Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 553–557
Bioorganic & Medicinal Chemistry Letters

Analogues of 2-crotonyloxymethyl-(4*R*,5*R*,6*R*)-4,5,6-trihydroxycyclohex-2-enone (COTC) with anti-tumor properties

Claire L. Arthurs,[a] Natasha S. Wind,[b] Roger C. Whitehead[a,*] 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 20 July 2006; revised 22 September 2006; accepted 22 September 2006
Available online 15 December 2006

Abstract—The syntheses of three novel analogues of the naturally occurring cytotoxic agent COTC are described and the results of bioassays of the target compounds against two lung cancer cell lines 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* and was demonstrated to possess cytotoxic and cancerostatic activity with low toxicity (Fig. 1). Subsequent investigations have demonstrated that both **1** and its structurally simplified synthetic analogue, 2-crotonyloxymethyl-cyclohex-2-enone (COMC, **2**), possess potent anti-tumor activity against murine and human tumors in culture as well as in tumor-bearing mice.[1–3]

The potent biological activity of these compounds has stimulated interest from synthetic chemists[4–6] as well as other investigators interested in their mechanism of anti-tumor activity.[7–15] Elegant investigations by Ganem, Creighton and collaborators have demonstrated that the latter may involve initial conjugation of glutathione (GSH) to **1** or **2** thereby generating a glutathionylated exocyclic enone of type **3**: this reaction is catalyzed by glutathione transferase (GST). Subsequent alkylation of intracellular proteins and/or nucleic acids by this reactive intermediate is believed to then lead to cell death (Scheme 1). A consequence of this proposed mechanism of action is the probability that cells which possess elevated levels of GST/GSH compared with normal cells (e.g., multidrug-resistant tumor cells) will exhibit enhanced sensitivity toward exposure to **1** or **2**.

Prompted by these findings, we have recently initiated a research program designed to probe the structural features necessary for optimal bioactivity of the cyclohex-2-enone derivatives. From the outset, our goal was to prepare an array of analogues of COTC with general

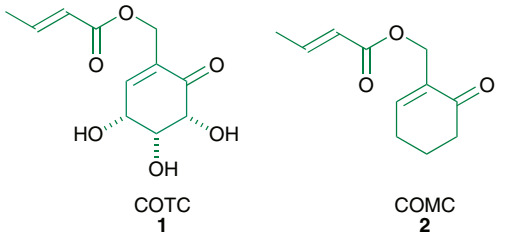
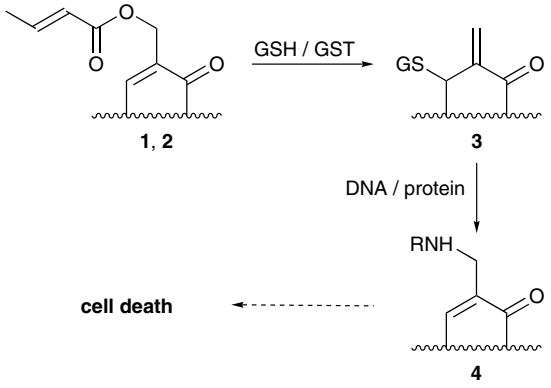


Figure 1.



Scheme 1.

Keywords: Anti-tumor; Natural product; Synthetic analogue; Glutathione transferase; Organo-fluorine.
* Corresponding author. Tel: +44 161 275 7856; fax: +44 161 275 4939; e-mail: roger.whitehead@manchester.ac.uk

0960-894X/$ - see front matter © 2006 Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2006.09.072

