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Cancer preventive agents, Part 2: Synthesis and evaluation of 2-phenyl-4-quinolone and 9-oxo-9,10-dihydroacridine derivatives as novel antitumor promoters☆

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Abstract—2-Phenyl-4-quinolone and 9-oxo-9,10-dihydroacridine derivatives were synthesized and screened as potential antitumor promoters by examining the ability of the compounds to inhibit Epstein–Barr virus early antigen (EBV-EA) activation induced by the tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in Raji cells. Interestingly, compounds **14**, **15**, and **17** showed similar inhibitory effects (89–92%, 66–69%, and 24–29% at 1000, 500, and 100 mol ratio to TPA, respectively) against EBV-EA with potencies comparable to those of glycyrrhetic acid, a known natural antitumor-promoter.
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1. Introduction

In the search for cancer chemopreventive agents from natural products, many plants and plant-derived compounds have been screened using the in vitro synergistic assay for inhibitory effects on the induction of Epstein–Barr virus early antigen (EBV-EA) by 12-*O*-tetradecanoylphorbol-13-acetate (TPA). Recently, we reported that myricanone (**1**), a cyclic diarylheptanoid derived from *Myrica rubra*, exhibited significant antitumor-promoting activity (100%, 75%, and 24% inhibition of activation at 1000, 500, and 100 mol ratio to TPA, respectively).[1,2] In addition, we have reported that several rotenoids showed potent antitumor-promoting activity and cytotoxicity against neoplastic cell lines.[3,4] Particularly, tephrosin (**2**), which has a biaryl structure consisting of rings A and B linked by interposed C and D rings, exhibited significant antitumor-promoting activity (74%, 40%, and 11% inhibition of activation at 500, 100, and 10 mol ratio to TPA, respectively) and cytotoxicity (ED_{50} values ranging from 0.001 to 0.36 μg/mL).

In addition, it was reported that flavone acetic acid (**3**, FAA), a synthetic biaryl compound composed of rings A and B linked by an interposed C ring, displayed remarkable activity against marine colon adenocarcinoma 38 and a broad spectrum of slow-growing solid tumors that are usually insensitive to most cytotoxic drugs.[5,6] Xanthenone-4-acetic acid (**5**, XAA) has also been shown to have an activity profile similar to that of FAA.[7,8] We have previously reported on the synthesis and evaluation of substituted 2-phenyl-4-quinolones, which are FAA analogues, for cytotoxicity against human tumor cell lines (HTCL).[9–12] For example, 2-phenyl-4-quinolone-8-acetic acid (**4**) displayed potent cytotoxicity, with ED_{50} values ranging from 0.04 to 0.44 μg/mL.[9] These data prompted us to investigate new FAA and XAA analogues as antitumor promoters and cytotoxic agents (see Fig. 1).

In this study, we prepared several 2-phenyl-4-quinolone and 9-oxo-9,10-dihydroacridine derivatives. These synthetic compounds were evaluated for antitumor-pro-

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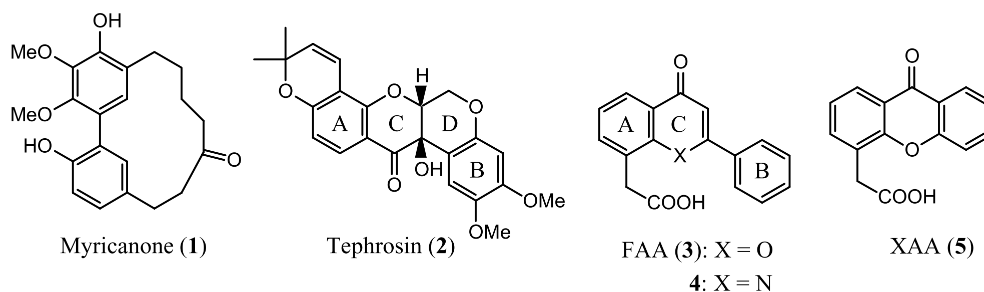


Figure 1. Structures of compounds **1**–**5**.

moting activity and for in vitro cytotoxic activity against HTCL.

