Synthesis of Novel Functionalized 4-Aza-2,3-didehydropodophyllotoxin Derivatives with Potential Antitumor Activity

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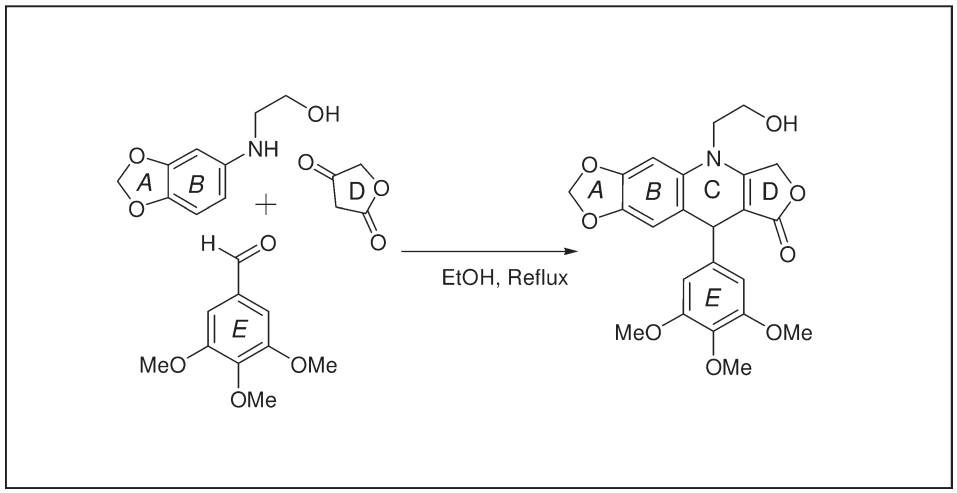

Novel arylamino alcohols were synthesized and these alcohols were used to prepare 12 novel *N*-(2-hydroxy-ethyl)-2,3-didehydroazapodophyllotoxins, in one step, by simple reflux in ethanol. Isolated yields in the range of 50–70% were obtained.

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INTRODUCTION

Podophyllotoxin (Fig. 1) is the starting material for the semi-synthesis of the anticancer drugs etoposide, etopophos and tenoposide. These antineoplastic pharmaceuticals block DNA topoisomerase II and have been used for the treatment of small and large cell lung, refractory testicular, stomach and pancreatic cancers, as well as myeloid leukemias [1a]. The podophyllotoxin core structure possesses a dual mode of action, i.e., inhibition of DNA topoisomerase II and of microtubule assembly through binding to tubulin, both of which are considered to be responsible for its antitumor activity. Podophyllotoxin is the precursor to a new derivative, CPH-82 or Reumacon®, which is being used to treat active rheumatoid or psoriatic arthritis in Europe. It is used to reduce inflammation. Podophyllotoxin is also the precursor of other derivatives used for the drug development of psoriasis and malaria [1b]. Several podophyllotoxin preparations are in the market for dermatological use to treat genital warts, e.g., imiquimod. Several new members of the podophyllotoxin derivatives have emerged as potentially superior chemotherapeutics, displaying improved water solubility and bioavailability, such as Nippon-Kayaku's NK-611 [2], GL-331 and Taiho's TOP-53 [3,4]. Podophyllotoxin derivatives also display anti-HIV-1 [5,6] and antibacterial [7] activities.

Currently, the commercial sources of podophyllotoxin are the rhizomes and roots of plants, such as *Podophyllum peltatum, Podophyllum emodi,* and American mayapple. *Podophyllum emodi* is an endangered species from the Himalayas [8]. Synthesis of podophyllotoxin is a multistep process, involving multicomponents and expensive reagents. The latest reported method for the synthesis of podophyllotoxin involves 5 steps and 20 reagents with 7.6% overall yield [9]. This tedious synthesis limits the new derivatives research of podophyllotoxin core. Podophyllotoxin contains 5 rings denoted as A, B, C, D, and E (Fig. 1). The basic structure of podophyllotoxin with rings A, B, C, D, and E was modified in different but limited ways since decades, in search of new chemical entities for drug discovery [10,11]. Derivatives of 4-aza-2,3-didehydropodophyllotoxin derivatives have also been synthesized as a strategy to develop podophyllotoxin analogs aimed at improving biological activity (Fig. 2) [12–14]. Some of those derivatives showed twice the cytotoxicity against P-388 leukemia cells as compared with podophyllotoxin [13].

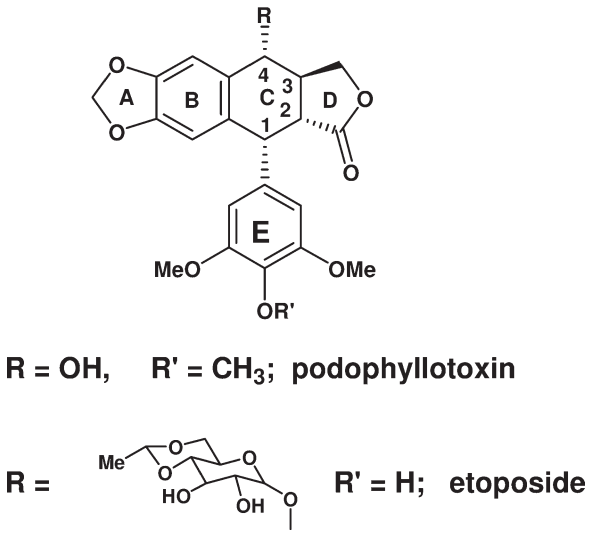


Figure 1. Structures of podophyllotoxin and etoposide.

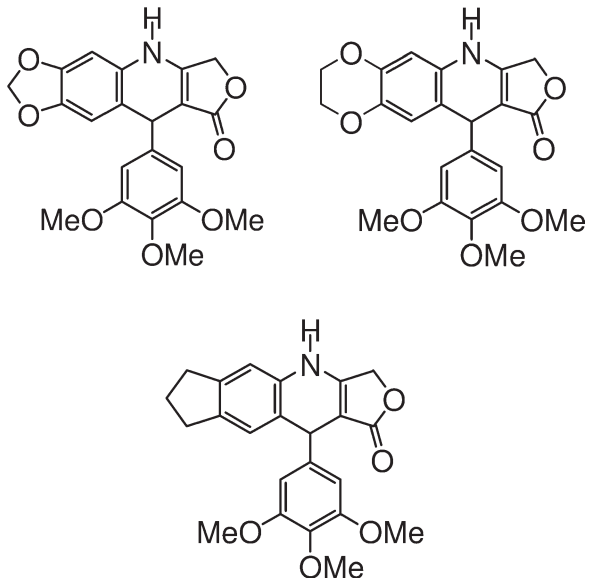


Figure 2. Structures of 4-aza-2,3-didehydropodo-phyllotoxin derivatives [13].

