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Cationic Porphyrins Are Reversible Proteasome Inhibitors

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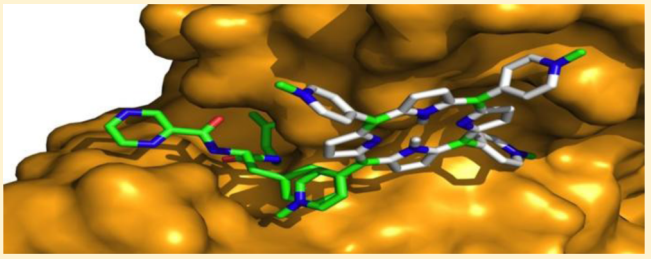
Supporting Information

ABSTRACT: The aim of this study is to verify if water-soluble porphyrins can be used as proteasome inhibitors. We have found that cationic porphyrins inhibit proteasome peptidase activities much more effectively than the corresponding anionic derivatives. The relevance of electrostatics in driving porphyin−proteasome interactions has been confirmed by the observation that the inhibitory efficiency of the cationic macrocycles decreases with the number of positive substituents. We have also investigated various metalloporphyrins, which differ due to the different propension of the central metal ion toward axial coordination. Our experimental results indicate that the naked cationic porphyrins are the most active in reversibly inhibiting the three main protease activities of the proteasome in the micromolar range. A spectroscopic characterization of porphyrin−proteasome interactions by UV−vis spectra parallels the results of inhibition assays: the higher the inhibitory effect the stronger the spectroscopic variations are. To interpret the action of porphyrins at a molecular level, we have performed calculations evidencing that cationic porphyrins may hinder the access to the canonical proteolytic site on the proteasome β5 subunit. In particular, an inspection of the top-scoring docking modes shows that the tetracationic porphyrin blocks the catalytic pocket, close to the N termini of the β5 proteasome subunit, more efficiently than its anionic counterpart. Proteasome inhibition activity of porphyrins unites their known anticancer properties making them suitable as a scaffold for the design of novel multitargeted molecules.



INTRODUCTION

Porphyrins are quite unique molecules. Their extended π system leads to a remarkably high extinction coefficient and make them highly hydrophobic. Yet, functionalization (mainly at the *meso*- positions) with charged substituents makes them water-soluble; the number and reciprocal dispositions of charged peripheral groups allows, in turn, to modulate their tendency to (self-)aggregate.[1,2] Porphyrins can be regarded as polytopic complexing agents because, in addition to the chemistry related to the periphery (which is tunable in terms of number, nature, and reciprocal disposition of substituents), the central core also has a manifold role in determining their physicochemical behavior.[3,4] In fact, besides protonation, insertion of different metal ions in the four-nitrogen core gives additional control of the spectroscopic features (especially concerning the emission properties) and permits us to tune the interactions of these macrocycles with templates as well as their self-assembly processes. For example, the presence of one or two water molecules axially coordinated to the central metal ions (penta- and hexa-coordinated cationic metalloporphyrins cannot intercalate but mainly interact with the external phosphates. Yet, the penta-coordinated ZnT4 is a very sensitive reporter for the left-handed Z-DNA), strongly influences the type of interactions with DNA.[5,6] All of these peculiar properties made this class of molecules suitable candidates for various applications ranging from material science to medicine.[7]

In oncology, porphyrins find extended application such as photosensitizers in photodynamic therapy (PDT),[8,9] an approved anticancer procedure in which a photosensitive molecule, upon exposure to radiation of appropriate wavelength, induces oxidative damage in tumor cells by means of singlet oxygen and ROS productions.[10] Also, carboranylated porphyrins have been used as boron carriers in boron neutron capture therapy (BNCT),[11] another anticancer therapy, in which the bombardment of ^{10}B, with thermal neutrons, triggers a nuclear reaction whose destructive potential covers a range of a cell diameter.[12]

In addition to this, it has been recently demonstrated that some porphyrins, including meso-tetrakis(4-*N*-methylpyridyl)-porphine (H_2T4), inhibit telomerase an enzyme which, in adult, is expressed in tumor cells only (the function of telomerase is correlated to senescence and cell death; in cancer cells, alterated telomerase activity restores the entire telomeric sequence after each division of the cell rendering it immortal). Szokalska and co-workers[16] demonstrated that cytotoxic effects

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caused by a porphyrinic photosensitizer in PDT could be potentiated through inhibition of the proteasome. This finding encouraged us to investigate the ability of porphyrins to inhibit the proteasome activity: the central idea is that instead of using a cocktail of active molecules it would be more convenient to use one single molecule having different biological targets but all converging toward the cure of tumors.

