# New Cytotoxic Naphthohydroquinone Dimers from Rubia alata 

Si-Meng Zhao, ${ }^{\dagger, \hbar, \S}$ Zhe Wang, ${ }^{\dagger, \ddagger, \delta}$ Guang-Zhi Zeng, ${ }^{, ~}{ }^{\dagger}$ Wei-Wu Song, ${ }^{\dagger}$ Xiao-Qiang Chen, ${ }^{\dagger}$ Xiao-Nian Li, ${ }^{\dagger}$ and Ning-Hua Tan*, ${ }^{\dagger}$<br>${ }^{\dagger}$ State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China<br>${ }^{\ddagger}$ University of Chinese Academy of Sciences, Beijing 100049, China

## (5) Supporting Information



ABSTRACT: Two novel naphthohydroquinone dimers with unprecedented skeletons, rubialatins A (1) and B (2), were isolated from the herbal plant Rubia alata together with their precursor, mollugin (3). The structures were elucidated on the basis of NMR spectra and crystal X-ray diffraction. Compound 1, a racemate, was separated by chiral column chromatography, and the absolute configurations of the enantiomers were determined by the computational methods. Cytotoxicity of $\mathbf{1 - 3}$ was evaluated as well as the effect on the NF- $\kappa$ B pathway. Compound (+)-1 showed cytotoxicity and could inhibit NF- $\kappa$ B pathway. Meanwhile, 2 showed cytotoxicity and a synergistic effect with TNF- $\alpha$ on NF- $\kappa$ B activation.

The roots and rhizomes of Rubia plants (Rubiaceae) are widely used for the treatment of menoxenia, rheumatism, contusion, and tuberculosis in China, Japan, Korea, and India. Bicyclic hexapeptides, ${ }^{1-8}$ quinones, ${ }^{9-14}$ and arborinane-type triterpenoids ${ }^{9,14,15}$ have been isolated from Rubia plants. To date, six naphthohydroquinone dimers have been reported from this genus, ${ }^{11-13}$ and some have attracted great interest from synthetic chemists, ${ }^{16,17}$ for their distinctive molecular architectures.

Morphologically, Rubia alata Roxb. has already been distinguished from other Rubia plants by having linear or lanceolate leafs, while other species typically have ovate leafs. The species distributes widely in South China and has been used as a folk medicine, but no chemical investigation on this plant has been reported. In our search for bioactive secondary metabolites, two novel naphthohydroquinone dimers with unprecedented skeletons, rubialatins A (1) and B (2) and their precursor, mollugin (3) (Figure 1), ${ }^{10}$ were isolated from the roots and rhizomes of R. alata. Compound 1 has a novel 6/6/ $5 / 6 / 6$ carbon skeleton coupled with a spirocycloisopentene group; 2 has a rearranged 6/7/6/6 tetracyclic system. To the best of our knowledge, no other structures with these skeletons have been reported.

Compound 1 was obtained as light yellowish crystals (acetone). Its molecular formula was determined by HREIMS $\left([\mathrm{M}]^{+}, 472.1148\right.$, calcd 472.1158$)$ as $\mathrm{C}_{27} \mathrm{H}_{20} \mathrm{O}_{8}$, which was in accordance with the ${ }^{1} \mathrm{H}$ and ${ }^{13} \mathrm{C}$ NMR spectroscopic data (Table 1). The IR spectrum showed the absorptions at 3525 , $3442,1732,1712,1599 \mathrm{~cm}^{-1}$, indicating the existence of hydroxyl, carbonyl, and phenyl groups. The ${ }^{1} \mathrm{H}$ NMR spectrum


