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Photoactivatable platinum (II) terpyridine derivatives for G-quadruplex DNA double anchoring

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ABSTRACT

Platinum (II) tolylterpyridine (ttpy) complexes, have been shown to generate monofunctional adducts with various G-quadruplex DNA forming sequences resulting from an efficient π-stacking mode on the top of the quadruplex structure. To further explore the potential of this series with regard to quadruplex recognition, classical photocrosslinking groups (benzophenone, tetraphenylazide) have been grafted on the tolylterpyridine ligand moiety, thereby generating two new derivatives Pt-ttpy-Bn and Pt-ttpy-N_3. Evaluation of their non-covalent binding for G-quadruplex DNA has been performed by FRET-melting and FID assays using two G-quadruplex matrices *i.e.* the telomeric sequence 22AG and the oncogene promotor sequence c-myc, which revealed high affinity and improved selectivity as compared to the parent compound. Subsequently the capacity of the compounds to establish one or two anchorage points (one by platination, one by photoinduced crosslinking) with the quadruplexes has been studied by gel electrophoresis with and without photoactivation. Interestingly both compounds do platinate the quadruplexes studied with high selectivity as the platination yield is poorly affected by the presence of duplex competitor. By contrast, only the azido derivative Pt-ttpy-N_3 was found to form a second covalent bond within the G-quadruplexes upon photoactivation indicating a higher confinement of the crosslinking moiety in this case. Finally the two compounds exhibit poor cytotoxicity in the dark on two cancer cell lines (A2780 and HT29), whereas that of the benzophenone derivative is significantly enhanced upon irradiation. Altogether the two new compounds represent novel prototypes usable for trapping G4 DNA alone or eventually G4-DNA protein interactions in complex *in vitro* systems or in cells.

Nucleic acid secondary structures called G-quadruplex are believed to play regulatory roles in the main functions related to DNA processing [1]. However, although the field is booming and quadruplex DNA is on the way to become a paradigm used to explain most DNA processing dysfunctions, there are still a number of essential questions unanswered. In particular, the number of quadruplex forming sequences (QFS) *in vivo* in the human genome is still a matter of controversy. This number was initially claimed to be around 300,000 based on bioinformatics analysis but recent *in vivo* studies in yeast based on quadruplex-induced genetic instability strongly suggest a drastic decrease of the number of PQS down to 20,000 [2] whereas *in vitro* sequencing identified up to 700,000 PQS [3]. Another crucial question is whether specific protein have been evolved to process quadruplex DNA or if this occurs via the standard machinery e.g. helicases [4]. For all these reasons it is of importance to provide new chemical biology tools for trapping and mapping PQS as well as identifying their protein partners.

Photoreactive species are extensively used for the study of DNA interactions with biological partners, and especially for trapping proteins [5]. Moreover, since the discovery of antitumor properties of cis-platin, numerous metal complexes have been synthesized for their coordination potential on DNA. With the use of a compound combining a photoreactive benzophenone and cis-platin analog, Lippard et al. were thus able to efficiently crosslink both duplex DNA and associated proteins, leading to identification of those proteins [6]. In this line we have recently shown that compounds combining a G-quadruplex stabilizing scaffold (i.e. bisquinolinum pyridodicarboxamide [7]), and cross linking moieties led to covalent trapping of G-quadruplex DNA, upon alkylation [8] and photoactivation [9]. In parallel, we developed the class of platinum (II) tolylterpyridine (Pt-ttpy) complexes and showed that these square planar complexes have the ability to π-stack on the top of G-quadruplexes which favor coordination to the surrounding nucleic bases resulting in highly specific and stable adducts [10].

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Therefore, based on the previous work outlined above, it was decided to introduce photocrosslinking function on the G-quadruplex binding platinum (II) complex (Pt-ttpy) in the aim to develop molecular tools with two anchoring possibilities. This double anchorage may occur inside the quadruplex and in this case should considerably strengthen the small molecule/quadruplex interaction and eventually raise preferences for quadruplex topologies. Alternatively these double connecting agents may allow trapping of quadruplex/protein interactions.

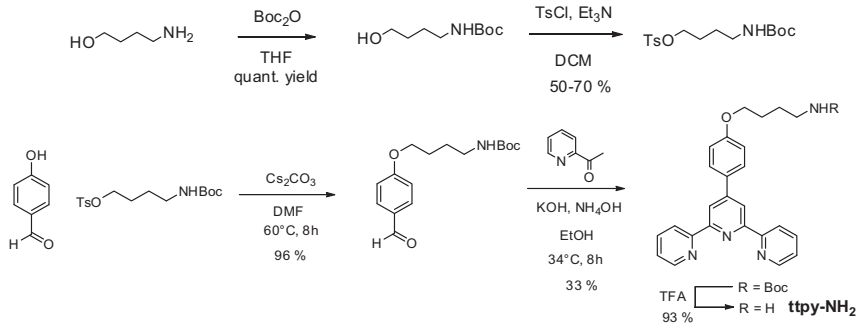
We therefore envisaged the preparation of two compounds (Pt-ttpy-Bn and Pt-ttpy-N_3), by grafting the two classical photoreactive groups, benzophenone (Bn) and 4-azido-2,3,5,6-tetrafluorobenzoic acid (N_3), on the tolylterpyridine scaffold. These photoreactive groups can be activated by irradiation at wavelengths close to UVA-visible (330–365 nm) to generate highly reactive radical intermediate species [11]. Both of these groups have been extensively used for protein–protein photolabelling [12] or for the study of protein–ligand [13] and DNA-ligand interactions [14].

