Metallo-Organic G-Quadruplex Ligands in Anticancer Drug Design

Y.-L. Jiang and Z.-P. Liu*

Department of Organic Chemistry, School of Pharmaceutical Sciences, Shandong University, Jinan 250012, P. R. China

Abstract: Guanine-rich DNA sequences can form planar four-stranded structures *via* Hoogsteen hydrogen bonds. These sequences in telomeric DNA and some oncogenes have been identified as targets for novel anticancer drugs. Consequently, there is great current interest in developing small molecules that can facilitate the formation of, and stabilize G-quadruplexes as potent antitumor chemotherapeutic agents. Metal complexes with planar geometries and π-delocalised ligands are emerging as a new class of quadruplex DNA ligand with unique features for optimal binding. This review will summarize the different types of metal-based compounds that target G-quadruplexes and provide useful information on the design of small ligands.

Keywords: G-quadruplexes, telomeric DNA, telomeres, targets, anticancer drug, metal complexes, ligand, stabilization.

1. INTRODUCTION

Deoxyribonucleic acid (DNA) forms a variety of less common structures in addition to the well-known anti-parallel double helix. These include hairpins and Holliday structures, triplexes and several different types of quadruplexes [1, 2]. The latter are formed by guanine rich DNA sequences and have been observed in the dimerisation region of HIV, centromeres, a portion of the insulin regulatory gene, fragile X-syndrome repeat regions [1, 3], the promoter regions of important oncogenes, such as c-Myc, c-Myb, c-Fos, and c-ABL [4], and in the single-stranded G-rich overhang of telomeres [5]. Telomeres have recently been considered as potential therapeutic targets, as a result of the differences between telomeres in normal and cancer cells. Telomeres function to protect the ends of chromosomes in normal somatic human cells, but each cell division is accompanied by an erosion of the telomeres, and eventually the telomeres become too short to protect the chromosome, leading to apoptosis and cell death. In contrast, many cancer cells are able to maintain the length of their telomere sequences by increasing the level of activity of the telomerase enzyme, conferring these cells the capacity to divide a large number of times. Telomerase, a ribonucleoprotein that maintains telomere length, is expressed in 85% of cancer cells, and this is one of the important features of their malignant character [6]. Consequently, the inhibition of telomerase has been identified as an attractive target for cancer therapy [7-11]. In many cases this has involved the synthesis of small molecules that can stabilize or induce the formation of quadruplex structures in telomere DNA, thus prevents telomerase elongation of telomeres by disrupting the interaction between the enzyme and its substrate, the unfolded G-rich single strand.

G-quadruplex structures (Fig. (**1**)) present a large π-surface, as a result the majority of small molecules that bind G-quadruplex themselves have a large π-surface, so as to maximize the π–π interactions they can form. Another design feature is that G-quadruplexes, like all nucleic acids, carry a high negative charge, and hence cationic ligands will generally bind more tightly to them. Recently, Neidle and others have shown that metal-complexes can be excellent stabilizers of quadruplex DNA [12-20]. A metal coordinated to heteroaromatic ligands with a square-planar geometry can have several advantages over more "classical" quadruplex DNA binders. The metal can play a major structural role in organizing the ligand into an optimal conformation for quadruplex DNA interaction. In addition, the electropositive metal can in principle be positioned at the center of the guanine quartet, increasing electrostatic stabilization by substituting the cationic charge of the potassium or sodium that would normally occupy this site. Another advantage is the electron-withdrawing properties of the metal, which reduce the electron density on the coordinated ligand, yielding a system that can display stronger π-π interactions [21].

There are excellent reviews on the developments of selective G4-ligands as potential anticancer agents [20, 22-25]. However, few highlights are concentrated on the metal-complexes as selective G-quadruplex binders. In this review we will focus on the metallo-organic G-quadruplex ligands and provide useful information for the design of novel anticancer agents.

2. METHODS FOR STUDYING SELECTIVE G4 LIGANDS

In the design and investigation of drug molecules targeting G-quadruplexes, effective assays have been developed for determining the selectivity of these compounds and studying their interactions with G4 structures. Fluorescence resonance energy transfer (FRET), surface plasmon resonance (SPR) and competition dialysis techniques have been most commonly used in screening selective G4 ligands [22].

FRET is a spectroscopic method that provides distance information of macromolecules in solution and has been successfully used to probe the secondary structure of guanine-

*Address correspondence to this author at the Department of Organic Chemistry, School of Pharmaceutical Sciences, Shandong University, Jinan 250012, P. R. China; Tel: +86-531-88382006; Fax: +86-531-88382548; E-mail: liuzhaop@sdu.edu.cn

1389-5575/10 $55.00+.00

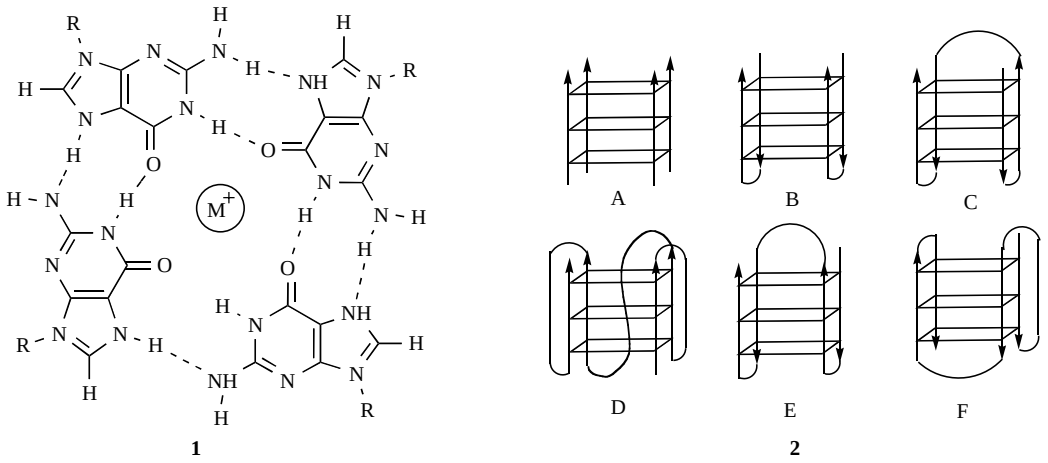


Fig. (1). Secondary structure of G-quadruplexes. **1.** An individual guanine quartet; **2.** G-quartets stack in various possible arrangements to form "G4 DNA".

rich sequences in which a donor and an acceptor are attached to one end of the oligonucleotide. The formation of a quadruplex in the G-rich sequence is often accompanied by a decrease in the distance between the donor and the acceptor, leading to a more efficient energy transfer from donor to acceptor. With ligand bound, the fluorescent quadruplex is often stabilized and shows an increased melting temperature. Thus, the melting temperature (*T*m) can be detected in the presence of different molecules by RT-PCR to estimate the interaction between a ligand and the quadruplex. In addition, the FRET assay may be performed in the presence of a large excess of other nonfluorescent DNA competitors (e.g., other G-quadruplexes, single strands and double strands), to screen out ligands that show preference for quadruplex over other structures.

The SPR method is an alternative choice for studying quadruplex–ligand interactions. In general, a quadruplex that interacts with ligands is immobilized onto the sensor chip, and the binding of the ligand to quadruplex results in a change in refractive index. SPR can monitor molecular binding interactions in a realtime manner and has found wide applications in determining the affinity and kinetics of drug–DNA interactions. Structural preference is determined based on the affinities of ligands with different DNA structures.

Competition dialysis is commonly used to probe the binding selectivity of ligands to various nucleic acid structures based on equilibrium dialysis. In this assay, the DNA-ligand solution is placed in a semipermeable dialysis membrane that allows only small ligands to pass through. When equilibrium is reached, the chemical potential of the free ligand is virtually equal inside and outside the dialysis membrane, and any excess ligand on the macromolecule side of the membrane can be attributed to binding to the macromolecule in question. Thus, the amount of ligands bound to each structure provides a direct measurement of the affinity for that structure, so that structural preferences can be readily compared.

