Bioorganic & Medicinal Chemistry 18 (2010) 1194–1203

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

β-Naphthoflavone analogs as potent and soluble aryl hydrocarbon receptor agonists: Improvement of solubility by disruption of molecular planarity

Yuji Fujita, Mitsuhiro Yonehara, Masashi Tetsuhashi, Tomomi Noguchi-Yachide, Yuichi Hashimoto, Minoru Ishikawa *

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

ARTICLE INFO

Article history:
Received 17 November 2009
Revised 10 December 2009
Accepted 11 December 2009
Available online 21 December 2009

Keywords:
AhR agonist
Naphthoflavone
Solubility

ABSTRACT

The physiological role of aryl hydrocarbon receptor (AhR) is not yet fully understood, and investigation is hampered by the limited solubility of reported AhR ligands in aqueous media. To achieve improved solubility, we focused on our previous finding that planarity-disruption of molecules leads to less efficient crystal packing and greater aqueous solubility. Here, we describe chemical modification of an AhR agonist, β-naphthoflavone, focusing on planarity-disruption. As expected, introduction of substituents at the *ortho*-positions of the phenyl group resulted in greater solubility. Among the compounds prepared, the fluoro analog showed more potent AhR agonistic activity and greater solubility than did β-naphthoflavone. Our results indicate that this strategy to improve aqueous solubility, that is, introduction of substituent(s) that disrupt planarity, may be generally applicable to bicyclic molecules.

1. Introduction

The aryl hydrocarbon receptor (AhR) is a ligand-dependent transcription factor which is known to mediate the toxicity of dioxin.[1] 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.[2] Following ligand binding, the cytosolic ligand–AhR complex translocates to the nucleus and dimerizes with AhR nuclear translocator (Arnt). The AhR–Arnt complex binds to dioxin-responsive elements (DRE), which are specific DNA sequences located upstream of AhR-responsive genes, including CYP1A1/2, and increases the gene transcription rate.[3] AhR expression is ubiquitous in vertebrate cells, but the physiological role of AhR is not yet fully understood. Although AhR is best known for mediating dioxin toxicity, knockout studies have indicated that AhR also plays a role in normal physiology, including certain immune responses.[4] More recently, two groups have reported that ligand-activated AhR regulates T_{reg} and Th17 cell development.[5,6]

Various polycyclic aromatic hydrocarbons (PAHs), including 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, dioxin), 2,3,7,8-tetrachlorodibenzofuran (TCDF), 3,3′,4,4′,5-pentachlorobiphenyl (PCB), β-naphthoflavone (**1a**), benzo[*a*]pyrene (**2**), 3-methylcholanthrene (MC) and indigo (**3**) (Fig. 1), have been identified as AhR ligands. Although these AhR ligands appear to have diverse structures, they possess some common features, that is, similar size, planar structure and hydrophobic character, which have been suggested to be crucial for high binding affinity with AhR.[4] However, these properties of currently known AhR ligands result in rather limited solubility (especially in aqueous solution), which is a great drawback in the use of these ligands as tools for investigating the physiological role of AhR.[7] Therefore, potent AhR ligands with improved solubility are needed.

In general, the aqueous solubility of drugs depends on their hydrophobicity (Log *P*).[8,9] Thus, the strategy of introducing hydrophilic group(s) into the molecule is widely used for increasing aqueous solubility. But this approach is not universally effective, because the introduced hydrophilic group(s) sometimes interrupts target protein–drug interaction. In addition, this strategy is not effective when both solubility and hydrophobicity need to be increased, for example, to improve oral bioavailability of highly hydrophilic compounds with insufficient solubility. Therefore, a novel and general strategy to increase the solubility of drug candidates would have a great impact on drug discovery and medicinal chemistry.

During previous research on integrin antagonists, we found that introduction of fluorine (**5**) or modification of the tetrahydropyrimidylamino group (**6**) resulted in an increase of aqueous solubility compared with that of the lead compound **4** (Table 1).[10,11] These analogs **5** and **6** are also more hydrophobic,[12] based on calculated Log *P* (*C* Log *P*) values and retention times on reversed-phase HPLC. On the other hand, equations for predicting

* Corresponding author. Tel.: +81 3 5841 7849; fax: +81 3 5841 8495.
E-mail address: m-ishikawa@iam.u-tokyo.ac.jp (M. Ishikawa).

0968-0896/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2009.12.036

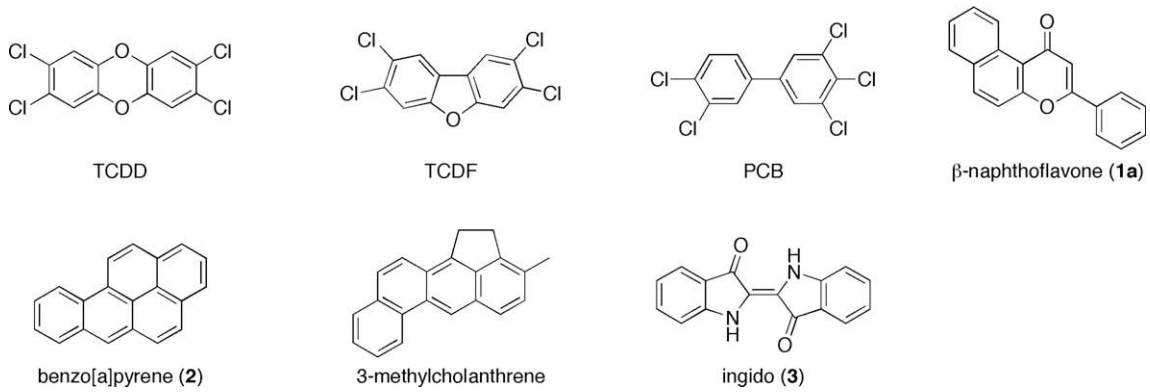


Figure 1. Chemical structure of AhR ligands.

solubility[13,14] include not only Log *P*, but also melting point: for example, Log[solubility (M)] = 0.5 − (Log *P*) − 0.01[[melting point (°C)] − 25].[14] The melting point itself is related to crystal lattice and crystal packing energy.[15] The melting points of **5** and **6** are lower than that of the lead compound **4**. Furthermore, the X-ray structure of **5** revealed a substantially increased dihedral angle between the piperidine ring and benzoyl group.[10] Thus, we speculated that the increase of aqueous solubility of **5** and **6** was caused not by decreased hydrophobicity, but by disruption of molecular planarity and/or symmetry, resulting in a decrease of crystal packing energy.[16] In other words, the increased solubility of **5** is the result of a larger dihedral angle and the consequent decrease of crystal packing energy.

In this report, we present substituted β-naphthoflavone analogs as AhR agonists with improved aqueous solubility. Our results indicate that the proposed strategy to improve aqueous solubility, that is, introduction of substituent(s) that modify the dihedral angle of bicyclic molecules, may be generally applicable.

2. Results

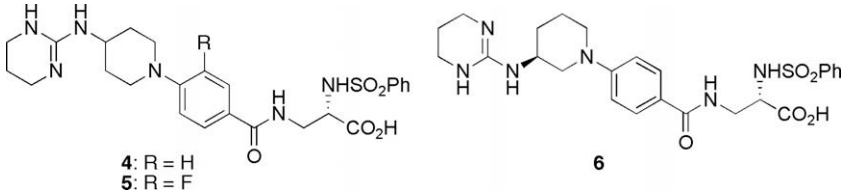
2.1. Molecular design

β-Naphthoflavone **1a** was reported to be a more potent AhR agonist than TCDD.[17] 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 **1a** to obtain AhR ligands with more potent activity and improved solubility. Because the structure of **1a** includes a rotatable biaryl moiety, we focused on decreasing the planarity of the molecule. The previous finding that the introduction of a fluorine atom into **4**, that is, **5**, resulted in an increase of aqueous solubility led us to expect that the introduction of substituent(s) on the phenyl group of **1a** would reduce planarity by causing an increase in the dihedral angle of the biaryl moiety, consequently decreasing the crystal packing energy and melting point, and thereby improving the aqueous solubility. In addition, the 2-pyridyl analog **1g**, in which the dihedral angle would be reduced due to lack of the hydrogen atom, was designed to further evaluate the relationship between solubility and the dihedral angle.

