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Mannich base derivatives of theophylline and 5-fluorouracil: syntheses, properties and topical delivery characteristics

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Summary

Mannich base prodrugs of theophylline and 5-fluorouracil have been prepared and tested for their ability to deliver their parent drugs through hairless mouse skin. The Mannich base derivatives were more effective than the previously described N-acyloxyalkyl derivatives. In the case of theophylline the Mannich base derivative was also found to be as effective as the previously described N-hydroxymethyl derivative. All of the Mannich bases reverted to their parent compounds in water, but some were relatively stable in aprotic solvents such as isopropyl myristate which was therefore used as a vehicle for the diffusion experiments with the prodrugs.

Introduction

A problem in the topical or oral delivery of polar, high-melting, heterocyclic drugs is that they are often relatively insoluble in lipids and in water. One approach to overcoming this problem has been to use N-acyloxyalkyl prodrug derivatives which are lower-melting and more lipophilic than the parent drugs. Recently, a number of examples of the application of this approach to modifying heterocyclic drugs have been described (Bodor and Sloan, 1977; Sloan and Bodor, 1982, for theophylline; Stella and Sloan, 1979, for diphenylhydantoin; Ozaki et al., 1981; Mollgaard et al., 1982, for 5-fluorouracil (5-FU); Sloan et al., 1983, for 6-thiopurines). In all of these examples the prodrug derivatives were more effective than the parent

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drug in delivering the parent drug across a topical membrane. Only the enhanced lipid solubilities of the derivatives were discussed, although it was well known that short-chain N-alkyl derivatives of heterocycles exhibit enhanced water solubilities as well (Pitman, 1981; Bansal et al., 1981). However, in the case of theophylline (Sloan and Bodor, 1982) it was shown that the N-acyloxyalkyl prodrugs actually exhibited slightly decreased water solubilities compared to theophylline. This was of particular significance since the theophylline paper also showed that the N-hydroxymethyl derivative of theophylline, which was probably more soluble in water than the parent drug (Bansal et al., 1981), but which was an order of magnitude less soluble in lipids (i.e. isopropyl myristate), outperformed the N-acyloxyalkyl derivatives in delivering theophylline across skin. This performance suggested that other prodrug derivatives which were designed to improve aqueous as well as lipid solubility of the polar, heterocylic drugs should be investigated, and it re-emphasized (Flynn and Yalkowsky, 1972) the importance of increased aqueous as well as lipid solubility in increasing delivery of polar drugs across topical membranes.

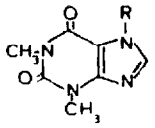
Although no examples of prodrugs have been reported where both water and lipid solubility have been optimized in order to obtain enhanced delivery of drugs across topical membranes, Johansen and Bundgaard (1980) have shown that the conversion of benzamide into its Mannich base derivatives resulted in log P values for the tertiary Mannich bases which were more positive than benzamide itself. Thus, although only the increased water solubilities of the derivatives were discussed, what was not mentioned was that there must also have been an even greater increase in lipid solubilities to give the more positive log P values [1]. The increased lipid as well as water solubility of the Mannich bases suggested that the Mannich base derivatives of amides and imides would be potentially attractive candidates for enhancing the delivery of amides and imides through topical membranes, but most especially through the skin where lack of water stability of the Mannich bases (Bundgaard and Johansen, 1981) might not be a hindrance. Therefore, the following investigation was undertaken to determine if Mannich base derivatives of heterocyclic amide and imide drugs were effective in delivering their parent drugs through skin.

Theophylline and 5-fluorouracil were chosen for derivatization and study because investigations of the effect of their N-acyloxyalkyl derivatives on delivery of the parent compounds relative to the parent compounds were already available in the literature (Sloan and Bodor, 1982; Mollgaard et al., 1982) for comparison with the Mannich base derivatives.

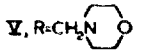
Methods and Materials

The hairless mice that were used were female SKH-hr-1 from Temple University Skin and Cancer Hospital. The Franz diffusion cells were obtained from Crown

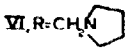
[1] The concomitant increase in the lipid solubility with increased water solubility can be attributed to the decrease in mp exhibited by the Mannich bases – Yalkowsky, S.H. and Valvani, S.C., J. Pharm. Sci., 69 (1980) 912–922.



I, R=H
II, R=$CH_2N(CH_3)_2$
III, R=$CH_2N(C_2H_5)_2$
IV, R=$CH_2N(C_3H_7)_2$



V, R=CH_2N O



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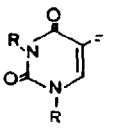
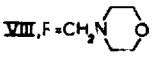


VII, R=H



VIII, R=CH_2N O

Glass, Somerville, NJ. TLC were run on Brinkman Polygram Sil G/UV 254. MP (corrected) were taken with a Thomas-Hoover Capillary apparatus. NMR spectra were recorded on a Varian T-60, IR spectra on a Beckman Accu Lab 1 spectrophotometer and UV spectra on a Beckman model 25 spectrophotometer. Microanalyses were obtained from Atlantic Microlab, Atlanta, GA. 5-Fluorouracil and theophylline were obtained from Sigma while the amines, propylene glycol [2], and formaldehyde were purchased from Aldrich. The bulk solvents were obtained from Fisher. The isopropyl myristate [2] was obtained from Givaudan, Clifton, NJ. The water bath used was a Fisher model 80 circulator bath.