Other techniques, such as electrospray ionization mass spectrometry (ESI-MS), competition polymerase stop assay, phage ELISA assay, circular dichroism (CD) spectroscopy, UV/Vis spectroscopy, calorimetric techniques, electrophoresis mobility shift assays, NMR spectroscopy and X-ray crystallographic methods, have also been used to screen selective G4 ligands [20].

3. METALLO-ORGANIC G-QUADRUPLEX LIGANDS AS ANTICANCER AGENTS

3.1. Metal-Porphyrins Complexes

The first reported examples of metallo-organic G-quadruplex ligands described the insertion of a metal in the central cavity of the tetra-N-methyl-pyridyl porphyrin molecule (TMPyP4, Fig. (**2**)) [26, 27]. TMPyP4 itself is an effective telomerase inhibitor with an IC_{50} of 6.5 μM in a cell-free assay [28, 29], and it has been extensively used as a probe of quadruplex structure, but its selectivity for quadruplexes over duplex DNA is not high [30]. Meunier's group reported the nickel(II)-, manganese(III)-porphyrins complexes as telomerase inhibitors [14, 31] (Fig. (**2**)). Both kinds of porphyrins complexes with different arms are active towards the telomerase enzyme in telomeric repeat amplification protocol (TRAP) assay, and the IC_{50} values are 5.0, 25.9, 7.3, 11.5 and 12.8 μM for compounds **3a**, **3b**, **4a**, **4b** and **5a**, respectively (Table **1**) [14, 31]. The G-quadruplex DNA binding properties of nickel(II) and manganese(III) porphyrins were studied by SPR method and the results are summarized in Table **1**. The best selectivity for G-quadruplex DNA is observed for Mn-TMPyP4 **3b**, which has one order of magnitude in favor of the quadruplex (Ka ~10^7 M^{-1}) over duplex (Ka ~10^6 M^{-1}). In contrast, the TMPyP4 ligand and other complexes exhibited little or no selectivity. The kinetics data of binding to DNA (K_{on}, K_{off}) in this series of metalloporphyrin derivatives indicate the important roles played by the metal ions. In fact, the nature of the metal influences the kinetics and the mode of interaction (stacking or external binding). In general, the kinetic process is faster for Ni than for Mn, and all the fast-binding molecules (TMPyP4, **3a**, **4a**, **5a**) have in common an aromatic moiety that is capable of interacting with DNA by stacking. Both **4a** and **4b** contain a quinoline substituent, which should be able to stack with a guanine tetrad and may govern the interaction of the conjugate with DNA. On the other hand, the presence of two

molecules of water (not shown in Fig. (**2**)) in the manganese(III) porphyrins as axial ligands changes the mode of their interaction and makes them unable to interact by stacking interaction with G-tetrads. It is supposed that these manganese(III) porphyrins bind the G-quadruplex by interaction in the grooves, thus slow kinetics are observed for **3b**, **5b** and **6b** [32]. However, the hindering stacking slows down the interaction and, hence, leads to better affinity and selectivity, as observed for the manganese porphyrin **3b**. This raises the possibility that stacking may not be the best means of achieving high affinity and selectivity for G-quadruplex structures and the external binding manner should be considered for excellent interaction. This study provides a good strategy in the design of selective G-quadruplex ligands as anticancer agents.

The pentacationic manganese(III) porphyrin **7**, containing a central aromatic core and four flexible cationic arms (Fig. (**3**)), exhibited an especially high affinity for the human telomeric quadruplex DNA 5'-$AG_3TTAG_3TTAG_3TTAG_3$ and a 10000-fold selectivity for this quadruplex sequence over two different duplex DNA sequences [33]. In the SPR test, the binding of **7** to GC and AT duplex DNA gives affinity constants in the 10^4 M^{-1} range, whereas it reaches 10^8 M^{-1}

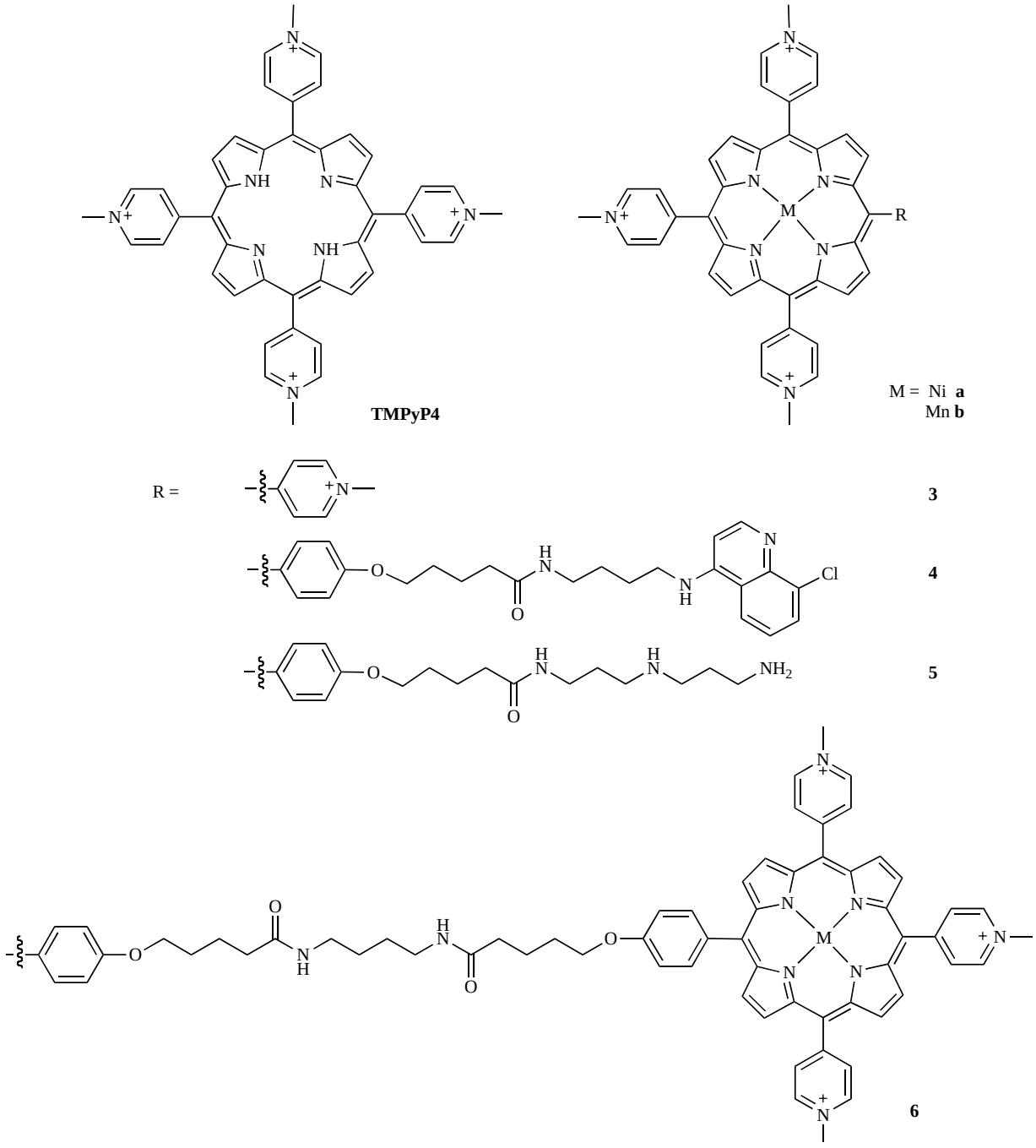


Fig. (2). Structures of TMPyP4 and nickel(II)-, manganese(III)-porphyrin complexes.

