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Structure–activity relationships of diverse annonaceous acetogenins against human tumor cells

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ABSTRACT

Twelve annonaceous acetogenins (ACGs) with different stereochemical structures and configuration were selected to test for their inhibitions on the growth of Hela, SMMC-7541, SGC-7901, MCF-7 and A-5408 tumor cell lines using MTT method. This was the first to simultaneously investigate effects of structural factors of stereochemical structures and configuration on cytotoxicities with structure–activity relationship. The present study showed that cytotoxic selectivities of ACGs with *threo/trans/threo/trans/erythro* stereochemical arrangement were gently more active than those with *threo/trans/threo/trans/threo* stereochemical arrangement, and ACGs with *cis* THF ring partly produced notable cytotoxic selectivities. Furthermore, ACGs with *S* configuration at C-24 exhibited gently more cytotoxic selectivities potency than those with *R* configuration at C-24.

Annonaceous acetogenins (ACGs) isolated from *Annonaceae* plants and synthesized successfully exhibit a broad range of pharmacological properties, such as cytotoxic, antitumoral, antiparasitic, pesticidal and immunosuppresive activities.[1–5] ACGs can reduce ATP levels via inhibition of complex I (NADH: ubiquinone oxidoreductase) in mitochondrial transport system of tumor cells,[6–9] exhibiting their potential cytotoxic activities. Therefore, ACGs may be judged as promising candidates for a future generation of drugs to fight against the current chemotherapy-resistant tumors.

ACGs are usually characterized by a long aliphatic chain bearing a terminal methyl-substituted α,β-unsaturated γ-lactone ring (sometimes rearranged to a ketolactone), with one-, two-, or three-tetrahydrofuran (THF) rings located along the hydrocarbon chain. According to the number and location of the THF along the hydrocarbon chain, ACGs could be best classified into mono-THF, adjacent bis-THF, nonadjacent bis-THF, tri-THF, and nonclassical acetogenins (tetrahydropyran and ring-hydroxylated THF ACGs).

A series of natural and synthetic ACGs have been used to investigate the structure–activity relationships (SAR).[10–14] The previous works of structure–activity relationships is summarized as following:[15–17] (1) The α,β-unsaturated γ-lactone at the end of the chain and the THF rings located along the hydrocarbon chain is crucial for activity; (2) The bis-adjacent-THF ACGs are the most potent compounds among this family; the nonadjacent bis-THF compounds are, in general but not always, superior to the mono-THF compounds, which, in turn, are more potent than the non-THF ACGs; (3) If all other structural features are identical, the shorter C-35 ACGs are more potent than the C-37 compounds; (4) The spacer, that is, the distance between the OH-flanked THF and the γ-lactone, is critical to the potency and selectivity of the ACGs; for example, a 13-carbon space is optimum for activity; (5) Neither the 4-OH group nor the 10-OH group in the hydrocarbon chains is essential for activity. However, ACGs have complex stereochemical features, and several chiral centers located along the hydrocarbon chain, which are common structural features of a large number of natural ACGs. It would be very useful to identify these crucial structural factors of acetogenins required for their bioactivities.

In the present study, 12 diverse ACGs with different stereochemical structures and configuration including asimicin[18] (**1**), squamocin[18] (**2**), squamocin-D[18] (**3**), desacetyluvaricin[18] (**4**), isodesacetyluvaricin[18] (**5**), squamostatin-D[18] (**6**), squamostatin-E[18] (**7**), squamostatin-B[18] (**8**), squamostatin-A[18] (**9**), 12,15-*cis*-squamostatin-A[19] (**10**), 4-deoxyannoreticuin[20] (**11**), and *cis*-4-deoxyannoreticuin[20] (**12**) were tested for their abilities to inhibit the growth of Hela, SMMC-7541, SGC-7901, MCF-7, and A-5408 tumor cell lines using MTT method. This is the first report on SAR for simultaneous investigation of stereochemical structures and configuration. The ACGs (Fig. 1) used to study were isolated from the seeds of *Annona squamosa* by column chromatography and HPLC method, and characterized by ^{1}H NMR, ^{13}C NMR, MS and HREIMS. The purity of each compound was determined to be above 98% by HPLC analysis and confirmed by LC–MS. Their stereochemistries

