Abstract: The present invention provides compounds of general formula (3) as useful potential antitumour agents against human cancer cell lines. The present invention further provides a process for the synthesis of 4-fluoroor 4-methoxy-3-nitrophenyl, 3-fluoro-4-methoxyphenyl, 2-fluoro-4-methoxyphenyl, 4-fluoro-3-methoxyphenyl, 4-methoxy-3-nitrophenyl, 4-nitrophenyl, 3-nitrophenol, 2-nitro phenol, 3-nitrophenol, 2-methoxyphenol, 3-methoxyphenol and 4-hydroxyphenyl.

(54) Title: SUBSTITUTED 4-BETA-ACRYLAMIDOPODOPHYLLOTOXIN CONGENERS AS ANTITUMOUR ANTIMICROBIALS AND THE PROCESS FOR PREPARATION THEREOF

(57) Abstract: The present invention provides compounds of general formula (3) as useful potential antitumour agents against human cancer cell lines. The present invention further provides a process for the synthesis of 4-fluoro-3-methoxyphenyl, 3-fluoro-4-methoxyphenyl, 2-fluoro-4-methoxyphenyl, 4-fluoro-3-methoxyphenyl, 3-fluoro-4-methoxyphenyl, 2-fluoro-5-methoxyphenyl, 2-fluoro-4-methoxyphenyl, 4-hydroxy-3-nitrophenyl, 4-methoxy-3-nitrophenyl, 4-nitrophenol, 3-nitrophenol, 2-nitro phenol, 3-nitrophenol, 2-methoxyphenol, 3-methoxyphenol and 4-hydroxyphenol.

(74) Agent: DHAWAN, Ramesh; Chander; Lalji Lahiri & Salhotra, Plot No. B-28, Sector-32, Institutional Area, Gurgaon 122 001, Haryana (IN).


(84) Designated States (unless otherwise indicated, for every kind of national protection available): ARIPO (BH, GW, GM, KE, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SL, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
FIELD OF THE INVENTION

The present invention relates to substituted 4P-acrylamidopodophyllotoxin congeners of general formula 3 as antitumour antibiotics. Present invention further relates to a process for the synthesis of 4P-acrylamidopodophyllotoxin congeners of general formula 3,

![Diagram of General formula 3]

wherein R and R1 are an aryl group and R is selected from 3, 4, 5-trimethoxyphenyl or 2-methoxy phenyl and R1 is selected from the group consisting of 4-hydroxy-3-methoxyphenyl, 3-hydroxy-4-methoxyphenyl, 4-fluoro-3-methoxyphenyl, 3-fluoro-4-methoxyphenyl, 2-fluoro-5-methoxyphenyl, 2-fluoro-4-methoxyphenyl, 4-hydroxy-3-nitrophenyl, 4-methoxy-3-nitrophenyl, 4-nitrophenyl, 3-nitrophenyl, 2-nitro phenyl, 4-methoxyphenyl, 3-methoxyphenyl and 4-hydroxyphenyl.

BACK GROUND OF THE INVENTION

Etoposide and tenyposide are semisynthetic podophyllotoxin derivatives that are in clinical usage as anticancer drugs Figure 1 (Chen. Y. Z.; Wang. Y. G.; Tian, X.; Li, J. X. Curr. Sci. 1990, 59, 517; Wang, J. Z.; Tian, X.; Tsumura, H.; Shimura, K.; Ito, H. Anti-cancer Drug Design, 1993, 8, 193). It is believed that analogues of 4'-demethyl epipodophyllotoxin exert their antitumour activity through stabilization of a cleavable complex between DNA and type-II DNA topoisomerase, this leads ultimately to inhibition of DNA catenation activity and produces single and double strand breaks (Satio, H.; Yoshikawa, H.; Nishimura, Y.; Kondo, S.; Takeuchi, T.; Umezawa, H. Chem Pharm. Bull. 1986, 34, 3733; Chen, Y. Z.; Wang, Y. G.; Li, J. X.; Tian, X.; Jia. Z. P.; Zhang, Z. Y. Life Sci. 1989, 45, 2569). A number of studies have been carried out on the structural

Figure-1

OBJECTIVE OF THE INVENTION
The main objective of the invention is to provide substituted 4p-acrylamidopodophyllotoxin congeners as useful antitumour antibiotics.
Another object of the present invention is to provide a process for the synthesis of 4p-acrylamido derivatives of podophyllotoxin as useful anticancer agents.
Another object of the present invention is to provide compounds based on the podophyllotoxin in good yields.

SUMMARY OF THE INVENTION
Accordingly, present invention provides substituted 4p-acrylamidopodophyllotoxin congener
compounds of general formula 3

\[ R \rightarrow R_1 \]

**General formula 3**

wherein \( R \) and \( R_1 \) are an aryl group and \( R \) is selected from 3, 4, 5-trimethoxyphenyl or 2-methoxyphenyl and \( R_1 \) is selected from the group consisting of 4-hydroxy-3-methoxyphenyl, 3-hydroxy-4-methoxyphenyl, 4-fluoro-3-methoxyphenyl, 3-fluoro-4-methoxyphenyl, 2-fluoro-5-methoxyphenyl, 2-fluoro-4-methoxyphenyl, 4-hydroxy-3-nitrophenyl, 4-methoxy-3-nitrophenyl, 4-nitrophenyl, 3-nitrophenyl, 2-nitrophenyl, 4-methoxyphenyl, 3-methoxyphenyl and 4-hydroxyphenyl.

In an embodiment of the present invention, chemical formula of the representative compounds are:

- \( 4p-(\xi)-3-(4-hydroxy-3-methoxyphenyl)-2-(3,4,5\text{-trimethoxyphenyl})\text{acrylamidopodophyllotoxin} \) (3a);
- \( 4p-(\xi)-3-(3-hydroxy-4-methoxyphenyl)-2-(3,4,5\text{-trimethoxyphenyl})\text{acrylamidopodophyllotoxin} \) (3b);
- \( 4p-(\xi)-3-(4-fluoro-3-methoxyphenyl)-2-(3,4,5\text{-trimethoxyphenyl})\text{acrylamidopodophyllotoxin} \) (3c);
- \( 4p-(\xi)-3-(3-fluoro-4-methoxyphenyl)-2-(3,4,5\text{-trimethoxyphenyl})\text{acrylamidopodophyllotoxin} \) (3d);
- \( 4p-(\xi)-3-(2-fluoro-5-methoxyphenyl)-2-(3,4,5\text{-trimethoxyphenyl})\text{acrylamidopodophyllotoxin} \) (3e);
- \( 4p-(\xi)-3-(2-fluoro-4-methoxyphenyl)-2-(3,4,5\text{-trimethoxyphenyl})\text{acrylamidopodophyllotoxin} \) (3f);
- \( 4p-(\xi)-3-(4-hydroxy-3-nitrophenyl)-2-(3,4,5\text{-trimethoxyphenyl})\text{acrylamidopodophyllotoxin} \) (3g);
- \( 4p-(\xi)-3-(4-methoxy-3-nitrophenyl)-2-(3,4,5\text{-trimethoxyphenyl})\text{acrylamidopodophyllotoxin} \) (3h);
- \( 4p-(\xi)-3-(4-nitrophenyl)-2-(3,4,5\text{-trimethoxyphenyl})\text{acrylamidopodophyllotoxin} \) (3i);
- \( 4p-(\xi)-3-(3-nitrophenyl)-2-(3,4,5\text{-trimethoxyphenyl})\text{acrylamidopodophyllotoxin} \) (3j);
- \( 4p-(\xi)-3-(2-nitrophenyl)-2-(3,4,5\text{-trimethoxyphenyl})\text{acrylamidopodophyllotoxin} \) (3k);
- \( 4p-(\xi)-2-(2\text{-methoxyphenyl})-3-(4\text{-nitrophenyl})\text{acrylamidopodophyllotoxin} \) (3l);
- \( 4p-(\xi)-2-(2\text{-methoxyphenyl})-3-(2\text{-nitrophenyl})\text{acrylamidopodophyllotoxin} \) (3m);
- \( 4p-(\xi)-3-(3\text{-methoxyphenyl})-2-(3,4,5\text{-trimethoxyphenyl})\text{acrylamidopodophyllotoxin} \) (3n);
- \( 4p-(\xi)-3-(3\text{-methoxyphenyl})-2-(3,4,5\text{-trimethoxyphenyl})\text{acrylamidopodophyllotoxin} \) (3o);
- \( 4p-(\xi)-3-(4\text{-hydroxyphenyl})-2-(3,4,5\text{-trimethoxyphenyl})\text{acrylamidopodophyllotoxin} \) (3p);
In another embodiment of the present invention, the structural formula of the representative compounds 3a-p are:
In yet another embodiment of the present invention, the said compounds are useful as antitumour antibiotics.

