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# Geobarrettin D, a Rare Herbipoline-Containing 6-Bromoindole Alkaloid from *Geodia barretti*

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**Abstract:** Geobarrettin D (**1**), a new bromoindole alkaloid, was isolated from the marine sponge *Geodia barretti* collected from Icelandic waters. Its structure was elucidated by 1D, and 2D NMR (including $^{1}$H-$^{15}$N HSQC, $^{1}$H-$^{15}$N HMBC spectra), as well as HRESIMS data. Geobarrettin D (**1**) is a new 6-bromoindole featuring an unusual purinium herbipoline moiety. Geobarrettin D (**1**) decreased secretion of the pro-inflammatory cytokine IL-12p40 by human monocyte derived dendritic cells, without affecting secretion of the anti-inflammatory cytokine IL-10. Thus, compound **1** shows anti-inflammatory activity.

**Keywords:** 6-bromoindole; herbipoline; *Geodia barretti*; anti-inflammatory activity; dendritic cells



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## 1. Introduction

The indole nucleus is an important element of many natural and synthetic molecules possessing significant biological activities. Indole has been termed a "privileged structure" in drug discovery and a common starting point for drug development or lead optimization [1,2]. Marine indole alkaloids comprise a large and complex class of natural products; most marine-derived indole metabolites are halogenated by bromine [2–4]. The presence of halogen substituents on the indole ring profoundly influences biological activity [2,3]. Interestingly, the Br substituent generally resides at C-5, less commonly at C-6, or at both C-5 and C-6 [4]. Additional modifications of the bromoindole core include C-substitutions by O, C (often prenyl) and *N* groups, pyrimidyl, 2-aminopyrimidyl groups or more complex polycyclic ring systems [5–11]. Bromoindoles have been reported to have anti-inflammatory, antibacterial, antifungal, antitumor, antioxidant, antifouling, and antiplasmodial activities [1,3,4,12,13].

The marine sponge *Geodia barretti* is the source of bromoindole alkaloids [14–18] and several *N*-methylated nucleosides [19]. In our previous study, three new 6-bromoindole derivatives were isolated from *G. barretti* collected in Icelandic waters [20]; of these, geobarrettins B and C exhibited anti-inflammatory activity [20]. As part of our ongoing investigation to find novel anti-inflammatory compounds, we report, here, the bromoindole geobarrettin D (**1**) (Figure 1, as the TFA salt) with potential anti-inflammatory effect measured by decreased pro-inflammatory cytokine secretion of human monocyte-derived dendritic cells (DCs).

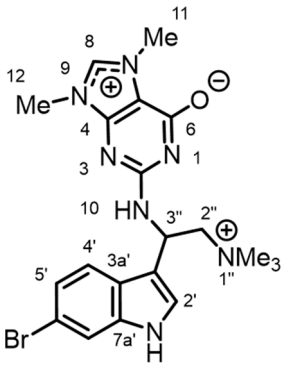


**Figure 1.** Structure of geobarrettin D (**1**).

## 2. Results

The lyophilized sponge *G. barretti* was extracted with $CH_2Cl_2$/MeOH (*v*/*v* 1:1). After removal of solvent, the resulting crude extract was resuspended in MeOH/$H_2O$ (9:1) and solvent-partitioned into five fractions of increasing polarity (hexane, $CHCl_3$, $CH_2Cl_2$, *n*-BuOH, and $H_2O$) using a modified Kupchan method [21,22]. The $CHCl_3$ and $CH_2Cl_2$ fractions were combined and purified by RP C18 HPLC to afford the 6-bromoindole derivative, geobarrettin D (**1**, Figure 1).