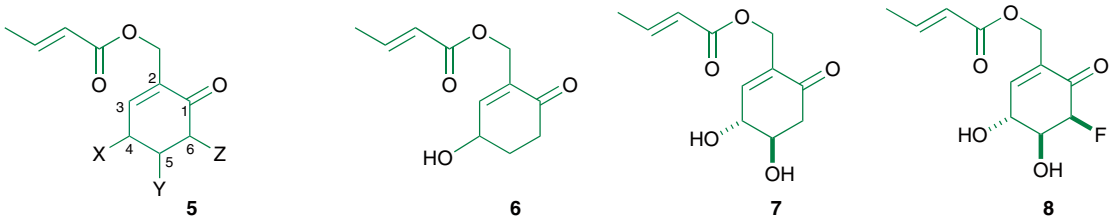
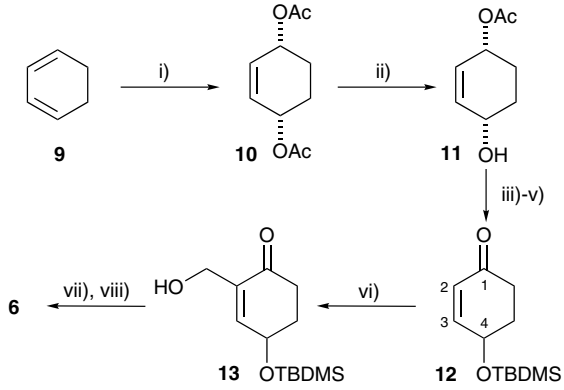


Figure 2.

structure **5**, wherein the C4, C5, and C6 substituents represent three potential loci for diversification. In this communication we report the outcome of our initial studies in this area which have concerned the preparation of three analogues of COTC (**6**–**8**) (Fig. 2). We also report the outcome of biological evaluation of our synthetic compounds against two lung cancer cell lines, A549 and H460. Compounds **6** and **7** were selected to probe the influence of degree of hydroxylation on anti-cancer activity: compound **8** was chosen to investigate whether incorporation of a highly electronegative and lipophilic fluorine atom adjacent to the carbonyl would improve potency compared to non-fluorinated compound **7**.

Our synthetic route to the monohydroxylated analogue **6** commenced with 1,3-cyclohexadiene (**9**) and is outlined in Scheme 2. Palladium-catalyzed *cis*-1,4-diacetoxylation of **9** using the conditions of Bäckvall[16] provided the *meso*-diacetate **10** which was enzymatically de-symmetrized using electric eel cholinesterase[17] to give an essentially racemic mono-acetate **11** (synthetic **11**: $[\alpha]_D^{21}$ −1.8 (*c* 2.21, CH_2Cl_2); (−)**11**: $[\alpha]_D$ −100.0 (*c* 1.3, CH_2Cl_2)).[18] Subsequent silylation with TBDMS-OTf, methanolysis of the allylic acetate followed by oxidation using the Ley–Griffith reagent (TPAP)[19] gave enone **12** in good yield (synthetic **12**: $[\alpha]_D^{19}$ −2.8 (*c* 1.29, CH_2Cl_2); (−)**12**: $[\alpha]_D$ −115.9 (*c* 1.06, $CHCl_3$)).[20] Introduction of a hydroxymethylene substituent at C2 of **12** was then accomplished using 'aqueous Baylis–Hillman reaction conditions.[21] Crotonylation of the primary hydroxyl of **13** was followed by removal of the silyl-protecting group under acidic conditions to give the target compound **6** in good yield.[22]



