The 18-h culture of the above was diluted in 5% (w/v) hog gastric mucin to obtain 100 times the LD_{50} and 0.5 mL was injected intraperitoneally into mice. The mice were treated subcutaneously (sc) or orally (po) with a specific amount of the test compound divided equally to be administered at 1 and 5 h after infection. A group of 10 animals each for at least three dose levels were thus treated and the deaths were recorded daily for six days. Ten mice were left untreated as infection control. Fifty percent effective dose values (ED_{50}) were calculated from the cumulative mortalities on the sixth day after infection by using the trimmed version of the Logit method.[13]

Acknowledgment. We thank the staff of the microbiological team for biological testings and the staff of Analytical Department for spectral measurement and elemental analyses.

Registry No. **2**, 70458-96-7; **5**, 85721-33-1; **11**, 94695-50-8; **11** (enol ether), 105859-06-1; **12**, 105859-07-2; **13**, 105859-08-3; **14**, 105859-09-4; **15**, 105859-10-7; **16**, 105859-11-8; **17**, 105859-12-9; **18**, 105859-13-0; **18** (free base), 105859-19-6; **19**, 105859-14-1; **19** (free base), 105859-20-9; **20**, 103994-87-2; **21**, 105859-15-2; **22**, 105859-16-3; **22** (free base), 103995-05-7; **23**, 105859-17-4; **24**, 103995-06-8; **25**, 105859-18-5; **26**, 98106-49-1; **27**, 98105-99-8; **28**, 98106-17-3; 3-acetamidopyrrolidine, 79286-74-1; 2,4-difluoroaniline, 367-25-9; triethyl orthoformate, 122-51-0; 4-fluoroaniline, 371-40-4; monoethyl malonate, 1071-46-1; 2,3,4,5-tetrafluorobenzoyl chloride, 94695-48-4; 2,3,4,5-tetrafluorobenzoic acid, 1201-31-6; piperazine, 110-85-0; 4-methylpiperazine, 109-01-3.

(13) Hamilton, M. A.; Russo, R. C.; Thurston, R. V. *Environ. Sci. Technol.* **1977**, *11*, 714.

Synthesis, Biological Evaluation, and Quantitative Structure–Activity Relationship Analysis of 2-Hydroxy-1*H*-isoindolediones as New Cytostatic Agents[1]

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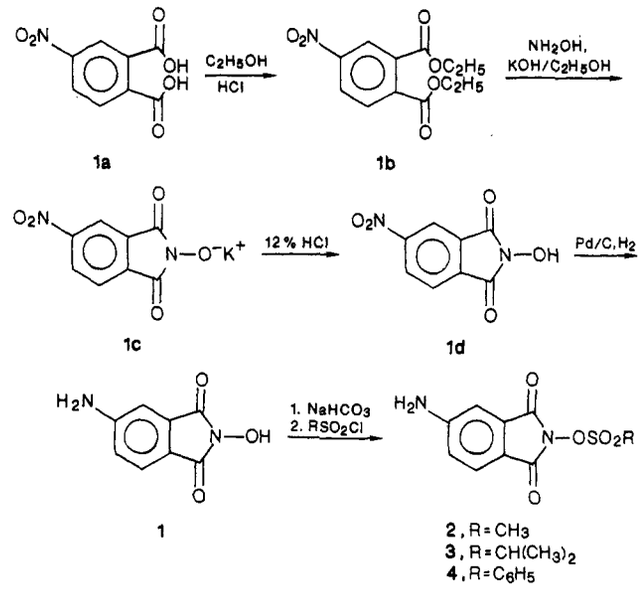
A series of 16 derivatives of 2-hydroxy-1*H*-isoindole-1,3-diones was designed and synthesized as potential antitumor agents. The cytostatic activity against L1210 cell growth of these compounds was studied, and their IC_{50} values were found to be in the range of 10^{-4} to 10^{-8} M. Quantitative structure–activity relationship analysis of these compounds showed that the inhibitory effect was well correlated with the electronic and the lipophilic parameters. Derivatives having a substituent with strongly electron-donating properties at the 6-position showed enhanced inhibitory activity while compounds having an electron-withdrawing group at the same position showed lower activity.

The necessary functional group in hydroxyurea for the inhibition of ribonucleotide reductase (RR) is known to be the =CNHOH.[2] RR is an important enzyme in DNA synthesis. This enzyme not only catalyzes one of the rate-determining steps in DNA synthesis but its activity is also positively correlated with the proliferation of cells.[3] This correlation is found to be particularly high in fast-growing or malignant cells.[4,5] The activities of three other major enzymes, which are also involved in DNA synthesis, namely, thymidylate synthetase, thymidine kinase, and DNA polymerase, were not increased to such an extent as that of RR.[6,7] Therefore selective inhibition of RR has been used as part of the overall strategy in the design of chemotherapeutic agents. Of all the major known RR inhibitors, only hydroxyurea is currently available for clinical use. Some RR inhibitors, for example, guanazoles and thiosemicarbazones, showed significant in vitro inhibitory activity against cell growth. However, their in vivo toxicities have prevented them from being used in clinical applications.[8,9]

The use of hydroxyurea is limited by its short half-life, which is due to its small molecular size and extremely polar nature. Frequent dosing regimen is required in order to circumvent the inherent problem of hydroxyurea. In the design of new potentially active compounds, the hydrophilic character of the hydroxyurea molecule should be modified such that the optimum balance of lipophilicity and hydrophilicity is achieved while the functional group, =CNHOH is maintained. On the basis of these criteria, a series of 2-hydroxy-1*H*-isoindole-1,3-diones were designed and synthesized. These molecules possess a variety of alkane- and arenesulfonate groups that serve as protective groups for the NOH moiety. The protective group is

Scheme I



[†] Department of Biochemistry.

