



Contents lists available at ScienceDirect

## Bioorganic &amp; Medicinal Chemistry Letters

journal homepage: [www.elsevier.com/locate/bmcl](http://www.elsevier.com/locate/bmcl)

## Bioreductively activatable prodrug conjugates of phenstatin designed to target tumor hypoxia

Blake A. Winn<sup>a</sup>, Zhe Shi<sup>a</sup>, Graham J. Carlson<sup>a</sup>, Yifan Wang<sup>a</sup>, Benson L. Nguyen<sup>a</sup>, Evan M. Kelly<sup>a</sup>, R. David Ross IV<sup>a</sup>, Ernest Hamel<sup>b</sup>, David J. Chaplin<sup>a,c</sup>, Mary L. Trawick<sup>a,\*</sup>, Kevin G. Pinney<sup>a,\*</sup>

<sup>a</sup> Department of Chemistry and Biochemistry, Baylor University, One Bear Place #97348, Waco, TX 76798-7348, United States

<sup>b</sup> Screening Technologies Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, Frederick National Laboratory for Cancer Research, National Institutes of Health, Frederick, MD 21702, United States

<sup>c</sup> Mateon Therapeutics, Inc., 701 Gateway Boulevard, Suite 210, South San Francisco, CA 94080, United States

## ARTICLE INFO

## Article history:

Received 3 October 2016

Revised 30 November 2016

Accepted 30 November 2016

Available online xxxxx

## Keywords:

Bioreductively activatable prodrug conjugates (BAPCs)

Phenstatin

Prodrug synthesis

Tumor-associated hypoxia

Inhibitors of tubulin polymerization

## ABSTRACT

A variety of solid tumor cancers contain significant regions of hypoxia, which provide unique challenges for targeting by potent anticancer agents. Bioreductively activatable prodrug conjugates (BAPCs) represent a promising strategy for therapeutic intervention. BAPCs are designed to be biologically inert until they come into contact with low oxygen tension, at which point reductase enzyme mediated cleavage releases the parent anticancer agent in a tumor-specific manner. Phenstatin is a potent inhibitor of tubulin polymerization, mimicking the chemical structure and biological activity of the natural product combretastatin A-4. Synthetic approaches have been established for nitrobenzyl, nitroimidazole, nitrofuranyl, and nitrothienyl prodrugs of phenstatin incorporating *nor*-methyl, *mono*-methyl, and *gem*-dimethyl variants of the attached nitro compounds. A series of BAPCs based on phenstatin have been prepared by chemical synthesis and evaluated against the tubulin-microtubule protein system. In a preliminary study using anaerobic conditions, the *gem*-dimethyl nitrothiophene and *gem*-dimethyl nitrofuranyl analogues were shown to undergo efficient enzymatic cleavage in the presence of NADPH cytochrome P450 oxidoreductase. Each of the eleven BAPCs evaluated in this study demonstrated significantly reduced inhibitory activity against tubulin in comparison to the parent anti-cancer agent phenstatin ( $IC_{50} = 1.0 \mu M$ ). In fact, the majority of the BAPCs (seven of the eleven analogues) were not inhibitors of tubulin polymerization ( $IC_{50} > 20 \mu M$ ), which represents an anticipated (and desirable) attribute for these prodrugs, since they are intended to be biologically inactive prior to enzyme-mediated cleavage to release phenstatin.

© 2016 Published by Elsevier Ltd.

Tumor-associated vasculature has emerged as a promising target for anti-cancer therapies due to its marked differences from vasculature feeding healthy tissue.<sup>1–10</sup> Vasculature associated with healthy tissue forms a well-organized delivery network for oxygen and nutrients to cells.<sup>1–10</sup> In contrast, tumor-associated vasculature is forced to develop rapidly to meet the enhanced demand for significant amounts of nutrients and oxygen required by

tumors.<sup>1–10</sup> The rapid growth of tumor-associated vasculature results in compromised structural integrity, which is characterized by weakened vessel walls, increased interstitial pressure, blind ends, and bulges.<sup>1–10</sup> Tumor-associated vasculature is generated rapidly, leading to groups of vessels spaced far apart from each other, and this results in regions of hypoxia that develop when this distance is greater than the diffusion distance of oxygen.<sup>1–10</sup>

One promising therapeutic option for targeting tumor-associated vasculature involves treatment with vascular targeting agents (VTAs), which include angiogenesis-inhibiting agents (AIAs) and vascular disrupting agents (VDAs).<sup>1–10</sup> AIAs, which represent a fairly well investigated therapeutic strategy, act by inhibiting angiogenesis, the formation of new tumor-associated vasculature, while leaving existing vessels intact.<sup>4,11,12</sup> Inhibition of angiogenesis limits tumor growth and also leads to increased blood flow in the remaining vasculature, allowing for increased delivery of

