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The effect of structure of Mannich base prodrugs of 6-mercaptopurine on their ability to deliver 6-mercaptopurine through hairless mouse skin

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Summary

Mannich base prodrugs of 6-mercaptopurine (6-MP) have been prepared and evaluated for their ability to deliver 6-MP through hairless mouse skin from isopropyl myristate (IPM) and dimethylformamide (DMF). For a series of straight chain dialkylaminomethyl derivatives (Mannich bases) of 6-MP their ability to enhance the delivery of 6-MP was not related to their solubility in the vehicle; the least soluble member of the series was the second most effective prodrug. The best derivative was the dipropylaminomethyl derivative which delivered 6-MP over 180 times faster than 6-MP from IPM or over 3 times faster than the best acyloxymethyl-6-MP derivatives from IPM. Although the Mannich bases were stable in IPM, when suspensions of the Mannich bases in IPM were applied to mouse skin in diffusion cells they underwent decomposition to 6-MP during the diffusion experiments. However, apparent steady-state rates of delivery of 6-MP by the Mannich bases could be determined by using very high suspension concentrations or by applying fresh suspensions every 6–12 hours. Although two of the Mannich bases were several orders of magnitude more soluble in DMF than in IPM, either only a small increase or a small decrease in rate of delivery of 6-MP from DMF compared to IPM was observed. The prodrugs/IPM did not, while the prodrugs/DMF did appear to cause more irreversible damage to the skin than the vehicles themselves as determined by second application experiments.

Introduction

6-Mercaptopurine (6-MP)(I) is a polar, heterocyclic, anti-mitotic drug that does not partition into skin well because of its poor solubility characteristics (Sloan et al., 1983; Waranis et al., 1987). As a result, although 6-MP is an effective anti-psoriasis agent when given orally (Kravetz and Balsam, 1961), it is ineffective when given topically. However, because of the desirability of minimizing systemic exposure to anti-mitotic drugs such as 6-MP during treatment of a non-life-threatening disease such as psoriasis, it is also desirable to develop approaches to enhancing the dermal delivery of 6-MP.

One approach to enhancing the dermal delivery of 6-MP is to optimize the vehicle in which it is delivered. The effect of a number of single and binary component vehicles on the delivery of 6-MP through hairless mouse skin has recently been

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described (Waranis et al., 1987). The results of that investigation suggested that the permeability coefficient (P) for the delivery of 6-MP from a vehicle through skin, which is the rate of delivery of 6-MP through skin (J) divided by the solubility of 6-MP in the vehicle (C^v), is inversely related to C^v (Eqn. 1). Those results and the results for other polar, heterocyclic drugs (Sloan et al., 1986, Sherertz et al., 1987) suggest that there are theoretical limits to the extent of enhancement of dermal delivery that can be expected from changes in vehicles. However, those theoretical limits hold only if the vehicle does not irreversibly affect the permeability of the skin.

$$J = PC^v \quad (1)$$

An alternate approach to enhancing the delivery of 6-MP through skin is to change transiently the physicochemical properties of 6-MP; or, to make prodrug forms of 6-MP. Reports of several series of homologous acyloxymethyl type prodrugs of 6-MP have recently appeared in the literature (Waranis and Sloan, 1987, Waranis and Sloan, 1988). However, in the cases of theophylline and 5-fluorouracil, Mannich base type prodrugs actually performed better than acyloxymethyl type prodrugs at delivering the parent drugs through hairless mouse skin (Sloan et al., 1984, 1988). Therefore, it was a logical progression to examine the potential of Mannich base type prodrugs of 6-MP to enhance the delivery of 6-MP through hairless mouse skin. The synthesis and the determination of the structure of the Mannich base prodrugs of 6-MP have been described elsewhere (Siver et al., 1988). This report describes the synthesis of several additional Mannich base prodrugs of 6-MP and the ability of these and the previously synthesized Mannich base prodrugs of 6-MP to enhance the delivery of 6-MP through hairless mouse skin from isopropyl myristate and dimethylformamide.

Materials and Methods

The mp (corrected) were taken with a Thomas–Hoover capillary apparatus. The ^{1}H NMR spectra were recorded on a Varian EM-390 or T-60 spectrometer while the UV spectra were recorded on a Cary 210 spectrophotometer. Microanalyses were obtained from Atlantic Microlab Inc., Atlanta, GA. Except for 6-MP, which was obtained from Sigma, the chemical starting materials were obtained from Aldrich. The bulk solvents were obtained from Fisher Scientific except for the isopropyl myristate (IPM) which was obtained from Givaudan, Clifton, NJ. The HPLC system consisted of a Beckman model 110A pump with a model 153 UV detector, a Rheodyne model 7125 injector with a 20 μl injector volume, and a Hewlett-Packard model 3392A integrator. The diffusion cells were Franz type cells (2.5 mm in diameter, 4.9 cm^2 = area, 20 ml receptor phase volume) which were obtained from Crown Glass of Somerville, NJ. The mice were female hairless mice (*Skh:hr-1*) obtained from Temple University Skin and Cancer Hospital and weighed 20–30 g.

