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Investigations on antimicrobial activity of cobaltabisdicarbollides

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Dedicated to Prof. Vladimir Bregadze on the occasion to his 75th anniversary in recognition to his outstanding contribution to Boron clusters compounds.

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ABSTRACT

Metallacarboranes have been identified as promising pharmacophores and building blocks in drug designed as well as inhibitors of HIV. The aim of this study was to evaluate the putative antimicrobial properties *in vitro* of cobaltabisdicarbollide [3,3′-Co(1,2-$C_2B_9H_{11}$)$_2$]$^-$, H[**1**], and its derivatives [3,3′-Co(8-R(CH_2CH_2O)$_2$-1,2-$C_2B_9H_{10}$)(1′,2′-$C_2B_9H_{11}$)]$^-$ (R = $-OOCCH_3$ (Na[**3**]); $-OCH_3$ (Na[**6**]); $-OCH_2CH_3$ (Na[**7**])). Pure cultures of 16 pathogenic bacterial strains (isolated from animals and humans as well as control strains) and 3 strains of *Candida* spp. were applied in antimicrobial studies that were performed by the agar-diffusion method of Bauer–Kirby and the method of minimum inhibitory concentrations. The obtained results revealed that among the compounds examined Na[**7**] and Na[**4**] exhibited the highest antimicrobial activity that was equal or even higher than those of the commercially available broad-spectrum antibiotic thiamphenicol. The cobaltabisdicarbollide H[**1**] was shown to express comparatively lower antibacterial and antifungal properties as compared to its derivatives. From a practical point of view it is important to emphasize that the meticillin-resistant strain of *Staphylococcus aureus* (TSA MRSA), the poliresistant strains of *Pseudomonas aeruginosa*, as well as of *Candida* spp., are sensitive to the compounds Na[**7**] and Na[**4**].

1. Introduction

Recently, there is an increasing interest in biological activity and medicinal application of boron and boron compounds [1]. Boron compounds (boronic acids, boron heterocycles, etc.) are reported to be promising antibacterials [2] and antifungal agents [3]. There are data that some curcumin boron complexes [4], boronic acid compounds [5], boron-modified polypeptides [6] and metallocarboranes [7] are specific and potent inhibitors of HIV protease.

The anionic *closo* icosahedral cobaltabisdicarbollide [3,3′-Co(1,2-$C_2B_9H_{11}$)$_2$], [**1**]$^-$, that show great chemical stability, electrochemical activity, high volume, low nucleophilic character, low charge density and amphiphilic properties [8] has been the object of many studies [9] since its discovery in 1968 [10]. Their derivatives are highly air-stable, very robust, withstanding strong acid, moderate base, high temperatures and intense radiation [9a]. The cobaltabisdicarbollide [**1**]$^-$ has been proposed in a wide range of applications such as extraction of radionuclides [11], conducting organic polymers [12] or use in medicine [13]. The use of polyhedral boron hydrides for cancer treatment is traditionally connected with a binary approach for cancer treatment named Boron Neutron Capture Therapy (BNCT) [14].

Polyhedral metallaborane and metallacarborane anions were proposed as carriers of radionuclide label for targeted radionuclide therapy/diagnostics of cancer [15] and several of them were found to demonstrate significant antitumor activity themselves [15,16].

There is a significant global need for new antibacterial and alternative mechanisms of action given the rise in resistance among bacteria [17]. The chemical and biological stability, low toxicity, and the possibility to introduce various modifications make boron clusters and specially H[**1**] attractive pharmacophores for potent and specific enzyme inhibition. The aim of this study is to expand the biomedical applications by evaluating the putative antimicrobial properties of the salts H[**1**], Na[**3**] to Li[**8**].

