Supporting Information

***Rec. Nat. Prod*. 16:6 (2022) 622-632**

A Novel Nitrogen-containing Glyceride from Fungal Saprobe *Tubeufia rubra* reverses MDR of Tumor Cell Lines to Doxorubicin

Xuebo Zeng [1,2], Shengyan Qian [2,3], Yongzhong Lu [4], Yongjie Li [2], Lizhuang Chen [2], Yixin Qian [2], Zhangjiang He [2*] and Jichuan Kang [2*].

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[3] School of Life Science, Guizhou University, Guiyang, Guizhou 550025, P. R. China
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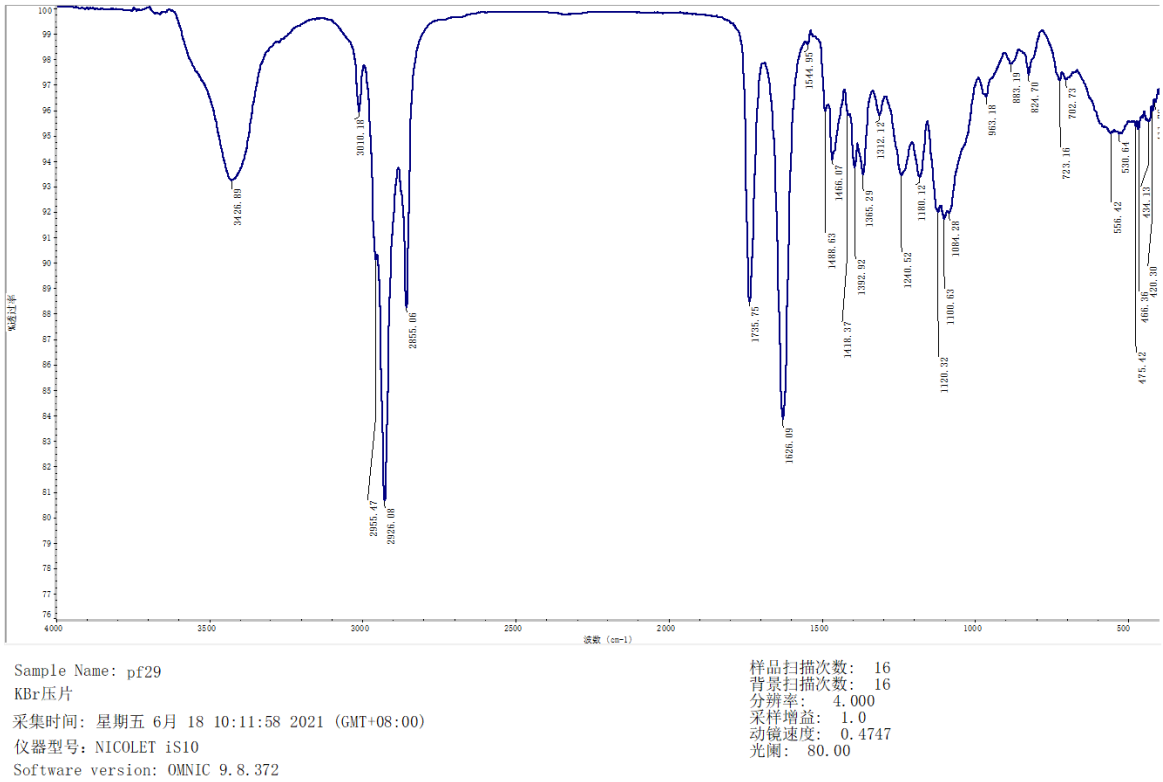

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仪器型号: NICOLET iS10
Software version: OMNIC 9.8.372

样品扫描次数: 16
背景扫描次数: 16
分辨率: 4.000
采样增益: 1.0
动镜速度: 0.4747
光阑: 80.00

Figure S1: IR spectrum of Rubracin A

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Qualitative Analysis Report

Data Filename	20200605ESIA12.d	**Sample Name**	pf29
Sample Type	Sample	**Position**	
Instrument Name	Agilent G6230 TOF MS	**User Name**	KIB
Acq Method	ESI.m	**Acquired Time**	6/4/2020 12:20:40 PM
IRM Calibration Status	Success	**DA Method**	ESI.m
Comment			

Sample Group	**Info.**
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User Spectra

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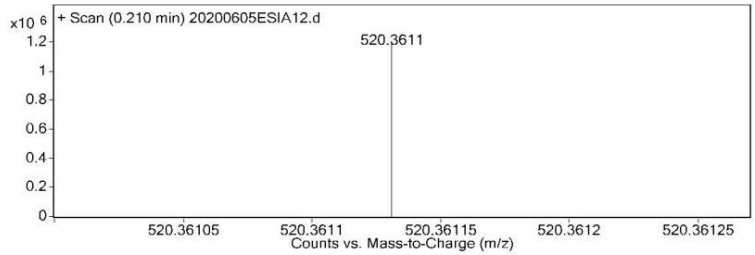

Peak List

m/z	z	Abund	Formula	Ion
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520.3611	1	1205406.88	C28 H51 N Na O6	M+
521.3648	1	342875.25	C28 H51 N Na O6	M+
1017.7316	1	1062040		
1018.7359	1	645095.31		
1019.7389	1	216519.41		
1033.7256	1	122494.33		
1049.7219	1	132093.2		
1515.1033	1	227966.75		
1516.1069	1	212686.09		

Formula Calculator Element Limits

Element	Min	Max
C	0	200
H	0	400
O	0	10
Na	1	1
N	0	2
D	0	1

Formula Calculator Results

Formula	CalculatedMass	Mz	Diff.(mDa)	Diff. (ppm)	DBE
C28 H51 N Na O6	520.3614	520.3611	0.3	0.6	3.5
C31 H47 D N2 Na O3	520.3625	520.3611	1.4	2.8	8.5
C28 H49 D N Na O6	520.3599	520.3611	-1.2	2.4	4.0

Figure S2: HR-ESI-MS spectrum of Rubracin A;

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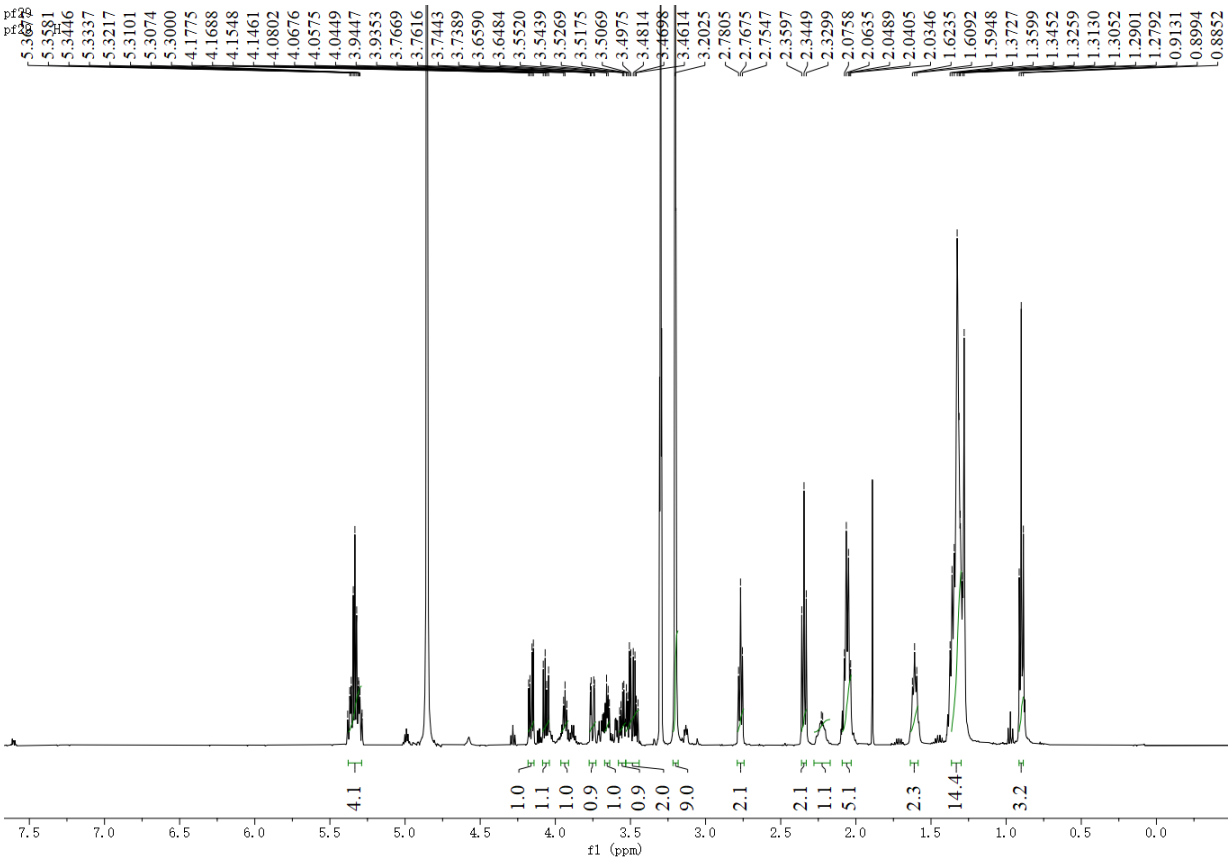

