Synthesis of Retinoid X Receptor-Specific Ligands That Are Potent Inducers of Adipogenesis in 3T3-L1 Cells

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A novel series of oxime ligands has been synthesized that displays potent, specific activation of the retinoid X receptors (RXRs). The oximes of 3-substituted (tetramethyltetrahydronaphthyl)carbonylbenzoic acids are readily available by condensation with hydroxyl- or methoxylamine; alkylation of the hydroxyl oxime provides a variety of analogues. Oximes and variously substituted oxime derivatives demonstrate high binding affinity for the RXRs and specific RXR activation and, hence, are called rexinoids. These oxime rexinoids are activators of the RXR:PPARγ heterodimer and are potent inducers of differentiation of 3T3-L1 preadipocytes to adipocytes. We have recently reported that ligands which activate the RXR:PPARγ heterodimer in this manner are effective in the treatment of type II diabetes (non-insulin-dependent diabetes mellitus, NIDDM). Thus, these new oxime rexinoids are potential therapeutic agents for the treatment of metabolic disorders, such as obesity and diabetes.

Introduction

The biological effects of retinoids (natural and synthetic vitamin A derivatives) are mediated through the family of intracellular receptors called the retinoid receptors.[1] The retinoid receptors are classified into two subfamilies: the retinoic acid receptors (RARα, RARβ, RARγ) and the retinoid X receptors (RXRα, RXRβ, RXRγ).[2] These receptors modulate gene expression by binding as heterodimers to specific DNA sequences, the retinoic acid response elements.[2c,3] The RXRs participate as partners in several hormone-response pathways and heterodimerize with RAR and other members of the intracellular receptor superfamily, including peroxisome proliferator-activated receptors (PPARs), vitamin D receptor (VDR), and thyroid hormone receptor (TR).[4] Hence, RXRs play a very critical role in the regulation of many biological activities that are responsive to a variety of structurally diverse hormones.

The retinoid receptors are ligand-activated transcription factors. The natural ligands for RARs (*all-trans*-retinoic acid, ATRA)[5] and RXRs (9-*cis*-retinoic acid, 9-*cis*-RA)[6] have been the basis for the design of many synthetic retinoid analogues. Potent, RAR-selective synthetic retinoids have been described,[7,8] and more recently, RXR-specific compounds, rexinoids, have been reported.[9] We have recently demonstrated that rexinoids function to activate the RXR:PPARγ signaling pathways and function as insulin sensitizers.[10] Because of this demonstrated in vivo activity in obesity and diabetes models, rexinoids are especially attractive clinical targets and may offer a new approach to the treatment of non-insulin-dependent diabetes mellitus (NIDDM or type II diabetes).[10]

We report herein an efficient and practical synthesis of a new class of potent rexinoids that are oxime derivatives of 3-substituted (tetramethyltetrahydronaphthyl)carbonylbenzoic acids. The new oximes are analogues of Targretin (LGD1069),[9c] an RXR-selective retinoid currently in phase II/III clinical trials for the treatment of various cancers and NIDDM. The ability of the synthetic compounds to interact with and alter RXR activity was evaluated employing a receptor/reporter cotransfection assay[9k,6a,11] and a competitive receptor binding assay.[9k,11,12] The new oxime ligands exhibit high affinity binding for the three RXR subtypes and specifically activate the transcriptional activity of RXRα, RXRβ, and RXRγ. Additionally, these RXR agonists were examined for their ability to activate the RXR:PPARγ heterodimer and induce differentiation of preadipocytes to adipocytes. These data suggest that the oxime rexinoids could offer novel treatment for diseases where the RXR:PPARγ heterodimer is the target for therapeutic action, such as the treatment of NIDDM.

Chemistry

The oximes are related to the previously reported RXR ligand **1** (3-methyl-TTNCB, see Chart 1) and are structurally similar to the first clinically submitted rexinoid, the methylidene-linked analogue Targretin (LGD1069, **2**).[9c] The oximes and methyloximes were prepared in a yield of 60−98% by a simple condensation reaction of 3-methyl-TTNCB, the corresponding ester, or the related 3-substituted-TTNCB analogue with hydroxylamine hydrochloride or an alkoxylamine hydrochloride (methoxy, ethoxy, or *tert*-butoxy), followed by, when necessary, saponification of the ester (see Scheme 1). Typically the reactions were performed at reflux temperature in ethanol with excess pyridine. The oximes and methyloximes were formed as a mixture of

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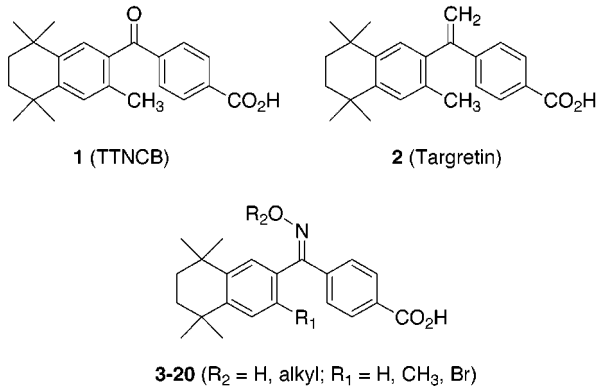
Chart 1. Synthetic RXR-Selective Retinoids



geometric isomers favoring the *cis* isomer (shown in structures **3**−**16**), in ratios generally ranging from 5:1 to 10:1 = *cis:trans*; the *tert*-butoxyoxime of 3-methyl-TTNCB was produced as a 1:1 mixture of *cis:trans* isomers. The crude oxime acids were solids which could be easily purified by recrystallization ($CHCl_3$/hexanes) or silica gel chromatography (1:1 = EtOAc:hexanes); one purification sequence (recrystallization or chromatography) provided the pure *cis* isomer. The minor, *trans*-oxime isomer was isolated by selective recrystallization or by preparative HPLC of the mother liquors.

The geometry of the oximes was confirmed with single-crystal X-ray analysis of the individual isomers **3** and **17**. The structures of the *cis* and *trans* isomers, as determined by X-ray, are shown in Figure 1. In the *cis* isomer **3**, the oxime is effectively coplanar with the benzoic acid and the conjugated system is approximately 70° out-of-plane with the tetramethyltetrahydronaphthyl ring system. In the *trans* isomer **17**, the oxime does not appear to be coplanar with either the benzoic acid ring or the tetramethyltetrahydronaphthylene. However, the aromatic systems are, again, nearly orthogonal to each other. The *cis*- and *trans*-oximes each have very distinctive ^{1}H NMR chemical shift patterns in the aromatic and upfield regions which permit empirical assignment of the geometric isomers (see Experimental Section). The alkyloximes were prepared from the *cis*-oxime esters by deprotonation with NaH at 0 °C, addition of the appropriate alkyl bromide at 0 °C, and warming to ambient temperature. After hydrolysis, the alkyloxime acids were isolated as crystalline products which were easily purified by recrystallization or silica gel chromatography.

