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# Synthesis and HSA binding characterisation of the water soluble 7-succinylpaclitaxel

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**Abstract**

A water soluble paclitaxel analogue, the 7-hemisuccinylpaclitaxel, was synthesised and its binding to human serum albumin (HSA) was characterised by difference circular dichroism and optical biosensor methodologies. The carboxylate group was introduced at paclitaxel C-7 position to improve the drug water solubility without significantly changing the biological activity. The paclitaxel analogue showed a relatively low affinity to HSA ($3.5 \times 10^4$ $M^{-1}$), while no significant interactions were evidenced with selective markers for the most characterised binding sites on the carrier, suggesting a non-selective binding to low affinity binding sites.
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*Keywords:* 7-Hemisuccinylpaclitaxel; HSA binding; Circular dichroism; Optical biosensor; IAsys; Affinity constant

## 1. Introduction

Paclitaxel (trade name Taxol®, PTX, Fig. 1) is a complex diterpene natural product, originally isolated in the late 1960s from the bark of the western yew, *Taxus Brevifolia* [1]. This compound has shown excellent antitumour activity in a wide variety of tumour models and has been found to be an active agent against drug refractory ovarian cancer. PTX is nowadays commonly used in therapy against ovarian and breast cancer [2,3]. Unfortunately, PTX has extremely low solubility in water, which makes it difficult to provide a suitable dosage form. For this reason, it is administered with emulsifiers causing severe allergic reactions. PTX is currently formulated in a vehicle composed of 1:1 blend of Cremophor EL (polyethoxylated castor oil) and ethanol, which is diluted with 5–20-fold in normal saline or dextrose solution (5%) for administration. Because of the relatively large volumes of the PTX formulation needed, the patients receive large doses of the highly toxic surfactant/ethanol mixture. A high incidence of idiosyncratic histamine release has been proved after exposure to cremophor [4]. The low solubility of PTX could be overcome by the development of a water soluble, chemically stable analogue. In this paper, we focused on the preparation, and successive binding studies to human serum albumin (HSA) of 7-succinylpaclitaxel (7-sPTX, Fig. 1), whose structure was selected to improve the water solubility of PTX because of the presence of the additional carboxylic group. The nature of the substituent at the C-7 position is not expected to affect significantly the biological activity of PTX: in fact, a large number of the structure-activity relationships studies have led to the general conclusions that the 'northern hemisphere' function groups at C-7, C-9 and C-10 have little effect on bioactivity [5–7]. Several PTX analogues have been obtained by chemical derivatisation at C-7 and the compounds have been shown to be as active as the parent drug in altering cell proliferation and microtubule polymerisation [8–10]. The synthesis of this type of

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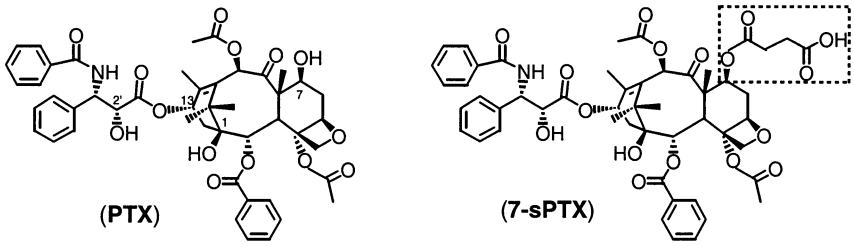


Fig. 1. Structure of paclitaxel (PTX) and 7-succinylpaclitaxel (7-sPTX).

taxoids is also important for checking their activity in PTX resistant tumour models. In fact the C-7 to C-10 region of PTX does not interact directly with tubulin, but it has been postulated that changes in this region of the molecule may affect the specific binding of PTX to P-glycoprotein. In particular it has been proposed that the ability of C-10 derivatives to overcome multidrug resistance in vitro is a result of reduced binding affinity for P-glycoprotein [11]. In addition, evidence for the proximity of C-7 to the P-glycoprotein binding site of PTX was suggested by the fact that attachment of a photoaffinity label to the C-7 hydroxyl group of PTX successfully labelled P-glycoprotein [12].

The activity of PTX and of its structural analogues involves several cellular pathways, these phenomena being essential for a better understanding of the biological profile of these compounds. HSA and other serum carriers are target proteins, being the free fraction of the drug in serum regulated by its binding to the carrier [13] and the binding process significantly affecting the solubility of the drug in the aqueous medium [14].

