2014 3rd International Conference on Environment Energy and Biotechnology
IPCBEE vol.70 (2014) © (2014) IACSIT Press, Singapore
DOI: 10.7763/IPCBEE. 2014. V70. 8

Controlled Red-Ox Reactions of Certain Cephalostatin analogs with anti-Cancer Activity

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Abstract. We broadened the transformation varieties of some bis-steroidal pyrazines as analogs to cephalostatine 1, which is a remarkable antineoplastic natural product isolated from the marine algae *Cephalodiscus gilchristi*. It is a small molecule with a unique cytotoxicity profile in the *in vitro* screen system of the National Cancer Institute, suggesting that it may affect novel molecular target(s). In this part of the work, the regioselectivity of F-ring reductive-opening was discovered for an analog and improved for another analog by using some borane-complexes. Looking for enhancement of biological activity, an α,β-unsaturated carbonyl was generated by oxidation of allylic position of a methylene group at C-12 to be as Michael receptor.

Keywords: cephalostatin1, F-ring reductive-opening process, regioselectivity, α, β-unsaturated carbonyl, Michael receptor.

1. Introduction

Cephalostatin 1 **1**, is a potent tumor inhibitory marine natural product isolated by Pettit's group at Arizona State University from *Cephalodiscus gilchristi* in addition to other 18 compounds belonging to the same family [1]-[4]. This group of compounds sharing the same backbone which consists of two steroids coupled to each other through pyrazine ring. Cephalostatin 1 is a small molecule suggested to affect novel molecular target(s) and with a unique cytotoxicity profile in the *in vitro* screen system of the National Cancer Institute.

Aiming at the synthesis of cephalostatin analogues with high biological activity, we reported here a new methods either to improve the regioselectivity of F-ring opening process, which was previously investigated [5], [6] or to generate α, β-unsaturated system on Ring-C as Michael receptor. This work is a part of a strategy aimed at understanding the chemistry of such bis-steroidal system, which we hope to enable us to achieve a total synthesis of one or more of the cephalostatin natural products. The first total synthesis of Cephalostatin 1 was reported by Fuchs and his coworkers in 1998 [7]. Moreover, through this work we were able to gather more information about structure-activity relationship, since most of the synthesized compounds were tested against three representative cancer cell lines (HM 02, HEP G2, MCF 7). In Jurkat leukemia T cells, cephalostatin 1 was found to induces a novel pathway of receptor-independent apoptosis that selectively uses Smac/ DIABLO as a mitochondrial signaling molecule. [8]

2. Results and Discussion

As a completion of our previous work, new routes were examined looking for selective reactions to desymmetrize the symmetrical diketone **2**, which can be prepared in gram scale using a well established method [9 and references therein]. The researcher is aiming at the synthesis of high biological active analogues to the cephalostatin 1 **1**.

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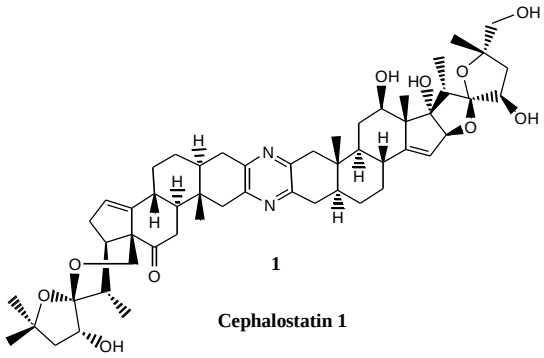
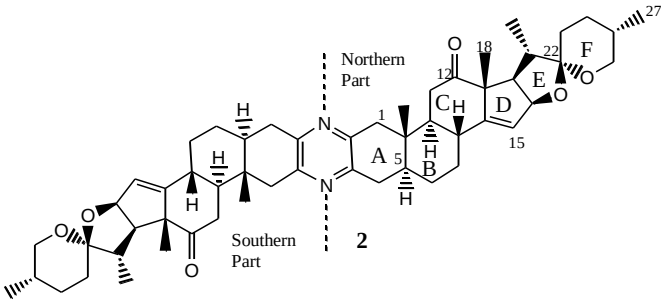
Herewith, new routes were investigated either to improve the selectivity of F-ring opening process, which was previously investigated by Hannover Group [5], [6], or to generate α, β-unsaturated system on Ring-C as Michael receptor aiming at enhancing the biological activities.



