Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 1397–1402

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

NEW SYNTHETIC INHIBITORS OF MICROTUBULE DEPOLYMERIZATION

Ulrich Klar,* Hermann Graf, Oliver Schenk, Bodo Röhr, and Horst Schulz

Research Laboratories of Schering AG, Müllerstrasse 170, D-13342 Berlin, Germany

Received 26 February 1998; accepted 22 April 1998

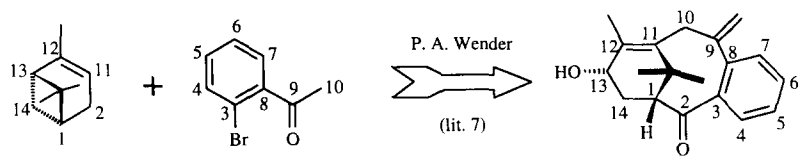
Abstract: A new class of borneol esters that might be considered as biological analogs of paclitaxel regarding their action on microtubules has been found. By structure-activity optimizations, compounds stabilizing microtubules much better than paclitaxel while showing a remarkably reduced cytotoxic activity were obtained. This dissoziation will open completely new therapeutic areas.

Since its first isolation from the bark extract of the Western yew *taxus brevifolia* in 1966 and elucidation of its structure by Wall and his collaborators[1] in 1971 it took about 18 years for paclitaxel (PT, taxol®) to enter the first clinical trial[2] as a potent anticancer drug.[3] In 1979 Susan Horwitz reported that PT accelerates the polymerization of tubulin to microtubules and stabilizes them by the inhibition of depolymerization.[4] PT and its closely related analogs represented the first class of compounds showing this type of mechanism, which has been strongly correlated with its antitumor properties.

Due to this unique mechanism of action and the complex and unusual structure, this diterpenoid alkaloid still stimulates research activities in pharmacology, chemistry, and medicine.

Although the first total syntheses[5] represent an ingenious masterpiece of synthetic organic chemistry it is unlikely that they will compete – due to their complexity – economically with the partial synthesis of PT and its closely related derivatives from readily available 10-deacetyl-baccatin III in the near future.

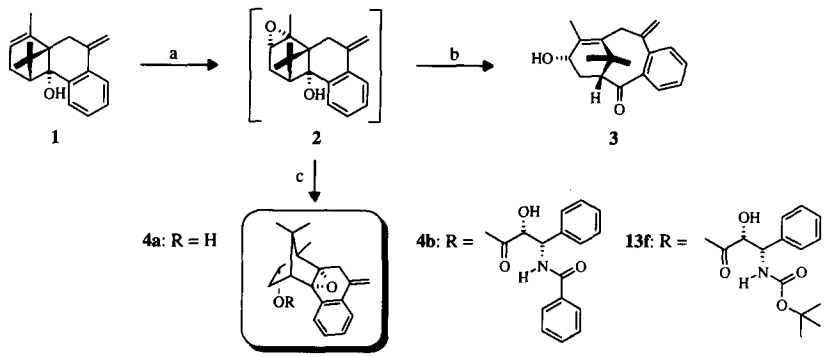
Several years ago we started a project to find biological analogs[6] of PT having a markedly simplified or even fundamentally different chemical structure. In one of our synthetic approaches we used the pinene route reported by Wender et al.[7] as a rapid and elegant entry for the preparation of a tricyclic taxane-skeleton like **I**.



A C ABC **I**

A key step in this convergent synthesis is the epoxidation of the chrysanthenone derivative **1** and the subsequent rearrangement of intermediate **2** to ketone **3** (Scheme 1). Our attempt to isolate and purify epoxide **2** resulted in the formation of a new rearrangement product, which was assigned the pentacyclic structure **4a** based on NMR-studies.[8] After esterification of the secondary hydroxyl group with the side chain present in docetaxel, compound **13f** showed a weak but significant inhibition of microtubule depolymerization, while the corresponding analog **4b**, bearing the PT chain proved to be inactive.

