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Identification of novel subtype selective RAR agonists

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ABSTRACT

Drugs targeting retinoid receptors have been developed to treat a variety of therapeutic indications, but their success has been limited in part due to lack of selectivity. A novel functional cell-based assay R-SAT™ was employed to screen a small molecule chemical library and identify a variety of novel RAR agonists with various subtype selectivities, including RARβ/γ and RARγ selective agonists. A novel class of synthetic compounds that distinguishes between the different RARβ isoforms is described. This pharmacophore displays anti-proliferative activity and induces differentiation in a neuronal cell line, consistent with a classical retinoid mechanism of action while providing unique subtype selectivity. These novel subtype selective RAR agonists could serve as powerful tools to probe into subtype and isoform-specific retinoid function.

1. Introduction

Natural and synthetic retinoids display pleiotropic effects that have led to their development as therapeutic drugs in a number of indications including acne, psoriasis and cancer. However, the prevalence of acute and chronic side effects has limited their wide applicability as therapeutic drugs of choice [1]. The observed toxicity effects are believed to originate from lack of selectivity, by acting indiscriminately at both the retinoid acid receptor (RAR) and retinoid X receptor (RXR) subtypes.

RAR and RXR receptors belong to the family of steroid hormone receptors, a class of ligand-activated transcription factors and multiple subtypes have been described for both RAR (RARα, NR1B1; RARβ, NR1B2; RARγ, NR1B3) and RXR (RXRα, NR2B1; RXRβ, NR2B2; RXRγ, NR3B3). Furthermore, isoforms for each of the subtypes have also been described. For example, the RARβ gene encodes several variants that are expressed from two different promoters (P1 and P2) [2]. To date, five transcriptional variants have been reported. RARβ1 and RARβ3 are under the regulation of P1 while RARβ2 and RARβ4 are transcribed from the proximal promoter P2. More recently, a novel isoform RARβ5 has been identified whose expression is directed from a distinct promoter P3 [3]. Functionally, these isoforms differ uniquely by the length and nature of their N-terminus ligand-independent transcriptional activation domain (AF-1). In humans, only the RARβ2, RARβ4 and RARβ5 are expressed in normal adult cells [4], while RARβ1 is mostly expressed in fetal tissues and some lung carcinomas. RARβ3 has not been detected in human tissues so far [5].

Such a breadth of receptor subtypes and isoforms most likely explains the adverse side effects that the current retinoid-like chemistries display, hence their limited use as therapeutics. Herein, we report on the identification of novel subtype and isoform selective RAR agonists using a novel functional cell-based assay.

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2. Materials and method

2.1. Materials

AM-580 and 9-*cis*-retinoic acid were purchased from Calbiochem. HEK-293, MCF-7 and NIH-3T3 cells were all from ATCC. NTERA-2 cells were acquired from DSMZ, Germany.

2.2. R-SAT™ assays

R-SAT™ (Receptor Selection and Amplification Technology) is a proprietary cell-based functional assay that allows one to monitor receptor-dependent proliferative responses and has been described elsewhere [6]. The technology has been validated for a number of receptors ranging from GPCRs [7], RTKs [F.P. not shown] and cytokine receptors [6]. Its principle resides in the genetic selection and amplification of the nuclear receptors in a ligand-dependent manner. This process is achieved by partial cellular transformation by overcoming contact inhibition and the loss of growth factor dependency. Monitoring activity is achieved by transfecting the cells with a β-galactosidase reporter gene vector whose expression is under a constitutively active promoter. Briefly, NIH-3T3 fibroblasts were plated overnight in DMEM 10% calf serum (Gibco BRL) and grown to 60–70% confluency prior to transfection. Transient transfections were performed using Superfect or Polyfect (Qiagen) according to manufacturer's instructions. Typically, a transfection mix would consist of the receptor and the β-galactosidase expression vectors. Sixteen hours post-transfection, cells were harvested and frozen, then subsequently incubated with different doses of ligand in DMEM containing 2% Cyto-SF3 (Kemp Technologies) to generate a dose–response curve. After 5 days, plates were developed by adding onto the washed cells a solution containing the β-galactosidase substrate *o*-nitrophenyl-d-galacto pyranoside ONPG (in phosphate-buffered saline with 5% Nonidet P-40 detergent) as described [6]. Plates were read using a microplate reader at 405 nm. Data from R-SAT™ assays were fit to the equation: $r = A + B(x/(x + c))$, where A = minimum response, B = maximum response minus minimum response, c = EC50, r = response, and x = concentration of ligand. Curves were generated using the curve fitting software packages Excel Fit and GraphPad Prism (San Diego, CA).

