Nitrogen Analogues of 1,4-Benzoquinones. Activities against the Ascitic Sarcoma 180 of Mice

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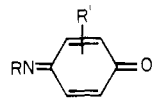
Compounds having the basic structure N-(R)-substituted ring-substituted 4-iminocyclohexadienone have been synthesized and tested as antitumor agents against the ascitic sarcoma 180 tumor in Swiss mice. Among these compounds, the dimethylindoanilines [R = 4-$(CH_3)_2NC_6H_4$] are most stable in water at pH 7.0 and at 25 °C, the oximes (R = OH) are less stable, and the *N*-halo compounds (R = Br and Cl) are least stable. The *N*-halo derivatives have the highest redox potentials under the conditions used, the greatest effect against ascitic sarcoma 180 in Swiss mice, and the greatest acute toxicity when injected ip in the Swiss mice. Discriminant analysis of the results indicates that substituents with positive values of F and negative values of π increase the antitumor activities, whereas those with positive values of σ and R should lower the toxicity. The redox potential, a molecular parameter, is the best single variable for discriminating between the groups based on antitumor activities.

Naturally occurring quinones, including coenzyme Q and vitamin K, have important biological functions, including a role in oxidative phosphorylation and electron transfer.[1] Some natural quinones, such as lapachol, and many synthetic quinones have the ability to inhibit the growth of tumors.[2]

Nitrogen analogues of the quinones have not been so extensively studied. About 200 of these have been screened by the National Cancer Institute for antitumor activities and seven have proven activities against mouse and rat cancers.[3] The nitrogen analogues of the quinones have many of the same chemical properties as the quinones and, in addition, have a group on the nitrogen atom which can be varied to provide different chemical and physical properties.

We decided to prepare compounds of the structure



in which R is Cl, Br, OH, or 4-$(CH_3)_2NC_6H_4$ (Table I) with substituents on the ring providing a variety of electronic and hydrophobic effects. Their stabilities in water at pH 7 and at 25 °C and their oxidation–reduction potentials in alcohol–water solutions were measured (Table I). Their acute toxicities and their abilities to increase the survival times in Swiss mice bearing ascitic sarcoma 180 were determined (Table II). Attempts to quantitatively correlate the toxicities and the antitumor activities of the compounds with substituent constants such as π and σ failed to give statistically significant correlations. Each of the four subseries, dimethylindoanilines, oximes, and *N*-chloro and *N*-bromo imines, includes some active compounds and some less active compounds. The effect of introducing a Cl atom in the dimethylindoanilines increases the activity compared to introduction of a OCH_3 group, but in the oxime series the opposite effect is observed. Hence, conventional linear regression analyses of substituent effects could not be used here. We decided to investigate the possibility that potencies and toxicities could be related to physical or chemical properties that depend on the molecular structure as a whole. Classification of compounds by these properties might give information which could prove useful in the design of new antitumor agents. We therefore subjected our data to discriminant analysis to uncover relationships based on physical and chemical properties of the molecules.

Discriminant Analysis of Antitumor Activities. The compounds were divided into two groups on the basis of their antitumor activities in the ascitic sarcoma 180 tumor system of Swiss mice. Compounds **18** and **19** were single examples of different series and were excluded. Nine compounds that gave T/C values of 175 or less were classed as moderately active (group I); the other eight compounds were classified as very active (group 2). These two groups were subjected to discriminant analysis by the BMDO7M Stepwise Discriminant Analysis Program, revised Dec 24, 1975, of the Health Sciences Computing Facility, University of California at Los Angeles.[4]

The substituent parameters π, σ, F, R, and MR shown in Table III are taken from the work of Hansch et al.[5] and are summed for all four positions of the ring since each position is adjacent to a carbonyl or an azomethine carbon atom. Values of MR are scaled by 0.1 to make them comparable in size to the other parameters. For groups on carbon 2 (adjacent to the carbonyl group), σ_m values were used; for substituents on carbon 3, σ_p values were used. Variables I_1, I_2, I_3, and I_4 were used to indicate the four series of compounds, the dimethylindoanilines, the oximes, the *N*-chloro imines, and the *N*-bromo imines, respectively. Values of π^2 and MR^2 were included in the discriminant analysis. The complete classification functions for the two groups are

moderately active compounds

$$-1.05\,\pi + 74.1\,\sigma - 126\,F + 11.5\,R + 115\,\mathrm{MR} - 45.0\,\mathrm{MR}^2 + 15.1\,\pi^2 - 5.40\,I_1 + 6.04\,I_2 - 0.36\,I_3 - 24.7$$

very active compounds

$$-2.46\,\pi + 52.8\,\sigma - 78.1\,F + 16.4\,R + 94.1\,\mathrm{MR} - 34.9\,\mathrm{MR}^2 + 7.65\,\pi^2 - 0.79\,I_1 + 3.23\,I_2 + 6.51\,I_3 - 21.8$$

