Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 5974–5977
Bioorganic & Medicinal Chemistry Letters

Arene *cis*-dihydrodiols: Useful precursors for the preparation of analogues of the anti-tumour agent, 2-crotonyloxymethyl-(4*R*,5*R*,6*R*)-4,5,6-trihydroxycyclohex-2-enone (COTC)

Claire L. Arthurs,[a] James Raftery,[a] Helen L. Whitby,[a] Roger C. Whitehead,[a,*] Natasha S. Wind[b] and Ian J. Stratford[b]

[a]*School of Chemistry, University of Manchester, Oxford Road, Manchester M13 9PL, UK*
[b]*School of Pharmacy and Pharmaceutical Sciences, University of Manchester, Oxford Road, Manchester M13 9PL, UK*

Received 22 June 2007; revised 18 July 2007; accepted 18 July 2007
Available online 21 August 2007

Abstract—The synthesis of 6-*epi*-COTC, a diastereoisomer of *Streptomyces* metabolite 2-crotonyloxymethyl-(4*R*,5*R*,6*R*)-4,5,6-trihydroxycyclohex-2-enone (COTC), is described. The anti-cancer activities of the novel analogue, in racemic and enantiomerically pure forms, are presented.

2-Crotonyloxymethyl-(4*R*,5*R*,6*R*)-4,5,6-trihydroxycyclohex-2-enone (COTC, **1**) was isolated in 1975 from cultures of *Streptomyces griseosporeus* (Fig. 1). It has been demonstrated that both **1** and its non-hydroxylated analogue, 2-crotonyloxymethyl-cyclohex-2-enone (COMC, **2**), display potent toxicity towards murine and human cancer cell lines.[1–3]

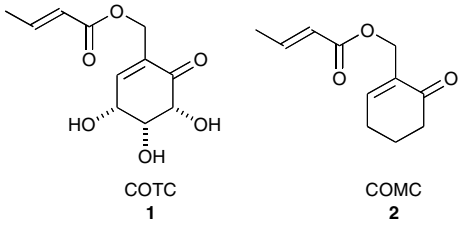
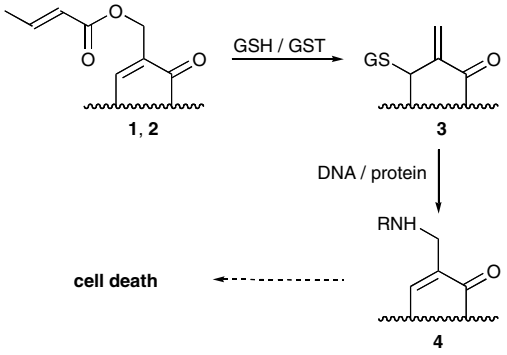


Figure 1. Structures of COTC and COMC.

Extensive investigations by several researchers[4] have demonstrated that the mechanism of anti-cancer activity of the cyclohexenones may involve conjugation of glutathione (GSH) to **1** or **2** thereby generating a glutathionylated exocyclic enone of type **3** (Scheme 1): this reaction can be catalysed by glutathione transferase (GST). Alkylation of intracellular proteins and/or nucleic acids by **3** is believed to then lead to cell death. A consequence of this mechanism of action is the probability that cells that possess elevated levels of GST/GSH will exhibit enhanced sensitivity towards exposure to **1** or **2**.



Scheme 1.

Recently, we initiated a synthetic chemistry programme designed to prepare analogues of COTC with general structure **5**, wherein the C4, C5 and C6 substituents represent three loci for structural variation (Fig. 2). Initial studies of a small array of these analogues have indicated that racemic compound **6** is up to four times more potent than COMC (**2**) towards lung cancer cell lines:[5] previously, comparative bioassays by Douglas

Keywords: Glutathione; Glutathione transferase; Anti-tumour; COTC.
* Corresponding author. Tel.: +44 161 275 7856; fax: +44 161 275 4939; e-mail: roger.whitehead@manchester.ac.uk