Figure S3: [1]H-NMR spectrum of Rubracin A in methanol-d4

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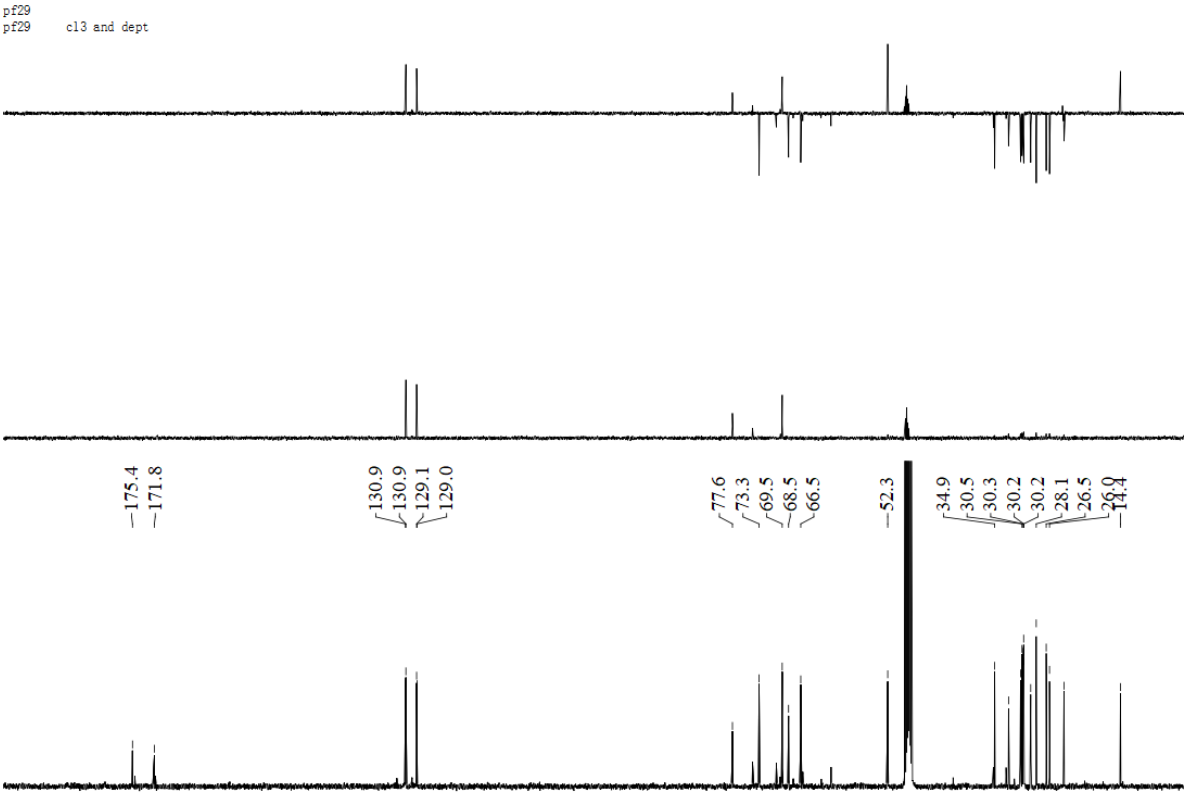

Figure S4: ^{13}C-NMR and DEPT spectrum of Rubracin A in methanol-d4

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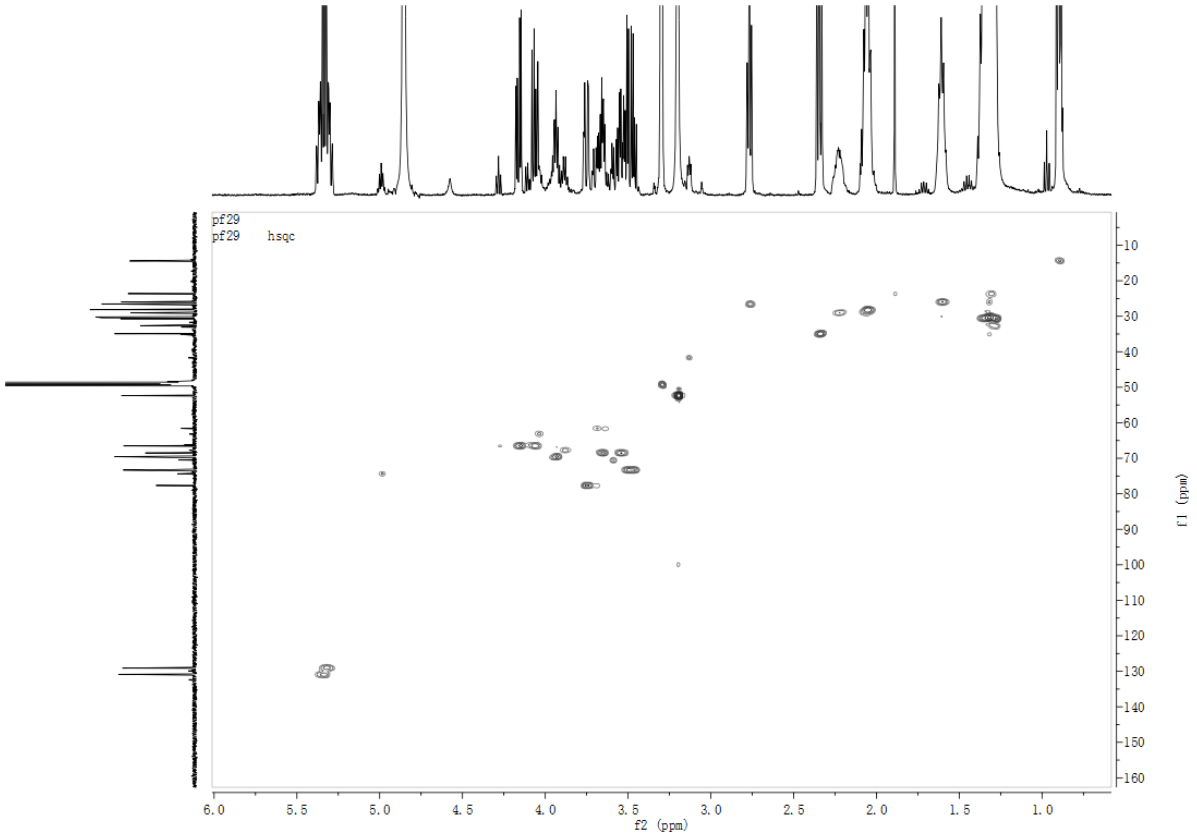