Use of all these variables allows the correct classification of eight of the nine moderately active compounds and seven of the eight very active compounds, although the confidence level is less than 80% ($F = 1.15 < F_{10,6,0,80} = 2.03$). These classification functions may be combined to form a discriminant function by using the differences in the coefficient of each term; the numbers in parentheses are $F_{1,6}$ values to remove each variable.

$$-1.41\,(0.06)\,\pi - 21.3\,(0.29)\,\sigma + 47.9\,(1.49)\,F + 4.9\,(0.11)\,R - 20.9\,(0.89)\,\mathrm{MR} - 10.1\,(0.98)\,\mathrm{MR}^2 - 7.45\,(0.37)\,\pi^2 + 4.61\,(0.69)\,I_1 - 2.81\,(0.24)\,I_2 + 6.87\,(2.75)\,I_3 + 2.9$$

The coefficients of σ and F are abnormally large because these variables have a high correlation as shown by the

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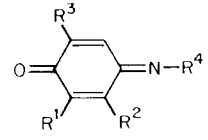
Table I. 1,4-Benzoquinone Derivatives



No.	R^1	R^2	R^3	R^4	Formula	Method	Yield, %	Mp, °C	Color	Redox potential,[a] V	Half-time of change,[b] h	Ref
1	H	H	H	4-$(CH_3)_2NC_6H_4$	$C_{14}H_{14}NO$	A	98	161–162	Dark purple	0.628	700	10
2	CH_3	H	H	4-$(CH_3)_2NC_6H_4$	$C_{15}H_{16}NO$	A	96	124–125	Dark purple[c]	0.604	2010	10
3	CH_3O	H	H	4-$(CH_3)_2NC_6H_4$	$C_{15}H_{16}NO_2$	A	92	167–168	Dark purple	0.561		11
4	Cl	H	H	4-$(CH_3)_2NC_6H_4$	$C_{14}H_{13}NOCl$	A	98	124–125 dec	Dark green	0.658	478	11
5	CH_3CONH	H	H	4-$(CH_3)_2NC_6H_4$	$C_{16}H_{17}N_2O_2$	A	94	185–186	Dark purple[d]	0.580		11
6	H	H	H	HO	C_6H_4NO	B	72	137–138 dec	Brown	0.544		12
7	Cl	H	H	HO	C_6H_4NOCl	B	79	142–144 dec		0.516	314	19
8	CH_3O	H	H	HO	$C_6H_7NO_2$	B	74	174–175 dec	Brown	0.521	298	20
9	H	Cl	H	HO	$C_6H_4NO_2Cl$	C	79	174–175	Yellow brown	0.537		13
10	H	Br	H	HO	$C_6H_4NO_2Br$	C	82	188–190	Yellow brown	0.532		13
11	H	I	H	HO	$C_6H_4NO_2I$	C	84	194–196 dec	Yellow orange	0.531		13
12	H	H	H	Cl	C_6H_4NOCl	D	72	83–84	Yellow	0.652	35.3	14
13	H	H	H	Br	C_6H_4NOBr	D	64	67–68 dec	Dark yellow	0.518		15
14	Cl	H	Cl	Cl	$C_6H_2NOCl_3$	E	85	65–66	Yellow	0.659	40.0	21
15	Br	H	Br	Br	$C_6H_2NOBr_3$	E	75	98–100	Brown	0.678	10.4	*e*
16	Br	H	Br	Cl	$C_6H_2NOBr_2Cl$	E	86	80–81	Yellow orange	0.665	23.3	14, 16
17	Cl	H	Cl	Br	$C_6H_2NOBrCl_2$	E	83	95–96	Yellow		3.7	*e*
18	H	H	H	4-$NaOC_6H_4$	$C_{12}H_8NO_2Na$				Purple			*f*
19	H	H	H	4-$CH_3COC_6H_4$	$C_{14}H_{11}NO_3$			115–117	Orange	0.633		*g*

[a] In alcohol–water. [b] In water at pH 7 and 25 °C. [c] Green reflection. [d] Gold reflection. [e] New compound: C, H. [f] Indophenol sodium salt, practical, P3487, Eastman Kodak Co. [g] Indophenyl acetate, 7739, Eastman Kodak Co.

