Physicochemical Characterization and Solubility Analysis of Thalidomide and Its *N*-Alkyl Analogs

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Received April 10, 2001; accepted September 27, 2001

Purpose. The present study was primarily aimed at exploring the feasibility of improving percutaneous delivery via chemical manipulation of the thalidomide molecule to form analogs with improved physicochemical properties. *N*-Alkyl analogs were synthesized with the belief that these would be suitably hydrophobic and far less crystalline than the reference compound. This article presents their physicochemical properties.

Methods. Thalidomide and three of its *N*-alkyl analogs were synthesized. Identification and levels of purity (>96%) were assured through element analysis, fast atom-bombardment mass spectrometry, nuclear magnetic resonance spectroscopy, and high-performance liquid chromatography. *N*-Octanol/water partition coefficients were determined at pH 6.4. Solubilities in water and a series of *n*-alkanols were obtained. Best-fit solubility parameters were determined from the solubilities of the respective compounds in London solvents and were also calculated from respective hexane solubilities, melting points and heats of fusion.

Results. Methylation of the thalidomide molecule at its acidic nitrogen led to an aqueous solubility about 6-fold higher than thalidomide but, because the alkyl chain length was further extended from methyl to pentyl, aqueous solubilities decreased essentially exponentially. The destabilization of the crystalline structure with increasing alkyl chain length led to an increased solubility in nonpolar media. The log partition coefficient increased linearly with increasing alkyl chain length and the solubility parameters declined systematically through this series. By adding a methyl group to the thalidomide structure, the melting point dropped by more than 100°C. Adding to the alkyl chain length led to further, more modest decreases. Heats of fusion decreased dramatically upon thalidomide's alkylation as well.

Conclusion. Alkylation of the thalidomide molecule resulted in compounds with physicochemical properties that appear to be markedly better suited for percutaneous delivery.

KEY WORDS: thalidomide; *N*-alkyl analogs; solubility parameter; lipophilicity; solubility.

INTRODUCTION

Synthesized in Germany in 1954, thalidomide was introduced into the pharmaceutical market in 1956 as a sedative and hypnotic drug. Soon after, it came into use as a tranquilizer and anti-emetic for pregnant women. Tragically, it was soon found to be teratogenic and had to be withdrawn from the market. However, it remained available in certain countries for research purposes. In 1964, Sheskin discovered that thalidomide was beneficial in the treatment of erythema nodosum leprosum (1). Clinical and immunologic similarities between the leprotic reaction of erythema nodosum leprosum and diffuse connective tissue diseases, particularly rheumatoid arthritis (RA), have been observed (2). Gutiérrez–Rodríguez (3) proved that the systemic administration of thalidomide to patients with RA results in rapid clinical improvement, although unacceptable side effects were observed. The action has been linked to the ability of thalidomide to suppress the synthesis of tumor necrosis factor alpha (TNFα). An alternative means of delivering thalidomide to minimize some of its adverse effects would be desirable. We reasoned that percutaneous delivery might allow the drug to be delivered into the joints and other affected areas of the extremities without raising systemic levels to a worrisome point.

For a drug to be taken seriously as a candidate for percutaneous delivery, it must have suitable physicochemical properties, particularly with respect to its absolute and relative solubilities and related partitioning tendencies (4,5). Based on thalidomide's physicochemical properties (high melting point and low lipophilicity), it appeared unlikely that it would be delivered percutaneously in amounts required for arresting the symptoms of RA. Given the potential clinical efficacy of TNFα inhibitors such as thalidomide in a wide range of immunologic disorders (1,6–8), especially RA (9), it seemed worthwhile to investigate what changes in thalidomide's structure might produce an active compound suitable for skin transport. It is for this reason that we have embraced the idea of synthesizing *N*-alkyl analogs of thalidomide (Fig. 1), which should exhibit decreased crystallinity and increased lipophilicity. Therefore, the solubilities and lipophilicities of thalidomide and several N-alkyl analogs were determined.

