Anticancer Drugs

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# Synthesis-Enabled Probing of Mitosene Structural Space Leads to Improved $IC_{50}$ over Mitomycin C**

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***Abstract:*** *A DNA crosslinking approach, which is distinct but related to the double alkylation by mitomycin C, involving a novel electrophilic spiro-cyclopropane intermediate is hypothesized. Rational design and substantial structural simplification permitted the expedient chemical synthesis and rapid discovery of MTSB-6, a mitomycin C analogue which is twice as potent as mitomycin C against the prostate cancer cells. MTSB-6 shows improvements in its selective action against noncancer prostate cells over mitomycin C. This hypothesis-driven discovery opens novel yet synthetically accessible mitosene structural space for discovering more potent and less toxic therapeutic candidates.*

Mitomycin C (MMC, Figure 1), a chemotherapeutic agent isolated from extracts of genus *Streptomyces*,[1] crosslinks DNA and possesses potent antitumor and antibiotic activities.[2] It has been studied since the 1960s for the treatment of many types of soft and solid tumors,[3] but its use has been restricted because of dose-limiting toxicity and delayed myelosuppression, among other side effects.[2c] Although MMC is readily available by fermentation,[1,4] efforts to improve its therapeutic index through direct modification have been largely limited to the C7,[5] C6,[6] C10, and N1a[7] positions and minor structural perturbations resulting from the delicate and generally sensitive structure, especially under acidic conditions. To date, there have been only a few derivatives with improved efficacy and/or decreased toxicity,[5a,b,6] but none has reached the market. In contrast, the chemical synthesis of MMC,[8] which has a challenging array of densely organized functional groups including the strained aziridine ring, is a daunting challenge. While several elegant total syntheses[9] have been achieved, access to new structural space pertaining to the MMC core and in particular structural analogues, made available by these synthetic routes, has been hampered by long synthetic sequences and/or low overall yields. In addition, analogous quinone-containing agents with dramatically simplified structures, but still maintaining reductive alkylating capacities, have also failed because of high toxicities or lost anticancer activity in vivo.[10] Herein, we report a succinct and efficient preparation of simplified structural analogues of MMC, in the forms of mitosenes, based on a novel mechanism-driven design. Importantly, one of these compounds is twice as potent as MMC against PPC prostate cancer cell lines, yet shows similar toxicity against RWPE-1 normal cell lines.

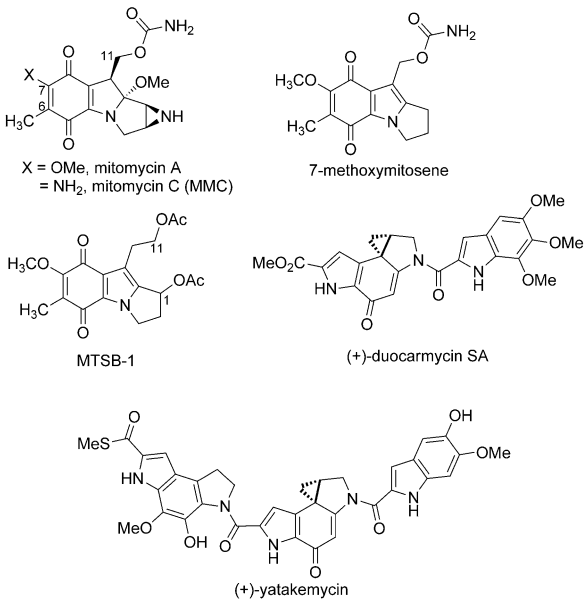


***Figure 1.*** Mitomycins, 7-methoxymitosene, the previously prepared mitosene analogue MTSB-1, and natural products employing spiro-cyclopropane as the DNA alkylating site.

