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Synthesis of Hydrazones and Oximes of Geldanaldehyde as Potential Polymerase Inhibitors[1,2]

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Derivatives of geldanamycin, hydrazones and oximes of geldanaldehyde analogous to rifampicin and its relatives in the rifamycin series, have been synthesized. The geldanamycin derivatives inhibited reverse transcriptase of Rauscher leukemia virus more strongly than did geldanamycin, and some were considerably less cytotoxic than geldanamycin.

The discovery of RNA-dependent DNA polymerase (reverse transcriptase; RT) in animal tumor viruses (*1–6*) and later in human tumor viruses (*7–9*) has stimulated considerable speculation on the role of these enzymes in tumor etiology (*10*). This speculation has been accompanied by a search for selective inhibitors of the enzymes, both as biological tools and as potential chemotherapeutic agents (*11–14*). The previously well-known potency of the ansamycins and their derivatives (Fig. 1) in inhibiting bacterial (though not mammalian) DNA-dependent RNA polymerase (*15–17*) suggested these compounds as candidates of high priority for testing as RT inhibitors. Accordingly, a remarkable number of derivatives of rifamycins SV (**1**) and S (**2**) (most of them already available from earlier antibacterial testing programs) (*18, 19*) were tested as RT inhibitors in a massive screening effort supported by the National Cancer Institute (*20, 21*).

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[1] This manuscript is dedicated to our fondly remembered friend, Morris Kupchan, whose interest in the antineoplastic maytansinoids paralleled our own interest in the biogenetically related geldanamycin.

[2] Presented in part at the 165th National Meeting of the American Chemical Society, Dallas, Texas, April 1973, Abstract MEDI 002; at the Annual Chemical Congress, London, April 1974; and at the 14th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, September 1974. Paper IV in the series on geldanamycin [Paper III: A. Haber, R. D. Johnson, and K. L. Rinehart, Jr., *J. Amer. Chem. Soc.* **99**, 3541 (1977)].

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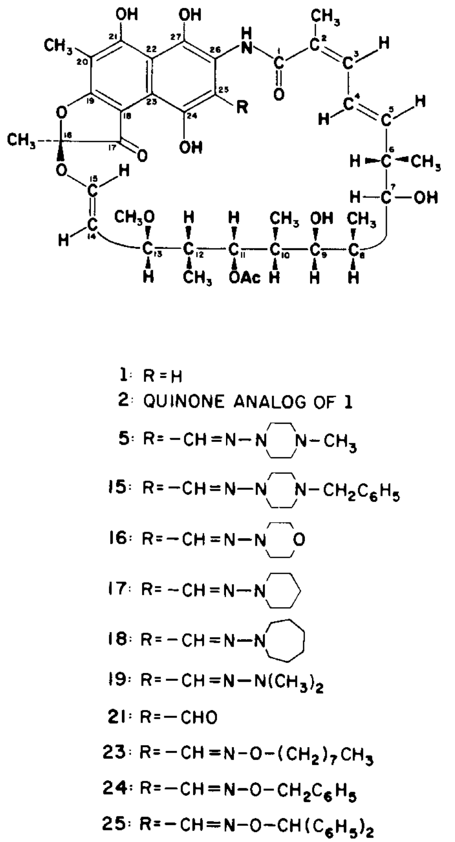


1: R = H

2: QUINONE ANALOG OF 1

5: R= −CH=N−N N−CH_3

15: R= −CH=N−N N−$CH_2C_6H_5$

16: R= −CH=N−N O

17: R= −CH=N−N

18: R= −CH=N−N

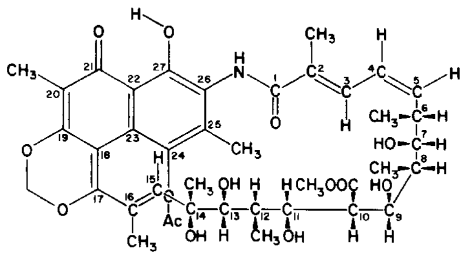
19: R= −CH=N−N$(CH_3)_2$

21: R= −CHO

23: R= −CH=N−O−$(CH_2)_7CH_3$

24: R= −CH=N−O−$CH_2C_6H_5$

25: R= −CH=N−O−$CH(C_6H_5)_2$



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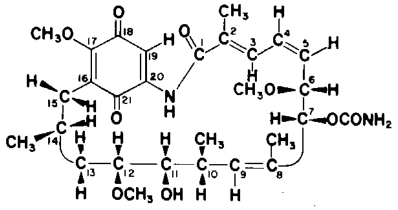

