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Synthesis and Biological Activity of Retinoic Acid Receptor-α Specific Amides

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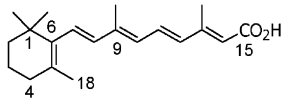
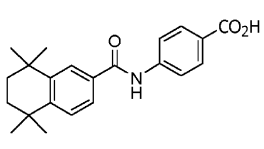
Abstract—Retinoids are analogues of all-*trans*-retinoic acid, a powerful hormone that mediates many fundamental biological processes. Cancer and other serious hyperproliferative diseases are attractive therapeutic targets for retinoids, but the therapeutic use of retinoids is limited due to severe toxicity. We report here the design of retinoid receptor-α specific ligands with growth inhibitory activity in breast cancer cell lines, and which do not cause the cutaneous toxicity associated with the currently available nonselective retinoid agonists.

Retinoids are analogues of all-*trans*-retinoic acid (ATRA), a powerful hormone that is capable of mediating many fundamental biological processes, including cell proliferation and differentiation and apoptosis.[1] Thus, retinoids have biological properties that suggest potential therapeutic uses in the treatment and prevention of cancer. Indeed, commercially available retinoids, including ATRA, have been approved for the treatment of some cancers.[2] Unfortunately, the longer term use of retinoids, particularly in the treatment of premalignant lesions, is severely limited by serious side effects such as mucocutaneous toxicity, hypertriglyceridemia, and headache.[3] In order for retinoids to reach their full therapeutic potential in treating cancer and other serious diseases, new compounds with reduced toxicity and improved therapeutic indices must be discovered.

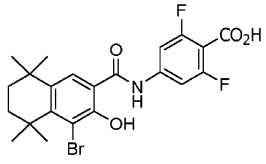
Retinoids induce their powerful biological effects by binding to and activating nuclear receptors. There are six known retinoid receptors, the retinoic acid receptors—RARα, β and γ—and the retinoid X receptors—RXRα, β, and γ. ATRA binds with high affinity to all three RARs but does not bind to the RXRs.[4] A geometric isomer of ATRA, 9-*cis*-retinoic acid, is the putative hormone for the RXRs, and it binds to both RXRs and RARs with high affinity.[5] In biological systems, RARs and RXRs form functional RAR–RXR heterodimers which are effectively activated by RAR-specific agonists but not by RXR-specific agonists. Upon binding an RAR agonist, these RAR–RXR heterodimers, which are associated with specific response elements in the promoter regions of retinoid responsive genes, recruit co-activator proteins to the receptor and induce gene transcription.



Retinoic Acid (ATRA)



Am 580



AGN 193836

It is now evident that each of the RAR subtypes can activate distinct sets of genes. In addition, it is known that the tissue distribution of the RAR subtypes is not uniform, and that distinct RAR subtypes are expressed in different tissues. For example, it is known that the

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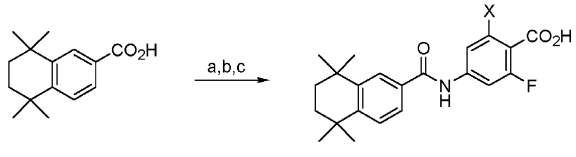
most abundant receptor in the skin is RAR_γ,[6] and studies strongly suggest that the mucocutaneous toxicity caused by retinoids is through activation of RAR_γ.[7] These factors imply that retinoids that specifically activate a single RAR subtype will elicit a narrower range of biological effects in fewer tissues than nonselective retinoids. Thus, it may be possible to design RAR subtype specific compounds that have increased beneficial biological effects and decreased toxicity relative to nonselective retinoids. Such compounds with improved therapeutic indices will be much more useful for the treatment of retinoid responsive diseases than the currently available nonselective retinoid drugs.

