Bioorganic & Medicinal Chemistry Letters 21 (2011) 3613–3615

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Design, synthesis of symmetrical bivalent mimetics of annonaceous acetogenins and their cytotoxicities

Qicai Xiao [a,b], Yongqiang Liu [c], Yatao Qiu [a], Zhiyi Yao [b], Guangbiao Zhou [c], Zhu-Jun Yao [d,*], Sheng Jiang [a,*]

[a] Laboratory of Regenerative Biology, Guangzhou Institute of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou 510530, China
[b] Shanghai Institute of Technology, Shanghai 210032, China
[c] 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
[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 19 February 2011
Revised 18 April 2011
Accepted 22 April 2011
Available online 28 April 2011

Keywords:
Annonaceous acetogenins
Mimicry
Bivalent analogue
Cytotoxicity
Selectivity

ABSTRACT

A new series of linear dimeric compounds mimicking naturally occurring annonaceous acetogenins have been synthesized by bivalent analogue design, and their cytotoxicities have been evaluated against the growth of cancer cells by MTT method. Most of these compounds show selective action favored to human cancer cell lines over normal cell lines, and compound **9** with bis-terminal benzoquinone functionality exhibits an IC_{50} = 0.40 μM against MCF7 cell lines. This work mentions that appropriate conformational constraints might be a useful optimizing tool for this unique class of anticancer compounds.

More than 400 annonaceous acetogenins have been isolated from the various plants of annonaceae family growing in tropical and subtropical regions.[1–3] These acetogenins exhibit a broad spectrum of biological activities, among which the most impressive is their anticancer activity (cytotoxicity). They were considered to be the most powerful inhibitors of complex I (NADH: ubiquinone oxidoreductase) in mitochondria,[4,5] and there is evidence that some members of the family induce apoptosis in cancer cells.[6] Due to their attractive biological activities and unique structures, annonaceous acetogenins have been attracting worldwide attention for several decades.[1,2,7] We have been engaged in modifying natural acetogenins into the corresponding mimetics with simpler structures for several years.[7–16] In our previous studies, we successfully invented AA005, a mimic of naturally occurring acetogenins, by replacement of the THF rings of natural bullatacin with an ethylene glycol ether unit. This compound exhibited very potent antitumor activity against a variety of human cancer cell lines in low to medium nanomolar range (Fig. 1).[9–11] Recently, we also developed a new mimic by replacement of the hydrophobic tail of AA005 with a biphenyl moiety, which was identified to show more potent inhibitory activity and higher selectivity against cancer cells over normal cells (in comparison with AA005).[17] These results mention that the introduction of appropriate groups into the linear AA005 skeleton could be a further optimizing tool for this unique class of anticancer agents.

Recently, bivalent ligand approach has attracted worldwide attention in the design of chemotherapeutic agents. It is adopted as a strategy to improve interactions of some designed small-molecule ligands with their corresponding receptors.[18] To explore the potential of dimeric symmetrical ligands in the development of potent mimicry of acetogenins, in this study, we assumed that α,β-unsaturated γ-methylbutyrolactone and quinone moieties were the essential structural requirement for the cytotoxicity of such acetogenin mimetics. Embedment of benzoquinone functionality into this series of acetogenin mimetics is due to some quinone-containing compounds serving as potent inhibitors of NADH: ubiquinone oxidoreductase in mitochondria.[7] Accordingly, these two subunits were applied in the construction of dimeric mimicking compounds, respectively. Additional consideration on the linker (bridge) property includes good physicochemical characters, little nonspecific binding affinity and low toxicity. Therefore, some diamine-fragments frequently appeared in the synthetic drugs were considered to employ as the bridge unit in this work. In this Letter, we want to report our recent results in the design, chemical synthesis of new bivalent compounds **2**–**9** and their biological evaluation (Fig. 1).

