Bioorganic & Medicinal Chemistry Letters 24 (2014) 1650–1653

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Identification of novel bivalent mimetics of annonaceous acetogenins via a scaffold-hopping strategy

Yanghan Liu [a], Yongqiang Liu [b], Zhen Li [c], Guang-Biao Zhou [b], Zhu-Jun Yao [d,*], Sheng Jiang [a,*]

[a] Laboratory of Medicinal Chemistry, Guangzhou Institute of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou 510530, China
[b] Division of Molecular Carcinogenesis and Targeted Therapy for Cancer, State Key Laboratory of Biomembrane and Membrane Biotechnology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China
[c] Department of Translational Imaging, The Houston Methodist Research Institute, Houston, TX 77030, United States
[d] State Key Laboratory of Coordination Chemistry, Nanjing National Laboratory of Microstructures, School of Chemistry and Chemical Engineering, Nanjing University, 22 Hankou Road, Nanjing 210093, China

ARTICLE INFO

Article history:
Received 11 November 2013
Revised 2 February 2014
Accepted 27 February 2014
Available online 12 March 2014

Keywords:
Annonaceous acetogenins
Homopiperazine
Bivalent analogues
Cytotoxicity
Selectivity

ABSTRACT

A series of novel bivalent mimetics of annonaceous acetogenins have been designed, synthesized, and evaluated. Among these, compound **7** bearing a homopiperazine ring in the middle region exhibited more potent growth inhibitory activity and higher selectivity against cancer cells over normal cells by comparison with AA005. This work indicates that modification of the middle piperazine ring is a useful optimizing tool for the simplified acetogenin mimetics.

Annonaceous acetogenins are a class of natural polyketides isolated from Annonaceous plants, and more than 400 members have been found and characterized in the past three decades.[1–3] They were found to show a broad range of biological activities, such as anticancer, antimalarial, anthelmintic, antiviral, and antimicrobial effects. It is generally accepted that annonaceous acetogenins serve as the blockers of complex **I** (NADH-ubiquinone oxidoreductase) in mitochondria and reduce the production of ATP.[4,5] Annonaceous acetogenins have been attracting worldwide attention for a long period because of their unique chemical structures and attractive biological activities especially for their anticancer activities.[1,2,6] During our efforts of simplifying natural annonaceous acetogenins into the corresponding mimetics,[7–15] we successfully invented a simple analogue AA005 by replacement of the bis-THF rings of natural bullatacin with an linear ethylene glycol ether functionality. AA005 exhibited significant potency of inhibiting the proliferation of several human cancer cell lines and selective action between human cancerous and healthy cells.[8–10] Subsequently, we further developed an improved mimicking compound having a biphenyl moiety in the left hydrocarbon chain, showing more potent inhibitory activity against cancer cell proliferation and higher cell selectivity.[16]

Discovery of bivalent ligand inhibitors has proven to be a useful protocol in medicinal chemistry and gained worldwide attention due to the potential high functional affinities through bivalent ligand–receptor interactions.[17] We recently applied this concept into synthesizing a new series of linear dimeric analogues mimicking natural annonaceous acetogenins.[18] Unfortunately, these simpler dimers were identified to exhibit 8–100 times less potency than AA005 against the growth of SGC7901 cells. In this study, we want to report a new series of bivalent analogues by a scaffold-hopping strategy, which have simple structures and potent growth inhibitory activity.

As shown in Figure 1, the newly designed mimetics are composed of three functionalities: diverse analogues of ubiquinone at the terminal(s) (P), hydrophilic piperazine ring in the middle and hydrophobic hydrocarbon chain(s) as the linkers. The common γ-lactone moiety in the natural acetogenins is suggested to be essential for binding at the quinone binding site of mitochondria complex **I**. Previously, several groups including us found that replacement of the γ-lactone moiety of natural acetogenins with the quinone portion of ubiquinone could maintain or increase the potency of complex **I** inhibition.[18–21] These results indicated that the quinone portion of ubiquinone is a good functional

* Corresponding authors. Fax: +86 20 32015318 (S.J.).
E-mail addresses: yaoz@nju.edu.cn (Z.-J. Yao), jiang_sheng@gibh.ac.cn (S. Jiang).

