Supporting Information

GPR18 Inhibiting Amauromine and the Novel Triterpene Glycoside Auxarthonoside from the Sponge-Derived Fungus *Auxarthron reticulatum*

Mamona Nazir[1], Henrik Harms[1], Irene Loef[1], Stefan Kehraus[1], Fayrouz El Maddah[1], Idris Arslan[2], Viktor Rempel[3], Christa E. Müller[3], Gabriele M. König[1]

Affiliations

[1]Institute for Pharmaceutical Biology, University of Bonn, Bonn, Germany

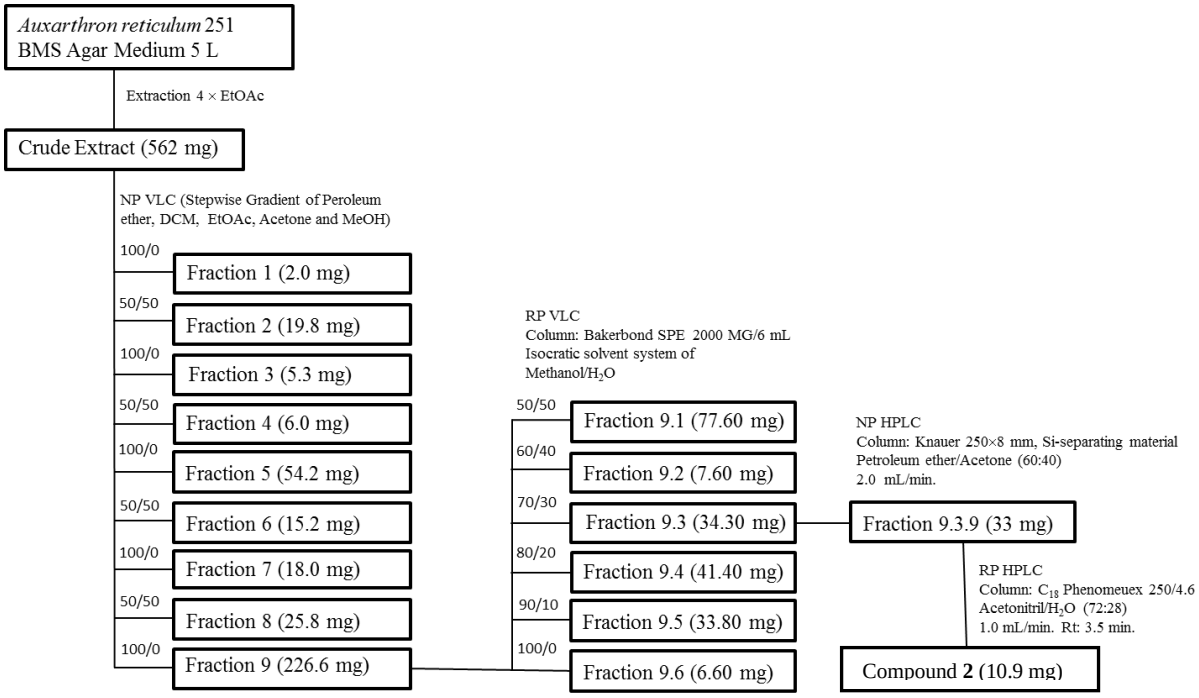
[2] Pamukkale University, Biomedical Engineering, Denizli, Turkey

[3]Pharma Center Bonn, Pharmaceutical Institute, Pharmaceutical Chemistry I, University of Bonn, Bonn, Germany

Contents

Extraction and Isolation Scheme

Isolation scheme for compound **2**.



Fig, S1.1: UV spectrum of compound **2** in MeOH.

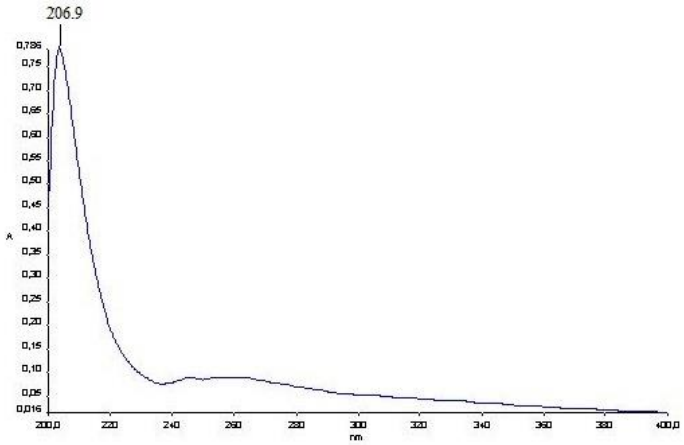


Fig. S1.2: IR spectrum of compound **2**.