General procedure for preparation of Mannich bases of 6-MP

To 4 equivalents of a secondary amine were added 4 equivalents of paraformaldehyde. The white suspension was stirred at room temperature for 1–5 min as an exothermic reaction ensued. The mixture was then stirred with 10 ml of ether overnight, filtered and allowed to react with 1.10 g (0.01 mol) of 6-MP hydrate for 2–4 h. The white suspension was diluted with 5 ml of tetrahydrofuran, stirred for an additional 5 min and filtered. The residue was washed once with an additional 5 ml of tetrahydrofuran and 3 times each with 5 ml of ether before being allowed to air dry to give the analytically pure Mannich base derivatives of 6-MP. Derivatives **II**, **III**, **IV** and **VI** had been previously isolated and completely characterized (Siver et al., 1988) and the derivatives isolated for use in this study were identical with those derivatives by ^{1}H NMR spectroscopy and by melting point. Two additional derivatives (**V** and **VII**) exhibited the following characteristics.

7-(Dibutylamino)methyl-6-mercaptopurine (V). 1.94 g (66%, mp 196 °C (d)) as a white solid; ^{1}H NMR (DMSO-d_6) δ 8.34 (s, 1, 2-H), 8.30 (s, 1, 8-H), 5.5-5.0 (broad s, 2, N–CH_2–N), 2.53 (t, J = 7

Hz, 4, N–C$\underline{H}_2$CH$_2$) 1.67–1.06 (m, 8, C$\underline{H}_2$), 0.87 (t, $\underline{J}$ = 7 Hz, 6, C$\underline{H}_3$–CH$_2$); UV (CH$_3$CN) λ max 330 nm ($\epsilon = 1.85 \times 10^4$ mol^{-1}) and 232 nm ($\epsilon = 6.76 \times 10^3$ mol^{-1}).

Anal. Calcd. for $C_{14}H_{23}N_5S$: C, 57.31; H, 7.90; N, 23.87. Found: C, 57.23; H, 7.94; N, 23.83.

7-(N-Pyrrolidyl)methyl-6-mercaptopurine (VII). 2.28 g (97% yield, mp 193° (d)) as an off-white solid; ^{1}H NMR (DMSO-d$_6$) δ 8.39 (s, 1, 2–$\underline{H}$), 8.30 (broad s, 1, 8–$\underline{H}$), 6.1–4.8 (broad m, 1, N–$\underline{H}$), 5.33 (broad s, 2, N–C$\underline{H}_2$–N), 2.63 (m, 4, N–C$\underline{H}_2$CH$_2$), 1.63 (m, 4, N–CH$_2$C$\underline{H}_2$); UV (CH$_3$CN) λ max 330 nm ($\epsilon = 1.61 \times 10^4$ mol^{-1}) and 299 nm ($\epsilon = 5.81 \times 10^3$ mol^{-1}).

Anal. Calcd for $C_{10}H_{13}N_5S$: C, 51.04; H, 5.57; N, 29.76. Found: C, 51.12; H, 5.60; N, 29.79.

Determination of solubilities

The solubilities of all the prodrugs in IPM and of **II** and **IV** in dimethylformamide (DMF) were determined in triplicate by stirring an excess of the prodrug in the solvent (2 ml) with a magnetic stirrer for 48 h at room temperature (23 ± 1° C). The flask was sealed and thermally insulated from the stirrer. The suspension was filtered through a 0.45 μm nylon membrane filter supported on a paper filter (Whatman no. 1). A measured volume of the clear filtrate was immediately taken and diluted with acetonitrile. The concentration of the prodrug in the acetonitrile solution was determined by measuring the intensity of the absorption at 330 nm.

Diffusion cell procedure

The general procedure that was used has been described previously (Sloan et al., 1986; Waranis and Sloan, 1987; Waranis et al., 1987). Briefly, the hairless mice were sacrificed and their dorsal skins were positioned dermis side down in diffusion cells in contact with pH 7.3 (at 32°) isotonic phosphate buffer (0.05 M, ionic strength = 0.11 M) containing 0.1% V/V formaldehyde (a preservative) as the receptor phase. The skins were kept in contact with the receptor phase for 48 h. The receptor phase was changed 3 times during this pre-application contact period. Control studies were run previously to show that the length of time (2–120 h) that the skins were in contact with the receptor phase did not significantly affect the flux of theophylline from propylene glycol through the skins (Sloan et al., 1986).

In all cases, the Mannich base prodrugs were applied as suspensions in IPM or DMF. In this way, the amount of prodrug in solution was maintained at a constant level (saturation) by the presence of the excess undissolved solid (the rate of dissolution was assumed to be greater than the rate of diffusion). This allowed for the determination of the effect of the prodrug structure on the rate of delivery of 6-MP from the vehicle since they were all tested at the same thermodynamic activity (Higuchi, 1960; Woodford and Barry, 1982). IPM was chosen as the vehicle in which to test all of the prodrugs because of the instability of the Mannich bases under aprotic conditions, and IPM is an aprotic, non-hygroscopic liquid. DMF, which is also aprotic, was chosen as a vehicle to examine the effect of increased solubility of the prodrugs in a vehicle on the ability of the prodrug to deliver 6-MP.

A suspension of the prodrug at a given total concentration (0.05 M to 0.80 M) was prepared by stirring the appropriate amount of the solid compound in 3 ml of vehicle for 48 h at room temperature (23 ± 1° C). The suspension was stirred with a magnetic stirrer in a sealed flask thermally isolated from the stirrer. After the 48 h preapplication contact period, a 0.5 ml aliquot of the suspension was applied to the donor side of the skin using an Eppendorf digital pipetter. The homogeneity of the suspension was maintained by stirring the suspension during the application of aliquots to the 3 cells.

