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Taxanes with high potency inducing tubulin assembly overcome tumoural cell resistances

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ABSTRACT

We have found that four taxanes with chemical modifications at positions C10 and C13 were active against all types of taxane resistant cell lines, resistant by P-gp overexpression, by mutations in the β-tubulin binding site or by overexpression of the highly dynamic βIII-tubulin isotype.

We have characterized the interaction of taxanes with high activity on chemotherapy resistant tumoural cells with microtubules, and also studied their cellular effects. The biochemical property enhanced in comparison with other taxanes is their potency at inducing tubulin assembly, despite the fact that their interactions with the microtubule binding sites (pore and luminal) are similar as studied by NMR and SAXS. A differential interaction with the S7–S9 loop (M-loop) is responsible for their enhanced assembly induction properties. The chemical changes in the structure also induce changes in the thermodynamic properties of the interaction, indicating a higher hydrophilicity and also explaining their properties on P-gp and βIII overexpressing cells and on mutant cells.

The effect of the compounds on the microtubular network is different from those observed with the classical (docetaxel and paclitaxel) taxanes, inducing different bundling in cells with microtubules being very short, indicating a very fast nucleation effect and reflecting their high assembly induction power.

1. Introduction

One of the main problems in the chemotherapeutic treatment of tumors is the development of resistance to the compounds used. Although many tumours show a favourable response in the early stages of treatment, after exposure to the chemotherapeutic agents, the tumour often develops resistance to the treatment. Antitumour agents targeting tubulin have a great efficacy in the treatment of solid (paclitaxel, docetaxel, ixabepilone) or blood (vinblastine, eribulin) cancers,[1–3] but, their highly systemic toxicity compromises the treatments based on these agents. Nevertheless, these compounds have also an advantage over the kinase inhibitors.[4,5] Since they block tubulin which is involved in several essential cell functions and is a major constitutive protein, there is no possible alternative pathway to substitute the functions of tubulin (the main reason for the development of resistance against kinase inhibitors[6,7]). Thus, resistance through alternative pathways is not possible in the case of these compounds.

Resistance to antitumoral agents targeting tubulin is possible through three different mechanisms:[8] (a) overexpression of membrane pumps, (b) mutations in the ligand binding site and (c) overexpression of tubulin isotypes less sensitive to the drug employed.

One of the membrane pumps usually involved in resistance to taxanes is P-glycoprotein (P-gp), a protein involved in detoxification mechanism. It is assumed that its major role is to restrict the entrance of toxics through the gastrointestinal trat or to prevent their access from blood stream to fetus and sensitive organs such as brain and testis.[9] In cells overexpressing P-gp, two opposite

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mechanisms control the intracellular concentration of drug, that is, P-gp decreases the internal concentration of a drug to reduce its effective cytotoxicity, but on the other hand, the target protein binds to a drug with high affinity to accumulate the drug inside the cell. The effectiveness of these resistance mechanisms depends on the relative affinity of a drug to P-gp and the target. An increased affinity to the target or a decreased affinity to P-gp will overcome P-gp-mediated resistance.[10]

The effects of variations in the affinity of taxanes on the resistance level of P-gp overexpressing cells have been previously studied in our group and reported.[10,11] These studies have shown that very high affinity taxanes (chitax-40) are able to overcome P-gp-mediated resistance. The same effect has been observed for other compounds which bind covalently to tubulin.[12–14]

Second-generation taxanes with modifications at C2, C3′N and C10 positions have also been shown to be extremely effective against cells resistant to taxanes by P-gp overexpression (Fig. 1).[15–17] Although there were previous studies on the relation of changes in the taxane structure with their biological activities,[18,19] measurements of the microtubule–taxane binding affinities have been performed only recently, which allows a rigorous examination of the relationship between binding affinity and cytotoxicity in drug-sensitive cells and drug-resistant cells overexpressing P-gp.[10,20,21] The study of the relationship between binding affinity and cytotoxicity (Fig. 5 of Matesanz et al. in 2008)[10] indicates that there are factors that modulate the cytotoxicity in cells although both variables are related, that is, there are compounds showing cytotoxicities on sensitive cells higher than expected from their binding affinity (paclitaxel, docetaxel, chitax-18), while some compounds exhibit higher cytotoxicity on resistant cells than those predicted from their binding affinity (chitax-19, chitax-21, chitax-35).