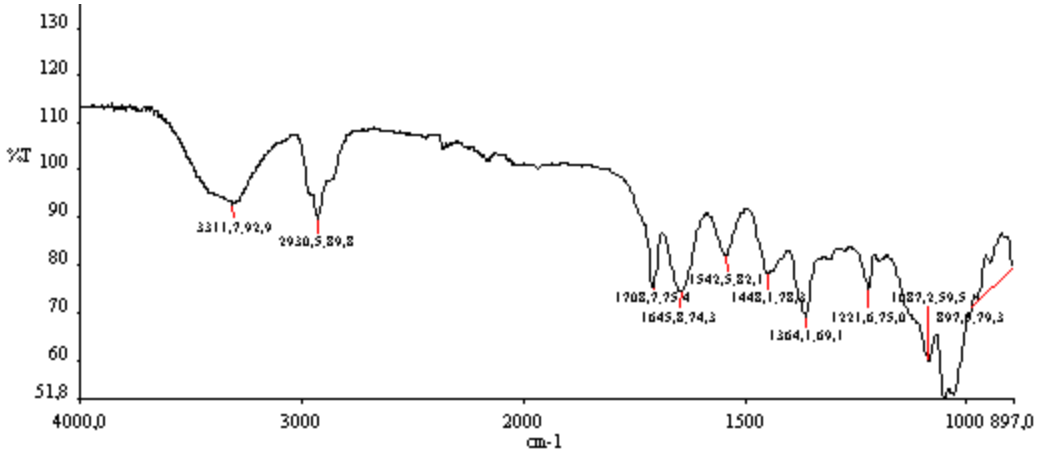


Fig. S1.3: ^{1}H NMR spectrum of compound **2** in acetone-d_6.

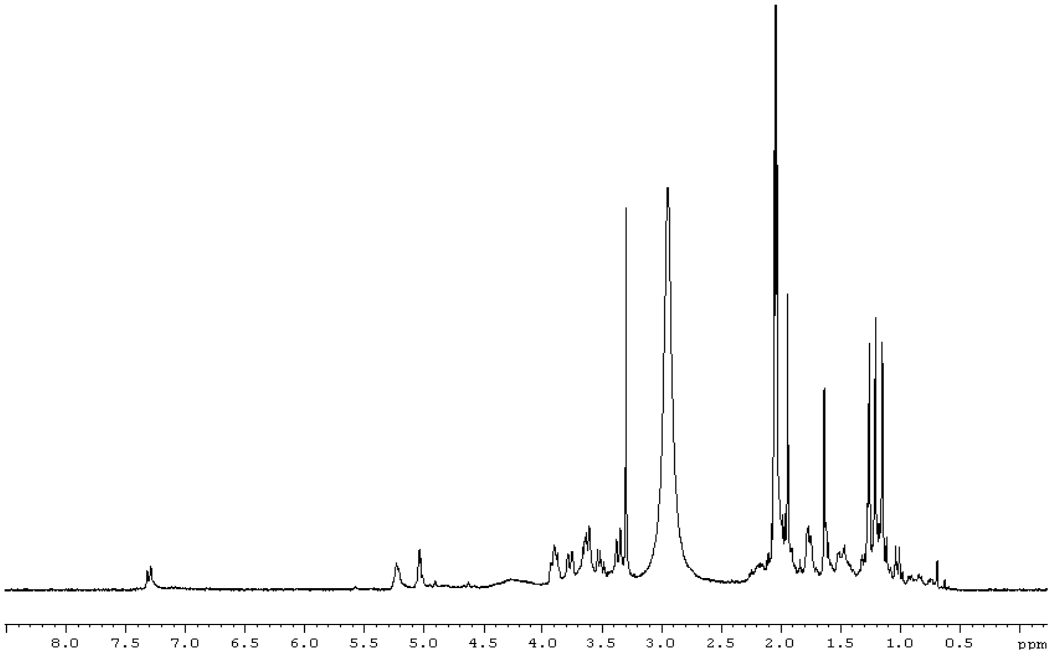


Fig. S1.4: ^{13}C NMR spectrum of compound **2** in acetone-d_6.

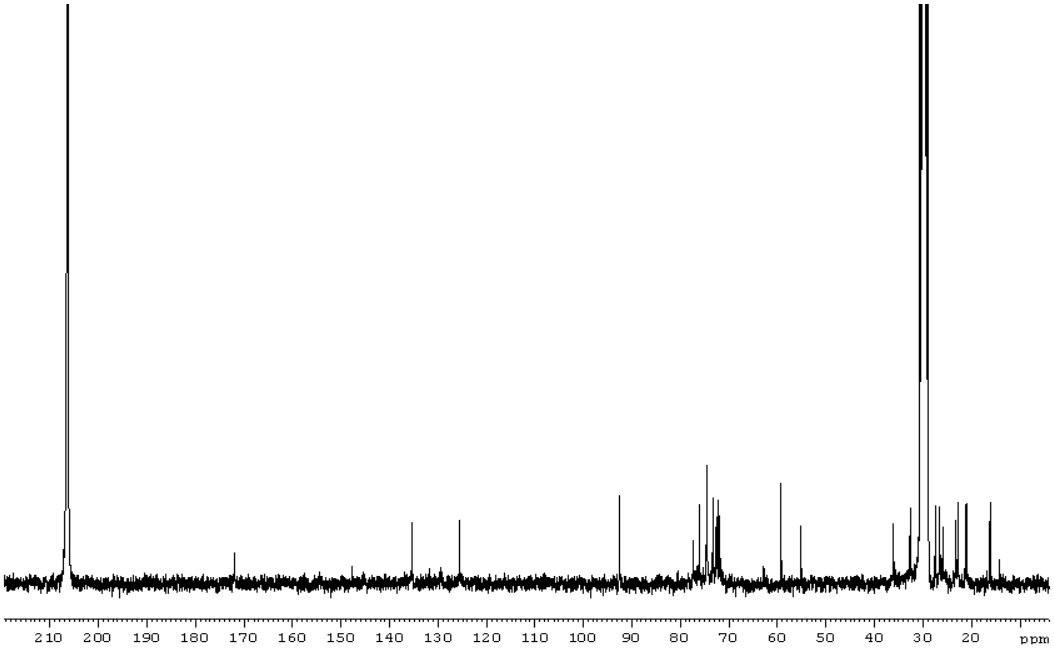


Fig. S1.5: DEPT 135 NMR spectrum of compound **2** in acetone-d_6.