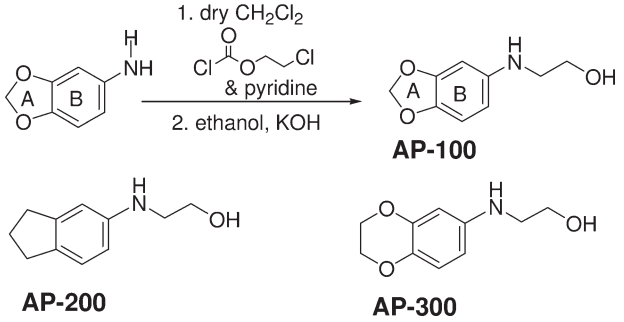
Many cytotoxic drugs have been conjugated to carriers for the selective targeting of organs and tissues [15]. To our best knowledge, no podophyllotoxin derivative has been conjugated to carriers for selective tissue targeting. In this work we report a facile and high-yielding synthetic procedure for the preparation of *N*-(2-hydroxy-ethyl)-2,3-didehydroazapodophyllotoxin derivatives with the potential to be conjugated to tissue-targeting carriers.

RESULTS AND DISCUSSION

Although there is an N—H bond at position 4 in the ''C'' ring of 4-aza-2,3-didehydropodophyllotoxin derivatives, nucleophilic substitution to electrophiles, such as acetic anhydride or an acyl chloride to form amides was not successful in our laboratory. Furthermore, attempts to use a strong base such as NaH or LDA to abstract the proton of the amine was not effective. Therefore, for the synthesis and biological activity studies of OH-functionalized derivatives of 4-aza-2,3-didehydropodophyllotoxin at the N atom in ring ''C,'' we synthesized some new arylamino alcohols (**AP-100** to **AP-300**, Scheme 1) to prepare N-(2-hydroxy-ethyl)-2,3-didehydroazapodophyllotoxins in one step, by simple refluxing in ethanol as reported previously [14]. The novelty in our work is the preparation of hydroxy-functionalized 2,3-didehydroazapodophyllotoxin derivatives, in two simple steps, which have a functional group which is available for further modifications. The novel arylamino alcohols were prepared by reacting commercially-available substituted anilines with 2-chloroethylchloroformate in dry dichloromethane in the presence of pyridine followed by reacting with KOH in ethanol (Scheme 1). These arylamino alcohols are not stable for long periods of time at room temperature and, thus, were synthesized freshly before used. Compounds **AP-101** to **AP-304** were prepared following the procedure reported by Tratrat et. al. [14] by reacting novel arylamino alcohol with tetronic acid and substituted aldehyde in ethanol (Fig. 3). Isolated yields were in the order of 60%. This is a straightforward one-step multicomponent synthesis, which involves simple isolation of the products by filtration and recrystallization. Isolated yields were in the 50–70% range.

Structures were corroborated with the help of ^{1}H, COSY, ^{13}C, DEPT45, DEPT90, DEPT135, HETCOR NMR, as well as ^{1}H-NMR coupled with deuterium exchange experiments, FTIR spectroscopy, HRMS and elemental analyses.

Scheme 1. Synthesis of arylamino alcohols. Structures of three examples of *N*-(2-hydroxy-ethyl)-2,3-didehydroazapodophyllotoxins are shown to indicate the structural variation of the arylamino alcohol precursor used.



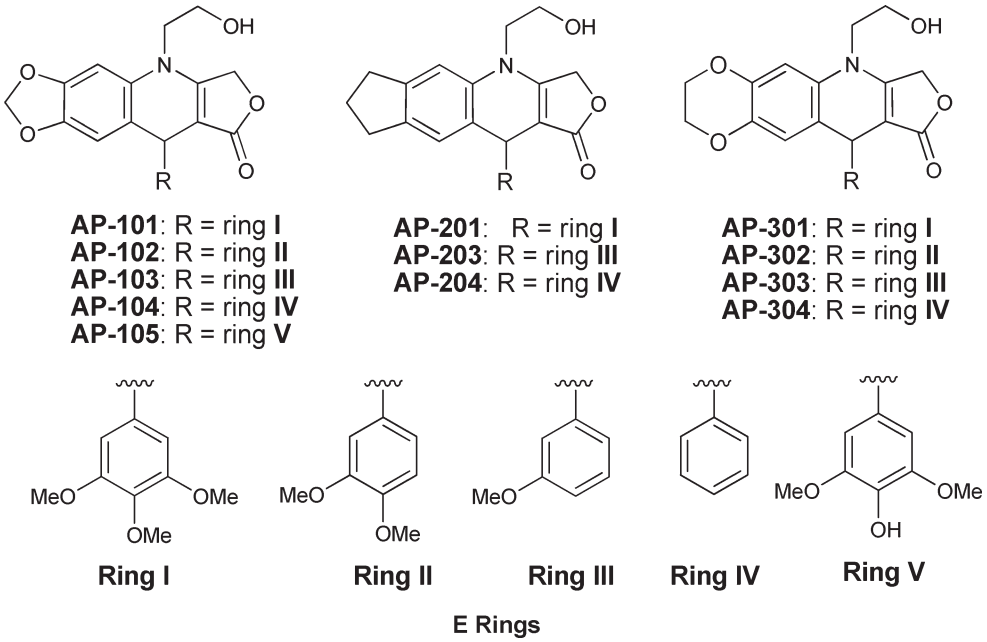


Figure 3. Structures of 4-aza-2,3-didehydropodo-phyllotoxin derivatives prepared in this work.

Compounds **AP-101** to **AP-304** were prepared following the procedure reported by Tratrat et al. [14] by reacting novel arylamino alcohol with tetronic acid and substituted aldehyde in ethanol (Fig. 3). The mechanism for the formation of **AP-101** is shown in Scheme 2. When the final ring closure is completed, the carbon atom, here assigned as **9**, has a unique ^{13}C-NMR chemical shift of ca. 39 ppm (Fig. 5). The proton attached to this carbon also has a unique chemical shift (s, 1H, 4.8 ppm, Fig. 4). Both types of chemical shifts for this carbon and proton were found uniform in the entire azapodophyllotoxin derivatives synthesized in this work. Some other specific carbons also corroborate the structure of the cyclisized scaffold, i.e. **8a** at 95 ppm and **5a** at 160 ppm. These chemical shifts were also uniformly found in the entire series of the azapodophyllotoxins synthesized in this work.

