Antimicrobial peptides from skin secretions of Chinese red belly toad

Bombina maxima

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Abstract

Two groups of antimicrobial peptides have been isolated from skin secretions of Bombina maxima. Peptides in the first group, named maximins 1, 2, 3, 4 and 5, are structurally related to bombinin-like peptides (BLPs). Unlike BLPs, sequence variations in maximins occurred all through the molecules. In addition to the potent antimicrobial activity, cytotoxicity against tumor cells and spermicidal action of maximins, maximin 3 possessed a significant anti-HIV activity. Maximins 1 and 3 were toxic to mice with LD\textsubscript{50} values of 8.2 and 4.3 mg/kg, respectively. Peptides in the second group, termed maximins H1, H2, H3 and H4, are homologous with bombinin H peptides. cDNA sequences revealed that one maximin peptide plus one maximin H peptide derived from a common larger protein. © 2002 Elsevier Science Inc. All rights reserved.

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1. Introduction

In recent years, it has been widely recognized that many organisms use peptides as part of their host defense systems against microorganisms invading [4,26]. Amphibian skin glands are rich resources of antimicrobial peptides. Up to now, based on their sequence/tridimensional structure characteristics, the microbicidal peptides from amphibian skin can be grouped into three broad families [2,5,6,9,19]. The first one contains linear amphipathic helix forming peptides such as magainins and related peptides from African clawed frog Xenopus laevis, bombinin-like peptides (BLPs) from Bombina orientalis and Bombina variegata, and dermaseptins from South American arboreal frog Phylomedusa sauvagei. Another family has four different groups of related peptides isolated from various species of the Ranidae family, namely brevinins-1 and -2, gaegurins, ranalexin, esculetins-1 and -2. Different from the peptides in the first family, they all contain 2 cysteine residues at the C-terminal part that form a disulfide bond. The third one includes temporins that were isolated from Rana temporaria. Temporins are composed of only 10–13 residues and they are the smallest antimicrobial peptides known from amphibian. Recently, another linear, cationic antimicrobial peptide, Kassinauterin-1, was isolated from the skin of African frog Kassina senegalensis [16]. Kassinauterin-1 exhibits no sequence similarity with previous characterized antimicrobial peptides from amphibian skin.

In addition to antimicrobial activity, it has been demonstrated that some antimicrobial peptides also possess other potent biological activities. Magainins and their analogues have been shown to have significant antitumor activity [1,7,20]. In addition, two synthetic magainins A and G were shown to have spermicidal activity. Transmission electron microscopic micrographs showed that both magainins altered the plasma membranes of sperm [21]. Moreover, It has been demonstrated that polyphemusins and tachyplesins, two antimicrobial peptides from horse crab, have anti-HIV activity [13]. In this report, we describe the isolation, characterization, cDNA cloning and biological activities of nine new antimicrobial peptides from skin secretions of Chinese red belly toad Bombina maxima. Based on their structural similarity, they can be divided into two groups. Five peptides in the first group, named maximins 1, 2, 3, 4 and 5, are...
structurally related with BLPs from *B. orientalis* and *B. variegata* skin [10,23,24]. Four peptides in the second group, named maximins H1, H2, H3 and H4, possess a high degree of sequence identity with bombinin H peptides from the same two *Bombina* species [24].

2. Materials and methods

2.1. Collection of toad skin secretions

Adult specimens of Chinese red belly toad *B. maixma* of both sexes (*n* = 30; weight range 30–50 g) were collected in Chuxiong County, Yunnan Province of China. Skin secretions were collected as follows: toads were put into a cylinder container. A piece of absorbent cotton immersed with anhydrous ether was put on the top of the container. The container was covered with a lid and permeated with anhydrous ether. After stimulated by anhydrous ether for 1–2 min, toad skin surface was seen to exude copious secretions. Skin secretions were collected by washing the dorsal region of each toad with 0.1 M NaCl solution, containing 0.01 M EDTA. The collected solutions (500 ml of total volume) were quickly centrifuged and the supernatants were lyophilized.

2.2. Structural analysis

Peptide sequencing was completed by Edman degradation method on an Applied Biosystems pulsed liquid-phase sequencer, model 491. Fast atom bombardment (FAB) mass spectrometry was carried out on an Autospec-3000 spectrometer, equipped with a high field magnet, using glycerol: 3-nitrobenzyl alcohol:dimethyl sulphoxide (1:1:1, v:v:v) as matrix. The ion gun was operated at 25 kV with a current of 1 μA, using Cs+ as the bombarding gas.

