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Synthesis and Medicinal Applications of N-Heterocyclic Carbene Complexes Based on Caffeine and Other Xanthines

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Xanthines are purine derivatives predominantly found in plants. These include compounds such as caffeine, theophylline, and theobromine and exhibit a variety of pharmacological properties, demonstrating efficacy in treating neurodegenerative disorders, respiratory dysfunctions, and also cancer. The versatile attributes of these materials render them privileged scaffolds for the development of compounds for various biological applications. Xanthines are N-heterocyclic carbene precursors that combine a pyrimidine and an imidazole ring. Owing to their biological relevance, xanthines have been employed as N-heterocyclic carbenes in the development of metallodrugs for anticancer and antimicrobial purposes. In this conceptual review, we examine key examples of N-heterocyclic carbene complexes derived from caffeine and other xanthines, elucidating their synthetic methods and describing their pertinent medicinal applications.

Introduction

Xanthines are alkaloids that can be produced by plants and animals.[1] These compounds are key components of metabolic processes involving purine nucleobases.[2] Xanthine is often considered the point of convergence for the purine base metabolism of adenine and guanine nucleotides and is involved in the production of uric acid.[3] Xanthines can bear methyl groups at several positions on the ring, and the position and number of N-methyl groups can differ. The most common are caffeine (1,3,7-trimethylxanthine), theophylline (1,3-dimethylxanthine) and theobromine (3,7-dimethylxanthine) (Figure 1). Given the similarity between xanthines and the nucleobases guanine and adenine, and the metabolic role these compounds can play, they are excellent candidates for developing novel drugs for a variety of pharmacological applications. Indeed, xanthines have been recognized as therapeutically potent compounds for the treatment of various diseases[4] and are effective in the treatment of neurodegenerative and respiratory diseases, diabetes and cancer.[5]

The most widely known methylxanthine is caffeine, which can be found in coffee, tea, cocoa, cola and other beverages and foods, being the most consumed psychostimulant worldwide.[6] Caffeine is used therapeutically to treat headache and respiratory depression in neonates, among other conditions.[7,8]

It is well accepted that caffeine is an antagonist of adenosine receptors and can also act as an inhibitor of phosphodiesterases.[9] In addition, caffeine can modulate $GABA_A$ receptors to inhibit acetylcholinesterase, which may be involved in its central nervous system effects.[6] Given the potential for medicinal applications that xanthines have shown thus far,[1,10,11] their use as ligands opens a variety of possibilities for drug development.

Xanthines are N-heterocyclic carbene precursors (NHCs); and therefore their role as ligands can be explored for their ability to bind as NHCs, in contrast to the readily available N-coordination. In the context of metallodrugs, NHCs are known to impart stability due to the strong donation abilities of this class of compounds.[12] Building NHCs from biologically relevant scaffolds[13] such as xanthines and combining them with a metal centre allows for the development of more biocompatible metallodrugs.[13] Indeed, the stability of metallodrugs in physiological media is often an issue, and in this regard, the stabilization that the NHC ligand can impart to the metal centre undoubtedly makes these ligands privileged candidates for the development of more stable metallodrugs.[14] The application of metal complexes bearing NHCs has been widely explored in catalysis.[15] However, their use in medicinal chemistry has been less explored but is a rapidly growing field.[149–12] A review focusing on metal complexes based on xanthines and their biological application has been recently published,[16] and a short review on xanthine based NHCs was published by Morales-Morales in 2018.[17] We considered that a more targeted review, specifically highlighting the role of the NHC, was due. In this conceptual review, we focus specifically on the development of metal complexes bearing NHCs based on xanthines, particularly caffeine, and on the known medicinal applications of these complexes, discussing key examples for their synthesis and biological applications.

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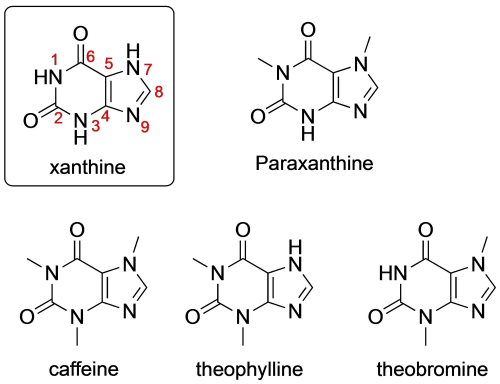


Figure 1. Types of Xanthines.

