EurJIC
European Journal of Inorganic Chemistry

Review
doi.org/10.1002/ejic.202400705

Chemistry Europe
European Chemical Societies Publishing

www.eurjic.org

DNA Binding Activity of Functionalized Schiff Base Metal Complexes

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We dedicate this work to professors Renato Barbieri (1930–2013) and Arturo Silvestri (1941–2009), that paved the way to our present research interests

Based on our recent research experience, this review highlights the DNA binding of salen, salphen and salnaphen metal complexes, with a focus on G-quadruplex (G4) DNA, which is crucial in peculiar genomic regions and in cancer regulation. Such metal complexes have in fact shown significant ability to bind and stabilize G4 structures. We will point out the role of the metal center and of the ligand substituents affecting their binding and selectivity toward G4s, supported by experimental and computational studies.

Since the discovery of the anticancer properties of several compounds, DNA has played a central role among the most extensively targeted biological molecules for anticancer chemotherapeutic drug development.[1] For example, the DNA-binding mechanism of cisplatin, the most prominent metal-based anticancer drug, is well-known,[2] leading to apoptosis by creating lesions and arresting DNA replication.[3,4]

Despite its effectiveness against several solid tumors, cisplatin, as well as most of the classical chemotherapeutical agents, has limitations due to its inability to differentiate between cancerous and healthy cells.[5] To overcome the limitations posed by the non-selectivity of cisplatin while retaining its antitumor activity, various metal-based drugs have been investigated. Ongoing research has sought to develop alternative strategies that maintain DNA as the primary target while mitigating the undesirable side effects associated with cisplatin. One approach has involved the design of platinum-based analogs with improved pharmacokinetic profiles and reduced toxicity.[6] Furthermore, researchers have explored non-platinum-based compounds that similarly target DNA but engage with it through distinct molecular interactions, potentially avoiding the pathways that lead to cisplatin resistance.[6,7] Another promising strategy includes the targeting of non-canonical structures of DNA, playing crucial roles in carcinogenesis, to selective drug delivery to cancer cells, thereby reducing damage to healthy tissues. This search for alternative strategies reflects the need to balance efficacy with tolerability in cancer treatment, driving innovation in the field of DNA-targeted therapies.[8]

DNA is a dynamic and flexible macromolecule. When processes such as replication and transcription occur, the canonical DNA double helix (B-DNA) is partially uncoiled into two single-stranded sequences. Some of these single-stranded sequences exhibit repetitive motifs and factors such as hydration, nucleotide sequence, ionic strength and the directionality of the glycosidic bonds, that can drive the folding into a non-canonical structure. Hairpins, triplexes, cruciforms, left-handed Z-DNA, tetraplexes, and poly (dA) duplexes (A-motif) are some of the DNA structures studied so far and many findings have shown their involvement in the regulation of biologically essential processes.[8–10]

Another important non-canonical DNA structure is the G4, formed when four guanine bases assemble into a planar structure known as G-tetrad via Hoogsteen hydrogen bonds (Figure 1).[11] In G4s, multiple G-tetrads stack on top of each other, stabilized by monovalent cations inside the central channel. G4s can form within a single DNA strand (intramolecular G4s) or across multiple strands (intermolecular G4s). Based on strand orientation, G4 topologies are classified as parallel (same direction), antiparallel (opposite direction), or hybrid (a combination of parallel and antiparallel strands) (Figure 1). G-rich sequences in DNA form G4s in living cells of various organisms,[12–14] and these sequences are non-randomly distributed. Many sequences able to fold in G4s in physiological conditions have been found in telomeres and oncogene promoters of the human genome.[15–17] For example, Balasubramanian and collaborators adapted next-generation sequencing techniques to map G4 structures (G4-seq) in the human genome, identifying over 700,000 G4 motifs, many of which had not been predicted by earlier computational methods.[18]

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Review
doi.org/10.1002/ejic.202400705

