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Synthesis and cytotoxicity of novel cholesterol–cobalt bis(dicarbollide) conjugates

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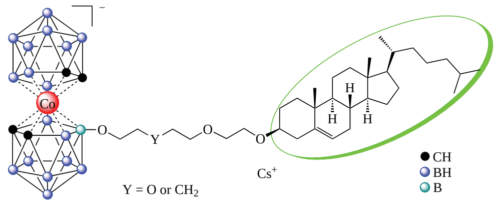
The novel conjugates of cholesterol with cobalt bis(dicarbollide) were synthesized by the ring-opening reactions of the cyclic oxonium derivatives of $[3,3'\text{-Co}(C_2B_9H_{11})_2]^-$ with the OH group of cholesterol 2-hydroxyethyl ether. The compounds obtained were tested for toxicity to glioblastoma U-87 MG cells and human embryo fibroblasts FECH-15 cells.



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The constant interest in cobalt bis(dicarbollide) anion, also known as COSAN, has made excellent contributions and important progress in chemistry of polyhedral boron hydrides during the past decades. The cobalt bis(dicarbollide) anion $[3,3'\text{-Co}(C_2B_9H_{11})_2]^-$ and its derivatives attract attention due to its unique electronic structure.[1] The presence of hydride BH vertices and charge delocalization over the whole structure confer a chemical stability, resistance to catabolism and amphiphilicity to COSAN.[2,3] Apart from the interest in its electronic properties, a burgeoning research efforts have been dedicated to $[3,3'\text{-Co}(C_2B_9H_{11})_2]^-$ application in medicinal chemistry since stable cobalt bis(dicarbollide)[4] shows low toxicity both *in vitro*[5,6] and *in vivo*[5,7] (it showed no signs of acute toxicity when intravenously[5] and intraperitoneally[7] injected into wild type mice). Bis(dicarbollide) cobalt can pass directly through synthetic lipid membranes[8] and accumulates in cells without disrupting membrane integrity.[9] These properties, along with the remarkable thermal and photochemical stabilities,[4] as well as the high content of boron in the molecule have led to the investigation of cobalt bis(dicarbollide) anion and its derivatives as boron neutron capture therapy (BNCT) agents.[10,11]

Boron neutron capture therapy is a binary method for treatment of cancers based on the selective accumulation of boron compounds in a tumour followed by its irradiation by a thermal neutron flux. As a result of the intracellular nuclear reaction, the selective destruction of tumour cells occurs with the preservation of the surrounding normal tissues. The promising requirements for the successful development of BNCT are the selective delivery of a large number of boron compounds to tumour cells, as well as a high accumulation of boron into them, while the concentration of boron in the cells of the surrounding normal tissues should be maintained at a low level to minimize damage to normal tissues.[12] Therefore, a growing interest in using cobalt bis(dicarbollide), containing about 1.5 times as much boron as in clinical used mercapto-*closo*-dodecaborate BSH and 18 times more boron atoms than in borylphenylalanine BPA, can be observed.

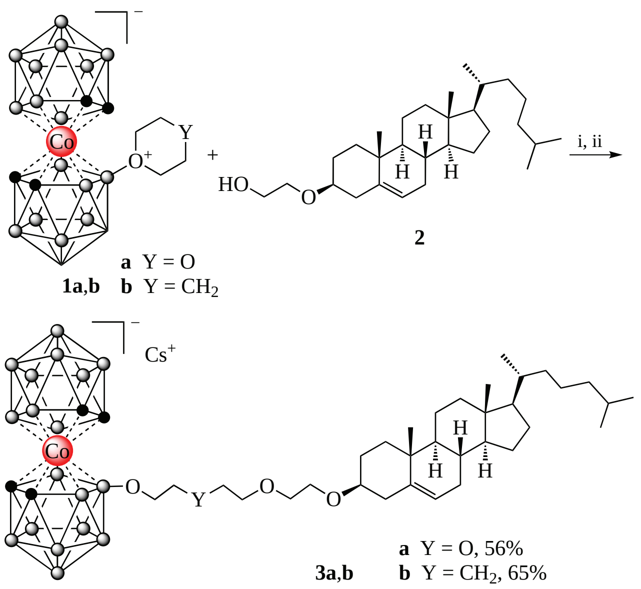
An important approach directed to selective delivery of the boron component into tumours is the development of liposomes, which can be used both as boron host molecules and as delivery agents.[13–15] One of the observed differences between tumour cells and their normal counterpart is rate metabolism of low-density lipoproteins. This difference is due to the increased need of tumour cells for cholesterol to form cell membranes; it is also often included in liposomal formulations in drug-delivery technology. Therefore, the development of boron-containing derivatives of cholesterol is potentially an effective approach for delivery of boron to cancer cells using liposomes. Currently, numerous examples of cholesterol derivatives containing boron clusters that have been synthesized as potential boron carriers for BNCT are presented.[16–24] It has been only recently shown that the inclusion of lipophilic boron-containing species in the liposome bilayer provides an attractive method to increase the gross boron content of the liposomes in the formulation.[25,26]

Recently, using 'click' reaction we have synthesized a series of cobalt bis(dicarbollide) with cholesterol bearing a 1,2,3-triazol fragment in a spacer.[27,28] In this contribution, we describe synthesis of a new series of cobalt bis(dicarbollide) derivatives of cholesterol through reaction of ring opening in cyclic ether–boron cluster adducts, as a result of which the cholesterol molecule is attached to the boron cluster through an ethylene glycol fragment. Poly(ethylene glycol) units are widely used as covalent modifiers of biological macromolecules and particulates as well as linkers for bioconjugates with various biologically relevant molecules. The oxonium derivatives of cobalt bis(dicarbollide) react with alcohols to give the corresponding substituted derivatives.[29]

