Chinikomycin A and B: Isolation, Structure Elucidation and Biological Activity of Novel Antibiotics from a Marine *Streptomyces* sp. Isolate M045 [1]

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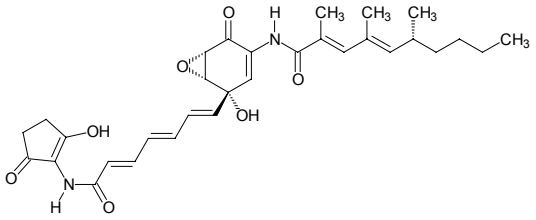
In our screening of marine Streptomycetes for bioactive principles, two novel antitumor antibiotics designated as chinikomycin A (**2a**) and B (**2b**) were isolated together with manumycin A (**1**), and their structure was elucidated by a detailed interpretation of their spectra. Chinikomycin A (**2a**) and B (**2b**) are chlorine containing aromatized manumycin derivatives of the type of 64-p-ABA-2 with an unusual *para* orientation of the side chains. They exhibited antitumor activity against different human cancer cell lines, however, were inactive in antiviral, antimicrobial, and phytotoxicity tests.

The manumycins constitute a class of antibiotics with antibiotic, cytotoxic and other biological activities; manumycin A (**1**) especially inhibits the Ras farnesyl transferase.[2] Structurally they contain a central six membered ring (*m*-C_7N unit) of biosynthetic origin from 3-amino-5-hydroxybenzoic acid, and two 1,3-arranged polyketide chains. A methyl-branched unsaturated fatty acid is linked to the central ring *via* an amide bond. The second side chain extends from the mC_7N unit as a structurally conserved all-*trans* triene (except in colabomycin A and D and U-62,162) and terminates with a carboxamide group linked to a 2-amino-3-hydroxycyclopent-2-enone moiety (C_5N unit).[3] The first member of the manumycin family, manumycin A (**1**), was isolated in 1963 by Zähner and co-workers from *Streptomyces parvulus* Tü 64.[4] Its constitution was determined in 1973,[5] the stereochemistry was reported in 1987 [2] and revised in 1998.[6]



1

In our screening program for bioactive principles from marine streptomycetes, two novel antibiotics belonging to the manumycin group were isolated, which we named chinikomycin A (**2a**) and B (**2b**) (niko in Nepali = to be cured). In addition, the strain also produced manumycin A (**1**). In this paper we report the isolation and the structure elucidation of **2a** and **2b** as well as the biological activity of these compounds.

Results and Discussion

The ethyl acetate extract of *Streptomyces* sp. isolate M045 drew our attention due to its biological activity against various microorganisms and microalgae. In the chemical screening of the ex-

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Dedicated to Prof. Dr. H.-G. Floss on the occasion of his 70th birthday
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tract, in addition to fats, fatty acids and prodigiosins, a striking middle polar yellow band was observed which was found to be responsible for the antibacterial activity.

On variation of the growth medium, temperature and starting pH, the production of the bioactive component was increased to about 15 - 25 mg/l in fish flour medium (FF-medium) with a pH starting at 6.5 and a cultivation temperature of 35 °C. The crude extract, obtained after usual work-up[7] of 60 of 1 l-Erlenmeyer flasks each containing 250 mL of FF medium, was subjected to flash chromatography on silica gel with a $MeOH/CH_2Cl_2$ gradient to yield three fractions. Repeated separation of fraction II delivered manumycin A (**1**) and chinikomycin A (**2a**) as yellow solids, and the red chinikomycin B (**2b**). The latter was produced by oxidation of **2a** during its isolation and is therefore not a primary natural product. Treatment of fraction III with silver oxide delivered a further crop of chinikomycin B (**2b**). Manumycin A (**1**) was easily identified by a substructure search in AntiBase[8] and by comparison of the NMR data with the literature values.[2]

