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SYNTHESIS AND ANTITUMOR ACTIVITY OF COMBRETASTATIN D-4

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Abstract – Combretastatin D-4 **1** was synthesized using electrochemical dimerization of phenols as the key step. Anodic oxidation of bromochloro-phenol **7** provided the corresponding diaryl ether **8**. Manipulation of the two hydroxyl groups differenciated, provided the seco-acid, which on Mitsunobu reaction gave the target molecule. Its biological assay showed new potent inhibitory activity against cellular proliferation of human HT-29 colon carcinoma cells.

A family of cyclic diarylheptanoids, such as galeon,[1] pterocarine,[2] and combretastatins,[3] exhibit a wide range of biological activity, such as antitumor, antibacterial, and antiplasmodial activities. Combined with these attractive activities, their structures often have a relatively simple structure, making them promising leads to new drugs. A number of synthetic approaches to these natural products, have been reported. Among them, construction of the diaryl ether[4] has generally been attained by the Ullmann and S_NAr protocols, and total synthesis of galeon and pterocarine was recently accomplished by the former.[5] Against such back ground, we have independently developed the phenolic oxidation methodology by thallium(III) salts.[6]

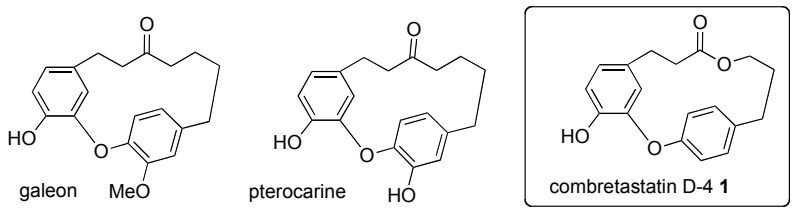
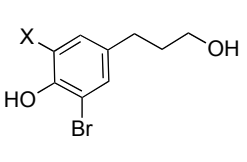


Figure 1. Natural diarylheptanoids

Table 1. Anodic oxidation of the phenols **2** and **7**

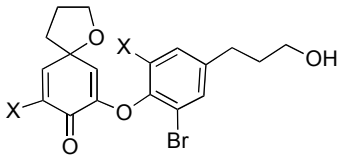
Entries	Substrates	Solvents	Concent. mM	F/mol	Products/yielfds (%)							
					3	**4**	**5**	**6**	**8**	**9**	**10**	**11**
1	**2**	$MeNO_2$	10	3	20							
2	**2**	MeOH	10	3	10	54	4					
3	**2**	CF_3CH_2OH	10	3		90						
4	**2**	CF_3CH_2OH*[1]	10	2	34		11	21				
5	**2**	CF_3CH_2OH*[1]	50	2	25		26					
6	**2**	CF_3CH_2OH*[1]	10	2	38		15	14				
7	**7**	MeOH*[1]	7	2					41	6	10	8
8	**7**	MeOH*[2]	10	2					34	10	6	2
9	**7**	CF_3CH_2OH	10	1.5					20	70		
10	**7**	CF_3CH_2OH*[1]	10	1.5					61			
11	**7**	CF_3CH_2OH*[1]	2	1.5					56			
12	**7**	CF_3CH_2OH*[1]	20	1.5					44			
13	**7**	CF_3CH_2OH*[3]	20	1.8					57			

Anode: Pt-net. Cathode: Pt-wire. Supporting salts: $LiClO_4$ 0.1 M and *n*-Bu_4NBF_4 0.1M for entry 1.
*[1]Addition of aq. NaOH. *[2]Addition of $HClO_4$. *[3]Addition of *n*-Bu_4NOH.



