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Dihydro-[1*H*]-quinolin-2-ones as retinoid X receptor (RXR) agonists for potential treatment of dyslipidemia

Bharat Lagu,* Rimma Lebedev, Barbara Pio, Maria Yang and Patricia D. Pelton

Johnson and Johnson Pharmaceutical Research and Development, Cranbury, NJ 08512, USA

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Abstract—A number of RXR modulators with novel structural features were synthesized and screened in the functional assays. The synthesis and the structure–activity relationship within the series of compounds will be presented. Some in vivo data generated in the models for dyslipidemia and diabetes will also be presented.

In the accompanying communication we have disclosed the structure-activity relationship in the compounds shown by a general structure **A** (Fig. 1) based on the structure of a known RXR modulator **1**.[1,2] Some compounds from the series showed higher potency for induction of ABCA1 mRNA, a target gene of the RXR–LXR heterodimeric complex over induction of aP2 mRNA, a target gene of RXR–PPARγ heterodimers. Encouraged by the results, we decided to explore the SAR in some detail within this series. Attention was focused on the trans double bond between the carboxylic acid and the phenyl ring. Almost all the RXR agonists reported thus far in the literature contain the carboxylic acid moiety conjugated to a double bond or to an aryl ring as evidenced by the structures of some of the known rexinoid agonists (**2**–**8**) shown in Figure 1.[3–10] A plausible explanation for this observation might be that most of the structures were inspired by the structure of the endogenous ligand for RXR, 9-*cis*-retinoic acid.[11] The cyclopropyl ring is generally considered a double bond surrogate by organic chemists. However to the best of our knowledge, RXR agonists incorporating cyclopropylcarboxylic acid in their structures such as **10** have not been reported. Structures of some compounds that contain a cyclopropyl group (**5** and **6**) with a '9-cis locked conformation' have been published.[10] The only example of RXR agonist containing a carboxylic acid without the α,β-unsaturated double bond (**8**) was reported by GlaxoSmithKline.[9]

In this communication the synthesis and structure–activity relationship in the analogs of **1** where the trans double bond was replaced with a cis double bond, alkyne (general structure **A**), a single bond (**9**), an alkoxy group and a cyclopropyl ring (**10**) is described.

Compound **13** containing a cis double bond was synthesized from the known intermediate **11** via Wittig olefination as shown in Scheme 1.[2] The cis isomer **13** was isolated from the trans isomer by a careful column chromatography, although the final compound was found to be contaminated with 5% of the compound with a trans double bond (**14**). The double bond of **14** was reduced by hydrogenation to obtain **15a**–**g**. Hydrogenation of compounds containing tri-substituted double bonds **16** and **17** yielded compounds **18** and **19**, respectively, as racemic mixtures. Sonogashira coupling of propargyl alcohols with **20** yielded intermediates **21** and **22**, which upon TEMPO oxidation gave the corresponding carboxylic acids **23** and **24**. In the case of **24**, the alkynyl bond was hydrogenated to give compound **25**. A Suzuki coupling reaction between 3,4-dihydro-1*H*-quinolin-2-one (**26b**) and acetic acid 3-bromo-4-trifluoroethoxytphenyl ester yielded phenol **27** with the acetoxy group having cleaved during the reaction and work-up. The phenol was then alkylated with the α-bromo esters to yield **28** and **29** upon hydrolysis.

The racemic cyclopropyl analogs were synthesized by the addition of an ylide generated from trimethylsulfoxonium iodide and sodium hydride in DMSO, to

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* Corresponding author at Present address. Novartis Institute for Biomedical Research, Cambridge, MA, USA. Tel.: +1 617 871 4257; fax: +1 617 871 4081; e-mail: bharat.lagu@novartis.com

