-Cl) 33

General Procedure B was followed using Cbz-Orn(*N*-Boc)-NCH_3-Ala-Sta-$NH(CH_2)_2$Ph(4'-Cl) **30** (56 mg, 0.075 mmol), and 4N HCl in dioxane (0.5 mL) to obtain the unprotected residue as a crude oil. The crude oil (45 mg, 0.066 mmol) was dissolved in DCM (2 mL) and Et_3N (12 μL, 0.086 mmol) was added. The solution was stirred vigorously for 5 min. *N,N'*-Bis-Boc-1-guanylpyrazole (27 mg, 0.086 mmol) was added and the solution was allowed to stir for 18 h at 20°C. The reaction mixture was concentrated to dryness *in vacuo* to obtain a crude residue. The crude residue was subjected to silica chromatography gradient eluting with 100% DCM to 10% MeOH/DCM to obtain **33** as an oil (50 mg, 85%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers) δ 11.48 (s, 1H), 8.39 (s, 1H), 7.38 – 7.27 (m, 5H), 7.24 – 7.17 (m, 2H), 7.14 – 7.03 (m, 2H), 7.00 – 6.66 (m, 1H), 6.51 – 6.20 (m, 1H), 6.01 – 5.75 (m, 1H), 5.14 – 5.01 (m, 2H), 5.01 – 4.88 (m, 1H), 4.79 – 4.45 (m, 1H), 4.03 – 3.79 (m, 3H), 3.55 – 3.27 (m, 4H),

3.04 – 2.70 (m, 5H), 2.34 – 2.11 (m, 2H), 1.80 – 1.29 (m, 28H), 0.97 – 0.77 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$, 325K, rotamers) δ 173.30, 172.76, 172.36, 172.09, 171.86, 171.08, 170.08, 163.48, 163.30, 156.38, 156.32, 153.50, 153.43, 137.65, 137.50, 136.50, 136.17, 132.46, 132.35, 130.20, 130.17, 128.81, 128.72, 128.69, 128.65, 128.37, 128.26, 128.13, 128.01, 83.47, 79.68, 79.56, 71.01, 70.79, 70.35, 70.21, 67.62, 67.37, 67.16, 55.94, 53.58, 53.02, 52.01, 51.52, 51.40, 51.15, 51.04, 50.67, 41.63, 41.04, 40.55, 40.30, 35.10, 31.36, 31.18, 31.04, 29.98, 29.76, 29.31, 28.43, 28.20, 25.58, 25.28, 25.06, 23.16, 23.09, 22.25, 15.19, 13.78, 13.67. IR (cm^{-1}) *v* 3324 (NH), 2956 (CH), 1638 (C=O). MS, *m/z* = 888.5 $[M + H]^+$.

Cbz-Arg(NH_2)-NCH_3-Ala-Sta-NH(CH_2)$_2$Ph(4'-Cl).TFA 3

General Procedure C was followed using Cbz-Arg(*N,N*-diBoc)-NCH_3-Ala-Sta-NH(CH_2)$_2$Ph(4'-Cl) **33** (40 mg, 0.045 mmol), to obtain **3** as a solid (35 mg, 97%). ^{1}H NMR (600 MHz, MeOD, rotamers) δ 7.43 – 7.13 (m, 9H), 5.16 – 4.90 (m, 3H), 4.66 – 4.53 (m, 1H), 4.04 – 3.85 (m, 2H), 3.46 – 3.33 (m, 2H), 3.26 – 3.05 (m, 4H), 2.82 – 2.70 (m, 3H), 2.31 – 2.18 (m, 2H), 1.82 – 1.27 (m, 10H), 1.01 – 0.81 (m, 6H). IR (cm^{-1}) *v* 3306 (NH), 2956 (CH), 1634 (C=O). MS, *m/z* = 688.5 $[M + H]^+$. HRMS found: (M+H) 688.3589; $C_{34}H_{50}ClN_7O_6$ requires (M+H), 688.3589.

Boc-Sta(oxazolidine)-OBz 44

A mixture of Boc-Sta(3*S*,4*S*)-OH (300 mg, 1.09 mmol), K_2CO_3 (196 mg, 1.42 mmol) and benzyl bromide (143 µL, 1.20 mmol) in DMF (2.5 mL) was allowed to stir for 3.5 h at 20°C. 10% Citric acid solution was added to the reaction mixture. The solution was extracted with EtOAc (2 x 20 mL). The combined organic layers were washed with saturated $NaHCO_3$ solution (2 x 10 mL). The organic layer was washed with water (20 mL), dried with $MgSO_4$ and the solvent was concentrated *in vacuo* to obtain Boc-Sta-OBz as a colourless oil (380 mg, 95%). A mixture of the crude Boc-Sta-OBz (370 mg, 1.01 mmol) and PTSA (17 mg, 0.101 mmol) in anhydrous toluene (6 mL) was heated to reflux for 4 h. Paraformaldehyde (50 mg) was added to the refluxing solution every 20 min, allowing the solution to clear before each subsequent addition. The reaction mixture was filtered while warm through a bed of Celite and the filtrate was concentrated to dryness *in vacuo* to obtain a crude residue. The crude residue was subjected to silica chromatography gradient eluting with 100% DCM to 10% MeOH/DCM to obtain **44** as an oil (180 mg, 47%). ^{1}H NMR (300 MHz, $CDCl_3$, 325K) δ 7.40 – 7.27 (m, 5H), 5.15 (s, 2H), 5.09 (d, *J* = 4.3 Hz, 1H), 4.68 (d, *J* = 4.5 Hz, 1H), 4.27 (td, *J* = 6.8, 2.9 Hz, 1H), 3.84 – 3.74 (m, 1H), 2.60 (qd, *J* = 15.4, 6.8 Hz, 2H), 1.67 – 1.50 (m, 2H), 1.47 (s, 9H), 1.41 – 1.34 (m, 1H), 0.93 (t, *J* =

6.8 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$, 325K) δ 170.26, 153.44, 135.96, 128.76, 128.48, 128.43, 80.59, 79.60, 77.82, 66.76, 58.85, 42.49, 38.92, 28.60, 25.21, 23.25, 22.53. IR (cm^{-1}) *v* 2958 (CH), 1737-1701 (C=O). MS, *m/z* = 278.3 $[M + H]^+$.

