SUPPLEMENTARY MATERIAL

Four cytotoxic annonaceous acetogenins from the seeds of *Annona squamosa*

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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

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Spectrum from DataSET13.wiff (sample 1) - Sample003, Experiment 10, +TOF MS² (100 - 1500) from 0.228 min
Precursor: 623.5 Da, CE: 35.0

Figure S13. Diagnostic ESIMS fragment ions (m/z) of **1-4**. Peaks in parentheses were not observed

Table S1. ¹H and ¹³C NMR spectroscopic data for compounds **1-2** recorded in CDCl₃.^a

Position	1		2	
	δ_C	δ_H (J in Hz)	δ_C	δ_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)

^a δ from TMS (ppm). ¹H-NMR [300 MHz, CDCl₃, m, J (Hz)] and ¹³C-NMR (75 MHz) spectroscopic data for Compounds **1** and **2**.

Table S2. ¹H and ¹³C NMR spectroscopic data for compounds **3-4** recorded in CDCl₃.^a

Position	3		4	
	δ_{C}	δ_{H} (<i>J</i> in Hz)	δ_{C}	δ_{H} (<i>J</i> in Hz)
1	174.0	-	174.6	-
2	134.3	-	131.2	-
3	31.8	2.26, t, (7.7 Hz)	31.9	2.40, m, 2.51, m
4	28.4	1.39-1.52, m	70.0	3.84, m
5	24.7-29.7	1.26-1.65, m	37.4	1.26-1.69, m
6	24.7-29.7	1.26-1.65, m	25.6-29.7	1.26-1.69, m
7	24.7-29.7	1.26-1.65, m	130.0	5.34, m
8	24.7-29.7	1.26-1.65, m	129.8	5.34, m
9	24.7-29.7	1.26-1.65, m	25.6-29.7	1.26-1.69, m
10	24.7-29.7	1.26-1.65, m	25.6-29.7	1.26-1.69, m
11	24.7-29.7	1.26-1.65, m	33.5	1.26-1.69, m
12	24.7-29.7	1.26-1.65, m	74.1	3.39, m
13	32.4	1.39-1.49, m	83.2	3.84, m
14	74.2	3.39, m	29.0	1.26-1.69, m
15	83.3	3.83-3.94, m	29.0	1.26-1.69, m
16	28.3	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	28.9	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, qd, (6.8, 1.3 Hz)	78.0	5.06, qd (6.8, 1.5 Hz)
37	19.2	1.41, d, (6.8 Hz)	19.1	1.43, d, (6.8 Hz)

^a δ from TMS (ppm). ¹H-NMR [400 MHz, CDCl₃, m, *J* (Hz)] and ¹³C-NMR (100 MHz) spectroscopic data for compounds **3** and **4**.