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.07.070

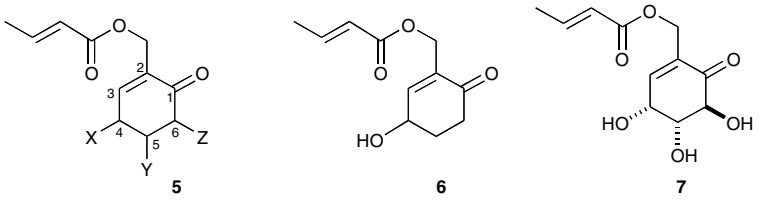
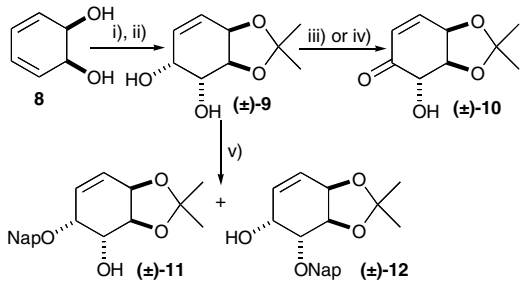


Figure 2. Analogues of COTC.

and co-workers of **1** and **2** against a range of cancer cell lines showed **2** to be more potent than **1** in almost all cases.[3] In order to gain further knowledge concerning the importance of relative and absolute stereochemistry, as well as the extent of hydroxylation, on observed bioactivity, we have now prepared 6-*epi*-COTC (**7**) in racemic and enantiomerically pure forms.[6] In this Letter, we provide details of these syntheses as well as the results of bioassay of the target compounds against lung cancer cell lines A549 and H460.

The synthesis of (±)-**7** commenced with the *meso* arene-dihydrodiol **8** derived from enzymatic dihydroxylation of benzene (Scheme 2). Acetonide formation followed by face-selective *cis*-dihydroxylation using Upjohn conditions[7] then provided diol (±)-**9**. We felt, initially, that selective oxidation of the allylic hydroxyl of (±)-**9** to give enone (±)-**10** would provide the basis for an expedient synthesis of the target compound. Unfortunately, however, oxidation of (±)-**9** using either PDC in DMF[8] or DDQ in THF[9] gave the enone in quite disappointing yields. We turned, therefore, to an alternative approach and were pleased to discover that selective protection of the allylic hydroxyl of (±)-**9** as its 2-naphthylmethyl (Nap) ether could be accomplished in good yield via an intermediate stannylene acetal.[10] Minor quantities (~10%) of readily separable regioisomer (±)-**12** were also generated in this reaction.

The structure of (±)-**11** was initially assigned by analysis of the ^{1}H NMR data of its 4-*O*-acetyl derivative and confirmation of the relative stereostructure was ultimately gained by X-ray analysis of a crystal of (±)-**11** obtained from petroleum ether/ethyl acetate (Fig. 3).[11,12]



Scheme 2. Reagents and conditions: (i) $(CH_3)_2C(OCH_3)_2$, *p*-TSA, acetone, 0 °C to rt, 3 h; (ii) OsO_4 (cat.), NMO, ^{t}BuOH, H_2O, rt, 24 h, 57% over two steps; (iii) PDC, DMF, rt, 2 h, 43%; (iv) DDQ, THF, 60 °C, 6 h, 20%; (v) Bu_2SnO, $C_6H_5CH_3$, CH_3OH, Δ, then 2-bromomethylnaphthalene, Bu_4NI, $C_6H_5CH_3$, 130 °C, 9 h, 76%.

Direct *trans*-ketalisation of (±)-**11** to give (±)-**13** proved to be an inefficient process (Scheme 3). This key transformation could be achieved, however, in acceptable yield via initial removal of the acetonide group followed by subsequent protection of the vicinal *trans*-diequatorial hydroxyls as their butanediacetal (BDA) derivative. Variable quantities of another acetal (tentatively assigned as (±)-**14** [15–20%]) together with a minor amount (~5%) of methoxylated compound (±)-**15** were also isolated from this two-stage procedure: the latter is presumed to arise from nucleophilic participation by the reaction solvent during introduction of the BDA group. Oxidation of the allylic hydroxyl of (±)-**13** proceeded smoothly to give enone (±)-**16** and subsequent introduction of a hydroxymethyl side-chain was accomplished using an imidazole-mediated Morita–Baylis–Hillman reaction.[13,14] Other conditions were also investigated for the latter reaction, including the use of alternative nucleophilic catalysts such as DMAP,[15] but these proved less effective than the imidazole-mediated process with regard to the isolated yield of (±)-**17** as well as reproducibility.

