Design and synthesis of potential new apoptosis agents: hybrid compounds containing perillyl alcohol and new constrained retinoids

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ARTICLE INFO

ABSTRACT

Novel retinoids **1–3** containing perillyl alcohol were synthesized through the addition of perillyl alcohol to the activated carboxylic acids (retinoids) promoted by DCC (N,N'-dicyclohexyl carbodiimide). A set of structurally and functionally diverse perillyl alcohol derivatives of retinoids were obtained in good yields (78–82%). Biological evaluation of these novel hybrid compounds (containing retinoids and perillyl alcohol) is currently underway in our laboratory.

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Apoptosis is an essential cellular process for normal development and homeostasis of multicellular organisms, to eliminate unwanted or damaged cells. Deregulated apoptosis plays a major role in many human diseases, including cancer.[1–4] Defects in the apoptosis machinery can confer apoptosis resistance of cancer cells to therapeutic agents, which can render current anticancer therapies less effective, and may lead ultimately to their failure in the clinic.[2–4] Accordingly, targeting critical regulators of apoptosis to overcome apoptosis resistance is a promising cancer therapeutic strategy. Perillyl alcohol and all-*trans*-retinoic acid (ATRA) have antitumour activity toward liver, colon and hematopoietic tumours, and are used as chemotherapeutic agents.[5] The chemo-preventive activity of perillyl alcohol and ATRA is associated with an increase in apoptosis of the tumour cells. Therefore, we hypothesized that hybrid molecules containing both perillyl alcohol and ATRA might further increase chemo-preventive activity through enhancing apoptosis. The hybrid concept to synthesize biological active molecules is a frontier area of research and this concept has successfully employed to synthesize new therapeutic agents for Alzheimer's diseases.[6]

Toward this goal, we first sought to synthesize compounds **4a–b** as new retinoic acid analogues by introducing a constrained phenyl ring system in the place of conjugated alkene backbone (spacers in ATRA) to inhibit the metabolism of ATRA into its isomers, 9-CRA and 13-CRA (Fig. 1).

Furthermore, we considered the synthesis of novel perillyl alcohol containing retinoids (1–3) for studying tumour cell-targeted apoptosis. To our surprise, a search of the literature failed to uncover any examples of perillyl alcohol containing retinoic acid derivatives. Here we report the first synthesis of perillyl alcohol containing retinoids by simple addition of perillyl alcohol to the activated carboxylic acids (retinoids), promoted by DCC (Scheme 1).
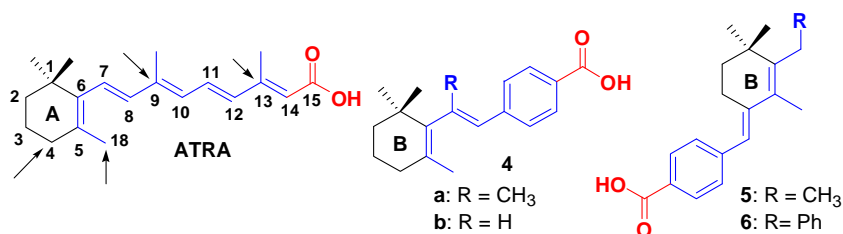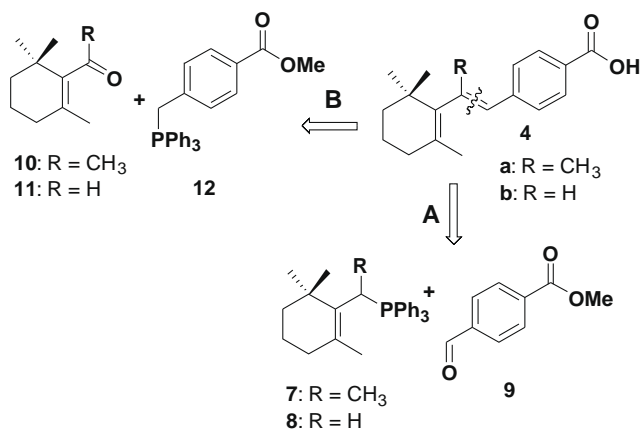

Figure 1. Structure of ATRA and our new retinoic acid analogues. Metabolic positions of all-*trans*-retinoic acid (ATRA) are shown by arrows.

