Medicinal chemistry

Hamad Elgazwy et al., Med chem 2013, 3:3
DOI: 10.4172/2161-0444.1000148

Research Article Open Access

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

Abdel-Sattar S Hamad Elgazwy[1]*, Mohamed R Shehata[2], Myssoune Y Zaki[3], Dalia H S Solima[4] and Marwa M Elbakkry[1,2]

[1]*Department of Chemistry, Faculty of Science, Ain Shams University, Abbassia 11566, Cairo, Egypt*
[2]*Department of Chemistry, Faculty of Science, Cairo University, Giza, Egypt*
[3]*National Organization for Drug Control and Research, Cairo, Egypt*
[4]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy (Girls' Branch), Al Azahar University, Nasser city, Cairo, Egypt*

Abstract

The reaction of 2-bromo-3,4,5-trimethoxybenzaldehyde 1 with $Pd(dba)_2$; dba=dibenzylideneacetone) 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-phenanthroline (Phen), should be added to with equimolar ratio in degassed acetone under nitrogen to give mononuclear σ-aryl palladium (II) complexes cis-[2-$Pd\{C_6H(CHO)$-6-$(OMe)_3$-3,4,5$\}BrL_2$] 3a-d, where L_2=TMEDA (3a); L_2=bpy (3b); L_2=dmbpy (3c); L_2=Phen (3d) in good yields 48-65%. The reaction of the synthesized five-membered *C,N*-palladacycle cis-[2-$Pd\{C_6H(CHO)$-6-$(OMe)_3$-3,4,5$\}BrL_2$] 3a-d, where L_2=TMEDA (3a); L=bpy (3b); L_2=dmbpy (3c); L_2=Phen (3d), with an1-naphthylisocyanates ($C_{10}H_7$-NCO) and an 1-naphthylisothiocyanates ($C_{10}H_7$-NCS), leads to the formation of novel palladacycle 4a-d and 5a-d, which was characterized in solution by ^{1}H NMR spectroscopy. The solid products were characterized by satisfactory elemental analysis and spectra 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 vivo* [1,21-23]. The promising results derived from palladacycles have prompted us to investigate the reaction of the palladacycles 3a-d with isocyanate ($C_{10}H_7$-NCO) and isothiocyanate ($C_{10}H_7$-NCS), in order to generate compounds for biological evaluation. The premised 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-phenanthrroline (Phen), and the druglikeness of the ligands scaffold, which may lead to favorable properties in the resulting of some palladacycles complexes.

Based on the results described above, in the present paper, we investigated the interactions of cathepsin B with some palladacycles derived from the reaction of 2-Bromo-3,4,5-trimethoxybenzaldehyde 1 with $[Pd_2(dba)_3]$.dba ($Pd(dba)_2$; dba = dibenzylideneacetone), in 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-phenanthrroline (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), sp^2 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), depend on the nature of substitution and the reaction conditions. The products of these

***Corresponding author:** Abdel-Sattar S Hamad Elgazwy, Department of Chemistry, Faculty of Science, Ain Shams University, Abbassia 11566, Cairo, Egypt, Tel/Fax: 002024831836; E-mail: elgazwy@sci.asu.edu.eg

Received July 22, 2013; **Accepted** August 29, 2013; **Published** August 30, 2013

Citation: Hamad Elgazwy ASS, Shehata MR, Zaki MY, Solima DHS, Elbakkry MM (2013) Synthesis of Novel Palladacycles Inhibitors of the Cathepsin B Activity and Antitumoral Agents. Med chem 3: 254-261. doi:10.4172/2161-0444.1000148

reactions are mono-inserted complexes and prepared in single solvent, and no diffraction- quality crystals were grown by slow diffusion of Et_2O into a CH_2Cl_2 solution.

In addition to that, the electron releasing methoxyl groups $(MeO)_3$- of the side chain could confer special properties to the formyl group, for example, facilitating its coordination to metallic centers to give cyclometalated 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 cytotoxic colchicines [48]. The cactus alkaloid, mescaline, beta-(3,4,5-trimethoxyphenyl)-ethylamine, 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 Spnth [49], a few other methods of preparation have been published [50,51].

The complexes exhibited growth inhibitory activity against L1210 mouse leukæmia 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 leukæmia 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 (^{1}H and ^{13}C{^{1}H})-NMR assignments were made with the help of DEPT techniques. Chromatographic separations were carried out by TLC on silica gel 60 ACC (70-230 mesh). Complex of "Pd- $(dba)_2$" ($[Pd_2(dba)_3]$ dba), was prepared as previously reported [70,71].

Synthesis of Cis-[Pd{C6H(CHO)-6-OMe$_3$-2,3,4}Br(L_2)] (3a-d)

General methods: 2-bromo-3,4,5-trimethoxybenzaldehyde 1 (1.19 mmol) was added to a suspension of "$Pd(dba)_2$"(288 mg, 0.5 mmol) and *N*-donor ligands (0.5 mmol) in degassed acetone (15 ml) under nitrogen, and the resulting mixture was stirred at 0°C for 30 mins, and continue stirring at room temperature for 3 h. The solvent was evaporated in vacuo, the residue extracted with CH_2Cl_2 (20 mL), and the resulting suspension filtered over anhydrous $MgSO_4$. The solvent was concentrated to dryness and the resulting solid was separated by filtration, washed with Et_2O (2×20 mL) and air-dried to give 3a-d as a yellow solid and air dried.

***Cis*-[Pd{C6H(CHO)-6-OMe$_3$-2,3,4}Br(tmeda)] (3a):** Yellow solid**,** Yield: 300 mg, 60%. Mp: 178-180°C dec. IR (cm^{-1}) (KBr), ν(CO), 1670, 1578, 1465, 1419, 1365, 1315, 1265, 1193, 1154, 1099, 1065, 1012, 805, 771, 725. ^{1}H NMR (300 MHz, DMSO): δ at 2.29 (S, 3H, -NMe), 2.54 (S, 3H, -NMe), 2.68(S, 3H, -NMe), 2.73 (S, 3H, -NMe), 2.55-2.80 (m, 4H, -CH_2-CH_2-), 3.83 (S, 3H, OMe), 3.95 (S, 3H, OMe), 4.22 (S, 3H, OMe), 7.19 (S, 1H, C_6H_1), 11.15 (S, 1H, CHO). ^{13}C-NMR (DMSO, 75 MHz); δ at 47.96, 48.32, 51.07, 52.10 $(NMe_2)_2$; 55.9, 58.74, 60.9, 61.02, 106.52, 136.36, 143.32, 146049, 151.14, 155.53, 195.89 (CHO). LRMS (EI): m/z: 498.02 [(M^++ 2), 100.0%)], 496.02 (73.5%), 500.02 (66.6%), 497.02 (50.1%), 495.02 (42.6%), 494.02 (21.1%), 502.02 (21.1%), 499.02 (16.9%), 501.02 (11.7%), 503.02 (3.7%), 492.02 (1.8%).; GC: t_R=7.914 min; column: DB-5 6 m×0.01 mm+1 m guard column: temp. prog. 50°C/2 min./20°C/1 min./325°C/10 min; Anal. Calcd for $C_{16}H_{27}N_2BrPdO_4$ (497.72): C, 38.61; H, 5.47; N, 5.63 Found: C, 38.31; H, 5.40; N, 5.62.