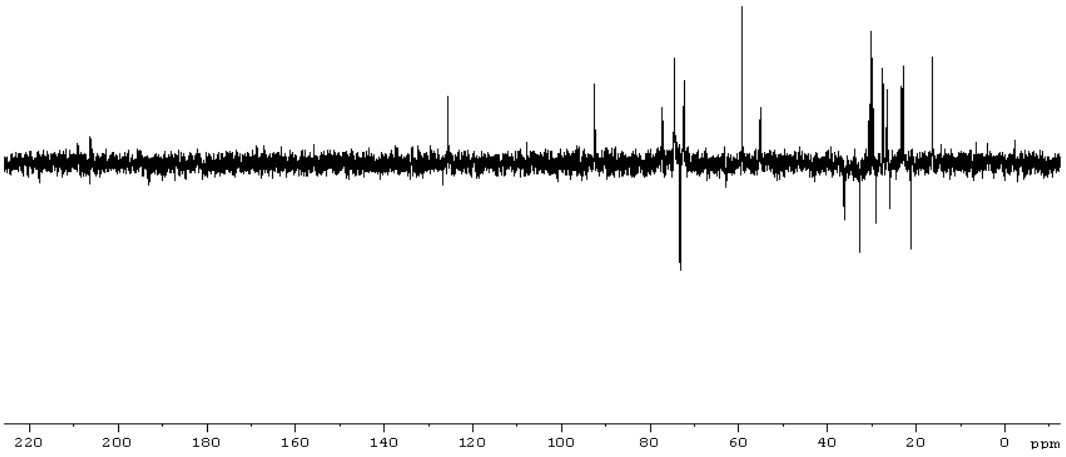


Fig. S1.6: ^{1}H-^{13}C HSQC NMR spectrum of compound **2** in acetone-d_6.