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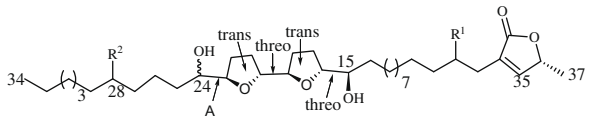
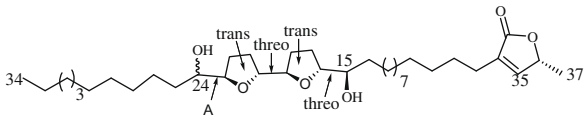
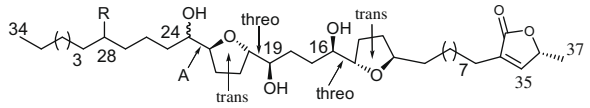


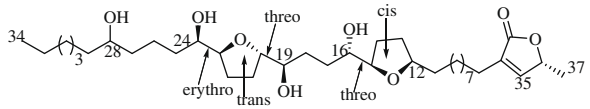
	R^1	R^2	A	Configuration
1. asimicin	OH	H	*threo*	4R, 15R, 24R
2. squamocin	H	OH	*erythro*	15R, 24S, 28R
3. squamocin-D	H	OH	*threo*	15R, 24R, 28R



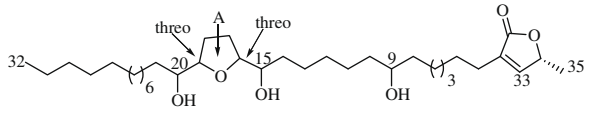
	A	Configuration
4. desacetyluvaricin	*threo*	15R, 24R
5. isodesacetyluvaricin	*erythro*	15R, 24S



	R	A	Configuration
6. squamostatin-D	H	*erythro*	16R,19R, 24S
7. squamostatin-E	H	*threo*	16R,19R, 24R
8. squamostatin-B	OH	*threo*	16R,19R, 24R, 28R
9. squamostatin-A	OH	*erythro*	16R,19R, 24S, 28R



10. 12,15-*cis*-squamostatin-A 16R, 19R, 24S, 28R



	A	Configuration
11. 4-deoxyannoreticuin	*trans*	9R, 15R, 20R
12. *cis*–4-deoxyannoreticuin	*cis*	9R, 15R, 20R

Figure 1. Structures of ACGs used for study.

and configuration were ascertained using combination of 1D and 2D NMR techniques and derivative preparations to their stereochemistries.[21]

The cytotoxicities of 12 ACGs and standard control adriamycin against the growth of five human tumor cell lines were assayed using classical MTT method[22] and the data were shown in Table 1.

Table 1
Cytotoxicity results of ACGs and standard control adriamycin

Compounds	IC_{50} (μM)				
	Hela	SMMC-7541	SGC-7901	MCF-7	A-5408
1	2.7×10^{-2}	1.1×10^{-2}	4.3×10^{-3}	1.3×10^{-2}	3.6×10^{-3}
2	9.7×10^{-3}	9.0×10^{-5}	7.3×10^{-4}	5.0×10^{-4}	8.7×10^{-4}
3	2.4×10^{-2}	1.2×10^{-2}	1.6×10^{-3}	2.8×10^{-3}	1.8×10^{-3}
4	1.6×10^{-1}	1.4×10^{-1}	4.3×10^{-2}	4.0×10^{-2}	4.0×10^{-1}
5	1.3×10^{-2}	1.6×10^{-2}	8.4×10^{-2}	2.1×10^{-2}	1.6×10^{-1}
6	3.8×10^{-2}	5.4×10^{-2}	1.8×10^{-1}	4.5×10^{-2}	1.4×10^{-3}
7	2.7×10^{-1}	1.8×10^{-1}	2.1×10^{-1}	9.0×10^{-1}	1.3×10^{-1}
8	3.8×10^{-1}	1.7×10^{-1}	1.7×10^{-1}	7.3×10^{-1}	4.3×10^{-2}
9	3.3×10^{-2}	5.0×10^{-2}	9.7×10^{-2}	9.7×10^{-1}	2.6×10^{-3}
10	1.3×10^{-3}	6.0×10^{-2}	9.3×10^{-2}	3.3×10^{-3}	1.6×10^{-2}
11	1.0	8.3×10^{-3}	2.3×10^{-1}	3.7×10^{-2}	3.2×10^{-1}
12	1.6×10^{-1}	9.0×10^{-2}	3.2	5.0×10^{-4}	1.5
Adriamycin	1.6×10^{-2}	3.7×10^{-3}	1.3×10^{-2}	4.9×10^{-2}	2.6×10^{-2}