### *2.1. Structural Elucidation*

The HRMS of geobarrettin D (**1**) exhibited molecular ion isotopomers *m*/*z* 458.1343/460.1316 ([M]+) in a 1:1 ratio, indicating the presence of one Br and a molecular formula $C_{20}H_{25}{}^{79}BrN_7O$, corresponding to 12 degrees of unsaturation. The $^{13}C$ NMR data (Table 1, see Supplementary Materials) showed 18 signals which were matched to the C content of the molecular formula, including five methyls ($\delta_C$ 54.8 (×3), 36.2, and 32.1), one $sp^3$ methylene ($\delta_C$ 69.3), one aliphatic methine ($\delta_C$ 45.4), five aromatic methines ($\delta_C$ 140.1, 125.9, 124.2, 120.8, and 115.9), four quaternary aromatic carbons ($\delta_C$ 139.1, 125.2, 117.0, and 113.4), four quaternary heteroatom-bonded $sp^2$ carbons ($\delta_C$ 155.0, 154.9, 151.0, and 110.0) (Table 1). The most intense MS peak at *m*/*z* 399.0603/401.0585 ([M-*N*$(CH_3)_3$]$^+$) (Figure S10) was derived from a neutral loss of trimethylamine (–*N*$(CH_3)_3$). IR bands (3254, 1182, and 1131 $cm^{-1}$) implied the presence of OH and/or NH functionalities. The $^1H$ NMR data of **1** in $CD_3OD$ (Table 1) exhibited signals due to five aromatic protons, five *N*-methyl groups, [$\delta_H$ 4.11(3H, s, 11-Me), 3.91(3H, s, 12-Me), and 3.27 (9H, s, 1″-*N*Me)], a methine proton, and a methylene group. Three aromatic signals at $\delta_H$ 7.65 (1H, d, *J* = 8.5 Hz, H-4′), 7.60 (1H, d, *J* = 1.6 Hz, H-7′) and 7.24 (1H, dd, *J* = 8.5, 1.6 Hz, H-5′) indicated the presence of a 1,2,4-trisubstituted benzene ring. The $^1H$ NMR spectrum, recorded in $D_2O/H_2O$ (1:9), showed the presence of a downfield exchangeable proton ($\delta_H$ 10.60), which is diagnostic of an *N*H proton in an indole ring, and confirmed by a weak coupling (*J* = 2.0 Hz) to the isolated aromatic proton at $\delta_H$ 7.53 in the pyrrole ring. Correlations observed in the HMBC spectrum (Figure 2) allowed the definement of the substitution pattern and NMR assignments of the indole: H-4′ to C-3′, C-6′, and C-7a′, from H-5′ to C-3a′ and C-7′, and from H-7′ to C-5′ and C-3a′. H-2′ also showed a correlation to C-3′, C-3a′, and C-7a′. The *N*H exchangeable proton H-1′ showed correlations to C-2′, C-3′, C-3a′, and C-7a′. The indole assignment was supported by the presence of several bands in the UV-vis spectrum ($\lambda_{max}$ 228, 261, and 287 nm).

Br-substitution at C-6′ was deduced by a comparison of the chemical shifts of the aromatic carbons of related 6-bromoindole alkaloids [16,18,23]. Thus, geobarrettin D (**1**) was defined as a 3-substituted 6-bromoindole alkaloid. The $^1H$-$^1H$ COSY correlations from H-3″ ($\delta_H$ 6.05 (1H, t, *J* = 6.3 Hz)) to H-2″ ($\delta_H$ 4.07 (1H, dd, *J* = 13.7, 5.9 Hz); 4.15 (1H, dd, *J* = 13.7, 6.8 Hz)) and HMBC correlations of $(CH_3)_3$-*N* ($\delta_H$ 3.30 (9H, s))/C-2″ ($\delta_C$ 69.3), H-2″/C-3″ ($\delta_C$ 45.4) and H-3″/C-2″ indicated the presence of 2,2-disubstituted *N*,*N*,*N*-trimethylethanaminium group; further support of the connectivity of 6-bromo-indol-

3-yl moiety and the *N*,*N*,*N*-trimethylethanaminium group were provided by additional HMBC correlations: H-3″/C-3′ ($\delta_C$ 113.4), H-3″/C-2′ ($\delta_C$ 125.9), H-3″/C-3a′ ($\delta_C$ 125.2), and H-2″/C-3′ (Figure 2).