Table 1. The Results of SPR and TRAP Assay Tests

Ligands	K_{on} (10^3 M^{-1} s^{-1})			K_{off} (10^{-3} s^{-1})			K_a (10^6 M^{-1})			IC_{50}/μM
	Quadruplex	GC duplex	AT duplex	Quadruplex	GC duplex	AT duplex	Quadruplex	GC duplex	AT duplex	
TMPyP4	4.75	339	44.6	6.60	201	24.5	0.72	1.69	1.82	6.5
3a	74.4	368	546	44.6	46.0	35.1	1.67	8.00	15.6	5.0
3b	27.2	0.95	1.67	3.35	2.72	1.74	8.12	0.35	0.96	25.9
4a	82.9	265	233	161	262	73.4	0.51	1.01	3.17	7.3
4b	174	3540	323	103	153	10.9	1.69	23.1	0.30	11.5
5a	0.01	65.8	2.03	0.003	33.3	1.27	3.66	1.98	1.60	12.8
5b	0.23	0.12	0.18	0.003	0.012	0.004	76.7	10.0	45.0	—
6b	2.36	0.35	5.27	2.07	2.52	3.24	1.14	0.14	1.63	—

K_{on}: association constant; K_{off}: dissociation constant ; K_a: affinity constant.

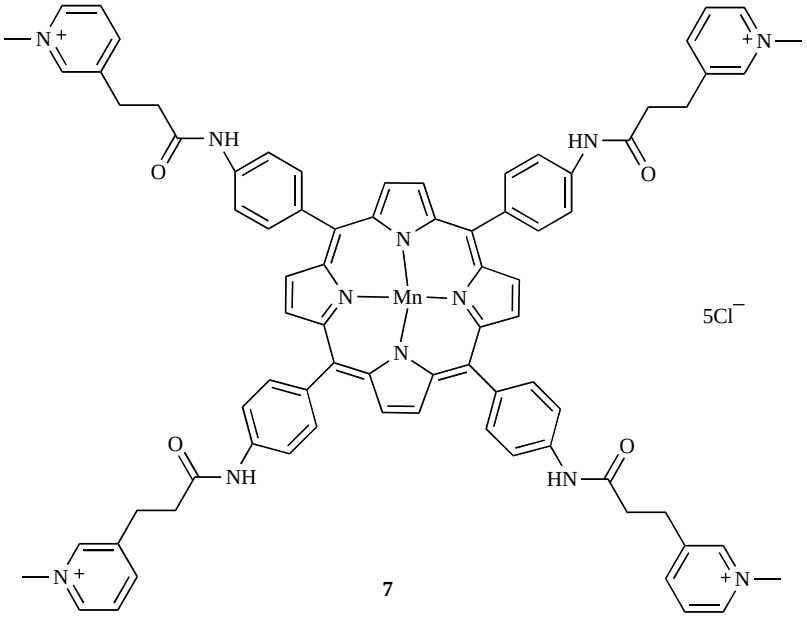
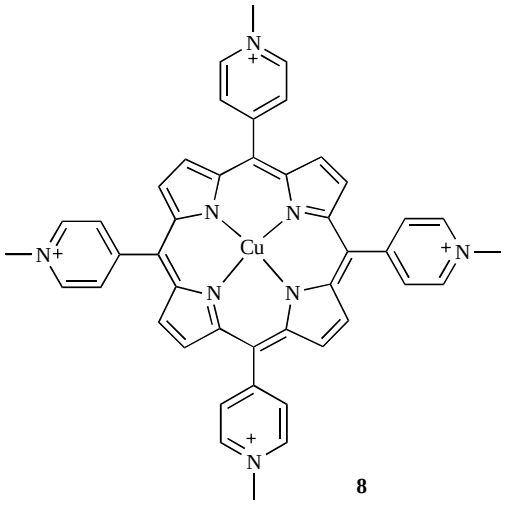


Fig. (3). Structure of pentacationic manganese(III) porphyrin.

with quadruplex DNA. The bulky cationic substituents surrounding the manganese(III) porphyrin, which preclude a close interaction with the double stranded DNA structures, seemed to decrease the affinity for duplex DNA. Compound **7** was the most efficient agent for the distinguishing between quadruplex and duplex DNA. It also displayed a good level of telomerase inhibition with an IC_{50} of 0.58 μM by TRAP assay. The very high affinity for the four-stranded DNA structure possibly originated from a combination of interactions between the G-quartet and the porphyrin core on the one hand, and between the grooves and/or loops and the flexible cationic arms on the other. This mode of interaction may be useful in the further design of selective metallo-organic G-quadruplex ligands.

Copper(II)-porphyrin complexes were reported to interact with the parallel stranded G-quadruplexes formed by $d(T_4G_nT_4)$ (n = 4 or 8) [34, 17]. Electron paramagnetic resonance spectroscopy showed the bound Cu-TMPyP4 (Fig. (**4**)) occupied magnetically non-interacting sites on the quadruplexes. Electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry revealed a maximum Cu-TMPyP4 to quadruplex stoichiometry of 2:1 for the shortest (n = 4) and longest (n = 10) quadruplexes. Consistent with their previous model for $d(T_4G_4T_4)$, Szalai *et al*. suggested that the two Cu-TMPyP4 molecules be externally stacked at each end of the run of guanines in all $d(T_4G_nT_4)$ (n = 4–10) quadruplexes [17].

Tetramethylpyridinium-porphyrazines 3,4-TMPyPz (Fig. (**5**)) were found to bind strongly and selectively to human telomeric G-quadruplex DNA (dissociation constant K_D = 0.2 ± 0.02 μM) in a 1:1 ratio and induced the formation of the antiparallel G-quadruplex conformation [35]. The effect



8

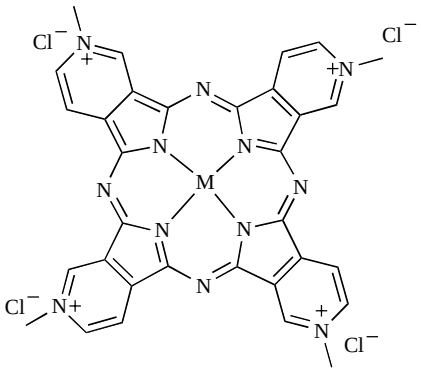
Fig. (4). Structure of Cu-TMPyP4.

of zinc(II) in the binding of 3,4-TMPyPz (K_D = 1.0 ± 0.7 μM) to G-quadruplex DNA was investigated. Though the zinc(II) complex was still selective for G-quadruplex over duplex DNA, the metal atom of zinc changed the binding stoichiometry between the complex and G-quadruplex DNA (4:1 ratio), possibly due to the aggregation [34]. In addition, these porphyrazines did not show any significant binding affinities against duplex DNA at a concentration up to 5 μM. Comparing with TMPyP4 (K_D = 14 μM), the stronger binding of the 3,4-TMPyPz with G-quadruplexes may be attributed to the more extended porphyrazine π-system, which might completely overlap the four guanines of the G-tetrad. Therefore, the dimensions of the aromatic core (π-surface) are important parameters in the design of high selective G-quadruplex binding ligands.

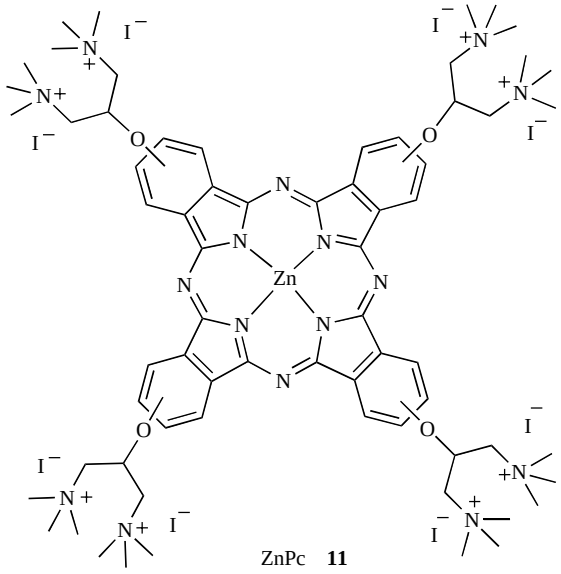
Zhou's group reported a cationic substituted zinc(II) phthalocyanine (ZnPc, **11**, Fig. (**5**)) as a potent and selective G-quadruplex binder and telomerase inhibitor with an IC_{50} value of 0.23 μM [36]. The affinity and selectivity (Δ*T*m = 4~25°C in K^+ and Na^+ solution) of this compound for G-quadruplex DNA over duplex DNA was better than that of TMPyP4. In contrast to 3,4-TMPyPz, ZnPc produced a transition from antiparallel to parallel G-quadruplex DNA, and moreover, it could induce the parallel structure formation in cation-deficient conditions (buffer without added ions). The planar structure of ZnPc and its positive charges (electrostatic factors) might favor its binding to guanine tetrad. The special ability of ZnPc to induce structure transition and formation of G-quadruplexes might be significance in telomere association and drug design.

