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Design and synthesis of tubulin ligands based on epothilones: a preliminary study

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Abstract—Starting from the X-ray structure of epothilone B, an original, non macrocyclic, structure has been designed to mimic the key structural features of this potent anticancer agent; the preparation of a key intermediate incorporating four stereogenic centers is described from cyclohexane-1,3-dione.

Epothilones A **1** and B **2** are the first members of a new class of fascinating and biologically active natural products isolated by Höfle from the myxobacterium *Sorangium cellulosum*[1] (Fig. 1). The current high interest for these 16-membered macrocyclic lactones arise from their potent microtubule binding property leading to significant in vivo cytotoxic activity against various cancer cell lines including multidrug-resistant ones.[2]

These compounds share the same tubulin binding site as paclitaxel[3] but have the advantage over the latter to be more water soluble. These properties and their interesting molecular structure have prompted numerous syntheses since 1996[4] allowing to gather interesting structure–activity relationships.

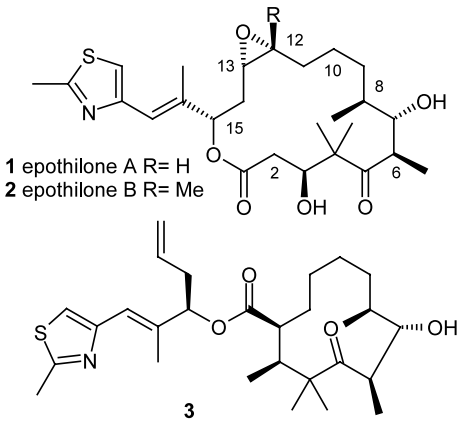

Figure 1.

Indeed, few efforts have been made to design analogues based on the X-ray structure and conformational analysis of **2** alone[5] and/or of paclitaxel either alone[6] or binded to tubulin.[7] Thus, Winckler and Axelsen have designed simpler analogs based on the introduction of an ethano bridge between C2 and C10 and synthetized compounds such as **3** which do not bind to tubulin.[8]

Starting from the X-ray structure of **2**, we searched for compounds having the same spatial arrangement of its key features: the C6–C8 aldol moiety, the C5 *gem*-dimethyl group, the C2–C4 β-hydroxyester group, the side chain with a thiazole ring and the C12–C13 epoxide or alkene residue (epothilone D which only differs from epothilone B by the presence of an alkene group instead of the epoxide has demonstrated a superior therapeutic index[9]). Selection of potential candidates was based on their conformational analysis and superposition with **2** and on their synthetic feasability. This study led to the design of a cyclohexane analogue which fulfill the above requirements (Fig. 2).[1]

Retrosynthetic analysis of **4** (possessing an alkene group insteed of an epoxide moiety) is based on the preparation of a cyclohexane derivative bearing 4 stereogenic centers and on the introduction at a proper stage the thiazole side chain (Scheme 1). We now disclose the diastereoselective synthesis of the racemic compound **4**. Starting from 2,2-dimethyl-1,3-cyclohex-

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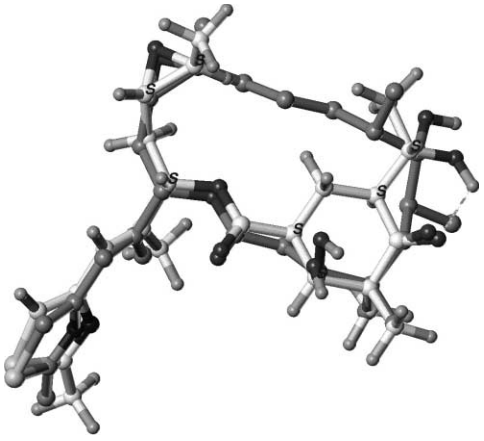
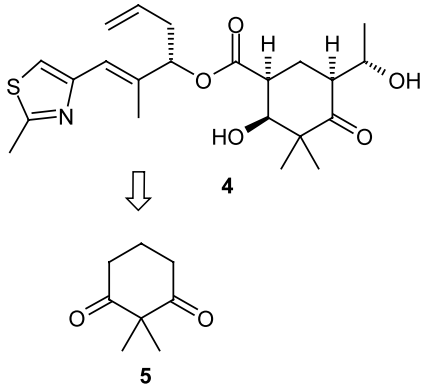


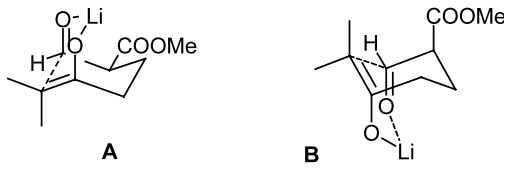
Figure 2. Dark grey: epothilone; light grey: analogue.



