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Synthesis of chiral hydroxylated enones as potential anti-tumor agents

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ABSTRACT

A series of chiral hydroxylated enones were synthesized as COTC ether analogues to investigate the structural features required for optimal and selective anti-tumor activity. The cytotoxicity of the seven COTC ether analogues against WRL-68 normal and HepG2, HL-60 cancer cell lines were measured. C-4 ether analogues with an aliphatic chain substituent were found to be more favorable than their aromatic counterparts. Inversion of the configuration at C-4 in **5e** to give **5f** only resulted in reduced selectivity towards cancer cells. These results show that 4-*O*-pentyl-gabosine D (**5e**) has optimum selectivity and cytotoxicity towards two cancer cell lines.

2-Crotonyloxymethyl-(4*R*,5*R*,6*R*)-4,5,6-trihydroxy-2-cyclohexenone (COTC, **1**) was isolated from cultures of *Streptomyces griseosporeus* in 1975 by Umezawa and co-workers.[1] Studies have demonstrated that COTC possesses potent activity against murine and human cancer cell lines in cultures as well as in tumor-bearing mice.[1,2]

The origin to the cytotoxicity of COTC aroused much interests and extensive studies were conducted.[3–11] Initially, the cytotoxicity of COTC was believed to originate from its inhibition of the detoxification enzyme glyoxalase I, thereby accumulating methylglyoxal which contributed to the resultant cytotoxicity. In 2002, however, an elegant investigation by Ganem and collaborators[8] has demonstrated that the cytotoxicity of COTC did not stem from the competitive inhibition of glyoxalase I; instead, its cytotoxicity was attributable to the conjugate addition of glutathione (GSH) to COTC under the catalysis of human glutathione transferase (hGSTs) (Fig. 1).

hGSTs is a family of GSH-dependent enzymes in phase II detoxification system, responsible for drug resistance processes by catalyzing the conjugation of GSH to a wide variety of anticancer drugs.[8,12] Under the catalysis of hGST, COTC undergoes an initial Michael addition by one equivalent of GSH to give the enol intermediate **2**. Upon departure from the enzyme, enol **3** collapses to expel crotonic acid and form the highly electrophilic exocyclic enone **4**, which is responsible for the cytotoxicity of COTC.[5,8] The exocyclic enone moiety in **4** reacts with the nucleophilic groups in nucleic acids and proteins inside cells, which directly accounts for COTC's cytotoxic activity; or with another equivalent of GSH. It was suggested that by depleting the amount of GSH, the cytotoxic methylglyoxal would accumulate due to the lack of the vital cofactor GSH for the detoxification glyoxalase system and therefore contributing indirectly to COTC's cytotoxicity.[11]

Intrigued by the potent biological activity of COTC, different strategies had been developed on the synthesis of COTC and its analogues, namely starting from (−)-quinic acid,[13–18] 1,3-cyclohexadiene,[17,18] *meso*-1,3-dihydrodiol,[19] sulfinylacrylates[20] and carbohydrates.[21,22] Recently, our group has prepared several COTC analogues from D-glucose and found that they work synergistically with cisplatin to achieve enhanced cytotoxicity towards A549 lung cancer cell line.[23] As a continuation of our studies, several COTC ether analogues bearing different aromatic and alkyl substituents C-4 were prepared in order to assess their selective cytotoxicity towards cancer cells.

Seven COTC analogues disclosed (**5a–g**, Fig. 2) in this paper were obtained from their own respective common intermediates: α-allylic alcohol **7** and β-allylic alcohol **8** by standard transformation. Previously, our group already published a promising synthetic route towards the two key allylic alcohols **7** and **8** using a L-proline mediated intramolecular direct aldol reaction of a D-glucose-derived diketone as the key step.[24] Conversion of **7** and **8** into the analogues were performed via a 5-step reaction sequence. Thus, the free hydroxyl group in **7** and **8** was first alkylated into their respective aliphatic or aromatic ethers. Deacetonation of the ethers gave their respective diols, which were then