2.3. Antimicrobial assays

Standard bacterial and fungal strains used in antimicrobial assays, Gram-positive bacterial strains *Staphylococcus aureus* (ATCC25922), *Bacillus megatherium*, Gram-negative bacterial strains *Escherichia coli* (ATCC25922), *Bacillus pyocyaneus* (CMCCB10104), *Bacillus dysenteriae*, *Klebsiella pneumoniae*, and fungal strains *Candida albicans* (ATCC2002), *Aspergillus flavus* (IFFI4015), and *Penicillium uticale* (IFFI2001), were obtained from Kunming Institute of Zoology, Chinese Academy of Sciences (CAS). The tested sample was injected i.p. into mice. For each tested sample, mice were divided into three groups (eight to ten mice in each group) and were injected with different doses of the sample. The tested animals were observed for 24 h after injection. Survival times were recorded and the LD50 value was calculated.

2.4. Acute toxicity on mice

Approximate intraperitoneal (i.p.) LD50 was determined mainly according to the procedure of Meier and Theakston [17]. Kunming mice weighing 20 ± 2 g (either sex) were donated by Kunming Institute of Zoology, Chinese Academy of Sciences (CAS). The tested sample was injected i.p. into mice. For each tested sample, mice were divided into three groups (eight to ten mice in each group) and were injected with different doses of the sample. The tested animals were observed for 24 h after injection. Survival times were recorded and the LD50 value was calculated.

2.5. Hemolysis assays

Hemolysis assay was tested with rabbit red cells in liquid medium as reported [3]. Serial dilutions of the peptides were used, and after incubation at 37°C for 30 min, the cells were centrifuged and the absorbance in the supernatant was measured at 595 nm. Maximum hemolysis was determined by adding 1% Triton X-100 to a sample of cells.

2.6. Cytotoxic activity

Human T cell lines Molt-4 and C8166 were donated by Chinese Type Culture Collection (Kunming Institute of Zoology, CAS); BIU-87 and T24 are bladder cancer cell lines and were from West China Medical University. All cell lines were maintained in RPMI-1640 (GIBCO) medium supplemented with 10% (v/v) heat-inactivated fetal calf serum (FCS), 2 mM glutamine (Sigma), 10 mM Hepes (Sigma), 50 mM 2-mercaptoethanol (Bio-Rad), 100 units/ml penicillin and streptomycin. 100 μl of Molt-4 or C8166 cells (3 × 10^5/ml) were seeded into a microtiter plate. For BIU-87 and T24 cell lines, 100 μl of trypsinized cells (2 × 10^5/ml) were seeded into a microtiter plate and allowed a preincubation period of overnight at 37°C for attachment. Various concentrations of the sample tested were added and incubated for 48 h. Cytotoxicity was measured by the MTT method [28]. IC50 was defined as the
concentration of the sample at which the absorbance at 490 nm was reduced by 50%.

2.7. Inhibition assay for the cytopathic effects of HIV-1

Antimicrobial peptides serially diluted with RPMI-1640 medium were added to triplicate wells of a 96-well flat-bottomed microtiter plate, then $3 \times 10^4$ C8166 cells and 200 TCID$_{50}$ (50% tissue culture infectious dose) of HIV-1 III$_B$ stock solution were added immediately to each well. After incubation at 37°C for 72 h without changing medium, syncytiotrophoblast cells from five different fields of each well were examined and counted under an inverted microscope (100 ×) [29]. 3'-Azido-3'-deoxythymidine (AZT, Sigma) and dextran sulfate were the reagents for positive control in each experiment. The inhibition percentage of syncytiotrophoblast cell formation was calculated by percentage of syncytiotrophoblast cell numbers in the sample treated culture to that in infected control culture. The concentration of the antiviral sample reducing HIV-1 replication by 50% (EC$_{50}$) was determined from the dose response curve. The selectivity index (SI) was calculated from the ratio of IC$_{50}$/EC$_{50}$.

