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Bioorganic & Medicinal Chemistry Letters 18 (2008) 1637–1641
Bioorganic & Medicinal Chemistry Letters

Synthesis of hybrid acetogenins, α,β-unsaturated-γ-lactone-free nitrogen-containing heterocyclic analogues, and their cytotoxicity against human cancer cell lines

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Received 17 December 2007; revised 9 January 2008; accepted 15 January 2008
Available online 19 January 2008

Abstract—A series of α,β-unsaturated-γ-lactone-free nitrogen-containing heterocyclic analogues of solamin, a natural mono-THF acetogenin, have been synthesized and their cytotoxicity was investigated against 39 tumor cell lines. One of them, 1-methylpyrazol-5-yl derivative, showed selective increase of cytotoxicity against NCI-H23 with 80 times higher potency than solamin.

Annonaceous acetogenins are polyketides featuring one to three tetrahydrofuran (THF) ring(s) connected an α,β-unsaturated-γ-lactone ring via a long hydrocarbon chain. More than 400 acetogenins have been isolated from *Annonaceous* species. They exhibit a broad range of biological activities, including cytotoxic, antitumor, immunosuppressive, pesticidal, antifeedant, and antimalarial activities.[1] Some acetogenins inhibit multidrug-resistant cancer cells with the ATP-driven transport system.[2] Many researchers are engaged in the synthesis of acetogenins[3] and their analogues[4] due to their attractive biological activities. The mechanism of action is considered to involve the inhibition of mitochondrial NADH ubiquinone oxidoreductase (complex I). The inhibition suppresses ATP production, leading to apoptosis of cancer cells.[5] McLaughlin and co-workers suggested that the hydrophilic THF moiety localizes at the surface of the inner membrane of mitochondria while the γ-lactone moiety interacts with the active site of the membrane.[6] Recently, Koert and co-workers showed that analogues whose γ-lactone moiety was replaced with a quinone exert inhibitory activities against mitochondrial complex I.[7] Furthermore, Miyoshi's group reported acetogenin-type complex I inhibitors possessing alkyl tails instead of a γ-lactone ring.[8] Thus, the role of the γ-lactone moiety of acetogenins has not been clarified yet.

There are a number of pesticides targeting mitochondrial complex I, the prime example of which is tebufenpyrad. Those pesticides have a nitrogen-containing heterocycle, such as pyrazole or pyrimidine, and a hydrophobic chain. We were interested in the synthesis of hybrid molecules featuring nitrogen-containing heterocycles and the THF-ring moiety of acetogenins, and their possible use as antitumor agents (Fig. 1).

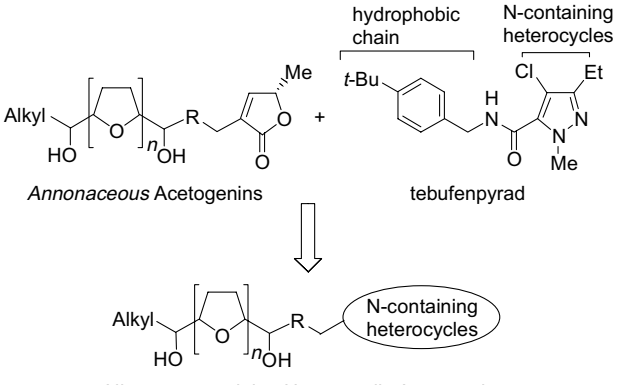

Figure 1. Design of nitrogen-containing heterocyclic analogues of *Annonaceous* acetogenins.

Keywords: *Annonaceous* acetogenins; Cytotoxity; Antitumor agents; Structure–activity relationships.
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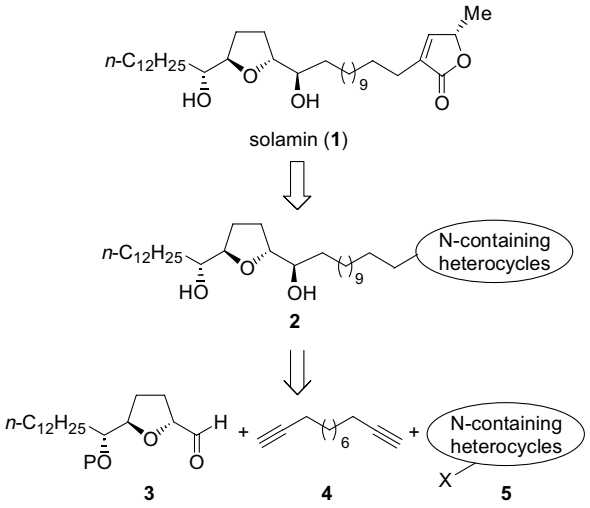
doi:10.1016/j.bmcl.2008.01.057

