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Improvement in Aqueous Solubility in Small Molecule Drug Discovery Programs by Disruption of Molecular Planarity and Symmetry

Minoru Ishikawa* and Yuichi Hashimoto

Institute of Molecular and Cellular Biosciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-0032, Japan

1. INTRODUCTION

Aqueous solubility is essential for drug candidates. Poor aqueous solubility is likely to result in poor absorption, even if the permeation rate is high, since the flux of a drug across the intestinal membrane is proportional to the concentration gradient between the intestinal lumen and the blood. The Biopharmaceutical Classification System (BCS) was introduced in the mid-1990s to classify drug substances with respect to their aqueous solubility and membrane permeability.[1] Drug substrates, for which solubility enhancement can improve the oral bioavailability, are classified in class 2 (poorly soluble/permeable) and class 4 (poorly soluble/poorly permeable).[2] The FDA regulations concerning oral medications require more extensive investigation of compounds with low solubility, and low solubility may have an even greater impact in the case of iv dosage forms. In addition, risk assessment of poorly soluble compounds is challenging because exposure may be difficult to define and test sensitivity may be reduced. Further, high concentrations of poorly soluble drugs in organisms may result in crystallization and acute toxicity, as in the case of uric acid and gout. Overall, poor solubility of drug candidates has been identified as the cause of numerous drug development failures.[3]

According to the simplest definition, the thermodynamic solubility of a compound in a solvent is the maximum amount of the most stable crystalline form of the compound that can remain in solution under equilibrium conditions.[4] The most stable form is invariably the form that has the highest melting point. The importance of thermodynamic assessment is greater in late discovery/early development, when it is useful to confirm earlier kinetic solubility results, to rule out potential artifacts, and to generate high-quality solubility data. The kinetic solubility of a molecule depends on its crystal form, and crystal polymorphs of a molecule can show different kinetic solubility. Therefore, crystal modification can produce an increase in dissolution rate and a temporary or apparent increase of solubility. However, it cannot produce a permanent alteration of solubility. Given sufficient time, the undissolved solute will revert to its most stable crystal form, and the solubility will approach the true thermodynamic solubility.[5,6] Therefore, the role of crystal modification is confined to increasing the dissolution rate of drugs.

Appropriate formulation can help in addressing these problems,[7] but the extent of absorption and solubility enhancement that can realistically be achieved is severely limited. Stability and manufacturing problems also have to be taken into account, since it is likely that an insoluble drug candidate may not be formulated as a conventional tablet or capsule and will require a less conventional approach such as, for example, a soft gel capsule.[8] Thus, it would be better to generate drug candidates with sufficient aqueous solubility at the drug discovery stage. In other words, it is much better to improve solubility by chemical means, i.e., by modification of the molecule itself. But on the other hand, application of combinatorial chemistry and high-throughput screening (HTS) systems has tended to change the profile of compound libraries in the direction of greater hydrophobicity and higher molecular weight, and this in turn has resulted in a profile of lower solubility.[9] This is a problem for medicinal chemists, and improvement of the aqueous solubility of bioactive molecules is a major and common issue in medicinal chemistry.

In general, the aqueous solubility of small molecules depends on their hydrophobicity ($\log P$).[10,11] The partition coefficient, $\log P$, is defined as follows: $\log P = \log[(\text{solute in } n\text{-octanol})/(\text{solute in water})]$. Increase of aqueous solubility leads to an increase of the denominator of the above equation and a decrease of $\log P$. Thus, decrease of $\log P$ by chemical modification, i.e., introduction of hydrophilic group(s) into molecules, is a classical and general strategy for improving aqueous solubility. But this approach is not universally effective because the introduced hydrophilic group(s) sometimes interferes with the target protein−drug interaction. In addition, this strategy is not effective when both solubility and hydrophobicity need to be increased, for example, to improve the oral bioavailability of highly hydrophilic compounds with insufficient solubility. Furthermore, compounds with poor solubility in both octanol and water sometimes retain poor absolute values of aqueous solubility despite a decrease of $\log P$ values, because $\log P$ values are just ratios. Therefore, a novel and general strategy to increase the aqueous solubility of drug candidates would have a great impact on drug discovery and medicinal chemistry. Here, we review an alternative strategy for improving aqueous solubility by means of disruption of molecular planarity and symmetry.

2. RATIONALE FOR A STRATEGY OF IMPROVING AQUEOUS SOLUBILITY BY DISRUPTION OF MOLECULAR PLANARITY AND SYMMETRY

In 1980, Yalkowsky presented general solubility equations (GSEs)[12] derived on the basis of semiempirical analysis. GSE includes not only $\log P$ but also melting point: for example, $\log[\text{solubility (M)}] = 0.5 - (\log P) - 0.01\{[\text{melting point (°C)}] - 25\}$.[13] The melting point itself is related to crystal lattice and crystal packing energies.[8] In other words, the solubility of a solid

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solute in water is dependent on two factors: the crystallinity of the solute and the ability of the solute to interact with water.[13] The underlying theory has been well established.[6,12−15] Therefore, disruption of crystal packing would be an alternative method for improving aqueous solubility. However, few chemical modifications focused on crystal packing have been reported. This might be because we do not have a sufficient understanding of the effects of chemical modification on crystal packing or melting point. Indeed, Gavezzotti noted that *the melting point is one of the most difficult crystal properties to predict*,[16] and Lipinski mentioned that *the prediction of crystal packing energies is at present extremely difficult*.[8] Therefore, a concrete and general strategy to decrease melting point and crystal packing would be extremely attractive to medicinal chemists.

Molecular planarity and symmetry are known to influence crystal packing, and disruption of molecular planarity would be expected to decrease the efficiency of crystal packing and the melting point. Indeed, the influence of saturation on melting point has been realized for years, due to the impact of hydrogenation on the melting point of unsaturated oils.[17] Very recently, Lovering analyzed the drug and clinical candidate database and reported that an increase in the fraction of sp^3-hybridized carbons is associated with a decrease in melting point.[18] Thus, the relationship between molecular planarity and melting point could provide the basis for a strategy to increase solubility. An example of the relationship between solubility and molecular planarity is provided by the case of polychlorinated biphenyls (PCBs). In the 1970s, the effects of PCBs on the environment were widely discussed, and the aqueous solubility of PCBs, including the effect of reduced chlorine substitution, was examined in order to understand the modes of transportation and attenuation of PCBs in the environment. It was found that ortho-substituted biphenyls, which would possess larger dihedral angles, showed higher solubility.[19,20] For example, 2,2′-dichlorobiphenyl (900 μg/mL) possessed greater aqueous solubility than 4-chlorobiphenyl (400 μg/mL) or 2,4′-dichlorobiphenyl (637 μg/mL). In other words, the substitution pattern of PCBs affects the solubility. As for molecular symmetry, Gavezzotti reported that ortho- and meta-disubstituted benzenes melt at lower temperature than the para-isomers in 1995.[21] He noted that *a very old rule of thumb says that symmetrical molecules pack in a three-dimensional periodic lattice more easily than less symmetrical ones and hence form more stable, higher-melting and less soluble crystals*. In 1996, Yalkowsky also reported a statistical study showing that the entropy of melting of organic compounds is related to molecular symmetry number.[22]

The above findings[12−22] were largely based upon examination of rigid, polycyclic and halogenated aromatic compounds and may not seem directly applicable to complex pharmaceutical compounds. In fact, there have been few reports of chemical modifications of pharmaceutical compounds focused on molecular planarity/symmetry, and most of the examples employed a combination of decrease of hydrophobicity and disruption of planarity, without exhaustive analysis of the mechanism of the solubility improvement. Further, reviews relating to solubility have been limited to prediction of solubility by means of theoretical and statistical studies.[6,8,9,23−25] In our work, we have focused on modification of bicyclic lead molecules in ways that would disrupt molecular planarity by increasing the dihedral angle or that would disrupt the molecular symmetry. The reasons why we have focused on the dihedral angle of bicyclic molecules

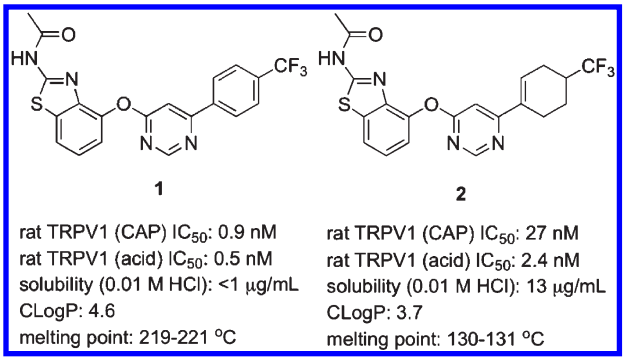


Figure 1. Improvement of thermodynamic aqueous solubility by removing aromaticity. CLogP values were calculated by us with ChemDraw Ultra, version 10.0. Melting points are from the literature.[26,27]

are the following: (i) little is known about the effect of increased dihedral angle on solubility, and (ii) dihedral angle is a convenient numerical parameter that can be obtained by calculation or X-ray crystal analysis among parameters of molecular planarity. To test the effectiveness of our strategy, we selected lead compounds for which attempts to decrease the hydrophobicity had resulted in loss or decrease of biological activity. To clarify the contributions of the different mechanisms, we carried out chemical modifications aimed at both increasing hydrophobicity and disrupting molecular planarity/symmetry and examined in detail the changes of physicochemical properties, including CLogP (calculated log *P*), retention time on reversed-phase HPLC, calculated dihedral angle, X-ray crystal structure, and melting point. In addition, we searched medicinal-chemistry-related journals for recent examples of improvement of aqueous solubility by disruption of molecular planarity/symmetry and found several examples, including substitution at the benzylic position and twisting of fused rings, which were confirmed by evaluation of physicochemical parameters, such as X-ray structure analysis and melting points. In the next section, we review this work according to the types of chemical modification that have been employed.