Several groups have reported compounds that specifically activate either the RAR or RXR families of receptors.[8] In addition, several compounds with moderate selectivity for two of the three RAR subtypes have been described.[8] Among the first RAR subtype selective retinoids discovered was the amide linked compound, Am-580, which was reported by Shudo and co-workers in 1988.[9] This compound has higher affinity for the RAR_α subtype than for RAR_β or RAR_γ. The amide linker group is a key structural feature for the RAR_α selectivity displayed by these arotinoids. This is presumably due to a favorable hydrogen-bonding interaction between the amide group of the ligand and the hydroxyl group on serine 232 residue present in the ligand binding pocket of RAR_α.[10] The space occupied by serine 232 in RAR_α is occupied by lipophilic alanine residues in RAR_β and RARγ, leading to destabilized protein–ligand interactions and decreased binding affinities. We recently reported on AGN 193836, an amide-linked compound, that has true pharmacological specificity (>1000-fold) in binding affinity for RAR_α over RAR_β and RAR_γ.[11] In cotransfection assays, AGN 193836 transactivates exclusively through RAR_α, and is completely inactive at RAR_β and RAR_γ. We have continued to develop this series of compounds and report here the discovery of **4** (AGN 195183), a compound with improved RAR_α binding selectivity relative to AGN 193836. The structure–activity relationships displayed by these compounds are also examined. Compound **4** inhibited the growth of breast cancer cell lines, and was inactive in an in vivo model of topical irritation.

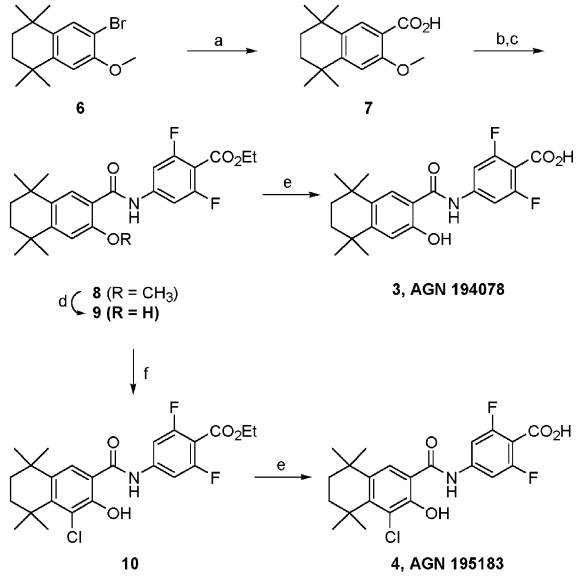
The mono-and di-fluorinated retinoids, **1** and **2**, were prepared from tetralin carboxylic acid **5**[9] by a three step protocol as shown in Scheme 1.[12] Tetralin **5** was refluxed in thionyl chloride to convert the carboxylic acid to an acid chloride. This compound was reacted immediately with either ethyl 2-fluoro-4-aminobenzoate or ethyl 2,6-difluoro-4-aminobenzoate[11] and pyridine to produce the corresponding amide derivatives. The ester group was then hydrolyzed under standard conditions (NaOH, ethanol) to produce the desired retinoid analogues, **1** (X = H) and **2** (X = F).



Scheme 1. Synthesis of mono- and difluorobenzoic acid compounds **1** and **2**: (a) $SOCl_2$, reflux; (b) ethyl 2-fluoro-4-aminobenzoate or ethyl 2,6-difluoro-4-aminobenzoate, pyridine, CH_2Cl_2, 82–90%; (c) NaOH, EtOH; H^+, 80–93%.

Scheme 2 shows the synthesis of compounds **3** and **4**. The known aryl bromide **6**[13] was subjected to a lithium-halogen exchange reaction, and the resulting anion was quenched with carbon dioxide to give carboxylic acid **7** after acidic workup. Compound **7** was treated as above with thionyl chloride and ethyl 2,6-difluorobenzoate to form amide analogue **8**. The methyl protecting group was then removed by treating **8** with boron tribromide, producing compound **9**. Compound **9** was then hydrolyzed under the usual conditions to give retinoid analogue **3**. Alternatively, compound **9** was chlorinated with sulfuryl chloride to give compound **10**, which was then hydrolyzed to carboxylic acid **4**.