Synthetic Approaches

Given the parallelism between xanthine rings and imidazoles, strategies for the synthesis of N-heterocyclic carbene complexes employ similar methods to those used for imidazolium salts.[13,14] However, the preparation of xanthine salts can present some drawbacks depending on the type of xanthine. For example, several reports point to the difficulty of functionalizing caffeine beyond methylation or benzylation[18–20][21] This difference has been attributed to the lower nucleophilicity of N9 of caffeine, which requires harsh reaction conditions to achieve quaternization, which is limited to methyl or benzyl substituents. To circumvent this, functionalization is generally performed starting from theophylline, for which quaternization is more facile since it can take place at N7. For this reason, many reports on NHC complexes, referred to as caffeine NHCs, concern compounds containing substituents at N7 other than a methyl group. Additionally, the difference in the nucleophilicity of the methylxanthines can also be reflected in their direct reactivity with metal complexes. In 1975, Taube reported the formation of N-bound and C-bound complexes of ruthenium based on xanthines under acidic conditions (Scheme 1).[22]

Differences in the methylation sites of xanthines have direct effects on the type of coordination to ruthenium. Caffeine and theobromine form only C8-bound complexes, theophylline forms both N7- and C8-bound complexes, and 9-alkylated xanthines form almost exclusively N-bound complexes (Figure 2).

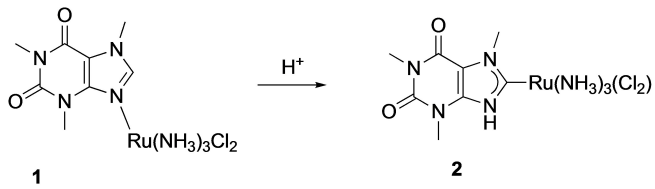
Importantly, these studies also demonstrated that the C8 tautomer is thermodynamically favoured, while N7 is kinetically favoured. In line with this, isocaffeine, methylated at N9, only formed the N-bound compounds. Examples of the synthesis of xanthine NHCs by tautomerization are, however, very limited.[23]

Giulia Francescato obtained her MSc in Medicinal Chemistry and Pharmaceutical Technology at the University of Pavia (Italy) in 2018. During her degree she did her thesis internship under the supervision of Prof. Lino Colombo and Massimo Serra, where she worked on the synthesis of lactam scaffolds as peptidomimetics for cancer and neurodegenerative disease treatment. Giulia is currently pursuing her PhD degree in Chemistry at the NOVA University of Lisbon, under the supervision of Dr. Ana Petronilho. Her research project concerns the synthesis of metal complexes stabilized by nucleobases and xanthines, and the study of their anticancer and antimicrobial properties.

After her BSc degree in Biochemistry at University of Porto and MSc in Medicinal Chemistry at University of Minho, Dr Maria Inês Leitão obtained her PhD in Molecular Biosciences from NOVA University in 2022. Her thesis, focusing on platinum and palladium N-Heterocyclic carbenes based on nucleosides and their anticancer activity, was developed under the supervision of Dr Ana Petronilho and Prof Federico Herrera. After working as postdoctoral fellow at University of Lisbon on ruthenium-peptide conjugates, she moved to CiQUS (Santiago de Compostela), where she is currently working on boron clusters as transport systems with Prof Montenegro as a postdoc associated researcher. Her research interests include organic and organometallic chemistry with medicinal applications

Giulia Orsini graduated from Università degli studi di Milano with a MSc degree in Medicinal Chemistry and Pharmaceutical Technology. Her thesis focused on the synthesis of N-oxyamide linked anionic glycoglycerolipids, guided by Prof. Diego Colombo. Following her graduation, she undertook a traineeship at the University of Vienna, where she worked on the synthesis of heterocyclic molecules as potential antiviral agents, under the supervision of Prof. Thierry Langer. Currently, Giulia is pursuing a PhD at ITQB NOVA, supervised by Dr. Ana Petronilho. Giulia's research is focused on the synthesis of modified nucleosides as metal complexes, aiming for a multimechanistic approach, for potential use as anticancer agents.

Ana Petronilho is the leader of the Bioorganometallic Chemistry Group at ITQB-NOVA. Ana obtained her PhD from the University of Seville in 2010 under the supervision of Prof. Carmona, working in synthetic methodologies for C-H activation of pyridines. She pursued her postdoctoral work in redox catalytic processes and artificial photosynthesis in the group of Prof. M. Albrecht at UCD, Ireland, In June 2015, after a short career break, she started her independent career at ITQB-NOVA, Portugal. Ana and her team work in the development of new methodologies for the synthesis of organometallic nucleosides and their applications in medicinal chemistry and catalysis.



Scheme 1. N- to C- tautomerization of ruthenium caffeine adducts catalysed by acid.

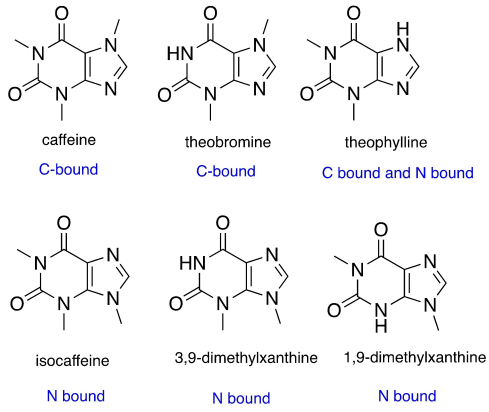


Figure 2. C and N tautomer formation as described by Taube for different xanthines.