MATERIALS AND METHODS

For the synthesis of thalidomide and its *N*-alkyl analogs, *N*-phthaloyl-DL-glutamic anhydride and urea were obtained from Aldrich (Milwaukee, WI) and methylamine, propylamine, and *n*-amylamine were obtained from Sigma Chemical Co. (St. Louis, MO). For solubility studies, reagent-grade organic solvents (Aldrich, Milwaukee, WI) were used as received. High-performance liquid chromatography (HPLC) grade acetonitrile and methanol (Fisher Scientific, Pittsburgh, PA) were used for the chomatography procedure and to dilute samples in preparation for HPLC analysis, respectively. Thalidomide and its analogs were synthesized according to literature methods (10,11) but with modification of the published purification procedures. Identification and levels of purity (>96%) were assured through element analysis, fast atom-bombardment mass spectrometry, nuclear magnetic resonance (^{1}H-NMR and ^{13}C-NMR) spectroscopy, HPLC,

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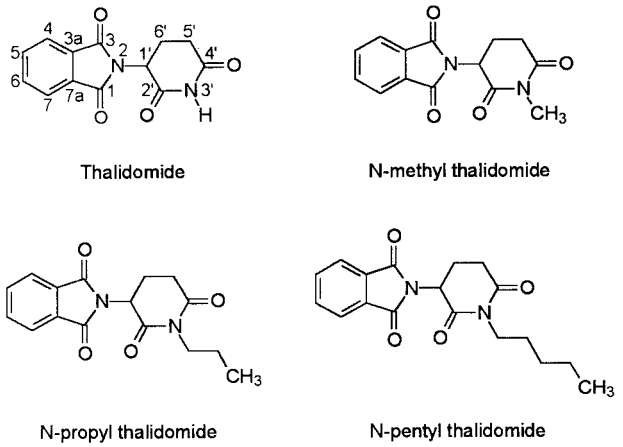

Fig. 1. Structures of thalidomide and its *N*-alkyl analogs.

and by the sharpness of melting points. Fast atom-bombardment mass spectra were recorded using xenon[13] as the bombardment gas (8 kV, 1 mA). The mass spectrometer was operated at 6 kV, and the magnetic analyser scanned at a rate of 5 s per decade over a mass range of 50–500 Da. These operating parameters applied to both positive- and negative-ion analysis. Samples were directly dissolved in the matrix and ionized on stainless steel targets at room temperature. ^{1}H- and ^{13}C-NMR spectra were performed at 30°C on a Varian Gemini-300 spectrometer operating at 400.08 and 75.46 MHz, respectively, using DMSO as a reference.

Synthesis Method

Thalidomide (Racemic Mixture)

One mol of *N*-phthaloyl-DL-glutamic anhydride was melted with 2 mol of urea in an oil bath at 170–180°C for 45 min. After cooling, the crude thalidomide was dissolved in 60 mL of ethanol (95%), heated to 60°C, and cooled. The crystallization was repeated three more times. Thalidomide was obtained as an almost white powder, with a melting point of 275°C and a yield of 70–75%. Anal ($C_{13}H_{10}N_2O_4$) calc. C 60.52% N 10.86% H 3.91%, found C 60.48% N 10.96% H 3.97%. ^{1}H-NMR (DMSO-d_6) δ: 7.89 (m, 4H, aromatic protons), 5.16 (1H, dd, J = 5.4, 12.8, H-1′), 2.08, 2.57, and 2.90 (3×m, 2×CH_2 of piperidinedione ring), 11.00 (1H, brs, NH); ^{13}C-NMR (DMSO-d_6) δ: 172.92, 170.00 and 167.33 (4×CO), 134.96, 131.35 and 123.49 (aromatic carbon atoms), 49.02 (C-1′), 30.91 (C-5′), 21.95 (C-6′), m/z 259 (M^+ + 1, 50), 154 (100), 136 (75), 120 (16), and 107 (27).

N-Methyl Thalidomide (Racemic Mixture)

One mol of *N*-phthaloyl-DL-glutamic anhydride and 1.2 mol of a 2 M methylamine solution in methyl alcohol were heated in an oil bath at 200°C for 8 h. After cooling, the crude imide was purified by recrystallization from 95% ethanol. The crude imide was dissolved in ethanol, heated to 60°C and then cooled at 4°C. This recrystallization process was repeated 5 times. De and Pal (11) purified *N*-methyl thalidomide by sublimation and a melting point of 131–133°C was reported. In this study, *N*-methyl thalidomide was purified by recrystallization from 95% ethanol and a melting point of 159°C was obtained and yields of 60–70%. According to HPLC, element analysis and the sharpness of the melting point, a high purity (≥98%) was obtained for *N*-methyl thalidomide. Anal ($C_{14}H_{12}N_2O_4$) calc. C 61.82% N 10.30% H 4.45%, found C 61.82% N 10.26 H 4.50%. ^{1}H-NMR (DMSO-d_6) δ: 7.85 (m, 4H, aromatic protons), 5.18 (1H, dd, J = 5.4, 12.8, H-1′), 2.95, 2.75, 2.55, and 2.05 (4×m, 2×CH_2 of piperidinedione ring), 3.05 (3H, s, CH_3); ^{13}C-NMR (DMSO-d_6) δ: 172.48, 170.48, and 168.03 (4×CO), 135.81, 132.11, and 124.36 (aromatic carbon atoms), 50.44 (C-1′), 31.97 (C-5′), 22.05 (C-6′), 27.50 (CH_3); m/z 272 (M^+, 53), 186 (8), 162 (100), 154 (41), 137 (40), and 107 (15).

