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SYNTHESIS AND BIOLOGICAL ACTIVITIES OF TRUNCATED ACRIDONE: STRUCTURE-ACTIVITY RELATIONSHIP STUDIES OF CYTOTOXIC 5-HYDROXY-4-QUINOLONE

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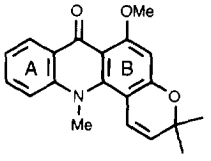
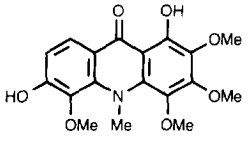
Abstract: A series of 5-hydroxy-4-quinolone (**3**) and 5-methoxy-4-quinolone (**4**) derivatives were synthesized as truncated acridone analogues and evaluated for antitumor and antiherpes activities. Among them 5-hydroxy-8-methoxy-quinolone showed potent antitumor activity (IC_{50} = 17.7 μM for HL60) which was greater than that of acronycine. © 1997 Elsevier Science Ltd.

The acridone alkaloid, acronycine (**1**) was isolated from *Acronychia baueri* in 1948[1] and was found to have potent antitumor activity (IC_{50} = 26.2 μM for HL60)[2]. The structure-activity relationships (SAR) of a series of naturally occurring acridone alkaloids have been studied to determine the effects of these compounds on the inhibition of cell growth and macromolecular biosynthesis of human promyelocytic leukemia cells[3]. Among 50 alkaloids tested, glyfoline (**2**) was found to be the most active compound (IC_{50} = 2.2 μM) and 22 alkaloids were found to be more active than acronycine. Further studies of glyfoline congeners by Watanabe and Su[4] revealed that the intramolecular hydrogen bonding between the 1-hydroxy and the peri-carbonyl function of the 1-hydroxy-9-acridone nucleus was an important determinant of their cytotoxicity. A positive inductive effect on this hydrogen-bonding by alkyl substitution on the nitrogen (NMe_2 > NHMe > NH_2) of 1-hydroxy acridones resulted in slightly increased cytotoxicity. Although the derivatives with substituents only on the B ring (i.e., normelicopicine, 1-hydroxy-10-methyl-2,3,4-trimethoxyacridine-9-one) were inactive[4a], this study did not determine conclusively whether substituents on the A ring affected cytotoxicity.



1. Acronycine



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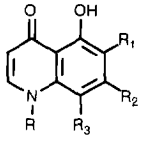

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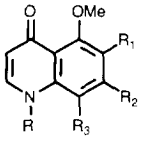

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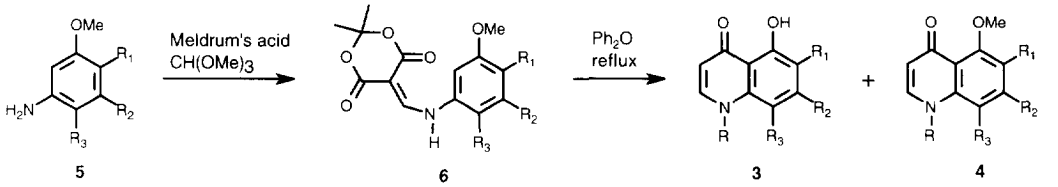
In order to elucidate further the pharmacophore of acridone congeners and to confirm the importance of the internal hydrogen bonding to cytotoxicity, we synthesized several A-ring truncated 1-hydroxy acridone analogs (**3**, i.e., 5-hydroxy-4-quinolone compounds) and their methoxy equivalents (**4**), and evaluated their cytotoxicity.

In this report, we describe the synthesis of various substituted 5-hydroxy quinolones and the assays used to determine their antitumor activity in an *in vitro* cell culture system (MTT assay and SRB assay). In addition, the antiviral activity of the compounds was tested using the herpes simplex virus because quinolone related compounds recently have shown excellent antiherpes activities[5].

