Accepted Manuscript

This is an Accepted Manuscript, which has been through the RSC Publishing peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, which is prior to technical editing, formatting and proof reading. This free service from RSC Publishing allows authors to make their results available to the community, in citable form, before publication of the edited article. This Accepted Manuscript will be replaced by the edited and formatted Advance Article as soon as this is available.

To cite this manuscript please use its permanent Digital Object Identifier (DOI®), which is identical for all formats of publication.

More information about Accepted Manuscripts can be found in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics contained in the manuscript submitted by the author(s) which may alter content, and that the standard Terms & Conditions and the ethical guidelines that apply to the journal are still applicable. In no event shall the RSC be held responsible for any errors or omissions in these Accepted Manuscript manuscripts or any consequences arising from the use of any information contained in them.
Diketopiperazine Alkaloids from a Mangrove Rhizosphere Soil Derived Fungus Aspergillus effuses H1-1

Huquan Gao,†a Weizhong Liu,a,b,c† Tianjiao Zhu,a Xiaomei Mo,a Attila Mándi,b Tibor Kurtán,b Jing Li,a Jing Ai,d Qianqu Gu,a and Dehai Li*a

1 Received (in XXX, XXX) Xth XXXXXXXXX 200X, Accepted Xth XXXXXXXXX 200X
DOI: 10.1039/b000000x

Effusin A (1), a spirobicyclic N,O-acetal derivative with an unprecedented 3',3a',5',6'-tetrahydrodrosperosperine-2',2'-pyran[2,3,4-de]chromene ring system, and a spiro-polyketide-diketopiperazine hybrid dihydrocryptoechinulin D (2) were isolated from a mangrove rhizosphere soil-derived fungus, Aspergillus effuses H1-1. Their structures were determined by detailed spectroscopic analysis. Effusin A (1) and dihydrocryptoechinulin D (2) occurred as racemates, the enantiomers of which were separated and characterized by online HPLC-ECD analysis and their absolute configurations were determined by the solution TDDFT ECD calculation approach. The cytotoxic effects of 1 and 2 were preliminarily evaluated and 2 showed potent activity on P388 cells with IC₅₀ value of 1.83 μM. The target of racemic 2 were also investigated and the (12R, 28S, 31S)-2 enantiomer showed selectivity against topoisomerase I.

Introduction

Fungi belonging to the genus Aspergillus are an attractive source of secondary metabolites with high structural diversities and interesting bioactivities, including pharmaceuticals such as the cholesterol-lowering drug lovastatin, psychoactive compounds such as xenovulene, and toxins such as aflatoxin B₁. In our ongoing search for bioactive novel compounds from marine-derived fungi, a strain identified as Aspergillus effuses H1-1 was isolated from the mangrove rhizosphere soil collected in the coast of Fujian province, China. The chemical study led to the isolation of two new spiro-polyketide-diketopiperazine alkaloids, named effusin A (1) and dihydrocryptoechinulin D (2) (Fig. 1). In this paper, the isolation, structural elucidation and bioactivities of compounds 1 and 2 are reported.

Results and Discussion

The fermented whole broth (70 L) gave a crude extract (76 g). The extract was separated by repeated silica gel column chromatography and finally semi-prep. ODS HPLC to yield compounds 1 (28 mg) and 2 (23 mg).

Effusin A (1) was obtained as a colorless solid. High-resolution electrospray mass spectrometry (HRESIMS) revealed ion at m/z 652.3377 ([M-H]⁻), indicating a molecular formula of C₁₀₉H₁₁₂N₂O₆ (calcld. for C₁₀₉H₁₁₀N₂O₆, 652.3387). Its IR absorptions suggested the presence of hydroxyl, secondary amine (3428, 3368, 3195 cm⁻¹) and secondary amide (1693 and 1624 cm⁻¹) groups. The UV-vis absorptions at λmax 358, 285 and 225 nm were characteristic of a dehydrotryptophan moiety.

