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Docking Study of Ligands into the Colchicine Binding Site of Tubulin

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Cancer is a major cause of mortality in developed countries, following only cardiovascular diseases. Death of cancerous cells can be achieved by stopping mitosis and the antimitotic class of drugs formed by the spindle poisons can be used for this purpose. Their role is to disorganize the mitotic spindle by targeting its main constituent, the microtubules, themselves made of heterodimers of α and β-tubulin. They disrupt the dynamics of the microtubules either by stabilizing them, as do paclitaxel or epothilones, or destabilizing them, as do colchicine. The binding site of colchicine seems to lie between the two units of the tubulin dimer. Here, we report on the characterization of this site by the docking of a series of reference compounds, and the subsequent docking of ligands prepared in our laboratory.

Keywords: Cancer; Antimitotic agents; Tubulin; Colchicine; Binding site; Docking

INTRODUCTION

Cancer is the second major cause of mortality in developed countries, responsible for nearly a third of the deceases in the United States in 2000.[1] It is characterized by a failure of the regulation of cell replication. A mutation-altered cell begins to divide anarchically. After some more mutations, its progeny can become truly cancerous cells, set apart from normal cells by three factors. First, they lose their normal functions; in the meantime, they divide more rapidly than non-cancerous cells and last but not least, they are no longer subject to apoptosis. On the other hand, they form tumors and can invade nearby tissues or tissues further away to create metastases.

An improved knowledge of the cell cycle has offered new insights into the replication mechanisms. This has led to specifically target the mitotic spindle, which is a major part of cell division, in order to develop new anticancerous agents. During mitosis, the microtubules forming the mitotic spindle extend from the centrosomes of the cell and fasten to the kinetochore of the chromosomes, thus permitting their gathering on the equatorial plan of the cell by a so-called tread-milling movement of the microtubules, which are lengthening at their extremity linked to the chromosomes, called + extremity, and shortening at the other end, namely the – extremity. The chromatides are then attracted to one of the centrosomes by the shortening of the microtubules at their + end. The mitotic spindle is thus of an outmost importance in cell replication, as it is involved in positioning and moving the chromosomes, practically dividing evenly the DNA between the two daughter cells. The mitotic spindle can be stabilized[2] in its polymerized state by paclitaxel or the epothilones or, on the contrary, destabilized[3] by colchicine. In both cases, disorganization of the dynamics of the microtubules results in stopping the mitosis, ultimately leading the cells to apoptosis.[4]

From a molecular point of view, microtubules are an assembly of protofilaments,[5] each made up of the axial association of heterodimers of α and β-tubulin. These dimers are arranged in the microtubules so that the + extremity is always formed by β-tubulin, the – end being of course made by α-tubulin. The structure of the heterodimer is well known owing to crystallographic studies.[6–8] α-Tubulin bears a binding site, named M, for GTP. β-tubulin has also a site, called E, binding GDP in the inactive

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state and exchanging it for GTP to activate.[9] The E site seems to have a role to play in the dynamics of the microtubules.[10]

The crystallographic data available include the binding site of paclitaxel and docetaxel, which block a conformation of the M loop involved in protofilaments association, thus stabilizing the polymerized state of the microtubule. Unfortunately, the binding site of colchicine is only known approximately. It would bind to sequences 1–36 and 216–243 of β-tubulin.[11] Moreover it would also sit its A ring (Figure 1) near Cys356 of the β monomer.[12] On the other hand, evidence of interaction with α-tubulin has been found.[13] Its binding is irreversible and appears to occur in two steps, producing many conformational changes of the heterodimer.[14] This binding site is common to structures such as the combretastatins and sulfonamides E7010 or ER34410 (Figure 1).[15,16]

Molecular modelling studies have given two putative binding sites for colchicine (Figure 2).[17,18] The first (site A) is delineated by Val23, Asn26, Tyr36, His229, Ala233, Phe244 and Phe272 of β-tubulin; however, it is included in the taxol binding site. The second (site B) is lying at the interface between the two monomers, with Arg2 and Tyr36 of β-tubulin and Thr73 and Asn76 of α-tubulin limiting it.

