SUPPLEMENTARY MATERIAL

One New Indolocarbazole Alkaloid from the *Streptomyces* sp. A22

Xiangwei Cheng [a, b], Biao Zhou [c, *], Huazhang Liu [a], Chao Huo [a, *] and Wanjing Ding [c, *]

[a] *College of Chemical Engineering, Zhejiang University of Technology, Hangzhou 310014, China;* [b] *Zhejiang Police College, Hangzhou, 310053 China;* [c] *Institute of Marine Biology, Ocean College, Zhejiang University, Zhoushan 316021, , China.*

***Corresponding author.** Biao Zhou, E-mail: zhoubiaotd@sian.com Chao Huo, E-mail: chaohc@zjut.edu.cn Wanjing Ding, E-mail: dwj1988555@126.com

Abstract: One new Indolocarbazoles Alkaloids, 12-N-methyl-k252c, together with eight known indolocarbazoles were isolated from the rice solid fermentation of the marine-derived *Streptomyces* sp. A22. Their structures were elucidated by analysis of NMR and MS spectroscopic data. All of these compounds were evaluated for BRD4 inhibitory activities and cytotoxic activity assay, respectively. Compounds **4** and **5** showed moderate cytotoxic activity with an IC_{50} value of 3.52 and 3.93 μM, respectively. Additionally, compound **1** also were tested for enzyme inhibition activities of protein kinases and showed moderate activity with IC_{50} values of 0.91-1.84 μM.

Key words: Indolocarbazoles; 12-N-methyl-k252c; enzyme inhibition activities.

Table S1. BRD4 inhibitory activities and cytotoxic activity assay for Compounds **1**-**9**.

Compounds	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
PC3 ($IC_{50,}$ μM) [a]	>20	>20	0.04	3.52	3.93	11.90	>20	>20	17.12
BRD4(% Inhibition rate [b])	36	2.3	-3	41	37	5	33	60	24

BRD4: Bromodomain-containing protein 4; [a] Staurosporine (**3**) was considered as a positive control; [b] Inhibition rate of BRD4 at 10 μg/mL and JQ-1was a positive control with IC_{50} of 515.6 nM.

Table S2. Enzyme inhibition activities of proteine kinases for Compounds **1** and **3**.

Compounds	IC_{50} (μM)		
	PKC	BTK	ROCK2
1	1.84	1.51	0.96
3	0.04	0.01	0.0009

PKC: Protein kinase C
BTK: Brution tyrosine kinase
ROCK2: Rho-associated protein kinase 2
Staurosporine (**3**) was considered as a positive control.

Table S3. ^{1}H and ^{13}C NMR Data of Compound **1**.

no.	δ_H [a]	δ_C [b]
1	7.80, d (7.8)	111.7, CH
2	7.42, t (7.7)	124.9, CH
3	7.22, t (7.5)	118.7, CH
4	9.26, d (8.0)	125.1, CH
4a		122.1, qC
4b		116.2, qC
4c		118.9, qC
5		172.4, qC
6	8.47, s	
7	4.95, s	45.2, CH_2
7a		132.7, qC
7b		111.1, qC
7c		121.1, qC
8	8.05, d (7.8)	121.5, CH
9	7.34, t (7.5)	120.2, CH
10	7.55, t (7.8)	124.8, CH
11	7.82, d (7.8)	109.8, CH
11a		138.3, qC
12a		128.1, qC
12b		124.6, qC
13	11.54, s	
13a		139.6, qC
12-NH	7.10, q (5.5)	
NH-CH_3	2.95, d (5.5)	37.8, CH_3

[a, b]Recorded at 400, 100 MHz, respectively .[a, b] Recorded in DMSO-d_6,

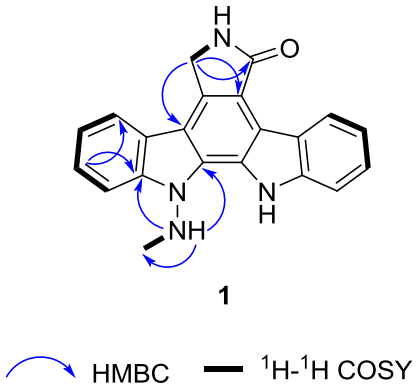

Figure S1. ^{1}H-^{1}H COSY and selected HMBC correlations for compound **1**.