2.2. Chemistry

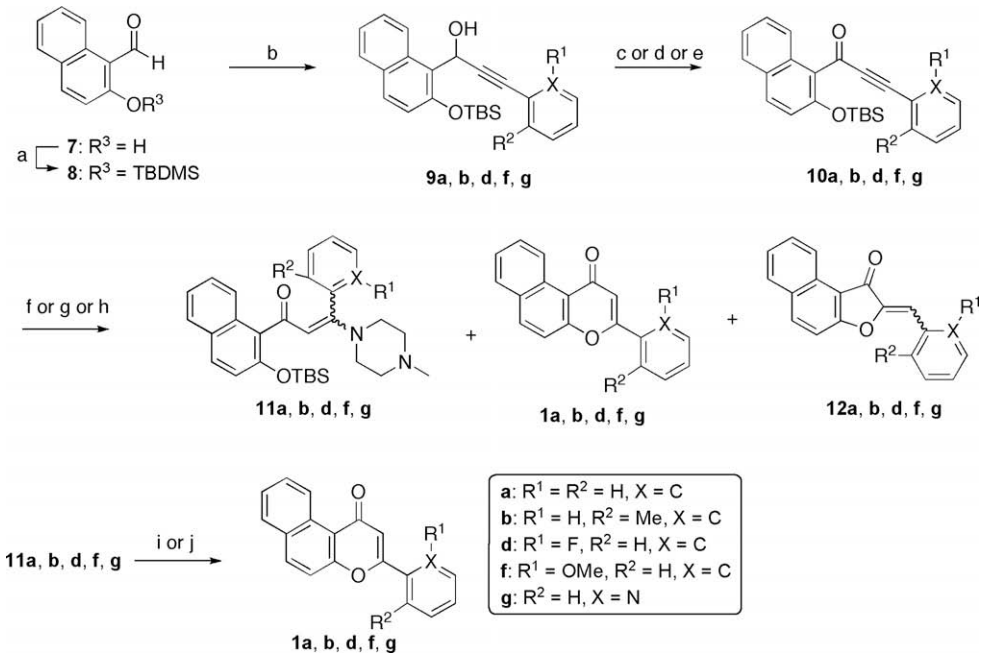
Monosubstituted β-naphthoflavone analogs were synthesized by using methods similar to those applied in the synthesis of flavone analogs,[18] as shown in Scheme 1. Briefly, after protection of phenol **7** with TBDMS, aldehyde **8** was reacted with aryl acetylide to give **9**. Oxidation, followed by deprotection of **10a** with TBAF gave the undesired five-membered ring **12a**[18] in 72% yield. Next, we examined the introduction of piperazine according to the reported method[19] for the preparation of a six-membered cyclic adduct. Michael addition of *N*-methylpiperazine to **10a** followed by reflux in MeOH gave **1a** in low yield (21%). Other major products were **11a** (46%), whose TBDMS group was not deprotected, and **12a** (5.9%). Therefore, another deprotection method was tried. Heating[20] (in DMSO/H_2O at 90 °C) both deprotected and cyclized **11a** to give **1a** in 78% yield. The ^{1}H NMR and ^{13}C NMR spectra of synthetic **1a** were in good accordance with literature data.[21,22]

Table 1
Physico-chemical data of integrin antagonists



Compound	Aqueous solubility[a] (mg/mL)	*C* Log *P*[b]	HPLC retention time[c] (min)	Melting point (°C)
4	<0.1	1.46	8.25	252–254
5	0.6	1.62	9.73	182–184
6	3.5	1.81	12.2	181–184

[a] Solubility in water.
[b] *C* Log *P* values were estimated by ChemDraw Ultra version 10.0.
[c] Inertsil ODS-2 reversed-phase column (4.6 mm × 250 mm).

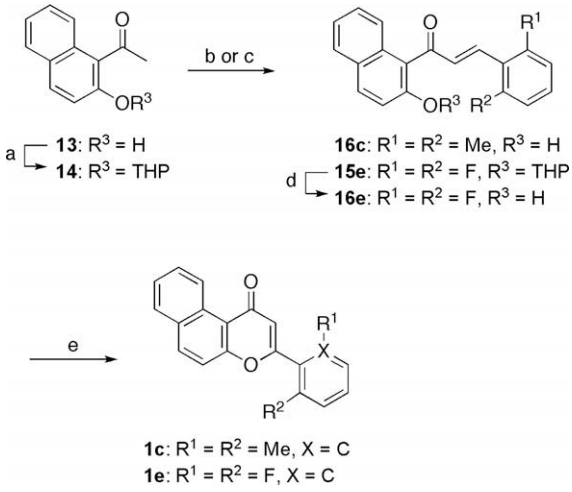


Scheme 1. Reagents and conditions: (a) TBDMSCl, imidazole, DMF, rt, 75%; (b) *n*-BuLi, ArCCH, THF, −78 °C, 92% (**9a**), 71% (**9b**), 67% (**9d**), 69% (**9f**), 47% (**9g**); (c) MnO_2, DCM, rt, 65% (**10a**); (d) Dess–Martin periodinane, DCM, rt, 65% (**10b**), 65% (**10d**); (e) Dess–Martin periodinane, *t*-BuOH, DCM, rt, 53% (**10f**), 72% (**10g**); (f) (i) *N*-methylpiperazine, THF, MeOH, rt, (ii) MeOH, reflux, **11a** (46%) + **1a** (21%) + **12a** (5.9%) in two steps; (g) (i) *N*-methylpiperazine, THF, MeOH, rt, (ii) EtOH, reflux, **11b** (58%) + **1b** (10%) + **12b** (10%) in two steps; (h) *N*-methylpiperazine, THF, MeOH, rt; (i) DMSO, H_2O, 90 °C, 78% (**1a**), 42% (**1d**) + 5.7% (**12d**) in two steps, 58% in two steps (**1g**); (j) (i) DMSO, H_2O, 90 °C, (ii) EtOH, reflux, 64% in two steps (**1b**), 64% in three steps (**1f**).

The five-membered ring **12a** was readily distinguishable from **1a** by comparison of their ^{1}H NMR and ^{13}C NMR spectra.

Similarly, **1b**, **1d**, **1f**, and **1g** were synthesized by means of the general procedure mentioned above, with slight modifications. In the oxidation step of **9**, Dess–Martin periodinane was used to shorten the reaction time. However, even with the reported improvement of *t*-BuOH-addition,[23] this was not effective in the case of **10f–g**. In the synthesis of **1b**, **11b** was readily deprotected simply by heating it in DMSO/H_2O in 84% yield (without cyclization under these experimental conditions). To achieve cyclization of the resultant phenol (deprotected **11b**), the solvent was changed to EtOH; heating under reflux gave **1b** in 64% yield. Thus, deprotection of **11b** with heating in DMSO/H_2O gave better yield than heating in alcohol. Then, for the synthesis of **1d**, **1f**, and **1g**, step (g) (ii) in Scheme 1 could be omitted. Instead, heating of **11d** and **11g** in DMSO/H_2O resulted in successive deprotection and cyclization in one-pot to give **1d** and **1g**, respectively. A small amount of **12d** accompanied **11d**. Consecutive heating in EtOH after heating in DMSO/H_2O was needed for the synthesis of **1f**. When taken together, we used two cyclization condition, one being 90 °C in DMSO/H_2O (for **1a**, **1d** and **1g**) and the other being reflux in EtOH (for **1b** and **1f**) for cases when the cyclization did not proceed under the first condition. The reason for the reactivity differences depending on structural difference of the aryl groups is not clear. However, steric effects of the bulky methyl or methoxy group, or electron-rich character of the aryl group might decrease the reactivity.

For disubstituted analogs **1c** and **1e**, the cyclization step in the above synthetic route was unsuccessful. Thus, the aldol reaction[24] was tried (Scheme 2). Ketone **13** or **14** with disubstituted benzaldehyde afforded **16**. For the synthesis of **16e**, phenol **13** was protected to give **14**,[25] because aldol reaction of **13** with difluorobenzaldehyde did not afford **16e**. Compounds **16c** and **16e** were treated with DDQ[26] to give **1c** and **1e**, respectively, in moderate yields.



Scheme 2. Reagents and conditions: (a) dihydropyran, PPTS, DCM, rt to 35 °C, 85%; (b) 2,6-dimethylbenzaldehyde, NaOH, EtOH, H_2O, rt to 70 °C, 53% (**16c**); (c) 2,6-difluorobenzaldehyde, $Ba(OH)_2$, MeOH, rt, 71% (**15e**); (d) TsOH, MeOH, rt, 38%; (e) DDQ, 1,4-dioxane, 110 °C, 51% (**1c**), 55% (**1e**).

2.3. Biological activity

To evaluate the AhR-agonistic activity of the prepared compounds, CYP1A1-dependent EROD activity in MCF-7 breast cancer cells was measured. CYP1A1 is the major enzyme that catalyses the de-ethylation of 7-ethoxyresorufin to resorufin, and its activity is induced by activation of AhR. The activity of 7-ethoxyresorufin *O*-deethylase (EROD) can be measured by using fluorescent resorufin. This EROD assay has been widely used to evaluate AhR agonists.[27–33] As positive controls, typical AhR agonists, **1a**, **2** and **3**, were used. Compounds **1a**, **2** and **3** exhibited potent EROD-induc-

ing activity with EC_{50} values of 1.4, 2.7, and 1.7 μM, respectively. Under the assay conditions used, both the monofluoro analog **1d** and difluoro analog **1e** showed at least four times stronger EROD-inducing activity than **1a** (Table 2). On the other hand, the EC_{50} values of monomethyl analog **1b** and dimethyl analog **1c** were higher than 10 μM. In particular, **1c** did not show any EROD-inducing activity at 10 μM. Introduction of a methoxy group (**1f**) and substitution of the pyridine ring (**1g**) led to increased EROD-inducing activity, with EC_{50} values of 0.27 and 0.45 μM, respectively. The difluoro analog **1e** had the strongest EROD-inducing activity with an EC_{50} value of 0.20 μM, which is seven times more potent than that of **1a**, among the compounds prepared.

None of compounds **1a**–**g** affected MCF-7 cell viability at 10 μM after 24 h incubation. Thus, these AhR agonists can be evaluated by cell-based assay.