Yet another embodiment of the present invention provides a process for the preparation of 4β-acrylamidopodophyllotoxin congeners of general formula 3 and the said process comprising the steps of:

i. dissolving 4P-acrylamidopodophyllotoxin of formula 1 in a solvent to obtain a solution;
ii. adding aromatic acrylic acid of formula 2a-p wherein R and R1 are an aryl group and R is selected from 3, 4, 5-trimethoxyphenyl or 2-methoxy phenyl and R1 is selected from the group consisting of 4-hydroxy-3-methoxyphenyl, 3-hydroxy-4-methoxyphenyl, 4-fluoro-3-methoxyphenyl, 3-fluoro-4-methoxyphenyl, 2-fluoro-5-methoxyphenyl, 2-fluoro-4-methoxyphenyl, 4-hydroxy-3-nitrophenyl, 4-methoxy-3-nitrophenyl, 4-nitrophenyl, 3-nitrophenyl, 2-nitro phenyl, 4-methoxyphenyl or 3-methoxyphenyl in the solution as obtained in step (i) with coupling reagents and acetic acid followed by stirring at temperature in the range of 25 to 30°C for a period in the range of 2 to 3 h;

\[ COOH \]

\[
\begin{align*}
\text{R} & \quad \text{R1} \\
\end{align*}
\]

2a-p

iii. filtering, washing with saturated solution of NaHCO₃, 10% hydrochloric acid and followed by water;

iv. drying the washed filterate as obtained in step (iii) over anhydrous Na₂SO₄ followed by chromatography through silica gel using an eluent to obtain the pure product.

In yet another embodiment of the present invention, the solvent used is selected from the group consisting of dichloromethane, chloroform and tetrahydrofuran, preferably dichloromethane.

In yet another embodiment of the present invention, the coupling reagents used are EDCI and HOBr both.

In yet another embodiment of the present invention, the eluent used is ethyl acetate/hexane in the ratio ranging between 1:1 to 1:4.

In yet another embodiment of the present invention, said compounds shows in-vitro anticancer activity against human tumour cells derived from five cancer types selected from the group consisting of breast cancer cell line (MCF-7, Zr-75-1), oral cancer cell line (KB, Gurav, DWD), colon cancer cell line (Colo 205), lung cancer cell line (A549, Hop 62) and ovarion cancer cell line (A-2780).

In yet another embodiment of the present invention, concentration of the Substituted 4β-acrylamidopodophyllotoxin congeners of general formula 3 used for in vitro activity against breast cancer cell lines (Zr-75-1) for GI₅₀ is in the range of 0.18-2.7 μM at an exposure period of at least 48 hrs.

In yet another embodiment of the present invention, concentration of the Substituted 4β-
acrylamidopodophyllotoxin congeners of general formula 3 used for *in vitro* activity against breast cancer cell lines (MCF-7) for GI<sub>50</sub> is in the range of 2.4-2.9 µM at an exposure period of at least 48 hrs.

In yet another embodiment of the present invention, concentration of the Substituted 4β-acrylamidopodophyllotoxin congeners of general formula 3 used for *in vitro* activity against oral cancer cell lines (KB) for GI<sub>50</sub> is in the range of 2.1-2.7 µM at an exposure period of at least 48 hrs.

In yet another embodiment of the present invention, concentration of the Substituted 4β-acrylamidopodophyllotoxin congeners of general formula 3 used for *in vitro* activity against oral cancer cell lines (Gurav) for GI<sub>50</sub> is in the range of 0.18-2.7 µM at an exposure period of at least 48 hrs.

In yet another embodiment of the present invention, concentration of the Substituted 4β-acrylamidopodophyllotoxin congeners of general formula 3 used for *in vitro* activity against oral cancer cell lines (DWD) for GI<sub>50</sub> is in the range of <0.1-2.9 µM at an exposure period of at least 48 hrs.

In yet another embodiment of the present invention, concentration of the Substituted 4β-acrylamidopodophyllotoxin congeners of general formula 3 used for *in vitro* activity against colon cancer cell lines (Colo205) for GI<sub>50</sub> is in the range of 0.17-2.7 µM at an exposure period of at least 48 hrs.

In yet another embodiment of the present invention, concentration of the Substituted 4β-acrylamidopodophyllotoxin congeners of general formula 3 used for *in vitro* activity against lung cancer cell lines (A-549) for GI<sub>50</sub> is in the range of <0.1-2.9 µM at an exposure period of at least 48 hrs.

In yet another embodiment of the present invention, concentration of the Substituted 4β-acrylamidopodophyllotoxin congeners of general formula 3 used for *in vitro* activity against lung cancer cell lines (Hop62) for GI<sub>50</sub> is in the range of 0.17-2.9 µM at an exposure period of at least 48 hrs.

In yet another embodiment of the present invention, concentration of the Substituted 4β-acrylamidopodophyllotoxin congeners of general formula 3 used for *in vitro* activity against ovarian cancer cell lines (A-2780) for GI<sub>50</sub> is in the range of <0.1-2.7 µM at an exposure period of at least 48 hrs.
BRIEF DESCRIPTION OF THE DRAWINGS
Scheme 1 discloses the process for the synthesis of new podophyllotoxin analogues as anticancer agents producing the novel derivatives of the podophyllotoxin in good yields. 4β-acrylamidopodophyllotoxin of formula 1 reacts with aromatic acrylic acid of formula 2(a) to 2(e) to yield substituted 4p-acrylamidopodophyllotoxin congener compounds of formula 3(a) to 3(e) respectively.

DETAILED DESCRIPTION OF THE INVENTION
The process of the present invention for the synthesis of C-4p-βV-linked derivatives of podophyllotoxin as anticancer agents produces the novel derivatives of the podophyllotoxin in good yields; where in the key step for the synthesis of these analogues is by C-4p-amino podophyllotoxin, which has been coupled with different types of aromatic acrylic acids to afford the 4p-acrylamido derivatives of podophyllotoxin.

Thus the present invention provides new class of podophyllotoxin analogues, which were synthesized.

In these efforts new 4p-acrylamido derivatives of podophyllotoxin have been synthesized and evaluated for their cytotoxicity and anticancer potency compared to adiramycin. The synthesis of these compounds has been carried out as described in the Scheme 1 using podophyllotoxin.

These new analogues of podophyllotoxin congeners coupled at C-4 position have shown promising anticancer activity in selected human cancer cell lines. This resulted in design and synthesis of new congeners as illustrated in Scheme 1.

Examples
The following examples are given by way of illustration and therefore should not be construed to limit the scope of the present invention.

Example 1
4p-(E)-3-(4-hydroxy-3-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)acrylamidopodophyllotoxin (3a).
4p-aminopodophyllotoxin (formula 1) (200 mg, 0.48 mmol) was dissolved in 20ml of dried dichloromethane, followed by addition of aromatic acrylic acid 2a (220 mg, 0.57 mmol) and EDCI (N-(3-Dimethylaminopropyl)-N'-ethyl-carbodiimide hydrochloride) (108 mg, 0.57 mmol) and catalytic amount of HOBt (1-HydroxyBenztriazole). The reaction mixture was
stirred at 27°C for 3 h. Acetic acid (0.4 ml) was added. The reaction mixture was filtered. The filtrate was washed with saturated solution of NaHCO₃, 10% hydrochloric acid and water respectively, dried over anhydrous Na₂SO₄ and chromatographed through silica gel using ethyl acetate/hexane (4:6) as an eluent to obtain the pure product. Yield 95%. Mp: 134-137°C, [α]₀²⁵ = -12.9 (c = 0.5 in CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ: 2.55-2.63 (dd, 1H, J = 4.5, 4.5 Hz), 2.86-3.00 (m, IH), 3.53 (s, 3H), 3.73 (s, 6H), 3.75 (s, 9H), 3.81 (s, 3H), 4.23-4.31 (m, 1H), 4.39-4.48 (m, 2H), 5.30 (t, 1H), 5.72 (d, 1H, J = 6.7 Hz), 5.95 (d, 2H, J = 3.0 Hz), 6.14-6.24 (m, 3H), 6.34-6.47 (m, 4H), 6.70-6.77 (m, 3H), 7.68 (s, 1H); ¹³C NMR (75 MHz, CDCl₃) δ: 36.8, 40.4, 43.1, 47.8, 54.6, 55.6, 55.8, 59.8, 60.0, 68.6, 101.1, 102.8, 107.2, 108.0, 109.1, 109.2, 109.4, 112.6, 115.0, 121.1, 124.7, 126.0, 130.1, 131.4, 131.6, 132.1, 132.4, 133.3, 135.2, 135.7, 136.2, 137.0, 146.5, 146.7, 147.1, 147.3, 151.9, 153.2, 167.6, 174.5; MS (ESI): 778 [M+Na].

