Iterative Design of an Emetine-based Prodrug Targeting Fibroblast Activation Protein (FAP) and Dipeptidyl Peptidase IV (DPPIV) Using A Tandem Enzymatic Activation Strategy

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SUPPORTING INFORMATION

EXPERIMENTAL PROCEDURES

General Information: All solvents and reagents used were bought from commercial sources and used without further purification. Peptide synthesis was done as solid-phase synthesis using AAPPTec Apex 396 40-well peptide synthesizer. The ^{1}H- and ^{13}C -NMR spectra were obtained on a Bruker Avance III 500 MHz NMR spectrometer at 500 MHz and 125 MHz, respectively in deuterated chloroform (CDCl3) or deuterated methanol (CD3OD). Chemical shifts are in δ units (ppm) with TMS (0.00 ppm), CHCl3 (7.27 ppm), or CH3OH (3.34 ppm) as the internal standard for ^{1}H-NMR, and CDCl3 (77.00 ppm) or CD3OD (49.90 ppm) for ^{13}C-NMR. Mass spectra were obtained on Bruker Esquire 3000 Mass Spectrometer equipped with ESI. Analytical thin-layer chromatography was performed using 0.25 mm precoated silica gel 60 F254 plates (Analtech Uniplates). Flash column chromatography was performed using silica gel 60 (200 X 400 mesh, Sorbent Technologies) with the indicated solvent. Purity of the compounds was determined with reverse phase-HPLC. The purity of all the compounds was determined to be >95%.

HPLC Methods

In this study, the reverse phase HPLC studies were carried out using the following methods.

HPLC Method 1

This method was used for analytical reversed-phase HPLC for the analysis of the synthetic emetine analogs and prodrugs (to determine purity) and for analysis of cleavage studies of emetine prodrugs.

HPLC Unit: Waters Delta 600 Controller equipped with a variable wavelength UV-vis detector (Waters 2487 Dual λ Absorbance Detector) set to detect at 215 nm and 285 nm and a Vydac 218TP54 (C18, 5 μm, 4.6 mm. i.d. X 250 mm) analytical column.

Software: Empower 2

HPLC Conditions: Flow rate: 1.3 mL/min; mobile phase A: 5% MeCN, 95% water and 0.1% TFA. Mobile phase B: 100% MeCN, 0.1% TFA. Gradient: 0-2min 100 % mobile phase A; 2-20 min gradual change to 100% mobile phase B; 20-25, 100% mobile phase B; 25-27min gradual change to 100% mobile phase A; 27-30 min, 100% mobile phase A. Injection volume: 100 μL

HPLC Method 2

This method was used for preparative purification of compounds **1-11**.

HPLC Unit: Waters Delta 600 Controller equipped with a variable wavelength UV-vis detector (Waters 2487 Dual λ Absorbance Detector) set to detect at 215 nm and 285 nm and a Luna (C18, 10 μm, 21.20 mm. i.d. X 250 mm) preparative column.

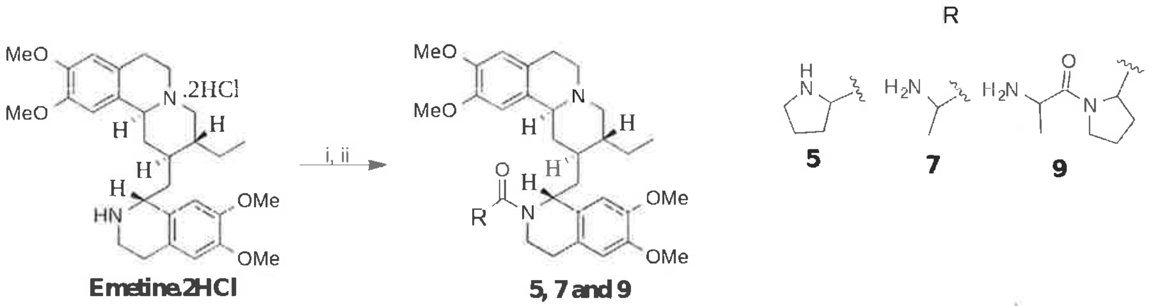
Software: Empower 2

HPLC Conditions: Flow rate: 25 mL/min; mobile phase A: 5% MeCN, 95% water and 0.1% TFA. Mobile phase B: 100% MeCN, 0.1% TFA radient: 0-4.0 min, 100% mobile phase A; 4.0- 20.0 min gradual change to 100% mobile phase B; 20.0-20.5 min 100% mobile phase B; 20.5- 21.0 min, sharp change to mobile phase A; 21.0-24.0 min mobile phase A. Injection volume: 1.0 mL

