Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 17, pp. 2313-2318, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00420-4

SYNTHESIS AND RECEPTOR BINDING AFFINITY OF CONFORMATIONALLY RESTRICTED RETINOIC ACID ANALOGUES

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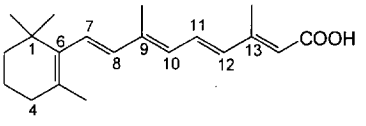
Abstract: In an effort to synthesize two conformationally restricted retinoids utilizing a cyclopropyl ring as bioisostere for the C9-C10 double bond, we describe the synthetic strategies and retinoid receptor binding affinity of the *trans*-cyclopropyl analogue and the unexpected epimerization of the *cis*-cyclopropyl analogue.

All-*trans*-retinoic acid (RA; **1**) binds selectively to the nuclear retinoic acid receptor family (RARs) while its geometric isomer, 9-*cis*-retinoic acid (9-*cis*-RA; **2**), binds to both the RARs and the nuclear retinoid X receptors (RXRs).[1] RXRs dimerize with RARs as well as other members of the steroid/thyroid hormone receptor superfamily and enhance the binding of receptors to their hormone response elements.[2] The importance of ligand binding in the function of RXR is, however, unclear. In addition, because 9-*cis*-RA can be converted to the more stable RA during biological studies,[3] and because of its lack of receptor binding specificity, this compound is of limited use in elucidating the role of the RAR and RXR receptor families in regulating specific cellular processes. Thus, we targeted the development of natural ligand-like conformationally restricted synthetic analogues of RA and 9-*cis*-RA, which may specifically activate the RAR or RXR receptor family to explore their functions, as well as study the ligand binding pocket.

A number of years ago we prepared the cyclopropyl analogues **3-6** of 13-*cis*-retinoic acid and RA in order to determine whether the 13-*cis* isomer had any unique biological activity.[4] While both methyl compounds **3** and **4** were inactive, we found that the desmethyl analogues **5** and **6** showed equivalent biological activity that was reduced relative to RA.[5] On the assumption that a similar observation would be obtained for RA and 9-*cis*-RA receptor binding, we have targeted cyclopropane analogues **7** and **8** for synthesis and evaluation. Others have recently used this strategy for more modified retinoids (**9** and **10**;[6] **11**[7]) and found the 9-*cis* analogues have greater than 100-fold selectivity for binding to the RXR's while the *trans* analogue (**9**) shows RAR specificity. Similar RXR specificity has been found for cyclohexene analogue **12**[8] suggesting this is a fruitful direction for further study.



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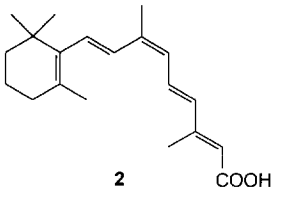

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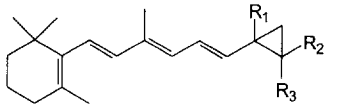
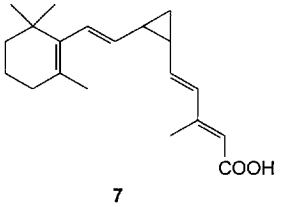


3 $R_1 = CH_3$, $R_2 = COOEt$, $R_3 = H$
4 $R_1 = CH_3$, $R_2 = H$, $R_3 = COOEt$
5 $R_1 = H$, $R_2 = COOEt$, $R_3 = H$
6 $R_1 = H$, $R_2 = H$, $R_3 = COOEt$



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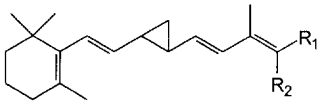
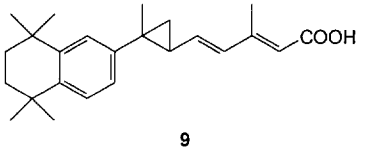


8 $R_1 = COOH$, $R_2 = H$
14 $R_1 = H$, $R_2 = COOH$



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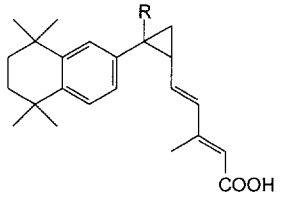
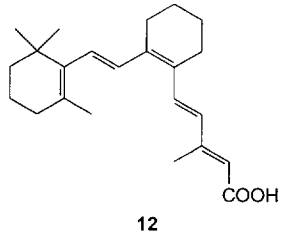