3. EXAMPLES OF IMPROVEMENT OF AQUEOUS SOLUBILITY BY DISRUPTION OF MOLECULAR PLANARITY AND SYMMETRY

3.1. Removal of Aromaticity. *Vanilloid Receptor 1 (TRPV1) Antagonists.* Wang et al. at Amgen Inc. have developed vanilloid receptor 1 (TRPV1) antagonists (Figure 1).[26] The clinical candidate AMG 517 (**1**) was found to have low thermodynamic aqueous solubility (<1 μg/mL in PBS or 0.01 M HCl), so they aimed to develop a second-generation clinical candidate with increased aqueous solubility and a shorter half-life. Their strategy was to introduce saturation into the 4-(trifluoromethyl)phenyl ring to reduce structural planarity and disrupt crystal-stacking capability. The partially saturated analogue **2** showed the anticipated improvement in thermodynamic solubility (13 μg/mL in 0.01 M HCl), being at least 13-fold more soluble than **1**, although this was accompanied with an approximately 30-fold decrease of potency in capsaicin-mediated assay and a 4-fold decrease in the acid-mediated assay. We estimated the CLogP values of compounds **1** and **2** to clarify the actual mechanism of the improvement of aqueous solubility. Compound **2** possessed a smaller CLogP value than **1**, suggesting that the solubility improvement might be due to both decrease of hydrophobicity and disruption

of planarity. The fact that **1** has a higher melting point than **2**[26,27] also supports the view that disruption of planarity contributes at least in part to the improved solubility of **2**. There are other reports suggesting that removal of aromaticity can improve aqueous solubility, although the mechanism involved was not established.[28,29]

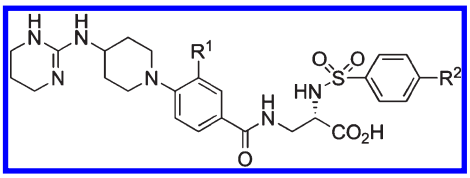
GVK BIO Database. Lovering et al. at Wyeth Research analyzed the GVK BIO database of compounds in various stages of drug developments (discovery, phases 1, 2, and 3, and commercial drugs).[18] They analyzed the relationship between the fraction of sp^3-hybridized carbons (Fsp^3), where Fsp^3 = (number of sp^3 hybridized carbons)/(total carbon count), and stage of development and found that Fsp^3 is correlated with success in compound transition from discovery through clinical testing to commercial drugs. In an attempt to explain these findings, they next retrieved solubility and melting point values from another database of more than 1000 compounds. Interestingly, Fsp^3 was found to positively correlate with solubility and negatively with melting point. This statistical study regarding the correlation between molecular planarity and solubility/melting point is very interesting, although they did not individually compare similar structures, and the investigated database contained few complex pharmaceutical compounds.

3.2. Increase of Dihedral Angle/Disruption of Molecular Symmetry. *Integrin $\alpha_v\beta_3/\alpha_{IIb}\beta_3$ Dual Antagonists.* We investigated non-peptide integrin $\alpha_v\beta_3/\alpha_{IIb}\beta_3$ dual antagonists.[30−34] To improve the treatment of acute ischemic diseases, a drug that possesses both suppressive activity against reperfusion injury and antithrombotic activity would be desirable. Leukocytes play a key role in reperfusion injury.[35−37] The Fab fragment of the human-murine monoclonal antibody abciximab,[38] which binds to the $\alpha_v\beta_3$ receptor and $\alpha_{IIb}\beta_3$ receptor, is already used to treat ischemic diseases. Therefore, dual antagonism of integrin $\alpha_v\beta_3$ (participating in the adhesion and migration of leukocytes) and integrin $\alpha_{IIb}\beta_3$ (participating in platelet aggregation) is likely to be of therapeutic value in the treatment of acute ischemic diseases. Both receptors $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ bind their ligand proteins, including vitronectin and fibrinogen, through recognition of the tripeptide RGD sequence.[39,40] In addition, the conformation of RGD-containing cyclic peptides has been reported to influence the $\alpha_v\beta_3/\alpha_{IIb}\beta_3$ selectivity.[41−43] On the basis of these reports, we generated a peptide-mimetic $\alpha_v\beta_3/\alpha_{IIb}\beta_3$ dual antagonist **3**.[30] The next issue was improvement of the aqueous solubility, which was very poor (<0.1 mg/mL).

The first attempt to improve solubility, that is, introduction of hydrophilic substituents, decreased the inhibitory activity in $\alpha_v\beta_3$-mediated cell adhesion assay using human vascular smooth muscle cells (VSMC) and human platelet rich plasma (hPRP) aggregation inhibition assay, as shown in Table 1. Specifically, introduction of a hydroxyl group at the 3-position of the central phenyl ring (**4**) decreased the activity in VSMC assay. Introduction of a carboxyl group at the benzenesulfonyl group (**5**) and introduction of two hydroxyl groups (**6**) led to decreased activities in both VSMC and hPRP assays. These results suggest that introduction of hydrophilic substituents is unsuitable in this case. In contrast, the second approach, that is, introduction of hydrophobic substituents (**8**−**10**), led to increased activity in receptor binding assays and VSMC and hPRP assays. Furthermore, we found that **8** and **10** showed increased aqueous solubility.[31,32]

We next analyzed the mechanism of the improved aqueous solubility of **8** and **10** (Table 2). The hydrophobicity parameters

Table 1. Structure−Activity Relationships of Our Integrin $\alpha_v\beta_3/\alpha_{IIb}\beta_3$ Dual Antagonists



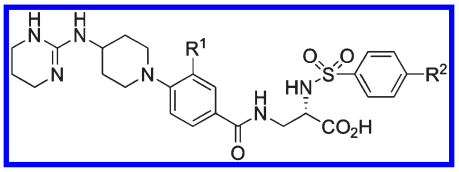
			IC$_{50}$ (nM)				
compd	R^1	R^2	$\alpha_v\beta_3$	$\alpha_{IIb}\beta_3$	VSMC[a]	hPRP[b]	CLogP[c]
3	H	H	1.3	3.0	190	290	1.5
4	OH	H	0.44	0.98	530	170	1.1
5	F	CO_2H	0.77	1.2	660	930	1.2
6	OH	OH	0.30	0.94	390	510	0.68
7	F	OH	0.14	0.18	53	230	1.2
8	F	H	0.36	0.21	48	37	1.6
9	Cl	H	0.17	0.023	72	90	2.0
10	OMe	H	0.19	0.44	110	130	1.3

[a] VSMC: $\alpha_v\beta_3$-mediated cell adhesion assay using human vascular smooth muscle cells (VSMC) and human vitronectin. [b] hPRP: human platelet aggregation inhibition assay. [c] CLogP values were estimated with ChemDraw Ultra, version 10.0.

(CLogP and retention time on reversed-phase HPLC[44]) indicate that **8** is more hydrophobic than **3**. In the case of the methoxy analogue **10**, there was an apparent discrepancy because the CLogP value was lower than that of **3**, whereas the retention time was larger than that of **3**. In addition, no clear relationship between aqueous solubility and hydrophobicity was observed among these compounds. On the other hand, there was a relationship between aqueous solubility and melting point: the rank order of aqueous solubility (**10** > **8** > **3**) corresponded to the order of melting points (**10** < **8** < **3**). Furthermore, the X-ray structure of **8** revealed a substantially increased dihedral angle between the piperidine ring and benzoyl group.[31] Therefore, we speculate that the increase of aqueous solubility of **8** (and **10**) was caused by disruption of molecular planarity without a decrease of hydrophobicity, resulting in a decrease of crystal packing energy.[45] On the other hand, compound **7** showed insufficient solubility despite its lower hydrophobicity compared with **8**. Crystal formation was observed during the evaluation of aqueous solubility, suggesting that the low aqueous solubility of **7** might be caused by new hydrogen bond formation, which might lead to tighter crystal packing. In this case, therefore, disruption of molecular planarity (**8** and **10**) improves the solubility more than a decrease of hydrophobicity (**7**).

As seen above, we showed that introduction of a fluorine atom (**8**) or a methoxy group (**10**) at the 3-position of the central aromatic ring of **3** improved aqueous solubility. The increase of aqueous solubility might be caused by disruption of molecular planarity as mentioned above, but another possible explanation would be disruption of molecular symmetry. Therefore, we designed a compound in which the molecular symmetry of **3** was disrupted. As shown in Table 3, modification of the tetrahydropyrimidylamino group (**11**) resulted in at least 35-fold increase of aqueous solubility compared with that of the lead

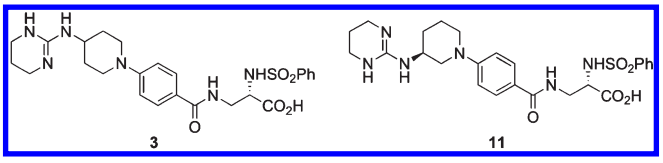
Table 2. Improvement of Aqueous Solubility of Our Integrin $\alpha_v\beta_3/\alpha_{IIb}\beta_3$ Dual Antagonists by Increasing the Dihedral Angle in the Bicyclic Structures



compd	R^1	R^2	aqueous solubility[a] (mg/mL)	CLogP[b]	HPLC retention time (min)[c]	melting point (°C)
3	H	H	<0.1	1.5	8.25	252−254
8	F	H	0.6	1.6	9.73	182−184
10	OMe	H	1.3	1.3	8.72	162−164
7	F	OH	0.1	1.2	6.16	193−197

[a] Solubility in water. [b] CLogP values were estimated with ChemDraw Ultra, version 10.0. [c] Inertsil ODS-2 reversed-phase column (4.6 mm × 250 mm).

