Impact of Physicochemical Profiling for Rational Approach on Drug Discovery

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Solid-state characterization plays a vital role in lead optimization and candidate selection with the appropriate physicochemical properties for proper oral dosage formulation. Aqueous solubility is an important parameter in the successful development of oral dosage formulation since poor aqueous solubility limits absorption. In this study, we summarized an efficient approach using a small amount of sample for solid-state characterization, including thermodynamic solubility, which is defined as physicochemical profiling. By using the physicochemical profiling results of 75 anti-cancer drugs and clinical candidates, we examined the relationship between thermodynamic solubility and molecular structural parameters and assessed the effects of thermodynamic solubility on pharmacokinetic profile for rational soluble drug design. The Log $D_{\text{pH } 7.4}$, aromatic ring count, and hydrogen bond count were good indicators for predicting sparingly soluble compounds that increase the lattice energy because of π – π stacking and hydrogen bonds, resulting in lowered thermodynamic solubility. The level of thermodynamic solubility in simulated intestinal fluid (pH 6.8) in the presence and absence of bile acid, which is required for minimal acceptable bioavailability (>30%), was 1 $\mu\text{g/mL}$ and 10 $\mu\text{g/mL}$, respectively. Physicochemical profiling, which includes thermodynamic solubility considering its solid-state properties, contributes to rational lead optimization and efficient candidate selection for drug development.

Key words physicochemistry; thermodynamic solubility; kinetic solubility; lipophilicity; bioavailability

The most common administration route of drugs entering the commercial market is oral administration due to its ease and convenience in terms of drug delivery.^{1,2)} Solubility is a crucial parameter that affects oral absorption as well as the permeability of solid dosage forms.^{3,4)} Low aqueous solubility results in poor absorption and variability in the drug plasma concentration, which results in an attrition rate and high cost of drug development.^{4,5)}

Many studies have reported on the importance of the solid form due to its effects on solubility and stability.^{6–9)} In general, the difference in solubility between the polymorphic or crystalline forms and the amorphous forms was less than 10 times or up to several hundred times, respectively.¹⁰⁾ Thus, an evaluation of the solubility taking into account its solid-state properties is essential during drug development.

Within the pharmaceutical industry, there are 2 different types of solubility measurements: kinetic solubility and thermodynamic solubility.⁵⁾ Kinetic solubility is a measurement of solubility that dilutes a highly concentrated compound organic solution into an aqueous solution. In contrast, thermodynamic solubility is equilibrium solubility of solid crystalline material in an aqueous solution. Although kinetic solubility is performed in high throughput and is preferably measured at the early discovery stage, kinetic solubility is sometimes overestimated because the precipitates are amorphous or in a meta-stable form, according to Ostwald's rule, because of fast-precipitation when the drug stock solution is added to the aqueous solution or by shaking.^{11,12)} Thus, the thermodynamic

solubility measurement on the basis of the solid form is a critical element for efficient and successful development in the solid dosage formulation.

During lead optimization and candidate selection, we evaluated the thermodynamic solubility measurement on the basis of the solid form using the following 3 main analyses: (1) powder X-ray diffraction (PXRD), (2) thermogravimetry (TG) and differential scanning calorimetry (DSC) analyses and (3) thermodynamic solubility measured by the shake-flask method, which is defined as physicochemical profiling. Physicochemical profiling enables the assessment of thermodynamic solubility by considering the solid-state properties in the early drug development stage. It also contributes to the batch analysis and evaluation of drug-likeness in drug discovery and is a key indicator of whether the solid-state properties of the drug are sufficient for development into an oral dosage form.

A decade has passed since the Rule of 5, which defined the molecular structural parameters for drug-like compounds using approximately 2000 candidates or late-stage trial compounds, according to Lipinski.^{5,13)} Given that the Rule of 5 was easy-to-use, it has been widely used for the assessment of drug-likeness. Variations of the Rule of 5, such as Rule of 3¹⁴⁾ and the Rule of 4,¹⁵⁾ have been proposed and many studies have recently attempted to analyze the relationship between molecular structural parameters and solubility.^{16,17)} These studies have gained much attention in the physicochemical field. The molecular structural parameters, such as the molecular weight, lipophilicity, polar surface area, and aromatic ring, have been reported as solubility parameters.^{3,18–20)} Although a few correlation studies between the molecular structural parameters and thermodynamic solubility have been reported, they used thermodynamic solubility without consideration of the crystallinity and crystal form.^{21–23)} Thermodynamic solubility without consideration of the crystallinity and crystal

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form was not suitable for the relationship analysis between the molecular structural parameters and solubility since thermodynamic solubility is affected by the crystallinity and crystal form. In addition, there has been no study about the factor creating the discrepancy between thermodynamic solubility and kinetic solubility and the correlation between thermodynamic solubility and oral absorption.