Syntheses:

The preparation of 1,3-bis(4'-morpholinyl)methyl-5-fluorouracil (VIII). Morpholine (0.44 g, 0.005 mole) was added to 0.4 g (0.005 mole) of 37% $H_2C=O$ in water and diluted with tetrahydrofuran (5 ml). The solution was then allowed to react with 0.33 g (0.0025 mole) of 5-FU (5-fluorouracil); the 5-FU gradually went into solution. After 16 h the solution was diluted with acetone (20 ml), and was concentrated in vacuo. This process was repeated twice; then the residue was dried in a desiccator overnight. The residue (0.60 g, 73% yield, m.p. 128–131°C) exhibited NMR and IR spectra consistent with the desired product VIII and gave the correct elemental analyses: ^1H NMR ($CDCl_3$)δ 7.4 (d, $J = 6$ Hz, 1, C$\underline{H}$ = C), 4.93 (s, 2, N–C$\underline{H}_2$–N), 4.50 (s, 2, N–C$\underline{H}_2$–N), 3.8–3.45 (m, 8, C–C$\underline{H}_2$–O) and 2.8–2.45 (m, 8, C–C$\underline{H}_2$–N); UV (CH_3OH) max 267 nm ($\epsilon = 6.45 \times 10^3$ l/mol); IR (KBr) 1710 and 1650 cm^{-1} (S) (C = O).

Anal. Calcd for $C_{14}H_{21}FN_4O_4$: C, 51.24; H, 6.44. Found: C, 51.12; H, 6.45.

The crude product could be triturated with ether to give a 56% recovery of the desired product (m.p. 137–139°C) which was identical with the analytically pure, crude product by NMR and IR spectroscopy. The IR spectrum of the crude product did not contain any N–H absorptions.

The preparation of Mannich base derivatives of theophylline. The reactions were run on 0.90 g (0.005 mole) of theophylline. The theophylline was treated first with 0.50 g (0.005 mole) of triethylamine then with 10 ml of tetrahydrofuran containing

[2] Isopropyl myristate will be abbreviated as IPM and propylene glycol as PG.

0.01 mole of the appropriate secondary amine and 0.81 g (0.01 mole) of 37% $H_2C=O$ in water. The reactions were allowed to stir at room temperature for 3 h; then they were diluted with 150 ml of CH_2Cl_2. The CH_2Cl_2 layers were separated, dried over Na_2SO_4 and concentrated in vacuo to give either oils or solids. These crude products were subsequently triturated with ether or petroleum ether, and the products were filtered and dried to give the following derivatives.

7-(Dimethylamino)methyltheophylline (II): 1.05 g, m.p. 121–123°C, 89% yield from petroleum ether; IR (KBr) 3130 cm^{-1} (M) (C–H), 1710 and 1665 cm^{-1} (S) (C=O); ^1H NMR ($CDCl_3$)δ 7.63 (s, 1, N=C<u>H</u>–N), 5.15 (s, 2, N–C<u>H</u>$_2$–N), 3.6 and 3.4 (two s, 6, O=CN–C<u>H</u>$_3$) and 2.37 (s, 6, C<u>H</u>$_3$–N); UV(CH_3OH) max 273 nm ($\epsilon = 9.24 \times 10^3$ l/mol).

Anal. Calcd for $C_{10}H_{15}N_5O_2$: C, 50.62; H, 6.37. Found: C, 50.55; H, 6.37.

7-(Diethylamino)methyltheophylline (III): 1.20 g, m.p. 122–124°C, 90% yield from petroleum ether [lit.(Burckhalter and Dill, 1959) m.p. 110°C in 38% yield or lit. (Rida et al., 1979) m.p. 114–115°C in 88% yield]; IR (KBr) 3130 cm^{-1} (M) (C–H), 1710 and 1665 cm^{-1} (S) (C=O); ^1H NMR ($CDCl_3$)δ 7.66 (s, 1, N=C<u>H</u>–N), 5.33 (s, 2, N–C<u>H</u>$_2$–N), 3.6 and 3.4 (two s, 6, O=C–N–C<u>H</u>$_3$), 2.71 (q, 4, <u>J</u> = 7 Hz, N–C<u>H</u>$_2$ CH_3) and 1.05 (t, 6, <u>J</u> = 7 Hz, N–CH_2C<u>H</u>$_3$); UV (CH_3OH) max 273 nm ($\epsilon = 9.56 \times 10^3$ l/mol).

