New cyclopalladated benzothiophenes: a catalyst precursor for the Suzuki coupling of deactivated aryl chlorides†

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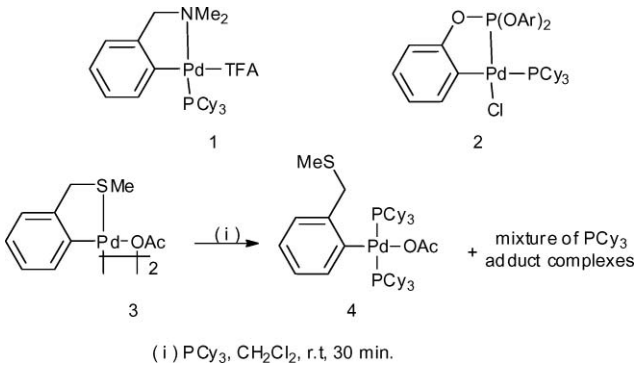
Dimeric benzothiophene-based palladacycles were synthesized from thioanisole-substituted perfluoroalkyl propargyl imines and palladium(II) salts *via* an intramolecular thiopalladation pathway. The treatment of benzothiophene-based palladacycles with an excess of phosphine ligands in benzene at room temperature selectively afforded *trans*-bis(phosphine) palladium complexes in good yields. The *trans*-bis(tricyclohexylphosphine) palladium complex was found to be an active catalyst in the Suzuki coupling of electron rich aryl chlorides. The complex was also employed in the catalytic synthesis of sterically hindered biaryls. The anticancer activity of palladacycles is also discussed.

Introduction

Palladacycles are a class of organometallic compounds of considerable interest due to their air and moisture tolerant character and also due to the variety of applications in catalysis, material science, organic synthesis, and in biological and medicinal chemistry as a potential anticancer agent.[1] In recent times, several novel palladacycles with PC, SC and NC donor ligands, and PCN, NCN, SCS and PCP pincer-type palladacycles have been successfully used in C–C coupling reactions with the advantages of their structural versatility, easy synthetic accessibility, high stability and high yield of products.[2] Similarly, *in situ*-generated palladium nanoparticles from palladacycles were also reported as excellent catalysts for coupling of activated aryl halides with arylboronic acids.[3] However, very few palladacycles are effective at activating less reactive aryl bromides substituted with electron donating groups or readily available aryl chlorides under relatively mild reaction conditions.[4] In this regard, the phosphine and *N*-heterocyclic carbene adduct palladacycles have emerged as active catalysts for C–C coupling, especially for the Suzuki coupling of aryl chlorides, and they are far more active than their parent dimeric palladacycles.[5,6] Among the phosphines, secondary and tertiary phosphine, $HP(^tBu)_2$, $HPCy_2$, or PCy_3, *etc.*, have been found to exhibit high activity in Suzuki coupling reactions of aryl chlorides.[7] Of particular note, in the synthesis of tricyclohexylphosphine (PCy_3) containing palladacycles, Bedford and coworkers selectively isolated mono phosphine adducts when phosphite-, imine-, and amine-based palladacycles were used (**1** and **2**) whereas thioether-based palladacycle, **3** afforded mixtures of tricyclohexylphosphine (PCy_3) adducts, predominantly *trans*-bis(phosphine) species (**4**).[7a,b,8] The bis(phosphine) PCP palladium complex is acyclic and does not contain a Pd–S bond. Notably, the *in situ*-prepared mixtures of products (**4** and a mixture of PCy_3 adduct complexes) from **3** and tricyclohexylphosphine have been found to be active in the Suzuki coupling of aryl chlorides.



Surprisingly, the closely related phosphino PCP palladium pincer complexes [Pd{OC(O)CF_3}{$C_6H_3(CH_2PPr^i_2)_2$-2,6}], [Pd{OC(O)CF_3}{C_6CH_2-1-$(CH_2PR_2)_2$-2,6-$(CH_2)_2$-3,5-H-4}] (R = Pr^i, Bu^t) were found to be inactive in the Heck coupling of aryl chlorides and pincer-type POCOP, bis (dicyclohexylphosphinito) palladium complexes afforded only low yields of biaryl product in coupling of 4-chloronitrobenzene with phenyl boronic acid.[9,10] In contrast, Shen observed that $PdCl_2(PCy_3)_2$ was an effective palladium catalyst for the Suzuki coupling of deactivated aryl chlorides.[11]