Example 2

4p-(£)-3-(3-hydroxy-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)acrylamido podophyllotoxin (3b). 4p-aminopodophyllotoxin (formula 1) (200 mg, 0.48 mmol) was dissolved in 20 ml of dried dichloromethane, followed by addition of aromatic acrylic acid 2b (220 mg, 0.57 mmol) and EDCI (108 mg, 0.57 mmol) and catalytic amount of HOBt. The reaction mixture was stirred at 28°C for 3 h. Acetic acid (0.4 ml) was added. The reaction mixture was filtered. The filtrate was washed with saturated solution of NaHCO₃, 10% hydrochloric acid and water respectively, dried over anhydrous Na₂SO₄ and chromatographed through silica gel using ethyl acetate/hexane (4:6) as an eluent to obtain the pure product. Yield 97%. Mp: 145-147°C, [α]₀²⁵ = - 6.9 (c = 0.5 in CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ: 2.52-2.63 (dd, 1H, J = 4.6, 4.6 Hz), 2.85-3.02 (m, 1H), 3.75 (s, 9H), 3.78 (s, 3H), 389 (s, 6H), 4.24-4.33 (dd, 1H, J = 4.6, 4.6 Hz), 4.43-4.52 (dd, 2H, J = 6.2, 6.2 Hz), 5.31 (t, 1H), 5.67 (d, 1H, J = 7.8 Hz), 5.97 (d, 2H, J = 4.6 Hz), 6.24 (s, 2H), 6.42 (s, 2H), 6.47 (s, 1H), 6.52-6.70 (m, 3H), 6.75 (s, 1H), 7.68 (s, 1H); ¹³C NMR (75 MHz, CDCl₃) δ: 36.7, 40.4, 43.1, 47.7, 55.3, 55.6, 55.7, 59.8, 60.0, 68.6, 101.1, 107.1, 108.0, 109.1, 109.2, 111.3, 116.6, 121.9, 127.5, 130.2, 131.1, 132.0, 133.5, 134.5, 135.7, 136.2, 137.1, 145.7, 146.5, 147.1, 148.0, 151.9, 152.9, 168.0, 174.5; MS (ESI): 778 [M+Na].
Example 3

4p-(£)-3-(4-fluoro-3-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)acrylamidopodophyllotoxin (3c).

4p-aminopodophyllotoxin (formula 1) (200 mg, 0.48 mmol) was dissolved in 20ml of dried dichloromethane, followed by addition of aromatic acrylic acid 2c (205 mg, 0.57 mmol) and EDCI (108 mg, 0.57 mmol) and catalytic amount of HOBt. The reaction mixture was stirred at 27°C for 3 h. Acetic acid (0.4 ml) was added. The reaction mixture was filtered. The filtrate was washed with saturated solution of NaHCO₃ 10 % hydrochloric acid and water respectively, dried over anhydrous Na₂SO₄ and chromatographed through silica gel using ethyl acetate/hexane (3:7) as an eluent to obtain the pure product. Yield 98 %. Mp: 144-146°C, [α]D²⁵ = -1.7 (c = 0.5 in CHCl₃); ³⁴ NMR (400 MHz, CDCl₃)δ : 2.52-2.63 (dd, 1H, J = 5.1, 5.1 Hz), 2.85-3.01 (m, 1H), 3.53 (s, 3H), 3.74 (s, 6H), 375 (s, 6H), 3.77 (s, 3H), 3.82 (s, 3H), 3.88 (t, 1H), 4.41- 4.50 (dd, 2H, J = 6.6, 5.8 Hz), 5.23- 5.30 (m, 1H), 5.73 (d, 1H, J = 6.6 Hz), 5.95 (d, 2H, J = 3.6 Hz), 6.22 (s, 2H), 6.43 (s, 2H), 6.44 (s, 1H), 6.48- 6.54 (dd, 1H, J = 1.4, 1.4 Hz), 6.72- 6.76 (m, 2H), 6.87- 6.98 (dd, 1H, J = 8.0, 8.0 Hz), 7.70 (s, 1H); ¹³C NMR (75 MHz, CDCl₃)δ : 36.7, 40.3, 43.1, 47.8, 55.1, 55.6, 55.7, 59.7, 59.8, 68.5, 101.1, 107.0, 108.0, 109.1, 109.2, 114.3, 115.4, 115.6, 123.1, 123.2, 130.0, 130.8, 131.8, 132.1, 133.5, 135.7, 135.8, 136.2, 137.2, 146.5, 147.1, 149.4, 151.9, 153.1, 167.4, 174.5; MS (ESI): 758 [M⁺+H].

Example 4

4p-(£)-3-(3-fluoro-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)acrylamidopodophyllotoxin (3d).

4p-aminopodophyllotoxin (formula 1) (200 mg, 0.48 mmol) was dissolved in 20ml of dried dichloromethane, followed by addition of aromatic acrylic acid 2d (205 mg, 0.57 mmol) and EDCI (108 mg, 0.57 mmol) and catalytic amount of HOBt; The reaction mixture was stirred at 29°C for 3 h. Acetic acid (0.4 ml) was added. The reaction mixture was filtered. The filtrate was washed with saturated solution of NaHCO₃ 10 % hydrochloric acid and water respectively, dried over anhydrous Na₂SO₄ and chromatographed through silica gel using ethyl acetate/hexane (3:7) as an eluent to obtain the pure product. Yield 94 %. Mp: 159-160°C, [α]D²⁵ = +13.9 (c = 0.5 in CHCl₃); ³⁴ NMR (400 MHz, CDCl₃)δ : 2.54-2.63 (dd, 1H, J =
2.87- 2.97 (m, 1H), 3.38 (t, 1H), 3.74 (s, 6H), 3.75 (s, 6H), 3.77 (s, 3H), 3.86 (s, 3H), 3.87 (s, 3H), 4.41- 4.49 (m, 2H), 5.26 (t, 1H), 5.74 (d, 1H, J = 6.7 Hz), 5.95 (d, 2H, J = 6.0 Hz), 6.22 (s, 2H), 6.40 (s, 2H), 6.44 (s, 1H), 6.62 (d, 1H, J = 12.8 Hz), 6.73 (s, 1H), 6.76- 6.87 (m, 2H), 7.66 (s, 1H); 13C NMR (75 MHz, CDC13): 36.7, 40.4, 43.1, 47.7, 55.6, 55.7, 59.7, 60.0, 68.5, 101.1, 106.9, 108.0, 109.1, 109.2, 113.2, 116.2, 116.5, 126.9, 127.8, 130.0, 130.7, 132.0, 133.1, 134.9, 135.7, 136.2, 137.3, 146.5, 147.1, 151.9, 152.2, 153.1, 167.5, 174.5; MS (ESI): 758 [M+H].

Example 5

4p-(-)-3-(2-fluoro-5-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)acrylamidopodophyllotoxin (3e).

4p-aminopodophyllotoxin (formula 1) (200 mg, 0.48 mmol) was dissolved in 20 ml of dried dichloromethane, followed by addition of aromatic acrylic acid 2e (205 mg, 0.57 mmol) and EDCI (108 mg, 0.57 mmol) and catalytic amount of HOBt. The reaction mixture was stirred at 27°C for 3 h. Acetic acid (0.4 ml) was added. The reaction mixture was filtered. The filtrate was washed with saturated solution of NaHCO₃, 10% hydrochloric acid and water respectively, dried over anhydrous Na₂SO₄ and chromatographed through silica gel using ethyl acetate/hexane (3:7) as an eluent to obtain the pure product. Yield 98%. Mp: 123-126 °C, [α]D²⁵ = -12.0 (c = 0.5 in CHCl₃); H NMR (400 MHz, CDCl₃) δ: 2.68-2.75 (dd, 1H, J = 5.2, 5.2 Hz), 2.95-3.08 (m, 1H), 3.40 (s, 3H), 3.74 (s, 6H), 375 (s, 6H), 3.80 (s, 3H), 3.84 (s, 3H), 3.90 (t, 1H), 4.49- 4.55 (dd, 2H, J = 60, 60 Hz), 5.32- 5.37 (dd, 1H, J = 4.5, 4.5 Hz), 5.96 (d, 2H, J = 9.0 Hz), 5.99 (d, 1H, J = 7.5 Hz), 6.20-6.25 (m, 1H), 6.28 (s, 2H), 6.45 (s, 2H), 6.48 (s, 1H), 6.72-6.77 (m, 1H), 6.78 (s, 1H), 6.93 (t, 1H), 7.94 (s, 1H); MS (ESI): 758 [M+H].

