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Design, Synthesis, and Biological Evaluation of Novel Transrepression-Selective Liver X Receptor (LXR) Ligands with 5,11-Dihydro-5-methyl-11-methylene-6*H*-dibenz[*b*,*e*]azepin-6-one Skeleton

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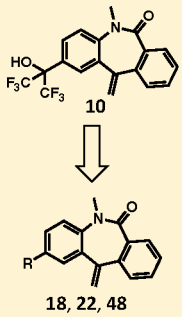
Supporting Information



Compd.	R	Percent inhibition of IL-6 production (%) at 10 μM	Transactivational agonistic activity (EC_{50} (μM)) LXRα (% efficacy)	LXRβ (% efficacy)
10	–	71%	1.7 ± 0.53 (45)	0.77 ± 0.102 (73)
18		51%	NA	NA
22		61%	NA	NA
48		78%	NA	NA

ABSTRACT: To obtain novel transrepression-selective liver X receptor (LXR) ligands, we adopted a strategy of reducing the transactivational agonistic activity of the 5,11-dihydro-5-methyl-11-methylene-6*H*-dibenz[*b*,*e*]azepin-6-one derivative **10**, which exhibits LXR-mediated transrepressional and transactivational activity. Structural modification of **10** based on the reported X-ray crystal structure of the LXR ligand-binding domain led to a series of compounds, of which almost all exhibited transrepressional activity at 1 or 10 μM but showed no transactivational activity even at 30 μM. Among the compounds obtained, **18** and **22** were confirmed to have LXR-dependent transrepressional activity by using peritoneal macrophages from wild-type and LXR-null mice. A newly developed fluorescence polarization assay indicated that they bind directly to LXRα. Next, further structural modification was performed with the guidance of docking simulations with LXRα, focusing on enhancing the binding of the ligands with LXRα through the introduction of substituents or heteroatom(s). Among the compounds synthesized, compound **48**, bearing a hydroxyl group, showed potent, selective, and dose-dependent transrepressional activity.

1. INTRODUCTION

Nuclear receptors (NRs) belong to the structurally conserved nuclear receptor superfamily of ligand-dependent transcription factors, which control diverse biological functions including reproduction, differentiation, homeostasis, and the immune system.[1] Forty-eight kinds of NRs have been identified. Among them, liver X receptors (LXRs) were originally orphan receptors, and their endogenous ligands were not known.[2] However, in 1996, Janowsky et al. indicated that several oxysterols, including 22(*R*)-hydroxycholesterol (**1**, Figure 1) and 24(*S*),25-epoxycholesterol (**2**), were endogenous ligands of LXRα, and they proposed that LXRα functions as an internal cholesterol sensor.[3] LXRs consist of two subtypes, LXRα and LXRβ, which have about 78% amino acid homology of the ligand binding domain (LBD); in particular, the surfaces of the ligand-binding pockets are almost identical.[4] LXRα is highly expressed in liver, intestine, and macrophages, while LXRβ seems to be ubiquitous in organs and tissues. LXRα and LXRβ are known to function coordinately. Oxysterols act on both LXRα and LXRβ and activate the transcription of LXR target genes, such as *abca1*, *abcg1*, *apoe*, and *glut4*, which have the LXR response element (LXRE) on their promoter region.[5] As the products of these genes are involved in lipid metabolism, reverse cholesterol transport, and glucose transport, it is thought that LXRs are promising drug targets for treatment of atherosclerosis, hyperlipidemia, or metabolic syndrome. This LXR-mediated activation of gene transcription by certain

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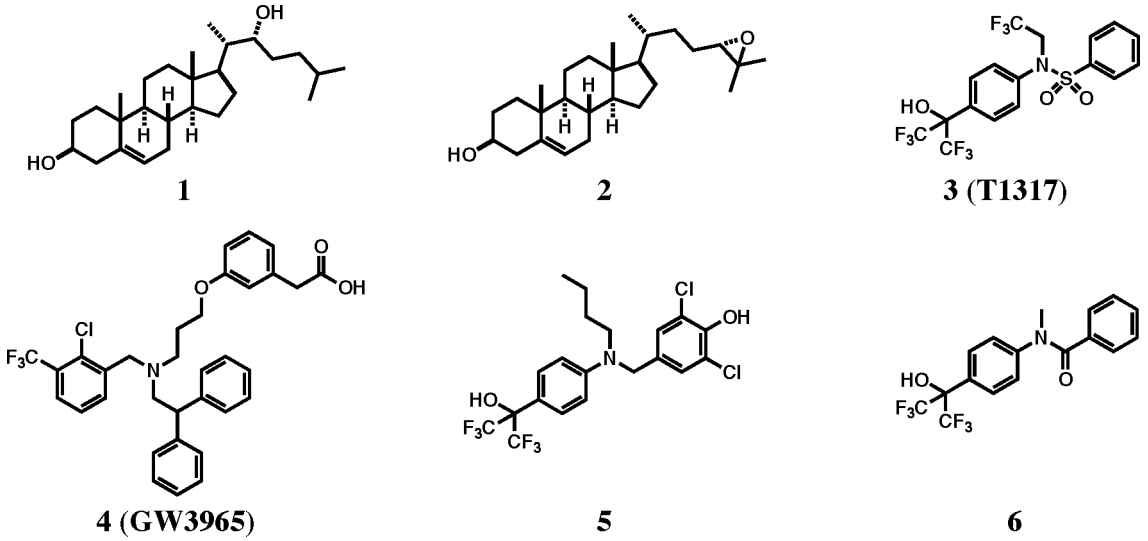

Figure 1. Chemical structures of endogenous LXR ligands (**1** and **2**), synthetic ligands (**3**, **4**, and **6**), and a transrepression-selective ligand (**5**).

oxysterols is called *transactivational* action. Currently known LXR ligands can be classified into two categories with respect to transactivational action, i.e., those that activate expression of the target genes and those that repress expression of the target genes. We define these as transactivational agonists and transactivational antagonists, respectively. It has been shown that the activities of LXR ligands depend upon their effect on recruitment of cofactors to helix 12 of the LXRs;[6] many transactivational agonists induce dissociation of corepressors (e.g., nuclear receptor corepressor (NCoR) and silencing mediator for retinoid and tyroid receptors (SMRT)) and association of coactivators (e.g., steroid receptor coactivator 1 (SRC1) and vitamin D receptor interacting protein (DRIP)), whereas many transactivational antagonists stabilize the binding of corepressors. Most of the reported LXR ligands, including *N*-2,2,2-trifluoroethyl-*N*-[4-(2,2,2-trifluoro-1-hydroxy-1-trifluoromethylethyl)phenyl]benzenesulfonamide (T1317, **3**)[7a] and 3-[3-[[[2-chloro-3-(trifluoromethyl)phenyl]methyl](2,2-diphenylethyl)amino]propoxy]benzeneacetic acid (GW3965, **4**)[7b] are transactivational agonists for both LXRα and LXRβ. However, transactivational agonists may induce serious hypertriglyceridemia because genes involved in lipid synthesis, such as *srebp-1c* and *fas*, have LXRE in their promoter region and are activated by LXR transactivational agonists.[8] This is a major barrier to medical application of LXR ligands.

Recent studies have revealed that LXRs also function in the regulation of inflammation in macrophages.[9] In vitro, several LXR ligands inhibit the expression of proinflammatory mediators such as interleukin-6 (IL-6), interleukin-1β (IL-1β), and inducible nitric oxide synthase (iNOS) in response to bacterial infection or lipopolysaccaride (LPS) stimulation. Studies in LXR knockout mice indicated that these actions are mediated by both LXRα and LXRβ. Therefore, it is speculated that LXRs are potential targets for treatment of not only atherosclerosis or hyperlipidemia but also inflammatory diseases such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and Castleman's disease.[10] This function of LXR is called *transrepressional* action. The promoter regions of *il-6*, *il-1β*, *inos*, and other proinflammatory genes do not contain LXRE, which is present in all of the genes showing LXR transactivation. Thus, the mechanisms of transactivational and transrepressional action are clearly different. Transrepressional activities of other NRs have also been reported. For example, in the case of peroxisome proliferator-activated receptor γ (PPARγ), an NR that is expressed at high levels in macrophages, SUMOylation of PPARγ by rosiglitazone (a PPARγ ligand) leads to stabilization of the transrepressional complex, which includes NCoR, on the promoter region of each proinflammatory gene, resulting in inhibition of gene expression under conditions of LPS stimulation.[11] In 2007, Ghisletti et al. proposed that an LXR agonist (**4**) showed transrepressional activity via a similar mechanism, i.e., through SUMOylation of LXR and prevention of NCoR clearance.[12] Compounds **3** and **4** exhibit both transactivational and transrepressional actions, and many reported LXR ligands are thought to have both activities. However, 25-hydroxycholesterol (an endogenous LXR ligand) and 27-hydroxycholesterol showed only transactivational activity, and this suggested that transactivational and transrepressional activities might be separable.[12] So far, only one series of transrepression-selective LXR modulators (a representative compound **5** is shown in Figure 1) has been reported, by GlaxoSmithKline in 2008.[13] Compound **5** has interesting properties (it has similar transrepressional activity to compound **3** without causing any increase of triglyceride (TG) accumulation), but it showed weak transactivational agonistic activity in a cell-based reporter assay (EC_{50} = 3−10 μM in our assay system).

In this paper, we describe the structure-based design, synthesis, and biological evaluation of a novel series of 2-substituted 5,11-dihydro-5-methyl-11-methylene-6*H*-dibenz-[*b*,*e*]azepin-6-one LXR ligands that exhibit potent and dose-dependent transrepression-selective activity.

2. RESULTS AND DISCUSSION

2.1. Identification of a Lead Compound Possessing Both Transrepressional and Transactivational Activity.

In our previous studies of LXR transactivational agonists and antagonists,[14] we measured transactivational activity using a Gal4-human LXR reporter system (transactivational agonistic activity was measured at a concentration of 0.1−30 μM test compound and antagonistic activity was measured under the same conditions in the presence of 0.1 μM compound **3**).[15] Percent efficacy of agonists was given relative to the positive control **6**.[14a,16] In the present work, we focused on the

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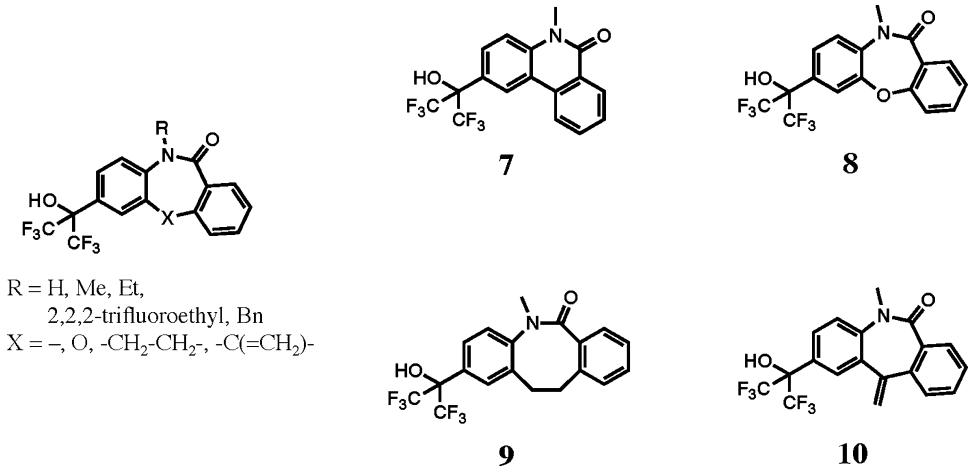

Figure 2. General structure and chemical structures of LXR transactivational antagonist (7) and transactivational agonists (**8**–**10**).

Table 1. Transactivational and Transrepressional Activities of Compounds 7–10 in Cell-Based Assays[a]

		LXR transcriptional assay			
		agonistic activity (EC_{50} (μM))		antagonistic activity (IC_{50} (μM))	
compd	% inhibition of IL-6 production at 10 μM	LXRα (% efficacy)	LXRβ (% efficacy)	LXRα	LXRβ
3	49	0.34 ± 0.17 (124)	0.09 ± 0.0059 (118)	NT	NT
6	NT	0.63 ± 0.029 (100)	0.18 ± 0.014 (100)	NA	NA
7	NA	NA	NA	4.1 ± 1.7	NA
8	54	4.2 ± 0.97 (12)	2.7 ± 0.36 (49)	NA	NA
9	20	0.91 ± 0.023 (117)	0.26 ± 0.0153 (108)	NA	NA
10	71	1.7 ± 0.53 (45)	0.77 ± 0.102 (73)	NA	NA

[a]Transactivational activity was measured on LXR-Gal4 chimeric receptors in transiently transfected HEK293 cells. The EC_{50} value is the molar concentration of test compound that affords 50% of the maximal reporter activity. Percent efficacy is given relative to the positive control **6**. The IC_{50} value is the molar concentration of test compound that affords a 50% decrease in the maximal reporter activity of 100 nM compound **3**. Transrepressional activity was measured in TPA-treated THP-1 cells. NT means not tested. NA means no activity at 30 μM.

transrepressional activity of phenanthridin-6-one, dibenz[*b*,*f*]-[1,4]oxazepin-11-one, 11,12-dihydrodibenz[*b*,*f*]azocin-6-one, and 5,11-dihydro-11-methylene-6*H*-dibenz[*b*,*e*]azepin-6-one derivatives (Figure 2). Transrepressional activity was evaluated by measuring the reduction in the amount of IL-6 induced by LPS in the presence of test compounds. Percent inhibition of IL-6 production of compounds (10 μM) in each table (vide infra) was measured at the same experiment side-by-side, and the dose-dependency of compounds was determined beforehand by separate experiments (Supporting Information). Compounds possessing strong inhibition of IL-6 production were selected, and their IC_{50} values were then determined (vide infra). Transactivational and transrepressional activities of representative compounds **7**–**10** (Figure 2) are shown in Table 1. Novel compound **10**, which has a 5,11-dihydro-5-methyl-11-methylene-6*H*-dibenz[*b*,*e*]azepin-6-one skeleton, showed the most potent transrepressional activity; it inhibited LPS-induced IL-6 production in a dose-dependent manner (Figure 3), showing 71% inhibition at 10 μM. It also exhibited transactivational agonistic activity, with EC_{50} values for LXRα and LXRβ of 1.6 μM and 0.77 μM, respectively. In our assay system, compound **10** showed a similar level of transrepressional activity to compound **5** (data not shown). Therefore, we selected compound **10** as a new lead compound.

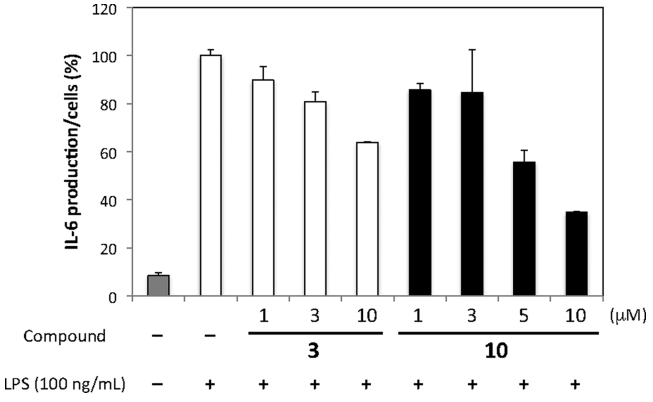

Figure 3. Dose-dependent transrepressional activity of compound **3** and **10**, evaluated in terms of IL-6 production by means of ELISA. Normalization was done according to the live cell count.