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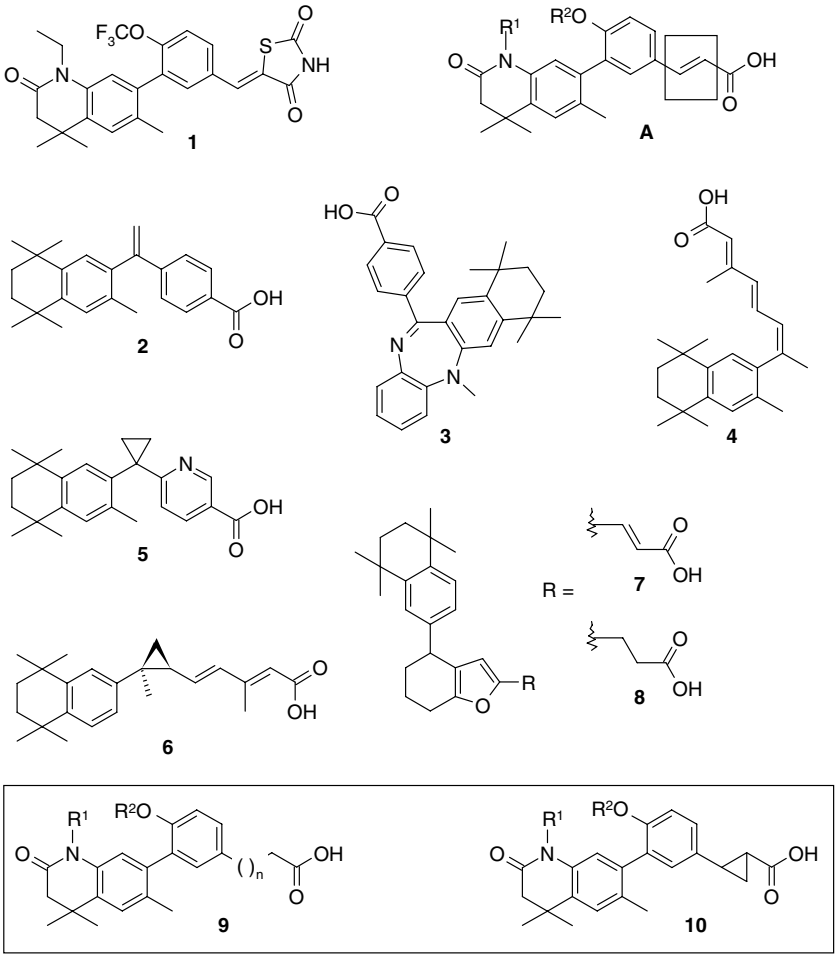
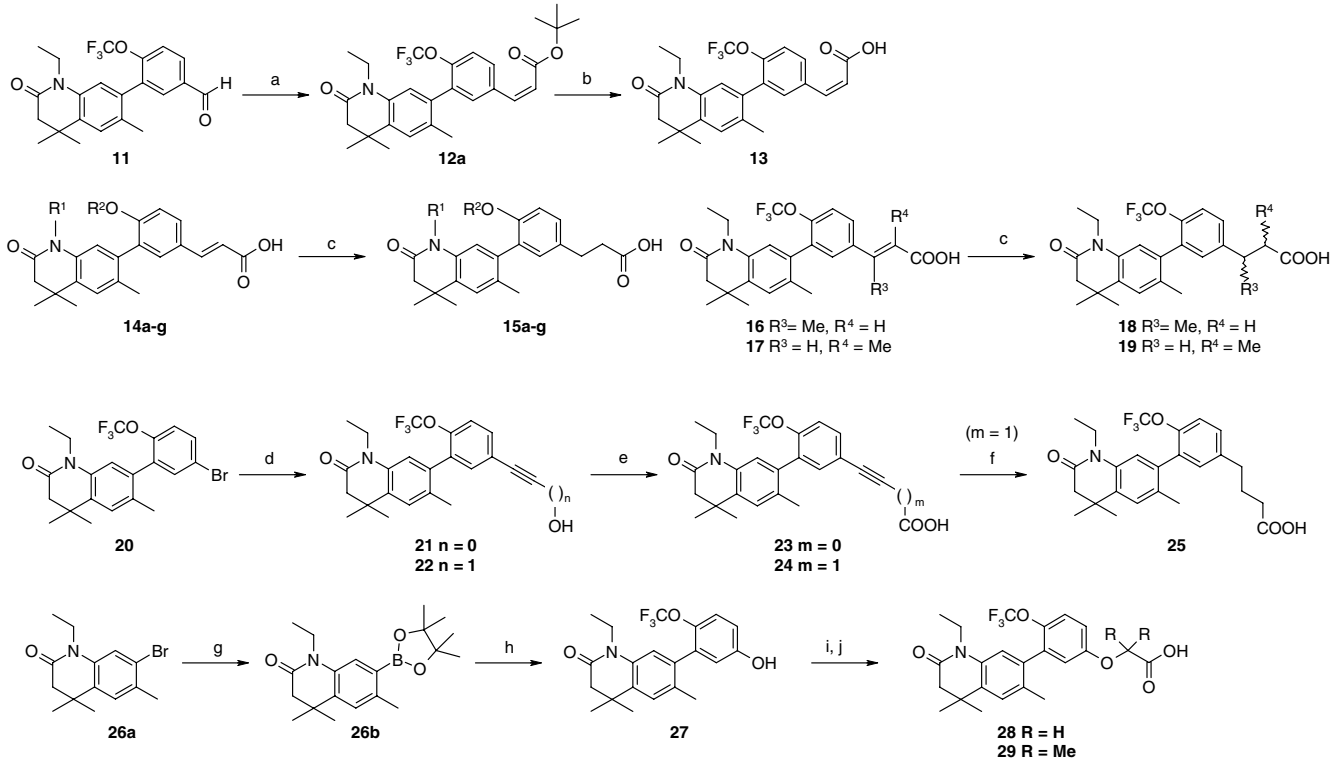