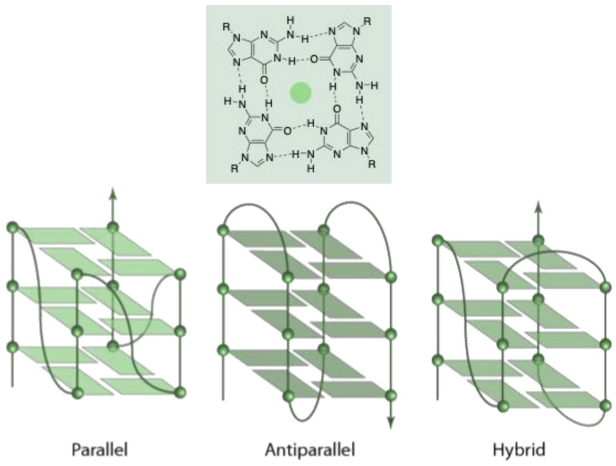


Figure 1. Schemes of a G-tetrad and of possible G4 topologies.

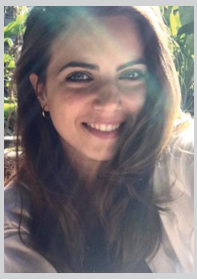
G4 key roles in cancer gene regulation have prompted a large community of scientists to the design and synthesis of G4-targeting compounds.[19,20] The precise mechanism of action is still unclear, although it is likely that, besides a simple stabilization of the G4 structure, also DNA damage processes at the site of the interaction are involved.[21] In the search for an effective binder able to interact with the G4 structure, thousands of compounds have been reported to selectively bind G4 over other DNA structures.[22] While most G4 binders are organic molecules, since the early 2000s there has been growing interest in metal-based compounds that bind to and stabilize G4s through interactions with the guanine tetrads.[23–25]

Typically, the rational design for molecules that interact with G4 motifs includes the incorporation of extended flat aromatic polycyclic systems, which facilitate stacking on the external G-quartet of G4s and positively charged substituents that promote electrostatic interactions with the DNA backbone. In addition, a metal center can organize ligands into specific geometries and orientations, optimizing G4 binding. The metal is often situated at the center of the complex, occupying the position normally held by K^+ ions in the G4 channel when binding through end-stacking with guanine tetrads.[26] Moreover, metal ions can impart unique electronic properties to the system, endowing the resulting metal complexes with intriguing optical, catalytic, or redox characteristics, which can be harnessed for imaging, catalysis or therapeutic purposes. Among the compounds investigated thus far, complexes of Schiff base salen-like and salphen-like ligands have garnered significant attention in a wide range of applications. The tunability of their properties makes them excellent catalysts[27] as well as promising G4 stabilizers.[22] Square planar and tetradentate Schiff base ligands (Figure 2) are formed through the condensation of 1,2-phenylenediamine (salphen) or 1,2-diaminoethane (salen) derivatives with substituted salicylaldehydes.

The imino nitrogen atoms and the deprotonated hydroxyl oxygen atoms enable these ligands to efficiently coordinate metal ions,[28] in an approximately square-planar geometry. This configuration is well-suited for the equatorial coordination of transition metals, while the two axial positions remain in principle available for the coordination of additional apical ligands. In 1889, Combes prepared the first salen-type ligand and its Cu complex.[29] Since then, the facile synthesis, versatility in coordinating with various metals, and high stability of Schiff-base metal complexes have facilitated their extensive use in transition-metal chemistry for diverse applications, particularly in catalysis.[30] Additionally, these complexes have shown significant potential in the biomedical field, functioning as anti-inflammatory and antiviral agents, as models for superoxide dismutase, and as biomarkers for various pathological conditions, including ovarian cancer.[31,32]

The use of Schiff bases as ligands for coordination compounds has a long history at the University of Palermo. It dates back to the activities of Luigi Sacconi (1911–1992), who was the first professor of Inorganic Chemistry at the University of Palermo, from 1954 to 1960. Sacconi presumably developed his idea of including imine ligands during his studies at the University of Florence, where Hugo Schiff first envisioned and developed such molecules. As a result, several transition metal complexes of salen derivatives were synthesized and characterized by Sacconi and collaborators,[33–36] with a particular focus on nickel(II) complexes.[37–40]

Barbieri and collaborators, starting from 1964, continued in this field by synthesizing and investigating the properties of several Schiff base metal complexes,[41,42] mainly based on salen derivatives.[43–47] Following the discovery of the properties of cisplatin, associated to its DNA binding, in the nineties, Barbieri research group started to investigate the effect of organotin(IV)



Luisa D'Anna obtained her master's degree in Chemistry and Pharmaceutical Technologies in 2021 at the University of Palermo, where she started her Ph.D. in Bioinorganic Chemistry. She's working on salphen metal complexes and their interaction with human and viral G-quadruplexes. Recently, she has been visiting researcher at the Université de Lorraine (Nancy, France) and at Université Paris Cité (Paris, France).