Scheme 1.

anedione **5**,[11] alkoxycarbonylation at C4 followed by reduction, alcohol protection, esterification and aldolisation should afford **4**.

Since alkoxycarbonylation was unsuccessful on **5** under different conditions, mono ketalisation to **6** (81%) was first carried out followed by condensation with benzylcyanoformate according to the procedure of Mander[12] (Scheme 2). The desired keto ester **7** (68%, 1/1 ketone–enol tautomeric mixture) was then reduced with $NaBH_4$ to give a 90% mixture of the two epimers **8a** and **8b** in variable ratios depending on experiments. Then, ketone deprotection was carried out on this mixture and, surprisingly, only **9** was isolated (97%). ^1H NMR of **9** is in agreement with a *cis* relationship of the hydroxyl and ester groups leading to a *pseudo*-axial conformation of



Scheme 3.

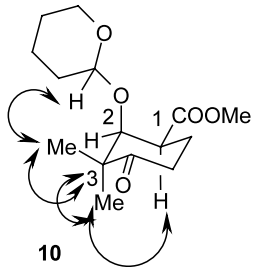
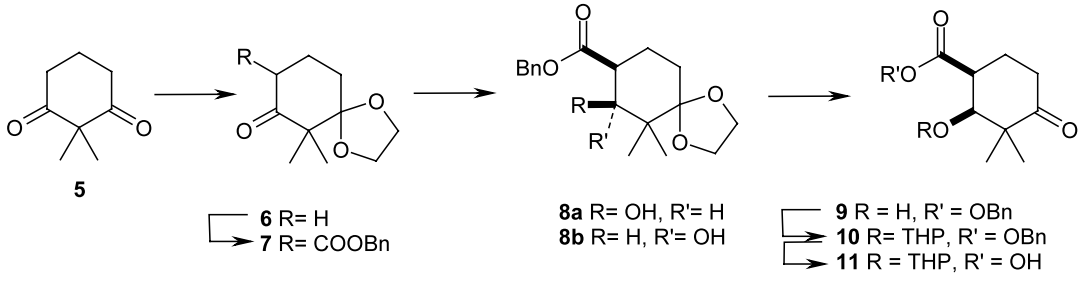


Figure 3.

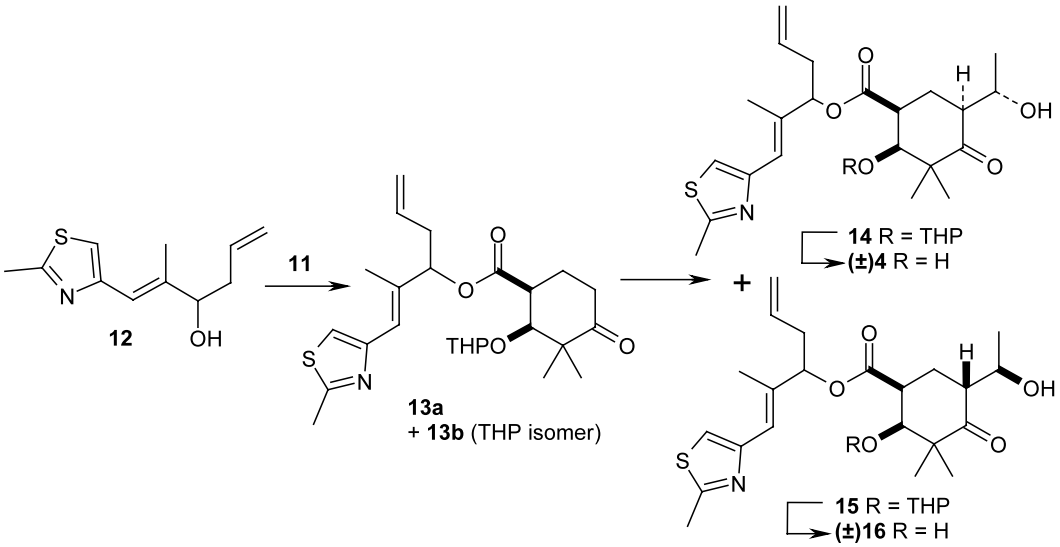
the former group ($J_{H1-H2}=1.7$ Hz). The exclusive formation of **9** is best explained by a retro-aldol reaction followed by recyclisation to afford the axial isomer.[13] Since molecular modelisation at the AM1[10] level reveals that **9** is slightly less stable than its equatorial epimer ($\Delta E=1.1$ kcal/mol), its formation is best explained by the more favorable chair–chair bicyclo[3,3,1]nonane-type transition state **A** compared to **B** (Scheme 3).