1

Cephalostatin 1

Fig. 1



2

Fig. 2

Concerning the first route, efforts were concentrated on the regioselectivity improvement to achieve a one-side opening dimer, aiming finally at reconstruction of the spiroketal moiety to be similar to that in the cephalostatin 1. The two space-demanded factors that influence this process, the substrate geometry and the geometrically and electronically suitable complex, were studied. Engineering such process was basing on previous results obtained [6]. To study the first factor, the diacetate derivatives **4** and **6** were investigated using catechol-borane complex. Upon applying the trivial procedure, it was noticed that, the process was failed when the substrate **4** was used and the starting material was unchanged during the reaction (TLC follow). In the beginning, it was a disappointed result for us. But, we were very satisfied when we found that the derivative 12β,12´β-diacetate **6** underwent F-ring opening process easily using the same complex and the same procedure (Scheme 1).

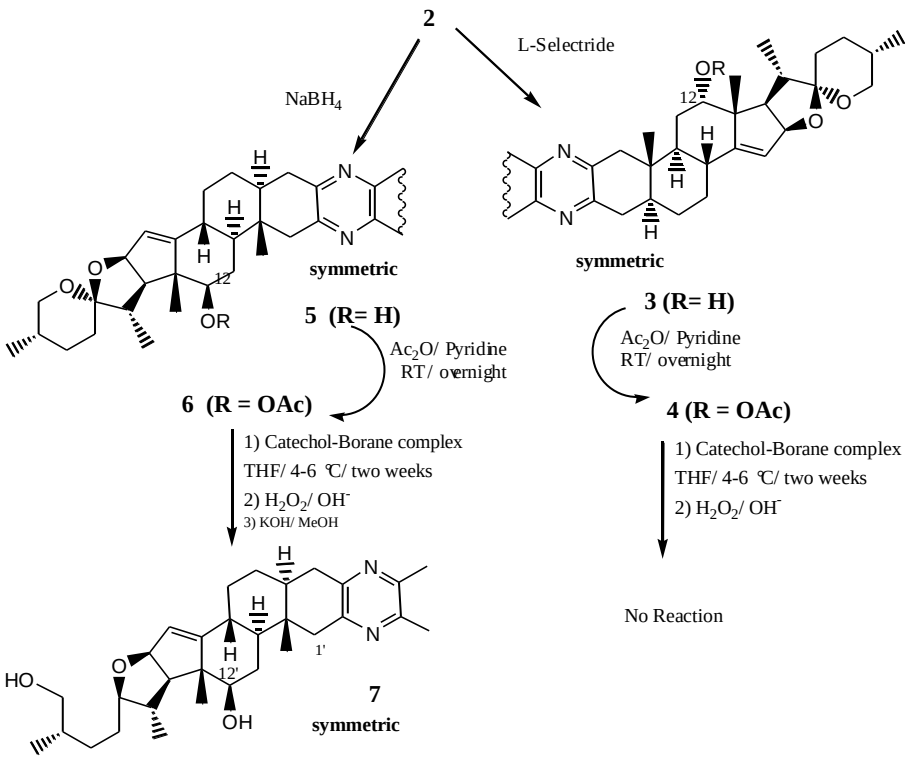


Fig. 3**:** Synthesis and reductive-opening test of symmetrical bis-acetoxy derivative α and β-diol.

The asymmetric diacetate **8** was the best candidate to get the mono-opened spiroketal. Treating compound **8** with excess of catechol-borane complex ***a*** at relatively low temperature (4-6 ˚C) yielded compound **9** in 74 % yield.