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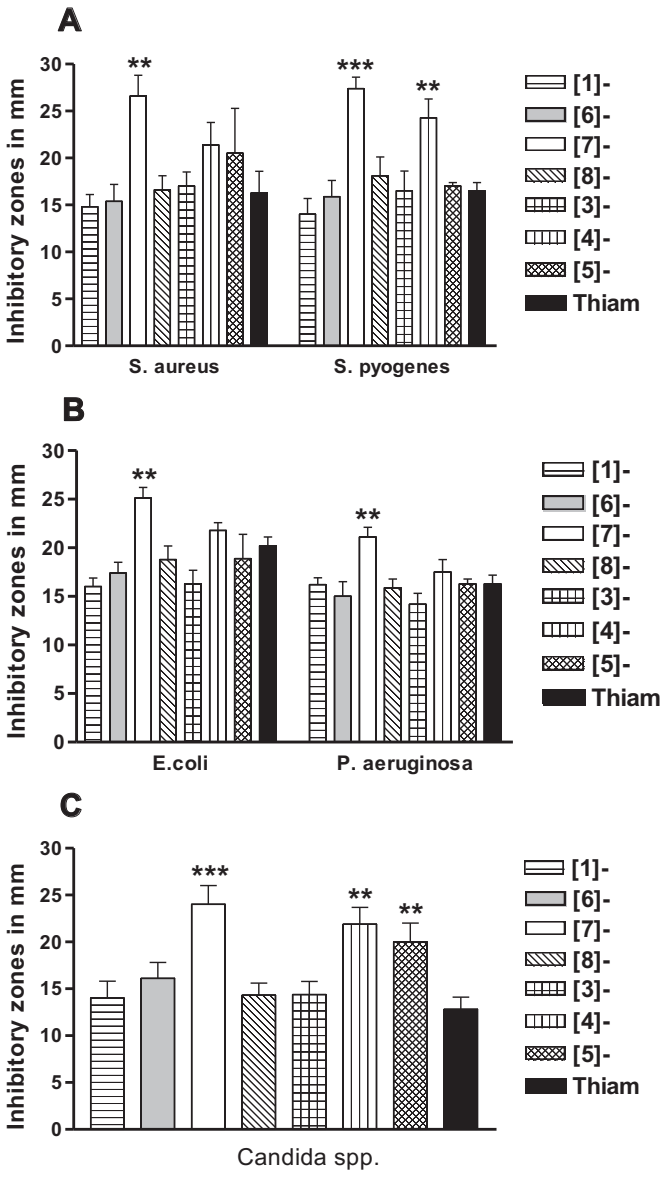

Fig. 1. Inhibitory effects of newly synthesized cobaltabisdicarbollides on pathogenic microorganisms: Gram-positive bacteria (A), Gram-negative bacteria (B) and *Candida* spp. fungi (C). Significant differences versus wide-spectrum antibiotic thiamphenicol (used as positive control) are presented: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ vs. thiamphenicol (Thiam); $n = 5$; being n the number of independent experiments performed *in vitro*.

2. Experimental section

2.1. Materials

2.1.1. Chemicals

Dimethyl sulfoxide (DMSO) neutral red and trypsin were purchased from AppliChem (Darmstadt, Germany); Mueller–Hinton's agar was from Scharlau Chemie S.A. (Barcelona, Spain). Dulbecco's modified Eagle's medium (D-MEM) and fetal bovine serum were from Gibco-Invitrogen (UK); thiazolyl blue tetrazolium bromide (MTT) was obtained from Sigma–Aldrich Chemie GmbH (Germany). All other chemicals of the highest purity commercially available were purchased from local agents and distributors. All sterile plasticware and syringe filters were from Orange Scientific (Belgium).

