# Tumor Inhibitory Triazenes. 2.[1] Variation of Antitumor Activity within an Homologous Series

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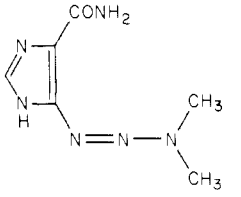
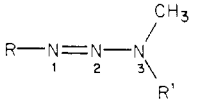
An homologous series of water-soluble, chemically stable analogues of 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (DTIC) has been prepared with activity comparable to DTIC in an experimental tumor system. The antitumor activity of this series of 3-alkyl-1-(4-carboxyphenyl)-3-methyltriazenes rapidly diminishes at alkyl chain lengths greater than pentyl. Partition coefficients were determined, but no relationship between these and antitumor activity could be established.

The continuing clinical interest in 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (DTIC, I),[2] particularly



I

in the treatment of malignant melanoma, despite its severe side effects, points to the need for a second-generation analogue which is better tolerated by the patient. The cause of the nausea and vomiting, leading in some cases to refusal of further treatment, is not certain. However the drug readily undergoes photodecomposition both in the solid state and in solution;[3] the diazonium compound so formed is very much more toxic than DTIC[4] and thus might be the cause of the observed toxicity. Our earlier structure–activity study[1] indicated that the basic structural requirements of the triazenes for antitumor activity are as shown in II. These consist of a carrying structure (R)

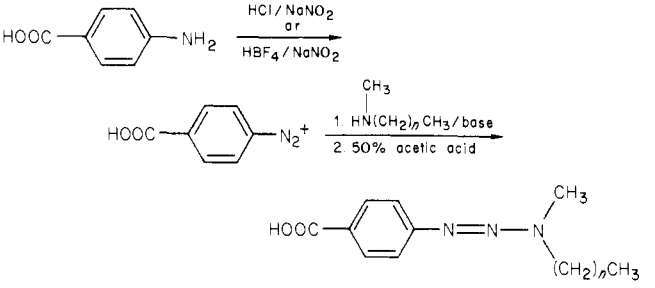


II

at $N^1$ and a methyl group at $N^3$, together with a preferentially metabolized group (R′). In particular, this study showed that an imidazole group was not a prerequisite for antitumor activity and, therefore, the problem of toxicity due to photodecomposition could be readily overcome without concomitant loss of activity. In fact, aryltriazenes proved to be stable at physiological pH and to have antitumor activity similar to DTIC.

The series of compounds described in this paper formed the next stage in our search for a potential clinical successor to DTIC. The 4-carboxyphenyl group was chosen as the carrying structure, so that the final drug should be soluble and stable at physiological pH and might thus be formulated at this pH for injection. Our previous results suggested that if R′ is an alkyl group, then lengthening of the chain increases the antitumor activity.[1] This led to the synthesis of the homologous series III. A number of analogous compounds were prepared with potential biologically dealkylatable groups. The physicochemical properties of these new compounds are shown in Table I; solubility and partition coefficient data were determined where sufficient material was available following antitumor assay.

**Chemistry.** The homologous series of 3-alkyl-1-(4-carboxyphenyl)-3-methyltriazenes (III), together with a number of analogous derivatives, was synthesized by standard methods shown in eq 1, by way of freshly prepared 4-carboxybenzenediazonium chloride or the previously isolated tetrafluoroborate salt.[5]



III

## Results and Discussion

The antitumor activities of the compounds against the TLX/5 lymphoma are shown in Table II. The parent compound in the series, 1-(4-carboxyphenyl)-3,3-dimethyltriazene (**1**), and DTIC were compared using 10% acetone in arachis oil as the vehicle in an attempt to overcome the ready photodecomposition of DTIC in aqueous solution. Compounds **1**–**9** of the carboxyphenyltriazene series were administered in saline at approximately pH 7.5. The activities of DTIC and **1** in either vehicle are comparable. As the length of the alkyl chain is increased some improvement in antitumor activity is apparent. This reaches a maximum with the pentyl derivative **5**, which produces an increase in life span of 108% at 25 mg/kg. However, the number of active dose levels has fallen to three from the more usual four. Analogues of greater chain length show progressively less activity, the hexyl compound