Samples (3 ml) were obtained of the receptor phase at various times during the following 48 h period. The samples were quantitated using UV spectroscopy (321 nm, $\epsilon = 1.94 \times 10^4$ mol^{-1}) to determine the amount of 6-MP present. A plot of the absorbance at 321 nm vs the concentration of 6-MP was linear ($r = 1.00$) over the entire range of concentrations that were found in the receptor phases from the delivery of 6-MP by the prodrug/IPM suspensions. In order to be certain that the absorbance in the UV spectra at 321 nm was due to 6-MP and not intact prodrug, a metabolite of 6-MP or some constituent of the

skin that had leached into the receptor phase, the receptor phase was also analyzed by HPLC (Adsorbasphere C-4 reverse-phase column (4.6 mm × 25 cm); (1 : 4) methanol/acetate buffer (0.01 M, pH 5) mobile phase) for the delivery of 6-MP from the application of the 0.40 M IPM suspension of **IV**. Only one peak in the chromatogram which corresponded to 6-MP (retention time of 3 min at a flow rate of 1.6 ml/min) was observed for all the samples obtained.

The timing of the acquisition of samples varied from compound to compound and was chosen to adequately cover the period of steady-state diffusion — a range of time which depended on the lag time and the length of steady-state diffusion. Fresh buffer (3 ml) was used to replenish the receptor phase after each sample was taken. This necessitated a correction for the calculation of the cumulative amount of 6-MP in each subsequent sample be made by adding 15% of the amount of 6-MP found in each previous sample to the amount of 6-MP found in the sample being analyzed.

In the repeat application studies the preparations of the cells and the initial application of prodrug/IPM were the same as the other diffusion cell experiments. However, after the initial applications the donor suspensions were removed and replaced with the same volume of fresh donor suspension at sufficient time intervals to ensure that intact, solid prodrug suspensions were present throughout the 48 h application period. The time intervals depended on the stability of each particular derivative. Care was taken during the reapplication process not to scrape or in any other way damage the skin when removing the spent donor suspension.

At the end of this 48 h diffusion period the donor side was washed 3 times with 10 ml of methanol taking care to remove all traces of solid particles and to keep the time of contact between the skin and the methanol to a minimum (< 3 min total). Control studies were run previously to show that the methanol wash had a small but significant ($P < 0.05$) effect (an increase of 1.7 times) on the flux of theophylline from propylene glycol (Sloan et al., 1986). The 3 methanol washes were combined and analyzed by UV spectroscopy to determine the amount of 6-MP (the methanol decomposed any residual Mannich base to 6-MP) that remained in the donor phase. After the methanol wash, the receptor phase was changed and the skin was kept in contact with the buffer for 23 h to allow any residual 6-MP in the skin to leach out. A sample was obtained at the end of the 23 h period and analyzed by UV spectroscopy to quantitate the amount of residual 6-MP leached from the skin. Control studies were run to show that the 23 h leach period was sufficient to allow most (> 90%) of the 6-MP present in the skin at the end of the 48 h application period to leach into the receptor phase. Analysis of the receptor phase after the 23 h postapplication leach period found less than 0.5% of the applied dose of each of the derivatives, in mg equivalents of 6-MP, had leached from the skin into the receptor phase. The receptor phase was then replaced with fresh buffer and contact was maintained with the skin for an additional hour before a baseline reading was obtained.

The total amount of 6-MP accounted for in the mass balance was determined by summation of the amount of 6-MP that leached from the skin during the 23 h post-application period, the cumulative amount of 6-MP delivered through the skin over the 48 h application period and the amount of 6-MP that had remained in the donor phase and had been removed with the methanol wash. The mass balance amounted to 87 ± 2%, 89 ± 2%, 90 ± 6%, 85 ± 1%, 81 ± 3%, and 84 ± 1% of the applied dose of **II**, **III**, **IV**, **V**, **VI**, and **VII**, respectively, in IPM.

The percent of the applied dose (0.4 M), in mg equivalents of 6-MP, delivered over the 48 h diffusion period varied depending on the derivative, ranging from 1.3 ± 0.7% for **VI** to 16 ± 2% for **III**. Compound **II** managed to deliver 10 ± 4% of the applied dose over a 48 h period while **IV**, **V** and **VII** delivered only 3.6 ± 0.1%, 2.2 ± 0.7% and 2.1 ± 0.8% of the applied doses, respectively.

Finally, in order to assess the degree of any irreversible effects of the compound/vehicle combination on the permeability of the skin, an aliquot (0.5 ml) of a theophylline suspension in propylene glycol (400 mg/6 ml) was applied to each cell and 3 ml samples of the receptor phases were obtained after 3, 6, 9, 12 and 24 h (Sloan et al., 1986). The

amount of theophylline in the samples was determined by UV spectroscopy (270 nm, $\epsilon = 1.02 \times 10^4$ mol^{-1}).

The rates of delivery of 6-MP from the prodrug/vehicle or 6-MP/vehicle suspension and theophylline from the theophylline/propylene glycol suspension were obtained in all cases by plotting the cumulative amount of 6-MP or theophylline measured in the receptor phase against time. Regression analysis of the linear portion of the plot ($r > 0.99$) was used to obtain the slope corresponding to the period of steady-state diffusion. The rate of delivery was obtained by dividing the above slope by the area of the diffusion cell (4.9 cm^2).

