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N-Mannich base derivatives of 5-fluorocytosine: a prodrug approach to improve topical delivery

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Summary

A series of *N*-Mannich bases of 5-fluorocytosine (5-FC) were synthesized and characterized. The prodrugs exhibited decreased melting points and increased lipid solubilities when compared to 5-FC. In addition, the prodrugs were found to hydrolyze readily in buffer, but were stable in isopropyl myristate (IPM) which was therefore used as the vehicle in the diffusion cell studies. The effect on delivery of 5-FC through hairless mouse skin by the Mannich bases was studied and it was found that improved delivery of the drug through skin was obtained. In addition, the effect of these compounds upon skin was investigated. After application of 5-FC and the prodrugs in IPM to the skin, a suspension of theophylline in propylene glycol (PG) was applied to the skins. The fluxes obtained from this second application were compared to the flux of theophylline from PG through skins which had not been previously treated with anything. It was found that 5-FC in IPM and IPM caused a significant increase in the flux of theophylline from PG. While there was also an increase in the flux of theophylline from PG through skins to which the prodrugs were applied, it was less than that seen for either 5-FC in IPM or IPM alone.

Introduction

5-Fluorocytosine (5-FC) is an antifungal antimetabolite with a narrow spectrum of action. In susceptible fungi, 5-FC is metabolized to 5-fluorouracil (5-FU) and/or 5-fluorodeoxyuridine monophosphate (5-FdUMP); both of these metabolites can lead to anomalies in fungal macromolecular synthesis (Polak, 1977; Waldorf and Polak, 1983). In man, 5-FC is not extensively metabolized and is excreted mostly unchanged. However, there is the possibility that some 5-FU may be generated in the intestines due to bacterial metabolism of 5-FC (Diasio et al., 1978). 5-FC is a polar, high-melting compound and, as such, is characterized by poor lipid and water solubilities, so that it does not exhibit adequate topical delivery characteristics. But, since 5-FC has been shown effective in the systemic treatment of certain cutaneous fungal infections (e.g. chromomycosis and mucocutaneous candidiasis) (Gringauz, 1983), a topical delivery form would be desirable.

The main barrier to topical delivery is the stratum corneum — the outer most layer of skin (Scheuplein, 1967). Diffusion across the stratum

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corneum is governed by Fick's law:

$$J = [(PC)(D)(C^v)]/H$$

The flux (J) is a measure of the steady-state rate of diffusion of a drug through the stratum corneum, in units of mass per area per time ($mg/cm^2/h$) (Martin et al., 1983). The partition coefficient (PC) is defined as the ratio of solubility of the drug in skin to its solubility in the vehicle (C^v) (Scheuplein, 1965). The concentration of drug in vehicle (C^v) and PC provide the driving force for passive diffusion. D is the diffusion coefficient and H is the membrane thickness. Further, for a given drug (prodrug)–vehicle-membrane combination, the terms D, H and PC can be combined into the term, K_p, the permeability constant (Poulson, 1973). Therefore, the simplified form of Fick's law can be written:

$$J = K_p(C^v)$$

There are two basic approaches to affect the flux of a compound: the first is by changing the vehicle used to deliver the compound (formulation), and the second is by changing the intrinsic solubility of the compound (prodrug). Both of these methods can affect the solubility and the PC of the compound. With the formulation approach, only the compound's solubility in the vehicle is changed. As the solubility of the drug in the vehicle is increased, the vehicle competes with the skin for the drug and partition coefficient, as well as flux, can actually decrease (Poulsen, 1973). Prodrugs, however, can increase the intrinsic solubility of the drug in both the skin and vehicle. Therefore, assuming that the increase in the solubility in the skin is proportionately greater than the increase in C^v, both PC and C^v, as well as flux, can increase. In light of this, the prodrug approach was chosen to increase the topical delivery of 5-FC.

As mentioned earlier, 5-FC exhibits poor biphasic solubility and biphasic solubility has been shown to be an important determinant of flux across skin (Sloan and Bodor, 1982; Bodor and Sloan, 1983). As shown with two other heterocyclic drugs — theophylline and 5-fluorouracil — *N*-Mannich base prodrugs can increase both lipid and water solubilities of the parent drug and lead to an increase in the delivery of the parent drug through the skin (Sloan et al., 1984). These *N*-Mannich bases are formed through the reaction of an NH-acidic group with formaldehyde and secondary amines:

$$\text{N–H} + \text{H}_2\text{C=O} + \text{HN–R}_2 \rightleftarrows \text{N–CH}_2\text{–NR}_2$$

The prodrug reverts to the parent compound by simple chemical hydrolysis (Bundgaard and Johansen, 1980). Thus, *N*-Mannich base prodrugs would seem to be especially useful for topical applications where their sensitivity to water would not necessarily be a liability. The present paper discusses the results of the use of an *N*-Mannich base prodrug approach to increase the topical delivery of 5-FC.

