Studies on the Mode of Action of Mitomycin C(7) Aminoethylene Disulfides (BMS-181174 and KW-2149): Reactivity of 7-*N*-(Mercaptoethyl)mitomycin C

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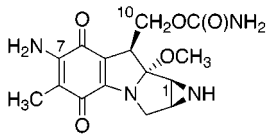
Mitomycin C (**1**) is extensively used in combination therapy to treat various neoplasms.[1] Its associated toxicities have led to an active drug development program[2] and the subsequent discovery of the two C(7) aminoethylene disulfides BMS-181174[3] (**2**) and KW-2149[4] (**3**). Both **2**[5a,b] and **3**[5c] exhibit improved pharmacological activity, compared with **1**, and **3** is currently undergoing clinical trials.[5c]



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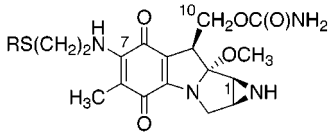


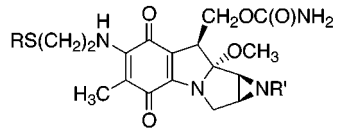
2 R = S–⟨⟩–NO_2 (BMS-181174)

3 R = $S(CH_2)_2N(H)C(O)(CH_2)_2CH(NH_2)CO_2H$ (KW-2149)

4 R = H

Compounds **2** and **3** only differ from mitomycin C (**1**) in the C(7) substituent. In **2** and **3,** a substituted aminoethylene disulfide unit replaces the C(7) amino group found in **1**. Novel mechanisms[6−8] proposed for **2** and **3** differ from the bioreductive activation pathway commonly accepted for mitomycin C.[9] A major contention of these hypotheses is that the C(7) aminoethylene disulfide unit in **2** and **3** undergoes thiol-mediated (e.g., R′SH = cysteine, glutathione (GSH)) disulfide exchange to give **4** and R′SSR.[6,7] Thiol **4** has never been identified. We present here preparative routes to **4** and related compounds and report on the reactivity of these species. The properties observed for thiol **4** require us to question these hypotheses[6,7] for **2** and **3,** and they lead us to suggest another pathway.

Our approach to **4** was to prepare mitomycins that rapidly and efficiently convert to **4** under mild conditions. Two strategies were used. The first entailed synthesizing C(7)-substituted mitomycins that undergo selective disulfide cleavage. We prepared **5**[10a,11] and **6**.[10b] Each contained a pyridyl disulfide group, which upon treatment with either D,L-dithiothreitol[12] (DTT) or *N*,*N*′-dimethyl-*N*,*N*′-bis(mercaptoacetyl)hydrazine[13] (DMH) underwent selective disulfide cleavage to give **7** and **8,** respectively. Subsequent intramolecular disulfide cleavage provided **4** and the oxidized cyclic disulfide. We expanded our study to include the two porfiromycin (R′ = CH_3) analogues **9** and **10**. In the second approach, we incorporated a C(7) terminal thiol ester unit to give **11**.[14] This route takes advantage of the relative ease with which thiol esters undergo base-mediated cleavage[15] and yielded the porfiromycin thiol **12**.



5 R = S–⟨⟩–N , R′ = H

6 R = S–⟨⟩(N, NO_2) , R′ = H

7 R = $SCH_2CH(OH)CH(OH)CH_2SH$, R′ = H

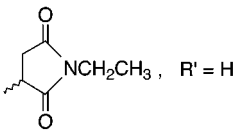
8 R = $SCH_2C(O)N(CH_3)N(CH_3)C(O)CH_2SH$, R′ = H

9 R = S–⟨⟩–N , R′ = CH_3

10 R = S–⟨⟩(N, NO_2) , R′ = CH_3

11 R = $C(O)CH_3$, R′ = CH_3

12 R = H, R′ = CH_3



13 R = ⟨⟩(O, NCH_2CH_3, O) , R′ = H

Thiol formation was monitored by HPLC (200−400 nm, photodiode array detection).[16] In Figure 1A we provide the HPLC profile (365 nm) obtained from a deaerated (Ar) methanolic (−78 °C) solution containing **6** and DTT (10 equiv). We observed the complete consumption of **6** (32.1 min) and the appearance of multiple peaks (3−4) between 22 and 26 min.[17] The same peaks were observed when we used DMH in place of DTT, acetone for methanol, and **5** in place of **6**. Figure 1B provides the corresponding HPLC chromatogram for a deaerated (Ar) methanolic NaOMe solution (−78 °C) containing **11**. Significantly, multiple peaks were observed between 23 and 29 min. The same peaks were observed for DTT-treated methanolic solutions containing either **9** or **10**.[17] We attributed the increased retention times for the porfiromycin multiple peaks, compared with the corresponding mitomycin peaks, to the effect of the N(1a) methyl group on the elution times.[18] These experiments indicated that a comparable set of intermediates is observed in the HPLC chromatograms independent of the activation procedure (DTT (DMH), NaOMe), the solvent used (methanol, acetone), and the structure of the starting mitomycins (**5**, **6**, **9**−**11**).

