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CHARACTERIZATION OF THE TAXOL STRUCTURE-ACTIVITY PROFILE FOR THE LOCUS OF THE A-RING SIDE CHAIN

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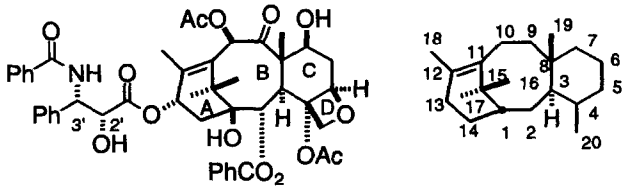
Abstract: Two series of taxol analogs with various *N*-acyl groups on 3'-phenylisoserine and isoserine A-ring side chains, respectively, were prepared, and their microtubule assembly activities and cytotoxicities toward J774.2 cells were evaluated. Biological activities were adversely affected by the structural modifications investigated, although the 3'-phenylisoserine series was significantly more active than the isoserine series.

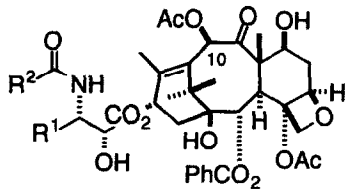
The clinically important[1] anti-tumor drug taxol[2] is a structurally novel diterpene that promotes the polymerization[3] of tubulin α,β-heterodimers by binding to[4] and stabilizing[5] the resulting microtubule polymer. It is thus unique among anti-mitotic drugs such as colchicine, podophyllotoxin, and the vinca alkaloids, which inhibit microtubule assembly.

The structure-activity profile of taxol has been the subject of considerable scrutiny.[6] Functionality and structural modification at C-7,[7,8] C-8,[9] C-9,[10] and C-10[8,11] have a relatively modest impact on biological activity, whereas the taxol C-2 benzoyloxy[12] and 3-oxetanyl acetate[13] substructures play crucial roles. One of the earliest observations regarding the taxol SAR was that the A-ring side chain is obligatory,[2a] and subsequent work demonstrated the 2'-OH to be crucial.[14] More recently, a number of taxol A-ring side chain analogs have been prepared and evaluated.[15] In general, these studies suggest the functionality and stereochemistry of the side chain to be reasonably well optimized with regard to biological activity. Herein we dis-



TAXOL

Table 1. Biological Activities of GTP, Taxol, Taxotere, and Taxol Analogs.

