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4-[3-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)phenyl]benzoic Acid and Heterocyclic-Bridged Analogues are Novel Retinoic Acid Receptor Subtype and Retinoid X Receptor α Agonists

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Abstract—Aromatic retinoids having a *meta*-substituted aromatic ring bridge, such as 4-[3-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)phenyl]benzoic acid and its 3,5-diaryl-substituted 4,5-dihydroisoxazole analogue, function as retinoid receptor panagonists by activating both retinoic acid and retinoid X receptors to induce gene transcription, and thereby provide novel scaffolds for retinoid drug development. Both classes of these ligand-inducible transcription factors are involved in mediating the inhibitory effects of retinoids on cancer cell growth.

The aromatic retinoids (*E*)-4-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)propenyl]benzoic acid (TTNPB or Ro13-7410, **1** in Table 1)[1] and 6-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-2-naphthalenecarboxylic acid (TTNN, **2**)[2,3] were leads for the development of the topical antipsoriatic drug tazarotene® (ethyl 6-[2-(4,4-dimethyl-2,3-dihydro-6-thiochromanyl)-ethynyl] nicotinate, AGN 190168)[4] and the topical antiacne drug adapalene® (6-[3-(1-adamantyl)-4-methoxyphenyl]-2-naphthalenecarboxylic acid, CD271[5]), respectively. Here, we report another novel retinoid scaffold—the teraryl retinoid 4-[3-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)phenyl]benzoic acid (TTNPB2, **3**) and analogue structures—that should be useful for constructing new retinoid receptor panagonists, which bind and activate both the retinoic acid receptor (RAR) and retinoid X receptor (RXR) classes of ligand-inducible transcription factors (reviewed in ref 6). Increasing evidence indicates that both RARs and RXRs are involved in regulating the inhibitory effects of retinoids on cancer cell growth and hyperproliferation.[7–11]

In the micromolar range TTNPB2 activates both receptor classes in CV-1 cells cotransfected with an expression vector for one of the receptors and the $(TREpal)_2$-*tk*-CAT reporter construct (Table 1). Transcriptional activation activities (as measured by AC_{50} values) for RARβ, RARγ, and particularly RXRα are enhanced by a methyl group at the 4-position of the 1,3-disubstituted benzene bridge between the 4-substituted benzoic acid and 2-substituted tetrahydronaphthalene rings (MM11387); whereas the AC_{50} value for RARα activation decreases by almost two-logs. Activation levels at 1 μM MM11387 resemble those at 1 μM *trans*-retinoic acid (*trans*-RA) for the RAR subtypes and at 1 μM 9-*cis*-RA for RXRα. A second methyl group at the 3-position of the tetrahydronaphthalene ring (MM11388) further reduces AC_{50} values for RARα and RXRα, although activation of all four receptors at 1 μM MM11388 is 45% to 96% of that at 1 μM *trans*-RA for the RARs and 70% of that at 1 μM 9-*cis*-RA for RXRα. Use of a 2,6-disubstituted pyridine bridge (MM11395) causes at least a one-log

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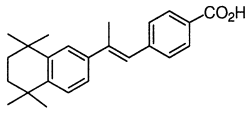
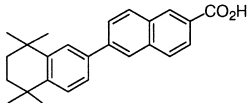
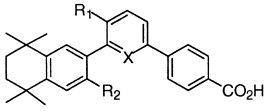
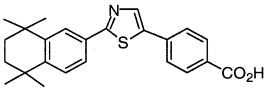
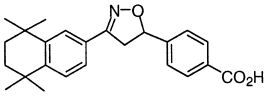
Table 1. Retinoic acid receptor (RAR) subtype and retinoid X receptor (RXR) α transcriptional activation (AC_{50}) on the $(TREpal)_2$-*tk*-CAT in transfected CV-1 cells by retinoids **1** to **9** compared to 1 μM *trans*-RA for the RARs and 1 μM 9-*cis*-RA for RXRα

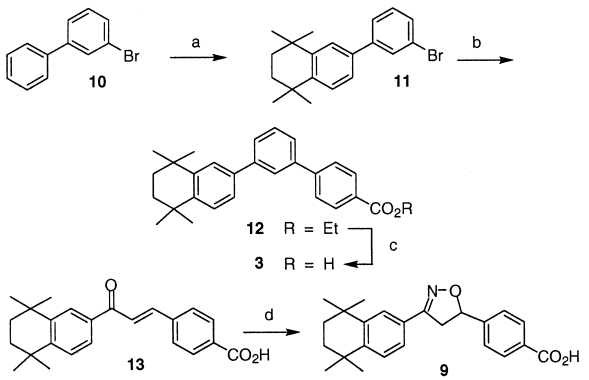
	Retinoid structure[a]	Name or code number	AC_{50} values (nM) (% activation at 1 μM)[b]			
			RARα	RARβ	RARγ	RXRα
1		TTNPB (Ro13-7410)[c]	200 (85)	22 (86)	26 (75)	>1000 (0)
2		TTNN[d]	>1000 (23)	52 (82)	68 (95)	>1000 (0)
						