2.4. Physico-chemical data

Thermodynamic aqueous solubility (solubility of a compound as a saturated solution in equilibrium) of **1a**–**g** was evaluated according to Avdeef and Testa.[34] Aqueous solubility of **1a** 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 **1a** was still poor (84.6 μg/mL). *ortho*-Substituted **1b**–**e** showed better solubility than **1a** (Table 2), as expected; indeed, dimethyl analog **1c** was 15 times more soluble (1270 μg/mL) than **1a**. Difluoro analog **1e** showed three times greater solubility (248 μg/mL) than **1a**. On the other hand, methoxy analog **1f** was less soluble than **1a**. Pyridine analog **1g** showed the second highest solubility among this series. When the synthesized compounds were analyzed in conjunction with EROD assay and solubility, compounds **1d**, **1e**, and **1g** were more potent AhR agonists with improved solubility. Among them, difluoro analog **1e** had the best overall profile, being seven times more potent and three times more soluble than **1a**.

Table 2 shows the dihedral angles of **1a**–**g** for optimized structures obtained by means of density functional theory (DFT) calculations (B3LYP/6-31G*),[35] together with melting points. *ortho*-Substituted **1b**–**c** and **1e** showed lower melting points and increased dihedral angles compared with **1a**, as expected. In contrast, however, **1d** showed lower melting point and decreased calculated dihedral angle compared with **1a**. A possible explanation of this calculated small dihedral angle of **1d** would be interaction between the fluorine lone pair and hydrogen at the 2-position. Methoxy analog **1f** showed a higher melting point and almost the same calculated dihedral angle, compared with those of **1a**. The reason for the relatively small dihedral angle may be similar to that in the case of **1d**, that is, interaction between the oxygen lone pair and hydrogen at the 2-position. Pyridine analog **1g**, which lacks a hydrogen atom, showed a higher melting point and decreased dihedral angle compared with **1a**, as expected.

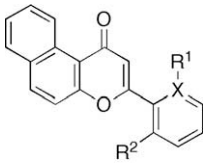
Hydrophobicity parameters, that is, *C* Log *P* and retention time on reversed-phase HPLC, are also summarized in Table 2. *ortho*-Substituted **1b**–**e** showed increased hydrophobicity, whereas pyridine analog **1g** showed reduced hydrophobicity, as expected. In the case of methoxy analog **1f**, there was an apparent discrepancy, because *C* Log *P* was lower than that of **1a**, whereas the retention time was larger than that of **1a**.

3. Discussion

As shown in Figure 2, physico-chemical data of the prepared compounds that were highly correlated to solubility were melting point (R^2 = 0.71) and dihedral angle (R^2 = 0.62), while *C* Log *P* and retention time did not correlate to the solubility (R^2 < 0.2). Interestingly, some compounds showed improved solubility despite having higher hydrophobicity than their mother compound. Next, exhaustive analyses of mutual correlations among parameters were performed. A high correlation (R^2 > 0.7) was found between dihedral angle and melting point (R^2 = 0.80). Thus, it appeared that the dihedral angle of these compounds influences the melting point, which is related to solubility. On the other hand, *C* Log *P* values and retention times of the compounds, which are both hydrophobicity parameters, did not show a high correlation (R^2 = 0.45). The reason for this may be the exceptionally large retention time of methoxy analog **1f**. In fact, *C* Log *P* and retention time showed a high correlation (R^2 = 0.86) when **1f** was excluded. The reason of the exceptional nature of the methoxy analog is not clear.

Among the highly soluble compounds **1b**, **1c**, **1d**, **1e**, and **1g**, **1b, 1c** and **1e** possess increased hydrophobicity, larger dihedral angle and lower melting point than those of **1a**. These results suggested that introduction of substituents into **1a** results in disruption of the planarity by increasing the dihedral angle, leading in turn to decreased crystal packing energy and lower melting point, and so increasing the solubility. On the other hand, the pyridine analog **1g** possesses reduced hydrophobicity, smaller dihedral angle and higher melting point than those of **1a**. Thus, we consider that the

Table 2
EROD activity and physico-chemical data of β-naphthoflavone analogs



1a-g

	R^1	R^2	X	EROD EC_{50} (μM)	Solubility[a] (μg/mL)	Melting point (°C)	Calculated dihedral angle[b] (°)	*C* Log *P*[c]	HPLC retention time[d] (min)
1a	H	H	C	1.4	84.6	165–167	17.8	4.65	7.70
1b	H	Me	C	>10	262	135–137	37.9	4.85	8.67
1c	Me	Me	C	>10	1270	92	70.0	5.05	9.68
1d	F	H	C	0.33	153	157	9.1	4.80	7.85
1e	F	F	C	0.20	248	150	40.5	4.94	7.78
1f	OMe	H	C	0.27	45.8	192–193	18.5	4.06	9.29
1g	—	H	N	0.45	299	187–188	0.0	3.40	5.09

[a] Solubility in an equal volume of EtOH and 1/15 M phosphate buffer (pH 7.4).
[b] Calculated dihedral angles were estimated by GAUSSIAN03.
[c] *C* Log *P* values were estimated by ChemDraw Ultra version 10.0.
[d] Waters μBondapak reversed-phase column (3.9 mm × 150 mm).

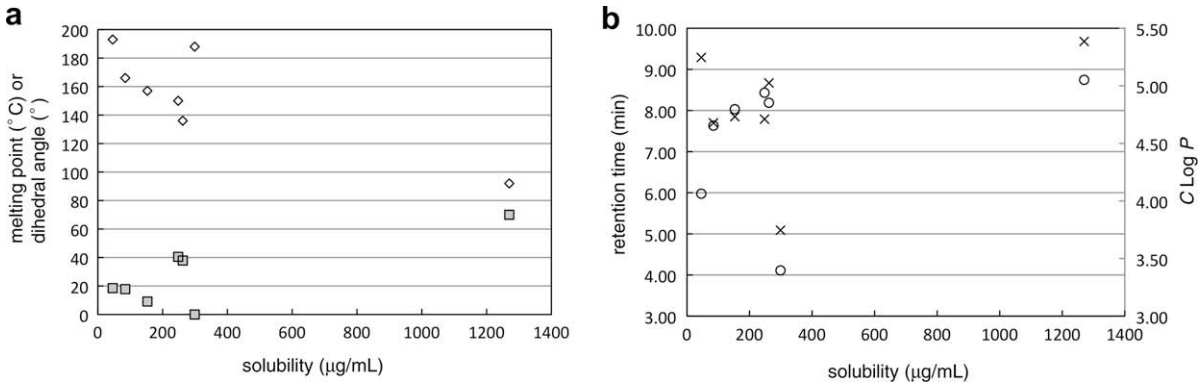


Figure 2. Relationships between solubility and physico-chemical data of **1a**–**g**. (a) Melting point (rhomboid) and dihedral angle (square), (b) *C* Log *P* (circle) and retention time (cross).

increased solubility of pyridine analog **1g** can be ascribed simply to a reduction of hydrophobicity. We believe that our novel strategy to improve solubility by focusing on dihedral angle, as presented in this paper, is quite distinct from the general/classical strategy based on decreasing the hydrophobicity of molecules.

However, the reason why **1d** showed improved solubility was not clear, because **1d** possessed a relatively small calculated dihedral angle and relatively high hydrophobicity. Lack of molecular symmetry of **1d** 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. On the other hand, it is known that halogen bonding[36] might influence the solubility. By elemental analysis, we confirmed fluoro analogs **1d** and **1e** are not hydrate(s) that is induced by fluorine atom(s). In addition, intermolecular or intramolecular halogen bonding leads to the increased crystal packing. Taken together, halogen bonding would not be the reason for the increased solubility.

Structure–activity relationships of naphthoflavone analogs were also investigated. Firstly, the best dihedral angle for EROD-inducing activity appeared to be between 0° (**1g**) and 40.5° (**1e**), when additional substituent effects were neglected. Although AhR ligand activity was thought to require a planar molecular shape, our results indicate that AhR shows some tolerance in this regard. Alternatively, planar conformations of these naphthoflavone analogs may be induced by interaction with AhR. Secondly, methyl analogs (**1b** and **1c**) showed weak EROD-inducing activity, suggesting that the methyl group may be too bulky. Fluoro and methoxy analogs exhibited stronger activity, suggesting that these substituents provide suitable dihedral angles for AhR-binding. Alternatively, fluoro and methoxy substituents might exhibit halogen bonding[36] or hydrogen bonding to the binding pocket of AhR.

Our results indicate that the strategy to improve aqueous solubility by introduction of substituent(s) that modify the dihedral angle of bicyclic molecules could have general applicability. Indeed, dimethyl analog **1c** was 15 times more soluble than **1a**. There are two other reports[37,38] suggesting that introduction of a halogen atom into biaryl molecules led to improvement of aqueous solubility. However, additional data regarding melting point or crystal structure would be needed to assess whether or not the increased solubility is associated with a change of dihedral angle. Further studies to verify the generality of our strategy to improve aqueous solubility are in progress.

4. Conclusion

To further investigate the physiological role of AhR, we require potent AhR agonists with improved solubility. We focused on our previous finding that an increase of the dihedral angle of molecules leads to decreased crystal packing energy, and thereby improves aqueous solubility. With the aim of modifying the dihedral angle of an AhR agonist, β-naphthoflavone (**1a**), the *ortho*-positions of the phenyl group were substituted. Fluoro-substituted **1d** and **1e**, methoxy-substituted **1f** and pyridine analog **1g** possessed enhanced EROD-inducing activity. Compounds **1b**, **1c**, and **1e** showed increased calculated dihedral angles, lower melting points, improved solubility, and increased hydrophobicity parameters (*C* Log *P* and retention time on HPLC). These results suggest that the increased solubility of **1b**, **1c** and **1e**, compared with **1a**, was caused by the increase of dihedral angle and the decrease of crystal packing energy, rather than by decrease of hydrophobicity. Among these compounds, difluoro analog **1e** had the best overall profile, being seven times more potent in terms of AhR-agonistic activity and three times more soluble than **1a**.