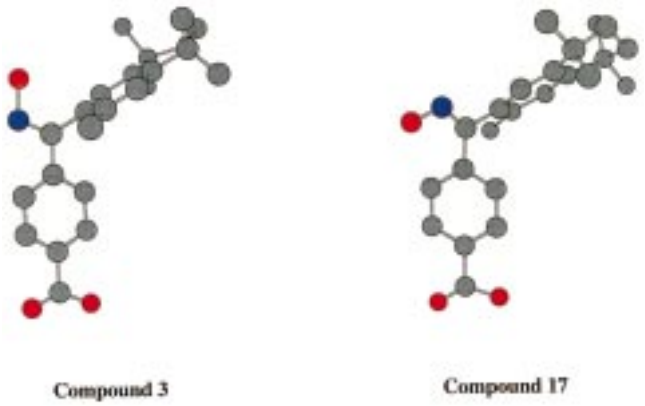


Figure 1. X-ray crystal structures of the *cis*-oxime **3** and the *trans*-oxime **17**. The benzoic acid ring is fixed in the X−Y plane. Hydrogen atoms have been omitted for clarity.

Biological Methods

The synthetic ligands were characterized for their ability to bind directly to the retinoid receptors employing competitive binding assays using recombinantly expressed RARα, RARβ, RARγ and RXRα, RXRβ, RXRγ in a baculovirus system as described previously.[9k,11,12] Binding data are shown in Tables 1 and 2. To examine transcriptional activity, cotransfection assays were performed in CV-1 cells transfected with an expression vector for each of the RAR and RXR subtypes, or RXRα was cotransfected into CV-1 cells along with an expression vector for hPPARγ. Cotransfection assay data are shown in Tables 1 and 2 and Figure 2. The ability of the rexinoids to induce adipogenesis was evaluated by monitoring triglyceride accumulation in 3T3-L1 cells. The dose−response curves for the triglyceride accumulation assay are shown in Figure 3.

Scheme 1. Synthesis of Oximes, Methyloximes, and Alkyloximes

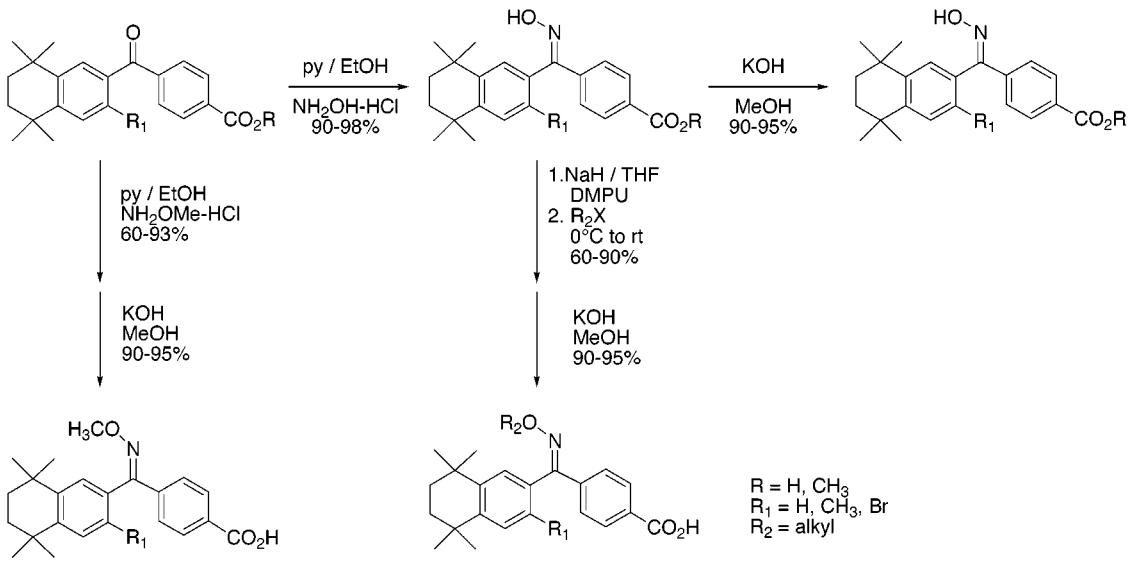
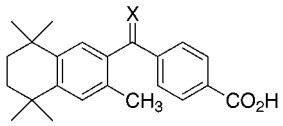


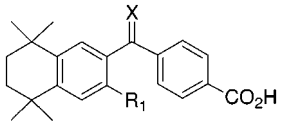
Table 1. Retinoid Binding and Cotransfection Data



		binding affinity[a] K_i (nM)			cotransfection activity[b] EC_{50} (nM)		
compd	X	RARα	RARβ	RARγ	RARα	RARβ	RARγ
1	O	>10 000	>10 000	>10 000	(4%)[c]	2068 (20%)	1604 (21%)
2	CH_2	5 453	5 353	3 206	(6%)	282 (22%)	648 (25%)
3	N-OH[d]	>10 000	>10 000	>10 000	(6%)	(10%)	(13%)
4	N-OMe[d]	>10 000	>10 000	>10 000	(2%)	(5%)	(2%)
compd	X	RXRα	RXRβ	RXRγ	RXRα	RXRβ	RXRγ
1	O	138	191	299	279 (80%)	213 (134%)	246 (74%)
2	CH_2	36	21	29	28 (83%)	25 (109%)	20 (85%)
3	N-OH[d]	6	5	5	7 (66%)	14 (81%)	7 (76%)
4	N-OMe[d]	9	8	8	5 (71%)	10 (90%)	4 (93%)

[a] Binding affinities (K_i values) were determined for the test retinoids by competition of 5 nM [^{3}H]ATRA (for RARs) and [^{3}H]Targretin (LGD1069, for RXRs). [b] The activity is reported as EC_{50} value (nM) which is the concentration of test compound required to elicit a response at half-maximal height on the dose−response curve. [c] Efficacy is calculated as a percent of maximal induction normalized to ATRA. EC_{50} is not calculated where the relative efficacy is less than 15%. [d] *cis*-Oxime isomer.

Table 2. Competitive Binding and Cotransfection Data for Oximes



			binding affinity[a] K_i (nM)			cotransfection activity EC_{50} (nM)[b]		
compd	R_1	X	RXRα	RXRβ	RXRγ	RXRα	RXRβ	RXRγ
3	Me	N-OH[c]	6	5	5	7	14	7
4	Me	N-OMe	9	8	8	5	10	4
5	Br	N-OH	12	6	57	27	32	26
6	H	N-OH	4234	3363	5051	2955	$>10^4$	2894
7	H	N-OMe	>1000	363	558	253	207	206
8	Me	N-OEt	26	58	42	5	12	9
9	Me	N-O*n*-Pr	6	61	57	32	37	28
10	Me	N-O*n*-Bu	177	283	224	86	157	99
11	Me	N-O*t*-Bu	484	623	445	$>10^4$	$>10^4$	$>10^4$
12	Me	N-Oallyl	7	15	80	6	18	7
13	Me	N-$OCH_2CH(CH_2)_2$	21	91	323	116	106	124
14	Me	N-OCH_2CN	11	14	14	12	25	11
15	Me	N-$O(CH_2)_2NH_2$	>1000	>1000	>1000	350	61	306
16	Me	N-OCH_2CO_2H	>1000	>1000	>1000	713	1384	1379
17	Me	*trans*-N-OH	60	120	209	46	165	34
18	Me	*trans*-N-OMe	394	>1000	238	384	377	254
19	Me	*trans*-N-OEt	620	>1000	467	$>10^4$	$>10^4$	$>10^4$
20	Me	*trans*-N-O*t*-Bu	>1000	>1000	>1000	$>10^4$	$>10^4$	$>10^4$

