Drugs of the Future 2001, 26(6): 533-544

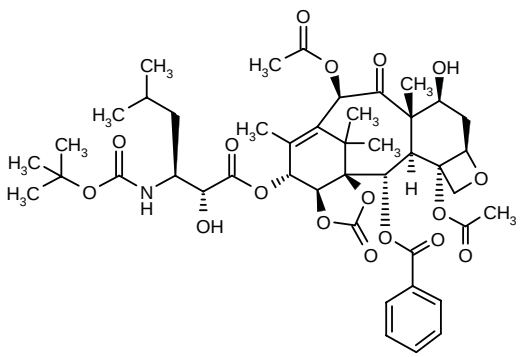
CCC: 0377-8282/2001

BAY 59-8862

Oncolytic

IDN5109
SB-T-101131

(2*R*,3*S*)-3-(*tert*-Butoxycarbonylamino)-2-hydroxy-5-methylhexanoic acid (3a*S*,4*R*,7*R*,8a*S*,9*S*,10a*R*,12a*S*,12b*R*,13*S*,13a*S*)-7,12a-bis(acetoxy)-13-(benzoyloxy)-9-hydroxy-5,8a,14,14-tetramethyl-2,8-dioxo,3a,4,7,8,8a,9,10,10a,12,12a,12b,13-dodecahydro-6,13a-methano-13a*H*-oxeto[2'',3'':5',6']benzo[1',2':4,5]cyclodeca[1,2-*d*]-1,3-dioxol-4-yl ester



$C_{44}H_{57}NO_{17}$ Mol wt: 871.9243

CAS: 186348-23-2

EN: 264502

Synthesis

BAY 59-8862 (1) was obtained from two synthons, one derived from 14β-hydroxy-10-deacetylbaccatin III and the second derived from (2*R*,3*S*)-3-(*N*-*tert*-butoxycarbonylamino)-2-hydroxy-5-methylhexanoic acid, each of which was prepared separately and then coupled. The synthesis involved several steps.

1) Semisynthesis of the first synthon

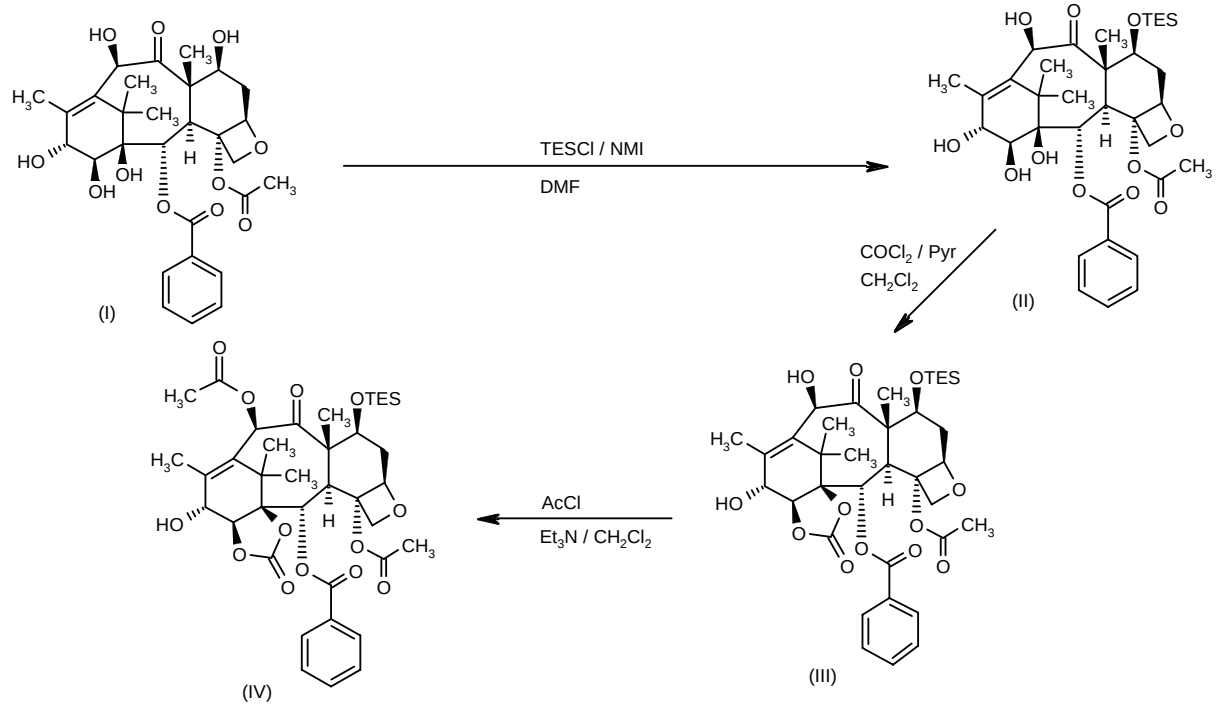
The diterpene 14β-hydroxy-10-deacetylbaccatin III (I) was extracted in a highly purified form from the needles of *Taxus wallichiana* cultivated in the Himalayan region (2, 3). It was then converted in 7-*O*-(triethylsilyl)-14β-hydroxybaccatin III-1,14-carbonate (IV) according to the following scheme: 14β-hydroxy-10-deacetylbaccatin III (I) was dissolved in anhydrous DMF and treated with *N*-methylimidazole (NMI) and chlorotriethylsilane to provide 7-*O*-(triethylsilyl)-10-deacetyl-14β-hydroxybaccatin III (II) in an almost quantitative yield. Compound (II) was dissolved in a mixture of methylene chloride and dry pyridine and added slowly to a cooled phosgene solution giving 7-*O*-(triethylsilyl)-10-deacetyl-14β-hydroxybaccatin III-1,14-carbonate (III). After acetylation of compound (III) in pyridine and acetyl chloride, the synthon (IV) was obtained and was ready for coupling (Scheme 1).