AhR ligands previously reported are highly hydrophobic, and introduction of hydrophilic substituents into these ligands generally causes a decrease of their activity. Our strategy described in this paper offers an alternative approach to obtaining potent AhR ligands with improved solubility.

5. Experimental

5.1. General methods

^{1}H NMR spectra were recorded on a JEOL JNMGX500 (500 MHz) spectrometer. Chemical shifts are expressed in parts per million relative to tetramethylsilane. Mass spectra were recorded on a JEOL JMS-DX303 spectrometer. Melting points were determined by using a Yanagimoto hot-stage melting point apparatus and are uncorrected. Analytical thin-layer chromatography (TLC) was performed on Merck 60 F254 pre-coated silica gel plates. Flash chromatography was performed on a column of Kanto Silica Gel N 60. HPLC analyses were performed on an analytical column (Waters μBondapak reversed-phase column (C18, 125 Å, 10 μm, 3.9 mm × 150 mm) eluted with a mobile phase consisting of 1/30 M phosphate buffer (pH 7.4) in 55% CH_3CN at a flow rate of 1.0 mL/min, with UV monitoring at 263–288 nm, at 40 °C.

5.2. Chemistry

5.2.1. 2-*tert*-Butyldimethylsilyloxy-1-naphthaldehyde (8)

A solution of **7** (1.63 g, 9.44 mmol), *t*-butyldimethylsilyl chloride (2.45 g, 16.3 mmol), and imidazole (944 mg, 13.9 mmol) in dry DMF (45 mL) was stirred at room temperature for 4 h under Ar. Saturated NH_4Cl solution was added and the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by

silica gel column chromatography (hexane/EtOAc = 20:1) to afford **8** (2.04 g, 7.12 mmol, 75% yield) as a colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 10.87 (s, 1H), 9.28 (br d, J = 8.5 Hz, 1H), 7.96 (d, J = 9.2 Hz, 1H), 7.77 (br d, J = 7.9 Hz, 1H), 7.62 (ddd, J = 8.5, 7.3, 1.5 Hz, 1H), 7.43 (ddd, J = 7.9, 7.3, 1.2 Hz, 1H), 7.06 (d, J = 9.2 Hz, 1H), 1.06 (s, 9H), 0.33 (s, 6H). FAB-MS m/z 287 $(M+H)^+$.

5.2.2. 1-(2′-*tert*-Butyldimethylsilyloxynaphthalen-1′-yl)-3-phenyl-2-propyn-1-ol (9a)

To a solution of phenylacetylene (0.150 mL, 1.37 mmol) in dry THF (2 mL) was slowly added *n*-BuLi (0.700 mL, 1.16 mmol) at −78 °C and the mixture was stirred for 40 min under Ar. Then, a solution of **8** (204 mg, 0.713 mmol) in dry THF (1.5 mL) was slowly added. Stirring was continued at −78 °C for 2 h under Ar, then the reaction was quenched with saturated NH_4Cl solution, and the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/EtOAc = 5:1) to afford **9a** (255 mg, 0.656 mmol, 92% yield) as a yellow oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.59 (d, J = 8.5 Hz, 1H), 7.79 (d, J = 7.9 Hz, 1H), 7.72 (d, J = 9.2 Hz, 1H), 7.54 (ddd, J = 8.5, 6.7, 1.2 Hz, 1H), 7.40–7.36 (m, 3H), 7.27–7.24 (m, 3H), 7.09 (d, J = 9.2 Hz, 1H), 6.63 (d, J = 5.5 Hz, 1H), 3.10 (d, J = 6.1 Hz, 1H), 1.09 (s, 9H), 0.33 (s, 3H), 0.32 (s, 3H). FAB-MS m/z 371 $(M-OH)^+$, 388 $(M)^+$, 389 $(M+H)^+$.

Compounds **9b**, **9d**, **9f**, and **9g** were prepared using the same procedure as described for preparing **9a** from **8**.

5.2.3. 1-(2′-*tert*-Butyldimethylsilyloxynaphthalen-1′-yl)-3-(2″-methylphenyl)-2-propyn-1-ol (9b)

2-Ethynyltoluene (0.160 mL, 1.23 mmol), *n*-BuLi (0.780 mL, 1.30 mmol), and **8** (315 mg, 1.10 mmol) afforded **9b** (313 mg, 0.777 mmol, 71% yield) as a yellow oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.68 (d, J = 8.5 Hz, 1H), 7.79 (d, J = 7.9 Hz, 1H), 7.72 (d, J = 8.5 Hz, 1H), 7.53 (ddd, J = 8.6, 6.7, 1.2 Hz, 1H), 7.40–7.37 (m, 1H), 7.34 (d, J = 7.9 Hz, 1H), 7.18–7.12 (m, 2H), 7.09–7.06 (m, 2H), 6.70 (d, J = 5.5 Hz, 1H), 2.91 (d, J = 5.5 Hz, 1H), 2.33 (s, 3H), 1.09 (s, 9H), 0.32 (s, 3H), 0.31 (s, 3H). FAB-MS m/z 385 $(M-OH)^+$, 402 $(M)^+$, 403 $(M+H)^+$.

5.2.4. 1-(2′-*tert*-Butyldimethylsilyloxynaphthalen-1′-yl)-3-(2″-fluorophenyl)-2-propyn-1-ol (9d)

1-Ethynyl-2-fluorobenzene (0.180 mL, 1.59 mmol), *n*-BuLi (0.900 mL, 1.49 mmol), and **8** (228 mg, 0.797 mmol) afforded **9d** (218 mg, 0.536 mmol, 67% yield) as a yellow oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.61 (d, J = 8.5 Hz, 1H), 7.79 (d, J = 7.9 Hz, 1H), 7.73 (d, J = 9.2 Hz, 1H), 7.55 (ddd, J = 8.6, 6.7, 1.2 Hz, 1H), 7.40–7.34 (m, 2H), 7.24–7.23 (m, 1H), 7.09 (d, J = 8.5 Hz, 1H), 7.04–7.00 (m, 2H), 6.67 (d, J = 5.5 Hz, 1H), 3.08 (d, J = 6.1 Hz, 1H), 1.09 (s, 9H), 0.33 (s, 3H), 0.32 (s, 3H). FAB-MS m/z 389 $(M-OH)^+$, 406 $(M)^+$, 407 $(M+H)^+$.

5.2.5. 1-(2′-*tert*-Butyldimethylsilyloxynaphthalen-1′-yl)-3-(2″-methoxyphenyl)-2-propyn-1-ol (9f)

2-Ethynylanisole (410 mg, 3.01 mmol), *n*-BuLi (1.75 mL, 2.78 mmol), and **8** (633 mg, 2.21 mmol) afforded **9f** (637 mg, 1.52 mmol, 69% yield) as a yellow oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.73 (d, J = 7.9 Hz, 1H), 7.78 (d, J = 7.9 Hz, 1H), 7.71 (d, J = 8.5 Hz, 1H), 7.53 (ddd, J = 8.6, 6.7, 1.2 Hz, 1H), 7.39–7.36 (m, 1H), 7.31 (dd, J = 8.0, 1.8 Hz, 1H), 7.26–7.22 (m, 1H), 7.07 (d, J = 9.2 Hz, 1H), 6.85–6.81 (m, 2H), 6.68 (d, J = 5.5 Hz, 1H), 3.81 (s, 3H), 2.94 (d, J = 5.5 Hz, 1H), 1.08 (s, 9H), 0.32 (s, 3H), 0.31 (s, 3H). FAB-MS m/z 401 $(M-OH)^+$, 418 $(M)^+$, 419 $(M+H)^+$.

5.2.6. 1-(2′-*tert*-Butyldimethylsilyloxynaphthalen-1′-yl)-3-(2″-pyridinyl)-2-propyn-1-ol (9g)

2-Ethynylpyridine (0.260 mL, 2.57 mmol), *n*-BuLi (1.60 mL, 2.54 mmol), and **8** (540 mg, 1.89 mmol) afforded **9g** (342 mg, 0.878 mmol, 47% yield) as a brown oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.59 (d, J = 8.5 Hz, 1H), 8.54–8.53 (m, 1H), 7.79 (d, J = 8.5 Hz, 1H), 7.73 (d, J = 8.5 Hz, 1H), 7.61–7.57 (m, 1H), 7.55–7.52 (m, 1H), 7.39–7.36 (m, 1H), 7.35–7.33 (m, 1H), 7.18 (ddd, J = 7.3, 4.9, 1.2 Hz, 1H), 7.09 (d, J = 8.5 Hz, 1H), 6.68 (d, J = 5.5 Hz, 1H), 3.18 (d, J = 5.5 Hz, 1H), 1.08 (s, 9H), 0.34 (s, 3H), 0.32 (s, 3H). FAB-MS m/z 372 $(M-OH)^+$, 389 $(M)^+$, 390 $(M+H)^+$.