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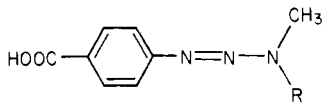
Table I. Physicochemical Properties of Some 3-Alkyl-1-(4-carboxyphenyl)-3-methyltriazenes



| no. | R | mp, °C | solvent[a] | anal.[b] | UV[c] $\lambda_{max}$, nm | UV[c] $\epsilon$ | solubility,[d] mol/L (pH 7.5 buffer) | log $P$[e] |
|---|---|---|---|---|---|---|---|---|
| 1 | methyl[f] | 176 | A | C, H, N | 321 | 20 900 | $1.59 \times 10^{-1}$ | 0.44 ± 0.01 |
| 2 | ethyl | 162 | A | C, H, N | 322 | 20 600 | $3.75 \times 10^{-2}$ | 0.04 ± 0.03 |
| 3 | propyl | 134-135 | B | C, H, N | 323 | 20 400 | $3.68 \times 10^{-2}$ | 0.46 ± 0.01 |
| 4 | butyl | 122-123 | B | C, H, N | 323 | 20 700 | $1.55 \times 10^{-2}$ | 0.97 ± 0.01 |
| 5 | pentyl | 132-134 | B | C, H, N | 323 | 20 500 | $4.28 \times 10^{-3}$ | 1.49 ± 0.01 |
| 6 | hexyl | 101-102 | C | C, H, N | 323 | 20 500 | $2.98 \times 10^{-3}$ | 1.95 ± 0.01 |
| 7 | heptyl | 103-105 | C | C, H, N | 323 | 20 500 | $4.10 \times 10^{-4}$ | 2.58 ± 0.05 |
| 8 | octyl[g] | 91.5-92.5 | C | C, H, N | 323 | 20 500 | $1.71 \times 10^{-4}$ | 2.77 ± 0.02 |
| 9 | dodecyl | 109-110 | D | C, H, N | 323 | 20 600 | $4.13 \times 10^{-6}$ | |
| 10 | octadecyl | 108-111 | C | H, N; C[h] | 323 | 21 000 | undetectable | |
| 11 | isopropyl | 120-122 | C | C, H, N | 323 | 21 100 | | 0.34 ± 0.01 |
| 12 | 2-methylbutyl | 117.5-119.5 | C | C, H, N | 324 | 21 200 | $1.62 \times 10^{-2}$ | 1.33 ± 0.01 |
| 13 | allyl | 128-130 | C | C, H, N | 321 | 20 800 | | 0.32 ± 0.01 |
| 14 | 2-propynyl | 116-118 | B | C, H, N | 313 | 14 300 | | 0.00 ± 0.03 |
| 15 | benzyl[i] | 156 | C | C, H, N | | | | |

[a] Solvents: A = ethyl acetate; B = cyclohexane; C = petroleum ether (bp 60-80 °C); D = benzene. [b] Elements cited were all within ±0.3% of the theoretical value. [c] UV spectra were determined in ethanol solution. [d] Solubilities were determined in Sorenson's phosphate buffer at pH 7.4 and 25 °C. [e] Partition was between octanol-saturated Sorenson's pH 7.4 phosphate buffer and buffer-saturated octanol. [f] Reference 5. [g] Reference 6; mp 91-92 °C. [h] C: calcd, 72.3; found, 72.8. [i] Reference 7; mp 149 °C.

Table II. Antitumor Activity of Some 3-Alkyl-1-(4-carboxyphenyl)-3-methyltriazenes

