Acetogenins as Selective Inhibitors of the Human Ovarian 1A9 Tumor Cell Line[1]

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Abstract: Several bioactive acetogenins were selective against 1A9 (ovarian cancer) cell replication but did not show a corresponding hyperactivity against other cell lines. The most active compound (**26**, molvizarin) was a selective inhibitor of 1A9 cell replication with a potency (ED_{50} = 5 pg/mL) of over 1 million times more than that for other cell lines.

Introduction. The Annonaceous acetogenins are a group of hyperbioactive C-35/C-37 natural products, which are derived from C-32/C-34 fatty acids that are combined with a 2-propanol unit. To date, over 350 Annonaceous acetogenins have been isolated from 37 species.[2] Most acetogenins are classified into three major groups: monotetrahydrofuran (THF), adjacent bis-THF, and nonadjacent bis-THF subclasses. Generally, the in vitro cytotoxic activity follows the order: adjacent bis-THF > nonadjacent bis-THF > mono-THF. However, structure−activity requirements are not extremely restrictive.[3,4] Tetrahydropyran (THP) ring compounds and acyclic compounds also occur. The Annonaceous acetogenins are powerful inhibitors of complex I (NADH, ubiquinone oxidoreductase) in mammalian and insect mitochondrial electron transport systems[5−7] and of NADH oxidase in the plasma membrane of cancer cells,[8] both of which are related to the production of adenosine triphosphate (ATP). Consequently, acetogenins have been found to be effective against multidrug (MDR) resistant cancer cell lines that require more ATP for the efflux pump.[3,9] Membrane localization and conformation of acetogenins may relate to their potent bioactivities.[10,11] In addition, acetogenins induce apoptosis (programmed cell death)[12] and stimulate Ca^{2+}-activated K^+ current in cultured smooth muscle cells.[13] In the present work, the in vitro antitumor activities of 28 known acetogenins isolated from Annonaceous plants were evaluated (Table 1).

Biological Results and Discussion. Compounds[14] were assayed for cytotoxic activity against a human tumor cell line panel using a reported procedure,[15,16] and the data are shown in Table 2. All compounds showed the highest activity against 1A9 cell replication. Compounds **2**, **19**, **21**, **26**, and **27** were hyperactive against 1A9 cell replication, but did not show a corresponding hyperactivity against any other cell line tested. The most active compounds (**19**, **26**, and **27**) belong to the adjacent bis-THF subclass.

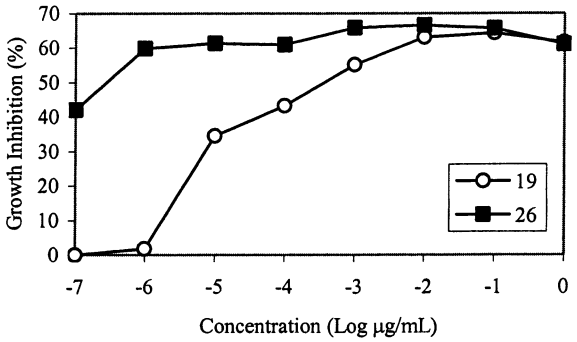

Figure 1. Effect of compounds **19** and **26** against 1A9 cell replication after 3-day exposure. The concentration values are log dose units in μg/mL.

Interestingly, all tested compounds did not show increased activity at higher concentrations; even compound **26**, which was markedly hyperactive against 1A9, showed a very shallow dose−response curve (the growth inhibition percentage of compound **26** at 1 μg/mL was approximately 62% and after a 100 000 times dilution, the growth inhibition percentage was unchanged) (Figure 1). Interestingly, Oberlies et al.[9] observed a similar inhibition pattern with bullaticin (**27**), one of the most potent acetogenins,[17] in MCF-7/Adr cells. Presumably, this behavior relates to the inhibitory action of acetogenins, but it is difficult to interpret without understanding the mechanism. The PTX10 subline of 1A9 was cross-resistant to all tested acetogenins. The PTX10 cell subline was selected from the parental line using paclitaxel and has a mutated β-tubulin gene.[18] The KB-VIN subline was not cross-resistant to the compounds, indicating that the acetogenins tested are not substrates of P-glycoprotein.