The most common routes[17] for the synthesis of NHC complexes derived from xanthines involve transmetalation and deprotonation. Base-assisted deprotonation can be performed using an external base or a "built-in-base", i.e., a base already present at the metal precursor. For external bases, two aspects can be considered: one involves the use of a strong base to generate free carbene from the azolium salt, and the other involves the use of a weak base, which does not lead to the free carbene and usually requires milder conditions.[24]

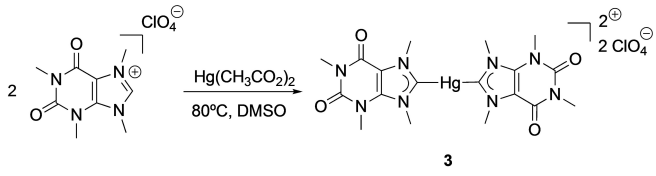
In 1976, Beck reported the synthesis of a mercury biscaffeine complex.[25] The reaction of 1,3,7,9-tetramethylxanthinium perchlorate (methylcaffeine) with Hg(II) acetate affords the C-8 mercurated complex. Acetate acts as an internal base able to deprotonate the caffeinium salt, yielding complex **3** (Scheme 2).

Herrman employed a similar methodology for the synthesis of caffeine complexes based on rhodium(I) and iridium(I)[19] and, subsequently, palladium.[26] Reaction of the metal precursors, containing ethoxide (the internal base) with methyl caffeine leads to the formation of complex **4** (Scheme 3). When four equivalents of methylcaffeine are used, and for prolonged times, biscarbene complexes are formed instead.

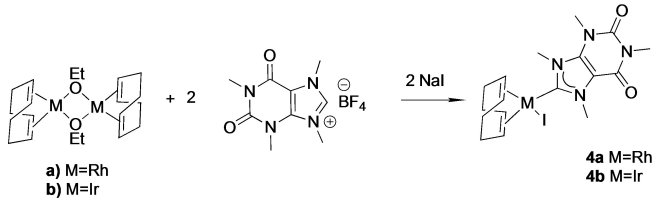
Another key example was described by Casini and co-workers in 2014, employing the free carbene route for the synthesis of a gold compound based on methyl caffeine.[21] The compound was synthesized using KHMDS, a strong base, to generate the free carbene from the caffeinium salt, which subsequently reacted with Au(tht)Cl to yield compound **5** (Scheme 4).

More recently, Tubaro, Visentin and Biffis described the synthesis of mononuclear and dinuclear gold complexes based on caffeine by employing K_2CO_3[27] (Scheme 5). The bisxanthinium ligand was reacted with the gold(I) precursor in the presence of K_2CO_3, a mild base, in acetonitrile. When the mixture is reacted for 3 h, a mononuclear complex is formed with the chelating dicarbene, the kinetic product. When the mixture is reacted for 72 h, a dinuclear gold(I) complex with two bridging dicarbene ligands is formed instead. The mononuclear compound spontaneously transforms into a dinuclear complex, the thermodynamically more stable species.

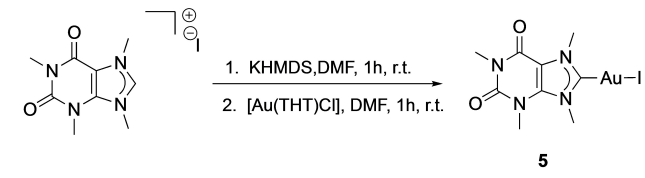
We[28] and the Szostak group[29] reported the synthesis of NHCs based on caffeine by reacting the corresponding



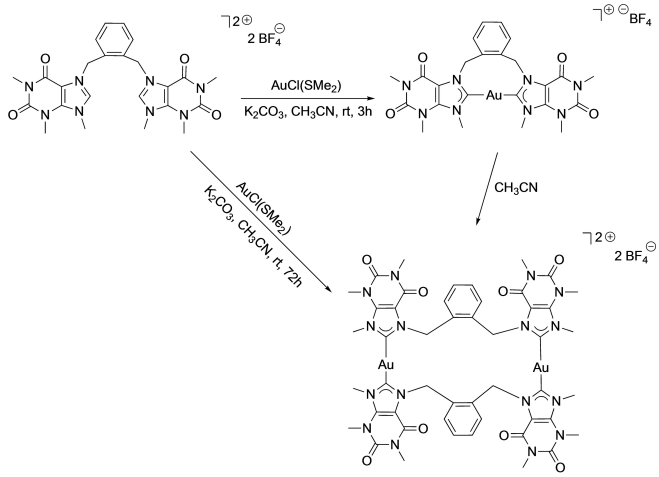
Scheme 2. Synthesis of mercury biscaffeine complex **3**.