2.2. Design of Transrepression-Selective Compounds. Our strategy to obtain transrepression-selective LXR ligands was to reduce the transactivational agonistic activity of compound **10**. Proper folding of C-terminal helix 12 is important for transactivational agonistic activity.[17] According to the reported X-ray crystal structures of compound **3** and hLXRs LBD,[18] the hydroxyl group of **3** forms a hydrogen bond with a histidine residue (His421 for LXRα and His435 for LXRβ) in helix 11, adjacent to helix 12, and this histidine residue interacts with a tryptophan residue (Trp443 for LXRα and Trp457 for LXRβ) in helix 12; these interactions induce proper folding of helix 12, affording transcriptionally active LXRs (Figure 4a). Compound **10** contains a hexafluoropropanol moiety, like compound **3**. Therefore, we initially focused on

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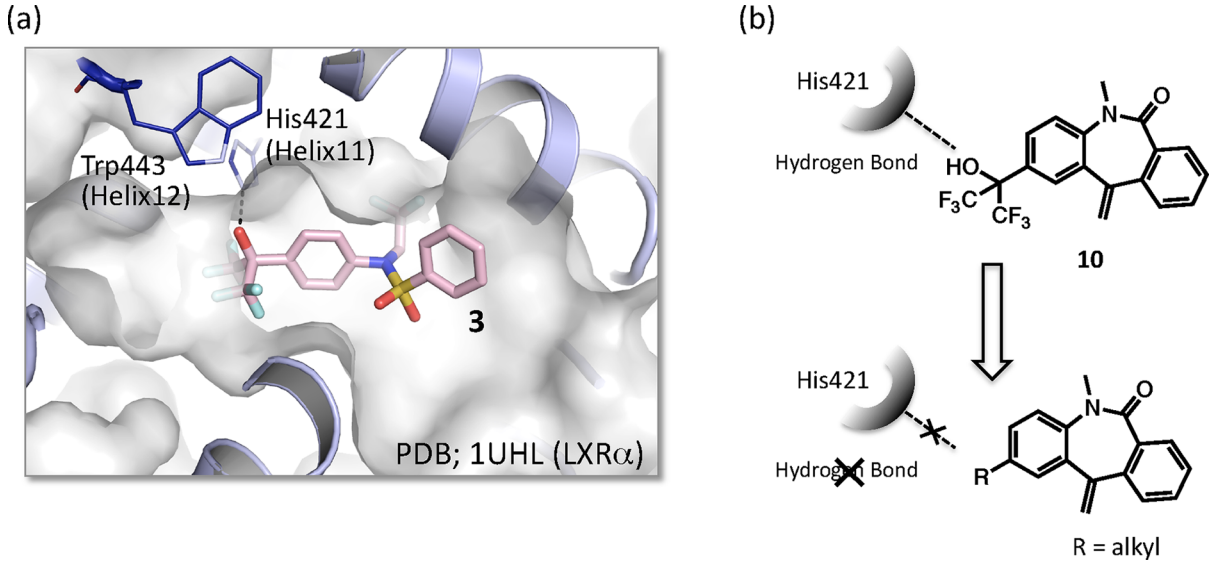
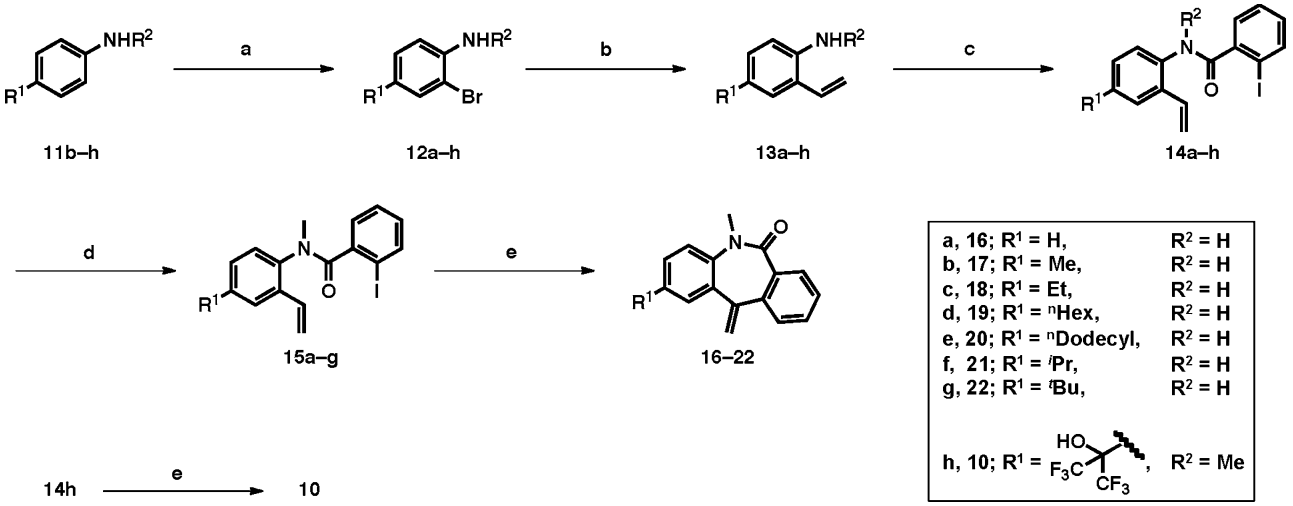


Figure 4. X-ray crystal structure of compound **3** and hLXRα LBD (a) and design strategy for our compounds (b).

Scheme 1. Synthesis of 10, 16−22[a]



[a]Reagents and conditions: (a) NBS, DCM, rt, 38−77%; (b) tributylvinyltin, $Pd(PPh_3)_4$, toluene, 100 °C or 2,4,6-trivinylcyclotriboroxane−pyridine complex, $Pd(PPh_3)_4$, K_2CO_3, DME/H_2O, reflux, 29−65%; (c) 2-iodobenzoyl chloride, Et_3N, DCM, rt, 46%−quant; (d) MeI, NaH, DMF, 0 °C to rt, 46−99%; (e) $Pd(OAc)_2$, PPh3, NaOAc, DMF, 100 °C, 34−88%.

replacement of the hexafluoropropanol moiety with alkyl groups (Figure 4b), which are expected to be unable to form the hydrogen bond with the histidine residue in LXRs so that the histidine residue may not be appropriately located to allow proper folding of helix 12 for transactivational agonistic activity.

2.3. Synthesis of compounds 16−22. 2-Substituted 5,11-dihydro-5-methyl-11-methylene-6*H*-dibenz[*b*,*e*]azepin-6-ones **16−22** were prepared via intramolecular Heck reaction as the key step, as shown in Scheme 1. 4-Substituted anilines **11** were monobrominated (**12**) and vinylated to give compounds **13**. Compounds **13** were amidated with 2-iodobenzoyl chloride in the presence of triethylamine to afford compounds **14** in 46%−quantitative yield. *N*-Methylation afforded **15**. The intramolecular Heck reaction proceeded smoothly with the use of $Pd(OAc)_2$ and PPh_3 in the presence of NaOAc as the base in DMF to afford compounds **16−22** (Scheme 1). Structure determination of representative compound **18** was performed by ^{1}H NMR, ^{13}C NMR, heteronuclear multiple quantum coherence (HMQC), and mass spectrometry.

2.4. Structure−Activity Relationship (SAR) of Alkyl Substituent. The synthesized compounds **16−22** all lacked transactivational agonistic activity at 30 μM, as expected, but showed weak transactivational antagonistic activity (Table 2). Next, we investigated the transrepressional activity of these compounds. Among compounds with various lengths of the alkyl substituent (**16−20**), the ethyl compound (**18**) showed the best transrepressional activity, while a shorter (**16**) or longer (**20**) alkyl substituent resulted in weaker activity. Among **16−18**, **21**, and **22**, we found that bulkier substituents tended to be associated with more potent transrepressional activity. On the basis of these results, we selected **18** and **22** as second-generation lead compounds.

2.5. Verification of Transrepression/Transactivation Selectivity. First, we examined in detail the transrepression/transactivation selectivity of the second-generation leads. To

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Table 2. Transactivational and Transrepressional Activities of Compounds 16−22[a]

			LXR transcriptional assay			
			agonistic activity (EC_{50} (μM))		antagonistic activity (IC_{50} (μM))	
compd	R^1	% inhibition of IL-6 production at 10 μM	LXRα (% efficacy)	LXRβ (% efficacy)	LXRα	LXRβ
3		49	0.34 ± 0.17 (124)	0.09 ± 0.0059 (118)	NT	NT
10	$HO(F_3C)_2C$	73	1.7 ± 0.53 (45)	0.77 ± 0.102 (73)	NA	NA
16	H	NA	NA	NA	>10	>10
17	Me	38	NA	NA	>10	>10
18	Et	51	NA	NA	>30	>30
19	*n*-hex	38	NA	NA	>10	>10
20	*n*-dodecyl	NA	NA	NA	>30	>30
21	*i*-Pr	44	NA	NA	1.7 ± 0.0	>10
22	*t*-Bu	61	NA	NA	5.4 ± 1.7	>10

[a]Transactivational activity was measured on LXR-Gal4 chimeric receptors in transiently transfected HEK293 cells. The EC_{50} value is the molar concentration of test compound that affords 50% of the maximal reporter activity. % Efficacy is given relative to the positive control **6**. The IC_{50} value is the molar concentration of test compound that affords a 50% decrease in the maximal reporter activity of 100 nM compound **3**. Transrepressional activity was measured in TPA-treated THP-1 cells. NT means not tested. NA means no activity at 30 μM.

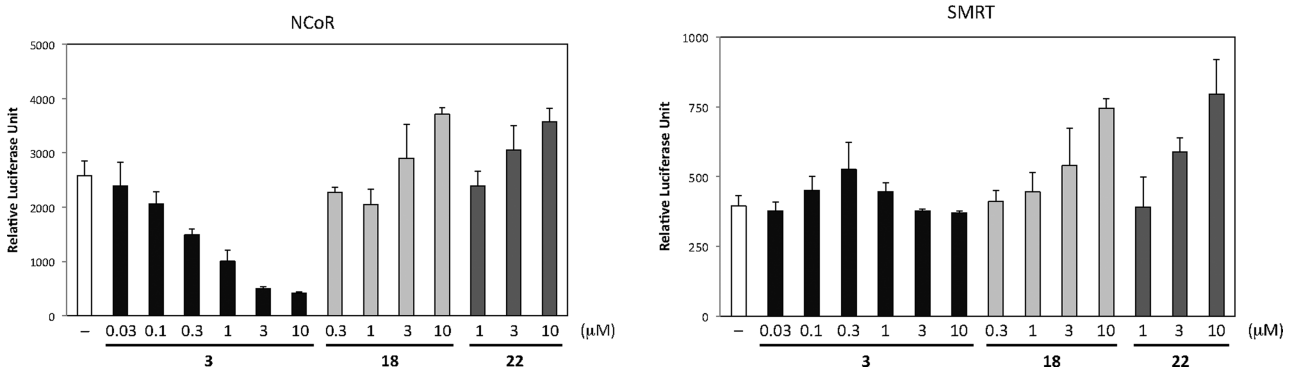

Figure 5. Dose dependence of transactivational antagonistic activity of compounds **3**, **18**, and **22** in cellular mammalian two-hybrid assays using pCMX-Gal4-NCoR and -SMRT.

investigate transactivational antagonistic activity, we performed mammalian two-hybrid (M2H) assay using pCMX-Gal4-SRC1 and -DRIP205 expression vectors to examine coactivator recruitment and -NCoR and -SMRT expression vectors to examine corepressor recruitment.[19] While compound **3** strongly recruited coactivators SRC1 and DRIP205 (data not shown), compound **18** did not recruit them at 10 μM and compound **22** did so only slightly. However, compounds **18** and **22** both dose-dependently stabilized corepressors NCoR and SMRT, whereas compound **3** did not (Figure 5). These results indicate that compounds **18** and **22** exerted transactivational antagonistic activity by stabilizing the interaction between LXRα and corepressors. Taking into account the experimental result that complex formation of LXRs and NCoR is important for transrepressional activity, compounds **18** and **22** might be possibly to suppress LPS-induced IL-6 production via LXRs-mediated transrepression. We next examined the transrepression/transactivation selectivity of the second-generation leads by means of mRNA expression analysis using wild-type mouse peritoneal macrophages and human hepatocellular liver carcinoma (Huh-7) cells. In mouse peritoneal macrophages, we examined the mRNA expression of *il-6*, *il-1β* (transrepressional genes) and *abca1* (transactivational gene). Compound **18** inhibited LPS-induced *il-6* and *il-1β* mRNA expression, as did compounds **3** and **10** (Figure 6a,b), but did not induce upregulation of *abca1* mRNA expression, unlike compounds **3** and **10** (Figure 6c). These results indicate that compound **18** is transrepression-selective. Moreover, we examined mRNA expression of *srebp-1c* and *fas*, which are associated with increased blood triglyceride and may be transactivated by LXR ligands. As shown in parts d and e of Figure 6, unlike compound **3** or the first-generation lead compound **10**, compound **18** did not increase the mRNA expression of *srebp-1c* or *fas* in Huh-7 cells. Therefore, compound **18** should not increase blood triglyceride and may be a better candidate as an anti-inflammatory medicine than ligands that have transactivational agonist activity.

2.6. Synthesized Compounds Showed Transrepressional Activity in an LXR-Dependent Manner. LPS transmits inflammatory signaling intracellularly through Toll-like receptor 4 (TLR4) in the plasma membrane, and this induces degradation of the transrepressional complex on the promoter region of proinflammatory genes (e.g., IL-6 and IL-1β), thereby activating gene transcription.[20] So, inhibitory activity toward LPS-induced IL-6 production is also exhibited by TLR4 antagonists[21] and TLR4-signaling inhibitors.[22] Therefore, we examined whether the IL-6 production−inhibitory activity of our second-generation lead compounds was LXR-dependent. To check this point, we used peritoneal macrophages from wild-type and LXR-null mice (LXR$\alpha^{-/-}$/$\beta^{-/-}$ (*Nr1h3*$^{-/-}$*Nr1h2*$^{-/-}$) or LXR$\alpha^{-/-}$ (*Nr1h3*$^{-/-}$)). As shown in Figure 7a, inhibition of LPS-induced IL-6 production by the LXR agonist **3** (3 μM), **10**, and **18** was entirely abolished in LXR$\alpha^{-/-}$/$\beta^{-/-}$ macrophages, as determined by ELISA (mouse

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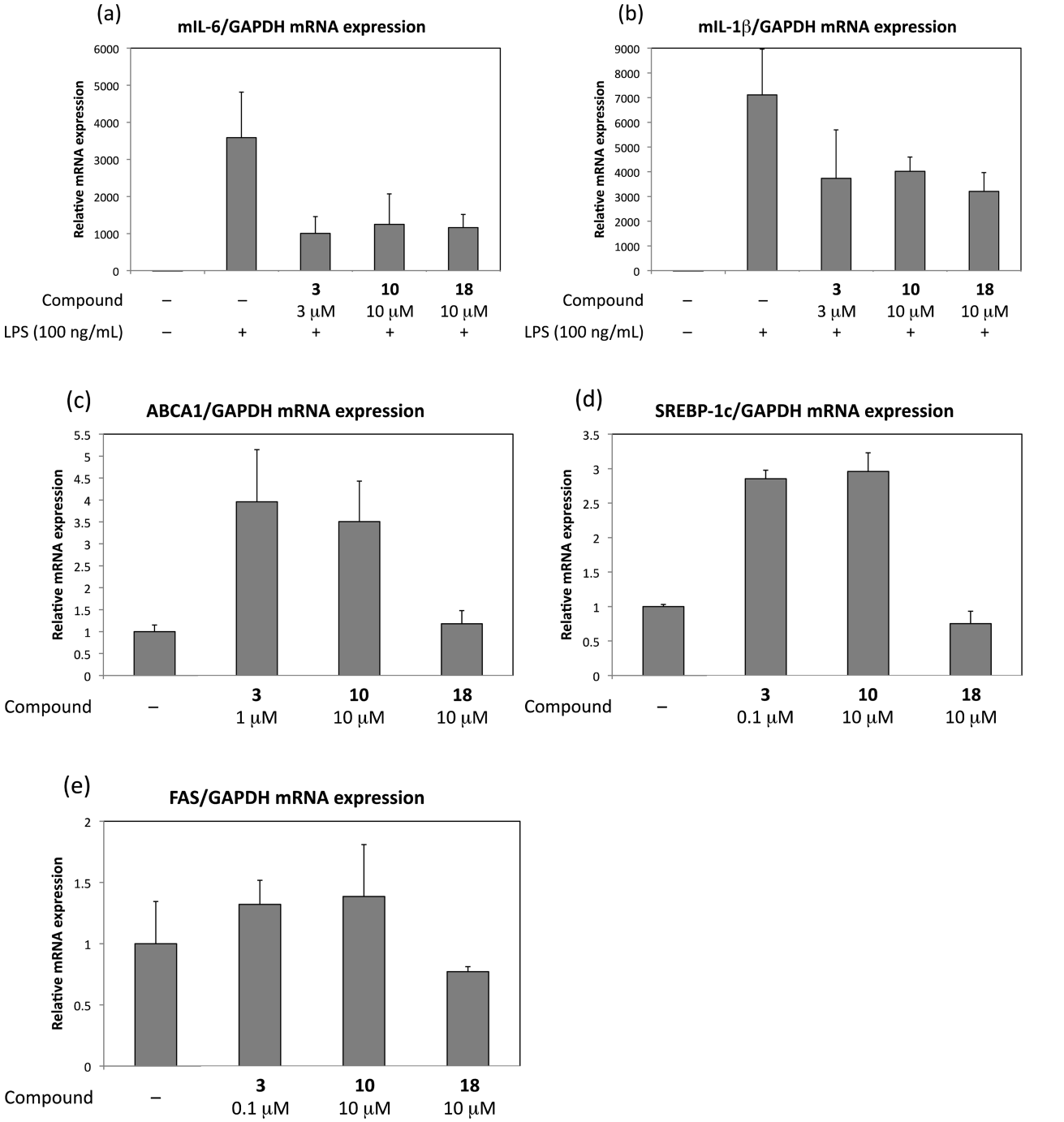

Figure 6. Effects of compounds **10** and **18** on mRNA expression of *il-6* (a), *il-1β* (b), and *abca1* (c) in wild-type mice peritoneal macrophages and on mRNA expression of *srebp-1c* (d) and *fas* (e) in human hepatocellular liver carcinoma (Huh-7) cells.