Our goal was to derive some rationales on the binding from a series of ligands prepared in our laboratory, which bind to the colchicine site.

MATERIALS AND METHODS

Biological Activity Determination

The cytotoxic activity of the compounds was determined by testing them on a L1210 cell line to provide their IC_{50} values. The cells were cultured on RPMI 1640 media with the addition of 10% of decompleted foetal serum, 2 mM of L-glutamine, 100 U/mL of penicillin, 100 μg/mL of streptomycin and 10 mM of Hepes buffer (pH = 7.4). Groups of 1500 cells were then exposed to various concentrations of the compounds to be tested and incubated for 48 h (4 cycles) at 37°C, in a 5% CO_2 – 95% air atmosphere. The activity was assessed by measuring the activity of the succinate dehydrogenase of the living cells transforming the yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide into crystals of blue colored formazan.

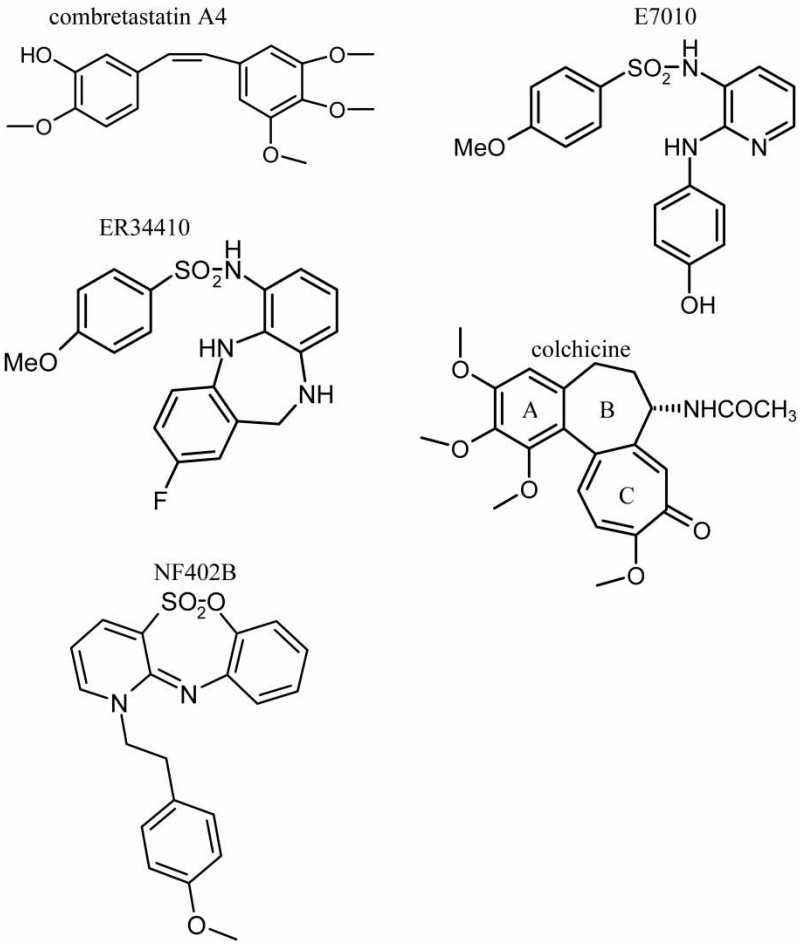

FIGURE 1 Structure of the reference ligands.