Cis-[Pd{C6H(CHO)-6-OMe3-2,3,4}Br(bpy)] (3b): Brownish green powder, Yield: 257 mg, 48%. Mp: 144-146°C dec.; IR (cm^{-1}) (KBr), ν(C=O, aldehyde), 1674; ^{1}H NMR (300 MHz, DMSO): δ, 11.12 (s, 1H, CHO), 9.13 (d, 1H, bpy, $^{1,3}J_{HH}$=5.1 Hz), 8.68 (d, 1H, bpy, $^{1,3}J_{HH}$ =5.1 Hz), 8.57 (d, 1H, bpy, $^{1,3}J_{HH}$ =6.9 Hz), 8.39-8.32 (m, 2H, bpy), 7.97-7.91 (m, 1H, bpy), 7.83-7.78 (m, 1H, bpy), 7.47-7.42 (m, 1H, bpy), 7.02 (s, C6H1, 1H), 4.12 (s, OMe, 3H), 3.77 (s, OMe, 3H), 3.73 (s, OMe, 3H); LRMS (EI): m/z: 535.96 [(M^+-1), 100.0%)], 537.96 [(M^++2), (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 (38.2%), 533.96 (37.2%), 535.95 (33.7%), 540.96 (26.0%), 542.96 (8.0%), 531.96 (3.1%), 538.95 (1.2%), 543.96 (1.1%). GC: t_R=6.814 min; column: DB-5 6 m×0.01 mm+1 m guard column: temp. prog. 50°C/2 min/20°C/1 min/325°C/10 min; Anal. Calcd for $C_{20}H_{19}BrN_2O_4Pd$ (537.70); C, 44.67; H, 3.56; N, 5.21; Found: C, 45.01; H, 4.08; N, 5.22.

***Cis*-[Pd{C6H(CHO)-6-OMe3-2,3,4}Br(dmbpy)] (3c):** Brown powder**,** Yield: 288 mg, 51%. Mp:282-284°C dec. IR (cm^{-1}) (KBr), ν(C=O, aldehyde), 1673, 1574, 1466, 1419, 1361, 1265, 1099, 1010, 802.; ^{1}H NMR (300 MHz, DMSO): δ 11.12 (s, 1H, CHO), 8.80 (d, 1H, Me_2bpy, $^{1,3}J_{HH}$=5.7 Hz), 8.36 (s, 2H, Me_2bpy), 7.45(d, 2H, Me_2bpy, $^{1,3}J_{HH}$=5.7 Hz), 7.13 (s, C6H1, 1H), 4.14 (s, OMe, 3H), 3.80 (s, OMe, 3H), 3.75 (s, OMe, 3H), 2.49 (s, 2Me, 6H); LRMS (EI): m/z: 565.99 [(M^++2), 100.0%)], 563.99 [(M^+-2), 73.9%)], 567.99 (66.1%), 564.99 (52.2%), 562.99 (42.4%), 566.99 (23.2%), 569.99 (20.9%), 561.99 (20.3%), 568.99 (15.4%), 570.99 (4.7%), 559.99 (1.7%), 565.00 (1.1%); GC: t_R=7.714 min; column: DB-5 6 m×0.01 mm+1 m guard column: temp. prog. 50°C/2 min/20°C/1 min/325°C/10 min; Anal. Calcd for $C_{22}H_{23}BrN_2O_4Pd$ (565.75); C, 46.71; H, 4.10; N, 4.95; Found: C, 47.20; H, 3.97; N, 4.99.

***Cis*-[Pd{C_6H(CHO)-6-OMe$_3$-2,3,4}Br(phen)] (3d):** Brown solid, Yield: 298 mg, 53%. Mp: 198-200°C dec. IR (cm^{-1}) (KBr), ν(C=O, aldehyde), 1672, 1542, 1422, 1340, 1148, 1106, 842, 711, 463.; ^{1}H NMR (DMSO, 300 MHz): 11.18 (a, CHO, 1 H), 9.57 (dd, phen, 1 H, $^{1,3}J_{HH}$=5 and 1.5 Hz), 8.57 (dd, phen, 1 H, $^{1,3}J_{HH}$=8 and 1.5 Hz), 8.48 (dd, phen, 1 H, $^{1,3}J_{HH}$=8 and 1.4 Hz), 7.9-8.1 (m, phen, 4 H), 7.60 (dd, phen, 1 MeO).; LRMS (EI): m/z: 559.96 [(M^++1), 100.0%)], 561.96 [(M^+-2), 99.9%)], 560.96 (95.8%), 561.95 (80.6%), 563.95 (77.7%), 558.96 (76.1%), 562.96 (41.4%), 563.96 (41.4%), 565.96 (38.6%), 557.96 (36.6%), 559.95 (33.2%), 564.96 (28.1%), 566.96 (8.6%), 555.96 (3.1%), 567.96 (1.3%), 562.95 (1.2%).; GC: t_R=7.925 min; column: DB-5 6 m×0.01 mm+1 m guard column: temp. prog. 50°C/2 min/22°C/1 min/325°C/10 min; Anal. Calcd for $C_{22}H_{19}BrN_2O_4Pd$ (561.72); C, 47.04; H, 3.41; N, 4.99. Found C, 46.89; H, 4.02; N, 4.88.

Reaction with bulky naphthylisocyanate and naphthylisothiocycnate

4.3.1) General methods reaction with naphthylisocyanate: Cis-[Pd{C_6H(CHO)-6-OMe$_3$-2,3,4}Br(L)2] 3a-d (0.1 mmol) was added to a suspension of naphthylisocyanate (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 Et_2O (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 [TMEDA] (4a): *Cis*-[Pd{C_6H(CHO)-6-OMe_3-2,3,4}Br(L)2] 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 Et_2O (2×20 mL) and air-dried to give a grey solid of 4a, yield; 41 mg, 70% .Mp: 210-212°C dec. IR (cm^{-1}) (KBr), ν(NH), 3274, ν(C=O, 1660, 1542, 1422, 1340, 1148, 1106, 842, 711, 463; ^{1}H NMR (DMSO, 300 MHz): δ 8.25 (d, naphthyl, 1H, $^{1,3}J_{HH}$=7.8 Hz), 8.17 (d, naphthyl, 1H, $^{1,3}J_{HH}$=7.5 Hz), 7.85 (d, naphthyl, 1H, $^{1,3}J_{HH}$=8.4 Hz), 7.67-7.30 (m, naphthyl, 4H), 6.98 (s, C6H1, 1H), 3.78 (S, 3H, OMe), 3.76 (S, 3H, OMe), 3.73 (S, 3H, -OMe), 3.68-3.64 (m, 4H, -CH_2-CH_2-), 2.9 (S, 12H,(-NMe_2)$_2$].; LRMS (EI): m/z: 585.15 [(M^++1), (100.0%)], 587.15 [(M^++2), (81.1%)], 584.15 (74.1%), 589.15 (38.1%), 583.15 (32.4%), 586.15 (27.2%), 588.15 (23.1%), 590.15 (10.4%), 581.15 (2.9%), 591.15 (1.9%).; GC: t_R=7.925 min; column: DB-5 6 m×0.01 mm+1 m guard column: temp. prog. 50°C/2 min/22°C/1 min/325°C/10 min; Anal. Calcd for $C_{27}H_{33}N_3O_5Pd$ (585.99): C, 55.34; H, 5.68; N, 7.17 Found: C, 55.45; H, 5.61; N, 7.19.