The ubiquitin−proteasome system (UPS) is the major cytosolic proteolytic system in prokaryotes and eukaryotes[17] with critical functions in cell cycle control, apoptosis, inflammation, transcription, signal transduction, protein quality control, and many other biological processes.[18] The end point of the UPS is the 26S proteasome, an endoprotease of about 2.5 megadaltons that functions primarily to degrade proteins that have been modified by the attachment of ubiquitin.[19] The 26S proteasome is composed of a catalytic core (also known as the 20S particle) and two 19S regulatory complexes.[20] The 19S caps contain a lid- and baselike structure: the lid component is responsible for recognizing polyubiquitinated substrates and deubiquitinating activity, which allows recycling of ubiquitin moieties. The 20S proteasome is a barrel-like structure whose subunits are arranged in four stacked seven-membered rings, each enclosing a central chamber. Seven different but related $\alpha1-\alpha7$ subunits form the two outer rings, whereas the two inner rings are composed of seven different $\beta1-\beta7$ subunits that contain the proteolytic sites. Each subunit uses the nucleophilic γ-hydroxy group of the N-terminal Thr to efficiently hydrolyze peptide bonds. They differ in substrate specificity: the $\beta1$ subunit possesses a postglutamyl peptide hydrolyzing (PGPH) or caspaselike activity; the $\beta2$ subunit has a trypsinlike (T-L) activity; the $\beta5$ subunit possesses a chymotrypsin-like (ChT-L) activity.[21] The proteasome regulates the activity of signal transduction pathways, such as the NF-κB pathway, degradation of tumor suppressor genes, such as p53, and oncogenes.[22] For these reasons, proteasome inhibition has become a new, promising strategy in cancer therapies.[23] Several compounds, both natural and synthetic, have been found to affect UPS functionality,[24−26] and some proteasome inhibitors are already efficient anticancer drugs. The biggest success so far, in targeting ubiquitin-dependent processes, has been the development of bortezomib, the first proteasome inhibitor to be approved for clinical use in human cancers.[27]

Synergic cytotoxic effect between proteasome inhibition and PDT probably arises from the severe oxidative damage of cellular macromolecules, including proteins that undergo multiple modifications such as fragmentation, cross-linking, and carbonylation, caused by photoirradiation of the sensitizers.[16] The major mechanism for elimination of carbonylated proteins is their degradation by proteasome. Therefore, inhibition of proteasome should result in the accumulation of molecules leading to fatal (cancer) cell damage.

Inspired by this scenario, we focused our attention on assessing the ability of porphyrins, highly versatile photosensitizers, to be active as proteasome inhibitors.

MATERIALS AND METHODS

Chemicals. The *meso*-tetrakis(4-N-methylpyridyl) porphyrin (H_2T4), *cis*-diphenyl-di-(*N*-methyl-4-pyridyl)-porphyrin (*cis*-T4), monophenyl-tri-(*N*-methyl-4-pyridyl)-porphyrin (tris-T4), *meso*-tetrakis(4-sulphonatophenyl)-porphyrin (H_2TPPS) were purchased from Midcentury, whereas the Mn(II), Zn(II), Cu(II) derivatives were obtained from metalation of H_2T4 as elsewhere reported.[28]

Purified rabbit proteasome 20S, fluorogenic substrates Suc-Leu-Leu-Val-Tyr-AMC, Z-Leu-Leu-Glu-AMC and Ac-Arg-Leu-Arg-AMC were purchased from Boston Biochem. Dulbecco's Minimum Essential Medium (DMEM), penicillin streptomycin, fetal calf serum were purchased from Invitrogen.

Preparation of Cell Lysates. To obtain cell lysates for proteasome activity assays, HeLa cells were grown in DMEM supplemented with Fetal Calf Serum 10% and were mantained at 37 °C in a humidified 5% CO_2 incubator. Whole cell lysates were prepared by freeze−thawing cycles in water containing 1 mM dithiothreitol (DTT).[29] The lysate was then centrifuged at 14,000 rpm for 30 min and the supernatant brought to 50 mM Hepes (pH 7.5) containing 5 mM $MgCl_2$ and 250 mM sucrose and 1 mM DTT (buffer A). We quantified the protein content of lysates using the Bradford method, (protein assay kit, BioRad), and then incubated equal amounts of protein with the indicated amounts of inhibitor, substrate, and buffer for 30 min at 37 °C.