Recently, Duval et al. reported the semisynthesis of the heterocyclic analogues of squamocin, a natural bis-THF-ring acetogenin, and the inhibitory activities of the analogues, such as the imidazole derivative, against mitochondrial complex I.[9] However, they did not mention the cytotoxicity of the compounds. Herein, we describe the synthesis of nitrogen-containing heterocyclic analogues of acetogenin based on our stereodivergent THF-ring formation reaction[10] and the evaluation of their cytotoxicity against 39 tumor cell lines.

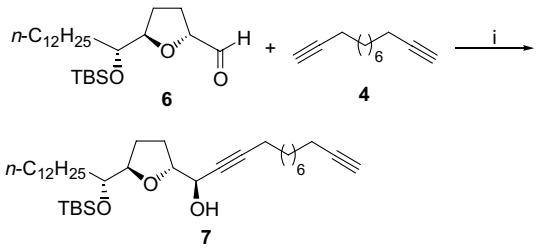
We adopted solamin (**1**),[11] a simple mono-THF acetogenin, as the lead compound, and designed the synthesis of α,β-unsaturated-γ-lactone-free nitrogen-containing heterocyclic analogues of solamin (Scheme 1). Nitrogen-containing heterocyclic analogues (**2**) can be prepared by asymmetric alkynylation of THF-ring moiety (**3**) with diyne segment (**4**), followed by Sonogashira coupling with nitrogen-containing heterocyclic halide (**5**).

THF-ring moiety (**6**) was prepared with our newly developed stereodivergent THF-ring formation reaction with high stereoselectivity (Scheme 2).[12] Reagent-controlled asymmetric alkynylation[13] of aldehyde (**6**) with 1,11-dodecadiyne (**4**) gave propargyl alcohol (**7**) with high stereoselectivity.[14]

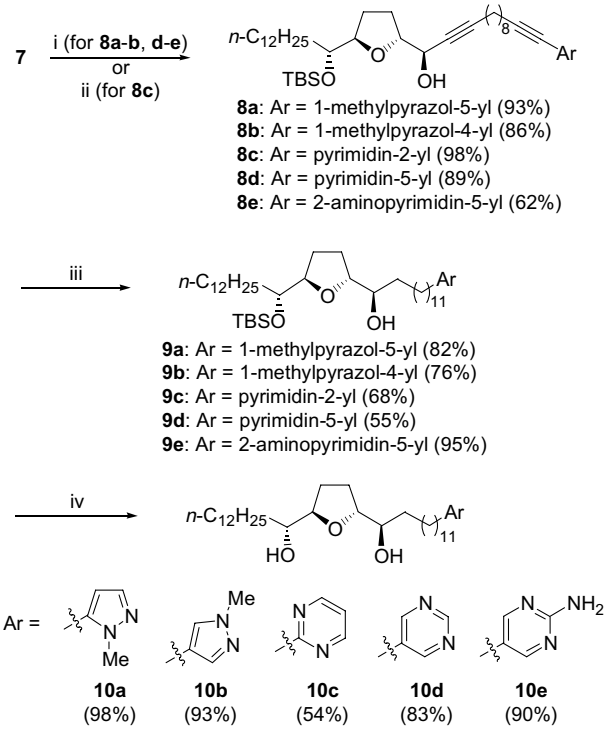
With key intermediate (**7**) in hand, we examined the introduction of nitrogen-containing heterocycles. Sonogashira coupling[15] of **7** with 5-iodo-1-methylpyrazole was carried out to give coupling product **8a** in good yield (Scheme 3). Next, hydrogenation of diyne was carried out with 10% Pd–C in EtOAc, giving compound **9a** in 82% yield. Finally, deprotection of the TBS group using aq HF in THF–CH_3CN afforded target analogue (**10a**) with the 1-methylpyrazol-5-yl moiety in high yield. On the other hand, other analogues (**10b**: 1-methylpyrazol-4-yl, **10c**: pyrimidin-2-yl, **10d**: pyrimidin-5-yl, and **10e**: 2-aminopyrimidin-5-yl) were also synthesized from **7** using the same procedure as that for **10a**.



