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9,10-Secosteroids, protein kinase inhibitors from the Chinese gorgonian *Astrogorgia* sp.

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ABSTRACT

Fourteen new 9,10-secosteroids designated as astrogorgols A–N (**1**–**14**) were isolated from a Chinese gorgonian *Astrogorgia* sp. together with eight known analogues. The structural patterns were characterized by the presence of a sterol-based 9,10-seco nucleus containing a 3-hydroxy-10-methylphenyl ring. Astrogorgol N (**14**) possessing a 1,4-dien-3-one unit in ring A was biogenetically considered as an intermediate to generate diverse 9,10-secosteroids. Five compounds showed significant inhibitory activities against human tumor related protein kinases, including ALK, AXL, FAK, IGF-1R, MET wt, SRC, and VEGF-R2.

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1. Introduction

Secosteroids are a class of natural products with unusual skeletons that are highly oxidized metabolites with bond cleavage in tetracyclic steroid nucleus.[1] Majority of secosteroids are found in marine organisms, such as sponges, gorgonians, soft corals, and ascidians. Based on the structural patterns, the reported marine secosteroids can be divided into six basic skeletons including 5,6-, 9,11-, 9,10-, 8,9-, 8,14-, and 13,17-secosteroids.[1] Although secosteroids were reported to be derived from 'normal' steroids by microorganism-mediated cleavage,[2] genuine biogenetic mechanism of the modified steroids still remain uncertain. 9,10-Secosteroids are a small group of secosterols (12 compounds have been reported), which were exclusively found from gorgonian genera of *Astrogorgia*,[3] *Calicogorgia*,[4] and *Muricella*[5,6] (family *Acanthogorgiidae*)[7], while astrogorgiadiol was the first 9,10-secosteroid isolated from an unidentified gorgonian *Astrogorgia* sp.[3] All of them contain a 3-hydroxy-10-methylaromatic ring with side-chain variation and backbone oxidation. Part of them possess cytotoxic, brine-shrimp lethal, antivirus, and anti-inflammatory activities, as well as the inhibition against the cell division of the fertilized starfish (*Asterina pectinifera*) eggs.[3–7] Due to the unusual structural patterns, 9,10-secosteroids also attracted the attention of chemists for total synthesis.[8–10] In the course of our investigation of chemical diversity and bioactive marine natural products from Chinese marine invertebrates,[11–15] an unidentified gorgonian specimen *Astrogorgia* sp. was collected off the coast of Beibuwan bay, Guangxi province of China. Chemical examination of this specimen resulted in the isolation of 21 9,10-secosterols and a biogenetic related sterol (**14**). This paper reports the structural elucidation of all new compounds, and the bioassay results of their inhibition against a profile of protein kinases.

2. Results and discussion

The ^{1}H NMR guided fractionation disclosed the EtOAc extract of gorgonian *Astrogorgia* sp. to show the spectral features of sterols, along with aromatic signals. In DAD-HPLC chromatographic spectrum of EtOAc extract, a series peaks with similar UV absorptions at 218 and 280 nm implied a group of analogs structurally closely related to astrogorgiadiol, a 9,10-secosterol bearing an aromatic moiety in ring A.[3–6] Further separation upon semi-preparative HPLC led to the isolation of 22 compounds with similar UV and NMR features.

Compounds **1**–**14** were determined as new 9,10-secosteroids, which were nominated as astrogorgols A–N. Eight known analogs were identical to calicoferols A–C, E, G, I (**15, 17–19, 21–22**),[4–6] 24-exomethylenecalicoferol E (**16**),[7] and astrogorgiadiol (**20**),[3,6] respectively, by the comparison of their NMR data and specific rotation ($[\alpha]_D$) values with those reported in literature.

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2.1. The structures of new 9,10-secosteroids

Astrogorgol A (**1**) was isolated as a colorless oil. HRESIMS (*m/z* 405.2762, $[M+Na]^+$) data assigned its molecular formula as $C_{26}H_{38}O_2$, indicating eight degrees of unsaturation. The IR absorptions suggested the presence of hydroxy (3565 cm^{-1}), ketone (1703 cm^{-1}), and aromatic (1655–1501 cm^{-1}) functionalities. The ^{13}C NMR and APT spectra exhibited a total of 26 carbon signals which were classified into five methyls, six methylenes, ten methines, and five quaternary carbons, involving a ketone and eight sp^2 carbons for an aromatic unit and an vinyl bond. These data were accounted for six sets of unsaturation. Thus, the molecule of **1** must possess a bicyclic nucleus in addition to an aromatic ring. In ^1H NMR spectrum, an aromatic ABX spin system at δ_H 6.98 (1H, d, J = 8.2 Hz, H-1), 6.57 (1H, dd, J = 2.1, 8.2 Hz, H-2) and 6.66 (1H, d, J = 2.1 Hz, H-4) was attributed to a tri-substituted aromatic ring. In addition, a tertiary methyl group at δ_H 0.99 (3H, s, H_3-18) and an aromatic methyl group at δ_H 2.25 (3H, s, H_3-19) were observed. These NMR data (Tables 1 and 3) were closely related to those of co-occurring calicoferol A.[4] The gross structure of **1** was further established by the analysis of 2D NMR (COSY, HMQC, and HMBC) spectra, indicating a 3-hydroxy-10-methylphenyl unit bearing 9,10-secosteroid. The location of a ketone at C-9 (δ_C 213.2) was confirmed by the HMBC relationships from H_2-7 (δ_H 1.58, 1.74) to C-9, C-14 (δ_C 55.3), and C-5 (δ_C 142.6). Obviously, **1** differed from calicoferol A due to the absence of a methylene group at side-chain, and this was evident from an olefinic proton H-23 (δ_H 5.30, dd, J = 6.7, 15.3 Hz) coupled to a methine H-25 (δ_H 2.18) instead of the methylene protons H_2-24 of calicoferol A. The overlapped H_3-26 and H_3-27 (δ_H 0.95, d, J = 6.8 Hz) correlated to C-23 (δ_C 135.5) and C-25 (δ_C 30.9) in HMBC, confirming the linkage of an isopropyl group to C-23. The $J_{H\text{-}22/H\text{-}23}$ value (15.3 Hz) was indicative of 22*E* geometry. The NOE correlations such as H_3-18/H-8 (δ_H 2.36, m), H_3-18/H-20, and H-17 (δ_H 1.23, m)/H-14 (δ_H 1.66, m) conducted to assign the relative configurations of **1** to be the same as those of calicoferol A. Since the absolute configuration of calicoferol A[4] was established by CD spectrum, the stereogenic centers of **1** were thus assumed to be 8*S*, 13*R*, 14*S*, 17*R* and 20*R*, based on the biogenetic consideration and similar NOE data and rotation sign ($[\alpha]_D$).

