Synergistic effects in Pt(II)-Porphyrinoid Dyes as candidates for a dual anticancer approach: a theoretical exploration

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Abstract: The combination of a photosensitizer (PS) with a cisplatin-like unit represents a new challenging strategy to increase the effectiveness of Photodynamic Therapy (PDT) and to afford a dual-approach anticancer treatment. Recently, new tetraPt^{II}-porphyrins conjugates have been proposed as promising potential multi-target agents. With the aim to reveal the effect of Pt^{II}-moiety on the crucial chemical and physical properties of the PS and to explore in turn, the effect of the PS on the activation mechanism of Pt^{II}-ligand before reaching its biological target, a careful first-principle investigation has been carried out on the above mentioned conjugates. Moreover, to propose a further advance in this novel field and give some insights useful for the design of new Pt^{II}-PSs with improved efficiency, we introduced structural modification into the porphyrin dye including in our exploration the analogous tetraPt^{II}-chlorines and tetraPt^{II}-bacteriochlorins dyes. Results show that the designed dyes better meet the criteria to be successful in a dual-approach therapy, possessing from one side improved optical properties and promoting, from the other side, a reduction of the hydrolysis rate of the Pt^{II} moiety, the latter being a desirable feature to avoid many side-reactions of the conjugate in their root to the biological target.

Introduction

To increase the effectiveness of anticancer therapy, the combination of multimodal treatment methods into a single synergistic system is a very promising approach. [1] One of the newest and most challenging strategy, still in its infancy, is to conjugate the photo-based treatment known as photodynamic therapy (PDT) [2] with classical Pt chemotherapy, thus functionalizing a photosensitizer (PS) with a cisplatin-like compound. [3-5] Combinational delivery of a therapeutic with different modalities of actions can eliminate the disadvantages of individual chemotherapy allowing at the same time a better Pt-mediated distribution of the PS within the tumour. In this field, a particular attention has been given to promising mixed-metals complexes [5], among which a very challenging Ru^{II}-Pt^{II} conjugate has been suggested to covalently bind DNA through its *cis*-$PtCl_2$ moiety and to induce DNA photocleavage through its Ru^{II}-chromophore. [6]

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This two-components system has been also shown to possess a very interesting synergistic effect between the metals, making it a promising multi-modal anticancer drug. [7]

Due to the extraordinary success of porphyrins in PDT and of platinum compounds in the cytostatic therapy, Pt^{II}-porphyrins conjugates appeared to be from the outset, an especially promising classes of molecules for a multi-target approach. [3,4]

The successful history of Pt^{II}-compounds as anticancer drugs in view of their effectiveness in binding DNA, once activated by hydrolysis, and in inhibiting transcription and replication is well known and cisplatin represents one of the great success stories in the field of cancer chemotherapy. [8,9]

On the other side, PDT is receiving an increasing attention worldwide as a less invasive treatment option for cancerous and not-cancerous diseases [2] due to its spatial and temporal control and high therapeutic efficacy. It produces a selective destruction of neoplastic tissue through the concomitant use of a PS, oxygen and light. The photochemical process leading to tumour cells necrosis and/or apoptosis starts with the excitation of the PS from its ground S_0 to the excited S_1 state, followed by non-radiative intersystem spin crossing (ISC) toward the triplet state T_1.

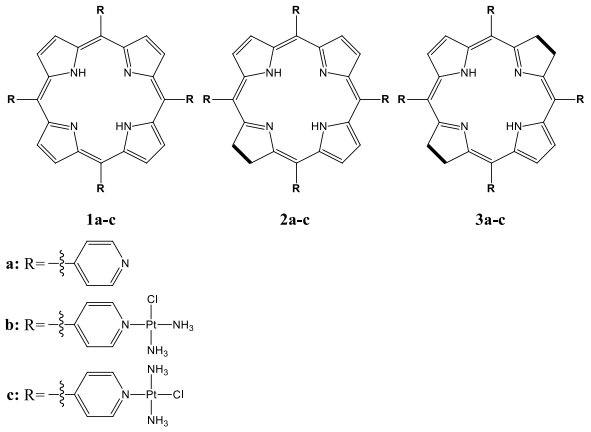
The direct energy-transfer from the photosensitizer relaxing back to its ground state to triplet molecular oxygen ($^3O_2$), known as Type II photoreaction, is able to produce the chemically highly active singlet oxygen $^1O_2$ species. The latter is able to react with many biological molecules, including lipids, proteins, and nucleic acids leading to cancer cell death representing, accordingly, the putative cytotoxic agent in PDT.