Materials and Methods

5-FC was obtained from Sigma; the paraformaldehyde, formaldehyde and the amines were all purchased from Aldrich. Isopropyl myristate (IPM) was obtained from Givaudan, Clifton, NJ. All other reagents were purchased from Fisher. The amines were all distilled over KOH before using, all other chemicals were used as received from the supplier.

NMR spectra were recorded on a Varian T-60, IR on a Beckman Acculab I and UV spectra on a Cary 210 spectrophotometer fitted with a constant temperature bath. All the UV spectra were run in dry acetonitrile; the acid and base shifts were obtained by adding 1 drop of either 12 N HCl or 12 N NaOH to the acetonitrile solutions (Leonard et al., 1965). Microanalyses were performed by Atlantic Microlabs, Atlanta, GA. MP (corrected) were taken on a Thomas-Hoover Capillary apparatus. TLC were run on Brinkman Polygram Sil G/UV 254.

The female, hairless mice (15–20 g, SKH-hr-1) were obtained from Temple University Skin and Cancer Hospital. The Franz diffusion cells were purchased from Crown Glass, Somerville, NJ. The water bath used was a Fisher model 80 circulator bath.

Syntheses

Preparation of bis-N^4,1-(1'-piperidinyl)methyl-5-fluorocytosine from aqueous formaldehyde (Ia)

To 0.39 g (0.003 mol) of 5-FC was added 0.97 g (0.012 mol) of 37% aqueous formaldehyde. The suspension was stirred until a clear solution had formed. The 1.02 g (0.012 mol) of piperidine was added and the reaction mixture was diluted with 10 ml of THF. After the mixture was stirred for 24 h, the suspension was triturated with ether to give a white solid (84% yield, mp > 220°C (d)) that was the desired adduct: IR (KBr) 3220 cm^{-1} (W) (NH), 1685 (M) and 1635 (S) (C=O and C=N); ^{1}H-NMR ($CDCl_3$) δ 7.40 (d, 1, J = 6 Hz, 6-C$\underline{H}$), 5.82–5.28 (m, 1, N–$\underline{H}$), 4.52–4.23 (broad s, 4, N–C$\underline{H}_2$–N), 2.75–2.38 (m, 8, C$\underline{H}_2$–N–C$\underline{H}_2$) and 1.77–1.25 (m, 12, C–C$\underline{H}_2$); UV (CH_3CN) max 289 nm ($\epsilon = 6.99 \times 10^3$ l/mol) max 242 nm ($\epsilon = 8.15 \times 10^3$ l/mol), UV (HCl) max 281 nm ($\epsilon = 7.92 \times 10^3$ l/mol) and UV (NaOH) max 289 nm ($\epsilon = 6.56 \times 10^3$ l/mol) max 244 nm ($\epsilon = 8.14 \times 10^3$ l/mol). *Anal.* Calcd for $C_{16}H_{26}FN_5O$: C, 59.42; H, 8.10; N, 21.66. Found: C, 59.38; H, 8.14; N, 21.63.

Reaction of 5-FC with excess paraformaldehyde and secondary amine

To 0.24 g (0.008 mol) of paraformaldehyde in 20 ml of dichloromethane was added 0.008 mole of the appropriate secondary amine. The suspension was stirred until a solution resulted (2–24 h). 5-FC (0.25 g, 0.002 mol) was added to the solution and the suspension that formed was stirred overnight. The resultant solution was concentrated in vacuo and the residue was triturated twice with ether or a mixture of ether and petroleum ether (1 : 1). The following *N*-Mannich bases were obtained:

Bis-N^4,1-(1'-piperidinyl)methyl-5-fluorocytosine (Ib): 181–183°C; 83% yield from ether/petroleum ether (1 : 1) and then ether; Ib was identical with Ia by IR, NMR and UV. *Anal.* Calcd for $C_{16}H_{26}FN_5O$: C, 59.42; H, 8.10; N, 21.66. Found: C, 59.23; H, 8.11.