IL-6 protein). LXR-independent induction of IL-6 in LXR$\alpha^{-/-}$/$\beta^{-/-}$ macrophages was observed in response to compounds **3** and **10**. The reason for this result is unclear at present, but the result for compound **3** is in agreement with reported data,[9a] so there may be another site of action. Compound **22** lacked transrepressional activity in LXR$\alpha^{-/-}/\beta^{-/-}$ macrophages. This result also indicates that LXRα/β contribute inhibition of IL-6 production by compounds **10**, **18**, and **22**, at least in part, although we cannot exclude the possibility that our compounds may inhibit LPS induced IL-6 production through other mechanism. We next focused on LXRα, which is highly expressed in immune cells such as macrophages. Inhibition of LPS-induced IL-6 production by compounds **3** and **10** was partially abolished in LXR$\alpha^{-/-}$ macrophages as determined at the mRNA level by means of real-time quantitative PCR analysis (Figure 7b). When these results were compared with those in wild-type mice, shown in Figure 6a, we surprisingly found that the inhibition by compound **18** in particular was almost entirely abrogated, which suggests that compound **18** might show transrepressional activity predominantly in an LXRα-dependent manner. The inhibition of LPS-induced IL-1β production by compounds **3**, **10**, and **18** was partially abolished at the mRNA level in LXR$\alpha^{-/-}$ macrophages (Supporting Information).

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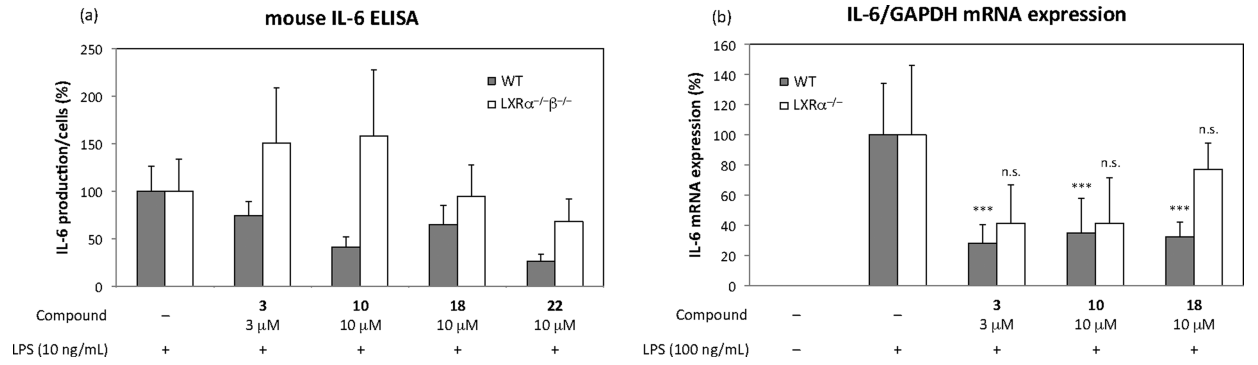

Figure 7. Inhibition of LPS-induced IL-6 production by compounds **10** and **18** (and also **22**) was abolished in LXR$\alpha^{-/-}\beta^{-/-}$ macrophages as determined at the protein level by ELISA (a) and in LXR$\alpha^{-/-}$ macrophages as determined at the mRNA level by real-time quantitative PCR analysis (b). Values represent means ± SE from at least three independent measurements. ***, $p < 0.001$ vs LPS+, n.s., not significant, ANOVA.

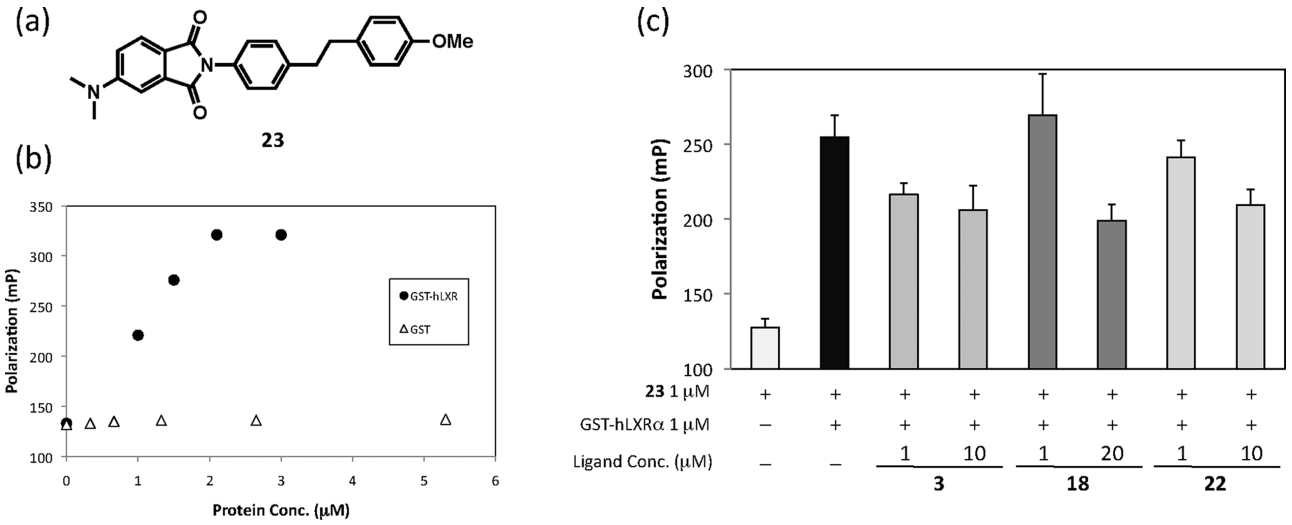

Figure 8. Structure of a reported fluorescent LXR ligand, 2-(4-(4-methoxyphenethyl)phenyl)-5- (dimethylamino)isoindoline-1,3-dione (**23**) (a). Compound **23** specifically binds to hLXRα (b). Compounds **18** and **22** specifically bind to GST-hLXRα (c).

2.7. Compounds 18 and 22 Bind Directly to LXRα. Experiments using the knockout mice confirmed that the synthesized compounds exhibited transrepressional activity in an LXR-dependent manner. Because **18** and **22** showed transactivational antagonistic action, we considered that they might bind to the same binding site as compound **3**. Therefore, we next investigated whether compounds **18** and **22** bind directly to LXR. Although scintillation proximity assay (SPA)[23] has been used to screen LXR ligands, there are no commercial available radioactive ligands at present. Because fluorescent ligands have been widely used for binding assay of target proteins,[24] we decided to employ fluorescence polarization (FP) assay using the reported fluorescent compound **23** (Figure 8a[25]). First of all, we investigated whether the fluorescent compound specifically binds to LXRα. A fixed concentration of the fluorescent compound (1 μM) was mixed with different concentrations of GST-hLXRα or GST protein (*X*-axis), and the FP value (*Y*-axis) was measured. As shown in Figure 8b, fluorescence polarization increased in a dose-dependent manner with GST-hLXRα but not GST. This result indicated the fluorescent compound binds specifically to hLXRα. Furthermore, compound **3**, which binds to LXRs LBD, dose-dependently decreased the FP value in the presence of fluorescent compound **23** (1 μM) and GST-hLXRα (1 μM) (Figure 8c). This result demonstrated that fluorescent compound and compound **3** competitively bind to hLXRα LBD. Under the conditions used, **18** (20 μM) and **22** (10 μM) both caused a dose-dependent decrease of the FP value, indicating that they also both bind directly to GST-hLXRα. These effective concentrations of compounds were predetermined by a separate experiment. The results also demonstrate that our fluorescence-based LXR LBD binding assay is effective. In summary, our second-generation leads **18** and **22** were confirmed to be transrepression-selective by means of reporter gene assay, IL-6 ELISA, M2H assay, and mRNA expression analysis, and we also demonstrated that inhibition of IL-6 production by these lead compounds is mediated by LXR via direct binding of the compounds to the LXR LBD.

2.8. Selectivity over Other Nuclear Receptors. We next confirmed the selectivity of the second-generation leads toward other NRs, that is, farnesoid X receptor (FXR), PPARγ, and retinoid X receptor α (RXRα). We selected three NRs based on the following knowledge. (1) LXR and FXR are categorized into the same subgroup of NRs based on their sequence homology, and compound **3** is known to bind to FXR.[26] (2) Disorders of lipid consumption and processing influence gene expression programs are orchestrated by PPARγ and LXR.[27] (3) LXRα and LXRβ form heterodimers with the obligate partner RXR. As far as we examined under experimental conditions used, the second-generation lead **18** did not show

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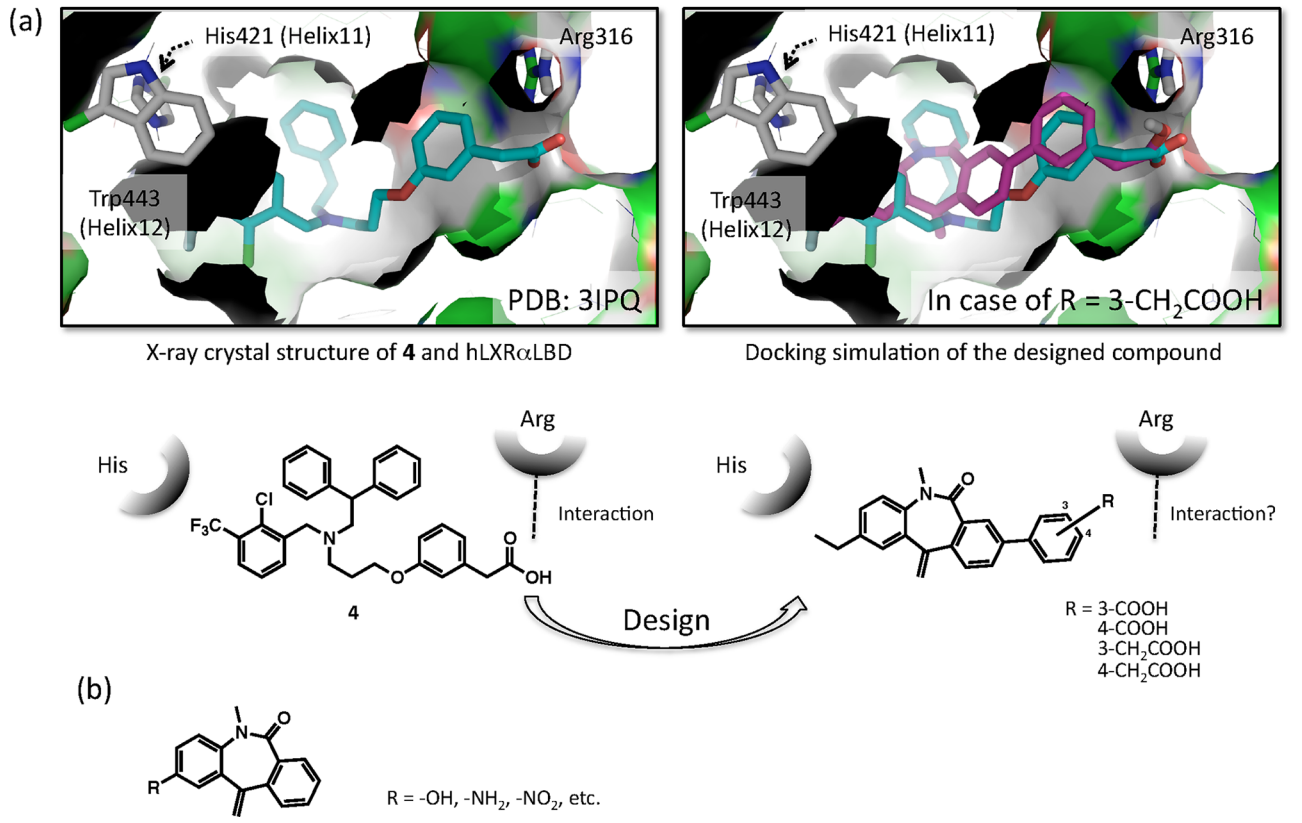

Figure 9. Design of candidate ligands interacting with Arg316 (a) and with heteroatom(s) in place of the alkyl group (b).

any FXR agonistic/antagonistic activities, PPARγ antagonistic activity, or RXRα agonistic/antaogonistic activities (Supporting Information). Compound **18** only showed very weak PPARγ agonistic activity (ca. 20% activation at 10 μM). These results indicate that compound **18** showed sufficient selectivity over other NRs, FXR, PPAR, and RXR.

2.9. Further Structural Development to Obtain More Potent Transrepression-Selective Ligands. Because we thought the low binding affinity for LXR of compounds **18** and **22** might account for their low transrepressional activity relative to compound **10**, we considered further structural modifications. One approach was to introduce a group that could interact with another amino acid residue in LXR based on the X-ray structure of compound **4** and LXRα LBD (PDB; 3IPQ),[28] and the other was to substitute the alkyl group with heteroatom(s). As shown in Figure 9a, compound **4** binds with LXRα by interacting with an arginine residue (Arg316). This position is rather far away from helix 12, and we guessed that this interaction with arginine, unlike that with histidine in helix 11, may not play a critical role in exerting transactivational agonistic activity. Therefore, we modified our compounds by introducing a substituent (R = 3-CH_2COOH), based on the results of docking simulation with AUTODOCK 4.2,[29] using the LXRα LBD (Figure 9).

2.10. Synthesis of Compounds 37−40 and 46−52. Intermediate **13c** was amidated with 2-iodo-5-methoxybenzoic acid (**27**), which was prepared from 5-hydroxyanthranilic acid (**24**) in three steps in the presence of Vilsmeier reagent to afford compound **28** (Scheme 2). *N*-Methylation was performed to obtain **29**. The intramolecular Heck reaction of **29** proceeded smoothly with the use of $Pd(OAc)_2$ and PPh_3 in the presence of NaOAc to afford compound **30**. Demethylation with boron tribromide and triflation with trifluoromethanesulfonic anhydride were performed to obtain compound **32**. Conversion of triflate **32** to borate ester **33** and subsequent phenylation with haloaryls **35a−d** were performed in moderate yield. Hydrolysis of **36a−d** proceeded to afford compounds **37−40**. Starting from commercially available **41a**, **41c**, and **41d**, and compound **41b** synthesized by the method reported in the literature,[30] we synthesized compounds **46**, **48**, **49**, and **52** in the same manner as illustrated in Scheme 1 (Scheme 3). Compound **46** was reduced with tin(II) chloride to obtain **47**. Compound **49** was reduced with lithium borohydride to obtain **50**. Compound **49** was also hydrolyzed to obtain **51**.