Boc- Sta(oxazolidine)-NH$(CH_2)_2$Ph(4'-Cl) 46

A mixture of Boc-Sta(oxazolidine)-OBz **44** (290 mg, 0.768 mmol), and LiOH hydrate (161 mg, 3.84 mmol) in a mixture of water (3 mL) and THF (9 mL) was allowed to stir for 18 h at 50°C. Water (20 mL) was added to the reaction mixture. The solution was extracted with EtOAc (2 x 10 mL). The aqueous layer was acidified with 10% citric acid solution and extracted with EtOAc (2 x 10 mL). The combined organic layers were washed with brine (20 mL) and dried with $MgSO_4$. The solvent was concentrated *in vacuo* to obtain Boc-Sta(oxazolidine)-OH **45** as a crude oil (219 mg, 99%). General Procedure A was then followed using the crude Boc-Sta(oxazolidine)-OH **45** (200 mg, 0.696 mmol), and 2-(4-chlorophenyl)-ethylamine (193 µL, 1.39 mmol) to obtain a crude residue. The crude residue was subjected to silica chromatography gradient eluting with 100% DCM to 10% MeOH/DCM to obtain **46** as a solid (185 mg, 63%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.29 – 7.22 (m, 2H), 7.15 – 7.08 (m, 2H), 6.08 (s, 1H), 5.09 (s, 1H), 4.59 (d, *J* = 4.8 Hz, 1H), 4.08 (td, *J* = 6.3, 4.2 Hz, 1H), 3.68 (s, 1H), 3.56 – 3.38 (m, 2H), 2.77 (t, *J* = 6.9 Hz, 2H), 2.40 (d, *J* = 6.3 Hz, 2H), 1.71 – 1.49 (m, 2H), 1.45 (s, 9H), 1.39 – 1.28 (m, 1H), 0.92 (t, *J* = 6.6 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$, 325K) δ 169.79, 153.56, 137.49, 132.61, 130.24, 128.88, 80.75, 80.52, 77.76, 58.92, 42.48, 40.82, 40.65, 35.22, 28.56, 25.09, 23.31, 22.49. IR (cm^{-1}) *v* 3262 (NH), 2951-2858 (CH), 1710 (C=O). MS, *m/z* = 425.3 $[M + H]^+$.

NH(CH_3)-Sta-NH$(CH_2)_2$Ph(4'-Cl).TFA 47

To a stirred solution of Boc-Sta(oxazolidine)-NH$(CH_2)_2$Ph(4'-Cl) **46** (95 mg, 0.224 mmol) in DCM (1.5 mL), was added Et_3SiH (140 µL, 0.876 mmol) and TFA (1.5 mL, 19.6 mmol). The reaction mixture was allowed to stir for 18 h at 20°C. The solvent was concentrated to dryness *in vacuo* to obtain **47** as an oil (97 mg, 98%). ^{1}H NMR (300 MHz, MeOD, rotamers) δ 7.31 – 7.17 (m, 4H), 4.09 – 4.00 (m, 1H), 3.43 (t, *J* = 7.1 Hz, 2H), 3.16 – 3.06 (m, 1H), 2.80 (t, *J* = 7.1 Hz, 2H), 2.74 – 2.42 (m, 5H)*, 1.77 – 1.41 (m, 3H), 1.05 – 0.91 (m, 6H). IR (cm^{-1}) *v* 2960 (CH), 1671 (C=O). *m/z* = 327.3 $[M + H]^+$. *The *N*-methyl was found to under intergate by 20%.

Cbz-Orn(*N*-Boc)-Ala-OMe 22

General Procedure A was followed using Cbz-Orn(*N*-Boc)-OH **21** (500 mg, 1.36 mmol) and HCl.NH_2-Ala-OMe (381 mg, 2.73 mmol) to obtain a crude residue. The crude residue was subjected to silica chromatography gradient eluting with 100% DCM to 10% MeOH/DCM to obtain **22** as a solid (531 mg, 86%). ^{1}H NMR (300 MHz, $CDCl_3$, 325K) δ 7.32 – 7.17 (m, 5H), 7.06 (s, 1H), 5.81 (d, J = 8.3 Hz, 1H, NH), 5.04 (s, 2H), 4.90 (s, 1H), 4.48 (p, J = 7.3 Hz, 1H), 4.29 (s, 1H), 3.65 (s, 3H), 3.23 – 2.95 (m, 2H), 1.88 – 1.57 (m, 2H), 1.56 – 1.46 (m, 2H), 1.38 (s, 9H), 1.31 (d, J = 7.2 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$, 325K) δ 173.01, 171.75, 156.42, 156.30, 136.45, 128.43, 128.02, 127.91, 79.11, 66.89, 54.05, 52.19, 48.05, 39.67, 30.17, 28.43, 26.04, 17.65. IR (cm^{-1}) v 3309 (NH), 2954 (CH), 1691-1656 (C=O). MS, m/z = 452.3 $[M + H]^+$.

Cbz-Arg(*N*,*N*-diBoc)-Ala-NCH$_3$-Sta-NH(CH$_2$)$_2$Ph(4'-Cl) 49

A mixture of Cbz-Orn(*N*-Boc)-Ala-OMe **22** (300 mg, 0.664 mmol), and LiOH hydrate (70 mg, 1.66 mmol) in a mixture of water (3 mL) and THF (9 mL) was allowed to stir for 3 h at 20°C. 10% Citric acid solution was added to the reaction mixture. The solution was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (20 mL) and dried with $MgSO_4$. The solvent was concentrated *in vacuo* to obtain Cbz-Orn(*N*-Boc)-Ala-OH **26** as an oil (288 mg, 99%). General Procedure A was then followed using Cbz-Orn(*N*-Boc)-Ala-OH **26** (80 mg, 0.183 mmol), and NH(CH_3)-Sta-NH(CH_2)$_2$Ph(4'-Cl).TFA **47** (97 mg, 0.219 mmol), to obtain a crude residue. The crude residue was subjected to silica chromatography gradient eluting with 100% DCM to 10% MeOH/DCM to obtain Cbz-Orn(*N*-Boc)-Ala-NCH$_3$-Sta-NH(CH_2)$_2$Ph(4'-Cl) **48** as an oil. The resulting oil (60 mg) was subsequently dissolved in 4N HCl in dioxane (1 mL) and allowed to stir for 5 h at 20°C. The reaction mixture was concentrated to dryness *in vacuo*. The oil was triturated with Et_2O and decanted off to obtain Cbz-Orn(NH_2.HCl)-Ala-NCH$_3$-Sta-NH(CH_2)$_2$Ph(4'-Cl) as a solid (50 mg, 91%). The solid was dissolved in DCM (2 mL) and Et_3N (13 µL, 0.095 mmol) was added. The solution was stirred vigorously for 5 min. *N,N'*-Bis-Boc-1-guanylpyrazole (30 mg, 0.095 mmol) was added and the solution allowed to stir for 18 h at 20°C. The reaction mixture was concentrated to dryness *in vacuo* to obtain a crude residue. The crude residue was subjected to silica chromatography gradient eluting with 100% DCM to 10% MeOH/DCM to obtain **49** as an oil (30 mg, 46%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers) δ 11.57 – 11.38 (m, 1H), 8.54 – 8.29 (m, 1H), 7.41 – 7.27 (m, 5H), 7.25 – 7.17 (m, 2H), 7.16 – 7.04 (m, 2H), 7.03 – 6.90 (m, 1H), 6.76 – 6.49 (m, 1H), 6.17 – 5.77 (m, 1H), 5.17 – 4.93 (m, 2H), 4.65 – 3.80 (m, 5H), 3.54 – 3.25 (m, 4H), 3.11 – 2.61 (m, 5H)*, 2.35 – 2.16 (m, 2H), 1.97 – 1.05 (m, 28H), 0.99 –

0.76 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$, 325K, rotamers) δ 174.00, 172.51, 172.37, 172.02, 156.49, 153.43, 137.71, 137.64, 137.48, 136.53, 136.34, 132.57, 132.43, 130.26, 130.21, 128.87, 128.81, 128.73, 128.67, 128.44, 128.38, 128.26, 128.22, 128.15, 128.05, 83.59, 83.38, 79.87, 79.53, 70.78, 70.69, 67.44, 67.33, 67.20, 55.91, 55.16, 51.48, 51.22, 50.42, 49.60, 46.17, 41.18, 40.90, 40.69, 40.63, 40.49, 40.35, 37.45, 35.18, 31.41, 29.32, 28.46, 28.25, 28.09, 26.52, 25.78, 25.25, 25.06, 24.87, 23.33, 23.24, 23.19, 22.37, 22.23, 18.09, 17.92. IR (cm^{-1}) *v* 3323 (NH), 2957 (CH), 1639 (C=O). MS, *m/z* = 888.4 $[M + H]^+$. *The *N*-methyl was found to under intergate by 20%.