Scheme 3. Synthesis of Rh(I) and Ir(I) NHC complexes **4**.



Scheme 4. Synthesis of gold(I) complex **5** using the free carbene route.



Scheme 5. Synthesis of mono- and dinuclear gold(I) complexes **6** and **7**.

caffeinium salts with nickelocene (Scheme 6). The Cp anion acts as an internal base, and cyclopentadiene is released. In the case of methylcaffeine, the formation of a biscarbene was also observed, as had already been reported for analogous imidazolylidene and triazolylidene complexes.[30,31]

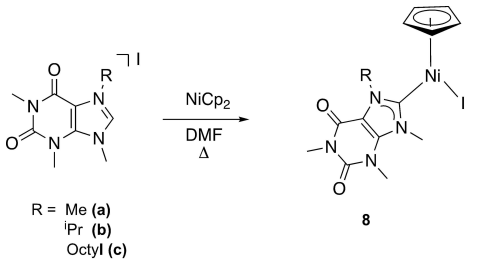
Transmetallation is perhaps the most prevalent synthetic method employed. Young's group reported the synthesis of silver complexes based on caffeine and theobromine.[18,32,33] The corresponding xanthinium salts were reacted with Ag_2O to yield the respective biscarbenes. For example, caffeinium salt was reacted with silver oxide to form the silver biscarbene **9**, which was subsequently transmetallated to rhodium(I), yielding compound **10** (Scheme 7).

A less explored route was described by Hanh's group, who reported the regioselective C8 metallation of purine bases via the oxidative addition of C8-halogenated caffeine to Pt(0).[34] 8-Chlorocaffeine reacts with $[Pt(PPh_3)_4]$ to yield the protic NHC **11**. This methodology was successfully extended to theophylline and theobromine.[35,36] Compound **11** can be obtained directly from 8-chlorocaffeine if a proton source is added to the initial reaction mixture or by performing the oxidative addition first, followed by protonation of the isolated compound (Scheme 8).

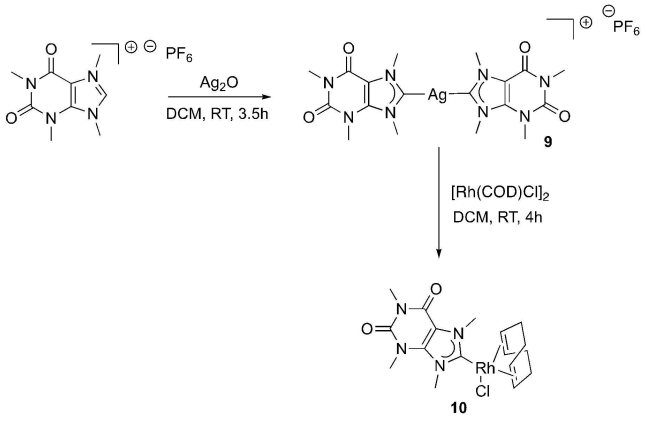
Notably, in the absence of a proton source, the formation of dinuclear species, typically obtained with other azoles, does not occur. This is also a point of contrast in xanthine reactivity compared to that of other azoles. This difference is probably due to the reduced electron density within the five-membered heterocycle due to the electron-withdrawing nature of the annelated ring system.[35]

More recently, our group reported the direct C–H oxidative addition of methyl caffeine to form platinum(II) N-heterocyclic carbenes.[37] This reactivity also contrasts with that previously described by Cavell for imidazole derivatives.[38] Indeed, for imidazolium salts, C–H oxidative addition is also possible, but reductive elimination becomes competitive, limiting the overall yield of the reaction. For the caffeinium salt this is not the case, and the compound can be formed easily and isolated in good yields (Scheme 9).

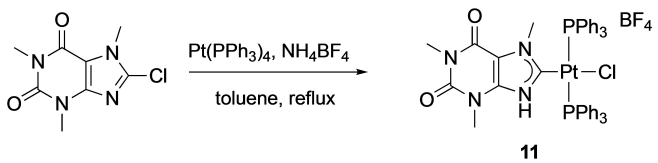
In summary, the different synthetic methodologies described above highlight the methods available for the synthesis of NHCs based on caffeine and other xanthines. While most methodologies are similar to those employed with imidazolium salts, in some cases, the reactivity of xanthine and xanthinium salts shows some differences, namely, on the C–H oxidative addition or the formation of dimeric species following C–X oxidative addition.



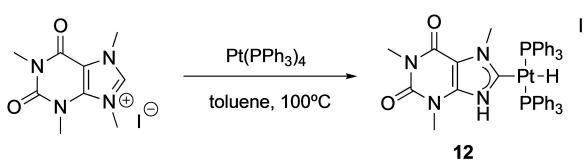
Scheme 6. Synthesis of nickel NHC complexes **8**.