Then the hydroxyl group of **9** was protected as a THP ether to give **10** (74%). Although a 70/30 mixture of diastereoisomers is produced, no other protecting group could be introduced due to steric hindrance (silyl ethers) or decomposition under basic conditions (MOM). At this stage, NOESY experiments confirmed the proposed stereochemistry with strong correlations between H1 and one C1 Me group (1,3 diaxial interaction), the methine proton of the OTHP moiety and the other C1 Me and H2 with both C1 Me groups (Fig. 3). Then, **10** was converted to acid **11** (93%) upon hydrogenolysis (H_2, Pd/C, EtOAc).

Compound **11** (2 equiv.) and the racemic alcohol **12** (1 equiv.) in presence of DCC (2 equiv.) and 4-DMAP (2 equiv.) in CH_2Cl_2 afforded esters **13a** and **13b** in a 7/3 ratio (68% isolated yield) (Scheme 4). ^1H and ^{13}C NMR for both esters revealed that they only differ by the configuration of the chiral stereogenic center of the THP protecting group (acid catalysed hydrolysis



Scheme 2.



Scheme 4.

afforded the same compound) and that only one diastereoisomer is formed during the esterification step, although its configuration could not be determined. This is in agreement with a kinetic resolution which may arise from a diastereofacial selectivity in the nucleophilic attack of **12** on the bulky intermediate resulting from the addition of acid **11** to DCC. Similar kinetic resolutions have been reported in the literature.[14] Finally, condensation of the lithium enolate of **13a** (LDA, THF, −78°C) with 1 equiv. of acetaldehyde afforded aldols **14** and **15** in a 59/41 ratio (36%).[11] ^{1}H NMR data for the first aldol is in agreement with a *cis* relationship between the CH_3CHOH and COOMe groups, the observed coupling constants between H5, H6ax ($J=13$ Hz) and H6eq ($J=6.8$ Hz) confirming the axial orientation of H5. In the case of **14**, larger modifications (with respect to **13a**) are noticed in the ^{1}H and ^{13}C NMR spectra (H5 appears as a quadruplet ($J=9.1$ Hz)). Since it has already been shown that cyclohexanone enolates afford *anti*-aldol with aliphatic aldehydes,[15] it may be assumed that **15** results from the other possible diastereofacial addition. The lack of selectivity may arise from 1,4 (with the OTHP group) and 1,3 (with a C1 Me) steric interactions in both transition states. Upon treatment with cat. PTSA in acetone, diols **4** (53%) and **16** (56%) were obtained from **14** and **15**, respectively.

In summary, comparison of a cyclohexane derivative (**4**) with the X-ray structure of epothilone B was found to result in similar spatial arrangement of the key features of this potent new anticancer agent. Synthesis of racemic compound **4** has been completed in eight steps from 1,3-cyclohexanedione. Indeed, no cytotoxicity was observed for **4** (and **16**) against L1210 leukemia cells.[16] In order to explain this result, it will be crucial to determine the relative configuration of the ester side chain resulting from the kinetic resolution step. Since all intermediates and the final compounds are oils, further work is under way to search for cristalline derivatives in order to get an X-ray analysis.