(1) Portions of this work were presented at APhA Acad. Pharm. Sci. Med. Chem. and Pharmacognosy Section, 132nd APhA Annual Meeting, Feb. 1985, San Antonio, TX.
(2) Hill, R. B.; Gordon, J. A. *Exp. Mol. Pathol.* **1968**, *9*, 71.
(3) Weber, G. *N. Engl. J. Med.* **1977**, *296*, 486.
(4) Takeda, E.; Weber, G. *Life Sci.* **1981**, *28*, 1007.
(5) Witt, L.; Yap, T.; Blakely, R. L. *Adv. Enzyme. Regul.* **1979**, *17*, 157.

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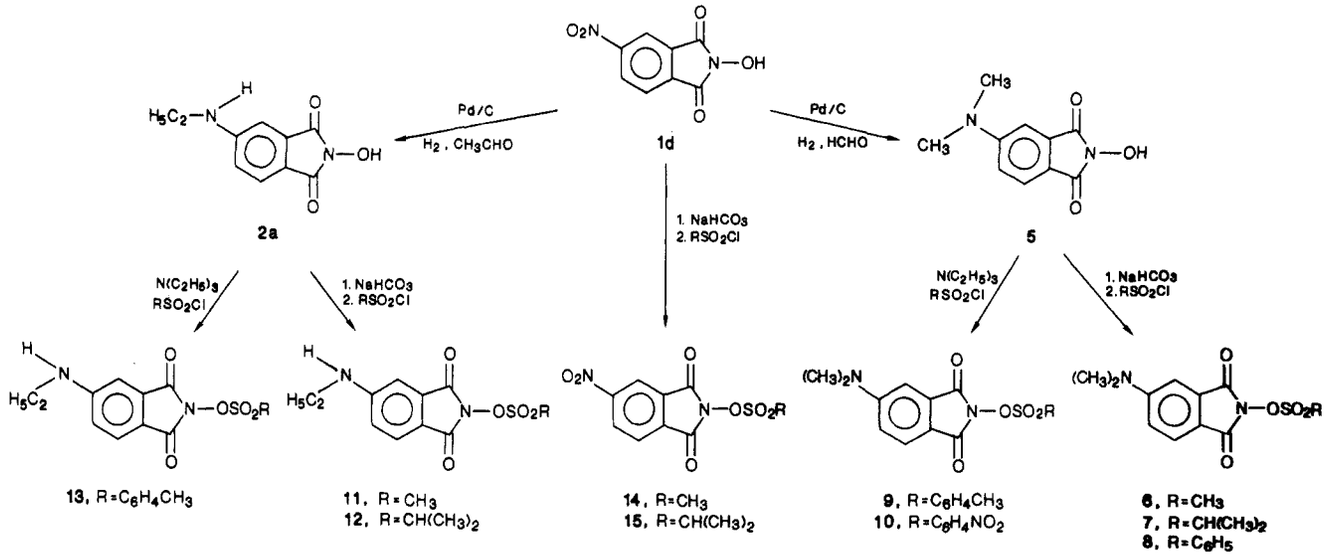
Scheme II



thought to prolong the half-life and thus increase the efficacy of the compound.[10]

The iron chelating property of hydroxyurea has been known to interfere with the iron-containing subunit of RR.[11,12] One of the compounds in this series, compound **5**, which has a free =NOH group, was found to chelate with ferric ion. Substitutions with electron-donating groups as well as electron-withdrawing groups at the 6-position of the molecule were carried out in order to investigate the overall electronic effects of these groups. Sixteen derivatives were synthesized, and the growth inhibition against L1210 cells (IC_{50}) of each compound was determined. Quantitative structure–activity relationship (QSAR) analysis for the correlation of the physicochemical properties with the growth-inhibitory activity was also studied.

Chemistry

The starting material for the synthesis of compound **1–15** was 4-nitrophthalic acid as shown in Scheme I. The phthalic acid was first converted to a diethyl ester and then cyclized with the addition of hydroxylamine in a basic solution to form a potassium salt, **1c**. The salt was acidified to form the NOH derivative at the 2-position, **1d**, which was then reduced in a Parr hydrogenator using Pd/carbon as the catalyst to give **1**. Compound **1** was converted to a sodium salt and then immediately reacted with methanesulfonyl chloride, 2-propanesulfonyl chloride, and benzenesulfonyl chloride to form compounds **2**, **3**, and **4**, respectively.

The procedure of Hurd et al.[13] was modified for the preparation of the major intermediate, 2-hydroxy-6-nitro-1*H*-isoindole-1,3-dione (**1d**). The cyclization of the diester **1b** was found to be most effective before the nitro group was reduced to the amino group. It appeared that the nitro group, being an electron-withdrawing group at the 4-position, was better able to facilitate the cyclization initiated by the addition of hydroxylamine. The product formed from the cyclization was the potassium salt **1c**, which would seem to be the ideal intermediate to react with the alkane- or arenesulfonyl chloride. However, when the 2-alkane- or 2-arenesulfonate derivatives of 2-hydroxy-6-nitro-1*H*-isoindole-1,3-diones were reduced, it was found that the RSO_3 moiety was cleaved. The successful synthetic routes for compounds **2–4** are illustrated in Scheme I. Dialkylating the amino group at the 6-position of **1** with trimethyl phosphate was our original approach to the synthesis of 6-*N*,*N*-dimethyl derivatives. However, the lack of sufficient basicity of the aromatic amino group did not favor the dialkylation on the nitrogen. By modification of the procedure of Romanelli and Becker,[14] compounds **6–13** were synthesized in good yield (Scheme II).