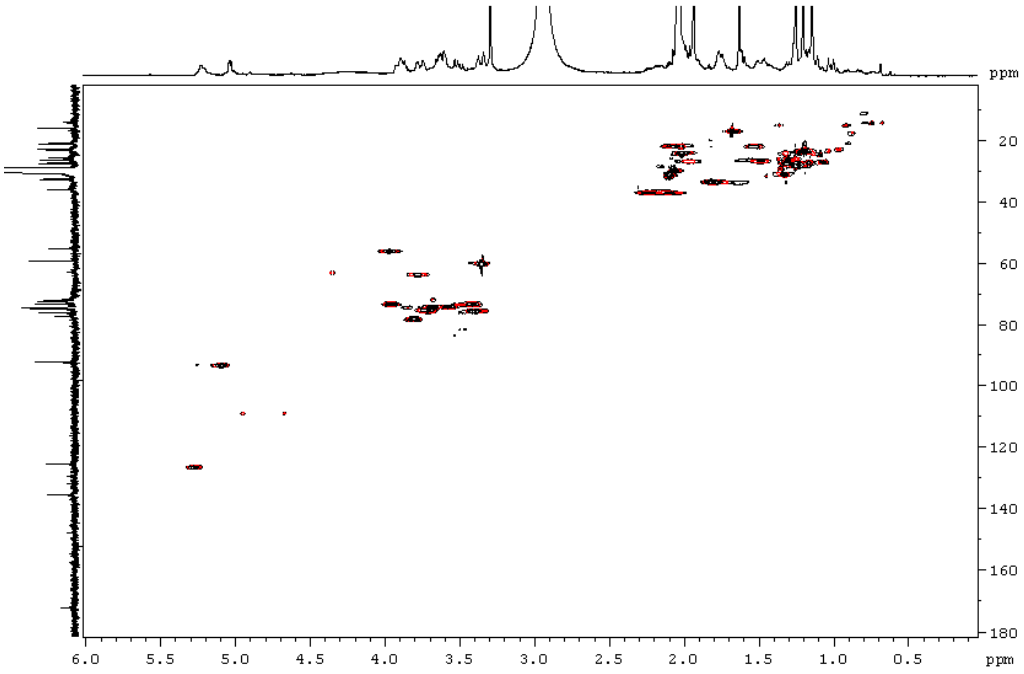


Fig. S1.7: ^{1}H-^{1}H COSY NMR spectrum of compound **2** in acetone-$d_{6.}$

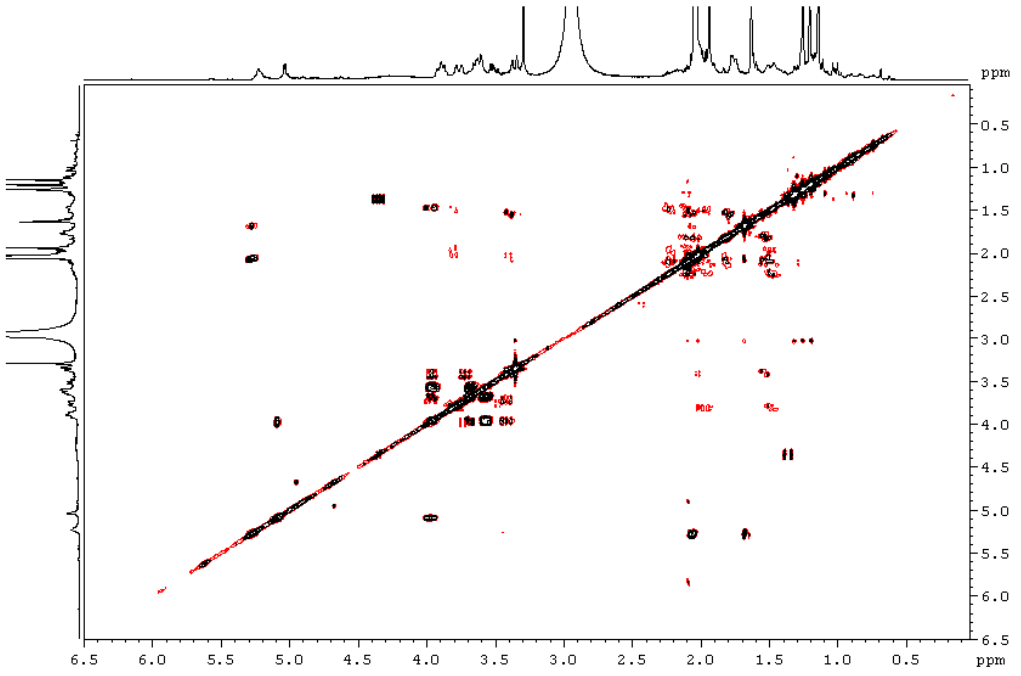


Fig. S1.8: ^{1}H-^{13}C HMBC NMR spectrum of compound **2** in acetone-$d_{6.}$

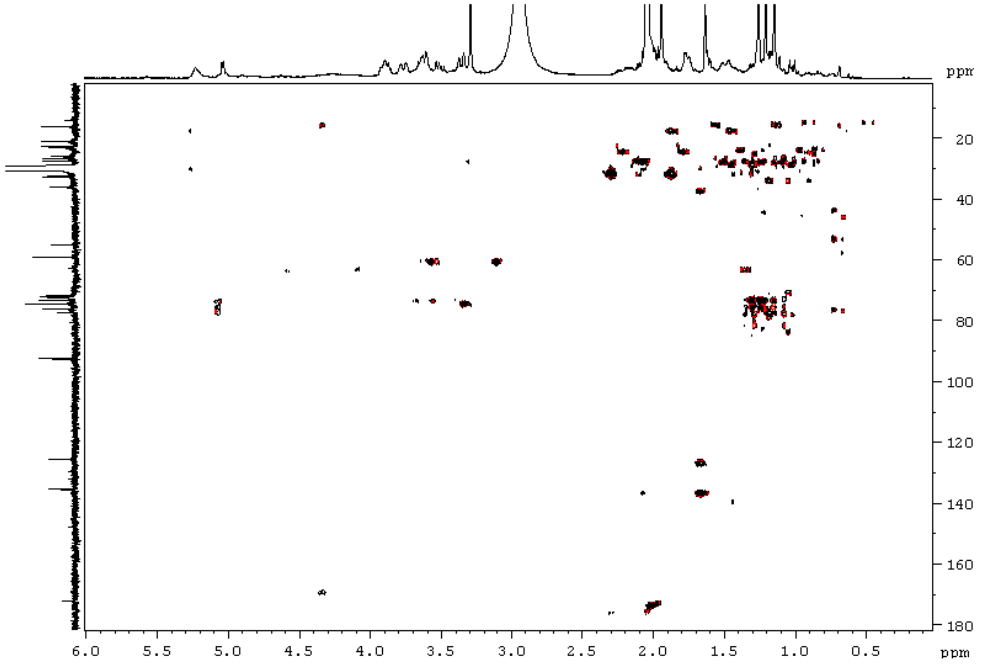


Fig. S1.9: ^{1}H-^{1}H NOESY NMR spectrum of compound **2** in acetone-d_6.

