Antitumor Activity of Some Quaternary Ammonium Compounds. Structure–Activity Relationship

FOTIOS M. PLAKOGIANNIS,*

The Research Institute, Brooklyn College of Pharmacy, Long Island University, Brooklyn, New York 11216

ERIC J. LIEN, AND JOHN A. BILES

School of Pharmacy, University of Southern California, Los Angeles, California 90007

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Several quinolinium derivatives were synthesized. Their antitumor activity against L-1210 lymphoid leukemia was studied. Among the compounds examined, 1,2,6-trimethylquinolinium iodide possessed the highest activity, a dosage of 37.5 mg/kg repeated for 9 days gave 6/6 survivor rate and about 13% increase in survival days as compared with that of the control group. When the dosage was increased to 150 mg/kg, 6/6 survivor rate and about 30% increase in survival days were obtained. The relationship between the biological activity and the physicochemical character, such as the apparent partition coefficient (log K_{app}, $CHCl_3$–water), π constant, and the surface area (A_w), is discussed.

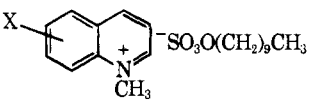
Since Ambrose and his coworkers[1] reported that tumor cells have a higher negative surface charge than the communal cells, many different types of basic compounds such as amidinium and quaternary ammonium compounds have been screened for their antitumor activity.[2] A number of nitro compounds, aromatic amines, and alkaloids have also been shown to have antitumor activity.[3–7] It is known that polycyclic aromatic compounds substituted at certain positions (*e.g.*, 1,2,5,6-dibenzanthracene) or with Me groups at position 6, 9, or 10 (*e.g.*, 9,10-dimethyl-1,2-benzanthracene) are highly carcinogenic, whereas substitution of a Me group at position 2 or 3 diminishes the carcinogenic effect. Recently Franke reported that hydrophobic interactions of polycyclic aromatic hydrocarbons with protein are necessary and favorable for the process of chemical carcinogenesis, although the role of hydrophobic interactions is only of secondary importance as compared with the chemical reactivity of the K–L region of the hydrocarbons.[8]

Certain benzacridines are carcinogenic (*e.g.*, 9-methyl-3,4-benzacridine) whereas replacement of the Me group in acridine by amino or nitro groups led to total loss of blastomogenic activity.[9] Bahner, *et al.*,[10] reported that 4-(*p*-dimethylaminostyryl)quinoline propiodide shared the activity of methiodide and ethiodide in producing repression of the lymphoma 8 tumor in rats. Cain and his coworkers[11] investigated the bisquaternary salts and stated that regardless of the mode of distribution, the lipohydrophilic balance is a prime factor for antitumor activity. They further stated that if a common transport-mediated distribution is accepted, there can be little specificity of the carrier system except a requirement for a cationic charge with proper lipohydrophilic balance.

The aim of the present work is to evaluate the activity of 11 quinolinium compounds against L-1210 lymphoid leukemia and to correlate their antitumor activity with some physicochemical parameters, such as the apparent partition coefficient (log K_{app}, $CHCl_3$–water), π constant, and the surface area of the molecule (A_w).

TABLE I

SOME PHYSICOCHEMICAL CONSTANTS OF THE QUATERNARY AMMONIUM SALTS AND THE APPARENT PARTITION COEFFICIENTS OF THE ALKYL SULFATES OF THE QUATERNARY AMMONIUM COMPOUNDS



N-Methiodide of	λ_{max}, mμ	π_x[a]	σ_x[b]	$A_w \times 10^{9}$[c] of x	Log K_{app} of the alkyl sulfate
3-Aminoquinoline	238	−1.23	−0.16	1.74	3.43[d]
8-Aminoquinoline	245.5	−1.23	−0.16	1.74	
2-Iodoquinoline	330	0.92	0.28	2.51	3.79[e]
6-Bromoquinoline	244	0.94	0.23	2.05	3.48[e]
6-Methoxyquinoline	250	0.12	−0.27	2.66	3.56[e]
2-Methylquinoline	321	0.50	−0.17	2.12	3.49[e]
2,6-Dimethylquinoline	322	1.00	−0.34	4.24	4.20[e]
Acridine	322	1.42	−0.48	3.54	4.96[e]

[a] From T. Fujita, J. Iwasa, and C. Hansch, *J. Amer. Chem. Soc.*, **86**, 5175 (1964). [b] Estimated values, using Hammet's σ constant from H. H. Jaffe, *Chem. Rev.*, **53**, 191 (1953). [c] From A. Bondi, *J. Phys. Chem.*, **68**, 441 (1964). [d] Determined in this study. [e] From J. A. Biles, F. M. Plakogiannis, B. J. Wong, and P. M. Biles, *J. Pharm. Sci.*, **55**, 909 (1966).