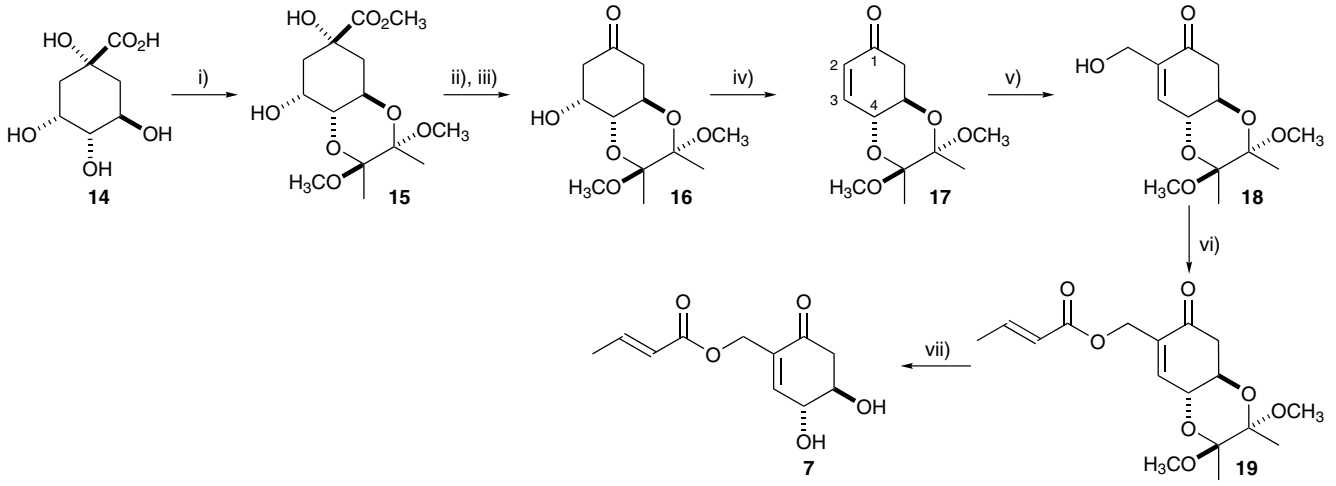
Scheme 2. Reagents and conditions: (i) MnO_2, LiCl, *p*-benzoquinone, $Pd(OAc)_2$, $LiOAc{\cdot}2H_2O$, AcOH, rt, 3 days, 69%; (ii) electric eel cholinesterase, NaN_3, phosphate buffer (pH 6.85), 20 °C, 30 h, 63%; (iii) TBDMSOTf, Et_3N, DMAP, CH_2Cl_2, 0 °C, 1 h, 82%; (iv) K_2CO_3, CH_3OH, rt, 3 h, 88%; (v) TPAP, NMO, 4 Å mol. sieves, CH_2Cl_2, rt, 7 h, 65%; (vi) DMAP (cat.), H_2CO, THF/H_2O (1:1), 40 °C, 24 h, 20% (63% based on recovered **12**); (vii) crotonic anhydride, pyridine, DMAP (cat.), CH_2Cl_2, rt, 1.5 h, 63%; (viii) TFA/H_2O (7:1), 0 °C, 30 min, 98%.

The synthetic route to the dihydroxylated compound **7** is outlined in Scheme 3. The pivotal intermediate in this sequence is the butane-diacetal (BDA)-protected dihydroxycyclohexenone **17**, the cyclohexane-diacetal (CDA)-protected variant of which was originally described in 1996 by Gebauer and Brückner.[23]

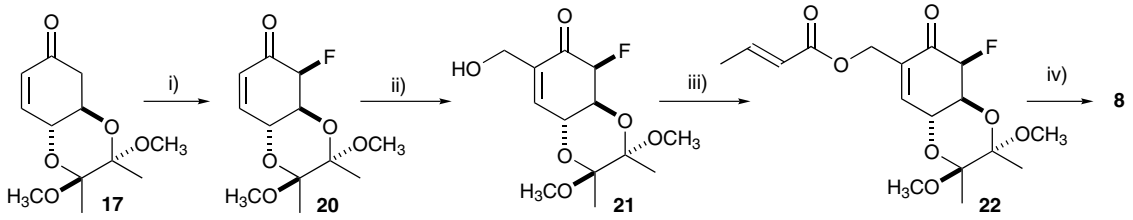
Using a modification of the synthetic scheme described by these authors, we prepared **17** in an overall yield of 72% from (−)-quinic acid (**14**). Thus, BDA-protected methyl quinate (**15**) was prepared in a single step and good yield from **14**. Reduction of the α-hydroxy ester moiety was then accomplished under mild conditions using $NaBH_4$ in methanol and subsequent oxidative cleavage of the resulting 1,2-diol was carried out using the silica-supported $NaIO_4$ reagent described by Zhong and Shing.[24] Dehydration of **16** to provide the α,β-enone **17** then proceeded smoothly, and in near-quantitative yield, using the conditions employed by the original authors. A hydroxymethylene substituent was introduced at C2 of **17** in a similar fashion to that described previously for the monohydroxylated analogue and crotonylation then provided ester **19**. Finally, removal of the BDA-protecting group proceeded smoothly under standard conditions to give the diol **7** which possesses 'unnatural' configuration at C5.[25]