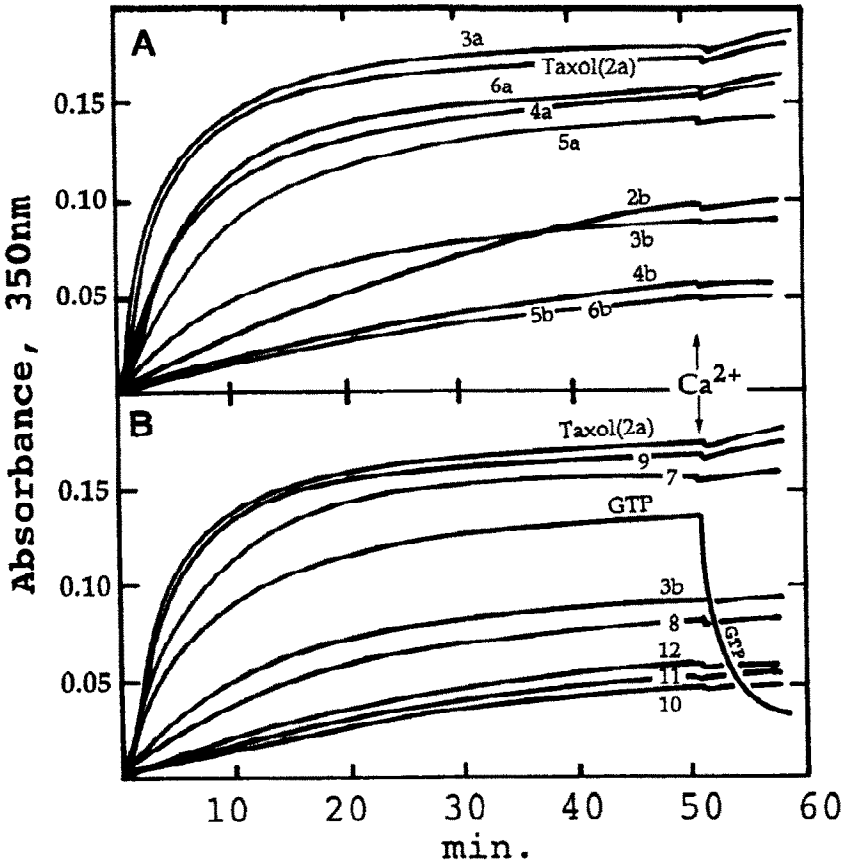


		R^1	R^2	Relative Initial Slope	Assembly (%)	ED_{50}[a] J774.2 (μM)
GTP	**1**			65	70	
Taxol	**2a**	Ph	Ph	100	100	0.09
	2b	H	Ph	6	60	20
Taxotere (10-OH)	**3a**	Ph	*t*-BuO	110	105	0.05
	3b	H	*t*-BuO	22	51	2
	4a	Ph	*p*-N_3-Ph	84	90	0.28
	4b	H	*p*-N_3-Ph	3	25	20
	5a	Ph	*p*-CF_3-Ph	50	80	0.90
	5b	H	*p*-CF_3-Ph	4	20	23
	6a	Ph	$PhCH_2O$	85	94	0.19
	6b	H	$PhCH_2O$	3	20	16
	7	Ph	*p*-CH_3CO-Ph	44	84	1.6
	8	Ph	*p*-PhCO-Ph	15	39	1.5
	9	Ph	*o*-OH-Ph	96	93	0.56
	10	H	1-adamantyl	3	24	11
	11	H	*t*-Bu	6	28	40
	12	H	cyclohexyl	8	33	16
	13	H	*n*-pentyl	11	34	26
	14	H	2-naphthyl	6	28	26

[a]ED_{50} = drug concentration that inhibits cell division by 50% after 72 h incubation.

close the results of a structure-activity study involving two series of taxol analogs with various *N*-acyl groups on 3'-phenylisoserine and isoserine A-ring side chains, respectively. While previous conclusions regarding the effect on biological activity of structural modification in the taxol A-ring side chain are essentially confirmed, several new trends are discerned, as well.

Figure 1. The assembly of MTP (1.5 mg/mL) in the presence of GTP (**1**, 1 mM), taxol (**2a**), or taxol analogs (20 μM). The assembly reactions were followed turbidimetrically at 350 nm. At the time denoted, 4 mM $CaCl_2$ was added to each experimental sample. (A) Microtubule assembly in the presence of 3'-phenylisoserine analogs **2a** - **6a** or their 3'-Ph-deleted isoserine analog counterparts **2b** - **6b**. (B) Microtubule assembly in the presence of selected 3'-phenylisoserine and isoserine analogs with variable 3'-*N*-acyl groups.



The substances evaluated in this study are indicated in Table 1. The 3'-phenylisoserine side chain analogs **4a**, **5a**, **6a**, and **7** - **9** were prepared by *N*-acylation of the corresponding amine[16] (analogs **4a**,[17] **5a**,[15c] and **8**[17] have been reported previously). New isoserine analogs **3b**, **4b**, **5b**, **6b**, and **10** - **14** were prepared similarly (analog **2b**[15b] has been reported previously). The biological activities for GTP, taxol, Taxotere,®,[15a] **2b**,[15b] **4a**,[17] **5a**,[15c] and **8**[17] are included for comparison.

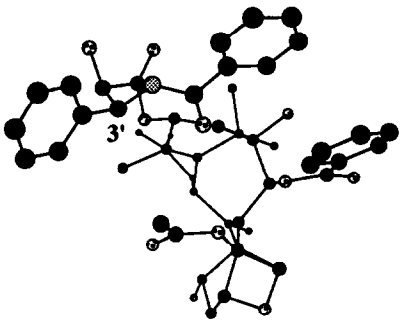
Drugs were dissolved in DMSO for biological evaluation. The maximum final concentration of DMSO in experimental samples in vitro was 1%, and in cell culture 0.2%. Calf brain microtubule protein (MTP) was purified by two cycles of temperature dependent assembly-disassembly through a procedure modified from Shelanski, *et al.*,[18] which has been described.[19] Microtubule assembly (assembly buffer: 0.1 M

MES, 1 mM EGTA, 0.5 mM $MgCl_2$, and 3 M glycerol, pH = 6.6) in the presence and absence of GTP (1 mM) and drugs (20 μM) was determined spectrophotometrically at 35 °C by monitoring changes in turbidity (representative of polymer mass[20]) at 350 nm. The content and amount of drug bound to microtubule polymers were inferred from the drug-microtubule pellet (centrifuged through a 50% sucrose cushion with a Beckmann 50 rotor at 45 000 rpm for 1 h) using HPLC analysis (see ref. 18 for details). Samples for electron microscopy were placed on carbon-on-Parlodion-coated grids, negatively stained with 2% uranyl acetate, and analyzed on a Philips 300 electron microscope. The murine macrophage-like cell line J774.2 was employed for the cytotoxicity studies.[19]