Figure 1. Structures of rubialatins $A$ and $B$ (1 and 2) and mollugin (3).
showed two pairs of $\mathrm{AA}^{\prime} \mathrm{BB}^{\prime}$ type aromatic protons at $\delta_{\mathrm{H}} 8.69$ $(1 \mathrm{H}, \mathrm{d}, J=7.7 \mathrm{~Hz}), 8.12(1 \mathrm{H}, \mathrm{d}, J=7.7 \mathrm{~Hz}), 7.62(1 \mathrm{H}, \mathrm{t}, J=$ $7.7 \mathrm{~Hz}), 7.19(1 \mathrm{H}$, overlap), and $7.77(2 \mathrm{H}$, overlap), $7.43(2 \mathrm{H}$, overlap); two cis olefinic protons at $\delta_{\mathrm{H}} 6.36(1 \mathrm{H}, \mathrm{d}, J=5.7 \mathrm{~Hz})$, $6.26(1 \mathrm{H}, \mathrm{d}, J=5.7 \mathrm{~Hz})$; one hydroxyl group at $\delta_{\mathrm{H}} 9.63(1 \mathrm{H}$, s); one methoxyl group at $\delta_{\mathrm{H}} 3.82(3 \mathrm{H}, \mathrm{s})$; and two methyl groups at $\delta_{\mathrm{H}} 1.35\left(6 \mathrm{H}, 3 \mathrm{H}\right.$ each, s). The ${ }^{13} \mathrm{C}$ NMR spectrum displayed three unsaturated ketonic carbonyl groups at $\delta_{\mathrm{C}} 187.6$ (s), $187.5(\mathrm{~s})$, and $186.8(\mathrm{~s})$; one carbonyl group at $\delta_{\mathrm{C}} 166.4$ (s); 12 aromatic carbons at $\delta_{\mathrm{C}} 144.3$ (s), 134.1 (d), 131.8 (s), 128.6 (d), 127.9 (d), 127.7 (d), and 134.9 (d), 134.8 (d), 133.0 (s), 132.7 (s), 127.4 (d), 127.1 (d); two olefinic carbons at $\delta_{\mathrm{C}}$ 142.3 (d), 125.7 (d); one methoxyl group at $\delta_{\mathrm{C}} 52.3$ (q); two methyl groups at $\delta_{\mathrm{C}} 28.9(\mathrm{q}), 27.5(\mathrm{q})$; as well as six quaternary carbons at $\delta_{\mathrm{C}} 102.8(\mathrm{~s}), 88.5(\mathrm{~s}), 81.6(\mathrm{~s}), 69.8(\mathrm{~s}), 68.8(\mathrm{~s})$,

[^0]Table 1. NMR Spectroscopic Data of Rubialatins A (1) and B (2)

