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4-Connected azabicyclo[5.3.0]decane Smac mimetics-Zn^{2+} chelators as dual action antitumoral agents

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ABSTRACT

Putative dual action compounds (DACs **3a**–**d**) based on azabicyclo[5.3.0]decane (ABD) Smac mimetic scaffolds linked to Zn^{2+}-chelating 2,2′-dipicolylamine (DPA) through their 4 position are reported and characterized. Their synthesis, their target affinity (cIAP1 BIR3, Zn^{2+}) in cell-free assays, their pro-apoptotic effects, and their cytotoxicity in tumor cells with varying sensitivity to Smac mimetics are described. A limited influence of Zn^{2+} chelation on in vitro activity of DPA-substituted DACs **3a**–**d** was sometimes perceivable, but did not lead to strong cellular synergistic effects. In particular, the linker connecting DPA with the ABD scaffold seems to influence cellular Zn^{2+}-chelation, with longer lipophilic linkers/DAC **3c** being the optimal choice.

Multiple pathophysiological mechanisms are connected with the development and the progression of cancer.[1] The ability of cancer cells to develop resistance to chemotherapeutics and target-directed therapies[2] demands for more effective treatments.[3] A *dual action compound* (*DAC*) should interfere with two validated mechanisms against cancer, and should thus increase efficacy and minimize resistance.[4]

Smac-DIABLO is a mitochondrial protein with multiple pro-apoptotic effects in cancer cells. It binds to inhibitor of apoptosis proteins[5] (IAPs), frees caspases-3, -7 and -9,[6] and induces degradation of cellular IAPs (cIAPs).[7] Zinc ions show anti-apoptotic effects in cancer cells. They prevent the autocatalytic conversion of procaspase-3 to active caspase-3 by interaction with its "safety catch" DDD sequence,[8] and their chelation causes serine protease-dependent depletion of anti-apoptotic X-linked IAP (XIAP).[9]

Substituted azabicyclo[5.3.0]decane Smac mimetics (ABDs) are potent, cytotoxic Smac mimetics targeted against IAPs.[10] Positions 4 and 10 of their bicyclic core (Fig. 1) can be substituted to increase their IAP affinity, and their drug-like profile.[11] We reasoned that zinc depletion by Zn^{2+}-chelating DACs built around ABDs should cause synergistic pro-apoptotic effects. We reported[12] 4-amidoalkyl ABDs substituted in position 10 with a Zn^{2+}-chelating moiety (i.e., 2,2′-dipicolylamine, DPA in **1**, Fig. 1). A (*S*)-PheGly linker connected DPA with Smac mimetic ABD scaffolds (i.e., 4-benzamidomethyl Smac mimetic **2**,[11] Fig. 1).

10-Connected Smac mimetic- Zn^{2+}-chelator DACs showed cell-free potency against IAPs and Zn^{2+}-chelating properties. Their cytotoxicity was moderate, and Zn^{2+} chelation-dependent effects in cellular assays were not observed.[12] We then targeted DPA-containing, ABD-based DACs connected through position 4, bearing an IAP affinity-best diphenylmethylamide substitution in position 10 (generic structure **3**, Fig. 2). In particular, we aimed to evaluate the influence of the linker between DPA and ABD scaffolds on cell-free properties, on biological activity and bioavailability in cancer cells.

Our synthetic strategy required access to gram quantities of N^3-Boc-protected 4-amino-10-diphenylmethylamido ABD **4** (Scheme 1). We optimized its synthesis from tricyclic butyl ester