Chemistry

We obtained 5-substituted quinolones by thermolysis of 5-arylaminomethylene-2,2-dimethyl-1,3-dioxane-4,6-diones (**6**, arylaminomethylene Meldrum's acid derivatives, Scheme 1) which was prepared by a reaction of properly-substituted arylamine (**5**) and Meldrum's acid in the presence of trimethyl orthoformate[6]. The cyclization of the Meldrum's acid derivatives was carried out in boiling diphenyl ether. Under the reaction conditions, partial demethylation of 5-methoxy-4-quinolones (**4**) resulted in formation of 5-hydroxy-4-quinolones (**3**) in some cases. Since some *N*-methyl quinolone derivative (R = CH_3) have been isolated from the thermolysis reaction, it is believed that the nitrogen on the quinolone ring is involved in the demethylation process[7].

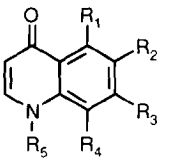
Scheme



Biological Activities

Human leukemia cell line, HL60, was used to measure antitumor activity. Activities against the murine leukemia cell line (L1210) was also tested. Both MTT assays and SRB assays were conducted for the synthesized quinolone compounds. The MTT assay was based on the reduction of the yellow colored 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) by mitochondrial dehydrogenases of metabolically -active cells to a blue formazan, as detailed by Carmichael et al[8]. We also determined the antitumor activity of these compound, following the protocols of the National Cancer Institute, for the sulforhodamine B assay[9]. Both assays gave similar IC_{50} values for the compounds tested. Antiherpes activity was determined by virus-induced cytopathic effect inhibition assay reported by Lee et al[10].

Table I. Antitumor and Antiherpes Activities of the Quinolone Compounds (μM)



No. (#)	R_1	R_2	R_3	R_4	R_5	L1210	HL-60	HSV-1(SI)	HSV-2(SI)
Acronycine (**1**)							26.2		
Glyfoline (**2**)						26.5	1.4 (2.2 #)		
Acyclovir								0.2 (1250)	2.1 (119)
Ara-C								0.3 (33)	2.0 (5)
1	OH	H	H	H	H	>40.0	>30.0	54 (1.8)	83 (1.2)
2	OH	H	H	OMe	H	18.9	17.7	82.6 (1.2)	>100 (NC)
3	OH	H	H	OMe	Me	>40.0	>30.0	>100 (NC)	>100 (NC)
4	OMe	H	H	OMe	H	>40.0	>30.0	>100 (NC)	>100 (NC)
5	OH	OMe	OMe	H	H	>40.0	>30.0	>100 (NC)	>100 (NC)
6	OMe	OMe	OMe	H	H	>40.0	>30.0	>100 (NC)	>100 (NC)
7	OH	OMe	OMe	H	Me	>40.0	>30.0	>100 (NC)	>100 (NC)
8	OMe	OMe	OMe	H	Me	>40.0	>30.0	>100 (NC)	>100 (NC)
9	H	H	OMe	H	H	>40.0	>30.0	>100 (NC)	>100 (NC)
10	H	OMe	OMe	H	H	>40.0	>30.0	>100 (NC)	>100 (NC)
11	H	OMe	OMe	H	Me	>40.0	>30.0	>100 (NC)	>100 (NC)
12	OMe	H	OMe	H	H	>40.0	>30.0		
13	OMe	H	OMe	H	Me	>40.0	>30.0	>100 (NC)	>100 (NC)
14	OH	H	OMe	H	H	>40.0	>30.0		
15	OH	H	H	Me	H	>40.0	>30.0	27.1 (3.7)	>100 (NC)
16	OMe	H	H	Me	H	>40.0	>30.0	>100 (NC)	>100 (NC)
17	OMe	H	H	Cl	H	>40.0	>30.0	>100 (NC)	>100 (NC)
18	OMe	H	CF3	H	H	>40.0	>30.0	>100 (NC)	>100 (NC)
19	H	OMe	OH	H	H	>40.0	>30.0	>100 (NC)	>100 (NC)
20	CF3	H	OMe	H	H	>40.0	>30.0	29.4 (<1)	29.4 (<1)
21	OH	H	CF3	H	H	>40.0	>30.0		

SI: Selectivity Index NC: Not Calculated

#: Reference 4b

Antitumor activities

IC_{50} values for the quinolone compounds tested are listed in Table I. None of the quinolones tested had greater cytotoxicity than glyfoline, the most potent cytotoxic compounds of the class. However, 5-hydroxy-8-methoxy-quinolone (compound #2) showed potent antitumor activity (IC_{50} = 17.7 μM for HL60) which was greater than that of acronycine. Although general structure activity relationship of quinolones to cytotoxicity was not able to be elucidated from these data, the A-ring in the acridone-related compounds did not seem to be indispensible for cytotoxicity and correlationship between internal hydrogen bonding and cytotoxicity in quinolones also was not found.