Scheme 1. Retrosynthesis of α,β-unsaturated-γ-lactone-free nitrogen-containing heterocyclic analogues of *Annonaceous* acetogenins.



Scheme 2. Reagents and condition: (i) $Zn(OTf)_2$, Et_3N, (1*R*,2*S*)-*N*-methylephedrine, toluene, rt, 87%.



Scheme 3. Reagents and conditions: (i) Ar–X (X = Br or I), $Pd(PPh_3)_4$, CuI, Et_2NH, 50 °C; (ii) Ar–Br, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, rt; (iii) 10% Pd–C, H_2 (3 atm), EtOAc, rt; (iv) 48% HF aq, THF/CH_3CN, rt.

Synthetic nitrogen-containing heterocyclic compounds (**10a**–**e**) and solamin[16] were tested for in vitro antiproliferative activity against a panel of 39 human cancer cell lines.[17] Table 1 shows the 50% growth-inhibitory concentration relative to control (GI_{50}). Among **10a**–**e**, two analogues (**10a** and **10d**, MG-MID (mean GI_{50} value in all cell lines tested) = 42 and 31 μM, respectively) exhibited almost two times higher growth inhibition than solamin (MG-MID = 76 μM), although the activities of other derivatives (**10b**, **10c**, and **10e**) were almost the same as that of solamin (60–71 μM). Pyrazole derivatives (**10a** and **10b**, range (differences in log GI_{50} value between the most sensitive cells and the MG-MID value) = 2.26 and 1.68, respectively) displayed more selective cytotoxicity than pyrimidine derivatives (**10c**–**e**, range = 0.93, 1.39, and 1.32, respectively). However, the two pyrazole derivatives, **10a** and **10b**, showed different selectivities. For example, **10a** exhibited 14.3 times higher cytotoxicity against NCI-H23 than **10b**. Next, we compared closely nitrogen-containing heterocyclic analogues (**10a** and **10d**) and solamin (Fig. 2).

Table 1. GI_{50} values of nitrogen-containing heterocyclic acetogenins (**10a**–**e**) against 39 human cancer cell lines

Human tumor cell line	Compound/cytotoxicity (GI_{50}[a] in μM)					
	10a	**10b**	**10c**	**10d**	**10e**	Solamin
Breast						
HBC-4	>100	>100	>100	56	>100	>100
BSY-1	32	>100	>100	34	>100	>100
HBC-5	69	>100	50	17	>100	>100
MCF-7	27	>100	79	34	>100	71
MDA-MB-231	32	>100	>100	>100	>100	>100
CNS						
U251	>100	>100	>100	61	>100	>100
SF-268	>100	>100	>100	49	>100	>100
SF-295	24	24	>100	25	56	40
SF-539	37	37	41	28	62	>100
SNB-75	>100	23	32	28	27	>100
SNB-78	>100	81	>100	52	>100	>100
Colon						
HCC2998	80	>100	>100	28	89	>100
KM-12	71	>100	>100	36	>100	>100
HT-29	76	>100	>100	33	90	>100
HCT-15	>100	>100	>100	47	>100	>100
HCT-116	9.2	70	>100	28	>100	>100
Lung						
NCI-H23	0.91	13	28	11	36	73
NCI-H226	52	56	30	22	44	>100
NCI-H522	3.8	12	17	13	22	58
NCI-H460	>100	>100	>100	40	>100	>100
A549	31	>100	>100	42	>100	>100
DMS273	49	>100	92	28	>100	>100
DMS114	0.55	2.1	16	4.1	4.8	4.3
Melanoma						
LOX-IMVI	>100	9.5	12	16	58	29
Ovarian						
OVCAR-3	>100	>100	>100	29	>100	>100
OVCAR-4	37	>100	38	22	>100	>100
OVCAR-5	>100	>100	>100	>100	>100	>100
OVCAR-8	>100	>100	>100	29	90	>100
SK-OV-3	77	>100	74	33	56	>100
Renal						
RXF-631L	>100	>100	>100	43	>100	>100
ACHN	88	>100	>100	40	>100	>100
Stomach						
St-4	65	>100	>100	39	>100	>100
MKN1	>100	>100	>100	32	>100	>100
MKN7	6.2	5.4	27	6.1	9.4	13
MKN28	4.4	19	48	13	40	14
MKN45	>100	>100	>100	50	>100	>100
MKN74	23	>100	69	38	>100	>100
Prostate						
DU-145	>100	>100	>100	90	>100	>100
PC-3	>100	>100	68	35	>100	>100
MG-MID[b]	42	60	68	31	71	76
Delta[c]	1.88	1.45	0.76	0.88	1.17	1.24
Range[d]	2.26	1.68	0.93	1.39	1.32	1.36

