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Benzimidazole derivatives related to 2,3-acrylonitriles, benzimidazo[1,2-*a*]quinolines and fluorenes: Synthesis, antitumor evaluation *in vitro* and crystal structure determination

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ABSTRACT

A synthesis and biological evaluation of new benzimidazole derivatives, related to 2,3-disubstituted acrylonitriles, benzimidazo[1,2-*a*]quinoline-6-carbonitriles and heteroaromatic fluorenes was described. The molecular and crystal structures of three compounds **4**, **16** and **17** reveal that non-fused fluoro derivative, **4**, deviates from planarity by 13.11(2)°, while fused methyl, **16**, and fluoro, **17**, derivatives are planar within 4° exhibiting a planar aromatic surface capable to intercalate into double-stranded DNA. Compound **4** exists as *E*-isomer.

The crystal structures confirmed that hydrogen bonding patterns are characterized dominantly by the weak C–H···N(F) bonds, except in the case of **4** where the presence of ethanol molecule of crystallization resulted in the N–H···O and O–H···N hydrogen bonds formation. In the crystal structures of **16** and **17** cyano group participates in hydrogen bonding formation, while in **4** this is not the case. All compounds, except **16** and **14** exerted pronounced antiproliferative activity on five tumor cell lines, whereby 2-benzimidazolyl-3-*N*-methylpyrolyl-acrylonitrile **13** and its fused analogue **23** exerted the highest activity on all cell lines ($IC_{50} = 0.8–30$ μM) and showed a special selectivity toward HeLa cells. There is no major difference in the biological activity between non-fused and fused analogues. Similarly, all compounds showed significant interaction with ct-DNA, supporting the fact that their antitumor activity could partially be the consequence of DNA-binding. The cyano moiety is important for the activity, but not the selectivity of tested compounds.

1. Introduction

Since the benzimidazole unit is the key building block for a variety of compounds which have crucial roles in the functions of biologically important molecules, there is a constant and growing interest over the past few years for the synthesis and biological studies of benzimidazole derivatives [1–3]. Benzimidazoles and their azino-fused cyclic derivatives have a wide range of well known biological activities such as anticancer [4–7], antimicrobial [8–10], antifungal [11], antiviral [12,13], etc. Benzoannulated benzimidazole analogues such as benzimidazo[1,2-*a*]quinolines or benzimidazo[1,2-*a*]quinazolines due to their planar chromophore have ability to become inserted between adjacent base pairs of a DNA molecule in intercalation process, or have potential application as fluorescent probes in homogeneous assays of biological molecules [14–18].

Furthermore, 2,3-disubstituted acrylonitriles have also received considerable attention because of their versatile biological activities [19–21]. For example, a group of authors has recently reported that some 3-heteroarylacrylonitriles with triazole or benzimidazole ring have been shown to possess cytotoxic activity on several human cancer cell lines [22,23]. More recently, some acrylonitriles were found to possess antibacterial and antituberculostatic activity as well as ability to inhibit tubulin polymerization [24,25].

As a part of our medicinal chemistry project aimed at the synthesis of potential anticancer agents related to benzimidazole derivatives, we have recently reported synthesis and strong

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inhibitory activities on several human cell lines of various amidino- and cyano-substituted styryl-2-benzimidazoles and benzimidazo[1,2-*a*]quinolines (Fig. 1) [26,27]. The benzimidazole compounds with cyano substituent in general strongly enhances the cytotoxic activity. Benzimidazo[1,2-*a*]quinoline-9(10)-carbonitrile has shown the extremely pronounced and selective activity on the HeLa cell line ($IC_{50} = 0.05$ μM) [26]. Such an extreme selectivity of heterocyclic cyano molecules on HeLa cells was previously observed by our group also for another heteroaromatic compounds like carboxanilides bearing cyano substituent on either anilide or benzothiophene part of the molecule as well as for methyl-2-cyano-naphtho[2,1-*b*]thiophen-5-carboxylate and 4-ethyl-7-cyano-thieno[2,3-*e*]-benzo[*b*]furan [28,29].

The above-mentioned results prompted us to synthesize novel non-fused heteroaromatic 2-benzimidazolyl substituted acrylonitriles **2**–**14** and fused benzimidazo[1,2-*a*]quinoline-6-carbonitriles **15**–**20** and fluorenes **21**–**23**. Full details about the synthesis, evaluation of antitumor activity *in vitro*, preliminary results of binding to ct-DNA and crystal structure determination are reported herein.

2. Chemistry

A novel series of benzimidazole derivatives **2**–**23** was prepared according to Scheme 1 and Table 1. Acyclic 2-benzimidazolyl substituted acrylonitriles **2**–**14** were prepared by condensation reaction of 2-cyanomethylbenzimidazole **1** with corresponding heteroaromatic aldehydes in absolute ethanol by adding a few drops of piperidine [30]. Compounds **2**–**14** were prepared in good yields (50–84%). Benzimidazo[1,2-*a*]quinoline-6-carbonitriles **15**–**20** and heteroaromatic fluorenes **21**–**23** were prepared photochemically, from non-fused compounds **2**–**14**, by reaction of dehydrocyclization. Photochemical dehydrocyclization was performed in ethanolic solution with addition of small amount of iodine, under oxidative conditions, using 400-W high-pressure mercury lamp and Pyrex filter for 6–15 h. Compounds **9**–**11** did not give desired fused products.