Anal. Calcd for $C_{12}H_{19}N_5O_2$: C, 54.32; H, 7.22; Found: C, 54.15; H, 7.24.

7-(Dipropylamino)methyltheophylline (IV): 1.33 g, m.p. 72–73°C, 90% yield from petroleum ether; IR (KBr) 3130 cm^{-1} (M) (C–H), 1705 and 1665 cm^{-1} (S) (C=O); ^1H NMR ($CDCl_3$)δ 7.63 (s, 1, N=C<u>H</u>–N), 5.27 (s, 2, NC<u>H</u>$_2$N), 3.6 and 3.4 (two s, 6, O=C–N–C<u>H</u>$_3$), 2.6 (t, 4, <u>J</u> = 8 Hz, NC<u>H</u>$_2$–CH_2–CH_3), 1.8–1.2 (m, 4, N–CH_2C<u>H</u>$_2$$CH_3$) and 0.9 (t, 6, <u>J</u> = 6 Hz, N–CH_2CH_2C<u>H</u>$_3$); UV (CH_3OH) max 273 nm ($\epsilon = 9.29 \times 10^3$ l/mol).

Anal. Calcd for $C_{14}H_{23}N_5O_2$: C, 57.32; H, 7.90. Found: C, 57.17; H, 7.95.

7-(4'-Morpholinyl)methyltheophylline (V): 1.13 g, m.p. 175–178°C, 80% yield from absolute ethanol [lit.(Burckhalter and Dill, 1959) m.p. 177°C in 92% yield]; IR (KBr) 3130 cm^{-1} (M) (C–H), 1705 and 1660 cm^{-1} (S) (C=O); ^1H NMR ($CDCl_3$) δ7.57 (s, 1, N=C<u>H</u>–N), 5.2 (s, 2, N–C<u>H</u>$_2$–N), 3.6 and 3.4 (two s, 6, O=C–N–C<u>H</u>$_3$), 3.8–3.6 (m, 4, C<u>H</u>$_2$O) and 2.8 – 2.55 (m, 4, N–C<u>H</u>$_2$); UV (CH_3OH) max 273 nm ($\epsilon = 9.58 \times 10^3$ l/mol).

7-(Pyrrolidyl)methyltheophylline (VI): 0.85 g, m.p. 114–115°C, 65% yield from ether [lit.(Burckhalter and Dill, 1959) m.p. 108°C in 61% yield]; IR (KBr) 3130 cm^{-1} (M) (C–H), 1710 and 1660 cm^{-1} (S) (C=O); ^1H NMR ($CDCl_3$) δ7.60 (s, 1, N=C<u>H</u>–N), 5.3 (s, 2, N–C<u>H</u>$_2$–N), 3.57 and 3.37 (two s, 6, O=C–N–C<u>H</u>$_3$), 2.85–2.5 (m, 4, N–C<u>H</u>$_2$) and 2.0–2.55 (m, 4, C<u>H</u>$_2$–CH_2–N); UV (CH_3OH) max 273 nm ($\epsilon = 9.49 \times 10^3$ l/mol).

Anal. Calcd for $C_{12}H_{17}N_5O_2$: C, 54.74; H, 6.51. Found: C, 54.65; H, 6.53.

Determination of delivery of drugs through hairless mouse skin by prodrugs and their parent drugs

Full thickness dorsal skin of 12–14-week-old female hairless mice was used. The mice were sacrificed by cervical dislocation. The excised skin was gently scraped to

remove fat and visceral debris, then gently secured over the diffusion cells with a rubber gasket. The receptor side of the cell (20 ml) was filled with pH 7.1 isotonic phosphate buffer containing 0.03% formaldehyde (1 ml of 37% $H_2C=O$ in 1000 ml water) and was stirred magnetically. The temperature of the cells was maintained at 32°C with a water bath.

Before the suspensions of the drug or prodrugs were applied to the membranes, background UV absorptions due to leaching of UV absorbing material from the skin into the receptor phases were determined for each cell by allowing the skin to be in contact with the receptor phase, and monitoring the UV spectra of the receptor phases until stable spectra were obtained. This usually took from 2 to 4 h. This absorption was then subtracted from the total absorption observed during the course of the diffusion run to give the absorption due to theophylline at 275 nm or 5-fluorouracil at 270 nm.

All the suspensions were prepared by sonicating mixtures of the drug or prodrugs in the appropriate solvent in sealed flasks for 3 h. The sealed suspensions that resulted were allowed to sit at room temperature for 24 h; then they were briefly (15 s) vortexed, and 0.50 ml samples were removed and applied to the membranes (area = 4.9 cm^2). After an additional 24 h the remaining sealed suspensions of the prodrugs were filtered. The filtrates and residues were analyzed by inspection of their NMR spectra [3] and TLC [3]; it was determined that no decomposition of the prodrugs had taken place. The filtrates were analyzed by UV spectroscopy and it was determined that the amount of prodrug still in solution corresponded to the solubilities determined in separate experiments. The remaining suspensions of the parent drugs were also filtered and the filtrates were analyzed by UV spectroscopy; it was determined that the amount of drug still in solution corresponded to the solubilities determined in separate experiments.

