Cytotoxic Effects of Pyridino[2,3-*f*]indole-4,9-diones on Human Tumor Cell Lines

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The cytotoxicities of pyridino[2,3-*f*]indole-4,9-dione derivatives were examined against human lung tumor cell lines (A 549), human ovarian tumor cell lines (SK-OV-3), human melanoma tumor cell lines (SK-MEL-2), human CNS tumor cell lines (XF 498) and human colon tumor cell lines (HCT 15) *in vitro* using a Sulforhodamine B assay. 3-Ethoxycarbonyl-1-(2-methoxyethyl)-2-methyl-1*H*-pyridino[2,3-*f*]indole-4,9-dione (5) showed excellent cytotoxicity against XF 498 and HCT 15. The ED_{50} values of 5 were 0.006 μg/ml against XF 498 and 0.073 μg/ml against HCT 15, while those of doxorubicin were 0.012 and 0.264 μg/ml, respectively. 1-Benzyl-3-ethoxycarbonyl-2-methyl-1*H*-pyridino[2,3-*f*]indole-4,9-dione (7) (ED_{50} value 0.065 μg/ml) was also significantly more cytotoxic against HCT 15 compared with doxorubicin.

Key words pyridino[2,3-*f*]indole-4,9-dione; human CNS tumor cell; human colon tumor cell

Heterocyclic aminoquinones have received attention because of their marked anticancer activities.[2,3)] In particular, streptonigrin is a potent antitumor agent, however, toxicity was found in its clinical use. 7-Amino-6-methoxy-5,8-quinolinedione is an essential part for the antitumor activity of streptonigrin,[4)] and we have already reported pyrido[1,2-α]imidazo[4,5-g]quinoxaline-6,11-dione as a heteroquinone analogue, which displayed significant cytotoxicity against human gastric adenocarcinoma cells (MKN 45).[5)] Planar tricyclic heteroquinones such as imidazoquinoxalinediones have been claimed to act as intercalating agents between DNA base pairs.[6)] Further research on tricyclic heteroquinone antitumor agents lead us to synthesize pyridino[2,3-*f*]indole-4,9-dione derivatives, which contain a quinolinedione moiety. We then examined the cytotoxicity of structurally diverse pyridino[2,3-*f*]indole-4,9-dione derivatives *in vitro* using a Sulforhodamine B (SRB) assay. We have already published the synthesis of pyridino[2,3-*f*]indole-4,9-dione derivatives elsewhere (Fig. 1).[7,8)] Interestingly, some of these derivatives showed considerable cytotoxicity towards XF498 and HCT15, compared with doxorubicin and *cis*-platin, which are used as anticancer drugs.

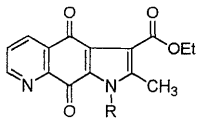
RESULTS AND DISCUSSION

Alkyl and aryl pyridino[2,3-*f*]indole-4,9-dione derivatives containing fixed 3-ethoxycarbonyl-2-methyl substituents and different 1-substituents were investigated for cytotoxic activity. The SRB assay,[9,10)] developed for measuring the cellular protein content of cultures, was applied for measurement of the cytotoxicity of the compounds against tumor cells. Human lung tumor cell lines (A 549), human ovarian tumor cell lines (SK-OV-3), human melanoma tumor cell lines (SK-MEL-2), human CNS tumor cell lines (XF 498) and human colon tumor cell lines (HCT 15) were used for *in vitro* cytotoxicity tests. The cytotoxic activity was evaluated by measuring the concentration of compound required to inhibit protein synthesis by 50% (ED_{50}) in comparison with the anticancer agents doxorubicin and *cis*-platin, and the data is summarized in Table 1.

3-Ethoxycarbonyl-1-(2-methoxyethyl)-2-methyl-1*H*-pyridino[2,3-*f*]indole-4,9-dione (**5**) was the most cytotoxic compound and showed marked activity, especially towards XF 498 and HCT 15. The ED_{50} value of **5** against XF 498 was 0.006 μg/ml and 0.073 μg/ml against HCT 15. These values are between two and three fold more potent than those of doxorubicin (ED_{50} values: 0.012 and 0.264 μg/ml, respectively). Compound **5** was also tolerably active against SK-MEL-2 compared with doxorubicin. 3-Ethoxycarbonyl-2-methyl-1-propyl-1*H*-pyridino[2,3-*f*]indole-4,9-dione (**3**), which contains a propyl group at the N-1 position, showed similar cytotoxicity to XF 498 and HCT 15 compared with doxorubicin. Against HCT 15, 1-benzyl-3-ethoxycarbonyl-2-methyl-1*H*-pyridino[2,3-*f*]indole-4,9-dione (**7**) was the most active compound. The ED_{50} value of **7** was 0.065 μg/ml, while that of doxorubicin was 0.264 μg/ml. On the other hand, the N-phenyl compound **9** showed comparable activity towards the HCT 15 (ED_{50} value: 0.110 μg/ml), however, addition of *p*-halogeno groups greatly decreased activity (ED_{50} values: 2.87—6.15 μg/ml) in the human tumor cell lines tested. Most compounds were more active than *cis*-platin. Cytotoxicity evaluation suggests that pyridino[2,3-*f*]indole-4,9-dione derivatives may be potential new antitumor agents.