20S, 26S Proteasome Activity. We have selected three specific fluorogenic peptides conjugated with the fluorophore amido-4-methyl coumarin (AMC). In particular, Suc-LLVY-7-amido-4-methyl coumarin, Z-Leu-LeuGlu-AMC, and Ac-Arg-Leu-Arg-AMC were used to test the ChT-L, PGPH-L, and T-L activity, respectively. Proteasome activity is monitored by measuring AMC fluorescence at 440 nm (infra). In the absence of proteasome activity, fluorescence is almost negligible and does not change with time. On the contrary, in the presence of some cleaving activity AMC emission at 440 nm increases with time and the slope of the emission versus time plot is correlated to proteasome activity. For the sake of a more intuitive approach, data are expressed as percentages of residual activity considering the slope of the control (peptide−AMC/proteasome in the absence of inhibitors) as 100% (Figure S1 of the Supporting Information). To rule out any misinterpretation of the inhibition assays due to a possible overlap of porphyrin and AMC fluorescence emissions, we have preliminarly collected spectra of AMC/porphyrins mixtures in the same experimental conditions adopted hereafter and found no interference.

Cell lysates (4 μg in proteins) or purified rabbit 20S proteasome (2 nM) were previously incubated in the presence of porphyrin in 200 μL buffer for 30 min at 37 °C. In particular, we used buffer A supplemented with 30 μM ATP for 26S proteasome activity and the reaction buffer (Hepes 20 mM, 0.5 mM EDTA) for 20S activity. In some 20S activity assays, the buffer was also supplemented with 0.03% SDS.[29]

In all assays, the reaction started with fluorogenic substrates addition (100 μM) and the AMC release was followed measuring fluorescence emission at 440 nm (excitation at 360 nm) for 20 min using a fluorescence plate reader (Varian Cary Eclipse). Concentration−response plots of the residual activity of the proteasome in the presence of the inhibitor may provide a quantitative estimate of its potency. The IC_{50} (i.e., the concentration of the inhibitor which causes the 50% reduction of activity) is identified from the midpoint of the concentration −response plot occurring at a fractional activity of 50% and corresponding to 50% inhibition of proteasome. The reversibility of inhibition was determined by measuring the recovery of enzymatic activity after a rapid and greater dilution of the proteasome porphyrin complex as previously reported.[30]

UV−vis and Fluorescence Spectra. UV−vis spectra were recorded by a JASCO V-530 spectrophotometer and fluorescence spectra were measured by Cary Eclypse Varian and by FL3 Jobin Yvon Horiba spectrofluorimeter. UV−vis spectra of porphyrin solutions in presence of 20S proteasome were performed in reaction buffer in the absence of SDS. Hypochromicity is calculated by subtracting the absorbance of the Soret band of the sample porphyrin/proteasome from the absorbance of the sample containing porphyrin alone.

Molecular Modeling. Cationic and anionic porphyrins were docked to the 20S proteasome cocrystallized with bortezomib (PDB code: 2F16) by using *AutoDock Vina*.[31,32] The binding site was defined as an area with a diameter of 40 Å around the coordinates of the mass center of bortezomib in PDB 2F16. Before docking calculations, ligands were removed and hydrogen atoms added to the β5 subunit of the 20S proteasome. Protein structure was kept fixed during docking simulations. Water was implicitely considered throughout all calculations assuming a dielectric constant of the medium of 80. All rotatable torsion angles of the porphyrin were allowed to rotate freely, and the 10 lowest energy structures were stored for further analysis. A total of 100 docking runs were computed for each ligand. Input files for the planar ligands were obtained from the Dundee PRODRG2 server. Nonplanar porphyrins were obtained by semiempirical geometry optimization using the AM1 methods in the *Hyperchem* software package.[33] The options selected for the optimization runs were: convergence limit 0.01, iteration limit 50, total charge 0, state lowest, minimization algorithm Polak-Ribiere.