The 6-*N*-ethylamino derivatives, compounds **11–13**, were synthesized by using a similar procedure as described for the preparation of the 6-*N*,*N*-dimethylamino derivatives except that acetaldehyde was used instead of formaldehyde. Both of these derivatives were converted to sodium salts and subsequently reacted with various alkane- and arenesulfonyl chlorides, giving the sulfonate derivatives, compounds **6–8**, **11**, and **12**.

The synthetic procedure for the preparation of compounds **9**, **10**, and **13** was slightly modified. Because of the poor solubility of the arenesulfonyl chlorides in an aqueous medium, the reactions involving *p*-toluenesulfonyl chloride as well as *p*-nitrobenzenesulfonyl chloride with **5** and **2a** were carried out in triethylamine instead of aqueous sodium bicarbonate (Scheme II).

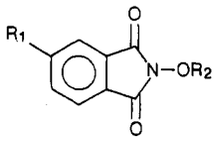
The 6-nitro derivatives **14** and **15** were prepared by converting compound **1d** directly to sulfonate derivatives as shown in Scheme II. Compound **16** was produced from the treatment of *N*-hydroxyphthalimide with methanesulfonyl chloride in a basic medium. The percent yields and melting points are summarized in Table I.

Results

Biological Study. In vitro growth inhibition of L1210 cells of the synthesized compounds was studied. The IC_{50}

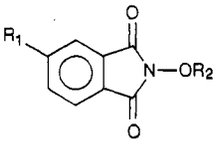
(6) Weber, G. *Adv. Enzyme Regul.* **1973**, *11*, 79.
(7) Weber, G.; Prajda, N.; Williams, J. C. *Adv. Enzyme Regul.* **1975**, *13*, 3.
(8) Thelander, L. *Annu. Rev. Biochem.* **1979**, *48*, 133.
(9) Elford, H. L.; Van't Riet, B.; Wampler, G. L.; Lin, A. L.; Elford, R. M. *Adv. Enzyme Regul.* **1981**, *19*, 151.
(10) Sinkula, A. A.; Yalkowsky, S. H. *J. Pharm. Sci.* **1975**, *64*, 181.
(11) Brockman, R. W.; Sidwell, R. W.; Arnett, G.; Shaddix, S. *Proc. Soc. Exp. Biol.* **1970**, *133*, 609.
(12) Remers, W. A. *Antineoplastic Agents*, Remers, W. A., Eds.; Wiley: 1984.
(13) Hurd, C. D.; Buess, C. M.; Bauer, L. *J. Org. Chem.* **1954**, *19*, 1140.
(14) Romanelli, M. G.; Becker, E. I. *Organic Syntheses*; Wiley: New York, 1973; Collect. Vol. V.

Table I. 2-Hydroxy-1*H*-isoindole-1,3-diones



no.	structure		formula	recrystn solvent	mp, °C	yield, %
	R_1	R_2				
1	NH_2	H	$C_8H_6N_2O_3$	MeOH	272–273	70
2	NH_2	SO_2CH_3	$C_9H_8N_2O_5S$	MeOH–benzene (1:1)	198–199	62
3	NH_2	$SO_2CH(CH_3)_2$	$C_{11}H_{12}N_2O_5S$	MeOH–benzene (1:1)	156–157	66
4	NH_2	$SO_2C_6H_5$	$C_{14}H_{10}N_2O_5S$	MeOH–benzene (1:1)	196–197	53
5	$(CH_3)_2N$	H	$C_{10}H_{10}N_2O_3$	MeOH	188–189	72
6	$(CH_3)_2N$	SO_2CH_3	$C_{11}H_{12}N_2O_5S$	MeOH–benzene (1:1)	152–154	63
7	$(CH_3)_2N$	$SO_2CH(CH_3)_2$	$C_{13}H_{16}N_2O_5S$	MeOH–benzene (1:1)	140–141	53
8	$(CH_3)_2N$	$SO_2C_6H_5$	$C_{16}H_{14}N_2O_5S$	MeOH–benzene (1:1)	161–162	65
9	$(CH_3)_2N$	$SO_2C_6H_4CH_3$	$C_{17}H_{16}N_2O_5S$	MeOH	178–179	54
10	$(CH_3)_2N$	$SO_2C_6H_4NO_2$	$C_{16}H_{13}N_3O_7S$	MeOH	145 dec	63
11	C_2H_5NH	SO_2CH_3	$C_{11}H_{12}N_2O_5S$	MeOH–benzene (1:1)	188–189	72
12	C_2H_5NH	$SO_2CH(CH_3)_2$	$C_{13}H_{16}N_2O_5S$	MeOH–benzene (1:1)	164–165	71
13	C_2H_5NH	$SO_2C_6H_4CH_3$	$C_{17}H_{16}N_2O_5S$	MeOH	150–152	66
14	NO_2	SO_2CH_3	$C_9H_6N_2O_7S$	acetone	205–206	75
15	NO_2	$SO_2CH(CH_3)_2$	$C_{11}H_{10}N_2O_7S$	acetone	196–197	70
16	H	SO_2CH_3	$C_9H_7N_1O_5S$	MeOH	144–145	70