Figure 1.

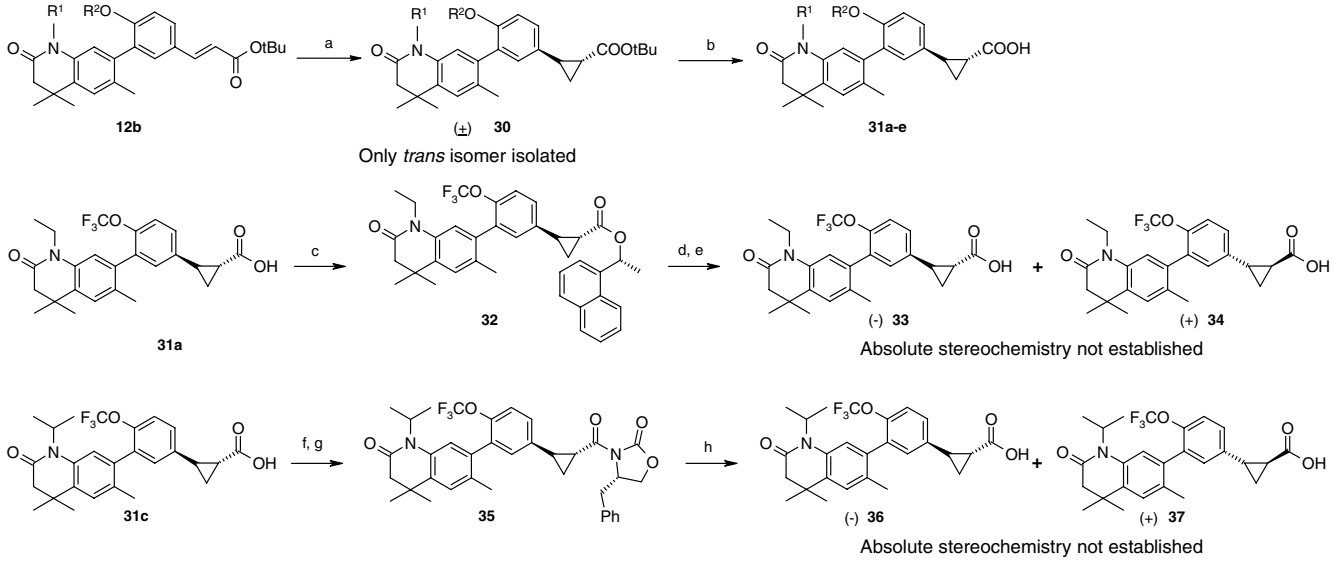
the cinnamate **12b**.[12] The removal of the protecting group under acidic conditions gave the compounds **31a**–**e**. In all cases, only the trans diastereomer was obtained as a racemic mixture.[13] Compounds **31a** and **31c** were converted into a mixture of diastereomers **32** and **35**, respectively, by attaching chiral auxiliaries as shown in Scheme 2. The diastereomers of **32** were separated by using chiral HPLC.[14] The chiral auxiliary was removed by hydrogenation to yield the cyclopropylcarboxylic acids **33** and **34** in the enantiomerically pure forms. In the case of **35**, the oxazolidinone moiety was removed by hydrolysis after separating the two diastereomers by flash column chromatography. At the present time, the absolute stereochemistry at the chiral centers for **33**, **34**, **36**, and **37** has not been established.

