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Synthesis and Antiproliferative Activity of Some Benzimidazole-4,7-dione Derivatives

Laura Garuti,[a,*] Marinella Roberti,[a] Monica Malagoli,[b] Tiziana Rossi[c]
and Mario Castelli[b]

[a]*Department of Pharmaceutical Science, University of Bologna, via Belmeloro 6, I-40126 Bologna, Italy*
[b]*Department of Biomedical Science, University of Modena, via Campi 287, I-41100 Modena, Italy*
[c]*Department of Pharmaceutical Science, University of Modena, via Campi 287, I-41100 Modena, Italy*

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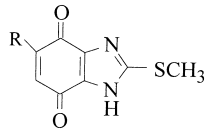
Abstract—A series of benzimidazole-4,7-diones bearing at the 2-position the thiomethyl group or the 2-pyridyl moiety has been synthesized and tested in vitro on three tumor cell lines. Two of them show a very good antiproliferative effect. Compounds **1** and **2d** are more active or equiactive, respectively, than MMC against human lymphoblastic leukemia. Both compounds exhibit high activity on human non-Hodgkin lymphoma. Compound **1** is non toxic at all the concentrations used in the antiproliferative assay and **2d** is toxic only at high concentration. © 2000 Elsevier Science Ltd. All rights reserved.

A great number of heterocyclic quinones display interesting antitumour activities. Their mechanism of action is very different for the various types of compounds. Many of them act as bioreductive alkylating agents. Compounds of note include mitomycin C (MMC),[1–4] mitosenes, such as EO9[5,6] and many indole analogues.[7] Other quinones exert their action via inhibition of DNA synthesis: these include streptonigrin,[8,9] several quinoline[10] and quinoxaline diones.[11] Significant biological properties appeared also in some benzimidazolediones.[12–14] 5-Amino-6-bromobenzimidazole-4,7-dione[12] shows good antitumor activity in vivo on P388 leukemia. Pyrrolo[1,2-*a*] benzimidazole and benzimidazole-based aziridinyl quinones[13–18] possess strong antitumor activity by virtue of their ability to cleave DNA. Moreover, studies on simple heterocyclic quinones containing nitrogen show that the number and position of nitrogens are important for the cytotoxicity.[18] On the basis of these considerations, a series of variously substituted benzimidazole-4,7-diones has been synthesized and tested for antiproliferative activity. The aim is to investigate the substituent effect at the 5(6) position of simplified quinone systems, related to general structure **1** and **2**. The 5(6) substituents, by varying the quinone reduction ease, influence its single-strand DNA cleavage capability and therefore its activity. Hence a range of compounds containing the thiomethyl group (**1**, **1a–c**, **1e–g**) or the pyridyl moiety (**2a**, **2c–e**) at C-2 is prepared. The C-5 position is unsubstituted or substituted with an amino function, with the methoxy group or with an electron withdrawing group as the bromine atom.



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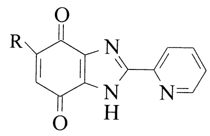

2

Chemistry

4,7-Dimethoxybenzimidazol-2-(3*H*)-thione[19] **3** and 2,3-diamino-1,4-dimethoxybenzene[20] **4** were reported in the literature. The hydroquinone **5** (Scheme 1) was obtained by heating **3** with 48% hydrobromic acid at 120 °C for 6 h. The *S*-methylation occurred during this hydrolysis and led to the 4,7-dihydroxy-2-methylthio benzimidazole as the main product in good yield. Oxidation of **5** with ferric chloride in aqueous solution at room temperature gave the 2-methylthiobenzimidazole-4,7-dione **1**. The 5(6)-substituted derivatives were obtained by nucleophilic addition reaction on the quinone. The reaction of **1** with primary and secondary amines in methanol solution resulted in the formation of 5(6)-amino-substituted-4,7-benzimidazolediones **1a** and **1b**.

