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SYNTHESIS AND BIOLOGICAL ACTIVITY OF *PARA*-SUBSTITUTED 3'-PHENYL DOCETAXEL ANALOGS

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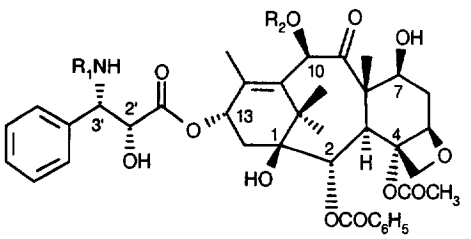
Abstract: An expedient route for semisynthetic *para*-modified 3'-phenyl docetaxel analogs is described using the initial regioselective iodination of the oxazolidinecarboxylate **3**. Palladium catalyzed coupling reactions of the iodo intermediate **4** with alkenyl and aryl boronic acids and alkynes led after alkaline hydrolysis to a series of N,O-protected *para*-substituted phenylisoserines **6**. Coupling with 10-deacetyl-7,10-O-diTroc-baccatin III followed by standard deprotections and acylation afforded the title analogs.

Considerable interest has been expressed recently by both the medical and scientific communities in taxane diterpenoids (taxoids)[1], a novel class of anticancer agents, the most powerful of which are natural paclitaxel (Taxol®) **1** and semisynthetic docetaxel (Taxotere®) **2**. As part of our own structure-activity relationships studies in the docetaxel series, we have been involved in the preparation of analogs bearing modifications on the side chain and particularly on the 3'-phenyl ring.



1, R_1=C_6H_5CO, R_2=Ac (paclitaxel)
2, R_1=t-BuOCO, R_2=H (docetaxel)

We have recently reported preliminary results concerning the preparation and biological activity of modified 3'-phenyl docetaxel analogs[2-5]. Thus, using different stereoselective approaches based on either an asymmetric aldol reaction[3] or a Staudinger [2+2] cycloaddition[2] as the key reactions, we have prepared various phenyl-modified phenylisoserines and then the corresponding docetaxel analogs possessing electron-withdrawing and electron-donating substituents at different positions of the 3'-phenyl group. We observed that, unlike the *ortho* and *meta* positions, the *para*-position tolerates substituents although with modulated activity. Our preliminary conclusion was that electronic effects are likely almost ineffective at *para* while bulky and basic substituents cause a loss in activity.

Recently Georg et al. reported biological results on substituted 3'-phenyl analogs of paclitaxel[6]. Their structure-activity relationship studies were directed by the Topliss Operational Scheme[7]. As in our

case, a relatively flat biological response was observed to the changes in electronic and hydrophobic effects. However it clearly appears that 3'-*para*-substituted analogs have activity similar to paclitaxel in *in vitro* experimental models.

These findings led us to investigate various new substituents at the *para*-position in the docetaxel series and especially alkenyl, alkynyl, hydroxyalkyl and keto-containing groups able to contribute to hydrophobic or electronic interactions with the binding site of taxoids on microtubules. The importance of an hydrophobic moiety in the 3'-position has been recently highlighted by the reported good biological activity of 3'-dephenyl-3'-aliphatic taxoids such as 3'-dephenyl-3'-isobutenyl-docetaxel[8] and 3'-dephenyl-3'-cyclohexyl-docetaxel[9a] and paclitaxel[9b]. Moreover NMR-based observations in protic solvents show the strong probability of a bioactive conformation of docetaxel and paclitaxel involving close hydrophobic interactions between the 3'-phenyl group of the C-13 side-chain, the benzoate at C-2 and the acetate at C-4[10].