[a] K_i values for the oximes on RARα, RARβ, and RARγ are >1000 nM. [b] EC_{50} values for the oximes on RARα, RARβ, and RARγ are >10 000 nM with efficacies of less than 25%. All EC_{50} values are determined from full dose−response curves ranging from 10^{-12} to 10^{-5} M in CV-1 cells. Retinoid activity is normalized relative to that of ATRA. [c] *cis*-Oxime isomer unless indicated otherwise.

Results and Discussion

The newly reported class of oxime rexinoids displays virtually complete RXR specificity in the competitive binding assay and the RXR:RXR homodimer cotransfection assay. The binding affinity reveals complete RXR specificity and the oximes have 10−100 times the binding affinity of the parent keto retinoid (see Table 1; **1** (X = O), K_i = 138−299 nM; **3** (X = N−OH), K_i = 5−6 nM). While the keto and olefinic linked systems possess some residual RAR activity, the oximes display complete RXR specificity (see Table 1). For both the hydroxyloxime **3** and the methoxyloxime **4**, the RAR K_i values are greater than 10 000 nM and the RXR K_i values range from 5 to 9 nM. The receptor binding specificity translates into transcriptional activity as well. The oximes are transcriptionally inactive on each of the RARs but display very potent, specific activation of the RXRs. The oximes are, in some cases, almost 2 orders of magnitude more potent than the parent ketone (**1**, 3-methyl-TTNCB, EC_{50} = 213−279 nM; **3**, oxime, EC_{50} = 7−14 nM; see Table 1) in activation of the RXRs. The oximes are almost 5 times more potent than the corresponding olefinic linked retinoid (**2**, Targretin, EC_{50} = 20−28 nM) which has been previously reported by our group.[9c] Among the most potent rexinoids of this oxime series are compounds **3** and **4**, each displaying 5−9 nM binding affinity for the RXRs and 4−14 nM potency in the cotransfection assays on the RXRs. The full retinoid activity profile for compounds **3** and **4** compared to the parent keto compound and the olefinic linked system is shown in Table 1. Data for the complete oxime series is shown in Table 2; all of the oximes reported here are RXR-specific ligands; thus the RAR data is omitted for clarity.

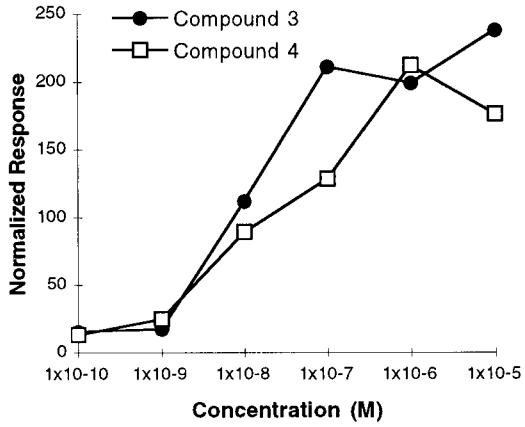


Figure 2. Activation of RXR:hPPARγ heterodimer by compounds **3** and **4**. hPPARγ was transfected with hRXRα in CV-1 cells for 5 h, followed by exposure to compound **3** or **4** at the indicated concentrations for 40 h prior to lysis and determination of luciferase and β-gal activity.

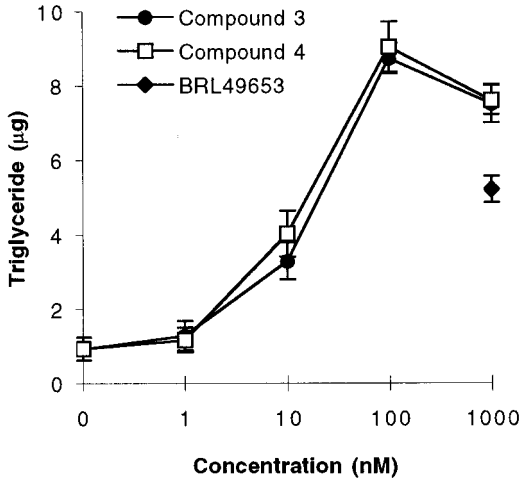


Figure 3. Triglyceride accumulation induced by compounds **3** and **4**. 3T3-L1 pre-adipocytes were differentiated to adipocytes with 7 days of rexinoid treatment. The extent of the differentiation is quantitated by measuring the accumulation of triglyceride produced within the cytoplasm of the adipocyte. The dose response for the rexinoids (**3** and **4**) is compared to a 1 μM concentration of the PPAR agonist, BRL49653. Numbers represent the mean triglyceride accumulation from triplicate wells. Error bars represent standard deviations.

Structural features which affect the RXR activation profile include substitution at the C-3 site of the tetramethyltetrahydronaphthalene ring system. Substitution at C-3 is essential for the oxime analogues to retain RXR activity; C-3 methyl and C-3 bromo (**5**) are both acceptable substitutions. The oximes which lack a C-3 substituent on the tetrahydronaphthalene system (**6**, **7**) are much less active (K_i >300 nM) than the C-3 methyl and C-3 bromo analogues. In the pentamethyltetrahydronaphthlene series, activity is retained in some of the *O*-alkylated oximes, particularly in those analogues with short alkyl substituents (**8**, **9**, **12**, **14**; K_i = 6−80 nM), while oximes bearing larger *O*-alkyl groups have slightly diminished RXR activity (**10**, **11**, **13**; K_i = 21−323 nM). Additional functionality (aminoethyl and carboxymethyl) on the oxime destroys retinoid activity (**15**, **16**; K_i > 1000 nM). Finally, the minor geometric isomers, the *trans*-oximes (**17**−**20**), display very weak or negligible retinoid activity. In all cases, the cotransfection activity parallels the binding affinity profile.