5.2.7. 1-(2′-*tert*-Butyldimethylsilyloxynaphthalen-1′-yl)-3-phenyl-2-propyn-1-one (10a)

A solution of **9a** (143 mg, 0.369 mmol) and MnO_2 (343 mg, 3.95 mmol) in dry CH_2Cl_2 (9 mL) was stirred at room temperature for 23 h and filtered through Celite. The filtrate was concentrated and the residue was purified by silica gel column chromatography (hexane/EtOAc = 10:1) to afford **10a** (92.9 mg, 0.240 mmol, 65% yield) as a yellow oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.00 (d, J = 8.5 Hz, 1H), 7.83 (d, J = 8.5 Hz, 1H), 7.80 (d, J = 7.9 Hz, 1H), 7.56–7.54 (m, 2H), 7.52–7.49 (m, 1H), 7.44–7.33 (m, 4H), 7.11 (d, J = 9.2 Hz, 1H), 0.99 (s, 9H), 0.26 (s, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) 181.3, 152.1, 133.1, 132.2, 131.1, 130.5, 128.9, 128.5, 128.1, 127.7, 126.0, 124.4, 123.7, 120.7, 120.4, 91.9, 90.6, 25.7, 18.3, −4.0. FAB-MS m/z 387 $(M+H)^+$.

5.2.8. 1-(2′-*tert*-Butyldimethylsilyloxynaphthalen-1′-yl)-3-(2″-methylphenyl)-2-propyn-1-one (10b)

To a solution of **9b** (313 mg, 0.777 mmol) in CH_2Cl_2 (1 mL) was added a solution of Dess–Martin periodinane (392 mg, 0.923 mmol) in CH_2Cl_2 (2.5 mL). The mixture was stirred at room temperature for 15 min, diluted with EtOAc (6 mL), quenched with 0.1 M $Na_2S_2O_3$ solution (6 mL), and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/EtOAc = 10:1) to afford **10b** (202 mg, 0.504 mmol, 65% yield) as a yellow oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.02 (d, J = 8.5 Hz, 1H), 7.82 (d, J = 9.2 Hz, 1H), 7.79 (d, J = 8.5 Hz, 1H), 7.52–7.48 (m, 2H), 7.40–7.37 (m, 1H), 7.32–7.29 (m, 1H), 7.20 (d, J = 7.9 Hz, 1H), 7.15 (dd, J = 7.6, 7.6 Hz, 1H), 7.11 (d, J = 8.5 Hz, 1H), 2.41 (s, 3H), 0.98 (s, 9H), 0.26 (s, 6H). FAB-MS m/z 401 $(M+H)^+$.

5.2.9. 1-(2′-*tert*-Butyldimethylsilyloxynaphthalen-1′-yl)-3-(2″-fluorophenyl)-2-propyn-1-one (10d)

The title compound **10d** (142 mg, 0.351 mmol, 65% yield) was synthesized from **9d** (218 mg, 0.536 mmol) and Dess–Martin periodinane (252 mg, 0.576 mmol) as a yellow oil following the same procedure as described for **10b** from **9b**.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.02 (d, J = 8.5 Hz, 1H), 7.84 (d, J = 8.5 Hz, 1H), 7.80 (d, J = 8.5 Hz, 1H), 7.55–7.50 (m, 2H), 7.44–7.38 (m, 2H), 7.14–7.08 (m, 3H), 0.99 (s, 9H), 0.27 (s, 6H). FAB-MS m/z 405 $(M+H)^+$.

5.2.10. 1-(2′-*tert*-Butyldimethylsilyloxynaphthalen-1′-yl)-3-(2″-methoxyphenyl)-2-propyn-1-one (10f)

To a solution of **9f** (637 mg, 1.52 mmol) and *t*-BuOH (0.150 mL, 1.64 mmol) in CH_2Cl_2 (1.2 mL) was added a solution of Dess–Martin periodinane (780 mg, 1.84 mmol) in CH_2Cl_2 (5.1 mL). The mixture was stirred at room temperature for 20 min, diluted with EtOAc (8.9 mL), quenched with $Na_2S_2O_3{\cdot}5H_2O$ (3.38 g, 13.6 mmol) in saturated $NaHCO_3$ solution (9.9 mL), and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, fil-

tered, and concentrated. The residue was purified by silica gel column chromatography (hexane/EtOAc = 10:1) to afford **10f** (338 mg, 0.811 mmol, 53% yield) as a yellow oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.04 (d, J = 8.5 Hz, 1H), 7.82 (d, J = 9.2 Hz, 1H), 7.79 (d, J = 8.5 Hz, 1H), 7.51–7.46 (m, 2H), 7.40–7.36 (m, 2H), 7.10 (d, J = 8.5 Hz, 1H), 6.92–6.89 (m, 1H), 6.87 (d, J = 8.5 Hz, 1H), 3.83 (s, 3H), 0.99 (s, 9H), 0.27 (s, 6H). FAB-MS m/z 417 $(M+H)^+$.

5.2.11. 1-(2′-*tert*-Butyldimethylsilyloxynaphthalen-1′-yl)-3-(2″-pyridinyl)-2-propyn-1-one (10g)

The title compound **10g** (25.8 mg, 66.6 μmol, 72% yield) was synthesized from **9g** (36.0 mg, 92.1 μmol), *t*-butanol (10.0 μL, 0.109 mmol), and Dess–Martin periodinane (64.7 mg, 0.153 mmol) as a yellow oil following the same procedure as described for **10f** from **9f**.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.64–8.62 (m, 1H), 8.01 (d, J = 8.5 Hz, 1H), 7.84 (d, J = 9.2 Hz, 1H), 7.79 (d, J = 7.9 Hz, 1H), 7.72–7.68 (m, 1H), 7.59–7.58 (m, 1H), 7.52–7.49 (m, 1H), 7.40–7.37 (m, 1H), 7.31 (ddd, J = 8.0, 4.9, 1.3 Hz, 1H), 7.09 (d, J = 8.5 Hz, 1H), 1.00 (s, 9H), 0.28 (s, 6H). FAB-MS m/z 388 $(M+H)^+$.

5.2.12. 1-(2′-*tert*-Butyldimethylsilyloxynaphthalen-1′-yl)-3-(*N*-methylpiperazinyl)-3-phenyl-2-propen-1-one (11a)

Step (i): A solution of **10a** (182 mg, 0.471 mmol) and *N*-methylpiperazine (70.0 μL, 0.636 mmol) in dry THF (2 mL) and dry MeOH (1 mL) was stirred at room temperature for 2 h under Ar and concentrated. Step (ii) To the residue was added MeOH (3 mL), and the solution was stirred at reflux for 7 h. Then water was added and the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/EtOAc = 10:1 and hexane/EtOAc = 5:1) to afford **11a** (106 mg, 0.218 mmol, 46% yield) as a yellow oil, **1a** (26.8 mg, 98.4 μmol, 21% yield), and **12a** (7.80 mg, 28.6 μmol, 5.9% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.72 (d J = 8.5 Hz, 1H), 7.55 (d, J = 7.9 Hz, 1H), 7.38–7.35 (m, 1H), 7.33 (d, J = 9.1 Hz, 1H), 7.24 (t, J = 7.3 Hz, 1H), 7.00–6.96 (m, 2H), 6.91 (t, J = 7.3 Hz, 1H), 6.82–6.77 (m, 2H), 6.62 (d, J = 9.2 Hz, 1H), 5.85 (s, 1H), 3.22–3.11 (m, 4H), 2.41–2.33 (m, 4H), 2.28 (s, 3H), 1.04 (s, 9H), 0.19 (s, 6H). FAB-MS m/z 487 $(M+H)^+$.

5.2.13. 2-Phenylmethylene-naphtho[2,1-*b*]furan-1(2*H*)-one (12a)

The title compound was obtained as a by-product in the synthesis of **11a**.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.88 (d, J = 8.5 Hz, 1H), 8.15 (d, J = 8.5 Hz, 1H), 7.98 (d, J = 7.3 Hz, 2H), 7.90 (d, J = 7.9 Hz, 1H), 7.74–7.71 (m, 1H), 7.55–7.52 (m, 1H), 7.50–7.48 (m, 3H), 7.43 (t, J = 7.3 Hz, 1H), 6.98 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) 184.4, 167.9, 147.4, 138.8, 132.2, 131.5, 129.9, 129.84, 129.78, 129.2, 128.8, 128.6, 125.7, 123.6, 113.1, 112.9. FAB-MS m/z 273 $(M+H)^+$.

5.2.14. 1-(2′-*tert*-Butyldimethylsilyloxynaphthalen-1′-yl)-3-(2″-methylphenyl)-3-(*N*-methylpiperazinyl)-2-propen-1-one (11b)

The title compound **11b** (145 mg, 0.290 mmol, 58% yield) was synthesized from **10b** (202 mg, 0.504 mmol) and *N*-methylpiperazine (70.0 μL, 0.636 mmol) as a yellow oil following the same procedure as described for **11a** from **10a**, except that EtOH was used as a solvent in step (ii). **1b** (13.8 mg, 48.2 μmol, 10% yield) and **12b** (14.0 mg, 48.9 μmol, 10% yield) were also obtained as a colorless solid and a yellow solid, respectively.

FAB-MS m/z 501 $(M+H)^+$.

