SUPPLEMENTARY MATERIAL

Four cytotoxic annonaceous acetogenins from the seeds of Annona squamosa

Yunjie Miao, Xiaofang Xu, Fei Yuan, Yeye Shi, Yong Chen, Jianwei Chen, Xiang Li*

a Nanjing University of Chinese Medicine, pharmaceutical institute, Nanjing 210046, P.R. China
b Nanjing University of Chinese Medicine Hanlin College, Taizhou 225300, P.R. China

* Corresponding authors. Tel.: +86 2585811512; fax: +86 2585811524. E-mail address: lixiang_8182@163.com

Abstract Four new annonaceous acetogenins, squamocin-I (1), II (2) and III (3) and squamoxinone-D (4), together with seven known annonaceous acetogenins (5-11) were isolated from the seeds of Annona squamosa. The structures of all isolates were elucidated and characterized by spectral and chemical methods. Compounds 1-4 were evaluated for their cytotoxicities against Hep G2, SMMC 7721, BEL 7402, BGC 803 and H460 human cancer cell lines. Compound 1 exhibited better potent activity than the positive compound and compound 3 shows selectively cytotoxic activity against H460 with IC50 values of 0.0492 μg/ml.

Keywords Annonaceous acetogenins, HPLC, NMR, Anti-tumor
Contents

S1. H NMR (300 MHz, CDCl3) spectrum of compound 1
S2. C NMR (75 MHz, CDCl3) spectrum of compound 1
S3. HRESIMS spectrum of compound 1
S4. H NMR (300 MHz, CDCl3) spectrum of compound 2
S5. C NMR (75 MHz, CDCl3) spectrum of compound 2
S6. HRESIMS spectrum of compound 2
S7. H NMR (400 MHz, CDCl3) spectrum of compound 3
S8. C NMR (100 MHz, CDCl3) spectrum of compound 3
S9. HRESIMS spectrum of compound 3
S10. H NMR (400 MHz, CDCl3) spectrum of compound 4
S11. C NMR (100 MHz, CDCl3) spectrum of compound 4
S12. HRESIMS spectrum of compound 4
S13. Diagnostic ESIMS fragment ions (m/z) of 1-4. Peaks in parentheses were not observed
S14. 1H and 13C NMR spectroscopic data for compounds 1-2 recorded in CDCl3.
S15. 1H and 13C NMR spectroscopic data for compounds 3-4 recorded in CDCl3.
S3. HRESIMS spectrum of compound 1

S4. 1H NMR (300 MHz, CDCl3) spectrum of compound 2
S5. 13C NMR (75 MHz, CDCl3) spectrum of compound 2

S6. HRMS/MS spectrum of compound 2
S9. HRESIMS spectrum of compound 3

S10. 1H NMR (400 MHz, CDCl3) spectrum of compound 4
Figure S13. Diagnostic ESIMS fragment ions (m/z) of 1-4. Peaks in parentheses were not observed.
Table S1. $^1$H and $^{13}$C NMR spectroscopic data for compounds 1-2 recorded in CDCl$_3$.

