Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 6138–6141
Bioorganic & Medicinal Chemistry Letters

Investigating the antiproliferative activity of quinoline-5,8-diones and styrylquinolinecarboxylic acids on tumor cell lines

B. Podeszwa,[a] H. Niedbala,[a] J. Polanski,[a,*] R. Musiol,[a] D. Tabak,[a] J. Finster,[a] K. Serafin,[a] M. Milczarek,[b] J. Wietrzyk,[b] S. Boryczka,[c] W. Mol,[c] J. Jampilek,[d,e] J. Dohnal,[d,e] D. S. Kalinowski[f] and D. R. Richardson[f]

[a]*Department of Organic Chemistry, Institute of Chemistry, University of Silesia, PL-40006 Katowice, Poland*
[b]*Department of Experimental Oncology, Ludwik Hirszfeld, Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, PL-53114 Wrocław, Poland*
[c]*Department of Organic Chemistry, Medical University of Silesia, PL-41200 Sosnowiec, Poland*
[d]*Zentiva a.s., CZ-10237 Prague 10, Czech Republic*
[e]*Department of Chemical Drugs, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences, CZ-61242 Brno, Czech Republic*
[f]*Department of Pathology, Iron Metabolism and Chelation Program, University of Sydney, Sydney, New South Wales 2006, Australia*

Received 25 July 2007; revised 7 September 2007; accepted 8 September 2007
Available online 14 September 2007

Abstract—The structure–activity relationships of new quinoline based compounds were investigated. Quinoline-5,8-dione and styrylquinoline scaffolds were used for the design of potentially active compounds. The novel analogues had comparable antiproliferative activity to cisplatin when evaluated in a bioassay against the P388 leukemia cell line. However, these compounds appeared far less efficient against SK-N-MC neuroepithelioma cells. Analogues without the 5,8-dione structure but containing the 8-carboxylic acid group were also found to induce antiproliferative activity. Hydrophobicity as measured by HPLC did not correlate with antiproliferative activity.

Quinoline-5,8-dione is a substantial molecular fragment of lavendamycin (**1**) and related compounds (**2**, **3**). Lavendamycin was originally isolated from the fermented broth of *Streptomyces lavendulae* and this class of compounds were identified in the 1970s as anti-tumor agents.[1]

The efficient synthesis of compound **2** made a number of analogues available and revealed interesting biological activity.[2–5] Their antiproliferative effects on cancer cells, including leukemia cells[6,7] and A549 carcinoma cells, have been reported.[8]

Although the toxicity of lavendamycin makes it unsuitable for clinical use, the activity of this compound has inspired several investigations.[9–12] Recently, the syntheses of a series of quinoline-5,8-diones and lavendamycin derivatives have been described. The evaluation of their antiproliferative activity against transformed cells and a normal rat kidney epithelial cell line validates their importance as potential anti-tumor agents.[3] In the current study, we investigated the potential anti-tumor activity of several known and novel quinoline-5,8-dione analogues and derivatives.

As reported recently, structural modification of lavendamycin to quinoline-5,8-diones having H or Me substitutions at position 2 retains high cytotoxicity. However, the resulting drugs are unselective and do not differentiate between tumor and normal cells, making their pharmaceutical use as anti-cancer agents problematic. On the other hand, the unmodified lavendamycin molecule showed improved selectivity against cancer cells. The highest selective toxicity being observed for analogues having a carboxylic acid group or its derivative at the C-2′ position (see **1**, **2** in Fig. 1).[3]

Keywords: Antiproliferative activity; Quinoline-5,8-diones; Quinolinecarboxylic acid.
* Corresponding author. E-mail: Polanski@us.edu.pl