2.3. Luciferase assays

HEK-293 cells were plated overnight into 96-well plates (10,000 cells/well). Cells were then transfected with each of the RAR receptor subtypes (15 ng/well) along with the 3 *RARE-Luciferase vector (Translucent) using Polyfect according to manufacturer's protocol (Qiagen). Sixteen hours post-transfection, cells were serum starved and incubated for 48 h in the presence of ligands as described in the text. Luciferase activity was then measured using a commercially available kit.

2.4. MCF7 cell proliferation inhibition assays

MCF7 breast cancer cells were plated overnight into six-well plates (50,000 cells/well). Cells were then incubated for 4 days in the presence of compounds as described in the text. Media and compounds were renewed every other day. Cells were then trypsinized and counted.

DNA synthesis was evaluated through [^{3}H] thymidine incorporation. Exponentially growing MCF7 cells were plated overnight into six-well plates. [^{3}H] thymidine was added to the media at 0.5 μCi/ml for 24 h in the presence or absence of compounds as described in the text. After 24 h, media was removed and cells washed twice in cold buffered PBS. Cells were treated and harvested in the presence of ice-cold solution I (NaOH 0.1 M, EDTA 5 mM) then 10% TCA. The extracts were then filtered onto GF/C filters and bound radioactivity measured using a scintillation counter.

2.5. Neurite outgrowth assay

Embryonal carcinoma NTERA-2 cells were plated overnight on chamber slides (2000 cells per chamber) in 10% FBS DMEM. Cells were then treated for 1 week with various compounds (10 μM) as described in the text. Media and compounds were renewed every other day. Slides were then fixed in PBS 4% formaldehyde for 10 min at room temperature then stored at 4 °C. Slides were then submitted to immuno-cytochemistry, thus incubated with either neuronal differentation marker GAP43 (Santa Cruz) or Neurofilament L (chemicon International) at recommended dilution in PBS 0.5% BSA overnight at 4 °C, washed then incubated in presence of a conjugated FITC secondary antibody (Santa Cruz) for an hour at room temperature.

3. Results

To identify novel and subtype selective small molecule agonists of the RAR receptors, a functional mammalian cell-based assay (R-SAT™, Receptor Selection and Amplification Technology) was developed for each of the three RAR subtypes (α, β, γ) [8]. NIH-3T3 mouse fibroblasts were transiently transfected with mammalian expression vectors encoding the different human RAR subtypes along with an expression vector encoding a constitutively expressed beta-galactosidase reporter gene. As summarized in Table 1, each RAR subtype tested, namely RARα, RARβ1, RARβ2 and RARγ, produces an agonist response that is consistent with previously published results [9]. As expected, the RAR subtypes respond to the natural ligand all-*trans*-retinoic acid (ATRA) with affinity in the nM range. Similarly, the RAR selective ligand AM-580 produces a potent response at these receptors in R-SAT™ assays, consistent with activity reported in binding and functional assays [10].

An ultra high-throughput (uHTS) screen of the RAR subtypes was performed using R-SAT™ against a library of about 160,000 small organic molecules. The compound library is diverse, sharing a significant overlap (67%) in chemical space with the reference MDDR (MDL Drug Data Report) library but also exhibiting unique chemical space properties. The library is also heavily biased in favor of compounds displaying drug likeness characteristics [11]. Upon screening, a number of novel chemical entities that displayed various selectivities towards the different RAR subtypes were uncovered. Selected scaffolds identified are presented in Table 2 including

Table 1 – Potency of retinoid ligands ATRA and AM-580 in the RSAT™ assay

		Binding pIC50	Activation pEC50	R-SAT pEC50
all-*trans*-RA (ATRA)	RARα	8.2	8.2	7.6 ± 0.3 (7)
	RARβ1	n.d.	n.d.	7.6 ± 0.4 (6)
	RARβ2	8.3	8.6	8.2 ± 0.4 (9)
	RARγ	8.2	8.5	7.3 ± 0.3 (5)
AM-580	RARα	7.4	8.5	7.1 ± 0.5 (26)
	RARβ1	n.d.	n.d.	7.3 ± 0.4 (21)
	RARβ2	6.1	7.6	7.7 ± 0.4 (42)
	RARγ	5.3	7.4	7.6 ± 0.5 (19)

Potencies in R-SAT™ are expressed as pEC50 (−log [EC50]). For comparison purposes, binding and activation data from the literature is presented [9]. R-SAT™ data represents the average ±S.D. The number into parentheses indicate the number of experiments performed in duplicate. n.d: not determined.

