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Communications to the Editor

Identification of a Retinoic Acid Receptor α Subtype Specific Agonist

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Retinoids are potent molecules that can effect a variety of fundamental biological processes including cell differentiation and proliferation and apoptosis.[1] However, in spite of their great therapeutic promise, the clinical use of retinoids is still restricted largely to dermatology[2] and some cancers.[3] This is largely due to the wide range of toxic effects that are associated with the currently available retinoids.[4] The more extensive clinical use of retinoids will occur only if new synthetic analogs with vastly improved therapeutic indices can be developed. Recent advances in the understanding of the molecular mechanisms of action of retinoids suggest that it would be possible to design such retinoids with improved therapeutic indices. Retinoids elicit their biological effects by activating a series of nuclear receptors which are ligand-inducible transcription factors belonging to the steroid receptor superfamily. The retinoid receptors are classified under two families, the retinoic acid receptors (RARs)[5] and the retinoid X receptors (RXRs),[6] each consisting of three distinct subtypes (α, β, and γ). The RARs function *in vivo* as RAR−RXR heterodimers[7] which bind to the promoter regions of retinoid responsive genes and mediate gene transcription upon ligand binding to the RARs.[8] It appears that each of the RARs regulates distinct genes, although there is some overlap.[9] Also, each of the RAR subtypes has a unique tissue distribution pattern.[10] Thus, a retinoid agonist which binds specifically to one RAR subtype would have limited biological effects because it would regulate only a subset of retinoid responsive genes, and these effects would be observed only in those tissues in which the receptor is substantially expressed. Such receptor specific retinoids will possess much better therapeutic indices than their nonselective counterparts, although each of the specific retinoids is likely to be effective in a narrower range of diseases.

Several classes of molecules that are specific for the RAR[11] or RXR[12] families of receptors have been described. However, only moderate selectivity has been achieved in terms of discriminating between the receptor subtypes.[13] An important advance was made with the internal amide analog Am 580 (Chart 1), which exhibited moderately selective (~30-fold) binding affinity for RAR_α relative to $RAR_{\beta/\gamma}$.[14] In this paper, we report on the identification of two retinoid analogs, **1** (AGN 193835) and **2** (AGN 193836), which have very greatly increased selectivity for the RAR_α subtype (Chart 2). Compound **2**, which has >2000-fold higher binding affinity for RAR_α relative to RAR_β with no measurable binding affinity for RAR_γ, is the first example of a true pharmacologically retinoid receptor subtype specific analog. These compounds will greatly facilitate the elucidation of the biology associated with RAR_α and the identification of RAR_α agonist responsive diseases. Also, these are the first examples of a new generation of receptor subtype specific retinoids that are likely to significantly advance the role of retinoid drugs in clinical medicine.

Compounds **1** and **2** were synthesized as outlined in Scheme 1. Bromination of **3**[15] under acidic conditions gave the bromophenol **4**. Compound **4** was protected as its MOM ether and then subjected to metal halogen exchange followed by CO_2 quench to afford the carboxylic acid **6**. Bromination of **6** under acidic conditions gave the bromo-substituted acid **7**. Compound **7** was protected as its MOM ether and subsequently coupled with either the mono- or difluoro-substituted aminobenzoate **13** or **16** to give the amide **9** or **10**. Compounds **9** and **10** were subjected to the same sequences of base hydrolysis of ester followed by acid-catalyzed deprotection of the MOM group to afford the retinoids **1** and **2**, respectively. The aminobenzoate **13** was synthesized as shown in Scheme 2 where the nitrotoluene **11** (Aldrich) was oxidized with $Na_2Cr_2O_7$ under acidic

