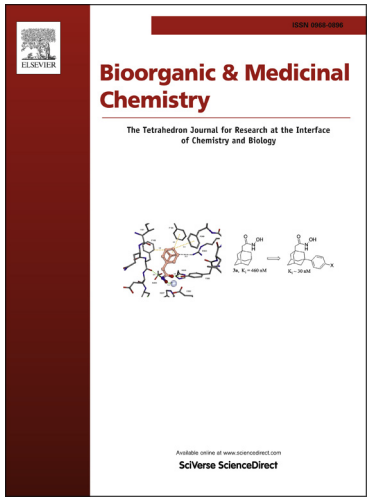

Design and synthesis of 6,7-methylenedioxy-4-substituted phenylquinolin-2*(1H)*-one derivatives as novel anticancer agents that induce apoptosis with cell cycle arrest at G2/M phase

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Design and synthesis of 6,7-methylenedioxy-4-substituted phenylquinolin-2*(1H)*-one derivatives as novel anticancer agents that induce apoptosis with cell cycle arrest at G2/M phase

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Abstract

Novel 6,7-methylenedioxy-4-substituted phenylquinolin-2-one derivatives **12a-n** were designed and prepared through an intramolecular cyclization reaction and evaluated for *in vitro* anticancer activity. Among the synthesized compounds, 6,7-methylenedioxy-4-(2,4-dimethoxyphenyl)quinolin-2(*1H*)-one (**12e**) displayed potent cytotoxicity against several different tumor cell lines at a sub-micromolar level. Furthermore, results of fluorescence-activated cell sorting (FACS) analysis suggested that **12e** induced cell cycle arrest in the G2/M phase accompanied by apoptosis in HL-60 and H460 cells. This action was confirmed by Hoechst staining and caspase-3 activation. Due to their easy synthesis and remarkable biological activities, 4-phenylquinolin-2(*1H*)-one analogs (4-PQs) are promising new anticancer leads based on the quinoline scaffold. Accordingly, compound **12e** was identified as a new lead compound that merits further optimization and development as an anticancer candidate.

Keywords

4-Phenylquinolin-2(*1H*)-one (4-PQ), Anticancer agent, Podophyllotoxin, Apoptosis, Structure-activity relationships (SAR).

1. Introduction

Cancer is the leading cause of death in economically developed countries and the second leading cause of death in developing countries.[1] Development of novel antitumor agents with cytotoxicity against cancer cell lines is an important focus of oncology. Natural products are often referred to as an inexhaustible source of lead compounds that are likely to exhibit multiple biological activities, including anticancer.[2,3] Because of their intrinsic biorelevance, natural products represent a significant source of inspiration for the design of structural analogues with new pharmaceutical profiles.

The microtubule network is an essential component of the cytoskeleton of eukaryotic cells. Podophyllotoxin (PPT, **1**) is a aryltetralin lignan isolated from the roots of *Podophyllum peltatum* L., P. *emodi* W., or P. *pleianthum* H..[4] PPT inhibits tubulin assembly and polymerization through interaction with the protein at the colchicine binding site, thus preventing the formation of the spindle, and arresting cell division in metaphase (G2/M stage).[5-7] Numerous efforts have been made to improve PPT's safety profile while maintaining its potency. Thus, its use as a lead agent in the development of new anticancer drugs has led to the discovery of semisynthetic derivatives, such as etoposide (**2**) and teniposide (**3**), and a more soluble prodrug of etoposide, named etopophos (**4**) (Fig. 1). Semisynthetic derivatives of PPT are currently in clinical use for the treatment of various malignancies.

[Figure 1 should be inserted here]

PPT's structure is complex due to the presence of four chiral carbons in ring C (Fig. 1), which creates challenges for SAR (structure–activity relationship) studies.

Research efforts to develop new analogues with simpler structures are ongoing ot identify the critical substructures for desired biological activity, and almost all rings (ring A to E) and positions on the cyclolignan skeleton have been modified.[8] Importantly, elimination of the stereogenic centers at C-2 and C-3 removes the possibility of epimerization at C-2. Epimerization of the natural lignan has plagued its clinical development, because the rapidly formed *in vivo cis*-lactone metabolite is inactive.[9] Numerous attempts have been made to obtain potent synthetically feasible analogues of PPT with a heteroatom incorporated in ring C to prevent epimerization. 4-Aza-podophyllotoxin (**5**) (Fig. 2) and related analogues were identified as structurally simpler derivatives of PPT with reported antiproliferative activity.[10-12] In addition, podophyllic aldehyde (**6**) was found to be a highly selective antitumor agent against HT-29 colon and A-549 lung carcinomas. Several aldehydes related to this compound but with different configurations have been synthesized and evaluated for cytotoxic activities in neoplastic cell lines.[13,14] These podophyllic aldehyde-related compounds lacked the lactone ring, but maintained cytotoxicity at, or under, micromolar level.

[Figure 2 should be inserted here]

Another naturally occurring *cis*-stilbene, combretastatin A-4 (CA-4, **7**) (Fig. 2) was isolated from the bark of the south African tree *Combretum caffrum* (Combretaceae).[15] CA-4 is a potent tubulin assembly inhibitor and vascular disrupting agent at low concentrations.[16] Its relative molecular simplicity suggests numerous practical approaches to the design of new antineoplastic agents, and several active stilbene-based compounds have been identified.[17] The main disadvantage of CA-4 is the ready isomerization of its *cis*-double bond to an inactive *trans*-form during storage

and administration.[18] Consequently, this double bond in the *cis*-stilbene motif has often been used as a modification site and replaced with various heterocyclic or carbonyl functional groups.[19-21]

According to previous SAR studies on PPT and related analogues, structurally modified compounds that lack the *trans*-γ-lactone ring (ring D) can show potent and selective cytotoxicity against cancer cells. Thus, the γ-lactone ring is not an essential feature for cytotoxicity of PPT analogues. These results encouraged us to select 4-aza-podophyllotoxin (**5**) as the template and design much simpler structures without a γ-lactone ring. In our previous investigation, 2-phenylquinolin-4(*1H*)-one analogs (2-PQs) (Fig. 2) were identified as antimitotic agents.[22] Recently, we shifted the phenyl ring on the quinolinone from the C-2 to C-4 position. Hence, a series of 4-phenylquinolin-2(*1H*)-one analogs (4-PQs) was designed that mimics the structures of **5**–**7** (Fig. 3). In addition, the two aromatic rings (B and E) of 4-phenylquinolin-2(*1H*)-one derivatives (4-PQs) adopt a conformation in which they are not coplanar, and the structural similarity between CA-4 and appropriately substituted 4-PQs might lead to interaction at the colchicine site to affect tubulin polymerization. Moreover, 4-PQ compounds would not be inactivated by *cis* to *trans* isomerization as with CA-4 derivatives.

[Figure 3 should be inserted here]

In this article, we describe the synthesis and cytotoxicity evaluation of 6,7-methylenedioxy-4-substituted phenylquinolin-2(*1H*)-one derivatives **12a-n** (Fig. 3). Also, additional biological studies have been performed to analyze how novel compounds of this class affect the cell cycle.

2. Chemistry

The synthetic route to 6,7-methylenedioxy-4-substituted phenylquinolin -2(*1H*)-one derivatives is illustrated in Scheme 1. The 4-phenylquinolin-2(*1H*)-one derivatives **12a-n** were prepared using the general Knorr quinoline synthesis (acid-catalysis).[23,24] The synthesis was initiated with alkoxycarbonylation of substituted acetophenones **8a-n** with a diethyl carbonate (**9**) to provide the corresponding benzoylacetates **10a-n**,[25-27] which were condensed with 3,4-methylenedioxy aniline to give benzoylacetanilide intermediates **11a-n**. The 4-phenylquinolin-2(*1H*)-one derivatives **12a-n** were obtained by cyclization of key intermediates **11a-n** with excess polyphosphoric acid (PPA) at 100-110 °C. The synthesized compounds **12a-n** are reported for the first time. All synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR and mass spectrometry. As an example, the target compound **12a** possessed a characteristic peak at 6.02 ppm, representative of the methylenedioxy protons. Three singlet peaks at 6.18, 6.63 and 6.87 ppm were assignable to C(3)H, C(5)H and C(8)H protons in the 2-quinolone core, and one broad singlet (11.78 ppm) to an exchangeable NH group. The protons of the 4-phenyl ring appeared in the region 7.36-7.51 ppm. In the ^{13}C NMR spectrum, compound **12a** possessed a characteristic absorption at δ 161.70 ppm for the 2-quinolone amide carbon (NHC=O). The IR spectrum of **12a** showed an absorption at 1653.07 cm^{-1} for the amide (NHC=O) carbonyl group.

[Scheme 1 should be inserted here]

3. Biological evaluation

3.1. Invitro cytotoxicity assay

The newly synthesized analogues **12a-n** were evaluated for cytotoxicity against Detroit 551 normal human cells and eight cancer cell lines: MCF-7 (breast adenocarcinoma), 2774 (ovarian carcinoma), SKOV-3 (ovarian carcinoma), HL-60 (leukemia), Hep 3B (hepatoma), H460 (non-small-cell-lung carcinoma), COLO205 (colorectal adenocarcinoma) and A498 (renal cell carcinoma). Etoposide and 5-FU was used as a positive control. The screening procedure was based on the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliun bromide (MTT) growth inhibition assay, and the results are summarized in Table 1.

Compound **12a**, which contains an unsubstituted 4-phenyl E-ring, displayed poor cytotoxicity with IC_{50} values from 18.5 to greater than 50 μM. The steric parameters of hydrogen and fluorine are extremely similar (van der Waals radii of 1.2 Å for hydrogen vs. 1.35 Å for fluorine), and fluoro-substituted **12l**, **12m**, and **12n** also displayed weak cytotoxicity against the cancer cell lines.

However, **12k**, whose ring E bears the same 3,4,5-trimethoxy substitution found in PPT, exhibited significant antiproliferative activity with sub-micromolar IC_{50} (0.93-7.6 μM) against several cancer cell lines. However, potency dropped when the 3,4,5-trimethoxy group (**12k**) was changed to a 2,4,6-trimethoxy group (**12j**). In addition, the effects of dimethoxy substitution on the phenyl E-ring is noteworthy. Generally, dimethoxy substitution on the 4-phenyl ring (**12e**, **12f**, **12h**, **12i**) led to more potent activity than monomethoxy substitution (**12b-d**). However, compound **12g** with 2,6-dimethoxy substitution was less potent than the other four dimethoxy substituted analogues. Among the four more active dimethoxy compounds, analogue **12e** with 2,4-dimethoxy substitution exhibited the most potent anticancer activity with IC_{50} less than or equal to 1 μM against five cell lines, 2774 (0.4 μM), SKOV-3 (0.4 μM), HL-60 (0.4 μM), Hep 3B (1.0 μM) and H460 (0.9 μM). Compound **12e** was also generally more potent than the trimethoxy substituted **12k**. Modification of the

E-ring with different numbers and positions of methoxy substituents may affect its spatial rotation and the molecular orientation, which could increase binding to the biological target and anticancer activity of the optimized compounds. Finally, **12e** showed only marginal toxicity against Detroit 551 normal human cells (IC_{50} **>** 25 μM). It is also noteworthy that the cell lines more resistant to **12e**, including MCF-7, COLO205, and A498, harbor wild-type p53.

[Table 1 should be inserted here]

It is generally accepted that PPT's cytotoxicity is attributable to inhibition of tubulin polymerization, while the target of etoposide is topoisomerase II. Recently, an alternative non-tubulin and non-antitopoisomerase mode of action has been proposed for some PPT derivatives. This mechanism is associated with cell death and sub-G1 apoptotic cell accumulation without any significant cell cycle arrest.[13] Thus, mechanistic studies on **12e** are underway in our laboratories.

3.2. Morphological changes and apoptosis

Apoptosis is one of the major pathways that lead to the process of cell death. Chromatin condensation, nuclear shrinking, and fragmented nuclei are known as classic characteristics of apoptosis. To examine the effect of **12e** on apoptotic induction, the morphology changes of HL-60 and H460 cells were studied using Hoechst 33258 stain, which confirmed apoptosis as the cause of reduced cell viability. Compound **12e** decreased cell viability and increased morphological changes in HL-60 and H460 cells. As shown in Figure 4, control cells with 0.1% DMSO treatment exhibited uniformly dispersed chromatin. Cells treated with compound **12e** for 24 h demonstrated typical apoptotic characteristics, including condensation of

chromatin and appearance of nuclear fragmentation and apoptotic bodies (the arrowhead indicates an apoptotic nucleus). These results clearly demonstrated that the compound **12e** is effective in inducing cellular apoptosis.

