

Synthesis and cytotoxic evaluation of uracil C-Mannich bases

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This study covers the design, synthesis, characterization, and cytotoxic activity of a series of uracil C-Mannich bases. Among them, one hybrid compound (**1**), a molecular combination of the potential antimetabolite substituted uracil and nitrogen mustard, having potential alkylating capability, was prepared as a Mannich base. The other compound was synthesized with the replacement of chlorines in the ethyl chains with hydroxyl groups for testing for anticancer activity. Some of Mannich bases having several amino groups with different pKa values were also synthesized and investigated in terms of cytotoxic activity. Their chemical structures were confirmed by means of their UV, IR, ¹H-NMR, ¹³C-NMR, and MS data. Compounds **6** with diethylamine and **8** with piperazine are reported for the first time in the literature and compounds **1**, **4**, and **5** containing nitrogen mustard, pyrrolidine, and diethanolamine, respectively, as amine function are reported for first time with detailed spectral data herein. Morpholine, piperidine, and dimethylamine were used in Mannich reactions for the synthesis of compounds **2**, **3** and **7**. We assessed their biological activities using MTT assays on 3 human cell lines: HeLa (cervix adenocarcinoma), MCF7 (breast adenocarcinoma), and A431 (skin epidermoid carcinoma). While compounds **2-8** have the potential to deaminate, forming *ortho*-quinone methides, which would be capable of alkylating cellular thiols, compound **1** has the potential to give aziridinium ion for nucleophilic alkylation. Our results are discussed in terms of the significance of these compounds in pharmaceutical use.

Key Words: Uracil, Mannich base, antiproliferative effect, anti-cancer compounds

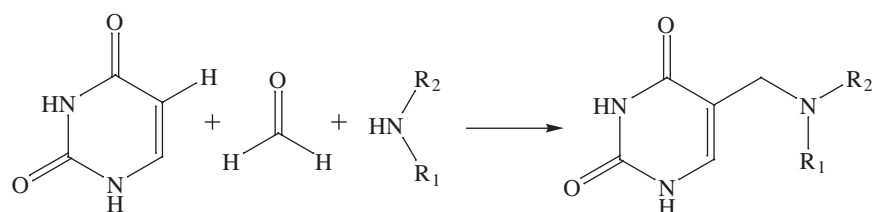
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Introduction

Mannich bases are pharmacologically important molecules as they have been reported to show a wide range of bioactivities such as antineoplastic,^{1–5} diuretic,⁶ antipsychotic,⁷ anticonvulsant,^{8–10} central acting muscle relaxant,¹¹ antibacterial,^{12–14} antimalarial,^{15,16} and antiviral¹⁷ activities. A number of modifications of Mannich bases gave rise to improved aqueous solubility and hydrolytic stability.¹⁸ Among the modifications, alkylation is of special importance as it plays an important role in the biogenesis of nucleic acids. Therefore, incorporation of alkyl groups received increasing attention for chemotherapeutic control of neoplastic diseases. In addition, uracil molecule occupies an important position inasmuch as it is a primary component of the nucleic acid moiety. Hence to combine uracil and alkylating compounds in a unique molecule seemed to us a logical approach for designing some new cytotoxic compounds. It has been reported that nitrogen mustard, known as an alkylating agent, varies in its alkylating properties depending on whether it is directly attached to an aromatic or pseudoaromatic ring system or to an aliphatic system.^{19,20} The results concerning the comparative alkylating activities of the studied Mannich bases clearly indicated that all the nitrogen mustards reacted similarly to mechlorethamine but differently from uracil mustard showing enamine conjugation.²¹ In the case of the uracil mustard, the reactivity of the alkylating functional group was similar to the findings reported by Bardos and co-workers for aromatic nitrogen mustards.²² They proposed that in the aromatic nitrogen mustard series the alkylating activity is strikingly dependent on the basicity of the nitrogen atom. It seems to us that the methylene bridge between uracil and mustard moieties will contribute to aziridinium ion formation. It has been reported that uracil-5-mustard having methylene bridge (C-Mannich base of uracil) between the alkylating nitrogen mustard group and uracil moiety (**1**) inhibits the mitotic division in HeLa cells by at least 2 mechanisms: (i) its incorporation into DNA, and (ii) its capacity to injure mitotic processes.^{23,24} As a thymine derivative 5-((*bis*(2-chloroethyl)amino)methyl)uracil hydrochloride exhibited a marked cytostatic activity.²⁵ These data prompted researchers to compare the alkylating activity of compounds with or without methylene bridge between the alkylating functional group and uracil moiety.

