Examination of Specificity of Retinoid-like Compounds for Human RXRs, RARs, and RORs

Prajakta Albrecht (palbrecht@indigobioscienes.com)[1], Koji Toyokawa[1], Ewa Maddox[1], Palmer Cramer[1], Jack Vanden Heuvel[1,2], and Bruce Sherf[1]

[1]INDIGO Biosciences • 1981 Pine Hall Road • State College, PA 16801 • USA; [2]Department of Veterinary and Biomedical Sciences • The Pennsylvania State University • University Park, PA • USA

Need more time to read?
Scan this code or visit:
http://www.indigobiosciences.com/resources-publication/examination-specificity-retinoid-compounds-human-RXRs-RARs-and-RORs

Abstract

Vitamin A (retinol) and its metabolites play many physiological roles including cell differentiation, cell proliferation, energy homeostasis, circadian rhythm and immune response. These compounds are known to act through Retinoid Acid Receptors (RARs), retinoid-related Orphan Receptors (RORs) and Retinoid X Receptors (RXRs). These receptors are also important drug targets, although the specificity of many retinoid-like compounds for the retinoid receptors has not been carefully explored due to the lack of robust and selective assays. In the present work we examined 25 retinoid-like compounds for their ability to regulate RARα, β, γ, RXRα, β, γ and RORα, β, γ. Compounds were tested for agonistic as well as antagonistic activities towards these receptors. Additionally, cytotoxicity analyses were performed to confirm true antagonistic activity. Many of these compounds show a broad range of receptor activity, while others show moderate selectivity. Only a few of these test compounds show significant antagonist activity to these retinoid receptors, illustrating the need to screen more broadly for drug candidates with improved specificity in inhibiting the human RXRs, RARs and RORs, as well as selectivity in targeting one receptor sub-type (α, β, or γ) over the others.

Introduction

Retinoic Acid Receptors (RARs), and Retinoid-related Orphan Receptors (RORs) belong to Thyroid hormone receptor-like subfamily, whereas the Retinoid X Receptors (RXRs) comprise their own subfamily. Each of the three receptor groups are comprised of three distinct variants (α, β and γ). Furthermore, each group shows a different mode of action: RARs form heterodimers with RXRs, RORs act as monomers, and RXRs partner with all of the other non-steroid hormone nuclear receptors to regulate gene transcription.

Dysfunctions of these receptors are associated with many disease conditions. For example, RARs are associated with cancer, neurodegenerative diseases, and schizophrenia; RORs are associated with metabolic disorders, inflammation, atherosclerosis and autoimmune diseases; and RXRs are associated with cancer. As such, all three groups of retinoid receptors present important targets for the development of new drugs that, ideally, would be *specific* to individual retinoid receptor groups and *selective* in targeting the activities of either the α, β or γ receptor variants.

The current study analyzes a library of 25 retinoid-like compounds for *specific* and *selective* bioactivities using commercially available cell-based assay systems for human RARα, β and γ, RXRα, β and γ, and RORγ (INDIGO Biosciences Inc; www.indigobiosciencs.com). RORα and β assays were developed for this study.

Materials and Methods

Test compounds- A portion of the test compounds analyzed in this study comprise the Retinoid Library offered by Enzo Life Sciences Inc.: Puerarin, ec23, ATRA, 9CRA, 13CRAm 4 OH-phenylretinamide, AM 580, TTNPB, Methoprene acid, Methoprene, Acitretin, 4-OH retinoic acid, RO 41-5253, adapalene, and AC-55469. DHA, EPA, and Ursolic Acid (Sigma-Aldrich Corp.), TO9013171, BMS961, ER50891, HX531, HX630, and MM11253 (Tocris Biosciences), and SR1001 (Cayman Chemicals) were also tested.

Retinoid Receptor Assays- Human RARα, β and γ , RXRα, β and γ, and RORγ Reporter Assay Systems are commercially available in kit formats from INDIGO Biosciences Inc. INDIGO's Retinoid Receptor Assays utilize division competent proprietary cells engineered to provide constitutive, high-level expression of the respective retinoid receptors, and include the luciferase reporter gene functionally linked to a responsive promoter. Luciferase gene expression occurs after ligand-bound receptor undergoes nuclear translocation, DNA binding, recruitment and assembly of the co-activators and accessory factors required to form a functional transcription complex, culminating in expression of the target gene. The Human RORα and β assays were developed for this study. The scheme for performing agonist-mode retinoid receptor assays is depicted in **Figure 1**. Antagonist-mode retinoid receptor assays were performed in combination with INDIGO's Live Cell Multiplex (LCM) Assay, as depicted in **Figure 2**.