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.09.040

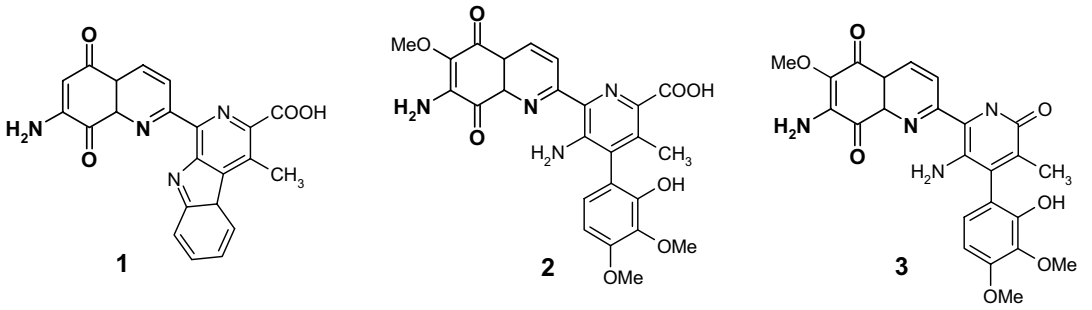


Figure 1. Structures of: lavendamycin (**1**), streptonigrin (**2**), and streptonigrone (**3**). In bold, the 7-aminoquinoline-5,8-dione moiety is shown.

In the current investigation, we examined the antiproliferative activity of a series of quinoline-5,8-diones against the mouse leukemia P388 line and human SK-N-MC cancer cell type. This includes both the analogues previously described and novel compounds **7**, **9** and **10**. Moreover, the effectiveness of the carboxylic substitution within the non-quinoline moiety of lavendamycin inspired us to investigate the importance of the carboxylic substitution by itself. Therefore, we synthesized a series of quinoline analogues **12**–**32** having carboxylic substitution(s) in the carbocyclic ring.

As shown in Figure 2, quinolines containing the carboxylic group were obtained according to Skraup protocol from appropriate aminobenzoic acids. Styrylquinolines were synthesized from 8-hydroxyquinaldine or quinaldic acids by the Skraup reaction, employing a microwave-assisted protocol.[13,14] Multi-step reactions lead us to **4** and **5**. Acylation with appropriate chlorides afforded amides **6**–**10**.

The antiproliferative activity of the synthesized compounds was tested against the P388 mouse leukemia and SK-N-MC human neuroblastoma cell lines.[15] Results are shown in Table 1. The clinically used cytotoxic agent, cisplatin, was used as a reference compound in this examination. Generally, the antiproliferative activity of the quinolinediones on P388 cells (Table 1, compounds **6**–**10**) was similar to that of cisplatin. The level of antiproliferative activity of compounds **6**–**10** against P388 cells was similar, irrespective of the individual 7-R(CO)N-substitution pattern. The N-unsubstituted compound **4** was also an efficient cytotoxic agent, although the activity of this compound was lower. It is noteworthy that the 7-methoxy substitution in compound **5** preserves the activity of analogue **4**. This indicates that although the 7-amido substitution (compounds **6**–**10**) is necessary for high cytotoxicity, the potent activity of the quinolinedione scaffold is not limited to the 7-amine substitution. The antiproliferative activity of the two quinoline-5,8-dione compounds, **5** and **6**, tested against SK-N-MC cells, was far less potent than that seen in P388.

We investigated if further modification of the quinoline scaffold by the removal of the 5,8-quinone function would retain activity. Therefore, 8-hydroxy-5,7-dinitroquinoline **11** was obtained. The presence of the nitro-

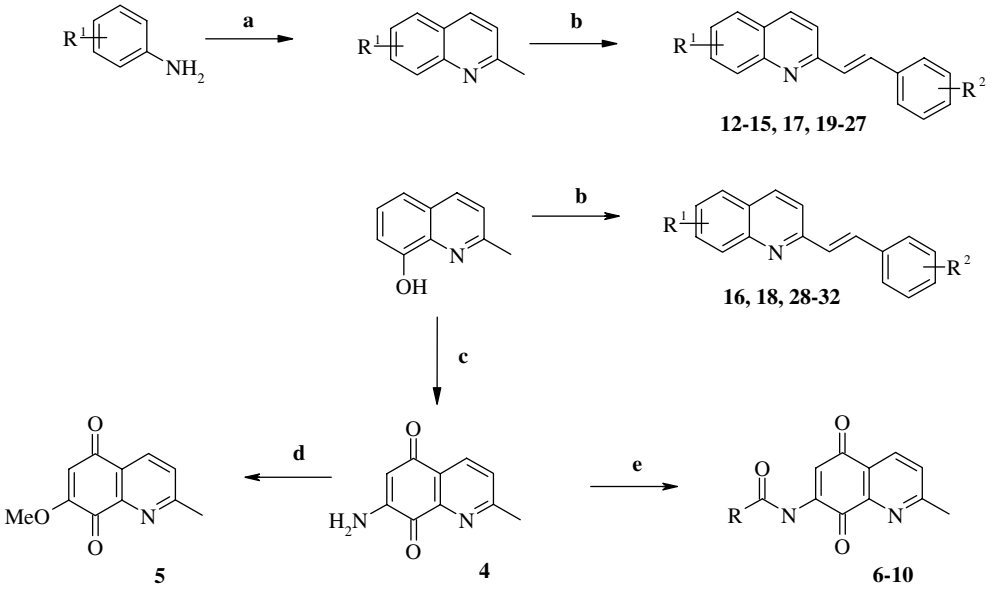
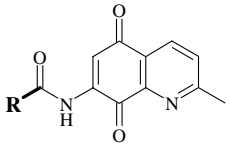
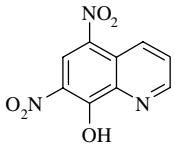
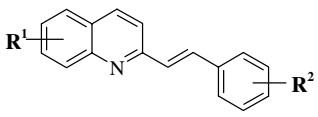