RESULTS AND DISCUSSION

Screening the Proteasome Inhibition Ability of Porphyrins in 20S Proteasome Preparations from Cell Lysates. The list of compounds that affect proteasome activity is already lengthy and several attempts have been made to summarize the actual knowledge about the most effective among these molecules.[34] Unfortunately, because of differences in the experimental conditions employed (e.g., buffer, proteasome source, methods) the values of the inhibiting potential, reported in different works for the same inhibitor, can vary significantly, thus making a safe comparison difficult. In an attempt to (partially) circumvent this drawback, we decided to quantify the proteolytic activity of the proteasome in the preparations identified as 20S cell lysates. Indeed, cell lysates are largely employed in studies aimed at probing the capability of many proteasome inhibitors and are, then, expected to provide the largest array of comparable data available in the literature to date.[29] Therefore, the capacity of different porphyrin derivatives to inhibit the ChT-L activity of 20S proteasome in cell lysates was first assayed.

Owing to the lack of studies concerning the activity of porphyrins as proteasome inhibitors, we started our studies with a quite crude screen involving only the tetracationic H_2T4 and the tetraanionic H_2TPPS. This initial experiment (performed at 1 μM of porphyrins) already gives a very clear indication: H_2T4 quenches ChT-L proteolitic activity but H_2TPPS does not. Figure 1 shows that the plot of the slope of the emission (related to proteasome activity) versus time − measured in the presence of the tetraanionic porphyrins − is almost identical to that shown by proteasome alone (90% vs 100%), whereas the presence of the tetracationic porphyrin H_2T4 causes quite a drastic reduction of the slope (65% of reduction) indicating a remarkable inhibition activity of this

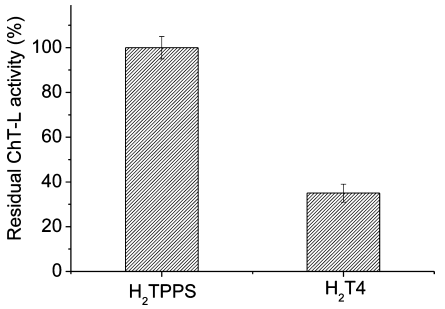
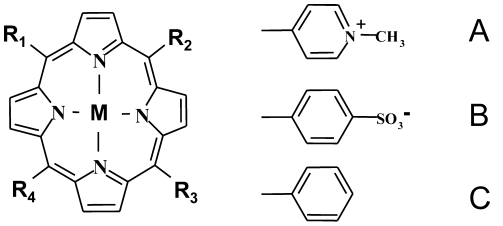


Figure 1. Residual chymotryptic-like activity of 20S proteasome in cell lysates in presence of H_2TPPS and H_2T4 at 1 μM. Data are referred to control sample normalized to 100%.

macrocycle. These coarse data give a first clear indication suggesting that electrostatics play a very important role in regulating porphyrin−proteasome interactions.

To focus better on this aspect, we performed proteasome activity assays using porphyrins with three or two cationic substituents, respectively (Chart 1). The outcome of these

Chart 1. Chemical Structures of All the Porphyrin Derivatives Used in This Study



	R_1	R_2	R_3	R_4	M
H2T4	A	A	A	A	2H
TrisT4	A	A	A	C	2H
cis-T4	A	A	C	C	2H
CuT4	A	A	A	A	Cu
ZnT4	A	A	A	A	Zn
MnT4	A	A	A	A	Mn
TPPS	B	B	B	B	2H

experiments (Figure 2) is perfectly in line with the expectations coming from the previous experiment. This shows that

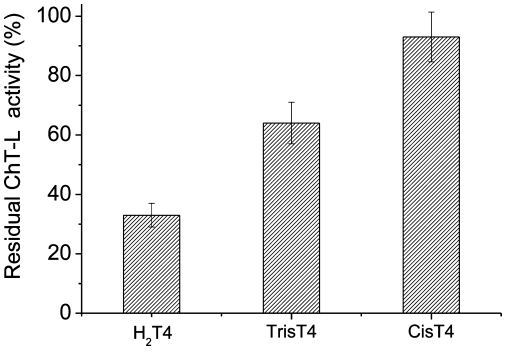


Figure 2. Residual chymotryptic-like activity of 20S proteasome in cell lysates with di-, tri- and tetracationic porphyrins at 1 μM. Data are referred to control sample normalized to 100%.