10 $R = CH_3$
11 $R = H$

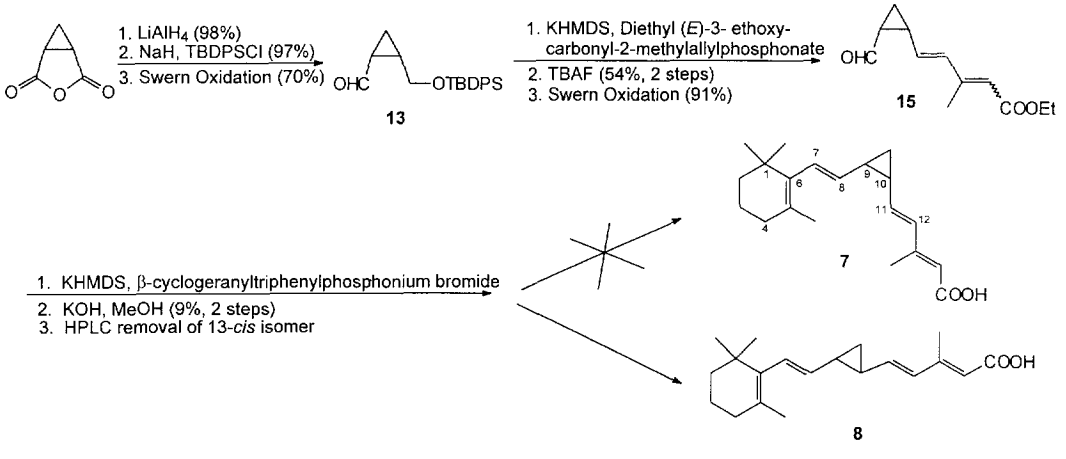


12

In this report, we describe our effort in synthesizing analogues **7** and **8**. Scheme 1 shows the initial attempt to synthesize analogue **7** which actually produced **8**. Commercially available 1,2-cyclopropanedicarboxylic anhydride was reduced (LAH) to *cis*-1,2-bis(hydroxymethyl)cyclopropane which was treated with *tert*-butyldiphenylsilyl chloride (NaH/THF) to form the monoprotected silyl diol. This alcohol was oxidized (Swern conditions) to aldehyde **13**. Horner-Emmons reaction with diethyl (*E*)-3- ethoxycarbonyl-2-methylallylphosphonate followed by desilylation and Swern oxidation gave formylester **15**. Wittig reaction of **15** with β-cyclogeranyltriphenylphosphonium bromide provided the cyclopropyl retinoid ester. Saponification followed by HPLC-separation surprisingly afforded the 13-*E* isomer **8** and 13-*Z* isomer **14** in an approximate 2:3 ratio. The overall yield from cyclopropane dicarboxylic anhydride was less than 5%. The preponderance of **14** can be attributed to the isomerization of base treated phosphonate in the Horner-Emmons reaction. Lower reaction temperatures (-100 °C) did not prevent this isomerization from occurring. Other side products were also observed in this step which could be due to rearrangement products contributing to the low retinoid yield (see below).

The receptor binding affinity of **8** and **14** was evaluated (Table 1) by published methods.[9] Briefly, recombinant receptor proteins were produced using insect cell- and *E. coli*-expression systems. Receptors were incubated with radiolabeled RAs (see Table 1) in the absence or presence of unlabeled retinoid analogues (1 x 10^{-10} - 1 x 10^{-5}M). Hydroxylapatite was used to separate radioligand bound to receptor from free ligand, and the bound ligand was measured by scintillation counting. The LIGAND program was used to determine binding affinities (Ki, inhibition constant). In cases where complete displacement of radiolabeled RA was not achieved at the highest competitor concentration tested, Ki was calculated from the IC_{50} value using the Cheng and Prushoff equation.[9] Analogues were evaluated together with the RAR-specific agonist (*E*)-4-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)propen-1-yl]benzoic acid (TTNPB)[10] and RXR-selective ligand 4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzoic acid (LGD 1069).[11] While **14** had the expected lack of affinity to both RAR[12] and RXR[1], compound **8** had good affinity for the RARs and little binding affinity towards the mRXRγ.[6-8] These results led us to suspect that we had, in fact, not obtained the *cis*-cyclopropyl analogue **7**. Thus, NOE experiments were performed and no expected enhancement was observed for the other presumably spatially close vinyl proton when either H-8 or H-11 was irradiated.

Scheme 1. Synthesis of *cis*-cyclopropyl retinoic acid analogue.

