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Tetrahedron Letters 49 (2008) 2410–2413
Tetrahedron Letters

(−)-Quinic acid: a versatile precursor for the synthesis of analogues of 2-crotonyloxymethyl-(4*R*,5*R*,6*R*)-4,5,6-trihydroxycyclohex-2-enone (COTC) which possess anti-tumour properties

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Received 15 January 2008; revised 6 February 2008; accepted 11 February 2008
Available online 14 February 2008

Abstract

Syntheses of three novel analogues of the *Streptomyces* metabolite COTC are described, using the versatile chiral pool starting material, (−)-quinic acid. The results of bioassays of the target compounds against two lung cancer cell lines, A549 and H460, are presented.

Keywords: Anti-cancer; Natural product; Quinic acid; Cyclopropane

2-Crotonyloxymethyl-(4*R*,5*R*,6*R*)-4,5,6-trihydroxycyclohex-2-enone (COTC, **1**) was isolated in 1975 from the cultures of *Streptomyces griseosporeus* and was demonstrated to possess cytotoxic and cyterostatic activity (Fig. 1). Both **1** and its synthetic analogue, 2-crotonyloxymethyl-cyclohex-2-enone (COMC, **2**), possess potent activity against murine and human cancer cell lines in culture as well as in tumour-bearing mice.[1–3] A principal mechanism of action of the cyclohexenones proceeds via enzyme-catalysed conjugation of glutathione (GSH) to the enone moiety of **1**/**2**, thereby generating an exocyclic enone of type **3**. Subsequent alkylation of intracellular proteins and/or DNA by this reactive entity then leads to cell death.[4]

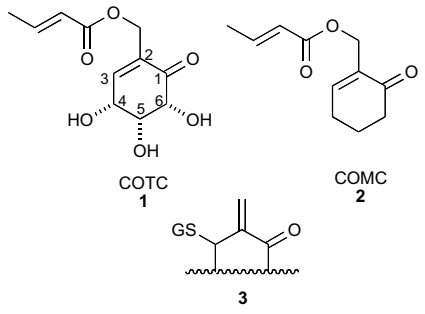

Fig. 1.

A focus of our recent research effort has been the preparation of analogues of **1** with general structure **4** wherein there are four loci for diversification. Initial studies have concentrated on analogues which differ from each other in the extent of oxygenation of the cyclohexenone core (Fig. 2). Thus, dihydroxylated compound **5**, which possesses inverted stereochemistry at C5 compared with COTC, was found to display low toxicity towards lung cancer cell lines A549 and H460. Tri-hydroxylated compound 6-*epi*-COTC (**6**) was equipotent with **5** towards the lung cancer cell lines whereas racemic mono-hydroxylated compound **7** was substantially more toxic than **5**, **6** and COMC (**2**), towards these cell lines.[5] Previously, COMC

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0040-4039/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2008.02.059

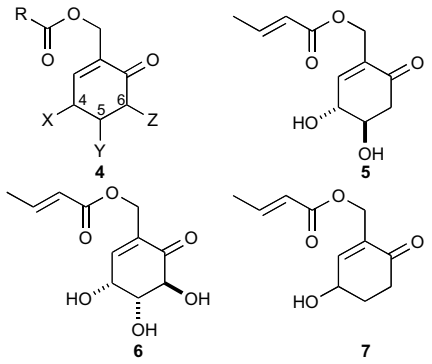


Fig. 2.

had been demonstrated to be the most potent anti-cancer agent belonging to this structural class, so these findings provide renewed stimulus for the synthesis and biological assessment of compounds related to **1/2**.

In this Letter, we describe the results of recent synthetic chemistry endeavours which demonstrate the versatility of the chiral pool material (−)-quinic acid (**8**) as a starting material for the preparation of analogues of COTC. Our target compounds were selected in order to probe several key structure activity features: thus, α,β-cyclopropyl ketone **9**, a *pseudo*-Michael acceptor, was chosen in order to ascertain the absolute requirement for a conjugated ketone moiety for anti-cancer activity. The unusual α,β-cyclopropyl-α′,β′-enone **10**, which has two potential sites available for nucleophilic attack by GSH, was selected to probe whether 'doubly-activated' molecules of this type would display enhanced anti-cancer properties compared with their mono-activated counterparts. Finally, the enantiomerically pure compound **11** was chosen to elucidate the influence of the absolute stereochemistry at C4 on in vitro anti-cancer activity (Fig. 3).