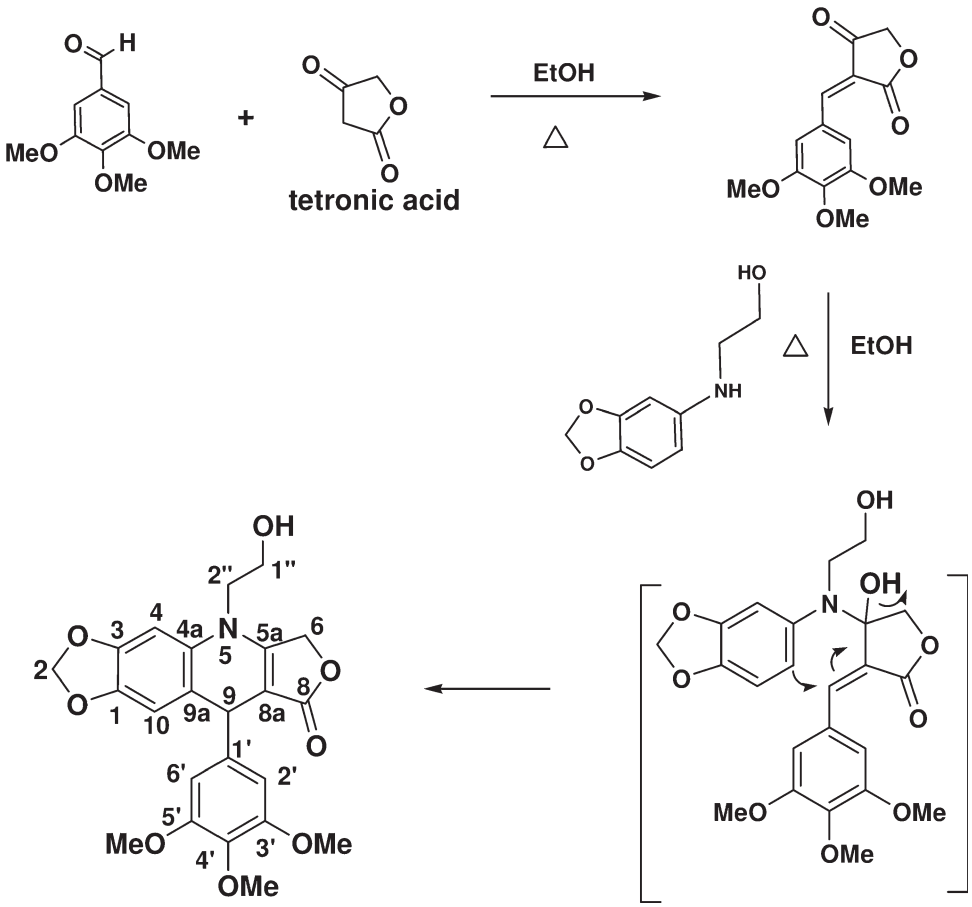
HETCOR NMR studies reveal other interesting findings (Fig. 5). Protons at carbon **2″** of all the azapodophyllotoxin derivatives are expected to be equivalent but these are not. One of them appears at ~3.6 ppm and the other at ~3.8 ppm and the latter may be interacting with protons at carbons **6** (Fig. 5). In fact, protons at position 6 are showing a broad peak probably due to that interaction. Also, protons at carbon **1″** are expected to be splitted into a triplet by two equal protons. However, these are unusually shaped as a doublet of doublets with J values of ~22 Hz and ~15 Hz, as protons at **2″** are not equivalent.

Itokawa and Takeya [12] have previously shown that (-)-4-aza-4-deoxypodophyllotoxin, which possess exactly the same enantiomeric form like podophyllotoxin, showed the same IC_{50} value of 0.0050 μg/mL against P388 leukemia cells. But interestingly, when strain between ring ''C'' and ''D'' was eliminated in 4-aza-2,3-didehydropodophyllotoxin analogue by dehydration at the 2,3 positions, the enhanced IC_{50} against P388 leukemia cells was observed, i.e., 0.0018 μg/mL as compare to 0.0043 μg/mL for podophyllotoxin [13]. Therefore, the synthesis of 4-aza-2,3-didehydro podophyllotoxin analogues with hydroxyl functionality, as in podophyllotoxin, has an excellent potential for the development of new drug entities not only as anti-tumor drugs but also for the treatment of psoriatic arthritis, HIV-1, genital warts, malaria, and bacterial infections as well.

In this work we have further modified the 4-aza-2,3-didehydropodophyllotoxin core by functionalizing position 4 at the ''C'' ring in a similar fashion as that occurring in podophyllotoxin derivatives. Libraries of these new heterocyclic compounds should be accessible by this straightforward one-step multicomponent synthesis, which involves simple isolation of the products by filtration and recrystallization and requires no further purification steps. Although the new compounds are racemic, we hope that the separation and testing of individual enantiomers may lead to more potent compounds.

This work will open a new area of 4-aza-2,3-didehydropodophyllotoxin analogues having almost similar structural functionalities to podophyllotoxin and will help to develop a library of new bioactive chemical entities which could be conjugated to carriers for tissue targeting. Whether these compounds may exert their

Scheme 2. Synthesis of **AP-101**.



cytotoxic effect through podophyllotoxin-like antitubulin mechanism, target topoisomerase II, or have a totally independent mode of action, constitutes the subject of further investigation, which will be reported in due course.

EXPERIMENTAL

Melting points were determined on a MEL-TEMP instrument and are uncorrected. IR spectra were recorded on a Perkin Elmer Spectrum 100 FTIR Spectrometer on ATS mode. ^{1}H, COSY, ^{13}C, DEPT45, DEPT90, DEPT135 and HETCOR NMR spectra were measured on a Bruker 400 Ultra shield Spectrometer using DMSO-d_6 as solvent. All chemical shifts are reported in parts per million relative to tetramethylsilane. Coupling constants (J) are reported in Hz. HRMS analyses were performed at the University of Florida (Gainesville) Mass Spectrometry facility. Absorption spectra were obtained in DMSO, using DMSO as blank, with an Agilent 8453 absorption spectrometer. Elemental analysis were performed at Atlanta Microlab GA.

Synthetic procedure for compounds AP-100 to AP-300.

2-[(3,4-Methylenedioxy)anilino]ethanol (AP-100). To a solution of 3,4-(-methylenedioxy)-aniline (5 g, 35.37 mmol) in dry dichloromethane (100 mL) and pyridine (3.6 mL, 44.57 mmol) at room temperature 2-chloroethylchloroformate (3.8 mL, 35.37 mmol) was slowly added. The mixture was stirred at room temperature (25°C) for 2.5 h and washed with water (5.0 mL × 4), dried over anhydrous magnesium sulphate and concentrated under vacuum. The residue was dissolved in ethanol (100 mL), treated with potassium hydroxide (8.6 g, 141.48 mmol) and heated at 90°C for 4 h. The mixture was dried under vacuum and the residue was dissolved in dichloromethane (150 mL), the precipitate was washed with dichloromethane twice (25 mL each). The combined organic phases were washed with water (5× mL) and brine. The organic phase was dried over anhydrous magnesium sulphate and concentrated under vacuum. The solid was purified by silica gel (120 g) flash chromatography with hexane-ethylacetate gradient to give compound **AP-100** (3.89 g, 61%) as brown needles. MP: 53–54°C, IR (cm^{-1}); 3283, 3162, 2869, 16360, 1496, 1232, 1190, 1122, 1073, 1031, 931, 883, 847, 782, 720, 692; ^{1}H-NMR (DMSO-*d*6, 400 MHz): δ (ppm) 3.01 (q, J = 11.84 Hz and 5.9 Hz, 2H), 3.54 (q, J = 11.64 Hz and 5.78 Hz, 2H), 4.64 (t, J = 5.71 Hz, 1H), 5.17 (t, J = 5.76 Hz, 1H), 5.82 (s, 2H), 6.00 (dd, J = 8.58 Hz and 2.16 Hz, 1H), 6.30 (d, J = 2Hz, 1H), 6.65 (d, J = 8.58 Hz, 1H); ^{13}C-NMR (DMSO-*d*6, 100 MHz): δ 59.42, 94.85, 99.65, 102.88, 108.18, 137.63, 144.66, 147.51; HRMS *m*/*z*: 182.0818 found (Calculated for $C_9H_{11}NO_3$, $[M+H]^+$ requires 182.0812).