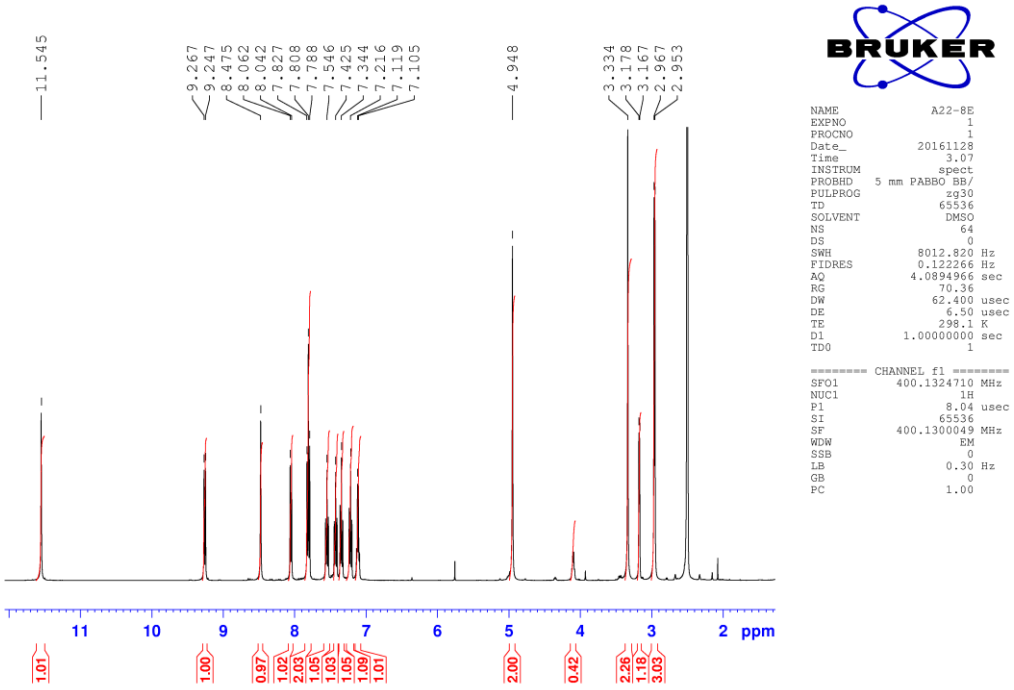

Figure S2. ^{1}H NMR spectrum of **1**.

