J. Antibiot. 58(9): 566–572, 2005

ORIGINAL ARTICLE

THE JOURNAL OF ANTIBIOTICS

Nocardimicins G, H and I, Siderophores with Muscarinic M3 Receptor Binding Inhibitory Activity from *Nocardia nova* JCM 6044

Yoshitaka Ikeda, Tamotsu Furumai, Yasuhiro Igarashi

Received: June 20, 2005 / Accepted: August 29, 2005
© Japan Antibiotics Research Association

Abstract In the screening for muscarinic M3 receptor binding inhibitors from microbial secondary metabolites, the extract of *Nocardia nova* JCM 6044 was found to be highly active. Bioassay-guided isolation led to the identification of three siderophores, nocardimicins G (**1**), H (**2**) and I (**3**). Their chemical structures were determined by spectroscopic analysis using NMR and MS. **1** and **2** inhibited the binding of tritium-labeled *N*-methylscopolamine to the muscarinic M3 receptor with K_i values of 0.44 μM and 0.37 μM, respectively, whereas **3** showed no inhibition at 10 μM. **1** and **2** also showed weak binding inhibitory activity to the M5 receptor but not to the M1, M2 and M4 receptors at 10 μM.

Keywords *Nocardia nova* JCM 6044, siderophore, muscarinic M3 receptor, binding assay

Introduction

Parasympatholytics block the acetylcholine-mediated transmission of impulses from postganglionic parasympathetic fibers to the organ of response by competitive antagonism at muscarinic receptors. Due to the resulting relaxing effect on smooth muscles, these substances are also called neurotropic spasmolytic agents. To date, five muscarinic acetylcholine receptor genes, *m*1∼*m*5, have been cloned [1]. Although four subtypes of muscarinic receptors (M1∼M4) have been pharmacologically defined, pharmacological characterization of the M5 receptor has not yet been accomplished. All the subtypes known to date belong to the superfamily of membrane-bound receptors coupled to G-proteins [2].

Muscarinic receptors are widely distributed in the central and peripheral nervous systems as well as in the parasympathetically innervated organs of response. All five subtypes occur in the central nervous system. The smooth muscle of the respiratory tract, gastrointestinal tract, and urinary bladder contains a heterogeneous population of M2 and M3 receptors. As contraction of smooth muscle generally reflected M3 pharmacology alone [3], the M3 receptor may represent a therapeutic target for the treatment of respiratory disorders such as chronic obstructive pulmonary disease, gastrointestinal disorders such as irritable bowel syndrome, and urinary tract disorders such as urinary incontinence [4].

We have previously reported the isolation of cremastrine, an alkaloid from *Cremastra appendiculata* (Orchidaceae) [5] and nocardimicins A∼F, siderophores from *Nocardia* sp. TP-A0674 [6], as selective muscarinic M3 receptor binding inhibitors. In our ongoing search for the muscarinic M3 inhibitors from natural products, three siderophores, nocardimicins G (**1**), H (**2**) and I (**3**) were identified in the culture broth of *Nocardia nova* JCM 6044. This paper describes the isolation, structure determination, and biological activity of **1**∼**3**.

Y. Ikeda (Corresponding author)**:** Pharmaceuticals Research Unit, Mitsubishi Pharma Corporation, 1000, Kamoshida-cho, Aoba-ku, Yokohama, Kanagawa 227-0033, Japan, E-mail: ikeda.yoshitaka@mc.m-pharma.co.jp

T. Furumai, Y. Igarashi: Biotechnology Research Center, Toyama Prefectural University, 5180, Kurokawa, Kosugi, Toyama 939-0398, Japan

Materials and Methods

Microorganism

The producing microorganism, strain *Nocardia nova* JCM 6044, was purchased from Japanese Collection of Microorganisms. It was maintained on an ISP medium No. 2 slant at 10°C for laboratory use.