http://dx.doi.org/10.1016/j.bmcl.2014.02.072
0960-894X/

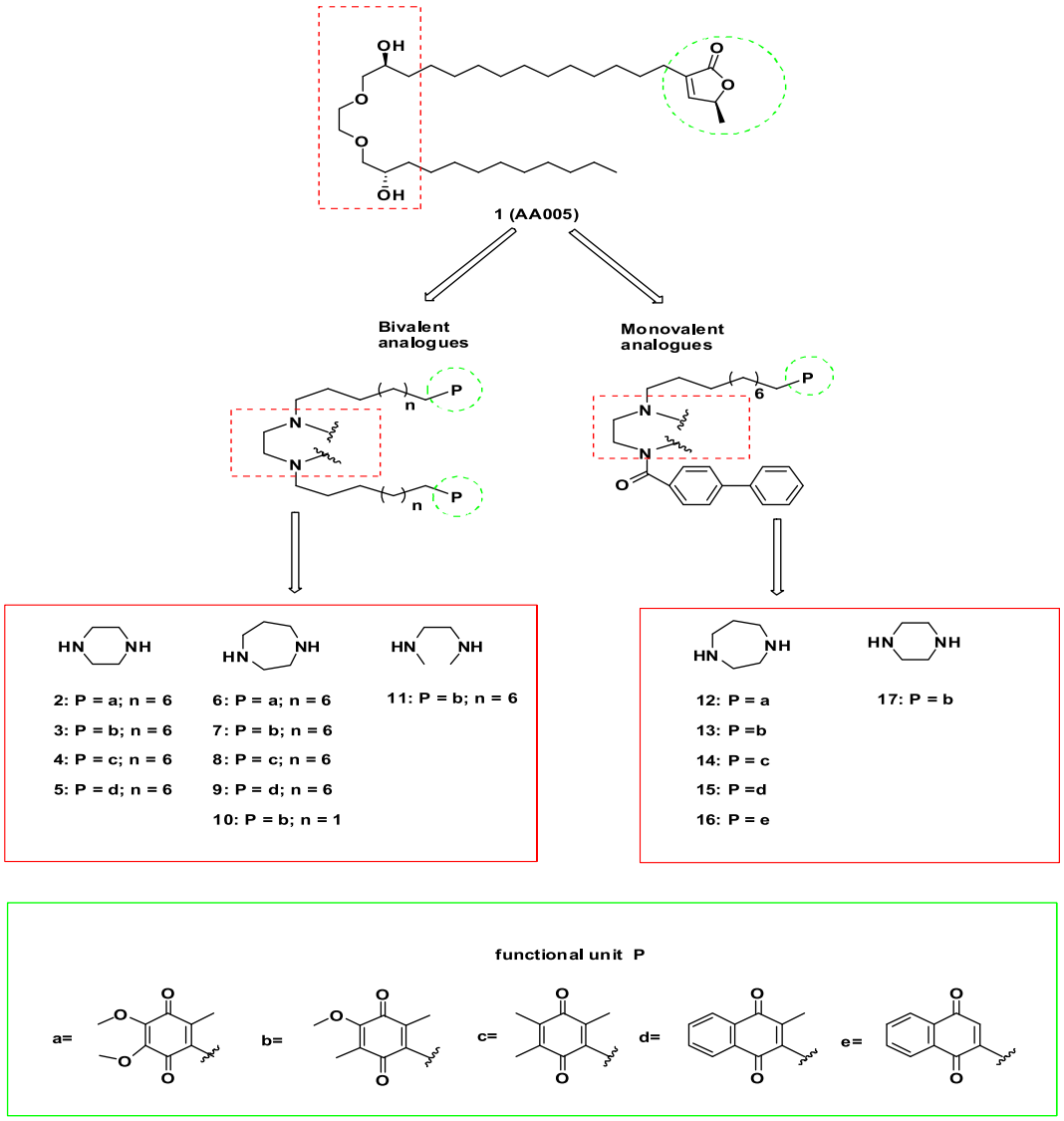


Figure 1. Design of new bivalent analogues of annonaceous acetogenins.

