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Synthesis and Antiproliferative Activity of Some Thiazolylbenzimidazole-4,7-diones

Laura Garuti,[a,*] Marinella Roberti,[a] Annalisa Pession,[b] Emanuela Leoncini[c] and Silvana Hrelia[c]

[a]*Department of Pharmaceutical Science, University of Bologna, via Belmeloro 6, I-40126 Bologna, Italy*
[b]*Department of Experimental Pathology, University of Bologna, via S. Giacomo, Bologna, Italy*
[c]*Department of Biochemistry "G. Moruzzi" University of Bologna, via Irnerio48, I-40126 Bologna, Italy*

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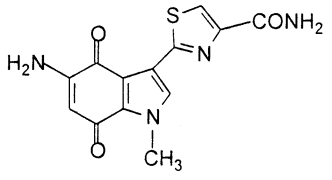
Abstract—Some thiazolylbenzimidazole-4,7-diones were synthesized and tested in vitro on two tumor cell lines. Compounds **2d** and **2e** show a very good activity on K562 cells, whereas compounds **2a** and **2b** are active on SW620 cells. The importance of the methoxy group on the quinone moiety is confirmed and the function at 4-position of the thiazole ring plays a determining role for the activity.

The thiazolylindolequinone BE 10988 **1**[1–5] was shown to be an inhibitor of topoisomerase II endowed with marked cytotoxic activities against a panel of human tumor cell lines. A number of analogues of BE have been evaluated for their cytotoxicity and action mechanism.[6] The quinone moiety and the thiazole ring appeared essential to biological activity. In view of our interest in benzimidazole-4,7-diones and their antiproliferative properties, we decided to investigate some benzimidazole quinones bearing the thiazole ring at the 2-position. In a previous paper,[7] we have found that in certain benzimidazole diones, the substituents on the quinone ring have a negative effect, while in others the presence of the methoxy group is an important feature. Therefore, we prepared a small series of thiazolylbenzimidazole diones bearing no substituent or the methoxy group at C-5(6). Moreover, studies on thiazole-containing antitumor agents showed that substituents on the thiazole ring might play a significant role in the biological acivity.[8] Thus, the presence of different functions at the thiazole 4-position is also evaluated with the aim to define the influence of some structural variants in the cytotoxic action. We report herein the synthesis of some thiazolylbenzimidazole diones related to general structure **2** and the preliminary results of their antiproliferative activity.



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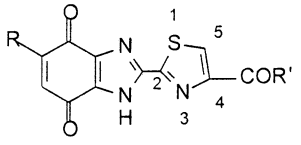

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Chemistry

The 2,3-diamino-1,4-dimethoxybenzene **3** was reported in the literature.[9] The reaction of this diamine with methyl trichloroacetimidate in acetic acid gave the 2-trichloromethylbenzimidazole **4**, which was converted into 2-cyano derivative **5** in anhydrous ammonia. A solution of the 2-cyano-4,7-dimethoxybenzimidazole in concentrated sulphuric acid at 0 °C, yielded benzimidazole-2-carboxamide **6**. The amide was converted into the thioamide **7** with Lawesson's reagent. The thiazole ring of the compound **8** was constructed by a modified Hantzsch

*Corresponding author. Tel.: +39-051-209-9715; fax: +39-051-209-9734; e-mail: lauga@alma.unibo.it

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reaction of the thioamide with ethyl bromopyruvate (Scheme 1).

Hydrolysis of **8** with 48% hydrobromic acid at 120 °C for 6 h resulted in the formation of the hydroquinone **9**. Oxidation of **9** with ferric chloride in aqueous solution at room temperature gave the quinone **2**. Treatment of **8** with methanolic ammonia afforded the amide **10** which was purified by column chromatography using ethyl acetate as eluent. The dimethoxy benzimidazoles **8** and **10** were oxidized with CAN in aqueous acetic acid to the quinones **2a** and **2b**. Finally, the reaction of the quinones **2**, **2a**, **2b** with ammonium hydroxide in methanol at room temperature yielded the corresponding 5(6)methoxy derivatives **2(c–e)** (Scheme 2).

Biology

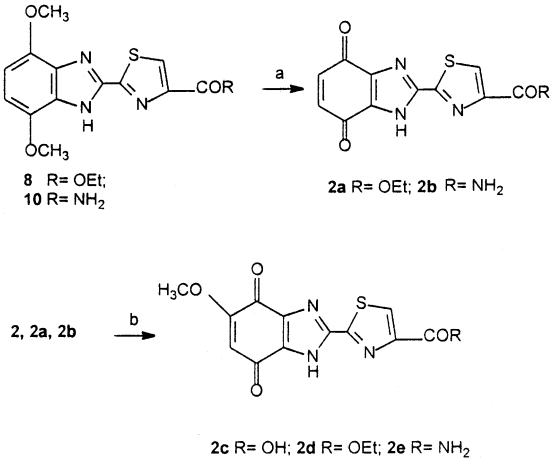
The cell lines used were derived from human tumors: the K562 cell line (from ATCC, Rockville, MA, USA) was from a erythroleukemia and the SW620 cell line (from ATCC, Rockville, MA, USA) was from a colon carcinoma. All cell lines were maintained in RPMI 1640 medium supplemented with 0.5% non essential aminoacids, 100UI/mL penicillin, 0.1 mg/mL streptomicin, 0.1 mg/mL L-glutammine and 10% fetal calf serum. Cells were incubated at 37 °C in an humidified atmosphere of 5% CO_2, 95% air. Cells of each line were seeded in six-well plates (5×10^5 cells/well) and after 24 h they were treated with the different compounds and doxorubicin, utilised as a known antiproliferative drug in these cell lines, dissolved in DMSO and diluted in medium containing 5% fetal calf serum to give final concentrations ranging from 1 to 100 μM (0.1–100 μM for doxorubicin). The final DMSO concentration was kept constant at 0.2% and was also added to control wells. After 24 h of incubation, cell proliferation analyses were performed. Daily observations were made by phase contrast microscopy.