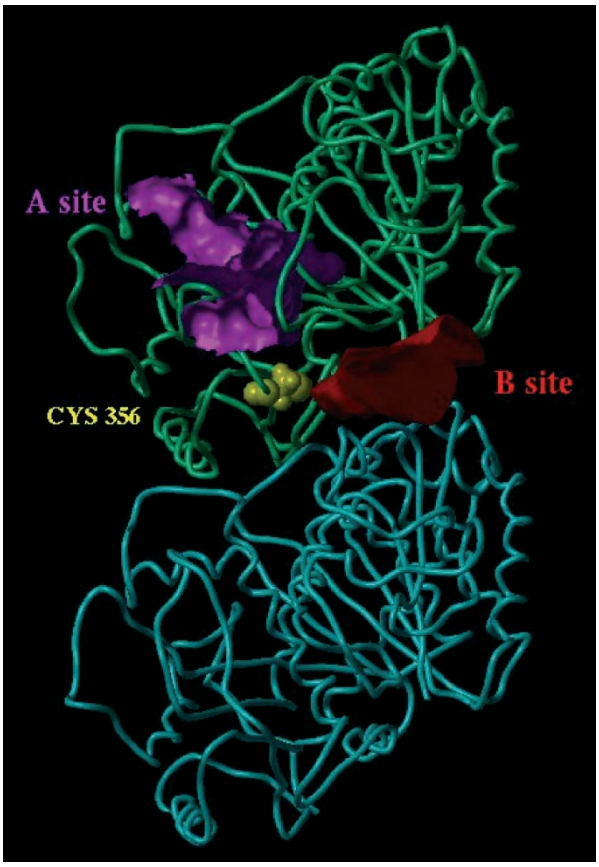

FIGURE 2 Putative A and B binding sites of colchicine (α-tubulin is figured by a blue ribbon and β-tubulin by a green ribbon).

These crystals were dissolved and the optical density determined, yielding the proportion of live cells.

Calculation Methods

All the calculations were conducted on a Silicon Graphics Octane 2 workstation, using the molecular modelling software Sybyl 6.9.[19] Unless otherwise noted, docking of the compounds was made using Gold 2.0.[20] All the minimizations have been carried out using the Maximin2 module of Sybyl after calculation of the Gasteiger-Hückel charges, to a gradient of 0.05 kcal/mol Å. The Tripos force field and a dielectric constant of 4 were used.

RESULTS AND DISCUSSION

Binding Site Research

The first step in our work has been to characterize with more precision the binding site of colchicine. Crystallographic data retrieved from the Protein Data Bank (entry 1JFF)[8] contain a dimer of α and β-tubulin in complex with paclitaxel. Therefore, after removing this ligand, to ensure that the structure of the dimer had not been altered by paclitaxel, we minimized the whole molecule. The colchicine was docked manually in each of the putative sites described in the literature and the complexes were again minimized.

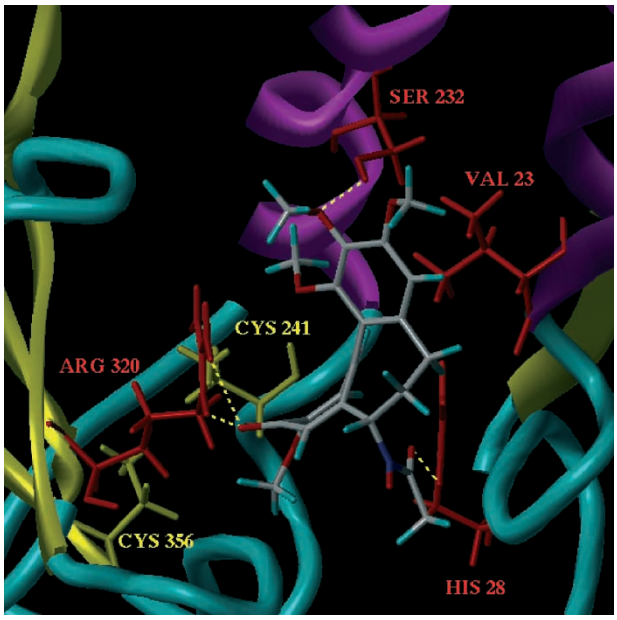

FIGURE 3 Docking of the colchicine in the A site.

In the A site, hydrogen bonds occur between colchicine and His28, Ser232 and Arg320 of β-tubulin. Moreover, Cys356β is fairly accessible (Figure 3), but colchicine shows its C ring close to it, instead of the A ring as previously suggested by experimental results.

In the B site, colchicine links to Thr33 and Tyr36 of β-tubulin and Asp76 of α-tubulin (Figure 4). In this site, Cys356β is slightly closer to the A ring of colchicine but the two are separated by a loop of β-tubulin.