3.2. Metal-Corroles Complexes

Corrole is a tetrapyrrolic macrocycle, akin to porphyrin, but with one less carbon atom in its outer periphery and one additional NH proton in its inner core, as a tri-anionic ligand. Gross and co-workers were the first to synthesize cationic corroles and found that they can interact with DNA [37]. Zhou *et al*. reported that some cationic corroles were capable of stabilizing G-quadruplex structures and inhibiting the activity of telomerase [38]. Some metallated corroles, in which the metal ions were in high-oxidation states, such as copper(III) and manganese(III), had also been investigated [39] Biochemical studies indicated that these copper and manganese corroles (Fig. (**6**)) could excellently stabilize G-quadruplex structures in the telomeric sequence and c-Myc region G-quadruplex sequence. The results of the PCR stop assay of two metal-corrole complexes toward the Telo system (human telomeric sequence 21G and mutated sequence 21Gmu) and c-Myc system (sequence Pu27 and mutated sequence Pu27mu) were shown in Table **2**. The IC_{50} value, which indicates the concentration of compound **12** required to achieve 50% inhibition of the PCR reaction, was found to be 2.74 μM in the c-Myc system (Pu27: 5'-TGGGGAGGGT GGGGAGGGTGGGGAAGG-3') and 3.51 μM in the Telo system (G21: 5'-GGGTTAGAATTAGGGTTAGGG-3'), and corrole **13** showed even better activity with an IC_{50} value

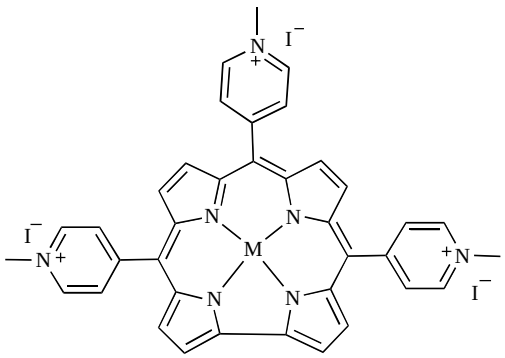


M = H 3,4-TMPyPz **9**
Zn(II) 3,4-TMPyPz-Zinc **10**



ZnPc **11**

Fig. (5). Structures of zinc(II)-expand porphyrin complexes.



M = Cu **12**
Mn **13**

Fig. (6). Structures of copper and manganese corroles complexes.

of 1.52 μM in the c-Myc system and 2.37 μM in the Telo system. These results were in consistent with the assumption that metal ions with high-oxidation states in the core of the corroles might strengthen the interaction between the corroles and G-quadruplexes [39]. The strong electron-withdrawing groups (i.e., pyridinium substituents on the periphery of corrole and the trivalent manganese) decrease the electron density of the corrole ring, thus allowing ready interaction with the electron rich guanine G-quadruplex.

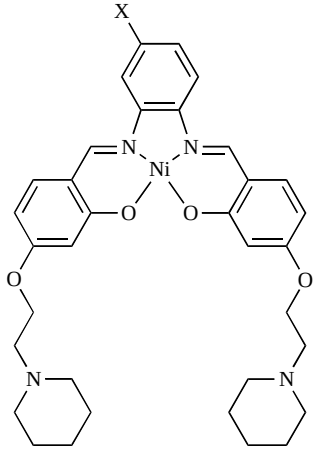
3.3. Nickel(II)-Salphen Complexes and Analogs

More recently, Neidle and Vilar group reported a new type of telomeric quadruplex DNA stabiliser based on nickel(II)-salphen complexes [12]. The Ni(II)–salphen complex (Fig. (**7**)) incorporates the main requirements for quadruplex stabilizing molecules, that is, a π-delocalized system prone to stacking on a G-quartet, a positive charge that is able to lie in the centre of the quartet, and finally, positively charged substituents which can interact with the grooves and loops of the quadruplex. Indeed, the Ni(II)–salphen complexes induce a high degree of quadruplex DNA stabilization (**14** and **15**, Δ*T*m = 33.2, 32.8°C at 1 μM, Table **3**) and telomerase inhibition with EC_{50} values in the range of 0.1 μM. More important, the selectivity of the Ni(II)–salphen complexes to quadruplex over duplex DNA is above 50-fold. The planar arrangement of the salphen rings (forced planar by coordination to the metal center) and their appropriate spacing make the complexes ideal to stack on top of the guanine tetrads. In addition, the protonated piperidine substituents are likely to interact with the grooves and loops of the quadruplex, which may be important for the quadruplex selectivity.

A series of square-planar and square-based pyramidal metal complexes of Ni(II), Cu(II), Zn(II) and V(IV) with salphen and salen (Fig. (**8**)) were prepared and studied [21]. The square-planar metal complexes of nickel(II) and copper(II) proved to be highly effective quadruplex DNA stabilizers (Δ*T*m = 22.9, 21.5°C at 1 μM, respectively, Table **3**), and had a high degree of selectivity for the quadruplex DNA over duplex DNA by FRET competition assays. The zinc(II) complex **18** showed bad affinity (Δ*T*m = 1.4°C at 1 μM), possibly due to the nonplanarity of the region around the zinc coordination and its penta-coordination. The vanadium complex **19** could also be assumed penta-coordinated, although here the pyramidal distortion was expected to be less pronounced than in the zinc(II) compound **18**. Therefore, complex **19** had a quasi-planar base which was likely to facilitate interaction with the guanine quartet of quadruplex DNA, and this was consistent with the FRET and FID results obtained (Δ*T*m = 10.5°C at 1 μM). As can be seen in Table **3**, the concentrations of metal complexes for the stabilization of quadruplex DNA (Δ*T*m = 20°C) are much lower than that for the stabilization of duplex DNA (Δ*T*m = 2°C). In addition, a displacement assay has been employed to measure the interaction of small molecules with quadruplex DNA [$^{G4}DC_{50}$: 50% displacement of the TO (Thiazole Orange)], and the results are overall consistent with those obtained by FRET. This study highligts the importance of both the geometry of the metallo-organic ligangs and the central metal ions. Thus, high Δ*T*m values are likely to be achieved through the optimal geometric arrangement of the aromatic rings around the central metal ion, which would result in

Table 2. Results of the PCR Stop Assay Toward the Telo System and c-Myc System

Compound	IC_{50} (μM)		Telo selectivity	IC_{50} (μM)		c-Myc selectivity
	21G	21Gmu		Pu27	Pu27mu	
12	3.51±2.14	7.09±1.70	2.02	2.74±0.40	4.19±2.68	1.53
13	2.37±1.01	6.46±1.92	2.72	1.52±0.31	3.23±1.70	2.13



14: X = H; **15**: X = F

Fig. (7). Structures of nickel(II)-salphen complexes.