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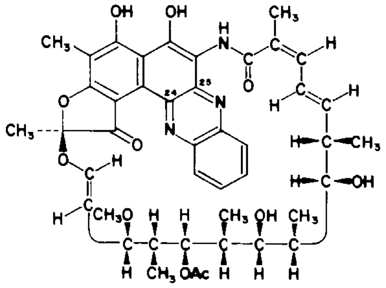

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FIG. 1. Ansamycins and their derivatives.

Concurrent with this enormous effort elsewhere on rifamycin derivatives, we have mounted a modest synthetic program directed toward preparing derivatives of the two ansamycin antibiotics whose structures and biosynthesis we have been studying for some time, i.e., streptovaricins (such as streptovaricin C, **3**) and geldanamycin (**4**) (*22*). Of these two, the streptovaricins are obviously much closer to rifamycin in structure, being naphthoquinone derivatives. However, the key position employed in substituting the rifamycin nucleus, C-25 (C-3 according to the original rifamycin numbering system) was unavailable, being blocked by a methyl group. For this reason, we turned to acetylation and oxidation products in seeking improvements in streptovaricin's activity, as has been and will be described elsewhere (*17, 23, 24*).

On the other hand, geldanamycin, although a derivative of a benzoquinone rather than a naphthoquinone, is unsubstituted at the key position (C-19) adjacent to the amide. Thus, many of the derivatives of rifamycin can be duplicated in the geldanamycin series. We chose to investigate two especially promising classes from the rifamycin studies, imines derived from an aldehyde substituent and heterocyclic condensation products derived from *o*-phenylenediamines and *o*-aminophenols. The former class is represented most successfully in the rifamycin series by rifampicin (rifampin, **5**), which is obtained from rifamycin SV (**1**) and is employed clinically throughout the world as the rifamycin derivative of choice for treatment of gram-positive infections, tuberculosis, and leprosy (*25*, *26*). The heterocyclic class would be represented by rifazine (**6**), obtained from rifamycin S (**2**) and an early clinical candidate in the rifamycin series (*27*). We describe in the present work the preparation of geldanamycin analogs of rifampicin and some of its relatives, while in an accompanying report we shall describe the synthesis of geldanamycin derivatives related to rifazine.

Synthesis of hydrazones. As noted above, geldanamycin derivatives were chosen for preparation according to prior experience elsewhere with rifamycin derivatives. Of obvious interest, by analogy with rifampicin (**5**), was the 1-amino-4-methylpiperazine derivative (**7**) which we call geldampicin (Fig. 2). Although rifampicin has little activity in inhibiting RT, it is clinically a highly successful antibacterial agent. Related derivatives, the hydrazones derived from 1-amino-4-benzylpiperazine (**8**), *N*-aminomorpholine (**9**), *N*-aminopiperidine (**10**), *N*-aminoperhydroazepine (**11**), *unsym*-dimethylhydrazine (**12**), *unsym*-di-*n*-butylhydrazine (**13**), and *unsym*-di-*n*-hexylhydrazine (**14**), were chosen because some of the analogous compounds in the rifamycin series (**15**, AF/ABP; **16**, NSC 143-447; **17**, NSC 143-448; **18**, NSC 144-103; and **19**, AF/DMI) were highly potent as inhibitors of RT (*19–21*).

Synthesis of the hydrazones **7** to **14** followed generally the routes employed previously in preparing hydrazones of rifaldehyde. Geldanamycin was subjected to a Mannich condensation to give the t-butylimine derivative (**20**) of geldanaldehyde. Although rifaldehyde (**21**) was prepared readily from the corresponding derivative by hydrolysis, attempts to isolate geldanaldehyde (**22**) were unsuccessful. On the other hand, the t-butylimine **20** underwent exchange with the requisite hydrazine directly to give the desired hydrazones (**7** to **14**) in yields of 21–66%. The hydrazones were stable, purple crystalline solids whose bioactivity will be discussed in brief below. Their structures were readily assigned from their molecular formulas (derived from elemental analyses and mass spectral data) and the absence of H-19 (at 7.02 ppm in the spectrum of geldanamycin) from their ^{1}H-nmr spectra. The imine protons are found near 9 ppm.