To promote type II reactions, a successful PS should possess: i) a red-shifted absorption wavelength, falling in the so-called therapeutic window ($600<\lambda<800$ nm), allowing for a deep penetration in human tissues; (ii) a singlet–triplet energy gap (ΔE_{S-T}) higher than the energy required to generate the singlet oxygen (0.98 eV); (iii) a consistent spin–orbit coupling from the excited S_1 to T_n electronic states to ensure an efficient ISC mechanism, that in turn, increases the production of singlet oxygen.[2]

Porphyrins and related tetrapyrrolic systems are among the most widely studied of all macrocyclic compounds and are the most useful photosensitizers for PDT in view of their excellent photophysical properties. [2,10] To improve the uptake and to influence the biodistribution of porphyrins, tetraplatinated porphyrins conjugates have been recently synthesized and studied for their anticancer properties. [4] The conjugates showed good phototoxicity upon irradiation at 575 nm and an exceptional one upon irradiation at 420 nm with the most prominent results observed for porphyrin **1c** which showed an over 5000-fold enhancement in the cytotoxicity after irradiation (See Scheme1). Compound **1c** also showed a higher quantum yield (Φ) of $^1O_2$. For that compound the authors have been also demonstrated a

cellular uptake and suggested a covalent binding to nitrogen atoms of guanosine even without the involvement of light.

While the properties of porphyrin-like dyes have been widely investigated in the past for their numerous roles in various biological processes [11] and for their application on photodynamic therapy [2,10,12], the effects of platinum moieties on their photochemical behaviour have not yet been rationalized. In order to give further information on these interesting conjugate assemblies, we undertaken a careful theoretical exploration of the photophysical properties of the recently synthesized tetraplatinated porphyrin complexes **1b** and **1c.** The approach, based on DFT and TDDFT, has been widely and successfully applied in previous investigations to elucidate the fundamental photochemical mechanisms of different classes of photosensitizers. [13] Herein, the influence of Pt^{II}-moiety on the crucial chemical and physical properties of the photosensitizer has been accurately described, giving insights on the Type II photoreactions also including the magnitude of spin orbit coupling (SOC) and comparing the obtained data with the metal-free porphyrin **1a**. On the other hand, the effect of the chromophore on the hydrolysis process of Pt^{II}-moiety leading to the release of the chloride ion, has been evaluated analysing the ligand substitution mechanisms by water.



Scheme 1. Investigated metal-free and tetraplatinated porphyrin-derivatives (**1a-c)**, chlorine derivatives (**2a-c)** and bacteriochlorine-derivatives (**3a-c)**.

To propose a further advance in this novel field and give some insights useful for the design of new PSs with improved efficiency, we decided to introduce structural modification into the porphyrin dye evaluating the consequent changes in the chemical and photophysical properties. We have then investigated the properties of the analogous tetraplatinated chlorines (**2a-c**) and tetraplatinated bacteriochlorins (**3a-c**) dyes, in order to spotlight common or different behaviours between them. Our results reveal that the designed dyes better meet the criteria to be successful in the application as multimodal anticancer candidates.

Results and Discussion

- **Photophysical Properties of TetraPt^{II}-porphyrinoids conjugates**

Due to their large aromatic nature, metal-free porphyrin-like compounds (e.g., chlorine and bacteriochlorin) exhibit a typical UV/Vis spectra characterized by a strong absorption in the Soret region (B bands, ~380–420 nm) and weaker satellite bands between 500 and 700nm, known as Q-bands. Despite the large molar absorption coefficient, the Soret band is not suitable for PDT of deeper tumor tissues, while the weaker satellite absorption bands (Q-bands) in the visible region are more likely to be used for PDT application. [2] The absorption spectra of porphyrinoids compounds have long been understood in terms of the highly successful "four-orbital model" first applied by Gouterman, [14] in which the principal excitation electronic transitions involve the occupied HOMO and

next-HOMO (H and H-1), and the unoccupied LUMO and next-LUMO (L and L+1) molecular frontier orbitals. It is well known that changes in the aromatic systems and symmetry of the porphyne ring can affect these levels and consequently their optical properties. [10,12]

In order to evaluate the influence of the Pt^{II}-moieties on the photophysical properties of the porphyrin dye, the electronic spectra of the recently synthesized tetraPt^{II}-conjugates **1b** and **1c** [4] have been computed in water, fully characterized and compared with the metal-free analogue **1a**. The optimized structures of all the investigated dyes are reported in Figure S1. Excitation energies, oscillator strength, main contributions, frontier molecular orbital (MOs) plots and computed UV-Vis spectra are reported in Figure S2. A plot of the computed wavelengths corresponding to B and Q bands for porphyrin-derivatives is reported in Figure 1. The comparison between the computed bands reveals that the presence of platinum does not sensibly modify the UV-Vis spectra. Actually, the strong Soret band arising from two equally intense transitions (namely B1 and B2) found at around 400 nm in the metal-free complex **1a** spectrum, it is just slightly shifted at higher wavelengths in the case of tetraplatinated conjugates **1b** and **1c** (See Figure 1 and S2). The red-shift of the very weak Q-bands in the presence of Pt^{II}-moieties is even more negligible. All the bands arise from transitions involving the four Gouterman orbitals, in both metal-free and metal-derivatives. The presence of platinum affects the orbital energy diagram with a stabilization of the frontier orbitals energy in the case of **1b** and **1c** (see S2) compared with the porphyrin dye **1a**, keeping however nearly unchanged the HOMO-LUMO gap. Their nature is not influenced by the platinum moieties, being localized on the central porphyrin ring in all the examined cases. Accordingly, all the transitions result $\pi \rightarrow \pi^*$ in nature, as it can be also deduced from the molecular orbitals plot reported in the SI section. Apparently, the optical properties of such systems do not benefit from the insertion of a heavy-metals, as occur instead in the case of other Pt^{II}-PS conjugates proposed for multi-modal anticancer approach. [6,7] As a consequence, although the proposed compounds have shown significant preliminary cytotoxicity, [4] the weak intense Q-band displayed by them lying

below 600 nm could be not highly suitable for PDT of deeper tumour tissues.