Crotonylation of the primary hydroxyl of (±)-**17** under standard conditions gave the fully protected target compound (±)-**18** (Scheme 4). One of the benefits of Nap protection for hydroxyl groups is the potential for facile deprotection under mildly oxidative conditions.[16] We were pleased, therefore, to discover that exposure of (±)-**18** to DDQ at rt for 6 h furnished allylic alcohol (±)-**19** in reasonable yield and removal of the BDA group under standard conditions then gave the target compound in racemic form.[17] An alternative sequence of deprotection steps involving acid-catalysed hydrolysis of (±)-**18** to give (±)-**20** followed by exposure to DDQ also proceeded smoothly to give 6-*epi*-COTC ((±)-**7**).

The asymmetric synthesis of (−)-**7** required the preparation of enantiopure diol (−)-**9**.[18] This was accomplished from the biotransformation product **21** of iodobenzene. Formation of the acetonide derivative of **21** followed by *cis*-dihydroxylation gave diol (+)-**22** and subsequent chemoselective reduction of the C–I bond[19] yielded (−)-**9** in good overall yield. An identical sequence of reactions to that described above gave the fully protected target compound (−)-**18** which was converted in two steps, via (+)-**19**, to (−)-**7**. For the purpose of comparative biological evaluation, diol (−)-**20** was also prepared by acidic hydrolysis of (−)-**18** (Scheme 5).

The results of bioassays of compounds **7**, **19** and **20** in both racemic and enantiomerically pure forms, as well

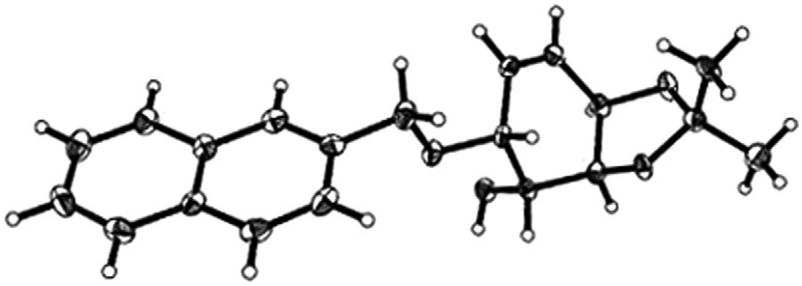
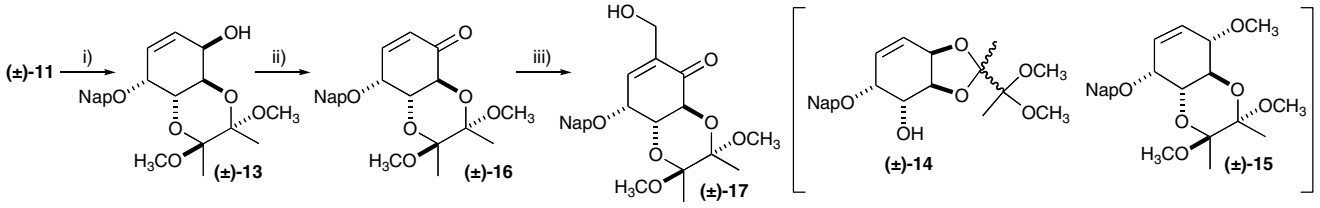
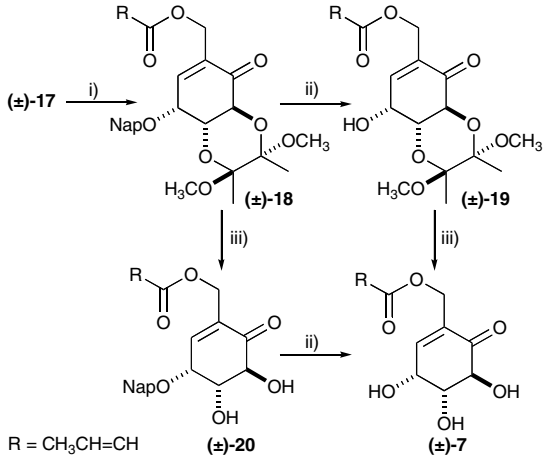


Figure 3. Crystal structure of (±)-**11** with ellipsoids at 50% probability.