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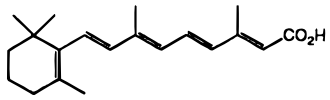
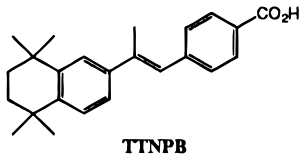
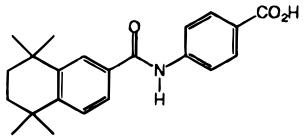
Chart 1



all-trans-RA

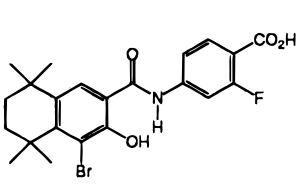


TTNPB

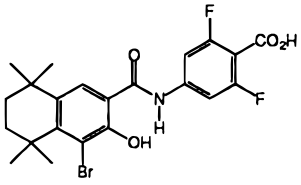


Am 580

Chart 2

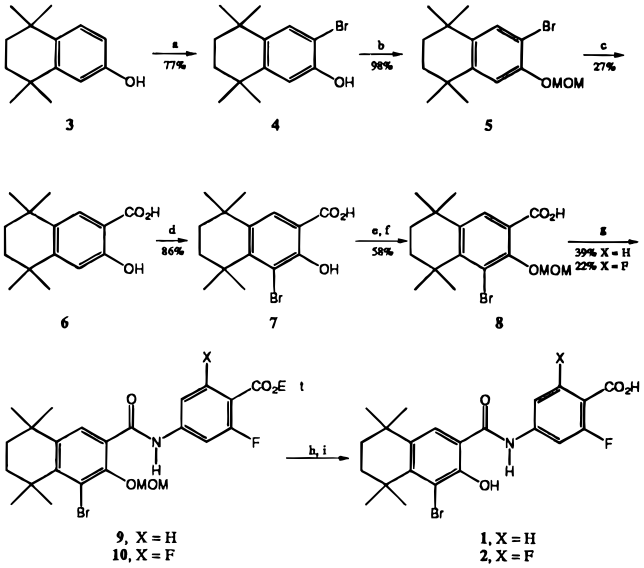


1 (AGN 193835)



2 (AGN 193836)

Scheme 1[a]

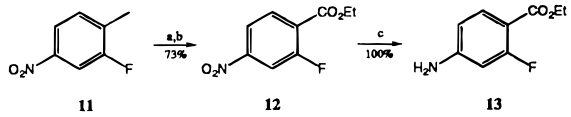


[a] (a) Br_2, HOAc; (b) chloromethyl methyl ether, diisopropylethylamine, Bu_4NBr, CH_2Cl_2, rt; (c) (i) *t*-BuLi, THF, −78 °C, (ii) CO_2, THF, −78 °C, (iii) 10% HCl, −78 °C; (d) Br_2, HOAc; (e) chloromethyl methyl ether, diisopropylethylamine, Bu_4NBr, CH_2Cl_2, rt; (f) NaOH, EtOH, rt, 2 h; (g) **13** or **16**, EDC, DMAP, CH_2Cl_2; (h) NaOH, EtOH; (i) HCl (conctd), MeOH (20% for **1** from **9**, 54% for **2** from **10**).

conditions and the resulting carboxylic acid then esterified to afford **12**. Palladium-catalyzed hydrogenation of **12** gave **13** in quantitative yield. The synthesis of the corresponding difluoroaminobenzoate **16** is outlined in Scheme 3. The benzoic acid **14** (Aldrich) was first esterified, and the 4-fluoro group of **15** was replaced with azide by refluxing with aqueous NaN_3 in acetonitrile. Palladium-catalyzed hydrogenation of the azide gave the desired aminobenzoate **16**.

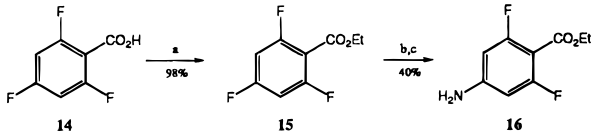
The binding affinities of the retinoids measured using

Scheme 2[a]



[a] (a) $Na_2Cr_2O_7$, HOAc, H_2SO_4, 90 °C; (b) $SOCl_2$, 80 °C/EtOH, Py, CH_2Cl_2; (c) H_2, Pd/C, ethyl acetate.

Scheme 3[a]



[a] (a) $SOCl_2$, 80 °C, 30 min/EtOH, triethylamine; (b) NaN_3, H_2O, CH_3CN, 80 °C, 72 h; (c) H_2, Pd/C, ethyl acetate, 6 h.