Bis-N^4,-1-(diethylamino)methyl-5-fluorocytosine (II): mp 129–131°C, 70% yield from ether; IR (KBr) 3210 cm^{-1} (broad W) (NH and OH), 1680 (M) and 1630 (S) (C=O and C=N); ^{1}H-NMR ($CDCl_3$) δ 7.53 (d, 1, J = 6 Hz, 6-C$\underline{H}$), 5.57–5.17 (m, 1, N–$\underline{H}$), 4.63–4.37 (broad s, 4, N–C$\underline{H}_2$–N), 2.68 (q, 8, J = 7 Hz, N–C$\underline{H}_2$–CH_3) and 1.08 and 1.05 (2 t, 12, J = 7 Hz, CH_2–C$\underline{H}_3$); UV (CH_3CN) max 289 nm ($\epsilon = 7.69 \times 10^3$ l/mol) max 244 nm ($\epsilon = 8.82 \times 10^3$ l/mol), UV (HCl) max 291 nm ($\epsilon = 1.16 \times 10^4$ l/mol) and UV (NaOH) max 289 nm ($\epsilon = 6.85 \times 10^3$ l/mol) max 244 nm ($\epsilon = 8.67 \times 10^3$ l/mol). *Anal.* Calcd for $C_{14}H_{26}FN_5O \cdot 0.25\ H_2O$: C, 55.33; H, 8.62; N, 23.05. Found: C, 55.43; H, 8.69; N, 23.06.

Bis-N^4,1-(dimethylamino)-methyl-5-fluorocytosine (III): mp 136–138°C, yield 46% from ether/petroleum ether (1 : 1); IR (KBr) 3190 cm^{-1} (M) and 3025 (W) (NH and OH), 1665 (M) and 1615 (S) (C=O and C=N); ^{1}H-NMR ($CDCl_3$) δ 7.37 (d, 1, J = 6 Hz, 6-C$\underline{H}$), 4.52–4.28 (broad s, 4, N–C$\underline{H}_2$–N) and 4.03 (s, 12, N–C$\underline{H}_3$); UV (CH_3CN) 289 nm ($\epsilon = 6.99 \times 10^3$ l/mol) max 243 nm ($\epsilon = 8.15 \times 10^3$ l/mol), UV (HCl) max 282 nm ($\epsilon = 7.96 \times 10^3$ l/mol) and UV (NaOH) max 289 nm ($\epsilon = 5.63 \times 10^3$ l/mol) max 242 nm ($\epsilon = 7.83 \times 10^3$ l/mol). *Anal.* Calcd for $C_{10}H_{18}FN_5O \cdot 0.25\ H_2O$: C, 48.47; H, 7.53. Found: C, 48.29; H, 7.21.

Bis-N^4,1-(1'-pyrrolidyl)methyl-5-fluorocytosine (IV): mp 140–142°C (foam, d); 85% yield from ether/petroleum ether (1 : 1); IR (KBr) 3220 cm^{-1} (broad W) (NH and OH), 1675 (S) and 1645 (S) (C=O and C=N); ^{1}H-NMR (DMSO-d_6) δ 7.72 (d, 1, J = 6 Hz, 6-C$\underline{H}$), 5.05–4.78 (m, 1, N-$\underline{H}$), 4.48 (s, 2, N–C$\underline{H}_2$–N), 4.35–4.10 (m, 2, NH–C$\underline{H}_2$–N), 2.82–2.32 (m, 8, C$\underline{H}_2$–N) and 1.92–1.42 (m, 8, C–C$\underline{H}_2$); UV (CH_3CN) max 289 nm ($\epsilon = 6.13 \times 10^3$ l/mol) max 243 nm ($\epsilon = 7.22 \times 10^3$ l/mol), UV (HCl) max 278 nm ($\epsilon = 7.35 \times 10^3$ l/mol) and UV (NaOH) max 289 nm ($\epsilon = 5.70 \times 10^3$ l/mol) max 243 nm ($\epsilon = 9.23 \times 10^3$ l/mol). *Anal.* Calcd for $C_{14}H_{22}FN_5O \cdot 0.75\ H_2O$: C, 54.70; H, 7.21; N, 22.78. Found: C, 54.59; H, 7.31; N, 22.45.

Bis-N^4,1-(4'-morpholinyl)methyl-5-fluorocytosine (V): mp 154–156°C, 71% yield from ether/petroleum ether (1 : 1) and then ether; IR (KBr) 3220 cm^{-1} (broad W) (NH and OH), 1680 (S) and 1630 (S) (C=O and C=N); ^{1}H-NMR ($CDCl_3$) δ 7.35 (d, 1, J = 6 Hz, 6-C$\underline{H}$), 5.92–5.39 (m, 1, N–$\underline{H}$), 4.58–4.25 (broad s, 4, N–C$\underline{H}_2$–N), 3.88–3.48 (m, 8, C$\underline{H}_2$–N) and 2.82–2.38 (m, 8, C$\underline{H}_2$–O); UV

(CH_3CN) max 289 nm ($\epsilon = 7.20 \times 10^3$ l/mol) max 246 nm ($\epsilon = 9.25 \times 10^3$ l/mol), UV (HCl) max 282 nm ($\epsilon = 8.82 \times 10^3$ l/mol) and UV (NaOH) max 289 nm ($\epsilon = 6.70 \times 10^3$ l/mol) max 246 nm ($\epsilon = 9.23 \times 10^3$ l/mol). *Anal.* Calcd for $C_{14}H_{22}FN_5O_3 \cdot 0.33\ H_2O$: C, 50.44; H, 6.98; N, 21.01. Found: C, 50.30; H, 6.72; N, 21.01.

