DEVELOPMENT OF A HYDRAZINE-MEDIATED SYSTEM FOR THE REDUCTIVE ACTIVATION OF MITOMYCIN C

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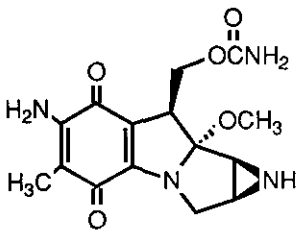
Abstract- The reactivity of mitomycin C (1) with hydrazines (2) has been examined. Monoarylhydrazines have been shown to efficiently reduce 1 leading to the activation of both proposed DNA binding sites (C-1 and C-10) within the drug.

Mitomycin C (1) is a clinically important antineoplastic agent.[1] The cytotoxicity of 1 is believed to be associated with the mono- and di- (cross-linking) functionalization of DNA. Drug binding at both the C-1 and C-10 positions in 1 is initiated by reduction of the quinone system. Several methods have been previously described for the in vitro reductive activation of 1. Chemical,[2a-c] catalytic,[2d-f] electrochemical,[2g-i] and enzymatic[2c,d,j,k] techniques have all been employed with varying success. Differences exist in the efficiency and reliability of these procedures, as well as the extent of mono- (i.e., C-1) versus di- (i.e., C-1, C-10) functionalization of the drug with nucleophiles. Several years ago we reported that use of large excesses (i.e., 50-fold) of either hydrazine (2a) or 1,1-dimethylhydrazine (2b) led to the activation of the C-1 position in 1.[3] Surprisingly, prior to this study few reports existed on the reduction of quinones by hydrazines.[4] In this paper, we detail the reactivity of mitomycin C with a variety of substituted hydrazines. Significantly, employment of select aryl-substituted hydrazines permitted modification of both proposed DNA binding sites (C-1 and C-10) in 1. Moreover, these reactions proceeded efficiently using near stoichiometric amounts of the hydrazine.



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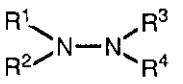


2

2a R^1, R^2, R^3, R^4=H
2b R^1, R^2=CH_3; R^3, R^4=H

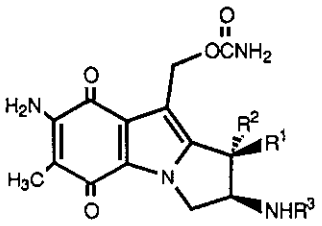
The hydrazines evaluated in this survey are listed in Table 1. Treatment of **1** with 50-fold or greater excess of **2c**-**2k** provided no detectable reaction. Prominent members within this group were the acyl-substituted hydrazines **2c**, **2d**, and **2i**, the nitrophenylhydrazines **2e** and **2f**, and the sterically congested hydrazines **2g**, **2h**, **2j**, and **2k**. Several of the hydrazines tested led to moderate activation of mitomycin C when present in 50-fold excess. Among these was **2a**, and the simple alkylhydrazines **2b** and **2n**. The most active reducing agents examined were the monoarylhydrazines **2o**-**2r**. Within this class, 4-methoxyphenylhydrazine (**2r**) and 4-fluorophenylhydrazine (**2q**) gave significant activation of **1** when present in 1.25 to 3-fold excess.

Table 1. Relative Reactivities of Substituted Hydrazines (2) for the Reductive Activation of Mitomycin C (1) [a]

No	R^1	R^2	R^3	R^4	EC[b]	No	R^1	R^2	R^3	R^4	EC[b]
2c	$CH_3C(O)$	H	H	H	NR[c]	**2l**	CH_3	H	CH_3	H	>50X
2d	$NH_2C(O)$	H	H	H	NR[c]	**2m**	C_6H_5	H	C_6H_5	H	>50X[g]
2e	3-$(O_2N)C_6H_4$	H	H	H	NR[d]	**2a**	H	H	H	H	50X
2f	4-$(O_2N)C_6H_4$	H	H	H	NR[c]	**2b**	CH_3	CH_3	H	H	50X
2g	CH_3	C_6H_5	H	H	NR[d]	**2n**	CH_3	H	H	H	<50X
2h	C_6H_5	C_6H_5	H	H	NR[d]	**2o**	2-C_5H_4N	H	H	H	10X
2i	$CH_3C(O)$	H	C_6H_5	H	NR[d]	**2p**	C_6H_5	H	H	H	3X
2j	C_6H_5	C_6H_5	2,4,6-$(O_2N)_3$-C_6H_2	H	NR[d,e]	**2q**	4-$(F)C_6H_4$	H	H	H	<3X
						2r	4-$(CH_3O)C_6H_4$	H	H	H	<1.25X
2k	CH_3	CH_3	CH_3	CH_3	NR[d,f]						

