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In vitro antileukemic activity of novel adenosine derivatives bearing boron cluster modification

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ABSTRACT

A series of adenosine derivatives bearing a boron cluster were synthesized and evaluated for their cytotoxicity against primary peripheral mononuclear cells from the blood of 17 patients with leukemias (16 CLL and 1 very rare PLL), as well as from 5 healthy donors used as a control. Among the tested agents, two, i.e., compounds **1** and **2**, displayed high in vitro cytotoxicity and proapoptotic potential on leukemic cells, with only scarce activity being seen against control cells. Biological tests related to apoptosis revealed the activation of the main execution apoptotic enzyme, procaspase-3, in CLL and PLL cells exposed to compounds **1** and **2**. Moreover, the above compounds indicated high activity in the proteolysis of the apoptotic markers PARP-1 and lamin B1, fragmentation of DNA, and the induction of some changes in the expression of the Mcl-1, protein apoptosis regulator in comparison with control cells.

1. Introduction

Chronic lymphocytic leukemia (CLL) is the most common type of adult leukemia in western countries. CLL cells are typically B-cells in origin, with the T-cell variant of the disease occurring rarely. CLL is characterized by an accumulation of transformed B lymphocytes in blood, bone marrow and lymphoid tissues.[1,2] It is characterized by a highly heterogeneous course and does not always require chemotherapy.[2,3] Nevertheless, it is thought to be incurable since relapse usually takes place after an initial remission. Although purine nucleoside analogs routinely used in CLL treatment (cladribine, fludarabine) have notably improved progression-free survival (PFS), overall survival (OS) has not altered significantly. Therefore, new molecules have been tested in search of better CLL treatment.[4–6] The CLL cells circulating in peripheral blood remain mostly in the G0/G1 phase of the cell cycle, with only a slight percentage of them dividing.[7,8] On the other hand, bone marrow and lymphoid follicles outside the primary lymphoid organs produce the leukemic cells which supply blood for CLL lymphocytes. The simultaneous coexistence of quiescent and cycling cells represents a special challenge in CLL therapy. Hence, the most efficient antileukemic strategy should target both of these cell fractions.[9,10]

It has been indicated that CLL may transform into more aggressive lymphomas (Richter's syndrome) or leukamias e.g. prolymphocytic leukemia (PLL). PLL is a very rare lymphoproliferative disorder accounting for 1–2% of all lymphocytic leukemias. The proportion of prolymphocytes among circulating lymphoid cells is often higher than 90%.[11] It is more aggressive than CLL and is characterized by poor prognosis. The average OS for PLL is approximately 3 years. Purine nucleoside analogs are usually ineffective in PLL treatment, which may be due to widespread loss of tumor protein p53 (TP53) expression in these cells. At present, the most effective PLL therapy is based on monoclonal antibodies such as Rituximab/MabThera and Alemtuzumab/Campath, which recognize B-lymphocyte antigen CD20 and CD52 molecules, respectively. Sometimes the distinction between CLL and PLL is impossible, in which case the condition is referred to as mixed CLL/PLL leukemia, with the number of prolymphocytes in the blood between 10% and 55%.[12]

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As mentioned above, among the anticancer drugs used to treat these leukemias, the majority are nucleoside derivatives applied alone or in combinations with other drugs/antibodies.[13–17] Examples of medicinal preparations based on nucleoside structure include cytarabine (arabinocytidine, ara-C, Cytosar®), cladribine (2-chloro-2′-deoxyadenosine, Litak®), clofarabine (2-chloro-2′-deoxy-2′-fluoroadenosine, Clolar®) and fludarabine (2-fluoro-arabinoadenosine 5′-phosphate/F-ara-AMP, Fludara®): the last three being adenosine derivatives.[18,19] Fludarabine, the soluble form of 2-fluoro-arabinoadenosine, is rapidly dephosphorylated to 2-fluoro-ara-A within minutes of intravenous infusion. It is generally accepted that these adenosine derivatives are within cells phosphorylated to mono-, di-, and triphosphate form by cytosolic kinases. The nucleoside triphosphates derived from drugs compete with natural nucleotides, thus disrupting cellular processes.[19] When incorporated into the DNA or RNA strands of the cancer cell, they promote the termination of DNA/RNA chains and/or inhibition of DNA/RNA polymerase activity. Another possible mechanism of the anticancer activity of these nucleoside analogs is the inhibition of enzymes participating in the metabolism of nucleotides and nucleic acids.[18,19] These drugs are also able to induce programmed cell death, i.e. apoptosis. The impairment of this process is of importance in the pathogenesis of numerous cancers, including CLL.[20–24]

At present, the gold standard for CLL therapy is the combination of purine nucleoside analogs, fludarabine or cladribine, with rituximab and cyclophosphamide, an alkylating agent, described as FCR (fludarabine, cyclophosphamide, and rituximab) or CCR (cladribine, cyclophosphamide, and rituximab), respectively.[14,15] In patients not eligible for such intensive treatment because of comorbidities, less effective drugs might be applied and several other treatment protocols for CLL are used in clinical practice. However, as with experimental anticancer preparations, a large number of currently available antileukemic drugs have several disadvantages that narrow their clinical utility, such as limited effectiveness, severe side effects and the emergence of drug-resistant cancer variants which pose an increasing problem for disease management.[25] Thus, there is still a strong need to identify new targets for antileukemic chemotherapy and to develop novel anticancer compounds and treatment strategies. Among new approaches assigned for CLL, much attention is paid to agents with the ability to turn on the apoptosis process.[22,23]