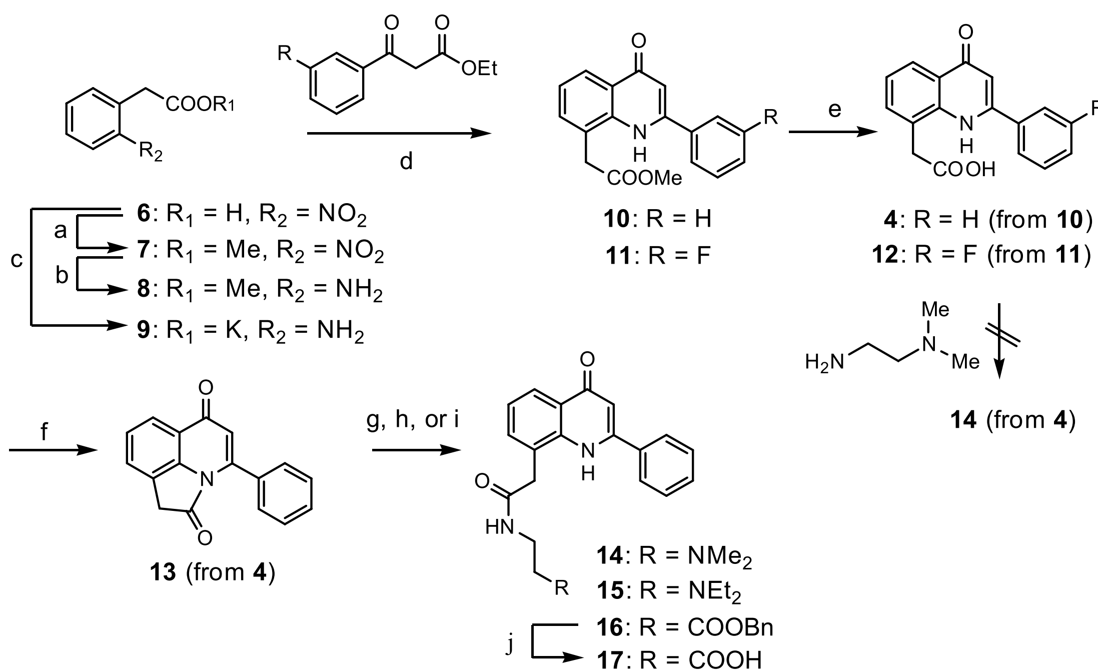
2. Chemistry

Scheme 1 shows the preparation of the 2-phenyl-4-quinolone derivatives. The esters **10**[9] and **11** were synthesized by condensation of benzoylacetate with amine **8**,[13] which was readily prepared from commercially available (*o*-nitrophenyl)acetic acid in two steps. Hydrolysis of esters **10** and **11** under basic conditions gave acids **4**[9] and **12**, respectively. Compound **12** smoothly underwent an intramolecular cyclization to give **13** using standard EDCI/DMAP conditions. Compound **13** was treated with *N*,*N*-dimethylethylenediamine to produce amide **14**. The product showed the characteristic carbonyl carbon signal in the ^{13}C NMR data and the structure of **14** was confirmed from ^{1}H NMR and mass spectral data. Similarly, compounds **15** and **16** were obtained by reacting **13** with *N*,*N*-diethylethylenediamine or β-alanine benzyl ester, respectively. On the other hand, it was difficult to prepare amide **14** directly from **11** because the intramolecular cyclization reaction proceeded readily. Subsequent reductive removal of the benzyl group of **16** by hydrogenation gave the acid **17**.