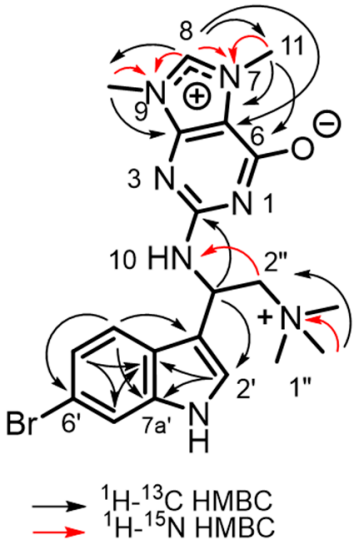


**Figure 2.** Key $^1$H-$^{13}$C HMBC and $^1$H-$^{15}$N HMBC correlations for compound **1**.

The balance of the molecular formula $C_{20}H_{25}{}^{79}BrN_7O$ of geobarrettin D (**1**), $C_7N_5H_8O$, after accounting for the 6-bromoindole and 2,2-disubstituted *N*,*N*,*N*-trimethylethanaminium moieties, required another six degrees of unsaturation. The HMBC cross-peak H-3″/C-2 ($\delta_C$ 154.9) revealed that the $C_7N_5H_8O$ unit was connected to C-3″ through a C-*N* bond, which explains the downfield chemical shift of C-3″ ($\delta_C$ 45.4). Analysis of the $^{13}$C NMR data revealed the seven remaining carbons as non-protonated $sp^2$ carbons with chemical shifts of $\delta_C$ 155.0, 154.9, 151.0, 140.1, 110.0 and two $sp^3$ carbons $\delta_C$ 36.2, 32.1 (Table 1). The $^1$H NMR chemical shift of the non-exchangeable $\delta_H$ 9.01 lacked an expected cross-peak in the HSQC spectrum, but strong symmetric 'satellite peaks' appearing in the HMBC, centered on $\delta_C$ 140.1, were due to $^1J_{CH}$ 'breakthrough' ($^1J_{H8\text{-}C8}$ = 220 Hz) [24,25]: the large magnitude is consistent with a five-membered heterocycle [26]. Long-range correlations were also seen from H-8 [$\delta_H$ 8.81 (1H, s, H-8)] to two *N*-methyl groups ($\delta_C$ 36.2 and 32.1 ppm) in addition to C-4 and C-5 ($\delta_C$ 151.0 and 110.0, respectively). These data are reconciled by an *N*,*N*-dimethyl imidazole ring.

H-detected $^{15}$N-heteronuclear 2D NMR experiments ($^1$H−$^{15}$N HSQC and $^1$H-$^{15}$N HMBC in $D_2O/H_2O$, 1:9) were also recorded. The correlations from H-8 [$\delta_H$ 8.81 (1H, s, H-8)] to *N*-9 ($\delta_N$ 157.3), *N*-7 ($\delta_N$ 156.4), from $CH_3$-12 ($\delta_H$ 3.91 (3H, s)) to *N*-9, from $CH_3$-11 ($\delta_H$ 4.11 (3H, s)) to *N*-7 in $^1$H−$^{15}$N HMBC and $^1$H−$^{13}$C HMBC spectra further supported a *N*,*N*-dimethyl imidazolinium ring. The latter partial structures, together with the last two degrees of unsaturation, were assembled with the remaining quaternary C and three N atoms to complete an *N*-quaternized guanininium nucleobase. This hypothesis was supported by the H-8/C-6 $^4J_{CH}$ correlation and the downfield chemical shift of C-4, and comparisons of $^{13}$C shifts of **1** with published data for similar purine bases, e.g., herbipoline (7,9-dimethyl-2-(*N*-amino)guaninium) [25,27–30] with the same C-2″–*N*-10 bond. The NMR data are in good agreement with other natural alkylpuriniums: 7,9-dimethyl-2-(*N*-methyl)guaninium chloride [25] and *N*,*N*-dimethyl-1,3-dimethylherbipoline [30].

Although **1** is chiral, the weak optical activity ($[\alpha]_{23}{}^D$ +2 (*c* 0.4, MeOH)) suggests a near-racemic mixture.