2) Synthesis of the second synthon

L-Leucinol (V) was converted into the protected derivative (VI) by treatment with *tert*-butoxycarbonyl anhydride in methylene chloride. Oxidation of compound (VI) with NaOCl and TEMPO in the presence of sodium bromide yielded the aldehyde (VII). This aldehyde (VII), when treated with sodium bisulfite overnight at –5 to 0 °C, produced the sodium bisulfite salt derivative (VIII), which was treated with KCN to obtain the nitrile derivative (IX). This nitrile derivative (IX) was heated under reflux with concentrated HCl, and after several crystallizations, afforded the desired amino acid (2*R*,3*S*)-3-amino-2-hydroxy-5-methylhexanoic acid (X). Amino acid (X) was dissolved in a mixture of water/dioxane and then treated with *tert*-butoxycarbonyl anhydride in the presence of TEA to yield the Boc derivative (XI), which was converted into the mixture of methyl esters (XII) in a conventional manner. Compound (XII) was heated with 2,4-dimethoxybenzaldehyde dimethyl acetal in THF in the presence of pyridinium *p*-toluensulfonate as catalyst to obtain the acetal (XIII). When hydrolyzed with potassium carbonate in aqueous methanol, acetal (XIII) yielded (4*S*,5*R*)-3-(*tert*-butoxycarbonyl)-2-(2,4-dimethoxyphenyl)-4-isobutyloxazolidine-5-carboxylic acid (XIV) (Scheme 2).

G. Pratesi*, D. Laccabue. Istituto Nazionale Tumori, Via Venezian 1, 20133 Milano, Italy. *Correspondence.

Scheme 1: Synthesis of Intermediate (IV) of BAY 59-8862



3) Coupling reaction of the two synthons

(4*S*,5*R*)-3-(*tert*-Butoxycarbonyl)-2-(2,4-dimethoxyphenyl)-4-isobutyloxazolidine-5-carboxylic acid (XIV) was dissolved in methylene chloride and added to 7-*O*-(triethylsilyl)-14β-hydroxybaccatin III-1,14-carbonate (IV) in toluene, in the presence of DMAP and DCC, to yield 7-*O*-(triethylsilyl)-14β-hydroxybaccatin III-1,14-carbonate 13-[3-(*tert*-butoxycarbonyl)-2-(2,4-dimethoxyphenyl)-4-isobutyl-5-oxazolidinecarboxylate] (XV) (Scheme 3).

4) Deprotection reaction of the coupling product

7-*O*-(Triethylsilyl)-14β-hydroxybaccatin III-1,14-carbonate 13-[3-(*tert*-butoxycarbonyl)-2-(2,4-dimethoxyphenyl)-4-isobutyl-5-oxazolidinecarboxylate] (XV) was dissolved in anhydrous methanol containing a catalytic amount of dry HCl at 0 °C. After work-up and crystallization from ethanol/water, 13-[*N*-(*tert*-butoxycarbonyl)-β-isobutylisoserinyl]-14β-hydroxybaccatin III-1,14-carbonate (BAY 59-8862) was obtained (Scheme 3).

Description

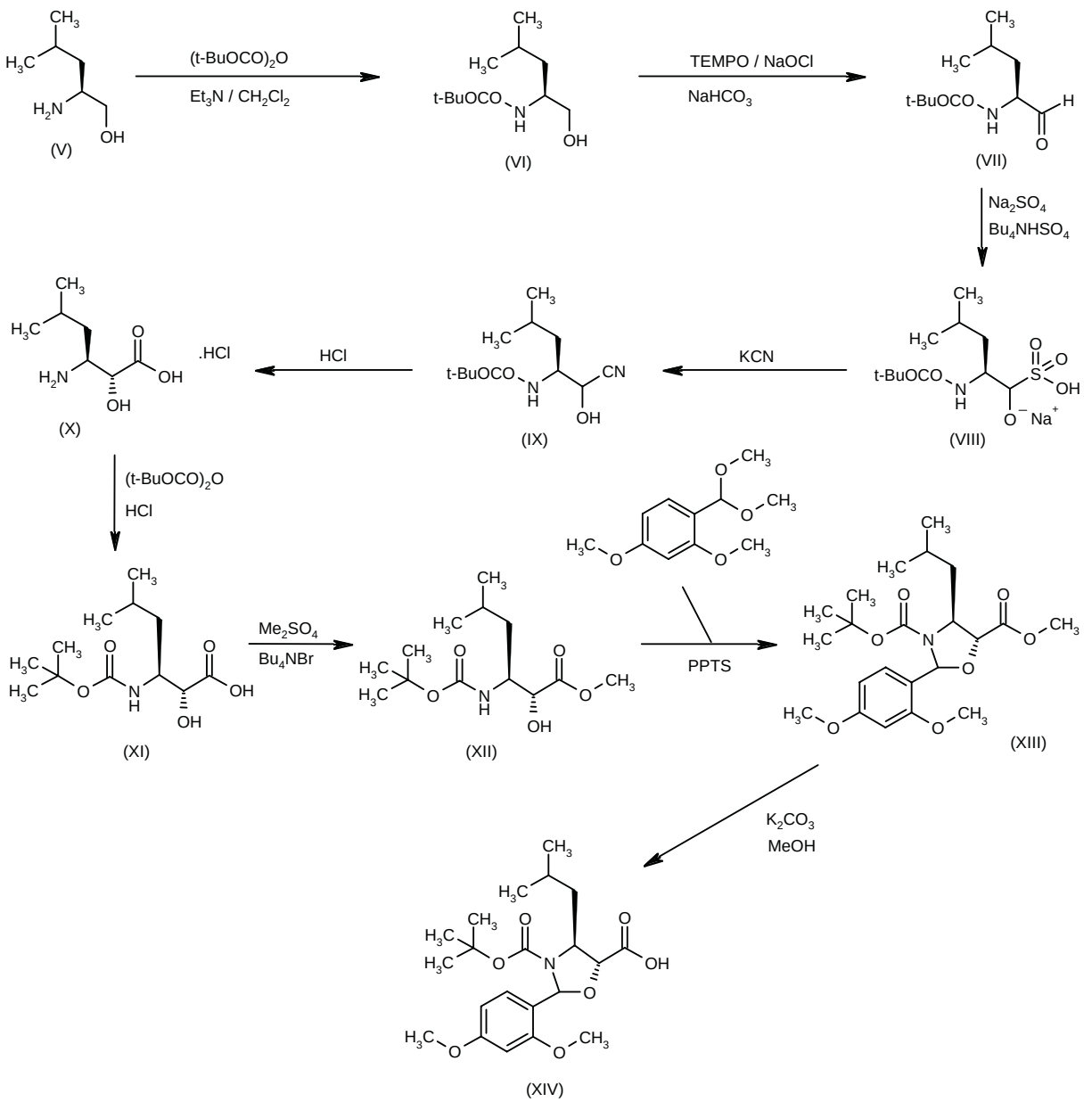
White crystalline solid, m.p. 245 °C; $[\alpha]_D$ –57.6° (c 0.1, $CHCl_3$).