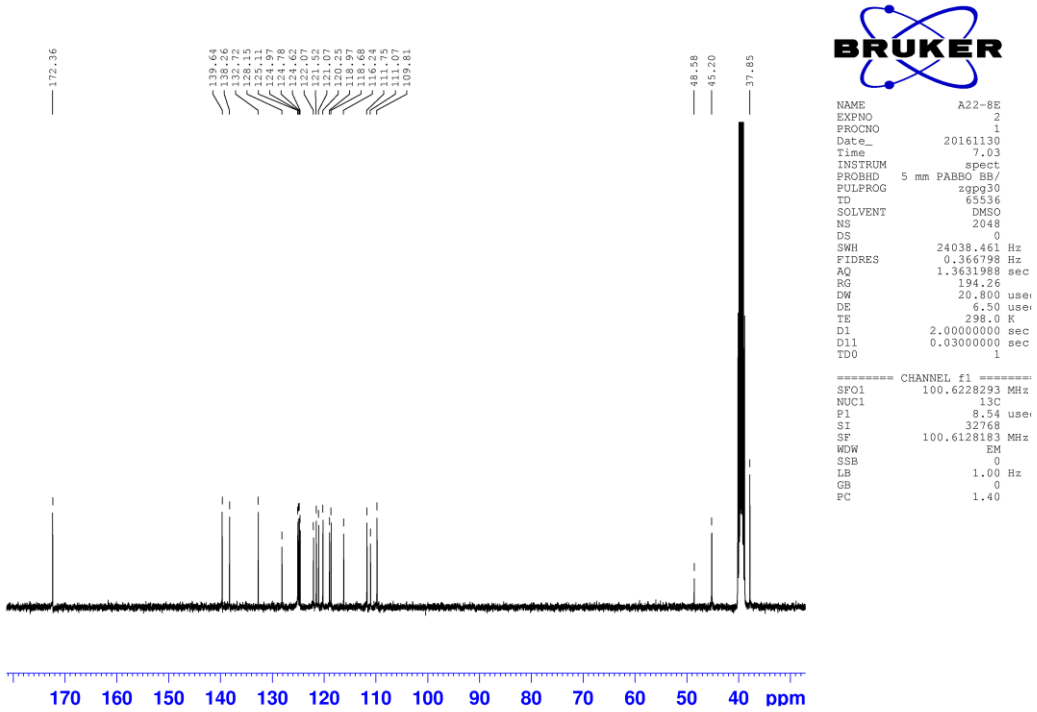

Figure S3. ^{13}C NMR spectrum of **1**.

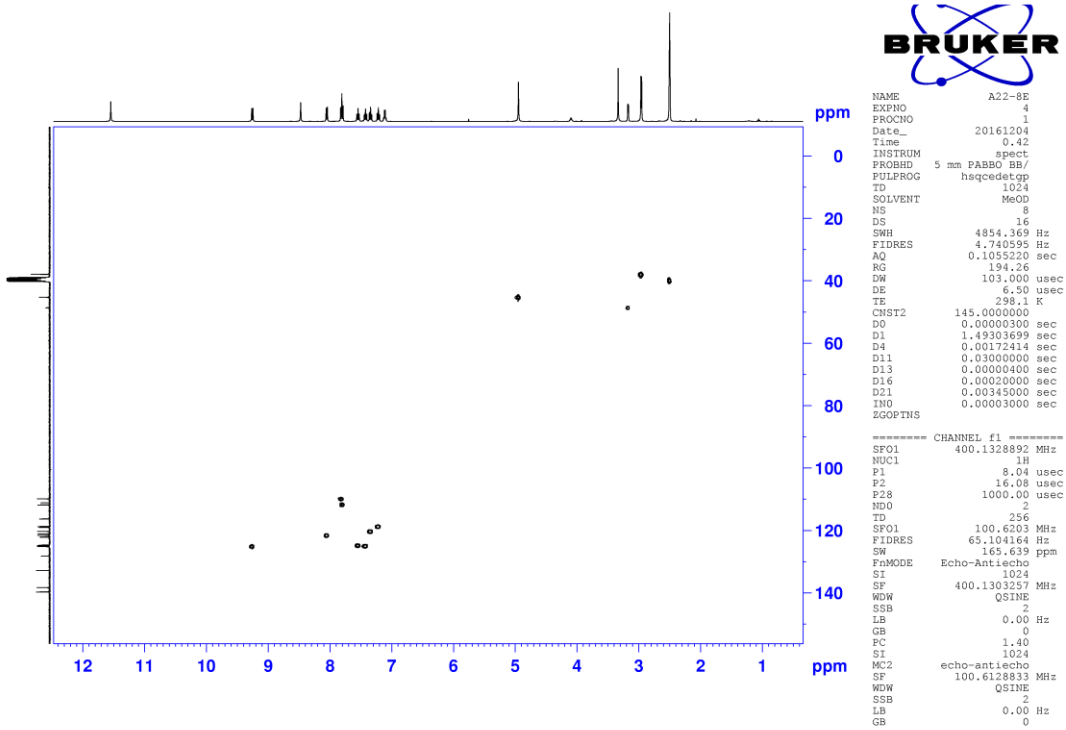

Figure S4. HSQC spectrum of **1**.