Chemical Synthesis General Procedure for the synthesis of amino acid coupled emetine analogs 5, 7 and 9 (synthesis scheme 1)

To synthesize each compound, respective Fmoc protected amino acid 1.0 molar equivalent was dissolved in 60 mL DMF, 8.0 molar equivalent of DIEA and 1.5 molar equivalent of HATU was added to the mixture. After stirring for 5 mins, 0.5 molar equivalent of emetine dihydrochloride was added and the mixture was stirred at room temperature for 5 h. Solvent was then evaporated under reduced pressure and the residue obtained was dissolved in EtOAc (100 mL) and washed with water (40 mL X 3), followed by saturated sodium chloride solution (40 mL X 2). The combined organic layer was dried over MgSO4 and solvent was evaporated. This crude product was dissolved in a 50 mL solution containing 25% piperidine in DMF and stirred for 25 mins to deprotect the Fmoc group. Solvent was evaporated from this mixture and the crude product was purified using flash column chromatography on silica gel using gradient elution with 100% ethyl acetate to elute the less polar impurities followed by elution with 1% triethyl amine (Et_3N) in MeOH to elute the partly pure product. This was finally purified by the reverse phase preparative HPLC using HPLC method 2 to obtain the desired compounds and these were lyophilized to remove all the solvents.

Scheme 1. Synthesis of compounds 5, 7 and 9



^{a}Reagents and conditions: (i). Fmoc-R-OH, HATU, DIEA, DMF; (ii) 25% piperidine in DMF.

(2*R*,3*R*,11b*S*)-2-(((1*R*)-6,7-dimethoxy-2-prolyl-1,2,3,4-tetrahydroisoquinolin-1-yl)methyl)-3-ethyl-9,10-dimethoxy-1,3,4,6,7,11b-hexahydro-2*H*-pyrido[2,1-*a*]isoquinoline (5): Yield: 80%, white powder ^1H NMR (500 MHz, CD_3OD) δ 0.92 (3H, t, J = 7.2 Hz), 1.20-1.32 (2H, m), 1.34-1.53 (2H, m), 1.62-1.73 (1H, m), 1.81-1.96 (3H, m), 2.00-2.14 (3H, m), 2.34-2.51 (3H, m), 2.58-2.64 (2H, m), 2.94-3.24 (5H, m), 3.29-

3.35 (1H, m), 3.43-3.55 (2H, m), 3.62-3.72 (1H, m), 3.79 (3H, s), 3.83 (6H, s), 3.85 (3H, s), 4.13 (1H, br d, J = 12.6 Hz), 5.07 (1H, dd, J = 4.0, 8.0 Hz), 5.68-5.79 (1H, m), 6.66 (1H, s), 6.69 (1H, s), 6.71 (1H, s), 6.94 (1H, s); ^{13}C NMR (125 MHz, CD_3OD) δ 11.2, 21.1, 23.9, 24.5, 28.7, 30.0, 36.4, 38.2, 39.7, 41.0, 47.2, 48.5, 52.4, 55.7 (2C), 56.0, 56.4, 57.3, 57.9, 61.6, 63.8, 109.0, 110.2, 111.3, 111.9, 125.3, 126.0, 130.6 (2C), 147.3 (2C), 147.9 (2C), 171.1. ESI-MS m/z 578.6 ($[C_{37}H_{52}N_4O_6 + H]^+$ calcd. 578.8) HPLC t_R is 12.0 min and purity is 98.8%.

2-amino-1-((*R*)-1-(((2*R*,3*R*,11b*S*)-3-ethyl-9,10-dimethoxy-1,3,4,6,7,11b-hexahydro-2*H*-pyrido[2,1-*a*]isoquinolin-2-yl)methyl)-6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)propan-1-one (7): Yield: 81%, white powder ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.92 (3H, t, J = 7.2 Hz), 1.21-1.37 (2H, m), 1.40-1.66 (5H, m), 1.70-1.85 (2H, m), 1.93-2.08 (2H, m), 2.32-2.51 (2H, m), 2.70-2.94 (2H, m), 2.96-3.16 (2H, m), 3.30-3.42 (1H, m), 3.44-3.57 (2H, m), 3.62-3.75 (3H, m), 3.80 (3H, s), 3.82 (6H, s), 3.83 (3H, s), 4.13 (1H, br d, J = 12.7 Hz), 5.09 (1H, dd, J = 4.0, 8.0 Hz), 5.78-5.88 (1H, m), 6.58 (1H, s), 6.68 (1H, s), 6.70 (1H, s), 6.98 (1H, s); ^{13}C NMR (125 MHz, CD_3Cl_3) δ 10.6, 17.5, 20.8, 24.1, 30.1, 36.6, 38.5, 39.4, 41.2, 47.4, 48.3, 52.3, 56.0 (2C), 56.3, 56.4, 57.4, 58.1, 62.1, 109.5, 110.1, 111.0, 111.6, 125.3, 125.8, 130.4 (2C), 147.8 (2C), 148.0 (2C), 170.4. ESI-MS m/z 552.5 ($[C_{37}H_{52}N_4O_6 + H]^+$ calcd. 552.7) HPLC t_R is 12.0 min and purity is 98.5%.