Introduction

Taxanes are inhibitors of microtubule depolymerization and thus represent an important class of antitumor drugs. The discovery and introduction of Taxol (paclitaxel) into clinical practice represents an important addition to cancer chemotherapy (4). Taxanes are characterized by a unique mechanism of action: they inhibit tubulin depolymerization and promote assembly of microtubules (5, 6). The process leads to a disruption in mitotic function and cell arrest in the M phase. Taxanes bind to tubulin at a different site than vinca alkaloids, which may explain some of the unique features of this novel class of tubulin inhibitors. After the discovery in 1980 that microtubules were the molecular target for paclitaxel (5), the compound was the only microtubule depolymerization inhibitor known in the literature for about 10 years. Analog synthesis and structure-activity studies were initially hampered by difficulties in obtaining an adequate drug supply. In fact, paclitaxel was initially extracted from the bark of *Taxus brevifolia*, a procedure that destroys the tree (7). During the 1990s, the analog Taxotere (docetaxel), obtained from a noncytotoxic precursor extracted from the needles of *Taxus baccata* (10-deacetylbaccatin III), was developed as a novel microtubule depolymerization inhibitor with antitumor activity (8, 9).

Paclitaxel and docetaxel are clinically useful agents for the treatment of ovarian, breast and lung carcinomas (10). Their efficacy is limited, however, by the natural

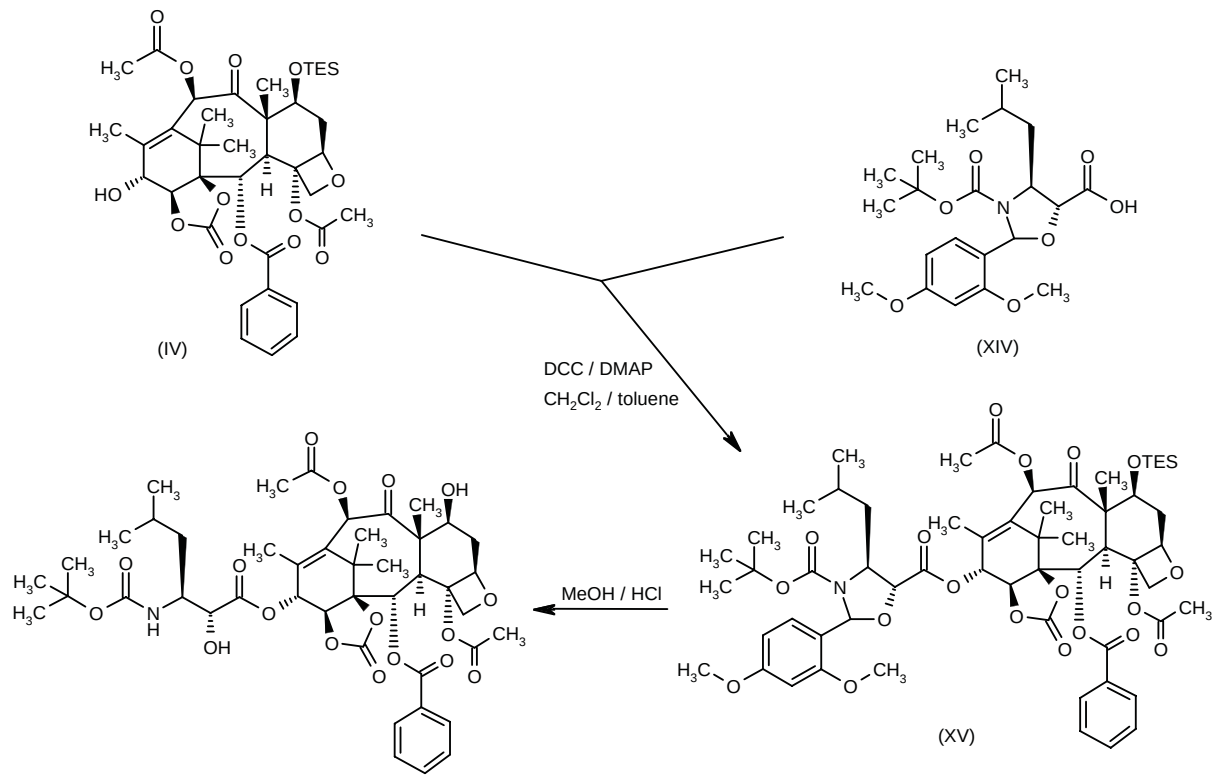
Scheme 2: Synthesis of Intermediate (XIV) of BAY 59-8862



