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Design and Synthesis of Novel RXR-Selective Modulators with Improved Pharmacological Profile

Pierre-Yves Michellys,[a,*,†] Marcus F. Boehm,[a,‡] Jyun-Hung Chen,[a] Timothy A. Grese,[b] Donald S. Karanewsky,[a,†] Mark D. Leibowitz,[c] Sha Liu,[c] Dale A. Mais,[d] Christopher M. Mapes,[a,§] Anne Reifel-Miller,[e] Katheen M. Ogilvie,[e,‖] Deepa Rungta,[d] Anthony W. Thompson,[a,¶] John S. Tyhonas,[a] Nathan Yumibe[e] and Robert J. Ardecky[a]

[a]*Department of Medicinal Chemistry, Ligand Pharmaceuticals, Incorporated, 10275 Science Center Drive, San Diego, CA 92121, USA*
[b]*Discovery Chemistry Research, Lilly Research Laboratories, Indianapolis, IN, USA*
[c]*Department of Pharmacology, Ligand Pharmaceuticals, Incorporated, 10275 Science Center Drive, San Diego, CA 92121, USA*
[d]*Department of New Leads Discovery, Ligand Pharmaceuticals, Incorporated, 10275 Science Center Drive, San Diego, CA 92121, USA*
[e]*Division of Endocrine Research, Lilly Research Laboratories, Indianapolis, IN, USA*

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Abstract—New RXR-selective modulators possessing a 6-fluoro trienoic acid moiety (6Z olefin) or a fluorinated/heterocyclic-substituted benzene core ring, were synthesized in an expedient and selective way. A subset of these compounds was evaluated for their metabolic properties (exposure in IRC male mice) and show a dramatic increase of exposure compared to our reference compound, **3** (LG101506).

RXR/PPAR heterodimers play a major role in the regulation of both glucose (RXR/PPARγ) and lipid (RXR/PPARα) metabolism.[1] Recent reports have shown that synthetic RXR agonist (rexinoids) such as **1** (LG100268) and **2** (AGN194204) (Fig. 1) are insulin sensitizing agents. They bind to RXR and functionally stabilize the RXR/PPAR heterodimers. These compounds exhibit the capacity to control both hyperglycemia and hyperinsulinaemia.[2] Unfortunately the rexinoid class of RXR agonists also produce various undesirable side effects. The most serious of these adverse effects is a severe increase of triglyceride levels and a suppression of the thyroid hormone axis[2b] that prohibits their use for chronic therapy of type 2 diabetes. We have previously demonstrated that **3** is capable of selectively activating the RXR/PPAR heterodimers versus RXR/RARs and RXR/LXR heterodimers while RXR agonists like **1** are not. Because of this selective profile against various RXR heterodimers, we have called compounds like **3** RXR modulators. Moreover, we also have shown that **3** can decrease plasma glucose levels by the same magnitude as seen for BRL49653 (rosiglitazone®) in the *db*/*db* mouse model[3] while displaying a distinct side-effect profile in a mouse and a rat model compared to classic RXR agonists like **1**.[3] **3** possesses a (2*E*,4*E*,6*Z*) trienoic acid moiety coupled to a 3,5-di-*tert*-butyl-6-(2,2-difluoroethoxy) phenyl core. Historical studies have established the critical role that the trienoic 6*Z* olefin geometry plays in determining the selectivity of these compounds for RXR over RAR.[4] SAR studies have demonstrated that a fluorinated alkoxy side chain improved the in vivo activity of this type of molecules.[3] Here, we report the synthesis of new RXR-selective modulators possessing fluorinated or heterocyclic substitutions on the 3- and 5-positions of the core benzene ring as well as on the potentially labile 6 olefin of the trienoic acid moiety.