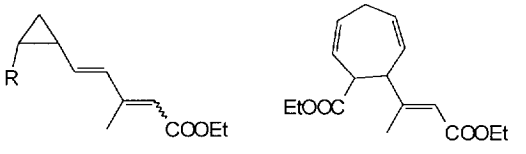


In order to help confirm **8** had been isolated in our efforts to prepare **7** we synthesized analogue **8** directly. The synthesis of **8** is shown in Scheme 2 and its ^{1}H NMR spectrum, NOE, and HPLC properties were found to be identical to that of the previously isolated **8**. When we conducted the NOE experiment for the alcohol precursor to **7** (**16**), the expected enhancement for the hydroxymethyl protons was observed when H-11 was irradiated. Since all the intermediates until the final Horner-Emmons reaction for preparing **7** and **8** are clearly different, it was concluded that **7** epimerized to **8** during the Horner-Emmons reaction.

Table 1. Receptor Binding Affinity of **8** and **14**.

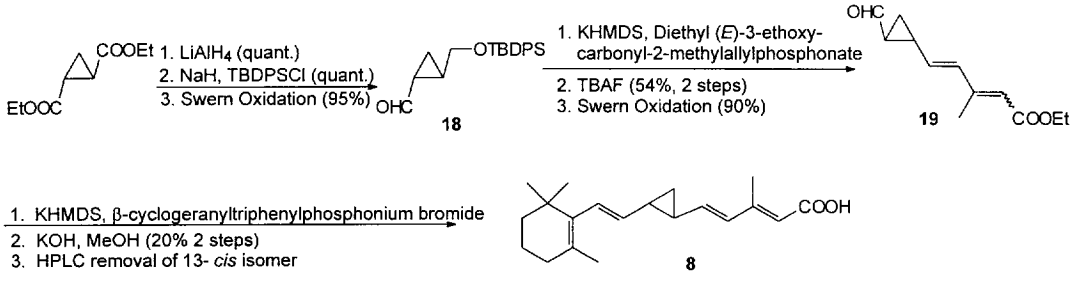
Analogue	K_i (nM)		
	cRARβ$_2$[a] (vs. 0.82nM ^{3}[H]-all-*trans*-RA)	cRARβ$_2$ (vs. 1.1 nM ^{3}[H]-9-*cis*-RA)	mRXRγ (vs. 3.4 nM ^{3}[H]-9-*cis*-RA)
RA	0.5 ± 0.05	0.4 ± 0.04	---[b]
9-*cis*-RA	0.7 ± 0.07	0.5 ± 0.08	27 ± 4
TTNPB	0.5 ± 0.08	0.5 ± 0.09	---
LGD 1069	>550	>800	75 ± 13
8	11 ± 0.6	13 ± 0.7	>7200
14	>1100	>1200	>2800

[a]similar results with RA, **8** and **14** were observed for hRARα (Ki = 0.1, 3.1, and >4000 respectively) and hRARγ (Ki = 0.6, 32, and >4000 respectively), [b]... = no binding competition.



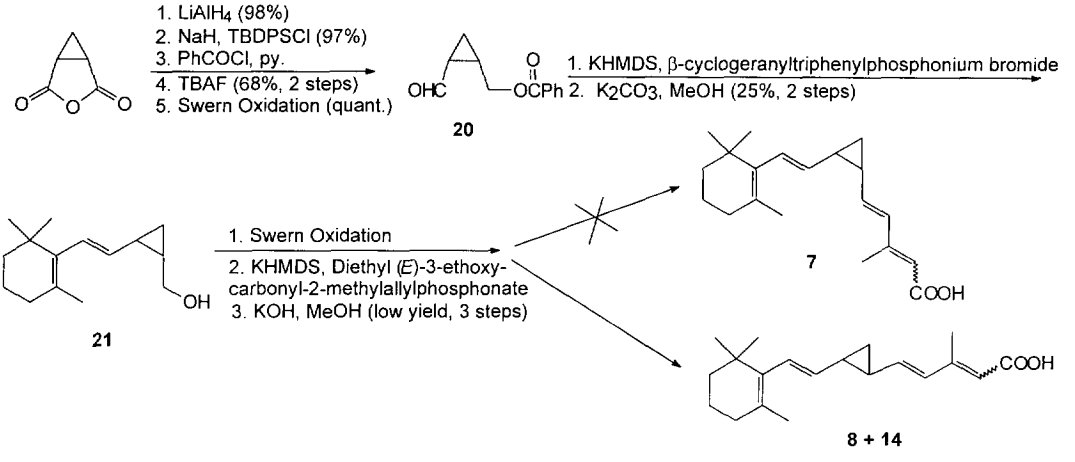
16 R = CH_2OH **17**

Scheme 2. Synthesis of *trans*-cyclopropyl retinoic acid analogue.



An alternative approach to synthesize **7** was developed in an attempt to minimize the possibility of epimerization and is shown in Scheme 3. The C-10 unit (β-cyclogeranyltriphenylphosphonium bromide) was successfully connected to the cyclopropyl ring via Wittig reaction conditions. No epimerization was observed for this intermediate. However, addition of the C-5 unit via a Horner-Emmons type reaction resulted in epimerization and the *trans*-cyclopropyl analogue **8** was again obtained in low yield instead of **7**.