Instrumental Analysis

Melting points were determined on a Yanaco micro melting point apparatus. Optical rotations were measured on a Jasco P-1020 digital polarimeter. NMR spectra were measured on a Bruker AMX-500 NMR spectrometer using standard Bruker pulse programs. Chemical shifts are given in δ values with reference to tetramethylsilane as an internal standard. IR spectra were recorded on a Perkin Elmer 1725X FT-TR spectrophotometer. LC-MS spectra were measured on an Agilent MSD spectrometer and high-resolution LC-MS spectra were measured on a JEOL JMS-700 spectrometer. The LC-MS/MS spectra were measured on a Finnigan TSQ QUANTUM Ultra spectrometer (Thremo electron, MA). UV spectra were recorded on a Hitachi U-3010 spectrophotometer.

Biological Assay

The binding affinities (K_i) to five receptor subtypes were determined according to the method previously described [5], by inhibition of specific binding of tritium labeled *N*-methylscopolamine ([^{3}H]-NMS) using membranes from insect Sf9 cells expressing human *m*1～*m*5 receptors at MDS Pharma Services (Taiwan).

Results and Discussion

Fermentation

A loopful of a mature slant culture of strain *Nocardia nova* JCM 6044 was inoculated into five 500-ml K-1 flasks containing 100 ml of the seed medium consisting of soluble starch 1%, glucose 0.5%, NZ-case 0.3%, yeast extract 0.2%, tryptone 0.5%, K_2HPO_4 0.1%, and $CaCO_3$ 0.3% (pH 7.0). The flask was incubated at 32°C for 5 days on a rotary shaker (200 rpm). Three-ml aliquots of the seed culture were transferred into fifty 500-ml K-1 flasks each containing 100 ml of the production medium consisting of glycerol 4%, NZ-case 0.5%, Pharmamedia 2%, $CaCO_3$ 0.5% and HP-20 1%. The pH of the medium was adjusted to 7.0 before sterilization. Fermentation was carried out at 32°C for 5 days on a rotary shaker (200 rpm).

Isolation

The fermented whole broth (5 liters) was centrifuged (5,000 rpm, 10 minutes) to separate the mycelium. The supernatant was discarded and the mycelium was extracted by agitating in acetone (4 liters). The mycelium was removed by centrifugation and acetone was removed by evaporation. The resultant aqueous solution was extracted with EtOAc (300 ml ×5) and the EtOAc layer was evaporated to dryness *in vacuo*. The EtOAc extract (3.06 g) was fractionated into three active fractions (fraction A～C) by preparative HPLC using an ODS column (Delta-Pak C18, 40 mm i.d. ×200 mm, Waters, MA) with a linear CH_3CN - 0.2% HOAc (aq) gradient from 50 to 100% CH_3CN over 20 minutes at a flow rate of 80 ml/minute. The fraction A (120.6 mg) was subjected to preparative HPLC using an ODS column (XTerra Prep RP18 C18, 19 mm i.d. ×150 mm, Waters, MA) with the eluent of CH_3CN - 10 mM NH_4OAc (aq) (pH 9.0, isocratic elution, 57 : 43) at a flow rate of 20 ml/minute. Active fractions were combined and evaporated *in vacuo* to give **1** (83.1 mg) as an active principle. Similarly, **2** (67.0 mg) and **3** (13.8 mg) were isolated from the fractions B (333.8 mg) and C (96.6 mg), respectively.

