Discovery of Novel and Potent Retinoic Acid Receptor α Agonists: Syntheses and Evaluation of Benzofuranyl-pyrrole and Benzothiophenyl-pyrrole Derivatives

Hiroyuki Yoshimura, Kouichi Kikuchi, Shigeki Hibi, Katsuya Tagami, Takashi Satoh, Toshihiko Yamauchi, Akira Ishibahi, Kenji Tai, Takayuki Hida, Naoki Tokuhara, and Mitsuo Nagai*

Tsukuba Basic Research Laboratories for Drug Discovery, Eisai Company, Ltd., 1-3, Tokodai 5-chome, Tsukuba-shi, Ibaraki 300-2635, Japan

Received March 2, 2000

In the course of our studies on retinoic acid receptor (RAR) agonists, we have designed and synthesized a series of benzofuran and benzothiophene derivatives. Some of these compounds (**1a**,**b**,**e**,**f**,**j**) markedly inhibited LPS-induced B-lymphocyte proliferation and exerted RARα selectivity. One of them, 4-[5-(4,7-dimethylbenzofuran-2-yl)pyrrol-2-yl]benzoic acid (**1b**), when orally administered significantly inhibited mouse antibody production and delayed type hypersensitivity (DTH) responses from a dose of 0.1 mg/kg.

Introduction

Retinoids are potent molecules that have a variety of biological activities, including induction of cellular proliferation, differentiation, apoptosis, and developmental changes.[1] These compounds also act as modulators for a variety of inflammatory and immunological events.[2] In immunologically related animal models, 13-*cis*-retinoic acid inhibited disease progress in both rat adjuvant arthritis[3] and rat experimental autoimmune encephalomyelitis,[4] which are known as models of human rheumatoid arthritis and multiple sclerosis, at doses of 100 and 75 mg/kg, respectively. These models are mainly dependent on T-cell activation. However, this compound was not effective in type II collagen (CII)-induced arthritis which is known to be an antibody-dependent disease.[5]

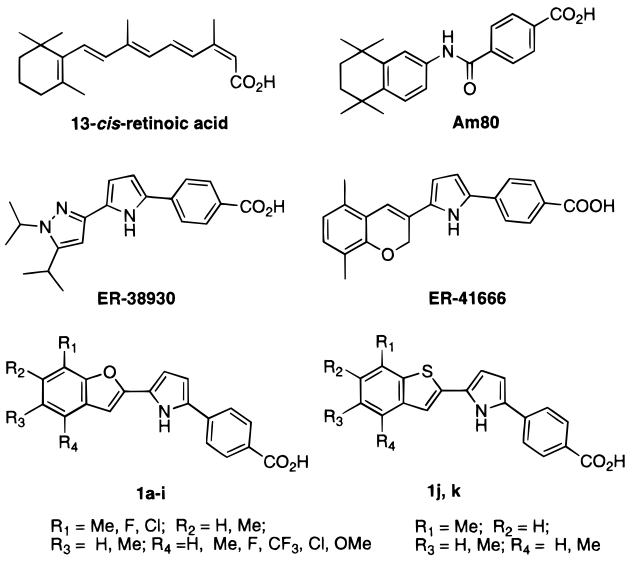
It has been shown that the biological effects of retinoids are mediated by activation of retinoic acid receptors (RARs), which are ligand-dependent gene transcription factors. There are three distinct receptor subtypes (RARα, -β, and -γ), which possess considerable homology in their ligand-binding domains.[6]

Although retinoids are thought to have great therapeutic potential, their clinical use is so far limited mainly to dermatological diseases[1a,7] and some cancers, for which retinoids may have both chemotherapeutic and chemopreventive applications.[8] The main reason for this limited use is the wide range of toxic effects of retinoids.[9] Thus, recent research has focused on the synthesis and development of subtype-selective retinoids in order to reduce their toxicity.[10]

There are few reports addressing the immunological effects and RAR subtypes both in vitro and in vivo. Apfel et al. reported that the RARα agonist Ro 40-6055 (Am80) (Chart 1) was a potent inhibitor of LPS-induced murine B-lymphocyte proliferation and that there was a correlation between the activity of RARα transcription and its potency to inhibit B-cell activation.[11] Am80 was also shown to inhibit inflammatory cytokine IL-6 production in vitro.[12] Furthermore, Am80 inhibited the increase of CII antibody from a dose of 0.3 mg/kg in rat CII-induced arthritis.[13] These results suggest that there is a strong correlation between the RARα agonistic activity and immunosuppressive effects, especially in the inhibition of antibody production. The therapeutic potential of RARα agonists is not only for immunological disorders but also in the treatment of cancer[14] and dermatological diseases.[15] In the course of our studies aimed at synthesizing novel retinoid analogues,[16] we focused our attention on RARα agonists which could be therapeutic agents of immunological disease with less toxicity than nonselective retinoids.

We reported previously that ER-41666, which possesses a flat structural moiety and a 2,5-disubstituted pyrrole group in the hydrophobic part and the linker, respectively, showed highly selective transactivation activity of the RARα receptor.[16e] ER-38930, which possesses a monocyclic five-membered ring moiety in

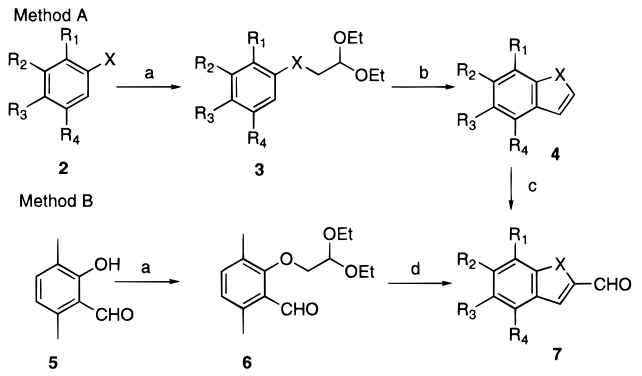
Chart 1



* To whom correspondence should be addressed. Tel: 81-298-47-5888. Fax: 81-298-47-2037. E-mail: m2-nagai@hhc.eisai.co.jp.

10.1021/jm000098s CCC: $19.00
Published on Web 06/14/2000

Scheme 1[a]



7a (X = O, R_1 = Me, R_2 = H, R_3 = H, R_4 = H), **7b** (X = O, R_1 = Me, R_2 = H, R_3 = H, R_4 = Me), **7c** (X = O, R_1 = Cl, R_2 = H, R_3 = H, R_4 = Cl), **7d** (X = O, R_1 = Me, R_2 = H, R_3 = H, R_4 = OMe), **7g** (X = O, R_1 = Me, R_2 = H, R_3 = Me, R_4 = H), **7h** (X = O, R_1 = Me, R_2 = H, R_3 = Me, R_4 = Me), **7i** (X = O, R_1 = Me, R_2 = Me, R_3 = H, R_4 = H), **7j** (X = S, R_1 = Me, R_2 = H, R_3 = H, R_4 = Me), **7k** (X = S, R_1 = Me, R_2 = H, R_3 = Me, R_4 = H)

[a] Reagents: (a) bromoacetaldehyde diethyl acetal, K_2CO_3, DMF; (b) polyphosphoric acid, toluene; (c) *n*-BuLi, THF, DMF; (d) AcOH.

the hydrophobic part, was also shown to markedly activate transactivation of the RARα receptor.[16d] We hoped that novel retinoids which possess a flat structural five,six-membered bicyclic ring, such as benzofuran and benzothiophene, might be more potent and selective than the retinoids we had previously obtained. Here we report the syntheses, structure−activity relationships (SAR), and in vivo results of such benzofuranyl-pyrrole and benzothiophenyl-pyrrole derivatives.

Chemistry

Three alternative routes for the synthesis of the important intermediate, the benzofuran **7**, are shown in Schemes 1 and 2. In the first method, the diethyl acetal **3**, derived from a commercially available phenol **2** and bromoacetaldehyde diethyl acetal, was treated with polyphosphoric acid to afford the benzofuran derivative **4**. Treatment of the benzofuran derivative **4** with *n*-butyllithium followed by formylation with *N*,*N*-dimethylformamide afforded the carbaldehyde **7**. The benzothiophene derivatives were prepared starting from the thiophenol instead of the phenol (method A).

The second method[17] of benzofuran synthesis involved the treatment of the salicylaldehyde **5** with bromoacetaldehyde diethyl acetal in the presence of potassium carbonate to afford the diethyl acetal **6**. Compound **6** was then cyclized with acetic acid to give the carbaldehyde **7** (method B).