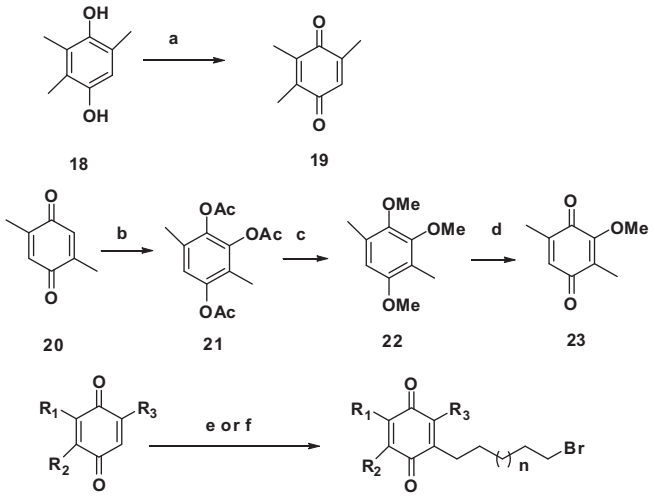
equivalent for the γ-lactone of natural acetogenins. Therefore, diverse analogues of ubiquinone were designed as an essential functionality for our bivalent mimicking. Hydrophilic piperazine ring was introduced in the middle because of its structural similarities with the bis-THF rings of natural acetogenins. We envisioned that modification of the conformational property of piperazine ring would affect inhibitory activity. Accordingly, a series of homopiperazine derivatives were also designed for comparison. The hydrophobic hydrocarbon chains of natural annonaceous acetogenins retain the amphiphilic nature of these acetogenin mimetics.

Syntheses of the ubiquinone analogues were shown in Scheme 1. Oxidation of 2,3,5-trimethylphenol **18** got **19** in 90% yield.[22] Treatment of 2,5-dimethyl-*p*-benzoquinone with acetic anhydride and boron trifluoride-etherate provided **21** in 92% yield. Then, compound **21** was treated with sodium hydroxide and dimethyl sulfate in methanol to afford **22** in 80% yield. Subsequent oxidation of compound **22** with phenyliodine diacetate (PIDA) provided **23** in 60% yield.[22] Finally, compounds **27**–**32** were synthesized in parallel via radical alkylation of the substituted quinones **19** and **23**–**26** in 20–56% yields,[23] via decarboxylation of the bromo-acid under silver nitrate and ammonium persulfate at 75 °C.

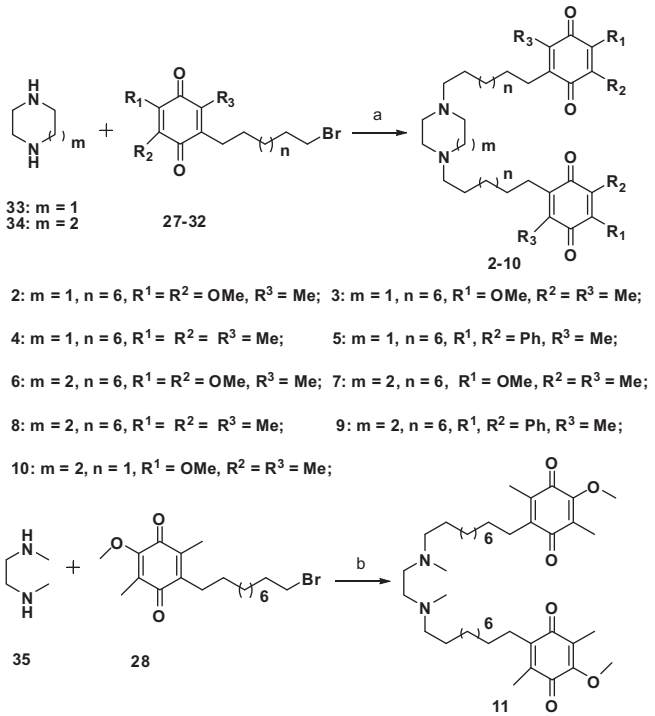
Syntheses of bivalent analogues **2**–**11** were shown in Scheme 2. Parallel treatment of piperazine or homopiperazine with corresponding quinones **27**–**32**, in the presence of catalytic amount of tetrabutylammonium iodide in acetonitrile under refluxing conditions, afforded the corresponding analogues **2**–**11**.

