Hydrotropic Solubilization of Paclitaxel: Analysis of Chemical Structures for Hydrotropic Property

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Purpose. To identify hydrotropic agents that can increase aqueous paclitaxel (PTX) solubility and to study the chemical structures necessary for hydrotropic properties so that polymeric hydrotropic agents can be synthesized.

Methods. More than 60 candidate hydrotropic agents (or hydrotropes) were tested for their ability to increase the aqueous PTX solubility. A number of nicotinamide analogues were synthesized based on the observation that nicotinamide showed a favorable hydrotropic property. The identified hydrotropes for PTX were used to examine the structure–activity relationship.

Results. *N,N*-Diethylnicotinamide (NNDENA) was found to be the most effective hydrotropic agent for PTX. The aqueous PTX solubility was 39 mg/ml and 512 mg/ml at NNDENA concentrations of 3.5 M and 5.95 M, respectively. These values are 5–6 orders of magnitude greater than the intrinsic solubility of 0.30 ± 0.02 μg/ml. *N*-Picolylnicotinamide, *N*-allylnicotinamide, and sodium salicylate were also excellent hydrotropes for PTX. Solubility data showed that an effective hydrotropic agent should be highly water soluble while maintaining a hydrophobic segment.

Conclusions. The present study identified several hydrotropic agents effective for increasing aqueous solubility of PTX and analyzed the structural requirements for this hydrotropic property. This information can be used to find other hydrotropic compounds and to synthesize polymeric hydrotropes that are effective for PTX and other poorly water-soluble drugs.

KEY WORDS: hydrotropic agents; solubilization; poorly water-soluble drug; paclitaxel; structure–activity relationship.

INTRODUCTION

Poor water solubility of many drugs and drug candidates causes significant problems in producing formulations with sufficiently high bioavailability (1–3). Paclitaxel (PTX) presents a good example of the importance of water solubility. Its use in cancer therapy has been hindered by its low water solubility (4), which has required special formulations utilizing ethanol and Cremophore EL (polyoxyethylated castor oil), which has significant side effects such as hypersensitivity reactions (5). Testing PTX in preclinical tumor model systems is also difficult (6). In addition, the cosolvent mixture is diluted in isotonic saline solution before intravenous administration, and the diluted solution remains stable for only several hours (7). For hydrophobic drugs with poor water solubility, including PTX, several methods have been used to increase their water solubility. Poorly water-soluble drugs have been formulated into micron- or submicron-size particulate preparations (3), liposomes and micelles (8), and solid dispersions (9,10). Cosolvent systems can increase the drug solubility significantly, but the choices of clinically used solvents are limited to ethylene glycol, dimethylsulfoxide, *N,N*-dimethylformamide, Cremophore, and ethanol (11).

In an attempt to find an alternative or supplementary method for increasing water solubility of poorly soluble drugs, we have examined the possibility of using hydrotropes. Hydrotropic agents (hydrotropes) have been used to increase the water solubility of poorly soluble drugs, and in many instances, the water solubility has increased by orders of magnitude (12). Hydrotropy is a collective molecular phenomenon describing an increase in the aqueous solubility of a poorly soluble compound by addition of a relatively large amount of a second solute (i.e., a hydrotrope) (1). Each hydrotropic agent is effective in increasing the water solubility of selected hydrophobic drugs, and no universal hydrotropic agent has been found to be effective with all hydrophobic drugs. Thus, finding the right hydrotropic agents for a particular hydrophobic drug requires the screening of a large number of candidate hydrotropes. In this study, we examined various candidate agents for their abilities to solubilize PTX so that the structures of effective agents can be used for identification of other hydrotropic agents and for synthesis of hydrotropic polymers.

MATERIALS AND METHODS

Materials

PTX was obtained from Samyang Genex Corp. (Taejeon, South Korea). 6-Hydroxynicotinic acid, 1,1′-carbonyldiimidazole (CDI), diethylamine, 3-picolylamine, nicotinoyl chloride hydrochloride, allylamine, acetic anhydride, pyridine, and triethylamine were purchased from Aldrich Chemical Company (Milwaukee, WI) and used without further purification. Methylene chloride was dried and distilled over calcium hydride. Tetrahydrofuran (THF) was distilled from sodium benzophenone before use. *n*-Hexane, diethyl ether, chloroform, and methanol were of reagent grade. All other chemicals were purchased from Fisher Scientific (Pittsburgh, PA). Freshly prepared distilled water was used throughout.

Synthesis and Characterization of Nicotinamide Analogues

Instrumental Analysis

^{1}H and ^{13}C NMR spectra were obtained using a Bruker ARX300 spectrometer at 300 MHz and 75 MHz, respectively. Elemental analysis was performed on a Perkin Elmer Series II CHNS/O Analyzer 2400. UV-VIS spectra were obtained by a Beckman DU® 640 spectrophotometer. Electrospray ionization mass spectrometry (ESI-MS) assay was done using a FinniganMAT LCQ (ThermoFinnigan Corp, San Jose, CA). The electrospray needle voltage was set at 4.5 kV, the heated capillary voltage was set to 10 V, and the capillary temperature to 225°C. Typical background source pressure was 1.2×10^{-5} torr. The sample flow rate was approximately 10 μl/min. Nitrogen gas was used for drying. The LCQ was scanned to 2,000 amu for these experiments.