Our synthetic approach to cyclopropane **9** was founded on a serendipitous observation arising during earlier investigations into the reactivity of ketone **12** towards various nucleophiles (unreported data): thus, exposure of **12** to Corey's ylid (dimethylsulfoxonium methylide) yielded compound **13** as the only isolable product. The formation of **13** was rationalised to occur via base-mediated elimination of *tert*-butyldimethylsilanoxide to generate enone **14** followed

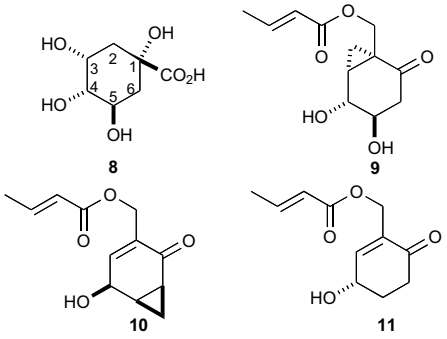
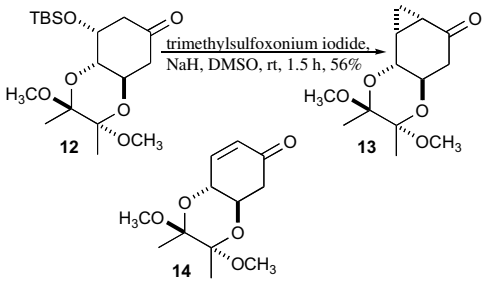


Fig. 3.

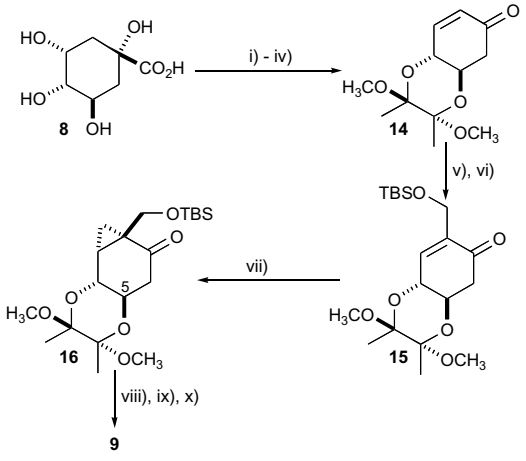


Scheme 1.

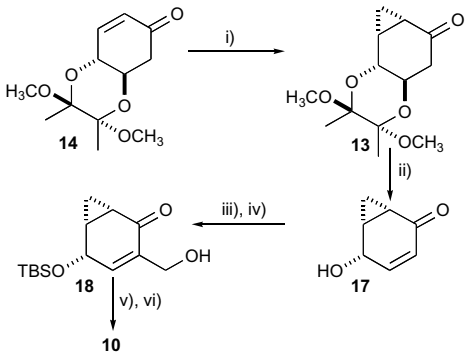
by stereoelectronically favoured 'axial attack' at C3 of **14** by the ylid (Scheme 1).

Prompted by this observation, we embarked on a synthesis of **9** proceeding via enone **14** as a key intermediate. This latter compound was prepared in 67% overall yield using a route modified slightly from that reported by Brückner and Gebauer for the synthesis of the cyclohexane-diacetal variant of **14** (Scheme 2).[6] Morita–Baylis–Hillman (M–B–H) reaction of the enone with formaldehyde using the conditions reported by Rezgui and El Gaied[7] proceeded in excellent yield to give the desired α-hydroxymethyl compound which was protected as its *tert*-butyldimethylsilyl (TBS) ether to give **15**. Pleasingly, exposure of **15** to an excess of Corey's ylid yielded a single cyclopropanated compound **16**, the stereochemical assignment of which was supported by a significant NOE from C(5)*H* (δ 3.86 ppm) to the cyclopropyl methylene *endo* hydrogen (δ 1.40 ppm). Deprotection of the primary hydroxyl group followed by crotonylation and acetal cleavage then yielded the target compound **9**.[8]

