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RXR–LXR heterodimer modulators for the potential treatment of dyslipidemia

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Abstract—A number of RXR agonists were synthesized and screened in functional assays. The synthesis and the structure–activity relationship (SAR) within the series of compounds will be presented. Some in vivo data in rodent models for dyslipidemia and diabetes will also be presented.

Retinoid X receptors are part of the nuclear receptor superfamily that function as ligand-activated transcription factors. RXRs modulate gene transcription through homodimers (RXR–RXR) or heterodimers such as RXR–LXRs, RXR–PPARs, RXR–FXR, RXR–TR, and RXR–VDR.[1,2] These receptors impact a number of metabolic pathways and the modulators of these receptors have shown efficacy in treating metabolic disorders. These modulators, however, also result in undesired side effects. Agonists of PPARγ such as Avandia™ and Actos™ improve insulin sensitivity in diabetic patients but also induce weight gain and edema in many patients. RXR modulators might provide an alternative pathway for treating a variety of metabolic diseases such as dyslipidemia and diabetes.[3,4] A pan RXR agonist, however, may also show untoward side effects in the clinic including hypertriglyceridemia and suppression of thyroid hormone.[5,6] Recent data with heterodimer selective RXR agonists such as LG1506 (**4**) indicate that it is possible to develop RXR modulators having beneficial metabolic effects without hypertriglyceridemia and thyroid suppression.[7] LG1506 selectively activates the PPAR subfamily of heterodimers and, unlike the selective PPARγ agonists, does not induce weight gain in rodents. These data indicate that RXR modulators with the appropriate tissue and gene selective profiles may be developed.

Structures of some of the known RXR agonists are shown in Figure 1.[8–13] A few compounds such as **1** and **4** were reported to selectively activate the RXR–PPARγ heterodimer over the RXR–LXR heterodimer.[10,13] Similarly, compound **3** was reported to activate the RXR–RXR homodimer and RXR–PPAR heterodimers but not the RXR–FXR and the RXR–LXR heterodimers.[12] The data reported for the tetrahydrobenzofurans **5a** and **5b** reveal that the binding affinity of the compounds increased when the thiazolidinedione (TZD) moiety was replaced by the carboxylic acid (K_i = 6 nM for **5b**; K_i = 20 nM for **5a**).[14] The collaborative research program between Johnson & Johnson PRD and Maxia Pharmaceuticals (now Incyte Corporation) resulted in the identification of novel TZDs such as **6**.[13] If a similar trend is observed, it might be possible to increase the potency of **6** by replacing the TZD moiety with a carboxylic acid **7**. Herein we disclose the synthesis and SAR for the series of compounds that are analogs of **7** (Fig. 1). Some of the compounds showed an unexpected selectivity for the RXR–LXR heterodimer partner, which could have beneficial effects for both cardiovascular disease and diabetes.[14,15]

A number of compounds with changes in the left hemisphere and the linker chain (Fig. 1) were synthesized by using the chemistry shown in Schemes 1 and 2. In all the analogs except **36** and **37**, the methyl group at the C-6-position was maintained in the left hemisphere. This methyl group is considered to be necessary for the compounds to attain the necessary L-shaped conformation ('α-methyl effect') thereby imparting selectivity for RXR over RAR.[16] In general, a number of benzo-fused cyclic amides were synthesized by known synthetic