Figure S5: HSQC spectrum of Rubracin A in methanol-d4

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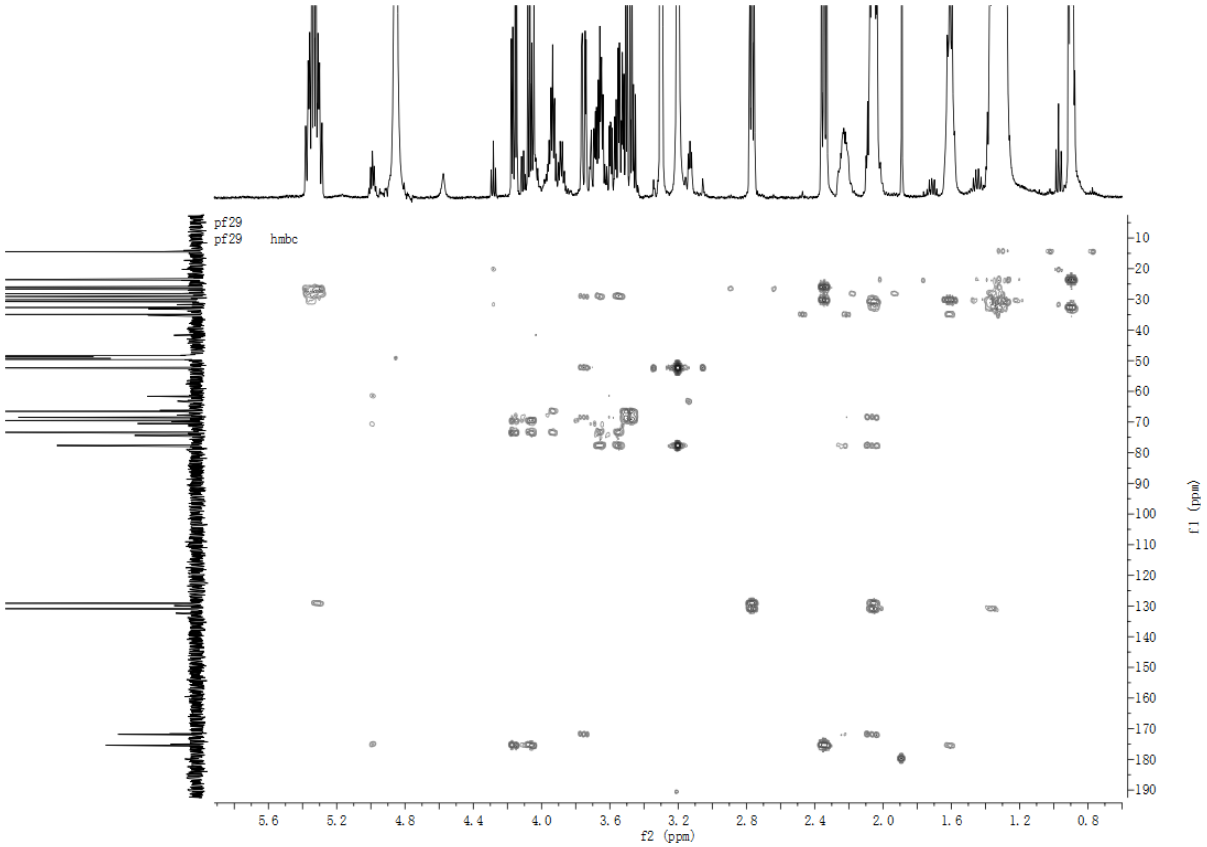

Figure S6: HMBC spectrum of Rubracin A in methanol-d4

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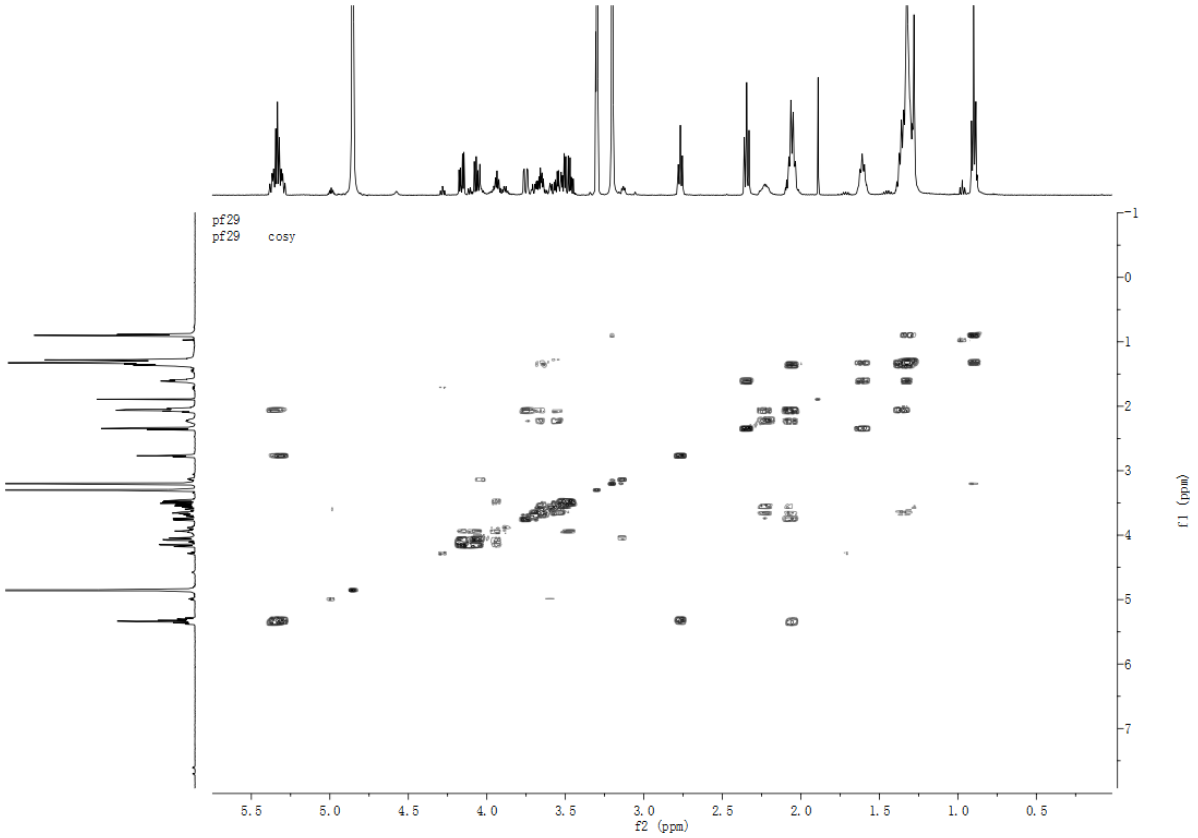

Figure S7: ^{1}H-^{1}H COSY spectrum of Rubracin A in methanol-d4

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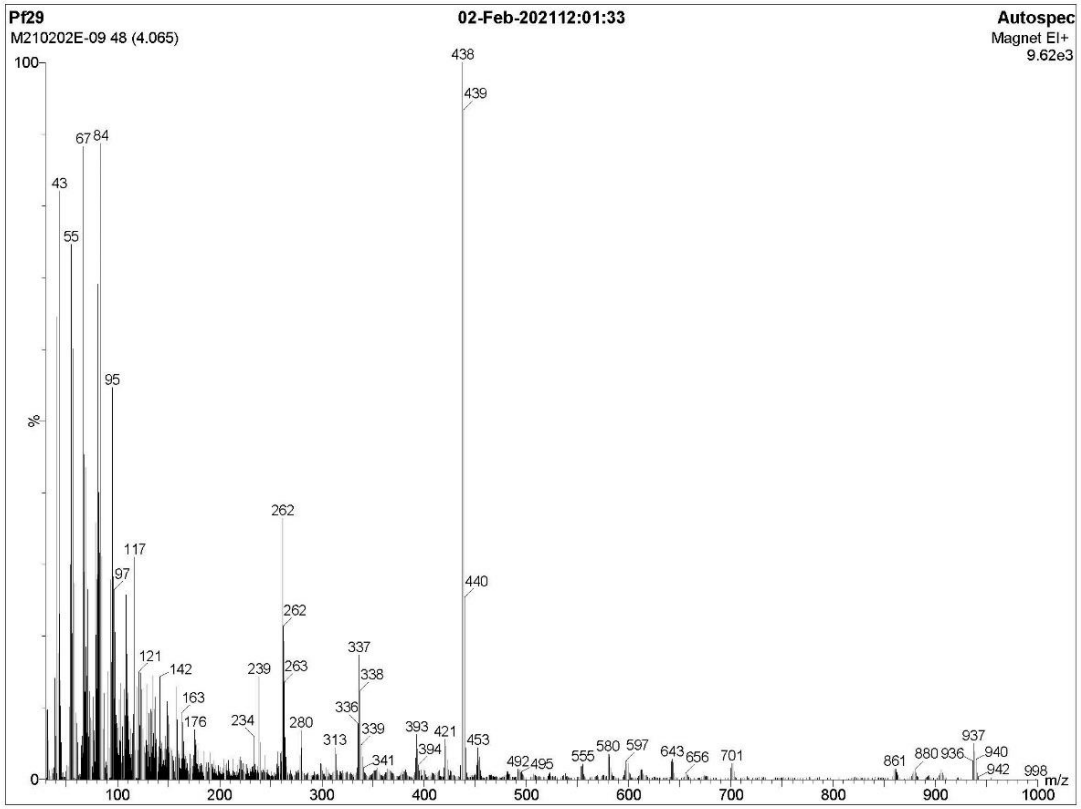