The compounds were initially screened for their ability to induce expression of ABCA-1 (ATP binding cassette protein), a key gene involved in the reverse cholesterol transport and known to be upregulated by LXR agonists.[15,16] A few compounds were screened in the aP2 gene induction assay indicative of the activation of RXR–PPARγ heterodimer (Tables 1–4).[17] In order to determine the activity at the RXR receptor (specifically RXRα), the compounds were tested in a co-transfection assay with the yeast GAL4 DNA binding domain fused to the ligand binding domain of RXRα and the yeast promoter driving luciferase expression Table 5.

In the ABCA-1 mRNA induction assay, the maximal efficacy of the newly synthesized RXR agonists was about half of that compared to the known LXR agonist TO-901317.[15] Compound **13** containing a cis double bond showed significantly reduced potency to activate the RXR–LXR heterodimer (EC_{50} = 886 nM) compared to **14b** (EC_{50} = 3.4 nM). The alkyne-containing compound **23** was found to be inactive in the functional assay. It is possible that the trajectory of the carboxylic acid is important in order to make the key contact with the Arg316 residue in the binding site of the RXR receptor.[9] Compounds **16** and **17** bearing β and α methyl group on the double bond with respect to the carboxylic acid showed completely different potency profiles. While **17** was found to be active in ABCA-1 gene induction assay and the RXR co-transfection assay, **16** was found to be inactive in both assays at the testing concentrations. Analog **31a** where the double bond was replaced by a cyclopropyl moiety showed potent activity in both assays (EC_{50} = 17.4 and 19.2 nM, respectively). This activity is quite interesting given the fact that these are



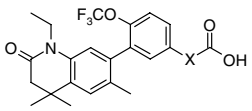
Scheme 1. Reagents and conditions: (a) THF, diphenylphosphonic acid ethyl ester, Triton B, −78 to 0 °C; (b) CH_2Cl_2, TFA, rt, 8 h, >90%; (c) 10% Pd-C, H_2, EtOAc or MeOH, >95%; (d) propargyl alcohol or 3-butyn-1-ol, NMP, $Pd(PPh_3)_4$, 120 °C 15 min, microwave, 26–40%; (e) TEMPO, CH_3CN, phosphate buffer, bleach, $NaClO_2$, 55%; (f) 10% Pd–C, H_2, rt, overnight, 100%; (g) $Pd(OAc)_2$, 2-(dicyclohexylphosphino)biphenyl, dioxane, Et_3N, pinacolborane, 85 °C, 4 h, 60%; (h) acetic acid-3-bromo-4-trifluoromethoxyphenyl ester, THF–DMPU (10:1), *n*-BuLi, dimethyl-*t*-butoxycarbonylmethylphosphonate, −78 to 0 °C, 54%; (i) ethyl-2-bromoisobutyrate, CH_3CN, Cs_2CO_3, 85 °C, 12 h; (j) THF–MeOH, aq NaOH, rt, overnight, 40%.