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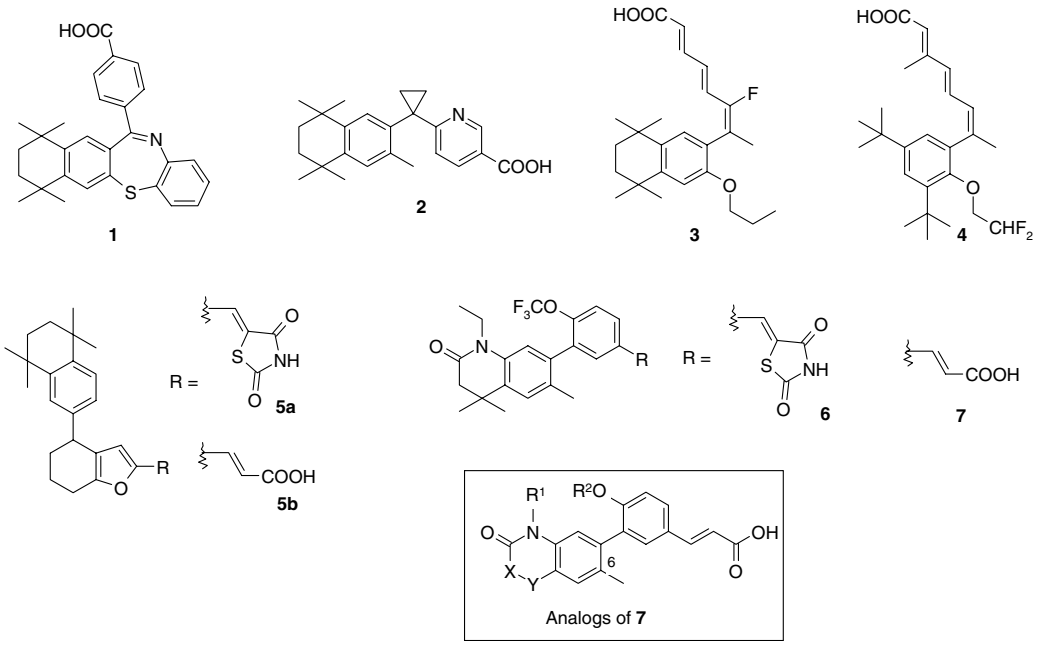
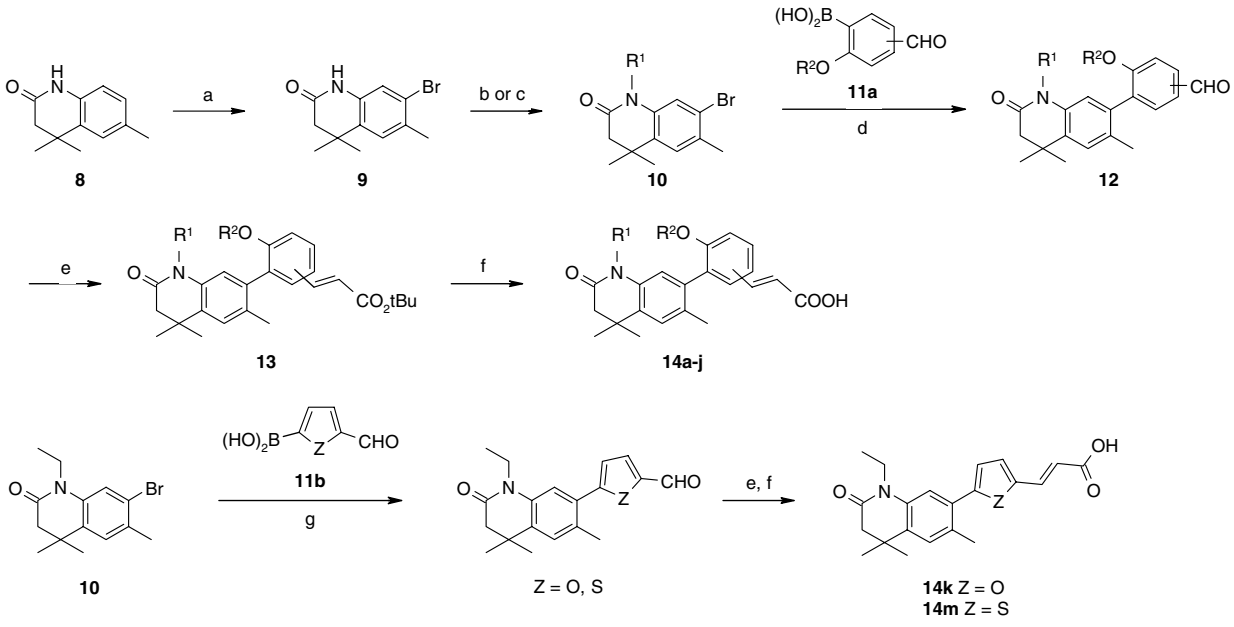