Recently, we have reported the synthesis of dimeric benzothiophene-based palladacycles *via* intramolecular thiopalladation of perfluoroalkyl propargyl imines.[12] In this paper, we report the selective synthesis of new acyclic bis(phosphine) palladium complexes from benzothiophene based palladacycles as precursors and their application to Suzuki coupling reactions. The air and moisture stable new acyclic bis(phosphine) palladium complexes have also found to be active for the synthesis of sterically

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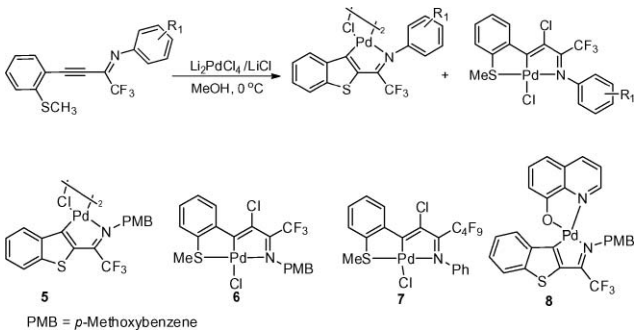
hindered biaryls. We also herein report the anticancer activity of benzothiophene-based palladacycles in human leukemia cell lines.

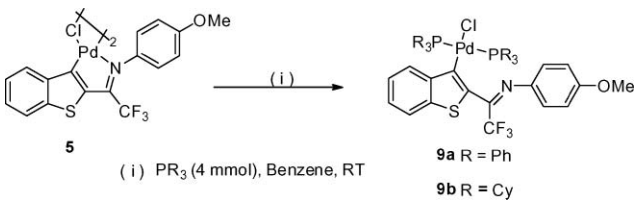
Results and discussion

The chloride-bridged benzothiophene-based palladacycles and SCN pincer palladacycles were isolated from thioanisole substituted perfluoroalkyl propargyl imines and Li_2PdCl_4 in MeOH at 0 °C (Scheme 1). Later the preparation of chloride bridged benzothiophene based palladacycle was selectively achieved using bis-benzonitrile palladium chloride.[12] All the palladacycles **5**, **6**, **7** and **8** were fully characterized by multi-nuclear NMR, IR, and mass spectroscopy.



Scheme 1

In an attempt to form monomers of the benzothiophene-based palladacycle **5**, various phosphorous based ligands were used with the expectation that they could serve as an active catalyst in the C–C coupling reactions. We found that trivalent phosphine ligands are most suitable ligands for the formation of adduct complex. Initially, **5** was treated with two equivalents of triphenylphosphine in benzene at room temperature to give **9a** as an air and moisture stable yellow bis(phosphine) adduct complex in 44% yield. In the reaction mixture, no trace of a monophosphine adduct was observed. By increasing the stoichiometry of PPh_3 to four equivalents, the yield of **9a** was increased to 91% (Scheme 2). The ^{1}H NMR spectrum and CHN analysis showed **9a** contains two molecules of phosphine per molecule of benzothiophene ligand. The presence of a single peak in the ^{31}P NMR at 22.1 ppm indicating the phosphine ligands are magnetically equivalent and coordinated in a *trans* arrangement about the palladium atom.[13]



Scheme 2

Similarly, the reaction of tricyclohexylphosphine with **5** yielded only the air and moisture stable bis(tricyclohexylphosphino) complex **9b** in 77% yield; there was no evidence for a monophosphine adduct. The phosphorous chemical shift [δ 22.49 (s)] is similar to that of *trans*-[$Pd(PCy_3)_2(C_6H_5)Cl$] compound [δ 22.9 (s)] obtained by the oxidative addition of chlorobenzene to [$Pd(PCy_3)_2dba$].[14] The ^{19}F NMR also showed a single peak which confirmed the presence of a single compound.