With this aim, we attempted to better characterise the HSA binding site of the water soluble 7-sPTX by displacement experiments. Competitive experiments using difference circular dichroism (CD) were performed and selective ligands were carefully chosen as markers for the different binding areas on the HSA protein, employing 7-sPTX as competitor. Furthermore, in order to define the affinity entity of this compound with the protein we carried out optical biosensor experiments by mean of an IAsys resonant mirror (RM)-based principle biosensor. This device allowed determination of the affinity constant of 7-sPTX to the immobilised protein, being suitable to monitoring biomolecular interactions on real-time. Where possible, the system permits as well to follow the kinetics of the binding process.

## 2. Results and discussion

### 2.1. Chemistry

The chemistry of PTX has been deeply investigated both on the diterpene moiety and on the side chain modification. 7-sPTX has been successfully synthesised following literature with slight modifications [10,16]. Protection at C2′–OH, which is normally the most reactive and only when protected, reaction proceeds selectively at the C-7 hydroxyl group, was quantitatively accomplished by following Rao et al. procedure [15]. The final product was obtained in accordance with Ojima and co-workers [16] and isolated pure and in good yield by mean of purification protocol used by Raybould and co-workers [10]: spectroscopic data are in agreement with those from literature. Reported values for PTX solubility give evidence of some discrepancies: this is probably due to the existence of a hydrate form with an aqueous solubility lower than the anhydrous [17]. In fact, dissolution studies have shown an initially higher apparent solubility of 6 μg $mL^{-1}$, with a decrease to 0.34 μg $mL^{-1}$ after a 24-h period. This effect could be related to an 'hydrophobic collapse' of PTX: a process whereby PTX changes its conformation in aqueous compared to organic solvents as a consequence of hydrophobic clustering of non-polar groups and loss of intramolecular hydrogen bonds [18]. The aqueous equilibrium solubility of the synthesised product was determined in 50 mM phosphate buffer, pH 7.4 by HPLC, which revealed that solubility is considerably higher than PTX. A value of 1.2 mg $mL^{-1}$ was determined by quantitative evaluation out of a calibration curve ($r^2 = 0.9987$).

### 2.2. Biology

#### 2.2.1. IAsys biosensor technology

For protein analyses optical biosensors are attractive advanced alternative tools for real-time monitoring biomolecular interactions [19–21]. The methodology developed a label-free system based on either surface plasmon resonance technology, BIAcore, (Sweden), or RM technology, IAsys, (England). The underlying principle of biosensors is 2-fold: the use of a ligand immobilised on a solid phase as an affinity surface to bind one or more soluble analytes and the measurement of mass migration on the affinity surface to quantify the binding properties. This technology represents an efficient improvement in the bioprocessing environment: specifically, for determining binding parameters of drug/

proteins interactions, it offers many advantages with respect to most of the conventional methodologies, usally labour-intensive and time-consuming assays, i.e. ELISA-based immunoassays, equilibrium dialysis, or chromatography. Above all, the methodology is a label-free system and the amount of both ligand and analyte needed to obtain informative results is very low, making this miniaturised system well suited for drug screening for their binding to target proteins as well as for target protein screening to a lead structure. Moreover, the sensor chip can be re-used many times and this leads to low running costs, obviously once the stability of the immobilised ligand is verified. The assay is rapid either in the ligand immobilisation (usually requiring 20–25 min) and in the ligand-analyte interaction study. In addition to this, there is a large variety of activated sensor chips commercially available permitting to choose the most suited immobilisation way in dependance to the biological system into exam. On the other hand, great attention should be paid to the data interpretation since there could be different technical problems with this methodology. Any change into the cell, i.e. viscosity of organic solvents, ionic strength, pH etc., leads to a high change in refractive index which must be distinguished from the real binding signal. This is particular true in detection of low molecular weight analytes where suitable blank subtraction and control experiments should undertaken. Associated to this, inactivation of the biological surface could happen if running and regeneration buffers are not chosen carefully enough. Furthermore the concentration of the ligand on the cell surface of the chip should be as low as possible in order to minimise mass transport effects during the experiment cycles.

