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Polybrominated diphenyl ethers from a sponge of the *Dysidea* genus that inhibit Tie2 kinase

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Abstract—Tie2 kinase, an enzyme that supports angiogenesis essential for tumor growth and survival, was selected as a target in a search for naturally occurring inhibitors of potential utility for antitumor therapy. Two polybrominated diphenyl ethers, 3,5-dibromo-2-(2′,4′-dibromophenoxy)phenol (**1**) and 4,6-dibromo-2-(2′,4′-dibromophenoxy)phenol (**2**) were isolated from an extract prepared from *Dysidea* sp. after bioassay-guided fractionation.

1. Introduction

It has been shown that the growth and metastasis of solid tumors is dependent on tumor angiogenesis.[1] For several types of solid tumors, clinical studies have suggested that there is a direct correlation between the density of tumor vessels and an adverse prognosis in patients. These include breast, colon, lung, kidney, bladder, and head and neck cancers.[2] The Tie2 pathway and the VEGF receptor pathway have been shown to be two independent mediators essential for angiogenesis in vivo.[3] Blocking Tie2 kinase activation by i.v. administration of a recombinant, soluble Tie2 receptor was shown to inhibit tumor growth and metastasis.[4] These findings demonstrated a role for the Tie2 pathway in the formation of the tumor vasculature and suggested that targeting proteins in the Tie2 kinase pathway might afford agents useful in anticancer therapy. Several Tie2 inhibitors are in clinical trials.[5]

A crude extract prepared from a sponge of the *Dysidea* genus was found to inhibit Tie2 kinase with an IC_{50} of 6.67 μg/mL. Previous studies of the chemical constituents of sponges in this genus have resulted in the isolation of structurally diverse secondary metabolites including bromophenols,[6] sesquiterpenes,[7] sesterterpenes,[8] sterols,[9] and polychlorinated compounds.[10] Although many biological activities have been reported for these compounds, no Tie2 kinase inhibitory activity has been reported previously for the isolated compounds. Reported herein is the isolation of two

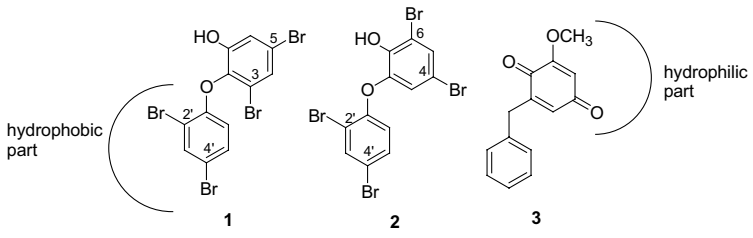

Figure 1. The structures of Tie2 inhibitors.

Keywords: Tie2 kinase inhibitor; Polybrominated diphenyl ethers; Dysidea; Sponge.
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polybrominated diphenyl ethers from the crude extract that inhibit Tie2 kinase (Fig. 1).

2. Results and discussion

The 1:1 CH_2Cl_2–CH_3OH crude extract prepared from *Dysidea* sp. was applied to a diol column. The column was washed successively with hexanes, 1:1 hexanes-CH_2Cl_2, CH_2Cl_2, 1:1 CH_2Cl_2–CH_3OH and CH_3OH. The CH_2Cl_2 fraction exhibited the strongest inhibition of Tie2 kinase. Crystallization from CH_3OH–hexanes afforded active compound **1** as colorless flakes. The 1:1 hexanes–CH_2Cl_2 fraction was fractionated further on a diol column. The column was washed successively with hexanes, 4:1 hexanes–CH_2Cl_2, 3:2 hexanes–CH_2Cl_2 and CH_2Cl_2. The 3:2 hexanes–CH_2Cl_2 fraction afforded active compound **2**.

The negative ESI-MS of **1** and **2** showed the same $[M-1]^-$ peak at *m/z* 501, but their ^1H NMR spectra were clearly different. Careful analysis of chemical shifts and coupling constants revealed that the two compounds had the same proton coupling pattern, but exhibited different chemical shifts. Their ^1H NMR spectra in DMSO-d_6 were same as 3,5-dibromo-2-(2′,4′-dibromophenoxy)phenol (**1**)[6b,11] and 4,6-dibromo-2-(2′,4′-dibromophenoxy)phenol (**2**),[6b,11] respectively (see Section 3). The ^{13}C NMR data (see Section 3) and X-ray diffraction measurement of the single crystal of **1** also supported the structure of **1**; therefore, the structure was unambiguously determined.