IC_{50} (μM) values of 12 diverse ACGs and standard control adriamycin against the growth of five human tumor cell lines for 3d MTT tests.

As shown in Table 1, Figures 2 and 3, all diverse ACGs exhibited different potent cytotoxic activity against the growth of different human tumor cell lines. Some of ACGs produced more selective cytotoxicity than adriamycin which has been used in clinical application extensively. As illustrated in Table 1 and Figure 2, squamocin (**2**) showed significant cytotoxicity against all five tumor cell lines, among of them, an IC_{50} value of squamocin (**2**) against MCF-7 cells was 5.0×10^{-4} μM, which was approximately 100 times more active than adriamycin with IC_{50} value of 4.9×10^{-2} μM. Furthermore, it was observed that the dose-effective relationships of bis-adjacent-THF ACGs including asimicin (**1**), squamocin (**2**), squamocin-D (**3**), desacetyluvaricin (**4**) and isodesacetyluvaricin (**5**) were conspicuous. In addition, Figure 3 also indicated that the dose-effective relationship of nonadjacent THF ACGs including squamostatin-E (**7**), squamostatin-B (**8**), squamostatin-A (**9**) and 12,15-*cis*-squamostatin-A (**10**), and single-THF ring ACGs including 4-deoxyannoreticuin (**11**) and *cis*-

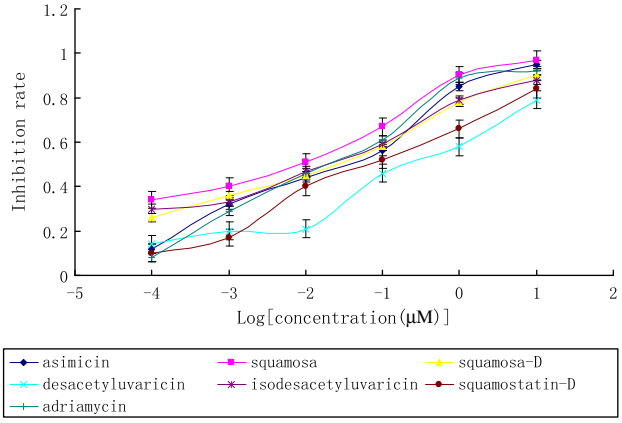


Figure 2. Comparison of the cell growth inhibition potential of asimicin (**1**), squamosin (**2**), squamocin-D (**3**), desacetyluvaricin (**4**), isodesacetyluvaricin (**5**), and squamostatin-D (**6**) versus the standard control adriamycin against Hela cells. Values were expressed as inhibition rate with each point representing the normalized average of three values and the error bars representing standard deviation about that average. The concentration values were Log [dose] with units of μM.