Although Hopp et al. reported that molvizarin (compound **26**) showed cytotoxic selectivity for the PC-3 human tumor cell line ($ED_{50} < 10^{-9}$ μg/mL),[19] we did not reproduce this result. In our study, compound **26** was hyperactive against 1A9 but not against PC-3 cells. Consequently, we explored whether the variation in results could be attributed to a difference in the experimental protocols. No details were described for the experimental protocol of Hopp et al., other than that the work was carried out at the Purdue Cancer Center;[20] however, the same group later used a MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay to evaluate cytotoxicities of acetogenins.[21] The principle of the MTT assay is reduction of MTT by mitochondrial enzymes such as succinate dehydrogenase.[22] In contrast, sulforhodamine B (SRB), which was used in the present work, is an anionic protein dye. Under mildly acidic conditions, SRB binds to basic amino acid residues in TCA-fixed cells to provide a sensitive index of cellular protein content. Acetogenins are known to inhibit mitochondrial enzymes,[5−7] which may explain the differences in activity reported for compound **26**. The length of treatment also varied between the bioassays. Hopp et al. used a 7-day assay

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Table 1

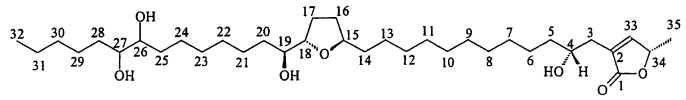
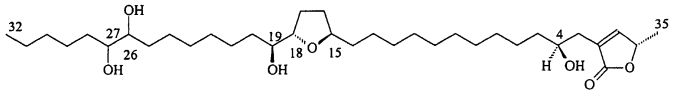
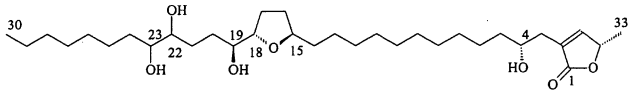
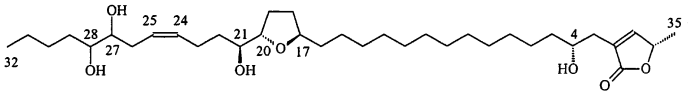
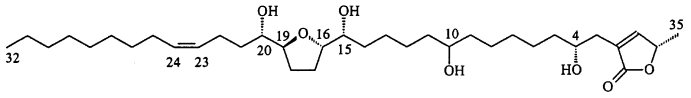
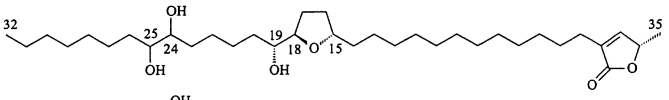
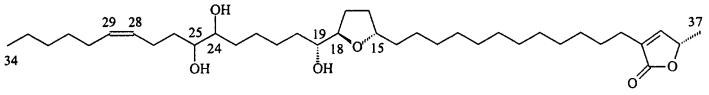
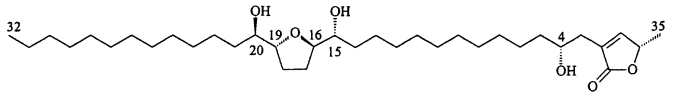
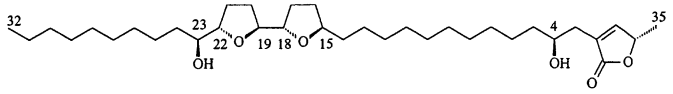
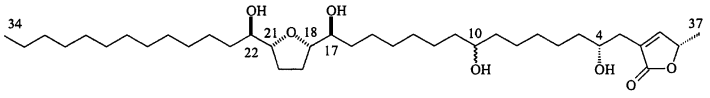
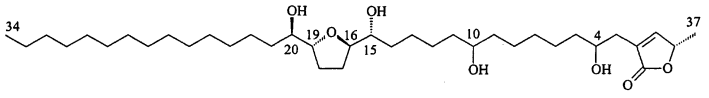
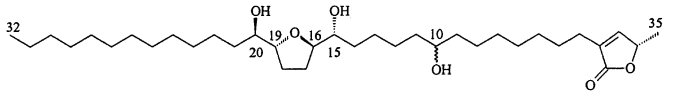
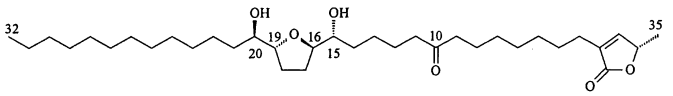
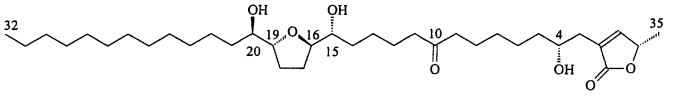
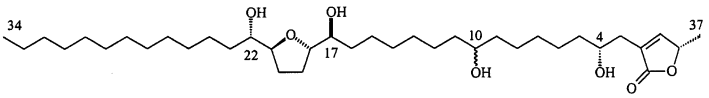
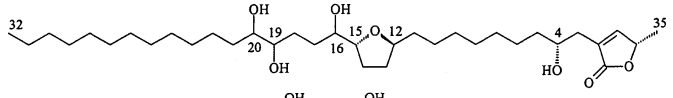
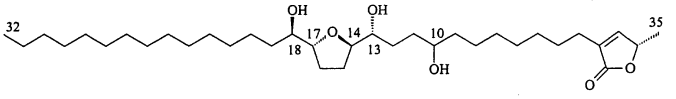
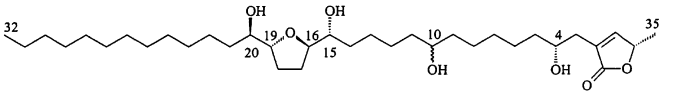
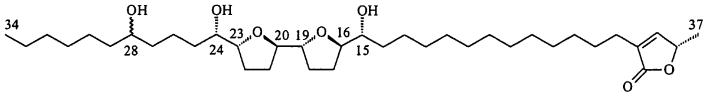
Compound	
Muricin A (**1**)	
Muricin B (**2**)	
Muricin D (**3**)	
Muricin F (**4**)	
Muricin G (**5**)	
Muricin H (**6**)	
Muricin I (**7**)	
Murisolin (**8**)	
Annocatacin A (**9**)	
cis-Annomontacin (**10**)	
Xylomaticin (**11**)	
Corossolin (**12**)	
Corossolone (**13**)	
Annonacinone (**14**) = Annonacin-10-one	
Annomontacin (**15**)	
Muricatetrocin A + Muricatetrocin B (**16**)	
Longifolicin (**17**)	
Annonacin (**18**)	
Squamocin (**19**)	