Synthesis of the fluorinated analogue of COTC **8** required introduction of a fluorine substituent at the C6 position of **17**. Thus, the trimethylsilyl-enolether derivative of **17** was prepared using conditions outlined by O'Brien and co-workers[26] and subsequent treatment of this intermediate with the 'F^+' source SelectFluor® furnished the axially fluorinated compound **20** in acceptable yield (Scheme 4).[27] Baylis–Hillman reaction of this compound gave allylic alcohol **21** and subsequent esterification followed by deprotection under standard conditions furnished the target compound **8** in good yield.[28] The stereochemistry at C6 of **21** was assigned by the use of 2D heteronuclear Overhauser effect spectroscopy (HOESY) as described previously by ourselves.[29]

In order to allow a comparative assessment of anti-cancer activity, the known compound COMC (**2**) was also



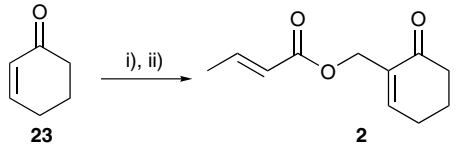
Scheme 3. Reagents and conditions: (i) butan-2,3-dione, $(CH_3O)_3CH$, camphorsulfonic acid, CH_3OH, Δ, 12 h, 98%; (ii) $NaBH_4$, CH_3OH, 0 °C to rt, 24 h; (iii) $NaIO_4$ on silica gel, CH_2Cl_2, 1.5 h, 75% over two steps from **15**; (iv) Et_3N, CH_3SO_2Cl, CH_2Cl_2, 3 h, 98%; (v) DMAP (cat.), H_2CO, THF/H_2O (1:1), 40 °C, 24 h, 80%; (vi) crotonic anhydride, pyridine, DMAP (cat.), CH_2Cl_2, rt, 1.5 h, 67%; (vii) TFA/H_2O (7:1), 0 °C, 30 min, 75%.



Scheme 4. Reagents and conditions: (i) KHMDS (0.5 M in toluene), TMS–Cl, THF, −78 to 0 °C, 1 h then SelectFluor®, CH_3CN, 0 °C, 30 min, 57%; (ii) DMAP (cat.), H_2CO, THF/H_2O (1:1), 40 °C, 24 h, 94%; (iii) crotonic anhydride, pyridine, DMAP (cat.), CH_2Cl_2, rt, 1.5 h, 39%; (iv) TFA/H_2O (7:1), 0 °C, 3 h, 95%.

prepared in two steps from cyclohex-2-enone (**23**) (Scheme 5).[9]

The results of bioassays of the synthetic analogues of COTC 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 tumor cell lines used in chemosensitivity testing.[30] Intracellular GSH concentrations are 16.9 and 22.3 mM, respectively. Furthermore, the A549 cells show high levels of cytosolic πGST. The cytotoxicity 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.[31] Values of IC_{50} are drug concentrations required to reduce cell number by 50% relative to untreated, control cells.



Scheme 5. Reagents and conditions: (i) DMAP (cat.), H_2CO, THF/H_2O (1:1), 40 °C, 24 h, 65%; (ii) crotonic anhydride, pyridine, DMAP (cat.), CH_2Cl_2, rt, 1.5 h, 38%.

The results allow several conclusions to be reached regarding the structural features influencing the anticancer properties of the COTC analogues toward the two cell lines: (i) the compounds bearing free hydroxyl groups at *both* C4 and C5 are substantially less potent than the non-hydroxylated analogue COMC (**2**); (ii) blockade of the vicinal diol functionality as its butanediacetal results in restoration of potency to levels comparable to COMC (**2**); (iii) incorporation of a lipophilic and electronegative fluorine atom at C6 has no beneficial effect on activity; (iv) introduction of a single allylic hydroxyl group at C4 results in a 2- to 4-fold improvement in potency compared with COMC (**2**).

