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# A novel bioassay for screening and quantification of taxanes

**Sergi Morais, P. C. Pandey†, Wilfred Chen and Ashok Mulchandani***
*Department of Chemical and Environmental Engineering, University of California, Riverside, CA 92521, USA. E-mail: adani@engr.ucr.edu; Fax: 909-787-5696; Tel: 909-787-6419*

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**We report a novel method for screening and quantifying tubulin-binding antimitotic agents that promote microtubule formation. The method is based on the shift in the peak of the fluorescence emission difference spectrum of tubulin complexed to metal free tetrakis(4-carboxyphenyl)porphyrin (TCPP) in the presence of antimitotic agents. Taxol® (paclitaxel), an anti-tumor drug approved for the treatment of a variety of cancers, caused the appearance of a new fluorescence peak at 645 nm at concentrations as low as 125 nM, the intensity of which was a function of the paclitaxel concentration. Cephalomannine caused the induction of a new fluorescence peak at 651 nm only above 1 μM. Baccatin did not induce the appearance of any new peak within detectable operating measurement conditions. These observations are in accordance with the biological activities/cytotoxicities of these compounds. Accordingly, it is proposed that the new method can be used for high throughput screening of antimitotic compounds.**

Antimitotic agents are a class of compounds having tumor inhibiting activity and chemotherapeutic significance.[1] Amongst them, Taxol® (a registered trademark of Bristol-Meyers Squibb Co.; the generic name of the drug is paclitaxel) has demonstrated anti-cancer activity against several classically refractory tumors. It has already been approved for the treatment of ovarian, breast and non-small lung cancers and AIDS related Kaposi's sarcoma and is under investigation for the treatment of other cancers.[2] The effectiveness of Taxol® as an anti-cancer agent has been attributed to its anti-microtubule activity, or more specifically to its binding to the protein tubulin with subsequent polymerization and stabilization of the polymerized tubulin. This causes the protein to lose its flexibility with the arrest of mitosis even in the absence of the microtubule associated proteins.[3,4] This knowledge has stimulated efforts for the search of alternative fully synthetic routes and renewable sources to cover the supply and demand gap of Taxol® and for new antimitotic agents with higher tumor inhibiting activity.

Current methods of screening the anti-tumor activities of potential antimitotic agents rely on slow, laborious and costly cell culture techniques. Additionally, chromatography-based analytical techniques currently used for quantitative determination of the selected drug in biological samples of patients undergoing treatment are unable to provide the much desired receptor level concentration. Therefore, novel analytical approaches to rapidly screen anti-tumor compounds and possibly determine the concentration of these compounds during the synthesis and subsequently in biological samples during treatment are highly desirable. The present investigation reflects research on this topic.

Amino acids, small peptides, proteins and small organic compounds are known to alter the spectrum of porphyrins and metalloporphyrins with the wavelength shifts being different for different molecules.[5] The shift is attributed to the conformational changes as a result of the binding interaction between the porphyrin and biomolecule. Porphyrins have been reported to reversibly inhibit acetylcholinesterase, a significant enzyme in the central nervous system.[6] Addition of a stronger competitive inhibitor such as tetracaine and procaine, displaced the porphyrin.[6a,b] Here we have taken advantage of the ability of porphyrin to form a complex with tubulin, and the subsequent recognition of the bound porphyrin by antimitotic agents to develop a simple, rapid and cost-effective method for the screening and quantification of these compounds. This represents, to our knowledge, the first example of a non-cell-based screening approach for such agents.

Tetrakis(4-carboxyphenyl)porphyrin (TCPP) (Fig. 1)[7] is an excellent photoactive porphyrin moiety that shows a sharp absorption at 412 nm (Fig. 2, inset A) and further emits fluorescence at 642 nm when excited at 412 nm (Fig. 2, trace 1). Because the emission peak falls within a novel wavelength

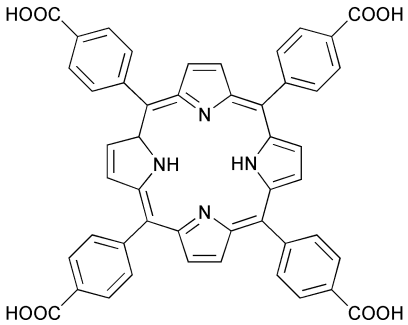


**Fig. 1** Chemical structure of *meso*-tetrakis(4-carboxyphenyl)porphyrin

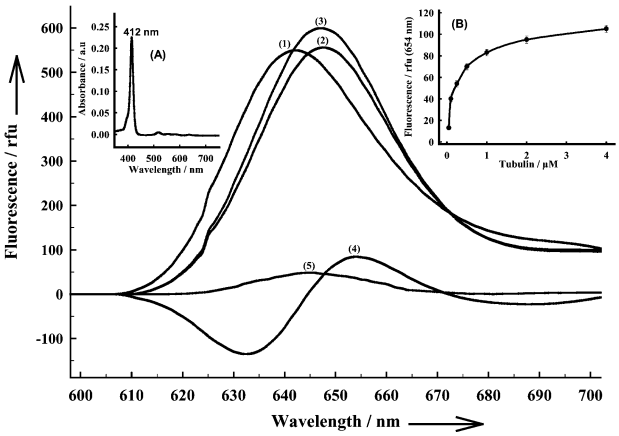


**Fig. 2** Spectra in 50 mM phosphate buffer, pH 7.5 of 0.4 μM TCPP alone (trace 1), 0.4 μM TCPP in the presence of 1.0 μM tubulin (trace 2), and 0.4 μM TCPP in the presence of 1.0 μM tubulin plus 10 μM Taxol® (trace 3). Trace 4 is the result of subtracting trace 1 from trace 2 (peak at 654 nm) while trace 5 is the result of the difference spectra between trace 3 and trace 2 (peak at 645 nm). Inset A shows the absorbance spectra of 0.4 μM TCPP. Inset B shows the dependence of the total fluorescence intensity of the peak at 654 nm on tubulin concentration in the difference spectrum 0.4 μM TCPP plus tubulin minus 0.4 μM TCPP. Taxol® and taxanes were dissolved in dimethyl sulfoxide (DMSO) before use. The maximum final concentration of DMSO in a sample was 0.5%.