Figure 1. Agonist-mode Nuclear Receptor assay scheme.

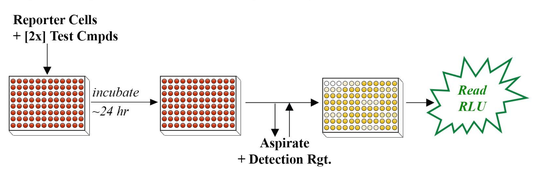
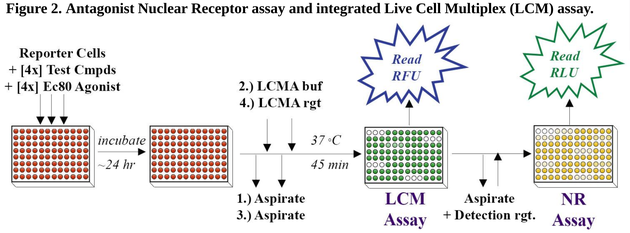


Figure 2. Antagonist Nuclear Receptor assay and integrated Live Cell Multiplex (LCM) assay.



Primary Screen

A panel of 25 'retinoid like' compounds were screened at 3 concentrations against each of the RARs, RORs and RXRs to assess their bioactivity and selectivity. Test compounds were screened at: • 5 μM, • 1.67 μM, and • 0.55 μM (except HX531 • 2.5 μM , • 0.83 μM, and • 0.27 μM); n=3

Figure 3. Human RAR alpha Assays.

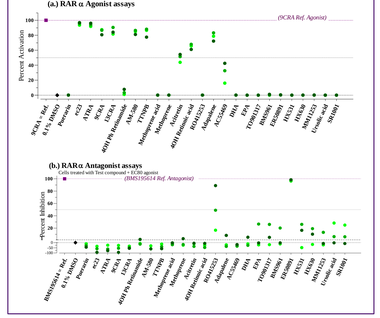


Figure 4. Human RAR beta Assays.

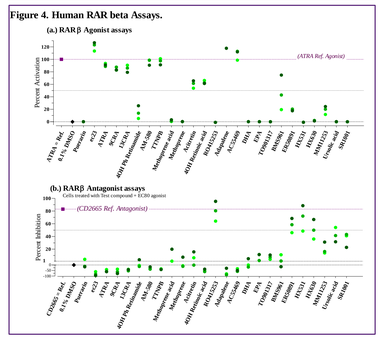


Figure 5. Human RAR gamma Assays.

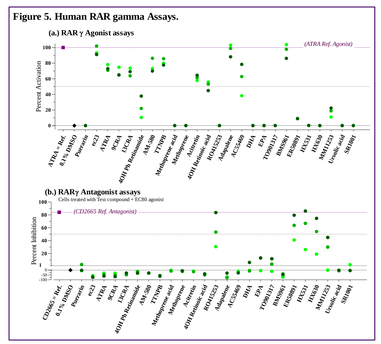


Figure 6. Human ROR alpha, Inverse-Agonist Assays.

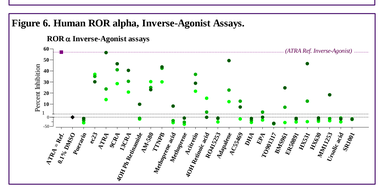


Figure 7. Human ROR beta, Inverse-Agonist Assays.

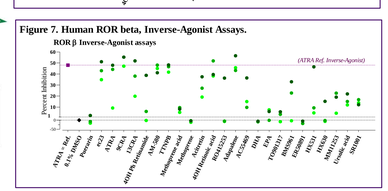


Figure 8. Human ROR gamma, Inverse-Agonist Assays.

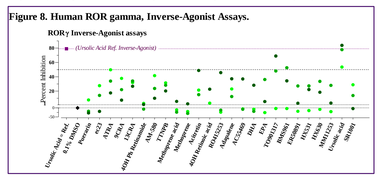


Figure 9. Human RXR alpha Assays.

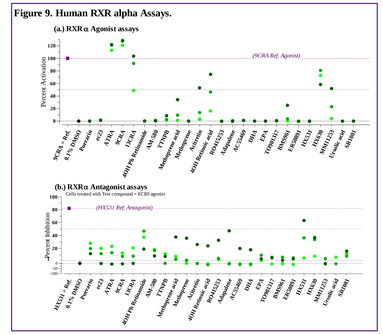


Figure 10. Human RXR beta Assays.