Scheme 3. Reagents and conditions: (i) H_2O:TFA:THF (5:2:1), rt, 2 h then butan-2,3-dione, $(CH_3O)_3CH$, CSA, CH_3OH, Δ, 16 h, 46%; (ii) PCC, CH_2Cl_2, rt, 2 h, 76%; (iii) H_2CO, imidazole, 1 M $NaHCO_3$(aq), THF, 40 °C, 32 h, 68%.



Scheme 4. Reagents and conditions: (i) Crotonic anhydride, pyridine, DMAP (cat.), CH_2Cl_2, rt, 3 h, 50%; (ii) DDQ, CH_2Cl_2:CH_3OH (4:1), rt, 6 h, 66% for **19**, 50% for **7**; (iii) TFA:H_2O (7:1), rt, 3 h, 98% for **7**, 50% for **20**.

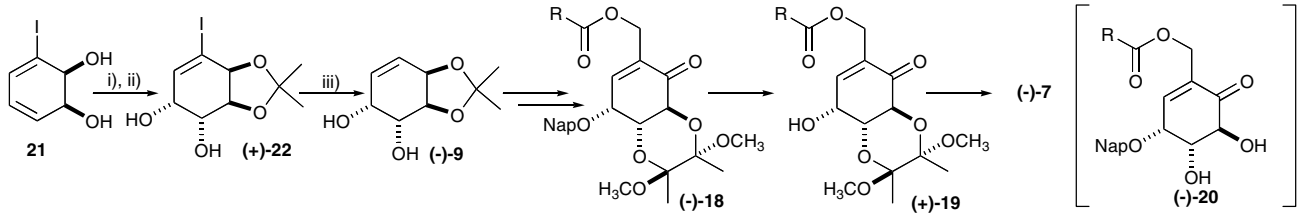
as of COMC (**2**),[5] against two lung cancer cell lines (A549 and H460) are shown in Table 1. These cell lines were chosen because they showed the highest level of GSH from among a panel of human tumour cell lines used in chemosensitivity testing.[20] The cytotoxicity assays were carried out by exposing cells to varying concentrations of each compound for 4 days and the number of surviving cells was then determined by the use of the MTT assay.[21]

Table 1. Bioactivity of COTC analogues towards lung cancer cell lines

Compound	IC_{50} (μM)	
	A549	H460
2	55	40
(±)-**7**	184	160
(−)-**7**	170	158
(±)-**19**	30	18
(+)-**19**	60	36
(±)-**20**	81	49
(−)-**20**	41	23

Experiments were repeated three times and data within individual experiments were derived from four separate observations: average values are given in the table.

The results allow a number of conclusions to be reached regarding the anti-cancer properties of the COTC analogues: (i) the trihydroxylated compound, 6-*epi*-COTC, in both racemic and enantiomerically pure forms, is the least toxic of all compounds tested towards the two cell lines; (ii) blockade of either one or two of the free hydroxyls with a lipophilic group results in a notable increase in potency, the extent of which is affected to a small but measurable degree by enantio-



Scheme 5. Reagents and conditions: (i) $(CH_3)_2C(OCH_3)_2$, *p*-TSA, CH_2Cl_2, rt, 1 h; (ii) OsO_4 (cat.), NMO, ^{t}BuOH, H_2O, rt, 36 h, 81% over two steps; (iii) H_2, 10% Pd on C, Et_3N, CH_3OH, rt, 3 h, 71%.

meric purity; (iii) all of the analogues of COTC display slightly enhanced potency towards the chemosensitive H460 cell line compared with the A549 cell line.

It is well documented that mammalian GSTs display broad substrate selectivity for hydrophobic compounds and the reduced potency of 6-*epi*-COTC compared with compounds **19** and **20** is consistent, therefore, with a GST mediated mechanism for anti-cancer activity. The observation that both mono- and di-hydroxylated analogues of (**1**) are more potent than COMC (**2**) towards lung cancer cell lines represents an interesting finding, which is made more intriguing by small variations in bioactivity associated with enantiomeric purity. Studies are currently under way to further investigate the influence of absolute stereochemistry, as well as the degree of hydroxylation of the cyclohexenone ring, on the anti-cancer activity of this class of compounds.