The synthesis of α,β-cyclopropyl-α′,β′-enone **10** proceeded via cyclopropane **13** which can be prepared by the treatment of either β-silyloxyketone **12** (vide supra) or



Scheme 2. Reagents and conditions: (i) butan-2,3-dione, $(CH_3O)_3CH$, camphorsulfonic acid, CH_3OH, Δ, 12 h, 98%; (ii) $NaBH_4$, CH_3OH, 0 °C to rt, 24 h; (iii) $NaIO_4$ on silica gel, CH_2Cl_2, 1.5 h, 70% over two steps; (iv) Et_3N, CH_3SO_2Cl, CH_2Cl_2, 3 h, 98%; (v) DMAP (cat.), H_2CO, THF/H_2O (1:1), 40 °C, 24 h, 80%; (vi) TBSCl, DMAP (cat.), Et_3N, CH_2Cl_2, 72 h, 98%; (vii) trimethylsulfoxonium iodide, NaH, DMSO, 48 h, 49%; (viii) TBAF, THF, 0 °C, 98%; (ix) crotonic anhydride, pyridine, DMAP (cat.), CH_2Cl_2, rt, 6 h, 46%; (x) TFA/H_2O (7:1), 0 °C, 30 min, then HPLC, 99%.



Scheme 3. Reagents and conditions: (i) trimethylsulfoxonium iodide, NaH, DMSO, rt, 1.5 h, 61%; (ii) TFA/H_2O (7:1), rt, 12 h, then K_2CO_3, H_2O, CH_3OH, rt, 30 min, 99%; (iii) TBSCl, DMAP (cat.), Et_3N, CH_2Cl_2, rt, 6 d, 78%; (iv) imidazole (cat.), H_2CO, THF, Na_2CO_3(aq), 40 °C, 7 d, 23%; (v) crotonic anhydride, pyridine, DMAP (cat.), CH_2Cl_2, rt, 24 h, 48%; (vi) TFA/H_2O (7:1), 0 °C, 1 h, 94%.

enone **14** with Corey's ylid (Scheme 3). Overnight exposure of **13** to aqueous TFA resulted in eliminative acetal removal to give alcohol **17** in good yield, and subsequent silylation of the free hydroxyl group in **17** followed by imidazole-catalysed M–B–H reaction[9] then gave hydroxymethyl compound **18**. Esterification of the primary hydroxyl of **18** followed by acid-catalysed removal of the silyl protecting group provided target compound **10** in ten steps from (−)-quinic acid (**8**).[10]

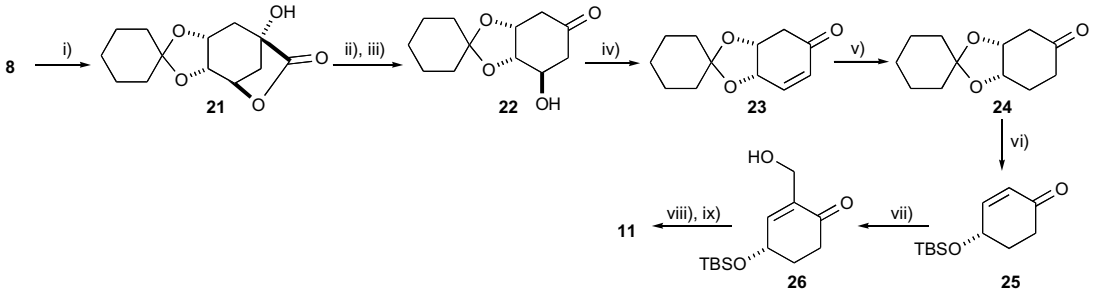
The key intermediate in the synthesis of our final target **11** was TBS-ether **25**, a large-scale synthesis of which has been described by Danishefsky and co-workers.[11] During the course of these workers' synthesis, isopropylidene protection was employed for the vicinal *cis*-diol in (−)-quinic acid. In our hands, although this synthetic route was successful, it proved to be a little capricious with regard to the reproducibility of yields as well as the ease of isolation of some intermediates. We opted, therefore, to modify the route to **25** and to utilise cyclohexylidene protection for the *cis*-diol moiety in **8** (Scheme 4).

