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Antitumor Agents. Part 226:† Synthesis and Cytotoxicity of 2-Phenyl-4-quinolone Acetic Acids and Their Esters

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Abstract—2-Phenyl-4-quinolone acetic acids and their esters were synthesized and evaluated for interaction with tubulin and for cytotoxicity against a panel of human tumor cell lines. 2-Phenyl- and 2-(2′-fluorophenyl)-4-quinolone-8-acetic acids (**11** and **12**) displayed potent cytotoxicity with ED_{50} values at nanomolar concentrations, but had minimal activity against tubulin polymerization. 2-(2′-Fluorophenyl)-4-quinolone-6-acetic acid (3) and 2-(2′-fluorophenyl)-4-quinolone-8-acetic acid methyl ester (**10**) moderately inhibited tubulin polymerization.

Flavone acetic acid (FAA, Fig. 1) is a synthetic flavone with a unique pattern of antitumor activity. Unlike conventional antitumor agents, it causes rapid tumor necrosis with little resultant toxicity in normal tissues. FAA has demonstrated excellent activity against murine colon adenocarcinoma 38 and a broad spectrum of slow-growing solid tumors that are usually insensitive to most cytotoxic drugs.[2,3] In contrast to its solid tumor activity, FAA shows poor activity against murine leukemia cell lines (P388 and L1210). Because of its unique pre-clinical solid tumor activity, FAA has been evaluated in clinical trials.[4] FAA's precise mechanism of anticancer action in experimental animals is poorly understood,[5,6] but undoubtedly is novel. Modification of FAA is continuing[7] as the unusual profile of FAA and its unclear mode of action make the development of structural analogues of great interest.

In our previous studies, numerous substituted 2-phenyl-4-quinolones, which are amino analogues of flavonoids (NH replacing O), were synthesized and evaluated for antimitotic and antitumor activities. Most compounds in this series showed promising in vitro activity in the NCI's human tumor cell lines (HTCL) assay with GI_{50} values in the low micromolar to nanomolar concentration range. In general, a good correlation was found between cytotoxicity and inhibition of tubulin polymerization.[8–11] Thirteen compounds in this series have been selected for in vivo xenograft testing, and to date, compound **1** (NSC 656158) is active in vivo. In the xenograft ovarian OVCAR-3 model, treated mice demonstrated a 130% increase in life span.

In our continuing studies, we designed, synthesized and evaluated a series of 2-phenyl-4-quinolone acetic acids with an acetic acid side chain at different positions of the A ring. These compounds combine the skeleton of the 2-phenyl-4-quinolones with the acetic acid side chain of FAA. 2-Phenyl-4-quinolone-8-acetic acid also is a bioisostere of FAA, in which NH replaces the O in the pyrone ring.

Scheme 1 shows the preparation of 2-phenyl-4-quinolone 5-, 6-, and 7-acetic acids. The 6-acetic acid (**3**) was

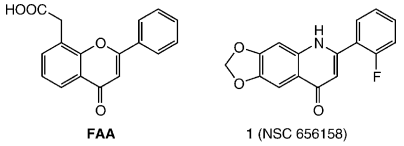

Figure 1.

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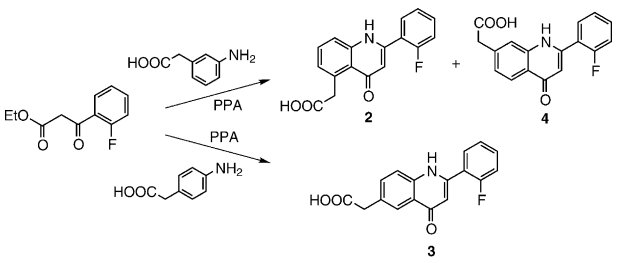
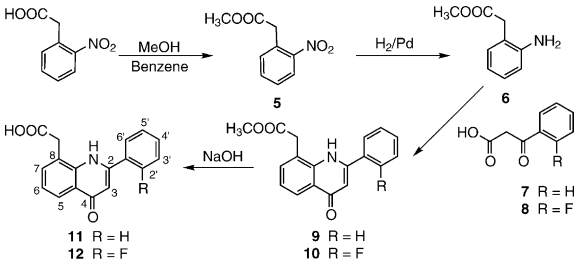


Scheme 1.



