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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.

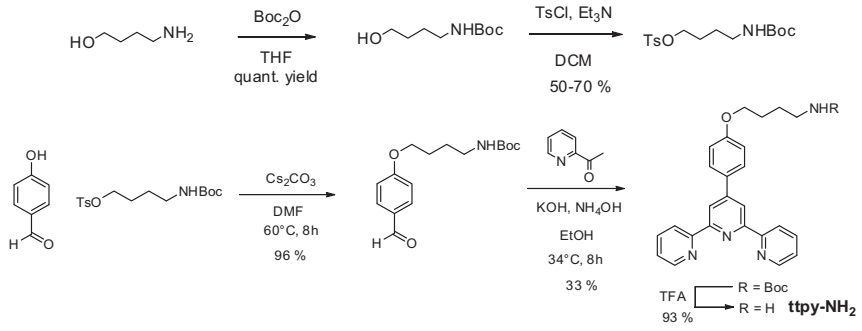
We therefore envisaged the preparation of two compounds (Pt-ttpy-Bn and Pt-ttpy-N_3), by grafting the two classical photoreactive groups, benzophenone (Bn) and 4-azido-2,3,5,6-tetrafluorobenzoic acid (N_3), on the tolylterpyridine scaffold. These photoreactive groups can be activated by irradiation at 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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