Table II. Toxicity and Inhibition of Ascitic Sarcoma 180 in Swiss Mice

Compd no.	LD_{50},[a] mg/kg	Ascitic sarcoma 180							
		Daily dose,[b] mg/kg	5-Day survivors	Av wt gain, %[c] Treated	Av wt gain, %[c] Controls	Cures[d]	Survival times, days av ± SD[g] Treated	Survival times, days av ± SD[g] Controls[e]	T/C,[f] %
1	80	30.0	6/6	−0.9	+7.8	0/6	19.2 ± 6.8	14.7 ± 2.0	132
2	27	6.0	6/6	−0.6	+8.4	0/6	18.0 ± 4.6	14.2 ± 3.3	127
3	25	10	6/6	+1.9	+9.6	0/6	19.3 ± 4.3	10.7 ± 3.0	181
4	100	20	6/6	+9.0	+17.6	0/6	33.3 ± 21.1	13.0 ± 1.6	256
5	70	3	6/6	+4.3	+9.0	1/6	26.8 ± 19.4	13.8 ± 2.5	194
6	260	150	6/6	−5.2	+3.6	0/6	17.8 ± 2.3	13.2 ± 3.8	135
7	240	70	6/6	+13.4	+14.9	0/6	12.2 ± 4.1	9.3 ± 2.5	130
8	120	60	6/6	+1.9	+9.6	0/6	18.5 ± 10.5	10.7 ± 3.0	173
9	380	90	6/6	−2.4	+5.7	1/6	39.0 ± 16.4	12.8 ± 5.2	304
10	1090	150	6/6	+6.4	+0.9	0/6	18.2 ± 5.3	11.5 ± 3.4	158
11	550	200	6/6	0.0	+7.8	1/6	23.7 ± 18.7	13.8 ± 2.9	171
12	12.5	5.0	6/6	−6.9	+6.7	2/6	40.5 ± 21.5	16.5 ± 4.9	246
13	40	15	6/6	−3.8	+9.6	0/6	15.5 ± 7.0	10.7 ± 3.0	145
14	20	10	6/6	−14.2	+26.2	0/6	25.3 ± 11.9	11.5 ± 3.5	220
15	25	9.0	6/6	−3.0	+8.5	0/6	22.8 ± 8.6	12.8 ± 1.9	178
16	63	22.5	5/6	−25.0	+4.6	2/6	41.0 ± 21.1	18.8 ± 2.1	218
17	20	8.0	6/6	−5.1	+5.2	0/6	27.2 ± 18.0	16.5 ± 2.4	165
18	50	15	6/6	−2.2	+6.8	1/6	32.8 ± 8.5	15.3 ± 5.8	214
19	90	20	6/6	−1.5	+14.3	0/6	19.2 ± 5.6	14.8 ± 3.7	130

[a] Single ip dose that will cause death within 5 days of 50% of the male Swiss mice tested. [b] Three ip doses were given at 24-h intervals, starting 24 h after ip injection of 10^6 ascitic sarcoma cells. [c] Mice were weighed 1 day after injection of cells and again 3 days later (24 h after the third dose of the compound). [d] Cures are recorded for those mice that are alive without any evidence of cancer cells 60 days after injection of the ascitic sarcoma 180 cells. [e] Controls were injected with isotonic salt solutions on the same schedule that was used to inject compounds into treated mice. [f] A value of T/C equal to or greater than 125% is considered statistically significant of the antitumor activity of the compound. [g] A survival time of 60 days is used in calculating the average times as well as the standard deviations for the mice that are alive 60 days after the injection of cells.