5.2.15. 2-(2′-Methylphenylmethylene)-naphtho[2,1-*b*]furan-1(2*H*)-one (12b)

The title compound was obtained as a by-product in the synthesis of **11b**.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.89 (d, J = 7.3 Hz, 1H), 8.29 (d, J = 7.9 Hz, 1H), 8.14 (d, J = 9.2 Hz, 1H), 7.90 (d, J = 8.5 Hz, 1H), 7.74–7.71 (m, 1H), 7.55–7.52 (m, 1H), 7.47 (d, J = 8.6 Hz, 1H), 7.37–7.29 (m, 3H), 7.23 (s, 1H), 2.55 (s, 3H). FAB-MS m/z 287 $(M+H)^+$.

5.2.16. 3-Phenyl-1*H*-naphtho[2,1-*b*]pyran-1-one (1a)

A solution of **11a** (93.3 mg, 0.192 mmol) in DMSO (1 mL) and H_2O (0.2 mL) was stirred at 90 °C for 9.5 h under Ar. Water was added and the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/EtOAc = 10:1) to afford **1a** (40.5 mg, 0.149 mmol, 78% yield) as a colorless solid. **1a** was crystallized from EtOAc/hexane.

Mp 165–167 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.10 (br d, J = 8.2 Hz, 1H), 8.14 (d, J = 9.2 Hz, 1H), 8.00–7.98 (m, 2H), 7.93 (br d, J = 7.9 Hz, 1H), 7.78 (ddd, J = 8.2, 6.7, 1.5 Hz, 1H), 7.66 (d, J = 9.2 Hz, 1H), 7.64 (ddd, J = 7.9, 6.7, 1.2 Hz, 1H), 7.57–7.55 (m, 3H), 7.00 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 180.4, 160.9, 157.5, 135.6, 131.5, 130.7, 130.6, 129.3, 129.2, 128.2, 127.3, 126.7, 126.2, 117.7, 117.4, 110.5. FAB-MS m/z 273 $(M+H)^+$. UV monitoring of HPLC at 280 nm.

5.2.17. 3-(2′-Methylphenyl)-1*H*-naphtho[2,1-*b*]pyran-1-one (1b)

Step (i): 1-(2′-Hydroxynaphthalen-1′-yl)-3-(2″-methylphenyl)-3-(*N*-methylpiperazinyl)-2-propen-1-one (94.5 mg, 0.245 mmol, 84% yield) was synthesized from **11b** (145 mg, 0.290 mmol) as a yellow solid following the same procedure as described for **1a** from **11a**. **1b** (5.90 mg, 20.6 μmol, 7.1% yield) was also obtained as a colorless solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.45 (d, J = 8.5 Hz, 1H), 7.74 (d, J = 7.9 Hz, 1H), 7.73 (d, J = 9.2 Hz, 1H), 7.50–7.47 (m, 1H), 7.38–7.28 (m, 4H), 7.21 (dd, J = 7.3, 1.2 Hz, 1H), 7.07 (d, J = 9.2 Hz, 1H), 6.07 (s, 1H), 3.58–3.08 (m, 4H), 2.45–2.36 (m, 4H), 2.36 (s, 3H), 2.31 (s, 3H). FAB-MS m/z 387 $(M+H)^+$.

Step (ii): The title compound **1b** (43.4 mg, 0.152 mmol, 68% yield) was synthesized from 1-(2′-hydroxynaphthalen-1′-yl)-3-(2″-methylphenyl)-3-(*N*-methylpiperazinyl)-2-propen-1-one (86.6 mg, 0.224 mmol) as a colorless solid following the method described in step (ii) for **11a** from **10a**, except for the use of EtOH as a solvent. **1b** was crystallized from EtOAc/hexane.

Mp 135–137 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.10 (br d, J = 8.5 Hz, 1H), 8.12 (d, J = 8.8 Hz, 1H), 7.93 (br d, J = 7.9 Hz, 1H), 7.79 (ddd, J = 8.5, 6.7, 1.5 Hz, 1H), 7.64 (br dd, J = 7.9, 6.7 Hz, 1H), 7.59 (br d, J = 7.3 Hz, 1H), 7.56 (d, J = 8.8 Hz, 1H), 7.44 (br dd, J = 7.3, 7.3 Hz, 1H), 7.36 (br d, J = 7.3 Hz, 1H), 7.35 (br dd, J = 7.3, 7.3 Hz, 1H), 6.67 (s, 1H), 2.54 (s, 3H). FAB-MS m/z 287 $(M+H)^+$. Anal. Calcd for $C_{20}H_{14}O_2$: C, 83.90; H, 4.93. Found: C, 83.61; H, 5.12. UV monitoring of HPLC at 263 nm.

Compounds **10d** and **10g** were prepared according to step (i) described for preparing **11a** from **10a** and the procedure described for preparing **1a** from **11a**.

5.2.18. 3-(2′-Fluorophenyl)-1*H*-naphtho[2,1-*b*]pyran-1-one (1d)

Compound **10d** (124 mg, 0.307 mmol) and *N*-methylpiperazine (50.0 μL, 0.454 mmol) afforded **1d** (37.3 mg, 0.128 mmol, 42% yield) and **12d** (5.10 mg, 17.6 μmol, 5.7% yield) as a pale yellow solid and a yellow solid, respectively. **1d** was crystallized from EtOAc/hexane.

Mp 157 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.08 (br d, J = 8.9 Hz, 1H), 8.13 (d, J = 9.2 Hz, 1H), 8.00 (ddd, J = 7.6, 7.6, 1.5 Hz, 1H), 7.93 (br d, J = 7.9 Hz, 1H), 7.79 (ddd, J = 8.9, 6.7, 1.5 Hz, 1H), 7.64 (ddd, J = 7.9, 6.7, 1.2 Hz, 1H), 7.62 (d, J = 9.2 Hz, 1H), 7.55–7.51 (m, 1H), 7.35 (br dd, J = 7.6, 7.6 Hz, 1H), 7.29–7.24 (m, 1H), 7.12 (s, 1H). FAB-MS m/z 291 $(M+H)^+$. Anal. Calcd for $C_{19}H_{11}FO_2$: C, 78.61; H, 3.82. Found: C, 78.31; H, 4.06. UV monitoring of HPLC at 271 nm.

5.2.19. 3-(2′-Pyridinyl)-1*H*-naphtho[2,1-*b*]pyran-1-one (1g)

Compound **10g** (32.5 mg, 83.9 μmol) and *N*-methylpiperazine (14.0 μL, 0.127 mmol) afforded **1g** (13.2 mg, 48.3 μmol, 58% yield) as a pale yellow solid. **1g** was crystallized from EtOAc/hexane.

Mp 187–188 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.11 (br d, J = 8.5 Hz, 1H), 8.79 (dd, J = 5.2, 1.8 Hz, 1H), 8.15 (d, J = 8.9 Hz, 1H), 8.14 (dd, J = 7.9, 1.2 Hz, 1H), 7.94 (br d, J = 7.9 Hz, 1H), 7.92 (ddd, J = 7.9, 7.9, 1.8 Hz, 1H), 7.79 (ddd, J = 8.5, 6.7, 1.5 Hz, 1H), 7.69 (d, J = 8.9 Hz, 1H), 7.65 (ddd, J = 7.9, 6.7, 1.2 Hz, 1H), 7.56 (s, 1H), 7.45 (ddd, J = 7.9, 5.2, 1.2 Hz, 1H). FAB-MS m/z 274 $(M+H)^+$. Anal. Calcd for $C_{18}H_{11}NO_2$: C, 79.11; H, 4.06; N, 5.13. Found: C, 78.98; H, 4.30; N, 5.19. UV monitoring of HPLC at 288 nm.

5.2.20. 2-(2′-Fluorophenylmethylene)-naphtho[2,1-*b*]furan-1(2*H*)-one (12d)

This compound was obtained as a by-product in the synthesis of **1d**.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.88 (d, J = 8.5 Hz, 1H), 8.38–8.35 (m, 1H), 8.16–8.11 (m, 1H), 7.91–7.87 (m, 1H), 7.74–7.68 (m, 1H), 7.55–7.50 (m, 1H), 7.47 (d, J = 9.2 Hz, 1H), 7.43–7.38 (m, 1H), 7.30–7.25 (m, 2H), 7.17–7.11 (m, 1H). FAB-MS m/z 291 $(M+H)^+$.

5.2.21. 3-(2′-Methoxyphenyl)-1*H*-naphtho[2,1-*b*]pyran-1-one (1f)

The title compound **1f** (183 mg, 0.605 mmol, 64% yield) was synthesized from **10f** (395 mg, 0.948 mmol) and *N*-methylpiperazine (0.160 mL, 1.45 mmol) as a yellow solid according to step (i) described for preparing **11a** from **10a**, the procedure for **1a** from **11a**, and step (ii) described for preparing **11a** from **10a**, except for the use of EtOH as a solvent. **1f** was crystallized from EtOAc/hexane.