The close similarity of NMR data indicated astrogorgol B (**2**) to possess a 9,10-secosteroid nucleus, the same as that of **1**. Further analysis of NMR data revealed the side-chain of **2** being consisted of 10 carbons, involving four methyls, two methylenes, three methines, and an olefinic quaternary carbon (δ_C 145.6, C-24). Analysis of COSY and HMBC data allowed to establish the side-chain unit to be identical to that of fucosterol.[16] The presence of an ethylidene group was evident from the COSY correlation between an olefinic proton (δ_H 5.11, q, J = 6.7 Hz, H-28) and the methyl protons at δ_H 1.59 (d, J = 6.7 Hz, H_3-29). The linkage of an isopropyl group to C-24 was based on the HMBC relationships of methyl protons H_3-26 and H_3-27 (δ_H 0.98, d, J = 6.8 Hz, 6H) with C-24 and C-25 (δ_C 28.6). The geometry of $\Delta^{24/28}$ was deduced as *Z* on the basis of more downfield shifted H-25 (δ_H 2.84, dq, J = 7.0, 7.0 Hz) than that of the corresponding proton (δ_H 2.20) of *E*-isomer[16] and the NOE relationship between H-28 and H_2-23 and between H_3-29 and H-25.

Astrogorgol C (**3**) was determined to be a 16-hydroxylated derivative of **1**, based on the interpretation of 2D NMR data and comparison with the NMR data of **1** and calicoferol I (**18**). The similar coupling constants of H-16 (δ_H 4.30, ddd, J = 5.0, 7.0, 7.5 Hz) in **3** compared to the same proton of calicoferol I[5] and the NOE interactions from H-16 to H-14 and H-17 allowed to assign H-16α.

The NMR data of astrogorgol D (**4**) indicated its partial structure in respect to 9,10-secosterol nucleus to be identical to that of **3**, while the NMR data of the side-chain in **4** were in agreement with those of calicoferol A (**15**) and cholesterol. The relative configuration of H-16 was determined to be the same as that of **3** based on the coupling constants of H-16 (δ_H 4.31, ddd, J = 4.4, 6.9, 7.8 Hz, **4**) and the NOE relationships of **4** to be closely similar to those of **3**.

Astrogorgol E (**5**) has a molecular formula of $C_{26}H_{40}O_2$ as provided by its ion peak at *m/z* 407.2931 ($[M+Na]^+$) in HRESIMS. A comparison of NMR data (Tables 1 and 3) revealed that the most signals of **5** were in accordance with those of **1**, except for C-9 which was substituted by a hydroxy group instead of a ketone. This assignment was supported by the presence of an oxymethine at δ_H 3.44 (ddd, J = 5.0, 10.5, 10.5 Hz, H-9), and the corresponding carbon resonated at δ_C 74.3 (CH-9), in association with the COSY relationships of H-9 with H-8 (δ_H 1.48) and H_2-11 (δ_H 1.58, 1.84). The coupling constants

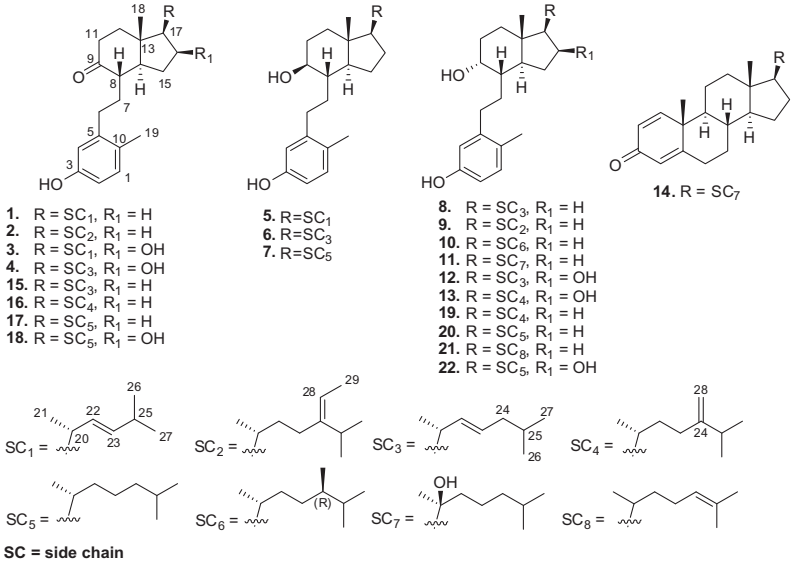


Table 1
^{1}H NMR data of astrogorgols A–G (**1**–**7**) in $CDCl_3$*

	1	2	3	4	5	6	7
1	6.98 d (8.2)	6.98 d (8.0)	6.98 d (8.1)	6.98 d (8.1)	6.98 d (8.1)	6.97 d (8.1)	6.97 d (8.1)
2	6.57 dd (2.1, 8.2)	6.58 dd (2.5, 8.0)	6.58 dd (2.3, 8.1)	6.58 dd (2.6, 8.1)	6.57 dd (2.5, 8.1)	6.56 dd (2.6, 8.1)	6.56 dd (2.4, 8.1)
4	6.66 d (2.1)	6.66 d (2.5)	6.66 d (2.3)	6.66 d (2.6)	6.65 d (2.5)	6.65 d (2.6)	6.66 d (2.4)
6	2.65 ddd (4.5, 12.2, 12.8) 2.40 ddd (5.4, 12.2, 12.8)	2.66 ddd (4.5, 12.5, 12.5) 2.41 ddd (5.0, 12.0, 12.5)	2.66 ddd (4.5, 12.3, 12.5) 2.43 m	2.66 ddd (5.0, 12.5, 12.5) 2.44 ddd (5.6, 12.5, 12.5)	2.58 ddd (4.9, 13.2, 13.2) 2.52 ddd (5.3, 13.2, 13.2)	2.58 ddd (5.1, 13.0, 13.5) 2.52 ddd (5.2, 13.0, 13.5)	2.59 ddd (5.1, 13.5, 13.5) 2.53 ddd (4.9, 13.1, 13.5)
7	1.74 m 1.58 m	1.76 m 1.58 m	1.78 m 1.59 m	1.79 dddd (5.0, 7.4, 12.5, 12.5) 1.61 m	1.72 m 1.64 m	1.73 m 1.64 m	1.74 m 1.64 m
8	2.36 m	2.36 m	2.46 m	2.46 m	1.48 m	1.49 m	1.48 m
9					3.44 ddd (5.0, 10.5, 10.5)	3.44 ddd (5.0, 10.4, 10.4)	3.44 ddd (5.2, 10.3, 10.3)
11	2.50 ddd (6.7, 14.2, 14.2) 2.32 ddd (1.0, 3.5, 14.2)	2.50 ddd (6.7, 14.5, 14.5) 2.31 ddd (2.0, 5.2, 14.5)	2.49 m 2.32 m	2.51 m 2.33 m	1.84 m 1.58 m	1.84 m 1.60 m	1.82 m 1.59 m
12	2.13 m 1.57 m	2.17 ddd (2.0, 6.5, 13.0) 1.58 m	2.12 m 1.54 m	2.12 ddd (1.4, 6.6, 12.9) 1.56 m	1.90 m 1.25 m	1.90 ddd (3.6, 3.6, 12.9) 1.26 m	1.92 m 1.23 m
14	1.66 m	1.68 m	1.49 m	1.50 m	1.27 m	1.27 m	1.25 m
15	1.66 m 1.26 m	1.70 m 1.29 m	2.29 m 1.44 m	2.29 m 1.43 ddd (4.4, 13.0, 13.0)	1.64 m 1.65 m	1.66 m 1.24 m	1.68 m 1.24 m
16	1.80 m 1.41 m	2.00 m 1.44 m	4.30 ddd (5.0, 7.0, 7.5)	4.31 ddd (4.4, 6.9, 7.8)	1.74 m 1.32 m	1.77 m 1.33 m	1.91 m 1.34 m
17	1.23 m	1.23 m	1.16 dd (7.0, 10.0)	1.17 dd (6.9, 11.2)	1.15 m	1.16 m	1.11 m
18	0.99 s	0.99 s	1.19 s	1.19 s	0.77 s	0.78 s	0.76 s
19	2.25 s	2.25 s	2.25 s	2.25 s	2.23 s	2.23 s	2.23 s
20	2.06 m	1.47 m	2.55 m	2.59 m	2.00 m	2.03 m	1.38 m
21	1.01 d (6.8)	0.96 d (6.8)	1.06 d (6.6)	1.08 d (6.6)	0.99 d (6.5)	1.00 d (6.6)	0.91 d (6.5)
22	5.16 dd (8.6, 15.3)	1.49 m 1.12 m	5.41 dd (9.3, 15.5)	5.44 dd (9.1, 15.3)	5.16 dd (8.5, 15.2)	5.20 dd (8.5, 15.1)	1.33 m 0.99 m
23	5.30 dd (6.7, 15.3)	1.99 m	5.59 dd (6.5, 15.5)	5.60 ddd (7.0, 7.0, 15.3)	5.28 dd (6.6, 15.2)	5.29 ddd (7.0, 7.0, 15.1)	1.33 m; 1.14 m
24				1.90 dd (6.8, 6.8)		1.83 m	1.12 m
25	2.18 m	2.84 dq (7.0, 7.0)	2.26 m	1.61 m	2.20 m	1.58 m	1.52 m
26	0.95 d (6.8)	0.98 d (6.8)	0.98 d (6.8)	0.88 d (6.6)	0.95 d (6.6)	0.87 d (6.6)[a]	0.873 d (6.6)[a]
27	0.95 d (6.8)	0.98 d (6.8)	0.98 d (6.8)	0.88 d (6.6)	0.95 d (6.6)	0.86 d (6.6)[a]	0.870 d (6.6)[a]
28		5.11 q (6.7)					
29		1.59 d (6.7)					