The benzophenone moiety is commercially available and the tetrafluorobenzoate azide was prepared in a well-described three-step synthesis [9a]. The two compounds, Pt-ttpy-Bn and Pt-ttpy-N_3, were synthesized from a tolyterpyridine precursor bearing an amino-terminated side chain on the tolyl ring (ttpy-NH_2) The preparation of this precursor was done in three steps of high efficiency from 4-hydroxybenzaldehyde (Scheme 1). Previously protected aminoalcohol spacer was substituted on 4-hydroxybenzaldehyde with 96% yield. The Kröhnke reaction [15] of this aldehyde with 2-acetylpyridine in the presence of potassium hydroxide and ammonium hydroxide afforded the tolylterpyridine product ttpy-NHBoc with 26% in one step. The deprotection of the amino group was performed with TFA and led to ttpy-NH_2 in a quantitative yield. The bifunctional compounds were prepared by coupling the photocrosslinking moieties with the starting material ttpy-NH_2, followed by a metallation step (Scheme 2). In the first step, the carboxylic acid derivatives of benzophenone and teraphenylazide were introduced under classical peptide-type coupling conditions using EDCI as coupling agent in the presence of DMAP and HOBt as catalyst, affording compounds ttpy-N_3 and ttpy-Bn with 79% and 84% yield respectively. These intermediates were platinated in presence of Pt(COD)Cl_2, in extra dry methanol under argon atmosphere. After filtering the solid from the reaction mixture and washing the platinum complexes Pt-ttpy-N_3 and Pt-ttpy-Bn were obtained in moderate to good yields.

1. Interaction measurements

The newly prepared tolylterpyridine derivatives were evaluated for their specific G-quadruplex DNA interactions using two quadruplex forming sequences 22AG and cmyc (myc22), corresponding to the human telomeric sequence and the oncogene promoter cmyc respectively (see sequences in Supporting Information). The affinity of these platinum complexes for G-quadruplex and duplex DNA was first investigated by fluorescent intercalator displacement (G4-FID) assay [16], which is based on the competitive displacement of thiazole orange (TO) from DNA by the compounds to be evaluated (Fig. 1). This semi-quantitative assay allows affinity ranking for series of compounds by determination of the DC_{50} value i.e. the compound concentration inducing 50% of probe fluorescence decrease ($DC_{50} < 0.5$ μM characterizes high affinity binders, $0.5 < DC_{50} < 1$ μM medium to moderate binders, $1 < DC_{50} < 2.5$ μM low affinity binders and $DC_{50} > 2.5$ μM no significant binding). For instance the non-functionalized complex Pt-ttpy used here as reference shows strong affinity for tested G-quadruplex DNA structures, oncogene cmyc and human telomeric 22AG sequences with a $DC_{50} \sim 0.2$ μM in both cases. This complex was found to displace TO from duplex DNA with a significantly more moderate efficacy as 7-fold higher DC_{50} is observed in this case ($DC_{50} = 1.5$ μM). In the same assay, the azido derivative binds the two G-quadruplex structures with a high affinity although the DC_{50} values are slightly lower: they fall in the range defining high affinity binders (DC_{50} Pt-ttpy-N_3-/22AG = 0.55 μM, DC_{50} Pt-ttpy-N_3/cmyc = 0.35 μM). On the opposite, for the benzophenone derivative the G-quadruplex affinity is a bit more affected with DC_{50} values of 1.33 μM and 0.61 μM for 22AG and cmyc respectively, but with a clear preference for cmyc. Most interestingly when evaluated in presence of duplex DNA, these complexes show quite no displacement of TO. ($DC_{50} > 2.5$ μM). Remarkably, comparison with the reference Pt-ttpy indicates that the selectivity for G-quadruplex structure is strongly increased when grafting a photocrosslinker moiety on the tolylterpyridine scaffold.

FRET-melting experiments were also performed using G-quadruplex DNA sequences doubly labelled with a donor–acceptor FRET pair namely F21T and FmycT [17] (F = fluorescein, T = TAMRA, see Supporting Information). This well-used assay is based on FRET principle to monitor the thermal stability of DNA alone or in presence of a binding compound. As compared to standard UV-melting, the FRET-melting enable to addition of unlabeled duplex DNA competitor thereby allowing evaluation of ligand selectivity for the G-quadruplex structure. Both compounds display strong stabilization of G-quadruplex structures, with ΔT_m values around 15–20 °C (Fig. 2) (for reference the benchmark compounds $PhenDC_3$ and PDS induce ΔT_m of 25–30 °C in the same conditions). Fig. 2A corresponds to results obtained with the telomeric quadruplex. The azido derivative exhibits a pattern very similar to that of the reference compound indicating a similar stabilization (first blue bar $\Delta T_m = 24$ °C) whereas the benzophenone derivative has a significantly lower stabilization effect ($\Delta T_m = 13$ °C) in consistency with



Scheme 1. Synthetic route for ttpy-NH_2. (a) Cs_2CO_3, DMF, 60 °C, 8 h, 96%; (b) 2-acetylpyridine, KOH, NH4OH, EtOH, 34 °C, 24 h, 26%; (c) TFA, DCM, RT, 4 h, quantitative yield. Nommer ttpy-NH_2 sur le schema R = H, ttpy NH_2.