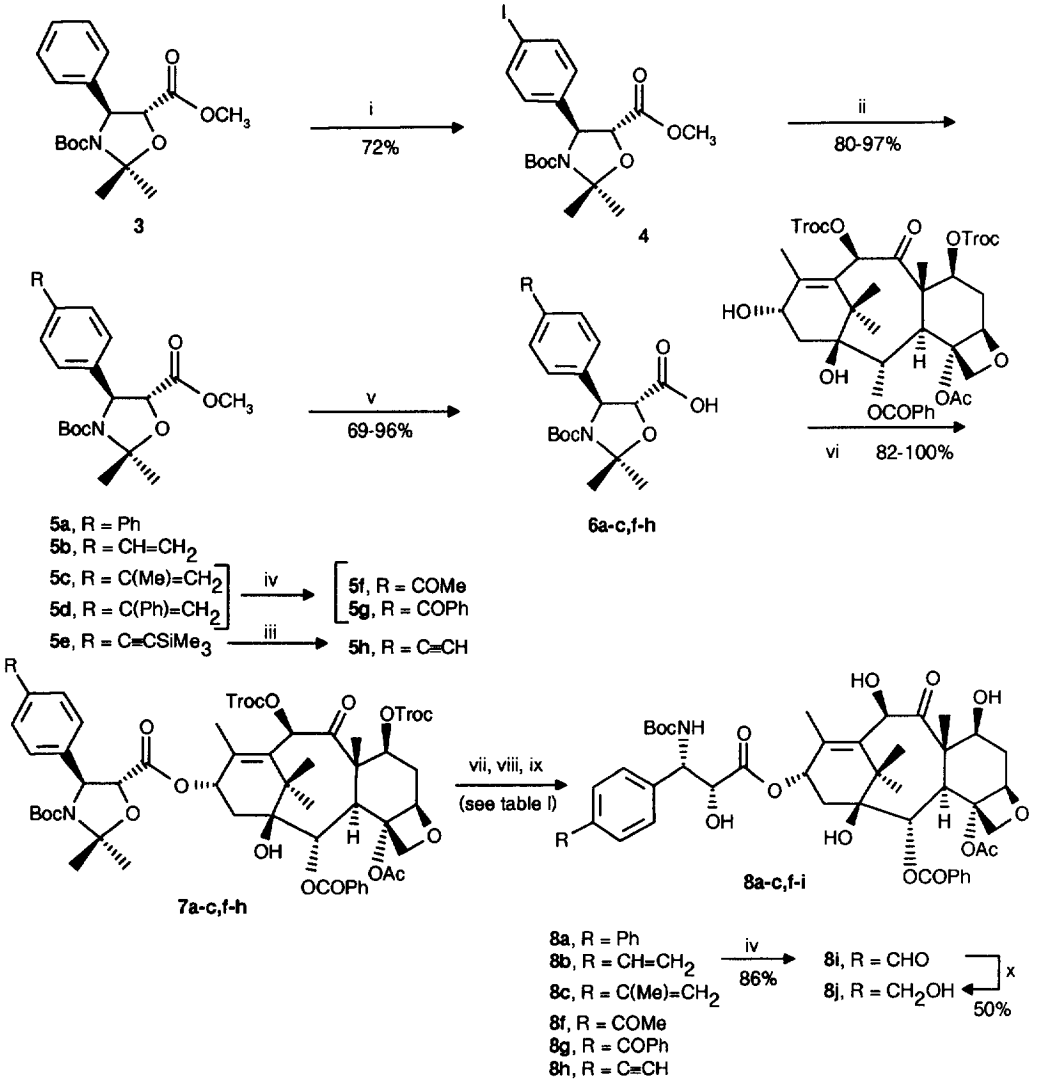
A convergent preparation of such 3'-*para*-substituted docetaxel analogs was achieved using the oxazolidine-type protected phenylisoserinate **3**[11] (Scheme I). This isoserine derivative, obtained by reaction of N-Boc-phenylisoserine methyl ester with methoxypropene, was regioselectively iodinated in *para* using [bis(trifluoroacetoxy)iodo]benzene (PIFA) and iodine to give the iodo-derivative **4** in good yield[12]. This iodo intermediate was submitted to palladium-catalyzed coupling reactions. Thus **4** reacts under Gronowitz conditions[13] with different alkenyl or aryl boronic acids to give esters **5a-d** in excellent yields (Table I). Ketones **5f,g** were obtained from alkenes **5c,d** by ozonolysis. Palladium catalyzed coupling of **4** with trimethylsilylacetylene provides the alkyne derivative **5e**[14]. This compound can be converted to **5h** by removing the trimethylsilyl group under standard conditions[15]. Alkaline hydrolysis of esters **5a-c,f-h** gave the corresponding acids in excellent yields. Esterification at C-13 of 10-deacetyl-7,10-O-diTroc-baccatin III[11,16] (Troc = 2,2,2-trichloroethoxycarbonyl) led to the expected esters again in very good yields. Oxazolidine cleavage with concomitant removal of the Boc group followed by reacylation of the 3'-amino group and removal of the Troc protecting groups led to the desired taxoids **8a-c,f-h** in satisfactory yields[17]. Aldehyde **8i** was obtained by ozonolysis of the corresponding alkenyl precursors **8b**. Such an oxidation has proven highly regioselective since the double bond located at C-11,C-12 displays low chemical reactivity. The *para*-hydroxymethyl analog **8j** was prepared by reduction of aldehyde **8i** with sodium cyanoborohydride at controlled pH.