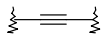
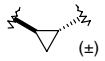


Scheme 2. Reagents and conditions: (a) trimethylsulfoxonium iodide, NaH, DMSO, CH_2Cl_2, rt, −50 °C, 3 h, 72–90%; (b) CH_2Cl_2, TFA, rt, 8 h, >90%; (c) (*R*)-(+)-methyl-2-naphthalene methanol, EDCI, HOBt, DMF, Et_3N, cat. DMAP, rt, 12 h; (d) separate diastereomers by chiral HPLC (see Ref. 14); (e) 10% Pd–C, EtOAc, H_2, rt, 8 h; (f) $SOCl_2$, CH_2Cl_2, rt, overnight. Then NaH, (*S*)-(−)-4-benzyl-2-oxazolidinone, THF, rt; (g) separate diastereomers by column chromatography; (h) LiOH, THF–MeOH–H_2O.

the first reported RXR agonists that contain cyclopropane-1-carboxylic acid. The activity of **31a** raised a possibility of reducing the double bond completely while retaining the ABCA-1 activity. The only example of such saturated bond to the carboxylic acid has been reported by GlaxoSmithKline, where compound **8** was found to be fivefold less potent than the parent compound **7**. The authors suggested that lower potency of

Table 1. Linker Chain Modification

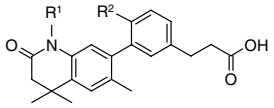


Compound	X	ABCA-1 EC_{50}[a] (nM)	RXRα co-transfection EC_{50}[a] (nM)
14b		3.6 (81%)	1.8 (115%)
13		886 (75%)	39.1 (97%)
23		>3000	ND
16		ND	>10,000
17		100 (49%)	27.9 (81%)
31a	 (±)	17.4 (ND)	19.2 (88%)
15b		47 (70%)	7.2 (79%)
25		>3000	527 (70%)
19		>3000	760 (85%)
18		347 (160%)	37.7 (99%)
28		>3000	ND
29		>3000	ND

ND, not determined.

[a] The numbers in parentheses represent %maximal activation in comparison to compound **1**.

Table 2. SAR for compounds **15a–g**



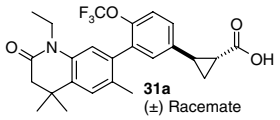
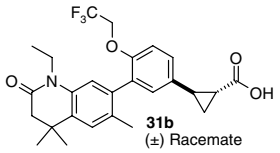
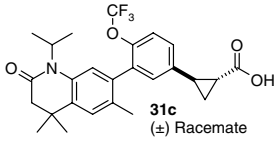
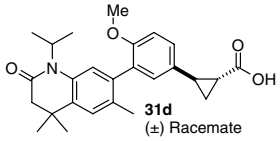
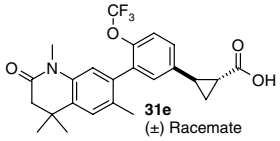
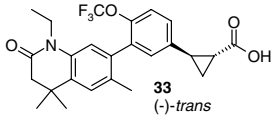
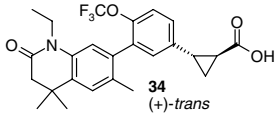
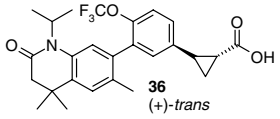
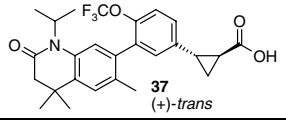
Compound	R^1	R^2	ABCA-1 EC_{50}[a] (nM)	RXR_α co-trans EC_{50}[a] (nM)
15a	Me	OCF_3	53	45.9 (91%)
15b	Et	OCF_3	47 (70%)	7.2 (79%)
15c	^{i}Pr	OCF_3	1 (59%)	3.4 (80%)
15d	CH_2CF_3	OCF_3	>3000	1.4 (100%)
15e	Et	OCH_2CF_3	>3000	70.2 (90%)
15f	^{i}Pr	OCH_2CF_3	369 (45%)	36.5 (79%)
15g	^{i}Pr	OMe	>3000	361(60%)

ND, not determined.

[a] The numbers in parentheses represent %maximal activation in comparison to compound **1**.

8 'presumably resulted from the entropic cost of a more flexible compound.' Similarly, in the present series, compound **15b** was found to be active but fourfold less potent than **14b** in both functional assays (EC_{50} = 47 and 7.2 nM in the ABCA-1 and RXR co-transfection assay, respectively).