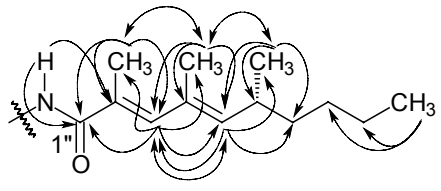
The molecular weights of **2a** and **2b** were determined by ESIMS to be 568 and 566, respectively. Isotopic peaks of the molecular ions indicated both to contain one chlorine atom each. ESI HR mass spectrometry of the $[M+Na]^+$ *quasi*-molecular signals led to the molecular formulae $C_{31}H_{37}ClN_2O_6$ and $C_{31}H_{35}ClN_2O_6$ for **2a** and **2b**, and thus the same number of carbon atoms is found as for manumaycin A (**1**). The proton NMR spectrum of compounds **2a** and **2b** showed both nine signals for protons at sp^2 carbon atoms, four methyl signals and further two multiplets of five and six protons at δ 2.6-2.4 and 1.4-1.2, respectively. In addition, the spectrum of **2a** contained five signals of acidic protons, whereas that of **2b** showed only three. This and the conversion of **2a** into **2b** with silver oxide and the reverse reaction with aqueous dithionite solution indicated the red **2b** to be the quinone of the hydroquinone **2a**. The ^{13}C NMR and APT spectra of **2a** showed only 27 instead of 31 signals. The spectra of compound **2b**, however, exhibited two further carbonyl signals and two additional methylene carbon signals as demanded by the molecular formula.

Interpretation of the 2D NMR spectra (H,H COSY, HMQC, HMBC and NOESY) led to 2,4,6-trimethyl-2E,4E-deca-2,4-dienoic acid amide as substructure **I** of both compounds **2a** and **2b** (Figure 1). This fragment is known as part of manumycin (**1**),[2] and was finally confirmed by comparison of the ^{13}C NMR data with the literature values (Table 1), and by the presence of **1** in the extracts.

Table 1. ^{13}C NMR (125.7 MHz, δ values) data of **1**, **2a** and **2b**

C No.	**2a**[a]	**2b**[b]	**1**[b]	C No.	**2a**[a]	**2b**[b]	**1**[b]	C No.	**2a**[a]	**2b**[b]	**1**[b]
1	136.8	176.3	189.0	12	121.0	123.7	121.7	5"	141.6	144.2	142.7
2	128.6	137.7	128.0	13	166.1	165.2	165.6	6"	32.1	32.9	32.9
3	107.6	114.7	126.6	1'	n.v.	197.5	197.6	7"	36.4	37.0	37.1
4	150.3	185.2	71.2	2'	114.9	115.1	115.5	8"	29.2	29.7	29.8
5	117.0	138.4	57.4	3'	n.v.	174.6	174.5	9"	22.2	22.8	22.8
6	122.2	135.0	52.8	4'	n.v.	32.1	32.2	10"	13.9	14.0	14.1
7	130.4	127.3	136.6	5'	n.v.	25.6	25.7	2"-Me	14.1	14.1	14.0
8	132.8	144.3	131.4	1"	168.3	168.3	168.8	5"-Me	16.3	16.4	16.5
9	143.0	141.5	139.6	2"	128.5	127.6	128.4	6"-Me	20.6	20.6	20.7
10	129.8	136.3	131.7	3"	138.7	141.8	140.2	-	-	-	-
11	142.6	142.8	143.4	4"	130.0	129.9	129.9	-	-	-	-

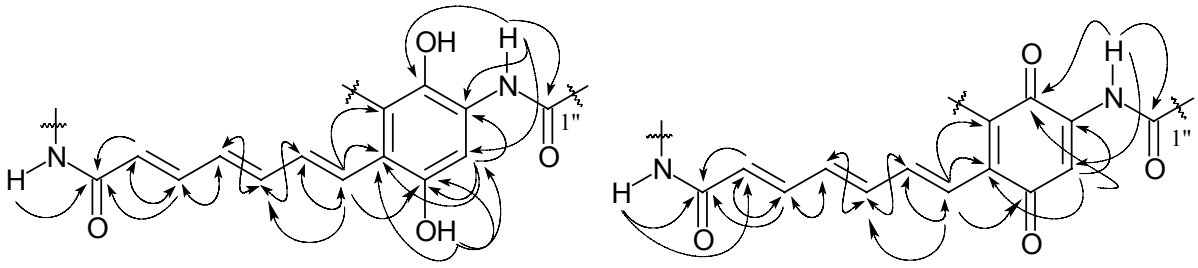
[a]DMSO-d_6, [b]$CDCl_3$, n.v. = not visible



I

Figure 1. (2E,4E)-2,4,6-Trimethyldeca-2,4-dienoic acid amide fragment (**I**) of chinikomycins A (**2a**), B (**2b**) and of manumycin A (**1**) derived from H,H COSY (not shown), HMQC (not shown), HMBC (→) and NOESY experiments (↔)