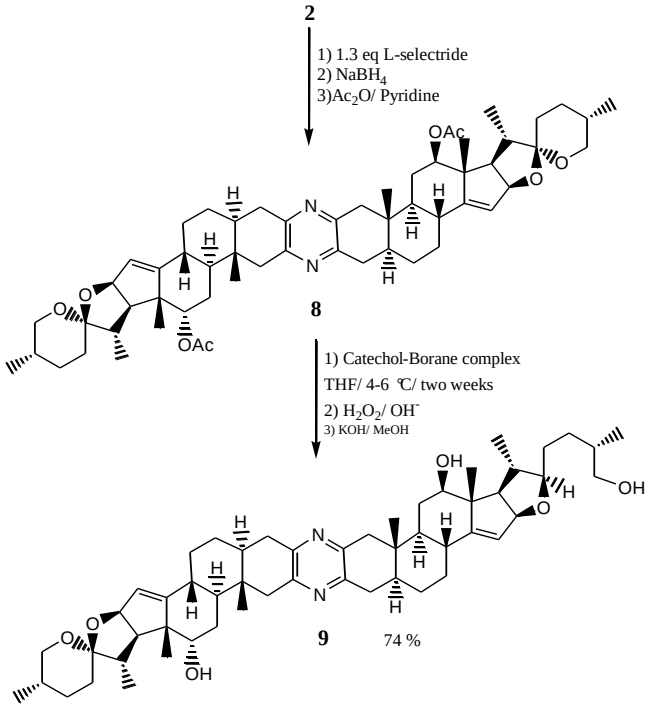


Fig. 4: Selective reductive-opening of unsymmetrical bis-acetoxy α, β-diol.

To understand more about the regioselective opening process, the substrate 11α-meythoxy diketone **10,** which was prepared easily from the diketone **2**,[9] and the modified complex, 3-methoxy catechol-borane **b**, were selected. The selection of the substrate was basing on the fact that the a-configurated methoxy group at position-11 prevents any nucleophilic attack on the adjacent carbonyl and hence the ketal moiety in this half will be free to be attacked from the complex. However, the other half contains the unprotected carbonyl group, which will be first reduced by the modified borane complex which will shield geometrically the ketal moiety from further attack by excess complex. Treating compound **10** with excess ***b*** at a relatively low temperature (4-6˚C), and after the usual alkaline-oxidative work up, gave compound **11** as a major product in addition to small amount of the mono-opened derivative **12** (Scheme 3). However, the double-opened spiroketal product was not isolated or detected. This indicates that the complex is electronically unsuitable to open the ketal moiety in the methoxy-half.

