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The effect of aminomethyl (N-Mannich base) derivatization on the ability of S^6-pivaloyloxymethyl-6-mercaptopurine prodrug to deliver 6-mercaptopurine through hairless mouse skin

Akram N. Saab and K.B. Sloan

Department of Medicinal Chemistry, University of Florida, Gainesville, FL 32610 (U.S.A.)

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Summary

A series of aminomethyl (N-Mannich base) derivatives of S^6-pivaloyloxymethyl-6-mercaptopurine (6-POM-6-MP) have been synthesized from the reaction of 6-POM-6-MP with formaldehyde and secondary amines. In contrast to the results from the aminomethylation of 6-mercaptopurine (6-MP), which gave 7-alkylation (7-AM-6-MP), the aminomethylation of 6-POM-6-MP gave 9-alkylation (9-AM-6-POM-6-MP). The 9-AM-6-POM-6-MP derivatives were much more soluble in isopropyl myristate (IPM) than 6-POM-6-MP itself or the corresponding 7-AM-6-MP derivatives. The 9-AM-6-POM-6-MP derivatives were all more effective (2.5–4 times) than 6-POM-6-MP and were more effective (1.5–15 times) than the corresponding 7-AM-6-MP derivatives in delivering 6-MP through hairless mouse skin, except for the 9-diethylaminomethyl-6-POM-6-MP derivative which was only 0.28 times as effective as 7-diethylaminomethyl-6-MP. In contrast to the 6-POM-6-MP derivative which delivered only 6-MP, the 9-AM-6-POM-6-MP derivatives also delivered comparable amounts of 6-POM-6-MP through hairless mouse skin from isopropyl myristate (IPM). There was a fairly good correlation between the log experimental permeability coefficients for the delivery of 6-MP ($r = 0.87$) or for the delivery of total 6-MP ($r = 0.81$) and the calculated solubility parameter values for the 9-AM-6-POM-6-MP prodrugs with the permeability coefficient generally decreasing as the calculated solubility parameter value for the prodrug approached that of the vehicle IPM.

Introduction

6-Mercaptopurine (6-MP; **I**) is an antiproliferative agent that is active orally (Kravets and Balsam, 1961) but inactive topically (Lowe et al., 1981) in the treatment of psoriasis. The apparent reason for its inactivity when given topically is its poor solubility properties (Sloan et al., 1983) that cause it to be inadequately absorbed. Since it would be advantageous to have a topically available form of 6-MP to reduce the potential for its systemic toxicity, a number of articles have recently appeared describing the effect of vehicles (Waranis et al., 1987) and of two prodrug approaches (Waranis and Sloan, 1987; Waranis and Sloan, 1988; Siver and Sloan, 1988) on enhancing the delivery of 6-MP through hairless mouse skin.

The two prodrug approaches that have been described are complementary to each other. The

Correspondence: K.B. Sloan, Department of Medicinal Chemistry, Box J-485, University of Florida, Gainesville, FL 32610, U.S.A.

first approach involves "soft" (Sloan et al., 1983) alkylation of 6-MP with α-(acyloxy)alkyl halides to give either S^6,9-bisacyloxymethyl derivatives (Waranis and Sloan, 1987) or S^6-acyloxymethyl derivatives (Waranis and Sloan, 1988) which are enzymatically reversible. In this approach either one or two of the polar functional groups in 6-MP are masked with a promoiety that primarily imparts enhanced lipid solubility to 6-MP. However, in both series of more lipid soluble prodrugs, the prodrugs that exhibit enhanced water solubility are also the more effective members of the series at enhancing the delivery of 6-MP through hairless mouse skin (about 60 times). Interestingly, a comparison of the fluxes achieved by the mono- with those achieved by the bis-acyloxymethyl prodrugs containing the same acyl group showed that the delivery of 6-MP from isopropyl myristate (IPM) was not significantly enhanced by adding a second lipophilic group which masked the second polar functional group in 6-MP. Apparently, the increase in lipophilicity in going from a mono-alkyl derivative where the more polar thionamide functional group is masked (Waranis and Sloan, 1988) to a bis-alkyl derivative where both polar functional groups are masked is more than offset by the decrease in water solubility caused by the addition of the second lipophilic promoiety.