2-amino-1-(2-(1-((3-ethyl-9,10-dimethoxy-1,3,4,6,7,11b-hexahydro-2*H*-pyrido[2,1-*a*]isoquinolin-2-yl)methyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-2-carbonyl)pyrrolidin-1-yl)propan-1-one (9). Yield: 70%, off-white powder; ^{1}H NMR

(500 MHz, CD_3OD) δ 0.90 (3H, t, J = 7.2 Hz), 1.07-1.25 (4H, m), 1.32-1.55 (3H, m), 1.57-1.73 (1H, m), 1.77-1.98 (3H, m), 2.00-2.14 (3H, m), 2.15-2.44 (3H, m), 2.61-2.78 (2H, m), 2.87-3.14 (5H, m), 3.19-3.27 (1H, m), 3.41-3.57 (2H, m), 3.59-3.73 (3H, m), 3.77 (3H, s), 3.78 (6H, s), 3.81 (3H, s), 4.13 (1H, br d, J = 12.8 Hz), 5.07 (1H, dd, J = 4.0, 8.0 Hz), 5.68-5.79 (1H, m), 6.66 (1H, s), 6.69 (1H, s), 6.71 (1H, s), 6.94 (1H, s); ^{13}C NMR (125 MHz, CD_3OD) δ 10.1, 17.8, 20.3, 23.4, 24.9, 28.5, 29.7, 36.2, 38.3, 39.2, 40.8, 47.1, 48.5, 49.2, 52.1, 55.9 (2C), 56.2, 56.4, 57.3, 57.9, 61.7, 63.7, 108.5, 110.1, 111.1, 111.9, 125.2, 125.9, 130.2 (2C), 147.6 (2C), 147.8 (2C), 170.6, 173.8. ESI-MS m/z 649.4 ($[C_{37}H_{52}N_4O_6 + H]^+$ calcd. 649.9) HPLC t_R is 13.0 min and purity is 99.8%.

General Procedure for the synthesis of the N-2' derived analogs of emetine with self-cleaving PABC linker (scheme 2, scheme 3)

Respective Fmoc-protected amino acid (1.2 molar equivalent) was dissolved in N, N dimethylformamide (DMF). To this was added 1.2 molar equivalent of N-ethoxycarbonyl-2- ethoxy-1,2-dihydroquinoline (EEDQ) followed by a quantity of dichloromethane (CH_2Cl_2) equal to the volume of DMF. The solution was stirred for 5 min at room temperature and 4-aminobenzyl alcohol (1.0 molar equivalent) was added which turned the colorless solution to yellow. The mixture was stirred at room temperature for 8 h. Solvent was evaporated under reduced pressure and the residue was purified with flash column chromatography on silica gel using gradient elution with 5% ethyl acetate in CH_2Cl_2 to elute less polar impurities followed by elution with 50%

EtOAc in CH_2Cl_2 to elute the desired product.

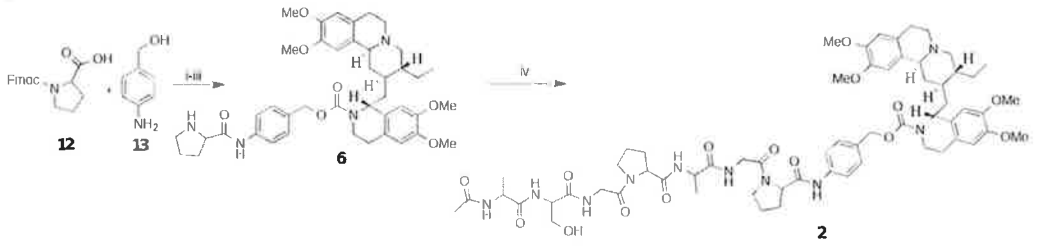
Conversion of emetine secondary amine to carbamate to afford compounds 6, 8 and 10 (scheme 2)