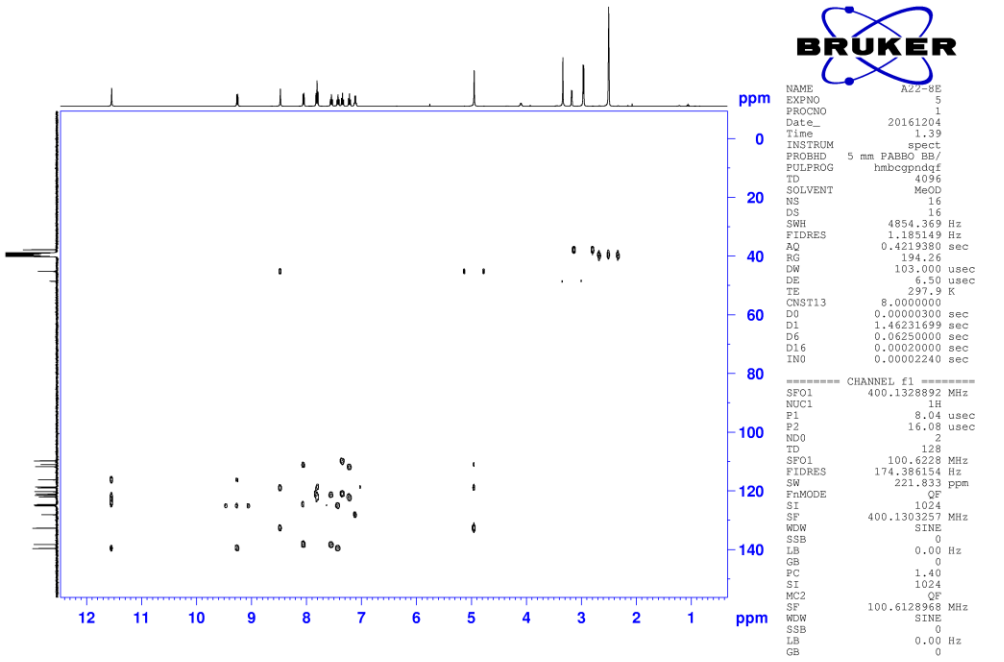

Figure S5. HMBC spectrum of **1**.