**Table 1.** $^{1}$H NMR (600 MHz) and $^{13}$C NMR (150 MHz) spectroscopic data for geobarrettin D (**1**).

| No. | $\delta_H$ [a] | $\delta_H$ [b] | $\delta_C$ [a] | $\delta_N$ [b] | $^{1}$H-$^{13}$C HMBC [a] | $^{1}$H-$^{15}$N HMBC [b] |
|---|---|---|---|---|---|---|
| N-1′ | | 10.60 (1H, d, 2.0) | | 131.3 | | |
| 2′ | 7.57 (1H, s) | 7.53 (1H, d, 2.0) | 125.9 | | C-1″, 3′, 5′, 3a′, 7a′ | |
| 3′ | | | 113.4 | | | |
| 3a′ | | | 125.2 | | | |
| 4′ | 7.65 (1H, d, 8.5) | 7.12 (1H, d, 8.5) | 120.8 | | C-3′, 6′, 3a′, 7a′ | |
| 5′ | 7.24 (1H, dd, 8.5, 1.6) | 7.45 (1H, dd, 8.5, 1.6) | 124.2 | | C-7′, 3a′ | |
| 6′ | | | 117.0 | | | |
| 7′ | 7.60 (1H, d, 1.5) | 7.61 (1H, d, 1.5) | 115.9 | | C-6′, 5′, 3a′ | |
| 7a′ | - | - | 139.1 | | | |
| N-1″ | | | | 47.9 [c] | | |
| 2″ | 4.07 (1H, dd, 13.7, 5.9)4.15 (1H, dd, *J* = 13.7, 6.8 Hz) | 4.07 (2H, m) | 69.3 | | C-2″, 3′, -N($CH_3$)$_3$ | N-10 |
| 3″ | 6.05 (1H, t, 6.3) | 6.05 (1H, t, 6.3) | 45.4 | | C-1″, 3′, 3a′, 2′, N-10 | |
| 1″-NMe | 3.30 (9H, s) | 3.27 (9H, s) | 54.8 | | C-2″, 1″ | N-3″ |
| N-1 | | | | | | |
| 2 | | | 154.9 | | | |
| N-3 | | | | | | |
| 4 | | | 151.0 | | | |
| 5 | | | 110.0 | | | |
| 6 | | | 155.0 | | | |
| N-7 | | | | 156.4 [c] | | |
| 8 | 9.01 (1H, s) | 8.81 (1H, s) | 140.1 | | C-11, 4, 5 | N-7, 9 |
| N-9 | | | | 157.3 [c] | | |
| N-10 | | | | 96.3 [c] | | |
| 11 | 4.11 (3H, s) | 3.84 (3H, s) | 36.2 | | C-5, 8 | N-7 |
| 12 | 3.91 (3H, s) | 3.92 (3H, s) | 32.1 | | C-4, 8 | N-9 |

[a] Recorded in $CD_3OD$. [b] Recorded in $D_2O/H_2O$ (1/9). [c] $^{15}$N $\delta$ obtained by indirect detection from $^{1}$H-$^{15}$N HMBC cross peaks.

### 2.2. Anti-Inflammatory Activity

When DCs were matured and activated in the presence of geobarrettin D (**1**), secretion of the pro-inflammatory cytokine IL-12p40 was diminished by 48%, whereas secretion of the anti-inflammatory cytokine IL-10 was not affected (Figure 3). On balance, these results indicate an overall anti-inflammatory effect of geobarrettin D (**1**).

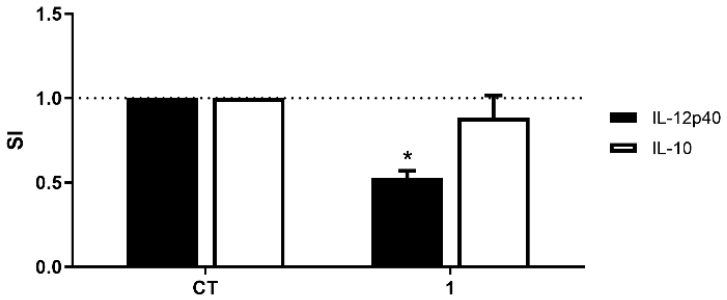


**Figure 3.** The effect of geobarrettin D (**1**) on DC secretion of IL-12p40 and IL-10. DCs were matured and activated with IL-1β, TNF-α and LPS for 24 h in the absence (solvent control (CT)) or presence of geobarrettin D (**1**) at 10 μg/mL. The concentrations of IL-12p40 and IL-10 in the supernatants were determined by ELISA. The data are presented as SI, i.e., the concentration of each cytokine in the supernatant of cells cultured in the presence of compound **1** divided by the concentration of the cytokine in the supernatant of cells cultured without compound **1**. The results are shown as mean ± SEM, $n = 3$. Different from CT: * $p < 0.05$.