† On-leave from the Department of Chemistry, Banaras Hindu University, Varanasi-221 005, India.

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range it reduces the possibility of any interference during investigation based on emission spectral measurements.

Addition of tubulin[8,9] to TCPP solution shifted the emission spectra and the peak was now observed at 648 nm (Fig. 2, trace 2). Investigation of the difference spectrum (TCPP + tubulin − TCPP) of the emission showed a peak at 654 nm (Fig. 2, trace 4). The intensity of this peak increased with tubulin concentration and reached a plateau above 1 μM tubulin (Fig. 2, inset B). This concentration of tubulin was used for further investigations.

The addition of Taxol®[10] to the TCPP–tubulin complex resulted in the appearance of a new peak at 648 nm (Fig. 2, trace 3). Investigation of the difference spectrum of the emission between TCPP + tubulin + Taxol® − TCPP + tubulin produced a new peak at 645 nm (Fig. 2, trace 5). The effect of Taxol® concentration on the intensity of the new fluorescence peak at 645 nm is shown in Fig. 3. A plot of the intensity *vs.* log[Taxol®]/μM was linear up to 10 μM ($r^2$ of 0.987) with a lower detection limit of 125 nM (defined as three times the standard deviation of the response for the blank) and a sensitivity (slope of the calibration plot) of 24.8 RFU per decade of [Taxol®]/μM (Fig. 3 inset).

The selectivity of the porphyrin–tubulin interactions for baccatin and cephalomannine, naturally occurring taxanes, was examined.[11] The results showed that while baccatin did not induce the occurrence of a new fluorescence peak even up to 38 μM, cephalomannine caused induction of a new fluorescence peak at 651 nm. This peak, however, only appeared above 1 μM and showed a linear relationship up to 10 μM cephalomannine. The sensitivity of the assay for cephalomannine was 10.6 RFU per decade of [cephalomannine]/μM (data not shown). The results obtained are in agreement with the biological activity data (cephalomannine is 3-fold less and baccatin is 1000-fold less active than Taxol®).[12] The above findings explicitly demonstrate the potential of this novel approach for assaying and screening antimitotic agents

Proteins are known to alter the absorbance and emission spectrum of porphyrins.[5] In order to ascertain the contribution/interference of porphyrin–protein interactions, the effect of BSA on the performance of the assay was studied. Difference emission spectrum between 0.4 μM TCPP plus 151 μM bovine serum albumin produced a peak at 654 nm. However, the addition of Taxol® to this mixture did not result in any peak shift of the difference spectrum as in the case of tubulin–TCPP–Taxol®. This finding exhibits the potential of the demonstrated assay for monitoring antimitotic agents in biological samples.

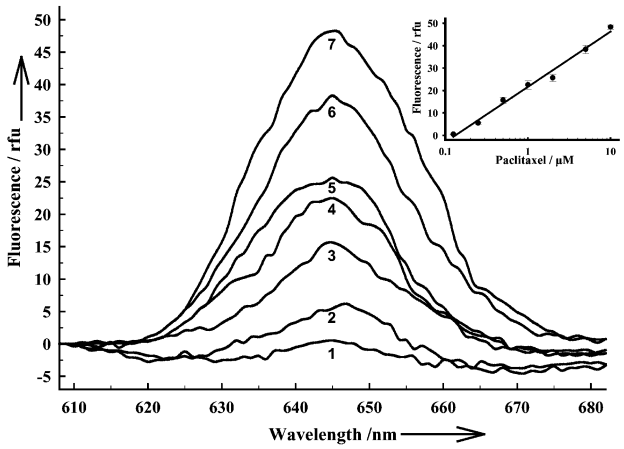


**Fig. 3** Difference spectra of TCPP + tubulin + Taxol® − TCPP plus tubulin. The Taxol® concentration ranges from 0.125 μM (trace 1) to 10 μM (trace 7). Traces 2, 3, 4, 5 and 6 represent 0.25, 0.5, 1.0, 2.0 and 5.0 μM Taxol®, respectively. The inset shows the linear relationship between the intensity of the peak at 645 nm and the Taxol® concentration.

In conclusion, the preliminary evidence provided in this Communication illustrates the potential of using a porphyrin–tubulin complex to screen and quantify antimitotic agents. This bioassay has several advantages such as a short assay time, small volume and the adaptability to high throughput analysis. Additionally, we believe that this bioassay can be used for high throughput screening of biological activity and quantification of the new generation antimitotic compounds that are analogs of paclitaxel. Since the bioassay utilizes tubulin, the receptor protein that is the target of antimitotic agents, as the biological recognition element, the use of cell lines are not necessary making this bioassay a rapid, sensitive and suitable tool for testing new antimitotic compounds. The availability of a wide-range of porphyrins combined with the fact that the excitation spectrum of the interaction between tubulin, porphyrins and antimitotic agents can also probed (data not shown), makes the demonstrated technique a powerful method for high throughput screening and quantifying antimitotic drugs at receptor level.

## Notes and references


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7 Tetrakis(4-carboxyphenyl)porphyrin (TCPP) was acquired from Frontier Scientific (Logan, UT) and used as delivered.

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