Cbz-Arg(NH_2)-Ala-NCH_3-Sta-$NH(CH_2)_2$Ph(4'-Cl).TFA 4

General Procedure C was followed using Cbz-Arg(*N,N*-diBoc)-Ala-NCH_3-Sta-$NH(CH_2)_2$Ph(4'-Cl) **49** (20 mg, 0.023 mmol), to obtain **4** as an oil (18 mg, 99%). ^{1}H NMR (600 MHz, MeOD, rotamers) δ 7.43 – 7.12 (m, 9H), 5.19 – 4.99 (m, 2H), 4.43 – 3.81 (m, 3H), 3.50 – 3.35 (m, 2H), 3.25 – 3.11 (m, 2H), 2.89 – 2.67 (m, 5H)*, 2.34 – 2.19 (m, 2H), 1.88 – 1.24 (m, 10H), 1.05 – 0.78 (m, 6H). IR (cm^{-1}) *v* 3291 (NH), 2963 (CH), 1651 (C=O). MS, *m/z* = 688.3 $[M + H]^+$. HRMS found: (M+H) 688.3565; $C_{34}H_{50}ClN_7O_6$ requires (M+H), 688.3589. *The *N*-methyl was found to under intergate by 20%.

Boc-Sta-$NCH_3(CH_2)_2$Ph(4'-Cl) 12

General Procedure A was followed using Boc-Sta(3*S*,4*S*)-OH (160 mg, 0.581 mmol), and 2-(4-chlorophenyl)-*N*-methylethanamine (197 mg, 1.16 mmol), to obtain **12** as a solid (122 mg, 50%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers) δ 7.33 – 7.24 (m, 2H), 7.19 – 7.07 (m, 2H), 4.78 (dd, *J* = 21.3, 9.9 Hz, 1H), 4.05 – 3.97 & 3.75 – 3.68 (2m, 1H), 3.66 – 3.40 (m, 4H), 2.95 & 2.87 (2s, 3H), 2.81 (t, *J* = 7.5 Hz, 2H), 2.47 – 2.40 (m, 1H), 2.28 – 2.20 (m, 1H), 1.74 – 1.50 (m, 2H), 1.45 (s, 9H), 1.39 – 1.22 (m, 1H), 1.00 – 0.87 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$, 325K, rotamers) δ 173.38, 173.15, 156.30, 137.53, 136.45, 133.09, 132.58, 130.35, 130.26, 130.00, 129.42, 129.17, 128.86, 79.13, 70.13, 69.98, 52.55, 51.46, 49.73, 42.25, 37.33, 36.52, 36.09, 34.23, 33.57, 33.28 , 28.61, 28.59, 25.02, 23.16, 23.12, 22.51, 22.42. IR (cm^{-1}) *v* 3339 (NH), 2956 (CH), 1702-1624 (C=O). MS, *m/z* = 427.4 $[M + H]^+$.

Cbz-Arg(*N,N*-diBoc)-Ala-Sta-$NCH_3(CH_2)_2$Ph(4'-Cl) 18

General Procedure B was followed using Boc-Sta-$NCH_3(CH_2)_2$Ph(4'-Cl) **12** (82 mg, 0.192 mmol), and 4N HCl in dioxane (0.5 mL) to obtain HCl.NH_2-Sta-$NCH_3(CH_2)_2$Ph(4'-Cl) **15** as an oil (53 mg, 76%). General Procedure A was then followed using Cbz-Arg(*N,N*-diBoc)-Ala-OH **10** (54 mg, 0.093 mmol) and HCl.NH_2-Sta-$NCH_3(CH_2)_2$Ph(4'-Cl) **15** (37 mg, 0.102 mmol) to obtain a crude residue. The crude

residue was subjected to silica chromatography gradient eluting with 100% DCM to 10% MeOH/DCM to obtain **18** as an oil (53 mg, 64%). ^{1}H NMR and MS data was identical to that previously described.[1] ^{13}C NMR (75 MHz, $CDCl_3$, 325K, rotamers) δ 173.24, 173.07, 172.88, 172.76, 172.03, 171.93, 171.88, 171.84, 171.51, 171.41, 163.39, 156.49, 156.42, 153.43, 153.38, 137.58, 137.53, 136.66, 136.51, 136.47, 132.94, 132.90, 132.47, 130.48, 130.25, 130.23, 129.09, 129.07, 128.81, 128.65, 128.30, 128.27, 128.20, 83.39, 79.55, 70.16, 70.00, 69.96, 69.81, 67.27, 67.20, 55.28, 55.01, 51.41, 51.34, 51.22, 51.03, 50.98, 50.00, 49.94, 49.71, 49.61, 41.78, 41.73, 41.60, 40.38, 37.30, 37.26, 36.43, 36.11, 36.05, 34.12, 34.06, 33.57, 33.53, 33.24, 33.20, 31.38, 28.45, 28.42, 28.23, 28.17, 25.03, 23.24, 23.21, 23.13, 22.49, 22.36, 22.24, 18.64, 18.59, 18.45. IR (cm^{-1}) *v* 3293 (NH), 2955 (CH), 1637 (C=O).

Cbz-Arg(NH_2)-Ala-Sta-$NCH_3(CH_2)_2$Ph(4'-Cl).TFA 5

General Procedure C was followed using Cbz-Arg(*N,N*-diBoc)-Ala-Sta-$NCH_3(CH_2)_2$Ph(4'-Cl) **18** (40 mg, 0.045 mmol), to obtain **5** as an oil (25 mg, 70%). The data was identical to that previously described.[1]

Cbz-Orn(*N*-Boc)-Val-OMe 24

A modified General Procedure A was followed using Cbz-Orn(*N*-Boc)-OH (600 mg, 1.64 mmol), and HCl.NH_2-Val-OMe (412 mg, 2.46 mmol). Once the coupling reaction was complete, it was quenched with 10% citric acid solution. The precipitate that formed was filtered off to give **24** as a solid (780 mg, 99%). The data was identical to that previously described.[1]

Boc-Sta-$NH(CH_2)_2$Ph 13

A modified General Procedure A was followed using Boc-Sta(3*S*,4*S*)-OH (200 mg, 0.726 mmol), and phenylethylamine (182 μL, 1.45 mmol). Once the coupling reaction was complete, it was quenched with 10% citric acid solution. The precipitate that formed was filtered off to give **13** as a solid (273 mg, 99%). The data was identical to that previously described.[1]

HCl.NH_2-Sta-$NH(CH_2)_2$Ph 16

General Procedure B was followed using Boc-Sta-$NH(CH_2)_2$Ph **13** (300 mg, 793 mmol), to obtain **16** as a solid (247 mg, 99%). The data was identical to that previously described.[1]