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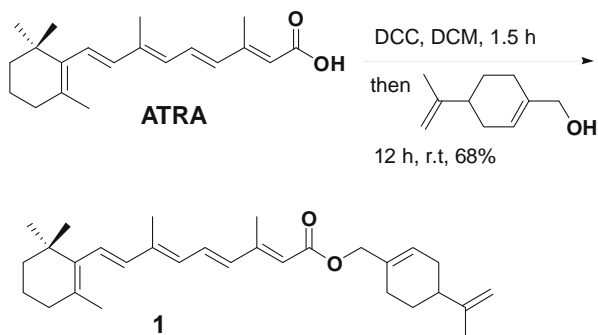
Scheme 1.


Scheme 2. Retrosynthetic analysis.

We next explored the synthesis of the constrained retinoids **4a**–**b** and the possible retro-synthetic analysis as shown in Scheme 2.

In the process of synthesizing **4a**, based on disconnection route A (Scheme 2) starting from the allylic alcohol **13**, we encountered the unusual Wittig salt **14** which led to the formation of the unusual compound **5** when the ylide **14** was treated with aldehyde **9** (Scheme 3).[7]

To explore the mechanism for forming the unusual Wittig salt **14**, we first tried to understand the substitution effect at the α-position of the allylic alcohol **13**. For that purpose we synthesized the allylic alcohol **15** from Grignard reaction of β-cyclocitral **11** with phenyl magnesium bromide in THF. The allylic alcohol **15** was converted into the corresponding ylide using PPh₃HBr followed by the Wittig reaction with aldehyde **9** furnishing the unusual product **6** with 83% yield (Scheme 4).

When the α-position of the allylic alcohol was substituted with hydrogen **17** (1° alcohol) we found both unusual and usual Wittig products **18** and **4b** with the ratio of 1:0.6, respectively (Scheme 4). From these experiments we found that when the α-position is substituted with methyl, phenyl gives exclusively the unusual Wittig salt, whereas when the α-position is substituted with hydrogen, both usual and unusual products are obtained.

To further explore the formation of the unusual Wittig product, we examined various solvents. The allylic alcohol **13** gave the unusual Wittig product (**5**) in methanol, DMF and acetonitrile (Table 1, entry 1). Interestingly, allylic alcohol **17** gave the normal Wittig product (**4b**) in DMF (Table 1, entry 2) but produced mixture of usual (**4b**) and unusual (**18**) Wittig products in both methanol and acetonitrile (Table 1, entry 2). This observation indicates that the α-methyl group plays an important role in the reaction. We next examined the mechanistic insight to study the unusual Wittig salt formation using the cyclic allylic alcoholic systems (the alcohol group is inside the ring system).
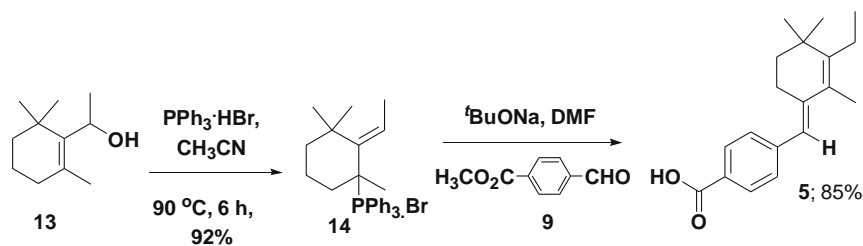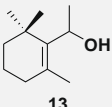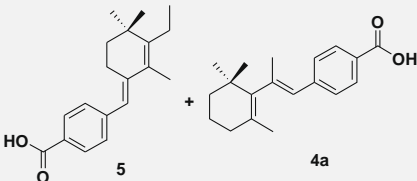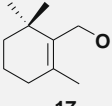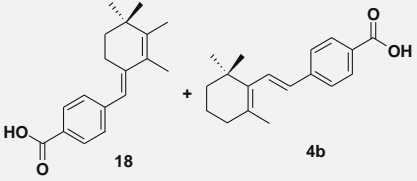

