JOURNAL OF
MEDICINAL
CHEMISTRY

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Volume 39, Number 26 December 20, 1996

Communications to the Editor

Identification of Retinoic Acid Receptor β Subtype Specific Agonists

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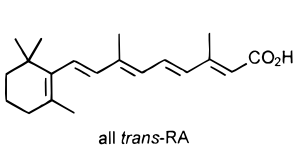
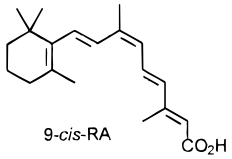
Received October 2, 1996

The many roles played by *all-trans*-retinoic acid (RA) in embryonic development and maintenance of life have generated considerable interest in this hormone and the corresponding synthetic analogs known as retinoids.[1] Retinoids elicit potent effects on cellular differentiation and proliferation by binding to nuclear receptors which mediate gene transcription.[2] The natural ligand for the retinoic acid receptors[3] (RARα, RARβ, and RARγ) is RA (Chart 1), while the 9-*cis*-isomer (9-*cis*-RA is a potent activator of the retinoid X receptors[4] (RXRs). As therapeutic agents, retinoids show considerable promise in the treatment of various human diseases such as psoriasis,[5] acne,[6] and cancer.[7] One limitation to their use however, has been the inherent toxicity associated with these compounds *in vivo*.

Chart 1

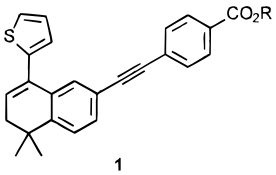


all *trans*-RA

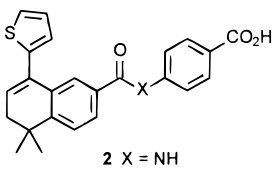


9-*cis*-RA

Chart 2

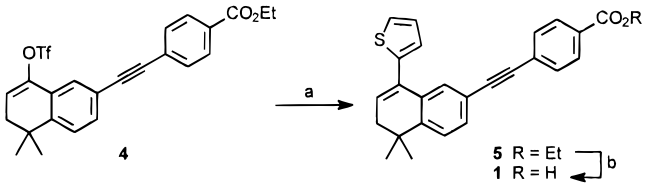


1



2 X = NH
3 X = O

Scheme 1[a]

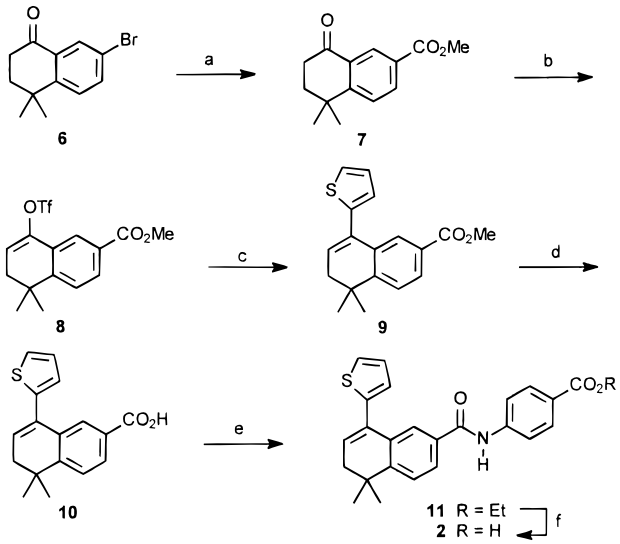


4

5 R = Et
1 R = H

[a] (a) Thiophene/*n*-BuLi/THF/0 °C/$ZnCl_2$, room temperature/$Pd(PPh_3)_4$/50 °C (67%); (b) LiOH/THF/H_2O (84%).

Scheme 2[a]



6 **7** **8** **9** **10**

11 R = Et
2 R = H

[a] (a) CO/MeOH/DMSO/Et_3N/$Pd(PPh_3)_2Cl_2$/dppp/70 °C (68%); (b) $NaN(SiMe_3)_2$/THF −78 °C/2-[*N*,*N*-bis(trifluoromethylsulfonyl)amino]-5-chloropyridine (59%); (c) thiophene/*n*-BuLi/THF/0 °C/$ZnCl_2$, room temperature/$Pd(PPh_3)_4$/THF/50 °C (92%); (d) NaOH/EtOH/THF/H_2O (93%); (e) 4-$H_2NC_6H_4CO_2Et$/1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride/DMAP/DMF/room temperature (80%); (f) NaOH/EtOH/THF/H_2O (80%).

