Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 1579–1584

BIOORGANIC &
MEDICINAL CHEMISTRY
LETTERS

NOVEL 1, 3-DISUBSTITUTED-5, 10-DIHYDRO-5, 10-DIOXO-1*H*-BENZO[G] ISOCHROMENE-3 CARBOXAMIDES AS POTENT ANTITUMOR AGENTS

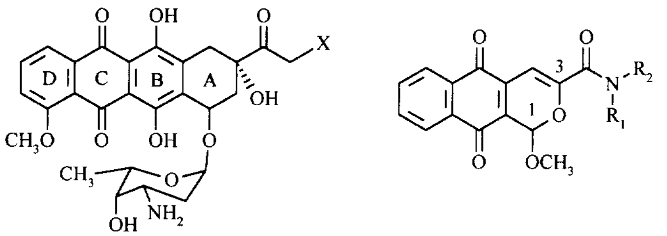
Wuyi Wang,[*,a] Tiechao Li,[b] Robert Milburn,[c] J. Yates,[d] E. Hinnant,[d]
Micheal J. Luzzio,[d] Stuart A. Noble,[d] and Giorgio Attardo[a]

[a]BioChem Therapeutic Inc., 275 Armand-Frappier Blvd. Laval, Québec, H7V 4A7, Canada
[b]Eli Lilly and Company, Lilly Research Laboratories, Indinapolis, Indiana , U. S. A.
[c]Waterloo Campus, Department of Chemistry, University of Waterloo, Ontario, N2L 3G1, Canada
[d]Glaxo-Welcome Inc. Five Moore Drive, Research Triangle Park, N.C., 27709, U. S. A.

Received 18 March 1998; accepted 1 May 1998

Abstract: Novel antitumor 5,10-dihydro-5,10-dioxo-1*H*-benzo[*g*]isochromene-3-carboxamides were discovered.

Quinone containing compounds play important roles in the area of tumor chemotherapy.[1,2] The anthracycline analogs adriamycin and daunomycin have been used in the clinic to treat various cancers. However, the therapeutic efficacy for solid tumors is limited and the clinical development of multi-drug resistance[3] to these drugs is common. In addition, these agents also suffer from serious side effects such as bone marrow depression and cardio toxicity.[4]



Doxorubicin (Adriamycin), X=OH
Daunomycin, X=H

BCH-2051, R_1=H, R_2=$(CH_2)_3N(CH_3)_2$

Figure 1

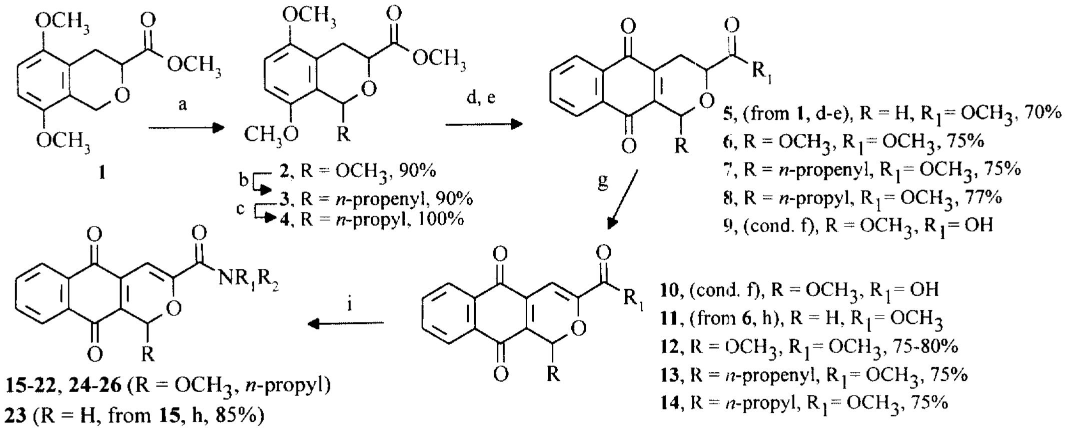
Mechanistically, anthracyclines exert their functions through the interaction with targets on the membrane and in the nucleus.[5a,b] Doxorubicin has been shown to stabilize topoisomerase II cleavable complex thereby leading to DNA damage.[5c] This action is dependent on protein kinase C activation, which is up-regulated by initial binding of doxorubicin to the plasma membrane.[6] Doxorubicin also interacts with microsomal P450 reductase and is subsequently oxidized by molecular oxygen to yield semiquinomethide and highly reactive superoxide radical species, which are implicated in cell membrane alterations, DNA cleavage and normal tissue toxicities.[7]

0960-894X/98/$19.00 © 1998 Elsevier Science Ltd. All rights reserved.
PII: S0960-894X(98)00274-1

Of the medicinal chemistry work conducted previously, 5-deoxydaunomycin having quinone function transposed from ring C to ring B showed comparable tumor responses as the parent molecule.[8] It would appear that a tricyclic version (with the maintenance of the key anthraquinone moiety) might as well possess the desired biological activity. We recently disclosed such tricyclic glycosides with a fused 3-ketopyran as ring A surrogate[9] and demonstrated good antitumor activities for these compounds.