2.8. Sperm immobilization assay

Sperm preparation was followed by the method described by DCruz et al. [8]. Semen was obtained by masturbation from healthy fertile men after 3 days sexual abstinence. After liquefaction, an aliquot was removed for a conventional semen analysis to confirm the motility of the sperm, the remainder was diluted with Biggers-Whitten-Whittingham (BWW) medium containing 0.3% BSA and the suspension was centrifuged at 600 g for 7 min. The sperm pellet was washed twice with BWW-0.3% BSA, resuspended in BWW-0.3% BSA and centrifuged at 500 g for 4 min. The pellet, containing 75–80% motile sperm, was diluted to a concentration of 5$ 	imes 10^7$/ml in Ham’s F-10 medium containing 0.2% BSA. Motile sperm was incubated with the treated sample dissolved in the medium. Rabbit serum of anti-sperm was used as positive control, and a corresponding volume of medium was added to negative control groups. After incubation for different time at 37°C, sperm aliquots were removed and observed for sperm motility by visual microscopic analysis, and the percentage of remaining motile sperm was recorded.

2.9. Construction and screening of a cDNA library

Standard recombinant DNA techniques were used as described [22]. mRNAs were prepared from the total RNA of B. maxima skin by oligo(dT) cellulose chromatography. A directional cDNA library was constructed with a plasmid cloning kit (SuperScript$^\text{TM}$ Plasmid System, GIBCO/BRL) following the instructions of manufacturer, producing a library of about $1.8 \times 10^3$ independent colonies.

A PCR-based method for high stringency screening of DNA libraries [12] was used for screening and isolating the clones with some modifications [27]. Two oligonucleotide primers, S$_1$ (5'-ATGAAATTTAGTACAATA-3', in the sense direction), a specific primer designed according to a conserved region of the cDNA sequence encoding signal peptide of BLPs [23], and a vector SP$_4$ promoter primer (5'-CATACGATTAGTGCACATAG-3', in the antisense direction) located in 3' of the cloned insert, were used in PCR reactions. All the oligonucleotide primers for PCR were prepared with a DNA synthesizer (Model 381A, Applied Biosystems). The presence of an insert sequence encoding maximin and its size were examined by PCR analysis, under the following conditions: 2 min at 94°C, followed by 30 cycles of 10 sec at 92°C, 30 sec at 50°C, 40 sec at 72°C. DNA sequencing was performed on an Applied Biosystems DNA sequencer, model ABI PRISM 377.

Computer analysis of protein sequences and nucleotide acid sequences were performed with the Clustal V sequence software package. The nucleotide sequence data reported in this paper are available from Genbank database with accession numbers AF378904, AF378905, AF378906, AF378907 and AF378908.

3. Results

3.1. Purification of antimicrobial peptides

Lyophilized skin secretion sample of B. maxima (1.8 g, total OD$_{280}$ of 300) was dissolved in 10 ml 0.05 M Tris-HCl buffer, pH 7.3, containing 5 mM EDTA. The sample was dialyzed against the same buffer at 4°C for 8 h, and then applied on a DEAE-Sephadex A-50 ion exchange chromatography column (2.6 × 50 cm) equilibrated with the same buffer. The elution was first performed with two column volumes of the same buffer without NaCl gradient, collecting fractions of 2.5 ml. The absorbance of the elute was monitored at 280 nm. The antimicrobial activity was predominantly concentrated in NaCl-free eluted fractions.

The antimicrobial fractions from DEAE-Sephadex A-50 column were collected and then applied to a Sephadex G-50 gel filtration column equilibrated with 0.15 M phosphate buffer solution, pH 7.8, resulted in the separation of five protein peaks (Fig. 1A), in which peak IV displayed antimicrobial activity. FAB mass spectrometry analysis revealed that it contained a peptide mixture with molecular masses ranging around 2500 to 2900 Da. Peak IV was pooled, lyophilized, dissolved in water and dialyzed for 12 h against 0.1 M phosphate buffer solution, pH 7.8. The sample was applied to a CM-Sephadex C-25 ion exchange column. Elution was achieved with a linear NaCl gradient (Fig. 1B). Three peaks were thus obtained. The antimicrobial activity was found concentrated in peak I and peak II. Peak I and peak II of CM-Sephadex C-25 were loaded finally on a Hypersil BDS C$_{18}$ reverse-phase HPLC (RP-HPLC) column. Five antimicrobial peptides, named maxi-
mins 1, 2, 3, 4 and 5 respectively, were purified, as marked M1 to M5 along the HPLC profiles reported in Fig. 2A and 2B. The final products obtained were 4.5, 1.6, 5.2, 2.6, and 1 mg for maximins 1 to 5, respectively. On the other hand, in the course of this study, the skin secretion sample (1.2 g, total OD \(280 \text{ nm} \) of 200) was first size-fractionalized on a Sephadex G-25 gel filtration column (Fig. 1C). Peak III in Fig. 1C with molecular masses around 2000 Da, as revealed by FAB mass spectrometry analysis, was associated with antimicrobial activity and hemolytic activity. Peak III was further subjected to HPLC purification. Four antimicrobial peptides, named maximins H1, H2, H3 and H4, were eluted at a high concentration of acetonitrile. Along the HPLC profile reported in Fig. 2C, the
elution positions of the various maximin H peptides, as marked with H1 to H4, are indicated. 2.8, 1.5, 2.7, and 1.1 mg final products were obtained for maximins H1 to H4, respectively.

