Biological activities of new secondary metabolite produced by *Streptomyces badius*

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Introduction

Search for bioactive compounds from nature play crucial role in fashioning new therapeutic agents. Especially secondary metabolites have major importance in drug discovery process. They are diverse and unusual in their chemical structures and may be used as scaffolds for further modifications. Actinomycetes are main producers of bioactive metabolites [1,2].

Actinomycetes are the most widely distributed groups of microorganisms in nature. They can be found in various environments such as soil and water. Their metabolites are active against bacteria, viruses, fungi, parasites and cancer cells [3].

References: [1] Dev S. (2010) Indian J. Exp. Biol. 48: 191-198.; [2] Berdy J. (2005) J. Antibiot. 58: 1-26; [3] Oskay M, Tamer AU, Azeri C (2004) Afr. J. Biotechnol. 3(9): 441-446.

Aim of the study

- Isolation and purification of a new metabolite from *Streptomyces badius* ATCC19888 fermentation broth.
- Determination of chemical structure of isolated compound.
- Evaluation of its biological activities.

Materials and Methods

Strain: *Streptomyces badius* ATCC 19888 was isolated from the soil in Kaukasus (Russia).

Fermentation and purification: fermenter (Sartorius Biostat A, Germany), HPLC (Knauer).

NMR analysis: The 1D ^{1}H and 13C NMR spectra as well as 2D homo- and heteronuclear spectra were collected on a 700MHz Bruker AVANCE III spectrometer, equipped with a QCI CryoProbe Experiments were performed at 25°C. Spectra were processed and prepared with TopSpin 3.0 Bruker Software.

HR MS analysis: MaldiSYNAPT G2-S HDMS (Waters) coupled with ACQUITY UPLC I-Class System (Waters).

Materials and Methods

Biological assays:

- The DD-peptidase 64-575 inhibition was measured spectrophotometrically according to the method previously described [4,5] with modifications.
- The DPPH and ABTS radicals scavenging activity was assayed based on methods previously described [6].

References: [4] Ch. Damblon et al.. Biochem. J. (1995), 309: 431-436; [5] M. Adam et al. Biochem J (1990) 270:525-529; [6] Solecka J. et al. Molecules (2014) 19:15866-15890.

Results: Fermentation

The *Streptomyces badius* ATCC 19888 was grown at 28°C for 10 days on yeast-malt agar medium. Then, *S. badius* spores were inoculated into a 500-ml Erlenmeyer flasks containing 35 ml of liquid medium M [7].

The inoculated flasks were incubated for 24h at 28°C in a rotary shaker at 220 rpm (Ecotron, Infors AG, Switzerland).
Then, 280 ml of seed culture was transferred to 4.5 l of the same medium in fermenter. Fermentation process was carried out 144h at 28°C with minimal aeration 30% of air. After that the supernatant was collected for purification.

References: [7] Rajnisz et al. (2016), Pol. J. Microbiol. 65(1):51-61

Results: Isolation and purification

***Streptomyces badius* ATCC 19888 supernatant (9 l)**

↓ 900g DowexWX40 (H^+) 100mesh
0-2M NH_3 (0,01M NH_3)

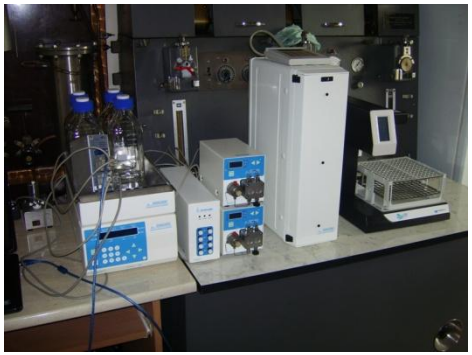
5.442g active fraction

↓ Solid Phase Extraction C18 Polar Plus columns J.T.Baker
CH_3CN-0.1% trifluoroacetic acid 15:85 v/v