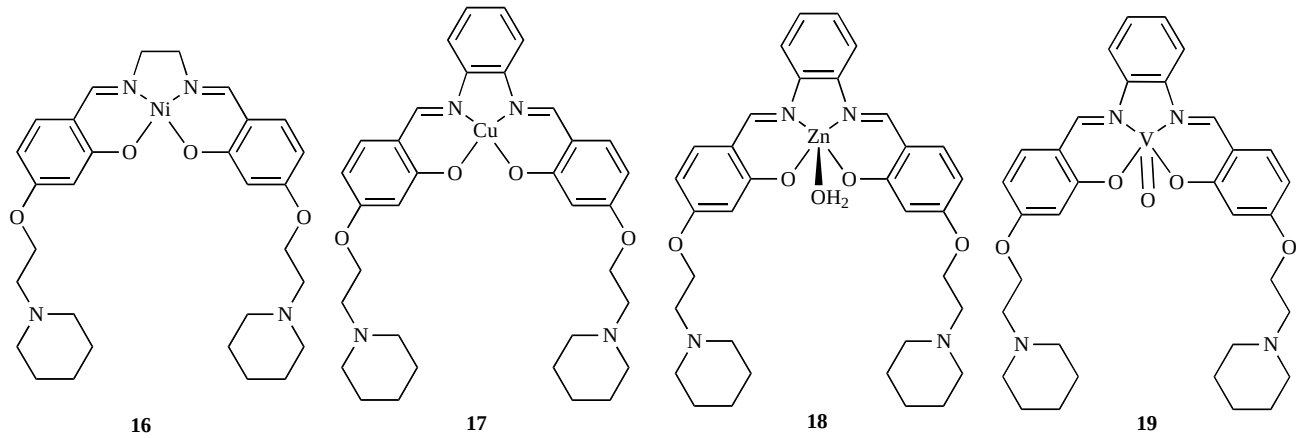


Fig. (8). The metal Ni(II), Cu(II), Zn(II), and V(IV)with salphen and salen complexes.

Table 3. Results of the FRET and FID Assay Tests

Compound	Δ*T*m 1 μM (°C)		[concn] μM Δ*T*m = 20 °C G4 DNA	[concn] μM Δ*T*m= 2 °C dsDNA	$^{tel}EC_{50}$ (μM)	$^{G4}DC_{50}$ (μM) G4 DNA
	G4 DNA	dsDNA				
14	33.2	0	0.2	4.1	0.14	
15	32.8	0	0.1	5.3	0.12	
16	22.9	0.4	0.4	3.6		1.70
17	21.5	0	0.7	3.7		0.56
18	1.4	0.1	4.0	9.8		<10.0
19	10.5	0	4.6	4.2		1.20

increased π-π stacking between the G-tetrads and these metal complexes. In addition, the coordination of the metal to the poly aromatic ligand results in a reduction of electron density on the ring, which gives a further enhancement of the π-π stacking interactions.

3.4. Platinum(II) Complexes with Monosubstituted Phenanthrolines

Following on their first report on nickel(II)-salphen complexes, Vilar and co-workers studied another class of quadruplex DNA stabilizer based on platinum(II) complex with monosubstituted phenanthroline (Fig. (**9**)) [13]. Complex **21** induced a high degree of stabilization for quadruplex DNA with an increase in melting temperature (Δ*T*m = 20°C at 1 μM), while for the ligand **20**, the Δ*T*m is 9°C at 1 μM, considerably lower than the Δ*T*m obtained with complex **21**. In addition, at the same concentration of the metal complex, the Δ*T*m for a duplex DNA sequence (5'-FAM-dTATAGCT ATA-HEG–TATAGCTATA–TAMRA-3') is negligible (Δ*T*m = 0.5°C at 1 μM), suggesting a 40-fold selectivity of complex **21** for quadruplex over duplex DNA. This result also underlines the importance of the central metal in the stablization of quadruplex DNA through the formation of planar complexes in favor of the π-π stacking interactions.

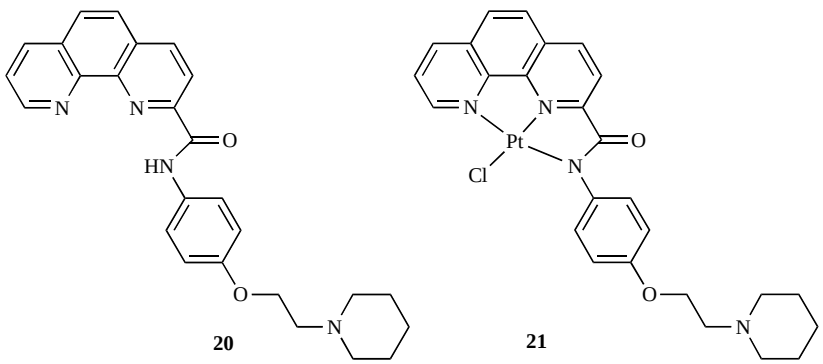


Fig. (9). Structures of phenanthroline ligand **20** and platinum(II) complex **21**.

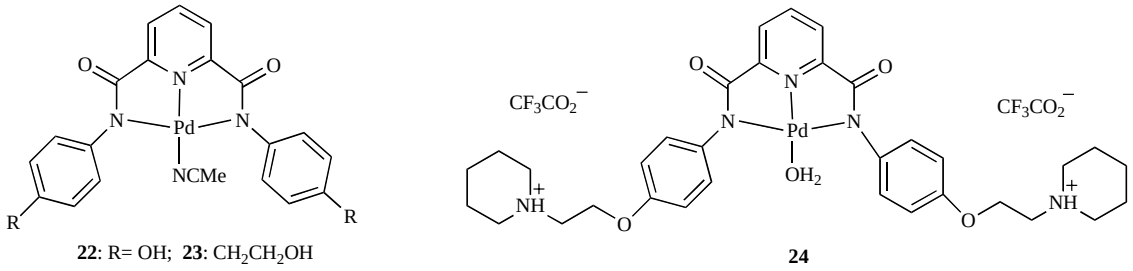


Fig. (10). Structures of palladium(II)-bis-carboxamidopyridine complexes **22-24**.

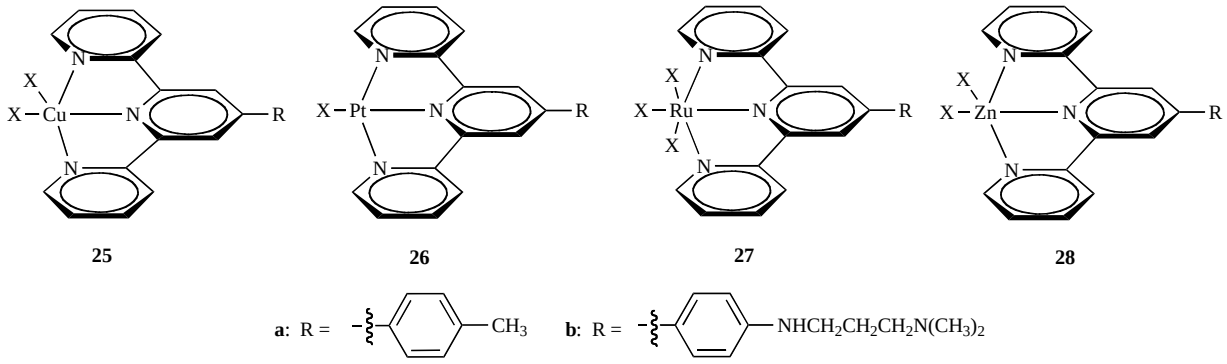


Fig. (11). Cu(II), Pt(II), Ru(III) and Zn(II) complexes with terpyridine-based ligands.

3.5. Palladium(II) Complexes with Bisubstituted Pyridines

Vilar and co-workers reported another similar class of quadruplex DNA stabilizer based on palladium(II) complexes with bisubstituted pyridines (Fig. (**10**)) [40]. However, compared to the phenanthroline complexes, these complexes based on the bis-carboxypyridine ligands were not very effective. Complex **24** yielded the highest quadruplex DNA stabilization, only with a Δ*T*m of 12°C at 1 μM, while complexes **22** and **23** showed poor affinity (with a Δ*T*m of 0 and 1°C at 1 μM). These results indicated that the piperidine substituent might play an important role in increasing the strength of the interaction between planar molecules, such as the phenanthrolines and pyridines under the study, and quadruplex DNA, possibly *via* the external interactions [40].