6,7,8-trimethoxy-2-naphthyl-2,3-dihydroisoquinoline-1,4-dione -3-Pd [bpy] (4b): Orang solid of 4b, yield; 55 mg, 60% .Mp: 250-252°C dec. IR (cm^{-1}) (KBr), ν(NH), 3277, ν(C=O, 1665, 1542, 1422, 1340, 1148, 1106, 842, 711, 463; ^{1}H NMR (DMSO, 300 MHz): δ 9.33 (d, 2H, bpy, $^{1,3}J_{HH}$=5.1 Hz), 9.18 (d, 2H, bpy, $^{1,3}J_{HH}$=5.1 Hz), 8.78 (d, 2H, bpy, $^{1,3}J_{HH}$=6.9 Hz), 8.59-8.32 (m, 2H, bpy), 7.94 (d, naphthyl, 1H, $^{1,3}J_{HH}$=7.8 Hz), 7.78 (d, naphthyl, 1H, $^{1,3}J_{HH}$=7.55 Hz), 7.94 (d, naphthyl, 1H, $^{1,3}J_{HH}$=8.4 Hz),7.72-7.52 (m, naphthyl, 4H), 7.05 (s, C6H1, 1H), 3.78 (S, 3H, OMe), 3.75 (S, 3H, OMe), 3.73 (s, 3H, OMe,).; LRMS (EI): m/z: 625.08 [(M^++1), (100.0%)], 627.08 [(M^++2), (96.2%)], 624.08 (81.3%), 629.08 (42.8%), 623.08 (40.3%), 626.09 (39.4%), 628.09 (33.6%), 625.09 (30.2%), 630.09 (15.4%), 624.09 (13.7%), 627.09 (7.4%), 629.09 (6.4%), 621.09 (3.7%), 631.09 (2.9%), 626.08 (1.4%), 622.09 (1.3%), 628.08 (1.1%).; GC: t_R=7.925 min; column: DB-5 6 m×0.01 mm+1 m guard column: temp. prog. 50°C/2 min/22°C/1 min/325°C/10 min; Anal. Calcd for $C_{31}H_{25}N_3O_5Pd$ (625.97): C, 59.48; H, 4.03; N, 6.71 Found: C, 59.54; H, 4.22; N, 6.75.

6,7,8-trimethoxy-2-naphthyl-2,3-dihydroisoquinoline-1,4-dione -3-Pd [dmbpy] (4c): Brown powder**,** Yield: 80 mg, 58%. Mp: 272-274°C dec. IR (cm^{-1}) (KBr), ν(NH), 3277, ν(C=O, 1673, 1574, 1466, 1419, 1361, 1265, 1099, 1010, 802.; ^{1}H NMR (300 MHz, DMSO): δ, 9.36 (s, 2H, Me_2bpy), 9.05 (d, 2H, Me_2bpy, $^{1,3}J_{HH}$=5.7 Hz), 8.65 (d, 2H, Me_2bpy, $^{1,3}J_{HH}$=5.7 Hz), 8.25 (d, naphthyl, 1H, $^{1,3}J_{HH}$=7.8 Hz), 8.07 (d, naphthyl, 1H, $^{1,3}J_{HH}$=7.5 Hz), 7.95 (d, naphthyl, 1H, $^{1,3}J_{HH}$=8.4 Hz), 7.67-7.49 (m, naphthyl, 4H), 7.13 (s, C6H1, 1H), 3.79 (s, OMe, 3H), 3.75 (s, OMe, 3H), 3.73 (s, OMe, 3H), 2.39 (s, 6H ,2Me); LRMS (EI): m/z: 653.11 [(M^++1), 100.0%)], 655.11 [(M^++2), (96.3%)], 652.12 (95.6%), 657.12 (50.0%), 654.12 (42.6%), 651.11 (40.4%), 656.12 (35.9%), 653.12 (32.5%), 658.12 (16.5%), 655.12 (7.7%), 649.12 (3.7%), 659.12 (3.3%), 650.12 (1.3%), 654.11 (1.1%), 656.11 (1.1%).; GC: t_R=7.925 min; column: DB-5 6 m×0.01 mm+1 m guard column: temp. prog. 50°C/2 min/22°C/1 min/325°C/10 min; Anal. Calcd for $C_{33}H_{29}N_3O_5Pd$ (654.02): C, 60.60; H, 4.47; N, 6.42 Found: C, 60.54; H, 4.62; N, 6.55.

6,7,8-trimethoxy-2-naphthyl-2,3-dihydroisoquinoline-1,4-dione -3-Pd [phen] (4d): Brown solid, Yield: 298 mg, 53%. Mp: 292-294°C dec. IR (cm^{-1}) (KBr), ν(C=O, 1672, 1542, 1422, 1340, 1148, 1106, 842, 711, 463.; ^{1}H NMR (DMSO, 300 MHz): δ 9.37 (dd, phen, 2 H, $^{1,3}J_{HH}$=5 and 1.5 Hz), 9.27 (dd, phen, 2 H, $^{1,3}J_{HH}$=8 and 1.5 Hz), 8.48 (dd, phen, 2 H, $^{1,3}J_{HH}$=8 and 1.4 Hz), 8.25 (d, naphthyl, 1H, $^{1,3}J_{HH}$=7.8 Hz), 8.07 (d, naphthyl, 1H, $^{1,3}J_{HH}$=7.5 Hz), 7.9-8.1 (m, 2 H, phen), 7.95 (d, naphthyl, 1H, $^{1,3}J_{HH}$=8.4 Hz), 7.67-7.49 (m, naphthyl, 4H), 7.27 (s, C6H1, 1H), 3.77 (s, OMe, 3H), 3.75 (s, OMe, 3H), 3.73 (s, OMe, 3H).; LRMS (EI): m/z: 649.08 [(M^++1), (100.0%)], 651.08 [(M^++2), (96.2%)], 648.08 (81.3%), 653.08 (42.8%), 650.09 (42.2%), 647.08 (40.3%), 652.09 (35.8%), 649.09 (32.2%), 654.09 (16.4%), 648.09 (14.6%), 651.09 (8.3%), 653.09 (7.1%), 645.09 (3.7%), 655.09 (3.2%), 650.08 (1.4%), 646.09 (1.3%), 652.08 (1.1%).; GC: t_R=7.925 min; column: DB-5 6 m×0.01 mm+1 m guard column: temp. prog. 50°C/2 min/22°C/1 min./325°C/10 min; Anal. Calcd for $C_{23}H_{25}N_3O_5Pd$ (649.99); C, 60.98; H, 3.88; N, 6.46. Found C, 60.89; H, 3.92; N, 6.58.

Reaction with naphthylisothiocycnate

General methods: Cis-[Pd{C_6H(CHO)-6-OMe_3-2,3,4}Br(L)2] 3a-d (0.1 mmol) was added to a suspension of naphthylisothiocyanate (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 Et_2O (2×20 mL), and air-dried to give 5a-d as a yellow solid and air dried.