Giampaolo Barone obtained his Ph.D. in Chemistry in 1998 from the University of Palermo, where he is currently Professor of Inorganic Chemistry. He has been a postdoc at the University of Bath, UK (1999), University of Salerno, Italy (1999–2001) and at the EMBL-Hamburg outstation, Germany (2001). He has been a visiting researcher in several European universities. His research interests are mainly focused on the synthesis of DNA-binding metal complexes and on the experimental and computational study of chemical and spectroscopic properties of metal compounds.

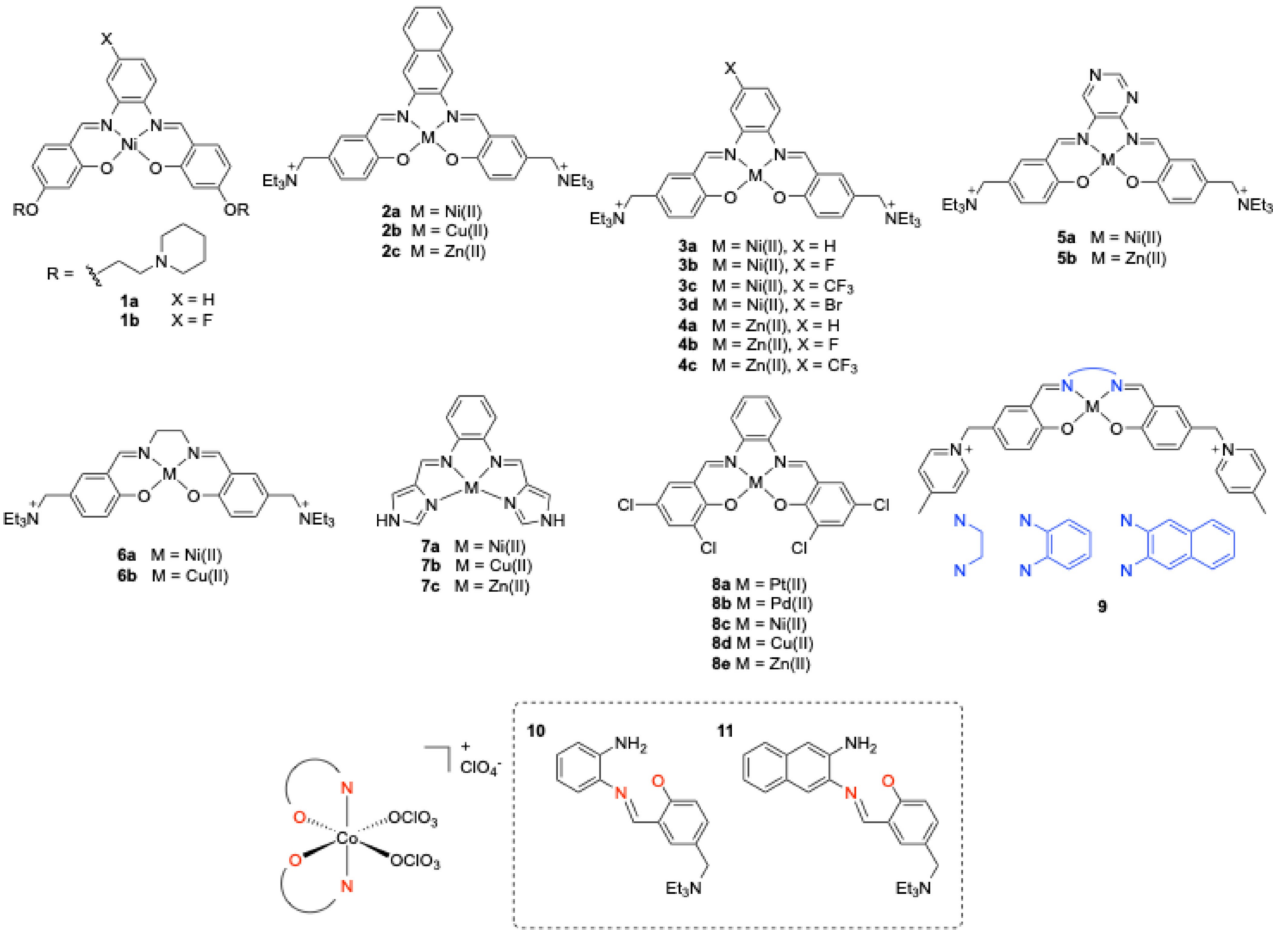


Figure 2. Examples of metal complexes of salen, salphen and salnaphen functionalized ligands, recently investigated.

compounds on the DNA structure.[48–54] Such activity was then continued by Silvestri and collaborators starting from 2004, who focused on 3d metal complexes of salen[55] and of salphen derivatives.[56–58] These studies were part of a focused research on the design of potential DNA metallo-intercalators, often followed by biological investigations to test their possible cytotoxicity *in vitro* against human cancer cell lines.[59–62] Soon after, the main interests of the group essentially became the design of potential G4 DNA binders.[63,64]

The first example of salphen-based metal complex binding to a G4 motif was documented by Neidle and Vilar in 2006.[65] The authors studied Ni(II) derivatives, such as **1**, that stabilize human telomeric G4s and inhibit telomerase. Since then, numerous derivatives have been developed with enhanced binding affinity and selectivity.