Taxol and the analogs listed in Table 1 were tested for their ability to promote tubulin assembly in the absence of GTP. 1.5 mg/mL MTP was assembled in the presence of the respective substances listed in Table 1, and the assembly processes characterized by their initial slopes and extents of final steady-state assembly relative to taxol (see Table 1 and Figure 1). Microtubule polymers formed in the presence of the analogs are stable against the depolymerizing effect of 4 mM $CaCl_2$, as are the polymers formed in the presence of taxol. Negatively stained electron micrographs of the steady-state polymers (not shown) demonstrated the existence of normal microtubules. In experiments involving potent assembly promoting analogs (**2a**, **3a**, **4a**, **5a**, **6a**, **7**, and **9**), an additional fraction of the polymers consists of non-microtubule forms such as hoops and ribbons.[4] Competition studies were performed using tritiated taxol and various concentrations of each analog. The microtubule polymers formed were centrifuged through a 50% sucrose cushion and the pellet was resuspended in water. Protein and taxol concentrations were measured using the Lowry[21] procedure and scintillation counting, respectively. All taxol analogs were observed to bind to microtubules competitively with taxol (data not shown). The effect of the taxol analogs on cell replication was examined and the ED_{50} values for the J774.2 cell line are presented in Table 1. The results indicate a correlation between cytotoxicity and ability to promote microtubule assembly.

Several trends emerge from a consideration of the data summarized in Table 1. First, the isoserine analogs with deleted 3'-Ph groups are consistently less active in the microtubule assembly assay and less cytotoxic than the 3'-phenylisoserine analogs that are closer in structure to taxol. Second, the varied substituents on the *N*-benzoyl phenyl group in the 3'-phenylisoserine analogs lead to reduced activity. Large para substituents (as in **7** and **8**) are the worst in this regard, whereas analog **9** with an *o*-OH group is one of the two 3'-phenylisoserine analogs most active in the microtubule assembly assay. The only side chain structural variation investigated in this study that improves activity is the well-known exchange of the taxol *N*-benzoyl group for the BOC group in Taxotere. Evidently, carbamate groups at this position are particularly favorable as CBZ analog **6a** is also quite active in the microtubule assembly assay and is quite cytotoxic. It is noteworthy that, among the simple isoserine analogs lacking 3'-Ph groups, BOC-modified **3b** is one of the two most active compounds investigated, along with taxol relative **2b**. However, mimicking the effect of the bulky *t*-BuO group in **3b** with the bulky amide alkyl groups in analogs **10** - **12** fails to raise their activities to the level exhibited by **3b**.

The examination of the five pairs of analogs with and without the 3'-Ph group (**2** - **6**) allows an assessment of cooperativity between this group and substitution on the *N*-acyl group. The conclusion is that the 3'-Ph group reduces the variation of biological activity with changes in *N*-acyl group structure. Among the



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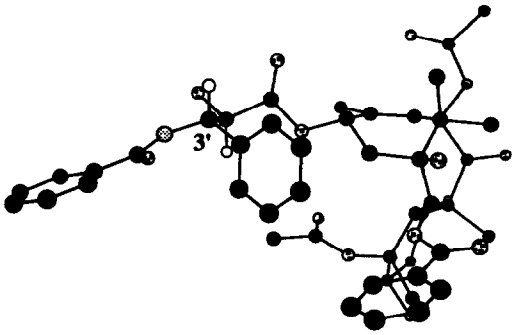

16

3'-phenylisoserine analogs (**2a**, **3a**, **4a**, **5a**, and **6a**), there is a two-fold variation of the initial rates of microtubule assembly, and a smaller variation in the steady state levels of microtubule assembly. In contrast, the analogous isoserine series (**2b**, **3b**, **4b**, **5b**, and **6b**) exhibits a seven-fold range of initial assembly rates, and a three-fold range of steady state assembly values. This observation underscores the previously detected importance of the 3'-Ph group in the taxol SAR.[15b] A simple explanation for these observations is that the more conformationally mobile isoserine side chain analogs[22] require a greater entropic loss in binding, and this feature exacerbates the (usually) deleterious effect of *N*-acyl group variation. An alternative is that the 3'-Ph group improves the sructural accomodation of the ligand by the microtubule binding domain, or *vice versa*.

Two tentative models for the conformation of taxol recognized by its microtubule binding site(s) have recently emerged. One is based on the conformation of (unbound) taxol that is dominant in chloroform and related hydrophobic solvents (**15**),[6b,15a,15b,23,24] and the other on the conformation of (unbound) taxol dominant in aqueous hydrophilic media (**16**).[24,25] The former appears to be supported by the correlation of biological[15b] and solution structural data[22] for a series of taxol analogs with deleted side chain substituents. The latter[25] is based on the concept of bound structure pre-organization determined by "hydrophobic collapse"[26] of the free taxol ligand in aqueous media. Although **15** and **16** present their *N*-benzoyl and 3'-Ph groups differently, it is nevertheless difficult to cast an interpretation of the SAR data disclosed herein in terms of one or the other of these bound drug structure models. Structural data on the taxol-microtubule binding site and its complex with the drug should clarify whether it is **15** or **16** or an unrelated drug conformation that combines with the taxol-microtubule binding site, thereby leading to a rational basis for the interpretation of the taxol structure-activity profile. Recent photoaffinity labeling results[17,27] represent the first step toward achieving that goal.

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