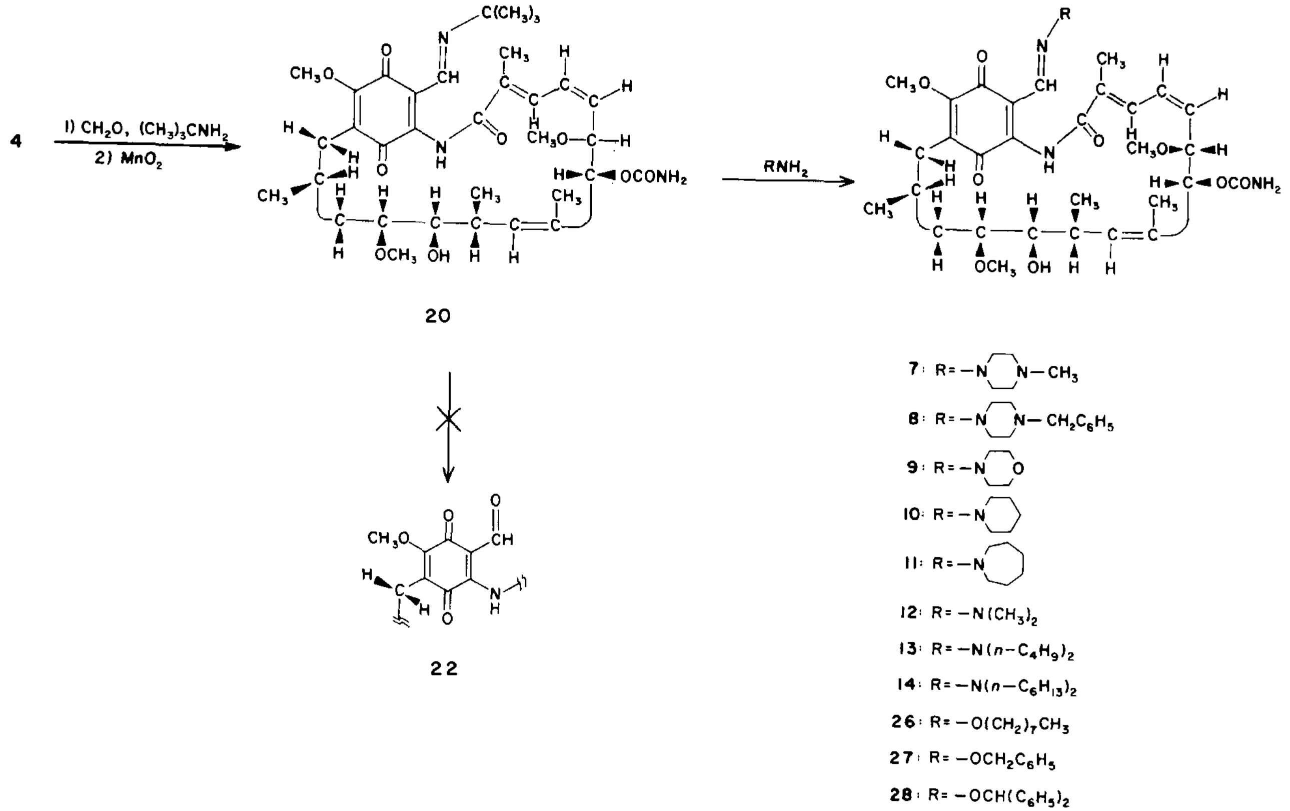

FIG. 2. Synthesis of hydrazones (**7** to **14**) and oximes (**26** to **28**) of geldanaldehyde (**22**).