In 2014, our research group reported the synthesis of Ni(II), Cu(II), and Zn(II) complexes of a new salphen-like ligand featuring an extended aromatic area provided by a naphthalene moiety, called salnaphen (**2**, Figure 1).[63,64] The binding affinity of these complexes to human telomeric (h-Telo) and *MYC* G4s DNA was studied using different spectrophotometric titrations, which revealed their ability to bind G4s with much higher affinity than B-DNA. Among them, the Ni(II) complex **2a** was the most effective stabilizer, able to induce G4 formation at room temperature even without K^+ ions in solution. The three compounds stabilize G4 structures and inhibit PCR product amplification, exhibit concentration and time-dependent cytotoxicity in HeLa and MCF-7 cancer cell lines, affecting cell cycle distribution. The order of effectiveness for both PCR inhibition and anticancer activity was Ni > Cu > Zn, correlating with their G4-DNA binding affinity.

One advantage of this research activity was the combination of experimental and computational approaches. In fact, crucial insights for the interpretation of experimental data were obtained by performing *in silico* investigations based on both molecular dynamics (MD) simulations, and quantum mechanics/molecular mechanics (QM/MM) calculations,[63,64] and using density functional theory (DFT) to describe the quantum layer. In top-stacking binding, the metal ion of the Schiff-base complexes typically aligns with two potassium cations in the central channel formed by stacked G-tetrads (Figure 3). Interestingly, *in vivo* studies of the effects of **2a** during sea urchin embryogenesis showed that it binds to a G4 structure in the promoter of *hbox12-a*, a key developmental gene, inducing its overexpression.[66]

The B-DNA and G4 binding of Ni(II) and Zn(II) complexes with water-soluble salphen ligands, featuring positively charged triethylammonium side chains (**3**–**4**),[68] was investigated with

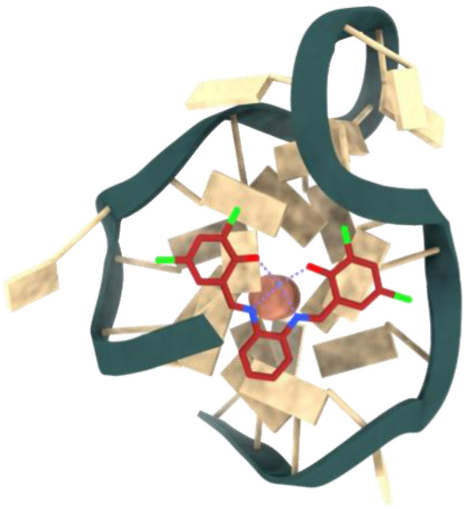


Figure 3. Example of top-stacking binding of a zinc(II) salphen complex with c-Kit1 G4 obtained by molecular docking. Adapted from [67].