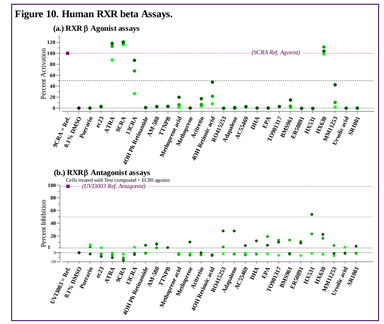
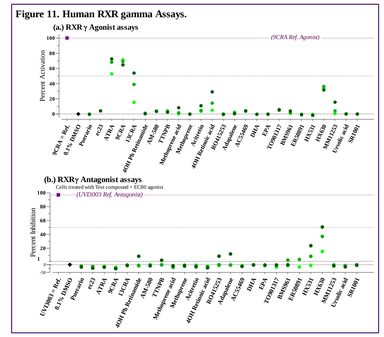


Figure 11. Human RXR gamma Assays.



POSITIVE HITS:
Any compound which shows 50% or more activation or inhibition in at least two out of the three doses tested were considered POSITIVE HIT and were analyzed further in Secondary screen for Dose Response Curves.

Positive compounds in AGONIST mode

Table 1a

Compound	[nM]	Percent (%) ACTIVATION					
		RARα	RARβ	RARγ	RXRα	RXRβ	RXRγ
Assay Reference	variable	9CRA Z' = 0.88	ATRA Z' = 0.87	ATRA Z' = 0.88	9CRA Z' = 0.83	9CRA Z' = 0.90	9CRA Z' = 0.90
ec23	*555*	94	113	93	1.7	3.3	3.9
	1,667	96	123	102	1.7	3.0	3.9
	5,000	97	127	91	1.2	2.6	4.0
ATRA	*555*	92	94	78	113	88	53
	1,667	94	89	71	122	113	68
	5,000	96	91	73	121	118	72
9 *cis*-Retinoic Acid	*555*	88	83	75	121	116	71
	1,667	87	88	65	129	121	69
	5,000	81	83	65	128	120	65
13 *cis*-Retinoic Acid	*555*	82	91	74	49	26	15
	1,667	90	86	64	92	68	39
	5,000	84	79	69	104	87	54
AM-580	*555*	85	99	73	1.3	2.9	4.0
	1,667	87	98	86	1.0	2.9	3.7
	5,000	81	91	70	0	2.5	3.3
TTNPB	*555*	87	101	80	1.5	2.6	4.1
	1,667	88	98	86	2.4	2.7	3.2
	5,000	77	91	78	8.6	3.3	2.4

Table 1b

Compound	[nM]	Percent (%) ACTIVATION					
		RARα	RARβ	RARγ	RXRα	RXRβ	RXRγ
Assay Reference	variable	9CRA Z' = 0.88	ATRA Z' = 0.87	ATRA Z' = 0.88	9CRA Z' = 0.83	9CRA Z' = 0.90	9CRA Z' = 0.90
Acitretin	*555*	44	54	58	3.1	3.7	3.5
	1,667	52	61	61	14	6.6	4.7
	5,000	54	66	64	53	17.1	11
4-OH Retinoic Acid	*555*	66	66	56	16	7.7	5.0
	1,667	68	62	53	46	22	14
	5,000	61	61	45	75	47	29
Adapalene	*555*	79	131	103	0	1.6	2.3
	1,667	83	130	99	0	1.0	1.6
	5,000	72	118	88	0	0	0
AC-55469	*555*	16	99	38	0	1.8	3.4
	1,667	33	113	63	1.0	2.4	3.9
	5,000	43	112	78	1.1	2.7	4.2
BMS961	*555*	0	19	104	0	1.0	0
	1,667	0	43	98	3.6	3.5	1.6
	5,000	1.1	75	86	25	15	3.9
HX630	*555*	0	2.1	0	73	99	34
	1,667	0	2.1	0	81	111	36
	5,000	0	1.3	0	58	104	32