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

Compound	IC_{50} (μM)	
	A549	H460
2	55	40
6	24	10
7	147	158
8	164	>200
19	32	60
22	31	43

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

Mammalian GSTs generally display selectivity for lipophilic substrates and the reduced potency of diols **7** and **8** compared with the more hydrophobic compounds **2**, **19**, and **22** is consistent, therefore, with a GST mediated mechanism for anti-cancer activity. Indeed, comparative bioassays by Douglas and co-workers of COTC (**1**) and COMC (**2**) against a range of cancer cell lines showed **2** to be more potent than **1** in almost all cases.[3,32] In this regard, the finding that the monohydroxylated compound **6** is more potent than COMC (**2**) toward the two lung cancer cell lines investigated here represents an interesting conundrum. Further studies are under way to investigate the influence of absolute stereochemistry, as well as the position and degree of hydroxylation of the cyclohexenone ring, on the anti-cancer activity of this intriguing class of compounds.

In conclusion, three analogues of the anti-tumor agent COTC (**1**) have been prepared from either 1,3-cyclohexadiene (**9**) or (−)-quinic acid (**14**) as starting material. The structures of these compounds differ from that of **1** with respect to the extent of hydroxylation of the cyclohexenone ring. Bioassay of the target compounds indicates that polar dihydroxylated compounds are only weakly active against lung cancer cell lines, whereas the most potent analogue bears a single hydroxyl group at C4 and is racemic. The findings indicate that further modification of the cyclohexenone core structure of COTC analogues will allow fine-tuning of the anti-cancer activity of this structural class.

Acknowledgments

We acknowledge, with thanks, the EPSRC for funding (C.L.A.) and the MRC for funding (I.J.S. [MRC Grant G0500366] and N.S.W.). We are also very grateful to Professor Gareth Morris of the School of Chemistry at the University of Manchester for invaluable advice concerning the NMR analysis of several of our target compounds and also to Rehana Sung for expert assistance with HPLC purification of compounds **7** and **8**.

