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Palladium–benzodiazepine derivatives as promising metallodrugs for the development of antiepileptic therapies

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ABSTRACT

We synthesized two organometallic diazepam–palladium(II) derivatives by C–H activation of diazepam (DZP) with palladium salts, i.e., $PdCl_2$ and $Pd(OAc)_2$ (OAc = acetate). Both compounds obtained are air stable and were isolated in good yields. The anticonvulsant potential of the complexes, labeled **[(DZP)PdCl]$_2$** and **[(DZP)PdOAc]$_2$**, was evaluated through two animal models: pentylenetetrazole (PTZ)- and picrotoxin (PTX)-induced convulsions. The organometallic DZP–palladium(II) acetate complex, **[(DZP)PdOAc]$_2$**, significantly increased ($p < 0.01$ or $p < 0.001$) latencies and protected the animals against convulsions induced by PTZ and PTX, while the analogous chloro derivative, **[(DZP)PdCl]$_2$**, was effective ($p < 0.01$) only in the PTZ model. These effects appear to be mediated through the GABAergic system. The possible mechanism of action of the DZP–palladium(II) complexes was also confirmed with the use of flumazenil (FLU), a $GABA_A$-benzodiazepine receptor complex site antagonist. Herein, we present the first report of the anticonvulsant properties of organometallic DZP–palladium(II) complexes as well as evidence that these compounds may play an important role in the study of new drugs to treat patients with epilepsy.

1. Introduction

Epilepsy is one of the most common serious neurological conditions worldwide, with an age-adjusted incidence of approximately 50 per 100,000 persons per year in developed countries [1]. Antiepileptic therapy can result in long-term remission in 60–70% of patients, but many patients require combination treatment to achieve optimal convulsion control, as monotherapy is ineffective at controlling seizures in 30–53% of patients. Despite the increase in available treatment options, patient outcomes have not improved significantly; thus, more effective therapies must be developed [2]. There is a clear need to continue to identify novel antiepileptic drugs (AEDs) that effectively control pharmacoresistant convulsions with minimal or no adverse events [3,4].

Shortly after their discovery in the late 1950s, benzodiazepines (BZDs) were implemented for use as anticonvulsants [5]. BZDs offered substantial advantages over previous medications [6], as noted by early clinicians, including high efficacy, rapid onset of action, and low toxicity [7]. Therefore, numerous advances in epilepsy treatment have been achieved with the use of BZDs. However, medicinal chemists have pursued the synthesis or semi-synthesis of new BZDs to improve their therapeutic activity and effectiveness and to reduce adverse events; for example, BZD consumption by the elderly can increase the

Abbreviations: AEDs, antiepileptic drugs; BSA, bovine serum albumin; DMSO, dimethyl sulfoxide; DZP, diazepam; **[(DZP)PdCl]$_2$**, *bis*[2-{(7-chloro-1-methyl-2-oxo-2,3-dihydro-1*H*-benzodiazepin-5-il)phenyl-κ^2-C,N}-μ_2-chloropalladium(II)]; **[(DZP)PdOAc]$_2$**, *bis*[2-{(7-chloro-1-methyl-2-oxo-2,3-dihydro-1*H*-benzodiazepin-5-il)phenyl-κ^2-C,N}-μ_2-acetatopalladium(II)]; FLU, flumazenil; GABA, γ-aminobutyric acid; i.p., intraperitoneal; *J*, coupling constant; MALDI-TOF-MS, Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectroscopy; OAc, acetate; PBS, phosphate-buffered saline; PTX, picrotoxin; PTZ, pentylenetetrazole; Py, pyridine; δ, chemical shift.

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risk of side effects or the use of potentially inappropriate medications, and BZDs are ineffective for certain types of epilepsy [4].

An important strategy in the development of new drugs involves the design and synthesis of bioactive species that contain metal elements in the molecular structure, i.e., metallodrugs [8,9]. A classic example of this type of compound is cisplatin and its derivatives, which are widely employed as anticancer agents [10–12]. One approach to the design and development of new efficient and potent metallodrugs is based on the association of well-known active organic drugs as ligands with metal elements, forming metal or organometallic complexes [9,12–16]. Utilizing this approach, in the present study, we pursued the synthesis of two BZD–palladium(II) organometallic complexes, *bis*[2-{(7-chloro-1-methyl-2-oxo-2,3-dihydro-1*H*-benzodiazepin-5-il)phenyl-κ^2-C,N}-μ_2-acetatopalladium(II)], **[(DZP)PdOAc]$_2$**, and *bis*[2-{(7-chloro-1-methyl-2-oxo-2,3-dihydro-1*H*-benzodiazepin-5-il)phenyl-κ^2-C,N}-μ_2-chloropalladium(II)], **[(DZP)PdCl]$_2$**, and evaluated them as potential AEDs.

Previous studies have reported the formation of metallacycle derivatives from BZDs with Pd(II) [16–19], Pt(II) [20], and Ru(II) [21]. All of these metallacycles were obtained via C–H activation reaction [22] and intramolecular coordination of the imine nitrogen. Moreover, Cinellu and coworkers [17] have already reported the synthesis of the **[(DZP)PdCl]$_2$** complex described in this work. However, to the best of our knowledge, there are no pharmacological studies related to the use of those BZD-metallacycle derivatives as potential AEDs.