Table III. Parameters Used in Discriminant Analysis

Compd no.[a]	π	σ	F	R	MR	MR^2	π^2	I_1	I_2	I_3	I_4	Groups: Based on T/C Obsd	Groups: Based on T/C Calcd[b]	Groups: Based on LD_{50} Obsd	Groups: Based on LD_{50} Calcd[c]
1	0.0	0.0	0.0	0.00	0.41	0.17	0.0	1	0	0	0	1	1	2	2
2	+0.56	−0.07	−0.04	−0.13	0.87	0.76	0.31	1	0	0	0	1	1	1	1
3	−0.02	+0.12	+0.26	−0.51	1.10	1.20	0.00	1	0	0	0	2	2	1	1
4	+0.71	+0.37	+0.41	−0.15	0.91	0.83	0.50	1	0	0	0	2	2	2	2
5	−0.97	+0.21	+0.26	−0.26	1.80	3.25	0.94	1	0	0	0	2	2	1	1
6	0.0	0.0	0.0	0.0	0.41	0.17	0.00	0	1	0	0	1	1	2	2
7	+0.71	+0.37	+0.41	−0.15	0.91	0.83	0.50	0	1	0	0	1	1	2	2
8	−0.02	+0.12	+0.26	−0.51	1.10	1.20	0.00	0	1	0	0	1	1	2	2
9	+0.71	−0.23	−0.41	−0.15	0.91	0.83	0.50	0	1	0	0	2	1	2	2
10	+0.86	+0.23	+0.44	−0.17	1.20	1.43	0.74	0	1	0	0	1	1	2	2
11	+1.12	+0.18	+0.40	−0.19	1.70	2.90	1.25	0	1	0	0	1	1	2	2
12	0.0	0.0	0.0	0.0	0.41	0.17	0.00	0	0	1	0	2	2	1	1
13	0.0	0.0	0.0	0.0	0.41	0.17	0.00	0	0	0	1	1	1	1	1
14	+1.42	+0.74	+0.82	−0.30	1.41	1.99	2.02	0	0	1	0	2	2	1	1
15	+1.72	+0.78	+0.88	−0.34	1.98	3.93	2.96	0	0	0	1	2	1	1	1
16	+1.72	+0.78	+0.88	−0.34	1.98	3.93	2.96	0	0	1	0	2	2	1	1
17	+1.42	+0.74	+0.82	−0.30	1.41	1.99	2.02	0	0	0	1	1	1	1	1

[a] Numbered as in Table I. [b] I_3, I_1, F, π. [c] I_2, I_1, R, σ.

Table IV. Within-Groups Correlation Matrix for Antitumor Activities

Parameter	π	σ	F	R	MR	MR^2	π^2	I_1	I_2	I_3	I_4
π	1.000										
σ	0.816	1.000									
F	0.831	0.971	1.000								
R	−0.214	−0.406	−0.507	1.000							
MR	0.519	0.680	0.765	−0.619	1.000						
MR^2	0.497	0.658	0.718	−0.510	0.974	1.000					
π^2	0.812	0.903	0.893	−0.330	0.814	0.847	1.000				
I_1	−0.498	−0.454	−0.484	−0.035	−0.193	−0.245	−0.426	1.00			
I_2	0.026	−0.075	0.073	−0.099	0.065	−0.001	−0.137	−0.456	1.00		
I_3	0.284	0.204	0.135	0.151	−0.048	0.007	0.243	−0.444	−0.156	1.000	
I_4	0.312	0.445	0.373	0.063	0.198	0.262	0.44>	−0.284	−0.451	−0.176	1.000

within-groups correlation matrix in Table IV. It is interesting to note that although σ and F are highly correlated, their relative effects on the antitumor activity are different as shown by the signs of the coefficients in the discriminant function.

Four variables, I_3, I_1, F, and π, are able to discriminate

Table V. Within-Groups Correlation Matrix for Toxicities

Parameter	π	σ	F	R	MR	MR^2	π^2	I_1	I_2	I_3	I_4
π	1.000										
σ	0.789	1.000									
F	0.806	0.977	1.000								
R	−0.217	−0.433	−0.535	1.000							
MR	0.515	0.697	0.785	−0.633	1.000						
MR^2	0.496	0.674	0.745	−0.526	0.977	1.000					
π^2	0.828	0.909	0.916	−0.339	0.820	0.846	1.000				
I_1	−0.485	−0.395	−0.411	0.006	−0.153	−0.187	−0.407	1.000			
I_2	0.086	0.003	0.111	−0.232	0.223	0.185	−0.081	−0.655	1.000		
I_3	0.283	0.259	0.224	0.097	0.005	0.044	0.235	−0.378	0.000	1.000	
I_4	0.283	0.259	0.244	0.097	0.005	0.044	0.235	−0.378	0.000	−0.500	1.000

between the two groups at a confidence level of 95% ($F = 3.80 > F_{4,12,0.95} = 3.26$). The discriminant function (with $F_{1,11}$ to remove in parentheses) is

$$-2.65\ (2.00)\ \pi + 9.58\ (5.41)\ F + 3.36\ (2.99)\ I_1 + 5.52\ (6.54)\ I_3 - 4.18$$

These four variables are able to classify all of the nine moderately active compounds correctly and six of the eight very active compounds.[6] The relative importance of the variables may vary with each class of compounds in these four series. However, in general, substituents with positive values of F and negative values of π should improve the antitumor activities of these compounds. The dimethylindoanilines and the *N*-chlorobenzoquinone imines are particularly active.