* All the ^{1}H NMR spectra were recorded at 500 MHz, except for **4** (at 400 MHz).
[a] Assignments may be interchanged in columns.

$J_{H\text{-}9,H\text{-}11ax}$ (10.5 Hz), $J_{H\text{-}9,H\text{-}8}$ (10.5 Hz), and $J_{H\text{-}9,H\text{-}11eq}$ (5.0 Hz) suggested H-9 to be located in *trans*-axial orientation toward the vicinal protons, while the NOE interactions between H-9/H-14 (δ_H 1.27), H-9/H_2-7 (δ_H 1.64, 1.72), and H-8 (δ_H 1.48)/H_3-18 (δ_H 0.77, s) confirmed H-9 to be α-oriented. A survey of literature led to the assignment of **5** to be an 9-epimer of calicoferol H.[5]

The NMR and HRESIMS data assigned the molecular formula of astrogorgol F (**6**) as $C_{27}H_{42}O_2$. Comparison of NMR data ascertained its structure closely similar to calicoferol A (**15**),[4] The difference was due to C-9, which presented as a hydroxymethine (δ_H 3.44, ddd, J = 5.0, 10.4, 10.4 Hz; δ_C 74.2). The similar J values of H-9 between **6** and **5** enabled to assign the relative configuration of H-9 in **6** to be the same as that of **5**.

HRESIMS data provided the molecular formula of astrogorgol G (**7**) as $C_{27}H_{44}O_2$, which was 2 amu more than that of **6**. Comparison of NMR data resulted in the partial structure of **7** in regard to 9,10-secosterol nucleus to be identical to that of **6**. The side-chain of **7** was determined to be the same as that of calicoferol E (**17**),[6] based on the close similar NMR data in association with 2D NMR analysis.

Analysis of 1D and 2D NMR (COSY, HSQC, HMBC, and NOESY) data revealed the gross structure of astrogorgol H (**8**) to be identical to **6**. The difference was recognized by the signal of H-9 (δ_H 4.04, br s) which presented as a broad singlet to replace a multi-coupling as in the case of **6**, suggesting H-9 of **8** to be in equatorial orientation. This assignment was further supported by the ^{13}C NMR data of C-9 (δ_C 67.3) in **8** shifted to upfield, around 7 ppm more than that **6**. In addition, the presence of NOE relationship between H-9 and H-8 and the absence of H-9/H-14 relationship as presented in **6** also evidenced β-orientation of H-9. Thus, the structure of **8** was determined as an 9-epimer of **6**.

Based on the 2D NMR data analysis and the comparison of NMR data with those of calicoferols as isolated from the same specimen, astrogorgol I (**9**) was determined as a 9α-hydroxylated analog of **2**.

For astrogorgol J (**10**), its partial NMR data in respect to those for 9,10-secosterol nucleus were in good agreement with those of **8** and **9**. The side-chain of **10** was identical to that of 24-methylcholesterol, based on the presence of four methyl doublets, two methylenes, and three methines. The COSY and HMBC relationships further confirmed the side-chain assignment. It was reported that the C-24 epimers of (24*R*)-24-methylcholest-5-en-3β-ol and (24*S*)-24-methylcholest-5-en-3β-ol could be distinguished by the chemical shift difference of C-26 and C-27 ($\Delta\delta_{C\text{-}26\text{-}C\text{-}27}$), for example, 2.8 ppm for 24*S*-isomer and 1.8 ppm for 24*R*-isomer. Meanwhile, C-25 of 24*S*-epimer shifted to upfield approximately 1.0 ppm more than that of 24*R*-epimer.[17] Thus, the $\Delta\delta_{C\text{-}26\text{-}C\text{-}27}$ value (2.0 ppm) of **10** was in accordance with 24*R*.

Astrogorgol K (**11**) was isolated as a minor compound. Its molecular formula was established as $C_{27}H_{44}O_3$ by HRESIMS data

Table 2
^{1}H NMR data of astrogorgols H–J (**8**–**10**) and L–N (**12**–**14**) in $CDCl_3$*

