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Aza-retinoids as novel retinoid X receptor-specific agonists

Luc J. Farmer,* Kristen S. Marron, Stacie S. Canan Koch,† C. K. Hwang, E Adam Kallel, Lin Zhi, Alex M. Nadzan, Dave W. Robertson and Youssef L. Bennani*

Ligand Pharmaceuticals Inc., 10255 Science Center Drive, San Diego, CA 92121, USA

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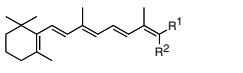
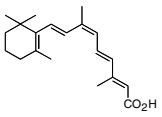
Dedicated to the memory of David W. Robertson in recognition of his outstanding accomplishments in drug discovery and research.

Abstract—A new structurally simple series of potent lipophilic aza-retinoids RXR agonists has been developed. SAR studies for the *N*-alkyl-azadienoic acids described here demonstrate that the RXR activity profile is sensitive to the *N*-alkyl chain length. Further, we have expanded the work to include azadienoic acids, which exhibited many accessible conformations leading to a better understanding of the SAR around the series.

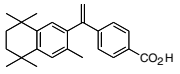
The nuclear receptors are members of a superfamily of eukaryotic transcription factors which regulate gene expression.[1] The retinoid receptors, which belong to this superfamily, are divided into three retinoic acid receptor (RARα, β, and γ) and three retinoid X receptor (RXRα, β, and γ) subtypes, distinguished by differences in amino acid sequences, responsiveness toward natural and synthetic ligands, and ability to modulate the expression of various target genes.[2] These receptors function as ligand-activated transcription regulators, transducing the pleiotropic effects of various retinoids on morphogenesis, differentiation, and homeostasis during development of the embryo and postnatal life.[3] The RARs are activated by both endogenous all-*trans*-retinoic acid **1** and 9-*cis*-retinoic acid **2**, while the RXRs are activated by 9-*cis*-RA.[4] ATRA **1**, 13-*cis*-retinoic acid **4**, and etretinate **5** are RAR selective agonists currently marketed for dermatological applications.[5] Widespread use of these agents has been limited because of undesirable side effects such as headaches and mucocutaneous toxicity.[6] It is hoped that the discovery of selective modulators of the RXRs will open new opportunities for the development of novel retinoids with an enhanced therapeutic profile. The potential utility of selective RXR activators may evolve from their ability to induce apoptosis[7] or possibly from regulation through heterodimer formation with RARs,[8] peroxisome proliferator-activated receptors (PPARs),[9] thyroid hormone receptor (TR),[10] and vitamin D_3 receptor (VDR).[11]



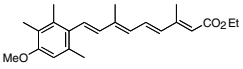
1 $R^1 = CO_2H$, $R^2 = H$; (ATRA)
4 $R^1 = H$, $R^2 = CO_2H$; (13-*cis*-RA)



2 9-*cis*-RA



3 (Targretin)



5 (Etretinate)

Recently, benzoic acids such as **3** (Targretin) have been reported as selective synthetic ligands for the RXRs.[12] Two retinoids, **2** and **3**, are currently in clinical trials for the treatment of a variety of cancers.[13] Most reported RXR selective activators, such as Targretin **3**, LG100268, **6a**[14] and SR11237 **6b**,[15] are retinoids with an aromatic acid motif. Vuligonda et al.[16] reported that cyclopropyl dienoic acid **7** is a potent RXR selective agonist. More recently, potent RXR selective retinoids with 6–7 locked trienoic acids[17] and 9-*cis* isosteres[18] were reported by us. An interesting trienoic acid replacement **8** was also published by Michellys et al.[19] which exhibited good RXR selectivity.

Keywords: RXR selective retinoids; RXR-agonists.
* Corresponding authors at present address: Vertex Pharmaceuticals Inc., 130 Waverly Street, Cambridge, MA 02139, USA. Fax: +1 617 444 6290; e-mail addresses: luc_farmer@vrtx.com; youssef_bennani@vrtx.com
† Present address: Pfizer, La Jolla, CA, USA.

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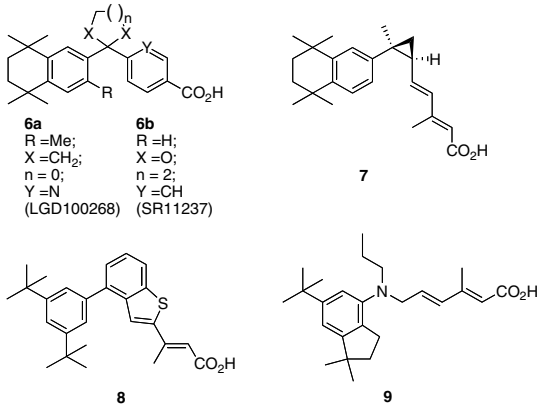


The RAR and RXR activation and binding abilities of representative RXR compounds are compiled in Table 1.

