Bioorganic & Medicinal Chemistry 24 (2016) 4023–4031

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Design, synthesis, and mode of action studies of a mitomycin tetramer inducing double activations with a single probe

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ARTICLE INFO

Article history:
Received 14 May 2016
Revised 21 June 2016
Accepted 21 June 2016
Available online 21 June 2016

Keywords:
Mitomycin tetramer
Double activations
Disulfide cleavage
DNA alkylation
DNA interstrand cross-link

ABSTRACT

We report design, synthesis, and mechanistic studies of a new mitomycin tetramer **9** along with a new mitomycin dimer **10**. Mitomycin **9** is a tetramer connected by the disulfide linker **11**, and easily undergoes disulfide cleavage to provide two dimeric structures **9r** that each contains a single thiol probe for activations. So, tetramer **9** as a precursor of **9r** was specifically targeted to undergo *double activations with a single probe*. A tetramer **9** was synthesized using **1** and key intermediate **11**, and a dimer **10** was synthesized from **1** and diamine **12**. Activation studies revealed that **9** underwent effective double activations with a single probe by nucleophiles while the reference **10** did not. Evaluations of DNA ISC formations showed that **9** generated substantial levels of DNA ISC by nucleophilic activation while the references **10** and **2** did not. The effectiveness of **9** in activation and formation of DNA ISC per probe was verified by comparing with dimers **5**–**8** of double activations with two probes. These findings highlighted the role of a single thiol in **9r** and demonstrated the intended *double activations with a single probe*, which marks the first case in mitomycin studies.

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1. Introduction

Mitomycins constitute a class of antitumor agents that display antiproliferative activity by DNA alkylation,[1,2] and recently, studies on synthesis and mode of actions of mitomycinoid alkaloids were broadly reviewed.[2b,c] Despite the reports of mitomycin A (MMA, **1**) and its numerous derivatives, only mitomycin C (MMC, **2**) has been a useful agent of clinical importance[1,2a] (Fig. 1). Continuing efforts to overcome the drug resistance and side effects of **2**[2a] led to the findings of disulfide mitomycins **3** (KW-2149)[3] and **4** (BMS-181174).[4] In general, mitomycins have to undergo proper activation processes by which they are converted to good electrophiles for chemical reactivity and biological activities. For **3** and **4**, it was believed that a different activation mechanism through the key function of the disulfide group[5–7] in **3** and **4** led to their improved pharmacological properties and DNA adduction[6–8] compared to **2**. The thiol generated by disulfide cleavage of **3** and **4** would trigger the activation of the mitomycin ring by intramolecular cyclization to a quinone ring, which leads to the generation of highly electrophilic C(1) site (activation) that proceeds to react with DNA (DNA adduction). Hence, the thiol group could serve as a good probe to activate the mitomycin ring by nucleophilic activation mechanisms that differ from traditional and/or reductive activation mechanisms.[2a,d]

Among several types of DNA adducts of mitomycin, the DNA interstrand cross-link (DNA ISC) adducts by bis-alkylation of DNA by mitomycin have drawn special interest because they are ~60 times more lethal than the corresponding monoadducts of **2**.[9] Considering that the reactivity of C(1) site toward DNA was 10–100 times higher than that of C(10) site,[10] we focused on the dimerization of mitomycin units that would provide two C(1) sites of higher reactivity, which could eventually provide higher levels of DNA ISC by bis-alkylation of DNA by both C(1) sites. So, aiming to enhance the formation of DNA ISC, we previously reported the design, synthesis, and activation studies of strategically-designed disulfide mitomycin dimers, **5**,[11] **6**,[12] **7**,[13,14] and **8**[15] (Scheme 1). Interestingly, dimers **5**–**8** all contained a medium-sized cyclic disulfide group as a linker to connect two mitomycin units. Cleavage of disulfide group in dimers **5**–**8** would provide **5r**–**8r** (reduced form) containing two thiols that could serve as good probes to initiate the mitomycin activation, leading to double activations with two thiol probes. As a result, dimers **5**–**8** underwent fast activations (k_{obs} (d^{-1}): 2.7–16) and produced high levels of DNA ISC (83–98%) adducts, which represented the important function of thiol probes. Based on the results of double activations with two thiol probes, we became interested in the strategy of *double activations with a single thiol probe*.

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http://dx.doi.org/10.1016/j.bmc.2016.06.041
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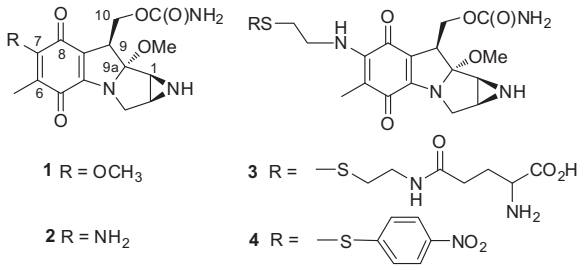


Figure 1. Structures of mitomycins **1**–**4**.