On the other hand, the second prodrug approach – aminomethylation of 6-MP – gives 7-aminomethyl-6-MP derivatives where the less polar imidazole functional group is masked with a polar promoiety containing a tertiary amine group that should primarily impart enhanced water solubility to the drug (Siver and Sloan, 1988), albeit transiently because of the chemical instability of the aminomethyl derivatives in water. This approach is also very effective at enhancing the delivery of 6-MP through hairless mouse skin (3–180 times). By contrast, an acyloxymethyl derivative of 6-MP in which only the imidazole functional group is masked (9-pivaloyloxymethyl-6-MP, Waranis and Sloan, 1988) and which exhibits lipid solubility comparable to that of the 7-aminomethyl derivatives is less effective (0.4 times) at delivering 6-MP than 6-MP itself from the same vehicle. Similar results are observed when comparing the effectiveness of acyloxymethyl and aminomethyl derivatives of theophylline and 5-fluorouracil (Sloan et al., 1984) at enhancing the delivery of their respective parent drugs through hairless mouse skin i.e., the aminomethyl derivative is more effective.

Although both previously described prodrug approaches are effective at enhancing the delivery of 6-MP through skin, it is of continuing interest to determine the factors involved in optimizing topical delivery by prodrug approaches. Since the introduction of another lipophilic acyloxymethyl promoiety into the S^6-acyloxymethyl-6-MP derivatives did not significantly increase the delivery of 6-MP through skin, and it was not possible to isolate sufficiently stable bisaminomethyl derivatives of 6-MP to test in diffusion cell experiments (Siver et al., 1988), the only remaining permutation to be examined for the two approaches is the effect of combinations of the acyloxymethyl and aminomethyl approaches. In this paper we describe the syntheses and characterizations of aminomethyl derivatives of 6-pivaloyloxymethyl-6-MP (9-AM-6-POM-6-MP), as well as report on their abilities to enhance the delivery of 6-MP through skin.

Experimental

Melting points were taken on a Thomas Hoover capillary melting point apparatus and are corrected. ^{1}H NMR spectra were recorded on a Varian EM-390 spectrometer. UV spectra were recorded on a Cary 210 or Shimadzu UV-265 spectrophotometer. The 6-mercaptopurine hydrate (6-MP) was obtained from Sigma. The isopropyl myristate (IPM) was obtained from Givaudan Corp., Clifton, NJ. The remaining vehicles and reagents were obtained from Aldrich except for the bulk solvents which were obtained from Fisher. The diffusion cells were Franz type cells (2.5 cm 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. The HPLC system consisted of a Beckman Model 110A pump with a model 153 detector, a Rheodyne 7125 injector

with a 20 μl loop, a Beckman C_8, 4.6 mm × 25 cm reverse phase column, and a 3392A Hewlett Packard integrator.

Syntheses

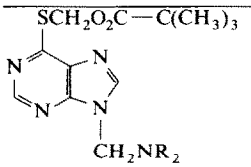
The preparation of S^6-pivaloyloxymethyl-6-MP (II). To an acetone (20 ml) solution of pivaloyloxymethyl chloride (1.5 g, 0.01 mol) was added 1.5 g (0.01 mol) of anhydrous sodium iodide. The suspension that began to form was stirred at room temperature for 3 h. At the same time, 1.7 g (0.01 mol) of 6-MP hydrate was added to a methanol (20 ml) solution of 85% potassium hydroxide (0.66 g, 0.01 mol). The thick white suspension that formed was allowed to stir at room temperature until the acetone suspension was added to it. When the acetone suspension was added to the methanol suspension the mixture became essentially clear. That solution was stirred at room temperature overnight. The suspension that resulted was concentrated to dryness. That residue was extracted with 100 ml of chloroform at room temperature for 30 min. The chloroform was filtered and the filtrate was concentrated to give a white solid which was fairly pure product: 2.18 g, 81% yield, mp 186–192 °C. The product was crystallized from dichloromethane to give a total of 1.98 g of pure product identical by mp, TLC and ^{1}H NMR with authentic product (Waranis and Sloan, 1988): 74% yield, mp 194–195 °.