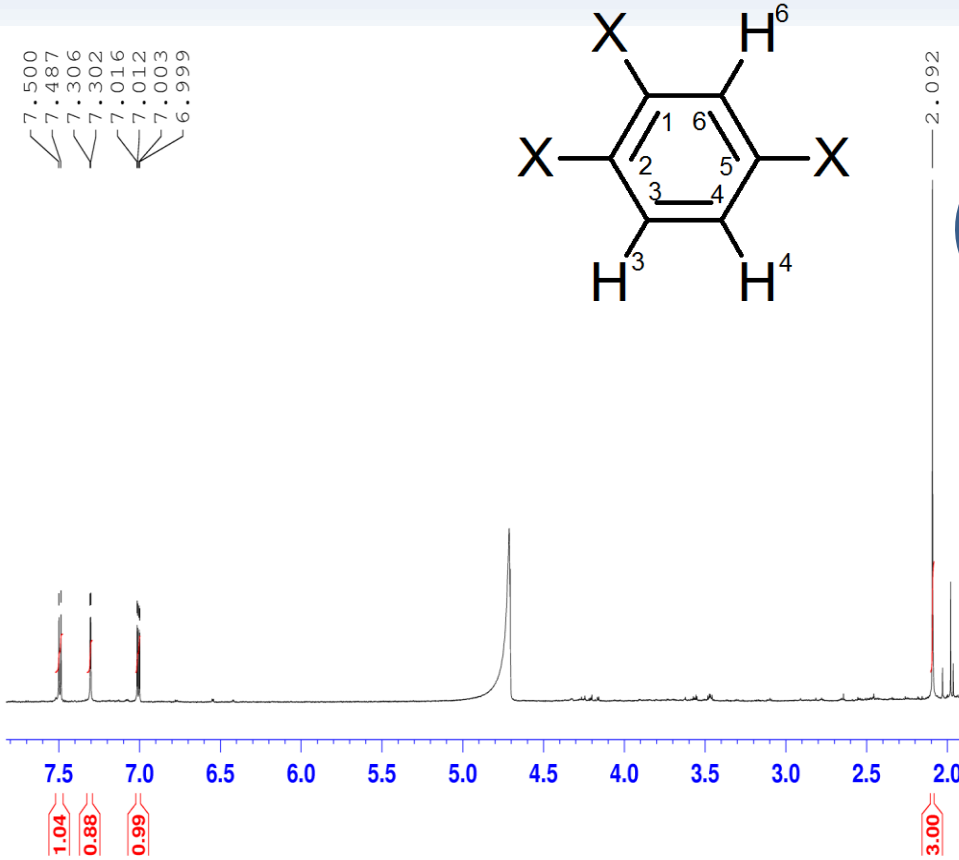
0.11368g active fraction

↓ HPLC, Atlantis dC18, Waters
34.22% phase B
(phase B, CH_3CN-0.1%TFA 20:80 v/v; phase A, 0.1%TFA)

1.389 mg of active metabolite (1)



Results: Structure determination



The ^{1}H NMR spectrum of **1** showed three signals in the aromatic region at 7.49 (d, 8.8 Hz, 1H), 7.30 (d, 2.9 Hz, 1H) and 7.01 ppm (dd, 8.8 Hz, 3 Hz, 1H) and a singlet at 2.09 ppm corresponding to the methyl group (3H). Signals at 7.49, 7.30 and 7.01 ppm were classified as ABM system and were assigned to H3, H6 and H4, respectively.

Figure 1. The ^{1}H NMR spectrum of 1 recorded in D_2O at 25°C.

Results: Structure determination

The ^{13}C NMR spectrum of **1** contains two signals in the carbonyl region, eleven in the aromatic region and one in aliphatic region. We observed five carbon signals more than expected from ESI-MS, thus they must have come from the contamination of the sample.