the aim to assess the impact of different substituents (H, F, CF_3) on the phenyl ring on the N,N′ bridge. The metal ion's nature significantly influenced the binding to polynucleotides in various conformations. UV-Vis and CD titrations showed that Ni(II) complexes **3** bind more tightly to DNA than the Zn(II) counterparts, **4**. Ni(II) complexes preferred binding the telomeric G4 sequence hTelo over duplex DNA, with binding constants (K_b) of about an order of magnitude higher. **3c** also induced the transition from hybrid to parallel G4-topology, stabilizing the latter. Zn(II) compounds **4** showed negligible binding to B-DNA but were highly selective for the G4 structure, despite being less active than **3**. This selectivity is likely due to the Zn(II) center's ability to coordinate solvent molecules in the apical position, hindering B-DNA intercalation while allowing end-stacking with G4. These findings were also supported by MD simulations.[68]

Similar results were obtained with other salphen-like Ni(II) and Zn(II) complexes featuring a pyrimidine ring on the N,N′ bridge (**5**).[69] The Zn complex was more selective for the telomeric G4 compared to its Ni(II) counterpart, despite the latter having a higher binding constant.[69] In the search of compounds with higher affinity toward G4s and less or no activity toward B-DNA, a weak point of the perfectly planar Ni(II) compounds was their ability to intercalate B-DNA. To address this issue, we designed, synthesized, and characterized Ni(II) and Cu(II) complexes **6** using the same ligands with positively charged triethylammonium side chains, but without an aromatic ring on the N,N′ bridge, i.e., a substituted salen ligand.[70] These compounds were tested against a panel of G4-forming sequences using UV-Vis, circular dichroism (CD), and FRET measurements. The nickel complex **6a** stabilized oncogene promoter G4s with high selectivity and showed no interactions with duplex DNA. Molecular docking studies revealed that **6a** selectively binds c-KIT G4 within its unique groove pocket (PDB entry 2O3M). This compound also exhibited dose-dependent cytotoxic activity (IC_{50} of 29 μM) on MCF-7 breast cancer cells after 72 hours, when combined with lipofectamine.[70]

In 2021 we synthesized three new salphen-like complexes (**7**) of Ni(II), Cu(II), and Zn(II) using an N4-donor ligand called "phenim", featuring an imidazole ring in the aldehydic core instead of the typical 6-membered aromatic ring.[71] Interestingly, the Ni(II) complex **7a** exhibited a distorted octahedral geometry in both solid-state and solution, as shown by its paramagnetic nature in ^{1}H-NMR spectra. These compounds were tested for binding to B-DNA, as well as to hTelo and c-MYC G4s, as models for hybrid and parallel G4 conformations, respectively. Only the Cu(II) complex **7b** showed selectivity for G4 over B-DNA, with a 10-fold higher K_b for non-canonical DNA structures and a preference for telomeric G4. MD simulations suggested that **7b** binds via π-π stacking with flanking bases A3 and T20 of the loop at the 3′-end. Ni(II) compound **7a** had low affinity for G4s, likely due to its octahedral geometry hindering stacking interactions.[71]

Very recently, we synthesized and characterized five non-charged metal complexes of Pt(II), Pd(II), Ni(II), Cu(II), and Zn(II) with a chlorine-substituted salphen ligand (**8**) and tested them against the three adjacent G4 units of the *KIT* proto-oncogene promoter, namely Kit2, SP, and Kit1.[67] FRET measurements showed that Pt(II) and Pd(II) compounds, although only sparingly water soluble, stabilize Kit1 and Kit2 G4s but not SP, telomeric, or double-stranded DNA. Spectroscopic studies identified the Cu(II) complex as the most G4-selective. Docking simulations revealed that these compounds fit into the groove binding pockets of both Kit1 and Kit2 G4s. Additionally, they exhibited dose-dependent cytotoxic activity against MCF-7, HepG2, and HeLa cancer cells.[67] In 2024, twelve novel metal complexes of salen, salphen, and salnaphen ligands with charged methylpyridinium side chains (compounds **9**) were reported.[72] These complexes stabilize specific G4 structures in oncogene promoters more effectively than B-DNA.