Cbz-Orn(*N*-Boc)-Val-Sta-NH$(CH_2)_2$Ph 31

A mixture of Cbz-Orn(*N*-Boc)-Val-OMe **24** (400 mg, 0.834 mmol), and LiOH hydrate (87 mg, 2.09 mmol) in a mixture of water (4 mL) and THF (12 mL) was allowed to stir for 3 h at 20°C. 10% Citric acid solution was added to the reaction mixture. The solution was extracted with EtOAc (3 x 15 mL). The combined organic layers were washed with brine (30 mL) and dried with $MgSO_4$. The solvent was concentrated *in vacuo* to obtain Cbz-Orn(*N*-Boc)-Val-OH **28** as an oil (385 mg, 99%). General Procedure A was then followed using Cbz-Orn(*N*-Boc)-Val-OH **28** (370 mg, 0.795 mmol), and HCl.NH_2-Sta-NH$(CH_2)_2$Ph **13** (300 mg, 0.954 mmol) to obtain a crude residue. The crude residue was subjected to silica chromatography gradient eluting with 100% DCM to 10% MeOH/DCM to obtain **31** as an oil (220 mg, 38%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers) δ 7.39 – 7.27 (m, 6H), 7.25 – 7.07 (m, 4H), 6.97 – 6.70 (m, 1H), 6.57 – 6.30 (m, 1H), 5.81 – 5.62 (m, 1H), 5.17 – 4.94 (m, 2H), 4.91 – 4.70 (m, 1H), 4.32 – 4.01 (m, 2H), 4.01 – 3.77 (m, 2H), 3.62 – 3.33 (m, 2H), 3.26 – 2.99 (m, 2H), 2.86 – 2.72 (m, 2H), 2.38 – 2.02 (m, 4H), 1.95 – 1.75 (m, 2H), 1.71 – 1.27 (m, 15H), 0.97 – 0.77 (m, 12H). ^{13}C NMR (151 MHz, $CDCl_3$, rotamers) δ 173.28, 172.69, 156.74, 156.55, 138.82, 138.65, 136.23, 128.86, 128.72, 128.69, 128.37, 128.34, 128.15, 128.09, 126.65, 70.74, 70.58, 67.26, 67.14, 60.24, 59.14, 54.86, 54.57, 51.54, 51.40, 41.47, 41.02, 40.82, 40.71, 40.16, 39.85, 35.53, 35.45, 30.35, 30.12, 29.83, 29.68, 28.58, 28.57, 24.89, 23.33, 23.30, 22.11, 21.98, 19.64, 18.32. IR (cm^{-1}) *v* 3288 (NH), 2962 (CH), 1640 (C=O). MS, *m/z* = 726.5 $[M + H]^+$.

Cbz-Arg(*N,N*-diBoc)-Val-Sta-NH$(CH_2)_2$Ph 34

General Procedure B was followed using Cbz-Orn(*N*-Boc)-Val-Sta-NH$(CH_2)_2$Ph **31** (220 mg, 0.303 mmol), and 4N HCl in dioxane (3.5 mL) to obtain Cbz-Orn(NH_2.HCl)-Val-Sta-NH$(CH_2)_2$Ph as a crude oil (199 mg, 99%). The crude residue (40 mg, 0.060 mmol) was dissolved in DCM (1 mL) and Et_3N (11 µL, 0.079 mmol) was added. The solution was stirred vigorously for 5 min. *N,N'*-Bis-Boc-1-guanylpyrazole (24 mg, 0.079 mmol) was added and the solution was allowed to stir for 18 h at 20°C. The reaction mixture was concentrated to dryness *in vacuo* to obtain a crude residue. The crude residue was subjected to silica chromatography gradient eluting with 100% DCM to 10% MeOH/DCM to obtain **34** as an oil (45 mg, 86%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers) δ 11.46 (s, 1H), 8.44 – 8.30 (m, 1H), 7.37 – 6.98 (m, 12H), 6.90 – 6.69 (m, 1H), 6.38 – 6.09 (m, 1H), 5.13 – 4.83 (m, 2H), 4.38 – 4.17 (m, 2H), 4.03 – 3.83 (m, 2H), 3.53 – 3.27 (m, 4H), 2.84 – 2.69 (m, 2H), 2.38 – 2.18 (m, 2H), 2.17 – 2.01 (m, 1H), 1.96 – 1.21 (m, 26H), 0.99 – 0.79 (m, 12H). ^{13}C NMR (75 MHz, $CDCl_3$, 325K, rotamers) δ 172.50, 172.32, 172.15, 172.06, 171.62, 171.32, 163.42, 156.75, 156.50, 156.38, 153.42,

139.15, 139.13, 136.34, 128.85, 128.81, 128.66, 128.64, 128.33, 128.28, 128.16, 128.04, 126.53, 126.51, 83.36, 79.56, 77.36, 70.65, 70.55, 67.31, 67.17, 59.86, 59.44, 55.65, 55.05, 51.43, 41.28, 41.17, 40.82, 40.74, 40.45, 40.29, 35.82, 35.78, 31.37, 30.68, 30.34, 29.90, 28.96, 28.44, 26.18, 25.52, 25.05, 25.02, 23.24, 23.20, 22.21, 22.13, 19.60, 19.57, 18.28, 18.11. IR (cm^{-1}) *v* 3287 (NH), 2961 (CH), 1636 (C=O). MS, *m/z* = 868.5 $[M + H]^+$.

Cbz-Arg(NH_2)-Val-Sta-NH(CH_2)$_2$Ph.TFA 6

General Procedure C was followed using Cbz-Arg(*N,N*-diBoc)-Val-Sta-NH(CH_2)$_2$Ph **34** (35 mg, 0.040 mmol), to obtain **6** as an oil (30 mg, 97%). ^{1}H NMR (600 MHz, MeOD, rotamers) δ 7.46 – 7.10 (m, 10H), 5.17 – 5.00 (m, 2H), 4.38 – 4.08 (m, 2H), 4.04 – 3.89 (m, 2H), 3.47 – 3.34 (m, 2H), 3.26 – 3.10 (m, 2H), 2.86 – 2.68 (m, 2H), 2.65 – 2.46 (m, 1H), 2.35 – 2.20 (m, 1H), 2.12 (br s, 1H), 1.90 – 1.46 (m, 6H), 1.40 – 1.22 (m, 1H), 1.05 – 0.78 (m, 12H). ^{13}C NMR (151 MHz, MeOD, rotamers) δ 174.73, 174.51, 174.01, 173.97, 173.60, 173.47, 158.61, 158.48, 158.40, 140.50, 138.05, 137.97, 129.89, 129.60, 129.37, 129.18, 128.97, 128.80, 127.47, 127.19, 71.65, 71.49, 71.41, 71.25, 67.83, 60.95, 60.82, 60.68, 60.54, 56.38, 56.26, 55.91, 55.77, 52.98, 52.82, 52.70, 52.55, 42.25, 42.13, 42.09, 41.97, 41.69, 41.61, 41.54, 41.28, 41.06, 40.97, 40.86, 36.72, 36.55, 36.52, 36.38, 31.74, 31.60, 31.42, 31.28, 30.36, 30.24, 30.11, 26.38, 26.26, 26.00, 25.90, 25.79, 23.80, 23.76, 23.71, 22.31, 22.28, 22.13, 22.10, 20.00, 19.96, 19.90, 19.86, 18.71, 18.67, 18.62, 18.58. IR (cm^{-1}) *v* 3268 (NH), 2964 (CH), 1637 (C=O). MS, *m/z* = 668.4 $[M + H]^+$. HRMS found: (M+H) 668.4133; $C_{35}H_{53}N_7O_6$ requires (M+H), 668.4136.