Scheme 7. Synthesis of the Rh(I) NHC complex **10** via transmetallation.



Scheme 8. Regioselective C8-metallation of caffeine



Scheme 9. Synthesis of compound **12** by C–H oxidative addition.

Medicinal Applications

The pharmaceutical utilization of xanthines mentioned earlier makes these compounds privileged candidates for their use as ligands in the development of metallodrugs. While metals provide a wide array of coordination numbers and redox states, the introduction of biologically relevant ligands such as xanthines can provide important additional properties, for example, to achieve higher selectivity. Thus far, relevant results have been described for antimicrobial and anticancer activities.

Antimicrobial Activity

In 2006, Youngs and co-workers reported the antimicrobial activity of methylated caffeine and its silver complex **13** against bacterial and fungal strains[32] (Figure 3). This work focused on developing complexes for the treatment of lung infections that develop in cystic fibrosis patients (CF). Cystic fibrosis leads to an increase in mucus viscosity in the lungs, creating a prone environment for bacterial growth. Compound **13** was tested

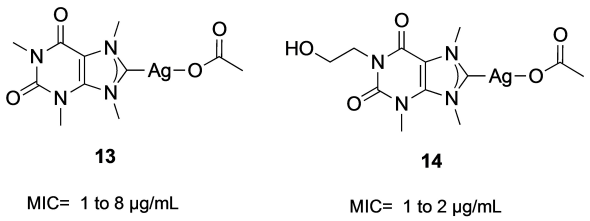


Figure 3. Structures of silver complexes **13** and **14**.

against pathogens relevant to cystic fibrosis-related infections. Specifically, the test organisms included *Escherichia coli* and *Pseudomonas aeruginosa* as representative gram-negative bacteria and *Staphylococcus aureus* as a gram-positive bacterium. *Candida albicans, Aspergillus niger* and *Saccharomyces cerevisiae* were used as representative fungal strains. Compound **13** was found to be highly active against *C. albicans*, *S. cerevisiae* and *A. niger*. Among the bacteria, **13** was very active against an array of bacterial strains, including strains isolated from patients with cystic fibrosis. In vivo studies using rat models showed that **13** has very low toxicity. To examine the role of silver, the pMG101 plasmid, known to confer resistance to silver, was also examined. The MIC value for the *E. coli* J53 strain lacking this plasmid was lower than that observed for the other strains, which confirmed the silver as the main site responsible for the antimicrobial activity.

Compound **14** (Figure 3) was also synthesized[33] and found to be more soluble than **13** by approximately 10-fold due to the presence of the hydroxyethyl group. Compound **14** was subsequently tested and found to be active against several virulent and multidrug-resistant pathogens obtained from patients with cystic fibrosis. As with compound **13**, for **14** the silver is key for antimicrobial activity. In vivo tests in a mouse pneumonia model were performed. *P. aeruginosa*-infected mice were treated with various doses of aerosolized compounds, and both **13** and **14** improved survival rates. Animals treated with **13** and **14** had significantly less dissemination of bacteria than the control animals, demonstrating their potential to treat MDR pulmonary infections associated with cystic fibrosis.

Our group examined the antifungal activity of half-sandwich nickel complexes bearing caffeine-based NHCs **15** and **16** (Figure 4).[28] The nickel complexes **15** were active against *C. albicans* and *C. glabrata*. Monocarbenes **15b** and **15c** were similarly active against both yeasts (39–78 μM *C. glabrata*; 78–156 μM *C. albicans*), while **15a** and **16** were more effective against *C. glabrata* (19–39 μM and 39–78 μM, respectively). All nickel NHC complexes had greater antifungal activity than their ligand precursors, which were generally not active.

Interestingly, biscarbene **16** was strongly active against *C. glabrata*, while for *C. albicans* was only slightly active or not active after 24 and 48 h. Seemingly, the presence of more than one NHC ligand and/or the influence of the overall charge of the nickel cation influences significantly the corresponding antifungal activity.

Anticancer Activity

The utilization of N-heterocyclic carbenes for the development of novel anticancer agents has grown considerably in recent years. In this regard, the use of bioactive compounds such as xanthines as NHC precursors is very appealing owing to their low toxicity and high versatility in terms of steric and electronic properties.

Gold NHCs have shown to be active antiproliferative compounds, being able to inhibit the selenoenzyme thioredoxin reductase (TrxR), which is important for redox homeostasis in cells.[39,40] Casini and co-workers subsequently reported a series of caffeine-based gold compounds with potent anticancer properties[21] (Figure 5). Compound **17** is a theophylline derivative with different substituents at N7, while **5** is a monocarbene gold compound with a methyl caffeine-8-ylidene substituent.

