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# Synthesis and characterization of a new alkyne functionalized bis(pyrazolyl)methane ligand and of its Pd(II) complexes: Evaluation of their *in vitro* cytotoxic activity

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ABSTRACT

A novel bis(pyrazolyl)methane ligand bearing alkyne substituents (—C≡C—$^t$Bu) in the C-4 position of the pyrazole rings has been synthesized together with its Pd(II) dichloride and diacetate complexes. Both the Pd(II) complexes are monomeric species stable in the solid state and in organic solution. The crystal structure of the Pd(II) dichloride complex displays a slightly distorted square-planar geometry and the molecules tend to form dimers associated through π–π stacking interactions between the pyrazolyl rings. The *in vitro* antitumor activities of the free ligand and of its corresponding Pd(II) complexes toward some human cancer cell lines, such as HeLa, SHSY-5Y and K562, have shown to be lower than *cisplatin* but with a better response in comparison to their parent free ligand.



## 1. Introduction

Among the wide range of bidentate chelating ligands currently used in metal coordination chemistry, bis(pyrazolyl)alkanes are one of the most fascinating family of stable and flexible ligands: they are isoelectronic and isosteric with the well-known bis(pyrazolyl)borates, and were first synthesized by Trofimenko in the seventies of the last century [1]. The coordinating properties of bis(pyrazol-1-yl)alkanes can be varied over wide range by introduction of various substituents into the pyrazole rings which are able to modify steric and electronic properties [2]. Their metal coordination chemistry with main group and transition metals has been mainly developed in the last twenty years [2] affording a number of transition metal systems with novel interesting properties in the fields of catalysis [3–8] and as prodrugs [9,10].

Bis(pyrazol)methanes have been widely used in palladium chemistry [11,12]. The first palladium complex was obtained by Trofimenko from reaction of $R_2C(pz)_2$ with $[(\pi\text{-allyl})PdX]_2$ (X = halide) dimer which yields cationic $[Pd\{R_2C(pz)_2\}(\pi\text{-allyl})]^+$ species [1]. Neutral $[Pd\{H_2C(pz)_2\}Cl_2]$, $[Pd\{H_2C(3,5\text{-}Me_2pz)_2\}Cl_2]$ and $[Pd\{Me_2C(pz)_2\}Cl_2]$ and cationic species $[Pd\{H_2C(pz)_2\}_2]^{2+}$, $[Pd\{H_2C(3,5\text{-}Me_2pz)_2\}_2]^{2+}$ and $[Pd\{Me_2C(pz)_2\}_2]^{2+}$ were reported by Minghetti et al. [13]. Coordination of $R_2C(pz^x)_2$ to Pd occurs to form a six-membered metallacycle that always adopts a boat conformation: however, when H or Me substituents are present in the bridging spacer between the pyrazolyl rings, boat-to-boat interconversion has been observed to occur. When more sterically hindered substituents are on the bridging carbon, boat-to-boat interconversion is absent and with methyl groups in the 3- and/or 5-position of the pyrazolyl rings, an enhancement of the rigidity of the boat conformation was observed [14]. Neutral and cationic Pd(II) Fréchet-type dendrimers $[PdClX\{RCH(3,5\text{-}Me_2pz)_2\}]$ (with X = Cl or Me) and $[PdMe(MeCN)\{RCH(3,5\text{-}Me_2pz)_2\}]\text{-}[BAr^f_4]$, (R = benzyl or benzyloxycarbonyl group or a poly(aryl-ether) Fréchet-type dendron; $Ar^f$ = 3,5-$(CF_3)_2C_6H_3$)), were recently reported and investigated as catalysts in the Heck reaction between *para*-iodotoluene and methyl acrylate [15,16]. Dinuclear Pd(II) dichloride and diacetate complexes were obtained with novel ditopic ligands containing two bis(pyrazolyl) chelating units

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connected by aromatic polycyclic spacers [17]. Interaction between $Pd(OAc)_2$ and several bis(arylpyrazolyl)methanes such as $H_2C(3\text{-}RC_6H_4pz)_2$ and $H_2C(3\text{-}RC_6H_4pz)(5\text{-}RC_6H_4pz)$ (where R = H, OMe or Br) afforded dinuclear palladacycles such as $H_2C(3\text{-}RC_6H_4pz)_2Pd_2$ and $[H_2C(3\text{-}RC_6H_4pz)(5\text{-}RC_6H_4pz)]Pd_2$, while dimeric species $[Pd_2(acac)_2\{H_2C(3\text{-}RC_6H_4pz)_2\}]$ and $[Pd_2(acac)_2H_2C(3\text{-}RC_6H_4pz)(5\text{-}RC_6H_4pz)\}]$ formed when the acetate-bridged palladacycles reacted with Tl(acac) [18].