N-Propyl Thalidomide (Racemic Mixture)

One mol of *N*-phthaloyl-DL-glutamic anhydride and 1.2 mol of 99% propylamine were heated in an oil bath at 200°C for 8 h. After cooling, the crude imide was purified by recrystallization from ethanol (95%). The crude imide was dissolved in ethanol, heated to 60°C and then cooled at −20°C. This recrystallization process was repeated five times. The final product was an off-white powder with a melting point of 136°C and yields of 50–60%. A 66–68°C melting point was previously reported for the compound by De and Pal (11). Anal ($C_{16}H_{16}N_2O_4$) calc. C 64.06% N 9.34% H 5.38, found C 64.09% N 9.31% H 5.56%. ^{1}H-NMR (DMSO-d_6) δ: 7.92 (m, 4H, aromatic protons), 5.25 (1H, dd, J = 5.4, 12.8, H-1′), 3.63 (m, 2H, N-CH_2), 2.96, 2.78, 2.54 and 2.09 (4×m, 2×CH_2 of piperidinedione ring), 1.45 (m, 2H, CH_2), 0.83 (3H, t, CH_3); ^{13}C-NMR (DMSO-d_6) δ: 172.46, 170.28, 168.02 (4×CO), 135.79, 132.11, 124.33 (aromatic carbon atoms), 50.47 (C-1′), 31.99 (C-5′), 22.19 (C-6′), 41.88 and 22.18 (2×CH_2 of propyl group), 11.97 (CH_3); m/z 300 (M^+, 100), 273 (6), 186 (38), 154 (23), 137 (28), and 126 (22).

N-Pentyl Thalidomide (Racemic Mixture)

One mol of *N*-phthaloyl-DL-glutamic anhydride was heated with 1.2 mol of *N*-amylamine (99%) in an oil bath at 200°C for 8 h. After unsuccessful attempts at distillation, the crude *N*-pentyl thalidomide was purified by silica gel column chomatography (diethyl either:hexane) using a stepwise gradient (10:90, 20:80, 30:70, 40:60; and 45:55). The volume of each mobile phase used was two times the column volume and *N*-pentyl thalidomide was collected during the 45% diethyl ether mobile phase elution. It was dried *in vacuo* and recrystallized once from diethyl ether, yielding white crystals of *N*-pentyl thalidomide and yields of 40–60%. According to the literature (11), *N*-pentyl thalidomide can be purified by distillation in vacuo and a 19–21°C melting point was reported. A melting point of 105°C was determined for *N*-pentyl thalidomide. Anal ($C_{18}N_{20}N_2O_4$) calc. C 65.91% N 8.54% H 6.15%, found C 65.55% N 8.45% H 6.08%. ^{1}H-NMR (DMSO-d_6) δ: 7.90 (m, 4H, aromatic protons), 5.22 (1H, dd, J = 5.4, 12.8, H-1′), 3.63 (m, 2H, N-CH_2), 2.98, 2.74, 2.50, and 2.07 (4×m, 2×CH_2 of piperidinedione ring), 1.40 and 1.22 (2×m, 2H and 4H, 3×CH_2), 0.83 (3H, t, CH_3); ^{13}C-NMR (DMSO-d_6) δ: 171.67, 169.47 and 167.30 (4×CO), 134.90, 131.35 and 123.51 (aromatic carbon atoms), 49.61 (C-1′), 39.42, 28.27, 26.87, and 21.24 (4×CH_2 of pentyl chain), 13.64 (CH_3), 31.09 (C-5′) and 21.66 (C-6′); m/z 328 (M^+, 100), 301 (6), 186 (44), 173 (5), 154 (16), and 130 (5).