After the suspensions of the drugs or prodrugs were applied to the membranes, samples (3 ml) were taken of the receptor phases at 3, 6, 9, 12 and 24 h. The receptor phases were immediately replaced with 3 ml of the buffer solution and the samples were analyzed by UV spectroscopy within 0.5 h.

TLC analyses of the receptor phases showed only the parent drugs were present, and when samples of the receptor phases were made basic the UV spectra underwent a shift of the UV max to longer wavelengths which is characteristic of the N-unsubstituted parent heterocycles. NMR spectroscopy also showed that the Mannich bases immediately reverted to their parent compounds in water. NMR spectra of the prodrugs in DMSO-d_6 showed that, upon addition of water to the NMR samples, there was an immediate loss of the N-$C\underline{H}_2$-N absorptions in the spectra; no hydroxymethyl N-$C\underline{H}_2$-OH absorptions (δ 5.6 for theophylline, and δ 5.2 and 5.1 for 5-FU) were observed.

In the diffusion cell experiments each combination of drug and vehicle was tested

[3] The filtrates were analyzed neat while the residues were dissolved in $CDCl_3$ and the spectra were analyzed for the appearance of the C_8-$\underline{H}$ absorption of theophylline at δ 7.77 and the C_6-$\underline{H}$ absorption of 5-FU at δ 7.7. TLC analyses were run using ether as the eluents.

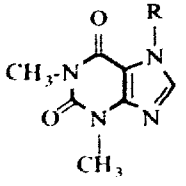
in parallel. Thus, during each experiment the first diffusion cell had parent drug in IPM, the second had the prodrug in IPM applied to the mouse skin membranes.

Determination of solubilities

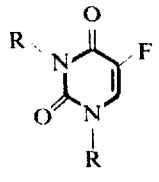
The solubilities in the solvents (see Table 1) were determined in two ways. The first way was under the same conditions as the samples that were applied to the diffusion cells were prepared, except that the samples were filtered and the filtrate was analyzed by UV spectroscopy. The second way was by stirring an excess of the drug or prodrug in the solvent for 24 h, allowing the suspension to sit at room temperature for 24 h, then filtering the suspension. All solubilities were determined in triplicate at least, and were reproducible within ±3% under the conditions that they were determined. The neat IPM filtrates were also analyzed by NMR spectroscopy by running each spectrum of each filtrate under exactly the same conditions and comparing the average of five integrations of the C_8–H absorptions due to each of the Mannich bases (the C_8–H for theophylline was not observable because of the

TABLE 1

SELECTED PROPERTIES OF PRODRUGS AND PARENT DRUGS



I. R = H
II–VI



VII, R = H
VIII

Drug or prodrug	Solubility (mg/ml)			m.p. (°C)
	Water	IPM [d]	PG [d]	
I: Theophylline	8.3 [a]	0.095 [c]	16.2 [c]	270–274 [a]
II: R = CH_2–$N(CH_3)_2$	> 100 [b]	7.6 [c,e]		121–123
III: R = CH_2–$N(C_2H_5)_2$	> 100 [b]	32.5 [e]		122–124
IV: R = $CH_2N(C_3H_7)_2$	> 100 [b]	27.6 [e]		72–73
V: R = CH_2–N⟨O⟩	> 100 [b]	1.3 [c]		175–178
VI: R = CH_2–N⟨ ⟩	> 100 [b]	10.7 [c]		114–115
VII: 5-Fluorouracil	26.2 [c]	0.0044 [c]	15.1 [c]	282–283 [a]
VIII: R = CH_2–N⟨O⟩	> 100 [b]	11.8 [c]		128–131

[a] The Merck Index, Windholz, M. (Ed.), 9th edn., Merck and Co., Rahway, NJ., 1976.

[b] Values obtained by adding 1.0 ml of water (pH 7.0) to 100 mg of Mannich base and sonicating the mixture for 10 s. This is not an accurate measure of solubilities due to the very rapid decomposition of the Mannich bases in water (Eqn. 3). Thus, the apparent increases in the solubilities of the prodrugs are probably due to the ability of the amines that are formed during the decomposition of the prodrugs to ionize the parent drugs that are also formed during the decomposition of the prodrugs.

[c] Values obtained by analysis of suspensions sonicated for 3 h.

[d] IPM = isopropyl myristate; PG = propylene glycol.

[e] Values obtained by analysis of suspensions stirred at room temperature for 24 h.

low solubility of theophylline). This gave the relative solubilities of the Mannich bases in IPM and verified that the prodrugs were intact in the IPM solutions.

