Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 24, pp. 2963-2966, 1996

0960-894X/96 $15.00 + 0.00

PII: S0960-894X(96)00555-0

A MODEL FOR THE TAXOL (PACLITAXEL)/ EPOTHILONE PHARMACOPHORE§

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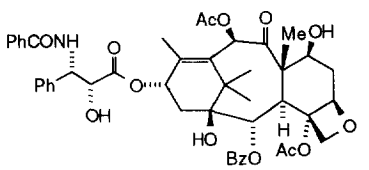
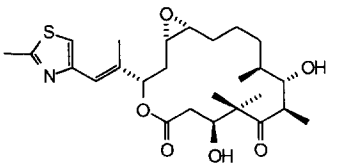
Abstract: Epothilone is a recently discovered compound that appears to bind at the same microtubule-binding site as the anticancer agent taxol. A model for the pharmacophore common to these structurally dissimilar compounds is presented.

The discovery of taxol (paclitaxel) **1** (Figure 1), with its unusually broad spectrum of potent antileukemic and tumor-inhibiting activity, has been an important breakthrough in cancer chemotherapy.[1] Its remarkable clinical efficacy against breast and ovarian cancer, coupled with its entirely novel mechanism of action,[2] have resulted in a prodigious effort directed towards both semi- and total synthesis of **1**,[3] which have recently culminated in the first three reported total syntheses of taxol.[4] While each of these efforts represent important milestones in organic synthesis, none of them would appear to materially contribute to the supply of taxol, based on the number of steps (ca 45-50) and the overall yields (0.0015-0.8%) of each of the respective total syntheses. The search for more efficient approaches to the synthesis of taxol and the discovery of other more readily accessible agents that operate by a similar mechanism of action continues unabated.[5]

Figure 1



1



2

At the time of its discovery, taxol was the only compound known to promote the formation of microtubules and to interfere with their disassembly by binding to β-tubulin.[6] In this way, the action of taxanes is distinct from that of the vinca alkaloids,[7] although the ultimate consequence of drugs in both families is interference with the mitotic spindle apparatus and the inhibition of mitosis. Recently, a Merck group found that another naturally occurring compound, epothilone (**2**), appears to bind to the same microtubule binding site

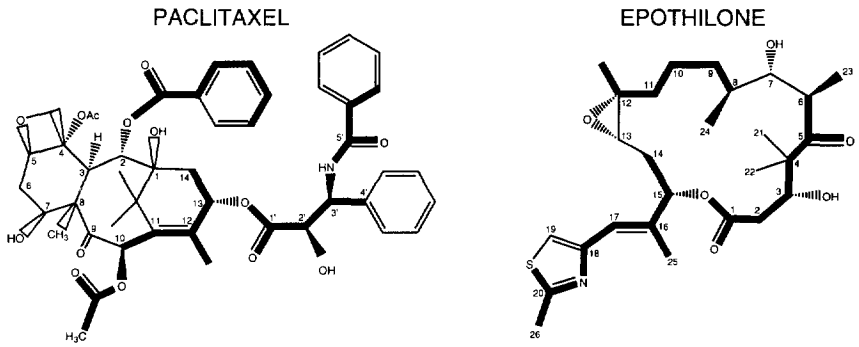
§ Dedicated to our colleague and friend Professor Madeleine M. Joullie in celebration of forty years of distinguished teaching and research at the University of Pennsylvania.

as taxol.[8,9] Competitive binding studies indicate that both compounds are complementary to the same binding site. This suggests that taxol (paclitaxel) and epothilone[10,11] share a common pharmacophore, although superficial comparison of the structures of taxol and epothilone reveal no obvious structural relationship. We describe herein the development of a model for the pharmacophore common to these structurally dissimilar substances, which could be of considerable importance in elucidating the mechanism of action of both substances, and in the design of novel chemotherapeutics that operate in the same manner.