Reaction of primary alcohol and amine to form carbamate moiety was utilized in this synthesis. Each Fmoc-amino acid coupled 4-aminobenzyl alcohol such as **16** was conjugated to emetine as described below: 4-(dimethylamino) pyridine (DMAP) (1 molar equivalent) was added to a solution of N, N'-disuccinimidyl carbonate (DSC) (1.2 molar equivalent) solution in 20% DMF/DCM mixture under argon atmosphere. The respective alcohol (1.0 molar equivalent) solution in dichloromethane was then added to this DMAP and DSC mixture and the reaction was stirred at room temperature for 15 to 20 min. TLC indicated the complete consumption of the alcohol between 15 to 20 min of the reaction. Then, emetine.2HCl (1.2 molar equivalent) solution in 15 mL CH_2Cl_2 and 4 molar equivalent of N,N-diisopropylethyl amine (DIEA) was added. The reaction mixture was stirred at room temperature under argon and the progress was monitored with TLC and ESI-MS. TLC indicated the completion of the reaction in about 1.5 h. Solvent was then evaporated completely from the reaction mixture under reduced pressure and the residue obtained was purified with flash column chromatography using gradient elution. 100% ethyl acetate eluted the impurities less polar than the desired compound followed by elution with 10% MeOH in EtOAc to obtain Fmoc protected product. This product was dissolved in a solution of 25% piperidine in dimethylformamide (DMF) and the mixture was stirred at room temperature for 15 minutes. Solvent was evaporated from the mixture under reduced pressure. A viscous solid was obtained and this was triturated in hexanes.

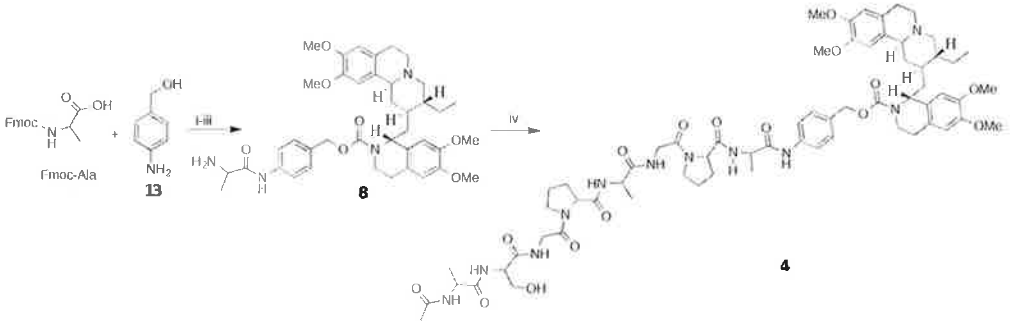
The final residue obtained was purified with preparative HPLC using method 2 to give the desired compound.

For the synthesis of compound **16**, compound **14** was first synthesized as described above: Yield 87%, white powder, ESI-MS m/z 443.1 ($[C_{27}H_{26}N_2O_4 + 1]^+$ calcd. 443.5; HPLC (method 1), t_R is 10.2 min, purity 97.8%. Deprotection of Fmoc from **14** was done by adding 25% piperidine in dimethylformamide (DMF) solution to **14**, and the mixture was stirred at room temperature for 10 minutes. Solvent was evaporated from the mixture under vacuum. 10.0 mL DMF was added to the oily residue and 200 mL hexanes was added to it. The mixture was stirred for 1.0 min in hexanes and allowed to settle. The hexane layer was removed by using separatory funnel. This treatment with hexanes was repeated 3 times and the DMF was evaporated from the DMF layer to give an oily residue. Pure product is then precipitated from cold diethyl ether to give compound **15**.

