

LC-MS (retention time: 1.52), MS m/z 486 ($M+1$).

Step 4:

To a solution of the acid (Example 451, Step 3) (0.32 g, 0.87 mmol) in
5 CH_3CN (10 mL) and DMF (2 mL) was added the diastereomeric mixture of 1(R)-
2(S) and 1(S)-2(R) 1-amino-2-vinyl-cyclopropanecarboxylic acid ethyl ester
hydrochloride (240 mg, 0.87 mmol) and TBTU (201 mg, 0.87 mmol) and DIPEA
(0.32 mL, 0.742 mmol) and the mixture stirred at 23°C for 12 h. The mixture was
concentrated and the residue partitioned between EtOAc and water. The organic
10 layer was separated, dried over MgSO_4 and concentrated. The residue was
chromatographed with 30% EtOAc/hexanes as eluant to give 240 mg (38%) of a light
yellow solid.

^1H NMR ($\text{DMSO}-d_6$, mixture of rotamers and diastereomers) δ 0.87 (s, 9H), 0.88 (s,
9H), 0.97-1.04 (m, 3H), 1.33 (s, 9H), 1.38 (s, 9H), 2.11-2.20 (m, 2H), 3.74-3.85 (m,
15 1H), 3.89-3.96 (m, 2H), 3.98-4.03 (m, 4H), 4.00-4.09 (m, 1H), 4.40-4.42 (m, 1H),
5.04-5.09 (m, 1H), 5.17-5.29 (m, 2H), 5.50-5.70 (m, 1H), 6.60-6.62 (m, 1H), 6.72-
6.75 (m, 1H), 7.50-7.56 (m, 1H), 7.56-7.72 (m, 2H), 7.70-7.80 (m, 1H), 7.92-8.00 (m,
1H), 8.00-8.06 (m, 1H), 8.29-8.40 (m, 1H), 8.65 (s, 1H), 8.79 (s, 1H);

LC-MS (retention time: 1.59), MS m/z 623 ($M+1$).

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Step 5:

The acid was prepared as previously described using LiOH in
THF/MeOH/ H_2O (4/2/1) in Example 451, Step 3, except using the product of
Example 451, Step 4 instead.

25 ^1H NMR ($\text{DMSO}-d_6$, mixture of rotamers and diastereomers) δ 0.90 (s, 9H), 1.17-
1.23 (m, 2H), 1.32-1.37 (m, 9H), 2.10-2.12 (m, 1H), 2.20-2.31 (m, 2H), 3.97-4.05 (m,
2H), 4.10-4.12 (m, 1H), 4.32-4.40 (m, 1H), 4.55-4.61 (m, 1H), 4.80-4.98 (m, 2H),
5.03-5.08 (m, 1H), 5.10-5.20 (m, 1H), 5.75-5.90 (m, 1H), 6.55-6.70 (m, 1H), 7.42-
7.57 (m, 1H), 7.60-7.64 (m, 2H), 7.70-7.72 (m, 1H), 7.80-7.97 (m, 1H), 7.96-7.99 (m,
30 1H), 8.20-8.50 (m, 2H);

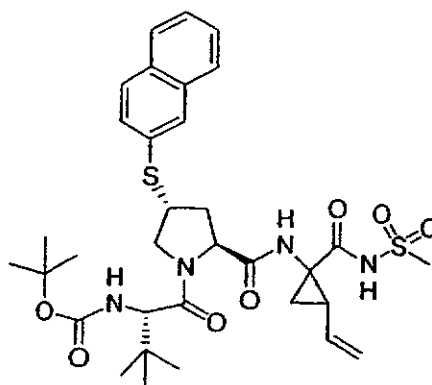
LC-MS (retention time: 1.52), MS m/z 595 ($M+1$).

Step 6:

A mixture of the acid (Example 451, Step 5) (172 mg, 0.29 mmol), methanesulfonamide (110 mg, 1.16 mmol), EDAC (110 mg, 0.58 mmol) and DMAP (71 mg, 0.58 mmol) was dissolved in THF (10 ml) and stirred for 12 h. DBU (0.087 mL, 0.58 mmol) was added and the mixture stirred for 48 h. The solvent is removed and the residue dissolved in EtOAc and washed with water and 1N HCl, dried over MgSO₄ and concentrated. The residue was purified by preparative thin layer chromatography to give 15 mg (8%) of Compound 451 as a tan solid.

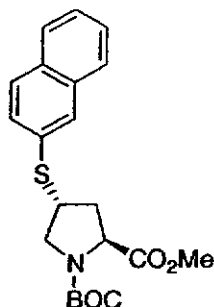
¹H NMR (DMSO-d₆, mixture of rotamers and diastereomers) δ 0.98 (s, 9H), 1.27-1.42 (m, 2H), 1.46 (s, 9H), 1.76-1.79 (m, 1H), 1.83-1.86 (m, 1H), 1.92-2.10 (m, 1H), 2.16-2.25 (m, 2H), 3.01-3.10 (m, 1H), 3.80-3.83 (m, 1H), 3.86-3.89 (m, 1H), 3.98-3.99 (m, 1H), 3.99-4.05 (m, 1H), 4.24-4.29 (m, 1H), 4.44-4.53 (m, 1H), 4.86 (s, 3H), 5.08-5.15 (m, 1H), 5.25-5.29 (m, 1H), 5.65-5.85 (m, 1H), 6.5-6.8 (m, 1H), 7.48 (t, $J = 7.7$ Hz, 1H), 7.54 (t, $J = 7.1$ Hz, 1H), 7.59 (t, $J = 7.1$ Hz, 1H), 7.75-7.77 (m, 1H), 7.88-7.91 (m, 2H), 8.46 (d, $J = 8.25$ Hz, 1H); LC-MS (retention time: 1.52), MS m/z 672 (M+1 minor), m/z 693 (M+Na Major).

Example 452: Preparation of Compound 452



Compound 452

20 Step 1:

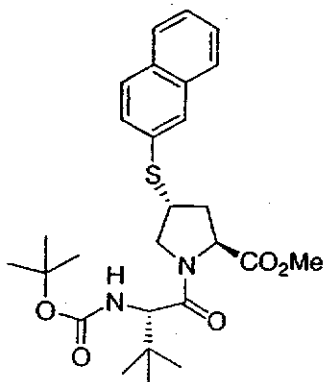


To a slurry of NaH (76 mg, 1.90 mmol) in DMF (20 mL) is added 2-thionaphthol (0.29 g, 1.80 mmol) and the mixture is stirred for 30 minutes. A solution of the tosylate (Example 451, Step 1) (0.61 g, 1.79 mmol) in DMF (2 mL) is added and the mixture stirred for 12 h at 23°C. The mixture is concentrated then partitioned between EtOAc/H₂O. The organic layer was washed with saturated NaHCO₃, dried (MgSO₄) and concentrated. The residue was chromatographed with 5% EtOAc/hexanes followed by 30% EtOAc/hexanes to give 261 mg (38%) of the product as a clear oil.

¹H NMR (DMSO-d₆) δ 1.32 (s, 9H), 2.29-2.35 (m, 2H), 3.33-3.47 (m, 2H), 3.66 (s, 3H), 3.71-3.81 (m, 1H), 4.29-4.32 (s, 1H), 7.49-7.55 (m, 3H), 7.70-7.80 (m, 1H), 7.81-7.97 (m, 3H);

LC-MS (retention time: 1.54), MS m/z 387 (M+1).

Step 2:

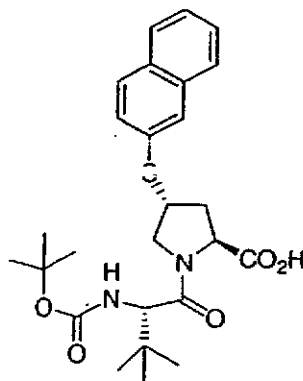


A mixture of 4-(naphthalen-2-ylsulfanyl)-pyrrolidine-1,2-dicarboxylic acid 1-tert-butyl ester 2-methyl ester (310 mg, 0.80 mmol) and 4N HCl in dioxane (1.49 mL, 2.69 mmol) was stirred for 2 h at 23°C then concentrated. The residue is dissolved in CH₃CN (10 mL) and N-Boc-t-butylglycine (196 mg, 0.85 mmol), TBTU (0.27 g,

0.85 mmol) and DIPEA (0.32 mL, 1.85 mmol) were added and the mixture stirred overnight. The mixture was concentrated and the residue dissolved in EtOAc, washed with 1 N HCl, saturated NaHCO₃, dried and concentrated to give 300 mg (90%) of the product as a yellow oil.

- 5 ¹H NMR (Methanol-d₄) δ 0.99 (s, 9H), 1.44 (s, 9H), 2.20-2.35 (m, 2H), 3.75 (s, 3H), 3.92-4.08 (m, 2H), 4.26 (d, J = 9.4 Hz, 1H), 4.57 (t, J = 9.5 Hz, 1H), 6.46 (d, J = 9.5 Hz, 1H), 7.48-7.60 (m, 3H), 7.83-7.90 (m, 3H), 8.02 (s, 1H)
LC-MS (retention time: 1.98), MS m/z 523 (M+Na).

10 Step 3:



- A solution of 4-(naphthalen-2-ylsulfanylmethyl)-pyrrolidine-1,2-dicarboxylic acid 1-tert-butyl ester 2-methyl ester (0.48 g, 0.96 mmol) is dissolved in MeOH (20 mL) and stirred with LiOH (0.2 g, 4.8 mmol) for 12 h. the solution is concentrated and
15 acidified and extracted with EtOAc. The organic extract was dried over MgSO₄ and concentrated to give 418 mg (91%) of a yellow solid.

- ¹H NMR (DMSO-d₆, 1:2 mixture of rotamers) δ 0.86, 0.93 (s, 9H) (1:2 mixture of rotamers), 1.35, 1.38 (s, 9H) (1:2 mixture of rotamers), 2.01-2.18, 2.25-2.35 (m, 2H), 3.25-3.40 (m, 2H), 3.70-3.80 (m, 1H), 4.00-4.20 (m, J = 9.4 Hz, 2H), 4.30-4.40 (s, 1H), 5.61-5.70, 6.42-6.50 (m, 1H) (1:2 mixture of rotamers), 7.50-7.54 (m, 3H), 7.87-7.89 (m, 3H), 7.98 (s, 1H);
20 LC-MS (retention time: 1.93), MS m/z 487 (M+1).

Step 4:

Scheme 1

**(1R,2S/1S,2R) Diastereomeric P1 Mixture**

A solution of 1-tert-butoxycarbonylamino-2-vinyl-cyclopropanecarboxylic acid ethyl ester (4.3 g, 17.8 mmol) in MeOH (50 mL) was treated with LiOH (0.84 g, 20.0 mmol) and water (5 mL) and the mixture stirred for 12 h. The solvent was removed and the residue acidified and extracted with EtOAc. The organic layers are dried over MgSO₄, filtered and concentrated to give the acid 2.1 g (52%) as a yellow oil.

The acid (2.1 g, 9.25 mmol) was dissolved in THF and treated with CDI (7.25 g, 13.8 mmol) and heated to reflux for 3 h then cooled to 23°C. Methanesulfonamide (1.76 g, 18.5 mmol) was added followed by DBU (2.77 ml, 18.5 mmol) and stirred for 72 h at 23°C. The mixture was concentrated and the residue acidified to pH 4 (1 N HCl) and extracted with EtOAc. The organic extracts were dried over MgSO₄ and concentrated to give 1.99g (71%) of a yellow oil that solidified on standing.

¹H NMR (DMSO-d₆) δ 1.16-1.23 (m, 1H), 1.43 (s, 9H), 1.65-1.75 (m, 1H), 2.15-2.25 (m, 2H), 3.16 (s, 3H), 5.08 (d, J = 9.9 Hz, 1H), 5.22 (d, J = 17.1 Hz, 1H), 5.40-5.52 (m, 1H);

LC-MS (retention time: 1.14), MS m/z 304 (M+1).

Step 5:

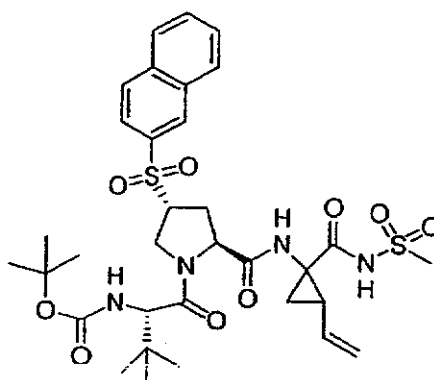
A solution of the product of Example 452, Step 4 in dioxane/4N HCl (2 ml) was stirred for 2 h then concentrated. The residue was dissolved in CH₃CN (5 mL) and added to a mixture of the acid (Example 452, Step 3) (120 mg, 0.25 mmol), TBTU (58 mg, 0.25 mmol) and DIPEA (0.06 ml, 0.35 mmol) was added and the mixture stirred for 12 h. The solvent was removed and the residue dissolved in EtOAc and washed with 1N HCl, saturated NaHCO₃ dried over MgSO₄ and concentrated. The residue is purified with preparative TLC (Analtech 20X40 cm, 1000 Å SiO₂) to give 128 mg (70%) of Compound 452 as a tan solid.

¹H NMR (DMSO-d₆, mixture of diastereomers) δ 0.97, 0.99 (s, 9H), 1.23-1.43 (m, 2H), 1.45 (s, 9 H), 1.82-1.83 (m, 1H), 2.00-2.51 (m, 2H), 2.90-2.99 (m, 1H), 3.33 (s,

3H), 3.90-3.99 (m, 1H), 4.01-4.20 (m, 2H), 4.25-4.30 (m, 1H), 4.45-4.55 (m, 1H), 4.95-5.10 (m, 1H), 5.12-5.25 (m, 1H), 5.71-5.85 (m, 1H), 6.4-6.8 (br m, 1H), 7.46-7.53 (m, 3H), 7.80-7.86 (m, 3H), 7.98-7.99 (m, 1H);
 LC-MS (retention time: 1.95), MS m/z 672 (M+1).

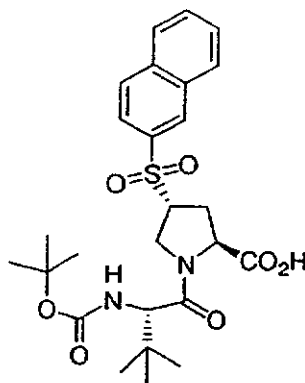
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Example 453: Preparation of Compound 453.



Compound 453

Step 1:



- 10 A mixture of Example 452, Step 3 (110 mg, 0.23 mmol) in DCM (20 ml), 3-chloroperbenzoic acid (121.6 mg, 0.57 mmol, 85% peracid), KHPO_4 (0.13g, 0.94 mmol) and K_2HPO_4 (0.18g, 1.05 mmol) are stirred at 23°C for 12 h. The solution is diluted with DCM, washed with water, saturated NaHCO_3 , dried over MgSO_4 and concentrated to give the product 110 mg, (92%) as a clear oil.
- 15 ^1H NMR (DMSO-d_6) δ 0.91 (s, 9H), 1.48 (s, 9H), 2.23-2.28 (m, 1H), 2.65-2.80 (m, 1H), 3.88-3.90 (m, 1H), 4.12 (t, $J = 8.0$ Hz, 1H), 4.20 (d, $J = 9.5$ Hz, 1H), 4.27 (d, $J = 9.9$ Hz, 1H), 6.76 (d, $J = 9.3$ Hz, 1H), 7.70-7.80 (m, 2H), 7.88-7.95 (m, 1H), 8.11 (d, $J = 8.1$ Hz, 1H), 8.17 (d, $J = 8.6$ Hz, 1H), 8.26 (d, $J = 8.1$ Hz, 1H), 8.71 (s, 1H);

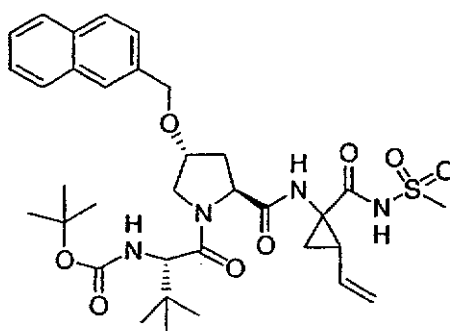
LC-MS (retention time: 1.72), MS m/z 519 ($M+1$).

Step 2:

A mixture of the product of Example 453, Step 1 (110 mg, 0.212 mmol),
5 amine (Example 452, Step 5a) (0.65 mg, 0.212 mmol), TBTU (48.5 mg, 0.21 mmol)
followed by DIPEA (60.8 ml, 0.35 mmol) and stirred for 12 h at 23°C. The solvent is
removed and the residue dissolved in EtOAc and washed with 1N HCl, saturated
NaHCO₃, dried over MgSO₄ and concentrated. The residue was purified by
preparative TLC (eluted with 10% MeOH/CH₂Cl₂) to give 25 mg (17%) of
10 Compound 453 as a white solid.

¹H NMR (DMSO-d₆, mixture of diastereomers) δ 0.93, 0.96 (s, 9H), 1.38-1.45 (m,
2H), 1.53, 1.55 (m, 9H), 1.76-1.85 (m, 1H), 2.21-2.40 (m, 2H), 3.13-3.15 (m, 2H),
3.34 (s, 3H), 3.91-3.99 (m, 1H), 4.15 (m, 1H), 4.25 (m, 1H), 4.30 (m, 1H), 5.09-5.12
(m, 1H), 5.26-5.31 (m, 1H), 5.72-5.76 (m, 1H), 6.65-6.68 (m, 1H), 6.71-6.76 (m,
15 1H), 7.67-7.76 (m, 2H), 7.91-7.95 (m, 1H), 8.03 (d, J = 7.8 Hz, 1H), 8.13 (d, J = 8.5
Hz, 1H), 8.20 (d, J = 7.6 Hz, 1H), 8.7 (s, 1H);
LC-MS (retention time: 1.76), MS m/z 705 ($M+1$).

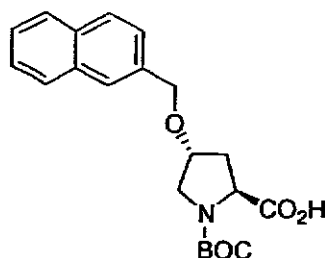
Example 454: Preparation of Compound 454.



Compound 454

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Step 1:

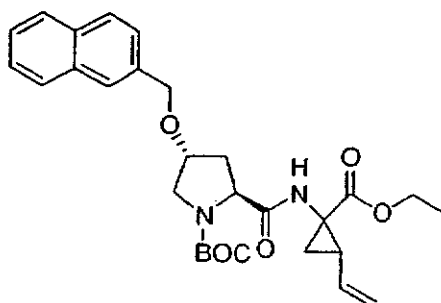


To slurry of the sodium hydride (0.91 g, 22.7 mmol) in THF(50 mL) was added N-BOC-trans-4(R)-hydroxy-L-proline (2.5 g, 10.8 mmol) and the mixture stirred at 23°C for 1 h. 2-Chloromethylnaphthalene (1.9 g, 10.8 mmol) was added and the mixture stirred for 12 h at room temperature. The solvent was removed and the residue poured into water and washed with hexanes. The aqueous layer was acidified (1 N HCl) and extracted with EtOAc. The EtOAc layer is separated, dried (MgSO₄), and concentrated to give a light yellow residue. The oil was purified by flash chromatography with 1:1 EtOAc/hexanes with 1% acetic acid added to give 1.56 g (59%) of the desired product as a thick oil.

¹H NMR (DMSO-d₆, 3:1 mixture of rotamers) δ 1.35, 1.37 (s, 9H, major and minor respectively), 1.92-2.02, 2.15-2.20 (m, 2H, major and minor respectively), 2.35-2.50 (m, 2H), 3.41-3.49 (m, 2H), 4.12-4.16, 4.20-4.21 (m, 2H), 4.65-4.68(m, 2H), 7.46-7.52 (m, 3H), 7.74-7.91 (m, 4H), (Acid OH not observed);

LC-MS (retention time: 1.44, YMC ODS-A C18 S7 3.0x 50 mm, gradient 10%MeOH/H₂O 0.1%TFA to 90% MeOH/H₂O 0.1% TFA), MS m/z 394 (M++1+Na).

Step 2:

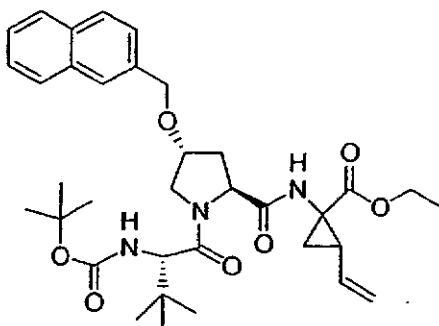


To a solution of the HCl salt of a 1:1 mixture of diastereoisomers (1R,2S/1S,2R where carboxy group is syn to vinyl moiety) of 2-(1-ethoxycarbonyl-2-vinyl -cyclopropylcarbamoyl)-4-(naphthalen-2-ylmethoxy)-pyrrolidine-1-carboxylic acid

tert-butyl ester (0.54 g, 1.3 mmole)[prepared by stirring the N-Boc amine with HCl (4N) in dioxane for 1 hr then removal of the solvent in vacuo] in CH₃CN (50 mL) is added Boc-4(R)-(2-methylnaphthyl)proline (0.5 g, 1.3 mmol), TBTU (0.45 g, 1.4 mmol) followed by DIPEA (0.78 mL, 4.5 mmol). The mixture is stirred for 12 h and concentrated. The residue was dissolved in EtOAc/H₂O and washed with saturated NaHCO₃, saturated NaCl, dried (MgSO₄) and concentrated to give a thick yellow oil (0.6 g, 91%) of the product as a mixture of diastereomers.

¹H NMR (DMSO-d₆) δ: 1.08-1.22 (m, 7 H), 1.23-1.39 (m, 9 H), 2.02-2.18 (m, 1H), 2.25-2.35 (m, 1H), 3.33-3.53 (m, 2H), 3.90-4.14 (m, 4H), 4.45-4.70 (m, 2H), 5.07-5.11 (m, 1H), 5.24-5.30 (m, 1H), 5.58-5.63 (m, 1H), 7.43-7.51 (m, 4H), 7.84-7.96 (m, 3H); MS m/z 531 (M++1+Na).

Step 3:



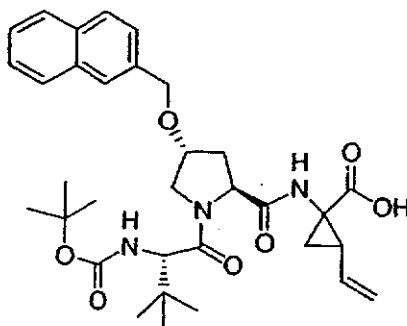
A solution of product of Example 454, Step 2 (600 mg, 1.18 mmol) was stirred with HCl (4N, 3 mL, 11.8 mmol) in dioxane for 1 hr then the solvent removed in vacuo. The residue is dissolved in CH₃CN (10 mL) treated with Boc-L-t-Bu-Gly (0.42 g, 1.38 mmol), TBTU (0.27 g, 1.18 mmol) followed by DIPEA (0.71 mL, 4.1 mmol). The mixture is stirred for 12 h and concentrated. The residue was dissolved in EtOAc/H₂O and washed with 1N HCl, saturated NaHCO₃, saturated NaCl, dried (MgSO₄) and concentrated to give a thick yellow oil. The product was purified by flash chromatography using gradient elution 5% EtOAc/Hexanes 10% EtOAc/Hexanes, 30% EtOAc/Hexanes and finally as eluant to give the product as a thick oil (0.243 g, 33%) of the product as a mixture of diastereomers and rotamers.

¹H NMR (DMSO-d₆) δ: 0.83-1.00 (m, 10H), 1.34 (s, 9H), 1.58-1.59, 1.65-1.67 (m, 2H), 1.95-1.99, 2.04-2.06, 2.10-2.19, 2.24-2.56 (m, 2H), 3.97-4.04 (m, 3H), 4.08-4.17

(m, 3H), 4.29-4.31 (m, 2H), 4.59-4.72 (m, 3H), 5.06-5.10 (m, 1H), 5.18-5.30 (m, 1H), 5.60-5.63 (m, 1H), 6.59-6.65, 6.70-6.74 (m, 1H), 7.43-7.51 (m, 4H), 7.84-7.96 (m, 3H), 8.66, 8.76 (s, 1H);
MS m/z 531 (M++1+Na).

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Step 4:



To a suspension of product of Example 454, Step 3 (240 mg, 0.39 mmol) in THF (15 mL), and H₂O (2 mL) was added LiOH (82 mg, 1.95 mmol). The reaction mixture
10 was stirred for 12 h then concentrated in vacuo until only the aqueous layer remained. The resulting aqueous residue was acidified to pH 3.0 by addition of 1.0 N aqueous HCl, and extracted with EtOAc (2x80mL). Combined organic extracts was dried (MgSO₄), filtered, and concentrated in vacuo to give the product as a tan solid (200 mg, 0.33 mmol, 85%):

15 ¹H NMR (DMSO-d₆) δ: 0.86, 0.94 (s, 9H minor and major respectively), 1.23-1.42 (m, 2H), 1.34 (s, 9H), 1.8-2.1 (m, 2 H), 2.18-2.30 (m, 1H), 3.59-3.73 (m, 3H), 4.0-4.09 (m, 1H), 4.18-4.34 (m, 3H), 4.56-4.62 (m, 1H), 4.66-4.67 (m, 1H), 4.82-4.92 (m, 1H), 5.0-5.20 (m, 1H), 5.91-6.08 (m, 1 H), 6.5-6.7 (m, 1H), 7.45-7.59 (m, 3H), 7.82-7.97 (m, 4H), 8.2-8.3, 8.3-8.4 (s, 1H);

20 LC-MS (retention time: 1.50), MS m/z 593 (M++1).

Step 5:

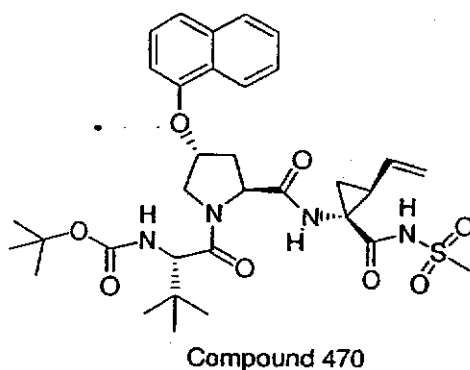
To a solution of product of Example 454, Step 4 (190 mg, 0.32 mmol) and EDAC (122 mg, 0.64 mmol) and 4-DMAP (78 mg, 0.64 mmol) in THF (20 mL) was
25 added commercially available methanesulfonamide (122 mg, 1.28 mmol). The resulting solution was stirred for 2 days, then DBU was added (95 μL, 0.64 mmol).