Binding Site Validation

In order to assess the validity of the potential binding sites of colchicine, a selection of reference ligands have been docked in both of them (Figure 1). We have chosen to employ the combretastatin A4, the sulfonamides E7010, ER34410 and the oxathiazepine NF402B, the most potent compound prepared in our laboratory.[21,22]

A Site

The docking of combretastin A4 did not produced meaningful results. This ligand is placed mostly outside the binding site. E7010 can be placed in the cavity, but forms only one hydrogen bond with

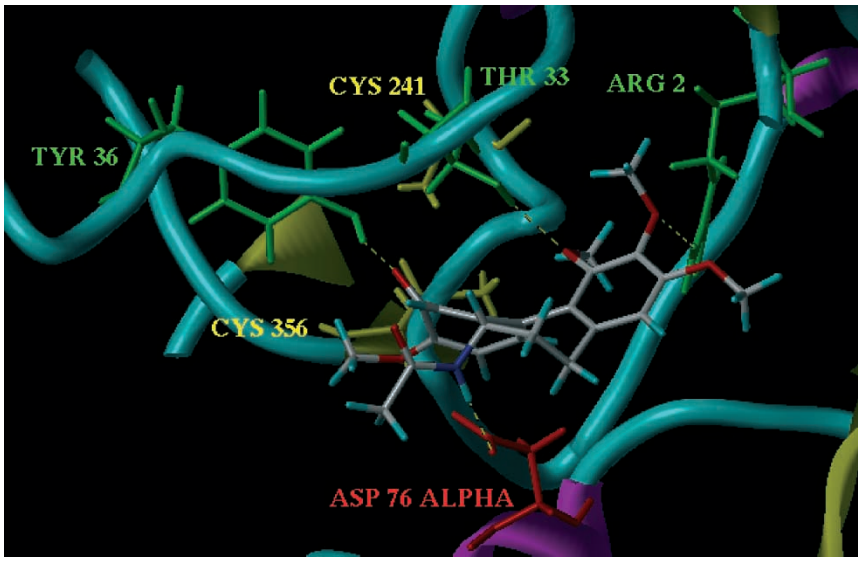

FIGURE 4 Docking of the colchicine in the B site.

Ser236 of β-tubulin. Lastly, neither ER34410 nor NF402B form hydrogen bonds with tubulin.

B Site

In this site, combretastatin A4 forms bonds with Thr80 of α-tubulin and Arg2 and Gly34 of β-tubulin. E7010 is placed in the site in a conformation that permits hydrogen bonding with Arg2, Thr33 and Asp130 of β-tubulin (Figure 5). In the same vein, the results achieved for ER34410 show interactions with Arg2β, Thr33β and Arg79α (Figure 6). NF402B gives equivalent results, as it links to Arg2 and His37 (Figure 7) of the β-tubulin.

A comparison between the results of the docking of reference ligands in the two sites clearly indicates that interactions would be in favour of a preferential binding to the B site, sitting at the interface of α and β-tubulin. Indeed, none of the chosen reference molecules could be placed correctly in the A site, as appears from the scarce hydrogen bonds, whereas all have several interactions with aminoacids when docked in the B site.

This is further confirmed by the superposition of the docked ligands. The aromatic moieties of the molecules are all superposed in the areas of the A and C rings of colchicine suggesting a possible pharmacophore based on the conserved position of the aromatic rings (Figure 8).

Considering the greater coherence of the results achieved at the level of the interfacial site, we have carried on our work exclusively on the B site.