A number of monovalent analogues **12**–**17** were also synthesized (Scheme 3). Coupling of 4-biphenylcarboxylic acid with benzyl piperazine-1-carboxylate followed by hydrogenation of **37** afforded compound **39** in 60% yield. Compound **40** was prepared by the same procedure starting from benzyl 1,4-diazepane-1-carboxylate. Parallel reactions of compound **39** or **40** with quinones **27**–**31**, respectively, in the presence of catalytic amount of tetrabutylammonium iodide in acetonitrile under refluxing conditions, afforded monovalent analogues **12**–**17**.

All the synthesized compounds **1**–**17** were evaluated with MTT assays for their inhibitory activity against the proliferation of human gastric cancer cell line (SGC7901), colorectal carcinoma cell line (HCT-116), human lung fibroblasts (HLF) and human bronchial epithelial cell line (16HBE). The results are summarized in Table 1. Among the compounds **2**–**5** featuring a common piperazine ring in the middle region, compound **2** bearing natural ubiquinone ring at

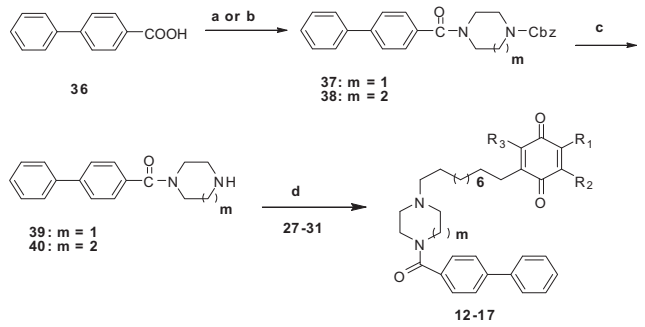


Scheme 1. Reagents and conditions: (a) I_2, H_2O_2, H_2SO_4, MeOH, 23 °C, 90%; (b) Ac_2O, $BF_3{\cdot}Et_2O$, 40 °C, 92%; (c) Me_2SO_4, aq NaOH, MeOH, 23 °C, 80%; (d) PIDA, 9:1 H_2O/MeOH, 60%; (e) 11-bromoundecanoic acid, $AgNO_3$, $(NH_4)_2S_2O_8$, 1:1 CH_3CN/H_2O, 75 °C, 20–56%. (f) 7-Bromoheptanoic acid, $AgNO_3$, $(NH_4)_2S_2O_8$, 1:1 CH_3CN/H_2O, 75 °C, 20%.



Scheme 2. Reagents and conditions: (a) TBAI, CH_3CN, reflux, 50–70%; (b) TBAI, CH_3CN, reflux, 52%.

the terminal showed 61–65 times less potency than AA005 in inhibition of the cell growth (SGC7901 and HCT-116), and no inhibition was found against HLF cell lines. Compound **3**, in which one



Scheme 3. Reagents and conditions: (a) benzyl piperazine-1-carboxylate, EDCI, HOBt, DCM, 82%; (b) benzyl 1,4-diazepane-1-carboxylate, EDCI, HOBt, DCM, 75%; (c) 10% Pd/C, H_2, MeOH, rt; (d) TBAI, CH_3CN, reflux, 50–70%.

