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# Cytotoxic Effects of Some N-Substituted-2-Amino-1H-Benzimidazoles

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## Abstract

The cytotoxic activity of previously synthesized (benzimidazol-2-yl) amines were evaluated on two cancer celllines: human epithelial colorectal carcinoma HT-29 (American Type Culture Collection HTB-38), breast cancer cells with epithelial-like morphology MDA-MB 231 (American Type Culture Collection ATCC HTB-26), and on normal spleen cells as well as. Indicative cytotoxic activity was ascertained for N-1H-benzimidazol-2-yl-1-propyl-1H-benzimidazol-2-amine **15,** 1-propyl-N-(1-propyl-1H-benzimidazol-2-yl)-1H-benzimidazol-2-amine **21** and 1-methyl-N-(1-propyl-1H-benzimidazol-2-yl)-1H-benzimidazol-2-amine**18** against the cellular line HT-29. The estimated $IC_{50}$values were 0.91, 1.92 and 1.98 μM respectively. The same compounds exhibited comparatively high antiproliferative activity against MDA-MB-231 cells. The realized $IC_{50}$ values were in the range 0.006 – 1.48 μM. Compounds **14**, **16**, **17**, **18** and **21**revealed stimulating activity to the normal spleen cells, where the $EC_{50}$ values were between 0.5 × $10^{-4}$ μM for compound **21** and 0.013 μM for compound **14**.

**Keywords:** 2-aminobenzimidazoles; Bis (benzimidazol-2-yl) amines; Synthesis; Cytotoxicity; Proliferative effect; HT-29; MDA-MB-231-cell lines

## Introduction

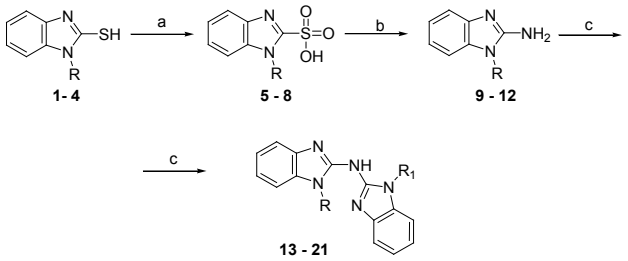
The 2-aminobenzimidazole represents a building block in the structure of several medicinally relevant small molecules. Therefore, the wide spectrum of biological activities (immunotropic, diuretic, anti histaminic, anti-inflammatory, antiviral) associated with the benzimidazolesis of great interest [1-6].The availability of the 2-aminobenzimidazolemoietyinthestructureofmanyantihelmithicand antiparasitic drugs support further the importance of the benzimidazole ring system in the development of new and better chemotherapeutical agents [7-11]. Nowadays many 2-aminobenzimidazole derivatives, which are known as microtubule inhibitors were evaluated for their anticancer activity and are appropriate as primary substances for the synthesis of novel anticancer drugs. It was established that benomyl and colchicine synergistically inhibits cell proliferation and mitosis of human cervical cancer (HeLa) cell line [12] while carbendazim inhibits proliferation of human cancer cells, including drug- and multidrug-resistant and p53-deficient cell lines [13]. Some albendazole derivatives demonstrated cytotoxic activity up to ten times higher than the parent drug (albendazole) against the HT-29-cell line and the definite prostate cancer cell line (PC-3). On the other hand many used in the praxis antiparasitic 2-aminobezimidazole derivatives revealed cytotoxicity against some leukemic and myeloma cells like SW707 (rectal), HCV29T (bladder), A549 (lung) and T47D (breast cancer) [14-18].

In the view of the above mentioned facts and as a continuation of our research in the field of benzimidazoles we undertook investigations on the effects of some bis (benzimidazol-2-yl) amines on human epithelial colorectal carcinoma HT-29, breast cancer cells, epithelial-like morphology MDA-MB 231 and normal spleen cells. Because of the structural similarity of benzimidazole nucleus to the purine basesof the DNA, we supposed that the benzimidazole derivatives would facile collaborate with biological substances. It could be anticipated that the incorporating of a second benzimidazole ring in the 2-aminobenzimidazole molecule can enhanced not only antiparasitic efficacy but can also lead to the appearance of proliferative or cytotoxic properties of the studied compounds. To find out appropriate medicines that would have specificity to cancer cells is indisputable.