Example 6

4p-(-)-3-(2-fluoro-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)acrylamidopodophyllotoxin (3f).

4p-aminopodophyllotoxin (formula 1) (200 mg, 0.48 mmol) was dissolved in 20 ml of dried dichloromethane, followed by addition of aromatic acrylic acid 2f (205 mg, 0.57 mmol) and EDCI (108 mg, 0.57 mmol) and catalytic amount of HOBt. The reaction mixture was stirred.
at 30°C for 3 h. Acetic acid (0.4 ml) was added. The reaction mixture was filtered. The filtrate was washed with saturated solution of NaHCO₃, 10% hydrochloric acid and water respectively, dried over anhydrous Na₂SO₄ and chromatographed through silica gel using ethyl acetate/hexane (3:7) as an eluent to obtain the pure product. Yield 96%. Mp: 123-125°C. [α]D²⁵ = +4.9 (c = 0.5 in CHCl₃); ¹H NMR (400 MHz, CDCl₃)δ : 2.61-2.66 (dd, 1H, J = 4.7, 4.7 Hz), 2.94-3.03 (m, 1H), 3.73 (s, 6H), 3.75 (s, 6H), 3.77 (s, 3H), 3.81 (s, 3H), 3.89 (s, 3H), 3.90-3.92 (m, 1H), 4.49-4.55 (m, 2H), 5.30-5.34 (m, 1H), 5.73 (d, 1H, J = 6.2 Hz), 5.96 (d, 2H, J = 11.7 Hz), 6.27 (s, 2H), 6.37-6.41 (m, 1H), 6.42 (s, 2H), 6.49 (s, 1H), 6.56-6.61 (m, 2H), 6.74 (s, 1H), 7.96 (s, 1H); ¹³C NMR (75 MHz, CDCl₃)δ : 36.7, 40.2, 40.3, 43.1, 47.8, 55.9, 59.8, 59.9, 68.6, 101.0, 101.2, 101.4, 106.6, 107.0, 108.0, 109.3, 110.3, 114.8, 115.0, 125.9, 130.1, 130.4, 130.7, 132.1, 135.7, 136.2, 137.2, 146.5, 147.1, 151.9, 152.9, 160.8, 162.8, 167.6, 174.5; MS (ESI): 758 [M⁺+H].

Example 7

4p-(E)-3-(4-hydroxy-3-isothienyl)-2-(3,4,5-trimethoxyphenyl)acrylamidopodophyllotoxin (3g).

4p-aminopodophyllotoxin (formula 1) (200 mg, 0.48 mmol) was dissolved in 20 ml of dried dichloromethane, followed by addition of aromatic acetic acid 2g (214 mg, 0.57 mmol) and EDCI (108 mg, 0.57 mmol) and catalytic amount of HOBt. The reaction mixture was stirred at 27°C for 3 h. Acetic acid (0.4 ml) was added. The reaction mixture was filtered. The filtrate was washed with saturated solution of NaHCO₃, 10% hydrochloric acid and water respectively, dried over anhydrous Na₂SO₄ and chromatographed through silica gel using ethyl acetate/hexane (4:6) as an eluent to obtain the pure product/Yield 98%. Mp: 184-187°C. [α]D²⁵ = -8.9 (c = 0.5 in CHCl₃); ¹H NMR (400 MHz, CDCl₃)δ : 2.54-2.63 (dd, 1H, J = 4.5, 4.5 Hz), 2.88-3.02 (m, 1H), 3.74 (s, 6H), 3.76 (s, 6H), 3.77 (s, 3H), 3.88 (s, 3H), 4.06-4.13 (m, 1H), 4.24-4.31 (dd, 1H, J = 3.7, 4.5 Hz), 4.41-4.49 (m, 2H), 5.30 (t, 1H), 5.78 (d, 1H, J = 6.7 Hz), 5.96 (d, 2H, J = 4.5 Hz), 6.22 (s, 2H), 6.40 (s, 2H), 6.43 (s, 1H), 6.73 (s, 1H), 7.00 (d, 1H, J = 9.0 Hz), 7.28 (d, 1H, J = 9.0 Hz), 7.70 (s, 1H), 7.74 (s, 1H); ¹³C NMR (75 MHz, CDCl₃)δ : 36.7, 40.4, 43.1, 47.7, 55.6, 55.8, 59.8, 60.0, 101.1, 106.9, 108.0, 109.1, 109.2, 118.7, 126.1, 126.2, 130.0, 130.4, 132.1, 135.7, 136.3, 136.4, 137.5, 146.5, 147.1, 151.9, 153.2, 167.4, 174.5; MS (ESI): 794 [M⁺+Na].
Example 8

4p-(E)-3-(4-methoxy-3-nitrophenyl)-2-(3,4,5-trimethoxyphenyl)acrylamidopodophyllotoxin (3h).

4p-aminopodophyllotoxin (formula 1) (200 mg, 0.48 mmol) was dissolved in 20ml of dried dichloromethane, followed by addition of aromatic acrylic acid 2h (221 mg, 0.57 mmol) and EDCI (108 mg, 0.57 mmol) and catalytic amount of HOBr. The reaction mixture was stirred at 27°C for 3 h. Acetic acid (0.4 ml) was added. The reaction mixture was filtered. The filtrate was washed with saturated solution of NaHCO₃, 10 % hydrochloric acid and water respectively, dried over anhydrous Na₂SO₄ and chromatographed through silica gel using ethyl acetate/hexane (3:7) as an eluent to obtain the pure product. Yield 97 %. Mp: 140-143°C, [a]D$_{25}^0$ = -23.9 (c = 0.5 in CHCl$_3$); ³⁄ NMR (400 MHz, CDC$_1$)$_3$δ : 2.56-2.63 (dd, 1H, J=5.3, 5.3 Hz), 2.87- 3.00 (m, 1H), 3.36 (t, 1H, J=7.0, 7.0 Hz), 3.73 (s, 6H), 3.75 (s, 6H), 3.76 (s, 3H), 3.94 (s, 3H), 3.95 (s, 3H), 4.39- 4.46 (m, 2H), 5.25 (t, 1H, J = 5.3, 6.2 Hz), 5.81 (d, 1H, J = 6.2 Hz), 5.94 (d, 2H, J = 5.3 Hz), 6.20 (s, 2H), 6.38 (s, 2H), 6.71 (s, 1H), 6.91 (d, 1H, J = 7.9 Hz), 7.25 (s, 1H), 7.27 (d, 1H, J = 7.9 Hz), 7.34 (s, 1H), 7.68 (s, 1H); ¹³C NMR (75 MHz, CDC$_1$)$_3$δ : 36.7, 43.1, 46.5, 47.8, 48.5, 55.7, 55.8, 56.7, 59.8, 60.0, 101.2, 106.9, 108.1, 109.2, 109.3, 114.0, 125.8, 127.4, 130.0, 130.4, 131.8, 132.1, 135.7, 136.2, 136.3, 137.6, 138.6, 146.5, 147.1, 151.6, 151.9, 153.3, 167.4, 169.1, 174.4; MS (ESI): 785 [M⁺+H].

Example 9

4p-(E)-3-(4-nitrophenyl)-2-(3,4,5-trimethoxyphenyl)acrylamidopodophyllotoxin (3i).