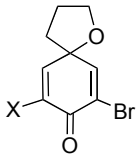
2: X = Br

7: X = Cl



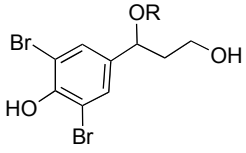
3: X = Br

8: X = Cl

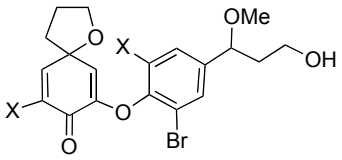


4: X = Br

9: X = Cl

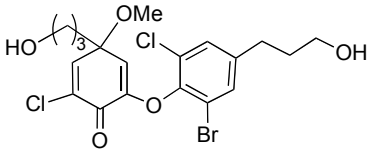


5: R = OMe or OH



6: X = Br

10: X = Cl



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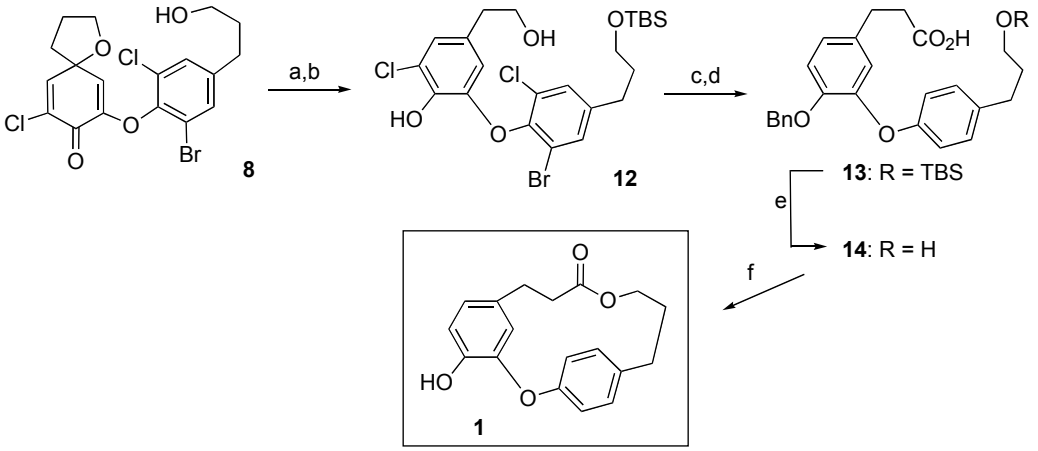
As part of our ongoing investigation, we attempted synthesis of galeon, pterocarine, and related compounds employing thallium(III)-mediated cyclization.[7] In their biological assessment, several synthetic intermediates showed more potent inhibitory activity against cellular proliferation of human HT-29 colon carcinoma cells than those of natural products,[13] although yields of the key oxidative cyclization were relatively lower than the reported data using the Ullmann protocol, owing to influenced by steric hindrance of the ring size and substitution patterns. To pursue an efficient synthetic methodology of cyclic diarylheptanoids, we attempted construction of the diaryl ether by dimerization of a phenol derivative under anodic oxidation conditions. The outstanding point of the electrochemical reaction would require no harmful metals, and usage of a single phenol may be chemo-economically efficient. With such investigation concept, synthesis of combretastatin D-4 **1**, isolated from the stem of *Getonia floribunda*, was undertaken, although any biological activity has not been reported.[3] However, natural products exhibiting low or no biological activity may have a potential as a new lead carrying

highly specific activity. In anodic oxidation, one-electron oxidation of phenol derivatives carrying a free hydroxyl group (ie **2** or **7**) provided the spiro-dimer **3** or **8**, in which the two hydroxyl groups was differentiated, making it possible to convert into an appropriate precursor of the desired lactone structure of **1**. We describe herein our research process.

With a synthetic plan mentioned above, we examined the anodic oxidation of halogenated phenols (Table 1). Upon use of the dibromophenol **2**,[8] two-electron oxidation, occurred along with expected one-electron oxidation, to give such by-products, as **4**-**6**, along with the desired **3** (entries 2-6). Among parameters of the oxidation reactions, solvents along with basic or acidic additives, rather than concentration and electricity, were effective to the reaction outcome. In contrast to $MeNO_2$ (entry 1), MeOH and CF_3CH_2OH solvents under neutral conditions, provided considerable amounts of the spiro compound **4**, produced through a cationic intermediate (entries 2 and 3). Reasons that can be considered for this result are: bromine substituents caused too low oxidation potential, which conducted unexpected two-electron oxidation process.[9] When the halogen substituents were changed to a Br-Cl pair, oxidation of bromochloro-phenol **7**[10] (entries 7 - 13) effected selective formation of the targeted spirodimer **8**, except entries 7-9. Moreover, addition of NaOH or *n*-Bu_4NOH as a base, modulated the oxidation potential, producing the radical species preferentially to form the dimmer **8** (entries 10 - 13).



Scheme 1. Synthesis of combretastatin D-4 **12**. *Reagents and conditions*: (a) TBSOTf, 2,6-lutidine/CH_2Cl_2, 80%. (b) Zn-AcOH/THF, 86%. (c) 10%Pd-C, HCO_2NH_4/EtOH, 60 °C, 86%. (d) i) BnBr, K_2CO_3/DMF, quant; ii) SO_3-pyr, Et_3N, DMSO/CH_2Cl_2, 83%; iii) $NaC1O_2$, 2-methyl-2-butene, $NaHPO_4$, *t*-BuOH, H_2O, 98%. (e) i) MeI, K_2CO_3/DMF, 70%; ii) TBAF/THF, 87%; iii) 1M aq. NaOH/MeOH, 95%. (f) i) PPh_3, DEAD/PhMe, 71%; ii) 10% Pd-C, HCO_2NH_4/EtOH, 60 °C, 89%.

