Synthesis and Biological Activity of Some Derivatives of Thiochroman-4-one and Tetrahydrothiapyran-4-one

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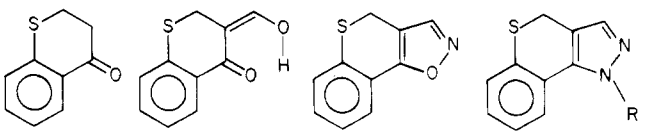
A small series of pyrazoles and isoxazoles derived from thiochroman-4-one has been synthesized and characterized. The compounds were examined for their in vitro inhibitory activity against *Bacillus subtilis* and *Pseudomonas fluorescens*. Among the tested compounds the pyrazole derivative from thiochroman-4-one was found to be the most effective inhibitor of growth of *B. subtilis*. Extensive ^{1}H NMR analysis was recorded for all compounds.

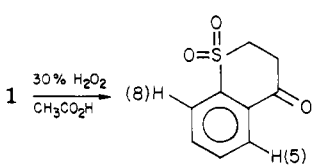
The chemistry and biological activity of pyrazole, isoxazole, and certain imine-ring functionalities in various polycyclic systems have been a major effort from this laboratory recently[1] and have resulted in the detection of a variety of bacteriostatic, bacteriocidal, and drug potentiation effects for certain derivatives.[1b,c,e,f,2] The area of hetero steroids and certain related model heterocyclic compounds is a very active one of interest to medicinal chemists.[3] This report concerns the preparation and antimicrobial activity of certain pyrazole and isoxazole derivatives of thiochroman-4-one and tetrahydrothiapyran-4-one. In view of the controversy over the structures of many pyrazoles both in solution and in the solid state,[1d,4-6] it was deemed necessary to examine the crystal structure of **8a** and this will be reported elsewhere.

Chemistry. Treatment of thiochroman-4-one (**1**) with ethyl formate and sodium methoxide gave hydroxymethylene ketone **2** in the usual manner.[7] Condensation of **2** with hydroxylamine hydrochloride or hydrazine (or substituted hydrazine) gave the isoxazole **3**[8] and pyrazole **4** (or **5**),[9] respectively. Formation of the sulfone derivatives



1 **2** **3** **4**, R = H; **5**, R = C_6H_5

6,[10] **7**, **8** (or **8a**), and **9**[11] was achieved by the reaction at room temperature of sulfides **1**, **3**, **4**, and **5**, respectively, with 30% hydrogen peroxide in glacial acetic acid. In the case of the phenyl derivative **5**, oxidation produced the trioxide (sulfone *N*-oxide) **9**.



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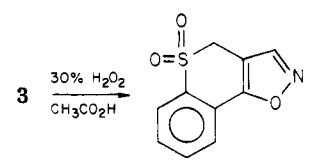

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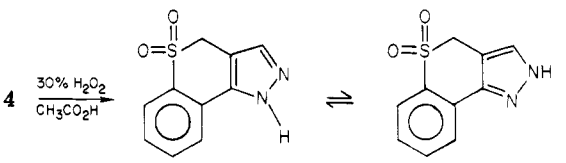
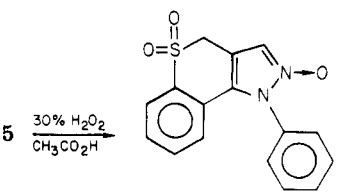


8 **8a**



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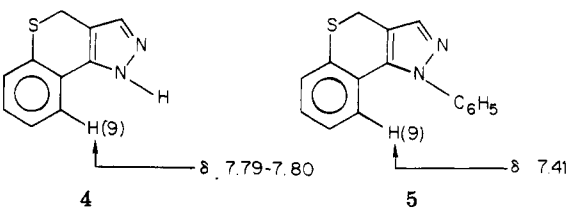
In pyrazole formation, the intermediate is presumably a hydrazone which might lead to the formation of N(1)- or N(2)-substituted pyrazole, perhaps depending upon the difference in nucleophilic character of the two nitrogen atoms in the substituted phenylhydrazine.[12,13] That the phenyl group is at N(1)in **5** is further supported by upfield shifts of the peri hydrogen [marked H(9)] (compared to the same proton in **4**), probably due to shielding by the suspected twisted phenyl ring on nitrogen. Fortunately, it was possible to obtain good crystals of one pyrazole in

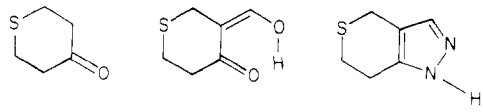


4 **5**

this study. A single crystal analysis will be reported later but it is sufficient to say that **8a** is the tautomer which exists in the solid state.