In this study, we present physicochemical profiling, which uses medium throughput²⁴⁾ and a systematic approach with a small amount of drugs in the early discovery stage. Using the physicochemical profiling results of 75 anti-cancer drugs and clinical candidates, we examined the correlation between molecular structural parameters and the thermodynamic solubility and discussed the effects of solubility on oral absorption. In addition, we discuss factors that cause differences between the kinetic and thermodynamic solubility. This is the first work which analyzes the relationship between thermodynamic solubility based on the crystallinity and crystal form and the molecular structural parameters, investigates the discrepancy between thermodynamic solubility and kinetic solubility and studies the correlation between thermodynamic solubility and oral absorption.

Experimental

Materials Seventy-five anti-cancer drugs and clinical candidates were selected as tool compounds in this study because most of them were poorly soluble and a wide range of solubility was covered. Their physicochemical profiling results and the pharmacokinetic (PK) parameters in rats are presented in Table 1. Some solids existed in hydrates or solvates, amorphous revealed by PXRD and the thermal analysis. These data were eliminated from the relationship analysis between thermodynamic solubility and the molecular structural parameters since they were not suitable due to the considerable influence of the solid state properties on solubility. The inequality sign of kinetic solubility data is sometimes used due to the dependency of the maximum solubility values on the molecular weight because the drug solution with a given concentration is diluted with aqueous media in the kinetic solubility measurement. To investigate the effect of thermodynamic solubility on oral absorption and oral absorption rate, the rat oral bioavailability and mean absorption time (MAT) data of the compounds with low permeability, large clearance or large volumes of distribution was not available for the analysis of the relationship between solubility and PK data.

All of the Takeda compounds were synthesized in the Medicinal Chemistry Research Laboratories and Chemical Development Laboratories at Takeda Pharmaceutical Co., Ltd. (TPC). Other anti-cancer drugs and clinical candidates were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), Sigma-Aldrich (St. Louis, MO, U.S.A.), Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan) and Tocris Bioscience (Bristol, U.K.).

Physicochemical Profiling

PXRD PXRD patterns were collected using a RINT UltimaIV powder X-ray diffractometer (Rigaku Corp., Tokyo, Japan) with CuK α radiation, generated at 40kV and 50mA. The drug substances were placed on a silicone plate at room temperature. The plates were then inserted into a 48-position Automatic Sample Changer, which enables an automatic con-

tinuous measurement of the 48 samples. The data were collected from 2 to 35° (2 θ) at a step size of 0.02° and scanning speed of 6°/min.

Crystallinity Calculation After subtracting the background pattern of the silicon plate from all of the PXRD patterns of the drug sample, the peak intensities of the crystalline (I_c) and non-crystalline (I_a) fractions were integrated from the baseline collection using Herman's method.^{25,26)} The crystallinity was calculated using the following equation:

$$\text{Crystallinity} = I_c \times 100 / (I_c + I_a) \times 100$$

To avoid any variability derived from daily and personal errors, the crystallinity was calculated using an auto-crystallinity calculation software (Rigaku, Tokyo, Japan).^{26,27)}

Thermal Analysis TG was performed using a TGA/DSC1 system (Mettler-Toledo International Inc., Greifensee, Switzerland). A TG curve was obtained in an open aluminum pan using *ca.* 0.60mg of the drug substance and a heating rate of 5°C/min under a nitrogen gas flow at 40mL/min. The DSC was measured using a DSC1 system (Mettler-Toledo International Inc., Greifensee, Switzerland). A DSC thermogram was obtained in a crimped aluminum pan using *ca.* 0.25mg of drug substance and a heating rate of 5°C/min under a nitrogen flow at 20mL/min.

Thermodynamic Solubility Measurement by the Shake-Flask Method To measure the thermodynamic solubility, 0.4mg of the drug substances was weighed into Thomson filter vials (Chrom Tech, Inc., Minnesota, U.S.A.) containing a 0.45 μ m polyvinylidene difluoride filter membrane. Four hundred microliters of JP1 (pH1.2), JP2 (pH6.8), and JP2-containing 20mM sodium glyco-chenodeoxycholate (GCDC), were added to the vials. The vials were incubated at 37°C with shaking at 500rpm for 18h and the resulting suspensions were filtrated by compressing the vials. The drug concentration of the filtrates was determined by HPLC.

High Performance Liquid Chromatography (HPLC) Analysis HPLC analysis was conducted using a UHPLC system (Prominence UFLC, Shimadzu Corp., Kyoto, Japan) with a photodiode array detector. A YMC-UltraHT Pro C18 packaged column (2 μ m, 2.0 $\times$ 3.0mm, YMC Co., Ltd., Kyoto, Japan) was used at 40°C at a flow rate of 0.7mL/min. The gradient mobile phase consisted of distilled water–50mM ammonium acetate buffer–acetonitrile (8:1:1, v/v/v) (A) and 50mM ammonium acetate buffer–acetonitrile (1:9, v/v) (B). The starting mobile phase consisted of 95% A and 5% B for 0.1min, changed gradually to 5% A and 95% B from 0.1 to 1.6min, and then returned to the initial condition after 1.3min and was re-equilibrated for an additional 0.6min.