A single crystal of **9a** was obtained upon slow evaporation of chloroform and carbon tetrachloride (9 : 1) at room temperature. The molecular structure of complex **9a** was determined by X-ray diffraction analysis, confirming disruption of chloride bridges and Pd–N bonds by coordinated PPh_3 (Fig. 1).[15] Complex **9a** adopts a distorted square planar geometry at the metal centre with the two triphenylphosphine ligands disposed *trans* relative to each other. The stability of the Pd–N bond in cyclopalladated derivatives is, in general, highly dependent on the basicity of the nitrogen atom. The basicity of the nitrogen atom in cyclopalladated compound **5** is reduced due to the presence of the electron withdrawing trifluoromethyl group, thus, facilitating ring opening by the more basic phosphines.

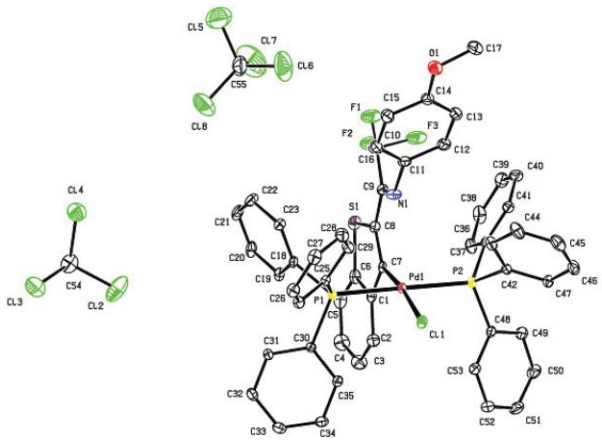
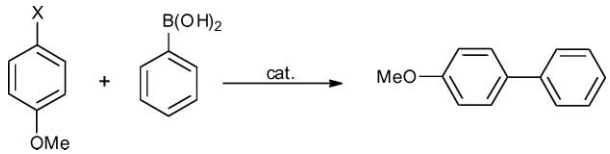


Fig. 1 The molecular structure of **9a**, with the atom-numbering scheme. Displacement ellipsoids are drawn at the 30% probability level. The minor components of the disordered F11/F21/F31 and H atoms have been omitted for clarity.

Next, we screened the catalytic activity of palladium complexes **9a** and **9b** for the Suzuki coupling of aryl halides. Complex **9a** was initially applied to the Suzuki coupling of 4-chloroanisole with phenylboronic acid using 1 mol% of the catalyst in 1,4-dioxane and in the presence of Cs_2CO_3 at 100 °C without any external additive such as [NBu_4Br], [PPh_4]Cl *etc.* The corresponding biaryl was only formed in low yields. Though the complex **9a** was not active enough for chloroanisole, excellent yields of biaryl were achieved using bromoanisole (Table 1, entry 2). With the reduction of catalyst loading to 0.2 mol%, there was no significant change in yield. Complex **9b** was also subjected to Suzuki coupling of chloroanisole with phenylboronic acid using 1 mol% of the catalyst under same conditions, and the corresponding biaryl was isolated in 94% yield; <2% biphenyl was observed as a homo-coupled product from phenylboronic acid after 8 h. On decreasing the catalyst loading to 0.5 mol%, no significant change in yield was observed, although the reaction time was extended up to 10 h for completion. The maximum yield was optimized in presence of Cs_2CO_3 as a base and 1,4-dioxane as a solvent at 100 °C.

The coupling of sterically hindered aryl chlorides is one of the more important and challenging area in organic synthesis. To explore the application of complex **9b** in the Suzuki coupling of sterically hindered substrates, the methodology was

Table 1 Catalytic activity of complexes **9a** and **9b** in the Suzuki coupling of chloro and bromoanisoles with phenyl boronic acid[a]



Entries	X	Catalyst (mol%)	Time/h	Isolated yield (%)
1	Cl	**9a** (1)	24	12
2	Br	**9a** (1)	5	96
3	Br	**9a** (0.2)	12	94
4	Cl	**9b** (1)	6	94
5	Cl	**9b** (0.5)	10	92

[a] Reaction Condition: aryl halide (2 mmol), phenyl boronic acid (3.0 mmol), Cs_2CO_3 (6 mmol), 1, 4-dioxane (5 mL), 100 °C.

extended to hindered *o*-tolylboronic acid, electron withdrawing *o*-formylphenyl boronic acid and 2-chlorotoluene. Excellent yield of respective Suzuki coupled product, **10a** and **10b** (Chart 1), were obtained. The sterically hindered 2-chloro-*m*-xylene was also tested with electron donating, electron withdrawing and hindered boronic acids and excellent yields of the respective coupled product, **10c**, **10d**, **10e** and **10f** was observed (Chart 1). The coupling of electron rich 2, 3 and 4-chloroanisoles gave the corresponding biaryls, **10g**, **10h**, **10i** and **10j** in 91–95% yields (Chart 1). The heterocyclic based palladium complexes derived from the annulated benzothiophene-based palladacycles shows excellent catalytic activity in the C–C coupling of deactivated aryl chlorides and boronic acids.