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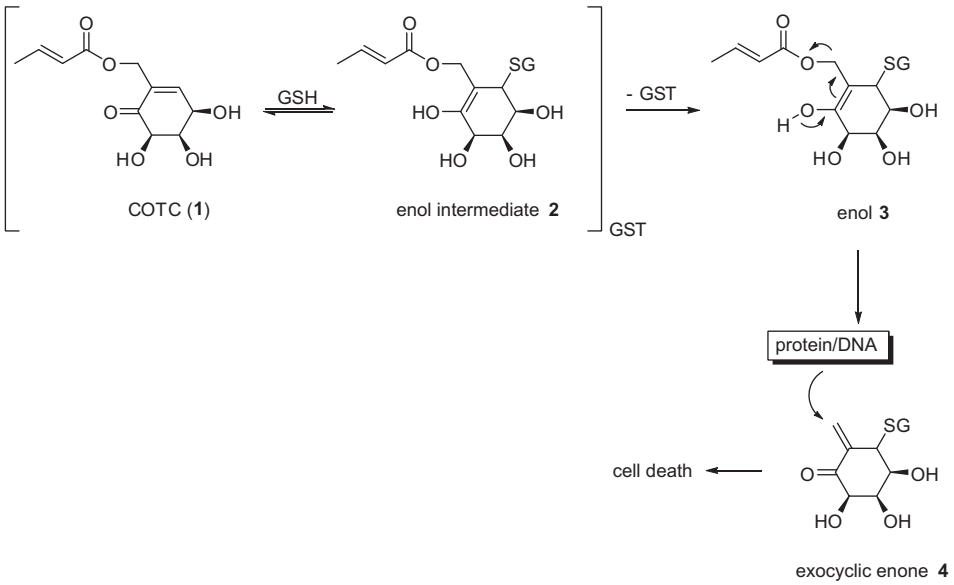


Figure 1. Mechanism for generation of exocyclic enone **4**.

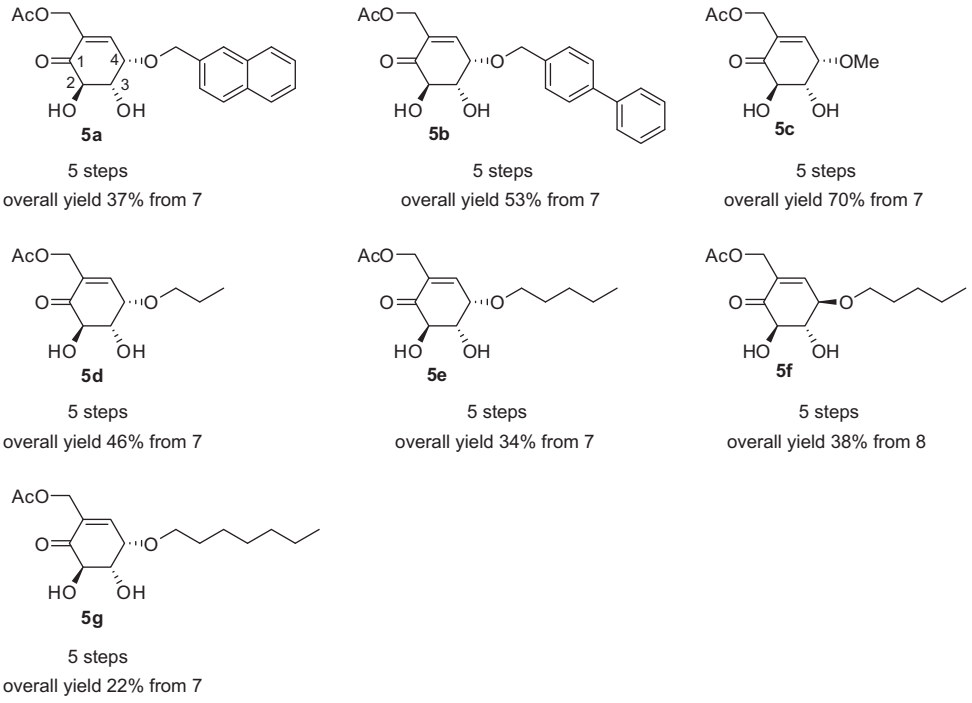


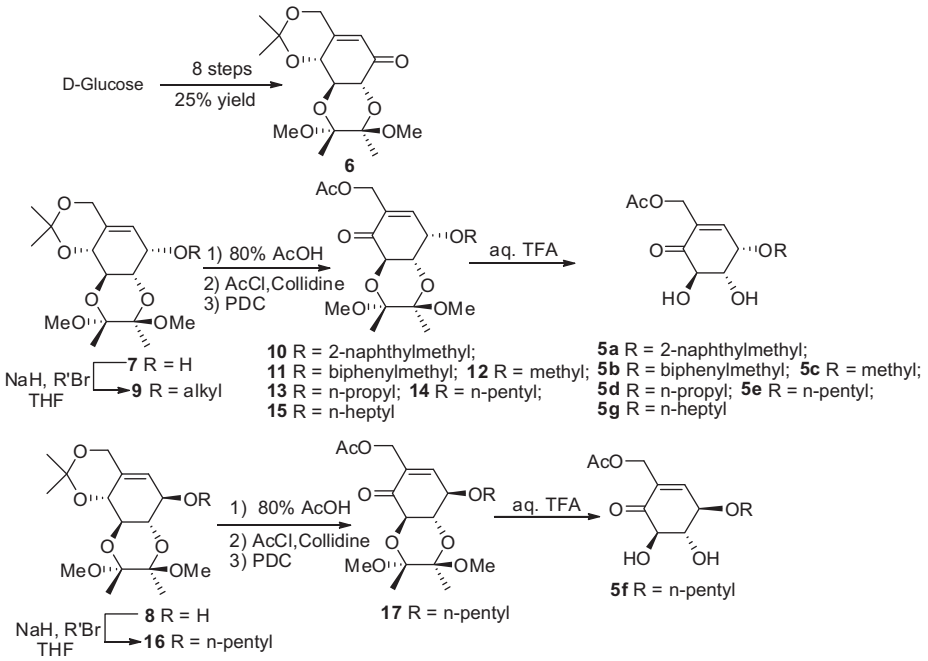
Figure 2. Synthesized COTC analogues **5a–g**.