0960-894X/98/$19.00
PII: S0960-894X(98)00226-1



Scheme 1

(a) $Ti(O^iPr)_4$, $HO_2{}^tBu$, toluene/ether 0 °C, 20 min, H_2O; (b) DABCO, CH_3CN, rf, 20 h; (c) H_2O, ether, rt; SiO_2.

It is now well established that an isoserine side chain at C-13, an ester group at C-2 as well as the oxetane moiety at C-4/C-5 in PT and its analogs are essential to maintain their activity on microtubules while modifications in the "northern" region (C-7 to C-10) have only minor effects. From these findings it can be assumed that the "northern" hemisphere of PT is not directly involved in the binding on microtubules. Against this background the question arose, if the new borneol-like lead structure **13f** will bind to the same site on microtubules as PT. A superposition of both frameworks shows only limited structural analogies regarding the A-ring region and equivalents for the 2-benzoate as well as the oxetane moiety of PT cannot be defined (Figure 1).

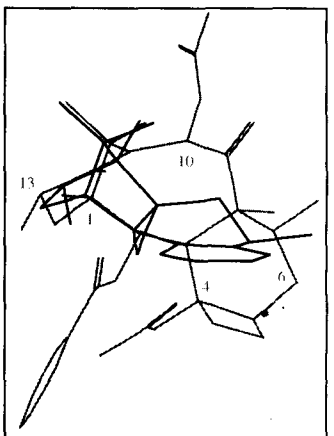


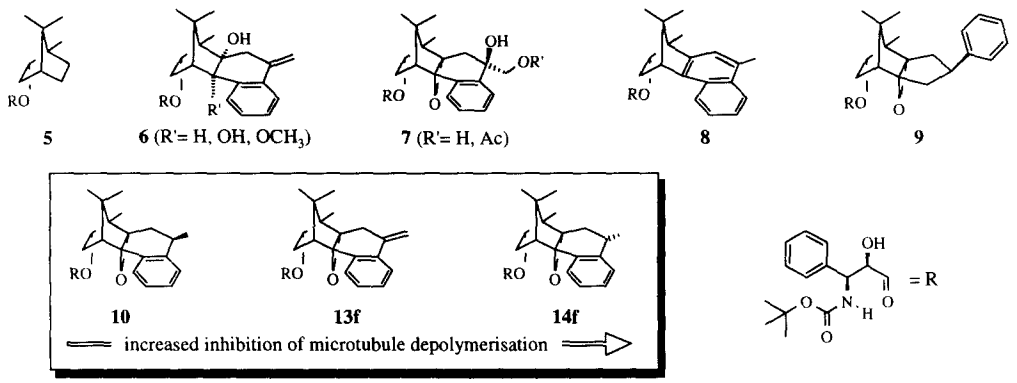
Figure 1
superposition of PT with **4a**; for simplicity, the isoserine chain and hydrogen atoms are not shown.

Therefore, our next activities focused on two objectives: (1) we had to enhance the potency to deduce structure-activity relationships and (2) we wanted to identify the structural elements in **4** which contribute to the tubulin binding.

Modifications at the pentacyclic framework

The most simplified modification of **13f**, the replacement of the complex polycyclic framework by borneol itself (**5**), led to a complete loss of activity. Even less severe changes like the reductive or nucleophilic opening of the tetrasubstituted epoxide (**6**), the hydroxylation of the styrene double bond (**7**), the aromatisation of ring B (**8**), or its contraction (**9**) resulted in inactive compounds.[9] Depending on the reaction sequence and the catalyst, it is possible to hydrogenate the double bond in **4a/13f** with good selectivity either from the α- or β- face. While the β-methyl isomer (**10**) was also completely inactive, its α-methyl epimer (**14f**) strongly improves the stabilization of microtubules (Table 1).