Table 3. Improvement of Aqueous Solubility of Our Integrin $\alpha_v\beta_3/\alpha_{IIb}\beta_3$ Dual Antagonists by Disrupting Molecular Symmetry



compd	aqueous solubility[a] (mg/mL)	CLogP[b]	HPLC retention time (min)[c]	melting point (°C)
3	<0.1	1.5	8.25	252−254
11	3.5	1.8	12.2	181−184

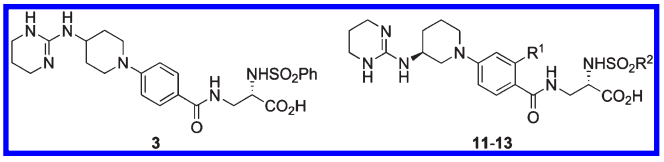
[a] Solubility in water. [b] CLogP values were estimated with ChemDraw Ultra, version 10.0. [c] Inertsil ODS-2 reversed-phase column (4.6 mm × 250 mm).

compound **3**.[33,34] Compound **11** is also more hydrophobic as judged from the CLogP values and retention times on reversed-phase HPLC. The melting point of **11** is lower than that of **3**. Thus, we speculate that the increase of aqueous solubility of **11** was not caused by decreased hydrophobicity but rather by disruption of molecular symmetry, resulting in a decrease of crystal packing energy.[45] Interestingly, **11** was more soluble and potent than **3** in VSMC and hPRP assays, apparently as a result of disruption of molecular symmetry without any increase of molecular weight.

Further chemical modification of **11** yielded potent dual antagonists **12** and **13** (Table 4). This class of antagonists commonly possessed excellent aqueous solubility. Thus, disruption of molecular symmetry is a promising strategy for improving aqueous solubility.

In an attempt to explain the mechanism of the improved aqueous solubility, the physicochemical parameters and log(solubility) values in Tables 2 and 3 were plotted. As shown in Figure 2, melting point was negatively correlated to log(solubility) (R^2 = 0.74), while both CLogP and retention time were weakly but *positively* correlated (R^2 = 0.28 and 0.51, respectively). Among a limited number of these analogues, therefore, log(solubility) was more highly correlated with melting point than with hydrophobicity parameters, and the increase of aqueous solubility was not caused by a decrease of hydrophobicity.

Table 4. Structure−Activity Relationship of Integrin $\alpha_v\beta_3/\alpha_{IIb}\beta_3$ Dual Antagonists



			IC$_{50}$ (nM)				
compd	R^1	R^2	$\alpha_v\beta_3$	$\alpha_{IIb}\beta_3$	VSMC[a]	hPRP[b]	aqueous solubility[c] (mg/mL)
3			1.3	3.0	190	290	<0.1
11	H	Ph	0.48	0.56	31	83	3.5
12	F	Ph	0.23	0.78	16	290	2.8
13	H	2-thienyl	0.25	0.40	52	99	3.3

[a] VSMC: $\alpha_v\beta_3$-mediated cell adhesion assay using human vascular smooth muscle cell (VSMC) and human vitronectin. [b] hPRP: human platelet aggregation inhibition assay. [c] Solubility in water.

These structural developments of the lead compound **3** provided drug candidates with improved activity and aqueous solubility. Scalable synthesis of the selected candidate was also

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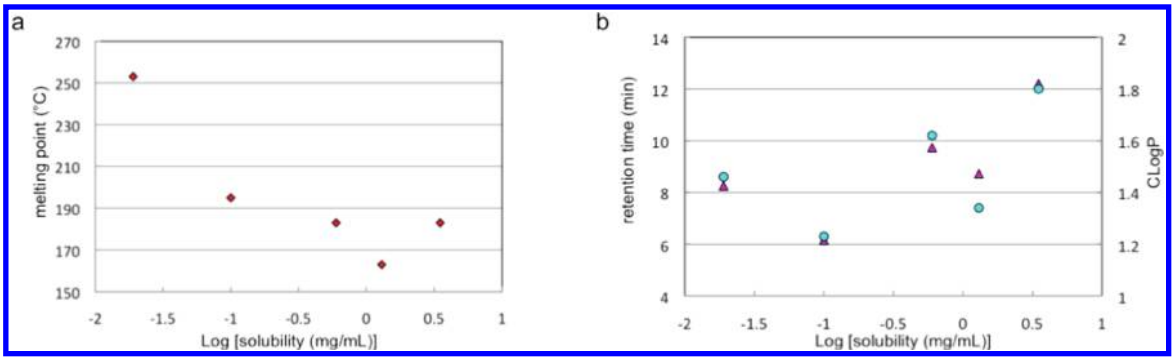


Figure 2. Relationships between solubility and physicochemical data of integrin dual antagonists: (a) melting point (red rhomboid); (b) CLogP (cyan circle) and retention time (magenta triangle).

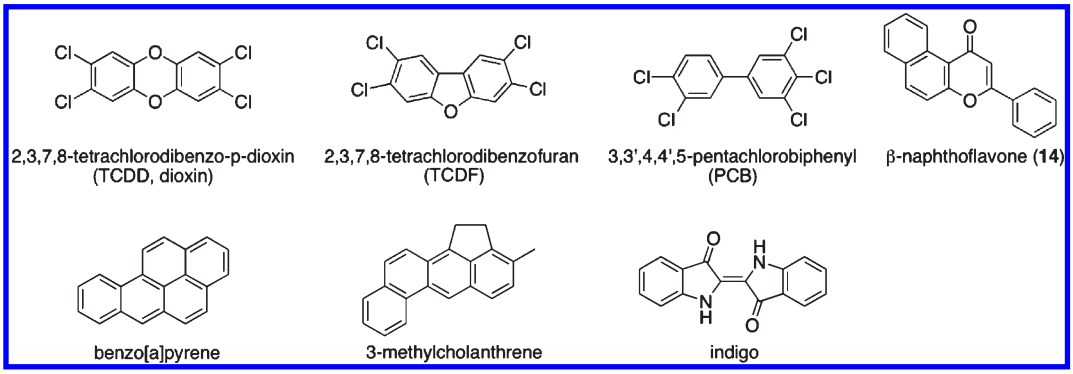


Figure 3. Chemical structures of AhR agonists.

developed.[46] The $\alpha_v\beta_3/\alpha_{IIb}\beta_3$ dual antagonists were reported to have significant inhibitory effects in several ischemia/reperfusion models compared to a selective $\alpha_v\beta_3$ antagonist or a selective $\alpha_{IIb}\beta_3$ antagonist.[47,48]

Aryl Hydrocarbon Receptor (AhR) Agonists. We next investigated improvement of the aqueous solubility of aryl hydrocarbon receptor (AhR) agonists.[49] AhR is a ligand-dependent transcription factor that is known to mediate the toxicity of dioxin.[50] Ligand binding to cytosolic AhR is considered to be the initial event leading to the manifestation of biological and toxicological responses, such as hepatotoxicity, immunotoxicity, tumor promotion, and induction of drug-metabolizing enzymes, including cytochrome P450 1A1 (CYP1A1), CYP1A2, aldehyde dehydrogenase-3, glutathione *S*-transferase, and xanthine dehydrogenase.[51] AhR expression is ubiquitous in vertebrate cells, but the physiological role of AhR is not yet fully understood. Although AhR is the best known for mediating dioxin toxicity, knockout studies have indicated that AhR also plays a role in normal physiology, including certain immune responses.[52]

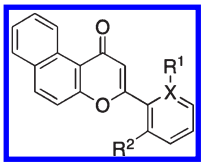
Various polycyclic aromatic hydrocarbons have been identified as AhR ligands (Figure 3). Although these AhR ligands appear to have diverse structures, they possess some common features, i.e., similar size, planar structure, and hydrophobic character, which have been suggested to be crucial for high binding affinity with AhR.[52] However, these properties of known AhR ligands result in rather limited solubility (especially in aqueous solution), which is a great drawback to the use of these ligands as tools for investigating the physiological role of AhR.[53] Therefore, potent AhR ligands with improved solubility are needed.

β-Naphthoflavone **14** was reported to be a more potent AhR agonist than TCDD.[54] Further, its hydrophobicity is lower than that of other AhR agonists, making it a potentially more useful tool for AhR research. Therefore, we planned structural development studies of **14** to obtain AhR ligands with more potent activity and improved solubility. Because the structure of **14** includes a rotatable biaryl moiety, we aimed to decrease the planarity of the molecule and introduced substituent(s) (**15**−**19**) on the phenyl group of **14**. To evaluate the effectiveness of the strategy of improving the solubility by disruption of planarity, the 2-pyridyl analogue **20** was also designed as a representative of the strategy of decreasing the hydrophobicity.