[Figure 4 should be inserted here]

3.3. Cell cycle analysis

Previous studies have indicated that PPT analogues induced DNA damage and caused G2/M arrest.[28-30] To evaluate the effect of **12e** on the cell cycle andgain further insight into the mode of action, we examined cell cycle accumulation at 24 h by propidium iodide staining and flow cytometry quantification in HL-60 and H460 cells treated with **12e**. In HL-60 cells treated with 0.25 μM, 0.5 μM, and 1.0 μM concentrations of **12e**, 11.87%, 25.02% and 40.54%, respectively, of cells were in G2/M phase, 10.51% of control (untreated) cells were in this phase (Fig. 5A). The percentage of G2/M cells also increased in H460 cells treated with **12e** (Fig. 5B). As shown in Figure 5, the DNA cell cycle analysis revealed typical G2/M arrest and apoptosis (a sub-G1 peak appeared) in response to treatment with **12e**.

[Figure 5 should be inserted here]

3.4. Mechanism of action

Cyclin B1 and CDK1 are intricately involved in cell cycle progression through the G2/M phase transition.[31] In our previous studies, the exposure of HL-60 cells to 2-PQ analogues led to G2/M phase arrest. In the Western blot analysis, a marked decrease in the expressions of cyclin B1 and CDK1 were detected in HL-60 cells.[32,33]

As shown in Figure 6, compound **12e** induced a transient increase followed by a decrease in cyclin B1 protein expression, whereas CDK1 protein expression level decreased in a concentration-dependent manner in HL-60 cells (Fig. 6A). However, CDK1 protein expression level increased in a concentration-dependent manner in H460 cells (Fig. 6B). It was reported that treatment with deoxypodophyllotoxin (DPPT) in Hela cells induced G2/M phase arrest. In the Western blot analysis, cyclin B1 expression was observed to have a rapid increase within 3 h of DPPT treatment that remained elevated for up to 24 h. After 48 h, the expression of cyclin B1 returned to near control levels.[34] Another study was reported that treatment of NTUB1 cells with higher concentration of 1-hydroxy-3-(3-alkylaminopropoxy)-9,10-anthraquinone (MHA) derivative for 24 h induced up-regulation of cyclinB1 and p21 expressions in NTUB1 cells while treatment with lower concentration of MHA for 24 h induced down-regulation of cyclinB1 and up-regulation of p21 expressions in NTUB1 cells.[35] These results suggest that cell cycle progression is well regulated by the timing of the expression of cell-specific cyclins and different concentrations of the compounds.

Apoptosis is associated with activation of caspases, an expanding family of cysteine proteases that play important roles in the execution phase of apoptosis triggered by various stimuli.[36] Among the capsases, caspase 3 is the best understood and mediates several apoptotic pathways. As shown in Figure 6, compound **12e** induced caspase 3 cleavage (17 kDa) in HL-60 and H460 cells. The above preliminary investigation of the mechanism of action of compound **12e** suggested that the anticancer effects of **12e** against HL-60 and H460 cells are mediated via G2/M arrest and caspase-dependent apoptosis.

[Figure 6 should be inserted here]

4. Conclusion

4-Phenylquinolin-2(*1H*)-one derivatives **12a-n** were synthesized and tested for antiproliferative activity against several cancer cell lines. Compound **12e**, containing 2,4-dimethoxy substitution on the 4-phenyl ring, demonstrated potent antiproliferative activities with IC_{50} values of 0.4, 0.4, 0.4 and 0.9 μM against 2774, SKOV-3, HL-60 and H460, respectively. The significant anticancer activity shown by **12e** prompted us to evaluate cell viability of HL-60 and H460 cells, with the observation of morphological features of apoptotic cells in these cell lines. Furthermore, **12e** could induce cell cycle arrest in the G2/M phase and apoptosis by activation of caspase-3 in both HL-60 and H460 cells. In conclusion, among the newly synthesized 4-PQ derivatives, compound **12e** was identified as a promising candidate; due to its excellent antiproliferative potency, together with remarkable apoptosis-inducing activity. Further structure optimization of **12e** is ongoing.

5. Experimental section

5.1. Materials and physical measurements

All solvents and reagents were obtained commercially and used without further purification. The progress of all reactions was monitored by TLC on 2 × 6 cm pre-coated silica gel 60 F_{254} plates of thickness 0.25 mm (Merck). The chromatograms were visualized under UV light at 254–366 nm. The following adsorbent was used for column chromatography: silica gel 60 (Merck, particle size 0.063–0.200 mm). Melting points (mp) were determined with a Yanaco MP-500D melting point apparatus and are uncorrected. IR spectra were recorded on Shimadzu IR-Prestige-21 spectrophotometers as KBr pellets. The NMR spectra were obtained on a Bruker Avance DPX-200 FT-NMR spectrometer at room temperature, and chemical shifts

are reported in ppm (δ). The following abbreviations are used: s, singlet; d, doublet; t, triplet; dd, double doublet; and m, multiplet. Low-resolution mass spectra were performed by Finnigan/Thermo Qust MAT95XL at National Chung Hsing University, Taichung, Taiwan.

5.2. Chemistry

5.2.1. General procedure for the synthesis of benzoylacetates 10a-n

The substrate β-ketoesters **10a-n** were either purchased or synthesized following published procedures. Some benzoylacetates were commercially available. Ethyl 3-oxo-3-phenyl propanoate (**10a**) was purchased. The reaction of benzoylacetates **10b-n** was prepared as described in previous reports.[25-27] A solution of a substituted acetophenone **8a-n** (0.05 mol) dissolved in toluene (50 mL) was added dropwise to a solution containing diethyl carbonate (**9**) (0.10 mol) and sodium hydride (0.15 mol 60% dispersion in mineral oil). The mixture was stirred at room temperature, and then refluxed for 30 min. The mixture was poured into ice water, acidified with glacial acetic acid, and extracted with EtOAc (3 × 100 mL). The EtOAc extract was then dried over anhydrous $MgSO_4$. After removal of the solvent *in vacuo*, the crude products were purified by silica gel column chromatography eluting with dichloromethane to afford benzoylacetates **10b-n**. All synthetic compounds were in agreement with ^{1}H NMR, ^{13}C NMR, IR and mass spectroscopic data.

5.2.1.1. Ethyl 3-(2-methoxyphenyl)-3-oxopropanoate (10b)

Yield 86% from **8b** as a yellow liquid; IR (KBr) ν (cm^{-1}): 1735.93, 1674.21 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.14 (t, J = 7.2 Hz, 3H, -O-$CH_2C\underline{H_3}$), 3.83 (s, 3H, -O$C\underline{H_3}$), 3.91 (s, 2H, -CO-$C\underline{H_2}$-CO-), 4.07 (q, J = 7.2 Hz, 2H, -O-$C\underline{H_2}CH_3$), 7.03 (t, J = 7.6 Hz, 1H, Ar-H), 7.14 (d, J = 8.2 Hz, 1H, Ar-H), 7.56 (t, J = 7.6 Hz, 1H, Ar-H), 7.70 (d, J = 7.8 Hz, 1H, Ar-H); ^{13}C NMR (50 MHz, DMSO-d_6): δ 14.37, 50.48,

56.03, 60.80, 112.95, 120.99, 126.11, 130.50, 135.37, 159.37, 168.20, 193.25; MS (EI, 70 eV) *m/z*: 222.1 (M^+); HRMS (EI) *m/z*: calculated for $C_{12}H_{14}O_4$: 222.0892; found: 222.0884.

5.2.1.2. Ethyl 3-(3-methoxyphenyl)-3-oxopropanoate (10c)

Yield 80% from **8c** as a yellow liquid; IR (KBr) ν (cm^{-1}): 1743.65, 1689.64 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.16 (t, *J* = 7.2 Hz, 3H, -O-$CH_2C\underline{H}_3$), 3.80 (s, 3H, -O$C\underline{H}_3$), 4.06-4.20 (m, 4H, -CO-$C\underline{H}_2$-CO- and -O-$C\underline{H}_2CH_3$), 7.19-7.25 (m, 1H, ArH), 7.40-7.57 (m, 3H, ArH); ^{13}C NMR (50 MHz, DMSO-d_6): δ 14.36, 46.12, 55.73, 61.05, 113.31, 120.18, 121.34, 130.38, 137.64, 159.91, 168.08, 193.67; MS (EI, 70 eV) *m/z*: 222.1 (M^+); HRMS (EI) *m/z*: calculated for $C_{12}H_{14}O_4$: 222.0892; found: 222.0898.

5.2.1.3. Ethyl 3-(4-methoxyphenyl)-3-oxopropanoate (10d)

Yield 87% from **8d** as a yellow liquid; IR (KBr) ν (cm^{-1}): 1735.93, 1674.21 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.16 (t, *J* = 7.2 Hz, 3H, -O-$CH_2C\underline{H}_3$), 3.82 (s, 3H, -O$C\underline{H}_3$), 4.05-4.15 (m, 4H, -CO-$C\underline{H}_2$-CO- and -O-$C\underline{H}_2CH_3$), 7.03 (d, *J* = 8.9 Hz, 2H, ArH), 7.93 (d, *J* = 8.9 Hz, 2H, ArH); ^{13}C NMR (50 MHz, DMSO-d_6): δ 14.36, 45.71, 55.95, 60.97, 114.40 (2C), 129.24, 131.24 (2C), 164.04, 168.25, 192.06; MS (EI, 70 eV) *m/z*: 222.1 (M^+); HRMS (EI) *m/z*: calculated for $C_{12}H_{14}O_4$: 222.0892; found: 222.0899.

5.2.1.4. Ethyl 3-(2,4-dimethoxyphenyl)-3-oxopropanoate (10e)

Yield 70% from **8e** as a yellow liquid; IR (KBr) ν (cm^{-1}): 1735.93, 1666.50 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.16 (t, *J* = 7.2 Hz, 3H, -O-$CH_2C\underline{H}_3$), 3.83-3.84 (m, 8H, 2 × -O$C\underline{H}_3$ and -CO-$C\underline{H}_2$-CO-), 4.08 (q, *J* = 7.2 Hz, 2H, -O-$C\underline{H}_2CH_3$), 6.58-6.62 (m, 2H, ArH), 7.75 (d, *J* = 9.4 Hz, 1H, ArH); ^{13}C NMR (50 MHz, DMSO-d_6): δ 14.39, 50.42, 56.03 (2C), 60.64, 98.59, 106.83, 119.11, 132.61,

161.57, 165.47, 168.46, 190.91; MS (EI, 70 eV) *m/z*: 252.1 (M^+); HRMS (EI) *m/z*: calculated for $C_{13}H_{16}O_5$: 252.0998; found: 252.0991.

5.2.1.5. Ethyl 3-(2,5-dimethoxyphenyl)-3-oxopropanoate (10f)

Yield 70% from **8f** as a yellow liquid; IR (KBr) ν (cm^{-1}): 1735.93, 1674.21 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.16 (t, *J* = 7.1 Hz, 3H, -O-$CH_2C\underline{H}_3$), 3.73 (s, 3H, -OC$\underline{H}_3$), 3.80 (s, 3H, -OC$\underline{H}_3$), 3.91 (s, 2H, -CO-C$\underline{H}_2$-CO-), 4.08 (q, *J* = 7.1 Hz, 2H, -O-C$\underline{H}_2CH_3$), 7.12-7.15 (m, 2H, ArH), 7.22-7.24 (m, 1H, ArH); ^{13}C NMR (50 MHz, DMSO-d_6): δ 14.38, 50.38, 55.90, 56.42, 60.75, 113.94, 114.51, 121.47, 126.35, 153.40, 153.81, 168.12, 192.75; MS (EI, 70 eV) *m/z*: 252.2 (M^+); HRMS (EI) *m/z*: calculated for $C_{13}H_{16}O_5$: 252.0998; found: 252.0996.

5.2.1.6. Ethyl 3-(2,6-dimethoxyphenyl)-3-oxopropanoate (10g)

Yield 78% from **8g** as a colorless liquid; IR (KBr) ν (cm^{-1}): 1743.65, 1705.07 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.14 (t, *J* = 7.1 Hz, 3H, -O-$CH_2C\underline{H}_3$), 3.75 (s, 8H, 2 × -OC$\underline{H}_3$ and -CO-C$\underline{H}_2$-CO-), 4.06 (q, *J* = 7.1 Hz, 2H, -O-C$\underline{H}_2CH_3$), 6.70 (d, *J* = 8.4 Hz, 2H, ArH), 7.35 (t, *J* = 8.4 Hz, 1H, ArH); ^{13}C NMR (50 MHz, DMSO-d_6): δ 14.30, 51.47, 56.26 (2C), 60.84, 104.71 (2C), 118.63, 132.07, 156.98 (2C), 166.82, 195.95; MS (EI, 70 eV) *m/z*: 252.2 (M^+); HRMS (EI) *m/z*: calculated for $C_{13}H_{16}O_5$: 252.0998; found: 252.1006.