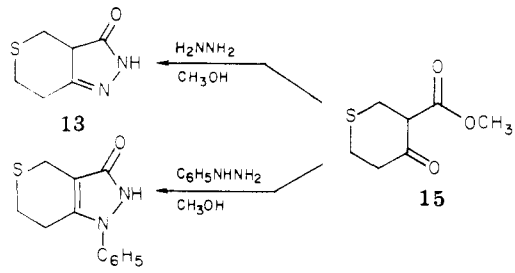
In view of the previous problems with structural assignments with pyrazoles, in solution and the compounds related to this work,[8,9] a careful ^{1}H NMR analysis was performed. The assignment for all protons can be found in the Experimental Section and is in accord with all proposed structures.[14]

Related simple systems were examined also. Tetrahydrothiapyran-4-one (**10**) was converted, in a manner similar to that described previously, to **11**, which was then



10 11 12

converted to pyrazole **12**. Related pyrazolones **13** and **14** were prepared in standard fashion from 3-carbomethoxytetrahydro-1,4-thiapyrone (**15**).[15]



13 15 14

Biological Results. Our interests for these heterocycles were focused on screening in vitro for growth inhibition against gram-positive and gram-negative bacteria. The primary screening was performed to study growth alteration of *Bacillus subtilis* at 91 μg/mL of test compound. In addition, screening was carried out with *P. fluorescens* and KB cells before an evaluation in mice was attempted. The data tabulated in Table I indicate that only is oxazole **3** and pyrazole **4** effected any significant inhibition of growth in *B. subtilis* and KB cells. Unfortunately, pyrazole **5** was essentially insoluble in aqueous medium and no influence on growth was determined. Interestingly, keto sulfone **6** displayed slight activity in *B. subtilis* and KB cells. However, the conversions of **4** → **8** (or **8a**) and **5** → **9** did *not* produce an active compound.

Pyrazole **12** was surprisingly inactive as were pyrazolones **13** and **14**. In view of the activity of **4** and related pyrazoles[1,2] the fused aromatic ring is apparently a necessary requirement.

Because of the potentiation of activity observed with selected antibiotics by several pyrazole systems from our Laboratory,[16] an investigation was made of the ability of pyrazole **4** to potentiate the action of mitomycin C and actinomycin D. The following protocol was used. Ten mice per group were used, and the mice were BDF_1 males (Sprague–Dawley, Madison, Wis.), 45 days old. On day 1, each mouse in each group received 10^5 L1210 cells. On days 2–6, each animal received ip injections (made up in distilled H_2O).

No potentiation affect of **4** on the action of actinomycin D was observed. A small affect on the action of mitomycin D of about 10% over the T/C of the animals treated with only mitomycin C was repeatable but was considered of only marginal value.

Experimental Section

General. Melting points were determined with a Thomas-Hoover capillary melting point apparatus and are uncorrected. Proton magnetic resonance spectra were obtained on a Varian XL-100(15) high-resolution NMR spectrometer (with a time averaging computer accessory, C-1024) operating at 100.0 MHz with tetramethylsilane used as the internal reference. IR spectra were taken on a Beckman-5A spectrophotometer with samples in potassium bromide pellets or films on sodium chloride plates. Mass spectral analyses were performed on a CEC Model 21 HR unit. Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn. Where analyses are indicated by symbols of elements, results are within ±0.4% or the theoretical values. Commercially available reagents were used without further purification unless otherwise specified. The biological assay techniques are known.[1e,f,2a,16,17]

Table I. Effect of Compounds on the Growth of Microorganisms and KB Cells (in H_2O)