Acknowledgements

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References

1. Höfle, G.; Bedorf, N.; Steinemetz, H.; Schomburg, D.; Gerth, K.; Reichenbach, H. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1567.
2. (a) Bollag, D. M.; McQueney, P. A.; Zhu, J.; Hensens, O.; Koupal, L.; Liesch, J.; Goetz, M.; Lazarides, E.; Woods, C. M. *Cancer Res.* **1995**, *55*, 2325; (b) Bollag, D. M. *Exp. Opin. Invest. Drugs* **1997**, *6*, 867; (c) Kowalski, R. J.; Giannakakou, P.; Hamel, E. *J. Biol. Chem.* **1997**, *272*, 2534.
3. (a) ter Har, E.; Kowalski, R. J.; Hamel, E.; Lin, C. M.; Longley, R. E.; Gunasakera, S. P.; Rosenkranz, H. S.; Day, B. W. *Biochemistry* **1996**, *35*, 243; (b) Hamel, E.; Sackett, D. L.; Vourloumis, D.; Nicolaou, K. C. *Biochemistry* **1999**, *38*, 5490.
4. (a) Biswas, K.; Lin, H.; Njardarson, J. T.; Chappell, M. D.; Chou, T.-C.; Guan, Y.; Tong, W. P.; He, L.; Horwitz, S. B.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2002**, *124*, 9825; (b) Nicolaou, K. C.; Roschangar, F.; Vourloumis, D. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 2014; (c) Mulzer, J. *Chem. Mon.* **2000**, *131*, 205.
5. Taylor, R. E.; Zajicek, J. *J. Org. Chem.* **1999**, *64*, 7224.
6. (a) Wang, M.; Xia, X.; Kim, Y.; Hwang, D.; Jansen, J. M.; Botta, M.; Liotta, D. C.; Snyder, J. P. *Org. Lett.* **1999**, *1*, 43; (b) Ojima, I.; Chakravarty, S.; Inoue, T.; Lin, S.; He, L.; Horwitz, S. B.; Kuduk, S. D.; Danishefsky, S. J. *Proc. Natl. Acad. Sci.* **1999**, *96*, 4256; (c) Giannakakou, P.; Gussio, R.; Nogales, E.; Downing, K. H.; Zaharewitz, D.; Bollbuck, B.; Poy, G.; Sackett, D.; Nicolaou, K. C.; Fojo, T. *Proc. Natl. Acad. Sci.* **2000**, *97*, 2904; (d) He, L.; Jagtap, P. G.; Kingston, D. G. I.; Shen, H.-J.; Orr, G. A.; Horwitz, S. B. *Biochemistry* **2000**, *39*, 3972.
7. Snyder, J. P.; Nettles, J. H.; Cornett, B.; Downing, K. H.; Nogales, E. *Proc. Natl. Acad. Sci.* **2001**, *98*, 5312.