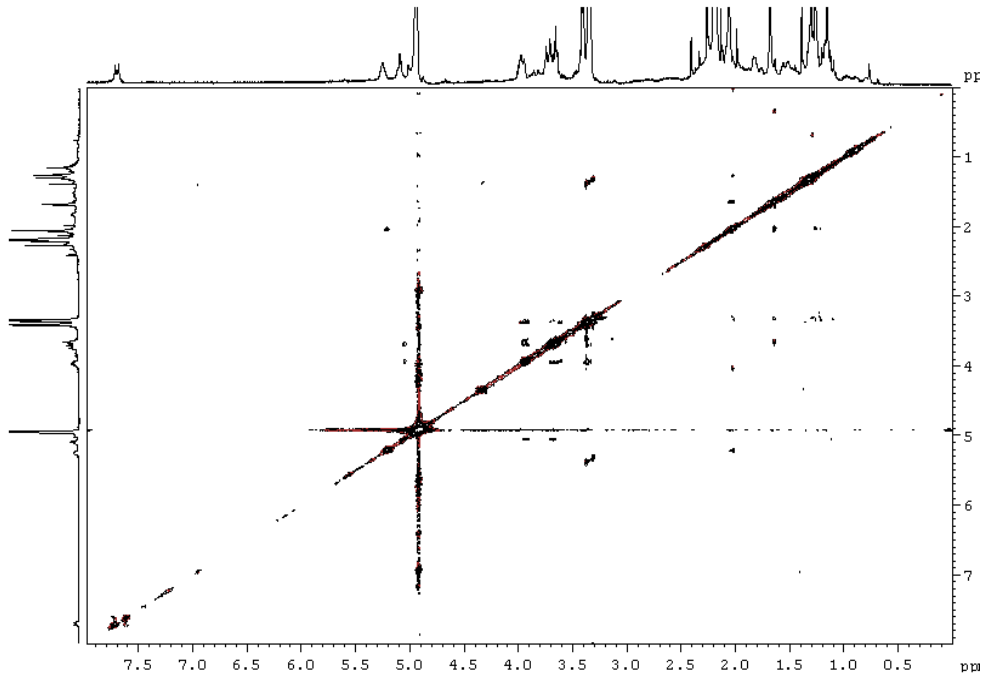
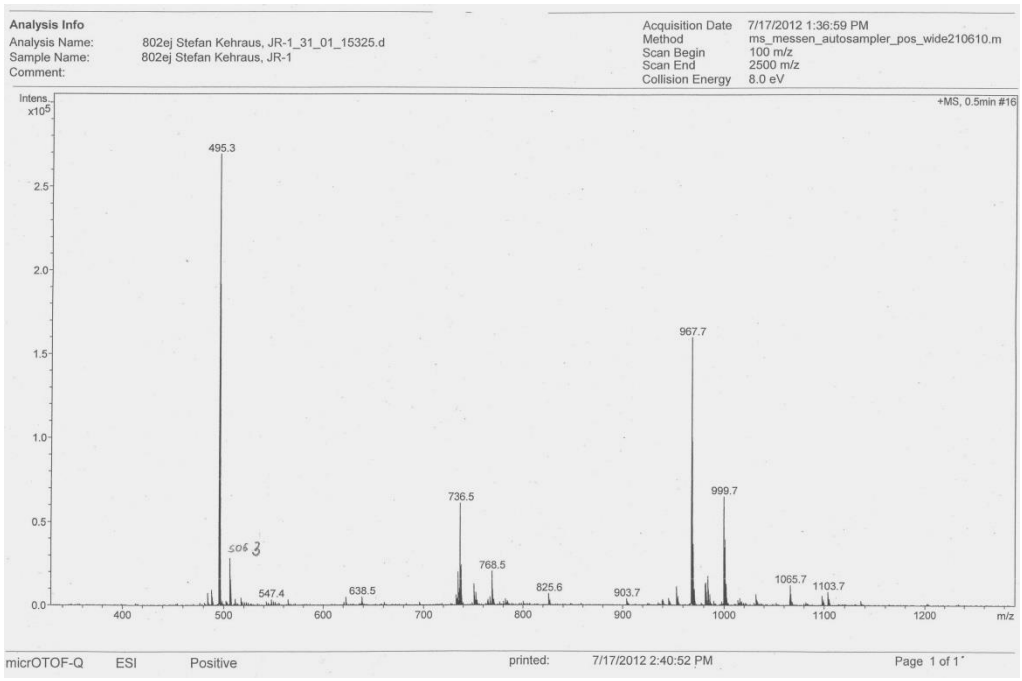


Fig. S1.10: HRESIMS spectrum of compound **2**.