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

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.04.095

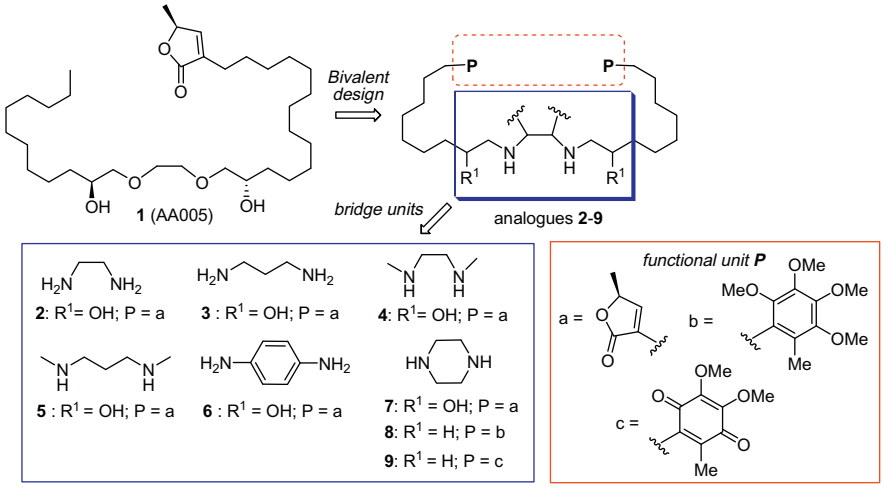
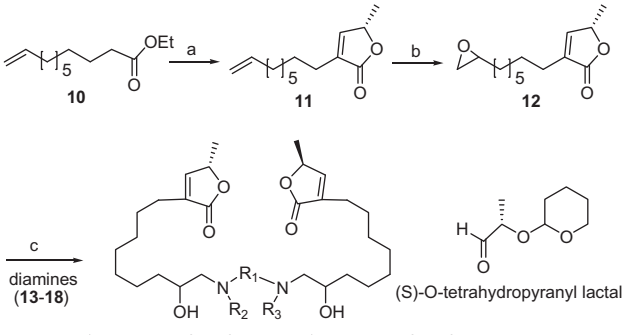


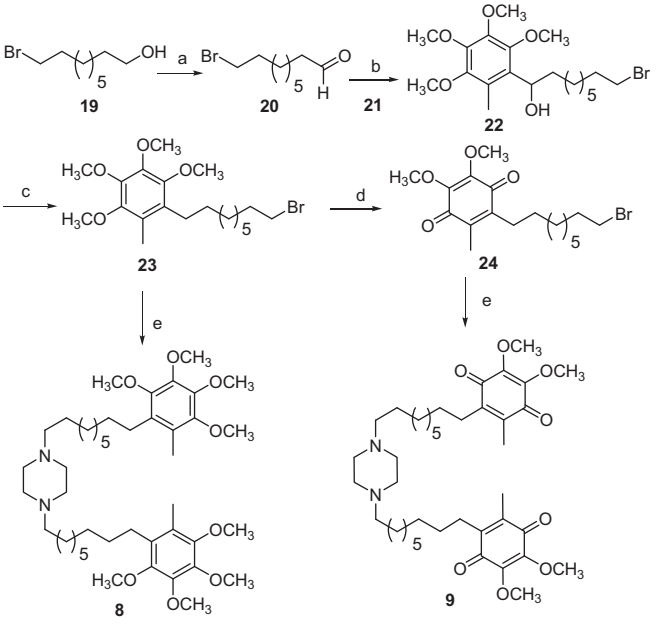
Figure 1. Design of new dimeric AA005-like molecules with conformational constraints.

To synthesize the bivalent AA005 analogues **2**–**7**, a parallel synthetic approach from the common precursor **12** was designed as shown in Scheme 1. Following our previous work,[11,14,19,20] butenolide **11** was obtained in a three-step sequence: (1) aldol condensation of ester **10** with freshly prepared (*S*)-*O*- tetrahydropyranyl lactal; (2) acid-catalyzed deprotection of the *O*-THP group and in situ lactonization, and (3) elimination of the β-hydroxy group with $(CF_3CO)_2O$ and Et_3N. Regioselective epoxidation of **11** was achieved by treatment with *m*-CPBA to give **12** in 83% yield. Subsequently, regioselective opening of epoxide **12** (2 equiv) with various diamines **13**–**18** in the presence of $ZnCl_2$ provided the final products **2**–**7** in 52–62% yield.