Kinetic Solubility Measurement by the Precipitation Method The kinetic solubility was evaluated by the precipitation method.^{28,29)} The compounds were dissolved in dimethylsulfoxide (DMSO) to yield 10mM DMSO stock solutions. To measure the kinetic solubility, a 96-well multi-filter plate (Millipore Corp., MA, U.S.A.) was used. DMSO stock solutions (5 μ L) were dispensed into a 96-well multi-filter plate, and 245 μ L of JP1 and JP2 and JP2-containing 20mM GCDC were added. The plate was incubated at 25°C for 24h. After filtration, the drug concentration was analyzed using HPLC.

Log $D_{\text{pH}7.4}$ (Log D) Determination Log D , which is a partition coefficient between 1-octanol and aqueous buffer pH7.4, of the compounds was measured using a chromato-

Table 1. Physicochemical and Pharmacokinetics Properties of Active Drugs in Cancer

Drug	Free base or salt	Kinetic solubility ($\mu\text{g/mL}$)		Thermodynamic solubility ($\mu\text{g/mL}$)		Log $D_{\text{pH } 7.4}$	Rat oral bioavailability (%)	cLog P^{a}	MAT (h)	Number of hydrogen bond donor and acceptor	Number of aromatic ring	$R_{\text{sp}}^{3, b)}$	Solid state properties ^{c)}
		JP2	GCDC	JP2	GCDC								
AZM-475271	Free base	75	>80	480	980	2.31	48.9	5.5	1.27	8	3	0.64	
PLK1 inhibitor (GSK)	Free base	0.91	26	0.062	5.1	2.00	n.a. ^{d)}	3.3	n.a. ^{d)}	13	3	0.18	
PD325901	Free base	70	>100	350	630	3.02	64.3	3.0	1.2	10	2	0.23	
Bicalutamide	Free base	35	72	2.1	19.9	1.97	n.t. ^{e)}	2.7	n.t. ^{e)}	8	2	0.29	
GSK3 β inhibitor (Janssen pharma)	Free base	0.14	46	0.19	66	2.66	n.a. ^{d)}	3.2	n.a. ^{d)}	12	4	0.47	
TO-901317	Free base	6.9	62	0.41	19	3.13	42.2	4.2	1.35	5	2	0.42	
ON-01910	Na salt	80	>96	>1000	1000	0.83	n.a. ^{d)}	-1.0	n.a. ^{d)}	9	2	0.40	Amorphous
Sumitinib	Free base	>76	>100	23	>1000	2.43	50.7	3.0	1.62	8	2	0.57	
Thiadiazolidinones derivatives (Neuropharma)	Free base	<0.07	12	<0.08	4.3	4.48	n.a. ^{d)}	6.1	n.a. ^{d)}	3	4	0.05	
T-1710502	Free base	0.08	77	<0.03	3.8	2.28	11.9	3.8	1.88	11	4	0.33	Hydrate or solvate
CHIR-265	Free base	<0.05	66	0.18	92	3.97	38	7.0	0.99	7	5	0.14	
Quinoline compound made by the workers at Kirin.	Free base	<0.03	81	<0.04	37	3.94	35.8	5.2	1.26	10	4	0.13	
Paclitaxel	Free base	0.78	23	<0.08	9.6	2.92	n.a. ^{d)}	4.7	n.a. ^{d)}	19	3	0.81	
Hydroxyflutamide	Free base	>59	>60	150	350	2.20	59	2.5	0.51	6	1	0.57	
Flutamide	Free base	38	>50	33	130	2.64	n.a. ^{d)}	3.3	n.a. ^{d)}	4	1	0.57	
Aurora kinase inhibitor (AstraZeneca)	Free base	<0.07	20	<0.09	0.81	3.24	n.t. ^{e)}	5.2	n.t. ^{e)}	4	3	0.26	
Sorafenib	Free base	0.15	>93	<0.04	8.1	3.70	34.9	5.5	1.12	10	3	0.11	
SP600125	Free base	2.5	38	0.66	21	2.40	n.t. ^{e)}	3.7	n.t. ^{e)}	3	3	0.00	
Chir99021	Free base	37	>92	4.8	3.8	2.46	n.a. ^{d)}	4.3	n.a. ^{d)}	10	4	0.16	
Chir98023	Free base	23	>86	0.5	5.6	2.42	n.t. ^{e)}	4.3	n.t. ^{e)}	10	4	0.11	
Topotecan	HCl salt	40	68	530	n.t. ^{e)}	0.92	n.a. ^{d)}	0.7	n.a. ^{d)}	9	3	0.53	
Rosiglitazone	Free base	49	62	3.4	21	2.17	87.9	3.0	0.79	8	2	0.38	
SB-216763	Free base	<0.07	28	<0.1	0.3	3.57	n.a. ^{d)}	5.0	n.a. ^{d)}	4	3	0.06	
KRN-633	Free base	1.2	39	0.12	12	2.72	n.t. ^{e)}	4.1	n.t. ^{e)}	10	3	0.33	
Lilly603281-31-8	Free base	74	89	1.7	17	1.08	n.a. ^{d)}	2.9	n.a. ^{d)}	9	4	0.35	
LY200125	HCl salt	42	>57	70	530	2.63	n.t. ^{e)}	3.0	n.t. ^{e)}	3	2	0.27	
Troglitazone	Free base	1.3	78	<0.23	48	3.30	16.5	5.6	0.72	9	2	0.71	
Thiadiazolidinones derivatives (Neuropharma)	Free base	<0.09	2.8	<0.11	1.8	3.47	n.a. ^{d)}	5.2	n.a. ^{d)}	2	3	0.06	
Sotrastaurin	Free base	65	>100	7.3	920	2.39	n.a. ^{d)}	2.8	n.a. ^{d)}	9	4	0.25	
BEZ235	Free base	<0.03	13	0.25	10	3.05	10.7	5.8	0.66	4	6	0.15	
HDAC inhibitor (Pfizer)	Free base	48	>54	94	120	0.38	82	0.7	0.40	8	2	0.07	
CP-724714	3/2 Succinate	7.6	>98	0.3	>1000	2.95	n.a. ^{d)}	4.8	n.a. ^{d)}	10	4	0.23	
PD0325901	Trifluoroacetate	<0.09	65	350	630	5.61	n.t. ^{e)}	7.7	n.t. ^{e)}	7	4	0.57	
Nutlin-3	Free base	53	>120	32	790	3.33	n.t. ^{e)}	8.0	n.t. ^{e)}	9	3	0.43	Amorphous
Tarceva	HCl salt	1.9	74	1.5	84	2.59	n.t. ^{e)}	4.3	n.t. ^{e)}	8	3	0.38	
Docetaxel	Free base	29	>150	1.5	27	3.14	n.t. ^{e)}	4.1	n.t. ^{e)}	20	2	1.26	
FK506	Free base	59	100	<0.14	37	6.41	n.t. ^{e)}	5.8	n.t. ^{e)}	16	0	3.40	Hydrate or solvate
TAK-715	Free base	0.11	>83	<0.05	19	4.25	n.a. ^{d)}	5.6	n.a. ^{d)}	5	4	0.20	