Synthesis of oximes. In addition to substituted hydrazones, substituted oximes have proven highly successful derivatives of rifaldehyde and we chose direct analogs of three of these—those with O-*n*-octyl, O-benzyl, and O-benzhydryl substituents [**23** (AF/013), **24** (AF/BO), and **25** (AF/05)] (*28*)—as targets. As with the hydrazones, the oximes were prepared by exchanging the t-butylimine **20** with the required O-alkylhydroxylamines. Yields of the oximes (**26** to **28**), which were orange–brown crystalline solids, were 37–54%. Biological activities will be discussed in the next section. As with the hydrazones, structures were assigned from the molecular formulas, the absence of H-19 in the ^{1}H-nmr spectra and the presence of aldiminic protons.

Biological activity. The principal antimicrobial activity noted previously (*29*) for geldanamycin (**4**) is its inhibition of protozoal growth, notably that of *Tetrahymena pyriformis* and *Crithidia fasciculata*, and of the fungal plant pathogens *Alternaria, Pythium, Botrytis,* and *Penicillium.* Some antibacterial activity is also observed, e.g., against *Staphylococcus aureus, Bacillus subtilis,* and *B. cereus.* Clearly, as seen in Table 1, conversion of geldanamycin to the geldanaldehyde derivatives described here eliminates antiprotozoal activity and (except for modest activity of the oximes **26** and **28** against *D. pneumoniae* and *S. hemolyticus*) whatever antibacterial activity was found for the parent antibiotic. Neither geldanamycin nor any of its hydrazones or oximes tested (including **27** as well as those in Table 1) shows significant inhibition of *E. coli.* Although the derivatives were not tested, geldanamycin itself showed no activity in

TABLE 1

ANTIMICROBIAL ACTIVITY AND BACTERIAL RNA POLYMERASE INHIBITION

	Antimicrobial activity (MIC, μg/ml)					
Test organism	**4**[a]	**7**	**8**	**20**	**26**	**28**
Tetrahymena pyriformis UC P4	2	1000	125	500	250	250
Staphylococcus aureus UC 74	12.5	>500	500	500	>500	>500
Escherichia coli UC 51	50	500	500	500	>500	>500
Streptococcus faecalis UC 151	50	>500	500	>500	>500	>500
Proteus vulgaris UC 93	50	500	500	500	>500	>500
Klebsiella pneumoniae UC 57	50	500	500	500	>500	>500
Diplococcus pneumoniae UC 41	—	500	62.5	250	7.8	62.5
Staphylococcus hemolyticus UC 152	—	500	125	250	7.8	31.2
Pseudomonas aeruginosa UC 95	>100	250	250	250	500	>500

Escherichia coli RNA polymerase activity (% of control)					
4	**7**	**8**	**20**	**26**	**28**
86[b] 92[d] 94[e]	106[c]	88[c]	99[c]	110[c]	84[c]

[a] Antimicrobial data for **4** from Ref. *29*.

[b] 0.36 μmol/ml.

[c] 0.5 μmol/ml.

[d] 0.27 μmol/ml.

[e] 0.09 μmol/ml.

TABLE 2

INHIBITION OF RDDP (RT) FROM RAUSCHER LEUKEMIA VIRUS AND BALB 3T3 CELL GROWTH INHIBITION BY GELDANAMYCIN DERIVATIVES

Compound	RDDP[a] (% inhibition)[b]	Cell growth inhibition[a]	
		ID$_0$ (nmol/ml)	ID$_{50}$ (nmol/ml)
4	4–19	≤0.0005	0.0019
7	46		
8	44–66	≤2.5	6.4
9	37	≤2.5	10.0
10	30		
11	27		
12	20		
13	26		
14	46		
20	41		
26	7		
27	81	≤0.25	0.40
28	0		

[a] Experimental conditions for the assays are described in Ref. *30*.
[b] At drug concentration of 0.25 μmol/ml.

inhibiting *E. coli* DNA polymerase I, rat liver RNA polymerase, or rat liver DNA polymerase at concentrations of 0.06–0.18 μmol/ml.