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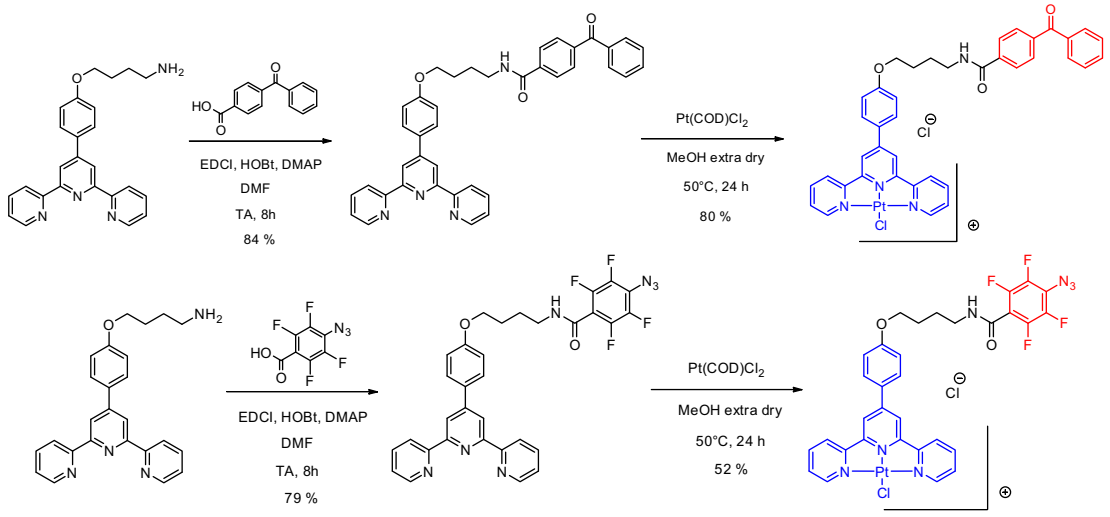

Scheme 2. Coupling conditions for the Pt-ttpy-N_3 and Pt-ttpy-Bn. (a) 4-Azidotetrafluorobenzoic acid, EDCI, HOBt, DMAP, DMF, RT, 8 h, 79%; (b) $Pt(COD)Cl_2$, dry MeOH, 50 °C, 24 h, 52%; (c) 4-benzoylbenzoic acid, EDCI, HOBt, DMAP, DMF, RT, 8 h, 84%; (d) $Pt(COD)Cl_2$, dry MeOH, 50 °C, 24 h, 81%. In blue, toly-terpyridine moiety. In red, photo-cross-linking agent. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

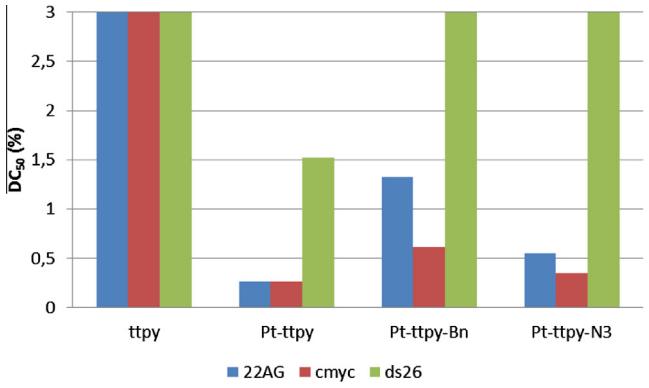

Fig. 1. DC_{50} (μM) values from G4-FID assay on G-quadruplex (22AG and cmyc) and duplex (ds26) DNA in 100 mM K^+ buffer.

the FID assay. Addition of 3–10 equivalents of duplex DNA competitor decreases the G-quadruplex binding for the three compounds but to a moderate extent. However, the strong improvement observed by FID assay is not reproduced here. Perfect agreement between the two assays cannot be expected as platination may occur to various degree during both experiments (unpublished results) [18].

In the case of cmyc (Fig. 2B), the three compounds show very similar profiles (high affinity with $\Delta T_m \sim 13$–15 °C) and excellent resistance to the competition revealing a high selectivity versus duplex (of note due to the high stability of the cmyc quadruplex, the assay is performed at low K^+ ionic force thus the ΔT_m values cannot be compared between F21T and FmycT experiments). In the whole, the two assays indicate that Pt-ttpy-Bn and Pt-ttpy-N_3 retained the high affinity for G-quadruplex DNA of the Pt-ttpy core. Equally, high selectivity for G-quadruplex versus duplex DNA is observed in all cases, especially in the case of cmyc. Nonetheless, all compounds displayed low affinity towards duplex DNA, assessing their strong structural preference for G-quadruplex DNA conformations. Of note and as previously shown [19], the tolyterpyridine ligand alone shows no binding activity in both assays thereby evidencing the crucial role of the metal that confers cationic charge, rigidity and square planar geometry suitable for quadruplex recognition.