As for further optimization of the template, we decide to explore the role of the carboxamide side chain, which, while potentially important in the interaction with DNA,[10] has not been explored previously. Chemically, carboxamides can be prepared efficiently therefore suitable for rapid establishment of SAR. We wish to describe hereafter a novel isochromene carboxamide series that displayed potent antitumor activity. (Figure 1)

Benzoisochromene 3-yl methyl carboxylates (**5**–**8**) have been prepared according to the following sequence. Starting from ester **1**,[11] oxidative methylation by DDQ[12] gave **2** from which 1-alkyl isochromans **3** and **4** were obtained in good yields. Cerium ammonium nitrate (CAN) oxidation converted these isochromans to the corresponding quinones, which added to 1-acetoxyl-1, 3-butadiene in Diels–Alder manner to give, after aromatization on silica gel during chromatographic purification, the required quinones. Conversion of ester **6** under basic conditions gave **9** and the 3, 4-unsaturated acid **10** as a mixture. Careful hydrolysis under anaerobic conditions increased the formation of **9** but its coupling with amines gave again a low quantity of the unsaturated carboxamide products. Since the elimination of **9** to **10** was facile and could complicate product purification step, it was decided that template **10** was needed for conversion to carboxamides for SAR study.[13]



(a) DDQ, methanol/CH_2Cl_2. (b) allylsilane, $BF_3.Et_2O$. (c) H_2, Pd/C. (d) CAN, CH_3CN. (e) 1-acetoxy- 1,3-butadiene, then aromatization on SiO_2 chromatography. (f) from **6**, NaOH, H_2O/THF, 70%, **9**:**10**=3:1. (g) O_2, Et_3N/Ph_3P. (h) Et_3SiH, TFA/CH_2Cl_2. (i) NaOH, H_2O/THF, then HCl; $(COCl)_2$, DMF (cat.)/CH_2Cl_2; amines or diamines, 60-70%.

Scheme 1

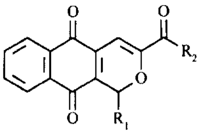
The optimal base-catalyzed dehydrogenation to the corresponding esters (**12–14**) comprised the use of two equivalents of triethylamine and four equivalents of triphenylphosphine.[14] In the absence of triphenylphosphine, the product still formed but the yield was not reproducible due to the formation of variable amount of epoxide. The standard conditions was less effective for conversion of **5** to **11**, which was better obtained from **12** by using Kraus reduction conditions.[15] Basic hydrolysis now led smoothly to acids that were activated for the coupling with amine side chains. Of various coupling conditions, acid chlorides gave the most satisfactory yields when condensed with amines or diamines. Compound **23** was optimally obtained from **15** by again using Kraus conditions. (Scheme 1).

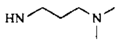
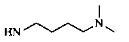
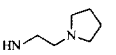
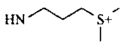
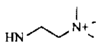
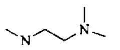
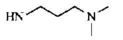
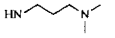
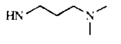
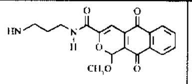
Biological screening was conducted using a microculture tetrazolium assay[16] against the human ovarian cancer cell line SKOV3, its P-170 glycoprotein mediated multi-drug resistant variant SKVLB and the colon carcinoma cell line HT-29. The MDR factor is expressed as the IC_{50} ratio of SKVLB over SKOV3.

As shown in Table 1, our novel carboxamide series displayed good to excellent activities against SKOV3. The majority of compounds exhibited *low* cross-resistance to doxorubicin . The activity against HT-29 was also measured and generally correlated with SKOV3 activity. **BCH-2051** was the most potent analog identified, showing better activity than doxorubicin in both sensitive and resistant cell lines. Other analogs with side chain termini capable of forming hydrogen bonds also gave good activity for the ovarion cell line, as seen with **17** and **18**. Compounds carrying permanently charged side chain were much less active (**20** and **21**). A secondary amide linkage was required for good activity whereas tertiary amide linkage (**22**) greatly reduced the activity. Alteration of C-1 substitution resulted in significant decrease in activity. For example, both **23** and **24** lacking cleavable group at C-1 dropped more than thirty folds in activity whereas hydroxyl analog **25** was almost inactive. Finally, dimer **26** displayed potent activity against SKOV3 and HT-29 but not with SKVLB. It is too early to derive conclusions regarding the structural requirement, but intramolecular hydrogen bonding appeared to be important for activity. (Figure 2) Indeed, the 0.7 ppm up-field shift in ^1H NMR of C-4 proton of the less active analog **22** as compared to **15** suggested a lack of planarity for the unsaturated carboxamide. With respect to chain length, 3-dimethylamino-propylamine side chain was optimal when compounds **15–17** were compared.