[a] **1** (1.25 mg) was dissolved in buffered aqueous ethanol (2.5 ml, 10% ethanol v/v, 0.1 M tris·acetic acid, pH 7.5) and Ar was bubbled through the solution (20 min). **2** was dissolved in the reaction buffer, degassed, and transferred into the reaction flask. The reactions were allowed to stand (2d) at ambient temperature under Ar. Samples of the reaction mixture were then injected directly into the hplc for analysis (See reference 2i for hplc conditions). The integrated area of the peaks in the hplc chromatograms at 313 nm was adjusted to account for differences in the absorption coefficients of the mitosane and mitosene products and then normalized to 100%. All reactions were run in duplicate. [b] EC = Effective concentration necessary for at least 50% consumption of **1**. [c] No detectable reaction at 250-fold excess of reducing agent (hplc analysis). [d] No detectable reaction at 50-fold excess of reducing agent (hplc analysis). [e] Reaction was run in unbuffered 50% aqueous tetrahydrofuran. [f] Reaction was run in 25% aqueous ethanol buffer (pH 7.5). [g] Reaction was run in 25% aqueous ethanol buffer (pH 7.5), and a trace amount of activation (5% consumption of **1**) was noted.

Detailed hplc analysis of the hydrazine-mediated reactions indicated that the primary products were trans-(**3a**) and cis-(**3b**) 1-hydroxy-2,7-diaminomitosenes, cis-1-hydroxy-2-acetamido-7-aminomitosene (**3c**),[2d,i] trans-1-acetoxy-2,7-diaminomitosene (**3d**)[5] and two hydrazine-derived compounds **A** and **B** (Table 2).



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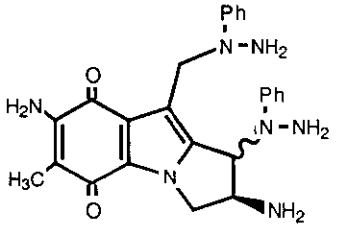

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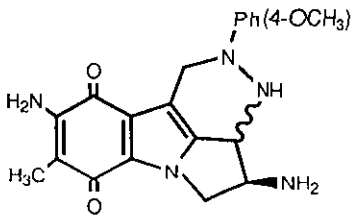

5

3a R^1=H; R^2=OH; R^3=H

3b R^1=OH; R^2=H; R^3=H

3c R^1=OH; R^2=H; R^3=$CH_3C(O)$

3d R^1=H; R^2=$CH_3C(O)O$; R^3=H

Table 2. Key Products Generated from the Reaction of Mitomycin C with Select Hydrazines[a]

Hydrazine	No. of Equiv.	1	3a	3b	3c	3d	A	B
2a	50	42	21[b]	24[c]	--	11	--	--
2o	3	52	8[b]	9	11	8	11[d]	--
2p	10	--	9	7	15	10	40[e]	19[f]
	3	37	9[b]	10	13	8	19[e]	3[f]
2q	3	28	9	18[c]	--	12	21[g]	4[h]
2r	3	2	4[b]	6	8	8	28[i]	43[j]
	1.25	34	12[b]	7	15	11	14[i]	--

[a]See Table 1, reference a for experimental conditions. The numbers reported are the calculated percent of each compound present at the conclusion of the reaction (hplc analysis). All reactions were performed in duplicate and averaged. [b]Resolution of hplc chromatogram did not permit digital integration. Graphical estimates of area were done using the triangulation method (base width measured @ 4% peak height). [c]Compounds 3b and 3c coeluted under the employed conditions. [d]hplc retention time: 20.5 min. [e]hplc retention time: 25.2 min. [f]hplc retention time: 33.4 min. [g]hplc retention time: 25.2 min. [h]hplc retention time: 33.1 min. [i]hplc retention time: 25.4 min. [j]hplc retention time: 33.2 min.

The relative amounts of **A** and **B** increased with increasing hydrazine concentration. Several semi-preparative scale reactions were conducted to identify compounds **A** and **B** generated in the **2p**- and **2r**-mediated reactions. Treatment of **1** with 15-fold excess of **2p** gave substantial amounts of **B**. Detailed ^{1}H Nmr and mass spectral analyses indicated that **B** was a 1,10-disubstituted phenylhydrazine mitosene adduct.[6] Key ^{1}H Nmr resonances observed for this product were the presence of a doublet (J = 4 Hz) at δ 4.90 for the C-1 methine proton and an AB quartet (J = 14.2 Hz) at δ 4.09 and 4.26 for the C-10 methylene protons.[7] FAB mass spectral analysis exhibited a protonated molecular ion at m/z 458. Addition of p-tolualdehyde and acetic