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N-*Picolylnicotinamide*

To a solution of 3-picolylamine (0.1 mol) and pyridine (0.2 mol) in dry methylene chloride (600 ml) was added nicotinoyl chloride hydrochloride (0.1 mol) at 0°C. The reaction mixture was stirred at room temperature for 24 h under nitrogen. After 24 h, the solvent was removed under reduced pressure, and the crude product was dissolved in water, neutralized with sodium bicarbonate, and extracted with chloroform (3 × 200 ml). The solution was dried over anhydrous magnesium sulfate. The solvent was removed at reduced pressure, and the product was isolated by column chromatography on a silica gel using THF/*n*-hexane. Yield was 80%; m.p. 105–107°C; λ_{max}(THF) 256 nm; ^{1}H NMR (DMSO-d_6) δ 4.52 (d, J = 5.8 Hz, 2H), 7.34 (dd, J = 4.8, 7.7 Hz, 1H), 7.49 (dd, J = 4.8, 8.1 Hz, 1H), 7.72–7.75 (m, 1H), 8.20–8.23 (m, 1H), 8.46 (dd, J = 1.0, 4.8 Hz, 1H), 8.58 (d, J = 2.4 Hz, 1H), 8.70 (dd, J = 1.0, 4.8 Hz, 1H), 9.06 (d, J = 2.0 Hz, 1H), 9.29 (t, J = 5.8 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 40.4, 123.4, 129.6, 134.7, 135.0, 135.1, 148.2, 148.3, 148.5, 148.9, 151.9, 164.9; ESI-MS, *m/z* 214 ([M+H]$^+$); analysis calculated for $C_{12}H_{11}N_3O$: C, 67.59; H, 5.20; N, 19.71. Found: C, 67.73; H, 5.10; N, 19.51.

N-*Allylnicotinamide*

To a stirred solution of allylamine (0.168 mol) in dry methylene chloride (500 ml), nicotinoyl chloride hydrochloride (0.112 mol) and triethylamine (0.225 mol) were added at 0°C. The reaction mixture was stirred at room temperature for 24 h under nitrogen. After 24 h, the solvent was removed under reduced pressure. The brown liquid was dissolved in distilled water and neutralized with sodium bicarbonate, followed by extraction with chloroform (3 × 200 ml). The solvent was removed at reduced pressure, and the crude product was column chromatographed with THF/*n*-hexane on a silica gel to produce a light yellow liquid. Yield was 85%; λ_{max}(THF) 260 nm; ^{1}H NMR (DMSO-d_6) δ 3.89–3.94 (m, 2H), 5.05–5.20 (m, 2H), 5.82–5.94 (m, 1H), 7.47 (dd, J = 5.0, 8.3 Hz, 1H), 8.20 (m, 1H), 8.68 (dd, J = 1.7, 5.0 Hz, 1H), 8.87 (t, J = 5.6 Hz, 1H), 9.04 (d, J = 1.7 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 41.5, 115.3, 123.3, 129.9, 134.9, 135.0, 148.5, 151.7, 164.6; ESI-MS, *m/z* 163 ([M+H]$^+$); analysis calculated for $C_9H_{10}N_2O$: C, 66.65; H, 6.21; N, 17.27. Found: C, 66.53; H, 6.07; N, 16.97.

6-Hydroxy-N,N-*Diethylnicotinamide*

To a stirred suspension of 6-hydroxynicotinic acid (0.108 mol) in THF (600 ml) was added CDI (0.108 mol) in one portion. The reaction mixture was stirred at reflux under nitrogen. After 24 h, diethylamine (0.216 mol) was added dropwise to the stirred suspension of *N*-(6-hydroxynicotinyl)-imidazole in THF at reflux. The reaction was further maintained for 24 h under nitrogen. After cooling of the reaction mixture to room temperature, 1N sodium hydroxide solution (120 ml) was added. THF was evaporated, and the aqueous solution of the crude product was washed with diethyl ether (5 × 200 ml). The aqueous solution was then neutralized with 1 N hydrochloric acid to pH 7 and extracted with chloroform (3 × 200 ml). The solution was dried over anhydrous magnesium sulfate. The solvent was removed at reduced pressure, and the product was isolated by column chromatography on a silica gel using THF/*n*-hexane. Yield was 65%; m.p. 113–115°C; λ_{max}(THF) 253 nm; ^{1}H NMR (DMSO-d_6) δ 1.09 (t, J = 7.2 Hz, 6H), 3.32 (q, J = 7.2 Hz, 4H), 6.34 (d, J = 9.1 Hz, 1H), 7.45 (dd, J = 2.4, 9.1 Hz, 1H), 7.50 (d, J = 2.4 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 13.3, 41.0, 114.4, 119.3, 135.5, 139.7, 161.9, 166.8; ESI-MS, *m/z* 195 ([M+H]$^+$); analysis calculated for $C_{10}H_{14}N_2O_2$: C, 61.84; H, 7.27; N, 14.42. Found: C, 61.73; H, 7.16; N, 14.47.