Antiherpes activities

Antiviral activity of the quinolone compounds was tested against the herpes simplex virus-1 (HSV-1) and herpes simplex virus-2 (HSV-2). Four of the compounds showed moderate activity against HSV-1 (IC_{50} ranged from 27.1 to 82.6 μM, Table I) and two showed activity against HSV-2 (IC_{50} = 29.4 - 83 μM). Any these compounds, however, were not comparable to acyclovir, which is a popular antiviral agent currently in clinical use.

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References and Notes

1. Hughs, G. K.; Lahey, F. N.; Price, J. R.; Webb, L. J. *Nature* **1948**, 162, 223.
2. (a) Garzen, K.; Svoboda, G. H. *The Alkaloids* vol 21, Academic Press, New York, **1983**, 1-28.
(b) Elomri, A.; Mitaku, S.; Michel, S.; Skaltsounis, A.-L.; Tillequin, F.; Koch, M.; Pierré, Guilbaud, N.; Léonce, S.; Kraus-Berthier, L.; Rolland, Y.; Atassi, G. *J. Med. Chem.* **1996**, 39, 4762.
3. Price, J. R. *The Alkaloids* vol 2, Academic Press, New York, **1983**, 353-368.
4. (a) Chou, T.-S.; Tzeng, C.-C.; Wu, T.-S.; Watanabe, K. A.; Su, T.-L. *Phytother. Res.* **1989**, 3, 237.
(b) Su, T.-S.; Köhler, B.; Chou, T.-C.; Chun, M. W.; Watanabe, K. A. *J. Med. Chem.* **1992**, 35, 2703.
5. Wentland, M. P.; Perni, R. B.; Dorff, P. H.; Brundage, R. P.; Castaldi, M. J.; Bailey, T. R.; Carabateas, P. M.; Bacon, E. R.; Young, D. C.; Woods, M. G.; Rosi, D.; Drozd, M. L.; Kullnig, R. K.; Dutko, F. J. *J. Med. Chem.* **1993**, 36, 1580.
6. Cassis, R.; Tapia, R.; Valderrama, J. *Syn. Commun.* **1995**, 15, 125.
7. *General method of preparation of Meldrum's acid derivative* (**6**)
Meldrum's acid (0.022 mol) and trimethyl orthoformate (0.024 mol) was added to a stirring solution of substituted aniline (**5**) (0.02 mol) in acetonitrile (30 ml), in succession. The mixture was heated to reflux (70 °C) for 2-4 hours and then cooled to room temperature. Solvent was evaporated and the residue was purified by recrystallization for aqueous methanol.
General method for thermolysis of Meldrum's acid derivative (**3**,**4**)
Meldrum's acid derivative (**6**) was added in small batches to boiling diphenyl ether (ca. 250 °C, 10-20 ml) so as to maintain a concentration of **6** under 20% by weight at any time. Gas evolution was observed instantly upon addition. Heating was continued for 0.5-1 hour after addition and the mixture was then cooled to room temperature. The product mixture was separated by column chromatography using 9% methanol/chloroform as eluent.
8. (a) Carmichael, J.; DeGraff, W. G.; Gazdar, A. F.; Minna, J. D.; Mitchell, J. B. *Cancer Res.* **1987**, 47, 936.
(b) Alley, M. C.; Scudiero, D. A.; Monks A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Shoemaker, R. H.; Boyd. M. R. *Cancer Res.* **1988**, 48, 589.
9. Skehan, P.; Streng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, 82, 1107.
10. Lee, C.-K.; Rha, Z. S.; Kim, H. S. *J. Of Kor. Soc. Of Virology* **1992**, 22, 69.

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