Synthesis of Novel Palladacycles Inhibitors of the Cathepsin B Activity and Antitumoral Agents

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Abstract

The reaction of 2-bromo-3,4,5-trimethoxybenzaldehyde 1 with Pd(dba)2 (dba=dibenzylideneacetoacetate) in the presence of a stoichiometric amount of nitrogen donor ligands, such as N,N,N',N'-tetramethyl-ethane-1,2-diamine (TMEDA), 2,2'-bipyridine (bpy), 4,4'-dimethyl-2,2'-bipyridine (dmbpy) and an 1,10-phenantrone (Phen), should be added to with equimolar ratio in degassed acetonitrile under nitrogen to give mononuclear α-aryl palladium (II) complexes cis-[2-Pd(C,HCHO)-6-(OMe)_3,4,5]BrL_1 3a-d, where L_1=TMEDA (3a); L=bdpy (3b); L=dmbpy (3c); L=Phen (3d) in good yield. The synthetic procedures for five-membered C,N-palladacycle cis-[2-Pd(C,HCHO)-6-(OMe)_3,4,5]BrL_1 3a-d, where L_1=TMEDA (3a); L=bdpy (3b); L=dmbpy (3c); L=Phen (3d), with an 1-naphthylisothiocyanates (C,H-NCO) and an 1-naphthylisothiocyanates (C,H-NCS), leads to the formation of novel palladacycles 4a-d and 5a-d, which was characterized in solution by 'H NMR spectroscopy. The solid products were characterized by satisfactory elemental analysis and spectro studies. All the resulting complexes 3a-d, 4a-d and 5a-d were tested in vitro against a number of cell lines. For example, it inhibited K562 leukaemia cells with an IC_{50} value in the range of (3.00 -4.3) µM (1 h exposure) and displayed cathepsin B inhibitory action with an IC_{50} value in the range of (0.045-0.055 µM).

Keywords: Palladium complexes; 1-naphthyl isocyanate; Isothiocyanate; Palladacycles

Introduction

Palladium (II) complexes show a discrete antitumor activity in vitro compared to the platinum-based drugs because of their extremely high liability in biologic fluids [1]. We have a long-standing interest in palladium chemistry, especially palladacycles, which are molecules containing a C–Pd bond stabilized intra-molecularly by a dative bond (e.g. N-, S-, O-, P-, Se- donor) [2–4]. Palladacycles are described in the literature as promising antitumor agents [5–16], but have yet to make sufficient progress to clinical trials for evaluation as drugs [17].

Nevertheless, a recent breakthrough from Caires et al. [18] has shown that a number of C,N-palladacycles were non-cytotoxic anticancer activity, and have notable cathepsin B inhibitory activity with the potential to treat metastatic cancer [19,20]. However, palladacycles are more stable, and, most important, less toxic, suggesting they could have a more specific antitumor activity in vitro [1,21–23]. The promising results derived from palladacycles have prompted us to investigate the reactivity of these aryl palladium complexes containing isocyanates and iso-thiocyanate (C_{n}H_{m}NCO) and isothiocyanate (C_{n}H_{m}NCS), in order to generate compounds for biological evaluation. The premises for such a study stems from the modular nature, i.e. ease of synthesis of analogues for structure activity studies (SAR) of the presence of N-donor ligands, such as N,N,N',N'-tetramethyl-ethane-1,2-diamine (TMEDA), 2,2'-bipyridine (bpy), 4,4'-dimethyl-2,2'-bipyridine (dmbpy) and 1,10-phenantrone (Phen). We concluded that the antitumor activity of the palladacycles compounds presented here can be attributed, at least in part, to the inhibitory properties of these complexes on the cysteine–protease activity, such as cathepsin B. Palladacycles are one of the most popular classes of organo palladium derivatives, which are widely applied in organic synthesis [24–39], organometallic catalysis [40–44], and new molecular materials [24]. Among them, the most investigated cyclic Pd-complexes are five- or six-membered rings fused with an aromatic ring, and the metallated carbon is usually an aromatic sp2 carbon [35–39]. However, six-member cyclopalladated with 1-naphthylisocyanate (–N=C=O) and 1-naphthylisothiocyanate (–N=C=S), sp2 carbon are rather rare. This is probably due to poor stability, which brings difficulties of preparation, isolation and characterization of these compounds. The limitation can be overcome by changing the nature of the metallated carbon atom, the type of donor groups and their substituents. Some of these reactions involve ortho-functionalized aryl complexes that after insertion of isocyanates gave heterocyclic, which the ortho group is included. A few examples of insertion of isocyanates into some other ortho-functionalized aryl palladium (II) complexes, leading to heterocyclic compounds have also been reported. The interest of this subject has prompted us to prepare aryl palladium complexes containing ortho–CHO functionalized. Herein, we report our results describing the preparation and full characterization of novel palladacycles 3a-d. In this context, the methodology of the synthesis of aryl palladium complexes 3a-d was studied. The reactivity of these aryl palladium complexes towards bulky of 1-naphthylisocyanate (–N=C=O) and 1-naphthylisothiocyanate (–N=C=S), sp2 carbon depend on the nature of substitution and the reaction conditions. The products of these