2-Hydroxy-N,N-*Diethylnicotinamide*

To a stirred suspension of 2-hydroxynicotinic acid (0.216 mol) in THF (700 ml) was added CDI (0.216 mol) in one portion. The reaction mixture was stirred at reflux under nitrogen. After 24 h, diethylamine (0.323 mol) was added dropwise to the stirred suspension of *N*-(2-hydroxynicotinyl)-imidazole in THF at reflux. The reaction was maintained for 24 h under nitrogen. After cooling of the reaction mixture to room temperature, the solution was concentrated under reduced pressure. The pale yellow precipitate was filtered, washed with diethyl ether, and dried *in vacuo*. Yield was 70%; m.p. 90–92°C; λ_{max}(THF) 313 nm; ^{1}H NMR (DMSO-d_6) δ 1.00 (t, J = 7.2 Hz, 3H), 1.07 (t, J = 7.2 Hz, 3H), 3.12 (q, J = 7.2 Hz, 2H), 3.35 (q, J = 7.2 Hz, 2H), 6.20 (m, 1H), 7.38 (dd, J = 2.4, 6.9 Hz, 1H), 7.41 (dd, J = 2.4, 6.9 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 12.8, 14.1, 38.4, 42.2, 104.6, 129.3, 136.1, 138.4, 159.2, 166.2; ESI-MS, *m/z* 195 ([M+H]$^+$); analysis calculated for $C_{10}H_{14}N_2O_2$: C, 61.84; H, 7.27; N, 14.42. Found: C, 62.14; H, 7.18; N, 14.42.

N-*Picolylacetamide*

To a stirred solution of acetic anhydride (0.069 mol) in THF (50 ml), a solution of 3-picolylamine (0.046 mol) in THF (20 ml) was added dropwise at room temperature. The reaction mixture was stirred for 5 h under nitrogen. After 5 h, an excess of water was added, and the aqueous solution was neutralized with 1 N sodium hydroxide. The solvent was removed at reduced pressure, and the product was isolated by column chromatography on a silica gel using THF/*n*-hexane. Yield was 85%; λ_{max}(THF) 257 nm; ^{1}H NMR ($CDCl_3$) δ 1.90 (s, 3H), 4.29 (d, J = 5.7 Hz, 2H), 7.16 (dd, J = 4.8, 8.2 Hz, 1H), 7.39 (s, 1H), 7.54 (m, 1H), 8.34 (dd, J = 1.4, 4.8 Hz, 1H), 8.35 (d, J = 1.4, 1H); ^{13}C NMR ($CDCl_3$) δ 22.7, 40.7, 123.4, 134.2, 135.5, 148.1, 148.6, 170.4; ESI-MS, *m/z* 151 ([M+H]$^+$); analysis calculated for $C_8H_{10}N_2O$: C, 63.98; H, 6.71; N, 18.65. Found: C, 63.85; H, 6.61; N, 18.54.

NMR Measurement

The ^{1}H NMR spectra of NNDENA in D_2O in the concentration range of 0.0025 to 1.36 M were obtained. The ratios of chemical shifts of nicotinamide protons to the chemical shift of HDO protons (4.63 ppm) were monitored with increasing NNDENA concentrations.

Solubility Study

Excess PTX was added to screw-capped vials containing a fixed volume of the hydrotrope solution. This mixture was stirred using a magnetic stirring bar for 24 h at 37°C. An aliquot of the sample was collected, and within 5 s, it was filtered through a 0.2-μm nylon membrane. This immediate filtering process prevented any possible formation of PTX

Table I. Paclitaxel Solubilities in the Presence of Various Hydrotropic Agents at 37°C[a]

Hydrotropic agent	Concentration used (M)[b]	PTX solubility (mg/ml)	Standard deviation
None (PTX solubility in pure water)	—	0.0003[c]	0.0000
N,N-Diethylnicotinamide	3.5	39.071	0.600
N-Picolylnicotinamide	3.5	29.435	1.205
N-Allylnicotinamide	3.5	14.184	0.385
Sodium salicylate	3.5	5.542	0.514
2-Methacryloyloxyethyl phosphorylcholine	2.9	3.199	0.037
Resorcinol	3.5	2.009	0.012
N,N-Dimethylnicotinamide	3.5	1.771	0.026
N-Methylnicotinamide	3.5	1.344	0.006
Butylurea	3.5	1.341	0.071
Pyrogallol	3.5	1.282	0.008
N-Picolylacetamide	3.5	1.084	0.003
Procaine HCl	2.5	0.720	0.005
Nicotinamide	3.5	0.694	0.031
Pyridine	3.5	0.658	0.080
3-Picolylamine	3.5	0.552	0.063
Sodium ibuprofen	1.5	0.500	0.070
Sodium xylenesulfonate	2.5	0.481	0.080
Ethyl carbamate	3.5	0.300	0.028
6-Hydroxy-*N,N*-diethylnicotinamide	2.0	0.241	0.004
Sodium *p*-toluenesulfonate	2.5	0.220	0.002
Pyridoxal hydrochloride	2.5	0.216	0.008
1-Methyl-2-pyrrolidone	3.5	0.071	0.002
Sodium benzoate	2.0	0.050	0.006
2-Pyrrolidone	3.5	0.038	0.002
Ethylurea	3.5	0.030	0.003
N,N-Dimethylacetamide	3.5	0.015	0.002
N-Methylacetamide	3.5	0.012	0.001
Isoniazid	1.0	0.009	0.002