## Materials and Methods

### Chemistry

The compounds 1-21 (Scheme 1) were synthesized as previously reported [11]. The tested compounds 13-21 were obtained by the reaction of the corresponding benzimidazole-2-sulfonic acids and appropriate benzimidazole-2-amines at 180°C for 30 minutes. After




**13**: R = H, $R_1$ = $CH_3$; **14**: R = H, $R_1$ = $C_2H_5$; **15**: R = H, $R_1$ = $C_3H_7$;
**16**: R = $CH_3$; $R_1$ = $CH_3$; **17**: R = $CH_3$, $R_1$ = $C_2H_5$; **18**: R = $CH_3$, $R_1$ = $C_3H_7$;
**19**: R = $C_2H_5$, $R_1$ = $C_2H_5$; **20**: R = $C_2H_5$, $R_1$ = $C_3H_7$; **21**: R = $C_3H_7$, $R_1$ = $C_3H_7$;

**Scheme 1: Synthesis of bis(benzimidazol-2-yl)amines; Regents andconditions:**
a) $KMnO_4$, 25% NaOH, reflux; b) 25% $NH_4OH$, 145°C, in welded ampoule; c) heating with 1-(un)substituted-*1H*-benzimidazol-2-sulphonic acid at 180°C

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cooling the fusion was diluted with ethanol. The filtered pellet was re-crystallized with ethanol.

### MTS test

The cells, incubated with the substances [19] were tested for proliferation ability by the MTS assay described in the protocol of "Promega" [20]. Percentage of untreated control cells (100% viability), was calculated for each concentration. In the controls the calculated data, received after incubation of each cell kind only with DMSO [19]. All data points represent an average of three independent assays.

### Statistics

After the Student's test the statistical errors were $p \leq 0.05$.

$EC_{50}$ and $IC_{50}$ were calculated with the "Origin" computer program.

## Result and Discussion

The synthesis of the studied compounds was accomplished as outlined in Scheme 1. The reaction of 1,2-diaminobenzene, carbon disulfide and sodium hydroxide in ethanol medium yielded 1H-benzimidazol-2-thiol **1**. The fusion of 1-alkyl-benzimidazoles with sulfur at 180°C yielded in 1-alkyl-benzimidazole-2-thiols **2-4**. The oxidation of the 1-(un)substituted-1*H*-benzimidazol-2-yl-tiols **1-4** with $KMnO_4$ in 25% water solution of sodium hydroxide led to the formation of 1- (un) substituted-1H-benzimidazol-2-yl-sulfonic acids**.** The reaction of benzimidazol-2-yl-sulfonic acids **5-8**, carried out with 25% ammonium hydroxide resulted in 2-aminobenzimidazoles **9-12**. The bis (benzimidazol-2-yl) amines **13-21** were synthesized by heating benzimidazol-2-yl-sulfonic acids **5-8** and 2-aminobenzimidazoles **9-12** at 180°C**.**

Compounds **13-21** were evaluated for their anti proliferative effect on human colorectal cancer cell lineHT-29, breast cancer cells MDA-MB-231 and normal spleen cells using the MTS test [20].

The conversation of the MTS tetrazolium compound into blue colored formazan is due to NADPH or NADH from the examined cells [21].

The bis (benzimidazol-2-yl) amines **13-21** were DMSO-dissolved at a concentration of 500 ml. The stock solution was diluted further 10, 100, 1000 and10000 times. Untreated cells, cultured only in culture medium were the controls. After 24 h of incubation the samples were used in the MTS assay for cell survival and proliferation. All results are given in Figures 1, 2 and 3. The calculated and plotted result and the values of $IC_{50}$ and $EC_{50}$ are represented in Table1.