Over the years there has been continuing interest in analogs of thymine that might have cytotoxic activity. Thus, compounds such as 5-fluorouracil,²⁶ 5-trifluoromethyluracil,²⁷ and 5-mercaptopomethyluracil²⁸ are effective as inhibitors of cell growth. In view of the biological significance of these 5-substituted uracils we became interested in extending the derivatization at the 5 position of uracil by the use of the Mannich reaction. There exists a close parallel between *in vivo* thymidine 5-phosphate synthesis and the Mannich reaction. The Mannich reaction has been applied to 6-methyluracil²⁹ in addition to uracil.^{30,31} Accordingly, we synthesized a series of 5-substituted aminomethyluracil derivatives (Scheme 1) in order to ascertain the scope of the reaction as well as any differences the side chain might have on biological activity.

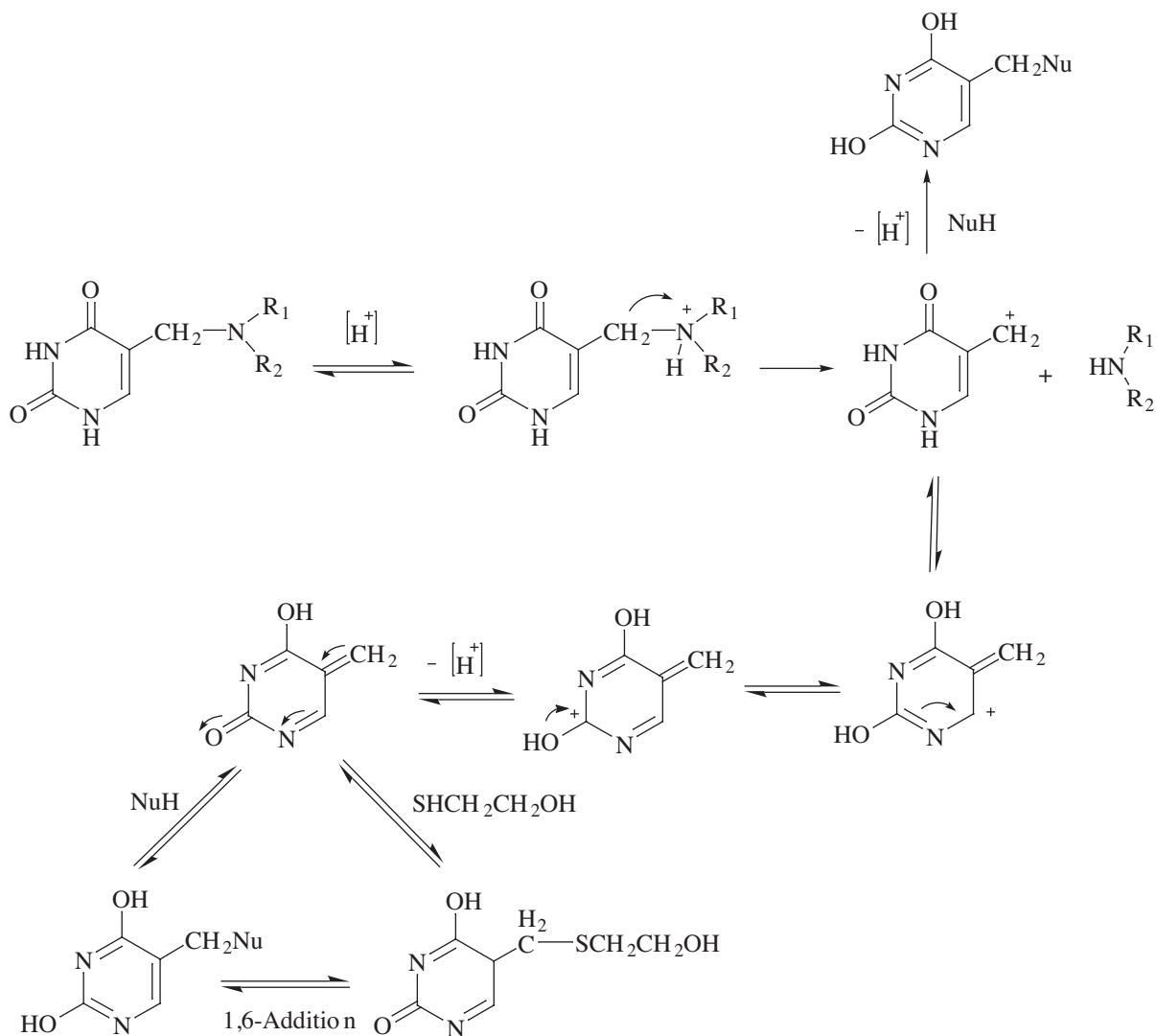
The aim of this study was to synthesize and biologically evaluate a number of Mannich bases derived from uracil ring (Scheme 1). Firstly, *bis*(2-chloroethyl)amine was chosen as an alkylating agent (**1**) since nitrogen mustard derivatives like mechlorethamine are very well known compounds having alkylating potency upon aziridinium ion.³² To preserve the aliphatic character of the nitrogen mustard group, we did not intend to attach the mustard nitrogen directly to the uracil ring. Several aminomethylation positions of the pyrimidine ring can be expected, since there are several possible alkylation sites that exhibit quite different acidities and basicities. C-aminomethylation is favored by acidic conditions, whereas N-Mannich bases are produced when free amine and formaldehyde in anhydrous solvents are employed as aminomethylating agents.³¹ Thus the