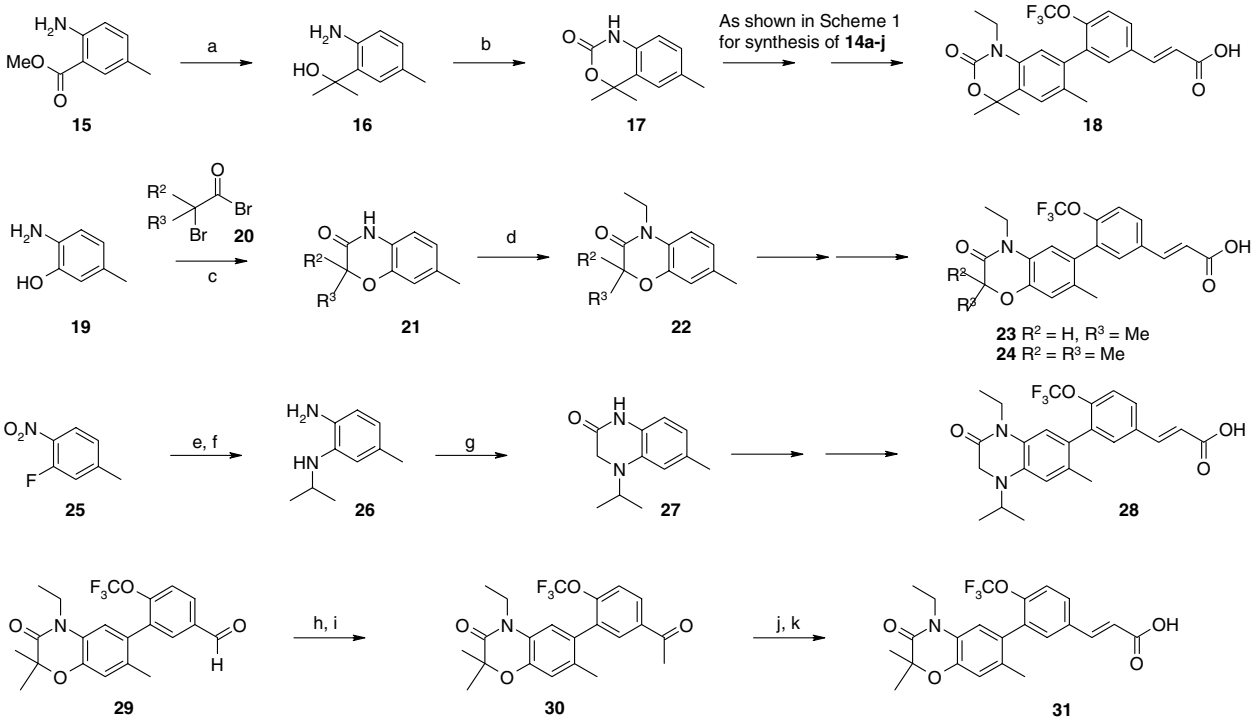
Figure 1. Known RXR ligands and **7**.