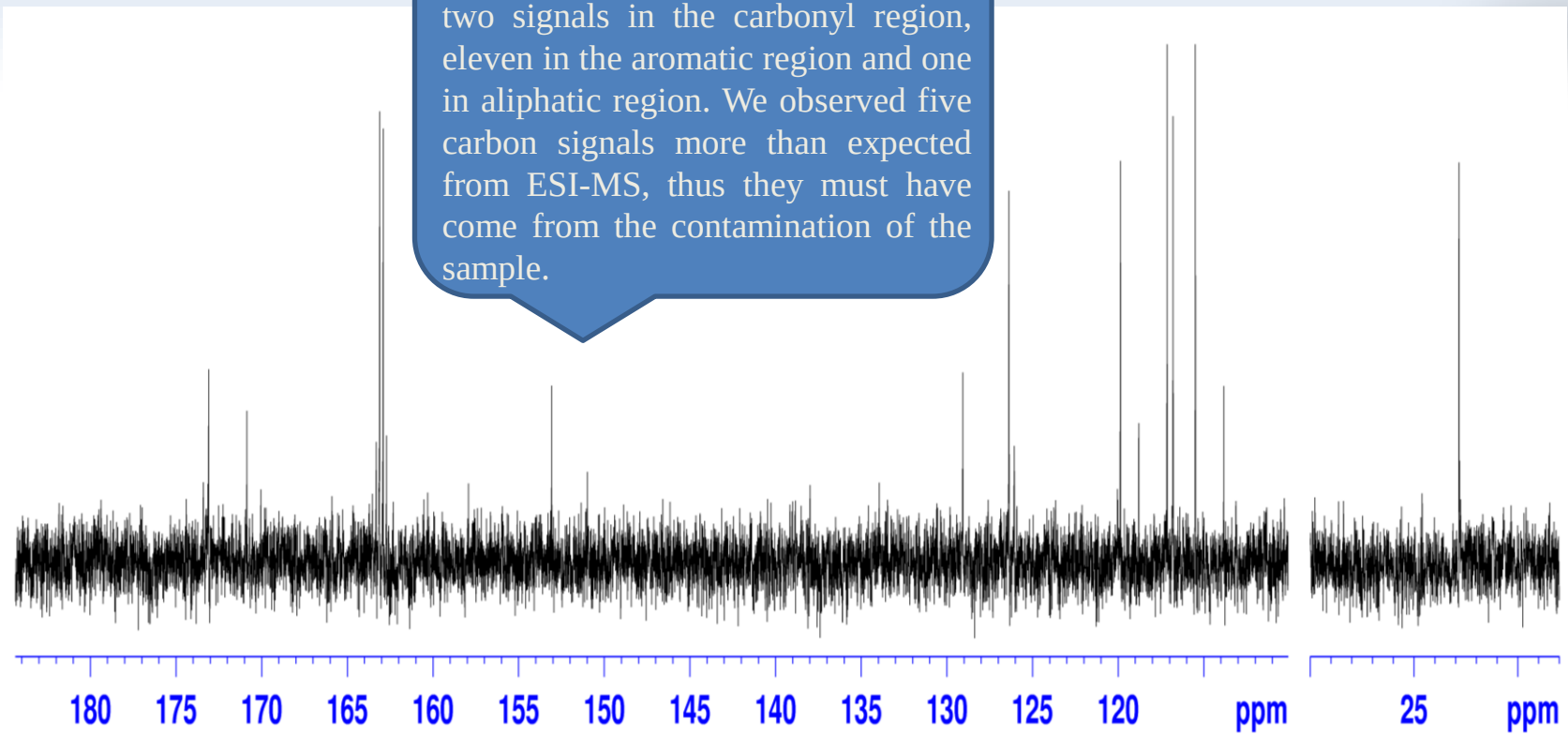


Figure 2. The ^{13}C NMR spectrum of 1 recorded in D_2O at 25°C.