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*Corresponding author. Tel.: +39-051-2099715; fax: +39-051-2099734; e-mail: mrobi@alma.unibo.it

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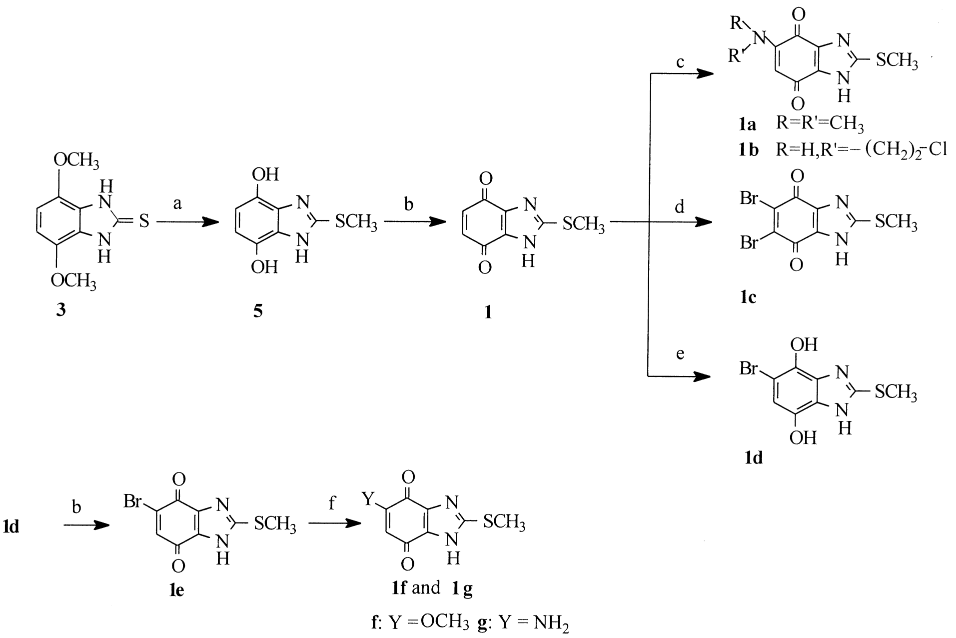
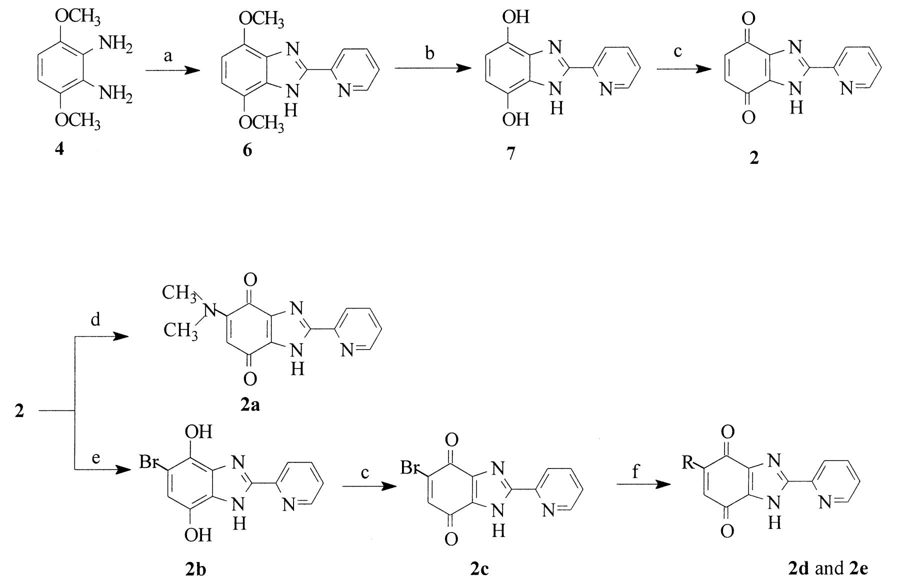


Scheme 1. (a) HBr, 120 °C; (b) Aq. $FeCl_3$; (c) dimethylamine or 2-chloroethylamine, MeOH; (d) Br_2, 1,2-DME; (e) HBr; (f) NH_4OH, MeOH.



Scheme 2. (a) 2-Pyridinecarboxaldehyde, nitrobenzene; (b) HBr, 120 °C; (c) Aq. $FeCl_3$; (d) dimethylamine, MeOH; (e) HBr; (f) NH_4OH, MeOH.