As for the mechanisms of action, several processes could take place. The N-(ω-aminoalkyl) carboxamide moiety has been used in anti-cancer agents having scaffolds as hycathone and derivatives.[17] More recently, imidazothioxanthone analogs carrying N-(ω-aminoalkyl) moiety were reported to display anticancer activities, which correlated with DNA binding property.[18] This mechanism was apparently supported by our observations that **15–18** and **22–24** having hydrogen bonding termini were much more potent than the bromide analog **19**. Compounds **20** and **21** were prepared in the aim of better charge interaction with DNA phosphate backbone, assuming that the side chain was situated in the minor groove of the DNA.[10] Thus the lack of activity might indicate a poor cellular penetration.

Table 1. Biological Activities of **BCH-2051** and the Analogs.



Compound	R_1	R_2	SKOV3 (IC$_{50}$, μM)	SKVLB/SKOV3	HT 29 (IC$_{50}$, μM)
15 (**BCH-2051**)	OCH_3		0.007	9.3	0.07
16	OCH_3		0.14	4.1	N.A.
17	OCH_3		0.05	10	0.17
18	OCH_3		0.06	6	1.54
19			1.06	2.60	2.76
20	OCH_3		1.3	13	N.A.
21	OCH_3		19.5	1.5	32.29
22	OCH_3		0.11	5.4	1.18
23	H		0.22	5.4	N.A.
24	*n*-Propyl		0.28	2	0.39
25	OH		3.7	6.3	6.30
26	OCH3		0.014	111	0.15
Doxorubicin	-	-	0.02	300	0.34

The quinone function in our template suggested a favorable possibility for the involvement of redox cycling. The cleavage of C-1 methoxy group was proved very facile once the quinone was reduced,[14] and the resulting benzoisochromylium species could serve as a strong alkylator. This hypothesis was in agreement with our observations that **BCH-2051** (**15**) was more active than both **23** and **24** lacking a cleavable linkage at C-1. The low activity of hydroxyl analog (**25**) was somewhat unexpected and might be the consequence of an internal hydrogen bonding between 1-hydroxyl and the neighboring carbonyl function thus rendering a less efficient quinone bio-reduction. Finally, the possible cell membrane activity of these carboxamides should be evaluated.

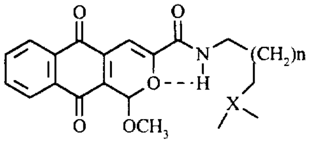


Figure 2

In summary, our novel carboxamide series display very encouraging antitumor activity and therefore warrant further studies with respect to the mode of action.[19] Given the structural difference of our series and doxorubicin, unique mechanisms underlining the observed biological activities could be expected. **BCH-2051** and several potent members exhibit comparable activity as doxorubicin in other cell lines such as human mammalian T47D and melanoma LOX tumor cell lines thereby showing potential for a broad spectrum of activity. One of the main features with this series is the improved activity against the doxorubicin resistant cell line. Studies suggest that the amino group of daunosamine is recognized by the MDR effector *p*170-glycoprotein.[20] The omission of daunosamine in our carboxamides series may be responsible for the observed low MDR, although solid evidence needs to be established. The effective synthesis is also beneficial. For example, dimeric carboxamide(s) (**26**) is prepared and shows potency equivalent to the linked anthracyclines[21] whose synthesis is more involved. Thus the readily available bis-carboxamides could be used as probes for a number of applications.[22]

References and Notes:

1. (a) *Anthracycline Antibiotics-New Analogs, Methods of Delivery and Mechanism of Action*, Priebe, W. , Ed.; ACS Symposium Series *574*, Washington, DC, **1995**. (b) *Bioactive Molecules V. 6: Anthracycline and Antracenedione-Based Anticancer Agents*; Lown, J. W., Ed.; Elsevier: New York, 1988.
2. (a) Tatsuta, K.; Ozeki, H.; Yamaguchi, M.; Okui, T. *Tetrahedron. Lett.* **1990**, *31*, 5495. (b) Kraus, G. A.; Li, J. *J. Org. Chem.* **1994**, *59*, 2219.
3. (a) Kessel, D. *Resistance to Antineoplastic Drugs*, CRC: New York, 1989. (b) Tsuruo, T.; Ogawa, M. Drug *Resistance as a Biochemical Target in Cancer Chemotherapy*, Academic: 1990.
4. (a) Gianni, L; Corden, B. J.; Myers, C. E. *Rev. Biochem. Toxicol.* **1983**, *5*, 1. (b) Blum, R. H.; Carter, S. K. 1974, *Annals of International Medicine* **1974**, *50*, 249. (c) Henderson, I. C.; Frei, E. *New Eng. J. Med.* **1979**, *300*, 310.