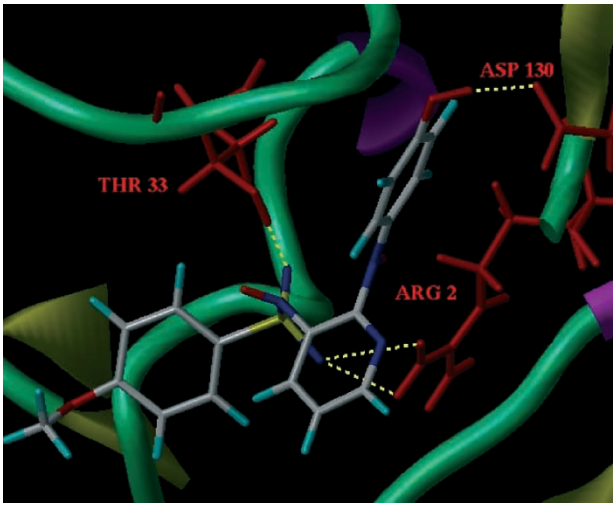

FIGURE 5 Interactions with E7010.

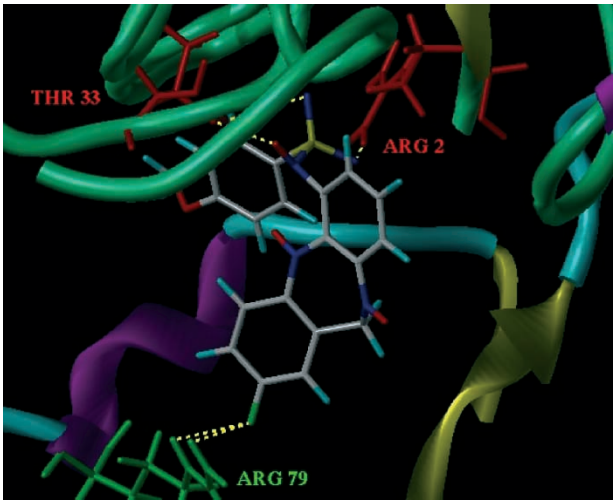

FIGURE 6 Interactions with ER34410.

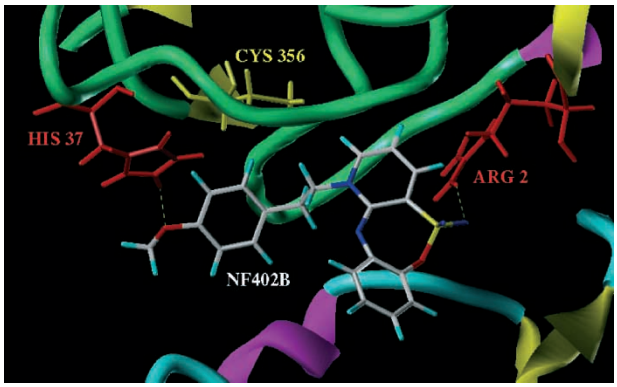

FIGURE 7 Interactions with NF402B.

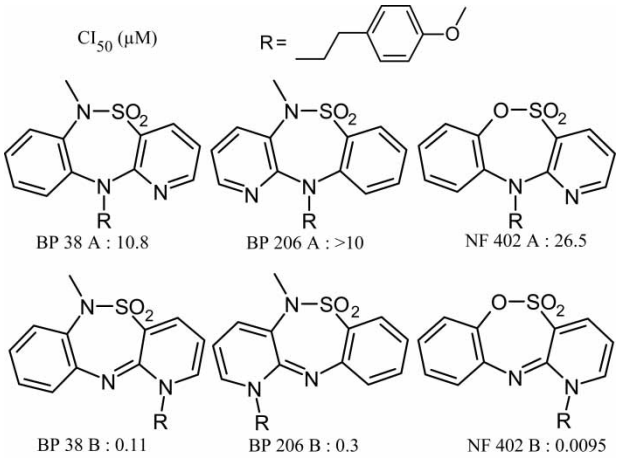

FIGURE 9 Structure and cytotoxic activity (L1210, IC_{50}: μM) of the benzopyridothiadiazepines and oxathiazepines.

Docking of Studied Compounds

Studied compounds can be divided into three series based on a structural point of view (Figure 9): the tricyclic head can be a benzo[*c*]pyrido[*f*]thiadiazepine, a benzo[*f*]pyrido[*c*]thiadiazepine or an oxathiazepine. Each series can be further split into two subseries (A and B), depending on the position of the side chain on the tricycle. Our goal is to achieve the potential bioactive conformation of the various prepared series, in an attempt to derive predictive QSAR models. Thus the molecule of each series showing the highest antiproliferative activity expressed in term of IC_{50}, has been docked.