Structure Determination

Physico-chemical properties of nocardimicins G (**1**), H (**2**) and I (**3**) are summarized in Table 1. Compound **2** was obtained as pale yellow amorphous solid. The molecular formula of **2** was established as $C_{42}H_{67}O_{10}N_5$ on the basis of the HR-LC/MS and ^{13}C NMR data. The ^{1}H NMR spectrum (Table 2) of **2** revealed the presence of two amide protons (δ 8.68 and 8.00), a 1,2-disubstituted benzene moiety (δ 7.68, 7.32, 7.05 and 6.82) and two methyl groups (δ 1.35 and δ 0.87). In the ^{13}C NMR and DEPT spectra of **2**, signals assignable to 42 carbons were detected, including four carbonyl carbons (δ 172.6, 172.3, 170.8 and 169.4), six aromatic carbons (δ 160.6, 134.2, 129.0, 119.2, 117.3 and 111.2), one quaternary sp^2 carbon adjacent to a heteroatom (δ 167.5), one oxymethine carbon (δ 77.1), one nitrogen bearing methylene carbon (δ 52.8), and two nitrogen bearing methine carbons (δ 53.7 and 52.0). Due to the existence of *cis* and *trans* conformations around the formamide bond, the NMR signals for some protons and carbons were observed in pairs at 27°C. These pairs of NMR signals were merged into one peak at 67°C except for the *N*-formyl and ε-methylene group. The remaining carbon signals were assigned to two methyl, 22 methylene, two methine carbons, and one quaternary carbon (Table 3).

2D-NMR analysis of **2** allowed the identification of four partial structures A～D: a hydroxyphenyloxazoline moiety, N_ε-formyllysine, 2-methyl-3-hydroxy hexadecanoate and ε-

Table 1 Physico-chemical properties of nocardimicins G (**1**), H (**2**) and I (**3**)

	1	**2**	**3**
Appearance	Pale yellow amorphous	Pale yellow amorphous	Pale yellow amorphous
MP	108∼112°C	152∼155°C	132∼136°C
$[\alpha]_D^{25}$	−3.9 (*c* 1.0, MeOH)	−5.8 (*c* 1.0, MeOH)	−9.5 (*c* 1.0, MeOH)
HR-LC-MS			
Found:	774.4649 $[M+H]^+$	802.4933 $[M+H]^+$	830.5243 $[M+H]^+$
Calcd:	774.4653 (for $C_{40}H_{64}O_{10}N_5$)	802.4966 (for $C_{42}H_{68}O_{10}N_5$)	830.5279 (for $C_{44}H_{72}O_{10}N_5$)
Molecular formula	$C_{40}H_{63}O_{10}N_5$	$C_{42}H_{67}O_{10}N_5$	$C_{44}H_{71}O_{10}N_5$
UV λ_{max} nm (log ε)	243 (4.02), 249 (4.03), 305 (3.64)	243 (4.03), 249 (4.04), 305 (3.66)	243 (4.02), 249 (4.03), 305 (3.65)
IR ν_{max} (cm^{-1})	2925, 2855, 2360, 1731, 1645, 1538, 1463, 1368	2924, 2854, 2360, 1732, 1645, 1538, 1466, 1368	2923, 2853, 2360, 1730, 1645, 1538, 1470, 1367

aminocaprolactam (Fig. 2). Connectivities of these partial structures were elucidated by the HMBC experiment. Important HMBC correlations are indicated in Fig. 2. The connectivity between the partial structures A and B was confirmed by the long-range coupling from H-13 to C-12. The HMBC correlation from H-17 to C-15 established the linkage between partial structures B and C through an ester bond and that from H-20 to C-19 revealed the linkage of the partial structures C and D through an amide bond. In consideration of the molecular formula, the remaining two hydroxyl groups were assigned as substituents at the nitrogen atoms N-23 and N-32. The positive-mode LC-MS/MS spectrum of **2** indicated that fragment ions corresponding to mycobactic acid [7] moiety and cobactine moiety at *m*/*z* 380 and 441, respectively (Fig. 3). Based on these spectral data, the structure of nocardimicin H (**2**) was established as shown (Fig. 1).

The molecular formulae of nocardimicin G (**1**) and I (**3**) were established as $C_{40}H_{63}O_{10}N_5$ and $C_{44}H_{71}O_{10}N_5$, respectively, on the basis of the HR-LC-MS and ^{13}C NMR data. These compounds showed ^1H and ^{13}C NMR spectra (Table 2 and 3) quite similar to those of nocardimicin H (**2**). In the LC-MS/MS spectra, the fragment ion derived from the mycobactic acid moiety was detected at *m*/*z* 380 as in the case of **2**, while the fragment mass derived from the cobactin moiety of **1** was 28 mass units smaller (*m*/*z* 413) and that of **3** was 28 mass units larger (*m*/*z* 469). These observations led to the confirmation that **1** and **3** are the congeners of **2** differing in the fatty acid chain length (Fig. 3).