Table II. Biological Activity and Physicochemical Parameters Used in the Regression Analysis



no.	structure		growth inhibition			parameters		
				log $(1/IC_{50})$				
	R_1	R_2	IC_{50}, M	obsd	calcd[a]	R_m	σ	μ
1	NH_2	H	3.5×10^{-4}	3.456	3.272	0.10	−0.66	1.53
2	NH_2	SO_2CH_3	3.3×10^{-6}	5.481	5.872	−0.17	−0.66	1.53
3	NH_2	$SO_2CH(CH_3)_2$	1.0×10^{-6}	6.000	5.748	−0.15	−0.66	1.53
4	NH_2	$SO_2C_6H_5$	3.1×10^{-6}	5.509	5.801	−0.15	−0.66	1.53
5	$(CH_3)_2N$	H	2.4×10^{-5}	4.620	5.055	−0.04	−0.83	1.61
6	$(CH_3)_2N$	SO_2CH_3	9.6×10^{-8}	7.036	6.654	−0.29	−0.83	1.61
7	$(CH_3)_2N$	$SO_2CH(CH_3)_2$	1.9×10^{-7}	6.721	6.245	−0.37	−0.83	1.61
8	$(CH_3)_2N$	$SO_2C_6H_5$	2.9×10^{-7}	6.538	6.710	−0.31	−0.83	1.61
9	$(CH_3)_2N$	$SO_2C_6H_4CH_3$	2.3×10^{-7}	6.638	6.608	−0.27	−0.83	1.61
10	$(CH_3)_2N$	$SO_2C_6H_4NO_2$	1.9×10^{-7}	6.721	6.818	−0.19	−0.83	1.61
11	C_2H_5HN	SO_2CH_3	3.4×10^{-7}	6.468	6.078	−0.21	−0.61	1.61
12	C_2H_5HN	$SO_2CH(CH_3)_2$	7.1×10^{-7}	6.149	6.327	−0.27	−0.61	1.61
13	C_2H_5HN	$SO_2C_6H_4CH_3$	6.8×10^{-7}	6.167	6.383	−0.29	−0.61	1.61
14	NO_2	SO_2CH_3	1.8×10^{-5}	4.745	4.715	−0.40	0.78	−4.13
15	NO_2	$SO_2CH(CH_3)_2$	2.2×10^{-5}	4.658	4.705	−0.37	0.78	−4.13
16	H	SO_2CH_3	3.0×10^{-6}	5.530	5.445	−0.25	0.00	0.03

[a] Calculated from eq 5 in Table III.

of each compound and the physicochemical parameters used in the correlation are listed in Table II. Of all the compounds tested, **6** and **7** exhibited the strongest inhibition against L1210 cells. The IC_{50} values of **6** and **7** were 8.9×10^{-8} and 1.9×10^{-7} M, respectively. Both **1** and **5**, which have the free OH group at the 2-position, showed the weakest inhibitory activity. The derivatization at the 2-position significantly enhanced the inhibitory activity. No major differences in the activity were noted when the derivatized group was changed from (methylsulfonyl)oxy to (isopropylsulfonyl)oxy or to the benzenesulfonyloxy group. In contrast to the derivatization at the 2-position of the molecule, the moiety at the 6-position had a greater effect on the inhibition. The 6-nitro derivatives **14** and **15** had IC_{50} values of 1.8×10^{-5} and 2.2×10^{-5} M, respectively. However, when the nitro group was replaced by an amino group, as in **2–4**, the activity was increased by about 10-fold. Further derivatization of the amino group to the *N,N*-dimethylamino group dramatically enhanced the activity to more than a 1000 times the activity observed for compound **1**. The *N*-ethylamino group at the 6-position also showed higher activity than the amino derivatives (**1-4**), although the activities of these compounds were not as high as those found in the *N,N*-dimethylamino derivatives (**5–10**).

QSAR. The correlations between the inhibitory activity on L1210 cells and the physicochemical parameters of 16 compounds were studied. The three parameters examined were the hydrophobic parameter, R_m, which is a chromatographic parameter related to partition coefficient; σ, which is a Hammett substituent constant and a measure of the electronic effect of a substituent on the reactivity of the molecule; and the dipole moment, μ, which is a measure of the separation of charges as well as a measure of drug receptor interaction involving charge–dipole, dipole–dipole, or dipole–induced dipole interactions.[15]

Table III. Correlation of Biological Activity and Physicochemical Parameters

eq no.	correlation equation	n	s	r
1	$\log (1/IC_{50}) = \underset{(0.992)}{4.916} - \underset{(3.839)^b}{3.806R_m}$	16	0.839	0.494
2	$\log (1/IC_{50}) = \underset{(0.751)}{4.669} - \underset{(17.028)}{28.275R_m^2} - \underset{(6.357)}{13.208R_m}$ $F_{1,13} = 12.77$	16	0.658	0.787
3	$\log (1/IC_{50}) = \underset{(0.668)}{5.343} - \underset{(0.932)}{0.881\sigma}$	16	0.903	0.676
4	$\log (1/IC_{50}) = \underset{(0.628)}{3.434} - \underset{(1.969)}{6.834R_m} - \underset{(0.473)}{1.618\sigma}$ $F_{1,13} = 54.20$	16	0.406	0.925
5	$\log (1/IC_{50}) = \underset{(0.501)}{3.595} - \underset{(9.440)}{13.597R_m^2} - \underset{(3.151)}{10.800R_m} - \underset{(0.525)}{1.312\sigma}$	*16*	*0.313*	*0.959*[a]
	ideal $R_m = -0.397$ (−0.997 to −0.285) $F_{1,13} = 9.85$			
6	$\log (1/IC_{50}) = \underset{(0.553)}{6.602} + \underset{(0.261)}{0.226\mu}$	16	0.920	0.446
7	$\log (1/IC_{50}) = \underset{(0.548)}{4.802} - \underset{(1.976)}{7.171R_m} + \underset{(0.130)}{0.456\mu}$ $F_{1,13} = 61.40$	16	0.400	0.927
8	$\log (1/IC_{50}) = \underset{(0.501)}{4.901} - \underset{(11.317)}{10.990R_m^2} - \underset{(3.630)}{10.247R_m} + \underset{(0.143)}{0.376\mu}$ $F_{1,13} = 4.48$	*16*	*0.354*	*0.948*[a]