The derivatives **22**, **23a**,**b** and **24** possessing a fluorine in the 6-position of the trienoic acid moiety (6′-position,

*Corresponding author. E-mail: pmichellys@gnf.org
†Current address: Genomic Institute of the Novartis Foundation, San Diego, CA, USA.
‡Current address: Conforma Therapeutics, San Diego, CA, USA.
§Current address: Johnson and Johnson, San Diego, CA, USA.
‖Current address: Pfizer Inc, San Diego, CA, USA.
¶Current address: Roche Bioscience, Palo Alto, CA, USA.

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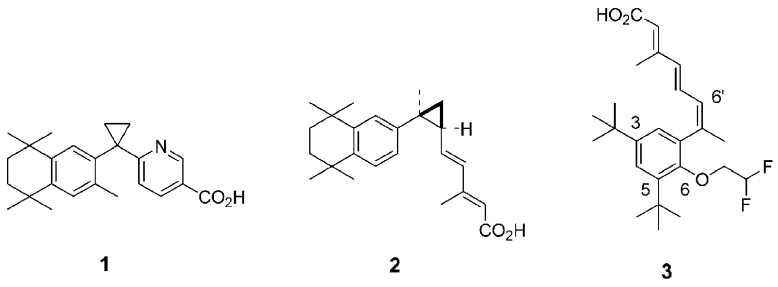
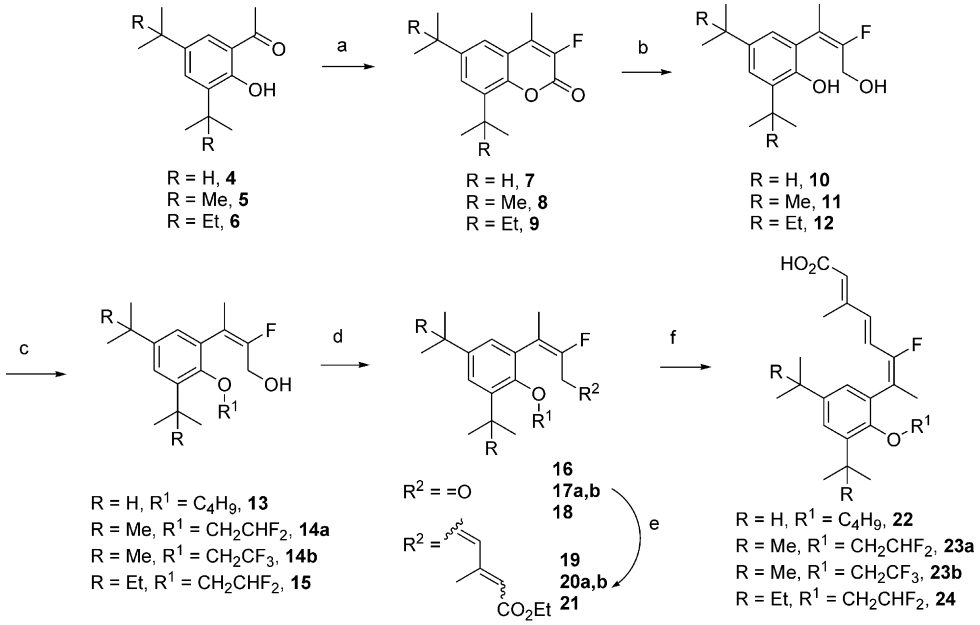
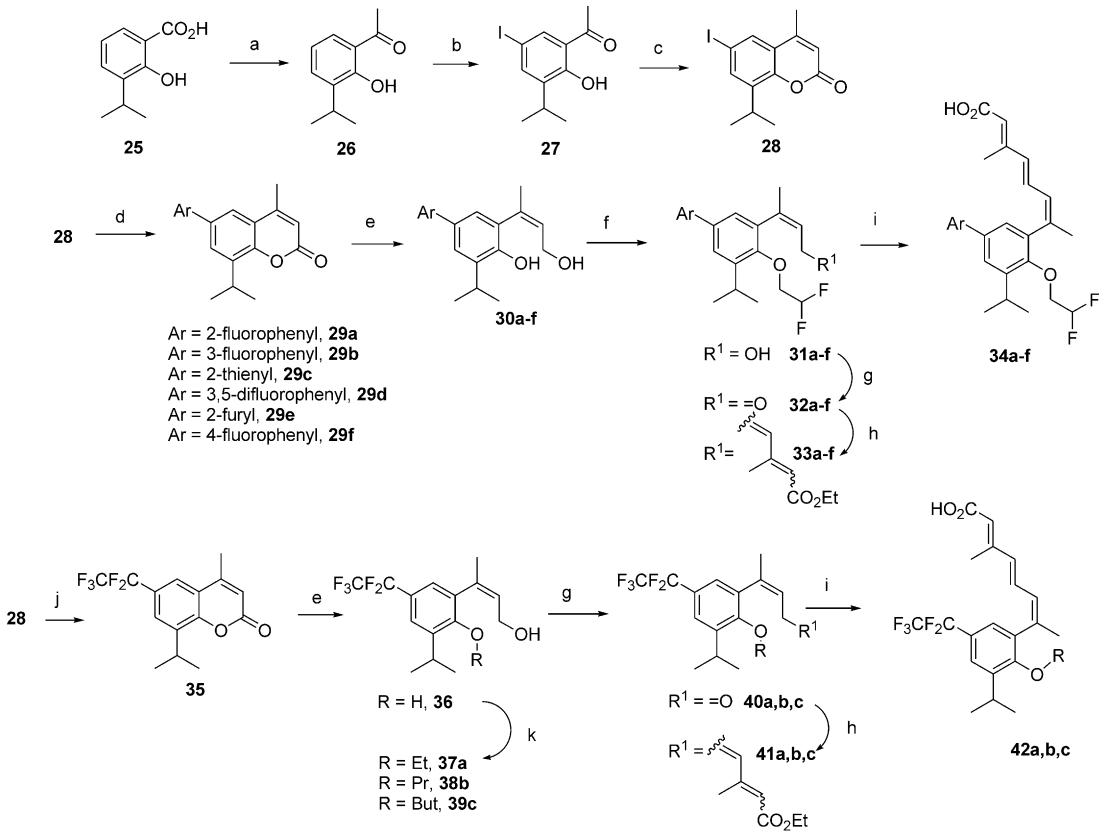