Scheme 2.

efficiently prepared by condensation of commercially available 4-aminophenylacetic acid and ethyl 2′-fluorobenzoylacetate when heated (90–100 °C) in polyphosphoric acid (PPA) for 2.5 h. The 5-acetic acid (**2**) and the 7-acetic acid (**4**) were obtained by condensation of 3-aminophenyl acetic acid and ethyl 2-fluorobenzoylacetate in PPA; the two isomers were separated by column chromatography.[14]

As shown in Scheme 2, the unsubstituted and 2-(2′-fluorophenyl)-4-quinolone-8-acetic acids (**11** and **12**) were prepared from the key intermediate, methyl 2-aminophenylacetate (**6**), and ethyl benzoylacetate (**7**) and ethyl 2-fluorobenzoylacetate (**8**). Compound **6** was synthesized by esterification of commercially available (*o*-nitrophenyl)acetic acid followed by reduction of the nitro group. However, **6** readily undergoes an intramolecular cyclization to the 2-oxindole via neutral and base catalyzed proton transfer.[12] Therefore, it was freshly made and condensed immediately with ethyl benzoylacetates **7** and **8**, to give esters **9**[15] and **10**, which then were hydrolyzed with aq NaOH to the corresponding acids **11** and **12**.

Compounds **2**–**4** and **9**–**12** were evaluated in a tubulin polymerization assay. As shown in Table 1,[16,17] 5- and 8-acetic acid substituted compounds were essentially inactive (**2**, **11**, and **12**) or showed little activity (**9**) in this assay. However, compound **10**, which is the methyl ester of analogue **12**, had moderate activity. In addition, the 6-substituted compound (**3**) also had moderate activity similar to that of compound **10** and the 7-acetic acid (**4**) had modest activity. Compounds **2**–**4** and **9**–**10** displayed moderate cytotoxicity in vitro against seven human tumor cell lines, including epidermoid, bone, ovarian, glioblastoma, melanoma, lung, and breast cancer cell lines. Among these five compounds, **10**, one of the two most potent compounds in the tubulin assay, was more active than the 5-, 6-, or 7-acetic acid quinolones. In addition, **10** showed selective activity against ovarian cancers. Interestingly, 2-phenyl-4-quinolone-8-acetic acid (**11**) and 2-(2′-fluorophenyl)-4-quinolone-8-acetic acid (**12**), which were inactive in the tubulin assay, were extremely potent in the cytotoxicity assay. The ED_{50} values of these two compounds were less than 0.1 μg/mL against most tested cell lines. A target other than tubulin is probably involved as the cause of the activities of **11** and **12**.

In summary, these findings with the 2-phenyl-4-quinolone acetic acid derivatives[13] showed unique and interesting results. 2-Phenyl-4-quinolone-8-acetic acid and 2-(2′-fluorophenyl)-4-quinolone-8-acetic acid are the most structurally similar to FAA and were the only highly active antitumor compounds in this series. Unlike most 2-phenyl-4-quinolones, they were not inhibitors of tubulin polymerization. 2-(2′-Fluorophenyl)-4-quinolone-6-acetic acid (**3**) and 2-(2′-fluorophenyl)-4-quinolone-8-acetic acid methyl ester (**10**) showed moderate inhibition of tubulin polymerization. Mechanism studies and further SAR evaluation are ongoing.

Table 1. Biological activities of 2-phenyl-4-quinolone acetic acids and their esters[13]

Compd	Cell line[a] ED_{50} (μg/mL)[b]							ITP[c] IC_{50} (μM) ± SD
	KB	HOS	1A9	U87-MG	SKMEL-2	A549	MCF-7	
2	NA	NA	NA	NA	>20 (9)	>20 (8)	>20 (7)	>40
3	>20 (36)[d]	>20 (15)	>20 (37)	>20 (9)	>20 (17)	>20 (24)	>20 (16)	5.2 ± 0.5
4	>20 (8)	NA	>20 (15)	NA	>20 (14)	>20 (21)	>20 (13)	10 ± 2
9	>20 (8)	>20 (7)	>20 (30)	NA	>20 (12)	NA	>20 (15)	30 ± 9
10	>10 (29)	>10 (18)	7.50	NA	>10 (11)	>10 (22)	>10 (14)	5.5 ± 0.4
11	0.04	0.07	0.04	0.12	0.06	0.11	0.44	>40
12	0.03	0.04	0.02	0.07	0.05	0.06	0.12	>40