These compounds were evaluated against different human cancer cell lines: A2780 (human ovarian cancer), A2780/R (human ovarian cancer), SKOV3 (human ovarian cancer), and A549 (human lung cancer) and noncancerous cells HEK-293T (human embryonic kidney). All compounds exhibited moderate antiproliferative activity against tested cancerous cell lines, with IC_{50} values in the micromolar range. The compounds showed some degree of selectivity and were not toxic to HEK-293T or A459 cells. Complex **17a** is the most promising compound but is less active than cisplatin in A2780 cells, while it is 2-fold more potent in A2780/R cells (cisplatin-resistant). The interactions of these compounds with quadruplex and duplex DNAs were also screened. Previous studies on the development of G-quadruplex

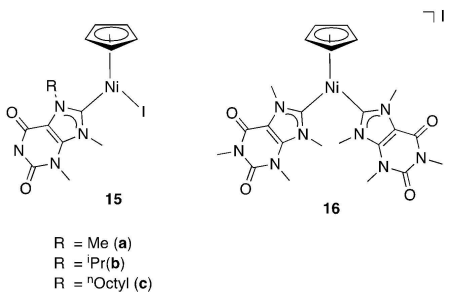


Figure 4. Half-sandwich Nickel caffeine complexes **15** and **16**.

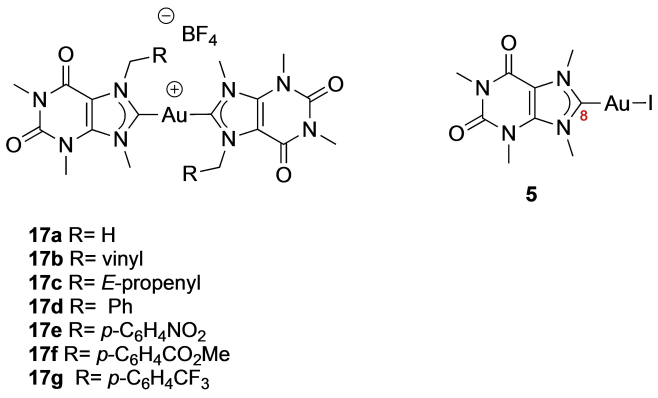


Figure 5. Mono and biscarbene gold(I) complexes **5** and **17**.

ligands revealed that the ideal ligand for G-quadruplex binding should mimic the naturally occurring self-assembly of G-quartets, the origin of quadruplex stability. This work identified complex **17a** as an effective G-quadruplex ligand. Next, complex **17a** was evaluated for interactions with G-quadruplexes by FRET. These results parallel those found for antiproliferative activity. Complex **17a** exhibited high affinity and selectivity for quadruplex DNA. Overall, these findings suggest that the use of caffeine as a guanine mimic is an excellent option for the construction of ligands that can bind G-quadruplex DNA. The compounds were examined ex-vivo in precision cut tissue slices (PCTS) of the liver, kidney, and colon, and the results obtained were in line with those observed in non-tumour cells regarding toxicity. These compounds were also evaluated for their interaction with the zinc-finger protein PARP-1 since these proteins are involved in cancer resistance to chemotherapies. Compound **17a** is also a modest PARP1 inhibitor and can impair the DNA damage response.

The Willans' group reported the anticancer activity of a series of silver(I)-N-heterocyclic carbenes based on caffeine, theophylline and theobromine (Figure 6.).[41,42] The in vitro cytotoxicities of compounds **19** and **14** were tested against A375 (malignant melanoma), HCT116 (colorectal carcinoma), HT29 (colorectal adenocarcinoma), LN229 (glioblastoma), Panc-1 (pancreatic carcinoma), SiHa (grade II, squamous cell carcinoma cervix), U87-MG (glioblastoma) and U251 (glioblastoma) cells.

Complexes **19** and **14** exhibited moderate cytotoxicity against all the cell lines, with IC_{50} values in the micromolar range, between 7 and 55 μM. The most active complex is **19d**, which has an N7-phenyl substituent, with IC50 ranging from 7 to 21 μM. The higher activity of **19d** is attributed to the possible stabilizing effect of the phenyl substituent on the silver–NHC bond, which could lead to a decreased silver release rate and enhanced cytotoxic profile. It is also noted that the presence of a hydroxyethyl group in the backbone of xanthine (compound **14**) improved the activity 2-fold, probably due to its higher solubility, as described previously by Youngs.[33] Hence, the combination of both steric effects of the ligand and the solubility of the silver complex contribute to the overall activity against cancer cells. Following on this, the antiproliferative abilities of silver complexes of tethered N-heterocyclic carbene-carboranyl ligands based on caffeine, namely compounds **20** and **21**, were also examined on colon cancer cell lines HCT116 $p53^{+/+}$ and HCT116 $p53^{-/-}$ (the later lacking the p53 gene). Both silver(I) NHC complexes were active against both cancer cell lines, with an IC_{50} of 17.6 μM (HCT116 $p53^{+/+}$) and 20.2 μM (HCT116 $p53^{-/-}$) for complex **20** and 11.6 μM for **21** (both cell lines). Moreover, the activity of both complexes is independent of the p53 gene, often mutated in cancer cells.