insensitivity of the tumors and the development of clinical resistance. Two mechanisms of acquired resistance to paclitaxel and docetaxel have been described. The first involves alteration in α- and β-tubulin (11-13) and the second involves the amplification of P-glycoprotein-170 (Pgp), a membrane phosphoglycoprotein that acts as a drug-efflux pump (14, 15). The mechanism of resistance is common to several clinically used anticancer agents belonging to different chemical classes, such as anthracyclines (doxorubicin, epirubicin, daunorubicin), epipodophyllotoxins (etoposide) and vinca alkaloids (vinblastine, vincristine). As a consequence, the development of multidrug resistance (MDR) is a frequent and usually lethal occurrence in human cancer progression.

From a large series of synthetic derivatives of 14β-hydroxy-10-deacetylbaccatin III, a novel analog, BAY 59-8862 (previously known as IDN 5109) was selected for preclinical development based on its ability to overcome MDR transport systems (16). BAY 59-8862 was shown to be effective against a wide spectrum of tumors,

Scheme 3: Synthesis of BAY 59-8862



including tumors presenting natural or acquired resistance to paclitaxel. It was also shown to be active by the oral route, in contrast to paclitaxel, and endowed with an increased ability to cross the blood-brain barrier, as well as increased water solubility compared with paclitaxel. Owing to the very favorable pharmacological preclinical profile, intraveously administered BAY 59-8862 is currently being evaluated in phase I clinical studies. The following summarizes the preclinical results showing that BAY 59-8862 is a very promising second-generation taxane.

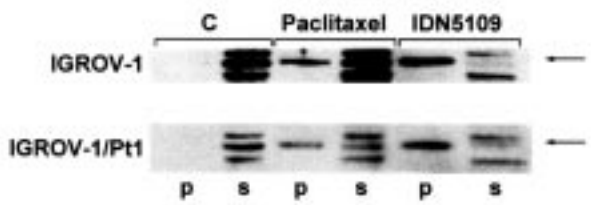


Fig. 1. Tubulin polymerization in taxane-treated cells (24 h exposure). p = polymerized form of tubulin; s = soluble form of tubulin. C = control cells; paclitaxel, 0.1 μM/ml; IDN5109 (BAY 59-8862), 0.1 μg/ml. Results from ref. 21.

Mechanism of Action

Paclitaxel was first described for its ability to induce the stabilization of microtubules as a consequence of its interaction with tubulin (5). When compared to paclitaxel, BAY 59-8862 was a more potent inducer of microtubule polymerization in the ovarian cell line IGROV-1 and its cisplatin-resistant cell subline (IGROV-1/Pt1) (Fig. 1). The effect was consistent with the greater cytotoxic activity of BAY 59-8862 compared to paclitaxel in such cell lines, thus suggesting that tubulin is the primary cellular target for BAY 59-8862.

Cellular Pharmacology

Cytotoxicity

The cytotoxic potency of BAY 59-8862 was compared to that of paclitaxel against a large panel of human tumor cell lines (Table I). The results indicated that BAY 59-8862 was as potent as paclitaxel in a variety of tumor cell lines and, like paclitaxel, displayed a wide spectrum of cytotoxicity. Similar results using other cell lines have been published (16).

The most striking result from initial investigations of BAY 59-8862 was the fact that among a series of

Table I: Cytotoxicity of paclitaxel and BAY 59-8862 in human tumor cell lines.[a]

Tumor type	IC_{50} (ng/ml) Paclitaxel	BAY 59-8862
Prostate carcinoma		
DU145	17 ± 4	17 ± 3
PC3	16 ± 2	16 ± 4
Melanoma		
665/2/21	17 ± 7	10 ± 6
665/2/60	18 ± 10	2.2 ± 2
Osteosarcoma		
SAOS	9.2 ± 3	4.5 ± 1.2
Glioblastoma		
U-87MG	8.3 ± 4.6	6.9 ± 2.7
SW1789	5.1 ± 1.5	2 ± 0.7
GBM	31 ± 8.5	14 ± 2.5
nonSCLC		
H460	7 ± 1	3.9 ± 1
CaLu-3	60 ± 40	14 ± 10
Colon carcinoma		
HT29	4 ± 1.3	5.4 ± 0.8

[a]Cytotoxicity was evaluated by cell growth inhibition after 72 h drug exposure. Mean ± SD. Results from the INT.

Table II: Cytotoxicity of paclitaxel and BAY 59-8862 on drug-resistant cells lines.[a]

Tumor type	IC_{50} (ng/ml) Paclitaxel	RI[2]	BAY 59-8862	RI[2]
Ovarian ca.				
A2780	31 ± 11		32 ± 17	
A2780/DX	360 ± 60	11.6	88 ± 50	2.75
A2780/CP	14 ± 4	0.45	48	1.5
1A9	4.1 ± 1.3		3.2 ± 0.6	
1A9/PTX22	92.5 ± 17.5	22.5	25 ± 8	7.8
IGROV-1	110 ± 18		27 ± 0.7	
IGROV-1/Pt1	29 ± 18	0.3	13 ± 0.7	0.5
Cervix ca.				
A431	10 ± 5		5.2 ± 2	
A431/Pt	16 ± 6	1.6	5.3 ± 1.4	1
Lung ca.				
POGB*	6.8 ± 2		6.2 ± 2	
POGB/DX*	4.8 ± 1	0.7	4.2 ± 0.9	0.7
POVD*	14 ± 0.7		10 ± 0	
POVD/DX*	2300	164	70 ± 17	7
Colon ca.				
LoVo	30 ± 2		26 ± 3	
LoVo/DX	13000 ± 300	433	150 ± 10	5.8
Osteosarcoma				
U2-OS	45 ± 18		4 ± 1	
U2-OS/Pt	60 ± 10	1.3	3.6 ± 0.8	0.9