In the NMR spectrum, the dehydrotryptophan moiety was confirmed by the HMBC correlations from H-8 (δH 7.12) to C-2 (δC 144.7), C-3a (δC 126.3), and C-10 (δC 162.8), and from NH-1...
(δH 11.13) to C-2 and C-7α (δC 135.1) (Fig. 2). The 1,1-dimethyl-2-propenyl group was attached to C-2 based on the HMBC correlations from H-18 (δH 1.47) and H-19 (δH 1.51) to C-2. A diketopiperazine ring was deduced based on the two amide carbonyl carbons (δC 162.8, C-1; 162.7, C-13) observed in 13C NMR spectrum and the HMBC correlations from NH-11 (δH 9.16) to C-9 (δC 124.0) and C-13 and from NH-14 (δH 9.67) to C-10 and the oxidized C-12 (δC 84.7) (Fig. 2). The isochroman moiety with a pentyl side-chain was deduced by the 1H-1H COSY correlations (H-28/H-29/H-30/H-31/H-32/H-33/H-34) and the HMBC correlations from H-28 (δH 4.39) to C-22 (δC 124.9) and C-26 (δC 149.7) and C-27 (δC 117.5), from OH-26 (δH 9.30) to C-26 and C-27, and from H-35 (δH 3.16) to C-23 (δC 140.3), C-24 (δC 130.2) and C-25 (δC 114.1), as well as the NOE enhancement of H-20b (δH 1.96) when H-29 (δH 3.49) was irradiated (Fig. 2). The prenyl group (from C-35 to C-39) was also deduced by the 1H-1H COSY correlations (H-35/H-36) and HMBC correlations from H-38 (δH 1.54) and H-39 (δH 1.60) to C-36 (δC 121.9) and C-37 (δC 132.1) (Fig. 2). The methoxyl was attached to C-28 evidenced by the HMBC correlation (Fig. 2). Then the planar structure of 1 was established by connection between C-12 and C-21 via C-20 based on the 1H-1H COSY (H-20/H-21) and HMBC correlations from H-20 (δH 2.73, 1.96) to C-12 (δC 84.7), C-13 (δC 162.7) and C-22, together with the connection of C-12 and C-23 via an oxygen according to the molecular formula, and the compound was named as effusin A.

![Fig. 2 Key 1H-1H COSY, HMBC and NOE correlations of 1 and 2.](image)

The relative stereochemistry of 1 was deduced as (12R*,21R*,28R*,29R*) on the basis of selective NOE difference experiments (Fig. 2). When H-29 (δH 3.49) was irradiated, the signals of H-28 (δH 4.39) and H-20b (δH 1.96) were enhanced, which revealed that these three hydrogens located at the same side. The enhancement of H-21 (δH 5.02) and NH-11 (δH 9.16) upon irradiation of H-20a (δH 2.73) indicated the cis relative configuration of H-20a, H-21 and the proximity of NH-11. Additionally, the Z-geometry of the double bond was deduced from the downfield shift of H-8 (δH 7.12) attributed to the deshielding effect of the carbonyl group on β-vinyl protons. This was also in agreement with the lack of NOE effect between H-8 and H-14.

<table>
<thead>
<tr>
<th>Atom no.</th>
<th>δC 1</th>
<th>δH mult (in Hz) 1</th>
<th>δC 2</th>
<th>δH mult (in Hz) 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11.13, s</td>
<td>11.05, s</td>
<td>1</td>
<td>144.7</td>
</tr>
<tr>
<td>2</td>
<td>104.0</td>
<td>103.6</td>
<td>3a</td>
<td>126.3</td>
</tr>
<tr>
<td>3</td>
<td>119.4</td>
<td>7.34, d (7.8)</td>
<td>5</td>
<td>119.4 6.96, dd (7.8, 7.3)</td>
</tr>
<tr>
<td>4</td>
<td>120.8</td>
<td>7.07, dd (7.8, 7.3)</td>
<td>6</td>
<td>111.5 7.42, d (7.8)</td>
</tr>
<tr>
<td>7</td>
<td>135.1</td>
<td>135.1</td>
<td>7a</td>
<td>113.7</td>
</tr>
<tr>
<td>8</td>
<td>124.0</td>
<td>124.4</td>
<td>9</td>
<td>162.8</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>168.3</td>
<td>11</td>
<td>9.16, s</td>
</tr>
<tr>
<td>12</td>
<td>84.7</td>
<td>59.7</td>
<td>13</td>
<td>162.7</td>
</tr>
<tr>
<td>14</td>
<td>9.67, s</td>
<td>8.86, s</td>
<td>15</td>
<td>39.7</td>
</tr>
<tr>
<td>16</td>
<td>145.1</td>
<td>6.09, dd (17.2, 10.8)</td>
<td>17</td>
<td>111.8 5.09, bd (10.8)</td>
</tr>
<tr>
<td>18</td>
<td>27.9</td>
<td>14.7, s</td>
<td>19</td>
<td>27.4</td>
</tr>
<tr>
<td>20</td>
<td>35.1</td>
<td>2.73, dd (12.4, 6.3) b:1.96, dd (12.4, 11.0)</td>
<td>21</td>
<td>64.7 5.02, dd (11.0, 6.3)</td>
</tr>
<tr>
<td>22</td>
<td>124.9</td>
<td>127.1</td>
<td>23</td>
<td>140.3</td>
</tr>
<tr>
<td>24</td>
<td>130.2</td>
<td>6.92, s</td>
<td>25</td>
<td>114.1 6.61, s</td>
</tr>
<tr>
<td>26</td>
<td>149.7</td>
<td>2.88, m</td>
<td>27</td>
<td>117.5</td>
</tr>
<tr>
<td>28</td>
<td>69.4</td>
<td>3.93, brs</td>
<td>29</td>
<td>73.5</td>
</tr>
<tr>
<td>30</td>
<td>31.0</td>
<td>1.65, m</td>
<td>31</td>
<td>25.0</td>
</tr>
<tr>
<td>32</td>
<td>31.3</td>
<td>1.33, m</td>
<td>33</td>
<td>22.1</td>
</tr>
<tr>
<td>34</td>
<td>14.0</td>
<td>0.88, t (6.9)</td>
<td>35</td>
<td>27.4</td>
</tr>
<tr>
<td>36</td>
<td>121.9</td>
<td>5.22, brd (7.3)</td>
<td>37</td>
<td>132.1</td>
</tr>
<tr>
<td>38</td>
<td>17.5</td>
<td>1.54, s</td>
<td>39</td>
<td>25.4</td>
</tr>
<tr>
<td>OCH3</td>
<td>35.2</td>
<td>3.26, s</td>
<td>OH-26</td>
<td>9.30, s</td>
</tr>
<tr>
<td>OH-21</td>
<td></td>
<td>11.76, s</td>
<td>OH-24</td>
<td>9.05, s</td>
</tr>
</tbody>
</table>