AC-41848 a potent RARβ1/γ agonist, AC-93253 a potent RARα agonist, and AC-55649 a potent RARβ2 isotype selective agonist.

Interestingly, the functional cell-based R-SAT™ assay identified permanently cation-charged molecules such as AC-93253 and AC-41848 with relatively high affinities for a subset of RAR subtypes. Because of the intracellular localization of nuclear hormone receptors (cytoplasm, nucleus), these molecules had to cross the cell membrane in order to complex with the RARs within the cells. Furthermore, the compounds showed a selective activity profile within the RAR subtypes, which rules out non-specific interactions. Despite the presence of the permanent cation, the physico-chemical properties of both compounds are within desired parameters, with clog *P* values around 2.0 and molecular weights below 400 Da. While AC-41848 appears completely novel from a structural perspective, AC-93253 is slightly reminescent of retinoic acid, especially when considering the *trans*-olefinic system. Therefore, the benzathiazolium motif of AC-93253 could be predicted to be either a carboxylic acid bioisoster or a prodrug for the corresponding carboxylic acid derivative; however, the 5,6-dimethyl-benzthiazolium analog of AC-93253 was also tested at the different RAR subtypes, but showed no activity (data not shown).

These structurally novel RAR ligands thus possess a permanent cation as opposed to the common anionic

Table 2 – Novel subtype selective RAR agonists

		AC-41848	AC-55649	AC-93253
RARα	Eff	16 ± 7	32 ± 18	**89 ± 28**
	pEC50	NA	5.6 ± 0.0	**6.3 ± 0.5**
	N	3	16	**4**
RARβ1	Eff	**51 ± 5**	29 ± 13	67 ± 21
	pEC50	**6.2 ± 0.1**	5.7 ± 0.1	NA
	N	3	13	2
RARβ2	Eff	24 ± 24	**99 ± 29**	35 ± 16
	pEC50	NA	**6.9 ± 0.4**	6.9 ± 0.0
	N	3	**17**	2
RARγ	Eff	**92 ± 31**	38 ± 16	11 ± 4
	pEC50	**5.9 ± 0.2**	5.2 ± 0.3	NA
	N	5	10	2

The data is expressed as averages of % efficacy (Eff) and potency (pEC50) (±S.D.) as obtained using R-SAT™. Efficacy is relative to AM-580 defined as 100%. *N* indicates the number of independent experiments. NA: not active at 10 μM. The selectivity of compounds is emphasized in bold.

ionizable carboxylic acid containing ligands. We infer that the permanent cation could have beneficial properties for topical administration having limited systemic exposure and fast elimination. Cations are usually more permeable via the paracellular route than uncharged compounds, which in turn are more permeable than anions. However, a transcellular route cannot be excluded, both AC-93253 and AC-41848 having highly conjugated pi-systems delocalising the charge.

Even though the physico-chemical properties of AC-55649 are unfavorable, especially with regards to the high lipophilicity ($C\log P > 9$) and low solubility (0.01 mg/mL), they corroborate well with the properties of known retinoid ligands currently on the market or under investigation. For instance, the average calculated $\log D$ and $\log P$ for the 348 retinoid ligands listed in the MDDR database were 5.6 and 7.6, respectively, compared with $C\,\log D$ and $C\,\log P$ values of 6.1 and $C\log P$ 7.6 for AC-55649, respectively. Experimental values were determined and are as follows: $\log D$ 4.5, $\log P$ 7.9 and solubility <0.01 mg/mL. The corresponding calculated values for Tretinoin, a drug marketed for systemic treatment, are $C\,\log D$ 6.1 and $C\log P$ 8.4.

Curiously, AC-93253 and AC-55649 were originally developed in the context of dyes and liquid crystals, respectively. To evaluate the potential use of screening such compounds that at a first glance are not "drug like" nor "hit like", AC-55649 was selected for further in vitro studies and SAR efforts in our laboratory. These efforts led to the identification of a 10-fold more potent chemical lead with excellent DMPK properties. The details of these findings are being reported elsewhere [12].