General method for the synthesis of the aminomethyl derivatives of S^6-pivaloyloxymethyl-6-MP

To one equivalent of S^6-pivaloyloxymethyl-6-MP (6-POM-6-MP) was added a mixture of 1.2 equivalents of the appropriate secondary amine and 1.2 equivalents of 37% aqueous formaldehyde. Enough water was added to give an easily stirred suspension which was stirred overnight at room temperature. The suspension was filtered and washed with a minimal amount of water. The residue was dried in vacuo at 30 °C until a constant weight was obtained to give the desired products. The NMR and UV spectral data are given in Tables 1 and 2, while the elemental analyses and % yields are reported here.

TABLE 1

^{1}H NMR data for 9-aminomethyl-6-POM-6-MP derivatives in $CDCl_3$



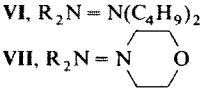
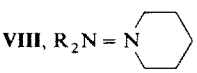
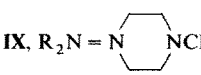
Derivative	Chemical shift, δ			
	$NC\underline{H}_2NR_2$	$SC\underline{H}_2O_2C$	2-<u>H</u>	8-<u>H</u>
III, $R_2N = N(CH_3)_2$	5.05	6.05	8.75	8.03
IV, $R_2N = N(C_2H_5)_2$	5.16	6.03	8.73	8.03
V, $R_2N = N(C_3H_7)_2$	5.16	6.03	8.73	8.03
VI, $R_2N = N(C_4H_9)_2$	5.2	6.1	8.76	8.06
VII, R_2N = 	5.11	6.1	8.83	8.1
VIII, R_2N = 	5.13	6.1	8.76	8.06
IX, R_2N = 	5.13	6.1	8.8	8.06

TABLE 2

Solubility, melting point and UV data for the 9-aminomethyl derivatives of 6-POM-6-MP

Derivative	mp (°C)	Solubility [a] mg/ml of solution	UV [b] λ_{max} (log ε)
I [c]	314(d)	–, –, 0.0030	
II [d]	195–196	–, 1.40, 0.80	
III	86– 88	38.2, 31.4, 17.9	277 (4.23), 213 (4.18)
IV	70– 72	111.3, 84.3, 48.2	277 (4.23), 213 (4.17)
V	87– 89	56.6, 39.7, 22.7	277 (4.47), 213 (4.41)
VI	58– 60	313.5, 204.9, 117.1	276 (4.83), 214 (4.77)
VII	134–136	5.6, 4.1, 2.3	276 (4.23), 213 (4.27)
VIII	91– 93	55.2, 40.4, 23.1	277 (4.31), 213 (4.27)
IX	104–106	24.0, 16.9, 9.7	276 (4.22), 212 (4.21)

[a] Solubilities in isopropyl myristate in mg of 9-aminomethyl-6-MOP-6-MP, equivalent mg of 6-POM-6-MP, and equivalent mg of 6-MP/ml of solution, respectively. [b] Ultraviolet absorbance in nm obtained in acetonitrile. [c] 6-MP. [d] 6-POM-6-MP.

TABLE 3

Calculated solubility parameters of 9-aminomethyl 6-POM-6-MP derivatives (δ_j), rates of delivery of 6-MP (J_i) and 6-POM-6-MP (J_j), and log experimental permeability coefficients for the delivery of 6-MP (P_i), of 6-POM-6-MP (P_j), and of total 6-MP (P_Σ) from IPM