In conclusion, expedient syntheses of the C6-diastereoisomer of the *Streptomyces* metabolite COTC, in both racemic and enantiomerically pure forms, have been developed using the arene-*cis*-dihydrodiols derived from benzene and iodobenzene as starting materials. Bioassay of the novel analogues, and partially protected variants thereof, indicates that blockade of either one or two of the free hydroxyl groups results in an increase in anti-cancer activity. Biological potency is also affected to a much lesser extent by the enantiomeric purity of the test compounds. These findings indicate that further modification of the cyclohexenone core of COTC analogues will allow optimisation of the anti-cancer activity of this intriguing structural class.

Acknowledgments

We acknowledge, with thanks, EPSRC for funding (C.L.A. and H.L.W.) and MRC for funding (I.J.S. and N.S.W). We are particularly grateful to Professor Derek Boyd of the Queens University in Belfast for the generous donation of diols **8** and **21** as well as for his invaluable advice regarding the preparation of compound **9** in racemic and enantiomerically pure forms.

References and notes

1. Takeuchi, T.; Chimura, H.; Hamada, M.; Umezawa, H.; Yoshioka, O.; Oguchi, N.; Takahashi, Y.; Matsuda, A. *J. Antibiot.* **1975**, *28*, 737.
2. Chimura, H.; Nakamura, H.; Takita, T.; Takeuchi, T.; Umezawa, H.; Kato, K.; Saito, S.; Tomisawa, T.; Iitaka, Y. *J. Antibiot.* **1975**, *28*, 743.
3. Aghil, O.; Bibby, M. C.; Carrington, S. J.; Double, J.; Douglas, K. T.; Phillips, R. M.; Shing, T. K. M. *Anti-Cancer Drug Des.* **1992**, *7*, 67.
4. (a) Sugimoto, Y.; Suzuki, H.; Yamaki, H.; Nishimura, T.; Tanaka, N. *J. Antibiot.* **1982**, *35*, 1222; (b) Huntley, C. F. M.; Hamilton, D. S.; Creighton, D. J.; Ganem, B. *Org. Lett.* **2000**, *2*, 3143; (c) Hamilton, D. S.; Ding, Z.; Ganem, B.; Creighton, D. J. *Org. Lett.* **2002**, *4*, 1209; (d) Zhang, Q.; Ding, Z.; Creighton, D. J.; Ganem, B.; Fabris, D. *Org. Lett.* **2002**, *4*, 1459; (e) Joseph, E.; Eiseman, J. L.; Hamilton, D. S.; Wang, H.; Tak, H.; Ding, Z.; Ganem, B.; Creighton, D. J. *J. Med. Chem.* **2003**, *46*, 194; (f) Hamilton, D. S.; Zhang, X.; Ding, Z.; Hubatsch, I.; Mannervik, B.; Houk, K. N.; Ganem, B.; Creighton, D. J. *J. Am. Chem. Soc.* **2003**, *125*, 15049; (g) Joseph, E.; Ganem, B.; Eiseman, J. L.; Creighton, D. J. *J. Med. Chem.* **2005**, *48*, 6549; (h) Zheng, Z.-B.; Zhu, G.; Tak, H.; Joseph, E.; Eiseman, J. L.; Creighton, D. J. *Bioconjugate Chem.* **2005**, *16*, 598.
5. Arthurs, C. L.; Wind, N. S.; Whitehead, R. C.; Stratford, I. J. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 553.