Discriminant Analysis of Toxicities. The same 17 compounds were separated into two new groups according to their LD_{50} for a single injection as follows: group 1, lower than 75 mg/kg; group 2, higher than 75 mg/kg. These compounds were subjected to the BMDO7M Stepwise Discriminant Analysis Program with the same parameters used previously. The complete classification functions for the two groups are

high-toxicity compounds, group 1

$$-11.6\ \pi - 43.5\ \sigma - 136\ F - 7.16\ R + 167\ MR - 67.2\ MR^2 + 31.5\ \pi^2 - 26.8\ I_1 - 31.7\ I_2 - 32.1$$

low-toxicity compounds, group 2

$$0.48\ \pi + 61.4\ \sigma - 75.3\ F + 21.6\ R + 79.5\ MR - 28.8\ MR^2 + 3.08\ \pi^2 + 1.98\ I_1 + 10.6\ I_2 - 21.2$$

Groups 1 and 2 are distinguished by the following function that is obtained by subtracting the coefficients in the classification function of group 2 from those of group 1 (with $F_{1,7}$ to remove in parentheses).

$$12.1\ (1.35)\ \pi + 17.9\ (0.06)\ \sigma + 60.7\ (0.76)\ F + 28.8\ (1.18)\ R - 87.5\ (4.89)\ MR + 38.4\ (4.43)\ MR^2 - 28.4\ (1.68)\ \pi^2 + 28.8\ (9.79)\ I_1 + 42.3\ (20.1)\ I_2 + 10.9$$

These variables are able to distinguish between members of group 1 and group 2 at a confidence level of 99.5% ($F = 7.70 > F_{9,7,0.995} = 6.87$) and to classify all 17 compounds correctly. However, four variables, I_2, I_1, R, and σ, can classify correctly all of the 17 compounds at a confidence level of 99.9% ($F = 12.0 > F_{4,12,0.999} = 10.35$). The function that discriminates between group 1 and group 2 using these four variables (with $F_{1,12}$ to remove in parentheses) follows.

$$9.17\ (2.54)\ \sigma + 18.9\ (4.74)\ R + 10.5\ (8.58)\ I_1 + 20.2\ (39.3)\ I_2 - 9.46$$

The variables σ and F, MR and MR^2, and F and π^2 have high correlations as the within-groups correlation matrix in Table V shows. The signs for the terms indicate that substituents with positive values of σ and R are desirable for low toxicity (large LD_{50}). However, each series may vary in its requirements so this relationship may depend on the relative number of compounds in each series. Dimethylindoanilines and oximes have the lowest toxicities.

Discriminant Analysis Using Molecular Parameters. When the redox potential is included as a discriminatory variable for 16 of the compounds for which redox potentials are known, this variable is shown to be very significant in separating the compounds into two groups based on T/C. This discriminant function for the four most significant variables (with $F_{1,11}$ to remove) is

$$-6.30\ (6.09)\ \pi + 15.1\ (4.22)\ F + 0.66\ (0.006)\ R + 75.1\ (13.8)\ \text{redox} - 44.2$$

$F = 7.43 > F_{4,11,0.995} = 6.88$;
15/16 classified correctly

These four variables discriminate between the groups much better ($F_{4,11} = 7.43$) than do the best set of variables for all 17 compounds ($F_{4,12} = 3.80$). It should be noted that the indicator variables have less significance when the redox potential, a molecular parameter, is used as a discriminatory variable. The redox potential alone can discriminate between the two groups at a confidence level of 99.5% ($F = 15.6 > F_{1,14,0.995} = 11.1$) and can classify 12 of the 16 compounds correctly. An increase in the redox potential should increase the antitumor activities of the compounds of these series.

When the redox potential is included as a discriminatory variable for the two groups of compounds based on LD_{50} (mg/kg), the redox potential is not a significant variable; however, the four variables, I_2, I_1, R, and σ, can discriminate between the groups at the 99.5% confidence level ($F = 10.2 > F_{4,11,0.995} = 6.88$) and classify all 16 compounds correctly. The redox potential alone classifies correctly only 12 of the 16 compounds. An increase in the redox potential may decrease the LD_{50} (increase the toxicity).

The ten compounds with known rates of change in water were also separated into two equal groups and subjected to discriminant analysis using the half-time of change as a discriminatory variable. This variable alone discriminates between the two groups based on T/C at the 80% confidence level ($F = 2.25 > F_{1,8,0.80} = 1.95$) and classifies eight of the ten compounds correctly. However, the half-time of change is not a significant variable for classifying the two groups based on the LD_{50} (mg/kg) ($F = 0.019 \ll F_{1,8,0.80} = 1.95$); it classifies only four of the ten compounds correctly. An increase in the half-time of change may decrease the antitumor activity but has little effect on the LD_{50}.