Compd	Concn, μg/mL	% inhibition *B. subtilis* (h)	% inhibition *P. fluorescens* (h)	% inhibition KB cells (h)
3	91	100 (24)	0 (24)	
	50			100 (24)
4	75	100 (24)	0 (24)	
	12.5			28 (24)
6	91	100 (12)	0 (24)	
	50			17 (24)
7	91	0 (24)		
8 (or 8a)	91	0 (24)		
9	91	0 (24)	0 (24)	
12	91	0 (24)	0 (24)	
13	91	0 (24)	0 (24)	
14	91	0 (24)	0 (24)	

2-(Hydroxymethylene)thiochroman-4-one (2). Commercial sodium methoxide [2.7 g (0.05 mol); Fisher Scientific Co., "Purified" grade] was suspended in 20 mL of anhydrous reagent-grade benzene in a dry system under N_2. Ethyl formate [3.7 g (0.05 mol); Matheson Coleman and Bell] was then added and the mixture was cooled to about 10 °C (ice bath) with magnetic stirring. Thiochroman-4-one (**1**) [Aldrich Chemical Co., 4.1 g (0.025 mol), in 25 mL of anhydrous benzene] was added dropwise to the reaction mixture, keeping the temperature between 10 and 15 °C. After the addition was completed, the reaction mixture was allowed to warm to room temperature at which time it turned to a reddish semisolid and stirring ceased. The mixture was kept overnight. Hydrolysis of the reaction mixture was effected with 100 mL of ice-cooled distilled water; the resulting organic layer was washed successively with distilled water and the aqueous 10% NaOH. This combined aqueous extracts were washed (ether 3 × 25 mL) and then acidified with dilute hydrocholoric acid (pH 6). A brown-colored liquid formed was extracted with ether (5 × 25 mL), washed (saturated NaCl, 25 mL), and then dried ($MgSO_4$). Evaporation of the ether gave 4.2 g (87.5%) of **2** as a waxy red oil (see ref 7) which was used in the following procedures without further purification.

4-*H*-[1]Benzothiopyrano[3,4-*d*]isoxazole (3). 2-(Hydroxymethylene)thiochroman-4-one (**2**) (0.18 g, 0.0094 mol) was dissolved in 30 mL of glacial acetic acid in standard apparatus. Hydroxylamine hydrochloride (1.0 g, 0.014 mol) in 5 mL of distilled water was then added dropwise at room temperature with constant stirring (magnetic stirrer). The reaction mixture was heated to a boil (0.5 h) and then cooled to room temperature. After stirring overnight, the mixture was triturated with cold water (75 mL). A crystalline solid separated and was filtered out by suction. Recrystallization (ethanol) gave 1.5 g (84.3%) of **3**: mp 71–73 °C (see also ref 8); NMR ($DCCl_3$) δ 4.00 (s, 2 H, CH_2), 7.10–7.80 (m, 4 H, ArH), 8.12 (s, 1 H, N=CH). Anal. ($C_{10}H_7NOS$) C, H, N, S; mol wt 189 (MS).

1,4-Dihydro[1]benzothiopyrano[4,3-*c*]pyrazole (4). 2-(Hydroxymethylene)thiochroman-4-one (**2**) (2.5 g, 0.013 mol) was dissolved in anhydrous methanol (40 mL) in standard apparatus with an N_2 inlet. Hydrazine (3 mL, 97%) in 10 mL of anhydrous methanol was added dropwise. An exothermic reaction ensued with darkening of the already reddish-brown methanol solution. The mixture was then heated to boil (15 min) and stirred at room temperature (4 h). Water (75 mL) was added to the reaction mixture which was heated to a boil with stirring (0.5 h). The reaction mixture was then cooled in ice water. Yellow crystals formed and were filtered and washed several times (water). Recrystallization (ethanol) yielded 2.3 g (94%) of pure **4**: mp 168.5–170 °C (see also ref 9); NMR (acetone-d_6) δ 2.70 (br s, 1 H, NH), 3.99 (s, 2 H, CH_2), 7.04–7.80 (m, 5 H, ArH, N=CH). Anal. ($C_{10}H_8N_2S$) C, H, N, S; mol wt 188 (MS).

1,4-Dihydro-1-phenyl[1]benzothiopyrano[4,3-*c*]pyrazole (5). The hydroxymethylene compound **2** (1.8 g, 0.0094 mol) was dissolved in 30 mL of glacial acetic acid in the common apparatus.