Since effusin A (1) had zero specific rotation and a baseline ECD curve, 1 was supposed to be a racemate, which was confirmed by the baseline separation of its enantiomers by chiral
HPLC using Chiralpak IC column (Fig. 3B). The separated enantiomers were characterized by their online HPLC-ECD spectra, which had been found an efficient tool to study stereoisomeric mixtures of natural products. The HPLC-ECD spectra of the enantiomers showed mirror image curves (Fig. 3C), and the first-eluting enantiomer (1a) had a broad negative Cotton effect (CE) at 349 nm, positive ones at 283 and 234 nm, and a negative one at 211 nm. The DFT reoptimization of the 30 (for computational procedure see Table S1, SI) initial MMFF conformers was carried out on the truncated model compound 1a' (C-29 n-pentyl group in 1a was replaced by methyl) at the B3LYP/6-31G(d) level, which afforded two major conformers with 64.8% (conf. A) and 30.6% population (conf. B) above 3% population. The two conformers differed in the orientation of the C-2 and C-24 alkenyl substituents. The ECD spectra of the arbitrarily chosen (12R, 21R, 28R, 29R) enantiomer (1a') were calculated with various functionals (B3LYP, BH&HLYP, PBE0) and 6-311G(d,p) basis set. All the Boltzmann-weighted ECD spectra reproduced well the experimental curve of the first-eluting enantiomer (1a) with PBE0 giving the best agreement (Fig. 3D), which allowed the determination of the absolute configuration of the first-eluting enantiomer (1a) as (12R, 21R, 28R, 29R). Consequently, the second-eluting enantiomer (1b) was also determined as (12S, 21S, 28S, 29S) by the ECD curve.

Dihydrocryptoechinuline D (2) was obtained as a yellow solid. Its molecular formula was determined as C_{36}H_{33}N_{2}O_{4} according to the positive HRESIMS peak at m/z 622.3256 [M+H]^{+} (calcld. 622.3281). It’s IR and UV-Vis spectra were very similar to those of cryptoechinuline D, which suggested the presence of dehydrotryptophan and diketopiperazine moieties.

The ^1H and ^13C NMR spectra of 2 were very similar to cryptoechinuline D, except the appearance of two sp^3 methylene signals (CH_2-26: δ_H 2.88, 3.07; δ_C 20.9 and CH_2-27: δ_H 1.54, δ_C 32.7) instead of two sp^2-hybridized carbons in cryptoechinuline D. This suggested that 2 was the dihydro analogue of cryptoechinuline D. This assumption was confirmed by ^1H-^1H COSY and HMBC (Fig. 2) spectra of 2, and it was named as dihydrocryptoechinuline D.