5.2.1.7. Ethyl 3-(3,4-dimethoxyphenyl)-3-oxopropanoate (10h)

Yield 76% from **8h** as a yellow liquid; IR (KBr) ν (cm^{-1}): 1735.93, 1674.21 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.16 (t, *J* = 7.1 Hz, 3H, -O-$CH_2C\underline{H}_3$), 3.80 (s, 3H, -OC$\underline{H}_3$), 3.83 (s, 3H, -OC$\underline{H}_3$), 4.04-4.15 (m, 4H, -CO-C$\underline{H}_2$-CO- and -O-C$\underline{H}_2CH_3$), 7.02 (d, *J* = 8.4 Hz, 1H, ArH), 7.43 (d, *J* = 1.4 Hz, 1H, ArH), 7.59 (dd, *J* = 8.4, 1.2 Hz, 1H, ArH); ^{13}C NMR (50 MHz, DMSO-d_6): δ 14.34, 45.62, 55.85, 56.10, 60.98, 110.78, 111.20, 123.85, 129.19, 149.11, 154.03, 168.29, 192.09; MS (EI, 70 eV)

m/z: 252.2 (M^+); HRMS (EI) *m/z*: calculated for $C_{13}H_{16}O_5$: 252.0998; found: 252.1003.

5.2.1.8. Ethyl 3-(3,5-dimethoxyphenyl)-3-oxopropanoate (10i)

Yield 81% from **8i** as a yellow liquid; IR (KBr) ν (cm^{-1}): 1735.93, 1689.64 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.17 (t, *J* = 7.1 Hz, 3H, -O-$CH_2C\underline{H}_3$), 3.79 (s, 6H, 2 × -O$C\underline{H}_3$), 4.06-4.15 (m, 4H, -CO-$C\underline{H}_2$-CO- and -O-$C\underline{H}_2CH_3$), 6.77 (s, 1H, ArH), 7.07-7.08 (m, 2H, ArH); ^{13}C NMR (50 MHz, DMSO-d_6): δ 14.35, 46.16, 55.87 (2C), 61.04, 105.95, 106.57 (2C), 138.21, 161.07 (2C), 168.05, 193.47; MS (EI, 70 eV) *m/z*: 252.2 (M^+); HRMS (EI) *m/z*: calculated for $C_{13}H_{16}O_5$: 252.0998; found: 252.0994.

5.2.1.9. Ethyl 3-oxo-3-(2,4,6-trimethoxyphenyl)propanoate (10j)

Yield 60% from **8j** as a white solid; mp: 69-71 °C; IR (KBr) ν (cm^{-1}): 1728.22, 1689.64 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.13 (t, *J* = 7.1 Hz, 3H, -O-$CH_2C\underline{H}_3$), 3.70 (s, 2H, -CO-$C\underline{H}_2$-CO-), 3.74 (s, 6H, 2 × -O$C\underline{H}_3$), 3.80 (s, 3H, -O$C\underline{H}_3$), 4.05 (q, *J* = 7.1 Hz, 2H, -O-$C\underline{H}_2CH_3$), 6.25 (s, 2H, ArH); ^{13}C NMR (50 MHz, DMSO-d_6): δ 14.41, 51.47, 55.95, 56.32 (2C), 60.72, 91.37 (2C), 111.65, 158.81 (2C), 163.17, 167.26, 194.47; MS (EI, 70 eV) *m/z*: 282.2 (M^+); HRMS (EI) *m/z*: calculated for $C_{14}H_{18}O_6$: 282.1103; found: 282.1106.

5.2.1.10. Ethyl 3-oxo-3-(3,4,5-trimethoxyphenyl)propanoate (10k)

Yield 69% from **8k** as a colorless liquid; IR (KBr) ν (cm^{-1}): 1743.65, 1681.93 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.18 (t, *J* = 7.1 Hz, 3H, -O-$CH_2C\underline{H}_3$), 3.75 (s, 3H, -O$C\underline{H}_3$), 3.84 (s, 6H, 2 × -O$C\underline{H}_3$), 4.07-4.19 (m, 4H, -CO-$C\underline{H}_2$-CO- and -O-$C\underline{H}_2CH_3$), 7.25 (s, 2H, ArH); ^{13}C NMR (50 MHz, DMSO-d_6): δ 14.36, 45.88, 56.41 (2C), 60.52, 61.01, 106.46 (2C), 131.51, 142.82, 153.24 (2C), 168.18, 192.68; MS (EI, 70 eV) *m/z*: 282.2 (M^+); HRMS (EI) *m/z*: calculated for $C_{14}H_{18}O_6$: 282.1103;

found: 282.1102.

5.2.1.11. Ethyl 3-(2-fluorophenyl)-3-oxopropanoate (10l)

Yield 73% from **8l** as a yellow liquid; IR (KBr) ν (cm^{-1}): 1743.65, 1689.64 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.15 (t, J = 7.1 Hz, 3H, -O-$CH_2C\underline{H_3}$), 4.04-4.15 (m, 4H, -CO-$C\underline{H_2}$-CO- and -O-$C\underline{H_2}CH_3$), 7.29-7.39 (m, 2H, ArH), 7.64-7.72 (m, 1H, ArH), 7.82-7.87 (m, 1H, ArH); ^{13}C NMR (50 MHz, DMSO-d_6): δ 14.36, 49.52 (d, $^{4}J_{CF}$ = 6.0 Hz, 1C), 61.09, 117.33 (d, $^{2}J_{CF}$ = 23.0 Hz, 1C), 124.77 (d, $^{2}J_{CF}$ = 11.5 Hz, 1C), 125.33, 130.89, 136.27 (d, $^{3}J_{CF}$ = 9.0 Hz, 1C), 161.74 (d, $^{1}J_{CF}$ = 253.0 Hz, 1C), 167.76, 191.09; MS (EI, 70 eV) *m/z*: 210.1 (M^+); HRMS (EI) *m/z*: calculated for $C_{11}H_{11}FO_3$: 210.0692; found: 210.0700.

5.2.1.12. Ethyl 3-(3-fluorophenyl)-3-oxopropanoate (10m)

Yield 69% from **8m** as a yellow liquid; IR (KBr) ν (cm^{-1}): 1743.65, 1689.64 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.16 (t, J = 7.1 Hz, 3H, -O-$CH_2C\underline{H_3}$), 4.11 (q, J = 7.1 Hz, 2H, -O-$C\underline{H_2}CH_3$), 4.20 (s, 2H, -CO-$C\underline{H_2}$-CO-), 7.46-7.64 (m, 2H, ArH), 7.68-7.83 (m, 2H, ArH); ^{13}C NMR (50 MHz, DMSO-d_6): δ 14.36, 46.08, 61.13, 115.35 (d, $^{2}J_{CF}$ = 22.5 Hz, 1C), 121.12 (d, $^{2}J_{CF}$ = 21.5 Hz, 1C), 125.06, 131.45 (d, $^{3}J_{CF}$ = 8.0 Hz, 1C), 138.45 (d, $^{3}J_{CF}$ = 6.5 Hz, 1C), 162.64 (d, $^{1}J_{CF}$ = 244.0 Hz, 1C), 167.90, 193.00; MS (EI, 70 eV) *m/z*: 210.1 (M^+); HRMS (EI) *m/z*: calculated for $C_{11}H_{11}FO_3$: 210.0692; found: 210.0695.

5.2.1.13. Ethyl 3-(4-fluorophenyl)-3-oxopropanoate (10n)

Yield 77% from **8n** as a yellow liquid; IR (KBr) ν (cm^{-1}): 1743.65, 1689.64 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.16 (t, J = 7.1 Hz, 3H, -O-$CH_2C\underline{H_3}$), 4.10 (q, J = 7.1 Hz, 2H, -O-$C\underline{H_2}CH_3$), 4.17 (s, 2H, -CO-$C\underline{H_2}$-CO-), 7.30-7.39 (m, 2H, ArH), 8.00-8.07 (m, 2H, ArH); ^{13}C NMR (50 MHz, DMSO-d_6): δ 14.36, 45.94, 61.08, 116.28 (d, $^{2}J_{CF}$ = 21.9 Hz, 2C), 131.43 (d, $^{3}J_{CF}$ = 9.6 Hz, 2C), 133.05, 165.80 (d, $^{1}J_{CF}$

= 251.0 Hz, 1C), 168.01, 192.53; MS (EI, 70 eV) *m/z*: 210.1 (M^+); HRMS (EI) *m/z*: calculated for $C_{11}H_{11}FO_3$: 210.0692; found: 210.0701.

5.2.2. General procedure for the synthesis of benzoylacetanilides 11a-n

A mixture of the substituted benzoylacetate (**10a-n**, 1 equiv) and 3,4-methylenedioxy aniline (1 equiv) was stirred in 150 mL toluene and then heated at reflux for 1-2 h. The mixture was cooled to the room temperature and partitioned with 10% NaOH (3 × 50 mL). The aqueous layer was acidified to pH 4-5 with dropwise addition of glacial acetic acid. The resulting precipitate was isolated by suction filtration, washed with water and EtOH, and then air-dried air to give the desired benzoylacetanilide (**11a-n**) of sufficient purity for the next reaction.

5.2.2.1. *N*-(Benzo[d][1,3]dioxol-5-yl)-3-oxo-3-phenylpropanamide (11a)

Yield 45% from **10a** as a light-yellow solid; mp: 149-151 °C; IR (KBr) ν (cm^{-1}): 1654.03, 1684.89 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6): δ 4.09 (s, 2H, -C$\underline{H_2}$-), 5.97 (s, 2H, -O-C$\underline{H_2}$-O-), 6.84 (d, *J* = 8.4 Hz, 1H, ArH), 6.94 (dd, *J* = 8.4, 2.0 Hz, 1H, ArH), 7.28 (d, *J* = 2.0 Hz, 1H, ArH), 7.51-7.58 (m, 2H, ArH), 7.63-7.74 (m, 1H, ArH), 8.00 (d, *J* = 7.2 Hz, 1H, ArH), 10.11 (s, 1H, NH); ^{13}C NMR (50 MHz, DMSO-d_6): δ 48.41, 101.43, 101.67, 108.53, 112.33, 128.81 (2C), 129.25 (2C), 133.83, 134.03, 136.72, 143.45, 147.53, 165.39, 195.04; MS (EI, 70 eV) *m/z*: 283.2 (M^+); HRMS (EI) *m/z*: calculated for $C_{16}H_{13}NO_4$: 283.0845; found: 283.0836.

5.2.2.2. *N*-(Benzo[d][1,3]dioxol-5-yl)-3-(2-methoxyphenyl)-3-oxopropanamide (11b)

Yield 38% from **10b** as a white solid; mp: 143-144 °C; IR (KBr) ν (cm^{-1}): 1656.92, 1672.36 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.85 (s, 3H, -OC$\underline{H_3}$), 3.97 (s, 2H, -C$\underline{H_2}$-), 5.96 (s, 2H, -O-C$\underline{H_2}$-O-), 6.83 (d, *J* = 8.4 Hz, 1H, ArH), 6.94 (dd, *J* = 8.4, 1.6 Hz, 1H, ArH), 7.04 (t, *J* = 7.4 Hz, 1H, ArH), 7.15 (d, *J* = 8.2 Hz, 1H,

ArH), 7.28 (d, *J* = 1.6 Hz, 1H, ArH), 7.56 (t, *J* = 7.8 Hz, 1H, ArH), 7.69 (d, *J* = 7.7 Hz, 1H, ArH), 10.01 (s, 1H, NH); ^{13}C NMR (50 MHz, DMSO-d_6): δ 52.96, 56.23, 101.39, 101.65, 108.51, 112.23, 113.01, 120.94, 126.91, 130.45, 133.99, 134.96, 143.29, 147.49, 159.23, 165.82, 195.04; MS (EI, 70 eV) *m/z*: 313.2 (M^+); HRMS (EI) *m/z*: calculated for $C_{17}H_{15}NO_5$: 313.0950; found: 313.0959.