To understand this effect, one has to consider the most favorable conformations the three borneol skeletons of **10**, **13f**, and **14f** will adopt. In all cases the chair-like conformation of ring B was preferred over the boat-like one with the styrene **4a** showing the smallest difference in energy (4.9 kJ/mol).[10] The superposition of these



conformations is shown in Figure 2. The fact, that compounds possessing the equatorial methyl group lose their activity can be explained by an unfavorable steric interaction with the protein. From the model represented by Figure 1 such an effect is not predictable because herein the equatorial methyl group would occupy the conformational space of the northern part of PT which is unlikely to interact with the protein. This might be taken as an indirect proof that the binding site of the new borneol-type compounds differs from the one of PT. As a consequence, the superposition of both frameworks is not permitted.

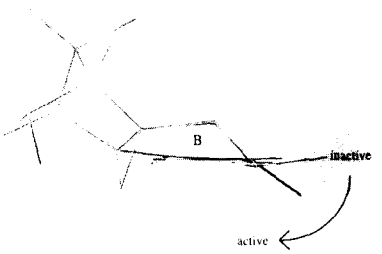


Figure 2

Modifications at the isoserine side chain

In the microtubule assay a substance concentration of 10 μM in the presence of 25 μM GTP with PT as reference was used. Stock solutions were 10% in DMSO, the final concentration in DMSO was 0.1%. Tubulin was isolated and purified from bovine brain.[11] Protein concentrations used were 2 mg/mL. To permit a quantitative assessment as well as a classification of the compounds, the relation of the decrease in microtubules represented by the change of UV absorbance, Δ_{ref} and Δ_{test}, respectively, multiplied with 100% is defined as rel(ative) stab(ility) (Figure 3). Thus, compounds that are more potent in stabilizing microtubules than PT are characterized by values >100%. Comparing the isosteric C-3' substituents at nitrogen given in Table 1 the most effective compounds (**13c**, **14b**, and **14c**) were obtained in the carbamate series. Thus, the 3'-ethoxycarbonylamino-group was chosen to investigate modifications at the 3'-aryl (Table 2). Compared with the unsubstituted phenyl group (**13c**, **14c**; see also Figure 4) a substitution by a methyl- or methyloxy-group or by halogen does not improve microtubule stabilization.

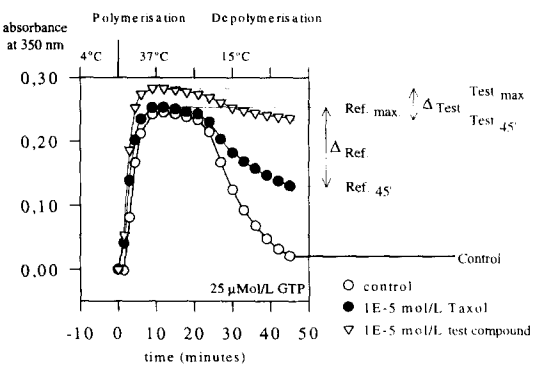
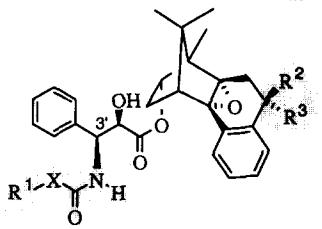


Figure 3

$$\frac{\Delta_{Ref}}{\Delta_{Test}} \bullet 100\% = rel.\ stab.$$

Table 1 Modifications at C-3'-nitrogen and their effect on microtubule stabilization.



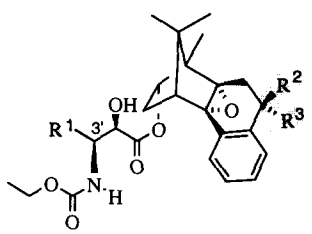
11, 13, 15 (a-g): R^2, $R^3 = CH_2$

12, 14, 16 (a-g): $R^2 = H$, $R^3 = CH_3$

compound		rel. stab. [%]					
		X = CH_2		X = O		X = NH	
R^1	No.	11	12	13	14	15	16
H	a	89					
Methyl	b	108		164	244		
Ethyl	c	114	168	228	209	ns	ns
n-Butyl	d			83	ns	68	130
i-Propyl	e	101				103	150
t-Butyl	f	ns		27	148	92	153
	g	ns	86				