Table 1 summarizes the RAR binding and transactivation data of these compounds, which were tested as previously described.[14] These compounds had no binding or transactivation activity at the RXRs (data not shown). Am-580 binds to RAR_α with about 35-fold higher affinity than to RAR_β and about 105-fold higher affinity than to RAR_γ. In transactivation assays, Am-580 is able to fully activate all three RAR subtypes, and it is moderately more potent at RAR_α ($EC_{50} = 2$ nM) than at RAR_β (9 nM) and RAR_γ (19 nM). A significant improvement in binding selectivity was achieved with the monofluorobenzoic acid, compound **1**. This compound is about 190-fold selective in binding to RAR_α than to RAR_β, and almost 500-fold selective in binding to RAR_α than to RAR_γ. Similar to Am-580, compound **1** is about 5-fold more potent in activating RAR_α than



Scheme 2. Synthesis of RAR_α-specific retinoid agonists **3** and **4**: (a) *t*-BuLi, THF, −78 °C; CO_2; H_3O^+, 93%; (b) $SOCl_2$, reflux; (c) ethyl 2,6-difluoro-4-aminobenzoate, pyridine, CH_2Cl_2, 85%; (d) BBr_3, CH_2Cl_2, −78 −25 °C, 92%; (e) NaOH, EtOH; H^+, 85–90%; (f) SO_2Cl_2, CH_2Cl_2, Et_2O, 25 °C, 70%.

Table 1. RAR transcriptional activation and competitive binding data for RAR_α-selective retinoid agonists[14]

Compd	RAR bind. K_d (nM)			RAR Trans. EC_{50} (nM) (% efficacy)		
	α	β	γ	α	β	γ
ATRA	14	11	16	240 (100)	38 (100)	6 (100)
Am 580	36	1400	3800	2 (101)	9 (105)	19 (80)
193836	4	6400	$>10^4$	290 (50)	NA	NA
1	2*	380*	990*	8 (69)	45 (78)	350 (85)
2	7*	1470*	5600*	94 (81)	>1000 (25)	NA
3	3	$>10^4$	5600	112 (77)	>1000 (10)	>1000 (10)
4	3	$>10^5$	$>10^5$	200 (80)	NA	NA

Values represent the mean of three determinations. Errors in this assay are approximately 15% of the mean value. NA indicates <10% efficacy at the highest dose tested (1 μM). Efficacy (%) is the percent transcriptional activity of a compound at the highest dose tested relative to transcriptional activity produced by 1 μM ATRA.

RAR_β in the transactivation assay. More significantly, compound **1** activates gene transcription through RAR_α with >40-fold higher potency than through RAR_γ. The difluorobenzoic acid, compound **2**, was even more selective than compound **1**, having K_d values of 7 nM at RAR_α and 1470 nM (210-fold selectivity) at RAR_β and 5600 nM (800-fold selectivity) at RAR_γ. In terms of transcriptional activity, compound **2** is a full agonist of RAR_α-mediated gene transcription, having an EC_{50} of 94 nM. This compound has only partial agonist activity at RAR_β at the highest dose tested (1000 nM), and it has no transcriptional activity at RAR_γ. Thus, the diflurobenzoic acid moiety is a key structural unit necessary for achieving high selectivity in the binding and transactivation assays.

We mentioned earlier that the hydrogen-bonding characteristics of the amide group in these compounds are responsible for maintaining strong binding to RAR_α while destabilizing binding to RAR_β and RAR_γ. We wished to examine if this property could be exploited further by introducing a hydrogen-bonding group adjacent to the amide linker in the tetrahydronaphthalene moiety. To this end, compound **3**, which has a hydroxyl group in the α-position relative to the amide group, was synthesized. Indeed, compound **3** binds to RAR_α with very high affinity, having a K_d value of 3 nM, while being unable to bind to RAR_β and binding only very weakly ($K_d = 5600$ nM) to RAR_γ. Furthermore, compound **3** maintained full transcriptional activity through RAR_α, with an EC_{50} value of 112 nM, but only activated RAR_β and RAR_γ with about 10% efficacy at the highest dose (1000 nM) tested. Another beneficial structural modification was to add halogens (Cl and Br) to the C18 position (ATRA numbering) to further improve RAR_α selectivity. Like AGN 193836, which is substituted with bromine in the C18-position, compound **4**, which is substituted with chlorine, has true pharmacological specificity for RAR_α over RAR_β and RAR_γ in the binding assays. Compound **4** binds to RAR_α with high affinity, having a K_d value of 3 nM, and it does not bind to either RAR_β or RAR_γ, even at doses as high as 100 μM. In the transactivation assays, compound **4** activates RAR_α exclusively, with an EC_{50} value of 200 nM. Compound **4** was unable to induce transcriptional activity through either RAR_β or RAR_γ at the doses tested. To our knowledge, compound **4** is the most selective RAR_α agonist known.