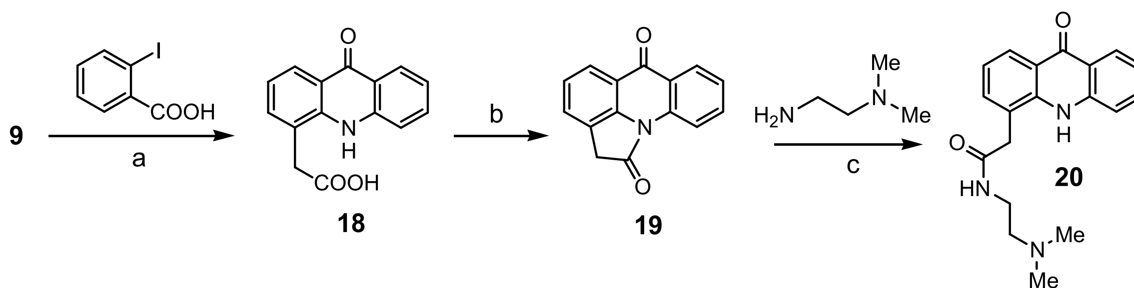
The synthesis of 9-oxo-9,10-dihydroacridine derivatives is outlined in Scheme 2. Compound **18** was synthesized according to a literature method.[8] Employing the same method shown in Scheme 1 furnished **19** and **20** from acid **18**.

3. Results and discussion

2-Phenyl-4-quinolone and 9-oxo-9,10-dihydroacridine derivatives **4**, **12**–**15**, and **17**–**20** were evaluated for their inhibitory effects on EBV-EA activation induced by



Scheme 1. Reagents and conditions: (a) MeOH, concd H_2SO_4, reflux. (b) H_2, 10% Pd–C, MeOH. (c) H_2, 10% Pd–C, K_2CO_3, MeOH. (d) Polyphosphoric acid (PPA), 90–100 °C, **11**; 12%. (E) 10% NaOH aq, EtOH, reflux, **12**; 58%. (f) 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI), 4-dimethylaminopyridine (DMAP), CH_2Cl_2, 93%. (g) *N*,*N*-Dimethyl ethylenediamine, toluene, reflux, **14**; 73%. (h) *N*,*N*-Dimethylethylenediamine, toluene, reflux, **16**; 55%. (h) H_2, 10% Pd–C, MeOH, 94%.



Scheme 2. Reagents and conditions: (a) (i) K_2CO_3, CuI, Cu, DMSO, 120 °C; (ii) H_2SO_4, 90 °C. (b) EDCI, DMAP, CH_2Cl_2, 73%. (c) Toluene, reflux, 83%.

Table 1. Inhibitory effects on TPA-induced EBV-EA activation

Compound	EBV-EA-positive cells (% viability)[a]				
	Compound concentration (mol ratio/32 pmol TPA)[b]				
	1000	500	100	10	IC_{50}
4	10.8 (60)	33.6 (>80)	66.1 (>80)	100 (>80)	452
12	17.2 (60)	39.4 (>80)	71.5 (>80)	100 (>80)	487
13	15.4 (60)	35.2 (>80)	64.5 (>80)	100 (>80)	460
14	8.8 (60)	31.4 (>80)	61.2 (>80)	97.6 (>80)	406
15	7.5 (70)	30.6 (>80)	60.9 (>80)	96.6 (>80)	405
17	8.3 (60)	30.2 (>80)	60.4 (>80)	97.0 (>80)	403
18	15.7 (60)	37.2 (>80)	69.7 (>80)	100 (>80)	480
19	19.6 (60)	45.7 (>80)	73.5 (>80)	100 (>80)	489
20	11.4 (60)	35.1 (>80)	68.4 (>80)	100 (>80)	456
Glycyrrhetic acid[c]	7.4 (60)	35.7 (>80)	83.2 (>80)	100 (>80)	413

[a] Values represent relative percentage to the control value (100%). Values in parentheses represent the surviving Raji cells measured through trypan blue staining. At least 60% surviving Raji cells 2 days after treatment with compounds is required for an accurate result.
[b] Mole ratio/TPA (32 pmol = 20 ng/mL), 1000 mol ratio = 32 nmol, 500 mol ratio = 16 nmol, 100 mol ratio = 3.2 nmol, and 10 mol ratio = 0.32 nmol.
[c] Positive control on TPA-induced EBV-EA activation.

