Bioorganic & Medicinal Chemistry 22 (2014) 1690–1699

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Synthesis and anticancer activity of focused compound libraries from the natural product lead, oroidin

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ARTICLE INFO

Article history:
Received 19 November 2013
Revised 7 January 2014
Accepted 15 January 2014
Available online 24 January 2014

Keywords:
Oroidin
Focused libraries
Cytotoxic compound
Natural product leads
Sponges
Synthesis

ABSTRACT

Oroidin (**1**), (*E*)-*N*-(3-(2-amino-1*H*-imidazol-4-yl)allyl)-4,5-dibromo-1*H*-pyrrole-2-carboxamide, is a pyrrole alkaloid isolated from the marine sponge *Agelas oroides*. Routine screening in a panel of twelve cancer cell lines revealed **1** to be poorly cytotoxic with the 50% growth inhibition concentration (GI_{50}) of 42 μM in MCF-7 (breast) cells and 24 μM in A2780 (ovarian) cells and >50 μM in all other cell lines tested. The development of eight focused libraries comprising thirty compounds total identified *N*-(biphenyl-4-ylmethyl)-1*H*-pyrrole-2-carboxamide (**4l**), *N*-benzyl-4,5-dibromo-1*H*-pyrrole-2-carboxamide (**5a**) and *N*-(biphenyl-4-ylmethyl)-4,5-dibromo-1*H*-pyrrole-2-carboxamide (**5l**) as potent inhibitors of cell growth in our panel of cell lines. Of these compounds GI_{50} values of <5 μM were observed with **4l** against HT29 (colon) and SW480 (colon); **5a** against HT29; and **5l** against HT29, SW480, MCF-7, A431 (skin), Du145 (prostate), BE2-C (neuroblastoma) and MIA (pancreas) cell lines. As a cancer class, colon cancer appears to be more sensitive to the oroidin series of compounds, with analogue **5l** being the most active.

1. Introduction

Natural products and their derivatives account for almost half of all approved drugs with the majority being of terrestrial origin.[1,2] Many marine origin compounds are finding applications as drugs,[3,4] potential drugs,[5] neutraceuticals,[6] molecular tools,[7] cosmeceuticals,[8] agrochemicals,[9] microbiological media (agar),[10] and in the food and pharmaceutical industries.[11] In terms of pharmaceuticals some have shown potential as anticancer agents,[5] antimicrobials,[12] antimalarials[13] and application in other medical conditions.[14–16] Our team has a long history of exploring a range of natural product sources as a means to identify and develop novel lead compounds against a range of human diseases.[17,18] Indeed, we have explored natural products targeting malaria, tuberculosis, epilepsy and cancer. In this present study we re-isolated oroidin (**1**), a natural product first isolated from the sponge *Agelas oroides* in 1971 and later from several other sponges (Fig. 1).[19,20]

Oroidin (**1**) belongs to the 'bromopyrrole' family of alkaloids, characterized by the presence of a brominated pyrrole–imidazole moiety.[22,23] While there are very few reports of the biological activity associated with **1**, it does display modest levels of anti-protozoal activity against *Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi*, *Leishmania donovani* and *Plasmodium falciparum*.[23]

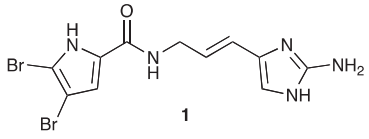

Figure 1. The chemical structure of oroidin (**1**), originally isolated from the marine sponge *Agelas oroides*.[21]

Oroidin has also been used as a lead compound in the development of inhibitors of bacterial biofilm formation.[24] We have held a long term interest in the discovery and development of cytotoxic small molecules ranging from cantharidin analogues,[25–28] cytotoxic nitriles through to mechanism based inhibition brought about by dynamin and clathrin inhibitors.[29–37] Given this interest, we screened **1** against our panel of twelve cancer cell lines for growth inhibition (see Table 1 for details). Oroidin displayed 42 and 24 μM potency against MCF-7 (breast) and A2780 (ovarian) carcinoma cell lines, respectively. While only moderately potent at inhibiting cell growth, oroidin's structural simplicity suggested that rapid elaboration might be possible.

While structurally simple, the reported synthetic routes to oroidin include Pd-mediated coupling with preformed imidazole moieties,[38–42] approaches requiring amino acid precursors[43–47] and olefination strategies.[48–52] More recently Rasapalli et al., reported the use of imidazo[1,2-*a*]pyrimidines as a heterocyclic

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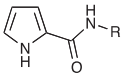
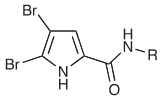
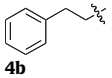
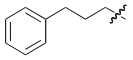
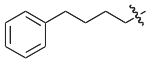
http://dx.doi.org/10.1016/j.bmc.2014.01.021
0968-0896/

Table 1
Percentage (%) growth inhibition in response to 25 μM of compounds in oroidin series from Library 1 and 2 (**4a–d** and **5a–d**)

Compound	HT29[a]	SW480[a]	MCF-7[b]	A2780[c]	H460[d]	A431[e]	Du145[f]	BE2-C[g]	SJ-G2[h]	MIA[i]	SMA[h]	U87[h]
	 **4a-d**						 **5a-d**					
1 Oroidin	31 ± 3	8 ± 1	27 ± 6	51 ± 6	7 ± 4	6 ± 2	23 ± 5	6 ± 5	<0	21 ± 2	15 ± 5	3 ± 3
												