4p-aminopodophyllotoxin (formula 1) (200 mg, 0.48 mmol) was dissolved in 20ml of dried dichloromethane, followed by addition of aromatic acrylic acid 2i (204 mg, 0.57 mmol) and EDCI (108 mg, 0.57 mmol) and catalytic amount of HOBr. The reaction mixture was stirred at 27°C for 3 h. Acetic acid (0.4 ml) was added. The reaction mixture was filtered. The filtrate was washed with saturated solution of NaHCO₃, 10 % hydrochloric acid and water respectively, dried over anhydrous Na₂SO₄ and chromatographed through silica gel using ethyl acetate/hexane (2:8) as an eluent to obtain the pure product. Yield 98 %. Mp: 187-190°C, [a]D$_{25}^0$ = +7.0 (c = 0.5 in CHCl$_3$); ³⁄ NMR (400 MHz, CDC$_1$)$_3$δ : 2.56-2.68 (dd, 1H, J = 5.1, 5.1 Hz), 2.91-3.10 (m, 1H), 3.73 (s, 6H), 3.75 (s, 6H), 381 (s, 3H), 3.91 (s, 3H), 4.25-4.35 (m, 1H), 4.46- 4.57 (m, 2H), 5.35 (t, 1H), 5.82 (d, 1H, J = 7.3 Hz), 5.97 (d, 2H, J = 5.1).
Hz), 6.27 (s, 2H), 6.38 (s, 2H), 6.73 (s, 1H), 7.22 (d, 2H, J = 8.8 Hz), 7.86 (s, 1H), 8.07 (d, 2H, J = 8.8 Hz); 1H NMR (75 MHz, CDCl₃) δ: 36.6, 43.0, 47.7, 55.6, 59.8, 60.0, 68.5, 101.2, 107.0, 109.0, 109.2, 123.1, 129.9, 130.5, 131.7, 132.1, 135.7, 136.2, 137.5, 138.0, 139.9, 142.5, 146.2, 146.5, 147.1, 147.5, 151.9, 152.9, 167.5, 174.5; MS (ESI): 777 [M+Na].

Example 10

4p-(E)-3-(3-nitrophenyl)-2-(3,4,5-trimethoxyphenyl)acrylamido podophyllotoxin (3j).

4p-aminopodophyllotoxin (formula 1) (200 mg, 0.48 mmol) was dissolved in 20 ml of dried dichloromethane, followed by addition of aromatic acrylic acid 2j (204 mg, 0.57 mmol) and EDCI (108 mg, 0.57 mmol) and catalytic amount of HOBt. The reaction mixture was stirred at 27°C for 3 h. Acetic acid (0.4 ml) was added. The reaction mixture was filtered. The filtrate was washed with saturated solution of NaHCO₃, 10 % hydrochloric acid and water respectively, dried over anhydrous Na₂SO₄ and chromatographed through silica gel using ethyl acetate/hexane (2:8) as an eluent to obtain the pure product. Yield 98 %. Mp: 181-184°C, [α]D²⁵ = + 8.5 (c = 0.5 in CHCl₃); 1H NMR (400 MHz, CDCl₃) δ: 2.54-2.62 (dd, 1H, J = 5.0, 5.0 Hz), 2.90-3.03 (m, 1H), 3.73 (s, 6H), 3.74 (s, 6H), 3.77 (s, 3H), 3.88 (s, 3H), 3.89-3.90 (m, 1H), 4.43-4.50 (m, 2H), 5.25-5.30 (m, 1H), 5.82 (d, 1H, J = 6.9 Hz), 5.94-5.98 (dd, 2H, J = 1.1, 1.1 Hz), 6.22 (s, 2H), 6.38 (s, 2H), 6.45 (s, 1H), 6.73 (s, 1H), 7.40-7.43 (m, 2H), 7.77-7.81 (m, 2H), 8.04-8.09 (m, 1H); MS (ESI): 755 [M+H].

Example 11

4p-(E)-3-(2-nitrophenyl)-2-(3,4,5-trimethoxyphenyl)acrylamido podophyllotoxin (3k).

4p-aminopodophyllotoxin (formula 1) (200 mg, 0.48 mmol) was dissolved in 20 ml of dried dichloromethane, followed by addition of aromatic acrylic acid 2k (204 mg, 0.57 mmol) and EDCI (108 mg, 0.57 mmol) and catalytic amount of HOBt. The reaction mixture was stirred at 26°C for 3 h. Acetic acid (0.4 ml) was added. The reaction mixture was filtered. The filtrate was washed with saturated solution of NaHCO₃, 10 % hydrochloric acid and water respectively, dried over anhydrous Na₂SO₄ and chromatographed through silica gel using ethyl acetate/hexane (3:7) as an eluent to obtain the pure product. Yield 97 %. Mp: 197-200°C, [α]D²⁵ = +1.7 (c = 0.5 in CHCl₃); 1H NMR (400 MHz, CDCl₃) δ: 2.61-2.69 (dd, 1H, J = 4.5, 4.5 Hz), 2.91-3.04 (m, 1H), 3.60 (s, 6H), 3.74 (s, 6H), 3.76 (s, 3H), 3.77 (s, 3H), 4.05-
4.14 (m, 1H), 4.23- 4.31 (m, 1H), 4.45- 4.54 (m, 2H), 5.35 (t, 1H), 5.88 (d, 1H, J = 6.7 Hz), 5.96 (d, 2H, J = 8.3 Hz), 6.22 (s, 2H), 6.25 (s, 2H), 6.47 (s, 1H), 6.82 (s, 1H), 6.93 (t, 1H), 7.35- 7.40 (m, 2H), 7.93 (s, 1H), 8.03 (t, 1H); 1H NMR (75 MHz, CDCl₃) δ : 36.6, 40.2, 40.3, 43.0, 47.7, 55.6, 55.6, 59.3, 64.5, 68.5, 101.2, 107.5, 108.0, 109.2, 124.2, 128.8, 129.6, 129.9, 131.3, 131.4, 132.1, 132.2, 133.3, 135.7, 136.2, 137.1, 138.3, 146.5, 147.1, 147.8, 151.9, 167.2, 174.4; MS (ESI): 755 [M+H].

Example 12

4p-(E)-2-(2-methoxyphenyl)-3-(4-nitrophenyl)acrylamidopodophyllotoxin (31).

4p-aminopodophyllotoxin (formula 1) (200 mg, 0.48 mmol) was dissolved in 20ml of dried dichloromethane, followed by addition of aromatic acrylic acid 21 (170 mg, 0.57 mmol) and EDCI (108 mg, 0.57 mmol) and catalytic amount of HOBT. The reaction mixture was stirred at 27°C for 3 h. Acetic acid (0.4 ml) was added. The reaction mixture was filtered. The filtrate was washed with saturated solution of NaHCO₃, 10% hydrochloric acid and water respectively, dried over anhydrous Na₂SO₄ and chromatographed through silica gel using ethyl acetate/hexane (2:8) as an eluent to obtain the pure product. Yield 98%. Mp: 181-183 °C, [α]₂°₀ = - 4.5 (c = 0.5 in CHCl₃); 1H NMR (400 MHz, CDCl₃) δ : 4.43-2.50 (dd, 1H, J = 4.7, 4.7 Hz), 2.88-3.01 (m, 1H), 3.74 (s, 6H), 3.77 (s, 3H), 383 (s, 3H), 4.06- 4.13 (m, 1H), 4.24- 4.30 (dd, 1H, J = 3.3, 3.3 Hz), 4.42- 4.49 (m, 2H), 5.61 (d, 1H, J = 6.7 Hz), 5.95 (s, 2H), 6.20 (s, 2H), 6.42 (s, 1H), 6.69 (s, 1H), 6.92- 7.02 (m, 3H), 7.12 (d, 2H, J = 8.8 Hz), 7.41 (t, 1H), 7.83 (s, 1H), 8.02 (d, 2H, J = 8.8 Hz); 13C NMR (75 MHz, CDCl₃) δ : 36.6, 43.1, 47.1, 55.2, 55.6, 59.3, 64.5, 68.3, 101.1, 108.0, 109.0, 109.3, 111.5, 119.2, 120.7, 123.3, 124.0, 130.3, 131.3, 132.0, 132.1, 135.7, 136.2, 137.9, 139.2, 142.4, 146.2, 146.5, 147.1, 151.9, 157.0, 174.6; MS (ESI): 696 [M+H].

Example 13

4p-(E)-2-(2-methoxyphenyl)-3-(2-nitrophenyl)acrylamidopodophyllotoxin (3m).