Scheme 3.
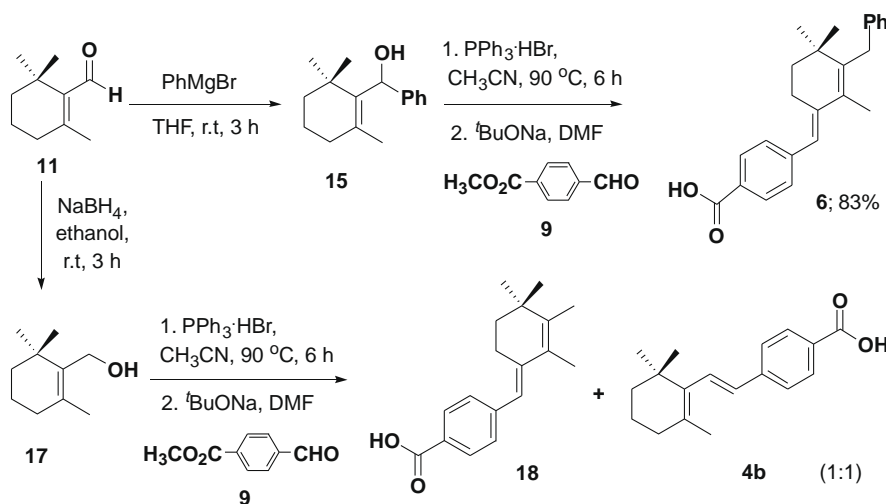
Scheme 4. Results obtained from route A.

Table 1
Studies of the solvent effect of the unusual Wittig reaction

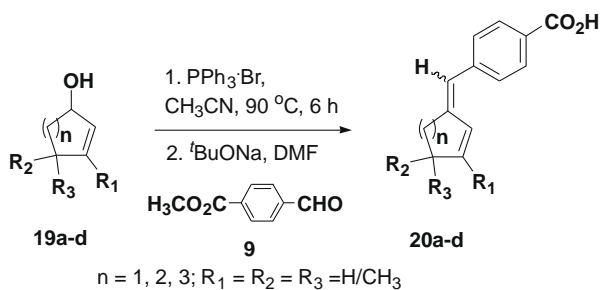| Entry	Allylic alcohol	Product	Yield[a] (%) (product ratio)[b]		
			MeOH	DMF	CH₃CN
1	**13**	**5** + **4a**	85 (**5:4a** = 1:0)	83 (**5:4a** = 1:0)	90 (**5:4a** = 1:0)
2	**17**	**18** + **4b**	80 (**18:4b** = 1:0.6)	78 (**18:4b** = 0:1)	76 (**18:4b** = 1:0.6)
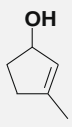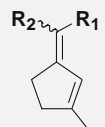
[a] Isolated yield refers to aldehyde.
[b] The ratios of the usual and unusual Wittig products were calculated from the ¹H NMR.

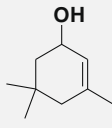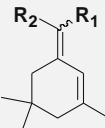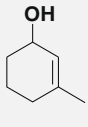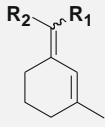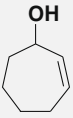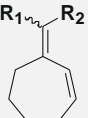To our surprise reactions utilizing allylic alcohols inside the ring system (Table 2, entries 1–4) generated the more normal Wittig

Table 2
Studies of the Wittig reactions alcohol attached to the ring system[a]

19a-d + 9 → 20a-d

n = 1, 2, 3; R₁ = R₂ = R₃ =H/CH₃

Entry	Allylic alcohol (**19a–d**)	Product	Yield (%)[b] (E:Z)[c]
1	**OH**	**20a**: (R₁ = H, R₂ = 4-HO₂CC₆H₄)	76 (99:1)[d]
2	**OH**	**20b**: (R₁ = H, R₂ = 4-HO₂CC₆H₄)	83 (85:15)
3	**OH**	**20c**: (R₁ = H, R₂ = 4-HO₂CC₆H₄)	85 (72:28)

Table 2 (*continued*)

Entry	Allylic alcohol (**19a–d**)	Product	Yield (%)[b] (E:Z)[c]
4	**OH**	**20d**: (R₁ = H, R₂ = 4-HO₂CC₆H₄)	69 (99:1)[d]

[a] All reactions were performed using 1 equiv of aldehyde and 3 equiv of ᵗBuONa in DMF for 8–12 h.
[b] Isolated yield (mixture of E and Z) refers to aldehyde.
[c] The E:Z ratios were determined by ¹H NMR.
[d] Trace amount of Z isomer was observed in ¹H NMR.

products (**20a–d**). We examined cyclic 5-, 6- and 7- member ring systems and in all cases we isolated the regular Wittig products.