Positive compounds in ANTAGONIST mode

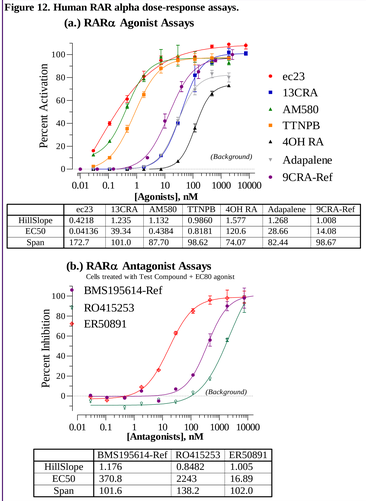
Table 2

Compound	[nM]	Percent (%) INHIBITION								
		RARα	RARβ	RARγ	RORα	RORβ	RORγ	RXRα	RXRβ	RXRγ
Assay Reference	variable	BMS 195614 Z' = 0.78	CD2665 Z' = 0.79	CD2665 Z' = 0.81	ATRA	ATRA	Ursolic Acid Z' = 0.77	HX531 Z' = 0.89	UVI3003 Z' = 0.70	UVI3003 Z' = 0.96
trans-Retinoic Acid	*555*	0	0	0	14	9	50	23	0	0
	1,667	0	0	0	24	44	34	13	0	0
	5,000	0	0	0	52	48	18	0	0	0
RO41-5253	*555*	17	64	31	0	0	0	2.4	0	0
	1,667	49	80	53	0	0	0	4.3	2.5	0
	5,000	89	95	84	0	36	46	33	28	8.5
ER50891	*555*	96	46	41	0	0	0	0	0	0
	1,667	98	58	64	0	0	27	5.2	11	3.8
	5,000	98	68	79	0	0	3.2	3.6	8.8	0.4
HX531	*278*	0	49	26	0	4.7	0	5.0	0	0
	833	26	72	67	13	9.3	27	36	23	8.3
	2,500	16	88	86	47	46	22	63	54	24
HX630	*555*	0	36	19	0	0	0	7.9	0	15
	1,667	19	50	54	0	0	34	34	16	37
	5,000	10	67	75	0	15	18	37	22	51
Ursolic Acid	*555*	28	54	0	0	12	54	6.1	2.2	0
	1,667	6.2	41	0	0	15	78	0	0	0
	5,000	0	32	0	0	22	84	0.7	0	0

Secondary Screen

Test compounds showing positive hits in 1° screen were tested at concentrations starting at 10 μM (serial dilutions of 1:3). The reference compounds in each assay are denoted by suffix "-Ref". Reference compounds were tested at same concentrations as the test compounds. Results from the Live Cell Multiplex Assay demonstrated that these test compounds did not show cytotoxicity at the highest dose tested in antagonist assays, indicating that the positive hits in antagonist mode are not 'False Positives' (results not shown).

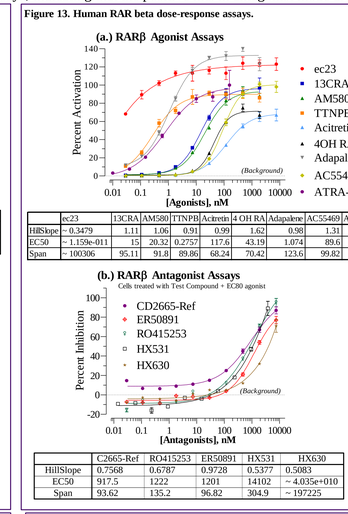
Figure 12. Human RAR alpha dose-response assays.



	ec23	13CRA	AM580	TTNPB	4OH RA	Adapalene	9CRA-Ref
HillSlope	0.4218	1.235	1.132	0.9860	1.577	1.268	1.008
EC50	0.04136	39.34	0.4384	0.8181	120.6	28.66	14.08
Span	172.7	101.0	87.70	98.62	74.07	82.44	98.67

	BMS195614-Ref	RO415253	ER50891
HillSlope	1.176	0.8482	1.005
EC50	370.8	2243	16.89
Span	101.6	138.2	102.0

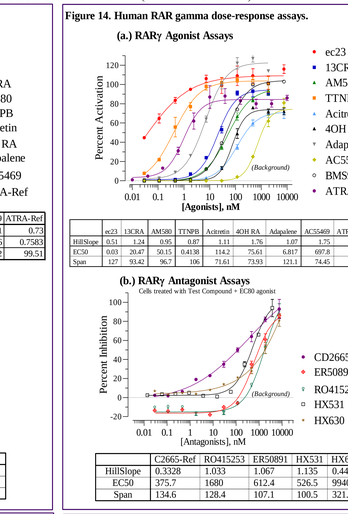
Figure 13. Human RAR beta dose-response assays.