2.11. Transrepressional and Transactivational Activities of the New Compounds. The transrepressional and transactivational activities of the newly synthesized compounds were evaluated. Compounds **37−40** broadly showed increased transactivational antagonistic activity (especially when a 4-substituent was present) (Table 3). However, the transrepressional activity of compounds **39** and **40** was greatly decreased relative to compound **18**, and compounds **37** and **38** lacked transrepressional activity. These results indicated that interaction with the arginine residue was disadvantageous for transrepressional activity. Further, because there was no clear correlation between transactivational antagonistic activity and transrepressional activity, the key interaction sites with LXR for the two activities might be different. Another possible explanation of weak transrepressional activity of this carboxylic acid series might be due to low penetration into THP-1 cells. As for compounds **46−52**, which were expected to be able to form a hydrogen bond with the histidine residue in LXR because of their heteroatom(s), they showed no transactivational agonistic activity at 30 μM (Table 4). Thus, a substituent

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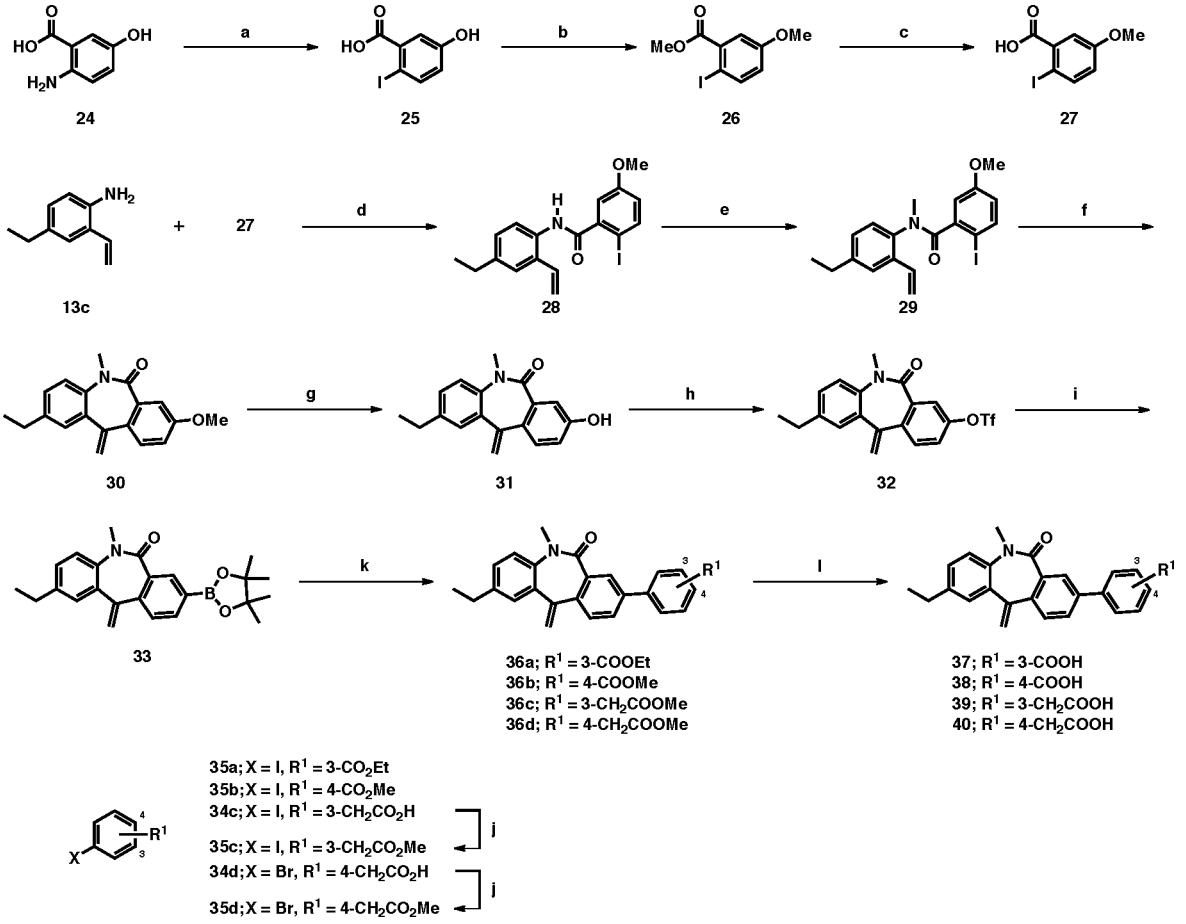
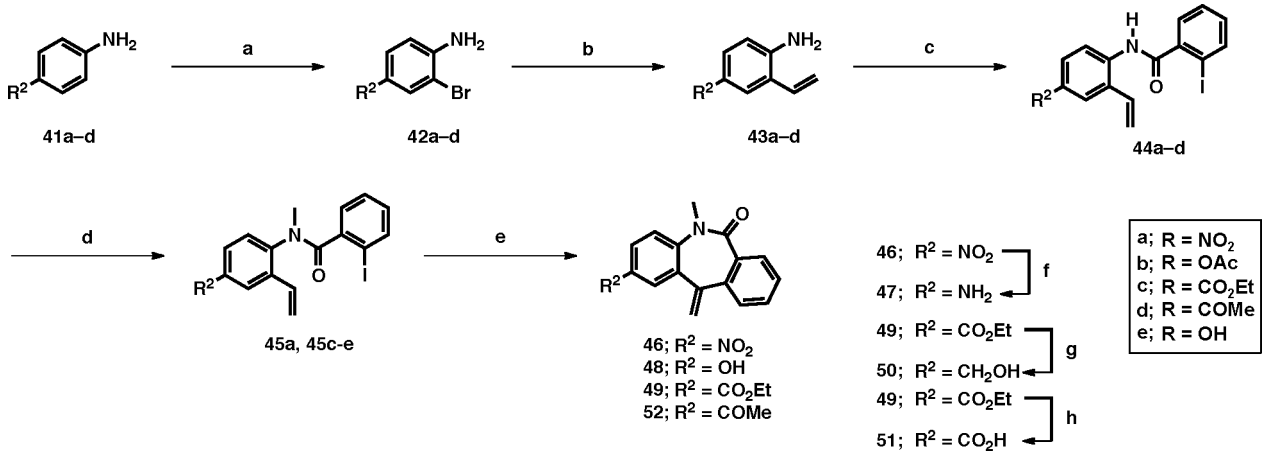
Scheme 2. Synthesis of 37−40[a]



[a]Reagents and conditions: (a) $NaNO_2$, conc HCl, 0 °C; KI, H_2O, 0 °C to reflux; (b) $(MeO)_2SO_2$, K_2CO_3, acetone, reflux; (c) 2 N NaOH aq, MeOH, reflux, 47% (3 steps); (d) **27**, chloromethylenedimethyliminium chloride, DCM, 0 °C to rt; Et_3N, DCM, 83%; (e) MeI, NaH, DMF, 0 °C to rt, 78%; (f) $Pd(OAc)_2$, PPh_3, NaOAc, DMF, 100 °C, 95%; (g) BBr_3, DCM, 0 °C to rt, 90%; (h) Tf_2O, py, DCM, 0 °C to rt, 47%; (i) bis(pinacolato)diboron, $(PPh_3)_2PdCl_2$, KOAc, 1,4-dioxane, 100−120 °C, 57%; (j) conc H_2SO_4, MeOH, reflux, 91−99%; (k) **35a−d**, $Pd(PPh_3)_4$, K_3PO_4, DMF, 25−83%; (l) 2 N NaOH aq, EtOH, 14−98%.

Scheme 3. Synthesis of 46−52[a]



[a]Reagents and conditions: (a) NBS, DCM, rt, 76−87%; (b) tributylvinyltin, $Pd(PPh_3)_4$, toluene, 100 °C or 2,4,6-trivinylcyclotriboroxane−pyridine complex, $Pd(PPh_3)_4$, K_2CO_3, DME/H_2O, reflux, 47−85%; (c) 2-iodobenzoyl chloride, Et_3N, DCM, rt, 88%−quant; (d) MeI, NaH, DMF, 0 °C to rt, from **44b** to **45e** (15%), **45a**, **45c**, **45d** (32−91%); (e) $Pd(OAc)_2$, PPh_3, NaOAc, DMF, 100 °C, 42−95%; (f) $SnCl_2 \cdot 2H_2O$, AcOEt, 70 °C, 75%; (g) $LiBH_4$, THF, rt, 26%; (h) 1 N NaOH aq, EtOH, rt, quant.

with appropriate acidity or hydrogen bond-forming ability is important for transactivational agonistic activity. We found that compound **48**, with a hydroxyl group, exhibited the most potent transrepressional activity, exceeding that of lead

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Table 3. Transactivational and Transrepressional Activities of Compounds 37−40[a]

				LXR transcriptional assay			
				agonistic activity (EC_{50} (μM))		antagonistic activity (IC_{50} (μM))	
compd	R^1	position	% inhibition of IL-6 production at 10 μM	LXRα	LXRβ	LXRα	LXRβ
18			78	NA	NA	>30	>30
37	COOH	3	NA	NA	NA	>30	>30
38	COOH	4	NA	NA	NA	1.3 ± 0.58	9.5 ± 2.7
39	CH_2COOH	3	27	NA	NA	21 ± 4.2	27 ± 0.12
40	CH_2COOH	4	9	NA	NA	2.2 ± 0.72	15 ± 0.61

[a]Transactivational activity was measured on LXR-Gal4 chimeric receptors in transiently transfected HEK293 cells. The EC_{50} value is the molar concentration of test compound that affords 50% of the maximal reporter activity. The IC_{50} value is the molar concentration of test compound that affords a 50% decrease in the maximal reporter activity of 100 nM compound **3**. Transrepressional activity was measured in TPA-treated THP-1 cells. NA means no activity at 30 μM.

Table 4. Transactivational and Transrepressional Activities of Compounds 46−52[a]

			LXR transcriptional assay			
			agonistic activity (EC_{50} (μM))		antagonistic activity (IC_{50} (μM))	
compd	R^2	% inhibition of IL-6 production at 10 μM	LXRα (% efficacy)	LXRβ (% efficacy)	LXRα	LXRβ
3		49	0.34 ± 0.17 (124)	0.09 ± 0.0059 (118)	NT	NT
10	$HO(F_3C)_2C$	73	1.7 ± 0.53 (45)	0.77 ± 0.102 (73)	NA	NA
46	O_2N	49	NA	NA	6.1 ± 0.050	13 ± 1.9
47	H_2N	58	NA	NA	19 ± 1.0	27 ± 5.2
48	HO	78	NA	NA	4.1 ± 0.045	11 ± 0.43
50	HOH_2C	29	NA	NA	>10	>10
51	HO_2C	27	NA	NA	14 ± 3.2	26 ± 1.8
52	Ac	53	NA	NA	4.9 ± 0.95	7.5 ± 1.0

[a]Transactivational activity was measured on LXR-Gal4 chimeric receptors in transiently transfected HEK293 cells. The EC_{50} value is the molar concentration of test compound that affords 50% of the maximal reporter activity. Percent efficacy is given relative to the positive control **6**. The IC_{50} value is the molar concentration of test compound that affords a 50% decrease in the maximal reporter activity of 100 nM compound **3**. Transrepressional activity was measured in TPA-treated THP-1 cells. NT means not tested. NA means no activity at 30 μM.

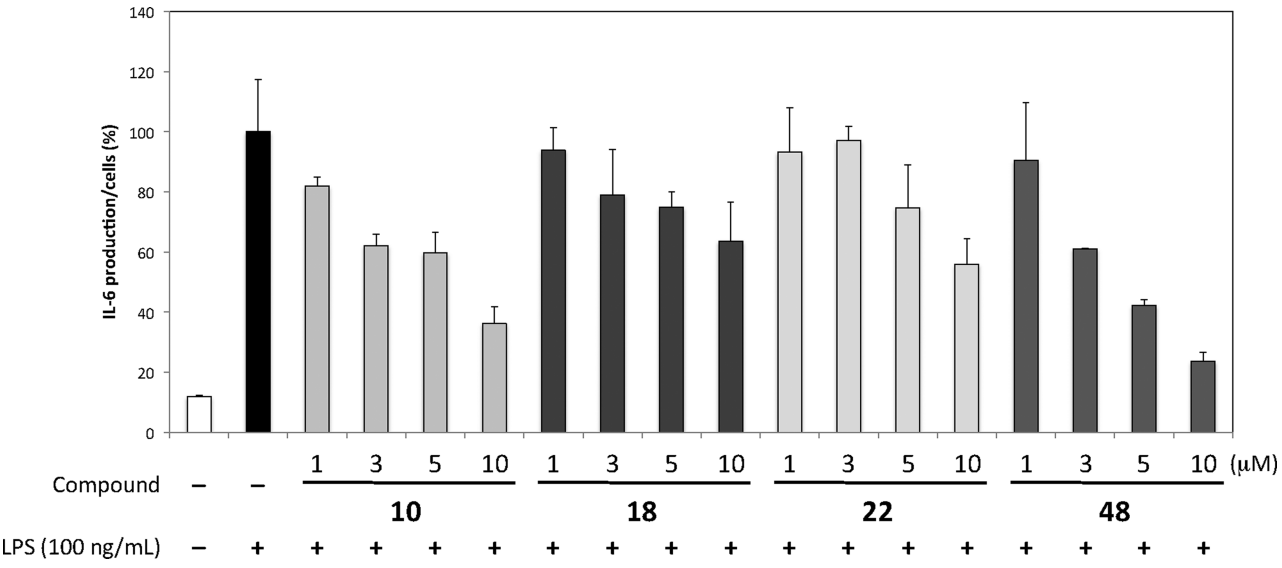

Figure 10. Dose dependence of transrepressional activity of compounds **10**, **18**, **22**, and **48** evaluated in terms of IL-6 production by means of ELISA. Normalization was done according to the live cell count.

compound **10**. Finally, we investigated the dose-dependency of transrepressional activity of compounds **18**, **22**, and **48**. As shown in Figure 10, all the compounds showed dose-dependent transrepressional activity and compound **48** was found to have the most potent transrepressional activity, as expected (its IC_{50} value was estimated to be 3.5 μM). Transactivational and transrepressional activities of representative compounds **3**, **5**, **10**, and **48** are summarized in Table 5. Relative efficacy (RE) of transrepressional activity and reported potency of **3** and **5** are also indicated.

2.12. Early in Vitro ADME of the Representative Compound. Compound **48** was further assessed for early in

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Table 5. Summary of Transrepressional and Transactivational Activities of Representative LXR Modulators

	transrepression (inhibition of IL-6 production)				transactivation (LXR agonistic activity EC_{50} (μM))		
compd	IC_{50} (μM)[a]	RE[a,b]	IC_{50} (nM)[c]	RE[c,d]	LXRα[a]	LXRβ[a]	LXRα/β[c]
3	>10	<0.6	100	1	0.34 ± 0.17	0.09 ± 0.0059	
5	>10	<1	15	1	7.1 ± 0.60	3.1 ± 1.72	3−5
10	5.8	1			1.7 ± 0.53	0.77 ± 0.102	
48	3.5	1.3			NA at 30 μM	NA at 30 μM	

[a]Data in our assay condition. [b]Relative efficacy compared with **10**. [c]Data taken from ref 13. [d]Relative efficacy with compound **3**.

vitro absorption−distribution−metabolism−excretion (ADME) properties. The permeability was evaluated in the Caco-2 intestinal epithelial cell line. In this assay, **48** was found to exhibit excellent permeability (mean A−B permeability = 84.3 × 10^{-6} cm/s), and it was classified as high permeability ($P_{app} \geq$ 20 × 10^{-6} cm/s). Next, metabolic stability was evaluated in human liver microsomes. The metabolic stability was measured as the percentage of the unmodified parent compound remaining in the mixture. Compound **48** was very stable and was almost recovered after incubation (mean compound remaining = 87.8%). Overall, compound **48** possessed both reasonable in vitro ADME properties and potent transrepressional activity and could be considered as a chemical tool for advanced studies.

3. CONCLUSION

We focused on novel transrepression-selective LXR ligands because of their potential value as tools for functional analysis of LXRs. On the basis of screening of transrepressional activity (inhibitory activity toward LPS-induced IL-6 production) of our LXR ligands, we selected tricyclic compound **10** as a lead compound. Because it possessed both transrepressional activity and transactivational agonistic activity, we next aimed to decrease the transactivational agonistic activity of **10**. We designed novel compounds that should be unable to form the hydrogen bond with a histidine residue on helix 11 in the LXR LBD, which is important for agonistic activity. All the synthesized compounds showed no transactivational agonistic activity at 30 μM, but showed weak transactivational antagonistic activity, and most of them had transrepressional activity. Compounds **18** and **22** were confirmed to be selective for transrepression over transactivation by means of M2H assay and mRNA expression analysis. We also showed that the transrepressional activity of these second-generation lead compounds was LXR-dependent, and we used a newly developed fluorescence polarization assay with fluorescent compound **23** as a nonradiolabeled LXR binding assay system to establish that the compounds bind directly to LXRα. Finally, further structural development directed at improving the binding affinity was performed. Among the compounds obtained, compound **48**, containing a hydroxyl group, showed potent, selective, and dose-dependent transrepressional activity.

4. EXPERIMENTAL SECTION

Chemistry. *General Methods.* ^{1}H NMR spectra were recorded on a JEOL ALPHA500 (500 MHz) spectrometer, and ^{13}C NMR spectra were recorded on a JEOL ALPHA500 (125 MHz) spectrometer. Chemical shifts (δ) are expressed in parts per million (ppm) relative to deuteriochloroform as an internal reference, with coupling constants in Hz. The abbreviations s, d, t, q, br, and m signify singlet, doublet, triplet, quartet, broad, and multiplet, respectively. Fast atom bombardment mass spectra (FAB-MS) and high-resolution mass spectra (HRMS) were recorded on a JEOL JMS-HX110 spectrometer with *m*-nitrobenzyl alcohol. Flash column chromatography was performed on silica gel 60 Kanto Kagaku (40−100 μm). The purity of each test compound was determined by HPLC (>95% purity; see Supporting Information).

*7-(1,1,1,3,3,3-Hexafluoro-2-hydroxypropan-2-yl)-10-methyldibenz[b,f][1,4]oxazepin-11(10H)-one (**8**).* Melting point 187.5−188.0 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.95 (s, 1H), 7.76 (dd, J = 7.8, 1.5 Hz, 1H), 7.64 (d, J = 1.5 Hz, 1H), 7.62−7.58 (m, 2H), 7.54 (d, J = 8.3 Hz, 1H), 7.45 (d, J = 7.8 Hz, 1H), 7.32 (dd, J = 8.3, 7.3 Hz, 1H), 3.50 (s, 3H). HRMS (FAB) calcd for $C_{17}H_{12}F_6NO_3$ 392.0721; found 392.0705 $(M + H)^+$.

*2-(1,1,1,3,3,3-Hexafluoro-2-hydroxypropan-2-yl)-5-methyl-11,12-dihydrodibenz[b,f]azocin-6-one (**9**).* Melting point 195.7−196.1 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44−7.40 (m, 2H), 7.18 (dd, J = 7.3, 1.8 Hz, 1H), 7.14 (d, J = 7.3 Hz, 1H), 7.11−7.04 (m, 2H), 6.89 (d, J = 7.3 Hz, 1H), 3.60 (s, 1H), 3.47−3.40 (m, 1H), 3.41 (s, 3H), 3.30−3.23 (m, 1H), 3.00−2.90 (m, 2H). HRMS (FAB) calcd for $C_{19}H_{16}F_6NO_2$ 404.1085; found 404.1046 $(M + H)^+$.