Results and Discussion

Synthesis

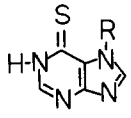
The synthesis of Mannich base derivatives of 6-MP had previously been described by Bryant and Harmon (1967). However, in that case only two examples were prepared (the morpholinyl- and piperidylmethyl derivatives) and the structure assigned was that of a 9-derivative. Recently, the synthesis of a series of Mannich bases including the piperidylmethyl derivative **VI** was described which employed non-protic solvents (instead of ethanol) in which to run the reactions because the Mannich base products would be more stable in them (Siver et al., 1988). This is a reflection of the fact that the formation of the Mannich bases is an equilibrium reaction which is reversible in the presence of protic solvents. In addition, evidence was presented which showed that the Mannich bases obtained were 7-derivatives. The Mannich bases prepared here were prepared in the same manner and exhibit similar or identical ^{1}H NMR and UV spectra; they are assumed to be 7-derivatives.

Solubility and stability

Three of the 6 Mannich bases that were prepared (**II**, **VI** and **VII**) were of the same order of magnitude of solubility in IPM (Table I) as 6-MP, in spite of the fact that the decomposition points of all the derivatives were 80 °C lower than that of 6-MP. A definite trend in the IPM solubilities was observed for the Mannich base prodrugs obtained from the open chain series of secondary amines (**II**–**V**). The dimethylamine derivative **II** exhibited the lowest solubility (1.7 times 6-MP) and the highest decomposition point. **III** was almost an order of magnitude more soluble than **II**, and **IV** was the most soluble (26 times 6-MP) in the series. Then the solubility decreased (**V** was only 6 times more soluble than 6-MP) and the decomposition point started to increase as the chain length increased to the dibutylamine derivative. Similar

TABLE 1

Solubilities and decomposition points of the Mannich base prodrugs of 6-MP

Compound 	DP [a]	IPM solubility Mean (±S.D.) [c]	DMF solubility Mean (±S.D.) [c]
I, R=H	314 [b]	0.0030 (0.001)	14.5 (0.015)
II, R=$CH_2N(CH_3)_2$	231	0.0058 (0.0008)	9.1 (0.76)
III, R=$CH_2N(C_2H_5)_2$	178	0.042 (0.006)	
IV, R=$CH_2N(C_3H_7)_2$	184	0.077 (0.010)	27.4 (4.5)
V, R=$CH_2N(C_4H_9)_2$	193	0.018 (0.005)	
VI, R=$CH_2N(CH_2)_5$	220	0.0050 (0.0010)	
VII, R=$CH_2N(CH_2)_4$	195	0.0024 (0.0006)	

[a] Decomposition point, corrected value.

[b] Windholz, 1983.

[c] Mg equivalent of 6-MP/ml.

TABLE 2

Rates of delivery of 6-MP from suspensions of Mannich base prodrugs in IPM or DMF through hairless mouse skin

Compound	Applied concentration (M [a])					Repeat application [b]
	0.05	0.10	0.20	0.40	0.80	
6-MP/IPM	0.66 (0.04) [c]	0.66 (0.4)	ND [d]	ND	ND	ND
II/IPM	0.85 (0.4)	5.6 (2.9)	11 (2)	23 (7)	35 (3)	25 (9)
III/IPM	ND	13 (5)	ND	120 (30)	110 (20)	110 (20)
IV/IPM	ND	2.5 (1.2)	ND	5.9 (0.2)	2.8 (0.2)	5.1 (1.1)
V/IPM	ND	2.0 (0.4)	ND	3.4 (1.6)	2.5 (0.7)	ND
VI/IPM	ND	1.3 (0.4)	ND	2.1 (0.8)	0.8 (0.3)	ND
VII/IPM	ND	1.7 (0.6)	ND	4.1 (0.8)	7.8 (1.1)	4.5 (1.4)
6-MP/DMF	ND	ND	ND	3.8 (0.7) [e]	ND	ND
II/DMF	ND	ND	ND	11 (1.0) [e]	ND	ND
IV/DMF	ND	ND	ND	10 (0.9)	ND	ND

Values are in $mg/cm^2\ h \times 10^3$, means (S.D.)

[a] Total concentration of compound includes solubilized and suspended material.

[b] Fresh donor suspension (0.1 M) applied at sufficient intervals to assure intact prodrug was present during the entire course of experiment.

[c] 0.04 M suspension.

[d] Not determined.

[e] 0.3 M suspension.

trends in a homologous series of derivatives of increasing lipid solubilities until a maximum is reached, then decreasing solubilities have been observed for other prodrugs of 6-MP (Waranis and Sloan, 1987; Waranis and Sloan, 1988) and of theophylline (Sloan and Bodor, 1982).

The solubilities of **II** and **IV** in DMF were also obtained. **II** and **IV** represent the extremes in solubility of the Mannich bases in IPM but there was only about a 3-fold difference in their solubilities in DMF. Part of this difference in solubility behavior may be due to the fact that the Mannich bases form stable pseudopolymorphs (Stagner and Guillory, 1979) when crystallized from DMF. In addition, the residues from the solubility determinations contained DMF by ^1H NMR spectroscopic analysis.

The stability of the prodrugs in IPM was determined by analysis of the filtrates and solid residues obtained from the filtration of the IPM suspensions used in determining the solubilities. No 6-MP was detected in either the filtrates or residues as determined by ^1H NMR spectroscopic analysis. However, the Mannich bases were unstable in the presence of water. The Mannich bases were dissolved in samples of freshly opened, anhydrous DMSO-d_6 and D_2O was added dropwise to the solution. The appearance of 6-MP [δ 8.47 (s, 1, 2–H) and 8.28 (s, 1, 8–H)] and disappearance of the Mannich base [δ 5.0–6.0 (broad s, 2, N–CH_2–N)] was followed by ^1H NMR spectroscopy. With each additional drop of D_2O a new equilibrium was quickly reached between the prodrug and 6-MP plus aminocarbinol until sufficient D_2O was added to drive the equilibrium completely to 6-MP.

