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# Skeletocutins M-Q, biologically active compounds from the fruiting bodies of the basidiomycete *Skeletocutis* sp. collected in Africa

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# Abstract

During the course of screening for new metabolites from basidiomycetes, we isolated and characterized five previously undescribed secondary metabolites skeletocutins M-Q (**1**-**5**) along with the known metabolite tyromycin A (**6**) from the fruiting bodies of the polypore *Skeletocutis* sp. The new compounds did not exhibit any antimicrobial, cytotoxic or nematicidal activities. However, compound **3** moderately inhibited the biofilm formation of *Staphylococcus aureus*, while compounds **3** and **4** performed moderately in the L-leucine-7-amido-4-methylcoumarin (L-Leu-AMC) inhibition assay. These compounds represent the first report of secondary metabolites occurring in the fruiting bodies of *Skeletocutis*. Interestingly, tyromycin A was found to be the only common metabolites in fruiting bodies and mycelial cultures of the fungus, and none of the recently reported skeletocutins from the culture of the same strain was detected in the basidiomes.



# Keywords

polyporaceae; secondary metabolites; basidiomycete; structure elucidation


# Introduction

Over the past years, we have been studying the secondary metabolites of African Basidiomycota, based on material collected during several expeditions to the rainforests and mountainous areas of Western Kenya, These species did not only often turn to be new to Science but also proved to be a prolific source of unprecedented compounds with prominent biological activities [1-5].

The present study deals with the comparison of the secondary metabolites located in the basidiomes (fruiting bodies) of another putatively new species belonging to the genus *Skeletocutis*, strain MUCL56074. We have recently reported the known metabolite tyromycin A (**6**) together with 12 unprecedented congeners, for which we proposed the trivial names, skeletocutins A-L, from a liquid culture of the same fungus [6]. A preliminary characterization of the producer organism suggested that it belongs to a new species because neither DNA sequence data in the public domain nor morphological characters matched the previously reported species as compared to the literature The genus *Skeletocutis* (family Polyporaceae), consists of approximately 40 species and their species grow as a crust on the surface of collapsing wood [7] and

mostly occur in the temperate climate zones. In our preceding study, the fungal specimen MUCL56074 has been assigned to the genus *Skeletocutis* by comparison of morphological and 5.8S/ITS rDNA sequences as reported previously [6]. Strain MUCL56074 represents a hitherto undescribed species, which will be formally described in a separate paper in a mycological journal, pending the examination of type material of other, related species. In view of a potential application of chemotaxonomic methodology, the basidiomes (i.e. the fruiting bodies) of the fungus were checked for the presence of secondary metabolites for later comparison with herbarium specimens of other species by HPLC-DAD/MS. Surprisingly, we detected further members of the skeletocutin family that were not present in the cultures. The current paper is dedicated to the description of their isolation, biological and physicochemical characterization.

# Results and Discussion

The fruiting bodies of the specimen MUCL56074 were extracted with acetone followed by purification via preparative HPLC, which led to the isolation of five previously undescribed secondary metabolites **1**-**5** and one one, tyromycin A (**6**) [8] (Figure 1, Figure 2).