[a] Mean ± SD, $n = 3$, except for PTX solubility in pure water, where $n = 10$.
[b] The concentrations less than 3.5 M represent the maximum solubilities of the hydrotropic agent.
[c] The aqueous PTX solubility is 0.30 ± 0.02 μg/ml.

particles as a result of the temperature decrease to ambient. The filtrate was diluted with acetonitrile (1:1), and the concentration of PTX was determined by an isocratic reverse-phase HPLC (Agilent 1100 series, Agilent Technologies, Wilmington, DE) using a Symmetry column (Waters Corp., Milford, MA) at 25°C. The mobile phase consisted of acetonitrile–water (45:55 v/v) with a flow rate of 1.0 ml/min. A diode array detector was set at 227 nm and linked to ChemStation software for data analysis. The PTX concentrations in the samples were obtained from a calibration curve.

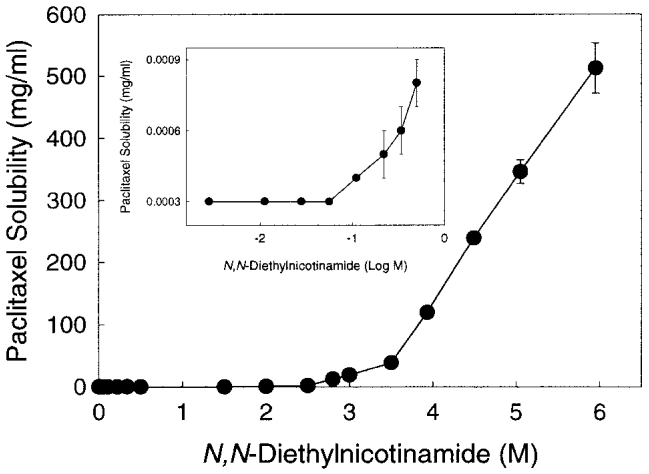


Fig. 1. Paclitaxel solubility as a function of the molar concentration of *N,N*-diethylnicotinamide. The solubility of paclitaxel at 5.95 M of *N,N*-diethylnicotinamide is 512.6 mg/ml. The inserted plot shows the paclitaxel solubility as a function of the log concentration of *N,N*-diethylnicotinamide.

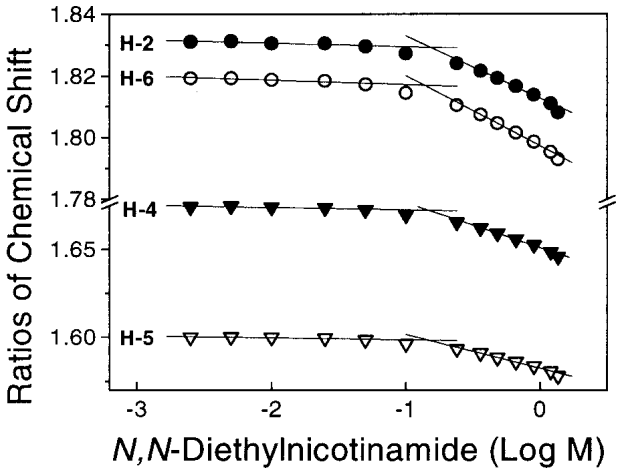


Fig. 2. The ratio of chemical shifts of nicotinamide protons to the chemical shift of HDO protons in D_2O as a function of the concentration of *N,N*-diethylnicotinamide. H-2, H-4, H-5, and H-6 indicate the proton position of the nicotinamide ring of *N,N*-diethylnicotinamide.

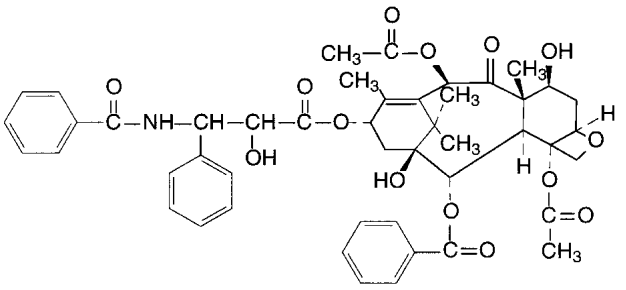


Fig. 3. Chemical structure of paclitaxel.

