Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 713–716

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

ANTITUMOR AGENTS 187:[1] SYNTHESIS AND CYTOTOXICITY OF SUBSTITUTED 8,8-DIMETHYL-2*H*,8*H*-PYRANO[6,5-*h*]QUINOLINE-2-ONE AND RELATED COMPOUNDS

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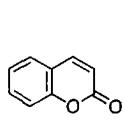
Received 8 September 1998; accepted 25 January 1999

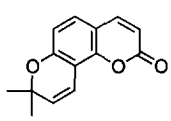
Abstract: Several substituted 8,8-dimethyl-2*H*,8*H*-pyrano[6,5-*h*]quinoline-2-ones and related compounds were synthesized and evaluated for their in vitro cytotoxicity against a panel of human tumor cell lines. The most active compound **(3)** showed significant cytotoxic activity with GI_{50} values in the micromolar range.

Coumarins constitute a major and widely distributed class of *O*-heterocyclic natural products with broad pharmacological profiles,[2] including antitumor activities.[3] The substitution pattern affects the pharmacological and biochemical properties and therapeutic applications of coumarines. Seselin **(2)**, an angular pyrancoumarin, displays various biological activities, including antifungal[4] and anti-HIV activities.[5] In particular, seselin exhibited moderate cytotoxicity in a mechanism-based anticancer bioassay employing DNA repair-deficient and repair-proficient yeasts.[6]

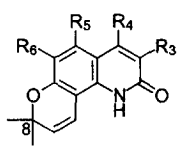
Potential anticancer drug design is highly dependent on information gained from structure–activity relationship (SAR) studies, which determine the structural features of a compound class that are essential for biological activity. Bioisosteric transformations of selected potent compounds can be used to obtain this data. Our laboratory used the principle of bioisosteric transformation in the synthesis of a series of 2-phenyl-4-quinolones **(PQ)**, which are the amino analogs of the plant-derived flavonoid **F1**. PQ derivatives showed extremely potent cytotoxic activity against a panel of human tumor cell lines with GI_{50} values in the low micromolar to nanomolar concentration range.[7–10] Therefore, we were prompted to synthesize a novel series of 8,8-dimethyl-2*H*,8*H*-pyrano[6,5-*h*]quinoline-2-ones **(DMPQ)** and related compounds. These compounds are bioisosteres of seselin where the O atom of the



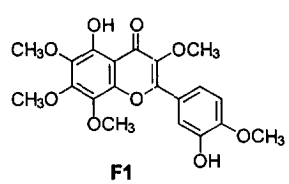
Coumarin (1)



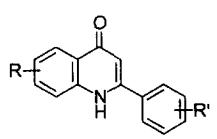
Seselin (2)



DMPQ



F1



PQ

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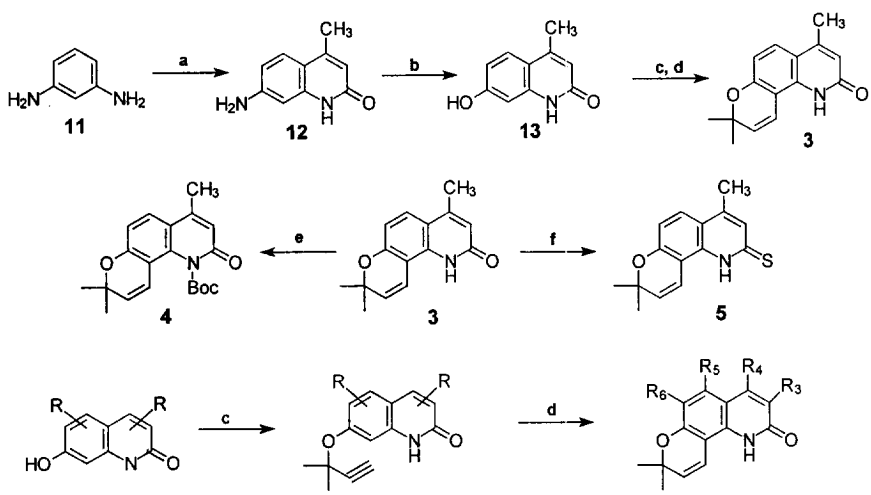
pyranocoumarin nucleus has been replaced by a N atom. Herein, we describe the first results on the potential cytotoxic activity of this class compound against human tumor cell lines. The synthesis and SAR study are also included.

Chemistry. The syntheses of **DMPQ** analogs **3–5** were accomplished by a 4- or 5-step sequence as illustrated in Scheme 1. 7-Amino-4-methyl-quinolin-2-one (**12**) was prepared from the *m*-phenylenediamine (**11**) and ethyl acetoacetate. Compound **12** was diazotized and hydrolyzed, yielding 7-hydroxy-4-methyl-quinolin-2-one (**13**). Compound **13** was converted in two steps to compound **3**. The nitrogen atom in **3** was converted to the carbamate **4** with di-*tert*-butyl dicarbonate in CH_2Cl_2 at room temperature. Treating **3** with Lawsesson's reagent in toluene gave thiolactam **5**.