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reactions are mono-inserted complexes and prepared in single solvent, and no differentiation-quality crystals were grown by slow diffusion of EtO into a CHCl₃ solution.

In addition to that, the electron releasing methoxyl groups (MeO)₂ of the side chain could confer special properties to the formyl group, for example, facilitating its coordination to metallic centers to give cyclometallated species. Finally, this aryl moiety is present in organic molecules of pharmaceutical interest. For example, the antileukemic lactones steganacin, steganangin [45,46], the antibacterial agent trimethoprim [47], and the cytototoxic colchicines [48]. The cactus alkaloid, mescaline, beta-(3,4,5-trimethoxyphenyl)-ethalamine, has been studied for some years, because of its most interesting effects on the psychic states of human subjects. Since the elucidation of the chemical structure of the alkaloid through the synthesis by Spn th [49], a few other methods of preparation have been published [50,51].

The complexes exhibited growth inhibitory activity against L1210 mouse leukemia cells in vitro over a wide concentration range; in general, the cyclometallated complexes were more active than the mixed ligand complexes, although one cyclometallated organoplatinum complex was less active than the mixed ligand analogue. Substitution around the periphery of the aromatic ligands also resulted in increased activity. One complex, derived from 1-(2'-pyridyl)indole, was tested in vivo and showed no significant antitumour inhibition against P388 leukemia at doses below toxic levels [52-68].

**Experimental Procedure**

**General**

Starting materials and commercial grade solvents were purchased from Sigma-Aldrich or Alfa Aesar and used without further purification. Reactions were carried out under nitrogen. Vero cells were from the American Tissue Culture Collection, The B16 cells were kindly donated by Dr. Nadia (Cancer Institute Hospital). Reactions were carried out without precautions to exclude atmospheric moisture, unless otherwise stated. The IR and C, H, N Elemental analyses and melting point determinations were carried out as described elsewhere [69]. NMR spectra were recorded on Varian Unity 300 and a Bruker Unity 200 instruments. Chemical shifts were referred to TMS ([69]. NMR spectra were recorded on Varian Unity 300 and a Bruker GC: [20.3%], 568.99 (15.4%), 570.99 (4.7%), 559.99 (1.7%), 565.00 (1.1%); LRMS (EI): m/z: 535.96 [(M++1), 100.0%], 537.96 (99.5%), 536.96 (94.6%), 537.95 (82.0%), 539.95 (79.0%), 534.96 (76.6%), 539.96 (41.2%), 541.96 (38.7%), 538.96 (32.8%), 533.96 (37.2%), 535.95 (33.7%), 540.96 (26.0%), 542.96 (8.0%), 531.96 (3.1%), 539.95 (1.2%), 543.96 (1.1%). GC: tR = 8.614 min; column: DB-5 6 mm × 0.01 mm + 1 mm guard column: temp. prog. 50°C/2 min/20°C/1 min/325°C/10 min; Anal. Calc. for C2H11BrNO3Pd (573.70); C, 44.67; H, 3.56; N, 2.51; Found: C, 45.01; H, 0.8; N, 2.22.

**Cis-[Pd(C6H(CHO)-6-OMe3-2,3,4)Br(L)] (3b)**: Brownish green powder, Yield: 257 mg, 48%. Mp: 144-146°C dec. IR (cm⁻¹) (KBr), ν(C=O) (aldehyde), 1674; H NMR (300 MHz, DMSO): δ, 6.12 (1H, CH, 1H), 9.13 (1H, CHO), 8.68 (1H, CH, 1H), 5.87 (1H, CH), 1.51 (2H, CH₂, 2H), 0.89 (3H, Me3, 3H).