| position | $1^{a}$ |  | $2^{\text {b }}$ |  |
| :---: | :---: | :---: | :---: | :---: |
|  | $\delta_{\mathrm{H}}(500 \mathrm{MHz})$ | $\delta_{\text {C }}(100 \mathrm{MHz})$ | $\delta_{\mathrm{H}}(600 \mathrm{MHz})$ | $\delta_{\text {C }}(150 \mathrm{MHz})$ |
| 1 |  | 186.8 (s) |  | 197.1 (s) |
| 2 |  | 102.8 (s) |  | 104.6 (s) |
| 3 |  | 69.8 (s) |  | 173.1 (s) |
| 4 |  | 81.6 (s) |  | 187.6 (s) |
| $4-\mathrm{OH}$ | 9.63 (1H, s) |  |  |  |
| 5 | 8.69 (1H, d, 7.7) | 127.9 (d) | 8.24 (1H, dd, 7.7, 1.3) | 130.8 (d) |
| 6 | 7.62 (1H, t, 7.7) | 134.1 (d) | 7.67 (1H, td, 7.7, 1.3) | 133.0 (d) |
| 7 | 7.19 (1H, overlap) | 128.6 (d) | 7.63 (1H, td, 7.7, 1.3) | 132.7 (d) |
| 8 | 8.12 (1H, d, 7.7) | 127.7 (d) | 7.94 (1H, dd, 7.7, 1.3) | 130.0 (d) |
| 9 |  | 131.8 (s) |  | 139.7 (s) |
| 10 |  | 144.3 (s) |  | 135.9 (s) |
| 11 | 6.36 (1H, d, 5.7) | 125.7 (d) | 7.33 (1H, d, 5.8) | 124.0 (d) |
| 12 | 6.26 (1H, d, 5.7) | 142.3 (d) | $6.91(1 \mathrm{H}, \mathrm{d}, 5.8)$ | 152.6 (d) |
| 13 |  | 88.5 (s) |  | 93.4 (s) |
| 14 | 1.35 (3H, s) | 28.9 (q) | 1.53 (3H, s) | 26.3 (q) |
| 15 | 1.35 (3H, s) | 27.5 (q) | 1.31 (3H, s) | 25.2 (q) |
| 16 |  | 166.4 (s) |  | 154.2 (s) |
| 17 | 3.82 (3H, s) | 52.3 (q) | 3.88 (3H, s) | 56.0 (q) |
| $1^{\prime}$ |  | 187.6 (s) or 187.5 (s) ${ }^{\text {c }}$ |  | 138.4 (s) |
| $2^{\prime}$ |  | 64.8 (s) |  | 119.4 (s) |
| $3^{\prime}$ |  | 68.8 (s) |  | 118.7 (s) |
| $4^{\prime}$ |  | 187.5 (s) or 187.6 (s) ${ }^{\text {c }}$ |  | 154.5 (s) |
| $4^{\prime}$ '-OH |  |  | 11.32 (1H, s) |  |
| $5^{\prime}$ | 7.77 ( 1 H , overlap) | 127.4 (d) or 127.1 (d) ${ }^{\text {c }}$ | 8.44 (1H, d, 8.3) | 124.7 (d) |
| $6{ }^{\prime}$ | 7.43 (1H, overlap) | 134.9 (d) or 134.8 (d) ${ }^{\text {c }}$ | 7.59 (1H, td, 8.3, 1.0) | 126.8 (d) |
| $7{ }^{\prime}$ | 7.43 (1H, overlap) | 134.8 (d) or 134.9 (d) ${ }^{\text {c }}$ | 7.69 (1H, td, 8.3, 1.0) | 130.4 (d) |
| $8^{\prime}$ | 7.77 (1H, overlap) | 127.1 (d) or 127.4 (d) ${ }^{\text {c }}$ | 7.92 (1H, d, 8.3) | 121.8 (d) |
| $9^{\prime}$ |  | 133.0 (s) or 132.7 (s) ${ }^{\text {c }}$ |  | 130.1 (s) |
| $10^{\prime}$ |  | 132.7 (s) or 133.0 (s) ${ }^{\text {c }}$ |  | 125.1 (s) |

${ }^{a}$ NMR data of $\mathbf{1}$ were recorded in $\mathrm{C}_{5} \mathrm{D}_{5} \mathrm{~N} .{ }^{b}$ NMR data of 2 were recorded in $\mathrm{CDCl}_{3}$. ${ }^{c}$ May be changeable for the signal overlapping.
and 64.8 (s). 1 was presumed to be a naphthoquinone dimer on the basis of these data.