(1Z)-6,7,8-trimethoxy-1-(naphthylimino)-1H-isothiochromen-4(3H)-one-3-Pd[TMEDA] (5a): Grey solid, yield; 41 mg, 70% .Mp: 282-284°C dec. IR (cm^{-1}) (KBr), ν(NH), 3274, ν(C=O, 1660, ν(C=N, 1580, ν(C=S, 1310; ν(C-S, 1100, 1542, 1422, 1340, 1148, 1106, 842, 711, 463; ^{1}H NMR (DMSO, 300 MHz): 8.02 (d, naphthyl, 1H, $^{1,3}J_{HH}$=7.8 Hz), 7.89 (d, naphthyl, 1H, $^{1,3}J_{HH}$=7.5 Hz), 7.75 (d, naphthyl, 1H, $^{1,3}J_{HH}$=8.4 Hz), 7.62-7.49 (m, naphthyl, 4H), 6.82 (s, C6H1, 1H), 3.77 [(S, 9H, (-OMe)$_3$], 3.68-3.66 (m, 4H, -CH_2-CH_2-), 2.93 [(S, 12H, (-NMe_2)$_2$].; ^{13}C-NMR (DMSO,75 MHz); δ, at 47.96, 48.32, 51.07, 52.10 (NMe_2)$_2$; 55.9, 58.74, 60.9, 61.02, 106.52, 136.36, 143.32, 146049, 151.14, 155.53, 195.89 (CHO).LRMS (EI): m/z: 601.12 [(M^++1), (100.0%), 603.12 [(M^++2), (99.3%)], 600.12 (79.7%), 605.12 (46.0%), 599.12 (39.5%), 602.13 (33.2%), 604.13 (28.7%), 601.13 (25.6%), 606.13 (13.0%), 600.13 (11.7%), 602.12 (6.2%), 603.13 (5.8%), 605.13 (5.2%), 597.12 (3.6%), 604.12 (3.2%), 607.13 (2.4%), 606.12 (2.1%), 607.12 (2.1%), 598.13 (1.1%). GC: t_R=7.925 min; column: DB-5 6 m×0.01 mm+1 m guard column: temp. prog. 50°C/2 min/22°C/1 min/325°C/10 min; Anal. Calcd for $C_{27}H_{33}N_3O_5PdS$ (602,05): C, 53.86; H, 5.52; N, 6.98; S, 5.33. Found: C, 53.85; H, 5.61; N, 6.99; S, 5.45.

(1Z)-6,7,8-trimethoxy-1-(naphthylimino)-1H-isothiochromen-4(3H)-one-3-Pd[bpy] (5b): Orang solid of 5b, yield; 55 mg, 60% .Mp: 297-299°C dec. IR (cm^{-1}) (KBr), ν(NH), 3277, ν(C=O,1665, 1542, 1422, 1340, 1148, 1106, 842, 711, 463; ^{1}H NMR (DMSO, 300 MHz): δ 9.35 (d, 2H, bpy, $^{1,3}J_{HH}$=6.9 Hz), 9.14 (dd, 2H, bpy, $^{1,3}J_{HH}$=6.9 and 5.1 Hz), 8.72 (d, 2H, bpy, $^{1,3}J_{HH}$=5.1 Hz), 8.53 (bs, 2H, bpy), 8.33 (d, naphthyl, 1H, $^{1,3}J_{HH}$=7.8 Hz), 8.22 (d, naphthyl, 1H, $^{1,3}J_{HH}$=7.55 Hz),7.85 (d, naphthyl, 1H, $^{1,3}J_{HH}$=8.4 Hz), 7.39 (bs, naphthyl, 4H), 6.85 (s, C6H1, 1H), 3.89 (S, 3H, OMe), 3.84 (S, 3H, OMe), 3.77 (s, 3H, OMe).; LRMS (EI): m/z: 643.06 [(M^++1), (100.0%), 641.06 [(M^+-2), (99.8%)], 640.06 (92.4%), 645.06 (47.0%), 639.06 (39.3%), 642.06 (38.8%), 644.06 (34.9%), 641.07 (28.8%), 646.07 (14.9%), 643.07 (6.2%), 642.07 (5.7%), 645.07 (5.3%), 637.06 (3.6%), 647.07 (2.8%), 647.06 (2.4%), 646.06 (2.3%), 644.07 (1.2%), 638.07 (1.2%) GC: t_R=7.925 min; column: DB-5 6 m×0.01 mm+1 m guard column: temp. prog. 50°C/2 min/22°C/1 min/325°C/10 min; Anal. Calcd for $C_{31}H_{25}N_3O_4PdS$ (642.03): C, 57.99; H, 3.92; N, 6.54; S, 4.99.; Found: C, 59.54; H, 4.22; N, 6.75; S, 5.01.

(1Z)-6,7,8-trimethoxy-1-(naphthylimino)-1H-isothiochromen-4(3H)-one-3-Pd[dmbpy] (5c): Brown powder**,** Yield: 80 mg, 58%.

Mp: >300°C dec. IR (cm^{-1}) (KBr), ν(NH), 3277, ν(C=O, 1673, 1574, 1466, 1419, 1361, 1265, 1099, 1010, 802.; ^{1}H NMR (300 MHz, DMSO): δ 8.80 (d, 1H, Me_2bpy, $^{1,3}J_{HH}$=5.7 Hz), 8.36 (s, 2H, Me_2bpy), 8.25 (d, naphthyl, 1H, $^{1,3}J_{HH}$=7.8 Hz), 8.07 (d, naphthyl, 1H, $^{1,3}J_{HH}$=7.5 Hz), 7.95 (d, naphthyl, 1H, $^{1,3}J_{HH}$=8.4 Hz), 7.67-7.49 (m, naphthyl, 4H), 7.45(d, 2H, Me_2bpy, $^{1,3}J_{HH}$=5.7 Hz), 7.03 (s, C6H1, 1H), 4.14 (s, OMe, 3H), 3.80 (s, OMe, 3H), 3.75 (s, OMe, 3H), 2.49 (s, 2Me, 6H). LRMS (EI): m/z: 669.09 [(M^++1),(100.0%)], 671.09 [(M^++2) (99.9%)], 668.09 (79.6%), 673.09 (46.6%), 670.09 (40.8%), 667.09 (39.5%), 672.10 (35.2%), 669.10 (31.3%), 674.10 (16.1%), 668.10 (14.3%), 671.10 (7.9%), 673.10 (6.9%), 670.10 (6.8%), 665.09 (3.6%), 672.09 (3.4%), 675.10 (3.2%), 675.09 (2.4%), 674.09 (2.4%), 666.10 (1.3%). GC: t_R=7.925 min; column: DB-5 6 m×0.01 mm+1 m guard column: temp. prog. 50°C/2 min/22°C/1 min/325°C/10 min; Anal. Calcd for $C_{33}H_{29}N_3O_4PdS$ (670.09): C, 59.15; H, 4.36; N, 6.27; S, 4.79.; Found: C, 60.54; H, 4.62; N, 6.55; S, 4.86.