	8	9	10	12	13	14
1	6.98 d (8.1)	6.98 d (8.2)	6.98 d (8.1)	6.99 d (8.0)	6.98 d (8.1)	7.06 d (10.1)
2	6.57 dd (2.6, 8.1)	6.57 dd (2.6, 8.2)	6.57 dd (2.5, 8.1)	6.58 dd (2.0, 8.0)	6.58 dd (2.5, 8.1)	6.23 dd (1.7, 10.1)
4	6.65 d (2.6)	6.65 d (2.6)	6.65 d (2.5)	6.65 d (2.0)	6.65 d (2.5)	6.07 d (1.7)
6	2.70 ddd (5.1, 10.9, 13.5)	2.70 ddd (4.9, 11.6, 12.7)	2.71 ddd (5.1, 11.8, 12.8)	2.69 ddd (5.2, 11.0, 13.5)	2.69 ddd (5.6, 11.2, 12.8)	2.47 ddd (4.8, 13.3, 13.3)
	2.42 ddd (5.4, 9.9, 13.5)	2.42 ddd (4.8, 11.1, 12.7)	2.41 ddd (5.5, 11.1, 12.8)	2.46 ddd (5.9, 10.0, 13.5)	2.44 ddd (5.5, 10.9, 12.8)	2.36 ddd (2.4, 4.1, 13.3)
7	1.52 m	1.53 m	1.54 m	1.56 m	1.54 m	1.95 m; 1.03 m
8	1.52 m	1.52 m	1.50 m	1.61 m	1.59 m	1.64 m
9	4.04 br s	4.04 br s	4.04 br s	4.06 br s	4.06 br s	1.03 m
11	1.74 m	1.73 m	1.71 m	1.79 ddd (3.6, 3.6, 14.0)	1.78 m	1.68 m
	1.57 m			1.69 m	1.69 m	
12	1.74 m	1.77 m	1.76 m	1.70 m	1.76 m	2.12 ddd (3.0, 3.0, 12.6)
	1.50 m	1.49 m	1.47 m	1.48 m	1.45 m	
						1.18 m
14	1.52 m	1.52 m	1.50 m	1.44 m	1.44 m	1.00 m
15	1.56 m	1.59 m	1.59 m	2.18 ddd (7.6, 7.6, 12.8)	2.23 m	1.64 m
	1.08 m	1.11 m	1.09 m	1.23 m	1.20 ddd (4.4, 13.4, 13.4)	1.19 m
16	1.68 m	1.86 m	1.82 m	4.21 ddd (5.1, 7.0, 7.6)	4.36 ddd (4.4, 7.6, 7.6)	1.68 m
	1.22 m	1.26 m	1.23 m			1.25 m
17	1.22 m	1.22 m	1.19 m	1.15 dd (7.0, 11.1)	1.11 dd (7.6, 11.0)	1.44 m
18	0.70 s	0.70 s	0.69 s	0.92 s	0.90 s	0.92 s
19	2.22 s	2.22 s	2.22 s	2.22 s	2.22 s	1.23 s
20	2.03 m	1.40 m	1.36 m	2.53 m	1.87 m	
21	1.01 d (6.7)	0.95 d (6.8)	0.92 d (6.5)	1.07 d (6.6)	1.020 d (6.3)	1.26 s
22	5.20 dd (8.3, 15.2)	1.46 m; 1.12 m	1.30 m; 1.05 m	5.44 dd (9.2, 15.3)	1.67 m; 1.23 m	1.42 m; 1.28 m
23	5.28 ddd (6.8, 6.8, 15.2)	1.99 m	1.20 m	5.56 ddd (7.0, 7.0, 15.3)	2.17 ddd (4.6, 10.8, 14.6)	1.76 m
		1.74 m	1.09 m		1.95 ddd (6.2, 10.4, 14.6)	1.24 m
24	1.82 dd (6.8, 6.8)		1.19 m	1.89 dd (6.8, 6.8)		1.13 m
25	1.58 m	2.83 dq (7.0, 7.0)	1.51 m	1.60 m	2.24 dq (6.8)	1.52 m
26	0.857 d (6.6)	0.97 d (7.0)	0.85 d (6.8)	0.87 d (6.7)	1.029 d (6.8)	0.86 d (6.8)
27	0.854 d (6.6)	0.97 d (7.0)	0.80 d (6.8)	0.87 d (6.7)	1.025 d (6.8)	0.86 d (6.8)
28		5.10 q (6.8)	0.77 d (6.5)		4.75 s, 4.70 s	
29		1.59 d (6.8)				

* All the ^{1}H NMR spectra were recorded at 500 MHz, except for **9** (at 400 MHz).

Table 3
^{13}C NMR data of astrogorgols A–J (**1**–**10**) and L–N (**12**–**14**) in $CDCl_3$*

	1	2	3	4	5	6	7	8	9	10	12	13	14
1	131.0 CH	131.0 CH	131.1 CH	131.1 CH	131.0 CH	131.0 CH	131.0 CH	131.0 CH	131.0 CH	131.0 CH	131.1 CH	131.1 CH	156.0 CH
2	112.5 CH	112.5 CH	112.6 CH	112.6 CH	112.4 CH	112.4 CH	112.4 CH	112.4 CH	112.4 CH	112.4 CH	112.5 CH	112.5 CH	127.5 CH
3	153.7 C	153.7 C	153.6 C	153.7 C	153.8 C[b]	153.7 C	153.7 C	153.7 C	153.7 C	153.6 C[b]	153.7 C	153.8 C	186.5 C
4	115.7 CH	115.7 CH	115.7 CH	115.7 CH	115.6 CH	115.6 CH	115.6 CH	115.5 CH	115.5 CH	115.5 CH	115.5 CH	115.5 CH	123.8 CH
5	142.6 C	142.5 C	142.4 C	142.3 C	142.9 C[b]	143.0 C	143.0 C	142.7 C	142.7 C	142.7 C	142.5 C	142.5 C	169.4 C
6	31.0 CH_2	31.0 CH_2	31.0 CH_2	31.0 CH_2	29.6 CH_2	29.6 CH_2	29.6 CH_2	30.9 CH_2	30.9 CH_2	30.9 CH_2	30.9 CH_2	30.8 CH_2	32.9 CH_2
7	27.6 CH_2	27.6 CH_2	27.7 CH_2	27.7 CH_2	30.2 CH_2	30.2 CH_2	30.2 CH_2	30.3 CH_2[a]	30.3 CH_2	30.3 CH_2[a]	30.3 CH_2	30.2 CH_2	33.6 CH_2
8	50.5 CH	50.4 CH	50.0 CH	50.0 CH	43.4 CH	43.4 CH	43.4 CH	40.9 CH	40.9 CH	40.9 CH	40.4 CH	40.4 CH	34.9 CH
9	213.2 C	213.2 C	212.5 C[b]	212.4 C	74.3 CH	74.2 CH	74.2 CH	67.3 CH	67.2 CH	67.2 CH	67.2 CH	67.1 CH	52.4 CH
10	128.0 C	128.0 C	128.1 C	128.0 C	127.9 C[b]	127.9 C	127.9 C	127.9 C	127.9 C	128.0 C[b]	127.9 C	127.8 C	43.6 C
11	38.4 CH_2[a]	38.3 CH_2	38.0 CH_2	38.0 CH_2	32.3 CH_2	32.3 CH_2	32.3 CH_2	30.2 CH_2[a]	30.1 CH_2	30.22 CH_2[a]	29.9 CH_2	29.9 CH_2	22.73 CH_2
12	38.3 CH_2[a]	38.5 CH_2	38.9 CH_2	38.9 CH_2	37.6 CH_2	37.5 CH_2	37.7 CH_2	34.1 CH_2	34.1 CH_2	34.1 CH_2	34.5 CH_2	34.2 CH_2	39.9 CH_2
13	42.7 C	42.8 C	42.6 C	42.6 C	42.9 C	42.9 C	43.0 C	42.8 C	42.9 C	42.9 C	42.8 C	42.8 C	43.0 C
14	55.3 CH	55.2 CH	52.3 CH	52.3 CH	52.8 CH	52.7 CH	52.7 CH	47.8 CH	47.8 CH	47.8 CH	45.0 CH	45.5 CH	55.6 CH
15	25.1 CH_2	25.1 CH_2	35.7 CH_2	35.7 CH_2	24.2 CH_2	24.3 CH_2	24.3 CH_2	24.5 CH_2	24.4 CH_2	24.4 CH_2	35.08 CH_2	36.5 CH_2	23.9 CH_2
16	29.3 CH_2	29.0 CH_2	73.1 CH	73.2 CH	28.9 CH_2	29.1 CH_2	28.7 CH_2	28.1 CH_2	27.7 CH_2	27.7 CH_2	72.2 CH	71.9 CH	22.3 CH_2
17	54.9 CH	54.9 CH	60.4 CH	60.3 CH	55.6 CH	55.5 CH	55.7 CH	56.0 CH	56.0 CH	56.1 CH	61.1 CH	61.3 CH	57.6 CH
18	11.8 CH_3	11.5 CH_3	12.9 CH_3	12.9 CH_3	12.2 CH_3	12.1 CH_3	12.0 CH_3	11.2 CH_3	11.0 CH_3	11.0 CH_3	12.4 CH_3	12.1 CH_3	13.7 CH_3
19	18.4 CH_3	18.3 CH_3	18.3 CH_3	18.3 CH_3	18.4 CH_3	18.4 CH_3	18.4 CH_3	18.3 CH_3	18.3 CH_3	18.3 CH_3	18.4 CH_3	18.3 CH_3	18.7 CH_3
20	39.8 CH	36.0 CH	35.0 CH	35.0 CH	39.8 CH	40.1 CH	35.7 CH	40.1 CH	36.1 CH	35.9 CH	35.14 CH	29.7 CH	75.1 C
21	20.7 CH_3	18.6 CH_3	21.1 CH_3	21.1 CH_3	20.7 CH_3	20.8 CH_3	18.6 CH_3	20.9 CH_3	18.8 CH_3	18.7 CH_3	21.4 CH_3	18.2 CH_3	26.4 CH_3
22	132.8 CH	35.6 CH_2	133.9 CH	138.2 CH	133.3 CH	137.8 CH	36.1 CH_2	138.0 CH	35.8 CH_2	33.7 CH_2	138.8 CH	34.9 CH_2	44.3 CH_2
23	135.5 CH	27.8 CH_2	137.1 CH	128.6 CH	135.1 CH	126.5 CH	23.8 CH_2	126.4 CH	27.7 CH_2	30.15 CH_2[a]	128.1 CH	31.1 CH_2	22.0 CH_2
24		145.6 C		41.8 CH_2		42.0 CH_2	39.5 CH_2	41.9 CH_2	145.8 C	38.9 CH	41.8 CH_2	157.0 C	39.6 CH_2
25	30.9 CH	28.6 CH	30.8 CH	28.4 CH	30.9 CH	28.5 CH	28.0 CH	28.6 CH	28.6 CH	32.4 CH	28.5 CH	34.0 CH	27.9 CH
26	22.7 CH_3	21.1 CH_3	22.5 CH_3	22.3 CH_3[a]	22.8 CH_3	22.32 CH_3	22.8 CH_3	22.3 CH_3	21.1 CH_3	20.2 CH_3	22.3 CH_3	21.9 CH_3	22.71 CH_3
27	22.7 CH_3	21.0 CH_3	22.5 CH_3	22.2 CH_3[a]	22.8 CH_3	22.26 CH_3	22.6 CH_3	22.3 CH_3	21.0 CH_3	18.2 CH_3	22.2 CH_3	21.8 CH_3	22.6 CH_3
28		116.6 CH							116.4 CH	15.4 CH_3		106.3 CH_2	
29		12.8 CH_3							12.7 CH_3				

