Bioorganic & Medicinal Chemistry Letters 25 (2015) 1765–1770

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

# AIMing towards improved antitumor efficacy

Matthew J. Weaver [a,†], Alison K. Kearns [a,†], Sascha Stump [a], Chun Li [b], Mariusz P. Gajewski [c], Kevin C. Rider [a], Donald S. Backos [d], Philip R. Reigan [d,*], Howard D. Beall [a,*], Nicholas R. Natale [a,*]

[a] Department of Biomedical and Pharmaceutical Sciences, University of Montana, Missoula, MT 59812, United States
[b] Department of Chemistry, Ithaca College, 953 Danby Road, Ithaca, NY 14850, United States
[c] Department of Chemistry, Arkansas Tech University, Russellville, AR 72801, United States
[d] Skaggs School of Pharmacy and Pharmaceutical Sciences, Anschutz Medical Campus, University of Colorado Denver, Aurora, CO 80045, United States

ARTICLE INFO

*Article history:*
Received 17 December 2014
Revised 20 February 2015
Accepted 23 February 2015
Available online 3 March 2015

Dedicated to the memory of Professor Albert I. Meyers

*Keywords:*
Isoxazole
Anthracene
Pyrrole
DNA Quadruplex
Glioblastoma
Tumor paint

ABSTRACT

Using the structure–activity relationship emerging from previous Letter, and guided by pharmacokinetic properties, new AIMs have been prepared with both improved efficacy against human glioblastoma cells and cell permeability as determined by fluorescent confocal microscopy. We present our first unambiguous evidence for telomeric G4-forming oligonucleotide anisotropy by NMR resulting from direct interaction with AIMs, which is consistent with both our G4 melting studies by CD, and our working hypothesis. Finally, we show that AIMs induce apoptosis in SNB-19 cells.



Gliomas represent 78% of new brain and CNS tumors in the United States each year and have a median survival rate of only 12–15 months due to limited treatment options.[1] There are difficulties in developing efficacious anticancer compounds that have favorable pharmacokinetic properties and cross the blood–brain barrier. In addition, surgical resection of gliomas has a poor success rate as a result of the inability to distinguish between cancerous and healthy tissue. Techniques using fluorescent compounds in the visualization of tumors during surgery, referred to as tumor paint, are actively being developed.[2–4] We have recently reported on the synthesis, bioactivity and structure–activity relationship (SAR) of fluorescent anthracenyl isoxazole amides (AIMs, **1**),[5] as well as their dimeric analogs (**2**)[6] depicted in Chart 1. The AIMs have shown promising activity in the National Cancer Institute's 60-cell line screening protocol (NCI 60) and could be exploited for tumor imaging. There are potential advantages to such agents that could be used both as tumor paint and exhibit antitumor activity.

It is often stated that as many as half of all investigational new drugs fail because of poor pharmacokinetic properties.[7,8] From our previous Letter on the SAR of AIMs, it became apparent that the presence of two dimethylamino propyl groups, or 'double tail', led to increased efficacy in each example studied. We considered the hypothesis that the enhanced activity was attributed to increased bioavailability arising from increased water solubility. We also noted that C(10) groups bearing lone pairs or π-density, chloro or phenyl, respectively, appeared to be superior as well. The combination of these two factors has not been previously studied; for this Letter, we synthesized, characterized, and studied three novel double tail AIMs substituted with bromo-, chloro- and phenyl at the anthracene's 10 position (Chart 1). Antitumor activity, cellular penetration of the AIMs and induction of apoptosis were studied in SNB-19 glioblastoma cells, and structural studies were carried out using NMR, circular dichroism spectroscopy (CD), X-ray crystallography and computational modeling.

Compounds **3a**, **3b** and **3c** (Chart 1) were prepared by the reaction of the previously reported isoxazole esters,[9,10] which were converted to the corresponding acid chlorides.[11] The acyl chlorides were then condensed with the bis-dimethylamino propyl pyrrole

* Corresponding authors. Tel.: +1 303 724 6431; fax: +1 303 724 7266 (P.R.R.); tel.: +1 406 243 5112; fax: +1 406 243 5228 (H.D.B.); tel.: +1 406 243 4132; fax: +1 406 243 5228 (N.R.N.).
*E-mail addresses:* philip.reigan@ucdenver.edu (P.R. Reigan), howard.beall@umontana.edu (H.D. Beall), nicholas.natale@umontana.edu (N.R. Natale).
† Authors (A.K.K. and M.J.W.) contributed equally to this manuscript.