Figure 1. Selected RXR-selective scaffold.

Fig. 1) were synthesized in a selective and straightforward manner. The 3-fluorocoumarins **7–9** were readily prepared from the acetylphenols **4–6** using 2.5 equiv of triethyl-2-fluoro-2-phosphonoacetate and NaH in DMF at 60 °C in good to moderate yield. Interestingly, the yield of the reaction decreases dramatically when the substitution of the benzene ring increases (*iso*-propyl > *tert*-butyl > *tert*-amyl). Reduction of the 3-fluorocoumarins **7–9** appeared to be delicate. None of the alcohol **10–12** was isolated when **7–9** was treated with common reductive agents like LAH, $NaAlH_4$ or Dibal-H. Instead, the only products observed were the over-reduced phenolic alcohols. However, when treated with a large excess of $NaBH_4$ in methanol at 0 °C, the coumarins **7–9** reduced smoothly into the desired phenolic alcohols **10–12** in good yields (> 70%) except for **9** that produced **12** in 35% yield. The phenol present in **10–12** was then selectively alkylated using standard conditions (Cs_2CO_3, DMF, rt). The remaining allylic alcohols were then oxidized into the aldehydes **16–18** with TPAP/NMO without isomerization of the double bond. Then, **16–18** were subjected to a Horner–Wadsworth–Emmons reaction with the anion of triethyl-3-methyl-phosphonocrotonate (generated from slow addition of *n*-BuLi to a solution of triethyl-3-methylphosphonocrotonate in THF–DMPU, −78 °C) to afford the corresponding esters **19–21** in excellent yields (> 95%). Hydrolysis of **19–21** using 2 N aqueous LiOH in refluxing THF/MeOH afforded the crude acids **22–24**. Recrystallization of crude **22–24** from CH_3CN released the isomerically pure 6′-fluorotrienoic acids in excellent purity (> 99%). Scheme 2 describes the introduction of various aromatic groups and a pentafluoroethyl moiety in the 3-position of the core aromatic ring (*para* position of the alkoxy side chain, Fig. 1). We chose to introduce the desired groups on a coumarin-based substrate for convenience of synthesis. Fluoro-aromatic or hetero-aromatic groups were introduced using Suzuki coupling while the pentafluoroethyl group was introduced using copper chemistry on the coumarin **28** (easily synthesized from the commercially available 2-hydroxy-3-*iso*-propyl benzoic acid **25**). **29a–f** were synthesized in good yield by treatment of **28** with various commercially available boronic acids in the presence of $Pd(PPh_3)_4$ (5%) in refluxing toluene/ethanol. Introduction of the pentafluoroethyl group was realized using condensation of pentafluoroethyl iodide and **28** in the presence of CuBr in DMF at 60 °C in a pressure tube.[5] When subjected to these conditions; the corresponding coumarin **35** was isolated in 80% yield. The remaining steps of the