2-(Indan-5-ylamino)-ethanol (AP-200). 2-(Indan-5-ylamino)-ethanol (AP-200) was synthesized from a similar reaction

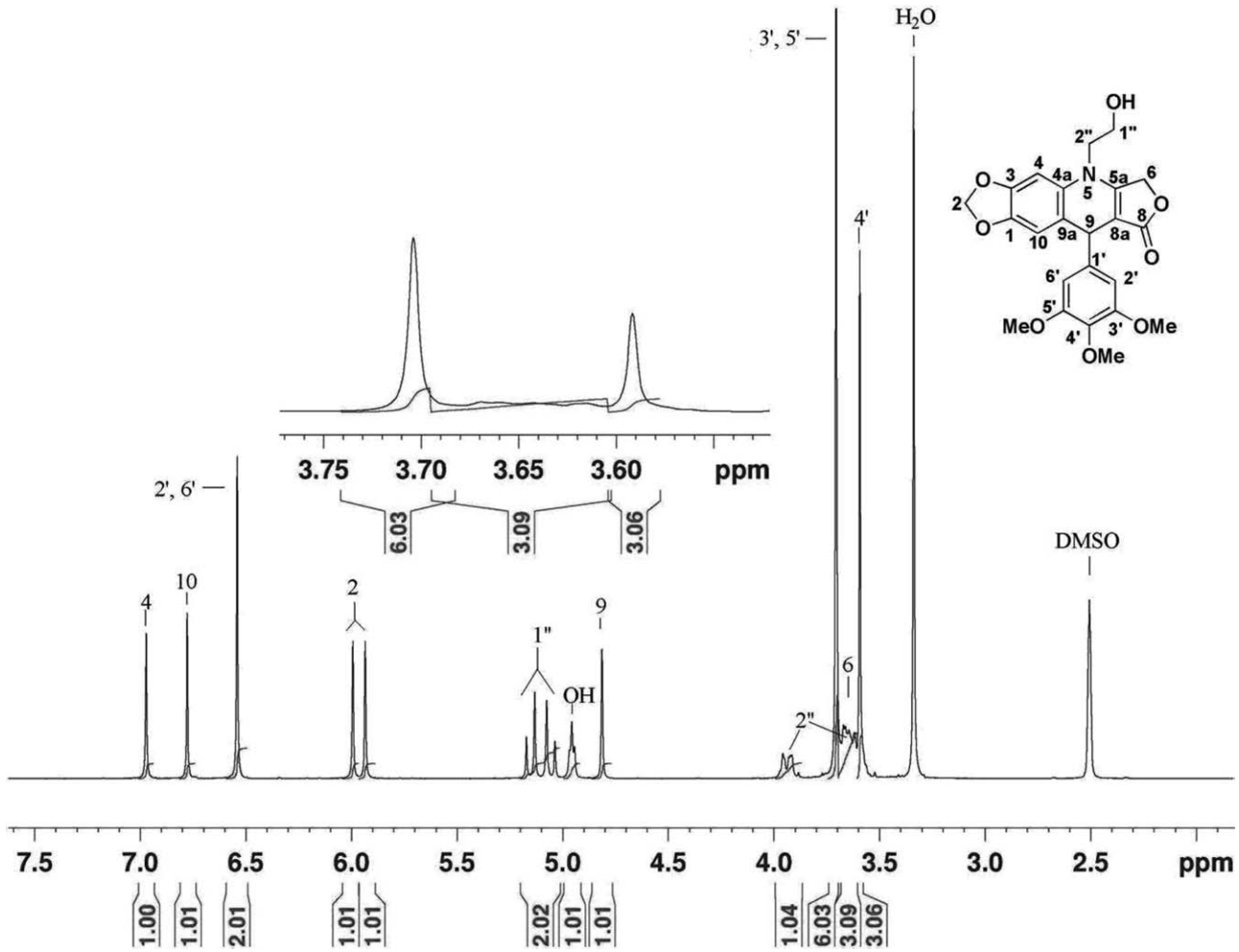


Figure 4. ^{1}H-NMR spectrum of AP-101 in DMSO-d_6.

between 5-aminoindan (9.04 gm, 64.50 mmol) and 2-chloroethylchloroformate (6.86 mL, 64.50 mmol). After flash chromatography, **AP-200** was obtained (6.79 g, 59% yield) as a dark brown oil showing: IR (cm^{-1}); 3345, 2940, 2837, 1615, 1494, 1456, 1329, 1290, 1260, 1212, 1157, 1119, 1058, 949, 840, 802, 703; ^{1}H-NMR ($CDCl_3$, 400 MHz),: δ (ppm) 2.01 (m, 2H), 2.78 (q, J = 15.43 Hz, and 7.72 Hz, 4H), 3.20 (t, J = 5.20 Hz, 2H), 3.27 (s, 2H), 3.74 (t, J = 5.22 Hz, 2H), 6.42 (dd, J = 8.03 Hz and 2.27 Hz, 1H), 6.53 (s, 1H), and 6.99 (d, J = 8.11 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 25.44, 31.69, 32.87, 46.56, 60.91, 109.43, 111.65, 124.53, 133.56, 145.22, 146.58; HRMS *m/z*: 178.1229 found (Calculated for $C_{11}H_{15}NO$, $[M+H]^+$ requires 178.1226).

2-(2,3-Dihydro-benzo[1,4]dioxin-6-ylamino)-ethanol (AP-300). 2-(2,3-Dihydro-benzo[1,4]dioxin-6-ylamino)-ethanol (AP-300) was synthesized from a similar reaction between 1,4-benzodioxan-6-amine (9.33 gm, 61.11 mmol) and 2-chloroethylchloroformate (6.5 mL, 61.11 mmol). The solid crude product was purified by silica gel (200 g) flash chromatography with hexane-ethyl acetate gradient to give the title compound (7.68 g, 64%) as a dark brown oil showing IR (cm^{-1}); 3373, 2928, 2876, 1625, 1595, 1502, 1460, 1325, 1276, 1205, 1171, 1062, 961, 919, 883, 828, 794, 745; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 3.10 (t, J = 5.19 Hz, 2H), 2.80 (m, 4H), 3.20 (t, 2H), 3.27 (br, 2H), 3.74 (t, J = 5.18 Hz, 2H), 6.43 (d, J = 2.0 Hz), 6.65 (d, J = 8.58 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 47.00, 61.09, 64.21, 64.76, 102.21, 107.37, 117.69, 136.03, 143.15, 144.06; HRMS *m/z*: 196.0968 found (calculated for $C_{10}H_{13}NO_3$, $[M+H]^+$ requires 196.0980).