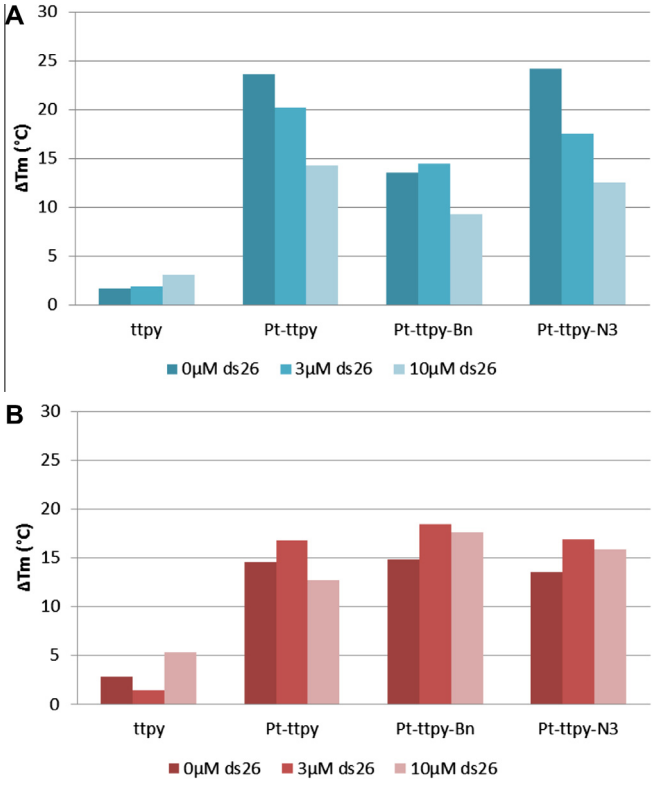

Fig. 2. ΔTm (°C) values from FRET-melting experiment on F21T (**A** – 10 mM K^+ buffer) and FmycT (**B** – 1 mM K^+ buffer) for compounds (5 eq.) in presence of 0, 3 and 10 μM of duplex DNA (ds26) as competitor.

2. Platination and photocrosslink of G-quadruplex DNA

To evaluate if the two compounds Pt-ttpy-Bn and Pt-ttyp-N_3 can cause double anchorage inside G-quadruplex DNA structures, we performed platination and photocrosslinking experiments with 22AG and cmyc oligonucleotides, which were subsequently analyzed by denaturing gel electrophoresis. After incubation with the oligonucleotides for 5 min, both compounds induce formation of retarded bands with 22AG (Fig. 3A and C – lines c) and of accelerated bands with cmyc (Fig. 3B and D – lines c) which can be