4p-aminopodophyllotoxin (formula 1) (200 mg, 0.48 mmol) was dissolved in 20ml of dried dichloromethane, followed by addition of aromatic acrylic acid 2m (170 mg, 0.57 mmol) and EDCI (108 mg, 0.57 mmol) and catalytic amount of HOBT. The reaction mixture was stirred at 27°C for 3 h. Acetic acid (0.4 ml) was added. The reaction mixture was filtered. The filtrate was washed with saturated solution of NaHCO₃, 10% hydrochloric acid and water.
respectively, dried over anhydrous Na₂SO₄ and chromatographed through silica gel using ethyl acetate/hexane (3:7) as an eluent to obtain the pure product. Yield 98%. Mp: 187-190°C, [α]D²⁵ = - 59.9 (c = 0.5 in CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ : 2.47-2.54 (dd, 1H, J = 5.2, 2.2 Hz), 2.86-3.00 (m, 1H), 3.73 (s, 6H), 3.73 (s, 6H), 3.76 (s, 3H), 3.83 (s, 3H), 4.02 (t, 1H, J = 9.8, 9.8 Hz), 5.94 (d, 2H, J = 3.0 Hz), 6.20 (s, 2H), 6.39 (s, 1H), 6.76 (s, 1H), 6.80-6.92 (m, 3H), 7.20-7.33 (m, 5H), 8.03 (s, 1H); ¹³C NMR (75 MHz, CDCl₃): δ : 36.6, 40.5, 43.1, 47.1, 55.2, 55.7, 59.8, 68.3, 101.2, 108.0, 108.9, 109.3, 111.0, 120.3, 124.3, 128.9, 129.8, 130.3, 131.2, 131.5, 131.6, 131.8, 132.0, 133.2, 135.7, 136.2, 136.4, 146.5, 147.1, 147.7, 151.9, 157.2, 167.6, 174.6; MS (ESI): 695 [M⁺+H].

Example 14

4p-(E)-3-(4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)acrylamidopodophyllotoxin (3n).

4p-aminopodophyllotoxin (formula 1) (200 mg, 0.48 mmol) was dissolved in 20ml of dried dichloromethane, followed by addition of aromatic acrylic acid 2n (196 mg, 0.57 mmol) and EDCI (108 mg, 0.57 mmol) and catalytic amount of HOBT. The reaction mixture was stirred at 27°C for 3 h. Acetic acid (0.4 ml) was added. The reaction mixture was filtered. The filtrate was washed with saturated solution of NaHCO₃, 10% hydrochloric acid and water respectively, dried over anhydrous Na₂SO₄ and chromatographed through silica gel using ethyl acetate/hexane (3:7) as an eluent to obtain the pure product. Yield 98%. Mp: 128-130°C, [α]D²⁵ = -0.9 (c = 0.5 in CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ : 2.55-2.66 (dd, 1H, J = 4.4, 5.1 Hz), 2.87-3.07 (m, 1H), 3.74 (s, 12H), 3.78 (s, 3H), 380 (s, 3H), 3.91 (s, 3H), 4.25-4.34 (m, 1H), 4.45-4.55 (m, 2H), 5.32 (t, 1H), 5.69 (d, 1H, J = 6.6 Hz), 5.96 (d, 2H, J = 4.4 Hz), 6.26 (s, 2H), 6.43 (s, 2H), 6.48 (s, 1H), 6.69-6.74 (m, 3H), 6.99 (d, 2H, J = 8.8 Hz), 7.78 (s, 1H); ¹³C NMR (75 MHz, CDCl₃): δ : 36.7, 40.2, 40.3, 43.1, 47.7, 55.0, 55.6, 59.7, 59.9, 68.6, 101.1, 104.1, 107.0, 108.0, 109.2, 113.5, 127.2, 130.1, 131.2, 132.0, 133.6, 134.2, 135.7, 136.2, 137.0, 146.5, 147.1, 151.9, 153.0, 159.2, 167.8, 174.5; MS (ESI): 740 [M⁺+H].

Example 15

4p-(E)-3-(3-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)acrylamidopodophyllotoxin (3o).

4p-aminopodophyllotoxin (formula 1) (200 mg, 0.48 mmol) was dissolved in 20ml of dried dichloromethane, followed by addition of aromatic acrylic acid 2o (196 mg, 0.57 mmol) and
EDCI (108 mg, 0.57 mmol) and catalytic amount of HOBt. The reaction mixture was stirred at 25 °C for 3 h. Acetic acid (0.4 ml) was added. The reaction mixture was filtered. The filtrate was washed with saturated solution of NaHCO₃, 10 % hydrochloric acid and water respectively, dried over anhydrous Na₂SO₄ and chromatographed through silica gel using ethyl acetate/hexane (3:7) as an eluent to obtain the pure product, yield 98 %. Mp: 187-190 °C, [α]D²⁵ = - 6.9 (c = 0.5 in CHCl₃); 3/4 NMR (400 MHz, CDCl₃) δ: 2.51-2.58 (dd, 1H, J = 4.8, 4.8 Hz), 2.85- 3.00 (m, 1H), 3.71 (s, 3H), 3.72 (s, 6H), 3.75 (s, 6H), 3.76 (s, 3H), 3.86 (s, 3H), 4.04- 4.10 (m, 1H), 4.24- 4.28 (dd, 1H, J = 4.0, 4.8 Hz), 4.43- 4.46 (m, 2H), 5.66 (d, 1H, J = 6.4 Hz), 5.93 (d, 2H, J = 8.0 Hz), 6.20 (s, 2H), 6.36-6.44 (m, 4H), 6.66 (d, 1H, J = 8.8 Hz), 6.72 (s, 1H), 6.93 - 7.02 (m, 2H), 7.70 (s, 1H); 13C NMR (75 MHz, CDCl₃) δ: 36.8, 40.4, 43.1, 47.8, 55.0, 55.7, 55.8, 59.8, 60.0, 68.6, 101.2, 106.5, 107.0, 108.0, 109.2, 109.3, 113.5, 113.7, 127.3, 130.1, 131.2, 131.3, 132.0, 132.1, 132.2, 133.7, 134.2, 135.7, 136.2, 137.1, 138.5, 146.5, 147.1, 151.9, 153.1, 159.2, 159.9, 167.9, 174.6; MS (ESI): 740 [M⁺+H].

Example 16

4p-(E)-3-(4-hydroxyphenyl)-2-(3,4,5-trimethoxyphenyl)acrylamidopodophyllDtoxiri (3p).

4P-aminopodophyllotoxin (formula 1) (200 mg, 0.48 mmol) was dissolved in 20ml of dried dichloromethane, followed by addition of aromatic acrylic acid 2p (188 mg, 0.57 mmol) and EDCI (108 mg, 0.57 mmol) and catalytic amount of HOBt. The reaction mixture was stirred at 27°C for 3 h. Acetic acid (0.4 ml) was added. The reaction mixture was filtered. The filtrate was washed with saturated solution of NaHCO₃, 10 % hydrochloric acid and water respectively, dried over anhydrous Na₂SO₄ and chromatographed through silica gel using ethyl acetate/hexane (1:1) as an eluent to obtain the pure product. Yield 98 %. Mp: 137-140 °C, [α]D²⁵ = - 4.9 (c = 0.5 in CHCl₃); 3/4 NMR (400 MHz, CDCl₃) δ: 2.52-2.64 (dd, 1H, J = 5.1, 5.1 Hz), 2.84-3.03 (m, 1H), 3.71 (s, 6H), 3.74 (s, 6H), 3.77 (s, 3H), 3.85 (s, 3H), 4.23- 4.32 (m, 1H), 4.40- 4.51 (m, 2H), 5.29 (t, 1H), 5.76 (d, 1H, J = 6.6 Hz), 5.96 (d, 2H, J = 2.9 Hz), 6.22 (s, 2H), 6.38 (s, 2H), 6.44 (s, 1H), 6.75 (s, 1H), 6.91 (d, 2H, J = 8.8 Hz), 7.03 (d, 2H, J = 8.8 Hz); MS (ESI): 764 [M⁺+K].

Example 17

Biological Activity

In vitro evaluation of anticancer activity

Compounds 3a-p have been evaluated for their in vitro cytotoxicity in selected human
cancer cell lines i.e., colon (Colo205), lung (Hop-62, A549), oral (KB, DWD, Gurav), Ovarian (A-2780) and breast (MCF7, Zr-75-1) origin by employing the sulforhodamine B (SRB) assay method (Skehn, P.; Storeng, R.; Scudiero, A.; Monks, J.; McMohan, D.; Vistica, D.; Jonathan, T. W.; Bokesch, H.; Kenney, S.; Boyd M. R. J. Natl. Cancer Inst. 1990, 82, 1107). The results are summarized with podophyllotoxin and standard drug Adriamycin in Table 1. All the new compounds were significantly cytotoxic towards the colon, breast, lung, oral and ovarian cell lines compared to the standard drug tested, with the concentration of the drug that produced 50% inhibition of cell growth (GI50).