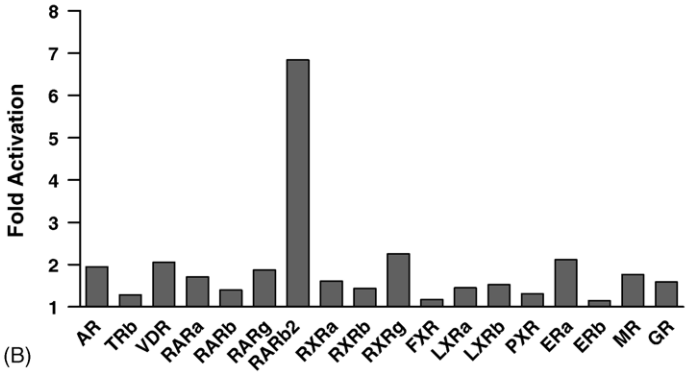
The chemotype defined by AC-55649 and structural analogs is of particular interest because of its ability to distinguish between RARβ2 and the other retinoid RAR subtypes including RARβ1. Profiling of AC-55649 and a few related analogs against the closely related retinoid X receptors (RXRs) has confirmed and expanded the selectivity nature of this class of compounds. Indeed, no activity is evident at any of the three RXR subtypes (α, β, γ) (Fig. 1A). Furthermore, a broader profiling effort against a large number of the nuclear receptor family members has revealed that this class of compounds is extremely selective for the RARβ2 subtype, with at least a 50–100-fold selectivity index (Fig. 1B).

To further characterize these novel compounds with retinoid-like activities, their ability to modulate the transcriptional properties of retinoid receptors was investigated. Human HEK-293 cells were thus transiently transfected with each of the RAR subtypes and the effects of the ligands on the transcriptional activation at the RAR response element (RARE) examined (Fig. 2). AC-41848 stimulated transcriptional activation through RARβ1 and RARγ. AC-93253 behaved as a selective RARα agonist. Finally, AC-55649 was a potent and selective activator of RARβ2 transcriptional activity.

In a number of cellular systems, retinoids display potent anti-proliferative properties. These effects are typically the result of growth arrest and subsequent apoptosis. For example, retinoids can promote apoptosis in neoplastic cells such as the human breast cancer cell line MCF7 [13]. In this setting, retinoids such as ATRA produce initially a dose–response inhibition of the proliferation of MCF7 cells that

		RARα	RARβ1	RARβ2	RARγ	RXRα	RXRβ	RXRγ
AC-55649	Eff	38+/-18	29+/-13	**99+/-29**	38+/-16	24+/-16	42+/-21	33+/-21
	pEC50	5.6+/-0.0	5.7+/-0.1	**6.9+/-0.4**	5.2+/-0.3	NA	5.6+/-0.3	6.1+/-0.2
	N	16	13	**17**	10	7	9	9
AC-55993	Eff	29+/-9	39+/-13	**93+/-22**	48+/-38	49+/-25	38+/-17	43+/-17
	pEC50	5.9+/-0.1	6.2+/-0.5	**7.4+/-0.4**	6.0+/-0.1	5.9+/-0.4	6.3+/-0.2	5.8+/-0.1
	N	19	16	**25**	12	10	9	8
AC-56061	Eff	40+/-9	48+/-13	**76+/-17**	17+/-12	38+/-8	30+/-13	41+/-5
	pEC50	5.9+/-0.1	5.9+/-0.1	**7.4+/-0.6**	NA	5.2+/-0.1	5.2+/-0.0	6.4+/-0.1
	N	5	4	**9**	3	3	3	8

(A)



(B)

Fig. 1 – AC-55649 and related analogs are RARβ2 isoform selective agonists (A) Selectivity of AC-556149 and analogs at the different RAR and RXR subtypes. R-SAT™ data is expressed as % efficacy and pEC50. Selectivity is highlighted in bold. Efficacy at the RAR subtypes is relative to the maximum activity seen with AM-580 (100%), while the efficacy at the RXR subtypes is relative to the maximum activation seen with 9-*cis*-retinoic acid (9-*cis*-RA) defined as 100%. (B) Selectivity profile of AC-55649 (10 μM) against a large panel of nuclear receptors as obtained using R-SAT™. Data is expressed as fold activation over baseline. Data presented is the average of at least three independent experiments.