RESULTS AND DISCUSSION

Paclitaxel Solubility by Hydrotropes

Because of its noted therapeutic potential and very low water solubility, PTX was chosen to examine hydrotropic properties of various agents in this study. The exact mechanisms involved in solubilization of PTX and other poorly soluble drugs by hydrotropic agents are not clearly understood, so it is difficult to predict the structural requirements of hydrotropes suitable for solubilizing PTX. For this reason, a large number of candidate agents were screened. Table I lists the agents tested and the corresponding water solubilities of PTX measured in the presence of those agents. Our preliminary study suggested that even good hydrotropes required a hydrotropic concentration of approximately 3 M. For this reason, a concentration of 3.5 M was used for all agents to compare their hydrotropic properties under the same condition. The concentrations of some agents listed in Table I are smaller than 3.5 M because of their limited solubility. Table I clearly identifies a number of hydrotropic agents effective for increasing the water solubility of PTX.

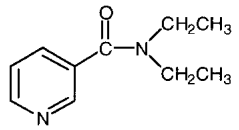
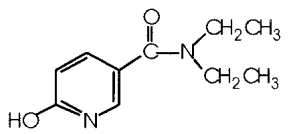
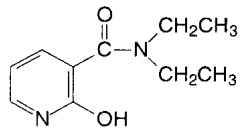
The aqueous solubility of PTX at 37°C was determined to be 0.30 ± 0.02 μg/ml. Thus, a PTX concentration of 39 mg/ml by *N,N*-diethylnicotinamide (NNDENA) in Table I indicates more than 100,000-fold increase in aqueous solubility. Equally effective was *N*-picolylnicotinamide. *N*-Allylnicotinamide, sodium salicylate, and 2-methacryloyloxyethyl phosphorylcholine increased the PTX solubility by four orders of magnitude. Other hydrotropes that resulted in an aqueous PTX solubility more than 1 mg/ml were resorcinol, *N,N*-dimethylnicotinamide, *N*-methylnicotinamide, butylurea, pyrogallol, and *N*-picolylacetamide. The PTX solubility of 0.3 mg/ml by ethyl carbamate appears to be much smaller than that by NNDENA but still represents a 1,000-fold increase.

Another 35 agents not listed in Table I showed paclitaxel solubilities of 0.005 mg/ml (or 5 μg/ml) or less. They are, in the descending order of solubilizing effect, nipecotamide (3.5 M), citric acid (2.0 M), sodium gentisate (1.0 M), *N*-isopropylacrylamide (1.5 M), methylurea (3.5 M), 1,3-diamino-2-hydroxypropane-*N,N,N′,N′*-tetramethylacetate (3.0 M), thiourea (2.5 M), 1-methylnicotinamide iodide (1.0 M), α-cyclodextrin (0.15 M), sodium thiocyanate (8.6 M), urea (6.0 M), caffeine (0.1 M), glyceryl triacetate (0.2 M), glycerin (3.5 M), adenosine (0.005 M), γ-cyclodextrin (0.17 M), β-cyclodextrin (0.02 M), diisopropylnicotinamide (0.05 M), pyridine-3-sulfonic acid (1.0 M), *o*-benzoic acid sulfimide (0.01 M), 2,6-pyridinedicarboxamide (0.0025 M), 3,4-pyridinedicarboxamide (0.025 M), 4-aminosalicylic acid (0.005 M), L-tryptophan (0.05 M), salicylaldoxime (0.1 M), sucrose (2.0 M), L-lysine (2.0 M), 4-aminobenzoic acid sodium salt (2.5 M), D-sorbitol (3.0 M), sodium L-ascorbate (3.0 M), sodium propionate (3.5 M), sodium acetate (4.0 M), 2-hydroxy-*N,N*-diethylnicotinamide (0.2 M), 2-hydroxy-*N*-picolylnicotinamide (0.0035 M), and 6-hydroxy-*N*-picolylnicotinamide (0.08 M).

Hydrotropic Property of NNDENA

The hydrotropic property of NNDENA was examined in more detail. Figure 1 shows the paclitaxel solubility as a function of the NNDENA concentration. NNDENA at 5.95 M increased the PTX concentration to 512 mg/ml (equivalent to 0.6 M because the molecular weight of PTX is 854 g/mol), and this corresponds to 10 NNDENA molecules per paclitaxel molecule dissolved. At the concentration of hydrotropes used in Table I, however, more than 100 hydrotropic agents are necessary for effective solubilization of PTX. The inserted plot in Fig. 1 shows the solubility increase of PTX as a function of the log concentration of NNDENA in the range of

Table II.

Hydrotropic agent	Conc. used (M)	PTX solubility (mg/ml)	Structure
N,N-Diethylnicotinamide	3.5	39.07	
	2.0	0.98	
	0.2	0.001	
6-Hydroxy-*N,N*-diethylnicotinamide	2.0[a]	0.24	
2-Hydroxy-*N,N*-diethylnicotinamide	0.2[a]	0.00	

[a] The maximum solubility of the hydrotropic agent.

0.0028 to 0.5 M. The water solubility of PTX begins to increase at 0.11 M of NNDENA, although the increase is small compared to higher concentrations of NNDENA.