Scheme 1. Reagents: (a) 2-fluorotriethylphosphono acetate, NaH, DMF, 60 °C; (b) $NaBH_4$, EtOH, rt; (c) Cs_2CO_3, R^1-Br, DMF, rt; (d) TPAP, NMO, CH_2Cl_2; (e) triethyl-3-methyl-phosphonocrotonate, *n*-BuLi, THF–DMPU, −78 °C; (f) LiOH, THF–MeOH, reflux then HCl and recrystallization from CH_3CN.



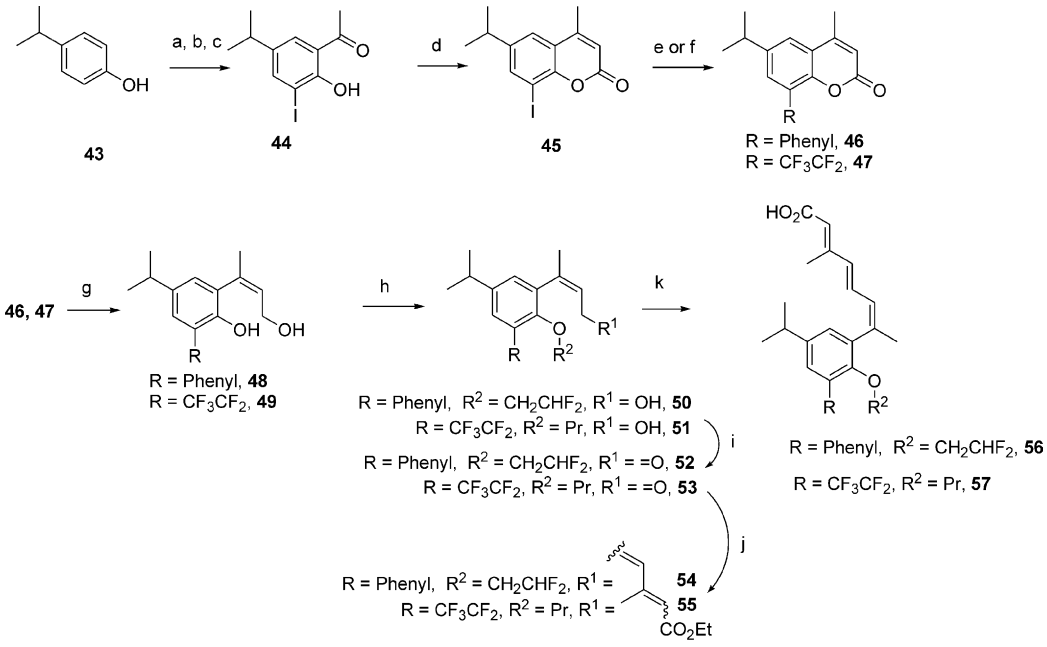
Scheme 2. Reagents: (a) MeLi, THF, 0 °C; (b) NIS, TsOH (cat.), CH_2Cl_2, reflux; (c) ethyltriphenyl phosphoranilidene acetate, toluene, reflux; (d) $ArB(OH)_2$, $Pd(PPh_3)_4$, toluene–ethanol, 2 N aq Na_2CO_3, reflux; (e) $NaAlH_4$, Et_2O, 0 °C; (f) Cs_2CO_3, CHF_2CH_2Br, DMF, rt; (g) TPAP, NMO, CH_2Cl_2; (h) triethyl-3-methyl-phosphonocrotonate, *n*-BuLi, THF–DMPU, −78 °C; (i) LiOH, THF–MeOH, reflux then HCl and recrystallization from CH_3CN; (j) CF_3CF_2I, CuBr, DMF, 60 °C, sealed tube; (k) Cs_2CO_3, R^1-Br, DMF, rt.