| Position | $\delta_C$ (ppm) | $\delta_H$ (J in Hz) | $\delta_C$ (ppm) | $\delta_H$ (J in Hz) |
|----------|------------------|----------------------|------------------|----------------------|
| 1        | 174.6            | -                    | 173.8            | -                    |
| 2        | 131.1            | -                    | 134.3            | -                    |
| 3        | 31.8             | 2.40, dd, 2.53, d (15.1 Hz) | 25.1             | 2.26, t (6.2 Hz) |
| 4        | 70.1             | 3.85-3.94, m         | 27.3             | 1.39-1.52, m         |
| 5        | 37.3             | 1.27-1.60, m         | 24.8-29.7        | 1.28-1.67, m         |
| 6        | 24.4-29.6        | 1.27-1.60, m         | 24.8-29.7        | 1.28-1.67, m         |
| 7        | 24.4-29.6        | 1.27-1.60, m         | 24.8-29.7        | 1.28-1.67, m         |
| 8        | 24.4-29.6        | 1.27-1.60, m         | 24.8-29.7        | 1.28-1.67, m         |
| 9        | 24.4-29.6        | 1.27-1.60, m         | 24.8-29.7        | 1.28-1.67, m         |
| 10       | 24.4-29.6        | 1.27-1.60, m         | 24.8-29.7        | 1.28-1.67, m         |
| 11       | 24.4-29.6        | 1.27-1.60, m         | 32.4             | 1.39-1.52, m         |
| 12       | 24.4-29.6        | 1.27-1.60, m         | 74.1             | 3.40, m             |
| 13       | 32.3             | 1.39-1.52, m         | 83.3             | 3.83-3.96, m         |
| 14       | 74.1             | 3.41, m              | 25.6             | 1.28-1.67, m         |
| 15       | 83.2             | 3.85-3.94, m         | 28.9             | 1.28-1.67, m         |
| 16       | 26.0             | 1.27-1.60, m         | 82.5             | 3.83-3.96, m         |
| 17       | 28.9             | 1.27-1.60, m         | 82.1             | 3.83-3.96, m         |
| 18       | 82.5             | 3.85-3.97, m         | 28.9             | 1.28-1.67, m         |
| 19       | 82.2             | 3.85-3.97, m         | 28.4             | 1.28-1.67, m         |
| 20       | 28.9             | 1.27-1.60, m         | 82.8             | 3.83-3.96, m         |
| 21       | 28.3             | 1.27-1.60, m         | 71.4             | 3.83-3.96, m         |
| 22       | 82.8             | 3.85-3.94, m         | 71.7             | 3.59, m             |
| 23       | 71.4             | 3.85-3.94, m         | 37.2             | 1.39-1.52, m         |
| 24       | 33.2             | 1.39-1.52, m         | 24.47-29.71      | 1.39-1.52, m         |
| 25       | 24.4-29.6        | 1.27-1.60, m         | 24.47-29.71      | 1.28-1.67, m         |
| 26       | 24.4-29.6        | 1.27-1.60, m         | 24.47-29.71      | 1.28-1.67, m         |
| 27       | 24.4-29.6        | 1.27-1.60, m         | 24.47-29.71      | 1.28-1.67, m         |
| 28       | 24.4-29.6        | 1.27-1.60, m         | 24.47-29.71      | 1.28-1.67, m         |
| 29       | 24.4-29.6        | 1.27-1.60, m         | 24.47-29.71      | 1.28-1.67, m         |
| 30       | 24.4-29.6        | 1.27-1.60, m         | 24.47-29.71      | 1.28-1.67, m         |
| 31       | 24.4-29.6        | 1.27-1.60, m         | 24.47-29.71      | 1.28-1.67, m         |
| 32       | 31.8             | 1.27-1.60, m         | 31.8             | 1.28-1.67, m         |
| 33       | 22.6             | 1.39-1.52, m         | 22.6             | 1.39-1.52, m         |
| 34       | 14.1             | 0.88, t (7.0 Hz)     | 14.1             | 0.88, t (6.7 Hz)     |
| 35       | 151.8            | 7.18, d (1.5 Hz)     | 148.8            | 6.99, d (1.5 Hz)     |
| 36       | 77.5             | 5.06, qd (7.0, 1.5 Hz)| 77.4             | 5.00, qd (6.8, 1.5 Hz)|
| 37       | 19.0             | 1.40, d (7.0 Hz)     | 19.2             | 1.41, d (6.8 Hz)     |

* $\delta$ from TMS (ppm). $^1$H-NMR [300 MHz, CDCl$_3$, m, J (Hz)] and $^{13}$C-NMR (75 MHz) spectroscopic data for Compounds 1 and 2.
Table S2. $^1$H and $^{13}$C NMR spectroscopic data for compounds 3-4 recorded in CDCl$_3$.$^*$