*General Procedure **1** (GP1). 2-Bromo-4-methylaniline (**12b**).* To a solution of *p*-toluidine (**11b**) (1.2 g, 11.2 mmol) and 10 mL of DCM was added *N*-bromosuccinimide (2.2 g, 12.4 mmol). The mixture was stirred for 20 h at room temperature, washed with water and brine, dried over anhydrous magnesium sulfate, and concentrated. The residue was purified by silica gel column chromatography (eluent: *n*-hexane/ethyl acetate = 9:1, v/v) to afford 924 mg (44%) of the title compound as a brown oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.23 (d, J = 1.2 Hz, 1H), 6.91 (dd, J = 7.9, 1.2 Hz, 1H), 6.68 (d, J = 7.9 Hz, 1H), 3.92 (br s, 2H), 2.22 (s, 3H).

*2-Bromo-4-ethylaniline (**12c**).* This compound was prepared from 4-ethylaniline (**11c**) by means of **GP1** as a brown oil (68%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.25 (d, J = 1.8 Hz, 1H), 6.94 (dd, J = 7.9, 1.8 Hz, 1H), 6.70 (d, J = 7.9 Hz, 1H), 3.94 (br s, 2H), 2.52 (q, J = 7.9 Hz, 2H), 1.18 (t, J = 7.9 Hz, 3H).

*2-Bromo-4-hexylaniline (**12d**).* This compound was prepared from **11d** by means of **GP1** as a brown oil (77%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.22 (s, 1H), 6.91 (d, J = 7.9 Hz, 1H), 6.69 (d, J = 7.9 Hz, 1H), 3.93 (br s, 2H), 2.46 (t, J = 7.9 Hz, 2H), 1.50 (br s, 2H), 1.28 (br s, 6H), 0.88 (t, J = 6.7 Hz, 3H). MS (FAB) 255, 257 $(M + H)^+$.

*2-Bromo-4-dodecylaniline (**12e**).* This compound was prepared from **11e** by means of **GP1** as a brown solid (66%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.23 (d, J = 1.8 Hz, 1H), 6.91 (dd, J = 7.9, 1.8 Hz, 1H), 6.69 (d, J = 7.9 Hz, 1H), 3.94 (br s, 2H), 2.46 (t, J = 7.9 Hz, 2H), 1.55−1.50 (m, 2H), 1.30−1.23 (m, 18H), 0.88 (t, J = 7.3 Hz, 3H). MS (FAB) 339, 341 $(M + H)^+$.

*2-Bromo-4-isopropylaniline (**12f**).* This compound was prepared from **11f** by means of **GP1** as a yellow oil (41%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33 (d, J = 1.8 Hz, 1H), 7.04 (dd, J = 7.9, 1.8 Hz, 1H), 6.99 (d, J = 7.9 Hz, 1H), 2.86−2.77 (m, 1H), 1.20 (d, J = 6.7 Hz, 6H).

*4-tert-Butyl-2-bromoaniline (**12g**).* This compound was prepared from **11g** by means of **GP1** as a brown oil (38%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40 (d, J = 1.8 Hz, 1H), 7.13 (dd, J = 7.9, 1.8 Hz, 1H), 6.72 (d, J = 7.9 Hz, 1H), 3.95 (br s, 2H), 1.26 (s, 3H).

*General Procedure **2** (GP2). 2-Vinylaniline (**13a**).* To a solution of 2-bromoaniline (**12a**) (182 mg, 1.06 mmol) and $Pd(PPh_3)_4$ (108 mg, 10 mol %) in 5 mL of toluene was added tributylvinyltin (350 μL, 1.20 mmol). The mixture was stirred at 100 °C under an argon atmosphere for 6 h and extracted with ethyl acetate. The extract was washed with water and brine, dried over anhydrous magnesium sulfate, and concentrated. The residue was purified by silica gel column

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chromatography (eluent: *n*-hexane/ethyl acetate = 9:1 to 5:1, v/v, silica gel/KF = 10:1, w/w) to afford 51.7 mg (41%) of the title compound as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.29 (d, J = 7.9 Hz, 1H), 7.09 (dd, J = 7.9, 7.9 Hz, 1H), 6.81−6.74 (m, 2H), 6.69 (d, J = 7.9 Hz, 1H), 5.63 (dd, J = 17, 1.2 Hz, 1H), 5.32 (dd, J = 11, 1.2 Hz, 1H), 3.75 (br s, 2H).

*4-Methyl-2-vinylaniline (**13b**).* This compound was prepared from **12b** by means of **GP2** as a brown oil (34%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.11 (d, J = 1.8 Hz, 1H), 6.90 (dd, J = 7.9, 1.8 Hz, 1H), 6.76 (dd, J = 17, 11 Hz, 1H), 6.60 (d, J = 7.9 Hz, 1H), 5.62 (dd, J = 17, 1.8 Hz, 1H), 5.29 (d, J = 11 Hz, 1.8H), 3.63 (br s, 2H), 2.25 (s, 3H).

*4-Ethyl-2-vinylaniline (**13c**).* This compound was prepared from **12c** by means of **GP2** as a brown oil (37%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.12 (d, J = 1.8 Hz, 1H), 6.94 (dd, J = 7.9, 1.8 Hz, 1H), 6.77 (dd, J = 17, 11 Hz, 1H), 6.63 (d, J = 7.9 Hz, 1H), 5.63 (dd, J = 17, 1.8 Hz, 1H), 5.30 (dd, J = 11, 1.8 Hz, 1H), 3.64 (br s, 2H), 2.55 (q, J = 7.9 Hz, 3H), 1.20 (t, J = 7.9 Hz, 3H).

*4-Hexyl-2-vinylaniline (**13d**).* This compound was prepared from **12d** by means of **GP2** as a yellow oil (57%). The resulting crude **13d** was used directly in the next step.

*4-Dodecyl-2-vinylaniline (**13e**).* This compound was prepared from **12e** by means of **GP2** as a yellow solid (65%). The resulting crude **13e** was used directly in the next step.

*General Procedure **3** (GP3). 4-Isopropyl-2-vinylaniline (**13f**).* To a solution of **12f** (429 mg, 2.00 mmol) in 10 mL of DME was added $Pd(PPh_3)_4$ (115 mg, 5 mol %), and the mixture was stirred at room temperature under an argon atmosphere for 15 min. Potassium carbonate (330 mg, 2.39 mmol), 3 mL of water, and 2,4,6-trivinylcyclotriboroxane−pyridine complex (496 mg, 2.06 mmol) were added, and the reaction mixture was heated under reflux for 4.5 h. The mixture was extracted with ethyl acetate, and the extract was washed with water and brine, dried over anhydrous magnesium sulfate, and concentrated. The residue was purified by silica gel column chromatography (eluent: *n*-hexane/ethyl acetate = 9:1, v/v) to afford 192 mg (59%) of the title compound as a brown oil. The resulting crude **13f** was used directly in the next step.

*4-tert-Butyl-2-vinylaniline (**13g**).* This compound was prepared from **12g** by means of **GP2** as a brown oil (29%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.29 (d, J = 1.8 Hz, 1H), 7.13 (dd, J = 7.9, 1.8 Hz, 1H), 6.79 (dd, J = 17, 11 Hz, 1H), 6.64 (d, J = 7.9 Hz, 1H), 5.63 (dd, J = 17, 1.8 Hz, 1H), 5.31 (dd, J = 11, 1.8 Hz, 1H), 3.66 (br s, 2H), 1.29 (s, 3H).

*General Procedure **4** (GP4). 2-Iodo-N-(2-vinylphenyl)benzamide (**14a**).* To a mixture of **13a** (51.7 mg, 0.434 mmol) and Et_3N (70 μL, 0.505 mmol) in 2 mL of DCM was added 2-iodobenzoyl chloride (177 mg, 0.664 mmol). The resulting mixture was stirred for 20 h at room temperature under argon atmosphere. The organic layer was washed with water and brine, dried over anhydrous magnesium sulfate, and concentrated. The residue was purified by silica gel column chromatography (eluent: *n*-hexane/ethyl acetate = 5:1, v/v) to afford 107 mg (71%) of the title compound as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.07 (d, J = 7.9 Hz, 1H), 7.93 (d, J = 7.9 Hz, 1H), 7.54 (d, J = 7.9 Hz, 1H), 7.56−7.52 (m, 2H), 7.49−7.43 (m, 2H), 7.22 (dd, J = 7.9, 7.9 Hz, 1H), 7.17 (dd, J = 7.9, 7.9 Hz, 1H), 6.92 (dd, J = 17, 11 Hz, 1H), 5.71 (d, J = 17 Hz, 1H), 5.43 (d, J = 11 Hz, 1H). MS (FAB) 350 $(M + H)^+$.

*2-Iodo-N-(4-methyl-2-vinylphenyl)benzamide (**14b**).* This compound was prepared from **13b** by means of **GP4** as a white solid (46%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.92 (d, J = 7.9 Hz, 1H), 7.89 (d, J = 7.9 Hz, 1H), 7.54 (dd, J = 7.9, 1.8 Hz, 1H), 7.45 (dd, J = 7.9, 7.9 Hz, 1H), 7.32−7.28 (m, 2H), 7.19−7.14 (m, 2H), 6.90 (dd, J = 17, 11 Hz, 1H), 5.70 (d, J = 17 Hz, 1H), 5.40 (d, J = 11 Hz, 1H), 2.36 (s, 3H). MS (FAB) 364 $(M + H)^+$.

*N-(4-Ethyl-2-vinylphenyl)-2-iodobenzamide (**14c**).* This compound was prepared from **13c** by means of **GP4** as a white solid (58%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.92 (d, J = 7.9 Hz, 1H), 7.92 (d, J = 7.9 Hz, 1H), 7.53 (dd, J = 7.9, 1.8 Hz, 1H), 7.45 (dd, J = 7.9, 7.9 Hz, 1H), 7.33−7.29 (m, 2H), 7.20 (dd, J = 7.9, 1.8 Hz, 1H), 7.16 (dd, J = 7.9, 7.9 Hz, 1H), 6.91 (dd, J = 17, 11 Hz, 1H), 5.71 (d, J = 17 Hz, 1H), 5.40 (d, J = 11 Hz, 1H), 2.66 (q, J = 7.9 Hz, 2H), 1.25 (t, J = 7.9 Hz, 3H). MS (FAB) 378 $(M + H)^+$.

*N-(4-Hexyl-2-vinylphenyl)-2-iodobenzamide (**14d**).* This compound was prepared from **13d** by means of **GP4** as a brown solid (quant). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.92 (dd, J = 7.9, 7.9 Hz, 2H), 7.53 (d, J = 7.9 Hz, 1H), 7.45 (dd, J = 7.9, 7.9 Hz, 1H), 7.33−7.27 (m, 2H), 7.20−7.14 (m, 2H), 6.90 (dd, J = 17, 11 Hz, 1H), 5.70 (d, J = 17 Hz, 1H), 5.40 (d, J = 11 Hz, 1H), 2.61 (t, J = 7.9 Hz, 2H), 1.65−1.57 (m, 2H), 1.38−1.27 (m, 6H), 0.89 (t, J = 7.3 Hz, 3H). MS (FAB) 434 $(M + H)^+$.

*N-(4-Dodecyl-2-vinylphenyl)-2-iodobenzamide (**14e**).* This compound was prepared from **13e** by means of **GP4** (95%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.92 (dd, J = 7.9, 7.9 Hz, 2H), 7.53 (d, J = 7.9 Hz, 1H), 7.45 (dd, J = 7.9, 7.9 Hz, 1H), 7.33−7.27 (m, 2H), 7.20−7.14 (m, 2H), 6.90 (dd, J = 17, 11 Hz, 1H), 5.70 (d, J = 17 Hz, 1H), 5.40 (d, J = 11 Hz, 1H), 2.60 (t, J = 7.9 Hz, 2H), 1.66−1.56 (m, 2H), 1.40−1.24 (m, 18H), 0.88 (t, J = 7.3 Hz, 3H). MS (FAB) 518 $(M + H)^+$.

*2-Iodo-N-(4-isopropyl-2-vinylphenyl)benzamide (**14f**).* This compound was prepared from **13f** by means of **GP4** as a white solid (99%). MS (FAB) 392 $(M + H)^+$.

*N-(4-tert-Butyl-2-vinylphenyl)-2-iodobenzamide (**14g**).* This compound was prepared from **13g** by means of **GP4** as a white solid (88%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.95 (d, J = 7.9 Hz, 1H), 7.92 (d, J = 7.9 Hz, 1H), 7.53 (d, J = 7.9 Hz, 1H), 7.49−7.43 (m, 2H), 7.40 (d, J = 7.9 Hz, 1H), 7.34 (s, 1H), 7.16 (dd, J = 7.9, 7.9 Hz, 1H), 6.92 (dd, J = 17, 11 Hz, 1H), 5.71 (d, J = 17 Hz, 1H), 5.41 (d, J = 11 Hz, 1H), 1.34 (s, 9H). MS (FAB) 406 $(M + H)^+$.

*General Procedure **5** (GP5). 2-Iodo-N-methyl-N-(2-vinylphenyl)-benzamide (**15a**).* To a solution of **14a** (107 mg, 0.308 mmol) in 2 mL of DMF were added sodium hydride (38.8 mg, 1.62 mmol) and iodomethane (40 μL, 0.643 mmol) at 0 °C. The mixture was stirred for 6.5 h at room temperature and extracted with ethyl acetate. The organic layer was washed with water and brine, dried over anhydrous magnesium sulfate, and concentrated. The residue was purified by silica gel column chromatography (eluent: *n*-hexane/ethyl acetate = 3:1, v/v) to afford 103 mg (92%) of the title compound as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.69 (d, J = 7.9 Hz, 1H), 7.49 (d, J = 7.9 Hz, 1H), 7.30 (d, J = 7.9 Hz, 1H), 7.16 (dd, J = 7.9, 7.9 Hz, 1H), 7.08 (dd, J = 7.9, 7.9 Hz, 1H), 7.01 (dd, J = 7.9, 7.9 Hz, 1H), 6.97−6.90 (m, 2H), 6.82 (dd, J = 7.9, 7.9 Hz, 1H), 5.80 (d, J = 17 Hz, 1H), 5.48 (d, J = 11 Hz, 1H), 3.41 (s, 3H). MS (FAB) 364 $(M + H)^+$.

*2-Iodo-N-methyl-N-(4-methyl-2-vinylphenyl)benzamide (**15b**).* This compound was prepared from **14b** by means of **GP5** as a colorless oil (99%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.69 (dd, J = 7.9, 1.8 Hz, 1H), 7.28 (d, J = 1.8 Hz, 1H), 7.18 (d, J = 7.9 Hz, 1H), 7.03 (dd, J = 7.9, 7.9 Hz, 1H), 6.97−6.87 (m, 3H), 6.83 (dd, J = 7.9, 7.9 Hz, 1H), 5.78 (d, J = 17 Hz, 1H), 5.44 (d, J = 11 Hz, 1H), 3.38 (s, 3H), 2.24 (s, 3H). MS (FAB) 378 $(M + H)^+$.

*N-(4-Ethyl-2-vinylphenyl)-2-iodo-N-methylbenzamide (**15c**).* This compound was prepared from **14c** by means of **GP5** as a colorless oil (94%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.69 (dd, J = 7.9, 1.8 Hz, 1H), 7.29 (d, J = 1.8 Hz, 1H), 7.20 (d, J = 7.9 Hz, 1H), 7.02 (dd, J = 7.9, 7.9 Hz, 1H), 6.96−6.88 (m, 3H), 6.82 (dd, J = 7.9, 7.9 Hz, 1H), 5.79 (d, J = 17 Hz, 1H), 5.44 (d, J = 11 Hz, 1H), 3.39 (s, 3H), 2.54 (q, J = 7.9 Hz, 2H), 1.16 (t, J = 7.9 Hz, 3H). MS (FAB) 392 $(M + H)^+$.

*N-(4-Hexyl-2-vinylphenyl)-2-iodo-N-methylbenzamide (**15d**).* This compound was prepared from **14d** by means of **GP5** as a brown oil (87%). The resulting crude **15d** was used directly in the next step.

*N-(4-Dodecyl-2-vinylphenyl)-2-iodo-N-methylbenzamide (**15e**).* This compound was prepared from **14e** by means of **GP5** as a colorless oil (97%). The resulting crude **15e** was used directly in the next step.

*2-Iodo-N-(4-isopropyl-2-vinylphenyl)-N-methylbenzamide (**15f**).* This compound was prepared from **14f** by means of **GP5** as a colorless oil (46%). MS (FAB) 406 $(M + H)^+$.