Fig. 3 DFT optimized conformational isomers of the truncated model compound of (12R, 21R, 28R, 29R)-1 (1a') above 3% and their Boltzmann populations obtained by B3LYP/6-31G(d) (A); HPLC spectrum of 1 on a chiral phase (B); HPLC-ECD spectra of the separated enantiomers of effusin A (1); first-eluting enantiomer (1a) (red curve) and second-eluting enantiomer (1b) (blue curve) (C) and solution ECD spectrum of 1a (peak 1, black curve) compared with the PBE0/6-311G(d,p) computed ECD spectrum for the truncated model of 1a' obtained as the Boltzmann-weighted average of the solution conformers. Bars represent the rotatory strengths of the lowest-energy conformer (D).

In the selective NOE experiments (Fig. 2), when H-33 (δ_H 1.10) was irradiated, the signals of H-32b (δ_H 1.74) and NH-11 (δ_H 7.71) were enhanced, indicating the β orientation of these protons. The
enhancement of H-32a ($\delta_H 2.08$) upon irradiation of H-31 ($\delta_H 2.58$) indicated that they were at the same side. Although no enhancement of hydrogen signals was observed when H-28 was irradiated, the relative stereochemistry of C-28 could be deduced the same as cryptoechinulin D, which was also isolated from this strain, from the biosyntheses opinions.

Similarly to effusin A (1), dihydrocryptoechinuline D (2) was also a racemic mixture as justified by the zero specific rotation and baseline ECD curve. The enantiomers were separated on a Chiralpak IC column and mirror image HPLC-ECD curves were recorded (Fig. 4B, 4C). Since the enantiomers of dihydrocryptoechinuline D (2) exhibited quite different ECD curves from those of effusin A (1), TDDFT ECD calculation was used again to determine the absolute configuration. The initial MMFF conformational search provided 72 conformers, the DFT reoptimization of which reduced the number of conformers to two above 3% population. The two conformers had 82.2% and 4.4% population (Fig. 4A), and they were used for the calculation of the ECD spectrum. The Boltzmann-weighted TDDFT ECD spectra of (12S, 28R, 31R)-2 were mirror image of the experimental HPLC-ECD of the first eluting enantiomer except for the lowest-energy transition, which suggested that the first eluting enantiomer (2a) has (12R, 28S, 31S) absolute configuration (Fig. 4D). A patent application for dihydrocryptoechinuline D (2) had been submitted in China describing the new structure and its cytotoxicities against P388, HL-60, BEL-7402 and A-549 cell lines but the separation, determination of absolute configuration and the molecular target of the two enantiomers were not mentioned.

Effusin A (1) contains a spirobicyclic N-O-acetal moiety, which could be obtained by a domino ring-closure reaction between the substituted salicylaldehyde moiety in aspergin and the eneamide moiety of diketopiperazine unit in neoechinul in B. In contrast, an enzyme-catalyzed regiospecific [4+2] Diels-Alder reaction produces the spirobicycle of 2 (Scheme 1). Similar Diels-Alder biosynthetic reaction has already been suggested for a few recent examples such as yaoshanenolides and lanceolatin.
Chiralpak IC column (5 μm, 150×4.6 mm, hexane/isopropanol eluent, 1 mL/min flow rate) and were recorded in stopped-flow mode on a JASCO J-810 electronic circular dichroism spectropolarimeter equipped with a 10 nm HPLC flow cell. TLC and column chromatography (CC) were performed on plates precoated with silica gel GF254 (10–40 μm) and over silica gel (200–300 mesh, Qingdao Marine Chemical Factory). Vacuum-liquid chromatography (VLC) was carried out over silica gel H (Qingdao Marine Chemical Factory).

**Fungal Material**

The working strain *Aspergillus effuses* H1-1 was isolated from the mud under mangrove along the coast of Fujian province, China. It was identified by Prof. Li Tian, the First Institute of Oceanography, SOA, Qingdao, China. The voucher specimen is deposited in the Key Laboratory of Marine Drugs, Chinese Ministry of Education.