Scheme 2

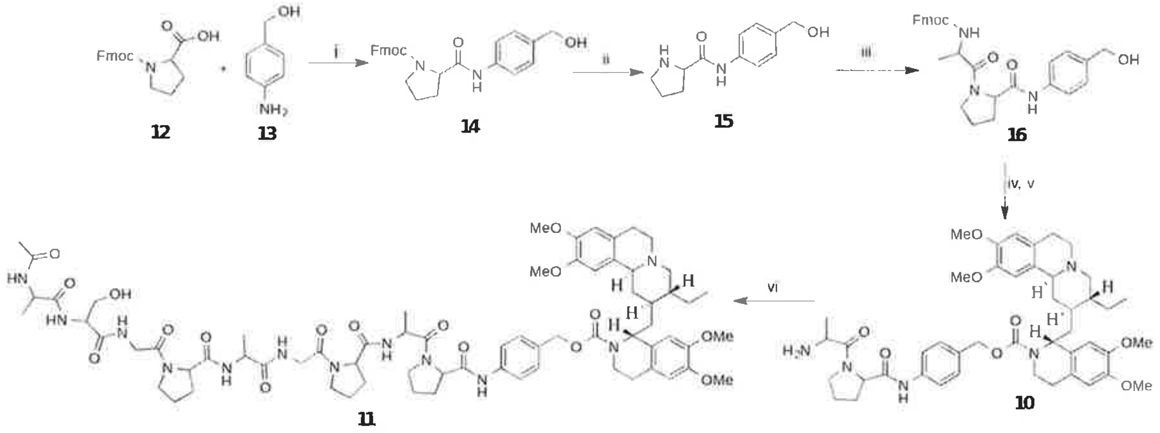


aReagents and conditions: (i) EEDQ, DCM-DMF, 84%; (ii) DMAP, DSC, DCM, Emetine.2HCl, DIEA, Argon;(iii) 25% Piperidine/DMF, 57%; (iv) HATU, **19**, DIEA, DMF, 35%.



aReagents and conditions: (i) EEDQ, DCM-DMF, 92%; (ii) DMAP, DSC, DCM, Emetine.2HCl, DIEA, Argon; (iii) 25% Piperidine/DMF, 50%; (iv) HATU, **22**, DIEA, DMF, 40%.

Scheme 3



aReagents and conditions: (i) EEDQ, DCM-DMF, 87%; (ii) 25% Piperidine/DMF, 65%; (iii) Fmoc-Ala-OH, EEDQ, DCM-DMF, RT, 80%; (iv) DMAP, DSC, **16**, DCM, Emetine.2HCl, DIEA, Argon; (v) 25% Piperidine/DMF, 50%; (vi) HATU, **19**, DIEA, DMF, 40%.

4-(pyrrolidine-2-carboxamido)benzyl (1*R*)-1-(((2*R*,3*R*,11b*S*)-3-ethyl-9,10-dimethoxy-1,3,4,6,7,11b-hexahydro-2*H*-pyrido[2,1-*a*]isoquinolin-2-yl)methyl)-6,7-dimethoxy-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (6):

Yield: 57%, off-white powder; ^{1}H NMR (500 MHz, CD_3OD) δ 0.89 (3H, t, J = 7.4 Hz), 1.09-1.36 (4H, m), 1.40-1.50 (3H, m), 1.52-1.62 (1H, m), 1.78-2.13 (2H, m), 2.18-2.32 (2H, m), 2.32-2.58 (4H, m), 2.60-2.71 (2H, m), 2.82-3.31 (4H, m), 3.41-3.60 (2H, m), 3.84 (3H, m), 3.86 (6H, s), 3.90 (3H, s), 3.99-4.10 (1H, m), 4.12-4.31 (3H, m), 5.05 (2H, s), 5.45 (1H, d, J = 12.5 Hz), 6.69 (2H, s), 6.74 (1H, s), 6.82 (1H, s), 7.36 (2H, d, J = 8.1 Hz), 7.58 (2H, d, J = 8.1 Hz); ^{13}C NMR (125 MHz, CD_3OD) δ 10.9, 20.3, 24.9, 26.6, 29.5, 31.0, 37.5, 38.2, 40.3, 42.6, 46.9, 50.4, 53.2, 56.6 (2C), 56.8(2C), 64.1(2C), 68.5, 73.5, 110.4 (2C), 111.4, 112.5, 122.4 (2C), 127.1, 127.9, 128.7 (2C), 132.1, 132.7, 138.3, 147.5 (2C), 149.6 (2C), 158.1, 172.1 ESI-MS m/z 727.5, ($[C_{42}H_{54}N_4O_7 + H]^+$ calcd. 727.9), HPLC t_R is 12.5 min and purity is 97.8%.