http://dx.doi.org/10.1016/j.bmcl.2015.02.063
0960-894X/

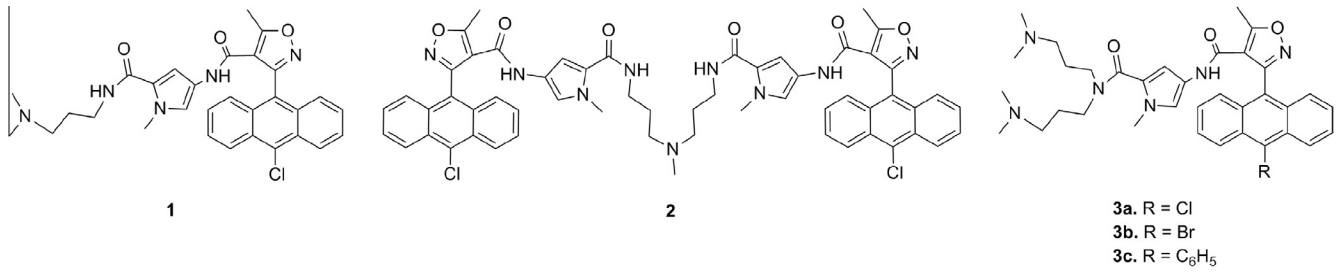


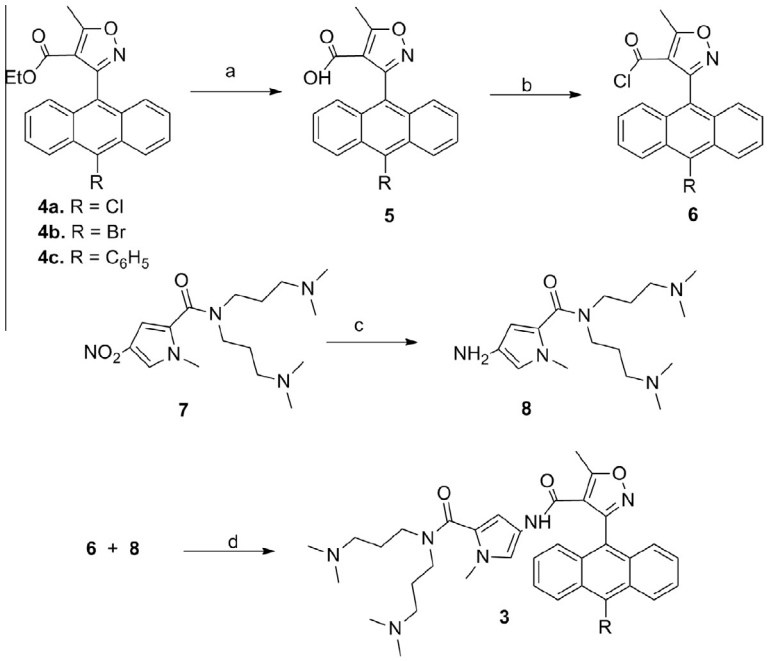

**Chart 1.** Structures of AIMs.

amine[6,11,12] under modified Schotten–Baumann conditions (Scheme 1 and Supplementary data). Purification was accomplished via preparative TLC on silica gel eluting with 10% ammonium hydroxide in methanol. Full characterization of the new AIMs is described in the Supplementary data.

A single crystal X-ray diffractometry (sc-xrd) of **4c** (Fig. 1A) shows the isoxazole of the AIM orients orthogonal to the 3-aryl group, with an observed dihedral angle between the isoxazole and the anthryl mean planes of 88.11°. This stereoelectronic effect was also previously observed in our crystallographic studies of isoxazole-3-anthracenes[9,13,10,14–16] and isoxazole-3-anthroquinones.[17] The isoxazole and ester are largely co-planar with the dihedral angle between the isoxazole mean plane and plane containing the ester carbonyl and ether atom being 10.89°. Two slightly different independent ethyl ester conformations were observed in the structure solution, both with the ethyl group endo to the anthracene; these were taken into account in order to arrive at the final value of $R = 0.040$. Full sc-xrd parameters and atomic coordinates are given in the Supplementary data.

Our working hypothesis is that AIMs exert their antitumor activity by binding a specific cellular target,[6,9] that is, G-quadruplex (G4) DNA.[18,19] The topology of the AIM by sc-xrd is similar to that observed in our computational docking study of the AIMs with human telomeric G4; Figure 1B illustrates AIM **3a** docked with coordinates from PDB accession 1KF1,[20] whereas Figure 1C shows the low energy interaction calculated for the solution conformation of the G4, PDB accession number 2JSM,[21–23] docked with **3c**. We considered a number of potential G4 coordinates and ligand binding modes; however, the lowest energy was calculated for a unique edge-to-face interaction of the AIM isoxazolyl-3-aryl moiety with the G-tetrad in both cases, which is in contrast to our predictions calculated with earlier programs, which suggested face-to-face π-stacking.[6,9]

Patel has noted that the telomeric G4 can adopt a variety of topologies in solution dependent on the conditions (counterion of either $Na^+$ or $K^+$), and the specific reading frame of the single strand telomeric repeat sequence. His group has provided evidence that there are two main physiologically relevant conformations present in $K^+$ solution, and that $(TTAGGG)_4$ predominantly adopts Form 1.[22] Form 1 possesses a (3+1) G-tetrad core, in which three of the G-tracts are positioned in one direction along the primary sequence, in opposition to the fourth, which requires two double-chain reversals. The natural telomeric sequence $d(T_2AG_3)_4$ formed a G4 in solution as verified by comparison with previously published spectra of the same sequence.[22] We studied the addition of compound **3a** to human telomeric G4 DNA which gives rise to select significant changes as evidenced by NMR spectroscopy (Fig. 2). Binding induces an upfield shift of key guanine imino proton signals in the region of 10–12 ppm. Tentative assignments of the imino signals can be made by comparison to the studies of analogous sequences by Patel.[22] The signals corresponding to the imino protons of G(16) and G(24) are up-field of the remaining




**Scheme 1.** Synthesis of AIMs. Reagents and conditions: (a) **4**, THF, MeOH, $KOH_{(aq)}$, (b) **5**, $SOCl_2$, (c) **7**, Pd/C, $H_2$, MeOH, (d) **6**, **8**, $CH_2Cl_2$, TEA.