* All the ^{13}C NMR spectra were recorded at 125 MHz, except those for **4** and **9** (at 100 MHz).
[a] Assignments within a column could be interchanged.
[b] Data deduced from HMBC spectrum.

based on a pseudomolecular ion peak at *m/z* 439.3181 $[M+Na]^+$. Its ^1H NMR data featured a 9α-hydroxy-9,10-secosterol, closely related to co-occurring astrogorgiadiol. However, the side-chain of **11** presented a singlet H_3-21 (δ_H 1.28) and absent a methine proton H-20 which was observed in astrogorgiadiol. The molecular composition of **11** showed one oxygen atom more than that of astrogorgiadiol, and this conducted to assume C-20 in **11** to be hydroxylated. This assignment was also supported by the EIMS fragments at *m/z* 331 $[M-C_6H_{13}]^+$ and *m/z* 289 $[M-C_8H_{17}O]^+$ for the typical side-chain cleavage.

Astrogorgol L (**12**) has a molecular formula of $C_{27}H_{42}O_3$ as determined by its NMR and HRESIMS data. Its NMR data featured a 9,10-secosteroid, closely resembled those of calicoferol B (**22**),[4] an analog isolated from the same fraction. COSY correlations in association with HMBC relationships guided to assign C-9 and C-16 to be hydroxylated. Like calicoferol B, the relative configuration of OH-9 and OH-16 was determined as 9α,16β-diol on the basis of the NOE relationships between H-9/H-8, H-8/H_3-18, and H-14/H-16. Further examination of NMR data revealed the identical side-chain between **12** and calicoferol A. The $J_{H\text{-}22/H\text{-}23}$ value (15.3 Hz) of olefinic protons H-22 (δ_H 5.44, dd, *J* = 9.2, 15.3 Hz) and H-23 (δ_H 5.56, ddd, *J* = 7.0, 7.0, 15.3 Hz) was indicative of 22*E* geometry.

Analysis of the NMR data of astrogorgol M (**13**) revealed that it differed from calicoferol B only in the side-chain, where the additional signals for an exomethylene [δ_H 4.75 (s, H-28a), 4.70 (s, H-28b); δ_C 106.3 (CH_2, C-28), 157.0 (qC, C-24)] were observed in ^1H and ^{13}C NMR spectra. This group was deduced to be located at C-24 based on the interpretation of the 2D NMR data (HSQC, COSY, HMBC), such as the HMBC interactions from H_2-28 to C-23 (δ_C 31.1), C-24 and C-25 (δ_C 34.0). Accordingly, the structure of **13** was determined as 24-exomethylene-bearing caliocoferol B.

Based on HRESIMS and NMR data, the molecular formula of astrogorgol N (**14**) was established as $C_{27}H_{42}O_2$, indicating seven degrees of unsaturation. The ^{13}C NMR and APT spectra exhibited a total of 27 carbons, involving five methyls, nine methylenes, eight methines, and five quaternary carbons. The NMR data of **14** featured a sterol, such as the tertiary methyl singlets for H_3-18 (δ_H 0.92, s) and H_3-19 (δ_H 1.23, s). The strong IR absorptions at 1662 and 1617 cm^{-1} suggested the presence of a α,β-unsaturated ketone. In addition, an olefinic ABX spin system at δ_H 7.06 (d, *J* = 10.1 Hz, H-1), 6.23 (dd, *J* = 1.7, 10.1 Hz, H-2), and 6.07 (d, *J* = 1.7 Hz, H-4), together with the carbon signals presented at δ_C 156.0 (CH, C-1), 127.5 (CH, C-2), 186.5 (qC, C-3), 123.8 (CH, C-4), and 169.4 (qC, C-5), indicated the presence of a 1,4-diene-3-one system in ring A.[18] Besides, a methyl singlet (δ_H 1.26, H_3-21) from side-chain correlated to C-17 (δ_C 57.6, CH), C-20 (δ_C 75.1) and C-22 (δ_C 44.3, CH_2) in HMBC clarified C-20 to be substituted by a hydroxy group. Further analysis of COSY and HMBC concluded the side-chain to be identical to that of **11**. Thus, the gross structure of **14** was established as 20-hydroxycholesta-1,4-diene-3-one. In regard to the stereogenic center C-20, the chemical shifts of Me-21 (δ_H 1.26, δ_C 26.4), and C-20 (δ_C 75.1), in association with the NOE correlation between H_3-21 and H-12eq (δ_H 2.12) were in accordance with 20*S* configuration.[18–20]