In the adult animal, the distribution of RARs is tissue specific.[8] For example, RARγ is found primarily in the skin[9] and has recently been associated with retinoid-induced topical irritation.[10] The RARβ subtype is located mainly in the heart, lung, and spleen. The

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S0022-2623(96)00687-5 CCC: $12.00 © 1996 American Chemical Society

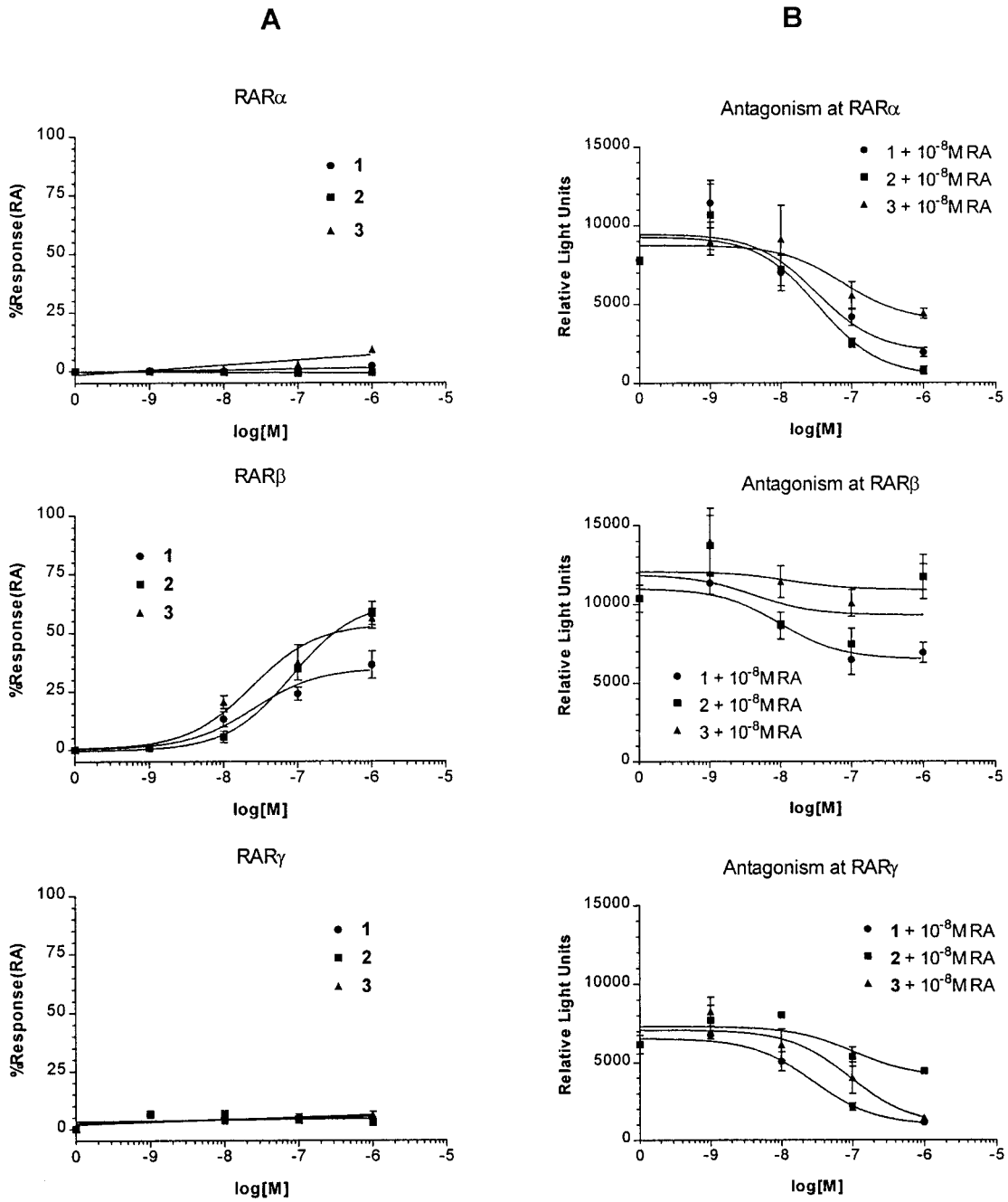


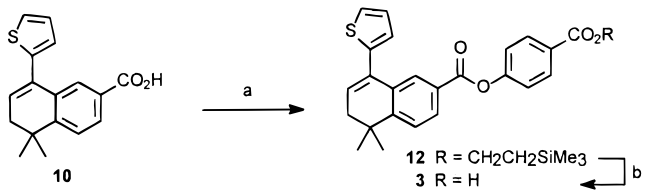
Figure 1. (A) Dose−response curves for retinoids **1**−**3** in CV-1 cells transfected with RAR holoreceptors and a MTV-4(R5G)-luciferase reporter plasmid at each RAR. (B) The antagonist effect of retinoids **1**−**3** on transactivation activity induced by RA (10^{-8} M) at each RAR. In each figure the horizontal scale is the log molar concentration of the retinoid.

RARα subtype, however, is ubiquitously expressed at low levels in the adult and embryo. In addition, transient expression of individual RARs in various embryonic tissues has also been observed, implying that each RAR has a discrete role in embryogenesis.[11] Taken together, these findings suggest that it may be possible to elicit only a particular aspect of retinoid biology in defined tissues by specific activation of an RAR subtype. As a result, an RAR subtype selective retinoid is likely to have a superior therapeutic index in a given disease compared to the currently available RAR *pan*-agonists. Recent efforts by several groups have enabled the identification of retinoids that are selective for receptor families,[12] RAR subtypes,[13] and antagonists of RAR-mediated activity.[14] In this communication, we present the synthesis and characterization of novel retinoids that display agonist activity only at RARβ.[15] We also show that these compounds can function as antagonists of RA activity at RARα and RARγ.