Detailed interpretation of HMBC and ${ }^{1} \mathrm{H}-{ }^{1} \mathrm{H}$ COSY correlations (Figure S6, Supporting Information) allowed the construction of the fragments. The HMBC correlations from $\delta_{\mathrm{H}} 8.12(\mathrm{H}-8)$ to $\delta_{\mathrm{C}} 144.3(\mathrm{C}-10)$ and $\delta_{\mathrm{C}} 186.8(\mathrm{C}-1)$; from $\delta_{\mathrm{H}}$ $8.69(\mathrm{H}-5)$ to $\delta_{\mathrm{C}} 131.8(\mathrm{C}-9)$ and $\delta_{\mathrm{C}} 81.6(\mathrm{C}-4)$; from $\delta_{\mathrm{H}} 9.63$ $(4-\mathrm{OH})$ to $\delta_{\mathrm{C}} 81.6(\mathrm{C}-4)$ and $\delta_{\mathrm{C}} 144.3$ (C-10), together with the ${ }^{1} \mathrm{H}-{ }^{1} \mathrm{H}$ COSY correlations of $\mathrm{H}-5 / \mathrm{H}-6 / \mathrm{H}-7 / \mathrm{H}-8$ gave a naphthohydroquinone moiety. Similarly, another naphthoquinone moiety was established based on the HMBC correlations from $\delta_{\mathrm{H}} 7.77$ ( $\mathrm{H}-5^{\prime}$ and $\mathrm{H}-8^{\prime}$ ) to $\delta_{\mathrm{C}} 133.0$ and 132.7 (C-9' and $\left.\mathrm{C}-10^{\prime}\right), \delta_{\mathrm{C}} 187.6$ and 187.5 (C-1 $1^{\prime}$ and $\left.\mathrm{C}-4^{\prime}\right)$, together with the ${ }^{1} \mathrm{H}-{ }^{1} \mathrm{H}$ COSY correlations of $\mathrm{H}-5^{\prime} / \mathrm{H}-6^{\prime} / \mathrm{H}-7^{\prime} / \mathrm{H}-8^{\prime}$. The HMBC correlations from $\delta_{\mathrm{H}} 1.35(\mathrm{H}-14$ and $\mathrm{H}-15)$ to $\delta_{\mathrm{C}}$ $88.5(\mathrm{C}-13)$ and $\delta_{\mathrm{C}} 142.3(\mathrm{C}-12)$; from $\delta_{\mathrm{H}} 6.26(\mathrm{H}-12)$ to $\delta_{\mathrm{C}}$ $69.8(\mathrm{C}-3)$; from $\delta_{\mathrm{H}} 6.36(\mathrm{H}-11)$ to $\delta_{\mathrm{C}} 102.8(\mathrm{C}-2)$, along with the ${ }^{1} \mathrm{H}-{ }^{1} \mathrm{H}$ COSY correlation of $\mathrm{H}-11 / \mathrm{H}-12$, indicated an oxygen-contained spirocycloisopentene group at $\mathrm{C}-3$ position. The molecular formula and the remaining degrees of unsaturation suggested that $\mathbf{1}$ should possess heptacyclic system. Though the key HMBC correlation from $\delta_{\mathrm{H}} 9.63$ (4$\mathrm{OH})$ to $\delta_{\mathrm{C}} 68.8$ ( $\mathrm{C}-3^{\prime}$ ) gave a linkage of the two naphthoquinone moieties, the complete structure of 1 could not be established by NMR study because of the crowded quaternary carbons. Fortunately, a single crystal suitable for Xray analysis was obtained after careful recrystallization in acetone. Thus, single-crystal X-ray diffraction was applied to
determine the final structure and absolute configurations of $\mathbf{1}$ (Figure 2). The results indicated that 1 occurs as a racemate,


Figure 2. X-ray crystal structure of rubialatin A (1).
which was also confirmed by the $[\alpha]$ of zero. Subsequent analysis by a chiralpak IC column allowed separation of the enantiomers, (+)-1 and (-)-1 (Figure S17, Supporting Information). Since the absolute configurations of the enatiomers must be $\left(2 R, 3 R, 4 S, 2^{\prime} R, 3^{\prime} S\right)-1$ or $(2 S, 3 S, 4 R$, $\left.2^{\prime} S, 3^{\prime} R\right)-1$ according to the X-ray analysis, the absolute configuration of each enatiomer was then determined by calculation of the ECD spectrum using the time-dependent density functional theory (TD-DFT) method of the Gaussian 03 program package. ${ }^{18}$ The geometry was optimized at the B3LYP/CC-pVDZ level on the basis of the crystal structure by the density functional theory (DFT) method. The harmonic vibrational frequency was then calculated at the same level to
confirm its stability. The ECD spectrum for $\left(2 R, 3 R, 4 S, 2^{\prime} R, 3^{\prime} S\right)$ 1 was further calculated at the B3LYP/CC-pVDZ level in methanol (Figure 3). The calculated ECD spectrum resembled


Figure 3. Calculated ECD spectrum of $\left(2 R, 3 R, 4 S, 2^{\prime} R, 3^{\prime} S\right)$ - $\mathbf{1}$ (solid); experimental ECD spectra of (+)-1 (dash) and (-)-1 (dot).
the experimental CD spectrum for $(-)-\mathbf{1}$, which was opposite to that for $(+)-1$. Accordingly, the absolute configurations of the enantiomers were determined as $(+)-\left(2 S, 3 S, 4 R, 2^{\prime} S, 3^{\prime} R\right)-\mathbf{1}$ and $(-)-\left(2 R, 3 R, 4 S, 2^{\prime} R, 3^{\prime} S\right)-\mathbf{1}$.