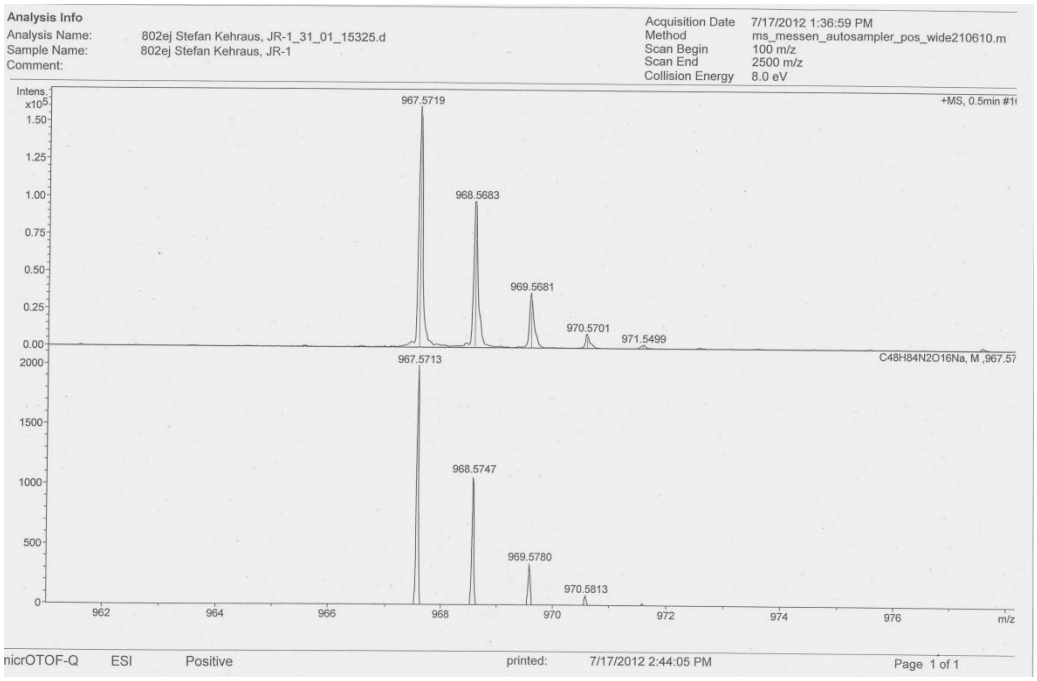
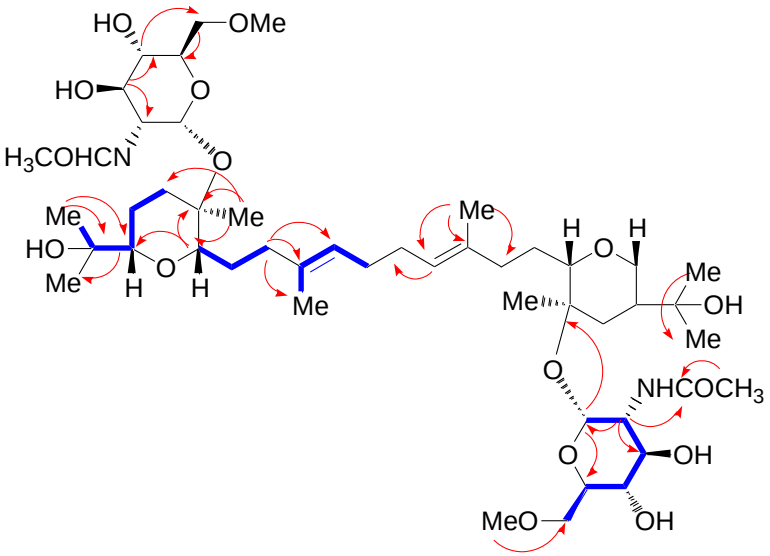


Fig. S1.11: ^{1}H-^{1}H COSY and ^{1}H-^{13}C HMBC correlations of compound **2**.



bold line: ^{1}H-^{1}H COSY key correlations

arrow: ^{1}H-^{13}C HMBC key correlations

Bioassays performed with compound **2**.

1. Antimicrobial Activity:

In agar diffusion assays, compound **2** was evaluated against a panel of microorganisms and found to be inactive.

Table S1.1: Antimicrobial activity of compound **2**. The sample concentration was 3 µL (µg) on paper disk. The assays were performed in the group of Prof. H.-G. Sahl, Pharmaceutical Microbiology, University of Bonn.

Strains	**Strain no.**	**Compound 2 (inhibition zone in mm)**
MSSA	5185	n.a.
MSSA	I-11574	n.a.
MRSA	LT-1334	n.a.
MRSA	LT-1338	n.a.
MRSE	LT-1324	n.a.
Candida albicans	I-11301	n.a.
Candida albicans	I-11134	n.a.
Citrobacter freundii	I-11090	n.a.
Klebsiella pneumoniae* subsp. *ozeanae	I-10910	n.a.
Enterococcus faecium	I-11305b	n.a.
Enterococcus faecium	I-11054	n.a.
Echerichia coli	I-11276b	n.a.
Echerichia coli	O-19592	n.a.
Stenotrophomonas maltophilia	O-16451	n.a.
Stenotrophomonas maltophilia	I-10717	n.a.
Pseudomonas aeruginosa	I-10968	n.a.
KNS	I-10925	n.a.
Staphylococcus simulans	22	n.a.
Micrococcus luteus	ATCC 4698	n.a.
Mycobacterium smegmatis	ATCC 70084	n.a.
Bacillus subtilis	168	n.a.
Arthrobacter crystallopoietes	DSM 20117	n.a.
Listeria welchimeri	DSM 20650	n.a.
Corynebacterium xerosis	Va167198	n.a.
Staphylococcus aureus	SG511	n.a.
Staphylococcus aureus	133	n.a.

n.a.: not active

Assays were performed according to:

Schäberle TF, Goralski E, Neu E, Erol Ö, Hölzl G, Dörmann P, Bierbaum G, König GM. Mar Drugs 2010; 8: 2466-2479

2. Inhibition of Enzymes:

Compound **2** was investigated with respect to cathepsin L, cathepsin B, bovine trypsin, human leukocyte elastase (HLE), and bovine chymotrypsin inhibition. The substance showed no inhibition of these enzymes.

Table S1.2: Enzyme inhibitory activity of compound **2**. The sample concentration was 20 μM.

Enzymes	Results
Human cathepsin L	n.i.
Human cathepsin B	n.i.
Bovine trypsin	n.i.
Human leukocyte elastase	n.i.
Bovine chymotrypsin	n.i.

n.i. = no inhibition

Assays were performed according to:

Pietsch M, Gütschow MJ. Med Chem 2005; 48: 8270-8288

Gütschow M, Pietsch M, Themann A, Fahrig J, Schulze BJ. Enz Inhib Med Chem 2005; 20*:* 341-347

3. Interaction with GPCRs

Compound **2** was investigated for of its interaction with human CB_1 and CB_2 receptors and evaluated for activity at the orphan receptors GPR18 and GPR55. It showed no affinity for cannabinoid receptors and no activity at the two orphan GPCRs, GPR55 and GPR18.