General synthesis of 4-aza-2,3-didehydropodophyllotoxin derivatives. An equimolar mixture of tetronic acid, a substituted aniline and an aromatic aldehyde dissolved in the minimum volume of ethanol was refluxed for 30 to 90 min. After cooling, the precipitate was filtered off, washed with minimal cold ethanol and then recrystallized from ethanol.

5-(2-Hydroxyethyl)-9-(3,4,5-trimethoxyphenyl)-6,9-dihydro-[1,3]dioxolo[4,5-g]furo[3,4-b]quinolin-8(5H)-one (AP-101). Reaction time: 30 min. The product was washed with cold ethanol and dried under high vacuum, recrystallization from ethanol yielded 70% as a white crystalline powder, MP: 241–243°C; UV–Vis λ_{max} (nm): 261, 322; IR (ν_{max}, cm^{-1}); 3498, 2936, 1727, 1652, 1589, 1504, 1476, 1320, 1232, 1192, 1117, 1033, 1011, 931, 787, 760, 686; 1 H-NMR (DMSO-d_6, 400 MHz): δ 3.59 (s, 3H, 4′C—OCH_3), 3.64 (m, 3H, 6C—H and 2″C—H), 3.70 (s, 6H, 3′-OCH_3 and 5′-OCH_3) 3.92 (m,1H, 2″C—H), 4.81 (s, 1H, 9C—H), 4.95 (t, 1H, OH),

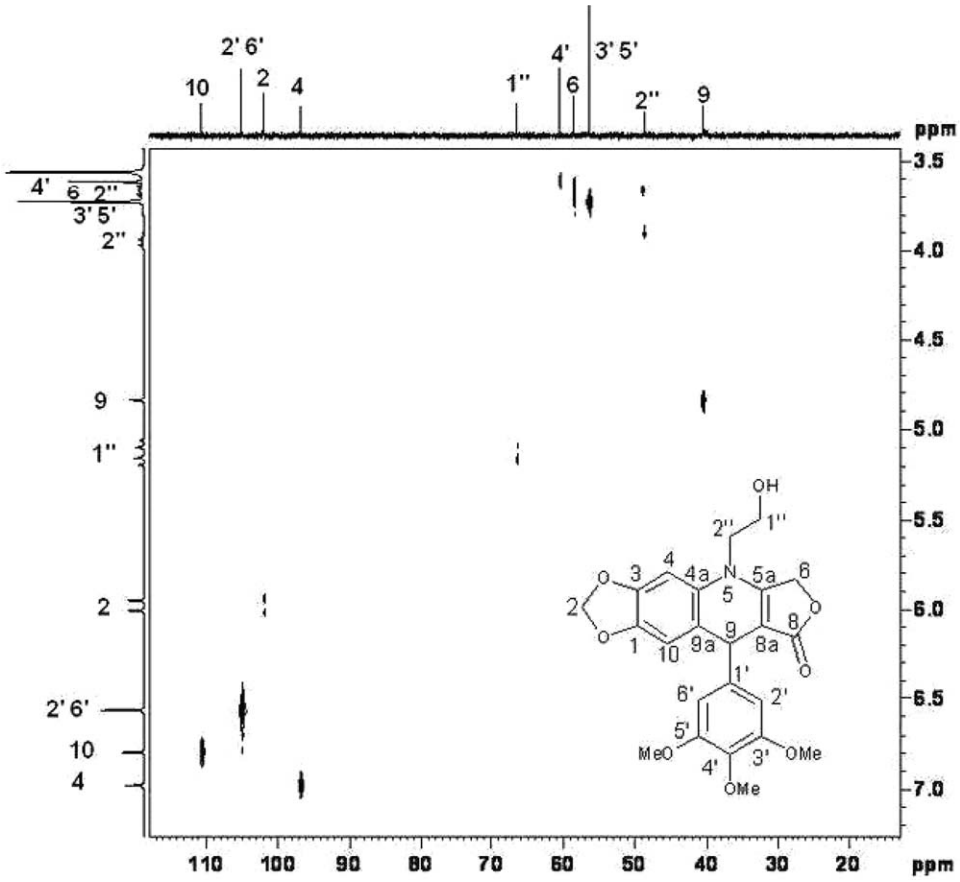


Figure 5. HETCOR spectrum of AP-101.

5.07 (d, J = 15.89 Hz, 1H, 1″C—H), 5.13 (d, J = 15.89 Hz, 1H, 1″C—H), 5.99 (d, J = 22.75 Hz, 2H, 2C—H), 6.519 (s, 2H, 2′C—H and 6′-H), 6.77 (s, 1H, 10C—H), 6.97(s, 1H, 4C—H); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 40.34, 48.44, 56.17, 56.29, 58.47, 60.22, 66.15, 95.23, 96.59, 101.73, 104.95, 104.95, 110.37, 119.80, 131.13, 136.38, 143.40, 143.62, 147.29, 153.19, 160.99, 172.67;HRMS m/z: 442.1491 found (calculated for $C_{23}H_{23}NO_8$, [M+H]+ requires 442.1496); *Anal*. Calcd. For [$C_{23}H_{23}NO_8$]; C, 62.58; H, 5.25; N, 3.17; O, 29.00. Found C, 62.55; H, 5.26; N, 3.20; O, 29.03

9-(3,4-Dimethoxyphenyl)-5-(2-hydroxyethyl)-6,9-dihydro-[1,3]dioxolo[4,5-g]furo[3,4-b]quinolin-8(5H)-one (AP-102). Reaction time: 30 min. The crude product was washed with cold ethanol and dried under high vacuum. Recrystallization from ethanol yielded 63% as a white crystalline powder; MP: 204–205°C; UV–Vis λ_{max} (nm): 261, 322; IR (ν_{max}, cm^{-1}); 3418, 2929, 1742, 1654, 1593, 1508, 1473, 1425, 1363, 1327, 1230, 1209, 1122, 1040, 1014, 989, 876, 847, 808, 759, 680; ^{1}H-NMR (DMSO-d_6, 400 MHz),: δ 3.68 (m, 9H, 2′,3′C—OCH_3, 1″C—H and 2″C—H), 3.85 (m,1H, 2″C—H), 4.81 (s, 1H, 9C—H), 4.99 (t, 1H, OH), 5.07 (d, J = 15.68 Hz, 1H, 1″C—H), 5.14 (d, J = 15.68 Hz, 1H, 1″C—H), 5.92 (s, 1H, 2C—H), 5.98 (s, 1H, 2C—H), 6.68 (s, 1H, 10C—H), 6.69 (dd, 1H J = 8.37 Hz and 2.4 Hz, 6′C—H), 6.79 (d, J = 8.15 Hz, 1H, 5′C—H) 6.86 (d, J = 7.5Hz, 1H, 2′C—H), 6.95 (s, 1H,4C—H); ^{13}C-NMR (DMSO- d_6, 100 MHz): δ 39.10, 47.86, 55.17, 55.27, 57.80, 65.48, 94.85, 95.89, 101.09, 109.85, 111.14, 111.60, 119.04, 119.39, 130.62, 139.88, 146.58, 147.10, 148.40, 160.04, 172.07; HRMS *m/z*: 412.1383 found (calculated for $C_{22}H_{21}NO_7$, [M+H]$^+$ requires 412.1391). *Anal*. Calcd. For [$C_{22}H_{21}$NO7]; C, 63.23; H, 5.14; N, 3.40. Found C, 64.19; H, 5.04; N, 3.38.

