Synthesis, Physicochemical Properties, and Antitumor Activities of Analogs of 1-Phenyl-3-benzyl-3-methyltriazenes

WILLIAM J. DUNN, III ˣ, and M. J. GREENBERG

Abstract □ To study the effect of substituents on the antitumor activities of analogs of 1-phenyl-3-benzyl-3-methyltriazenes, a series of compounds was designed and synthesized in which the substituent on the 1-phenyl was an electron-withdrawing group and the substituent on the 3-benzyl had a broad range of physicochemical properties. Of the 13 analogs prepared and tested against Sarcoma-180 in the mouse, five showed significant activity. The results were submitted to discriminant analysis to determine structure–activity relationships.

Keyphrases □ Triazenes, substituted—synthesized, antitumor activity evaluated, mice □ Antitumor activity—evaluated in various substituted triazenes, mice □ Structure–activity relationships—various substituted triazenes evaluated for antitumor activity

For 1-phenyl-3,3-dimethyltriazenes (I) (1), activity against Sarcoma-180 in the mouse recently was reported to be a function of the Hammett sigma constant for X, the phenyl group substituent. This relationship is given by:

$$pC_{130} = 3.41\ (\pm 0.03) - 0.69\ (\pm 0.09)\sigma \qquad \text{(Eq. 1)}$$

$$n = 13 \qquad r^2 = 0.85 \qquad s = 0.09$$

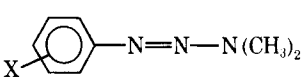
where pC_{130} is $-\log C_{130}$, and C is the molar concentration per kilogram of mouse required to increase the lifespan of the treated group 30% over the control. The derivatives on which the equation is based were designed using the cluster analysis scheme proposed by Hansch *et al.* (2).

The unusual aspect of this relationship is that activity is a function of the electronic nature of X. In a study of the structure–activity requirements of triazenes of Structure II, Hansch *et al.* (3) proposed that lipophilicity is significant in determining the level of activity against L-1210 in the mouse.

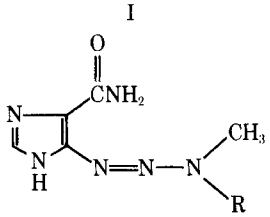
This report describes the synthesis and evaluation, as antitumor agents, of a series of triazenes of general Structure III.

EXPERIMENTAL

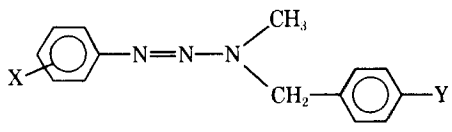
Synthesis—The triazenes were prepared by coupling the appropriate benzylamine with a diazonium cation using the procedure of Rondestvedt and Davis (4). The benzylamines were obtained commercially or were prepared using a reported procedure (5). The procedure is illustrated here for the synthesis of III*b*. The structures were verified by IR, PMR, and mass spectrometric methods (Table I).



I



II



III

For III*b*, *p*-chlorobenzaldehyde (35.1 g) in dry benzene (75 ml) was placed in a three-necked flask equipped with a distilling trap. An aliquot of 200 ml of benzene saturated with methylamine was added, and the mixture was refluxed for 24 hr. Benzene was distilled at reduced pressure, and the imine that resulted was dissolved in absolute methanol. To this mixture was added sodium borohydride (14 g) in 70 ml of methanol.

After refluxing for 4 hr, the mixture was cooled to 0° and acetone was added to decompose the excess hydride. The mixture was extracted with 3 *N* HCl and ether to remove unreduced starting material. The amine was regenerated with 6 *N* sodium hydroxide and extracted with ether. The amine was dried, taken up in ether, and purified as the hydrochloride. The yield was 16.4 g (34%), mp 204° [lit. (5) mp 205°].

The amine (9.08 g) was reacted with the diazonium cation generated from *p*-aminobenzoic acid (5.48 g) and sodium nitrite (2.76 g) at −5°. The coupling reaction with the benzylamine at 0° yielded 6.20 g (51%) of triazene, mp 168° (methanol).

Anal.—Calc. for $C_{15}H_{14}ClN_3O_2$: C, 59.31; H, 4.99; N, 13.84. Found: C, 59.70; H, 4.76; N, 14.11.