Figure 2. (a) Skraup synthesis, crotonaldehyde; (b) microwave irradiation[13] and aromatic aldehyde; (c) multi-step synthesis described earlier[2]; (d) MeOH; (e) Py/acid chlorides.

Table 1. Log *K* determinations using HPLC and antiproliferative activity assay results of the compounds examined in this study

Compound[a]		Log *K*	Antiproliferative activity	
			P388	SK-N-MC
	R		IC_{50} (μM)	IC_{50} (μM)
4	See Figure 2	ND	10.31 ± 6.80	ND
5	See Figure 2	0.0907	6.10 ± 2.80	4.26
6	CH_3–	0.2227	1.61 ± 0.87	> 6.25
7	CH_3CH_2–	ND	1.43 ± 1.06	ND
8	$CH_3CH_2CH_2$–	ND	1.51 ± 0.85	ND
9	$PhCH_2CH_2$–	ND	1.44 ± 0.28	ND
10	(*E*)-PhCH=CH–	ND	2.39 ± 0.34	ND
11		0.7154	7.14 ± 3.78	> 6.25
				
	R^1	**R^2**		
12	8-COOH	2-OH	1.6787	13.56 ± 1.24	ND
13	5,8-COOH	2-Cl	1.2047	113.72 ± 14.70	ND
14	5,8-COOH	4-Cl	1.2107	109.68 ± 10.54	ND
15	5,8-COOH	3-Cl	1.2163	113.81 ± 32.42	ND
16	5,7-NO_2-8-OH	4-OMe	ND	79.11 ± 10.64	ND
17	6-COOH	4-Cl	1.6043	116.29 ± 46.91	ND
18	8-OH	3-Cl	1.5395	15[b]	ND
19	6-COOH	2-OH	1.3842	51.15 ± 31.44	ND
20	5,8-COOH	2-Br	1.2062	89.40 ± 30.43	ND
21	5-COOH	3-OMe	1.2263	100.02 ± 24.3	ND
22	8-COOH	4-Cl	1.8165	15.33 ± 1.26	ND
23	6-COOH	3-Cl	1.6315	109.77 ± 53.91	ND
24	5-COOH	2-OH	1.2550	30[b]	ND
25	5,8-COOH	3-OMe	1.1811	25[b]	ND
26	5-COOH	4-Br	1.4397	109.12 ± 18.21	ND
27	7-COOH	2-OH	1.3678	30[b]	ND
28	5,7-NO_2-8-OH	4-Cl	ND	7.14 ± 3.78[c]	ND
29	5,7-NHAc-8-OAc	2,4-OMe	ND	20[b]	ND
30	5-NO_2-7-COOH-8-OH	2-Cl	ND	158.38 ± 45.53	ND
31	5-NO_2-7-COOH-8-OH	4-OMe	ND	147.63 ± 49.44	ND
32	5,7-NHAc-8-OAc	2-Br	ND	6.58 ± 4.43	ND
	cisplatin			**3.39 ± 1.54**	ND

The results are means ± standard deviation of 3–7 experiments. ND, not determined.

