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Note

Cytotoxic Activity toward KB Cells of 2-Substituted Naphtho[2,3-*b*]furan-4,9-diones and Their Related Compounds

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We investigated the cytotoxic activity of 2-substituted naphtho[2,3-*b*]furan-4,9-diones. We have previously synthesized 33 types of 2-substituted and related compounds, and the cytotoxic activity of these compounds was then examined by a KB cell culture assay. 2-(3-Furanoyl)benzoic acids and 1,4-naphthoquinones had no activity. 2-Acetyl-4,9-dimethoxynaphtho[2,3-*b*]furan 4 showed low activity. However, parent naphtho[2,3-*b*]furan-4,9-dione 2 and most 2-substituted derivatives exhibited cytotoxic activity. The parent structure was therefore for cytotoxicity. 2-Formylnaphtho[2,3-*b*]-furan-4,9-dione 11 had particularly potent activity (ED_{50} = 0.09 μg/ml).

Key words: cytotoxic activity; KB cell culture assay; 2-substituted naphtho[2,3-*b*]furan-4,9-dione

Several naphtho[2,3-*b*]furan-4,9-diones with interesting biological activities have been isolated from plants.[1] For example, 2-acetylnaphtho[2,3-*b*]furan-4,9-dione **1** isolated from *Tabebuia cassinoides* (Lam.) DC (*Bignoniaceae*) exhibited cytotoxic activity.[2] Hayashi *et al.*[3] have reported that the cytotoxic activity of 2-methylnaphtho[2,3-*b*]furan-4,9-dione was three times that of **1**. The activity of 2-substituted naphtho[2,3-*b*]furan-4,9-diones varies with the type of substituent on parent naphtho[2,3-*b*]furan-4,9-dione **2**. Although organic chemists are interested in synthesizing these compounds, a convenient method to directly introduce substituents on to the 2-position of **2** has not previously been elucidated.

We recently began to focus on the synthesis of 2-substituted compounds, and have reported the preparation of parent compound **2**,[4] natural product **1**,[5,6] 2-trimethylsilylnaphtho[2,3-*b*]furan-4,9-dione **9**,[6] 2-(2-methyl-1,3-dioxolan-2-yl)naphtho[2,3-*b*]furan-4,9-dione **10**,[6] and 2-formylnaphtho[2,3-*b*]furan-4,9-dione **11**.[6] Halodesilylation and nitrodesilylation of **9** have been reported in connection with studies to prepare the 2-substituted compounds. 2-Chloronaphtho[2,3-*b*]furan-4,9-dione **12**, 2-bromonaphtho[2,3-*b*]furan-4,9-dione **13**, 2-iodonaphtho[2,3-*b*]furan-4,9-dione **14**, and 2-nitronaphtho[2,3-*b*]furan-4,9-dione **15** have subsequently been obtained in good yields.[6] Furthermore, **12** has been treated with carbon,[7,8] oxygen,[7] sulfur,[7,8] and nitrogen[8,9] nucleophiles to give desired compounds **16**–**42**. We are interested in the cytotoxic activity of the obtained 2-substituted derivatives. We examine in the present study the activity of the derivatives by using a KB cell culture assay according to the NIH protocol.[10]

We were also interested in the activity of related compounds without the parent structure, so we tested the activity of the following compounds in the same manner: 4,9-dimethoxynaphtho[2,3-*b*]furan **3**[6] and 2-acetyl-4,9-dimethoxynaphtho[2,3-*b*]furan **4**,[6] intermediates with a reduced parent structure that were formed during the synthesis of 2-substituted naphtho[2,3-*b*]-furan-4,9-dione; 2-(3-furanoyl)benzoic acid **5**[4] and 2-(2-acetyl-4-furanoyl)benzoic acid **6**,[6] intermediates without a tricyclic structure that were formed during the synthesis of parent **1** and the short synthesis of the parent structure; and 1,4-naphthoquinones **7** and **8**,[7] decomposition products lacking a furan ring that were isolated during the reaction of compound **12** with an oxygen nucleophilic agent. Tables 1, 2 and 3 show the structures of all the compounds tested in the present study and their cytotoxicity (ED_{50}).

There is only one report regarding the biological activity of parent **2**. It has been reported that **2** exhibited inhibitory activity against the Epstein-Barr virus early antigen (EBA-VA) activation introduced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA).[11] The cytotoxic activity of **2** was initially investigated, and it was found to exhibit cytotoxic activity in the KB cell culture assay (ED_{50} = 0.6 μg/ml). We then investigated the activity of related compounds **3**–**8** without the parent structure, and the results showed that, while compounds **3**, **5**, **6**, **7** and **8** were not cytotoxic, compound **4** was mildly cytotoxic (ED_{50} = 6.4 μg/ml) (Table 1).

