N-Heterocyclic Carbenes Derived from Guanosine: Synthesis and Evidences of Their Antiproliferative Activity

Maria Inês P. S. Leitão, Federico Herrera,* and Ana Petronilho*†

Instituto de Tecnologia Química e Biológica António Xavier, Av. da Republica, 2780-157 Oeiras, Portugal

ABSTRACT: Palladium(II) and platinum(II) complexes bearing N-heterocyclic carbenes derived from guanosine are synthesized via oxidative addition, followed by protonation in the presence of acid. Cytotoxicity of the compounds is evaluated in several cell lines. Compounds 2a, 2b, and 3a are selective for glioblastoma U251 cells and are nontoxic toward healthy human embryonic kidney (HEK293) cells.

INTRODUCTION

N-heterocyclic carbenes (NHCs) are widely used as ligands for transition metals, with extensive applications in catalysis and medicinal chemistry. Nucleobases and their alkylated adducts are NHC precursors, and their ability to form ylides has enabled their use as biomarkers in certain types of cancer. Notably, only a few examples of NHCs stabilized by transition metals derived from nucleobases have been described. For purines, NHC formation employs oxidative addition of the corresponding halogenated nucleobases or cyclometalation supported by a chelating unit tether. However, these procedures are restricted to unnatural adducts of adenine and to caffeine and cannot be easily transferred to metallated nucleosides. The difficulty of doing so relies mostly on finding a suitable protection/deprotection methodology. While this is a common practice in nucleoside chemistry, it cannot be easily transferred to metallated nucleosides. This is due to the harsh conditions employed for deprotection, which compromise the integrity of the compounds in two fundamental ways: the stability of the M–C bond and of the glycosidic bond.

The use of nucleobase derivatives as therapeutic agents suggests that the combination with a metal complex may provide a wide array of pharmaceutical applications, particularly when bound to the metal center as NHC. Metal–NHC systems have been employed as anticancer agents, and one of the main challenges at present is to develop compounds capable of targeting cancerous cells while being nontoxic to healthy ones. For instance, cisplatin is used in more than 50% treatments of cancer patients but it presents major drawbacks, such as poor selectivity, intrinsic or acquired resistance, and severe side effects. A suitable approach to overcome this problem is to increase selectivity by utilizing ligands derived from biomolecules that are able to induce selectivity by molecular recognition. As such, complexes that contain tailor-made ligands forming metal bioconjugates could facilitate targeting cancerous cells and achieve a higher selectivity. For purines, coordination to a metal as NHC via C-8 (Scheme 1) provides a connectivity that enables base-pairing interactions, as all sites involved in Watson–Crick base pairing remain intact. This feature constitutes an excellent tool for the development of metal complexes capable of performing targeted molecular recognition.

RESULTS AND DISCUSSION

The synthesis employs oxidative addition of brominated nucleosides to platinum(0) and palladium(0). Accordingly, the reaction of 2′,3′,5′-triacetyl-8-bromoguanosine with M(PPh3)4 in refluxing toluene (M = Pd, Pt) affords the corresponding guanosine complexes 1a and 1b in good yields (Scheme 1). Protection of the hydroxyl groups is required, as the reaction with unprotected 8-bromo-guanosine leads to a mixture of compounds. Complexes 1a and 1b can be protonated under acidic conditions at room temperature,

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affording the corresponding protic NHCS 2a and 2b. For 2a, protonation is induced in methanolic solutions of 1a with aqueous HBF₄. Earlier attempts to synthesize the corresponding platinum compound from 1b using HBF₄ were unsuccessful. Thus, compound 2b was synthesized using ethanolic solutions of HCl. The synthesis of the BF₄⁻ derivative can also be achieved using ethanoic solutions of aqueous HBF₄ but in moderate yields. Under acidic conditions similar to those of deprotection but for longer reaction times, compounds 1a and 1b undergo deprotection of the hydroxyl groups of the ribose. This deprotection methodology induces concomitant protonation of the nitrogen atom N7 of the guanosine ligand, a process that does not affect the integrity of the M–C bond. Complexes 1–3 are stable under air and moisture for prolonged periods of time. When a solution of 1b in CDCl₃ was kept under air for several weeks, no decomposition was observed by ¹H NMR spectroscopy. Complexes 1–3 were characterized by NMR spectroscopy in dimethyl sulfoxide-d₆ (DMSO-d₆). As a general trend, in the ¹H spectra, the H1 resonates around 3.7 ppm, irrespectively of the metal atom, and the N7 undergoes a slight upfield shift of ca. 1 ppm upon protonation. In the ¹³C{¹H} NMR spectra, the ribose ring gives rise to five resonances between 60 and 90 ppm for all compounds. For complexes 1, the metalled C-8 is observed at 166.9 ppm (1a) and 139.9 ppm (1b). Upon protonation, the ylidene derivatives 2 and 3 show some differences in the ¹³C{¹H} NMR spectra, specifically a downfield shift of C-8, (cf. 6 166.9 ppm (2a); δ = 153.0 ppm (2b)). The ³¹P{¹H} NMR spectra present two singlets at around 21–22.00 ppm for palladium complexes and 17–19.00 ppm for platinum complexes. For compounds 1b, 2b, and 3, the ³¹P{¹H} spectra show the presence of a minor compound (presumably the enol-tautomer) that we were unable to identify. In ¹H NMR, the signals are mostly overlapped (e.g., 2b) or not detected (1b), whereas for ¹³C{¹H}, the minor product is not observed. For 1b, the ratio between the minor and the major compound varies when changing from CDCl₃ to DMSO-d₆ further supporting the presence of a tautomeric form of the ligand. Of note, all compounds were characterized by microanalysis to further confirm their purity. Guanine derivatives can aggregate via base-pairing. As an example, aggregation of compound 1a was monitored by ¹H NMR, evidencing that the NH and the NH₂ groups undergo a slight high-field shift (Supporting Information, SI) upon dilution. Aggregation measurements for these complexes are currently being carried out via NMR and will be reported in due course.