Compound	R Groups	Compound	R Groups
I	$R_1=R_2= -CH_2CH_2Cl$	V	$R_1=R_2= -CH_2CH_2OH$
II	$R_1=R_2= -C_2H_4OC_2H_4 -$	VI	$R_1=R_2= -CH_2CH_3$
III	$R_1=R_2= -C_5H_{10} -$	VII	$R_1=R_2= -CH_3$
IV	$R_1=R_2= -C_4H_8 -$	VIII	$R_1=R_2= -C_2H_4NHC_2H_4 -$

Scheme 1. The synthesis pathway of the compounds by Mannich reaction.

corresponding heterocyclic C-Mannich base can be obtained by arranging reaction time, reaction solvent, and temperature. Our investigations were not extended to N-1, N-3 substituted Mannich base considering these derivatives cannot form hydrogen bonds with the uracil-specific carriers or enzymes. Secondly, we prepared different Mannich bases not only including mustard function giving aziridinium ion as an alkylating agent but also using some other secondary amines that were not able to give aziridinium ions. In this case the action mechanism of the compounds should be different from that of nitrogen mustard derivatives. The deamination reaction might be observed in the case of practically all C-Mannich bases; it possesses both theoretical and practical interest. Usually Mannich base derived aliphatic or aryl aliphatic ketones give rise to activated α, β -unsaturated ketones by deamination in the first step, which is very sensitive for Michael addition to alkylate certain cellular constituents like thiol function.³³ In addition, deamination of Mannich bases on the phenyl ring to the corresponding *ortho*-quinone methides is conceivable.¹ These enones and *ortho*-quinone methides have high affinities for thiols, which are absent in nucleic acids. Hence in contrast to currently available alkylating agents used in cancer chemotherapy, interactions with nucleic acids may be absent and side effects such as mutagenicity and carcinogenicity may be prevented. Another reason for undertaking this study was selective toxicity, because certain cancer cells have a lower pH than the corresponding normal cells and hence molecules that form alkylating species under acidic conditions may have selective toxicity towards neoplastic tissues.³⁴ The compounds described in this report were designed as prodrugs of alkylators except compounds **1** and **2**. Our proposed alkylation mechanism of the compounds is illustrated in Scheme 2. According to the mechanism, compounds might generate carbocation and quinone methide intermediates having alkylator properties. It can be expected that pKa values of amine functions might have an effect on the deamination process of Mannich bases. Finally, another objective of the present investigation was to evaluate their cytotoxic efficacy.



Scheme 2. The putative pathways for the formation of alkylating species carbocation and quinone-methide of uracil-Mannich bases in acidic medium.

Experimental

Chemistry

Melting points were determined with a capillary melting point apparatus (Buchi 510, BUCHI, Switzerland) and are uncorrected. UV spectra were recorded on a spectrophotometer in methanol solution (UV-160, Shimadzu, Japan). The IR spectra were obtained using a Jasco FT/IR-430 spectrophotometer (JASCO, Japan) as potassium bromide pellets. The NMR spectra (400 MHz for ^1H and 100 MHz for ^{13}C) were recorded in appropriate solvents without TMS (AS 400 Mercury Plus NMR Varian, USA). Chemical shifts were measured as parts per million (δ) and the J values given in Hz. A Memmert water bath with shaker attachment

was used in the stability study. The MS was determined on an LC/MS Waters 2695 Alliance Micromass ZQ. TLC was developed routinely using Merck silica-gel plates (Kieselgel 60F₂₅₄) and the solvent systems of chloroform/methanol/ammonium hydroxide (4:2:0.1) was used.