As described previously, NF402B interacts with Arg2 and His37 of β-tubulin (Figure 7). NF402A adopts a conformation characterized by a position of its aromatic groups nearly perpendicular to those of its regioisomer. Being placed in the binding site closer to the Arg2, it loses the interaction with the His37β, which is replaced by a link with the Thr80 of the α-tubulin (Figure 10). However, its activity is 2500-fold lower than that of its regioisomer, hinting that linking with both the α and β-tubulin could contribute to a sharply decreased activity. Moreover, the 4-methoxyphenethyl side chain sits deeper in the binding site when borne by the pyridinyl nitrogen, thus occupying more fully the pocket and probably contributing to a higher activity.

The second most active molecule is BP38B, which adopts a conformation very close to that of NF402B (RMS = 0.369 Å). The higher steric hindrance due to the methyl group branched on its sulfonamide function results in the loss of its link with Arg2 (Figure 11). Even if the interaction with His37 is conserved, it is 10-fold less active than NF402B. This suggests a certain importance of the hydrogen bond with the Arg2 for good antiproliferative activity. The importance of a link with His37 is further demonstrated by BP38A (Figure 12). This compound is 100-fold less potent than BP38B and does not interact with either Arg2 or His37, but forms links with Thr33 and Tyr36 of the β-tubulin, thus confirming the role of His37.

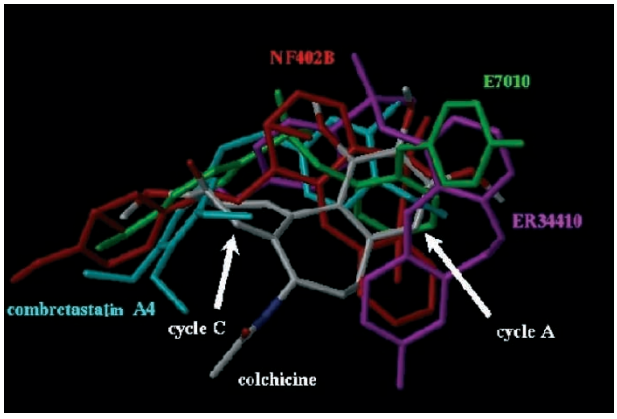

FIGURE 8 Superposition of the reference ligands docked into the B site.

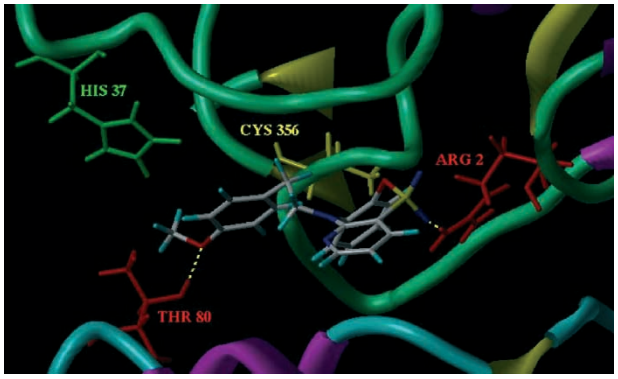

FIGURE 10 Interactions with NF402A.

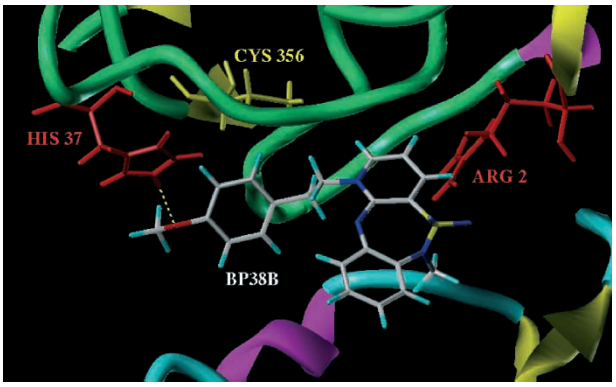

FIGURE 11 Interactions with BP38B.