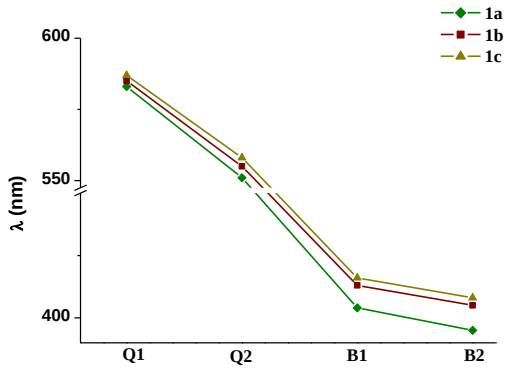


Figure 1. Plot of the computed wavelengths (nm) corresponding to B and Q bands for porphyrin-derivatives (**1a-c).** See SI for more detailed informations.

With the aim to propose new agents with improved optical properties, we decided to introduce structural modification into the porphyrin dye and analyse the consequent change in their photophysical behaviours. Actually, two of the peripheral double bonds in opposite porphyrin pyrrolic rings are cross conjugated and are not required to maintain aromaticity. Accordingly, reduction of one or both these double bonds (to give chlorins and bacteriochlorins, respectively) maintains much of the aromaticity, but the change in symmetry should results in bathochromically shifted Q bands with high extinction coefficients.

In order to give insights on the influence of the central chromophore on the absorption properties of Pt^{II}-porphyrinoids analogues, the UV-Vis spectra of tetraplatinated-Chlorins (**2b** and **2c**) and of tetraplatinated-Bacteriochlorins (**3b** and **3c**), have been computed and compared with the porphyrin analogues (**1b** and **1c)**.

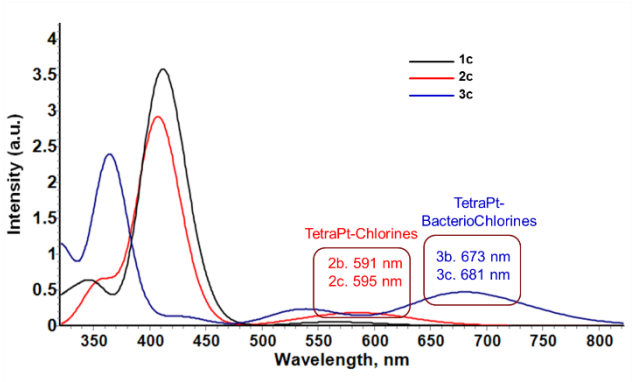


Figure 2. Superposition of the computed absorption spectra of tetraplatinated porphyrinoids **1c, 2c, 3c,** in water solvent. All the other spectra are reported in SI section.

Superposition of the computed absorption spectra of tetraplatinated porphyrinoids is reported in Figure 2. For clarity, just one conformer for each family is reported in the picture (**1c**,**2c**,**3c**). Complete spectra superposition, MOs plots and other detailed information are reported in the Supplementary Information.

Spectra reported in Figure 2 show a significant red-shift of the wavelength maxima and a hyperchromic shift in the Q-bands as one goes from tetraplatinated porphyrins to chlorines- and bacteriochlorines-derivatives. Platinated chlorins **2b** and **2c** show the lowest energy band at 591nm and 595nm, respectively, while a more significant batochromic shift is observed in the presence of two reduced pyrroles. Actually, wavelengths higher than 650 nm are reached in the cases of **3b** and **3c** which indeed absorb at 673 nm and 681 nm, respectively. Such red-shifted absorption wavelengths could make these compounds more suitable for the PDT application since light sources in that region could better penetrate into tissues allowing a more efficient treatment of deeper malignancies.

In all the cases the λ_{max} transition is $\pi \rightarrow \pi^*$ in nature and no intervention of platinum is observed in the involved molecular orbitals (See Fgures S3-S5). The significant red-shift of the lowest energy Q band is a direct consequence of the reduced H-L gap observed as the number of pyrrole rings decreases, as shown in Figure 3.