Table 1. Binding Affinity K_d (nM) of Retinoids to RAR α/β/γ

	RAR		
entry	α	β	γ
retinoic acid	15 ± 1.7	13 ± 2.5	18 ± 1
TTNPB	72 ± 37	5 ± 2	26 ± 20
Am 580	36 ± 1.5	1361 ± 321	3824 ± 2644
1 (AGN 193835)	4.4 ± 2.3	3037 ± 859	>30 000
2 (AGN 193836)	8.4 ± 1.3	17 374 ± 6347	>30 000

[a] K_d values (mean ± SEM of triplicate determinations) were determined via competition of [^{3}H]-(*all-E*)-retinoic acid (5 nM) binding with unlabeled test retinoid at baculovirus-expressed RARs and application of the equation of Cheng and Prussof.[16c]

baculovirus-expressed RARs and RXRs[16] are shown in Table 1. Retinoic acid, the natural hormone for the RARs, binds with approximately equal affinity to all three RAR subtypes. In contrast, Am 580 binds with approximately 30-fold selectivity to RAR_α relative to $RAR_{\beta/\gamma}$. However, Am 580 still has measurable affinity to RAR_β and RAR_γ with K_d values around 1 μM. Thus, pharmacological doses of Am 580 will activate RAR_β and RAR_γ as well as RAR_α, and the biology of Am 580 will be accordingly complicated. Compound **1** is much more selective for RAR_α having around 700-fold higher affinity for RAR_α than for RAR_β with no measurable binding affinity for RAR_γ. Interestingly, compound **1** binds to RAR_α with slightly higher affinity than the natural hormone retinoic acid. Compound **2** shows true pharmacological specificity in that it binds with greater than 2000-fold higher affinity to RAR_α than to RAR_β and has no measurable binding affinity to RAR_γ. Am 580, **1**, and **2** showed no measurable binding affinity to any of the RXR subtypes (data not shown).

The gene transcriptional properties of these compounds were determined in transactivation assays using an estrogen receptor−RAR chimera (ER-RAR). These chimeric receptor assays provide a very clean readout of only the transfected receptor subtype by circumventing contribution by the endogenous RARs.[17] Am 580 transactivates effectively through all three RARs with some selectivity for RAR_α (Figure 1A). However, Am 580 has essentially full agonist activity at all three RARs at 1 μM concentration. In contrast, compound **1** strongly transactivates only through RAR_α, having activity at RAR_β only at 1 μM and being inactive at RAR_γ (Figure 1B). Compound **2** is completely inactive at $RAR_{\beta/\gamma}$ and transactivates exclusively through RAR_α, albeit with somewhat reduced efficacy (Figure 1C).

It is likely that the H-bond donor properties of the internal amide linkage play an important role in the