1 possesses a planar structure identical with BMS-199687 [8] and A-77543 [9]. BMS-199687 has been isolated from *Actinomadura ferruginea* and shown to have *in vitro* and *in vivo* antitumor activity. A-77543 is a metabolite of a *Nocardia* and shows antitumor activity *in vivo* and anti-HIV activity. Although these three compounds show similar NMR spectra and optical rotations {**1**: $[\alpha]_D^{22}$ −6.9° (*c* 1.0, $CHCl_3$) and $[\alpha]_D^{25}$ −3.9° (*c* 1.0, MeOH), BMS-199687: $[\alpha]_D^{25}$ −11.4° (*c* 1.0, $CHCl_3$), A-77543: $[\alpha]_D^{20}$ −7.1° (*c* 1.0, MeOH)}, it is uncertain whether they are the same compound. The planar structure of **2** is identical with that of brasilibactin A, a cytotoxic compound isolated from *Nocardia brasilieneis* [10]. The optical rotations of these compounds showed significant differences {**2**: $[\alpha]_D^{22}$ −10.7° (*c* 1.0, $CHCl_3$), brasilibactin A: $[\alpha]_D^{22}$ −31° (*c* 1.0, $CHCl_3$)}, suggesting that they are stereoisomers. Elucidation of the stereochemistry of **1**∼**3** is under investigation.

Biological Properties

The inhibitory activities of **1**∼**3** to muscarinic M1∼M5 receptor binding are summarized in Table 4. **1** and **2** inhibited the binding of [^{3}H]-NMS to the muscarinic M3 receptor with a K_i value of 0.44 μM and 0.37 μM, respectively, while **3** showed no inhibition. This finding suggest that the fatty acid chain moiety play a role in the interaction of these compounds with the muscarinic M3 receptor like nocardimicins A∼F [6]. They also showed inhibitory activity to M5 receptor binding but not to binding to the M1, M2 and M4 receptors at 10 μM (Table 4). It is noteworthy that nocardimicins exhibit high subtype-selectivity against the M3 receptor. Currently used parasympatholytic drugs can be chemically divided into two classes: compounds derived from tropane alkaloids and synthetic quaternary ammonium compounds with diverse chemical structures. None of these substances possesses the desirable property of sufficient subtype-selective blockage of M3 receptors that mediate contraction. The largely nonselective blockage of all muscarinic receptor subtypes explains some of the side effects such as increase in the

Table 2 ^{1}H NMR Data (δ) for **1**～**3** in pyridine-d_5 (67°C)