Table 1
Antiproliferative activity of AA005 (**1**) and new mimetics **2**–**17**[a–c]

Compd	GI_{50} (μM)			
	SGC7901	HCT116	HLF	16HBE
1 (AA005)	0.16	0.03	9.715	3.34
2	9.77	1.95	>20	2.63
3	0.14	0.18	>20	>20
4	>20	0.07	>20	3.85
5	>20	0.26	>20	3.52
6	8.69	1.98	>20	1.14
7	0.04	0.04	>20	>20
8	0.14	0.3	0.36	3.32
9	0.12	0.09	4.95	2.7
10	>20	>20	>20	>20
11	9.76	1.48	>20	>20
12	5	1.36	>20	10.31
13	1.56	0.89	>20	3.15
14	1.39	1.78	>20	4
15	5.57	0.97	>20	2.85
16	>20	>20	>20	>20
17	5.27	2.29	>20	3.19

[a] AA005 was used as a positive control.
[b] Inhibition of cell growth by the listed compounds was determined by using MTT assay.
[c] Standard error of the GI_{50} was generally less than 10%.

methoxy group of ubiquinone ring was replaced by methyl group, is 10 and 70 times more potent than **2** against SGC7901 and HCT-116 cell lines, respectively. Compound **4**, in which two methoxy groups of ubiquinone ring were replaced by methyl group, is 28 times more potent than **2** against HCT-116 cell lines, but it lost its antiproliferative activity against SGC7901 cell lines. Similarly, compound **5** bearing benzo-quinone ring at the terminal lost its antiproliferative activity against SGC7901 cell lines. These results indicate that replacement of the methoxy groups of ubiquinone ring by methyl groups may be an effective strategy for activity improvement. Replacement of piperazine of compounds **2**–**5** with homopiperazine resulted in compounds **6**–**9**. They were found to exhibit more potent inhibition of cell growth than the corresponding compounds **2**–**5** against SGC7901 and HCT-116 cell lines, respectively. For instance, compound **9** exhibits 2 and 167 times more potency than the corresponding compound **5** against SGC7901 and HCT-116 cell lines, respectively. We are pleased to find that compound **7** bearing a homopiperazine ring and 3-methoxy-2,5-dimethyl quinone was identified as the best compound in

this series and showed 4 times more potent inhibitory activity against SGC7901 cell lines than reference compound AA005 (**1**). Meanwhile, compound **7** displayed higher selectivity between cancerous and normal cell lines than that of **1**. Compound **11** bearing a *N*, *N*′-dimethyl ethylenediamine in the middle is 244 times less potent than compound **7** against SGC7901 cell lines, indicating that the homopiperazine ring is a more preferred functionality. These results indicate that modification of the conformational property in the middle region of these mimetics could significantly enhance the growth inhibitory activity. Compound **10** with a shorter hydrocarbon chain lost its cytotoxicities, indicating that the moderate distance between the middle part and terminal moiety is favorable for the bioactivity.[24] Monovalent analogues are much less potent than the corresponding bivalent analogues (**13** vs **7**, **14** vs **8**, and **15** vs **9**), indicating that the second quinone is favorable for antiproliferative activities.

In summary, a series of new mimetics of annonaceous acetogenin have been designed, synthesized, and evaluated in this work. Compound **7** was identified to exhibit more potent growth inhibitory activity and higher selectivity against cancer cells over normal cells by comparison with AA005. This work indicates that replacement of the methoxy groups of ubiquinone ring by methyl groups and modification of the conformational property of the piperazine ring in the middle region may be useful optimizing tools for improving these analogues. In addition, easier synthesis of compound **7** will be advantageous for supporting more future applications and further investigations. The inhibitory study on complex **I** and metastatic tumors in animal models is currently underway in our laboratory and will be reported in due course.

Acknowledgments

This work was supported by the National Key Program for Basic Research (2010CB529201, 2010CB833202, 2012CB910800, and 2009CB940900), the National Natural Science Foundation (8107 1930, 81171925, and 21172220), the Special Foundation of President, and the Strategic Leading Science & Technology Program of the Chinese Academy of Sciences.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.02.072.

