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ScienceDirect
Tetrahedron Letters 49 (2008) 4186–4188
Tetrahedron Letters

Asteropterin, an inhibitor of cathepsin B, from the marine sponge *Asteropus simplex*

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Received 17 March 2008; revised 3 April 2008; accepted 7 April 2008
Available online 10 April 2008

Abstract

A new pteridine derivative, asteropterin (**1**), was isolated as a cathepsin B inhibitor from the marine sponge *Asteropus simplex*. The structure of asteropterin (**1**) was elucidated by the analysis of spectral data.
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Keywords: Sponge; Inhibitor; Lumazine; Cathepsin B

Cathepsins, collectively known as a class of lysosomal protein-degrading enzymes, have been shown to possess specific physiological activities including cancer progression.[1] The contribution of cathepsin B in tumor invasion is well documented and the inhibition of its activity resulted in a decreased invasiveness of tumor cells.[2] Therefore, the inhibitors of cathepsin B have a potential as anticancer agents.

In the screening of cathepsin B inhibition of the extract of Japanese marine invertebrates, we found that the extract of the marine sponge *Asteropus simplex* collected off Shikine Island exhibited a potent activity. Bioassay-guided fractionation afforded a new pteridine derivative named asteropterin (**1**). In this Letter, we describe the isolation and structure elucidation of this compound.

The sponge (2 kg, wet weight) was extracted with MeOH, EtOH, and acetone. The combined extracts were concentrated and partitioned between MeOH–H_2O (9:1) and *n*-hexane. The aqueous MeOH fraction was separated by ODS flash chromatography (MeOH–H_2O) and gel filtration. The active fractions were combined and repeatedly purified by reversed-phase HPLC to furnish 5.6 mg of asteropterin (**1**) as a yellowish powder.

Asteropterin (**1**) had a molecular formula of $C_{12}H_{13}N_7O_2$ as determined by HR-ESIMS [*m*/*z* 288.12060 $(M+H)^+$, Δ −0.30 mmu] and NMR data. UV spectrum in MeOH showed absorptions at λ_{max} 279 (ε 1360) and 416 nm (ε 290).

The analysis of the ^1H NMR spectrum of asteropterin (**1**) in conjunction with the HSQC data indicated the presence of two methylenes [δ_H/δ_C 2.92/22.4 (C-3) and 3.82/48.7 (C-2)], an *N*-methyl (3.06/36.0), three hydrogen-bearing sp^2 carbons [8.70/135.8 (C-2′), 8.30/134.0 (C-7″), and 7.35/116.6 (C-4′)], and two NH [δ 11.44 (H-3″) and 11.48 (H-1″)]. Six non-hydrogenated sp^2 carbon signals [δ 122.7 (C-4a″), 131.4 (C-5″), 140.7 (C-8a″), 149.5 (C-2″), 151.0 (C-6″), and 161.6 (C-4″)] were observed in the ^{13}C NMR spectrum.

The COSY spectrum showed that the two methylene signals were mutually coupled and two aromatic protons (H-2′ and H-4′) were long-range coupled. The latter protons were attributed to a 5-substituted imidazole ring on the basis of $^1J_{CH}$ values [216 Hz (C-2′) and 199 Hz (C-4′)] and carbon chemical shift values.[3] An HMBC cross peak

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0040-4039/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2008.04.043

between the N-methyl protons and C-2 showed that C-2 was attached to a methylamino group. HMBC cross peaks from H_2-3 to C-4′ and C-5′ revealed that C-3 was connected to the imidazole ring at C-5′. From these data, partial structure **a** was deduced (Fig. 1a).

The remaining portion had a composition of $C_6H_3N_4O_2$ and contained six sp^2 carbons, only one of which was hydrogen-bearing, and two NH. Deuterium-induced carbon chemical shifts were observed for C-2″, C-4″, and C-8a″ indicating that these three carbons were adjacent to one or two of the NH group(s).[4] HMBC cross peaks were observed from both 1″-NH and 3″-NH to C-4a″. Because C-4a″ did not experience a deuterium-induced shift, these correlations were assigned as three-bond couplings, that is, there must be a carbon atom between C-4a″ and each NH group. Therefore, the remaining carbon that experienced a deuterium exchange shift should be placed between the two hydrogen-bearing nitrogen atoms to form partial structure **b** (Fig. 1b), in which substituents X and Y were both assigned as an oxygen atom considering the carbon chemical shifts, leaving a unit with a composition of C_2HN_2 to link between partial structures **a** and **b**.

