Substituent Effect on the Thermodynamic Solubility of Structural Analogs: Relative Contribution of Crystal Packing and Hydration

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ABSTRACT: Thermodynamic analysis of the solubility of benzoylphenylurea (BPU) derivatives was conducted to investigate the relative importance of crystal packing and hydration for improving solubility with minor structural modification. The contribution of crystal packing to solubility was evaluated from the change in Gibbs energy on the transition from the crystalline to liquid state. Hydration Gibbs energy was estimated using a linear free-energy relationship between octanol–water partition coefficients and gas–water partition coefficients. The established solubility model satisfactorily explained the relative thermodynamic solubility of the model compounds and revealed that crystal packing and hydration equally controlled solubility of the structural analogs. All hydrophobic substituents were undesirable for solubility in terms of hydration, as expected. On the other hand, some of these hydrophobic substituents destabilized crystal packing and improved the solubility of the BPU derivatives when their impact on crystal packing exceeded their negative influence on hydration. The replacement of a single substituent could cause more than a 10-fold enhancement in thermodynamic solubility; this degree of improvement was comparable to that generally achieved by amorphous formulations. Detailed analysis of thermodynamic solubility will allow us to better understand the true substituent effect and design drug-like candidates efficiently. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci

Keywords: solubility; crystal packing; calorimetry; hydration; log *P*; thermodynamics; drug design

INTRODUCTION

Improving the aqueous solubility of drugs is one of the major challenges in current drug discovery.[1,2] Poor aqueous solubility of a drug candidate often causes nonlinear oral absorption in preclinical and clinical studies and prevents sufficient evaluation of its efficacy and safety profile.[3] The aqueous solubility of a compound might be improved without modification of the chemical structure by utilizing metastable solid forms such as salts, cocrystals, and amorphous forms.[4–7] These solid forms can induce supersaturation and transiently show higher solubility than thermodynamic solubilty.[8] However, drug supersaturation cannot solve all solubility problems.[8,9] Solubility and oral absorbability of drugs in metastable forms will be determined predominantly by the thermodynamic solubility of their stable forms (typically the crystalline free forms) if they cannot maintain a high degree of supersaturation.[8,9] In addition, metastable forms tend to show lower physical and chemical stability than stable crystalline forms and thus may present challenges to formulation developers.[10–12] Therefore, it is still important to improve equilibrium solubility by structure optimization. A drug candidate that shows good thermodynamic solubility will assure sufficient oral exposure and mitigate future risks in formulation development.

It has been reported that hydrophobicity and crystal packing energy are the major factors that determine thermodynamic solubility.[1,13] The reduction of hydrophobicity is a classic and general strategy for improving aqueous solubility.[1,2,13] The octanol–water partition coefficient ($\log P_{oct}$) is a useful hydrophobicity index as it is often a good predictor variable for intrinsic activity, membrane permeability, and metabolic stability, as well as solubility.[14–17] A reduction in hydrophobicity would help enhance aqueous solubility by promoting drug hydration. Using a different strategy, the disruption of crystal packing has been shown effective for improving the aqueous solubility of crystalline materials.[1,18–20] An increase in molecular flexibility can interfere with rigid crystal packing and increase solubility.[21–23] On the contrary, planar structures comprising fused aromatic rings and biaryls tend to form strong intermolecular π−stacking interactions and have a negative impact on solubility.[18–20]

The simultaneous contribution of these two factors, crystal packing and hydration, may complicate structure–solubility relationships in drug discovery, where many structural analogs are synthesized to find the best clinical candidate. Medicinal chemists can easily predict the substituent effect on $\log P_{oct}$ values before synthesis by using packaged software,[13,16,17,21] and they expect a negative correlation between solubility and $\log P_{oct}$. However, the introduction of a substituent sometimes causes unexpected results. For example, the addition of a hydrophobic substituent does not always decreases solubility, and the addition of a hydrophilic substituent occasionally decreases solubility.[18–20,23] In these cases, an alteration in the crystal packing pattern, induced by the substituents, likely plays an important role in determining drug solubility.

Yalkowsky and coworkers[24,25] have proposed a general solubility equation for estimating the impact of crystal packing and hydration on solubility from the melting point and $\log P_{oct}$ values of the compound.[24,25] The equation can roughly predict the solubility of structurally diverse compounds.[25] However, the

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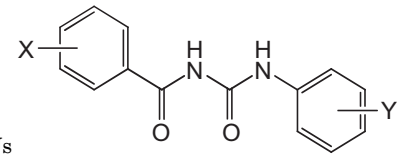