The results obtained up to now allow to conclude that the metal center of the title complexes significantly influences nucleic acid folding affinity and stabilization, with Ni(II) compounds always showing the strongest effects. Increasing the size of the π-conjugated moiety on the N,N′ bridge enhances G4 stabilization, typically by top-stacking binding, with the following order of effectiveness: salnaphen > salphen > salen. Molecular modeling and experimental data confirm persistent binding between G4s and these metal complexes, especially with Ni complexes with salphen and salnaphen ligands, and revealed that no structural deformations in the nucleic acid are induced. In cell line tests, all metal complexes exhibited limited cytotoxicity, indicated by high IC_{50} values, though partial inhibition of cell proliferation was observed. Ni(II) compounds increased the number of nuclear G4s in treated cells in a dose-dependent manner, potentially modulating the G4 landscape and downregulating oncogenes. The study suggests that our metal complexes can modulate gene expression and achieve specific selectivity, such as Pt(II) salphen with *BCL2* and Pd(II) salen with *RET*. These properties, along with low cytotoxicity, are crucial for reducing secondary treatment effects. Overall, modulating interactions with loops and tetrads could enhance G4 selectivity in drug design.[72] Recently, two octahedral cobalt(III) complexes of half salphen (**10**) and

half salnaphen (**11**) ligands, both bearing two perchlorate groups, exhibited significant stability in presence of physiological glutathione levels and promising anticancer activity in SW-1353 cell lines.[73] They showed moderate stabilization of G4s and molecular modelling suggested external DNA binding for both compounds, highlighted the notable role of the perchlorate ligand in hydrogen bonding.

Other research groups have extensively studied properties and applications of metal salphen complexes. In particular, Vilar and collaborators have sensibly contributed to the field, exploring the G4 affinity and selectivity of nickel(II) salphen complexes connected by polyether chains of varying lengths (**12**, Figure 4). Using their earlier G4 stabilizer **13**, as a benchmark, they examined sequences forming both single G4 units and dimers separated by TTA spacers, mimicking the single-stranded overhangs of telomeric DNA that form multiple adjacent G4 structures. The length of the polyether linker proved crucial, with the longest variant (**12c**) demonstrating the greatest efficacy. **12c** exhibited strong affinity for sequences forming consecutive antiparallel and mixed-type G4s, and facilitated the conversion of mixed hybrid G4 dimers into parallel G4s in potassium ion environments. Despite having lower overall affinity compared to **13**, **12c** showed superior selectivity for dimeric G4s linked by short TTA sequences. Gel electrophoresis and emission spectroscopy further supported these findings, suggesting that the spacing between the nickel-salphen units aligns well with the distance between consecutive G4s, allowing simultaneous interaction with selected telomeric sequences.[74] Furthermore, a continuous flow platform was developed, for the "one-pot" synthesis of extensive libraries of compounds. This platform was integrated with a microfluidic system for continuous flow G4 FRET melting assays, enabling the synthesis of numerous salphen-like Ni(II) compounds. Among these, **14** emerged as the most active and selective, particularly for a parallel G4 structure formed in the *KIT* promoter, while exhibiting no binding to B-DNA.[75] Very recently, the same research group has contributed significantly to the understanding of dinuclear metal-salphen complex (**15**) interactions with G4 DNA structures. They explored the influence of linker length and flexibility on binding selectivity for monomeric and dimeric G4s. The shorter polyethylene glycol linkers showed better selectivity for dimeric G4s, while longer peptide linkers provided flexibility and retained selectivity for dimeric G4 structures. Despite limited cellular uptake, these complexes showed nuclear localization, highlighting their potential in selectively targeting G4s.[76]