**Fermentation and Extraction**

A small spoon of spore growing on potato dextrose agar slant was inoculated into 250 mL Erlenmeyer flask containing 75 mL culture medium consisting of glucose 2%, maltose 2%, cornstarch 0.03% (in sea water) and cultured at 28˚C for two days on a rotary shaker at 160 rpm. Then 10 mL resultant seed culture was inoculated into 500 mL Erlenmeyer flask containing 150 mL above culture medium and incubated at 28˚C for seven days on a rotary shaker at 160 rpm. The fermented whole broth (70 L) was filtrated through cheesecloth to separate into broth supernatant and mycelium. The former was extracted three times with ethyl acetate to give an ethyl acetate solution. The latter was extracted three times with methanol, which was evaporated under reduced pressure to remove methanol to afford an aqueous solution. The aqueous solution was extracted three times with ethyl acetate to give another ethyl acetate solution. Both the ethyl acetate solutions were combined and concentrated in vacuo to give a crude extract (76 g).

**Purification**

The crude extract (76 g) was subjected to silica gel (200–300 mesh) column packed in petroleum ether, and was separated into seven fractions (Fr.1 - Fr.7) using a step gradient elution of petroleum ether-chloroform and chloroform-methanol. The fraction (Fr.4), eluted with chloroform-methanol (100:1) solution from the silica gel column was further chromatographed gradiently on silica gel using chloroform-methanol (100:1–10:1) as elution and divided into 3 subfractions(Fr.4-1 - Fr.4-3). Subfraction Fr.3-1 yielded compound 3 (150 mg). The fraction (Fr.1), eluted with petroleum ether-chloroform (1:1) solution from the silica gel column was further chromatographed gradiently on silica gel using petroleum ether-chloroform (2:1–1:1) as elution and divided into 4 subfractions (Fr.1 - 1-Fr.1-4). Subfraction Fr.4-1 yielded compound 4 (26 mg), and subfraction Fr.4-2 yielded compound 5 (98 mg).

_**Effusin A (1):** _colorless solid (actone); UV (MeOH) $\lambda_{\text{max}}$ (log ε) 358 (3.62), 285 (3.60), 225 (4.12), 203 (4.38) nm; IR (KBr) $\nu_{\text{max}}$ 3428, 3368, 3195, 3079, 2961, 2969, 1693, 1624, 1442, 1377, 1079, 1036 cm$^{-1}$; $^1$H NMR and $^{13}$C NMR data, Table 1; HRESIMS m/z 652.3377 [M-H]$^-$ (calcd for C$_{39}$H$_{40}$N$_{2}$O$_4$ 652.3387).

(12R, 21R, 28R, 29R)-1 (1a): retention time (t$_R$) 4.92 min (Chiralpak IC, hexane/isopropanol 85:15); ECD data were recorded as $\lambda_{\text{max}}$ (ϕ) by stopping the flow of the eluent at (hexane/isopropanol 85:15) at the maximum concentration: 349 (–13.87), 283 (5.75), 266sh (3.15), 245sh (3.38), 234 (12.09), 211 (–25.17).

(12S, 21S, 28S, 29S)-1 (1b): retention time (t$_R$) 5.58 min (Chiralpak IC, hexane/isopropanol 85:15); ECD $\lambda_{\text{max}}$ (ϕ) in hexane/isopropanol 85:15: 346 (7.49), 279 (–3.46), 267sh (–2.69), 241sh (–2.78), 211 (12.99).

_Dihydrocryptoechinulin D (2):_ yellow solid (actone); UV (MeOH) $\lambda_{\text{max}}$ (log ε) 348 (3.76), 275 (3.86), 224 (4.21) nm; IR (KBr) $\nu_{\text{max}}$ 3350, 3240, 3044, 2987, 2928, 2872, 1670, 1637, 1428, 1380, 1271, 1253, 747 cm$^{-1}$; $^1$H NMR and $^{13}$C NMR data, Table 1; HRESIMS m/z 622.3256 [M+H]$^+$ (calcd for C$_{39}$H$_{40}$N$_{2}$O$_6$ 622.3281).

(12R, 28S, 31S)-2 (2a): $\phi^{20}_D +204.8$ (c 0.15, MeOH); retention time (t$_R$) 5.48 min (Chiralpak IC, hexane/isopropanol 70:30); ECD $\lambda_{\text{max}}$ (ϕ) in hexane/isopropanol 70:30: 402sh (1.07), 382 (1.60), 335 (–2.96), 280sh (2.30), 249 (9.37), 223 (–7.09), 209 (–7.16).

(12S, 28R, 31R)-2 (2b): $\phi^{20}_D –192.8$ (c 0.15, MeOH); retention time (t$_R$) 7.00 min (Chiralpak IC, hexane/isopropanol 70:30); ECD $\lambda_{\text{max}}$ (ϕ) in hexane/isopropanol 70:30: 408sh (–0.83), 385 (–1.43), 342 (2.14), 282sh (–1.86), 248 (–7.35), 221 (4.91), 210 (4.65).