Because the dissolution of PTX in NNDENA is expected to occur through association of NNDENA molecules, self-association of NNDENA molecules was examined using NMR. Figure 2 shows the NNDENA concentration dependence of the ratio of chemical shifts of nicotinamide protons to the chemical shift of HDO protons in D_2O. As the concentration of NNDENA increased to about 0.1 M, the ratios of chemical shifts of all protons of the nicotinamide ring started to decrease. The data indicate that NNDENA self-associates via the vertical plane-to-plane interaction of the aromatic rings. The crossover point in Fig. 2 can be described as the minimum hydrotropic concentration (MHC), which is the threshold concentration of self-aggregate formation. The MHC value of NNDENA in the aqueous media was estimated to be 0.12 M. Interestingly, this MHC value is almost the same as the concentration of 0.11 M where NNDENA begins to exhibit the solubilizing ability for PTX in aqueous solutions.

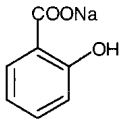
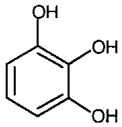
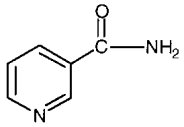
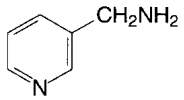
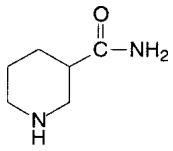
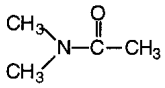
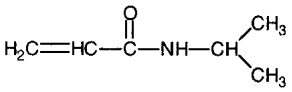
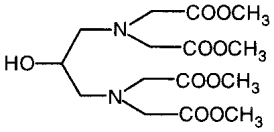
Structural Analysis of Hydrotropic Property for PTX

To gain insights into the structural requirements necessary for hydrotropy, chemical structures of various agents listed in Table I were analyzed for their ability to increase aqueous PTX solubility. The structure of PTX is shown in Fig. 3. A few common features of good hydrotropes for PTX were identified.

High Water Solubility of Hydrotropic Agents

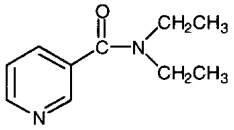
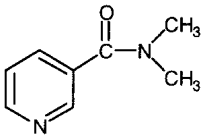
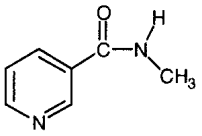
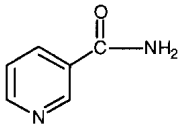
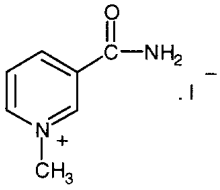
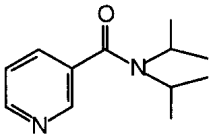
The main criterion for effective hydrotropy is high water solubility of the hydrotropic agent. If the water solubility is low (e.g., less than 2 M), the hydrotropic property is not observed to be significant. At 2.0 M, PTX solubility was higher in NNDENA than in 6-hydroxy-*N,N*-diethylnicotinamide. The PTX solubility in NNDENA was greatly increased with increasing the NNDENA concentration. 2-Hydroxy-*N,N*-diethylnicotinamide with the maximum water solubility of only 0.2 M did not have any PTX-solubilizing effect. The following example shows the importance of water solubility of hydrotropic agents on increasing aqueous PTX solubility (Table II).

Table III.

Hydrotropic agent	Conc. used (M)	PTX solubility (mg/ml)	Structure
Sodium salicylate	3.5	5.54	
Pyrogallol (1,2,3-trihydroxybenzene)	3.5	1.28	
Nicotinamide	3.5	0.69	
3-Picolylamine	3.5	0.55	
Nipecotamide	3.5	0.005	
N,N-Dimethylacetamide	3.5	0.015	
N-Isopropylacrylamide	1.5[a]	0.004	
1,3-Diamino-2-hydoxypropane-*N,N,N′,N′*-tetramethylacetate	3.0[a]	0.004	

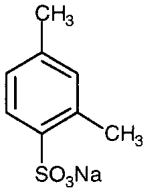
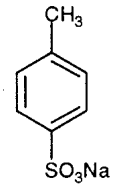
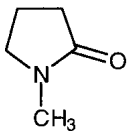
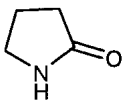
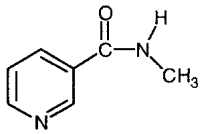
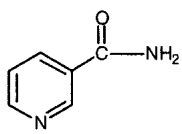
[a] The maximum solubility of the hydrotropic agent.

Table IV.

Hydrotropic agent (Conc. used)	Conc. used (M)	PTX solubility (mg/ml)	Structure
N,N-Diethylnicotinamide	3.5	39.07	
N,N-Dimethylnicotinamide	3.5	1.77	
N-Methylnicotinamide	3.5	1.34	
Nicotinamide	3.5	0.69	
1-Methylnicotinamide iodide	1.0[a]	0.003	
N,N-Diisopropylnicotinamide	0.05[a]	0.001	

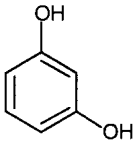
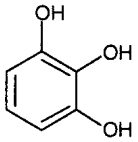
[a] The maximum solubility of the hydrotropic agent.