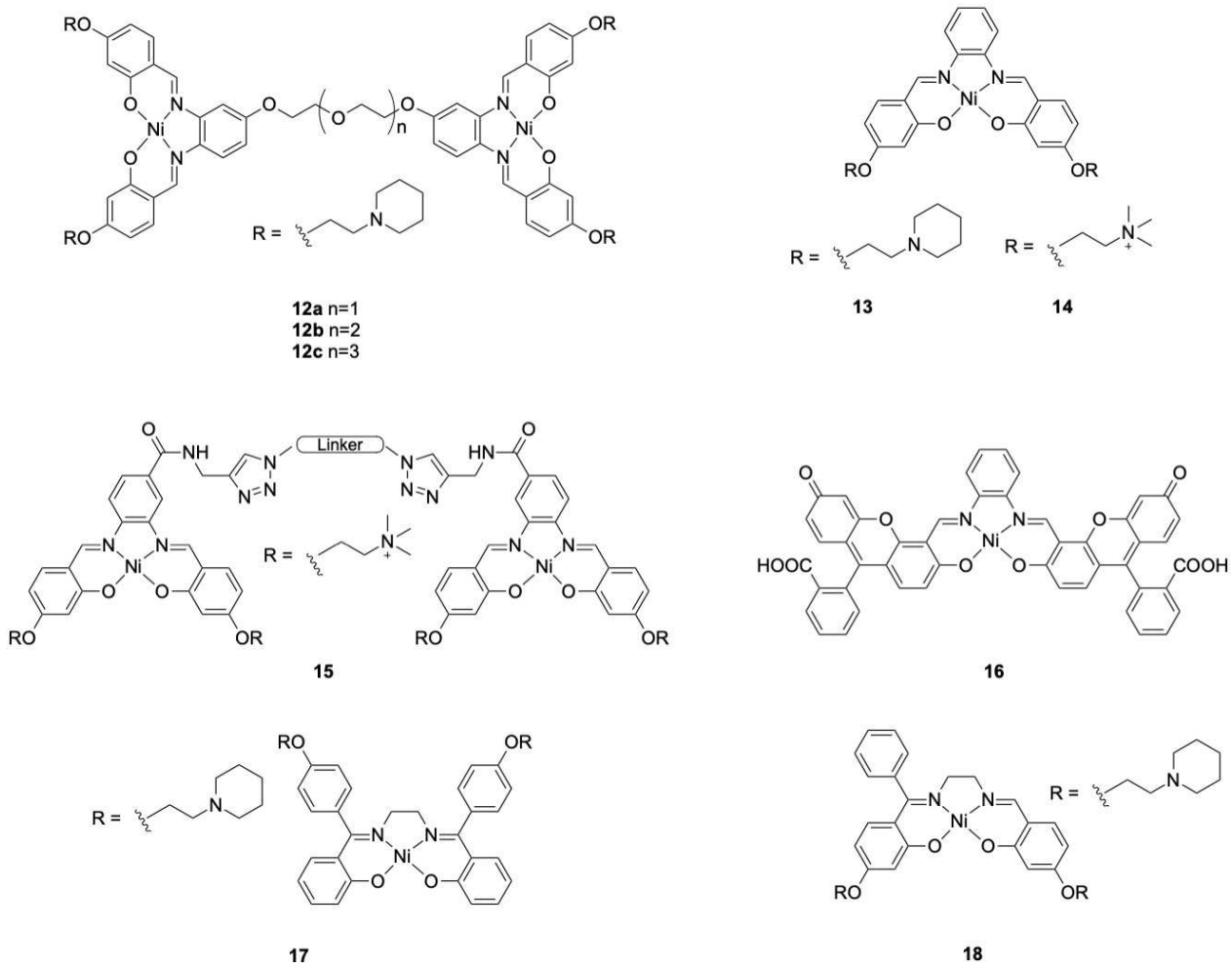


Figure 4. Structures of nickel(II) complexes with salen and salphen derivatives, investigated by other research groups.

A recent study by Bhattacharya and collaborators reported on the development of a novel Ni(II) complex, **16**, designed to track the cellular localization of salphen-like metal complexes. Integrating fluorescein moieties, **16** retained the G4-stabilizing properties of salphen and the fluorescent characteristics of fluorescein. UV-Vis and CD titrations, along with thermal denaturation studies, demonstrated that **16** effectively binds telomeric G4 structures in both mixed hybrid and antiparallel conformations. **16** outperformed its Pd(II) and Ni(II) salen counterparts in terms of binding and fluorescence in buffered solutions, with fluorescence quenching upon binding to G4-forming DNA sequences. Additionally, **16** inhibited telomerase activity, showing cytotoxicity with an IC_{50} of 14 μM in HEK 293 T cells after a 72-hours period of incubation. Long-term inhibition of cell proliferation across various cancer cell lines, consistent with telomerase suppression, was observed. The complex also allowed localization within HEK 293 T cells, where it was found in the mitochondria and nucleus.[77]