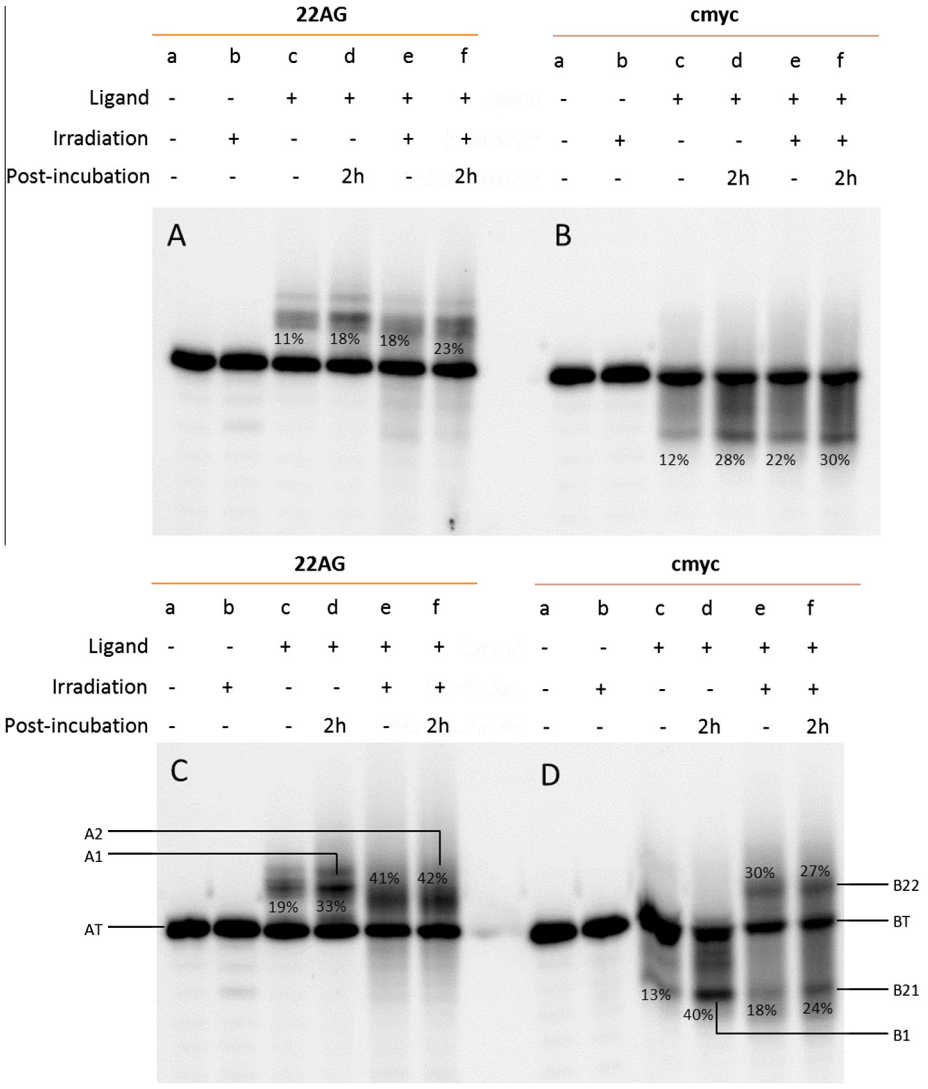

Fig. 3. Denaturating gel electrophoresis (15% acrylamide) of incubation of 2 eq. of compounds Pt-ttpy-Bn (A and B) and Pt-ttpy-N_3 (C and D) with cmyc and 22AG (10 μM) in the presence of K^+-buffer (50 mM) after 5 min incubation at 37 °C. Lane a = DNA only, b = DNA only, irradiated by 60 flashes, c = 5 min incubation of DNA and ligand, d = 5 min incubation of DNA and ligand, followed by a 2 h post-incubation, e = 5 min incubation with ligand, followed by photoactivation and f = 5 min incubation with ligand, followed by photoactivation and a 2 h post-incubation.

attributed to platinum adduct. Herein the adducts are formed in yields similar to the ones already found for (photo)-alkylating agents [8,9] varying from 11% to 19% for 22AG to 30% for cmyc, and are significantly increased with longer incubation time (up to 18–33% with 22AG and 40% with cmyc, Fig. 3A–D – lines d). Of note these adducts migration characteristics i.e. retarded bands with 22AG and accelerated with cmyc are similar to those observed with the model compound Pt-ttpy (Fig. S1). When the incubation is followed by photoactivation, no significant change in the number and migration level of bands is observed in the case of Pt-ttpy-Bn, irrespective of the incubation time (Fig. 3A and B – lines e, f) suggesting either that photocrosslinking is not occurring or that band migration is not modified by photoalkylation. By contrast, strong modification of the adducts profile is induced in the case of Pt-ttpy-N_3. The faster migrating bands corresponding to platination adducts of cmyc are strongly decreased in favour of new retarded bands (Fig. 3D – lines e, f). Similarly the migration of the retarded bands observed upon platination of 22AG is modified (Fig. 3C – lines e, f). As irradiation alone does not affect DNA integrity (Fig. 3A–D – lines b), the newly appearing bands induced in these conditions account likely for photocrosslinking adducts generated from the already formed platination products. (Fig. 3D – lines d, f). It can thus be assumed that the new bands observed upon photoactivation correspond to double anchorage of the bifunctional compound Pt-ttpy-N_3 inside the cmyc quadruplex.

To locate the platination and photocrosslinking sites, bands were isolated from the gels and subjected to different sequencing procedures. However partial deplatination of adducts during the analysis procedure occurred (Fig. 3A–D – products A1 and B1), therefore only photoalkylation sites could be identified. As shown in our previous studies, benzophenone adducts revert to starting material at high temperature [20], thus sequencing of Pt-ttpy-Bn adducts had been done by 3′-exonuclease, whose digestion stops at crosslinked sites [21]. Treatment of Pt-ttpy-Bn photoactivated products by 3′-exonuclease showed no digestion arrests, indicating that Pt-ttpy-Bn forms no photocrosslink adducts on G-quadruplex DNA (Data not shown). Pt-ttpy-N_3 photoadducts (Fig. 3C and D – products A2, B21 and B22) have been treated by piperidine at high temperature, which induces cleavage on the 5′-side of the photocrosslinked bases. Treatment of products obtained after photoactivation of Pt-ttpy-N_3 (Fig. 3C and D – A2, B21 and B22) highlights defined DNA

crosslinking sites (Fig. S2). Cleavage sites allowed us to determine the location of photocrosslinking adducts on both 22AG and cmyc by comparing the migration of those fragments with the DMS-piperidine treated non-alkylated oligonucleotide.

With 22AG, the azido derivative forms covalent adducts on guanines located within the two external 3′ and 5′ quartets of the G-quadruplex structure (G8-G10-G14-G16-G22). This crosslinking pattern is consistent with stacking of the compound on the top of the parallel and antiparallel conformations identified by NMR spectroscopy and X-rays for the human telomeric sequence [22] (Fig. 4B and C). In the case of cmyc, Pt-ttpy-N_3 also binds to the external quartets of the G-quadruplex structure [23], as shown by crosslinking of guanines situated mainly on the 5′-end (G17-G13-G8-G4) and to a lesser extent on the 3′ end (G19-G15-G10-G6) (Fig. 4A).

