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Short communication

1,2,3,4-Tetrahydro-2-thioxopyrimidine analogs of combretastatin-A4

Lauren Lee [a], Ryan Davis [a], Jenna Vanderham [a], Patrice Hills [b], Hilary Mackay [a], Toni Brown [a], Susan L. Mooberry [b], Moses Lee [a,*]

[a] *Department of Chemistry and the Division of Natural and Applied Sciences, Hope College, 35E, 12th street, Holland, MI 49423, United States*
[b] *Department of Physiology and Medicine, Southwest Foundation for Biomedical Research, San Antonio, TX 78227, United States*

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Abstract

Eleven 1,2,3,4-tetrahydro-2-thioxopyrimidine analogs of combretastatin-A4 (CA-4) were synthesized and their cytotoxicity against the growth of two murine cancer cell lines (B16 melanoma and L1210 leukemia) in culture was determined using an MTT assay. Two 2-thioxopyrimidine analogs **8f** and **9a** exhibited significant activity ($IC_{50} < 1$ μM for L1210 and <10 μM for B16 cells). Exposure of A-10 cells to **8f** and **9a** produced a significant reduction in cellular microtubules in interphase cells, with an EC_{50} value of 4.4 and 2.9 μM, respectively, for microtubule loss. Molecular modeling studies using MacSpartan indicated that the two active 2-thioxopyrimidine analogs preferably adopt a twisted conformation, similar to CA-4, affirming that conformation and structure are connected to activity.

Keywords: Tubulin; Combretastatin; Chalcone; Thiourea; Cytotoxicity

Vascular targeting agents (VTAs), such as combretastatin-A4 (CA-4, **1**, Fig. 1), are effective antitumor agents. VTAs rapidly and specifically disrupt the abnormal tumor vasculature, resulting in vascular collapse and tumor necrosis [1]. There are data to suggest that the antivascular actions might be mediated through the vascular endothelial-cadherin signaling pathway [1b]. CA-4 (**1**) is a natural product extracted from the African Willow Tree, *Combretum caffrum* and it inhibits tubulin polymerization by interacting with the colchicine binding site on tubulin [2]. This alters the morphology of endothelial cells and causes vascular shutdown and regression of tumor vasculature. However, the use of CA-4 (**1**) as a clinical antitumor agent is limited by its low bioavailability and poor aqueous solubility [3]. These drawbacks have led to the development of water-soluble derivatives and analogs as depicted in Fig. 1, which include a phosphate-containing pro-drug (CA-4P, **2**), an amino analog **3** [5a] and an amino acid derivative (AVE-8062, **4**) [4]. These structural analogs have proven to be effective VTAs and they have antitumor actions alone and in combination with current cancer treatments such as cytotoxic chemotherapy, radiation, radio-immunotherapy, and anti-angiogenic agents [1].

Further examples of cytotoxic analogs of CA-4, **1**, that have been reported include furanones [5b,6a], isoxazoles [6b], imidazoles [6c], triazoles [6d], azetidinones [6e], pyrazoles (e.g., **5**) [6f], pyrazolines (e.g., **6**) [7], and cyclohexenones (e.g., **7**) [8]. The latter three heterocyclic derivatives of CA-4 (**1**) were synthesized in the author's laboratory and their structures are given in Fig. 1. The compounds showed varying degrees of cytotoxic potency, but the pyrazole-compound **5** showed a significant loss in potency against murine cancer cells growing in culture. The X-ray crystallography structure of a close analog of pyrazole **5** (in which the −OH group is replaced with an $-OCH_3$ group) [6f] revealed that the compound adopted a planar conformation, lacking the twisted geometry of CA-4 needed to bind optimally to tubulin [9]. The planar conformation of 3,5-diarylpyrazoles was also predicted from molecular modeling studies [7,8]. Interestingly, the pyrazoline **6** and cyclohexenone **7** compounds have significant cytotoxicity and they cause a major loss of cellular microtubules

* Corresponding author. Tel.: +1 616 392 7190; fax: +1 616 392 7923.
E-mail address: lee@hope.edu (M. Lee).