To evaluate the AhR-agonistic activity of the prepared compounds, CYP1A1-dependent 7-ethoxyresorufin *O*-deethylase (EROD) activity[55] in MCF-7 breast cancer cells was measured. Compound **14** exhibited potent EROD-inducing activity with an EC_{50} value of 1.4 μM under the assay conditions used (Table 5). Both the monofluoro analogue **17** and difluoro analogue **18** showed at least 4 times stronger EROD-inducing activity than **14**. On the other hand, the EC_{50} values of the monomethyl analogue **15** and dimethyl analogue **16** were higher than 10 μM. Introduction of a methoxy group (**19**) and substitution of the pyridine ring (**20**) led to increased EROD-inducing activity, with EC_{50} values of 0.27 and 0.45 μM, respectively. The difluoro analogue **18** had the strongest EROD-inducing activity, with an EC_{50} value of 0.20 μM, which is 7 times more potent than that of **14**, among the compounds prepared.

The thermodynamic aqueous solubility of **14**−**20** was evaluated. The aqueous solubility of **14** in phosphate buffer (pH 7.4) was quite low (<0.15 μg/mL). So a mixture of an equal volume of phosphate buffer (pH 7.4) and EtOH was used as an aqueous medium for the evaluation of thermodynamic solubility. Even under this condition, the solubility of **14** was still poor (84.6 μg/mL)

Table 5. Improvement of Thermodynamic Aqueous Solubility of our AhR Agonists by Increasing the Dihedral Angle of the Bicyclic Structures



compd	R^1	R^2	X	EROD EC_{50} (μM)	solubility[a] (μg/mL)	melting point (°C)	calcd dihedral angle[b] (deg)	CLogP[c]	HPLC retention time (min)[d]
14	H	H	C	1.4	84.6	165−167	17.8	4.7	7.70
15	H	Me	C	>10	262	135−137	37.9	4.9	8.67
16	Me	Me	C	>10	1270	92	70.0	5.1	9.68
17	F	H	C	0.33	153	157	9.1	4.8	7.85
18	F	F	C	0.20	248	150	40.5	4.9	7.78
19	OMe	H	C	0.27	45.8	192−193	18.5	4.1	9.29
20		H	N	0.45	299	187−188	0.0	3.4	5.09

[a] Solubility in an equal volume of EtOH and 1/15 M phosphate buffer (pH 7.4). [b] Calculated dihedral angle was estimated with Gaussian 03.[56] [c] CLogP values were estimated with ChemDraw Ultra, version 10.0. [d] Waters μBondapak reversed-phase column (3.9 mm × 150 mm).

(Table 5). Ortho-substituted **15**−**18** showed better solubility than **14**, as expected. Indeed, dimethyl analogue **16** was 15 times more soluble (1270 μg/mL) than **14**. Difluoro analogue **18** showed 3 times greater solubility (248 μg/mL) than **14**. On the other hand, methoxy analogue **19** was less soluble than **14**. Pyridine analogue **20** showed the second highest solubility (299 μg/mL) in this series.

To clarify the reason for the better solubility of ortho-substituted naphthoflavones, physicochemical parameters, including CLogP, retention time on reversed-phase HPLC, melting point, and calculated dihedral angle, were determined. The dihedral angles of **14**−**20** for the optimized structures were obtained by means of density functional theory (DFT) calculations (B3LYP/6-31G*).[56] Compounds **15**, **16**, and **18** possess increased hydrophobicity, larger dihedral angle, lower melting point, and improved aqueous solubility compared with **14**. These results suggest that introduction of substituents into **14** disrupted the planarity by increasing the dihedral angle, leading in turn to decreased crystal packing energy and lower melting point and so increasing the solubility. In contrast, pyridine analogue **20**, which lacks a hydrogen atom, showed a higher melting point and decreased dihedral angle compared with **14**, though **20** showed better aqueous solubility because of its reduced hydrophobicity. Thus, we believe that this alternative strategy to improve solubility by focusing on dihedral angle is quite distinct from the general/classical strategy based on decreasing the hydrophobicity of molecules. It is noteworthy that disruption of molecular planarity (**16**) afforded greater solubility improvement than decrease of hydrophobicity (**20**) in this case. On the other hand, **17** showed higher hydrophobicity, lower melting point, and improved aqueous solubility but a decreased calculated dihedral angle compared with **14**. A possible explanation of the small calculated dihedral angle of **17** would be interaction between the fluorine lone pair and hydrogen at the 2-position. Compared to the optimized structure of **17**, the second lowest energy conformation of **17** (~1.5 kcal/mol less stable) possessed a larger dihedral angle (38.4°). The reason why this rotamer showed a torsional structure is probably the lack of any significant interaction of the lone pair on fluorine. Methoxy analogue **19** showed a higher melting point and almost the same calculated dihedral angle compared with **14**. The reason for the relatively small dihedral angle may be similar to that in the case of **17**, that is, interaction between the oxygen lone pair and hydrogen at the 2-position. Lack of molecular symmetry of **17** might lead to a lower melting point and greater solubility, or the changes of electron density arising from the introduction of fluorine might have resulted in increased solubility.

Next, the physicochemical parameters and log(solubility) were plotted. As shown in Figure 4, melting point and dihedral angle (R^2 = 0.66 and 0.44, respectively) were correlated to solubility, while CLogP and retention time were not (R^2 = 0.10 and 0.00, respectively). The weaker correlation of dihedral angles would be mainly due to the inclusion of the pyridine analogue **20** (small dihedral angle and relatively high solubility). In fact, log(solubility) was rather highly correlated to both dihedral angle and melting point (R^2 = 0.86 and 0.96, respectively) when **19** and **20** were excluded.

The dimethyl analogue **16** was the most soluble, being 15 times more soluble than **14**. The difluoro analogue **18** had the best overall profile, being 7 times more potent in EROD assay and 3 times more soluble than **14**, and it is expected to be a useful chemical tool for investigating the physiological role of AhR.

Peroxisome Proliferator-Activated Receptor (PPAR) δ Partial Agonists. Our third target was peroxisome proliferator-activated receptor (PPAR) δ partial agonists.[57] PPARs are ligand-activated transcription factors belonging to the nuclear receptor superfamily.[58] In the presence of a ligand, PPARs heterodimerize with retinoid X receptor (RXR), and the heterodimers modulate transcription of target genes by binding to PPAR response elements in the promoter region. Three different PPAR genes (α, β/δ referred to as δ, and γ) have been identified so far. Each PPAR isotype displays a distinct pattern of tissue distribution and a distinct pharmacological profile, suggesting that each has unique functions in different cell types.[59] PPARδ is involved in fatty acid metabolism, insulin resistance, reverse cholesterol transport, and other biological pathways.[60−62] Recently, a PPARδ partial agonist showed full efficacy on free fatty acid oxidation in vitro and corrected the plasma lipid parameters and

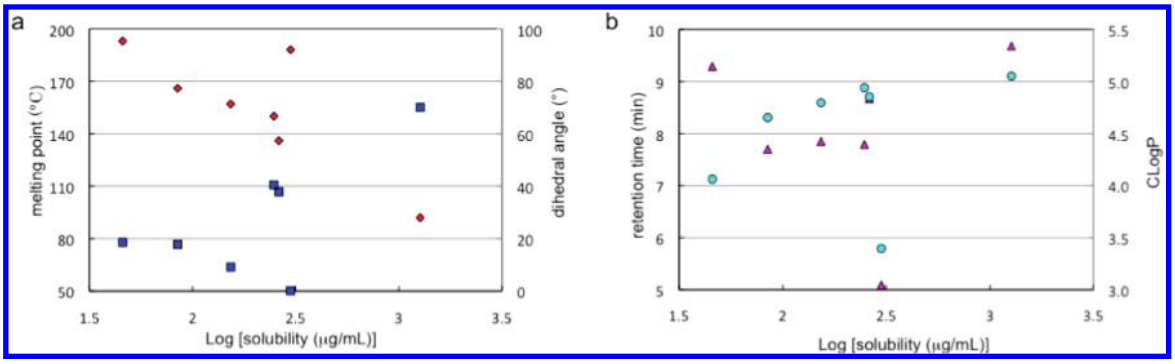
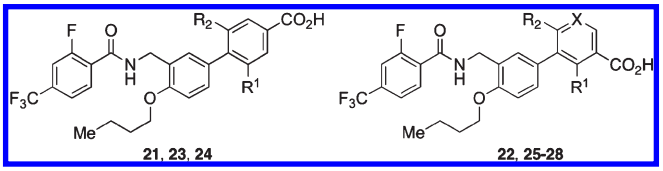


Figure 4. Relationships between solubility and physicochemical data of **14**−**20**: (a) melting point (red rhomboid) and dihedral angle (blue square); (b) CLogP (cyan circle) and retention time (magenta triangle).