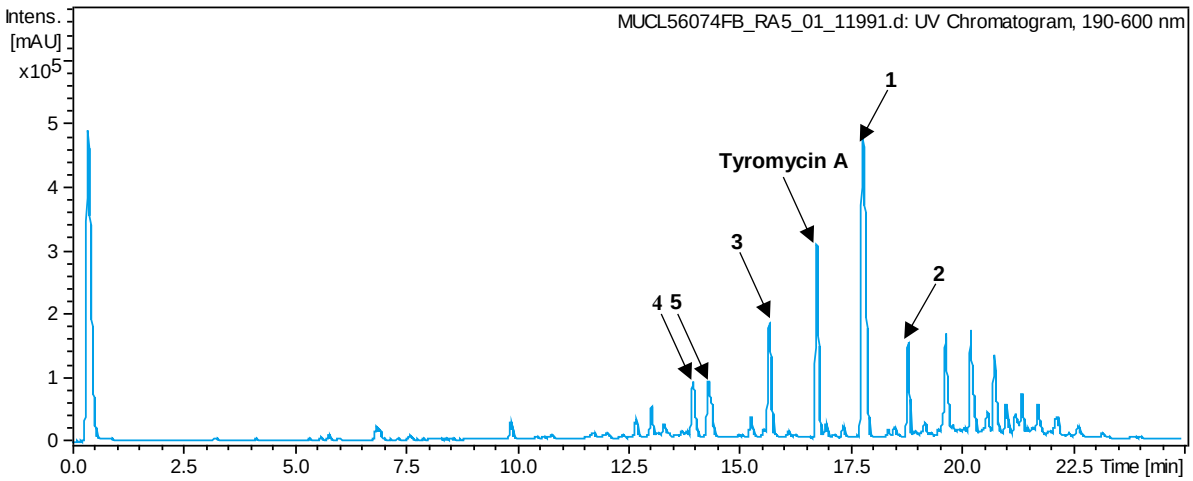


**Figure 1:** HPLC chromatogram profile of the extract from fruity bodies

Compound **1**, named skeletocutin M, was isolated as yellow solid. Its molecular formula was determined to be $C_{28}H_{42}O_6$ with eight degrees of unsaturation by HRESIMS. Peaks *m/z* 475.3054, 497.2868 and 971.5839 corresponding to the ions $[M+H]^+$, $[M+Na]^+$ and $[2M+Na]^+$ respectively were also recorded in the HRESIMS spectrum. Methyl singlet

resonating at ∂ 2.08 ($H_3$-6') and methylene groups triplet and quintet occurring at ∂ 2.50 and 1.59 were recorded in the $^1$H NMR spectrum. Further, the $^{13}$C NMR spectrum revealed only 14 signals instead of 28 as indicated in the molecular formula suggesting that the molecule consisted of two identical halves.

The HMBC correlations of C-6' methyl protons occurring at ∂ 2.08 to C-3'/4'/5' and $H_2$-1/$H_2$-18 to C-2'/C-3 '/C-4' confirmed the presence of the maleic anhydride moiety in the molecule. Integration of the C-6' methyl singlet signal in the $^1$H NMR spectrum gave an integral value of 6 confirming the two maleic anhydride groups. The methylene groups multiplet occurring between ∂ 1.28-1.36 was assigned to the remaining 14 methylene groups making up the carbon chain. Integration of this multiplet gave an integral value of 28 confirming the size of the chain. The connection of the chain to the two maleic anhydride was confirmed by the HMBC correlation of the $H_2$-1/$H_2$-18 and $H_2$-2/$H_2$-17 to C-3' (144.9). The long range COSY correlation of the $H_2$-1 to $H_3$-6' confirmed the orientation of the methyl group ($H_3$-6') in position 4'. The structure of **1** was therefore concluded unambiguously to be 1,18-bis [4′-methyl-2′,5′-dioxo-3′-furyl] octadecane.