More voluminous 3'-substituents like β-naphtyl (data not shown) or benzyloxyphenyl (**17g/18g**) lead to inactive compounds. A pronounced stabilization was observed with the phenol-derivative **18e**, which might be able to form an additional hydrogen bridge to the protein and the 2-thienyl derivatives **17h/18h**. A further increase was observed with the 3-thienyl-group (**17i/18i**) while the corresponding furane analogs (**17k/18k**) were unobtrusive. The most impressive change in activity was found by switching from 3-pyridyl (**17l/18l**) to 4-pyridyl (**17m/18m**) the latter belonging to the most active compounds identified in the borneol series.

Table 2 Modifications at C-3'-aryl and their effect on microtubule stabilization.



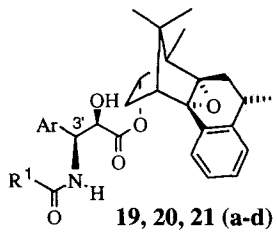
17 (a-m): R^2, $R^3 = CH_2$

18 (a-m): $R^2 = H$, $R^3 = CH_3$

compound		rel. stab. [%]		compound		rel. stab. [%]	
R^1	No.	17	18	R^1	No.	17	18
	a	142	152		g	ns	ns
	b	106	139		h	215	311
	c	152	139		i	788	788
	d	163	193		k	119	190
	e	130	306		l	65	119
	f	95	125		m	423	1300

This effect might be explained in terms of a favorable hydrogen-bridge from the protein to the 4-pyridyl nitrogen atom. Next we addressed the question if a combination of the identified favorable structural elements can further enhance microtubule stabilization (Table 3). For the first time we observed with compounds **19a** (see also Figure 4) and **19b** the effect that the amount of microtubules increased although the depolymerisation phase has already been induced. In terms of our evaluation (Figure 3) this will result in negative values for the relative stability (Test $_{45'}$ > Test $_{max}$). So far, all active compounds have shown a similar or even enhanced rate in tubulin polymerisation. Compound **19d** possesses a quiet different profile, which is characterized by a significantly delayed polymerisation although the formed microtubules are still stabilized (Figure 4).

Table 3 Matched combinations of structural elements and their effect on microtubule stabilization.



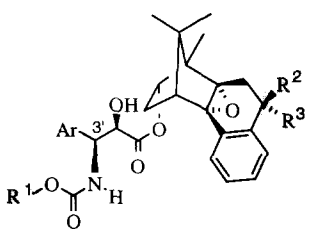
19, 20, 21 (a-d)

*see text

compound		rel. stab. [%] Ar = 	Ar = 	Ar = 
R^1	No.	**19**	**20**	**21**
Methoxy	**a**	-5*	316	293
n-Butoxy	**b**	-3*		80
n-Propyl	**c**	862	91	
i-Propylamino	**d**	(Figure 4)	56	

In Table 4, some optimized isoserine-chain analogs were combined with the less favorable borneol skeletons of **7** and **10**. The 4-pyridyl-substituent in compounds **22b/22c** and **22e**, respectively, compensates these unfavorable situations in a very impressive manner. The same tendency is observed with the borneol skeleton of **6** (data not shown). Bis-epoxide **22a** represents a conformation that can be considered as a combination of the borneol skeletons from **13f** and **14f**. This is confirmed by the high microtubule stabilizing effect.

Table 4 Mismatched combinations of structural elements and their effect on microtubule stabilization.