Chromatographic Procedure

The HPLC system consisted of a Beckman 114M solvent delivery system and a Spectraflow 783 variable wavelength ultraviolet detector, operated at 220 nm and a flow rate of 1.2 mL/min. A Perkin–Elmer ISS-100 autoinjector was used to inject samples (20 μL) onto a Spheri-5 (RP-8, 5 μm, 220 × 4.6 mm) column (Alltech Associates, Inc., Deerfield, IL) and a NewGuard precolumn (RP-18, 7 μ, 15 × 3 mm) insert was used. The mobile phase comprised of acetonitrile:water (25% acetonitrile for thalidomide and *N*-methyl thalidomide and 35% and 45% for *N*-propyl and *N*-pentyl thalidomide, respectively). The pH was adjusted to 2 with ortho-phosphoric acid (Fluka Chemical Co., Ronkonkoma, NY). Calibration curves established that excellent linearity existed over the entire concentration range from 0.01 μg/mL to 10 μg/mL.

Solubility Determination

The 25°C solubilities of thalidomide and its *N*-alkyl analogs in hexane, cyclohexane, carbon tetrachloride, toluene, benzene and phosphate buffer (pH 6.4) and the 32°C solubilities in phosphate buffer (pH 6.4) and an extended series of n-alkanols were obtained by equilibrating excess amounts of each of the compounds with these solvents. Samples were filtered (PTFE filter media with Polypropylene housing, 0.45-μm pore size, Whatman Inc., Haverhill, MA) and measured with respect to volume. The solvent from each individual sample was then evaporated and reconstructed in an appropriate amount of methanol or directly diluted with methanol and assayed by HPLC.

Differential Thermal Analysis

The heat (ΔH_f) and entropy (ΔS_f) of fusion of thalidomide and its *N*-alkyl analogs were determined with a Perkin–Elmer DSC7 Differential Scanning Calorimeter (DSC). Heating curves were recorded at 10°C/min. All tracings were repeated four times. There were no appreciable differences (±4%) in the thermograms for any compound from run to run.

Melting Point Determination

The melting points of thalidomide and its *N*-alkyl analogs were determined by two methods: (1) differential thermal analysis (as described above) and (2) controlled-heating thermal microscopy (Mettler Hot Stage with FPS Temperature Regulator and a Zeiss Standard Microscope). The heating rate for both methods was 10°C/min.

Partition Coefficient Determination

Solutions of each compound in this study were prepared with phosphate buffer (pH 6.4)-saturated octanol and transferred to assay tubes containing equal volumes of phosphate buffer. These mixtures were agitated for 2 h and after centrifugation at 2000 g for 10 min, the n-octanol and buffer phases were analyzed by HPLC. Partition coefficients (K_{oct}) were calculated as the ratio of drug concentration in the n-octanol phase to that in the buffer phase.

RESULTS

Table I summarizes the physicochemical properties of thalidomide and its *N*-alkyl analogs. Enthalpies of fusion, ΔH_f, and entropies of fusion, ΔS_f, are also shown in Table I. Thalidomide, *N*-propyl thalidomide and *N*-pentyl thalidomide exhibited only one thermal transition each. The endotherms at 275, 136, and 105°C, correspond to the melting of these crystals, respectively. *N*-Methyl thalidomide showed a major endotherm at 159°C and a small endotherm at 165°C.

Table I. Physicochemical Properties of Thalidomide and Its *N*-alkyl Analogs

Physical parameter	Thalidomide	*N*-Methyl thalidomide	*N*-Propyl thalidomide	*N*-Pentyl thalidomide
Molecular weight (g/mol)	258	272	300	328
Crystalline density (g/mL)	1.48	1.43	1.35	1.28
Molar volume, V_2 (mL/mol)	174	191	223	255
Melting Temperature, T_f(°C) ± SD	275 ± 0.11	159 ± 0.11	136 ± 0.90	105 ± 0.26
Heat of fusion, ΔH_f (kcal/mol) ± SD	8.61 ± 0.27	4.33 ± 0.06	6.52 ± 0.25	5.73 ± 0.08
Entropy of fusion, ΔS_f (cal/mol/K)	15.71	10.02	15.94	15.16
Activity of solid phase a_2^s, $\delta C_p = 0$*	1.29×10^{-3}	1.03×10^{-1}	5.18×10^{-2}	1.29×10^{-1}
Activity of solid phase a_2^s, $\delta C_p = \Delta S_p$*	8.07×10^{-3}	1.54×10^{-1}	7.89×10^{-2}	1.64×10^{-1}
Solubility parameter, δ_2 $(cal/cm^3)^{1/2}$, $\delta C_p = 0$	13.2	12.2	11.4	11.2
Solubility parameter, δ_2 $(cal/cm^3)^{1/2}$, $\delta C_p = \Delta S_p$	13.7	12.3	11.5	11.2
Ln $X_{2,\ ideal}$ ($\Delta C_p = 0$)	–6.65	–2.27	–2.96	–2.05
Ln $X_{2,\ ideal}$ ($\Delta C_p = \Delta S_p$)	–4.82	–1.87	–2.54	–1.81

* Ideal activity of solid phase, a_2^s estimated from Eq. 1 (15) with one or the other assumption.