[a] Compounds **4**–**6** and **11** were described previously in Ref. 2, compound **8** in Ref. 4, compounds **12**, **15**, **16**, **18**, **19**, **21**, **24**, **27** in Ref. 3, **28**, **29**, **31** in Ref. 16, **23** in Ref. 17.

[b] Antiproliferative activity could be measured only as percent of inhibition of cancer cells proliferation at 0.1 μM.

[c] Under poor solubility in the test medium.

substituents makes the 8-hydroxy group highly acidic which should mimic the 8-quinolinone structure. In fact, it appeared that this compound was an efficient antiproliferative agent against P388 cells, having activity similar to that of the unsubstituted 7-aminequinaldine-5,8-dione (compound **4**). The SAR observed in compounds **6**–**10** indicated that the cytotoxic activity of these analogues depended only to a limited extent upon the individual structure of the amide group. It is of interest to note that there is a marked difference in the antiproliferative activity of compounds **5**, **6**, and **11** when comparing P388 leukemia cells and the SK-N-MC neuroepithelioma line. Similarly to compound **6**, analogue **11** showed very limited activity against the SK-N-MC line, while compound **5** was active against both line types.

In the styrylquinoline series (compounds **12**–**32**), some interesting relationships between structural properties and P388 cell line activity were observed. Compound **28** having the 8-OH function of the acidity enhanced by 5,7-dinitro substitution showed the activity similar to that of compounds **4** and **11**, which can be probably explained by the effect similar to that discussed for compound **11**.

The carboxylic group located at the C-8 position was found to induce antiproliferative effects, as observed in compounds **12** and **22**. If we compare the activity of compounds **12** and **22** to the high activity of compound **11**, where the 8-hydroxy group mimics the C=O through nitro substitution, the antiproliferative efficacy was probably due to the close proximity of the C=O and the heterocyclic nitrogen. This explanation is thought to be more probable to explain the activity rather than implicating the ionizable properties of the 8-COOH group. This feature is reminiscent of the original lavendamycin **1** structure, featuring a carbonyl with a neighboring heterocyclic nitrogen.

On the other hand, the CO/N proximity rule was no longer true if an additional COOH group was present within the quinoline moiety, e.g., the activity of compounds **13**–**15** was much lower than that observed for compound **12** or the activity of compounds **30** and **31** was much lower than that observed for **22**.

The same effect was observed for all compounds having the COOH substitution accompanied by an additional electroaccepting group. Thus, compound **32** having high activity (CO/N proximity can be here identified by the heterocyclic N and 8-O(CO)Me substitution) can be compared to low activity compounds **30**–**31** which have an additional 5-COOH/7-NO_2 system. Since such a substitution increases the ionization of COOH, this suggests that this effect is disadvantageous for antiproliferative activity.

The attempt to explain the antiproliferative activity of this series of compounds by hydrophobicity measured by HPLC (column 3, Table 1) was unsuccessful. This parameter did not closely correlate with antiproliferative activity.

Acknowledgments

This study has been supported by Polish Ministry of Science Grant No. 3T09A 01127 and by the Ministry of Education of the Czech Republic MSM 6215712403. D.R.R. thanks the National Health and Medical Research Council (NHMRC) of Australia for Fellowship and Project Grant funding and the Australian Research Council (ARC) for a Discovery Grant. D.S.K. appreciates the support of an Australian Post-Graduate Award.