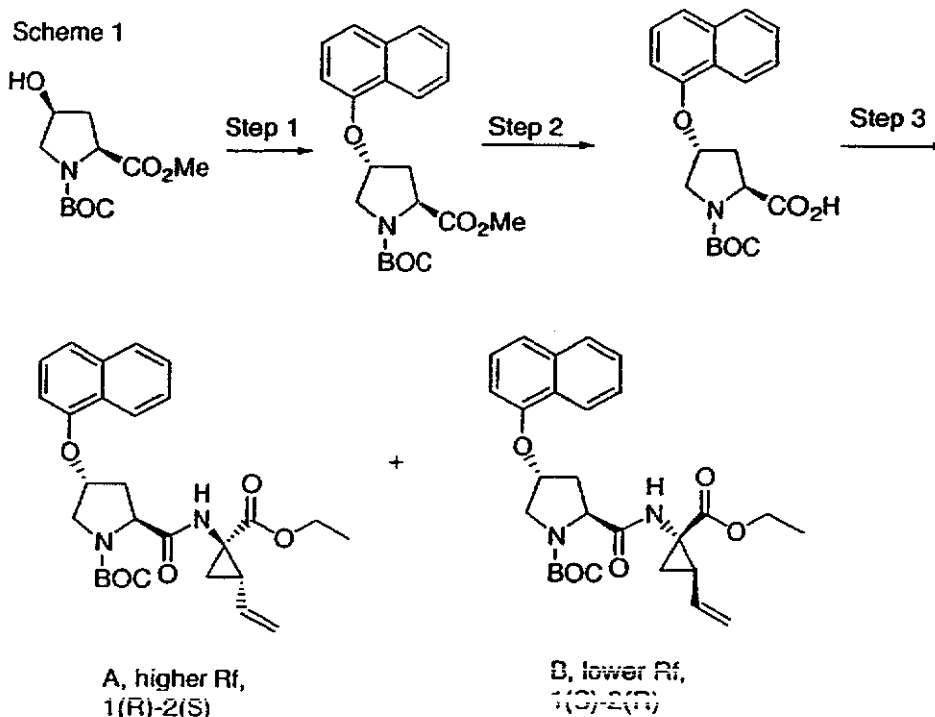
The reaction was stirred for 24 h then concentrated. The residue was partitioned between EtOAc (80mL) and water and washed with 1 N HCl), aqueous NaHCO₃ (2x30 mL), dried (MgSO₄) and purified by preparative HPLC (65-90% MeOH/Water/0.1% TFA) which gave 56 mg of a mixture of product and material in which the BOC group was removed. The material was further purified by preparative TLC (eluted with 10% MeOH/CH₂Cl₂ using 20X40cm plates from Analtech) to give Compound 454 as a tan solid (12 mg, 6%).

¹H NMR (MeOD-d₄ 50/50 mixture of P1 diastereomers) δ 0.88-0.99 (m, 2H), 1.01, 1.02 (s, 9H minor and major diastereomers respectively), 1.23-1.42 (m, 2H), 1.38 (s, 9H), 1.72-1.79 (m, 1H), 1.86-1.88 (m, 1H), 2.00-2.10 (m, 2H), 2.10-2.23 (m, 1H), 2.3-2.5 (m, 1H), 3.12, 3.17 (s, 3H), 3.72-3.79 (m, 1H), 4.26-4.41 (m, 3H), 4.72 (d, J = 8.2 Hz, 1H), 4.76 (d, J = 8.2 Hz, 1H), 5.09-5.12 (t, J = 9.3 Hz, 1H), 5.28 (dd, J = 3.5, 17.6 Hz, 1H) 5.7-5.8 (m, 1H), 6.55-6.80 (m, 1H), 7.45-7.47 (m, 3H), 7.79-7.83 (m, 4H);

LC-MS (retention time: 1.48), MS m/z 670 (M++1).

Example 470: Preparation of Compound 470.





Step 1:

To a solution of commercially available N-Boc-(4S)-(cis)-Hydroxyproline-OMe (200mgs, 0.82 mmole), triphenylphosphine (320mgs, 1.22 mmole) and 1-naphthol (176mgs, 1.22 mmole) in 2.5 mL tetrahydrofuran was added dropwise a solution of diethyldiazodicarboxylate (190 μ L, 1.22 mmole) in 1.0 mL THF over 10 minutes. After stirring for 5.5 days, the reaction was concentrated in vacuo. The crude yellow oil was chromatographed on a 20X40cm preparative TLC plate (Analtech SiO₂) eluting with 6-1 hexanes-ethyl acetate to yield the desired product as a pale yellow oil (150mgs, 33%).

¹H NMR (CDCl₃, 500MHz) δ 1.44 (s, 9H) 2.33 (1H, m), 2.72 (1H, m), 3.77 and 3.38 (2s, 3H, rotamers), 3.88 (dd, 1H, J= 4.3, 12.4 Hz), 3.97 (bd, 1H), 4.53 and 4.62 (2t, 1H, J=7.8Hz, rotamers), 5.10 (bd, 1H), 6.76 (t, 1H, J=9.5 Hz), 7.37 (m, 1H), 7.46 (m, 3H), 7.80 (d, 1H, J=7.7 Hz), 8.18 (m, 1H);

LC-MS A (retention time: 1.86; MS m/z 394 (M+Na)⁺

Step 2:

To a stirred solution of Boc-(4R)-naphthal-1-oxo)-Pro-OEt (150mgs, 0.40 mmole) in 1.5mL THF and 0.5mL water was added lithium hydroxide (10mgs). The

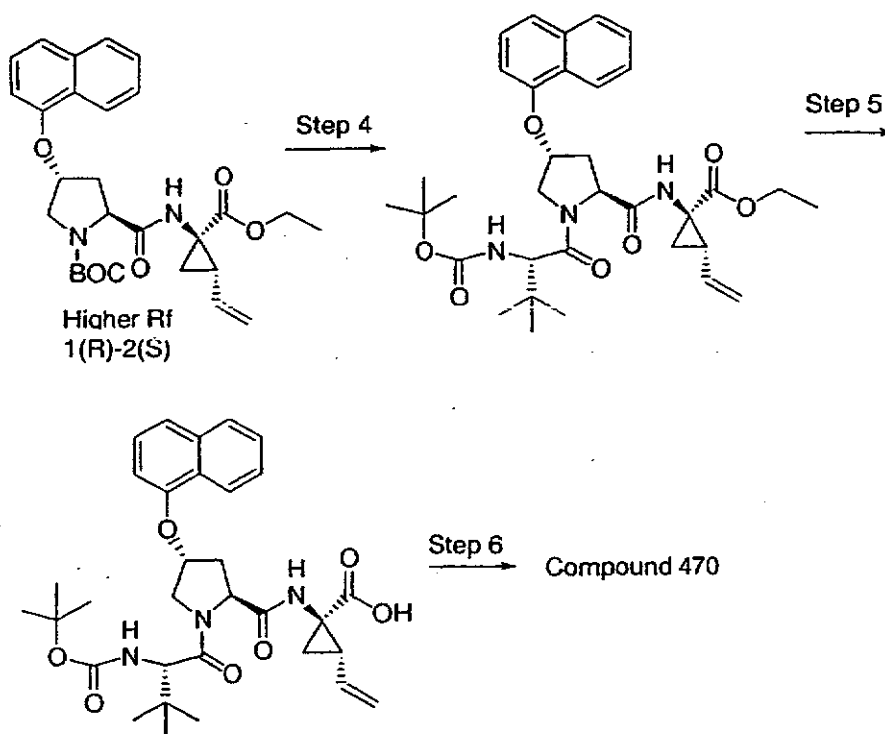
solution was stirred for 21 hours at room temperature and then diluted with 0.5N NaHCO₃. The basic solution was extracted with ethyl acetate and then the aqueous layer was acidified to pH 2 with the dropwise addition of conc. HCl. This acidified layer was then extracted again with ethyl acetate. This second ethyl acetate layer was
5 dried with magnesium sulfate, filtered and then concentrated in vacuo to yield Boc-(4R)-naphthal-1-oxo)-Pro-OH as pale-pink crystals (147mgs, 100%).
¹H NMR (CDCl₃, 500MHz) δ 1.47 and 1.48 (2s, 9H, rotamers), 2.40 and 2.52 (2m, 1H), 2.68 and 2.78 (2m, 1H), 3.78-4.07 (m, 2H), 4.57 and 4.69 (2t, 1H, J=7.6 and 8.0 Hz, rotamers), 5.12 (bd, 1H), 6.77 (dd, 1H, J=7.6, 21.2 Hz), 7.37 (m, 1H), 7.46 (m,
10 3H), 7.81 (t, 1H, J=5.8 Hz), 8.19 (m, 1H);
LC-MS A (retention time: 1.79 ; MS m/z 358 (M+H)⁺

Step 3:

To a solution of Boc-((4R)-naphthal-1-oxo)-Pro-OH (147mgs, 0.41 mmole)
15 and racemic (1R/2S)/(1S/2R)-1-amino-2-vinylcyclopropane carboxylic acid ethyl ester hydrochloride salt (79mgs, 0.41 mmole) in 2.8mL methylene chloride was added DIPEA (250 μ L, 1.44 mmole) and TBTU (158mgs, 0.49 mmole). The resulting solution was stirred under nitrogen for 20 hours and then diluted with 40mL methylene chloride. The organic layer was washed with water, 1N NaHCO₃, 1N
20 HCl, water and brine. The solution was then dried with sodium sulfate and concentrated in vacuo. Purification by preparative TLC yielded two separate diastereomers, higher R_f diastereomer A (P2[Boc(4R)-(naphthal-1-oxo)proline]-P1(1R, 2S Vinyl Acca)-OEt, 78 mgs, 38%) and lower R_f diastereomer B (P2[Boc(4R)-(naphthal-1-oxo)proline]-P1(1S, 2R Vinyl Acca)-OEt, 91mgs, 45%) as
25 off white solids:
Diastereomer A: P2[Boc(4R)-(naphthal-1-oxo)proline]-P1(1R, 2S Vinyl Acca)-OEt:
¹H NMR (CDCl₃, 500MHz) δ 1.24 (t, 3H), 1.43 (s, 9H), 1.52 (m, 1H), 1.84 (m, 1H), 2.02 (m, 1H), 2.14 (m, 1H), 2.81 (m, 1H), 3.88 (m, 2H), 4.11 (q, 1H, J=7.15), 4.19 (m, 1H), 4.54 (m, 1H), 5.15 (m, 1H), 5.31 (dd, 1H, J=17, 0.8 Hz), 5.77 (m, 1H), 6.83
30 (m, 1H), 7.36 (t, 1H, J=7.8 Hz), 7.46 (m, 3H), 7.78 (d, 1H, J=7.6 Hz), 8.14 (d, 1H, J=8.15Hz);
LC-MS B (retention time: 1.85 ; MS m/z 495 (M+H)⁺

Diastereomer B, Example 10B: P2[Boc(4R)-(naphthal-1-oxo)proline]-P1(1S, 2R Vinyl Acca)-OEt: ^1H NMR ($\text{d}_1\text{-CHCl}_3$, 500MHz) δ 1.24 (t, 3H), 1.42 (s, 9H), 1.85 (m, 1H), 2.15 (q, 1H, $J=8.9\text{Hz}$), 2.40 (m, 1H), 2.78 (m, 1H), 3.78 (m, 1H), 4.12 (m, 2H), 4.52 (m, 1H), 5.15 (m, 1H), 5.31 (m, 1H), 5.79 (m, 1H), 6.80 (m, 1H), 7.35 (t, 1H, $J=7.6\text{ Hz}$), 7.46 (m, 3H), 7.78 (d, 1H; $J=7.6\text{ Hz}$), 8.14 (d, 1H, $J=8.10\text{ Hz}$).
 LC-MS B (retention time: 1.85 ; MS m/z 495 ($\text{M}+\text{H}$)⁺)

Scheme 2



Step 4:

To P2[Boc(4R)-(naphthal-1-oxo)proline]-P1(1R, 2S Vinyl Acca)-OEt (A, higher Rf) (78mg, 0.16 mmol) was added 4N HCl in dioxane (2.0mL) and the solution was allowed to stir for 30 minutes. Concentration in vacuo yielded the HCl salt of P2[(4R)-(naphthal-4-oxo) proline]-P1(1R,2S Vinyl Acca)-OEt as a yellow oil which was taken on to the next step directly without further purification.

To a solution of BOC L-tBuGly (73mgs, 0.32 mmole) and the HCl salt of P2[(4R)-(naphthal-4-oxo) proline]-P1(1R,2S Vinyl Acca)-OEt (0.16 mmole) in 11mL acetonitrile was added DIPEA (140 μL , 0.79 mmole) and HATU (132mgs, 0.35 mmole). The resulting solution was stirred under nitrogen for 17 hours and then diluted with 100mL ethyl acetate. The organic layer was washed with water, 1N

NaHCO₃, 1N HCl, water and brine. The solution was then dried with sodium sulfate and concentrated in vacuo to yield the title compound as a pale-yellow oily film (92mgs, 96%).

¹H NMR (CDCl₃, 500MHz) δ 1.06 (s, 9H), 1.22 (t, 3H, J=7.1), 1.38 (s, 9H), 1.41 (m, 1H), 1.82 (m, 1H), 2.13 (m, 1H), 2.42 (m, 1H), 2.79 (m, 1H), 3.92-4.2 (m, 1H), 4.12 (q, 2H, J=6.6 Hz), 4.38 (bt, 1H), 5.12 (d, 1H, J=10.3 Hz) 5.2-5.39 (m, 3H), 5.75 (m, 1H), 6.82 (d, 1H, J=7.5 Hz), 7.34-7.46 (m, 4H), 7.59 (bs, 1H, NH), 7.76 (d, 1H, J=7.9 Hz), 8.13 (d, 1H, J=8.3 Hz);

LC-MS C (retention time: 2.82 ; MS m/z 608 (M+H)⁺

10

Step 5:

To a solution of product of Example 470, Step 4 (92mgs, 0.15mmole) in 750mL tetrahydrofuran and 250 mL water was added lithium hydroxide (4mgs). The resulting solution was stirred for 28.5 hours worked up as usual and then resubjected to the same conditions except adding twice as much lithium hydroxide (8mgs). After 24 hours the reaction was diluted with ethyl acetate and washed with water. The organic layer was dried with sodium sulfate and concentrated in vacuo. The resulting semisolid was purified by flash chromatography eluting with 3-1 hexanes-ethyl acetate to yield BocNH-P3(t-BuGly)-P2[(Boc (4R)-(naphthal-1-oxo) proline)]-PI(1R,2S Vinyl Acca)-OH as a clear semisolid (30mgs, 34%).

¹H NMR (d₄-MeOH, 500MHz) δ 1.04 (s, 9H), 1.24 (t, 1H, J=3.9 Hz), 1.32 (s, 9H), 1.66 (m, 1H), 2.07 (m, 1H), 2.40 (m, 1H), 2.71 (m, 1H), 4.04-4.07 (m, 1H), 4.28 (m, 1H), 4.42 (m, 1H), 4.55 (m, 1H), 5.02 (m, 1H), 5.18-5.29 (m, 2H), 5.90 (m, 1H), 6.54 (m, 1H), 6.92 (m, 1H), 4.26 (m, 4H), 7.77 (m, 1H), 8.15 (m, 1H);

LC-MS C (retention time: 2.65 ; MS m/z 580 (M+H)⁺

25

Step 6:

To a solution of BocNH-P3(t-BuGly)-P2[(Boc (4R)-(naphthal-1-oxo) proline)]-PI(1R,2S Vinyl Acca)-OH (Example 470, Step 5) (65mgs, 0.11 mmole) in 3.7 mL tetrahydrofuran was added 1,1'-carbonyl diimidazole (22mgs, 0.135 mmole). The resulting mixture was refluxed for 30 minutes and then cooled to room temperature. At this point, methanesulfonamide (27mgs, 0.28 mmole) and DBU

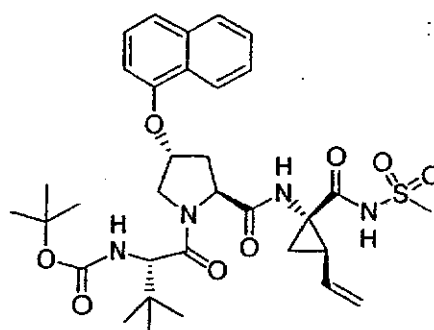
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(34 μ L, 0.224 mmole) were added. The reaction was stirred for 2 days and then more DBU (10 μ L) and methanesulfonamide (9 mgs) were added. After 24 hours, the reaction was diluted with 50 mL ethyl acetate and washed with 50 mL 0.25N HCl and 50 mL brine. The solution was dried with sodium sulfate and concentrated in vacuo. The crude material was purified by preparative TLC (3-2 ethyl acetate-hexanes) to give Compound 470 (21mgs, 28%) as a white filmy solid.

¹H NMR (d₄-MeOH, 500MHz) δ 1.04 (s, 9H), 1.36 (s, 9H), 1.88 (t, 1H), 2.18 (m, 1H), 2.31 (m, 1H), 2.63 (m, 1H), 3.11 (bs, 3H), 4.076 (m, 1H), 4.30 (bd, 1H), 4.41 (bd, 1H), 4.52 (apparent t, 1H), 5.07 (m, 1H), 5.24-5.30 (m, 2H), 5.80 (m, 1H), 6.92 (d, 1H, J=7.45 Hz), 7.35-7.46 (m, 4H), 7.76 (d, 1H, J=8.1 Hz), 8.13 (d, 1H, J=8.3 Hz);

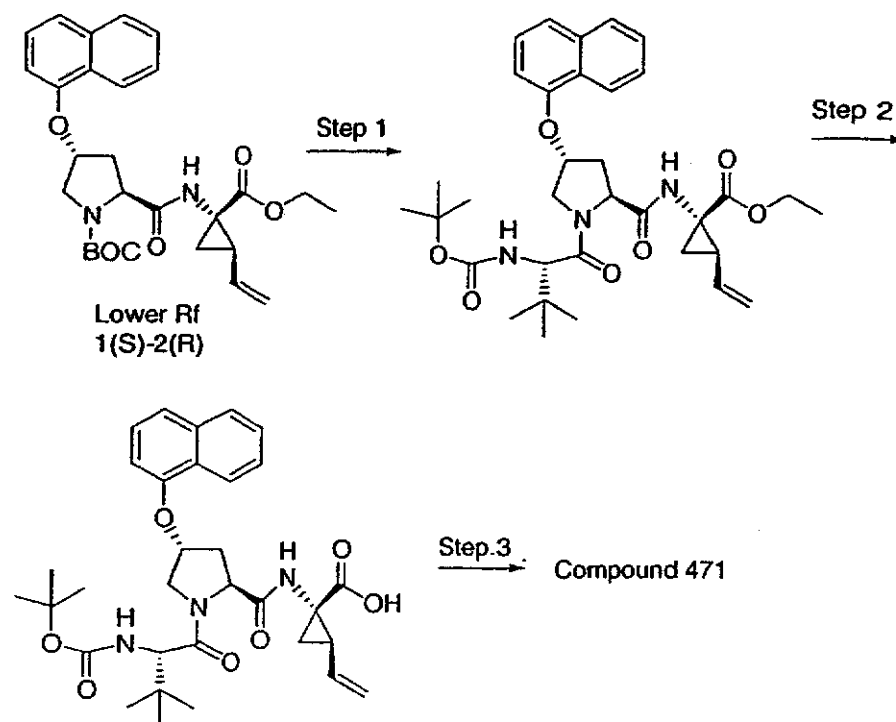
LC-MS C (retention time: 2.57 ; MS m/z 657 (M+H)⁺

Example 471: Preparation of Compound 471.



Compound 471

Scheme 2



Step 1:

To P2[Boc(4R)-(naphthal-1-oxo)proline]-P1(1S, 2R Vinyl Acca)-OEt (Example 470, Step 3, lower Rf) (91mgs, 0.18 mmole) was added 4N HCl in dioxane (2.0mL) and the solution was allowed to stir for 30 minutes. Concentration in vacuo yielded the HCl salt of P2[(4R)-(naphthal-1-oxo) proline]-P1(1S,2R Vinyl Acca)-OEt as a yellow oil which was taken on to the next step directly without further purification.

To a solution of N-Boc-L-tert-leucine-OH or BOC L-tBuGly (85mgs, 0.37 mmole) and the HCl salt of P2[(4R)-(naphthal-1-oxo) proline]-P1(1S,2R Vinyl Acca)-OEt (product obtained from reaction mentioned above) (0.18 mmole) in 13 mL acetonitrile was added DIPEA (160 μ L, 0.92 mmole) and HATU (154mgs, 0.41 mmole). The resulting solution was stirred under nitrogen for 17 hours and then diluted with 100 mL ethyl acetate. The organic layer was washed with water, 1N NaHCO₃, 1N HCl, water and brine. The solution was then dried with sodium sulfate and concentrated in vacuo to yield the title compound as a clear film (53mgs, 47%).

¹H NMR (d₁-CHCl₃, 500MHz) δ 1.02 (s, 9H), 1.22 (t, 3H, J=7.0 Hz), 1.39 (s, 9H), 1.47 (m, 1H), 1.88 (dd, 1H, J=8.0, 5.5 Hz), 2.07 (m, 1H), 2.42 (m, 1H), 2.80 (dt,

J=13.8, 6.0 Hz, 1H), 3.96 (m, 1H), 4.14 (m, 2H), 4.34 (m, 2H), 4.77 (t, 1H, J= 7.2 Hz), 5.09-5.33 (m, 3H), 5.72 (m, 1H), 6.82 (d, 1H, J= 7.6 Hz), 7.34-7.50 (m, 4H), 7.77 (d, 1H, J= 8.0 Hz), 8.15 (d, 1H, J= 8.25 Hz);

LC-MS C (retention time: 2.81; MS m/z 608 (M+H)⁺)

5

Step 2:

This product was prepared according procedure described in Example 470, Step 5 (5mg, 10%), except using the product of Example 471, Step 1 instead.

¹H NMR (d₄-MeOH, 500MHz) δ 0.99 (s, 9H), 1.28 (m, 1H), 1.37 (s, 9H), 1.60 (m, 1H), 2.06 (m, 1H), 2.28 (m, 1H), 2.66 (m, 1H), 3.91 (m, 1H), 4.33 (m, 2H), 4.61 (bt, 1H), 4.97 (d, 1H, J=11.0 Hz), 5.19 (m 2H), 6.09 (m, 1H), 6.88 (d, 1H, J= 7.1 Hz), 7.35-7.46 (m, 4H), 7.78 (d, 1H, J= 8.2 Hz), 8.12 (d, 1H, J=8.3 Hz);

LC-MS C (retention time: 2.60 ; MS m/z 580 (M+H)⁺)

15

Step 3:

To a solution of BocN-P3(L-tBuGly)- P2[(Boc (4R)-(naphthal-1-oxo proline)]-P1(1S,2R Vinyl Acca)-COOH (38mgs, 0.066 mmole) (Example 471, Step 2) in 2.2 mL tetrahydrofuran was added 1,1'-carbonyl diimidazole (13mgs, 0.079 mmole). The resulting mixture was refluxed for 30 minutes and then cooled to room temperature. At this point, methanesulfonamide (16mgs, 0.16 mmole) and DBU (20μL, 0.13 mmole) were added. The reaction was stirred for 2 days and then more DBU (10μL) and methanesulfonamide (9 mgs) were added. After 24 hours, the reaction was diluted with 50 mL ethyl acetate and washed with 50 mL 0.25N HCl and 50 mL brine. The solution was dried with sodium sulfate and concentrated in vacuo. The crude product was purified using one 20X40cm preparative TLC plate from Analtech (eluent 3-2 ethyl acetate-hexanes) to give Compound 471 (25mgs, 58%) as a white filmy solid.

¹H NMR (d₄-MeOH, 500MHz) δ 1.03 (s, 9H), 1.34 (s, 9H), 1.80 (m, 1H), 2.18 (m, 1H), 2.31 (m, 1H), 2.68 (m, 1H), 3.09 (bs, 3H), 4.04 (m, 1H), 4.20-4.44 (m, 2H), 4.51 (apparent t, 1H), 5.08 (m, 1H), 5.25-5.31 (m, 2H), 5.77 (m, 1H), 6.93 (d, 1H, J=7.6 Hz), 7.36-7.45 (m, 4H), 7.77 (d, 1H, J=8.0 Hz), 8.15 m, 1H);

LC-MS C (retention time: 2.57 ; MS m/z 657 (M+H)⁺)

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Section L:**Example 472: Biological Studies**

5

Recombinant HCV NS3/4A protease complex FRET peptide assay

The purpose of this in vitro assay was to measure the inhibition of HCV NS3 protease complexes, derived from the BMS, H77C or J4L6S strains, as described
10 below, by compounds of the present invention. This assay provides an indication of how effective compounds of the present invention would be in inhibiting HCV proteolytic activity.

Serum from an HCV-infected patient was obtained from Dr. T. Wright, San
15 Francisco Hospital. An engineered full-length cDNA (complement deoxyribonucleic acid) template of the HCV genome (BMS strain) was constructed from DNA fragments obtained by reverse transcription-PCR (RT-PCR) of serum RNA (ribonucleic acid) and using primers selected on the basis of homology between other genotype 1a strains. From the determination of the entire genome sequence, a
20 genotype 1a was assigned to the HCV isolate according to the classification of Simmonds et al. (See P Simmonds, KA Rose, S Graham, SW Chan, F McOmish, BC Dow, EA Follett, PL Yap and H Marsden, J. Clin. Microbiol., 31(6), 1493-1503 (1993)). The amino acid sequence of the nonstructural region, NS2-5B, was shown to be >97% identical to HCV genotype 1a (H77C) and 87% identical to genotype 1b
25 (J4L6S). The infectious clones, H77C (1a genotype) and J4L6S (1b genotype) were obtained from R. Purcell (NIH) and the sequences are published in Genbank (AAB67036, see Yanagi,M., Purcell,R.H., Emerson,S.U. and Bukh,J. Proc. Natl. Acad. Sci. U.S.A. 94(16),8738-8743 (1997); AF054247, see Yanagi,M., St Claire,M., Shapiro,M., Emerson,S.U., Purcell,R.H. and Bukh,J, Virology 244 (1), 161-172.
30 (1998)).