Position	**1**	**2**	**3**
3	7.05 (1H, br.d, *J*=8.3 Hz)	7.05 (1H, br.d, *J*=8.3 Hz)	7.05 (1H, br.d, *J*=8.4 Hz)
4	7.32 (1H, dt, *J*=7.8, 1.7 Hz)	7.32 (1H, br.t, *J*=7.8 Hz)	7.32 (1H, br.t, *J*=7.1 Hz)
5	6.82 (1H, br.t, *J*=7.6 Hz)	6.82 (1H, br.t, *J*=7.6 Hz)	6.81 (1H, br.t, *J*=7.5 Hz)
6	7.68 (1H, dd, *J*=7.8, 1.8 Hz)	7.68 (1H, dd, *J*=7.8, 1.1 Hz)	7.68 (1H, dd, *J*=7.8, 1.2 Hz)
9	4.88 (1H, m)[a]	4.89 (1H, m)[a]	4.89 (1H, m)[a]
	4.52 (1H, dd, *J*=10.2, 8.5 Hz)	4.53 (1H, dd, *J*=10.1, 8.7 Hz)	4.53 (1H, dd, *J*=10.1, 8.7 Hz)
10	5.17 (1H, dd, *J*=10.2, 7.7 Hz)	5.17 (1H, dd, *J*=10.2, 7.8 Hz)	5.18 (1H, dd, *J*=10.2, 7.8 Hz)
13(NH)	8.70 (1H, d, *J*=7.3 Hz)	8.68 (1H, d, *J*=7.2 Hz)	8.79 (1H, d, *J*=6.5 Hz)
14	4.94 (1H, br.dd, *J*=13.6, 7.9 Hz)	4.94 (1H, br.dd, *J*=13.7, 8.0 Hz)	4.94 (1H, br.dd, *J*=13.7, 8.0 Hz)
17	5.47 (1H, dt, *J*=12.3, 6.1 Hz)	5.47 (1H, dt, *J*=12.3, 6.1 Hz)	5.49 (1H, dt, *J*=12.3, 6.1 Hz)
18	2.92 (1H, dq, *J*=6.8, 6.5 Hz)	2.92 (1H, dq, *J*=6.8, 6.5 Hz)	2.92 (1H, dq, *J*=6.8, 6.5 Hz)
20(NH)	8.01 (1H, d, *J*=6.6 Hz)	8.00 (1H, d, *J*=6.3 Hz)	8.07 (1H, d, *J*=6.5 Hz)
21	4.88 (1H, m)[a]	4.89 (1H, m)[a]	4.89 (1H, m)[a]
24	3.82～3.72 (2H, m)	3.82～3.72 (2H, m)	3.82～3.72 (2H, m)
25	1.60～1.50 (2H, m)[b]	1.60～1.50 (2H, m)[b]	1.60～1.50 (2H, m)[b]
26	1.80～1.70 (1H, m)[c]	1.80～1.70 (1H, m)[c]	1.80～1.70 (1H, m)[c]
	1.70～1.60 (1H, m)[d]	1.70～1.60 (1H, m)[d]	1.70～1.60 (1H, m)[d]
27	2.16～2.10 (1H, m)[e]	2.16～2.10 (1H, m)[e]	2.16～2.10 (1H, m)[e]
	1.60～1.50 (1H, m)[b]	1.60～1.50 (1H, m)[b]	1.60～1.50 (1H, m)[b]
28	2.16～2.10 (1H, m)[e]	2.16～2.10 (1H, m)[e]	2.16～2.10 (1H, m)[e]
	2.02～1.91 (1H, m)	2.02～1.91 (1H, m)	2.02～1.91 (1H, m)
29	1.70～1.60 (2H, m)[d]	1.70～1.60 (2H, m)[d]	1.70～1.60 (2H, m)[d]
30	1.80～1.70 (2H, m)[c]	1.80～1.70 (2H, m)[c]	1.80～1.70 (2H, m)[c]
31	3.70～3.50 (2H, br.s)	3.70～3.50 (2H, br.s)	3.70～3.50 (2H, br.s)
33	8.79 (0.6H, br.s)[f]	8.79 (0.6H, br.s)[f]	8.79 (0.6H, br.s)[f]
	8.18 (0.4H, br.s)[f]	8.18 (0.4H, br.s)[f]	8.18 (0.4H, br.s)[f]
34	1.87～1.80 (2H, m)	1.87～1.80 (2H, m)	1.87～1.80 (2H, m)
35	1.60～1.50 (2H, m)[b]	1.60～1.50 (2H, m)[b]	1.60～1.50 (2H, m)[b]
36	1.32～1.20 (2H, m)[g]	1.32～1.20 (2H, m)[g]	1.32～1.20 (2H, m)[g]
37	1.32～1.20 (2H, m)[g]	1.32～1.20 (2H, m)[g]	1.32～1.20 (2H, m)[g]
38	1.32～1.20 (2H, m)[g]	1.32～1.20 (2H, m)[g]	1.32～1.20 (2H, m)[g]
39	1.32～1.20 (2H, m)[g]	1.32～1.20 (2H, m)[g]	1.32～1.20 (2H, m)[g]
40	1.32～1.20 (2H, m)[g]	1.32～1.20 (2H, m)[g]	1.32～1.20 (2H, m)[g]
41	1.32～1.20 (2H, m)[g]	1.32～1.20 (2H, m)[g]	1.32～1.20 (2H, m)[g]
42	1.32～1.20 (2H, m)[g]	1.32～1.20 (2H, m)[g]	1.32～1.20 (2H, m)[g]
43	1.32～1.20 (2H, m)[g]	1.32～1.20 (2H, m)[g]	1.32～1.20 (2H, m)[g]
44	1.32～1.20 (2H, m)[g]	1.32～1.20 (2H, m)[g]	1.32～1.20 (2H, m)[g]
45	1.40～1.30 (2H, m)	1.32～1.20 (2H, m)[g]	1.32～1.20 (2H, m)[g]
46	0.87 (3H, br.t, *J*=6.9 Hz)	1.32～1.20 (2H, m)[g]	1.32～1.20 (2H, m)[g]
47	—	1.40～1.30 (2H, m)	1.32～1.20 (2H, m)[g]
48	—	0.87 (3H, br.t, *J*=6.9 Hz)	1.32～1.20 (2H, m)[g]
49	—	—	1.40～1.30 (2H, m)
50	—	—	0.86 (3H, br.t, *J*=7.0 Hz)
51	1.35 (3H, d, *J*=7.0 Hz)	1.35 (3H, d, *J*=6.9 Hz)	1.35 (3H, d, *J*=7.0 Hz)