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. Mirza, S.; Molleyres, L.-P.; Vasella, A. *Helv. Chim. Acta* **1985**, *68*, 988.
5. Shing, T. K. M.; Tang, Y. *Tetrahedron* **1990**, *46*, 6575.
6. Huntley, C. F. M.; Wood, H. B.; Ganem, B. *Tetrahedron Lett.* **2000**, *41*, 2031.
7. Sugimoto, Y.; Suzuki, H.; Yamaki, H.; Nishimura, T.; Tanaka, N. *J. Antibiot.* **1982**, *35*, 1222.
8. Huntley, C. F. M.; Hamilton, D. S.; Creighton, D. J.; Ganem, B. *Org. Lett.* **2000**, *2*, 3143.
9. Hamilton, D. S.; Ding, Z.; Ganem, B.; Creighton, D. J. *Org. Lett.* **2002**, *4*, 1209.
10. Zhang, Q.; Ding, Z.; Creighton, D. J.; Ganem, B.; Fabris, D. *Org. Lett.* **2002**, *4*, 1459.
11. 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.
12. 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.
13. Joseph, E.; Ganem, B.; Eiseman, J. L.; Creighton, D. J. *J. Med. Chem.* **2005**, *48*, 6549.
14. Zheng, Z.-B.; Zhu, G.; Tak, H.; Joseph, E.; Eiseman, J. L.; Creighton, D. J. *Bioconjugate Chem.* **2005**, *16*, 598.
15. Kamiya, D.; Uchihata, Y.; Ichikawa, E.; Kato, K.; Umezawa, K. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1111.
16. Bäckvall, J.-E.; Byström, S. E.; Nordberg, R. E. *J. Org. Chem.* **1984**, *49*, 4619.
17. Deardorff, D. R.; Matthews, A. J.; McMeekin, D. S.; Craney, C. L. *Tetrahedron Lett.* **1986**, *27*, 1255.
18. Kazlauskas, R. J.; Weissfloch, A. N. E.; Rappaport, A. T.; Cuccia, L. A. *J. Org. Chem.* **1991**, *56*, 2656.
19. Griffith, W. P.; Ley, S. V.; Whitcombe, G. P.; White, A. D. *J. Chem. Soc., Chem. Commun.* **1987**, 1625.
20. Audia, J. E.; Boisvert, L.; Patten, A. D.; Villalobos, A.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1989**, *54*, 3738.
21. Rezgui, F.; El Gaied, M. M. *Tetrahedron Lett.* **1998**, *39*, 5965.
22. Selected analytical data for compound **6**. ν_{max} (film)/cm^{-1} 3434br s (O–H), 1716s (C=O, ester), 1657s (C=O, enone); δ_H (300 MHz; $CDCl_3$) 1.93 (3H, dd, *J* 6.9, 1.8, CH_3CH=CHC(=O)), 2.35–2.72 (4H, m, C(5)H_2 and C(6)H_2), 4.62–4.69 (1H, m, C(4)*H*), 4.80–4.92 (2H, m, CH_2O(C=O)), 5.92 (1H, dq, *J* 15.3, 1.8, CH$_3$CH=C*H*C(=O)), 6.92–6.93 (1H, m, C(3)*H*), 7.06 (1H, dq, *J* 15.3, 6.9, CH$_3$C*H*=CHC(=O)); δ_C (75 MHz; $CDCl_3$) 18.4 (*C*H$_3$CH=CHC(=O)), 32.7 (*C*(5)H$_2$ or *C*(6)H$_2$), 35.8 (*C*(5)H$_2$ or *C*(6)H$_2$), 60.7 (*C*H$_2$O(C=O)), 66.7 (*C*(4)H), 122.4 (CH$_3$CH=*C*HC(=O)), 134.5 (*C*(2)), 146.0 (*C*(3)H), 148.9 (CH$_3$*C*H=CHC(=O)), 166.3 (CH$_3$CH=CH*C*(=O)), 197.4 (*C*(1)O); *m/z* (CI/NH$_3$) 228 ([M+NH$_4$]$^+$, 50%) 211 ([M+H]$^+$, 100) (Found 228.1239, $C_{11}H_{18}O_4N$ ([M+H]$^+$) requires 228.1230).
23. Gebauer, O.; Brückner, R. *Liebigs Ann.* **1996**, 1559.
24. Zhong, Y.-L.; Shing, T. K. M. *J. Org. Chem.* **1997**, *62*, 2622.
25. Selected analytical data for compound **7**. $[\alpha]_D^{18}$ +11.74 (*c* 2.5, CH_2Cl_2); ν_{max} (film)/cm^{-1} 3402br s (O–H), 2963w (C–H), 1719m (C=O, ester), 1678m (C=O, enone); δ_H (300 MHz; $CDCl_3$) 1.94 (3H, dd, *J* 6.9, 1.8, CH_3CH=CHC(=O)), 2.52 (1H, dd, *J* 16.5, 12.3, C(6)H_β), 2.92 (1H, dd, *J* 16.5, 4.8, C(6)H_α), 4.01 (1H, ddd, *J* 12.3, 8.4, 4.8, C(5)*H*), 4.58 (1H, dq, *J* 8.4, 2.4, C(4)*H*), 4.85 (2H, br s, CH_2O(C=O)) 5.90 (1H, dq, *J* 15.6, 1.8, CH$_3$CH=C*H*C(=O)), 6.84 (1H, d, *J* 2.4, C(3)*H*), 7.05 (1H, dq, *J* 15.6, 6.9, CH$_3$C*H*=CHC(=O)); δ_C (75 MHz; $CDCl_3$) 18.4 (*C*H$_3$CH=CHC(=O)), 44.8 (*C*(6)H$_2$), 60.3 (*C*H$_2$O(C=O)), 72.9 (*C*(5)H), 73.3 (*C*(4)H), 122.2 (CH$_3$CH=*C*HC(=O)), 135.2 (*C*(2)), 146.4 (*C*(3)H), 146.8 (CH$_3$*C*H=CHC(=O)), 166.4 (CH$_3$CH=CH*C*(=O)), 195.8 (*C*(1)O); *m/z* (CI/NH$_3$) 244 ([M+NH$_4$]$^+$, 90%), 227 ([M+H]$^+$, 20) (Found 244.1181, $C_{11}H_{18}O_5N$ ([M+NH$_4$]$^+$) requires 244.1179).
26. Murray, L. M.; O'Brien, P.; Taylor, R. J. K. *Org. Lett.* **2003**, *5*, 1943.
27. Banks, R. E. *J. Fluorine Chem.* **1998**, *87*, 1.
28. Selected analytical data for compound **8**. $[\alpha]_D^{18}$ −4.41 (*c* 1.4, H_2O); ν_{max} (film)/cm^{-1} 3402br s (O–H), 2900w (C–H), 1708s (C=O, ester), 1680s (C=O, enone); δ_H (400 MHz; $CDCl_3$)