Herein, we propose the use of carboranes, members of the vast boron cluster family of compounds, for modification of selected nucleoside designs. The use of carboranes as pharmacophores and modifying units was made popular by the pioneering work of Endo and colleagues.[26] However, the hydrophobic properties of carboranes have already been used to modify the biological activity of biomolecules, for example, to influence the synthesis and biological properties of enkephalin-like peptides containing carboranylalanine in place of phenylalanine.[27] Due to their spherical shape and lipophilicity, carboranes are used in most cases as surrogates for heterocycles, annulated carbon rings, or most popularly, for substituted or unsubstituted phenyl rings.[28–31]

Numerous modifications of biomolecules with boron clusters have been previously synthesized as the carriers of boron for the boron neutron capture therapy (BNCT) of cancers.[32–35] However, the unique properties of carboranes are not dependent on BNCT, as they change the properties of molecules to which they are bound, affecting their solubility, stability or ligand–receptor interactions. Recently it has been shown that some adenosine derivatives can act as modulators of purinergic receptor activity[36–38] or can be used to modify antiviral drugs.[39,40]

The recent appeal of boron clusters for the pharmaceutical industry lies in the knowledge that these clusters are abiotic and therefore chemically and biologically orthogonal to native cellular components, i.e. they are stable and inert in biological environments, and they are resistant to catabolism, which is a desirable property for biological applications. In addition, boron clusters may be used to target biological receptors that appear to be unaffected by nonboron-containing organic molecules by interacting with these receptors through diverse mechanisms.[29–31] Several reviews on current status and developments of boron cluster medicinal chemistry published recently are available.[30,31,41,42]

Hence, greater knowledge of whether boron cluster modified adenosine molecules are able to interfere with biological processes going in the leukemic cells would be valuable. It seems plausible that boron cluster-bearing adenosine might induce apoptosis in the similar way as cladribine or fludarabine, which are used in the therapy of leukemias.[18,20] The following report presents results supporting this hypothesis and confirming the considerable antileukemic activity of selected adenosine-boron cluster conjugates in comparison with known drugs.

2. Results and discussion

2.1. Synthesis of adenosine derivatives bearing a boron cluster

The present study investigates the cytotoxicity and proapoptotic potential of 2′-deoxyadenosine, adenosine and arabinoadenosine modified with a carborane cluster ($C_2B_{10}H_{11}$) against peripheral blood mononuclear cells (PBMCs) of CLL and PLL patients, as well as those of healthy volunteers. The nucleoside derivatives substituted at C-2, C-8 and N-6 positions of the purine nucleobase and 2′position of the sugar residue of the adenosine or 2′-deoxyadenosine, compounds **1**–**5**, **9** and **10** were tested (Chart 1).

Previously described compounds (**1**, **2**, **4** and **5**) and newly synthesized adenosine derivatives containing 1,12-dicarba-*closo*-dodecaborane boron cluster (**3**, **9** and **10**), Chart 1, were evaluated. The Sonogashira-type cross coupling between 2-iodo-adenosine (**6**) or 2-iodo-arabinoadenosine (**7**) and 2-ethynyl-1,12-dicarba-*closo*-dodecaborane (**8**), a boron cluster donor containing a triple bond was used to tether the modification to the nucleoside acceptor (compounds **1**, **2** and **9**), Scheme 1.[37,43]

Compounds **3** and **4** modified at 2′ position of the nucleoside sugar residue were prepared according to the 'click chemistry' coupling method between boron cluster acceptor, 2′-*O*-(1-propyn-3-yl)adenosine or 2′-*O*-(1-pentyn-5-yl)adenosine and boron cluster donor, 1-(3-azidopropyl)-1,12-dicarba-*closo*-dodecaborane, as described.[37,44,45] Tethering of the negatively-charged 7,8-dicarba-*nido*-undecaborane to the nucleobase moiety of the adenosine at position N-6 (compound **5**) was achieved via dioxane ring opening of the oxonium adduct of the boron cluster and cyclic ether as described.[36,45,46] Compound **10** was obtained from **9** via palladium catalyzed reduction of the triple bond (Scheme 1).[47] 2-Iodo-arabinoadenosine (**7**) was synthesized in four steps procedure from 2-iodo-adenosine (**6**) with total yield 31%. The procedure was carried out according to Yamada et al. with some modifications.[48]

2.2. Biological evaluation

2.2.1. Cytotoxicity and apoptosis induction

Cytotoxicity tests were first performed to select the most active molecules and to choose the optimal compound concentrations for consequent comparative analyses of the novel agents modified with carborane cluster, as well as the conventional anticancer drugs, i.e. cladribine and fludarabine. The cytotoxicity of the tested agents used at increasing concentrations was evaluated cytometrically in leukemic and normal PBMC cells after 24 and 48 h exposure. The viability of PBMCs from the blood of CLL patients and