Bis-N^4,1-(4'-methyl-1'-piperazinyl)methyl-5-fluorocytosine (VI): mp 163–165°C, 73% yield from ether/petroleum ether (1 : 1); IR (KBr) 3210 cm^{-1} (broad W) (NH and OH), 1675 (S) and 1625 (S) (C=O and C=N); ^{1}H-NMR ($CDCl_3$) δ 7.35 (d, 1, J = 6 Hz, 6-CH), 5.95–5.35 (m, 1, N–H), 4.58–4.32 (broad s, 4, N–CH_2–N), 2.85–2.57 (m, 8, CH_2–N), 2.57–2.35 (m, 8, CH_2–N–CH_3) and 2.27 (s, 6, N–CH_3); UV (CH_3CN) max 289 nm ($\epsilon = 6.49 \times 10^3$ l/mol) max 245 nm ($\epsilon = 8.07 \times 10^3$ l/mol), UV (HCl) max 290 nm ($\epsilon = 8.46 \times 10^3$ l/mol) and UV (NaOH) max 289 nm ($\epsilon = 6.46 \times 10^3$ l/mol) max 245 nm ($\epsilon = 7.99 \times 10^3$ l/mol). *Anal.* Calcd for $C_{16}H_{28}FN_7O \cdot 0.75\ H_2O$: C, 52.37; H, 8.10; N, 26.72. Found: C, 52.32; H, 7.78; N, 26.69.

Determination of solubilities

The lipid solubilities of 5-FC and its prodrugs were obtained in IPM. Three suspensions of the drug or prodrugs were allowed to stir for 48 h; the suspensions were then filtered. The filtrates were then diluted with either methanol, for 5-FC, or dry acetonitrile, for the prodrugs. The amount of compound in solution was determined by monitoring the UV absorption at 278 nm ($\epsilon = 4.98 \times 10^3$ l/mol) for 5-FC or at 289 nm for the prodrugs. Further the prodrugs were stable in IPM.

Determination of half-lives and rates of hydrolysis

The hydrolysis of the prodrugs in pH 7.1 buffer was monitored by UV spectroscopy at 278 nm, by measuring the appearance of 5-FC. The hydrolyses were determined to be first-order by plotting log ($A_t - A_\infty$) versus time. The hydrolyses were then run in triplicate at a constant temperature of 24°C with a water bath fitted to the spectrophotometer. The half-lives and rates of hydrolysis were obtained using the Guggenheim method (Jencks, 1969).

Diffusion cell studies

The diffusion cells were run in triplicate for each compound. Three female, hairless mice were sacrificed by cervical dislocation, and the full dorsal skin thickness was used. The skins were secured with a rubber gasket to the diffusion cell, with the dermis in contact with the receptor phase. The receptor side of the cell (20 ml) was filled with isotonic phosphate buffer (pH 7.3 at 32°C) with 0.1% formaldehyde added to inhibit microbial growth. The receptor phase was stirred magnetically and was kept at a constant temperature of 32°C with a circulating water bath.

The skins were placed in contact with the receptor phase for 24–48 h to remove any water-soluble, UV-absorbing compounds, then the receptor phase was replaced with fresh buffer. The compounds were applied as suspensions (0.5 ml, 0.1 M). The suspensions were allowed to stir for 48 h prior to application to the skin surface. Samples (3 ml) were removed from the receptor phase periodically over 48 h, with each sample being replaced by an equal amount of fresh buffer. The UV absorption of the samples was monitored at 278 nm ($\epsilon = 5.94 \times 10^3$ l/mol) — the UV max of 5-FC in this buffer.

The cumulative amount of 5-FC in the receptor phase at any time was calculated by adding 15% (3 ml sample from 20 ml receptor phase) of the mg of 5-FC in the previous samples to the sample being analyzed. This takes into account the amount of 5-FC removed from the receptor phase when the previous 3 ml samples were taken for analysis. The fluxes were calculated by plotting the cumulative mg of 5-FC in the receptor phase against the time of sampling. The slopes for each phase of these plots were determined by linear regression ($r > 0.99$), and the slopes were divided by the surface area of the diffusion cells (4.9 cm^2) to give flux in mg/cm^2/h. The reported fluxes are the averaged values for the three diffusion cells.

