J. Med. Chem. **1997**, *40*, 937−941

Hydrazide-Containing Inhibitors of HIV-1 Integrase†

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Received October 31, 1996⊗

Inhibitors of HIV integrase are currently being sought as potential new therapeutics for the treatment of AIDS. A large number of inhibitors discovered to date contain the *o*-bis-hydroxy catechol structure. In an effort to discover structural leads for the development of new HIV integrase inhibitors which do not rely on this potentially cytotoxic catechol substructure, NSC 310217 was identified using a three-point pharmacophore search based on its assigned structure *N*-(2-hydroxybenzoyl)-*N*-(2-hydroxy-3-phenoxypropyl)hydrazine (**1**). When a sample of NSC 310217 was obtained from the NCI repository, it was shown to exhibit potent inhibition of HIV-1 integrase (3′-processing $IC_{50} = 0.6$ μg/mL). In work reported herein, we demonstrate that NSC 310217, rather than containing **1**, which has no inhibitory potency against HIV-1 integrase, is comprised of roughly a 1:1 mixture of *N*-(2-hydroxybenzoyl)-*N*′-(2-hydroxy-3-phenoxypropyl)hydrazine (**6**) and *N*,*N*′-bis-salicylhydrazine **7**, with all inhibitory potency residing with compound **7** ($IC_{50} = 0.7$ μM for strand transfer). In subsequent structure−activity studies on **7**, it is shown that removing a single amide carbonyl (compound **14**, $IC_{50} = 5.2$ μM) or replacing one aromatic ring system with a naphthyl ring (compound **19**, $IC_{50} = 1.1$ μM) can be accomplished with little loss of inhibitory potency. Additionally, replacing a single hydroxyl with a sulfhydryl (compound **23**, $IC_{50} = 5.8$ μM) results in only moderate loss of potency. All other modifications examined, including the replacement of a single hydroxyl with an amino group (compound **22**), resulted in complete loss of potency. Being potent, structurally simple, and non-catechol-containing, compounds such as **7** and **14** may provide useful leads for the development of a new class of HIV integrase inhibitor.

A variety of anti-HIV agents rely on inhibition of key enzymes involved with the virus life cycle. The utility of compounds directed at single targets, such as reverse transcriptase, is potentially limited by the development of resistance. Recent advances have demonstrated that targeting multiple enzymes may provide one means of circumventing this problem, with combination therapies directed against both the HIV reverse transcriptase and protease enzymes showing promising results. HIV integrase is recognized as an important addition to the list of enzymes whose inhibition may be efficacious in anti-AIDS therapy,[1] since this enzyme is required for viral replication,[2−5] yet it is not indigenous to the human host. HIV integrase functions in a two-step manner by initially removing a dinucleotide unit from the 3′-ends of the viral DNA (termed 3′-processing). The 3′-processed strands are then transferred from the cytoplasm to the nucleus where they are introduced into the host DNA following 5-base pair offset cleavages of opposing host strands (termed strand transfer). In the development of HIV integrase inhibitors, considerable use has been made of radiolabeled oligonucleotide-based assays which allow the *in vitro* determination of IC_{50} values for inhibition of both 3′-processing and strand transfer.[6,7] A significant number of compounds exhibiting good potency in these assays contain two aryl systems joined through a central linker, with at least one of the aryl units frequently possessing the 1,2-bis-hydroxy (catechol) substitution pattern.[8−13] Although examples exist where catechol-containing compounds have been shown to exert protective effects against HIV infection in cellular systems,[13,14] one potential *in vivo* limitation of catechol-containing inhibitors is collateral cytotoxicity resulting from oxidation to quinone species.[15] Efforts have therefore been undertaken to develop non-catechol-based integrase inhibitors.[16,17]

O N NH2 OH OH O

1

Figure 1. Assigned structure of NSC 310217 indicating the three-point pharmacophore on which it was selected for integrase testing.[19]

In support of this endeavor, computer-assisted molecular modeling has been employed to identify potential inhibitors from among the 206 876 open compounds contained within the National Cancer Institute (NCI) Drug Information System (DIS) database.[18] Using a three-point pharmacophore (Figure 1), NSC 310217 was identified in one such search, based on its assigned structure **1**, and a sample of NSC 310217 obtained from the NCI repository was subsequently shown to exhibit potent inhibition of HIV-1 integrase (3′-processing $IC_{50} = 0.6$ μg/mL).[19] Compound **1** was of particular interest because it lacked the characteristic catechol substructure. Surprisingly, when an authentic sample of **1** was obtained by direct synthesis, it was found to differ from NSC 310217. As will be reported herein, further studies leading to the elucidation of the composition of NSC

† Part 3 in a series of papers describing pharmacophore-based development of HIV-1 integrase inhibitors.