[a] Best equations. [b] Numbers in parentheses are 95% confidence intervals.

Eight regression equations were obtained by using either one parameter or the combination of R_m^2 and one or two other parameters as shown in Table III. When the correlation involved only one parameter, either μ, σ, or R_m, the r value was in the range of 0.44–0.47 (eq 1, 3, and 6 and in Table II). The correlation was greatly enhanced when an additional parameter was included in the equations; for example, R_m gave a very small correlation; however, when μ or σ was included as shown in eq 4 and 7 of Table III, the r values were 0.925 and 0.927, respectively. The largest correlation was observed in eq 5 in which R_m^2, R_m, and σ were included. In comparing eq 5 and 8, it is obvious that the replacement of σ by μ did not change the r value considerably.

Discussion

Biological Study. Compounds having the free hydroxy group at the 2-position (**1** and **5**) showed the weakest growth inhibitory activity, while compounds having alkane- or arenesulfonate groups at the same position showed significant enhancement in the inhibition of cell growth. The purposes of attaching the alkane- and arenesulfonate groups at the 2-position was to have them serve as protective groups for the NOH moiety. The data obtained showed that the protective group was necessary for a high activity. This may be due to the more lipophilic nature of the derivatized molecule as compared to the underivatized molecule which lacked the lipophilic property to partition across the cell membrane. The stability of compound **7** was studied in the culture medium at 37 °C. The $t_{1/2}$ of the compound was found to be 40 ± 1.3 h for the hydrolysis of the 2-propanesulfonate group at the 2-position. Metal chelation as well as free-radical mechanisms have been proposed for the action of hydroxyurea.[16,17] Of these compounds studied, the free-NOH derivatives (**1** and **5**) were significantly less active than the corresponding methanesulfonate derivatives (**2** and **6**). This evidence as well as the hydrolysis data seem to suggest that the sulfonate groups are acting as protective groups.

Various groups at the 6-position also showed different inhibition activity of cell growth. Derivatives having an *N,N*-dimethyl group at the 6-position (**6–10**) had 10 times greater activity than that of compounds having the amino group attached to the same position (**2–4**). The amino derivatives, in turn, were 10 times more active than the nitro derivatives (**14** and **15**). These results seemed to suggest that a more electron-donating group at the 6-position of the molecule increased the inhibitory activity of the compound while an electron-withdrawing group at the same position had the opposite effect. However, other factors must be considered in the assessment of the activity of a compound. For example, the lipophilicity, dipole moments, and electronic nature of the functional group should also be considered.

The *N*-ethylamino derivatives at the 6-position did not show a significant difference in the activity when compared to that of the *N,N*-dimethylamino derivatives. The small difference may be due to the small variation in the substituent group.

QSAR. The QSAR study showed a large correlation between the activity and the two parameters used in the study, namely, the hydrophobic and the electronic parameters. The three parameters used in the regression equation were the hydrophobic parameter, R_m; the Hammett constant, σ, and the dipole moment, μ. R_m is derived from R_f by the equation $R_m = \log [(1/R_f - 1)]$.[18] The R_f values were obtained from TLC. According to Boyce et al.,[19] R_m for a substituent is a free energy based constant that is similar to that used by Hansch et al. The σ constants were derived from the comparison of the pK_a of the substituted benzoic acid to that of benzoic acid. These constants are measurements of the magnitude of *intramolecular* electronic effects of the substituents on the reactive center attached to the benzene ring. In QSAR analysis, if the drug and receptor interaction is influenced directly by the electronic nature of the substituent on the benzene ring, then the use of σ constant as a parameter would be appropriate. However, frequently, the *intramolecular* electronic effects due to the variation of sub-

(15) Lien, E. J.; Guo, Z.-R.; Li, R.-L. *J. Pharm. Sci.* **1982**, *71*, 641.
(16) Ehrenberg, A.; Reichard, P. *J. Biol. Chem.* **1972**, *247*, 3485.
(17) Atkin, C. L.; Thelander, L.; Reichard, P.; Land, G. *J. Biol. Chem.* **1973**, *248*, 7464.
(18) Tomlinson, E. *J. Chromatogr.* **1975**, *113*, 1.
(19) Boyce, C. B. C.; Milborrow, B. V. *Nature (London)* **1965**, *208*, 537.