In efforts to explore the medicinal chemistry and biology of novel retinoids, we have discovered and wish to report on a new class of structurally simple lipophilic aza-retinoids which exhibited a potent RXR selective agonist profile and displayed novel conformational features. Thus, in the search for novel isosteres for the tetraenoic acid chain and the cyclohexyl moiety in ATRA **1** and 9-*cis*-RA **2**, we investigated the introduction of a nitrogen atom between a variety of aromatic or bicyclic groups and side chain bearing a dienoic acid moiety. The SAR studies consisted of a systematic variation of the aryl or bicyclic moieties; the *N*-alkyl groups and the dienoic acid spacer group. These studies resulted in the discovery of the substituted indane-derived *N*-propyl dienoic acid **9** as a potent and highly selective RXR agonist. In addition, we have found that the dienoic acid chain flexibility, coupled with the appropriate N-substituent, provides compounds which expand the definition of the RXR agonist pharmacophore.

The simple and efficient preparation of these *N*-alkyl retinoids was achieved by either the formation of an amide bond or by reductive amination of an aldehyde. The amine substrates **12a–c** were prepared by Friedel–Crafts alkylation of the appropriately substituted benzene rings **10a–c** with 2,5-dichloro-2,5-dimethyl hexanes to give tetrahydro naphthalenes **11a–c** in good yields (Scheme 1). Nitration of **11a–c** followed by palladium catalyzed hydrogenation afforded the desired bicyclic amines **12a–c** in good yields.

3,5-Di-*tert*-butyl benzoic acid **13** was treated with methyllithium to give the corresponding ketone **14**, which was sequentially reacted with hydroxylamine hydrochloride in ethanol, and the resulting oxime was converted to the corresponding *N*-acyl aniline **15** through a Beckman rearrangement. Deacetylation to the primary amine **16** was accomplished by acid hydrolysis in ethanol in 85% yield (Scheme 2).

Aminoindane **20** was prepared starting from *tert*-butylbenzene **17**, which was treated with isoprene in the presence of $AlCl_3$ to give the corresponding indane **18** in 50% yield.[20] The latter was acylated under standard Friedel–Crafts conditions to give methyl ketone **19** in 62% yield. In a sequence similar to that described above, ketone **19** was transformed into amine **20** in two steps and 81% overall yield (Scheme 3).

The dienoic acid moiety was prepared from readily available ethyl 3-methyl-4-oxocrotonate **21**, which upon treatment with triphenylphosphoranylacetaldehyde in refluxing benzene gave *trans-trans*-aldehyde **22** in 47% yield (Scheme 4). Jones oxidation of **22** afforded ethyl 6-mono-carboxylic acid dienoate **23**. Both aldehyde **22** and carboxylic acid **23** served as coupling fragments leading to the desired retinoids.

Amines **12a–c**, **16**, and **20** were coupled with carboxylic acid **23** in a standard DCC amide coupling to give the corresponding amides **24a–c**, which were subjected to N-alkylation conditions followed by saponification to give the corresponding retinoid-like *N*-alkyl-amidocarboxylic acids of type **25** in good overall yields (Scheme 5). Alternatively, the amines were coupled via reductive amination with aldehyde **22**[21] to give allylic amines of type **26** in generally good yields. Alkylation with a variety of alkyl halides under standard conditions gave the corresponding *N*,*N*-dialkyl retinoates, which were saponified to the desired carboxylic acid of type **27**.

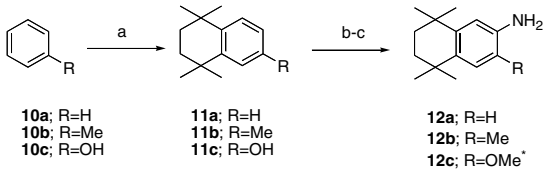
The proline-based retinoids, **48** and **49**, were prepared from the bromotetrahydronaphthalene **28**[22] according to Scheme 6. Aromatic amination of the aryl bromide with (*R*)- or (*S*)-prolinol in the presence of potassium carbonate and copper oxide in DMF produced the corresponding naphthyl prolinol intermediate **29**. The primary alcohol was oxidized under Swern conditions with oxalyl chloride/DMSO to afford the aldehyde, which was used directly without further purification.