Figure S8 : EI-MS fragment ions of Rubracin A

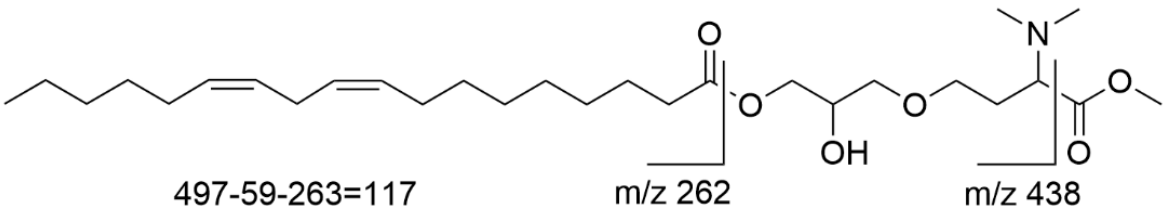

Figure S9: MS Fragmentation of Rubracin A

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光谱峰值检测报告

2021/07/14 13:50:55

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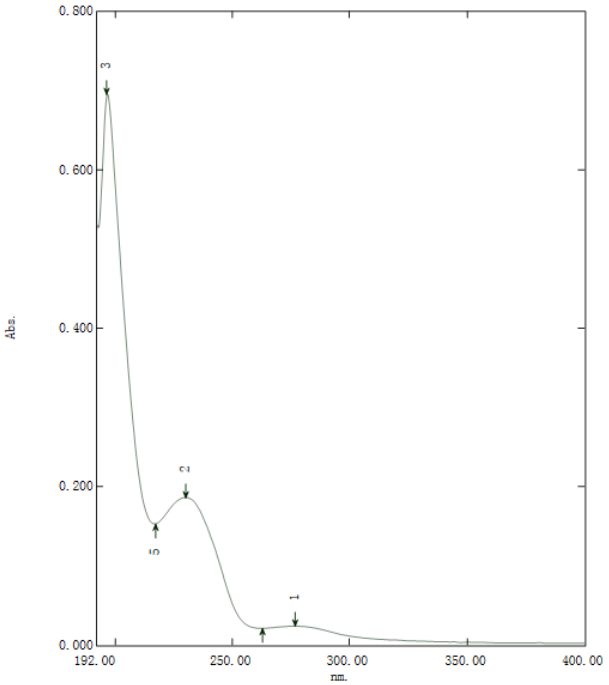

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采样间隔: 0.5
自动采样间隔: 停用
扫描模式: 单个

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测定方式: 吸收值
狭缝宽: 5.0 nm
积分时间: 0.1 秒
光源转换波长: 323.0 nm
检测器单元: 直接
S/R 转换: 标准
阶梯校正: OFF

[附件属性]
附件: 无

[数据处理参数]
阈值: 0.0010000
点: 4
内插: 停用
平均: 停用

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重量:
体积:
稀释:
光程长: 10mm
附加信息: 样品浓度: 0.0312毫克/毫升
溶剂: 甲醇

No.	P/V	波长(nm)	Abs.	描述
1	↑	276.50	0.024	
2	↑	230.00	0.186	
3	↑	196.50	0.695	
4	↓	262.50	0.021	
5	↓	217.00	0.153	

Figure S10: UV (Methanol) spectrum for Rubracin A

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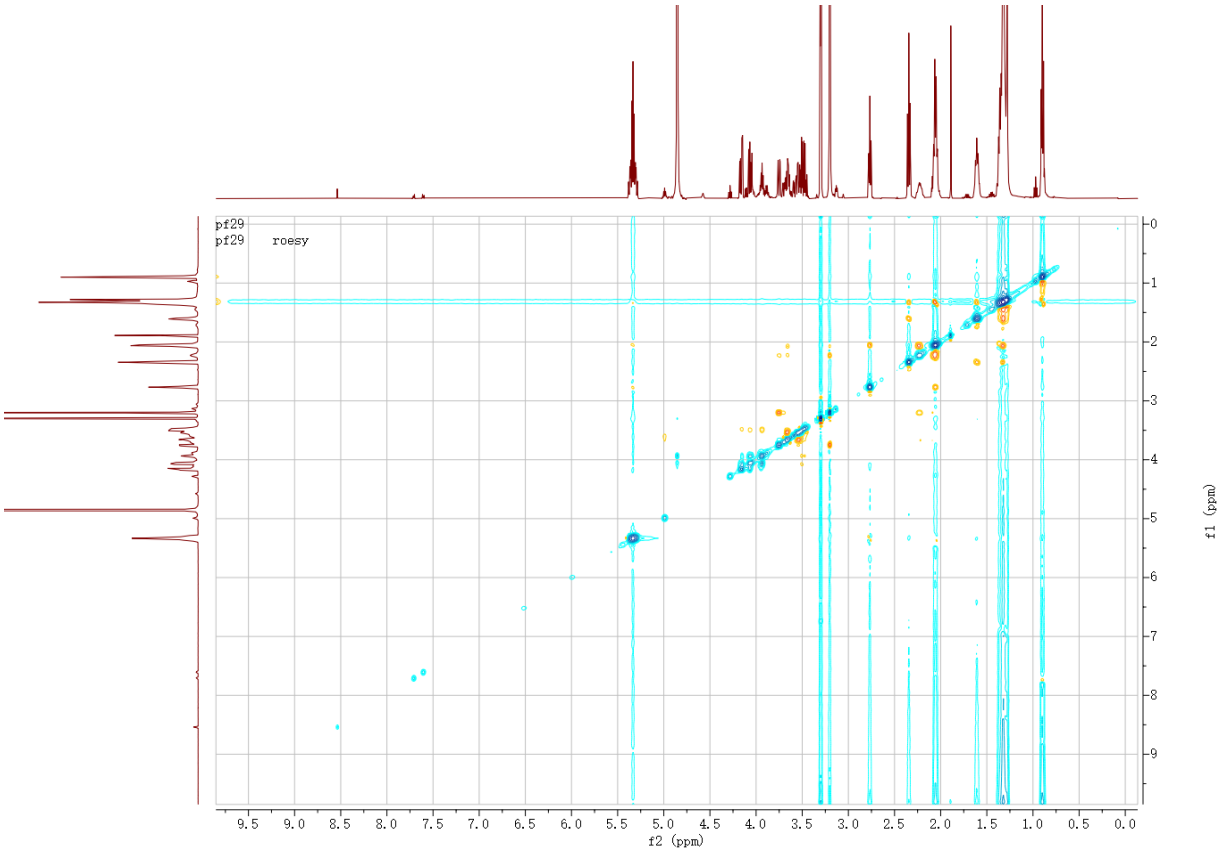

Figure S11: ROESY spectrum of Rubracin A

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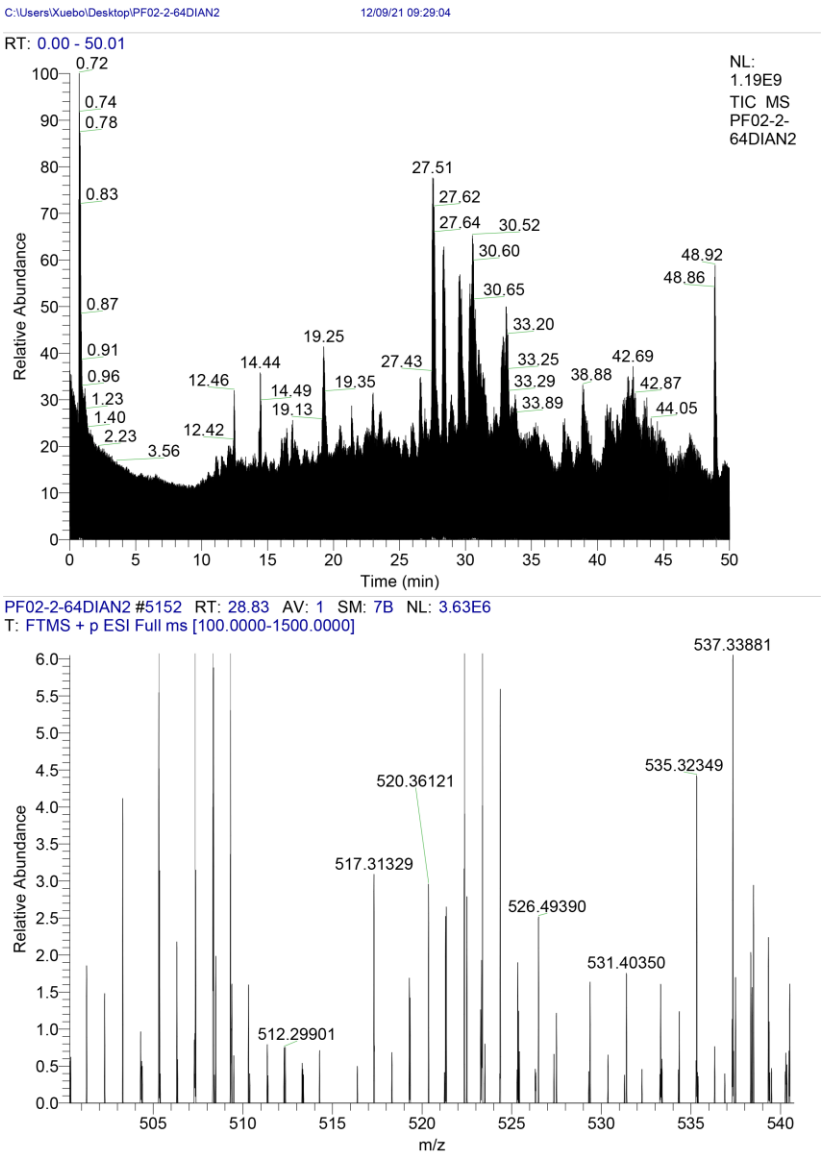

Figure S12: The UHPLC/MS chromatogram of Rubracin A (m/z 520.3611 $[M + Na]^+$) was isolated from EtOAc extract (35.77g).