We also examined the ability of the two most potent oxime rexinoids, **3** and **4**, to activate the RXR:PPARγ heterodimer in a cotransfection assay. The RXR:PPARγ heterodimer is a dual-ligand responsive dimer and can be activated by either a PPAR agonist (such as a thiazolidinedione, TZD) or an RXR ligand, or both.[13] The ability of TZDs to activate the RXR:PPARγ heterodimer correlates with their ability to reduce hyperglycemia in animal models of NIDDM and obesity,[14] and previous reports by our group demonstrate that activation of the RXR:PPARγ heterodimer with rexinoids translates into favorable in vivo activity in diabetic and obese animal models.[10] In the cotransfection assay, compounds **3** and **4** produce a concentration-dependent increase in transactivation of the RXR:PPARγ heterodimer, with EC_{50} values of 14 and 39 nM, respectively (Figure 2).[15] Further, we examined a cell-based model of RXR:PPARγ activation, differentiation of 3T3-L1 cells to adipocytes.[16] The 3T3-L1 assay of adipocyte metabolism displays insulin-induced differentiation, and for instance, PPARγ ligands such as thiazolidinediones (TZDs) act as insulin-sensitizing agents and are able to enhance the rate and percent of differentiation of fibroblasts to adipocytes in this assay.[14,17] Since RXR ligands can activate the RXR:PPARγ heterodimer, we examined whether the transcriptional activity of oxime rexinoids would translate into differentiation activity in the 3T3-L1 cell-based assay. In fact, the oximes **3** and **4** are capable of producing a concentration-dependent increase in triglyceride accumulation, with efficacies similar to that of the TZD, BRL49653 (Figure 3). These results imply that the oxime rexinoids, alone, are activating the RXR:PPARγ heterodimer in a cell-based assay and supply further evidence that the new rexinoids could be effective agents for the treatment of metabolic disorders.

Conclusion

The newly reported series of RXR oxime agonists reported here display specific, potent activation and specific binding for all three RXR subtypes. The analogues were designed based on earlier studies of related keto retinoids, and the present series represents a second generation of novel, more potent, and specific RXR ligands, or rexinoids. The binding affinity and cotransfection profile define key structural limitations for RXR activity. First, 3-substituted tetramethyltetrahydronaphthalene derivatives display 10−100 times greater activity (both binding affinity and transactivation) on the RXRs than the C-3 unsubstituted tetrahydronaphthalene analogues. Second, more highly *O*-functionalized oxime derivatives display diminished agonist activity. Finally, the geometry of the oxime influences the RXR activity. The major *cis*-oxime is a potent, specific activator of RXR homodimers, while the minor isomer, the *trans*-oxime, has a much weaker RXR activation profile. The most potent rexinoids of this series are the oxime of 3-methyl-TTNCB (**3**) and the methyloxime of 3-methyl-TTNCB (**4**). These oxime rexinoids are also able to activate the RXR:PPARγ heterodimer in cotransfection experiments. Further, the transcriptional control translates into cell-based activ-

ity, and the oximes **3** and **4** are potent inducers of adipogenesis in a 3T3-L1 differentiation assay. The RXR binding affinity, transcriptional response, and 3T3-L1 cell-based activity demonstrate that the oxime rexinoids activate the RXR:PPARγ heterodimer and, based on previous reports by our group,[10] implicate these new rexinoids as potential treatment for metabolic disorders such as diabetes and obesity.

Experimental Section

General Experimental. Proton and carbon nuclear magnetic resonance spectra (^{1}H and ^{13}C NMR) were obtained on a Bruker ACP 400 spectrometer using the solvent indicated. Infrared spectra (IR) were recorded on a Mattson Galaxy Series model 3020 FT-IR spectrophotometer. High-resolution mass spectra (HRMS) and fast atom bombardment mass spectra (FAB) were recorded on a Finnigan 4000 spectrometer at the University of Colorado Health Science Center. Melting points were taken on a Laboratory Devices or Electrothermal melting point apparatus and are reported uncorrected. Combustion analyses were performed by Galbraith Laboratories, Inc. (Knoxville, TN) or by Quantitative Technologies, Inc. (Whitehouse, NJ). Single-crystal X-ray analysis was performed at the University of California, Irvine. As required, starting materials were azeotropically dried prior to reaction and reactions were conducted with standard precautions taken to exclude moisture. The diaryl ketones, 4-[(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoic acid (TTNCB) and 4-[(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)-carbonyl]benzoic acid (3-Me-TTNCB), were prepared as described.[9c]

The oximes, methyloximes, and alkyloximes were prepared by the following general methods:

Method A. Synthesis of Oximes from Keto Acids. A solution of the diaryl ketone[9c] (TTNCB or 3-methyl-TTNCB) in EtOH (1.0−0.5 M) was treated with hydroxylamine hydrochloride (2 equiv) and pyridine (5 equiv), and the mixture was heated at reflux for 6 h. The mixture was cooled to room temperature, and the ethanol was removed in vacuo. The residue was taken up in water, and the aqueous layer was adjusted to pH = 4−5 with 1 M HCl and extracted with EtOAc (3×). The organic layers were combined and washed with water (2×) and brine. The organic solution was dried (Na_2SO_4), filtered, and concentrated to give a solid. The product was recrystallized (CH_2Cl_2/hexanes, Et_2O/hexanes, or $CHCl_3$/hexanes) to give the pure *cis*-oxime acid.

Method B. Synthesis of Oximes from Keto Esters. A solution of the diaryl ketone methyl ester (TTNCB methyl ester, 3-methyl-TTNCB methyl ester, or 3-bromo-TTNCB methyl ester) in EtOH (1.0−0.5 M) was treated with hydroxylamine hydrochloride (1.5−2 equiv) and pyridine (2.1 equiv), and the mixture was heated at reflux for 6 h. The mixture was cooled to room temperature, and the ethanol was removed in vacuo. The residue was taken up in water, and the aqueous layer was extracted with EtOAc (3×); the combined organic extract was washed with water (2×) and brine. The organic solution was dried (Na_2SO_4), filtered, and concentrated. The crude product was hydrolyzed in excess KOH/MeOH at ambient temperature for 24 h. The methanol was removed in vacuo. The residue was taken up in water, and the aqueous layer was adjusted to pH = 4−5 with 1 M HCl and extracted with EtOAc (3×). The organic layers were combined and washed with water (2×) and brine. The organic solution was dried (Na_2SO_4), filtered, and concentrated to give a solid. The product was recrystallized (CH_2Cl_2/hexanes, Et_2O/hexanes, or $CHCl_3$/hexanes) to give the pure *cis*-oxime acid.

Method C. Synthesis of Methyloximes from Keto Acids. A solution of the diaryl ketone (TTNCB or 3-methyl-TTNCB) in EtOH (1.0−0.5 M) was treated with methoxylamine hydrochloride (2 equiv) and pyridine (5 equiv), and the mixture was heated at reflux for 6 h. The mixture was cooled to room temperature, and the ethanol was removed in vacuo. The residue was taken up in water, and the aqueous layer was adjusted to pH = 4−5 with 1 M HCl and extracted with EtOAc (3×). The combined organic extract was washed with water (2×) and brine. The organic solution was dried (Na_2SO_4), filtered, and concentrated to give a solid. The product was recrystallized (CH_2Cl_2/hexanes, Et_2O/hexanes, or $CHCl_3$/hexanes) to give the pure *cis*-*O*-methyloxime acid.