## 3. Discussion

Marine sponges have proven to be a rich source of naturally occurring modified nucleosides: these exist as free bases, nucleotides, and within polynucleotides [31,32]. The first natural purinium salt found in nature, herbipoline, was isolated from the sponge *Geodia gigas* [32]. Subsequently, several related herbipoline salts were characterized from

tropical marine sponges: l-methylherbipoline from *Jaspis* sp. [25]], 1-methylherbipoline salts of halisulfate-1 and suvanine from *Coscinoderma mathewsi* [27] and suvanine (*N*,*N*-dimethyl-1,3-dimethylherbipoline salt) from *Coscinoderma* sp. [30]. A literature survey revealed that **1** is the first herbipoline-containing indole [32], and a rare bis-quaternized alkaloid.

Most natural product alkaloids are derived from the aromatic amino acids including tryptophan, tyrosine and phenylalanine. Compound **1** is likely biosynthetically derived from 6-bromotryptamine—an alkaloid known from other marine invertebrates [33]—through a fusion of an oxidized tryptamine intermediate and a guanine equivalent (Figure 4) through a 1,4-conjugate addition, followed by extensive methylation reactions involving *S*-adenosylmethionine (SAM). Oxidation and conjugate addition is necessary and sufficient to explain formation of many C–C and C–heteroatom bonds in alkaloids [34], including **1**. For example, one of us (T.F.M) recently reported two cyclic guanidines, aiolochroiamides A and B, whose formation may also be rationalized by an oxidation–conjugate addition mechanism [35]. It is likely that the oxidation reaction is enzyme-mediated as spontaneous autoxidation seems unlikely, however the subsequent conjugate addition may be spontaneous given the low specific rotation (and therefore enantiomeric excess) observed for **1**. As with other complex highly-methylated quaternized alkaloids, the ordering of *N*-methylation and condensation reactions is uncertain. Further biosynthetic studies are necessary for understanding the biosynthesis of **1**, but these are beyond the scope of this study.

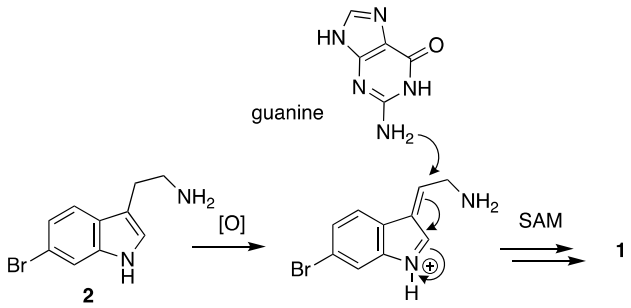


**Figure 4.** Putative biosynthesis of geobarrettin D (**1**).

Purines have found antiviral, antibiotic, and anticancer activities [27,31,36,37], and have the potential to regulate myocardial oxygen supply and cardiac blood flow [38]. In addition, evidence supports their role in biological evolution, differentiation, and ecological processes [31]. Purines are also involved in various inflammatory responses which underscores the significant attention given to purine natural products and their synthetic mimetics for the development of anti-inflammatory agents [20,39,40].

The anti-inflammatory properties of compound **1** were investigated in an in vitro model of human monocyte-derived DCs [20]. DCs matured and activated in the presence of compound **1** secreted less IL-12p40 than DCs cultured without **1**, whereas **1** had no effect on their secretion of IL-10. The pro-inflammatory cytokine IL-12p40 (one of the two chains that form the structures of IL-12 and IL-23 cytokines) is a major determinant of the differentiation of naïve T cells into Th1 or Th17 phenotypes [41], whereas the anti-inflammatory cytokine IL-10 drives polarization of naïve T cells into a T regulatory phenotype [42]. Thus, suppression of IL-12p40 secretion by DCs in the presence of **1** indicates that geobarrettin D (**1**) has anti-inflammatory activity.