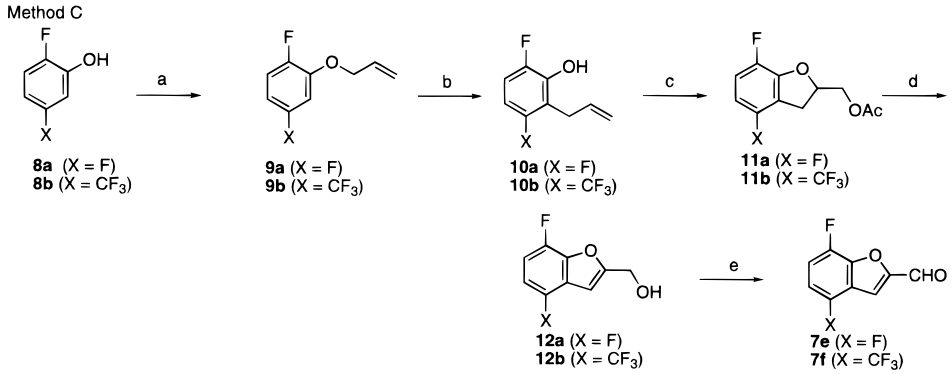
The benzofuran derivatives **7e**,**f**, which possess a strong electron-withdrawing substituent such as fluoro and trifluoromethyl, could not be obtained by acid-catalyzed cyclization of the diethyl acetal derivatives. Therefore, **7e**,**f** were synthesized by Nelson's method[18] as shown in Scheme 2. The Claisen rearrangement of the allyl ether **9** was carried out in *N*,*N*-dimethylaniline at 190 °C to afford the phenol **10**. The construction of the dihydrobenzofuran was achieved by epoxidation of compound **10** with 3-chloroperoxybenzoic acid (MCPBA) followed by intramolecular opening of the epoxide by the phenolate anion to give the alcohol which was then transformed to the acetate **11**. Conversion of the dihydrobenzofuran derivative **11** to the benzofuran derivative **12** was accomplished in three steps. The dihydrobenzofuran **11** was subjected to bromination with *N*-bromosuccinimide followed by dehydrobromination to afford the benzofuran derivative, which was deacylated with potassium carbonate to give the alcohol **12**. This compound **12** was oxidized under Swern's condition to give the aldehydes **7e,f** (method C).

The syntheses of the benzofuranyl-pyrrole derivatives **1a**−**k** were performed by the method described for the synthesis of ER-34617.[16c] The key intermediate diketone **14** was synthesized by condensation of the aldehyde **7** with the enone **13** in the presence of 3-benzyl-5-(2-hydroxyethyl)-4-methylthiazolium chloride and triethylamine. The pyrrole formation was conducted with ammonium acetate in methanol to give the pyrrole derivatives, which were then hydrolyzed to the final target compounds **1a**−**k** (Scheme 3).

Results and Discussion

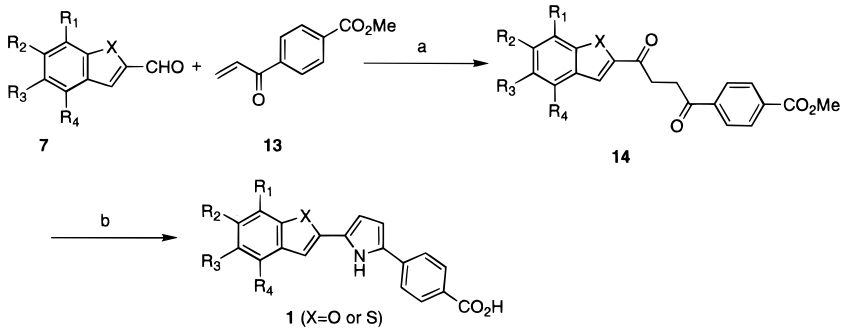
The above novel retinoids were synthesized and evaluated in vitro for their ability to bind to individual RARs and to induce gene transcription in the cotransfection assay. Cotransfection assays were performed as described in the Experimental Section, and relative EC_{30} values are reported (see footnotes to Table 1). Binding assays for RAR receptor subtypes were performed in a manner similar to that described previously[16c] using

Scheme 2[a]



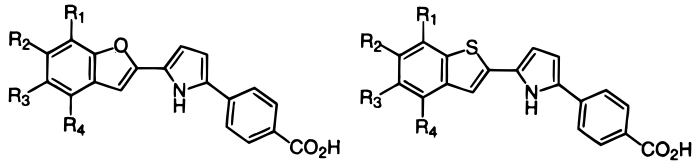
[a] Reagents: (a) allyl bromide, K_2CO_3, DMF; (b) 190 °C, *N*,*N*-dimethylaniline; (c) (i) *m*-CPBA, CH_2Cl_2, (ii) aq KOH, DMSO, (iii) Ac_2O, pyridine; (d) (i) *N*-bromosuccinimide, AIBN, CCl_4, (ii) *t*-BuOK, *t*-BuOH, (iii) K_2CO_3, MeOH; (e) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2.

Scheme 3[a]



[a] Reagents: (a) 3-benzyl-5-(2-hydroxyethyl)-4-methylthiazolium chloride, Et_3N, DMF; (b) (i) $AcONH_4$, MeOH, (ii) aq NaOH, EtOH.

Table 1. Competitive Binding, Transactivation, and B-Cell Proliferation Inhibition



1a-i **1j, k**

					binding affinity,[a] relative IC_{50}[b]			subtype-specific transactivation,[e] relative EC_{30}[f]			proliferation inhibition[h]
compd	R_1	R_2	R_3	R_4	RAR-α	RAR-β	RAR-γ	RAR-α	RAR-β	RAR-γ	relative IC_{50}[i]
1a	Me	H	H	H	1.6 ± 0.1	nd[c]	nd[c]	0.80 ± 0.14	92 ± 38	1000 ± 300	0.98 ± 0.11
1b	Me	H	H	Me	1.4 ± 0.3	490 ± 60	nd[c]	0.37 ± 0.02	25 ± 12	240 ± 30	0.76 ± 0.28
1c	Cl	H	H	Cl	1.5 ± 0.3	690 ± 300	nd[c]	0.70 ± 0.15	110 ± 30	820 ± 290	1.3 ± 0.4
1d	Me	H	H	OMe	15 ± 3	nd[c]	nd[c]	6.0 ± 2.1	730 ± 340	3700 ± 210	4.7 ± 1.2
1e	F	H	H	F	2.5 ± 0.3	nd[c]	nd[c]	0.80 ± 0.17	210 ± 40	1750 ± 750	0.93 ± 0.11
1f	F	H	H	CF_3	1.9 ± 0.4	500 ± 50	nd[c]	0.29 ± 0.04	8.5 ± 0.4	110 ± 20	0.37 ± 0.04
1g	Me	H	Me	H	16 ± 3	nd[c]	nd[c]	1.0 ± 0.4	380 ± 130	890 ± 90	1.2 ± 0.1
1h	Me	H	Me	Me	9.4 ± 5.0	nd[c]	nd[c]	2.0 ± 0.5	200 ± 70	480 ± 40	2.2 ± 0.5
1i	Me	Me	H	H	12 ± 8	nd[c]	nd[c]	3.1 ± 1.1	160 ± 80	2000 ± 500	2.4 ± 0.4
1j	Me	H	H	Me	6.1 ± 0.4	nd[c]	nd[c]	1.3 ± 0.6	130 ± 60	840 ± 360	0.75 ± 0.02
1k	Me	H	Me	H	62 ± 21	nd[c]	nd[c]	2.4 ± 0.5	1300 ± 500	2900 ± 50	3.6 ± 0.2
ATRA					1.0	1.0	1.0	1.0	1.0	1.0	1.0
					0.76 ± 0.11[d]	0.47 ± 0.05[d]	0.41 ± 0.04[d]	3.4 ± 0.4[g]	2.2 ± 0.2[g]	0.26 ± 0.03[g]	0.65 ± 0.10[j]

[a] Specific binding affinity was defined as total binding minus nonspecific binding, and the 50% inhibitory dose (IC_{50}) values were obtained from logarithmic plots. The selectivity of test compounds for each receptor is indicated as relative IC_{50}, where the IC_{50} value for each receptor was divided by that of the natural ligand (ATRA). [b] Means of IC_{50}/ATRA IC_{50} ± SEM. [c] nd: not detectable (relative IC_{50} > 1000). [d] Means of ATRA IC_{50} (nM) ± SEM. [e] Subtype-specific activity is expressed in terms of relative EC_{30}, which is the concentration of retinoid required to produce 30% of the maximal observed response, normalized relative to that of ATRA. [f] Means of EC_{30}/ATRA EC_{30} ± SEM. [g] Means of ATRA EC_{30} (nM) ± SEM. [h] The inhibition of LPS-induced mouse B-lymphocyte proliferation of each compound is indicated as relative IC_{50}. [i] Means of IC_{50}/ATRA IC_{50} ± SEM. [j] Means of ATRA IC_{50} (nM) ± SEM.

[^{3}H]ATRA. RXRα transactivation was also studied, but none of these compounds activated RXRα (data not shown). Inhibition of LPS-induced mouse B-lymphocyte proliferation was studied by using the method reported by Apfel.[11] The results are summarized in Table 1.

We initially synthesized the 4,7-dimethylbenzofuran derivative **1b** which was thought to possess a similar hydrophobic structure to both the benzopyranyl-pyrrole derivative (ER-41666) and the pyrazolyl-pyrrole derivative (ER-38930). In the cotransfection assay, compound **1b** was more potent than ATRA at RARα and 25−240-fold less potent at RAR*β* and RAR*γ*. It also showed very weak affinity for RAR*β* and RAR*γ*, whereas its binding affinity at RARα was comparable to that of ATRA. Furthermore, this compound (**1b**) showed stronger inhibitory activity for LPS-induced mouse B-lymphocyte proliferation than ATRA (Figure 1).