Structure of Compounds. The structures of all compounds prepared were verified by elemental analysis, infrared spectroscopy, nuclear magnetic resonance studies, and/or by comparison with known compounds. The

compounds that were prepared for the first time were studied most completely in order to corroborate the expected structures.

The IR spectra of these compounds have certain absorption peaks in common, two of which in particular were used as evidence of structure. One is a medium-to-strong absorption in the region between 1618 and 1668 cm^{-1} which is due to the C=O stretching mode and the other is a medium-to-strong absorption in the region between 1520 to 1580 cm^{-1} which is absent in the spectra of the corresponding quinones. Thus it is associated to some extent with the C=N group, and the smaller wavenumber (in comparison with that for this group in some other compounds) is explained[7] in terms of a strong conjugation with the quinonoid double bonds and subsequent reduction of the double-bond character of the C=N bond. The bonds that are characteristic of aromatic structures at 1660–2000 cm^{-1} could not be identified in quinone oximes and quinone imines. These data strongly suggest that the structure of these compounds in the solid state is quinonoid. Jaffe[8] has calculated by molecular orbital methods that the oxime form should be more stable than the *p*-nitrosophenol form.

The NMR spectrum of 2-chloro-1,4-benzoquinone 4-oxime in dioxane shows a singlet at δ 3.5 and seven doublets around δ 6.4–8.0. The singlet at δ 3.5 contains four protons and is due to the solvent (dioxane). The doublet at δ 7.95 represents the proton at C-3 when it is syn to the hydroxyl group and the doublet at δ 7.45 represents the proton on C-3 when it is anti to the hydroxyl group. The two doublets centered around δ 7.25 represent the proton on C-5 when it is syn to the hydroxyl group, and the two doublets centered around δ 7.75 represent the proton on C-5 when it is anti to the hydroxyl group. This splitting pattern of the proton on C-5 is due not only to the syn and anti isomerism but also to the presence of the adjacent proton (proton on C-6). The doublet at δ 6.55 represents the proton at C-6 and the splitting is caused by the adjacent proton (proton at C-5). The NMR spectrum substantiates the quinonoid structure of this compound.

Assignment of the NMR spectra of the quinone imines is based on the fact that the lone pair of electrons of the nitrogen atom causes a separation between syn and anti proton signals.[9] The NMR spectrum of *N*-bromo-2,6-dichloro-1,4-benzoquinone 4-imine in acetone-d_6 shows a multiplet around δ 2.1, a singlet at δ 2.65, and two doublets around δ 7.5–8.0. The multiplet around δ 2.1 and singlet at δ 2.65 are both due to a small amount of undeuterated solvent used. The doublet at δ 7.62 represents the proton on C-5 when it is anti to the bromoimino group. The doublet at δ 7.95 represents the proton on C-3 when it is syn to the bromoimino group. The most reasonable interpretation of the downfield shift is based on the formation of a hydrogen bond between the proton ortho to the imino group and the aprotic solvent.[9]

When a hydrogen bond is formed with the approach of the base (proton acceptor) to the ortho proton, the base on the syn side suffers from the steric hindrance due to the halogen atom bonded to the nitrogen atom, while there is no such effect on the anti side.

Experimental Section

Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Ultraviolet absorption studies were made on a Cary 14 spectrophotometer. Infrared spectra of the compounds were determined on mineral oil mulls on a Beckman IR-5A spectrophotometer with sodium chloride optics. For the nuclear magnetic resonance studies, a solution of about 10 wt % was prepared for each compound in a suitable solvent (acetone-d_6 or dioxane). Tetramethylsilane (Me_4Si) was used as the reference standard either internally or externally. The spectra were obtained on a Varian XL-100 analytical nuclear magnetic resonance spectrometer. Elemental analyses, when indicated by atomic symbols, differed from the calculated values by no more than 0.4%.

Syntheses. Method A. Indoaniline Derivatives. To an aqueous suspension of 220 mmol of finely divided AgCl were added solutions of 143 mmol of sodium carbonate, 25 mmol of the appropriate phenol in alcohol, and 25 mmol of *N,N*-dimethyl-*p*-phenylenediamine hydrochloride in water. After 30 min of stirring, the mixture was filtered to remove Ag and residual AgCl, and the filtrate was extracted with ethyl acetate. Workup of the ethyl acetate extract gave 90–98% yields of the desired products. Attempts to prepare compounds from catechol and from resorcinol gave unrecognizable products.[10,11]