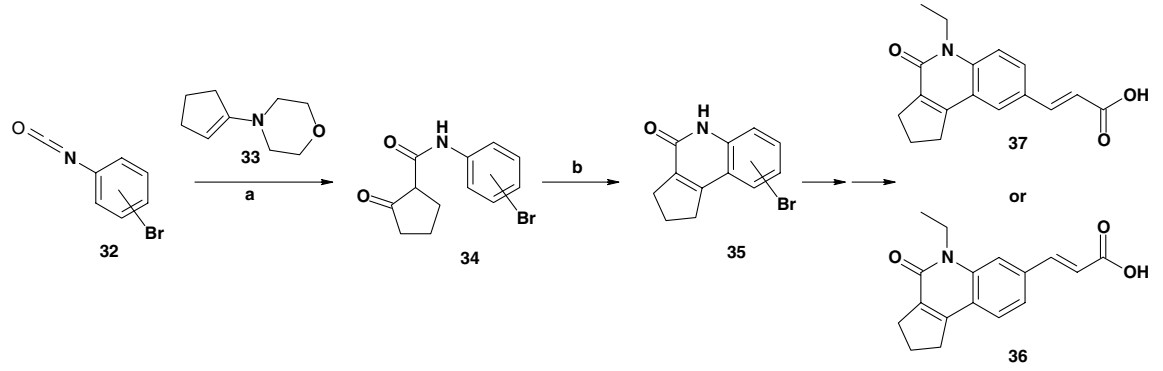
Scheme 1. Reagents and conditions: (a) NBS, CH_2Cl_2, rt, 12 h, 80–95%; (b) KOH, DMSO, alkyl iodide, rt, 12 h, 65–94%; (c) Boc_2O, NaH, THF, 98%; (d) **11a**, $Pd(PPh_3)_4$, toluene–EtOH, 2M K_2CO_3, reflux, 47–80%; (e) THF–DMPU (10:1), *n*-BuLi, dimethyl-*tert*-butoxycarbonylmethylphosphonate, −78 °C to 0 °C, 41–75%; (f) CH_2Cl_2, TFA, rt, 8 h, >90%; (g) **11b**, $Pd(PPh_3)_4$, toluene–EtOH, 2M K_2CO_3, reflux, 47–80%.

routes and brominated by the treatment with NBS.[17–19] The amide nitrogen was then alkylated under standard conditions in high yield. Suzuki coupling with an appropriate aryl boronic acid yields aldehyde, which was then converted into the cinnamate ester using Horner–Emmons conditions in good overall yields. The final carboxylic acids were obtained by the removal of the *tert*-butyl group by treatment with TFA (Scheme 3).

In order to identify compounds that would have potential to increase reverse cholesterol transport and plasma HDL-C as potential novel therapy for the treatment of dyslipidemias characterized by low circulating HDL-C, we initially screened all the compounds for their ability to induce expression of ABCA-1 (a member of the ATP binding cassette protein family), a key gene involved in reverse cholesterol transport and known to be upregulated by LXR agonists.[20,21] Compounds were screened using branched DNA technology to measure induction of ABCA1 mRNA in the human monocytic cell line, THP-1.[22] In this assay, the maximal efficacy of the newly synthesized RXR agonists was about half



Scheme 2. Reagents and conditions: (a) MeMgBr, THF, −78 °C to 0 °C, 12 h, 58%; (b) phosgene in toluene, THF, Et_3N, −0 °C to rt, 3 h, 95%; (c) CH_2Cl_2, rt, 3 h; (d) KOH, DMSO, alkyl iodide, rt, 12 h; (e) isopropylamine, CH_2Cl_2, 80 °C, 12 h; (f) 5% Pd–C (cat.), EtOH, ammonium formate, rt; (g) DCC, CH_2Cl_2, bromoacetic acid, rt, 1 h, 7%; (h) MeMgBr, THF, Et_2O, −78 °C to rt; (i) Dess–Martin reagent, CH_2Cl_2, rt, overnight, 46% over two steps; (j) THF–DMPU (10:1), *n*-BuLi, dimethyl-*tert*-butoxycarbonyl-methylphosphonate, −78 °C to 0 °C; (k) CH_2Cl_2, TFA, rt, 8 h,96%.

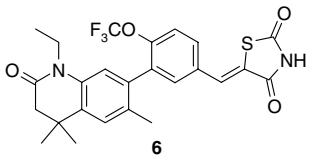


Scheme 3. Reagents and conditions: (a) and (b) Ref. 19.

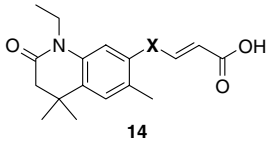
of that compared to the known LXR agonist TO-901317.[23] In addition, all RXR agonists screened showed additive activity when screened in combination with an LXR agonist using the bDNA assay but did not have LXR agonist activity as evidenced in the LXR transactivation reporter assays (data not shown). These data lend additional support that the compounds activated the RXR/LXR heterodimers through an interaction with the RXR receptor. To determine the activity at the RXR receptor (subtype alpha), 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. A few selected compounds were also tested for their ability to induce the adipocyte fatty acid binding protein (aP2) mRNA in primary human preadipocytes, which would be indicative of activation of the RXR–PPARγ heterodimer. The results are summarized in Tables 1–3.