Attempts to determine the rates of hydrolysis of the Mannich bases at pH 7.4 were unsuccessful because the hydrolyses occurred too rapidly to be measured by ordinary UV spectrophotometric techniques. These results support the empirically based predictions of stability by Bundgaard and Johansen (1981) based on pK_as of the amide and amine groups that form the Mannich base. Assuming a pK_a of the secondary amine to be between 8 and 9 and the pK_a of the 7–H in 6-MP to be about 11, half-lives on the order of 10^{-2}–10^{-3} seconds would have been predicted.

Diffusion experiments

Single application of prodrugs in IPM. The results of the diffusion cell experiments for the

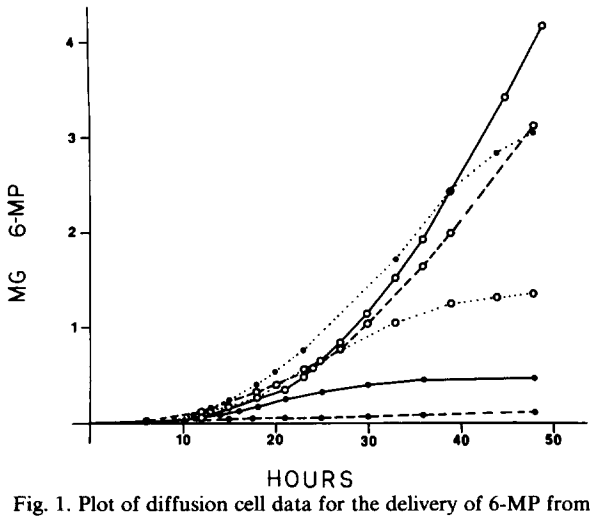


Fig. 1. Plot of diffusion cell data for the delivery of 6-MP from IPM suspensions of **II** at concentrations of 0.05 M (●– – –●), 0.10 M (●———●), 0.20 M (○ · · · ○), 0.40 M (●· · · ·●) and 0.80 M (○———○) and for the repeat application study at 0.10 M (○– – –○).

delivery of 6-MP from IPM suspensions of the Mannich base prodrugs are shown in Table 2 and Figs. 1–4. The effect of increasing the suspension concentration of **II** (total dissolved and undissolved) on the rate of delivery of 6-MP from IPM is shown in Fig. 1. A steady increase in the rate was seen as the total concentration of the suspension was increased from 0.05 M to 0.80 M. This trend does not appear to be due to the rate determining step for diffusion being dissolution of prodrug in the vehicle instead of the assumed partitioning into and through the skin. If dissolution of the prodrug were rate determining, a steady-state rate would still have been reached for each concentration of applied suspension. As can be seen from Fig. 1 that was not the case for IPM suspensions of **II** and similar results were seen for the remaining prodrugs. In the case of **II** only the 0.8 M suspension and the repeat applications of 0.1 M suspensions appeared to have reached a steady-state rate for the delivery of 6-MP. The plots of cumulative mg of 6-MP versus time for the remaining suspension concentrations were S-shaped curves where the delivery of 6-MP leveled off after 22–38 h depending on the concentration. The values for the rates of delivery of 6-MP by **II** and the other Mannich bases obtained from the S-shaped curves, and listed in Table 2, were taken from the slopes of lines defined by the most linear section about the inflection point of the curves.

Even the lowest suspension concentration (0.05 M) represented a greater than 1300 fold excess of solid in the suspension given the low solubility of **II** in IPM (Table 1). In addition, **II** as well as all the Mannich base prodrugs were stable while being stirred in IPM for 48 h and the suspensions that were obtained were stable weeks later as determined by ^{1}H NMR spectroscopy. However, ^{1}H NMR spectroscopic analysis of the donor solid collected from the diffusion cells 48 h after application of the suspensions of **II**/IPM showed that no prodrug was present in the 0.05–0.4 M suspensions. All of **II** had decomposed to 6-MP. Only when the donor suspension concentration was raised to 0.8 M was any **II** detected in the IPM suspension that remained in the donor phase after 48 h of skin contact and then the ratio of **II** : 6-MP was only 1 : 3. Thus, the apparent dependence of flux on concentration results from the instability of **II** in contact with skin. Similar instability in IPM suspensions of the Mannich bases in contact with skin in diffusion cells was found with all the other Mannich base prodrugs to a greater or lesser degree depending on the particular derivative. These results are the consequence of the diffusion of water, which is capable of hydrolyzing the Mannich bases, from the receptor phase into the donor phase.

Compound **III**, the diethyl Mannich base, proved to be the least stable in IPM when exposed to the skin. A 0.40 M suspension of **III** in IPM was converted completely to 6-MP after only 15 h of contact with the skin; see Fig. 2. However, **III** also gave the greatest apparent steady-state rate of delivery of 6-MP from IPM. In a ^{1}H NMR spectroscopy experiment involving a 0.10 M suspension of **III** in IPM, the percent of intact prodrug found in the suspended solid after 0, 2, 8 and 12 h of contact with the skin was 100, 85, 14 and 0%, respectively. In addition, the rate of delivery of 6-MP from a 0.10 M suspension of **III** in IPM after the first 12 h of contact with the skin was not significantly different than the delivery of 6-MP from 6-MP in IPM over the same time interval.