On the other hand, a number of the derivatives showed considerable activity in inhibiting RTs from tumor viruses (Table 2) (*30*). Whereas geldanamycin itself was essentially inactive in this test, several of the geldanaldehyde hydrazones (**7**, **8**, **9**, **14**) inhibited RT from RLV to the extent of 40–70% at 0.025 μmol/ml. The O-benzyloxime of geldanaldehyde (**27**) was even more active, giving 81% inhibition of RLV RT at this level.

Accompanying these observations of enhanced activity (relative to geldanamycin) of the derivatives was the observation that the derivatives were considerably less cytotoxic than geldanamycin. Thus, the hydrazones **8** and **9** were 3000 to 25 000 times less toxic to BALB 3T3 cells than geldanamycin,.while the oxime **27** was 200 to 500 times less cytotoxic.

The combination of enhanced activity and diminished toxicity has stimulated a much more extensive testing of some of the derivatives as virucidal agents, both *in vitro* and *in vivo*. As is reported elsewhere (*30*), both *in vitro* and *in vivo* inhibition of viral infectivity are observed, but therapeutic activity *in vivo* is lacking.

EXPERIMENTAL

Melting points are uncorrected and were determined on a Kofler micro hot-stage apparatus. Infrared (ir), ultraviolet (uv), and ^{1}H-nmr spectra were obtained by Mr. R. L. Thrift and associates: ir spectra (KBr pellets or $CHCl_3$ solutions) on Perkin–Elmer

Model 521 spectrophotometer; uv spectra on Perkin–Elmer Model 202 spectrophotometer or Cary Model 15 spectrophotometer, ^{1}H-nmr spectra in appropriate solvents on Varian Associates Model T-60, A-60A, A56/60, HA-100, and HR 220 spectrometers. Chemical shift values are reported in parts per million from internal tetramethylsilane. Mass spectra were obtained on Varian MAT Model 731 and CH-5 DF mass spectrometers, by Mr. J. C. Cook, Jr., and Mr. J. Wrona. Microanalyses were obtained at the University of Illinois by Mr. J. Nemeth and associates.

Column chromatographic separations were performed with Brinkmann silica gel (0.05–0.25 m) or Merck aluminum oxide. Thin-layer chromatographic plates were prepared with silica gel GF 254 (E. Merck), preparative tlc plates with silica gel PF 254 (E. Merck).

Geldanaldehyde t-butylimine (**20**). A mixture of 2.5 g (4.5 mmol) of geldanamycin, 50 ml of tetrahydrofuran, 1.42 ml (13.5 mmol) of t-butylamine, and 0.8 ml (9.5 mmol) of 37% formaldehyde was stirred for 16 hr at 45–50°C, then evaporated to dryness. The residue was dissolved in 50 ml of benzene and solvent was evaporated to dryness again to remove moisture, then the residue, 50 ml of benzene and 3.5 g of manganese dioxide [prepared by the procedure of Mancera and Rosenkranz (*31*)], was stirred at 45–50°C for 1.5 hr. Excess manganese dioxide was filtered and the filtrate was evaporated to dryness. The crude product was dissolved in a small amount of acetone and chromatographed on a silica gel column (4 × 40 cm, containing 196 g of silica gel) with chloroform–methanol–benzene (8 : 1 : 1) as solvent. After elution, the second yellow–brown fraction was filtered and concentrated to dryness slowly; the yellow–brown product was dried under vacuum at 60°C for 6 hr to give 1.52 g (53%) of **20**. Purification over EM silica gel 60 (Brinkmann) using chloroform–methanol (7 : 1) as solvent gave an orange solid, mp 116–119°C, which contained less than 1% impurities by HPLC (1-ft × 1/4-in. micro-Porasil C_{18}, uv 254 nm, acetonitrile : water: 1 : 1). The mass spectrum (70 eV) of **20** contained, in addition to the molecular ion, a more intense peak at *m/e* 600 (M − HNCO). The ^{1}H-nmr spectrum lacked the quinone proton (H-19) at 7.02 ppm and contained an imine proton (—CH=N—) at 8.77 ppm and t-butyl protons at 1.48 ppm.

Anal. Calcd for $C_{34}H_{49}N_3O_9$: C, 63.43; H, 7.67; N, 6.52; mol wt, 643. Found: C, 63.25; H, 7.71; N, 6.59; mol wt, 643 (mass spectrum).