[a]Cytotoxicity was evaluated by cell growth inhibition or the MTT assay (*) after 72 h drug exposure. Mean ± SD. [2]RI = resistance index, ratio between the IC_{50} of resistant and sensitive cells. Results from the INT.

paclitaxel analogs, BAY 59-8862 showed the best activity against the MCF7/DX (MDR phenotype) cell lines (16). Following that finding, the effects of BAY 59-8862 on drug-resistant cancer cell lines, together with its effects against the corresponding parental cell lines, were investigated (Table II). The results indicated that, in contrast to paclitaxel, BAY 59-8862 was a weak substrate for Pgp in A2780/DX, POVD/DX, and LoVo/DX cell lines (*i.e.*, as shown by the much lower resistance index values). In addition, a lower resistance index for BAY 59-8862 than for paclitaxel was achieved in the 1A9/PTX22 cell line, which is characterized by a tubulin mutation. Moreover, both taxanes overcame the MRP (multidrug-resistance protein) mechanism of resistance in the POGB/DX cell line, as well as mechanisms involved in cisplatin resistance.

The cytotoxicity of BAY 59-8862 was also compared to that of paclitaxel against a panel of colon tumor cell lines that varied in their Pgp expression (Table III). BAY 59-8862 had equivalent potency against all three cell lines, whereas the IC_{50}s of paclitaxel increased in parallel with increased Pgp expression (more than 10-fold between HCT-15 and SW-620 cell lines). Additional studies showing that BAY 59-8862 was very effective in inhibiting the growth of human cancer cells overexpressing Pgp have been published by another research group (17).

Drug efflux studies

Specific experiments were carried out to confirm the hypothesis that the activity of BAY 59-8862 in taxane-resistant tumor cells was due to the low sensitivity of the novel analog towards the Pgp-drug efflux pump. Specifically, flow cytometry studies were performed in MDA-MB-435 mammary tumor cell sublines to test the effects of several agents on the accumulation and retention of compounds that are substrates for Pgp, such as Rh-123 and doxorubicin. The MDR-expressing cell subline (18) demonstrated efflux of Rh-123 and doxorubicin. The efflux of Rh-123 was effectively blocked by verapamil and by BAY 59-8862, but not by paclitaxel or docetaxel. The inhibitory effects of BAY 59-8862 on the efflux of other Pgp substrates suggest that the compound blocks the pump activity. In addition to achieving activity against MDR tumor cells, the effect may have significant clinical implications, where taxanes may be combined with other agents that are normally substrates for this mechanism of drug efflux. In fact, such properties may result in improvement of the therapeutic efficacy of such combinations or modulation of the toxicity of the combination without affecting the therapeutic index. The effects of verapamil and various taxanes on the efflux of Rh-123 were also evaluated by flow cytometry in colon tumor cell lines, which are characterized by varying levels of Pgp expression. BAY 59-8862 completely blocked the efflux of Rh-123 from the SW-620 and the DLD-1 cells, and partially blocked the efflux from the HCT-15 cells. Neither

Table III: Response to taxanes of colon tumor cell lines with varying Pgp-170 expression levels.

Cell line	Pgp-170 expression	IC_{50} (nM)[a] Paclitaxel	IC_{50} (nM)[a] BAY 59-8862	Xenograft[b] Paclitaxel	Xenograft[b] BAY 59-8862
HCT-15	457	290 ± 0.9	16 ± 0.9	R	S
DLD-1	84	37 ± 2.7	14 ± 0.7	R	S
SW-620	19	24 ± 2.1	12 ± 0.8	R	HS

[a]Cytotoxicity was evaluated by the sulforhodamine B assay, after 72 h drug exposure. Mean ± SD. [b]Tumors were implanted s.c. and treated i.v. q4dx4. BAY 59-8862, 60 mg/kg/inj; paclitaxel, 25 mg/kg/inj. For scoring system, see Table IV. Results from the RPCI and ref. 25. R = resistant; S = sensitive; HS = highly sensitive.

Table IV: Criteria for evaluating tumor response to drug.

Score[a]			
R	TWI%[b] < 70	and/or	LCK[c] <1
S	TWI% > 70	and/or	LCK > 1
HS	TWI% > 90	and/or	LCK > 2

[a]R = resistant; S = sensitive; HS = highly sensitive. [b]TWI% = percentage tumor weight inhibition in treated *versus* controls. [c]LCK = $\log_{10}$ cell kill induced by the treatment.

paclitaxel nor docetaxel inhibited the efflux of Rh-123 from any of these tumor lines (19).

Cellular response to damage

The effect of BAY 59-8862 on cell cycle progression was comparable to that of paclitaxel against the CEM-VBL-resistant human leukemia cell line. After 24 h of drug exposure, the cell cycle was blocked in G_2/M in a concentration-dependent manner (17).

Comparative studies were performed with paclitaxel and BAY 59-8862 on ovarian carcinoma cell lines and prostate carcinoma cell lines. BAY 59-8862 induced Raf1 and Bcl2 phosphorylation and p34cdc2 dephosphorylation, consistent with mitotic arrest and activation of the spindle checkpoint. BAY 59-8862-induced apoptosis resulted in early cell death after mitotic arrest, or in a slow and delayed cell death following DNA reduplication, depending on the functionality of the checkpoints activated by microtubule damage. Such findings indicate that BAY 59-8862 acts on the same pathways as those activated by paclitaxel (20-22).