With the aid of the coupling constants of the olefinic protons and their H,H COSY correlations, a system of three conjugated *trans* double bonds was constructed. By careful interpretation of the H,H COSY, HMQC and HMBC couplings, substructures **II** and **III** were developed for compounds **2a** and **2b**, respectively (Figure 2). The carbon skeleton of substructure **II** is part of 64-p-ABA-2 (**2c**),[9] which was obtained by feeding of *Streptomyces parvulus* with 4-aminobenzoic acid, however, in **2a** two additional hydroxyl groups and the chlorine atom are present.



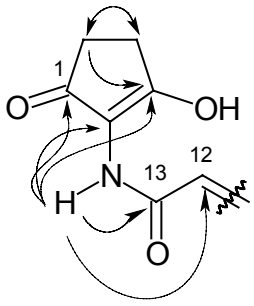
II **III**

Figure 2. Correlation of the HMBC (→, only selected) and H,H COSY (↔) experiment in the fragments **II** and **III** of chinikomycin A (**2a**) and B (**2b**), respectively

In the substructures **I** and **II** of **2a** and **I** and **III** of **2b**, all expected 26 carbon atoms can be assigned easily. As in **2a** not all carbon signals were visible, further interpretation was done on **2b**. The resulting substructure **IV** (Figure 3) was confirmed by comparison of the ^{13}C NMR data with the respective part of manumycin A (**1**). Also in the latter, the carbon signals of the cyclopentan-1,3-dione moiety are broad,[10] which explains the missing signals in **2a**. The sub-structure

IV is additionally proven by the similar fragmentation pattern on ESI MS-MS of manumycin A (**1**) and chinikomycin A (**2a**): The *quasi*-molecular signals at *m/z* 57

3 ($[M+Na]^+$) and 591 ($[M+Na]^+$) of **1** and **2a** delivered ions at *m/z* 460 and 478, respectively, by loss of the 2-amino-cyclopentan-1,3-dione fragment.

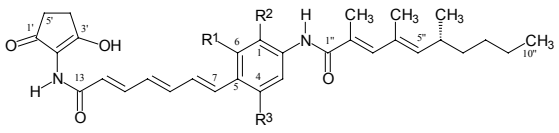


IV

Figure 3. Cyclopentan-1,3-dione fragment (**IV**) of the chinikomycins derived from H,H COSY (↔), HMQC (not shown) and HMBC (→) spectra of **2a**/**2b**

As the amide carbonyl group C-1" in **I** is overlapping with the anilide structure in **II** and **III**,

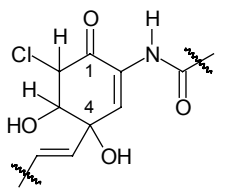
and as **IV** must be connected with the triene chain of **II**/**III** as in **1** (see Tab. 1), the chlorine atom must be attached to the free valence at C-5 of the fragments **II**/**III** to complete the structures **2a** and **2b** of chinikomycin A and B.



2a: R_1 = Cl, R_2 = R_3 = OH
2b: quinone form of **2a**
2c: R_1 = R_2 = R_3 = H

Whereas 64-p-ABA-2 (**2c**) and a few related compounds have been obtained in a precursor-directed biosynthesis by feeding of *p*-aminobenzoic acid,[9] the chinikomycins are the first truly natural manumycin antibiotics with a *para* orientation of the side chains. We realized however, that a compound with identical shift values as for **2a** had been isolated by one of the authors previously from *Streptomyces parvulus* Tü 64.[11] The isolated amount had been insufficient to solve the structure with the technical capabilities at that time.