Table 1. Cotransfection data in CV-1 cells[a] and binding data for known retinoids

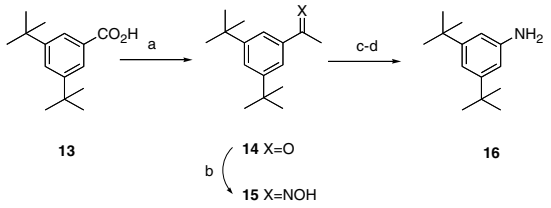
Compound	EC_{50} (nM)/K_d (nM)					
	RARα	RARβ	RARγ	RXRα	RXRβ	RXRγ
1	388/15	87/17	21/17	997/53	1240/306	1100/306
2	208/93	31/97	50/148	219/8	131/15	150/14
3	NA	NA	NA	28/36	25/21	20/29
7	NA	NA	NA	4.5/3	3.0/3	4.0/3.0
8	NA	NA	NA	1.5/1.5	2.0/2.5	1.0/1.8

NA, not active.

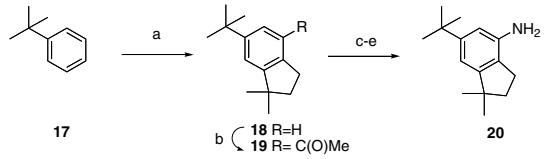
[a] EC_{50} values were determined from a full dose–response curve ranging from 10^{-12} to 10^{-15} M. Retinoid activity was normalized relative to that of ATRA and is expressed as potency (EC_{50}), which is the concentration of retinoid required to produce 50% of the maximal observed response.



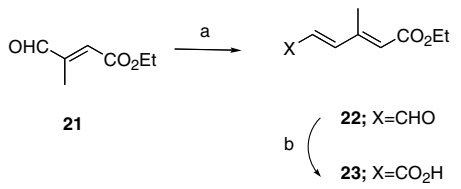
Scheme 1. Reagents and conditions: (a) 2,5-Dichloro-2,5-dimethylhexanes, $AlCl_3$, CH_2Cl_2 (40–83%); (b) HNO_3 (60–99%); (c) H_2, Pd/C, EtOH (quant.). *Nitronaphthol **11c** was O-methylated (K_2CO_3, MeI, acetone) prior to reduction.



Scheme 2. Reagents and conditions: (a) MeLi (2.2 equiv), THF, −78 °C (62%); (b) NH_2OH–HCl, Py, EtOH (98%); (c) $SOCl_2$, DCM (62%); (d) HCl, EtOH, reflux (98%).



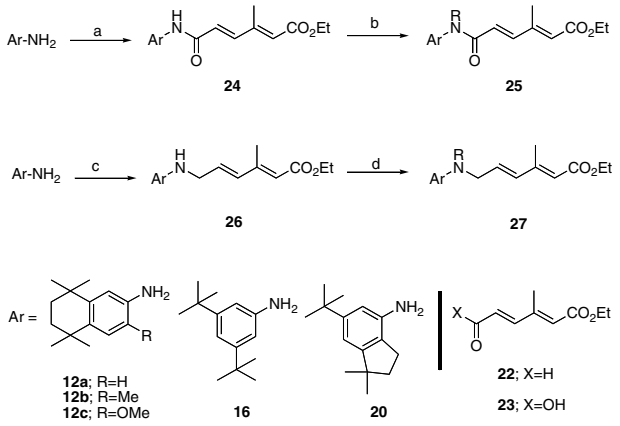
Scheme 3. Reagents and conditions: (a) Isoprene, $AlCl_3$, CH_3NO_2 (50%); (b) AcCl, $AlCl_3$, DCM (62%); (c) NH_2OH–HCl, Py, EtOH; (d) $SOCl_2$, DCM; then (e) HCl, EtOH, reflux (81% three steps).



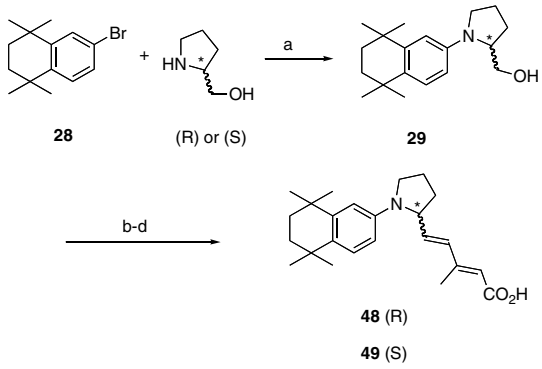
Scheme 4. Reagents and conditions: (a) Ph_3CHCHO, PhH, cat. benzoic acid, reflux (38%); (b) Jones oxidation (98%).