General procedure for synthesis of the compounds

Compounds were synthesized as described before²¹. A suspension of uracil (0.01 mol), amine (0.02 mol), and formalin (0.022 mol) in THF was stirred at room temperature for 24 h. The reaction mixture was heated under reflux at 70 °C for 72 h. Removal of the solvent in vacuo produced the crude residue, which was recrystallized from methanol/ether or ethyl acetate/ether to yield the desired Mannich bases.

5-((bis(2-chloroethyl)amino)methyl)uracil hydrochloride (1)

Yield 35%; mp 222 °C (lit. 224-226 °C);³⁵ UV λ_{max} (nm) (log ϵ) 211 (3.921), 263 (4.066); IR ν_{max} (cm⁻¹) 2978, 1714, 1675, 638, 1464, 918; ¹H-NMR (CDCl₃) δ 7.79 (s, 1H, CCHNH), 4.25 (s, 2H, CCH₂N), 4.04 (t, 4H, J =5.9 Hz, CH₂CH₂Cl), 3.66 (t, 4H, J =5.9 Hz, CH₂CH₂Cl); ¹³C-NMR (CDCl₃) δ 166.2 (NHCO), 152.6 (NHCONH), 146.8 (CCHNH), 101.6 (COCH), 55.0 (CH₂CH₂Cl), 52.0 (CCH₂N), 37.7 (CH₂CH₂Cl); ESI-MS 266.10 [M+1]⁺.

5-(morpholinomethyl)uracil (2)

Yield 52%; mp 208 °C (lit. 217 °C);³⁰ UV λ_{max} (nm) (log ϵ) 207 (4.295), 263 (3.972); IR ν_{max} (cm⁻¹) 3215, 3039, 2813, 1722, 1670, 1450, 1111, 867; ¹H-NMR (CD₃OD) δ 7.39 (s, 1H, CCHNH), 3.67 (t, 4H, J =4.8 Hz, CH₂OCH₂), 3.23 (s, 2H, CCH₂N), 2.47 (t, 4H, J =4.8 Hz, CH₂NCH₂); ¹³C-NMR (CD₃OD) δ 165.5 (NHCO), 152.2 (NHCONH), 141.4 (CCHNH), 108.4 (COCH), 66.6 (CH₂OCH₂), 53.3 (CCH₂N), 53.2 (CH₂NCH₂); ESI-MS 212.10 [M+1]⁺.

5-(piperidinomethyl)uracil (3)

Yield 61%; mp 149 °C (lit. >300 °C);³⁰ UV λ_{max} (nm) (log ϵ) 208 (4.013), 263 (3.752); IR ν_{max} (cm⁻¹) 3282, 2938, 1732, 1706, 1669, 1447, 1067; ¹H-NMR (DMSO-d₆) δ 7.39 (s, 1H, CCHNH), 3.24 (s, 2H, CCH₂N), 2.46 (t, 4H, J =4.8 Hz, CH₂NCH₂), 1.62-1.44 (m, 6H, CH₂CH₂CH₂CH₂CH₂); ¹³C-NMR (DMSO-d₆) δ 165.7 (NHCO), 152.5 (NHCONH), 142.3 (CCHNH), 108.2 (COCH), 53.8 (CH₂NCH₂), 53.2 (CCH₂N), 25.6 (CH₂CH₂CH₂), 25.3 (CH₂CH₂CH₂); ESI-MS 210.00 [M+1]⁺.

5-(pyrrolidinomethyl)uracil (4)

Yield 32%; mp 210 °C (lit. 201 °C);³⁶ UV λ_{max} (nm) (log ϵ) 215.6 (2.477), 263.8 (1.930); IR ν_{max} (cm⁻¹) 3037, 2962, 1731, 1668, 1450, 1207, 856; ¹H-NMR (CD₃OD) δ 7.41 (s, 1H, CCHNH), 3.40 (s, 2H, CCH₂N), 2.61 (p, 4H, J_1 =3.2, J_2 =7.2 Hz, CH₂NCH₂), 1.81 (p, 4H, J_1 =3.2, J_2 =7.2 Hz, CH₂CH₂CH₂CH₂); ¹³C-NMR (CD₃OD) δ 165.6 (NHCO), 152.8 (NHCONH), 142.5 (CCHNH), 109.1 (COCH), 53.4 (CH₂NCH₂), 50.1 (CCH₂N), 23.0 (CH₂CH₂CH₂CH₂); ESI-MS 196.20 [M+1]⁺.