Table 1. Continued

Drug	Free base or salt	Kinetic solubility ($\mu\text{g/mL}$)		Thermodynamic solubility ($\mu\text{g/mL}$)		Log $D_{\text{pH } 7.4}$	Rat oral bioavailability (%)	cLog P^{a}	MAT (h)	Number of hydrogen bond donor and acceptor	Number of aromatic ring	R $sp^{3,b}$	Solid state properties ^{c,f}
		JP2	GCDC	JP2	GCDC								
T-1085501	Free base	0.2	15	0.033	5.6	3.26	n.t. ^e	4.7	n.t. ^e	4	3	0.31	
T-1111813	Free base	0.15	>98	<0.1	2.3	3.73	11.5	4.4	2.04	5	4	0.14	
T-1337297	Free base	>55	>64	85	310	1.42	n.a. ^d	1.8	n.a. ^d	7	4	0.06	
T-1365965	Free base	45	75	3.3	61	1.45	n.a. ^d	2.1	n.a. ^d	10	4	0.44	
T-1369607	Free base	0.23	>82	<0.04	92	3.07	n.a. ^d	3.9	n.a. ^d	8	5	0.09	
T-1387074	Free base	13	>110	0.41	41	3.13	n.t. ^e	5.0	n.t. ^e	8	4	0.28	
T-1580652	Free base	0.26	>73	<0.07	28	2.51	23.5	3.6	1.77	9	4	0.19	
T-1586455	2HCl salt	0.25	49	7.6	51	3.05	n.t. ^e	4.0	n.t. ^e	7	5	0.09	
VX745	Free base	13	>83	4.4	46	2.55	33.5	5.5	1.26	4	4	0	
Crizotinib	Free base	57	>86	220	>1000	2.37	n.a. ^d	4.3	n.a. ^d	7	3	0.50	
PF-573228	Free base	3.6	53	0.52	32	1.56	n.t. ^e	3.2	n.t. ^e	11	3	0.29	
T-1761249	Sulfate	0.52	20	0.07	28	2.07	n.t. ^e	2.6	n.t. ^e	6	5	0.04	
Anastrozole	Free base	>57	>62	940	>1000	1.85	44.3	1.3	-0.17	4	2	0.70	
Idarubicin	Free base	44	>100	25	27	3.02	n.t. ^e	0.9	n.t. ^e	15	2	0.73	Hydrate or solvate
Letrozole	Free base	>60	>65	48	77	1.04	105.8	1.2	n.a. ^d	4	3	0.06	
Levofolinate	Free base	>140	20	>1000	>1000	0.17	n.t. ^e	0.5	n.t. ^e	21	2	0.43	Amorphous
Melphalan	Free base	n.a. ^e	12 ^e	4.7 ^e	3.1 ^e	1.53	n.a. ^d	-0.2	n.a. ^d	6	1	0.86	
Tamoxifen citrate	Citrate	1.4	52	1.8	>1000	5.08	n.t. ^e	6.8	n.t. ^e	2	3	0.30	
Ubenimex	Free base	n.t. ^e	n.t. ^e	>1000	>1000	n.t. ^e	89.5	-0.3	1.22	10	1	1.00	
(S)-Thalidomide	Free base	36	38	60	75	0.47	n.a. ^d	0.5	n.a. ^d	7	1	0.30	
Nilutamide	Free base	>60	>70	66	140	2.13	n.t. ^e	1.7	n.t. ^e	6	1	0.50	
Vorinostat	Free base	43	42	250	320	0.89	n.t. ^e	1.0	n.t. ^e	8	1	0.75	
Fulvestrant	Free base	<1.28	96	<0.78	29	4.95	n.t. ^e	7.3	n.t. ^e	5	1	4.33	
Dexamethasone	Free base	50	67	80	170	2.04	n.t. ^e	1.8	n.t. ^e	8	0	2.67	
Exemestane	Free base	51	>63	26	110	2.58	n.t. ^e	3.3	n.t. ^e	2	0	1.50	
Etoposide	Free base	86	>110	110	300	0.74	n.t. ^e	0.0	n.t. ^e	16	2	1.23	Hydrate or solvate
Megastrol acetate	Free base	2.2	66	0.19	40	3.36	n.t. ^e	3.6	n.t. ^e	4	0	2.43	
Methotrexate	Free base	81	81	>1000	1000	n.a. ^d	n.a. ^d	-0.5	n.a. ^d	18	3	0.33	Hydrate or solvate
Oxaliplatin	Free base	>150	>84	>1000	960	0.25	n.t. ^e	-0.6	n.t. ^e	9	0	3.00	
Toremifene citrate	Citrate	8.1	67	<0.11	>1000	4.87	n.t. ^e	6.5	n.t. ^e	2	3	0.30	
Vinorelbine citrate	Free base	140	>150	>930	>1000	3.41	n.t. ^e	5.9	n.t. ^e	13	3	1.14	Amorphous
