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Original research article

Biological approach of anticancer activity of new benzimidazole derivatives

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ABSTRACT

Background: A series of new benzimidazole derivatives, earlier synthesized, was tested in vitro as new bioreductive prodrugs with the potential anticancer activity. Their effect on the DNA destruction and growth inhibition into selected tumor cell lines at normoxia and hypoxia conditions was determined.
Methods: The human lung adenocarcinoma A549 cell line was used to determine the anticancer activity of the analyzed compounds by using WST-1 assay. The apoptosis test (caspase 3/7 assay) was used to define the cytotoxic way of tumor cells death. Additionally test In situ DNA Damage Assay Kit was applied to recognize the DNA destruction.
Results: Four of the examined compounds (**1, 3, 7, 9**) show a very good antiproliferative effect and three of them are specific for hypoxia conditions (**2, 4, 8**).
Conclusion: Compound **8** is the most cytotoxic against human lung adenocarcinoma A549 cells at hypoxic conditions. Hypoxia/normoxia cytotoxic coefficient of compound 8 (4.75) is close to hypoxia/normoxia cytotoxic coefficient of tirapazamine (5.59) – reference substance in our experiments and this parameter locates it between mitomycin C and 2-nitroimidazole (misonidazole). The screening test of the caspase-dependent apoptosis proved that the exposure of compounds **1**–**2** and **7**–**8** against A549 cells for a 48 h promote apoptotic cell death. Additionally, the test of the DNA damage established that compounds **1**, **2**, **7**, **8** are specific agents for the hypoxia-selective cytotoxicity of nitrobenzimidazoles [6,26].
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Introduction

Hypoxia is the leading targeting in cancer therapy. The poor oxygen concentration, which is characteristic for solid tumors affects many processes such as angiogenesis, erythropoiesis and alteration of cellular metabolism at tumor cells [5]. Hypoxia, for sure, can influence the survival of tumor cells by different changes in the gene expression that reduce apoptosis and increase autophagy, vasculogenesis, metastasis, immune reactivity and activity of receptor tyrosine kinase. Generally tumor cells under hypoxia lose genomic stability by generating the reactive oxygen species (ROS) and suppress regulation of DNA repair pathways [8,10,21,23,25].

In order to minimize those survival effects of tumor cells, scientists conduct research into targeted therapy with the use of specific substances which have a bioreductive mechanism of action at hypoxia conditions [1,6,13]. This concept of approaching new chemical classes of pro-drugs activated to selective cytotoxins, was started by using derivatives of aniline nitrogen mustard as the first class of bioreducive prodrugs. Now different chemical bonds such as nitro group, quinones, heterocyclic N-oxides (CB 1954, tirapazamina, AQ4N) are currently radical prodrugs useful for cancer therapy (Fig. 1) [11,12,14,20]. The mechanisms of the metabolic activation of bioreductive prodrugs were shown at Scheme 1 [25]. The common feature of all these new chemical compounds is their ability to generate cytotoxic agents for DNA damage. A new group of benzimidazole derivatives, i.e. potential new agents of the DNA destruction, should be particularly paid attention to [2–4]. These compounds are intensively being worked on as they might have new anticancer properties [7,16,17,22,24]. It was the reason for initiating our experiments in the group of new benzimidazole derivatives and N-oxide benzimidazole derivatives. Therefore, we analyzed a series of benzimidazole derivatives **(1–12)** to elucidate their contribution to the antyproliferation activity at normoxia and hypoxia conditions. The particularly selective activity of N-oxide benzimidazole derivatives into hypoxia was very interesting for us. Additionally we determined their cytotoxic activity by necrosis or apoptosis. The main reason for our experiments concerned their effect of DNA damage at hypoxia and normoxia cancer cells.

Abbreviations: T, tirapazamine; TPZ, tirapazamine; WST, 1 water-soluble tetrazolium salt; DMSO, dimethyl sulfoxide; FBS, fetal bovine serum; CTR, control sample.

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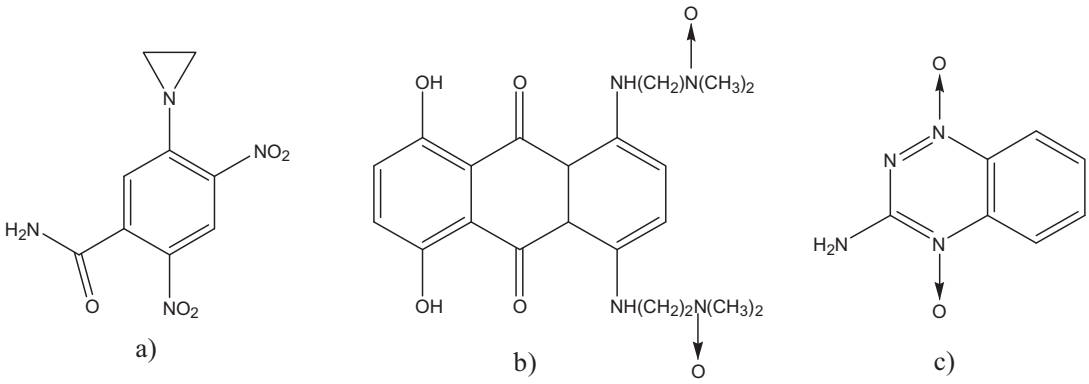


Fig. 1. The known bioreductive prodrugs: (a) CB 1954, (b) AQ4N, and (c) tirapazamine.