(1Z)-6,7,8-trimethoxy-1-(naphthylimino)-1H-isothiochromen-4(3H)-one-3-Pd[phen] (5d): Brown solid**,** Yield: 298 mg, 53%. Mp: >300°C dec. IR (cm^{-1}) (KBr), ν(C=O, 1672, 1542, 1422, 1340, 1148, 1106, 842, 711, 463.; ^{1}H NMR (DMSO, 300 MHz): 9.27 (dd, phen, 1 H, $^{1,3}J_{HH}$=5 and 1.5 Hz), 9.08 (dd, phen, 1 H, $^{1,3}J_{HH}$=8 and 1.5 Hz), 8.48 (dd, phen, 1 H, $^{1,3}J_{HH}$=8 and 1.5 Hz), 8.25 (d, naphthyl, 1H, $^{1,3}J_{HH}$=7.8 Hz), 7.9-8.1 (m, phen, 4 H), 8.07 (d, naphthyl, 1H, $^{1,3}J_{HH}$=7.5 Hz), 7.95 (d, naphthyl, 1H, $^{1,3}J_{HH}$=8.4 Hz), 7.67-7.49 (m, naphthyl, 4H), 7.03 (s, C6H1, 1H), 4.14 (s, OMe, 3H), 3.80 (s, OMe, 3H), 3.75 (s, OMe, 3H).; LRMS (EI): m/z: 667.06 [(M^++1),(100.0%)], 665.06 (99.7%), 664.06 (93.1%), 669.06 (47.1%), 666.06 (40.9%), 663.06 (39.2%), 668.06 (37.0%), 665.07 (30.8%), 670.07 (15.9%), 667.07 (7.0%), 666.07 (6.3%), 669.07 (6.0%), 661.06 (3.6%), 671.07 (3.1%), 671.06 (2.5%), 670.06 (2.4%), 668.07 (1.4%), 662.07 (1.3%).; GC: t_R=7.925 min; column: DB-5 6 m×0.01 mm+1 m guard column: temp. prog. 50°C/2 min/22°C/1 min/325°C/10 min; Anal. Calcd for $C_{33}H_{25}BrN_3O_4PdS$ (666.05); C, 59.51; H, 3.78; N, 6.31; S, 4.81.; Found C, 46.89; H, 4.02; N, 4.88; S, 5.05.

Cell culture

B16 and Vero cells were maintained in an atmosphere of 5% (v/v) CO_2 at 37°C. IC_{50} values were obtained using published methodologies. Briefly, 5×10^3 cells/wellwere used to seed 96 well cell culture treated plates (Sigma). Compounds were dissolved at 5 mg ml-1 in sterile DMSO, and further diluted with the appropriate complete cell culture medium. After 72 h, cell viability was assessed using MTT(Sigma), also following published protocols [20,72-74].

Growth inhibitory activity against the human K562 cell line

K562 human chronic myeloid leukemia cells were maintained in RPM1 1640 medium supplemented with 10% fetal calf serum and 2 mM glutamine at 37°C in a humidified atmosphere containing 5% CO_2, and were incubated with a specified dose of test agent for 1 h at 37°C in the dark. The incubation was terminated by centrifugation (5 min, 300 g), and the cells were washed once with drug-free medium. Following the appropriate drug treatment, the cells were transferred to 96-well microtitre plates. Plates were then kept in the dark at 37°C in a humidified atmosphere containing 5% CO_2. The assay is based in the ability of viable cells to reduce a yellow soluble tetrazolium salt, 3-(4,5 dimethylthiazol-2-yl)-2,5- diphenyl-2*H*-tetrazolium bromide (MTT, Sigma Chemical Co.) to an insoluble purple formazan precipitate. The optical density was then read at a wavelength of 550 nm on a plate reader, and a doseresponse curve was constructed. For each curve, an IC_{50} value was read as the dose required reducing the final optical density to 50% of the control value.

Results and Discussion

In our present work, we describe the synthesis of several palladacycles in the form of TMEDA, bipyridyl 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 IC_{50} of range (3.02-4.3 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).

Compound	N-N	E	IC_{50} / μM[a]
3a	TMEDA		4.33 ± 0.05
3b	bpy		4.34 ± 0.05
3c	dmbpy		4.35 ± 0.05
3d	phen		4.36 ± 0.05
4a	TMEDA	**O**	3.02 ± 0.04
4b	bpy	**O**	3.03 ± 0.04
4c	dmbpy	**O**	3.05 ± 0.04
4d	phen	**O**	3.14 ± 0.04
5a	TMEDA	**S**	3.014 ± 0.04
5b	bpy	**S**	3.022 ± 0.04
5c	dmbpy	**S**	3.012 ± 0.06
5d	phen	**S**	3.027 ± 0.05

[a]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.

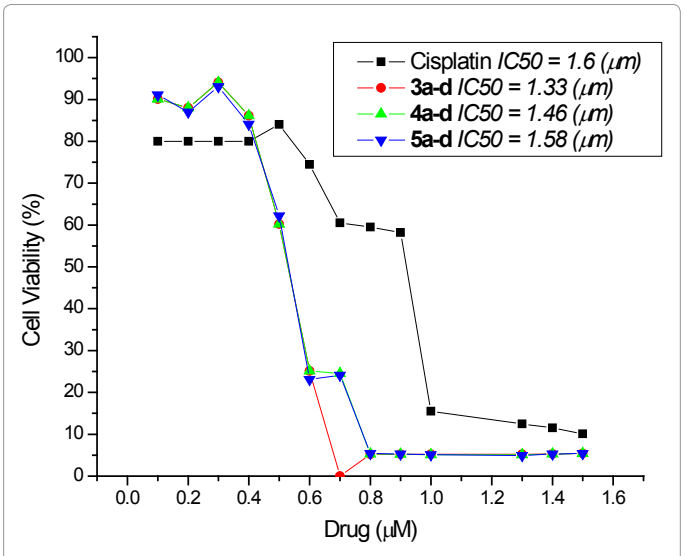


Figure 1: Toxicity of palladacycles **3a-d** and cisplatin against Vero cell lines.

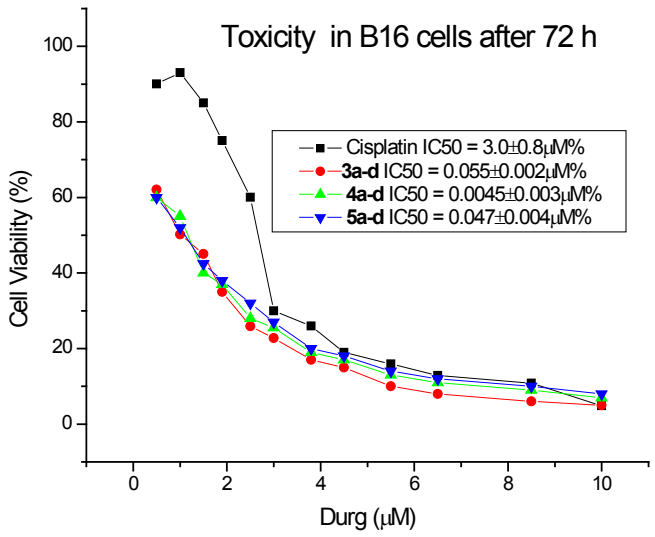


Figure 2: Toxicity of palladacycles **3a-d** and cisplatin against B16 cell lines.

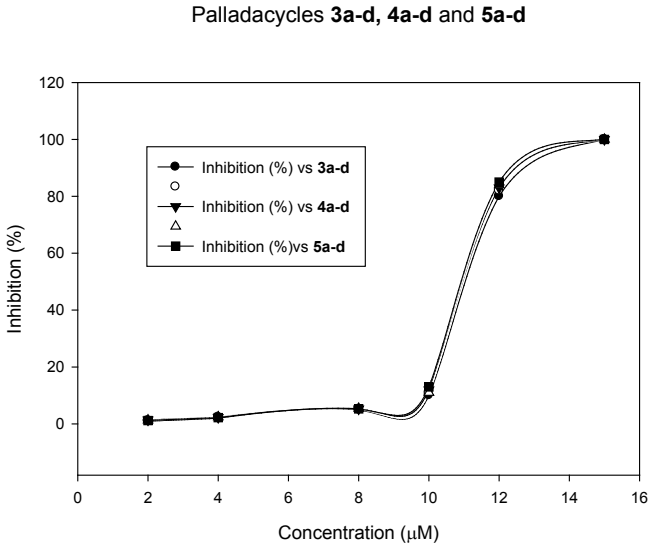


Figure 3: Inhibitory action of Palladacycles (**3-5)a-d** towards (human liver) cathepsin B.