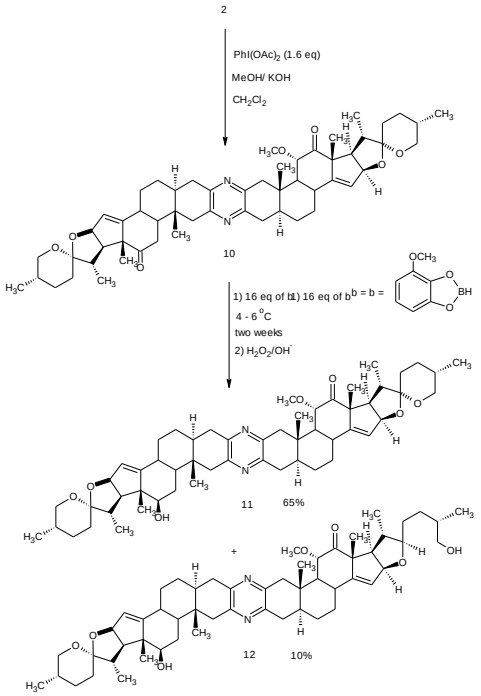


Fig. 5: Selective reductive-opening of 11α-methoxy ketone using complex **a**

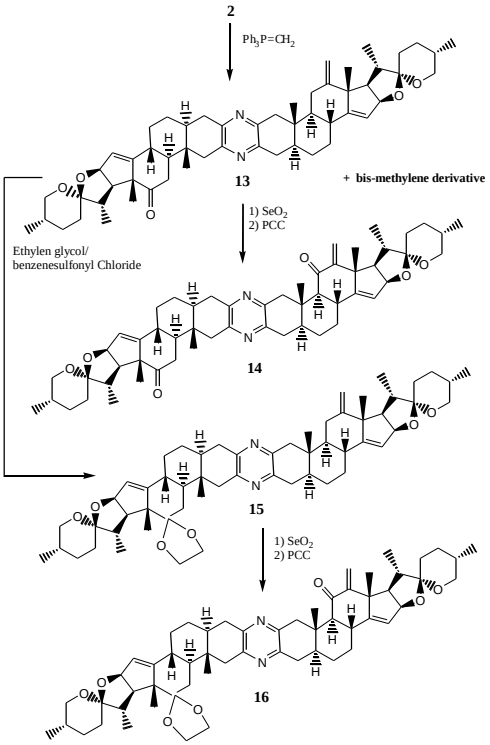


Fig. 6: Synthesis of α,β-unsaturated carbonyl derivatives derived from **13**

The second studied route however, was the generation of α,β-unsaturated carbonyl on Ring-C as a Michael receptor which may enhance the biological activity. Starting from the diketone **2**, the Wittig-product **13** was oxidized firstly with SeO_2 and after work-up, the resulted crude material (without chromatography) was oxidized with PCC yielding finally the keto-enone **14** (Scheme 4). Compound **14** was found to be much more active than both precursors **2** and **13**. The protected analogue with ethylene glycol compound **16** was also tested and found to have moderate to weak activity but still more active than its both precursors **13** and **15**.

Biological Activity: Some of the synthesized compounds were tested in Hannover Medical School against three cancer cells; HM 02 (stomach adenocarcinoma), HEP G2 (hepatocellular carcinoma) and MCF7 (breast adenocarcinoma) using a trivial procedure [6]. It was noticed that compound **9** has less activity than its ring- closed analogue (the deacetelated derivative of **8**). The same result was noticed in the case of compounds **11** and **12**. Compound **11** showed less activity compared to **12** which has a relatively good activity against the three tested cell lines (GI_{50} = 1-2 1μM). This may emphasize the importance of the ketal moiety to the biological activity. Furthermore, we have noticed that the generation of Michael acceptor on the Ring-C enhances the biological activity sharply. For example, compound **14**, (GI_{50} = 0.8μM) was much more active than its weakly active precursors **2** and **13**. Similar results were noticed in the case of compound **16**, which was more active than its precursor **15**. Comparatively, compound **14** showed better activity than **16**.

3. Experimental

3.1. Preparation of mono-opened 12β, 12´α-diol 9:

To 90 mg of **8** (0.093 mmol, 1 eq) dissolved in 4.0ml abs. THF at 0˚C, a solution of catechol-borane complex (210 mg (1.91 mmol, 20 eq) catechol dissolved in 2.0 ml abs. THF at 0˚C and 1.9 ml BH_3.THF (1.0 M solution)) was slowly added under Argon atm. The reaction mixture was further stirred for 30 min, before conducting it in the Refrigerator (4-6˚C) for two weeks. The reaction was quenched with slow addition of 0.2 ml of 10 % KOH and 2 ml of each EtOH and DEE were added. At RT 0.5 ml of 35 % H_2O_2 was added and vigorously stirred for two hours. The reaction solution was extracted with chloroform and the organic phase was washed several times with $NaHCO_3$ solution to remove the unreacted chatechol. The crude material, after removing the solvent under low pressure, was dried and dissolved in small amount of chloroform and 2

ml of saturated KOH/methanol solution was added. Refluxing the reaction solution, extraction with chloroform and purification of the resulted crude material using column chromatography (silica gel, EA:PE 1:2) resulted in 58 mg of the mono-opend diol **9** (74 %).