Mp 192–193 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.11 (br d, J = 8.5 Hz, 1H), 8.10 (d, J = 8.9 Hz, 1H), 7.98 (dd, J = 7.3, 1.8 Hz, 1H), 7.91 (br d, J = 8.2 Hz, 1H), 7.77 (ddd, J = 8.5, 6.7, 1.5 Hz, 1H), 7.62 (ddd, J = 8.2, 6.7, 1.2 Hz, 1H), 7.61 (d, J = 8.9 Hz, 1H), 7.50 (ddd, J = 8.9, 7.3, 1.8 Hz, 1H), 7.32 (s, 1H), 7.14 (ddd, J = 7.3, 7.3, 1.2 Hz, 1H), 7.07 (br d, J = 8.9 Hz, 1H), 3.98 (s, 3H). FAB-MS m/z 303 $(M+H)^+$. Anal. Calcd for $C_{20}H_{14}O_3$: C, 79.46; H, 4.67. Found: C, 79.21; H, 4.89. UV monitoring of HPLC at 271 nm.

5.2.22. (*E*)-1-(2′-Hydroxynaphthalen-1′-yl)-3-(2″,6″-dimethylphenyl)-2-propen-1-one (16c)

To a solution of **13** (610 mg, 3.28 mmol) in EtOH (10 mL) was added a 3.3 M NaOH solution (10 mL). The resultant solution was cooled to 0 °C in an ice bath and 2,6-dimethylbenzaldehyde (530 mg, 3.95 mmol) was slowly added. The mixture was stirred at room temperature for 3.5 h, at 50 °C for 1.25 h, at 70 °C for 2.5 h, and room temperature for 16 h, then quenched with saturated NH_4Cl solution and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/EtOAc = 10:1). The obtained solid was washed with hexane and EtOAc (20:1) to afford **16c** (526 mg, 1.74 mmol, 53% yield) as a yellow solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 12.64 (s, 1H), 8.14 (d, J = 15.9 Hz, 1H), 8.04 (d, J = 8.5 Hz, 1H), 7.92 (d, J = 8.5 Hz, 1H), 7.80 (d, J = 7.9 Hz, 1H), 7.51–7.48 (m, 1H), 7.40–7.37 (m, 1H), 7.20 (d, J = 8.5 Hz, 1H), 7.18 (t, J = 7.3 Hz, 1H), 7.16 (d, J = 15.9 Hz, 1H), 7.11 (d, J = 7.3 Hz, 2H), 2.44 (s, 6H). FAB-MS m/z 303 $(M+H)^+$.

5.2.23. 2-(2′-Tetrahydropyranyloxy)-1-acetonaphthone (14)

To a solution of **13** (707 mg, 3.80 mmol) and pyridinium *p*-toluenesulfonate (24.5 mg, 97.0 μmol) in CH_2Cl_2 (15 mL) was added a solution of 3,4-dihydro-2*H*-pyran (2.00 mL, 21.9 mmol) in CH_2Cl_2 (3 mL). The mixture was stirred at room temperature for 23 h and at 35 °C for 5 h, then washed with saturated $NaHCO_3$ solution, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/EtOAc = 10:1) to afford **14** (874 mg, 3.23 mmol, 85% yield) as a colorless solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.84 (d, J = 9.2 Hz, 1H), 7.79 (d, J = 7.9 Hz, 1H), 7.75 (d, J = 8.5 Hz, 1H), 7.49–7.46 (m, 1H), 7.47 (d, J = 9.2 Hz, 1H), 7.40–7.36 (m, 1H), 5.61 (t, J = 3.1 Hz, 1H), 3.93–3.88 (m, 1H), 3.67–3.64 (m, 1H), 2.70 (s, 3H), 1.98–1.90 (m, 3H), 1.74–1.69 (m, 2H), 1.65–1.62 (m, 1H). FAB-MS m/z 271 $(M+H)^+$.

5.2.24. (*E*)-1-(2′-Tetrahydropyranyloxynaphthalen-1′-yl)-3-(2″,6″-difluorophenyl)-2-propen-1-one (15e)

To a solution of **14** (865 mg, 3.20 mmol) and $Ba(OH)_2{\cdot}8H_2O$ (1.36 g, 4.31 mmol) in MeOH (15 mL) was added 2,6-difluorobenzaldehyde (0.400 mL, 3.75 mmol). The mixture was stirred at room temperature for 17 h, water (15 mL) was added, then the reaction was quenched with 2 M HCl (2.5 mL), and the whole was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/EtOAc = 10:1 and hexane/EtOAc = 5:1) to afford **15e** (890 mg, 2.26 mmol, 71% yield) as a yellow amorphous solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.90 (d, J = 9.2 Hz, 1H), 7.82 (d, J = 7.9 Hz, 1H), 7.79 (d, J = 8.5 Hz, 1H), 7.60 (d, J = 16.8 Hz, 1H), 7.52 (d, J = 9.2 Hz, 1H), 7.49 (d, J = 17.1 Hz, 1H), 7.47–7.45 (m, 1H), 7.40–7.37 (m, 1H), 7.30 (tt, J = 8.5, 6.4 Hz, 1H), 6.92 (dd, J = 8.5, 8.5 Hz, 2H), 5.63 (t, J = 2.7 Hz, 1H), 3.91–3.86 (m, 1H), 3.65–3.60 (m, 1H), 1.88–1.80 (m, 3H), 1.70–1.62 (m, 1H), 1.58–1.49 (m, 2H). FAB-MS m/z 395 $(M+H)^+$.

5.2.25. (*E*)-1-(2′-Hydroxynaphthalen-1′-yl)-3-(2″,6″-difluorophenyl)-2-propen-1-one (16e)

A solution of **15e** (482 mg, 1.21 mmol) and *p*-TsOH monohydrate (17.7 mg, 93.0 μmol) in MeOH (7 mL) was stirred at room temperature for 61 h, then water (7 mL) was added. The solution was alkalized with saturated $NaHCO_3$ solution (3.5 mL), and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/EtOAc = 10:1) to afford **16e** (144 mg, 0.464 mmol, 38% yield) as an orange solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 12.72 (s, 1H), 8.11 (d, J = 8.5 Hz, 1H), 8.01 (d, J = 16.5 Hz, 1H), 7.93 (d, J = 9.2 Hz, 1H), 7.85–7.80 (m, 2H), 7.57–7.53 (m, 1H), 7.43–7.40 (m, 1H), 7.35 (tt, J = 8.5, 6.3 Hz, 1H), 7.19 (d, J = 9.2 Hz, 1H), 6.98 (dd, J = 8.5, 8.5 Hz, 2H). FAB-MS m/z 311 $(M+H)^+$.

5.2.26. 3-(2′,6′-Dimethylphenyl)-1*H*-naphtho[2,1-*b*]pyran-1-one (1c)

To a solution of **16c** (235 mg, 0.777 mmol) in 1,4-dioxane (96 mL) was added DDQ (361 mg, 1.59 mmol) and the mixture was stirred at 110 °C for 3 h. Water was added, and the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/EtOAc = 10:1) to afford **1c** (119 mg, 0.396 mmol, 51% yield) as a pale yellow amorphous solid. **1c** was crystallized from EtOAc/hexane.

Mp 92 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.11 (br d, J = 8.5 Hz, 1H), 8.11 (d, J = 9.2 Hz, 1H), 7.93 (br d, J = 7.9 Hz, 1H), 7.79 (ddd, J = 8.5, 7.3, 1.5 Hz, 1H), 7.65 (ddd, J = 7.9, 7.3, 1.2 Hz, 1H), 7.52 (d, J = 9.2 Hz, 1H), 7.32 (t, J = 7.9 Hz, 1H), 7.18 (d, J = 7.9 Hz, 2H), 6.52 (s, 1H), 2.32 (s, 6H). FAB-MS m/z 301 $(M+H)^+$. Anal. Calcd for $C_{21}H_{16}O_2$: C, 83.98; H, 5.37. Found: C, 84.01; H, 5.53. UV monitoring of HPLC at 263 nm.

5.2.27. 3-(2′,6′-Difluorophenyl)-1*H*-naphtho[2,1-*b*]pyran-1-one (1e)

The title compound **1e** (77.8 mg, 0.251 mmol, 55% yield) was synthesized from **16e** (142 mg, 0.457 mmol) and DDQ (211 mg, 0.930 mmol) according to the same procedure as described for **1c** from **16c**, as a pale yellow solid. **1e** was crystallized from EtOAc/hexane.

Mp 150 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.07 (br d, J = 8.5 Hz, 1H), 8.12 (d, J = 9.2 Hz, 1H), 7.93 (br d, J = 8.2 Hz, 1H), 7.79 (ddd, J = 8.5, 6.7, 1.5 Hz, 1H), 7.65 (ddd, J = 8.2, 7.3, 1.2 Hz, 1H), 7.56 (d, J = 9.2 Hz, 1H), 7.50 (tt, J = 8.5, 6.1 Hz, 1H), 7.09 (dd, J = 8.5, 8.5 Hz, 2H), 6.79 (s, 1H). FAB-MS m/z 309 $(M+H)^+$. Anal. Calcd for $C_{19}H_{10}F_2O_2$: C, 74.03; H, 3.27. Found: C, 73.74; H, 3.49. UV monitoring of HPLC at 263 nm.