## 4. Materials and Methods

### *4.1. General Procedures*

The UV spectrum was recorded on a NanoVueTM spectrophotometer (GE Healthcare Life Sciences, Little Chalfont, UK) with a 0.2 mm path length. Optical rotation was measured on a P-2000 polarimeter (Jasco, Oklahoma City, OK, USA), with a quartz cell (10 mm path length). The infrared spectrum was measured on a Spectrum Two TM FTIR spectrometer (Perkin Elmer®, Waltham, MA, USA) of samples as thin films. NMR spec-

tra were recorded on a Bruker Avance 600 spectrometer (Billerica, MA, USA) ($^1$H and $^{13}$C frequencies: 600.13 MHz and 150.76 MHz, respectively) in $CD_3OD$ and $D_2O/H_2O$ (1:9). The residual solvent signals were used as internal references: $\delta_H$ 3.30/$\delta_C$ 49.0 ppm ($CD_3OD$) and $\delta_H$ 4.79 ($D_2O$). For $^1$H-$^{15}$N 2D NMR spectroscopy, the nominal $^{15}$N standard was liquid ammonia, $NH_3$ (l) ($\delta$ = 0 ppm). Samples (1.6–6.0 mg) were introduced into Shigemi tubes and their 2D NMR spectra measured as illustrated by the following $^1$H{$^{13}$C} heteronuclear HSQC and HMBC spectra using modifications of the Bruker pulse sequences hsqcedetgpsisp2.4 and hmbcgplpndqf, respectively: Spectra were acquired at near ambient temperature (T = 300.0 K) in the specified solvent with an rf pulse calibrated to 1H $\pi/2$ = 8.75 µs, with appropriate gradient field strengths, and dwell times corresponding to $^1$H (F2) and $^{13}$C (F1) spectral widths of 8417.5 Hz and 33112.6 Hz and centered at $\delta_H$ 7.01 and $\delta_C$ 110.4 ppm, respectively. Accumulated scans ($n$ = 8 for HMBC and $n$ = 32 for HSQC) for each T1 increment were averaged between a relaxation delay, D1 =1.5 s. The acquired matrix ($^1$H and $^{13}$C, 1024 × 256 increments for HMBC, and 672 × 256 for HSQC) was zero-filled in each dimension to a final size of 2048 × 1024, and processed, after standard apodizations, by Fourier transform.The high-resolution mass spectrum was measured on an Acquity UPLC I-Class System coupled to Xevo G2-XS QTof Mass Spectrometer (Waters®, Milford, MA, USA) using Acquity UPLC® HSS T3 column (High Strength Silica C18, 1.8 µm, 2.1 × 100 mm, Waters®, (Milford, MA, USA) operating at 60 °C). The MS and $MS^n$ spectra were recorded in positive mode and data were acquired using MassLynx® Software (version 4.1, Waters Crop., Milford, MA, USA). VLC chromatography on $C_{18}$ adsorbent (LiChroprep RP-18, 40–63 µm, Merck Inc., Darmstadt, Germany) and Dionex 3000 HPLC system armed with a G1310A isopump, a G1322A degasser, a G1314A VWD detector (210 nm), a 250 × 21.2 mm Phenomenex Luna C18(2) column (5 µm), and a 250 × 4.6 mm Phenomenex Gemini-NX C18 column (5 µm) were conducted for separation and purification of pure compounds.

### *4.2. Animal Materials*

In short, the sponge material *Geodia barretti* was collected in Iceland, identified by. Hans Tore Rapp, University of Bergen (Norway), and deposited at University of Iceland. For a complete description of the samples, see Xiaxia Di et al. [20].

### *4.3. Extraction and Isolation*

Frozen sponge was cut into approximately 1 $cm^3$ pieces and freeze-dried. The dried tissue was extracted with a mixture of $CH_2Cl_2$:$CH_3OH$ ($v/v$, 1:1) for 3 times (2 L, each for 24 h) at room temperature. The combined $CH_2Cl_2$-MeOH extracts were dried under reduced pressure then the residue (1.8 g) was suspended in MeOH:$H_2O$ ($v/v$, 9:1) and subjected to a modified Kupchan partition, as previously described [21,22], to yield five fractions: hexane (fraction A), chloroform (fraction B), dichloromethane (fraction C), *n*-butanol (fraction D), and $H_2O$ (fraction E). Using a VLC RP-18 CC (MeOH-$H_2O$, 10:90→100:0) technique, the mixture of the fractions B and C was separated into nice fractions (F2.1−F2.9). Fraction F2.2 (75.0 mg) was purified by preparative HPLC (28:72:0.1 $CH_3CN$-$H_2O$-TFA, 8.0 mL/min) and then re-chromatographed by semi-preparative HPLC ($CH_3CN$-$H_2O$-TFA, 31:69:0.1) to give geobarrettin D (**1**) (3.3 mg).