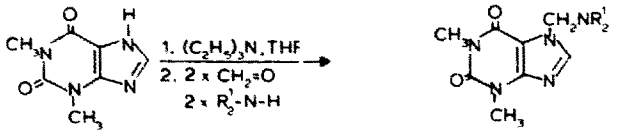
Analysis by UV and NMR spectroscopy of samples of suspensions of drugs or prodrugs used in diffusion cells experiments gave the same solubilities as these solubility determinations.

Results and Discussions

Synthesis

The Mannich bases were prepared according to modifications of literature procedures in the case of theophylline (Rida et al., 1979; Burckhalter and Dill, 1959). However, only in the case of the morpholinylmethyl derivative V was it possible to obtain the desired pure product using only one equivalent of formaldehyde and secondary amine in absolute ethanol as described in the literature. In the other cases it was found that the use of the combination of an equivalent of the tertiary amine – triethylamine – and two equivalents of formaldehyde and secondary amine in the reaction was necessary to give complete conversion of theophylline to its Mannich bases (Eqn. 1). It was particularly important in the processing of

equation 1



these reactions to separate the water and dry the CH_2Cl_2 layer carefully with Na_2SO_4. Otherwise, significant amounts of 7-(hydroxymethyl)theophylline ($HOC\underline{H}_2$–N at δ 5.57 in $CDCl_3$) were formed during the isolation process and it could not be separated from the Mannich base by crystallization or chromatography. The consistently low melting points previously reported for the Mannich bases of theophylline (Rida et al., 1979; Burckhalter and Dill, 1959) were probably due to contamination by 7-(hydroxymethyl)theophylline.

The UV (Gulland et al., 1934), IR and NMR spectra of the theophylline Mannich bases were consistent with their assigned structures; in particular no C-alkylated impurities ($CC\underline{H}_2N$ at δ 3.48; Rida et al., 1979) were observed.

N-Mannich bases of 5-fluorouracil (5-FU) have not been previously reported, although C-alkylation of uracil itself has been reported using the conditions under which the Mannich reaction is usually run (Burckhalter et al., 1960). In this study only the 1,3-bis(4'-morpholinyl)methyl-5-FU derivative (VIII) was characterized; no mono-derivatives were observed spectroscopically even when only one equivalent of amine and formaldehyde was used. The lack of previous reports of the synthesis of 5-FU N-Mannich bases was probably due to the fact that the Mannich base derivatives of 5-FU obtained from other amines and formaldehyde typically were not solids and could not be isolated from reactions mixtures without decomposition occurring. For instance, the reaction of pyrrolidine and formaldehyde with 5-FU (Eqn. 2) gave an oil which exhibited two N–$C\underline{H}_2$–N type absorptions in its NMR

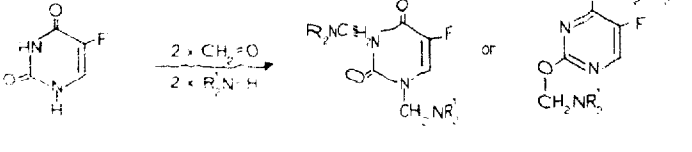
spectrum, no N–H absorptions in its IR spectrum and was homogeneous by TLC analysis. However, the oil would not crystallize and would not give correct elemental analyses.

The spectral properties of VIII clearly identified it as an N-Mannich base. First, the C<u>H</u> = C absorption was still intact indicating no C-alkylation had taken place. Second, the X–C<u>H</u>$_2$–N absorption was too far upfield for a O–C<u>H</u>$_2$–N absorption compared to a N–C<u>H</u>$_2$–N absorption. Usually a O–C<u>H</u>$_2$–N absorption such as in IX is about 60 Hz downfield from a comparable N–C<u>H</u>$_2$–N absorption, and since the X–C<u>H</u>$_2$–N absorptions for VIII were upfield from the comparable N–C<u>H</u>$_2$–N absorptions in the theophylline derivatives, they could not be N–C<u>H</u>$_2$–O absorptions, but must be N–C<u>H</u>$_2$–N absorptions [4].

Stability and solubility studies

The N-Mannich bases were not stable in the presence of water. This result is consistent with results obtained using equations developed for the hydrolysis of Mannich bases (Bundgaard and Johansen, 1981) and the pK_as of the parent compounds (5-FU, pK_as 8.0 and 13.0, Florey, 1973; theophylline, pK_a 8.6, Florey, 1975) which suggested $t_{1/2}$ values of about 1.3×10^{-5} min (N-3) and 7 min (N-1) for the 5-FU (VIII), and 5×10^{-8} min for the theophylline (VI) Mannich bases. NMR experiments also supported the low stability of the Mannich bases in water. Solutions of the N-Mannich bases were prepared in dry DMSO-d_6 and then D_2O was added stepwise to the solutions. As soon as the first drops of D_2O were added the ratio of the N–C<u>H</u>$_2$–N absorptions to the C_8–<u>H</u> or C_6–<u>H</u> absorptions in theophylline or 5-FU, respectively, immediately decreased until a new equilibrium was obtained between the amide (or imide) plus the carbinolamine (formaldehyde plus amine), and the Mannich base plus water (Eqn. 3). NMR spectra of the

equation 2



IX

equation 3 $RCNHCH_2NR'_2 + H_2O \rightleftharpoons RCNH_2 + HOCH_2NR'_2$

Mannich bases in D_2O showed that immediate and complete decomposition had occurred, as determined by the complete loss of the N–C<u>H</u>$_2$–N absorptions. Similar decomposition of the Mannich bases was also observed in other protic solvents such as methanol and propylene glycol. Thus, the Mannich base prodrugs are chemically labile and do not require enzymatic assistance to regenerate the parent drugs under the conditions of the diffusion cell experiments.