| compd | R | vehicle | % increase in life span of TLX/5 tumor bearing animals at the following dose level, mg/kg, daily × 5: 12.5 | 25 | 50 | 100 | 200 | 400 | min dose to give 50% ILS, mol/kg |
|---|---|---|---|---|---|---|---|---|---|
| 1 | methyl | saline[a] | 40 | 72 | 83 | 34 | 0 | -34 | $6.2 \times 10^{-5}$ |
| 2 | ethyl | saline | 44 | 79 | 79 | 67 | 27 | -21 | $7.0 \times 10^{-5}$ |
| 3 | propyl | saline | 13 | 69 | 69 | 69 | 57 | -3 | $9.4 \times 10^{-5}$ |
| 4 | butyl | saline | 69 | 82 | 86 | 47 | 20 | -31 | $5.3 \times 10^{-5}$ |
| 5 | pentyl | saline | 10 | 108 | 75 | 61 | -61 | -61 | $7.1 \times 10^{-5}$ |
| 6 | hexyl | saline | 0 | 0 | 18 | 73 | -8 | -53 | $3.0 \times 10^{-5}$ |
| 7 | heptyl | saline | 3 | 1 | 5 | 9 | -19 | -48 | |
| 11 | isopropyl | saline | 37 | 90 | 64 | 60 | 45 | 35 | $7.0 \times 10^{-5}$ |
| 12 | 2-methylbutyl | saline | 7 | 11 | 52 | 62 | 60 | 57 | $2.0 \times 10^{-4}$ |
| 13 | allyl | saline | 1 | 11 | 96 | 76 | 56 | -25 | $1.7 \times 10^{-4}$ |
| 14 | 2-propynyl | saline | 0 | 36 | 76 | 70 | 40 | -18 | $1.5 \times 10^{-4}$ |
| 15 | benzyl | saline | 6 | 6 | 7 | 49 | -59 | -61 | |
| 1 | methyl | 10% acetone/oil | 19 | 51 | 70 | 49 | -4 | -36 | |
| DTIC | | 10% acetone/oil | 55 | 55 | 59 | 55 | 61 | -11 | |

[a] An aqueous solution for injection was produced by dissolving 400 mg of drug in the minimum of 1 N NaOH, adding 7 mL of saline, adjusting the pH to 7.5 with 1 N HCl, and making the volume up to 10 mL with saline. Lower doses were prepared by serial dilution.

**6** being active at only 100 mg/kg and the heptyl derivative **7** is devoid of activity. The octyl and dodecyl compounds **8** and **9** were also inactive, and the octadecyl derivative **10** was not tested. The branched and unsaturated chain analogues **11**–**14** show average activity, while **15** is surprisingly inactive.

A number of suggested explanations for this sudden loss of activity may be made: change in transport characteristics and tissue distribution as lipophilicity increases with chain length; alteration in extent or type of metabolism; change in stability of metabolic intermediates. As a preliminary step toward establishing an explanation for this unexpected change in antitumor activity, the in vivo antitumor results were compared with the solubility and partition coefficient data obtained for the homologous series **1**–**10** (Table I). The solubility data produce a straight-line semilog plot, of molar solubility against alkyl chain length, of slope $-5.8 \times 10^{-2}$. A similar plot for the partition data has a slope of 2.5.

Neither of these sets of data show any sudden changes similar to that observed in the antitumor results. The minimum dose producing a 50% increase in life span was calculated for each drug, the results being shown in Table II. The marked differences between the active and inactive antitumor agents are in no way paralleled by the physicochemical data. The solubilities and partition coefficients have an even spread as would be expected from such an homologous series, and therefore no direct correlation can be expected between these parameters and the observed biological activity. However, the unsaturated and branched chain derivatives require treatment at higher

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doses to produce a 50% increase in life span.

As a first step toward determining the possible metabolic fate of these compounds in vivo, the extent of demethylation of compound 1 by liver microsomes from phenobarbitone-pretreated CBA/LAC mice was investigated by the method of Cochin and Axelrod.[8] As reported previously, no detectable formaldehyde was formed under these conditions.[9] The reason for this apparent lack of metabolism in vitro is currently the subject of further investigation.

## Experimental Section

Melting points were determined on a Reichert, Kofler micro hot stage apparatus and are uncorrected. Ultraviolet spectra were recorded in ethanol solution on a Pye SP800 spectrometer. Elemental analyses were obtained from Butterworth Laboratories Ltd., Teddington, England, or from Dr. F. B. Strauss, Oxford, England.

**Triazene Synthesis.** All the amines used were obtained commercially and used without further purification, with the exception of *N*-methylpentylamine which was prepared by the method of Lucier and co-workers.[10] The physicochemical properties of the new compounds are described in Table I. The ultraviolet absorbance in the region of 323 nm is characteristic of the triazene structure. The preparation of compound 5 is typical.