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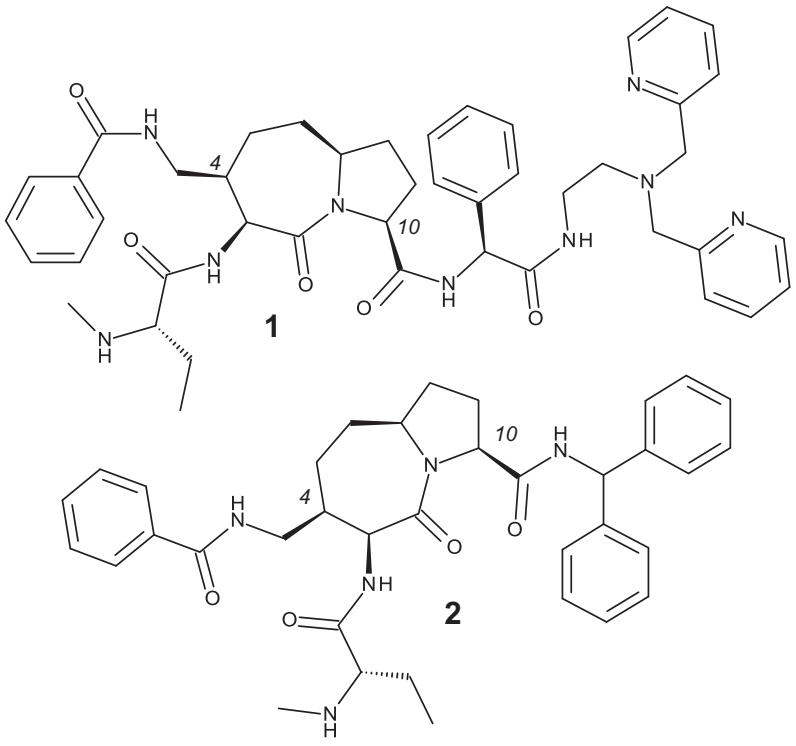

Fig. 1. Structure of ABD-based compounds **1** and **2**.

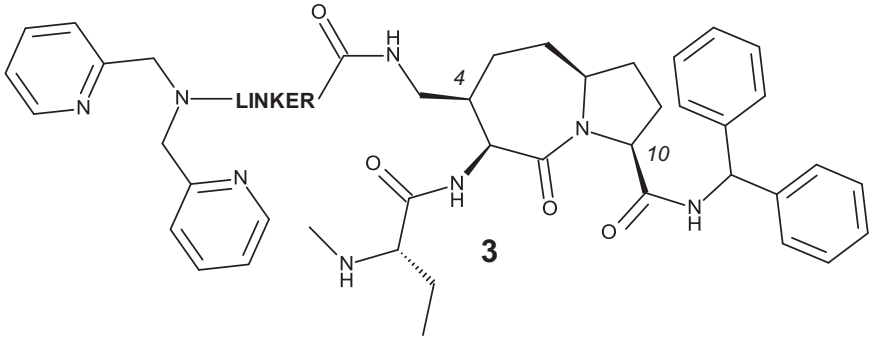

Fig. 2. General structure of 4-connected Smac mimetic- Zn^{2+}-chelator DACs **3**.

5,[11] available in large quantity in our lab, by improving yields and simplifying known experimental protocols.[11] Scheme 1 shows the 10-step optimized synthesis of amine **4** in an overall ≈20% yield from **5**.

We selected *N*,*N*-bis(2-pyridinylmethyl)glycine **6** as a key DPA-containing synthon (boxed structure, Fig. 3), and we coupled it to key Smac intermediate **4** either as such (compound **3a**, LINKER = CH_2, "**no linker**") or through three linkers with varying length and lipophilicity. Namely, β-alanine (compound **3b**, LINKER = $CH_2CONH(CH_2)_2$, "**short lipophilic**"), 11-aminoundecanoic acid (compound **3c**, LINKER = $CH_2CONH(CH_2)_{10}$, "**long lipophilic**"), and 8-amino-3,6-dioxaoctanoic acid (compound **3d**, LINKER = $CH_2CONH(CH_2)_2O(CH_2)_2OCH_2$, "**hydrophilic**") were used respectively as short lipophilic, long lipophilic and long hydrophilic linkers (Fig. 3).

DPA-containing synthon **6** was prepared following a published procedure.[13] Nucleophilic substitution on DPA **7** with ethyl 2-chloroacetate **8** in mild conditions (step a, Scheme 2) led to ester **9**, then hydrolyzed in aqueous KOH and neutralized (step b) to acid **6** in a good, overall ≈60% yield. Coupling of carboxylate **6** with amine **4** in basic peptide coupling conditions (step c) led to *N*-Boc-protected **10a**, that was then deprotected in acidic conditions, yielding target "**no linker**" DAC **3a** (step d, Scheme 2) in a good ≈76% yield from **4**.[14]

We reasoned that time- and cost-expensive amine **4**should be used as late as possible in a synthetic scheme, to minimize its consumption. Thus, target DACs **3b** and **3c**, where the Zn^{2+}-chelating moiety and the Smac mimetic portion are connected by lipophilic linkers, were prepared by coupling of pre-formed acid DPA-linker constructs **11a,b** with amine **4** (Scheme 3). Commercially available