Synthesis of cis-o-formylaryl palladium complexes [Pd(R)Br(L_2)] 2a-d; (R=6-formyl-2,3,4-trimethoxyphenyl

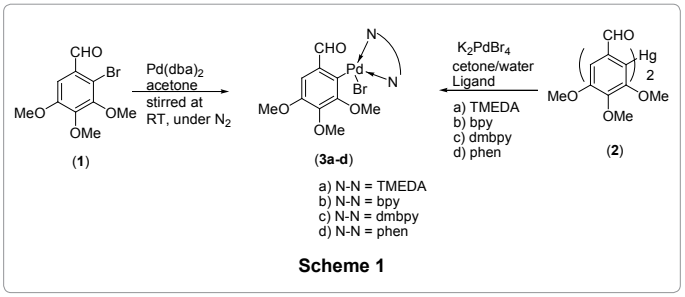
Reactions of 2-bromo-3,4,5-trimethoxybenzaldehyde 1 with [Pd_2(dba)$_3$].dba ("Pd(dba)$_2$"; dba = dibenzylideneacetone) the presence of a stoichiometric amount of nitrogen donor ligands, such as *N,N,N',N'*-tetramethyl-ethane-1,2-diamine (TMEDA), 2,2'-bipyridine (bpy) 4,4'-dimethyl-2,2'-bipyridine (dmbpy) and 1,10-phenanthroline (Phen) with equimolar ratio in degassed acetone under nitrogen. The resulting mixture was stirred at 0°C for 30 min and at room temperature for 3 hrs to give mononuclear σ-aryl palladium (II) complexes 2a-d in good yields 48-65%, as outlined in Scheme 1.

It is better to present a generalized picture of mechanistic aspects of this processes begin with the oxidative addition of the organic halide to a Pd(0) substrate. These reaction seem to take place through a S_N^2 mechanism which is consistent with other group [56-58], and the resulting *cis*-aryl Pd(II) complexes afforded as described in Scheme 2.

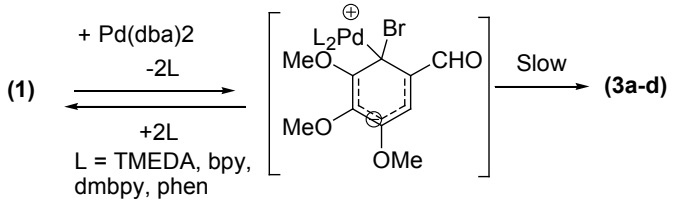
Another piece of evidence to support our configuration and structure determination of the resulting *cis*- aryl Palladium(II) complexes (3a-d) was presented by the reactions of (aryl)$_2$ mercury, and aryl mercury bromide with a variety of nitrogen ligands have been studied [59]. The presence of the 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 [HgR_2] (R=C_6H(CHO)-6,(OMe)$_3$-2,3,4 with Q_2[Pd_2Br_6] [Q=($PhCH_2$)Ph_3P] in acetone results in the precipitation of Q_2[$Pd_2R_2Br_2$(μ-Br)$_2$], which is easily separated from the byproduct RHgBr. reacts with TMEDA, 2,2'-bipyridine or 4,4'-dimethyl-2,2'-bipyridine or 1,l0-phenanthroline or PPh_3 to give [PdRBr(TMEDA)] (3a) or [PdRBr(bpy)] (3b) or [PdRBr(dmbpy)] (3c) or [PdRBr(phen)] (3d) respectively, which are difficult to separate from the byproduct QBr; however, a better method to access these compounds free of impurities starts from K_2[$PdBr_4$]. To allow both reagents to be in solution, we reacted an aqueous solution of K_2[$PdBr_4$] with an acetone solution of [HgR_2]. To our surprise, the reaction occurs without precipitation of any of the reaction products, and if acetone is removed and more water added, the byproduct RHgBr precipitates quantitatively leaving a yellow aqueous solution of some water-soluble arylpalladium(II) complexes. The dichloromethane solutions of 2,2'-bipyridine (bpy) or 4,4'-dimethyl-2,2'-bipyridine (dmbpy) or 1,l0-phenanthroline (phen) added and extraction of the water solution with more dichloromethane added, allow the isolation of complexes 3b-d in moderate yields (48, 51,53) %, respectively. All of these compounds show in their IR spectra a strong band at ca. 1630-1660 cm^{-1} assignable to ν(CO) of the formyl gzroup. This frequency is similar to that observed in [HgR_2], HgRBr, or 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

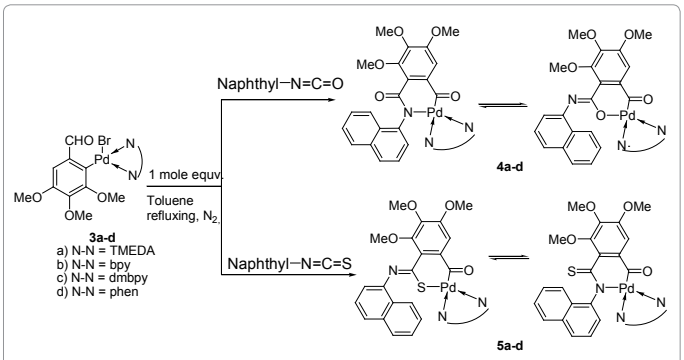
Synthesis of cis-o-formylaryl palladium complexes 3a-d: As part of a systematic investigation to discover new organometallic approach, thus the insertion of 1- naphthylisocyanate (-N=C=O) and 1-naphthylisothiocyanate (-N=C=S) into the Pd-C bond, resulting in the formation of imidoyl palladium complexes, constitutes a key step 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.



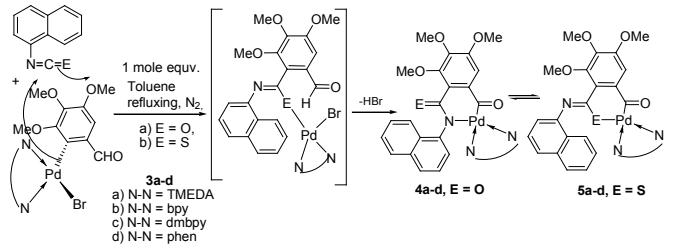
Scheme 1



Scheme 2: S_N^2 mechanism of oxidative addition of aryl bromides with Pd(0) compounds.



Scheme 3



Scheme 4: Schematic representation the synthesis of palladacycles (**4a-d**) and (**5a-d**).

One might believe that palladium complexes as intermediates for the formation of complex 4a-d and/or 5a-d. This suggested that the presence of naphthylisocyanate or naphthylisothiocycanate as ligands, could be responsible for the interchange of the ligands and existence of complexes 4a-d or 5a-d in solution derived from two *cis* form.