synthesis of **34a–f** and **42a–c** follows exactly the same synthetic route described in Scheme 1. The final trienoic acids **34a–f** and **42a–c** were recrystallized from CH_3CN and isolated in excellent isomeric purity (>99%).

Scheme 3 describes the synthesis of analogues possessing similar functional groups in the 5-position of the core aromatic moiety (*ortho* position of the alkoxy side chain, Fig. 1). Again, the iodo-coumarin (**46** and **47**) route proved to be very useful and allowed a quick and efficient synthesis of compounds **56** and **57**.

The competitive binding to RXRα, β and γ and RARα, β and γ of each compound has been characterized by using ^{3}[H]-9-*cis*-retinoic acid for RXRs and ^{3}[H]-all-*trans*-retinoic acid for RARs (shown as K_i, Table 1). The RXRα transcriptional activation profile of each compound was determined in CV-1 cells (the AOX response element used as the reporter).[6] For RXR, the efficacy is measured relative to LGD1069 (Table 2). We have already reported that **1** efficiently activates the RXR/PPARγ and RXR/RAR heterodimers alone or in combination (synergy assay) with 100 nM of BRL49653 (RXR/PPARγ synergy assay) or 3 nM of TTNPB (RXR/RAR synergy assay).[3,7] In the case of structures like **3**, the activation of the RXR:PPARγ heterodimer is of much lower amplitude and generally the use of a PPARγ agonist (e.g., BRL49653) amplifies the response of the modulator alone. The same protocol for characterizing activity at both RXR/PPARγ or RXR/RAR heterodimers[7] has been used to characterize the compounds described in this communication (**22**, **23a**,**b**, **24**, **34a–f**, **42a–c**, **56**, and **57**). The results are summarized in Table 2.

All the compounds from Table 1 show very good selectivity for RXRs over RARs. They bind with high affinity (K_i < 45 nM) to all RXR receptor sub-types, and show only weak RARα, RARβ or RARγ binding (Table 1). In some cases, a trend of selectivity for a specific RXR sub-type could be noted. For example, **22**, **23a**, **23b**, **34a**, and **34c** (Table 1, entries 3, 4, 5, 7, and 9) bind better to the RXRα receptor over RXRβ and RXRγ, **23c** and **42c** show better binding to RXRβ (Table 1, entries 6 and 15) while **34d** has the same binding affinity for all three RXR sub-types (Table 1, entry 10). Table 2 shows co-transfection data for the compounds **22**, **23a**,**b**, **24**, **34a–f**, **42a–c**, **56**, and **57**. All the compounds exhibit RXR homodimer antagonist activity (except **42a**, Table 2, entry 13) that has little RXR agonist activity. Previous SAR on similar structures possessing a 6-substituted trienoic acid moiety has shown that the RXR activity of such compounds can be altered by elongation of the 6-alkoxy side chain.[3] As this side chain gets bulkier, RXR homodimer agonist activity decreases and the RXR homodimer antagonist activity increases. While the same paradigm applies to the compounds presented in this communication (data



Scheme 3. Reagents: (a) NIS, TsOH (10%), CH_2Cl_2, rt; (b) *n*-BuLi, THF, then *N*,*N*-dimethylacetamide, −78 °C to rt; (c) NIS, TsOH (10%), CH_2Cl_2, reflux. Steps (d), (e), (f), (g), (h), (i), and (j) identical to Scheme 2.