Table S1.3: Activity of compound **2** at human CB receptors and orphan receptors GPR18 and GPR55

	Radioligand binding vs. [^{3}H]CP55,940 K_i ± SEM (µM)[a]		*β*-Arrestin assays	
	CB_1	**CB_2**	**GPR18**	**GPR55**
Compound **2**	**> 10 (33 ± 3%)[d]**	**> 10 (12 ± 7%)[d]**	**> 10 (0 ± 6%)[b]**	**> 10 (16 ± 14%)[c]**

[a]Efficacy related to the maximum effect of the full agonist CP55,940 (1 µM = 100%).

[b]%Inhibition of THC (10 µM)-mediated *β*-arrestin recruitment.

[c]%Inhibition of LPI (1 µM)-mediated *β*-arrestin recruitment.

[d]%Inhibition of [^{3}H]CP55,940 binding.

Assays were performed according to:

Harms H, Rempel V, Kehraus S, Kaiser M, Hufendiek P, Müller CE, König GM. J Nat Prod 2014; 77: 673-677

4. Inhibition of Sulfotransferase

Compound **2** was tested for inhibitory activity on sulfotransferase (CST) at concentrations of 100 µM and 200 µM, and showed no inhibition of CST. The assays were performed by the group of Prof. Gieselmann, University of Bonn according to: Zech I. Substratreduktionstherapie der Metachromatischen Leukodystrophie: Expression der Cerebrosid-Sulfotransferase und Etablierung einer hochdurchsatzfähigen Aktivitätsbestimmung, PhD thesis, Universität Bonn, 2013.

Purity Analysis of Compound 2

The purity of compound **2** was assessed by three different HPLC methods:

Method A:

A Waters HPLC system equipped with a 996 PDA detector, a 600 pump, and 717 plus autosampler; column: Macherey-Nagel, EC 250 x 4.6 mm Nucleodur Sphinx; RP-18, 5 µm, mobile phase: MeOH/H_2O (80/20); flow: 2.0 mL min^{-1}.

Method B:

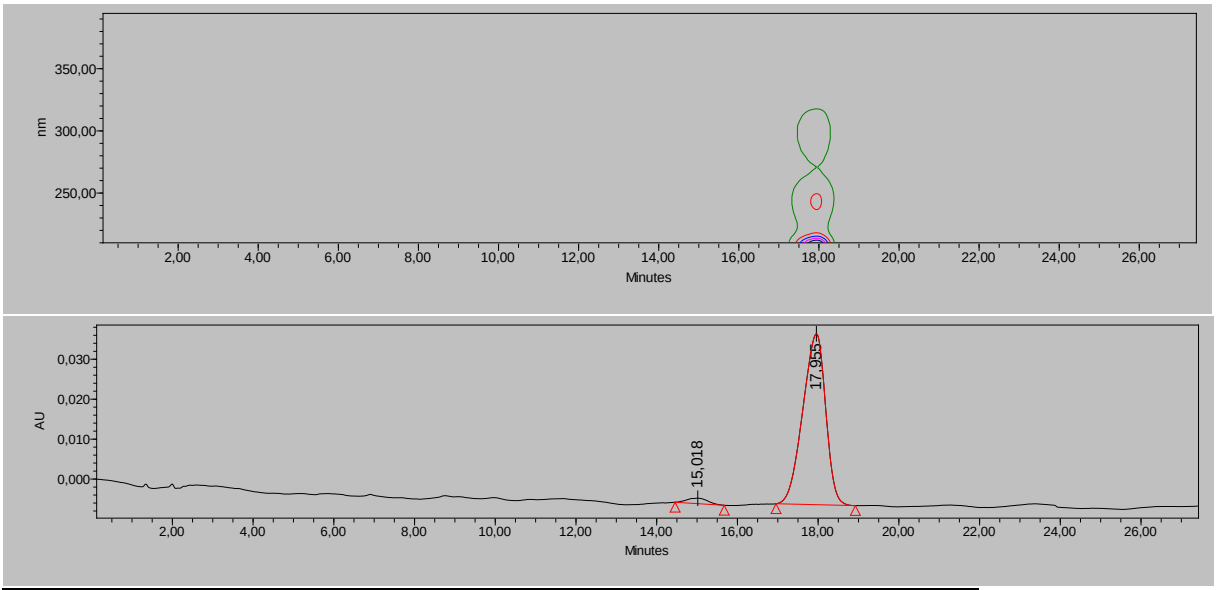
A Waters HPLC system equipped with a 996 PDA detector, a 600 pump and 717 plus autosampler; column: Atlantis RP-18, 5 um, EC 250 x 4.6 mm; mobile phase: MeOH/H_2O (80/20); flow: 2.0 mL min^{-1}.

Method C:

A Waters HPLC system equipped with a 996 PDA detector, a 600 pump and 717 plus autosampler; column: Macherey-Nagel; EC 250 x 4.6 mm; Nucleodur Isis, RP-18, 5 um, mobile phase: MeOH/H_2O (80/20); flow: 2.0 mL min^{-1}.