Yields of **20** in three additional runs on this scale were 45–52%; in a run on a double scale, 40%; and in a run on a one-tenth scale, 58%.

1-Amino-4-methylpiperazine. Dinitrogen trioxide (generated according to a standard procedure) (*32*) was bubbled into a cooled, stirred solution of 20.0 g of *N*-methylpiperazine in 100 ml of water at such a rate that the temperature was maintained at 10°C. After the solution turned yellow, the nitroso compound was salted out using potassium carbonate in slight excess. The mixture was extracted with three 100-ml portions of ether and concentrated. The residue was distilled at 77–78°C (2 torr) [lit. (*33*) 76°C (1.5 torr)] to give 23.4 g (90%) of 4-methyl-1-nitrosopiperazine, which was reduced with 10.0 g of lithium aluminum hydride in 200 ml of ether to give 18.7 g (90%) of 1-amino-4-methylpiperazine, bp 171–173°C [lit. (*34*) 172–175°C].

Geldanaldehyde 1-amino-4-methylpiperazine hydrazone (**7**, *geldampicin*). A mixture of 4.29 g (6.66 mmol) of geldanaldehyde t-butylimine (**20**), 0.90 g (8.00 mmol) of 1-amino-4-methylpiperazine, and 50 ml of methanol was stirred at 35°C for 8 hr, then concentrated *in vacuo*. The residue was dissolved in a minimal amount of chloroform, applied to a silica gel column, and eluted with 3 liters of chloroform followed by 1 liter of 3% methanol in chloroform to give 2.25 g (48%) of deep purple crystals of **7**, mp 146–148°C.

Anal. Calcd for $C_{35}H_{51}N_5O_9$: C, 61.29; H, 7.50; N, 10.21; mol wt, 685.3686. Found: C, 60.87; H, 7.67; N, 9.80; mol wt, 685.3679 (HRMS).

When the synthesis was repeated using 1.67 g (2.6 mmol) of geldanaldehyde t-butylimine (**20**) and 0.43 g (3.74 mmol) of 1-amino-4-methylpiperazine in 50 ml of ethyl acetate, the yield was 0.90 g (51%), while the yield from 0.167 g of **20** (ethyl acetate solvent) was 0.074 g (42%).

1-Amino-4-benzylpiperazine. N-Benzylpiperazine was prepared in 69% yield according to the procedure of Craig and Young (*35*), then was nitrosated as described above for *N*-methylpiperazine to give 8.2 g (100%) of 4-benzyl-1-nitrosopiperazine, bp 133–135°C (0.1 torr) [lit. (*36*) 144°C (0.8 torr)]. The nitroso compound was reduced with lithium aluminum hydride to give 7.6 g (99%) of 1-amino-4-benzylpiperazine, bp 124–125°C (1.8 torr) [lit. (*36*) 126–130°C (1.2 torr)].

Geldanaldehyde 1-amino-4-benzylpiperazine hydrazone (**8**). A mixture of 1.67 g (2.6 mmol) of geldanaldehyde t-butylimine (**20**) and 0.715 g (3.74 mmol) of 1-amino-4-benzylpiperazine in 50 ml of ethyl acetate was agitated at 55°C for 4 hr, then evaporated to dryness. The crude residue was dissolved in a small amount of acetone and chromatographed twice over a column (4 × 40 cm) containing 196 g of silica gel, using chloroform–methanol–benzene (8 : 1 : 1) then chloroform–amyl alcohol (9 : 1) to give 0.85 g (43%) of **8**, mp 134–136°C.

Anal. Calcd for $C_{41}H_{55}N_5O_9$: C, 64.63; H, 7.27; N, 9.19; mol wt, 761. Found: C, 64.49; H, 7.58; N, 9.06; mol wt, 761 (mass spectrum).

When the reaction was repeated on a one-tenth scale, the yield was also 43%.