Cell proliferation

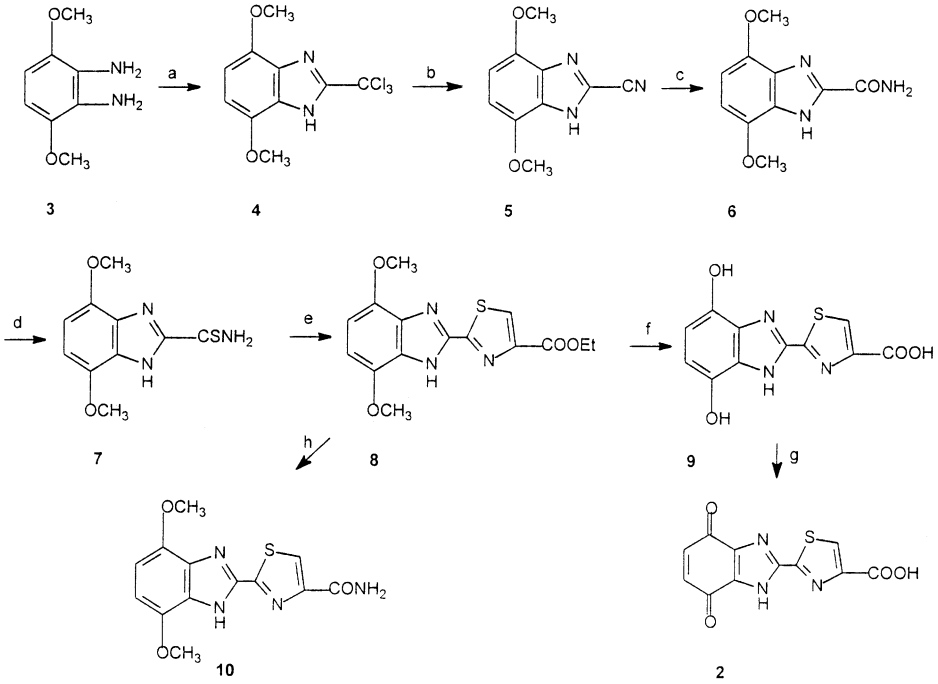
Evaluation of cell proliferation was performed by measuring DNA synthesis by means of methyl-[^{3}H]-thymidine incorporation as previously reported.[10]

Results and Discussion

The effect of the administration of the different compounds on DNA synthesis is reported in Table 1 as IC_{50} value (IC_{50} represents the concentration that causes



Scheme 2. Reagents and conditions: (a) CAN, AcOH/H_2O; (b) NH_4OH, MeOH.



Scheme 1. Reagents and conditions: (a) Methyl trichloroacetimidate/AcOH, (75%); (b) NH_3/MeOH (70%); (c) H_2SO_4, 0 °C (50%); (d) Lawesson's reagent/THF (48%); (e) ethyl bromopyruvate/EtOH, (50%); (f) HBr, 120 °C (65%); (g) aq $FeCl_3$ (57%); (h) 30% NH_4OH (20%).

Table 1. Antiproliferative activity of derivatives **2**, **2a**–**e** against K562 and SW620 cell lines

Compd	IC_{50} (μM)	
	K562	SW620
Doxorubicin	0.62±0.08	0.75±0.09
2	17.97±2.71	8.76±1.13
2a	16.32±2.45	1.42±0.27
2b	7.70±1.06	3.03±0.96
2c	15.08±2.34	12.06±1.92
2d	1.16±0.18	13.07±2.06
2e	1.32±0.33	15.88±2.34

IC_{50} represents the drug concentration that causes 50% inhibition of DNA synthesis in comparison to control cells.
Values are means±SD of at least four independent experiments.

50% inhibition of DNA synthesis in comparison to control cells). From the data, it is evident that the antiproliferative activity is variously manifested in the two cell lines. The compounds **2d** and **2e** are the most active on K562 cells, confirming the importance of the methoxy group on the quinone ring. The derivatives **2a** and **2b** display a significant activity on SW620 cells. It appears that the function at the thiazole 4-position plays a significant role in the biological activity. The presence of the carboxylate and carboxamide moieties leads to active compounds, while the free carboxylic function reduces the activity. The different antiproliferative activity of these compounds on the two cell lines is difficult to ascribe to a single physicochemical parameter; the introduction of a methoxy group in the quinone ring and/or modification of thiazole-4-substituent could significantly alter properties as acidity, solubility, reduction potential and also their partition coefficient, influencing, in turn, their ability to partition into cell membranes and to enter the cell by passive diffusion. It has been reported that tumor cell membranes differ in composition from normal counterparts, tumor cells show an increased membrane molecular order and microviscosity in a fashion which is inversely related to the growth rate and malignacy of the tumor.[11] Membrane microvisity is related to the fatty acid composition and unsaturation index of the membrane phospholipids that is a fingerprint of tumor cells[12] and of the growth rate of tumor itself.[13] So different drugs may partition into and cross through cell membranes according both to the physicochemical properties of the molecule and the fatty acid composition and unsaturation index of the tumor cell membranes. In order to demonstrate the validity of these speculations, further experiments are in progress to elucidate the existence of a relationship between drug antiproliferative effect and membrane microviscosity/phospholipid fatty acid composition of tumor cells. In conclusion, although other significant structural changes within the common molecule framework are necessary to derive meaningful SARs, data here reported clearly demonstrated that both the C-5 methoxy group and the function at 4-position of the thiazole ring have a considerable effect on antiproliferative properties.

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