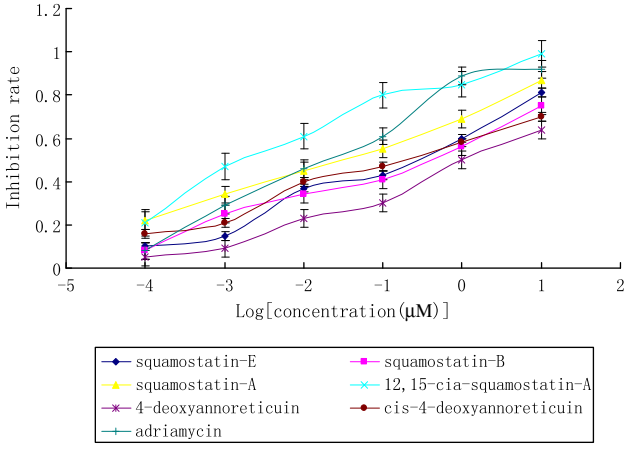


Figure 3. Comparison of the cell growth inhibition potential of squamostatin-E (**7**), squamostatin-B (**8**), squamostatin-A (**9**), 12,15-*cis*-squamostatin-A (**10**), 4-deoxyannoreticuin (**11**), and *cis*-4-deoxyannoreticuin (**12**) versus the standard control adriamycin against Hela cells. Values were expressed as inhibition rate with each point representing the normalized average of three values and the error bars representing standard deviation about that average. The concentration values were Log [dose] with units of μM.

4-deoxyannoreticuin (**12**) showed a good linear. Generally, the study results showed the bis-adjacent-THF ACGs were the most potent among ACGs tested; the nonadjacent bis-THF ACGs were, in general but not always, superior to the mono-THF ACGs, which was in agreement with the results of the previous study.[15–17] On the other hand, the stereochemistry of the THF rings had an effect on activity in this test system. Regarding the bis-adjacent THF ACGs, squamocin (**2**) and squamocin-D (**3**) are differ from each other only in the stereochemistry of one of the THF rings, squamocin (**2**) with stereochemical arrangement of *threo/trans/threo/trans/erythro* across positions C-15–C-24 was confirmed to be slightly more potency than squamocin-D (**3**) with stereochemical arrangement of *threo/trans/threo/trans/threo*. It is also noted that the difference between desacetyluvaricin (**4**) and isodesacetyluvaricin (**5**) is the stereochemistry of one-THF rings (Fig. 1). The present study showed that isodesacetyluvaricin (**5**) with *threo/trans/threo/trans/erythro* stereochemical structure was gently more potency than desacetyluvaricin (**4**) with *threo/trans/threo/trans/threo* stereochemical structure. This regularity could be further supported by comparing the activity of nonadjacent bis-THF ACGs such as squamostatin-D (**6**), squamostatin-E (**7**), squamostatin-B (**8**), and squamostatin-A (**9**), for these four compounds are differ from each other only in the stereochemistry of one of the THF rings (Fig. 1). The nonadjacent bis-THF acetogenin 12,15-*cis*-squamostatin-A (**10**) with *cis/threo/threo/trans/erythro* stereochemistry exhibited better selective cytotoxicity against Hela cells (IC_{50} 1.3×10^{-3} μM) and MCF-7 (IC_{50} 3.3×10^{-3} μM) than squamostatin-A (**9**) with *trans/threo/threo/trans/erythro* stereochemistry against Hela cells (IC_{50} 3.3×10^{-2} μM) and MCF-7 cells (IC_{50} 9.7×10^{-1} μM). From the above analysis we presume that the *threo/trans/threo/trans/erythro* stereochemical structure of the annonaceous acetogenins might play a more important role to interact with the glycerol backbone region of lipids in liposomal membranes, and it also might serve a more important role to optimize the location and the configuration of the other functional group(s) of the acetogenins, such as hydroxyls, than *threo/trans/threo/trans/threo* stereochemical structure. It was found that the two mono-THF ACGs had different selective cytotoxicity. 