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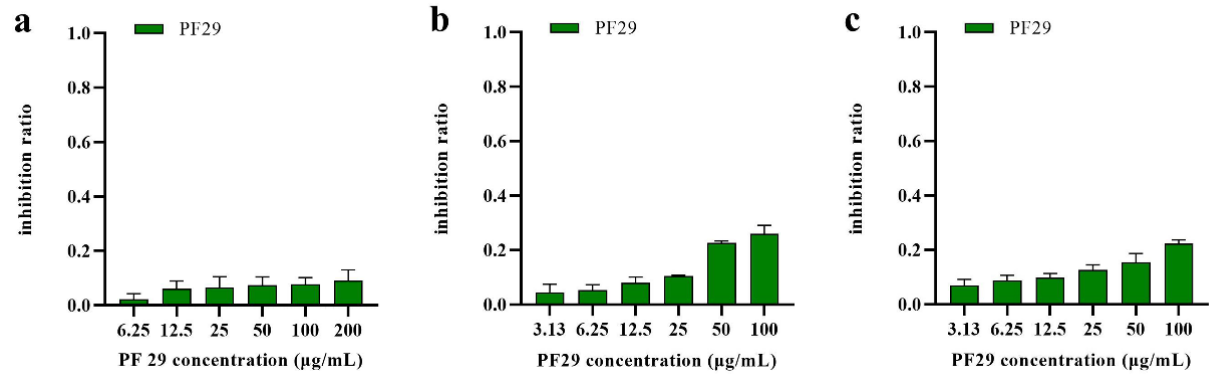

17	A549/ADR-PF29	0ug/ml	1.6ug/ml	3.125ug/ml	6.25ug/ml	12.5ug/ml	25ug/ml	50ug/ml	100ug/ml	对照孔
18	重复1	1.873	1.813	1.795	1.787	1.752	1.695	1.459	1.415	0.111
19	重复2	1.824	1.795	1.801	1.766	1.715	1.645	1.456	1.444	0.156
20	重复3	1.884	1.805	1.756	1.756	1.705	1.702	1.503	1.386	0.174
21	OD值	1.860333333	1.804333333	1.784	1.769666667	1.724	1.680666667	1.472666667	1.415	0.147
22	抑制率	0	0.032684825	0.044552529	0.052918288	0.079571984	0.104863813	0.226264591	0.259922179	

	K562/ADR-PF29	0ug/ml	1.6ug/ml	3.125ug/ml	6.25ug/ml	12.5ug/ml	25ug/ml	50ug/ml	100ug/ml	对照孔
	重复1	1.985	1.862	1.865	1.812	1.812	1.715	1.695	1.579	0.123
	重复2	1.998	1.867	1.825	1.807	1.785	1.762	1.655	1.559	0.158
	重复3	1.923	1.919	1.837	1.808	1.768	1.733	1.712	1.549	0.175
	OD值	1.968666667	1.882666667	1.842333333	1.809	1.788333333	1.736666667	1.687333333	1.562333333	0.152
	抑制率	0	0.04733945	0.069541284	0.087889908	0.099266055	0.127706422	0.154862385	0.223669725	

7	MCF7/ADR-PF29	0ug/ml	6.25ug/ml	12.5ug/ml	25ug/ml	50ug/ml	100ug/ml	200ug/ml	400ug/ml	对照孔
8	重复1	2.164	2.095	1.969	1.936	1.939	1.965	1.916	1.939	0.102
9	重复2	2.072	2.003	1.989	1.967	1.972	1.904	1.984	1.874	0.104
0	重复3	2.019	2.061	1.936	1.955	1.901	1.925	1.817	1.818	0.108
1	OD值	2.085	2.053	1.964666667	1.952666667	1.937333333	1.931333333	1.905666667	1.877	0.10467
2	抑制率	0	0.016158896	0.060764181	0.066823767	0.074566571	0.077596364	0.090557145	0.105032823	

Figure S13: The raw data and Cytotoxic activities of Rubracin A against MCF-7/Dox (a), A549/Dox (b), and K562/Dox (c).

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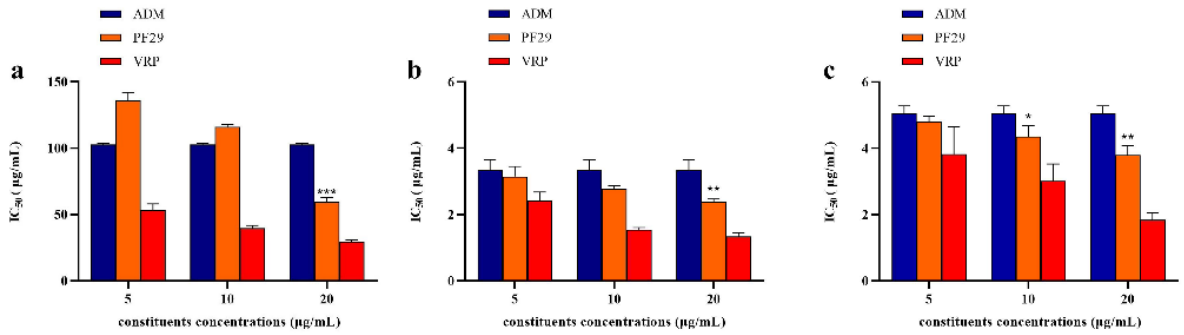

Figure 14: IC_{50} values of MCF-7/Dox (a), A549/Dox (b), and K562/Dox (c) of Rubracin A combined with doxorubicin

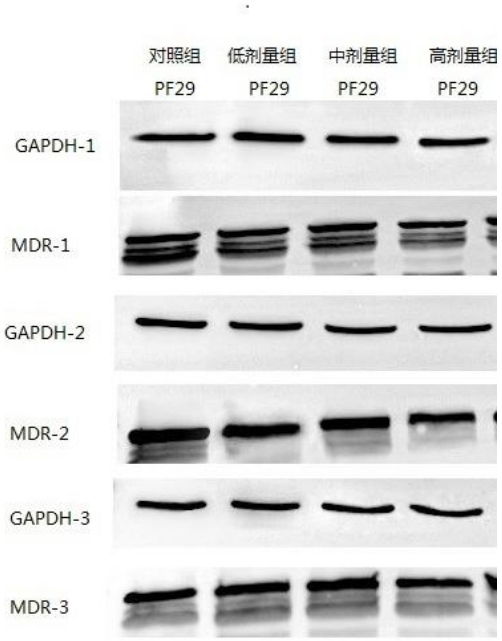

Figure 15: Western Blot results of Rubracin A

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Table S1: The IC_{50} of doxorubicin against the sensitive cell lines and MDR tumor cell lines

Cell lines	IC_{50}(μg/mL)	RF[a]
MCF-7	2.661±0.353	
MCF-7//Dox	102.762±0.953	38.6
A549	0.131±0.006	
A549//Dox	3.330±0.327	25.4
K562	0.104±0.031	
K562//Dox	5.053±0.226	48.6

[a] Reversal fold (RF) = IC_{50} (Drug-resistant tumor cells)/IC_{50}(Sensitive cells).