Table 6. Structure−Activity Relationships of Our PPARδ Partial Agonists



				PPARδ		PPARα		PPARγ	
compd	R^1	R^2	X	EC_{50}[a] (nM)	% efficacy[a]	EC_{50}[a] (nM)	% efficacy[a]	EC_{50}[a] (nM)	% efficacy[a]
21	H	H		170	8	NA[b]		NA[b]	
23	Me	H		11	9	NA[b]		NA[b]	
24	F	H		53	11	NA[b]		NA[b]	
22	H	H	CH	29	48	790	100	NA[b]	
25	Me	H	CH	1.6	40	130	100	NA[b]	
26	F	F	CH	5.7	68	NA[b]		NA[b]	
27	H	H	N	220	62	4000		NA[b]	
28	Me	Me	N	76	55	4200	100	NA[b]	

[a] EC_{50} values and % efficacy are given relative to the positive controls, GW501516 for PPARδ, fenofibric acid for PPARα, and ciglitazone for PPARγ. [b] NA means not active at 1 μM as an agonist.

insulin sensitivity in vivo.[63] On the basis of the X-ray crystal structure of PPARδ, biphenylcarboxylic acids **21** and **22** were designed and synthesized as PPARδ-selective partial agonists/antagonists.[64] Compound **21** shows antagonistic activity with EC_{50} of 170 nM and 8% maximum efficacy, whereas **22** shows partial agonistic activity with EC_{50} of 29 nM and 48% maximum efficacy. The molecular mechanism of PPARδ partial agonism remains unclear. We thought that it might be clarified by comparing the X-ray crystal structures of PPARδ−partial agonist complexes with those with PPARδ−full agonist and PPARδ−antagonist complexes. Unfortunately, the reported PPARδ partial agonists, biphenylcarboxylic acids **21** and **22**, showed insufficient aqueous solubility to allow the preparation of suitable crystals.

As the first approach for increasing the aqueous solubility of **21** and **22**, we tried (i) introduction of an oxygen atom into the *n*-butyl group and (ii) replacement of the phenyl ring with hetero rings. Unfortunately, these approaches to decrease the hydrophobicity of the molecules led to decreased PPARδ partial agonistic activity. Then we examined an alternative approach to increase aqueous solubility, that is, disruption of molecular planarity (Table 6). Introduction of a methyl group (**23**) or fluorine atom (**24**) at the ortho-position of **21** led to improved PPARδ partial agonistic activity with similar efficacy. In particular, methyl analogue **23** showed 15 times stronger PPARδ partial agonistic activity than **21**. The effect of substitution with a *m*-carboxyl group (**22**, **25**−**28**) was similar to that with a *p*-carboxyl group (**21**, **23**, and **24**). Methyl analogue **25** and difluoro analogue **26** showed 18 and 5 times stronger activity than **22**, respectively. These results indicate that hydrophobic biaryl structures are preferable for PPARδ partial agonistic activity compared with hydrophilic biaryl structures. Pyridyl analogue **27** showed increased aqueous solubility (vide infra), although the PPARδ agonistic activity was weak. We hypothesized that introduction of methyl group(s) into the biaryl moiety in the pyridyl analogue **27** would improve both the activity and aqueous solubility. Indeed, as expected, dimethylpyridyl analogue **28** showed 3 times stronger activity than **27** and comparable activity to **22**.

The thermodynamic aqueous solubility of the potent partial agonists **21**−**28** was evaluated. The aqueous solubility of **21** and **22** in 1/15 M phosphate buffer (pH 7.4) was quite low

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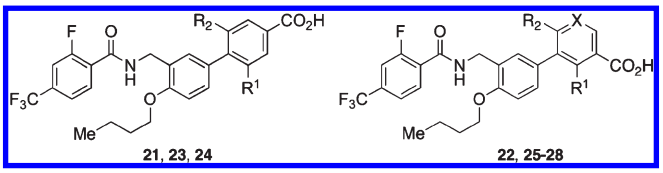
Table 7. Improvement of Thermodynamic Aqueous Solubility of Our PPARδ Partial Agonists by Increasing the Dihedral Angle of the Bicyclic Structures



				solubility (mg/mL)					
compd	R^1	R^2	X	50% EtOH[a]	phosphate buffer (pH 7.4)	melting point (°C)	calcd dihedral angle[b] (deg)	CLogP[c]	HPLC retention time (min)[d]
21	H	H		0.375	<0.001	259−262	43.5	7.1	7.98
23	Me	H		0.985	<0.001	241−243	52.5	7.3	9.46
24	F	H		3.22	<0.001	221−223	36.1	7.3	8.72
22	H	H	CH	1.35	<0.001	177−178	36.9	7.1	7.79
25	Me	H	CH	9.95	<0.001	146−149	57.5	7.0	8.65
26	F	F	CH	10.4	0.0217	177	46.2	7.1	7.42
27	H	H	N	9.03	0.00762	152	37.4	6.0	3.61
28	Me	Me	N	17.7	2.70	104−106	78.1	6.1	4.36

[a] Solubility in a mixture of equal volumes of EtOH and 1/15 M phosphate buffer (pH 7.4). [b] Calculated dihedral angles of simplified model **29** (Figure 5) were estimated by using Gaussian 03.[56] [c] CLogP values were estimated with ChemDraw Ultra, version 10.0. [d] Waters μBondapak reversed-phase column (3.9 mm × 150 mm).

(<0.001 mg/mL). So a mixture of equal volumes of 1/15 M phosphate buffer (pH 7.4) and EtOH was also used as an aqueous medium for the evaluation of thermodynamic solubility. All the compounds shown in Table 7 had higher solubility than the parent compounds. In the *p*-carboxyl series **21**, **23**, and **24**, introduction of a methyl group (**23**) or fluorine atom (**24**) into the biphenyl moiety resulted in better solubility than that of **21**, as expected. The fluoro analogue **24** was 9 times more soluble (3.22 mg/mL) than **21** in aqueous EtOH. Although compounds **23** and **24** were more soluble in aqueous EtOH than **21**, they were still essentially insoluble in phosphate buffer. In the case of the *m*-carboxyl series **22** and **25**−**28**, introduction of a methyl group (**25**) or two fluorine atoms (**26**) resulted in 7 times greater solubility than for **22** in aqueous EtOH. Furthermore, **26** showed moderate solubility in phosphate buffer (0.0217 mg/mL) for the first time among these PPAR ligands. Pyridyl analogue **27** showed better solubility in both 50% EtOH and phosphate buffer than the parent compound **22**. Surprisingly, disruption of molecular planarity (**26**) resulted in greater solubility in phosphate buffer than decrease of hydrophobicity (**27**) in this case, too. Dimethylpyridyl analogue **28** showed 2 times greater solubility than **27** in aqueous EtOH. It is noteworthy that **28** also has greatly improved solubility in phosphate buffer (2.70 mg/mL); that is, it is at least 2700 and 350 times more soluble than **22** and **27**, respectively.

Among the synthesized compounds, **23** and **24** were more potent and more soluble PPARδ-selective partial agonists/antagonists than **21**. In the *m*-carboxyl series, **25** and **26** were more potent PPARδ-selective partial agonists than **22**, with improved solubility. Two PPAR partial agonists, **26** and **28**, showed excellent overall profiles and appeared to be suitable for preparing crystals for X-ray crystallographic study. Difluoro analogue **26** was 5 times more potent and 8 times more soluble than **22** in aqueous EtOH and was soluble in phosphate buffer

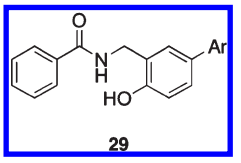


Figure 5. Simplified model of PPARδ modulators for calculation of dihedral angle.

(0.0217 mg/mL). Dimethylpyridyl analogue **28** had activity comparable to that of **22** and was highly soluble in phosphate buffer (2.70 mg/mL).

To confirm the mechanism of the improved solubility of the biaryl analogues, melting point, calculated dihedral angle, CLogP, and retention time on reversed-phase HPLC were evaluated (Table 7). Dihedral angles in optimized structures of a simplified model **29** (Figure 5) were obtained by means of DFT calculations. All the compounds with methyl group(s) introduced at the ortho-position of the biaryl moiety (**23**, **25**, and **28**) possessed increased hydrophobicity, larger dihedral angle, and lower melting point compared with the parent compounds **21**, **22**, and **27**, respectively. The most soluble analogue **28** showed the lowest melting point and the largest dihedral angle in this series. The only exception was the smaller CLogP of **25** compared with **22**, although the other hydrophobicity parameter, retention time, was larger than that of **22**. These results suggest that introduction of methyl group(s) into biaryl molecules results in disruption of the planarity, increasing the dihedral angle and leading in turn to decreased crystal packing energy and lower melting point, with a consequent increase of solubility.

When **28** was compared with **22**, two kinds of modifications (replacement of the phenyl group with a pyridine ring and introduction of methyl groups) had resulted in more than 2700 times higher solubility in phosphate buffer. We consider

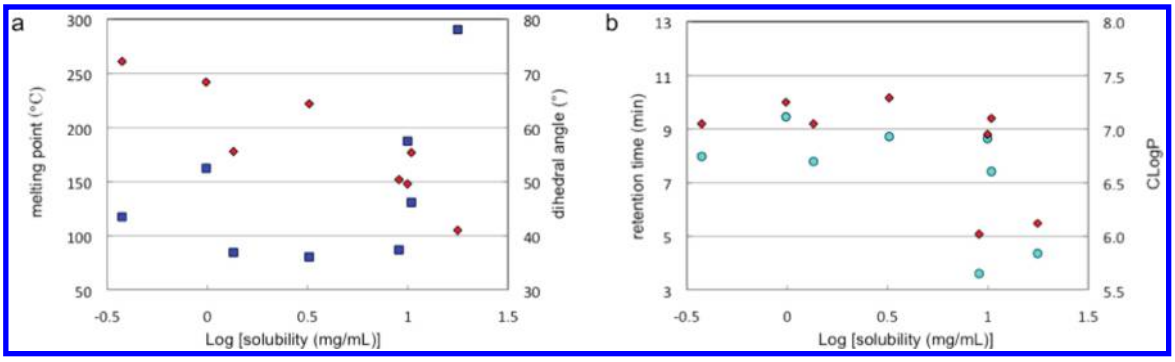


Figure 6. Relationships between solubility and physicochemical data of **21**−**28**: (a) melting point (red rhomboid) and dihedral angle (blue square); (b) CLogP (cyan circle) and retention time (magenta triangle).

that this improvement of solubility can be ascribed to two factors, i.e., decrease of hydrophobicity by introduction of the pyridine ring (**22** vs **27**) and disruption of molecular planarity by introduction of the methyl groups (**27** vs **28**). Thus, these results indicated that a combination of strategies for improving aqueous solubility is an effective approach.