Derivative	δ_j [a]	Steady state [b]		Steady state [c]		
		$J_i \times 10^3$ (±S.D.) mg/cm^2 h	log P_i cm/h	$J_j \times 10^3$ (±S.D.) mg/cm^2 h	log P_j [d] cm/h	log P_Σ [e] cm/h
I [f]	14.40	0.6 (0.3)	−0.73			
II [f]	12.60	9.4 (1.4)	−1.94			
III	11.81	37.0 (0.90)	−2.68	75.7 (0.6) [43.3]	−2.62	−2.35
IV	11.47	33.9 (3.20)	−3.16	32.5 (2.2) [18.6]	−3.41	−2.97
V	11.20	24.5 (1.2)	−2.97	70.0 (3.3) [40.0]	−2.75	−2.55
VI	10.98	30.3 (3.1)	−3.59	35.4 (2.1) [20.2]	−3.76	−3.37
VII	11.99	38.6 (1.8)	−1.78	29.6 (3.7) [16.9]	−2.14	−1.62
VIII	11.78	33.3 (0.50)	−2.84	25.6 (6.3) [14.6]	−3.20	−2.68
IX	11.74	29.0 (7.1)	−2.52	52.6 (9.0) [30.1]	−2.51	−2.21

[a] Solubility parameter in $(cal/cm^3)^{1/2}$. [b] Rates of delivery in terms of mg of 6-MP/cm^2 h, $n = 3$. [c] Rates of delivery in terms of mg of 6-POM-6-MP/cm^2 h and [equivalent mg of 6-MP/cm^2 h]. [d] Permeability coefficient determined either from (mg of 6-POM-6-MP/cm^2 h)/(equivalent mg of 6-POM-6-MP/ml of IPM from Table 2) or (equivalent mg of 6-MP/cm^2 h)/(equivalent mg of 6-MP/ml of IPM from Table 2). [e] Permeability coefficient obtained by adding P_i and P_j. [f] Solubility parameter, flux and permeability coefficient for 6-POM-6-MP (**II**) and 6-MP (**I**) using IPM as a vehicle.

9-Dimethylaminomethyl-6-POM-6-MP (**III**): 66% yield.

Anal. Calcd for $C_{14}H_{21}N_5O_2S$: C, 51.99; H, 6.54; N, 21.65.

Found: C, 52.05; H, 6.55; N, 21.57.

9-Diethylaminomethyl-6-POM-6-MP (**IV**): 85% yield.

Anal. Calcd for $C_{16}H_{25}N_5O_2S$: C, 54.67; H, 7.16; N, 19.92.

Found: C, 54.59; H, 7.20; N, 19.87.

9-Dipropylaminomethyl-6-POM-6-MP (**V**): 91% yield.

Anal. Calcd for $C_{18}H_{29}N_5O_2S$: C, 56.96; H, 7.69; N, 18.45.

Found: C, 57.03; H, 7.75; N, 18.40.

9-Dibutylaminomethyl-6-POM-6-MP (**VI**): 85% yield.

Anal. Calcd for $C_{20}H_{33}N_5O_2S$: C, 58.94; H, 8.16; N, 17.18.

Found: C, 59.05; H, 8.18, N, 17.18.

9-(Morpholin-4′-yl)methyl-6-POM-6-MP (**VII**): 80% yield.

Anal. Calcd for $C_{16}H_{23}N_5O_3S$: C, 52.58; H, 6.34; N, 19.16.

Found: C, 52.56; H, 6.37; N, 19.08.

9-(Piperidin-1′-yl)methyl-6-POM-6-MP (**VIII**): 88% yield.

Anal. Calcd for $C_{17}H_{25}N_5O_2S$: C, 56.13; H, 6.93; N, 19.26.

Found: C, 56.13; H, 6.95; N, 19.22.

9-(4′-Methylpiperazin-1′-yl)methyl-6-POM-6-MP (**IX**): 63% yield.

Anal. Calcd for $C_{14}H_{21}N_5O_2S$: C, 51.99; H, 6.54; N, 21.65.

Found: C, 52.05; H, 6.55; N, 21.57.