Method D. Synthesis of Methyloximes from Keto Esters. A solution of diaryl ketone methyl ester (TTNCB methyl ester or 3-methyl-TTNCB methyl ester) in EtOH (1.0−0.5 M) was treated with methoxylamine hydrochloride (1.5−2 equiv) and pyridine (2.1 equiv), and the mixture was heated at reflux for 6 h. The mixture was cooled to room temperature, and the ethanol was removed in vacuo. The residue was taken up in water, and the aqueous layer was extracted with EtOAc (3×); the organic layers were combined and washed with water (2×) and brine. The organic solution was dried (Na_2SO_4), filtered, and concentrated. The crude product was hydrolyzed in excess KOH/MeOH at ambient temperature for 24 h. The methanol was removed in vacuo. The residue was taken up in water, and the aqueous layer was adjusted to pH = 4−5 with 1 M HCl and extracted with EtOAc (3×). The organic layers were combined and washed with water (2×) and brine. The organic solution was dried (Na_2SO_4), filtered, and concentrated to give a solid. The product was recrystallized (CH_2Cl_2/hexanes, Et_2O/hexanes, or $CHCl_3$/hexanes) to give the pure *cis*-*O*-methyloxime acid.

Method E. Synthesis of Alkyloximes from Oximes. A solution of the *cis*-oxime of the diaryl ketone methyl ester (0.3−5 mmol) in THF (1 M) and DMPU (1 M) was added at 0 °C to a suspension of NaH (3 equiv) in THF (1 M). The suspension was allowed to warm to room temperature with stirring over 30 min and then cooled again to 0 °C. A solution of the appropriate alkyl bromide (3 equiv) in THF (1 M) was added. The solution was allowed to warm to room temperature and stirred for 15 h. Aqueous, saturated NH_4Cl (5.0 mL) was added, and the aqueous layer was adjusted to pH = 5−6 with 1 M HCl and extracted with EtOAc (3×). The organic layers were combined and washed with water (2×) and brine. The organic solution was dried ($MgSO_4$), filtered, and concentrated to give a white solid. The crude product was hydrolyzed in excess KOH/MeOH at ambient temperature for 24 h. The methanol was removed in vacuo. The residue was taken up in water, and the aqueous layer was adjusted to pH = 4−5 with 1 M HCl. The aqueous solution was extracted with EtOAc (3×). The organic layers were combined and washed with water (2×) and brine. The organic solution was dried (Na_2SO_4), filtered, and concentrated to give a solid. The product was recrystallized (CH_2Cl_2/hexanes or Et_2O/hexanes) to give the pure *cis*-*O*-alkyloxime acid.

***cis*-4-[(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoic Acid Oxime (3).** Prepared as described in method A; 3-methyl-TTNCB served as the starting material. Recrystallization ($CHCl_3$/hexanes) gave 4.05 g (88%) of a white solid: mp 214−218 °C dec; ^{1}H NMR ($CDCl_3$/MeOH-d_4) δ 7.99 and 7.53 (d of ABq, J = 8.4 Hz, 4H, ArH), 7.20 (s, 1H, ArH), 6.99 (s, 1H, ArH), 2.11 (s, 3H, $ArCH_3$), 1.69 (s, 4H, 2 × CH_2), 1.32 (s, 6H, 2 × CH_3), 1.22 (s, 6H, 2 × CH_3); HRMS (CI, isobutane) calcd for $C_{23}H_{28}NO_3$ (MH^+) 366.2069, found 366.2060 (MH^+). Anal. ($C_{23}H_{27}NO_3$) C, H, N.

***cis*-4-[(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoic Acid *O*-Methyloxime (4).** Prepared as described in method C; 3-methyl-TTNPB served as the starting material. Recrystallization (CH_2Cl_2/hexanes) gave a white solid, 564 mg (93%): mp 228−228.5 °C; ^{1}H NMR ($CDCl_3$) δ 8.04 and 7.57 (d of ABq, J = 8.4 Hz, 4H, ArH), 7.18 (s, 1H, ArH), 6.95 (s, 1H, ArH), 4.01 (s, 3H, OCH_3), 2.08 (s, 3H, CH_3), 1.69 (s, 4H, 2 × CH_2), 1.31 (s, 6H, 2 × CH_3), 1.22 (s, 6H, 2 × CH_3); ^{13}C NMR (100.8 MHz, $CDCl_3$) δ 171.3, 156.0, 145.4, 142.3, 141.4, 132.6, 130.1, 129.4, 127.9, 127.1, 126.3, 62.7, 35.1, 35.0, 34.1, 33.9, 31.8, 19.4; LRMS (CI, isobutane) *m*/*e* 380 (MH^+). Anal. ($C_{24}H_{29}NO_3$) C, H, N.

***cis*-4-[(3-Bromo-5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl)carbonyl]benzoic Acid Oxime (5).** Prepared as described in method B; methyl 4-[(3-bromo-5,6,7,8-tetrahydro-

5,5,8,8-tetramethyl-2-naphthyl)carbonyl]benzoate served as the starting material. Recrystallization (CH_2Cl_2/ether/hexanes) gave a white solid, 330 mg (83%): mp 240−244 °C dec; ^{1}H NMR ($CDCl_3$) δ 8.20 and 7.92 (d of ABq, 4H, J = 8.3 Hz, ArH), 7.54 (s, 1H, ArH), 7.30 (s, 1H, ArH), 1.72 (s, 4H, 2 × CH_2), 1.33 (s, 6H, 2 × CH_3), 1.26 (s, 6H, 2 × CH_3); HRMS (EI) calcd for $C_{22}H_{24}BrNO_3$ (M^+) 429.0939, found 429.0918.

***cis*-4-[(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthyl)carbonyl]benzoic Acid Oxime (6).** Prepared as described in method A; TTNCB served as the starting material. Recrystallization (CH_2Cl_2/hexanes) gave a white solid, 111 mg (72%): ^{1}H NMR ($CDCl_3$) δ 8.06 and 7.65 (d of ABq, J = 8.2 Hz, 4H, ArH), 7.59 (d, J = 1.7 Hz, 1H, ArH), 7.40 (dd, J = 1.7, 8.0 Hz, 1H, ArH), 7.25 (d, J = 8.0 Hz, 1H, ArH), 1.71 (s, 4H, 2 × CH_2), 1.27 (s, 6H, 2 × CH_3), 1.21 (s, 6H, 2 × CH_3).

***cis*-4-[(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthyl)carbonyl]benzoic Acid *O*-Methyloxime (7).** Prepared as described in method C; TTNCB served as the starting material. Recrystallization (CH_2Cl_2/hexanes) gave a pale yellow solid, 85 mg (66%): mp 183−186 °C; IR (neat) 2962 s, 2930 s, 1866 s, 1694 s, 1460 w, 1422 m, 1283 m, 1053 m cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.16 and 7.45 (d of ABq, J = 8.2 Hz, 4H, ArH), 7.39 (app s, 1H, ArH), 7.25 (m, 1H, ArH), 7.16 (d, J = 8.1 Hz, 1H, ArH), 3.97 (s, 3H, OCH_3), 1.67 (s, 4H, 2 × CH_2), 1.27 (s, 6H, 2 × CH_3), 1.21 (s, 6H, 2 × CH_3); MS (FAB) *m/e* 366 (MH^+); HRMS (FAB, MH^+) calcd for $C_{23}H_{28}NO_3$ 366.2069, found 366.2077.