The BMS, H77C and J4L6S strains were used for production of recombinant NS3/4A protease complexes. DNA encoding the recombinant HCV NS3/4A protease complex (amino acids 1027 to 1711) for these strains were manipulated as described by P. Gallinari et al. (see Gallinari P, Paolini C, Brennan D, Nardi C, Steinkuhler C, De Francesco R. *Biochemistry*. 38(17):5620-32, (1999)). Briefly, a three-lysine solubilizing tail was added at the 3'-end of the NS4A coding region. The cysteine in the P1 position of the NS4A-NS4B cleavage site (amino acid 1711) was changed to a glycine to avoid the proteolytic cleavage of the lysine tag. Furthermore, a cysteine to serine mutation was introduced by PCR at amino acid position 1454 to prevent the autolytic cleavage in the NS3 helicase domain. The variant DNA fragment was cloned in the pET21b bacterial expression vector (Novagen) and the NS3/4A complex was expressed in *Escherichia coli* strain BL21 (DE3) (Invitrogen) following the protocol described by P. Gallinari et al. (see Gallinari P, Brennan D, Nardi C, Brunetti M, Tomei L, Steinkuhler C, De Francesco R, *J Virol*. 72(9):6758-69 (1998)) with modifications. Briefly, NS3/4A expression was induced with 0.5mM Isopropyl β -D-1-thiogalactopyranoside (IPTG) for 22hr at 20°C. A typical fermentation (10L) yielded approximately 80g of wet cell paste. The cells were resuspended in lysis buffer (10mL/g) consisting of 25mM N-(2-Hydroxyethyl)Piperazine-N'-(2-Ethane Sulfonic acid) (HEPES), pH7.5, 20% glycerol, 500mM Sodium Chloride (NaCl), 0.5% Triton-X100, 1ug/ml lysozyme, 5mM Magnesium Chloride (MgCl₂), 1ug/ml DnaseI, 5mM β -Mercaptoethanol (β ME), Protease inhibitor - Ethylenediamine Tetraacetic acid (EDTA) free (Roche), homogenized and incubated for 20 mins at 4°C. The homogenate was sonicated and clarified by ultra-centrifugation at 235000g for 1hr at 4°C. Imidazole was added to the supernatant to a final concentration of 15mM and the pH adjusted to 8.0. The crude protein extract was loaded on a Nickel - Nitrilotriacetic acid (Ni-NTA) column pre-equilibrated with buffer B (25mM HEPES, pH8.0, 20% glycerol, 500mM NaCl, 0.5% Triton-X100, 15mM imidazole, 5mM β ME). The sample was loaded at a flow rate of 1mL/min. The column was washed with 15 column volumes of buffer C (same as buffer B except with 0.2% Triton-X100). The protein was eluted with 5 column volumes of buffer D (same as buffer C except with 200mM Imidazole).

NS3/4A protease complex-containing fractions were pooled and loaded on a desalting column Superdex-S200 pre-equilibrated with buffer D (25mM HEPES, pH7.5, 20% glycerol, 300mM NaCl, 0.2% Triton-X100, 10mM β ME). Sample was loaded at a flow rate of 1mL/min. NS3/4A protease complex-containing fractions
5 were pooled and concentrated to approximately 0.5mg/ml. The purity of the NS3/4A protease complexes, derived from the BMS, H77C and J4L6S strains, were judged to be greater than 90% by SDS-PAGE and mass spectrometry analyses.

The enzyme was stored at -80°C, thawed on ice and diluted prior to use in
10 assay buffer. The substrate used for the NS3/4A protease assay was RET S1 (Resonance Energy Transfer Depsipeptide Substrate; AnaSpec, Inc. cat # 22991)(FRET peptide), described by Taliani et al. in Anal. Biochem. 240(2):60-67 (1996). The sequence of this peptide is loosely based on the NS4A/NS4B natural cleavage site except there is an ester linkage rather than an amide bond at the
15 cleavage site. The peptide substrate was incubated with one of the three recombinant NS3/4A complexes, in the absence or presence of a compound of the present invention, and the formation of fluorescent reaction product was followed in real time using a Cytofluor Series 4000.

20 The reagents were as follow: HEPES and Glycerol (Ultrapure) were obtained from GIBCO-BRL. Dimethyl Sulfoxide (DMSO) was obtained from Sigma. β -Mercaptoethanol was obtained from Bio Rad.
Assay buffer: 50mM HEPES, pH7.5; 0.15M NaCl; 0.1% Triton; 15% Glycerol; 10mM β ME. Substrate: 2 μ M final concentration (from a 2mM stock
25 solution in DMSO stored at -20°C). HCV NS3/4A type 1a (1b), 2-3 nM final concentration (from a 5 μ M stock solution in 25mM HEPES, pH7.5, 20% glycerol, 300mM NaCl, 0.2% Triton-X100, 10mM β ME).

The assay was performed in a 96-well polystyrene black plate from Falcon. Each well
30 contained 25 μ l NS3/4A protease complex in assay buffer, 50 μ l of a compound of the present invention in 10% DMSO/assay buffer and 25 μ l substrate in assay buffer. A

control (no compound) was also prepared on the same assay plate. The enzyme complex was mixed with compound or control solution for 1 min before initiating the enzymatic reaction by the addition of substrate. The assay plate was read immediately using the Cytofluor Series 4000 (Perspective Biosystems). The
5 instrument was set to read an emission of 340nm and excitation of 490nm at 25°C. Reactions were generally followed for approximately 15 minutes.

The percent inhibition was calculated with the following equation:

10
$$100 - [(\delta F_{inh}/\delta F_{con}) \times 100]$$

where δF is the change in fluorescence over the linear range of the curve. A non-linear curve fit was applied to the inhibition-concentration data, and the 50% effective concentration (IC_{50}) was calculated by the use of Excel XI-fit software using
15 the equation, $y=A+((B-A)/(1+((C/x)^D)))$.

All of the compounds tested were found to have IC_{50} s of 10 μM or less. Further, compounds of the present invention, which were tested against more than one type of NS3/4A complex, were found to have similar inhibitory properties though the
20 compounds uniformly demonstrated greater potency against the 1b strains as compared to the 1a strains.

Specificity Assays

25 The specificity assays were performed to demonstrate the selectivity of the compounds of the present invention in inhibiting HCV NS3/4A protease as compared to other serine or cysteine proteases.

The specificities of compounds of the present invention were determined against a
30 variety of serine proteases: human leukocyte elastase (HLE), porcine pancreatic elastase (PPE) and human pancreatic chymotrypsin and one cysteine protease: human liver cathepsin B. In all cases a 96-well plate format protocol using colorimetric p-

nitroaniline (pNA) substrate specific for each enzyme was used as described previously (Patent WO 00/09543) with some modifications to the serine protease assays. All enzymes were purchased from Sigma while the substrates were from Bachem.

5

Each assay included a 2hr enzyme-inhibitor pre-incubation at RT followed by addition of substrate and hydrolysis to ~30% conversion as measured on a Spectramax Pro microplate reader. Compound concentrations varied from 100 to 0.4 μ M depending on their potency.

10

The final conditions for each assay were as follows:

50mM Tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) pH8, 0.5M Sodium Sulfate (Na_2SO_4), 50mM NaCl, 0.1mM EDTA, 3% DMSO, 0.01% Tween-
15 20 with:

133 μ M succ-AAA-pNA and 20nM HNE or 8nM PPE; 133 μ M succ-AAV-pNA and 15nM HLE; 100 μ M succ-AAPF-pNA and 250pM Chymotrypsin.

100mM NaHPO_4 (Sodium Hydrogen Phosphate) pH 6, 0.1mM EDTA, 3% DMSO,
20 1mM TCEP (Tris(2-carboxyethyl)phosphine hydrochloride), 0.01% Tween-20,
30 μ M Z-FR-pNA and 5nM Cathepsin B (enzyme stock activated in buffer containing 20mM TCEP before use).

The percentage of inhibition was calculated using the formula:

25

$$[1 - ((\text{UV}_{\text{inh}} - \text{UV}_{\text{blank}}) / (\text{UV}_{\text{ctl}} - \text{UV}_{\text{blank}}))] \times 100$$

A non-linear curve fit was applied to the inhibition-concentration data, and the 50% effective concentration (IC_{50}) was calculated by the use of Excel XI-fit software.

30

HCV Replicon Cell-based Assay

- An HCV replicon whole cell system was established as described by Lohmann V, Korner F, Koch J, Herian U, Theilmann L, Bartenschlager R., Science 285(5424):110-3 (1999). This system enabled us to evaluate the effects of our HCV Protease compounds on HCV RNA replication. Briefly, using the HCV strain 1B sequence described in the Lohmann paper (Assession number:AJ238799), an HCV cDNA was generated encoding the 5' internal ribosome entry site (IRES), the neomycin resistance gene, the EMCV (encephalomyocarditis viurs)-IRES and the HCV nonstructural proteins, NS3-NS5B, and 3' non-translated region (NTR). In vitro transcripts of the cDNA were transfected into the human hepatoma cell line, Huh7. Selection for cells constitutively expressing the HCV replicon was achieved in the presence of the selectable marker, neomycin (G418). Resulting cell lines were characterized for positive and negative strand RNA production and protein production over time.
- Huh7 cells, constitutively expressing the HCV replicon, were grown in Dulbecco's Modified Eagle Media (DMEM) containing 10% Fetal calf serum (FCS) and 1mg/ml G418 (Gibco-BRL). Cells were seeded the night before (1.5×10^4 cells/well) in 96-well tissue-culture sterile plates. Compound and no compound controls were prepared in DMEM containing 4% FCS, 1:100 Penicillin / Streptomycin, 1:100 L-glutamine and 5% DMSO in the dilution plate (0.5% DMSO final concentration in the assay). Compound / DMSO mixes were added to the cells and incubated for 4 days at 37°C. After 4 days, plates were rinsed thoroughly with Phosphate-Buffered Saline (PBS) (3 times 150µl). The cells were lysed with 25µl of a lysis assay reagent containing the FRET peptide (RET S1, as described for the in vitro enzyme assay). The lysis assay reagent was made from 5X cell Luciferase cell culture lysis reagent(Promega #E153A) diluted to 1X with distilled water, NaCl added to 150 mM final, the FRET peptide diluted to 10 µM final from a 2 mM stock in 100% DMSO. The plate was then placed into the Cytofluor 4000 instrument which had been set to 340nm excitation / 490 emission, automatic mode for 21 cycles and the plate read in a kinetic mode. EC₅₀ determinations were carried out as described for the IC₅₀ determinations.

As a secondary assay, EC₅₀ determinations from the replicon FRET assay were confirmed in a quantitative RNA assay. Cells were lysed using the Rneasy kit (Qiagen). Purified total RNA was normalized using RiboGreen (Jones LJ, Yue ST, Cheung CY, Singer VL, Anal. Chem., 265(2):368-74 (1998)) and relative

5 quantitation of HCV RNA expression assessed using the Taqman procedure (Kolykhalov AA, Mihalik K, Feinstone SM, Rice CM, Journal of Virology 74, 2046-2051 (2000)) and the Platinum Quantitative RT-PCR Thermoscript One-Step kit (Invitrogen cat # 11731-015). Briefly, RNA made to a volume of 5µl (≤ 1ng) was added to a 20µl Ready-Mix containing the following: 1.25X Thermoscript reaction

10 mix (containing Magnesium Sulfate and 2-deoxynucleoside 5'-triphosphates (dNTPs)), 3mM dNTPs, 200nM forward primer (sequence: 5'-gggagagccatagtggctctgc-3'), 600nM reverse primer (5'-cccaaattctccaggcattga-3'), 100nM probe (5'-6-FAM-cggaattgccaggacgaccgg-BHQ-1-3')(FAM: Fluorescein-aminohexyl amidite; BHQ: Black Hole Quencher), 1µM Rox reference dye

15 (Invitrogen cat # 12223-012) and Thermoscript Plus Platinum Taq polymerase mixture. All primers were designed with ABI Prism 7700 software and obtained from Biosearch Technologies, Novato, CA. Samples containing known concentrations of HCV RNA transcript were run as standards. Using the following cycling protocol (50°C, 30 min; 95°C, 5 min; 40 cycles of 95°C, 15 sec, 60°C, 1

20 min), HCV RNA expression was quantitated as described in the Perkin Elmer manual using the ABI Prism 7700 Sequence Detector.

Biological Examples

25 Representative compounds of the invention were assessed in the HCV replicon cell assay and/or in several of the outlined specificity assays. For example, Compound 34 was found to have an IC₅₀ of 23 nanomolar (nM) against the NS3/4A BMS strain in the enzyme assay. Similar potency values were obtained with the published H77C (IC₅₀ of 3 nM) and J4L6S (IC₅₀ of 2.9 nM) strains. The EC₅₀ value

30 in the replicon assay was 166 nM.

In the specificity assays, the same compound was found to have the following activity: HLE > 100 μM ; PPE > 200 μM ; Chymotrypsin > 200 μM ; Cathepsin B > 200 μM . These results indicate this family of compounds are highly specific for the NS3 protease and many of these members inhibit HCV replicon replication.

5

The compounds of the current invention were tested and found to have activities in the ranges as follow:

IC50 Activity Ranges (NS3/4A BMS Strain): A is 10 – 100 micromolar (μM); B is 1 – 10 μM ; C is 0.1 – 1 μM ; D is <0.1 μM

10 EC50 Activity Range (for compounds tested): A is 10 - 100 μM ; B is 1 - 10 μM ; C is 0.1 - 1 μM ; D is <0.1 μM

Note that by using the Patent example number and the Patent compound number shown in the table the structures of compounds can be found herein.

15 In accordance with the present invention, preferably the compounds have a biological activity (EC_{50}) of 10 μM or less, more preferably 1 μM or less and most preferably 100 nM or less.

20

Table 1

Biological Activity

IC50 range	EC50 range	Patent Example Number	Patent Cmpd Number
D	D	1	1

D	C	2	2
D	D	3	3
D	D	4	4
D	D	5	5
C	C	6	6
D	C	7	7
C	B	8	8
D	C	9	9
D	D	10	10
D	D	11	11

D	D	12	12
D	D	13	13
D	D	14	14
C	B	15	15
D	C	16	16
D	D	17	17
D	D	18	18
D	D	19	19
D	C	20	20
D	D	21	21

D	C	22	22
D	D	23	23
D	D	24	24
D	D	25	25
D	D	26	26
D	D	27	27
D	D	28	28
D	D	29	29
D	D	30	30
D	D	31	31

D	D	32	32
D	D	33	33
D	C	34	34
D	D	35	35
D	D	36	36
D	D	37	37
D	D	38	38
D	D	39	39
D	C	40	40
D	D	41	41

D	D	42	42
C	B	45	45
C	B	46	46
D	C	47	47
B		48	48
B		49	49
C	B	50	50
B		52	52
C	B	53	53

D	C	55	55
D	D	56	56
D	C	57	57
D	D	58	58
D	C	59	59
D	B	60	60
C	B	61	61
D	D	62	62
D	B	63	63
D	D	64	64

D	C	65	65
D	C	66	66
D	D	67	67
D	D	68	68
D	D	69	69
D	D	70	70
D	D	71	71
D	C	72	72
D	C	73	73
D	D	74	74

D	D	75	75
D	D	76	76
D	C	77	77
D	B	78	78
D	C	79	79
D	B	80	80
D	C	81	81
D	C	82	82
D	C	83	83
D	C	84	84

C	B	85	85
B	A	86	86
B	A	87	87
B	A	88	88
D	D	89	89
D	C	91	91
D	D	92	92
C	C	93	93
D	D	94	94
D	C	95	95

D	D	96	96
D	D	97	97
B		99	99
C	B	100	100
D	D	101	101
D	D	102	102
D	D	103	103
D	D	104	104
D	D	105	105
D	D	106	106

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D	C	107	107
D	D	108	108
D	D	109	109
D	C	110	110
D	C	120	120
D	C	121	121
D	C	122	122
D	C	123	123
C	B	124	124
D	D	125	125

D	C	126	126
D	C	127	127
D	C	128	128
C	C	129	129
D	C	130	130
C	B	131	131
D	B	132	132
C	B	133	133
D	C	134	134
D	C	135	135

D	C	136	136
D	D	137	137
D	D	138	138
D	C	139	139
D	B	140	140
D	D	141	141
D	D	142	142
D	D	143	143
D	C	144	144
D	C	145	145

D	D	146	146
D	B	147	147
D	D	148	148
D	D	149	149
D	D	150	150
D	D	151	151
D	D	152	152
D	D	153	153
D	C	154	154
D	D	155	155

D	C	180	180
D	C	181	181
D	C	182	182
D	D	183	183
D	D	185	185
D	D	186	186
D	C	187	187
D	C	188	188
D	C	189	189

D	C	190	190
D	C	191	191
D	D	192	192
D	C	193	193
D	C	194	194
D	D	195	195
D	B	196	196
C	A	197	197
D	C	198	198
D	C	199	199

D	D	200	200
D	D	201	201
D	B	202	202
D	C	204	204
D	D	206	206
D	D	207	207
D	C	209	209
D	D	210	210
D	D	211	211
D	D	212	212

D	D	213	213
D	D	215	215
D	D	219	219
D	D	220	220
D	D	223	223
D	C	224	224
D	C	225	225
D	D	227	227
D	D	229	229
C	C	230	230

B		231	231
D	D	232	232
C	C	233	233
D	C	235	235
D	C	237	237
D	C	238	238
D	D	239	239
D	D	240	240
D	D	241	241
D	B	242	242

C	A	243	243
D	A	244	244
C		245	245
D	D	250	250
D	D	251	251
D	D	252	252
D	D	253	253
D	D	254	254
D	C	255	255

A		256	256
D	D	257	257
D	D	258	258
D	D	259	259
D	C	260	260
D	D	261	261
D	D	262	262
C	C	263	263
C	B	264	264
D	D	265	265

D	D	266	266
D	D	267	267
D	D	268	268
D	C	269	269
D	C	270	270
D	C	271	271
D	D	272	272
D	D	273	273
D	C	274	274
D	D	275	275

D	D	276	276
D	D	277	277
D	D	278	278
D	D	279	279
D	D	280	280
D	D	281	281
D	C	282	282
D	C	283	283
C	A	284	284
D	D	285	285

D	D	286	286
D	C	287	287
D	B	288	288
D	C	289	289
D	D	290	290
D	D	291	291
D	C	292	292
D	C	293	293
D	C	294	294
C	B	295	295

D	C	296	296
C	B	297	297
D	D	298	298
D	C	299	299
D	C	300	300
D	D	320	320
D	C	321	321
D	D	322	322
D	C	323	323

D	C	324	324
D	D	325	325
D	D	326	326
D	D	327	327
D	D	328	328
D	D	329	329
D	D	330	330
D		331	331
C	C	334	334
D	B	335	335

C	B	336	336
C	B	337	337
D	B	338	338
D	B	339	339
D	A	340	340
B		341	341
B		342	342
B		343	343
C		344	344
B	A	345	345

B		346	346
C	A	347	347
A		348	348
B		349	349
C	A	350	350
B		351	351
A		352	352
A		353	353
B		354	354
C	A	355	355

C	B	356	356
D	C	357	357
C	B	370	370
D	D	371	371
D	D	372	372
D	D	373	373
D	C	374	374
D	D	375	375
D	D	376	376

D	C	377	377
D	D	378	378
D	D	379	379
D	C	380	380
D	C	381	381
D	C	382	382
D	D	383	383
D	D	384	384
D	D	385	385
C	B	386	386

D	D	410	410
D	C	411	411
D	D	412	412
D	D	413	413
D	D	414	414
D	D	415	415
D	C	420	420
D	C	421	421
D	B	422	422

C		423	423
D	C	424	424
C	B	425	425
D	C	426	426
D	C	427	427
D	C	428	428
D	D	429	429
D	C	430	430
D	C	431	431
D	C	432	432

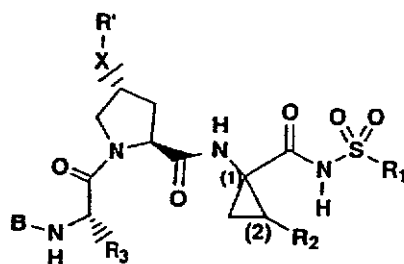
C	B	433	433
D	C	434	434
D	C	435	435
D	C	436	436
D	C	437	437
D	C	438	438
D	C	450	450
B		451	451
B		452	452

		453	453
C	B	454	454
C		470	470
B		471	471

Section M:

Table 2

- 5 The following compounds that can be made using the methods described herein and specifically in sections A through K of the exemplification section and more specifically in sections B, E, F and G. Moreover it should be made clear that each of the groups B, R₃, R₂ and R₁ shown below can be replaced by any of the groups exemplified in sections A through K and elsewhere herein or designated in
- 10 Formula I. For example the R₃ group in Table 2 is shown as a t-butyl group but one skilled in the art would recognize that for each of the entries cited below this group could be replaced with an isopropyl group or a C₁₋₆ alkyl substituted with an alkoxy. Or the B group shown below could be replaced with a tert-butyl urea moiety for each of the entries cited below.



Entry	B	R ₃	X	R'	R ₂	C(1,2)-Cycloprop- ane stereochem- istry	R ₁
A			O			(1R, 2S)	
B			O			(1R, 2S)	
C			O			(1R, 2S)	
D			O			(1R, 2S)	
E			O			(1R, 2S)	
F			O			(1R, 2S)	
G			O			(1R, 2S)	
H			O			(1R, 2S)	
I			O			(1R, 2S)	

Entry	B	R ₃	X	R'	R ₂	C(1,2)-Cyclopropane stereochemistry	R ₁
J			O			(1R, 2S)	
K			O			(1R, 2S)	
L			O			(1R, 2S)	
M			O			(1R, 2S)	
N			O			(1R, 2S)	
O			O			(1R, 2S)	
P			O			(1R, 2S)	
Q			O			(1R, 2S)	
R			O			(1R, 2S)	
S			O			(1R, 2S)	
T			O			(1R, 2S)	
U			O			(1R, 2S)	

Entry	B	R ₃	X	R'	R ₂	C(1,2)-Cyclopropane stereochemistry	R ₁
V			O			(1R, 2S)	
W			O			(1R, 2S)	
X			O			(1R, 2S)	
Y			O			(1R, 2S)	
Z			O			(1R, 2S)	
Aa			O			(1R, 2S)	
Bb			O			(1R, 2S)	
Cc			O			(1R, 2S)	
Dd			O			(1R, 2S)	
Ee			O			(1R, 2S)	
Ff			O			(1R, 2S)	
Gg			O			(1R, 2S)	
Hh			O			(1R, 2S)	

Entry	B	R ₃	X	R'	R ₂	C(1,2)-Cyclopropane stereochemistry	R ₁
Ii			O			(1R, 2S)	
Jj			O			(1R, 2S)	
Kk			O			(1R, 2S)	
Li			O			(1R, 2S)	
Mm			O			(1R, 2S)	

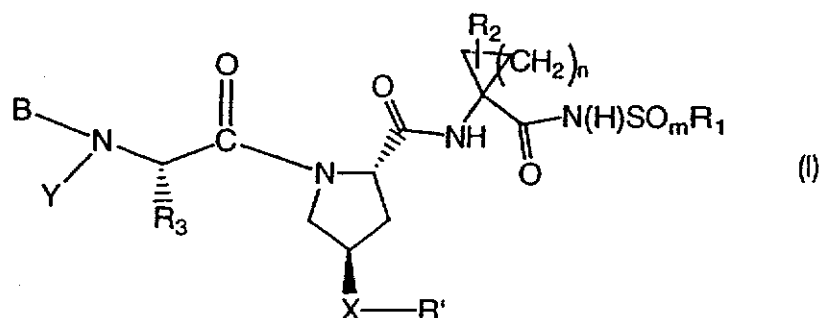
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CLAIMS

1. A compound having the formula



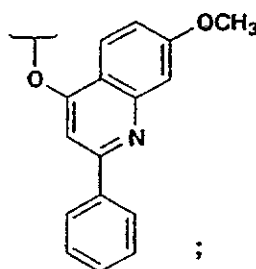
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wherein:

- (a) R_1 is C_{1-8} alkyl, C_{3-7} cycloalkyl, or C_{4-10} alkylcycloalkyl;
- (b) m is 1 or 2;
- (c) n is 1 or 2;
- (d) R_2 is H, C_{1-6} alkyl, C_{2-6} alkenyl or C_{3-7} cycloalkyl, each optionally substituted with halogen;
- (e) R_3 is C_{1-8} alkyl optionally substituted with halo, cyano, amino, C_{1-6} dialkylamino, C_{6-10} aryl, C_{7-14} alkylaryl, C_{1-6} alkoxy, carboxy, hydroxy, aryloxy, C_{7-14} alkylaryloxy, C_{2-6} alkylester, C_{8-15} alkylarylester; C_{3-12} alkenyl, C_{3-7} cycloalkyl, or C_{4-10} alkylcycloalkyl, wherein the cycloalkyl or alkylcycloalkyl are optionally substituted with hydroxy, C_{1-6} alkyl, C_{2-6} alkenyl or C_{1-6} alkoxy; or R_3 together with the carbon atom to which it is attached forms a C_{3-7} cycloalkyl group optionally substituted with C_{2-6} alkenyl;
- (f) Y is H, phenyl substituted with nitro, pyridyl substituted with nitro, or C_{1-6} alkyl optionally substituted with cyano, OH or C_{3-7} cycloalkyl; provided that if R_4 or R_5 is H then Y is H;
- (g) B is H, C_{1-6} alkyl, $R_4-(C=O)-$, $R_4O(C=O)-$, $R_4-N(R_5)-C(=O)-$, $R_4-N(R_5)-C(=S)-$, R_4SO_2- , or $R_4-N(R_5)-SO_2-$;
- (h) R_4 is (i) C_{1-10} alkyl optionally substituted with phenyl, carboxyl, C_{1-6} alkanoyl, 1-3 halogen, hydroxy, $-OC(O)C_{1-6}$ alkyl, C_{1-6} alkoxy, amino

25

- optionally substituted with C_{1-6} alkyl, amido, or (lower alkyl) amido;
(ii) C_{3-7} cycloalkyl, C_{3-7} cycloalkoxy, or C_{4-10} alkylcycloalkyl, each
optionally substituted with hydroxy, carboxyl, (C_{1-6} alkoxy)carbonyl,
amino optionally substituted with C_{1-6} alkyl, amido, or (lower alkyl)
5 amido; (iii) C_{6-10} aryl or C_{7-16} arylalkyl, each optionally substituted
with C_{1-6} alkyl, halogen, nitro, hydroxy, amido, (lower alkyl) amido,
or amino optionally substituted with C_{1-6} alkyl; (iv) Het; (v)
bicyclo(1.1.1)pentane; or (vi) $-C(O)OC_{1-6}$ alkyl, C_{2-6} alkenyl or C_{2-6}
alkynyl;
- 10 (i) R_5 is H; C_{1-6} alkyl optionally substituted with 1-3 halogens; or C_{1-6}
alkoxy provided R_4 is C_{1-10} alkyl;
(j) X is O, S, SO, SO_2 , OCH_2 , CH_2O or NH;
(k) R' is Het; or C_{6-10} aryl or C_{7-14} alkylaryl, optionally substituted with R^a ;
and
- 15 (l) R^a is C_{1-6} alkyl, C_{3-7} cycloalkyl, C_{1-6} alkoxy, C_{3-7} cycloalkoxy, halo- C_{1-6}
alkyl, CF_3 , mono- or di- halo- C_{1-6} alkoxy, cyano, halo, thioalkyl,
hydroxy, alkanoyl, NO_2 , SH, , amino, C_{1-6} alkylamino, di (C_{1-6})
alkylamino, di (C_{1-6}) alkylamide, carboxyl, (C_{1-6}) carboxyester, C_{1-6}
alkylsulfone, C_{1-6} alkylsulfonamide, di (C_{1-6}) alkyl(alkoxy)amine, C_{6-}
20 $_{10}$ aryl, C_{7-14} alkylaryl, or a 5-7 membered monocyclic heterocycle;
with the proviso that X- R' is not



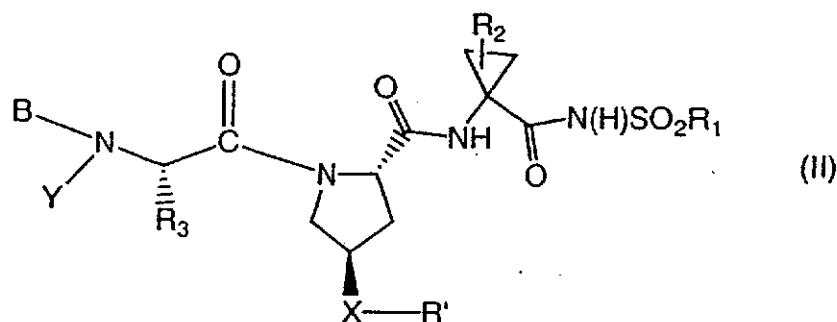
or a pharmaceutically acceptable salt, solvate or prodrug thereof.

