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Design, synthesis, and biological evaluation of water-soluble amino acid prodrug conjugates derived from combretastatin, dihydronaphthalene, and benzosuberene-based parent vascular disrupting agents

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ABSTRACT

Targeting tumor vasculature represents an intriguing therapeutic strategy in the treatment of cancer. In an effort to discover new vascular disrupting agents with improved water solubility and potentially greater bioavailability, various amino acid prodrug conjugates (AAPCs) of potent amino combretastatin, amino dihydronaphthalene, and amino benzosuberene analogs were synthesized along with their corresponding water-soluble hydrochloride salts. These compounds were evaluated for their ability to inhibit tubulin polymerization and for their cytotoxicity against selected human cancer cell lines. The amino-based parent anticancer agents **7**, **8**, **32** (also referred to as KGP05) and **33** (also referred to as KGP156) demonstrated potent cytotoxicity (GI_{50} = 0.11–40 nM) across all evaluated cell lines, and they were strong inhibitors of tubulin polymerization (IC_{50} = 0.62–1.5 μM). The various prodrug conjugates and their corresponding salts were investigated for cleavage by the enzyme leucine aminopeptidase (LAP). Four of the glycine water-soluble AAPCs (**16**, **18**, **44** and **45**) showed quantitative cleavage by LAP, resulting in the release of the highly cytotoxic parent drug, whereas partial cleavage (<10–90%) was observed for other prodrugs (**15**, **17**, **24**, **38** and **39**). Eight of the nineteen AAPCs (**13**–**16**, **42**–**45**) showed significant cytotoxicity against selected human cancer cell lines. The previously reported CA1-diamine analog and its corresponding hydrochloride salt (**8** and **10**, respectively) caused extensive disruption (at a concentration of 1.0 μM) of human umbilical vein endothelial cells growing in a two-dimensional tubular network on matrigel. In addition, compound **10** exhibited pronounced reduction in bioluminescence (greater than 95% compared to saline control) in a tumor bearing (MDA-MB-231-luc) SCID mouse model 2 h post treatment (80 mg/kg), with similar results observed upon treatment (15 mg/kg) with the glycine amino-dihydronaphthalene AAPC (compound **44**). Collectively, these results support the further pre-clinical development of the most active members of this structurally diverse collection of water-soluble prodrugs as promising anticancer agents functioning through a mechanism involving vascular disruption.

1. Introduction

Tumor vasculature is as an attractive target for the treatment of cancer due, in part, to its distinct characteristics, such as rapid and disorganized proliferation of endothelial cells.[1–5] Both small-molecules and biologics that specifically interact with tumor vasculature are referred to as vascular targeting agents (VTAs), which are further sub-classified as angiogenesis-inhibiting agents (AIAs) and vascular disrupting agents (VDAs).[6,7] AIAs are typically represented by compounds that target vascular endothelial growth factors to prevent the development of new tumor vasculature.[7] Conversely, VDAs are comprised of compounds that disrupt and directly damage established tumor vasculature, by affecting

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rapidly growing endothelial cells, to suppress tumor blood flow. One class of VDAs interacts with the colchicine (Fig. 1) site located on the α,β-heterodimer of tubulin, causing cell retraction, rounding, and ultimately detachment from the aggregated sheet of cells.[1,4,8,9]

The natural products combretastatin A-4 (CA4) and combretastatin A-1 (CA1) (Fig. 1), originally isolated by Pettit and co-workers (Arizona State University) from the South African bush-willow tree, *Combretum caffrum* Kuntze, are among the colchicine site class of tubulin binding VDAs.[10–13] This family of natural products, along with a number of synthetic derivatives and analogs,[6,14–17] significantly inhibit microtubule assembly in endothelial cells lining tumor-feeding vasculature, leading to a series of cell signaling events that ultimately result in endothelial cell morphology changes and blood flow reduction.[1,7,17] The inhibition of tubulin polymerization results in activation of RhoA, an intracellular coordinator of the cytoskeletal rearrangement of microtubules and actin, and leads to rapid vascular collapse.[15,18,19] Administration of these VDAs in amounts significantly lower than their maximum tolerated doses has resulted in tumor necrosis in treated laboratory mice.[3,12]

The combretastatin water-soluble phosphate prodrugs, combretastatin A-4 phosphate (CA4P also known as Zybrestat™, Fig. 1) and combretastatin A-1 diphosphate (CA1P also known as OXi4503, Fig. 1) are among a group of VDAs which have demonstrated promising efficacy in human clinical trials.[18–27] They undergo enzyme-mediated dephosphorylation and, as their parent compounds (CA4 and CA1), they bind to tubulin and interfere with the tubulin-microtubule protein system. This causes pronounced morphological effects in the tumor vasculature.[8,22–28] Pre-clinical and pharmacokinetic evaluations of these combretastatin prodrugs in patients bearing advanced tumors indicate their efficacy as VDAs.[22,29–31] Interestingly, CA1P has dual mechanistic capability, functioning as both a VDA and as a cytotoxic agent based on its in vivo mediated conversion to a highly reactive ortho-quinone.[18,29–31] The relative structural simplicity of the combretastatins has motivated synthetic chemists to develop libraries of structurally inspired analogs through alteration of the A-ring, the B-ring, and the ethylene bridge.[6,15,16,32–35]