Partition Coefficients—1-Octanol–water partition coefficients were determined according to a reported procedure (6). Those for the carboxylic acids were determined at pH 7.4 and corrected for ionization using the pKa = 4.20 determined for *p*-(3,3-dimethyl-1-triazeno)benzoic acid by the method of Bordwell and Cooper (7).

Hydrolysis—Each *p*-(3-benzyl-3-methyl-1-triazeno)benzoic acid was dissolved in methanol (10 ml). A 1-ml aliquot was taken from this solution and placed in a 100-ml volumetric flask. The volume was adjusted to 100 ml with pH 7.0, 0.01 *M* phosphate buffer, preheated to 37°. The disappearance of the maximum absorption for each triazene was followed spectrophotometrically.

Rate constants were calculated from the absorbance data using the technique of Guggenheim (8), and $t_{1/2}$ values are given in Table I. Rate constants also were determined as a function of pH for triazenes III*a* and III*c* (Fig. 1).

Biological Testing—The triazenes were tested for activity against Sarcoma-180 ascitic tumor in the mouse using a modification of the procedure of Sartorelli *et al.* (9). Donar mice were sacrificed by asphyxiation with chloroform, followed by removal of the top skin layer of the intraperitoneal cavity, and fluid was removed. A threefold dilution of this fluid was made with sterile saline, and 0.1 ml (approximately 2×10^6 cells) was injected into 15–18-g female albino Swiss mice. Each test group was composed of six mice; a control group of six mice was determined for each test.

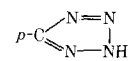
The triazenes were suspended in sterile saline and administered on alternate sides of the intraperitoneal cavity 24 hr after implantation. This dosing was repeated daily for 3 days. Initial dosage levels were set at 0.25, 0.38, and 0.50 of the LD_{50} for that particular drug; other dosage levels were in this range. The mice were observed daily, and the day of death was recorded.

A dose–response curve was generated from which the log dose (milligrams per kilogram) required to give T/C = 130% was obtained. The error in such a determination can be as high as 25%. Compound III*d*, although active, did not give a suitable dose–response curve from which a standard response curve could be obtained.

The LD_{50} values were determined by the procedure of Turner (10) using suspensions prepared as previously described. The LD_{50} values reported were the moles per kilogram of animal lethal to 50% of a group of five mice in 1 week. Each LD_{50} was determined from a dose–response curve derived from at least four test groups. The activities are reported in Table I.

Discriminant Analysis—Analyses were carried out using a standard statistical package. The physicochemical constants were either deter-

Table I—Physical and Biological Properties of 1-Phenyl-3-benzyl-3-methyltriazenes