The other two bivalent quinone compounds **8** and **9** were synthesized using the procedure described in Scheme 2. The commercially available 9-bromononan-1-ol **19** was firstly transformed into the aldehyde **20** by Swern oxidation. *ortho*-Lithiation of 2,3,4,5-tetramethoxytoluene **21** followed by reaction with aldehyde **20** gave the alcohol **22**. The newly born hydroxyl group of alcohol **22** was then removed by reduction with $Et_3SiH/BF_3{\cdot}Et_2O$. The resulting intermediate **23** was further oxidized with ceric ammonium nitrate (CAN) to yield the quinone **24**. Subsequently, parallel assembly of intermediate **23** and **24** (2 equivalents) with piperazine was carried out in MeCN under refluxing conditions, affording target compounds **8** and **9** in 65% and 66% yield, respectively.



Scheme 1. Reagents and conditions: (a) (i) LDA, THF–HMPA, (*S*)-*O*-tetrahydropyranyl lactal, −78 °C; (ii) 10% H_2SO_4, THF, rt; (iii) $(CF_3CO)_2O$, Et_3N, CH_2Cl_2, 67%. (b) *m*-CPBA, CH_2Cl_2, 0 °C, 83%. (c) $ZnCl_2$, *i*-PrOH, 52–62%.



Scheme 2. Reagents and conditions: (a) $(COCl)_2$, DMSO, NEt_3, DCM, −78 °C, 86%; (b) 2,3,4,5-tetramethoxytoluene **21**, *n*-BuLi, TMEDA, THF, 0 °C, 52%; (c) Et_3SiH, $BF_3{\cdot}Et_2O$, DCM, −78 °C to rt, 82%; (d) CAN, CH_3CN/H_2O, RT, 83%. (e) CH_3CN, TBAI, reflux, 65–66%.

Cytotoxicity of compounds **1**–**9** was evaluated with MTT assays by inhibition of cell growth against several human solid tumor cell lines, such as SGC7901, MCF7, HCT-116, and HT-29, and human normal cell lines, such as HLF and Beas-2B. The results are summarized in Table 1. Some of these compounds were found to show good to excellent selectivity between human cancer cell lines (SGC7901, MCF7, HCT-116, and HT-29) and normal cell lines (HLF and Beas-2B). For instance, compound **2** (1,2-ethanediamine as the linkage) shows significant action preference to human cancer cell lines HCT-116 and HT-29 and no cytotoxicities were observed against HLF and Beas-2B cell lines, though it is 40–50 times less potent than AA005 in inhibition of cell growth (HCT-116 and HT-29).

Compared with **2**, compound **4** with an N-methylated 1,2-ethanediamine linker shows opposite selectivity among the four human cancer cell lines (SGC7901 and MCF7, HCT-116 and HT-29), and it is inactive against HCT-116 and HT-29 cell lines. Compound

Table 1
Inhibitory activity of AA005 and analogues **2**–**9** against cell growth[a]

Compounds	GI_{50} (μM)[b,c,d,e]					
	SGC7901	MCF7	HCT116	HT29	HLF	Beas-2B
1 (AA005)	0.06	0.26	0.11	0.35	>10	>10
2	>10	>10	5.48	14.3	NA	NA
3	NA	NA	NA	NA	NA	NA
4	6.22	3.16	NA	NA	>10	>10
5	4.12	2.38	>10	1.5	4.42	10.6
6	4.40	7.37	2.49	0.87	NA	NA
7	1.67	2.19	3.72	>10	>10	NA
8	NA	NA	NA	NA	NA	NA
9	0.49	0.40	0.50	>10	>10	>10

[a] AA005 was used as a positive control, and compounds **2**–**7** were measured in distereomeric mixtures.
[b] SGC7901: human gastric cancer cell line; MCF7: human breast cancer cell line; HCT116: colorectal carcinoma cell line; HT29: human colon cancer cell line; HLF: human lung fibroblasts; Beas-2B: human bronchial epithelial cell.
[c] Inhibition of cell growth by the listed compounds was determined by using MTT assay.
[d] NA, not active.
[e] Standard error of the GI_{50} was generally less than 10%.