4-(2-aminopropanamido)benzyl (1*R*)-1-(((2*R*,3*R*,11b*S*)-3-ethyl-9,10-dimethoxy-1,3,4,6,7,11b-hexahydro-2*H*-pyrido[2,1-*a*]isoquinolin-2-yl)methyl)-6,7-dimethoxy-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (8): Yield: 50%, off-white powder; ^{1}H NMR (500 MHz, CD_3OD) δ 0.91 (3H, t, J = 7.4 Hz), 1.32-1.36 (1H, m), 1.47 (3H, s), 1.49-1.52 (1H, m), 1.60-1.73 (2H, m), 1.80-2.04 (2H, m), 2.07-2.18 (2H, m), 2.30-2.55 (2H, m), 2.77 (1H, d, J = 13.2 Hz), 2.97-3.16 (2H, m), 3.25-3.34 (3H, m), 3.38-3.50 (2H, m), 3.56-3.83 (4H, m), 3.88 (3H, s), 3.89 (3H, s), 3.91 (3H, s), 3.93 (3H, s), 4.13-4.22 (1H, m), 4.98 (2H, s), 5.39 (1H, d, J = 12.5 Hz), 6.76 (2H, s), 6.84 (1H, s), 7.07 (1H, s), 7.39 (2H, d, J = 8.1 Hz), 7.60 (2H, d, J = 8.1 Hz); ^{13}C NMR (125 MHz, CD_3OD) δ 11.1, 17.5, 24.2, 29.8, 36.9, 37.4, 38.3, 39.1, 39.5, 40.2, 42.0, 49.5, 50.9, 52.5, 56.2 (2C), 56.5(2C), 63.1, 63.5, 67.4, 74.2, 110.3 (2C), 111.2 (2C), 122.6 (2C), 127.5, 128.7 (2C), 129.5, 132.5, 138.5, 147.4 (2C), 147.9 (2C), 157.5, 173.2 ESI-MS m/z 701.3, ($[C_{40}H_{52}N_4O_7 + H]^+$ calcd. 701.9), HPLC t_R is 13.1 min and purity is 98.7%.

4-(1-alanylpyrrolidine-2-carboxamido)benzyl 1-((3-ethyl-9,10-dimethoxy-1,3,4,6,7,11b-hexahydro-2*H*-pyrido[2,1-*a*]isoquinolin-2-yl)methyl)-6,7-dimethoxy-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (10):

Yield: 50%, pale yellow powder; ^1H NMR (500 MHz, CD_3OD) δ 0.90 (3H, t, J = 7.4 Hz), 1.08-1.24 (3H, m), 1.23-1.42 (6H, m), 1.52-1.68 (1H, m), 1.92-2.09 (2H, m), 2.11-2.35 (3H, m), 2.51-2.82 (3H, m), 2.82-2.96 (1H, m), 3.02-3.18 (4H, m), 3.2-3.38 (3H, m), 3.62-3.73 (4H, m), 3.81 (3H, s), 3.83 (3H, s), 3.87 (3H, s), 3.99 (3H, s), 4.21-4.38 (1H, m), 4.45-4.65 (1H, m), 4.90-5.12 (1H, m), 5.30 (1H, d, J = 12.5 Hz), 5.38 (1H, d, J = 10.8 Hz), 6.71 (2H, s), 6.77 (1H, s), 6.80 (1H, s), 6.91 (1H, s), 7.34 (2H, d, J = 8.0 Hz), 7.50 (2H, d, J = 8.0 Hz); ^{13}C NMR (125 MHz, CD_3OD) δ 10.1, 19.2, 24.5, 26.1, 30.1, 31.0, 31.5, 35.2, 35.8, 37.1, 38.2, 39.9, 42.2, 47.9, 50.1, 51.2, 56.2 (2C), 56.9 (2C), 62.1, 63.2, 64.6, 70.5, 111.1, 112.3, 112.9, 113.8, 121.8 (2C), 127.0, 127.8, 128.0 (2C), 130.1, 131.9, 135.0, 139.8, 147.1 (2C), 149.2 (2C), 157.8, 172.7, 175.2. ESI-MS m/z 798.8, ($[C_{45}H_{59}N_5O_8 + H]^+$ calcd. 799.0), HPLC t_R is 13.9 min and purity is 98.6%

N-(4-((hydroxymethyl)phenyl)pyrrolidine-2-carboxamide (15)

Compound **15**. Yield 65%, pale yellow solid, ^1H NMR (500 MHz, $CDCl_3$) δ 1.78-1.86 (1H, m), 1.87-1.94 (1H, m), 2.18-2.27 (2H, m), 2.85 (1H, s), 2.95-3.04 (1H, m), 3.05-3.12 (1H, m), 3.39-3.35 (1H, m), 3.79 (1H, dd, J = 2.5, 6.3 Hz), 4.52 (2H, s), 4.58 (1H, s), 7.33 (2H, d, J = 7.5 Hz), 7.58 (2H, d, J = 7.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 29.6, 34.8, 50.7, 64.5, 67.5, 123.6 (2C), 131.2 (2C), 141.1, 141.6, 177.9. HPLC (method 1) purity 98.6%.

