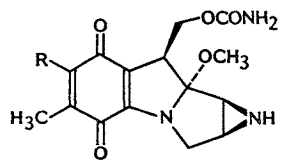
SYNTHESIS AND *IN VIVO* ANTITUMOR ACTIVITY OF NOVEL MITOMYCIN A DISULFIDE ANALOGS

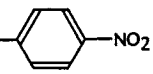
Sir:

Recent research efforts directed towards identifying a more active and a less myelosuppressive, second generation mitomycin C (MMC; **1**) analog has led to the discovery of new chemistry[1] and novel analogs[2] of MMC. In this regard, of particular significance is the synthesis of a novel disulfide MMC analog, namely 7-(*para*-nitrophenyl)dithioethylamino-MMC (**2**)[3]. Its favorable antitumor biological profile[4] has led to its selection as a candidate for clinical evaluation. On the basis of limited literature precedent[5] that analogs in the mitomycin A (MMA) series (*e.g.* **3**) are more potent and potentially more efficacious than their MMC series (*e.g.* **4**) counterparts, we embarked on the synthesis of **5** and other analogs in this general class. SAMI *et al.*[5] have demonstrated in one instance that the alcoholysis procedure (R_1OH - KOH) of URAKAWA *et al.*[6] can be successfully employed in the synthesis of a MMA disulfide analog **3** ($R_1=CH_2CH_2SSCH_2CH_2OH$) from MMA (**3**, $R_1=CH_3$). The main limitation of this technology as mentioned by the authors is that the displacing alcohol (R_1OH) has to be a liquid. Considering the ease with which unsymmetrical disulfides undergo disproportionation reactions[7] to symmetrical disulfides under basic conditions, coupled with the fact that MMA (**3**, $R_1=CH_3$) and thiols are incompatible in a reaction due to their oxidation-reduction reaction[8], we sought a milder and a more general method to synthesize **5** and other analogs in this class. In 1986 we reported a new synthetic approach[9] involving a triazene assisted *O*-alkylation of 7-hydroxy-MMC (**3**, $R_1=H$)[6] to prepare MMA (**3**, $R_1=CH_3$) and other selected 7-alkoxy derivatives in this series. In this communication, we report a successful extension of this technology to the synthesis of a series of MMA disulfide analogs including **5** and their *in vivo* antitumor biological activity against P388 murine leukemia.

For the successful application of the triazene mediated *O*-alkylation procedure, the key reagent, 3-nitro-2-pyridyldithioethyltriazene **8** was synthesized by reacting a known disulfide **6**[10] with 3-nitro-2-mercaptopyridine[11] using the BROIS procedure[10] followed by subsequent treatment of the product (**7**) with 4-methylphenyldiazonium chloride[12]. With the reasonably stable triazene **8** in hand, synthesis of MMA disulfide analogs **5**, **10**~**15** was readily accomplished in three steps from MMC (**1**) as outlined in Scheme 1. All new compounds synthesized in this study were fully characterized analytically. The salient

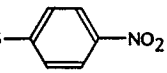


1 R = NH_2

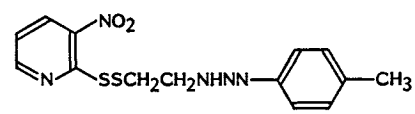
2 R = $NHCH_2CH_2SS$—  —NO_2

3 R = OR_1

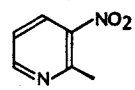
4 R = NHR_1

5 R = OCH_2CH_2SS—  —NO_2

$RSSCH_2CH_2NH_2 \cdot HCl$



6 R = $COOCH_3$

7 R = 

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Scheme 1.