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⊗ Abstract published in *Advance ACS Abstracts,* February 15, 1997.

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Scheme 1

3 R = NHBoc
4 R = NH_2

Scheme 2

Scheme 3

7, 15, 16, 19, 22, 23, 26

310217 were undertaken and have resulted in the identification of a new class of potent hydrazide-containing HIV integrase inhibitors.

Synthesis

In order to identify the chemical structure of NSC 310217 and further study its biological activity, we prepared its assigned structure **1** as shown in Scheme 1. The direct alkylation of hydrazine is often difficult because of polyalkylation; however, protecting groups largely solve this difficulty. Treatment of (±)-1,2-epoxy-3-phenoxypropane (**2**) with *tert*-butyl carbazate gave the *N,N'*-substituted compound **3**, which upon acid-catalyzed deprotection provided monosubstituted hydrazine **4**. In acylating hydrazines, the regiochemistry is dependent on both electronic as well as steric factors. For example, in the case of methylhydrazine, acylation with acyl chlorides occurs primarily at the *N*-methyl nitrogen, providing the terminally unsubstituted acylhydrazine.[20] With other monosubstituted hydrazines, mixtures of regioisomers are observed.[21] Reaction of **4** with salicylic acid pentafluorophenyl ester[22] gave the desired *N*-(2-hydroxy-3-phenoxypropyl)-*N'*-(2-hydroxybenzoyl)hydrazine (**1**). In contrast, when commercially available salicylhydrazine (**5**) was treated with salicylic acid pentafluorophenyl ester, acylation occurred at the *N'*-position, giving the isomeric *N,N'*-disubstituted hydrazine **6** (Scheme 2). The preparation of *N,N'*-bis-aroylhydrazines, including *N,N'*-bis-salicylhydrazine **7,** was achieved using a mild Pfp ester-mediated synthesis as shown in Scheme 3.[22]

In order to undertake structure–activity relationship studies of *N,N'*-disalicyloylhydrazine (**7**), we prepared *N*-(2-hydroxybenzyl)-*N'*-salicyloylhydrazine (**14**) and the related hydrazone **10** (Scheme 4). Treatment of salicylaldehyde (**8**) with hydrazine monohydrate or salicylhydrazide yielded hydrazones **9** and **10**, respectively. Hydrogenation of **9** failed to give the desired *N*-(2-hydroxybenzyl)hydrazine (**13**), which would be a logical

Scheme 4

precursor to final target **14**. Likewise, direct synthesis of **14** by hydrogenation of **10** was not possible due to the insolubility of the latter in normal solvents suitable for hydrogenation. In a more lengthy approach, treatment of salicylaldehyde (**8**) with *tert*-butyl carbazate provided the hydrazone **11**, which was then hydrogenated to yield *N*-(2-hydroxybenzyl)-*N'*-(*tert*-butyloxycarbonyl)hydrazine **12** in quantitative yield. Deprotection of **12** provided the monosubstituted hydrazine **13**, which was immediately reacted with salicyl Pfp ester to provide **14** in 33% yield.

Results and Discussion

A sample of NSC 312017, selected through the three-point pharmacophore search of the DIS database (Figure 1), and subsequently obtained from the NCI repository, had shown potent inhibition of HIV-1 integrase.[19] Surprisingly, synthetic **1**, prepared as part of this study, was inactive in the integrase assay within the concentration range tested (Table 1) and provided spectral data inconsistent with that obtained from the sample of NSC 310217, indicating that the original assignment of structure **1** to NSC 310217 was not correct. Therefore isomeric hydrazide **6** was prepared in an effort to identify the actual composition of NSC 310217. Although synthetic **6** also proved to be inactive in the integrase assay (IC_{50} >100 μM), it provided spectral data quite similar to that of NSC 310217. Comparison of NMR spectra showed that in addition to protons assignable to **6**, NSC 310217 also contained protons consistent with what one would expect from symmetric hydrazide **7**. Compound **7**, which has previously been reported as a metal chelator,[23,24] was therefore prepared and shown to provide an NMR spectrum, which when combined with the spectrum of **6** accounted for the observed NMR spectrum of NSC 310217. Furthermore, HPLC analysis of NSC 310217 indicated the presence of two major components in an approximately 1:1 ratio, which cochromatographed with synthetic **6** and **7**, respectively. Finally, isolation of the two components of NSC 310217 by preparative HPLC showed that they were indeed identical with synthetic **6** and **7**. It was therefore not surprising that synthetic **7** showed potent inhibition of HIV integrase, with a strand transfer IC_{50} value of 1 μM. This is consistent with the observed IC_{50} value for NSC 310217, when considered to be an approximate 1:1 mixture of **7** and inactive **6**.