In the case of compounds containing fluorine atom(s), the reason for the improvement of aqueous solubility is not clear. Compound **24** possessed a smaller calculated dihedral angle, lower melting point, and higher hydrophobicity than **21**. A possible explanation of the small dihedral angle would be interaction between the fluorine lone pair and hydrogen at the 2′-position. Lack of molecular symmetry of **24** might have led to a lower melting point and greater solubility, or the changes of electron density arising from the introduction of fluorine might have resulted in increased solubility. Compound **26** showed the same melting point as **22** but had a larger calculated dihedral angle and higher hydrophobicity. Fluorinated β-naphthoflavone **17** showed improved solubility, a relatively small calculated dihedral angle, and relatively high hydrophobicity (Table 5). Taken together, these results indicate that introduction of fluorine atom(s) might improve aqueous solubility not only by disrupting molecular planarity but also via other mechanism(s). A recent report noted that a larger difference between the highest occupied and lowest unoccupied molecular orbitals (HOMO and LUMO, respectively), or larger molecular polarizability, is associated with lower solubility.[65] Among the PPARδ partial agonists reported here, however, the aqueous solubility was not related to the difference between calculated HOMO and LUMO or calculated dipole moment.

We next analyzed the relationship between aqueous solubility and molecular symmetry, focusing on the position of the carboxyl group in our PPARδ partial agonists. When the position of the carboxyl group was changed from para to meta, the thermodynamic solubility increased (4-fold, **21** vs **22**; 10-fold, **23** vs **25**) (Table 7). The melting points of *m*-carboxylic acids **22** and **25** were lower than those of the corresponding para-derivatives **21** and **23**, respectively. These results suggest that disruption of molecular symmetry results in decreased crystal packing energy and lower melting point, leading to increased solubility. It is noteworthy again that more soluble compounds (**22** and **25**) were generated by disruption of molecular symmetry without any increase of molecular weight.

In an attempt to understand the mechanism of the improved aqueous solubility, the physicochemical parameters and log(solubility) in Table 7 were plotted. As shown in Figure 6, log(solubility) was more highly correlated with melting point (R^2 = 0.79) than were dihedral angle, CLogP, and retention time (R^2 = 0.50, 0.44, and 0.48, respectively). Overall, we analyzed the relationships between solubility and physicochemical parameters of three kinds of compounds, as shown in Figures 2, 4, and 6. In all cases, log(solubility) was more highly correlated with melting point than were dihedral angle, CLogP, and retention time. In other words, these analyses indicate that melting point can be considered as a parameter of aqueous solubility, at least in these three kinds of lead molecules modified in ways that would disrupt molecular planarity and symmetry.

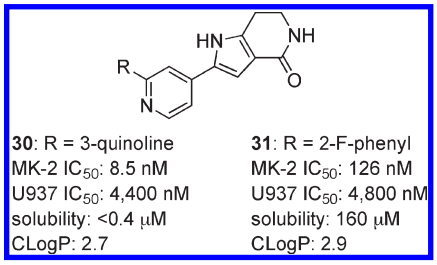


Figure 7. Improvement of aqueous solubility by ortho-substitution of bicyclic structure. CLogP values were estimated by us, using ChemDraw Ultra, version 10.0.

Inhibitors of Mitogen-Activated Protein Kinase-Activated Protein Kinase 2 (MK-2). Anderson and co-workers at Pfizer reported inhibitors of mitogen-activated protein kinase-activated protein kinase 2 (MK-2).[66] Many of the compounds bearing a pyrrolopyridine skeleton have low aqueous solubility. For example, quinoline analogue **30** has an aqueous solubility of <0.4 μM (Figure 7). Substitution of fluorophenyl group (**31**) resulted in 15 times weaker MK-2 inhibitory activity. However, 2-fluorophenyl analogue **31** has an aqueous solubility of 160 μM, at least 400-fold better than that of **30**. The authors suggested that the improved solubility of **31** might be related to a decrease in the planarity of the compound due to the slightly larger fluorine atom in the ortho position. We estimated the CLogP values of **30** and **31** and confirmed that the improvement of solubility is not due to decreased hydrophobicity. It would be interesting to determine the melting points and dihedral angles of compound **31** and the parent compound. Analogue **31** has moderate to high permeability, as judged by Caco-2 monolayer assay (A−B = 9.45×10^{-6} cm/s) and also shows reasonably low rat plasma protein binding (92%). Protein binding of **31** in the medium used in the cell assay, containing 10% fetal calf serum, was determined to be 31%. Compound **31** inhibited >80% of tumor

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necrosis factor α (TNFα) production when orally dosed 2 h prior to lipopolysaccharide (LPS) challenge in a rat acute model of inflammation. It was detected in the plasma at a high level, most likely due to reduced clearance or other improved pharmacokinetic properties. Thus, it shows many desirable properties as a candidate drug.

Matrix Metalloproteinase (MMP) 12 Inhibitors. Dublanchet et al. at Pfizer reported novel non-zinc chelating matrix metalloproteinase (MMP) 12 inhibitors.[67] They introduced a hydroxyl group into the biphenyl structure of MMP-12 inhibitor **32** (Figure 8). The affinity and thermodynamic aqueous solubility data show that compound **33** displays at least 130-fold better solubility than the nonsubstituted biphenyl compound **32** while retaining potency similar to that of compound **32**. Dihedral angle and melting point would be required to clarify the mechanism of solubility improvement because the lone pair of the 2-hydroxyl group and hydrogen at the 2′-position might form a hydrogen bond that would lead to a planar structure.

AstraZeneca Database. Leach et al. at AstraZeneca Pharmaceuticals analyzed the AstraZeneca database of thermodynamic aqueous solubility measurements, rat plasma protein binding measurements, and rat oral exposure measurements, searching for occurrences of pair fitting.[68] In this large-scale study, they focused on matched pairs resembling those in Figure 9. There were 13 examples for Y = F with a mean *Z*-score (the number of standard deviations away from the mean difference) of +0.54 (range = −0.44 to +1.45) and there were five examples for Y = Cl with a mean *Z*-score of +1.93 (range = +1.34 to +2.42), although concrete structures and their solubility were not reported. These results indicate that when a conformational effect causes compounds to prefer a less planar structure, solubility tends to increase more than expected (or to decrease less than expected). This statistical study is noteworthy, because ortho-substitution was found to affect aqueous solubility in 18 pairs of compounds. If measured dihedral angles, actual solubility data, and melting points or crystal structures of more molecules were available, it would be possible to investigate the relationship between dihedral angle and aqueous solubility in more detail.

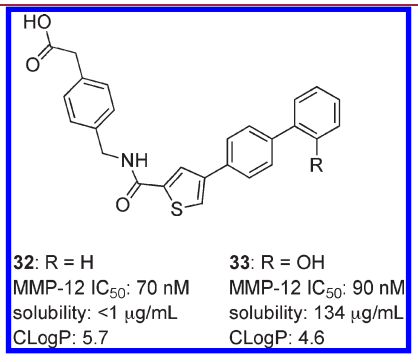


Figure 8. Improvement of thermodynamic aqueous solubility by ortho-substitution of a bicyclic structure. CLogP values were estimated by us, using ChemDraw Ultra, version 10.0.

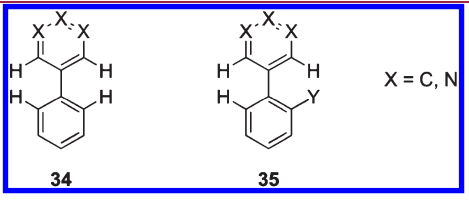


Figure 9. Matched pairs of compounds in the AstraZeneca database, used to examine the effect of substitution on thermodynamic aqueous solubility.

Increase of dihedral angle was also useful for benzamide, anilide, or phenylurea structures. They also reported that transformation from **36** to **37** by adding chlorine caused an increase in thermodynamic solubility by 1.5 log units, which is far from expectation (*Z*-score of 3.1) (Figure 10a).[68] This can be understood in terms of a conformational effect constraining the urea group to be orthogonal to the aromatic ring in **37**, whereas more planar conformations that may stack better in the solid state are permitted in the case of **36**. Furthermore, they studied the generality of the effect in a small number of pairs in which a substituent is added ortho to an anilide already bearing a substituent (F, Cl, Br, Me, OH, etc.) at the other ortho-position and for which data were available, as illustrated by **38** and **39** in Figure 10b. There are three examples for X = F in the solubility data set with a mean *Z*-score for the change in log(solubility) of +1.49 (range = +0.8 to +2.6), one example for X = Cl with a *Z*-score of +3.10, and three examples for X = Me with a mean *Z*-score of +1.56 (range = +0.59 to +2.41). These examples suggest that the solubility does not decrease by as much as would be expected (or increases) upon the addition of each of these substituents.