5.2.2.3. *N*-(Benzo[d][1,3]dioxol-5-yl)-3-(3-methoxyphenyl)-3-oxopropanamide (11c)

Yield 37% from **10c** as a pale gray solid; mp: 141-142 °C; IR (KBr) ν (cm^{-1}): 1656.92, 1685.86 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.81 (s, 3H, -OC<u>H</u>$_3$), 4.08 (s, 2H, -C<u>H</u>$_2$-), 5.97 (s, 2H, -O-C<u>H</u>$_2$-O-), 6.84 (d, *J* = 8.4 Hz, 1H, ArH), 6.93 (dd, *J* = 8.4, 1.8 Hz, 1H, ArH), 7.21-7.50 (m, 4H, ArH), 7.59 (d, *J* = 7.6 Hz, 1H, ArH), 10.12 (s, 1H, NH); ^{13}C NMR (50 MHz, DMSO-d_6): δ 48.57, 55.84, 101.44, 101.68, 108.53, 112.34, 113.36, 119.98, 121.34, 130.43, 133.81, 138.11, 143.46, 147.54, 159.91, 165.35, 194.79; MS (EI, 70 eV) *m/z*: 313.2 (M^+); HRMS (EI) *m/z*: calculated for $C_{17}H_{15}NO_5$: 313.0950; found: 313.0958.

5.2.2.4. *N*-(Benzo[d][1,3]dioxol-5-yl)-3-(4-methoxyphenyl)-3-oxopropanamide (11d)

Yield 38% from **10d** as a light-brown solid; mp: 144-145 °C; IR (KBr) ν (cm^{-1}): 1655.96, 1674.28 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.84 (s, 3H, -OC<u>H</u>$_3$), 4.02(s, 2H, -C<u>H</u>$_2$-), 5.97 (s, 2H, -O-C<u>H</u>$_2$-O-), 6.84 (d, *J* = 8.4 Hz, 1H, ArH), 6.93 (dd, *J* = 8.4, 1.8 Hz, 1H, ArH), 7.06 (d, *J* = 8.8 Hz, 2H, ArH), 7.27 (d, *J* = 1.6 Hz, 1H, ArH), 7.97 (d, *J* = 8.8 Hz, 2H, ArH), 10.09 (s, 1H, NH); ^{13}C NMR (50 MHz, DMSO-d_6): δ 48.15, 56.06, 101.42, 101.64, 108.52, 112.30, 114.45 (2C), 129.70, 131.23 (2C), 133.87, 143.40, 147.50, 163.89, 165.57, 193.34; MS (EI, 70 eV) *m/z*: 313.1 (M^+); HRMS (EI) *m/z*: calculated for $C_{17}H_{15}NO_5$: 313.0950; found: 313.0944.

5.2.2.5. *N*-(Benzo[d][1,3]dioxol-5-yl)-3-(2,4-dimethoxyphenyl)-3-oxopropanamide (11e)

Yield 44 % from **10e** as a white solid; mp: 164-166 °C; IR (KBr) ν (cm^{-1}): 1652.10, 1676.21 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.83 (s, 3H, -OC<u>H</u>$_3$), 3.85 (s, 3H, -OC<u>H</u>$_3$), 3.89 (s, 2H, -C<u>H</u>$_2$-), 5.96 (s, 2H, -O-C<u>H</u>$_2$-O-), 6.61-6.64 (m, 2H, ArH), 6.83 (d, *J* = 8.4 Hz, 1H, ArH), 6.93 (dd, *J* = 8.4, 1.8 Hz, 1H, ArH), 7.27 (d, *J* = 1.6 Hz, 1H, ArH), 7.73 (d, *J* = 9.4 Hz, 1H, ArH), 9.96 (s, 1H, NH); ^{13}C NMR (50 MHz, DMSO-d_6): δ 52.90, 56.13, 56.29, 98.76, 101.36, 101.60, 106.78, 108.50, 112.16, 119.73, 132.62, 134.09, 143.21, 147.48, 161.52, 165.20, 166.19, 192.68; MS (EI, 70 eV) *m/z*: 343.2 (M^+); HRMS (EI) *m/z*: calculated for $C_{18}H_{17}NO_6$: 343.1056; found: 343.1049.

5.2.2.6. *N*-(Benzo[d][1,3]dioxol-5-yl)-3-(2,5-dimethoxyphenyl)-3-oxopropanamide (11f)

Yield 48% from **10f** as a light-brown solid; mp: 144-146 °C; IR (KBr) ν (cm^{-1}): 1646.32, 1668.50 (C=O); ^{1}H NMR (200 MHz, $CDCl_3$-d_1): δ 3.73 (s, 3H, -OC<u>H</u>$_3$), 3.80 (s, 3H, -OC<u>H</u>$_3$), 3.95(s, 2H, -C<u>H</u>$_2$-), 5.96 (s, 2H, -O-C<u>H</u>$_2$-O-), 6.83 (d, *J* = 8.6 Hz, 1H, ArH), 6.89-6.95 (m, 1H, ArH), 7.08-7.31 (m, 4H, ArH), 10.00 (s, 1H, NH); ^{13}C NMR (50 MHz, $CDCl_3$-d_1): δ 52.88, 56.00, 56.68, 101.38, 101.61, 108.53, 112.19, 114.04, 114.61, 121.01, 127.11, 133.98, 143.27, 147.49, 153.34, 153.65, 165.80, 194.58; MS (EI, 70 eV) *m/z*: 343.2 (M^+); HRMS (EI) *m/z*: calculated for $C_{18}H_{17}NO_6$: 343.1056; found: 343.1051.

5.2.2.7. *N*-(Benzo[d][1,3]dioxol-5-yl)-3-(2,6-dimethoxyphenyl)-3-oxopropanamide (11g)

Yield 59% from **10g** as a white solid; mp: 131-133 °C; IR (KBr) ν (cm^{-1}): 1654.03, 1709.97 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.72 (s, 6H, -OC<u>H</u>$_3$),

3.74 (s, 2H, -C<u>H</u>$_2$-), 5.96 (s, 2H, -O-C<u>H</u>$_2$-O-), 6.69 (d, *J* = 8.4 Hz, 2H, ArH), 6.82 (d, *J* = 8.4 Hz, 1H, ArH), 6.92 (dd, *J* = 8.4, 1.8 Hz, 1H, ArH), 7.25 (d, *J* = 1.8 Hz, 1H, ArH), 7.33 (t, *J* = 8.4 Hz, 1H, ArH), 9.94 (s, 1H, NH); ^{13}C NMR (50 MHz, DMSO-*d*$_6$): δ 53.89, 56.36 (2C), 101.40,101.76, 104.81 (2C), 108.46, 112.43, 119.26, 131.84, 133.89, 143.37, 147.44, 156.95 (2C), 164.02, 197.56; MS (EI, 70 eV) *m/z*: 342.9 (M^+); HRMS (EI) *m/z*: calculated for $C_{18}H_{17}NO_6$: 343.1056; found: 343.1062.

5.2.2.8. *N*-(Benzo[d][1,3]dioxol-5-yl)-3-(3,4-dimethoxyphenyl)-3-oxopropanamide (11h)

Yield 39% from **10h** as a pale gray solid; mp: 191-192 °C; IR (KBr) ν (cm^{-1}): 1653.07, 1676.21 (C=O); ^{1}H NMR (200 MHz, DMSO-*d*$_6$): δ 3.80 (s, 3H, -OC<u>H</u>$_3$), 3.84 (s, 3H, -OC<u>H</u>$_3$), 4.03(s, 2H, -C<u>H</u>$_2$-), 5.96 (s, 2H, -O-C<u>H</u>$_2$-O-), 6.84 (d, *J* = 8.4 Hz, 1H, ArH), 6.94 (dd, *J* = 8.3, 1.8 Hz, 1H, ArH), 7.08 (d, *J* = 8.4 Hz, 1H, ArH), 7.27 (d, *J* = 1.6 Hz, 1H, ArH), 7.47 (d, *J* = 1.6 Hz, 1H, ArH), 7.67 (dd, *J* = 8.4, 1.8 Hz, 1H, ArH), 10.12 (s, 1H, NH); ^{13}C NMR (50 MHz, DMSO-*d*$_6$): δ 48.15, 56.01, 56.25, 101.43, 101.65, 108.52, 110.90, 111.39, 112.30, 123.86, 129.67, 133.88, 143.42, 147.52, 149.08, 153.86, 165.57, 193.34; MS (EI, 70 eV) *m/z*: 343.1 (M^+); HRMS (EI) *m/z*: calculated for $C_{18}H_{17}NO_6$: 343.1056; found: 343.1050.

5.2.2.9. *N*-(Benzo[d][1,3]dioxol-5-yl)-3-(3,5-dimethoxyphenyl)-3-oxopropanamide (11i)

Yield 61% from **10i** as a light-brown solid; mp: 158-160 °C; IR (KBr) ν (cm^{-1}): 1653.07, 1695.50 (C=O); ^{1}H NMR (200 MHz, DMSO-*d*$_6$): δ 3.79 (s, 6H, -OC<u>H</u>$_3$), 4.06 (s, 2H, -C<u>H</u>$_2$-), 5.97 (s, 2H, -O-C<u>H</u>$_2$-O-), 6.77-6.86 (m, 2H, ArH), 6.92 (dd, *J* = 8.4, 2.0 Hz, 1H, ArH), 7.10 (d, *J* = 2.2 Hz, 2H, ArH), 7.26 (d, *J* = 2.0 Hz, 1H, ArH), 10.12 (s, 1H, NH); ^{13}C NMR (50 MHz, DMSO-*d*$_6$): δ 48.65, 56.03 (2C), 101.44, 101.64, 105.80, 106.60 (2C), 108.56, 112.31, 133.78, 138.67, 143.46, 147.53, 161.08

(2C), 165.30, 194.59; MS (EI, 70 eV) *m/z*: 342.9 (M^+); HRMS (EI) *m/z*: calculated for $C_{18}H_{17}NO_6$: 343.1056; found: 343.1059.

5.2.2.10. *N*-(Benzo[d][1,3]dioxol-5-yl)-3-oxo-3-(2,4,6-trimethoxyphenyl)propanamide (11j)

Yield 51% from **10j** as a white solid; mp: 124-126 °C; IR (KBr) ν (cm^{-1}): 1652.10, 1706.11 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.69 (s, 2H, -C<u>H</u>$_2$-), 3.72 (s, 6H, -OC<u>H</u>$_3$), 3.79 (s, 3H, -OC<u>H</u>$_3$), 5.96 (s, 2H, -O-C<u>H</u>$_2$-O-), 6.24 (s, 2H, ArH), 6.82 (d, *J* = 8.4 Hz, 1H, ArH), 6.92 (dd, *J* = 8.4, 1.8 Hz, 1H, ArH), 7.25 (d, *J* = 1.8 Hz, 1H, ArH), 9.90 (s, 1H, NH); ^{13}C NMR (50 MHz, DMSO-d_6): δ 53.90, 55.95, 56.36 (2C), 91.41 (2C), 101.38, 101.69, 108.46, 112.33 (2C), 133.96, 143.29, 147.42, 158.72 (2C), 162.91, 164.57, 197.17; MS (EI, 70 eV) *m/z*: 372.6 (M^+); HRMS (EI) *m/z*: calculated for $C_{19}H_{19}NO_7$: 373.1162; found: 373.1166.

5.2.2.11. *N*-(Benzo[d][1,3]dioxol-5-yl)-3-oxo-3-(3,4,5-trimethoxyphenyl)propanamide (11k)

Yield 27% from **10k** as a white solid; mp: 186-188 °C; IR (KBr) ν (cm^{-1}): 1649.21, 1672.36 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.74 (s, 3H, -OC<u>H</u>$_3$), 3.83 (s, 6H, -OC<u>H</u>$_3$), 4.08 (s, 2H, -C<u>H</u>$_2$-), 5.97 (s, 2H, -O-C<u>H</u>$_2$-O-), 6.84 (d, *J* = 8.4 Hz, 1H, ArH), 6.93 (dd, *J* = 8.4, 2.0 Hz, 1H, ArH), 7.26-7.29 (m, 3H, ArH), 10.16 (s, 1H, NH); ^{13}C NMR (50 MHz, DMSO-d_6): δ 48.42, 56.59 (2C), 60.65, 101.45, 101.67, 106.48 (2C), 108.56, 112.36, 131.98, 133.76, 142.65, 143.47, 147.53, 153.25 (2C), 165.46, 193.82; MS (EI, 70 eV) *m/z*: 373.0 (M^+); HRMS (EI) *m/z*: calculated for $C_{19}H_{19}NO_7$: 373.1162; found: 373.1170.