TPA in Raji cells as a primary screening test for antitumor-promoters (Table 1).[1,2,14] Glycyrrhetic acid, a known natural antitumor promoter, was used as the reference standard.[14] In this assay, all of the compounds tested showed inhibitory effects on EBV-EA activation without cytotoxicity on Raji cells, and the effects of **14**, **15**, and **17** were almost equivalent to that of glycyrrhetic acid. In particular, compound **15** exhibited remarkable inhibitory effects (92, 69, and 29 at 1000, 500, and 100 mol ratio to TPA, respectively) on EBV-EA induction. In addition, the following conclusions were drawn: (a) The presence of an alkyl amide moiety in the side chain, such as in compounds **14**, **15**, and **17**, is important for activity. (b) Compared with 9-oxo-9,10-dihydroacridine **20**, 2-phenyl-4-quinolone **14** showed enhanced activity. (c) Lactam compounds **13** and **19** showed reduced activity.

Compounds **11**–**20** were also tested for cytotoxicity against a HTCL panel.[15] Compounds **11**–**16** and **18**–**20** were essentially inactive at 16 μg/mL, with compound **17** having the lowest mean IC_{50} of 4.2 ± 0.7 μg/mL and at least fourfold lower activity against KB-VIN, suggesting that it was a substrate for pgP (P-glycoprotein).

4. Conclusion

2-Phenyl-4-quinolone and 9-oxo-9,10-dihydroacridine derivatives, including 11 newly synthesized compounds, were prepared and evaluated as cytotoxic agents and antitumor promoters. Although target compounds **11**–**20** were not cytotoxic against HTCL, compounds **14**, **15**, and **17** displayed significant antitumor-promoting activity. Further investigation of this series of compounds is ongoing in our laboratory.

5. Experimental

All chemicals and solvents were used as purchased. Melting point was measured on a Fisher–Johns melting point apparatus without correction. ^1H NMR and ^{13}C NMR spectra were recorded on Varian Gemini 2000 (300 MHz) NMR spectrometer with TMS as the internal standard. All chemical shifts are reported in parts per million. NMR spectra were referenced to the residual solvent peak; chemical shifts δ in parts per million; apparent scalar coupling constants J in Hz. Mass spectral data were obtained on a TRIO 1000 or a JEOL JMS BU-20 mass spectrometer, respectively. IR spectra were recorded on Perkin-Elmer 1320 spectrophotometer. Analytical thin-

layer chromatography (TLC) was carried out on Merck precoated aluminum silica gel sheets (Kieselgel 60 F-254). All target compounds were characterized by ^{1}H and IR spectral analyses and MS analyses.

5.1. [2-(3-Fluorophenyl)-4-oxo-1,4-dihydroquinolin-8-yl]acetic acid methyl ester (11)

Methyl 2-aminophenylacetate (283 mg, 1.71 mmol) was suspended in 1.5 mL of polyphosphoric acid (PPA). The mixture was warmed to 90–100 °C, and ethyl (3-fluorobenzoyl)acetate (300 mg, 1.43 mmol) was added dropwise. The resulting mixture was further stirred for 1 h. Water was added, then aqueous NaOH (10%) was added slowly until pH 6 and the solution was extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography eluting with EtOAc/*n*-hexane = 1:1 to afford **11** (51 mg, 12%); brown amorphous solid; IR (KBr) 3000, 1725, 1609, 1583, 1514, 1266, 756 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 3.78 (s, 2H, $COOCH_3$), 3.96 (s, 2H, $ArCH_2$), 6.62 (d, 1H, J = 1.7, Ar-H), 7.21–7.34 (m, 2H, Ar-H), 7.50–7.63 (m, 4H, Ar-H), 8.36 (d, 1H, J = 7.1, Ar-H), 10.20 (s, 1H, NH); ESI-MS *m*/*z* 311 (M^+).

5.2. [2-(3-Fluorophenyl)-4-oxo-1,4-dihydroquinolin-8-yl]acetic acid (12)

Acetate **11** (45 mg, 0.15 mmol) was suspended in 50% aqueous EtOH (2 mL) containing 2 mL of aqueous NaOH (10%). The reaction mixture was heated to reflux for 18 h. After cooling, the solution was slowly acidified with aqueous HCl (10%). The precipitate was collected and washed with water to provide **12** (25 mg, 58%); yellow amorphous solid; ^{1}H NMR (DMSO-d_6): δ 4.11 (s, 2H, CH_2), 7.30–7.46 (m, 3H, Ar-H), 7.55–7.65 (m, 2H, Ar-H), 7.84–8.00 (m, 2H, Ar-H), 8.07 (dd, 1H, J = 1.4, 8.2, Ar-H); ESI-MS *m*/*z* 298 (M^++1).

