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Research paper

Synthesis and evaluation of thymidine kinase 1-targeting carboranyl pyrimidine nucleoside analogs for boron neutron capture therapy of cancer

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ABSTRACT

A library of sixteen 2nd generation amino- and amido-substituted carboranyl pyrimidine nucleoside analogs, designed as substrates and inhibitors of thymidine kinase 1 (TK1) for potential use in boron neutron capture therapy (BNCT) of cancer, was synthesized and evaluated in enzyme kinetic-, enzyme inhibition-, metabolomic-, and biodistribution studies. One of these 2nd generation carboranyl pyrimidine nucleoside analogs (**YB18A** [**3**]), having an amino group directly attached to a *meta*-carborane cage tethered *via* ethylene spacer to the 3-position of thymidine, was approximately 3–4 times superior as a substrate and inhibitor of hTK1 than **N5-2OH (2**), a 1st generation carboranyl pyrimidine nucleoside analog. Both **2** and **3** appeared to be 5′-monophosphorylated in TK1(+) RG2 cells, both *in vitro* and *in vivo*. Biodistribution studies in rats bearing intracerebral RG2 glioma resulted in selective tumor uptake of **3** with an intratumoral concentration that was approximately 4 times higher than that of **2**. The obtained results significantly advance the understanding of the binding interactions between TK1 and carboranyl pyrimidine nucleoside analogs and will profoundly impact future design strategies for these agents.

Abbreviations: 3CTAs, 3-carboranyl thymidine analogs; ^{10}B, boron-10; ATCC, American type culture collection; AZT, zidovudine; B, boron; BNCT, boron neutron capture therapy; *Ba*TK, *Bacillus anthracis* thymidine kinase; DEAE, diethylaminoethanol; DMEM, Dulbecco's modified eagle's medium; dThd, thymidine; d4T, stavudine; ESI+ or ESI−, electrospray ionization positive or negative ion mode, respectively; FBS, fetal bovine serum; HRP, horseradish peroxidase; hTK1, human thymidine kinase 1; ICP-OES, inductively coupled plasma-optical emission spectroscopy; IACUC, institutional animal care and use committee; LET, linear energy transfer; MP, monophosphate; PEI, polyethylenimine; PTA, phosphate transfer assay; RP-18, reversed-phase 18; rPR, relative phosphorylation rate; *Sa*TK, *Staphylococcus aureus* thymidine kinase; siRNA, small interfering ribonucleic acid; SM, supplementary materials; TBDMS, *tert*-butyldimethylsilyl; TK1, thymidine kinase 1.

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1. Introduction

TK1-like enzymes, including human thymidine kinase 1 (hTK1), *Bacillus anthracis* TK (*Ba*TK), and *Staphylococcus aureus* TK (*Sa*TK), play essential roles in the biosynthetic salvage pathways of nucleotide synthesis, complementing the *de novo* pathways particularly in proliferating cells and bacteria [1–6]. In normal human cells, TK1 activity is high only in S-phase. In cancer cells, however, TK1 activity can remain also high in other phases of the cell cycle [7–9].

Clinically relevant agents that rely on TK1 activity include the HIV/AIDS prodrugs zidovudine (AZT) and stavudine (d4T) [10]. Both compounds are analogs of the endogenous TK1 substrate, thymidine (dThd, **1**, Fig. 1). They are effectively 5′-monophosphorylated by TK1. Their ultimate mechanism of action, however, is based on the subsequent formation of a cytotoxic triphosphate metabolite by other kinases. Other biomedically relevant analogs of **1** that possibly rely on 5'-monophosphorylation by TK1 are 3-carboranyl thymidine analogs (3CTAs). The agents were developed as boron delivery agents for boron neutron capture therapy (BNCT) of brain tumors [11,12]. Other types of carboranyl nucleoside derivatives were developed for the same and other purposes [13–17]. BNCT is a binary cancer treatment system that relies on the accumulation of boron-10 (^{10}B) in tumor cells followed by external neutron irradiation. Subsequent capture of neutrons by ^{10}B results predominantly in high linear energy transfer (LET) ionizing radiation, i.e., $^{4}He^{2+}$ (α-particle) and $^{7}Li^{3+}$ nuclei. These particles can selectively destroy tumor cells because of their limited path lengths of <10 μm in biological tissue. Prerequisite for the success of BNCT is the selective accumulation of ^{10}B in tumor vs. normal cells [18,19].