As shown in Table 1, compound **14a** where the double bond resided at the *para* position with respect to the OCF_3 group in the linker phenyl group showed EC_{50} comparable to that of **6** in the RXR co-transfection assay (1.8 nM vs 0.9 nM for **6**). The compound was significantly more potent (EC_{50} = 3.6 nM) for activating RXR–LXR heterodimer than **6** (EC_{50} = 180 nM). In comparison, **14b** where the double bond was attached at the *meta* position with respect to the OCF_3 group in the linker phenyl group was not active in the ABCA-1 gene induction assay. Replacement of the trifluoromethoxy group with a triflouroethoxy group resulted in **14d**

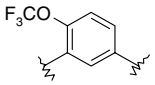
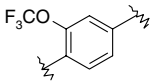
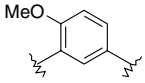
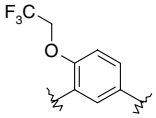
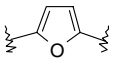
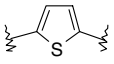
Table 1. Modifications in the linker chain in **14**



6



14

Compound	X	ABCA-1 EC_{50}[a] (nM)	RXR_{α} co-trans EC_{50}[a] (nM)
6	N/A	180 (100%)	0.9 (100%)
14a		3.6 (81%)	1.8 (115%)
14b		>3000	ND
14c		975	ND
14d		296 (60%)	67.3 (76%)
14k		>3000	>10,000
14m		>3000	1200

ND, not determined.
[a] The numbers in parentheses represent % maximal activation in comparison to compound **6**.

with decreased potency and efficacy while replacement of the OCF_3 group with a methoxy (**14c**) dramatically decreased the activity. The compounds **14k** and **14m** where the phenyl ring was replaced by a furan or a thiophene were found to be weakly active in the functional assays Table 4.

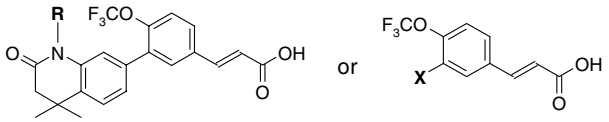
We next turned our attention to the dihydro-[1*H*]-quinolin-2-one core of the compounds. The results are shown in Table 2.

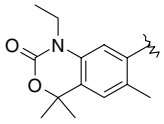
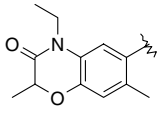
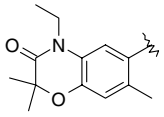
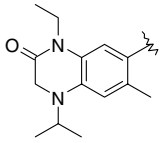
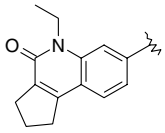
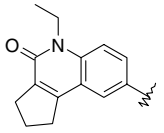
The presence and the nature of an alkyl group on the nitrogen of the dihydro-[1*H*]-quinolin-2-one core were found to be important in the functional assays for compounds **14a** and **14f**–**i**. The absence of an alkyl group in **14e** resulted in loss of activity. Interestingly, the presence of ethyl, propyl or trifluoroethyl gave compounds with high and comparable potency (EC_{50} = 1–2 nM) in the RXR transactivation assay but a wider range of potency observed in the ABCA-1 gene induction assay. The presence of methyl or an isopropyl group on the nitrogen gave compounds with lower potency in the co-transfection assay. However, **14h** was significantly more potent in the ABCA-1 gene induction assay (EC_{50} = 10 nM) than **14f** (EC_{50} = 200 nM) suggesting that the compounds may be showing some degree of selectivity. The compounds **18**, **23**, and **28** where the dihydro-quinolin-2-one core was modified showed no activity at the testing concentration, however compound **24** was found to have reasonable potency in the RXR co-transfection (EC_{50} = 173.1 nM) and the ABCA-1 (EC_{50} = 39.7 nM) assay. Both **36** and **37** were found to be inactive at the testing concentration in the ABCA-1 assay, but **37** was found to be substantially more potent (EC_{50} = 96 nM) than **36** (EC_{50} = 1600 nM) in the RXR transactivation assay.