After the 48 h, the remaining donor phase was removed by washing the skin surface with methanol (2×10 ml) and the washings were analyzed by UV spectroscopy to determine the amount of 5-FC remaining in the donor phase (methanol decomposes the prodrugs). The receptor phases were then replaced with fresh buffer

and the skins were allowed to remain in contact with the buffer for 24 h. The receptor phase was then sampled and analyzed by UV spectroscopy to determine the amount of 5-FC that had leached from the skin. These last two steps were used to determine the mass balance. This part of the diffusion cell study constitutes the first application.

After the above sampling the receptor phase was changed. The skins were allowed to remain in contact with the buffer for 30 min and then a 3 ml sample was taken and analyzed by UV spectroscopy to ensure that all of the 5-FC had leached from the skin. Theophylline in propylene glycol (PG, 0.5 ml, 0.4 M) was applied to each donor side. This suspension had been allowed to stir for 48 h prior to application. The receptor phase was again sampled over a 48 h period. The UV absorption of the samples was measured at 270 nm ($\epsilon = 1.02 \times 10^4$ l/mol) to determine the amount of theophylline in the receptor phase. The cumulative amount of theophylline and the fluxes were determined as before. This phase of the diffusion cell studies constituted the second application.

Several control studies were also performed. Following the same timing as the first application, 3 hairless mouse skins were allowed to remain in contact with pH 7.3 (at 32°C) buffer for a total of 120 h (nothing applied for the first application). Then theophylline in PG was applied as in the second application and the flux of theophylline was determined. In a second study, 3 skins were again placed in contact with buffer for 96 h, washed twice with 10 ml of methanol, and put into contact with buffer for an additional 24 h. Theophylline in PG was then applied and the flux of theophylline measured to assess the effect of the methanol wash (used to remove the remaining donor phase from the first application) on the skin's permeability. In the third experiment, 3 mouse skins were allowed to leach for 48 h and then 0.5 ml of IPM was added to each donor side. After 48 h, the IPM was washed off with methanol. After a 24 h leach period, theophylline in PG was applied and the flux of theophylline was measured. Finally, in an attempt to assess the effect of the components of the delivery system itself on subsequent diffusion, 3 skins were leached for 48 h with buffer, then 0.5 ml of a 0.2 M suspension of paraformaldehyde and piperidine in IPM was applied. After 48 h, the skins were washed with methanol and allowed to remain in contact with buffer for an additional 24 h. The receptor phase was changed. Then theophylline in PG was applied and the flux of theophylline was measured.

Results and Discussion

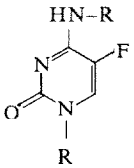
Syntheses and structure determination

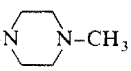
These *N*-Mannich base derivatives of cytosine-based drugs are novel prodrugs (Table 1). The first method (Method I) used to synthesize the *N*-Mannich base prodrugs of 5-FC consisted of allowing 5-FC to react with excess 37% formalin and a secondary amine. With this method only the piperidinylmethyl derivative could be isolated, and its melting point was still rather high (> 220°C). Since 5-FC itself is rather hygroscopic, the water in the formalin solution made product isolation difficult. In an effort to design a more 'anhydrous' procedure (Method II) to synthesize these prodrugs, paraformaldehyde, a solid polymer of formaldehyde, was used as the methylene donor. With this method *N*-Mannich bases of 5-FC could be formed with a wide variety of secondary amines. Upon comparison of the piperidinylmethyl derivatives formed by the two methods, the prodrug formed with paraformaldehyde (Ib) had a lower melting point (181–183°C). This was felt to be due to different crystal polymorphs caused by the different workup procedures, since the two forms could be interconverted by using the other workup procedure.