Beyond its versatility as ligand for several metal transition based systems, the pyrazole nucleus has been widely used as core motifs for a large number of compounds with various applications such as agro-chemicals, building blocks of other key intermediates, and in medicine. In medicine, pyrazole has found application, among other, as a pharmacophore in some of the active biological molecules [19]. Thus, while pyrazole derivatives have been largely studied for many applications including anticancer, antimicrobial, anti-inflammatory, antiglycemic, anti-allergy and antiviral, much less has been reported on their corresponding metal complexes, in spite of the known ability of metals to modify the properties and activity of ligands. Few complexes with pyrazole ligands showing an antitumor activity similar to that of *cisplatin* have been reported so far, but the effect produced by the substituents on the heterocycle has not been well clarified [20]. Moreover, related to the metal, it could be noted that the solubility of palladium complexes is generally better compared to platinum-based complexes [21]. This could be also an important and attractive possibility for the treatment of cancer.

Having in mind to clarify this point and to give our contribute to elucidate the influence of mode of binding of this family of ligands in the biological activity of the corresponding palladium complexes [22], here we report the preparation and full characterization of a novel bis(pyrazolyl)methane ligand bearing alkyne substituents in the 4-position of the pyrazole ring, named bis [4-(3,3-dimethylbut-1-ynyl)-1H-pyrazol-1-yl]methane ($\mathbf{L_2}$), starting from the parent bis[4-iodo-1H-pyrazol-1-yl]methane ($\mathbf{L_1}$) (Scheme 1).

To the best of our knowledge, no attempts were previously made to functionalize the pyrazole rings of such type of ligands with alkynyl groups, only a heteroscorpionate (2-propargyloxyphenyl) bis(pyrazolyl)methane being reported by Mohr and co-workers,where the alkynyl functionality was however introduced in the 2-hydroxyphenyl substituent on the bridging methylene of the ligand [23]. Then, we have studied the coordination chemistry of ligand $L_2$ toward $PdCl_2$ and $Pd(OAc)_2$ acceptors. Finally, the ligand $L_2$ and both its Pd(II) complexes 1 and 2 have been studied to verify their antitumor activity, *in vitro*, toward three well-known human cancer cell lines such as HeLa, SHSY-5Y and K562.

## 2. Materials and methods

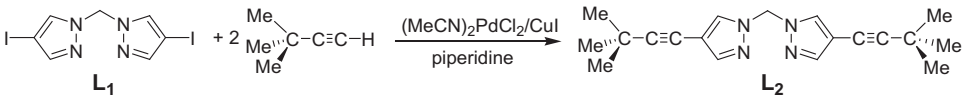
All reagents were purchased (Aldrich) and used without further purification. All solvents were purified by conventional methods and stored under nitrogen. All reactions and manipulations for the syntheses of ligands $\mathbf{L_1}$ and $\mathbf{L_2}$ were carried out under an atmosphere of argon, while their interactions with metal salts were carried out in the air. The samples for microanalyses were dried *in vacuo* to constant weight (20 °C, ca. 0.1 Torr). Dulbecco's modified Eagle's medium (DMEM), RPMI 1640 medium and foetal bovine serum were from Euroclone. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], were purchased from Aldrich. Cisplatin were purchased from Alexis Biochemicals. Stock solutions of palladium complexes **1**, **2** and ligand $\mathbf{L_2}$ were prepared in DMSO and stored in the dark at −20 °C. The HeLa (cervix adenocarcinoma), SHSY-5Y (neuroblastoma) and K562 (chronic myeloid leukemia) human cell lines were obtained from the American Type Culture Collection. Elemental analyses (C, H, N) were performed in-house with a Fisons Instruments 1108 CHNS-O Elemental Analyser. IR spectra were recorded from 4000 to 400 $cm^{-1}$ with a Perkin-Elmer Spectrum 100 FT-IR instrument. $^1H$ and $^{13}C\{^1H\}$ NMR spectra were recorded on a 400 Mercury Plus Varian instrument operating at room temperature (400 MHz for $^1H$ and 100 MHz for $^{13}C$). H and C chemical shifts ($\delta$) are reported in parts per million (ppm) from $SiMe_4$ ($^1H$ and $^{13}C$ calibration by internal deuterium solvent lock). Positive ESI-Mass Spectra were obtained with a Series 1100 MSI detector HP spectrometer, using MeOH as mobile phase. Solutions for electrospray ionization mass spectrometry (ESI–MS) were prepared using reagent grade methanol and obtained data (masses and intensities) were compared to those calculated by using the IsoPro isotopic abundance simulator version 2.1 [24]. Peaks containing palladium(II) ions were identified as the highest peak of an isotopic cluster. TGA-DTA spectra were obtained with a STA 6000 Simultaneous Thermal Analyzer Perkin-Elmer.