Information concerning the identity of the multiple HPLC peaks was gathered through thiol trapping-experiments (4,4′-dipyridyl disulfide (DPDS), 2,2′-dithiobis(5-nitropyridine) (DTNP), *N*-ethylmaleimide

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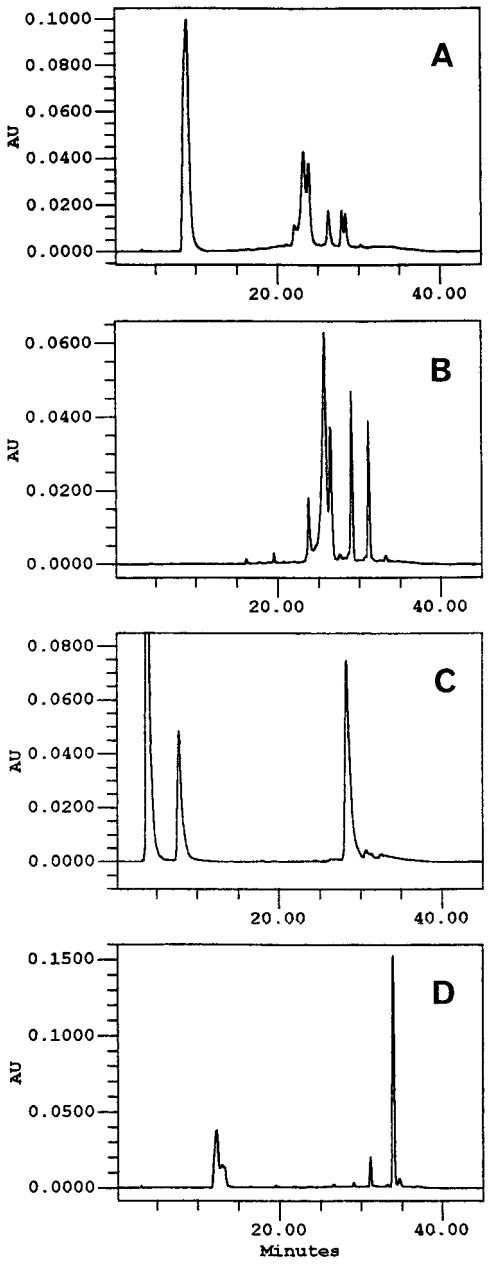
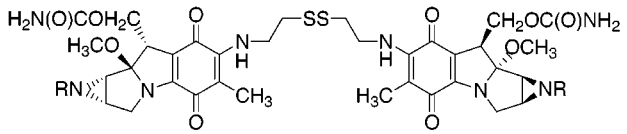


Figure 1. HPLC (365 nm) of mitomycin **6** and porfiromycin **11** activated reactions. (A) Mitomycin **6** treated with DTT (10 equiv) in MeOH at −78 °C. Major peaks (min): t_R 8.3, 5-nitro-2-thiopyridone; 21.9, 23.0, 23.6, **4**; 26.6, **7**; 28.4, 2:1 **6**:DTT adduct; 28.8, **15**. (B) Porfiromycin **11** treated with NaOMe in MeOH at −78 °C. Major peaks (min): t_R 23.9, 25.9, 26.6, 29.1, **12**; 31.2, **14**. (C) Mitomycin **6** sequentially treated with DTT (10 equiv) in MeOH at −78 °C (Figure 1A) followed by DPDS. Major peaks (min): t_R 3.8, 4-thiopyridone; 8.3, 5-nitro-2-thiopyridone; 28.5, **5**. (D) Porfiromycin **11** sequentially treated with NaOMe in MeOH at −78 °C (Figure 1C) followed by DTNP. Major peaks (min): t_R 12.3, 5-nitro-2-thiopyridone byproduct(s); 31.2, **14**; 33.6, **10**. The identities of **5** and **10** in Figure 1C,D, respectively, were confirmed by co-injection (cospot) of an authentic sample with the reaction solution in the HPLC (TLC).