Table 3. SAR in cyclopropane-containing RXR agonists

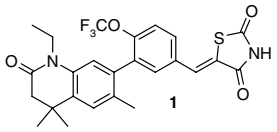
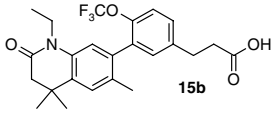
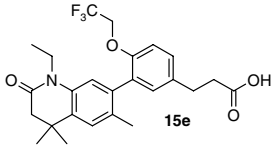
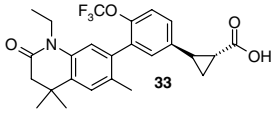
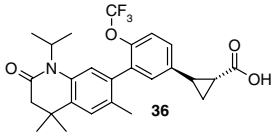
Compound	ABCA-1 EC_{50} nM	RXRα co-trans EC_{50}[a] (nM)
 **31a** (±) Racemate	17.4	19.2 (158%)
 **31b** (±) Racemate	>3000	115.8 (87%)
 **31c** (±) Racemate	>3000	5.0 (85%)
 **31d** (±) Racemate	1524.0	207.5 (50%)
 **31e** (±) Racemate	>3000	144.0 (108%)
 **33** (-)-*trans*	>3000	2.3 (85%)
 **34** (+)-*trans*	>3000	361 (92%)
 **36** (+)-*trans*	>3000	10 (70%)
 **37** (+)-*trans*	>3000	198 (50%)

ND, not determined.

[a] The numbers in parentheses represent %maximal activation in comparison to compound **1**.

Increasing the tether length from two to three carbons resulted in a significant loss of potency for compound **25**. Compound **19** bearing a methyl group on the α carbon with respect to the carboxylic group was less potent than the compound **18** bearing methyl group on the β carbon. This observation is in contrast to the results observed for the double bond containing analogs **16** and **17**. Interestingly, compounds **28** and **29**, which bear the same number of atoms as **15b** but contain an oxygen atom in the linker chain, were not active up to 3 μM concentration.

Table 4. Selectivity for the RXR agonists

Compound	ABCA-1 EC_{50}[a] (nM)	aP2 EC_{50} (nM)	RXR co-trans EC_{50}[a](nM)
 **1**	180 (100%)	139	0.9 (100%)
 **15b**	47 (70%)	1200	7.2 (79%)
 **15e**	>3000	>3000	70.2 (90%)
 **33**	>3000	581	2.3 (85%)
 **36**	>3000	266	10 (70%)

[a] The numbers in parenthesis represent %maximal activation in comparison to compound **1**.

Table 5. Effects of **15b** on serum cholesterol and triglyceride levels following 8 days of treatment in Sprague–Dawley rats fed a high cholesterol diet

Dose (mg/kg)	HDL-C (mg/dL)	LDL-C (mg/dL)	Total Cholesterol (mg/dL)	Triglycerides (mg/dL)
Vehicle	21 ± 2	52 ± 5	321 ± 20	218 ± 20
0.3 mpk	29 ± 1**	53 ± 5	343 ± 18	320 ± 38
1 mpk	30 ± 2**	46 ± 4	313 ± 18	279 ± 32
3 mpk	37 ± 1**	46 ± 4	302 ± 14	253 ± 24
10 mpk	39 ± 2**	35 ± 2**	228 ± 18**	270 ± 42
30 mpk	42 ± 1**	38 ± 2*	252 ± 24*	322 ± 34
10 mpk **1** (0.5% methocel)	47 ± 1**	41 ± 1	267 ± 10*	286 ± 12

Data are expressed the means ± SEM and were analyzed using one-way ANOVA and Dunnet's multiple comparison test ($^{*}p < 0.05$, $^{**}p < 0.001$). All comparisons are made relative to the Vehicle controls.

Similar to **15b**, a number of analogs with differing R^1 and R^2 groups (**15a**–**g**) were active in the RXR co-transfection assay with EC_{50} values ranging from 1.4 to 361 nM (Table 2). Some of the trends were common in the compounds with or without a double bond, e.g. for R^1 group, the ethyl or isopropyl groups were found to give compounds that were more potent than when R^1 = Me or for R^2 group, compounds with trifluoroethoxy group showed more functional activity than the compounds with trifluoroethoxy group at the R^2 position.[1] Compounds **15a**–**c** had high potency in both the ABCA-1 induction and RXR co-transfection assays, particularly compound **15c**. Although **14b** is about four times more potent in the RXRa co-transfection assay than **15b** (an observation similar to the one reported for **7** and **8** by the researchers from Glaxo SmithKline),[9] it must be pointed out that other analogs such as **15c** and **15d** are almost as potent as **14b** in the functional assays.