The endotherm at 159°C corresponds to the melting point of *N*-methyl thalidomide, suggesting that the small subsequent energy absorption involved relaxation of structure in the liquid state. Melted samples of all the compounds, assayed by HPLC showed only trace amounts (less than 0.5%) of decomposition, and it was assumed that the endotherms primarily represent energy consumed on melting.

A physical parameter that is necessary for solubility analysis is the molar volume of liquid solute. The molar volume, V_2, of thalidomide was determined from its molecular weight divided by its crystalline density (12). Because the crystalline densities of the *N*-alkyl analogs were not known, their molar volumes were estimated, starting with thalidomide's true molar volume, by summation and subtraction of functional-group molar volumes that distinguish the individual compounds from thalidomide (13). The essentials of estimation of the molar volumes for the *N*-alkyl analogs are presented in Table II.

The aqueous and hexane solubilities of thalidomide and its *N*-alkyl analogs are listed in Table III along with their octanol/water partition coefficients (K_{oct}). The solubilities of these compounds in a series of *n*-alkanols are summarized in Table IV on a molar scale. It can be seen that for every solute the molar solubilities across the homologous series of solvents decrease systematically with increasing alkanol chain length.

The hexane solubilities of the compounds can be seen in Table III. The assumption that the volume fraction of hexane in the saturated solutions (ϕ_1), is unity, was made (14). Using this surmise, the solubility parameters for all the solutes were calculated according to Eq. 3 (15) and the assumption that $\Delta C_p = 0$ or $\Delta C_p = \Delta S_f$. The mol fraction solubilities (ln X) of thalidomide and its *N*-alkyl analogs in several organic solvents at 25°C are listed in Table V, as well as the values of the solubility parameters of the solvents. To show the extent to which thalidomide's and the analogs' solubility behavior might conform to regular solution behavior, the regular solution solubility parabolas for all four compounds were calculated according to Roy and Flynn (15) (Eq. 3) (with the assumption, $\Delta C_p = \Delta S_f$ and $\Delta C_p = 0$) where in each case the solutions are considered ideal (Fig. 2 A–D).

DISCUSSION

Structures of Thalidomide and Its *N*-Alkyl Analogs

Thalidomide

In the ^{1}H-NMR spectrum of thalidomide, the four protons of the aromatic ring resonate as a symmetrical multiplet at δ 7.89. The signal from H-1′ appears as a doublet of doublets (J 5.4 and 12.8) at δ 5.16. The remaining four protons on the piperidinedione ring appear as three sets of multiplets at δ 2.08, 2.57, and 2.90. The amine proton resonates as a broad singlet at ca. δ 11.00. In the ^{13}C-NMR spectrum of thalidomide three signals at δ 172.92, 170.00, and 167.33 attributable to the four carbonyl carbon atoms and three signals at 134.96, 131.35, and 123.49 attributable to the six aromatic carbon atoms are clearly discernable. The three remaining carbon atoms of the piperidinedione ring resonate at 49.02 (C-1′), 30.91 (C-5′), and 21.95 (C-6′), respectively.

N-Methyl Thalidomide

The ^{1}H-NMR spectrum of *N*-methyl thalidomide is identical to that of thalidomide except for the signal from the methyl group at δ 3.05 in the ^{1}H-NMR spectrum and at δ 27.50 in the ^{13}C-NMR spectrum.

N-Propyl Thalidomide

The ^{1}H-NMR spectrum of the *N*-propyl analog shows in addition to the signals of the thalidomide nucleus a multiplet at each of δ 3.63 and 1.45 due to the methylene protons and a triplet at δ 0.83 due to the methyl group of the propyl moiety. In addition to the signals of the thalidomide nucleus the ^{13}C-NMR spectrum of the *N*-propyl analog shows signals at δ 41.88 and 21.53 due to the methylene carbon atoms and 11.97 arising from the methyl group of the propyl moiety.

N-Pentyl Thalidomide

The ^{1}H-NMR spectrum of the *N*-pentyl analog shows in addition to the signals of the thalidomide nucleus a multiplet at δ 3.63 and 1.40 for the terminal methylene groups of the pentyl moiety whereas the signal from the two central methylene groups appears as a multiplet at δ 1.22 and the methyl group resonate as a triplet at δ 0.83. In addition to the signals from the thalidomide nucleus the ^{13}C-NMR spectrum of the N-pentyl analog shows signals due to methylene carbon atoms at δ 39.42, 28.27, 26.87, and 21.24 whereas the methyl group resonates at δ 13.64.