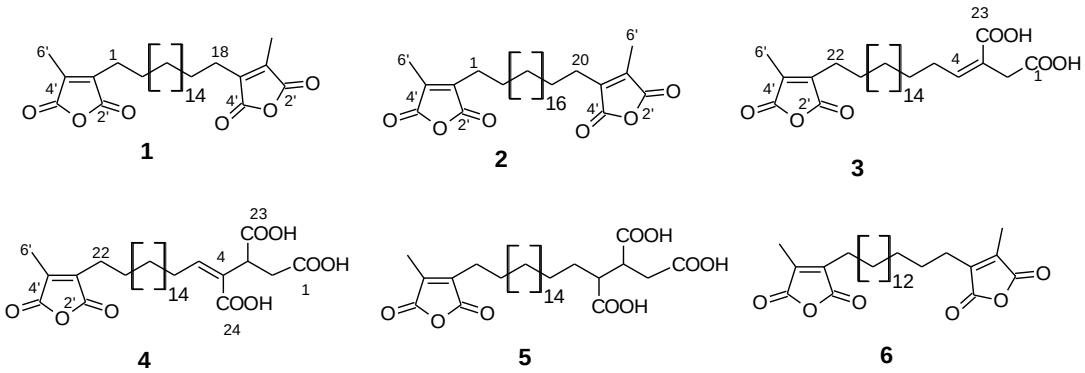


**Figure 2:** Chemical structures of compound **1**-**6**

Compound **2** (skeletocutin N) was obtained as a white solid with the molecular formula $C_{30}H_{46}O_6$ and eight degrees of unsaturation determined from HRESIMS data. The 1 and 2D NMR data of **2** revealed a similar structure as **1** with the difference being the size of the carbon chain in the molecule. The integral value of 32 obtained from the integration of the C-3-C-18 multiplet with resonance between ∂ 1.26-1.32 led to the conclusion that **2** had an icosane chain instead of the octadecane chain elucidated for **1**.

**Table 1:** NMR data ($^{1}$H 500, $^{13}$C 125 MHz) in acetone-$d_6$ for **1** and ($^{1}$H 500, $^{13}$C 125 MHz) in $CDCl_3$ for **2**

| | **1** | | **2** | |
|---|---|---|---|---|
| NO. | $^{13}$C | $^{1}$H/HSQC | $^{13}$C | $^{1}$H/HSQC |
| 1/18/20 | 24.8, $CH_2$ | 2.50 (t), J=7.63Hz | 24.4, $CH_2$ | 2.46 (t), J=7.60Hz |
| 2/17/19 | 28.2, $CH_2$ | 1.59 (p), J=7.63Hz | 27.6, $CH_2$ | 1.58 (p), J=7.60Hz |
| 3-16/18 | 29.4-29.8, $CH_2$ | 1.28-1.36 (m) | 29.2-29.7, $CH_2$ | 1.26-1.32 (m) |
| 2’ | 167.1, C | | 165.9, C | |
| 3’ | 144.9, C | | 144.8, C | |
| 4’ | 141.7, C | | 140.4, C | |
| 5’ | 167.4, C | | 166.3, C | |
| 6’ | 9.6, $CH_3$ | 2.08 (s) | 9.5, $CH_3$ | 2.08 (s) |

Compound **3** (skeletocutin O) isolated as a yellow oil had the molecular formula $C_{28}H_{44}O_7$ and seven degrees of unsaturation deduced from the HRESIMS data. The peak *m/z* 493.3163 for $[M+H]^+$, 515.2980 for $[M+Na]^+$, 475.3058 for $[M+H-H_2O]^+$, and 1007.6067 for $[2M+Na]^+$ were also recorded in the HRESIMS spectrum. A methyl singlet with resonance at *∂* 2.08 (H-$_3$-6'), along with a singlet (*∂* 3.37 ($H_2$-2)), quintet (*∂* 1.58 ($H_2$-21)) and a triplet (*∂* 2.45 ($H_2$-22)) for three methylene groups and a methine triplet at *∂* 7.13 (H-4) were observed in the $^{1}$H NMR of **3**. Analysis of the 1 and 2D data of **3** indicated a similar structure as **1** with difference being the absence of one of the maleic anhydride moiety which was replaced by a dicarboxylic acid moiety at one end of the chain and the oleifinic bond between C-3 (*∂* 124.5) and C-4 (*∂*148.9). The absorption peaks of the two carbons of the carboxylic acid moieties were occurring at *∂* 176.8 (C-1) and *∂* 172.0 (C-23). The dicarboxylic acid moiety could be elucidated from the HMBC correlations of $H_2$-2 to C-1/C-3/C-4/C-23, H-4 to C-23/C-3 and $H_2$-5 to C-3. Further cross peaks between $H_2$-5 and $H_2$-6/ H-4 were observed in the COSY spectra which confirmed the linkage of this moiety to the chain at C-3. The configuration of the C-3-C4 oleifinic bond was assigned the *Z* configuration because of the small coupling constant recorded for H-4 (7.56 Hz). Therefore, the structure of **3** was concluded to be (3*Z*)-3-[22-(4′-methyl-2′,5′-dioxo-2′,5′-dihydrofuran-3′-yl) nonadec-3-ene] butanedioic acid.