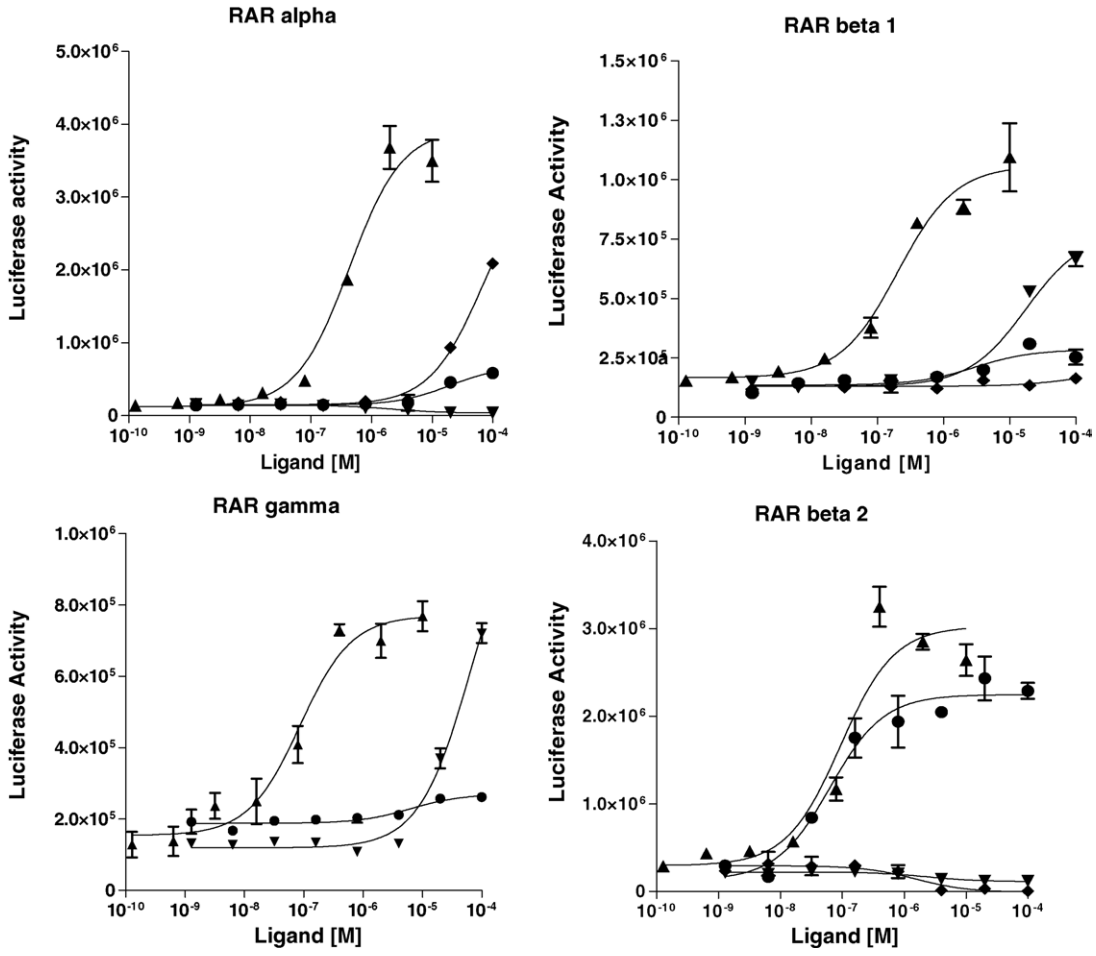


Fig. 2 – Transcriptional activation by novel subtype selective RAR agonists. Human HEK-293 cells were transiently transfected with each of the RAR subytpes in the presence of the RARE-Luc reporter gene. Compounds were added at the doses indicated and the luciferase activity quantified 48 h post-treatment. Data represents an example of a representative experiment. ▲: 9-*cis*-RA; ▼: AC-41848; ◆: AC-93253; ●: AC-55649.

ultimately evolves towards programmed cell death [14]. The RARβ subtype appears to play a critical role in the mediation of these inhibitory and apoptotic effects: treatment of MCF7 cells with retinoic acid leads to increased expression of RARβ which correlates with growth arrest and apoptosis [15]. The effects of our RARβ2 small molecule agonists in this setting were therefore investigated. Reference compounds ATRA and AM-580 produce a dose dependent inhibition of MCF7 proliferation (Fig. 3A). Similarly, AC-55649 and AC-55993 significantly inhibit the cellular growth of MCF7 cells in a manner consistent with their relative potencies as defined in the functional R-SAT™ assay. Furthermore, both compounds appear to act through inhibition of DNA synthesis as seen in the [^{3}H] thymidine incorporation experiments (Fig. 3B). The extent of the inhibition is comparable to the one seen in the presence of all-*trans*-retinoic acid.