All structures of novel non-fused **2**–**14** and fused derivatives **15**–**23** were determined by NMR analysis, based on the analysis of H–H coupling constants as well as chemical shifts. The photocyclization reaction leads to a downfield shift of the signal of most aromatic protons, along with a disappearing of one aromatic proton and proton of NH group on benzimidazole ring thus confirming fused structure formation.

3. Biological results and discussion

3.1. Antitumor evaluation in vitro

Compounds **3**–**23** were screened for their antiproliferative ability on five tumor cell lines, derived from different tumor types: cervical carcinoma (HeLa), pancreatic carcinoma (MiaPaCa-2), colon carcinoma (SW 620), breast carcinoma (MCF-7) and lung carcinoma (H 460). Three compounds (**2**, **11** and **12**) were previously prepared and tested for the cytotoxic activity on tumor cell lines by Saçzewski et al. [23] and similar growth inhibitory results were obtained.

All compounds, except 2-methylbenzimidazo[1,2-*a*]quinoline-6-carbonitrile **16** and non-fused 2-benzimidazolyl-3-(5-imidazolyl)-acrylonitrile **14** exerted pronounced antiproliferative activity. In general, there are no major differences in the antiproliferative activity among all compounds, apart from a few exceptions. Thus, 2-benzimidazolyl-3-*N*-methylpyrolyl-acrylonitrile **13** and its fused analogue **23** exerted the highest activity on all cell lines ($IC_{50} = 0.8$–30 μM) and also showed a special selectivity toward HeLa cells ($IC_{50} = 0.8$ μM). Similar activity was also obtained by dimethoxy derivatives **6** and **19**, but without selectivity to HeLa cells. The presence of acetamido group on phenyl ring in non-fused compound **7** leads to significantly better selectivity toward HeLa and MCF-7 cell lines, while its fused analogue **20** showed better activity with the lack of selectivity. The attachment of thiophene ring to 3 position of 2-benzimidazolyl acrylonitrile in compound **12** leads to selectivity toward HeLa cells, which is again lost in its fused analogue **22**. Apart from these differences, there is in general no major difference between non-fused and fused analogues with the exception of **3** and **16**, whereby non-fused analogue **3** exerted significantly higher activity. The explanation for this could be either in the ability of both non-fused and fused compounds to interact (bind) with cellular macromolecules (proteins and/or DNA) and to induce similar response in tumor cells (e.g. DNA-damage response), or the most responsible functionality for this activity is the cyano moiety either in quinoline ring, or as an acrylonitrile moiety. Namely, acrylonitrile is known to react nonenzymatically with the cysteine thiol groups present in proteins [31], but it can also be metabolized by P-450 isoenzyme CYP2E1, to 2-cyanoethylene oxide, which can bind irreversibly to nucleic acids as well as proteins. Also, a very toxic metabolite – cyanide is released. Epoxide hydrolase and glutathione S-transferase (GST) may have protective roles, whereby GST isoenzymes responsible for this pathway are not completely known; the polymorphic GSTT1 and GSTM1 are predominantly involved after massive intoxications with alkylating agents [32]. Therefore, as was also nicely elaborated in Saçzewski et al., like acrylonitrile, the here-presented compounds may act by inhibiting sulfhydryl-dependent enzymes of intermediary metabolism by cyanoethylation of sulfhydryl groups or by liberation of cyanide and subsequent inhibition of cytochrome c oxidase [23]. Thus, either different P-450, or GST polymorphisms could be responsible (because of a lower, or a higher capacity for detoxication) for divergent responses of various tumor (and other) cells to acrylonitrile, or other cyano-substituted compounds.

Saçzewski et al. stated that even though the cyano group of the acrylonitrile moiety was not an absolute requirement for activity, compounds lacking this group were less active compared to their acrylonitrile counterpart. Also, replacement of the nitrile group for hydrogen had less of an effect than replacement for a methyl group. Although, we did not check the potential activity of analogues lacking the cyano group, our results and the results published by

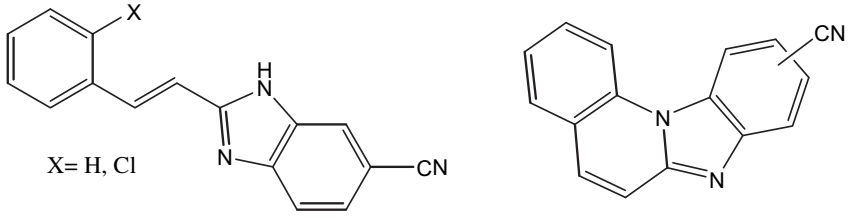

Fig. 1. Structures of earlier prepared cyano-substituted benzimidazoles and benzimidazo[1,2-*a*]quinolines.