	ec23	13CRA	AM580	TTNPB	Acitretin	4 OH RA	Adapalene	AC55469	ATRA-Ref
HillSlope	~ 0.3479	1.11	1.06	0.91	0.99	1.62	0.98	1.31	0.73
EC50	~ 1.159e-011	15	20.32	0.2757	117.6	43.19	1.074	89.6	0.7583
Span	~ 100306	95.11	91.8	89.86	68.24	70.42	123.6	99.82	99.51

	C2665-Ref	RO415253	ER50891	HX531	HX630
HillSlope	0.7568	0.6787	0.9728	0.5377	0.5083
EC50	917.5	1222	1201	14102	~ 4.035e+010
Span	93.62	135.2	96.82	304.9	~ 197225

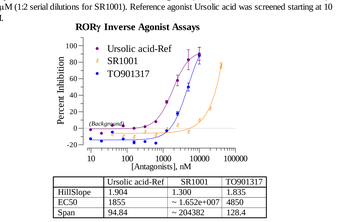
Figure 14. Human RAR gamma dose-response assays.



	ec23	13CRA	AM580	TTNPB	Acitretin	4OH RA	Adapalene	AC55469	ATRA-Ref	BMS961
HillSlope	0.51	1.24	0.95	0.87	1.11	1.76	1.07	1.75	1.28	1.103
EC50	0.03	20.47	50.15	0.4138	114.2	75.61	6.817	697.8	0.9859	46.98
Span	127	93.42	96.7	106	71.61	73.93	121.1	74.45	81.22	104.6

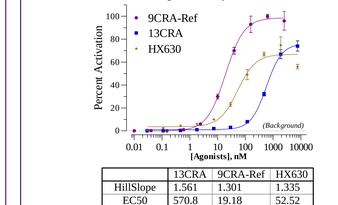
	C2665-Ref	RO415253	ER50891	HX531	HX630
HillSlope	0.3328	1.033	1.067	1.135	0.4453
EC50	375.7	1680	612.4	526.5	99400
Span	134.6	128.4	107.1	100.5	321.8

Figure 15. Human ROR gamma inverse-agonist dose-response assays. Test compounds were screened at concentrations starting at 10 μM (1:2 serial dilutions for TO9013171) & 40 μM (1:2 serial dilutions for SR1001). Reference agonist Ursolic acid was screened starting at 10 μM.



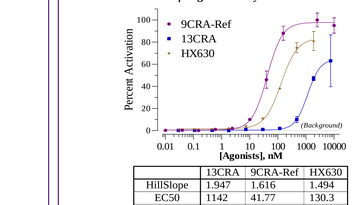
	Ursolic acid-Ref	SR1001	TO901317
HillSlope	1.904	1.300	1.835
EC50	1855	~ 1.652e+007	4850
Span	94.84	~ 204382	128.4

Figure 16. Human RXR alpha Agonist dose-response assays.



	13CRA	9CRA-Ref	HX630
HillSlope	1.561	1.301	1.335
EC50	570.8	19.18	52.52
Span	74.95	98.66	63.22

Figure 17. Human RXR beta Agonist dose-response assays.



	13CRA	9CRA-Ref	HX630
HillSlope	1.947	1.616	1.494
EC50	1142	41.77	130.3
Span	64.12	97.18	82.07

Conclusions

Considering all the compounds that hit any receptor at percent activation or inhibition of more than 50% , they can be classified as:

Non Specific Compounds : **ATRA, 9CRA,** and **13CRA** are not specific agonists as they show activity against all 6 receptors. Additionally, ATRA shows inverse-agonistic activity against RORα and RORγ.

Moderately Specific Compounds: **4-hydroxy retinoic acid** show agonistic activity against all three RARs and RXRα. **HX630** shows agonistic activity against RXRβ and RXRγ and antagonistic activity against RARα, RARβ and RXRγ. **HX531** shows antagonistic activity against RARα, RARβ and RXRα, RXRβ.

Specific Compounds: **ec23, AM580, TTNPB, Acitretin,** and **Adapalene** show agonistic activity against RARs, whereas **AC55469** and **BMS961** show agonistic activity against RARβ and RARγ with little or no activity against RARα, respectively. **RO415253** and **ER50891** show antagonistic activity only against the RARs. **Ursolic acid** shows inverse-agonistic (or antagonistic activity) against RORγ and RARβ.