Prednisone	Free base	>74	>76	140	250	1.52	n.t. ^e	1.7	n.t. ^e	7	0	2.00	
Vindesine sulfate	Sulfate	>140	>180	>1000	>1000	3.04	n.t. ^e	3.4	n.t. ^e	16	3	1.39	Amorphous
VEGFR2 inhibitor (Kirin)	Free base	<0.03	33	<0.03	14	2.76	n.t. ^e	5.3	n.t. ^e	10	4	0.14	
Tegafur	Free base	29	30	>1000	>1000	-0.24	n.t. ^e	-0.1	n.t. ^e	4	1	1.00	
Lapachol	Free base	n.t. ^e	n.t. ^e	58	93	n.t. ^e	n.t. ^e	3.7	n.t. ^e	4	1	0.25	

a) Calculated value (Daylight).

b) Ratio of number of sp^3 -hybridized carbons to number of sp - and sp^2 -hybridized carbons

c) n.t.: not tested.

d) n.a.: not available.

e) Degradation in aqueous solution

f) Some solids existed in hydrates or solvates, amorphous revealed by PXRD and the thermal analysis. These data were eliminated from the relationship analysis between thermodynamic solubility and the molecular structural parameters since they were not suitable due to the considerable influence of the solid state properties on solubility.

Fig. 1. TPC Workflow for Physicochemical Profiling

graphic method as previously described.³⁰⁾ A Waters Alliance 2795 HPLC system with a 2487 UV-Vis detector was used (Milford, MA, U.S.A.).

Data Visualization and Statistical Analysis A TIBCO spotfire (TIBCO Software Inc., Somerville, MA, U.S.A.) was used to visualize and conduct the statistical analysis.

PK Study in Rats All animal experiments were carried out in accordance with the guidelines of the Institutional Animal Care and Use Committee (Takeda Pharmaceutical Co., Ltd.). The PK parameters were obtained by cassette dosing of the compounds in rats.^{31,32)} Fasted Jcl:SD male rats (8 weeks old) were used. Prior to dosing, rats were anesthetized with a femoral vein injection of isoflurane. A mixture of no more than 10 drugs was administered at doses of 0.1 mg/kg (intravenous or i.v.) and 1 mg/kg (oral or *per os* (p.o.)). Blood samples were then collected from the tail vein using a Pasteur pipette with heparin in regular intervals for 8 h. The concentration of all of the plasma samples was determined using the LC-MS/MS method. The HPLC apparatus comprised an SIL-10AHC (Shimadzu, Kyoto, Japan). The analytical column was a Inertsil C8 column (2.1×33 mm) and the mobile phase consisted of acetonitrile and acetonitrile–10 mM aqueous ammonium formate (15:85, v/v) with a flow rate of 0.2 mL/min in a run time of 8 min. The HPLC apparatus was coupled to a Sciex API 3000 mass spectrometer (MDS SCIEX, Concord, Ontario, Canada), which was operated by an ESI source in the positive mode. Positive ion mode with turbo spray was used in the multiple reaction monitoring.