Table 1. Binding data of RXR modulators (CV-1 cells). K_i calculated using [^{3}H]-9-*cis*-RA for RXR and [^{3}H]-ATRA for RAR[6] (all data shown in nM)

Entries	Compd	RXRα (K_i)	RXRβ (K_i)	RXRγ (K_i)	RARα (K_i)	RARβ (K_i)	RARγ (K_i)
1	**1**	12.9	75(17)	12	1550	9901	9901
2	**3**	1.5	4	84(6.0)	54(8.6)	9513	9513
3	**22**	4.4±2.1	24.4±12.6	27.8±14.1	3730	4623	>10,000
4	**23a**	7.8±1.2	11.5±2.3	13.2±2.6	8300	5681	>10,000
5	**23b**	4.1±2.4	15.5±3.1	12.4±3.5	4663	6008	>10,000
6	**24**	28.7±1.3	5.2±2.3	40.3±4.5	6592	5325	>10,000
7	**34a**	2.3±1.5	16.0±5.3	10.9±4.1	6756	3927	8764
8	**34b**	1.0±0.2	7.4±3.2	2.9±0.2	>10,000	3597	>10,000
9	**34c**	0.8±0.2	3.9±2.1	5.6±2.2	4555	2112	>10,000
10	**34d**	2.6±1.2	2.4±1.4	2.8±1.5	>10,000	1956	>10,000
11	**34e**	3.0±1.3	NT	NT	NT	NT	>10,000
12	**34f**	0.8±0.3	3.9±1.5	5.6±2.4	4555	2112	>10,000
13	**42a**	6.3±2.1	8.5±3.1	7.7±2.5	>10,000	>10,000	>10,000
14	**42b**	25.6±15.2	17.2±3.1	27.6±10.3	2500	1984	>10,000
15	**42c**	21.4±12.3	8.2±1.3	45.7±14.2	2750	2270	>10,000
16	**56**	6.1±4.2	12.3±6.5	22.5±10.3	4659	2612	>10,000
17	**57**	85.8±18.3	9.9±2.5	23.6±5.6	>10,000	>10,000	>10,000

not shown), these are close analogues to our lead compound **3**, and the in vitro profile of compound **3** was used as reference for their profiling and only RXR homodimer antagonists are shown in Table 2. All the compounds described in Table 2 strongly activate the RXR/PPARγ heterodimer in combination with BRL46953. Some compounds like **34a**, **34b** and **42a** have remarkable sub-nanomolar EC_{50}s in this assay. No RXR/RAR synergy was observed when tested in combination with 3 nM TTNPB except for **38a** (Table 1). This compound possesses a partial agonist profile, explaining the synergy observed for RXR/RAR. Previous experimental results have already shown that RXR/RAR synergy was generally correlated with the degree of RXR agonist activity observed with the tested compounds. We have described extensively in a previous communication the pharmacological relevance of compounds possessing the in vitro profile of **3**.[3] Here, all the compounds described have an in vitro profile as good as or better than the reference compound **3**.