Next we examined the effects of substituents at the 4- and 7-positions. Although the 4-desmethyl-7-methylbenzofuran derivative **1a** and 4,7-dichlorobenzofuran derivative **1c** showed high selective binding affinity for RARα, both of these compounds had less potent transactivation of RARα than the 4,7-dimethylbenzofuran **1b**. The 4-methoxy-7-methylbenzofuran derivative **1d**, which possesses an electron-donating substituent and a group larger than methyl at the 4-position, had less potent inhibitory activity on B-lymphocyte proliferation. Introduction of an electron-withdrawing substituent and a smaller or equivalent size group with a methyl substituent such as fluoride and trifluoromethyl did not reduce the selectivity and inhibitory activity of B-lymphocyte proliferation (**1e**,**f**). In particular, the 7-fluoro-4-trifluoromethylbenzofuran derivative **1f** showed marked potency in inhibitory activity of B-lymphocyte proliferation. The 4,5,7-trimethylbenzofuran derivative **1h** and the 6,7-dimethylbenzofuran derivative **1i** showed weak inhibitory activity. The benzothiophene derivative **1j**, in which a sulfur atom was used to replaced an oxygen of the benzofuran, showed comparable inhibitory activity of B-lymphocyte proliferation to the 4,7-dimethyl-

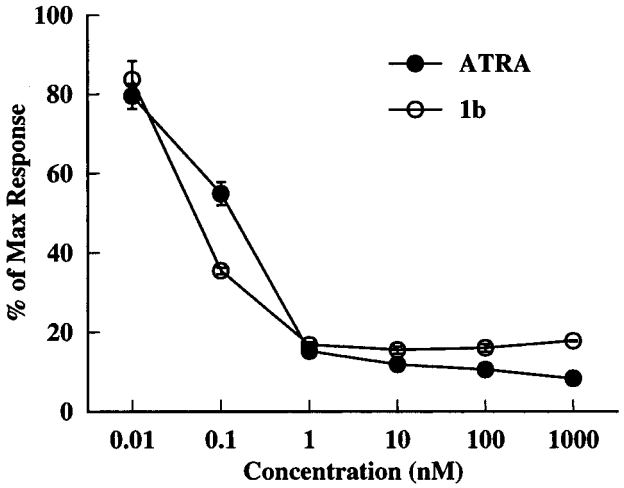


Figure 1. Inhibition of LPS-induced mouse B-cell proliferation with **1b** and ATRA.

benzofuran derivative **1b**. However, the 5,7-dimethylbenzothiophene derivative **1k** had less potent RARα activity and inhibitory activity than the 5,7-dimethylbenzofuran derivative **1g**.

From the SAR of these benzofuranyl derivatives and benzothiophenyl derivatives, it was found that both types of derivatives have transactivation activity and inhibitory activity of B-lymphocyte proliferation. The 4,7- and 5,7-disubstituents of benzofuran were found to be important for RARα selectivity. In particular, the 4,7-disubstituted benzofuranyl derivatives showed strong inhibitory activity. Methyl, fluoride, and trifluoromethyl appear to be the most suitable substituents to support this action**.** Some of these benzofuranyl derivatives (**1a**,**b**,**e**,**f**) and the benzothiophenyl derivative (**1j**) inhibited LPS-induced B-lymphocyte proliferation more potently than ATRA and exerted RARα selectivity.

Next, we studied the oral immunosuppressive activity of compound **1b**, which exhibited high RARα selectivity and potent inhibitory activity of LPS-induced B-lymphocyte proliferation, as well as improved pharmacokinetic characteristics (data not shown). The results of inhibition of mouse antibody production[19] and mouse DTH[20] for this compound are summarized in Figures 2 and 3. The former model is mainly mediated by activated B lymphocytes, and the latter one is mediated by T lymphocytes. Compound **1b** suppressed anti-DNP IgG2a antibody production in DNP-KLH-immunized mice dose-dependently in the antibody production model. **1b** did not cause any obvious toxicity at the doses tested. Furthermore, **1b** suppressed the response in a dose-dependent manner in the mouse DTH model. Oral treatment of compound **1b** significantly inhibited both mouse antibody production and delayed type hypersensitivity (DTH) responses from a dose of 0.1 mg/kg. Moreover, **1b** was effective in various immunological disease models. In both adjuvant- and collagen-induced arthritis models in rats, we observed that **1b** markedly inhibited paw swelling. It also ameliorated the disease-related symptoms in mouse graft versus host disease.[21] In addition, **1b** exerted a profound improvement in experimental and spontaneous nephritis models.[22] The details of pharmacological studies on compound **1b** and other potent compounds will be the subject of further reports from our laboratory.

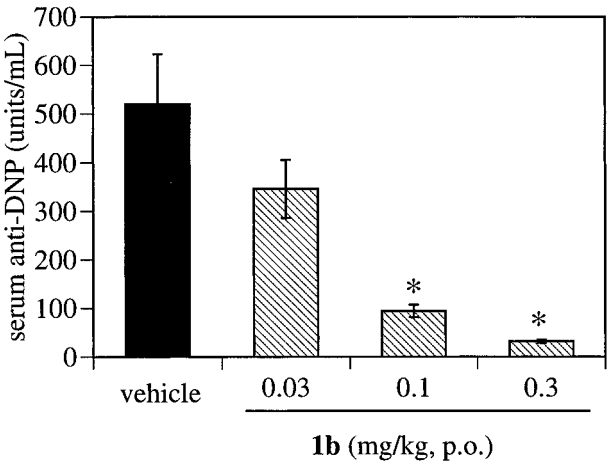


Figure 2. Effect of **1b** on antibody production in BALB/c mice. BALB/c mice were immunized and treated with **1b,** and their serum antibody titers were determined as indicated in the Experimental Section. Data represent the mean ± SEM of serum anti-DNP IgG2a titers in 5 animals. *p < 0.05, **1b**-treated compared with vehicle control by one-way ANOVA followed by Dunnett multiple comparison.

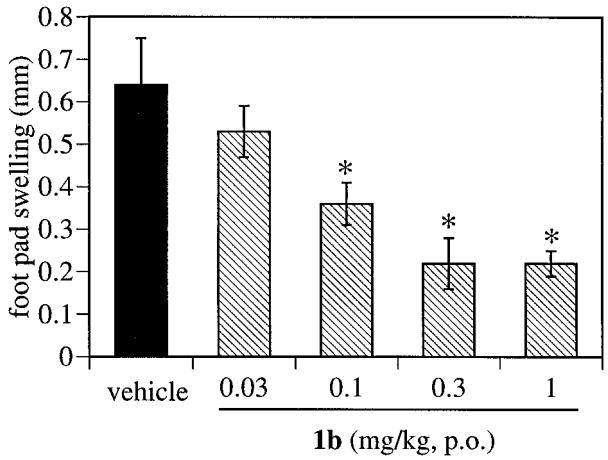


Figure 3. Effect of **1b** on DTH responses in BALB/c mice. BALB/c mice were immunized, treated with **1b,** and challenged with antigen as indicated in the Experimental Section. Data represent the mean ± SEM of right foot pad swelling in 5 animals. *p < 0.05, **1b**-treated compared with vehicle control by one-way ANOVA followed by Dunnett multiple comparison.

In conclusion, we have described for the first time the synthesis and SAR of a new series of benzofuranyl-pyrrole derivatives and benzothiophenyl-pyrrole derivatives. Some of these compounds (**1a**,**b**,**e**,**f**,**j**) markedly inhibited LPS-induced B-lymphocyte proliferation and exerted RARα selectivity. One of them, compound **1b** (ER-38925), when orally administered significantly inhibited DTH response at fairly low doses, i.e., less than 0.1 mg/kg, and was effective in various immunological disease models. This compound (**1b**) and other potent analogues (**1a**,**e**,**f**,**j**) are currently being evaluated with regard to immunosuppressive activity, pharmacokinetics, and safety to select a candidate which may be used clinically as an immunosuppressive agent.

Experimental Section

Chemistry. Reagents and solvents were purchased from usual commercial sources. Silica gel (Kieselgel 60, Merck) was used for column chromatography and silica gel (Kieselgel 60

F_{254}, Merck) for analytical thin-layer chromatography (TLC). Compounds were detected on TLC plates by exposure to UV light (254 nm). Melting points were measured on a Yanagimoto micromelting point apparatus without correction. ^{1}H NMR spectra were recorded on a Varian Unity 400 spectrometer, and chemical shifts are expressed in ppm downfield of tetramethylsilane (TMS), which was used as an internal reference. Mass spectra (MS) were obtained on a JEOL JMS-HX100 mass spectrometer. All organic extracts were dried over $MgSO_4$, and the solvents were removed with a rotary evaporator under reduced pressure.

General Procedure for Preparation of Benzofuran-2-carbaldehyde (Method A). 4,7-Dimethylbenzofuran-2-carbaldehyde (7b). To a solution of 2,5-dimethylphenol (**2b**) (10 g, 81.8 mmol) in *N*,*N*-dimethylformamide (100 mL) were added successively potassium carbonate (22.6 g, 163.5 mmol) and bromoacetaldehyde diethyl acetal (14.8 mL, 98.4 mmol) at room temperature. This mixture was stirred at 140 °C for 2.5 h and then cooled to room temperature. The mixture was diluted with water and extracted with ethyl acetate. The organic phases were washed with brine, dried, and evaporated to afford 2,2-diethyl-2,4-dimethylphenyl ether (18 g) as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.22−1.26 (m, 6H), 2.18 (s, 3H), 2.29 (s, 3H), 3.63−3.69 (m, 2H), 3.76−3.81 (m, 2H), 3.97−3.99 (m, 2H), 4.85 (t, J = 5.2 Hz, 1H), 6.38 (d, J = 1.6 Hz, 1H), 6.68 (dd, J = 1.6, 7.2 Hz, 1H), 6.99 (d, J = 7.2 Hz, 1H).

To a solution of this crude diethyl acetal (18 g, 75.5 mmol) in toluene (100 mL) was added polyphosphoric acid (50 g), and this mixture was stirred at 90 °C for 1 h under a nitrogen atmosphere and then cooled and poured into ice−water. The mixture was extracted with ethyl acetate, and the organic phases were washed with brine, dried, and evaporated. The crude residue was purified by flash column chromatography on silica gel, eluting with *n*-hexane, to afford 4,7-dimethylbenzofuran (**4b**) (3.5 g) as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 2.50 (s, 6H), 6.77 (d, J = 2.0 Hz, 1H), 6.93 (d, J = 8.0 Hz, 1H), 6.99 (d, J = 8.0 Hz, 1H), 7.62 (d, J = 2 Hz, 1H).