We subsequently studied the effects of substituents at the 2-position for cytotoxic activity. First, the activity of those compounds having a carbon bond at the 2-position

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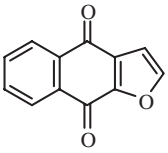
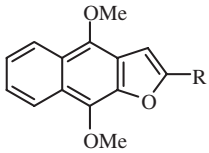
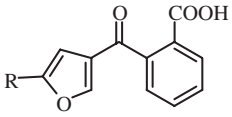
Table 1. ED_{50} Values (μg/ml) of Naphtho[2,3-*b*]furan-4,9-dione, 4,9-Dimethoxynaphtho[2,3-*b*]furans, 2-(3-Furanoyl)benzoic Acids and 1,4-Naphthoquinones



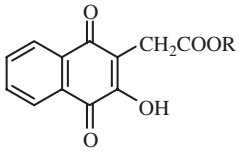
Compound No.	ED_{50} (μg / ml)
2	0.6



Compound No.	R	ED_{50} (μg / ml)
3	H	>10
4	COMe	6.4

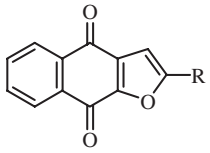


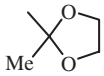
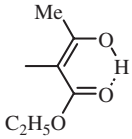
Compound No.	R	ED_{50} (μg / ml)
5	H	>10
6	COMe	>10



Compound No.	R	ED_{50} (μg / ml)
7	Et	>10
8	Me	>10

Table 2. ED_{50} Values (μg/ml) of 2-Substituted Naphtho[2,3-*b*]furan-4,9-diones (1)



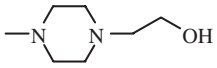
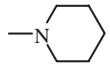
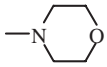
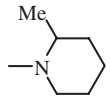
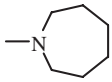
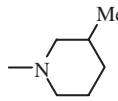
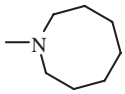
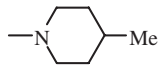
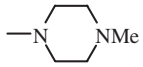
Compound No.	R	ED_{50} (μg/ml)	Compound No.	R	ED_{50} (μg/ml)
1	COMe	1.0[2)]	**18**	OPh	2.2
9	$SiMe_3$	3.7	**19**	SPh	2.5
12	Cl	0.5	**20**	SMe	0.8
13	Br	0.6	**15**	NO_2	0.2
14	I	0.7	**21**	N_3	1.0
10		0.4	**22**	NHPr	0.6
			23	NH*i*-Pr	1.1
11	CHO	0.09	**24**	NHBu	0.7
16	$CH(COOEt)_2$	3.7	**25**	NH*i*-Bu	0.8
			26	NH*sec*-Bu	1.6
17		3.7	**27**	NMe_2	1.0
			28	NEt_2	3.9
			29	NPr_2	>10

was studied. Compound **10**, which has the acetyl group of **1** protected by a ketal, showed strong activity ($ED_{50} = 0.4\,\mu g/ml$), while 2-formyl compound **11** exhibited stronger activity ($ED_{50} = 0.09\,\mu g/ml$). Compounds **16** and **17**, which possess bulky substituent groups, showed moderate activity.

2-Phenoxy compound **18** and 2-phenylthio compound **19** showed similar activities; however, the activity of 2-methylthio compound **20** was three times stronger than that of **19**. The bulky substituent group at the 2-position may thus have decreased the cytotoxic activity. Halogen derivatives **12**–**14** were also examined, with 2-chloro compound **12**, which has the smallest group among the derivatives, showing the strongest activity, while 2-trimethylsilyl compound **9** had moderate activity (Table 2).

The activity of those compounds having a nitrogen bond at the 2-position was also studied. 2-Nitro compound **15** had strong activity ($ED_{50} = 0.2\,\mu g/ml$). When the nitro group was reduced to a hydroxyamino group, it

Table 3. ED_{50} Values (μg/ml) of 2-Substituted Naphtho[2,3-*b*]furan-4,9-diones (2)

Compound No.	R	ED_{50} (μg / ml)	Compound No.	R	ED_{50} (μg / ml)
30		1.4	**37**		1.6
31		>10	**38**		0.6
32		1.8	**39**		0.7
33		1.7	**40**		6.7
34		>10	**41**		>10
35		4.3	**42**		0.2
36		1.1			