As summarized in Table 1, the azido derivative is able to establish both platination and photocrosslinks in the two G-quadruplex structures studied whereas the activity of the benzophenone derivative is limited to platination. As thymines are the targets of benzophone radical [9a], we can speculate that the benzophenone moiety in this case adopts an unfavorable positioning with regard to the loop thymines of both quadruplexes thereby explaining absence of reactivity.

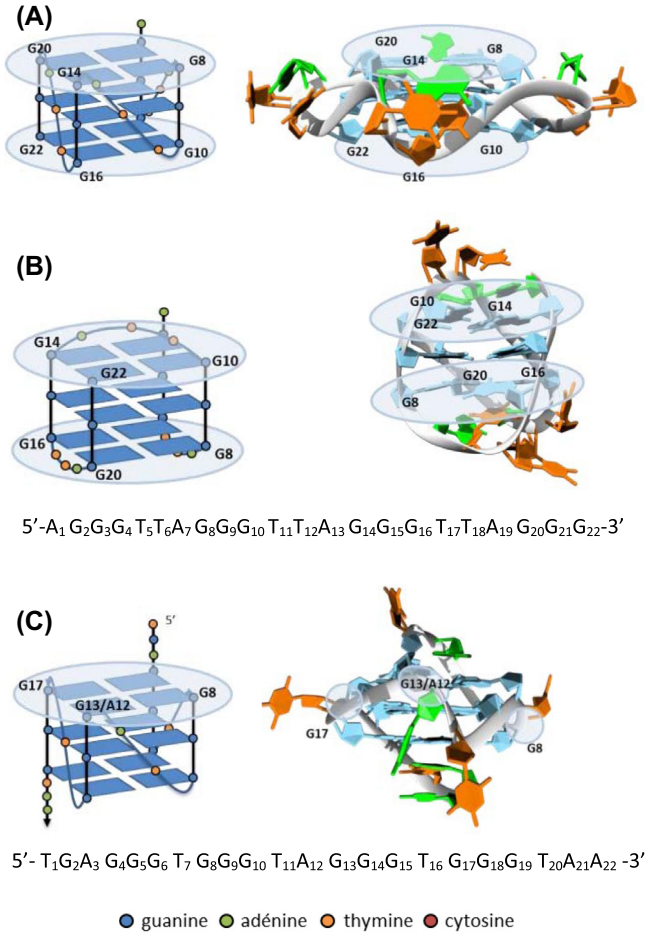

Fig. 4. Photocrosslinking sites on human telomeric repeat sequence 22AG G-quadruplex forming sequence ((A) parallel form and (B) antiparallel form) for Pt-ttpy-N_3 and (C) major photocrosslinking sites on cmyc G-quadruplex forming sequence. (Of note only parallel and antiparallel forms of 22AG are shown here as an illustration of photocrosslinking sites but do not account for all conformations in equilibria in aqueous solution.)

Table 1
Platination and photocrosslinking adducts determined for Pt-ttpy-Bn and Pt-ttpy-N_3 in the presence of cmyc and 22AG G-quadruplex forming sequences.

		Pt-ttpy-N_3	Pt-ttpy-Bn
CMYC	Platination	+	+
	Photocrosslink	+	–
22AG	Platination	+	+
	Photocrosslink	+	–

Finally the selectivity of platination of Pt-ttpy-Bn and Pt-ttpy-N_3 was assessed by gel electrophoresis. To this end, formation of platinated adducts was followed as a function of time (Fig. S3A–D). When adding an excess of duplex DNA as a competitor, longer incubation times are required to reach platination maxima but these total amounts of adducts are not affected in the case of Pt-ttpy-N_3 with both cmyc and 22AG, which confirm the high selectivity of this compound for G-quadruplex DNA (Fig. 5). Platination by Pt-ttpy-Bn is more affected by the presence of duplex competitor, as shown by the significant decrease of adducts from 20% to 50% observed in this case (Fig. 5).

Encouraged by these results, we went one step ahead and evaluated the cytotoxicity of these new platinum complexes on two human cancer cell lines: human ovarian cancer cell line (A2780) and human colon carcinoma cell line (HT29). The two compounds showed poor toxicity in the dark (IC_{50} > 20 μM) (Table 2) whereas toxicity of Pt-ttpy-Bn increased significantly upon UV/Vis irradiation (320–420 nm) on both cell lines, which was not observed for Pt-ttpy-N_3. Whether this difference in activity results from poor cellular uptake or low stability of nitrene intermediates in cellular environment remains to be determined, in particular in the light of the extent of platination of the cellular DNA induced by each compound. Nonetheless the phototoxicity observed for Pt-ttpy-Bn already suggests the formation of the reactive form of the benzophenone moiety inducing trapping of macromolecular targets in its vicinity.