These new derivatives **20a–d** also have the hydrophobic cycloalkene ring and constrained phenyl ring system in the place of conjugated alkene backbone in ATRA, and the carboxylic acid group remains intact. We compared the structural relation of our original target-constrained retinoids **4a–b** with the new unusual products **5–6** and these new compounds **20a–d**. Having an extension of the double bond conjugation via the 4th position of the cycloalkene ring unit may block or limit retinoic acid metabolism caused by cytochrome P450 enzymes (CYPs). This could be very advantageous, since it is believed that the most prominent physiological degradation pathway starts with the rate-limiting hydroxylation at the C-4 position of the cyclohexenyl ring, leading to the formation of 4-hydroxy-ATRA, followed by a reductase enzyme into 4-oxo-ATRA and further transformation by CYP(s) into more polar metabolites.[8]

Then we tried to synthesize our original target-constrained retinoids **4a–b** through the disconnection route B (Scheme 1). First we tried with the ylide 4-[(triphenyl-phosphanyl)-methyl]-benzoic acid methyl ester **12** with β-cyclocitral **11** with different reaction conditions and the reactions failed to obtain the constrained retinoid **4a**. We then prepared the reactive ylide 4-(diethoxy-phosphorylmethyl)-benzoic acid methyl ester **21**, and treating with β-cyclocitral **11** using ᵗBuOK as a base yielded the final product **4b** with 79% yield (Table 3, entry 3).

Table 3
Results obtained from route B
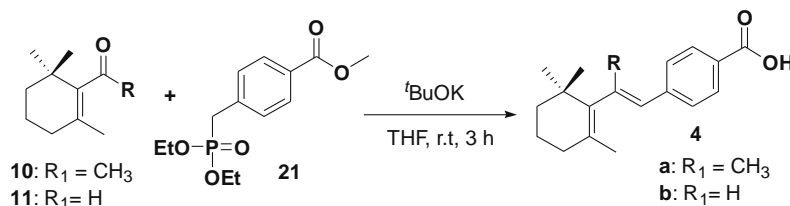
Entry	R	Reaction condition	Yield[a] (%)
1	H	*t*BuONa in DMF	—
2	H	*t*BuONa in THF	16
3	**H**	***t*BuOK in THF**	**79**
4	CH$_3$	*t*BuONa in DMF	—
5	CH$_3$	*t*BuONa in THF	—
6	CH$_3$	*t*BuOK in THF	—
7	CH$_3$	TMG, THF	—

[a] Isolated yield.
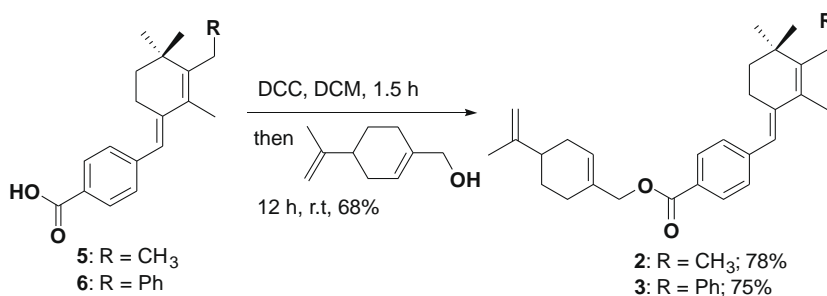
Scheme 5.

However, very surprisingly the Wittig reaction of ylide 4-(diethoxy-phosphorylmethyl)-benzoic acid methyl ester **21** with ketone **10** clearly failed to give the olefination product **4a**.

Encouraged by these results and having established a new strategy for synthesizing the new retinoid derivatives we synthesized the perillyl alcohol containing constrained retinoids **2–3**.[9] These were synthesized from the acids **5** and **6** using DCC as a carboxylic acid activating agent in DCM. The activated carboxylic acid was treated with perillyl alcohol in DCM at room temperature and generated the products **2** and **3** with 78% and 75% yields, respectively (Scheme 5).