A common feature of the RARβ specific agonists described herein is the presence of a dihydronaphthalene nucleus bearing a 2-thienyl group at C8 (Chart 2). This structural feature appears to be responsible for the RARβ specificity observed in transactivation activity, but has little influence on the selectivity of ligand binding.

Acetylene **1** (AGN 193174) is readily available from vinyl triflate **4**[16] by Pd(0)-catalyzed coupling with 2-thienylzinc chloride in THF (Scheme 1).[17] The intermediate ester **5** was hydrolyzed using LiOH in THF/H_2O to afford **1** as a colorless solid. Preparation of the amide analog **2** (AGN 193639) began with tetralone **6** (Scheme 2). Exposure of **6** to CO and MeOH in the presence of a palladium catalyst provided keto ester **7**

Scheme 3[a]



[a] (a) 4-$HOC_6H_4CO_2CH_2CH_2SiMe_3$/1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride /DMAP/DMF, room temperature (71%); (b) TBAF/THF/(35%).

in 68% yield.[18] Vinyl triflate **8**, prepared from **7** via the sodium enolate,[19] was converted to dihydronaphthalene **9** following the Pd-catalyzed coupling protocol used to prepare **1**. Hydrolysis of methyl ester **9** using aqueous NaOH in EtOH/THF afforded carboxylic acid **10** in excellent yield. Coupling of **10** with ethyl 4-aminobenzoate was carried out using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI) and DMAP in DMF at room temperature to give amide ester **11** in 80% yield. Retinoid **2** was obtained from **11** by ester hydrolysis under standard conditions. The internal ester **3** (AGN 193676) was prepared from carboxylic acid **10** by esterification using EDCI and the 2-(trimethylsilyl)ethyl ester of 4-hydroxybenzoic acid to afford diester **12** (71%), followed by selective hydrolysis of the benzoate ester using tetrabutylammonium fluoride (TBAF) in THF (Scheme 3).