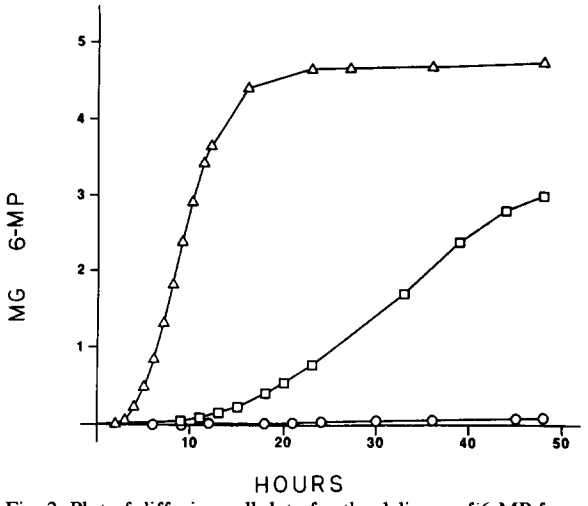


Fig. 2. Plot of diffusion cell data for the delivery of 6-MP from 0.40 M IPM suspensions of **II** (□) and **III** (△) and a 0.10 M IPM suspension of 6-MP (○).

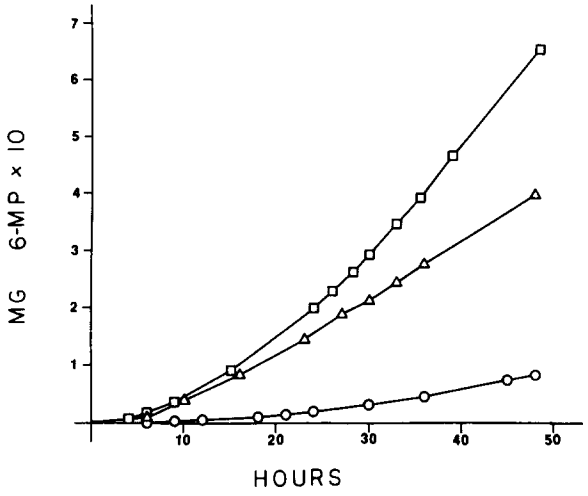


Fig. 4. Plot of diffusion cell data for the delivery of 6-MP from 0.40 M IPM suspensions of **VII** (□) and **VI** (△) and a 0.10 M IPM suspension of 6-MP (○).

Compound **IV**, the dipropyl Mannich base, was the only derivative other than **II** and **III** to show complete degradation to 6-MP after 48 h of contact with the skin when a 0.40 M suspension was applied. As seen in Fig. 3, the rate of delivery of 6-MP from the 0.40 M suspension of **IV** in IPM had decreased at the 48 h sample.

Compounds **V**, **VI** and **VI** all had prodrug remaining in the suspended solid (2, 42 and 58%, respectively) by ^{1}H NMR spectroscopy after 48 h of contact with the skin when applied as 0.40 M suspensions in IPM. It is not surprising that these are the only derivatives that did not show a leveling off of the rate at the end of 48 h when applied as 0.40 M suspensions, see Figs. 3 and 4. Only **VI** and **VII** (the two Mannich bases made with cyclic secondary amines) were sufficiently stable in IPM to show prodrug present after 48 h of contact with the skin when applied as 0.10 M suspensions. On the other hand, compound **V** was completely degraded to 6-MP after 48 h of skin contact when applied as a 0.10 M suspension in IPM.

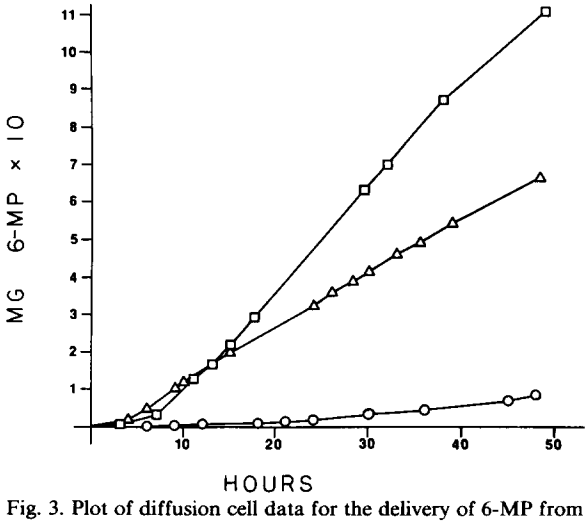


Fig. 3. Plot of diffusion cell data for the delivery of 6-MP from 0.40 M IPM suspensions of **IV** (□) and **V** (△) and a 0.10 M IPM suspension of 6-MP (○).

The apparent steady-state rate of delivery of 6-MP by the 0.40 M suspension of **IV** in IPM was also determined by quantitating the amount of 6-MP in the receptor phase by HPLC analysis. The steady-state rate obtained, $(6.1 \pm 0.1) \times 10^{-3}$ mg/cm^{2}h, was not significantly different from the steady-state rate obtained using UV spectroscopic analysis, $(5.9 \pm 0.2) \times 10^{-3}$ mg/cm^{2}h. The lag times for the establishment of the apparent steady-state rate of delivery were also the same for the rates obtained by HPLC analysis and UV spectroscopic analysis, 7.5 ± 2.2 h and 7.5 ± 1.8 h, respectively.