In this work, we used cobalt bis(dicarbollide) derivatives with different oxonium cycles **1a,b** and the cholesterol derivative modified with the hydroxy group, namely, 3β-(2-hydroxyethoxy)cholest-5-ene **2**, which was synthesized according to the reported procedure by the nucleophilic substitution of cholesterol tosylate with ethylene glycol.[30] The novel cholesterol cobalt bis(dicarbollide) conjugates **3a**,**b** were obtained by nucleophilic ring opening reactions of the 1,4-dioxane and tetrahydropyran oxonium derivatives of cobalt bis(dicarbollide) **1a**,**b** with the OH group of modified cholesterol **2** in the presence of twofold molar excess of NaH (Scheme 1). This afforded the cholesterol derivatives of $[3,3'\text{-Co}(C_2B_9H_{11})_2]^-$ featuring different spacers between the boron cage and the biological macromolecule. Product **3a** bears hydrophilic $(CH_2CH_2O)_2$ spacer, whereas analogue **3b** contains lipophilic $(CH_2)_5$ spacer between the boron cage and the bioactive part of the molecule. These spacers can be considered as long units featuring high flexibility degrees and biocompatibility, which can be introduced using a simple synthetic procedure.

Assessment of the cytotoxicity of compounds **3a**,**b** was carried out in the MTT test. To this end, a 50% inhibitory concentration (IC_{50}) of compounds **3a** and **3b** was determined for human embryo fibroblasts FECH-15 [non-transformed or normal healthy cells, Figure 1, part (*a*)] and for human glioblastoma cells U-87 MG [part (*b*)]. Additional titration was required to determine IC_{50} of compound **3a** [part (*c*)]. It was found that for compound **3a**, IC_{50} against tumor cells U-87 MG is detected at a concentration of 0.06 mg ml^{-1}, and for normal human cells FECH-15 only at a higher concentration of 1.56 mg ml^{-1}. The concentration of IC_{50} for compound **3b** against U-87 MG cells was 1.56 mg ml^{-1}, and for normal FECH-15 cells was 39.5 mg ml^{-1}.

The selective cytotoxicity index (CC_{50}) against tumour cells U-87 MG, which was defined as the ratio of IC_{50} for cells of the normal phenotype FECH-15 to IC_{50} for U-87 MG cells, was approximately 26 and 25.3 for compounds **3a**,**b**, respectively.[31] The recommended cytotoxic concentration for new chemotherapeutic agents should be at least 10^{-4} mM, while selectivity index of some antitumor drugs sometimes is 6–8.[31,32] Consequently, compounds **3a**,**b** may be promising not only as a potential agent for BNCT, but also as an independent antitumor agent.



Scheme 1 *Reagents and conditions*: i, NaH, THF, reflux, 8 h; ii, CsCl, acetone–water.

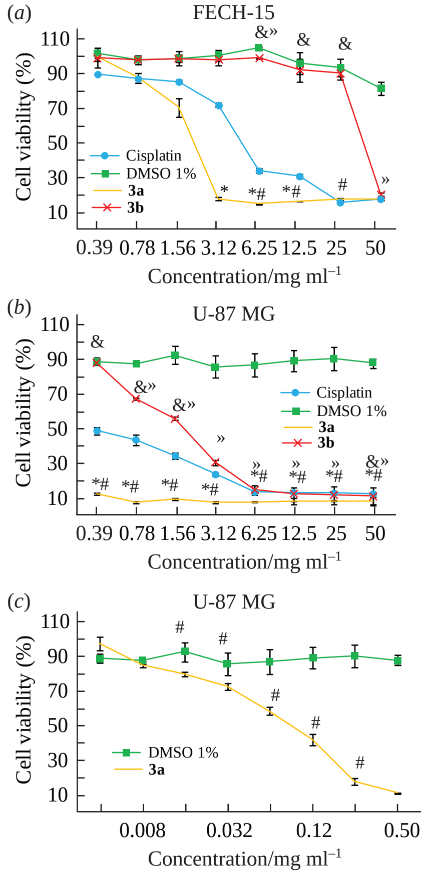


Figure 1 *In vitro* cytotoxicity of compounds **3a**,**b** on (*a*) FECH-15 cells and (*b*, *c*) U-87 MG cells, $n = 5$. CC_{50} of cisplatin was determined before as equal to 0.75–1.05 μg mol^{-1} (0.6–3.5 μmol dm^{-3}) that agree with our data.[33] The starting concentration of cisplatin was 0.05 mg ml^{-1}, the starting DMSO solution was 1%. Compound **3a** had initial concentrations of (*a*, *b*) 50 and (*c*) 0.5 mg ml^{-1}, which corresponded to 9.9 and 0.09 mg ml^{-1} of total boron or 2 and 0.02 mg ml^{-1} of 10 boron, respectively. Compound **3b** had a starting concentration of 50 mg ml^{-1}, which corresponded to 10.01 mg ml^{-1} of total boron or 2 mg ml^{-1} of 10 boron, respectively. Statistically significant differences at $p < 0.05$ (T-test): * between **3a** and cisplatin; # between **3a** and DMSO 1%; & between **3b** and cisplatin; » between **3b** and DMSO 1%.

In conclusion, two novel conjugates of cholesterol with cobalt bis(dicarbollide) were synthesized and their toxicity to glioblastoma U-87 MG cells and human embryo fibroblasts FECH-15 cells was assessed. These compounds may be promising not only as a potential agent for BNCT, but also as an independent antitumor agent. In the future, the boron-containing lipids obtained are planned to be evaluated for their liposome formulation that can be used for the selective introduction of boron into tumor cells for cancer therapy with the capture of boron neutrons.

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This work does not involve human participants and animal subjects.

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2022.05.021.

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