**1-(4-Carboxyphenyl)-3-methyl-3-pentyltriazene (5).** A solution of 5.5 g (0.04 mol) of 4-aminobenzoic acid in 100 mL (0.1 mol) of hydrochloric acid was diazotized at 0 °C by the addition of 2.76 g (0.04 mol) of sodium nitrite in 10 mL of water. After 0.5 h, excess nitrite was decomposed with sulfamic acid, and a mixture of 5.47 g (0.04 mol) of *N*-methylpentylamine hydrochloride and 6.0 g (0.15 mol) of sodium hydroxide in 25 mL of water was added. When the reaction was complete (no coloration of alkaline α-naphthol solution), 50% aqueous acetic acid was added to reduce the pH to 4–5. The mixture was extracted with ethyl acetate, the organic phase was dried over $Na_2SO_4$, and the solvent was removed under reduced pressure. Crystallization of the residue from petroleum ether (bp 60–80 °C), followed by recrystallization from cyclohexane, yielded 6.3 g (63%), mp 132–134 °C. Anal. ($C_{13}H_{19}N_3O_2$) C, H, N.

**Solubility Determination.** Solubilities were determined by shaking an excess of the compound in 0.1 M Sorenson's pH 7.5 phosphate buffer in a water bath at 25 °C for 1 h. The mixture was rapidly filtered, and an aliquot was diluted to a known volume with ethanol. The resulting solution was passed through a Millex 25 (Millipore) disposable filter and the absorbance measured at the $\lambda_{max}$. The solubility was then calculated from the standard spectral data.

**Partition Coefficient Determination.** The compound was shaken at room temperature for 1 h in 0.1 M Sorenson's pH 7.5 phosphate buffer previously saturated with octanol. A known volume of this filtered solution was shaken with a known volume of buffer saturated octanol for 1 h at room temperature. Aliquots of the aqueous phase and the initial filtrate were independently treated in the same manner as the filtrate in the solubility determination. The partition coefficient, *P*, was calculated from the expression:

$$P = \text{vol of aqueous phase} \times (\text{initial absorbance} - \text{final absorbance}) / \text{vol of octanol phase} \times \text{final absorbance of aqueous phase}$$

The results in Table I are the mean of at least two determinations.

**Antitumor Activity.** The TLX/5 lymphoma was transplanted subcutaneously in female CBA/LAC mice as previously described.[9] The triazenes were administered intraperitoneally in saline (Table II, footnote *a*) for 5 consecutive days, commencing 3 days after tumor transplantation. Groups of five animals were used for each dose level, and their survival time was compared with ten untreated control mice. An increase in life span of 20% or greater is statistically significant.

**Acknowledgment.** This work was supported by grants to the Institute of Cancer Research: Royal Cancer Hospital, from the Cancer Research Campaign and the Medical Research Council. We are grateful to Professor W. C. J. Ross and Dr. K. R. Harrap for their interest.

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# Antileukemic Compounds Derived from the Chemical Modification of Macrocyclic Trichothecenes. 1. Derivatives of Verrucarin A

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Verrucarin A (2) was epoxidized to give the β-9,10-epoxide 7 (major product) and α-9,10-epoxide 9 (minor product). The β-epoxide 7 and its acetate 8 exhibit high in vivo antileukemic activity against P-388 mouse leukemia, whereas 2 and 9 are inactive. Epoxidation of verrucarin B (3) and roridin A (1) to their respective β-9,10-epoxides (11 and 12, respectively) also yields compounds with substantially increased activity. Allylic alcohols derived from 2, α-C8 (20), β-C8 (14), and C16 (15), were synthesized and tested; only 15 exhibited substantial in vivo activity.

The search for biologically active compounds from the extracts of higher plants, microbial fermentations, and marine animals has yielded a vast array of new and interesting natural products. Through a random search of a large number of these extracts, a significant number of materials have been isolated and identified which show potential for use as antineoplastic agents.[1] Some of these have served as useful prototypes for further chemical modification which potentially could lead to more effective drugs.[1]

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