### 2.2.2. IAsys instrumentation

All analyses were performed using IAsys Plus optical biosensor (Labsystems Affinity Sensors, Cambridge, England). This instrument employs a dual-well stirred cuvette, where the sample is added in a single step, to deliver the material under investigation to the immobilised ligand. The optical biosensor was operated at 25 °C. The data were collected at the fastest data collection rate (0.3 $s^{-1}$) and a 100% stirring in order to minimise mass transport effects. Biosensor data were analysed using GRAFIT V. 5.0.0.36 software. GRAPHPAD PRISM 3.0 software from GRAPHPAD PRISM was also used to fit within a linear regression approach Scatchard Plots.

IAsys is based on the RM principle [22,23]: a RM is integrated with the micro-cuvette device and permits to follow the optical phenomenon of an evanescent field. Changes in refractive index at the surface of the device, or the biological layer, change the angle at which light can be made to propagate in the waveguide: conditions for propagation are met at only one discrete angle (resonant angle). The change in angle is linear with respect to mass.

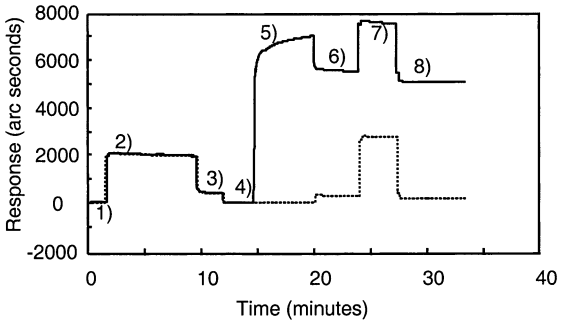


Fig. 2. Sensorgram of the HSA immobilisation to the CMD matrix: (1) buffer baseline stabilisation; (2) EDC/NHS mixture add (7 min of reaction); (3) buffer washes to remove unreacted molecules; (4) acetate buffer re-equilibration; (5) HSA in acetate buffer add (7 min of reaction); (6) buffer washes to allow dissociation; (7) block of non-coupled activated CMD sites with ethanolamine (3 min of reaction); (8) baseline stabilisation. The light line indicates the reference channel signal.

### 2.2.3. Determination of the equilibrium dissociation constant ($K_D$) of 7-sPTX/HSA complex

*2.2.3.1. Immobilisation of HSA.* The amine coupling method was used to immobilise HSA to the carboxydextran matrix. A typical sensorgram of the immobilisation process is shown in Fig. 2. The amount of the immobilised protein is determined by measuring the biosensor response upon ligand binding between phases eight and three and considering that 1 ng $mm^{-2}$ HSA gives a 200 arcsecond signal. In the case of the present investigation the immobilised HSA was 23 ng $mm^{-2}$. The carboxydextran matrix of the reference channel was reacted as the experiment channel, but without anchoring the protein. This procedure is important in order to follow non-specific binding phenomena on the biosensor surface.

*2.2.3.2. Binding experiments.* 7-sPTX was investigated for its reversible binding to the serum carrier. A steady state was reached in a few seconds, after the drug was added to the cuvette with the HSA modified Carboxymethyl dextran (CMD) surface. Then 7-sPTX dissociated very rapidly when the ligate solution was replaced by running buffer. The overlaid sensorgrams in Fig. 3 were obtained using different concentrations of 7-sPTX ($3.0 \times 10^{-6}$ to $2.0 \times 10^{-4}$ M) in the cuvette. As dissociation process was practically immediate after running buffer washes, only association phase of the experiment was worthy to be studied, the steady state value (at a selected time, i.e. always after 2 min binding) at every different concentration being considered the $R_{eq}$ (response at equilibrium). These values were then

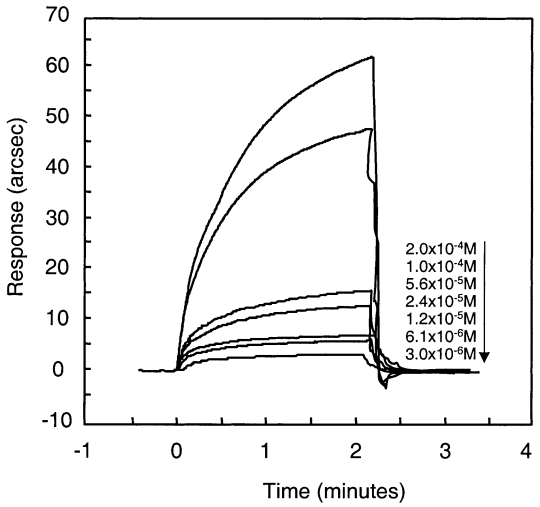


Fig. 3. Corrected sensorgram overlays for 7-sPTX binding to HSA. Binding and dissociation processes at the indicated 7-sPTX concentrations.

corrected by subtracting the reference channel response and plotted against concentrations.