First generation 3CTAs, such as **N5-2OH (2**, Fig. 1), were found to be good substrates of TK1, despite having a bulky and highly lipophilic carboranyl substituent [20] tethered *via* alkyl spacer to the 3-position. Preferential uptake of **2** by TK1(+) vs. TK1(−) cells *in vitro*, presumably involving intracellular trapping of its 5′-monophosphate, was observed [21,22]. Consequently, a favorable *in vivo* biodistribution profile of **2** in tumor bearing rodents, possibly encompassing tumor-selective accumulation by intracellular accumulation of the 5′-monophosphate metabolite, led to promising preclinical BNCT of rats with brain tumors [21,22]. However, the need for improvement of 1st generation 3CTAs became apparent during these studies. They had moderate capacity to inhibit TK1 activity, i.e., to compete with endogenous **1** for phosphorylation at the substrate-binding site [11,23].

In order to make further progress in achieving the crucial objective of improved TK1 substrate and inhibitory capacities, the design, synthesis, and biological evaluation of carboranyl pyrimidine nucleoside analogs with amino- or amido functionalities in *meta* (1,7)-carborane cluster-containing side chains either at the 3- or 4-position will be described in this paper (see Fig. 1 for the atom numbering of pyrimidine nucleosides).

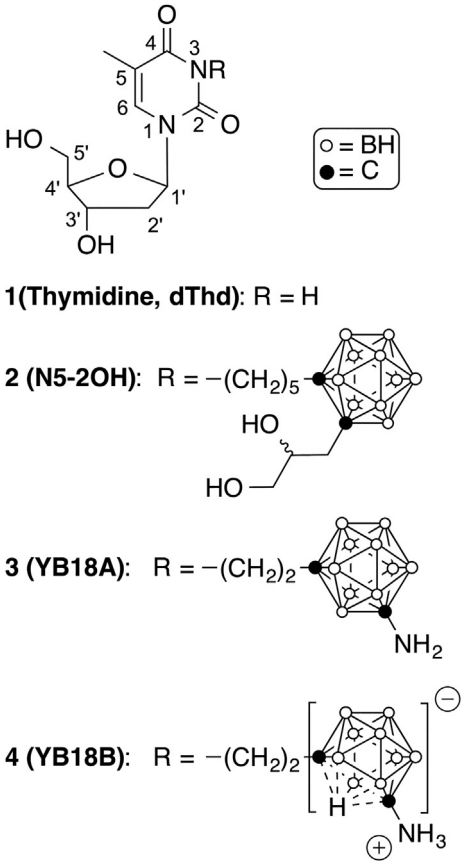

Fig. 1. Structures of **1**, the first generation 3CTA **2**, and the second generation 3CTAs **3** and **4**.

2. Results

2.1. Design and chemistry

All TK1-like enzymes have similar overall 3-D fold including the presence of a structural component that has been designated as the 'lasso-loop' [6,24]. The enzymes undergo significant conformational changes upon binding of **1** and ATP [25,26]. In the apo state, the lasso loop is folded away from the substrate-binding site whereas in the holo state, the lasso loop covers the substrate-binding site tightly by forming hydrogen bonds with **1**, primarily *via* main chain atoms.

Preliminary computational docking of 1st generation 3CTAs, such as **2**, to TK1 crystal structures led to the hypothesis that initial substrate binding results in incomplete closure of the lasso loop, leaving the bulky carborane cage outside of the active site, and that some hydrogen bonds are lost because of the 3-substitution [23,27,28]. This model may explain the moderate TK1 inhibitor characteristics of 1st generation 3CTAs because endogenous **1** may still have ample competitive access to the substrate binding site. It is also conceivable that a location of the highly hydrophobic carborane cage outside of the enzyme is disrupting the hydrogen bond network of water molecules and that this is contributing to the lack of binding of 3CTAs to TK1. It needs to be emphasized, however, that the exact molecular interactions between 3CTAs and TK1 amino acid residues remain unknown.

Based on the current hypothesis on 3CTA-TK1 interactions, previous efforts in our laboratories have focused on the introduction of hydrogen bond donor/acceptors, including hydroxyl-, amidino-, and guanidino groups, in a spacer element between the carborane cluster and the scaffold of **1** in 2nd generation 3CTAs to re-establish hydrogen bonds between substrate and enzyme that were lost due to the 3-substitution [29,30]. These groups were mostly placed in close proximity to the 3-position. Unfortunately, these 3CTAs did not prove to be superior to **2** as TK1 substrates and inhibitors. In the case of some compounds, lack of stability was another problem [29]. The design concept for carboranyl pyrimidine nucleosides discussed in this paper explores the introduction of hydrogen bond donor/acceptor amino- or amido groups in spacer elements between *meta*-carborane cluster and pyrimidine nucleoside scaffold, both at the 3-(Schemes 1–3) and the 4-(Scheme 4) position. In addition, **3** (**YB18A**) and **4** (**YB18B**) (Fig. 1), both containing an amino group directly attached to a *meta*-carborane cluster tethered *via* ethylene spacer to the 3-position, were prepared for detailed enzyme kinetic and inhibition studies. The syntheses of both compounds and their