***cis*-4-[(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoic Acid *O*-Ethyloxime (8).** Prepared as described in method C, except that ethoxylamine hydrochloride was condensed with 3-methyl-TTNCB. Purification by radial chromatography (1:1 = EtOAc:hexanes) afforded a white solid, 147 mg (67%): mp 202−205 °C; IR (neat) 2961 s, 2924 s, 2361 m, 1694 s, 1420 m, 1285 m, 1051 m, 1024 m cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.98 and 7.50 (d of ABq, J = 8.3 Hz, 4H, ArH), 7.14 (s, 1H, ArH), 6.94 (s, 1H, ArH), 4.25 (q, J = 7.0 Hz, 2H, OCH_2), 2.03 (s, 3H, $ArCH_3$), 1.67 (s, 4H, 2 × CH_2), 1.31 (s, 6H, 2 × CH_3), 1.28 (t, J = 7.0 Hz, 3H, CH_3), 1.20 (s, 6H, 2 × CH_3); ^{13}C NMR (100.8 MHz, $CDCl_3$) δ 172.1, 156.0, 145.4, 142.4, 142.0, 132.8, 130.5, 130.4, 129.6, 128.1, 127.2, 126.8, 70.6, 35.4, 35.3, 34.4, 34.2, 32.1, 19.7, 15.2; MS (FAB) *m/e* 394 (MH^+). Anal. ($C_{25}H_{31}NO_3$) C, H, N.

***cis*-4-[(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoic Acid *O*-Propyloxime (9).** Prepared as described in method E; 3-methyl-TTNCB oxime methyl ester served as the starting material, and *n*-propyl bromide served as the alkyl bromide. Recrystallization (ether/hexanes) gave a white solid, 99 mg (73%): mp 178−180 °C; IR (neat) 3021 s, 2963 s, 2928 s, 2876 m, 1692 s, 1458 w, 1420 m, 1265 m, 969 w cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 and 7.57 (d of ABq, J = 8.5 Hz, 4H, ArH), 7.16 (s, 1H, ArH), 6.97 (s, 1H, ArH), 4.16 (t, J = 6.7 Hz, 2H, OCH_2), 2.05 (s, 3H, $ArCH_3$), 1.73 (m, 2H, CH_2), 1.69 (s, 4H, 2 × CH_2), 1.35 (s, 6H, 2 × CH_3), 1.26 (s, 6H, 2 × CH_3), 0.91 (t, J = 7.4 Hz, 3H, CH_3); ^{13}C NMR (100.8 MHz, $CDCl_3$) δ 172.2, 155.7, 145.1, 142.2, 141.7, 132.5, 130.3, 130.1, 127.8, 126.9, 126.4, 76.5, 35.2, 35.1, 34.1, 33.9, 31.9, 22.7, 19.4, 10.3; MS (FAB) *m/e* 408 (MH^+); HRMS (FAB, MH^+) calcd for $C_{26}H_{34}NO_3$ 408.2539, found 408.2525. Anal. ($C_{26}H_{33}NO_3$) C, H, N.

***cis*-4-[(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoic Acid *O*-Butyloxime (10).** Prepared as described in method E; 3-methyl-TTNCB oxime methyl ester served as the starting material and *n*-butyl bromide served as the alkyl bromide. Recrystallization (ether/hexanes) gave a white solid, 82 mg (58%): mp 195−198 °C; IR (neat) 2961 s, 2932 s, 2872 m, 1694 s, 1420 m, 1265 m, 1024 w, 735 w cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 and 7.57 (d of ABq, J = 8.4 Hz, 4H, ArH), 7.15 (s, 1H, ArH), 6.96 (s, 1H, ArH), 4.20 (t, J = 6.7 Hz, 2H, OCH_2), 2.05 (s, 3H, $ArCH_3$), 1.72 (m, 2H, CH_2), 1.69 (s, 4H, 2 × CH_2), 1.34 (m, 2H, CH_2), 1.31 (s, 6H, 2 × CH_3), 1.22 (s, 6H, 2 × CH_3), 0.90 (t, J = 7.4 Hz, 3H, CH_3); ^{13}C NMR (100.8 MHz, $CDCl_3$) δ 171.8, 155.7, 145.1, 142.1, 141.7, 132.5, 130.3, 130.1, 129.3, 127.8, 126.9, 126.4, 74.7, 35.2, 35.1, 34.1, 33.9, 31.9, 31.5, 19.4, 19.1, 13.9; MS (FAB) *m/e* 422 (MH^+); HRMS (FAB, MH^+) calcd for $C_{27}H_{36}NO_3$ 422.2695, found 422.2678. Anal. ($C_{27}H_{35}NO_3$) C, H, N.

***cis*-4-[(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoic Acid *O*-*tert*-Butyloxime (11).** Prepared as described in method C except that *tert*-butoxylamine hydrochloride was condensed with 3-methyl-TTNPB. Recrystallization (benzene) gave a white solid, 112 mg (40%): ^{1}H NMR ($CDCl_3$) δ 8.01 and 7.59 (d of ABq, J = 8.4 Hz, 4H, ArH), 7.12 (s, 1H, ArH), 6.96 (s, 1H, ArH), 2.01 (s, 3H, CH_3), 1.69 (s, 4H, 2 × CH_2), 1.33 (s, 9H, 3 × CH_3), 1.31 (s, 6H, 2 × CH_3), 1.22 (s, 6H, 2 × CH_3); ^{13}C NMR (100.8 MHz, $CDCl_3$) δ 170.5, 154.1, 144.8, 142.6, 141.7, 132.7, 130.4, 130.0, 127.6, 127.0, 126.8, 79.6, 35.2, 35.1, 34.1, 33.9, 31.9, 29.7, 26.0, 19.7; HRMS (EI) calcd for $C_{27}H_{35}NO_3$ (M^+) 421.2616, found 421.2602. Anal. ($C_{27}H_{35}NO_3$) C, H, N.

***cis*-4-[(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoic Acid *O*-Allyloxime (12).** Prepared as described in method E; 3-methyl-TTNCB oxime methyl ester served as the starting material, and allyl bromide served as the alkyl bromide. Recrystallization (ether/hexanes) gave a white solid, 87 mg (78%): mp 177−179 °C; IR (neat) 2961 s, 2924 s, 2662 w, 1694 s, 1420 m, 1016 m cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 and 7.57 (d of ABq, J = 8.2 Hz, 4H, ArH), 7.16 (s, 1H, ArH), 6.97 (s, 1H, ArH), 6.02 (m, 1H, =CH), 5.27 (dd, J = 17.2, 1.5 Hz, 1H, $=CH_{cis}$), 5.19 (dd, J = 10.4, 1.5 Hz, 1H, $=CH_{trans}$), 4.71 (d, J = 5.6 Hz, 2H, OCH_2), 2.06 (s, 3H, $ArCH_3$), 1.69 (s, 4H, 2 × CH_2), 1.31 (s, 6H, 2 × CH_3), 1.21 (s, 6H, 2 × CH_3); ^{13}C NMR (100.8 MHz, $CDCl_3$) δ 172.3, 156.2, 145.2, 142.1, 141.4, 134.4, 132.4, 130.0, 129.2, 127.7, 126.9, 126.2, 117.3, 75.5, 35.0, 34.9, 34.0, 33.8, 31.7, 19.3; MS (FAB) *m/e* 406 (MH^+); HRMS (FAB, MH^+) calcd for $C_{26}H_{32}NO_3$ 406.2382, found 406.2341.