25

2. The compound of Claim 1 wherein R_1 is C_{3-7} cycloalkyl.

3. The compound of Claim 1 wherein R_2 is C_{1-6} alkyl, C_{2-6} alkenyl or C_{3-7} cycloalkyl.
- 5 4. The compound of Claim 3 wherein R_2 is C_{2-6} alkenyl.
5. The compound of Claim 1 wherein R_3 is C_{1-8} alkyl optionally substituted with C_6 aryl, C_{1-6} alkoxy, carboxy, hydroxy, aryloxy, C_{7-14} alkylaryloxy, C_{2-6} alkylester, C_{8-15} alkylarylester; C_{3-12} alkenyl, C_{3-7} cycloalkyl, or C_{4-10} alkylcycloalkyl.
- 10 6. The compound of Claim 5 wherein R_3 is C_{1-8} alkyl optionally substituted with C_{1-6} alkoxy; or C_{3-7} cycloalkyl.
7. The compound of Claim 1 wherein Y is H.
- 15 8. The compound of Claim 1 wherein B is H, C_{1-6} alkyl, $R_4-(C=O)-$, $R_4O(C=O)-$, $R_4-N(R_5)-C(=O)-$, $R_4-N(R_5)-C(=S)-$, R_4SO_2- , or $R_4-N(R_5)-SO_2-$.
9. The compound of Claim 8 wherein B is $R_4-(C=O)-$, $R_4O(C=O)-$, or
- 20 $R_4-N(R_5)-C(=O)-$.
10. The compound of Claim 9 wherein B is $R_4O(C=O)-$ and R_4 is C_{1-6} alkyl.
11. The compound of Claim 1 wherein R_4 is (i) C_{1-10} alkyl optionally substituted
- 25 with phenyl, carboxyl, C_{1-6} alkanoyl, 1-3 halogen, hydroxy, C_{1-6} alkoxy; (ii) C_{3-7} cycloalkyl, C_{3-7} cycloalkoxy, or C_{4-10} alkylcycloalkyl; or (iii) C_{6-10} aryl or C_{7-16} arylalkyl, each optionally substituted with C_{1-6} alkyl or halogen.
12. The compound of Claim 11 wherein R_4 is (i) C_{1-10} alkyl optionally
- 30 substituted with 1-3 halogen or C_{1-6} alkoxy; or (ii) C_{3-7} cycloalkyl or C_{4-10} alkylcycloalkyl.

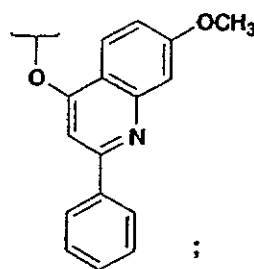
13. The compound of Claim 1 wherein R_5 is H or C_{1-6} alkyl optionally substituted with 1-3 halogens.
14. The compound of Claim 13 wherein R_5 is H.
- 5 15. The compound of Claim 1 wherein X is O or NH.
16. The compound of Claim 1 wherein R' is Het; or C_{6-10} aryl optionally substituted with R^a .
- 10 17. The compound of Claim 16 wherein R' is Het.
18. The compound of Claim 1 wherein the heterocycle contains 1 or 2 nitrogen atoms and optionally a sulfur atom or an oxygen atom in the ring.
- 15 19. The compound of Claim 18 wherein the heterocycle is substituted with at least one of C_{1-6} alkyl, C_{1-6} alkoxy, halo, C_{6-10} aryl, C_{7-14} alkylaryl, or a 5-7 membered monocyclic heterocycle.
- 20 20. The compound of Claim 1 wherein R^a is C_{1-6} alkyl, C_{3-7} cycloalkyl, C_{1-6} alkoxy, halo- C_{1-6} alkyl, halo, amino, C_6 aryl, or a 5-7 membered monocyclic heterocycle.
21. A compound having the formula
- 25



wherein:

- (a) R_1 is C_{3-7} cycloalkyl;
- (b) R_2 is C_{1-6} alkyl, C_{2-6} alkenyl or C_{3-7} cycloalkyl;
- (c) R_3 is C_{1-8} alkyl optionally substituted with C_6 aryl, C_{1-6} alkoxy,
 5 carboxy, hydroxy, aryloxy, C_{7-14} alkylaryloxy, C_{2-6} alkylester, C_{8-15}
 alkylarylester, C_{3-12} alkenyl, C_{3-7} cycloalkyl, or C_{4-10} alkylcycloalkyl;
- (d) Y is H;
- (e) B is H, C_{1-6} alkyl, $R_4-(C=O)-$, $R_4O(C=O)-$, $R_4-N(R_5)-C(=O)-$,
 $R_4-N(R_5)-C(=S)-$, R_4SO_2- , or $R_4-N(R_5)-SO_2-$;
- 10 (f) R_4 is (i) C_{1-10} alkyl optionally substituted with phenyl, carboxyl, C_{1-6}
 alkanoyl, 1-3 halogen, hydroxy, C_{1-6} alkoxy; (ii) C_{3-7} cycloalkyl, C_{3-7}
 cycloalkoxy, or C_{4-10} alkylcycloalkyl; or (iii) C_{6-10} aryl or C_{7-16}
 arylalkyl, each optionally substituted with C_{1-6} alkyl or halogen;
- (g) R_5 is H or C_{1-6} alkyl optionally substituted with 1-3 halogens;
- 15 (h) X is O or NH;
- (i) R' is Het; or C_{6-10} aryl optionally substituted with R^a ; and
- (j) R^a is C_{1-6} alkyl, C_{3-7} cycloalkyl, C_{1-6} alkoxy, halo- C_{1-6} alkyl, halo,
 amino, C_6 aryl, or a 5-7 membered monocyclic heterocycle;

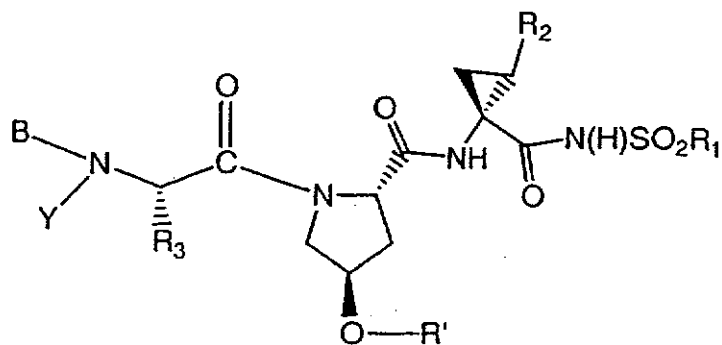
with the proviso that X_a-R' is not



or a pharmaceutically acceptable salt, solvate or prodrug thereof.

- 22. The compound of Claim 21 wherein R' is a bicyclic heterocycle.
- 25 23. The compound of Claim 22 wherein the heterocycle contains 1 or 2 nitrogen
 atoms and optionally a sulfur atom or an oxygen atom in the ring.

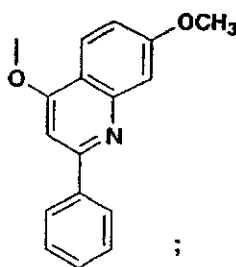
24. The compound of Claim 22 wherein the heterocycle is substituted with at least one of C_{1-6} alkyl, C_{1-6} alkoxy, halo, C_6 aryl, and a 5-7 membered monocyclic heterocycle.
- 5 25. The compound of Claim 21 wherein R' is a bicyclic heterocycle containing 1 nitrogen atom and substituted with methoxy and at least one of a C_6 aryl and a 5-7 membered monocyclic heterocycle.
26. The compound of Claim 21 wherein R' is a monocyclic heterocycle.
- 10 27. The compound of Claim 26 wherein the heterocycle contains 1 or 2 nitrogen atoms and optionally a sulfur atom or an oxygen atom in the ring.
28. The compound of Claim 26 wherein the heterocycle is substituted with at least one of C_{1-6} alkyl, C_{1-6} alkoxy, halo, C_{6-10} aryl, C_{7-14} alkylaryl, or a 5-7 membered monocyclic heterocycle.
- 15 29. The compound of Claim 21 wherein R' is a monocyclic heterocycle containing 1 or 2 nitrogen atoms and substituted with methoxy and at least one of a C_6 aryl and a 5-7 membered monocyclic heterocycle.
- 20 30. A compound having the formula



(III)

wherein:

- (a) R_1 is C_{3-7} cycloalkyl;
- (b) R_2 is C_{2-6} alkenyl;
- (c) R_3 is C_{1-8} alkyl;
- 5 (d) Y is H;
- (e) B is $R_4O(C=O)-$, or $R_4-N(R_5)-C(=O)-$;
- (f) R_4 is C_{1-10} alkyl;
- (g) R_5 is H;
- (h) R' is a bicyclic heterocycle optionally substituted with R^a ; and
- 10 (i) R^a is C_{1-6} alkyl, C_{1-6} alkoxy, halo, C_6 aryl, or a 5-7 membered monocyclic heterocycle; with the proviso that O- R' is not



or a pharmaceutically acceptable salt, solvate or prodrug thereof.

- 15 31. The compound of Claim 30 wherein R_1 is cyclopropyl or cyclobutyl.
- 32. The compound of Claim 30 wherein R_2 is vinyl.
- 33. The compound of Claim 30 wherein R_3 is t-butyl.
- 20 34. The compound of Claim 30 wherein R_4 is t-butyl.
- 35. The compound of Claim 30 wherein R' is quinoline or isoquinoline optionally substituted with R^a .
- 25 36. The compound of Claim 30 wherein R_1 is cyclopropyl, R_2 is vinyl, R_3 is t-butyl, R_4 is t-butyl, and R' is isoquinoline substituted with R^a .

37. The compound of Claim 36 wherein R^a is C_{1-6} alkoxy.
38. The compound of Claim 37 wherein R^a further includes at least one of C_6 aryl
5 or a 5-7 membered monocyclic heterocycle.
39. A method of treating an HCV infection in a patient, comprising administering
to the patient a therapeutically effective amount of the compound of claim 1 or a
pharmaceutically acceptable salt, solvate or prodrug thereof.
10
40. A method of inhibiting HCV NS3 protease comprising administering to a
patient in need thereof a therapeutically effective amount of the compound of claim 1
or a pharmaceutically acceptable salt, solvate or prodrug thereof.
- 15 41. A composition comprising the compound of claim 1 or a pharmaceutically
acceptable salt, solvate or prodrug thereof and a pharmaceutically acceptable carrier.
42. The composition of claim 41 further comprising an additional
immunomodulatory agent.
20
43. The composition of claim 42 wherein the additional immunomodulatory agent
is selected from the group consisting of α -, β -, and δ -interferons.
44. The composition of claim 41 further comprising an antiviral agent.
25
45. The composition of claim 44 wherein the antiviral agent is selected from the
group consisting of ribavirin and amantadine.
46. The composition of claim 41 further comprising an inhibitor of HCV protease
30 other than the compound of claim 1.
47. The composition of claim 46 further comprising an inhibitor of a target in the

HCV life cycle other than HCV NS3 protease.

48. The composition of claim 47 wherein the other target is selected from the group consisting of helicase, polymerase, metalloprotease and mixtures thereof.

5

49. Use of the composition of claim 41 for the manufacture of a medicament for treating a hepatitis C viral infection in a patient.

10

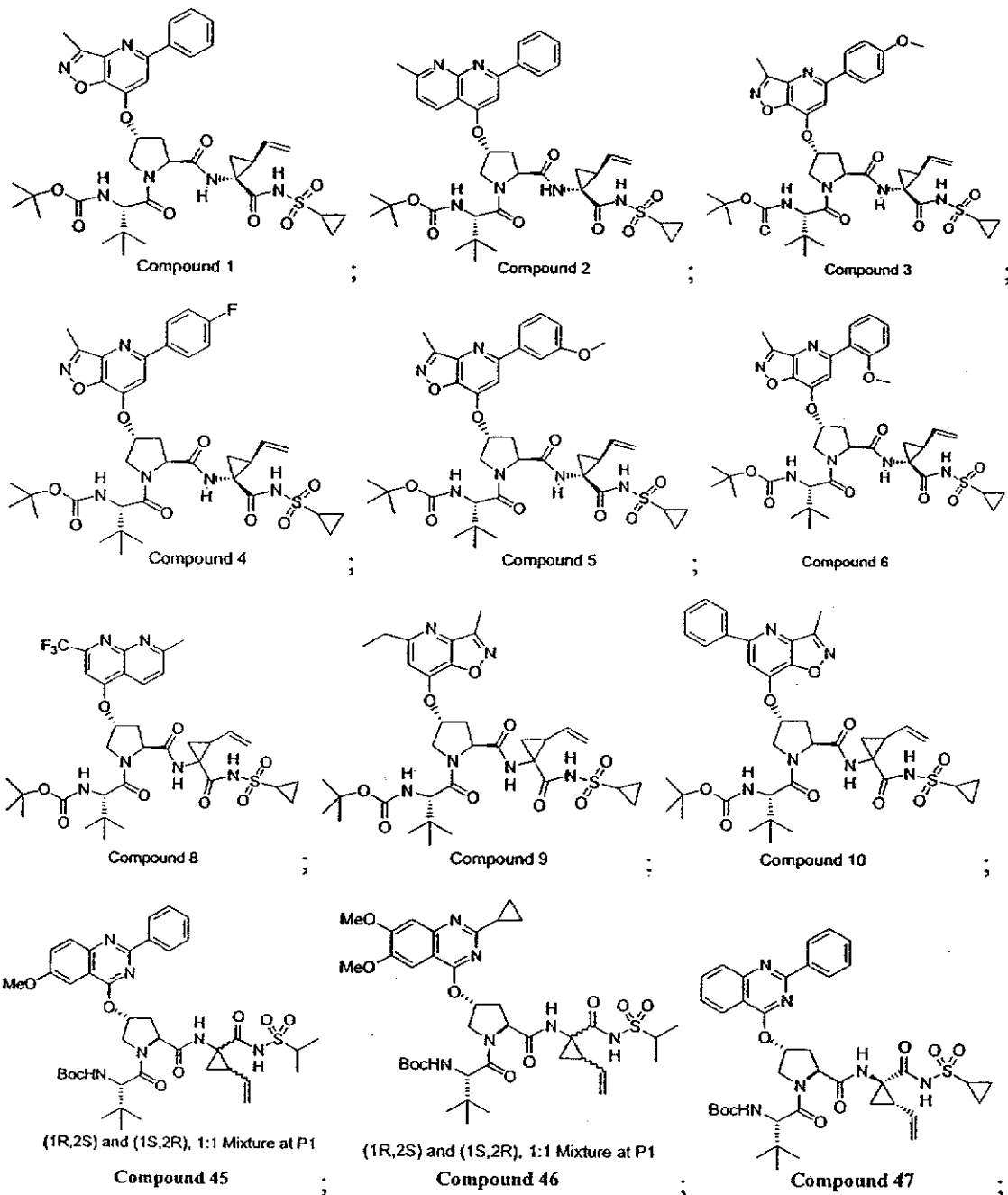
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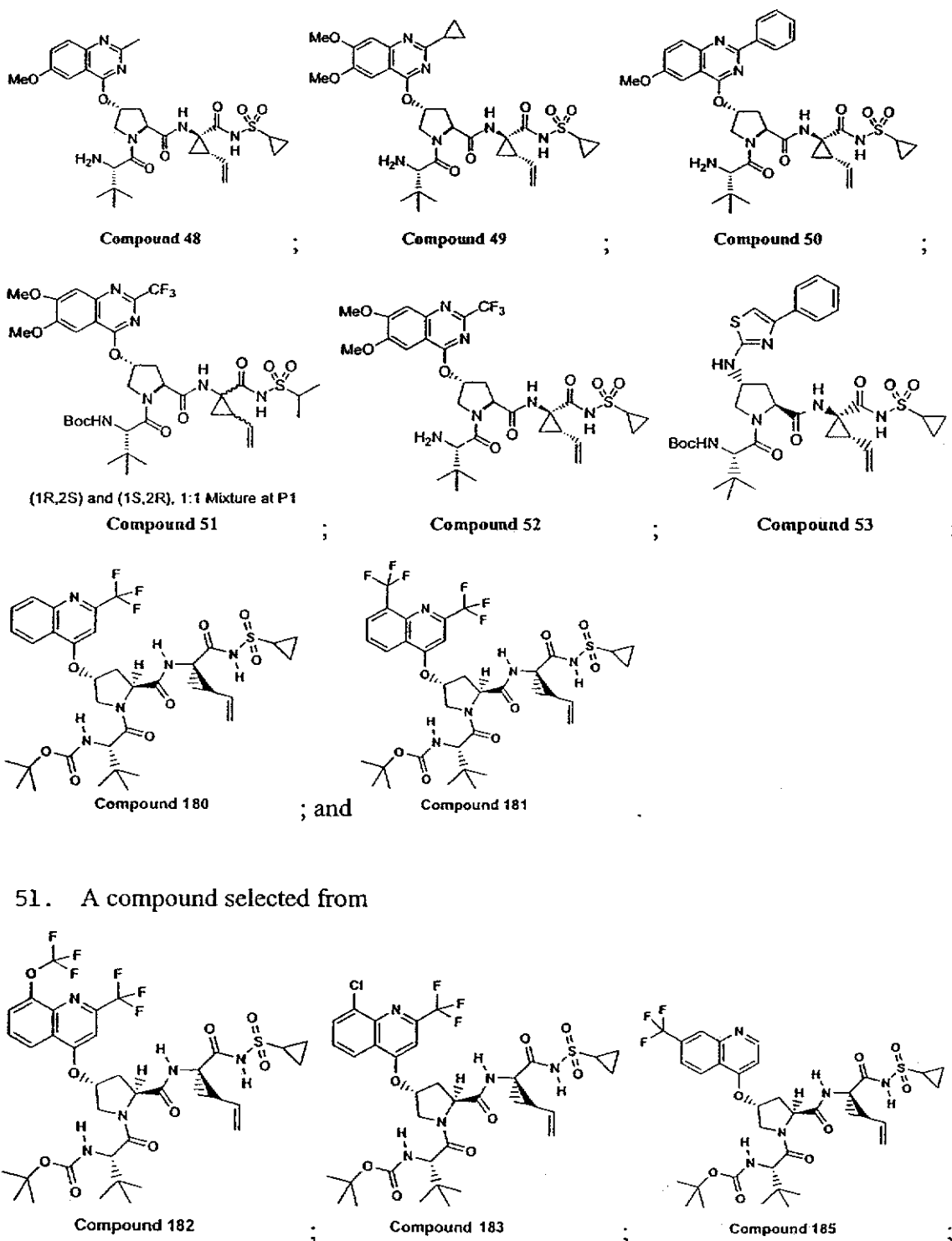
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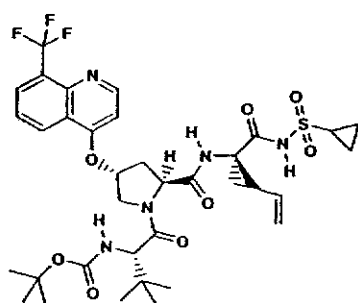
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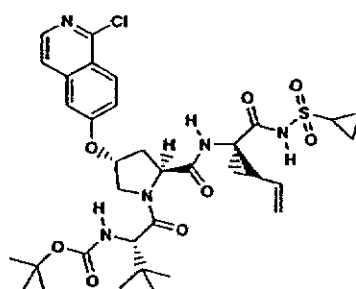
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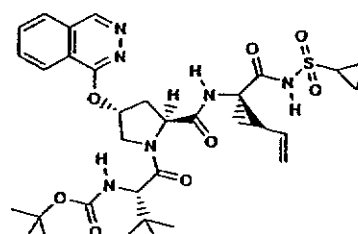
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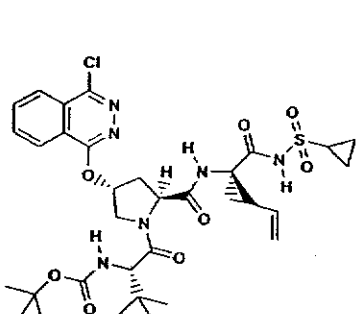
Compound 186



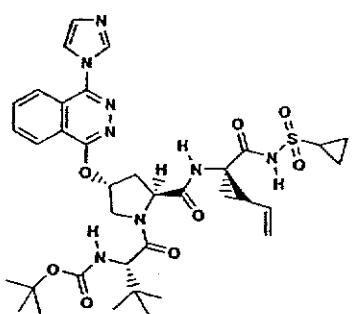
Compound 188



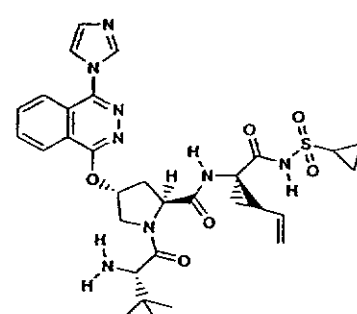
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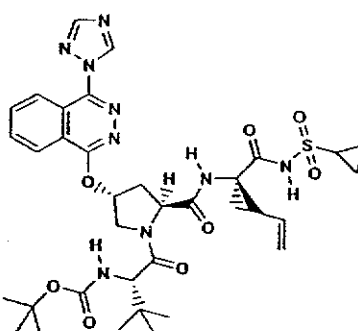
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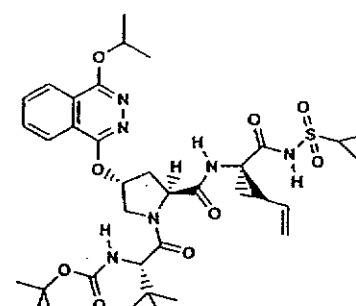
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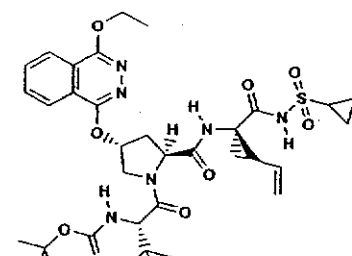
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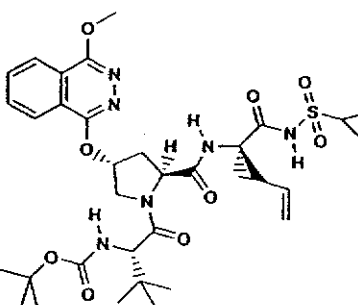
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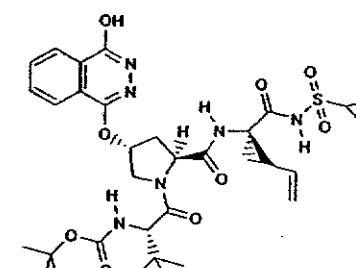
Compound 199



Compound 200

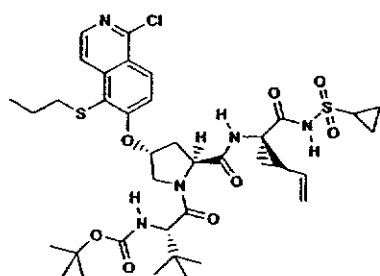


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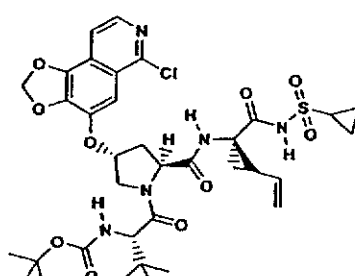


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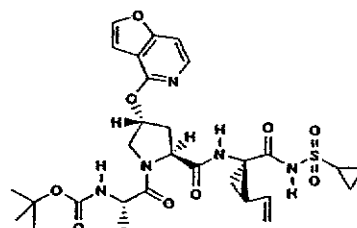
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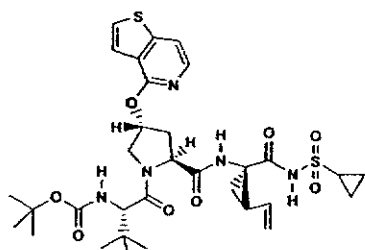
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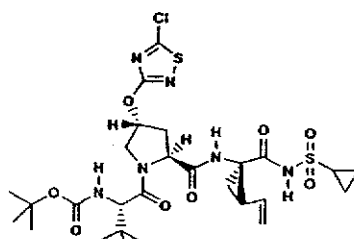
Compound 212



Compound 233

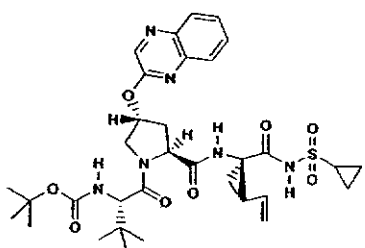


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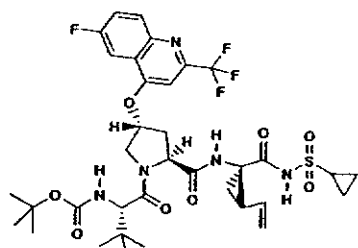
Compound 236