Compound **4**, named skeletocutin P, was isolated as a white solid. Its molecular formula was established to be $C_{29}H_{44}O_9$ with eight degrees of unsaturation from the HRESIMS data. The peak *m/z* 537.3058 for $[M+H]^+$, 559.2877 for $[M+Na]^+$, 519.2953 for $[M+H-H_2O]^+$ and 1095.5860 for $[2M+Na]^+$ were observed in the HRESIMS spectrum. The 1 and 2D NMR data of **4** were similar to **3** with the difference being the presence of a tricarboxylic acid moiety instead of the dicarboxylic acid moiety at one end of the chain. The three carboxylic acid moieties for the tricarboxylic acid part of the molecule had resonances at *∂* 174.5 (C-1), 173.8 (C-23) and 167.8 (C-24) in the $^{13}C$ NMR spectrum. The HMBC correlations of $H_2$-2 to C-1/C-3/C-4/C-23 and H-5 to C-3/C-24 and the COSY correlations of $H_2$-2 to H-3 confirmed the tricarboxylic acid moiety in the molecule. COSY correlations of $H_2$-6 to $H_2$-7/H-5 confirmed the linkage of this moiety to the rest of the molecule. The small coupling constant of H-5 (7. 36 Hz) led to the assignment of the C-4-C-5 double bond as having a *Z* configuration. Therefore, the structure of **4** was unambiguously elucidated as (4*Z*)-22-(4′-methyl-2′,5′-dioxo-2′,5′-dihydrofuran-3′-yl) docos-4-ene-1,2,3-tricarboxylic acid.

Compound **5** (skeletocutin Q) with molecular formula be $C_{29}H_{46}O_9$ and seven degrees of unsaturation established from the HR-MS data was obtained as a yellow solid. Analysis of the 1 and 2D NMR data of **5** indicated a similar structure as **4** with the C-4-C-5 olefinic bond missing. In the $^{13}C$ NMR spectrum of **5** the peaks occurring at *∂* 144.7 and 131.9 recorded for compound **4** were missing and instead a methylene signal resonance at *∂* 28.5 (C-5) and methine signal at *∂* 46.1 (C-4) were recorded. HMBC correlations were observed between H-4 (*∂* 2.50) to C-2/C-3/C-23/C-24 and $H_2$-5 (*∂* 1.56) to C-6/C-4/C-24. Furthermore, COSY correlations between H-3 and $H_2$-2/H-4 and $H_2$-5 and H-4/$H_2$-6 were recorded. Hence the structure of 5 was concluded to be 22-(4′-methyl-2′,5′-dioxo-2′,5′-dihydrofuran-3′-yl)docosane-1,2,3-tricarboxylic acid.
tyromycin A (6) , a closely related compound to the metabolites **1**-**5** has been reported before from the cultures of the same fungus (i.e. the corresponding mycelial culture of the specimen that was the subject of the present study [6]) and originally from *Tyromyces lacteus* [8]. In these two cases, tyromycin A was reported to be the major component of the culture extracts. Even though this compound is occurring in fruiting bodies, skeletocutin M (**1**) is the major component but not tyromycin A. The two compounds differ in the size of the chain with the latter having 18 carbon chain instead of the 16 carbon chain in tyromycin A.