$^1J_{CH}$ value of 174 Hz for CH-7″ (δ_H/δ_C 8.30/134.0) and the carbon chemical shift of C-7″ suggested that this carbon was part of a six-membered ring and attached to a nitrogen atom.[3] An HMBC cross peak between H-7″ and C-8a″ confined the position of C-7″ to be within two bonds from C-8a″. HMBC cross peaks, N-CH_3/C-6″ and H_2-2/C-6″, indicated that partial structure **a** was connected to C-6″. An HMBC cross peak between H-7 and C-6″ and ROESY cross peak between N-CH_3 and H-7″ permitted to link C-6″ and C-7″. In order to satisfy these requirements, asteropterin (**1**) should be represented either by a pyridazine or by a pyrazine ring system (partial structures **c** and **d**, respectively) (Fig. 2). The ^{15}N-HMBC spectrum of asteropterin (**1**) displayed a correlation of H-7 to a nitrogen at 300 ppm, which suggested the presence of a pyrazine ring rather than a pyridazine ring (δ_N 335 for pyrazine and δ_N 397 for pyridazine).[5–7] Therefore, asteropterin (**1**) has a lumazine skeleton. The NMR data of lumazine supported this idea[8] (Table 1).

Asteropterin (**1**) inhibited cathepsin B with an IC_{50} value of 1.4 μg/mL. We examined the activity of lumazine (**2**), xanthopterin (**3**), isoxanthopterin (**4**), histamine (**5**), and mixture of lumazine and histamine, all of which were inactive against cathepsin B (Fig. 3). It suggests that the linkage of these lumazine and histamine units makes inhibition.

Only a limited number of pteridines have been reported from sponges.[9–12] Most complex examples among them are pseudoanchynazines from *Clathria* sp. Several pteridine derivatives including lumazine and xanthopterin are known to inhibit xanthine oxidase.[13] Asteropterin (**1**) is a unique

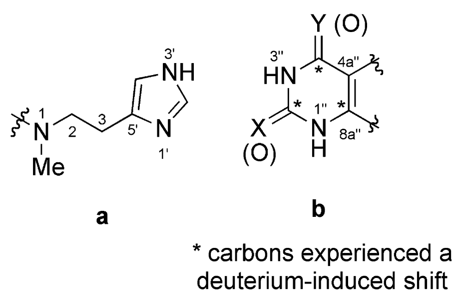

Fig. 1. Partial structures of asteropterin (**1**).

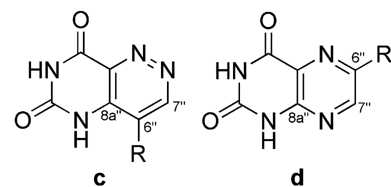

Fig. 2. Partial structures of asteropterin (**1**).

Table 1
^{1}H and ^{13}C NMR spectral data in DMSO-d_6 at 600/150 MHz for asteropterin (**1**)[a]

No.	δ_C	δ_N	δ_H mult	COSY	HMBC
1		70			
2	48.7		3.82 t	3	3,5′,6″,1N-Me
3	22.4		2.97 t	2	1,2,1′,4′,5′
1N-Me	36.0		3.06 s		1,2,6″
1′		182	—		
2′	135.8		8.70 s		1′,3′,4′,5′
3′		173			
4′	116.6		7.35 s		1′,2′,3′,5′
5′	131.4				
1″		120	11.48 s		3″,4a″
2″	149.5				
3″		150	11.44 s		1″,4a″
4″	161.6				
4a″	122.7				
5″		—[b]			
6″	151.0				
7″	134.0		8.30 s		4″,[c]4a″,[c] 6″,8″,8a″
8″		300			
8a″	140.7				

[a] Measured in DMSO-d_6.
[b] Not observed.
[c] Very weak cross peak.

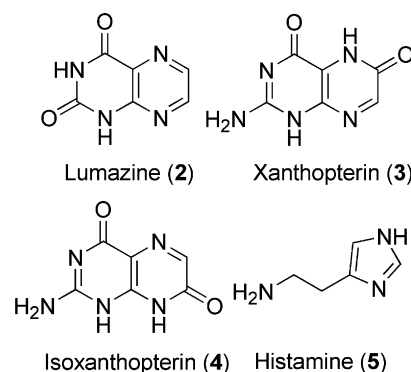

Fig. 3. Related compounds of asteropterin (**1**).

molecule with a combination of lumazine and *N*-methyl histamine with cathepsin B inhibitory activity.

Acknowledgments

We thank Dr. Kazuo Furihata of the University of Tokyo for his assistance in measuring NMR spectra. We also acknowledge Professor Rob W. M. van Soest, Zoological Museum of University of Amsterdam for the identification of the sponge (ZMAPOR 16718). This work was partly supported by Grant-in-Aid for Scientific Research on Priority Areas 16073207 from MEXT.

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