4-deoxyannoreticuin (**11**) showed better bioactivity against SMMC-7541 (IC_{50} 8.3×10^{-3} μM) than bis-adjacent-THF acetogenins squamocin-D (**3**), desacetyluvaricin (**4**), and isodesacetyluvaricin (**5**). Furthermore, the *cis*-4-deoxyannoreticuin (**12**) with *cis* THF ring surprisingly elicited selective cytotoxicity against human breast cancer (MCF-7) with an IC_{50} value of 5.0×10^{-4} μM. Interestingly, there are several chiral centers in the middle of chain of ACGs, and the chiral center is a considerable factor for bioactivity. As shown in Figure 1 and Table 1, the bis-adjacent-THF ACGs squamocin (**2**) with *S* configuration at C-24 produced gently more potential activities against the five human tumor cell lines than squamocin-D (**3**) with *R* configuration at C-24, and isodesacetyluvaricin (**5**) with *R* and *S* configuration at C-15 and C-24, respectively, also exhibited better bioactivity than desacetyluvaricin (**4**) with both *R* configuration at C-15 and C-24. This observation also could be supported by comparison of the IC_{50} values against five tumor cell lines of squamostatin-D (**6**), squamostatin-E (**7**), squamostatin-B (**8**), squamostatin-A (**9**) and analogical ACGs tested with different configuration. Therefore, we supported a hypothesis that the *S* configuration at C-24 might assist acetogenins to accelerate membrane transport or intracellular transport, and the *S* configuration at C-24 might facilitate the ACGs to access mitochondrial enzyme and combine receptor binding sites more convenient than ACGs with *R* configuration at C-24, In addition, the present study also provided obvious evidence that the ACGs with three hydroxyls, such as asimicin (**1**), squamocin (**2**) and squamocin-D (**3**), were more potent than the ACGs with two hydroxyls, such as desacetyluvaricin (**4**) and isodesacetyluvaricin (**5**). It was noted that the positions of OH groups failed to significantly affect the activities, indicating that this median polarity provided by three hydroxyls might be required for passage across lipophilic membranes, where excessive hydrophilicity or low polarity might result in exclusion. It is curious to note that the acetogenins are essentially C-35 or C-37 linear compounds, and lipid bilayers are typically 36 carbons across, this observation suggests a linear stretching of the acetogenins pass through the lipid membrane. Drug-membrane interactions for a series of acetogenins in liposomes are currently under investigation.