The established mechanism of action for MMC (Scheme 1 A) is initiated by a cellular reduction of the quinone moiety, followed by the formation of 7-aminoleucoaziridinomitosene upon elimination of MeOH, and culminates with mono- or bisalkylation of DNA, where bisalkylation often results in crosslinking of complementary DNA strands and is the irreversible lethal lesion.[2b] To deviate from the details and yet stay true to the essence of this mechanism, we contrived an approach based on a biologically unprecedented, but chemically relevant modification. As shown in Scheme 1 B, a mitosene with a generic structure (**1**) should, similar to that of MMC, undergo an initial reduction of its quinone moiety to trigger the expulsion of the leaving group (LG) at C1 (mitosene numbering), thereby generating a highly electrophilic site at the same position. A subsequent reaction with DNA would result in the first DNA alkylation. A second alkylation, however, would be mechanistically distinct from that of MMC. Instead of an eniminium intermediate (i.e., **A**),

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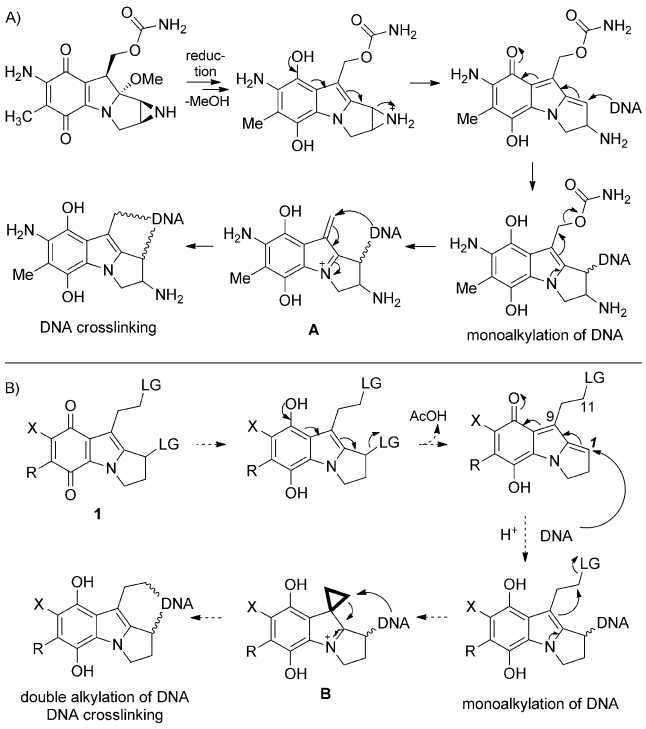



***Scheme 1.*** A) Established mechanism of DNA crosslinking by MMC. B) Our hypothesized mechanism of action for the mitosene derivative MTSB-1.

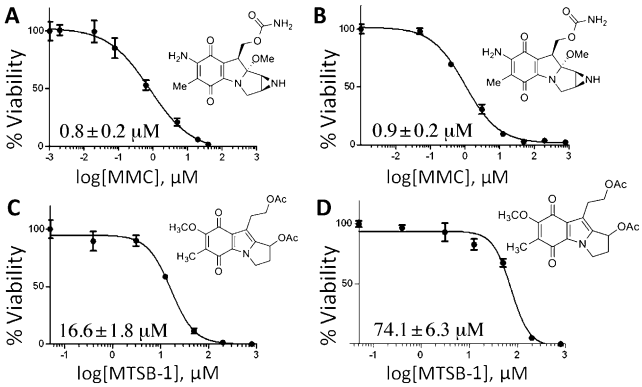



***Figure 2.*** $IC_{50}$ curves and values for MMC against PPC-1 cells (A) and RWPE-1 cells (B), and MTSB-1 against PPC-1 cells (C) and RWPE-1 cells (D).

the expulsion of the second LG, if feasible, would generate a spiral electrophilic cyclopropane species (e.g., **B**). We reasoned that the strained ring could behave like a Michael-type receptor and react with another base of the same DNA molecule, thereby realizing a second alkylation. It needs to be pointed out that the order of these two alkylation events could be reversed.[11] Nevertheless, the introduction of a new alkylation mechanism via the cyclopropane intermediate **B**, while maintaining the reductive initiation, would make hitherto unexplored chemical space around the MMC/mitosene skeleton available for addressing the issues associated with MMC.