3.2. Structural characterization and sequence homology

Purified antimicrobial peptides were subjected to amino acid sequence analysis by automated Edman degradation and their amino acid sequences are reported in Fig. 3. Maximins 1, 2, 3, 4 and 5 are composed of 27 amino acid residues, and they are found to be structurally related with BLPs purified from B. orientalis and B. variegata [10,23]. Maximins 1 and 2 differ only by one residue substitution at position 26 (Ala to Val). Sequence identities among maximins 2, 3, 4 and 5 are around 40–78% and substitutions occurred all through the molecules. Furthermore, sequence identities among maximins and BLPs are only around 40–70%. Maximins H1, H2, H3 and H4, being 20 amino acids long, are highly homologous with bombinin H peptides [24]. The proposed amino acid sequences were confirmed by FAB mass spectrometry analysis (Fig. 3). FAB mass spectrometry also indicated that, except maximins 3 and 5, all other peptides contain a C-terminally amided residue.

3.3. Maximin cDNA cloning

Ten positive clones, which contain an insert around 650-base pairs, were identified and isolated. Both strands of these ten clones were sequenced. Clone 73, 1, 118, 96 and 80 were found to contain inserts that encode the relevant purified antimicrobial peptides. As a representative, the nucleotide sequence of clone 118 that contains an insert encoding maximin 3 is shown in Fig. 4. The cDNA structure of maximins is similar to that of BLPs as reported [23]. It
was found to have an open reading frame which encodes a polypeptide composed of a signal peptide, an acidic peptide, a maximin peptide, spacer octapeptide, another acidic peptide plus finally a C-terminal maximin H peptide. Clones 73, 1, 118, 96 and 80 encode maximin 1/maximin H1, maximin 2/maximin H1, maximin 3/maximin H2, maximin 4/maximin H3, and maximin 5/maximin H4, respectively. In clones 73, 1 and 96, the sequence of the maximin is preceded by a single arginine and followed by the sequence Gly-Lys-Arg. These are typical processing sites for endopeptidase cleavage and formation of the C-terminal amide. However, in clones 118 and 80, the sequence of the maximin is followed by the sequence Arg-Arg-Arg and Arg-Lys-Arg, respectively. In all five clones, the sequence of the maximin H is preceded by a dibasic site (Lys-Arg), which forms the cleavage site for the releasing of the mature maximin H. The cDNA sequences clearly confirmed the amino acid sequences of maximins and maximin H peptides obtained by biochemical methods.

3.4. Antimicrobial activity

Maximins 1, 2, 3, 4, 5 exhibited a broad-spectrum of antibacterial activity, as they inhibited the growth of most tested bacterial strains (Table 1). The only exception was the resistance of *E. coli* to maximin 5. However, significant differences in potency on different bacterial strains among maximins were observed. Of the fungal strains, only *C. albicans* was sensitive to maximins, while the other two species were found to be resistant to maximins 1, 2, 3 and 4, but sensitive to maximin 5. The antibiotic activity of maximins proved to be lethal for some of the sensitive strains, the examples included *S. aureus*, *E. coli*, *B. dysenteriae*, *K. pneumoniae* and *C. albicans*. The sensitive strains were not capable of resuming growth on agar plates after a 6 h treatment with concentrations above the corresponding MICs. Maximin H peptides also possessed potent antimicrobial activity against four tested strains, including three of bacteria, and one of fungi, but *B. dysenteriae* was found to be resistant to maximin H peptides (Table 2).