We then determined the growth inhibitory properties of compound **4** in two human breast cancer cell lines, T-47D, which is estrogen receptor (ER) positive, and SK-BR-3, which is ER negative. Both of these cell lines are known to express relatively high levels of RAR_α and should be responsive to RAR_α-specific agonists.[15] Compound **4** inhibited of the growth of T-47D cells by approximately 60% with an IC_{50} value of 1.4 nM. By comparison, ATRA inhibited the growth of T-47D cells by about 70% with an IC_{50} value of 200 nM. In SK-BR-3 cells, **4** was even more efficacious showing 93% growth inhibition with an IC_{50} value of 11 nM. This compares well with ATRA, which induced 99% growth inhibition with an IC_{50} value of approximately 2 nM. These data indicate that RAR_α-specific ligands such as **4** are potentially useful for the treatment of breast cancer.

With regard to retinoid toxicity, we wanted to determine if RAR_α-specific agonists such as **4** had any advantage over RAR_α-selective compounds such as Am-580. We tested **4** and Am-580 in our previously described topical irritation assay, a clinically relevant in vivo model of retinoid toxicity.[3b] In this assay, the retinoids were applied at a dose of 1000 nmol/25 g to the backs of hairless mice for an 8 day period. The severity of the induced topical toxicity was scored from 0 to 21 (0 = no irritation, 21 = severe irritation) based on the amount of flaking, abrasion and body weight loss observed in each animal. As can be seen from the data summarized in Table 2, Am-580 caused severe topical irritation at this dose whereas **4** caused no topical irritation whatsoever. Thus, the topical toxicity caused by RAR_α subtype specific agonists such as **4** is very different from that caused by compounds with only moderate RAR_α subtype selectivity.

In summary, we have described the synthesis and structure–activity relationships of new RAR_α-selective agonists which led to the discovery of the RAR_α-specific agonist **4**. We have shown that this compound is a potent growth inhibitor of the ER positive and ER negative human cancer breast cell lines, T-47D and SK-BR-3, respectively. In addition, in a hairless mouse model of retinoid induced topical irritation, compound **4** was non-toxic while Am-580 caused severe topical irritation. This confirms our hypothesis that compounds with true RAR subtype specificity will have very different biological properties than RAR subtype selective or

Table 2. Compound **4** and ATRA inhibit growth of the human breast cancer cell lines, T-47D and SK-BR-3,[15] compound **4** does not cause the topical irritation induced by the RAR_{α}-selective retinoid, Am-580[3b]

Compd	T-47D IC_{50} (nM) (% efficacy)	SK-BR-3 IC_{50} (nM) (% efficacy)	Topical irritation score (0–21)
ATRA	200±71 (74)	1.7±0.5 (99)	ND[a]
Am 580	ND[a]	ND[a]	16±1
4	1.4±1.8 (57)	11±7 (93)	0±0

[a]ND, not determined. Efficacy (%) is the percent inhibition of BrdU incorporated in dividing cells at the highest dose tested (1 μM) relative to vehicle control.

non-selective retinoid agonists. Such compounds will have improved therapeutic indices relative to the non-selective retinoids currently available and greater utility in treating cancers and other serious retinoid responsive diseases. Compound **4** (AGN 195183) is currently in Phase I/IIA clinical trials in cancer patients.