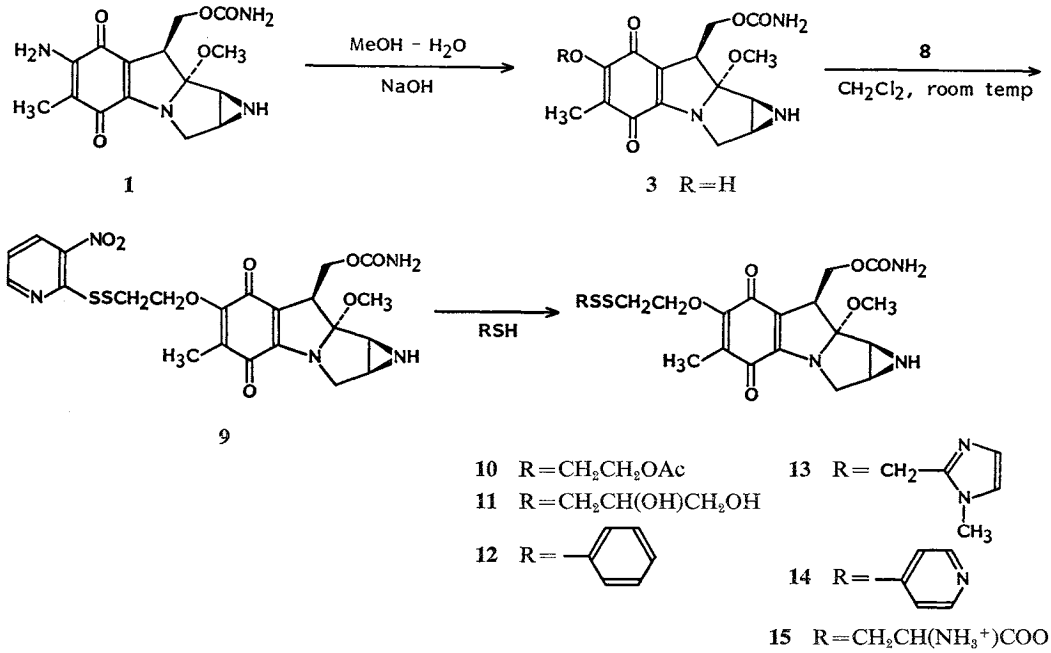

Table 1. Activity of MMA disulfide analogs against P388 leukemia in mice[a].

Compound	Expt No.	Dose (mg/kg/injection)[b]	Max (%T/C[c]) (MMC)	T/C ratio (analog/MMC)
5	A	12.8	178 (>333[d])	≦0.53
9	A	6.4	206 (>333[d])	≦0.61
10	B	3.2	156 (233)	0.66
11	C	12.8	167 (172)	0.97
12	D	12.8	155 (200[e])	0.78
13	D	12.8	>232[d](200[e])	≧1.16
14	D	6.4	127 (200[e])	0.64
15	D	12.8	168 (200[e])	0.84

[a] P388 leukemia, 10^6 cells, were implanted ip on day-0 into CDF_1 mice.

[b] Optimum dose (achieving highest T/C), at non-toxic levels, administered ip on day-1 post-leukemia implant.

[c] % T/C: Median survival time (MST) in days of treated animals/MST of controls, ×100. The MST of each control group was 9.0 days except for 11.0 days in expt No. D.

[d] Three or four mice of six alive at experimental termination (day-30) but each with residual leukemia determined by autopsy.

[e] Includes one of six mice cured (free of overt leukemia) as of day-30.

features of this new process are a) the disulfide mitosane intermediate **9** is obtained directly from **3** (R=H) by circumventing the intermediacy of MMA (**3**, R=CH_3); and b) intermediate **9** readily facilitated the synthesis of disulfide MMA analogs **5**, **10**~**15** *via* the well precedented thiol-exchange reaction[13] which involved the displacement of the nypsl (3-nitro-2-thiopyridyl) group with appropriate thiols. It is noteworthy in these thiol-exchange reactions that the displacement reaction is faster than the redox reaction[8] between thiol and the quinone moiety. The reaction medium for the thiol-exchange reaction depended upon the nature of the reacting thiol and the final product. Thus for compounds **5**, **10**~**14** which involved the use of lipophilic thiols, the reaction was performed in acetone containing triethylamine. In contrast, for the water soluble analog **15**, 2~

5% methanol in acetone containing aqueous sodium bicarbonate was employed as the reaction medium. Thus, in comparison to the alcoholysis process[5], the methodology reported herein offers a mild and a very general approach to the synthesis of novel disulfide MMA analogs.

The antitumor activity of disulfide MMA analogs synthesized in this study *versus* P388 leukemia in mice are shown in Table 1. The assays were not performed concurrently but each compound was evaluated in a full dose-response manner against MMC (**1**) which was also evaluated at several dose levels in each experiment; % T/C values shown in the parentheses were those recorded concomitantly for **1** at its optimal dose. Perusal of the activities (% T/C values) revealed that none of the compounds with the possible exception of **13**, showed superior activity to the parent drug **1**. Moreover, the expected increase in potency for this class (MMA type) of compound was not observed. In particular, although not evaluated in parallel, the activity and potency of compound **5** when compared to the reported activity and potency of **2** (% T/C >331 achieved at 12.8 mg/kg)[3,4] was surprising. A full biological structure-activity relationship evaluation of the compounds in the present series in comparison to their counterparts in the MMC series will be reported separately elsewhere.

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