As previously mentioned, some reported compounds such as **1** and **4** selectively activated the RXR–PPARγ heterodimer. A few compounds from this series were screened for their ability to activate RXR–PPARγ heterodimer (induction of aP2) using the bDNA assay[22] and the results are shown in Table 3. The maximal activity for compound **6** in this assay was 25–35% compared to the PPARγ agonist Rosiglitazone.

While **6** was equipotent for activating RXR–LXR and RXR–PPARγ heterodimers, compounds **14d**, **14j**, and **31** were found to be more specific for the activation of RXR–LXR over RXR–PPARγ heterodimers. Compounds **14j**, and **31** had low potency but high efficacy for the induction of aP2 as evidenced by the high maximal activation values relative to compound **6**. To the best of our knowledge, these are the first reported examples of RXR agonists that show greater selectivity in the in vitro assay for the activation of

Table 2. Modifications in the left hemisphere



Compound	R or X	ABCA-1 EC_{50}[a] (nM)	RXR_α co-trans EC_{50}[a] (nM)
14e	H	>3000	NA
14f	Me	200 (56%)	24.3
14a	Et	3.6 (81%)	1.8 (94%)
14g	*n*-Pr	>3000	2.1 (83%)
14h	*i*-Pr	10 (70%)	16.3
14i	CH_2CF_3	>3000	1.8 (94%)
18		>3000	ND
23		>3000	ND
24		39.7 (79%)	173.1(78%)
28		>3000	ND
36		>3000	1600
37		>3000	96.6 (125%)

NA, not active. ND, not determined.

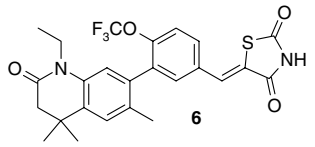
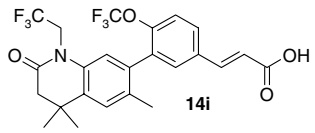
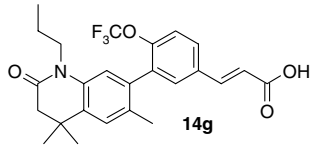
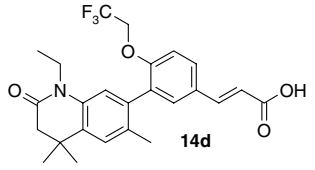
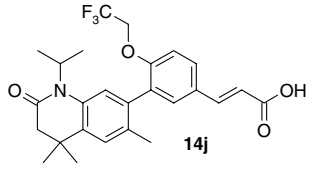
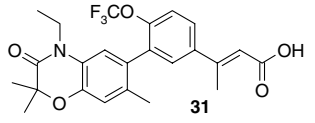
[a] The numbers in parentheses represent % maximal activation in comparison to compound **6**.

the RXR–LXR heterodimer complex over RXR–PPARγ complex.

While the origin of such selectivity is not well understood at the present time, one of the compounds, **14d**, was evaluated in rodent models of diabetes and dyslipidemia. The compound showed a satisfactory pharmacokinetic profile in Sprague–Dawley rats when dosed at 3 mg/kg (10% hydroxypropyl β-cyclodextrin (pH 10) with an oral bioavailability of 51%, C_{max} = 844 ng/mL, AUC = 5519 ng h/mL; $T_{1/2}$ = 4.6 h).