acid to **B** gave the corresponding dihydrazone.[8] Accordingly, compound **B** has been assigned as 2,7-diamino-1,10-bis(phenylhydrazino)mitosene (**4**). Treatment of **1** with lower amounts of **2p** (5 equiv) led to the formation of **3a**-**3d**, **A**, and a small amount of **B**. Attempts to either isolate or trap **A** proved unsuccessful. Substitution of **2r** (1.5 equiv.) for **2p** led to comparable results (Table 2). Efforts to isolate **A** in this reaction proved unsuccessful as well, but led to the isolation of a compound which eluted slightly earlier than **A** in the hplc chromatogram. The ^{1}H Nmr chemical shift for the C-1 methine proton for this adduct appeared significantly upfield (0.77 ppm) from that of **4** while the average chemical shift for the C-10 methylene hydrogens (δ 4.15) remained unchanged in the two compounds.[7] These combined observations led us to tentatively assign the **A**-derived product from the **2p**-mediated reaction as the cyclized adduct **5**.[9,10]

The determination that the primary products of these reactions were **3a**-**d**, **A**, and **B** supports the notion that these transformations proceed through initial reduction of the quinone ring in **1** by hydrazine **2**,[4] followed by nucleophilic attack of the solvent, buffer, or excess hydrazine on the reductively activated mitomycin C. This notion was reinforced by the observation that introduction of oxygen into the reaction system led to a noticeable decrease in the consumption of **1**. Significantly, the efficiency of the monoarylhydrazine-mediated mitomycin C transformations suggests that this technique may find use in the reductive activation of other quinone-containing medicinal agents.[11]

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5. Compound **3d** is unstable. Structural assignment is based on the ^{1}H Nmr spectrum obtained for **3d** after chromatographic purification (SiO_2:$CHCl_3$(9):CH_3OH(1)) of a semi-preparative reaction of **1**+**2r**, conversion of **3d** to **3a** upon treatment with either base or acid, and the absence of **3d** in reactions of **1**+**2a** in non-acetate buffer systems. ^{1}H Nmr ($CDCl_3$-CD_3OD, 9:1) δ 1.75 (s, 3H), 2.06 (s, 3H), 4.14-4.19 (m, 1H), 4.26 (br d, 1H, J = 13.9 Hz), 4.58 (dd, 1H, J = 6.4, 13.9 Hz), 5.18 (s, 2H), 6.09 (d, 1H, J = 1.9 Hz).
6. Key spectral data for **4** (**B**): ^{1}H Nmr (CD_3OD) δ 1.72 (s, 3H), 3.87 (dd, 1H, J = 4.8, 12.8 Hz), 4.00-4.10 (m, 1H), 4.09, 4.26 (ABq, 2H, J = 14.2 Hz), 4.52 (dd, 1H, J = 6.9, 12.8 Hz), 4.90 (d, 1H, J= 4.0 Hz), 6.70-6.80 (m, 4H), 6.95-7.19 (m, 6H). FAB ms m/z 458 (m+H, 16), 350 (85), 242 (100). Upon standing (CD_3OD, 72 h) **4** rearranged to give one major new product and several minor adducts.
7. *The ^{1}H Nmr assignments were supported by the corresponding COSY spectrum.*
8. Key spectral properties for dihydrazone: ^{1}H Nmr ($CDCl_3$) δ 1.92 (s, 3H), 2.31 (s, 3H), 2.32 (s, 3H), 4.06-4.08 (m, 1H), 4.08 (d, 1H, J = 12.9 Hz), 4.43 (dd, 1H, J = 2.1, 12.9 Hz), 5.26, 5.37 (ABq, 2H, J = 18.8 Hz), 5.35 (br s, 1H), 6.80-7.45 (m, 18 H). FAB ms m/z 700 (m+K, 4), 684 (m+Na, 6), 662 (m+H, 8), 452 (42), 333 (48), 242 (66), 119 (100).
9. Key spectral properties for **5**: ^{1}H Nmr (CD_3OD) δ 1.80 (s, 3H), 3.68, 4.59 (ABq, 2H, J = 12.5 Hz), 3.75 (s, 3H), 4.02 (dd, 1H, J = 4.8, 11.8 Hz), 4.14 (d, 1H, J = 4.3 Hz), 4.45-4.51 (m, 1H), 4.66 (dd, 1H, J = 6.7, 11.8 Hz), 6.85 (d, 2H, J = 8.9 Hz), 7.01 (d, 2H, J = 8.9 Hz).
10. In agreement with this contention, the thermospray LC/ms spectra of the **2p**-mediated reaction indicated the presence of a compound with m/z 349 at a retention time anticipated for the cyclized adduct.
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