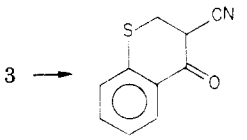
melting point of 230–231 °C does not correspond to a product we could obtain from our conditions or from using hydrazine with the *O*-methyl ether of **2** as was cited in the work of Fravolini. Since we were able to obtain N and S analysis as well as IR, MS, and ^{1}H NMR analysis of **3**, we believe our structure is on firm ground. Moreover, since we are able to ring open **3** with $NaOCH_3$ to give the known ketonitrile (reported in ref 7), the validity of our structure



3 is secure. We must tentatively conclude that the compound of Fravolini must be of a different structure since our MS analysis gave a molecular weight of 189 for **3** (calcd mol wt is 189) obtained in our procedure.

(9) Again pyrazoles **4** and **5** are reported by Fravolini (ref 8) but the melting point values were 157 and 209–210 °C, respectively. Our values of 168.5–170 and 169–171 °C were obtained from highly purified samples, the IR, MS, and ^{1}H NMR analyses of which are in total support of our structures. The MS analysis gave molecular weights of 188 and 264, respectively. Since the melting point values of our **4** and **5** were very close, a mixture melting point determination was taken in an effort to determine if a common product had resulted (although spectral data refuted this). The melting range obtained was 120–140 °C confirming the structures to be different. Possibly, Fravolini has obtained dimers but we have new evidence for this under our conditions. Reference to **4** has been made also by Pagani [G. Pagani and S. Maiorana, *Chim. Ind. (Milan)*, **53**, 469 (1971); *Chem. Abstr.*, **75**, 48829c (1971)] but no properties were reported in the abstract.

(10) Sulfone **6** has been reported [(a) T. Nambara, *Yakugaku Zasshi*, **78**, 624 (1958); (b) I. W. J. Still and M. T. Thomas, *J. Org. Chem.*, **33**, 2730 (1968); (c) A. G. Harrison, M. T. Thomas, and I. W. J. Still, *Org. Mass Spectrom.*, **3**, 899 (1970)] with slight variations in melting point. Our value was 131–133 °C and a mass spectral analysis gave a mol wt of 196.

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Preparation and Antitumor Activity of 1-Aryl-3,3-dimethyltriazene Derivatives

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Several 1-aryl-3,3-dimethyltriazene derivatives have been synthesized and tested for their antitumor activity against the TLX5 lymphoma in mice. These compounds are characterized by the presence of a carbonyl group bound to the benzene nucleus in the para position to the triazene function. Three *p*-sulfamoyl derivatives have also been included and proved to be inactive. Among the carbonyl derivatives compounds **1** and **20**, which can be used as reference, cause ILS of about 50%, respectively, at four and three dose levels. Compound **16**, the *o*-nitrophenylhydrazone of the hydrazide **1**, is active at all six dose levels studied. The adduct **19**, obtained from the same hydrazide and *p*-nitrobenzaldehyde, is active at four dose levels, and the ILS values at two optimum doses are significantly greater than those caused by compound **1**.

An imidazole triazene, DIC or 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (NSC-45388), has good activity against malignant melanoma.[1] The antitumor activity is not peculiar to imidazole derivatives, since aryldimethyltriazenes have also been shown to inhibit the growth of rodent tumors. Since the earlier studies of Clarke et al.[2] and Burchenal et al.,[3] a larger series of aryltriazene derivatives has been recently synthesized and tested for antineoplastic activity. The examination of the structure–activity relationships reveals for these compounds that a carbonyl group bound to the aromatic nucleus in the para position to the triazene substituent is present in those compounds which are most active against the TLX5 lymphoma[4,5] and the L1210 leukemia.[6,7] Therefore a series of substituted hydrazides and other carbonyl derivatives, and a few related compounds, carrying a triazene functional group, have now been synthesized and tested for anticancer activity.