Horner–Wadsworth–Emmons olefination of the aldehyde with 3-ethoxycarbonyl-2-methyl-2-enyl phosphonate in THF/DMPU mixture gave the dienoate ester, which was hydrolyzed under standard conditions to give the corresponding aza-retinoids **48** and **49**.

Cotransfection potency[23] (EC_{50}, nM) and binding affinity[24] (K_i, nM) for the described aza-retinoids (**9**, **30**–**49**) on each of the RXR isoforms (α, β, and γ) are compiled in Table 2. The new aza-retinoids are RXR-specific agonists, thus RAR data are not shown for clarity. All of the reported compounds shown in Table 2 have RAR-binding affinities >1000 nM. The secondary amide **30** and various N-alkylated dienoic amides (*N*-methyl, -benzyl, -heptyl, etc.); some data shown, such as **34**, did not bind to or activate the retinoid X receptors; with the exception of *N*-propyl amide **32**, which displayed moderate RXR-binding affinity (K_i = 299–644 nM) and



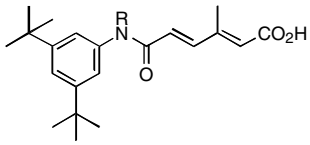
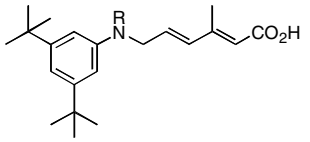
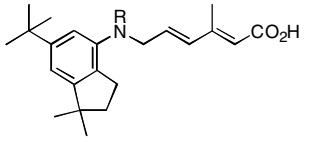
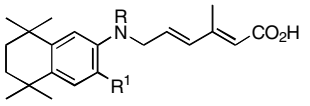
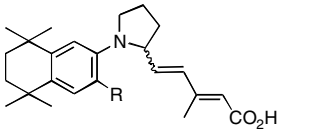
Scheme 5. Reagents and conditions: (a) DCC, DCM, DMAP cat. **23** (78–90%); (b) NaH, THF, RX then KOH, MeOH (30–83% two steps); (c) $NaCNBH_3$, $ZnCl_2$, MeOH, **22** (50–94%); (d) K_2CO_3, DMA, RX then KOH, MeOH (73–90% two steps).



Scheme 6. Reagents and conditions: (a) K_2CO_3, (*R*) or (*S*)-prolinol, CuO, DMF, reflux (23%); (b) oxalyl chloride, DMSO, DCM, TEA; (c) 3-ethoxycarbonyl-2-methylpropenylphosphonate, *n*-BuLi, THF/DMPU (1:1), −78 °C (35% two steps); (d) 2 M LiOH, EtOH, 60 °C (82%).

variable activation of the RXRs (EC_{50} = 151–1000 nM). The initial results prompted us to examine the *n*-alkyl series of compounds further. It is clear that the presence of the amide carbonyl is either not tolerated in the binding pocket of these receptors or does not allow for sufficient chain flexibility of the ligand. We reasoned that the reduction of the amide carbonyl group would create a more flexible dienoic acid chain which could adopt a favourable conformation. We prepared secondary and tertiary *N*-alkyl aminoretinoids-derived from 3,5-di-*tert*-butyl aniline **16**. The biological results for six selected compounds **33**–**38** in this series are displayed in Table 2. While we investigated many *N* alkyl groups (some data not shown), the *N*-propyl retinoid **36** displayed the most potent RXR-specific profile with K_i = 17–38 nM and EC_{50} = 63–99 nM in the three RXRs. In this series, activation of RXRs is sensitive to *N*-alkyl chain length, with the three-carbon chain being the best. Elaboration with longer alkyl chains resulted in analogues with only weak RXR binding. We also investigated the effects of aromatic group variation, maintaining the *N*-alkyl chain length at 2–5 carbons long. A series of 6-*tert*-butyl-1,1-dimethylindane-based

Table 2. Cotransfection potency and binding affinity data for amido- and aza-retinoids