With the desired spiro-dimer **8** in hand, the existing primary hydroxyl group was protected as a silyl ether, followed by reduction with Zn/AcOH, to furnish the diaryl ether **12**. The halogen atoms were removed by a hydrogen transfer method, and the phenol group was protected as a benzyl ether. The free hydroxyl

group was oxidized in two steps to form carboxylic acid **13**. Compound **13** was transformed to a methyl ester temporarily during the desilylation process, and the following hydrolysis provided seco acid **14**. Mitsunobu reaction[11] of **14** smoothly proceeded, and successive reductive removal of the benzyl group produced combretastatin D-4 **1**, spectroscopic data of which was superimposable to the reported data.[3,12]

We also examined IC_{50} values (μg/mL) of synthetic **1**, along with galeon and several synthetic compounds carrying closely related cyclic diaryl ether structures,[7] against proliferation of human HT-29 colon carcinoma cells (Fig. 2).[13] It was noteworthy that combretastatin D-4 **1**, synthesized in this investigation, exhibited inhibitory activity (IC_{50} 18.4 μg/ml), although **1** has been reported to exhibit no remarkable biological activity. Further structure - activity relationship studies of **1** and the biologically active chlorinated derivatives are in progress.

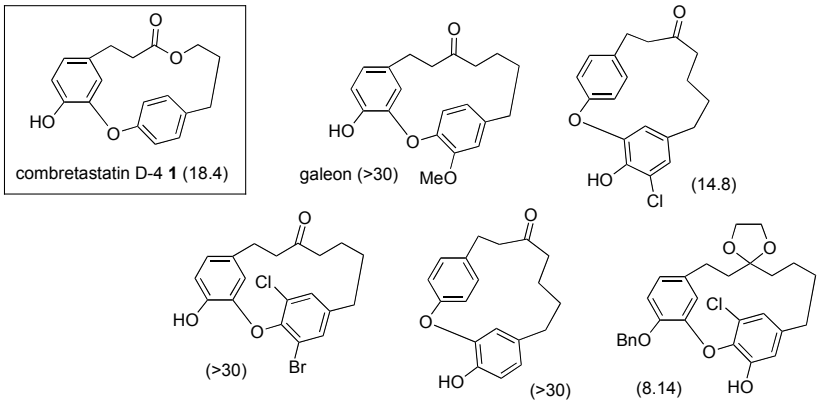


Figure 2. Inhibitory activity (IC_{50} μg/mL) against proliferation of human HT-29 colon carcinoma cells

In conclusion, we have completed the synthesis of combretastatin D-4 **1**. In addition, biological assessment of this compound newly showed the inhibition of cell proliferation of colon carcinoma cells. Further investigation will be conducted to acquire promising leads to new chemotherapeutic agents, despite relatively simple cyclic diaryl ether structures.

ACKNOWLEDGEMENTS

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12. IR (film) 1504, 1518, 1597, 1730, 2925, 3422 cm^{-1}; ^{1}H-NMR δ 2.10 (2H, m), 2.25 (2H, t, *J* = 5.2 Hz), 2.82 (4H, m), 4.06 (2H, t, *J* = 4.8 Hz), 5.29 (1H, d, *J* = 2 Hz), 5.53 (1H, s), 6.60 (1H, d, *J* = 6 Hz), 6.93 (1H, d, *J* = 8 Hz), 7.01 (2H, d, *J* = 8.4 Hz), 7.30 (2H, d, *J* = 8.8 Hz); ^{13}C-NMR δ 27.1, 28.7, 32.8, 34.0, 63.9, 112.5, 114.9, 121.4, 123.5, 131.0, 132.6, 137.8, 142.4, 149.0, 154.1, 173.8. HREIMS found *m/z* 298.1199, calcd for $C_{18}H_{18}O_4$: M, 298.1205.
13. The inhibition of human HT29 colon carcinoma cells was measured by MTT assay. The cells were seeded at 2 x 10^4 cells well in 96-well plates. After overnight incubation, cells were treated for 48 h with increasing concentrations of compound (up to 30 μg/mL). Then MTT (5 mg/mL in PBS)

was added to the cultures and incubated for another 4 h. The growth medium as removed and DMSO was added to each well to dissolve purple crystals of formazan. The absorbance was measured in a spectrophotometer at a wave length of 570 nm. The IC_{50} value was defined as the compound concentration needed to inhibit 50% of growth relative to the untreated control.