	R_1 R_2 X					
3	H H CH	TTNBP2 (MM11256)	1.0 (127)	4.3 (109)	2.3 (99)	>1000 (27)
4	Me H CH	MM11387	79 (87)	< 1 (113)	< 1 (93)	135 (85)
5	Me Me CH	MM11388	>1000 (43)	10 (96)	1.0 (87)	430 (70)
6	H H N	MM11395	370 (61)	140 (68)	>1000 (20)	>1000 (17)
7	H Me N	MM11396	>1000 (26)	>1000 (42)	1000 (5)	>1000 (0)
8		MM11176	>1000 (6)	740 (36)	220 (69)	>1000 (8)
9		MM11391	14 (86)	< 1 (94)	< 1 (88)	100 (65)

[a]New compounds were fully characterized (IR, ^1H NMR, mp) and passed analysis (elemental or HRMS).
[b]Activation (50%) for RARα, β, γ, and RXRα on the $(TREpal)_2$-*tk*-CAT by retinoids in monkey kidney CV-1 cells transfected with expression vectors for each of these receptors, compared to that of 1 μM *trans*-RA for the RARs and 1 μM 9-*cis*-RA for RXRα as 100%. Two copies of the TREpal response element, which is activated by RARs and RXRs, were linked to the chloramphenicol acetyl transferase (CAT) reporter containing the thymidine kinase promotor (*tk*) (ref 11). The β-galactosidase expression vector was used to normalize for transfection efficacy. Data points are the means of triplicate experiments. AC_{50} values were calculated by interpolation of concentration–response curves. Assays were conducted at the Burnham Institute under license from Ligand Pharmaceuticals for use of this patented technology.
[c]Ref 13.
[d]Ref 12.

increase in the AC_{50} values for RARα and β, more than a two-log increase for RARγ, and a decrease in RXRα activation. The MM11395 analogue having a methyl group at the 3-position of the tetrahydronaphthalene ring (MM11396) is almost devoid of activity. The 2,5-disubstituted 1,3-thiazole bridge (MM11176) also produces low activity, with only RARγ being moderately activated at 1 μM MM11176. Potent activity is regained by using a 3,5-disubstituted 4,5-dihydroisoxazole bridge (MM11391) with activations for the RAR subtypes comparable to those by TTNPB2 and RXRα activation enhanced. MM11391 may behave as a panagonist because of its structural similarities to the RAR-selective *Z*-oxime of 6-(4,5,6,7-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenylcarbonyl)-2-naphthalenecarboxylic acid[11] and the RXR-selective methyloxime of 4-(4,5,6,7-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenylcarbonyl)benzoic acid.[13]

Moreover, the 10.6 Å 8-tetrahydronaphthalenyl carbon and carboxylic acid carbon distance for the low-energy conformer of MM11391 is between the 11.3 Å and 9.6 Å C–C distances found for the low-energy conformers of these RAR- and RXR-selective analogues using CAChe software (Oxford Molecular Ltd) and the MM3 force field.

TTNPB2 gave IC_{50} values of 2.7 μM for inhibiting the growth of retinoid-sensitive NIH:OVCAR-3 ovarian cancer cells and 3.0 μM for inhibiting the growth of retinoid-resistant MDA-MB-231 breast cancer cells compared to IC_{50} values of 2.3 μM and greater than 12.5 μM,



Scheme 1. Synthesis of TTNPB2 (**3**): (a) 2,5-dimethyl-2,5-dichlorohexane (ref 12), $Cl(CH_2)_2Cl$, $AlCl_3$, 0 °C: 6-(3-bromophenyl)-1,2,3,4-tetrahydro-1,1,4,4-tetramethylnaphthalene (**11**) (97%); (b) $[Pd[P(C_6H_5)_3]_4$, anhyd DME], 4-carbethoxyphenylboronic acid (ref 12), EtOH; aq Na_2CO_3, reflux: ethyl 4-[3-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)phenyl]benzoate (**12**) (79%); (c) KOH, 75% aq MeOH, (70 °C); (1 N HCl): **3** (99%). Synthesis of MM11391 (**9**); (d) **13** (ref 15), $NH_2OH{\cdot}HCl$, NaOAc, MeOH, 70 °C, 15 h; aq HCl (51%). (See ref 16 for mp and spectral data.).