Experimental Section

Chemicals.—The following quinoline derivatives were purchased from commercial sources and used without further purification: 3- and 8-aminoquinolines (Fisher Chemicals), 2-iodo-, 6-bromo-, 6-methoxy-, 2-methyl-, 2,6-dimethylquinolines, and acridine (Matheson Coleman and Bell Co.). $CHCl_3$ (U.S.P.) and distd H_2O were used as the solvents.

Synthesis of Quaternary Ammonium Compounds.—The

(1) E. J. Ambrose, A. M. James, and J. H. B. Lovich, *Nature (London)*, **177**, 576 (1956).

(2) A. Burger in "Fundamental Concepts in Drug–Receptor Interactions," J. F. Danielli, J. F. Moran, and D. J. Triggle, Ed., Academic Press, New York, N. Y., 1970, p 10.

(3) Z. Eckstein, *Oesterr. Chem.-Z.*, **66**, 111 (1965).

(4) M. Movrin, *Farm. Glas.*, **18**, 68 (1962).

(5) K. Miura, M. Ideda, T. Oohashi, I. Okada, and Y. Igarashi, *Yakugaku Zasshi*, **84**, 341 (1964).

(6) P. B. Ghosh and M. W. Whithouse, *J. Med. Chem.*, **11**, 305 (1968).

(7) C. C. J. Culvenor, *J. Pharm. Sci.*, **57**, 1112 (1968).

(8) R. Franke, *Mol. Pharmacol.*, **5**, 640 (1969).

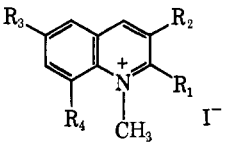
(9) N. N. Petrov, "Cancer," Macmillan Co., New York, N. Y., 1963, pp 157, 316.

(10) C. T. Bahner, J. Dale, J. Fain, E. Franklin, J. C. Goan, W. Stump, M. West, and J. Wilson, *J. Org. Chem.*, **22**, 1110 (1957).

(11) B. F. Cain, G. T. Atwell, and R. N. Seelye, *J. Med. Chem.*, **12**, 199 (1969).

TABLE II

ANTITUMOR ACTIVITY OF SOME QUINOLINIUM DERIVATIVES AND ACRIDINIUM IODIDE AGAINST LYMPHOID LEUKEMIA L-1210 IN MICE (BDF_1)



No.	R_1	R_2	R_3	R_4	Dose,[a] mg/kg	Survivors	Animal weight diff, $T - C$[b]	Survival days—Test	Survival days—Control	% T/C[c]
1	CH_3	H	CH_3	H	400	2/6	−2.4	9	8.9	101
					300	4/6	−0.4	9.5	9	105
					300[d]	0/6				
					300[d]	0/6	−1.0	9.6	9.4	102
					150	6/6	1	9.8	9	108
					150[d]	6/6	−1.8	11.2	8.6	130
					150[d]	6/6	−1.4	11.5	8.4	136
					150[d]	6/6	−1.8	11.2	8.4	133
					75	6/6	1.5	9	9	100
					75[d]	6/6	−1.5	10.2	8.4	121
					75[d]	6/6	0.0	12.5	9.4	132
					75[d]	6/6	−0.4	11.1	9.4	118
					37.5[d]	6/6	−0.5	9.3	8.4	110
					37.5[d]	6/6	−0.6	11.0	9.4	117
					37.5	6/6	−0.8	10.6	9.4	112
2	CH_3	H	H	H						
					150	0/6				
					75[d]	6/6	e			e
					75	0/6	e			
3	H	H	OCH_3	H						
					300	0/6				
					150	3/6	1.4			e
					75	6/6	e			e
4	H	H	Br	H	80	0/6				
					40	6/6	1			e
					40[d]	0/6				
					20	6/6	e			e
					5	6/6	e			e
5	H	NH_2	H	H	100	0/4				
6	H	H	H	NH_2	400	0/4				
					200	1/4	2.1	13	9.3	139
					100	1/4	1.2			e
7	I	H	H	H	400	4/4	e			e
					100	4/4	e			e
8	Acridinium iodide				400	1/6	e			e
					75	5/6	e			e
					75[d]	0/6				
					18.8	6/6	e			e

[a] Route of administration: ip injection, vehicle: saline, carboxymethylcellulose or Me_2CO. [b] Average wt change of the test group minus average change of control animals in grams. [c] The ratio of survival time of test to control animals, expressed as per cent. [d] The dose was repeated for 9 days. [e] No significant change.

quaternary ammonium compds were synthesized as previously described.[12,13] The melting points were uncorrected.