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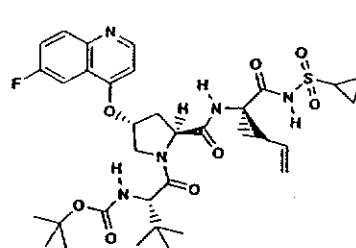


Compound 237

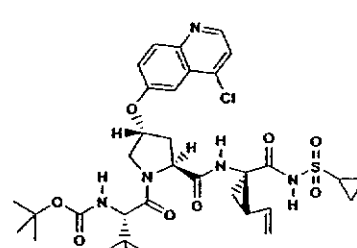
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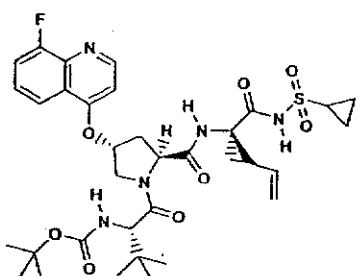
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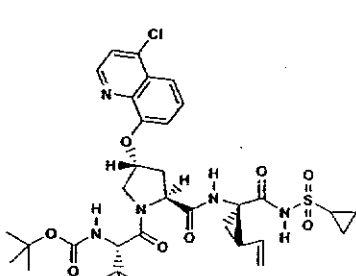
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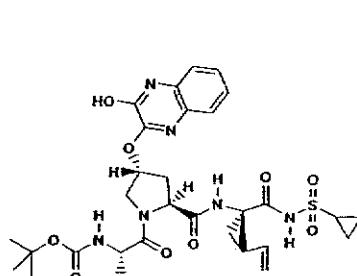
Compound 240



Compound 241

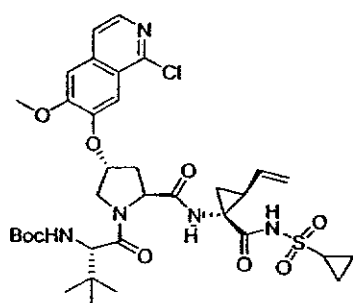


Compound 242

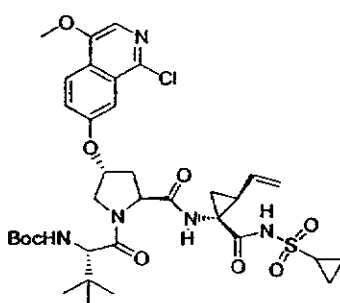


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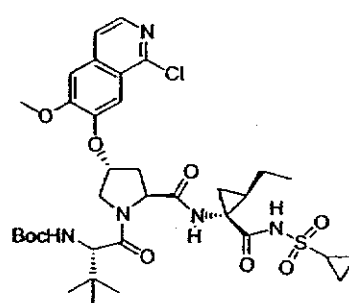
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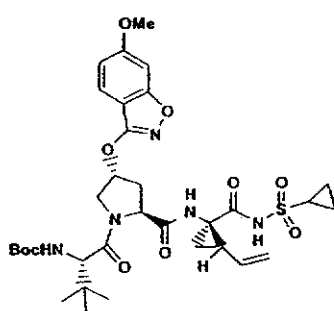
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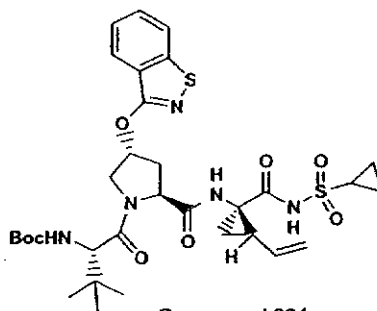
Compound 283



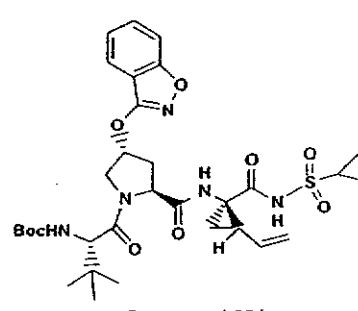
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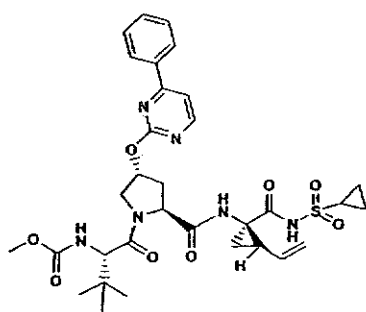
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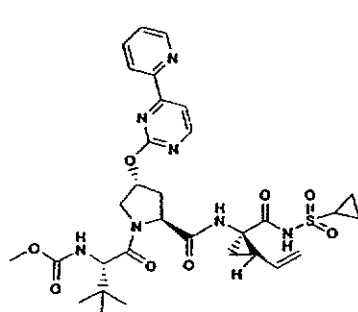
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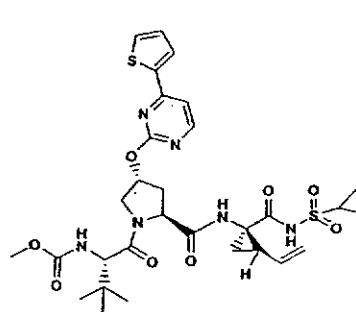
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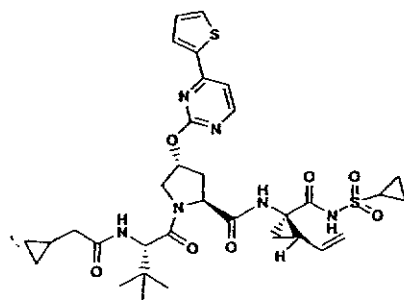
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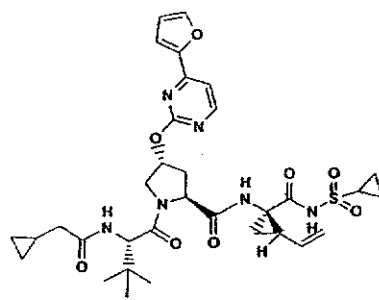
Compound 336



Compound 337

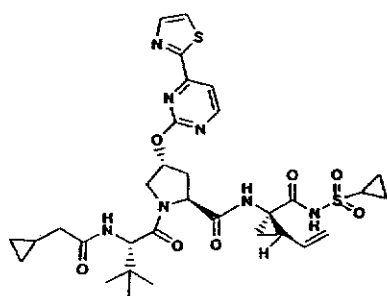


Compound 338

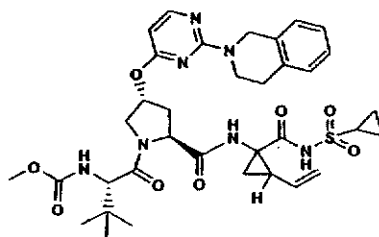


Compound 339

679

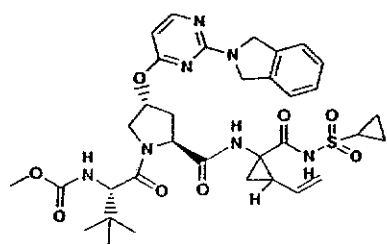


Compound 340



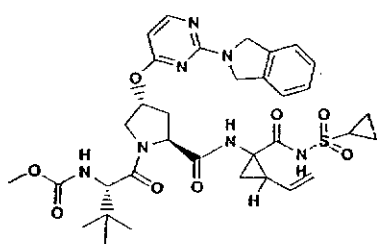
Compound 341

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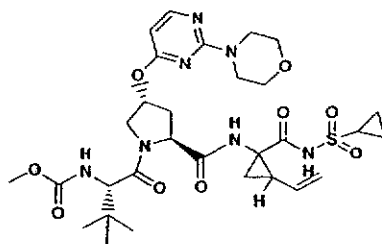


Compound 342

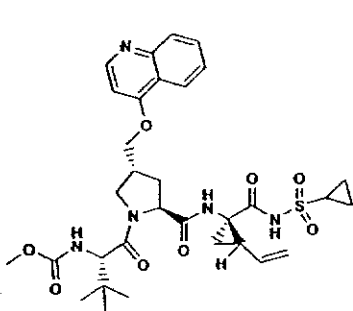
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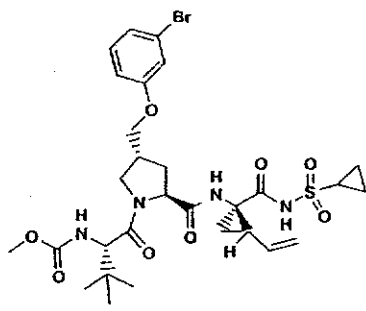
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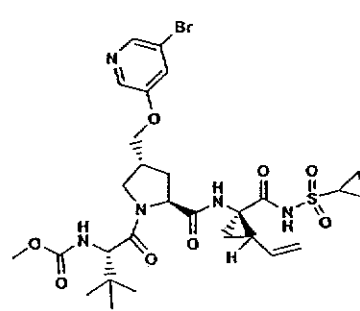
Compound 343



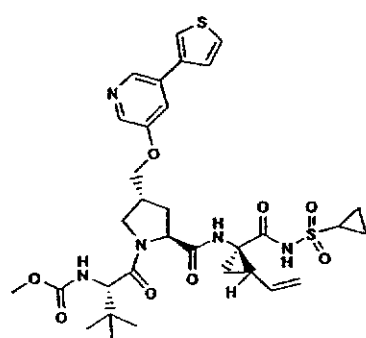
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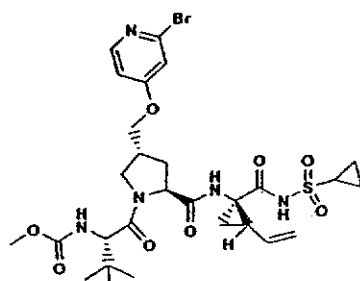
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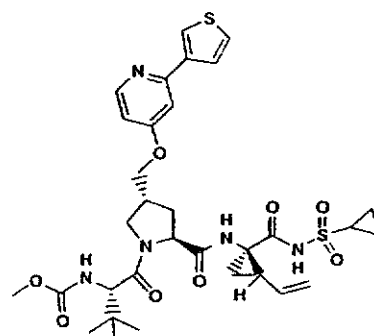
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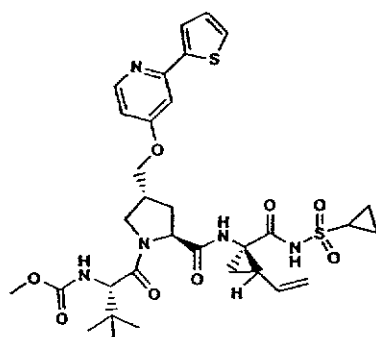
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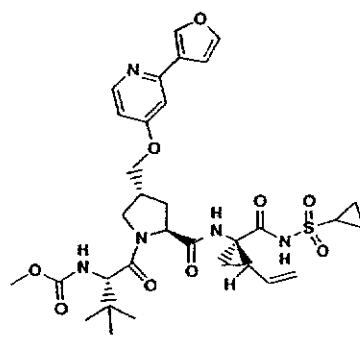
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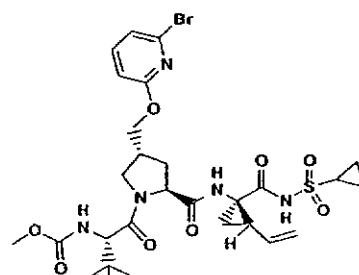
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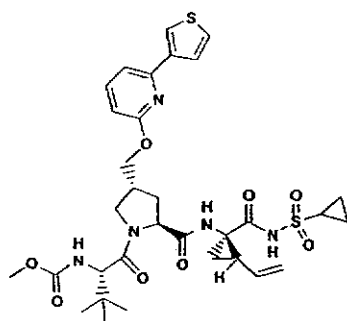
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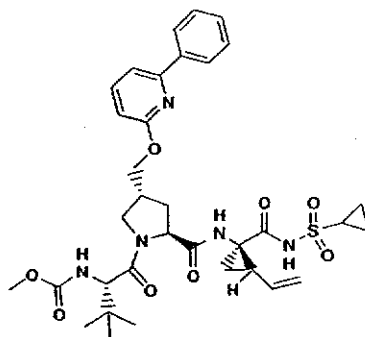
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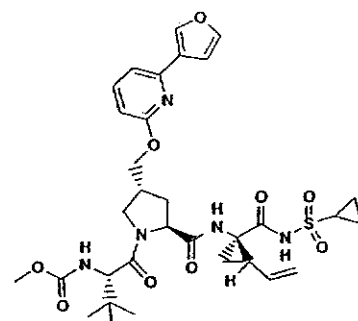
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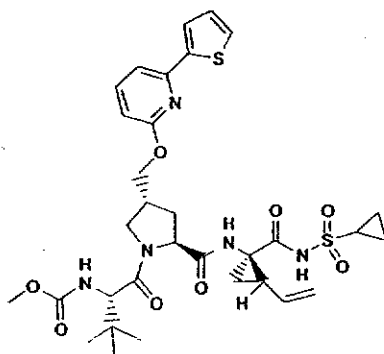
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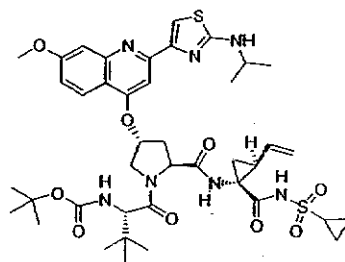
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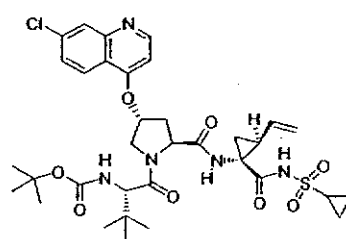
Compound 355 ;



Compound 356 ;

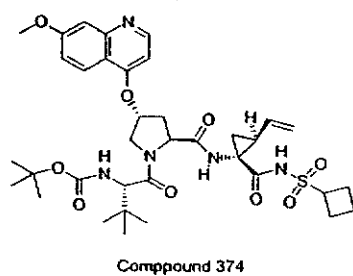
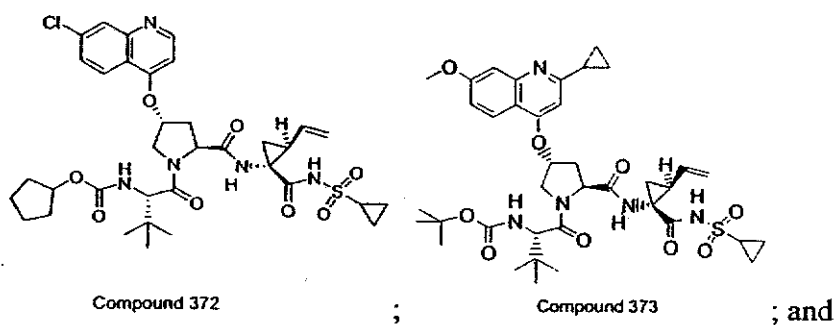


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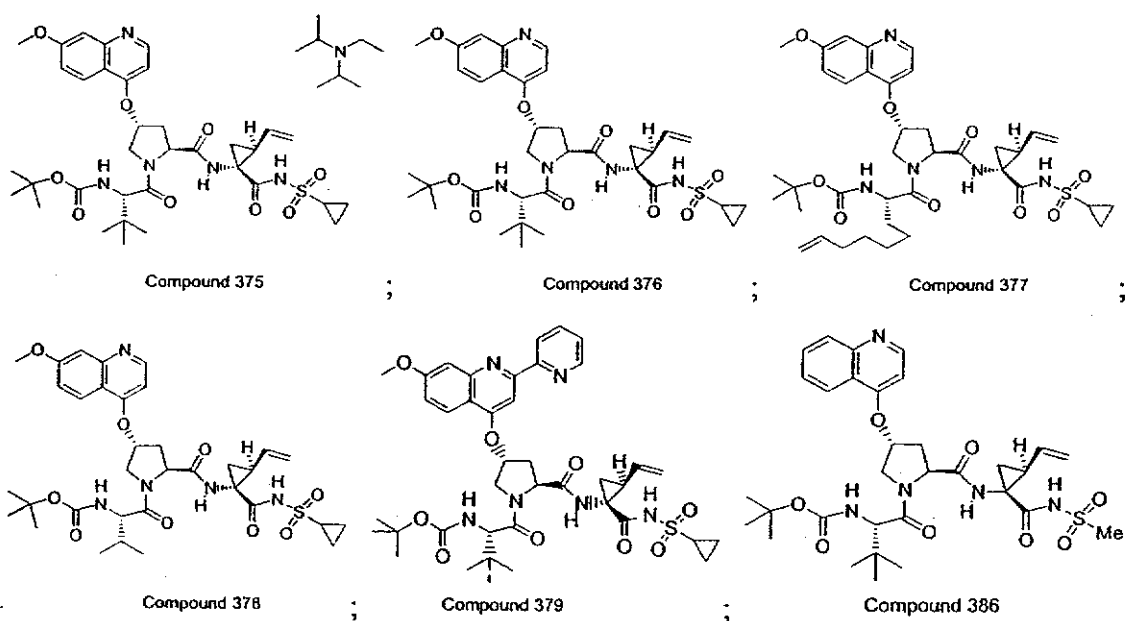


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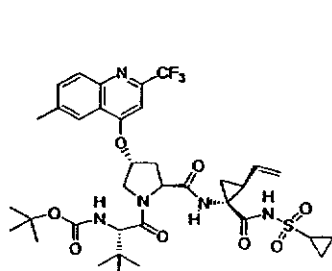
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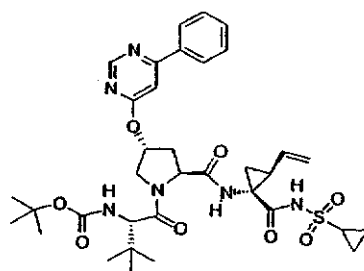
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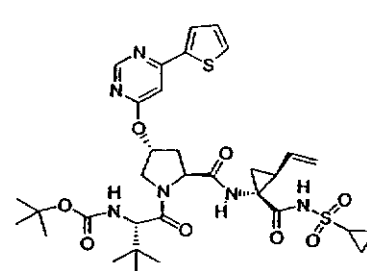
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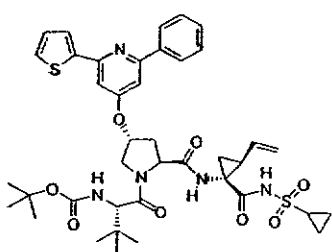
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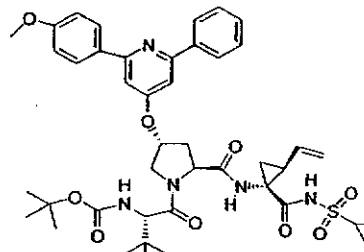
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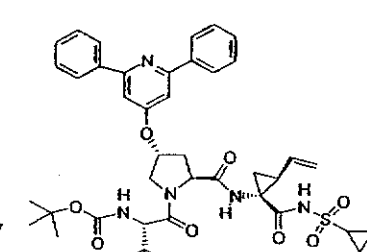
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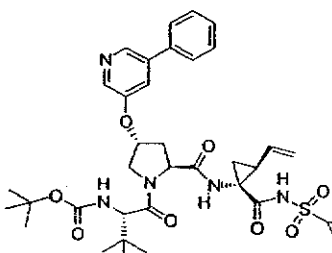
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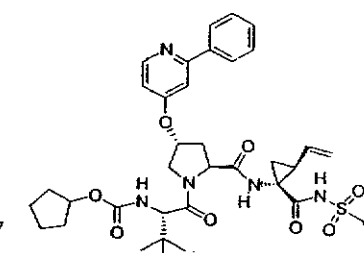
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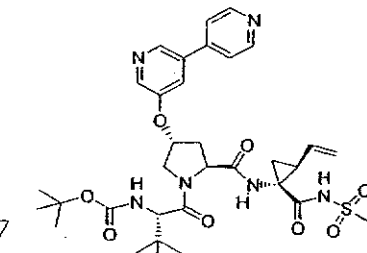
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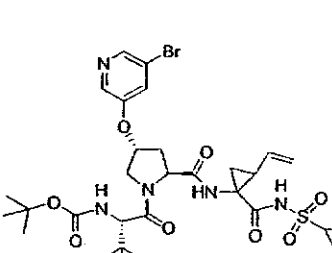
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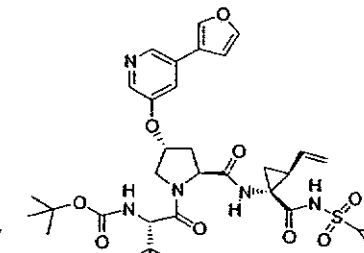
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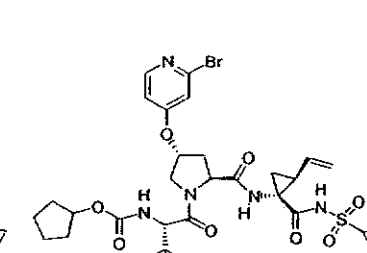
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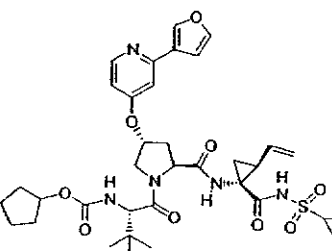
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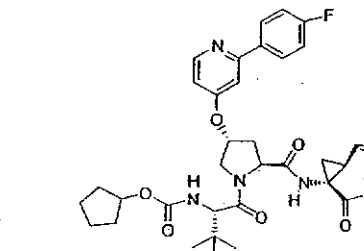
Compound 424



Compound 425



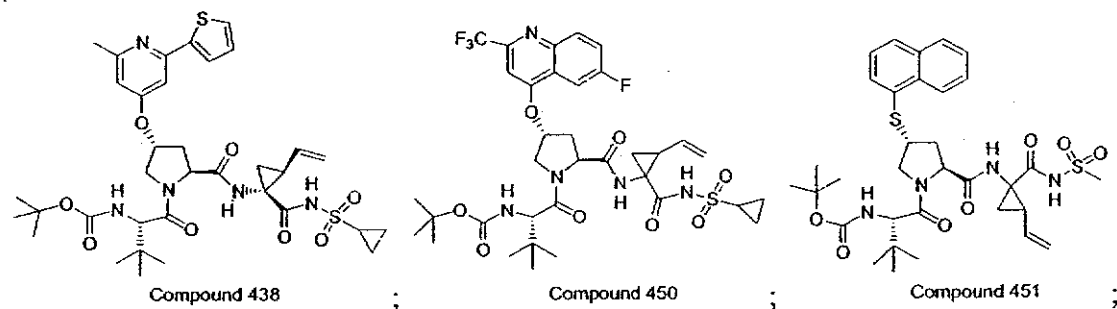
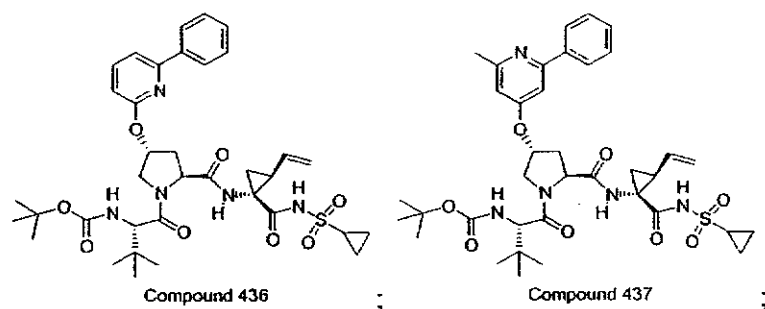
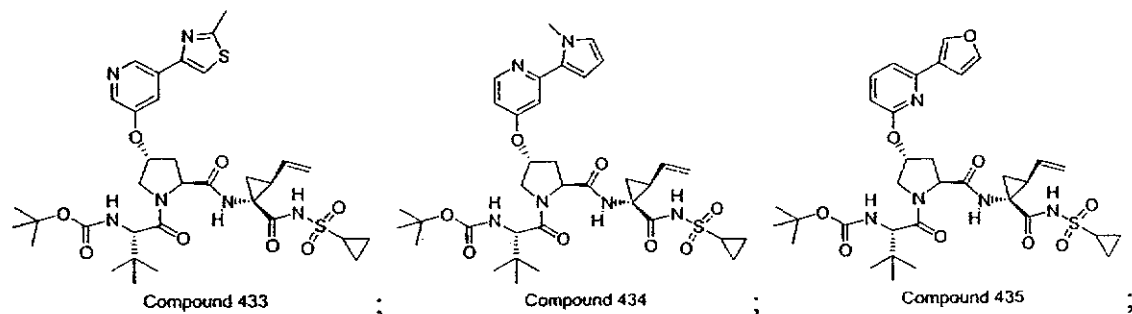
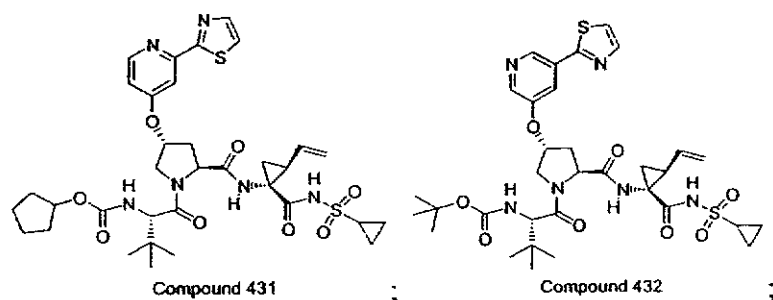
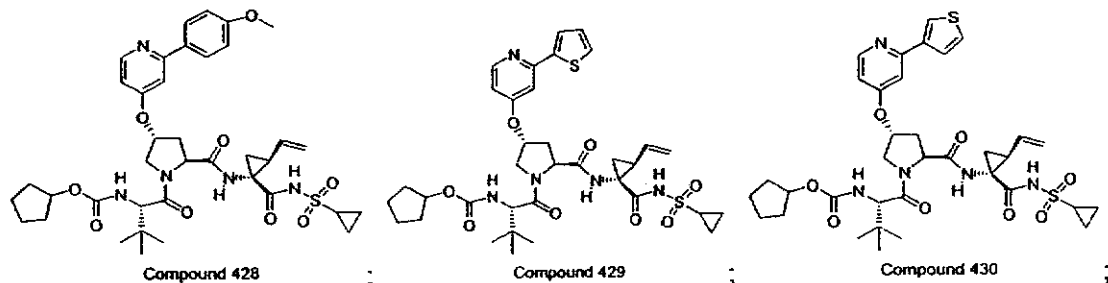
Compound 426