8. Winckler, J. D.; Holland, J. M.; Kasparec, J.; Axelsen, P. H. *Tetrahedron* **1999**, *55*, 8199.
9. (a) Chou, T.-C.; Zhang, X.-G.; Balog, A.; Su, D.-S.; Meng, D.; Savin, K. A.; Bertino, J. R.; Danishefsky, S. J. *Proc. Natl. Acad. Sci.* **1998**, *95*, 9642; (b) Chou, T.-C.; Zhang, X.-G.; Harris, C. R.; Kuduk, S. K.; Balog, A.; Savin, K. A.; Bertino, J. R.; Danishefsky, S. J. *Proc. Natl. Acad. Sci.* **1998**, *95*, 15798.
10. Structures discussed in this study were modeled using standard bond lengths and angles, using the molecular modeling software SYBYL® 6.41, Tripos Inc., 1699 South Hanley Road, Saint-Louis, Missouri 63144, USA, running on an Indigo^2 R4400 Extreme Silicon Graphics workstation. Geometries and energies were obtained by AM1 semi-empirical calculations (Dewar M. J. S.; Zoebish E. G.; Healy, E. F.; Stewart, J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902–3909) with the Gaussian92® package (*Gaussian 92/DFT*, *Revision F.3*; Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Gill, P. M. W.; Johnson, B. G.; Wong, M. W.; Foresman, J. B.; Robb, M. A.; Head-Gordon, M.; Replogle, E. S.; Gomperts, R.; Andres, J. L.; Raghavachari, K.; Binkley, J. S.; Gonzalez, C.; Martin, R. L.; Fox, D. J.; Defrees, D. J.; Baker, J.; Stewart, J. J. P.; Pople, J. A. Gaussian, Inc.: Pittsburgh, PA, 1993).
11. All new compounds have been characterized by ^{1}H, ^{13}C NMR and HRMS. Selected data: Compound **4**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.18 (s, 3H), 1.21 (d, 3H, $J=6.8$ Hz), 1.25 (s, 3H), 1.95–2.04 (m, 5H), 2.50–2.65 (m, 3H), 2.71 (s, 3H), 3.24 (ddd, 1H, $J=1.8$, 4.5, 12.9 Hz), 3.36 (sl, 1H), 3.79 (sl, 1H), 3.99 (quint., 1H, $J=6.8$ Hz), 4.10 (s, 1H), 5.09 (dd, 1H, $J=1.7$, 10.1 Hz), 5.13 (dd, 1H, $J=1.7$, 17.0 Hz), 5.37 (t, 1H, $J=6.4$ Hz), 5.74 (ddt, 1H, $J=6.9$, 10.1, 17.0 Hz), 6.56 (s, 1H), 6.98 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.08, 19.12, 19.68, 20.67, 24.27, 26.34, 37.69, 42.20, 50.14, 50.90, 67.54, 77.97, 78.64, 116.41, 118.13, 120.89, 133.15, 136.84, 152.02, 164.99, 173.39, 216.97; MS (m/z) (CI): MS (m/z) 422.1999 $[M+H]^+$. Compound **16**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.13 (s, 3H), 1.15 (s, 3H), 1.25 (s, 3H), 1.60–1.68 (m, 1H), 2.10 (s, 3H), 2.40–2.59 (m, 3H), 2.71 (s, 3H), 2.85–2.95 (m, H),3.53 (d, 1H, $J=3.5$ Hz), 3.85–3.98 (m, 2H), 5.10 (dd, 1H, $J=1.7$, 10.1 Hz), 5.15 (dd, 1H, $J=1.7$, 17.2 Hz), 5.43 (t, 1H, $J=6.5$ Hz), 5.73 (ddt, 1H, $J=6.9$, 10.1, 17.2 Hz), 6.55 (s, 1H), 6.99 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.61, 19.31, 19.85, 21.83, 22.95, 24.33, 37.64, 42.28, 49.80, 50.89, 68.10, 77.98, 79.08, 116.29, 117.83, 123.12, 133.45, 136.81, 152.44, 164.70, 171.60, 219.20; MS (m/z) (CI): 346 $[M+H]^+$, MS (m/z) 422.2005.
12. Mander, L. N.; Sethi, S. P. *Tetrahedron Lett.* **1983**, *24*, 5425.
13. (a) Quesnel, Y.; Toupet, L.; Duhamel, L.; Duhamel, P.; Poirier, J.-M. *Tetrahedron: Asymmetry* **1999**, *10*, 1015 and references cited therein; (b) Yang, W.; Digits, C. A.; Hatada, M.; Narula, S.; Rozamus, L. W.; Huestis, C. M.; Wong, J.; Dalgarno, D.; Holt, D. A. *Org. Lett.* **1999**, *1*, 2033.
14. Eliel, E. L.; Wilen, S. H.; Mander, L. N. *Stereochemistry of Organic Compounds*; John Wiley: New York, 1994; pp. 395–405.
15. (a) Dubois, J.-E.; Dubois, M. *Bull. Soc. Chim.* **1969**, 3120; (b) Gesson, J.-P.; Renoux, B.; Schulten, E. *Tetrahedron Lett.* **1997**, *38*, 5811.
16. Rodier, S.; Le Huérou, Y.; Renoux, B.; Doyon, J.; Renard, P.; Pierre, A.; Gesson, J.-P.; Greé, R. *Anti-Cancer Drug Des.* **2001**, *16*, 109–117.