### 2.1. Bis(4-iodo-pyrazol-1-yl)methane ($\mathbf{L_1}$)

To a suspension of bis(pyrazol-1-yl) methane (0.4450 g, 3.0 mmol, previously prepared according to Claramunt's procedure [25], of $I_2$ in powder form (0.6100 g, 2.4 mmol) and of $HIO_3$ (0.2100 g, 1.2 mmol) in 30 ml of acetic acid, $H_2SO_4$ (0.375 mL, 30% pp) was added. The mixture was stirred for half an hour at 70 °C, until the purple colour of iodine disappeared. The mixture was then poured into 50 ml of $H_2O$ affording a colourless precipitate, which was filtered off, washed with $H_2O$ and identified as $\mathbf{L_1}$. Yield: 91%. It is soluble in acetone, acetonitrile, DMSO, DMF and chloroform. *Anal.* Calc. for $C_7H_6I_2N_4$: C, 21.1; H, 1.51; N, 14.01. Found: C, 21.22; H, 1.58; N, 13.75%. M.p. 158–162 °C. IR ($cm^{-1}$): 3118 m, 3006w ν(Carom-H), 2961w ν(Caliph-H), 1538w, 1511 m 1475w, 1448 m, 1407 s, ν(C=N, C=C), 1696w, 1718w, 1672w, 1637w, 1612w, 1391 m, 1358 s, 1228w, 1022 m, 914w. $^1H$ NMR ($CD_3CN$, 293 K) $\delta$ (ppm): 6.23 s (2H, —$CH_2$—), 7.55 s (2H, $H_5$), 7.68 s (2H, $H_3$). $^{13}C$ NMR ($CD_3CN$, 293 K) $\delta$ (ppm): 63.5 (*C*4 of pz), 67.4 (—$CH_2$—), 135.8 (*C*5 of pz), 153.1 (*C*3 of pz). TGA (mg% vs °C): heating from 30 to 300 °C with a speed of 7 °C/min, stable up to 100 °C, melts around 163 °C, then decomposes leaving a black residue corresponding to a 5% of the original weight.

### 2.2. Bis[4-(3,3-dimethylbut-1-ynyl)-pyrazol-1-yl]methane ($\mathbf{L_2}$)

Glass equipment was previously dried through three alternative cycles based on argon fluxing and high vacuum aspiration. Then, the following procedure was followed: bis(4-iodopyrazol-1-yl) methane (0.500 g, 1.25 mmol), CuI (0.0119 g, 0.0625 mmol), and $[(MeCN)_2PdCl_2]$ (0.0048 g, 0.0185 mmol) were dissolved in 5 mL of piperidine. Then 3,3-dimethylbut-1-yne (0.246 g, 3.00 mmol) was added. The reaction mixture was stirred at 100 °C for 12 h




**Scheme 1.** Synthesis of ligand $\mathbf{L_2}$ from ligand $\mathbf{L_1}$.