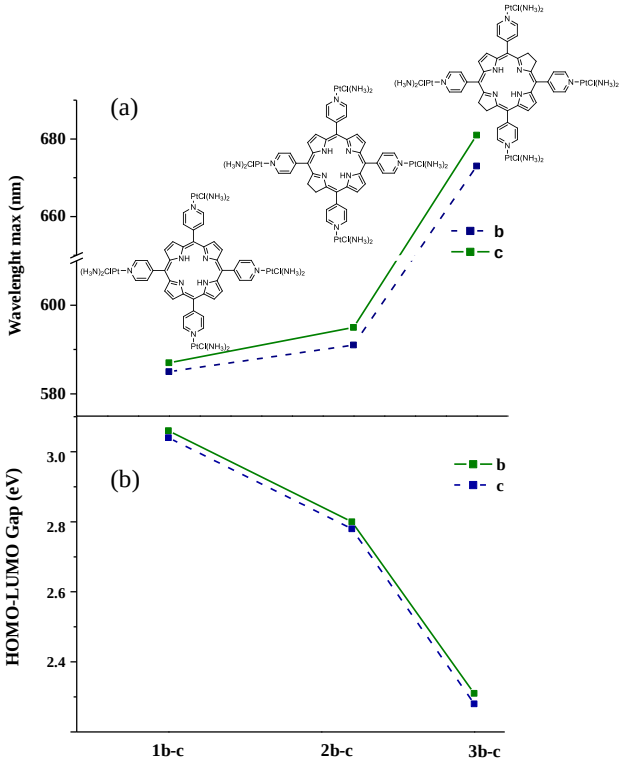


Figure 3. (a) Wavelenght maxima variation (b) HOMO-LUMO energy gap (eV), for all the investigated Pt-porphyrinoids conjugates (**1b**,**1c**,**2b**,**2c**,**3b**,**3c**) (see also SI)

Actually, the HOMO and LUMO+1 energies increase while just minor variations are observed for HOMO-1 and LUMO, the latter keeping quite constant in energy whatever the molecule is (See S5). The result is a substantial reduction of H-L gap as direct consequence of the conjugation breakdown. The influence of the platinum moieties on chlorine and bacteriochlorines derivatives has been also evaluated computing the spectra for the metal-free **2a** and **3a**, in analogy to what previously done for porphyrins dyes. Also in these cases platinum ligand does not substantially affect the optical properties producing just a slight shift of the λ_{max} toward higher wavelengths. All the computed spectra for chlorins and bacteriochlorins are reported in the Supplementary information.

- **Type II Photoreaction**

To promote Type II photoreactions, a good photosensitizer must be able to generate the cytotoxic singlet oxygen species promoting the O_2 $^3\Sigma_g^- \rightarrow {}^1\Delta_g$ transition. To permit the energy transfer process to take place and to achieve a good singlet oxygen quantum yield, the PS must possess a singlet-triplet energy gap ($\Delta_{S\text{-}T}$) larger than that of O_2, in conjunction with an efficient ISC process.

The amount of energy required to excite the molecular oxygen has been previously computed to be 0.90 eV at the same level of theory herein used, [12c] in good agreement with the experimental value of 0.98 eV. To evaluate thus the feasibility of the energy transfer process, we computed the singlet-triplet energy gap for all the investigated porphyrinoids dyes. Results are reported in Figure 4a. From the plot it emerges that porphyrins and chlorins derivatives possess a $\Delta_{S\text{-}T}$ sensibly higher than the energy required to promote the O_2 $^3\Sigma_g^- \rightarrow {}^1\Delta_g$ transition, in their tetraplatinated forms but also in the metal free derivatives. On the contrary, bacteriochlorins-ones display a T1 state lower in energy than those of their analogues, but still sufficient to promote the formation of singlet oxygen.

Obviously, the production of the cytotoxic singlet oxygen is possible only if an efficient intersystem crossing to triplet states occurs from S1. From a theoretical point of view, the rate of the ISC mechanism (K_{isc}) depends, not uniquely, on the relative magnitude of the spin–orbit matrix elements between the singlet (Sn) and triplet (Tn) wavefunctions involved in the transition. According to the Fermi golden rule, the K_{isc} is indeed proportional to the square module of the SOC elements and roughly to the energy difference between the electronic states involved (the energy gap rule). To select the possible states of interest for the Sn - Tn transition analysis, the energetic diagram for the Sn and Tn vertical excitation states with respect to the S0 ground state has been drawn and reported in Figure 4b and in the SI section. These compounds show a different number of triplet states lying below S1, so that the ISC process could involve decay to higher triplet electronic states followed by internal conversion to the first excited triplet state. To establish the feasibility of the intersystem crossing, spin orbit coupling values (SOC) between S_1 and S_2 and lower triplet states have been computed and reported in Figure 4c-e. More details can be also found in the Supporting Information. As shown in Figure 4b, four triplet states have been found below S1, while with the reduction of pyrrole units, the number of triplet states below S1 decreases. Indeed, only three triplet states are accessible from S1 for metal- and metal-free chlorines and decrease to two for platinated and metal-free bacteriochlorins. In the two latter cases, also S2-T4 and S2-T3 channel could be accessible for chlorins and bacteriochlorins, respectively. However, for all the considered ISC channels from S1 and S2 excited states, we found matrix elements comprised by 0 and 6 cm^{-1}. The small computed SOC values, although large enough to induce ISC on a nanosecond time scale [16], are a direct consequence of the ππ* character of the involved orbitals in the radiationless transition which lack any significant metal contribution, so that the well-known "heavy atom effect" [17] is quite irrelevant. Actually, according to the El-Sayed rules, the rate of the intersystem spin crossing sensibly increases if the radiationless transition involves a change in the orbital type. [14]