Since there are 3 possible sites of alkylation in 5-FC (endocyclic amino groups at positions 1 and 3; exocyclic amino group at position 4), it was necessary to determine the exact sites of alkylation. The ^{1}H-NMR of the prodrugs suggested that dialkylation had occurred. When the spectra were run in $CDCl_3$ only a broad singlet was obtained for the N–C$\underline{H}_2$–N protons, however, when the spectra were run in DMSO-d_6 two separate N–C$\underline{H}_2$–N resonances could be seen. For example, when the spectrum of the morpholinyl derivative was obtained, these resonances were located at δ 4.42 (singlet) and 4.19 (multiplet). The multi-

TABLE 1

Melting points and solubilities of the N-Mannich base prodrugs of 5-fluorocytosine



Compound (R)	mp (°C)	Solubility in IPM (± S.D.)	
		mg/ml ($\times 10^2$)	M($\times 10^4$)
5-FC: -H	295–297 [a]	0.50 (0.03)	0.39
Ia: 	> 220 [b]	5.0 (0.9)	1.6
Ib: 	181–183	12.0 (0.5)	3.7
II: $-CH_2-N(C_2H_5)_2$	129–131	271 (6.0)	91
III: $-CH_2-N(CH_3)_2$	136–138	23.0 (0.1)	9.5
IV: 	140–142	18.0 (0.3)	6.1
V: 	154–156	18.0 (0.7)	5.5
VI: 	163–165	7.1 (0.5)	2.2

[a] Literature value, Windholz, M. (Ed) Merck Index, 10th edn., Merck and Co., Rahway, NJ, 1983.

[b] Synthesized with aqueous formaldehyde (Method I), all others were synthesized with paraformaldehyde (Method II).

plet collapsed to a singlet upon addition of D_2O, therefore one site of alkylation was assigned to the exocyclic amino group at position 4. When the spectra in $CDCl_3$ were expanded, the broad singlet that corresponded to these resonances also resolved into a singlet and a multiplet. The assignment of a dialkylated structure to these derivatives corresponds to that given for the Mannich base adducts of cytosine itself (Sloan and Siver, 1984).

The assignment of the N^4-position as a site of alkylation has literature precedent. McGhee and von Hippel (1975) found that the reaction of formaldehyde with cytidine formed the *N*-hydroxymethyl group at the exocyclic amine, and identified the structure by ^{1}H-NMR. Another group trapped the N^4-hydroxymethyl derivative of cytosine with bisulfite and used X-ray crystallography to confirm its structure (Hayatsu et al., 1982).

The remaining singlet was assigned to the 1-position and ^{13}C-NMR was used to confirm this assignment. The spectrum of the *bis*-adduct of cytosine with morpholine was obtained in DMSO-d_6 and compared to that of cytidine, a 1-substituted cytosine. The spectra were found to be similar (Sloan and Siver, 1984). Therefore, these aminomethylated derivatives of 5-FC were assigned a *bis*-N^4,1-alkylated structure by analogy to the results obtained for cytosine itself.

Solubility and kinetic data

As expected, these Mannich base adducts showed improved lipid solubility over that of the parent compound as measured in IPM (Table 1). It should be noted that a decrease in melting point accompanied the increase in solubility. *N*-alkylation of the drug removes sites of hydrogen-bonding which helps to decrease the strong crystal

TABLE 2

Half-lives of hydrolysis of the N-Mannich bases of 5-FC in pH 7.1 phosphate buffer (0.05 M, μ = 0.15) at 24°C

Compound	Half-life [± S.D. (min)]
II	0.1 (0.0)
III	0.74 (0.00)
Ia	0.89 (0.03)
Ib	0.90 (0.01)
IV	1.05 (0.01)
V	9.10 (0.20)
VI	26.30 (1.00)

lattice forces present in the parent compound (Pitman, 1981; Repta et al., 1975). This relation between melting point and solubility is exemplified by the two piperidine derivatives (Ia and Ib), the lower melting compound (Ib) has a solubility in IPM twice that of the higher melting one (Ia).

The hydrolyses of these prodrugs was followed by UV spectroscopy in phosphate buffer (0.05 M, pH 7.1). The prodrugs exhibited a wide range of stabilities, with the diethyl derivative having the shortest half-life and the *N*-methylpiperazine derivative the longest (Table 2). The hydrolysis of these compounds was also followed by ^{1}H-NMR spectroscopy. A sample of prodrug was dissolved in DMSO-d_6 and D_2O was added dropwise. The methylene protons at the 1-position disappeared first, followed by the slower disappearance of the N^4-methylene protons.

Diffusion cell experiments

First application. Hairless mouse skin was used to test the delivery of 5-FC through skin (Table 3). It had been found to be a good approximation of human skin for similar poorly soluble drugs (Stoughton, 1975). The prodrugs were applied as suspensions in IPM to the donor side of the diffusion cell. The suspensions were the same concentration (0.5 ml, 0.1 M; equivalent to 6.46 mg of applied 5-FC), except for the high-melting piperidine derivative (0.05 M suspension, equivalent to 3.23 mg of applied 5-FC). The amount of 5-FC left in the skin and donor phases were determined and added to the amount that diffused to obtain a mass balance; the mass balances were 90 ± 5% of the applied doses. The donor phases were checked by NMR during the diffusion experiment, and the prodrugs were found to be intact by comparing the intensities of the 6-C<u>H</u> and N–C<u>H</u>$_2$–N resonances.