Having in this fashion identified **7** as a very potent, non-catechol-containing integrase inhibitor, it was of

Table 1. HIV-1 Integrase Inhibitory Potencies Determined as Described in the Experimental Section

Compound	IC_{50} Values (μM)	
	3'-Processing	Integration
1	>200	>200
6	>200	>200
7	2.07 ± 0.75	0.73 ± 0.13
14	6.7 ± 0.8	5.2 ± 1.5
10	>100	>100
15	>100	>100
16	>100	>100
17	>100	>100
5	46 114	33 43
18	>100	>100
19	2.3 ± 0.3	1.1 + 0.15
20	>200	>200
21	>100	>100
22	>100	>100
23	9.1 ± 3.7	5.8 + 1.3
24	70.0 79.6	57.5 50.0
25	>100	>100
26	>100	>100

interest to examine what structural features were required for its activity. As shown in Table 1, a systematic dissection of **7** was therefore undertaken. Removal of one amide carbonyl (compound **14**) had essentially little effect on inhibitory potency. Besides eliminating the oxygen, this modification had the secondary effect of transforming the original carbonyl sp^2-hybridized carbon to an sp^3-hybridized center. It therefore was somewhat unexpected that reintroducing the lost sp^2 geometry at this center (compound **10**) was extremely deleterious to inhibitory potency. Eliminating one or both hydroxyl groups or one entire aromatic ring all gave inactive compounds (**15**−**17**, respectively); however, when the structure was further simplified to monohydrazide **5**, some of the lost potency was regained. Finally, further simplification to salicyl amide (**18**) again gave an inactive agent (Table 1).

This first series of analogues had been designed to elucidate critical components of parent hydrazide **7** by constructing simplified variants through the elimination

of structural elements. A further study was subsequently undertaken to evaluate effects of replacing or altering portions of **7**. The results of this second study are shown in Table 1. Addition of a second aryl ring to form an unsymmetrical naphthyl/phenylhydrazide (**19**) had no effect on inhibitory potency, while separating the two halves of **7** by means of a propyl linker (compound **20**) eliminated inhibitory potency. This is in contrast to the good inhibitory potency previously observed for a series of bis-arylpropylamides.[12] Replacement of the two aromatic hydroxyl groups with amines gave the inactive **21**, which remained inactive when one of the hydroxyls was re-introduced (compound **22**). In contrast, replacement of one hydroxyl of **7** with a sulfhydryl group (compound **23**) resulted in only a moderate loss of potency. For substituents at the 2-position this observed effect on potency, OH > SH ≫ NH (inactive), was also reflected in the monohydrazides, with potencies having the rank order: hydroxyl-bearing **5** > sulfhydryl-bearing **24** > amino-containing **25** (inactive). Finally, replacement of the hydroxyls with a pyridyl nitrogen also gave an inactive compound (**26**).

In summary, except for extending the aromatic ring system (compound **19**), removing a single amide carbonyl (compound **14**), or replacing a single hydroxyl with a sulfhydryl (compound **23**), alteration of the parent bishydrazide **7** was accompanied by substantial or complete loss of inhibitory potency. As a new structural class of non-catechol-containing integrase inhibitors, bisaroylhydrazines may provide valuable avenues for the development of HIV integrase inhibitors.