Materials and methods

Procedures of biochemistry experiments

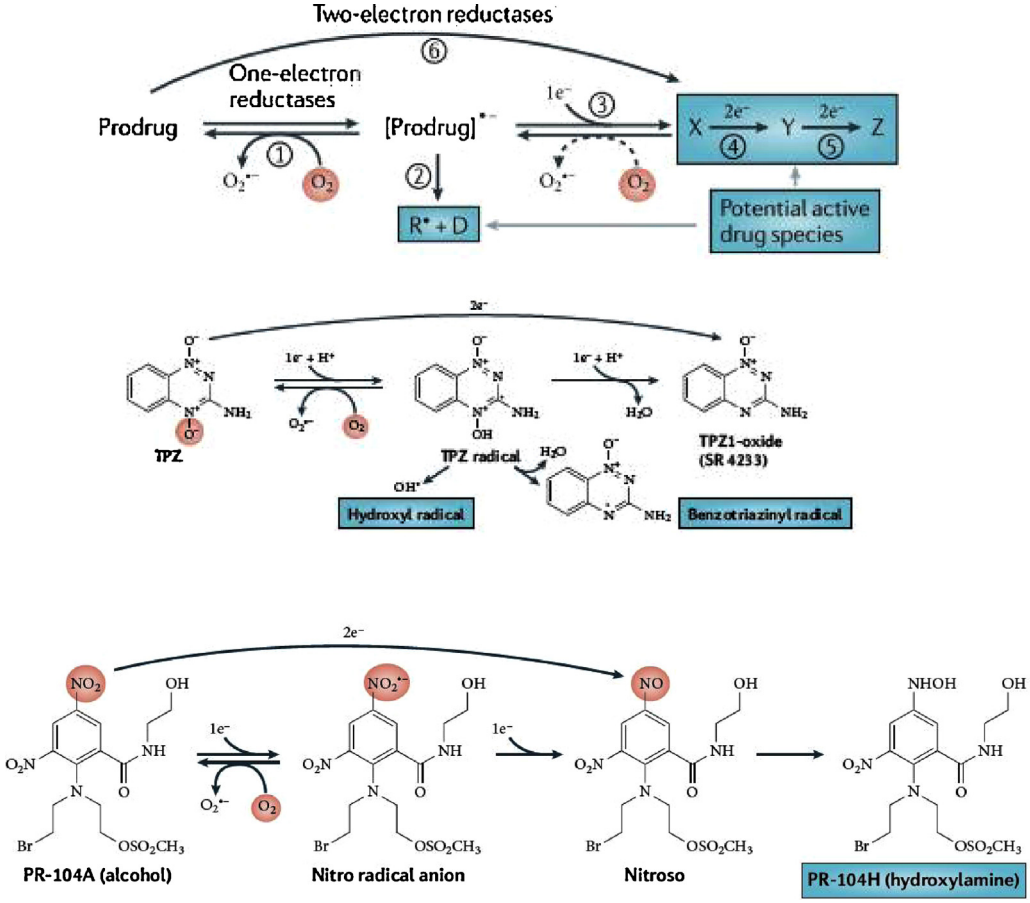
Cell culture

A549 (human lung adenocarcinoma) cell line was purchased from Health Protection Agency Culture Collections (ECACC, Salisburg, UK), were cultured in F12K medium (HyClone, UK) supplemented with 10% heat-inactivated fetal bovine serum (FBS), penicillin (10,000 U/ml) and streptomycin (10,000 μg/ml) in 5% CO_2 at 37 °C.

Hypoxic cells were created by culture of A549 cells in hypoxic incubator in 1% O_2 and 5% CO_2 at 37 °C for 24 h before treatment.

DNA damage assay

The effect of compounds on DNA damage was determined based on measuring phosphorylation of histone H2AX on serine 139 using the EpiQuick in situ DNA damage assay kit [Epigentek]. A549 cells were seeded in 96-well plates at a density of 5000 cells/well and cultured in normoxic or hypoxic condition for 24 h before treatment. Next the cells were treated with vehicle or indicated compounds at concentration of IC_{50} range and were later cultured in the same conditions. DNA damage in normoxic and hypoxic cells was measured after 4 h incubation with tested compounds. After this time, cells were fixed and assay was performed according to the manufacture protocol. The amount of DNA damage was proportional to the intensity of color development. The absorbance



Scheme 1. One-electron versus two-electron reduction of bioreductive prodrug into the cytotoxic metabolites (in blue) [25]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

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