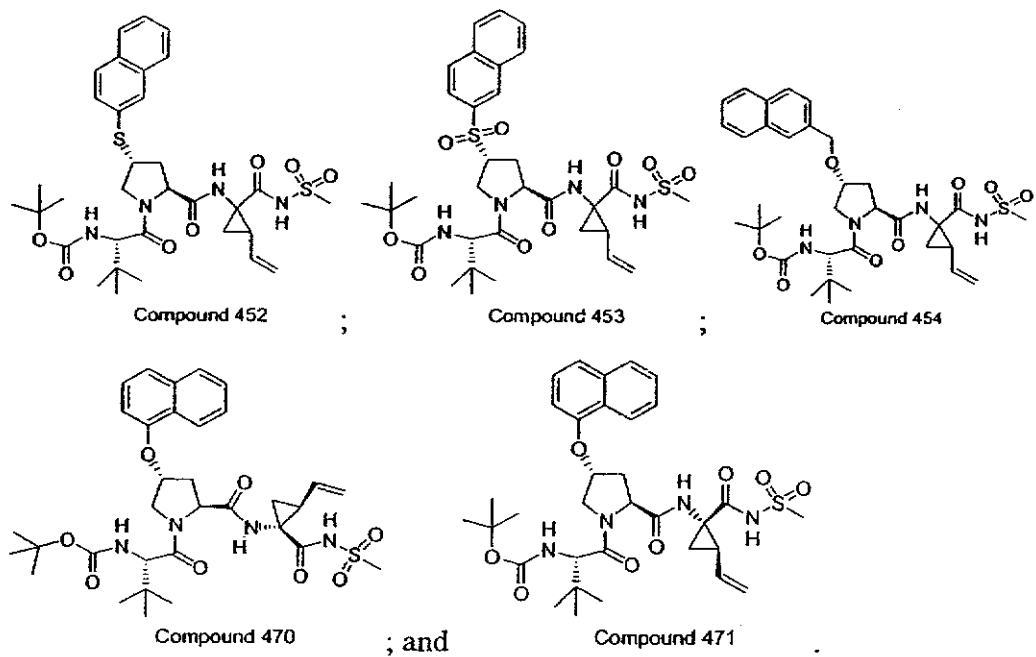
Compound 427

; and

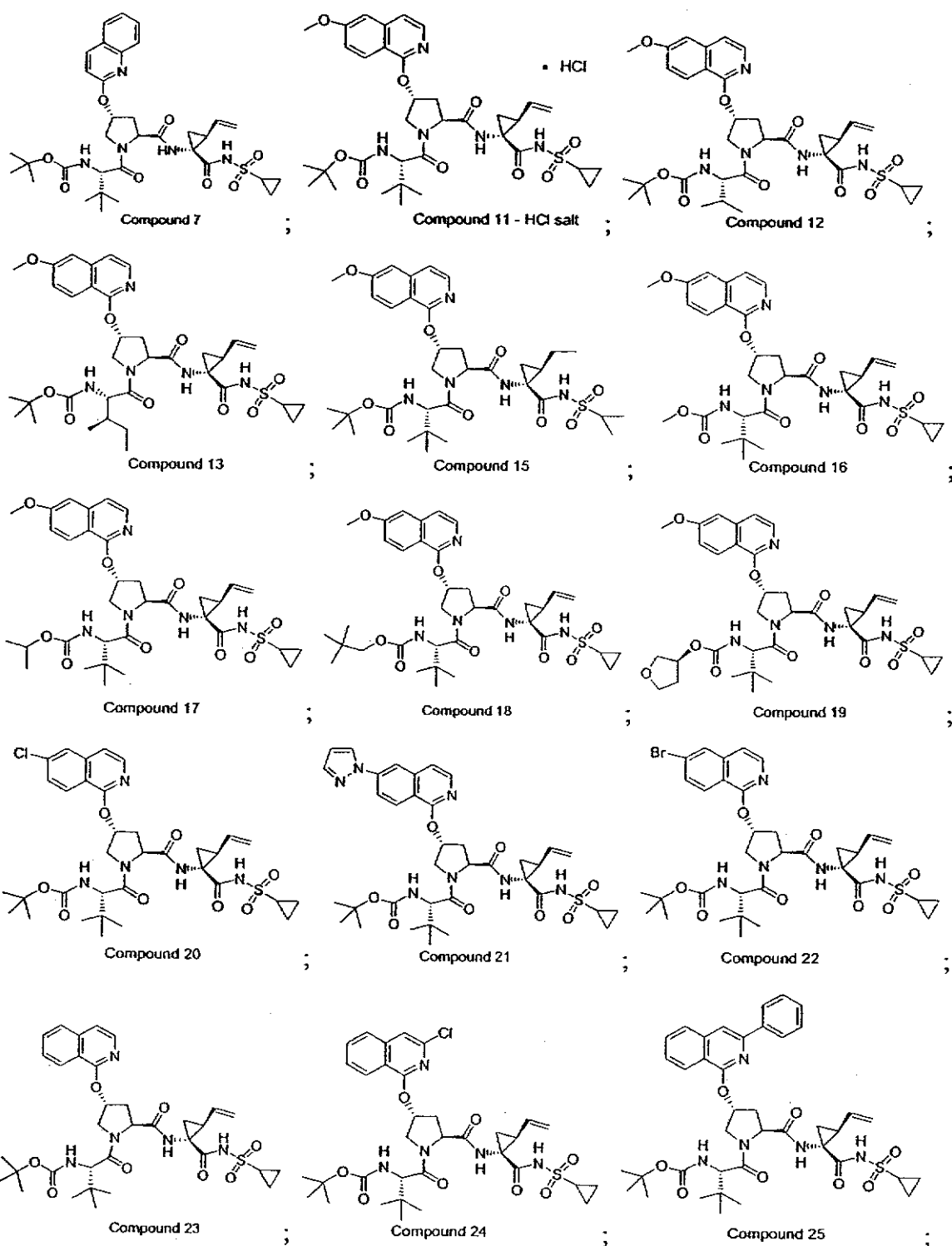
55. A compound selected from



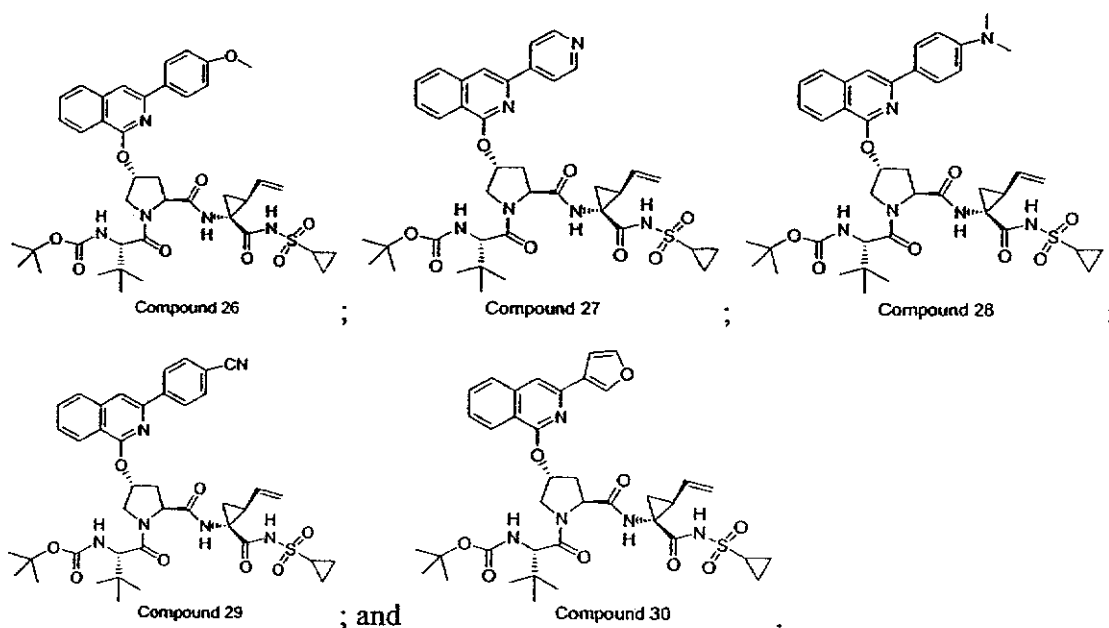
684



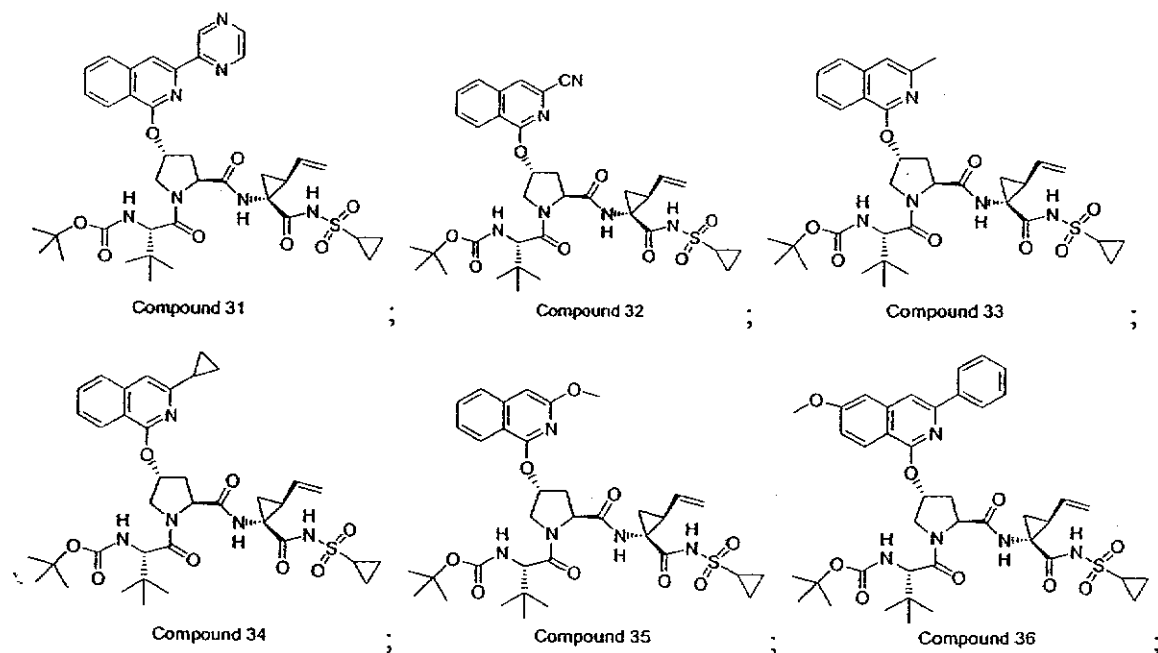
56. A compound selected from



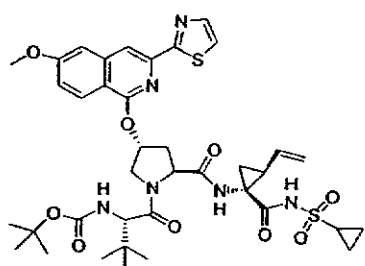
686



57. A compound selected from

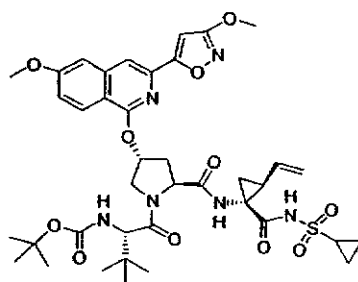


687



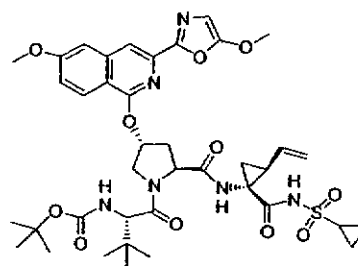
Compound 37

;



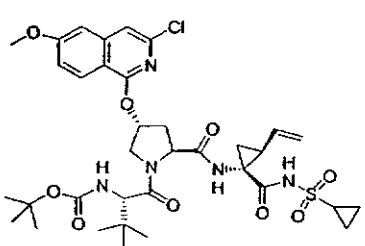
Compound 38

;



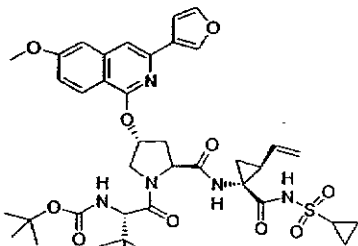
Compound 39

;



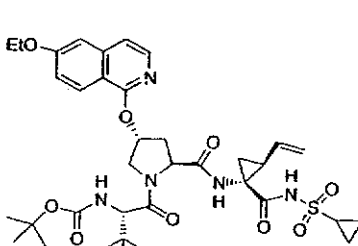
Compound 40

;



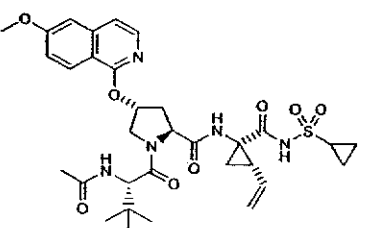
Compound 41

;



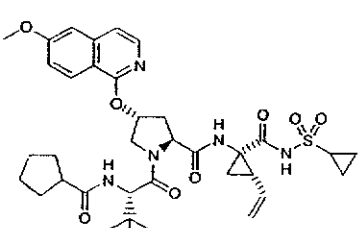
Compound 42

;



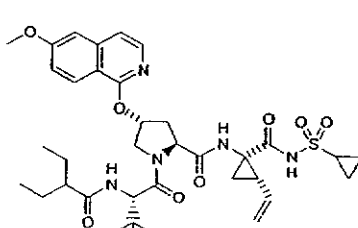
Compound 55

;



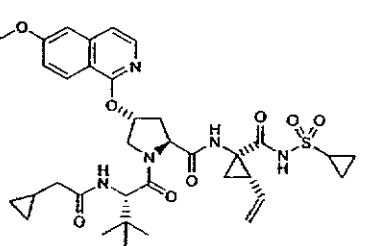
Compound 56

;



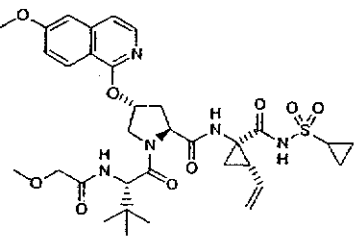
Compound 57

;



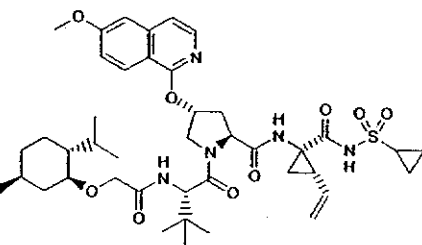
Compound 58

;



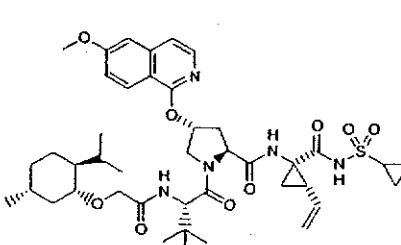
Compound 59

;



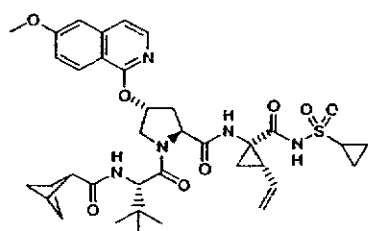
Compound 60

;



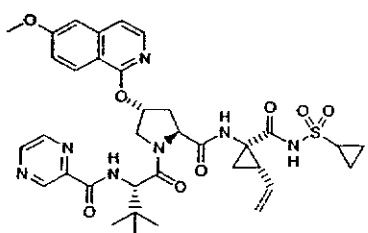
Compound 61

; and

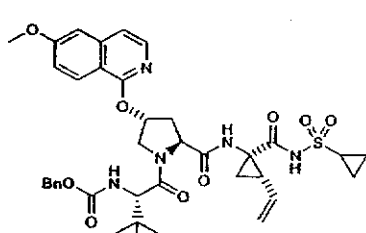


Compound 62

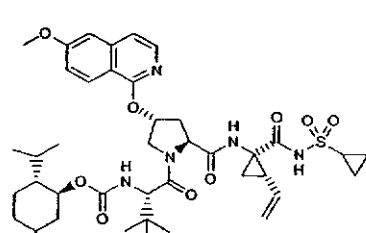
58. A compound selected from



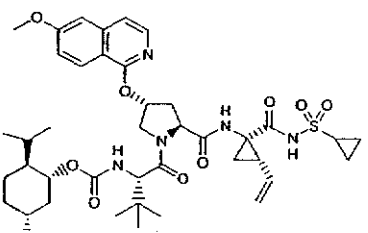
Compound 63



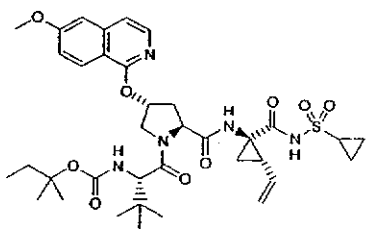
Compound 64



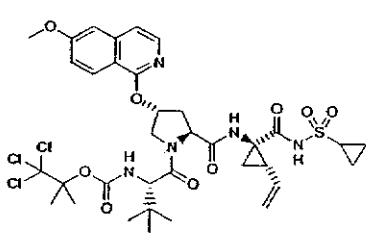
Compound 65



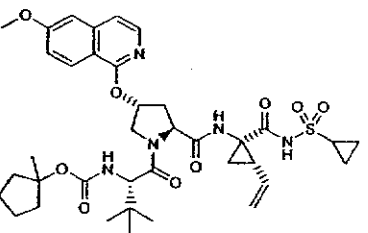
Compound 66



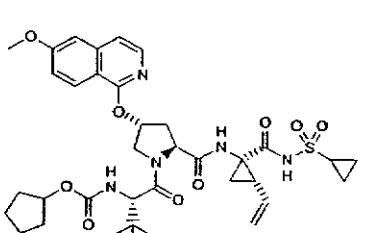
Compound 67



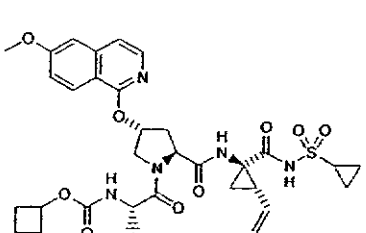
Compound 68



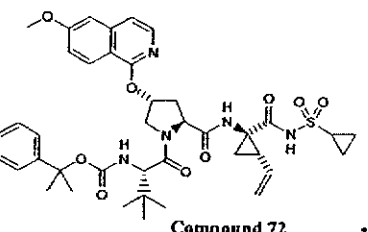
Compound 69



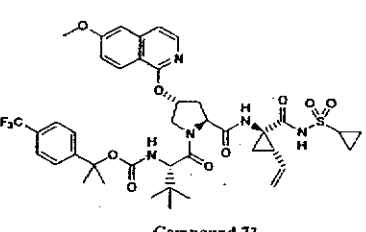
Compound 70



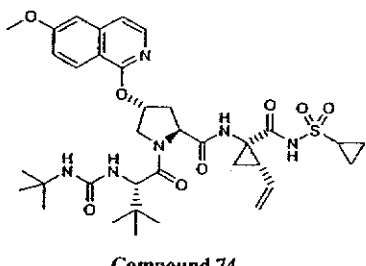
Compound 71



Compound 72

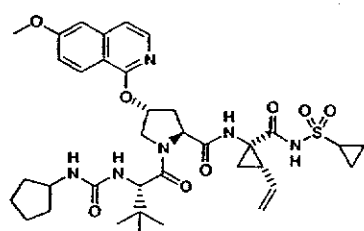


Compound 73



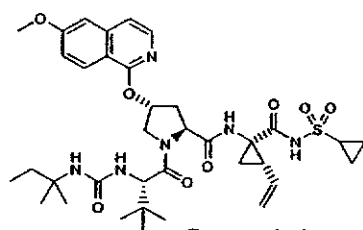
Compound 74

689



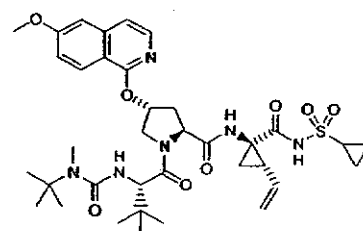
Compound 75

;



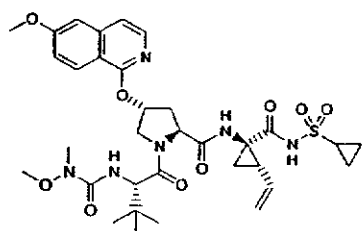
Compound 76

;



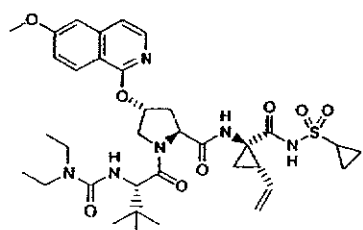
Compound 77

;



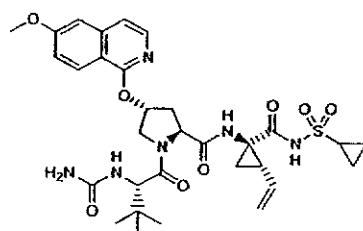
Compound 78

;



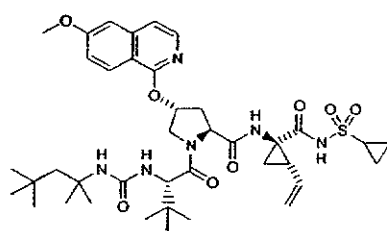
Compound 79

;



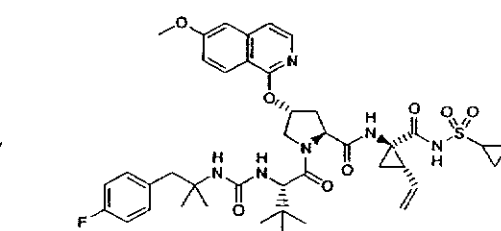
Compound 80

;



Compound 81

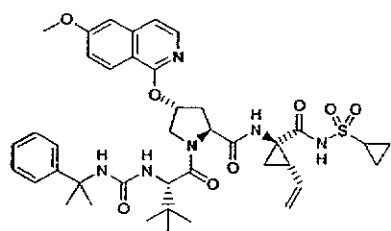
;



Compound 82

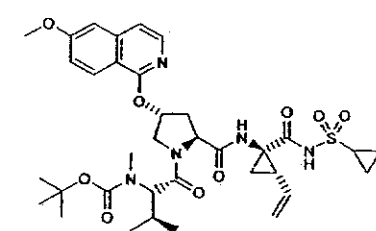
; and

59. A compound selected from



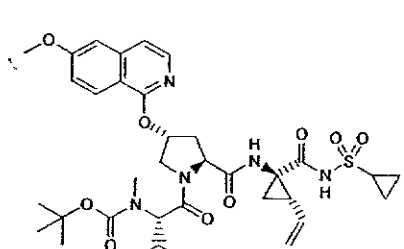
Compound 83

;



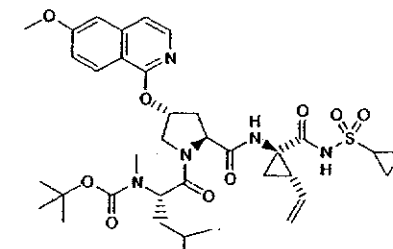
Compound 84

;



Compound 85

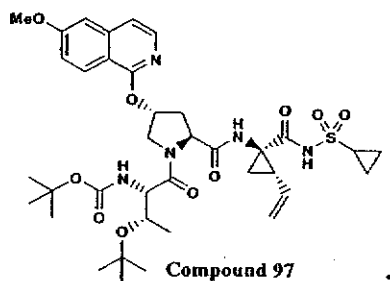
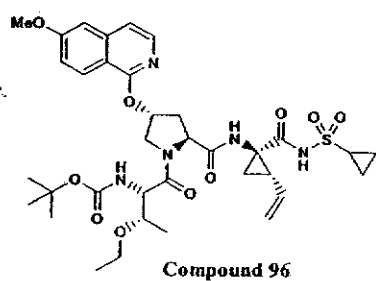
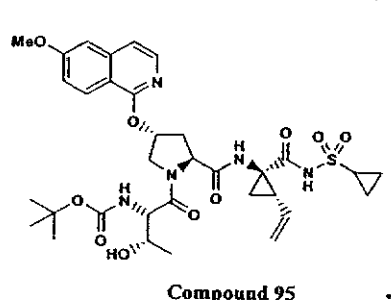
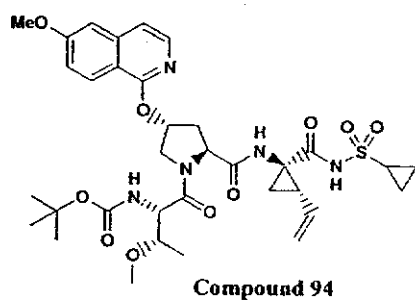
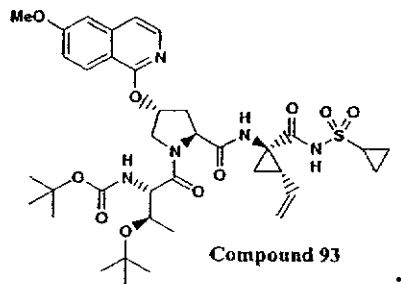
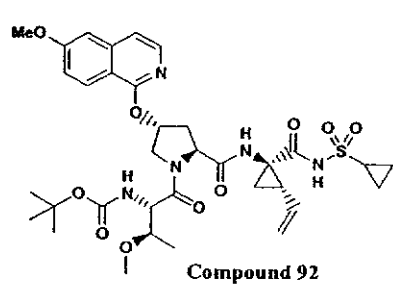
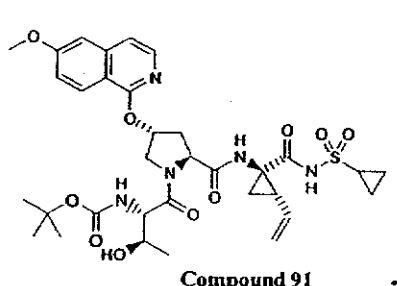
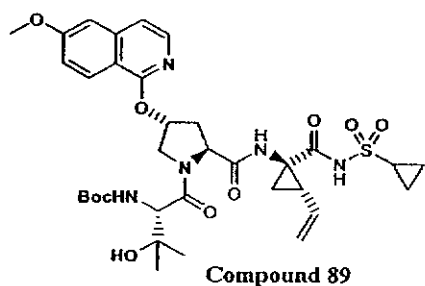
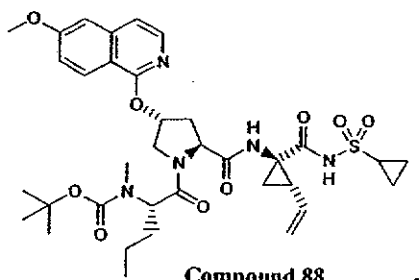
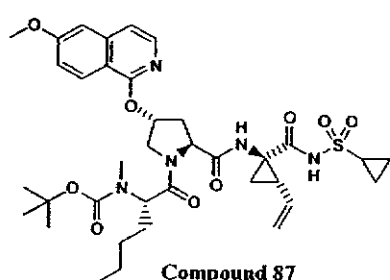
;