2.2. Inhibitory activity against protein kinases

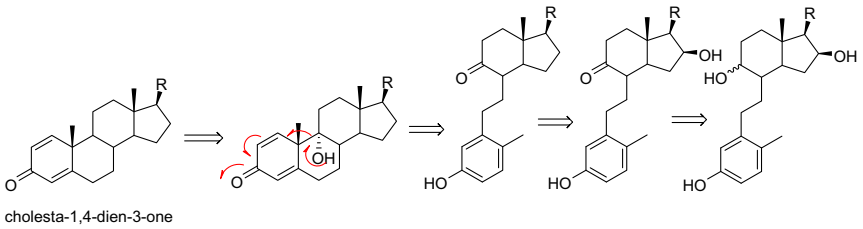
9,10-Secosteroids such as astrogorgiadiol and calicoferols (F–I) were reported to exhibit significant cytotoxicity against human leukemia cell line.[5] In order to investigate the possible anti-tumor mechanisms of 9,10-secosteroids, part of the isolated compounds representing various ring-oxygenated patterns were selected for bioassay in vitro for the inhibitory activities toward 16 different human tumor related protein kinases. The bioassay results informed that 9-oxo-9,10-secosteroids such as calicoferols A (**15**) and E (**17**), and 24-exomethylenecalicoferol E (**16**), 9β-hydroxy-9,10-secosteroid astrogorgol F (**6**) and 9α-hydroxy-9,10-secosteroid astrogorgiadiol (**20**) showed significant inhibition against protein kinases ALK, AXL, FAK, IGF1-R, MET wt, SRC, and VEGF-R2, whereas they exhibited weak inhibition against other protein kinases (Table 4). These findings indicated that 9,10-secosteroids selectively inhibited protein kinases. Meanwhile, 9,16-dioxygenated compounds such as calicoferols I (**18**) and B (**22**) were weakly reacted with all 16 protein kinases, indicating C-16 oxygenation dramatically decreased inhibitory activity. Variation of side-chain patterns slightly affected the inhibitory activity. Thus, we concluded that the inhibitory activity of 9,10-secosteroids against protein kinase mainly relied on 9-oxgyenated 9,10-secosterol nucleus. It is noted that 9,10-secosteroids containing 9-ketone showed stronger inhibition than those with 9-hydroxy group.

Anaplastic lymphoma kinase (ALK) is an orphan receptor tyrosine kinase. ALK inhibitors are targeted therapy against tumors harboring oncogenic fusions or activating mutations.[21] Receptor tyrosine kinase AXL is a key regulator of multiple angiogenic behaviors including endothelial cell migration, proliferation, and tube formation in vitro. Thus inhibiting AXL signaling will simultaneously affect tumor and stromal cell compartments and thus represents a unique approach for cancer therapeutic development.[22] FAK (focal adhesion kinase) and IGF-1R (insulin-like growth factor receptor-1) directly interact with each other and, thereby, activate crucial signaling pathways that benefit cancer cells. Inhibition of FAK and IGF-1R function have shown to significantly decrease cancer cell proliferation and increase sensitivity to chemotherapy and radiation treatment.[23] Kinase VEGF-R2 plays an important role in tumor angiogenesis, and its relevance as pharmacological target for the treatment of a large variety of solid cancers has been extensively described in the literature.[24] The potent inhibitory activity of 9,10-secosteroids toward VEGF-R2 indicated that they may induce the inhibition of tumor angiogenesis. SRC family kinases play a critical role in cell adhesion, invasion, proliferation, survival, and angiogenesis during tumor development. Therefore, these kinases are currently regarded as the very important therapeutic targets for caner.[25–27] Moreover, targeting IGF-1R tyrosine kinase for treatment of related cancers has been extensively studied.[28,29] It was reported that the three kinds of kinases (SRC, VEGF-R2, and IGF-1R) involving in the different signaling pathway are influenced by crosstalk and interaction with each other.[28,30] The potent activities of 9,10-secosteroids toward SRC and IGF-1R suggested that they could possibly be developed as promising kinase inhibitors for anticancer.

Table 4
Inhibitory activity of astrogorgols toward protein kinases*

Compds	IC_{50} (μM)															
	AKT1	ALK	ARK5	Aurora-B	AXL	FAK	IGF1-R	MEK1 wt	MET wt	NEK2	NEK6	PIM1	PLK1	PRK1	SRC	VEGF-R2
6	>100	9.33	>100	38.1	21.9	16.9	3.16	>100	34.0	>100	>100	>100	>100	>100	2.40	4.95
15	>100	4.16	>100	>100	14.7	9.92	2.42	>100	47.6	>100	>100	>100	>100	>100	2.24	4.60
16	>100	4.36	>100	>100	20.2	10.7	2.30	>100	27.5	>100	>100	>100	100	>100	1.48	4.85
17	>100	4.73	>100	>100	32.6	9.63	2.46	>100	71.5	>100	>100	>100	>100	>100	2.17	6.01
20	>100	7.55	>100	25.1	16.9	13.2	2.77	48.9	78.0	>100	>100	>100	>100	>100	1.91	4.35

* IC_{50} values of 18, 19, and 22 (>100 μM).



Scheme 1. Postulated biogenetic conversion to form 9,10-secosteroids.

2.3. Conclusion

Co-existence of 9,10-secosteroids with **14** led to assumption that the 1,4-diene-3-oxo-sterol maybe a precursor to generate 9,10-secosteroids (Scheme 1). It was depicted that oxidation firstly occurred at C-9 to form a 9-OH intermediate, which was followed by C-9/C-10 cleavage and then double bond rearrangement. Since the microbial hydroxylation of steroids was extensively studied, the oxygenation of 9,10-secosteroids at C-9 and C-16 was postulated to be induced by gorgonian endophytic microorganisms.

This paper reported a series new 9,10-secosteroids, which enriched the family members of marine derived secosterols. Apart from astrogorgiadiol as previously reported from gorgonian genus *Astrogorgia*, this is the second paper to introduce a series of 9,10-secosterols from this coral genus. 24-Norcholestanes such as **1**, **3**, and **5** were rarely found from nature. In addition, 9,10-secosterols were only found from gorgonians, thus this structural pattern is assumed to be the chemotaxonomic markers of genus *Astrogorgia*. Although 9,10-secosterols are able to be generate from 'normal' sterols by microorganisms,[4] the wide distribution of 9,10-secosteroids in various gorgonian genera collected from different geographical locations conducted to depict that 9,10-cleavage of sterol was induced by enzymatic reaction rather than microorganism. The significant inhibition of 9,10-secosteroids against a range array of tumor-related protein kinases and the reported cytotoxic activities toward tumor cells implied that 9,10-secosteroids are possible to be developed as protein kinase targeted inhibitors for treatment of cancers.