The present SAR study clearly indicated that the stereochemical factor was essential for potent activity irrespective of bis-adjacent THF ACGs or nonadjacent bis-THF ACGs. In this study, the structure–activity profile of ACGs revealed that the ACGs with stereochemical arrangement of *threo/trans/threo/trans/erythro* were gently more active than those with *threo/trans/threo/trans/threo*, and the ACGs with *cis* THF ring partly produced notable selective cytotoxicity. Moreover, the ACGs with *S* configuration at C-24 had more selective cytotoxicity than the ACGs with *R* configuration at C-24. More importantly, ACGs processed significant activities on the growth of various tumor cell lines. Interestingly, ACGs were reported to possess inhibitory activities towards multiple drug resistant (MDR) tumor cell lines.[23,24] These results suggested that ACGs might be promising antitumor candidates for future clinical application.

Acknowledgments

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References and notes

1. Yazbak, A.; Sinha, S. C.; Keinan, E. *J. Org. Chem.* **1998**, *63*, 5863.
2. Alali, F. Q.; Rogers, L.; Zhang, Y.; McLaughlin, J. L. *J. Nat. Prod.* **1999**, *62*, 31.
3. Takahashi, S.; Kubota, A.; Nakata, T. *Tetrahedron Lett.* **2002**, *36*, 453.
4. Makabe, H.; Higuchi, M.; Konno, H.; Murai, M.; Miyoshi, H. *Tetrahedron Lett.* **2005**, *46*, 4671.
5. Hwang, C. H.; Keum, G.; Sohn, K.; Lee, D. H.; Lee, E. *Tetrahedron Lett.* **2005**, *46*, 6621.
6. Londershausen, M.; Leicht, M.; Lieb, F.; Moeschle, H.; Weiss, H. *Pestic. Sci.* **1991**, *33*, 427.
7. Lewis, M. A.; Arnason, J. T.; Philogene, B. J.; Rupprecht, J. K.; McLaughlin, J. L. *Pestic. Biochem. Physiol.* **1993**, *45*, 15.
8. Ahammadsahib, K. I.; Hollingworth, R. M.; McGovern, J. P.; Hui, Y. H.; McLaughlin, J. L. *Life Sci.* **1993**, *53*, 1113.
9. Hollingworth, R. M.; Ahmmadsahib, K. I.; Gadelhak, G.; McLaughlin, J. L. *Biochem. Soc. Trans.* **1994**, *22*, 230.
10. Rodier, S.; Huerou, Y. L.; Renoux, B.; Julien Doyon, J.; Renard, P.; Pierre, A.; Gessona, J. P.; Gree, R. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1373.
11. Motoyama, T.; Yabunaka, H.; Miyoshi, H. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2089.
12. Yabunaka, H.; Abe, M.; Kenmochi, A.; Hamada, T.; Nishioka, T.; Miyoshi, H. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2385.
13. Abe, M.; Kenmochi, A.; Ichimaru, N.; Hamada, T.; Nishioka, T.; Miyoshi, H. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 779.
14. Marshall, J.; Sabatinia, J.; Valeriote, F. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2434.
15. Miyoshi, H.; Ohshima, M.; Shimada, H.; Akagi, T.; Iwamura, H.; McLaughlin, J. L. *Biochim. Biophys. Acta* **1998**, *1365*, 443.
16. Oberlies, N. H.; Chang, C.-J.; McLaughlin, J. L. *J. Med. Chem.* **1997**, *40*, 2102.
17. He, K.; Zeng, L.; Ye, Q.; Shi, G.; Oberlies, N. H.; Zhao, G. X.; Njoku, C. J.; McLaughlin, J. L. *Pestic. Sci.* **1997**, *49*, 372.
18. Zafra-Polo, M. C.; Gonzalez, M. C.; Estornell, E.; Sahpaz, S.; Cortes, D. *Phytochemistry* **1996**, *42*, 253.
19. Chang, F. R.; Chen, J.-L.; Lin, C.-Y.; Chiu, H.-F.; Wu, M.-J.; Wu, Y.-C. *Phytochemistry* **1999**, *51*, 883.
20. Hopp, D. C.; Alali, F. Q.; Gu, Z.-M.; McLaughlin, J. L. *Phytochemistry* **1998**, *47*, 803.
21. Hoye, T. R.; Hanson, P. R.; Hasenwinkel, L. E.; Ramirez, E. A.; Zhuang, Z. P. *Tetrahedron Lett.* **1994**, *35*, 8529.
22. The cell lines used for the assay, human uterine cervix cancer (Hela), human liver cancer (SMMC-754), human stomach cancer (SGC-7901), huamn breast cancer (MCF-7), and huamn lung cancer (A-5408) were purchased from Shanghai Institute of Pharmaceutical Industry (Shanghai, China), MTT and dimethyl sulphoxide (DMSO) was purchased from Sigma Chemical Co. (St Louis. MO). The cells were cultured in RPMI-1640 and DMEM supplemented

with 10% neonatal bovine serum (NBS), penicillin (100 IU/ml), and streptomycin (100 μg/ml). The cells were then incubated in a 96-well microtitre plate at a density of 8000–10,000 cell/ml. The ACGs with different concentrations, 20 μl/well were added after 12 h seeding using taxol as a standard control. The microtitre plate incubated for 72 h in a humidified atmosphere of 5% CO_2 at 37 °C. The optical densities were read on an enzyme-labeled detector (BR680) at a wavelength of 490 nm, the IC_{50} values were averaged from three parallel experiments.

23. Oberlies, N. H.; Jones, J. L.; Corbett, T. H.; Fotopoulos, S. S.; McLaughlin, J. L. *Cancer Lett.* **1995**, *96*, 55.
24. Oberlies, N. H.; Croy, V. L.; Harrison, M.; McLaughlin, J. L. *Cancer Lett.* **1997**, *115*, 73.