5-((bis(2-hydroxyethyl)amino)methyl)uracil (5)

Yield 20%; mp >300 °C (lit. >300 °C);³⁵ UV λ_{max} (nm) (log ϵ) 214.8 (1.979), 266 (0.564); IR ν_{max} (cm⁻¹) 3415, 2866, 1716, 1668, 1448, 1240, 1079, 1027; ¹H-NMR (CD₃OD) δ 7.48 (s, 1H, CCHNH), 3.61 (t, 4H, $J=5.9$ Hz, CH₂CH₂OH), 3.39 (s, 2H, CCH₂N), 2.64 (t, 4H, $J=5.9$ Hz, NCH₂CH₂); ¹³C-NMR (CD₃OD) δ 166.0 (NHCO), 152.4 (NHCONH), 140.9 (CCHNH), 110.4 (COCCH), 59.5 (CH₂CH₂OH), 55.9 (NCH₂CH₂), 50.1 (CCH₂N); ESI-MS 230.30 [M+1]⁺.

5-((diethylamino)methyl)uracil (6)

Yield 48%; mp 245 °C; UV λ_{max} (nm) (log ϵ) 218.0 (1.818), 265.2 (1.107); IR ν_{max} (cm⁻¹) 3430, 2931, 1716, 1670, 1446, 1151; ¹H-NMR (CD₃OD) δ 7.40 (s, 1H, CCHNH), 3.36 (s, 2H, CCH₂N), 2.58 (q, 4H, $J=3.2$ Hz, NCH₂CH₃), 1.08 (t, 6H, $J=3.2$ Hz, CH₂CH₃); ¹³C-NMR (CD₃OD) δ 165.7 (NHCO), 152.8 (NHCONH), 142.3 (CCHNH), 109.0 (COCCH), 54.8 (CCH₂N), 46.4 (CH₂CH₃), 10.3 (NCH₂CH₃); ESI-MS 198.20 [M+1]⁺.

5-((dimethylamino)methyl)uracil (7)

Yield 72%; mp 204 °C (lit. 203-206 °C);³⁷ UV λ_{max} (nm) (log ϵ) 262 (3.964); IR ν_{max} (cm⁻¹) 3113, 2971-2947, 1730, 1702, 1674, 1450, 818, 781; ¹H-NMR (CD₃OD) δ 7.22 (s, 1H, CCHNH), 3.01 (s, 2H, CCH₂N), 2.08 (s, 6H, NCH₃); ¹³C-NMR (CD₃OD) δ 164.9 (NHCO), 151.8 (NHCONH), 140.8 (CCHNH), 109.2 (COCCH), 54.3 (CCH₂N), 45.2 (NCH₃); ESI-MS 170.20 [M+1]⁺.

5-(piperazinomethyl)uracil (8)

Yield 18%; mp 222 °C; UV λ_{max} (nm) (log ϵ) 207 (3.005), 263 (2.547); IR ν_{max} (cm⁻¹) 3415, 2935, 1705, 1660, 1455, 831; ¹H-NMR (DMSO-d₆) δ 7.21 (s, 1H, CCHNH), 4.02 (s, 2H, CCH₂N), 3.67 (t, 2H, $J=4.8$ Hz, CH₂CH₂NH), 3.55 (t, 2H, $J=4.8$ Hz, CH₂CH₂NH), 2.93 (bs, 1H, NH), 2.96 (t, 2H, $J=4.8$ Hz, NHCCH₂CH₂), 2.76 (t, 2H, $J=4.8$ Hz, NHCCH₂CH₂); ¹³C-NMR (DMSO-d₆) δ 165.6 (NHCO), 152.4 (NHCONH), 141.8 (CCHNH), 108.5 (COCCH), 54.3 (CCH₂N), 52.4 (CH₂NCH₂), 51.3 (CH₂NHCCH₂); ESI-MS 211.20 [M+1]⁺.

Biological activity assays

Antiproliferative effects of the test compounds were measured in vitro on 3 human cell lines: HeLa (cervix adenocarcinoma), MCF7 (breast adenocarcinoma), and A431 (skin epidermoid carcinoma), by using the MTT assay.³⁸ Briefly, cancer cells (5000/well) were seeded onto a 96-well microplate and attached to the bottom of the well overnight. On the second day, 200 μ L of new medium containing the test substances was added. After incubation for 72 h, the living cells were assayed by the addition of 20 μ L of 5 mg/mL MTT solution. MTT was converted by intact mitochondrial reductase and precipitated as blue crystals during a 4 h contact period. The medium was then removed, and the precipitated crystals were dissolved in 100 μ L of DMSO during a 60 min period of shaking. Finally, the reduced MTT was assayed at 545 nm, using a microplate reader; wells with

untreated cells were utilized as controls. All in vitro experiments were carried out on 2 microplates with at least 5 parallel wells. Stock solutions of the tested substances (30 mM) were prepared with DMSO. The highest DMSO concentration (0.3%) of the medium did not have any significant effect on the cell proliferation. Their antiproliferative effects were determined at 2 final concentrations (30 and 90 μ M). Doxorubicin was used as reference antiproliferative agent. It caused 50% inhibition of proliferation at 0.15, 0.28, and 0.16 μ M on HeLa, MCF7, and A431 cells, respectively.