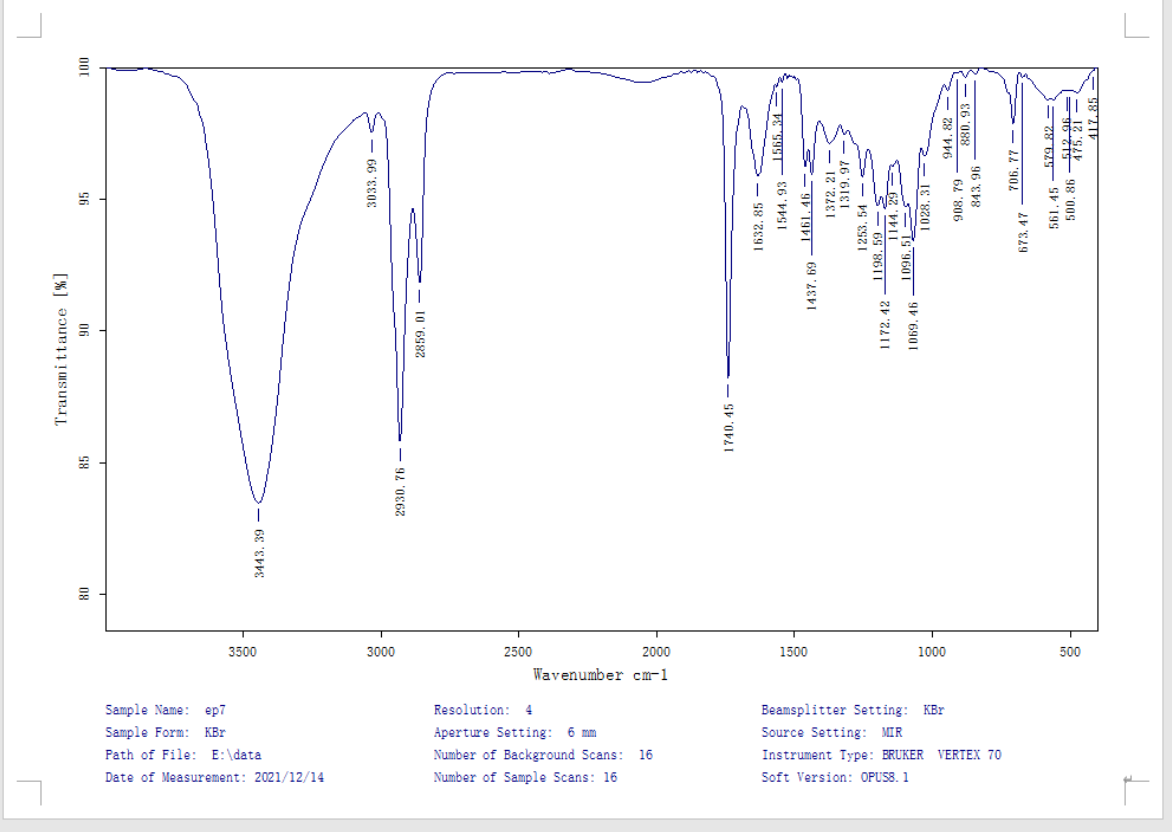

Figure S16: IR spectrum of Rubracin H

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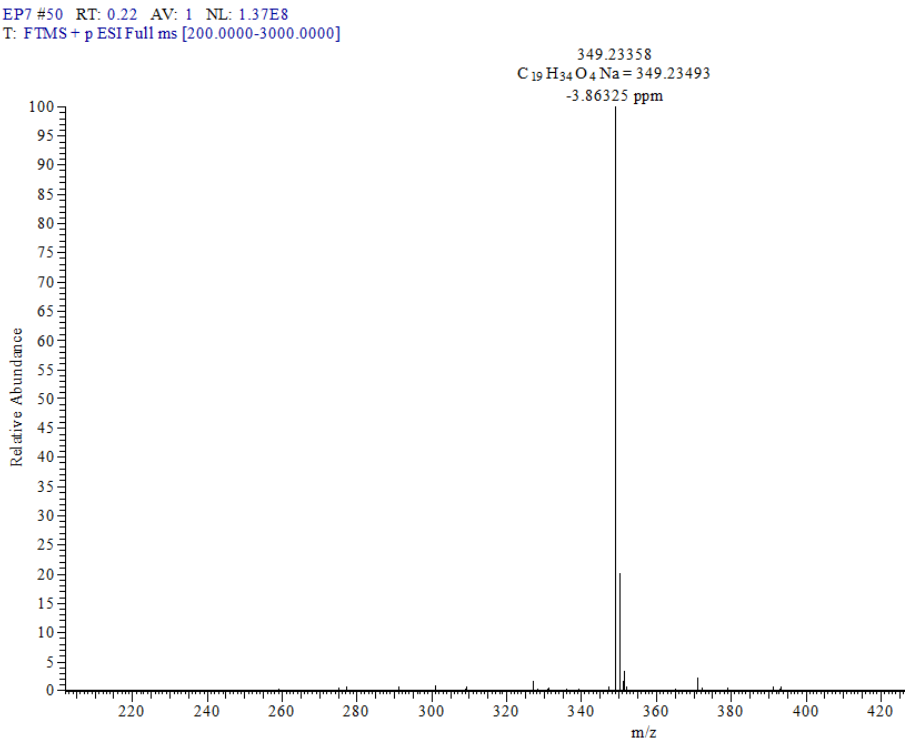