3.6. Metal-Terpyridine Complexes

Teulade-Fichou recently reported an interesting study to issue the importance of the metal complex's geometry for quadruplex vursus duplex DNA binding [14]. A series of copper(II), platinum(II), ruthenium(III) and zinc(II) complexes (Fig. (**11**)) with terpyridine-based ligands were prepared and their binding to DNA studied [41, 42]. The copper(II) complexes (with a distorted square-based pyramidal geometry) exhibited high-affinity and selectivity for quadruplex versus duplex DNA ($\Delta T_{1/2}$ = 15.3°C, selectivity ~22 fold), and the platinum(II)-terpyridine complexes showed relatively good affinity and selectivity ($\Delta T_{1/2}$ = 11.3°C, selectivity ~10 fold) [16]. In contrast, zinc(II) and ruthenium(III) complexes (with trigonal bipyramidal and octahedral geometry respectively) demonstrated to be poor quadruplex stabilizers. These results were in consistent with the idea that a planar and highly delocalized aromatic system was necessary for optimal π-π interactions with guanine-quartets. These results also stress the influences of the geometry of the metal complexes and the nature of the metals in the recognition of G-quadruplex-DNA.

3.7. Platinum(II)-Dipyridophenazine Complexes

More recently, Platinum(II) complexes with dipyridophenazine ligands as human telomerase inhibitors and luminescent probes for G-quadruplex DNA have been developed [43]. As shown in Fig. (**12**), complex **29** contains a dipyridophenazine (dppz) ligand with a pendant COOH functional group, which may be involved in the H-bonding interaction with the guanine in the external tetrad of G-quadruplex DNA. This may account for its high binding selectivity and affinity (the binding constant K ~10^7 dm^3 mol^{-1}) of this complex toward the G-quadruplex, as well as its nanomolar potency against telomerase (IC_{50} 760 nM). G-quadruplex DNA binding is accompanied by up to a 293-fold increase in the intensity of photoluminescence at λ_{max} 512 nm.

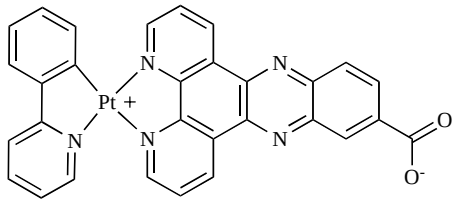
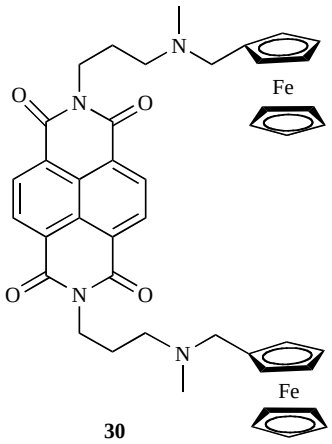


Fig. (12). Structure of complexe **29**.

3.8. A Metal Moiety Introduced into the Periphery

The use of a metal moiety grafted in the periphery of the central aromatic core of a G-quadruplex ligand has also been

reported for various purposes [44, 45]. Fe(II) terminated appendages linked to a perylene have been used as probes devoted to quadruplex-selective chemical cleavage [44]. In addition, Fe(II) linked to a naphthalene diimide core such as ferrocenylnaphthalene diimide (Fig. (**13**)) proved to be a tetraplex DNA-specific binder by electrochemical telomerase assay without relying on PCR. The *T*m value increased by 14°C upon addition of 1 equiv of **32** and increased by 30°C in the presence of 3 equiv of **30** [45].



30

Fig. (13). Ferrocenylnaphthalene diimide complexe **30**.

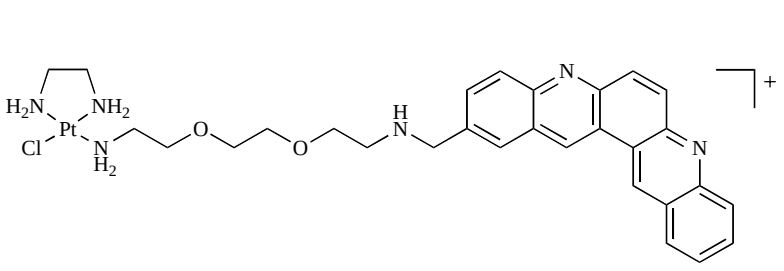
Stimulated by the discovery that the terminal G-quartets could be platinated [46-48], Teulade-Fichou and coworkers studied the Pt(II) complexes to provide additional anchorage of a G-quadruplex binding motif inside the DNA target [49]. The platinum-quinacridine hybrid agent (Pt-MPQ, **31**) showed in Fig. (**14**), interacted with quadruplex-DNA *via* a dual covalent-noncovalent binding mode, targeting preferentially guanines constitutive of exeternal G-quartets. This unprecedented synergism between π-stacking-directed association and a covalent trapping mediated by a mono-functional Pt complex opens up new perspectives for the development of novel quadruplex-binding modes.

In addition, a platinum-acridine hybrid agent, Pt-ACRAMTU **32** (Fig. (**15**)) is worth to be noted. It exhibited excellent activity in a variety of cancers including cisplatin sensitive and resistant ovarian, non-small cell lung, colon, leukemic, glioblastoma and pancreatic cell lines (for example, the IC_{50} values for Pt-ACRAMTU was 0.13 μM in HL-60 leukemia cells) [50-53]. More recently, Pt-ACRAMTU was found to interact with G4 DNA significantly faster ($t_{1/2}$ = 1.2 h) than with dsDNA ($t_{1/2}$ = 2~3 h), indicating it could be developed as a G4-specific ligand [54].

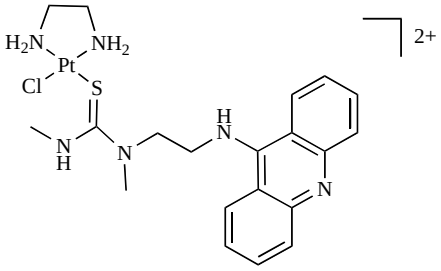
4. CONCLUSION

Overall research in the field of quadruplex ligands is getting more and more exciting as knowledge increases and progress is also being made in developing ligands that can discriminate between different quadruplexes, essential for pharmaceutical success. Metal complexes with planar geometries and π-delocalised ligands are emerging as a new class of quadruplex DNA stabilisers with unique features for optimal binding. Most of this kind of metallo-organic ligands bind to G-quadruplexes through terminal stacking or external binding, with high affinity and selectivity [14], in some cases depending on their metal-mediated conformations [55]. Among the metal-porphyrins (such as Ni(II)-, Mn(III)-, Cu(II)-, Fe(III)-, Zn(II)-, Pt(II)-complexes) reported, pentacationic manganese(III)-porphyrin is the most efficient quadruplex stabilizer, with a 10000-fold selectivity over duplex DNA. Ni(II)-salphen complexes also can induce a high degree of quadruplex DNA stabilization with good selectivity. These achievements have enlightened the promising prospects of G4 ligands as anticancer agents with reduced side effect and toxicity. Further studies are currently being undertaken to benefit from the selective recognition of quadruplex-DNA by the metallo-organic complexes.

Some principles for the design of selective metallo-organic G-quadruplex ligands as anticancer agents have been established. In general, ionic interactions between cationic ligand species and the anionic backbone of the G-quadruplex, along with the appropriate spacing for two or more ionic interactions, will be considered. In addition, inside intercalation between tetrads, outside- or endstacking, groove binding, or a combination of two or more of these modes could give rise to binding specificity. Therefore, an effective and high selective metallo-organic ligand should interact with G-quadruplex in multiple modes, with consideration of electrostatic and geometric factors, π-π stacking, external or groove/lope bindings. With a better understanding of the biological role and structures of G-quadruplex DNA, we can anticipate that more effective G4 ligands with high affinity ($K_d \leq 1$ nM) and high specificity (10000-fold or lower affinity to all other nucleic acids) will be developed for cancer therapy in the near future.



31: Pt-MPQ



32: PT-ACRAMTU

Fig. (14). Structures of Pt-MPQ and Pt-ACRAMTU complexes.

ACKNOWLEDGEMENTS

This work was supported by the Natural Science Foundation of China (Grant No. 30873134) and Natural Science Foundation of Shandong Province (Y2007C125).