Incubation of **1** and **7** with 2-mercaptoethanol

2-Mercaptoethanol (0.005 mol) was added to solutions of **1** and **7** (0.005 mol) in phosphate buffer solution (pH 7.4, 6.9 and 6.4, 10 mL). After incubation at 37 °C for 72 h on a shaking constant-temperature bath the aqueous phase was extracted with chloroform. The chloroform layer was separated and dried over anhydrous sodium sulfate. Removal of the organic solvent afforded a product shown by TLC (CHCl_3 -MeOH (4:1)) to be principally unchanged **1** and **7** along with very small quantities of other compounds. The residue was eluted on a silica gel column with chloroform-methanol gradually. The starting compounds were mainly obtained. TLC of the aqueous phase also revealed only the presence of the unchanged **1** and **7**.

Results and discussion

We synthesized compounds **1-8** by traditional Mannich reaction using uracil, formalin solution, and appropriate amines. It was noteworthy that all Mannich reactions to prepare nitrogen mustard with uracil (**1**) did not take place when ethanol was used as a solvent. The Elderfield method²⁹ was referred to for the preparation of **1** under hard reaction conditions, namely, by using glacial acetic acid as a solvent and heating under reflux for 3 h. In our study compound **1** was prepared under relatively soft conditions via stirring of the starting materials in tetrahydrofuran for 24 h at room temperature.

Structures of dimethylamino (**7**), piperidino (**3**), and morpholino (**2**) Mannich bases at the 5 position of uracil were characterized by their elemental and ¹H-NMR properties by Burckhalter et al.³⁰ and Asherson et al.³⁷ However, Bombardieri et al.³⁹ reported that the reaction with uracil, formalin, and morpholine gave N-morpholinomethyluracil (N-Mannich base) depending on nitrogen analyses of the compound. This report was in disagreement with Burckhalter et al.'s and Asherson et al.'s findings. As seen, it is difficult to understand which structural isomer (C-5 or N-1 and/or N-3 substituted) of the Mannich bases occurred without interpretation of NMR data. The other compounds derived, diethylamine (**6**) and piperazine (**8**), were not reported clearly in the literature. Moreover, there are no spectral data for compound **4**, which is a pyrrolidine Mannich base. The structural assignment of diethanolamine derivative (**5**) was only based on UV and elemental analyses in the literature.³⁵ Compound **1** containing mustard moiety was characterized by UV, IR, and elemental properties without giving any NMR data. Consequently, especially high resolution NMR findings were necessary for legal structure elucidation since the uracil ring has 3 convenient positions for aminomethylation. In our study, the high resolution ¹H-NMR spectra of these compounds exhibited only one aromatic singlet and disappearance of the C-5 proton of the uracil ring, indicating formation of the C-Mannich bases at the 5 position. UV, IR, ¹H-NMR, ¹³C-NMR, and MS spectral data of each compound are given in the experimental section in detail.

In summary, compounds **6** and **8** are reported for first time in the literature and compounds **1**, **4**, and **5** are reported for first time with detailed spectral data herein. At pH values of 7.4 and 6.9 representative compounds **1** and **7** were stable and hence the deamination process envisaged in Scheme 2 appears unlikely. No reaction occurred with a model nucleophile 2-mercaptoethanol and test compounds at pH 7.4 and 6.9. The data in the Table indicate that the cytotoxic activity was more remarkable in the mustard derivative (**1**) among the cell lines covered in our study. As also seen in the Table, this compound yielded a percent inhibition of 34.38 μ M, which was comparable to the effect manifested by the control compound, Etoposide (31.64%), on breast carcinoma cell lines in 30 μ M concentration. This compound was followed by diethanolamine derivative **5** on breast carcinoma cells with a percent inhibition of 10.75% at the same concentration. The effect of diethanolamine derivative was comparable to that of diethyl **6** and dimethylamine **7** derivatives on skin dermoid carcinoma cells. The action mechanism of diethanolamine and mustard derivatives might share similarities. However, the highest inhibitor activity was obtained with mustard derivative **1** on all test carcinoma cells, while mustard derivative **1** was followed by diethanolamine derivative **5** on skin dermoid carcinoma cells. This finding might suggest that Cl rather than OH was required for activity. There was no relation between pKa values and inhibitor activities of the compounds tested in this study. Stability test results do not appear to support our hypothesis based on the elimination-nucleophilic addition mechanism under biomimetic test conditions (37 °C and pH 7.4) for alkylation.