Compound 2 was obtained as yellow crystals (acetone). Its molecular formula was determined by HREIMS ([M] ${ }^{+}$, 456.1194, calcd 456.1209) as $\mathrm{C}_{27} \mathrm{H}_{20} \mathrm{O}_{7}$, which was in accordance with the ${ }^{1} \mathrm{H}$ and ${ }^{13} \mathrm{C}$ NMR spectroscopic data (Table 1). The IR spectrum showed the absorptions at 3441, $3432,1630,1461 \mathrm{~cm}^{-1}$, indicating the existence of hydroxyl, carbonyl, and phenyl groups. The ${ }^{1} \mathrm{H}$ NMR spectrum showed two pairs of $\mathrm{AA}^{\prime} \mathrm{BB}^{\prime}$ type aromatic protons at $\delta_{\mathrm{H}} 8.24(1 \mathrm{H}, \mathrm{dd}$, $J=7.7,1.3 \mathrm{~Hz}), 7.94(1 \mathrm{H}, \mathrm{dd}, J=7.7,1.3 \mathrm{~Hz}), 7.67(1 \mathrm{H}, \mathrm{td}, J=$ $7.7,1.3 \mathrm{~Hz}), 7.63(1 \mathrm{H}, \mathrm{td}, J=7.7,1.3 \mathrm{~Hz})$, and $8.44(1 \mathrm{H}, \mathrm{d}, J=$ $8.3 \mathrm{~Hz}), 7.92(1 \mathrm{H}, \mathrm{d}, J=8.3 \mathrm{~Hz}), 7.69(1 \mathrm{H}, \mathrm{td}, J=8.3,1.0 \mathrm{~Hz})$, $7.59(1 \mathrm{H}, \mathrm{td}, J=8.3,1.0 \mathrm{~Hz})$; two cis olefinic protons at $\delta_{\mathrm{H}} 7.33$ $(1 \mathrm{H}, \mathrm{d}, J=5.8 \mathrm{~Hz}), 6.91(1 \mathrm{H}, \mathrm{d}, J=5.8 \mathrm{~Hz})$; one hydroxyl group at $\delta_{\mathrm{H}} 11.32(1 \mathrm{H}, \mathrm{s})$; one methoxyl group at $\delta_{\mathrm{H}} 3.88(3 \mathrm{H}$, $\mathrm{s})$; and two methyl groups at $\delta_{\mathrm{H}} 1.53(3 \mathrm{H}, \mathrm{s}), 1.31(3 \mathrm{H}, \mathrm{s})$. The ${ }^{13} \mathrm{C}$ NMR spectrum displayed two unsaturated ketonic carbonyl groups at $\delta_{\mathrm{C}} 197.1(\mathrm{~s}), 187.6(\mathrm{~s})$; one carbonyl group at $\delta_{\mathrm{C}}$ $154.2(\mathrm{~s}) ; 16$ aromatic carbons at $\delta_{\mathrm{C}} 154.5$ (s), 139.7 (s), 138.4 (s), 135.9 (s), 133.0 (d), 132.7 (d), 130.8 (d), 130.4 (d), 130.1 (s), 130.0 (d), 126.8 (d), 125.1 (s), 124.7 (d), 121.8 (d), 119.4 (s), 118.7 (s); two olefinic carbons at $\delta_{\mathrm{C}} 152.6$ (d), 124.0 (d); one methoxyl group at $\delta_{\mathrm{C}} 56.0(\mathrm{q})$; two methyl groups at $\delta_{\mathrm{C}}$ 26.3 (q), 25.2 (q); as well as three quaternary carbons at $\delta_{\mathrm{C}}$ 173.1 (s), 104.6 (s), 93.4 (s). Compound 2 was also presumed to be a naphthoquinone dimer on the basis of these data.