Acknowledgements

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References and Notes

1. Review: Sporn, M. B., Roberts, A. B., Goodman, D. S., Eds. *The Retinoids: Biology, Chemistry, and Medicine*; 2nd ed.; Raven: New York, 1994.
2. (a) Huang, M. E.; Ye, Y. C.; Chen, S. R.; Chai, J. R.; Lu, J. X.; Zhao, L.; Gu, L. J.; Wang, Z. Y. *Blood* **1988**, *72*, 567. (b) Motzer, R. J.; Schwartz, L.; Law, T. M.; Murphy, B. A.; Hoffman, A. D.; Albino, A. P.; Vlamis, V.; Nanus, D. M. *J. Clin. Oncol.* **1995**, *13*, 1950. (c) Duvic, M.; Hymes, K; Heald, P.; Breneman, D.; Martin, A. G.; Myskowski, P.; Crowley, C.; Yocum, R. C. *J. Clin. Oncol.* **2001**, *19*, 2456.
3. (a) Agnish, N. D.; Kochhar, D. M. In *Retinoids and Clinical Practice*; Korean, G., Ed.; Mercel Dekker: New York, 1992; p 47. (b) Standeven, A. M.; Johnson, A. T.; Escobar, M.; Chandraratna, R. A. S. *Toxicol. Appl. Pharmacol.* **1996**, *138*, 169.
4. (a) Petkovich, M.; Brand, N. J.; Krust, A.; Chambon, P. *Nature* **1987**, *330*, 444. (b) Giguere, V.; Ong, E. S.; Segui, P.; Evans, R. M. *Nature* **1987**, *330*, 624. (c) Krust, A.; Kastner, P.; Petkovich, M.; Zelent, A.; Chambon, P. *Proc. Natl. Acad. U.S.A.* **1989**, *86*, 5310.
5. (a) Heyman, R. A.; Mangelsdorf, D. J.; Dyck, J. A.; Stein, R. B.; Eichele, G.; Evans, R. M.; Thaller, C. *Cell* **1992**, *68*, 397. (b) Levin, A. A.; Sturzenbecker, L. J.; Kazmer, S.; Bosakowski, T.; Huselton, C.; Allenby, G.; Speck, J.; Kratzeisen, C.; Rosenberger, M.; Lovey, A.; Grippo, J. F. *Nature* **1992**, *355*, 359.
6. Elder, J. T.; Fisher, G. J.; Zhang, Q.-Y.; Eisen, D.; Krust, A.; Kastner, P.; Chambon, P.; Voorhees, J. J. *J. Invest. Dermatol.* **1991**, *96*, 425.
7. Reczek, P. R.; Ostrowski, J.; Yu, K.-L.; Chen, S.; Hammer, L.; Roalsvig, T.; Starrett, J. E., Jr.; Driscoll, J. P.; Whiting, G.; Spinazze, P. G.; Tramposch, K. M.; Mansuri, M. M. *Skin Pharmacol.* **1995**, *8*, 292.
8. Beard, R. L.; Chandraratna, R. A. In *Handbook of Experimental Pharmacology, Retinoids The Biochemical and Molecular Basis of Vitamin A and Retinoid Action*; Nau, H., Blaner, W. S., Eds.; Springer: Berlin Heidelberg, 1999; Vol. 139, p 185.
9. Kagechika, H.; Kawachi, E.; Hashimoto, Y.; Himi, T.; Shudo, K. *J. Med. Chem.* **1988**, *31*, 2182.
10. Renaud, J.-P.; Rochel, N.; Ruff, M.; Vivat, V.; Chambon, P.; Gronemeyer, H.; Moras, D. *Nature* **1995**, *378*, 681.
11. Teng, M.; Duong, T. T.; Klein, E. S.; Pino, M. E.; Chandraratna, R. A. S. *J. Med. Chem.* **1996**, *39*, 3035.
12. All new compounds gave ^{1}H NMR, ^{13}C NMR, IR, and HRMS data consistent with their structures.
13. Qing, F.-L.; Fan, J.; Sun, H.-B.; Yue, X.-J. *J. Chem. Soc., Perkin. Trans. 1* **1997**, *20*, 3053.
14. (a) Transactivation assay: Benbrook, D.; Lernhardt, E.; Pfahl, M. *Nature* **1988**, *333*, 669. (b) Graupner, G.; Malle, G.; Maignan, J.; Lang, G.; Pruniéras, M.; Pfahl, M. *BBRC* **1991**, *179*, 1554. Binding assay: Allegretto, E. A.; McClurg, M. R.; Lazarchik, S. B.; Clemm, D. L.; Kerner, S. A.; Elgrot, M. G.; Boehm, M. F.; White, S. K.; Pike, J. W.; Heyman, R. A. *J. Biol. Chem.* **1993**, *268*, 26625.
15. Fitzgerald, P.; Teng, M.; Chandraratna, R. A. S.; Heyman, R. A.; Allegretto, E. A. *Cancer Res.* **1997**, *57*, 2642.