The two polybrominated diphenyl ethers were reasonably potent inhibitors of Tie2 kinase (Table 1) exhibiting IC_{50} values of 2.1 and 6.2 μM for **1** and **2**, respectively. Other biological activities for compounds of this type have been noted, including antibacterial and fungicidal activity,[6c] brine shrimp toxicity,[6c] enzyme inhibition of inosine monophosphate dehydrogenase, guanosine monophosphate synthetase, and 15-lipoxygenase,[6b] inhibition of the cell division of fertilized sea urchin eggs,[6e] and cytotoxity.[6d] Compound **1** has also been reported to inhibit contractile activity in the guinea pig ileum.[12]

Tie2 kinase is a receptor tyrosine kinase. Waldmann and co-workers[13] have described Tie2 kinase inhibitor **3**, which has a structure not dissimilar to those of polybrominated diphenyl ethers **1** and **2**. It was suggested that a hydrophobic moiety, which may interact with a hydrophobic pocket close to the binding site, and a hydrophilic moiety, which may participate in hydrogen bonding to the ATP binding site of kinases are necessary for inhibitory activity. It has been suggested[13] that a quinone flanked by a hydrophobic group may be important for binding, but the present results indicate that a brominated phenol system may bind more efficiently to the ATP binding site because the diphenyl ethers **1** and **2** showed better Tie2 kinase inhibitory activity than quinone **3**. The discovery of the Tie2 kinase inhibitory activity of the two polybrominated diphenyl ethers provide good lead structures for further modification to afford improved Tie2 kinase inhibitors.

Table 1. IC_{50} values of compounds **1**–**3** as inhibitors of Tie2 kinase

Compound	IC_{50} (μM)
1	2.1
2	6.2
3	18[a]

[a] Data from Ref. 13a.

3. Experimental

3.1. General experimental procedures

Lichroprep diol (40–63 μm) is a product of EM Industries, Inc. ^1H NMR were measured on General Electric QE 300, GN-300 NMR spectrometers or a Varian Unity INOVA-500 spectrometer. Mass spectra were recorded on a Finnigan MAT 4600 mass spectrometer.

3.2. Bioassay

The bioassay for Tie2 kinase inhibitory activity was carried out by methods similar to those described previously.[14]

3.3. Fractionation and structure identification

The crude extract was prepared from a sponge of *Dysidea* sp. by soaking with 1:1 CH_2Cl_2–CH_3OH. The crude extract (42 mg, IC_{50} 6.67 μg/mL) was applied to a 20 g diol column. The column was washed successively with hexanes, 1:1 hexanes–CH_2Cl_2, CH_2Cl_2, 1:1 CH_2Cl_2–CH_3OH and CH_3OH. The 1:1 hexanes–CH_2Cl_2 fraction (2.8 mg, IC_{50} 10.75 μg/mL), CH_2Cl_2 fraction (30.1 mg, IC_{50} 6.67 μg/mL), and 1:1 CH_2Cl_2–CH_3OH fraction (5.7 mg, IC_{50} 3.14 μg/mL) exhibited strong inhibition of Tie2 kinase. The CH_2Cl_2 fraction (20 mg) was crystallized from CH_3OH–hexanes to afford compound **1** as colorless flakes (5.5 mg, IC_{50} 1.06 μg/mL) and its mother liquor (14.3 mg, IC_{50} 4.66 μg/mL). TLC analysis revealed that the CH_2Cl_2–CH_3OH fraction also consisted mainly of **1**. The 1:1 hexanes–CH_2Cl_2 fraction (2.8 mg) was fractionated further on a 10 g diol column. The column was washed successively with hexanes, 4:1 hexanes–CH_2Cl_2, 3:2 hexanes–CH_2Cl_2, and CH_2Cl_2. The 3:2 hexanes–CH_2Cl_2 fraction afforded compound **2** (0.3 mg, IC_{50} 3.33 μg/mL).

Compound **1:** ^1H NMR (DMSO-d_6, 300 MHz): δ 6.42 (1H, d, J = 8.9 Hz, H-6′), 7.12 (1H, d, J = 2.3 Hz, H-6), 7.36 (1H, dd, J = 8.9, 2.3 Hz, H-5′), 7.37 (1H, d, J = 2.3 Hz, H-4), 7.85 (1H, d, J = 2.3 Hz, H-3′), ^{13}C NMR ($CDCl_3$, 125 MHz): δ 113.4 (C-2′), 116.6 (C-6), 116.8 (C-3), 118.4 (C-4′), 120.3 (C-6′), 120.5 (C-5), 128.5 (C-4), 132.2 (C-5′), 136.9 (C-3′), 139.3 (C-2), 150.9 (C-1), and 152.7 (C-1′); negative ion ESI-MS, *m/z* 1002 $[2M-2]^-$ and 501 $[M-1]^-$. The above spectral data were same as those reported previously for compound **1**.[6b,11]

Compound **2:** ^1H NMR (DMSO-d_6, 300 MHz): δ 6.90 (1H, d, J = 8.8 Hz, H-6′), 6.91 (1H, d, J = 2.3 Hz, H-3),

7.51 (1H, d, $J = 2.3$ Hz, H-5), 7.52 (1H, dd, $J = 8.8$, 2.3 Hz, H-5′), 7.93 (1H, d, $J = 2.3$ Hz, H-3′); negative ion ESI-MS *m*/*z* 1002 $[2M-2]^-$ and 501 $[M-1]^-$. These spectral data were same as those reported previously for compound **2**.[6b,11]

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