5-(2-Hydroxyethyl)-9-(3-methoxyphenyl)-6,9-dihydro-[1,3]dioxolo[4,5-g]furo[3,4-b]quinolin-8(5H)-one (AP-103). Reaction time: 30 min. The crude product was washed with cold ethanol and dried under high vacuum. A white crystalline powder was obtained, which was purified by recrystallization from ethanol; yield 54%, MP: 140–141°C; UV–Vis λ_{max} (nm): 261, 322; IR (ν_{max}, cm^{-1}); 3422, 2924, 1743, 1654, 1593, 1507, 1473, 1425, 1327, 1230, 1209, 1121, 1041, 1014, 988, 876, 848, 808, 759, 680; ^{1}H-NMR (DMSO-d_6, 400 MHz),: δ 3.68 (m, 6H, 3′C—OCH_3, 1″C—H and 2″C—H), 3.85 (m,1H, 2″C—H), 4.87 (s, 1H, 9C—H), 5.01 (t, J = 4.85 Hz, 1H, OH), 5.03 (d, J = 15.48 Hz, 1H, 1″C—H), 5.10 (d, J = 15.48 Hz, 1H, 1″C—H), 5.92 (s, 1H, 2C—H), 5.98 (s, 1H, 2C—H), 6.65 (s, 1H, 2′C—H), 6.71 (br, 1H, 10C—H), 6.78 (d, J = 8.26Hz, 1H, 4′C—H), 6.78 (d, J = 8.15 Hz, 1H, 5′C—H) 6.80 (s, 1H, 6′C—H), 6.95 (s, 1H,4C—H), 7.15(t, J = 8.08Hz, 1H); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 39.95, 48.12, 54.85, 57.96, 65.72, 94.77, 96.18, 101.33, 110.04, 111.30, 113.49, 119.04, 119.76, 129.30, 130.99, 143.16, 146.88, 148.66, 159.23, 160.39, 172.18; HRMS *m/z*: 382.1288 found (calculated for $C_{21}H_{19}NO_6$, [M+H]$^+$ requires 382.1285). *Anal*. Calcd. For

$[C_{21}H_{19}NO6]$; C, 66.13; H, 5.02; N, 3.67. Found C, 66.09; H, 5.00; N, 3.81.

5-(2-Hydroxyethyl)-9-phenyl-6,9-dihydro-[1,3]dioxolo[4,5-g]furo[3,4-b]quinolin-8(5H)-one (AP-104). Reaction time: 30 min. The crude product was washed with cold ethanol and dried under high vacuum yielding a white crystalline powder which was purified by recrystallization from ethanol; yield 62 %, MP: 180–181°C; UV–Vis λ_{max} (nm): 261, 322; IR (ν_{max}, cm^{-1}); 3278, 2880, 1709, 1641, 1480, 1438, 1369, 1339, 1242, 1198, 1020, 936, 876, 742, 700; ^{1}H-NMR (DMSO-d_6, 400 MHz),: δ 3.64 (m, 3H, 1″C—H and 2″C—H), 3.79 (m,1H, 2″C—H), 4.88 (s, 1H, 9C—H), 5.01 (t, J = 4.84 Hz, 1H, OH), 5.05 (br 2H, 1″C—H), 5.90 (s, 1H, 2C—H), 5.96 (s, 1H, 2C—H), 6.59 (s, 1H, 10C—H), 6.94 (s, 1H, 4C—H) 7.14 (br, 1H, 4′C—H), 7.21(br, 4H, 2′C—H, 3′C—H, 5′C—H and 6′C—H); ^{13}C-NMR (DMSO- d_6, 100 MHz): δ 40.43, 48.62, 58.43, 66.24, 95.35, 96.71, 101.83, 110.60, 119.65, 126.75, 127.99, 128.77, 131.54, 143.67, 147.36, 147.60, 160.88, 172.69; HRMS *m/z*: 352.1178 found (calculated for $C_{20}H_{17}NO_5$, $[M+H]^+$ requires 352.1179).

9-(4-Hydroxy-3,5-dimethoxyphenyl)-5-(2-hydroxyethyl)-6,9-dihydro-[1,3]dioxolo[4,5-g]furo[3,4-b]quinolin-8(5H)-one (AP-105). Reaction time: 30 min. The crude product was washed with cold ethanol and dried under high vacuum yielding a white crystalline powder which was purified by recrystallization from ethanol; yield 69%, MP: 237–238°C; UV–Vis λ_{max} (nm): 261, 322; IR (ν_{max}, cm^{-1}); 3551, 3345, 2940, 1708, 1638, 1605, 1509, 1481, 1456, 1367, 1322, 1242, 1198, 1108, 1068, 1016, 919, 860, 818, 763, 687; ^{1}H-NMR (DMSO-d_6, 400 MHz),: δ 3.66 (m, 9H, 5′and 3′C—OCH_3, 1″C—H and 2″C—H), 3.92 (m, 1H, 2″C—H), 4.75 (s, 1H, 9C—H), 4.95 (t, J = 4.85 Hz, 1H, OH), 5.02 (d, J = 15.43 Hz, 1H, 1″C—H), 5.12 (d, J = 15.43 Hz, 1H, 1″C—H), 5.92 (s, 1H, 2C—H), 5.98 (s, 1H, 2C—H), 6.48 (s, 2H, 2′and 6′ C—H), 6.74 (s, 1H, 10C—H), 6.95 (s, 1H,4C—H), 8.10(s, 1H, 4′C—OH); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 40.16, 48.50, 56.40, 58.53, 66.15, 95.50, 96.56, 101.75, 105.27, 110.47, 120.35, 131.13, 134.58, 138.19, 143.60, 147.21, 148.32, 160.86, 172.79; HRMS *m/z*: 428.1345 found (calculated for $C_{22}H_{21}NO_8$, $[M+H]^+$ requires 428.1340).; *Anal*. Calcd. For $[C_{22}H_{21}NO_8]$; C, 61.82; H, 4.95; N, 3.28. Found C, 61.74; H, 4.84; N, 3.24.