Compound	X	Y	Yield, %	Melting Point	Formula	Analysis, %		Chemical Shifts, ppm[a]			Biological Properties			
						Calc.	Found	3-Methyl	3-Benzyl	1-Phenyl	pC_{130}	pLD_{50}	Log P (Ion Corrected)	$t_{1/2}$, days
III*a*	*p*-CO_2H	H	53	149°	$C_{15}H_{15}N_3O_2$	C 66.88 H 5.63 N 15.61	66.76 5.57 15.69	3.15	4.93	7.25–8.10	Inactive	2.80	4.04	2.55
III*b*	*p*-CO_2H	Cl	51	168°	$C_{15}H_{15}ClN_3O_2$	C 59.31 H 4.99 N 13.84	59.70 4.76 14.11	3.15	4.98	7.28–7.93	3.19	2.65	4.85	3.05
III*c*	*p*-CO_2H	NO_2	51	156°	$C_{15}H_{14}N_4O_4$	C 57.31 H 4.50 N 17.83	57.23 4.54 18.07	3.30	5.20	7.47–8.30	Inactive	2.69	4.03	4.18
III*d*	*p*-CO_2H	CN	62	165°	$C_{16}H_{15}N_4O_2$	C 65.29 H 5.15 N 19.04	65.12 4.90 18.93	3.25	5.17	8.03–8.30	Active	2.62	3.60	3.96
III*e*	*p*-CO_2H	CH_3	72	166°	$C_{16}H_{17}N_3O_2$	C 67.82 H 6.06 N 14.83	67.86 6.03 15.07	3.20	5.02	7.23–8.02	3.25	2.75	4.75	2.65
III*f*	*p*-CO_2H	OCH_3	69	157°	$C_{16}H_{17}N_3O_3$	C 64.19 H 5.74 N 14.04	64.01 5.78 14.13	3.20	4.97	6.93–8.02	Inactive	2.77	4.43	2.64
III*g*	*p*-CO_2H	$NHCOCH_3$	49	193°	$C_{17}H_{18}N_4O_3$	C 62.55 H 5.57 N 17.17	62.40 5.60 17.23	3.22	5.02	7.23–8.02	Inactive	2.21	3.35	2.55
III*h*	*p*-CO_2H	$C(CH_3)_3$	37	146°	$C_{19}H_{23}N_3O_2$	C 70.12 H 7.14 N 12.91	70.15 7.18 12.99	3.20	4.98	7.34–7.96	3.20	2.75	6.09[b]	—
III*i*	*p*-CN	H	84	84°	$C_{15}H_{14}N_4$	C 71.97 H 5.65 N 22.39	71.89 5.73 22.28	3.12	4.93	7.27–7.47	Inactive	2.59	3.82	—
III*j*	*p*-CN	Cl	81	58°	$C_{15}H_{13}ClN_4$	C 63.21 H 4.61 N 19.68	63.39 4.74 19.96	3.31	4.90	7.18–7.48	Inactive	2.71	4.44[b]	—
III*k*	*m*-Cl	Cl	55	Dec.	$C_{14}H_{13}Cl_2N_3$	C 57.15 H 4.46 N 14.29	57.16 4.48 14.30	3.15	4.90	7.01–7.50	3.08	2.24	5.03[b]	—
III*l*	*p*-$CONH_2$	H	39	133°	$C_{15}H_{16}N_4O$	C 67.13 H 6.02 N 20.88	66.91 6.03 20.87	3.18	4.98	7.25–7.90	Inactive	2.98	2.76	—
III*m*	*p*- 	H	46	168°	$C_{15}H_{15}N_7$	C 61.41 H 5.16 N 33.43	61.05 5.23 33.20	3.19	5.06	7.62–8.10	Inactive	3.08	2.37[b]	—

[a] Relative to tetramethylsilane at 0 ppm. Solvents were deuterochloroform for III*a*, dimethyl sulfoxide-d_6 for III*b*–III*h*, III*l*, and III*m*; and carbon tetrachloride for III*i*–III*k*. [b] Estimated value.

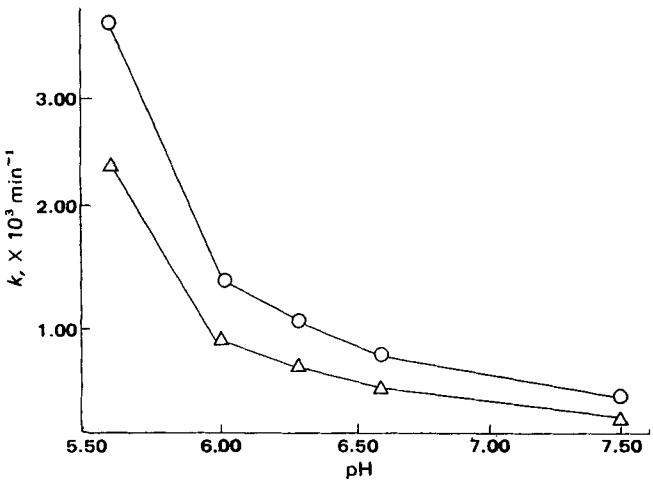


Figure 1—*The pH-rate profile for IIIa (○) and IIIc (△).*

mined or obtained from published compilations (11, 12) (Table II). In the analysis, prior probabilities for activity or inactivity were set as being equal.