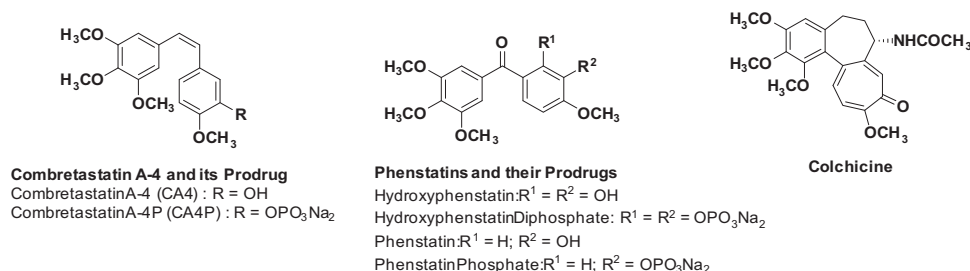
**Abbreviations:** BAPC, bioreductively activatable prodrug conjugate; CA1, combretastatin A-1; CA4, combretastatin A-4; Et<sub>3</sub>N, triethylamine; HPLC, high performance liquid chromatography; HRMS, high resolution mass spectrometry; MeOH, methanol; NaBH<sub>4</sub>, sodium borohydride; TLC, thin layer chromatography; THF, tetrahydrofuran; EtOAc, ethyl acetate; POR, NADPH cytochrome P450 oxidoreductase; DMP, Dess-Martin periodinane; DEAD, diethylazodicarboxylate; DIAD, diisopropyl azodicarboxylate; ADDP, 1,1'-(azodicarbonyl)dipiperidine.

\* Corresponding authors.

E-mail addresses: [Mary\\_Lynn\\_Trawick@baylor.edu](mailto:Mary_Lynn_Trawick@baylor.edu) (M.L. Trawick), [Kevin\\_Pinney@baylor.edu](mailto:Kevin_Pinney@baylor.edu) (K.G. Pinney).

<http://dx.doi.org/10.1016/j.bmcl.2016.11.093>

0960-894X/© 2016 Published by Elsevier Ltd.



**Fig. 1.** Colchicine, phenstatin, and combretastatin natural products and their corresponding phosphate salts.

chemotherapy and potentially enhanced tumor damage from radiotherapy to an otherwise difficult therapeutic target due to hypoxia.<sup>4,11,12</sup> VDAs impact tumor vasculature from a mechanistic approach that is distinct from AIAs. One subset of VDAs is comprised of small-molecule inhibitors of tubulin polymerization that target existing tumor-associated vasculature by causing rapid morphology changes (flat to round) of the endothelial cells lining these vessels.<sup>1–10</sup> This leads to irreversible vessel damage and tumor necrosis.<sup>1–10</sup>

A representative clinically relevant small-molecule VDA that disrupts microtubule formation is the natural product combretastatin A-4 (CA4) (Fig. 1). First isolated from the African bush willow tree *Combretum caffrum* Kuntze by the Pettit group, CA4 is a potent inhibitor of tubulin polymerization, functioning through a binding interaction at the colchicine site on tubulin.<sup>13–17</sup> Its corresponding water-soluble phosphate prodrug salt [combretastatin A-4P (CA4P)] has reached advanced clinical trials as a promising VDA.<sup>18–22</sup> However, no small-molecule VDA has yet been approved by the FDA.

Phenstatin, originally synthesized by the Pettit group in 1998, is another potent inhibitor of tubulin polymerization with pronounced anti-cancer activity against human cancer cell lines (Fig. 1).<sup>23,24</sup> Phenstatin was discovered serendipitously by the Pettit group during an attempt to prepare a CA4 analog bearing an epoxide moiety as a replacement for the ethylene bridge.<sup>23</sup> The ketone functionality of phenstatin was the surprising result of a Jacobsen epoxidation reaction intended to form an epoxide from the corresponding olefin.<sup>23</sup> Phenstatin mirrors the biological mechanism of action of CA4, disrupting microtubule assembly through a binding interaction with the colchicine site on tubulin.<sup>24</sup> Phenstatin and its corresponding water-soluble phosphate salt prodrug counterpart demonstrate pronounced cytotoxicity against human cancer cell lines.<sup>24</sup> While hydroxyphenstatin, the diol analog of phenstatin, displays potent inhibition of tubulin polymerization, its diphosphate salt analog is less active against *in vitro* cancer cell lines and as an inhibitor of tubulin polymerization than its phenstatin phosphate counterpart.<sup>24</sup>