Cbz-Orn(*N*-Boc)-NCH_3-Val-OMe 25

General Procedure A was followed using Cbz-Orn(*N*-Boc)-OH **21** (200 mg, 0.546 mmol), and HCl.NH(CH_3)-Val-OMe **20** (149 mg, 0.819 mmol) to obtain a crude residue. The crude residue was subjected to silica chromatography gradient eluting with 100% DCM to 10% MeOH/DCM to obtain **25** as an oil (200 mg, 74%). ^{1}H NMR (300 MHz, MeOD, 325K) δ 7.43 – 7.21 (m, 5H), 5.10 (s, 2H), 4.77 (d, *J* = 8.8 Hz, 1H), 3.75 – 3.55 (m, 3H), 3.18 – 2.82 (m, 5H), 2.25 (s, 1H), 1.89 – 1.51 (m, 5H), 1.45 (d, *J* = 3.0 Hz, 9H), 1.12 – 0.72 (m, 6H). ^{13}C NMR (75 MHz, MeOD, 325K) δ 175.38, 172.42, 158.46, 138.22, 129.43, 128.97, 128.78, 108.92, 79.99, 67.69, 63.87, 52.32, 40.92, 32.29, 30.01, 28.81, 28.41, 27.08, 20.21, 19.28. IR (cm^{-1}) *v* 3307 (NH), 2968 (CH), 1703 (C=O). MS, *m/z* = 494.4 $[M + H]^+$.

Cbz-Orn(*N*-Boc)-NCH_3-Val-Sta-$NH(CH_2)_2Ph$ 32

A mixture of Cbz-Orn(*N*-Boc)-NCH_3-Val-OMe **25** (170 mg, 0.344 mmol), and LiOH hydrate (87 mg, 2.07 mmol) in a mixture of water (1.7 mL) and THF (5.1 mL) was allowed to stir for 18 h at 20°C. 10% Citric acid solution was added to the reaction mixture. The solution was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (20 mL) and dried with $MgSO_4$. The solvent was concentrated *in vacuo* to obtain Cbz-Orn(*N*-Boc)-NCH_3-Val-OH **29** as an oil (163 mg, 99%). General Procedure A was then followed using Cbz-Orn(*N*-Boc)-NCH_3-Val-OH **29** (90 mg, 0.188 mmol), and HCl.NH_2-Sta-$NH(CH_2)_2$Ph **16** (118 mg, 0.375 mmol) to obtain a crude residue. The crude residue was subjected to silica chromatography gradient eluting with 100% DCM to 10% MeOH/DCM to obtain **32** as an oil (68 mg, 50%). ^{1}H NMR (300 MHz, MeOD) δ 7.39 – 7.10 (m, 10H), 4.99 (q, *J* = 12.5 Hz, 2H), 4.59 (d, *J* = 11.1 Hz, 1H), 4.53 – 4.45 (m, 1H), 4.04 – 3.91 (m, 2H), 3.50 – 3.34 (m, 2H), 3.15 – 2.97 (m, 5H), 2.74 (t, *J* = 7.4 Hz, 2H), 2.34 – 2.15 (m, 3H), 1.77 – 1.48 (m, 6H), 1.43 (s, 9H), 1.36 – 1.27 (m, 1H), 0.99 – 0.80 (m, 12H). ^{13}C NMR (75 MHz, MeOD, 325K) δ 175.97, 173.82, 171.55, 158.73, 158.51, 140.52, 137.98, 129.75, 129.50, 129.42, 129.02, 128.68, 127.29, 80.06, 71.52, 67.86, 64.38, 53.05, 52.38, 41.93, 41.70, 40.83, 36.51, 31.34, 29.59, 28.81, 27.37, 27.27, 26.05, 23.61, 22.32, 19.83, 19.02. IR (cm^{-1}) *v* 3305 (NH), 2961 (CH), 1632 (C=O). MS, *m/z* = 740.6 $[M + H]^+$.

Cbz-Arg(*N,N*-diBoc)-NCH_3-Val-Sta-$NH(CH_2)_2Ph$ 35

General Procedure B was followed using Cbz-Orn(*N*-Boc)-NCH_3-Val-Sta-$NH(CH_2)_2$Ph **32** (54 mg, 0.073 mmol), and 4N HCl in dioxane (0.5 mL) to obtain the unprotected residue as a crude oil (49 mg, 99%). The crude oil (43 mg, 0.064 mmol) was dissolved in DCM (0.9 mL) Et_3N (11.5 µL, 0.083 mmol) was added. The solution was stirred vigorously for 5 min. *N,N'*-Bis-Boc-1-guanylpyrazole (26 mg, 0.083 mmol) was added and the solution was allowed to stir for 18 h at 20°C. The reaction mixture was concentrated to dryness *in vacuo* to obtain a crude residue. The crude residue was subjected to silica chromatography gradient eluting with 100% DCM to 10% MeOH/DCM to obtain **35** as an oil (48 mg, 86%). ^{1}H NMR (300 MHz, MeOD) δ 7.40 – 7.13 (m, 10H), 5.14 – 4.93 (m, 2H), 4.66 – 4.49 (m, 2H), 4.09 – 3.90 (m, 2H), 3.48 – 3.38 (m, 3H), 3.15 & 3.02 (2s, 3H), 2.87 – 2.71 (m, 2H), 2.35 – 2.17 (m, 3H), 1.83 – 1.41 (m, 25H), 1.37 – 1.27 (m, 1H), 1.02 – 0.81 (m, 12H). ^{13}C NMR (75 MHz, MeOD, 325K) δ 175.85, 173.82, 171.53, 164.57, 158.72, 157.60, 154.26, 140.53, 137.99, 129.76, 129.50, 129.43, 129.01, 128.67, 127.30, 105.52, 84.56, 80.41, 71.51, 67.87, 64.40, 52.99, 52.38, 41.92, 41.71, 41.11, 36.52, 31.37, 29.52, 28.64, 28.28, 27.27, 26.62, 26.05, 23.61, 22.32, 19.84, 19.02. IR (cm^{-1}) *v* 3331 (NH), 2961 (CH), 1635 (C=O). MS, *m/z* = 882.7 $[M + H]^+$.