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proteasome inhibition decreases linearly with the number of positive charges (the inhibiting ability of the dicationic porphyrin is indeed comparable to that shown from the tetraanionic derivative) substantiating the key role of the electrostatics in tuning proteasome−porphyrin interactions.

As mentioned before, porphyrins can be considered polytopic complexing molecules with quite a defined center and a tunable periphery. Therefore, once assessed the relevance of the peripheral charge, we thought that investigating the influence of the central metal ion on the inhibition activity would be useful. In fact, as previously discussed, the type and extent of porphyrin interactions with surrounding molecules are deeply influenced from the presence of central metal ions that axially coordinate water molecule(s). Quite notably, it turns out that the inhibitory efficiency of metalloporphyirns depends on the coordination properties of the central metal ion (Figure 3).

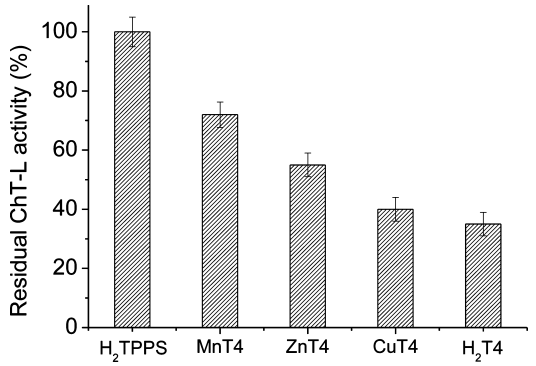

Figure 3. Residual chymotryptic-like activity of 20S proteasome in cell lysates with different metalated porphyrins (MnT4, ZnT4, and CuT4) at 1 μM. Data are referred to control sample normalized to 100%.

In particular, a regular increase of inhibition of proteasome activity is observed going from hexa-(MnT4) to penta-coordinated (ZnT4) to planar (CuT4) porphyrins suggesting that axially coordinated water molecules get in the way of porphyrin−proteasome interactions.

To fully understand the system, we have performed inhibition measurements also with various metalloderivatives of the tetra-anionic porphyrin: consistently with the data previously discussed also the anionic metalloderivatives do not inhibit protasome activity (data not shown).

To assess whether the spectroscopic features of porphyrins were modified upon proteasome binding, we performed UV−vis measurements of the cationic (H_2T4) and anionic (H_2TPPS) porphyrins in similar experimental conditions, (buffer, porphyrin, and proteasome concentrations) adopted for the activity assay. In this case, to rule out any possible interaction with nonproteasomal components, only purified proteasome 20S samples were utilized. UV−vis spectra (left panel of Figure 4) show that addition of 20S to a solution of the tetraanionic H_2TPPS does not induce any change. On the contrary, titration of H_2T4 with purified proteasome 20S induces a dose-dependent hypochromic effect of the Soret band of H_2T4 (right panel of Figure 4 and the insert). Noticeably these changes are stable over 24 h.

Hypochromic effect is observed for all metalloderivatives of the cationic porphyrins. Intriguingly, the trend of the proteasome inhibition activity observed for the different porphyrins tested, both in terms of charges and role of the central metal, is paralleled by the UV−vis binding measurements (Figure 5). The degree of hypocromicity, in fact, correlates with the inhibitory activity of the various porphyrins

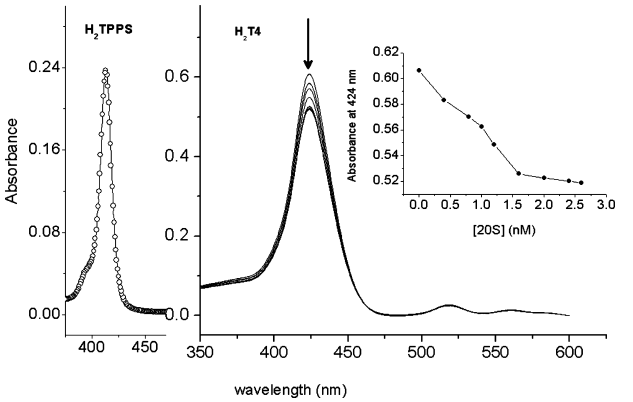

Figure 4. UV−vis spectra of H_2TPPS (left panel) at 4 μM (path length 0.1 cm) in reaction buffer in absence (solid line) and in presence of purified proteasome 20S (open circles). Right panel: UV−vis titration of 4 μM H_2T4 (path length 1 cm) with increasing amounts of 20S proteasome. The insert reports a plot of the hypochromic effect vs proteasome concentration.