Cyclin-Dependent Kinase (CDK) Inhibitors. Jones and co-workers at AstraZeneca Pharmaceuticals described cyclin-dependent kinase (CDK) inhibitors.[69] Introduction of a methyl group (**41**) at the ortho-position of dimethylbenzamide **40** led to a 230-fold increase of themodynamic aqueous solubility, despite the increase of hydrophobicity compared to **40** (Figure 11). Compound **41** exhibited comparable CDK inhibitory activity to **40**. They hypothesized that the improved solubility was due to a steric clash between the methyl group and dimethylamide adversely affecting packing in the solid state.

Met Kinase Inhibitors. Schroeder and co-workers at Bristol-Myers Squibb identified selective Met kinase inhibitors.[70] They introduced an ethoxy group into the pyridone 4-position of the highly planar molecule **42** (Figure 12). Compound **43** possessed

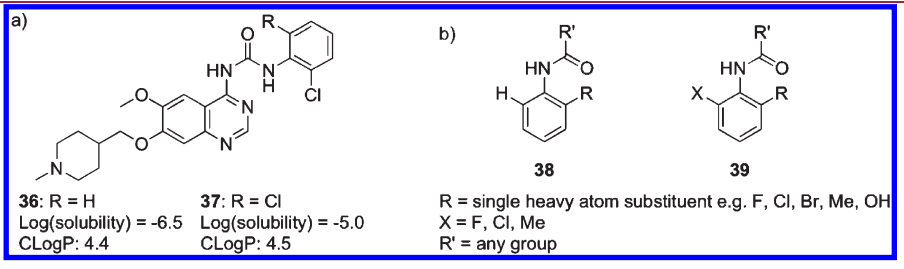


Figure 10. Improvement of thermodynamic aqueous solubility by ortho-substitution of a phenylurea. ClogP values were estimated by us with ChemDraw Ultra, version 10.0.

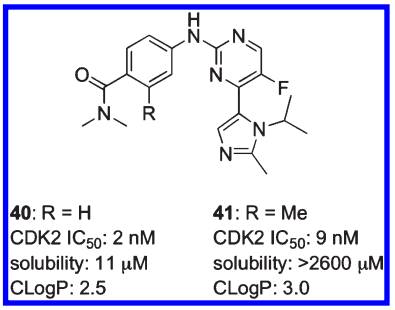


Figure 11. Improvement of thermodynamic aqueous solubility by ortho-substitution of benzamide. CLogP values were estimated by us with ChemDraw Ultra, version 10.0.

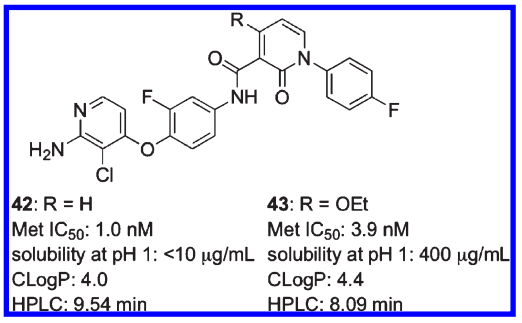


Figure 12. Improvement of aqueous solubility by ortho-substitution of pyridone carboxamide. CLogP values were estimated by us with ChemDraw Ultra, version 10.0. Retention times on reversed-phase HPLC were taken from Supporting Information of Schroeder et al.[70]

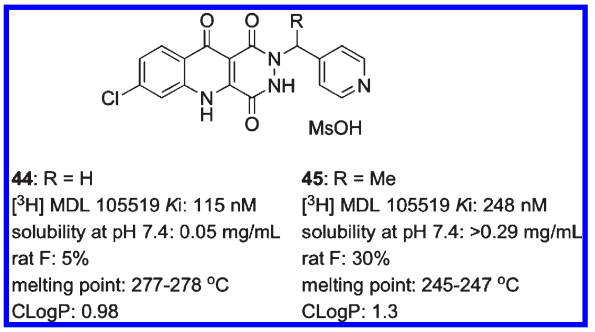


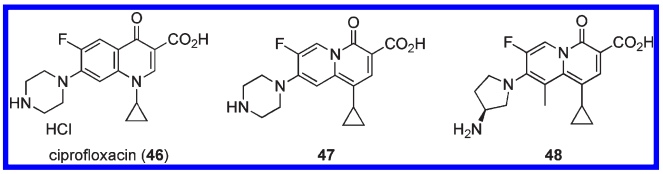
Figure 13. Improving aqueous solubility by α-substitution. Biological activity was evaluated in terms of displacement of a radioligand for the NMDA glycine site ([^{3}H]MDL 105519) in rat brain membranes. CLogP values were estimated by us with ChemDraw Ultra, version 10.0.

in vitro activity comparable to that of **42** but showed >40-fold improvement in aqueous solubility at pH 1.0 (aqueous solubility of 400 μg/mL). This dramatic improvement in aqueous solubility can be attributed to a deviation from planarity induced by the R substituent which results in disruption of crystal packing. We estimated CLogP values and collected retention times on reversed-phase HPLC. However, these hydrophobicity parameters of **42** and **43** were discrepant, so we could not judge which compound is more hydrophobic. Concerning the pharmacokinetic parameters in mouse (10 mg/kg), rat (10 mg/kg), and dog (5 mg/kg), compound **43** was well absorbed after oral administration of a solution formulation, with a favorable half-life ($t_{1/2}$) and mean residence time (MRT). Furthermore, the measured oral bioavailabilities (F_{po}) were excellent (100%) in all three species. Antitumor activity was examined in a GTL-16 xenograft model in which tumor cells were implanted subcutaneously and staged to approximately 125 mm^3 prior to commencement of once-daily oral dosing of compound **43** for 14 consecutive days. Compound **43** was active at all dose levels tested, as defined by >50% tumor growth inhibition (TGI) over one tumor volume doubling time (TVDT). The minimum efficacious dose of 6.25 mg/kg corresponded to a TGI of 66%, and complete tumor stasis was observed throughout the dosing period at doses of 25 and 50 mg/kg (TGI = 95% and 102%, respectively).

3.3. Introduction of Substituents into Benzylic Position. *N-Methyl-D-aspartate (NMDA) Antagonists.* Brown and co-workers at AstraZeneca Pharmaceuticals identified 7-chloro-2,3-dihydro-2-(phenyl)pyridazino[4,5-*b*]quinoline-1,4,10 (5*H*)-triones (PQTs) as potent and selective *N*-methyl-D-aspartate (NMDA) antagonists.[71] It has been recognized that a major hurdle in designing efficacious NMDA glycine-site antagonists is overcoming poor physical properties, such as solubility, cell permeability, plasma protein binding, and brain penetration. They introduced alkyl groups into the α-position to improve solubility by disrupting molecular planarity. In general, the solubility of the substituted analogues was improved, compared with that of the lead compound **44**. In some cases, the putative mechanism is supported by a corresponding lowering of melting points between the α-alkyl substituted and unsubstituted analogues. For example, the melting point of **44** was higher than that of α-methyl analogue **45** (Figure 13). The CLogP values indicated that introduction of substituents into **44** also results in increased hydrophobicity. Concerning the biological activity, **45** showed activity similar to that **44**. In accordance with the above results, the unsubstituted compound **44** possessed the lowest rat oral bioavailability (5%) among the compounds studied. In contrast, the α-substituted analogue **45** had the best oral bioavailability (30%). An interesting extension of this approach would be stereoselective synthesis of compounds with asymmetric benzylic substitution. There are also several other reports suggesting that introduction of substituents into benzylic positions improves aqueous solubility.[72−75] It would be of interest to determine the melting points or crystal structures of these compounds.

3.4. Twisting of Fused Rings. *DNA Gyrase Inhibitors.* Li and co-workers at Abbot Laboratories synthesized a 2-pyridone series of DNA gyrase inhibitors.[76] Starting from quinolone structures, subtle interchange of the nitrogen with a carbon atom yielded two novel heterocyclic nuclei that had not previously been evaluated as antibacterial agents. Almost all of the compounds tested exhibited enhanced thermodynamic aqueous solubility relative to ciprofloxacin (**46**). The isostere of ciprofloxacin, **47**, showed about 3 times greater thermodynamic aqueous solubility and bioavailability, with similar activity (Table 8). This example is indicative of the differences between quinolones and 2-pyridones. Compound **48** showed 3 times greater solubility than **46** with 8 times more potent activity. Apparently, transposition of the nitrogen of a quinolone to the bridgehead position results in a change to the overall polarity of the molecule and perhaps a perturbation of the planarity of the core structure that would influence molecular packing. These changes are associated with a favorable change in the solubility and pharmacokinetics of the 2-pyridones. They also confirmed the planarity of **48** by X-ray structure analysis of a single crystal. The steric congestion created by the interaction of the methyl group with the cyclopropane

Table 8. Improvement of Thermodynamic Aqueous Solubility by Twisting of Fused Rings



compd	CC_{50} [a] (μg/mL)	aqueous solubility[b] (mg/mL)	rat F (%)	CLogP[c]
ciprofloxacin (**46**)	0.24	0.08	16	−1.2
47	0.15	0.25	46	−0.89
48	0.03	0.25	32	0.09

[a] CC_{50} is defined as the drug concentration that causes 50% inhibition of the maximal gyrase (*E. Coli* H560) mediated DNA cleavage. [b] Thermodynamic solubility in phosphate buffer (0.05 M), pH 7.4, at 37 °C. [c] CLogP values were estimated by us with ChemDraw Ultra, version 10.0.

forces the methyl and cyclopropyl groups out of the plane of the pyridone ring with a twist that was estimated to be approximately 30°. They suggested that this deviation from planarity is responsible for the enhanced solubility of the 2-pyridones.