Lastly, Ralph and collaborators synthesized twelve symmetric and asymmetric Ni(II) salen-like complexes bearing benzophenone groups. They discovered that the symmetric complex **17** selectively binds parallel unimolecular G4 structures, while the asymmetric **18** exhibited stronger interactions with antiparallel G4-forming sequences. Neither complex showed binding to B-DNA models, demonstrating that small core structure changes can substantially influence G4 selectivity.[78]

Summary and Outlook

Over the past decade, metal complexes of Schiff base ligands have garnered significant attention due to their peculiar structural and electronic properties. Their ease of synthesis, stability, and ability to involve various metal centers render these complexes highly tunable for diverse applications, also in catalysis. These features have enabled the development of an extensive library of compounds, spurring further research exploration. In this review, we provide a focused overview of the design and synthesis of functionalized salen, salphen and salnaphen metal complexes and their interaction with G4 DNA. We have reported several examples of metal complexes synthesized in our laboratory, highlighting the relationship between their structural properties and their ability to interact with G4s. Moreover, we also have applied distinct *in silico* methods with an increasing level of accuracy (e.g., molecular docking, MD simulations, QM/MM calculations) to characterize G4 structural arrangements,[79,80] their involvement in the molecular recognition processes of small molecules, and, in particular, of functionalized salen, salphen and salnaphen metal complexes. The main conclusion is that the presence of more extended aromatic groups on the N,N′ bridge increases the DNA binding strength but reduces the G4/B-DNA selectivity. The absence of aromatic groups on the N,N′ bridge sensibly reduces the DNA binding strength but favors G4 over B-DNA binding. It will be tuning the size and shape of the moiety on the N,N′ bridge that will probably sort out more efficient G4 binding title compounds. An essential role is played by the presence of positively charged groups on the salicylaldehyde moieties, increasing both water solubility and electrostatic attraction with the DNA backbone. In this respect, tuning the shape, length and charge of pendant groups protruding out from the central aromatic core, could also enhance the affinity/selectivity toward different G4 structures, due to their possible peculiar interactions with the grooves and/or with the loops. Finally, the central metal ion affects the overall affinity by non-straightforward but rational ways, attributable to its peculiar coordination chemistry properties. Similar metal complexes have been developed by other research groups, focusing on their interactions with G4s and their potential anticancer properties. It is clear from the presented results that salphen metal complexes are optimal molecules to interact with the aforementioned DNA structures for anticancer applications. On the other hand, our research group, using both experiments and computation, contributed to the structural identification of G4 motifs in the genome of viruses like SARS-CoV-2,[81,82] reporting also the first structure of the TMPRSS2 RNA–G4,[83] implicated in viral entry into host cells.[84] As a consequence, the antiviral activity of Schiff base metal complexes is a field to be deepened in the close future, together with other G4-related diseases. Another possible future direction to improve the anticancer activity of salphen metal complexes is to conjugate them to nanostructures. For example, in a recent publication, carbon nanodots functionalized with Ni(II) complex **3 a** stabilize G4 structures more effectively than either the metal complex alone or non-functionalized carbon nanodots.[85] Interestingly, these nano-conjugates exhibited greater potency and selectivity against cancer cells, suggesting a synergistic effect stemming from G4 interaction and the generation of reactive oxygen species.[85] Finally, the integration of machine learning approaches could accelerate the discovery and design of new salphen derivatives, opening up opportunities for targeted therapeutic interventions and advancing our understanding of G4 properties.

Acknowledgements

Open Access publishing facilitated by Università degli Studi di Palermo, as part of the Wiley - CRUI-CARE agreement.

Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

Keywords: G-quadruplex · Salen · Salnaphen · Salphen · Transition metals

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Manuscript received: October 22, 2024
Revised manuscript received: January 29, 2025
Accepted manuscript online: January 30, 2025
Version of record online: February 10, 2025