Experimental Section

Integrase Assay. Determination of IC_{50} values was as reported.[11,19]

Synthesis. Melting points were taken on a Mel Temp II melting point apparatus and are uncorrected. Elemental analyses were obtained from Atlantic Microlab Inc., Norcross, GA. IR (KBr) spectra were recorded on a Perkin-Elmer 1600 FT-IR spectrometer, and ^{1}H NMR data were obtained on a Bruker AC250 (250 MHz) instrument. Fast atom bombardment mass spectra (FABMS) were acquired with a VG Analytical 7070E mass spectrometer under the control of a VG 2035 data system. Analytic and preparative HPLC were performed on a Waters PrepLC 4000 System controlled using Waters Millennium software, with detection by a Waters 996 photodiode array UV spectrophotometer. Either a Vydac C_{18} peptide and protein semiprep column (10 mm i.d. × 25 cm long) run at a flow rate of 2 mL/min or a Vydac C_{18} peptide and protein preparative column (22 mm i.d. × 25 cm long) run at a flow rate of 20 mL/min was utilized using a two-component solvent system consisting of 0.05% TFA in H_2O and 0.05% TFA in acetonitrile (solvent B). Flash column chromatography was performed with E. Merck silica gel 60 (particle size, 0.04−0.063 mm).

***N*-(*tert*-Butyloxycarbonyl)-*N'*-(2-hydroxy-3-phenoxypropyl)hydrazine (3).** A mixture of *tert*-butyl carbazate (6.6 g, 50 mmol) and (±)-1,2-epoxy-3-phenoxypropane (**2**) (7.5 g, 50 mmol) in 2-propanol (250 mL) was refluxed (overnight). Solvent was removed under reduced pressure, and the residue was crystallized from EtOAc−hexane to yield **3** as a white solid (5.65 g, 40% yield), mp 117.5−119 °C: ^{1}H NMR (DMSO-d_6) δ 8.25 (σ, 1H), 7.3−7.24 (m, 2H), 6.93−6.88 (m, 3H), 4.91 (d, J = 4.2 Hz, 1H), 4.54−4.48 (m, 1H), 4.0−3.92 (m, 1H), 3.89−3.8 (m, 2H), 2.8−2.76 (m, 2H), 1.38 (s, 9H); FABMS *m/z* 227 (MH^+). Anal. ($C_{14}H_{22}N_2O_4$) C, H, N.

***N*-(2-Hydroxybenzoyl)-*N*-(2-hydroxy-3-phenoxypropyl)hydrazine (1).** A solution of **3** (564 mg, 2 mmol) in TFA acid (6 mL) was stirred at room temperature (1 h), then solvent was evaporated under reduced pressure, and the residue was dried *in vacuo* to provide crude *N*-(2-hydroxy-3-phenoxypropyl)hydrazine (**4**). This crude material was immediately dissolved in chloroform (20 mL), a solution of salicylic acid pentafluorophenyl ester[22] (608 mg) in chloroform (10 mL) was added dropwise over 1 h at 0 °C, then triethylamine (300 mg, 3 mmol) was added over 10 min, and the mixture was stirred at room temperature (overnight). Solvent was removed under reduced pressure, and the residue was purified by silica gel chromatography (EtOAc:hexane, 1:1) to provide **1** as a white solid (456 mg, 75% yield), mp 117.5−119 °C (EtOAc−hexane): ^{1}H NMR (DMSO-d_6) δ 9.95 (br s, 1H), 7.3−7.17 (m, 4H), 6.94−6.76 (m, 5H), 5.24 (br s, 1H), 4.92 (br s, 2H), 4.19 (m, 1H), 4.01 (m, 2H), 3.43 (m, 2H); IR (KBr) 3327, 1654, 1578, 748 cm^{-1}; FABMS *m/z* 303 (MH^+). Anal. ($C_{16}H_{18}N_2O_4$) C, H, N.

***N*-(2-Hydroxybenzoyl)-*N'*-(2-hydroxy-3-phenoxypropyl)hydrazine (6).** A mixture of commercially available salicylhydrazide (**5**) (1.52 g, 10 mmol) and (±)-1,2-epoxy-3-phenoxypropane (**2**) (1.5 g, 10 mmol) in 2-propanol (30 mL) was stirred at reflux (overnight). Solvent was evaporated under reduced pressure, and the residue was purified by silica gel chromatography (EtOAc:hexane:NH_3, 1:1:trace) to provide **6** as a white solid (782 mg, 26% yield), mp 112−122 °C (EtOAc−hexane): ^{1}H NMR (DMSO-d_6) δ 12.15 (s, 1H), 10.32 (s, 1H), 7.8−7.77 (m, 1H), 7.42−7.24 (m, 3H), 6.95−6.84 (m, 5H), 4.01−3.91 (m, 2H), 3.48−3.38 (m, 4H), 3.03−2.96 (m, 1H); IR (KBr) 3246, 1638, 1598, 1243, 753 cm^{-1}; FABMS *m/z* 303 (MH^+).