The biological activity of each retinoid **1**−**3** was evaluated in RAR and RXR binding and transactivation assays. Binding affinities were determined using baculovirus-expressed RARs and RXRs as previously described.[20] The ability to transactivate each of the RARs was determined in CV-1 cells transiently cotransfected with a RAR expression plasmid and the RAR responsive MTV-4(R5G)-luciferase reporter plasmid as previously described.[21] This reporter uses a DR-5 response element analogous to the natural retinoic acid response element (RARE) found in the promoter regions of RA responsive genes. In a similar manner, either a CRBP-tk-luciferase reporter with RXRα or -γ or a CPRE-tk-luciferase reporter with RXRβ expression plasmids[22] were used to measure the activity of the retinoids at each of the RXRs. Compounds **1**−**3** were found to be inactive at the RXRs in both the binding and transactivation assays (data not shown).

The results of these experiments (Table 1, Figure 1A) clearly show that retinoids **1**−**3** transactivate only through RARβ and are inactive at RARα and -γ. Further, compounds **1** and **3** appear more potent than RA at RARβ but have only 30−60% of the efficacy of the natural hormone. The amide **2** is of particular interest. Preparation of retinoids using an amide group to link the hydrophobic region to the carboxylate bearing moiety has been shown to confer RARα selectivity in binding and transactivation.[13a] In the case of **2**, the observed binding affinity at RARα is consistent with these results, being 10-fold higher relative to RARβ. In contrast, however, this compound exhibits transactivation activity only at RARβ. These findings support the earlier suggestion that the 2-thienyl group at C8 of the dihydronaphthalene ring selects exclusively for RARβ transactivation over the remaining RAR subtypes.

The possibility that retinoids **1**−**3** may act as RAR antagonists for RARα and -γ mediated activity was tested in the cotransfection assay using a constant dose of RA (10^{-8} M). As shown in Figure 1B, the inhibition of RA activity in a dose dependent manner demonstrates the ability of these compounds to act as RARα and -γ antagonists. In addition, these results are in complete accord with the observed K_d values at RARα and -γ (Table 1) and the ability of these compounds to act as partial agonists at RARβ (Figure 1A).

Table 1. EC_{50} and K_d Values (nM) for RA and Compounds **1**−**3**

		RAR		
retinoid		α	β	γ
RA	EC_{50}[a] (% eff)[b]	459	87	20
	K_d[c]	15 ± 2	13 ± 3	18 ± 1
1	EC_{50}[a] (% eff)[b]	NA[d]	25 ± 4 (32)	NA[d]
	K_d[c]	129 ± 6	20 ± 2	104 ± 30
2	EC_{50}[a] (% eff)[b]	NA[d]	115 ± 37 (61)	NA[d]
	K_d[c]	94 ± 15	996 ± 148	$>10^4$
3	EC_{50}[a] (% eff)[b]	NA[d]	26 ± 2 (47)	NA[d]
	K_d[c]	303 ± 32	189 ± 41	1490 ± 106

[a] EC_{50} values (effective concentration at 50% of maximum retinoid response) are the mean ± SEM of at least three experiments performed in triplicate using CV-1 cells cotransfected with the luciferase reporter plasmid MTV-4(R5G)-Luc and an expression vector of the indicated retinoic acid receptor. [b] % eff = normalized to the maximum RA response and reported as an average of three experiments. [c] K_d values are reported as the mean value of three determinations by competition of [^{3}H]-(*all-E*)-retinoic acid (5 nM) binding with unlabeled test retinoid using baculovirus expressed RARs. [d] NA = not active.

In summary, the incorporation of the 2-thienyl moiety into the nucleus of various classes of retinoids resulted in the specific activation of gene transcription through RARβ.[23] Further, compounds **1**−**3** were shown to be functional antagonists of RA at RARα and -γ. These compounds will be useful in delineating the biology associated with RARβ activation and in identifying specific diseases that are responsive to RARβ agonists. Testing of these agents in various biological systems is in progress, and the results of these experiments will be the subject of a future report.

Acknowledgment. We thank D. Mais, E. Berger, and K. Flatten for providing the ligand binding data and Mr. H. Nguyen for the mass spectral analyses.

Supporting Information Available: Experimental details for the synthesis of compounds **1**, **2**, and **3** and procedures used in the biological assays (8 pages). Ordering information is given on any current masthead page.

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(23) We have also found the C8 phenyl analog of compound **1** to exhibit the same profile as **1** in the RAR transactivation assay, though the efficacy at RARβ is somewhat lower.

JM960687R