Scheme 3. Attempted alternative approach to **7**.



It is well known that *cis*-divinylcyclopropane undergoes Cope rearrangement to 1,4-cycloheptadiene at room temperature.[13] The presence of a *cis*-divinylcyclopropane moiety in our target molecule **7** might have initiated the Cope rearrangement and contributed to the low yield of only **8**. However, we were not able to identify such a rearrangement product among the complex side product mixture. A model reaction utilizing the aldehyde **16** and triethyl phosphonoacetate was thus performed in order to investigate the possibility of rearrangement occurring in our system. The product was isolated and partially purified, and while the ^{1}H NMR spectrum was complex it was consistant with **17** and was clearly not the Horner-Emmons product even though FAB MS indicated that the product had identical mass as the expected rearranged cycloheptadiene. This assumption is further confirmed by UV-Vis spectrophotometry in which the starting material **15** has a conjugated diene system with λ_{max} at 277nm while the product isolated did not show absorbance above 230nm indicating the disappearance of the conjugated diene. Thus, it appears that the Cope rearrangement product **17** was obtained and that similar processes occurred in efforts to prepare compound **7**.[14]

In conclusion, receptor binding studies of the 9-*trans*-cyclopropyl analogue of RA (**8**) shows RAR selectivity while the 13-*cis* isomer shows no binding to either RAR or RXR. It should be noted that analogue **8** was studied as a racemic mixture, others have demonstrated that utilizing the individual enantiomer of *cis*-cyclopropyl moiety improved the binding affinity of the resulting retinoid analogue.[15] We also anticipate a similar observation for **8** and it may prove desirable to prepare optically pure **8**. Although the attempted synthesis of **7** lead mainly to unidentified products and the unexpected epimerization to **8**, further efforts are underway in synthesizing a related minimally modified 9-*cis* analogue.

Acknowledgement

Partial financial support from Public Health Service grants CA49837 and DK14881 is gratefully acknowledged.

References and Notes

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14. Scientists at Allergan Pharmaceutical (Vuligonda, V. and Chandraratna, R. A. S.) have demonstrated the rearrangement occurs in a system closely related to **7** (personal communication).
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16. For **8**: IR (KBr) 3437, 2924, 1675 cm^{-1}; UV (CH_3OH) λ_{max} 282 nm (ε 13400); ^1H NMR ($CDCl_3$) δ 0.96 (br s, 8H), 1.23 (br s, 2H), 1.43 (m, 2H, retinoid H-2), 1.56 (m, 2H, retinoid H-3), 1.63 (s, 3H, retinoid 5-CH_3), 1.94 (t, 2H, retinoid H-4), 2.24 (s, 3H, retinoid 13-CH_3), 5.0 (m, 1H, retinoid H-8), 5.70 (m, 2H, retinoid H-11 and H-13), 5.92 (d, 1H, J = 16.2 Hz, retinoid H-7), 6.21 (d, 1H, J = 15.4 Hz, retinoid H-12); RP-HPLC (CH_3OH/H_2O 85:15, both with 10 mM NH_4OAc, flow rate 0.75 mL/min) t_R = 11.3 min; FAB-MS, *m/e* (relative intensity) 300 (29.5), 285 (20.8), 147 (100).
17. For **14**: IR (KBr) same as **8**; UV (CH_3OH) λ_{max} 282 nm (ε 5033); ^1H NMR ($CDCl_3$) δ 0.96 (br s, 8H, $C(CH_3)_2$ and cyclopropyl H), 1.23 (br s, 2H, cyclopropyl H), 1.40 (m, 2H, retinoid H-2), 1.56 (m, 2H, retinoid H-3), 1.65 (s, 3H, retinoid 5-CH_3), 1.92 (t, 2H, retinoid H-4), 1.98 (s, 3H, retinoid 13-CH_3, irradiation showed NOE to resonances at 5.56 and 5.73 ppm), 5.0 (m, 1H, retinoid H-8), 5.56 (s, 1H, retinoid H-14), 5.73 (m, 1H, retinoid H-11), 5.93 (d, 1H, J = 17.5 Hz, retinoid H-7), 7.64 (d, 1H, J = 16.7 Hz, retinoid H-12); RP-HPLC (CH_3OH/H_2O 85:15, both with 10 mM NH_4OAc, flow rate 0.75 mL/min) t_R = 9.7 min.

(*Received in USA* 19 *May* 1997; *accepted* 11 *August* 1997)