Geobarrettin D (**1**): Light yellowish oil; $[\alpha]_{23}^{D}$ +2 (*c* 0.4, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) nm: 212 (3.90), 228 (4.04), 261 (3.81), 287 (3.65); IR $\nu_{max}$ $cm^{-1}$: 3255, 1679, 1607, 1478, 1444, 1385, 1203, 1131; For $^1$H and $^{13}$C NMR data, see Table 1; HRESIMS $m/z$ 458.1343 $[M]^+$ ($C_{20}H_{25}ON_7Br$, 458.1298).

### *4.4. Maturation and Activation of DCs*

DCs were differentiated from human monocytes as previously described [20]. Peripheral blood mononuclear cells (PBMCs) were isolated from peripheral blood obtained from healthy human donors (approval #06-068-V1 by National Bioethics Committee of Iceland (Visindasidanefnd) from 15$^{th}$ of December 2015) by density centrifugation

using Histopaque-1077 (Sigma-Aldrich, Munich, Germany). Then $CD14^+$ monocytes were isolated from the PBMCs using magnetic cell sorting and CD14 Microbeads (Miltenyi Biotech, Bergisch Gladbach, Germany). The $CD14^+$ monocytes were cultured at $5 \times 10^5$ cells/mL in RPMI 1640 medium, supplemented with 10% fetal calf serum and 5% penicillin/streptomycin (all from Gibco®, Thermo Fisher Scientific, UK) in 48 well tissue culture plates for seven days. In order to differentiate $CD14^+$ monocytes into immature DCs, IL-4 at 12.5 ng/mL and GM-CSF at 25 ng/mL (both from R&D Systems, Bio-Techne, Abingdon, England) were added to the cells. After seven days the monocytes had differentiated into immature DCs which were harvested and cultured for 24 h in 48 well tissue culture plates at $2.5 \times 10^5$ cells/mL. The immature DCs were matured and activated by culturing them with IL-1β at 10 ng/mL, TNF-α at 50 ng/mL (both from R&D Systems), and lipopolysaccharide (LPS) at 500 ng/mL (Sigma-Aldrich, Munich, Germany). Geobarrettin D (**1**) was dissolved in DMSO and added to the DCs at 10 µg/mL at the same time as the cytokines and LPS. DMSO was used as a control. After 24 h the mature and activated DCs were harvested and the concentrations of IL-12p40 and IL-10 in the supernatants were measured by sandwich ELISA using DuoSets from R&D Systems according to the manufacturer's protocol. The results are expressed as a secretion index (SI). Data are presented as the mean values ± SEM, $n = 3$. As the data were not normally distributed, Mann–Whitney U test was used to determine statistical differences between the groups (SigmaStat 3.1, Systat Software, San Jose, CA, USA) and $p < 0.05$ was considered as statistically significant.

## 5. Conclusions

Geobarrettin D (**1**) is a newly detected bromoindole alkaloid possessing an unusual brominated and fused-herbipoline-dimethylguaninium heterocycle. Geobarrettin D (**1**) decreased DC secretion of the pro-inflammatory cytokine IL-12p40, indicating its potential as an anti-inflammatory agent.

**Supplementary Materials:** The following are available online at https://www.mdpi.com/article/10.3390/molecules28072937/s1, Figure S1. 1H NMR spectrum of geobarrettin D (**1**), recorded in CD3OD, 600 MHz; Figure S2. 13C NMR spectrum of geobarrettin D (**1**), recorded in CD3OD, 150 MHz; Figure S3. DEPT-135 NMR spectrum of geobarrettin D (**1**); Figure S4. HSQC spectrum of geobarrettin D (**1**); Figure S5. HMBC spectrum of geobarrettin D (**1**); Figure S6. COSY spectrum of geobarrettin D (**1**); Figure S7. 1H NMR spectrum of geobarrettin D (**1**), recorded in D2O/H2O 10/90, 600 MHz; Figure S8. 1H-15N HSQC spectrum of geobarrettin D (**1**), recorded in D2O/H2O 10/90; Figure S9. 1H-15N HMBC spectrum of geobarrettin D (**1**), recorded in D2O/H2O 10/90; Figure S10. ESI spectrum of geobarrettin D (**1**); Figure S11. IR spectrum of geobarrettin D (**1**).

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**Sample Availability:** Samples of the compound geobarrettin (**1**) are available from the authors.

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