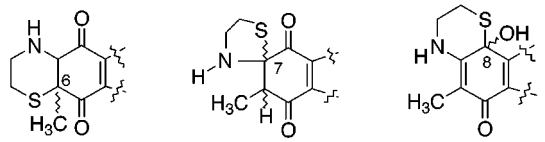
(NEM)). Figure 1C shows that adding DPDS to a DTT-treated solution of **6** (Figure 1A) completely eliminated the multiple HPLC peaks and the production of **5** (28.5 min). Correspondingly, treating the methanolic NaOMe solution-containing **11** (Figure 1B) with DTNP gave **10** (33.6 min) as the major product (Figure 1D). Similarly, we found that **5** was converted to **6**, **9** to **10**, and **10** to **9** upon successive treatment with DTT and the appropriate disulfide (DPDS, DTNP) and that **11** was converted to **9** upon sequential treatment with methanolic NaOMe and DPDS. Replacing disulfides DPDS and DTNP with NEM in the thiol-trapping reactions beginning with either **5** or **6** gave diastereomeric **13**. These collective experiments demonstrated that the multiple HPLC peaks are likely to be free thiol **4** (**12**) and isomeric forms of **4** (**12**).[19]

Additional experiments supported this notion. Elevating the reaction temperature of a deaerated methanolic NaOMe solution of **11** from −78 °C to room temperature led to near quantitative production of disulfide **14**.[20a] Correspondingly, treatment of deaerated methanolic ("pH" 5.5, 6.5, 7.4) solutions containing either **5** or **6** at room temperature with DTT (1 equiv) gave disulfide **15**[20a,b] as the major product. Absent in these reactions was the production of noticeable amounts of aziridine ring-opened mitosenes (HPLC analysis).



14 R = CH_3
15 R = H

From where did the multiple peaks in the HPLC chromatograms (Figures 1A,B) come? Without NMR structural evidence we attributed the multiple peaks, in part, to **4** (**12**) and isomeric forms of the free thiol **4** (**12**), which can include C(6)-**16**,[6] C(7)-**17**, and C(8)-**18**[7] cyclized adducts.



16 **17** **18**

A significant finding of this study was the observation of multiple peaks in the HPLC chromatograms corresponding to thiol **4** (**12**). Furthermore, we found that **4** (**12**) generation did *not* lead to aziridine ring-opened mito*senes*. This finding was surprising since it has been proposed that the terminal thiol unit in **4** (**12**) initiates conversion of the mitomycin ring system to an activated mitosene and subsequent nucleophilic (DNA) attack at the C(1) and C(10) sites.[6,7] Last, we found that **4** and **12** were efficiently converted to dimeric mitomycins **15** and **14**, respectively, at room temperature. The efficiency of **14** and **15** production may be clinically significant. Compound **15** is the major metabolite produced upon administration of **3** to normal and tumor-bearing mice.[21] Several mechanisms exist for the anaerobic dimerization of **4** to either the bis-semiquinone or mixed hydroquinone−quinone species corresponding to **15**. Formation of *reduced* **15** from either **2** or **3** leads to a novel DNA cross-linking agent in which DNA modification of complementary strands can initially proceed at the C(1) aziridine sites on the *two* mitomycin subunits. By comparison, mitomycin C cross-linking transformations require activation of the C(1) aziridine site and the C(10) position. Chemical studies have

documented the diminished reactivity of the C(10) site in reductively activated **1** versus the C(1) position.[22] Accordingly, activated dimeric mitomycins, such as reduced **15** (**14**), may permit DNA cross-linking reactions to proceed more efficiently than conventional monomeric mitomycins. Studies on the mechanistic details of this projected pathway and the sequence selectivity and structure of dimeric mitomycin−DNA adduct(s) are in progress.

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(11) Satisfactory spectral data was obtained for all previously prepared (**5**,[10a] **6**,[10b] **11**,[14] **14**,[20a] **15**[20a,b]) and new (**5**, **6**, **13**) compounds. Compounds **7** and **8** were too unstable to isolate. Treatment of either **5** or **6** with GSH provided the corresponding GSH−mitomycin mixed disulfide 7-*N*-[2-(glutathionedithio)-ethyl]mitomycin C. Additional details can be found in Wang, S. Ph.D. Thesis, University of Houston, Houston, TX, 1998.

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(16) The HPLC analyses were conducted with the following Waters Associate Units: 515 pump (pump A), 515 pump (pump B), Millennium chromatography manager, Waters 996 photodiode array detector, Rehodyne 7725i manual injector, C_{18} μBondapak (stainless steel) column (3.9 × 300 mm) using the following linear gradient 90% A (aqueous 0.025 M triethylammonium acetate, pH 6.5), 10% B (acetonitrile) isocratic for 5 min, then from 90% A, 10% B to 45% A, 55% B in 30 min. The flow rate was 1 mL/min, and the eluent was monitored from 200 to 400 nm.

(17) The identities of the major remaining peaks were determined after PTLC isolation, spectroscopic elucidation, and then subsequent confirmation of the product peak in the reaction mixture by co-injection (cospot) of the authentic sample with the reaction solution in the HPLC (TLC).

(18) A similar difference in the HPLC retention times was observed for mitomycin C (16.7 min) and porfiromycin (18.1 min).

(19) The HPLC data does not exclude multimeric adducts of **4** (**12**).

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