Geldanaldehyde N-aminomorpholine hydrazone (**9**). A solution of 2.14 g (3.33 mmol) of geldanaldehyde t-butylimine (**20**), 350 mg (3.40 mmol) of *N*-aminomorpholine, and 40 ml of methanol was kept at 55°C for 4 hr, then concentrated *in vacuo*. The residue was dissolved in a minimal amount of chloroform and chromatographed over a column (21 × 400 mm) containing 90 g of silica gel, eluting with 2 liters of chloroform, followed by 500 ml of 2% methanol in chloroform. The purple fractions were combined, concentrated, and chromatographed over a neutral alumina column (J. T. Baker) (21 × 425 mm). Elution with 1.5 liters of chloroform followed by 2% methanol in chloroform (500 ml) gave a grape violet solid which was crystallized from acetone–hexane to give 600 mg (26%) of **9**, mp 145–147°C.

Anal. Calcd for $C_{34}H_{48}N_4O_{10}$: mol wt, 672.3370. Found: mol wt, 672.3374 (HRMS).

Geldanaldehyde N-aminopiperidine hydrazone (**10**). The preparation followed the procedure for **9**, employing 2.14 g (3.33 mmol) of geldanaldehyde t-butylimine (**20**) and 350 mg (3.40 mmol) of *N*-aminopiperidine. The product was dissolved in a minimal amount of acetone and hexane was added to precipitate 650 mg (30%) of **10**, mp 143–145°C.

Anal. Calcd for $C_{35}H_{50}N_4O_9$: mol wt, 670.3597. Found: mol wt, 670.3565 (HRMS).

In a second run employing 129 mg (0.2 mmol) of geldanaldehyde t-butylimine (**20**) and 30 mg (0.3 mmol) of 1-aminopiperidine in 4 ml of dry ethyl acetate the yield of **10** was 43 mg (32%).

Geldanaldehyde N-aminoperhydroazepine hydrazone (**11**). The preparation was according to that of **9** employing 3.2 g (5.0 mmol) of geldanaldehyde t-butylimine (**20**) and 600 mg (5.0 mmol) of *N*-aminoperhydroazepine. Precipitation from ether–pentane gave 350 mg (21%) of **11** as navy blue crystals, mp 142–143°C.

Anal. Calcd for $C_{36}H_{52}N_4O_9$: mol wt, 684.3734. Found: mol wt, 684.3737 (HRMS).

Geldanaldehyde N′,N′-dimethylhydrazone (**12**). A solution of 304 mg (0.50 mmol) of geldanaldehyde t-butylimine (**20**), 40 mg (0.66 mmol) of *unsym*-dimethylhydrazine, and 25 ml of methanol was kept at 25°C for 45 min. The dark brown solution was concentrated *in vacuo* and the residue was purified by preparative tlc (silica GF_{254}, 2 mm) using chloroform–benzene–methanol (8 : 1 : 1) as elution solvent. The purple band was removed and extracted with acetone to give an oily product, which was crystallized from ether–hexane to give 195 mg (66%) of **12**, mp 146–147°C.

Anal. Calcd for $C_{32}H_{46}N_4O_9$: mol wt, 630.3265. Found: mol wt, 630.3268 (HRMS).

In a second run employing 1.52 g (2.5 mmol) of geldanaldehyde t-butylimine (**20**), 180 mg (3.0 mmol) of *unsym*-dimethylhydrazine, and 50 ml of methanol the yield of crystalline, dark purple **12** was 710 mg (45%).

Geldanaldehyde N′,N′-di-n-butylhydrazone (**13**). The preparation followed the procedure of **9**, employing 2.15 g (3.33 mmol) of geldanaldehyde t-butylimine (**20**), 500 mg (3.87 mmol) of *unsym*-di-*n*-butylhydrazine, and eluting with chloroform only to give 1.52 g (62%) of **13**, mp 120–121°C.

Anal. Calcd for $C_{38}H_{58}N_4O_9$: mol wt, 714.4203. Found: mol wt, 714.4201 (HRMS).

Geldanaldehyde N′,N′-di-n-hexylhydrazone (**14**). The preparation was according to that of **12**, employing 161 mg (0.25 mmol) of geldanaldehyde t-butylimine (**20**) and 50 mg (0.25 mmol) of *unsym*-di-*n*-hexylhydrazine at 55°C for 5 hr to give 40 mg (22%) of dark blue **14**, mp 80°C.