5.3. General procedure for synthesizing target compounds 13 and 19

A solution of **4** or **18** in anhydrous CH_2Cl_2 at room temperature under argon was treated with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) and 4-dimethylaminopyridine (DMAP). After 4 h, the mixture was diluted with CH_2Cl_2 and washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography eluting with $CHCl_3$ to $CHCl_3$/MeOH = 10:1 to afford **13** or **19**.

5.3.1. 4-Phenyl-1*H*-pyrrolo[3,2,1-*ij*]quinoline-2,6-dione (13). Starting with 95 mg (0.34 mmol) of **4**, EDCI (78 mg, 0.41 mmol), and DMAP (4 mg, 0.034 mmol); yield 83 mg, 93%; pale yellow powder; mp 224.0–226.0 °C ($CHCl_3$–MeOH–*n*-hexane); IR (KBr) 1772, 1639, 1606, 1492, 1406, 1384, 1186 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 3.93 (s, 2H, $ArCH_2$), 6.30 (s, 1H, Ar-H), 7.41–7.51 (m, 6H, Ar-H), 7.61 (dd, 1H, J = 0.8, 7.1, Ar-H), 8.12 (d, 1H, J = 8.0, Ar-H); ^{13}C NMR ($CDCl_3$): δ 36.5, 118.4, 122.1, 123.5, 123.6, 125.8, 127.3, 128.0, 128.5, 129.9, 132.2, 143.8, 147.4, 170.6, 179.0; EI-HRMS *m*/*z*: Calcd for $C_{17}H_{11}NO_2$: M, 261.0790. Found: M^+, 261.0782.

5.3.2. 2*H*-Pyrrolo[3,2,1-*de*]acridine-1,6-dione (19). Starting with 632 mg (2.50 mmol) of **18**, EDCI (574 mg, 2.99 mmol), and DMAP (31 mg, 0.25 mmol); yield 428 mg, 73%; pale yellow powder; IR (KBr) 1743, 1649, 1599, 1497, 1153 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 3.96 (s, 2H, $ArCH_2$), 7.41 (dd, 1H, J = 7.1, 8.0, Ar-H), 7.51 (ddd, 1H, J = 0.8, 7.1, 8.0, Ar-H), 7.63 (dd, 1H, J = 0.8, 7.1, Ar-H), 7.81 (ddd, 1H, J = 1.7, 7.1, 8.5, Ar-H), 8.18 (dd, 1H, J = 0.8, 8.0, Ar-H), 8.52 (dd, 1H, J = 1.7, 8.0, Ar-H), 9.08 (dd, 1H, J = 0.8, 8.5, Ar-H); ESI-MS *m*/*z* 236 (M^++1).

5.4. General procedure for synthesizing target compounds 14 and 15

To a solution of **13** in toluene was added the appropriate diamine. The reaction mixture was heated to reflux for 4 h, quenched with saturated NH_4Cl aq, and extracted with $CHCl_3$. The extract was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography eluting with $CHCl_3$ to $CHCl_3$/MeOH = 3:1.