Determination of solubilities

The solubilities of the aminomethyl derivatives of 6-POM-6-MP were determined in triplicate (S.D. ± 3%) by stirring an excess of each derivative

in IPM (3 ml) with a magnetic stirrer for 48 h at room temperature ($23 \pm 1°C$) in a sealed flask which was thermally insulated from the stirrer surface (Table 2). The 48 h was sufficient to ensure that the solution was saturated. The suspension was filtered through a 0.45 μm nylon membrane filter and a portion of the filtrate was immediately diluted with acetonitrile. The concentration of the derivative in the acctonitrile solution was determined by measuring the UV absorbance at 277 nm (ϵ from Table 2). The residue from each filtration was dissolved in $CDCl_3$ and analyzed by ^1H NMR spectroscopy to determine if the aminomethyl derivative was intact based on the intensity of the N-$C\underline{H}_2$-N absorption and the position and intensity of the C_8-$\underline{H}$ absorption.

Calculation of solubility parameters of N-Mannich base prodrugs

The calculated solubility parameters for the N-Mannich base derivatives of 6-POM-6-MP were obtained using the group contribution method of Fedors (1974) as illustrated by Waranis and Sloan (1987) and Waranis et al. (1987). The values for the solubility parameters are given in Table 3.

Diffusion cell experiments

The diffusion cell experiments were run in essentially the same way as previously described (Waranis and Sloan, 1987 and 1988; Siver and Sloan, 1988). Briefly, the hairless mice were sacrificed and the dorsal portion of each mouse skin was placed in contact with the receptor phase (pH 7.3 phosphate buffer, 0.05 M, $\mu = 0.11$ M containing 1 ml 36% formaldehyde per liter of buffer as preservative) at 32°C for 66 h. The receptor phase was changed at least three times during this preapplication leaching-conditioning period to remove any water-soluble UV absorbing materials that could interfere with the UV analysis, and to remove any water-soluble material with esterase activity. Control experiments were run which showed that essentially no UV absorbing material was leaching into the receptor phase after 24–48 h.

After the preapplication period a 0.5 ml aliquot of a suspension (1.16 M for the dibutyl derivative **VI** and 0.3 M for the rest of the derivatives) of each derivative in IPM was applied to the donor side of each of the three diffusion cells. The donor suspensions were prepared by stirring each derivative in IPM for 48 h. The donor phases were removed after 12 and 24 h, and fresh suspensions were applied to each diffusion cell. The donor phases that were removed were dissolved in $CDCl_3$ and were analyzed by ^1H NMR spectroscopy. The intensity and position of the C_8-$\underline{H}$ absorption at δ 8.0-8.20 was used to determine to what extent the prodrug was intact.

Samples of the receptor phase (3 ml) were removed at 3, 6, 9, 12, 15, 24, 27, 30, 33 and 48 h after application and immediately analyzed by UV spectroscopy. The remainder of the receptor phase was then discarded and the receptor phase replenished with fresh buffer. The amount of 6-MP in each sample was determined from the UV absorption at 320 nm ($\epsilon = 1.94 \times 10^4$ l/mol) and the amount of 6-POM-6-MP from the UV absorption at 277 nm ($\epsilon = 1.76 \times 10^4$ l/mol) less the contribution from 6-MP at that wavelength (0.118 times the absorbance at 320 nm). The diffusion cell samples were apparently fairly stable under these conditions as no change in the relative or absolute absorption intensities of the components was observed after 12–48 h at room temperature.

In one diffusion cell experiment the rate of delivery of intact 6-POM-6-MP by the dimethylaminomethyl derivative was also determined by HPLC using 50% methanol-water, a 280 nm filter and a flow rate of 1.2 ml/min. Under those conditions the retention times of 6-MP and 6-POM-6-MP were 2.3 and 6.3 min, respectively.

In all cases the rates of delivery of 6-MP or 6-POM-6-MP through skin were obtained by plotting the cumulative mg of 6-MP or 6-POM-6-MP measured in the receptor phase against time and dividing the slopes of the steady-state portions of those plots by the area of the diffusion cells. The permeability coefficients for the delivery of 6-MP or 6-POM-6-MP were then obtained by dividing the rates of delivery of 6-MP or 6-POM-6-MP by the solubilities of the corresponding 9-AM-6-POM-6-MP derivatives in terms of equivalent mg of 6-MP/ml of IPM or equivalent mg of 6-POM-6-MP/ml of IPM, respectively. The rates of de-

livery and permeability coefficients are given in Table 3.