Nevertheless, tetraplatinated derivatives display higher SOC values compared to metal-free precursors (see Figure 4c-e and SI) and they are several order of magnitude higher than those computed for the currently approved PDT drug Foscan® [18,19] usually used as reference. It is worth of note that comparable small values have been also previously computed for metallic PSs for which the accessible ISC channels involve excited states with nearly identical orbital transition contribution or characterized by a lack of metal-contribution. [13a,20] In the case of porphyrins-derivatives, the most accessible triplet state from S1 and S2 is T4, showing the smallest singlet-triplet energy splitting (≈0.2 eV for S1→T1 and ≈0.3eV for S2→T4). The highest SOC values for both these ISC channels have been computed for porphyrin **1c**. Chlorins- and bacteriochlorines-derivatives, show similar behaviors. Derivatives having the chloride leaving group in trans with respect to the pyridyl group (namely **2c** and **3c**) show the highest SOC values for the most accessible ISC channels (S1-T3 for **2c** and S2-T3 for **3c**).

Remarkably, in the case of chlorines derivatives, the metal-free dye **2a** also shows a not-negligible SOC value for the accessible S2-T4 channel. However, due to the absence of metal contribution in the orbitals involved in the examined channels, the computed SOC are far from the very high values obtained in Pt- and other metal-complexes. [7,21]

Nevertheless, it should be emphasized that the wavelength used experimentally for the evaluation of the singlet oxygen quantum yield of platinated porphyrins (around 400 nm), [4] promotes the population of the higher energy singlet states S3 and S4. It is upon irradiation in that region that platinated compounds 1b and 1c exhibited excellent values of Φ. [4] For this reason, we explored the possibility of ISC from S3 and S4 computing SOC values between them and any triplet states energetically accessible lying below. Actually, the inspection of the higher Tn states nature reveals a more pronounced contribution of the metal orbitals and, therefore, an enhancement of the ISC mechanisms involving these higher triplet states it could be expected. Indeed, for all the platinated conjugates we found larger values for the coupling involving higher excited states compared to those obtained for S1 and S2 and the lower triplet states, which can give a rationale for the high $^1O_2$ quantum yield experimentally determined at 401nm. [4] (See Figures S6-S9)