^{1}H-NMR: (400 MHz, $CDCl_3$); δ = 5.58 (br s, 1H, 15 ´-H), 5.45 (br s, 1H, 15-H), 4.90 (br d, J_{16-17} = 7.9 Hz, 1H, 16 ´-H), 4.78 (dd, J_{16-17} = 8.2 Hz, J_{16-15} = 1.5 Hz, 1H, 16-H), 3.77 (br s, 1H, 12 ´-H), 3.32-3.56 (m, 4H, 26a /26b/26 ´a/26 ´b-H), = 3.23-3.35 (m, 2H, 22/12-H), 2.75-3.01 (m, 4H, 1a/4a/1 ´a/4 ´a -H), 2.45-2.71 (m, 5H, 1b/4b /1 ´b/4 ´b/17 ´-H), 2.33 (tr, J_{17-20} = J_{17-16} = 8.0 Hz, 1H, 17-H), 2.18 (m, 1H, 8 ´-H), 1.15 (s, 3H, 18 ´-H), 1.06 (d, J_{21-20} = 8.0 Hz, 3H, 21-H), 1.03 (s, 3H, 18-H), 1.01 (d, $J_{21´-20´}$ = 6.5 Hz, 3H, 21 ´-H), 0.92 (d, J_{27-25} = 8.0 Hz, 3H, 27-H), 0.88 (s, 3H, 19-H), 0.87 (s, 3H, 19 ´-H), 0.81 (d, $J_{27´-25´}$ = 6.0 Hz, 3H, 27 ´-H). **^{13}C-NMR:** (100 MHz, $CDCl_3$); δ = 160.34 (C, 14-C), 153.73 (C, 14 ´-C), 148.76, 148.60, 148.59, 148.37 (all C, pyrazine-C), 121.37 (CH, 15 ´-C), 119.82 (CH, 15-C), 106.67 (C, 22 ´-C), 87.17 (CH, 22-C), 86.01 (CH, 16-C), 85.14 (CH, 16 ´-C), 79.31 (CH, 12-C), 75.87 (CH, 12 ´-C), 68.11 (CH_2, 26-C), 67.20 (CH_2, 26 ´-C), 59.37 (CH, 17-C), 53.71 (CH, 17 ´-C), 52.93 (C, 13-C), 52.22 (C, 13 ´-C), 51.95 (CH), 49.24 (CH), 45.64 (CH_2, 1-C), 45.24 (CH_2, 1 ´-C), 44.82 (CH), 41.60 (CH), 41.43 (CH), 41.19 (CH), 35.98 (C, 10-C), 35.85 (C,10 ´-C), 35.80 (CH), 35.18, 35.10 (CH_2, 4/4 ´-C), 34.87 (CH), 34.29 (CH), 32.35 (CH_2), 30.82 (CH_2), 30.44 (CH, 25 ´-C), 30.25 (CH_2), 31.18 (CH_2), 29.44 (CH_2), 29.73 (CH_2), 28.82 (CH_2), 28.72 (CH_2), 28.06 (CH_2), 27.86 (CH_2), 25.42 (CH_3, 18-C), 18.61 (CH_3, 18 ´-C), 17.15 (CH_3, 27 ´-C), 16.87, 16.63, (both CH_3, 21/27-C), 14.17 (CH_3, 21 ´-C), 13.98 (CH_3, 18-C), 11.86, 11.85 (CH_3, 19/19 ´-C).

3.2. Preparation of spiro-enone 16:

To a 2-necks round bottom flask containing 0.040 g (0.0451 mmol, 1.0 eq) of spiro-methylene **15** dissolved in 5 ml of abs. CH_2Cl_2, 0.051 g (0.0946 mmol, 2.0 eq) of SeO_2 and 1 ml of *t*-BuOOH (98 %) were added. The reaction mixture was stirred at RT for 96 h. After the reaction completion (TLC follow), it was quenched with 1 ml of a saturated solution of $NaHCO_3$ and diluted with 10 ml CH_2Cl_2. Extracting the reaction solution with saturated solution of sodium hydrogencarbonate followed by washing the organic phase with brine solution and drying it over $MgSO_4$ gave a crude material after removing the solvent under reduced pressure and then high vacuum. The resulting crude material was subjected to flash-chromatography (silica gel, PE: EA elution) gave 15 mg of the keto-enon **16** (38 %) as a pure substance in addition to two more polar side products (4 mg and 7 mg).

IR: $CHCl_3/\nu_{max}/cm^{-1}$; 2931 s (C-H), 1709 s (C=O), 1650 w (C=C), 1458 m (C-H), 1400 s (pyrazine), 1230 s (C-O).