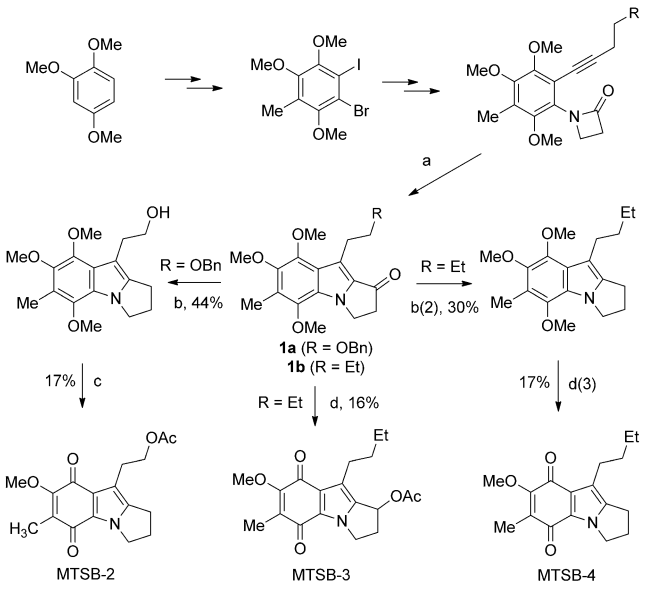
We recently reported a formal synthesis of 7-methoxymitosene and an expedient preparation of a related bis(acetate), that is, MTSB-1 (Figure 1).[12] While the former compound was previously shown to only possess antibacterial activities,[13] the latter structure, as a new entry of mitosenes, is suitable for evaluating our hypothesis outlined in Scheme 1 B as AcO might behave as a LG. We chose the prostate cancer cell line (PPC-1) and the normal prostate cell line (RWPE-1), which are frequently used to study the potency and toxicity, respectively, of chemotherapeutics.[14] Much to our delight, MTSB-1 inhibits proliferation of the PPC-1 prostate cancer cell at reasonably low concentrations ($IC_{50}$: $16.6 \pm 1.8$ μM, Figure 2 C), while its $IC_{50}$ value against the RWPE-1 normal prostate cell line [$(74.1 \pm 6.3)$ μM, Figure 2 D] is more than four times higher. We selected RWPE-1 because these immortalized normal cells divide more slowly than cancer cell lines and can provide information about a compound's off-target toxicity. In comparison, MMC is over an order of magnitude more potent against the PPC-1 prostate cancer cell line with an $IC_{50}$ value of $(0.8 \pm 0.2)$ μM (Figure 2 A), which is similar to other reported values.[15] However, little difference could be observed for MMC against the normal RWPE-1 prostate cells [$IC_{50}$: $(0.9 \pm 0.2)$ μM, Figure 2 B], which is consistent with the considerable off-target toxicity demonstrated by MMC in vivo. Of interest is that MTSB-1 did not show much antibiotic activity (for details, see the Supporting Information).

As MTSB-1 is the first of its kind, these hypothesis-driven initial results, especially considering its substantially lower off-target cytotoxicity, are very encouraging, and offer circumstantial support for the proposed mechanism (Scheme 1 B), despite its notable structural deviations and simplification compared to that of MMC. The structural changes include replacing the strained aziridine ring with a readily installable 1-acetoxy group and inserting an additional $CH_2$ group at the C9 side chain.