[a] Concentration for 50% inhibition of cell growth relative to control. Cell growth was determined according to the sulforhodamine B assay.
[b] Mean GI_{50} value in all cell lines tested.
[c] Differences in log GI_{50} value between the most sensitive cells and the MG-MID value.
[d] Differences in log GI_{50} value between the most and least sensitive cells.

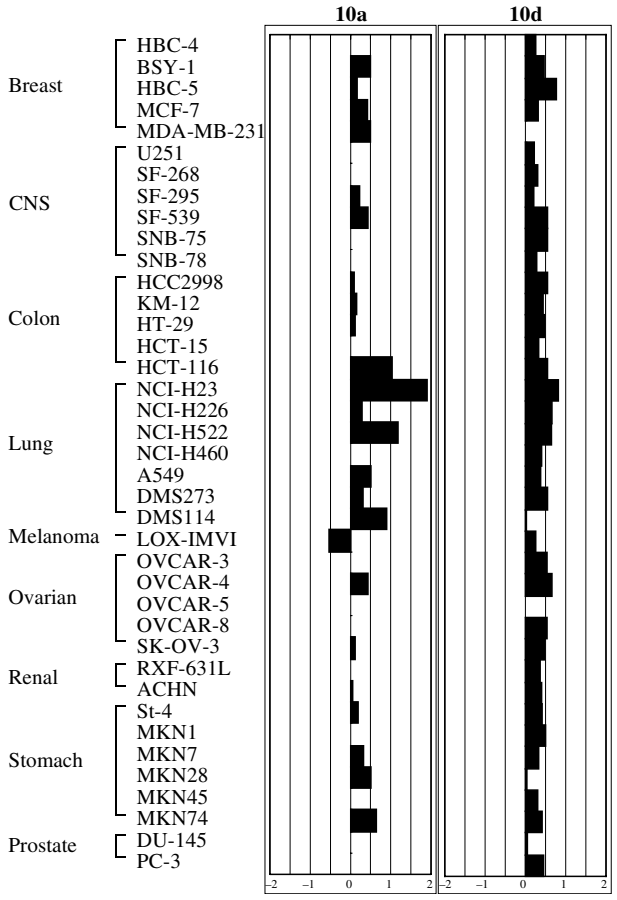


Figure 2. Differences between $\log GI_{50}$ of **10a** and **10d** tested against 39 cancer cell lines and those of solamin. The x axis represents $\delta\Delta$ ($\log GI_{50}$ of solamin—$\log GI_{50}$ of **10a** or **10d**), respectively.

Figure 2 shows that the inhibitory activities of **10d** against almost all cancer cell lines were higher than those of solamin. **10a** showed selective increases of cytotoxicity against human lung cancer cell lines. In particular, **10a** displayed strong cytotoxicity against NCI-H23 and NCI-H522 with potencies that were 80.2 and 15.3 times, respectively, than those of solamin. Using COMPARE analysis,[18] we compared the fingerprint of **10a** (Fig. 2) with those of more than 60 standard anticancer drugs currently in use, and found that the fingerprint of **10a** did not show any significant correlation with those of standard anticancer drugs, suggesting that **10a** had a unique mechanism of action. Thus, we confirmed that the unstable γ-lactone moiety[19] can be replaced with nitrogen-containing heterocycles and found two compounds with unique profiles. Further synthesis of other nitrogen-containing heterocyclic analogues and investigation of their cytotoxicity are under way.

Acknowledgments

Biological activities of the compounds were examined by the Screening Committee of New Anticancer Agents supported by a Grant-in-Aid for Scientific Research on Priority Area 'Cancer' from The Ministry of Education, Culture, Sports, Science and Technology, Japan. We acknowledge a Grant-in-Aid for Scientific Research on Priority Area from The Ministry of Education, Culture, Sports, Science and Technology, Japan.

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