^{1}H-NMR: (400 MHz, $CDCl_3$); δ = 5.61 (br s, 1H, 15-H), 5.52 (s, 1H, 28a-H), 5.48 (br s, 1H, 15 ´-H), 5.12 (s, 1H, 28b-H), 4.95 (dd, J_{16-17} = 8.8 Hz, J_{16-15} = 1.8 Hz, 1H, 16-H), 4.88 (br d, $J_{15´-17´}$ = 8.2 Hz, 1H, 16 ´-H), 3.94-4.13 (m, 5H, 28 ´/29 ´/1a-H), 3.75 (m, 1H, 17-H), 3.40-3.55 (m, 4H, 26a/26b/26 ´a/26 ´b-H), 2.40-2.95 (m, 12H), 1.22 (s, 3H, 18 ´-H), 1.14 (s, 3H, 18-H), 1.09 (d, J_{21-20} = 6.8 Hz, 21-H), 1.06 (s, 3H, 19-H). 1.02 (d, $J_{21´-20´}$ = 8.2 Hz, , 3H, 21 ´-H), 0.86 (s, 3H, 19 ´-H), 0.81 (d, J_{27-25} = $J_{27´-25´}$ = 6.3 Hz, 6H, 27/27 ´-H).

^{13}C-NMR: (100 MHz, $CDCl_3$); δ = 202.32 (C, 11-C), 157.35 (C, 12-C), 156.07 (C, 14 ´-C), 154.02 (C, 14-C), 149.35, 148.28, 147.92, 147.71 (all C; pyrazine-C), 120.52 (CH,15-C),120.44 (15 ´-C), 113.41 (C, 12 ´-C), 107.41 (CH_2, 28-C), 107.06 (2 x C, 22/22 ´-C), 86.52 (CH, 16 ´-C), 84.39 (CH, 16-C), 68.17 (CH_2, 26 ´-C), 67.22 (CH_2, 26-C), 65.19, 65.03 (both CH_2, 28 ´/29 ´-C), 64.54 (CH), 55.56 (C), 54.59 (CH), 53.72 (C), 52.72 (C), 50.48 (CH, 9 ´-C), 45.19, 45.55 (2 x CH_2, 1/1 ´-C), 41.76 (CH, 20-C), 41.30 (CH, 20 ´-C), 36.37, (C), 35.87 (CH), 35.22, 35.10 (2 x CH_2, 4/4 ´-C), 33.98, 33.72 (2 x CH), 31.28 (CH_2), 31.18 (CH_2), 30.45 (CH_2), 30.39, 30.33 (2 x CH), 30.14 (CH_2), 29.89 (CH_2), 29.17 (CH_2), 29.03 (CH_2), 28.78 (CH_2), 28.06 (CH_2), 27.87 (CH), 25.23 (CH), 18.98 (CH_3, 18 ´-C), 17.16, 17.10, 16.66 (3 x CH_3, 18/27/27 ´-C), 13.87 (2 x CH_3, 21/21 ´-C), 11.58, 11.87, (both CH_3, 19/19 ´-C).

4. Acknowledgment

The Author would like to acknowledge Professor Helmut Duddeck at Hannover University for offering all facilities needed to accomplish the work. The Author also thanks the DFG for the financial support.

5. References

[1] G. R. Pettit, J. Xu, Y. Ichihara, M. Williams and M. Boyd, *Can. J. Chem.* 1994, **72**, 2260.

[2] G. R. Pettit, J.P. Xu, J.M. Schmidt, M. R. Boyd, *Bioorg. and Med. Chem. Lett.* 1995, **5** (17), 2027.

[3] G. R. Pettit, M. Inoue, Y. Kamano, C. Dufresne, M. Christie, M. Niven and D. L. Herald. *J. Chem. Soc., Chem. Comm.* 1988, 865.

[4] G.R. Pettit, R. Tau, J.P. Xu, Y. Ichihara, M.D. Williams and M.R. Boyd, *J. Nat. Prod.*, 1998, **61**, 955.

[5] Ba ̈sler, S., Brunck, A., Jaktelat, R., Winterfeldt, E. *Helv. Chim. Acta*, 2000, **83**, 1854.

[6] M. Nawasreh, *Bioorganic and Medicinal Chemistry*, 2008, **16** (1), 255.

[7] LaCcour, T. G.; Guo, C.,Bhandaru, S., Boyd, M. R., Fuchs, P. L. *J. Am. Chem. Soc.* 1998, **120**, 692.

[8] Verena M. Dirsch, Irina M. Mu ̈ller, So ̈ren T. Eichhorst, George R. Pettit, Yoshiaki Kamano, Masuo Inoue, *Cancer Research*, 2003, **63**, 8869.

[9] M. Nawasreh and E. Winterfeldt, *Curr. Org. Chem.*, 2003, **7** (7), 649.