On the other hand, the stability of the Mannich bases in IPM was variable. On

[4] C_4H_8N–C<u>H</u>$_2$–OC_2H_5 at δ 4.17 compared to C_4H_8N–C<u>H</u>$_2$–NC_4H_8 at δ 3.20; Sloan, K.B., unpublished work.

the basis of analyses of NMR spectra of the solid phases and the solutions, derivatives II, V, VI and VIII were found to be stable in IPM either in the solid phase or in solution 24 h after they had been sonicated for 3 h. Under those conditions derivatives III and IV were stable in solution but the IPM insoluble material was theophylline. This result is likely due to decomposition in solution by the Mannich base followed by precipitation of theophylline since theophylline is so much less soluble in IPM than any of the derivatives. Support for such an interpretation follows from the fact that solutions of the Mannich bases prepared by stirring overnight instead of by sonication resulted in higher observed solubilities for III and IV but not for II. In the case of III, the analysis of the NMR spectrum of the IPM-insoluble portion showed only the prodrug was present while in the case of IV mostly theophylline remained [5].

When the IPM filtrates, which were used to determine the IPM solubilities of II and III, were exposed to atmospheric moisture at room temperature for 2 days, 50–80% decomposition of the Mannich base prodrugs in solution occurred and precipitation of theophylline resulted as determined by analysis of NMR spectra of the solutions and precipitates [5]. The IPM solutions of the other Mannich bases eventually decomposed 1–10% upon prolonged exposure (2 weeks) to atmospheric moisture. Although no quantitative data were determined, the qualitative order of stability of the theophylline Mannich bases on exposure to atmospheric moisture in IPM solution was $V = VI \gg IV > III > II$, as determined by analysis of NMR spectra of their IPM solutions and precipitates from IPM solutions [5].

The trend in the IPM solubility data generated by UV for the Mannich bases of theophylline by either method (c) or (d) in Table 1 was found to be consistent with the trend observed by analysis of the NMR spectra of the neat IPM solutions of the prodrugs immediately after filtration, i.e. $III > IV > VI > II > V$. The fact that there was agreement between the solubility data obtained for II by all 3 methods suggests that for thermally stable Mannich bases either method (c) or (d) in Table 1 can be used to determine solubilities. Two additional trends in the solubility data were also apparent. First, the relatively polar 5-FU and theophylline molecules were quite soluble in protic solvents such as water and propylene glycol but were relatively insoluble in IPM (as expected because of their polar structures). Second, the Mannich base prodrugs exhibited significantly improved water and lipid (IPM) solubilities relative to 5-FU and theophylline. This effect is probably due to the decrease in melting points (decreased hydrogen bonding) exhibited by the derivatives (Table 1) which tends to increase the thermodynamic activity of the derivatives in their solid state and their solubility in general (Repta et al., 1975).

Diffusion studies

One Mannich base derivative each of theophylline and 5-FU was chosen for study in diffusion cell experiments using hairless mouse skin. The pyrrolidylmethyl

[5] The filtrates were analyzed neat while the residues were dissolved in $CDCl_3$ and the spectra were analyzed by comparing the integrations of the C_8-H absorption of theophylline at δ 7.77 with the C_8-H absorptions of the intact Mannich bases at δ 7.57–7.06.

derivative VI of theophylline was chosen for study because it was the most lipid soluble of the derivatives that were thermally stable and relatively stable to atmospheric moisture. The bis-(4′-morpholinyl)methyl derivative VIII of 5-FU was chosen for study because it was stable and it was the best characterized derivative of 5-FU.

The diffusion data (Tables 2 and 3, and Figs. 1 and 2) suggest that the Mannich base prodrugs are more effective as prodrugs for the delivery of theophylline and 5-FU through skin than the acyloxyalkyl derivatives of the same drugs. In order to support that conclusion, it was determined that for the parent drugs the diffusion data generated in this study were consistent with previous studies using diffusion cells. The previous study of prodrugs of theophylline (Sloan and Bodor, 1982)