MATERIALS AND METHODS

Materials 3-Ethoxycarbonyl-1,2-dimethyl-1*H*-pyridino-[2,3-*f*]indole-4,9-dione (**1**), 3-ethoxy-carbonyl-1-ethyl-2-methyl-1*H*-pyridino[2,3-*f*]indole-4,9-dione (**2**), 3-ethoxycarbonyl-2-methyl-1-propyl-1*H*-pyridino[2,3-*f*]indole-4,9-dione



R= **1**; CH_3 **2**; C_2H_5 **3**; C_3H_7 **4**; cyclopropyl

5; $CH_2CH_2OCH_3$ **6**; CH_2CH_2OH **7**; benzyl **8**; furfuryl

9; phenyl **10**; 4-fluorophenyl **11**; 4-chlorophenyl **12**; 4-bromophenyl **13**; 4-iodophenyl

Fig. 1

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Table 1. The Cytotoxicity Data on Human Lung Tumor Cell Lines (A 549), Human Ovarian Tumor Cell Lines (SK-OV-3), Human Melanoma Tumor Cell Lines (SK-MEL-2), Human CNS Tumor Cell Lines (XF 498) and Human Colon Tumor Cell Lines (HCT 15)

Compounds	A549 ED_{50} (μg/ml)	SK-OV-3 ED_{50} (μg/ml)	SK-MEL-2 ED_{50} (μg/ml)	XF498 ED_{50} (μg/ml)	HCT15 ED_{50} (μg/ml)
cis-Platin	1.670	1.180	0.430	0.620	3.520
Doxorubicin	0.086	0.018	0.035	0.012	0.264
1	1.207	2.170	0.720	0.377	0.920
2	0.930	1.170	0.930	0.110	1.490
3	0.340	0.140	0.160	0.014	0.220
4	0.265	0.540	0.933	0.241	0.739
5	0.370	0.089	0.064	0.006	0.073
6	0.335	0.769	0.611	0.376	1.849
7	0.315	0.170	0.070	0.069	0.065
8	1.115	0.410	0.275	1.032	0.353
9	0.680	1.670	0.240	0.740	0.110
10	4.574	2.867	5.282	5.836	1.560
11	5.098	4.733	5.294	5.705	1.267
12	6.149	5.145	6.049	5.570	1.974
13	4.556	5.762	5.026	5.608	1.354

(**3**), 1-cyclopropyl-3-ethoxycarbonyl-2-methyl-1*H*-pyridino-[2,3-*f*]indole-4,9-dione (**4**), 3-ethoxycarbonyl-1-(2-methoxy-ethyl)-2-methyl-1*H*-pyridino[2,3-*f*]indole-4,9-dione (**5**), 1-ethanol-3-ethoxycarbonyl-2-methyl-1*H*-pyridino[2,3-*f*] indole-4,9-dione (**6**), 1-benzyl-3-ethoxycarbonyl-2-methyl-1*H*-pyridino[2,3-*f*]indole-4,9-dione (**7**), 3-ethoxycarbonyl-1-furfuryl-2-methyl-1*H*-pyridino[2,3-*f*]indole-4,9-dione (**8**), 3-ethoxy-carbonyl-2-methyl-1-phenyl-1*H*-pyridino[2,3-*f*]indole-4,9-dione (**9**), 3-ethoxycarbonyl-1-(4-fluorophenyl)-2-methyl-1*H*-pyridino[2,3-*f*]indole-4,9-dione (**10**), 1-(4-chlorophenyl)-3-ethoxy-carbonyl-2-methyl-1*H*-pyridino[2,3-*f*]indole-4,9-dione (**11**), 1-(4-bromophenyl)-3-ethoxy-carbonyl-2-methyl-1*H*-pyridino[2,3-*f*]indole-4,9-dione (**12**) and 3-ethoxycarbonyl-1-(4-iodo-phenyl)-2-methyl-1*H*-pyridino-[2,3-*f*]indole-4,9-dione (**13**) were synthesized as previously described.[7,8]

Cell Lines and Culture Conditions The SK-MEL-2 human melanoma, A 549 non-small cell lung, SKOV-3 ovarian, HCT-15 colon, and XF-498 CNS tumor cell lines were maintained as stocks in RPMI 1640 (Gibco) supplemented with 10% fetal bovine serum (Gibco). Cell cultures were passaged once or twice weekly using trypsin-EDTA(Ethylenediaminetetraacetic acid) to detach the cells from their culture flasks.

SRB Cytotoxicity Assay Rapidly growing cells were harvested, counted, and inoculated at appropriate concentrations (1—2×10^4 cells/well) into 96 well microtiter plates. After incubation for 24 h, the compounds dissolved in culture medium were applied to the culture wells in triplicate followed by incubating for 48 h at 37 °C under 5% CO_2 atmosphere. The cultures fixed with cold trichloroacetic acid (TCA) were stained by 0.4% SRB dissolved in 1% acetic acid. After solubilizing the bound dye with 10 mM unbuffered tris base using a gyrotory shaker, the absorbance at 520 nm was measured with a microplate reader (Dynatech Model MR 700). Fifty percent inhibitory concentration (ED_{50}) was defined as the concentration which reduced absorbance to 50% of untreated wells as control.

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