Synthesis of Cobaltabisdicarbollide compounds: Following our studies on cobaltabisdicarbollides' direct substitution, we recently reported on the high yield synthesis of polyanionic species as novel high-boron content molecules with enhanced water solubility [18]. The synthetic ways were based on the use of carboxylates and alkoxides as nucleophiles in the ring-opening reaction of cyclic oxonium [3,3′-Co(8-$C_4H_8O_2$-1,2-$C_2B_9H_{10}$)(1′,2′-$C_2B_9H_{11}$)], [**2**] compound [19]. We have synthesized sodium salts that incorporate a PEG chain and the [3,3′-Co(1,2-$C_2B_9H_{11}$)$_2$]$^-$ moiety. Chart 1 shows the species Na(H_2O)[3,3′-Co(8-O(CH_2CH_2O)$_2$C(O)CH_3-1,2-$C_2B_9H_{10}$)(1′,2′-$C_2B_9H_{11}$)], Na[**3**]; Na[1″-{3,3′-Co(8-O(CH_2CH_2O)$_2$C(O)-1,2-$C_2B_9H_{10}$)(1′,2′-$C_2B_9H_{11}$)}-2″−OH−C_6H_4}], Na[**4**]; Na_3[1″,3″,5″-{3,3′-Co(8-O(CH_2CH_2O)$_2$-1,2-$C_2B_9H_{10}$)(1′,2′-$C_2B_9H_{11}$)}$_3$-C_6H_3], Na_3[**5**]; Na[3,3′-Co(8-O(CH_2CH_2O)$_2$$CH_3$-1,2-$C_2B_9H_{10}$)(1′,2′-$C_2B_9H_{11}$)], Na[**6**]; Na[3,3′-Co(8-O(CH_2CH_2O)$_2$$CH_2CH_3$-1,2-$C_2B_9H_{10}$)(1′,2′-$C_2B_9H_{11}$)], Na[**7**] that were synthesized from the zwitterionic [**2**] according to Scheme 1 following the reported procedure [18a,19a]. The chelating ligand derivative Li[1,1′-(PPh_2)$_2$-3,3′-Co(1,2-$C_2B_9H_{10}$)$_2$], Li[**8**], was synthesized (see Scheme 2) in one pot reaction in very good yield and with an easy working up process according to the method reported at the literature [20].

The cobaltabisdicarbollide species were dissolved in DMSO and diluted in sterile PBS for antibacterial tests or in culture medium for cytotoxicity investigations. The final concentration of DMSO in the stock solutions (where the concentration of the tested compound was 1 mg/mL) was 2%. It has been recently reported [21] that metallacarboranes are often poorly soluble in water and have the tendency to aggregate in aqueous media but it has not been observed in this study.

The commercially available broad spectrum antibiotic thiamphenicol was applied in antimicrobial investigations as a positive control. The dilutions of the antibiotic were prepared in sterile PBS.

2.1.2. Microorganisms

The antimicrobial activity of the compounds was tested on a broad range of Gram-positive and Gram-negative pathogenic bacterial strains (isolated either from animals or humans as well as the control strains) and on *Candida* spp. In our experiments we used 4 strains of *Staphylococcus aureus* (*S. aureus* TSA MRSA, *S. aureus* Kowan, *S. aureus* 131, *S. aureus* 230), 4 of *Streptococcus pyogenes* (*S. pyogenes* 1, *S. pyogenes* 2, *S. pyogenes* 184, *S. pyogenes* 383), 4 of *Escherichia coli* (*E. coli* 045, *E. coli* 075-20, *E. coli* 16 − B, *E. coli* 0621 − C) and 4 of *Pseudomonas aeruginosa* (*P. aeruginosa* 1, *P. aeruginosa* 177, *P. aeruginosa* 318, *P. aeruginosa* 357). The bacterial strains were isolated from patients with infections of different location (skin, ears, conjunctiva, respiratory and urogenital tracts) that were subject to continuous treatment with various antibacterial means. The strains showed *in vitro* a high drug resistance mainly to streptomycin, penicillin, oxacillin, ampicillin, and some of them also to amoxicillin but are sensitive to amphenicols (established by the agar-diffusion method of Bauer–Kirby in the Laboratory of Microbiology, Faculty of Veterinary Medicine, Forest Technical University, Sofia, Bulgaria).

Pure cultures of 3 pathogenic strains of *Candida albicans* (*C. albicans* 1, *C. albicans* 332, *C. albicans* 398) and 1 of *Candida tropicalis* (*C. tropicalis* 324) were also included in the experiments.

2.1.3. Animal cell cultures

Madin–Darby bovine kidney (MDBK) non-tumor permanent cell line was used in our investigations. The cells were grown as monolayer culture in D-MEM medium, supplemented with 5–10% fetal bovine serum, 100 U/mL penicillin and 100 μg/ml streptomycin. The cultures were maintained at 37 °C in a humidified CO_2 incubator. For routine passages, adherent cells were detached using a mixture of 0.05% trypsin and 0.02% EDTA. The experiments were performed during the exponential phase of cell growth.