Results and Discussion

Synthesis, solubility and stability

The aminomethylation of 6-POM-6-MP (**II**) gave high yields of products (63–91% yield) typical of most aminomethylation reactions. Although apparently only a single product was obtained, aminomethylation could have taken place at either the 7- or the 9-position, so it was important to determine the position of the alkylation. Previously it had been shown that aminomethylation of S^6-acetyloxymethyl-6-MP had taken place on the 9-position by converting its piperidinylmethyl derivative to the known S^6,9-bisacetyloxymethyl-6-MP derivative using acetic anhydride (Siver et al., 1988). No S^6,7-bisacetyloxymethyl-6-MP was observed. Since the UV and ^{1}H NMR spectra of the aminomethyl derivatives of 6-POM-6-MP in Tables 1 and 2 were essentially identical with those of the 9-aminomethyl derivatives of S^6-acetyloxymethyl-6-MP (K.B. Sloan and A.N. Saab, unpublished data), the aminomethyl derivatives of 6-POM-6-MP also have been assigned 9-alkyl structures (9-AM-6-POM-6-MP).

The melting points and solubilities of the 9-AM-6-POM-6-MP derivatives are given in Table 2. There is a good correlation between the melting points of both the homologous acyclic series and the cyclic series of derivatives and their solubilities in IPM; as the melting point decreased there was an increase in solubility. The solubilities of the 9-AM-6-POM-6-MP derivatives in IPM were three to four orders of magnitude greater than those of the corresponding 7-aminomethyl derivatives of 6-MP (Siver and Sloan, 1988). In that series the maximum solubility in equivalent mg of 6-MP/ml of solution was only 0.077 mg/ml for the dipropylaminomethyl derivative and the least soluble derivative in the present series is the morpholinylmethyl derivative which exhibits a solubility of 2.3 mg/ml. This result emphasizes the importance of masking the thionamide functional group of 6-MP to improve its lipid solubility properties. On the other hand, the 9-AM-6-POM-6-MP derivatives were also three times to one hundred and sixty times more soluble in IPM than 6-POM-6-MP itself which emphasizes the importance of masking both functional groups in 6-MP for maximizing the solubility of 6-MP in lipid solvents. These results are consistent with the results obtained for the S^6,9-bisacyloxymethyl-6-MP and S^6-acyloxymethyl-6-MP series (Waranis and Sloan, 1987 and 1988, respectively). The S^6,9-bisacyloxymethyl derivatives were much more soluble in the lipid vehicle IPM than the corresponding S^6-derivatives which in turn were much more soluble than the one 9-derivative examined.

The solubilities of the 9-AM-6-POM-6-MP derivatives in water were not determined because they are too unstable in water at pH 7.4 to obtain meaningful data. This very rapid decomposition of these aminomethyl derivatives (Bundgaard and Johansen, 1981) also precludes the measurement of their rates of hydrolyses by ordinary UV spectroscopic techniques. However, based on the empirically based predictions of Bundgaard and Johansen (1981) half-lives on the order of 10^{-2} to 10^{-3} s would have been predicted.

On the other hand, the 9-AM-6-POM-6-MP derivatives were very stable in IPM in tightly sealed flasks, and all except the diethylaminomethyl derivative also were stable in IPM when applied to mouse skin in the diffusion cell experiments. The donor phase sample of the diethylaminomethyl derivative **IV** in IPM that was removed at the 12 h diffusion cell sample was about 50% decomposed while the sample that was removed at 24 h was about 90% decomposed based on the intensity of the C_8-H absorption of intact **IV** at δ 8.03 compared to the intensity of the C_8-H absorption of **II** at δ 8.17 in their ^{1}H NMR spectra. The corresponding 7-diethylaminomethyl derivatives of 6-MP and theophylline were also the least stable members of the homologous series of acyclic aminomethyl derivatives of 6-MP (Siver and Sloan, 1988) and of theophylline (Sloan et al., 1988). In fact, in the latter case the diethylaminomethyl derivative was unstable in IPM before contact with hydrated mouse skin.