The lag times for establishment of the apparent steady-state rates of delivery of 6-MP by the pro-

drug/IPM suspensions varied considerably (data not given, but see Figs. 1–4). Compound **II** exhibited a lag time more closely related to that of 6-MP, 19 ± 7 h and 19.8 ± 0.2 h, respectively. The lag time for compound **III** was only 5 ± 1 h. Only **V**, which also exhibited an extended period of apparent steady-state delivery of 6-MP (see Fig. 3), exhibited a shorter lag time (3.2 ± 0.7 h) than **III**. Compounds **IV**, **VI** and **VII** exhibited lag times of 8 ± 2 h, 16 ± 16 h and 10 ± 5 h, respectively, from 0.4 M suspensions.

Compounds **II** and **III** gave the highest, apparent steady-state rates of delivery of 6-MP from IPM $(23 \pm 7) \times 10^{-3}$ mg/cm^{2}h and $(120 \pm 30) \times 10^{-3}$ mg/cm^{2}h, respectively. The rate of delivery of 6-MP through hairless mouse skin by a 0.40 M IPM suspension of **III** represents an increase in the rate of over 180-fold compared to the rate of delivery of 6-MP by 6-MP itself from IPM, and was a little over 3 times as effective as the best acyloxymethyl derivatives of 6-MP (Waranis and Sloan, 1987; Waranis and Sloan, 1988). The rate of delivery of 6-MP by the worst prodrug from IPM, compound **VI**, was over 3 times faster than by 6-MP from IPM. Finally, **III**/IPM delivered about 6.5 times as much 6-MP as the best 6-MP/vehicle (1-octanol) combination even though 1-octanol caused about 4 times as much damage to the skin as IPM (Waranis et al., 1987a).

The prodrug with the highest solubility in IPM, **IV**, did not give the highest, apparent steady-state rate of delivery of 6-MP from IPM. Compound **III**, which was about half as soluble in IPM as **IV** (see Table 1), gave a rate of delivery of 6-MP from IPM that was more than 20 times the rate produced by **IV** from IPM. One explanation for these results is that **III** may have a higher solubility in the stratum corneum than **IV**. The partitioning of the drug from the vehicle into the skin is more favorable in the case of **III**, the result being an increase in the rate of delivery over that given by **IV** even though **IV** is present in the vehicle at a higher concentration. The same situation is seen with compounds **VII** and **VI**. Compound **VII** attained nearly twice the apparent steady-state rate of delivery of 6-MP that **VI** attained, yet **VII** was only half as soluble in the vehicle as **VI**. Thus, increased lipid (vehicle) solubility does not appear to be the determining factor in how effective these Mannich base prodrugs are in enhancing the delivery of 6-MP. In fact **II**, which is only 1.7 times more soluble in IPM than 6-MP, was next to the most effective derivative in terms of delivering 6-MP from IPM (30 times more effective). As with the acyloxymethyl prodrugs (Waranis and Sloan, 1987) the first several members of a homologous series were generally the most effective.

Increasing the concentration to 0.80 M did not prove to be a good way to verify that apparent steady-state rates of delivery of 6-MP had been achieved at 0.40 M. Both **II** and **VII** showed a significant increase in the rate upon going from a 0.40 M to a 0.80 M suspension, even though the repeat application study suggested that the apparent steady-state rate was reached at 0.40 M. In the case of **VII**, this increase was especially surprising since intact, solid prodrug was present in the donor suspension after 48 h when a 0.40 M suspension was applied. Compound **III** showed no significant difference in the rate of delivery between the 0.40 M, 0.80 M or the repeat application study experiments. Compound **V** also showed no significant difference between the rates obtained from the 0.40 M and the 0.80 M IPM suspensions. On the other hand, a significant decrease in the rate of delivery was seen with **IV** and **VI** in IPM when the concentration was increased from 0.40 M to 0.80 M. This disparity in the rates associated with the 0.8 M suspension is most likely due to the viscosity of the suspension at such a high concentration where the limiting step for delivery may have become movement of the compound through the donor phase. The 0.80 M suspensions were so thick they were difficult to stir with a magnetic stirring bar and magnetic stirrer. This viscosity also did not allow an accurate measurement of the amount applied when applying the donor phase as when the less viscous pipettable suspensions of lower concentration were used.

Repeat application of prodrugs in IPM. As a result of the findings on the stability of the Mannich base prodrugs in IPM in contact with the skin, repeat application studies were run to insure that the apparent steady-state rates of delivery of 6-MP were being determined for the Mannich

base prodrugs. Theoretically, as long as there is one molecule of intact prodrug remaining as suspended solid to replenish the drug that has partitioned from the vehicle into the skin, the concentration and thus the driving force for partitioning will remain constant and apparent steady-state rates of delivery should be reached. In order to achieve those conditions, a 0.1 M donor suspension was applied initially, then removed and replaced with the same volume of fresh 0.1 M donor suspension at sufficient time intervals (6–12 h, depending on the stability characteristics of the particular derivative) to insure that some intact, undissolved prodrug was present in the donor phase throughout the 48 h of the experiment.