2. Results and discussion

2.1. Chemistry

The synthesis of the two palladacycles was quite simple and easy to accomplish. Two air stable solids were isolated in good yields (ca. 80%). Both DZP–palladium derivatives were obtained after cyclopalladation of DZP, via coordination of the imine nitrogen group and C–H activation of the phenyl substituent of the benzodiazepine moiety at the *ortho* position, using either $PdCl_2$ or $Pd(OAc)_2$ salts as palladium sources (Fig. 1). Structurally, both compounds were isolated as dimers with planar and open-book shapes for **[(DZP)PdCl]$_2$** and **[(DZP)PdOAc]$_2$**, respectively [23,24]. In solution, the two dimer complexes were present as a mixture of two possible isomers, the *cisoid* and *transoid* forms (Fig. 2), clearly detected by NMR studies [23,25].

^{1}H NMR spectra of **[(DZP)PdCl]$_2$** (in DMSO-d_6) and **[(DZP)PdOAc]$_2$** (in $CDCl_3$), both at room temperature, indicated the anticipated presence of two isomers (Fig. 2), the *transoid* and *cisoid*, in 4:1 and 1:1 ratios, respectively. Notably, for the **[(DZP)PdCl]$_2$** complex, the *transoid* and *cisoid* isomers each clearly display one pair of diastereotopic signals attributable to the methylene hydrogens of the benzodiazepine ring, as well as one singlet related to the hydrogens of the NCH_3 moiety. For the **[(DZP)PdOAc]$_2$** complex, similar patterns were observed for the analogous group of hydrogens in the *transoid* isomer. However, for the *cisoid* counterpart of **[(DZP)PdOAc]$_2$**, two pairs of diastereotopic signals for the methylene hydrogens and two singlets for the methyl-N hydrogens were observed. Moreover, we verified that the hydrogens of the CH_3COO-bridges for the *transoid* isomer gave rise to one singlet, whereas two singlets for these hydrogens were observed for the *cisoid* counterpart. This relative enhancement of the complexity of the *cisoid*-**[(DZP)PdOAc]$_2$** isomer is related to the geometry of the acetate-bridged dimer, commonly referred to as an open-book arrangement. In this case, the steric hindrance between the two planes formed by the square planar geometry of the palladium moieties provokes a twist of the two mirror images of the dimeric complex. This lowers the symmetry of the molecule; thus, most of the equivalent hydrogens of the two moieties become magnetically different.

However, the complexity of the NMR data of the dimeric DZP–palladium(II) derivatives was drastically reduced with the formation of the monomeric counterparts in solution via simple addition, in situ, of Py-d_5 to a solution of the respective dimers in the NMR tube (see Fig. 3 and experimental section). Only one isomer was observed for both the acetate and the chloro palladium derivatives. In fact, we observed and labeled all hydrogens of the DZP fragment (except the hydrogen replaced after *ortho*-metalation reaction), and in the case of the palladium acetate derivative, only one signal at 2.28 ppm was observed for the CH_3COO ligand.

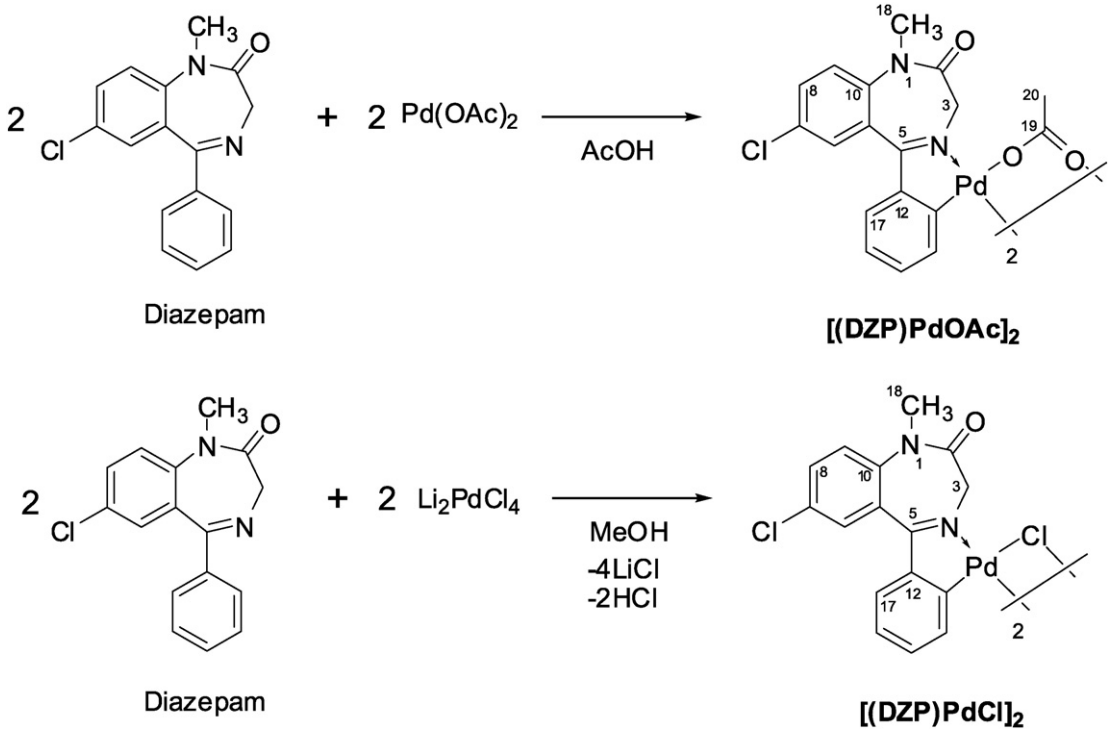

Fig. 1. Cyclopalladation reactions of diazepam (DZP) in the presence of different sources of Pd(II), forming **[(DZP)PdCl]$_2$** and **[(DZP)PdOAc]$_2$**.