Results: Structure determination

The set of 2D NMR data was used to establish the structure of compound **1**. The assignment of aromatic protons was confirmed using 2D COSY spectrum

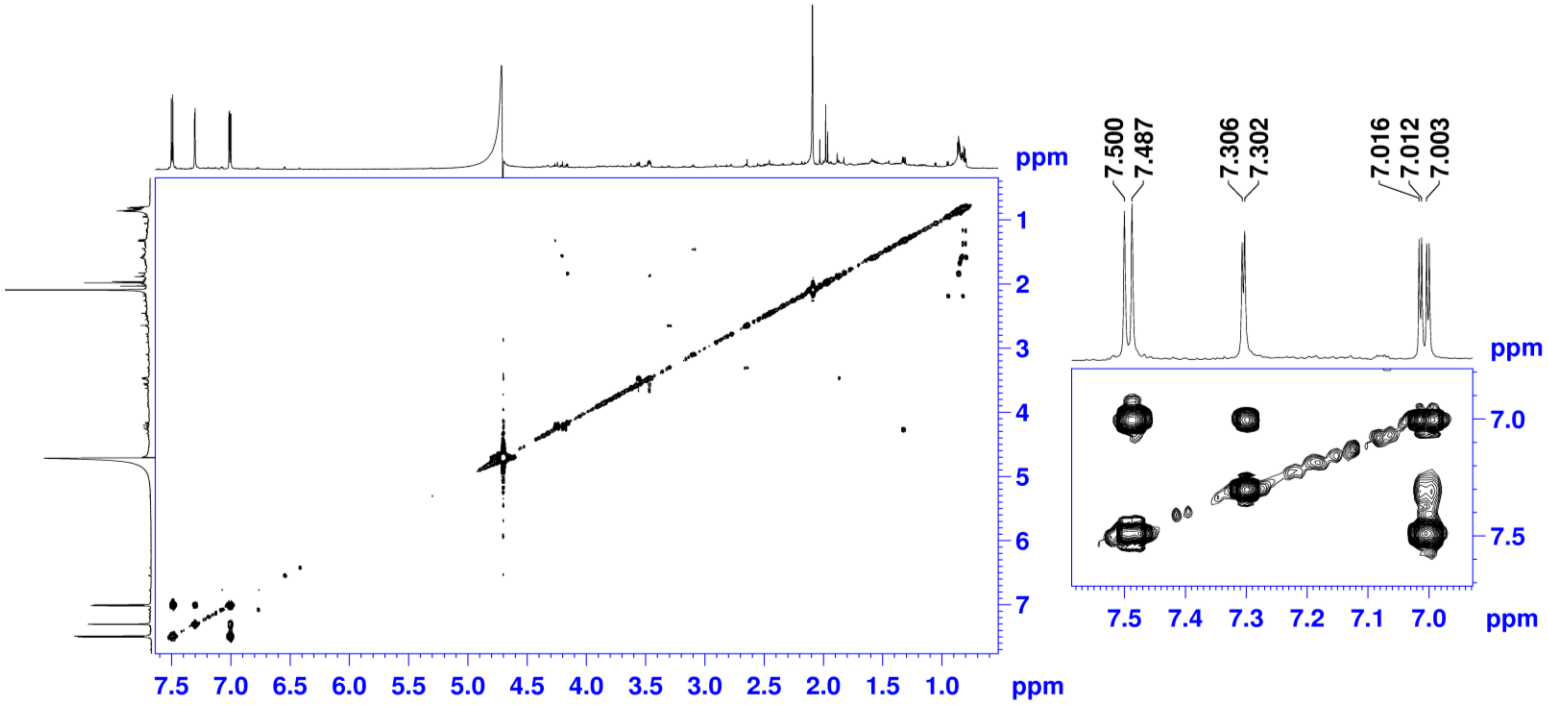
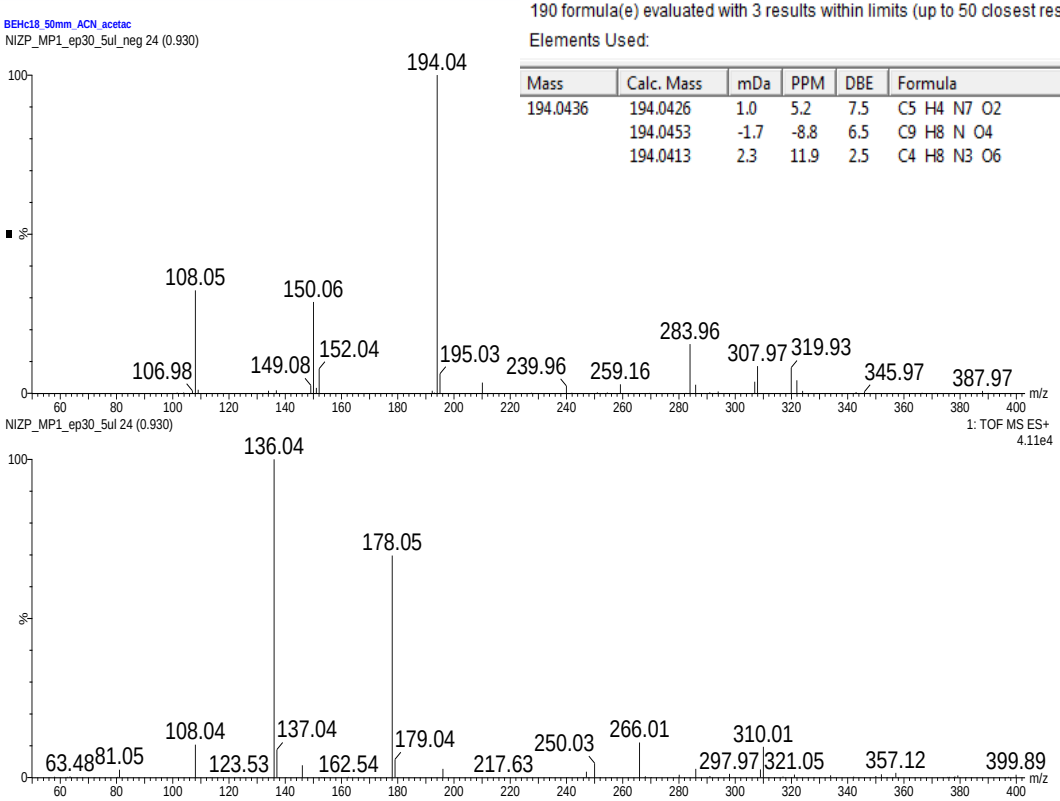