Compound **15** was coupled to Fmoc-Ala-OH using the same amide bond formation method with EEDQ to produce **16**.

(9*H*-fluoren-9-yl)methyl (1-(2-((4-(hydroxymethyl)phenyl)carbamoyl)pyrrolidin-1-yl)-1-oxopropan-2-yl)carbamate (16): Yield 80%, white powder; ^{1}H NMR (500 MHz, CD_3OD) δ 1.37 (3H, d, J = 7.5 Hz), 1.93-2.09 (2H, m), 2.10-2.21 (1H, m), 2.23-2.35 (1H, m), 3.31-3.35 (2H, m), 3.60-3.69 (1H, m), 3.78-3.87 (1H, m), 4.20-4.27 (2H, m), 4.31-4.50 (2H, m), 4.58 (3H, br s), 7.25-7.32 (4H, m), 7.34-7.49 (4H, m), 7.56 (2H, d, J = 7.5 Hz), 7.81 (2H, d, J = 7.5 Hz), 7.99 (1H, s). ^{13}C NMR (125 MHz, CD_3OD) δ 17.4, 26.3, 30.8, 48.2, 49.8, 50.2, 62.5, 64.8, 68.1, 120.9 (2C), 121.2 (2C), 126.3 (2C), 128.1, 128.3, 129.0 (2C), 129.3 (2C), 138.6, 138.9, 142.7 (2C), 145.1, 145.4, 158.4, 172.8, 174.1. ESI-MS m/z 513.1 ($[C_{30}H_{31}N_3O_5 + 1]^+$ calcd. 513.6 and m/z 536.3 ($[C_{30}H_{31}N_3O_5 + Na]^+$ calcd. 536.6); Purity was determined by HPLC to be 99%.

General procedure for peptide synthesis

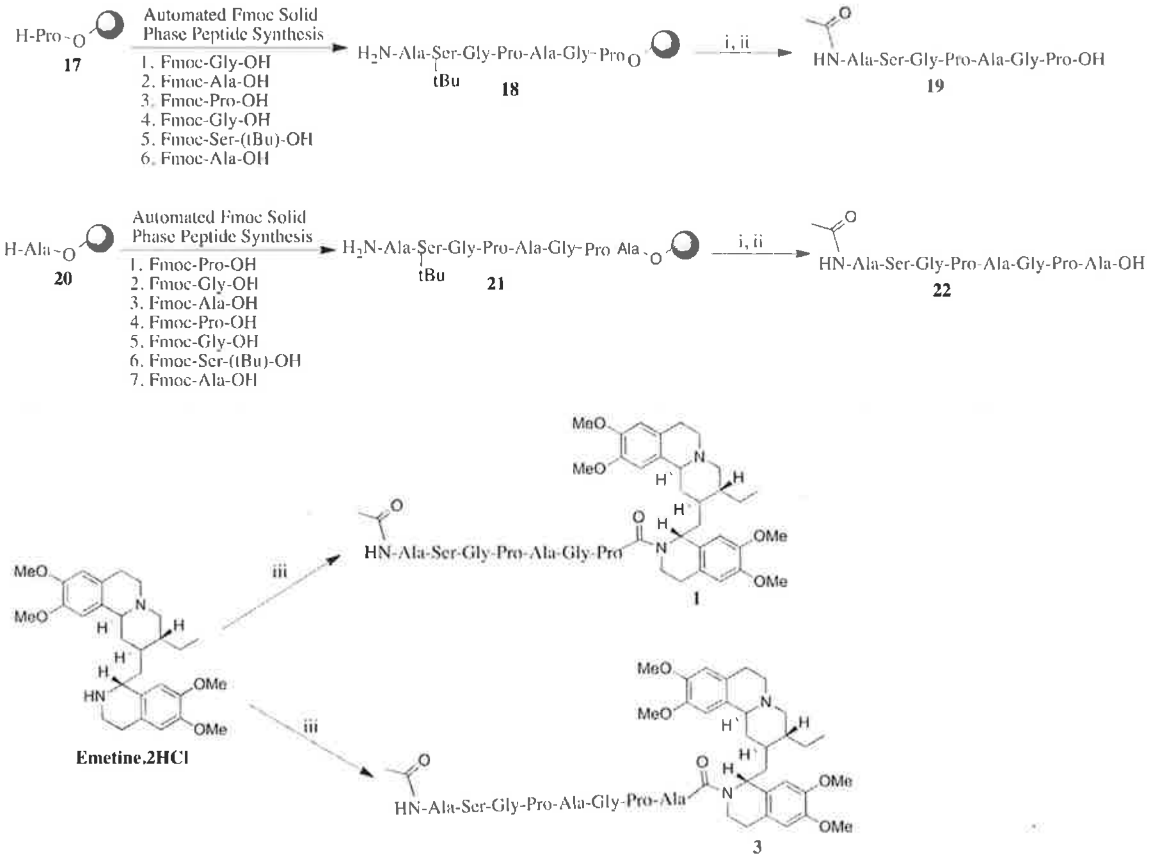
Peptides were prepared by solid-phase synthesis using AAPPTec Apex 396 40-well peptide synthesizer. The product was obtained using standard Fmoc protocol, starting with preloaded H- Pro-OH onto a 2-ClTrt resin. The amino acids (0.1 mmol) were coupled for 1 h using double coupling conditions. HOBt (5 equiv) used as activating reagent; 5 equiv of Fmoc-protected amino acids were used with 0.5 M DIC in NMP.