TABLE 2

COMPARISON OF DELIVERY OF THEOPHYLLINE (TH) THROUGH HAIRLESS MOUSE SKIN BY VI AND THEOPHYLLINE

Drug or prodrug (vehicle)	mg present as parent drug in solution in donor phase	mg (±S.D., n = 3) present as parent drug in receptor phase after 12 h	Flux (mg/cm^2·h ±S.D., n = 3)	Lag time (h)
I: Theophylline [a] (IPM) [b]	0.047	1.41 ± 0.89	$2.85 \pm 1.79 \times 10^{-2}$ [d]	2.6
VI: TH-7-CH_2N⟨morpholine⟩ [a] (IPM) [b]	3.7 [c]	9.17 ± 0.98	$19.5 \pm 1.78 \times 10^{-2}$ [d]	2.2

[a] 0.50 ml of a 0.36 M suspension, or the equivalent of 32.4 mg of theophylline applied.
[b] Isopropyl myristate.
[c] 5.4 mg of prodrug in solution.
[d] Calculated based on linear regression analysis of data for 3, 6, 9 and 12 h.

TABLE 3

COMPARISON OF DELIVERY OF 5-FLUOROURACIL (5-FU) THROUGH HAIRLESS MOUSE SKIN BY VIII AND 5-FLUOROURACIL

Drug or prodrug (vehicle)	mg present as parent drug in solution in donor phase	mg (±S.D., n = 3) present as parent drug in receptor phase after 12 h	Flux (mg/cm^2·h ±S.D., n = 3)	Lag time (h)
VII: 5-Fluorouracil [a] (IPM) [b]	0.0022	0.25 ± 0.023	$5.56 \pm 3.55 \times 10^{-3}$ [d]	2.7
VIII: 5-FU-1,3-CH_2N⟨morpholine⟩O [a] (IPM) [b]	2.33 [c]	1.59 ± 0.13	$3.0 \pm 0.14 \times 10^{-2}$ [d]	0.8

[a] 0.50 ml of a 0.04 M suspension, or the equivalent of 2.6 mg of 5-FU applied.
[b] Isopropyl myristate.
[c] 5.9 mg of prodrug in solution.
[d] Calculated based on linear regression analysis of data for 3, 6, 9 and 12 h.

presented data for the delivery of theophylline from IPM for 3, 6, 9 and 12 h from which a flux of $1.72 \pm 0.13 \times 10^{-2}$ mg/cm^2 · h ($r = 0.999$) could be determined. The flux of $2.85 \pm 1.79 \times 10^{-2}$ mg/cm^2 · h ($r = 0.982$) obtained in this study was not significantly different from the previously determined flux ($P < 0.5 > 0.2$), thus suggesting that the results of the two studies are consistent with each other.

The Mannich base VI was about 6.9 times (19.5/2.85) more effective than theophylline in delivering theophylline from IPM while the 7-(hydroxymethyl)theophylline prodrug, which was the best prodrug for the delivery of theophylline previously described (11.1×10^{-2} mg/cm^2 · h), was about 6.5 times more effective than theophylline (11.1/1.72). In addition, the best acyloxymethyl prodrug derivative [the 7-(butyryloxymethyl)theophylline derivative, 7.25×10^{-2} mg/cm^2 · h] was only 4.2 times (7.25/1.72) better than theophylline in delivering theophylline from IPM. Therefore, the Mannich base VI is qualitatively comparable to the best prodrug of theophylline previously described and is significantly better than the best acyloxyalkyl derivative in delivering theophylline from IPM across skin.

Although diffusion data for 3, 6, 9 and 12 h have been used in the comparison of this work with the previous work on delivery of theophylline from IPM, when the 24

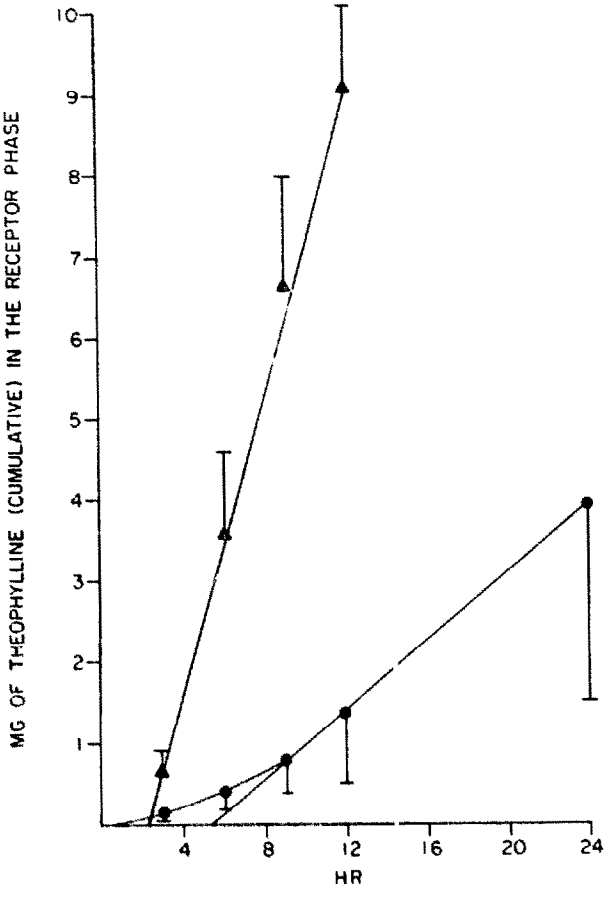


Fig. 1. The delivery of theophylline through hairless mouse skin by theophylline in isopropyl myristate (●) and by the prodrug VI in isopropyl myristate (▲).