The prodrugs were found to significantly increase the delivery of 5-FC. There was usually a lag time before a steady-state delivery of 5-FC was obtained (Figs. 1 and 2 and Table 3). The *bis*-dimethylaminomethyl-5-fluorocytosine prodrug (III) exhibited the best delivery rate, with a steady-state rate of delivery of 5-FC approximately 10 times that of 5-FC itself delivered from IPM. The two

TABLE 3

Flux and permeability coefficient for steady-state phase of delivery of 5-fluorocytosine through hairless mouse skin

Compound/ IPM [a]	Flux (mg/cm^2/h) (± S.D. × 10^3)	K_p (cm/h) (× 10^3)	X-intercept (h)
5-FC	5.0 (1.5)	111	6.0
V	15.10 (0.97)	213	9.9
VI	26.1 (8.41)	919	14.2
Ia [b]	34.6 (19.9)	1671	2.8
Ib	34.20 (1.50)	716	7.3
III	44.7 (2.7)	365	4.7

[a] Supension of 0.1 M.
[b] Suspension of 0.05 M.

TABLE 4

Steady-state flux of theophylline from propylene glycol after application of 5-fluorocytosine and its N-Mannich base prodrugs (second application)

First application	Flux(mg/cm^2/h) (± S.D. × 10^3)	X-intercept (h)
Controls:		
No methanol wash	1.41 (0.09)	3.5
Methanol wash	2.42 (0.4)	4.7
IPM [a]	184 (23)	1.6
Aminomethylating agent/IPM [b]	162 (27)	1.4
Compound/IPM:		
5-FC	261 (5)	1.0
III	81.3 (15.3)	1.0
Ib	177 (52)	1.7
V	58.4 (6.6)	0.6
VI	76.6 (10.2)	1.0

[a] Sloan et al., 1986.
[b] Aminomethylating agent formed through the reaction of paraformaldehyde with piperidine.

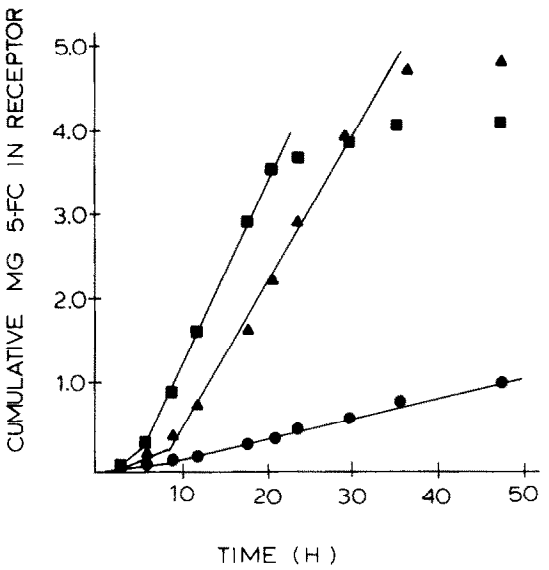


Fig. 1. Graph of diffusion cell data for 5-FC (●), III (■) and Ib (▲).

crystal polymorphs of compound I exhibited the same flux in spite of their different solubilities. The most soluble prodrug (II) was not tested because it was not stable in IPM exposed to atmospheric moisture.

The rate of delivery of 5-FC from III and Ib

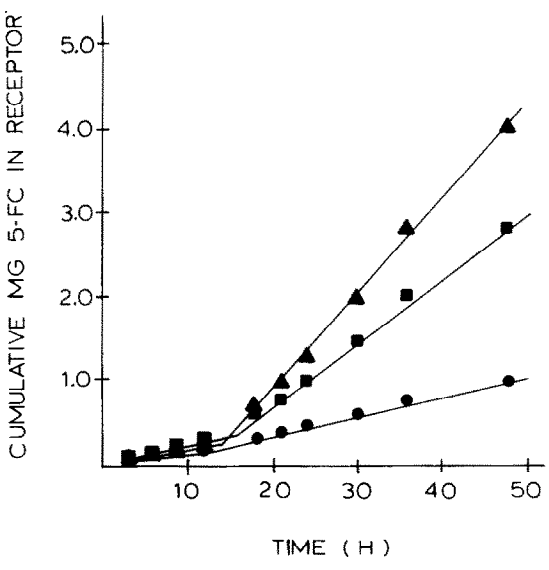


Fig. 2. Graph of diffusion cell data for 5-FC (●), VI (▲) and V (■).

leveled off after 20 and 35 h, respectively. This was due to depletion of suspended prodrug in the donor phase so the donor phase became a soution with a concomitant decrease in activity. The maximum amount of 5-FC delivered was only about 4–5 mg compared to an expected delivery of about 6 mg. That is because the configuration of the Franz cell sample port necessitated removal of the donor phase (by pipet) each time a sample was taken and fresh buffer added. Thus, some of the initial suspension clung to the walls of the pipet and was not available to dissolve, partition into the skin and deliver 5-FC.