**Synthesis of Cis-[Pd(C6H(CHO)-OMe2,3,4)Br(L)] (3a-d)**

**General methods** reaction with naphthylisocyanate: Yellow solid, Yield: 288 mg, 51%. Mp: 282-284°C dec. IR (cm⁻¹) (KBr), ν(C=O) (aldehyde), 1672, 1574, 1466, 1419, 1361, 1265, 1091, 1010, 802; H NMR (300 MHz, DMSO): δ, 11.12 (1H, CH), 8.80 (d, 1H, MeO), 7.84 (2H, MeO), 7.4-7.8 (m, phen, 4 H), 7.6-7.8 (m, phen, 4 H); GC: tR = 7.92 min; column: DB-5 6 mm × 0.01 mm + 1 mm guard column: 8.97 min; column: DB-5 6 mm × 0.01 mm + 1 mm guard column; temp. prog. 50°C/2 min/20°C/1 min/325°C/10 min; Anal. Calc. for C16H27N2Br(PdO4) (557.73); C, 38.61; H, 5.47; N, 7.52; Found: C, 38.34; H, 5.04; N, 7.56.

**Cis-[Pd(C6H(CHO)-OMe2,3,4)Br(bpy)] (3a)**: Yellow solid, Yield: 298 mg, 53%. Mp: 198-200°C dec. IR (cm⁻¹) (KBr), ν(C=O) (aldehyde), 1674, 1574, 1466, 1419, 1361, 1265, 1091, 1010, 802; H NMR (300 MHz, DMSO): δ, 11.12 (1H, CH), 8.80 (d, 1H, MeO), 7.84 (2H, MeO), 7.4-7.8 (m, phen, 4 H), 7.6-7.8 (m, phen, 4 H); GC: tR = 8.12 min; column: DB-5 6 mm × 0.01 mm + 1 mm guard column: temp. prog. 50°C/2 min/20°C/1 min/325°C/10 min; Anal. Calc. for C16H27N2Br(PdO4) (557.73); C, 38.61; H, 5.04; N, 7.56.

**Reaction with bulky naphthylisocyanate and naphthylisothiocyanate**

4.3.1) General methods reaction with naphthylisocyanate:

Cis-[Pd(C6H(CHO)-OMe2,3,4)Br(bpy)] (3a) was made to a suspension of naphthylisocyanate (14 μl, 0.1 mmol) in dry toluene
6,7,8-trimethoxy-2-naphthyl-2,3-dihydroisoquinoline-1,4-dione -3-Pd [TMEDA] (4a): cis-[Pd(C6H(CHO)-6-OMe)3-2,3,4]Br.[L2] 3a (50 mg, 0.1 mmol) was added to a suspension of naphthyl-1-isocyanate (14 μl, 0.1 mmol) in dry toluene (15 ml), and the resulting mixture was refluxing for 3 h. The solvent was concentrated to dryness and the resulting solid was separated by filtration, washed with Et2O (2×20 ml) and air-dried to give 4a-d as a yellow solid and air dried.

6,7,8-trimethoxy-2-naphthyl-2,3-dihydroisoquinoline-1,4-dione -3-Pd [bpy] (4b): cis-[Pd(C6H(CHO)-6-OMe)3-2,3,4]Br.[L2] 3b (50 mg, 0.1 mmol) was added to a suspension of naphthyl-1-isocyanate (14 μl, 0.1 mmol) in dry toluene (15 ml), and the resulting mixture was refluxing for 3 h. The solvent was concentrated to dryness and the resulting solid was separated by filtration, washed with Et2O (2×20 ml) and air-dried to give 4a-d as a yellow solid and air dried.

6,7,8-trimethoxy-2-naphthyl-2,3-dihydroisoquinoline-1,4-dione -3-Pd [dmbpy] (4c): cis-[Pd(C6H(CHO)-6-OMe)3-2,3,4]Br.[L2] 3c (50 mg, 0.1 mmol) was added to a suspension of naphthyl-1-isocyanate (14 μl, 0.1 mmol) in dry toluene (15 ml), and the resulting mixture was refluxing for 3 h. The solvent was concentrated to dryness and the resulting solid was separated by filtration, washed with Et2O (2×20 ml) and air-dried to give 4a-d as a yellow solid and air dried.