Compounds where the cinnamic acid double bond was replaced with a cyclopropyl group were also screened in the ABCA-1 gene induction and RXR co-transfection assays. The results are summarized in Table 3. In general, the compounds (**31a**–**e**, **33**–**37**) showed good activity (EC_{50} = 2.3–361 nM) in the RXR co-transfection assay. However, the compounds showed significant differences in the activation of the RXR–LXR heterodimer (EC_{50} = 17.4 nM to >3 μM). The individual enantiomers were synthesized for compounds **31a** and **31c**. The (−)-enantiomers (i.e. compounds giving negative value for optical rotation) **33** and **36** were found to be more potent than the (+)-enantiomers (**34** and **37**) and the racemates (**31a** and **31c**) in the co-transfection assay. At the present time the absolute stereochemistry shown for these enantiomers has not been assigned.

With the active compounds in hand, we screened a few compounds in the selectivity screen for their ability to activate the RXR–LXR (ABCA-1 mRNA induction) and RXR–PPARγ aP2 mRNA induction) heterodimer complexes. The results are summarized in Table 4. While **1** is equipotent in activating both RXR–LXR and RXR–PPARγ heterodimer complexes, **15b** showed some

selectivity for activating the RXR-LXR complex (EC_{50} = 47 nM vs EC_{50} = 1200 nM for RXR–PPARγ). Compound **15e** had weak activity while compounds **33** and **36** were found to be selective for activating RXR–PPARγ heterodimer over the RXR–LXR heterodimer. Compound **15b** was selected as a representative example for evaluation in the in vivo models for diabetes and dyslipidemia based on the satisfactory pharmacokinetic profile in rats (3 mg/kg oral dose in 0.5 methocel; oral bioavailability = 71%; C_{max} = 678 ng/mL; AUC = 6129 ng h/mL; $T_{1/2}$ = 24 h; CL = 4.6 mL/min/kg).

As a model of dyslipidemia, Sprague–Dawley male rats were fed a high cholesterol, atherogenic diet ad libitum, which typically induces a fivefold increase in serum cholesterol after 14 days. Compound **15b** was orally administered after 6 days on the diet in 0.5% methocel for a total of 8 days. Serum cholesterol, HDL-C, LDL-C and triglycerides were measured. Treatment with compound **15b** elicited a statistically significant increase in HDL-C and a decrease in LDL-C levels as compared to the vehicle control animals. The C_{max} values ranged from an average of 58.5 ng/mL (0.3 mg/kg) to 7510 ng/mL (30 mg/kg). A significant increase in serum triglyceride levels was not observed. First generation rexinoid agonists such as LGD1069 (Tagretin™) elevate the triglyceride levels in both rat and human.[18,19] On the other hand, **15b** failed to lower blood glucose in the db–db mouse model for diabetes following 11 days of treatment (data not shown). These results are in agreement with the observed selectivity of the compound to activate RXR–LXR heterodimer over the RXR–PPARγ heterodimer.

We have reported some structurally unique and potent RXR agonists where the double bond conjugated with the carboxylic acid was replaced with either a single bond or a cyclopropyl group. Some of the compounds were found to selectively activate the RXR–LXR heterodimer complex over the RXR–PPARγ heterodimer complex.