3 is inactive to all tested cell lines, however, its analogue **5** by simple N-methylation of the 1,3-propanediamine moiety exhibits low micromolar potencies against the human breast cancer cells (SGC7901, MCF7 and HT-29) as well as both tested normal cell lines. A similar but more rigid analogue **7** with a 1,4-piperazine moiety could not afford a better record, though it is 2–6 times more potent than two against SGC7901, MCF7, and HCT116. More interestingly, approximately 16 times enhancement of activity against HT29 cell lines was observed when the 1,2-ethanediamine moiety was replaced by benzene-1,4-diamine (compound **6**). In addition, compound **8**, in which the α,β-unsaturated γ-methylbutyrolactone was replaced with 2,3,4,5-tetramethoxytoluene, leads to completely loss of activity. To our surprise, bis-quinone compound **9**, the oxidation product of the 2,3,4,5-tetramethoxytoluene derivative **8**, was identified as the best compound in this series and exhibits 10–25 times more inhibitory potency than compound **2** against SGC7901, MCF7, and HCT116.

In summary, we have successfully designed and synthesized a series of symmetrical bivalent mimetics of annonaceous acetogenins by introduction of conformational constraints to the linear skeleton of AA005. Biological evaluation of these bivalent analogues shows that most dimeric compounds retain the action selectivity favored to human cancer cell lines. Compound **9** with a 1,4-piperazine linker and two benzoquinone terminals exhibits the most potent inhibitory activities in this series of compounds based on the MTT assay. Furthermore, application of the bivalent analogue strategy in the mimicry of annonaceous acetogenins makes the AA005-based anticancer agents much simpler and more flexible for future further development.

Acknowledgments

We thank the National Major Scientific and Technological Program for Drug Discovery (2009ZX09103-101, 2009ZX09501), NSFC (Grant No. 20802078, 20972160, 20921091), Shanghai University Distinguished Professor (Eastern scholars) Program (DF2009-02), Pujiang Talent Plan Project (09PJ1409200) and MOST (Grant No. 2009CB940900, 2010CB529201, 2010CB833200).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.04.095.

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. Chih, H. W.; Chiu, H. F.; Tang, K. S.; Chang, F. R.; Wu, Y. C. *Life Sci.* **2001**, *69*, 1321.
7. For recent reviews see: Hu, T.-S.; Wu, Y.-L.; Yao, Z.-J. In *Medicinal Chemistry of Bioactive Natural Products*; Liang, X.-T., Fang, W.-S., Eds.; John Wiley Sons Inc.: New Jersey, 2006. pp 399; (b) Kojima, N.; Tanaka, T. *Molecules* **2009**, *14*, 3621.
8. Yao, Z.-J.; Wu, H.-P.; Wu, Y.-L. *J. Med. Chem.* **2000**, *43*, 2484.
9. 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.
10. 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.
11. 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.
12. Jiang, S.; Wu, Y.-L.; Yao, Z.-J. *Chin. J. Chem.* **2002**, *20*, 1393.
13. Huang, G.; Jiang, S.; Wu, Y.-L.; Yao, Z.-J.; Wu, J. *ChemBioChem* **2003**, *4*, 1216.
14. 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..
15. Liu, H.-X.; Huang, G.-R.; Zhang, H.-M.; Jiang, S.; Wu, J.-R.; Yao, Z.-J. *ChemBioChem* **2007**, *8*, 172.
16. Liu, H.-X.; Shao, F.; Li, G.-Q.; Xun, G.-L.; Yao, Z.-J. *Chem. Eur. J.* **2008**, *14*, 8632.
17. Xiao, Q.; Liu, Y.; Qiu, Y.; Zhou, G.; Mao, C.; Li, Z.; Yao, Z.-J.; Jiang, S. *J. Med. Chem.* **2011**, *54*, 525.
18. (a) Gestwicki, J. E.; Cairo, C. W.; Strong, L. E.; Oetjen, K. A.; Kiessling, L. L. *J. Am. Chem. Soc.* **2002**, *124*, 14922; (b) Li, F.; Jas, G. S.; Qin, G.; Li, K.; Li, Z. *Bioorg Med. Chem. Lett.* **2010**, *20*, 6577.
19. Yao, Z.-J.; Wu, Y.-L. *Tetrahedron Lett.* **1994**, *35*, 157.
20. Yao, Z.-J.; Wu, Y.-L. *J. Org. Chem.* **1995**, *60*, 1170.