To a solution of **4b** (3.5 g, 23.9 mmol) in anhydrous tetrahydrofuran (50 mL) was added *n*-butyllithium (1.6 M solution in *n*-hexane; 18.4 mL, 28.7 mmol) at −30 °C under a nitrogen atmosphere. After being stirred for 15 min at this temperature, the mixture was treated by dropwise addition of *N*,*N*-dimethylformamide (5.6 mL, 81.5 mmol). This mixture was allowed to warm to room temperature for 30 min, and then was quenched with saturated aqueous ammonium chloride and extracted with ethyl acetate. The organic extract was washed with brine, dried, and evaporated. The resulting solid residue was washed with *n*-hexane to afford **7b** (2.3 g, 16%; three steps) as a pale yellow solid: mp 84−85 °C; ^{1}H NMR ($CDCl_3$) δ 2.53 (s, 6H), 7.02 (d, J = 6.8 Hz, 1H), 7.20 (d, J = 6.8 Hz, 1H), 7.59 (s, 1H), 9.85 (s, 1H).

General Procedure for Preparation of Benzofuran-2-carbaldehyde (Method B). 4,7-Dimethylbenzofuran-2-carbaldehyde (7b). To a solution of 3,6-dimethylsalicylaldehyde (**5**) (17.4 g, 116 mmol) in *N*,*N*-dimethylformamide (200 mL) were added bromoacetaldehyde diethyl acetal (17.8 mL, 118 mmol) and potassium carbonate (32 g, 232 mmol) at room temperature. The mixture was stirred for 2.5 h at 150 °C. After cooling to room temperature, the mixture was diluted with water and then extracted with ethyl acetate. The organic extract was washed with brine, dried, and evaporated. The crude residue was purified by flash column chromatography on silica gel, eluting with 10% ethyl acetate in *n*-hexane, to afford **6** as a pale yellow oil (23.4 g).

A solution of **6** (23.4 g, 98.2 mmol) in AcOH (120 mL) was refluxed for 8 h under a nitrogen atmosphere. After cooling to room temperature, the mixture was poured into saturated aqueous sodium hydrogen carbonate and then extracted with ethyl acetate. The organic extract was washed with brine, dried, and evaporated. The resulting solid residue was washed with *n*-hexane to afford **7b** (7.8 g, 39%) as a pale yellow solid. The proton NMR spectra, mp and R_f value were identical with those of **7b** prepared by method A.

General Procedure for Preparation of Benzofuran-2-carbaldehyde (Method C). Synthesis of 7-Fluoro-4-trifluoromethylbenzofuran-2-carbaldehyde (7f). 2-Allyloxy-1-fluoro-4-trifluoromethylbenzene (9b). To a solution of 2-fluoro-5-trifluoromethylphenol (**8b**) (50 g, 139 mmol) in *N*,*N*-dimethylformamide (300 mL) were added allyl bromide (31 mL, 361 mmol) and potassium carbonate (57 g, 412 mmol) at room temperature. The mixture was stirred for 2 h at 80 °C under a nitrogen atmosphere. After the reaction cooled to room temperature, brine was added and the mixture was then extracted with ethyl acetate. The combined organic phases were washed with brine, then dried and evaporated. The residue was purified by flash column chromatography on silica gel, eluting with 10% ethyl acetate in hexane to give **9b** (56 g, 92%) as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 4.63−4.66 (m, 2H), 5.35 (dd, J = 1.2, 11.2 Hz, 1H), 5.45 (dd, J = 1.6, 17.2 Hz, 1H), 6.00−6.12 (m, 1H), 7.14−7.21 (m, 3H).

2-Allyl-6-fluoro-3-trifluoromethylphenol (10b). 9b (56 g, 254 mmol) was dissolved in *N*,*N*-dimethylaniline (120 mL) at room temperature, and the mixture was stirred for 28 h at 190 °C under a nitrogen atmosphere. After the reaction cooled to 0 °C, the mixture was poured into 10% aqueous hydrochloric acid and then extracted with ethyl acetate. The combined organic phases were washed with brine, then dried and evaporated. The residue was purified by flash column chromatography on silica gel, eluting with 10% ethyl acetate in *n*-hexane to give **10b** (54 g, 96%) as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 3.59 (d, J = 6 Hz, 2H), 5.02−5.09 (m, 2H), 5.52−5.56 (m, 1H), 5.90−6.00 (m, 1H), 7.04 (t, J = 9.2 Hz, 1H), 7.20 (dd, J = 4.8, 8.4 Hz, 1H).

(7-Fluoro-4-trifluoromethyl-2,3-dihydrobenzofuran-2-yl)methyl Acetate (11b). To a solution of **10b** (54 g, 245 mmol) in dichloromethane (300 mL) was added 3-chloroperoxybenzoic acid (84 g, 343 mmol) for 10 min at room temperature. The resulting mixture was stirred for 6 h at the same temperature under a nitrogen atmosphere. The mixture was diluted with ethyl acetate, washed with saturated aqueous sodium hydrogen carbonate, saturated aqueous sodium thiosulfate, and brine, and then dried and evaporated to give 6-fluoro-2-(2-oxiranylmethyl)-3-trifluoromethylphenol (53 g) as a crude yellow oil: ^{1}H NMR ($CDCl_3$) δ 2.80−2.86 (m, 2H), 2.97 (d, J = 4.0 Hz, 1H), 3.33 (dd, J = 4.0, 7.2 Hz, 1H), 3.42−3.48 (m, 1H), 7.08 (t, J = 8.8 Hz, 1H), 7.21 (dd, J = 5.2, 9.2 Hz, 1H).

To a solution of 6-fluoro-2-(2-oxiranylmethyl)-3-trifluoromethylphenol (53 g, 224 mmol) in dimethyl sulfoxide (280 mL) was added potassium hydroxide (18.8 g, 269 mmol) in water (50 mL) for 15 min. at 0 °C. The mixture was stirred for 14 h at room temperature under a nitrogen atmosphere and then diluted with water and extracted with ethyl acetate. The combined organic phases were washed with brine, then dried and evaporated to give (7-fluoro-4-trifluoromethyl-2,3-dihydrobenzofuran-2-yl)methanol (41 g) as a crude pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 3.29 (dd, J = 7.6, 16.4 Hz, 1H), 3.44 (dd, J = 9.2, 16.8 Hz, 1H), 3.78 (dd, J = 5.2, 12.0 Hz, 1H), 3.96 (dd, J = 2.0, 12.0 Hz, 1H), 5.03−5.13 (m, 1H), 6.99 (t, J = 8.8 Hz, 1H), 7.07 (dd, J = 4.0, 8.8 Hz, 1H).

To a solution of (7-fluoro-4-trifluoromethyl-2,3-dihydrobenzofuran-2-yl)methanol (41 g, 173 mmol) in pyridine (130 mL) was added acetic anhydride (21 mL, 225 mmol) at room temperature, and the mixture was stirred for 4 h at the same temperature under a nitrogen atmosphere. The reaction mixture was poured into 10% aqueous hydrochloric acid, and the mixture was extracted with ethyl acetate. The combined organic phases were washed with brine, then dried and evaporated. The residue was purified by flash column chromatography on silica gel, eluting with 10% ethyl acetate in *n*-hexane to give **11b** (37.1 g, 66%, three steps) as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 2.08 (s, 3H), 3.20 (dd, J = 6.8, 16.4 Hz, 1H), 3.52 (dd, J = 9.6, 16.4 Hz, 1H), 4.30 (dd, J = 6.4, 12.4 Hz, 1H), 4.36 (dd, J = 3.6, 12.4 Hz, 1H), 5.14−5.24 (m, 1H), 7.02 (t, J = 8.8 Hz, 1H), 7.10 (dd, J = 4.8, 8.8 Hz, 1H).

(7-Fluoro-4-trifluoromethylbenzofuran-2-yl)methanol (12b). To a solution of **11b** (37.1 g, 133 mmol) in carbon

tetrachloride (320 mL) were added *N*-bromosuccinimide (23.7 g, 133 mmol) and 2,2′-azobis(isobutyronitrile) (285 mg, 1.7 mmol) at room temperature. The mixture was stirred for 105 min at 60 °C under a nitrogen atmosphere. After the reaction mixture cooled to 0 °C, the solid was filtered through a glass filter and was washed with hexane. The organic filtrates were combined and concentrated under reduced pressure to afford a residue, which was diluted with ethyl acetate, washed with saturated aqueous sodium thiosulfate, brine, and then dried and evaporated to give (3-bromo-7-fluoro-4-trifluoromethyl-2,3-dihydrobenzofuran-2-yl)methyl acetate (44 g) as a brown oil.

To a stirred solution of (3-bromo-7-fluoro-4-trifluoromethyl-2,3-dihydrobenzofuran-2-yl)methyl acetate (44 g, 123 mmol) in 160 mL of 2-methyl-2-propanol was added potassium *tert*-butoxide (1.0 M in 2-methyl-2-propanol solution; 135 mL, 135 mmol) at room temperature under a nitrogen atmosphere. This mixture was stirred for 90 min at 60 °C. After cooling to room temperature, the reaction mixture was poured into iced water and extracted with ethyl acetate. The combined organic phases were washed with brine, then dried and evaporated to give crude (7-fluoro-4-trifluoromethylbenzofuran-2-yl)methyl acetate (34 g) as a brown oil.