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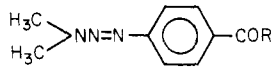
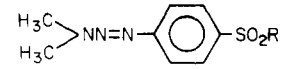
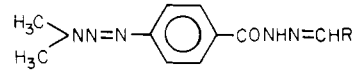
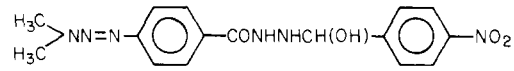
Experimental Section

Melting points were determined in open glass capillaries using a Büchi apparatus and are uncorrected. IR spectra (as Nujol mulls) were recorded on a Perkin-Elmer Model 225 spectro-

Table I

Compd no.	R	Formula	Recrystn solvent	Yield, %	Mp, °C
					
1[a]	$NHNH_2$	$C_9H_{13}N_5O$	Ethanol	64	155 dec
2	$NHN(C_3H_7)_2$	$C_{15}H_{25}N_5O$	Acetone	58	150 dec
3	$NHNHCONHC_3H_7$	$C_{13}H_{20}N_6O_2$	Ethanol	38	167 dec
4	$NHNHCSNHC_2H_5$	$C_{12}H_{18}N_6OS$	Ethanol	64	196 dec
5	N_3	$C_9H_{10}N_6O$	Ether	50	110 dec
6		$C_{11}H_{13}N_7O$	Ethanol	36	219 dec
					
7		$C_{10}H_{13}N_7O_2S$	Ethanol	20	184
8[b]	$N(CH_3)_2$	$C_{10}H_{16}N_4O_2S$			123
9[c]	$N(CH_2CH_2Cl)_2$	$C_{12}H_{18}Cl_2N_4O_2S$			104-106
					
10	2,4-$(CH_3O)_2$-C_6H_3	$C_{18}H_{21}N_5O_3$	Methanol	42	185 dec
11	4-C_2H_5O-C_6H_4	$C_{18}H_{21}N_5O_2$	Methanol	50	198 dec
12	C_6H_5	$C_{16}H_{17}N_5O$	Methanol	53	211 dec
13	4-Cl-C_6H_4	$C_{16}H_{16}ClN_5O$	Methanol	36	216 dec
14	4-Br-C_6H_4	$C_{16}H_{16}BrN_5O$	Methanol	53	217 dec
15	4-I-C_6H_4	$C_{16}H_{16}IN_5O$	Methanol	41	219 dec
16	2-NO_2-C_6H_4	$C_{16}H_{16}N_6O_3$	Ethanol	35	217 dec
17	4-NO_2-C_6H_4	$C_{16}H_{16}N_6O_3$		100	264 dec
18	OC_2H_5	$C_{12}H_{17}N_5O_2$	Ethyl acetate	46	139 dec
					
19		$C_{16}H_{18}N_6O_4$	Washed with cold ethanol	72	256-257 dec

[a] Y. F. Shealy et al.[7] reported mp 136-137 °C; NMR (Me_2SO-d_6-Me_4Si) 3.35 (br s, 6 H), 4.65 (s, 2 H), 8.07-7.42 (m, 4 H), 9.90 (s, 1 H); IR max ν (Nujol) 3290 cm^{-1} (NH). Anal. ($C_9H_{13}N_5O$) C, H, N. [b] L. Lalezari and F. Afghahi[11] reported mp 118-120 °C. Anal. ($C_{10}H_{16}N_4O_2S$) C, H, N. [c] Lit.[11] mp 100-102 °C. Anal. ($C_{12}H_{18}Cl_2N_4O_2S$) C, H, N.

photometer. UV spectra were determined on a Hitachi Perkin-Elmer Model 124 and NMR spectra on a JEOL C60 HL spectrophotometer. Kieselgel HF 254 + 366 (Type 60, Merck) and methanol–ethyl acetate–ligroine (3:2:1) were used for TLC.

The compounds synthesized are reported in Table I. The hydrazones **10**–**16** and compound **19** have been prepared by addition of a solution of the aldehyde in hot ethanol to an equimolar solution of hydrazide **1** in the same solvent and warming for a few minutes. As it is seen from Table I, *p*-nitrobenzaldehyde does not give the corresponding hydrazone, like the other aromatic aldehydes, but a different product is formed to which structure **19** has been assigned in accordance with the elemental analysis and on the basis of the following data. First, compound **19** heated for 2 h at 120 °C loses a molecule of water originating compound **17**, whose elemental analysis is in agreement with a hydrazone structure. Second, TLC mobilities and UV spectra of compounds **17** and **19** are quite different, and therefore compound **19** cannot be a monohydrate of hydrazone **17**. Furthermore, compound **19** exhibits a strong characteristic band at 1040 cm^{-1} which disappears after heating; this band is attributable to C–O stretching. Since both compound **17** and **19** are very poorly soluble in the common solvents, no further evidence for the assigned structures could be obtained by means of NMR studies. It should also be noted that the UV spectrum in ethanol of compound **19** corresponds to the sum of the spectra of the starting hydrazide **1** and *p*-nitrobenzaldehyde. Additionally, TLC on silica gel shows that this substance at the very low attainable concentrations is split, giving two spots corresponding to the starting reagents. The hydrazone **17** has been obtained only by heating compound **19** for 2 h at 120 °C. The hydrazone **18** has been obtained by addition of a solution of hydrazide **1** in hot ethanol to an excess of ethyl orthoformate (three times in volume), heating at 60 °C for 30 min, and allowing the product to separate by standing. The substituted hydrazide **2** has been prepared according to a general procedure,[8] precipitating the product by addition of water to the reaction mixture, after standing for about 15 h.