**Bioassays**

_Cell proliferation/viability assays:_ The maintance of HL-60, P388, A-549, and BEL-740 cells, compound treatment, and MTT (HL-60 or P388)$^{16}$ and SRB (A-549 or BEL-740)$^{17}$ assays for cell proliferation/viability were the same as those described previously.

_Topoisomerase I mediated DNA cleavage assay._ Topoisomerases I were assayed by relaxation of supercoiled plasmid DNA. Relaxation of 250 ng of supercoiled by...
topoisomerase I (2 U) was performed in 20 μL of topoisomerase I relaxation buffer [10 mM Tris-HCl, pH 7.9, 1 mM EDTA, 150 mM NaCl, 0.1% (v/v) BSA, 0.1 mM spermidine, 5% (v/v) glycerol] in the presence and absence of varying amounts of the test compounds dissolved in dimethyl sulfoxide (5% (v/v) final concentration). Reactions were started by addition of DNA. Control groups were either DNA alone or DNA treated with topoisomerase. After 30 min at 37°C, the reaction was terminated by addition of 1% (w/v) SDS and digested with 50 mg/mL proteinase K at 55°C for 30 min. DNA was extracted with an equal volume of chloroform/isoamyl alcohol (24:1) and separated on 1% (w/v) agarose gel in Tris-acetate-EDTA (TAE) buffer (40 mM Tris-acetate, pH 8.0, and 2 mM EDTA) at 2 V/cm for 3.5 h. Gels were stained with 5 mg/mL ethidium bromide, destained, and photographed using Polaroid 665 film or a gel-imaging system for numerical quantification by densitometry scanning (Herolab, Wiesloch, Germany).

The effect of racemic 2, (12R,28S,31S)-2 (2a) and (12S, 28R, 31R)-2 (2b) on topoisomerases was investigated using a conventional plasmid DNA relaxation assay. HCPT, a well-known Topo I inhibitor, was employed as a positive control. (12R, 28S, 31S)-2 (2a) leads to the observed moderate inhibited the DNA relaxation activity of Topo I at the concentration of 100 μM.

25 Computational section

Conformational searches were carried out by means of the Macromodel 9.7.211 software using Merck Molecular Force Field (MMFF) with implicit solvent model for octanol. In order to decrease the number of conformers (246 and 405 for the truncated model of 1 and compound 2, respectively, within 21 kJ/mol energy window), the MMFF geometries were reclustered for all heavy atoms except for carbons 16-19 and 36-39. Geometry reoptimizations at B3LYP/6-31G(d) level of theory followed by TD-DFT calculations using various functionals (B3LYP, BH&HLYP, PBE0) and 6-311G(d,p) basis set were performed by the Gaussian 03 package. Boltzmann distributions were estimated from the ZPVE corrected B3LYP/6-31G(d) energies. ECD spectra were generated as the sum of Gaussians test compounds dissolved in dimethyl sulfoxide (5% (v/v) final glycerol) in the presence and absence of varying amounts of the relaxation buffer [10 mM Tris-HCl, pH 7.9, 1 mM EDTA, 150 mM NaCl] in the presence and absence of varying amounts of the relaxation buffer [10 mM Tris-HCl, pH 7.9, 1 mM EDTA, 150 mM NaCl].

Conclusions

Effusin A (1) and dihydrocryptoechinuline D (2), two new spiro-polyketyide-diketopiperazines were isolated from the fungus Aspergillus effusus H1-1. Effusin A (1) possessed an unprecedented 3’,3a’,5’,6’-tetrahydropispiro[piperazine-2,2’-pyrano[2,3,4-de]chromene] skeleton, and 2a showed antitumor activity targeted to the topoisomerase I.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (Nos. 41171620, 41171620), the State Key Laboratory of Drug Research (No. SIMM1203KF-14), the Promotive research fund for excellent young and middle-aged scientists of Shandong Province (No. BS2010HZ027), the Public Projects of State Oceanic Administration (No. 2010418022-3), and the Program for Changjiang Scholars and Innovative Research Team in University (No. IRT0944). T.K. and A.M. thank the HURO/0901/274/2.2.2 project (websites: www.huro-cbc.eu and www.hungary-romania-cbc.eu) and the National Information Infrastructure Development Institute (NIIFI 10038).

Notes and references

21 U. Varetto, MOLEKEL 5.4., 2009, Swiss National Supercomputing Centre: Manno, Switzerland.