Equilibrium data ($R_{eq}$), as obtained at fixed time of binding, may be analysed by two related approaches, Scatchard analysis and binding curve analysis: a comparison of equilibrium constants derived by these two different approaches provides a useful check on the validity of the method. In the Scatchard analysis a plot of $R_{eq}/[L]$ vs. $R_{eq}$ yields a slope of $-K_A$ with an $x$ axis intercept of $R_{max}$. The $y$ intercept is equivalent to $K_A R_{max}$ (Fig. 4). For the binding curve analysis approach a binding curve was made: if the binding curve is Langmuirian, $K_D$ can be obtained directly from the binding curve, as being equal to the ligate concentration at $R_{max}/2$ (Fig. 5). To generate this plot, experimental data were fitted to the equation

$$R_{eq} = \frac{R_{max}[L]}{K_D + [L]}$$

by mean of GRAFIT v. 5.0.0.36 software. The equation fits equilibrium response ($R_{eq}$) data at different ligate concentrations to give the dissociation equilibrium

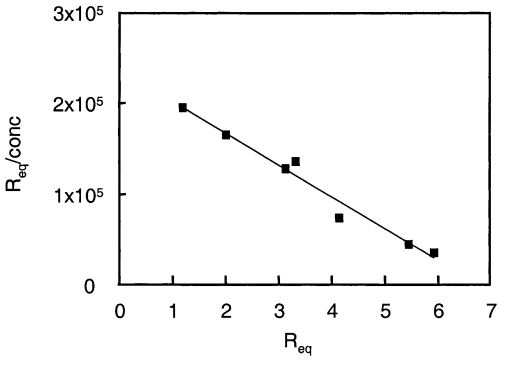


Fig. 4. Scatchard Plot of the 7-sPTX binding to HSA.

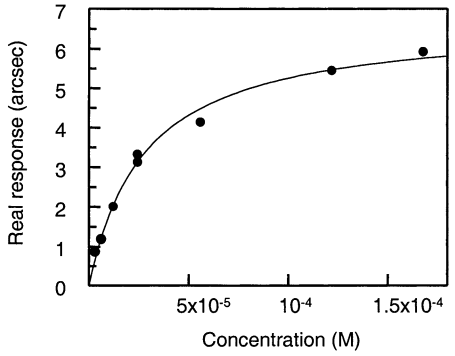


Fig. 5. Response data at equilibrium vs. 7-sPTX concentration.

constant ($K_D$) where $R_{max}$ is the maximum capacity of the ligand for the ligate.

Based on this methodology, the equilibrium dissociation constant $K_D$ of 7-sPTX for HSA, was found to be $2.79 \times 10^{-5} \pm 2.82 \times 10^{-6}$ M. The same data were also analysed by using Scatchard regression method: this analysis yielded value for $K_A$ of $3.51 \times 10^4 \pm 2.60 \times 10^3$ $M^{-1}$ ($K_D$: $2.85 \times 10^{-5}$ M). Regression value ($r^2$) for the linear fit of the data was 0.973. $K_D$ value is essentially the same as that obtained from the binding isotherm analysis. In order to determine the reproducibility of the assay, the experiment was performed several times as well as on different HSA cuvettes.

One of the applications of this technique is to utilise biosensor as a tool for screening libraries of drugs with the ambitious aim to develop new targets considering their binding affinity to HSA. In this way, it is very important to determine the HSA binding affinity as well as the percentage of bound values, considering a fixed concentration of protein and ligate. Reports from Refs. [24,25] demonstrated the potential of the methodology for screening drugs for their binding to HSA, demonstrating a throughput of 100 componds/24 h, with extremely low consumption of ligates [25]. Taking into exam some considerations by Myszka and co-workers [24], we attempted to evaluate the bound percentage of 7-sPTX. We assumed HSA concentration to be 0.68 mM to approximate the physiological concentration of albumin in serum, and drug concentration equal to 0.01 mM reproducing the drug concentration used in standard dialysis assay. Under these conditions, a 96 bound percentage of ligate was evaluated. It is worth mentioning that the optical biosensor has been successfully applied to the screening of drugs also to other target biomolecules [19,25–27], by measuring directly the binding of the small molecule to the biopolymer or by amplifying the signal by indirect monitoring. Indeed, the low molecular weight of the drug could limit the applicability of the biosensor to get information on the biorecognition mechanism, being this technique based on the measurement of a mass change on the biosensor surface. However, this limit is going to be overcome by