Cbz-Arg(NH_2)-NCH_3-Val-Sta-$NH(CH_2)_2$Ph.TFA 7

General Procedure C was followed using Cbz-Arg(*N,N*-diBoc)-NCH_3-Val-Sta-$NH(CH_2)_2$Ph **35** (40 mg, 0.045 mmol), to obtain **7** as an oil (35 mg, 97%). ^{1}H NMR (600 MHz, MeOD, rotamers) δ 7.41 – 7.10 (m, 10H), 5.13 – 4.91 (m, 2H), 4.69 – 4.49 (m, 2H), 4.39 – 3.85 (m, 2H), 3.45 – 3.34 (m, 2H), 3.27 – 3.07 (m, 5H), 2.84 – 2.67 (m, 2H), 2.47 – 2.13 (m, 3H), 1.83 – 1.42 (m, 6H), 1.36 – 1.20 (m, 1H), 1.04 – 0.78 (m, 12H). ^{13}C NMR (151 MHz, MeOD, rotamers) δ 175.47, 175.31, 173.89, 172.03, 171.60, 171.51, 171.16, 163.02, 162.79, 158.74, 158.66, 140.47, 140.32, 137.92, 129.91, 129.59, 129.43, 129.33, 129.19, 128.98, 128.82, 128.64, 128.58, 128.36, 127.45, 127.22, 71.60, 71.44, 67.88, 67.70, 64.25, 63.90, 52.88, 52.78, 52.43, 52.28, 52.20, 42.22, 42.08, 42.02, 41.97, 41.89, 41.79, 41.45, 37.89, 36.69, 36.53, 36.42, 31.41, 31.14, 29.45, 27.69, 27.36, 27.29, 26.38, 26.07, 25.96, 25.89, 25.77, 23.82, 23.71, 22.21, 21.73, 19.75, 19.12, 18.99. IR (cm^{-1}) *v* 3337 (NH), 2963 (CH), 1635 (C=O). MS, *m/z* = 682.6 $[M + H]^+$. HRMS found: (M+H) 682.4285; $C_{36}H_{55}N_7O_6$ requires (M+H), 682.4292.

1.2 Modeling

A model of *P. falciparum* PMV (PfPMV) in complex with **1** was constructed from a homology model described previously. That model used the X-ray crystal structures of Plasmepsin II from *P. falciparum* (2BJU),[3] Plasmepsin from *P. vivax* (1QS8),[4] human BACE-1 (2VIE),[5] and the secreted aspartic protease (3PVK)[6] as templates. The initial homology model was refined using molecular dynamics (MD). The structure of the ligand in this model was converted into that of **1**, and the model further refined with MD. MD simulations were performed using the GROMACS (v4.5.5) program[7] employing the OPLS-aa force field.[8] The system was solvated in a box of water (TIP4P). Ionizable residues were fixed in their charged state, and the system neutralized and the ionic concentration adjusted to 0.1 M by including Na^+ and Cl^- ions. Protein and ligand with water and ions were coupled separately to a thermal bath at 300K using velocity rescaling[9] applied with a coupling time of 0.1 ps, and the pressure was coupled to an isotropic barostat using a time constant of 1 ps and compressibility of 4.5×10^{-5} bar^{-1}. All simulations were performed with a single non-bonded cutoff of 10 Å and applying a neighbor-list update frequency of 10 steps (20fs). The particle-mesh Ewald method[10] was used to account for long-range electrostatics, applying a grid width of 1.2 Å and a fourth-order spline interpolation. Bond lengths were constrained using the LINCS algorithm.[11] The system was initially minimized prior to MD simulation, followed by positional restrained MD, with all protein non-hydrogen atoms restrained to their original positions for 0.1 ns. This was followed by 1 ns of unrestrained MD.

1.3 Biology

Plasmepsin V fluorogenic PEXEL cleavage assays.

Plasmepsin V fluorogenic PEXEL cleavage assays were performed as described previously.[1,12] Briefly, PMV-agarose was prepared by purification of HA-tagged *P. falciparum* PMV from transgenic *P. falciparum* parasite lysates using affinity chromatography with goat anti-HA agarose. The digest was obtained as described above and was used at a final assay concentration of 0.2 μL/20 μL. The KAHRP PEXEL peptide substrate DABCYL-RNKRTLAQKQ-E-EDANS was obtained commercially and used at a final assay concentration at the enzyme Km (5-10 μM). The end-point for all assays was set within the linear range of activity (approximately 2 h). Tween-20 was used at 0.005% final assay concentration. Final assay buffer concentration was as follows: 25 mM Tris HCl, 25 mM MES (pH 6.4). A nine-point 1/2 serial dilution of compounds was generated using DMSO as a vehicle (final assay concentration of 1%). Assay reaction was incubated for 120 min at 37°C and read using a fluorescence plate reader (Ex 340 nm, Em 495 nm). IC_{50} values were determined using a nonlinear regression four-parameter fit analysis, using GraphPad Prism software, where two of the parameters were constrained to 0 and 100%. It is noted that the fluorogenic PEXEL cleavage assay is conducted in heterogenous conditions, whereby plasmepsin V is bound to a Sepharose-bead. Under these conditions, achieving homogeneity between assay points is relatively difficult and results in compound IC_{50} curves with variable Hill slope coefficients. This is a reflection of the technical nature of the assay rather than an inherent change of the inhibition mechanism.

HepG2 cytotoxicity assay.

The cytotoxicity assays were performed as described by Sleebs *et al.*[1,12] Briefly, HepG2 cells were cultured in Dulbecco's Modified Eagles Medium (DME) supplemented with 10% heat inactivated fetal calf serum (FCS) in a humidified incubator at 37°C and 5% CO_2. Eleven-point compound titration assays were performed by treating cells (1×10^4) for 48 h in 96-well plates. Cytotoxicity was determined using CellTiter Glo (Promega) and calculated as a percentage of DMSO control. Etoposide was used as a control compound, and obtained an IC_{50} of 9.8 μM.

Parasite viability assays.

Parasite viability assays were performed as described by Sleebs *et al.*[1,12] *P. falciparum* 3D7 were cultured in human O^+ erythrocytes at 4% hematocrit in RPMI 1640 medium supplemented with 25 mM HEPES, pH 7.4, 0.2% sodium bicarbonate, and 0.5% Albumax II (Invitrogen) in culture gas (5% CO_2,

5% O_2, 90% N) at 37°C. Early ring-stage *P. falciparum* 3D7 parasites were obtained by sorbitol synchronization and treated in 96-well plates with compounds dissolved in ethanol (not greater than 2% final to limit toxicity) or DMSO (not greater than 0.2% final to limit toxicity) in nine-point titrations for 72 h at 37 °C in culture gas. Parasitemia was then determined by flow cytometry and expressed relative to vehicle-treated controls. Parasitemia was qualitatively assessed by Giemsa smears.

Parasite PEXEL processing assay, immunoblot, and densitometry.

Parasite viability assays were performed as described by Sleebs *et al.*[1,12] Transgenic P. falciparum expressing PfEMP3-GFP from the CRT promoter were generated previously[13] and treated with compounds as described previously.[12] Briefly, 30−34 h old trophozoites were purified from uninfected erythrocytes by passing through a Vario Macs magnet column (Miltenyi Biotech) and treated with inhibitor for 5 h at 37°C in culture gas. Parasites were treated with 0.1% saponin and pellets solubilized in 4× Laemmli sample buffer before protein separation via SDS-PAGE. Proteins were transferred to nitrocellulose using an iBlot (Invitrogen), blocked in 10% skim milk/PBS-T and probed with mouse anti-GFP (Roche; 1:1000), rabbit anti-HSP70 (1:4000), or rabbit anti-Aldolase (1:1000) antibodies followed by horseradish peroxidase-conjugated secondary antibodies (Silenius; 1:2000) and visualized using enhanced chemiluminescence (Amersham). Densitometry of blots exposed within the linear range were scanned at 400 dpi using a GS-800 calibrated densitometer (Bio-Rad) and quantified in Quantity One v4.6.3 software (Bio-Rad).