Method B. Quinone 4-Oximes. To a cold (−3 °C) solution of 64 mmol of the appropriate phenol, 68 mmol of NaOH, and 78 mmol of KNO_2 in water was added 142 mmol of concentrated H_2SO_4 with stirring and cooling. After an additional hour of stirring and cooling, the crystalline product was filtered out, washed with cold water, and dried. The yields were 72–84% of theory.[12]

Method C. 3-Halo-1,4-benzoquinone 4-Oximes. A solution of 16 mmol of the appropriate 3-halophenol in glacial acetic acid was added to a solution of 30 mmol of $NaNO_2$ in H_2SO_4 kept below 20 °C. The mixture was kept at 0 °C for 10 min and poured on ice. The compound precipitated as a solid in theoretical yields of 79–85%.[13]

Method D. *N*-Halo-1,4-benzoquinone 4-Imines. Treatment of 24 mmol of 4-aminophenol hydrochloride in water with sodium hypohalite (290 mmol of NaOH and 152 mmol of halogen) gave the desired products.[14,15]

Method E. *N*,2,6-Trihalo-1,4-benzoquinone 4-Imines. The bromo compounds were prepared from 200 mmol of 4-nitrophenol by treatment with 470 mmol of bromine in acetic acid to give the 2,6-dibromo-4-nitrophenol; 50 mmol of the latter was reduced with 100 mmol of tin and 360 mmol of hydrochloric acid to the amine which was halogenated with sodium hypohalite (290 mmol of NaOH and 152 mmol of halogen) to give the desired *N*-halo compound.[16] The chloro compounds were prepared by the same procedure except that 29 mmol of 4-nitrophenol reacted with 5 mL of fuming sulfuric acid to introduce a sulfonic acid group ortho to the hydroxyl group. This sulfonic acid group was subsequently displaced by 58 mmol of chlorine in the formation of 2,4-dichloro-4-nitrophenol.[17]

Conditional Oxidation–Reduction Potentials. The method of Fieser and Fieser[18] was used to determine oxidation–reduction potentials in alcohol–water solutions. Because of the limited solubility of these compounds in water, the solvent was ethyl alcohol and water in a 1:1 ratio by volume and 0.1 N in HCl and 0.2 N in LiCl. A bright platinum electrode was used for measurement of the potential; the reference electrode was a saturated calomel electrode. Approximately 0.1 mmol of the quinone analogue was dissolved in 60 mL of the alcohol–water solution and titrated with 0.025 M ascorbic acid as the reducing agent. The emf at the midpoint of the titration was added to the emf of the saturated calomel electrode in order to compare these conditional redox potentials with the standard hydrogen electrode. Although the values of the conditional redox potentials shown in Table I are valid only for the conditions used in their determination (nature of solvent, temperature, hydrogen ion activity, and ionic strength), their values can be compared with those for other compounds in the same table.

Stabilities in Water. The stabilities in water at pH 7 and 25 °C were determined spectrophotometrically for representative samples of each class of compounds. The compounds were dissolved in absolute ethyl alcohol and their spectra determined. A small amount of this solution was dissolved in a buffer solution which was 0.01 M in 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris) and brought to pH 7 with concentrated HCl. The optical density at the wavelength of maximum absorption was measured at various times (hours or days) depending on the rate of change. When the logarithms of the absorbances were plotted vs. time, straight lines were obtained, the slopes of which were calculated by the least-squares method. From the slope of each line both

the rate constant for the change as well as the half-time for the change were found. The latter is listed in Table I for the compounds studied in this way.

Acute Toxicities. The single ip dose that will kill half the animals tested was used as a measure of the toxicities of these compounds. The protocol and the results are shown in Table II.

Inhibition of Ascitic Sarcoma 180 in Mice. Swiss mice obtained from Mid-Continent Research Animals, Inc., Shawnee Mission, Kan., were used in these experiments. The ascitic sarcoma 180 cells were obtained from Frederic A. French, Chemotherapy Laboratory, Mount Zion Hospital, San Francisco, Calif. The LD_{50} provides an estimate for the first dose used in the determination of ascitic sarcoma 180 activity; subsequent determinations are made with smaller or larger doses of the compounds to avoid toxicity and yet retain activity. The values given for the daily dose in Table II are those that gave the largest values of T/C.