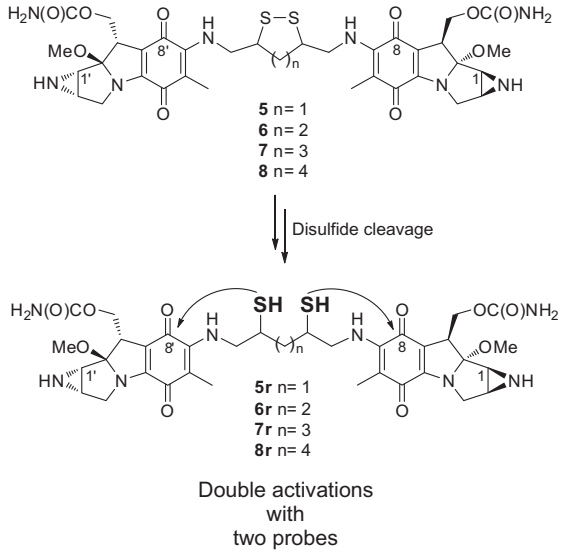
2. Results and discussion

2.1. Design of 9 and study objectives

In continuing efforts to identify the function of a thiol probe in mitomycin activations, we wished to see if the intended double activations with a single probe indeed occurred, and if so, to assess the effectiveness of the activations and DNA adductions. In addition, we were interested in whether the distance and flexibility of the linker would have any effect on those activations. As part of our studies for this purpose, we report herein new mitomycins **9** and **10** in the context of the strategy of double activations with a single probe (Scheme 2), and their comparisons with the precedent examples (e.g., **5**–**8**). Compound **9** contains several structural features associated with mechanistic aspects. First, although compound **9** is a tetramer, it could be seen as a 'dimeric dimer' since it can easily undergo disulfide cleavage (or reduction) to provide two dimeric species **9r** with a single thiol. The thiol–disulfide exchange and, in particular, the disulfide cleavage by a proper nucleophile were observed in our previous studies[11–15] and thus, expected to occur in compound **9**. As mentioned above, our primary interest lay in double activations with a single probe and we therefore aimed to create an appropriate mitomycin dimer with a single thiol probe (e.g., **9r**). However, mitomycin **9r** was believed to be very unstable due to the facile and reversible attack of the thiol to quinone ring and it was very difficult to isolate, which was consistent with the previous observation that the thiol species generated by disulfide cleavage of **3** or **4** was also very unstable.[7] Accordingly, instant generation from a disulfide precursor could be one of the best ways to create the specifically-designed dimer (e.g., **9r**) for our purpose. As a result, we envisioned dimerization of the same dimeric unit **9r** to afford dimeric dimer **9**. Second, compound **9** (and **9r**) contained a 2-mercaptoethylamino unit at C(7), which is an important structural requirement for mitomycin activation. Only in this structural condition could the thiol easily attack the quinone ring by forming a six-membered cyclic structure. Taken together, compound **9** was aimed to undergo efficient nucleophilic double activations with a single probe and corresponding facile DNA adduction. On the other hand, hydroxy mitomycin **10** contains hydroxy linker instead of disulfide linker with the same carbon skeleton as in **9r** and could therefore serve as a good reference to identify the net effect of thiol probe (or disulfide) in **9r** (or **9**). This is the first case in mitomycin studies where double activations with a single thiol probe were tested.

We also attempted to compare target mitomycin **9** with **5**–**8** in terms of the function of thiol probes and the distances and flexibilities of the linker. Although compounds **5r**–**8r** with two thiol probes seemed to be more advantageous for double activations of mitomycin rings, the two thiols in **5r**–**8r** could easily revert back to disulfide to form **5**–**8**, which might retard the activations because activations could be induced by thiol itself. In this regard, it was simply presumed that compound **9r** with a single probe could be sufficient for double activations as there was no possibility of intramolecular disulfide formation. In addition, the flexibility and distance between the two C(1) sites in **9r** would be lower and shorter, respectively, than those in **5r**–**8r**, which might affect the mitomycin activations and subsequent DNA adductions.

So, the objectives of this work were studies on design, synthesis, activation mechanism, and DNA ISC formations for **9** compared to **10**, and elucidation of the strategy of double activations with a single probe in comparison with **5**–**8**. Notably, considering **9** and **10** still contained units of mitomycin C (**2**), it was believed that these mitomycins would undergo activations under reductive[16] and acidic conditions,[17] and thus exhibit basic levels of cytotoxicity. Furthermore, since our main interest lay in the activation mechanism of these peculiar mitomycins, the cytotoxicity data of **9** and **10** were not included in the scope of our present studies. Despite comprehensive investigations of mitomycin activations, much still remains unknown about the precise activation processes and the structures of intermediates and activated products.



Scheme 1. Disulfide cleavage of mitomycin dimers **5**–**8** to give **5r**–**8r**.

2.2. Chemistry

2.2.1. Synthesis of disulfide intermediate 11

Prior to synthesis of the target mitomycins **9** and **10**, we established the synthesis of the required key intermediate, disulfide **11**[18] as shown in Scheme 3.

At first, we treated amine **12** with di-*t*-butyl dicarbonate (Boc_2O) to afford Boc-protected derivative **13**[19] in 99% yield. Next, we reacted **13** with methanesulfonyl chloride (MsCl) to obtain mesyl derivative **14**[19] in 92% yield. Subsequent replacement of the mesyl group with acetylthio group using potassium thioacetate (KSAc) in DMF gave acetylthio derivative **15**[20] along with substantial formation of a side product with higher R_f value (0.80, 1:2 EtOAc/hexanes) than that (0.70) of product **15**. The optimized yield of this step was 61% and further efforts to improve the yield and identify the side product were not successful. It was believed that the adjacent Boc-amino group might interfere with the replacement process, which was also observed in the previous example of 1,5-bis (*tert*-butyloxycarbonylamino)-2,4-pentanediol dimethanesulfonate.[11] Hydrolysis of **15** by potassium carbonate (K_2CO_3) was then conducted to afford thiol derivative **16**.[20] Although the reaction seemed to proceed smoothly, the yield was generally low (less than 50%) probably due to the instability of thiol species, which is consistent with our previous results that thiol could undergo easy

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