Crystals of 2a were obtained by slow evaporation of a saturated chloroform solution, allowing their characterization by single-crystal X-ray analysis (Figure 1). The crystal structure indicates a trans-orientation for the two phosphines. The Pd–C-8 bond length is 1.981(8) Å, which correlates well with related N-heterocyclic carbene compounds of palladium. The sugar adopts a puckered conformation, with the C2 turned out of the plane formed by C1′–O4−C4′ by 109.8(5)°, following the trend found for purine nucleosides with bulky substituents at the C-8 (C2′ endo). The torsion angle around the glycosidic bond (χ, defined by O4′−C1′−N9−C4) is 77.5(9)°, corresponding to a synclinal orientation. It reflects a distortion of the ring to accommodate the acetate groups and minimize steric crowding imposed by the phosphines.

The cytotoxicity of the compounds, including the ligand precursor I, was tested at concentrations ranging between 0.1 and 80 µM in human embryonic kidney (HEK293) cells, prostate cancer (PC3) cells, cervical cancer (HeLa) cells, and glioblastoma (U251) cells for 48 h. Cytotoxicity was evaluated by the ability of cells to metabolize 3-(4,5-dimethylthiazol-2-
yl)-2,5-diphenyltetrazolium bromide (MTT), as described elsewhere. Accordingly, healthy human embryonic kidney cells, HEK293, were not sensitive to any of the compounds (cf. SI). For cancerous cell lines PC3 and HeLa, compounds 1−3 showed no significant antiproliferative activity. By contrast, glioblastoma U251 cells revealed sensitivity to compounds 2a, 2b, and 3a (Figure 2). Palladium NHC compounds 2a and 3a show a higher antiproliferative activity than their corresponding platinum derivative 2b.

These compounds induce a significant antiproliferative activity when compared with cisplatin for glioblastoma U251. The antiproliferative activity of compound 1a presents a large variability between experiments and thus is not found statistically significant. This is probably a result of its low solubility, as 1a precipitates at higher concentrations upon addition to liquid cell culture medium. In contrast, the measurements for 2a and 3a are consistent between experiments. For example, the antiproliferative activity of compound 3a reaches ca. 50% for a range of concentrations between 20 and 80 μM. For platinum complexes, the higher cytotoxic activity is observed for compound 2b, the acetate-protected platinum NHC. Compound 2b induces a significant decrease in cancer cell growth at a concentration as low as 0.1 μM, but in general, the maximum percentage of decrease was less significant than that of compounds 2a and 3a. The ligand precursor I, the brominated and protected guanosine, does not show antiproliferative activity.

**CONCLUSIONS**

In summary, we have disclosed a new methodology for the synthesis of N-heterocyclic carbenes derived from guanosine with palladium(II) and platinum(II) precursors. For the reaction to be effective, the guanosine derivative requires protection of its hydroxyl groups with acetate. This constitutes the first example of a purine NHC derived from a nucleoside. The deprotected NHC can subsequently be obtained under acidic conditions. Antiproliferative measurements with a number of cell lines show that the compounds are nontoxic for healthy cells HEK293. Compounds 2a, 2b, and 3a are active cytotoxic agents for glioblastoma cell line U251 and show a significant antiproliferative activity when compared with cisplatin. These are promising results, as glioblastoma multiform is a very aggressive form of brain cancer in adults. Studies on other glioblastoma cell lines and examination of the mechanism of action of these compounds are currently being pursued.

**ASSOCIATED CONTENT**

- Supporting Information
  The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b02387.
  - Crystallographic data (CIF)
  - Experimental procedures, NMR and mass spectra, and cytotoxic studies (PDF)

**AUTHOR INFORMATION**

*Corresponding Authors

- E-mail: Ana.petronilho@itqb.unl.pt (A.P.).
- E-mail: fherrera@itqb.unl.pt (F.H.).

**ORCID**

Ana Petronilho: 0000-0002-3296-1522

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Notes

The authors declare no competing financial interest.

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DEDICATION

Dedicated to Professor Ernesto Carmona on the occasion of his 70th birthday.

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