The last compound BP206B is 30-fold less active than NF402B. Once again, this compound suffers from steric hindrance due to the methyl borne by the sulfonamide function. However, the hydrogen bond with the Arg2 is maintained and the result of the hindrance is the loss of interaction with His37, replaced by an interaction with Thr80 of the α-tubulin (Figure 13); however, its IC_{50} remains at a micromolar level, even if its conformation is largely different from that of NF402B. This confirms two hypotheses. The first is the importance of a bond with Arg2 for good activity. The second is the importance of the position of the side chain on the tricycle, as this compound forms the same interactions as NF402A but is nonetheless 100-fold more potent. The B subseries, bearing the side chain on the nitrogen of an outer ring, shows a better activity than its A counterpart, for which the side chain is placed on the nitrogen of the central ring. This difference in activity could be explained by better occupation of the binding site by the 4-methoxyphenethyl substituent, as described for the NF402 regioisomers. On the contrary, BP206A seems to lose all the aforementioned interactions; its IC_{50} has not been determined exactly and it cannot be compared with the other molecules.

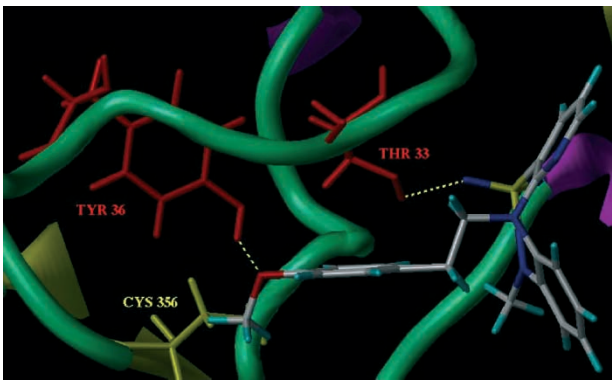

FIGURE 12 Interactions with BP38A.

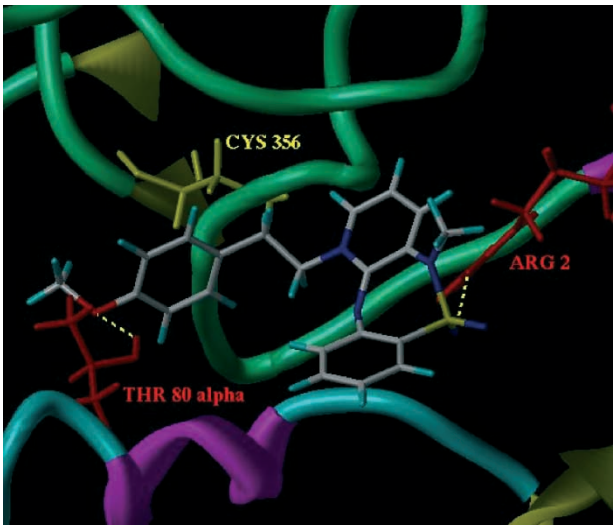

FIGURE 13 Interactions with BP206B.

CONCLUSION

In conclusion, the docking of colchicine in the two putative binding sites known in the literature has been carried out to fully characterize the more probable one. We have used for this purpose a set of reference ligands to validate each of the hypothetical sites. The results achieved by such docking have confirmed the validity of the binding site lying at the interface between the α and β-tubulin but have also invalidated the other site, which coincides with the binding site of paclitaxel. This characterization of the binding site of colchicine has permitted a docking study of a series of highly potent ligands prepared in our laboratory. The reward for this work is two fold; on the one hand, we have determined the potentially bioactive conformation of each series of compounds; thus we are now able to generate QSAR models based on these conformations. On the other hand, we have uncovered the importance of Arg2 and His37 of the β-tubulin for a good antimitotic activity as well as explained the observed difference in potency between the A and B regioisomers by a difference in occupancy of the binding site. Mutagenesis studies on these amino acids could support this hypothesis.