Results and Discussion

Takeda Pharmaceutical Co., Ltd. (TPC) Approach for Physicochemical Profiling The investigation of solid-state properties is essential to successful development due to its influences on the chemical and physical properties of the drug including crystallinity, solubility, and stability.⁸⁾ Physicochemical profiling is employed at TPC *via* lead optimization

using medium throughput screening with a sample amount of 7 mg, which provides crucial information on the formulation strategy and the candidate developability. The solid-state characteristics are investigated using analytical techniques such as PXRD and thermal analysis (TG/DSC), and the thermodynamic solubility measurement by the shake-flask method.

The schematic TPC approach for physicochemical profiling is shown in Fig. 1. Physicochemical profiling was performed on an equivalent of 7 mg in the powder state. First, the crystal form and crystallinity were identified by PXRD, enabling rapid and non-destructive analysis. Subsequently, thermal characterization (TG and DSC), which investigates the melting point, fusion enthalpy, solid phase transition, and dehydration/desolvation during heating, and thermodynamic solubility measurement by the shake-flask method using the Thomson filter vials, which are designed to minimize drug adsorption on to the filter components compared to the Mini-uni prepTM (Whatman PLC, Maidstone, U.K.), were performed.³³⁾

Measuring the solubility in gastrointestinal fluids provides a good prediction of the *in vivo* oral absorption behavior. Although it is well known that a solubility comparison in FaSSIF and FeSSIF can help predict food effects *in vivo*,³⁴⁾ we used fed-state simulated intestinal fluids such as JP2, which contains 20 mM GCDC, since GCDC is one of the major components in human bile acid³⁵⁾ and is suitable for HPLC analysis.

Previous reports have described that thermodynamic solubility measurements with the crystal form determination was performed during the late stage candidate development²⁴⁾ since most compounds obtained at the lead optimization process are amorphous, have low purity or the crystal form may not be characterized due to the large sample consumption and low throughput. However, using our TPC approach to physicochemical profile enables the measurement of the thermodynamic solubility including the crystal form and crystallinity as a medium throughput screening with a small amount of sample at the optimization process, which contributes to

Fig. 2. Correlation between Thermodynamic Solubility and Measured $\text{Log } D_{\text{pH}7.4}$ Value (a); Measured $\text{Log } D_{\text{pH}7.4}$ Value Including the Compounds Whose Solids Were Amorphous and Solvate or Hydrate (b)

Fig. 3. Correlation between Thermodynamic Solubility and Calculated $\text{cLog } P$ Value

Fig. 4. Correlation between Measured $\text{Log } D_{\text{pH}7.4}$ Value and Calculated $\text{cLog } P$ Value

the lead optimization and candidate selection for oral dosage formulation.

Contribution of Lipophilicity to Aqueous Solubility Lipophilicity is a well-known parameter that affects aqueous solubility and permeability. Previous studies have shown that increases in lipophilicity results in a high risk for poor oral absorption and variation in drug plasma concentration due to low aqueous solubility.^{7,8)} Although the importance of lipophilicity has been proposed using the relationship between lipophilicity and solubility, they have been mainly used in kinetic solubility data.^{36,37)} In this study, we examined the effect of lipophilicity on thermodynamic solubility. The $\text{Log } D$ was used as a measure for lipophilicity of the compounds. The relationship between thermodynamic solubility and $\text{Log } D$ in this data set is illustrated in Fig. 2a. Based on the lipophilicity of $\text{Log } D$, the thermodynamic solubility may have different propensities. The compounds were categorized in $\text{Log } D$ as follows, (1) compounds with $\text{log } D < 1$, (2) compounds with $1 \leq \text{Log } D \leq 3.5$, and (3) compounds with $\text{Log } D > 3.5$. All of the compounds of category (1) showed a high solubility ($> 40 \mu\text{g/mL}$). In addition, the category (3) compounds were poorly soluble ($< 1 \mu\text{g/mL}$). Furthermore, the compounds of category (2) showed low-, medium- and high-solubility and a

lipophilicity-independent tendency.

Figure 2b showed the relationship between thermodynamic solubility and lipophilicity. No correlation was observed including the compounds whose solids were amorphous and solvate or hydrate. These findings indicated that it is important to measure thermodynamic solubility based on the crystal form and crystallinity by TPC approach.