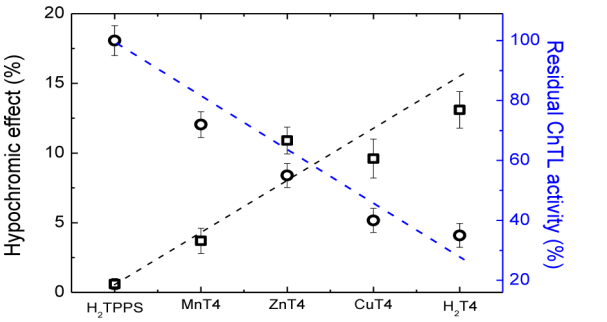

Figure 5. Black line, hypochromic effect of Soret band in UV−vis spectra of different porphyrins at 4 μM following addition of 2 nM 20S proteasome (square points, left *Y* axis); blue line, percentage of residual ChT-L activities of 20S proteasome (circle points, right *Y* axis).

and, as shown in Figure 5, in turn depends on the coordination features of the central metal.

The UV−vis variations observed in these experiments exclude porphyrins aggregation. Porphyrin aggregation leads, in fact, to broadening of the Soret band accompanied from noticeable hypochromicity: comparison of the spectrum of the free and proteasome bound H_2T4 does not show any of these signs. Moreover, Resonance Light Scattering (RLS) of proteasome-bound H_2T4 (Figure S2 of the Supporting Information) ruled out, definitively, the presence of aggregated species.

Proteasome Activity Assays in Cell Lysates and Purified 20S Proteasome Preparations. All of the assays described above were performed adopting the experimental conditions most frequently used in studies in which proteasome activity in crude biological samples was measured. However, because nonproteasomal peptidases (e.g., cathepsins) could also hydrolyze the fluorogenic peptides under these experimental conditions, these assays are expected to be nonspecific and variations in fluorescence emission may be difficult to be rationalized in terms of proteasomal activity alone. Examples of such questionable interpretations have already been reported

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and include the significant variations in proteasomal activity of the cytosolic fractions of homogenized tissues, which were assessed by fluorogenic substrates.[35,36] Consequently, only a comparison of the activity assays performed in cell lysates and in isolated 20S proteasome preparations may account for the specificity of the inhibitor. To address this point, the inhibitory efficiency of all porphyrins studied in this work has been tested, in a concentration range going from 0.5 to 3 μM, against purified 20S (the dose−response curves are shown in Figure S3 of the Supporting Information). Also, these experiments underline the major activity of H_2T4 with respect to all the other porphyrins. For this reason, in the following part of the manuscript we will discuss only the data concerning the activity of H_2T4 that was assayed (always in a concentration range 0.5 to 3 μM) by probing the ChT-L activity either in purified proteasome samples and in cell lysates (Figure 6).

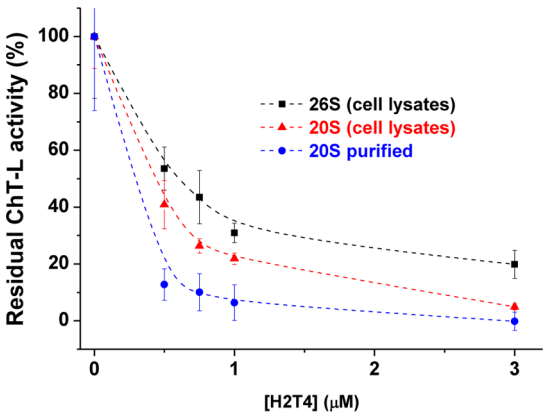

Figure 6. Concentration−response plot of H_2T4 for chymotryptic-like activity on 26S and 20S (either from cell lysates or purified 20S proteasome).

The peptidease activity of purified 20S proteasome and of cell lysates are inhibited by porphyrins to a similar extent; the small differences observed can then be ascribed to the presence of nonproteasomal peptidases in crude biological samples. In fact, previous studies have demonstrated that immunoprecipitation of the proteasome from crude biological samples resulted in 70−80% reduction in ChT-L activity.[37] This means that the residual peptidase activity (20−30%) may be ascribed to nonproteasomal peptidases that may not be inhibited by specific proteasome inhibitors.