References

[1] Samuels, A., Ghafoor, A., Cardinez, C., Cokkinides, V., Jemal, A., Harris, A. and Ward, E. (2003) Cancer statistics: a presentation from the American Cancer Society, http://www.cancer.org.
[2] Li, Q., Sham, H.L. and Rosenberg, S.H. (1999) *Annu. Rep. Med. Chem.*
[3] Hamel, E. (1998) *Med. Res. Rev.* **18**, 259–295.
[4] Wilson, L., Panda, D. and Jordan, M.A. (1999) *Cell Struct. Funct.* **24**, 329–335.
[5] Tilney, L.G., Bryan, J., Bush, D.J., Fujiwara, K., Mooseker, M.S., Murphy, D.B. and Snyder, D.H. (1973) *J. Cell Biol.* **59**, 267–275.

[6] Nogales, E., Wolf, S.G. and Downing, K.H. (1998) *Nature* **391**, 199–203.
[7] Gigant, B., Curmi, P.A., Martin-Barbey, C., Charbaut, E., Lachkar, S., Lebeau, L., Siavoshian, S., Sobel, A. and Knossow, M. (2000) *Cell* **102**, 809–816.
[8] Löwe, J., Li, H., Downing, K.H. and Nogales, E. (2001) *J. Mol. Biol.* **313**, 1045–1057.
[9] Michitson, T.J. (1993) *Science* **261**, 1044–1047.
[10] Downing, K.H. and Nogales, E. (1998) *Curr. Opin. Cell Biol.* **10**, 16–22.
[11] Uppuluri, S., Knipling, L., Sackett, D.L. and Wolff, J. (1993) *Proc. Natl Acad. Sci. USA* **90**, 11598–11602.
[12] Bai, R., Pei, X.F., Boye, O., Getahun, Z., Grover, S., Bekisz, J., Nguyen, N.Y., Brossi, A. and Hamel, E. (1996) *J. Biol. Chem.* **271**, 12639–12645.
[13] Chaudhuri, A.R., Seethramalu, P., Schwarz, P.M., Hauusheer, F.H. and Luduen, R.H. (2000) *J. Mol. Biol.* **303**, 679–692.
[14] Pyles, E.A. and Hastie, S.B. (1993) *Biochemistry* **32**, 13560–13565.
[15] Xia, Y., Yang, Z.Y., Xia, P., Bastow, K.F., Tachibana, Y., Kuo, S.C., Hamel, E., Hackl, T. and Lee, K.H. (1998) *J. Med. Chem.* **41**, 1155–1162.
[16] Yoshino, H., Veda, N., Niijima, J., Sugumi, H., Kotake, Y., Koyaganagi, N., Yoshimatsu, K., Asada, M., Watanabe, T., Nagasu, T., Tsukahasa, K., Iijima, A. and Kitoh, K. (1992) *J. Med. Chem.* **35**, 2496–2497.
[17] Bai, R., Covell, D.G., Pei, X.F., Ewell, J.B., Nguyen, N.Y., Brossi, A. and Hamel, E. (2000) *J. Biol. Chem.*, 40443–40452.
[18] Pal, D., Mahapatra, P., Manna, T., Chakrabarti, P., Bhattacharyya, B., Banerjee, A., Basu, G. and Roy, S. (2001) *Biochemistry* **40**, 15512–15519.
[19] SYBYL 6.9 – Molecular Modeling Software (2001) Tripos Inc., 1699 South Hanley Road, St. Louis, MO 63144-2913, U.S.A.
[20] Jones, G., Willett, P., Glen, R., Leach, A.R. and Taylor, R. (1997) *J. Biol. Chem.* **267**, 727.
[21] Gallet, S., Berthelot, P., Lebègue, N., Carato, P., Flouquet, N., Hickman, J., Pierre, A., Pfeiffer, B. and Renard, P. (2004) International patent PCT/FR04/00234.
[22] Gallet, S., Berthelot, P., Lebègue, N., Carato, P., Flouquet, N.,Hickman, J., Pierre, A., Pfeiffer, B. and Renard, P. (2004) International patent PCT/FR04/00235.