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sp2-Bridged Diaryl Retinoids: Effects of Bridge-Region Substitution on Retinoid X Receptor (RXR) Selectivity

Marcia I. Dawson,[a,*] Peter D. Hobbs,[b] Ling Jong,[b] Dongmei Xiao,[c] Wan-ru Chao,[b] Chin Pan[a] and Xiao-kun Zhang[c]

[a]*Medicinal Chemistry Department, Molecular Medicine Research Institute, 325 East Middlefield Road, Mountain View, CA 94043, USA*
[b]*Retinoid Program, SRI International, 333 Ravenswood Avenue, Menlo Park, CA 94025, USA*
[c]*The Burnham Institute, 10901 North Torrey Pines Road, La Jolla, CA 92037, USA*

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Abstract—RXR class selectivity and RXR transcriptional activation activity compared to those for the retinoic acid receptor subtypes were enhanced on the 4-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenylethenyl)benzoic acid scaffold and its 3-methyl analogue by replacing their 1,1-ethenyl bridge by a 1,1-(2-methylpropenyl) or cyclopropylidenylmethylene group.

The RXRs are unique members of the nuclear receptor superfamily of ligand-inducible transcription factors because they heterodimerize with the retinoic acid receptors (RARs), vitamin D receptor, thyroid hormone receptor, and orphan receptors, such as the peroxisome proliferator-activated receptors α and γ, and TR3/nur77 (reviewed in ref 1). Increasing evidence indicates that the RXR heterodimeric partner, its ligand, and the response element to which the retinoid-heterodimer complex binds influence whether RXR will be occupied by ligand to further modulate gene transcription.[2–9] The ability of RXR-selective retinoids to enhance transactivation by RAR-selective retinoids[2–9] suggests that they may also enhance the therapeutic efficacy of these and other ligands that activate RXR dimeric partners.

4-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenylcarbonyl)benzoic acid (SR11004, **1** in Fig. 1),[10] an analogue of the RAR-selective 6-substituted 2-naphthalenecarboxylic acid CD567 (**2**),[11] and 4-[2-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-naphthalenyl)propenyl] benzoic acid (3-methyl-TTNPB, **3**)[12] provided the leads to RXR-selective synthetic analogues of RAR/RXR panagonist 9-*cis*-retinoic acid (9-*cis*-RA, **6** in Table 1).[13,14] Retinoid receptor transcriptional activation assays using the $(TREpal)_2$-*tk*-CAT reporter construct[15–17] in CV-1 cells cotransfected with an expression vector for one of the RAR subtypes α, β, and γ, or RXRα indicated that (1) decreasing the distance between the hydrophobic and polar termini in **2** by replacing its 2,6-disubstituted naphthalenyl moiety by a 1,4-disubstituted phenyl ring, (2) increasing the hydrophobicity of the sp2 bridge linking the aryl rings of **1**, and (3) including a 3-methyl group on the tetrahydronaphthalene ring of **4** enhanced RXRα transactivation, as demonstrated by **1**, **4**, and **5**, respectively.[10,15,18]

RXR-selective **5** (LGD1069)[19] prevented mammary tumor formation in rats treated with the carcinogen *N*-methyl-*N*-nitrosourea[20] and showed anticancer efficacy in clinical trials.[21] The potent, more RXR-selective 2-[1-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-naphthalenyl) cyclopropyl]-5-pyridinecarboxylic acid (LGD100268)[22] was reported to lack cancer preventive efficacy when administered alone under these conditions.[23] These and other results[8,17,24,25] suggest that the cancer preventive and therapeutic effects of RXR-selective retinoids require concomitant activation of RARs, as occurs by using the panagonist 9-*cis*-RA alone or the combination of an RXR-selective retinoid with an RAR-selective retinoid[24] or with another agent, such as interferon α.[26]

Enhancing alkyl bulk on the bridge between the aryl rings in sp3-carbon-bridged RXR analogues improved RXR selectivity.[10,15] Here, we report the effects on RXRα activation and selectivity on the $(TREpal)_2$-*tk*-CAT reporter construct, compared to those of the RAR subtypes, by replacing the hydrogens on the ethenyl bridges of **4** and

*Corresponding author. Tel.: +1-650–237-7456; fax: +1-650–237-7455; e-mail: mdawson@mmrx.org

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5 by alkyl substitutents. Substitution with methyl groups produced 4-[1-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-2-methylpropenyl]benzoic acid (**7**)[10,15] and its more potent 3-methyl homologue (**8**)[17,24] (see Table 1). However, introducing a sp2 cyclopropylidenylmethylene bridge between the aryl rings provides selective RXR/RAR-subtype panagonists, in which further modifications of the aryl rings may modulate RAR-subtype activity. For example, **9** activates both RARβ and RXRα on the $(TREpal)_2$-*tk*-CAT, whereas at 10 nM **10** is more selective and more potent than **9** in activating RXRα (108% compared to 2%).