5.4.1. *N*-(2-Dimethylaminoethyl)-2-(4-oxo-2-phenyl-1,4-dihydroquinolin-8-yl)-acetamide (14). Starting with 25 mg (0.096 mmol) of **13** and 0.5 mL *N*,*N*-dimethylethylenediamine; yield 24 mg, 73%; pale yellow amorphous solid; IR (KBr) 3177, 3007, 2901, 1628, 1603, 1575, 1544, 1507, 1443, 1325, 767 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.22 [s, 6H, $N(CH_3)_2$], 2.42 (t, 2H, J = 5.8, CH_2CH_2N), 3.33 [dt, 2H, J = 5.8, 6.0, $NHCH_2CH_2N$, after addition of D_2O, 3.33 (t, J = 5.8)], 3.85 (s, 2H, $ArCH_2CO$), 6.68 (s, 1H, Ar-H), 6.93 (br s, 1H, NH, D_2O exchange), 7.23–7.28 (m, 1H, Ar-H), 7.45–7.59 (m, 4H, Ar-H), 7.91 (dd, 2H, J = 1.4, 8.0, Ar-H), 8.32 (dd, 1H, J = 1.1, 8.1, Ar-H), 11.76 (br s, 1H, NH, D_2O exchange); ^{13}C NMR ($CDCl_3$): δ 37.2, 42.6, 45.0, 57.2, 108.3, 123.2, 123.4, 125.9, 126.3, 126.8, 129.4, 130.5, 133.4, 134.7, 139.9, 150.4, 171.1, 179.0; EI-HRMS *m*/*z*: Calcd for $C_{21}H_{23}N_3O_2$: M, 349.1790. Found: M^+, 349.1789.

5.4.2. *N*-(2-Diethylaminoethyl)-2-(4-oxo-2-phenyl-1,4-dihydroquinolin-8-yl)-acetamide (15). Starting with 25 mg (0.096 mmol) of **13** and 0.2 mL *N*,*N*-diethylethylenediamine; yield 27 mg, 75%; pale yellow amorphous solid; ^{1}H NMR ($CDCl_3$): δ 1.07 (t, 6H, J = 7.1, $CH_2CH_3 \times 2$), 2.68 (t, 4H, J = 7.1, $NCH_2CH_3 \times 2$), 2.71 (t, 2H, J = 5.2, $CH_2CH_2NEt_2$), 3.41 (dt, 2H, J = 5.2, 5.8, $NHCH_2CH_3$), 3.90 (s, 2H, $ArCH_2$), 6.67 (s, 1H, Ar-H), 7.26 (t, 1H, J = 7.6, Ar-H), 7.52–7.59 (m, 4H, Ar-H), 7.91 (dd, 2H, J = 1.4, 7.4, Ar-H), 8.32 (dd, 1H, J = 1.4, 8.2, Ar-H), 11.70 (br s, 1H, NH); ESI-MS *m*/*z* 378 (M^++1).

5.5. 3-[2-(4-Oxo-2-phenyl-1,4-dihydroquinolin-8-yl)-acetylamino]propionic acid benzyl ester (16)

To a solution of **13** (50 mg, 0.191 mmol) of toluene (2 mL) were added β-alanine benzyl ester *p*-toluenesulf-

onate salt (202 mg, 0.574 mmol) and Et_3N (0.08 mL, 0.574 mmol). The reaction mixture was refluxed for 4 h and concentrated in vacuo. The residue was diluted with $CHCl_3$ and washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo. The crude product was purified by silica gel column chromatography eluting with $CHCl_3$/MeOH = 50:1 to afford **16** (46 mg, 55%); white powder; IR (KBr) 3228, 3064, 1734, 1629, 1580, 1512, 1171, 753 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.60 (t, 2H, J = 6.0, CH_2CH_2COOBn), 3.56 (dt, 2H, J = 6.0, 6.0, $NHCH_2CH_2$), 3.85 (s, 2H, $ArCH_2CONH$), 5.08 (s, 2H, $COOCH_2Ar$), 6.68 (d, 1H, J = 1.7, Ar-H), 7.21 (dd, 1H, J = 7.1, 8.0, Ar-H), 7.26–7.36 (m, 4H, Ar-H), 7.42 (dd, 1H, J = 1.4, 7.1, Ar-H), 7.51–7.58 (m, 4H, Ar-H), 7.88–7.91 (m, 2H, Ar-H), 8.32 (dd, 1H, J = 1.4, 8.0, Ar-H), 11.82 (s, 1H, NH); ESI-MS *m/z* 441 (M^++1).