Table 1. Antiproliferative activity of compounds.

Compound		Inhibition (%) \pm SEM		
		Hela	MCF7	A431
1	30 μ M	19.54 \pm 1.19	34.38 \pm 1.79	47.82 \pm 0.69
	90 μ M	28.70 \pm 1.82	44.64 \pm 1.34	63.35 \pm 1.22
2	30 μ M	12.28 \pm 1.13	5.12 \pm 1.76	15.59 \pm 2.84
	90 μ M	3.49 \pm 2.70	2.64 \pm 1.69	4.05 \pm 2.57
3	30 μ M	8.11 \pm 3.57	3.81 \pm 1.60	10.23 \pm 2.78
	90 μ M	4.70 \pm 2.57	11.54 \pm 1.53	3.23 \pm 2.87
4	30 μ M	2.05 \pm 2.62	-1.00 \pm 0.85	15.23 \pm 2.78
	90 μ M	-3.84 \pm 1.30	0.98 \pm 1.18	20.64 \pm 2.47
5	30 μ M	21.01 \pm 1.05	10.75 \pm 0.84	21.79 \pm 2.09
	90 μ M	15.61 \pm 1.68	7.46 \pm 1.43	11.88 \pm 1.93
6	30 μ M	7.88 \pm 0.69	5.81 \pm 0.47	19.13 \pm 0.91
	90 μ M	9.65 \pm 2.23	4.78 \pm 2.68	24.54 \pm 1.12
7	30 μ M	9.61 \pm 2.43	3.47 \pm 1.57	28.40 \pm 1.19
	90 μ M	17.06 \pm 1.95	6.09 \pm 0.28	19.08 \pm 2.72
8	30 μ M	9.33 \pm 1.87	2.81 \pm 1.52	15.80 \pm 0.86
	90 μ M	6.64 \pm 3.76	1.29 \pm 3.31	6.30 \pm 1.21
Etoposide	30 μ M	95.01 \pm 0.73	31.64 \pm 2.02	93.85 \pm 0.59

It is conceivable therefore that the bioactivities of the compounds are due to the molecules per se or that activity does not involve thiols. Thus future molecular modifications should be aimed at producing N-substituted

Mannich bases rather than C-substituted Mannich bases. Such derivatives may have marked cytotoxicity and value as candidate antineoplastic agents.

Taken together, the results that emerged from this investigation will guide the future expansion of these series of compounds.

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References

1. Dimmock, J. R.; Erciyas, E.; Kumar, P.; Hetherington, M.; Quail, J. W.; Pugazhenth, U.; Arpin, S. A.; Hayes, S. J.; Allen, T. M.; Halleran, S.; de Clercq, E.; Balzarini, J.; Stables, J. B. *Eur. J. Med. Chem.* **1997**, *32*, 583-594.
2. Gul, H. I.; Gul, M.; Erciyas, E. *Arzneim. Forsch.* **2002**, *52*, 628-635.
3. Cogan, P. S.; Koch, T. H. *J. Med. Chem.* **2003**, *46*, 5258-5270.
4. Gul, M.; Mete, E.; Atalay, M.; Arik, M.; Gul, H. I. *Arzneim. Forsch.* **2009**, *59*, 364-369.
5. Kucukoglu, K.; Gul, M.; Atalay, M.; Mete, E.; Kazaz, C.; Hanninen, O.; Gul, H. I. *Arzneim. Forsch.* **2011**, *61*, 366-371.
6. Lee, C. M.; Plattner, J. J.; Ours, C. W.; Horrom, B. W.; Smital, J. R.; Martin, Y. C.; Pernet, A. G.; Bunnell P. R.; el Masry, S. E.; Dodge, P. W. *J. Med. Chem.* **1984**, *27*, 1579-1587.
7. Scott, M. K.; Martin, G. E.; Di Stefano, D. L.; Fedde, C. L.; Kukla, M. J.; Barrett, D. L.; Baldy, W. J.; Elgin Jr., R. J.; Kesslick, J. M. *J. Med. Chem.* **1992**, *35*, 552-558.
8. Dimmock, J. R.; Jonnalagadda, S. S.; Phillips, O. A.; Erciyas, E.; Shyam, K.; Semple, H. A. *J. Pharm. Sci.* **1992**, *81*, 436-440.
9. Vashishtha, S. C.; Zello, G. A.; Nienaber, K. H.; Balzarini, J.; de Clercq, E.; Stables, J. P.; Dimmock, J. R. *Eur. J. Med. Chem.* **2004**, *39*, 27-35.
10. Selvakumar, V.; Duraipandi, S.; Devdas, S. *J Pharm Res.* **2011**, *4*, 3168-70.
11. Shiozawa, A.; Narita, K.; Izumi, G.; Kurashige, S.; Sakitama, K.; Ishikawa, M. *Eur. J. Med. Chem.* **1995**, *30*, 85-94.
12. Gul, H. I.; Denizci, A. A.; Erciyas, E. *Arzneim. Forsch.* **2002**, *52*, 773-777.
13. Erciyas, E.; Erkalcli, H. I.; Cosar, G. *J. Pharm. Sci.* **1994**, *83*, 545-548.
14. Muthumani, P.; Neckmohammed, M. R.; Venkataraman, S.; Chidambaranathan, N.; Devi, P.; Suresh Kumar, C. A. *Int. J. Pharm. Biomed. Res.* **2010**, *1*, 78-86.
15. Miroshnikova, O. V.; Hudson, T. H.; Gerena, L.; Kyle, D. E.; Lin, A. J. *J. Med. Chem.* **2007**, *50*, 889-896.
16. Friebolin, W.; Jannack, B.; Wenzel, N.; Furrer, J.; Oeser, T.; Sanchez, C. P.; Lanzer, M.; Yardley, V.; Becker, K.; Davioud-Charvet, E. *J. Med. Chem.* **2008**, *51*, 1260-1277.
17. Edwards, M. L.; Ritter, H. W.; Stemerick, D. M.; Stewart, K. T. *J. Med. Chem.* **1983**, *26*, 431-436.