Previous studies in our laboratories (unpublished data) have shown that molecules like **3** possessing a trienoic acid moiety have sometimes low PK exposure and the use of the Na salt was generally required to ensure a minimal exposure. We have evaluated the PK exposure of a subset of compounds described here in IRC mice. Each compound was dosed as the free acid in CMC/SLS/Povidone at 30 mg/kg (single dose). Table 2 shows the AUC results obtained with compounds **20a**, **31a** and **37a** compared to **3** as well as their respective T_{max} (data given in h) and C_{max} (expressed in μg/mL of plasma or

Table 2. In vitro evaluation of RXR modulators (CV-1 cells)[a]

Entries	Compds	RXRα Agonist Eff	RXRα Agonist EC_{50}	RXRα Antagonist Eff	RXRα Antagonist IC_{50}	RXRα/PPARγ Agonist Eff (EC_{50})	RXRα/PPARγ Agonist (EC_{50})	RARγ Agonist Syn.
1	**1**	75±5	17±3	12		161±25	18.6±5.1	6.9
2	**2**	4	NC	84±12	6.0±2.1	54±12	8.6±3.2	1.9
3	**22**	1	NC	92±10	4.7±1.6	47±15	4.1±2.2	1.5
4	**23a**	2	NC	87±8	6.8±2.3	63±18	6.4±5.3	1.8
5	**23b**	1	NC	92±11	7.2±2.5	30±11	12.2±5.0	1.5
6	**24**	2	NC	90±11	13.7±3.0	51±14	2.7±1.9	1.5
7	**34a**	1	NC	91±9	3.1±1.0	46±17	0.4±0.2	1.5
8	**34b**	1	NC	91±11	3.9±1.1	36±9	0.8±0.5	1.6
9	**34c**	3	NC	91±14	1.9±0.5	15±5	4.7±2.4	1.4
10	**34d**	2	NC	92±9	4.6±1.5	40±11	2.7±1.2	0.8
11	**34e**	3	NC	89±6	3.6±1.5	37±18	7.4±5.6	1.2
12	**34f**	2	NC	94±13	2.2±1.2	32±8	1.3±1.0	1.1
13	**42a**	44±13	92±15	39±8	5.3±2.0	99±13	0.7±0.4	2.8
14	**42b**	3	NC	91±12	5.1±2.2	59±11	2.7±1.9	1.4
15	**42c**	1	NC	93±14	4.0±1.2	71±14	7.4±4.3	1.2
16	**56**	5	NC	83±15	57.9±8.3	78±12	16.3±16.3	1.0
17	**57**	5	NC	80±11	10.7±6.4	29±11	15.1±9.4	1.0

[a]Efficacy for RXRα activity was measured against LGD1069.[6] RXR/PPARγ synergy mode calculated using 100 nM BRL49653, efficacy relative to BRL49653. RXR/RAR synergy calculated using 3 nM TTNPB, fold elevation over DMSO background[7] (all data shown in nM).

Table 3. Oral exposure study of selected RXR modulators in male ICR mouse[a]

Compd	Oral AUC (0–8 h) (μg × h/mL)	T_{max} (h)	C_{max}	
			(μg/mL)	(μM)
3	3.8±1.20	1	0.76±0.46	1.81±1.09
23a	17.7±4.95	3	3.69±1.89	8.41±4.31
34a	15.17±2.97	1	4.14±0.51	9.31±1.15
42a	14.41±3.35	1	4.43±2.82	10.24±6.52

[a]Dose formulation of the free acid in CMC/SLS/Povidone (30 mg/kg). Timepoints: 1, 3, and 8 h (serial sacrifice, $n = 3$/timepoint).

μM). All the compounds displayed in Table 2 show a dramatic increase in exposure (>3 times) and compound **20a** has a longer half-life (3 h instead of 1 h for **3**).

In conclusion, we have expediently synthesized a new series of RXR-selective modulators possessing a fluorinated trienoic moiety or various fluorinated, aromatic or heteroaromatic-substituted core ring systems from inexpensive starting materials. All the compounds selectively bind with high affinity to RXRs versus RARs. They also possess little or no RXR/RAR synergy (<2-fold) and activate the RXR/PPARγ heterodimer when used in combination with a PPARγ agonist (BRL49653). The PK profiles (AUC calculation) for a subset of this new series of compounds have been examined in IRC mice. The exposure data presented in Table 3 show a dramatic exposure increase compared to our lead compound **3**.

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