**Table 2:** NMR data ($^{1}$H 500, $^{13}$C 125 MHz) in $CDCl_3$ for **3** and ($^{1}$H 500, $^{13}$C 125 MHz) in DMSO for **4-5**

| | **3** | | **4** | | **5** | |
|---|---|---|---|---|---|---|
| **No.** | $^{13}$C | $^{1}$H/HSQC | $^{13}$C | $^{1}$H/HSQC | $^{13}$C | $^{1}$H/HSQC |
| 1 | 176.8, C | | 174.5, C | | 174.3, C | |
| 2 | 32.1, $CH_2$ | 3.37 (s) | 35.8, $CH_2$ | 2.23 (dd), *J*=16.33, 5.80 Hz<br>2.92 (dd), *J*=16.33, 7.78 Hz | 33.6, $CH_2$ | 2.29 (dd), *J*=16.56, 2.37 Hz<br>[a]2.53 (m) |
| 3 | 124.5, C | | 39.6, CH | 3.86 (dd), *J*=7.78, 5.80 Hz | 42.8, CH | 2.83 (ddd), *J*=10.54, 6.67, 3.44 Hz |
| 4 | 148.9, CH | 7.13 (t), *J*=7.56 Hz | 131.9, C | | 46.1, CH | [a]2.50 (m) |
| 5 | 29.12, $CH_2$ | 2.23 (m) | 144.7, CH | 6.74 (t), *J*=7.36 Hz | 28.6, $CH_2$ | 1.56 (m) |
| 6 | 28.3, $CH_2$ | 1.48 (m) | 28.5, $CH_2$ | 2.19 (q), *J*=7.36 Hz | 28.6, $CH_2$ | 1.35 (m) |
| 7 | | | 28.5, $CH_2$ | 1.38 (m) | | |
| 8-20 | 29.3-29.7, $CH_2$ | 1.26-1.31 (m) | 29.1-29.5, $CH_2$ | 1.23-1.26 (m) | 28.82-29.0, $CH_2$ | 1.23-1.26 (m) |
| 21 | 27.6, $CH_2$ | 1.58 (p), *J*=7.63 Hz | 27.3, $CH_2$ | 1.48 (p), *J*=7.48 Hz | 26.9, $CH_2$ | 1.49 (p), *J*=7.96 Hz |
| 22 | 24.4, $CH_2$ | 2.45 (t), *J*=7.63 Hz | 24.1, $CH_2$ | 2.39 (t), *J*=7.48 Hz | 23.6, $CH_2$ | 2.40 (t), *J*=7.96 Hz |
| 23 | 172.0, C | | 173.8, C | | 172.9, C | |
| 24 | | | 167.8, C | | 174.7, C | |
| 2’ | 165.9, C | | 166.7, C | | 166.2, C | |
| 3’ | 144.8, C | | 143.9, C | | 143.4, C | |
| 4’ | 140.4, C | | 141.3, C | | 140.8, C | |
| 5’ | 166.3, C | | 166.9, C | | 166.4, C | |
| 6’ | 9.3, $CH_3$ | 2.08 (s) | 9.7, $CH_3$ | 1.99 (s) | 9.2, $CH_3$ | 2.00 (s) |

The isolated compound **1**-**6** were evaluated for antimicrobial, cytotoxic and nematicidal activities as described in the experimental section but **1**-**5** were devoid of activity in these assays. Tyromycin A (**6**) had been reported before to be active against several Gram-positive bacteria [6] namely *Bacillus subtilis*, *Staphylococcus aureus*, MRSA *Staphylococcus aureus* and *Micrococcus luteus*. In the antimicrobial assay, compound **3** and **5** were observed to interfere with the formation of biofilm commonly associated with *S. aureus*. Consequently, they were evaluated for biofilm inhibition activity against *S. aureus*, compound **5** and **3** showed weak activity with only 20% and 56% inhibition of the biofilm at concentration 256 µg/mL respectively. Tyromycin A (**6**) [8] was previously reported to be the inhibitor of leucine aminopeptidase in HeLa S3 cells, accordingly all the compounds **1**-**5** were tested for the inhibition activity of hydrolyze $_{L}$-Leu-AMC. Compound **4** exhibited moderate activity with $IC_{50}$ of 71.1 µg/mL when 50 µM of the substrate was used. Compounds **3** and **5** exhibited weak activities with $IC_{50}$ values > 80 µg/mL at both substrate concentrations (Figure 3; Table 3).