[a),b),c),d),e),g)] Overlapped each other.

[f)] Observed as broadened signals at 67°C. Described chemical shifts were recoded at 27°C.

Table 3 ^{13}C NMR data (δ) for **1**∼**3** in pyridine-d_5 (67°C)

Position	**1**	**2**	**3**
1	111.2 (s)	111.2 (s)	111.1 (s)
2	160.6 (s)	160.6 (s)	160.6 (s)
3	117.3 (d)	117.3 (d)	117.3 (d)
4	134.2 (d)	134.2 (d)	134.2 (d)
5	119.2 (d)	119.2 (d)	119.1 (d)
6	129.0 (d)	129.0 (d)	128.9 (d)
7	167.5 (s)	167.5 (s)	167.5 (s)
9	69.9 (t)	69.9 (t)	69.9 (t)
10	68.9 (d)	68.9 (d)	68.9 (d)
12	170.8 (s)	170.8 (s)	170.8 (s)
14	53.8 (d)	53.7 (d)	53.7 (d)
15	173.7 (s)[a)]	172.6 (s)[a)]	172.6 (s)[a)]
17	77.1 (d)	77.1 (d)	77.1 (d)
18	45.0 (d)	45.0 (d)	45.0 (d)
19	172.7 (s)[a)]	172.3 (s)[a)]	172.3 (s)[a)]
21	52.1 (d)	52.0 (d)	52.0 (d)
22	169.4 (s)	169.4 (s)	169.4 (s)
24	52.8 (t)	52.8 (t)	52.8 (t)
25	26.5 (t)	26.5 (t)	26.1 (t)
26	28.0 (t)	27.9 (t)	27.9 (t)
27	31.7 (t)[b)]	31.7 (t)[b)]	31.7 (t)[b)]
28	31.9 (t)[b)]	31.9 (t)[b)]	31.9 (t)[b)]
29	23.4 (t)	23.4 (t)	23.4 (t)
30	27.2 (t)	27.2 (t)	27.2 (t)
31	50.0, 46.6 (t)[c)]	50.0, 46.6 (t)[c)]	50.0, 46.6 (t)[c)]
33	163.0, 157.3 (d)[c)]	163.0, 157.3 (d)[c)]	163.0, 157.3 (d)[c)]
34	31.9 (t)[b)]	31.9 (t)[b)]	31.9 (t)[b)]
35	26.1 (t)	26.1 (t)	26.1 (t)
36	30.0∼29.6 (t)[d)]	30.0∼29.6 (t)[d)]	30.0∼29.6 (t)[d)]
37	30.0∼29.6 (t)[d)]	30.0∼29.6 (t)[d)]	30.0∼29.6 (t)[d)]
38	30.0∼29.6 (t)[d)]	30.0∼29.6 (t)[d)]	30.0∼29.6 (t)[d)]
39	30.0∼29.6 (t)[d)]	30.0∼29.6 (t)[d)]	30.0∼29.6 (t)[d)]
40	30.0∼29.6 (t)[d)]	30.0∼29.6 (t)[d)]	30.0∼29.6 (t)[d)]
41	30.0∼29.6 (t)[d)]	30.0∼29.6 (t)[d)]	30.0∼29.6 (t)[d)]
42	30.0∼29.6 (t)[d)]	30.0∼29.6 (t)[d)]	30.0∼29.6 (t)[d)]
43	30.0∼29.6 (t)[d)]	30.0∼29.6 (t)[d)]	30.0∼29.6 (t)[d)]
44	32.2 (t)[b)]	30.0∼29.6 (t)[d)]	30.0∼29.6 (t)[d)]
45	22.9 (t)	30.0∼29.6 (t)[d)]	30.0∼29.6 (t)[d)]
46	14.2 (q)	32.2 (t)[b)]	30.0∼29.6 (t)[d)]
47	—	22.9 (t)	30.0∼29.6 (t)[d)]
48	—	14.2 (q)	32.1 (t)[b)]
49	—	—	22.9 (t)
50	—	—	14.1 (q)
51	14.0 (q)	13.9 (q)	14.0 (q)