3.5. Lethal activity on mice

The mice injected i.p. with maximins 1 and 3 exhibited typical poisoned symptoms, such as gasp, jerk, and tension. No hemorrhagic spots were found in the mice injected subcutaneously (s.c.) a dose of 6 µg/g body weight, but strong and widespread hyperaemia of s.c. capillary vessel
was observed. The LD_{50} values of maximins 1 and 3 were 8.2 and 4.3 mg/kg, respectively.

3.6. Assays of other biological activities

As listed in Table 3, maximins 1 and 3 possessed significant cytotoxicity on all the cell lines tested. MTT assays of the four human tumor cell lines revealed an average IC_{50} values of 26 μg/ml (range, 15.3–35 μg/ml) for maximin 1, 20 μg/ml (range, 11.4–28 μg/ml) for maximin 3. Comparatively, human tumor cells especially those of solid tumor cell lines were less sensitive to maximins 4 and 5. Generally, C8166 cells were more sensitive than other cell lines to maximins.

The results of anti-HIV-1 activity were listed in Table 4. Interestingly, only maximin 3 showed significant anti-HIV activity in all the peptides tested. Maximin 3 exerted its anti-HIV activity with a selectivity index of 7.6 at the concentration of 1.5 μg/ml.

Spermicidal efficacy of maximins 1 and 3 has also been studied under in vitro conditions using human spermatozoa. The data showed that 80% sperm motility was inhibited with a concentration of the maximin of 100 μg/ml within 30 min as compared to the control. The action of maximins on sperm motility was observed to be dose-dependent (data not shown).

Different from maximins that had little hemolytic activity to red blood cells even with the concentration of the peptides up to 50 μg/ml, maximin H peptides showed strong hemolytic activity. All the maximin H peptides can lyse 90–100% rabbit red blood cells at the concentration of 50 μg/ml.

4. Discussion

BLPs isolated from the skin of *B. orientalis* and *B. variegata* [10,23], which have a variable N-terminal region and an identical C-terminal region, differ from each other by only one or a few amino acids. The sequence heterogeneity is mainly due to replacements at positions 4–5, and 13–14. On the contrary, in maximins, the conserved residues are found at positions 2–3, 7, 11, 15–16, 19, 21–22, and 25 of the peptides, and other positions conferred residue substitutions. Although in most cases sequence differences are conserved substitutions, several significant replacements are observed among maximins. A remarkable substitution occurred in the C-terminus of maximin 3, ending in histidine rather than usual Asn-amide. Different from other maximins, maximin 4 is characterized with a two-residue replacement (Ser^{23}-The^{24} to Glu^{23}-Lys^{24}, Maximin 5 differs by amino acid substitutions with a unique N-terminus serine residue, and two phenylalanines at positions 13–14. An
The lectivity index (SI) is expressed as IC50, the concentration of antiviral agent reducing HIV-1 replication by 50%. Section of conserved parts of maximins to their overall biological activities such as the BLPs precursors from B. variegata and B. orientalis are known to contain a D-amino acid in the second position, in addition to their corresponding all L-isomers, resulting in distinctive antimicrobial activities [14, 18]. Weather in maximin H peptides such as post-translational modification occurs remains to be determined.

Sequence analysis of cDNA clones encoding maximins and maximin H peptides revealed that the precursors each encodes one copy of a maximin plus one copy of a maximin H peptide. The structures of the precursors for maximins are the same like those of the BLPs precursors from B. variegata [23], but different from those of the BLPs precursors from B. orientalis [10]. Substitution of glycine to arginine in clones 118 and 80 might be a reason to explain the absence of a C-terminal amide of maximin 3 and maximin 5 encoded.

In addition to a broad-spectrum of antimicrobial activity, at the μM concentrations, maximins displayed cytotoxic activity on human tumor cells. This is consistent with the antitumor activity of magainins and their analogues [1, 7, 20]. It can be also seen in Table 3 that maximins 1 and 3 possessed more potent cytotoxicities on tumor cells than those of maximins 4 and 5. The contribution of the variable and conserved parts of maximins to their overall biological functions is unknown. This might be explained by their structure differences, especially the significant residue replacements Ser22-The24 to Glu22-Lys24 in maximin 4, and Gly1 to Ser1, Ala13-Leu14 to Phe13-Phe14 in maximin 5.