As a model of dyslipidemia, Sprague–Dawley male rats were fed a high cholesterol, atherogenic diet (C13002 Research Diets, New Brunswick, NJ) ad libitum, which typically induces a fivefold increase in serum cholesterol after 14 days. Compound **14d** was orally administered (0.3, 1, 3, and 10 mpk) after 6 days on the diet in 15% hydroxylpropyl β-cyclodextrin at pH 10) for a total of 8 days. Serum cholesterol, HDL-C, LDL-C, and triglyceride levels were determined. Treatment with compound **14d** elicited a statistically significant increase in HDL-C and a small decrease in LDL-C levels as compared to the vehicle control animals. Serum triglycerides were not increased to a statistically significant level. The C_{max} concentrations ranged from an average of 61 ng/ml (0.3 mg/kg) to 2124 ng/ml (10 mg/kg). On the other hand, the compound failed to lower blood glucose in the db/db mouse model for diabetes following 11 days of treatment (data not shown). These results seem to be

Table 3. Selectivity for the RXR agonists

Compound	ABCA-1 EC_{50}[a] (nM)	aP2 EC_{50} (nM)	RXR_{α} co-trans EC_{50}[a] (nM)
 **6**	180 (100%)	139	0.9 (100%)
 **14i**	>3000	126	1.8 (94%)
 **14g**	>3000	131	2.1 (83%)
 **14d**	296 (60%)	>3000	67.3 (80%)
 **14j**	44 (32%)	>3000[b]	18.3 (64%)
 **31**	163 (76%)	>3000[c]	61.2 (86%)

[a] The numbers in parentheses represent % maximal activation in comparison to compound **6**.
[b] 149% maximal activation compared to **6**.
[c] 240% maximal activation compared to **6**.

Table 4. Effects of **14d** and **6** on serum cholesterol and triglycerides 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	15 ± 3	144 ± 14	421 ± 26	227 ± 36
0.3	16 ± 5	134 ± 17	382 ± 38	341 ± 31
1	38 ± 4*	151 ± 8	440 ± 24	182 ± 24
3	34 ± 2*	102 ± 9	302 ± 27	249 ± 38
10	41 ± 2**	126 ± 13	349 ± 26	175 ± 28
1 (mpk) **6**	41 ± 2**	126 ± 13	371 ± 38	216 ± 9

Data are expressed as 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.

consistent with the in vitro finding that **14d** did not activate the RXR–PPARγ heterodimer but did activate the RXR–LXR heterodimer. In contrast, **6** was found to potently activate both RXR–LXR and RXR–PPARγ heterodimers and showed glucose-lowering effects in addition to the beneficial effects on the cholesterol levels (data not shown). These observations suggest that it may be possible to synthesize RXR modulators that selectively activate different permissive heterodimers other than RXR–PPARγ. While these results are intriguing, one would need to study a large number of RXR agonists with different selectivity profiles (in the in vitro assays) in these models of dyslipidemia and diabetes before a firm conclusion is reached.

We have synthesized analogs of **6** where the TZD group was replaced with a carboxylic acid. The SAR in the series suggest a possibility of finding RXR modulators that

are selective for activation of different permissive heterodimers of RXR other than the previously reported RXR–PPARγ modulators. These compounds could be used as tools to further ascertain the roles of various heterodimers for the treatment of metabolic disorders.