Physicochemical Properties

N-Alkylation of the glutarimide ring in the thalidomide molecule replaces the imido hydrogen atom, which is responsible for strong hydrogen and dipolar bonding within the crystalline state. The melting points for the *N*-alkyl analogs in this series are all at least 100°C lower than thalidomide's melting

Table II. Estimation of Molar Volumes for the *N*-Alkyl Analogs

Functional group or atom	Partial molar volume contribution per group (mL/mol)	*N*-Methyl thalidomide	*N*-Propyl thalidomide	*N*-Pentyl thalidomide
Thalidomide	174.33	174.33	174.33	174.33
H	3.1	–3.1	–3.1	–3.1
CH2	16.2	—	+32.4	+64.8
CH3	19.3	+19.3	+19.3	+19.3
Estimated molar volume (mL/mol)		191	223	255

Table III. Solubility and Partition Coefficients of Thalidomide and Its *N*-Alkyl Analogs

Compound	Solubility			K_{oct} ± SD
	25°C water (pH 6.4) μg/mL ± SD	32°C water (pH 6.4) μg/mL ± SD	25°C hexane μg/mL ± SD	
Thalidomide	52.1 ± 1.49	61.4 ± 2.03	0.1 ± 0	3.09 ± 1.03
N-Methyl thalidomide	275.9 ± 6.39	370.4 ± 4.27	90 ± 0	14.1 ± 1.05
N-Propyl thalidomide	57.3 ± 1.46	59.4 ± 1.63	220 ± 10	129 ± 1.05
N-Pentyl thalidomide	6.54 ± 0.52	9.0 ± 0.21	530 ± 10	1023 ± 1.06

point, illustrating the remarkable impact of eliminating the acidic imido hydrogen atom of the thalidomide molecule.

Table I contains estimates of the thermodynamic activities of thalidomide and its *N*-alkyl analogs at 25°C. These were obtained using Equation 1 according to Roy and Flynn (15) in conjunction with the experimental values for ΔH_f and T_f. These values also represent the mol fractional ideal solubilities. It can be seen that the inherent thermodynamic activity increases dramatically when the thalidomide structure is alkylated. However, there is no simple pattern to the thermodynamic activities of the analogs as a result of extending the alkyl chain. Although it is inappropriate to directly relate the thermodynamic activity of one compound to that of another, as there is no provision in classical thermodynamics for doing so, it is still clear from these data that a high level of crystallinity is associated with low activity and vice versa. It must always be kept in mind that it is the thermodynamic activity of a drug in a solution that establishes its maximum practical driving force for its escape from the solution.

At 52 μg/mL (Table III), the 25°C aqueous solubility of thalidomide is exceptionally low. Its low solubility in water is undoubtedly due in part to the exceptionally high level of crystallinity reflected in its high melting point and enthalpy of fusion. There may be other factors here that also have bearing on the value. By way of contrast, the aqueous solubility of *N*-methyl thalidomide, 276 μg/mL, is quite high. The loss of the imido hydrogen's ability to H-bond with water is more than compensated for by the reduced crystallinity of the compound. Based on this general behavior of amines (17,18), one can also speculate that the change in structure also slightly increases the strength of the nitrogen atom as an H-bond receptor. In the instances of the *N*-propyl and *N*-pentyl analogs, further reductions in compound crystallinity are insufficient to overcome the impact of making the compounds incrementally more hydrophobic and the aqueous solubility drops dramatically.

Table IV. Solubilities of Thalidomide and Analogs in Normal Alkanols

n-Alkanol	Solubility (μmol/mL) at 32°C			
	Thalidomide	*N*-Methyl thalidomide	*N*-Propyl thalidomide	*N*-Pentyl thalidomide
Methanol	4.38	50.66	62.60	129.88
Ethanol	1.55	25.40	44.00	90.34
n-Propanol	1.01	21.25	35.17	81.25
n-Butanol	0.74	16.43	34.50	79.60
n-Pentanol	0.62	14.63	28.53	73.41
n-Hexanol	0.47	11.99	24.20	66.40
n-Heptanol	0.35	10.77	21.50	62.01
n-Octanol	0.27	9.71	20.70	61.55
n-Nonanol	0.23	8.86	19.43	48.38
n-Decanol	0.19	8.75	12.90	38.20
n-Undecanol	0.16	8.42	12.43	35.58
n-Dodecanol	0.16	4.38	10.60	35.24

N-alkylation of the glutarimide ring in the thalidomide molecule results in compounds that are more lipophilic. This is evident from the systematically declining solubility parameters through the series (Table I) but is even better demonstrated in the octanol/water partition coefficients (Table III).