Antitumor Activity

The antitumor activity of BAY 59-8862 has been investigated in several research centers, including Istituto Nazionale Tumori (INT) of Milano, Italy, Istituto Ricerche Farmacologiche Mario Negri (IRFMN) of Bergamo, Italy and Roswell Park Cancer Institute (RPCI) of Buffalo, New York.

Intravenous treatment

The efficacy of BAY 59-8862 administered i.v. was compared to that of paclitaxel using a large panel of human tumor xenografts grown s.c. in nude athymic mice. The antitumor efficacy was evaluated as percentage tumor weight inhibition (TWI%) in treated versus control mice, and as $\log_{10}$ cell kill (LCK) induced by drug treatment (23). Tumors were classified as resistant (R), sensitive (S) or highly sensitive (HS) according to the criteria reported in Table IV (23).

Table V: Antitumor activity of i.v. paclitaxel and BAY 59-8862 against human tumor xenografts characterized by acquired or natural resistance to paclitaxel.

Tumor	Paclitaxel[a] TWI%	Paclitaxel[a] LCK	Paclitaxel[a] Score[b]	BAY 59-8862[a] TWI%	BAY 59-8862[a] LCK	BAY 59-8862[a] Score[b]
Ovarian ca.						
INT-ACP/PTX	8	0.1	R	63	1.8	S
IGROV-1	30	0.2	R	85	1.4	S
IGROV-DDP	45	0.4	R	94	1.7	HS
Glioma						
U-87MG	59	0.6	R	64	0.5	R
Colorectal						
CoBA	67	0.5	R	92	1.2	HS
Lung ca.						
POVD/DX	–13	0	R	37	0.5	R

[a]Drugs were delivered i.v., q4dx4. Paclitaxel, 54 mg/kg/inj; BAY 59-8862, 90 mg/kg/inj. [b]For scoring system, see Table IV. Results from the INT and ref. 23.

Table VI: Antitumor efficacy of i.v. paclitaxel and BAY 59-8862 against human tumor xenografts characterized by acquired or natural resistance to cisplatin and/or doxorubicin.

Tumor type	No.	Paclitaxel[a] S[b]	Paclitaxel[a] HS[b]	BAY 59-8862[a] S[b]	BAY 59-8862[a] HS[b]
Gynecological ca. (A2780/DDP, SKOV, A431, MX-1, A2780/DX, 1A9, HOC18)	7	2	5	1	6
Lung ca. (CaLu-3, A549)	2	2		1	1
Gastrointestinal ca. (LoVo)	1	1			1
Prostate ca. (DU145)	1	1			1
Total	11	6	5	2	9

[a]Drugs were delivered i.v. q4dx4. The effects reported were achieved by the optimal dose in each experiment. [b]For scoring system see Table IV. Results from the INT, the IRFMNB and ref. 23.

Table VII: Comparison of tumor weight inhibition between similar doses of i.v. paclitaxel and BAY 59-8862.

Tumor	Drug	Dose (mg/kg)	TWI%	Statistical significance[a]
IGROV/DDP	Paclitaxel	54	45	
	BAY 59-8862	54	82	$p < 0.05$
CaLu-3	Paclitaxel	54	94	
	BAY 59-8862	60	99	$p < 0.01$
A2780/DDP	Paclitaxel	54	98	
	BAY 59-8862	60	90	$p < 0.001$
LoVo	Paclitaxel	54	88	
	BAY 59-8862	60	93	$p = 0.05$

[a]Calculated by Student's t test. Results from the INT and ref. 23.

The first series of experiments investigated the antitumor efficacy of BAY 59-8862 against various tumors presenting natural or acquired resistance to paclitaxel (various resistance mechanisms have been characterized in the latter cells). Using the schedule of 4 times every 4th day, BAY 59-8862 at its MTD of 90 mg/kg overcame drug resistance in 4 of 6 tumor lines (INT-ACP/PTX, IGROV, IGROV/DDP and CoBA) (Table V). In an ovarian tumor line poorly sensitive to paclitaxel (MNB-PTX1), BAY 59-8862 showed marked antitumor activity (24). Superior antitumor efficacy of BAY 59-8862 compared to paclitaxel has also been reported against resistant human colon carcinoma xenografts expressing various Pgp levels (25). Moreover, oral BAY 59-8862 was efficacious (TWI>70%) against 3 paclitaxel-resistant renal carcinoma xenografts (26) (Table VIII).

A second panel included tumors selected for resistance (natural or acquired) to doxorubicin and/or to cisplatin (two clinically active drugs for mammary and ovarian carcinomas, tumor types in which paclitaxel has been approved for clinical use) but responsive to paclitaxel. Table VI summarizes the results of these studies. BAY 59-8862 was effective against all 11 of the tumors investigated, being as effective as paclitaxel in most tumor lines and more effective in 4 of them: the A2780/DX ovarian carcinoma, the CaLu-3 non-small cell lung cancer, the LoVo colon carcinoma and the DU145 hormone-independent prostate carcinoma.

The superior antitumor efficacy of BAY 59-8862 compared to paclitaxel was seen not only at the highest (MTD) dose tested, but also when equal doses of the two drugs were administered (Table VII).

Oral activity

The current taxanes in clinical use, paclitaxel and docetaxel, are both substrates for the Pgp responsible for the MDR phenotype (15, 27, 28). In addition to being involved in mechanims of tumor cell resistance, Pgp is overexpressed in the intestinal mucosa and may have a role in biliary excretion and fecal elimination. This may be the cause of the limited oral bioavailability of paclitaxel (29). In fact, cotreatment with Pgp inhibitors enhances the oral absorption of paclitaxel (30, 31).