[a),b)] Assignments may be interchangeable.

[c)] Observed as broadened signals at 67°C. Described chemical shifts were recoded at 27°C.

[d)] Overlapped each other.

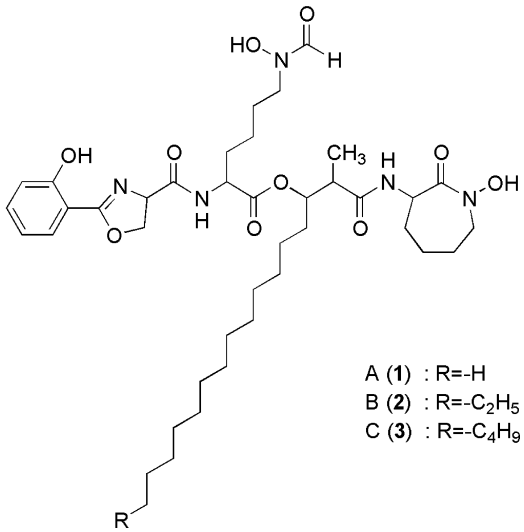

Fig. 1 Structures of nocardimicins A (**1**), B (**2**) and C (**3**).

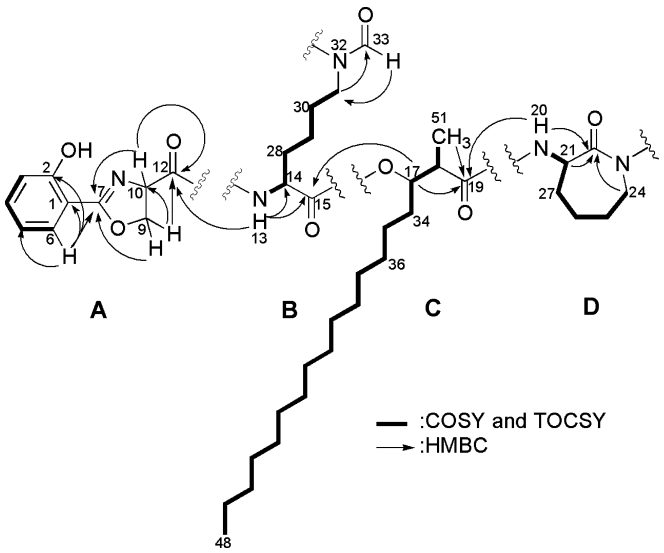

Fig. 2 Key COSY and HMBC correlations for **2**.

cardiac rate that might be caused by the blockage of cardiac M2 receptors. For control of smooth muscle spasms, the development of subtype selective antagonists with fewer side effects is necessary. Nocardimicins are the new lead compound for the design of selective M3 antagonists. Further studies on the pharmacological functions of the nocardimicins are in progress.