All these new 3'-*para*-modified docetaxel analogs were evaluated in experimental models. Biological activities were measured in three assay systems, i.e. inhibition of microtubule disassembly, *in vitro* cytotoxicity against murine P388 leukemia cells and *in vivo* antitumor activity against B16 melanoma known as sensitive to docetaxel. Results are summarized in Table II.

Structure-activity relationships can be established from the results obtained with small alkyl, alkenyl and alkynyl groups at the *para*-position (entries 2, 4, 8). *In vitro* cytotoxicity values clearly show that these small groups are well tolerated. However, while all these compounds exhibit comparable activity *in vivo*, an alkenyl analog possessing a bulkier substituent, i.e. iso-propenyl (entry 5), is significantly less cytotoxic against P388 leukemia cells and is inactive *in vivo*. Other bulky substituents have an equally opposing effect on cytotoxicity and microtubule depolymerization properties as demonstrated by the results obtained with the phenyl and benzoyl groups (entries 3, 7). Electron-withdrawing substituents such as acetyl or formyl retain *in vitro* activity (entries 6, 9). Further interesting information follows from the *in*

vivo evaluation of these two compounds: the acetyl analog **8f** which has a higher maximal tolerated dose than docetaxel displays high antitumor activity while the formyl analog **8i** exhibits toxicity which is probably due to the difficulty in formulating the compound for i.v. administration. Lastly the *para*-hydroxymethyl analog **8j** exhibits potent properties *in vitro* but delayed toxicity in animal models (entry 10).

Scheme I



Reagents: i) **3** (1 equiv.), PIFA (1.1 equiv.), I_2 (1 equiv.), CH_2Cl_2, 20°C, 50 min. ii) $RB(OH)_2$, $Pd[P(C_6H_5)_3]_4$ cat., Na_2CO_3, H_2O, MeOH, toluene, reflux, 4 h.; for the preparation of **5e**: $HC{\equiv}CSiMe_3$, $Pd[P(C_6H_5)_3]_4$ cat., Et_2NH, CuI, 20°C, 15 h. iii) $AgNO_3$, EtOH, H_2O, 20°C, 2 h. then KCN, H_2O, 20°C, 18 h. iv) O_3, CH_2Cl_2, MeOH, -70°C, 3 h. then Me_2S. v) LiOH, EtOH, H_2O, 20°C, 1 h. vi) DCC, DMAP, toluene, reflux, 2.5 h. vii) HCOOH, 20°C, 3.5 h. viii) Boc_2O, CH_2Cl_2, $NaHCO_3$, 20°C, 16 h. ix) Zn powder, AcOH, 60°C, 5 min. x) $NaBH_3CN$, MeOH, Et_2O, HCl (pH = 6).