***cis*-4-[(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoic Acid *O*-Methylcyclopropyloxime (13).** Prepared as described in method E; 3-methyl-TTNCB oxime methyl ester served as the starting material, and bromomethylcyclopropane served as the alkyl bromide. Recrystallization (EtOAc/hexanes) gave a white solid, 84 mg (70%): mp 178−182 °C; IR (neat) 2961 s, 2924 s, 2861 s, 1692 s, 1422 m, 1263 m, 1024 m, 1005 s, 968 w, 941 w, 866 w, 765 w cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 and 7.57 (d of ABq, J = 8.4 Hz, 4H, ArH), 7.17 (s, 1H, ArH), 6.99 (s, 1H, ArH), 4.03 (d, J = 7.0 Hz, 2H, OCH_2), 2.08 (s, 3H, $ArCH_3$), 1.69 (s, 4H, 2 × CH_2), 1.32 (s, 6H, 2 × CH_3), 1.28 (m, 1H, CH), 1.23 (s, 6H, 2 × CH_3), 0.54 (dd, J = 12.7, 4.6, 2H, 2 × CH), 0.31 (dd, J = 10.4, 4.6, 2H, 2 × CH); ^{13}C NMR (100.8 MHz, $CDCl_3$) δ 171.2, 155.6, 145.2, 142.1, 141.8, 132.7, 130.2, 130.7, 127.8, 127.0, 126.5, 79.4, 35.2, 35.1, 34.1, 31.9, 19.4, 10.7, 3.1; MS (FAB) *m/e* 420 (MH^+); HRMS (FAB, MH^+) calcd for $C_{27}H_{34}NO_3$ 420.2539, found 420.2514. Anal. ($C_{27}H_{33}NO_3$) C, H, N.

***cis*-4-[(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoic Acid *O*-Cyanomethyloxime (14).** Prepared as described in method E; 3-methyl-TTNCB oxime methyl ester served as the starting material, and cyanobromomethane served as the alkyl bromide. Purification by radial chromatography (1:1 = Et_2O:hexanes) gave a white solid, 96 mg (76%): mp 210−212 °C; IR (neat) 2961 s, 2926 s, 2863 m, 1696 s, 1420 m, 1285 m, 1269 m, 1067 m, 1020 m cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 and 7.61 (d of ABq, J = 8.0 Hz, 4H, ArH), 7.19 (s, 1H, ArH), 6.97 (s, 1H, ArH), 4.81 (s, 2H, OCH_2, 2.08 (s, 3H, $ArCH_3$), 1.70 (s, 4H, 2 × CH_2), 1.32 (s, 6H, 2 × CH_3), 1.23 (s, 6H, 2 × CH_3); ^{13}C NMR (100.8 MHz, $CDCl_3$) δ 172.1, 160.0, 146.0, 142.6, 139.8, 132.3, 130.3, 129.0, 128.3, 128.0, 127.7, 126.0, 116.1, 59.3, 35.0, 35.0, 34.2, 34.0, 31.8, 19.3; MS (FAB) *m/e* 405 (MH^+). Anal. ($C_{25}H_{28}N_2O_3$) C, H, N.

***cis*-4-[(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoic Acid *O*-(1-Aminoethyl)oxime (15).** Prepared as described in method E; 3-methyl-TTNCB oxime methyl ester served as the starting material, and 2-amino-1-bromoethane served as the alkyl bromide. Purification by radial chromatography (1:1 = Et_2O:hexanes) gave a white solid, 84 mg (60%): mp 172−176 °C dec; IR (neat) 3420 broad, 2922 s, 2855 s, 1696 s, 1591 m, 1456 w, 1364 m, 1231 m, 1016

s cm^{-1}; ^1H NMR (400 MHz, $CDCl_3/CD_3OD$) δ 7.75 and 7.51 (d of ABq, $J = 8.5$ Hz, 4H, ArH), 7.20 (s, 1H, ArH), 6.97 (s, 1H, ArH), 3.73 (t, $J = 5.3$ Hz, 2H, OCH_2), 3.54 (t, $J = 5.3$ Hz, 2H, NCH_2), 2.10 (s, 3H, $ArCH_3$), 1.69 (s, 4H, 2 × CH_2), 1.32 (s, 6H, 2 × CH_3), 1.21 (s, 6H, 2 × CH_3); ^{13}C NMR (100.8 MHz, $CDCl_3/CD_3OD$) δ 168.4, 156.9, 145.2, 142.2, 139.5, 134.1, 132.6, 130.0, 127.8, 126.9, 126.0, 61.0, 42.4, 34.9, 33.9, 33.7, 31.6, 19.1; MS (FAB) *m/e* 409 (MH^+); HRMS (FAB, MH^+) calcd for $C_{25}H_{33}N_2O_3$ 409.2489, found 409.2493.

***cis*-4-[(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoic Acid *O*-Methylcarboxyoxime (16).** Prepared as described in method E; 3-methyl-TTNCB oxime methyl ester served as the starting material, and methyl α-bromoethyl acetate served as the alkyl bromide. Recrystallization (EtOAc/hexanes) gave a white solid, 40 mg (40%): mp 269−273 °C dec; IR (neat) 2961 m, 2924 m, 2896 m, 1694 s, 1422 m, 1281 m, 1098 m, 1032 m cm^{-1}; ^1H NMR (400 MHz, $CDCl_3/CD_3OD$) δ 7.98 and 7.53 (d of ABq, $J = 8.4$ Hz, 4H, ArH), 7.18 (s, 1H, ArH), 7.09 (s, 1H, ArH), 4.71 (s, 2H, OCH_2), 2.11 (s, 3H, $ArCH_3$), 1.70 (s, 4H, 2 × CH_2), 1.32 (s, 6H, 2 × CH_3), 1.23 (s, 6H, 2 × CH_3); ^{13}C NMR (100.8 MHz, $CDCl_3/CD_3OD$) δ 172.1, 168.3, 157.6, 145.3, 142.0, 139.8, 132.4, 130.8, 129.7, 129.6, 127.7, 126.9, 126.2, 70.8, 34.9, 34.8, 33.9, 33.7, 31.5, 19.0; MS (FAB) *m/e* 424 (MH^+). Anal. ($C_{25}H_{29}NO_5$) C, H, N.