[a]Cell lines include human epidermoid carcinoma of the nasopharynx (KB), bone carcinoma (HOS), human ovarian cancer (1A9), glioblastoma carcinoma (U-87-MG), human melanoma cancer (SKMEL-2), human lung cancer (A549), and human breast cancer (MCF-7).
[b]Cytotoxicity, ED_{50} for each cell line, is the concentration of compound that causes a 50% reduction in adsorbance at 562 nm relative to untreated cells using the SRB assay.[20]
[c]Tubulin polymerization was evaluated as described in ref 11. A minimum of two independent experiments was performed with each compound. The IC_{50} value is defined as the concentration that inhibits the extent of assembly by 50% after 20 min at 26 °C.
[d]Numbers in parentheses represent the percentage of inhibition at tested concentration.

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13. Tubulin polymerization assays were performed as described in ref 11. Cytotoxic assays were performed as described in: Rubinstein, L. V.; Shoemaker, R. H.; Paull, K. D.; Simo, R. M.; Tosini, S.; Skehan, P.; Scudiero, P. A.; Monks, A.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1113.
14. Melting points were determined on a Fisher-Johns melting point apparatus without correction. ^{1}H NMR spectra were measured on a Bruker AC-300 spectrometer with TMS as internal reference and $CDDl_3$ or DMSO-d_6 as solvent. Flash chromatography was performed on silica gel (mesh 25–150 μm).
15. **General procedure for the synthesis of 2-phenyl-4-quinolone acetic acids and their esters**. Methyl 2-aminophenylacetate (**6**) (1.65 g, 10 mmol) was suspended in 8 g of polyphosphoric acid (PPA). The mixture was warmed at 90–100 °C, and 1.92 g (10 mmol) of ethyl benzoylacetate (**7**) was added dropwise. The resulting mixture was further stirred for 1 h. After cooling, water was added, then aqueous NaOH (10%) was added slowly until pH = 6 and the solution extracted with $CHCl_3$. The organic layer was dried over sodium sulfate and concentrated in vacuo. Chromatography using $CHCl_3/CH_3OH$ (30:1) as eluant afforded 2.93 g of compound **9**, yield 65.5%; mp 76–78 °C; ^{1}H NMR ($CDCl_3$) δ 3.77 (s, 3H, CH_3), 3.95 (s, 2H, CH_2), 6.65 (s, 1H, H-3), 7.28 (m, 2H, H-6, H-7), 7.56 (m, 3H, H-3′, H-4′, and H-5′), 7.82 (m, 2H, H-2′, H-6′), 8.34 (d, 1H, J = 8.0 Hz, H-5), 10.1 (s, 1H, NH).
16. **2-Phenyl-4-quinolone-8-acetic acid (11)**. 2-Phenyl-4-quinolone-8-methyl acetate (**9**) (150 mg, 0.51 mmol) was suspended in 50% aq EtOH (10 mL) containing NaOH (100 mg). The mixture was heated under reflux for 2 h. After cooling, the solution was slowly acidified with aqueous HCl. The precipitate was collected, and washed with water to provide 140 mg of **11**, yield 98%; mp 238–240 °C; ^{1}H NMR (DMSO-d_6) δ 4.17 (s, 2H, CH_2), 7.40 (s, 1H, H-3), 7.56 (m, 2H, H-6, H-7), 7.61–8.01 (m, 5H, aromatic), 8.14 (d, 1H, J = 8.0 Hz, H-5), 11.9 (s, 1H, NH), 12.9 (s, 1H, COOH).
17. **2-(2′-Fluorophenyl)-4-quinolone-8-acetic acid (12)**. Obtained by hydrolysis of **10** with aqueous 50% EtOH containing NaOH using the same synthetic procedure as for **11**, yield 78.9%; mp 243–245 °C; ^{1}H NMR (DMSO-d_6) δ 4.07 (s, 2H, CH_2), 7.35 (s, 1H, H-3), 7.35–7.40 (m, 4H, H-6, H-7, H-3′ and H-5′), 7.53–7.63 (m, 2H, H-4′, H-6′), 8.08 (d, 1H, J = 9.0 Hz, H-5), 12.0 (br s, 1H, NH), 12.4 (S, 1H, COOH).