From all tested substances compounds **15, 18** and **21** expressed toxic effect against HT-29 cells. The unsubstituted at 1-th position in the one of the two benzimidazole rings compound **15** showed the most pronounced toxic effect to HT-29 cells, $IC_{50}$ = 0.91 μM, followed by the compound **21,** $IC_{50}$ = 1,92 μM and **18** - $IC_{50}$ = 1,98 μM. Compounds **15, 18, 19, 20** and **21** exhibited relatively high anti proliferative activity to MDA-MB-231 cells. Most toxic were 1-propyl-N-(1-propyl-1H-benzimidazol-2-yl)-1H-benzimidazol-2-amine **21** and N-1H-benzimidazol-2-yl-1-propyl-1H-benzimidazol-2-amine **15** with $IC_{50}$–0.0006 μM and $IC_{50}$–0.135 μM respectively.

From the results, given in Table 1, it can be seen that compounds **14**, **16**, **17**, **18** and **21** showed definitely stimulating effects to the normal

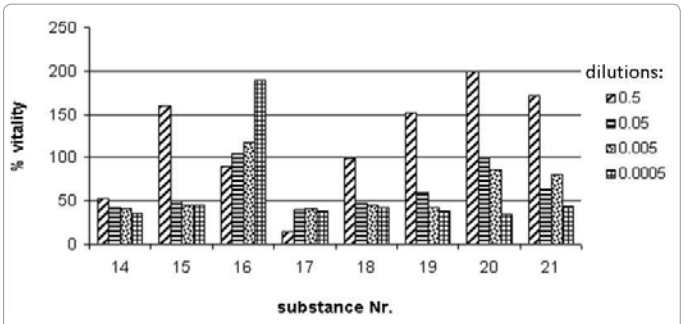


**Figure 1:** HT-29 cells vitality (%).

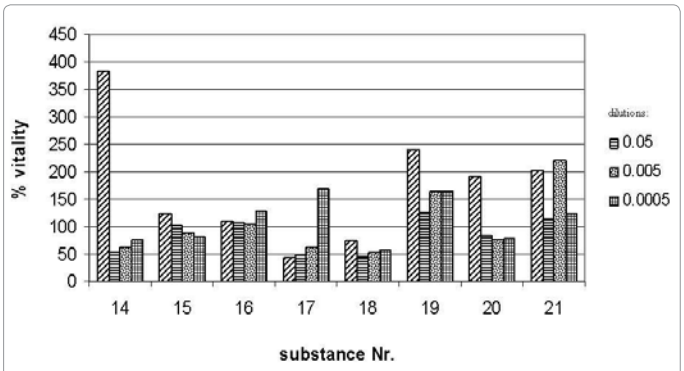


**Figure 2:** MDA-MB-231 cells vitality (%).

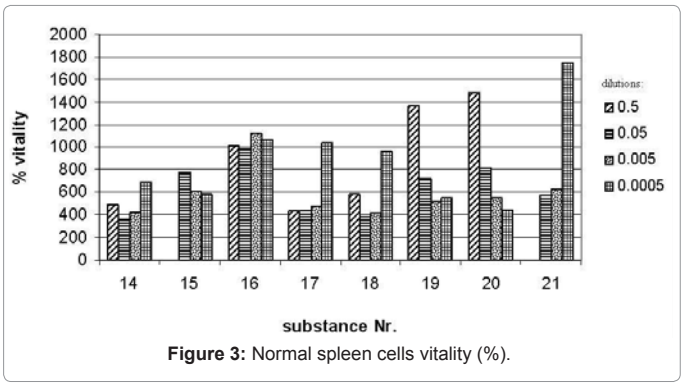


**Figure 3:** Normal spleen cells vitality (%).

spleen cells. The $EC_{50}$ data varied from $0.05 \times 10^{-3}$ μM for 1-propyl-N-(1-propyl-1H-benzimidazol-2-yl)-1H-benzimidazol-2-amine **21** to 0.013 μM for N-1H-benzimidazol-2-yl-1-ethyl-1H-benzimidazol-2-amine **14.** If the result for compound **21** is taken in mind it must be emphasized that 1-propyl-N-(1-propyl-1H-benzimidazol-2-yl)-1H-benzimidazol-2-amine exhibit proliferative effect to the normal spleen cells even at higher dilutions in comparison to this at which the compound displays cytotoxic effect on HT-29 cells and MDA-MB-231 cells. As far as the properties of 1-methyl-N-(1-propyl-1H-benzimidazol-2-yl)-1H-benzimidazol-2-amine 18 should be pointed out that this compound possessed a relative high cytotoxicity both to HT-29 cells ($IC_{50}$ = 1.9 μM) and to MDA-MB-231 ($IC_{50}$ = 1.4 μM) cell line, but against normal spleen cells the same compound displayed proliferative activity at lower concentration ($EC_{50}$ = 0.004 μM). The last result is important showing the selective capacity of substance **18**. Surprising results demonstrated N-1H-benzimidazol-2-yl-1-

| Compound | $IC_{50}$ ± SE (μM) | | | $EC_{50}$ ± SE (μM) | | |
|---|---|---|---|---|---|---|