Table IV. Squared Correlation Matrix of the Three Parameters

	R_m	σ	μ
R_m	1	0.202	0.236
σ		1	0.952
μ			1

stituents on the benzene ring do not contribute directly to the enhancement of the *intermolecular* interaction between the drug and the receptor. Various substituents may affect the binding force of the drug and the receptor by directly interacting with the receptor by charge–dipole, dipole–dipole, dipole–induced dipole, or induced dipole–induced dipole interaction.[15] In some instances, a correlation, between dipole moment, μ, and σ may exist, especially in small congeneric series. In cases when larger substituents are involved, the correlation may be small.[20] In comparing eq 5 and 8 in Table III, it is obvious that the replacement of σ by μ did not considerably change the r value in this series of compounds. The intramolecular electronic effect as measured by σ may be more important as it may affect the stability of the molecule. For example, the nitro group may enhance the hydrolysis of the sulfonate group. However, it is difficult to assess from limited data the role of drug–receptor interaction as compared to the stability of the molecule. A selection of more divergent substituents with lower intercorrelation between σ and μ may shed some light on this differentiation. For the compounds examined, there is a high degree of covariance between σ and μ ($r^2 = 0.95$), as reflected by the squared correlation matrix. The covariance between R_m and σ (or μ) is relatively low ($r^2 = 0.24$). The R_m and σ (or μ) can be considered as independent variables (Table IV).

Experimental Section

Chemistry. Elemental analyses were performed by C.F. Geiger, Ontario, CA. IR spectra of the compounds were obtained from a Beckman IR 4210 spectrometer. NMR spectra were obtained from a 90-MHz Varian 390 NMR spectrometer. Mass spectra of the compounds obtained for the purposes of structure confirmation were obtained from a Hewlett-Packard Model 59858 mass spectrometer. Chemical ionization with ammonia as the reagent gas was used for the analyses. The corrected melting point of the compounds were determined with a Thomas-Hoover melting point apparatus. The TLC was carried out on precoated silica gel F254 chromatographic plastic sheets. The solvent system consisted of methanol–benzene (4:6).

Diethyl 4-Nitrophthalate (1b). 4-Nitrophthalic acid (100 g, technical grade containing 80% 4-nitrophthalic acid and 20% 3-nitrophthalic acid) was dissolved in 150 mL of absolute ethanol. Hydrogen chloride gas was bubbled through a $CaCl_2$ drying tube into the alcoholic solution. After the solution had been fully saturated with HCl gas, the mixture was refluxed for 24 h, and the solvent was subsequently removed by a rotary evaporator. The oily residue was washed with water three times and once with 10% Na_2CO_3 solution. The oily solution was dissolved in anhydrous ether and dried over Na_2SO_4. The solution was filtered and evaporated under reduced pressure to dryness. The light yellow crude product was recrystallized from cold ethanol. The resulting cream colored crystals were dried in a vacuum desiccator overnight and gave diethyl 4-nitrophthalate (83 g, 65%): mp 31–32 °C. The purified compound was used for the following synthesis.

2-Hydroxy-6-nitro-1*H*-isoindole-1,3-dione (1d). Hydroxylamine hydrochloride (6.95 g, 0.01 mol) was suspended in 50 mL of ethanol with stirring at room temperature. A methanol solution of KOH (5.6 g, 0.01 mol in 50 mL of solvent) was slowly added to the suspension, which was warmed to 50 °C. The mixture was stirred until the solution had a pH of 7. The potassium chloride formed was filtered immediately, and the filtrate was put into a 500-mL round-bottom flask equipped with a magnetic stirrer. To the freshly prepared hydroxylamine solution was added 26.7 (0.10 mol) of diethyl 4-nitrophalate (**1b**) while the flask was kept at 0–4 °C in an ice bath. A change of color from yellow to orange to purple was noted within 6 h. The mixture was allowed to react further at room temperature for 48 h. The residue was filtered and dried under reduced pressure. This dried product was then acidified with 12% HCl. The resulting yellow crystals were filtered and washed with water. Pure crystals were obtained by recrystallization from methanol (yield 14.1 g, 68%): mp 168–170 °C. The compound was immediately used for the following synthesis.

6-Amino-2-hydroxy-1*H*-isoindole-1,3-dione (1). 2-Hydroxy-6-nitro-1*H*-isoindole-1,3-dione (**1d**) (5 g, 0.024 mol) was placed in 100 mL of methanol in the presence of 0.50 g 10% palladium on carbon. This mixture was placed in a Parr hydrogenator at a pressure of 30 psi of H_2. The reduction required approximately 45 min until the calculated amount of hydrogen was consumed. The reduced product with the catalyst was warmed gently and then filtered. The filtrate was evaporated to dryness under reduced pressure. Yellow crystals were obtained by recrystallization from methanol. Anal. ($C_8H_6N_2O_3$) C, H, N.

6-Amino-2-[(methylsulfonyl)oxy]-1*H*-isoindole-1,3-dione (2). The crystals of compound 1 (0.85 g, 0.0048 mol) was suspended in 20 mL of water and 0.4 g of sodium bicarbonate was added. The resulting sodium salt was stirred and kept cold in an ice bath. When the temperature of the suspension reached 4 °C, methanesulfonyl chloride (0.86 g, 0.0048 mol) was added dropwise. The mixture was stirred for 2 h in an ice bath. At the end of the 2 h, the solid was filtered and washed with water several times. The product was recrystallized from methanol–benzene (1:1), giving bright yellow crystals. Anal. ($C_9H_8N_2O_5$) C, H, N.