5.2.2.12. *N*-(Benzo[d][1,3]dioxol-5-yl)-3-(2-fluorophenyl)-3-oxopropanamide (11l)

Yield 61% from **10l** as a white solid; mp: 147-148 °C; IR (KBr) ν (cm^{-1}):

1653.07, 1691.64 (C=O); ^{1}H NMR (200 MHz, $CDCl_3$-d_1): δ 4.10 (d, *J* = 2.4 Hz, 2H, -C<u>H</u>$_2$-), 5.93 (s, 2H, -O-C<u>H</u>$_2$-O-), 6.73 (d, *J* = 8.2 Hz, 1H, ArH), 6.86 (dd, *J* = 8.2, 2.2 Hz, 1H, ArH), 7.11-7.22 (m, 2H, ArH), 7.26 (d, *J* = 2.2 Hz, 1H, ArH), 7.53-7.60 (m, 1H, ArH), 7.84-7.92 (m, 1H, ArH), 9.00 (s, 1H, NH); ^{13}C NMR (50 MHz, $CDCl_3$-d_1): δ 49.45 (d, $^{4}J_{CF}$ = 8.0 Hz, 1C), 101.27, 103.11, 108.05, 113.51, 117.01 (d, $^{2}J_{CF}$ = 23.0 Hz, 1C), 124.56 (d, $^{2}J_{CF}$ = 21.5 Hz, 1C), 124.77, 130.75, 131.79, 135.84 (d, $^{3}J_{CF}$ = 9.5 Hz, 1C), 144.46, 147.79, 161.98 (d, $^{1}J_{CF}$ = 254.5 Hz, 1C), 163.62, 194.75; MS (EI, 70 eV) *m/z*: 301.0 (M^+); HRMS (EI) *m/z*: calculated for $C_{16}H_{12}FNO_4$: 301.0750; found: 301.0760.

5.2.2.13. *N*-(Benzo[d][1,3]dioxol-5-yl)-3-(3-fluorophenyl)-3-oxopropanamide (11m)

Yield 62% from **10m** as a pale gray solid; mp: 130-132 °C; IR (KBr) ν (cm^{-1}): 1655.96, 1695.50 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6): δ 4.10 (s, 2H, -C<u>H</u>$_2$-), 5.96 (s, 2H, -O-C<u>H</u>$_2$-O-), 6.84 (d, *J* = 8.4 Hz, 1H, ArH), 6.92 (dd, *J* = 8.4, 1.8 Hz, 1H, ArH), 7.25 (d, *J* = 1.8 Hz, 1H, ArH), 7.51-7.86 (m, 4H, ArH), 10.13 (s, 1H, NH); ^{13}C NMR (50 MHz, DMSO-d_6): δ 48.51, 101.41, 101.71, 108.56, 112.35, 115.31 (d, $^{2}J_{CF}$ = 22.5 Hz, 1C), 120.94 (d, $^{2}J_{CF}$ = 21.0 Hz, 1C), 125.09, 131.39, 133.75, 138.88, 143.48, 147.55, 162.69 (d, $^{1}J_{CF}$ = 244.0 Hz, 1C), 165.13, 194.09; MS (EI, 70 eV) *m/z*: 300.9 (M^+); HRMS (EI) *m/z*: calculated for $C_{16}H_{12}FNO_4$: 301.0750; found: 301.0747.

5.2.2.14. *N*-(Benzo[d][1,3]dioxol-5-yl)-3-(4-fluorophenyl)-3-oxopropanamide (11n)

Yield 59% from **10n** as a white solid; mp: 164-165 °C; IR (KBr) ν (cm^{-1}): 1633.78, 1690.68 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6): δ 4.09 (s, 2H, -C<u>H</u>$_2$-), 5.96 (s, 2H, -O-C<u>H</u>$_2$-O-), 6.83 (d, *J* = 8.4 Hz, 1H, ArH), 6.93 (dd, *J* = 8.4, 1.8 Hz, 1H, ArH), 7.27 (d, *J* = 1.8 Hz, 1H, ArH), 7.32-7.41 (m, 2H, ArH), 8.04-8.11 (m, 2H, ArH), 10.11

(s, 1H, NH); ^{13}C NMR (50 MHz, DMSO-d_6): δ 48.36, 101.44, 101.68, 108.53, 112.35, 116.29 (d, $^{2}J_{CF}$ = 21.5 Hz, 2C), 131.90 (d, $^{3}J_{CF}$ = 9.5 Hz, 2C), 133.47, 133.77, 143.47, 147.53, 165.30, 165.69 (d, $^{1}J_{CF}$ = 250.5 Hz, 1C), 193.71; MS (EI, 70 eV) *m/z*: 300.9 (M^+); HRMS (EI) *m/z*: calculated for $C_{16}H_{12}FNO_4$: 301.0750; found: 301.0755.

5.2.3. General procedure for the synthesis of 6,7-methylenedioxy-4-substituted phenylquinolin-2-one derivatives 12a-n

A mixture of the benzoylacetanilide (**11a-n**) and PPA (10 g) was heated at 100-110 °C for 0.5 h to 1 h. The mixture was cooled after TLC monitoring indicated that the reaction was completed, and then the reaction mixture diluted with ice water, and extracted with $CHCl_3$ (3 × 50 mL). The combined organic layers were dried over anhydrous $MgSO_4$, and the solvent was removed *in vacuo*. The crude products were purified by column chromatography (silica gel, chloroform/ethyl acetate = 2/1) to give the corresponding pure 4-phenylquinolin-2-ones **12a-n**.

5.2.3.1. 6,7-Methylenedioxy-4-phenylquinolin-2(*1H*)-one (12a)

Compound **12a** (0.16 g, 0.60 mmol) was obtained by cyclization of **11a** (0.27 g, 0.95 mmol) with PPA (10 g); Yield: 63%; white solid; mp: 274-275 °C; IR (KBr) ν (cm^{-1}): 1653.07 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6): δ 6.02 (s, 2H, -O-C<u>H$_2$</u>-O-), 6.18 (s, 1H, Ar-H), 6.63 (s, 1H, Ar-H), 6.87 (s, 1H, Ar-H), 7.36-7.51 (m, 5H, Ar-H), 11.78 (br. s, 1H, NH); ^{13}C NMR (50 MHz, $CDCl_3$-d_1): δ 45.27, 101.25, 103.00, 108.04, 113.43, 128.57 (2C), 128.95 (2C), 131.81, 134.34, 136.05, 144.42, 147.76, 163.61, 196.51; MS (EI, 70 eV) *m/z*: 265.1 (M^+); HRMS (EI) *m/z*: calculated for $C_{16}H_{11}NO_3$: 265.0739; found: 265.0733.

5.2.3.2. 6,7-Methylenedioxy-4-(2-methoxyphenyl)quinolin-2*(1H)*-one (12b)

Compound **12b** (0.19 g, 0.64 mmol) was obtained by cyclization of **11b** (0.29 g, 0.93 mmol) with PPA (10 g); Yield: 68%; white solid; mp: 269-271 °C; IR (KBr) ν

(cm^{-1}): 1663.07 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.65 (s, 3H, -OC<u>H</u>$_3$), 5.99, 6.01 (s, each 1H, -O-C<u>H</u>$_2$-O-), 6.13 (s, 1H, Ar-H), 6.30 (s, 1H, Ar-H), 6.84 (s, 1H, Ar-H), 7.02 (t, *J* = 7.4 Hz, 1H, Ar-H), 7.10-7.17 (m, 2H, Ar-H), 7.43 (t, *J* = 7.4 Hz, 1H, Ar-H), 11.71 (br. s, 1H, NH); ^{13}C NMR (50 MHz, DMSO-d_6): δ 55.89, 95.79, 102.26, 103.90, 112.00, 113.69, 119.44, 121.17, 126.27, 130.37, 130.77, 136.09, 143.71, 149.69, 150.35, 156.41, 161.85; MS (EI, 70 eV) *m/z*: 295.1 (M^+); HRMS (EI) *m/z*: calculated for $C_{17}H_{13}NO_4$: 295.0845; found: 295.0835.

5.2.3.3. 6,7-Methylenedioxy-4-(3-methoxyphenyl)quinolin-2(*1H*)-one (12c)

Compound **12c** (0.17 g, 0.58 mmol) was obtained by cyclization of **11c** (0.30 g, 0.96 mmol) with PPA (10 g); Yield: 60%; white solid; mp: 278-279 °C; IR (KBr) ν (cm^{-1}): 1653.07 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.75 (s, 3H, -OC<u>H</u>$_3$), 6.02 (s, 2H, -O-C<u>H</u>$_2$-O-), 6.19 (s, 1H, Ar-H), 6.66 (s, 1H, Ar-H), 6.85 (s, 1H, Ar-H), 6.90-6.93 (m, 2H, Ar-H), 7.00 (dd, *J* = 8.2, 2.2 Hz, 1H, Ar-H), 7.38 (t, *J* = 8.2 Hz, 1H, Ar-H), 11.73 (br. s, 1H, NH); ^{13}C NMR (50 MHz, DMSO-d_6): δ 55.65, 96.01, 102.38, 103.75, 112.87, 114.37, 114.75 118.75, 121.09, 130.23, 136.79, 138.98, 143.78, 150.49, 151.57, 159.80, 161.62; MS (EI, 70 eV) *m/z*: 295.2 (M^+); HRMS (EI) *m/z*: calculated for $C_{17}H_{13}NO_4$: 295.0845; found: 295.0851.

5.2.3.4. 6,7-Methylenedioxy-4-(4-methoxyphenyl)quinolin-2(*1H*)-one (12d)

Compound **12d** (0.19 g, 0.64 mmol) was obtained by cyclization of **11d** (0.29 g, 0.93 mmol) with PPA (10 g); Yield: 68%; white solid; mp: 269-271 °C; IR (KBr) ν (cm^{-1}): 1663.07 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.82 (s, 3H, -OC<u>H</u>$_3$), 6.06 (s, 2H, -O-C<u>H</u>$_2$-O-), 6.19 (s, 1H, Ar-H), 6.75 (s, 1H, Ar-H), 6.90 (s, 1H, Ar-H), 7.07 (d, *J* = 8.6 Hz, 1H, Ar-H), 7.37 (d, *J* = 8.6 Hz, 1H, Ar-H), 11.69 (br. s, 1H, NH); ^{13}C NMR (50 MHz, DMSO-d_6): δ 55.70, 96.06, 102.37, 103.86, 113.13, 114.64 (2C), 118.60, 129.76, 130.32 (2C), 136.81, 143.82, 150,46, 151.53, 160.04, 161.73; MS (EI,

70 eV) *m/z*: 295.2 (M^+); HRMS (EI) *m/z*: calculated for $C_{17}H_{13}NO_4$: 295.0845; found: 295.0851.

5.2.3.5. 6,7-Methylenedioxy-4-(2,4-dimethoxyphenyl)quinolin-2(*1H*)-one (12e)

Compound **12e** (0.11 g, 0.34 mmol) was obtained by cyclization of **11e** (0.19 g, 0.55 mmol) with PPA (10 g); Yield: 61%; white solid; mp: 251-252 °C; IR (KBr) ν (cm^{-1}): 1654.03 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.67 (s, 3H, -OC<u>H</u>$_3$), 3.81 (s, 3H, -OC<u>H</u>$_3$), 6.02, 6.04 (s, each 1H, -O-C<u>H</u>$_2$-O-), 6.12 (s, 1H, Ar-H), 6.39 (s, 1H, Ar-H), 6.61-6.69 (m, 2H, Ar-H), 6.85 (s, 1H, Ar-H), 7.09 (d, *J* = 8.2 Hz, 1H, Ar-H), 11.70 (br. s, 1H, NH); ^{13}C NMR (50 MHz, DMSO-d_6): δ 55.79, 55.95, 95.76, 99.16, 102.24, 104.07, 105.74, 114.07, 118.73, 119.56, 131.07, 135.98, 143.69, 149.72, 150.28, 157.53, 161.57, 161.99; MS (EI, 70 eV) *m/z*: 325.1 (M^+); HRMS (EI) *m/z*: calculated for $C_{18}H_{15}NO_5$: 325.0950; found: 325.0954.

