New platinum(II) complexes targeting the loops of the human telomeric G-quadruplex.

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ABSTRACT

Two novel series of platinum(II) complexes have been designed and shown to interact with the human telomeric G-quadruplex-DNA via different binding modes: t-terpyridine-platinum (Pt-tpy) complexes covalently interact with quadruplex-DNA via selective platination of adenine residues of the loops, their interaction being driven by the aromatic surface of the ligand; ii- platinum-quinacridine hybrid (Pt-MPQ) interacts with quadruplex-DNA via a dual non-covalent/covalent binding mode, targeting preferentially guanines constitutive of external G-quartets. Therefore, platinum complexes presented herein constitute potential agents for irreversible trapping of G-quadruplex-DNA.

INTRODUCTION

Telomeric DNA of human cells comprises tandem repeats of sequence 5'-TTAGGG-3' in the strand that contains the 3' end, which protrudes to form a single-strand overhang (the G-tail). This G-rich overhang has demonstrated its ability to fold into G-quadruplex in vitro. G-quadruplex-DNA is a four-stranded structure that is held together by square planes of four guanines (G-quartet), associated through Hoogsteen base pairing. Although there is no direct proof of G-quadruplex in vivo, recent biological evidences support their existence at the telomeres of ciliate Styloynchia and human. Molecules able to stabilize the G-quadruplex structure of DNA can lead to an arrest of proliferation of cancer cells and thus could lead to the discovery of new anticancer agents. Initially, quadruplex-stabilization was considered as an indirect approach for inhibiting the telomerase, an enzyme deeply involved in the immortalization process of cancer cells. Actually, stabilizing quadruplex structure was thought to sequester the telomeric DNA under its folded form whereas the enzyme requires the linear conformation for its activity. However, recent evidences suggest that the mechanism underlying the drug action is drastically more complex than anticipated. It is now proposed that biological effects of quadruplex ligands might originate in the displacement of protective proteins normally associated with telomeres (shelterin complex) thus disrupting telomere function that in turn impedes the action of telomerase.

The quadruplex-stabilization occurs, in most cases, via π-stacking interactions between the ligand (usually a flat aromatic molecule) and the G-quartet constitutive of the external-face of the quadruplex. G-quadruplex ligands are usually positively charged, either with a formal charge (via the N-methylation of an aza-aromatic moiety for example) or by in-situ protonation of amine sidearms, with the exception of the telomestatin. Electrostatic and π-stacking interactions thus govern the efficiency of the ligand-quadruplex association. These non-covalent interactions are, by nature, weak and reversible and it is thus likely that the introduction of covalent linkage would result in a higher stabilization of the quadruplex-structure, which might in turn improve the anti-proliferative effect of the ligand.

RESULTS AND DISCUSSION

Platination studies of human telomeric G-quadruplex structures by cisplatin and related complexes highlighted that these DNA structures can be efficiently platinated by platinum complexes. Indeed, apart from the adenines in the loops, some of their guanines can flip out of the G-quartet, thus rendering their N7 atom accessible to platinum complexes. These results lead us to design new platinum complexes combining G-quadruplex ligand and platinum(II) complexes.

Scheme 1. Chemical structures of Pt-tpy complexes and schematic representation of their platination sites.

We firstly designed a series of platinum(II)-terpyridines complexes (Pt-tpy, Scheme 1). Their ability to interact with G-quadruplex-DNA was firstly evaluated in non-platinating conditions via two biophysical assays, the G4-FID and FRET-melting assays. It was concluded from that
series of experiments that Pt-tpy complexes are high-affinity quadruplex-ligands, with a robust quadruplex-over duplex-DNA selectivity.\(^7\)

Having demonstrated that Pt-tpy complexes efficiently recognize quadruplex-DNA, their covalent interactions with the quadruplex-structure derived from human telomeric sequence (22AG) were investigated. Platination of 22AG was conducted at 37°C for 16hrs; the platination sites determined via enzymatic footprinting experiments. It was shown that Pt-tpy complexes selectively trap adenine residues localized in the loops of the antiparallel G-quadruplex structure. Interestingly, the platination pattern was found dependent on the nature of Pt-tpy complex: simple terpyridine-platinum complex (Scheme 1, left) platinates preferentially A7 of a lateral loop whereas the more extended p-tolyl-terpyridine-platinum complex (Scheme 1, right) interacts predominantly with A13 of the diagonal loop. These results indicate that the aromatic part of the ligand governs the ligand/quadruplex interaction, and then orient the platination site. Platination being kinetically slower compared to π-stacking-directed association, it should occur with the accessible adenines of the structure once the ligand is fixed.

![Scheme 2](image)

Scheme 2. Chemical structure of Pt-MPQ and a proposed model for its interaction with 22AG quadruplex.

Another approach was also developed, in which the quadruplex ligand and the platinum complex are spatially separated, linked together by a flexible linker (Scheme 2). The length of this linker, approximately 12Å, is defined for spanning the thickness of three stacked G-tetrads, thus enabling both parts of the conjugate to interact concomitantly with both faces of the quadruplex.\(^8\)

The first prototype of this series, Pt-MPQ (Scheme 2), is comprised of a monofunctional Pt moiety and a quinacridine (mono-para-quinacridine or MPQ), previously demonstrated as efficient G-quadruplex ligand. Alike Pt-tpy complexes, Pt-MPQ/quadruplex interactions were monitored by denaturing gel electrophoresis and the platination sites determined by both chemical and enzymatic experiments. Herein, platination preferentially took place at G2 and G22 sites (Scheme 2), the anti-parallel structure of 22AG being the preferred target of Pt-MPQ.

These results confirm that guanines involved in G-tetrads are indeed platinable. Additionally, the localisation of platination sites on a single face of quadruplex implies that the binding mode is driven by the preferential recognition by MPQ of the tetrad surrounded by two lateral loops which direct Pt complex on the opposite tetrad. The concomitant presence of both moieties was demonstrated to be crucial for insuring quadruplex binding thus enabling an unusual interaction mode, combining both covalent and non covalent binding.

**CONCLUSION**

Pt-tpy and Pt-MPQ series, because they interact with G-quadruplex via two different binding modes, demonstrate that a selective trapping of either loops or quartet residues is feasible. These complexes are thus able to lock irreversibly the human telomeric-derived oligonucleotide into its G-quadruplex form, therefore potentially increasing their ability to disrupt the telomere/protein supramolecular assembly. Consequently, these novel Pt complexes represent a solid basis for innovative design of G-quadruplex ligands acting through an original binding mode.

**REFERENCES**


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