6-Amino-2-[(isopropylsulfonyl)oxy]-1*H*-isoindole-1,3-dione (3) and 6-Amino-2-(benzenesulfonyloxy)-1*H*-isoindole-1,3-dione (4). The same procedure described above was followed except that 2-propanesulfonyl chloride and benzenesulfonyl chloride were used for compounds **3** and **4**, respectively, instead of the methanesulfonyl chloride. The yield for compound **3** was 0.90 g. Anal. ($C_{11}H_{12}N_2O_5S$) C, H, N, S. The yield for compound 4 was 1.4 g. Anal. ($C_{14}H_{10}H_2O_5S$) C, H, N, S.

2-Hydroxy-6-(*N*,*N*-dimethylamino)-1*H*-isoindole-1,3-dione (5) and 6-(*N*,*N*-Dimethylamino)-2-[(methylsulfonyl)oxy]-1*H*-isoindole-1,3-dione (6). 2-Hydroxy-6-nitro-1*H*-isoindole-1,3-dione (**1d**; 1.66 g, 0.008 mol) was suspended in 100 mL of methanol containing 0.167 g of palladium on carbon (10%) and 0.020 mol of formaldehyde. This mixture was placed in a Parr hydrogenator with the pressure of H_2 adjusted to 30 psi of H_2. After 1 h, the reaction mixture was filtered and the filtrate was evaporated under reduced pressure. The residue was recrystallized from methanol, giving compound **5**. Compound **5** (0.800 g, 0.004 mol) was suspended in 40 mL of water, and $NaHCO_3$ (0.70 g) was added until the solution turned basic (pH 8). The basic solution was stirred in an ice bath. Methanesulfonyl chloride (1.04 g, 0.009 mol) was slowly added to the cooled mixture. The reaction mixture was stirred for an additional 45 min and then filtered. The resulting solid was recrystallized from methanol–benzene (1:1). Anal. ($C_{11}H_{12}N_2O_5S$), C, H, N, S.

2-[(Isopropylsulfonyl)oxy]-6-(*N*,*N*-dimethylamino)-1*H*-isoindole-1,3-dione (7) and 2-(Benzenesulfonyloxy)-6-(*N*,*N*-dimethylamino)-1*H*-isoindole-1,3-dione (8). The same procedure as described above was followed except that 2-propanesulfonyl chloride and benzenesulfonyl chloride were used for **7** and **8**, respectively instead of methanesulfonyl chloride. Compound **7**, anal. ($C_{13}H_{16}N_2O_3S$) C, H, N, S. Compound **8**, anal. ($C_{16}H_{14}N_2O_5S$) C, H, N, S.

6-(*N*,*N*-Dimethylamino)-2-(*p*-toluenesulfonyloxy)-1*H*-isoindole-1,3-dione (9). Compound **5** (0.82 g, 0.0040 mol) and *p*-toluenesulfonyl chloride, (0.78 g, 0.0041 mol) was suspended in 20 mL of chloroform. As the mixture was stirred in an ice bath, triethylamine (0.6 mL) was added dropwise until the suspension was dissolved in chloroform. The reaction mixture was then stirred for 4 h at room temperature. The solvent was evaporated to dryness and the residue was washed with water. The resulting compound was dried in vacuum overnight. The compound was recrystallized from methanol. Anal. ($C_{17}H_{16}N_2O_5S$) C, H, N, S.

6-(*N*,*N*-Dimethylamino)-2-[(*p*-nitrobenzenesulfonyl)-oxy]-1*H*-isoindole-1,3-dione (10). The same procedure described above was followed except that *p*-nitrobenzenesulfonyl chloride

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was used instead of *p*-toluenesulfonyl chloride. Anal. ($C_{14}H_{18}N_3O_7S$) C, H, N, S.

6-(*N*-Ethylamino)-2-[(methylsulfonyl)oxy]-1*H*-isoindole-1,3-dione (11). The starting material for the synthesis of compound **11** was 6-(*N*-ethylamino)-2-hydroxy-1*H*-isoindole-1,3-dione (**2a**), which was prepared according to the general procedure used for the synthesis of compound **5**. In this procedure acetaldehyde was used instead of formaldehyde. The crystals of **2a** (2.29 g, 0.008 mol) were suspended in 40 mL of water. $NaHCO_3$ was added until the mixture became basic (pH 8). While this solution was being stirred in an ice bath, methanesulfonyl chloride (0.9 g 0.008 mol) was slowly added. This reaction mixture was continuously stirred for 2 h, and then the residue was filtered and recrystallized from methanol/benzene (1:1). Anal. ($C_{11}H_{12}N_2O_5S$) C, H, N, S.

6-(*N*-Ethylamino)-2-[(isopropylsulfonyl)oxy]-1*H*-isoindole-1,3-dione (12) and 6-(*N*-Ethylamino)-2-(toluenesulfonyloxy)-1*H*-isoindole-1,3-dione (13). The same procedure was used for the synthesis of compound **12** as for compound **11** except that 2-propanesulfonyl chloride was used instead of methanesulfonyl chloride. The general procedure used for the synthesis of compound **9** was followed for the preparation of compound **13** as shown in Scheme II. Compound **12**, anal. ($C_{13}H_{16}N_2O_5S$) C, H, N, S. Compound **13**, anal. ($C_{17}H_{16}N_2O_5S$) C, H, N, S.

2-[(Methylsulfonyl)oxy]-6-nitro-1*H*-isoindole-1,3-dione (14). Compound **1d** (1.66 g, 0.008 mol) was suspended in a 10% $NaHCO_3$ solution (5 mL) until the sodium salt was formed. Methanesulfonyl chloride (1.14 g, 0.01 mol) was added slowly while the mixture was stirred in an ice bath. After stirring for 45 min, the mixture was filtered. The resulting solid was recrystallized from acetone. Anal. ($C_9H_6N_2O_7S$) C, H, N, S.