5.2.3.6. 6,7-Methylenedioxy-4-(2,5-dimethoxyphenyl)quinolin-2(*1H*)-one (12f)

Compound **12f** (0.10 g, 0.31 mmol) was obtained by cyclization of **11f** (0.17 g, 0.50 mmol) with PPA (10 g); Yield: 62%; white solid; mp: 240-241 °C; IR (KBr) ν (cm^{-1}): 1669.46 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.59 (s, 3H, -OC<u>H</u>$_3$), 3.69 (s, 3H, -OC<u>H</u>$_3$), 5.99, 6.02 (s, each 1H, -O-C<u>H</u>$_2$-O-), 6.14 (s, 1H, Ar-H), 6.33 (s, 1H, Ar-H), 6.74 (d, *J* = 2.8 Hz, 1H, Ar-H), 6.84 (s, 1H, Ar-H), 6.98 (dd, *J* = 8.9, 2.8 Hz, 1H, Ar-H), 7.06 (d, *J* = 9.2 Hz, 1H, Ar-H), 11.71 (br. s, 1H, NH); ^{13}C NMR (50 MHz, DMSO-d_6): δ 55.94, 56.38, 95.77, 102.27, 103.96, 113.22, 113.60, 115.31, 116.03, 119.38, 127.04, 136.06, 143.73, 149.50, 150.39 (2C), 153.61, 161.85; MS (EI, 70 eV) *m/z*: 325.2 (M^+); HRMS (EI) *m/z*: calculated for $C_{18}H_{15}NO_5$: 325.0950; found: 325.0959.

5.2.3.7. 6,7-Methylenedioxy-4-(2,6-dimethoxyphenyl)quinolin-2(*1H*)-one (12g)

Compound **12g** (0.49 g, 1.51 mmol) was obtained by cyclization of **11g** (0.75 g,

2.18 mmol) with PPA (10 g); Yield: 69%; white solid; mp: 280-281 °C; IR (KBr) ν (cm^{-1}): 1654.03 (C=O); ^{1}H NMR (200 MHz, $CDCl_3$-d_1): δ 3.68 (s, 6H, 2 × -OC<u>H</u>$_3$), 5.93 (s, 2H, -O-C<u>H</u>$_2$-O-), 6.48 (s, 2H, Ar-H), 6.65 (d, *J* = 8.4 Hz, 2H, Ar-H), 6.99 (s, 1H, Ar-H), 7.35 (t, *J* = 8.4 Hz, 1H, Ar-H), 12.99 (br. s, 1H, NH); ^{13}C NMR (50 MHz, $CDCl_3$-d_1): δ 55.91 (2C), 96.48, 101.53, 103.72, 104.05 (2C), 114.71, 115.38, 119.94, 130.12, 135.68, 144.23, 146.92, 150.38, 157.62 (2C), 164.53; MS (EI, 70 eV) *m/z*: 325.3 (M^+); HRMS (EI) *m/z*: calculated for $C_{18}H_{15}NO_5$: 325.0950; found: 325.0954.

5.2.3.8. 6,7-Methylenedioxy-4-(3,4-dimethoxyphenyl)quinolin-2(*1H*)-one (12h)

Compound **12h** (2.29 g, 7.04 mmol) was obtained by cyclization of **11h** (3.00 g, 8.74 mmol) with PPA (10 g); Yield: 80%; white solid; mp: 272-273 °C; IR (KBr) ν (cm^{-1}): 1647.28 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.77 (s, 3H, -OC<u>H</u>$_3$), 3.80 (s, 3H, -OC<u>H</u>$_3$), 6.05 (s, 2H, -O-C<u>H</u>$_2$-O-), 6.23 (s, 1H, Ar-H), 6.81 (s, 1H, Ar-H), 6.88 (s, 1H, Ar-H), 6.93 (dd, *J* = 8.2, 1.8 Hz, 1H, Ar-H), 6.98 (d, *J* = 1.8 Hz, Ar-H), 7.06 (d, *J* = 8.2 Hz, 1H, Ar-H), 11.73 (br. s, 1H, NH); ^{13}C NMR (50 MHz, DMSO-d_6): δ 56.00 (2C), 96.00, 102.33, 103.94, 112.15, 112.61, 113.14, 118.59, 121.35, 129.97, 136.74, 143.78, 149.17, 149.56, 150.41, 151.71, 161.76; MS (EI, 70 eV) *m/z*: 325.1 (M^+); HRMS (EI) *m/z*: calculated for $C_{18}H_{15}NO_5$: 325.0950; found: 325.0954.

5.2.3.9. 6,7-Methylenedioxy-4-(3,5-dimethoxyphenyl)quinolin-2(*1H*)-one (12i)

Compound **12i** (0.41 g, 1.26 mmol) was obtained by cyclization of **11i** (0.75 g, 2.18 mmol) with PPA (10 g); Yield: 57%; white solid; mp: 283-284 °C; IR (KBr) ν (cm^{-1}): 1654.03 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.74 (s, 6H, 2 × -OC<u>H</u>$_3$), 6.03 (s, 2H, -O-C<u>H</u>$_2$-O-), 6.20 (s, 1H, Ar-H), 6.49-6.57 (m, 3H, Ar-H), 6.71 (s, 1H, Ar-H), 6.86 (s, 1H, Ar-H), 11.73 (br. s, 1H, NH); ^{13}C NMR (50 MHz, DMSO-d_6): δ 55.83 (2C), 96.00, 100.87, 102.38, 103.79, 106.96 (2C), 112.87, 118.56, 136.73, 139.59, 143.82, 150.54, 151.70, 160.97 (2C), 161.64; MS (EI, 70 eV) *m/z*: 325.2 (M^+);

HRMS (EI) *m/z*: calculated for $C_{18}H_{15}NO_5$: 325.0950; found: 325.0954.

5.2.3.10. 6,7-Methylenedioxy-4-(2,4,6-trimethoxyphenyl)quinolin-2(*1H*)-one (12j)

Compound **12j** (0.12 g, 0.34 mmol) was obtained by cyclization of **11j** (0.22 g, 0.59 mmol) with PPA (10 g); Yield: 57%; white solid; mp: 287-288 °C; IR (KBr) ν (cm^{-1}): 1653.07 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.60 (s, 6H, 2 × -OC$\underline{H}_3$), 3.80 (s, 3H, -OC$\underline{H}_3$), 5.99, 6.03 (s, each 1H, -O-C$\underline{H}_2$-O-), 6.26 (s, 1H, Ar-H), 6.32 (s, 2H, Ar-H), 6.81 (s, 1H, Ar-H), 8.26 (s, 1H, Ar-H), 11.62 (br. s, 1H, NH); ^{13}C NMR (50 MHz, DMSO-d_6): δ 55.81, 56.17 (2C), 91.46 (2C), 95.73, 102.14, 103.52, 106.78, 114.72, 121.07, 136.01, 143.64, 146.02, 150.15, 158.19 (2C), 161.92, 162.10; MS (EI, 70 eV) *m/z*: 355.3 (M^+); HRMS (EI) *m/z*: calculated for $C_{19}H_{17}NO_6$: 355.1056; found: 355.1048.

5.2.3.11. 6,7-Methylenedioxy-4-(3,4,5-trimethoxyphenyl)quinolin-2(*1H*)-one (12k)

Compound **12k** (0.17 g, 0.48 mmol) was obtained by cyclization of **11k** (0.25 g, 0.67 mmol) with PPA (10 g); Yield: 71%; white solid; mp: 275-276 °C; IR (KBr) ν (cm^{-1}): 1647.28 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.68 (s, 3H, -OC$\underline{H}_3$), 3.75 (s, 6H, 2 × -OC$\underline{H}_3$), 6.03 (s, 2H, -O-C$\underline{H}_2$-O-), 6.23 (s, 1H, Ar-H), 6.65 (s, 2H, Ar-H), 6.79 (s, 1H, Ar-H), 6.86 (s, 1H, Ar-H), 11.72 (br. s, 1H, NH); ^{13}C NMR (50 MHz, DMSO-d_6): δ 56.48 (2C), 60.53, 96.00, 102.37, 103.97, 106.36 (2C), 113.07, 118.65, 133.12, 136.68, 138.03, 143.87, 150.52, 151.91, 153.39 (2C), 161.75; MS (EI, 70 eV) *m/z*: 355.2 (M^+); HRMS (EI) *m/z*: calculated for $C_{19}H_{17}NO_6$: 355.1056; found: 355.1050.

5.2.3.12. 4-(2-Fluorophenyl)-6,7-methylenedioxyquinolin-2(*1H*)-one (12l)

Compound **12l** (0.11 g, 0.39 mmol) was obtained by cyclization of **11l** (0.21 g,

0.70 mmol) with PPA (10 g); Yield: 55%; white solid; mp: 251-252 °C; IR (KBr) ν (cm^{-1}): 1653.07 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6): δ 6.06 (s, 2H, -O-C$\underline{H_2}$-O-), 6.29 (s, 1H, Ar-H), 6.45 (d, J = 2.0 Hz, 1H, Ar-H), 6.91 (s, 1H, Ar-H), 7.33-7.47 (m, 3H, Ar-H), 7.51-7.62 (m, 1H, Ar-H), 11.87 (br. s, 1H, NH); ^{13}C NMR (50 MHz, DMSO-d_6): δ 95.99, 102.47, 103.42, 112.96, 116.38 (d, $^{2}J_{CF}$ = 21.5 Hz, 1C), 120.11, 124.89 (d, $^{2}J_{CF}$ = 15.8 Hz, 1C), 125.52, 131.39, 131.67 (d, $^{3}J_{CF}$ = 8.0 Hz, 1C), 136.46, 144.04, 146.18, 150.73, 159.02 (d, $^{1}J_{CF}$ = 245.0 Hz, 1C), 161.47; MS (EI, 70 eV) *m/z*: 283.1 (M^{+}); HRMS (EI) *m/z*: calculated for $C_{16}H_{10}FNO_3$: 283.0645; found: 283.0641.

5.2.3.13. 4-(3-Fluorophenyl)-6,7-methylenedioxyquinolin-2(*1H*)-one (12m)

Compound **12m** (0.13 g, 0.46 mmol) was obtained by cyclization of **11m** (0.21 g, 0.70 mmol) with PPA (10 g); Yield: 65%; white solid; mp: 249-251 °C; IR (KBr) ν (cm^{-1}): 1654.03 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6): δ 6.07 (s, 2H, -O-C$\underline{H_2}$-O-), 6.25 (s, 1H, Ar-H), 6.67 (s, 1H, Ar-H), 6.90 (s, 1H, Ar-H), 7.24-7.37 (m, 3H, Ar-H), 7.51-7.62 (m, 1H, Ar-H), 11.81 (br. s, 1H, NH); ^{13}C NMR (50 MHz, DMSO-d_6): δ 96.08, 102.44, 103.64, 112.62, 115.97 (d, $^{2}J_{CF}$ = 23.0 Hz, 2C), 119.08, 125.22, 131.28 (d, $^{3}J_{CF}$ = 8.5 Hz, 1C), 136.83, 139.84 (d, $^{3}J_{CF}$ = 8.0 Hz, 1C), 143.94, 150.38, 150.64, 161.51, 162.54 (d, $^{1}J_{CF}$ = 243.5 Hz, 1C); MS (EI, 70 eV) *m/z*: 283.1 (M^{+}); HRMS (EI) *m/z*: calculated for $C_{16}H_{10}FNO_3$: 283.0645; found: 283.0648.

5.2.3.14. 4-(4-Fluorophenyl)-6,7-methylenedioxyquinolin-2(*1H*)-one (12n)

Compound **12b** (0.17 g, 0.60 mmol) was obtained by cyclization of **11b** (0.21 g, 0.70 mmol) with PPA (10 g); Yield: 85%; white solid; mp: 251-252 °C; IR (KBr) ν (cm^{-1}): 1668.50 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6): δ 6.02 (s, 2H, -O-C$\underline{H_2}$-O-), 6.19 (s, 1H, Ar-H), 6.62 (s, 1H, Ar-H), 6.86 (s, 1H, Ar-H), 7.25-7.34 (m, 2H, Ar-H), 7.40-7.47 (m, 2H, Ar-H), 11.76 (br. s, 1H, NH); ^{13}C NMR (50 MHz, DMSO-d_6): δ 96.06, 102.41, 103.67, 112.90, 116.11 (d, $^{2}J_{CF}$ = 22.0 Hz, 2C), 119.02, 131.15 (d, $^{3}J_{CF}$

= 8.5 Hz, 2C), 133.94, 136.79, 143.88, 150.56, 150.74, 161.58, 162.70 (*d*, $^{1}J_{CF}$ = 244.5 Hz, 1C); MS (EI, 70 eV) *m/z*: 283.1 (M^{+}); HRMS (EI) *m/z*: calculated for $C_{16}H_{10}FNO_3$: 283.0645; found: 283.0636.