Table V.

Hydrotropic agent (Conc. used)	PTX solubility (mg/ml)	Structure
Sodium xylenesulfonate (2.5 M)[a]	0.48	
Sodium *p*-toluenesulfonate (2.5 M)[a]	0.22	
1-Methyl-2-pyrrolidone (3.5 M)	0.07	
2-Pyrrolidone (3.5 M)	0.04	
N-Methylnicotinamide (3.5 M)	1.34	
Nicotinamide (3.5 M)	0.69	

[a] The maximum solubility of the hydrotropic agent.

Table VI.

Hydrotropic agent	Conc. used (M)	PTX solubility (mg/ml)	Structure
Resorcinol (1,3-dihydroxybenzene)	3.5	2.009	
Pyrogallol (1,2,3-trihydroxybenzene)	3.5	1.282	

The agents that did not show any appreciable hydrotropic properties have low water solubilities, as expected. Examples are glyceryl triacetate (0.2 M), caffeine (0.1 M), salicylaldoxime (0.1 M), 3,4-pyridinedicarboxamide (0.025 M), *o*-benzoic acid sulfimide (0.01 M), 4-aminosalicylic acid (0.005 M), adenosine (0.005 M), and 2,6-pyridinedicarboxamide (0.0025 M). Those agents have low water solubility and thus showed almost no hydrotropic effect.

High Hydrophobicity of Hydrotropic Agents

For those agents having high water solubilities, the hydrotropic property increases as the hydrophobicity of the molecule increases. Poorly soluble organic drugs are all hydrophobic, i.e., nonpolar, and do not interact with water molecules through hydrogen bonding. Thus, the presence (or insertion) of hydrophobic drug molecules in water (known as hydrophobic hydration) causes a direct perturbation of water, i.e., an alteration in the hydrogen-bonding state of water molecules. Water structure formers, such as sucrose and sorbitol, inhibit dissolution of poorly soluble drugs, whereas water structure disruptors such as nicotinamide increase the solubility by destroying clusters of associated water molecules and releasing water of solvation (13). Thus, effective hydrotropic agents are expected to destabilize water structure and at the same time interact with poorly soluble drugs. Hydrotropic agents lacking a significant hydrophobic component are not effective at all. The following examples show the importance of hydrophobic groups in promoting hydrotropic properties.

Importance of Pyridine and Benzene Rings

Almost all highly effective hydrotropic agents listed in Table I have either a pyridine or a benzene ring in their structures. Molecules without such rings in their structures were not as effective as those containing the ring structures. As shown in Table III, nicotinamide and 3-picolylamine displayed about the same hydrotropic property, while nipecotamide, which has a saturated ring structure, is less than 1% effective as nicotinamide. Other agents without pyridine or benzene ring that had very small hydrotropic effect are urea and its alky derivatives (methyl-, ethyl-, and butylurea), glycerin, thiourea, *N*-isopropylacrylamide, *N*-methylacetamide, *N,N*-dimethylacetamide, sodium thiocyanate, and 1,3-diamino-2-hydroxypropane-*N,N,N',N'*-tetramethylacetate.

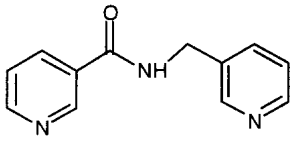
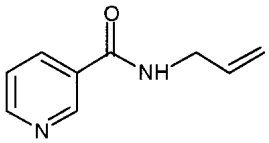
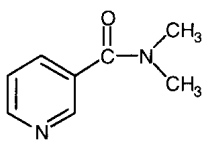
Maximum Hydrophobicity without Losing Water Solubility

The hydrotropic properties of nicotinamide derivatives show a positive correlation with the hydrophobicity of molecules as long as water solubility is not lost. As shown in Table IV, NNDENA showed more than a 20 times higher hydrotropic property than *N,N*-dimethylnicotinamide at the same concentration. *N,N*-Dimethylnicotinamide, in turn, was more effective than *N*-methylnicotinamide, and *N*-methylnicotinamide was twice as effective as nicotinamide. 1-Methylnicotinamide iodide was too hydrophilic to be hydrotropic. The poor hydrotropic property of *N,N*-diisopropylnicotinamide results from its poor water solubility, which is only 0.05 M.

Increase in the Hydrotropic Property by a Factor of Two with a Methyl Group on the Ring

At the same concentration, sodium xylenesulfonate was more hydrotropic than sodium *p*-toluenesulfonate (Table V). A similar trend was seen with 1-methyl-2-pyrrolidone and 2-pyrrolidone. In both examples, the presence of one methyl group increased the hydrotropic property of the molecule by

Table VII.

Hydrotropic agent	Conc. used (M)	PTX solubility (mg/ml)	Structure
N-Picolylnicotinamide	3.5	29.44	
N-Allylnicotinamide	3.5	14.18	
N,N-Dimethylnicotinamide	3.5	1.77	

a factor of 2. The same result was observed for *N*-methylnicotinamide and nicotinamide.