The $\text{Log } D$ was particularly useful in providing a more clear differentiation among the poorly soluble compounds compared to $\text{cLog } P$.³⁷⁾ We investigated the differences in the tendency between $\text{Log } D$ and $\text{cLog } P$ calculated by $\text{cLog } P$ ver. 4.82 (Daylight Chemical Information Systems Inc.) on thermodynamic solubility. The $\text{Log } D$ enhanced the discrimination between the soluble and poorly soluble compounds compared to $\text{cLog } P$ (Fig. 3). The relationship between $\text{Log } D$ and $\text{cLog } P$ was shown in Fig. 4. $\text{Log } D$ was different from $\text{cLog } P$ because $\text{cLog } P$ is a constant for the molecule under its neutral form and $\text{Log } D$ takes into account all neutral and charged forms of the molecule. The poor correlation between $\text{cLog } P$ and thermodynamic solubility was observed among the compounds with the large discrepancy between $\text{cLog } P$ and $\text{Log } D$ since they have dissociable groups. These results suggested that $\text{Log } D$, but not $\text{cLog } P$, was a good indicator of

poorly soluble compounds.

These findings suggested that $\text{Log } D$ is very useful to identify compounds with very high (category (1)) and very poor (category (3)) solubility, in which the solvation process plays an important role in thermodynamic solubility. In these two regions, thermodynamic solubility depends on lipophilicity due to the very smaller value of the enthalpy change of the crystal lattice breaking in the dissolution process than the enthalpy change of the solvation.

The prediction regarding aqueous solubility based on lipophilicity was challenging because most of the drugs have a $\text{Log } D$ between 1 and 3.5.³⁸⁾ It may be presumed that the breakage of the crystal lattice can affect thermodynamic solubility of the compounds with a $\text{Log } D$ between 1 and 3.5 since the enthalpy change of the solvation in the dissolution process compared to the enthalpy change of the crystal lattice breaking.

These results suggested that thermodynamic solubility among these compounds is considerably affected by the crystal packing and other molecular structural parameters, except for lipophilicity, may contribute to thermodynamic solubility.

Molecular Structural Parameters That Affect on Thermodynamic Solubility Recent studies have indicated that the combination of $\text{Log } D$ values together with the aromatic ring count in a given molecule may be a powerful tool to predict kinetic solubility.²²⁾ We also examined the relationship between thermodynamic solubility in JP2, and the sum of the $\text{Log } D$ and aromatic ring count in category (2) compounds (Fig. 5). Compounds with a sum of $\text{Log } D$ and aromatic ring count of less than 5 were very soluble (Fig. 5). In addition, we analyzed the relationship between thermodynamic solubility and other molecular structural parameters aside from the aromatic ring count. In the partial region of $\text{Log } D$ between 1 and 3.5, there is an increasing sum in the hydrogen bond donor and acceptor counts, resulting in a lowered thermodynamic solubility (Fig. 6).

These findings suggested that the aromatic ring and the sum of the hydrogen bond donor and acceptor could contribute to thermodynamic solubility of $\text{Log } D$ ranging from 1 to 3.5.

Molecular Structural Parameters That Affect on the Discrepancy between Kinetic Solubility and Thermodynamic Solubility A comparison between kinetic solubility and thermodynamic solubility in this study was performed. The relationship between thermodynamic solubility and kinetic

solubility in the JP2 and JP2-containing GCDC, respectively, are presented in Figs. 7a and b. The line in both of these figures indicates the $y=x$ relationship (variable x and y means thermodynamic solubility and kinetic solubility, respectively). In the compounds indicated above the line, the thermodynamic solubility was lower than the kinetic solubility. Thus, there were variable extents in the discrepancy between kinetic solubility and thermodynamic solubility. In particular, the discrepancy in JP2 was much larger in the range of $\text{Log } D$ from

Fig. 5. Distribution of Thermodynamic Solubility in Measured $\text{Log } D_{\text{pH}7.4}$ and Aromatic Ring Count

Fig. 6. Distribution of Thermodynamic Solubility in Hydrogen Bond Donor and Acceptor Count

Fig. 7. Difference between Kinetic Solubility and Thermodynamic Solubility in JP2 (a); JP2-Containing GCDC (b)

1 to 3.5 (Fig. 8a). In contrast, no discrepancy was observed in the compounds from categories (1) and (3). The discrepancy was larger as the crystal lattice energy was increasing because the crystal lattice breaking process is considered in thermodynamic solubility, but not in the kinetic solubility measurement.

We further investigated which molecular structural parameters, except for lipophilicity, govern the discrepancy between kinetic solubility and thermodynamic solubility in JP2 among the compounds in category (2) since the studies about the factor of the discrepancy between thermodynamic solubility and kinetic solubility have never reported. Several studies have reported the effects of the aromatic ring and the fraction of sp^3 hybridized carbon atoms on solubility.^{22,39)} First, we explored the relationship between the discrepancy and ratio sp^3 (R_{sp^3}), which are as follows: $R_{sp^3} = sp^3$ hybridized carbon count/ sp and sp^2 hybridized carbon count.