References and notes

1. Rao, K. V. *Cancer Chemother. Rep.* Part 2 **1974**, *4*, 11.
2. Behforouz, M.; Haddad, J.; Cai, W.; Arnold, M. B.; Mohammadi, F.; Sousa, A. C.; Horn, M. A. *J. Org. Chem.* **1996**, *61*, 6552.
3. Behforouz, M.; Cai, W.; Mohammadi, F.; Stocksdale, M. G.; Gu, Zh.; Ahmadian, M.; Baty, D. E.; Etling, M. R.; Al-Anzi, Ch. H.; Swiftney, T. M.; Tanzer, L. R.; Merriman, R. L.; Behforouz, N. C. *Bioorg. Med. Chem.* **2007**, *15*, 495.
4. Behforouz, M.; Cai, W.; Stocksdale, M. G.; Lucas, J. S.; Jung, J. Y.; Briere, D.; Wang, A.; Katen, K. S.; Behforouz, N. C. *J. Med. Chem.* **2003**, *46*, 5773.
5. Behforouz, M.; Gu, Z.; Cai, W.; Horn, M. A.; Ahmadian, M. *J. Org. Chem.* **1993**, *58*, 7089.
6. Balitz, D. M.; Bush, J. A.; Bradner, W. T.; Doyle, T. W.; O'Herron, F. A.; Nettleton, D. E. *J. Antibiot. (Tokyo)* **1982**, *35*, 259.
7. Abe, N.; Nakakita, Y.; Nakamura, T.; Enoki, N.; Uchida, H.; Takeo, S.; Munekata, M. *J. Antibiot. (Tokyo)* **1993**, *46*, 1672.
8. Fang, Y.; Linardic, C. M.; Richardson, D. A.; Cai, W.; Behforouz, M.; Abraham, R. T. *Mol. Cancer Ther.* **2003**, *2*, 517.
9. Doyle, T. W.; Balitz, D. M.; Grulich, R. E.; Nettleton, D. E.; Gould, S. J.; Tann, C.-H.; Meows, A. E. *Tetrahedron Lett.* **1981**, *22*, 4595.
10. Hackethal, C. A.; Golbey, R. B.; Tan, C. T. C.; Karnofsky, D. A.; Burchenal, J. H. *Antibiot. Chemother.* **1961**, *11*, 178.
11. Boger, D. L.; Yasuda, M.; Mitscher, L. A.; Drake, S. D.; Kitos, P. A.; Thompson, S. C. *J. Med. Chem.* **1987**, *30*, 1918.
12. Wilson, W. L.; Labra, C.; Barrist, E. *Antibiot. Chemother.* **1961**, *11*, 147.
13. Musiol, R.; Podeszwa, B.; Finster, J.; Niedbala, H.; Polanski, J. *Monatsh. Chem.* **2006**, *137*, 1211.
14. Musiol, R.; Jampilek, J.; Kralova, K.; Richardson, D. R.; Kalinowski, D. S.; Podeszwa, B.; Finster, J.; Niedbala, H.; Palka, A.; Polanski, J. *Bioorg. Med. Chem.* **2007**, *15*, 1280.
15. The mouse P388 leukemia cell line and human SK-N-MC neuroepithelioma cell type were obtained from the American Type Culture Collection (Rockville, MD, U.S.A.). The P388 cell line was cultured in RPMI 1640 medium with 2 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, and 1.0 mM sodium pyruvate, 10% fetal bovine serum (FBS) at 37 °C in 5% CO_2. The SK-N-MC cell type was cultured in minimum essential medium (MEM; Gibco, Melbourne Australia) containing 10% (v/v) FBS, 1.0 mM sodium pyruvate (Gibco), 1% (v/v) non-essential amino acids (Gibco), 2 mM L-glutamine (Gibco), 100 U/mL penicillin (Gibco), streptomycin (Gibco), and 0.28 μg/mL fungizone (Squibb Pharmaceuticals, Montreal, Canada). Twenty-four hours before addition of the tested compounds, the cells were plated in 96-well plates. The assay was performed after 72 h exposure to varying concentrations of the tested agents. The results were calculated as IC_{50} values. Each concentration of each individual compound was tested in triplicate in a single experiment, with each experiment being repeated 3–7 times. After 72 h of incubation with tested compounds, 20 μL of MTT solution (MTT: stock solution: 5 mg/mL) was added to each well and incubated for 4 h at 37 °C. After this incubation, 80 μL of the lysis mixture was added to each well (lysis mixture: 225 mL dimethylformamide, 67.5 g sodium dodecyl sulphate, and 275 mL of distilled water). The optical densities of the samples were read after an incubation of 24 h at 570 nm.
16. Polanski, J.; Niedbala, H.; Musiol, R.; Podeszwa, B.; Tabak, D.; Palka, A.; Mencel, A.; Mouscadet, J.-F.; Le Bret, M. *Lett. Drug Des. Discov.* **2007**, *4*, 99.
17. Polanski, J.; Niedbala, H.; Musiol, R.; Tabak, D.; Podeszwa, B.; Gieleciak, R.; Bak, A.; Palka, A.; Magdziarz, T. *Acta Pol. Pharm., Drug Res.* **2004**, *61*, 3.