A typical result is shown in Fig. 1 for the delivery of 6-MP by repeat applications of a 0.1 M suspension of **II**. Similar results were obtained from the repeat applications of 0.1 M suspensions of **III**, **IV** and **VII**. In each case the apparent steady-state rate of delivery of 6-MP by the Mannich base prodrugs at 0.4 M concentration of suspension was not significantly different ($P < 0.05$) from the rate obtained from the repeat application experiments. Thus, those derivatives that exhibited a significant difference in the rate of delivery between the 0.10 M and the 0.40 M suspensions were checked to insure that the apparent steady-state rate of delivery had indeed been obtained by going from a 0.10 M to a 0.40 M suspension.

Application of prodrugs in DMF. The results from diffusion cell experiments conducted to determine the effect of changing the vehicle from IPM to dimethylformamide (DMF) on the rate of delivery of 6-MP from suspensions of 6-MP, **II**, and **IV** are also shown in Table 2. The rate of delivery of 6-MP from 6-MP in DMF was 5.8 times faster than the rate from 6-MP in IPM even though the combination of 6-MP/DMF was less damaging to the skin than the combination of 6-MP/IPM as determined by the flux of theophylline from a subsequently applied suspension of theophylline in PG. The solubility of 6-MP was also much greater in DMF than in IPM (Table 1).

Both prodrugs that were examined also exhibited much higher solubilities in DMF than in IPM. Compounds **II** and **IV** were approximately 1600 and 350 times more soluble, respectively, in DMF than in IPM. However, this increase in solubility did not translate directly to an increase of the same magnitude in the rate of delivery of 6-MP. The steady-state rate of delivery of 6-MP from a 0.40 M suspension of **IV** in DMF was only twice that of a 0.40 M suspension of **IV** in IPM. In contrast to that, the steady-state rate of delivery of 6-MP from **II** in DMF was half the rate from **II** in IPM. A 1600 fold increase in the solubility of **II** in the vehicle resulted in a reduction in the rate of delivery of 6-MP by a factor of two. This illustrates the antagonism that can exist between the partition coefficient and the solubility in the vehicle when enhancement of the delivery of a compound through the skin is attempted by an approach which involves only the composition of the vehicle. Changing the vehicle would have no effect on the intrinsic solubility of the compound in the skin. Therefore, increasing the concentration of the compound in the vehicle by changing the vehicle would result in a decrease in the partition coefficient. In the case of the dimethyl *N*-Mannich base in DMF, this resulted in a net decrease in the rate of delivery of 6-MP.

Application of theophylline in propylene glycol to assess skin damage. A second donor phase was applied to the skin 1 h after the 23 h post-application leach period to assess skin damage. It consisted of a suspension of theophylline in propylene glycol. An increase in the flux of theophylline compared to controls was an indication that the barrier function of the skin had been irreversibly affected by the first drug/vehicle combination. The higher the flux of theophylline from the application of this second donor phase the greater the amount of damage that had been done to the skin by the first application (Sloan et al., 1986).

As shown in Table 3, application of IPM alone increased the flux of theophylline by factor of 75 over that of the control. None of the drug/vehicle combinations involving either 6-MP or the *N*-Mannich base prodrugs had an effect on the skin significantly greater than the effect of IPM alone. However, 3 of the combinations, **II**/IPM, **VII**/IPM and **VI**/IPM, resulted in second application fluxes of theophylline that were significantly lower than that after IPM alone. IPM alone

TABLE 3

Steady-state flux of theophylline through hairless mouse skin from the application of a propylene glycol suspension of theophylline after the initial prodrug/vehicle application

Initial combination	Theophylline flux $mg/cm^2 h \times 10^3$, mean (S.D.) [a]	Relative flux [b]
Control [c]	2.4 (0.4)	1
IPM [d]	180 (20)	75
6-MP/IPM [e]	160 (20)	67
II/IPM	120 (30)	50
III/IPM	180 (30)	75
IV/IPM	190 (50)	79
V/IPM	190 (30)	79
VI/IPM	120 (20)	50
VII/IPM	80 (20)	34
DMF	8 (4)	3
6-MP/DMF [f]	21 (16)	9
II/DMF [f]	110 (70)	46
IV/DMF	200 (100)	83

[a] $n = 3$.
[b] Relative to control.
[c] Control is 96 h leach, MeOH wash and 24 h leach prior to application.
[d] 48 h leach, 48 h IPM, MeOH wash and 24 h leach prior to application.
[e] All initial combination concentrations were 0.10 M.
[f] 0.3 M concentration.

had twice the effect on the skin that **VII**/IPM had. It was also found (results not shown) that combinations of amines and formaldehyde that would result from the decomposition of the *N*-Mannich bases in IPM had no effect on the flux of theophylline in the second application studies (Koch and Sloan, 1987). It seems evident that whatever damage was sustained by the skin from the application of the initial drug/IPM combination can be attributed to the IPM in which the compounds are applied and there is no significant drug/vehicle effect involved in the enhanced delivery of 6-MP by the Mannich bases of 6-MP in IPM.

The flux of theophylline from the second application study showed no difference in the effect of **II**/IPM from that of **II**/DMF on the barrier function of the skin. The same held true for **IV**/IPM and **IV**/DMF. The fluxes of theophylline after **II**/DMF and **IV**/DMF were both significantly higher than the control even though DMF alone has been shown in similar studies to have a low level effect on the barrier function of the skin (Sherertz et al., 1987).

Conclusions

The Mannich base derivatives of 6-MP are more effective at enhancing the delivery of 6-MP through hairless mouse skin than any other prodrug or vehicle approach. The fact that they decompose on contact with skin moisture over a 48-h period should not be a practical problem if an anhydrous formulation of the Mannich base is applied topically 2 or 3 times a day as is usually the case.

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