Compound stability assays.

Stability in pancreatin

Pancreatin (Porcine pancreas; Sigma Aldrich catalogue # P7545, Lot # 061M1822V; compose of a mixture of enzymes including trypsin, chymotrypsin, aminopeptidases, carboxypeptidases, lipase and amylase) was prepared in phosphate buffer (0.1 M, pH 7.4) to a concentration of 50 mg/mL. Aliquots of pancreatin solution were spiked with acetonitrile/water solutions of each test compound to a nominal compound concentration of 5000 ng/mL. The spiked pancreatin solution was vortex mixed and aliquots (50 µL) were transferred into fresh micro centrifuge tubes and maintained at 37°C. At various time points over the 24 hour incubation period, duplicate samples were taken and snap-frozen in dry ice. All samples were stored frozen at -80°C until analysis by LCMS. Samples were analyzed using a Waters Micromass Xevo G2 QTOF coupled to a Waters Acquity UPLC. Detection was positive electrospray ionisation under MSE mode; the column Ascentis Express RP amide column (50x2.1 mm, 2.7 µm); LC

conditions used: gradient cycle time: 4 min; injection vol: 3 μL; flow rate: 0.4 mL/min; mobile phase acetonitrile-water gradient with 0.05% formic acid. The positive control, Leucine enkephalin, degraded rapidly indicating the presence of proteinase activity in the pancreatin preparation used.

Stability in human serum

Human plasma (pooled, n=3 donors) was separated from whole blood procured from the Australian Red Cross Blood Service. Plasma was stored frozen at -80°C and thawed in a 37°C water bath on the day of the experiment. Aliquots of human plasma were spiked with acetonitrile/water solutions of each test compound to a nominal compound concentration of 5000 ng/mL. The spiked plasma was vortex mixed and aliquots (50 μL) were transferred into fresh micro centrifuge tubes and maintained at 37°C. At various time points over the 55 hours incubation period, duplicate samples were taken and snap-frozen in dry ice. All samples were stored frozen at -20°C until analysis by LCMS. LCMS analysis used is the same as for the stability in pancreatin. The positive control, Leucine enkephalin, degraded rapidly indicating the presence of proteinase activity in the human plasma used.

Minimal degradation of compounds **1-5** was observed in human plasma at 37°C, over 55 hrs, indicating that that they are not susceptible to hydrolytic enzymes present in plasma (Table S1).

1.4 Supplementary Figures

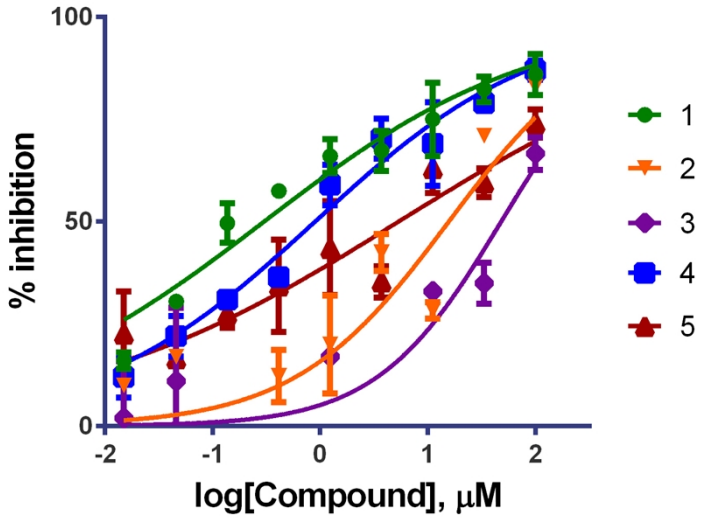


Figure S1. Dose response curves of compounds **1-5**. A 9-point dilution in three independent fluorogenic substrate (wtKAHRP) cleavage experiments of each compound was incubated with *P. falciparum* (Pf) PMVHA isolated from parasites. Error bars represent ±SEM.

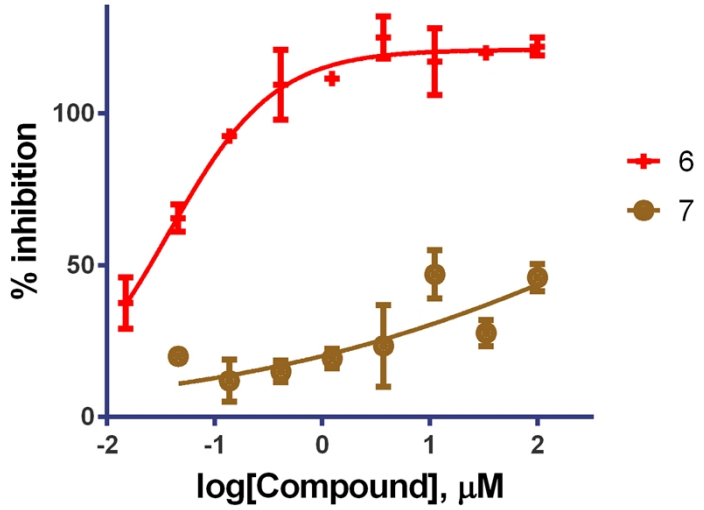


Figure S2. Dose response curves of compounds **6** and **7**. A 9-point dilution in three independent fluorogenic substrate (wtKAHRP) cleavage experiments of each compound was incubated with *P. falciparum* (Pf) PMVHA isolated from parasites. Error bars represent ±SEM.

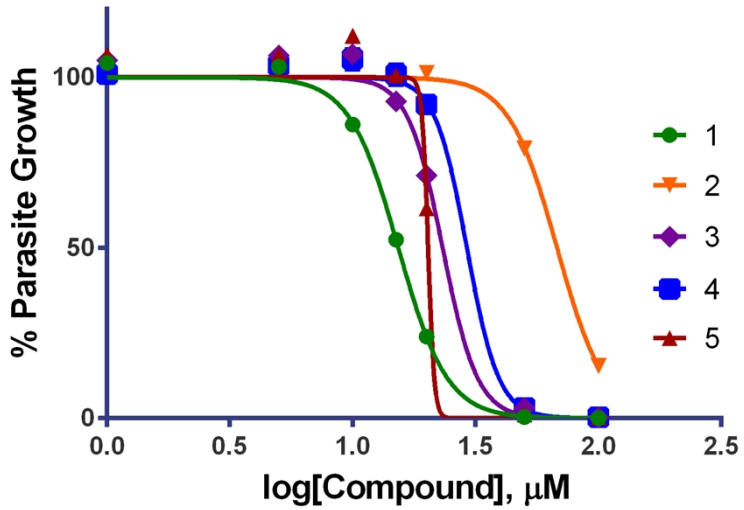


Figure S3. Dose response curves of compounds **1-5** against *P. falciparum* 3D7. Data shown are the mean ±SEM of three replicate experiments measuring parasitemia relative to vehicle-treated controls by flow cytometry following exposure to compounds in 7-point dilution series for 72 h.