On the basis of the equation for flux, it may seem that the increased solubility of the derivatives in the vehicle, caused by the decrease in crystal lattice forces compared to 5-FC, is causing the increase in flux seen with the prodrugs. However, the expected concomitant increase in solubility in the skin (also caused by the decrease in crystal lattice forces in 5-FC upon its conversion to the prodrugs) must also be responsible for the higher fluxes, since the compound's ability to partition into the skin is part of the driving force for diffusion. Further, if a drug's solubility in the vehicle is increased to a point that the vehicle competes with the skin for the drug, flux will be decreased (Sloan et al., 1986). Therefore, while these *N*-Mannich base derivatives obviously increase the equivalent solubility of 5-FC in the vehicle, they are probably also increasing the solubility in the skin, which may be the more important factor. This is one advantage of using a prodrug to increase delivery into skin over a formulation approach — a prodrug can actually increase the compound's intrinsic solubility.

Second application. In an effort to assess the effects of these compounds on the skin, a second application of theophylline in propylene glycol (Th/PG) was performed in each diffusion cell experiment (Table 4). The steady-state fluxes obtained from the second application were used as a measure of the impairment of the skin's barrier function (Sloan et al., 1986). Several studies were performed to serve as baseline values. When the skins were kept in contact with buffer for 120 h (with no methanol wash), then a Th/PG suspension was applied, a flux of $1.41 \pm 0.09 \times 10^3$

mg/cm^2/h for Th/PG was obtained (Table 4). If the skins were again kept in contact with buffer for 120 h but were washed with methanol 24 h before application of Th/PG, the flux increased by slightly more than one-half. While the methanol does have some effect on the permeability of the skin, the wash is necessary to remove the donor phases from the first application. The remaining fluxes were compared to this latter value. It should be mentioned that there does not appear to be much difference between the flux of Th/PG obtained upon application of Th/PG after 120 h contact of the skin with buffer compared to the flux of Th/PG after 2 or 24 h contact (Sloan et al., 1986).

Application of IPM alone or 5-FC in IPM had the most detrimental effect on the skin, as evidenced by the large increases in the flux of Th/PG compared to the controls (76 and 107 times greater, respectively). 5-FC seems to have an effect in addition to that of IPM, since the flux of Th-PG is significantly greater after application of 5-FC/IPM than after IPM alone ($P < 0.01$). The application of the aminomethylating agent (solution of paraformaldehyde and piperidine) in IPM seemed to have no significant effect over that of IPM—the flux of Th/PG in this experiment was not significantly different ($P < 0.20$) from that seen after application of IPM alone. Interestingly, the prodrugs, except for Ib, caused significantly decreased fluxes of Th/PG when compared to those obtained after application of IPM or 5-FC in IPM ($P < 0.01$).

While IPM does seem to affect skin permeability, this alone cannot account for the total increase in delivery of 5-FC into skin that was seen from the application of the prodrugs, since the prodrug/IPM combinations caused less damage to the skins than IPM alone or 5-FC/IPM. Also, IPM is a commonly used vehicle in pharmaceutical formulations and has been found to have a low potential for irritancy when applied to normal skin (Frosch and Kligman, 1977). Finally, since these *N*-Mannich base prodrugs are not stable in protic solvents, IPM presented the best choice as a vehicle to use in these studies.

These novel *N*-Mannich base prodrugs apparently are excellent derivatives to increase the topical delivery of nitrogen-containing heterocycles. They are chemically, as opposed to enzymatically, hydrolyzed and the degree of stability can be modified according to the secondary amine used to synthesize the prodrug. The lipophilicity of the prodrugs can also be adjusted with the choice of the secondary amine, so there is a great deal of flexibility in terms of the solubility and the stability of the prodrugs that can be synthesized.

Finally, it should be mentioned that these prodrugs liberate formaldehyde upon hydrolysis. The Cosmetic Ingredient Review (Cosmetic, Toiletry and Fragrance Association) has set a maximum allowable level of formaldehyde in dermatological formulations at 0.2% (Elder, 1984). Even at a prodrug concentration of 0.1 M, only 0.3% formaldehyde would be liberated upon complete hydrolysis.

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