Ott's group confirmed the importance of the NHC connectivity of xanthine ligands, by evaluation a set of platinum(II) terpyridine complexes based on caffeine[43] (Figure 7) Thus, complexes **22** and **23** bearing NHCs based on caffeine and complexes **24** and **25** with a N5-coordinated theobromine as ligand were examined for their antiproliferative activity towards MCF7 and MDA-MB-231 breast cancer and HT29 colon adenocarcinoma cells. Organometallic complexes **22** and **23** showed IC_{50} values ranging between 0.27 and 0.83 μM and exhibit a considerably higher activity than complexes **24** and **25** ($IC_{50} > 100$ μM). Complexes **22**–**25** induced neural differentiation-like morphological changes in MCF7 cells, but complexes **24** and **25** required considerably greater concentrations to achieve these changes. Antiangiogenic behaviour was inferred from the screening of **22**–**25** via the tube formation assay. Inhibition by complexes **22** and **24** was evaluated as a possible mode of action.

Moderate TrxR activity was also confirmed, indicating that this mechanism might be a contributing factor to overall TrxR activity, but the enzyme is not a major target for the compounds. These results also indicate that the greater charge of **22** and **23** and their organometallic nature, provided by the caffeine NHC ligand, might be responsible for the different biological effects.

The same group also described the anticancer properties of compounds **26** and **27** based on rhodium(I) and ruthenium(I) (Figure 8), demonstrating, in this case, the specificity of the role of the metal centre.[44] The compounds were examined against IMR-32 (human neuroblastoma cell line), HepG2 (human

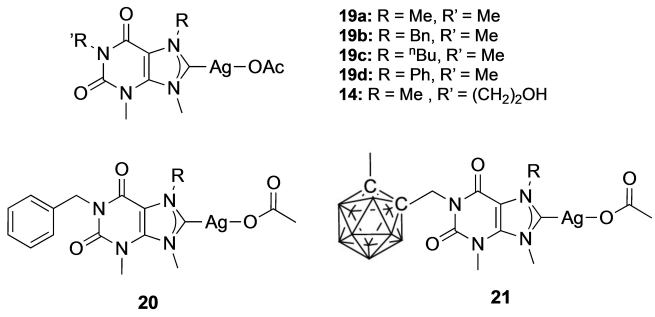


Figure 6. Synthesis of silver NHC complexes **19**–**21**.

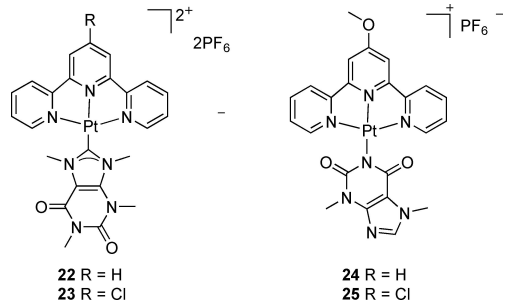


Figure 7. Platinum terpyridine NHC complexes **22**–**25**.

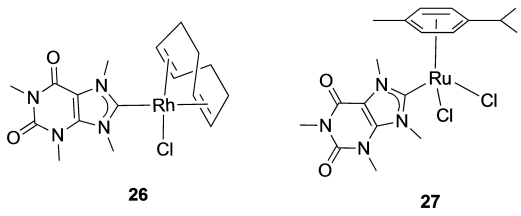


Figure 8. Compounds **26** and **27** bearing a caffeine derived NHC.

hepatoma cell line), MCF-7 and MDA-MB-231 (human breast cancer cell lines), HCT116 (human colon carcinoma cell line), Panc-1 (human pancreatic cell line), LNCaP (human prostate adenocarcinoma cell line) and JoPaca-1 (human pancreatic cell line). Complex **26** exhibited a wide variety of cytotoxic effects (IC_{50} values ranging from 8.8 to 76.7 μM; 96 h) and was particularly active against HCT-116 cells (IC_{50} values 2–8 times lower than those in other cancer cell lines). In this last cell line, DNA damage and cell cycle arrest were also induced in the sub-G1 fraction, eventually leading to apoptosis. In contrast, compound **27** and its ligand precursor did not exhibit any anticancer activity, which indicates that the presence of rhodium is essential for the activity.

Complex **26** can induce significant accumulation of ROS, which is most likely due to inhibition of TrxR. A decrease in the mitochondrial membrane potential was also detected, leading to apoptosis accompanied by a decrease in the levels of pro-caspase 9 and pro-caspase 3 and in the cleavage of PARP.