| | HT-29 | MDA-MB-231 | Normal spleen cells | HT-29 | MDA-MB-231 | Normal spleen cells |
| **14** | $3.5.10^3$ ± 0.35 | 1,65 ± 0.07 | - | - | - | 0.013 ± 0.07 |
| **15** | 0.91 ± 0.11 | 0.135 ± 0.06 | 0.11 ± 0.62 | - | - | - |
| **16** | - | - | - | 0.01 ± 0.046 | 0.002 ± 0.05 | 0.047 ± 0.32 |
| **17** | - | - | - | 0.48 ± 0.009 | 1.48 ± 0.3 | 0.005 ± 0.15 |
| **18** | 1.98±0.43 | 1.48 ± 0.06 | - | - | - | 0.004 ± 0.13 |
| **19** | - | 1.87 ± 0.24 | 0.23 ± 1.96 | 0.005 ± 0.38 | - | - |
| **20** | $7.10^3$ ± 0.05 | 16.7 ± 0.28 | 1.67 ± 0.02 | - | - | - |
| **21** | 1.92 ± 0.08 | 0.006 ± 0.27 | - | - | - | $0.5.10^{-4}$ ± 0.6 |

**Table 1:** *In vitro* cytotoxicity and proliferative effects on HT-29, MDA-MB-231and normal spleen cells.

ethyl-1H-benzimidazol-2-amine **14** revealing to be almost non-toxic to HT-29 and toxic to MDA-MB-231, but possessing a proliferative effect on normal spleen cells at a lower concentration ($EC_{50}$-0.013 μM). Proliferative influence on all the experimental cells manifested 1-methyl-N-(1-methyl-1H-benzimidazol-2-yl)-1H-benzimidazol-2-amine **16** and 1-ethyl-N-(1-methyl-1H-benzimidazol-2-yl)-1H-benzimidazol-2-amine **17**.

The significant statistical deviations in all examinations were determined ($p \leq 0.05$).

## Conclusion

The preliminary screening *in vitro* showed that the studied compounds **15**, N-1H-benzimidazol-2-yl-1-propyl-1H-benzimidazol-2-amine **18** and 1-propyl-N-(1-propyl-1H-benzimidazol-2-yl)-1H-benzimidazol-2-amine **21** possessed comparatively high cytotoxic effect on HT-29 cells. $IC_{50}$ values were in the range 0.91-1.98 μM. Strongest anti proliferative effect on MDA-MB-231 cells had compounds **15** and **21** with $IC_{50}$–0.0006 μM and $IC_{50}$–0.135 μM respectively. Simultaneously compounds **18** and **21**exerted proliferative activity to the normal spleen cells. On the base of these promising screening results, it may be concluded that the selectivity of compounds **18** and **21** will be essential for anticancer drug development.The received experimental data substantiate the hypothesis that the introduction of another benzimidazole ring at 2nd position in the structure of 2-aminobenzimidazole as well as the presence of a butyl group at 1st place are favorable to the reciprocal action of these molecules with thebiological agents.

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