Salicylaldehyde Hydrazone (9). A mixture of salicylaldehyde (**8**) (1.22 g, 10 mmol) and hydrazine monohydrate (1 mL) in toluene was stirred at reflux (overnight), then diluted with hexane (30 mL), and cooled to room temperature. A white solid was deposited, which was collected by filtration and dried, providing **9**[26] (1.01 g, 74% yield), mp 96.0−96.5 °C: ^{1}H NMR ($CDCl_3$) δ 11.05 (s, 1H), 7.85 (s, 1H), 7.24−7.16 (m, 1H), 7.1−7.06 (m, 1H), 6.94−6.81 (m, 2H), 5.41 (br s, 2H). Anal. ($C_7H_8N_2O$) C, H, N.

Salicylaldehyde *N*-Salicylhydrazone (10). A solution of commercially available salicylhydrazide (**5**) (1.52 g, 10 mmol), salicylaldehyde (**8**) (1.22 g, 10 mmol), and acetic acid (15 mg) in anhydrous EtOH (60 mL) was refluxed (overnight). The reaction mixture was cooled to room temperature to provide **10** as a white solid, which was collected by filtration and dried (2.45 g, 95% yield), mp 285−286 °C: ^{1}H NMR ($CDCl_3$) δ 12.02 (s, 1H), 11.76 (s, 1H), 11.86 (s, 1H), 8.68 (s, 1H), 7.9−7.88 (m, 1H), 7.57−7.54 (m, 1H), 7.49−7.42 (m, 1H), 7.35−7.28 (m, 1H), 7−6.9 (m, 4H); IR (KBr) 3507, 1618, 1560, 1308, 1237, 750 cm^{-1}. Anal. ($C_{14}H_{13}N_2O_3$) C, H, N.

Salicylaldehyde *N*-(*tert*-Butyloxycarbonyl)hydrazone (11). A solution of salicylaldehyde (**8**) (2.44 g, 20 mmol) and *tert*-butyl carbazate (2.64 g, 20 mmol) in hexane was heated to reflux (5 h) and then cooled to room temperature. The resulting white solid was collected by filtration and dried to provide **11** (3.96 g, 84% yield), mp 149.5−150 °C: ^{1}H NMR ($CDCl_3$) δ 10.96 (s, 1H), 7.93 (s, 1H), 7.28−7.22 (m, 1H), 7.16−7.13 (m, 1H), 6.99−6.95 (m, 1H), 6.89−6.83 (m, 1H), 1.54 (s, 9H). Anal. ($C_{12}H_{16}N_2O_3$) C, H, N.

***N*-(2-Hydroxybenzyl)-*N'*-(*tert*-butyloxycarbonyl)hydrazine (12).** A solution of **11** (1.18 g, 5 mmol) in EtOAc (40 mL) was hydrogenated over 10% Pd·C (120 mg) under 40 psi of hydrogen in a Parr apparatus (room temperature, 24 h). The reaction mixture was filtered through silica gel, concentrated, dried *in vacuo*, and crystallized (hexane) to provide **12** as a white solid in quantitative yield, mp 78−80 °C: ^{1}H NMR ($CDCl_3$) δ 7.23−7.16 (m, 1H), 7.03−7.01 (m, 1H), 6.89−6.77 (m, 2H), 6.17 (br s, 1H), 4.15 (s, 2H), 1.45 (s, 9H). Anal. ($C_{12}H_{18}N_2O_3$) C, H, N.

***N*-(2-Hydroxybenzyl)-*N'*-salicylhydrazine (14).** A solution of **12** (236 mg, 1 mmol) in anhydrous dichloromethane (4 mL) was treated with TFA (2 mL), and the mixture was stirred overnight at room temperature. The volatiles were removed, and the residue was dried *in vacuo* (2 h) to provide crude *N*-(2-hydroxybenzyl)hydrazine (**13**) as syrup which was immediately treated with salicylic acid pentafluorophenyl ester[22] (608 mg, 2.0 mmol) in DMF (6 mL). After stirring overnight at room temperature, solvent was removed under reduced pressure and