6. It should be noted that enantiomerically pure compound, 6-*epi*-COTC ((−)-**7**), possesses the same absolute stereostructure as the ketocarbasugar, (−)-Gabosine A: (a) Bach, G.; Breiding-Mack, S.; Grabley, S.; Hammann, P.; Huetter, K.; Thiericke, R.; Uhr, H.; Wink, J.; Zeeck, A. *Liebigs Ann. Chem.* **1993**, 241; (b) Banwell, M. G.; Bray, A. M.; Wong, D. J. *New J. Chem.* **2001**, *25*, 1351.
7. VanRheenen, V.; Kelly, R. C.; Cha, D. F. *Tetrahedron Lett.* **1976**, *17*, 1973.
8. Zamir, L. O.; Luthe, C. *Can. J. Chem.* **1984**, *62*, 1169.
9. McKittrick, B. M.; Ganem, B. *J. Org. Chem.* **1985**, *50*, 5897.
10. Simas, A. B. C.; Pais, K. C.; da Silva, A. A. T. *J. Org. Chem.* **2003**, *68*, 5426.
11. Crystallographic data (excluding structure factors) for (±)-**11** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 630924. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 0 1223336033 or e-mail: deposit@ccdc.cam.ac.uk].
12. Crystal structure data for (±)-**11** $C_{20}H_{22}O_4$, $M = 326.38$, monoclinic spacegroup C2, $a = 23.915$ (5), $b = 5.572$ (12), $c = 24.964$ (5) Å, $U = 3275.9$ Å^3, d_{calcd} 1.323 = mgM^3. Independent reflections (12,286), $R1 = 0.0576$, $wR2 = 0.0821$ for 1364 reflections with $I > 2\sigma(I)$.
13. Luo, S.; Wang, P. G.; Chen, J.-P. *J. Org. Chem.* **2004**, *69*, 555.
14. Basavaiah, D.; Rao, A. J.; Satynarayana, T. *Chem. Rev.* **2003**, *103*, 811.
15. Rezgui, F.; El Gaied, M. M. *Tetrahedron Lett.* **1998**, *39*, 5965.
16. Xia, J.; Abbas, S. A.; Locke, R. D.; Piskorz, C. F.; Alderfer, J. L.; Matta, K. L. *Tetrahedron Lett.* **2000**, *41*, 169.
17. Selected analytical data for compound (−)-**7**: $[\alpha]_D^{20}$ − 15.7 (*c* 0.28, CH_3OH); ν_{max} (film)/cm^{-1} 3432br (O–H), 2966w (C–H), 1647 s (C=O, enone); δ_H (300 MHz; D_2O) 1.76 (3H, dd, *J* 6.9, 1.8, CH_3CH=CH), 3.81 (1H, dd, *J* 10.5, 4.2, C(5)*H*), 4.35 (1H, d, *J* 10.5, C(6)*H*), 4.44–4.48 (1H, m, C(4)*H*), 4.55–4.70 (2H, m, CH_2OC(=O)), 5.81 (1H, dq, *J* 15.6, 1.8, CH_3CH=C*H*), 6.90–7.03 (2H, m, C(3)*H* and CH_3CH=CH); δ_C (75 MHz; D_2O) 19.3 (CH_3CH=CH), 60.3 (CH_2OC(=O)), 62.7 (*C*(5)H), 68.6 (*C*(6)H), 70.9 (*C*(4)H), 121.9 (CH_3CH=*C*H), *C*(2) not observed, 139.3 (*C*(3)H), 152.1 (CH_3CH=CH), 166.5 (CH_2OC(=O)), 192.6 (*C*(1)O); *m*/*z* (+ve ion electrospray) 265 ([M+Na]$^+$,100%) (Found: 265.0678, $C_{11}H_{14}O_6Na$ ([M+Na]$^+$) requires 265.0683).
18. (a) Hudlicky, T.; Price, J. D.; Rulin, F.; Tsunoda, T. *J. Am. Chem. Soc.* **1990**, *112*, 9439; (b) Hudlicky, T.; Luna, H.; Olivo, H. F.; Andersen, C.; Nugent, T.; Price, J. D. *J. Chem. Soc., Perkin Trans. 1* **1991**, 2907.
19. Personal communication: Boyd, D. R.; Sharma, N. D. (unpublished data).
20. Robertson, N.; Haigh, A.; Adams, G. E.; Stratford, I. J. *Eur. J. Cancer* **1994**, *30A*, 1013.
21. Carmichael, J.; DeGraff, W. G.; Gazdar, A. F.; Minna, J. D.; Mitchell, J. B. *Cancer Res.* **1987**, *47*, 936.