A chlorohydrin **3** is not an intermediate in the biosynthesis of **1**,[3] however, may be formed from the latter by ring opening with chloride and could easily loose water at C-5. The carbocation thus formed could undergo a sextet rearrangement to deliver **4**, and deprotonation at OH-4 yielding a carbonyl group and enolisation at C-1/C-4 would end up with **2a**. It is obvious, that the assumption of an alternative chlorohydrin **5** would finally deliver the isomer **6** even without a rearrangement of the carbon skeleton. There is, however, little doubt that **2a** is the preferable structure: According to the HMBC data (Figure 2), the OH group at δ 10.04 must be connected with the carbon atom at δ 150.3 and be placed in between the aromatic CH (C-3) and the ring atom bearing the conjugated chain (C-5). In **6**, these correlations are substituted by less favorable 4- and 5-bond couplings of OH-6. Furthermore, H-7 shows a cross signal with the signal at δ 150.3 (C-4) which is due to a ^3J coupling in **2a**, but would request a ^4J coupling in **6**. Similar correlations supporting structure **2b** are found in the HMBC spectrum of the corresponding quinone. Diagnostic is the strong cross signal between H-7 (δ 6.83) and δ 185.2 as expected for CO-4 in **2b**; in **6**, the corresponding coupling should be much weaker (^4J). The cross signal between H-3 and C-7 is very weak as expected for **2b**, but should be strong in **6**. An NOE signal between H-3 and H-7/8 is not visible (but would be expected for **6**), and a strong interaction between the OH-4 group (signal at δ 10.04) and H-3 is a final proof for their *ortho* orientation as in **2a**. Also do the experimental values agree better with the calculated ^{13}C shifts[12] on the basis of **2a** than for **6**. It should be mentioned additionally that the formation of chinikomycin B by air oxidation is explained better by **2b** than by an *ortho*-quinone derived from **6** with its higher oxidation potential.



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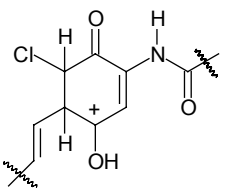

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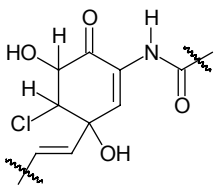

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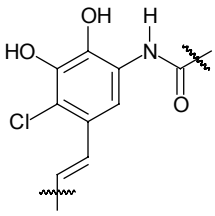

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Biological Activity. Chinikomycin A (**2a**) and B (**2b**) both exhibited moderate antitumor activity, **2a** being the more potent one. The hydroquinone **2a** selectively inhibited proliferation in cell lines of mammary cancer (MAXF 401NL, IC_{50} = 2.41 µg/mL,), melanoma (MEXF 462NL, IC_{50} = 4.15 µg/mL), and renal cancer (RXF 944L, IC_{50} = 4.02 µg/mL). The quinone **2b** showed selective antitumor activity[13] against the mammary cancer cell line MAXF 401NL (IC_{50} = 3.04 µg/mL) (see Table 2). Cell lines of non small cell lung cancer (LXFA 629L, LXFL 529L), and uterus cancer (UXF 1138L) were not sensitive to **2a** and **2b**. The chinikomycins exhibited not antimicrobial and antiviral activities.

Table 2. Antitumor activity of **2a** and **2b** in human tumor cell lines

	IC_{50}[a]	
Tumor cell line	**2a**	**2b**
LXFA 629L	>6.0	>6.0
LXFL 529L	>6.0	>6.0
MAXF 401NL	2.41	3.04
MEXF 462NL	4.15	>6.0
RXF 944L	4.02	>6.0
UXF 1138L	>6.0	>6.0

[a] IC_{50} values in [µg/mL].

Experimental Section

General Experimental Procedures. Material & methods and antimicrobial tests were used as described earlier[7]. ESI-HRMS was measured on Micromass LCT mass spectrometer coupled with a HP 1100 HPLC with a Diode Array Detector. Reserpin (MW = 608) and Leucin-Enkephalin (MW = 555) were used as standards in positive and negative mode. R_f-values were measured on Polygram SIL G/UV_{254} (Macherey-Nagel & Co., Düren, Germany) with dichloromethane/10 % methanol when not stated otherwise.

FF-medium. Glucose (21 g), fish powder (5 g), wheat flour (10 g), yeast extract (1 g), magnesium sulfate (0.5 g), sodium chloride (1 g), calcium chloride (0.5 g) and trace element solution (10 mL) were dissolved in artificial sea water (0.5 L) and tap water (0.5 L). Trace element solution: Ferrous sulfate (10.2 g), cobalt chloride (0.04 g), calcium chloride (0.04 g), manganese chloride (0.04 g), zinc sulfate (0.08 g), sodium borate (0.08 g), and sodium molybdate (0.74 g) were dissolved in 500 mL water.

M_2^+ medium. Malt extract (10 g), yeast extract (4 g) and glucose (4 g) were dissolved in artificial seawater (0.5 L) and tap water (0.5 L). The medium is set to pH 7.8 prior to sterilization.