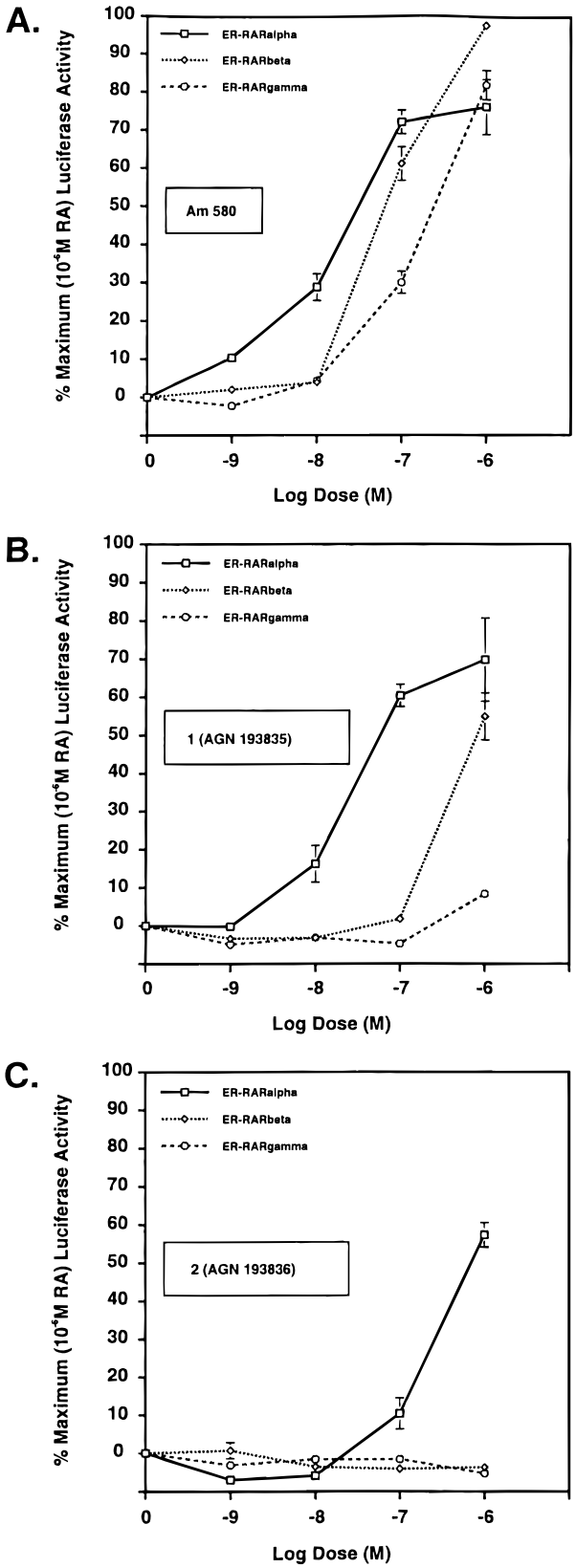


Figure 1. Dose−response curves for Am 580, **1**, and **2**. CV-1 cells were transiently transfected with chimeric ER−RAR receptor expression plasmids and an ER responsive luciferase reporter plasmid as indicated in methods. ER−RAR$_\alpha$-, ER−RAR$_\beta$-, and ER−RAR$_\gamma$-mediated luciferase activity was measured for Am 580 (panel A), **1** (panel B), and **2** (panel C). Luciferase activity (vertical scale, mean ± SEM of triplicate determinations) is expressed as a percentage relative to that measured in similarly transfected cells treated with 10^{-6} M ATRA. The horizontal scale is the log molar concentration of the retinoid.

RAR$_\alpha$ selectivity of these analogs. The introduction of the polar, hydrophilic amide linkage into the tether region of the arotinoid skeleton of TTNPB as in Am 580 would be expected to lead to reduced affinity for retinoid receptors. This is indeed observed for RAR$_\beta$ and RAR$_\gamma$ which bind Am 580 with much lower affinity than they bind TTNPB. However, in spite of its reduced lipophilicity, Am 580 binds equally well to RAR$_\alpha$ as does TTNPB, suggesting that the internal amide of Am 580 selectively stabilizes its interaction with RAR$_\alpha$. One possible explanation for these data is that a H-bond is formed between the amide proton of Am 580 and a residue in the binding pocket of RAR$_\alpha$, while such interactions are not possible for RAR$_\beta$ and RAR$_\gamma$. We systematically explored substitutions on the aromatic rings that could potentially affect the H-bond donor properties of the internal amide linkage and found that the substitutions present in **1** and **2** were optimal in achieving RAR$_\alpha$ selectivity.

In summary, we have identified retinoid analogs that bind with high affinity and great selectivity to RAR$_\alpha$. Compound **2** is the first example of a truly retinoid receptor subtype *specific* (>1000-fold selectivity in binding) analog. These analogs will be invaluable tools in determining the physiological role of RAR$_\alpha$ and in identifying therapeutic applications that are unique to RAR$_\alpha$ agonists. In addition, such subtype selective agonists are very likely to be associated with fewer toxic effects and hence would be useful drugs of improved therapeutic index in the treatment of a subset of retinoid responsive diseases.

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Supporting Information Available: Experimental procedures and analytical data for compounds **1**, **2**, **4**−**10**, **12**, **13**, **15**, and **16** along with descriptions of binding and transactivation assays (4 pages). Ordering information is given on any current masthead page.

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