Although literature data may significantly vary depending on the biological source, the kind of assay employed and the concentration of inhibitor used, our data are in line with those indications and suggest that H_2T4 is a quite specific proteasome inhibitor.

A comparative study of the 26S proteasome activity (black squares in Figure 6) has also been performed in the presence of H_2T4 and in the same experimental conditions previously adopted for the 20S assay. The lower inhibitory potency exerted by H_2T4 for these proteasome preparations may be, thus, related to the presence of the regulatory lids that may (slightly) hinder the entrance of the inhibitor in the catalytic chamber. Yet, the similar inhibition effect found for H_2T4 with 20S and 26S substantiates the hypothesis that the mechanism of inhibition foresees porphyrin interaction with the catalytic core of the proteasome.

The ChT-L activity of the proteasome is mostly probed to evaluate proteasomal activity in crude biological samples, or in the isolated proteasome, due to previous reports suggesting that the chymotryptic substrate is the most proteasome-specific of those examined.[29] Nevertheless, the three activities of the proteasome are allosterically regulated[38] and measuring only chymotriptic-like activity may not fully account for total proteasome activity. To address this issue, we decided to probe all the three proteolytic activities of the proteasome.

Quantifying the Inhibiting Potential of H_2T4. Figure 7 illustrates the concentration−response plots for the inhibition

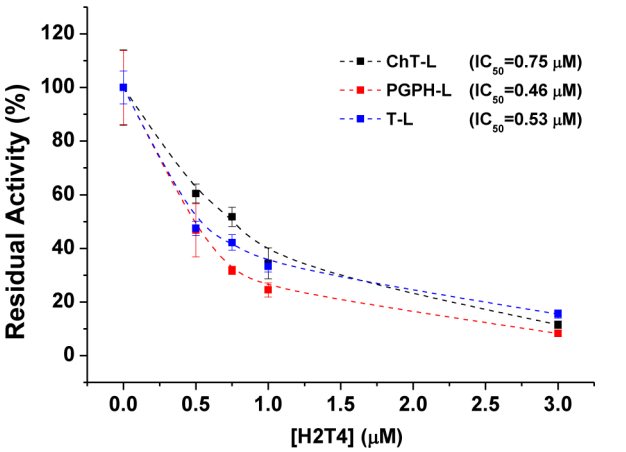

Figure 7. Concentration−response plot of H_2T4 for chymotryptic-like, caspaselike, and trypticlike residual activies on 20S from cell lysates.

of the three 20S proteasome activities in the presence of H_2T4. The insert of Figure 7 reports the IC_{50} values of the inhibitor.

To understand the mechanism of interaction betweeen porphyrins and proteasome active sites, we checked for the reversibility of H_2T4-mediated proteasome inhibition on chymotryptic-like activity. A convenient method for determining reversibility consists in incubating proteasome solution at 100-fold concentration (2×10^{-7} M) over that required for the activity assay ($2\times$ 10^{-9} M), with H_2T4 solution at concentration equivalent to 10-fold the IC_{50} ($1\times$ 10^{-5} M).[30] After 30 min, this mixture was diluted 100-fold into reaction buffer containing the substrate of chymotryptic-like activity to start the reaction; the activity was then measured and compared to that of a similar sample of proteasome incubated and diluted in the absence of H_2T4 porphyrin. Proteasome chymotryptic-like activity is fully recovered after dilution showing fast reversibility of binding between H_2T4 and proteasome active binding site.

Molecular Modeling of the Proteasome−Porphyrin Complex. On the basis of the evidence that cationic porphyrin inhibits the proteolytic active site on the proteasome β5 subunit, which is also the target of lactacystin and Bortezomib,[39,40] we set out to generate a tentative model of the porphyrin/proteasome complex by molecular modeling. Indeed, because the different proteasome proteolytic sites have a common, evolutionary origin (and cationic porphyrins inhibit all catalytic functions comparably well), we expect a common mode of binding to all three pockets as already observed for other proteasome inhibitors.[41] The first binding mode of planar porphyrins for the β5 subunit is very similar to the following two lower-affinity poses, therefore, we propose them to be