18. Sloan, K. B. *United States Patent* US 4845081, 1989.
19. Lyttle, D. A.; Petering, H. G. *J. Am. Chem. Soc.* **1958**, *80*, 6459-6460.
20. Larionov, L. F. *Brit. J. Cancer* **1956**, *10*, 26-32.
21. Fabbrissin, S.; De Nardo, M.; Nisi, C.; Morasca, L.; Dolfini, E.; Franchi, G. *J. Med. Chem.* **1976**, *19*, 639-642.
22. Bardos, T. J.; Datta-Gupta, N.; Hebborn, P.; Triggle, D. J. *J. Med. Chem.* **1965**, *8*, 167-174.
23. Margot, T.; Fakan, F.; Muchnova, Z. *Neoplasma* **1969**, *16*, 249-255.
24. Slotova, J.; Karpfel, Z. *Biol. Plantarum* **1969**, *11*, 49-59.
25. Farkas, J.; Sorm, F. *Collect. Czech. Chem. Commun.* **1961**, *26*, 893-895.
26. Stone, J. E.; Potter, Van R. *Cancer Res.* **1957**, *17*, 800-803.
27. Reyes, P.; Heidelberger, C. *Mol. Pharmacol.* **1965**, *1*, 14-30.
28. Giner-Sorolla, A. L.; Medrek, L. *J. Med. Chem.* **1966**, *9*, 97-101.
29. Elderfield, R. C.; Wood, J. R. *J. Org. Chem.* **1961**, *26*, 3042-3043.
30. Burckhalter, J. H.; Seiwald, R. J.; Scarborough, H. C. *J. Am. Chem. Soc.* **1960**, *82*, 991-994.
31. Tramontini, M.; Angiolini, L. *Tetrahedron* **1990**, *46*, 1791-1837.
32. Pratt, W. B.; Ruddon, R. W. *The Anticancer Drugs*, Oxford University Press, New York, 1979.
33. Dimmock, J. R.; Erciyas, E.; Kirkpatrick, D. L.; King, K. M. *Pharmazie* **1988**, *43*, 614-616.
34. Wike-Hooley, J. L.; Haveman, I.; Reinhold, H. S. *Radiother. Oncol.* **1984**, *2*, 343-366.
35. Ross, L. O.; Lee, Wm. W.; Schelstraete, M. G. M.; Goodman, L.; Baker, B. R. *J. Org. Chem.* **1961**, *26*, 3021-3022.
36. Asano, S.; Kitamura, J.; Takatori, K. *Yakugaku Zasshi*, **1972**, *92*, 1162-1165.
37. Asherson, J. L.; Bilgic, O.; Young D. W. *J. Chem. Soc. Perk. T. 1.* **1980**, *2*, 522-528.
38. Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55-63.
39. Bombardieri, C. C.; Taurins, A. *Can. J. Chem.* **1955**, *33*, 923-928.

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