the increasing of the new instrument sensibility, or by following different strategies for the experiments. The observed signal can be amplified by developing displacement experiments with a reference compound in the presence of its specific antibody. Alternatively, the experiment can be performed in an upside down manner, i.e. immobilising the smaller molecule and so measuring the binding signal of a protein or enzyme or nucleic acid, which indeed gives an higher signal.

### 2.2.4. HSA binding of 7-sPTX by difference CD spectroscopy

Difference CD spectroscopy was used to characterise the binding properties of 7-sPTX to HSA. To this end, the induced CD spectra of three ligands (bromophenol blue, *rac*-ketoprofen and bilirubin) complexed to HSA were measured. The change of the induced spectra upon the adding of increasing concentrations of 7-sPTX was then monitored. The selected ligands are known to bind to specific binding areas on HSA, as highest affinity binding sites [13,28]. In particular bromophenol blue binds at site I, and *rac*-ketoprofen to site II, following the notation by Sudlow et al. [29]. The last ligand, bilirubin, is known to bind to the bilirubin binding site, also reported as site III [30]. This allowed to get information on the type of interaction of 7-sPTX occurring at any of these binding areas, which account of the binding of most of the drugs. Actually, no significant changes have been observed in the value of the induced CD spectra upon increasing the concentration of 7-sPTX added to the solution of the marker/HSA complexes. The PTX analogue, used as competitor, has been added up to [7-sPTX]/[marker] 8/1 ratio in the case of bromophenol blue (Fig. 6a), and up to [7-sPTX]/[marker] 15/1 ratio in the case of *rac*-ketoprofen and bilirubin (Fig. 6b and c). Thus 7-sPTX did not affect significantly the binding at the studied binding area, this suggesting that none of the most important binding sites, i.e. sites I, II and III, represents its high affinity binding site. This behaviour is in agreement with the relatively low $K_A$ value determined for 7-sPTX by optical biosensor measurements, suggesting a non-selective binding at low affinity binding sites. Further support to this hypothesis arises from the observed low selectivity in the binding of 7-sPTX to HSA. Indeed, no significant differences have been observed in the CD spectrum of the free and the HSA bound 7-sPTX (Fig. 7), while the binding to sites I, II and III usually determines some extent of stereoselectivity [31].

Thus, even if a binding of 7-sPTX to sites I, II, and III on HSA cannot be excluded, all the obtained data indicate a low affinity binding process at several binding sites.

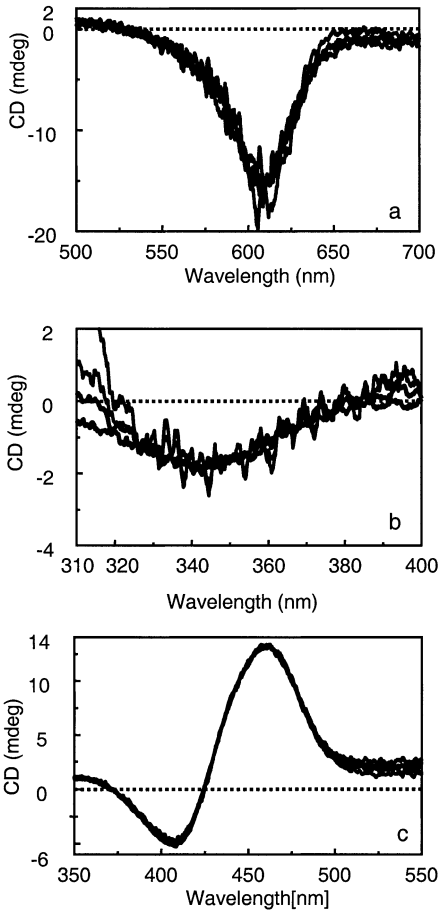


Fig. 6. Induced CD spectra of the [drug]/[HSA] 1/1 complexes in the presence of increasing concentrations of 7-sPTX. [HSA] 0.015 mM, phosphate buffer 50 mM, pH 7.4, 1 cm cell. (a) [7-sPTX]/[bromophenol blue] 0/1, 1/1, 3/1, 8/1; (b) [7-sPTX]/[*rac*-ketoprofen] 0/1, 5/1, 10/1, 15/1; (c) [7-sPTX]/[bilirubin] 0/1, 1/1, 3/1, 6/1, 15/1.