The discrepancy between the kinetic solubility and thermodynamic solubility in JP2 tended to increase as the R_{sp^3} value decreased (Fig. 8b). A smaller R_{sp^3} value, which indicated that the molecule would be more flat, increased the opportunity for π - π stacking. Several studies have reported that the hydrogen bond donor and acceptor stabilize the crystal packing and increase the melting point of the molecule.^{24,40,41)} The solubility was increased by reducing the crystal lattice energy, which resulted in a reduced number of strong hydrogen bonds. We evaluated the effect of the hydrogen bond donor and ac-

ceptor on the discrepancy between kinetic solubility and thermodynamic solubility. The discrepancy was much larger when the sum of hydrogen bond donor and acceptor count was greater than 8 (Fig. 8c).

Influence of Thermodynamic Solubility on Oral Absorption

The effect of thermodynamic solubility on rat oral absorption was investigated. These results are shown in Figs. 9a and b. Thermodynamic solubility in JP2 was lower, resulting in poorer absorption. Despite poor thermodynamic solubility in JP2, the compounds with good solubility in JP2-containing GCDC showed sufficient bioavailability. This is due to the bile acid in the gastrointestinal tract, which plays a key role in the solubilization of poorly soluble compounds and enhances oral absorption.⁴²⁾ Thus, it is important to evaluate the aqueous solubility contained in the bile acid for poorly soluble compounds because there is the risk that their absorption is strongly affected by the effect of food. In addition, these results demonstrated that JP2-containing 20mM GCDC was suitable to simulate fluid from fed-state intestine fluid. Compared to thermodynamic solubility in JP2, thermodynamic solubility in JP2-containing 20mM GCDC have a better relationship with bioavailability because a few bile acids were secreted in rats during the fasting state.

We further investigated the minimum thermodynamic solubility sufficient for oral absorption. The minimum thermodynamic solubility in JP2 and JP2-containing GCDC required

Fig. 8. Discrepancy between the Kinetic Solubility and Thermodynamic Solubility in JP2 as a Function of Measured Log $D_{pH7.4}$ (a); R_{sp^3} (b); Hydrogen Bond Donor and Acceptor Count (c)

Fig. 9. Correlation between Rat Oral Bioavailability and Thermodynamic Solubility in JP2 (a); Thermodynamic Solubility in JP2-Containing GCDC (b); Kinetic Solubility in JP2 (c); Kinetic Solubility in JP2-Containing GCDC (d)

for acceptable bioavailability ($>30\%$) was more than $1\text{ }\mu\text{g/mL}$ and $10\text{ }\mu\text{g/mL}$, respectively. Compared to thermodynamic solubility, kinetic solubility in both the JP2 and JP2-containing GCDC exhibited a poorer correlation with oral bioavailability (Figs. 9c, d). Specifically, kinetic solubility in JP2-containing GCDC showed no correlation with oral bioavailability. The crystal lattice must be broken up and the separate molecules must be solvated in order to be absorbed since the drug suspension in 0.5% aqueous methylcellulose is administered to rats. Thus, thermodynamic solubility based on the process of crystal lattice break and the solvation of molecules had better correlation with oral absorption than kinetic solubility.

Solubility is an important factor that affects the absorption rate. The MAT is used as a parameter to measure the absorption rate, which is calculated by subtracting mean residence time after i.v. administration from mean residence time after oral administration. In this study, we evaluated the effect of the thermodynamic solubility on the MAT. A higher thermodynamic solubility in both the JP2 and JP2-containing GCDC resulted in a decrease in MAT (Figs. 10a, b). In contrast, MAT was independent of kinetic solubility (Figs. 10c, d).

These results indicated that the dissolution parameter measured *in vitro*, that is the thermodynamic solubility, was positively correlated with the oral absorption and MAT. In addition, the bioavailability determinations based on the kinetic solubility may be misleading in the lead optimization and candidate selection process. Overall, the thermodynamic solubility is a very useful parameter to predict the oral absorption

and provides valuable information for the solid dosage form.

Conclusion

In this study, we have described the medium throughput approach for physicochemical profiling using a small amount of drug substance, which contributes to the developability assessment of early drug discovery. Our investigation of the relationship between thermodynamic solubility and lipophilicity demonstrated that thermodynamic solubility may be classified into 3 groups according to their $\text{Log } D$; a very soluble group is defined as a $\text{Log } D$ value below 1, slightly soluble is within the $\text{Log } D$ range of 1 to 3.5 and poorly soluble is defined as a $\text{Log } D$ value above 3.5. In $\text{Log } D$ between 1 and 3.5, the thermodynamic solubility reflects the sum of the $\text{Log } D$, aromatic ring count and the hydrogen bond acceptor and donor count, suggesting that the π - π stacking and hydrogen bond may be the major interactions that influence crystal packing, which affects thermodynamic solubility. Furthermore, thermodynamic solubility is useful for the criteria of acceptable bioavailability evaluation. Thus, thermodynamic solubility with regard to its solid-state properties as measured by physicochemical profiling enables the rational lead optimization and efficient candidate selection in early drug discovery.

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Fig. 10. Correlation between MAT and Thermodynamic Solubility in JP2 (a); Thermodynamic Solubility in JP2-Containing GCDC (b); Kinetic Solubility in JP2 (c); Kinetic Solubility in JP2-Containing GCDC (d)

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