Figure S17: HR-ESI-MS spectrum of Rubracin H

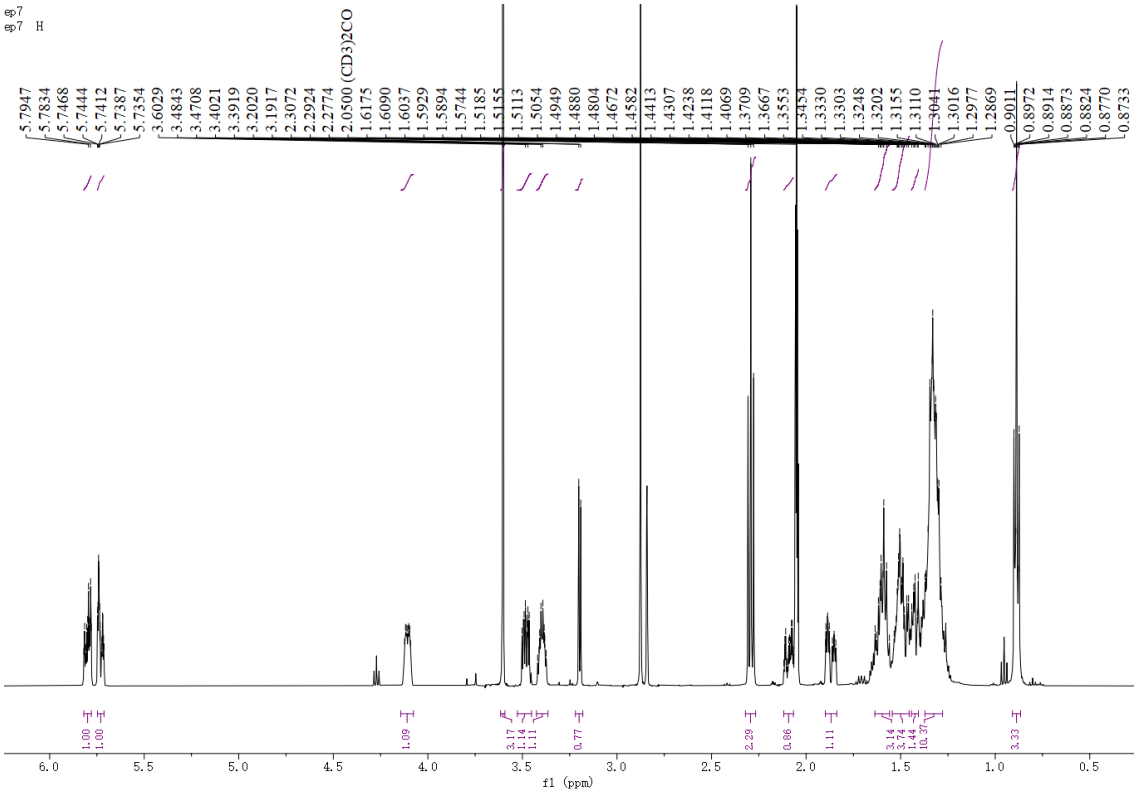

Figure S18: ^{1}H-NMR spectrum of Rubracin H in Acetone-d6

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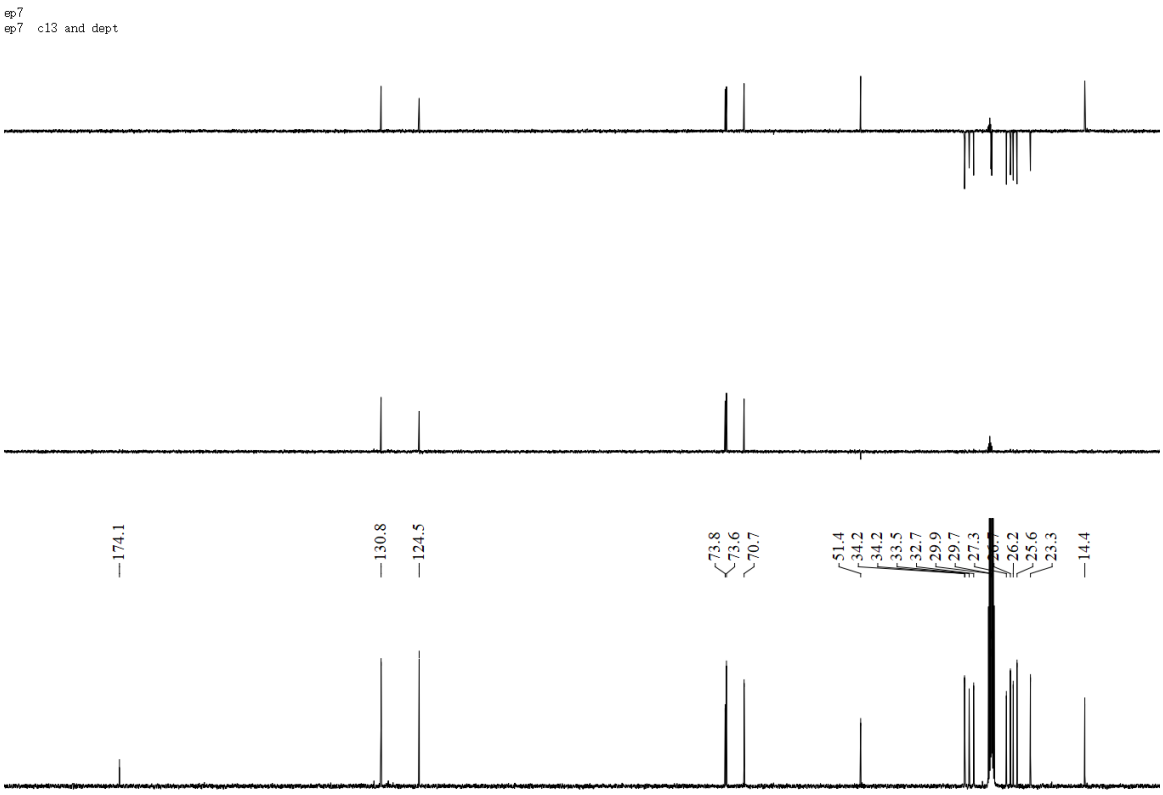

Figure S19: ^{13}C-NMR and DEPT spectrum of Rubracin H in Acetone-d6

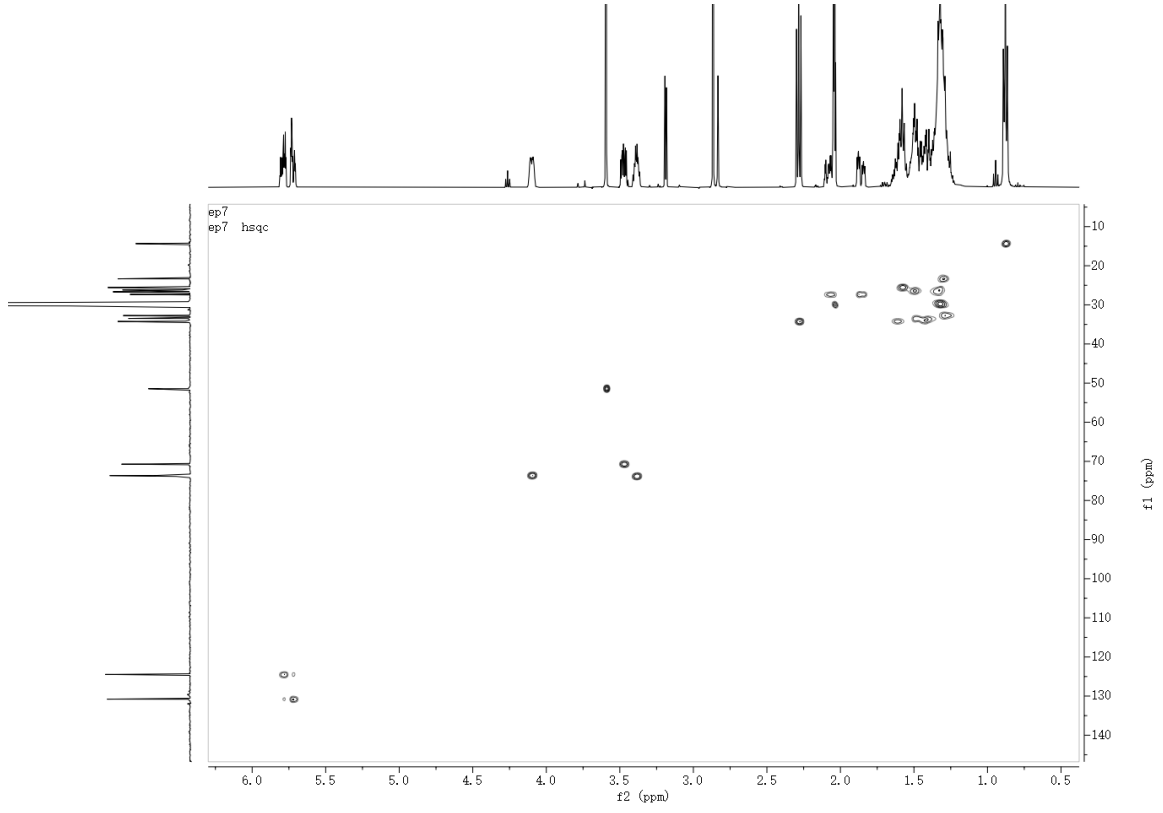

Figure S20: HSQC spectrum of Rubracin H in Acetone-d6

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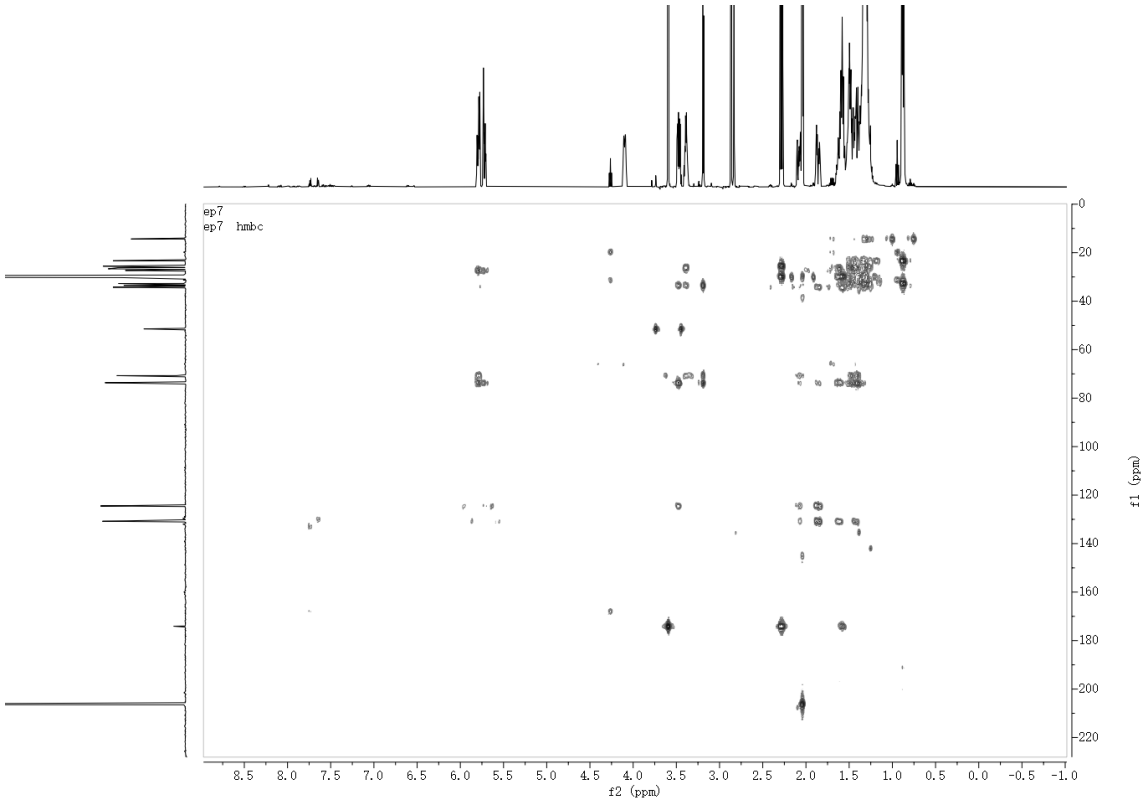