Similar to 1,2D NMR also allowed the construction of the fragments rather than the complete structure (Figure S25, Supporting Information). The HMBC correlations from $\delta_{\mathrm{H}}$ $7.94(\mathrm{H}-8)$ to $\delta_{\mathrm{C}} 135.9(\mathrm{C}-10)$ and $\delta_{\mathrm{C}} 197.1(\mathrm{C}-1)$; from $\delta_{\mathrm{H}}$ $8.24(\mathrm{H}-5)$ to $\delta_{\mathrm{C}} 139.7(\mathrm{C}-9)$ and $\delta_{\mathrm{C}} 187.6(\mathrm{C}-4)$, together with the ${ }^{1} \mathrm{H}-{ }^{1} \mathrm{H}$ COSY correlations of $\mathrm{H}-5 / \mathrm{H}-6 / \mathrm{H}-7 / \mathrm{H}-8$ gave a 1,2-dicarbonyl substituted benzene moiety. The HMBC correlations from $\delta_{\mathrm{H}} 1.53(\mathrm{H}-14)$ and $\delta_{\mathrm{H}} 1.31(\mathrm{H}-15)$ to $\delta_{\mathrm{C}}$ $93.4(\mathrm{C}-13)$ and $\delta_{\mathrm{C}} 152.6(\mathrm{C}-12)$; from $\delta_{\mathrm{H}} 7.33(\mathrm{H}-11)$ and $\delta_{\mathrm{H}}$ $6.91(\mathrm{H}-12)$ to $\delta_{\mathrm{C}} 93.4(\mathrm{C}-13)$ and $\delta_{\mathrm{C}} 173.1(\mathrm{C}-3)$, along with the ${ }^{1} \mathrm{H}-{ }^{1} \mathrm{H}$ COSY correlation of $\mathrm{H}-11 / \mathrm{H}-12$, indicated the existence of an oxygen-contained cycloisopentene group. The HMBC correlations from $\delta_{\mathrm{H}} 7.92$ ( $\mathrm{H}-8^{\prime}$ ) to $\delta_{\mathrm{C}} 125.1$ (C-10') and $\delta_{\mathrm{C}} 138.4\left(\mathrm{C}-1^{\prime}\right)$; from $\delta_{\mathrm{H}} 8.44$ (H-5') to $\delta_{\mathrm{C}} 154.5$ (C-4 $\left.{ }^{\prime}\right)$; from $\delta_{\mathrm{H}} 11.32\left(4^{\prime}-\mathrm{OH}\right)$ to $\delta_{\mathrm{C}} 154.5\left(\mathrm{C}-4^{\prime}\right), \delta_{\mathrm{C}} 118.7\left(\mathrm{C}-3^{\prime}\right)$ and $\delta_{\mathrm{C}} 125.1$ ( $\mathrm{C}-10^{\prime}$ ), together with the ${ }^{1} \mathrm{H}-{ }^{1} \mathrm{H}$ COSY correlations of $\mathrm{H}-5^{\prime} / \mathrm{H}-6^{\prime} / \mathrm{H}-7^{\prime} / \mathrm{H}-8^{\prime}$ built a naphthohydroquinone moiety. Besides the correlations mentioned above, the

HMBC correlation from a methoxyl to a carbonyl group, $\delta_{\mathrm{H}}$ $3.88(\mathrm{H}-17)$ to $\delta_{\mathrm{C}} 154.2$ ( $\mathrm{C}-16$ ), could be observed. The complete structure could not be elucidated by the NMR analysis for the lack of the linkage information on the fragments. Fortunately, single crystal was obtained after recrystallization in acetone. The single-crystal X-ray diffraction analysis showed the final structure (Figure 4).