Compounds **3** and **4** have been prepared by addition of equivalent amounts respectively of *n*-propyl isocyanate or ethyl isothiocyanate to solutions of hydrazide **1** in warm ethanol, stirring the reaction mixtures for 1 h at room temperature.

The azide **5** has been prepared according to the usual procedure,[9] by slow addition under stirring of a solution of sodium nitrite to a cooled suspension of hydrazide **1** in 2 N HCl and extracting the product with ether. Compound **6** has been prepared by diazotizing and coupling the amine **6b**, obtained by reduction of the corresponding nitro derivative **6a**. Compound **7** has been prepared in the same way starting from 4-(*p*-aminobenzenesulfonamido)-4*H*-1,2,4-triazole.[10]

4-(*p*-Nitrobenzoyl)amino-4*H*-1,2,4-triazole (6a). *p*-Nitrobenzoyl chloride (5.55 g, 30 mmol) was slowly added to a suspension of 4-amino-4*H*-1,2,4-triazole (2.52 g, 30 mmol) in 5 mL of pyridine cooled in an ice bath. The resulting solid mixture was then stirred with 20 mL of water, filtered, and washed with water. The product was recrystallized from ethanol and dried at 120 °C to yield 2 g (28%) of light-yellow crystals: mp 238–241 °C. In order to obtain the analytical sample the raw material (1 g) was suspended in 5 mL of water and Na_2CO_3 (about 0.5 g) was added in small portions under stirring to dissolve the product. The solution was filtered and the product precipitated by dropwise addition of 2 N HCl was recrystallized from ethanol and dried

at 120 °C. Anal. ($C_9H_7N_5O_3$) C, H, N.

4-(*p*-Aminobenzoyl)amino-4*H*-1,2,4-triazole (6b). A mixture of 2 g (8.6 mmol) of **6a** and 0.2 g of 10% Pd/C catalyst in 200 mL of ethanol was hydrogenated at 50 psi at room temperature for 1 h. The mixture was filtered and concentrated to a small volume under reduced pressure and the precipitated product was recrystallized from ethanol to yield 0.5 g (28%) of white crystals: mp 281 °C dec. The analytical sample was obtained as above for compound **6a**: white crystals from ethanol; UV (EtOH) 298 nm; IR 3440 and 3360 cm^{-1} (NH_2). Anal. ($C_9H_9N_5O$) C, H, N.

4-[*p*-(3,3-Dimethyl-1-triazeno)benzoyl]amino-4*H*-1,2,4-triazole (6). To a suspension of the amine **6b** (2.03 g, 10 mmol) in 2.5 mL of concentrated HCl, while cooling in an ice bath, a solution of sodium nitrite (0.7 g, 10 mmol) in water (3 mL) was slowly added. At the end of the diazotization the reaction mixture was dropwise added to 5 mL of a 40% aqueous solution of dimethylamine. The pH was adjusted at 7.5–8 with diluted HCl and the precipitated triazene was recrystallized from ethanol.

4-[*p*-(3,3-Dimethyl-1-triazeno)benzenesulfonamido]-4*H*-1,2,4-triazole (7). To a suspension of 4-(*p*-aminobenzenesulfonamido)-4*H*-1,2,4-triazole[10] (1.2 g, 5 mmol) in 8 mL of concentrated HCl, while cooling in an ice bath, a solution of sodium nitrite (0.43 g, 5 mmol) was slowly added. At the end of the diazotization the solution was slowly added to 14 mL of a 40% aqueous solution of dimethylamine. The pH was lowered to about 4 with diluted HCl and the precipitated triazene was recrystallized from ethanol.