Table IV summarized a display of the solubilities of thalidomide and analogs in the simple straight chain alkanols. The impact of extending the alkyl chain length is greatest on thalidomide, which exhibits a 28-fold decrement in solubility from methanol to dodecanol. As might be expected, the impact declines as the lipophilicity of the compounds is increased. Thus, as the compounds are made increasingly hydrophobic, the impact of increasing the hydrophobicity of the solvents is lessened.

The demonstrated loss of capacity of the alkanols to dissolve each of the compounds is relatively important. There are two identifiable "forces" acting here. The alkanol solvents are becoming more hydrophobic as their chain lengths are lengthened. The increased hydrophobicity in principle can have a positive or negative effect on solubility depending on whether the direction makes the solvents more like or less like the respective solutes (whether the cohesive energy densities of the solvents are moving towards or away from the cohesive energy densities of the supercooled liquid form of the respective solutes). The second "force" derives from the fact that, as the alkyl chain length of the alkanols is extended, the numbers of hydroxyl groups they offer for H-bonding per unit volume decreases proportionally. These hydroxyl groups interact with the polar, H-bonding centers of thalidomide and its *N*-alkyl analogs. One can read into this pattern of behavior that the intermolecular interactions of these collective solvents with the highly polar keto and imido functionalities of the thalidomide molecule is most determinative of solution phase activities. Sloan and co-workers (19) determined the solubility of theophylline in a series of n-alcohols and the trend in the solubilities was an odd-higher, even-lower molar solubility pattern up to the C_5 alcohol, then a drop in solubility for the C_7–C_{11} alcohols. This sort of odd-higher, even-lower trend in solubilities was also previously reported for the solubilities of levonorgestrel in C_1–C_8 straight chain alcohols (20).

As suggested in the introduction, there are two simplifications of Eq. 1 (15) that have been offered by previous in-

Table V. Mol Fraction Solubility of Thalidomide and Its *N*-Alkyl Analogs

Solvent	δ_1[a] $(cal/cm^3)^{1/2}$	Mol fraction solubility (ln X) at 25°C			
		Thalidomide	*N*-Methyl thalidomide	*N*-Propyl thalidomide	*N*-Pentyl thalidomide
n-Hexane	7.27	–16.80	–10.05	–9.25	–8.46
Cyclohexane	8.19	–16.25	–9.44	–8.31	–7.25
Carbon tetrachloride	8.55	–12.60	–6.34	–4.75	–2.98
Toluene	8.93	–10.02	–4.58	–3.72	–2.47
Benzene	9.16	–9.68	–4.40	–3.17	–2.13
Water (pH 6.4)	23.0	–12.53	–10.91	–12.58	–14.84

[a] See Hoy (16).

vestigators. Neau and Flynn (21) evaluated the merits of the assumptions regarding ΔC_p. They found that, for compounds that are rigid such as benzene and polycyclic aromatic hydrocarbons, ΔC_p is indeed closer to zero than to the entropy of fusion. However, for compounds that are not rigid, such as *n*-alkyl para-aminobenzoates, the value of ΔC_p is better approximated by the entropy of fusion. The thalidomide molecule contains a flat phthalimide moiety and an almost equally flat glutarimide moiety. The latter is not constrained within a plane but must rotate to a certain extent about its connection to the phthaloyl portion of the molecule so that the two portions are not coplanar. Therefore, thalidomide and its *N*-alkyl analogs do not appear to be as rigid as the polycyclic aromatic hydrocarbons but are clearly somewhat more geometrically constrained than the alkyl-para-aminobenzoates. It is therefore hard to place them into the best category represented by these two choices. The influences of heat capacity assumptions on the estimation of solubility parameters and ideal solubility were specifically studied by Neau and co-workers (22). In this study the estimates were found to be essentially insensitive to the chosen ΔC_p assumption ($\Delta C_p = 0$ or $\Delta C_p = \Delta S_f$) under the circumstances that 87°C was the largest difference between the melting point and the solution temperature. However, based on the physicochemic properties of hydrocortisone and its solubilities in London solvents reported by Hagen (23), Neau and coworkers (22) notes that the heat capacity assumption might play a significant role in the evaluation of the ideal solubility under circumstances where the difference between the melting point and the solution temperature is large. The influence of these two assumptions on the values of the ideal solubility and solubility parameters of thalidomide and its *N*-alkyl analogs, can be seen in Table I. Thalidomide has an extremely high melting point (275°C), and its solubility parameter is clearly sensitive to the heat capacity assumption. However, as anticipated based on previous work reported (21), the heat capacity assumption had far less effect on the values of the solubility parameters of the three *N*-alkyl analog as they all exhibit melting points ≤159°C. As can be seen in Table I, the assumption, ΔC_p equals zero, leads to a value of ln $X_{2,ideal}$ for thalidomide of –6.65. The alternate assumption, $\Delta C_p = \Delta S_f$, gives a value of ln $X_{2,ideal}$ of –4.82. Thus, on a mol fraction basis, the ideal solubility of thalidomide differs by a factor of about six based on the assumption used. However, the mol fraction ideal solubility of the *N*-methyl and *N*-propyl analogs were only 1.5 times higher when ΔC_p was taken to be equal to the entropy of fusion. The factor was only 1.3 for the *N*-pentyl analog. Thus, the estimated ideal solubility is influenced by the ΔC_p assumption more profoundly as the difference in melting and experimental temperature increases. Since thalidomide and its *N*-alkyl analogs are not fully rigid molecules, ΔC_p would seem to be better approximated by the entropy of fusion than by a value of zero in these cases. We have adopted this approach in further analysis.