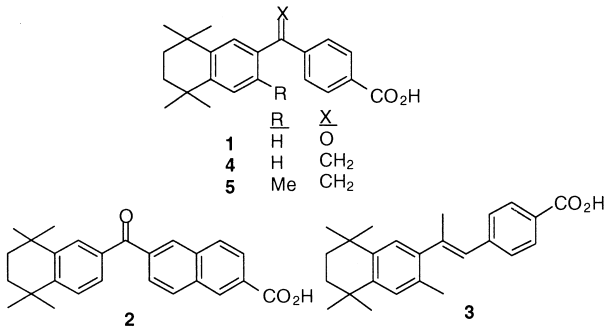
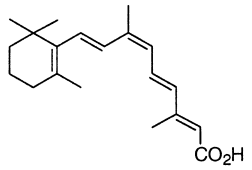
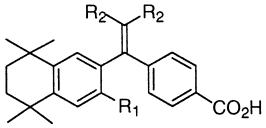
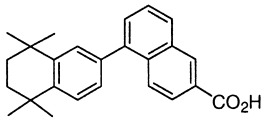
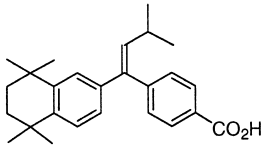


Figure 1. Structures of SR11004 (**1**), CD567 (**2**), 3-MeTTNPB (**3**), MM11225 (**4**), and MM11247 (**5**), which were initial leads to more potent RXR-selective retinoids.

In contrast, analogue **11**, in which the bonds corresponding to the ethenyl bridge and the 4-substituted benzoic acid ring of **4** are coplanar by inclusion in a 5-substituted 2-naphthalenecarboxylic acid ring, and the 2-methylpropylidene analogue **12** were inactive. We previously found that removal of the *Z*-methyl group of **7** only slightly decreased RXRα activity.[1] The loss of activity by replacing the remaining *E*-methyl group by an *E-i*-propyl group (**12**) suggests that further steric constraints are present in the region of the RXRα

Table 1. Retinoic acid receptor (RAR) subtype α, β, and γ and retinoid X receptor (RXR) α 50% transcriptional activation (AC_{50}) on the $(TREpal)_2$-*tk*-CAT by retinoids **7** to **12** compared to 1 μM *trans*-RA for the RARs and 1 μM 9-*cis*-RA (**6**) for RXRα[a,b]

Structure	Name or code number	50% Activation values (AC_{50}, nM) (% activation at 1 μM compared to 1 μM *trans*-RA for RARs or 1 μM 9-*cis*-RA for RXRα)			
		RARα	RARβ	RARγ	RXRα
					
6	9-*cis*-RA	19 (105)	2 (112)	3 (126)	7 (100)
					
7 R_1 = H, R_2 = Me	MM11217	>1000 (0)	>1000 (21)	>1000 (0)	160 (71)
8 R_1 = Me, R_2 = Me	MM11345	>1000 (0)	>1000 (−6)	>1000 (−12)	22 (107)
9 R_1 = H, R_2 = (CH_2)–	MM11346	>1000 (19)	17 (85)	>1000 (16)	5 (118)
10 R_1 = Me, R_2 = (CH_2)–	MM11173	>1000 (−23)	>1000 (−19)	>1000 (12)	< 10 (120)
					
11	MM11258	>1000 (0)	>1000 (1)	>1000 (18)	> 1000 (12)
					
12	MM11344	>1000 (0)	>1000 (−6)	>1000 (−10)	> 1000 (−1)

[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 *trans*-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 reporter (CAT) containing the thymidine kinase promoter (*tk*).[10] 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.

ligand-binding domain that interacts with the retinoid bridge.