Considering that the original selection of BAY 59-8862 was based on its ability to overcome multidrug resistance in tumor cell lines (16), it seemed to be a very promising candidate to investigate for antitumor efficacy after oral delivery. The first experiment was performed at the INT and compared the efficacy by i.v. and oral route of paclitaxel and BAY 59-8862 against the very sensitive MX-1 breast carcinoma. BAY 59-8862 was equally active by either route, whereas paclitaxel completely lost its activity after oral delivery (Fig. 2). After this finding, many other tumors were investigated. Table VIII summarizes the antitumor activity of BAY 59-8862 after i.v. and oral

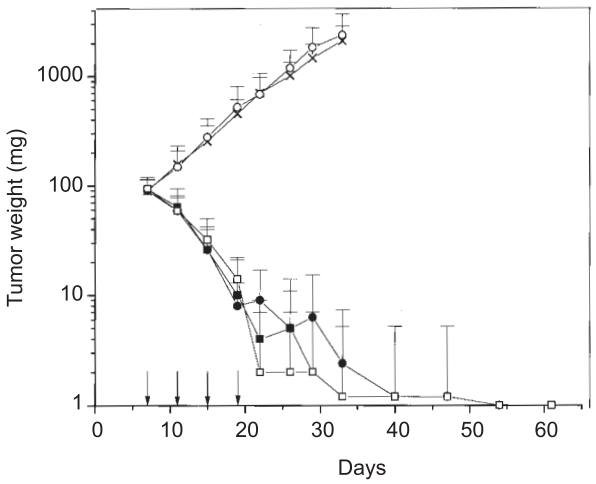


Fig. 2. Growth curves of the human breast carcinoma, MX-1, xenografted in nude mice after taxane treatment (every 4 days for 4 times). X, control, ○, 90 mg/kg oral paclitaxel; ●, 54 mg/kg i.v. placlitaxel; □, 90 mg/kg oral BAY 59-8862, ■, 90 mg/kg i.v. BAY 59-8862. Arrows, days of treatment. Results from the INT.

administration (24, 25, 26, 32). As can be seen, orally administered BAY 59-8862 was highly effective in all 11 tumor lines investigated. In order to achieve comparable efficacy by the i.v. route, 1.5- to 2-fold higher doses had to be administered p.o. Such findings correlate well with the bioavailability and pharmacokinetic properties of the molecule.

Activity against central nervous system tumors

The development of effective chemotherapy for CNS tumors is hampered by the blood-brain barrier, which impedes drug penetration into the brain (33). Although hydrophobicity has been regarded as a relevant prerequisite for a drug to cross the blood-brain barrier, several very hydrophobic substances (such as vincristine and etoposide) are unable to access the brain (34). Many of these agents have recently been shown to be substrates for Pgp (35).

BAY 59-8862 was tested against a human glioma xenograft growing orthotopically (intracranial) in nude mice. The preliminary results of the study showed that histological sections of brains of tumor-bearing mice treated i.v. with BAY 59-8862 presented few or no tumor foci 14 days after the end of treatment, whereas untreated or paclitaxel-treated mice presented many tumor foci of variable size which were widespread in the cerebral and cerebellar tissue. Such findings were supported by the pharmacokinetic analysis, which showed a much more favorable penetration of BAY 59-8862 in mice brain as compared to paclitaxel (Table IX) (36).

Pharmacokinetics

BAY 59-8862 plasma levels were measured by using a recently developed HPLC assay which is able to determine levels of the compound and its epiform with a high

Table VIII: Comparison of antitumor activity of BAY 59-8862 after p.o. and i.v. delivery (q4dx3/4).

	BAY 59-8862 i.v.		BAY 59-8862 p.o.	
Tumor	Dose (mg/kg/inj)	TWI%	Dose (mg/kg(inj)	TWI%
Breast ca.				
MX-1	90	100	90	100
Colon ca.				
LoVo	60	93		
	90	97	90	94
CoBA	90	92	90	87
Glioblastoma				
U-87MG	60	47		
	90	64	90	52
Ovarian ca.				
IGROV/DDP	60	82	60	76
	90	94	90	82
1A9	60	99	120	99
HOC-18	60	97	120	100
MNB-PTX1	60	72	120	80
Renal ca.				
CAKI	60	65	120	78
SN12KI	60	65	120	78
786-0	60	92	120	76

For scoring system, see Table IV. Results from the INT, the IRFMNB and refs. 24, 26, 32.

Table IX: Distribution of BAY 59-8862 and paclitaxel in nude athymic mice.

	BAY 59-8862	Paclitaxel
Dose (mg/kg) i.v.	90	54
Plasma $AUC_{0\text{-}6h}$ (μg/ml·h)	36.0	116.6
Brain $AUC_{0\text{-}6h}$ (μg/g·h)	6.3	1.4
R[a]	0.175	0.012

[a]R, brain AUC/plasma AUC. Results from the INT, the IRFMN and ref. 36.

degree of sensitivity, precision and accuracy (37). The pharmacokinetics of BAY 59-8862 were investigated in euthymic and athymic mice (24,32).

The main pharmacokinetic parameters in the plasma of euthymic mice after a single i.v. or oral administration of 3 dose levels of the drug are presented in Table X. After i.v. treatment, the drug disappeared in a biphasic fashion with a terminal half-life of 3.5-3.9 h. Drug disappearance was slower after oral administration, with an elimination half-life of 6.2-8.8 h. Drug bioavailability after oral administration was 37-66%. Comparable results were achieved in a parallel study performed in nude athymic mice (the same mice used for antitumor activity studies). Bioavailability of the oral dose was found to be 48% (24). After i.v. or oral administration, the 7-epiform of the drug was detectable up to 8 h after administration, and its levels were approximately 10-15% of those of the parent compound (24).