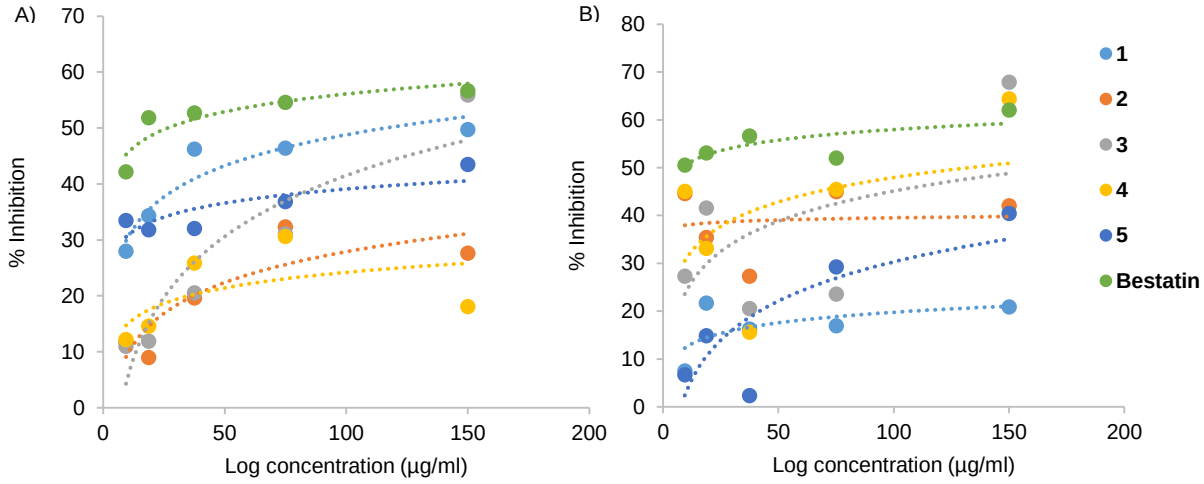


**Figure 3:** Inhibition of hydrolysis of leu-AMC

A) concentration of $_{L}$-Leu-AMC: 100µM B) concentration of $_{L}$-Leu-AMC: 50µM

**Table 3:** Inhibition of $_{L}$-Leu-AMC by the metabolites

| | $IC_{50}$ (µg/ml) | | | | | |
|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | Bestatin |
| L-Leu-AMC (50 µM ) | - | - | 89.6 | 71.1 | 153.2 | 10.8 |
| L-Leu-AMC (100 µM ) | - | - | 130.4 | 102.3 | 225.3 | 40.9 |

### Physicochemical data for compounds 1-5

Skeletocutin M (**1**): Yellow solid; UV (MeOH) $\lambda_{max}$ (log ε) 220 (4.26); HRESIMS *m/z* 475.3054 $[M + H]^+$, calcd for $C_{28}H_{43}O_6$, 475.3059.

Skeletocutin N (**2**): White solid; UV (MeOH) $\lambda_{max}$ (log ε) 214 (4.84), 254 (5.02); HRESIMS *m/z* 503.3374 $[M + H]^+$, calcd for $C_{30}H_{47}O_6$, 503.3373.

Skeletocutin O (**3**): Yellow oil; UV (MeOH) $\lambda_{max}$ (log ε) 220 (3.87); HRESIMS *m/z* 493.3163 $[M + H]^+$, calcd for $C_{28}H_{45}O_7$, 493.3165.

Skeletocutin P (**4**): White solid; UV (MeOH) $\lambda_{max}$ (log ε) 222 (5.14); HRESIMS *m/z* 537.3058 $[M + H]^+$, calcd for $C_{29}H_{45}O_9$, 537.3064.