acted as an aromatic amidation agent for nucleic acid. 2-Azido compound **21** also showed cytotoxic activity ($ED_{50} = 1.0\,\mu g/ml$). Compounds **22**–**26**, which have aliphatic secondary amino residues, were also tested. 2-Propylamino compound **22**, having the smallest substituent group among the derivatives, showed the strongest activity. Branched isomer **23** had lower activity than **22**. Among the butylamino derivatives, branched derivatives **25** and **26** had lower activities than straight-chain derivative **24**. Compounds **27**–**29**, having aliphatic tertiary amino residues, were then tested. 2-Dimethylamino compound **27** was more active than 2-diethylamino compound **28**, while 2-diisopropylamino compound **29** had no activity. The activities of compounds **30**–**41**, which have cyclic amino residues were also investigated. The activity was highest in 5-member ring **30**, followed by 6-member ring **32**, 7-member ring **40** and 8-member ring **41**. It is interesting that 2-(3-pyrrolin-1-yl) compound **31** showed no cytotoxic activity, despite the presence of a 5-member ring. 2-(2-Methylpiperidino) compound **33** showed similar activity to **32**. 2-(4-Methylpiperidino) compound **35** had lower activity than **32**, while 2-(3-methylpiperidino) compound **34** had no activity. *N*-Substituted piperazinyl compounds **36**–**38** had cytotoxic activity, with **38** showing the strongest activity among the derivatives ($ED_{50} = 0.6\,\mu g/ml$). 2-Morpholino compound **39** showed high activity ($ED_{50} = 0.7\,\mu g/ml$). The activities of **38** and **39** were stronger than those of the other cyclic amino compounds. 2-(1-Imidazolyl) compound **42** also showed strong activity ($ED_{50} = 0.2\,\mu g/ml$) (Table 2, Table 3).

Compounds **3**, **5**, **6**, **7** and **8** had no cytotoxic activity, while **4** showed only low activity in our examination. Parent **2** and almost all of its derivatives possessed cytotoxic activity, and parent structure **2** thus appears to be necessary for cytotoxicity. We found that the activity of the 2-substituted compounds varied with the type of substituent group, although both electron-withdrawing groups and electron-donating groups at the 2-position exhibited cytotoxic activity. We demonstrated that neither an inductive effect nor resonance effect at the 2-position had any effect on cytotoxicity. Some compounds were more active than parent **2**. It was found that some substituent groups at the 2-position increased the activity. However, bulky substituent groups at the 2-position decreased the activity. Chang and Chen[12] have reported that the carcinostatic mechanism of morindaparvin-A, which has an anthraquinone structure, was intercalation to DNA. Intercalation was thus assumed to be the cytotoxic mechanism of naphtho[2,3-*b*]furan-4,9-diones. However, among the compounds with bulky substituent groups, the activities of compounds **10**, **38** and **39**, which possess an oxygen atom, were marked, while those of compounds **16** and **17** were moderate. Hydrogen bonding to DNA thus appears to be involved in cytotoxicity. Kim *et al.*[13] have reported that the formyl and nitro groups on the benzimidazoles acted as hydrogen bond acceptors, and as a result, hydrogen bonding of formyl-derivative **11** and nitro-derivative **15** to DNA may have enhanced the cytotoxicity.

On the other hand, Lown *et al.*[14] have reported that the activity of carcinostatic agents having an anthraquinone structure was inhibited by superoxide dismutase (SOD). They suggested that the hydroxyl radical was the active intermediate of these agents. When **3**, which has a quinone moiety, was reduced, it exhibited no cytotoxic activity, and thus the free radicals were concluded to be

involved in the cytotoxic mechanism of naphtho[2,3-*b*]furan-4,9-diones. Furthermore, Bhashyam *et al.*[15] have reported that the presence of basic nitrogen enhanced the activity of drugs that intercalate DNA. This supports the results of the present study; the activity of compound **42**, which possesses an imidazole group, was high, while almost all the compounds synthesized by nitrogen nucleophilic substitution were cytotoxic. Coste *et al.*[16] have reported that such aldehydes as formaldehyde and 2-furaldehyde formed DNA-protein cross-links, and as a result, the same action mechanism may be involved with compound **11**, possessing a formyl group, which exhibited the strongest activity in this study.

In conclusion, intercalation to DNA and free radicals are thought to be involved in the cytotoxicity of naphtho[2,3-*b*]furan-4,9-diones. The derivatives have such potent activity because these mechanisms act together. To obtain a more active compound, further studies on the preparation of compounds having suitable substituent groups (size and hydrogen bonds) are in progress, and the results will be reported in due course.

Experimental

Cytotoxic screening of the 42 compounds was conducted according to NIH protocol. KB cells were incubated with 2×10^4 tumor cells grown in MEM + 10% fetal calf serum at 37 °C at a 10° angle for 72 h. Each drug was dissolved in dimethyl sulfoxide (0.01 ml), which did not inhibit normal growth, and was tested at 0.04 to 10 μg/ml (N = 4). Protein determinations were made for all the test and control tubes, standards and medium blanks according to the method of Oyama and Eagle.[17] ED_{50} is the calculated effective dose that inhibited growth to 50% that of the control.

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