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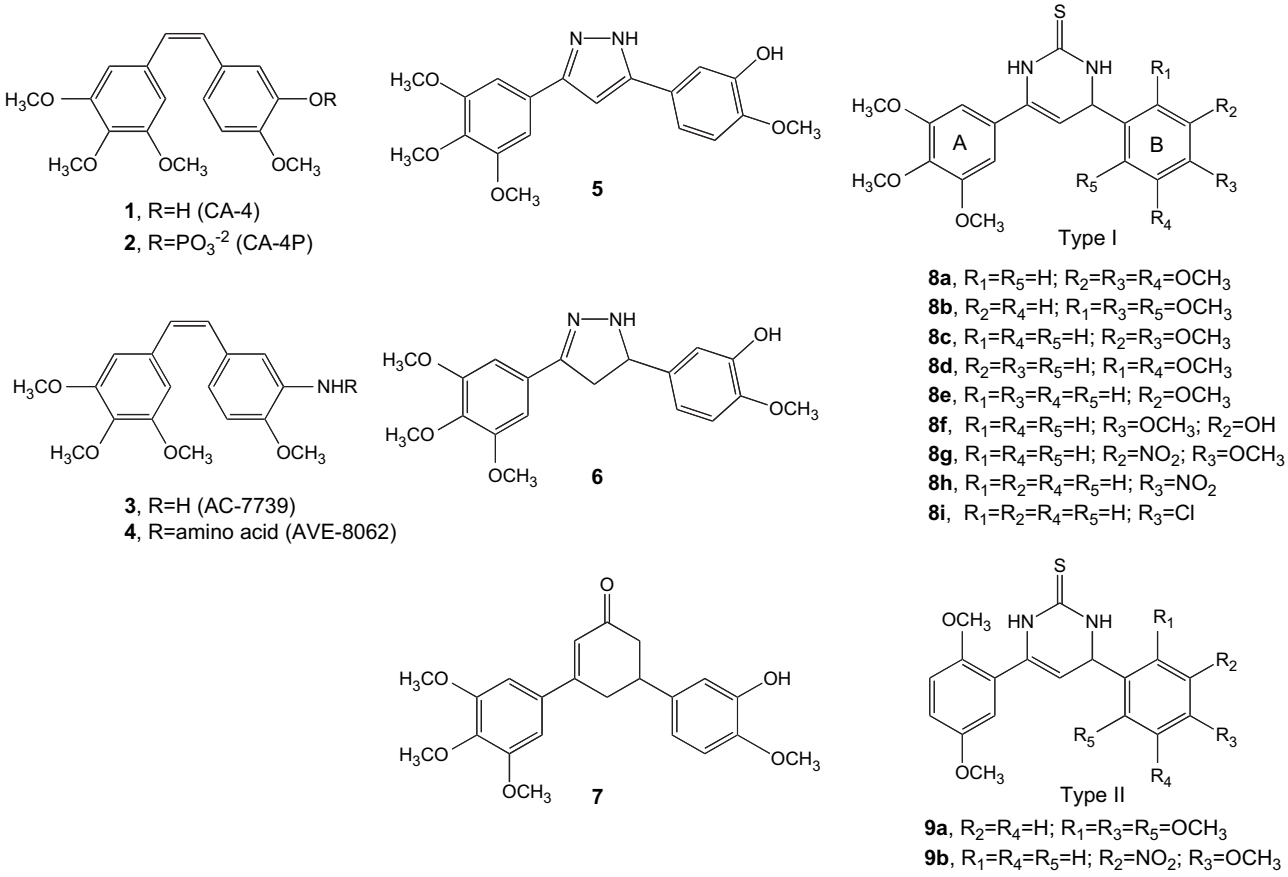

Fig. 1. Structures of combretastatin-A4 (CA-4, **1**) and its water-soluble derivatives **2**–**4** and analogs **5**–**7**, including pyrazole, pyrazoline and cyclohexenone derivatives of chalcones, **5**–**7**, respectively. Two general types of 1,2,3,4-tetrahydro-2-thioxopyrimidine, I for compounds **8a**–**i** and II for **9a**,**b**.

in A-10 cells [7,8]. Molecular modeling studies confirmed that both compounds **6** and **7** preferred twisted geometries [2].

Despite past successes in designing analogs of CA-4, **1**, that are more soluble in biological media, the task of creating molecules that are equally potent as an anticancer agent as CA-4 has been far more challenging. As part of a program to develop novel heterocyclic analogs of CA-4, a series of novel 1,2,3,4-tetrahydro-2-thioxopyrimidine analogs was designed with the purpose of examining their cytotoxic properties and to correlate the results with the conformational ''twist'' of the molecules. An additional objective for synthesizing this class of compounds is to search for analogs of CA-4 that have good water solubility and are biologically active. 1,2,3,4-Tetrahydro-2-thioxopyrimidine analogs are attractive for our studies because only a small handful of 1,2,3,4-tetrahydro-2-thioxopyrimidine have been made and reported [10a], and none has been

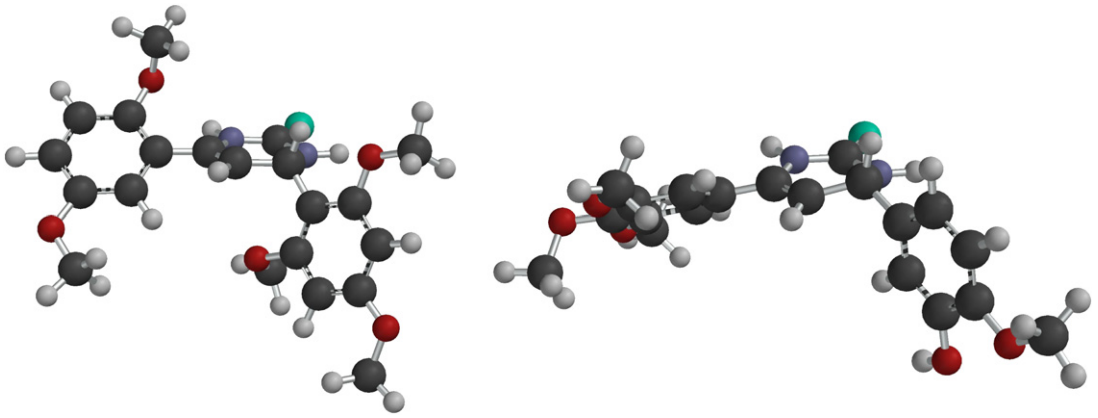

Fig. 2. Energy optimized structures of two 1,2,3,4-tetrahydro-2-thioxopyrmidine analogs: **9a** (left) and **8f** (right), type II and I, respectively.