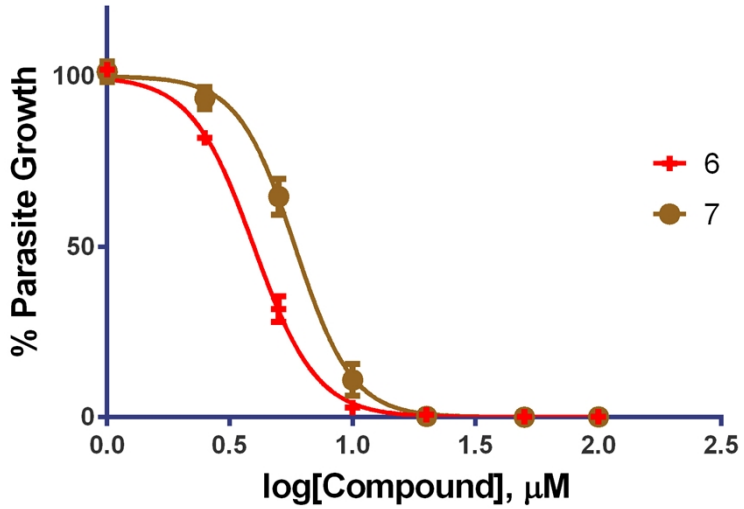


Figure S4. Dose response curves of **6** and **7** against *P. falciparum* 3D7. Data shown are the mean ±SEM of three replicate experiments measuring parasitemia relative to vehicle-treated controls by flow cytometry following exposure to compounds in 7-point dilution series for 72 h.

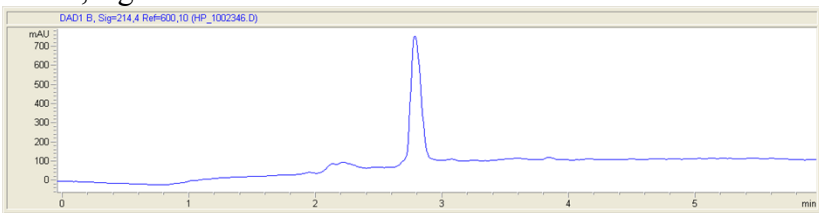
Table S1. Stability of compounds **1-5** incubated at 37°C in human plasma.

Sampling time (hrs)	% Remaining [a]				
	1	2	3	4	5
0	100	100	100	100	100
7	107	110	98	86	99
24	103	111	93	84	92
35	103	104	98	85	95
48	105	97	95	90	106
55	100	92	98	95	92

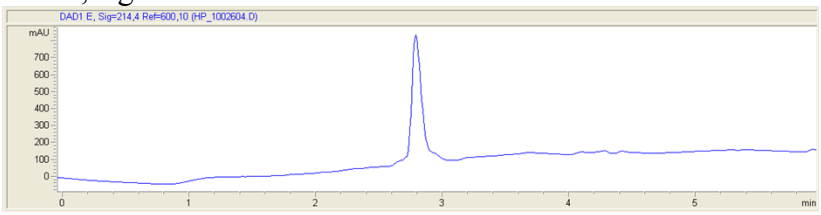
[a] Data presented are averages of duplicate measurements and expressed as percentages relative to the average concentration of the initial time point; the maximum variability of duplicate measurements determined as the deviation from the mean were within 12%.

1.5 HPLC traces of compounds 1-7

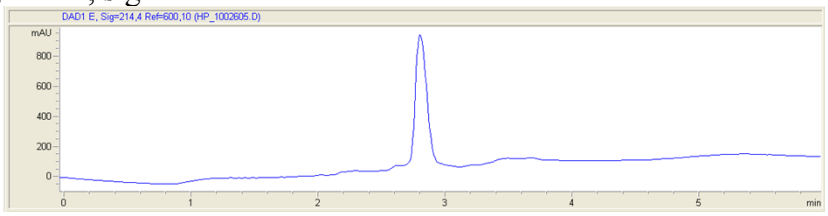
Compound 1, 1 mM, sig=214 nm



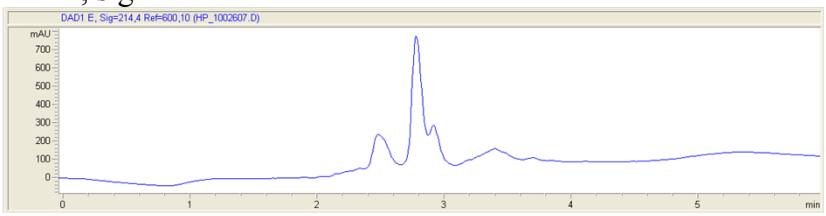
Compound 2, 1 mM, sig=214 nm



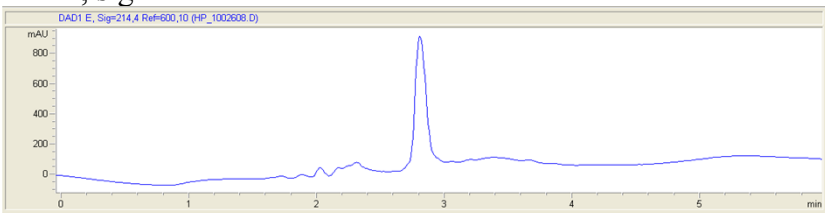
Compound 3, 1 mM, sig=214 nm



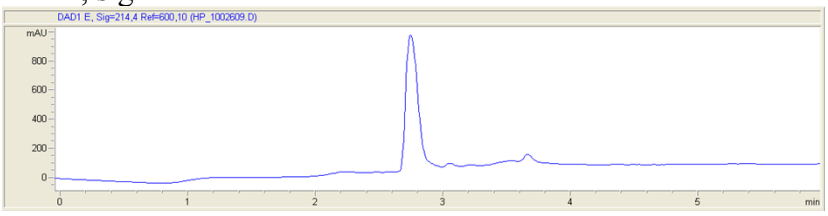
Compound 4, 1 mM, sig=214 nm



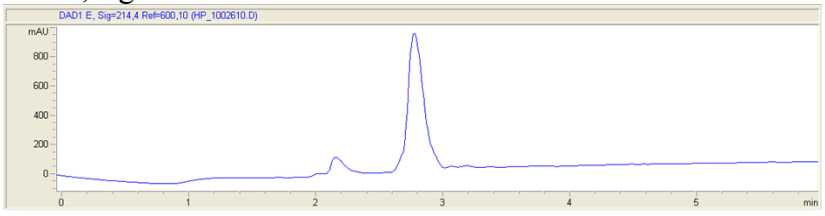
Compound 5, 1 mM, sig=214 nm



Compound 6, 1 mM, sig=214 nm



Compound 7, 1 mM, sig=214 nm



1.6 Abbreviations

DCM	dichloromethane
DIPEA	*N,N*-diisopropylethylamine
DMF	*N,N*-dimethylformamide
PfEMP3	*Plasmodium falciparum* erythrocyte membrane protein 3
GFP	green fluorescent protein
HBTU	2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
PTSA	*p*-toluenesulfonic acid
Sta	statine
TFA	trifluoroacetic acid
TFFH	fluoro-*N,N,N′,N′*-tetramethylformamidinium hexafluorophosphate
THF	tetrahydrofuran

1.7 References

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