ABBREVIATIONS

TMPyP4	=	5,10,15,20-Tetrakis(N-methylpyridinium-4-yl)-21H,23H-porphyrin
IC_{50}	=	concentration causing half-maximal inhibition
TRAP	=	thrombin receptor-activating peptide
SPR	=	surface plasmon resonance
Ka	=	affinity consistent
H_2TMPyP4	=	5,10,15,20-Tetrakis(1-methyl-4-pyridyl)-21H,23H-porphine
Cu-TMPyP4	=	Cu^{2+} derivative of 5,10,15,20-tetrakis(1-methyl-4-pyridyl)-21H,23H -porphine
3, 4-TMPyPz	=	3,4-tetramethylpyridiniumporphyrazine
ZnPc	=	zinc(II) porphyrazines
*T*m	=	melting temperature
EC_{50}	=	concentration for half-maximal contraction
FRET	=	fluorescence resonance energy transfer
FID	=	flame ionization detector
Dppz	=	dipyrido[3,2-a:2',3'-c]phenazine
PCR	=	protochlorophyllide reductase
Pt-MPQ	=	platinum-(mono-para-quinacridine)
ACRAMTU	=	1-[2-(acridin-9-ylamino)ethyl]-1,3-dimethylthiourea
Pt-ACRAMTU	=	$[PtCl(en)(ACRAMTU)](NO_3)_2$

REFERENCES

[1] Gilbert, D.E.; Feigon, J. Multistranded DNA structures. *Curr. Opin. Struct. Biol.*, **1999**, *9*, 305-314.

[2] Nelson, S.M.; Ferguson, L.R.; Denny, W.A. DNA and the chromosome-varied targets for chemotherapy. *Cell Chromosome*, **2004**, *3*, 2-27.

[3] Han, H.; Langley, D.R.; Rangan, A.; Hurley, L.H. Selective interactions of cationic porphyrins with G-quadruplex structures. *J. Am. Chem. Soc.*, **2001**, *123*, 8902-8913.

[4] Lemarteleur, T.; Gomez, D.; Paterski, R.; Mandine, E.; Mailliet, P.; Riou, J.F. Stabilization of the c-myc gene promoter quadruplex by specific ligands' inhibitors of telomerase. *Biochem. Biophys. Res. Commun.*, **2004**, *323*, 802-808.

[5] Gomez, D.; Paterski, R.; Lemarteleur, T.; Shinya, K.; Mergny, J.L.; Riou, J.F. Interaction of telomestatin with the telomeric single-strand overhang. *J. Biol. Chem.*, **2004**, *279*, 41487-41494.

[6] Kim, N.W.; Piatyszek, M.A.; Prowse, K.R.; Harley, C.B.; West, M.D.; Ho, P.L.C.; Coviello, G.M.; Wright, W.E.; Weinrich, R.L; Shay, J.W. Specific association of human telomerase activity with immortal cells and cancer. *Science*, **1994**, *266*, 2011-2015.

[7] Olaussen, K.A.; Dubrana, K.; Domont, J.; Spano, J.P.; Sabatier, L.; Soria, J.C. Telomeres and telomerase as targets for anticancer drug development. *Crit. Rev. Oncol. Haematol.*, **2006**, *57*, 191-214.

[8] Blasco, M.A. Telomeres and human disease: ageing, cancer and beyond. *Nat. Rev. Genet.*, **2005**, *6*, 611-622.

[9] Neidle, S.; Parkinson, G. Telomere maintenance as a target for anticancer drug discovery. *Nat. Rev. Drug Discov.*, **2002**, *1*, 383-393.

[10] Mergny, J.L.; Riou, J.F.; Mailliet, P.; Teulade-Fichou, M.P.; Gilson, E. Natural and pharmacological regulation of telomerase. *Nucleic Acids Res.*, **2002**, *30*, 839-865.

[11] Rezler, E.M.; Bearss, D.J. Hurley, L.H. Telomeres and telomerases as drug targets. *Curr. Opin. Pharmacol.*, **2002**, *2*, 415-423.

[12] Reed, J.E.; Arnal, A.A.; Neidle, S.; Vilar, R. Stabilization of G-quadruplex DNA and inhibition of telomerase activity by square-planar nickel(II) complexes. *J. Am. Chem. Soc.*, **2006**, *128*, 5992-5993.

[13] Reed, J.E.; Neidle, S.; Vilar, R. Stabilisation of human telomeric quadruplex DNA and inhibition of telomerase by a platinum-phenanthroline complex. *Chem. Commun.*, **2007**, 4366-4368.

[14] Dixon, I.M.; Lopez, F.; Esteve, J.P.; Tejera, A.M.; Blasco, M.A.; Pratviel, G.; Meunier, B. Porphyrin derivatives for telomere binding and telomerase inhibition. *ChemBioChem*, **2005**, *6*, 123-132.

[15] Bertrand, H.; Bombard, S.; Monchaud, D.; Teulade-Fichou, M.P. A platinum–quinacridine hybrid as a G-quadruplex ligand. *J. Biol. Inorg. Chem.*, **2007**, *12*, 1003-1014.

[16] Bertrand, H.; Monchaud, D.; De Cian, A.; Guillot, R.; Mergny, J.L.; Teulade-Fichou, M.P. The importance of metal geometry in the recognition of G-quadruplex-DNA by metal-terpyridine complexes. *Org. Biomol. Chem.*, **2007**, *5*, 2555-2559.

[17] Evans, S.E.; Mendez, M.A.; Turner, K.B.; Keating, L.R.; Grimes, R.T.; Melchoir, S.; Szalai, V.A. End-stacking of copper cationic porphyrins on parallel-stranded guanine quadruplexes. *J. Biol. Inorg. Chem.*, **2007**, *12*, 1235-1249.

[18] Kieltyka, R.; Fakhoury, J.; Moitessier, N.; Sleiman, H.F. Platinum phenanthroimidazole complexes as G-quadruplex DNA selective binders. *Chem. Eur. J.*, **2008**, *14*, 1145-1154.

[19] Monchaud, D.; Teulade-Fichou, M.P. A hitchhiker's guide to G-quadruplex ligands. *Org. Biomol. Chem.*, **2008**, *6*, 627-636.

[20] Ou, T.M.; Lu, Y.J.; Tan, J.H.; Huang, Z.S.; Wong, K.Y.; Gu, L.Q. G-quadruplexes: targets in anticancer drug design. *ChemMedChem*, **2008**, *3*, 690-713.

[21] Arnal, A.A.; Buchholz, J.B.; Neidle, S.; Vilar, R. Effects of metal coordination geometry on stabilization of human telomeric quadruplex DNA by square-planar and square-pyramidal metal complexes. *Inorg. Chem.*, **2008**, *47*, 11910-11919.

[22] Tana, J.H.; Gu, L.Q.; Wub, J.Y. Design of selective G-quadruplex ligands as potential anticancer agents. *Mini Rev. Med. Chem.*, **2008**, *8*, 1163-1178.

[23] Arola, A.; Vilar, R. Stabilisation of G-quadruplex DNA by small molecules. *Curr. Top. Med. Chem.*, **2008**, *8*, 1405-1415.

[24] Franceschin, M. G-quadruplex DNA structures and organic chemistry: more than one connection. *Eur. J. Org. Chem.*, **2009**, 2225-2238.

[25] Chen, B.; Liang, J.L.; Tian, X.P.; Liu, X.C. G-quadruplex structure: a target for anticancer therapy and a probe for detection of potassium. *Biochemistry* (*Moscow*), **2008**, *73*, 853-861.

[26] Izbicka, E.; Wheelhouse, R.T.; Raymond, E.; Davidson, K.K.; Lawrence, R.A.; Sun, D.; Windle, B.E.; Hurley, L.H.; Von Hoff, D.D. Effects of cationic porphyrins as G-quadruplex interactive agents in human tumor cells. *Cancer Res.*, **1999**, *59*, 639-644.

[27] Shi, D.F.; Wheelhouse, R.T.; Sun, D.; Hurley, L.H. Quadruplex-interactive agents as telomerase inhibitors: synthesis of porphyrins and structure-activity relationship for the inhibition of telomerase. *J. Med. Chem.*, **2001**, *44*, 4509-4523.