Table I : Chemical yields obtained in the preparation of docetaxel analogs (Scheme I).

Entry	Substituent -R	5 (%)	6 (%)	7 (%)	8a-c,f-h (%)[d] (from 7)	8i (%) (from 8b)	8j (%) (from 8i)
a	$-C_6H_5$	97	87	75	77, 95, 64		
b	$-CH=CH_2$	82	96	84	91, 92, 64		
c	$-C(CH_3)=CH_2$	85	87	96	59, 85, 33		
d	$-C(C_6H_5)=CH_2$	85					
e	$-C\equiv CSiMe_3$	75					
f	$-COCH_3$	86[a]	69	88	54, 79, 53		
g	$-COC_6H_5$	89[b]	71	100	30, 61, 40		
h	$-C\equiv CH$	47[c]	76	95	76, 65, 65		
i	-CHO					86	
j	$-CH_2OH$						50

[a]Obtained from **5c**. [b]Obtained from **5d**. [c]Prepared from **5e**. [d]Yields for steps vii, viii and ix of the synthesis.

Table II : Biological activities of modified 3'-phenyl analogs of docetaxel.

Entry (compound)	Substituent -R	Tubulin assay[18] IC50/IC50 (**1**)	P388 IC50[19] (μg/ml)	B16 Melanoma[20] MTD (mg/Kg/day)	T/C (%)	NCI Score
1 (**2**)	-H	0.64	0.04	13.4	0	++
2	$-CH_3$[a]	1.3	0.04	15.5	8	++
3 (**8a**)	$-C_6H_5$	2.0	0.65	35	40	+
4 (**8b**)	$-CH=CH_2$	0.7	0.06	38.3	19	+
5 (**8c**)	$-C(CH_3)=CH_2$	1.8	0.35	20	105	-
6 (**8f**)	$-COCH_3$	0.95	0.19	35.3	10	++
7 (**8g**)	$-COC_6H_5$	20	>1	20	95	-
8 (**8h**)	$-C\equiv CH$	0.6	0.085	21.7	15	+
9 (**8i**)	-CH=O	0.75	0.13	<13.4	N.E.[b]	
10 (**8j**)	$-CH_2OH$	0.4	0.06	<4.8	N.E.[c]	

[a]Prepared as described in ref.3. [b]Non evaluable: immediate death after injection (insoluble compound). [c]Non evaluable: delayed toxicity.

Our results reinforce the hypothesis of a rather well-defined demand by the docetaxel receptor on microtubules for the 3'-hydrophobic group. While the natural 3'-phenyl group and semisynthetic 3'-alkenyl and 3'-cyclohexyl groups are well accepted, we observe the possibility of regioselective modifications at the *para*-position of the 3'-benzene ring with several selected substituents without dramatic decrease of activity. Moreover the biological results obtained with our *para*-modified analogs show a good correlation between the ability to inhibit microtubules depolymerization and cytotoxicity and highlight the importance of strong hydrophobic interactions of the isoserine side-chain with microtubules.

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    MTD (maximal tolerated dose) is the daily dose which can be administered without toxicity and without causing a body weight loss > 20%. Docetaxel was highly active at the MTD of 13.4 mg/kg/day with a T/C of 0%. Mice were checked daily and adverse clinical reactions were noted.

    The T/C value expresses the tumor growth inhibition which is the most widely used criterion for the determination of antitumor activity for early stage disease. The tumor weight was determined simultaneously for the treated and the control groups: T is the median tumor weight of the treated mice and C is the median tumor weight of the control mice. T/C values were generally determined when C reached approximately 750 to 1500 mg. According to NCI standards, a T/C ≤42% is the minimum level for activity. A T/C <10% is considered as a high antitumor activity level which justifies further development. For further details see: Bissery, M-C.; Guénard, D.; Guéritte-Voegelein, F.; Lavelle, F. *Cancer Res.* **1991**, *51*, 4845-4852.

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