Discussion

The data reported in Table II show the effects of the various triazenes against the TLX5 lymphoma in mice. The comparison of the activity among the tested compounds has been performed on the basis of the number of dose levels at which an ILS of about 50% is observed and considering also the maximum ILS values obtained. Compound **20**, which has been included as reference because of its activity against L1210 leukemia[6] and TLX5 lymphoma,[5] is effective at three dose levels. Compound **1**, in addition to its reported activity against the L1210 leukemia,[7] is active also against TLX5, at four dose levels. The alkylation of its hydrazide function causes a loss of activity as seen with compound **2**. The semicarbazone **3** is effective at three dose levels, whereas the analogue thiosemicarbazone **4** causes significant effects only at one dose level. The azide **5**, possessing a *p*-carbonyl group and a cytotoxic function, has only moderate activity, causing a significant ILS only at two dose levels.

The three sulfonyl derivatives, **7–9**, are inactive, in spite of the presence in compound **9** of a nitrogen mustard group. The observed inactivity against TLX5 for compounds **8** and **9** is in accord with their reported inactivity against the L1210 leukemia.[11] At least for compound **7** the lack of activity might be related to the presence of the sulfonyl group, since the corresponding carbonyl derivative **6** is active.

A series of arylhydrazones of compound **1** has been prepared (**10–17**) and the influence of substituents producing different electronic effects has been examined. For all these compounds no correlation can be found between the nature of substituents and the observed activity.

Of particular interest is compound **16**, active at all the six dose levels employed, whereas the corresponding para isomer **17** is inactive. Furthermore, the adduct **19**, structurally related to the inactive compound **17**, shows marked activity at three dose levels, in duplicate experiments. The analysis of variance indicates that the ILS values at 200 and 400 mg/kg/day, when compared with those obtained with compound **1**, are significantly greater ($p < 0.0001$).[12] Further investigation, concerning the evaluation of activity in other experimental tumor systems and the mechanism of action, is in progress for the hydrazone **16**, the adduct **19**, and analogous derivatives.

Table II. Antitumor Activity of Compounds Reported in Table I against TLX5 Lymphoma in Mice[a]

Compd no.	Dose, mg/kg/day	% ILS over controls	Compd no.	Dose, mg/kg/day	% ILS over controls
1	10	59	11	12.5	0.0
	20	55		25	13
	40	57		50	29
	80	44		100	50
	160	−46		200	65
2	2.5	−3.7		400	63
	5	−3.7	12	12.5	26
	10	0.0		25	24
	20	20		50	49
	40	−32		100	36
3	12.5	−1.0		200	1.8
	25	10		400	−26
	50	61	13	12.5	4.6
	100	44		25	14
	200	49		50	40
	400	−44		100	44
4	12.5	−1.0		200	51
	25	16		400	−23
	50	18	14	12.5	7.6
	100	55		25	21
	200	−31		50	52
5	6.25	−1.0		100	52
	12.5	4.6		200	60
	25	21		400	−35
	50	46	15	12.5	11
	100	51		25	15
	200	−72		50	40
6	12.5	−1.0		100	54
	25	32		200	−38
	50	47	16	12.5	44
	100	49		25	40
	200	28		50	49
	400	−14		100	51
7	12.5	0.9		200	57
	25	4.6		400	59
	50	0.9	17	12.5	−1.0
	100	4.6		25	−1.0
	200	16		50	8.7
	400	14		100	16
8	12.5	0.0		200	26
	25	7.5		400	20
	50	9.4	18	12.5	13
	100	34		25	30
	200	−36		50	43
9	3.12	−3.7		100	−27
	6.25	−3.7	19	25	9.6, 6.5
	12.5	−1.0		50	35, 12
	25	0.0		100	90, 74
	50	2.9		200	102, 83
	100	−3.7		400	71, 79
10	12.5	1.9		800	60, 44
	25	19	20[b]	12.5	14
	50	48		25	55
	100	52		50	51
	200	35		100	47
	400	−33		200	3.9
				400	−61

[a] 10^5 TLX5 cells were injected subcutaneously in the inguinal region of CBA/LAC female mice. The drugs were used as a solution freshly prepared in acetone–arachis oil 10/90 (v:v); the treatment was performed as a daily intraperitoneal administration from day 3 to 7 from tumor inoculation. For each dose level, groups of five animals and ten controls were used. [b] Values obtained using (3,3-dimethyl-1-triazeno)-*p*-benzamide.[5]

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Hospital) from the Medical Research Council (Grant No. 973/787/K).