Phenylhydrazine (1.2 g, 0.01 mol) was then added to the solution at room temperature, and the reaction mixture was heated to a boil and then allowed to cool at room temperature. The crystalline solid separated was filtered and recrystallized (dilute acetic acid) to yield 2.2 g (88.7%) of **5**: mp 169–171 °C; NMR ($DCCl_3$) δ 3.90 (s, 2 H, CH_2), 6.82–7.56 (m, 10 H, ArH, N=CH). Anal. ($C_{16}H_{12}N_2S$) C, H, N; mol wt 264 (MS).

Thiochroman-4-one 1,1-Dioxide (6). Thiochroman-4-one (1) (2.05 g, 0.014 mol) was dissolved in 25 mL of glacial acetic acid and to this solution was added 10 mL of 30% hydrogen peroxide. The mixture was diluted with 25 mL of cold distilled water. A crystalline solid formed and was filtered. It was recrystallized (dilute acetic acid) to yield 1.8 g (67.5%) of **6**: mp 131–133 °C (lit.[10a] 131.5 °C); NMR ($DCCl_3$) δ 3.36 [t, 2 H, C(=O)CH_2], 3.66 (t, 2 H, $-SO_2CH_2$), 7.60–8.17 (m, 4 H, ArH). Anal. ($C_9H_8O_3S$) S.

4*H*-[1]Benzothiopyrano[3,4-*d*]isoxazole 5,5-Dioxide (7). To a solution of 0.2 g (0.001 mol) of isoxazole **3** in 5 mL of glacial acetic acid was added 3 mL of 30% hydrogen peroxide, and the reaction mixture was allowed to stand at room temperature (30 h). The mixture was diluted with 25 mL of cold distilled water and cooled (ice bath). The white crystalline solid separated and was filtered and recrystallized (dilute acetic acid) to yield 0.22 g (95%) of **7**: mp 170–172 °C; NMR ($DCCl_3$) δ 4.43 (s, 2 H, CH_2), 7.63–8.32 (m, 5 H, ArH, N=CH). Anal. ($C_{10}H_7NO_3S$) N, S; mol wt 221 (MS).

1,4-Dihydro[1]benzothiopyrano[4,3-*c*]pyrazole 5,5-Dioxide (8a). Pyrazole **4** (0.2 g, 0.001 mol) was dissolved in 5 mL of glacial acetic acid and to this solution was added 3 mL of 30% hydrogen peroxide, and the reaction mixture was allowed to stand at room temperature (50 h). The mixture was concentrated on a rotary evaporator to a small volume, which was diluted with 25 mL of cold distilled water. Crystallization (dilute acetic acid) yielded 0.15 g (65%) of **8a**: mp 249–250 °C (see also ref 9); NMR (Me_2SO-d_6) δ 3.26 (br s, 1 H, NH), 4.68 (s, 2 H, CH_2), 7.46–8.40 (m, 5 H, ArH, N=CH). Anal. ($C_{10}H_8N_2O_2S$) N; mol wt 220 (MS).

1,4-Dihydro-1-phenyl[1]benzothiopyrano[4,3-*c*]pyrazole 2,5,5-Trioxide (9). Pyrazole **5** (0.3 g, 0.001 mol) was dissolved in 10 mL of glacial acetic acid, and to this solution was added 7 mL of 30% hydrogen peroxide. The reaction mixture was kept at room temperature (50 h). The mixture was then diluted with 25 mL of distilled water and cooled. A crystalline solid separated and was filtered off under suction. It was washed several times with water and recrystallized (dilute acetic acid) to give 0.35 g (98.8%) of **9**: mp 211–212 °C; NMR ($DCCl_3$) δ 4.41 (s, 2 H, CH_2), 6.84–7.70 (m, 10 H, ArH, N=CH). Anal. ($C_{16}H_{12}N_2O_3S$) N.

2,4,6,7-Tetrahydrothiopyrano[4,3-*c*]pyrazole (12). A solution of 7.4 g (0.1 mol) of ethyl formate in 50 mL of dry benzene was added to a cooled suspension of sodium methoxide (5.4 g, 0.1 mol) in 100 mL of dry benzene under N_2. While the mixture was magnetically stirred, a solution of 5.80 g (0.05 mol) of tetrahydrothiopyran-4-one (**10**)[15] in 50 mL of dry benzene was added dropwise. The reaction mixture was left overnight and was hydrolyzed with 300 mL of ice water. The organic layer was separated and washed with water and then NaOH (10%). The combined aqueous extracts were washed (ether), and the ether was discarded. The aqueous extracts were cooled and acidified with 6 N HCl. The oil that separated was extracted with ether and evaporated to yield the hydroxymethylene derivative **11**, 2.5 g (35%).