Table 1. Physicochemical Properties of the BPUs

	Substituents		Solubility (μM)[a]	$R_{s,\,obs\text{-}i}$	$\Delta\Delta G^{\circ}_{obs\text{-}i}$ (kJ/mol)	log P_{oct}	T_m (K)	ΔH_f (kJ/mol)
	X	Y						
BPU-1	H	4-Cl	0.19 (±0.02)	1	0	3.32	510	39.1
BPU-2	2-F	4-Cl	0.59 (±0.00)	3.13	−2.82	3.37	465	32.9
BPU-3	3-F	4-Cl	0.08 (±0.01)	0.42	2.14	3.52	510	39.1
BPU-4	4-F	4-Cl	0.08 (±0.02)	0.42	2.14	3.45	532	41.6
BPU-5	2-Me	4-Cl	0.32 (±0.08)	1.68	−1.29	3.53	474	35.2
BPU-6	4-Me	4-Cl	0.03 (±0.01)	0.16	4.57	3.77	511	38.9
BPU-7	2-Cl	4-Cl	0.37 (±0.01)	1.95	−1.65	3.44	471	35.5
BPU-8	4-Cl	4-Cl	0.08 (±0.01)	0.42	2.14	3.88	518	40.8
BPU-9	2-OMe	4-Cl	0.47 (±0.03)	2.45	−2.22	3.88	438	26.0
BPU-10	2-OEt	4-Cl	0.15 (±0.00)	0.79	0.59	4.39	420	27.0
BPU-11	2-Et	4-Cl	0.45 (±0.03)	2.37	−2.14	3.94	436	23.9
BPU-12	H	H	2.30 (±0.04)	12.12	−6.18	2.72	477	32.1

[a]Mean (±SD), $n = 3$; $R_{s,\,obs\text{-}i}$, observed relative solubility; $\Delta\Delta G^{\circ}_{obs\text{-}i}$, observed difference in Gibbs energy change on dissolution; log P_{oct}, logarithm of octanol–water partition coefficient; T_m, melting temperature; ΔH_f, enthalpy of fusion.

relative importance of crystal packing and hydration on the solubility of structural analogs has not been investigated in detail. A better understanding of the quantitative relationship between crystal packing, hydration, and solubility will allow the efficient design of soluble molecules and allow complete evaluation of their physicochemical properties. If the impact of crystal packing on solubility is comparable to that of hydration, we should pay as much attention to the substituent effect on crystal structure as on log P_{oct} to address solubility problems during the molecular optimization process. Exploration of the stable crystal form earlier in the discovery stage will become important for evaluating thermodynamic solubility and prioritizing drug candidates.[26]

In the present study, the aqueous thermodynamic solubility of a family of drug-like model compounds, benzoylphenylurea (BPU) derivatives, was measured. These derivatives have been studied as anticancer, antidiabetic, and anti-inflammatory agents.[27–29] In agricultural chemistry, a variety of analogs have been synthesized for the quantitative structure–activity studies of their larvicidal potency.[30] The substituent effect on hydration was estimated from log P_{oct} values and a linear free-energy relationship (LFER), and that on crystal packing was evaluated by thermal analysis. A thermodynamic model that considers both crystal packing and hydration was constructed to explain the relative solubility of the model compounds. The relative importance of crystal packing and hydration in determining thermodynamic solubility was estimated and strategies for improving solubility during drug discovery are discussed.

MATERIALS AND METHODS

Chemicals

Benzoylphenylurea derivatives listed in Table 1 were synthesized as previously reported.[30] Antipyrine and caffeine were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Sodium nitrate, ethylbenzene, propylbenzene, and butylbenzene were purchased from Tokyo Chemical Industry Company, Ltd. (Tokyo, Japan). All other reagents were of reagent grade and were used without further purification. The water used was filtered through a Milli-Q Water Purification System (Millipore, Bedford, Massachusetts) prior to use.

Measurement of Thermodynamic Solubility

The thermodynamic aqueous solubility of BPUs was determined at 25°C using a shake flask method. Excess solid was added to glass tubes containing 5 mL of water. The test solutions were placed in a shaker incubator (Personal Lt-10, Taitec Company, Saitama, Japan) for 24 h and then transferred to HPLC glass vials. The solutions were left for 48 h in a temperature-controlled autosampler (model G1329; Agilent Technologies, Inc., Santa Clara, California) at 25°C to allow the solids to sediment. The concentration of each compound remaining in solution was determined by HPLC using UV detection (model 1100 series HPLC system; Agilent Technologies, Inc.) and an YMC-Triart C18 column (4.6 mm i.d., 100 mm length, 3 μm; YMC Company, Ltd., Kyoto, Japan). Mobile phase A consisted of 0.1% (v/v) $HClO_4$ and 1% (v/v) acetonitrile in water. Mobile phase B consisted of 0.1% (v/v) $HClO_4$ and 10% (v/v) water in acetonitrile. The analytes were eluted with a linear gradient by ramping mobile phase B from 60% to 100% over 3 min at a flow rate of 1 mL/min. The sample injection volume was 50 μL and the detection wavelength was set at 254 nm. HPLC analysis of each vial was repeated three times periodically to confirm complete solid separation. All solubility measurements were performed in triplicate.

Differential Scanning Calorimetry Measurement

The thermodynamic properties of BPUs were determined by differential scanning calorimetry (DSC, model Q2000; TA Instruments Inc., New Castle, Delaware). The instrument was calibrated for temperature and cell constant using high purity indium. Enthalpy of fusion and melting temperature were measured by placing crystalline samples in aluminum pans and heating at 1°C/min. All experiments were performed in

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