Table 1 (Continued)

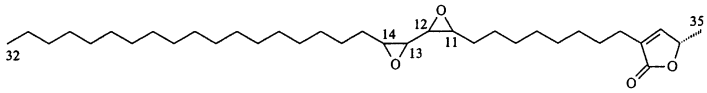
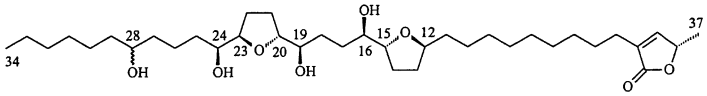
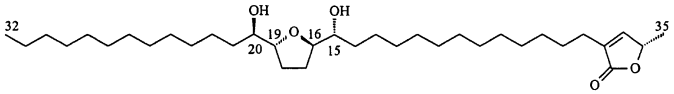
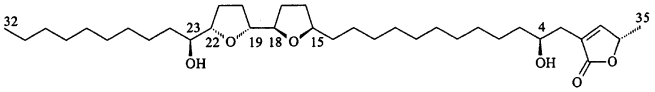
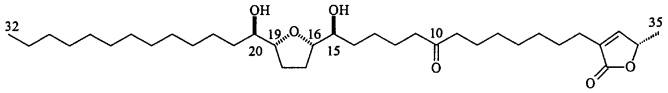
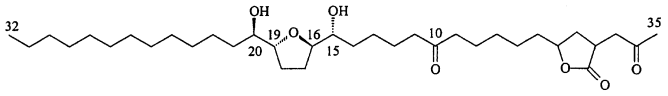
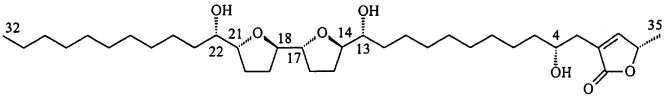
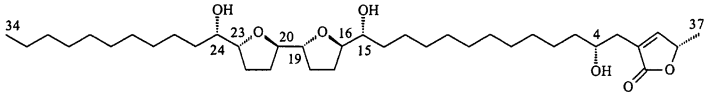
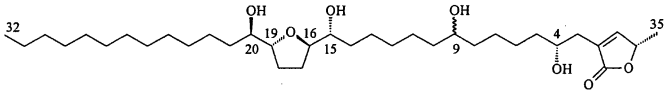
Compound	
Diepoxymontin (**20**)	
Squamostatin-A (**21**)	
Solamin (**22**)	
Annocatacin B (**23**)	
cis-Corossolone (**24**)	
Isoannonacin-10-one A (**25**)	
Molvizarin (**26**)	
Bullatacin (**27**)	
Annoreticuin (**28**)	