To a solution of (7-fluoro-4-trifluoromethylbenzofuran-2-yl)-methyl acetate (34 g, 118 mmol) in 160 mL of methanol was added potassium carbonate (24 g, 175 mmol) at room temperature under a nitrogen atmosphere. This mixture was stirred for 1 h at 60 °C. After cooling, the reaction mixture was diluted with ethyl acetate, washed with brine, and then dried and evaporated to give a crude brown oil. The residue was purified by flash column chromatography on silica gel, eluting with 10−20% ethyl acetate in *n*-hexane to give the (7-fluoro-4-trifluoromethylbenzofuran-2-yl)methanol (**12b**) 7.1 g (36%, three steps) as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 4.85 (d, J = 7.0 Hz, 2H), 6.91 (brs, 1H), 7.10 (t, J = 9.2 Hz, 1H), 7.47 (dd, J = 4.0, 8.8 Hz, 1H).

7-Fluoro-4-trifluoromethylbenzofuran-2-carbaldehyde (7f). To a stirred solution of oxalyl chloride (7.48 mL, 85 mmol) in 300 mL of dichloromethane was added dimethyl sulfoxide (12.1 mL, 170 mmol) for 5 min at −78 °C under a nitrogen atmosphere. **12b** (10.0 g, 43 mmol) in 20 mL of dichloromethane was added to the reaction mixture at −78 °C, and the mixture was stirred for 40 min at the same temperature. Triethylamine (35 mL, 251 mmol) was added to the reaction mixture at −78 °C and stirred 15 min at the same temperature. The reaction temperature was allowed to rise to room temperature for 30 min. After the mixture was cooled to −78 °C, water was added to the reaction mixture, and the temperature was raised to room temperature. The mixture was diluted with ethyl acetate, washed with water, and then dried and evaporated to give a crude yellow oil (11 g). The residue was purified by flash column chromatography on silica gel, eluting with 10% ethyl acetate in *n*-hexane to give **7f** (7.2 g, 73%) as a pale yellow solid: mp 58−59 °C; ^{1}H NMR ($CDCl_3$) δ 7.34 (t, J = 8.8 Hz, 1H), 7.62 (ddd, J = 0.8, 4.0, 8.4 Hz, 1H), 7.74 (dd, J = 1.6, 2.8 Hz, 1H), 9.98 (s, 1H).

7-Methylbenzofuran-2-carbaldehyde (7a). Compound **7a** was synthesized from 2-methylphenol (**2a**) following the representative procedure described as method A and obtained as a pale yellow solid in 9% yield (three steps): mp 58−59 °C; ^{1}H NMR ($CDCl_3$) δ 2.58 (s, 3H), 7.24 (t, J = 7.6 Hz, 1H), 7.32 (d, J = 7.6 Hz, 1H), 7.56 (s, 1H), 7.57 (d, J = 7.6 Hz, 1H), 9.88 (s, 1H). The proton NMR spectral data were identical with those reported.[17]

4,7-Dichlorobenzofuran-2-carbaldehyde (7c). Compound **7c** was synthesized from 2,5-dichlorophenol (**2c**) following the representative procedure described as method A and obtained as a pale yellow solid in 31% yield (three steps): mp 155−159 °C; ^{1}H NMR ($CDCl_3$) δ 7.29 (d, J = 8.4 Hz, 1H), 7.46 (d, J = 8.4 Hz, 1H), 9.84 (s, 1H).

7-Methyl-4-methoxybenzofuran-2-carbaldehyde (7d). Compound **7d** was synthesized from 2-methyl-5-methoxyphenol (**2d**) following the representative procedure described as method A and obtained as a pale yellow solid in 20% yield (three steps): mp 100−101 °C; ^{1}H NMR ($CDCl_3$) δ 2.24 (s, 3H), 3.86 (s, 3H), 6.60 (d, J = 8.4 Hz, 1H), 7.22 (d, J = 8.4 Hz, 1H), 7.65 (s, 1H), 9.80 (s, 1H).

4,7-Difluorobenzofuran-2-carbaldehyde (7e). Compound **7e** was synthesized from 2,5-difluorophenol (**2e**) following the representative procedure described as method C and obtained as a pale yellow oil in 3% yield (nine steps): ^{1}H NMR ($CDCl_3$) δ 6.96 (dt, J = 2.8, 8.8 Hz, 1H), 7.21 (ddd, J = 4.0, 8.8, 9.6 Hz, 1H), 7.66 (d, J = 2.4 Hz, 1H), 9.92 (s, 1H).

5,7-Dimethylbenzofuran-2-carbaldehyde (7g). Compound **7g** was synthesized from 2,4-dimethylphenol (**2g**) following the representative procedure described as method A and obtained as a pale yellow solid in 32% yield (three steps): mp 72−73 °C; ^{1}H NMR ($CDCl_3$) δ 2.21 (s, 3H), 2.52 (s, 3H), 7.14 (s, 1H), 7.32 (s, 1H), 7.50 (s, 1H), 9.82 (s, 1H).

4,5,7-Trimethylbenzofuran-2-carbaldehyde (7h). Compound **7h** was synthesized from 2,4,5-trimethylphenol (**2h**) following the representative procedure described as method A and obtained as a pale yellow solid in 53% yield (three steps): mp 121−122 °C; ^{1}H NMR ($CDCl_3$) δ 2.33 (s, 3H), 2.43 (s, 3H), 2.50 (s, 3H), 7.13 (s, 1H), 7.57 (s, 1H), 9.83 (s, 1H).

6,7-Dimethylbenzofuran-2-carbaldehyde (7i). Compound **7i** was synthesized from 2,3-dimethylphenol (**2i**) following the representative procedure described as method A and obtained as a pale yellow solid in 9.8% yield (three steps): mp 81−82 °C; ^{1}H NMR ($CDCl_3$) δ 2.42 (s, 3H), 7.24 (t, J = 7.6 Hz, 1H), 7.32 (d, J = 7.6 Hz, 1H), 7.56 (s, 1H), 7.57 (d, J = 7.6 Hz, 1H), 9.88 (s, 1H).

4,7-Dimethylbenzothiophene-2-carbaldehyde (7j). Compound **7j** was synthesized from 2,5-dimethylthiophenol (**2j**) following the representative procedure described as method A and obtained as a pale yellow solid in 10% yield (three steps): mp 97−98 °C; ^{1}H NMR ($CDCl_3$) δ 2.54 (s, 3H), 2.64 (s, 3H), 7.15 (d, 1H), 7.21 (d, 1H), 10.21 (s, 1H).

5,7-Dimethylbenzothiophene-2-carbaldehyde (7k). Compound **7k** was synthesized from 2,4-dimethylthiophenol (**2k**) following the representative procedure described as method A and obtained as a pale yellow solid in 14% yield (three steps): mp 66−67 °C; ^{1}H NMR ($CDCl_3$) δ 2.44 (s, 3H), 2.56 (s, 3H), 7.14 (s, 1H), 7.58 (s, 1H), 7.99 (s, 1H), 10.16 (s, 1H).

General Procedure for Preparation of Pyrrolylbenzoic Acid 1. Methyl 4-[4-(4,7-Dimethylbenzofuran-2-yl)-4-oxobutanoyl]benzoate (14b). To a solution of **7b** (5.5 g, 31.6 mmol) in 100 mL of *N*,*N*-dimethylformamide were added methyl 4-acryloylbenzoate (6.0 g, 31.6 mmol) (**13**), triethylamine (5.3 mL, 37.9 mmol) and 3-benzyl-5-(2-hydroxyethyl)-4-methylthiazolium chloride (1.7 g, 6.3 mmol) at room temperature. The mixture was stirred for 30 min at 80 °C. After cooling to room temperature, the reaction mixture was diluted with water, extracted with ethyl acetate, washed with brine, and then dried and evaporated to give a crude solid. It was recrystallized with methanol to give **14b** (9.5 g, 83%) as a pale yellow solid: mp 131 °C (methanol); ^{1}H NMR ($CDCl_3$) δ 2.50 (s, 3H), 2.51 (s, 3H), 3.45−3.55 (m, 4H), 3.94 (s, 3H), 7.00 (d, J = 6.8 Hz, 1H), 7.16 (d, J = 6.8 Hz, 1H), 7.62 (s, 1H), 8.09 (d, J = 8.4 Hz, 2H), 8.14 (d, J = 8.4 Hz, 2H).

4-[5-(4,7-Dimethylbenzofuran-2-yl)pyrrol-2-yl]benzoic Acid (1b). To a suspension of **14b** (9.5 g, 26 mmol) in methanol (40 mL) was added ammonium acetate (20 g, 260 mol) at room temperature. After stirring under reflux for 2 h, the mixture was diluted with water and cooled to 0 °C. The precipitate was collected by filtration to give methyl 4-[5-(4,7-dimethylbenzofuran-2-yl)pyrrol-2-yl]benzoate (8.7 g, 97%) as a pale yellow solid: mp 177−178 °C; ^{1}H NMR ($CDCl_3$) δ 2.48 (s, 3H), 2.55 (s, 3H), 3.93 (s, 3H), 6.72−6.77 (m, 2H), 6.83 (s, 1H), 6.93 (d, J = 6.8 Hz, 1H), 6.97 (d, J = 6.8 Hz, 1H), 7.63 (d, J = 8.4 Hz, 2H), 8.07 (d, J = 8.4 Hz, 2H).