LB medium. Tryptone (10 g), yeast extract (5 g), sodium chloride (10 g) and glucose (5 g) were dissolved in artificial seawater (0.5 L) and tap water (0.5 L).

SM medium. Defatted soybean flour (20 g) and mannitol (20 g) were dissolved in artificial seawater (0.5 L) and tap water (0.5 L).

ME medium. Meat extract (1 g), glucose (10 g), peptone (2 g) and yeast extract (1 g) were dissolved in artificial seawater (0.5 L) and tap water (0.5 L).

CL medium. Calcium chloride (45 g), glucose (5 g) and yeast extract (40 g) were dissolved in artificial seawater (0.5 L) and tap water (0.5 L).

Gause's starch medium. Soluble starch (20 g), potassium nitrate (1 g), potassium hydrogen phosphate (0.5 g), magnesium sulfate (0.5 g), sodium chloride (0.5 g), ferrous sulfate (0.01 g) and agar (18 g) were dissolved in 1 L water containing 50 % natural sea water and 0.1 mg/mL $K_2Cr_2O_7$. The pH of the solution was set to 7.2 before sterilization.

Taxonomy of the producing strain. The strain M045 has been derived from sediment of Jiaozhou Bay in China. It was isolated on Gause's starch medium with incubation at 28 °C. The pure culture was maintained on Gause's starch agar medium with $K_2Cr_2O_7$ at 4 °C. The strain forms red vegetative mycelium and white aerial mycelium. The substrate mycelium does not have transverse septa and not fragment. The aerial mycelium has a lot of branches. The strain forms spiral sporophores (*Spirales*). The spores are oval with smooth surface. Melanin pigment is not produced on tyrosine agar[14] and water-soluble pigment is not produced on other media. The temperature optimum is at ~28 °C. The strain does not grow at 45 °C or at 10 °C. Gelatin and starch are degraded. Hydrogen sulfide is not produced. The strain is catalase positive and nitrate reductase is not formed. Cell walls are purified and amino acids analyzed using thin-layer chromatography as described by Lechevalier and Lechevalier.[15] The peptidoglycan cell wall of the strain contains major amounts of L-diaminopimelic acid

(L-DAP) and glycine but no diagnostic sugars in whole cell hydrolysates of the strain (cell wall chemotype I).[16] Due to its chemical and morphological features ans well as the 16S rRNA (GenBank accession number AY644669), the strain can be assigned to the genus *Streptomyces*. The strain is deposited in the culture collection of marine actinomycetes at the Institute of Oceanology, Chinese Academy of Sciences, Nanhai Road 7, 266071 Qingdao, China.

Optimisation of chinikomycin A (2a) production by *Streptomyces* sp. isolate M045. In M_2^+ medium using our standard conditions for the screening of streptomycetes, the strain *Streptomyces* sp. isolate M045 produced chinikomycin A (**2a**) only in a very small amount (<<1 mg/L). In order to increase the production of **2a**, the strain was grown in different media under four different conditions. The **2a** concentration was determined by integration of the HPLC signal and by comparison with a pure sample. The results of the optimization are listed in Table 3.

Table 3. Results of the optimization of chinikomycin A (**2a**) production (mg/L) by *Streptomyces* sp. isolate M045 (pH values were adjusted by adding dilute NaOH prior to sterilization)

Medium	pH 6.5		pH 8.0	
	28 °C	35 °C	28 °C	35 °C
M_2^+ medium	0	0	<<1	0
SM medium	0	0	0	0
ME medium	0	0	<<1	0
CL medium	0	0	0	0
LB medium	0	0	0	0
FF medium	0	15-25	0	<<1

Fermentation of *Streptomyces* sp. isolate M045 and work-up. The marine strain *Streptomyces* sp. isolate M045 was inoculated from its slant agar culture on M_2^+ agar plates with 50 % seawater. It formed red colonies with a white aerial mycelium and a red coloration of the agar on incubation at 28 °C while shaking for 72 h. With fresh agar cultures of the strain, 60 of 1 L Erlenmeyer flasks each containing 250 mL of fish flour medium (set to the pH 6.5 before sterilization) were inoculated and grown for 4 days at 35 °C while shaking at 120 rpm. A dark brown culture broth with a yellowish gray mycelium was obtained.