To further probe the mechanism of action of MTSB-1, and hence lay the groundwork for improving its efficacy, we synthesized three of its structural variants, that is, MTSB-2, MTSB-3, and MTSB-4, as mechanistic probes by following a similar synthetic strategy (Scheme 2).[12] Hence, MTSB-2, which has the AcO group at C1 removed, was prepared from the previously reported tricyclic, fully substituted indole **1a**[12] by a four-step sequence: debenzylative hydrogenolysis, the Wolff–Kishner deoxygenation, acetylation of the free OH group, and finally oxidative quinone formation. MTSB-3, which has an ethyl group replacing the AcO at C10, was readily prepared from the requisite tricyclic indole **1b** upon sequential reduction, acetylation, and oxidative quinone formation. MTSB-4, which does not have any AcO group, was accessed in two steps from **1b**. Notably, in all the preparations, the final oxidation of the electron-rich benzene ring suffered from low efficiencies as a result of a myriad of side reactions, despite many efforts to improve it.

We reasoned that if MTSB-1 exerts its cytotoxicity by crosslinking DNA (as proposed in Scheme 1 B), these mechanistic probes would be rendered much less cytotoxic because of the removal of one or both alkylating site(s). The $IC_{50}$ values of the three analogues were determined against




***Scheme 2.*** Synthesis of several mechanistic probes of MTSB-1. Reaction conditions: a) $PtCl_2$ (0.3 equiv), $O_2$ (1 atm), DCE, 80°C, 12 h. B) 1. Pd/C, $H_2$ (1 atm), THF, RT, 12 h, 88%; 2. $N_2H_4$ (2 equiv), $K_2CO_3$ (15 equiv), ethylene glycol, 180°C, 6 h, 50%. c) 1. AcCl (1.5 equiv), $Et_3N$ (3 equiv), $CH_2Cl_2$, 0°C to RT, 83%; 2. $NaNO_2$ (0.1 M, 1.5 equiv), HCl (1 M), $CHCl_3$, RT, 16 h, 20%. d) 1. $NaBH_4$ (2 equiv), MeOH, RT, 2 h, 91%; 2. AcCl (1.5 equiv), $Et_3N$ (3 equiv), $CH_2Cl_2$, 0°C to RT, 84%. 3. AgO (3 equiv), $HNO_3$ (6 M), THF, RT, 5 min. DCE = 1,2-dichloroethane, THF = tetrahydrofuran.

the PPC-1 prostate cancer cell line. As shown in Figure 3, MTSB-2, MTSB-3, and MTSB-4 have $IC_{50}$ values of (166.5 ± 28.6) μM, (183.0 ± 25.7) μM, and > 200 μM, respectively, which are all tenfold, or more, less potent than MTSB-1, and hence are all largely ineffective in decreasing cell viability. Likewise,

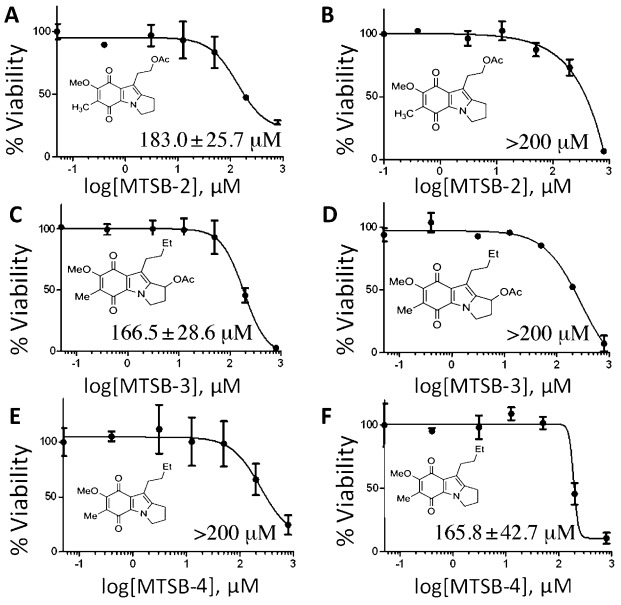
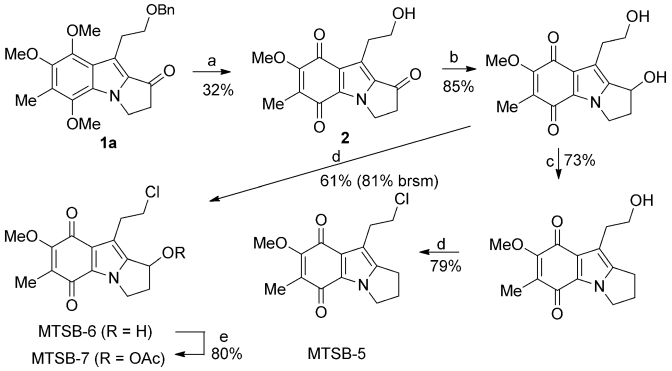