Cyclohexylidene quinide **21** was prepared from **8** in standard fashion and subsequent reductive ring-opening of the γ-lactone followed by oxidative cleavage of the resulting vicinal diol gave β-hydroxy ketone **22**. Dehydration of **22** to give enone **23** could be achieved at slightly elevated temperature using methanesulfonyl chloride and Et_3N, however the reaction was sluggish and the transformation was better accomplished at room temperature via an intermediate trifluoromethanesulfonate and in the presence of pyridine. Catalytic hydrogenation of **23** proceeded with complete chemoselectivity to give cyclohexanone **24** and subsequent base-mediated elimination of the cyclohexylidene protecting group with concomitant silylation gave **25**, which was identical in all respects to the material reported by Danishefsky and co-workers.[11] DMAP-catalysed Morita–Baylis–Hillman reaction of **25** with formaldehyde yielded hydroxymethyl compound **26** which was converted in the usual manner (vide supra) to target compound **11** ($[\alpha]_D^{20}$ −23.0 (*c* 0.45, CH_2Cl_2)). The enantiomeric purity of this material was confirmed by chiral GC analysis using the essentially racemic compound **7**[5a] for comparison.

The results of bioassays of the analogues of COTC against two lung cancer cell lines (A549 and H460) are shown in Table 1. The assays were carried out by exposing cells to varying concentrations of each compound for 4 days. The number of surviving cells was then determined by the use of the MTT assay.[12]

The results of bioassay of the novel compounds **9**–**11** allow several conclusions to be reached regarding the structural features influencing the anti-cancer properties of COTC analogues towards the two cell lines: (i) an α,β-enone moiety appears to be vital for anti-cancer activity (data for **9**); (ii) the absolute configuration at C4 has no observable influence on potency (comparative data for **7** and **11**); (iii) incorporation of an additional electrophilic site does not improve potency (comparative data for **10** and **7**/**11**).

In conclusion, we have demonstrated the versatility of (−)-quinic acid as a starting material for the synthesis of analogues of the anti-tumour agent COTC. Three novel target compounds have been prepared which were selected in order to probe several important structure activity features. The results of bioassay of the analogues provide useful information which will guide future researchers in



Scheme 4. Reagents and conditions: (i) cyclohexanone, DMF/C_6H_6 (1:1), Amberlite IR 120 (H)®, reflux (Dean and Stark), 5 h, 60%; (ii) $NaBH_4$, CH_3OH, 0 °C to rt, 24 h; (iii) $NaIO_4$, H_2O, CH_3OH, 0 °C, 1.5 h, 58% from **21**; (iv) $(CF_3SO_2)_2O$, pyridine, CH_2Cl_2, 0 °C to rt, 12 h, 82%; (v) H_2, 10% Pd on C, EtOAc, rt, 17 h, 92%; (vi) DBU, TBSCl, C_6H_6, reflux, 6 h, 80%; (vii) DMAP (cat.), H_2CO, THF/H_2O (1:1), 40 °C, 24 h, 52%; (viii) crotonic anhydride, pyridine, DMAP (cat.), CH_2Cl_2, rt, 1.5 h, 68%; (ix) TFA/H_2O (7:1), 0 °C, 1 h, 98%.

Table 1
Bioactivity of COTC analogues towards lung cancer cell lines

Compound	IC_{50} (μM)	
	A549	H460
COMC (**2**)[5a]	55	40
5[5a]	147	158
6[5b]	170	158
7[5a]	24	10
9	>200	>200
10	18	20
11	17	11

the design and synthesis of compounds belonging to this structural class with improved anti-cancer activity.