5.3. Biology

5.3.1. EROD assay

EROD assay was carried out with MCF-7 cells as described by Lee and Safe with some modifications. [30]MCF-7 cells were cultured in D-MEM (High Glucose) containing L-glutamine and Phenol red (Wako Pure Chemical Industries, Ltd) with 10% (v/v) fetal bovine serum (Gibco) and penicillin–streptomycin Mixed Solution (Nacalai tesque). Trypsinized cells (5×10^5 cells/mL) were plated in a 96-well plate at 125 μL/well and incubated for 4.5 h (37 °C, 5% CO_2). Fresh medium and DMSO containing test compound (3% DMSO, 25 μL) were added and incubation was continued for 24 h. The cells were washed with PBS (160 μL). PBS (40 μL) was added to each well and the cells were incubated in a water bath at 37 °C for 2 min. The reaction was started by adding ethoxyresorufin (24 μM in PBS, 40 μL), dicoumarol (30 μM in PBS, 40 μL), and NADPH (7 mM in PBS, 20 μL). Cells were incubated in a water bath at 37 °C for 150 min. Resorufin was measured at excitation/emission wavelengths of 540/590 nm. Fluorescamine was added (1 mM in acetonitrile, 30 μL) to measure proteins. The fluorescence difference (excitation/emission wavelengths of 355/460 nm) between before and immediately after the addition of fluorescamine was measured, because NADPH showed fluorescence at the wavelengths. Fluorescence was measured using a Wallac 1420 ARVO sx (Perkin–Elmer). All data points were measured in triplicate. The AhR activity was determined by dividing resorufin fluorescence by protein fluorescence.

5.3.2. Cell viability

Cell viability was measured following the same procedure as described for EROD assay. After incubation for 24 h, the medium was removed. Trypsin/EDTA (0.25%, 100 μL) was added and cells were counted.

5.3.3. Thermodynamic aqueous solubility

The thermodynamic solubility determination was based on the method of Avdeef and Testa.[34] Briefly, about 1 mg of compound was ground with an agate mortar and taken up in 1.0 mL of an equal volume of a mixture of 1/15 M phosphate buffer (pH 7.4) and EtOH. The suspension was shaken for 48 h at 25 °C. An aliquot was filtered through a Millipore DIMEX-13 (0.22 μm). The filtrate was diluted in DMF and injected into an HPLC with UV detection; peak areas were recorded at 263–288 nm. The concentration of the sample solution was calculated using a previously determined calibration curve, corrected for the dilution factor of the sample.

5.3.4. DFT calculation

All calculations were performed at the DFT level, by means of the hybrid Becke3LYP[39–42] (B3LYP) function as implemented in GAUSSIAN 2003.[35] The 6-31G* basis set was used for the H, C, N, O and F atoms. Geometry optimization and vibrational analysis were performed at the same level. All stationary points were optimized without any symmetry assumptions and characterized by normal coordinate analysis at the same level of the theory (number of imaginary frequencies, Nimag, 0).

Acknowledgments

The work described in this paper was partially supported by Grants-in-Aid for Scientific Research from The Ministry of Education, Culture, Sports, Science and Technology, Japan, and the Japan Society for the Promotion of Science.

References and notes

1. Denison, M. S.; Pandini, A.; Nagy, S. R.; Baldwin, E. P.; Bonati, L. *Chem. Biol. Interact.* **2002**, *141*, 3.
2. Mimura, J.; Fujii-Kuriyama, Y. *Biochim. Biophys. Acta.* **2003**, *1619*, 263.
3. Birnbaum, L. S.. In *Progress in Clinical and Biological Research*; Spitzer, H. L., Slaga, T. J., Greenlee, W. F., McClain, M., Eds.; Wiley-Liss: New York, 1994; Vol. 387, pp 139–154.
4. Esser, C.; Rannug, A.; Stockinger, B. *Trend Immunol.* **2009**, *30*, 447.
5. Quintana, F. J.; Basso, A. S.; Iglesias, A. H.; Korn, T.; Farez, M. F.; Bettelli, E.; Caccamo, M.; Oukka, M.; Weiner, H. L. *Nature* **2008**, *453*, 65.
6. Veldhoen, M.; Hirota, K.; Westendorf, A. M.; Buer, J.; Dumoutier, L.; Renauld, J.-C.; Stockinger, B. *Nature* **2008**, *453*, 106.
7. Marple, L.; Brunck, R.; Throop, L. *Environ. Sci. Technol.* **1986**, *20*, 180.
8. Hansch, C.; Quinlan, J. E.; Lawrence, G. L. *J. Org. Chem.* **1968**, *33*, 347.
9. Valvani, S. C.; Yalkowsky, S. H.; Roseman, T. J. *J. Pharm. Sci.* **1981**, *70*, 502.
10. Ishikawa, M.; Kubota, D.; Yamamoto, M.; Kuroda, C.; Iguchi, M.; Koyanagi, A.; Murakami, S.; Ajito, K. *Bioorg. Med. Chem.* **2006**, *14*, 2109.
11. Ishikawa, M.; Hiraiwa, Y.; Kubota, D.; Tsushima, M.; Watanabe, T.; Murakami, S.; Ouchi, S.; Ajito, K. *Bioorg. Med. Chem.* **2006**, *14*, 2131.
12. Valkó, K. *J. Chromatogr., A* **2004**, *1037*, 299.
13. Banerjee, S.; Yalkowsky, S. H.; Valvani, S. C. *Environ. Sci. Technol.* **1980**, *10*, 1227.
14. Jain, N.; Yalkowsky, S. H. *J. Pharm. Sci.* **2001**, *90*, 234.
15. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Delivery Rev.* **2001**, *46*, 3.
16. Ishikawa, M.; Ajito, K. In *Soyaku Shien Kenkyu no Tenbo; Torisawa*; Y: Ed.; CMC Publishing, Tokyo, 2008. pp 3–13.
17. Sugihara, K.; Okayama, T.; Kitamura, S.; Yamashita, K.; Yasuda, M.; Miyairi, S.; Minobe, Y.; Ohta, S. *Arch. Toxicol.* **2008**, *82*, 5.
18. Harkat, H.; Blanc, A.; Weibel, J.-M.; Pale, P. *J. Org. Chem.* **2008**, *73*, 1620.
19. Bhat, A. S.; Whetstone, J. L.; Brueggemeier, R. W. *J. Comb. Chem.* **2000**, *2*, 597.
20. Maiti, G.; Roy, S. C. *Tetrahedron Lett.* **1996**, *38*, 495.
21. http://riodb01.ibase.aist.go.jp/sdbs/(National Institute of Advanced Industrial Science and Technology, April 2009).
22. Joseph-Nathan, P.; Santillan, R. L. *Spectrochem. Acta, Part A* **1984**, *40*, 1077.
23. Anies, C.; Pancrazi, A.; Lallemand, J.-Y.; Prange, T. *Bull. Soc. Chim. Fr.* **1997**, *134*, 203.
24. Minatti, A.; Zheng, X.; Buchwald, S. L. *J. Org. Chem.* **2007**, *72*, 9253.
25. Nam, N.-H.; Kim, Y.; You, Y.-J.; Hong, D.-H.; Kim, H.-M.; Ahn, B.-Z. *Eur. J. Med. Chem.* **2003**, *38*, 179.
26. Rao, Y. K.; Fang, S.-H.; Tzeng, Y.-M. *Bioorg. Med. Chem.* **2005**, *13*, 6850.
27. Holth, T. F.; Beylich, B. A.; Skarphedinsdottir, H.; Liewenborg, B.; Grung, M.; Hylland, K. *Environ. Sci. Technol.* **2009**, *43*, 3329.
28. Petrulis, J. R.; Chen, G.; Benn, S.; LaMarre, J.; Bunce, N. J. *Environ. Toxicol.* **2001**, *16*, 177.
29. Willett, K. L.; Gardinali, P. R.; Sericano, J. L.; Wade, T. L.; Safe, S. H. *Arch. Environ. Contam. Toxicol.* **1997**, *32*, 442.
30. Lee, J.-E.; Safe, S. *Toxicol. Sci.* **2000**, *58*, 235.
31. Peters, A. K.; van Londen, K.; Bergman, Å.; Bohonowych, J.; Denison, M. S.; van den Berg, M.; Sanderson, J. T. *Toxicol. Sci.* **2004**, *82*, 488.
32. Han, E. H.; Kim, J. Y.; Jeong, H. G. *Arch. Pharm. Res.* **2006**, *29*, 570.
33. Sanderson, J. T.; Aarts, J. M. M. J. G.; Brouwer, A.; Froese, K. L.; Denison, M. S.; Giesy, J. P. *Toxicol. Appl. Pharmacol.* **1996**, *137*, 316.
34. Avdeef, A.; Testa, B. *Cell Mol. Life Sci.* **2002**, *59*, 1681.
35. All calculations were carried out with a GAUSSIAN 03 program package. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.;

Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. *GAUSSIAN 03, Revision E.01*: Gaussian: Wallingford, CT, 2004.

36. Metrangolo, P.; Resnati, G. *Chem. Eur. J.* **2001**, *7*, 2511.
37. Leach, A. G.; Jones, H. D.; Cosgrove, D. A.; Kenny, P. W.; Ruston, L.; MacFaul, P.; Wood, J. M.; Colclough, N.; Brian Law, B. *J. Med. Chem.* **2006**, *49*, 6672.
38. Anderson, D. R.; Meyers, M. J.; Vernier, W. F.; Mahoney, M. W.; Kurumbail, R. G.; Caspers, N.; Poda, G. I.; Schindler, J. F.; Reitz, D. B.; Mourey, R. J. *J. Med. Chem.* **2007**, *50*, 2647.
39. Becke, A. D. *Phys. Rev.* **1988**, *A38*, 3098.
40. Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 1372.
41. Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648.
42. Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev.* **1988**, *B37*, 785.