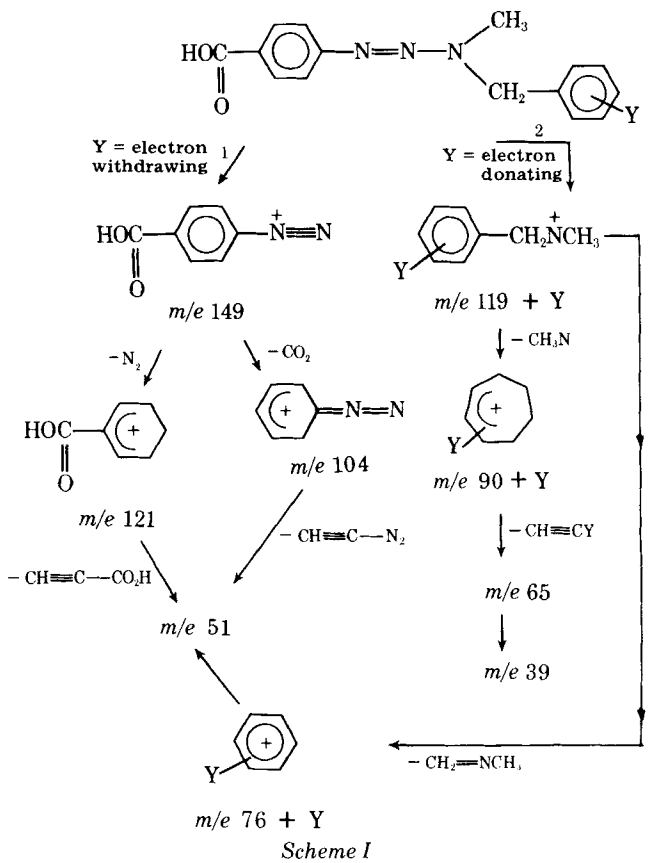
RESULTS AND DISCUSSION

The synthetic procedures used to prepare this class of compounds generally gave good yields. The partition coefficients show that they were very lipophilic; the *tert*-butyl analog was too lipophilic to obtain a reliable measurement. At pH 7, the triazenes were relatively stable to hydrolysis, with $t_{1/2}$ in the range of 2.5–4.0 days. Hydrolysis is acid catalyzed, as can be seen from the pH-rate profile in Fig. 1.

The mass spectral fragmentation for the triazenes in this study produced fragments resulting mainly from cleavage of the 2–3-nitrogen bond of the triazene group. Thus, the fragmentation patterns were those of the diazonium cation and the benzylamine that resulted from such cleavage. For analogs in which X = COOH and Y varies, the fragmentation is given in Scheme I. When Y is electron withdrawing, the base peak is the benzoic acid cation; it is the substituted tropylium ion when Y is electron donating.

There was very little difference in the antitumor activities on a molar basis. The same was true for the toxicities; the analogs were essentially equitoxic. Since the compounds showed little variation in activity, they could not be used in a Hansch analysis.

The 13 analogs were classified as active and inactive and subjected to discriminant analysis. This technique was recently shown to be useful in classifying inhibitors of monoamine oxidase (10) on the basis of their



Scheme I

physicochemical properties. Ideally, the procedure results in a function that classifies the molecules into an active group and an inactive group. The results are given in Table III. The analysis classifies 11 of the 13 compounds correctly; one from each category is misclassified.

An examination of the discriminant functions shows that these results are consistent with a previous report (1) in that activity appears to be associated with electron-donating groups for the substituent X. Variation

Table II—Physicochemical Parameters

Compound	f_x	f_y	σ_x[a]	σ_y[a]
IIIa	−0.40[b]	0.19[b]	0.00	0.00
IIIb	−0.40[b]	0.81	0.00	0.23
IIIc	−0.40[b]	−0.01	0.00	0.78
IIId	−0.40[b]	−0.44	0.00	0.66
IIIe	−0.40[b]	0.71	0.00	−0.17
IIIf	−0.40[b]	0.39	0.00	−0.27
IIIg	−0.40[b]	−0.68	0.00	0.00
IIIh	−0.40[b]	2.24[b]	0.00	−0.20
IIIi	−0.22	0.19	0.66	0.00
IIIj	−0.22	0.81	0.66	0.23
IIIk	0.81	0.81	0.37	0.23
IIIl	−1.28	0.11	0.36	0.00
IIIm	−1.23[a]	0.19[b]	0.50	0.00

Correlation Matrix

	ACT	f_x	f_y	σ_x	σ_y
ACT	1.00	0.42	0.48	−0.37	0.09
f_x	0.42	1.00	0.22	0.01	0.18
f_y	0.48	0.22	1.00	0.02	0.38
σ_x	−0.37	−0.01	0.02	1.0	−0.05
σ_y	0.09	0.18	−0.38	−0.05	1.00

[a] Reference 12. [b] Reference 11.