Scheme 1



a: $CH_3COCH_2COOC_2H_5$; b: $NaNO_2$ / H_2SO_4, H_2O;
c: 3-Chloro-3-methyl-1-butyne, KI, K_2CO_3 in DMF;
d: *N,N*-Diethylaniline, reflux; e: Di-*tert*-butyl dicarbonate, Et_3N;
f: Lawesson reagent, toluene

6 $R_3 = R_4 = R_5 = R_6 = H$
7 $R_3 = R_4 = CH_3$; $R_5 = R_6 = H$
8 $R_3 = R_4 = R_6 = CH_3$; $R_5 = H$
9 $R_3 = R_5 = R_6 = H$; $R_4 = C_3H_7$
10 $R_3 = R_5 = R_6 = H$; $R_4 = C_6H_5$

Compounds **6–10** were prepared by the following general methods: A mixture of the substituted 7-hydroxy-quinolin-2-one, K_2CO_3, KI, and 3-chloro-3-methyl-1-butyne was in DMF heated at 60 °C under stirring for 24 h. After cooling, the mixture was filtered and concentrated to dryness. The residue was then dissolved in *N,N*-diethylaniline and refluxed for 2 h. The products were further purified by silica gel chromatography, yields were 60–70%. All **DMPQ** compounds were characterized by HRMS and ^1H NMR spectrocopy.[11]

Cytotoxicity assays The in vitro cytotoxicity assay was carried out according to procedures described in Rubinstein et al.[12] The human tumor cell line panel includes epiderimoid carcinoma of the nasopharynx (KB), lung carcinoma (A-549), ileocecal carcinoma (HCT-8), renal cancer (CAKI-1), breast cancer (MCF-7) and ovarian cancer (1A9). The results are presented in Table 1.

The most active compound **3** was further tested in the National Cancer Institute's in vitro disease-oriented antitumor screen, which determines a test agent's effect on growth parameters against a panel of approximately 60 human tumor cell lines. The cytotoxic effects of each compound are obtained as TGI or GI_{50} values, which represent the molar drug concentration required to cause total growth inhibition and half growth inhibition, respectively. The results are expressed in Table 2 as $\log GI_{50}$ values.

Table 1. In Vitro Cytotoxicities of 3,4,5,6-Substituted DMPQ and Related Compounds

	ED_{50} (μg / mL) [a]					
Compound	**KB [b]**	**A 549 [b]**	**HCT-8 [b]**	**CAKI-1 [b]**	**MCF-7 [b]**	**1A9 [b]**
3	1.6	2.2	1.7	2.5	1.7	ND[c]
4	2.5	4.3	3.6	4.5	2.4	ND
5	23.0	30.3	28.8	NA[d]	13.8	12.0
6	13.1	18	19.3	> 40(30)[e]	14.7	5.8
7	23.0	25.0	32.0	35.5	19.0	ND
8	37	41	41	43	38	ND
9	>40(45)	>40(29)	NA	NA	>40(31)	>40(44)
10	>40(45)	36.3	28.0	>40(35)	30.0	10.0

[a] Cytotoxicity as ED_{50} for each cell line, the concentration of compound that causes a 50% reduction in adsorbance at 562 nm relative to untreated cells using the SRB assay.[11] [b] Human ileocecal carcinoma (HCT-8), human breast cancer (MCF-7), human lung carcinoma (A-549), human epidermoid carcinoma of the nasopharynx (KB), human renal cancer (CAKI-1) and human ovarian cancer (1A9). [c] ND = not determined. [d] NA = no activity. [e] Inhibition was less than fifty percent at the highest concentration tested. The percent inhibition observed in such cases is given in the parentheses.

Table 2. Inhibition of In Vitro Tumor Cell Growth[a] by Compound **3**

Cytotoxicity log GI_{50} (M)[b]				
HL-60[c]	**NCI-H226[c]**	**COLO205[c]**	**HCT-116[c]**	**SF295[c]**
-6.37	> -4.00	-5.56	-5.31	-4.52
SK-MEL-28[c]	**OVCAR-3[c]**	**CAKI-1[c]**	**PD-145[c]**	**MDA-MB-435[c]**
> -4.00	-5.22	-5.21	-5.44	-5.76

[a]Data obtained from NCI's in vitro disease oriented human tumor cells screen; [b]Log concentrations that reduced cell growth to 50% of level at start of experiment; [c] HL-60, leukemia cell lines; NCI-H226, non-small cell lung cancer; COLO 205 and HCT-116, colon cancer cell lines; SF-295, CNS; SK-MEL-28, melanoma; OVCAR-3 and CAKI-1, ovarian and renal cancer cell lines; PD-145, prostate cancer cell lines; MDA-MB-435, breast cancer cell line.