3. Experimental section

3.1. General experimental procedures

Optical rotations were measured on a Rudolph Autopol III automatic polarimeter. IR spectra were recorded on Thermo Nicolet Nexus 470 FT-IR spectrometer. ^{1}H, ^{13}C, and 2D NMR spectra were measured on Bruker Avance 400 NMR and Bruker Avance 500 NMR spectrometers (400/500 MHz for ^{1}H and 100/125 MHz for ^{13}C, respectively). Chemical shifts are expressed in δ (ppm) referring to the solvent peaks δ_H 7.26 and δ_C 77.0 for $CDCl_3$, and coupling constants in Hertz. High resolution ESIMS and EIMS were obtained from Thermo Scientific LTQ Orbitrap XL and Finnigan TRACE 2000 GC–MS instruments, respectively. Si gel (160–200 and 200–300 mesh, Qingdao Marine Chemistry Co., Ltd), ODS (50 μm, YMC), and Sephadex LH-20 (18–110 μm, Pharmacia Co.) were used for column chromatography. Precoated Si gel plates (Merck, Kieselgel 60 F_{254}, 0.25 mm) were used for TLC analyses. Reversed-phase HPLC chromatography was performed upon an Alltech instrument (426-HPLC pump) equipped with Alltech uvis-200 diode array detector at 210 nm using semipreparative HPLC column (YMC-packed C_8, 5 μm, 250 × 10 mm). Normal-phase HPLC chromatography was performed on a LC-3000 instrument (Beijing ChuangXinTongHeng Science and Technology Co., Ltd) equipped with P3000A Pump, UV3000 UV/Vis detector and a normal-phased column (10 μm, 250 × 10 mm).

3.2. Animal material

The gorgonian *Astrogorgia* sp. was collected from the inner coral reef at a depth of 12 m in Beibuwan Bay, Guangxi province of China, in June 2009. Fresh samples were frozen immediately. The specimen was identified by Leen van Ofwegen (National Museum of Natural History Naturalis). The gorgonian (GWB-32) was deposited at the State Key Laboratory of Natural and Biomimetic Drugs, Peking University, PR China, and also deposited at the National Museum of Natural History Naturalis, The Netherlands.

3.3. Extraction and separation

The frozen gorgonian *Astrogorgia* sp. (wet weight: 1.26 kg) was homogenized and then extracted with MeOH. The concentrated extract was desalted in MeOH to yield a residue (49.7 g). This residue was partitioned between H_2O and EtOAc, while the EtOAc layer was concentrated in vacuum to yield a EtOAc fraction (12.8 g). Part of EtOAc fraction (11.5 g) was subjected to Si gel vacuum liquid chromatography (VLC), eluting with acetone–petroleum ether (PE, 60–90 °C) gradient to obtain 14 subfractions (SF1–SF14). ^{1}H NMR detection indicated secostreols mainly concentrated to SF5–SF7. SF5 (85 mg) was subsequently separated upon semipreparative HPLC (ODS) with a mobile phase (MeOH–H_2O = 89:11) to obtain **15** (22.0 mg), **16** (4.6 mg), **17** (9.5 mg), and **2** (4.8 mg), respectively. Following by the same protocol as for SF5, SF6 (194 mg) was separated upon semipreparative NP-HPLC with a mobile phase of petroleum ether–EtOAc (83:17) to yield **3** (0.5 mg), **12** (1.5 mg), **4** (6.7 mg), **18** (5.8 mg), **21** (0.8 mg), **8** (0.8 mg), **19** (3.1 mg), **6** (5.6 mg), **7** (2.9 mg), **20** (10.7 mg), **9** (2.0 mg), and **10** (1.3 mg). From SF7 (124 mg), **11** (0.5 mg), **13** (3.0 mg), **22** (24.5 mg), **5** (1.3 mg), **1** (3.7 mg), and **14** (1.9 mg) were purified upon semipreparative HPLC (ODS) (MeOH–H_2O = 80:20 as a mobile phase).

3.3.1. Astrogorgol A (1)

Colorless oil; $[\alpha]_D^{24}$ +13.6 (*c* 0.17, MeOH); UV(MeOH) λ_{max} 204, 219, 279 nm; IR (KBr) ν_{max} 3565, 2958, 2871, 1703, 1655, 1618, 1501, 1463, 1369, 1298, 1234, 1193 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3; HRESIMS *m/z* 405.2762 $[M+Na]^+$ (calcd for $C_{26}H_{38}O_2Na$, 405.2770), 787.5637 $[2M+Na]^+$ (calcd for $C_{52}H_{76}O_4Na$, 787.5641).

3.3.2. Astrogorgol B (2)

Colorless oil; $[\alpha]_D^{25}$ +28.2 (*c* 0.21, MeOH); UV(MeOH) λ_{max} 203, 219, 279 nm; IR (KBr) ν_{max} 3305, 2958, 2869, 1695, 1611, 1588, 1503, 1463, 1376, 1298, 1234, 1159, 1100 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3; HRESIMS *m/z* 447.3226 $[M+Na]^+$ (calcd for $C_{29}H_{44}O_2Na$, 447.3239), 871.6567 $[2M+Na]^+$ (calcd for $C_{58}H_{88}O_4Na$, 871.6580).

3.3.3. Astrogorgol C (3)

Colorless oil; $[\alpha]_D^{21}$ +16.0 (*c* 0.02, MeOH); UV(MeOH) λ_{max} 202, 218, 278 nm; IR (KBr) ν_{max} 3420, 2958, 2930, 2866, 1603, 1385,

1124, 1046 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; HRESIMS *m/z* 421.2711 $[M+Na]^+$ (calcd for $C_{26}H_{38}O_3Na$, 421.2719).

3.3.4. Astrogorgol D (4)

Colorless oil; $[\alpha]_D^{24}$ +17.7 (*c* 0.24, MeOH); UV(MeOH) λ_{max} 203, 220, 279 nm; IR (KBr) ν_{max} 3439, 2956, 2870, 1711, 1659, 1622, 1527, 1500, 1433, 1327, 1296, 1184, 1158, 1115 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; HRESIMS *m/z* 435.2866 $[M+Na]^+$ (calcd for $C_{27}H_{40}O_3Na$, 435.2875), 847.5844 $[2M+Na]^+$ (calcd for $C_{54}H_{80}O_6Na$, 847.5853).

3.3.5. Astrogorgol E (5)

Colorless oil; $[\alpha]_D^{23}$ +9.3 (*c* 0.08, MeOH); UV(MeOH) λ_{max} 202, 220, 278 nm; IR (KBr) ν_{max} 3444, 2954, 2928, 2863, 1618, 1500, 1461, 1377, 1290, 1248, 1162, 1119, 1049 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; HRESIMS *m/z* 407.2931 $[M+Na]^+$ (calcd for $C_{26}H_{40}O_2Na$, 407.2926).

3.3.6. Astrogorgol F (6)

Colorless oil; $[\alpha]_D^{24}$ +14.2 (*c* 0.18, MeOH); UV(MeOH) λ_{max} 203, 218, 278 nm; IR (KBr) ν_{max} 3419, 2953, 2931, 2869, 1613, 1589, 1502, 1461, 1380, 1293, 1249, 1159, 1116, 1076 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; HRESIMS *m/z* 421.3081 $[M+Na]^+$ (calcd for $C_{27}H_{42}O_2Na$, 421.3083), 819.6274 $[2M+Na]^+$ (calcd for $C_{54}H_{84}O_4Na$, 819.6267).

3.3.7. Astrogorgol G (7)

Colorless oil; $[\alpha]_D^{23}$ +22.2 (*c* 0.11, MeOH); UV(MeOH) λ_{max} 204, 218, 278 nm; IR (KBr) ν_{max} 3386, 2951, 2929, 2868, 1611, 1589, 1502, 1463, 1380, 1294, 1159, 1118, 1076 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; HRESIMS *m/z* 423.3232 $[M+Na]^+$ (calcd for $C_{27}H_{44}O_2Na$, 423.3239), 823.6574 $[2M+Na]^+$ (calcd for $C_{54}H_{88}O_4Na$, 823.6580).