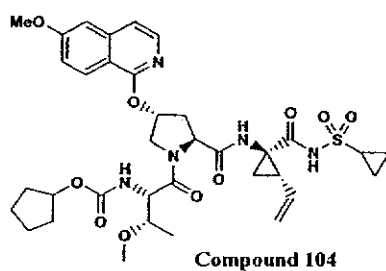
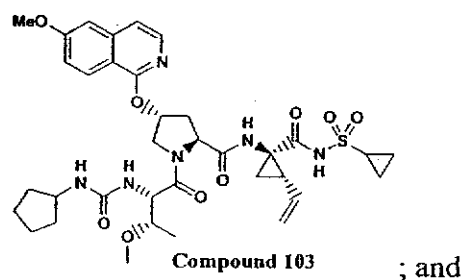
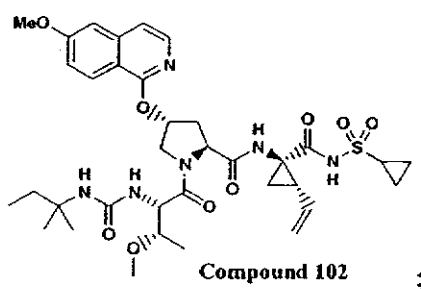
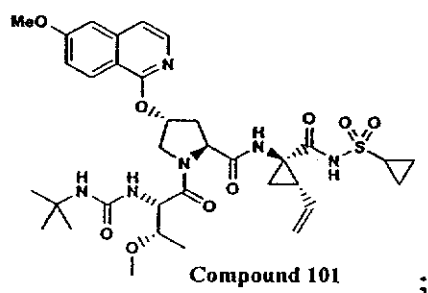
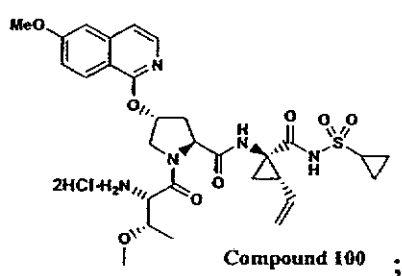
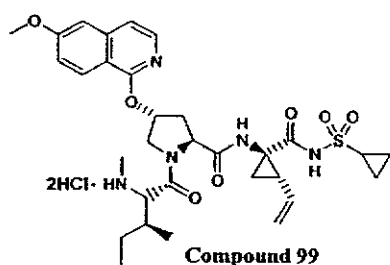
Compound 86

;

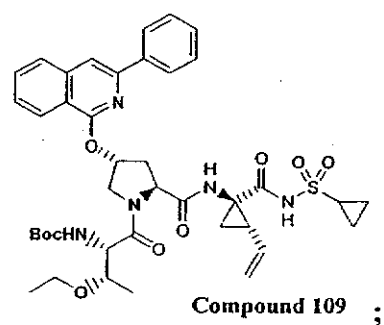
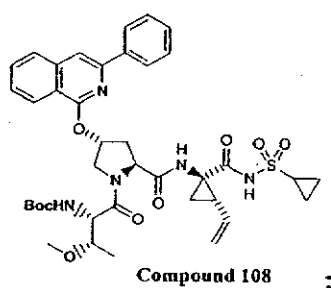
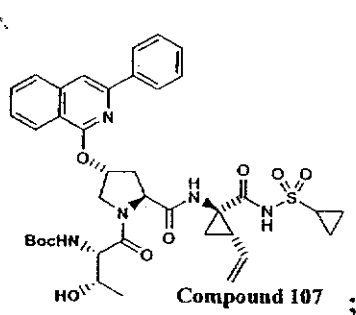
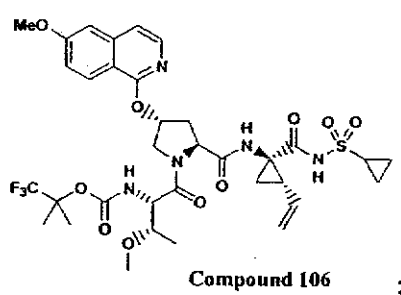
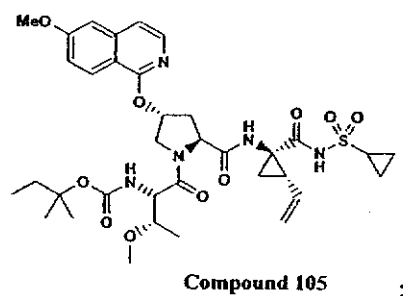
690



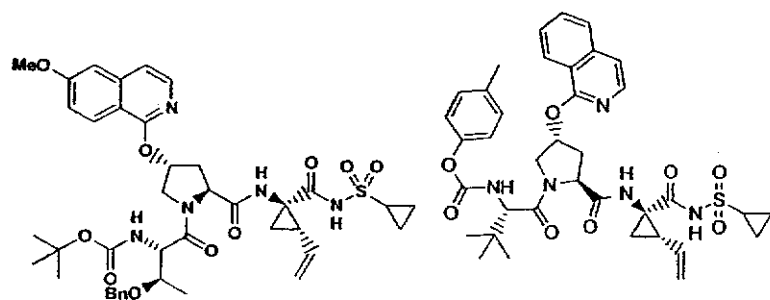
691



60. A compound selected from

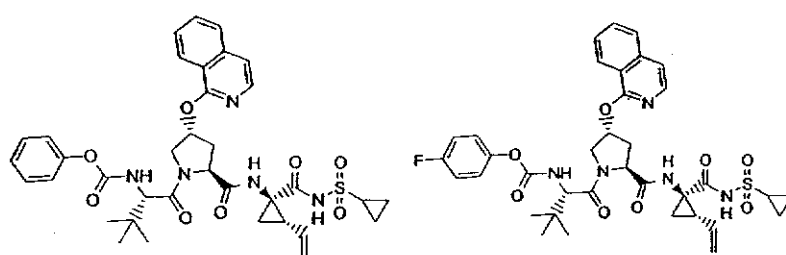


692



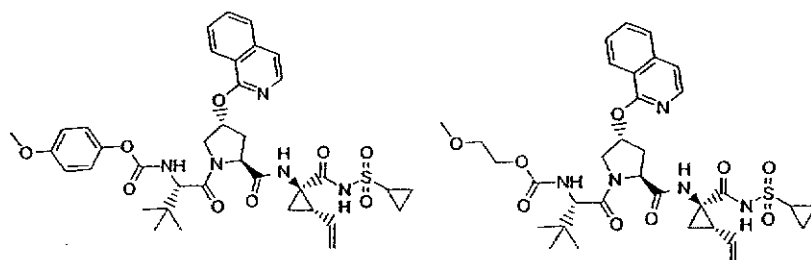
Compound 110

Compound 120



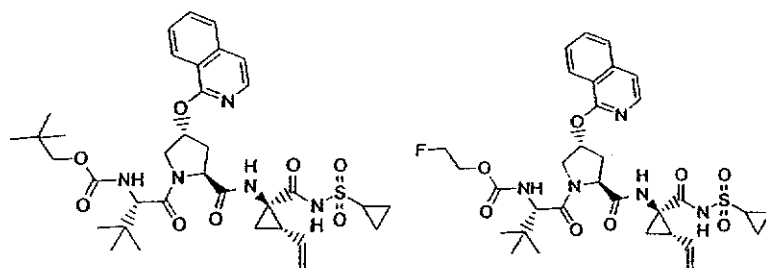
Compound 121

Compound 122



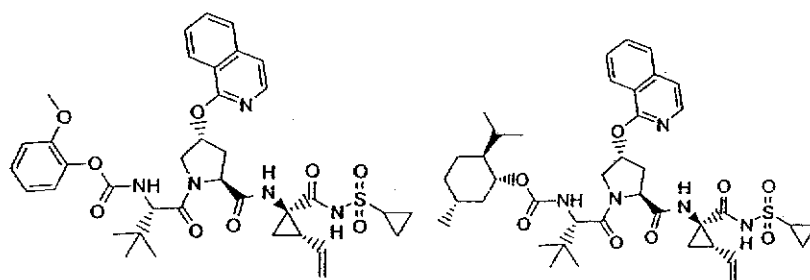
Compound 123

Compound 124



Compound 125

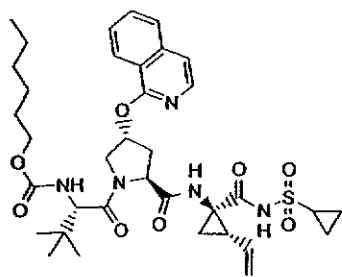
Compound 126



Compound 127

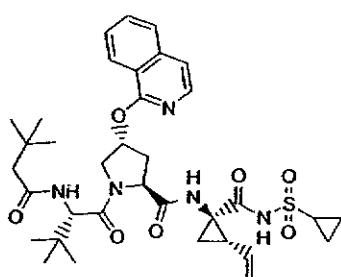
Compound 128

693



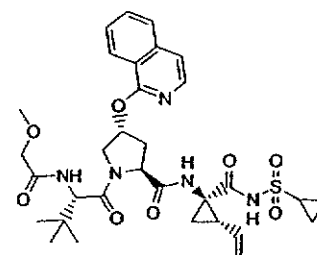
Compound 129

;



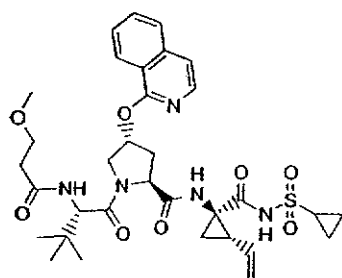
Compound 130

;



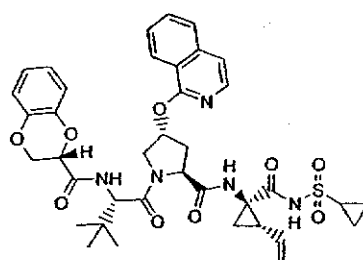
Compound 131

;



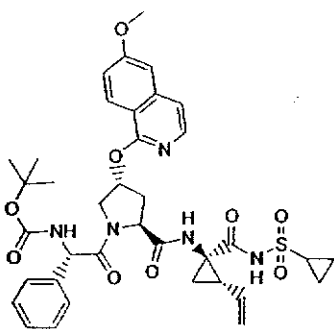
Compound 132

; and



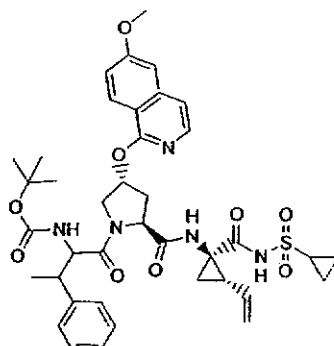
Compound 133

61. A compound selected from



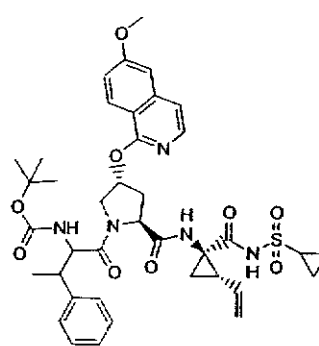
Compound 134

;



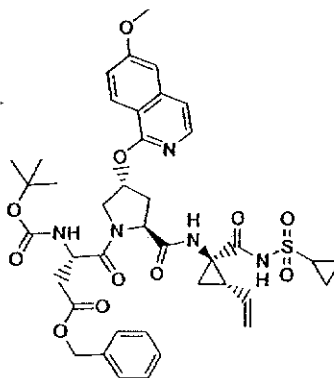
Compound 135

;



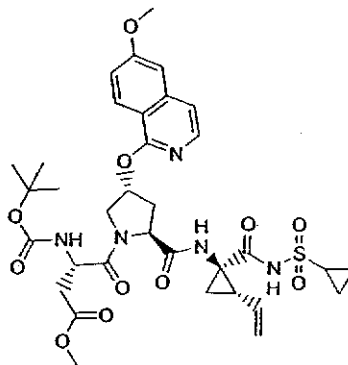
Compound 136

;



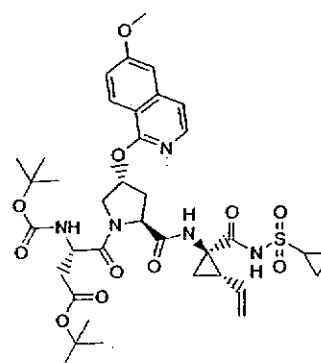
Compound 137

;



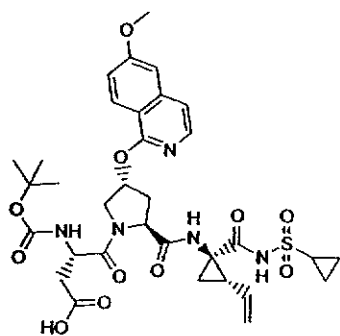
Compound 138

;



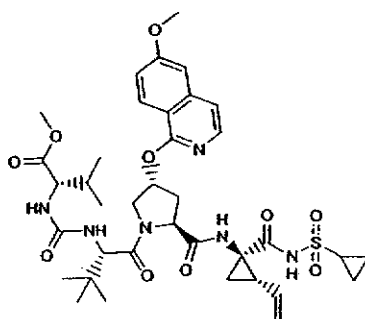
Compound 139

;



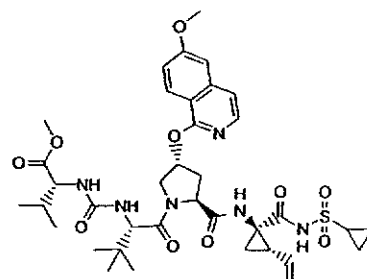
Compound 140

;



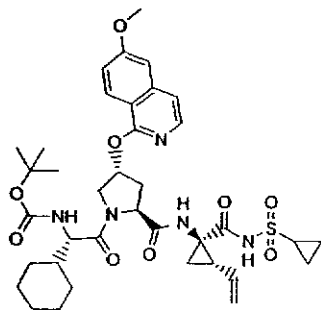
Compound 141

;



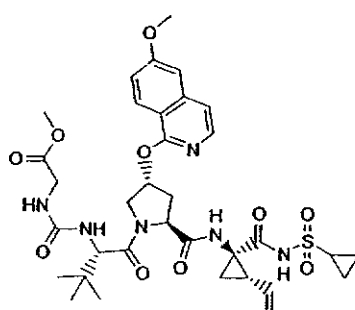
Compound 142

;



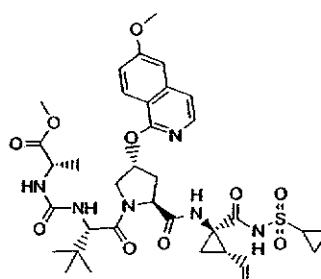
Compound 143

;



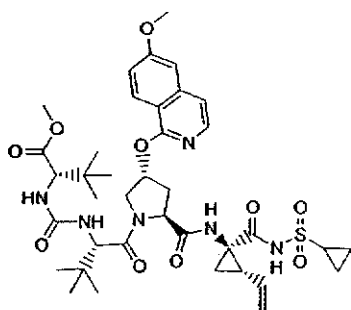
Compound 144

;



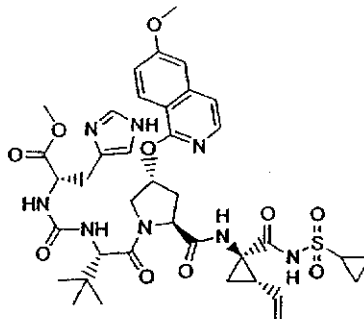
Compound 145

;



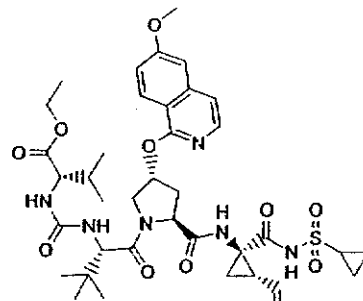
Compound 146

;



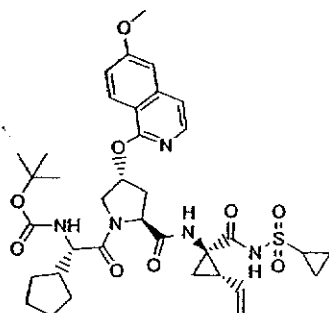
Compound 147

;



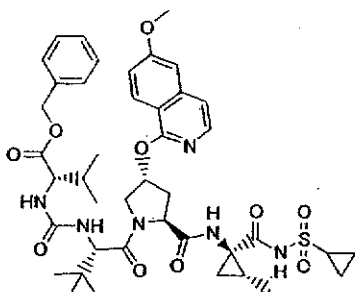
Compound 148

;



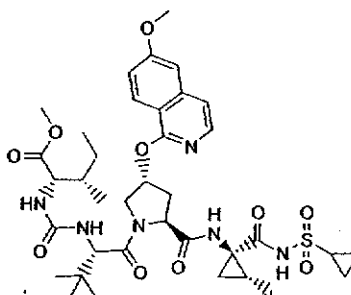
Compound 149

;



Compound 150

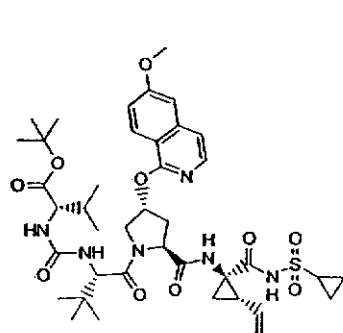
;



Compound 151

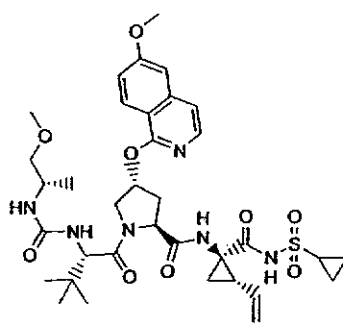
;

695



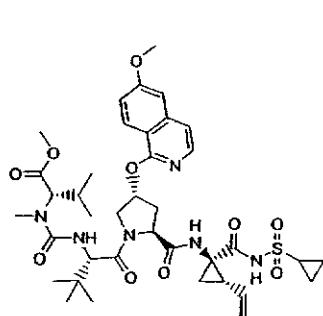
Compound 152

; and



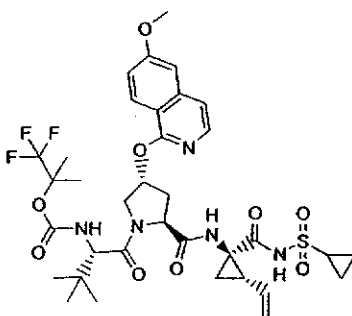
Compound 153

62. A compound selected from



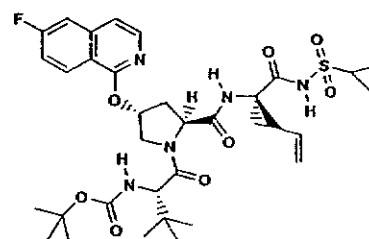
Compound 154

;



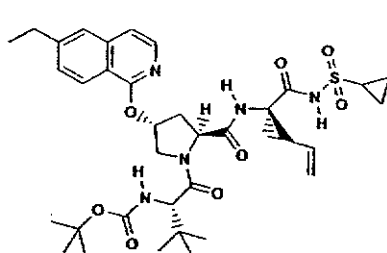
Compound 155

;



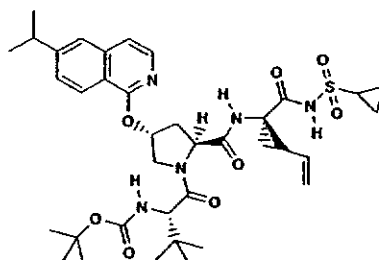
Compound 187

;



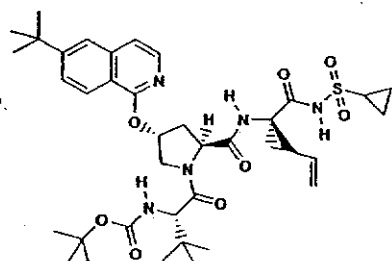
Compound 189

;



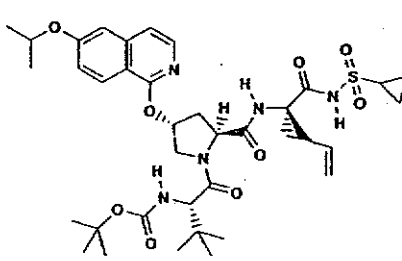
Compound 190

;



Compound 191

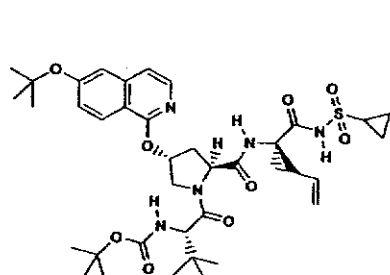
;



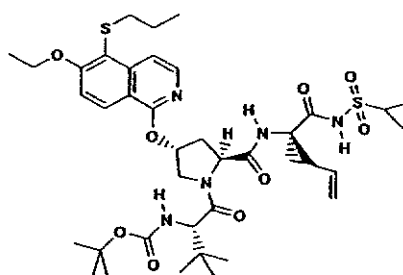
Compound 192

;

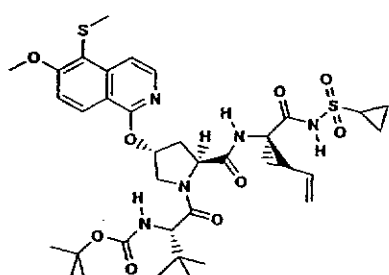
696



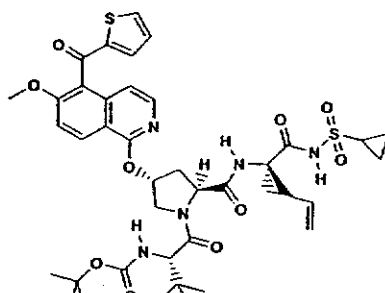
Compound 193



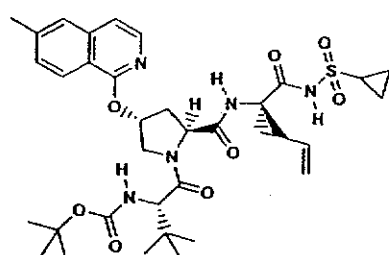
Compound 206



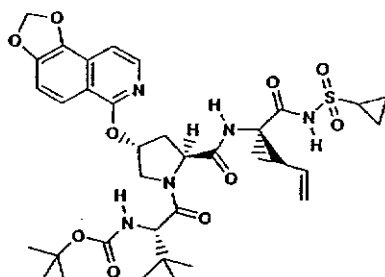
Compound 207



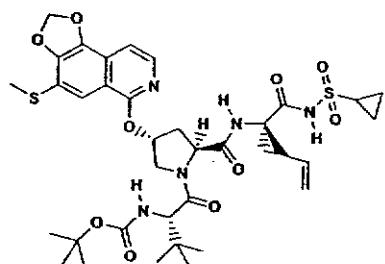
Compound 209



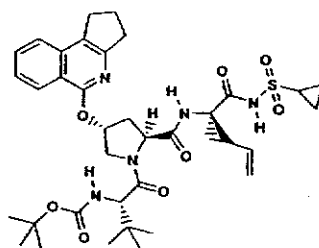
Compound 210



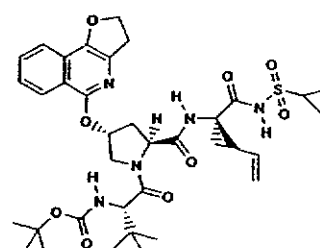
Compound 211



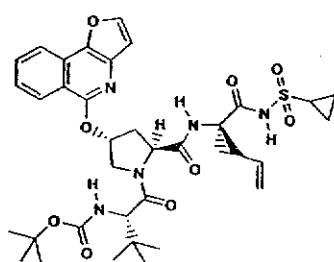
Compound 213



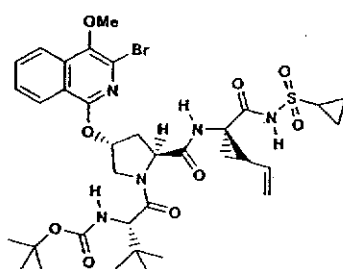
Compound 215



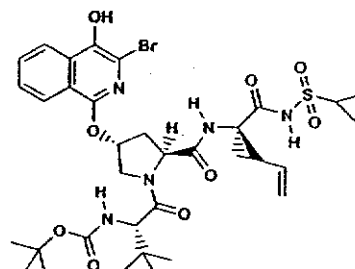
Compound 219



Compound 220

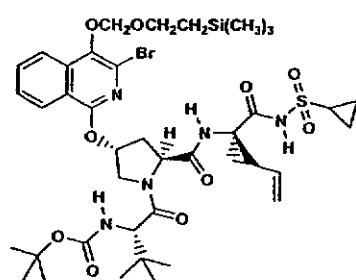


Compound 223



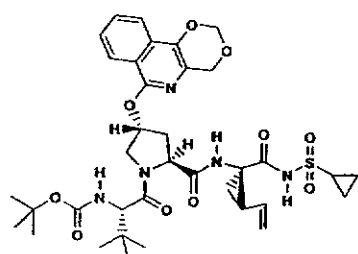
Compound 224

; and

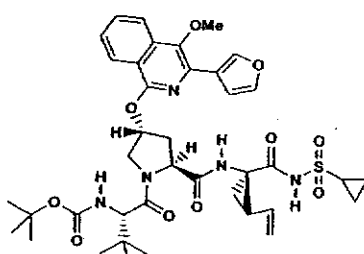


Compound 225

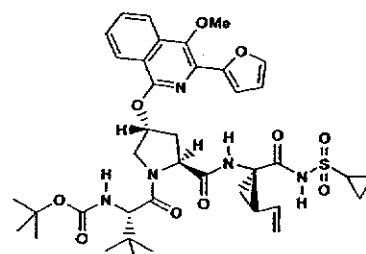
63. A compound selected from



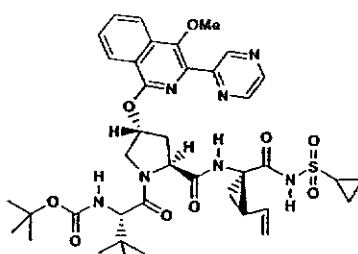
Compound 227



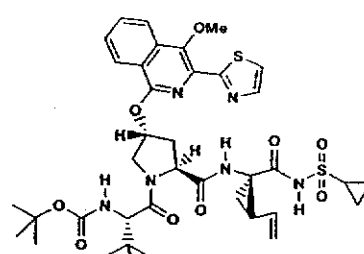
Compound 229



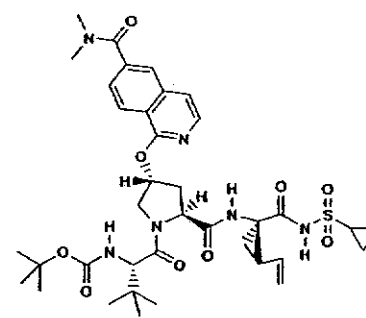
Compound 230



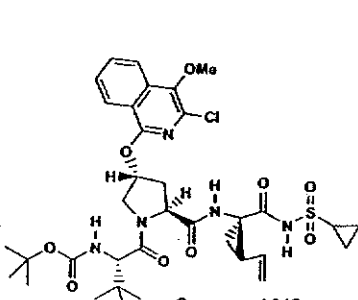
Compound 231



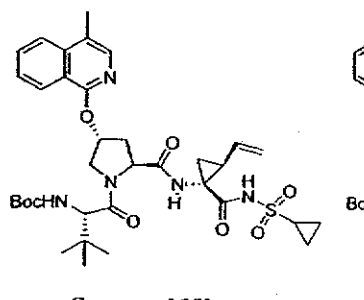
Compound 232



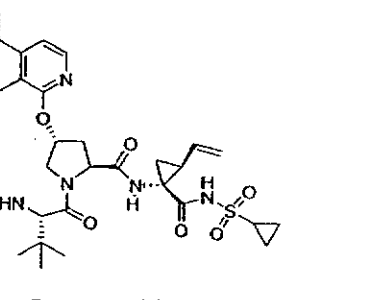
Compound 244



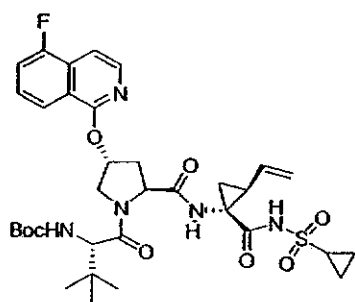
Compound 245



Compound 250

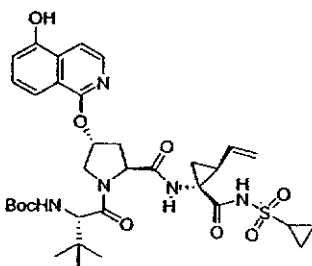


Compound 252



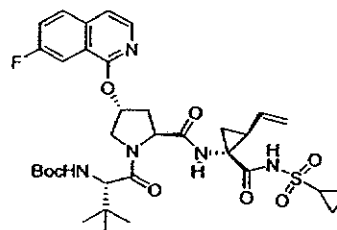
Compound 255

;