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representative of the porphyrin/proteasome complexes. However, although planar structures of H_2T4 have been inferred from X-rays diffractions studies[42] nonplanar distortions are largely documented. So, we carried out docking simulations using also saddlelike conformations obtained by semiempirical optimization runs. We note that saddlelike conformations of H_2T4 may hinder the access to the active site (bottom left part of Figure 8). Notably, parallel docking calculations, performed

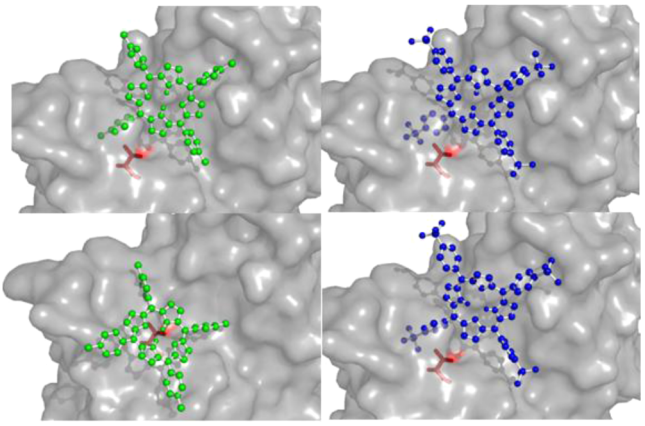

Figure 8. Representative top-scoring binding poses of planar (top) and nonplanar (bottom) of H_2T4 (left) and H_2TPPS (right) with the $\beta5$ subunit of the proteasome (gray surface). The catalytic site of the proteasome is evidenced in red.

with the inactive anionic porphyrin H_2TPPS, never evidenced binding poses close to the N-termini of the $\beta5$ subunit (as reported in the bottom right of Figure 8). Although we are aware that it may be unwise to overemphasize the outcome of docking runs, we have to remark that they reconcile with the results of inhibition studies.

CONCLUSIONS

Over the past decade, it has been increasingly recognized that cancer is a complex disease that is caused by multiple genetic and environmental factors. It is thus quite conceivable that the traditional reductionist model one drug−one target may not hold in this case opening the way to new drugs that address multiple targets.[43] Inspired by these scenarios, and, bearing in mind that photosensitizers are already used in many cancer treatments, we have screened a number of porphyrin derivatives to assess their efficiency as proteasome inhibitors. Among all porphyrins, cationic derivatives have attracted particular attention since they interact with anionic phosphate groups of DNA thus causing additional cell damage upon irradiation.[44−47] Furthermore, cationic porphyrins are known to interact with the negatively charged lipid membranes of the cell, facilitating their diffusion across the lipid bilayer and thus enhancing their efficacy.[48] Our experiments demonstrate that tetracationic porphyrins, and some of its metallo derivatives, may significantly inhibit all the three catalytic functions of the 20S and the 26S proteasome either in crude biological samples or in isolated 20S proteasome preparations. The most active compound, H_2T4, has a similar inhibitory ability for all the three catalytic sites and comparable with other inhibitors such as lactacystin (IC_{50} in the micromolar range) although with a faster off-rate from the proteasome. Notably, reversible proteasome inhibitors have been proposed to result in better antitumor activity by exhibiting a better tolerability in affected tissues.[49,50] Therefore, on the basis of molecular modeling we probed the binding mode of H_2T4 to the active site of the $\beta5$ subunit. The low toxicity of porphyrins and their effective proteasome inhibition encourage us to continue our evaluation of this class of compounds.

ASSOCIATED CONTENT

Supporting Information

Time-dependent increase of the emission of AMC following digestion of fluorogenic substrate at different H_2T4 concentrations, RLS spectra of H_2T4 at 6 μm in absence and in presence of 20S proteasome, curves dose response of all porphyrins used in this work. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

PDT, photodynamic therapy; BNCT, boron neutron capture therapy; UPS, ubiquitin−proteasome system; DTT, dithiothreitol; AMC, fluorophore amido-4-methyl coumarin; PGPH-L, caspaselike; ChT-L, chymotrypsin-like; T-L, trypsinlike; H_2T4, *meso*-tetrakis(4-*N*-methyl pyridyl) porphyrin; *cis*-T4, *cis*-diphenyl-di-(*N*-methyl-4-pyridyl)-porphyrin; tris-T4, monophenyl-tri-(*N*-methyl-4-pyridyl)-porphyrin; H_2TPPS, *meso*-tetrakis(4-sulphonatophenyl)-porphyrin

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