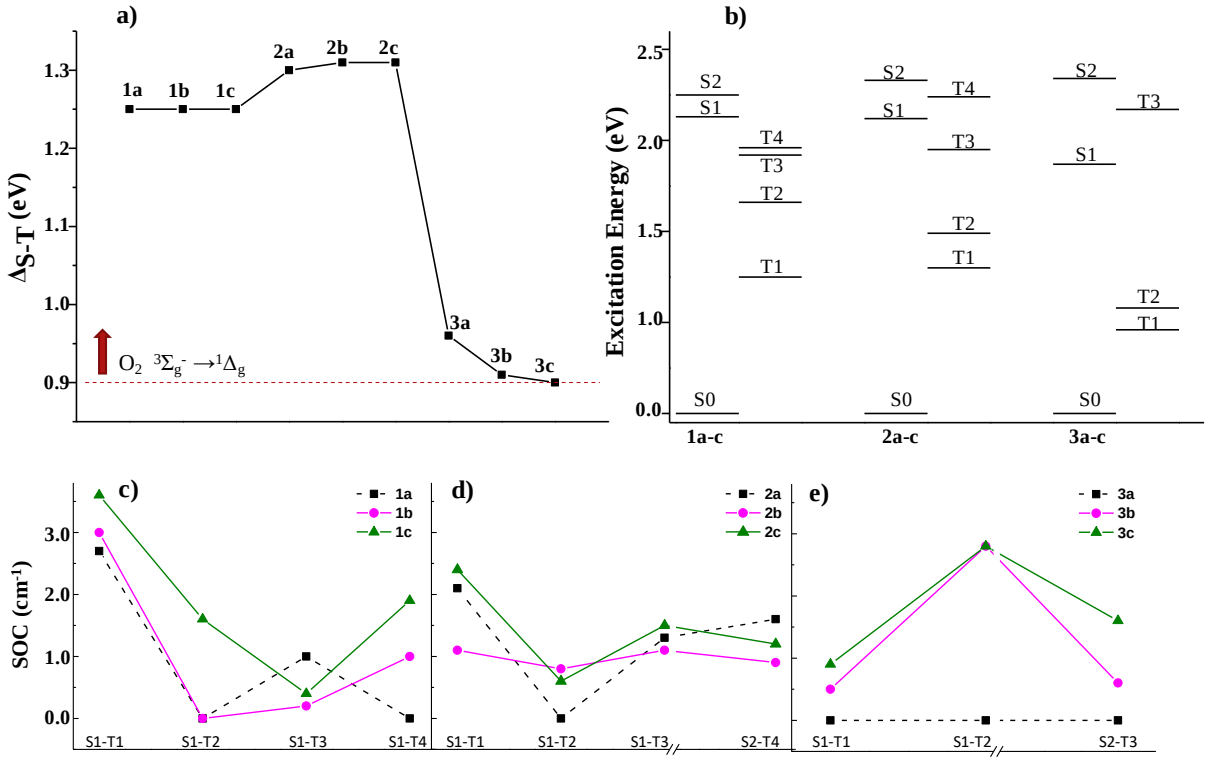


Figure 4: (a) Singlet-Triplet energy gap (eV); b) Diagram of S_n and T_n vertical excitation states (eV); c) Plots of the computed SOC values between S1, S2 and the triplet states lying below them (cm^{-1}), for all the investigated Pt-porphyrinoids conjugates (see also SI)

Interestingly, compound **2c** show very high SOC values (up to 580 cm^{-1}) between S3 and S4 and the very close in energy triplet states (T9-T12). On the contrary, neither bacteriochlorins-derivatives nor porphyrins ones display comparable values and so many deactivation channels like in the case of **2c** compound. ISC mechanism involving these higher states could be sufficiently faster to compete with internal conversion within the singlet manifold. Such kind of mechanism, has been previously suggested for Pt^{II} compounds [22] and other metal-complexes [15]. As expected, no high SOC values have been computed instead between higher states in metal-free dyes, due to the lack of any metal contribution in the involved orbitals.

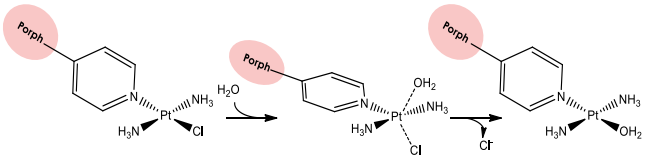
Such result show that the insertion of platinum into these dyes, although does not have a great influence on the absorption spectra and on the ISC mechanism involving S1 and S2 state, can provide an efficient pathway to populate the lowest triplet state by internal conversion from higher triplet ones. In addition, such mechanism seems to be particularly feasible in the case of tetraplatinated chlorine derivative **2c**, allowing us to hypothesize new prospects for such kind of conjugate, which would deserve further experimental investigation.

- **Pt^{II} Activation Mechanism**

As previously mentioned, DNA has been experimentally suggested as main target for tetraplatinated porphyrin **1c** even without the involvement of light. [4] A covalent binding to nitrogen atom of guanosine has been indeed hypothesized for such compound encouraging further investigation on the activation mechanism of it before reaching its biological target. Actually, in analogy to the other Pt^{II} anticancer drugs widely investigated so far,[9] a ligand substitution process by water could take place in the dark producing activated form of the complex likely to react with DNA bases. Accordingly, the hydrolysis of Pt^{II} moiety in these new conjugates is also expected to play an important role in their activation before reaching DNA.

From a mechanistic point of view, it is broadly accepted that the Pt^{II} anticancer drugs undergo a hydrolysis process through second-order nucleophilic substitution (SN2) reactions. [8,9] In such a process, the equatorial plane of the five-coordinated TS structure plays an important role in determining the hydrolysis behaviour. Pt^{II}-porphyrinoids compounds are supposed to react with water molecules following the same reaction mechanism. It is noteworthy that only one labile group is bound to the Pt^{II} metal center, so that such compounds are expected to form only one covalent bond to DNA. Actually, the precursor *cis*$[Pt(NH_3)_2(pyridine)Cl]^+$, known as pyriplatin, has been shown to induce monofunctional lesion to DNA, strongly inhibiting transcription both in cell extracts and in live cells [8a]

In order to give insights on the hydrolysis process, the chloride substitution by water has been investigated not only for the recently synthesized porphyrin **1c** (Scheme 1) but also for all the other Pt-porphyrinoids compounds herein proposed, in order to spotlight similar or different behaviors between them.



Scheme 2. Investigated Hydrolysis Process for TetraPt^{II}-porphyrinoids complexes (**1b**,**1c**,**2b**,**2c**,**3b**,**3c**) *(porphyrinoid dyes are illustrated by a coloured circle for clarity; See SI for the reaction mechanism and free energy profiles of each complex).*

The exploration of the hydrolysis process of the considered systems allows us to evaluate from one side, the ligand-exchange kinetics as a function of the geometric features of Pt^{II}-moiety, comparing cis and trans isomers (**1**-**2**-**3 b** vs **1**-**2**-**3 c**), and from the other side, the influence of the chromophore dye on their ligand-exchange kinetics.

The activation energy barrier for the chloride-water substitution process are schematically reported in Figure 5. The optimized structures located along the reaction path and more detailed information can be found in the Supporting Information.