4-(2-Hydroxyethyl)-10-(3,4,5-trimethoxyphenyl)-3,4,6,7,8,10-hexahydro-1H-cyclopenta[g]furo[3,4-b]quinolin-1-one (AP-201). Reaction time: 30 min. The crude product was washed with 1:1 ethylacetate-hexane (V/V) and dried under high vacuum yielding a white crystalline powder which was purified by recrystallization from ethanol: ethylacetate mixture 1:1; yield 49%, MP: 153–154°C; UV–Vis λ_{max} (nm): 261, 322; IR (ν_{max}, cm^{-1}); 3422, 2929, 1743, 1655, 1620, 1593, 1508, 1473, 1426, 1363, 1328, 1293, 1231, 1210, 1123, 1066, 1043, 1015, 989, 876, 848, 823, 808, 791, 759, 744, 681; ^{1}H-NMR (DMSO-d_6, 400 MHz),: δ 1.96 (m, 2H, 7C—H), 2.71 (m, 2H, 6C—H), 2.81 (m, 2H, 8C—H), 3.58 (s, 3H, 4′-OCH_3), 3.66 (m, 9H, 3′and 5′, C—OCH_3, 1″C—H and 2″C—H), 3.98 (m, 1H, 2″CH), 4.86 (s, 1H, 10C—H), 4.98 (t, J = 4.85 Hz, 1H, OH), 5.05 (d, J = 15.98 Hz, 1H, 1″C—H), 5.15 (d, J = 15.98 Hz, 1H, 1″C—H), 6.53 (s, 2H, 2′ and 6′ C—H), 7.06 (s, 1H, 9C—H), 7.10 (s, 1H, 5C—H); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 25.17, 31.52, 32.32, 39.94, 47.68, 55.74, 55.74, 57.83, 59.77, 65.73, 95.29, 104.53, 109.92, 124.76, 126.33, 134.48, 135.85, 138.83, 143.32, 143.33, 152.73, 160.81, 172.27; HRMS *m/z*: 438.1903 found (calculated for $C_{25}H_{27}NO_6$, $[M+H]^+$ requires 438.1911). *Anal*. Calcd. For $[C_{25}H_{27}NO_6]$; C, 68.63; H, 6.22; N, 3.20. Found C, 68.51; H, 6.31; N, 3.16.

4-(2-Hydroxyethyl)-10-(3-methoxyphenyl)-3,4,6,7,8,10-hexahydro-1H-cyclopenta[g]furo[3,4-b]quinolin-1-one (AP-203). Reaction time: 30 min. The crude product was washed with 1:1 ethylacetate-hexane (V/V) and dried under high vacuum yielding a white crystalline powder which was purified by recrystallization from ethanol: ethylacetate mixture 1:1; yield 51%, MP: 158–159°C; UV–Vis λ_{max} (nm): 261, 322; IR (ν_{max}, cm^{-1}); 3378, 2933, 1718, 1643, 1597, 1476, 1431, 1366, 1347, 1321, 1262, 1243, 1202, 1172, 1161, 1095, 1020, 1001, 877, 845, 813, 780, 765, 746, 699, 671; ^{1}H-NMR (DMSO-d_6, 400 MHz),: δ 1.95 (m, 2H, 7C—H), 2.68 (m, 2H, 6C—H), 2.81 (m, 2H, 8C—H), 3.66 (m, 6H, 3′C—OCH_3, 1″C—H and 2″C—H one), 3.84 (m, 1H, 2″C—H one), 4.90 (s, 1H, 10C—H), 5.01 (t, J = 4.85 Hz, 1H, OH), 5.04 (d, J = 13.32 Hz, 1H, 1″C—H), 5.12 (d, J = 13.32 Hz, 1H, 1″C—H), 6.69 (m, 1H, 4′C—H), 6.78 (m, 2H, 2′and 6′C—H), 6.95 (s, 1H, 9C—H), 7.08 (s, 1H, 5C—H), 7.12(t, 1H, 5′C—H); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 25.66, 31.98, 32.82, 40.23, 48.32, 55.33, 58.25, 66.22, 95.77, 110.42, 111.57, 114.15, 120.35, 124.96, 126.99, 129.79, 135.26, 139.28, 143.89, 149.46, 159.68, 161.08, 172.69; HRMS *m/z*: 378.1700 found (calculated for $C_{23}H_{23}NO_4$, $[M+H]^+$ requires 378.1700). *Anal*. Calcd. For $[C_{23}H_{23}NO_4]$; C, 73.19; H, 6.14; N, 3.71. Found C, 72.88; H, 6.14; N, 3.76.

4-(2-Hydroxyethyl)-10-phenyl-3,4,6,7,8,10-hexahydro-1H-cyclopenta[g]furo[3,4-b]quinolin-1-one (AP-204). Reaction time: 30 min. The crude product was washed with 1:1 ethylacetate-hexane (V/V) and dried under high vacuum yielding a white crystalline powder which was purified by recrystallization from ethanol: ethylacetate mixture 1:1; yield 52%, MP: 191–192°C;); UV–Vis λ_{max} (nm): 261, 322; IR (ν_{max}, cm^{-1}); 3386, 2952, 2857, 1730, 1637, 1477, 1444, 1413, 1355, 1323, 1203, 1059, 1034, 1015, 994, 880, 852, 812, 754, 737, 701; ^{1}H-NMR (DMSO-d_6, 400 MHz),: δ 1.95 (m, 2H, 7C—H), 2.68 (m, 2H, 6C—H), 2.81 (m, 2H, 8C—H), 3.69 (m, 3H, 1″C—H and 2″-H), 3.86 (m, 1H, 2″C—H), 4.94 (s, 1H, 10C—H), 5.04 (t, J = 4.85 Hz, 1H, OH), 5.09 (d, J = 15.55 Hz, 1H, 1″C—H), 5.15 (d, J = 15.55 Hz, 1H, 1″C—H), 6.92 (s, 1H, 9C—H), 7.09 (s, 1H, 5C—H), 7.15(m, 4H, 2′,3′,4′and 6′C—H); ^{13}C-NMR (DMSO- d_6, 100 MHz): δ 25.65, 31.96, 32.82, 40.31, 48.33, 58.25, 66.23, 95.77, 110.44, 125.09, 126.63, 127.07, 128.06, 128.74, 135.35, 139.31, 143.88, 147.92, 161.08, 172.69; HRMS *m/z*: 348.1597 found (calculated for $C_{22}H_{21}NO_3$, $[M+H]^+$ requires 348.1594). *Anal*. Calcd. For $[C_{22}H_{21}NO_3]$; C, 76.06; H, 6.09; N, 4.03. Found C, 75.73; H, 6.06; N, 4.06.

6-(2-Hydroxyethyl)-10-(3,4,5-trimethoxyphenyl)-2,3,7,10-tetrahydro-[1,4]dioxino[2,3-g]furo[3,4-b]quinolin-9(6H)-one (AP-301). Reaction time: 60 min. The crude product was washed with cold ethanol and dried under high vacuum yielding a white crystalline powder which was purified by recrystallization from ethanol; yield 61%, MP: 256–257°C; UV–Vis λ_{max} (nm): 261, 322; IR (ν_{max}, cm^{-1}); 3506, 2936, 1733, 1650, 1591, 1506, 1474, 1367, 1295, 1203, 1120, 1065, 993, 894, 755, 686; ^{1}H-NMR (DMSO- d_6, 400 MHz): δ 3.59 (s, 3H, 4′C—OCH_3), 3.70 (m, 9H, 3′and 5′, C—OCH_3, 1″C—H and 2″C—H), 3.89 (m, 1H, 2″C—H), 4.16 (m, 4H, 2and3C—H)

4.80 (t, J = 4.85 Hz, 1H, OH), 4.96 (s, 1H, 10C—H), 5.07 (d, J = 15.29 Hz, 1H, 1″C—H), 5.13 (d, J = 15.29 Hz, 1H, 1″C—H), 6.53 (s, 2′and6′C—H), 6.71 (s, 1H, 11C—H), 6.75 (s, 1H, 5C—H); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 39.67, 48.32, 56.24, 58.32, 60.29, 64.42, 64.72, 66.23, 95.14, 103.38, 104.95, 119.07, 120.33, 130.49, 136.36, 139.87, 142.89, 143.52, 153.24, 161.12, 172.79; HRMS m/z: 456.1647 found (calculated for $C_{24}H_{25}NO_8$, $[M+H]^+$ requires 356.1653). *Anal.* Calcd. For $[C_{24}H_{25}NO_8]$; C, 63.29; H, 5.53; N, 3.08. Found C, 63.04; H, 5.44; N, 3.04.