Acknowledgment We thank Mr. T. Ogawa for HR-LC/MS and LC-MS/MS measurements.

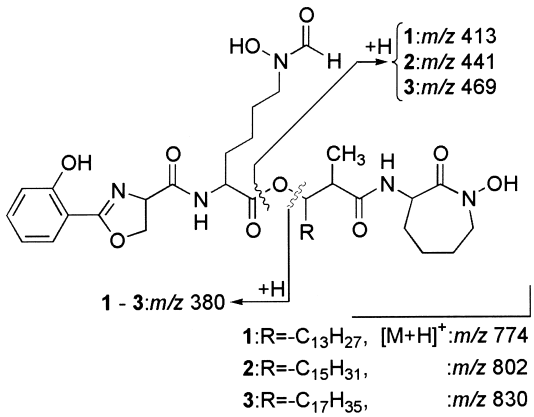

Fig. 3 Fragment ions of **1**~**3** in LC-MS/MS (positive mode).

Table 4 IC_{50} values (μM) of **1**~**3** to muscarinic M1~M5 receptors

Receptor	1	2	3
M1	>10	>10	>10
M2	>10	>10	>10
M3	2.06 (0.44)[a)]	1.75 (0.37)[a)]	>10
M4	>10	>10	>10
M5	2.07 (1.49)[a)]	4.00 (2.87)[a)]	>10

[a)] K_i values (μM) are in parentheses.

References

1. Kubo T, Fukuda K, Mikami A, Maeda A, Takahashi H, Mishina M, Haga T, Haga K, Ichiyama A, Kanagawa K, Kojima M, Matsuno H, Hirose T, Numa S. Cloning, sequencing and expression of complementary DNA encoding the muscarinic acetylcholine receptor. Nature 323: 411–416 (1986)
2. Cockcroft S, Gomperts GD. Role of guanine nucleotide binding protein in the activation of polyphosphoinositide phosphodiesterase. Nature 314: 534–536 (1985)
3. Eglen RM, Hegde SS, Watson N. Muscarinic receptor subtypes and smooth muscle function. Pharmacol Rev 48: 531–565 (1996)
4. Wallis RM. Pre-clinical and clinical pharmacology of selective muscarinic M3 receptor antagonists. Life Sci 56: 861–868 (1995)
5. Ikeda Y, Nonaka H, Furumai T, Igarashi Y. Cremastrine, a pyrrolozidine alkaloid from Cremastra appendiculata. J Nat Prod 68: 572–573 (2005)
6. Ikeda Y, Nonaka H, Furumai T, Onaka H, Igarashi Y. Nocardimicins A, B, C, D, E, and F, siderophores with muscarinic M3 receptor inhibiting activity from *Nocardia*

sp. TP-A0674. J Nat Prod 68: 1061–1065 (2005)
7. Snow G. A. Mycobactions: Iron-Chelating Growth Factors from Mycobacteria. Bacteriol Rev 34: 99–125 (1970)
8. Tunakawa M, Chang L, Mamber SW, Bursuker I, Hugill R. (Bristol-Myers Squibb Company) Antitumor antibiotic BMS-199687. U.S. 5,811,440, September 22 (1998)
9. Wagastuma T, Kizuka M, Kurakata S, Shiozawa H, Nakajima M, Furukawa H. (Sankyo Co., Ltd.) Japan Pat. 00,344,768, December 12 (2000)
10. Tsuda M, Yamakawa M, Oka S, Tanaka Y, Hoshino Y, Mikami Y, Sato A, Fujiwara H, Ohizumi Y, Kobayashi J. Brasilibactin A, a cytotoxic compound from actinomycete *Nocardia brasiliensis*. J Nat Prod 68: 462–464 (2005)