| Position | $\delta$ H | $\delta$ C | $\delta$ H | $\delta$ C |
|----------|------------|------------|------------|------------|
| 1        | 7.40       | -          | 7.46       | -          |
| 2        | 7.34       | -          | -          | -          |
| 3        | 3.18       | 2.26, t (7.7 Hz) | 3.19 | 2.40, m, 2.51, m |
| 4        | 2.84       | 1.39-1.52, m | 7.00 | 3.84, m |
| 5        | 2.47-2.97  | 1.26-1.65, m | 37.4 | 1.26-1.69, m |
| 6        | 2.47-2.97  | 1.26-1.65, m | 25.6-29.7 | 1.26-1.69, m |
| 7        | 2.47-2.97  | 1.26-1.65, m | 130.0 | 5.34, m |
| 8        | 2.47-2.97  | 1.26-1.65, m | 129.8 | 5.34, m |
| 9        | 2.47-2.97  | 1.26-1.65, m | 25.6-29.7 | 1.26-1.69, m |
| 10       | 2.47-2.97  | 1.26-1.65, m | 25.6-29.7 | 1.26-1.69, m |
| 11       | 2.47-2.97  | 1.26-1.65, m | 33.5 | 1.26-1.69, m |
| 12       | 2.47-2.97  | 1.26-1.65, m | 74.1 | 3.39, m |
| 13       | 3.24       | 1.39-1.49, m | 83.2 | 3.84, m |
| 14       | 7.42       | 3.39, m | 29.0 | 1.26-1.69, m |
| 15       | 8.33       | 3.83-3.94, m | 29.0 | 1.26-1.69, m |
| 16       | 2.83       | 1.63, m, 1.87, m | 81.8 | 3.84, m |
| 17       | 29.0       | 1.63, m, 1.87, m | 74.1 | 3.39, m |
| 18       | 82.6       | 3.83-3.94, m | 33.5 | 1.26-1.69, m |
| 19       | 82.3       | 3.83-3.94, m | 25.6-29.7 | 1.26-1.69, m |
| 20       | 2.89       | 1.63, m, 1.87, m | 25.6-29.7 | 1.26-1.69, m |
| 21       | 25.7       | 1.63, m, 1.87, m | 25.6-29.7 | 1.26-1.69, m |
| 22       | 82.8       | 3.83-3.94, m | 25.6-29.7 | 1.26-1.69, m |
| 23       | 71.3       | 3.83-3.94, m | 25.6-29.7 | 1.26-1.69, m |
| 24       | 32.4       | 1.39-1.49, m | 25.6-29.7 | 1.26-1.69, m |
| 25       | 24.5-29.7  | 1.26-1.65, m | 25.6-29.7 | 1.26-1.69, m |
| 26       | 24.5-29.7  | 1.26-1.65, m | 25.6-29.7 | 1.26-1.69, m |
| 27       | 24.5-29.7  | 1.26-1.65, m | 25.6-29.7 | 1.26-1.69, m |
| 28       | 33.3       | 1.26-1.65, m | 25.6-29.7 | 1.26-1.69, m |
| 29       | 71.8       | 1.26-1.65, m | 25.6-29.7 | 1.26-1.69, m |
| 30       | 33.3       | 1.26-1.65, m | 25.6-29.7 | 1.26-1.69, m |
| 31       | 24.5-29.7  | 1.26-1.65, m | 25.6-29.7 | 1.26-1.69, m |
| 32       | 31.9       | 1.26-1.65, m | 31.9 | 1.26-1.69, m |
| 33       | 22.6       | 1.40-1.53, m | 22.7 | 1.26-1.69, m |
| 34       | 14.1       | 0.89, t (6.9 Hz) | 14.1 | 0.88, t (6.7 Hz) |
| 35       | 148.9      | 6.99, d (1.3 Hz) | 151.8 | 7.19, d (1.1 Hz) |
| 36       | 77.5       | 5.00, q, (6.8, 1.3 Hz) | 78.0 | 5.06, q (6.8, 1.5 Hz) |
| 37       | 19.2       | 1.41, d (6.8 Hz) | 19.1 | 1.43, d (6.8 Hz) |

* $\delta$ from TMS (ppm). $^1$H-NMR [400 MHz, CDCl$_3$, m (Hz)] and $^{13}$C-NMR [100 MHz] spectroscopic data for compounds 3 and 4.