*N-(4-tert-Butyl-2-vinylphenyl)-2-iodo-N-methylbenzamide (**15g**).* This compound was prepared from **14g** by means of **GP5** as a white solid (85%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.69 (dd, J = 7.9, 1.8 Hz,

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1H), 7.46 (d, J = 1.8 Hz, 1H), 7.19 (d, J = 7.9 Hz, 1H), 7.09 (dd, J = 7.9, 1.8 Hz, 1H), 7.01 (ddd, J = 7.9, 7.9, 1.8 Hz, 1H), 6.93 (dd, J = 7.9, 1.8 Hz, 1H), 6.92 (dd, J = 17, 11 Hz, 1H), 6.81 (ddd, J = 7.9, 7.9, 1.8 Hz, 1H), 5.78 (dd, J = 17, 1.2 Hz, 1H), 5.45 (dd, J = 11, 1.2 Hz, 1H), 3.39 (s, 3H), 1.23 (s, 9H). MS (FAB) 420 $(M + H)^+$.

*General Procedure **6** (GP6). 5,11-Dihydro-5-methyl-11-methylene-6H-dibenz[b,e]azepin-6-one (**16**).* A mixture of **15a** (103 mg, 0.282 mmol), $Pd(OAc)_2$ (16.8 mg, 26 mol %), PPh_3 (34.7 mg, 50 mol %), and NaOAc (44.0 mg, 0.369 mmol) in 2 mL of DMF was stirred for 8 h at 100 °C under an argon atmosphere. After filtration through Celite, the mixture was extracted with ethyl acetate. The extract was washed with water and brine, dried over anhydrous magnesium sulfate, and concentrated. The residue was purified by silica gel column chromatography (eluent: *n*-hexane/ethyl acetate = 5:1, v/v) to afford 48.3 mg (73%) of the title compound as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.94 (dd, J = 7.9, 1.8 Hz, 1H), 7.45 (ddd, J = 7.9, 7.9, 1.8 Hz, 1H), 7.36 (ddd, J = 7.9, 7.9, 1.8 Hz, 1H), 7.32−7.21 (m, 4H), 7.15 (ddd, J = 7.9, 7.9, 1.8 Hz, 1H), 5.49 (d, J = 1.2 Hz, 1H), 5.44 (d, J = 1.2 Hz, 1H), 3.58 (s, 3H). HRMS (FAB) calcd for $C_{16}H_{14}NO$ 236.1075; found 236.1034 $(M + H)^+$.

*5,11-Dihydro-2,5-dimethyl-11-methylene-6H-dibenz[b,e]azepin-6-one (**17**).* This compound was prepared from **15b** by means of **GP6** as a colorless oil (45%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.93 (dd, J = 7.9, 1.8 Hz, 1H), 7.42 (ddd, J = 7.9, 7.9, 1.8 Hz, 1H), 7.35 (ddd, J = 7.9, 7.9, 1.8 Hz, 1H), 7.23 (dd, J = 7.9, 1.8 Hz, 1H), 7.11−7.10 (m, 2H), 7.07 (s, 1H), 5.46 (d, J = 1.2 Hz, 1H), 5.42 (d, J = 1.2 Hz, 1H), 3.56 (s, 3H), 2.32 (s, 3H). HRMS (FAB) calcd for $C_{17}H_{16}NO$ 250.1232; found 250.1233 $(M + H)^+$.

*5,11-Dihydro-2-ethyl-5-methyl-11-methylene-6H-dibenz[b,e]azepin-6-one (**18**).* This compound was prepared from **15c** by means of **GP6** as a colorless oil (53%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.94 (dd, J = 7.9, 1.8 Hz, 1H), 7.43 (ddd, J = 7.9, 7.9, 1.8 Hz, 1H), 7.35 (ddd, J = 7.9, 7.9, 1.8 Hz, 1H), 7.26−7.23 (m, 1H), 7.14−7.12 (m, 2H), 7.08 (s, 1H), 5.47 (d, J = 1.2 Hz, 1H), 5.43 (d, J = 1.2 Hz, 1H), 3.56 (s, 3H), 2.62 (q, J = 7.9 Hz, 2H), 1.23 (t, J = 7.9 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.28, 148.04, 143.49, 141.73, 138.39, 137.66, 131.68, 131.24, 131.17, 127.92, 127.68, 127.15, 126.24, 122.11, 116.35, 38.32, 28.05, 15.36. HRMS (FAB) calcd for $C_{18}H_{18}NO$ 264.1388; found 264.1357 $(M + H)^+$.

*5,11-Dihydro-2-hexyl-5-methyl-11-methylene-6H-dibenz[b,e]azepin-6-one (**19**).* This compound was prepared from **15d** by means of **GP6** as a yellow oil (88%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.94 (d, J = 7.9 Hz, 1H), 7.43 (t, J = 7.9 Hz, 1H), 7.35 (t, J = 7.9 Hz, 1H), 7.25 (d, J = 7.9 Hz, 1H), 7.14−7.08 (m, 2H), 7.06 (s, 1H), 5.46 (s, 1H), 5.42 (s, 1H), 3.56 (s, 3H), 2.56 (t, J = 7.9 Hz, 2H), 1.62−1.52 (m, 2H), 1.37−1.27 (m, 6H), 0.88 (t, J = 6.7 Hz, 3H). HRMS (FAB) calcd for $C_{22}H_{26}NO$ 320.2014; found 320.2006 $(M + H)^+$.

*5,11-Dihydro-2-(1-dodecyl)-5-methyl-11-methylene-6H-dibenz[b,e]azepin-6-one (**20**).* This compound was prepared from **15e** by means of **GP6** as a colorless oil (76%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.94 (d, J = 7.9 Hz, 1H), 7.43 (t, J = 7.9 Hz, 1H), 7.35 (t, J = 7.9 Hz, 1H), 7.25 (d, J = 7.9 Hz, 1H), 7.13−7.08 (m, 2H), 7.06 (s, 1H), 5.46 (s, 1H), 5.42 (s, 1H), 3.56 (s, 3H), 2.56 (t, J = 7.9 Hz, 2H), 1.62−1.55 (m, 2H), 1.34−1.22 (m, 18H), 0.88 (t, J = 7.3 Hz, 3H). HRMS (FAB) calcd for $C_{28}H_{38}NO$ 404.2953; found 404.2919 $(M + H)^+$.

*5,11-Dihydro-2-isopropyl-5-methyl-11-methylene-6H-dibenz[b,e]azepin-6-one (**21**).* This compound was prepared from **15f** by means of **GP6** as a colorless oil (34%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.94 (dd, J = 7.9, 1.2 Hz, 1H), 7.43 (ddd, J = 7.9, 7.3, 1.2 Hz, 1H), 7.36 (ddd, J = 7.9, 7.3, 1.2 Hz, 1H), 7.26 (dd, J = 7.3, 1.2 Hz, 1H), 7.16−7.13 (m, 2H), 7.09 (d, J = 1.2 Hz, 1H), 5.47 (d, J = 1.2 Hz, 1H), 5.43 (d, J = 1.2 Hz, 1H), 3.57 (s, 3H), 2.92−2.85 (m, 1H), 1.24 (dd, J = 6.6, 1.8 Hz, 6H). HRMS (FAB) calcd for $C_{19}H_{20}NO$ 278.1545; found 278.1536 $(M + H)^+$.

*5,11-Dihydro-2-tert-butyl-5-methyl-11-methylene-6H-dibenz[b,e]azepin-6-one (**22**).* This compound was prepared from **15g** by means of **GP6** as a colorless oil (63%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.95 (d, J = 7.9 Hz, 1H), 7.44 (dd, J = 7.9, 7.9 Hz, 1H), 7.36 (dd, J = 7.9, 7.9 Hz, 1H), 7.31 (dd, J = 7.9, 1.8 Hz, 1H), 7.28−7.25 (m, 1H), 7.23 (d, J = 1.8 Hz, 1H), 7.15 (d, J = 7.9 Hz, 1H), 5.47 (s, 1H), 5.43 (s, 1H), 3.57 (s, 3H), 1.31 (s, 9H). HRMS (FAB) calcd for $C_{20}H_{22}NO$ 292.1701; found 292.1748 $(M + H)^+$.

*2-(1,1,1,3,3,3-Hexafluoro-2-hydroxypropan-2-yl)-5-methyl-11-methylene-6H-dibenz[b,e]azepin-6-one (**10**).* This compound was prepared from **11h** by means of a series of procedures similar to those used for **16** as a white solid (16% in 4 steps); Mp 205.0−206.2 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.96 (dd, J = 7.9, 1.8 Hz, 1H), 7.65−7.60 (m, 2H), 7.48 (ddd, J = 7.9, 7.9, 1.8 Hz, 1H), 7.39 (ddd, J = 7.9, 7.9, 1.8 Hz, 1H), 7.29 (d, J = 7.9 Hz, 1H), 7.30−7.27 (m, 1H), 5.55 (s, 1H), 5.48 (s, 1H), 3.61 (s, 1H), 3.59 (s, 3H). HRMS (FAB) calcd for $C_{19}H_{14}F_6NO_2$ 402.0929; found 402.0924 $(M + H)^+$.

*2-Iodo-5-methoxybenzoic Acid (**27**).* A solution of $NaNO_2$ (0.71 g, 10.3 mmol) in 4 mL of water was added slowly to a cold (0 °C) stirred solution of 5-hydroxyanthranilic acid (1.5 g, 9.8 mmol) and 8 mL of concentrated HCl in 20 mL of water. After the addition was completed, the solution was stirred for an additional 30 min at 0 °C. A solution of KI (2.5 g, 14.8 mmol) in 4 mL of water was added slowly. The resulting mixture was stirred for 20 min at 0 °C and then heated to 90 °C for 30 min to remove N_2. The mixture was cooled to room temperature and extracted with ethyl acetate. The organic extract was washed with satd $NaHSO_3$ aq and water and dried over anhydrous magnesium sulfate. The solvent was removed in vacuo to give **25** as a yellowish−red solid, which was used directly for the next step. To a solution of **25** and K_2CO_3 (2.78 g, 20.1 mmol) in 50 mL of anhydrous acetone was added dimethyl sulfate (1.9 mL, 20.0 mmol). The reaction mixture was heated under reflux for 5 h, cooled to room temperature, and filtered. The filtrate was removed in vacuo to give **26** as a yellow oil, which was used directly for the next step. To a solution of **26** in 30 mL of MeOH was added 10 mL of 2 N NaOH aq, and the mixture was heated under reflux for 2 h. The solvent was removed in vacuo, and the residue was dissolved in water and extracted with ethyl acetate. The aqueous layer was acidified with 2 N HCl and extracted with ethyl acetate. The combined extract was dried over anhydrous magnesium sulfate and concentrated in vacuo. The residue was purified by silica gel column chromatography (eluent: *n*-hexane/ethyl acetate = 1:1, v/v) to afford 1.27 g (47%) of the title compound as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.90 (d, J = 8.5 Hz, 1H), 7.55 (d, J = 3.1 Hz, 1H), 6.81 (dd, J = 8.5, 3.1 Hz, 1H), 3.84 (s, 3H).

*N-(4-Ethyl-2-vinylphenyl)-2-iodo-5-methoxybenzamide (**28**).* To a solution of **27** (280 mg, 1.01 mmol) in 2 mL of DCM was added chloromethylenedimethyliminium chloride (130 mg, 1.02 mmol) at 0 °C, and the mixture was stirred for 30 min at room temperature. To a solution of **13c** (117 mg, 0.795 mmol) in 2 mL of DCM were added 130 μL of Et_3N and the above solution at 0 °C, and stirring was continued for 12 h at room temperature. The reaction mixture was washed with water and brine, dried over anhydrous magnesium sulfate, and concentrated. The residue was purified by silica gel column chromatography (eluent: *n*-hexane/ethyl acetate = 5:1 to 4:1, v/v) to afford 258 mg (80%) of the title compound as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.94−7.89 (m, 1H), 7.76 (d, J = 7.9 Hz, 1H), 7.36−7.30 (m, 2H), 7.20 (d, J = 7.9 Hz, 1H), 7.10 (d, J = 3.1 Hz, 1H), 6.91 (dd, J = 17, 11 Hz, 1H), 6.75 (dd, J = 7.9, 3.1 Hz, 1H), 5.71 (d, J = 17 Hz, 1H), 5.41 (d, J = 11 Hz, 1H), 3.83 (br s, 3H), 2.66 (q, J = 7.9 Hz, 2H), 1.25 (t, J = 7.9 Hz, 3H).

*N-(4-Ethyl-2-vinylphenyl)-2-iodo-5-methoxy-N-methylbenzamide (**29**).* This compound was prepared from **28** by means of **GP5** as a yellow oil (78%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.51 (d, J = 7.9 Hz, 1H), 7.31 (d, J = 1.8 Hz, 1H), 7.23 (d, J = 7.9 Hz, 1H), 6.95−6.88 (m, 2H), 6.53 (d, J = 3.1 Hz, 1H), 6.42 (dd, J = 7.9, 3.1 Hz, 1H), 5.80 (d, J = 17 Hz, 1H), 5.45 (d, J = 11 Hz, 1H), 3.56 (s, 3H), 3.39 (s, 3H), 2.56 (q, J = 7.9 Hz, 2H), 1.17 (t, J = 7.9 Hz, 3H).

*2-Ethyl-5,11-dihydro-8-methoxy-5-methyl-11-methylene-6H-dibenz[b,e]azepin-6-one (**30**).* This compound was prepared from **29** by means of **GP6** as a yellow oil (95%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.46 (d, J = 2.4 Hz, 1H), 7.17 (d, J = 7.9 Hz, 1H), 7.12 (s, 2H), 7.07 (s, 1H), 6.98 (dd, J = 7.9, 2.4 Hz, 1H), 5.41 (s, 1H), 5.38 (s, 1H), 3.82 (s, 3H), 3.56 (s, 3H), 2.62 (q, J = 7.9 Hz, 2H), 1.23 (t, J = 7.9 Hz, 3H).

*2-Ethyl-5,11-dihydro-8-hydroxy-5-methyl-11-methylene-6H-dibenz[b,e]azepin-6-one (**31**).* To a solution of **30** (120 mg, 0.410

mmol) in 2 mL of dehydrated DCM was added dropwise 1.6 mL of boron tribromide (1.0 M solution in dichloromethane) at 0 °C under an argon atmosphere. The mixture was stirred for 3 h and poured into ice−water. The organic layer was washed with water and brine, dried over anhydrous magnesium sulfate, and concentrated. The residue was purified by silica gel column chromatography (eluent: *n*-hexane/ethyl acetate = 3:1 to 1:1, v/v) to afford 103 mg (90%) of the title compound as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.80−7.78 (m, 2H), 7.16−7.12 (m, 3H), 7.08 (s, 1H), 6.98 (dd, J = 8.5, 2.4 Hz, 1H), 5.41 (s, 1H), 5.38 (s, 1H), 3.57 (s, 3H), 2.63 (q, J = 7.9 Hz, 2H), 1.23 (t, J = 7.9 Hz, 3H). MS (FAB) 279 $(M)^+$.

*2-Ethyl-5,11-dihydro-5-methyl-11-methylene-6-oxodibenz[b,e]-azepin-8-yl trifluoromethanesulfonate (**32**).* To a solution of **31** (10.0 mg, 0.0358 mmol) and 8 μL of pyridine in 1 mL of DCM was added dropwise trifluoromethanesulfonic anhydride (8 μL, 0.0474 mmol) at 0 °C. The mixture was stirred for 2 h at room temperature and poured into ice−water. The organic layer was washed with satd $NaHCO_3$ aq, water, and brine, dried over anhydrous magnesium sulfate, and concentrated. The residue was purified by silica gel column chromatography (eluent: *n*-hexane/ethyl acetate = 3:1 to 1:1, v/v) to afford 7.0 mg (47%) of the title compound as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85 (s, 1H), 7.34 (s, 2H), 7.20−7.14 (m, 2H), 7.08 (s, 1H), 5.51 (s, 1H), 5.51 (s, 1H), 3.56 (s, 3H), 2.64 (q, J = 7.9 Hz, 2H), 1.24 (t, J = 7.9 Hz, 3H). MS (FAB) 412 $(M + H)^+$.

*2-Ethyl-5,11-dihydro-5-methyl-11-methylene-8-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-6H-dibenz[b,e]azepin-6-one (**33**).* A mixture of **32** (20.1 mg, 0.0489 mmol), bis(pinacolato)diboron (26.4 mg, 0.104 mmol), $PdCl_2(PPh_3)_2$ (16.4 mg, 48 mol %), and KOAc (19.5 mg, 0.199 mmol) in 1 mL of dioxane was stirred for 3 h at 120 °C under an argon atmosphere. The mixture was filtered through Celite, and the filtrate was extracted with ethyl acetate. The extract was washed with water and brine, dried over anhydrous magnesium sulfate, and concentrated. The residue was purified by silica gel column chromatography (eluent: *n*-hexane/ethyl acetate = 5:1, v/v) to afford 10.9 mg (57%) of the title compound as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.38 (s, 1H), 7.83 (d, J = 7.9 Hz, 1H), 7.24 (d, J = 7.9 Hz, 1H), 7.11 (s, 2H), 7.06 (s, 1H), 5.48 (s, 1H), 5.44 (s, 1H), 3.55 (s, 3H), 2.60 (q, J = 7.9 Hz, 2H), 1.31 (s, 6H), 1.30 (s, 6H), 1.21 (t, J = 7.9 Hz, 3H). MS (FAB) 390 $(M + H)^+$.