10 Procedure of the SRB-assay

Single cell suspension of the tumour cells grown in tissue culture were made, cells counted and cell count adjusted to 1×10^5 to 5×10^5 cells/ml. Ninety six (96) well plates were seeded with this cell suspension, each well receiving 100 µl of it. The plate was then be incubated at 37 ºC temperature in CO2 incubator for 24 hours. Drugs were added at concentrations after 24-hour incubation followed by further incubation for 48 hours. Experiment was terminated by gently layering the cells in the wells with 30% TCA and plates were kept in refrigerator for 1 hour following which they were washed thoroughly with tap water, dried attained with 0.4% SRB in 1% acetic acid and finally, the bound SRB eluted with 0.1M tris. Absorbance was read at 540 nm, in the microtitre-plate reader. Optical density of drug-treated cells was compared with that of control cells and cell inhibition was calculated as percent values. Each compound was tested at 10, 20, 40 and 80 µg/ml in triplicate on human malignant cell lines.

Table 1. In vitro anticancer activity (GI50 µM) data for some representative compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>Breast</th>
<th>Oral</th>
<th>Colon</th>
<th>Lung</th>
<th>Ovarian</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zr-75-1</td>
<td>MCF7</td>
<td>KB</td>
<td>Gurav</td>
<td>DWD</td>
</tr>
<tr>
<td>3a</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.4</td>
<td>2.5</td>
</tr>
<tr>
<td>3b</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.9</td>
<td>2.9</td>
</tr>
<tr>
<td>3c</td>
<td>2.2</td>
<td>-</td>
<td>-</td>
<td>2.4</td>
<td>-</td>
</tr>
<tr>
<td>3d</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3e</td>
<td>2.7</td>
<td>-</td>
<td>2.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3f</td>
<td>2.5</td>
<td>2.4</td>
<td>2.6</td>
<td>0.18</td>
<td>0.19</td>
</tr>
<tr>
<td>3g</td>
<td>0.18</td>
<td>2.7</td>
<td>2.2</td>
<td>0.19</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>3h</td>
<td>2.2</td>
<td>-</td>
<td>2.6</td>
<td>2.9</td>
<td>2.7</td>
</tr>
<tr>
<td>3i</td>
<td>2.2</td>
<td>-</td>
<td>2.1</td>
<td>2.4</td>
<td>2.1</td>
</tr>
</tbody>
</table>
Table:

<table>
<thead>
<tr>
<th></th>
<th>2.7</th>
<th>2.1</th>
<th>&lt;0.1</th>
<th>2.4</th>
<th>0.17</th>
<th>2.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>3i</td>
<td>0.11</td>
<td>0.13</td>
<td>0.13</td>
<td>0.13</td>
<td>0.13</td>
<td>0.13</td>
</tr>
<tr>
<td>3k</td>
<td>2.5</td>
<td>2.7</td>
<td>2.7</td>
<td>2.7</td>
<td>2.7</td>
<td>2.7</td>
</tr>
<tr>
<td>3l</td>
<td>2.1</td>
<td>2.4</td>
<td>2.4</td>
<td>2.4</td>
<td>2.4</td>
<td>2.4</td>
</tr>
<tr>
<td>3m</td>
<td>2.2</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>3n</td>
<td>2.9</td>
<td>2.9</td>
<td>2.9</td>
<td>2.9</td>
<td>2.9</td>
<td>2.9</td>
</tr>
<tr>
<td>3o</td>
<td>0.18</td>
<td>2.1</td>
<td>2.1</td>
<td>2.1</td>
<td>2.1</td>
<td>2.1</td>
</tr>
<tr>
<td>3p</td>
<td>2.7</td>
<td>2.7</td>
<td>2.7</td>
<td>2.7</td>
<td>2.7</td>
<td>2.7</td>
</tr>
<tr>
<td>ADR</td>
<td>0.11</td>
<td>0.13</td>
<td>0.13</td>
<td>0.13</td>
<td>0.13</td>
<td>0.13</td>
</tr>
</tbody>
</table>

ADR = adriamycin is the control drug

Etoposide = GI50 µM range 0.80-116 (values from NCI database)

In conclusion, the main advantages of the present inventions are that these new 4β-acrylamidopodophyllotoxin congeners of podophyllotoxin have exhibited promising in vitro cytotoxic activity. Further, these compounds have been prepared from podophyllotoxin upon coupling with a variety of substituted-acrylic acids in the presence of EDCI and HOBr at room temperature (25 to 30°C) provides the new 4p-acrylamidopodophyllotoxin congeners in very good yields.

ADVANTAGES OF THE INVENTION

1. The present invention provides 4p-acrylamidopodophyllotoxin congeners useful as antitumour agents.

2. It also provides a process for the preparation of new 4p-acrylamidopodophyllotoxin congeners useful as antitumour agents.
We claim

1. Substituted 4p-acrylamidopodophyllotoxin congener compounds of general formula 3

\[
\begin{align*}
\text{Formula 3}
\end{align*}
\]

wherein R and R1 are an aryl group and R is selected from 3, 4, 5-trimethoxyphenyl or 2-methoxy phenyl and R1 is selected from the group consisting of 4-hydroxy-3-methoxyphenyl, 3-hydroxy-4-methoxyphenyl, 4-fluoro-3-methoxyphenyl, 3-fluoro-4-methoxyphenyl, 2-fluoro-5-methoxyphenyl, 2-fluoro-4-methoxyphenyl, 4-hydroxy-3-nitrophenyl, 4-methoxy-3-nitrophenyl, 4-nitrophenyl, 3-nitrophenyl, 2-nitro phenyl, 4-methoxyphenyl, 3-methoxyphenyl and 4-hydroxyphenyl.

2. The compounds as claimed in claim 1, wherein chemical formulae of the representative compounds are:

- 4p-(£)-3-(4-hydroxy-3-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)acrylamidopodophyllotoxin (3a);
- 4p-(E)-3-(3-hydroxy-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)acrylamidopodophyllotoxin (3b);
- 4p-(£)-3-(4-fluoro-3-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)acrylamidopodophyllotoxin (3c);
- 4p-(£)-3-(3-fluoro-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)acrylamidopodophyllotoxin (3d);
- 4p-(£)-3-(2-fluoro-5-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)acrylamidopodophyllotoxin (3e);
- 4p-(E)-3-(2-fluoro-4-methoxyphenyl)-2-(3,4,5,54trimethoxyphenyl)acrylamidopodophyllotoxin (3f);
- 4p-(£)-3-(4-hydroxy-3-nitrophenyl)-2-(3,4,5-trimethoxyphenyl)acrylamidopodophyllotoxin (3g);
4p-(E)-3-(4-methoxy-3-nitrophenyl)-2-(3,4,5-trimethoxyphenyl)acrylamidopodophyllotoxin (3h);
4p-(E)-3-(4-nitrophenyl)-2-(3,4,5-trimethoxyphenyl)acrylamidopodophyllotoxin (3i);
4p-(E)-3-(3-nitrophenyl)-2-(3,4,5-trimethoxyphenyl)acrylamidopodophyllotoxin (3j);
4p-(E)-3-(2-nitrophenyl)-2-(3,4,5-trimethoxyphenyl)acrylamidopodophyllotoxin (3k);
4p-(E)-2-(2-methoxyphenyl)-3-(4-nitrophenyl)acrylamidopodophyllotoxin (3l);
4p-(E)-2-(2-methoxyphenyl)-3-(2-nitrophenyl)acrylamidopodophyllotoxin (3m);
4p-(E)-3-(4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)acrylamidopodophyllotoxin (3n);
4p-(E)-3-(3-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)acrylamidopodophyllotoxin (3o);
4p-(E)-3-(4-hydroxyphenyl)-2-(3,4,5-trimethoxyphenyl)acrylamidopodophyllotoxin (3p);

3. The compounds as claimed in claim 1, wherein the structural formula of the representative compounds 3a-p are:
4. The compounds as claimed in claim 1, wherein the said compounds are useful as antitumour antibiotics.
5. A process for the preparation of 4p-acrylamidopodophyllotoxin congeners compounds of general formula 3, the said process comprising the steps of:

   (i) dissolving 4p-aminopodophyllotoxin of formula 1 in a solvent to obtain a solution;

   (ii) adding aromatic acrylic acid of formula 2a-p in the solution as obtained in step (i) with coupling reagents and acetic acid followed by stirring at temperature in the range of 25 to 30°C for a period in the range of 2 to 3 h;

   (iii) filtering, washing with saturated solution of NaHCO₃, 10% hydrochloric acid and followed by water;

   (iv) drying the washed filtrate as obtained in step (iii) over anhydrous Na₂SO₄ followed by chromatography through silica gel using an eluent to obtain the pure product.