***Figure 3.*** $IC_{50}$ curves and values for MTSB-2 against PPC-1 cells (A) and RWPE-1 cells (B), MTSB-3 against PPC-1 cells (C) and RWPE-1 cells (D), and MTSB-4 against PPC-1 cells (E) and RWPE-1 cells (F).

they were ineffective against the RWPE-1 normal prostate cell line, with $IC_5$ values close to or over 200 μM (Figure 3). These data suggest that MTSB-1 most likely double alkylates DNA, and in turn supports our hypothesis that the spirocyclopropyliminium **B** would be a key intermediate in DNA alkylation.

To provide further support for the involvement of the spiro-cyclopropane of type **B** in DNA alkylation, we designed a chloro analogue, that is, MTSB-5, wherein the AcO at C1 of MTSB-1 is removed and the AcO at C10 is replaced by a chloride. Its synthesis is outlined in Scheme 3. To improve




***Scheme 3.*** Synthesis of the chloride derivatives MTSB-5–MTSB-7. Reaction conditions: a) 1. Pd/C, $H_2$ (1 atm), THF, RT, 80%; 2. $NaNO_2$ (0.1 M, 1.5 equiv), HCl (1 M), $CHCl_3$, RT, 16 h, 40%; b) $NaBH_4$ (3 equiv), MeOH, RT, 2 h, then add sat. aq. $NH_4Cl$, stir in air; c) $Et_3SiH$ (2 equiv), $CF_3COOH$ (5 equiv), $CH_2Cl_2$, 0°C, 1 h, then TBAF, 73%; d) triphosgene (0.5 equiv), $Et_3N$ (2.5 equiv), $CH_2Cl_2$, 0°C to RT; e) AcCl (1.5 equiv), pyridine (3 equiv), $CH_2Cl_2$, 0°C to RT, 80%. TBAF = tetra-*n*-butylammonium fluoride.

the low efficiencies encountered in the previous oxidative quinone formations, it was attempted after the debenzylation of **1a** and before reductive manipulation of the electron-withdrawing C1 carbonyl group. An improved 40% yield (two-step, 32% yield from **1a**), upon isolation, of the corresponding indoloquinone **2** was achieved. Subsequent two-step reductive deoxygenation of the ketone moiety followed by the treatment with triphosgene smoothly delivered the target molecule.

As chloride is a better leaving group than acetate, an alkylating species of type **B** would be formed more readily, in accordance to our hypothesis. Hence, MTSB-5 should at least show a lower $IC_{50}$ value than that of MTSB-2. Much to our delight, MTSB-5 has a low $IC_{50}$ value of (0.65 ± 0.15) μM against PPC-1 prostate cancer cells (Figure 4 A), thus showing much higher potency than MTSB-2 and even slightly improved potency over MMC. While monoalkylation of DNA by mitomycin may be repairable and thus nonlethal, this result is nevertheless in line with the high anticancer potency of (+)-yatakemycin[16] and (+)-duocarmycin SA[17] (Figure 1), which monoalkylate DNA through a reactive spiro-cyclopropane ring. This reasoning, in turn, is consistent with the involvement of a spiro-cyclopropane intermediate, related to **B**, in the hypothesized mechanism of action and implicates a DNA alkylating site different from those of