Solid tumors represent inherently challenging therapeutic targets due, in part, to the significant differences between vasculature feeding healthy tissue versus tumor-associated vasculature, which is a contributing factor to the profound regions of hypoxia that often characterize tumors.<sup>3,8,25–36</sup> A combination of blind ends, leaky vessel walls, occlusions, and kinked vessels, along with the increased average distance between capillaries, leads to hypoxic regions where many common radiotherapies and chemotherapeutic options are less effective.<sup>8,25–36</sup> The challenges created by hypoxia and large diffusion distances offer a unique opportunity for targeted therapeutic intervention.<sup>8,25</sup> Bioreductively activatable prodrug conjugates (BAPCs) represent a possible hypoxia-activated treatment method.<sup>8,25</sup> BAPCs are designed to be activated by reductase enzymes, such as NADPH cytochrome P450 oxidoreductase (POR), in regions of hypoxia in the tumor microenvironment,

releasing a potent anti-cancer agent in a tumor-specific manner.<sup>8,25</sup>

A series of BAPCs utilizing CA4 as the parent anticancer agent and incorporating *nor*-, *mono*-, and *gem*-dimethyl nitrothiophene triggers was reported in 2006 by Davis and co-workers.<sup>37,38</sup> The CA4-BAPC bearing the *gem*-dimethyl nitrothiophene trigger proved to be the most active of the trio, demonstrating the greatest resistance to cleavage under normoxic conditions *in vitro*, in effect displaying a high selectivity for low-oxygen tumor environments.<sup>37</sup> While the *nor*-methyl nitrothiophene BAPC was only activated at very low oxygen concentrations (<0.01% O<sub>2</sub>), the bioreductive triggers for *mono*-methyl and *gem*-dimethyl BAPCs were cleaved over a greater range of oxygen concentrations.<sup>37</sup> The *gem*-dimethyl CA4-BAPC was significantly more effective in hypoxic environments *in vitro* compared to the *nor*- and *mono*-methyl CA4-BAPCs, releasing approximately 50% CA4 at 0.5% O<sub>2</sub> with the aid of POR.<sup>37</sup>

The mechanism of cleavage (under hypoxia) for the nitrothiophene trigger begins with a one electron reductase such as POR reducing the nitro group on the trigger (Fig. 2).<sup>37</sup> Once the nitro group on the trigger has been reduced, an electron cascade through the thiophene ring leads to trigger detachment, releasing the active VDA (CA4 in this example).<sup>37</sup> In comparison, under normal oxygen tension, the species obtained after the initial one-electron reduction is simply re-oxidized (by molecular oxygen) and thus does not lead to cleavage.<sup>37</sup>

Although no BAPC has yet been approved by the FDA, two BAPCs that have reached advanced clinical trials are TH-302 and PR-104. TH-302 (from Threshold Pharmaceuticals) is a 2-nitroimidazole based BAPC attached to the DNA alkylating agent bromoisophosphoramidate (Fig. 3).<sup>39,40</sup> The prodrug is activated by one electron reductases such as POR, reducing the nitro group on the nitroimidazole trigger in a similar mechanistic pathway to the nitrothienyl trigger cleavage.<sup>8</sup> Highly effective in *in vitro* studies and early *in vivo* studies in mice with hypoxia cytotoxicity ratios (HCR) as high as 600, TH-302 has advanced to Phase III clinical trials after successful Phase I and II studies, although the results of the first Phase III trial were not statistically significant.<sup>41–43</sup> PR-104 (Fig. 3), synthesized and biologically evaluated by Wilson et al. at the University of Auckland, is a nitroaromatic prodrug of PR-104A, eventually being reduced to the active forms PR104H and PR-104M.<sup>8,44–46</sup> The cytotoxicity of PR-104M derives from its ability to form interstrand DNA crosslinks.<sup>8</sup> PR-104 was taken into Phase I and II clinical trials, yielding promising results in Phase I trials but stalled at Phase II due to dose-limiting toxicity and overall efficacy issues.<sup>8,47,48</sup>

Intrigued by the concept of targeting tumor hypoxia with BAPCs, a series of such prodrugs were prepared by chemical synthesis based on the unique tubulin-active anticancer agent, phenstatin. Utilizing a combination of synthetic pathways previously described in the literature along with our modifications designed to improve yield and reaction efficiency, a selected subset of *nor*-, *mono*-, and *gem*-dimethyl nitrothiophene, nitrobenzyl,

Download English Version:

<https://daneshyari.com/en/article/5155784>

Download Persian Version:

<https://daneshyari.com/article/5155784>

[Daneshyari.com](https://daneshyari.com)