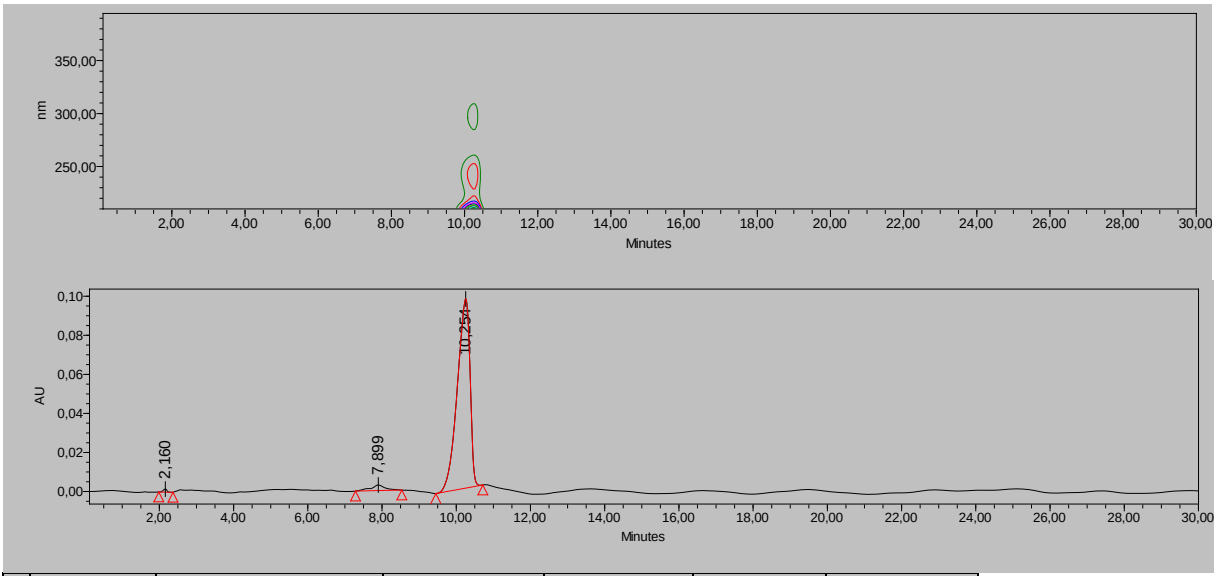
Table S1.4: Purity analysis of compound **2** with HPLC.

Method	**Wavelength**	**Retention Time**	**Purity**
A	240 nm	17.95 min	97%
B	240 nm	10.25 min	96%
C	240 nm	9.28 min	97%



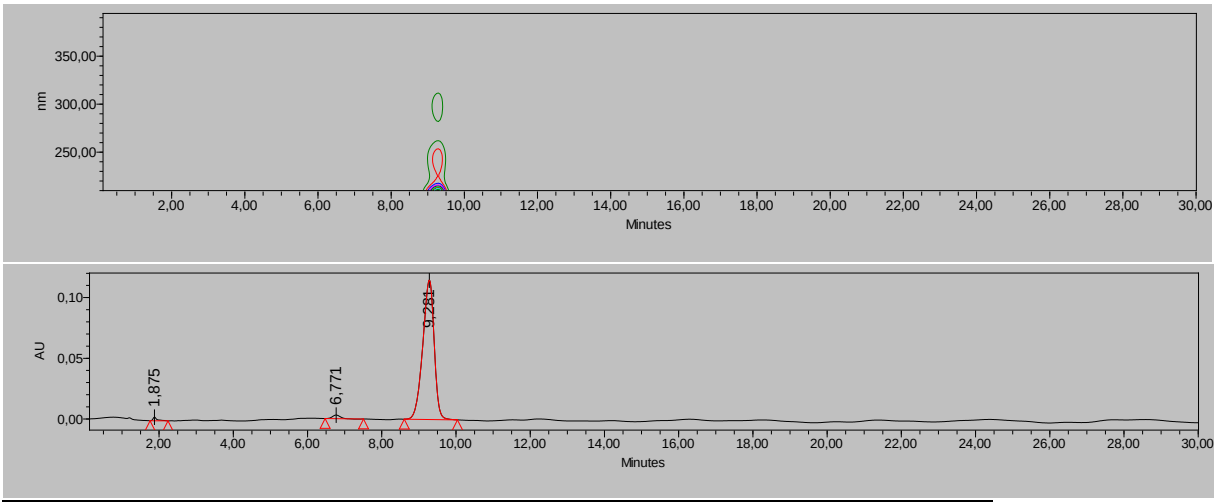
	Name	Retention Time	Area	% Area	Height	Int Type
2		17,955	1682501	97,19	42769	bb
1		15,018	48609	2,81	1381	bb

Fig. S1.12: HPLC chromatogram (contour plot and detection at 240 nm) of compound **1** using method A.



	Name	Retention Time	Area	% Area	Height	Int Type
3		10,254	2312829	96,24	96897	bb
2		7,899	79277	3,30	2880	bb
1		2,160	11205	0,47	1616	bb

Fig. S1.13: HPLC chromatogram (contour plot and detection at 240 nm) of compound **1** using method B.



	Name	Retention Time	Area	% Area	Height	Int Type
3		9,281	2452727	97,26	114947	bb
2		6,771	45149	1,79	2976	bb
1		1,875	24078	0,95	3003	bb

Fig. S1.14: HPLC chromatogram (contour plot and detection at 240 nm) of compound **1** using method C.