Measurement of the Apparent Partition Coefficients.—The determination of the apparent partition coefficient was based on eq 1 as previously reported.[13,14]

$$\underset{(aq)}{B^+Me} + \underset{(aq)}{SO_3^-OR} \underset{}{\overset{K_{app}}{\rightleftharpoons}} \underset{(CHCl_3)}{MeBSO_3OR} \qquad (1)$$

$$\underset{c_1}{} \quad \underset{c_2}{} \quad \underset{c_3}{}$$

$$K_{app} = c_3/c_1c_2 \text{ (1/mole)}$$

The uv absorption maximum, given in Table I, was determined in a Coleman-Hitachi 124 double beam spectrophotometer or in a Perkin-Elmer uv spectrophotometer. Two of the apparent partition coefficients listed in Table I were measured in this study, the others had been reported previously.[14]

Antitumor Test.—The testing of the compounds was carried by the Cancer Chemotherapy National Service Center, National Cancer Institute, according to previously described protocols.[15] The results are summarized in Table II.

Results and Discussion

The mp of compounds **3**–**5** and **7** agreed with the reported values.[12,16,17] The mp of compound **6** was 196–198°. The analytical values of C and H of this

(12) I. A. Vogel, "Practical Organic Chemistry," 3rd ed, Wiley, New York, N. Y., 1962, p 662.

(13) J. A. Biles, F. M. Plakogiannis, B. J. Wong, and P. M. Biles, *J. Pharm. Sci.*, **55**, 909 (1966).

(14) F. M. Plakogiannis, E. J. Lien, C. Harris, and J. A. Biles, *ibid.*, **57**, 197 (1970).

(15) *Cancer Chemother. Rep.*, **25**, 1 (1962).

(16) W. H. Mille and W. H. Watson, *J. Chem. Soc.*, **97**, 746 (1910).

(17) G. Harris, Ed., "Dictionary of Organic Compounds," Vol. 3, Oxford University Press, New York, N. Y., 1965, p 1880.

new compound as well as the other compounds were within ±0.4% of the calculated.

Due to the inherent complex nature of the antileukemia test, the biological variation, and the relatively low potency of most of the compds examined, no meaningful quantitative structure–activity correlation could be obtained from the limited amount of data available. From Table II it is clear that the only compd which showed promising results is 1,2,6-trimethylquinolinium iodide (*N*-methiodide of 2,6-dimethylquinoline) which gives 6/6 survival rate and about 13% increase in survival days at 37.5 mg/kg level, and 6/6 survival rate and about 30% increase in survival days at 150 mg/kg level. It is interesting to note that this compd has π_x and log K_{app} values higher than all the other quinoline derivatives but lower than these of the acridine derivative (see Table I). Unfortunately, no quantitative correlation could be obtained at the present. With the exception of *N*-methiodide of acridine, the surface of the substituent (A_w) parallels π_x and log K_{app}. All of the compds examined, with the exception of *N*-methiodide of 2-iodoquinoline, possessed toxicity at elevated dosages (80–400 mg/kg).

The electronic effect of the substituent on the activity is difficult to assess, since only 2 compds with slightly positive σ values (see Table I) were studied and these 2 compds did not show significant antileukemia activity.