Previously, we demonstrated that RXR-selective retinoids inhibit the growth of retinoid-resistant MDA-MB-231 human breast cancer cells in part by activating RXRα-nur77 heterodimers on the βRARE to induce the expression of RARβ,[25] which is lost in many cancers. We found that *trans*-RA had no effect on the growth of MDA-MB-231 cells, largely due to their lack of RARβ,[25] and **8** alone did not inhibit growth. However, *trans*-RA plus **8** produced significant inhibition, as did the RAR/RXR panagonist **9** alone (Fig. 2).

Inhibition by **9** is likely due to its induction of RARβ expression through the RXR-nur77,[25] followed by its activation of the synthesized RARβ protein that forms the RXR-RARβ. Our finding that growth inhibition by the far more RXR-selective **8** requires the presence of an RAR agonist suggests that activation of RXRα alone is not sufficient for inhibiting the growth of these cells. These data also suggest that RXR/RARβ panagonists, such as **9**, alone or in combination with another therapeutic agent, may be useful in cancer therapy, whereas more RXR-selective retinoids, such as **8** and **10**, may only be useful in combination with an RAR-selective retinoid or another agent. Interestingly, we found that **9** inhibited MDA-MB-231 cell growth after seven days in culture with an IC_{50} value of 1.1 μM, whereas 12.5 μM 9-*cis*-RA gave only 45% inhibition.

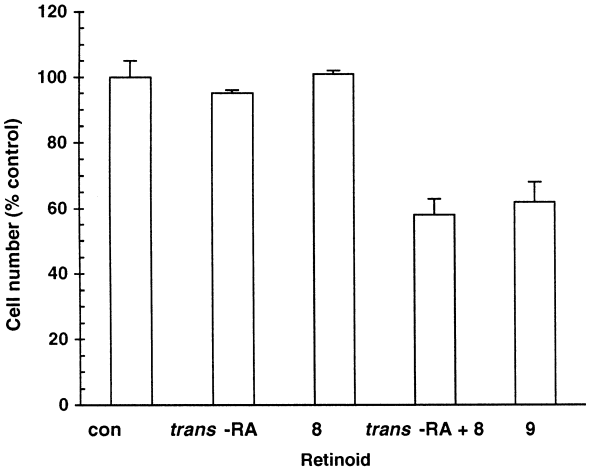
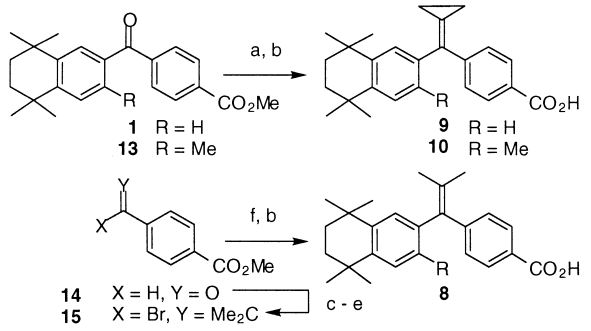


Figure 2. Inhibition of MDA-MB-231 breast cancer cell growth by 0.1 μM *trans*-retinoic acid (*trans*-RA), 1 μM **8**, 0.1 μM *trans*-RA plus 1 μM **8**, and 1 μM **9** after 10 days, with medium containing 10% fetal bovine serum and retinoid solution or Me_2SO vehicle alone changed every 48 h. The number of viable cells was determined by the MTT assay.[25] Results represent the average of triplicate experiments ± the standard error. Only growth inhibition by *trans*-RA plus **8** and by **9** was statistically significant ($P < 0.001$).



Scheme 1. Syntheses of retinoids **9** and **10** from diaryl ketones **1** and **13** (ref 10): (a) [cyclopropyltriphenylphosphonium bromide, $KN(SiMe_3)_2$, toluene]; $(MeOCH_2CH_2OCH_2CH_2)_3N$, reflux (66% for **9**, 69% for **10**); (b) KOH, aq EtOH; aq citric acid (90% for **9**, 83% for **10**, 80% for **8**). Synthesis of **8** from **14**: (c) [*i*-$Pr(C_6H_5)_3PI$, $KNSi(Me_3)_2$, THF]; (d) Br_2, CH_2Cl_2; (e) DBU, THF; (66% overall); (f) 5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-naphthaleneboronic acid, $[(C_6H_5)_3P]_4Pd$, Na_2CO_3, aq DME (41%).

The synthesis of **7** from diaryl ketone **1** was reported.[10] Retinoids **9** and **10** were similarly prepared in high yields from ketones **1** and **13** as outlined in Scheme 1, whereas the more sterically hindered **8** required a stepwise procedure involving a vinyl bromide-aryl bromide coupling.

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