5.3. Biological evaluation

5.3.1. Antiproliferative assay

Human tumor cell lines of the cancer screening panel were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum (GIBCO/BRL), penicillin (100 U/mL)/streptomycin (100 g/mL) (GIBCO/BRL) and 1% L-glutamine (GIBCO/BRL) at 37 °C in a humidified atmosphere containing 5% CO_2. Human hepatoma Hep 3B and normal skin Detroit 551 cells were maintained in DMEM medium supplemented with 10% fetal bovine serum (GIBCO/BRL), penicillin (100 U/mL)/streptomycin (100 g/mL) (GIBCO/BRL) and 1% L-glutamine (GIBCO/BRL) at 37 °C in a humidified atmosphere containing 5% CO_2. Logarithmically growing cancer cells were used for all experiments. The human tumor cell lines were treated with vehicle or test compounds for 48 h. Cell growth rate was determined by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliun bromide] reduction assay.[37,38] After 48 h treatment, cell growth rate was measured by scanning with an ELISA reader with a 570 nm filter and the IC_{50} values of test compounds were calculated.

5.3.2. Hoechst 33258 staining

HL-60 cells were plated at a density of 1×10^5 cells per well in 24-well plates and then incubated with 0.25 μM, 0.5 μM, and 1.0 μM, of compound **12e** for 24 h. H460 cells were plated at a density of 5×10^4 cells per well in 24-well plates and then incubated with 1.0 μM, 2.5 μM, and 5.0 μM, of compound **12e** for 24 h. Cells were examined directly and photographed under a phase contrast microscope. Nuclei were stained with Hoechst 33258 (bis-benzimide, Sigma) to detect chromatin condensation

or nuclear fragmentation, morphological characteristics of apoptosis. Compound **12e** treated cells were stained with 5 μg/mL Hoechst 33258 for 10 min. After washing twice with PBS, cells were fixed with 4% paraformaldehyde (PFA) in PBS for 10 min at 25 °C. Fluorescence of the soluble DNA (apoptotic) fragments was measured in a Varian Fluorometer at an excitation wavelength of 365 nm and emission wavelength of 460 nm.[39]

5.3.3. Cell cycle distribution analysis

Cell cycle analysis by FACS® was performed as described in the previous paper. [40] HL-60 cells were co-treated with compound **12e** (0.25 μM, 0.5 μM, and 1.0 μM) for 24 h, and H460 were also co-treated with compound **12e** (1.0 μM, 2.5 μM, and 5.0 μM). After treatment, the cells were washed once with PBS and fixed with 70% ice-cold ethanol at −20 °C overnight. Then the cells were stained with a solution containing 0.1% Triton-X 100 (Sigma), 0.2 mg/mL RNase (Sigma) and 20 μg/mL propidium iodide (PI, Sigma) in the dark for 30 min. Cell cycle distribution were measured using a FACScan flow cytometer (Becton Dickinson, San Jose, CA, USA) and all histograms were analyzed by ModFit software.

5.3.4. Western blot assay

The treated cells were collected and washed with PBS. After centrifugation, cells were lysed in a lysis buffer. The lysates were incubated on ice for 30 min and centrifuged at 12,000 *g* for 20 min. Supernatants were collected, and protein concentrations were then determined using the Bradford Assay. After adding a 5× sample loading buffer containing 625 mM Tris–HCl, pH = 6.8, 500 mM dithiothreitol, 10% SDS, 0.06% bromophenol blue, and 50% glycerol, protein samples were electrophoresed on 10% SDS-polyacrylamide gels and transferred to a nitrocellulose membrane. Immunoreactivity was detected using the Western blot

chemiluminescence reagent system (PerkinElmer, Boston, MA). β-Actin was used as a loading control.

Acknowledgments

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Figure legends

Figure 1. Structures of podophyllotoxin and related compounds: podophyllotoxin (**1**) and its semi-synthetic derivatives in clinical use, etoposide (**2**), teniposide (**3**) and etopophos (**4**).

Figure 2. Stuctures of 4-aza-PPT analogues (**5**), podophyllic aldehyde (**6**), combretastatin A-4 (**7**), 2-PQ derivatives and the target compounds (**12a-n**).

Figure 3. Rationally designed 4-PQ analogs mimic structures of PPT analogues and CA-4.

Figure 4. Fluorescent images of Hoechst staining showing compound **12e** induced cell death. (A) Treatment of HL-60 cells with **12e** for 24 h. The arrows indicate the formation of apoptotic bodies. (B) Treatment of H460 cells with **12e** for 24 h. The arrows indicate the formation of apoptotic bodies. Scale bar = 50 μm.

Figure 5. Compound **12e** affected the cell cycle distribution. (A) HL-60 cells treated with **12e** for 24 h. (B) H460 cells treated with **12e** for 24 h.

Figure 6. Regulation of mitotic phase- and apoptosis-associated proteins expression by compound **12e.** (A) HL-60 cells treated with the indicated concentration of **12e** for 24 h. (B) H460 cells treated with the indicated concentration of **12e** for 24 h. After treatment, the cells were harvested and subjected to Western blot. The relative amounts of cyclin B1, CDK1, and caspase 3 proteins were quantified and normalized to the corresponding β-actin protein amount. The quantitative data are shown under each protein, respectively.

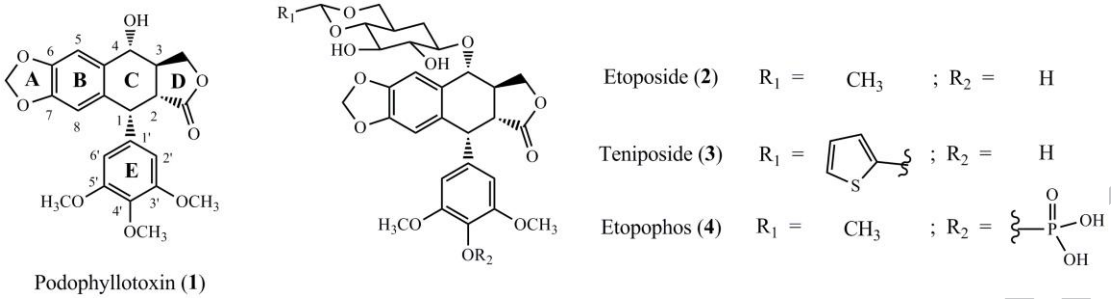
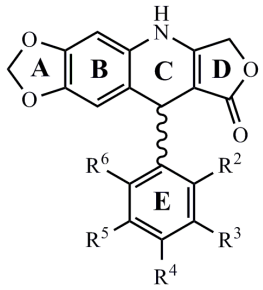
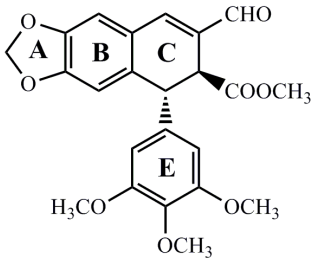


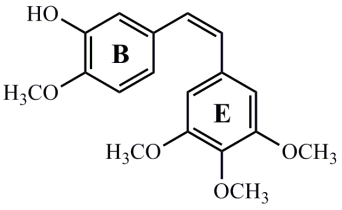
Figure 1.



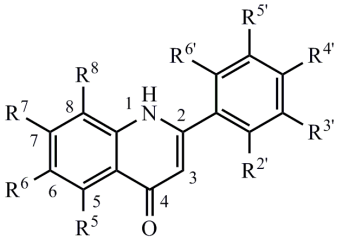
4-Aza-podophyllotoxin (**5**)



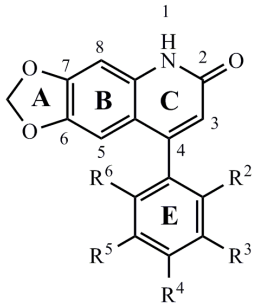
Podophyllic aldehyde (**6**)



Combretastatin A-4 (**7**)



2-Phenylquinolin-4-one analogs
(2-PQs)



4-Phenylquinolin-2-one analogs
(4-PQs ; **12a-n**)

Figure 2.

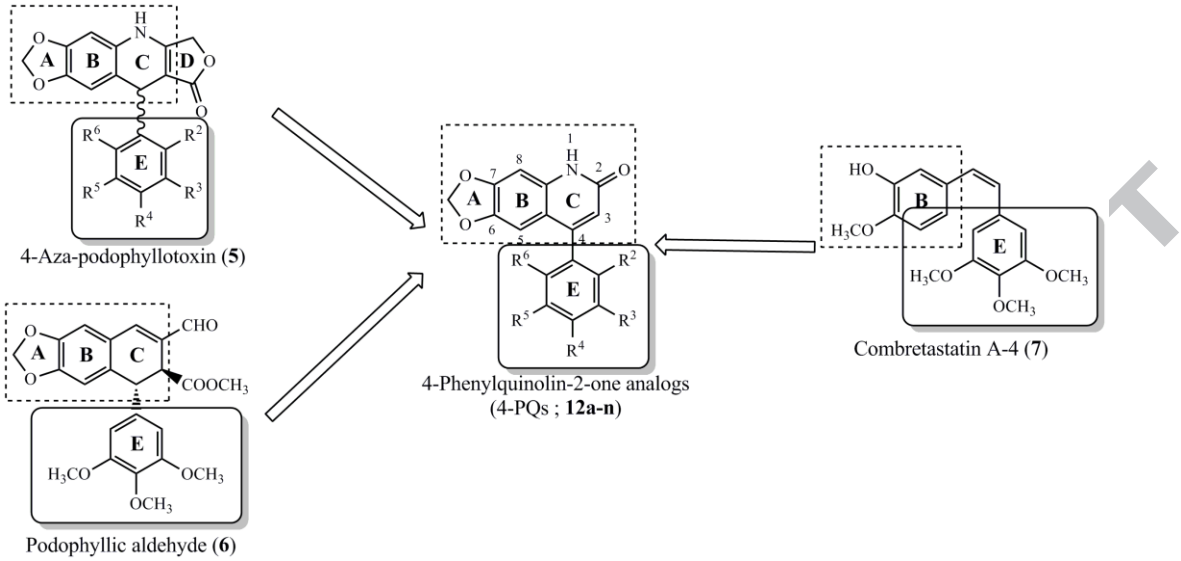


Figure 3.

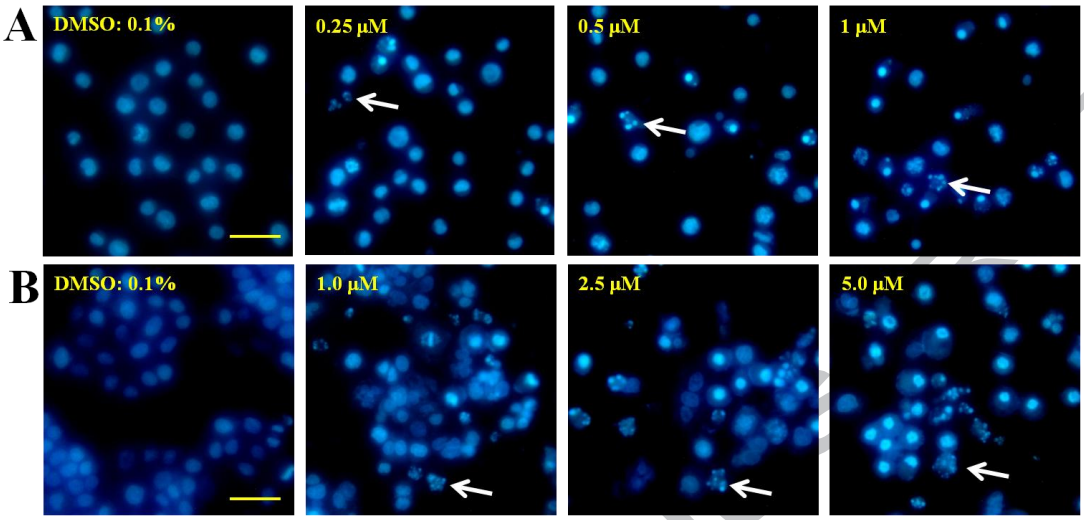
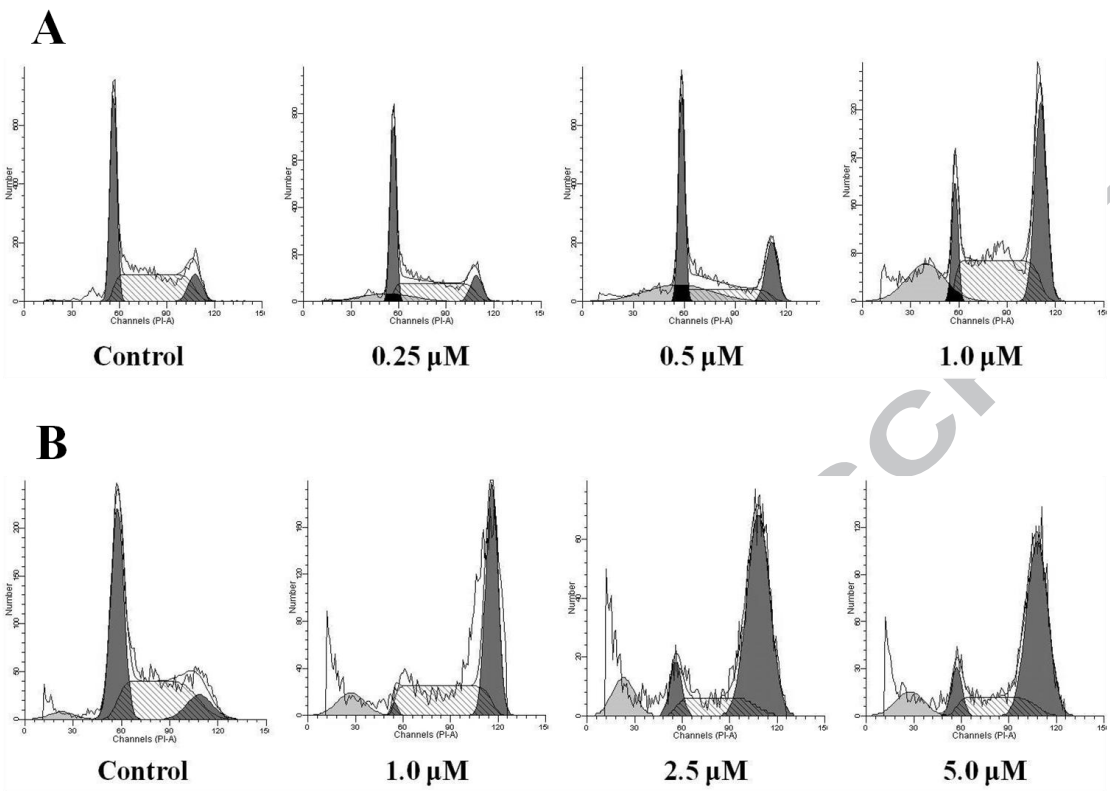