h data of this study were included in the calculation (see Fig. 1) a flux of $4.32 \pm 2.58 \times 10^{-2}$ mg/cm^2 · h was obtained (using 9, 12 and 24 h data) with a lag time of 5.3 h. No data for 24 h were available from the previous study so a comparison including the 24 h flux data was not possible. The flux for the Mannich base of theophylline from 12 to 24 h decreased considerably since well over 42% of the total applied theophylline had been delivered at 24 h.

When the data for flux of 5-FU from IPM in Table 3 were used, the prodrug VIII was 5.5 times more effective than 5-FU in delivering 5-FU from IPM. When the data for the diffusion of the best prodrug of 5-FU (1-butyryloxymethyl derivative) described in Mollgaard et al., (1982) were used to calculate a flux for the prodrug from PG, a flux of 17.2×10^{-4} mg/cm^2 · h was obtained while for 5-FU itself a flux of 7.8×10^{-4} mg/cm^2 · h was obtained. Therefore, the acyloxymethyl prodrug of 5-FU was only 2.2 times better than 5-FU in delivering 5-FU from PG (17.2/7.8). Although it is not possible to compare delivery from two such different vehicles, the Mannich base derivative is much more effective in delivering 5-FU from IPM (3.0×10^{-2} mg/cm^2 · h) than the best acyloxymethyl derivative reported is in delivering 5-FU from PG.

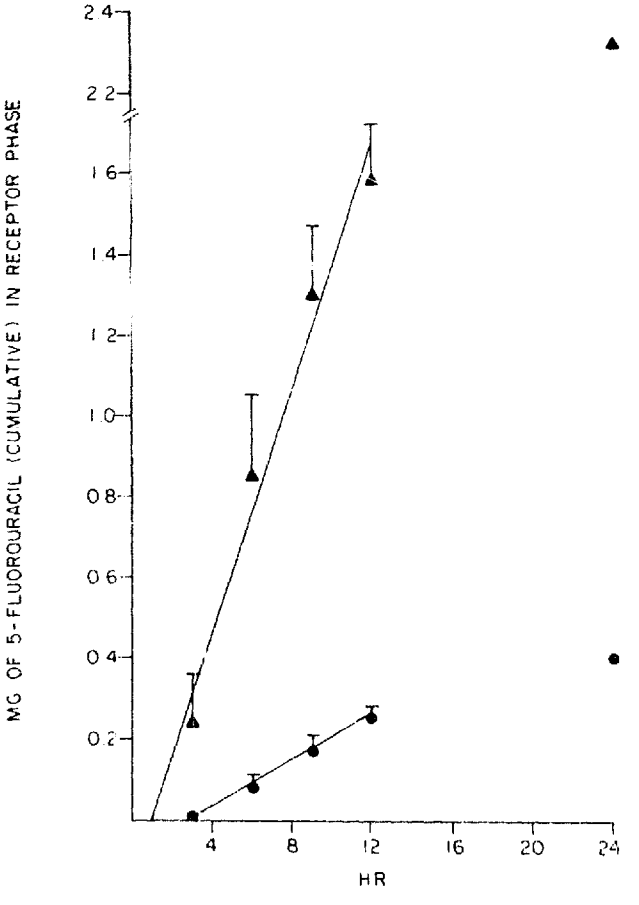


Fig. 2. The delivery of 5-fluorouracil (5-FU) through hairless mouse skin by 5-FU in isopropyl myristate (●), and by the prodrug VIII in isopropyl myristate (▲).

The Mannich base prodrug derivatives of theophylline and 5-FU as well as the hydroxymethyl derivatives of theophylline (Sloan and Bodor, 1982) are significant compared to other prodrugs of amides and imides which have been designed to enhance delivery of their parent drugs through skin because they exhibit enhanced water as well as enhanced lipid solubilities. These more water-soluble (polar) prodrugs are also more effective in improving topical delivery than prodrugs that have been designed to incorporate only lipid solubilizing groups into the structure of the parent drugs. Although this result should not be surprising in view of the fact that skin and other biological membranes present lipid–water (biphasic) barriers to absorption, increased water solubility has not been a design factor when developing prodrugs (especially of polar heterocyclic drugs) for improved topical delivery. Regardless, then, of the molecular mechanism by which the present result is accomplished, water as well as lipid solubility should be a design goal in future development of prodrugs for improved topical delivery; and the Mannich bases appear to be attractive candidates for consideration to accomplish that goal, especially since they are chemically labile and do not require enzymatic assistance to regenerate the parent drugs under protic conditions.

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