The second way that tumour cells resists paclitaxel and its derivatives is by changing their tubulin. Although it is difficult for human cells to mutate in response to a chemical agent, it is possible for them to use the genetic diversity of the tubulin isotypes to reduce their sensitivity to certain compounds. Most vertebrate isotypes fall into six categories, that is, βI, βII, βIII, βIV, βV and βVI,[22] with most of the differences in the clusters 124–129 and 237–240, as well as the C-terminal sequence. Although all 6 isotypes are able to carry out two of the three canonical functions of microtubules (forming the mitotic spindle and the interphase network),

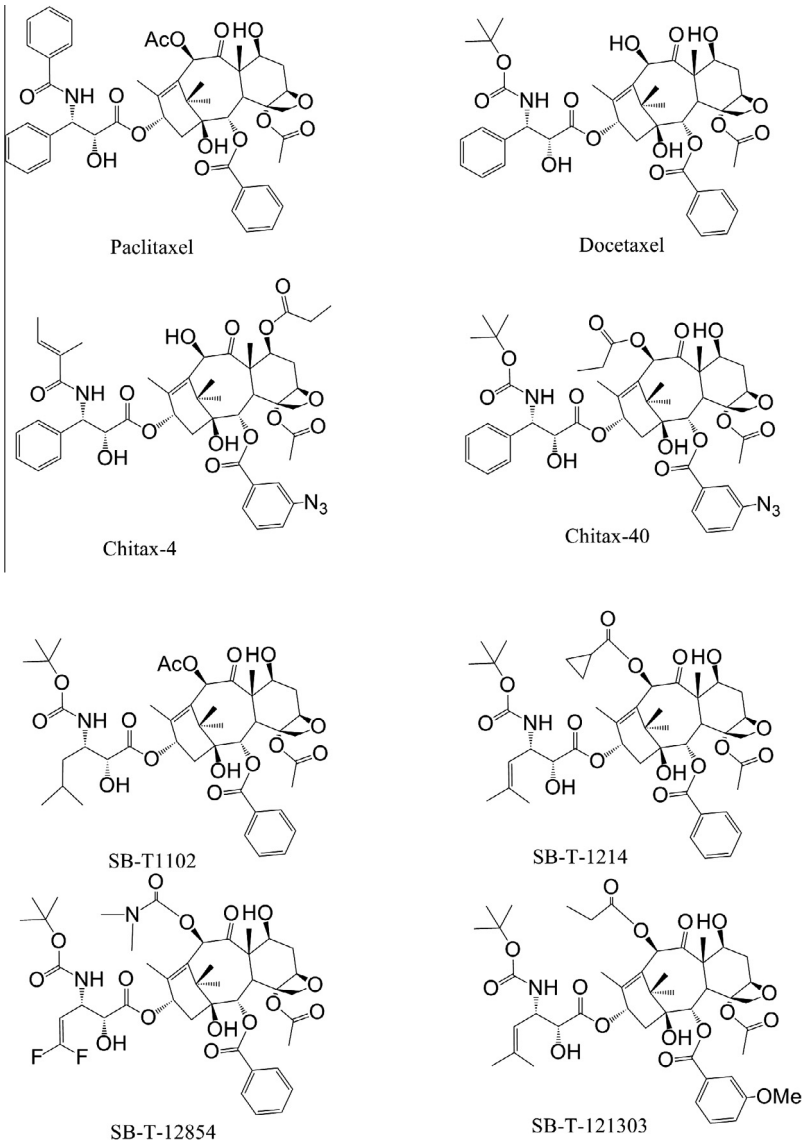

Figure 1. Chemical structures of the compounds employed in this study.