The 5,6-dibromo derivative **1c** was obtained by addition of bromine to **1** in 1,2-dimethoxyethane. Treating of **1** with 48% hydrobromic acid gave 5(6)-bromo-4,7-benzimidazolediol hydrobromide **1d** which was oxidized to quinone **1e** in aqueous ferric chloride solution. The reaction of **1e** with ammonium hydroxide in methanol at room temperature yielded a mixture of **1f** (24.4%) and **1g** (15%), which was separated by column chromatography. Refluxing **4** with 2-pyridinecarboxaldehyde in nitrobenzene gave 2-(2-pyridyl)-4,7-dimethoxybenzimidazole **6**, which was purified by column chromatography. The remaining compounds in this series, namely **7**, **2**, **2a–e** were prepared (Scheme 2) by methods similar to those used for the 2-methylthio series.[21]

Results and Discussion

The series of benzimidazole-4,7-diones **1**, **1a–c**, **1e–g** and **2a**, **2c–e** were screened for activity against Molt-3 cells, from human lymphoblastic leukemia, SupT-1 cells, from human non-Hodgkin lymphoma and MCF-7 cells from human breast adenocarcinoma and comparison was made with mitomycin C (MMC).[22,23] The results, reported in Table 1, show that compounds **1** and **2d** possess the best antiproliferative effect. Such activity is strong against SUPT1 cells, although lower than that of the MMC. Moreover, compound **1** is more active than MMC on human lymphoblastic leukemia, whereas

Table 1. The antiproliferative activities of benzimidazole-4,7-diones and MMC

Compound	ID_{50} (μM)[a]		
	SupT1	Molt3	MCF7
1	2.87	1.32	62.65
1a	38	18.32	>90
1b	15.31	30.6	>90
1c	6.26	11.99	40.3
1e	5.03	32.14	62.8
1f	5.07	20.82	36
1g	10.8	60.4	>90
2a	17.44	22.45	>90
2c	5.57	7.52	>90
2d	2.57	2.63	26.4
2e	29.30	39.67	>90
MMC	< 0.7	2.66	42.92

[a] ID_{50} drug dosage required to inhibit the cancer cell growth by 50%.

Table 2. Cytotoxicity of **1** and **2d** on VERO cells

Compound	Conc. μM	Dye test[a]	Lowry's test[b]
Control		100	100
1	90	73.6	68.9
	11.2	100.7	86.4
	0.7	99.3	102.9
2d	90	16.4	16.2
	11.2	71.2	81.3
	0.7	113.7	97.2

[a] Percent cellular growth.
[b] Percent protein content.

compound **2d** and MMC are equiactive. Compounds **1e** and **1f** have a moderate inhibitor activity. In contrast, all derivatives show no activity on breast adenocarcinoma; the MMC standard also is not active in this assay. Because of their interesting antiproliferative activity, the in vitro toxicity of derivatives **1** and **2d** on VERO cells has been evaluated.[24] As reported in Table 2 the data from the dye test and the Lowry's method for protein content, show that compound **1** is well tolerated at all the concentrations used in antiproliferative assay (90–0.7 μM), while compound **2d** is toxic only at high concentration (90 μM). The results obtained do not permit an analysis of structure–activity relationships, however, it is evident that, in the first series of compounds (**1**, **1a**–**c**, **1e**–**g**) the substituents on the quinone ring have a negative effect, perhaps due to steric hindrance: the most active derivative is unsubstituted. Among the derivatives bearing the 2-pyridyl moiety at C-2 (**2a**, **2c**–**e**) **2d** is the most active with an ID_{50} 2.57 and 2.63 μM on SupT1 and Molt3, respectively. In this case, the presence of the methoxy group is an important feature, as it appears in many indole and quinoline quinones. All derivatives bearing amine substituents at C-5(6) (**1a**, **1b**, **1g**, **2a**, **2e**) are not active. Considering the high antiproliferative activity of **1** and **2d** on leukemia and lymphoma cells, also at small doses, and the fact that **1** is not toxic and **2d** is toxic only at the higher concentration (90 μM), we believe that these two compounds could be interesting candidates for further in vivo testing.

Acknowledgements

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