Retinoids are also known to mediate survival and differentiation of certain cell types, including neurons [16,17]. Several studies of embryonic neuronal cells have indicated that retinoic acid stimulate neurite outgrowth [18]. In particular, this phenomenon appears to be mediated through activation of the RARβ2 receptor isoform as demonstrated by RARβ2 upregulation following RA treatment [19], as well as by the use of relatively RARβ selective agonists and antagonists [20]. The ability of RARβ2 isotype selective agonist chemistries to promote neurite outgrowth was thus investigated in the retinoid responsive embryonal carcinoma cell line NTERA-2 [21]. Cells were stimulated either with retinoic acid or AC-55649 and AC-55993 for 1 week before neurite outgrowth was assessed through staining with the neuronal differentiation marker GAP43. In contrast to untreated cells, retinoic acid treated cells display a large increase in neurite outgrowth as revealed by immunocytochemistry (Fig. 4). The RARβ2 selective agonists AC-55649 and AC-55993 also induce neurite outgrowth of NTERA-2, indicating that they mediate neuronal differentiation in a manner similar to that seen with retinoic acid. Similar results were evident with another differentiation marker, Neurofilament L (data not shown).

4. Discussion

We have developed a novel way of assessing functional activity of retinoid receptors, a subgroup of the nuclear

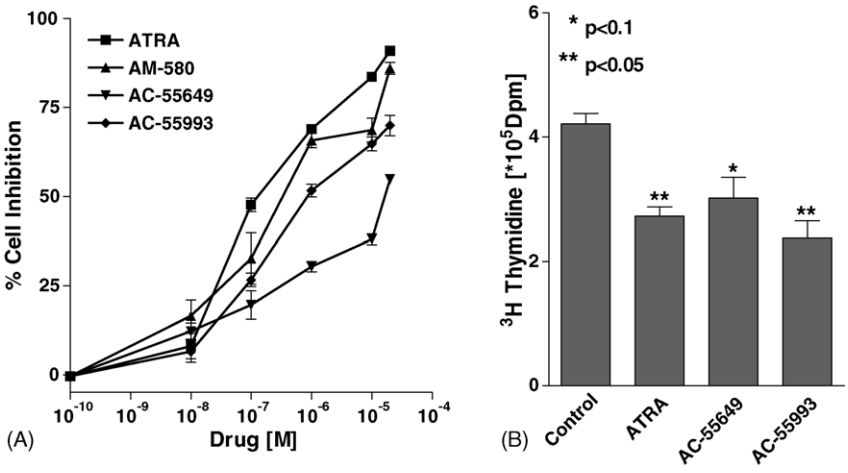


Fig. 3 – Growth inhibition of MCF7 cells by selective RARβ2 agonists. (A). Dose dependent inhibition of MCF7 proliferation. Cells were treated for 4 days with either ATRA, AM-580, AC-55649 and AC-55993 and the cell number evaluated with a cell coulter. Data is expressed as a percentage of cell inhibition relative to untreated cells. Results are the average of three independent experiments. (B). DNA synthesis was evaluated through incorporation of [^{3}H] thymidine. Exponentially growing MCF7 cells were incubated for 24 h with [^{3}H] thymidine and treated with the reference retinoid agonist ATRA (10 μM) or AC-55649 and AC-55993 (10 μM). Cumulative [^{3}H] thymidine is reported.

hormone receptors. This strategy, R-SAT™, has a broad applicability and can be extended to the other members of the nuclear receptor family. R-SAT™ offers a number of unique features that distinguish it from other commonly used HTS technologies. For instance, the length of the assay and the large biological separation between stimulus and response offer several distinct advantages including simple, low-cost detection, improved assay enablement, signal amplification and ultra high-throughput. Having the measured response located well downstream of the ligand–receptor interaction allows for the capture and integration of multiple signaling responses into a single homogeneous output. Therefore, when dealing with assay enablement, it becomes possible to employ a large number of signaling intermediates to enable and augment functional assays for nuclear hormone receptors. Moreover, the high sensitivity to constitutive responses (i.e. in