To a solution of methyl 4-[5-(4,7-dimethylbenzofuran-2-yl)-pyrrol-2-yl]benzoate (8.7 g, 25.2 mmol) in ethanol (100 mL) was added 5 N aqueous sodium hydroxide (20 mL) solution at room temperature, and this mixture was refluxed for 30 min. After cooling to room temperature it was diluted with water (150 mL) and acidified with 5 N aqueous hydrochloric acid. The precipitate was collected by filtration and dried to give crude **1b** as a yellow solid (8.1 g, 98%). It was recrystallized

from ethanol−water to give a yellow solid: mp 271 °C dec; ^{1}H NMR (DMSO-d_6) δ 2.43 (s, 3H), 2.46 (s, 3H), 6.71 (t, J = 2.4 Hz, 1H), 6.84 (t, J = 2.4 Hz, 1H), 6.92 (d, J = 7.2 Hz, 1H), 6.96 (d, J = 7.2 Hz, 1H), 7.23 (s, 1H), 7.89 (d, J = 8.4 Hz, 2H), 7.95 (d, J = 8.4 Hz, 2H), 11.81 (brs, 1H), 12.85 (brs, 1H). Anal. ($C_{21}H_{17}NO_3 \cdot 0.2H_2O$) C, H, N.

4-[5-(7-Methylbenzofuran-2-yl)pyrrol-2-yl]benzoic Acid (1a). Compound **1a** was synthesized from 7-methylbenzofuran-2-carbaldehyde (**7a**) following the general procedure described previously and obtained as a pale yellow solid in 53% yield (three steps): mp 264−266 °C (ethanol−water); ^{1}H NMR (DMSO-d_6) δ 2.52 (s, 3H), 6.71−6.74 (m, 1H), 6.83−6.86 (m, 1H), 7.06 (d, J = 7.2 Hz, 1H), 7.12 (t, J = 7.2 Hz, 1H), 7.18 (s, 1H), 7.43 (d, J = 7.2 Hz, 1H), 7.89 (d, J = 8.4 Hz, 2H), 7.95 (d, J = 8.4 Hz, 2H), 11.83 (s, 1H), 12.82 (brs, 1H). Anal. ($C_{20}H_{15}NO_3 \cdot 0.2H_2O$) C, H, N.

4-[5-(4,7-Dichlorobenzofuran-2-yl)pyrrol-2-yl]benzoic Acid (1c). Compound **1c** was synthesized from 4,7-dichlorobenzofuran-2-carbaldehyde (**7c**) following the general procedure described previously and obtained as a yellow solid in 38% yield (three steps): mp 276−279 °C (ethanol−water); ^{1}H NMR (DMSO-d_6) δ 6.83 (t, J = 2.4 Hz, 1H), 6.89 (t, J = 2.4 Hz, 1H), 7.35 (d, J = 7.2 Hz, 1H), 7.38 (d, J = 7.2 Hz, 1H), 7.39 (s, 1H), 7.91 (d, J = 8.4 Hz, 2H), 7.97 (d, I = 8.4 Hz, 2H), 12.02 (brs, 1H), 12.86 (brs, 1H). Anal. ($C_{19}H_{11}NO_3Cl_2 \cdot 0.3H_2O$) C, H, N.

4-[5-(7-Methyl-4-methoxybenzofuran-2-yl)pyrrol-2-yl]-benzoic Acid (1d). Compound **1d** was synthesized from 7-methyl-4-methoxybenzofuran-2-carbaldehyde (**7d**) following the general procedure described previously and obtained as a yellow solid in 55% yield (three steps): mp 262−263 °C (ethanol−water); ^{1}H NMR (DMSO-d_6) δ 2.63 (s, 3H), 3.92 (s, 3H), 6.77−6.82 (m, 3H), 7.24 (d, J = 8.0 Hz, 1H), 7.86−7.95 (m, 5H), 8.13 (d, J = 8.8 Hz, 1H), 8.28 (s, 1H), 11.62 (s, 1H). Anal. ($C_{21}H_{17}NO_4 \cdot 0.3H_2O$) C, H, N.

4-[5-(4,7-Difluorobenzofuran-2-yl)pyrrol-2-yl]benzoic Acid (1e). Compound **1e** was synthesized from 4,7-difluorobenzofuran-2-carbaldehyde (**7e**) following the general procedure described previously and obtained as a yellow solid in 47% yield (three steps): mp >300 °C (ethanol−water); ^{1}H NMR (DMSO-d_6) δ 6.82 (dd, J = 2.4, 3.6 Hz, 1H), 6.86 (dd, J = 2.4, 3.6 Hz, 1H), 7.08 (dd, J = 3.2, 8.8 Hz, 1H), 7.19 (dd, J = 3.2, 8.8 Hz, 1H), 7.42 (d, J = 2.4 Hz, 1H), 7.92 (d, J = 8.4 Hz, 2H), 7.96 (d, J = 8.4 Hz, 2H), 12.08 (s, 1H). HRMS Calcd for $C_{19}H_{11}NO_3F_2$ (MH^+): 339.0707. Found: 339.0698.

4-[5-(7-Fluoro-4-trifluoromethylbenzofuran-2-yl)pyrrol-2-yl]benzoic Acid (1f). Compound **1f** was synthesized from 7-fluoro-4-trifluoromethylbenzofuran-2-carbaldehyde (**7f**)-following the general procedure described previously and obtained as a pale yellow solid in 44% yield (three steps): mp 215−217 °C (acetone−water); ^{1}H NMR (DMSO-d_6) δ 6.90 (brs, 2H), 7.35 (t, J = 8.8 Hz, 1H), 7.49 (brs, 1H), 7.63 (dd, J = 3.6, 8.8 Hz, 1H), 7.91 (d, J = 8.4 Hz, 2H), 7.97 (d, J = 8.4 Hz, 2H), 12.08 (brs, 1H). Anal. ($C_{20}H_{11}F_4NO_3 \cdot 0.5H_2O$) C, H, N.

4-[5-(5,7-Dimethylbenzofuran-2-yl)pyrrol-2-yl]benzoic Acid (1g). Compound **1g** was synthesized from 5,7-dimethylbenzofuran-2-carbaldehyde (**7g**) following the general procedure described previously and obtained as a pale yellow solid in 41% yield (three steps): mp 262−263 °C (ethanol−water); ^{1}H NMR (DMSO-d_6) δ 2.32 (s, 3H), 2.45 (s, 3H), 6.68−6.71 (m, 1H), 6.80−6.83 (m, 1H), 6.88 (d, J = 1.2 Hz, 1H), 7.10 (s, 1H), 7.20 (d, J = 1.2 Hz, 1H), 7.86 (d, J = 8.4 Hz, 2H), 7.93 (d, J = 8.4 Hz, 2H), 11.78 (s, 1H), 12.80 (brs, 1H). Anal. ($C_{21}H_{17}NO_3 \cdot 0.2H_2O$) C, H, N.

4-[5-(4,5,7-Trimethylbenzofuran-2-yl)pyrrol-2-yl]benzoic Acid (1h). Compound **1h** was synthesized from 4,5,7-trimethylbenzofuran-2-carbaldehyde (**7h**) following the general procedure described previously and obtained as a pale yellow solid in 71% yield (three steps): mp 267 °C dec (ethanol−water); ^{1}H NMR (DMSO-d_6) δ 2.26 (s, 3H), 2.35 (s, 3H), 2.43 (s, 3H), 6.67−6.71 (m, 1H), 6.81−6.85 (m, 1H), 6.87 (s, 1H), 7.21 (s, 1H), 7.88 (d, J = 8.4 Hz, 2H), 7.94 (d, J = 8.0 Hz, 2H), 11.78 (brs, 1H). Anal. ($C_{21}H_{17}NO_3 \cdot H_2O$) C, N; H: calcd, 5.85; found, 5.29. HRMS Calcd for $C_{22}H_{19}NO_3$ (MH^+): 345.1365. Found: 345.1378.

4-[5-(6,7-Dimethylbenzofuran-2-yl)pyrrol-2-yl]benzoic Acid (1i). Compound **1i** was synthesized from 6,7-dimethylbenzofuran-2-carbaldehyde (**7i**) following the general procedure described previously and obtained as a pale yellow solid in 43% yield (three steps): mp 278−280 °C (ethanol−water); ^{1}H NMR (DMSO-d_6) δ 2.30 (s, 3H), 2.42 (s, 3H), 6.69−6.72 (m, 1H), 6.81−6.84 (m, 1H), 7.02 (d, J = 8.4 Hz, 1H), 7.11 (s, 1H), 7.30 (d, J = 8.4 Hz, 1H), 7.86 (d, J = 8.4 Hz, 2H), 7.94 (d, J = 8.4 Hz, 2H), 11.78 (s, 1H), 12.80 (brs, 1H). Anal. ($C_{21}H_{17}NO_3 \cdot 0.2H_2O$) C, H, N.

4-[5-(4,7-Dimethylbenzothiophene-2-yl)pyrrol-2-yl]benzoic Acid (1j). Compound **1j** was synthesized from 4,7-dimethylthiophene-2-carbaldehyde (**7j**) following the general procedure described previously and obtained as a pale yellow solid in 14% yield (three steps): mp 280−281 °C (ethanol−water); ^{1}H NMR (DMSO-d_6) δ 2.42 (s, 3H), 2.54 (s, 3H), 6.56−6.59 (m, 1H), 6.78−6.81 (m, 1H), 7.02 (d, J = 6.8 Hz, 1H), 7.08 (d, J = 6.8 Hz, 1H), 7.89 (s, 1H), 7.90 (d, J = 8.4 Hz, 2H), 7.94 (d, J = 8.4 Hz, 2H), 11.76 (s, 1H), 12.83 (brs, 1H). Anal. ($C_{21}H_{17}NO_2S \cdot 0.2H_2O$) C, H, N.