Other examples of the disruption of crystal packing include modification of intermolecular and/or intramolecular hydrogen bonding in the crystal, although this approach is outside the scope of this review. Several reports have examined the relationship between solubility and crystal packing or melting point.[77−80]

4. POSSIBLE ADVANTAGES OF A STRATEGY FOCUSED ON MOLECULAR PLANARITY/SYMMETRY

As mentioned above, a strategy focused on disruption of molecular planarity and symmetry is quite distinct from the general/classical strategy to improve solubility by decreasing the hydrophobicity of molecules and offers several advantages as follows.

First, the introduction of hydrophilic substituents often interferes with protein−drug interactions. In such cases, the strategy described in this review would provide an alternative approach for improving aqueous solubility. Actually, introduction of hydrophilic substituents into integrin antagonists led to a decrease of the inhibitory activity in a cell-based assay. Reported AhR agonists are generally highly hydrophobic, and introduction of hydrophilic substituents usually causes a decrease of their activity. Introduction of hydrophilic substituents into PPARδ partial agonists also caused a decrease of their activity. In contrast, disruption of molecular planarity and symmetry of integrin antagonists, AhR agonist, and PPARδ partial agonists increased both biological activity and aqueous solubility. These results suggest that the alternative approach to improving aqueous solubility is particularly attractive when introduction of hydrophilic substituents is unsuccessful.

Second, we found that disruption of molecular planarity coupled with an increase of hydrophobicity resulted in better aqueous solubility than a decrease of hydrophobicity in three cases (**8** vs **7**, **16** vs **20**, and **26** vs **27**). Introduction of hydrophilic groups may decrease aqueous solubility if the introduced hydrophilic groups form novel hydrogen bonds that induce tighter crystal packing. We found an example of a less hydrophobic compound with worse aqueous solubility (**8** vs **7**).

Third, medicinal chemists sometimes find that both solubility and hydrophobicity need to be increased, for example, to improve the oral bioavailability of highly hydrophilic compounds with insufficient solubility. Experimentally there is almost certainly a lower (hydrophilic) log P limit to absorption and permeation. Therefore, we hypothesized that if compounds soluble in both organic solvents and water are generated, aqueous solubility would be improved even if the log P value is increased; that is, the solubility ratio in octanol is increased. Indeed, we were able to obtain integrin antagonists, AhR agonists, and PPARδ partial agonists that showed improved solubility despite having higher hydrophobicity than their parent compounds. It is expected that the solubility of these compounds in organic solvents would also be increased.

Introduction of substituents often interferes with protein−drug interactions because of steric restrictions of binding pockets in proteins. Fluorine has the smallest van der Waals radius of any substituent except for hydrogen. Thus, fluorine substitution should have the least impact on target protein−drug interaction, and so fluorine might be the most versatile substituent to increase dihedral angle without loss of biological activity. In fact, fluorinated integrin antagonist **8**, AhR agonists **17** and **18**, and PPARδ partial agonists **24** and **26** showed increased solubility and activity. On the other hand, it appears that introduction of fluorine atom(s) might improve aqueous solubility not only by disrupting molecular planarity but also via other mechanism(s). Further study is needed.

Disruption of molecular symmetry also provides a means to increase aqueous solubility without an increase of molecular weight, which may have adverse consequences for the pharmacokinetics. Here, we have described some examples of compounds whose aqueous solubility was increased without any increase of molecular weight, that is, integrin antagonist **11** and PPARδ partial agonists **22** and **25**.

Finally, multiple strategies to improve aqueous solubility are likely to be superior to a single strategy. We showed that two kinds of modifications (**28**, substitution to pyridine ring and introduction of methyl groups) of PPARδ partial agonist **22** led to more than 2700 times higher solubility in phosphate buffer, owing to the combination of reduction of hydrophobicity and disruption of molecular planarity. Other combinations such as disruption of both molecular symmetry and molecular planarity are also attractive. For example, two kinds of modifications (**25**, changing the position of the carboxyl group from para to meta

and introducing a methyl group) of PPARδ partial agonist **21** led to 27 times higher solubility in aqueous EtOH. The disruption of molecular symmetry by changing the position of the carboxyl group contributes in part to the improved solubility (**21** vs **22**), as does the disruption of molecular planarity by introduction of the methyl group (**22** vs **25**). In addition, we reviewed examples of disruption of molecular planarity achieved by several kinds of modification: increase of dihedral angle, removal of aromaticity, substitution of benzylic positions, and twisting of fused rings. Combinations of these chemical modifications might also be useful for improving aqueous solubility.

5. CONCLUSION

Aqueous solubility is essential for drug candidates, and improvement of the aqueous solubility of small molecules is a major issue for medicinal chemists. The strategy of introducing hydrophilic group(s) into a molecule is generally used for improving aqueous solubility but is not universally effective. Thus, we have focused on an alternative strategy for improving aqueous solubility, that is, disrupting molecular planarity/symmetry, and investigated chemical modifications focused on the molecular planarity/symmetry of bicyclic structures. To demonstrate the validity of this strategy, we selected lead compounds whose biological activity was impaired by substitutions that decreased hydrophobicity. To clarify the mechanisms of improvement of aqueous solubility, we carried out chemical modifications that would both increase hydrophobicity and disrupt molecular planarity and symmetry and we examined the changes in physicochemical properties of the compounds. Our results indicate that disruption of molecular planarity/symmetry is effective to increase aqueous solubility; at least 350-fold improvement of aqueous solubility by disruption of molecular planarity, despite increased hydrophobicity, was achieved in one example. A search of the literature indicated that increase of dihedral angle, removal of aromaticity, substitution of benzylic positions, and twisting of fused ring are all effective modifications for disrupting molecular planarity/symmetry, as indicated by consideration of changes in CLogP, retention time on reversed-phase HPLC, melting point, and so on. In these analyses, we have shown that melting point can be considered as a parameter of molecular planarity and symmetry. In today's medicinal chemistry generally, there is less focus on crystallization and melting point of bioactive molecules, but we hope that the present review will bring to medicinal chemists a renewed sense of the importance of melting point.

We emphasize that the strategy of improving aqueous solubility by focusing on molecular planarity/symmetry is quite distinct from the general/classical strategy based on decreasing the hydrophobicity of molecules. Possible advantages include the following: (i) if introduction of hydrophilic substituents is inappropriate, hydrophobic substituents can be used; (ii) disruption of molecular planarity/symmetry is more effective than decrease of hydrophobicity in some cases; (iii) both hydrophobicity and aqueous solubility can be increased simultaneously; (iv) solubility can be improved with little or no increase of molecular weight; (v) combinations of the strategies may be superior to a single strategy. For example, we found that the combination of disruption of molecular planarity and decrease of hydrophobicity increased solubility by at least 2700-fold in one example. We believe that that further work in this area will provide sophisticated, flexible, and general strategies for improving the solubility of molecules of pharmaceutical interest.

AUTHOR INFORMATION

Corresponding Author

*Phone: +81.3.5841.7849. Fax: +81.3.5841.8495. E-mail: m-ishikawa@iam.u-tokyo.ac.jp.

BIOGRAPHIES

Minoru Ishikawa received his M.Sc. (1996) from Tokyo Institute of Technology, then became a Researcher at the Medicinal Chemistry Research Labs in Meiji Seika Kaisha, Ltd., Japan (1996−2008). During that time, he received his Ph.D. degree from the University of Tokyo under the direction of Professor Yuichi Hashimoto (2006). He subsequently joined the Institute of Molecular and Cellular Biosciences, The University of Tokyo, as an Assistant Professor (2008 to present). His research interests are in the areas of medicinal chemistry and bioorganic chemistry, including improvement of physicochemical properties of drug candidates, regulators of protein stability, peptide mimetics, and regulators of protein−protein interactions.

Yuichi Hashimoto received his B.Sc. and Ph.D. in Pharmaceutical Sciences from the University of Tokyo (1977 and 1982, respectively). He became Assistant Professor of the Faculty of Pharmaceutical Science, University of Tokyo (1983−1989), then moved to the Institute of Applied Microbiology, University of Tokyo, as Associate Professor (1989−1997), and finally joined the Institute of Molecular and Cellular Biosciences, University of Tokyo, as Full Professor (1997 to present). His major research interests span the fields of basic molecular medicinal chemistry and bioorganic chemistry and include studies of the structural development of bioresponse modifiers, regulators of protein trafficking/stability, action mechanisms of the small molecular regulators of gene expression, and signal transduction.

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ABBREVIATIONS USED

GSE, general solubility equation; PCB, polychlorinated biphenyl; TRPV, transient receptor potential vanilloid; VSMC, vascular smooth muscle cell; hPRP, human platelet rich plasma; HPLC, high performance liquid chromatography; AhR, aryl hydrocarbon receptor; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; EROD, 7-ethoxyresorufin *O*-deethylase; DFT, density functional theory; PPAR, peroxisome proliferator-activated receptor; HOMO, highest occupied molecular orbital; LUMO, lowest unoccupied molecular orbital; MK-2, mitogen-activated protein kinase-activated protein kinase 2; MMP, matrix metalloproteinase; CDK, cyclin-dependent kinase; NMDA, *N*-methyl-D-aspartate

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