ABSTRACT

The research summarized in this doctoral dissertation (performed in the Screening Laboratory of Anticancer Compounds existing at the Department of Bioorganic Chemistry, CMMS PAS in Lodz), has been focused on evaluation of anticancer properties of eighty derivatives of benzo[b]furans and dicarboximides, originally designed and synthesized by the group of Prof. J. Kossakowski at the Department of Medical Chemistry, Medical University of Warsaw. The studies aimed at elucidation of molecular mechanisms of the observed toxicity in several tumor cell lines, and at selection of candidates for the development of new anticancer drugs.

In the first stage of the study, the cytotoxic properties of the screened compounds were determined in the MTT assay. Eleven compounds occurred to be toxic for HeLa and/or K562 cancer cells and exhibited IC_{50} values in the range of 1-100 μM. Importantly, these compounds were not toxic to normal endothelial cells (HUVEC). Moreover, some of these compounds exhibited selective cytotoxic activity (IC_{50} 1-30 μM) toward leukemic cells (K562, MOLT-4, HL-60) but were non-toxic to adherent cancer (HeLa, CFPAC) and normal cells (HUVEC). Selective cytotoxicity toward leukemia cells has been observed for a compound MKN (**14**, a benzo[b]furan derivative), and for dicarboximide derivatives BK 176.1, BK 176.2, BK 176.4, BK 176.5 and BK 124.1 (#**1**,**2**,**4**,**5** and **6,** respectively). Cytotoxic activity of MKN (**14**), BK 124.1 (**6**) and BK 176.4 (**4**), was compared to those of the reference drugs, i.e. cytarabine, bortezomib, sorafenib, CPT-11 and doxorubicin, which are used in the treatment of various types of cancer, in particular of leukemias. The results show that the cytotoxicity and selectivity of benzo[b]furane and dicarboximide derivatives in leukemia cells surpass the reference cytostatics. Importantly, in contrast to the reference compounds (except for cytarabine), the benzo[b]furane and dicarboximide derivatives are not toxic for normal cells (HUVEC).

For the selected compounds of high cytotoxic effect (IC_{50} 1-100 μM), further studies were performed to determine whether apoptosis or necrosis is a cause of the observed cell death. Increased activity of executive caspases, i.e. caspase 3 and 7, in the examined cancer cells K562, MOLT-4, and HeLa indicates that the tested compounds induced apoptosis. Particularly interesting was a significant increase of caspase 3 and 7 activity in leukemic cells K562 and MOLT-4 treated with MKN and BK 176.4. Measurement of the level of phosphatidylserine (a marker of apoptosis) on the surface of cells after incubation with MKN and BK 176.4 showed an increased number of both early- and late- apoptotic leukemic cells K562 and MOLT-4.

In order to identify apoptotic pathway(s) induced in leukemia cells by MKN and BK 176.4, the activities of caspase 8 and caspase 9 were measured. Caspase 8 gets activated in the course of receptor apoptosis pathway, while activation of caspase 9 is characteristic for the mitochondrial pathway. The studies performed within the PhD thesis have demonstrated that **14** and **4** induce, both, receptor and mitochondrial apoptosis pathways in leukemia cells (K562, MOLT-4). This is evidenced by strong activation of caspase 8 and 9, respectively.

In the next stage of the research, I analyzed the expression of genes involved in apoptosis in leukemia cells treated with MKN and BK 176.4. This was to identify the genes specific for the receptor

and/or mitochondrial apoptosis pathway, the expression of which is changed in chronic myelogenous leukemia cells upon incubation with MKN and BK 176.4. The expression levels of 93 pro- and anti-apoptotic genes in K562 cells were determined using DNA microarrays. It was found that in the presence of the tested compounds the expression of several proapoptotic genes involved in, both, the receptor pathway (including *TNFRSF 10A, TNFRSF 10B, TNFRSF 21, CASP8, CASP10, RIPK1*) and he mitochondrial pathway (such as *BAX, BAD, BID, BAK, BIM, PUMA, NOXA, Smac/DIABLO, APAF1*), is significantly upregulated. The upregulation of selected proapoptotic genes was further confirmed by a *real-time* RT-PCR.

In the last part of my research I tried to identify direct mechanism of the observed cytotoxicity of the tested benzo[b]furan and dicarboximide derivatives. Taking into account the structure of the test compounds (flat, aromatic molecules), the literature reports on intercalation of similar derivatives to nucleic acids, as well as the presence of bromobenzyl moiety (as in MKN) I examined whether MKN and BK 176.4 affect the structure and function of DNA. I have found that they inhibit the hydrolysis of plasmid DNA by the restriction enzyme *Bam*HI. Furthermore, I also confirmed the interaction of test compounds with DNA by a circular dichroism (CD) technique. These results suggest that MKN (**14**) and BK 176.4 (**4**) may exert cytotoxic activity be alkylation and/or intercalation into DNA.

SAR (*structure-activity relationship*) analysis was also carried out for the studied benzo[b[furan and dicarboximide derivatives. The most desirable elements in their structure were identified which are required for antitumor activity.

As found in these studies, new derivatives of benzofurans and dicarboximides, particularly MKN and BK 176.4 show promising anticancer activity. This observation may be of a great value in finding new pharmaceuticals used in the treatment of leukemias or other proliferative diseases.