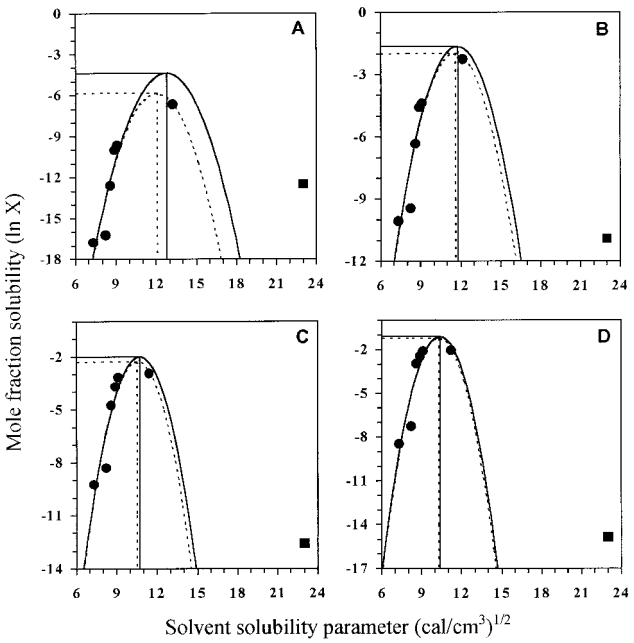

Fig. 2. Regular solution parabola for (A) thalidomide, (B) *N*-methyl thalidomide, (C) *N*-propyl thalidomide, and (D) *N*-pentyl thalidomide. (—— $\Delta C_p = \Delta S_p$ and ------ $\Delta C_p = 0$) (● = London solvents; ■ = water).

Regular solution theory predicts a parabolic relationship between the mol fraction solubility of a solute and the solubility parameters of "regular" (essentially nonpolar) solvents. As anticipated, the solubilities of the compounds in London solvents, closely fit to the respective curves. Figure 2 (A–D) also demonstrates that regular solution theory is totally inappropriate for solubility estimation in solvents like water that are capable of extensive hydrogen bonding and/or other strong, orienting bonding with the solute. Specifically, the mol fraction water solubilities of thalidomide and its *N*-alkyl analogs are greatly displaced from the regular solution scale. Figure 2 (A–D) also reflects the differences in solubility parameters, as well as the ideal solubility of the compounds, when

using one or the other of the ΔC_p simplifying assumptions. It is graphically clear that the heat capacity assumption does have an appreciable impact on the estimations of solubility parameters and ideal solubilities.

In conclusion, one can clearly see that alkylation of the thalidomide molecule results in less crystalline compounds of increased lipophilicity—physicochemical properties that appear to make them better suited for percutaneous delivery. When using the equation given by Potts and Guy (24), the permeability coefficients of thalidomide and its *N*-alkyl analogs can be estimated from K_{oct} and mw, and based on those estimates it is expected that the *N*-alkyl analogs of thalidomide will be more easily delivered through the skin than thalidomide itself. In continuing investigations we will determine the extent to which these basic expectations are actually met.

ACKNOWLEDGMENT

This study was supported in part by an Upjohn grant awarded by the University of Michigan, College of Pharmacy and by financial support for the lead author provided by Potchefstroom University for Christian Higher Education.

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