Acknowledgments

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References and notes

1. Lagu, B.; Pio, B.; Lebedev, R.; Yang, M.; Pelton, P. D. *Bioorg. Med. Chem. Lett.* (accompanying paper).
2. Pfahl, M.; Tachdjian, C.; Al-Shamma, H.; Giachino, A. F.; Jakubowicz-Jaillardon, K.; Guo, J.; Boudjelal, M.; Zapf, J. W. PCT Int. Appl. WO 2003075924 A1, **2003**.
3. Boehm, M. F.; Zhang, L.; Baeda, B. A.; White, S. K.; Mais, D. E.; Berger, E.; Suto, C. M.; Goldman, M. E.; Heyman, R. A. *J. Med. Chem.* **1994**, *37*, 2930.
4. Vuligonda, V.; Lin, Y.; Chandraratna, R. A. S. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 213.
5. Michellys, P. Y.; Ardecky, R. J.; Chen, J. H.; Crombie, D. L.; Etgen, G. J.; Faul, M. M.; Faulkner, A. L.; Grese, T. A.; Heyman, R. A.; Karanewsky, D. S.; Klausiing, K.; Leibowitz, M. D.; Liu, S.; Mais, D. A.; Mapes, C. M.; Marschke, K. B.; Reifel-Miller, A.; Ogiliveie; Rungta, D.; Thompson, A. W.; Tyhonas, J. S.; Boehm, M. F. *J. Med. Chem.* **2003**, *46*, 2683.
6. Canan Koch, S. S.; Dardashti, L. J.; Cesario, R. M.; Croston, G. E.; Boehm, M. F.; Heyman, R. A.; Nadzan, A. M. *J. Med. Chem.* **1999**, *42*, 742.
7. Faul, M. M.; Ratz, A. M.; Sullivan, K. A.; Trankle, W. G.; Winneroski, L. L. *J. Org. Chem.* **2001**, *66*, 5772.
8. Kagechica, H. *Curr. Med. Chem.* **2002**, *9*, 591.
9. Haffner, C. G.; Lenhard, J. M.; Miller, A. B.; McDougald, D. L.; Dwornik, K.; Itoop, O. R.; Gampe, R. T.; Xu, H. E.; Blanchard, S.; Montana, V. G.; Consler, T. G.; Bledsoe, R. K.; Ayscue, A.; Croom, D. *J. Med. Chem.* **2004**, *47*, 2010.
10. Vuligonda, V.; Thacher, S. M.; Chandraratna, R. A. S. *J. Med. Chem.* **2001**, *44*, 2298.
11. Levin, A. A.; Sturzenbecker, L. J.; Kazmer, S.; Bosakowski, T.; Huselton, C.; Allenby, G.; Speck, J.; Kratzeisen, C.; Rosenberg, M.; Lovey, A.; Grippo, J. F. *Nature* **1992**, *355*, 359.
12. Corey, E. J.; Chykovsky, M. *J. Am. Chem. Soc.* **1965**, *87*, 1353.
13. Relative stereochemistry assigned based on ^{1}H NMR. Solladie-Cavallo, A.; Isarno, T. *Tetrahedron Lett.* **1999**, *40*, 1579.
14. The diastereomers were separated by using a Varian Prep Star 218 HPLC instrument with Chiralpak-AD (21.2 × 250 mL) column as the stationary phase and 80% *n*-hexane-20% EtOH with 0.05% TFA as the eluent. With the flow rate of 5 mL/min, injection volume of 0.5 mL, the retention times (detector at 254 nm) for the two diastereomers were 18.3 min and 21.4 min, respectively.
15. Repa, J. J.; Turley, S. D.; Lobaccaro, J. M. A.; Medina, J.; Li, L.; Lustig, K.; Shan, B.; Heyman, R. A.; Dietschy, J. M.; Mangelsdorf, D. J. *Science* **2000**, *289*, 1524.
16. Attie, A. D.; Kastelein, J. P.; Hayden, M. R. J. *Lipid Res.* **2001**, *42*, 1717.
17. Burris, T. P.; Pelton, P. D.; Zhou, L.; Osborne, M. C.; Cryan, E.; Demarest, K. T. *Mol. Endocrinol.* **1999**, *13*, 410.
18. Bershad, S. A.; Rubinstein, J. R.; Paterniti, N. A.; Le, S. C.; Poliak, B.; Heller, H. N.; Ginsberg, R.; Fleischmajer, R.; Brown, W. V. *N. Engl. J. Med.* **1985**, *313*, 981.
19. Miller, V. A.; Benedetti, F. M.; Rigas, J. R.; Verret, A. L.; Pfister, D. G.; Straus, D.; Kris, M. G.; Crisp, M.; Heyman, R.; Loewen, G. R.; Truglia, J. A.; Warrell, R. P., Jr. *J. Clin. Oncol.* **1997**, *15*, 790.