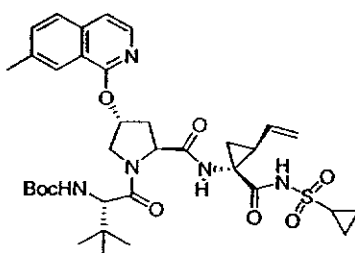
Compound 256

;



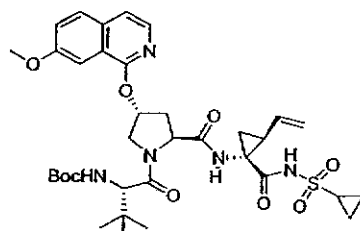
Compound 257

;



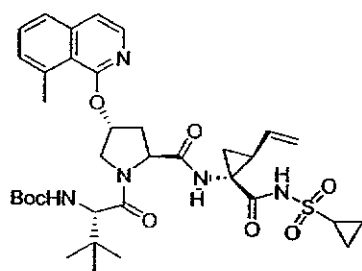
Compound 259

;



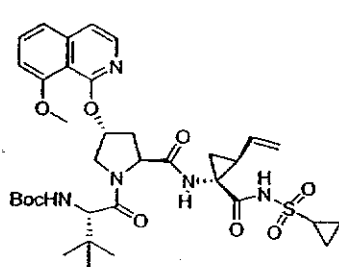
Compound 260

; and



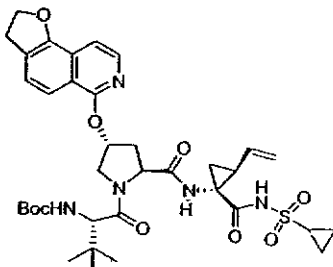
Compound 263

64. A compound selected from



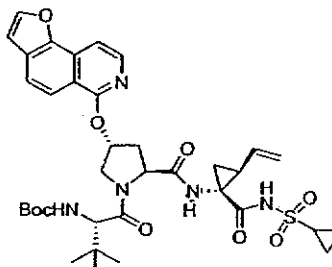
Compound 264

;



Compound 265

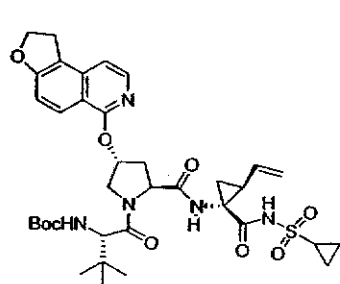
;



Compound 266

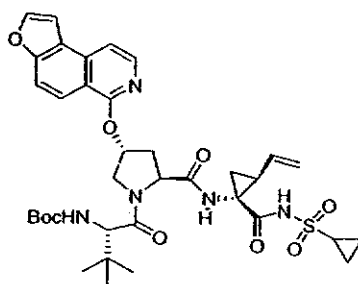
;

699



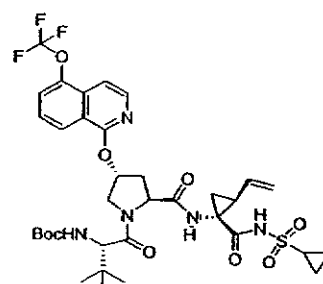
Compound 267

;



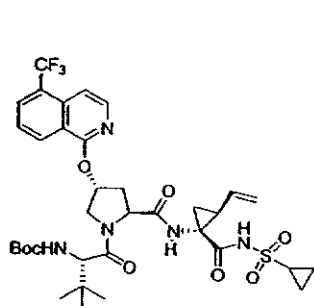
Compound 268

;



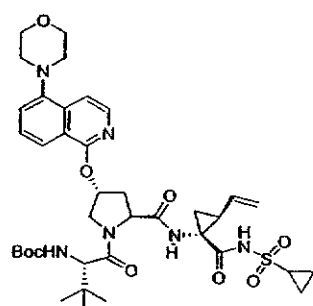
Compound 269

;



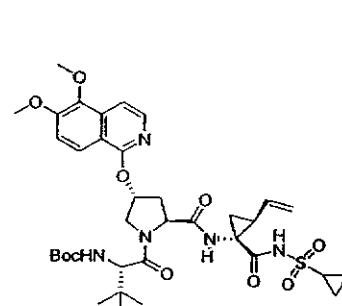
Compound 270

;



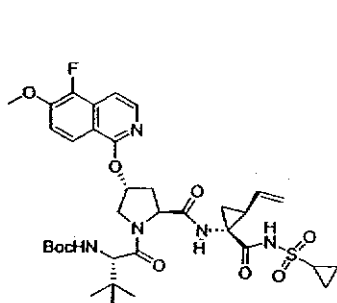
Compound 271

;



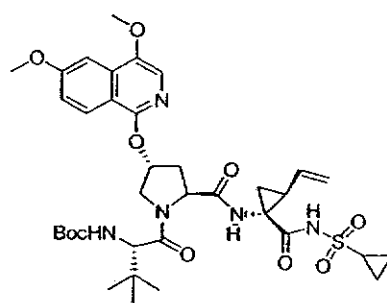
Compound 272

;



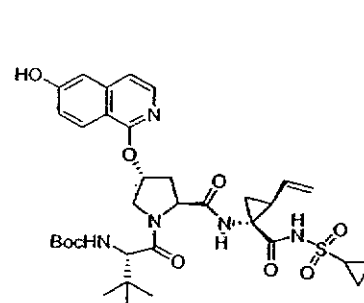
Compound 274

;



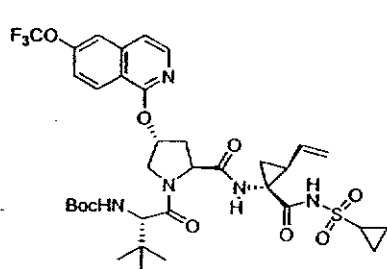
Compound 279

;



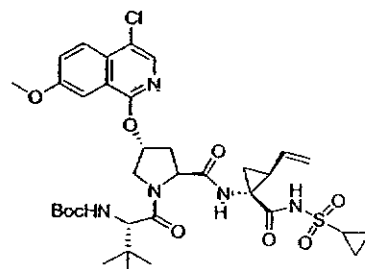
Compound 280

;



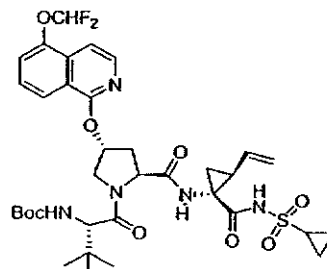
Compound 282

;



Compound 284

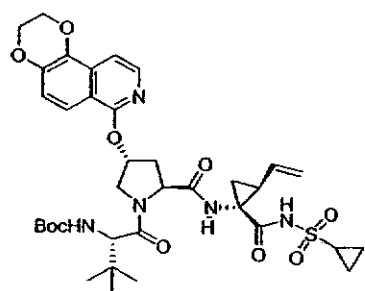
;



Compound 285

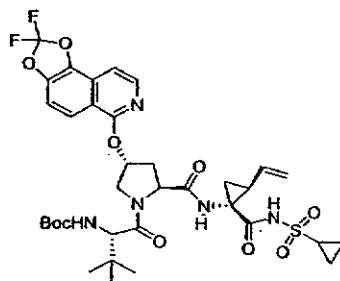
;

700



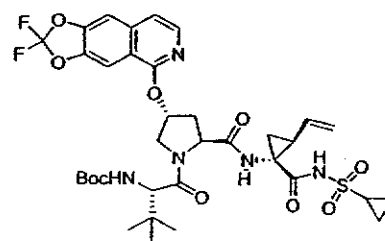
Compound 286

;



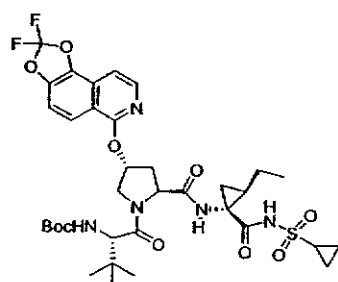
Compound 287

;



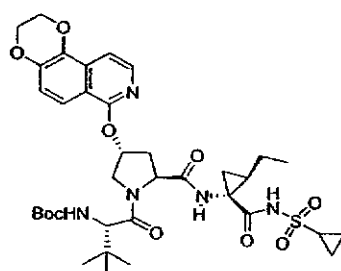
Compound 288

;



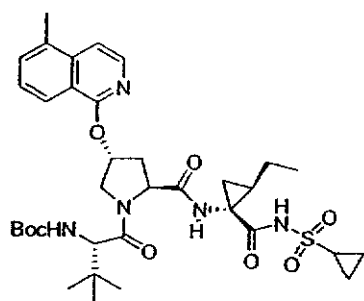
Compound 289

; and



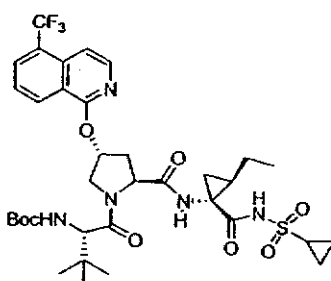
Compound 290

65. A compound selected from



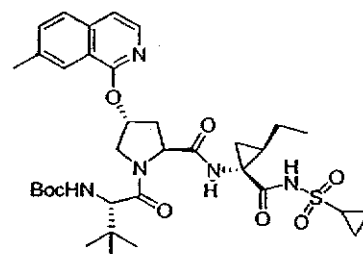
Compound 293

;



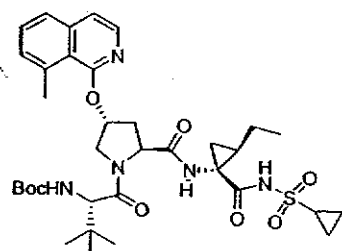
Compound 295

;



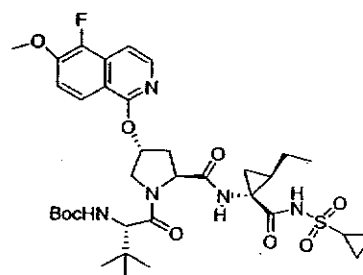
Compound 296

;



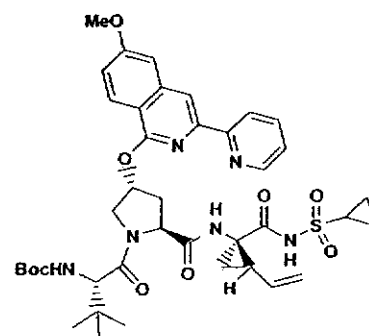
Compound 297

;



Compound 299

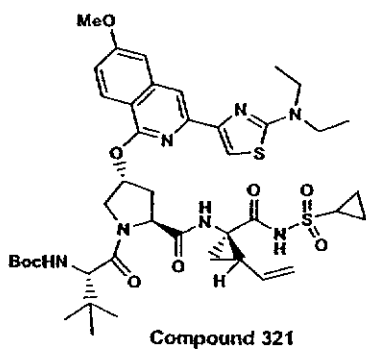
;



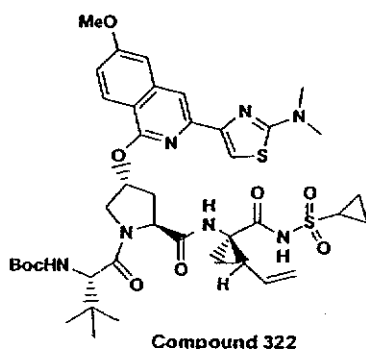
Compound 320

;

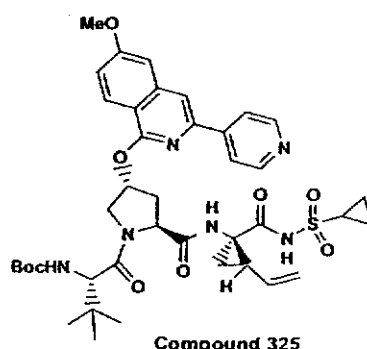
701



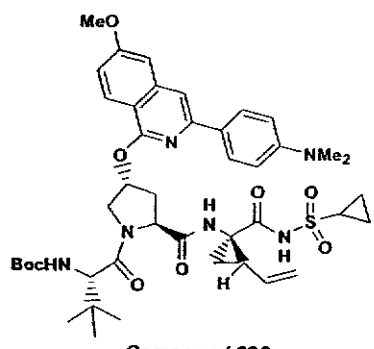
Compound 321



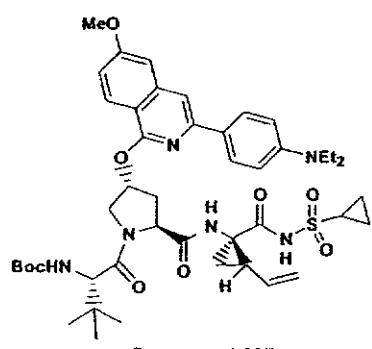
Compound 322



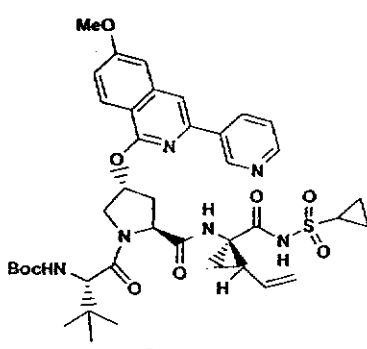
Compound 325



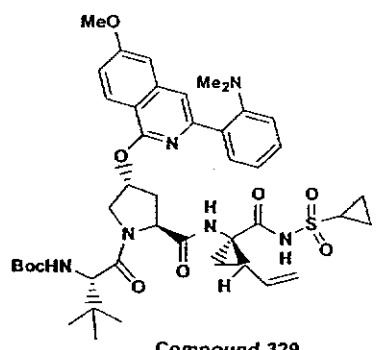
Compound 326



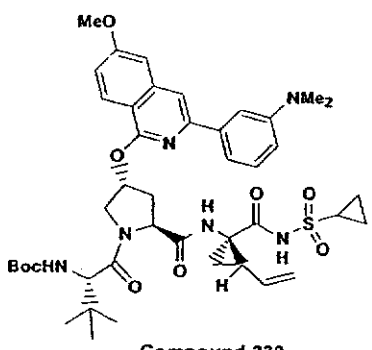
Compound 327



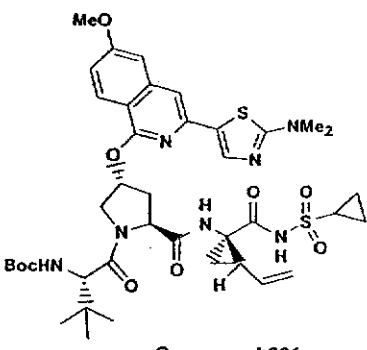
Compound 328



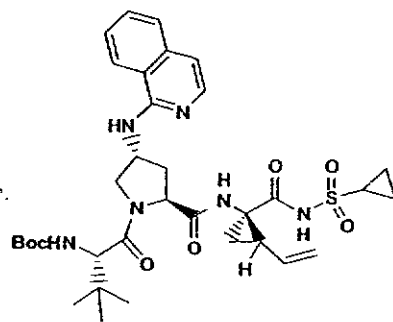
Compound 329



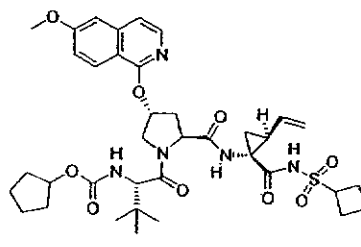
Compound 330



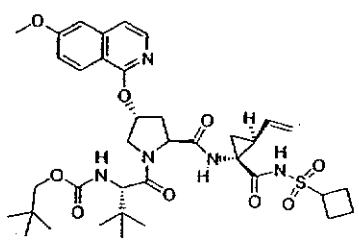
Compound 331



Compound 357

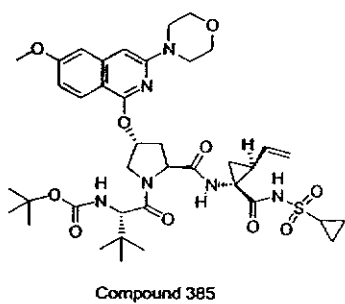
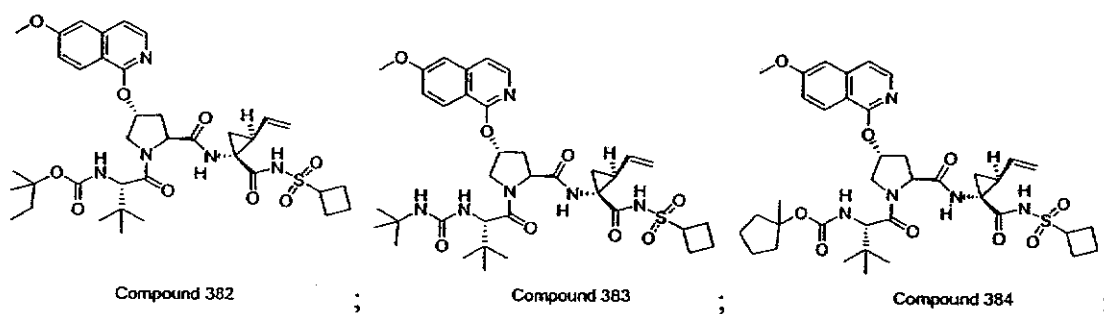


Compound 380

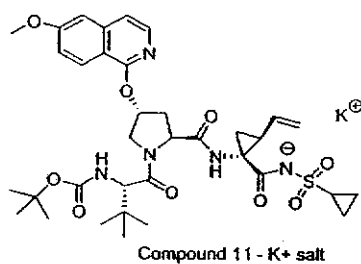


Compound 381

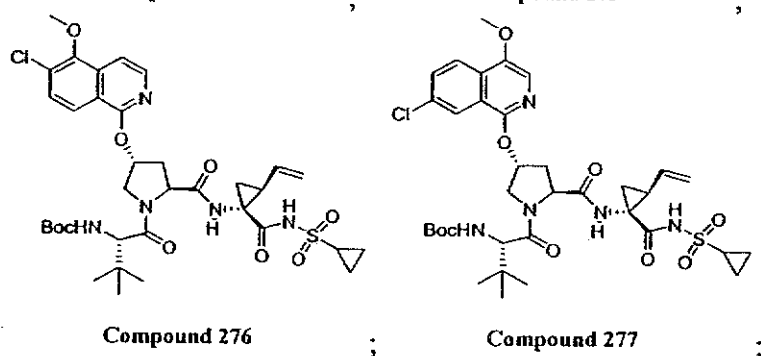
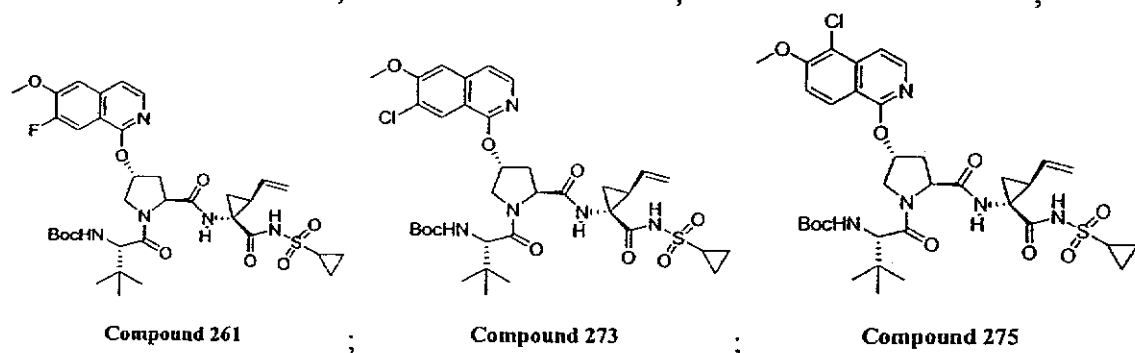
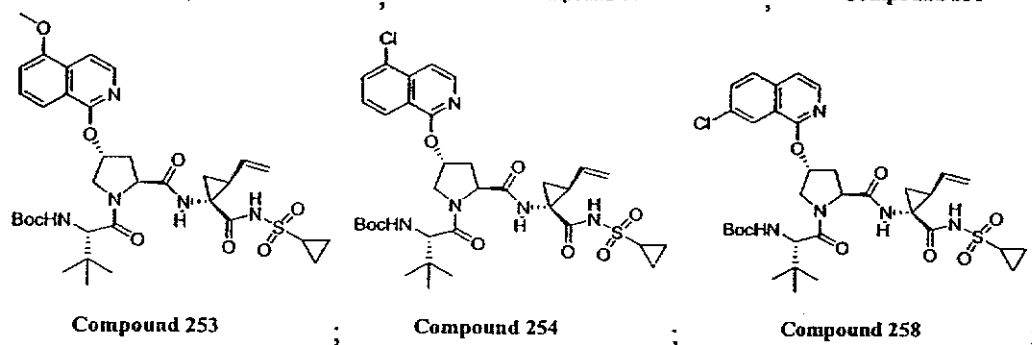
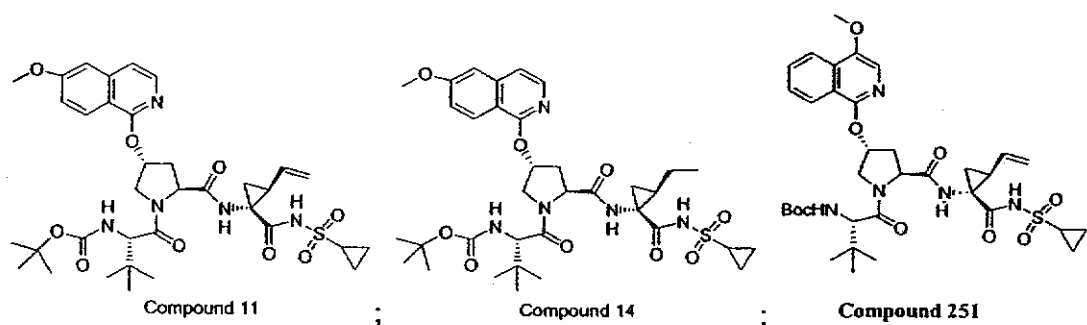
702



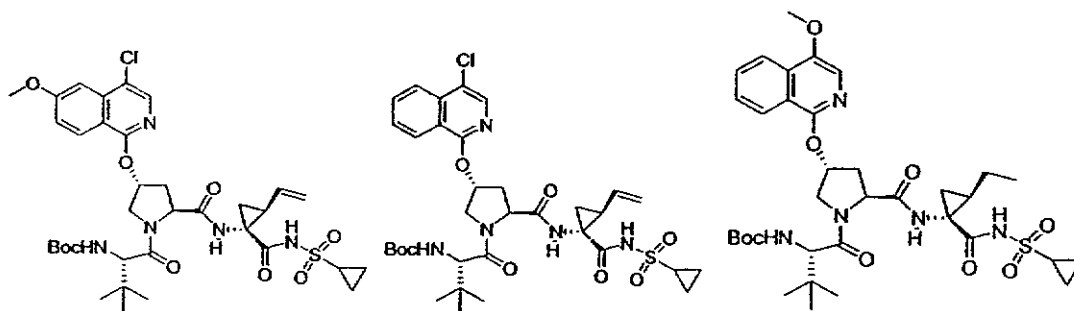
66. A compound which is



67. A compound selected from



704



Compound 278

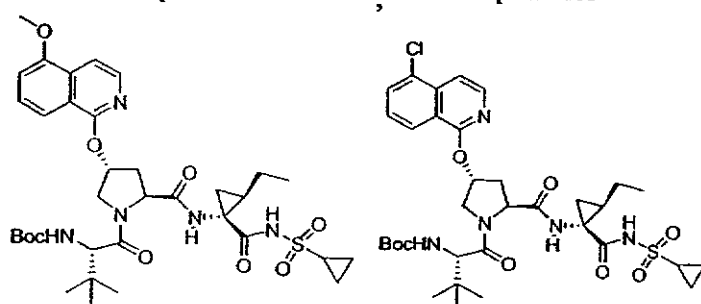
;

Compound 281

;

Compound 291

;



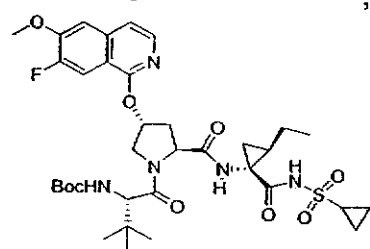
Compound 292

;

Compound 294

;

and



Compound 298