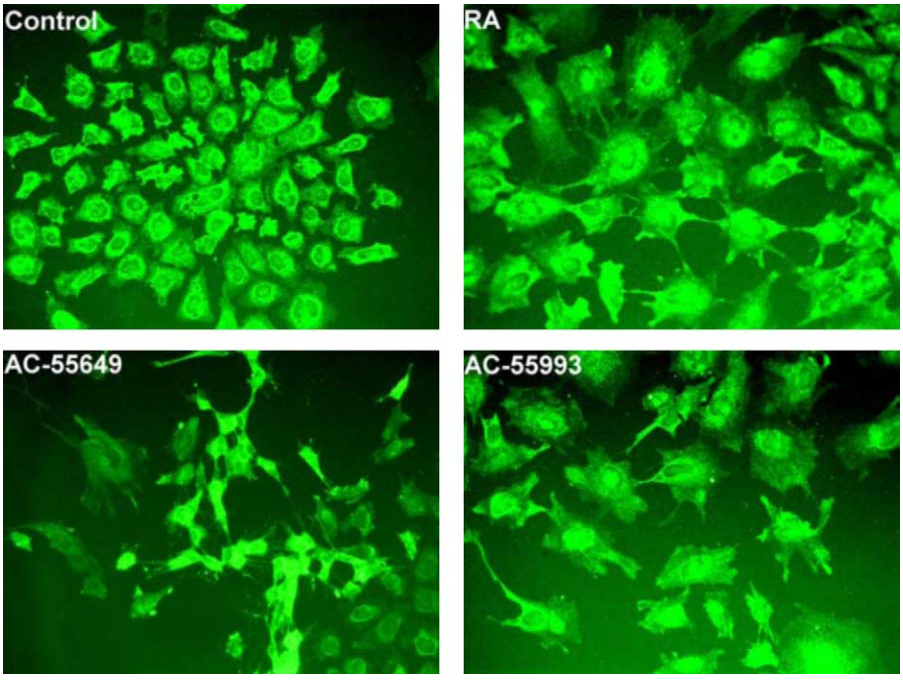


Fig. 4 – Neuronal differentiation of NTERA-2 cells by selective RARβ2 agonists. NTERA-2 cells were exposed for one week to either retinoic acid (RA), AC-55649 or AC-55993 (10 μM). Cells were then fixed and stained with antibodies directed against the neuronal differentiation marker Gap43. Similar results were obtained with another marker Neurofilament L (not shown).

absence of ligand) paves the way for rapidly developing nuclear orphan receptor assays. Limitations also exist primarily relating to the nature of the cell-based assay (cell toxicity, non-selective effects at high drug concentration). Using such strategies, it is possible to build a nearly homogeneous functional screening platform within and across genetic families to support a chemical genomics approach to drug discovery. Indeed, we have sucessfully developed R-SAT™ assays for all the reported (known and orphan) human nuclear hormone receptors (data not shown). Overall, R-SAT™ applied to the nuclear receptors constitutes a powerful tool to identify and follow up with novel chemistries.

We have uncovered novel and subtype selective agonist chemistries for the RAR subtypes using our proprietary platform technology R-SAT™. Because of their subtype selectivities, such chemistries would be more attractive than the classical retinoids by (1) providing selective tools to probe into the specific functions of retinoid receptor subtypes and (2) developing drugs that avoid noxious side effects that have hampered the broad use of retinoids and derivatives as therapeutics. These novel chemistries also display more drug-like properties than retinoids.

Of particular interest is the discovery of potent and isoform selective small molecule agonists that distinguish between RARβ2 and the other RAR and RXR subtypes, including the related isoform RARβ1. This novel pharmacophore displays properties associated with classical retinoids including anti-proliferative effects on neoplastic cells as well as the ability of inducing differentiation of neuronal cells, and more generally properties associated with modulation of transcriptional activity common to nuclear receptors. A recent publication by Chambon and co-workers emphasized that retinoid receptors are involved in the regulation of CNS functions [22]. Especially, the heterodimers RARβ–RXRβ and RARβ–RXRγ were found to be associated to the dopamine signaling pathway, thus having a potential to affect pathologies such as Parkinson's disease and schizophrenia. Hence, in addition to chemotherapy a RARβ2 selective agonist with the potential use for treatment of CNS disorders would be attractive.