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Aminobenzoic Acid Diuretics. 2.[1] 4-Substituted-3-amino-5-sulfamylbenzoic Acid Derivatives

PETER W. FEIT

Leo Pharmaceutical Products, 2750 Ballerup, Denmark

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The synthesis of 42 N-alkylated 4-substituted-3-amino-5-sulfamylbenzoic acids is detailed. Reasons for the interest in these new aminobenzoic acid derivatives are discussed. Diuretic screening results in the dog assay for the compounds are summarized and compared with 3-benzylamino-4-chloro-5-sulfamylbenzoic acid (**76**) and the corresponding *N*-Bu compd (**77**) recently described as diuretics. For the most active 4-R_1-3-R_2NH-5-sulfamylbenzoic acids (**85**, R_1 = NHC_6H_5; R_2 = $CH_2C_6H_5$; **86**, R_1 = NHC_6H_5; R_2 = *n*-Bu; **97**, R_1 = SC_6H_5; R_2 = $CH_2C_6H_5$; **98**, R_1 = SC_6H_5; R_2 = *n*-Bu; **103**, R_1 = OC_6H_5; R_2 = $CH_2C_6H_5$; **110**, R_1 = OC_6H_5; R_2 = $CH_2CBr{=}CH_2$; **111**, bumetamide (pINN), R_1 = OC_6H_5; R_2 = *n*-Bu; **114**, R_1 = OC_6H_5; R_2 = 2-furylmethyl; **118**, R_1 = OC_6H_4-4-OH; R_2 = $CH_2C_6H_5$) the results at different doses after both iv and oral administration are given. Comparison with 4-chloro-*N*-(2-furylmethyl)-5-sulfamylanthranilic acid (furosemide) has revealed that these new highly efficacious compounds possess a level of activity hitherto unknown for "high-ceiling" diuretics.

Except for some carbonic anhydrase inhibitors, all benzenesulfonamide diuretics with saluretic action (including the thiazides and related bicyclic compounds), which have found clinical application, have as a common structural feature Cl or CF_3 in the ortho position to a sulfonamide group.[2-4] It is generally held that outstanding sulfonamide saluretics should possess halogen or pseudohalogen in this position besides an electronegative group meta to the sulfonamide group or as part of a condensed ring. Even 4-chloro-*N*-(2-furylmethyl)-5-sulfamylanthranilic acid[5] (furosemide), a well-established, nonthiazide type, high-ceiling diuretic,[4] was found to fit this broad generalization.[3] On the other hand, this empirical rule, given with some reservations,[3] had been based mainly on varied structures of thiazide type diuretics and consequently the question still remained, whether it was of general validity and applicable to sulfonamide diuretics with a different type of action.

In the preceding paper of this series some 3-amino-4-halogeno-5-sulfamylbenzoic acid derivatives have been described as powerful high-ceiling diuretics.[1] At this stage in pursuing the structure–activity relationships of highly efficacious diuretics and for reasons given above efforts have been directed towards an alteration of the 4 substituent of these aminobenzoic acid derivatives.

Chemistry.—The principal synthetic route for the preparation of the 4-R_1-3-R_2NH-5-sulfamylbenzoic acids[6] (Table IV) is outlined in Scheme I and detailed in the Experimental Section. The starting material, 4-chloro-3-nitro-5-sulfamylbenzoic acid[1] (**1**), was easily available. In the first step the relatively high reactivity of the chloro substituent of **1** was utilized for the alkylation of various amines, phenols, thiophenols, mercaptans, and alcohols to yield most of the described 4-R_1-3-nitro-5-sulfamylbenzoic acids (Table I). Special reaction conditions were required in those cases where the introduced substituent was reactive itself. For example, the phenoxy compd **22** is easily hydrolyzable in 1 *N* NaOH to yield the phenol **26**. The *n*-butylsulfinyl compd **18** was prepared by oxidation of the

(1) Part 1: P. W. Feit, H. Bruun, and C. Kaergaard-Nielsen, *J. Med. Chem.*, **13**, 1071 (1970); in this ref the term metanilic acid has been used erroneously for 3-aminobenzoic acid throughout.

(2) See G. de Stevens, "Medicinal Chemistry," Vol. I, Academic Press, New York, N. Y., 1963, Chapters V and VI.

(3) See J. M. Sprauge, "Topics in Medicinal Chemistry," Vol. II, J. L. Rabinowitz and R. M. Myerson, Ed., Wiley, New York, N. Y., 1968, pp 22–24.

(4) See R. Muschaweck and K. Sturm, "Arzneimittel," G. Ehrhart and H. Ruschig, Ed., Vol. I, Verlag Chemie, Weinheim, West Germany, 1968, Chapter 16, pp 694–703.

(5) K. Sturm, W. Siedel, R. Weyer, and H. Ruschig, *Chem. Ber.*, **99**, 328 (1966).

(6) Løvens Kemiske Fabrik Produktionsaktieselskab, Belgian Patent 743,744 (1970).