In conclusion, we have synthesized novel constrained retinoids (**4b**, **5**, **6** and **20a–d**) and found when alcohols are outside the ring produce unusual Wittig salts, whereas when alcohols are inside the ring produce usual Wittig salts. These acids were further esterified using perillyl alcohol to synthesize new perillyl alcohol containing retinoids through DCC coupling. This is based on the addition of perillyl alcohol to the activated carboxylic acids, promoted by DCC. A novel set of structurally and functionally diverse perillyl alcohol containing retinoids were obtained in moderate to good yields. Biological evaluation of these novel hybrid retinoids is currently underway in our laboratory.

Acknowledgments

Supplementary data

Supplementary data (copies of ^{1}H, ^{13}C NMR and Mass spectra) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.01.003.

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9. *General procedure for synthesis of perillyl alcohol containing new retinoids* (**1–3**): A mixture of all *trans*-retinoic acid (100 mg, 0.35 mmol) in dry CH$_2$Cl$_2$ (5 mL) was stirred under nitrogen atmosphere was added DCC (79 mg, 0.38 mmol) and cat. DMAP at room temperature. The reaction mixture was stirred for 2 h at room temperature under nitrogen atmosphere. To this stirred solution perillyl alcohol

(58.6 mg, 0.38 mmol) was added drop wise. The reaction mixture was stirred at room temperature until TLC analysis indicated none remaining (about 10–12 h). The reaction was quenched with saturated NH_4Cl and extracted with ethyl acetate. The extracts were washed with H_2O and brine, then dried overage Na_2SO_4, and evaporated. The residue was purified by flash column chromatography using hexane/ethyl acetate (8:1) as the eluent to give **1** as colourless oil.

Compound **1**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.05 (s, 6H), 1.45–1.52 (m, 2H), 1.58–1.67 (m, 2H), 1.73 (s, 3H), 1.76 (s, 3H), 1.97–2.10 (m, 6H), 2.10–2.20 (m, 4H), 2.38 (s, 3H), 4.53 (s, 2H), 4.76 (m, 2H), 5.78–5.82 (m, 2H), 6.12–6.15 (m, 1H), 6.20 (s, 1H), 6.25–6.31 (2H, d, J = 18 Hz), 6.92–7.05 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.3, 14.3, 19.6, 21.2, 22.1, 26.8, 27.7, 29.4, 30.9, 33.5, 34.6, 39.9, 41.3, 68.2, 109.1, 118.7, 125.8, 129.1, 129.8, 130.4, 131.4, 133.4, 135.5, 137.6, 138.1, 140.0, 150.1, 153.4, 167.5.

Compound **2**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.07–1.15 (m, 9H), 1.23–1.38 (m, 2H), 1.50–1.54 (m, 3H), 1.77 (s, 3H), 1.94 (s, 4H), 2.18–2.30 (m, 6H), 2.60–2.68 (m, 2H), 4.70–4.79 (m, 4H), 5.59 (s, 1H), 6.48 (s, 1H), 7.33–7.36 (d, J = 9 Hz, 2H), 8.00–8.03 (d, J = 9 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.3, 15.7, 21.0, 23.5, 24.2, 25.1, 26.0, 27.1, 27.8, 28.0, 31.1, 35.8, 36.4, 39.2, 41.7, 69.4, 109.2, 120.8, 125.9, 127.8, 128.0, 129.8, 130.0, 133.8, 142.6, 144.7, 149.2, 150.8, 166.0. ESI MS: $[M+H]^+$ 419.2946, calcd 419.2950 for $C_{29}H_{39}O_2$.

Compound **3**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.05 (s, 6H), 1.58–1.65 (m, 5H), 1.77 (s, 4H), 1.87 (s, 4H), 2.15–2.30 (m, 5H), 2.70–2.76 (m, 2H), 3.71 (s, 2H), 4.73–4.77 (m, 4H), 5.88 (br s, 1H), 6.57 (s, 1H), 7.17–7.25 (m, 3H), 7.29–7.33 (m, 2H), 7.38–7.41 (d, J = 9 Hz, 2H), 8.02–8.05 (d, J = 9 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 16.8, 21.2, 24.4, 25.0, 25.8, 27.0, 27.7, 28.2, 30.9, 35.3, 35.8, 36.1, 39.0, 41.6, 56.0, 69.7, 110.0, 121.8, 125.4, 128.7, 129.6, 129.7, 130.8, 133.0, 140.3, 142.5, 144.2, 145.0, 150.1, 166.8. ESI MS: $[M+H]^+$ 481.3160, calcd 481.3107 for $C_{34}H_{40}O_2$.