6. A process as claimed in step (i) of claim 5, wherein the solvent used is selected from the group consisting of dichloromethane, chloroform and tetrahydrofuran.

7. A process as claimed in step (ii) of claim 5, wherein the coupling reagents used are N-(3-Dimethylaminopropyl)-N'-ethyl-carbodiimide hydrochloride (EDCI) and 1-HydrybxyBenztiazole (HOBt).

8. A process as claimed in step (iv) of claim 5, wherein the eluent used is ethyl acetate/hexane in the ratio ranging between 1:1 to 1:4.
9. The compounds as claimed in claim 1, wherein the said compounds show in-vitro anticancer activity against human tumour cells derived from five cancer types selected from the group consisting of breast cancer cell line (MCF-7, Zr-75-1), oral cancer cell line (KB, Gurav, DWD), colon cancer cell line (Colo 205), lung cancer cell line (A549, Hop 62) and ovarion cancer cell line (A-2780).

10. The compounds as claimed in claim 1, wherein concentration of the compound used for in-vitro activity against breast cancer cell lines (Zr-75-1) for GI50 is in the range of 0.18-2.7 µM at an exposure period of at least 48 hrs.

11. The compounds as claimed in claim 1, wherein concentration of the compound used for in-vitro activity against breast cancer cell lines (MCF-7) for GI50 is in the range of 2.4-2.9 µM at an exposure period of at least 48 hrs.

12. The compounds as claimed in claim 1, wherein concentration of the compound used for in-vitro activity against oral cancer cell lines (KB) for GI50 is in the range of 2.1-2.7 µM at an exposure period of at least 48 hrs.

13. The compounds as claimed in claim 1, wherein concentration of the compound used for in-vitro activity against oral cancer cell lines (Gurav) for GI50 is in the range of 0.18-2.7 µM at an exposure period of at least 48 hrs.

14. The compounds as claimed in claim 1, wherein concentration of the compound used for in-vitro activity against oral cancer cell lines (DWD) for GI50 is in the range of <0.1-2.9 µM at an exposure period of at least 48 hrs.

15. The compounds as claimed in claim 1, wherein concentration of the compound used for in-vitro activity against colon cancer cell lines (Colo205) for GI50 is in the range of 0.17-2.7 µM at an exposure period of at least 48 hrs.

16. The compounds as claimed in claim 1, wherein concentration of the compound used for in-
vitro activity against lung cancer cell lines (A-549) for GI_{50} is in the range of <0.1-2.9 μM at an exposure period of at least 48 hrs.

17. The compounds as claimed in claim 1, wherein concentration of the compound used for in vitro activity against lung cancer cell lines (Hop62) for GI_{50} is in the range of 0.17-2.9 μM at an exposure period of at least 48 hrs.

18. The compounds as claimed in claim 1, wherein concentration of the compound used for in vitro activity against ovarian cancer cell lines (A-2780) for GI_{50} is in the range of <0.1-2.7 μM at an exposure period of at least 48 hrs.
Scheme 1

2a: R = 3,4,5-trimethoxyphenyl, R¹ = 4-hydroxy-3-methoxyphenyl
2b: R = 3,4,5-trimethoxyphenyl, R¹ = 3-hydroxy-4-methoxyphenyl
2c: R = 3,4,5-trimethoxyphenyl, R¹ = 4-fluoro-3-methoxyphenyl
2d: R = 3,4,5-trimethoxyphenyl, R¹ = 3-fluoro-4-methoxyphenyl
2e: R = 3,4,5-trimethoxyphenyl, R¹ = 2-fluoro-5-methoxyphenyl
2f: R = 3,4,5-trimethoxyphenyl, R¹ = 2-fluoro-4-methoxyphenyl
2g: R = 3,4,5-trimethoxyphenyl, R¹ = 4-hydroxy-3-nitrophenyl
2h: R = 3,4,5-trimethoxyphenyl, R¹ = 4-methoxy-3-nitrophenyl
2i: R = 3,4,5-trimethoxyphenyl, R¹ = 4-nitrophenyl
2j: R = 3,4,5-trimethoxyphenyl, R¹ = 3-nitrophenyl
2k: R = 3,4,5-trimethoxyphenyl, R¹ = 2-nitrophenyl
2l: R = 2-methoxyphenyl, R¹ = 4-nitrophenyl
2m: R = 2-methoxyphenyl, R¹ = 2-nitrophenyl
2n: R = 3,4,5-trimethoxyphenyl, R¹ = 4-methoxyphenyl
2o: R = 3,4,5-trimethoxyphenyl, R¹ = 3-methoxyphenyl
2p: R = 3,4,5-trimethoxyphenyl, R¹ = 4-hydroxyphenyl;

3a: R = 3,4,5-trimethoxyphenyl, R¹ = 4-hydroxy-3-methoxyphenyl
3b: R = 3,4,5-trimethoxyphenyl, R¹ = 3-hydroxy-4-methoxyphenyl
3c: R = 3,4,5-trimethoxyphenyl, R¹ = 4-fluoro-3-methoxyphenyl
3d: R = 3,4,5-trimethoxyphenyl, R¹ = 3-fluoro-4-methoxyphenyl
3e: R = 3,4,5-trimethoxyphenyl, R¹ = 2-fluoro-5-methoxyphenyl
3f: R = 3,4,5-trimethoxyphenyl, R¹ = 2-fluoro-4-methoxyphenyl
3g: R = 3,4,5-trimethoxyphenyl, R¹ = 4-hydroxy-3-nitrophenyl
3h: R = 3,4,5-trimethoxyphenyl, R¹ = 4-methoxy-3-nitrophenyl
3i: R = 3,4,5-trimethoxyphenyl, R¹ = 4-nitrophenyl
3j: R = 3,4,5-trimethoxyphenyl, R¹ = 3-nitrophenyl
3k: R = 3,4,5-trimethoxyphenyl, R¹ = 2-nitrophenyl
3l: R = 2-methoxyphenyl, R¹ = 4-nitrophenyl
3m: R = 2-methoxyphenyl, R¹ = 2-nitrophenyl
3n: R = 3,4,5-trimethoxyphenyl, R¹ = 4-methoxyphenyl
3o: R = 3,4,5-trimethoxyphenyl, R¹ = 3-methoxyphenyl
3p: R = 3,4,5-trimethoxyphenyl, R¹ = 4-hydroxyphenyl;
**INTERNATIONAL SEARCH REPORT**

**A. CLASSIFICATION OF SUBJECT MATTER**

INV. C07D493/04 A61K31/365 A61P35/00

ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
</table>

[X] Further documents are listed in the continuation of Box C.  

[X] See patent family annex.

* Special categories of cited documents:
  - "A" document defining the general state of the art which is not considered to be of particular relevance
  - "E" earlier document but published on or after the international filing date
  - "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another document or other special reason (as specified)
  - "O" document referring to an oral disclosure, use, exhibition or other means
  - "P" document published prior to the international filing date but later than the priority date claimed
  - "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
  - "X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
  - "Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
  - "Z" document member of the same patent family

**Date of the actual completion of the international search**

28 June 2011

**Date of mailing of the international search report**

05/07/2011

Name and mailing address of the ISA: European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016

Authorized officer: Leclal Ilo, Jennifer
<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patent document cited in search report</td>
<td>Publication date</td>
<td>Patent family member(s)</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>-----------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td>WO 2004073375 A2</td>
<td>02-09-2004</td>
<td>EP 1599485 A2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 2007066837 A1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AT 402938 T</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AU 2005233382 A1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BR PI0509912 A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CA 2562617 A1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CN 1946727 A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DK 1742952 T3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ES 2311985 T3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HK 1095812 A1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HR 20080542 T3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 2005100363 A1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PT 1742952 E</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SI 1742952 T1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 2009170843 A1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 2011053967 A1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ZA 200609055 A</td>
</tr>
<tr>
<td>WO 2008136018 A2</td>
<td>13-11-2008</td>
<td>GB 2461667 A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 2010526058 A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 2008275248 A1</td>
</tr>
</tbody>
</table>