4-[5-(5,7-Dimethylbenzothiophene-2-yl)pyrrol-2-yl]benzoic Acid (1k). Compound **1k** was synthesized from 5,7-dimethylthiophene-2-carbaldehyde (**7k**) following the general procedure described previously and obtained as a pale yellow solid in 26% yield (three steps): mp 261−262 °C (ethanol−water); ^{1}H NMR (DMSO-d_6) δ 2.36 (s, 3H), 2.42 (s, 3H), 6.54−6.56 (m, 1H), 6.77−6.79 (m, 1H), 6.96 (s, 1H), 7.43 (s, 1H), 7.71 (s, 1H), 7.88 (d, J = 8.4 Hz, 2H), 7.93 (d, J = 8.4 Hz, 2H), 11.76 (s, 1H), 12.76 (brs, 1H). Anal. ($C_{21}H_{17}NO_2S \cdot 0.5H_2O$) C, H, N.

Biology. 1. Binding Study. Binding assays were performed as described in a previous report.[16c] Compounds were tested in log dilutions of 5.0×10^{-7} to 5.0×10^{-11} M with duplicate determinations at each concentration. Binding in the presence of a 1000-fold excess of unlabeled ligand was defined as nonspecific binding. Specific binding was defined as the total binding minus the nonspecific binding, and the 50% inhibitory dose (IC_{50}) values were obtained from logarithmic plots. The selectivity of compounds for each receptor is indicated as relative IC_{50}, where the IC_{50} value of each compound for a receptor was divided by that of ATRA. The values given in Table 1 are the average of at least two experiments.

2. Transactivation Assay. Transient transactivation assays for each receptor were also performed by the method previously reported.[16c] Compounds were tested in log dilutions of 3.0×10^{-6} to 1.0×10^{-10} M with duplicate determinations at each concentration. Receptor and reporter vectors were transfected into COS-1 cells by the Lipofection method. After 4 h of incubation, the medium was replaced with DMEM supplemented with 10% FBS and incubation was continued for an additional 20 h. The cells were then suspended in DMEM supplemented with 10% FBS and seeded at 3×10^4/well in 96-well plates. After 6 h of incubation, compounds at various concentrations were added to duplicate wells. The plates were incubated for a further 48 h, and then the cell supernatants were assayed for PLAP activity. The values given in Table 1 are the average of at least two experiments.

3. Inhibition of LPS-Induced Mouse B-Lymphocyte Proliferation Assay. Mouse splenocyte proliferation assay induced by *E. coli* lipopolysaccharide (LPS), a well-known murine B-lymphocyte proliferation inducer, was performed according to the method previously reported with slight modifications.[11] Compounds were tested in log dilutions of 1.0×10^{-6} to 1.0×10^{-12} M with triplicate determinations at each final concentration. BALB/c spleen cell suspensions were placed at 1.2×10^5 cells/180 μL/well in 96-well culture plates, in RPMI-1640 medium containing antibiotics, 50 μM 2-mercaptoethanol, 10% FBS and 5 μg/mL LPS. Then 20 μL of each compound serially diluted was added to the wells and the plates were incubated for 3 days at 37 °C, 5% CO_2. Splenocytes were pulsed with 20 μL of 0.5 μCi of [^{3}H]thymidine for further 6 h. After incubation, cells were harvested on glass fiber filters

and processed for β-plate counting. The 50% inhibitory concentration (IC_{50}) values were calculated with a nonlinear regression method. The inhibition of each compound is indicated as relative IC_{50}, where the IC_{50} value of the compound was divided by that of ATRA. The values given in Table 1 are means of relative $IC_{50} \pm$ SEM from triplicate determinations.

4. Mice. Female BALB/c mice were obtained from Charles River Japan and used at 8−10 weeks of age. They were bred in the company's animal facility under SPF conditions and allowed access to food and water ad libitum during the study.

5. Antibody Production in Mice. BALB/c mice were divided into groups each consisting of 5 animals. They were immunized by intraperitoneal injection of 100 μg of dinitrophenyl-conjugated keyhole lympet hemocyanin (DNP-KLH) emulsified in Freund's complete adjuvant on day 0. Compounds were orally administered to the mice at various doses from day 0 to 9 post-immunization. Control mice were given vehicle only during the same period. On day 10, mice were bled from the orbital vein, and anti-DNP antibody (IgG2a class) titer in the serum was determined by DNP-specific ELISA assay.

6. DNP-Specific ELISA. A 96-well ELISA plate was coated with 5 μg/mL of DNP-condjugated bovine serum albumin (BSA) for 1 h at 37 °C. After blocking with 300 μL of blocking buffer (1% BSA−phosphate-buffered saline (PBS)) for 2 h at 37 °C, 50 μL of either samples or standard serum, previously diluted with ELISA buffer (1% BSA, 0.1% Tween 20-PBS), was added and incubated for 1 h at 37 °C. After washing, plates were added with 50 μL of peroxidase-conjugated anti-mouse IgG2a, diluted with ELISA buffer at 1:2500, and incubated further for 1 h at 37 °C. After washing, plates were developed with 50 μL of 400 μg/mL *o*-phenylenediamine dihydrochloride. Thirty minutes later, the reaction was stopped with 50 μL of 1 N sulfuric acid and the plates were read at 490 nm with an automated microplate reader (BIO-TEK). The units of anti-DNP titers in each sample were calculated with reference to standard serum obtained from DNP-KLH-immunized mouse.

7. Delayed-Type Hypersensitivity (DTH). Female BALB/c mice were divided into groups each consisting of 5 animals. The animals were immunized with subcutaneous injection of 1×10^8 of sheep red blood cells (SRBC) in their back. Compounds were orally administered to the mice at various doses from day 3 to 5 post-immunization. Control mice were given vehicle only during the same period. On day 6, mice were subcutaneously injected with 7×10^7 of SRBC in their right food pad. Twenty-four hours later, thickness of both foot pads was measured and paw swelling in each mice was calculated by subtracting left from right foot pad thickness.

References

(1) For reviews, see: (a) Orfanos, C. E.; Ehlert, R.; Gollnick, H. The Retinoids. A Review of Their Clinical Pharmacology and Therapeutic Use. *Drugs* **1987**, *34*, 459−503. (b) Linney, E. Retinoic Acid Receptors: Transcription Factors Modulating Gene Regulation. Development, and Differentiation. *Current Topics in Developmental Biology*; Academic Press: Orlando, FL, 1992; pp 309−350. (c) Sporn, M. B., Roberts, A. B., Goodman, D. S., Eds. *The Retinoids: Biology, Chemistry, and Medicine*, 2nd ed.; Raven Press Ltd.: New York, 1994.

(2) Ross, A. C.; Hammerling, U. G. Retinoids and the Immune System. *The Retinoids: Biology, Chemistry, and Medicine*, 2nd ed.; Raven Press Ltd.: New York, 1994; pp 521−543.

(3) Brinckerhoff, C. E.; Coffey, J. W.; Sullivan, A. C. Inflammation and Collagenase Production in Rats with Adjuvant Arthritis Reduced with 13-*cis*-Retinoic Acid. *Science* **1983**, *221*, 756−758.

(4) Massacesi, L.; Castigli, E.; Vergelli, M.; Olivotto, J.; Abbamondi, A. L.; Sarlo, F.; Amaducci, L. Immunosuppressive Activity of 13-*cis*-Retinoic Acid and Prevention of Experimental Autoimmune Encephalomyelitis in Rats. *J. Clin. Invest.* **1991**, *88*, 1331−1337.

(5) Trentham, D. E.; Brinckerhoff, C. E. Augmentation of Collagen Arthritis by Synthetic Analogues of Retinoic Acid. *J. Immunol.* **1982**, *129*, 2668.

(6) (a) Mangelsdorf, D. J.; Umesono, K.; Evans, R. M. The Retinoid Receptors. *The Retinoids: Biology, Chemistry, and Medicine*, 2nd ed.; Raven Press Ltd.: New York, 1994; pp 319−349. (b) Leid, M.; Kastner, P.; Chambon, P. Multiplicity Generates Diversity in the Retinoic Acid Signaling Pathways. *Trends Biochem. Sci.* **1992**, *17*, 427−433.

(7) Orfanos, C. E.; Zouboulis, C. C.; Almond-Roesler, B.; Geilen, C. C. Current Use and Future Potential Role of Retinoids in Dermatology [Review]. *Drugs* **1997**, *53*, 358−388.

(8) (a) Smith, M. A.; Parkinson, D. R.; Cheson, B. D.; Friedman, M. A. Retinoids in Cancer Therapy. *J. Clin. Oncol.* **1992**, *10*, 839−864. (b) Vokes, E. E.; Weichselbaum, R. R.; Lippman, S. M.; Hong, W. K. Head and Neck Cancer. *N. Engl. J. Med.* **1993**, *328*, 184−194. (c) Nagpal, S.; Chandraratna, R. A. S. Retinoids as Anti-Cancer Agents [Review]. *Curr. Pharm. Des.* **1996**, *2*, 295−316. (d) Lotan, R. Retinoids in Cancer Chemoprevention. *FASEB J.* **1996**, *10*, 1031−1039. (e) Hong, W. K.; Sporn, M. B. Recent Advances in Chemoprevention of Cancer. *Science* **1997**, *278*, 1073−1077.