Diffusion cell experiments

The protocol for the diffusion cell experiments was designed to ensure quantitation of any intact

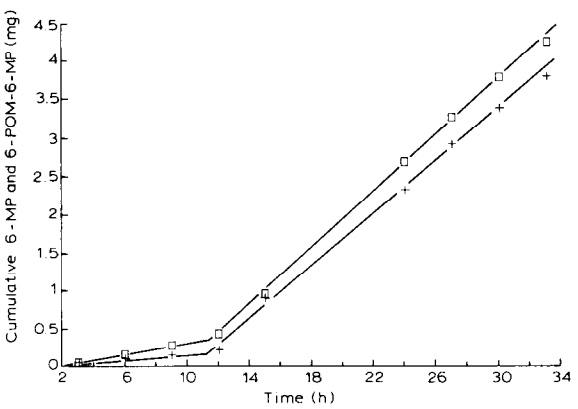


Fig. 1. Plots of cumulative mg of 6-MP (□) and 6-POM-6-MP (+) delivered through hairless mouse skin from a suspension of 9-morpholinylmethyl-6-POM-6-MP in IPM versus time.

6-POM-6-MP that was being delivered through the skin – immediate analyses and replacement of the receptor phase after each sample (Waranis and Sloan, 1987), and to ensure maintenance of intact prodrug in the donor phase suspension – replacement of the donor phase after the 12 h and 24 h sample (Siver and Sloan, 1988). Generally the rates of delivery of 6-MP and 6-POM-6-MP were determined from UV spectroscopic analyses of the receptor phase samples. In a separate experiment the rate of delivery of 6-POM-6-MP was also determined from HPLC quantitation of the amount of 6-POM-6-MP delivered by the dimethylaminomethyl derivative from IPM into the receptor phase. The rate of delivery of 6-POM-6-MP determined by HPLC was identical with the rate determined by UV so it was assumed that UV spectroscopic determination was sufficiently accurate for the remainder of the experiments.

The results from the diffusion cell experiments are given in Table 3 (see Fig. 1 for a representative plot of cumulative mg versus time). In all the cases studied a steady-state rate of delivery of both 6-MP and 6-POM-6-MP was obtained. Although data for lag times are not given, generally the lag time for development of steady-state delivery of 6-POM-6-MP was 6–13 h while for 6-MP it varied with the type of derivative – about 1–3 h for the acyclic derivatives and 6–10 h for the cyclic ones.

There was not much difference in the rates of delivery of 6-MP by each of the 9-AM-6-POM-6-MP derivatives. The range was from 0.0245 for the dipropylaminomethyl derivative **V** to 0.0386 mg of 6-MP/cm^2 h for the morpholinylmethyl derivative **VII**. Although 6-POM-6-MP itself did not deliver intact 6-POM-6-MP from IPM through hairless mouse skin (Waranis and Sloan, 1988), the aminomethyl derivatives of 6-POM-6-MP delivered 6-POM-6-MP at rates that were comparable to or greater than the rates at which they delivered 6-MP – the range was from 0.0256 for the piperidinylmethyl derivative **VIII** to 0.0757 mg of 6-POM-6-MP/cm^2 h for the dimethylaminomethyl derivative **III**. These results may be due to the high rates of delivery of the aminomethyl derivatives of 6-POM-6-MP into the skin which give levels of 6-POM-6-MP which are too high for the enzymes responsible for the hydrolysis of 6-POM-6-MP to completely hydrolyze the 6-POM-6-MP to 6-MP.

There was no apparent correlation between lipid (IPM) solubility properties of the aminomethyl 6-POM-6-MP derivatives and their abilities to deliver 6-MP or intact 6-POM-6-MP. However, in the homologous series of acyclic derivatives (**III–VI**) and in the series of cyclic derivatives (**VII–IX**) the least soluble derivatives **III** and **VII**, respectively, were the most efficient at delivering 6-MP. On the other hand, there was a good correlation between the rank order of the IPM solubilities of the aminomethyl derivatives and their permeability coefficients for the delivery of 6-MP (P_i), 6-POM-6-MP (P_j) or total 6-MP ($P_\Sigma = P_i + P_j$) with the least soluble derivative **VII** giving the highest P_i, P_j or P_Σ values and vice versa. Similar trends are apparent from the results of diffusion cell experiments with the aminomethyl derivatives of theophylline and 5-fluorouracil (Sloan et al., 1988) and 6-MP itself (Siver and Sloan, 1988).