The entire culture broth was mixed with ca. 1 kg diatomaceous earth, pressed through a pressure filter and both filtrate and residue were extracted with ethyl acetate. The combined extracts were evaporated *i. vac*. at 30 °C to dryness to yield 14 g of the crude extract, which contained yellow **2a**, prodigiosins and a large amount of fat and fatty acids. The extract was triturated with cyclohexane (250 mL) and the insoluble solid was separated by filtration through a sintered glass funnel to get extract A (cyclohexane soluble part) and the precipitate B (insoluble material). The solid B was subjected to silica gel column chromatography (3 × 60 cm, 270 g silica gel, dichloromethane/methanol gradient, 1.0 L CH_2Cl_2, 2.0 L CH_2Cl_2/0.5 % MeOH, 1.0 L CH_2Cl_2/2 % MeOH) and separated under TLC control into fraction I (80 mg), II (325 mg) and III (414 mg). Fraction I contained only fats and prodigiosins and was not further analyzed.

Fraction II was washed with cyclohexane/25 % dichloromethane (2 × 25 mL) to get a yellow solid (fraction IIa) containing mainly **2a**. On TLC, the yellow zone of **2a** (R_f = 0.50) turned to red with sodium hydroxide. On silica gel and Sephadex LH 20, **2a** is partially oxidized forming red **2b**. The crude material was separated twice by PTLC (20 × 20 cm, CH_2Cl_2/5 % MeOH) and quickly recovered while the plates were still wet to deliver 50 mg of chinikomycin A (**2a**). The red zone that appeared during the separation yielded 3.5 mg of red chinikomycin B (**2b**). The cyclohexane/25 % dichloromethane soluble part (fraction IIb) showed strong antibacterial activity and contained a light yellow band on TLC, which was more stable than **2a** and showed no color reaction with dilute sodium hydroxide but turned to blue violet on spraying with anisaldehyde/sulfuric acid. Purification of fraction IIb by PTLC (20 × 20 cm, CH_2Cl_2/7 % MeOH) followed by Sephadex LH-20 yielded 90 mg of manumycin A (**1**, R_f = 0.50).

Extract A was similarly purified on silica gel columns first with a dichloromethane-methanol gradient and then with cyclohexane/70 % ethyl acetate. The product was finally washed twice with cyclohexane (2 × 50 mL) to yield another 100 mg **2a** and 30 mg of **1**.

Chinikomycin A (**2a**). Yellowish brown solid; R_f = 0.34 (CH_2Cl_2/5 % MeOH); UV/VIS (MeOH) λ_{max} (log ε) 260 (4.46), 335 (s, 4.46), 395 (4.62) nm; OR $[\alpha]_D^{20}$ –86° (c 0.02, CHCl3); no CD effect visible; IR (KBr) ν_{max} 3425, 2965, 2924, 2851, 1657, 1620, 1538, 1501, 1383, 1319, 1228, 1173, 1084, 1008, 873, 662, 546 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 13.90 (1 H, s br, 3'-OH), 10.04 (1 H, s, 4-OH), 9.86 (1 H, s, 13-NH), 9.12 (1 H, s, 2-NH), 9.06 (1 H, s, 1-OH), 7.47 (1 H, s, H-3), 7.45 (1 H, dd, ^3J = 15.5, 11.1 Hz, H-8), 7.26 (1 H, dd, ^3J = 15.1, 11.2 Hz, H-11), 6.97 (1 H, d, ^3J = 15.5 Hz, H-7), 6.86 (1 H, dd, ^3J = 15.1, 11.1 Hz, H-9), 6.82 (1 H, s, H-3"), 6.51 (1 H, d, ^3J = 14.6 Hz, H-12), 6.49 (1 H, dd, ^3J = 14.6, 11.2 Hz, H-10),

5.36 (1 H, d, ^3J = 9.5 Hz, H-5"), 2.53 (4 H, m, H_2-4',5'), 2.51 (1 H, m, H-6"), 2.03 (3 H, s, 2"-Me), 1.82 (3 H, s, 4"-Me), 1.4-1.2 (6 H, m, H_2-7",8",9"), 0.96 (3 H, d, ^3J = 6.8 Hz, 6"-Me), 0.88 (3 H, t, ^3J = 6.8 Hz, H_3-10"); ^{13}C NMR (DMSO-d_6, 500 MHz) δ see table 1. (+)-ESI-MS *m/z* (%) 615 ($[M^1+2Na-H]^+$, 5), 613 ($[M^2+2Na-H]^+$, 25), 593 ($[M^1+Na]^+$, 32), 591 ($[M^2+Na]^+$, 100); (-)-ESI-MS *m/z* (%) 569 ($[M^1-H]^-$, 52), 567 ($[M^2-H]^-$, 100). (+)-ESI HRMS *m/z* 591.2237 (calcd for $[M^2+Na]^+$, $C_{31}H_{37}ClN_2O_6Na$, 591.2237712).