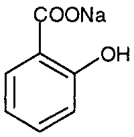
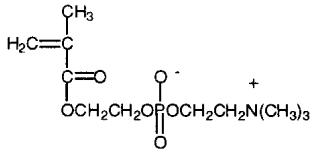
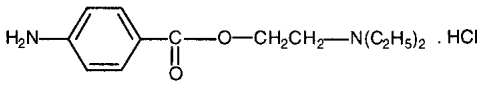
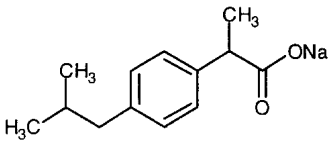
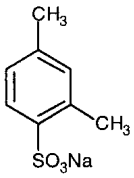
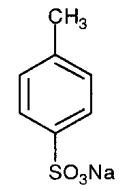
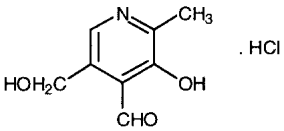
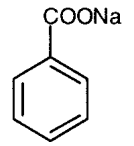
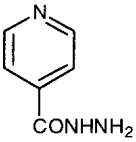
Decrease in Hydrotropic Property with a Hydroxyl Group

The hydrophilicity of a molecule can be increased by attaching hydroxyl groups to the molecule. Increase in hydrophilicity comes with a reduction in the hydrotropic properties of the molecule. For example, pyrogallol, which is more hydrophilic than resorcinol, has lower hydrotropic property (Table VI).

More Effective Hydrotropic Property by One Long Hydrophobic Chain Than by Two Shorter Hydrophobic Chains

As shown in Table VII, the high hydrotropic properties of *N*-picolylnicotinamide and *N*-allylnicotinamide suggest that one longer carbon chain is better than two shorter carbon chains, e.g., one allyl group vs. two methyl groups. A significant increase in PTX solubility by *N*-picolylnicotinamide may be partly related to the presence of another pyridine ring that

Table VIII.

Hydrotropic agent	Conc. used (M)	PTX solubility (mg/ml)	Structure
Sodium salicylate	3.5	5.54	
2-Methacryloyloxyethyl phosphorylcholine	2.9[a]	3.20	
Procaine · HCl	2.5[a]	0.72	
Sodium ibuprofen	1.5[a]	0.50	
Sodium xylenesulfonate	2.5[a]	0.48	
Sodium *p*-toluenesulfonate	2.5[a]	0.22	
Pyridoxal hydrochloride	2.5[a]	0.22	
Sodium benzoate	2.0[a]	0.05	
Isoniazid	1.0[a]	0.01	

[a] The maximum solubility of the hydrotropic agent.

is shown to be essential for hydrotropic property, as discussed above.

Hydrophobic Interaction between Hydrotropic Agent and Solute

Aliphatic derivatives of urea were studied for their effects on increasing the water solubility of PTX. Butylurea shows the highest solubilizing effect among the analogues studied, which suggests that as the hydrophobicity decreases, the hydrotropic property also decreases. Urea is known to break up the hydrogen-bonded water molecule clusters surrounding nonpolar solute molecules. The poor hydrotropic property of urea suggests that disruption of water structure alone, without substantial interaction with solute, is not enough for effective hydrotropy.

Separation of Hydrophilic and Hydrophobic Domains

A good hydrotropic agent appears to have hydrophilic and hydrophobic domains on the same molecule. Examples are shown in Table VIII. This is reasonable because hydrotropic agents are expected to have nonbonded hydrophobic interactions with hydrophobic solute molecules while maintaining hydrophilic property for high water solubility. It is interesting to note that sodium salicylate is highly effective in dissolving PTX. The carboxyl and hydroxyl groups are located on the same side of the molecule, resulting in clear separation of the hydrophilic domain from the hydrophobic domain. The same may be true with 2-methacryloyloxyethyl phosphorylcholine (2.9 M). Procaine·HCl (2.5 M), sodium ibuprofen (1.5 M), sodium xylenesulfonate (2.5 M), and sodium *p*-toluenesulfonate (2.5 M) show easily identifiable separation of hydrophilic and hydrophobic parts.

Polymeric Hydrotropic Agents

Although hydrotropic agents can increase the water solubility of poorly soluble drugs by several orders of magnitude, they have not been explored extensively in the pharmaceutics field. The main reason for this may be the concern that the use of low-molecular-weight hydrotropic agents at high concentrations may result in undesirable side effects, including toxicity and cell damages. This concern, however, may be alleviated by preparing polymeric forms of hydrotropes. For this reason, we synthesized polymers of hydrotropic agents effective for PTX, and hydrotropic polymers were as effective as the low-molecular-weight counterpart in increasing the water solubility of PTX. For example, a polymeric form of *N*-picolylnicotinamide maintains their hydrotropic properties (14). Table I suggests several candidate hydrotropic agents that can be synthesized into polymers. NNDENA, *N*-picolylnicotinamide, *N*-allylnicotinamide, and sodium salicylate appear to be good candidates for making polymeric hydrotropic agents for PTX.

ACKNOWLEDGMENTS

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