Skeletocutin Q (**5**): Yellow solid; UV (MeOH) $\lambda_{max}$ (log ε) 206 (4.49), 256 (4.65); HRESIMS *m/z* 539.3215 $[M + H]^+$, calcd for $C_{29}H_{47}O_9$, 539.3220.

# Conclusion

In summary, five previously undescribed tyromycin A derivatives could be isolated from *Skeletocutis* sp. fruiting bodies. These metabolites are closely related to the skelotocutins previously reported from liquid cultures. Compound **3** and **5** were observed to weakly inhibit the biofilm formation of *Staphylococcus aureus*, and constrain activity of hydrolyzing $_{L}$-Leu-AMC in KB 3.1 cells as well. There have been relatively few studies on the production of secondary metabolites in mycelial cultures vs. fruiting bodies in higher fungi, but so far there are only few examples where the same compounds are predominant in both. For instance, in most species hitherto studied of the ascomycete order Xylariales, the fruiting bodies and cultures mostly showed a complementary secondary metabolite production [9], In the current case, it appears likely that the basidiomes of *Skeletocutis* can be used for chemotaxonomic studies, and investigations of herbarium specimens may not even be helpful for the

taxonomic revision of the genus but may even lead to the discovery of further previously undescribed members of the tyromycin/skeletocutin type.

# Experimental

## General experimental procedures

NMR spectra were recorded with 700 spectrometer with 5mm TXI cryoprobe ($^{1}$H 700 MHz, $^{13}$C 175 MHz, $^{15}$N 71 MHz) and Bruker AV Ⅱ-600 ($^{1}$H 600 MHz, $^{13}$C 150 MHz) spectrometers. HRESIMS mass spectra were recorded with Agilent 1200 series HPLC-UV system (column 2.1×50 mm, 1.7µm, C18 Acquity UPLC BEH (waters), solvent A: $H_2O$ + 0.1% formic acid; solvent B: AcCN +0.1% formic acid, gradient: 5% B for 0.5 min increasing to 100% in 19.5 min and then maintaining 100% B for 5 min, flow rate 0.6 mL/min, uv/vis detection 200-600nm combined with ESI-TOF-MS (Maxis, Bruker)) [scan range 100-2500 m/z, capillary voltage 4500 V, dry temperature 200 °C ]. UV spectra were recorded by using a Shimadzu UV-2450 UV−vis spectrophotometer.

## Fungal material

The fungal specimen was collected by C. Decock and J. C. Matasyoh from Mount Elgon National Reserve [6] Dried specimen and corresponding cultures were deposited at MUCL, Louvain-la-Neuve, Belgium, under the accession number MUCL 56074.

## Extraction of the crude extract

9.8 g fruiting bodies were extracted with 500 mL of acetone overnight, the extract was filtered and another 500 mL of acetone was added and extracted in ultrasonic bath for 30 minutes. The extracts were combined and the solvent evaporated to afford 226 mg of crude extract.

## Isolation of compounds 1-6

The crude extract was filtered using SPME Strata-X 33 u Polymeric RP cartridge (Phenomenex, Inc., Aschaffenburg, Germany). The extract was fractionated by preparative reverse phase liquid chromatography (PLC 2020, Gilson, Middleton, USA). VP Nucleodur 100-5C 18 ec column (25 x 40 mm, 7µm: Macherey-Nagel) used as stationary phase. Deionized water (Milli-Q, Millipore, Schwalbach, Germany) with 0.05% TFA (solvent A) and acetonitrile with 0.05% TFA (solvent B) were used as the

mobile phase. Elution gradient: 50% solvent B for 3 minutes followed by increasing to 100% in 60 min and finally isocratic condition at 100% solvent B for 5 min. Flow rate was 40 mL/min and UV detection was carried out at 210, 254, 350nm. Eight fractions F1-F8 were collected according to the observed peaks.