6,7,8-trimethoxy-2-naphthyl-2,3-dihydroisoquinoline-1,4-dione -3-Pd [phen] (4d): cis-[Pd(C6H(CHO)-6-OMe)3-2,3,4]Br.[L2] 3d (50 mg, 0.1 mmol) was added to a suspension of naphthyl-1-isocyanate (14 μl, 0.1 mmol) in dry toluene (15 ml), and the resulting mixture was refluxing for 3 h. The solvent was concentrated to dryness and the resulting solid was separated by filtration, washed with Et2O (2×20 ml) and air-dried to give 4a-d as a yellow solid and air dried.
In present work, we describe the synthesis of several palladacycles in the form of TMEDA, bipyrindyl and phenanthroline complexes, as well as the screening of these drugs for antitumor activity, depending on the cyclopalladated fine structure, different antitumor properties were observed involving inhibition of the respiratory activity.

Palladium Complexes 3a-d, 4a-d and 5a-d were tested for in vitro anticancer activity against a K562 human leukaemic cell line via a medium throughput screen. For comparison, a number of palladacycles 3a-d, 4a-d and 5a-d are presented in Table 1. However, complexes 3a-d, 4a-d and 5a-d display good in vitro activity, with an IC50 range of (3.02-3.4 µM). Having established palladium complexes (3-5)a-d as a “hit” from this primary screen, we have evaluated it in other immortal cell lines namely B16 (Murine Melanoma) and Vero (African Green Monkey Kidney Epithelia) [52-54]. Preliminary data show 3a-d, 4a-d and 5a-d to have submicromolar activity (Figures 1 and 2). Furthermore, some related organo palladium complex has been tested for cathepsin B inhibitory activity [55] and registered an IC50 value of 2.98 µM, which was in the same range as that of the presented palladium complexes (3-5)a-d (Figure 3).

<table>
<thead>
<tr>
<th>Compound</th>
<th>N-N</th>
<th>E</th>
<th>IC50 (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>TMEDA</td>
<td>O</td>
<td>4.33 ± 0.05</td>
</tr>
<tr>
<td>3b</td>
<td>bpy</td>
<td>O</td>
<td>4.34 ± 0.05</td>
</tr>
<tr>
<td>3c</td>
<td>dmbpy</td>
<td>O</td>
<td>4.35 ± 0.05</td>
</tr>
<tr>
<td>3d</td>
<td>phen</td>
<td>O</td>
<td>4.36 ± 0.05</td>
</tr>
<tr>
<td>4a</td>
<td>TMEDA</td>
<td>O</td>
<td>3.02 ± 0.04</td>
</tr>
<tr>
<td>4b</td>
<td>bpy</td>
<td>O</td>
<td>3.03 ± 0.04</td>
</tr>
<tr>
<td>4c</td>
<td>dmbpy</td>
<td>O</td>
<td>3.05 ± 0.04</td>
</tr>
<tr>
<td>4d</td>
<td>phen</td>
<td>O</td>
<td>3.14 ± 0.04</td>
</tr>
<tr>
<td>5a</td>
<td>TMEDA</td>
<td>S</td>
<td>3.014 ± 0.04</td>
</tr>
<tr>
<td>5b</td>
<td>bpy</td>
<td>S</td>
<td>3.022 ± 0.04</td>
</tr>
<tr>
<td>5c</td>
<td>dmbpy</td>
<td>S</td>
<td>3.02 ± 0.06</td>
</tr>
<tr>
<td>5d</td>
<td>phen</td>
<td>S</td>
<td>3.027 ± 0.05</td>
</tr>
</tbody>
</table>

*MTT assay from DMSO stock solution on human leukaemic K562 cells. (1h exposure)

Table 1: In vitro activity of palladacycles against a K562 cell line.

![Figure 1: Toxicity of palladacycles 3a-d and cisplatin against Vero cell lines.](image-url)
Synthesis of cis-o-formylaryl palladium complexes [Pd(R)Br(L)] 2a-d: (R=6-formyl-2,3,4-trimethoxyphenyl)

Reactions of 2-bromo-3,4,5-trimethoxybenzaldehyde 1 with [Pd(dba)₂],dba ("Pd(dba)₂; dba = dibenzylideneacetone) the presence for 3 hrs to give mononuclear σ-aryl palladium (II) complexes 2a-d. The resulting mixture was stirred at 0°C for 30 min and at room temperature (bpy) 4,4'-dimethyl-2,2'-bipyridine (dmbpy) and 1,10-phenanthroline-N,N,N',N' of a stoichiometric amount of nitrogen donor ligands, such as of this processes begin with the oxidative addition of the organic halide -aryl Pd(II) complexes afforded as described in Scheme 2.