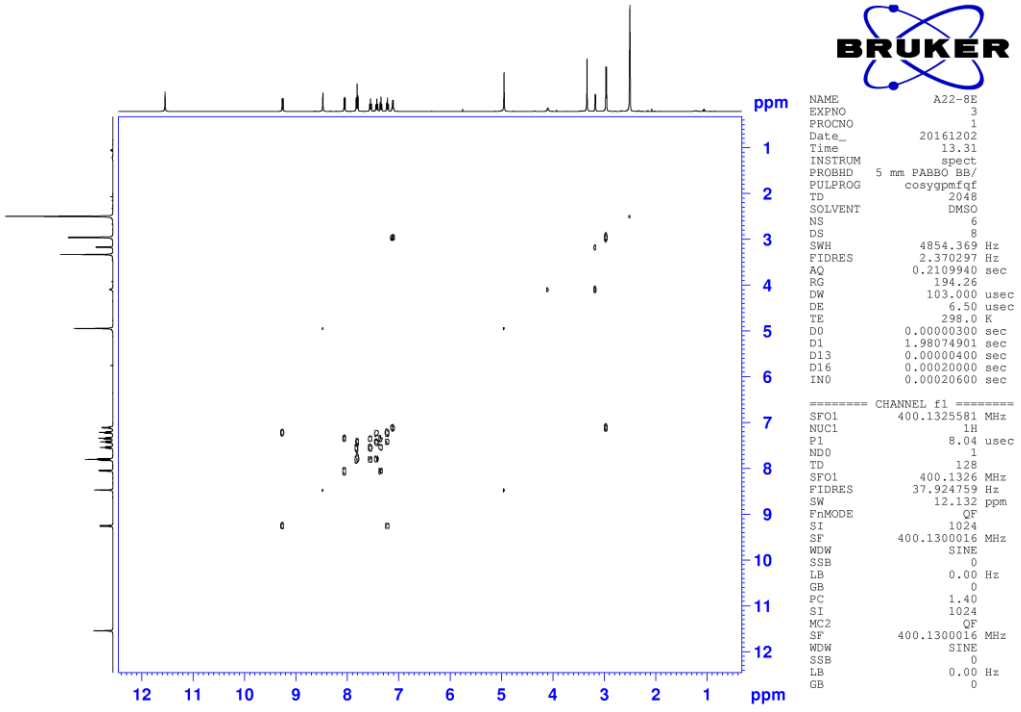

Figure S6. ^{1}H-^{1}H COSY spectrum of **1**.

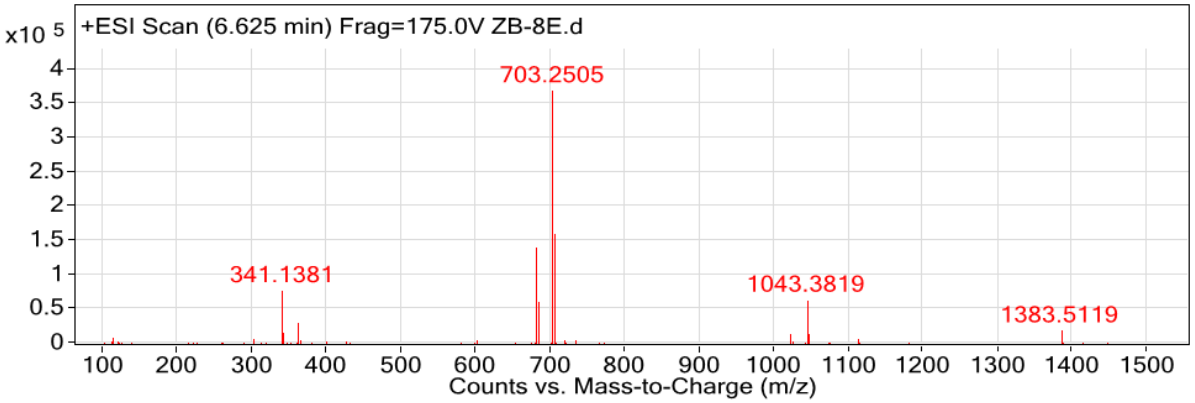

Figure S7. HRESIMS spectrum of **1**.