In a recent study performed in nude mice to investigate the distribution of BAY 59-8862 in comparison with paclitaxel (38), drug levels were determined in tissues and plasma by HPLC at different times after treatment. The results indicated that BAY 59-8862 was distributed more in brain, kidney and heart and less in liver than paclitaxel. This different distribution, and particularly the penetration in brain, may have important implications in the activity of BAY 59-8862 against CNS tumors (see Table IX).

As reported for paclitaxel and docetaxel, BAY 59-8862 is cleared mainly by metabolism. Metabolic investigations on the biliary excretion of BAY 59-8862 in rat, carried out using the HPLC-MS technique, indicated the formation of monohydroxy and dihydroxy derivatives of BAY 59-8862. The oxidative process involves the isobutyl group at the C3' side chain of the taxane ring (Dr. M. Zucchetti, IRFMN, personal communication).

Toxicology

All toxicology studies were carried out at RPCI. For i.v. administration, a formulation of BAY 59-8862 solubilized in a mixture of Tween 80:ethanol (1:1) at a concentration of 30 mg/ml at pH 4.2 was used. In the solution, BAY 59-8862 was physically and chemically stable for 1 year. Safety studies of BAY 59-8862 given i.v. were carried out in rats and dogs after single and multiple (daily for 5 consecutive days) dosage regimens.

In the single-dose rat study, two patterns of lethality were seen. The first was due to infusional toxicity, with death occurring within minutes of infusion, and was partially ascribed to the vehicle or to the low drug solubility. The other pattern of toxicity was clearly due to the cytotoxic moiety of BAY 59-8862, with death or moribund condition occurring between days 3 and 6, a time span usually associated with gastrointestinal toxicity. There were no delayed patterns of toxicity. In the multiple-dose rat study, the data indicated the tendency for greater than additive toxicity (*i.e.*, lethality) when high nonlethal single doses were split and given daily for 5 consecutive days.

In the dog studies, two patterns of physical abnormalities were noted. The first pattern consisted of peracute infusional toxicities seen in dogs treated with BAY 59-8862 at all doses, and in control dogs treated with vehicle alone. Such signs were most likely due to histamine release induced by the vehicle in which BAY 59-8862 was solubilized. The second pattern of dose-related toxicity was the onset of diarrhea and myelosuppression.

In summary, the highest nonlethal single dose of BAY 59-8862 in rats was 25 mg/kg, which corresponds to 148 mg/m2. The highest nonlethal single dose in dogs was 2.5 mg/kg, corresponding to 50 mg/m2. The starting dose in humans, therefore, should be based on the data in dogs as they are the most sensitive species.

Table X: Main pharmacokinetic parameters of BAY 59-8862 in CDF-1 euthymic mice.

Dose (mg/kg)	Route of injection	C_{max} (μg/ml ± SD)	$t_{1/2}$ (h)	$AUC_{(0\text{-}\infty)}$ (μg·h/ml)	F(%)[a]
30	p.o.	8.5 ± 0.5	7.9	33.5	66
	i.v.	24.6 ± 1.65	3.9	50.6	
60	p.o.	12 ± 0.3	8.8	50.9	49
	i.v.	112 ± 16.8	3.5	103.9	
120	p.o.	15.6 ± 3	6.2	76.1	37
	i.v.	363 ± 55	3.9	293.6	

[a]F = AUC oral/AUC i.v. Results from refs. 23, 24.

Conclusions

Paclitaxel and docetaxel have represented the only available taxanes for a long time. In recent years, improvements in synthetic chemical procedures and extensive studies in structure-activity relationship in large series of analogs have allowed the discovery of new taxanes for potential clinical use.

Currently, other taxanes have entered phase I clinical trials, including BMS-184476 (39), BMS-188797 (40) and RPR116258A (41, 42). In the meantime, other classes of cytotoxic natural products acting as microtubule depolymerization inhibitors have been discovered and some are currently in preclinical development. They include discodermolide (43), eleutherobin (44, 45), sarcodictyns (45), laulimalides (46) and epothilones (47). Of these compounds, epothilone B and an analog have further progressed to clinical investigation (48-50).

BAY 59-8862 is one of the most promising new taxane analogs. The results presented here demonstrate that the drug is able to overcome resistance mediated by overexpression of transport systems such as MDR and MRP in cell culture systems. This was also documented by in vivo studies using tumor cells growing as tumor xenografts. The pharmacological properties of the compound are also supported by the preclinical profile of antitumor activity in a panel of human tumor models representative of a variety of tumor types. Many gynecological tumors were included in the panel in consideration of the high activity of paclitaxel in breast and ovarian cancers. Investigations using human xenografts in different laboratories showed BAY 59-8862 to have significantly better antitumor efficacy than paclitaxel in most tumors resistant to paclitaxel, and comparable or superior efficacy in responsive tumors. Moreover, the high bioavailability and efficacy of BAY 59-8862 shown after oral delivery, in contrast to the lack of efficacy of oral paclitaxel, opens the way to the clinical use of oral taxanes. Another favorable pharmacological property of BAY 59-8862 is its improved tolerability *in vivo*. Finally, the accumulation of i.v. BAY 59-8862 was substantially better than that of i.v. paclitaxel in the CNS, resulting in good activity against intracranial tumors. This observation suggests that BAY 59-8862 may be active in the treatment of brain metastases or primary brain cancers.

A phase I study of i.v. BAY 59-8862 has already begun at the RPCI and a study of oral administration will follow.

Manufacturer

Indena SpA (IT); licensed to Bayer AG (DE).

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