(9) (a) Amstrong, R. B.; Ashenfelter, K. O.; Eckhoff, C.; Levin, A. A.; Shapio, S. S. General and Reproductive Toxicology of Retinoids. *The Retinoids: Biology, Chemistry, and Medicine*, 2nd ed.; Raven Press Ltd.: New York, 1994; pp 545−572. (b) Standeven, A. M.; Beard, R. L.; Johnson, A. T. Boehm, M. F. Escobar, M.; Heyman, R. A.; Chandraratna, R. A. S. Retinoid-Induced Hypertriglyceridemia in Rats Is Mediated by Retinoic Acid Receptors. *Fund. Appl. Toxicol.* **1996**, *33*, 264−271. (c) Soprano, D. R.; Soprano, K. J. Retinoids as Teratogens [Review]. *Annu. Rev. Nutr.* **1995**, *15*, 111−132. (d) Willhite, C. C.; Dawson, M. I. Structure−Activity Relationships of Retinoids in Developmental Toxicology IV. Planar Cisoid Conformational Restriction. *Toxicol. Appl. Pharmacol.* **1990**, *103*, 324−344.

(10) (a) Kagechika, H.; Kawachi, E.; Hashimoto, Y.; Himi, T.; Shudo, K. Retinobenzoic Acids. 1. Structure−Activity Relationships of Aromatic Amides with Retinoidal Activity. *J. Med. Chem.* **1988**, *31*, 2182−2192. (b) Teng, M.; Duong, T. T.; Klein, E. S.; Chandraratna, R. A. S. Identification of a Retinoic Acid Receptor α Subtype Specific Agonist. *J. Med. Chem.* **1996**, *39*, 3035−3038. (c) Johnson, A. T.; Klein, E. S. Wang, L.; Pino, M. E.; Chandraratna, R. A. S. Identification of Retinoic Acid Receptor β Subtype Specific Agonists. *J. Med. Chem.* **1996**, *39*, 5027−5030. (d) Esgleyes-Ribot, T.; Chandraratna, R. A. S.; Lew-Kaya, D.; Sefton, J.; Duvic, M. J.; Response of Psoriasis to a New Topical Retinoid, AGN190168. *J. Am. Acad. Dermatol.* **1994**, *30*, 581−590. (e) Delescluse, C.; Cavey, M. T.; Martin, B.; Bernard, B. A.; Reichert, U.; Maignan, J.; Darmon, M.; Shroot, B. Selective High Affinity Retinoic Receptor α or β-γ Ligands. *Mol. Pharmacol.* **1991**, *40*, 556−562. (f) Charpentier, B.; Bernardon, J.-M.; Eustache, J.; Millois, C.; Martin, B.; Michel, S.; Shroot, B. Synthesis, Structure−Activity Relationships, and Biological Activities of Ligands Binding to Retinoic Acid Receptor Subtypes. *J. Med. Chem.* **1995**, *38*, 4993−5006. (g) Yu, K.-L.; Spinazze, P.; Ostrowski, J.; Currier, S. J.; Pack, E. J.; Hammer, L.; Roalsvig, T.; Honeyman, J. A.; Tortolani, D. R.; Reczek, P. R.; Mansuri, M. M.; Starret, J. E., Jr. Retinoic Acid Receptor β, γ-Selective Ligands: Synthesis and Biological Activity of 6-Substituted 2-Naphthoic Acid Retinoids. *J. Med. Chem.* **1996**, *39*, 2411−2421. (h) Vuligonda, V.; Lin, Y.; Thacher, S. M.; Standeven, A. M.; Kochar, D. M.; Chandraratna, R. A. S. A. New Class of RAR Subtype Selective Retinoids: Correlation of Pharmacological Effects with Receptor Activity. *Bioorg. Med. Chem.* **1999**, *7*, 263−270. (i) Dhar, A.; Liu, S.; Klucik, J.; Berlin, K. D.; Madler, M. M.; Lu, S.; Ivey, R. T.; Zacheis, D.; Brown, C. W.; Nelson, E. C.; Birckbichler, P. J.; Benbrook, D. M. Synthesis, Structure−Activity Relationships, and RARγ-Ligand Interactions of Nitrogen Heteroarotinoids. *J. Med. Chem.* **1999**, *42*, 3602−3614.

(11) Apfel, C.; Bauer, F.; Crettaz, M.; Forni, L.; Kamber, M.; Kaufmann, F.; LeMotte, P.; Pirson, W.; Klaus, M. A. Retinoic Acid Receptor α Antagonist Selectively Counteracts Retinoic Acid Effects. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 7129−7133.

(12) Kagechika, H.; Kawachi, E.; Fukasawa, H.; Saito, G.; Iwanami, N.; Umemiya, H.; Hashimoto, Y.; Shudo, K. Inhibition of IL-1 Induced IL-6 Production by Synthetic Retinoids. *Biochem. Biophys. Res. Commun.* **1997**, *231*, 243−248.

(13) Kuwabara, K.; Shudo, K.; Hori, Y. Novel Synthetic Retinoic Acid Inhibits Rat Collagen Arthritis and Differentially Affects Serum Immunoglobulin Subclass Levels. *FEBS Lett.* **1996**, *378*, 153−156.

(14) Tobita, T.; Takeshita, A.; Kitamura, K.; Ohnishi, K.; Yanagi, M.; Hiraoka, A.; Karasuno, T.; Takeuchi, M.; Miyawaki, S.; Ueda, R.; Naoe, T.; Ohno, R. Treatment With a New Synthetic Retinoid, Am80, of Acute Promyelocytic Leukemia Relapsed From Complete Remission Induced by All-trans Retinoic Acid. *Blood* **1997**, *90*, 967−973.

(15) (a) Ishibashi, Y.; Harada, S.; Ohkawara, A.; Tagami, H.; Iizuka, H.; Toda, K.; Takigawa, M.; Shimizu, M.; Nakashima, M. Clinical Phase II Study on Am-80 Ointment −Investigation of Systemic Effect. *Rinshouiyaku* **1995**, *11*, 721−732. (b) Ishibashi, Y. Efficacy of Am-80 Ointment on Psoriasis -A Bilateral-paired Comparative Study with Betamethasone Valerate Ointment (Phase III Study). *Rinshouiyaku* **1995**, *11*, 733−759.

(16) (a) Yoshimura, H.; Nagai, M.; Hibi, S.; Kikuchi, K.; Abe, S.; Hida, T.; Higashi, S.; Hishinuma, I.; Yamanaka, T. A Novel Type of Retinoic Acid Receptor Antagonist: Synthesis and Structure−

Activity Relationships of Heterocyclic Ring-Containing Benzoic Acid Derivatives. *J. Med. Chem.* **1995**, *38*, 3163−3173. (b) Hibi, S.; Kikuchi, K.; Yoshimura, H.; Nagai, M.; Tai, K.; Hida, T. Syntheses and Structure−Activity Relationships of Novel Retinoid X Receptor Agonists. *J. Med. Chem.* **1998**, *41*, 3245−3252. (c) Kikuchi, K.; Hibi, S.; Yoshimura, H.; Tokuhara, N.; Tai, K.; Hida, T.; Yamauchi, T.; Nagai, M. Syntheses and Structure−Activity Relationships of 5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-quinoxaline Derivatives with Retinoic Acid Receptor α Agonistic Activity. *J. Med. Chem.* **2000**, *43*, 409−419. (d) Kikuchi, K.; Hibi, S.; Yoshimura, H.; Tai, K.; Hida, T.; Tokuhara, N.; Yamauchi, T.; Nagai, M. Novel Retinoic Acid Receptor α Agonists: Syntheses and Evaluation of Pyrazole Derivatives. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 619−622. (e) Hibi, S.; Tagami, K.; Kikuchi, K.; Yoshimura, H.; Tai, K.; Hida, T.; Tokuhara, N.; Yamauchi, T.; Nagai, M. Syntheses and Evaluation of Naphthalenyl- and Chromenyl-pyrrolyl-benzoic Acids as Potent and Selective Retinoic Acid Receptor α Agonists. *Bioorg. Med. Chem. Lett.*, **2000**, *10*, 623−625.

(17) Ansar, M.; Akoum Ebrik S. A.; Mouhoub, R.; Berthelot, P.; Vaccher, C.; Vaccher, M. P.; Flouquest, N.; Caignard, D. H.; Renartd, P.; Pirard, B.; Rettori, M. C.; Evrard, G.; Durant, F.; Debaert, M. 3-Benzo[*b*]furyl- and 3-benzo[*b*]thienylaminobutyric acids as $GABA_B$ ligands. Synthesis and structure−activity relationship studies. *Eur. J. Med. Chem.* **1996**, *31*, 449−460.

(18) Weerawarna, S. A.; Guha-Biswas, M.; Nelson, W. L. Benzofurans. Improved Syntheses of Bufuralol, 7-Ethyl-2-(2-*tert*-butylamino-1-hydroxyethyl)benzofuran, and 1″-Oxobufuralol, 7-Acetyl-2-(2-*tert*-butylamino-1-hydroxyethyl)benzofuran. *J. Heterocycl. Chem.* **1991**, *28*, 1395−1403.

(19) McKnight, A.; Zimmer, G.; Fogelman, I.; Wolf, S.; Abbas, A. Effects of IL-12 on Helper T Cell-Dependent Immune Responses In Vivo. *J. Immunol.* **1994**, *152*, 2172−2179.

(20) Lagrange, P. Regulation mechanisms of the activity of T Lymphocytes. Applications to infective and tumor pathology. I. Delayed Hypersensitivity and Humoral Response. *Pathol. Biol.* **1976**, *24*, 67−74.

(21) Yamauchi, T.; Ishibashi, A.; Tokuhara, N.; Nagai, M. Methods for Preventing, Inhibiting or Treating Graft Rejection Reactions in Graft-Versus-Host Disease (GVHD) and Organ Transplantation. WO 98/14214.

(22) Yamauchi, T.; Ishibashi, A.; Tokuhara, N.; Nagai, M. Retinoic Acid Agonists as Preventive and Therapeutic Agents for Nephritis. WO 99/20309.

JM000098S