The reaction proceeds *via* an associative mechanism through a penta-coordinated trigonal bipyramid transition state, in agreement with the hydrolysis mechanism displayed by anticancer Pt^{II} complexes, previously investigated. [9]

The first thing that should be emphasized is that the hydrolysis process proceeds slower when the leaving group is on the opposite side with respect to the pyridyl-group, that is trans-isomers. Actually, as shown in Figure 3, derivatives **1c**, **2c** and **3c** require a greater amount of energy for the hydrolysis process to take place compared with cis-isomers **1b**, **2b** and **3b**. This finding is really interesting since a slow rate of aquation is generally related to reduced side effects displayed by Pt^{II} containing anticancer drugs, and accordingly, is essential to have candidate compounds with no too much labile ligands. Research on platinum compounds with *trans* geometry was marginalized for decades due to their kinetic instability which could facilitate undesired reactions on the way to the biological target with a consequent lack of anticancer activity. In these specific Pt^{II}-chromophore compounds instead, trans isomers show a slower rate of conversion to the reactive aquated species than cis-analogues and, accordingly, they should be preferred to avoid many side reactions with other DNA-competitor nucleophiles. In addition, Figure 5 reveals that the nature of the chromophore dye bound to Pt^{II} moiety affects the ligand-exchange kinetics in the case of trans-isomers. Actually, while the activation barriers computed for cis-isomers are not sensibly affected by the chromophore, for trans isomers instead, a reduction of the hydrolysis rate is predicted when porphyrin is replaced by chlorine or bacteriochlorine dyes. This effect can be explicated in view of the enhanced electro-donor character of the reduced porphyrinoids compared with porphyrin, with a drop in the positive charge on the Pt^{II} ligand.

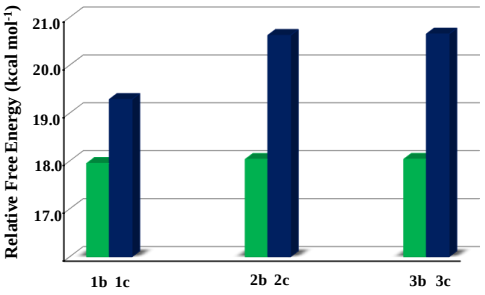


Figure 5. Free energy barriers in solution (kcal/mol) for the hydrolysis process of tetraplatinated-porphyrinoids, in water environment

As a consequence, the bond between Pt and the leaving chloride in trans position results to be less polarized and slightly less prone to substitution. Natural Bond Orbital charges analysis confirm the decreased positive charge localized on Pt^{II} fragment in both chloride- and bacteriochloride- transition states structure (see SI). Our data reveal, therefore, that the introduction of reduced dyes not only improve the optical properties, compared with platinated-porphyrin systems, but has also an influence on the hydrolysis process, reducing the chloride-water exchange which is a desirable feature to avoid many side-reactions.

Conclusions

A careful DFT, TDDFT and QR-TDDFT investigation has been carried out on recently synthesized TetraPt^{II}-porphyrins conjugates [4] (**1b**, **1c**) proposed as promising multi-target agents in view of the combination of PDT and cytostatic activity into a single synergistic system. Moreover, to propose a further advance in this novel field and give some insights useful for the design of new PSs with improved efficiency, we introduced structural modification into the porphyrin dye evaluating the consequent changes in the chemical and photophysical properties of analogous tetraPt^{II}-chlorines and tetraPt^{II}-bacteriochlorins dyes. (**2b**,**c** and **3b**,**c**).

For all the compounds we explored the effect of Pt^{II}-moiety on the crucial properties of each chromophore investigating also the metal-free dyes (**1**-**3a**) and, in turn, we evaluated the effect of the chromophore on the activation mechanism of Pt^{II}-ligand required before reaching its biological target.

Inspection of UV-Vis electronic spectra, Type II photoreactions and spin-orbit coupling (SOC) computations reveals that the designed dyes better meet the criteria to be successful in the PDT application. Actually, although all the conjugates possess $\Delta_{S\text{-}T}$ gap higher enough to ensure the O_2 $^3\Sigma_g^- \rightarrow {}^1\Delta_g$ transition, combined with not-negligible SOC values, tetraPt^{II}-chlorines and tetraPt^{II}-bacteriochlorines show a significant red-shifted λ_{max} which could better penetrate into tissues allowing a more efficient treatment of deeper malignancies. All tetraplatinated derivatives

display higher SOC values compared to metal-free precursors and they are several order of magnitude higher than those computed for the currently approved PDT drug Foscan®[18,19] usually used as reference value. Interestingly, slightly higher SOC values have been computed for trans isomers (**1-3c)**. Furthermore, an enhanced ISC mechanism has been found from higher singlet and triplet states, which could compete with internal conversion within the singlet manifold, especially in the case of Pt^{II}-chlorin derivative **2c**.