While it is difficult to establish a common pharmacophore with any certainty, due to the large amount of conformational flexibility in both the taxol side chain and in epothilone, we base our overlap of the structures of **1** and **2** on (1) the SAR data that is available for taxol, indicating that the C-13 sidechain (and the C2' and C3' sidechain stereochemistry), as well as the C-2 benzoate and the C-4/C-5 hydroxyoxetane moiety are all necessary for biological activity[2] and (2) our observation that the absolute stereochemistry of the C-13 carbinol of the ester side chain and the C-15 carbinol of the lactone bond of epothilone share the same absolute stereochemistry.

From this starting point, we have used molecular mechanics software to search for a common pharmacophore in taxol/epothilone.[12] In the absence of structural information about the drug binding site, the computational problem is a search for regions of steric and functional similarity in the conformational space of two flexible molecules. We found that it is possible to superimpose thirteen of the fifteen ring atoms of epothilone and most of the side chain atoms in epothilone onto corresponding atoms in taxol-- all with the proper stereochemistry. We have reduced this 3-D model to a schematic 2-D representation, as outlined in Figure 2, using thick lines to represent the bonds in both molecules which superimpose.

Figure 2

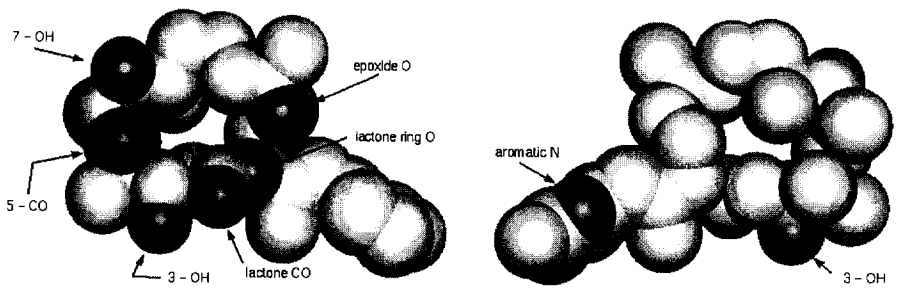


This model leads to several experimentally testable hypotheses. First, it is known that the C-10 acetate of taxol is not essential for activity because this group is lacking in the active congener, taxotere (docetaxel). Therefore, it predicts that epothilone should be active after removal of its corresponding group, i.e., its aromatic ring. Second, features such as the C3-OH, the C7-OH and the geminal dimethyl group should not be essential for activity since they do not correspond to similar groups in taxol. We suggest that the hydroxyoxetane moiety of taxol, which is critical for biological activity but not included in the proposed pharmacophore, may be

operating at a recognition site distant from the one associated with this model. Third, space-filling models of epothilone in this configuration (Figure 3) indicate that one aspect of the molecule is distinctly hydrophobic, and the other is highly polar. X-ray crystallographic studies indicate that the interface between proteins and ligands tend to be hydrophobic.[13] Therefore, it should be possible to add polar substituents to the hydrophilic aspect of epothilone without interfering with the protein-ligand interface.

There are other plausible configurations of the two compounds that can be superimposed, and steric complementarity between taxol and epothilone in this model is not ideal. The latter is a consequence of using an optimization stategy that emphasizes bond geometry rather than molecular shape or chemical properties. Experimental testing of the proposed pharmacophore as outlined above is currently underway and our results will be reported in due course.

Figure 3



EPOTHILONE – Hydrophilic aspect EPOTHILONE – Hydrophobic aspect

Acknowledgments. We warmly thank Professor G. Hofle (Gesellschaft fur Biotechnologische Forschung, Braunschweig, Germany) for supplying the X-ray coordinates of epothilone prior to publication, and Professors David Myles (University of California, Los Angeles) and Gunda Georg (University of Kansas) for valuable discussions. Support from the NIH [CA40250 to J. D. W. and GM50805 and HL47469 to P. H. A.] and a grant-in-aid from the American Heart Association of Southeastern Pennsylvania (P. H. A.) is gratefully acknowledged.

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(*Received in USA* 31 *October* 1996; *accepted* 8 *November* 1996)