Table 2. Activity of Acetogenins against Human Tumor Cell Line and Drug-Resistant Cell Line Replication

	cell line/ED_{50} (μg/mL)[a,b]										
compd	SK-MEL-2	MCF-7	PC-3	HOS	HCT-8	CAKI	A549	1A9	PTX10	KB	KB-VIN
1	18.0	6.0	14.1	18.4	15.0	>20	16.8	0.33	8.2	12.8	15.0
2	>20	15.5	>20	>20	>20	NA	>20	0.52	>10 (26)	>20	>20
3	>20	15.5	>20	>20	>20	>20	>20	0.68	>10 (27)	18.0	>20
4	>20	14.3	>20	NA	>20	>20	>20	0.87	>10 (25)	>20	>20
5	17.0	7.6	15.3	17.0	14.4	19.0	17.0	0.93	>10 (40)	13.0	17.5
6	17.5	10.5	17.5	>20	>20	>20	>20	0.77	>10 (37)	15.1	>20
7	NA	>20	>20	NA	>20	NA	NA	>10 (39)	>10 (13)	>20	>20
8	>20	>20	>20	NA	>20	>20	NA	0.96	>10 (21)	>20	>20
9	15.3	7.6	14.5	16.0	13.8	17.8	17.2	1.93	>10 (30)	13.5	>20
10	15.1	6.6	14.0	15.0	15.0	17.3	14.5	0.08	8.7	10.8	14.0
11	15.0	5.6	12.2	13.6	12.7	16.0	11.5	0.50	7.2	9.0	13.3
12	14.2	<5 (55)	8.1	9.8	10.8	13.9	9.8	0.10	5.0	7.6	12.5
13	>20	9.5	>20	NA	>20	NA	>20	0.76	>10 (31)	>20	>20
14	>20	11.5	>20	>20	>20	>20	>20	0.74	>10 (36)	18.3	>20
15	14.1	<5 (51)	9.2	14.1	13.7	15.3	11.8	0.078	6.8	9.2	13.7
16	>20	11.5	>20	>20	>20	>20	>20	0.07	>10 (37)	>20	>20
17	>20	9.0	16.8	>20	20.0	>20	20.0	0.81	>10 (43)	17.3	>20
18	13.8	<5 (62)	8.2	8.2	7.9	9.8	7.8	0.07	6.0	7.5	10.3
19	16.5	6.0	11.1	8.5	9.8	13.5	9.3	0.00032	7.0	8.1	12.0
20	>20	>20	>20	>20	>20	NA	>20	8.8	>10 (15)	>20	>20
21	NA	8.0	>20	>20	>20	>20	>20	0.0087	>10 (40)	>20	>20
22	NA	>20	NA	>20	>20	NA	>20	7.8	>10 (18)	>20	>20
23	NA	>20	>20	>20	>20	NA	>20	0.10 (47)	>10 (23)	>20	>20
24	18.8	7.9	13.5	>20	>20	>20	19.0	0.82	>10 (37)	18.0	>20
25	NA	>20	>20	NA	>20	NA	>20	2.2	>10 (19)	>20	>20
26	16.4	7.7	12.9	9.9	14.9	16.4	12.9	0.000005	8.7	10.0	13.9
27	17.1	8.4	14.3	11.9	14.9	16.8	13.3	<0.00008 (55)	8.0	10.0	16.1
28	13.1	<5 (57)	7.8	13.1	14.2	16.3	12.0	0.5	9.3	11.8	17.0
Paclitaxel	ND	ND	ND	ND	0.011	ND	0.005	0.002	0.041	0.001	ND

[a] Cell line/ED_{50} in μg/mL after 3-day continuous exposure—if inhibition is <50% at the highest concentration, the percent observed is the bracketed value. [b] NA = not active (inhibition ≤ 5%) at the highest concentration. ND = not determined.

period, while cells were treated for 3 days in the current work. Although the mechanism of action of acetogenins against 1A9 cell replication is not known, 1A9 cells proliferate very quickly, and any difference in assay period needs to be considered. On the basis of this possibility, compound **26** was tested against PC-3 and 1A9 cells side-by-side, and activities over a 3-day or 7-day period were recorded using the SRB and MTT

assays. Neither the duration of treatment nor the endpoint assay method altered the finding that 1A9 cells displayed hypersusceptibility, and activity against the PC-3 cell line was not significant (>4 μg/mL; data not shown). Thus, the difference between work published by the McLaughlin group and the present study remains unexplained.

Conclusions. All tested compounds were not good substrates of P-glycoprotein (KB vs KB-VIN) and were less active against the PTX10 subline of 1A9. We have previously reported cross-resistance of PTX10 cells to other anticancer agents that are not microtubule-active.[23] Based on a prior literature study,[3,9] one mechanism of cross-resistance to acetogenins is possibly related to the depletion of adenosine triphosphate (ATP) and the ATP requirement of the efflux pump.

In our current findings, three acetogenins (**19**, **26**, and **27**) of the adjacent bis-THF subclass are selective inhibitors of an ovarian cancer cell line in vitro, with picomolar activity. This result is consistent with studies showing that, in athymic mice, bullatacin (**27**) was significantly active against transplants of A2780 human ovarian carcinoma.[6] It will be important to establish a mechanism for this potent activity and to determine which variable(s) can account for the dramatic differences in activity between independent studies.

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