Acknowledgements

We acknowledge, with thanks, the EPSRC (C.L.A.), BBSRC (M.P.) and the MRC (I.S. and N.S.W) for funding and AstraZeneca for the generous provision of a vacation bursary (K.F.L.).

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8. *Selected analytical data for compound* **9**: A clear oil; $[\alpha]_D^{22}$ +101.5 (*c* 1.3, CH_2Cl_2); ν_{max} (film)/cm^{-1} 3434 (br s, OH), 2099 (w, CH), 1644 (br s, C=O); δ_H (300 MHz; $CDCl_3$) 1.22 (1H, dd, *J* 8.4, 6.3, cyclopropyl CH_{exo}), 1.43 (1H, t, *J* 6.0, cyclopropyl CH_{endo}), 1.91 (3H, dd, *J* 6.9, 1.8, CH_3CH=CH), 2.05–2.11 (1H, m, C(3)*H*), 2.31 (1H, dd, *J* 18.0, 10.2, C(6)H_β), 2.35 (2H, br, 2 × O*H*), 2.82 (1H, dd, *J* 18.0, 5.7, C(6)H_α), 3.79–3.87 (1H, m, C(5)*H*), 3.95 (1H, d, *J* 11.7, one of CH_2OC=O), 4.14 (1H, q, *J* 4.2, C(4)*H*), 4.73 (1H, d, *J* 11.7, one of CH_2OC=O), 5.86 (1H, dq, *J* 15.6, 1.8, CH_3CH=C*H*), 7.00 (1H, dq, *J* 15.6, 6.9, CH_3C*H*=CH); δ_C (75 MHz; $CDCl_3$) 13.8 (cyclopropyl *C*H_2), 18.0 (*C*H_3CH=CH), 26.5 (*C*(3)H), 34.8 (*C*(2)), 43.4 (*C*(6)H_2), 64.7 (*C*H_2OC=O), 68.4 (*C*(5)H), 70.4 (*C*(4)H), 122.2 (CH_3CH=*C*H), 145.4 (CH_3*C*H=CH), 166.3 (CH_2O*C*=O), 222.1 (*C*(1)O); *m*/*z* (CI/NH_3) 258 (MNH_4^+, 10%), 241 (MH^+, 100); (Found: 241.2523, $C_{12}H_{17}O_5$ requires 241.2524).
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10. *Selected analytical data for compound* **10**: A clear oil; $[\alpha]_D^{20}$ −84.2 (*c* 0.57, CH_2Cl_2); ν_{max}(film)/cm^{-1} 3424 (br, OH), 1713 (s, C=O), 1666 (s, C=O); δ_H (300 MHz; $CDCl_3$) 1.11–1.34 (2H, m, cyclopropyl CH_2), 1.93 (3H, dd, *J* 6.0, 3.0, CH_3CH=CH), 2.07–2.12 (2H, m, C(5)*H* and C(6)*H*), 2.60 (1H, br, O*H*), 4.72–4.87 (2H, m, CH_2OC=O), 4.96–4.98 (1H, m, C(4)*H*), 5.90 (1H, dq, *J* 15.0, 3.0, CH_3CH=C*H*), 6.35–6.36 (1H, m, C(3)*H*), 7.05 (1H, dq, *J* 15.0, 6.0, CH_3C*H*=CH); δ_C (75 MHz; $CDCl_3$) 11.3 (cyclopropyl *C*H_2), 18.4 (*C*H_3CH=CH), 18.9 (*C*(5)H or *C*(6)H), 24.3 (*C*(5)H or *C*(6)H), 60.7 (*C*H_2OC=O), 64.9 (*C*(4)H), 122.3 (CH_3CH=*C*H), 130.7 (*C*(2)), 141.9 (*C*(3)H), 146.1 (CH_3*C*H=CH), 166.3 (CH_2O*C*=O), 195.6 (*C*(1)O); *m*/*z* (CI/NH_3) 240 (MNH_4^+, 15%), 223 (MH^+, 100); (Found: 223.0969, $C_{12}H_{15}O_4$ requires 223.0965).
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