Structure	Compound	R	EC_{50} (nM); K_i (nM)		
			RXRα	RXRβ	RXRγ
	**30**	H	NA; NA	NA; NA	NA; NA
	31	CH_2Ph (benzyl)	NA; NA	NA; NA	NA; NA
	32	C_3H_7 (*n*-propyl)	1000; 644	151; 605	669; 299
	**33**	H	NA; 529	NA; NA	NA; NA
	34	CH_3	378; 37	95; 88	322; 131
	35	C_2H_5	221; 31	235; 24	231; 35
	36	C_3H_7 (*n*-propyl)	99; 17	69; 34	63; 38
	37	CH_2Ph (benzyl)	NA; 121	NA; 238	NA; 207
	38	C8H17 (*n*-octyl)	NA; 122	NA; 126	35; 221
	**9**	C_3H_7 (*n*-propyl)	22; 8	21; 12	19; 10
	39	C_4H_9 (*n*-butyl)	53; 21	52; 60	50; 31
	40	C_3H_7 (iso-propyl)	252; 67	216; 301	262; 121
	41	C_4H_7 (methylcyclopropyl)	123; 63	98; 105	100; 150
	42	C_5H_8 (prenyl)	NA; 254	NA; 327	NA; 309
	**43**	C_2H_5; R1 = H	216; 103	239; 185	128; 162
	44	C_2H_5; R1 = CH_3	64; 42	117; 152	125; 99
	45	C_3H_7; R1 = H	152; 47	206; 72	170; 110
	46	C_3H_7; R1 = CH_3	231; 18	212; 161	223; 211
	47	C_3H_7; R1 = OCH_3	400; 257	169; 721	NA; 576
	**48** (*R*)-	H	NA; 103	1291; 188	1263; 202
	49 (*S*)-		179; 21	161; 63	147; 100

compound were prepared; the biological results on the most active analogues (**9**, **39**–**42**), are compiled in Table 2.

Our results show that the substituted indane is a suitable isosteric replacement for the dialkylaryl group. *N*-Alkyl substituent modification is again a significant element for retaining RXR activity, with *N*-propyl indane **9** and *N*-butyl indane **39** being the most potent compounds of the series. Chain branching from *N*-isopropyl **40**, *N*-methylcyclopropyl **41** to *N*-prenyl **42** resulted in a systematic decrease in receptor activation and binding affinity. It appears that the *N*-propyl substitution, as exemplified by indane-based retinoid **9**, combines the best structural elements in terms of pharmacophore requirements for potent activation of the RXRs. Further variation of the aromatic moiety utilizing the classic 3-substituted 5,5,8,8-tetramethyl-tetrahydronaphthyl group gave compounds **43**–**47**, which display overall diminished RXR activity when compared to retinoid **9**. This class of compounds, however, showed less dependence on *N*-alkyl substitution for RXR activity with both *N*-ethyl **44** and *N*-propyl **46** analogues displaying moderate activities. The proline-derived cycloazaretinoids **48** and **49** were synthesized to evaluate the configurational restrictions for RXR activity.

The (*R*)-proline analogue has very little activity on the retinoid receptors, while the (*S*)-proline-derived analogue has moderate RXR activity (EC_{50} = 147–179 nM, K_i = 21–100 nM). In light of previously reported variations of RXR activity for cyclopropane-based retinoids **7** and its enantiomer by Vuligonda et al.[16], and for the diastereomeric pairs of cyclopentane-based retinoids reported by us,[25] these results strongly suggest that the chirality of prolines **48** and **49** played a role in the 7- to 9-fold discrepancy in potency for RXRs observed. These analogues confirm that the receptor specificity is dependent on the spatial configuration of the dienoic acid chain. Based on new insights into the role of helix H12 in hRXRβ–LG268 complex[26] and the specificity for retinoid X receptor, structural information on complex with the ligand binding domain of hRXRβ with retinoids such as **9** and **49** would certainly be informative to further improve RXR selectivity and activity of these novel modulators.

In conclusion, we have developed a new and structurally simple series of lipophilic aza-retinoids. The 6-*tert*-butyl-1,1-dimethylindanyl-*N*-propylazadienoic acid **9** exhibits a potent RXR-specific profile. Other *N*-alkyl chain substituents (longer alkyl, branched and unsaturated alkyl, benzyl, etc.) on this indanyl series do not retain potent RXR agonist profiles. The RXR-specific agonist activity can also be extended to the other classes of azadienoic retinoids prepared in this study (for example, compounds **36** and **44**). The SAR studies for the *N*-alkyl chain length (small alkyl, i.e., ethyl and propyl, being the best). Further, we have expanded the RXR

agonist pharmacophore model and redefined it by including azadienoic acids which exhibit many accessible conformations.

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