Figure 4. X-ray crystal structure of rubialatin B (2).
Compounds 1 and 2 showed unprecedented skeletons in natural products. Compound 1 has a novel 6/6/5/6/6 carbon skeleton coupled with a spirocycloisopentene group; 2 has a rearranged 6/7/6/6 tetracyclic system. They shared the same substructures, indicating that these compounds might originate from the same precursor, mollugin (3) (Figure S33, Supporting Information). The oxidation of 3 yielded ( $\pm$ )-5. Then the key intermediates $( \pm)-6$ were derived from a series of nucleophilic coupling reactions between ( $\pm$ )-5 and 4 . Compound 1 was produced by further oxidation of $( \pm)-6$, and 2 was formed from $( \pm)-6$ by intramolecular rearrangements.

Compounds 1-3 were evaluated for their cytotoxicity against three human tumor cell lines (A549, SGC-7901, and HeLa) by the SRB method (Table 2). ${ }^{19}$ Compound 2 showed

Table 2. Cytotoxicity of Compounds $\mathbf{1 - 3}\left(\mathrm{IC}_{50}, \boldsymbol{\mu} \mathrm{M}\right.$, Mean $\pm \mathrm{SD}$ )

|  | A549 | SGC-7901 | HeLa |
| :--- | :--- | :--- | :--- |
| $( \pm)-\mathbf{1}$ | $\mathrm{ND}^{a}$ | ND | ND |
| $(+) \mathbf{- 1}$ | ND | $45.90 \pm 1.12$ | ND |
| $(-)-\mathbf{1}$ | ND | ND | ND |
| $\mathbf{2}$ | $25.63 \pm 0.74$ | $10.74 \pm 0.65$ | $13.08 \pm 0.38$ |
| $\mathbf{3}$ | ND | $53.37 \pm 3.20$ | $43.63 \pm 0.04$ |
| cisplatin | $5.10 \pm 0.07$ | $3.83 \pm 0.07$ | $3.43 \pm 0.09$ |
| ${ }^{\mathbf{a}} \mathrm{ND}:$ no detected $(>60 \mu \mathrm{M})$. |  |  |  |

cytotoxicity against these cell lines with the $\mathrm{IC}_{50}$ values at $25.63,10.74$, and $13.08 \mu \mathrm{M}$. Interestingly, neither the racemic 1 nor ( - )-1 showed cytotoxicity, while (+)-1 showed weak activity against SGC-7901 cell line with the $\mathrm{IC}_{50}$ value at 45.90 $\mu \mathrm{M}$. 3 showed weak cytotoxicity against SGC-7901 and HeLa cell lines too.

Compounds $\mathbf{1}$ and $\mathbf{2}$ were also evaluated for their effects on the NF- $\kappa$ B pathway (Figure 5). ${ }^{20}$ Only compound (+)-1 could inhibit TNF- $\alpha$ induced NF- $\kappa$ B activation at $40 \mu \mathrm{M}$ (Figure 5A). But 2 could activate the NF- $\kappa$ B pathway with the existence of TNF- $\alpha$, which revealed the synergistic effect of 2 with TNF- $\alpha$ on NF- $\kappa$ B activation (Figure 5B). Both NF- $\kappa$ B inhibition and activation have been reported to be relative to tumorigenesis. ${ }^{21,22}$ These results showed that ( + )-1 and 2 showed


Figure 5. Effects of compounds 1 and 2 on TNF- $\alpha$ induced NF- $\kappa$ B activation; $* p<0.05 ; * * p<0.01$.
cytotoxicity by different mechanisms, which need to be further investigated.

## ASSOCIATED CONTENT

## (s) Supporting Information

Experimental procedures, bioactivity assays, 1D and 2D NMR, MS, UV, IR, $[\alpha]^{\mathrm{D}}, \mathrm{CD}$ spectra, and X-ray crystal data (CIF) of rubialatins $A$ (1) and $B(2)$. This material is available free of charge via the Internet at http://pubs.acs.org.

## AUTHOR INFORMATION

## Corresponding Authors

*E-mail: nhtan@mail.kib.ac.cn.
*E-mail: gzh_zeng@mail.kib.ac.cn.

## Author Contributions

${ }^{\S}$ These authors contributed equally.

## Notes

The authors declare no competing financial interest.