2-[(Isopropylsulfonyl)oxy]-6-nitro-1*H*-isoindole-1,3-dione (15). The same procedure described above for compound **14** was followed except that 2-propanesulfonyl chloride was used instead of methanesulfonyl chloride. Anal. ($C_{11}H_{10}N_2O_7S$) C, H, N, S.

2-[(Methylsulfonyl)oxy]-1*H*-isoindole-1,3-dione (16). The procedure described for the synthesis of compound **14** was followed except that the starting material used was 2-hydroxy-1*H*-isoindole-1,3-dione instead of compound **1d**. Anal. ($C_9H_7NO_5S$) C, H, N, S.

In Vitro Growth Inhibition Study. The following experiments were performed under sterile conditions. Murine leukemia cell line L1210 grown in media RPMI 1640 (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (Biocell, Compton, CA) (5×10^5 cells/mL) was used to test the growth inhibition activity of the synthesized compounds. The concentrations of the compounds ranging from 10^{-3} to 10^{-8} M were prepared in phosphate buffer saline (PBS). Each compound was initially solubilized in dimethyl sulfoxide (Me_2SO), however, each final dilution contained less than 1% Me_2SO. Solutions of different concentrations (0.20 mL) were pipeted into separate test tubes (1 × 7.5 cm) in duplicates. Cell culture (1.8 mL) containing a cell population of 6×10^4 cells/mL was pipeted into test tubes. Controls, containing only PBS and Me_2SO at identical dilutions, were also prepared in the same manner. These cultures were incubated in a humidified incubator at 37 °C. The incubator was supplied with 95% air and 5% carbon dioxide. After 72 h, cells in each test tube was diluted 10 times with saline and counted by using a Coulter counter (Coulter Electronics Inc., Hialeah, FL). The counts were corrected for the dilution.

Chemical Stability Experiment. The stability of compound **7** was investigated. The compound was incubated at 37 °C in medium RMPI 1640, pH 7.4. The sample was analyzed by HPLC (Beckman Model 210) at 1-h intervals for 72 h. A C_{18} column (5 μm, 1.8 × 25 cm) and a variable-wavelength detector set at 268 nm were used in this analysis. The $t_{1/2}$ of compond **7** was determined to be 40 ± 1.3 h. The hydrolyzed product, postulated to be 2-hydroxy-6-(*N*,*N*-dimethylamino)-1*H*-isoindole-1,3-dione (**5**) appeared as an extra peak along with the peak for **7** in the high-pressure liquid chromatogram. Spiking the sample with **5** gave an enhanced peak height, providing further evidence that the hydrolyzed product and **5** were probably the same.

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Registry No. **1**, 105969-84-4; **1a**, 610-27-5; **1b**, 2050-19-3; **1c**, 105970-00-1; **1d**, 105969-98-0; **2**, 105969-85-5; **2a**, 105969-99-1; **3**, 105969-86-6; **4**, 91517-75-8; **5**, 105969-87-7; **6**, 105969-88-8; **7**, 105969-89-9; **8**, 105969-90-2; **9**, 105969-91-3; **10**, 105969-92-4; **11**, 105969-93-5; **12**, 105969-94-6; **13**, 105969-95-7; **14**, 105969-96-8; **15**, 105969-97-9; **16**, 57212-70-1; 2-hydroxy-1*H*-isoindole-1,3-dione, 524-38-9; 3-nitrophthalic acid, 603-11-2; hydroxylamine hydrochloride, 5470-11-1; methanesulfonyl chloride, 124-63-0; 2-propanesulfonyl chloride, 10147-37-2; benzenesulfonyl chloride, 98-09-9; formaldehyde, 50-00-0; *p*-toluenesulfonyl chloride, 98-59-9; *p*-nitrobenzenesulfonyl chloride, 98-74-8; acetaldehyde, 75-07-0.

Synthesis and 3′-Substituent Effects of Some 7α-Methoxy-1-oxacephems on Antibacterial Activity and Alkaline Hydrolysis Rates

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Relationships between intrinsic antibacterial activity and β-lactam chemical reactivity of 7β-(phenylacetamido)-7α-methoxy-1-oxacephems with various 3′-substituents (**1–9**) were studied in order to clarify the effect of the 3′-substituent on the antibacterial activity. The chemical reactivity of the β-lactam ring estimated by pseudo-first-order rate constants log k_{obsd}^{NMR} of alkaline hydrolysis at pD 10.4 and 35.0 °C correlates well linearly with ^{13}C NMR chemical shift differences ($\Delta\delta(4-3)$), infrared stretching frequencies of the β-lactam carbonyl ($\nu_{C=O}$), and σ_I values. Values of log ($1/C_N$), averaged for the MIC values for *Escherichia coli*, *E. coli* NIH JC-2, *E. coli* EC-14, and *Klebsiella pneumoniae* SRC-1, were taken as an estimation of the intrinsic antibacterial activity. The log ($1/C_N$) values of the compounds without good leaving groups (**1**, **2**, **4**, **5**, and **8**) correlated fairly well with log k_{obsd}^{NMR} values. The comparatively high antibacterial activity of compounds with good leaving groups (**6**, **7**, and **9**) may be attributable to the different course of decomposition of these compounds.

β-Lactam antibiotics inhibit the synthesis of bacterial cell walls in bacteria by acylating the active center of the target transpeptidases, which play an important role in constructing the three-dimensional network of the cell

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