In this context, a first experiment, as assayed by inhibition of the cytopathic effects of HIV-1, has demonstrated that maximin 3, but not other maximins, possesses significant anti-HIV activity. This provides the first evidence that some of these linear cationic peptides may have anti-HIV potency. It is noteworthy that maximin 3 possesses a unique histidine C-terminus, which might contribute importantly in this specific biological activity. As previously reported, antimicrobial peptides polyphemusins and tachyplesins from the horse crab have anti-HIV activity with a selectivity index of 3 and 17 at the concentrations of 1.9 and 8.4 μg/ml, respectively [15]. Natural polyphemusins and tachyplesins are 17–18 amino acid cyclic peptides, characterized with two disulfide bonds in their primary structures. The structure differences among polyphemusins, tachyplesins and maximin 3 may result in different anti-HIV mechanisms. Substantial spermicidal activity was also observed for maximins 1 and 3 tested. Maximins are linear cationic peptides and easy to form membrane pore and they may induce a detergent-type disruption of sperm membrane like in the case of magainins [21].

Even though lots of amphibian skin antimicrobial peptides have been documented, their toxicities on mammals have not been well studied [11]. In our study, strong and widespread hyperaemia of s.c. capillary vessel was observed after i.p. injection of maximins 1 and 3. How did these peptides exert their lethal toxicity on mice remains to be clarified. However, northern blot had revealed low but significant expression of BLPs mRNA in Bombina brain and stomach [10], implying that antimicrobial peptides from Bombina species have other physiological functions in central nervous system and gastrointestinal tract system.

In conclusion, maximins, a family of linear cationic peptides from skin secretions of B. maxima, differ both in their structures and biological activity potency from previously reported BLPs from different Bombina species. Because of their distinct and broad biological activity spectrum (antimicrobes, antitumor, anti-HIV, spermicidal, potential novel targets in mammals), they appear to have an interesting potential for therapeutic application.

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### Table 3

Cytotoxicity of maximins on human tumor cell lines

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>maximin 1</th>
<th>maximin 3</th>
<th>maximin 4</th>
<th>maximin 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>C8166</td>
<td>15.3</td>
<td>11.4</td>
<td>24.2</td>
<td>34.4</td>
</tr>
<tr>
<td>Molt-4</td>
<td>24.3</td>
<td>25.2</td>
<td>35.4</td>
<td>&gt;50</td>
</tr>
<tr>
<td>BIU-87</td>
<td>20.5</td>
<td>28</td>
<td>&gt;50</td>
<td>&gt;50</td>
</tr>
<tr>
<td>T24</td>
<td>35.4</td>
<td>18</td>
<td>&gt;50</td>
<td>&gt;50</td>
</tr>
</tbody>
</table>

Maximin’s ability to inhibit the growth of C8166, Molt-4, BIU-87 and T241 cell lines was assayed by MTT methods. IC50 means the concentration that can inhibit the 50% growth of the cells. These concentrations represent mean values (±25%) of three independent experiments performed in duplicates.

### Table 4

Inhibition assay for the cytopathic effects of HIV-1

<table>
<thead>
<tr>
<th></th>
<th>IC50 (μg/ml)</th>
<th>EC50 (μg/ml)</th>
<th>SI</th>
</tr>
</thead>
<tbody>
<tr>
<td>maximin 1</td>
<td>15.5</td>
<td>21.4</td>
<td>0.72</td>
</tr>
<tr>
<td>maximin 3</td>
<td>11.4</td>
<td>1.5</td>
<td>7.6</td>
</tr>
<tr>
<td>maximin 4</td>
<td>24.2</td>
<td>21.9</td>
<td>1.1</td>
</tr>
<tr>
<td>maximin 5</td>
<td>34.4</td>
<td>39.8</td>
<td>0.88</td>
</tr>
</tbody>
</table>

Anti-HIV activity was tested by inhibition assay for the cytopathic effects of HIV-1, as described in the “Method”. IC50 means the concentration that can inhibit the 50% growth of the cells. EC50 was defined as the concentration of antiviral agent reducing HIV-1 replication by 50%. Selectivity index (SI) is expressed as IC50/EC50.
References