5.6. 3-[2-(4-Oxo-2-phenyl-1,4-dihydroquinolin-8-yl)-acetylamino]propionic acid (17)

A solution of **16** (40 mg, 0.09 mmol) in MeOH (20 mL) at room temperature under argon was treated with 40 mg Pd–C (10%) and placed under hydrogen (30 psi). The mixture was shaken in a Parr apparatus for 15 h and then the content was filtered to remove the catalyst and evaporated to dryness in vacuo to give **17** (30 mg, 94%); pale yellow amorphous solid; ^1H NMR (DMSO-d_6): δ 2.40 (t, 2H, J = 6.0, CH_2CH_2COOH), 3.27 (dt, 2H, J = 6.0, 6.0, $NHCH_2CH_2$), 3.98 (s, 2H, $ArCH_2$), 6.47 (s, 1H, Ar-H), 7.28–7.34 (m, 1H, Ar-H), 7.56–7.61 (m, 3H, Ar-H), 7.96–8.00 (m, 1H, Ar-H), 8.04 (d, 1H, J = 8.2, Ar-H), 8.10–8.20 (m, 1H, Ar-H), 8.72–8.78 (m, 1H, Ar-H); ESI-MS *m/z* 351 (M^++1).

5.7. *N*-(2-Dimethylaminoethyl)-2-(9-oxo-9,10-dihydroacridin-4-yl)-acetamide (20)

To a solution of **19** (50 mg, 0.213 mmol) of toluene (4 mL) was added 0.1 mL *N*,*N*-dimethylethylenediamine. The reaction mixture was refluxed for 7 h and concentrated in vacuo. The residue was filtered with $CHCl_3$/MeOH and the filtrate was evaporated to dryness in vacuo. The product was collected and washed with EtOAc to provide **20** (57 mg, 83%); pale yellow amorphous solid; ^1H NMR ($CDCl_3$): δ 2.22 [s, 6H, $N(CH_3)_2$], 2.41 [t, 2H, J = 5.8, $CH_2CH_2N(CH_3)_2$], 3.33 [dt, 2H, J = 5.8, 6.3, $NHCH_2CH_2N(CH_3)_2$, after addition of D_2O, 3.33 (t, J = 5.8)], 3.85 (s, 2H, $ArCH_2$), 6.67 (br s, 1H, NH, D_2O exchange), 7.19 (dd, 1H, J = 7.1, 8.2, Ar-H), 7.24–7.29 (m, 1H, Ar-H), 7.49–7.52 (m, 2H, Ar-H), 7.66 (ddd, 1H, J = 1.4, 6.9, 8.2, Ar-H), 8.45 (br d, 2H, J = 8.2, Ar-H), 11.86 (br s, 1H, NH, D_2O exchange); ESI-MS *m/z* 324 (M^++1).

5.8. Inhibition of EBV-EA activation

The inhibition of EBV-EA activation was assayed using Raji cells (virus non-producer type), EBV genome-carrying human lymphoblastoid cells, which were cultivated in 10% fetal bovine serum (FBS) RPMI 1640 medium. The indicator cells (Raji, 1×10^6/mL) were incubated at 37 °C for 48 h in 1 mL medium containing *n*-butyric acid (4 mM, inducer), TPA (32 pM), and various amounts of test compounds dissolved in 5 μL DMSO. Smears were made from the cell suspension. The EBV-EA inducing cells were stained with high titer EBV-EA-positive serum from NPC patients and detected by an indirect immunofluorescence technique. In each assay, at least 500 cells were counted, and the number of strained cells (positive cells) was recorded. Triplicate assays were performed for each data point. The EBV-EA inhibitory activity of the test compounds was expressed by comparison with that of the positive control experiment (100%), which was carried out with *n*-butyric acid (4 mM) plus TPA (32 nM). In the experiments, the EBV-EA induction was normally around 35%, and this value was taken as the positive control (100%). *n*-Butyric acid (4 mM) alone induced 0.1% EA-positive cells. The viability of treated Raji cells was assayed by the trypan-blue staining method.

5.9. Cytotoxicity assay

The in vitro cytotoxicity assay was carried out according to procedures described by Rubinstein et al.[15] The human tumor cell line panel consists of ovarian carcinoma (1A9), lung carcinoma (A549), breast cancer (MCF-7), epidermoid carcinoma of nasopharyngeal cancer (KB), and multi-drug resistant KB subclone expressing P-glycoprotein (KB-Vin).

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