Table 2. Effects of 1 μM all-*trans*-retinoic acid (*trans*-RA), 9-*cis*-RA, TTNBP2, MM11387, and MM11391 on the growth of retinoid-sensitive T-47D and ZR-75-1 and retinoid-resistant MDA-MB-231 breast cancer cells after 10 days[a]

Retinoid	Cell numbers (% relative to nontreated control)		
	T-47D	ZR-75-1	MDA-MB-231
Control	100±4	100±3	100±4
trans-RA	39±2*	70±4*	94±2
9-*cis*-RA	35±3*	59±5*	69±2*
TTNPB2 (**3**)	32±4*	47±4*	80±5*
MM11387 (**4**)	35±5*	49±1*	75±5*
MM11391 (**9**)	23±4*	42±3*	65±4*

[a]Cells were cultured at 37 °C in medium containing 10% fetal bovine serum and either 1.0 μM retinoid or Me_2SO vehicle alone, with medium and retinoid solution replaced every 48 h. Cell numbers were determined using the MTT assay.[9] Results (% growth) represent the average of three experiments ± the standard error and are statistically significant (*) relative to the control ($P < 0.001$).

respectively, for *trans*-RA after treatment for seven days at 37 °C in medium containing 10% fetal bovine serum with viable cell numbers determined spectrophotometrically. After 10 days, growth inhibition by 1 μM TTNPB2, MM11387, or MM11391 on retinoid-sensitive ZR-75-1 and T-47D breast cancer cells or on retinoid-insensitive MDA-MB-231 breast cancer cells was comparable to or greater than that of 1 μM *trans*-RA or 9-*cis*-RA (see Table 2).

The teraryl scaffold of TTNPB2 is suitable for combinatorial analogue synthesis using Suzuki-type palladium-catalyzed biaryl-couplings[14] to introduce 1,3-disubstituted aryl or heterocyclic ring bridging groups. The syntheses of TTNPB2 and MM11391 are shown in Scheme 1 and described in the accompanying legend. MM11387, MM11388, MM11395, MM11396, and MM11176, the structures of which are shown in Table 1, were readily prepared using similar methodologies.

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16. Characterization data, **11**: white solid, mp 78–80 °C (hexane); R_f 0.45 (hexane); IR (KBr) 2956, 1591, 1551, 1469, 1386, 1361, 793 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.32 (s, 6, CMe_2), 1.34 (s, 6, CMe_2), 1.72 (s, 4, CH_2CH_2), 7.28 (dd, $J = 7.8$, 7.8 Hz, 1, ArH), 7.31 (dd, $J = 2.0$, 8.1 Hz, 1, ArH), 7.38 (d, $J = 8.1$ Hz, 1, ArH), 7.47 (m, 3, ArH), 7.70 (dd, $J = 1.7$, 1.7 Hz, 1, ArH). **12**: White solid, mp 148–149 °C (CH_2Cl_2/hexane); R_f 0.35 (50% CH_2Cl_2/hexane); IR (KBr) 2956, 1712, 1272, 1102, 771 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.33 (s, 6, CMe_2), 1.35 (s, 6, CMe_2), 1.42 (t, $J = 7.1$ Hz, 3, $CO_2CH_2CH_3$), 1.73 (s, 4, CH_2CH_2), 4.41 (q, $J = 7.1$ Hz, 2, $CO_2CH_2CH_3$), 7.40 (br s, 2, ArH), 7.56 (m, 4, ArH), 7.71 (d, $J = 8.5$ Hz, 2, ArH), 7.79 (dd, $J = 1.4$, 1.4 Hz, 1, ArH), 8.13 (d, $J = 8.5$ Hz, 2, ArH). **3**: White crystalline solid, mp 232–233 °C (CH_2Cl_2/hexane); IR (KBr) 2955, 1685, 1609, 1422, 1298, 771 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.34 (s, 6, CMe_2), 1.36 (s, 6, CMe_2), 1.74 (s, 4, CH_2CH_2), 7.41 (br s, 2, ArH), 7.58 (m, 4, ArH), 7.75 (d, $J = 8.6$ Hz, 2, ArH), 7.81 (dd, $J = 1.4$, 1.4 Hz, 1, ArH), 8.21 (d, $J = 8.6$ Hz, 2, ArH). **9**: White solid; IR (KBr) 2900–3500, 1700, 1614, 1460, 1293, 1020, 829 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.16, 1.19, 1.21 (3s, 12, 2 CMe_2), 1.61 (s, 4, CH_2CH_2), 2.89 (dd, $J = 14$, 9 Hz, 1, CH_2CH *anti* to Ar), 3.18 (dd, $J = 14$, 7 Hz, 1, CH_2CH *syn* to Ar), 4.25 (m, 1, CH_2CH), 7.2–7.3 (dd, 3, NapH), 7.35 (d, $J = 9$ Hz, 2, ArH *meta* to CO_2H), 7.38 (d, $J = 9$ Hz, 2, ArH *ortho* to CO_2H).