22, 23, 24 (a-e)

compound				rel. stab. [%] Ar = 	Ar = 	Ar = 
R^1	R^2	R^3	No.	**22**	**23**	**24**
CH_3	-O-CH_2-		**a**	423		
CH_3	OH	CH_2OAc	**b**	116		
C_2H_5	OH	CH_2OH	**c**	327		20
C_2H_5	OH	CH_2OAc	**d**		ns	22
C_2H_5	CH_3	H	**e**	227	58	ns

Compound **19a** was chosen for further characterization in the NCI's *in vitro* disease-oriented primary antitumor screen with a panel of 60 cell lines.[12] In Table 5 the log GI_{50}-values for some tumor cell lines are compared with those of PT.[13] Despite its excellent microtubule stabilizing potential, which is superior to PT, unexpectedly high concentrations of **19a** are needed to inhibit tumor cell growth. Thus, with the new class of borneol esters we succeeded, at least in part, in a separation of the tubulin mechanism from a cytostatic/cytotoxic action.

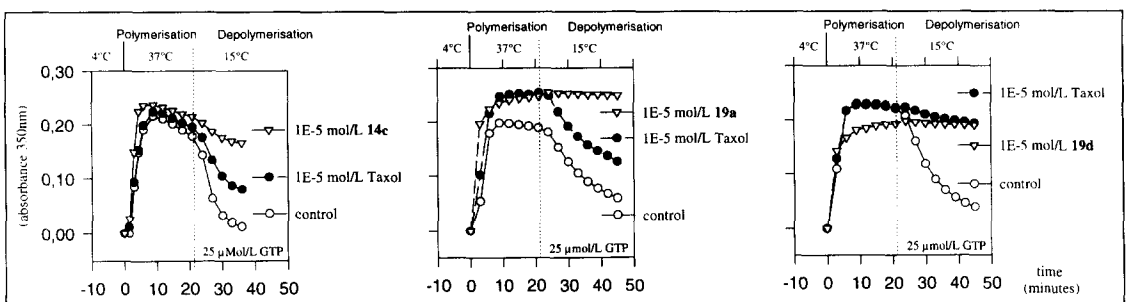


Figure 4 Tubulin polymerisation/microtubule depolymerisation of compounds **14c**, **19a**, and **19d**.

Although the application of these compounds as monotherapeutics in tumor indications will be limited, they might be of value as tools and wherever the stabilization of microtubules without other cell-toxic effects are advantageous. One of these potential applications might be the treatment of Alzheimer disease.[14] It has been demonstrated very recently that PT protects very efficiently against β-amyloid toxicity in primary neurons.[15] This will open completely new therapeutic areas for compounds which are able to stabilize microtubules.

Table 5

cell line	PT log-GI_{50}	19a log-GI_{50}	cell line	PT log-GI_{50}	19a log-GI_{50}	cell line	PT log-GI_{50}	19a log-GI_{50}	cell line	PT log-GI_{50}	19a log-GI_{50}
HL-60 (TB)	-8.30	-6.06	HT29	8.60	-6.35	SK-MEL-2	-8.30	-5.59	UO-31	-6.00	-6.25
MOLT-4	-8.40	-5.33	KM12	-8.50	-6.03	SK-MEL-5	-8.40	-5.63	MCF7	-8.50	-5.55
SR	-8.60	-5.84	SF-268	-8.10	-5.42	UACC-62	-8.40	-5.58	MCF7/ADR	-5.50	-5.72
HOP-62	-7.80	-5.21	SF-295	-7.80	-5.68	OVCAR-3	-8.50	-5.73	HS 578T	-8.50	-5.83
NCI-H226	-7.50	-6.01	SF-539	-8.50	-5.33	OVCAR-8	-8.30	-5.61	MDA-MB-435	-8.60	-6.23
NCI-H522	-8.50	-6.09	SNB-19	-8.00	-5.66	SK-OV-3	-8.00	-5.72	BT-549	-8.20	-4.64
Colo 205	-8.50	-5.73	U251	-8.40	-5.40	786-0	-7.70	-5.56			
HCC-2998	-8.40	-6.28	PC-3	-8.40	-5.52	A498	-7.10	-4.91	MID (lines shown):	-7.51	-5.68
HCT-15	-6.70	-6.01	M14	-8.00	-5.76	RXF 393	-8.10	-5.34	MID (whole panel):	-7.53	-5.50

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