It is better to present a generalized picture of mechanistic aspects of palladacycles with the reactivity towards 1-naphthylisocyanate and 1-naphthylisothiocyanate as models, these reactions of palladium complexes with isocyanates (NCO) and isothiocyanates (RNCS) have been investigated [65,66]. The reaction of new cis-[Pd{C6H(CHO)-OMe_3-3,4,5}Br(N-N)] (3a-d), while (N–N =TMEDA (3a), bpy (3b), dmbpy (3c); phen, (3d)) with RNCE (E=O, S) yields the novel compounds (4a-d) (E=O and R=Naphthyl and (5a-d), E=S and R=Naphthyl); the formation of these complexes results from an addition reaction followed by the migration of a proton from the aldehyde group in the aryl fragment to the bromide in the (L2)PdBr moieties.

These resulting compounds having 3,4,5-trimethoxyphenyl group similar to analogues of cactus alkaloid, mescaline [beta-(3,4,5-trimethoxyphenyl)-arylmethylamine], that were used for induced changes of brain-cortex ribosomes [67]. In complexes 4a-d show in their IR spectra, a strong band at 1665 cm^{-1} assignable to ν(CO) group. This frequency is similar to that observed in iso-indenone, indicating that there is coordination of the formyl group to the isocyanate inserted. A piece of evidence to support our assumption that the reasons of inhibition the insertion of naphthyl isocyanate into bulky substrates, this caused by the steric hindrance of bulky reactants. The ^{1}H NMR spectrum at room temperature indicates that formation of the complexes seems to be favored by the potentially great steric hindrance and their ligands were in a square planar disposition. In this case, such steric hindrance should monitored by UV during the reaction process. All the reactions of aryl palladium complexes with 1-naphthylisocyanate (NCO) and 1-naphthylisothiocyanate (NCS) were investigated, and some of these results were consistent with the results reported recently [62-64,68]. The band assignable to ν(aryl), ν(bpy), ν(dmbpy) and ν(phen) in the IR spectra of the palladacycles are observed within the range 1515-1731 cm^{-1} and two bands at 1716 and 1615 cm^{-1}; one of them may be due to the ν(C=C), and the other remaining band may be assignable to the ν(C=N) mode, corresponding to the ligands coordinated to the palladium atom. 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 were synthesized and tested (Table 1). However, complexes of palladacycles 4a-d and 5a-d displays good *in vitro* activity, with an IC_{50} 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 2.98-3.20 μM, which is in the same range as that of a dimeric dppf bridged C,N-palladacycle (dppf=1,1'-*bis*(diphenylphosphine)-ferrocene) [55].

Conclusion

Novel Palladacycles 3-5 give a good result of inhibitory activity against cathepsin B and leukimia cells *in vitro* over a wide concentration range. Palladacycles 3a-d is cytotoxic and inhibits cathepsin B with IC_{50} 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.

Acknowledgements

The authors are grateful to Cancer Research Technology and Robin Leach, MDS Pharma Services, are thanked for helpful discussions. This paper is dedicated to Professor José Vicente Soler for his contributions to Organometallic Chemistry and exploiting palladacycles Chemistry.

References

1. Navarro-Ranninger C, Lopez-Solera I, Perez JM, Masaguer JR, Alonso C (1993) Appl Organomet Chem 7: 57- 61.
2. Dupont J, Consorti CS, Spencer J (2005) The potential of palladacycles: more than just precatalysts. Chem Rev 105: 2527-2571.
3. Dupont J, Pfeffer M, Spencer J (2001) Eur J Inorg Chem 1917-1927.
4. Dupont J, Pfeffer M, Ryabov (2008) in Palladacycles, ed. Wiley-VCH, Weinheim ch 13: 307–339.
5. Higgins JD 3rd, Neely L, Fricker S (1993) Synthesis and cytotoxicity of some cyclometallated palladium complexes. J Inorg Biochem 49: 149-156.
6. Navarro-Ranninger C, Lopez-Solera I, Gonzales VM, Perez JM, Alvarez-Valde´s A, et al. (1996) Inorg Chem 35: 5181.
7. Navarro-Ranninger C, López-Solera I, Pérez JM, Rodríguez J, García-Ruano JL, et al. (1993) Analysis of two cycloplatinated compounds derived from N-(4-methoxyphenyl)-alpha-benzoylbenzylidenamine. Comparison of the activity of these compounds with other isostructural cyclopalladated compounds. J Med Chem 36: 3795-3801.

8. Quiroga AG, Pérez JM, López-Solera I, Masaguer JR, Luque A, et al. (1998) Novel tetranuclear orthometalated complexes of Pd(II) and Pt(II) derived from p-isopropylbenzaldehyde thiosemicarbazone with cytotoxic activity in cis-DDP resistant tumor cell lines. Interaction of these complexes with DNA. J Med Chem 41: 1399-1408.

9. Perez AG, Quiroga AG, Montero EI, Alonso C (1999) Navarro-Ranninger CJ. Inorg Biochem 73: 235.

10. Rodrigues EG, Silva LS, Fausto DM, Hayashi MS, Dreher S, et al. (2003) Cyclopalladated compounds as chemotherapeutic agents: antitumor activity against a murine melanoma cell line. Int J Cancer 107: 498-504.

11. Bhandarkar SS, Bromberg J, Carrillo C, Selvakumar P, Sharma RK, et al. (2008) Tris (dibenzylideneacetone) dipalladium, a N-myristoyltransferase-1 inhibitor, is effective against melanoma growth in vitro and in vivo. Clin Cancer Res 14: 5743-5748.

12. Iacobuzio-Donahue CA, Shuja S, Cai J, Peng P, Murnane MJ (1997) Elevations in cathepsin B protein content and enzyme activity occur independently of glycosylation during colorectal tumor progression. J Biol Chem 272: 29190-29199.

13. Sloane BF, Rozhin J, Johnson K, Taylor H, Crissman JD, et al. (1986) Cathepsin B: association with plasma membrane in metastatic tumors. Proc Natl Acad Sci U S A 83: 2483-2487.

14. Almeida PC, Nantes IL, Rizzi CCA, Judice WAS, Chagas JR, et al. (1999) Biol Chem 274: 30433.

15. Almeida PC, Nantes IL, Chagas JR, Rizzi CC, Faljoni-Alario A, et al. (2001) Cathepsin B activity regulation. Heparin-like glycosaminogylcans protect human cathepsin B from alkaline pH-induced inactivation. J Biol Chem 276: 944-951.

16. Heck RF (1985) Palladium Reagents in Organic Synthesis; Academic Press: New York.

17. Bincoletto C, Tersariol IL, Oliveira CR, Dreher S, Fausto DM, et al. (2005) Chiral cyclopalladated complexes derived from N,N-dimethyl-1-phenethylamine with bridging bis(diphenylphosphine)ferrocene ligand as inhibitors of the cathepsin B activity and as antitumoral agents. Bioorg Med Chem 13: 3047-3055.

18. Caires ACF, Almeida ET, Mauro AE, Hemerly JP, Valentini SR (1999) Quimica Nova 22: 329-334.

19. Spencer J, Sharratt DP, Dupont J, Monteiro AL, Reis VI, et al. (2005) Organometallics 24: 5665–5672.

20. Spencer J, Chowdhry BZ, Mallet AI, Rathnam RP, Adatia T, et al. (2008) Tetrahedron 64: 6082–6089.

21. Wunberg T, Hendrix M, Hillisch A, Lobell M, Meier H, et al. (2006) Drug Discovery Today 11: 175–180.

22. Proudfoot JR (2002) Drugs, leads, and drug-likeness: An analysis of some recently launched drugs. Bioorg Med Chem Lett 12: 1647-1650.

23. Higgins JD 3rd, Neely L, Fricker S (1993) Synthesis and cytotoxicity of some cyclometallated palladium complexes. J Inorg Biochem 49: 149-156.