Anal. Calcd for $C_{42}H_{66}N_4O_9$: mol wt, 770.4829. Found: mol wt, 770.4845 (HRMS).

O-(n-Octyl)hydroxylamine hydrochloride. A solution of 13.0 g (0.08 mol) of *N*-hydroxyphthalimide [prepared in 62% yield according to the procedure of Mazur and Plume (*37*), mp 238.0–240.5°C (lit. (*37*) 237–240°C)], 17.8 g (0.18 mol) of triethylamine, 19.2 g (0.08 mol) of *n*-octyl iodide, bp 106°C (20 mm) [lit. (*38*) 108°C at 18 mm; prepared by a standard procedure from *n*-octyl chloride], and 120 ml of dimethylformamide was heated in 30 min to 90°C, kept at that temperature for 60 min, cooled, and worked up by the procedure of Schumann et al. (*39*) to give 14.9 g (54%) of *N*-(n-octyloxy)phthalimide. A portion of the substituted imide (7.3 g, 0.026 mol) was then converted with hydrazine to 1.9 g (48%) of O-(*n*-octyl)hydroxylamine hydrochloride, mp 149–152°C [lit. (*40*) 150°C].

Geldanaldehyde O-(n-octyl)hydroxylamine oxime (**26**). The preparation followed the procedure for **8**, employing 1.67 g (2.6 mmol) of geldanaldehyde t-butylimine (**20**) and 0.68 g (3.74 mmol) of O-(*n*-octyl)hydroxylamine hydrochloride, and eluting with chloroform–methanol-benzene (8 : 1 : 1) to yield 0.86 g (45%) of **26**, mp 65–67°C.

Anal. Calcd for $C_{38}H_{57}O_{10}N_3$: C, 63.75; H, 8.02; N, 5.87; mol wt, 715. Found: C, 64.04; H, 8.25; N, 5.98; mol wt, 715 (mass spectrum).

When the reaction was carried out with 0.167 g (0.26 mmol) of geldanaldehyde t-butylimine (**20**) and 0.068 g (0.374 mmol) of O-(*n*-octyl)hydroxylamine hydrochloride the yield was 0.084 g (45%).

Geldanaldehyde O-benzylhydroxylamine oxime (**27**). The preparation was according to that for **8**, employing 1.67 g (2.6 mmol) of geldanaldehyde t-butylimine (**20**) and 0.6 g (3.76 mmol) of O-benzylhydroxylamine hydrochloride to give 0.67 g (37%) of **27**, mp 105–107°C.

Anal. Calcd for $C_{37}H_{47}N_3O_{10}$: C, 64.05; H, 6.83; N, 6.06; mol wt, 693. Found: C, 63.86; H, 7.09; N, 5.99; mol wt, 693 (mass spectrum).

When the preparation was carried out with 0.167 g (0.26 mmol) of geldanaldehyde t-butylimine (**20**) and 0.06 g (0.376 mmol) of O-benzylhydroxylamine hydrochloride the yield was 0.067 g (36%).

Geldanaldehyde O-benzhydrylhydroxylamine oxime (**28**). *N*-Hydroxyphthalimide was converted to O-benzhydrylhydroxylamine hydrochloride in 84% yield, mp 179.8–180.0°C, according to Schumann et al. (*39*). The preparation of **28** followed the procedure for **8**, employing 0.336 g (0.52 mmol) of geldanaldehyde t-butylimine (**20**) and 0.164 g (0.74 mmol) of O-benzhydrylhydroxylamine hydrochloride, and using chloroform–methanol–benzene (8 : 1 : 1) as solvent. The orange–brown fraction was removed from the column, filtered, and taken to dryness to yield 0.217 g (54%) of **28**, mp 205–207°C.

Anal. Calcd for $C_{43}H_{51}O_{10}N_3$: C, 67.08; H, 6.68; N, 5.46; mol wt, 769. Found: C, 67.08; H, 6.86; N, 5.65; mol wt, 769 (mass spectrum).

When the procedure was repeated using 1.37 g (2.13 mmol) of geldanaldehyde t-butylimine (**20**) and 0.68 g (3.1 mmol) of O-benzhydrylhydroxylamine hydrochloride the yield was 0.7 g (37%).

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