Morales group reported the antiproliferative activity of theophylline based Ir(I) NHC complexes, including fluorinated thiolate ligands[45] (Figure 9). The complexes were evaluated against six human cancer cell-lines: glia cells of nervous central system (U251), prostate (PC3), leukemia (K562), colon (HCT15), breast (MCF7) and lung (SKLU1).

Complex **28** shows the best performance against PC-3 and SKLU-1 with IC_{50} values of 7.8 ± 0.4 μM and 10.7 ± 0.7 μM, respectively, being more active than cisplatin. The thiolate derivatives **29** were more active than **28**, but less selective.

Our last example concerns the work developed by Visentin[46] and co-workers on a series of palladium NHC complexes derived from caffeine, theophylline and theobromine with different substitution patterns. These comprise monocarbenes bearing different phosphine ligands, and biscarbene complexes featuring an allyl moiety. These complexes were examined for their cytotoxic activity in human ovarian cancer cell lines A2780 (cisplatin-sensitive) and SKOV3 (cisplatin-resistant). All the complexes, except **33a** displayed good antiproliferative effects on cisplatin-sensitive A2780 cells, although only **30c**, **30d** and **31d** displayed better activity than cisplatin. In the cisplatin-resistant cell line SKOV-3, compounds **30b–d**, **31a**, **32b** and **32d** exhibited greater activity than cisplatin. Compound **30d** was the most active compound, with an IC_{50} value 3.5 times lower than that of cisplatin (Figure 10).

Importantly, representative compounds of the different subclasses were almost inactive against fibroblasts. For example, compound **30d** is the most active compound in the A2780 and SKOV-3 cancer cell lines but has an $IC_{50} > 100$ μM in fibroblasts. Complexes were also evaluated for their apoptotic effects. The complexes that showed the highest pro-apoptotic activity for the A2780 cells, were **30c--d**, **33b--d**, **34b--d**, with **30c** showing the greatest total pro-apoptotic activity. On the cisplatin-resistant SKOV-3 cell line, derivatives **30b--d**, **31d**, **32**, **33** and **34** showed to be particularly active, especially **30c**. This work was later expanded to other palladium complexes,[47,48] and in general complexes bearing NHC derivatives show good antiproliferative activity. The presence of phosphines such as PTA leads to highly soluble compounds and it was also noted that less hindered compounds are more effective cytotoxic agents.

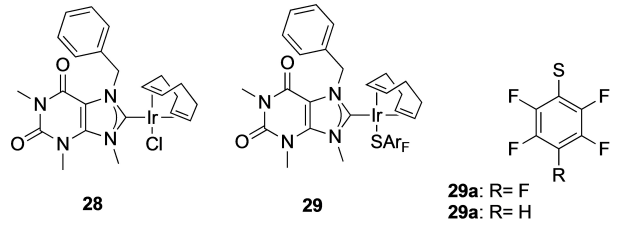


Figure 9. Compounds **26** and **27** bearing a caffeine derived NHC.

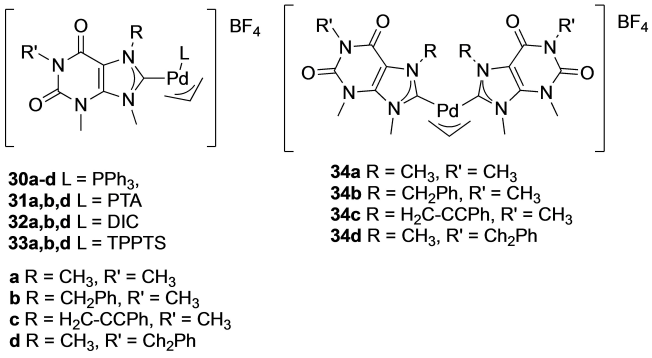


Figure 10. Palladium complexes **30**–**34**.

Summary and Outlook

The synthetic approaches described above demonstrate that the development of NHCs derived from xanthines is diverse and easily accessible. While many of these methodologies parallel those used for imidazole-based NHCs, subtle distinctions arise in the reactivity of xanthines, particularly in processes such as direct C–H oxidative addition or the formation of dimeric species subsequent to C–X oxidative addition. Moreover, the medicinal properties of xanthine-based NHC complexes have led to important advances, specifically through their ability to act as antimicrobial agents with a high degree of selectivity for different fungal strains and effectiveness in both gram-positive and gram-negative bacterial strains. The anticancer effects of these compounds are also highly significant, namely their ability to interact with G-quadruplexes, for which the purine core is key. As mentioned throughout this manuscript, the versatility of xanthines for further functionalization, their low toxicity and pharmacological properties, combined with the potential of NHC complexes for medicinal applications, suggests that the development of these compounds is an emerging field for bioorganometallic medicinal applications.

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Conflict of Interests

The authors declare no conflict of interest.

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