regioselectively acetylated with the use of 2,4,6-collidine to give their respective primary acetates. Pyridinium dichromate (PDC) oxidation, followed by acid hydrolysis of the *trans*-diacetal group then yielded the desired COTC analogues (Scheme 1).

With the COTC analogues successfully prepared, their biological activities against a normal human liver cell line (WRL-68) and two cancer cell lines (hepatoma HepG2 and leukemia HL-60) were examined (Table 1). The optimal selective cytotoxic activity should demonstrate low IC_{50} values for the cancer cells but a high IC_{50} value for normal cells.

Initially, the cytotoxicity of diols **5a** and **5b** against normal cell line was measured. For the 2-naphthalenylmethyl ether **5a**, it showed low cytotoxicity against both normal and cancer cell lines. Disappointingly, biphenylmethyl ether **5b** was non-selective and toxic against normal cell line, therefore it was abandoned from further investigations. These results suggest that arylmethyl substituents are not desirable structural features for a selective anticancer agent.

Prompted by our findings, several alkyl ether analogues were then prepared with a 1-, 3-, 5- and 7-carbon unit in their respective ether chains. For enone **5c** (R = Me), its cytotoxicity against both normal and cancer cell lines were low. When the carbon chain length was changed to 3 (**5d**), it was found that although a minor drop in the IC_{50} value in normal cell line was observed, its cytotoxicity against both cancer cell lines was improved. Further increasing the ether chain length to a 5-carbon unit (**5e**) resulted in an



Scheme 1. Synthesis of COTC analogues.

Table 1
IC_{50} values for COTC analogue **5a**–**g** and **18**

Compd.	R	IC_{50} (μM) (WRL-68)	IC_{50} (μM) (HepG2)	IC_{50} (μM) (HL-60)
5a	2-Naphthylmethyl	>200	>50	>50
5b	Biphenylmethyl	71.3	—	—
5c	Methyl	>200	>50	>50
5d	*n*-Propyl	191	33.6	10.1
5e	*n*-Pentyl	169	16.1	3.1
5f	*n*-Pentyl	48.3	10.1	2.6
5g	*n*-Heptyl	152	>50	39.8
18	—	>200	>50	>50

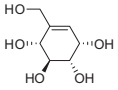


Figure 3. Structure of Streptol **18**.

even lower, yet still acceptable, IC_{50} value for the normal cell line; however, the IC_{50} value against cancer cell lines was lowered by at least two-folds: 16.1 μM (HepG2) and 3.1 μM (HL-60). However, when the ether chain length was further increased to a 7-carbon unit (**5g**), the cytotoxicities against both cancer cell lines were reduced. These results suggest that currently, the analogue **5e** with the *n*-pentyl ether chain displays the best selectivity and cytotoxicity.

With the optimized results obtained from **5e**, its C-4 epimer **5f** was constructed in order to study the stereochemical effect of the ether chain. Interestingly, the configuration inversion at C-4 in **5f** caused the IC_{50} values for both normal and cancer cell lines to decrease dramatically. This result renders the analogue **5f** cytotoxic to normal cells and hence the desired selectivity was lost.

As a reference, streptol (**18**, Fig. 3) was also synthesized following our established strategies[25,26] and its biological activities against the normal and cancer cell lines have now been determined. Since **18** possess an allylic alcohol functionality at C-1 rather than a ketone functionality, it showed no cytotoxicity against the normal and cancer cell lines, which gives support to the aforesaid mechanism.

In summary, seven COTC analogues were synthesized from D-glucose and their direct cytotoxic activities towards WRL-68, HepG2 and HL-60 cell lines were assessed. Aliphatic ether analogues were found to display more selective cytotoxicity towards cancer cells and greater potency than the arylmethyl ether analogues. Configurational inversion of the stereochemistry at C-4 from alpha to beta drastically decreased the cytotoxic selectivity towards cancer cells. Finally, alpha *n*-pentyl ether **5e** was found to be the most cancer cell-selective cytotoxic agent amongst the seven analogues tested.

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