Another piece of evidence to support our configuration and structure determination of the resulting cis-aryl Palladium(II) complexes (3a-d) was presented by the reactions of (aryl), mercury, and aryl mercury bromide with a variety of nitrogen ligands have been studied [59]. The presence of the electron-withdrawing heteroatoms results in these mercurials being stronger acceptors than the corresponding phenylmercury compounds. The most common routes to organomercury compounds involve the direct reaction of mercury with an alkyl bromide, to form the mercury analog of a Grignard Reagent (GR). The subsequent reaction of RHgBr with potassium cyanide yields the appropriate dialkyl mercury derivative. In order to obtain insight into the pathway of the reaction, we have prepared these palladacycles (3a-d) [60-64] via a method similar to the one described recently by the reaction of prepared [HgR₂] (R=C₆H(CHO)-6,(OMe)₃-2,3,4 with Q₁[Pd₂Br₆] [Q=(PhCH₃)₅P] in acetonitrile in the precipitation of Q₂[Pd₂Br₆] in acetone/water, 2-bromo-3,4,5-trimethoxybenzaldehyde (1), indicating that there is no coordination of the formyl group to the metal atom.

Reaction with bulky 1-naphthyl isocyanate and isothiocyanate

Synthesis of cis-o-formylaryl palladium complexes 3a-d: As part of a systematic investigation to discover new organometallic approach, thus the insertion of 1- naphthylisocyanate (-N=C=O) and 1-naphthylisothiocyanate (-N=C=S) into the Pd-C bond, resulting in the palladium-catalyzed of organic substrates in laboratory synthesis and also in industrial processes. The palladacycles 3a-d was examined their reactivity towards naphthyl isocyanate in different molars in refluxing toluene afforded the corresponding spiro metalo-complexes of cis-arylpalladium complexes 4a-d and cis-arylpalladium complexes 5a-d in good yields. as described in Scheme 3. This is probably due to the result of the interchange between nitrogen donor of ligand and the bulky naphthyl isocyanate, which is a very well-unknown process.
OMe OMe OMe Br H OMe OMe OMe

Their IR spectra, a strong band at 1665 cm⁻¹ changes of brain-cortex ribosomes [67]. In complexes group similar to analogues of cactus alkaloid, mescaline [β-(3,4,5-

aldehyde group in the aryl fragment to the bromide in the (L2)PdBr addition reaction followed by the migration of a proton from the R=Naphthyl); the formation of these complexes results from an (3b), dmbpy (3c); phen, (3d)) with RNCE (E=O, S) yields the novel (4a-d) and/or 5a-d were tested for in vitro anticancer activity against a K562 human leukaemic cell line via a medium throughput screen. For comparison, a number of palladacycles 3a-d, 4a-d and 5a-d were synthesized and tested (Table 1). However, complexes of palladacycles 4a-d and 5a-d displays good in vitro activity, with an IC₅₀ of 4.3 μM. Having established 5a-d as a “hit” from this primary screen, we have evaluated it in other immortal cell lines namely B16 (Murine Melanoma) and Vero (African Green Monkey Kidney Epithelia) [52-54]. Preliminary data show 3a-d to have submicromolar activity (Figures 1-3). Furthermore, similar palladacycles has been tested for cathepsin B inhibitory activity and registered an IC50 value of 2.98-3.30 μM, which is in the same range as that of a dimeric dppf bridged C,N-palladacycle (dpfp=1,1'-bis(diphenylphosphine)-ferrocene) [55].

Conclusion
Novel Palladacycles 3-5 give a good result of inhibitory activity against cathepsin B and leukemia cells in vitro over a wide concentration range. Palladacycles 3a-d is cytotoxic and inhibits cathepsin B with IC₅₀ values in the low μM range [52-54]. This study are aiming to address some complexes can be used as biological probes for proteins and biomolecules, e.g. cysteine, selenocysteine proteases [73-75]. All these exciting aspects of palladacycle chemistry will be divulged in due course.

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methoxyphenyl)-alpha-benzoylbenzylidenamine. Comparison of the activity of these compounds with other isosteric cyclopalladinated compounds. J Med Chem 36: 3795-3801.


55. Commercial IC50 determinations were performed by MDS Pharma Services (http://www.mdps.com). assay 112260. CTSB (Cathepsin B). Brief details: substrate 20 mM Boc-Leu-Arg-Arg-AMC. 1% DMSO vehicle by spectrofluorimetric quantitation of AMC.