Figure 4.



Cell line	Conc. (µM)	Cell cycle phase (%)			
		Sub G1	G0/G1	S	G2/M
Control	0	0.35	39.80	49.69	10.51
HL-60	0.25	13.88	41.36	46.76	11.87
	0.5	27.86	44.82	30.16	25.02
	1.0	19.65	12.46	46.99	40.54
Control	0	3.07	46.19	43.08	10.73
H460	1.0	10.80	1.57	44.06	54.37
	2.5	11.65	9.60	18.82	71.58
	5.0	11.15	9.37	22.41	68.22

Figure 5.

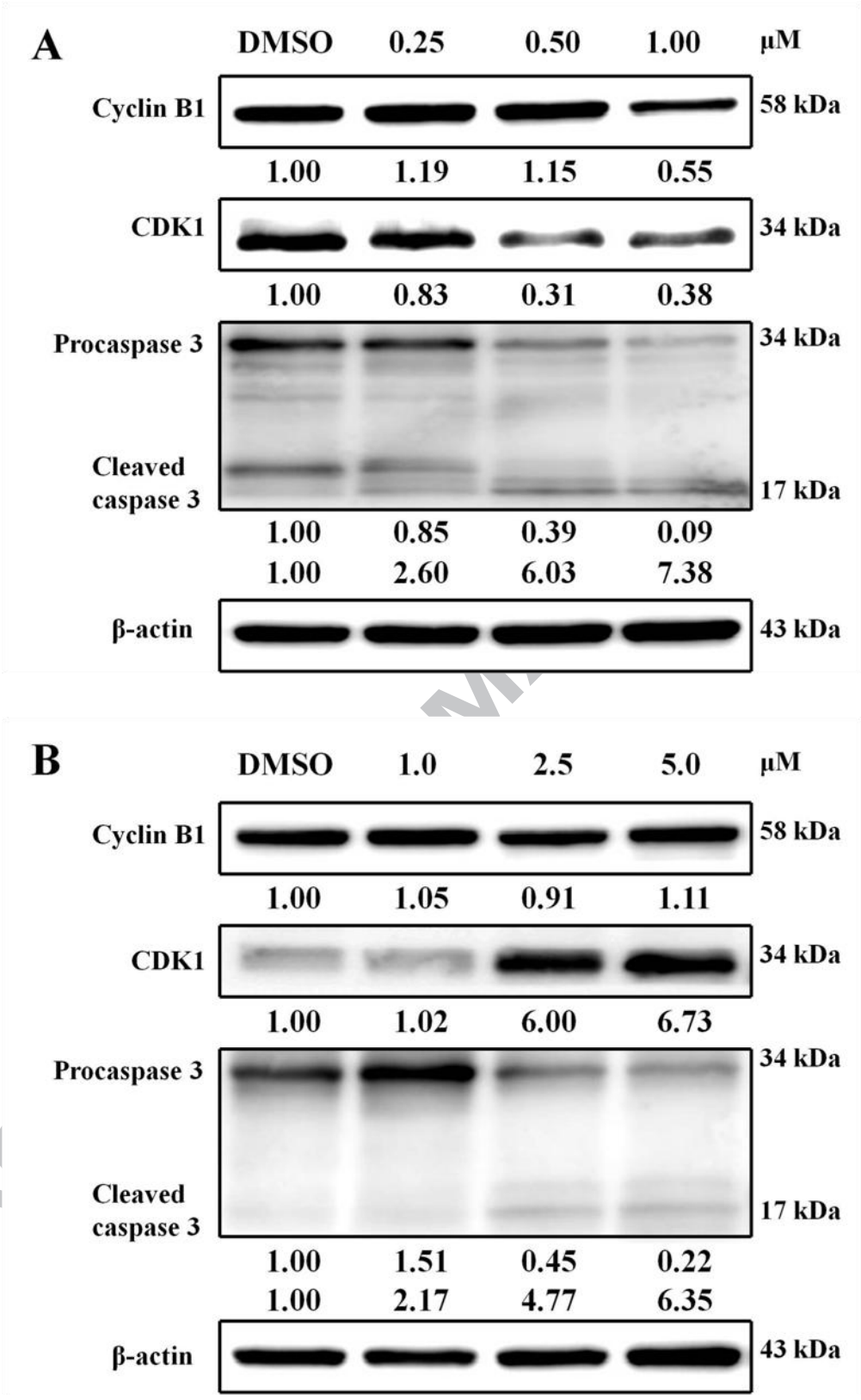
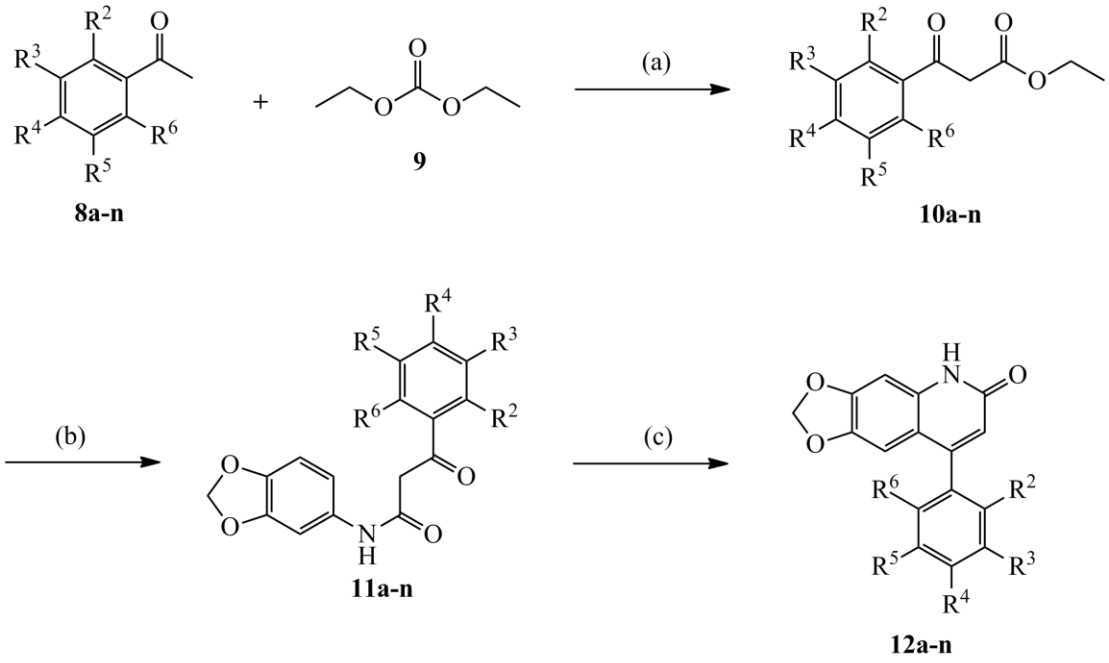


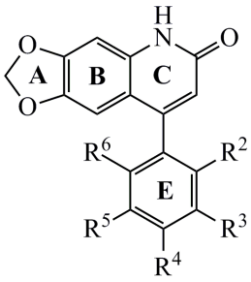
Figure 6.



	R^2	R^3	R^4	R^5	R^6
a :	H	H	H	H	H
b :	OCH_3	H	H	H	H
c :	H	OCH_3	H	H	H
d :	H	H	OCH_3	H	H
e :	OCH_3	H	OCH_3	H	H
f :	OCH_3	H	H	OCH_3	H
g :	OCH_3	H	H	H	OCH_3
h :	H	OCH_3	OCH_3	H	H
i :	H	OCH_3	H	OCH_3	H
j :	OCH_3	H	OCH_3	H	OCH_3
k :	H	OCH_3	OCH_3	OCH_3	H
l :	F	H	H	H	H
m :	H	F	H	H	H
n :	H	H	F	H	H

Scheme 1. Reagents and conditions: (a) NaH, toluene, reflux; (b) 3,4-methylenedioxy aniline, toluene, reflux; (c) PPA, 100-110 °C.

Table 1. Cytotoxicity of compounds **12a-n**.



Compd	R^2	R^3	R^4	R^5	R^6	$IC_{50}(\mu M)^a$								
						MCF-7	2774	SKOV-3	HL-60	Hep 3B	H460	COLO 205	A498	Detroit 551
Un-substituted														
12a	H	H	H	H	H	18.5	>30	>30	>50	>50	>50	>50	42.7	>50
Mono-substituted														
12b	OCH_3	H	H	H	H	27.1	18.8	26.6	10	23.3	>25	18.1	9.8	>25
12c	H	OCH_3	H	H	H	14.9	15.6	24.6	>10	>10	>10	39.6	>50	>10
12d	H	H	OCH_3	H	H	>30	>30	26.8	>20	>20	>20	>50	>25	>20
Di-substituted														
12e	OCH_3	H	OCH_3	H	H	6.0	0.4	0.4	0.4	1.0	0.9	7.4	48	>25
12f	OCH_3	H	H	OCH_3	H	3.7	6.5	20	3.5	10	10	6.1	28.9	>100
12g	OCH_3	H	H	H	OCH_3	29.8	>30	>30	>100	>100	>100	>50	>50	>100
12h	H	OCH_3	OCH_3	H	H	14.3	6.6	6.4	4.1	14.6	14.4	16.8	25.6	23.6
12i	H	OCH_3	H	OCH_3	H	>30	8.3	>30	2.9	>10	>10	>50	11.7	>10
Tri-substituted														
12j	OCH_3	H	OCH_3	H	OCH_3	21	>30	>30	75	>100	67.6	37.4	>50	>100
12k	H	OCH_3	OCH_3	OCH_3	H	4.5	2.1	0.93	2.3	5.0	7.6	>50	>50	>25
Fluoro-substituted														
12l	F	H	H	H	H	>30	26.4	>30	>100	>100	>100	>50	>50	>100
12m	H	F	H	H	H	>30	24.5	>30	>20	>20	>20	>50	>50	>20
12n	H	H	F	H	H	>30	17.6	>30	>60	>20	>20	>50	>50	>20
Etoposide									5.48		1.0			
5-FU									22.3		26.7			

Human tumor cells were treated with different concentrations of samples for 48 h (n = 3 independent experiments).

[a]Data are presented as IC_{50} (μM, the concentration of 50% proliferation-inhibitory effect).

Graphical Abstract

Design and synthesis of 6,7-methylenedioxy-4-substituted phenylquinolin-2*(1H)*-one derivatives as novel anticancer agents that induce apoptosis with cell cycle arrest at G2/M phase

Yi-Fong Chen[a], Yi-Chien Lin[b], Po-Kai Huang[b], Hsu-Chin Chan[c], Sheng-Chu Kuo[b], Kuo-Hsiung Lee[d,e*], Li-Jiau Huang[a,b*]