Fraction F1 was further purified by reverse phase HPLC (solvent A/solvent B), elution gradient: 45% solvent B for 3 minutes followed by 45%-100% solvent B for 18 minutes and thereafter isocratic conditions at 100% for 4 minutes with a preparative HPLC column (Kromasil, MZ Analysentechnik, Mainz, Germany; 250 x 20 mm, 7 µm C-18) as stationary phase and the flow rate was 15 mL/min, contributed to the identification of compound **4** (2.5 mg). Used the same column and elution gradient, compound **5** (2.87 mg) was purified from fraction F2; compound **3** (4.26 mg) was separated from F4; compound **1** (4.84 mg) was resulted from F6; compound **2** (2.26 mg) was identified from F7; tyromycin A (**6**, 2.15 mg) was carried out from F8.

## Antimicrobial assay

Minimum inhibitory concentration (MIC) were determined in serial dilution assays by using several microorganisms, as described previously [5,6]: Gram– positive bacteria: *Bacillus subtilis* DSM10, MRSA Methicillin resistant *Staphylococcus aureus* DSM11822, *Staphylococcus aureus* DSM346, *Micrococcus luteus* DSM20030 and *Mycobacterium smegmatis* ATCC700084; Gram– negative bacteria: *Escherichia coli* DSM498, *Chromobacterium violaceum* DSM30191 and *Pseudomonas aeruginosa* PA14; Filamentous fungi: *Mucor plumbeus* MUCL49355; Yeasts: *Candida tenuis* MUCL29892, *Pichia anomala* DSM6766 and *Candida albicans* DSM1665. The assays were conducted in 96-well plates in Mueller-Hinton Broth (MHB) for bacteria and in YMG medium for filamentous fungi and yeasts.

## Cytotoxicity assay

In vitro cytotoxicity ($IC_{50}$) was evaluated against mouse fibroblasts cell line L929 and HeLa (KB3.1) was carried out according to our previous papers [10,11].

## Inhibition of biofilm formation

The assay was performed in 96-well flat bottom plates (Falcon $^{TM}$ Microplates, USA) as previously described [12]. *Staphylococcus aureus* DSM1104 was enriched

overnight to reach 0.5 McFarland standard turbidity in casein-peptone soymeal-peptone (CASO) medium containing 4% glucose with PH7.0 for biofilm forming. Methanol was used as negative control while tetracycline was used as positive control. All experiments were made in triplicates.

### Nematicidal activity assay

The nematicidal activity of isolated compounds against *Caenorhabditis elegans* was performed in 24-well microtiter plates as previously described [13]. Ivermectin was used as positive control and methanol was used as negative control. The results were expressed as $LD_{90}$.

### Inhibition of leucine aminopeptidases

Hydrolysis activity of $_{L}$-Leucine-7-amido-4-methylcoumarin ($_{L}$-Leu-AMC) by the surface bound aminopeptidases of KB3.1 cells was assayed based on the method from Weber et al [8] with slightly modification. KB3.1 cells were grown as monolayer cultures in Dulbecco's Modified Eagle (DMEM) medium containing 10% of fetal calf serum at 37 °C in 24-well multidishes. After three days the confluent monolayers were washed with phosphate buffered saline (PBS) for twice, added reaction mixture (450 µl Hank's buffer PH 7.2 containing 50 µM and 100 µM substrate $_{L}$-Leu-AMC, and compounds dissolved in 50 µl DMSO). After incubated at 23 °C for 30 minutes, 1 ml cold 0.2 M glycine-buffer PH 10.5 was added. The amount of hydrolyzed 7-amino-4-methylcoumarin (AMC) was measured in a fluorescence spectrophotometer (excitation/emission: 365/440 nm; Tecan Infinite M200 PRO). Bestatin [14] and DMSO were used as positive and negative control, respectively.

## Supporting Information

Supporting Information File 1

HRESIMS data, $^{1}H$, $^{13}C$, COSY, HSQC, HMBC NMR spectra of metabolites, media composition for incubating microorganisms and ITS sequences of the producing strain.

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