Acknowledgments

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References and notes

1. Mangelsdorf, D. J.; Evans, R. M. *Cell* **1995**, *83*, 841.
2. Rastinejad, F. *Curr. Opin. Struct. Biol.* **2001**, *11*, 33.
3. Lenhard, J. M.; Lancaster, M. E.; Paulik, M. A.; Weiel, J. E.; Binz, J. G.; Sundseth, S. S.; Gaskiill, B. A.; Lightfoot, R. M.; Brown, H. R. *Diabetologia* **1999**, *42*, 545.
4. Mukherkjee, R.; Davies, P. J. A.; Crombie, D. L.; Bischoff, E. D.; Cesario, R. M.; Jow, L.; Hamann, L. G.; Boehm, M. F.; Mondon, C. E.; Nazdan, A. M.; Paterniti, J. R.; Heyamn, R. A. *Nature* **1997**, *386*, 407.
5. 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.
6. 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.
7. Leibowitz, M. D.; Ardecky, R. J.; Boehm, M. F.; Broderick, C. L.; Carfagma, M. A.; Crombie, D. L.; D'Arrigo, J.; Etgen, G. J.; Faul, M. M.; Grese, T. A.; Havel, H.; Hein, N. I.; Heyman, R. A.; Jolley, D.; Klausing, K.; Liu, S.; Mais, D. E.; Mapes, C. M.; Marschke, K. B.; Michellys, P. Y.; Montrose, Y.; Rafizadeh, C.; Ogilvie, K. M.; Pascual, B.; Rungta, D.; Tyhonas, J. S.; Urcan, M. S.; Wardlow, M.; Yumibe, N.; Reifel-Miller, A. *Endocrinology* **2006**, *147*, 1044.
8. Kagechika, H.; Shudo, K. *J. Med. Chem.* **2005**, *48*, 5875.
9. Takahashi, B.; Ohta, K.; Kawachi, E.; Fukasawa, H.; Hashimoto, Y.; Kagechica, H. *J. Med. Chem.* **2002**, *45*, 3327.
10. Deng, T.; Shan, S.; Li, Z.; Wu, Z.; Liao, C.; Ko, B.; Lu, X.; Cheng, J.; Ning, Z. *Biol. Pharm. Bull.* **2005**, *28*, 1192.
11. 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, K. M.; Rungta, D.; Thompson, A. W.; Tyhonas, J. S.; Boehm, M. F. *J. Med. Chem.* **2003**, *46*, 2683.
12. Haffner, C. G.; Lenhard, J. M.; Miller, A. B.; McDougald, D. L.; Dwornik, K.; Ittoop, 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.
13. Pfahl, M.; Tachdjian, C.; Al-Shamma, H.; Giachino, A. F.; Jakubowicz-Jaillardon, K.; Guo, J.; Boudjelal, M.; Zapf, J. W. (Maxia Pharmaceuticals, Inc., USA). PCT Int. Appl. WO 2003075924 A1, **2003**.
14. Cao, G.; Liang, Y.; Broderick, C. L.; Oldham, B. A.; Beyer, T. P.; Schmidt, R. J.; Zhang, Y.; Stayrook, K. R.; Suen, C.; Otto, K. A.; Miller, A. R.; Dai, J.; Foxworthy, P.; Gao, H.; Ryan, T. P.; Jiang, X. C.; Burris, T. P.; Eascho, P. I.; Etgen, G. J. *J. Biol. Chem.* **2003**, *278*, 1131.
15. Joseph, S. B.; McKilligin, E.; Pei, L.; Watson, M. A.; Collins, A. R.; Laffitte, B. A.; Chen, M.; Noh, G.; Goodman, J.; Hagger, G. N.; Tan, J.; Tippin, T. K.; Wang, X.; Lusis, A. J.; Hsueh, W. A.; Law, R. E.; Collins, J. L.; Wilson, T. M.; Tontonoz, P. *Proc. Natl. Acad. Sci.* **2002**, *99*, 7604.
16. Sussman, F.; de Lara, A. R. *J. Med. Chem.* **2005**, *48*, 6212.
17. Grubb, G. S.; Zhang, P.; Terfenco, E. A.; Fensome, A.; Wrobel, J. E.; Fletcher, H.; Edwards, J. Ptete.; Jones, T. K.; Tegley, C. M.; Zhi, L. US 6444668B1, **2000**.
18. Higuchi, R.; Arienti, K. L.; Mani, N.; Pio, B.; Zhi, L.; Chen, P.; Caferro, T. R. PCT Int. Appl. WO 2001016139, **2001**.
19. Jaroch, S.; Hölscher, P.; Rehwinkel, H.; Sülzle; Burton, G.; Hillman, M.; McDonald, F. M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2561.
20. Tontonoz, P.; Mangelsdorf, D. *J. Mol. Endocrinol.* **2003**, *17*, 985.
21. Attie, A. D.; Kastelein, J. P.; Hayden, M. R. *J. Lipid Res.* **2001**, *42*, 1717.
22. Burris, T. P.; Pelton, P. D.; Zhou, L.; Osborne, M. C.; Cryan, E.; Demarest, K. T. *Mol. Endocrinol.* **1999**, *13*, 410.
23. 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.