Results and Discussions. The substituted 8,8-dimethyl-2*H*,8*H*-pyrano[6,5-*h*]quinoline-2-ones (**3–10**) were tested for cytotoxicity against a panel of human tumor cell lines. From the ED_{50} values summarized in Table 1, compounds **3** and **4** showed significant ($ED_{50} \leq 4.0$ μg/mL) cytotoxic activity. Compounds **5–8** showed moderate or weak activity, while **9** and **10** were inactive. Compound **3**, where the methyl group was at the 4-position, proved to be the most active compound in this study.

By comparing the cytotoxic activities of compounds with no, single, or multiple substitutions (**3–10**), the following conclusions were reached: (a) Introduction of a methyl group at the 4-position (**3**) led to enhanced cytotoxic activity compared with the unsubstituted compound **6**. (b) Replacing the hydrogen atom of the amine with a Boc group led to slightly decreased activity, while converting the 2-ketone to a 2-thione analog (**5**) decreased activity more than ten fold. Thus, these two functional moieties are essential for retaining cytotoxicity. (c) Increasing the number of methyl groups to two (**7**) or three (**8**) dramatically reduced the

cytotoxicity. (d) Larger or bulky groups at the 4-position (**9** and **10**) abolished the cytotoxic activity. In addition, the results obtained from NCI's in vitro disease-oriented antitumor screen shown in Table 2 are in agreement with our results in Table 1. Compound **3** showed strong cytotoxic effects against leukemia cell lines, including HL-60. It also showed fairly good activity against colon, ovarian, renal, and prostate cell lines as well as increased activity against breast cancer. However, it had low activity against non-small cell lung and melanoma cancer cell lines, with $\log GI_{50}$ values > -4.00. In summary, the position and size of the substitutents in the pyranoquinoline-2-one nucleus seems to be important for the antitumor activity in this compounds class. Compound **3** with a methyl group at the 4-position is a lead compound with potent cytotoxic activity. Synthesis of additional analogs and mechanism of action studies are ongoing to develop more potent compounds.

Acknowledgement: This investigation was supported in part by grant CA 17625 from Cancer Institute, NIH awarded to K. H. Lee.

References and notes:

1. For Part 186, see Guan, J.; Zhu, X. K.; Tachibana, Y.; Bastow, K. F.; Brossi, A.; Hamel, E.; Lee, K. H. *Biorg. & Med. Chem.*, in press.
2. Murray, R. D. H. *Progress in the Chemistry of Organic Natural Products* **1991** *58*, 84.
3. Padmawinata, K. *Acta Pharm.* **1973** *40*, 1.
4. Bandara, B. M. R.; Gunatilaka, A. A. L.; Wijeratne, E. M. K.; Macleod, J. K. *Phytochemistry* **1990,** *29*, 297.
5. Huang, L.; Kashiwada, Y.; Consentino, L. M.; Fan,S.; Chen, C.; McPhail, A. T.; Fujioka, T.; Mihashi, K.; Lee, K. H. *J. Med. Chem.* **1994**, *37*, 3947.
6. Gunatilaka, A. A. L.; Kingston, D. G. I.; Wijeratne, E. M. K.; Bandara, B. M. R.; Hofmann, G. A.; Johnson, R. K. *J. Nat. Prod.* **1994**, *57*, 518.
7. Kuo, S. C.; Lee, H. Z.; Juang, J. P.; Lin, Y. T.; Wu, T. S.; Chang, J. J.; Lednicer, D.; Paull, K. D.; Lin, C. M.; Hamel, E.; Lee, K. H. *J. Med. Chem.* **1993**, *36*, 1146.
8. Li, L.; Wang, H. K.; Kuo, S. C.; Wu, T. S.; Lednicer, D.; Lin, C. M.; Hamel, E.; Lee, K. H. *J. Med. Chem* **1994**, *37*, 3400.
9. Li, L.; Wang, H. K.; Kuo, S. C.; Wu, T. S.; Lednicer, D.; Lin, C. M.; Hamel, E.; Lee, K. H. *J. Med. Chem* **1994**, *37*, 1126.
10. Xia, Y.; Yang, Z. Y.; Xia, P.; Bastow, K. F.; Tachibana, Y.; Kuo, S. C.; Hamel, E.; Hackl, T.; Lee, K. H. *J. Med. Chem.* **1998**, *41*, 1155.
11. For example: **4,8,8-Trimethylhydro-2*H*-pyrano[6,5-*h*]quinline-2-one (3)**: mp: 256–258 °C. ^{1}H NMR (300MHz, $CDCl_3$) δ : 1.46 (s, 6H, $2XCH_3$), 2.45 (s, 3H, CH_3), 5.80 (d, J = 9.6Hz, 1H, H-9), 6.40 (s, 1H, H-3), 6.47 (d, J = 8.76 Hz, 1H, H-6), 6.86 (d, J = 9.6 Hz, 1H, H-10), 7.46 (d, J = 8.76 Hz, 1H, H-5), 9.96 (dr, 1H, NH); HRMS calcd for $C_{15}H_{15}NO_2$ 241.1103, Found 241.1110.
12. 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.