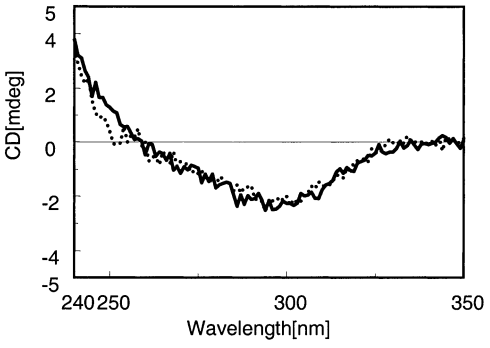


Fig. 7. CD spectra of free (—) and HSA bound (------) 7-sPTX in phosphate buffer 50 mM, pH 7.4, 1 mm cell, [7-sPTX] and [HSA] 0.075 mM.

## 3. Experimental section

### 3.1. Chemistry

$^1$H- and $^{13}$C-NMR spectra were recorded on a Varian VXR-400 MHz spectrometer with $Me_4Si$ (in $CDCl_3$) as

internal standards. Chloroform-*d* was used as solvent; $Me_4Si$ ($\delta$ 0.00 ppm) was used as an internal standard. Anhydrous conditions were achieved by flame-drying flasks and equipments. Reactions were monitored by TLC (thin layer chromatography) on Merck 60 F254 (0.25 mm) plates. Commercially available reagents and solvents were used without further purification, unless otherwise noted. Methylene chloride ($CH_2Cl_2$) was dried by distillation from calcium hydride ($CaH_2$). Paclitaxel was provided by Indena S.p.A., Milan, Italy.

#### 3.1.1. Water solubility determination

2.20 mg of 7-sPTX was suspended in 50 mM phosphate buffer, pH 7.4. The suspension was sonicated (Sonorex Super RK-103H) for 10 min at 25 °C and then centrifuged (5160 g, 8 min). The HPLC analysis was performed by a Jasco PU-980 pump interfaced with a Jasco multi wavelength detector (MD-910, Tokyo, Japan) using a waters spherisorb 5 μm –CN, 4.6 × 250 mm, analytical column. Quantitative determination was obtained by mean of a calibration curve ($r^2 = 0.9987$): standards were diluted in mobile phase mixture (50 mM phosphate buffer, pH 7.4/MeOH/$CH_3CN$: 65/18.5/16.5) within a concentration range of 0.1 up to 2.20 mg $mL^{-1}$.

### 3.2. Biology

#### 3.2.1. IAsys experiments

CMD dual-well hydrogel cuvettes were from Labsystems Affinity Sensors. Ethanolamine, *N*-hydroxysuccinimide (NHS), and 1-ethyl-3-(3-*N*,*N*-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) were purchased as NHS coupling kit from Affinity Sensors. The solutions were prepared and stored as explained into the protocol. Phosphate buffered saline (PBS/T), final pH 7.4, was prepared using Sigma dry powder in foil pouches. Each pouch dissolved in 1 l of deionised water yield 0.01 M phosphate buffered saline, NaCl 138 mM, KCl 2.7 mM, Tween 20 0.05, pH 7.4 at 25 °C. Acetate buffer (Sigma Aldrich, Milan, Italy), 10 mM, was adjusted to pH 5 with glacial acetic acid (Sigma). All solutions were made using ultra high purity water. HSA, essentially fatty acids free, was supplied by Sigma Aldrich and it was used without further purification. All reagents were analytical grade and were used without further purification.

#### 3.2.2. HSA immobilisation

The protein was immobilised through its surface amine groups *via* amide bonds with the CMD. The running buffer was PBS/T: 10 mM phosphate buffered saline pH 7.4, 0.05% Tween 20, (NaCl 136 mM, KCl 2.7 mM, 0.05% w/v Tween 20). CMD hydrogel in the cells was activated with a 1:1 EDC/NHS mixture (100 mM NHS and 400 mM EDC) for 7 min. HSA was added at a 1 mg $mL^{-1}$ concentration in sodium acetate 10 mM, pH 5 and let reacted for 7 min. Unreacted NHS-esters were blocked by washing with 1 M ethanolamine pH 8.5 for 3 min. PBS/T was added to stabilise the baseline. To determine the reproducibility of the assays, the entire experiments were replicated over different HSA surfaces: the protein was immobilised on different cuvettes for several times leading to different immobilised density values averaging from 1700 to 4700 arcsecond corresponding to 8–23 ng $mm^{-2}$ of anchored protein respectively.