*General Procedure **7** (GP7). Methyl 3-(3-Iodophenyl)propanoate (**35c**).* To a solution of **34c** (50.0 mg, 0.191 mmol) in 1 mL of MeOH was added 0.1 mL of concentrated H_2SO_4, and the mixture was refluxed for 4 h. The organic solution was extracted with DCM, and the extract was washed with water and brine, dried over anhydrous magnesium sulfate, and concentrated to obtain 48.1 mg (91%) of the title compound as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.64 (s, 1H), 7.61 (d, J = 7.9 Hz, 1H), 7.25 (d, J = 7.9 Hz, 1H), 7.06 (t, J = 7.9 Hz, 1H), 3.70 (s, 3H), 3.57 (s, 2H).

*Methyl 3-(4-Bromophenyl)propanoate (**35d**).* This compound was prepared from **34d** by means of **GP7** as a colorless oil (99%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.45 (d, J = 7.9 Hz, 2H), 7.16 (d, J = 7.9 Hz, 2H), 3.70 (s, 3H), 3.58 (s, 2H).

*General Procedure **8** (GP8). Ethyl 3-(5,11-Dihydro-2-ethyl-5-methyl-11-methylene-6-oxodibenz[b,e]azepin-8-yl)benzoate (**36a**).* A mixture of **33** (12.7 mg, 0.0326 mmol), ethyl 3-iodobenzoate (**35a**) (6 μL, 0.0356 mmol), $Pd(PPh_3)_4$ (30.6 mg, 80 mol %), and K_3PO_4 (15.0 mg, 0.0710 mmol) in 1 mL of DMF was stirred for 3 h at 120 °C under an argon atmosphere. After filtration through Celite, the mixture was extracted with ethyl acetate and the extract was washed with water and brine, dried over anhydrous magnesium sulfate, and concentrated. The residue was purified by silica gel column chromatography (eluent: *n*-hexane/ethyl acetate = 5:1, v/v) to afford 8.2 mg (61%) of the title compound as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.26 (s, 1H), 8.21 (d, J = 1.8 Hz, 1H), 8.03 (d, J = 7.9 Hz, 1H), 7.78 (d, J = 7.9 Hz, 1H), 7.70 (dd, J = 7.9, 1.8 Hz, 1H), 7.50 (dd, J = 7.9, 7.9 Hz, 1H), 7.36 (d, J = 7.9 Hz, 1H), 7.17−7.14 (m, 2H), 7.11 (s, 1H), 5.53 (s, 1H), 5.48 (s, 1H), 4.40 (q, J = 7.3 Hz, 2H), 3.60 (s, 3H), 2.64 (q, J = 7.3 Hz, 2H), 1.40 (t, J = 7.3 Hz, 3H), 1.24 (t, J = 7.3 Hz, 3H). MS (FAB) 412 $(M + H)^+$.

*Methyl 4-(5,11-Dihydro-2-ethyl-5-methyl-11-methylene-6-oxodibenz[b,e]azepin-8-yl)benzoate (**36b**).* This compound was prepared from **35b** by means of **GP8** as a yellow oil (79%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.21 (d, J = 1.8 Hz, 1H), 8.09 (d, J = 7.9 Hz, 2H), 7.69 (dd, J = 7.9, 1.8 Hz, 1H), 7.66 (d, J = 7.9 Hz, 2H), 7.35 (d, J = 7.9 Hz, 1H), 7.18−7.13 (m, 2H), 7.11 (s, 1H), 5.53 (s, 1H), 5.48 (s, 1H), 3.93 (s, 3H), 3.59 (s, 3H), 2.63 (q, J = 7.9 Hz, 2H), 1.24 (t, J = 7.9 Hz, 3H).

*Methyl 2-(3-(5,11-Dihydro-2-ethyl-5-methyl-11-methylene-6-oxodibenz[b,e]azepin-8-yl)phenyl)acetate (**36c**).* This compound was prepared from **35c** by means of **GP8** as a brown oil (83%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.17 (d, J = 1.8 Hz, 1H), 7.65 (dd, J = 7.9, 1.8 Hz, 1H), 7.52−7.46 (m, 2H), 7.39 (t, J = 7.9 Hz, 1H), 7.32 (d, J = 7.9 Hz, 1H), 7.27 (d, J = 7.9 Hz, 1H), 7.17−7.12 (m, 2H), 7.11 (s, 1H), 5.52 (s, 1H), 5.46 (s, 1H), 3.69 (s, 3H), 3.67 (s, 2H), 3.59 (s, 3H), 2.63 (q, J = 7.9 Hz, 2H), 1.24 (t, J = 7.9 Hz, 3H). MS (FAB) 412 $(M + H)^+$.

*Methyl 2-(4-(5,11-Dihydro-2-ethyl-5-methyl-11-methylene-6-oxodibenz[b,e]azepin-8-yl)phenyl)acetate (**36d**).* This compound was prepared from **35d** by means of **GP8** as a colorless oil (25%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.16 (d, J = 1.8 Hz, 1H), 7.64 (dd, J = 7.9, 1.8 Hz, 1H), 7.55 (d, J = 7.9 Hz, 2H), 7.36−7.30 (m, 3H), 7.17−7.10 (m, 3H), 5.51 (s, 1H), 5.46 (s, 1H), 3.71 (s, 3H), 3.66 (s, 2H), 3.59 (s, 3H), 2.63 (q, J = 7.3 Hz, 2H), 1.23 (t, J = 7.3 Hz, 3H). MS (FAB) 412 $(M + H)^+$.

*General Procedure **9** (GP9). 3-(5,11-Dihydro-2-ethyl-5-methyl-11-methylene-6-oxodibenz[b,e]azepin-8-yl)benzoic Acid (**37**).* To a solution of **36a** (8.2 mg, 0.020 mmol) in 1 mL of ethanol was added 0.5 mL of 2 N aqueous NaOH. The mixture was stirred for 3 h at 80 °C and extracted with ethyl acetate. The extract was washed with 2 N HCl, water, and brine, dried over anhydrous magnesium sulfate, and concentrated to afford 4.9 mg (64%) of the title compound as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.35 (s, 1H), 8.24 (d, J = 1.8 Hz, 1H), 8.09 (d, J = 7.9 Hz, 1H), 7.84 (d, J = 7.9 Hz, 1H), 7.72 (dd, J = 7.9, 1.8 Hz, 1H), 7.53 (dd, J = 7.9, 7.9 Hz, 1H), 7.37 (d, J = 7.9 Hz, 1H), 7.17−7.15 (m, 2H), 7.12 (s, 1H), 5.54 (s, 1H), 5.49 (s, 1H), 3.61 (s, 3H), 2.64 (q, J = 7.3 Hz, 2H), 1.24 (t, J = 7.3 Hz, 3H). HRMS (FAB) calcd for $C_{25}H_{22}NO_3$ 384.1600; found 384.1582 $(M + H)^+$.

*4-(5,11-Dihydro-2-ethyl-5-methyl-11-methylene-6-oxodibenz-[b,e]azepin-8-yl)benzoic Acid (**38**).* This compound was prepared from **36b** by means of **GP9** as a white solid (14%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.22 (d, J = 2.4 Hz, 1H), 8.14 (d, J = 7.9 Hz, 2H), 7.72−7.67 (m, 3H), 7.37 (d, J = 7.9 Hz, 1H), 7.17−7.15 (m, 2H), 7.11 (s, 1H), 5.54 (s, 1H), 5.49 (s, 1H), 3.60 (s, 3H), 2.64 (q, J = 7.3 Hz, 2H), 1.24 (t, J = 7.3 Hz, 3H). HRMS (FAB) calcd for $C_{25}H_{22}NO_3$ 384.1600; found 384.1643 $(M + H)^+$.

*2-(3-(5,11-Dihydro-2-ethyl-5-methyl-11-methylene-6-oxodibenz-[b,e]azepin-8-yl)phenyl)acetic Acid (**39**).* This compound was prepared from **36c** by means of **GP9** as a white solid (98%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.16 (d, J = 1.8 Hz, 1H), 7.65 (dd, J = 7.9, 1.8 Hz, 1H), 7.52−7.49 (m, 2H), 7.39 (t, J = 7.9 Hz, 1H), 7.32 (d, J = 7.9 Hz, 1H), 7.28 (d, J = 7.9 Hz, 1H), 7.17−7.12 (m, 2H), 7.10 (s, 1H), 5.51 (s, 1H), 5.46 (s, 1H), 3.70 (s, 2H), 3.59 (s, 3H), 2.63 (q, J = 7.9 Hz, 2H), 1.23 (t, J = 7.9 Hz, 3H). HRMS (FAB) calcd for $C_{26}H_{24}NO_3$ 398.1756; found 398.1774 $(M + H)^+$.

*2-(4-(5,11-Dihydro-2-ethyl-5-methyl-11-methylene-6-oxodibenz-[b,e]azepin-8-yl)phenyl)acetic Acid (**40**).* This compound was prepared from **36d** by means of **GP9** as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.17 (d, J = 1.8 Hz, 1H), 7.64 (dd, J = 7.9, 1.8 Hz, 1H), 7.55 (d, J = 7.9 Hz, 2H), 7.36−7.30 (m, 3H), 7.17−7.12 (m, 2H), 7.10 (s, 1H), 5.51 (s, 1H), 5.46 (s, 1H), 3.69 (s, 2H), 3.59 (s, 3H), 2.63 (q, J = 7.9 Hz, 2H), 1.23 (t, J = 7.9 Hz, 3H). HRMS (FAB) calcd for $C_{26}H_{24}NO_3$ 398.1756; found 398.1709 $(M + H)^+$.

*2-Bromo-4-nitroaniline (**42a**).* This compound was prepared from **41a** by means of **GP1** as a yellow solid (81%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.38 (d, J = 1.8 Hz, 1H), 8.04 (dd, J = 7.9, 1.8 Hz, 1H), 6.74 (d, J = 7.9 Hz, 1H), 4.82 (br s, 2H).

*4-Amino-3-bromophenyl Acetate (**42b**).* This compound was prepared from **41b** by means of **GP1** as a white solid (83%). ^{1}H NMR

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(500 MHz, $CDCl_3$) δ 7.19 (d, J = 1.8 Hz, 1H), 6.86 (dd, J = 7.9, 1.8 Hz, 1H), 6.76 (d, J = 7.9 Hz, 1H), 2.26 (s, 3H).

*Ethyl 4-Amino-3-bromobenzoate (**42c**).* This compound was prepared from **41c** by means of **GP1** as a yellow solid (87%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.12 (d, J = 1.8 Hz, 1H), 7.80 (dd, J = 7.9, 1.8 Hz, 1H), 6.74 (d, J = 7.9 Hz, 1H), 4.32 (q, J = 6.7 Hz, 2H), 1.37 (t, J = 6.7 Hz, 3H).

*1-(4-Amino-3-bromophenyl)ethanone (**42d**).* This compound was prepared from **41d** by means of **GP1** as a brown solid (76%) and used for the next step without purification.

*4-Nitro-2-vinylaniline (**43a**).* This compound was prepared from **42a** by means of **GP2** as a yellow solid (85%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.19 (d, J = 1.8 Hz, 1H), 8.00 (dd, J = 7.9, 1.8 Hz, 1H), 6.70−6.63 (m, 2H), 5.76 (d, J = 18 Hz, 1H), 5.50 (d, J = 11 Hz, 1H), 4.44 (br s, 2H).

*4-Amino-3-vinylphenyl Acetate (**43b**).* This compound was prepared from **42b** by means of **GP2** as a yellow oil (69%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.00 (d, J = 2.4 Hz, 1H), 6.81 (dd, J = 7.9, 2.4 Hz, 1H), 6.71 (dd, J = 17, 11 Hz, 1H), 6.66 (d, J = 7.9 Hz, 1H), 5.62 (d, J = 17 Hz, 1H), 5.34 (d, J = 11 Hz, 1H), 3.75 (br s, 2H), 2.27 (s, 3H).

*Ethyl 4-Amino-3-vinylbenzoate (**43c**).* This compound was prepared from **42c** by means of **GP3** as a white solid (47%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.00 (d, J = 1.8 Hz, 1H), 7.80 (dd, J = 7.9, 1.8 Hz, 1H), 6.79−6.72 (m, 2H), 5.73 (d, J = 18 Hz, 1H), 5.42 (d, J = 11 Hz, 1H), 4.34 (q, J = 6.7 Hz, 2H), 1.38 (t, J = 6.7 Hz, 3H).

*1-(4-Amino-3-vinylphenyl)ethanone (**43d**).* This compound was prepared from **42d** by means of **GP2** as a yellow solid. The resulting crude **43d** was used directly in the next step.

*2-Iodo-N-(4-nitro-2-vinylphenyl)benzamide (**44a**).* This compound was prepared from **43a** by means of **GP4** as a yellow oil (quant). The resulting crude **44a** was used directly in the next step.

*4-(2-Iodobenzamido)-3-vinylphenyl Acetate (**44b**).* This compound was prepared from **43b** by means of **GP4** as a white solid (94%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.07 (d, J = 7.9 Hz, 1H), 7.93 (d, J = 7.9 Hz, 1H), 7.53 (d, J = 7.9 Hz, 1H), 7.46 (dd, J = 7.9, 7.9 Hz, 1H), 7.34 (s, 1H), 7.20 (d, J = 1.8 Hz, 1H), 7.17 (dd, J = 7.9, 7.9 Hz, 1H), 7.09 (dd, J = 7.9, 1.8 Hz, 1H), 6.87 (dd, J = 17, 11 Hz, 1H), 5.71 (d, J = 17 Hz, 1H), 5.46 (d, J = 11 Hz, 1H), 2.32 (s, 3H). MS (FAB) 408 $(M + H)^+$.

*Ethyl 4-(2-Iodobenzamido)-3-vinylbenzoate (**44c**).* This compound was prepared from **43c** by means of **GP4** as a white solid (88%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.35 (d, J = 8.5 Hz, 1H), 8.11 (d, J = 2.4 Hz, 1H), 8.03 (d, J = 8.5 Hz, 1H), 7.94 (d, J = 8.5 Hz, 1H), 7.60−7.51 (m, 2H), 7.47 (dd, J = 8.5, 7.3 Hz, 1H), 7.19 (dd, J = 7.3, 6.7 Hz, 1H), 6.88 (dd, J = 17, 11 Hz, 1H), 5.79 (d, J = 17 Hz, 1H), 5.53 (d, J = 11 Hz, 1H), 4.39 (q, J = 7.3 Hz, 2H), 1.41 (t, J = 7.3 Hz, 1H). MS (FAB) 422 $(M + H)^+$.

*N-(4-Acetyl-2-vinylphenyl)-2-iodobenzamide (**44d**).* This compound was prepared from **43d** by means of **GP4** as a white solid (50% in 2 steps). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.40 (d, J = 7.9 Hz, 1H), 8.03 (s, 1H), 7.95 (d, J = 7.9 Hz, 2H), 7.62 (s, 1H), 7.54 (d, J = 7.9 Hz, 1H), 7.47 (dd, J = 7.9, 7.9 Hz, 1H), 7.19 (dd, J = 7.9, 7.9 Hz, 1H), 6.88 (dd, J = 17, 11 Hz, 1H), 5.79 (d, J = 17 Hz, 1H), 5.55 (d, J = 11 Hz, 1H), 2.62 (s, 3H). MS (FAB) 392 $(M + H)^+$.

*2-Iodo-N-methyl-N-(4-nitro-2-vinylphenyl)benzamide (**45a**).* This compound was prepared from **44a** by means of **GP5** as a yellow viscous oil (53%). MS (FAB) 409 $(M + H)^+$.

*Ethyl 4-(2-Iodo-N-methylbenzamido)-3-vinylbenzoate (**45c**).* This compound was prepared from **44c** by means of **GP5** as a colorless oil (91%). MS (FAB) 436 $(M + H)^+$.

*N-(4-Acetyl-2-vinylphenyl)-2-iodo-N-methylbenzamide (**45d**).* This compound was prepared from **44d** by means of **GP5** (32%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.08 (d, J = 1.8 Hz, 1H), 7.71 (dd, J = 7.9, 1.8 Hz, 1H), 7.65 (dd, J = 7.9, 1.8 Hz, 1H), 7.37 (d, J = 7.9 Hz, 1H), 7.03 (ddd, J = 7.9, 7.9, 1.8 Hz, 1H), 6.98−6.91 (m, 2H), 6.85 (ddd, J = 7.9, 7.9, 1.8 Hz, 1H), 5.93 (d, J = 18 Hz, 1H), 5.58 (d, J = 12 Hz, 1H), 3.41 (s, 3H), 2.54 (s, 3H). MS (FAB) 406 $(M + H)^+$.