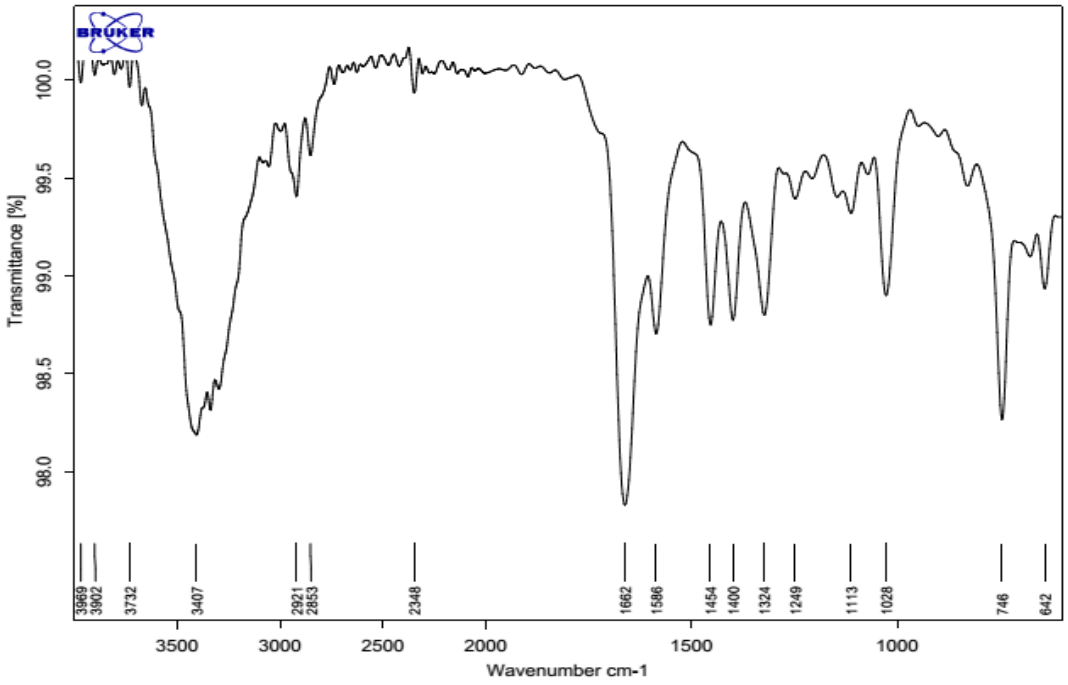

Figure S8. IR spectrum of **1**.

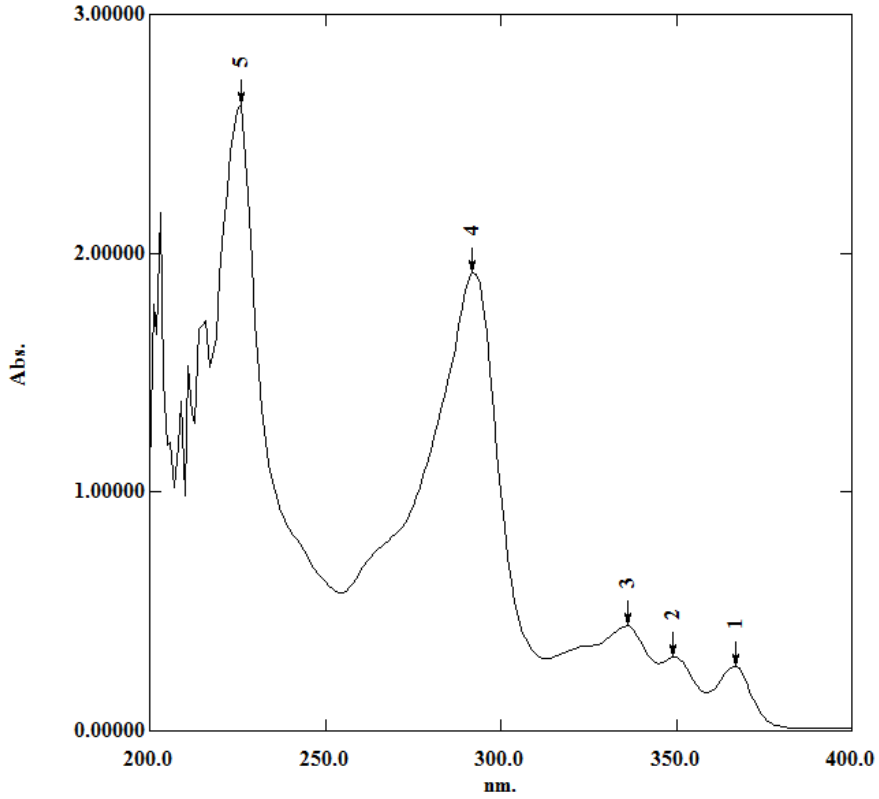

Figure S9. DAD UV-Vis spectrum of **1**.