Figure S21: HMBC spectrum of Rubracin H in Acetone-d6

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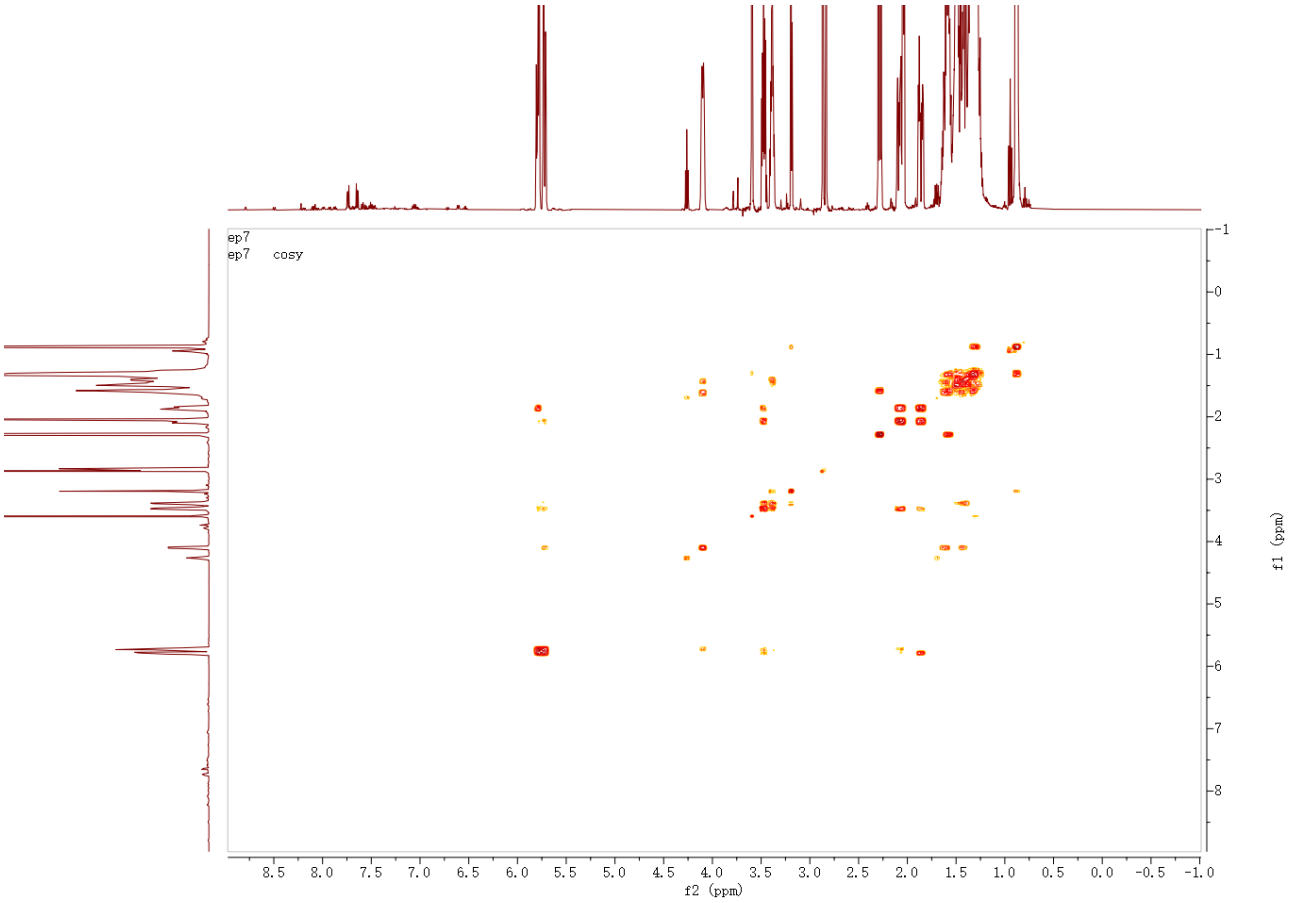

Figure S22: ^{1}H-^{1}H COSY spectrum of Rubracin H in Acetone-d6

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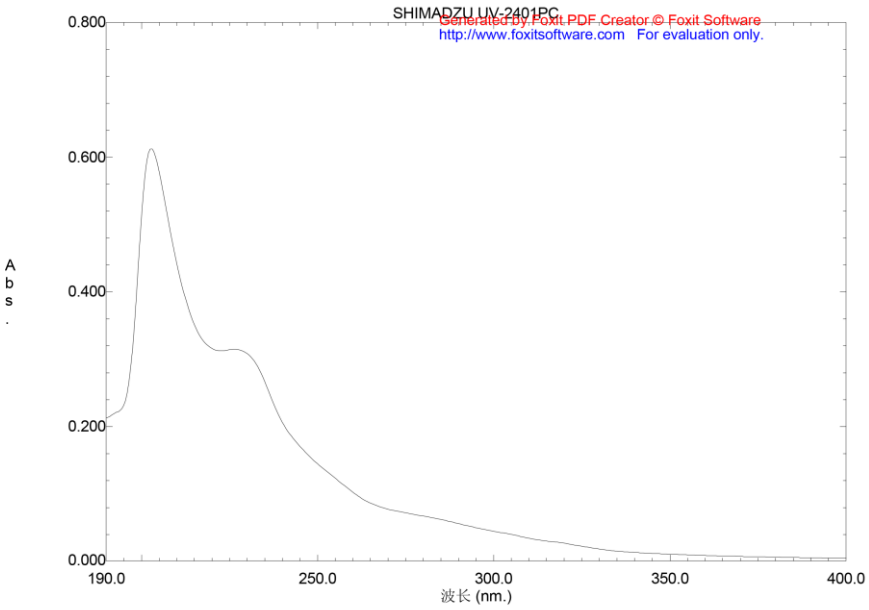

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采样间隔: 0.5

否.	波长 (nm.)	Abs.
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Figure S23: UV (Methanol) spectrum for Rubracin H

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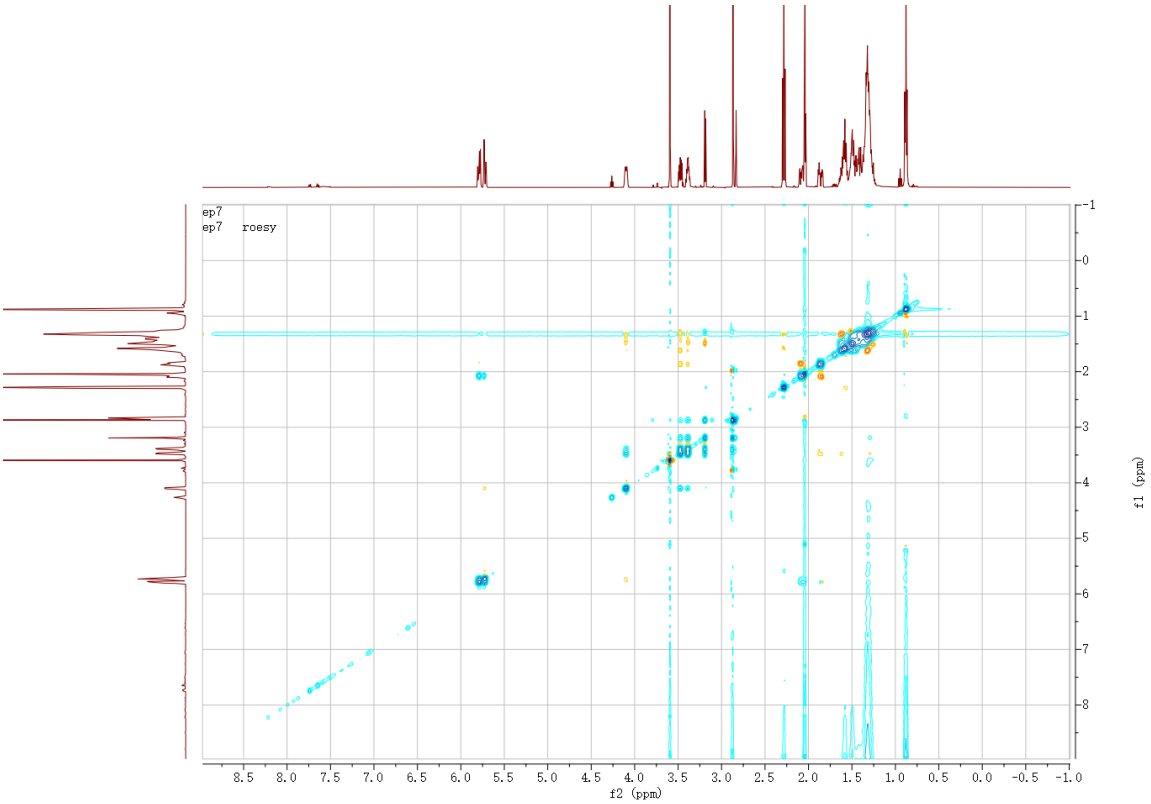

Figure S24: ROESY spectrum of Rubracin H

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