Chinikomycin B (**2b**). Red solid; R_f = 0.58 (CH_2Cl_2/5 % MeOH); UV/VIS (MeOH) λ_{max} (log ε) 261 (4.68), 344 (4.72), 500 (3.98) nm; IR (KBr) ν_{max} 3425, 2963, 2923, 2857, 1655, 1620, 1502, 1457, 1382, 1318, 1297, 1233, 1173, 1121, 1083, 1035, 1008, 874, 851, 653, 546 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 13.64 (1H, s br, 3'-OH), 8.60 (1H, s, 2-NH), 8.13 (1 H, s, 13-NH), 7.80 (1 H, dd, ^3J = 15.6, 10.0 Hz, H-8), 7.60 (1 H, s, H-3), 7.37 (1 H, dd, ^3J = 14.8, 10.3 Hz, H-11), 6.86 (1 H, s, H-3"), 6.83 (1 H, d, ^3J = 15.6 Hz, H-7), 6.73 (1 H, dd, ^3J = 14.8, 10.1 Hz, H-9), 6.68 (1 H, dd, ^3J = 14.8, 10.3 Hz, H-10), 6.28 (1 H, d, ^3J = 14.8 Hz, H-12), 5.40 (1 H, d, ^3J = 9.7 Hz, H-5"), 2.62 (2 H, s br, H_2-5'), 2.56 (2 H, s br, H_2-4'), 2.45 (1 H, m, H-6"), 2.08 (3 H, s 2"-Me), 1.83 (3 H, s, 4"-Me), 1.4-1.2 (6 H, m, H_2-7",8",9"), 0.97 (3 H, d, ^3J = 6.5 Hz, 6"-Me), 0.86 (3 H, t, 7.0 Hz, H_3-10"); ^{13}C NMR ($CDCl_3$, 500 MHz) δ see table 1. (+)-ESI-MS *m/z* (%) 611 ($[M^2+2Na-H]^+$); (-)-ESI-MS *m/z* (%) 567 ($[M^1-H]^-$, 29), 565 ($[M^2-H]^-$, 100); (-)-ESI HRMS *m/z* 565.2104 (calcd for $[M^2-H]^-$, $C_{31}H_{34}ClN_2O_6$, 565.2105274).

Oxidation of chinikomycin A (2a). Fraction III was dissolved in 50 mL dichloromethane and stirred under TLC control for 15 min with ~1 g silver oxide until all yellow **2a** was transformed into red **2b**. Purification by PTLC (20 × 20 cm, CH_2Cl_2/5 % MeOH) afforded 7 mg of chinikomycin B (**2b**).

Reduction of chinikomycin B (2b). A red solution of chinikomycin B (**2b**, <1 mg) in dichloromethane (2 mL) and 0.1 N aqueous sodium dithionite solution (1 mL) was shaken in a test tube until the color of the organic phase had changed from red to yellow. The organic phase was separated and evaporated, the residue was identified by TLC as chinikomycin A (**2a**).

Antimicrobial and antiviral activity. The chinikomycins (**2a**/**2b**) were inactive against *Bacillus subtilis*, *Streptomyces viridochromogenes* (Tü 57), *Staphylococcus aureus*, and *Escherichia coli*, the microalgae *Chlorella vulgaris*, *Chlorella sorokiniana* and *Scenedesmus subspicatus*, the fungus *Mucor miehei* and *Candida albicans* at a concentration of 40 µg/test plate, while manumycin (**1**) gave inhibition zones of 16-28 mm at 10 µg/test plate. The respective activities of the crude extract were due to the manumycin A (**1**) content. In an antiviral assay with the EMC virus, the chinicomycins had at 100 µg/ml no protective activity on A549 cells against the cytopathic effects of a viral infection[17].

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