***trans*-4-[(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoic Acid Oxime (17).** Isolated by successive recrystallization of the mother liquor from preparation of compound **3**: mp 202.5−203.5 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 8.05 and 7.64 (d of ABq, $J = 8.4$ Hz, 4H, ArH), 7.20 (s, 1H, ArH), 7.08(s, 1H, ArH), 2.03 (s, 3H, $ArCH_3$), 1.68 (s, 4H, 2 × CH_2), 1.27 (s, 6H, 2 × CH_3), 1.25 (s, 6H, 2 × CH_3); HRMS (EI) calcd for $C_{23}H_{27}NO_3$ (M^+) 365.1991, found 365.1997. Anal. ($C_{23}H_{27}NO_3$) C, H, N.

***trans*-4-[(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoic Acid *O*-Methyloxime (18).** Isolated by HPLC purification of the mother liquor from preparation of compound **4**: ^1H NMR ($CDCl_3$) δ 8.10 and 7.59 (d of ABq, $J = 8.1$ Hz, 4H, ArH), 7.16 (s, 1H, ArH), 7.08 (s, 1H, ArH), 4.01 (s, 3H, OCH_3), 2.10 (s, 3H, CH_3), 1.68 (s, 4H, 2 × CH_2), 1.27 (s, 6H, 2 × CH_3), 1.23 (s, 6H, 2 × CH_3); ^{13}C NMR (100.8 MHz, $CDCl_3$) δ 170.6, 156.1, 145.9, 142.4, 138.9, 133.6, 132.9, 129.9, 129.8, 128.9, 128.3, 62.4, 35.0, 34.1, 33.9, 31.8, 31.7, 20.2; HRMS (EI) calcd for $C_{24}H_{29}NO_3$ (M^+) 379.2147, found 379.2147. Anal. ($C_{24}H_{29}NO_3$) C, H, N.

***trans*-4-[(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoic Acid *O*-Ethyloxime (19).** Isolated by HPLC purification of the mother liquor from preparation of compound **8**: ^1H NMR ($CDCl_3$) δ 8.03 and 7.55 (d of ABq, $J = 8.0$ Hz, 4H, ArH), 7.12 (s, 1H, ArH), 7.07 (s, 1H, ArH), 4.25 (q, $J = 7.1$ Hz, 2H, OCH_2), 2.07 (s, 3H, $ArCH_3$), 1.65 (s, 4H, 2 × CH_2), 1.30 (t, $J = 7.0$ Hz, 3H, CH_3), 1.25 (s, 6H, 2 × CH_3), 1.21 (s, 6H, 2 × CH_3); HRMS (EI) calcd for $C_{25}H_{31}NO_3$ (M^+) 393.2304, found 393.2315. Anal. ($C_{25}H_{31}NO_3$) C, H, N.

***trans*-4-[(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoic Acid *O*-*tert*-Butyloxime (20).** Isolated by HPLC purification of the mother liquor from preparation of compound **11**: IR (thin film) 3021 m, 2965 s, 2928 s, 2866 m, 1694 s, 1120 m, 1364 m, 1285 m, 1190 m, 964 s, 760 s cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.07 and 7.57 (d of ABq, $J =$ 8.4 Hz, 4H, ArH), 7.11 (s, 1H, ArH), 7.06 (s, 1H, ArH), 2.18 (s, 3H, CH_3), 1.66 (s, 4H, 2 × CH_2), 1.35 (s, 9H, 3 × CH_3), 1.28 (s, 6H, 2 × CH_3), 1.18 (s, 6H, 2 × CH_3); ^{13}C NMR (100.8 MHz, $CDCl_3$) δ 170.6, 154.0, 145.3, 142.1, 139.8, 133.9, 130.2, 129.5, 128.8, 128.3, 79.4, 35.1, 35.0, 34.1, 33.9, 31.8, 31.7, 29.9, 27.7, 20.8; HRMS (CI, isobutane) calcd for $C_{27}H_{36}NO_3$ (MH^+) 422.2695, found 422.2675. Anal. ($C_{27}H_{35}NO_3$) C, H, N.

Biology. Binding Studies. The binding affinities (K_i values) were determined for the test compounds by competition of 5 nM [^{3}H]-*all-trans*-RA (for RARs) or [^{3}H]Targretin ([^{3}H]-LGD1069, Ligand Pharmaceuticals, Inc.; for RXRs). K_i values were determined by application of the Cheng−Prussof method.[12a]

Cotransfection Assay. Cotransfection assays were performed in CV-1 cells transfected with an expression vector for each of the RAR and RXR subtypes and a luciferase reporter gene under the control of the appropriate RAR or RXR response elements (RARE and RXRE, respectively).[6a,11] A ΔMTV-TREp-Luc reporter construct was used for the RARs, a CRBPII-tk-Luc reporter was used for RXRα and RXRγ, and a CPRE-tk-Luc reporter was used for RXRβ. Similarly, for the heterodimer assay, RXRα was cotransfected into CV-1 cells along with an expression vector for hPPARγ; activation of the RXR:hPPARγ heterodimer was tested on the PPRE-tk-Luc reporter as described.[4f,10,13e] The luciferase reporter for the heterodimer assay contains three copies of the AOX PPRE driving luciferase expression. Compounds were tested in three separate experiments in log dilutions from 1×10^{-5} to 1×10^{-12} M with triplicate determinations at each concentration. Typically, CV-1 cells were transiently cotransfected with the receptor expression vector, the reporter plasmid, and a β-galactosidase (RSV-β-Gal) internal control (used to calculate normailized luciferase response). The cells were incubated in the presence of the test compound and assayed for luciferase and β-galactosidase activity as described previously.[6a] The luciferase data are presented as percent normalized response with the maximal response (100%) elicited by the control retinoid (*all-trans*-RA for the RAR and RXR assays and LG100268[12h] for the RXR:hPPARγ heterodimer assay). Standard errors for this assay system were, on average, ca. 15% of the mean value. The activity is reported as EC_{50} value (nM) which is the concentration of test compound required to elicit a response at half-maximal height on the dose−response curve.

Adipogenesis Assay. Triglyceride accumulation was quantified employing a modified method of Sigma Diagnostics procedure no. 339. The 3T3-L1 cells obtained from American Type Culture Collection (CCL-92.1) were plated at 3500 cells per well in a 96-well plate. Two days postconfluence 3T3-L1 cells were treated with the thiazolidinedione, BRL49653 (1 μM), **3** (1 nM−1 μM), or **4** (1 nM−1 μM) in Delbecco's modified Eagle medium supplemented with 10% fetal bovine serum and 10 μg/mL insulin. After 7 days of treatment the cells were rinsed with PBS and lysed with 0.1% NP-40 in PBS. To the lysate was added 100 μL/well of the Sigma Diagnostics triglyceride assay solution, and the plates were incubated for 1 h at 37 °C. The enzymatic color reaction was read at 540 nm. All treatments were done in triplicate and standard deviations calculated.

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Supporting Information Available: Crystal data, refinement parameters, and X-ray coordinates for compounds **3** and **17**. This material is available free of charge via the Internet at http://pubs.acs.org.

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