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Antiinflammatory Activity of 17-Esters of 6α,9α-Difluoro-21-deoxyprednisolone

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Several 17-monoesters of 6α,9α-difluoro-21-deoxyprednisolone were prepared and tested for their antiinflammatory activity. Propionate **11** and butyrate **12** displayed a high topical activity.

The availability of corticosteroid 17-monoesters, via 17,21-orthoesters,[2] allowed us to develop a general route to their 21-deoxy analogues by reductive elimination of the 21-hydroxyl group.[3] In spite of the lack of a function considered essential for the corticoid activity, some 21-deoxycorticosteroids have been reported to display topical antiinflammatory activity,[4] which is markedly increased by the presence of protective groups at C-16 and C-17 like acetonides[5] and esters at C-17.[6]

In a previous paper we described the high antiinflammatory activity of 17,21-alkyl orthoesters, 17-monoesters, and 17,21-diesters of 6α,9α-difluoroprednisolone.[7] Here we wish to report the synthesis and some biological properties of 17-esters of 6α,9α-difluoro-21-deoxyprednisolone.

The compounds were obtained from 6α,9α-difluoroprednisolone 17-monoesters according to the already published procedure[3] involving the preparation of the 21-tosylates and the subsequent reduction in situ through the corresponding 21-iodo derivatives.

Yields, melting points, specific optical rotations, and analytical data of the compounds are given in Table I.

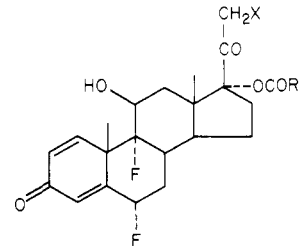
Biology and Evaluation. The 17-esters of 6α,9α-difluoro-21-deoxyprednisolone **10–13** have been assayed for their antiexudative activity by the granuloma pouch test according to Selye.[8] The compound was injected into the pouch of rats on day 5 or injected subcutaneously daily from day 2 to day 10. Autopsy was performed on day 11.

The compounds have been assayed also in the vasoconstriction test on volunteers according to the modification described by Falconi and Rossi.[9] In all cases reference compounds have also been tested. The results are shown in Tables II and III.

With the exception of acetate **10**, the 21-deoxy-17-esters displayed a high local antiexudative activity, greater than that of free deoxydifluoroprednisolone **14**[10] and of the corresponding 21-hydroxy esters investigated, propionate **2** and benzoate **4**. Evaluation of **13** vs. **4** in the same test after daily subcutaneous treatment revealed that the 21-deoxy derivative displayed a lower systemic antiexudative activity.

In the vasoconstriction test, compounds **10–12** proved

Table I



No.	R	X	Yield,[a] %	Mp, °C	$[\alpha]_D$, deg	Formula	Analyses
4	C_6H_5	OH	70	228–231	+14.2	$C_{28}H_{30}F_2O_6$	C, H
7	C_2H_5	OTs	89	205–207	+14	$C_{31}H_{36}F_2O_8S$	C, H, S
8	C_3H_7	OTs	98	125[b]	−12.2	$C_{32}H_{38}F_2O_8S$	C, H, S
9	C_6H_5	OTs	85	204–206	−21	$C_{35}H_{36}F_2O_8S$	C, H, S
10	CH_3	H	45[c]	258–260	+24	$C_{23}H_{28}F_2O_5$	C, H
11	C_2H_5	H	72	235–237	+20.5	$C_{24}H_{30}F_2O_5$	C, H
12	C_3H_7	H	68	219–221	−19.7	$C_{25}H_{32}F_2O_5$	H[d]
13	C_6H_5	H	54	282–284	−6.6	$C_{28}H_{30}F_2O_5$	C, H

[a] Yield is of analytically pure material. [b] With decomposition. [c] Overall yield. Intermediate 21-tosylate **6** was not isolated. [d] C: calcd, 66.65; found, 66.20.