On the other hand, the exploration of the hydrolysis process of the considered systems allowed us to evaluate from one side, the ligand-exchange kinetics as a function of the geometric features of Pt^{II}-moiety, comparing cis and trans isomers (**1-2-3 b** vs **1-2-3 c**), and from the other side, the influence of the chromophore dye on their ligand-exchange kinetics.

Remarkably, we found that trans isomers (**1c**, **2c**, **3c**) show a slower rate of conversion to the reactive aquated species than cis-analogues and, accordingly, they should be preferred to avoid many side reactions with other DNA-competitor nucleophiles. More importantly, results show that the nature of the chromophore dye interestingly affects the hydrolysis rate of the Pt^{II}-moiety. In particular, chlorines and bacterio-chlorines derivatives show a slower chloride-water exchange as a function of the geometric features of Pt^{II}-unit compared to porphyrin dye. Actually, while the activation barriers computed for cis-isomers are not sensibly affected by the chromophore, for trans isomers instead, a reduction of the hydrolysis rate is predicted when porphyrin is replaced by chlorine or bacteriochlorine dyes. This effect can be explicated in view of the enhanced electro-donor character of the reduced porphyrinoids compared with porphyrin, with a drop in the positive charge on the Pt^{II} ligand. As a consequence, the bond between Pt and the leaving chloride in trans position results to be less polarized and slightly less prone to substitution.

In summary, our data help to shed light on new and promising agents for a combined approach against cancer. They also reveal that the substitution of porphyrin dye by chlorine or bacteriochlorine in the Pt-porphirinoid systems, could be useful not only to improve the optical properties of the conjugates but it is also likely to modulate the Pt^{II}-hydrolysis rate, reducing the chloride-water exchange which is a desirable feature to avoid many side-reactions.

We believe that our data can stimulate further investigations on these interesting assemblies and contribute to the common effort to derive rational guidelines to support the design of new successful candidates for multimodal anticancer approach.

Experimental Section

All the calculations herein presented have been performed at DFT and its time-dependent TD-DFT formulation [23] by using the Gaussian 09 program code. [24] Ground state geometry optimizations have been performed in water without constrains by using the B3LYP exchange-correlation functional[25-26] in conjunction with the 6-31G(d,p) basis set for all atom except for Pt one, which was described by the quasi-relativistic Stuttgart-Dresden pseudopotential.[27] Water environment has been simulated by means of the integral equation formalism polarizable continuum model (IEFPCM),[28] which corresponds to a linear response in non-equilibrium solvation. The hydrolysis process of all the Pt^{II}-porphyrinoids conjugates has been explored in implicit solvent. Nevertheless, to better account for the effects of hydrogen bonding and provide a more appropriate solvation of the anionic leaving group, two explicit water molecules, beside the catalytic one, have been also added in the system. To confirm proper convergence to equilibrium and transition state geometries, vibrational frequency analysis has been carried out on the basis of analytical second derivatives of the Hamiltonian at this level of theory. To obtain more accurate activation energy barrier for the hydrolysis process of all the investigated conjugates, single-point calculations were also carried out with the larger basis set 6-31++G(2df,2pd).

Absorption spectra have been obtained in water, as vertical electronic excitations on the ground-state structures, employing M06[29] XC functional. The good performances of M06 in the reproduction of optical properties of Pt and other metals-containing systems has been widely tested. [7,20,30] Spin–orbit matrix elements were computed by using the quadratic-response QR-TDDFT approach, [31-32] as implemented in the Dalton code,[33] at their ground-state optimized geometries, using the approximate 1-electron spin-orbit operator with scaled nuclear charges.[34] For this purpose, B3LYP was used coupled with the cc-pVDZ basis set for all atoms and SDD pseudopotential on metal ions. The spin–orbit couplings (SOCs) have been defined according to the following formula:

$$SOC_{ij} = \sqrt{\sum_{n} \left|\langle \psi_{S_i} | \hat{H}_{SO} | \psi_{T_{j,n}} \rangle\right|^2}; \quad n = x, y, z$$

where $\hat{H}_{SO}$ is the spin–orbit Hamiltonian.

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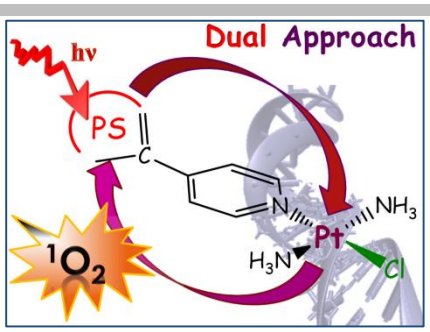
Entry for the Table of Contents

FULL PAPER

The combination of a photosensitizer (PS) with a cisplatin-like unit represents a new challenging strategy to afford a dual approach anticancer treatment. The synergistic effect between the two functionalities have been herein explored investigating promising Pt^{II}–Porphyrinoids conjugates by a careful first-principle investigation.



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Synergistic effects in Pt(II)-Porphyrinoid Dyes as candidates for a dual anticancer approach: a theoretical exploration