All of the aminomethyl derivatives of 6-POM-6-MP were much more effective than 6-POM-6-MP itself at delivering 6-MP – 2.5 to four times. Also, except for the 7-diethylaminomethyl derivative of 6-MP, the aminomethyl derivatives of 6-POM-6-MP delivered more 6-MP than the corresponding aminomethyl derivatives of 6-MP – 1.5 times for the dimethylaminomethyl to 15 times for the piperidinylmethyl derivative. The diethylaminomethyl-6-POM-6-MP derivative was only

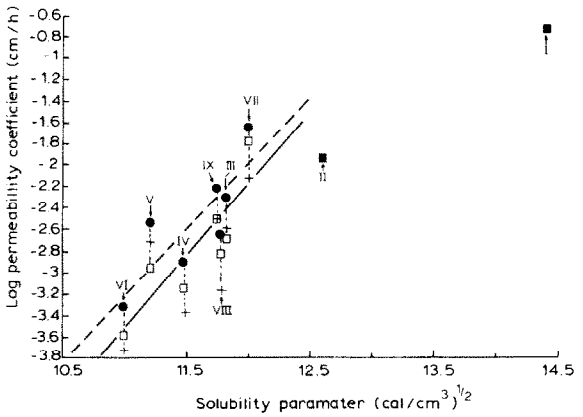


Fig. 2. A plot of log experimental permeability coefficients for the delivery of 6-MP (P_i, □, ———), 6-POM-6-MP (P_j, +) and total 6-MP (P_Σ, ●, - - - - - -) from suspensions of 9-aminomethyl-6-POM-6-MP derivatives in IPM, and including P_i data for the delivery of 6-MP from IPM suspensions of 6-MP and 6-POM-6-MP (■), versus calculated solubility parameters.

0.28 times as effective as the 7-diethylaminomethyl-6-MP derivative. However the latter was also twenty times more effective than the next most effective dialkylaminomethyl derivative of 6-MP. The reason for the markedly superior performance of the diethylaminomethyl derivative in the 6-MP series is not obvious.

Reasonably good correlations were found between the calculated solubility parameters of the prodrugs (δ_i) and the log experimental permeability coefficients for the delivery of 6-MP (P_i, □, ———) and for the delivery of total 6-MP (P_Σ, ●, - - - - - -), $r = 0.86$ and 0.81 respectively. The plots of the data are shown in Fig. 2. A poorer correlation was found between δ_1 and P_j ($r = 0.72$) and that plot is not shown in Fig. 2. In each plot the lower the value of the calculated solubility parameter of the prodrug and the more similar the value was to IPM, the higher the solubility of the prodrug in IPM and the lower the value of the permeability coefficient.

Conclusion

Although the introduction of a second more lipophilic promoiety into S^6-acyloxymethyl-6-MP prodrugs did not significantly enhance their abilities to deliver 6-MP through hairless mouse skin (Waranis and Sloan, 1987), in this work the introduction of a second promoiety that theoretically enhanced the water solubility of 6-MP has significantly enhanced the ability of one S^6-acyloxymethyl-6-MP prodrug (6-POM-6-MP) to deliver not only 6-MP but also 6-POM-6-MP.

These results confirm the importance of increased water as well as increased lipid solubility as criteria for optimizing a prodrug approach to enhancing delivery through skin.

Also, as previously shown (Sloan et al., 1988; Waranis and Sloan, 1987, 1988) enhanced solubility in a lipoidal vehicle is not a good predictor of the relative abilities of a homologous series of lipoidal prodrugs to enhance the delivery of the parent drug from that vehicle through skin. In fact, the less soluble members in a series of prodrugs are usually the more effective derivatives for delivering the parent drugs.

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