10-(3,4-Dimethoxyphenyl)-6-(2-hydroxyethyl)-2,3,7,10-tetrahydro-[1,4]dioxino[2,3-g]furo[3,4-b]quinolin-9(6H)-one (AP-302). Reaction time: 90 min. The crude product was washed with cold ethanol and dried under high vacuum yielding a white crystalline powder which was purified by recrystallization from ethanol; yield 65%, MP: 210–211°C; UV–Vis λ_{max} (nm): 261, 322; IR (ν_{max}, cm^{-1}); 3497, 2932, 1726, 1647, 1504, 1474, 1440, 1347, 1268, 1206, 1133, 1067, 1025, 1004, 892, 812, 758, 706; ^{1}H-NMR (DMSO- d_6, 400 MHz): δ 3.68 (m, 9H, 3′and 4′, C—OCH_3, 1″C—H and 2″C—H), 3.82 (m, 1H, 2″C—H), 4.16 (m, 4H, 2and3C—H), 4.99 (t, J = 4.85 Hz, 1H, OH), 4.80 (s, 1H, 10C—H), 5.06 (d, J = 15.07 Hz, 1H, 1″C—H), 5.10 (d, J = 15.07 Hz, 1H, 1″C—H), 6.61 (s, 1H, 11C—H), 6.66 (dd, J = 7.99 Hz and 1.91 Hz, 1H, 6′C—H), 6.73 (s, 1H, 5C—H), 6.79 (t, J = 8.38 Hz, 1H, 5′C—H), 6.84 (d, J = 1.91 Hz, 1H, 2′C—H); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 39.05, 48.38, 55.90, 55.99, 58.28, 64.43, 64.73, 66.15, 95.39, 103.27, 111.86, 112.34, 119.20, 119.72, 120.55, 130.65, 139.82, 140.57, 142.80, 147.74, 149.05, 160.75, 172.78; HRMS m/z: 426.1553 found (calculated for $C_{23}H_{23}NO_7$, $[M+H]^+$ requires 426.1547). *Anal.* Calcd. For $[C_{23}H_{23}NO_7]$; C, 64.93; H, 5.45; N, 3.29. Found C, 64.92; H, 5.34; N, 3.32.

6-(2-Hydroxyethyl)-10-(3-methoxyphenyl)-2,3,7,10-tetrahydro-[1,4]dioxino[2,3-g]furo[3,4-b]quinolin-9(6H)-one (AP-303). Reaction time: 90 min. The crude product was washed with cold ethanol and dried under high vacuum yielding a white crystalline powder which was purified by recrystallization from ethanol; yield 60%, MP: 175–176°C; UV–Vis λ_{max} (nm): 261, 322; IR (ν_{max}, cm^{-1}); 3424, 2936, 1720, 1641, 1581, 1483, 1442, 1367, 1296, 1274, 1250, 1206, 1155, 1060, 1036, 1004, 907, 868, 806, 772, 753, 713, 695; ^{1}H-NMR (DMSO- d_6, 400 MHz): δ 3.68 (m, 6H, 3′C—OCH_3, 1″C—H and 2″C—H), 3.78 (m, 1H, 2″C—H), 4.16 (m, 4H, 2 and 3 C—H), 4.85 (s, 1H, 10C—H), 5.01 (t, J = 4.82 Hz, 1H, OH), 5.07 (d, J = 15.63 Hz, 1H, 1″C—H), 5.14 (d, J = 15.63 Hz, 1H, 1″C—H), 6.59 (s, 1H, 11C—H), 6.71 (br, 1H, 6′C—H), 6.74 (s, 1H, 5C—H), 6.76 (s, 1H, 2′C—H), 6.78 (br, 1H, 4′C—H), 7.15 (t, J = 8.09 Hz, 1H, 5′C—H); ^{13}C-NMR (DMSO- d_6, 100 MHz): δ 39.48, 48.45, 55.35, 58.25, 64.43, 64.73, 66.21, 95.13, 103.37, 111.65, 114.05, 119.22, 120.03, 120.26, 129.79, 130.77, 139.83, 142.91, 149.17, 159.71, 160.89, 172.72; HRMS m/z: 396.1452 found (calculated for $C_{22}H_{21}NO_6$, $[M+H]^+$ requires 396.1442). *Anal.* Calcd. For $[C_{22}H_{21}NO_6]$; C, 66.83; H, 5.35; N, 3.54. Found C, 66.68; H, 5.27; N, 3.52.

6-(2-Hydroxyethyl)-10-phenyl-2,3,7,10-tetrahydro-[1,4]dioxino[2,3-g]furo[3,4-b]quinolin-9(6H)-one (AP-304). Reaction time: 90 min. The crude product was washed with cold ethanol and dried under high vacuum yielding a white crystalline powder which was purified by recrystallization from ethanol; yield 68%, MP: 229–230°C; UV–Vis λ_{max} (nm): 261, 322; IR (ν_{max}, cm^{-1}); 2956, 2876, 1719, 1637, 1581, 1475, 1437, 1363, 1292, 1250, 1203, 1149, 1065, 1007, 923, 893, 832, 806, 753, 734, 695; ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 3.66 (m, 3H, 1″C—H and 2″C—H), 3.79 (m, 1H, 2″C—H), 4.14 (m, 4H, 2and3C—H), 4.88 (s, 1H, 10C—H), 5.04 (br, 1H, OH), 5.08 (d, J = 15.48 Hz, 1H, 1"C—H), 5.13 (d, J = 15.48 Hz, 1H, 1"C—H), 6.59 (s, 1H, 11C—H), 6.74 (s, 1H, 5C—H), 7.21 (br, 2H, 2′and6′C—H), 7.24 (br, 2H, 3′ and 5′C—H), 7.14 (t, J = 6.87 Hz, 1H, 4′C—H); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 39.57, 48.46, 58.25, 64.43, 64.72, 66.21, 95.25, 103.39, 119.30, 120.16, 126.67, 127.99, 128.75, 130.85, 139.86, 142.72, 147.60, 160.86, 172.71; HRMS m/z: 366.1338 found (calculated for $C_{21}H_{19}NO_5$, $[M+H]^+$ requires 366.1336). *Anal.* Calcd. For $[C_{21}H_{29}NO_5]$; C, 69.03; H, 5.24; N, 3.83. Found C, 68.64; H, 5.29; N, 3.84.

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