5. (a) Neidle, S. *Prog. Med.Chem.* **1979**, *16*, 151. (b) Tritton, T. R. *Pharmacol. Therap.* **1991**, *49*, 293. (c) Tewey, K. M.; Rowe, T. C.; Yang, L.; Halligan, B. D.; Liu, L. F. *Science* **1984**, *226*, 466.

6. In reference 1a, Vichi, P.; Song, J. Hess, J.; Tritton, T. R. *Chapter 15*, pp 241–247.

7. (a) Gaudiano, G.; Koch, T. H. *Chem. Res. Toxicol.* **1991**, *4*, 2, and references therein. (b) Sato, S.; Iwaizumi, M.; Handa, K. *Gann*, **1977**, *68*, 103. (c) Bachur, N. R.; Gordon, S. L.; Gee, M. V. *Cancer Res.* **1977**, *38*, 1745. (d) Mikkelsen, R. B.; Lin, P.-S.; Wallach, D. F. H. *J. Mol. Med.* **1977**, *2*, 33. (e) Tritton, T. R.; Murphee, S. A.; Sartoreti, A. C. *Biochem. Biophy. Res. Comm.* **1978**, *84*, 802.

8. Cameron, D. W.; Feutrill, G. I.; Griffiths, P. G. *Tetrahedron Lett.* **1988**, *29*, 4629.

9. Attardo, G.; Breining, T.; Courchesne, M.; Lamothe, S.; Lavallee, J.-F.; Nguyen, D.; Rej, R.; St. Denis, Y.; Wang, W.; Xu, Y.-C.; Barbeau, F.; Lebeau, E.; Kraus, J.-L. U.S. Patent 5,606,037, 1996.

10. Wang, A. H. J. *Nucleic Acids and Molecular Biology*, **1987**, *1*, 32.

11. Lavallée, J.-F.; Rej, R.; Courchesne, M.; Nguyen, D.; Attardo, G. *Tetrahedron Lett.* **1993**, *34*, 3519.

12. Xu, Y.-C.; Lebeau, E.; Attardo, G.; Myers, P. L.; Gillard, J. *J. Org. Chem.* **1994**, *59*, 4868.

13. Retrospectively, the choice of template **10** over **9** was correct. Data showed that **10** was 10 times as potent comparing with **9**. Compound **26** was about 10^3 more potent than its (1, 1′, 3, 3′)-tetrahydro analog.

14. Wang, W.; Breining, T.; Li, T.; Milburn, R.; Attardo, G. *Tetrahedron Lett.* **1998**, *39*, 2459.

15. (a) Kraus, G. A.; Molina, M. T.; Walling, J. A. *J. Chem. Soc. Comm.* **1986**, 1568; (b) Kraus, G. A.; Frazier, K. A.; Roth, B. D.; Taschner, M. J.; Neuenschenwarder, K. *J. Org. Chem.* **1981**, *46*, 2417.

16. Plumb, J. A.; Milroy, R.; Kaye, S. B. *Cancer Res.* **1989**, *49*, 4435.

17. Archer, S.; Pica-Mattoccia, L.; Cioli, D.; Seyed-Mozaffari, A.; Zayed, A.-H. *J. Med. Chem.* **1988**, *31*, 254.

18. Varvarsesou, A.; Tsotinis, A.; Siatra-Papastaikoudi, T.; Papadaki-Valiraki, A.; Thurston, D. E.; Jenkins, T. C.; Kelland, L. R. *Bioorg. Med. Chem. Lett.* **1996**, *6*, No. *7*, 865.

19. Mechanistic study and in vivo data of selected compounds will be communicated in due course.

20. a Priebe, W.; Van, N.T.; Burke, T. G.; Petez-Soler, R. *Anti-Cancer Drugs*, **1993**, *4*, 37; (b) Solary, E.; Ling, Y.-H.; Perez-Solar, R.; Priebe, W.; Pommier, Y. *Int. J. Cancer* **1994**, *58*, 85.

21. Henry, D. W.; Tong, G. L. U. S. Patent 4112217, 1978.

22. (a) Griffin, J. H.; Dervan, P. B. *J. Am. Chem. Soc.* **1987**, *109*, 6840. (b) Wang, W.; Lown, J. W. *J. Med. Chem.* **1992**, *35*, 2890.