Upon reaction completion, the peptide on the resin was washed consecutively with NMP, methanol, and CH_2Cl_2. The N-terminal of the peptide was then capped with Ac_2O (6 molar equivalent) in the presence of DIEA (6 molar equivalent) in 10 mL CH_2Cl_2. The product obtained was washed with CH_2Cl_2. Cleaved from the resin together with the de-protection of tBu was accomplished with 95% TFA in water. The TFA solution was evaporated, and the peptide was precipitated in cold diethyl ether. The residue obtained was purified by the reverse phase preparative HPLC using the HPLC method 2. Eluent fraction was lyophilized to give desired compounds as off-white powder.

General procedure for the synthesis of peptidyl-emetine prodrugs with and without the linker (schemes 2, 3 and 4)

Respective peptide (3 molar equivalent) was dissolved in dimethyl formamide and DIEA (8 molar equivalents) was added. This was followed by HATU (3 molar equivalents). The mixture was stirred for 5 mins at room temperature and 1 molar equivalent of either emetine or any of compounds **6**, **8** and **10** was added. The reaction mixture was stirred at room temperature overnight. Solvent was evaporated from the mixture under reduced pressure and the residue obtained was purified by the reverse phase preparative HPLC using the HPLC method 2 to afford the desired compounds (**1**, **2**, **3**, **4** or **11)** which was lyophilized to give powdered products.

Scheme 4



[a]Reagents and conditions: (i) Ac_2O, DIEA, DCM; (ii) 95% TFA in H_2O (iii) HATU, DIEA, DMF

Ac-Ala-Ser-Gly-Pro-Ala-Gly-Pro-Emetine (1)

Yield 65%, pale yellow powder; ESI-MS m/z 1061.1, ($[C_{54}H_{77}N_9O_{13} + H]^+$ calcd. 1061.3) HPLC t_R is 15.5 min and purity is 98.2%.

Ac-Ala-Ser-Gly-Pro-Ala-Gly-Pro-PABC-Emetine (2): Yield 35%, yellow powder ESI-MS m/z 1210.0, ($[C_{62}H_{84}N_{10}O_{15} + H]^+$ calcd. 1210.4) HPLC t_R is 16.0 min and purity is 97.6%.

Ac-Ala-Ser-Gly-Pro-Ala-Gly-Pro-Ala-Emetine (3) Yield 70%, yellow powder ESI-MS m/z 1132.1, ($[C_{57}H_{82}N_{10}O_{14} + H]^+$ calcd. 1132.3) HPLC t_R is 16.0 min and purity is 98.0%.

Ac-Ala-Ser-Gly-Pro-Ala-Gly-Pro-Ala-PABC-Emetine (4) Yield 40%, light ESI-MS m/z 1281.2 ($[C_{65}H_{89}N_{11}O_{16} + H]^+$ calcd. 1281.5) HPLC t_R is 16.0 min and purity is 98.5%.

Ac-Ala-Ser-Gly-Pro-Ala-Gly-Pro-Ala-Pro-PABC-Emetine (11): Yield 40%, light yellow powder; ESI-MS m/z 1378.8, ($[C_{70}H_{96}N_{12}O_{17} + H]^+$ calcd. 1378.6) HPLC t_R is 16.5 min and purity is 99.2%.