24. Navarro-Ranninger C, López-Solera I, Pérez JM, Rodríguez J, García-Ruano JL, et al. (1993) Analysis of two cycloplatinated compounds derived from N-(4-methoxyphenyl)-alpha-benzoylbenzylidenamine. Comparison of the activity of these compounds with other isostructural cyclopalladated compounds. J Med Chem 36: 3795-3801.

25. Hartwig JF (1998) Chem Int Ed 37: 2047.

26. Elgazwy ASSH (2003) Tetrahedron 59: 7445-7463.

27. Tsuji J (1995) Palladium Reagents and Catalysis. Wiley: Chich-ester, UK.

28. Wolfe JP, Wagaw S, Marcoux JF, Buchwald SL (1998) Acc Chem Res 31: 805.

29. Shibasaki M, Vogl EM (1999) J Organomet Chem 576: 1.

30. Loiseleur O, Hayashi M, Keenan M, Schmees N, Pfaltz A (1999) J Organomet Chem 576: 16.

31. Herrmann WA, Bohm VPW, Reisinger CP (1999) J Organomet Chem 576: 23.

32. Cacchi S (1999) J Organomet Chem 576: 42.

33. Larock RC (1999) J Organomet Chem 576: 111.

34. Suzuki J (1999) Organomet Chem 576: 147.

35. Dupont J, Pfeffer M, Spencer J ((2001)) Eur J Inorg Chem 1917.

36. Cope AC, Friedrich EC (1968) J Am Chem Soc 90: 909.

37. Alper HJ (1973) Organomet Chem 61: C62.

38. Dehand J, Mauro A, Ossor H, Pfeffer M, Santos RHD, et al. (1983) J Organomet Chem 250: 537.

39. McPherson HM, Wardell JL (1983) Inorg Chim Acta 75: 37.

40. Elgazwy ASSH (2007) Applied Organometallic Chemistry 21: 1041-1053.

41. Elgazwy ASSH (2008) Monatshefte für Chemie 139: 1285-1297.

42. Dupont J, Pfeffer M, Wiley-V CH, RyabovA (2008) in Palladacycles. Weinheim 13: 307-339.

43. Elgazwy ASSH (2009) Polyhedron 28: 349-359.

44. Elgazwy ASSH (2009) Polyhedron 28: 3667-3674.

45. Ziegler FE, Chliwner 1, Fowler KW, Kanfer SJ, Kuo SJ, et al. (1980) J Am Chem SOC 102: 790.

46. Tomioka K, Ishiguro T, Mizuguchi H, Komeshima N, Koga K, et al. (1991) Absolute structure-cytotoxic activity relationships of steganacin congeners and analogues. J Med Chem 34: 54-57.

47. Chan JH, Roth B (1991) 2,4-Diamino-5-benzylpyrimidines as antibacterial agents. 14. 2,3-Dihydro-1-(2,4-diamino-5-pyrimidyl)-1H-indenes as conformationally restricted analogues of trimethoprim. J Med Chem 34: 550-555.

48. Ringel I, Jaffe D, Alerhand S, Boye O, Muzaffar A, et al. (1991) Fluorinated colchicinoids: antitubulin and cytotoxic properties. J Med Chem 34: 3334-3338.

49. Spnth E (1919) Monatsh 40: 129.

50. Tsao MU (1951) J Am Chem Soc 73: 5495.

51. Reti L (1953) In: Manske & Holmes (Ed.), The Alkaloids, Vol. III 22: 313.

52. Spencer J, Mendham AP, Kotha AK, Richardson SC, Hillard EA, et al. (2009) Structural and biological investigation of ferrocene-substituted 3-methylidene-1,3-dihydro-2H-indol-2-ones. Dalton Trans 918-921.

53. Richardson SC, Winistorfer SC, Poupon V, Luzio JP, Piper RC (2004) Mammalian late vacuole protein sorting orthologues participate in early endosomal fusion and interact with the cytoskeleton. Mol Biol Cell 15: 1197-1210.

54. Richardson S, Ferruti P, Duncan R (1999) Poly(amidoamine)s as potential endosomolytic polymers: Evaluation in vitro and body distribution in normal and tumour-bearing animals. J Drug Target 6: 391-404.

55. Commercial IC50 determinations were performed by MDS Pharma Services (http://www.mdsps.com): assay 112250, CTSB (Cathepsin B). Brief details: substrate 20 mM Boc-Leu-Arg-Arg-AMC, 1% DMSO vehicle by spectrofluorimetric quantitation of AMC.

56. Tsao MU, Van Dyke E (1955) J Am Chem Soc 77: 6693.

57. Grove DM, van Koten G, Louwen JN, Noltes JG, Speck AL, et al. (1982) J Am Chem Soc 104: 6609.

58. Rietveld MHP, DM Grove, van Koten G (1997) New J Chem 21: 751.

59. Tu ek-Bo L, D'Alpaos M (1998) Polyhedron 17: 1481-1493.

60. Hirai Y, Igashira-Kamiyama A, Kawamoto T, Konno T (2007) Chem Lett 36: 434.

61. Gilman H, Barnett MM (1936) des Travaux. C des Pays-Bas 55: 563–566.

62. VicenteJ, Abad JA, Stiakaki MA, Jones PG (1991) J Chem SOC Chem Commun 137.

63. Vicente J, Abad JA, Jones PGn (1992) Organometallics 11: 3612.

64. Vicente JJ, Abad A Lahoz FJ, Plou FJ (1990) J Chem Soc Dalton Trans 1459.

65. Gareth R Owen, Andrew JP (2009) White and Ramon Vilar Organometallics 28: 5783-5793.

66. Gareth R Owen, Ramón Vilar, Andrew JP White, David J Williams (2003) Organometallics 22: 4511-4521.

67. Gilman H, Barnett MM (1936) Recueil des Travaux Chimiques des Pays-Bas 55: 563.

68. Shimizu D, Takeda N, Tokitoh N (2007) J Organomet Chem 692: 2716.

69. Takahashi Y, Ts Ito, Sakai S, Ishii Y (1970) Journal of the Chemical Society D: Chemical Communications 1065.

70. Paquette LA (1996) Encyclopedia of Reagents for Organic Synthesis. J Wiley and Sons, Sussex, England, UK.

71. Pierpont CG, Mazza MC (1974) Inorg Chem 13: 1891.

72. Bincoletto C, Tersariol IL, Oliveira CR, Dreher S, Fausto DM, et al. (2005) Chiral cyclopalladated complexes derived from N,N-dimethyl-1-phenethylamine with bridging bis(diphenylphosphine)ferrocene ligand as inhibitors of the cathepsin B activity and as antitumoral agents. Bioorg Med Chem 13: 3047-3055.

73. Spencer J, Sharratt DP, Dupont J, Monteiro AL, Reis VI, et al. (2005) Organometallics 24: 5665–5672.

74. Casini A, Gabbiani C, Sorrentino F, Rigobello MP, Bindoli A, et al. (2008) Emerging protein targets for anticancer metallodrugs: inhibition of thioredoxin reductase and cathepsin B by antitumor ruthenium(II)-arene compounds. J Med Chem 51: 6773-6781.

75. Elgazwy AS, Ismail NS, Atta-Allah SR, Sarg MT, Soliman DH, et al. (2012) Palladacycles as antimicrobial agents. Curr Med Chem 19: 3967-3981.