In summary, we developed two platinum (II) tolylterpyridine derivatives, equipped with photoreactive groups, Pt-ttpy-N_3 and Pt-ttpy-Bn. Interestingly these compounds retained the high binding affinity of Pt-ttpy scaffold for G-quadruplex DNA but with

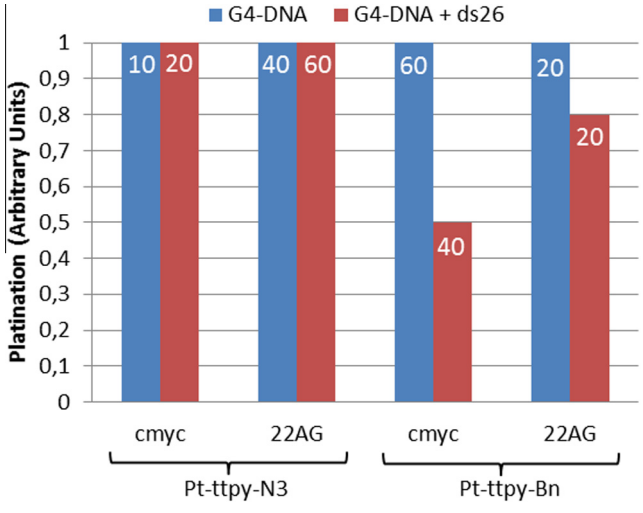

Fig. 5. Amount of platinated adducts reached by Pt-ttpy-Bn and Pt-ttpy-N_3 (2 eq.) on cmyc and 22AG G-quadruplex forming sequences alone (blue bars) and in the presence of an excess of duplex DNA competitor (red bars – ds26, 10 eq.). Time (min) needed to reach the maximum of platination is indicated on each bar. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Cytotoxicity (μM) in the dark and upon photoactivation with UV–Vis irradiation (5 J/cm^2) of Pt-ttpy-Bn and Pt-ttpy-N_3 on A2780 and HT29 cell lines.

		A2780		HT29	
		Dark	UVA	Dark	UVA
Cellular viability (%)	Untreated cells	100%	80%	98%	88%
	Cells treated by DMSO 0.1%	104%	78%	102%	84%
IC_{50} (μM)	Pt-ttpy-N_3	>20	20	>20	20
	Pt-ttpy-Bn	>20	9.20	>20	14.49

improved selectivity likely due to the steric hindrance afforded by the photocrosslinking moieties that should block insertion into duplex DNA. Both complexes show efficient and selective platination of the two G-quadruplex structures studied, derived from the cmyc oncogene promoter and from the human telomeric sequences. Upon photoactivation, double anchoring into the quadruplex structure has been shown for the azido derivative Pt-ttpy-N_3 resulting from platination and photoinduced alkylation. The benzophenone derivative appeared unable to establish cross-links with the two quadruplexes studied, which might be dependant on the poor accessibility of thymines residues. Thus this point remains to be explored and rationalized with a larger set of G-quadruplex matrices of different sequence and topology. Whether these compounds are able to perform double anchorage of G-quadruplex DNA or can trap proteins interacting on G-quadruplex DNA in complex environments like cells or cellular extracts is still an open question, but already they represent molecular tools with great potential for studying G-quadruplex DNA biology.

3. Experimental

Stock solution of these complexes (2 mM in DMSO, stored at −10 °C) were used for G4-FID and FRET-melting assays and aliquoted to avoid freeze–thaw cycles. Stock solution of thiazole orange (2 mM in DMSO, stored at −10 °C) was used for G4-FID assay. Oligonucleotides purified by reversed-phase HPLC were purchased from Eurogentec (Belgium). Fluorescence measurements were performed on a FluoroMax-3 spectrophotometer.

3.1. G4-FID experiments

Experiments were performed in 1 mL cuvettes. K^+100 buffer was used, containing 10 mM lithium cacodylate and 100 mM KCl. G4-FID oligonucleotides, 22AG (5′-$AG_3T_2AG_3T_2AG_3T_2AG_3$-3′), cmyc (5′-$T_2GAG_3TG_3TAG_3TG_3TA_2$-3′) and ds26 (5′-$CA_2TCGATCGA_2$ $TTCGATC_2GAT_2G$-3'), were purchased at Eurogentec. 0.25 μM pre-folded DNA target was mixed with thiazole orange (0.5 μM for G4-DNA and 0.75 μM for ds26). Each ligand addition to the cuvette was followed by a 3 min equilibration time, after which the fluorescence spectrum was recorded. Thiazole orange displacement (%) was calculated as $TOD(\%) = 100 - [(FA/FA_0) \times 100]$, where FA is the fluorescence area of TO bound to DNA after each ligand addition and FA_0 before any ligand addition. By plotting the TOD as a function of the concentration of added ligand, DC_{50} values are measured for 50% of TOD and are means of 2–4 experiments.

3.2. FRET-melting experiments

Experiments were performed in 400 μL cuvettes. K^+10 and K^+1 buffers were used, containing 10 mM lithium cacodylate, 10 or 1 mM KCl, and 90 or 99 mM LiCl. FRET-melting oligonucleotides, F21T (FAM-$G_3[T_2AG_3]_3$-TAMRA) and FmycT (FAM-$T_2GAG_3TG_3$ $TAG_3TG_3TA_2$-TAMRA), were purchased at Eurogentec. Labelled oligonucleotides (0.2 μM, 1 eq.) and ligand (1 μM, 5 eq.) are degassed by sonication. After a first equilibration step (25 °C, 5 min), a stepwise increase of 0.5 °C per minute to reach 95 °C was performed. Final data were analyzed with Excel (Microsoft Corp.) and Origin Pro 8.6 (OriginLab Corp.). The emission of 6-FAM was normalized (0–1) and $T_{½}$ was defined as the temperature for which the normalized emission is 0.5. ΔT_m values are means of 2–4 experiments.