Table III—Discriminant Analysis Results

Linearized Discriminant Function

	ACT 0	ACT 1
Constant	−0.59	−1.52
ACT	0.00	0.00
f_y	0.26	2.96
σ_x	3.79	−0.62
σ_y	1.19	4.32

Probability of Membership in ACT

Observed	From ACT	Classified into ACT	0	1
IIIa	0	0	0.60	0.40
IIIb	1	1	0.12	0.88
IIIc	0	1	0.19[a]	0.81
IIId	1	0	0.52[a]	0.48
IIIe	1	1	0.39	0.61
IIIf	0	0	0.67	0.33
IIIg	0	0	0.94	0.06
IIIh	1	1	0.01	0.99
IIIi	0	0	0.96	0.03
IIIj	0	0	0.72	0.28
IIIk	1	1	0.41	0.58
IIIl	0	0	0.90	0.10
IIIm	0	0	0.93	0.07

[a] Misclassified observation.

at Y does affect activity, with the more lipophilic and electron-withdrawing groups being classified as active.

The detection of the role of lipophilicity is consistent with the report of Hansch *et al.* (3), who found that the activity of analogs of II was a function of the lipophilicity of the substituents at the 3-nitrogen.

The interpretation of these results in terms of the mode of action of the triazenes can only be viewed as suggestive at this point. It was suggested previously that the triazenes must be demethylated to form an active metabolite (13). The compounds in this study are subject to such activation, but the physicochemical effect on metabolism of substituents removed from the site of *N*-demethylation has not been studied for triazenes. Therefore, further work is necessary before conclusions can be drawn.

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Rapid and Sensitive Direct TLC Fluorometric Method for Evaluation of Impurities in Oxytetracycline

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Abstract □ A rapid partition TLC method for the determination of oxytetracycline and its degradation products is described. Quantitative determination of the substances is performed by direct TLC fluorometry in 2 hr using plates prepared previously.

Keyphrases □ Oxytetracycline and impurities—TLC fluorometric analyses in prepared solutions □ TLC fluorometry—analyses, oxytetracycline and impurities in prepared solutions □ Fluorometry, TLC—analyses, oxytetracycline and impurities in prepared solutions □ Antibacterials—oxytetracycline and impurities, TLC fluorometric analyses in prepared solutions

Tetracyclines are sensitive to both acidic and alkaline degradation media (1). Storage under adverse moisture and temperature conditions gives rise to acidic degradation products. The first stage of acidic degradation is the formation of anhydro compounds, which have attracted a great deal of attention because of their potential toxicity (2, 3).

Although the most likely degradation products of oxytetracycline (I) are not toxic, an accurate and convenient analytical method is needed to investigate the purity of commercial I samples. Anhydrooxytetracycline (II), epioxytetracycline (III), α-apooxytetracycline (IV), and β-apooxytetracycline (V) occur as impurities in I, but little information has been published about their quantitative evaluation.

Compound I was determined by UV spectrophotometry (4) and fluorometry (5). Degradation products were separated from I by paper chromatography (6, 7). Some acidic degradation products of I were determined chromatographically using paper impregnated with 10% (w/v) urea in pH 5.0 McIlvaine's buffer (8). After elution, I was quantitated by spectrophotometry.

Compounds II, IV, V, and terrinolide (VI) were separated on a kieselguhr layer (9). Compound I also was separated from its impurities on a diatomaceous earth column, and I and V were evaluated by spectrophotometry (10).

The use of paper chromatography is undesirable (6–8) because the procedure is lengthy and not reproducible. A TLC separation of II, IV, and V from I on kieselguhr (9) could not be achieved in this laboratory.

The TLC procedure presented is rapid and allows the quantitation of traces of II–V in I by direct TLC fluorometry. The method is reproducible and is sensitive to 10^{-2} μg of II–V in a 1% (w/v) solution of I.

EXPERIMENTAL

Reagents—Disodium ethylenediaminetetraacetate (VII), ethylene glycol, acetone, ethyl formate, ethyl acetoacetate, and sodium hydroxide were analytical grade. Kieselguhr G[1] was used.

Preparation of Plates—A slurry of 20 g of kieselguhr and 47 ml of 5% (w/v) aqueous VII, previously adjusted to pH 9 with 20% NaOH, was prepared[2] and spread on glass plates at a thickness of 0.25–0.30 mm. The plates were dried in air for a minimum of 2 hr at room temperature (25°); a transverse channel of approximately 1-mm width was drawn in the layer

[1] Macherey, Nagel & Co, D-516 Düren, Germany.

[2] Desaga applicator, Heidelberg, Germany.