*N-(4-Hydroxy-2-vinylphenyl)-2-iodo-N-methylbenzamide (**45e**).* This compound was prepared from **44b** by means of **GP5** as a white solid (62%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.68 (d, J = 7.9 Hz, 1H), 7.20 (d, J = 7.9 Hz, 1H), 7.03 (d, J = 7.9 Hz, 1H), 6.95−6.81 (m, 4H), 6.54 (dd, J = 7.9, 2.4 Hz, 1H), 5.75 (d, J = 17 Hz, 1H), 5.46 (d, J = 11 Hz, 1H), 3.37 (s, 3H). MS (FAB) 380 $(M + H)^+$.

*5,11-Dihydro-5-methyl-11-methylene-2-nitro-6H-dibenz[b,e]-azepin-6-one (**46**).* This compound was prepared from **45a** by means of **GP6** as a brown oil (88%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.19−8.16 (m, 2H), 7.96 (d, J = 7.9 Hz, 1H), 7.51 (dd, J = 7.9, 7.9 Hz, 1H), 7.42 (dd, J = 7.9, 7.9 Hz, 1H), 7.35 (d, J = 7.9 Hz, 1H), 7.29 (d, J = 7.9 Hz, 1H), 5.64 (s, 1H), 5.56 (s, 1H), 3.62 (s, 3H). HRMS (FAB) calcd for $C_{16}H_{13}N_2O_3$ 281.0926; found 281.0925 $(M + H)^+$. Anal. Calcd for $C_{16}H_{12}N_2O_3$: C, 68.56; H, 4.32; N, 9.99. Found: C, 68.75; H, 4.51; N, 9.73.

*5,11-Dihydro-2-hydroxy-5-methyl-11-methylene-6H-dibenz[b,e]-azepin-6-one (**48**).* This compound was prepared from **45e** by means of **GP6** (95%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.93 (d, J = 7.9 Hz, 1H), 7.43 (dd, J = 7.9, 7.9 Hz, 1H), 7.36 (dd, J = 7.9, 7.9 Hz, 1H), 7.22 (d, J = 7.9 Hz, 1H), 7.09 (d, J = 7.9 Hz, 1H), 6.77 (dd, J = 7.9, 2.4 Hz, 1H), 6.74 (d, J = 2.4 Hz, 1H), 5.46 (d, J = 1.2 Hz, 1H), 5.43 (d, J = 1.2 Hz, 1H), 5.24 (br s, 1H), 3.54 (s, 3H). HRMS (FAB) calcd for $C_{16}H_{14}NO_2$ 252.1025; found 252.1003 $(M + H)^+$. Anal. Calcd for $C_{16}H_{13}NO_2 \cdot 1/4H_2O$: C, 75.13; H, 5.32; N, 5.48. Found: C, 75.43; H, 5.30; N, 5.45.

*Ethyl 5,11-Dihydro-5-methyl-11-methylene-6-oxodibenz[b,e]-azepine-2-carboxylate (**49**).* This compound was prepared from **45c** by means of **GP6** as a colorless oil (42%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.99−7.93 (m, 3H), 7.47 (ddd, J = 7.9, 7.9, 1.8 Hz, 1H), 7.38 (ddd, J = 7.9, 7.9, 1.8 Hz, 1H), 7.30−7.26 (m, 2H), 5.55 (s, 1H), 5.51 (s, 1H), 4.42−4.35 (m, 2H), 3.60 (s, 3H), 1.39 (t, J = 7.3 Hz, 3H). MS (FAB) 308 $(M + H)^+$.

*2-Acetyl-5,11-dihydro-5-methyl-11-methylene-6H-dibenz[b,e]-azepin-6-one (**52**).* This compound was prepared from **45d** by means of **GP6** as a brown viscous oil (22% in 2 steps). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.95 (dd, J = 7.9, 1.2 Hz, 1H), 7.90 (dd, J = 7.9, 1.2 Hz, 1H), 7.87 (d, J = 1.2 Hz, 1H), 7.48 (ddd, J = 7.9, 7.9, 1.2 Hz, 1H), 7.39 (ddd, J = 7.9, 7.9, 1.2 Hz, 1H), 7.31−7.27 (m, 2H), 5.56 (s, 1H), 5.51 (s, 1H), 3.60 (s, 3H), 2.59 (s, 3H). HRMS (FAB) calcd for $C_{18}H_{16}NO_2$ 278.1181; found 278.1198 $(M + H)^+$. Anal. Calcd for $C_{18}H_{15}NO_2 \cdot 8/9H_2O$: C, 73.70; H, 5.77; N, 4.78. Found: C, 73.51; H, 5.38; N, 4.45.

*2-Amino-5,11-dihydro-5-methyl-11-methylene-6H-dibenz[b,e]-azepin-6-one (**47**).* To a solution of **46** (207 mg, 0.737 mmol) in 5 mL of ethyl acetate was added tin(II) chloride dihydrate (860 mg, 3.81 mmol). The mixture was heated to reflux for 3.5 h. After cooling, the mixture was poured into ice water, then satd $NaHCO_3$ aq was added. The organic layer was washed with water and brine, dried over anhydrous magnesium sulfate, and concentrated. The residue was purified by silica gel column chromatography (eluent: *n*-hexane/ethyl acetate = 1:1, v/v, triethylamine 0.1%, v/v) to afford 138 mg (75%) of the title compound as a yellow−white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.92 (d, J = 7.9 Hz, 1H), 7.42 (dd, J = 7.9, 7.9 Hz, 1H), 7.35 (dd, J = 7.9, 7.9 Hz, 1H), 7.20 (d, J = 7.9 Hz, 1H), 7.03 (d, J = 7.9 Hz, 1H), 6.70−6.62 (m, 2H), 5.43 (s, 1H), 5.41 (s, 1H), 3.52 (s, 3H). HRMS (FAB) calcd for $C_{16}H_{15}N_2O$ 251.1184; found 251.1196 $(M + H)^+$. Anal. Calcd for $C_{16}H_{14}N_2O \cdot 1/4H_2O$: C, 75.42; H, 5.74; N, 10.99. Found: C, 75.20; H, 5.61; N, 10.63.

*5,11-Dihydro-2-hydroxymethyl-5-methyl-11-methylene-6H-dibenz[b,e]azepin-6-one (**50**).* To a solution of **49** (35.7 mg, 0.116 mmol) in 1.5 mL of dehydrated THF was added $LiBH_4$ (13.0 mg, 0.597 mmol) at 0 °C. The mixture was stirred for 10.5 h at room temperature and extracted with ethyl acetate. The organic layer was washed with water and brine, dried over anhydrous magnesium sulfate, and concentrated. The residue was purified by silica gel column chromatography (eluent: *n*-hexane/ethyl acetate = 1:1, v/v) to afford 8.0 mg (26%) of the title compound as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.93 (dd, J = 7.9, 1.2 Hz, 1H), 7.43 (ddd, J = 7.9, 7.3, 1.2 Hz, 1H), 7.35 (ddd, J = 7.9, 7.3, 1.2 Hz, 1H), 7.31−7.27 (m, 2H), 7.24 (dd, J = 7.3, 1.2 Hz, 1H), 7.21 (d, J = 7.9 Hz, 1H), 5.49 (s, 1H), 5.45 (s, 1H), 4.67 (s, 2H), 3.56 (s, 3H). HRMS (FAB) calcd for $C_{17}H_{16}NO_2$ 266.1181; found 266.1173 $(M + H)^+$.

*5,11-Dihydro-5-methyl-11-methylene-6-oxodibenz[b,e]azepine-2-carboxylic Acid (**51**).* To a solution of **49** (33.8 mg, 0.110 mmol) in 2 mL of ethanol was added 1 mL of 1 N aqueous NaOH. The mixture was stirred for 2 h at room temperature, then extracted with diethyl ether. The extract was washed with 2 N HCl, water, and brine, dried over anhydrous magnesium sulfate, and concentrated to afford 30.7 mg (quant) of the title compound as a brown solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.04 (dd, J = 7.9, 1.8 Hz, 1H), 8.01 (d, J = 1.8 Hz, 1H), 7.96 (dd, J = 7.9, 1.2 Hz, 1H), 7.48 (ddd, J = 7.9, 7.9, 1.2 Hz, 1H), 7.39 (ddd, J = 7.9, 7.9, 1.2 Hz, 1H), 7.31 (d, J = 7.9 Hz, 1H), 7.29 (dd, J = 7.9, 1.2 Hz, 1H), 5.57 (s, 1H), 5.52 (s, 1H), 3.62 (s, 3H). HRMS (FAB) calcd for $C_{17}H_{14}NO_3$ 280.0974; found 280.0951 (M + H)$^+$. Anal. Calcd for $C_{17}H_{13}NO_3 \cdot 1/2H_2O$: C, 70.82; H, 4.89; N, 4.86. Found: C, 70.54; H, 4.91; N, 4.51.

Biology. *Cell Culture Conditions.* Human acute monocytic leukemia THP-1 cells were cultured in RPMI 1640 containing 10% fetal bovine serum (FBS), penicillin, and streptomycin mixture at 37 °C in a humidified atmosphere of 5% CO_2 in air. Human embryonic kidney (HEK) 293 cells were cultured in D-MEM containing 5% FBS, penicillin, and streptomycin mixture at 37 °C in a humidified atmosphere of 5% CO_2 in air. Human hepatocellular liver carcinoma HuH-7 cells were cultured in D-MEM containing 5% FBS, penicillin, and streptomycin at 37 °C in a humidified atmosphere of 5% CO_2 in air.

Transient Transfection Assays. HEK293 cells were plated at a density corresponding to 20% confluence in a 96-well plate 24 h prior to transfection. Cells were cotransfected with 15 ng of CMX-Gal4N-hLXRα, CMX-Gal4N-hLXRβ, CMX-VP16N-hLXRα, CMX-Gal4N-SRC1, CMX-Gal4N-DRIP205, CMX-Gal4N-NCoR, or CMX-Gal4N-SMRT expression plasmid, 50 ng of a tk-MH100 × 4-luc reporter, and 10 ng of CMX-β-galactosidase expression vector. Transfections were performed by the calcium phosphate coprecipitation method. After 8 h, transfected cells were treated with test compound or dimethyl sulfoxide (DMSO) for 16 h. Treated cells were assayed for luciferase activity in a luminometer. The luciferase activity of each sample was normalized by the level of β-galactosidase activity. Each transfection was carried out in triplicate.

Measurement of hIL-6. THP-1 cells were plated (2×10^6 cells per 10 cm dish) and stimulated with 10 nM TPA for 48 h. The differentiated cells were plated at a density of 3×10^4 cells/well (96-well plate) and stimulated with or without samples 6 h prior to treatment with or without LPS (100 ng/mL). After 18 h, the concentration of IL-6 in the culture supernatant was determined with an ELISA kit according to the manufacturer's protocol (GE Healthcare).

Mice and Isolation of Murine Peritoneal Macrophages. C57BL/6J mice were obtained from Nihon CLEA, LXR-null mice were kindly provided by Dr. Mangelsdorf, University of Texas Southwestern Medical Center at Dallas, TX,[31] and these mice were maintained under controlled temperature (23 ± 1 °C) and humidity (45−65%) with free access to water and chow (Lab. Animal Diet MF; Oriental Yeast Co., Ltd., Tokyo, Japan). Eight-to-10-week-old *Nr1h3(LXRα)$^{-/-}$* and *Nr1h3(LXRα)$^{-/-}$Nr1h2(LXRβ)$^{-/-}$* mice on a C57BL/6J background were maintained on standard chow and injected intraperitoneally with 1 mL/20 g weight of 3% thioglycollate solution (thioglycollate medium; GIBCO) 4 days prior to harvesting of the macrophages. Briefly, the mice were sacrificed, and ice-cold phosphate-buffered saline (PBS) was injected into the peritoneal cavity of each mouse. This fluid was carefully withdrawn and centrifuged, and the cell pellet was resuspended in RPMI 1640 containing 10% fetal bovine serum (FBS), penicillin, and streptomycin mixture. The cells were pooled and plated at 2.0 million cells/mL, and the macrophages were allowed to adhere for 2−6 h. The medium was then replaced, and the cells were incubated for 24 h. The experimental protocol adhered to the Guidelines for Animal Experiments of the Nihon University School of Medicine and was approved by the Ethics Review Committee for Animal Experimentation of Nihon University School of Medicine.

Compound Treatment. Murine peritoneal macrophages were plated at a density of 4×10^6 cells/well (6-well plate) for mRNA measurement and 2×10^5 cells/well (96-well plate) for ELISA and incubated for 24 h. Cells were stimulated with or without test sample 6 h prior to treatment with or without LPS (100 ng/mL for mRNA measurement and 10 ng/mL for ELISA). After 18 h, cells were harvested or the concentration of mIL-6 in the culture supernatant was determined with an ELISA kit according to the manufacturer's protocol (R&D Systems). HuH-7 cells were plated at a density of 2×10^5 cells/well (6-well plate) 24 h prior to treatment with test compounds. Then, cells were treated with test compounds or with ethanol for 24 h.

Real-Time Quantitative RT-PCR Analysis. Total RNAs from samples were prepared by the acid guanidine thiocyanate-phenol/chloroform method. cDNAs were synthesized using the ImProm-II reverse transcription system (Promega, Madison, WI). Real-time PCR was performed on the ABI PRISM 7000 sequence detection system (Applied Biosystems, Foster City, CA) using Power SYBR Green PCR Master Mix (Applied Biosystems). Some primer sequences are listed in Supporting Information. The mRNA values were normalized to the expression level of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA.

Expression and Purification of GST Fusion Proteins. Glutathione *S*-transferase (GST) fusion proteins were expressed in transformed Rosetta (DE3) competent cells derived from *Escherichia coli* BL21 grown in LB by induction with 0.4 mM IPTG (isopropyl-β-D-thiogalactopyranoside) and 2% ethanol at 15 °C for 24 h. Cell suspensions were centrifuged at 8000 rpm for 5 min. Bacterial pellets were resuspended in 50 mM Tris-HCl buffer containing 500 mM NaCl, 1 mM dithiothreitol (DTT), and 1 mM phenylmethylsulfonyl fluoride (PMSF). Cell suspensions were sonicated and incubated for 20 min after adding 1% Triton-X 100 (v/v) and centrifuged at 12000*g* for 30 min. Supernatants were purified by GSTrap FF according to the manufacturer's protocol (GE Healthcare). To ensure that equal amounts of proteins were used for fluorescence polarization assay, eluted proteins were quantified by Coomassie Blue staining (96-well scale) and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with SYPRO Orange staining.

LXRα Binding Assay (Fluorescence Polarization Assay). Recombinant GST-hLXRα (full-length) and GST proteins were produced and purified as described above. Serial dilutions of GST-hLXRα (full-length) or GST were incubated in the presence of compound **23** (1 μM) at 0 °C for 1 h in 50 mM Tris-HCl buffer (50 mM Tris-HCl, 10 mM reduced glutathione, pH 8.0). After incubation, the fluorescence polarization of compound **23** was measured on fluorescence spectrometer (Jasco FP-6500) with excitation at 400 nm and emission detection at 500 nm. In competitive displacement studies, compounds **3**, **18**, or **22** was incubated with 1 μM GST-hLXRα (full-length) or GST for 1 h in the presence of compound **23**.

ADME Study. Caco-2 A-B permeability (pH6.5/7.4) analysis was carried out at Cerep (www.cerep.com). Test compounds were applied at standard concentrations (10 μM). Data are expressed in P_{app} (apparent permeability) in 10^{-6} cm/s. Colchicine (mean A−B = 0.1×10^{-6} cm/s) and ranitidine (mean A−B = 0.4×10^{-6} cm/s) were used as low permeability controls, propranolol (mean A−B = 35.7×10^{-6} cm/s) was used as a high permeability control, and labetalol (mean A−B = 3.9×10^{-6} cm/s) was used as a P-gp substrate control. Metabolic stability assay with human liver microsomes was also carried out at Cerep. Test compounds were applied at standard concentrations (0.1 μM). LC/MS/MS was utilized to quantitate remaining test compounds. Imipramine (mean compound remaining = 92.2%), propranolol (mean compound remaining = 88.6%), terfenadine (mean compound remaining = 3.7%), and verapamil (mean compound remaining = 36.7%) were used as positive controls.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The dose-dependency of compounds, the inhibition of LPS-induced IL-1β production by compounds **3**, **10**, and **18**, selectivity of **18** over other NRs, primer sequences, and purity of the synthesized compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

LXR, liver X receptor; NR, nuclear receptor; LBD, ligand binding domain; LXRE, LXR response element; NCoR, nuclear receptor corepressor; SMRT, silencing mediator for retinoid and tyroid receptors; SRC1, steroid receptor coactivator 1; DRIP, vitamin D receptor interacting protein; IL-6, interleukin-6; IL-1β, interleukin-1β; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccaride; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; PPARγ, peroxisome proliferator-activated receptor γ; TG, triglyceride; M2H, mammalian two-hybrid; FP, fluorescence polarization

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