4a	9 ± 7	6 ± 4	3 ± 5	<0	3	<0	5 ± 9	<0	<0	<0	<2	3 ± 5
5a	>100	33 ± 4	>100	84 ± 2	43	56 ± 7	52 ± 3	>100	95 ± 2	33 ± 4	61 ± 2	31 ± 8
												
4b	9 ± 5	9 ± 7	7 ± 7	6 ± 4	5 ± 7	5 ± 2	5 ± 11	<0	2 ± 9	<0	7 ± 3	9 ± 4
5b	46 ± 2	1 ± 5	70 ± 1	58 ± 3	19 ± 10	7 ± 0.4	<0	62 ± 6	22 ± 3	19 ± 6	53 ± 7	<0
												
4c	16 ± 5	7 ± 5	21 ± 2	10 ± 5	13 ± 6	1 ± 4	<0	<0	0 ± 4	10 ± 0.2	8 ± 2	11 ± 1
5c	41 ± 1	<0	63 ± 5	48 ± 4	25 ± 8	5 ± 2	<0	<0	8 ± 3	21 ± 2	45 ± 9	<0
												
4d	16 ± 2	<0	23 ± 4	13 ± 3	11 ± 3	12 ± 2	10 ± 6	<0	8 ± 3	15 ± 5	6 ± 1	<0
5d	41 ± 2	<0	70 ± 1	56 ± 5	17 ± 6	24 ± 3	19 ± 5	<0	14 ± 8	34 ± 4	40 ± 5	<0

[a] Colon.
[b] Breast.
[c] Ovarian.
[d] Lung.
[e] Skin.
[f] Prostate.
[g] Neuroblastoma.
[h] Glioblastoma.
[i] Pancreas.

surrogate for the polyfunctionalised 2-amino-1*H*-imidazole nucleus of oroidin, hymenidin and clathordin.[53] Takale et al. have also recently reported the synthesis and anti-bacterial activity of oroidin analogues.[54] The biosynthesis and synthesis of this natural product containing the pyrrolic ring has been reviewed by Al-Mourabit[55] and Young.[56] To date all methods of oroidin synthesis found within the literature have been either complex, not stereospecific and typically low yielding.

2. Results

As the initial activity of **1** was modest we examined structural modifications; however, current literature approaches were viewed as too complex for focused library development, validation and potential enhancement of the cytotoxicity of oroidin-based analogues, even though the recent synthesis by Rasapalli now allowed for more rapid access into the oroidin scaffold.[53] Adopting a simpler approach we viewed **1** as comprising three modifiable regions (**A**) the pyrrole moiety tail group, (**B**) a central linker, and (**C**) the aminoimidazole head group connected via a central linker containing an olefinic moiety (Fig. 2).

In our initial design approaches we viewed the C=C as only serving to increase synthetic complexity. Thus in the synthesis of our focused libraries, only linker saturated analogues were considered. In this initial report we restricted the choice of pyrrole moiety to the 4,5-dibromo and the parent pyrrole, a Br to H isosteric modification. We envisaged a simple nucleophilic displacement approach to the coupling of a family of amines with either the 2,2,2-trichloro-1-(1*H*-pyrrol-2-yl)ethanone (**2**) or 2,2,2-trichloro-1-(4,5-dibromo-1*H*-pyrrol-2-yl)ethanone (**3**) which are commercially available.[44,45] Judicious choice of amine would allow investigation of Region B and C: the linker and tail group. In the assembly of the focused libraries associated with the chosen Region A moieties, the amines were characterized as: unsubstituted phenyl analogues (Library 1 and 2), substituted phenyl analogues (Library 3 and 4), sterically bulky analogues (Library 5 and 6), and imidazole analogues (Library 7 and 8) where Libraries 1, 3, 5, 7 and 2, 4, 6, 8 correspond to the pyrrole and 4,5-dibromopyrrole head groups respectively (Fig. 3).

Our initial synthetic efforts investigated the displacement of the trichloroethanone moiety by treatment of **2** with benzylamine in DMF with two equivalents of Et_3N at room temperature. This

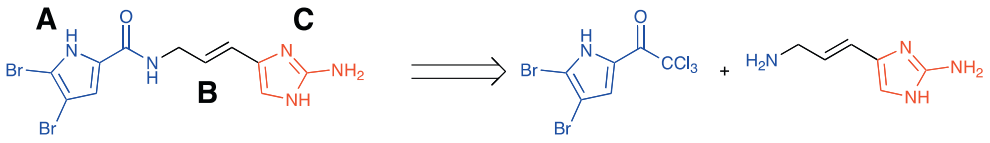


Figure 2. The three modifiable regions and retrosynthetic analysis of oroidin: the oroidin molecule, divided into three sections: **Region A**—the 4,5-dibromo-1*H*-pyrrole tail group; **Region B**—the alkenyl linker; and **Region C**—the head group. Oroidin analogues can be accessed via simple coupling of an amine with the pyrrole trichloromethyl ketone.