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## REFERENCES

(1) Tan, N. H.; Zhou, J. Chem. Rev. 2006, 106, 840-895.
(2) Morita, H.; Takeya, K. Heterocycles 2010, 80, 739-764.
(3) Zhao, S. M.; Kuang, B.; Fan, J. T.; Yan, H.; Xu, W. Y.; Tan, N. H. Chimia 2011, 65, 852-856.
(4) Fan, J. T.; Chen, Y. S.; Xu, W. Y.; Du, L.; Zeng, G. Z.; Zhang, Y. M.; Su, J.; Li, Y.; Tan, N. H. Tetrahedron Lett. 2010, 51, 6810-6813.
(5) Fan, J. T.; Su, J.; Peng, Y. M.; Li, Y.; Li, J.; Zhou, Y. B.; Zeng, G. Z.; Yan, H.; Tan, N. H. Bioorg. Med. Chem. 2010, 18, 8226-8234.
(6) Huang, M. B.; Zhao, S. M.; Zeng, G. Z.; Kuang, B.; Chen, X. Q.; Tan, N. H. Tetrahedron 2014, 70, 7627-7631.
(7) Hitotsuyanagi, Y.; Kusano, J. I.; Kim, I. H.; Hasuda, T.; Fukaya, H.; Takeya, K. Phytochemistry Lett. 2012, 5, 335-339.
(8) Hitotsuyanagi, Y.; Odagiri, M.; Kato, S.; Kusano, J. I.; Hasuda, T.; Fukaya, H.; Takeya, K. Chem.-Eur. I. 2012, 18, 2839-2846.
(9) Singh, R.; Geetanjali; Chauhan, S. M. S. Chem. Biodiversity 2004, 1, 1241-1264.
(10) Itokawa, H.; Mihara, H.; Takeya, K. Chem. Pharm. Bull. 1983, 31, 2353-2358.
(11) Qiao, Y. F.; Takeya, K.; Itokawa, H.; Iitaka, Y. Chem. Pharm. Bull. 1990, 38, 2896-2898.
(12) Itokawa, H.; Ibraheim, Z. Z.; Qiao, Y. F.; Takeya, K. Chem. Pharm. Bull. 1993, 41, 1869-1872.
(13) Ibraheim, Z. Z.; Gouda, Y. G. Bull. Pharm. Sci. Assiut Univ. 2010, 33, 225-233.
(14) Fan, J. T.; Kuang, B.; Zeng, G. Z.; Zhao, S. M.; Ji, C. J.; Zhang, Y. M.; Tan, N. H. I. Nat. Prod. 2011, 74, 2069-2080.
(15) Kuang, B.; Han, J.; Zeng, G. Z.; Chen, X. Q.; He, W. J.; Tan, N. H. Nat. Prod. Bioprospect. 2012, 2, 166-169.
(16) Lumb, J. P.; Trauner, D. I. Am. Chem. Soc. 2005, 127, 28702871.
(17) Lumb, J. P.; Choong, K. C.; Trauner, D. I. Am. Chem. Soc. 2008, 130, 9230-9231.
(18) Frisch, M. J. Gaussian 03, Revision C.2; Gaussian, Inc., Wallingford, CT, 2004 (see the Supporting Information for the full reference).
(19) Zeng, G. Z.; Tan, N. H.; Ji, C. J.; Fan, J. T.; Huang, H. Q.; Han, H. J.; Zhou, G. B. Phytother. Res. 2009, 23, 885-891.
(20) Zhao, Y.; Su, J.; Goto, M.; Morris-Natschke, S. L.; Li, Y.; Zhao, Q. S.; Yao, Z. J.; Lee, K. H. I. Med. Chem. 2013, 56, 4749-4757.
(21) Sunwoo, J. B.; Chen, Z.; Dong, G.; Yeh, N.; Bancroft, C. C.; Sausville, E.; Adams, J.; Elliott, P.; Waes, C. V. Clin. Cancer Res. 2001, 7, 1419-1428.
(22) Kasperczyk, H.; Ferla-Brühl, K. L.; Westhoff, M. A.; Behrend, L.; Zwacka, R. M.; Debatin, K. M.; Fulda, S. Oncogene 2005, 24, 69456956.


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