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References and Notes

(1) R. A. Morton, Ed., "Biochemistry of Quinones", Academic Press, New York, N.Y., 1965.
(2) J. S. Driscoll, G. F. Hazard, H. B. Wood, and A. Goldin, *Cancer Chemother. Rep., Part 2*, **4**, 1 (1974).
(3) Private communication from G. F. Hazard.
(4) The Health Sciences Computing Facility is sponsored by NIH Special Research Resources Grant RR-3. For a description of this program see "BMD-Biomedical Computer Programs", W. J. Dixon, Ed., University of California Press, Berkeley, Calif., 1971.
(5) C. Hansch, A. Leo, S. H. Unger, K. H. Kim, D. Nitaitani, and E. J. Lien, *J. Med. Chem.*, **16**, 1207 (1973).
(6) Upon the suggestion of a reviewer we substituted σ^2 for F and R in the discriminant analysis for T/C and LD_{50} and obtained the following incomplete discriminant functions: for T/C, $6.09\,I_3 + 3.75\,I_1 + 17.8\,\sigma - 17.4\,\sigma^2 - 4.58$ ($F = 3.45 > F_{4,12,0.95} = 3.26$; 15/17 classified correctly); for LD_{50}, $22.9\,I_2 + 12.0\,I_1 - 9.10\,MR + 17.0\,\sigma - 6.88$ ($F = 13.6 > F_{4,12,0.999} = 9.63$; 17/17 classified correctly). The function for T/C indicates that σ may well have an optimum (maximum) value for these series of compounds.
(7) D. Hadzi, *J. Chem. Soc.*, 2725 (1956).
(8) H. H. Jaffé, *J. Am. Chem. Soc.*, **77**, 4448 (1955).
(9) H. Saito and K. Nukada, *Can. J. Chem.*, **46**, 2989 (1968).
(10) L. F. Fieser and H. T. Thompson, *J. Am. Chem. Soc.*, **61**, 376 (1939).
(11) P. W. Vittum and G. H. Brown, *J. Am. Chem. Soc.*, **68**, 2235 (1946).
(12) N. J. Leonard and J. W. Curry, *J. Org. Chem.*, **17**, 1071 (1952).
(13) H. H. Hodgson and D. E. Nicholson, *J. Chem. Soc.*, 1808 (1939).
(14) N. Tarugi and F. Lenci, *Boll. Chim. Farm.*, **50**, 907 (1911); *J. Chem. Soc.*, **102** (ii), 397 (1912).
(15) S. I. Burmistrov and N. A. Zuikova, *Zh. Obshch. Khim.*, **20**, 1852 (1950); *Chem. Abstr.*, **45**, 2924*b* (1951).
(16) W. W. Hartmen, J. B. Dicket, and J. G. Stampfli, "Organic Syntheses", Collect. Vol. II, Wiley, New York, N.Y., 1943, p 175.
(17) R. L. Datta and H. K. Mitter, *J. Am. Chem. Soc.*, **41**, 2028 (1919).
(18) L. F. Fieser and M. Fieser, *J. Am. Chem. Soc.*, **57**, 491 (1935).
(19) J. L. Bridge, *Justus Liebigs Ann. Chem.*, **277**, 79 (1893); *Chem. Central.*, **26**, 868 (1893).
(20) H. L. Bolker, F. L. Kung, and M. L. Kee, *Tappi*, **50**, 199 (1967): *Chem. Abstr.*, **67**, 12705e (1967).
(21) G. I. Mikhailov, *Tr., Vses. Nauchno-Issled. Inst. Khim. Reakt. Osobo Chist. Khim. Veshchestv.*, **16**, 83 (1939); *Chem. Abstr.*, **34**, 3707 (1940).

Structure–Activity Relationships in Antitumor Aniline Mustards[1]

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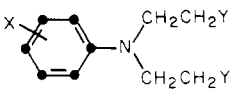
Quantitative structure–activity relationships (QSAR) have been formulated for the hydrolysis of aniline mustards and their antitumor activity against Walker 256 tumor and L1210 and P388 leukemia. In general, the antitumor activity parallels hydrolysis under the conditions defined by Ross; toxicity (LD_{50}) parallels antitumor efficacy. Chlorambucil is an exception. A most important finding is that ideal lipophilicity for effectiveness against Walker tumor appears to be much higher than for the leukemias which suggests that solid tumors may, in general, require more lipophilic drugs than leukemias.

It has become clear in recent years that even that most difficult area of chemotherapy, cancer chemotherapy, can be studied using the techniques of QSAR.[2] In this report we wish to analyze data from the literature as well as those collected over the years by the National Cancer Institute on aniline mustards of type I. It is our hope in making such retrospective studies that some generalizations can be uncovered which will be of help in the design of better antitumor drugs.



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These compounds, referred to as alkylating agents, fall into the must studied class of antitumor compounds and are one of the few classes of cancer drugs about which we have a general understanding of the mechanism of action.[3] A large amount of evidence has accumulated to suggest that the antitumor as well as the carcinogenic activity of the alkylating agents is brought about by their interaction with DNA and RNA.[4]

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