References and notes

1. McLaughlin, J. L. *J. Nat. Prod.* **2008**, *71*, 1311.
2. Bermejo, A.; Figadère, B.; Zafra-Polo, M.-C.; Barrachina, I.; Estornell, E.; Cortes, D. *Nat. Prod. Rep.* **2005**, *22*, 269.
3. McLaughlin, J. L. *Nat. Prod. Rep.* **1996**, *13*, 275.
4. Landolt, J. L.; Ahammadsahib, K. I.; Hollingworth, R. M.; Barr, R.; Crane, F. L.; Buerck, N. L.; McCabe, G. P.; McLaughlin, J. L. *Chem. Biol. Interact.* **1995**, *98*, 1.
5. JosM, R.; Tormo, T.; Gallardo, R.; AragNn, D.; Cortes, E. E. *Chem. Biol. Interact.* **1999**, *122*, 171.
6. Kojima, N.; Tanaka, T. *Molecules* **2009**, *14*, 3621.
7. Yao, Z.-J.; Wu, H.-P.; Wu, Y.-L. *J. Med. Chem.* **2000**, *43*, 2484.
8. Zeng, B.-B.; Wu, Y.; Yu, Q.; Wu, Y.-L.; Li, Y.; Chen, X.-G. *Angew. Chem.* **2000**, *112*, 2010. *Angew. Chem. Int. Ed.* **2000**, *39*, 1934.
9. Zeng, B.-B.; Wu, Y.; Jiang, S.; Yu, Q.; Yao, Z.-J.; Liu, Z.-H.; Li, H.-Y.; Li, Y.; Chen, X.-G.; Wu, Y.-L. *Chem. Eur. J.* **2003**, *9*, 282.
10. Jiang, S.; Liu, Z.-H.; Sheng, G.; Zeng, B.-B.; Chen, X.-G.; Wu, Y.-L.; Yao, Z.-J. *J. Org. Chem.* **2002**, *67*, 3404.
11. Jiang, S.; Wu, Y.-L.; Yao, Z.-J. *Chin. J. Chem.* **2002**, *20*, 1393.
12. Huang, G.; Jiang, S.; Wu, Y.-L.; Yao, Z.-J.; Wu, J. *ChemBioChem* **2003**, *4*, 1216.
13. Jiang, S.; Li, Y.; Chen, X.-G.; Hu, T.-S.; Wu, Y.-L.; Yao, Z.-J. *Angew. Chem. Int. Ed.* **2004**, *43*, 329. *Angew. Chem.* **2004**, *116*, 333.
14. Liu, H.-X.; Huang, G.-R.; Zhang, H.-M.; Jiang, S.; Wu, J.-R.; Yao, Z.-J. *ChemBioChem* **2007**, *8*, 172.
15. Liu, H.-X.; Shao, F.; Li, G.-Q.; Xun, G.-L.; Yao, Z.-J. *Chem. Eur. J.* **2008**, *14*, 8632.
16. Xiao, Q.; Liu, Y.; Qiu, Y.; Zhou, G.; Mao, C.; Li, Z.; Yao, Z.-J.; Jiang, S. *J. Med. Chem.* **2011**, *54*, 525.
17. Gestwicki, J. E.; Cairo, C. W.; Strong, L. E.; Oetjen, K. A.; Kiessling, L. L. *J. Am. Chem. Soc.* **2002**, *124*, 14922.
18. Xiao, Q.; Liu, Y.; Qiu, Y.; Yao, Z.; Zhou, G.; Yao, Z.-J.; Jiang, S. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 3613.
19. Hoppen, S.; Emde, U.; Friedrich, T.; Grubert, L.; Koert, U. *Angew. Chem. Int. Ed.* **2000**, *39*, 2099.
20. Arndt, S.; Emde, U.; Bäurle, S.; Friedrich, T.; Grubert, L.; Koert, U. *Chem. Eur. J.* **2001**, *7*, 993.
21. Yabunaka, H.; Abe, M.; Kenmochi, A.; Hamada, T.; Nishioka, T.; Miyoshi, H. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2385.
22. Duveau, D. Y.; Arce, P. M.; Schoenfeld, R. A.; Raghav, N.; Cortopassi, G. A. *Bioorg. Med. Chem.* **2010**, *18*, 6429.
23. Pei, Z.; Gustavsson, T.; Roth, R.; Frejd, T.; Hägerhäll, C. *Bioorg. Med. Chem.* **2010**, *18*, 3457.
24. Abe, M.; Kubo, A.; Yamamoto, S.; Hatoh, Y.; Murai, M.; Hattori, Y.; Makabe, H.; Nishioka, T.; Miyoshi, H. *Biochemistry* **2008**, *47*, 6260.