[28] Wheelhouse, R.T., Sun, D., Han, H., Han, F.X.; Hurley, L.H. Cationic porphyrins as telomerase inhibitors: the interaction of tetra-(N-methyl-4-pyridyl) porphine with quadruplex DNA. *J. Am. Chem. Soc.*, **1998**, *120*, 3261-3262.

[29] Hurley, L.H.; Wheelhouse, R.T.; Sun, D.; Kerwin, S.M.; Salazar, M.; Federoff, O.Y.; Han, F.X.; Han, H.; Izbicka, E.; Von Hoff, D.D. G-quadruplexes as targets for drug design. *Pharmacol. Ther.*, **2000**, *85*, 141-158.

[30] Ren, J.; Chaires, J.B. Sequence and structural selectivity of nucleic acid binding ligands. *Biochemistry*, **1999**, *38*, 16067-16075.

[31] Maraval, A.; Franco, S.; Vialas, C.; Pratviel, G.; Blasco, M.A.; Meunier, B. Porphyrin-aminoquinoline conjugates as telomerase inhibitors. *Org. Biomol. Chem.*, **2003**, *1*, 921-927.

[32] Prince, S.; Kçrber, F.; Cooke, P.R.; Lindsay Smith, J.R.; Mazid, M.A. Structure of monomeric hydrated manganese(III) tetra(4-N-

methylpyridyl)porphyrin pentachloride. *Acta Crystallogr.*, **1993**, C*49*, 1158-1160.

[33] Dixon, I.M.; Lopez, F.; Tejera, A.M.; Esteve, J.P.; Blasco, M.A.; Pratviel, G.; Meunier, B. A G-quadruplex ligand with 10000-fold selectivity over duplex DNA. *J. Am. Chem. Soc.*, **2007**, *129*, 1502-1503.

[34] Keating. L.R.; Szalai, V.A. Parallel-stranded guanine quadruplex interactions with a copper cationic porphyrin. *Biochemistry*, **2004**, *43*, 15891-15900.

[35] Goncalves, D.P.; Rodriguez, R.; Balasubramanian, S.; Sanders, J.K. Tetramethylpyridiniumporphyrazines-a new class of G-quadruplex inducing and stabilising ligands. *Chem. Commun.*, **2006**, 4685-4687.

[36] Ren, L.; Zhang, A.; Huang, J.; Wang, P.; Weng, X.; Zhang, L.; Liang, F.; Tan, Z.; Zhou, X. Quaternary ammonium zinc phthalocyanine: Inhibiting telomerase by stabilizing G-quadruplexes and inducing G-quadruplex structure transition and formation. *ChemBioChem*, **2007**, *8*, 775-780.

[37] Gershman, Z.; Goldberg, I.; Gross, Z. DNA binding and catalytic properties of positively charged corroles. *Angew. Chem. Int. Ed.*, **2007**, *46*, 4320-4324.

[38] Fu, B.; Huang, J.; Ren, L.; Weng, X.; Zhou, Y.; Du, Y.; Wu, X.; Zhou X.; Yang, G. Cationic corrole derivatives: a new family of G-quadruplex inducing and stabilizing ligands. *Chem. Commun.*, **2007**, 3264-3266.

[39] Fu, B.Q.; Zhang, D.; Weng, X.C.; Zhang, M.; Ma, H.; Y.Z.; Ma, and Zhou, X. Cationic metal–corrole complexes: design, synthesis, and properties of guanine-quadruplex stabilizers. *Chem. Eur. J.*, **2008**, *14*, 9431-9441.

[40] Reed, J.E.; White, A.J.P.; Neidle, S.; Vilar, R. Effect of metal coordination on the interaction of substituted phenanthroline and pyridine ligands with quadruplex DNA. *Dalton Trans.*, **2009**, 2558-2568.

[41] Schubert, U.S.; Hofmeier, H.; Newkome, G.R. *Modern Terpyridine Chemistry*, Wiley-VCH: Weinheim, **2006**.

[42] Constable, E.C. 2,29,69,20-Terpyridines: from chemical obscurity to common supramolecular motifs. *Chem. Soc. Rev.*, **2007**, *36*, 246-253.

[43] Ma, D.L.; Che, C.M.; Yan, S.C. Platinum(II) complexes with dipyridophenazine ligands as human telomerase inhibitors and luminescent probes for G-quadruplex DNA. *J. Am. Chem. Soc.*, **2009**, *131*, 1835-1846.

[44] Tuntiwechapikul, W.; Lee, J.T.; Salazar, M. Design and synthesis of the G-quadruplex-specific cleaving reagent perylene-EDTA. Iron (II). *J. Am. Chem. Soc.*, **2001**, *123*, 5606-5607.

[45] Sato, S.; Kondo, H.; Nojima, T.; Takenaka, S. Electrochemical telomerase assay with ferrocenylnaphthalene diimide as a tetraplex DNA-specific binder. *Anal. Chem.*, **2005**, *77*, 7304-7309.

[46] Redon, S.; Bombard, S.; Elizondo-Riojas, M.A.; Chottard, J.C. Platinum cross-linking of adenines and guanines on the quadruplex structures of the $AG_3(T_2AG_3)_3$ and $(T_2AG_3)_4$ human telomere sequences in Na^+ and K^+ solutions. *Nucleic Acids Res.*, **2003**, *31*, 1605-1613.

[47] Ourliac-Garnier, I.; Elizondo-Riojas, M.A.; Redon, S.; Farrell, N.P.; Bombard. S. Cross-links of quadruplex structures from human telomeric DNA by dinuclear platinum complexes show the flexibility of both structures. *Biochemistry*, **2005**, *44*, 10620-10634.

[48] Ourliac-Garnier, I.; Bombard, S. GG sequence of DNA and the human telomeric sequence react with cis-diammine-diaquaplatinum at comparable rates. *J. Inorg. Biochem.*, **2007**, *101*, 514-524.

[49] Bertrand, H.; Bombard, S.; Monchaud, D.; Teulade-Fichou, M.P. New platinum(II) complexes targeting the loops of the human telomeric G-quadruplex. *Nucleic Acids Symp. Ser.*, **2008**, No. *52*, 163-164.

[50] Martins, E.T.; Baruah, H.; Kramarczyk, J.; Saluta, G.; Day, C.S.; Kucera, G.L.; Bierbach, U. Design, synthesis, and biological activity of a novel non-cisplatin-type platinum-acridine pharmacophore. *J. Med. Chem.*, **2001**, *44*, 4492-4496.

[51] Ackley, M.C.; Barry, C.G.; Mounce, A.M.; Farmer, M.C.; Springer, B.E.; Day, C.S.; Wright, M.W.; Berners-Price, S.J.; Hess, S.M.; Bierbach, U. Structure–activity relationships in platinum–acridinylthiourea conjugates: effect of the thiourea nonleaving group on drug stability, nucleobase affinity, and *in vitro* cytotoxicity. *J. Biol. Inorg. Chem.*, **2004**, *9*, 453-461.

[52] Hess, S.M.; Mounce, A.M.; Sequeira, R.C.; Augustus, T.M.; Ackley, M.C.; Bierbach, U. Platinum-acridinylthiourea conjugates show cell line-specific cytotoxic enhancement in H460 lung carcinoma cells compared to cisplatin. *Cancer Chemother. Pharmacol*, **2005**, *56*, 337-343.

[53] Hess, S.M.; Andersona, J.G.; Bierbachb, U. A non-crosslinking platinum–acridine hybrid agent shows enhanced cytotoxicity compared to clinical BCNU and cisplatin in glioblastoma cells. *Bioorg. Med. Chem. Lett.*, **2005**, *15*, 443-446.

[54] Lu, R.; Bierbach, U. Kinetically favored platination of adenine in the G-rich human telomere repeat. *J. Am. Chem. Soc.*, **2007**, *129*, 15764-15765.

[55] Monchaud, D.; Yang, P.; Lacroix, L.; Teulade-Fichou, M.P.; Mergny, J.L. A metal-mediated conformational switch controls G-quadruplex binding affinity. *Angew. Chem. Int. Ed.*, **2008**, *47*, 4858-4861.

Received: March 21, 2010 Revised: May 17, 2010 Accepted: May 18, 2010