#### 3.2.3. Preparation of sample solutions

7-sPTX was dissolved in 50 mM phosphate buffer (PB) and then sonicated for 5 min at 25 °C (Sonorex Super RK-103H). The same buffer in which the solutions were prepared was used as running buffer for the experiments. The solution was freshly prepared as a concentrated stock solution and immediately prior to analysis was diluted with running buffer: a suitable range of concentrations was diluted into the cuvette. Addition of 7-sPTX by dilution into the cuvette (usually 1–10) avoided disturbance of the baseline.

### 3.3. Circular dichroism measurements

CD spectra were recorded using a Jasco J-810 spectropolarimeter (Jasco, Tokyo, Japan). The instrument was interfaced to personal computer to acquire and elaborate data. All measurements were carried out at room temperature using 1 cm and 1 mm pathlength cells. The same instrumental parameters were employed to reduce the errors: time constant 4 s, scan speed 20 nm $min^{-1}$, resolution 0.2 nm, sensitivity 20 mdeg, sbw 1. Solutions of the protein were prepared in phosphate buffer (pH 7.4, 50 mM) and actual concentrations were determined from the absorbance at 280 nm ($\varepsilon_{280}$ 32180 calculated as optical density per mol of protein) [32]. Bromophenol blue, *rac*-ketoprofen and bilirubin were purchased from Sigma Aldrich. The solutions of the protein and of the ligands were prepared immediately before their use. Competition experiments were carried out on [marker]/[HSA] 1/1 complexes. HSA concentration was kept constant (0.015 mM), while the concentration of the competitor was varied according to the required ratio. Difference CD methodology were carried out selecting achiral or racemic markers, thus the interaction with the protein is responsible for the observed induced CD signal, which arises only from the complexed ligand and it will selectively reflect the steroselective binding. The competitor (7-sPTX) does not show any electronic transitions, and then any induced CD signal, in the spectral range where the induced CD of bromophenol blue and bilirubin was measured (350–700 nm). On the contrary a CD band is observable for 7-sPTX at about 300 nm, this making

necessary to subtract its contribution when analysing the displacement experiments of complexed *rac*-ketoprofen.

## 4. Conclusions

The synthesised 7-sPTX showed much higher solubility with respect to the parent compound. A 96 bound percentage of ligate to HSA was evaluated for PTX analogue under protein physiologic conditions, with an affinity constant of $3.5 \times 10^4$ $M^{-1}$. However, no significant interactions have been evidenced with the most important binding sites on HSA, as studied by displacement experiments using difference circular dichroism spectroscopy.