To the crude hydroxymethylene derivative **11** (2.5 g, 0.017 mol) was added 5 mL of hydrazine in 50 mL of methanol and boiled for 5 h. The methanol was stripped off, and the resulting low-melting solid was recrystallized (hexane) to give 1.2 g (49.3%) of **12**: mp 112–113 °C; NMR ($DCCl_3$) δ 2.98 (s, 4 H, $-SCH_2CH_2-$), 3.66 (s, 2 H, $-SCH_2-$), 7.32 (s, 1 H, N=CH), 11.2–12 (br s, 1 H, –NH). Anal. ($C_6H_8N_2S$) N, S.

1,4,6,7-Tetrahydrothiopyrano[4,3-*c*]pyrazol-3(2*H*)-one (13). 3-Carbomethoxytetrahydro-1,4-thiapyrone (**15**)[15] (1.62 g, 0.01 mol) was dissolved in 15 mL of anhydrous methanol and treated with 3.2 g (0.1 mol) of 95% hydrazine. The mixture was boiled for 3 h with stirring. Evaporation of the methanol and pouring the concentrate into ice water gave a white pyrazolone **13**. Recrystallization (aqueous ethanol) gave pure, colorless pyrazolone **13** [1.4 g (89.7%)]: mp 288–290 °C dec; NMR (Me_2SO-d_6) δ 2 66–2.84 (m, 4 H, $-SCH_2CH_2-$), 3.38 (s, 2 H, $-SCH_2-$), 8.20–9.12 (br s, 2 H, NH, –HCC=O). Anal. ($C_6H_8N_2OS$) C, H.

1,4,6,7-Tetrahydro-1-phenylthiopyrano[4,3-*c*]pyrazol-3(2*H*)-one (14). 3-Carbomethoxytetrahydro-1,4-thiapyrone (**15**) (0.81 g, 0.005 mol) was dissolved in 15 mL of anhydrous methanol and treated with 1.09 g (0.01 mol) of phenylhydrazine. The reddish-brown solution obtained was stirred under nitrogen with gentle heating for 2 h. Cooled to room temperature and diluted with about 150 mL of ice-cold water, the reaction mixture deposited nearly pure pyrazolone **14**. Recrystallization (aqueous methanol) yielded 1.52 g (95%) of **14**: mp 220–221 °C (lit.[18] 219 °C); NMR (Me_2SO-d_6) δ 2.64–2.94 (m, 4 H, $-SCH_2CH_2$), 3.98 (s, 2 H, SCH_2), 7.18–7.76 (m, 5 H, ArH), 10.24–11.18 (br s, 1 H, –CHC=O).

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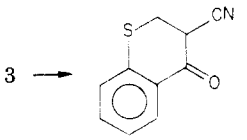
(7) T. Moriwake, *J. Med. Chem.*, **9**, 163 (1966). Compound **2** was reported to have bp 157–158 °C (4 mm) and did give a correct C and H analysis.

(8) Although **3** was reported by Moriwake (ref 7), the heterocycle was only described as a "dark reddish viscous condensation product". Another reference to **3** can be found from the work of Fravolini [A. Fravolini, A. Martani, and G. Grandolini, *Boll. Sci. Fac. Chim. Ind. Bologna*, **26**, 269 (1968); *Chem. Abstr.*, **70**, 106482u (1969)]. However, the

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.

(11) A record of pyrazole **8** was made by Pagani and Maiorana, cited in ref 9. No properties were given in the abstract nor was mention made that tautomers (such as **8** ⇄ **8a**) could exist.

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(14) Unfortunately, we could not uncover a ^{13}C NMR study on any related pyrazole with which to make comparison of proton distribution on N(1) and N(2) with the influence on the ^{13}C chemical shift. See ref 6 for a study on simple pyrazoles and ref 1d and 5 for summaries of work in this area.

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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-