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REFERENCES

[1] Nagpal S, Chandraratna RA. Recent developments in receptor-selective retinoids. Curr Pharm Design 2000;6(9):919–31.
[2] Zelent A, Mendelsohn C, Kastner P, Krust A, Garnier JM, Ruffenach F, et al. Differentially expressed isoforms of the mouse retinoic acid receptor beta generated by usage of two promoters and alternative splicing. Embo J 1991;10(1):71–81.
[3] Peng X, Maruo T, Cao Y, Punj V, Mehta R, Das Gupta TK, et al. A novel RARbeta isoform directed by a distinct promoter P3 and mediated by retinoic acid in breast cancer cells. Cancer Res 2004;64(24):8911–8.
[4] Sommer KM, Chen LI, Treuting PM, Smith LT, Swisshelm K. Elevated retinoic acid receptor beta(4) protein in human breast tumor cells with nuclear and cytoplasmic localization. Proc Natl Acad Sci USA 1999;96(15):8651–6.
[5] Chen LI, Sommer KM, Swisshelm K. Downstream codons in the retinoic acid receptor beta-2 and beta-4 mRNAs initiate translation of a protein isoform that disrupts retinoid-activated transcription. J Biol Chem 2002;277(38):35411–2.
[6] Piu F, Magnani M, Ader ME. Dissection of the cytoplasmic domains of cytokine receptors involved in STAT and Ras dependent proliferation. Oncogene 2002;21(22):3579–91.
[7] Brauner-Osborne H, Brann MR. Pharmacology of muscarinic acetylcholine receptor subtypes (m1–m5): high-throughput assays in mammalian cells. Eur J Pharmacol 1996;295(1):93–102.
[8] Piu F, Gauthier NK, Wang F, Beta Arrestin 2 modulates the activity of Nuclear Receptor RAR beta 2 through activation of ERK2 kinase. Oncogene 2005, doi:10.1038/sj.onc.1209024.
[9] Toma S, Isnardi L, Riccardi L, Bollag W. Induction of apoptosis in MCF-7 breast carcinoma cell line by RAR and RXR selective retinoids. Anticancer Res 1998;18(2A):935–42.
[10] Delescluse C, Cavey MT, Martin B, Bernard BA, Reichert U, Maignan J, et al. Selective high affinity retinoic acid receptor alpha or beta–gamma ligands. Mol Pharmacol 1991;40(4):556–62.
[11] Lipinski CA. Drug-like properties and the causes of poor solubility and poor permeability. J Pharmacol Toxicol Meth 2000;44(1):235–49.
[12] Lund BV, Piu F, Gauthier NK, Eeg A, Sherbukhin V, Brann MR, Hacksell U and Olsson R, Discovery of potent and isoform selective retinoic acid b2 receptor agonists. J Med Chem, 2005, in press.
[13] Shao ZM, Dawson MI, Li XS, Rishi AK, Sheikh MS, Han QX, et al. p53 independent G0/G1 arrest and apoptosis induced by a novel retinoid in human breast cancer cells. Oncogene 1995;11(3):493–504.
[14] Toma S, Isnardi L, Raffo P, Dastoli G, De Francisci E, Riccardi L, et al. Effects of all-*trans*-retinoic acid and 13-*cis*-retinoic acid on breast-cancer cell lines: Growth inhibition and apoptosis induction. Int J Cancer 1997;70(5):619–27.
[15] Liu Y, Lee MO, Wang HG, Li Y, Hashimoto Y, Klaus M, et al. Retinoic acid receptor beta mediates the growth-inhibitory effect of retinoic acid by promoting apoptosis in human breast cancer cells. Mol Cell Biol 1996;16(3):1138–49.
[16] Quinn SD, De Boni U. Enhanced neuronal regeneration by retinoic acid of murine dorsal root ganglia and of fetal murine and human spinal cord in vitro. In Vitro Cell Dev Biol 1991;27(1):55–62.
[17] Wuarin L, Sidell N. Differential susceptibilities of spinal cord neurons to retinoic acid-induced survival and differentiation. Dev Biol 1991;144(2):429–35.
[18] Maden M. Retinoids in neural development. In: Balner HNaW, editor. Handbook of experimental pharmacology. Heidelberg: Springer-Verlag; 1999. p. 399–442.
[19] Corcoran J, So PL, Barber RD, Vincent KJ, Mazarakis ND, Mitrophanous KA. Retinoic acid receptor beta-2 and neurite outgrowth in the adult mouse spinal cord in vitro. J Cell Sci 2002;115(Pt 19):3779–86.
[20] Corcoran J, Shroot B, Pizzey J, Maden M. The role of retinoic acid receptors in neurite outgrowth from different populations of embryonic mouse dorsal root ganglia. J Cell Sci 2000;113(Pt 14):2567–74.
[21] Andrews PW. Retinoic acid induces neuronal differentiation of a cloned human embryonal carcinoma cell line in vitro. Dev Biol 1984;103(2):285–93.
[22] Krezel W, Ghyselinck N, Samad TA, Dupe V, Kastner P, Borrelli E, et al. Impaired locomotion and dopamine signaling in retinoid receptor mutant mice. Science 1998;279(5352):863–7.