3.3.8. Astrogorgol H (8)

Colorless oil; $[\alpha]_D^{20}$ −12.8 (*c* 0.04, MeOH); UV(MeOH) λ_{max} 203, 217, 278 nm; IR (KBr) ν_{max} 3413, 2953, 2926, 2868, 1612, 1589, 1501, 1462, 1380, 1293, 1250, 1157, 1080, 1057 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; HRESIMS *m/z* 421.3088 $[M+Na]^+$ (calcd for $C_{27}H_{42}O_2Na$, 421.3083), 819.6288 $[2M+Na]^+$ (calcd for $C_{54}H_{84}O_4Na$, 819.6267).

3.3.9. Astrogorgol I (9)

Colorless oil; $[\alpha]_D^{20}$ −5.6 (*c* 0.5 CHCl3); UV(MeOH) λ_{max} 203, 219, 279 nm; IR (KBr) ν_{max} 3413, 2931, 2869, 1614, 1501, 1462, 1379, 1295, 1240, 1158, 1002 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; HRESIMS *m/z* 449.3388 $[M+Na]^+$ (calcd for $C_{29}H_{46}O_2Na$, 449.3396), 875.6887 $[2M+Na]^+$ (calcd for $C_{58}H_{92}O_4Na$, 875.6893).

3.3.10. Astrogorgol J (10)

Colorless oil; $[\alpha]_D^{22}$ +9.6 (*c* 0.08, MeOH); UV(MeOH) λ_{max} 203, 222, 278 nm; IR (KBr) ν_{max} 3449, 2959, 2931, 2869, 1623, 1500, 1462, 1378, 1262, 1161, 1107, 1030 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; HRESIMS *m/z* 437.3405 $[M+Na]^+$ (calcd for $C_{28}H_{46}O_2Na$, 437.3396), 851.6923 $[2M+Na]^+$ (calcd for $C_{56}H_{92}O_4Na$, 851.6893).

3.3.11. Astrogorgol K (11)

Colorless oil; $[\alpha]_D^{22}$ +12.8 (*c* 0.03, MeOH); UV(MeOH) λ_{max} 201, 218, 278 nm; IR (KBr) ν_{max} 3423, 2930, 2865, 1631, 1462, 1375, 1249, 1147, 1116 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.99 (1H, d, J = 8.1 Hz, H-1), 6.65 (1H, d, J = 2.7 Hz, H-4), 6.57 (1H, dd, J = 2.7, 8.1 Hz, H-2), 4.04 (1H, br s, H-9), 2.70 (1H, m, H-6a), 2.43 (1H, m, H-6b), 2.22 (3H, s, H3–19), 1.88–1.37 (14H, m, H_2-7, H-8, H_2-11, H_2-12, H-14, H-15a, H-16a, H-17, H-22a, H-23a, and H-25), 1.32–1.20 (3H, m, H-16b, H-22b, and H-23b), 1.28 (3H, s, H3-21), 1.18–1.09 (3H, m, H-15b and H_2-24), 0.88 (3H, s, H_3-18), 0.87 (6H, d, J = 6.8 Hz, H_3-26 and H_3-27); EIMS *m/z* (rel. int.%) 416 $[M]^+$ (0.3), 398 $[M-H_2O]^+$ (6.7), 380 $[M-2H_2O]^+$ (3.8), 365 $[M-2H_2O-CH_3]^+$ (3.4), 331 $[M-C_6H_{13}]^+$ (3.0), 313 $[M-C_6H_{13}-H_2O]^+$ (6.8), 295 $[M-C_6H_{13}-2H_2O]^+$ (9.8), 285 $[M-C_6H_{13}-H_2O-CO]^+$ (11.3), 267 $[M-C_6H_{13}-2H_2O-CO]^+$ (21.2), 259 (5.5), 242 (4.0), 173 (11.9), 147 (42.6), 134 (72.0), 121 (100), 111 (25.0), 91 (22.0), 81 (15.7); HRESIMS *m/z* 439.3181 $[M+Na]^+$ (calcd for $C_{27}H_{44}O_3Na$, 439.3188), 855.6471 $[2M+Na]^+$ (calcd for $C_{54}H_{88}O_6Na$, 855.6479).

3.3.12. Astrogorgol L (12)

Colorless oil; $[\alpha]_D^{24}$ −3.2 (*c* 0.09, MeOH); UV(MeOH) λ_{max} 202, 218, 278 nm; IR (KBr) ν_{max} 3439, 2928, 1656, 1532, 1431, 1327, 1284, 1135, 1054 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; HRESIMS *m/z* 437.3022 $[M+Na]^+$ (calcd for $C_{27}H_{42}O_3Na$, 437.3032), 851.6170 $[2M+Na]^+$ (calcd for $C_{54}H_{84}O_6Na$, 851.6166).

3.3.13. Astrogorgol M (13)

Colorless oil; $[\alpha]_D^{24}$ +6.6 (*c* 0.18, MeOH); UV(MeOH) λ_{max} 202, 217, 278 nm; IR (KBr) ν_{max} 3431, 2931, 2870, 1614, 1589, 1501, 1461, 1380, 1294, 1242, 1158, 1100, 1030 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; HRESIMS *m/z* 451.3191 $[M+Na]^+$ (calcd for $C_{28}H_{44}O_3Na$, 451.3188), 879.6495 $[2M+Na]^+$ (calcd for $C_{56}H_{88}O_6Na$, 879.6479).

3.3.14. Astrogorgol N (14)

Colorless oil; $[\alpha]_D^{22}$ +16.9 (*c* 0.11, MeOH); UV(MeOH) λ_{max} 245 nm; IR (KBr) ν_{max} 3417, 2944, 2870, 1662, 1617, 1460, 1376, 1300, 1241, 1177 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; HRESIMS *m/z* 421.3074 $[M+Na]^+$ (calcd for $C_{27}H_{42}O_2Na$, 421.3083), 819.6258 $[2M+Na]^+$ (calcd for $C_{54}H_{84}O_4Na$, 819.6267).

3.4. Assay for protein kinase inhibition[31]

The bioassay for compounds to inhibit protein kinases was performed in 96-well FlashPlates from Perkin–Elmer/NEN (Boston, MA) in a 50 μL reaction volume, which contained 20 μL of assay buffer, 5 μL of test compound (in 10% DMSO), 5 μL of ATP solution (in H_2O), 10 μL of substrate, and 10 μL of purified recombinant protein kinase in the reaction cocktail. It also contained 60 mM HEPES-NaOH, pH 7.5, 3 mM $MgCl_2$, 3 mM $MnCl_2$, 3 mM Na-orthovanadate, 1.2 mM DTT, 50 μg/mL PEG-20000, and 1 μM [γ-33P]-ATP (ca. 5×10^5 cpm/well) in the assay for all enzymes. The substrates used in this study were listed below: AKT1; ALK, ARK5, Aurora-B, AXL, FAK, IGF-1R, MEK1 (wt), MEK (wt), NEK2, NEK6, PIM1, PLK1, PRK1, SRC, and VEGF-R2.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.09.028.

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