1.74 (1H, br s, O*H*), 1.91 (3H, d, *J* 6.8, C*H*$_3$CH=CHC(=O)), 3.10 (1H, br s, O*H*), 4.30 (1H, ~d, *J* 18.4, C(5)*H*), 4.67 (1H, br s, C(4)*H*), 4.86 (1H, d, *J* 14.8, one of C*H*$_2$O(C=O)), 4.90 (1H, d, *J* 14.8, one of C*H*$_2$O(C=O)), 5.22 (1H, br d, *J* 50.0, C(6)*H*F), 5.89 (1H, d, *J* 14.0, CH$_3$CH=C*H*C(=O)), 6.86 (1H, br s, C(3)*H*), 6.99–7.09 (1H, m, CH$_3$C*H*=CHC(=O)); δ_C (100 MHz; $CDCl_3$) 18.1 (*C*H$_3$CH=CHC(=O)), 59.7 (*C*H$_2$O(C=O)), 68.1 (d, *J* 7.6, *C*(4)H), 74.1 (d, *J* 17.5, *C*(5)H), 90.7 (d, *J* 182.8, *C*(6)H), 121.9 (CH$_3$CH=*C*HC(=O)), 134.0 (*C*(2)), 143.0 (*C*(3)H), 146.3 (CH$_3$*C*H=CHC(=O)), 165.9 (CH$_3$CH=CH*C*(=O)), 190.7 (*C*(1)O); δ_F (376 MHz; $CDCl_3$) −206.79 (dd, *J* 50.0, 18.4, C(6)H*F*); *m*/*z* (CI/NH_3) 262 ($[M+NH_4]^+$, 100%), 245 ($[M+H]^+$, 30) (Found 262.1077, $C_{11}H_{17}O_5NF$ ($[M+NH_4]^+$) requires 262.1085).

29. Begum, L.; Box, J. M.; Drew, M. G. B.; Harwood, L. M.; Humphreys, J. L.; Lowes, D. J.; Morris, G. A.; Redon, P. M.; Walker, F. M.; Whitehead, R. C. *Tetrahedron* **2003**, *59*, 4827.
30. Robertson, N.; Haigh, A.; Adams, G. E.; Stratford, I. J. *Eur. J. Cancer A* **1994**, *30*, 1013.
31. Carmichael, J.; DeGraff, W. G.; Gazdar, A. F.; Minna, J. D.; Mitchell, J. B. *Cancer Res* **1987**, *47*, 936.
32. It is of note that the magnitudes of the IC_{50} values reported here for all compounds are generally greater than those reported previously for either **1** or **2** against other cancer cell lines. This may be due, at least in part, to differing durations of the respective assays or to variation in GSH/GST levels between the cell lines.