the residue was purified by silica gel chromatography (EtOAc: hexane, 1:1). Crystallization (EtOAc–hexane) provided **14** as a white solid (86 mg, 33% yield): ^{1}H NMR (DMSO-d_6, 1:1 mixture of rotamers) δ 12.2 (s, 0.5H), 11.81 (s, 0.5H), 10.9 (s, 0.5H), 10.31 (s, 0.5H), 9.61 (s, 0.5H), 7.95–7.9 (m, 0.5H), 7.79–7.76 (m, 0.5H), 7.49–7.35 (m, 1H), 7.21–7.19 (m, 0.5H), 7.12–7.02 (m, 0.5H), 7–6.71 (m, 5H), 5.67 (m, 0.5H), 3.96 (s, 2H); IR (KBr) 3284, 1654, 1618, 1560, 1458, 1245, 750 cm^{-1}; FABMS m/z 259 (MH$^+$). Anal. ($C_{14}H_{14}N_2O_3$) C, H, N.

***N,N'*-Bis-aroylhydrazines.** Bis-aroylhydrazines **15**, **16**, **19**, and **22–26** were prepared as reported.[22]

***N*-Acetyl-*N'*-salicyloylhydrazine (17).** This compound has been previously prepared.[24]

1,3-Bis(salicylamido)propane (20). A solution of salicylic acid pentafluorophenyl ester (708 mg, 2 mmol) and 1,3-diaminopropane (74 μL, 1 mmol) in DMF (3 mL) was stirred at room temperature (overnight); then solvent was removed under reduced pressure to provide crude **20.** Purification by silica gel chromatography (EtOAc:hexane, gradient from 1:1 to 1:0) yielded **20** as a white solid (283 mg, 45% yield), mp 178.5–180.0 °C (EtOAc): ^{1}H NMR (DMSO-d_6) δ 12.58 (s, 2H), 8.85 (t, J = 5.3 Hz, 2H), 7.84–7.81 (m, 2H), 7.42–7.35 (m, 2H), 6.91–6.85 (m, 4H), 3.4–3.32 (m, 4H), 1.79 (quintuplet, J = 6.9 Hz, 2H); IR (KBr) 3376, 1654, 1648, 1594, 1540, 1250, 747 cm^{-1}; FABMS m/z 315 (MH$^+$). Anal. ($C_{17}H_{18}N_2O_4$) C, H, N.

***N,N'*-Bis(2-aminobenzoyl)hydrazine (21).** *N*-[2-[(*tert*-Butyloxycarbonyl)amino]benzoyl]-*N'*-(2-aminobenzoyl)hydrazine[22] (185 mg, 0.5 mmol) in anhydrous dichloromethane (4 mL) was treated with trifluoroacetic acid (1 mL) overnight at room temperature. The solvent was removed under reduced pressure, and the residue was dissolved in hot EtOAc. Upon cooling to room temperature a white solid formed, which was collected by filtration and dried *in vacuo* to provide to provide **21** in 96% yield, mp 175–177 °C; ^{1}H NMR (DMSO-d_6) δ 10.08 (s, 2H), 7.64–7.6 (m, 2H), 7.24–7.18 (m, 2H), 6.78–6.74 (m, 2H), 6.61–6.55 (m, 2H), 3.99 (s, 4H); FABMS m/z 391.6 (MH$^+$); IR (KBr) 2966, 1701, 1624, 1560, 1208, 740 cm^{-1}. Anal. ($C_{14}H_{14}N_4O_2 \cdot 2CF_3CO_2$) C, H, N.

HPLC Analysis of NSC 310217. A 30 mg sample of NSC 310217, obtained from the NCI repository, was dissolved in DMSO (400 μL) and diluted with 4.4 mL of MeOH:H_2O (10:1), then three separate runs of 1.6 mL (10 mg) were performed using a preparative column (linear gradient of 25–55% B over 20 min). Two components were present (retention times of 9.18 and 11.00 min) having respective relative areas of 45% and 55% (λ_{254}). These were collected, pooled, and taken to dryness, providing white solids (6 and 17 mg, respectively). Examination of both solids by analytical HPLC (linear gradient of 25–55% B over 20 min) indicated that each was >99% pure (λ_{254}; retention times of 19.7 and 22.7 min, respectively). The two components were identified as hydrazides **7** and **6**, respectively, based on NMR spectra and coelution with synthetic reference samples.

Acknowledgment. Appreciation is expressed to Ms. Pamela Russ and Dr. James Kelley of the LMC for mass spectral analysis.

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JM960755+