3.3. Gel electrophoresis

The oligonucleotides were 5′-end-labelled using a polynucleotide kinase and [γ32P]-ATP (Perkin Helmer). The reaction products were purified by electrophoresis on 20% acrylamide denaturing gel. Samples platination were prepared by folding a mixture of 5′-end-radiolabeled DNA and 10 μM of non-radiolabeled material to a total volume of 18 μL in 50 mM $KClO_4$ buffered solution. The folding was achieved by heating the samples at 90 °C for 5 min, followed by slow cooling to room temperature over the course of 2 h to induce the formation of the quadruplex structure. It was then incubated with 2 equivalents of platinum complexes for 5 min or 2 h, and platinated products were separated by electrophoresis on 15% denaturing gel. Half of the samples were photoactivated. Bands obtained from Pt-ttpy-Bn were eluted from gel, precipitated, treated by 3′-exonuclease at 37 °C for 30 min and loaded on a 20% denaturing gel. Bands obtained from Pt-ttpy-N_3 were eluted from gel, precipitated and treated with piperidine. Gels were scanned using a STORM860 (GE Healthcare). Gel analysis was done using Image Quant Software.

3.4. Photoactivation experiments

Samples for irradiations were prepared by folding for G-quadruplex DNA. A mixture of 5′-end-radiolabeled DNA and 10 μM of nonradiolabeled material to a total volume of 18 μL each in 50 mM $KClO_4$ buffered solution. Folded oligonucleotides were then incubated with 2 equivalents of platinum complexes for 5 min at 37 °C. Samples were irradiated in centrifuge tubes (2 mL, Eppendorf). The tubes were placed into a reflector bowl filled with ice and the samples were irradiated with a studio flash lamp (Alienbees B 1600 640 WS without UV filter, 3 cm distance between flashtube and samples). Half of the samples were loaded on a 15% denaturing gel electrophoresis right after photoactivation and the other half of samples was loaded after 2 h of post-incubation at 37 °C.

3.5. Cell culture conditions

HT-29 cells (ATCC HTB38), a human colon carcinoma cell line, were purchased from the American Type Culture Collection (ATCC) and cultured in Dulbecco's modified Eagle's medium GlutaMAX (DMEM, Life Technologies, Saint-Aubin, France) supplemented with 10% fetal calf serum (FCS, South America origin, BioWhittaker Lonza, Verviers, Belgium) and 1% Penicillin/Streptomycin (Life Technologies) in humidified atmosphere under 5% CO_2 in air at 37 °C. A2780 cells, a human ovarian cancer cell line, were purchased from the European Collection of Cell Cultures (ECACC) and cultured in RPMI-1640 medium (Life Technologies) supplemented with 10% fetal calf serum and 1% Penicillin/Streptomycin in humidified atmosphere under 5% CO_2 in air at 37 °C. Cells were subcultured twice a week by dispersal with TrypLE Express Enzyme (Life Technologies).

3.6. Photocytotoxicity assays

HT-29 and A2780 cells were seeded in 24-well plates at a density of 5×10^4 cells/well in 1 mL of culture medium. After 24 h of incubation at 37 °C, compound to test, in stock solution in dimethyl sulfoxide (Sigma–Aldrich), was diluted in culture medium in the dark at the following final concentrations 5, 10 and 20 μM or 0.1, 0.5 and 1 μM in duplicate. 1 mL of culture medium was replaced with the culture medium containing the compound to test in each well. Two plates of each compound and for each cell line were realized, one for dark cytotoxicity and the other for photocytotoxicity. After 24 h of incubation with compound at 37 °C in the dark, the cells were washed with phosphate buffered saline (PBS, Life Technologies). Irradiation was performed in PBS with a UVA1 Sellamed sytem, emitting in the spectral range from 340 to 420 nm as described by Bracchitta et al. [24]. Irradiation was carried out during 150 s at a final irradiance of 33.33 mW/cm^2 to reach a delivery fluence of 5 J/cm^2 UVA. Different controls were realized as follow, unirradiated and untreated cells, unirradiated and 0.1 0.2 and 0.4% DMSO treated cells, irradiated and untreated cells and finally irradiated and DMSO treated cells.

After irradiation, PBS was removed and fresh medium free of drug was added. Plates were left to incubate at 37 °C in the dark for 3 days before evaluation of the cell viability by determination of mitochondrial activity using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide assay (MTT, Sigma). At the time of counting, 50 μL of a MTT solution at 5 mg/mL was added to each well. After 30 min of incubation and removal of the medium, formazan crystals were taken up with 600 μL of DMSO and absorbance at 562 nm was measured with a Fluostar microplate reader (BMG Labtech).

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ica.2016.02.033.

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