Figure 3. The 2D COSY spectrum of 1 recorded in D_2O at 25°C.

Results: Structure determination

Single Mass Analysis
Tolerance = 3.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2
Monoisotopic Mass, Even Electron Ions
190 formula(e) evaluated with 3 results within limits (up to 50 closest results for each mass)
Elements Used:

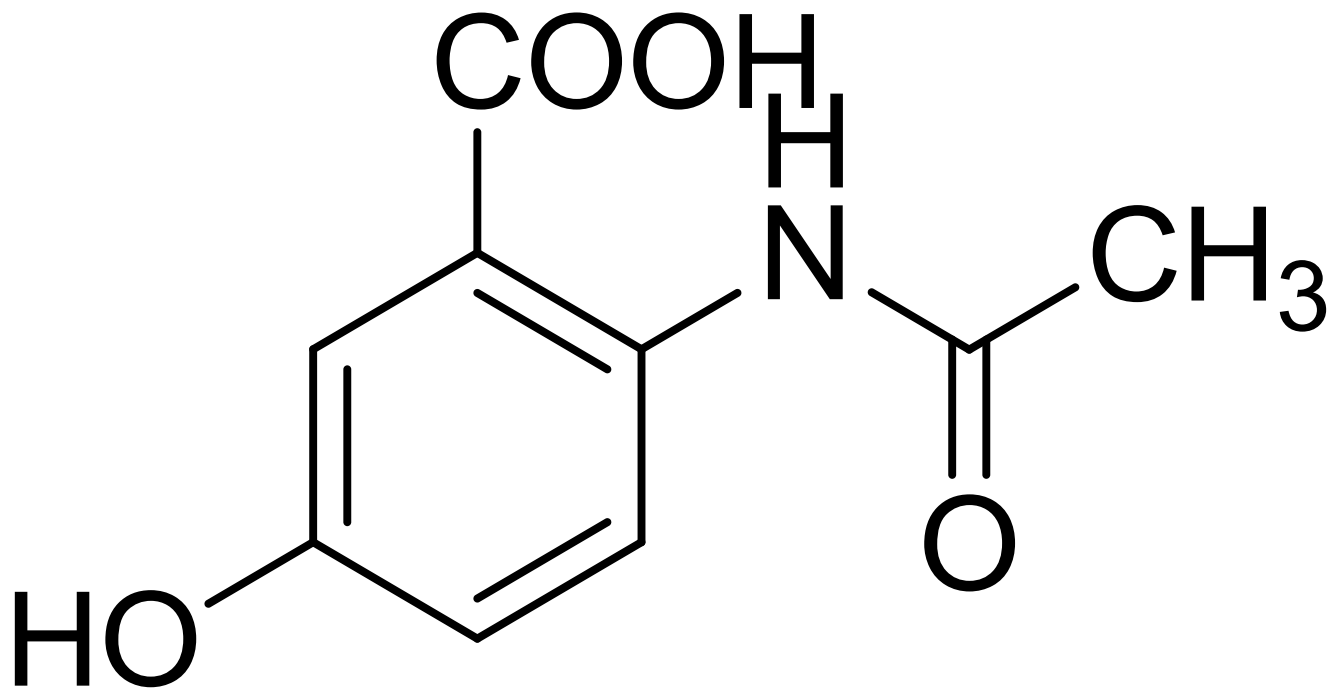
Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	N	O
194.0436	194.0426	1.0	5.2	7.5	C5 H4 N7 O2	22.4	4.864	0.77	5	4	7	2
	194.0453	-1.7	-8.8	6.5	C9 H8 N O4	25.0	7.514	0.05	9	8	1	4
	194.0413	2.3	11.9	2.5	C4 H8 N3 O6	17.5	0.008	99.17	4	8	3	6



The molecular formula of **1** ($C_9H_9NO_4$) was determined by high resolution ESI-MS [ESI-MS spectra for the peak with the RT 0.92 min of the sample **1** ep.30 – negative ion mode (top) and positive ion mode (bottom)]. The molecular formula prediction for the deprotonated molecule (*m/z* 194) in negative ion mode is shown in the table.

Results: Structure determination

The molecular formula of **1** was determined to be 2-acetamido-5-hydroxybenzoic acid ($C_9H_9NO_4$)



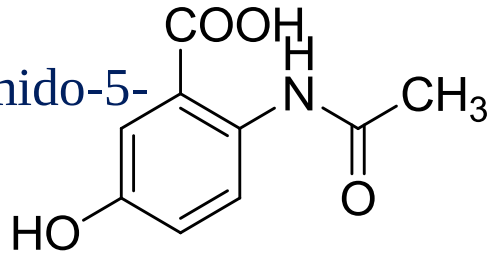
Results: Biological activities

Biological activity	2-acetamido-5-hydroxybenzoic acid ($C_9H_9NO_4$)
DD-peptidase 64-575 inhibition	IC_{50} = 0.21±0.04 mmol/l
DPPH radical scavenging t=4h	IC_{50} = 34.18±1.31 μg/ml
ABTS radical scavenging t=1h	IC_{50} = 3.93±0.10 μg/ml

Conclusions

❖ New metabolite **1** was isolated from *S. badius* fermentation broth and purified by chromatography methods.

❖ The molecular formula of **1** was determined to be 2-acetamido-5-hydroxybenzoic acid ($C_9H_9NO_4$).



❖ This compound shows DD-peptidase 64-575 inhibitory activity as well as prolonged antioxidative activity.

❖ Such chemical moiety may serve as model compound for further modern drug discovery and be a source of active substance in anti-ageing cosmetics.