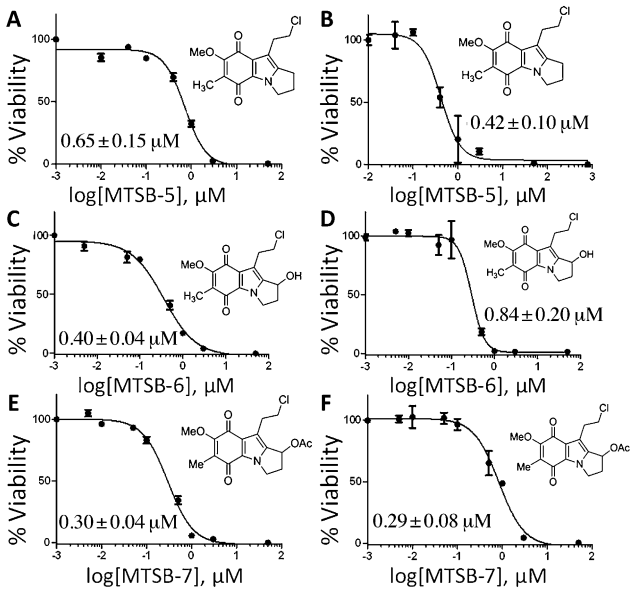



***Figure 4.*** $IC_{50}$ curves and values for MTSB-5 against PPC-1 cells (A) and RWPE-1 cells (B), MTSB-6 against PPC-1 cells (C) and RWPE-1 cells (D), and MTSB-7 against PPC-1 cells (E) and RWPE-1 cells (F).

MMC. Like MMC, MTSB-5 shows similar potencies against both PPC-1 and RPWE-1 cell lines (Figure 4B).

Because DNA crosslinking may be more lethal to cells than the related monoalkylation in the cases of MMC and MTSB-1, installation of an additional alkylating site to MTSB-5 could enhance cytotoxicity. Two such compounds, MTSB-6 and MTSB-7, with an OH group and an AcO group, respectively, at C1 of the MTSB-5 structure were readily prepared (Scheme 3). Indeed, MTSB-6 and MTSB-7 demonstrated increasing efficacy against PPC-1 cells with improved $IC_{50}$ values of $(0.40 \pm 0.04)$ μM and $(0.30 \pm 0.04)$ μM, respectively (Figures 4C and E). In contrast to to MTSB-7, which showed no difference in potency against the RWPE-1 cell line [$IC_{50}$ of $(0.29 \pm 0.08)$ μM, Figure 4F], MTSB-6 was twofold less cytotoxic against RWPE-1 [$IC_{50}$: $(0.84 \pm 0.20)$ μM, Figure 4D] than against PPC-1. These results may form the basis of rationally improving the potency of these analogues while lowering the toxicity. Notably, in contrast to the over tenfold increase of potency of MTSB-1 over MTSB-2 by the incorporation of 1-OAc, the improvement was very moderate in the case of MTSB-7 over MTSB-5. It is possible that with a much better leaving group, such as chloride, a cyclopropyl intermediate related to **B** would first alkylate DNA, and is highly cytotoxic as evident in the case of MTSB-5. As such, a second alkylation enabled by the 1-OAc group might only lead to the observed marginal improvement.

In conclusion, we have advanced a hypothesis that, different from the mode of DNA double alkylation by mitomycin C, structurally related yet novel mitosene derivatives could likewise crosslink DNA via a novel electrophilic spiro-cyclopropane intermediate. Rational design and substantial structural simplification permits rapid access to some preliminary examples of the mitosenes, that is, MTSB-1–MTSB-7. Their cytotoxicity assays against the PPC-1 prostate cancer cell line and the RWPE-1 normal cell line yield $IC_{50}$ values which are consistent with the hypothesis. Among them, MTSB-6 exhibits twice as high a potency against PPC-1 as does mitomycin C, but similar toxicity against RPWE-1. The facile synthesis of these mitosenes and the promising potency and toxicity data open up novel mitosene structural space for systematic optimization and thus the potential for developing a new class of anticancer drugs.

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