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## References

[1] M.C. Wani, H.L. Taylor, M.E. Wall, P. Coogan, A.T. McPhail, J. Am. Chem. Soc. 93 (1971) 2325–2326.
[2] Holmes F.A., Kudelka A.P., Kavanagh J.J., Huber M.H., Ayani J.A., Valero V., Taxane Anticancer Agents: Basic Science and Current Status, in: Georg G.I., Chen T.T., Ojima I., Vyas D.M. (Eds.), ACS Symposium Series 583, American Chemical Society, Washington, DC, 1995, pp. 31–57.
[3] M. Suffness (Ed.), Taxol: Science and Applications, CRC Press, New York, 1995, p. 1995.
[4] A.K. Singla, A. Garg, D. Aggarwal, Int. J. Pharm. 235 (2002) 179–192.
[5] S.H. Chen, V. Farina, in: V. Farina (Ed.), The Chemistry an Pharmacology of Taxol and Related Compounds, Elsevier, New York, 1995, pp. 165–253.
[6] K.C. Nicolaou, W.-M. Dai, R.K. Guy, Angew. Chem., Int. Ed. Engl. 33 (1994) 15.
[7] G.I. Georg, T.C. Boge, Z.S. Cheruvallath, J.S. Clowers, G.C.B. Harriman, M. Hepperle, H. Park, in: M. Suffness (Ed.), Taxol: Science and Applications, CRC Press, Inc, Boca Raton, FL, USA, 1995, pp. 317–375.
[8] A.E. Mathew, M.R. Mejillano, J.P. Nath, R.H. Himes, V.J. Stella, J. Med. Chem. 35 (1992) 145–151.
[9] F. Guéritte-Voegelein, D. Guénard, F. Lavelle, M. Le Goff, L. Mangatal, P. Potier, J. Med. Chem. 34 (1991) 992–998.
[10] P.G. Grothaus, T.J.G. Raybould, G.S. Bignami, C.B. Lazo, J.B. Byrnes, J. Immunol. Methods 158 (1993) 5–15.
[11] T.J. Altstadt, C.R. Fairchild, J. Golik, K.A. Johnston, J.F. Kadow, F.Y. Lee, B.H. Long, W.C. Rose, D.M. Vyas, H. Wong, M. Wu, M.D. Wittman, J. Med. Chem. 44 (2001) 4577–4583.
[12] I. Ojima, S.D. Kuduk, S. Chakravarty, Recent advances in the medicinal chemistry of taxoid anticancer agents, in: B.E. Maryanof, A.B. Reitz (Eds.), Adv. Med. Chem. Greenwich, CT: JAI Press, 1998, pp. 69–124.
[13] T. Peters, Jr., All About Albumin, Biochemistry, Genetics, and Medical Applications, Academic Press, New York, 1996.
[14] M.A. Lovich, C. Creel, K. Hong, C.W. Hwang, E.R. Edelman, J. Pharm. Sci. 90 (2001) 1324–1335.
[15] C.S. Rao, J. Chu, R. Liu, Y. Lai, Bioorg. Med. Chem. 6 (1998) 2193–2204.
[16] S. Lin, K. Fang, M. Hashimoto, K. Nakanishi, I. Ojima, Tetrahedron Lett. 41 (2000) 4287–4290.
[17] R.T. Liggins, W.L. Hunter, H.M. Burt, J. Pharm. Sci. 86 (1997) 1458–1463.
[18] D.G. van der Velde, G.I. Georg, G.L. Grunewald, C.W. Gunn, L.A. Mitscher, J. Am. Chem. Soc. 115 (1993) 11650–11651.
[19] R. Gambari, Curr. Med. Chem. 1 (2001) 277–291.
[20] K.N. Baker, M.H. Rendall, A. Patel, P. Boyd, M. Hoare, R.B. Freedman, D.C. James, Trends Biotech. 20 (2002) 149–156.
[21] W.D. Wilson, Science 295 (2002) 2103–2105.
[22] R. Cush, J.M. Cronin, W.J. Stewart, C.H. Maule, J. Molloy, N.J. Goddard, Biosensors Bioelectron. 8 (1993) 347–353.
[23] P.E. Buckle, R.J. Davies, T. Kinning, D. Yeung, P.R. Edwards, D. Pollard-Night, C.R. Lowe, Biosensors Bioelectron. 8 (1993) 355–363.
[24] R.L. Rich, Y.S. Day, T.A. Morton, D.G. Myszka, Anal. Biochem. 296 (2001) 197–207.
[25] A. Frostell-Karlsson, A. Remaeus, K. Andersson, P. Borg, M. Hämäläinen, R. Karlsson, J. Med. Chem. 43 (2000) 1986–1992.
[26] E. Danelian, A. Karlén, R. Karlsson, S. Winiwarter, A. Hansson, S. Löfås, H. Lennernas, M.D. Hämäläinen, J. Med. Chem. 43 (2000) 2083–2086.
[27] M. Alterman, H. Sjöbom, P. Säfsten, P. Markgren, H. Danielson, M.D. Hämäläinen, S. Löfås, J. Hultén, B. Classon, B. Samuelsson, A. Hallberg, Eur. J. Pharm. Sci. 13 (2001) 203–212.
[28] U. kragh-Hansen, Pharmacol. Rev. 33 (1981) 17–53.
[29] G. Sudlow, D.J. Birkett, D.N. Wade, Mol. Pharmacol. 11 (1975) 824–832.
[30] C. Bertucci, Chirality 13 (2001) 372–378.
[31] C. Bertucci, E. Domenici, Curr. Med. Chem. 9 (2002) 1463–1481.
[32] M.L. Elwell, J.A. Shellman, Biochim. Biophys. Acta 494 (1977) 367–383.