Incorporation of the NH_2 substituent within either ring A or ring B in the combretastatin family resulted in new analogs that exhibited important biological activity.[15,36,37] In 2006, we reported the initial design and synthesis of the 2′ CA4-amine (Fig. 1) and described its potent inhibition of tubulin assembly and its activity as a VDA.[15] Later work by others confirmed the potency of this 2′ CA4-amine analog and related compounds.[34] Subsequently, our studies showed that the di-amino variant of combretastatin A-1 (CA1-diamine, Fig. 1)[16] was strongly cytotoxic against human cancer cell lines (average GI_{50} = 13.9 nM) and also demonstrated potent activity in regard to inhibition of tubulin assembly (IC_{50} = 2.8 μM).[16] It is fairly common for compounds that interact with tubulin in the low μM range (cell free assay) to demonstrate nM cytotoxicity against human cancer cell lines; a variety of factors are postulated to influence this activity differential.[38] The trimethoxyphenyl moiety, the *p*-methoxyphenyl moiety, the *Z*-configuration of the two aryl rings, and the optimal 4–5 Å aryl–aryl distance all proved important for enhanced tubulin binding activity of the combretastatin analogs.[17,39–41] Inspired, in part, by the SAR studies associated with the combretastatins and their close structural analogs, we were the first to report the synthesis and biological activity of related dihydronaphthalene tubulin-binding agents [for example, OXi6196 and KGP05, Fig. 1],[42–44] followed by the discovery of a phenolic-based benzosuberene (KGP18, Fig. 1) analog and its corresponding amino congener (KGP156, Fig. 1).[40,45–48] These compounds have emerged as potential pre-clinical candidates due to their robust in vitro cytotoxicity

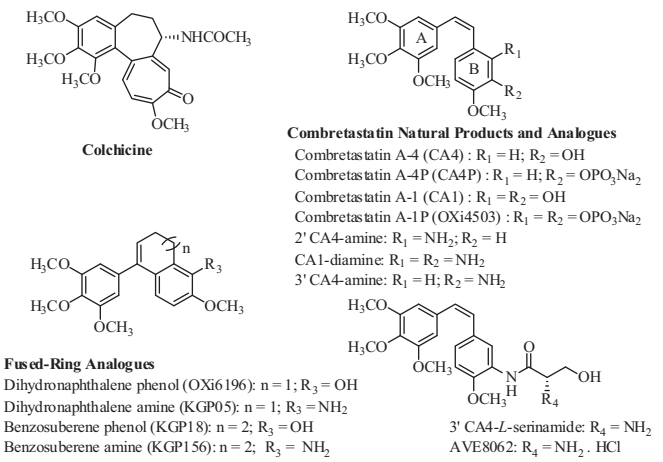

Figure 1. Colchicine and natural and synthetic combretastatin, dihydronaphthalene, and benzosuberene analogs.

(sub-nanomolar to picomolar GI_{50} values) against selected human cancer cell lines and strong tubulin inhibitory activities.[45–47] Certain of these dihydronaphthalene analogs have subsequently been reported by another group.[49] In our previous work,[46] we also demonstrated robust tubule disruption [in a human umbilical vein endothelial cell (HUVEC) tube disruption assay] and cell rounding capability of KGP156, which is one of the parent compounds for several of the prodrugs designed and synthesized in this study.

The issue of limited water solubility associated with these aniline based anti-cancer agents can be addressed through prodrug strategies.[50,51] Amino acid prodrugs, like glycine and serine, with shorter hydrocarbon or polar side chains, are reported to be more readily water soluble and more likely to be cleaved because of their structural simplicity.[50] Another advantage of using amino acids is that they are capable of undergoing quantitative cleavage by hydrolytic enzymes, likely an aminopeptidase.[51,52] The synthesis and biological evaluation of a series of water-soluble amino acid prodrugs of amino-combretastatin were previously reported.[15,35,53] A water-soluble serinamide prodrug of 3′ CA4-amine (Fig. 1) known as AVE8062 (Ombrabulin, synthesis[36] is described in Supplementary data), showed significant promise as a VDA in phase III human clinical trials[54,55] and enhanced antitumor activity and decreased toxicity (for normal cells) in both in vitro and in vivo models.[36,53,55–57] The parent drug is generated after amide bond cleavage by a hydrolytic enzyme.[51,53] Leucine aminopeptidase (LAP)[58–60] is one of a widely distributed group of aminopeptidases that exhibit broad specificity[61–63] and that catalyzes the hydrolysis of amino acids from the amino terminus of polypeptide chains.[64] There are also a number of literature reports of amino acid prodrugs cleaved by LAP.[50,53] In humans, LAP is found primarily in the cytosol of liver cells, which makes the serum leucine aminopeptidase a marker of hepatic disorders.[65,66] The increase of LAP in human sera can also be diagnostically indicative of a number of cancers such as carcinoma of the pancreas and head and neck cancer.[67–69]

Inspired by these developments, herein we report the synthesis of eight glycine and serine AAPCs of highly potent amino-bearing structural variants incorporated within the combretastatin, dihydronaphthalene, and benzosuberene molecular scaffolds.[42,44,70,71] Furthermore, in order to enhance water solubility (and potentially bioavailability)[72] of these newly synthesized AAPCs, their eleven hydrochloride salts were synthesized.[51,53] The synthesis of compounds **13**, **15**, **37**, **39**, 3′ CA4-L-serinamide and AVE8062 were previously reported,[15,36,47,53] and they were re-synthesized as a part of our ongoing biological studies. Each of the parent amino-based