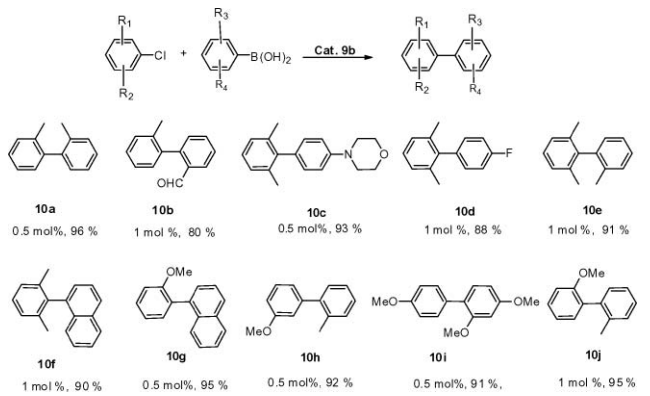


Chart 1 Reaction conditions: aryl chloride (1 mmol), phenyl boronic acid (1.5 mmol), catalyst **9b**, Cs_2CO_3 (3 mmol), 1,4-dioxane (3 mL), 100 °C, 10–12 h.

Anticancer activity of cyclopalladated compounds

Research interest towards palladium complexes has increased considerably in order to obtain metal-based drugs that possess reduced toxicity compared to *cis*platin and analogous compounds. However, palladium complexes have a tendency to undergo fast hydrolysis, to the similar platinum based complexes. This problem has been over come by the introduction of cyclopalladated complexes that are more stable, less toxic and show specific anticancer activity. Therefore, cyclopalladated compounds are receiving much attention in the field of medicinal chemistry.

Table 2 IC_{50} of compounds represented in μg ml^{-1} in 3 million cells by MTT assay

Compounds	HL60	K562	CCRF
Compound **5**	14.8	10.7	14.7
Compound **6**	4.8	5.1	4.9
Compound **7**	19.9	9.9	19.9
Compound **8**	25.2	5.2	15.0

We have tested the palladacycles (**5–8**) for anticancer activity using three human leukemia cell lines. The cytotoxicity of the compounds was evaluated by MTT assay.[16] The modified cytotoxicity assay was performed on different panels of human leukemic cell lines HL60 (*Human promyelocytic leukemia cells*), K562 (*Chronic myelogenous leukemia*) and CCRF-CEM (*Human acute lymphocytic leukemia*). The cells were grown in RPMI 1640 medium supplemented with 10% fetal calf serum under standard contentions (37 °C temperature with 5% CO_2). For cytotoxicity assay two million cells were plated in a 96 well plates containing 100 μL of medium and pre-incubated for 24 h at 37 °C with 5% CO_2. After 24 h the cyclopalladated compounds were introduced with respective concentration including DMSO and cyclohexamide in a separate well as a vehicle control and positive control respectively. Each treatment was performed in triplicate in three different experiments. To evaluate the number of viable cells at the end of compound incubation, MTT solution (sigma 10μ/well) was added to the cells. The plates were incubated for 3 h at 37 °C with 5% CO_2. After 3 h, DMSO (200 μl) was added and then the absorbance was measured at 550 nm using Spectra Max. 1909 plate reader. The results are expressed ±SD of the percentage of viable cells at each compound concentration compared to untreated control cells and the concentrations that inhibited cell growth by 50% (IC_{50}) were calculated on the means of three experiments. The concentrations of all compounds required achieving the 50% inhibition of cell growth (IC_{50}) with the exposure of the compounds for 24 h were determined. The IC_{50} values of the compounds are summarized in the bar diagram (Table 2 and Fig. 2). Compound **6** displayed the greatest inhibition to three human leukemia cell lines, HL60 (*Human promyelocytic leukemia cells*), K562 (*Chronic myelogenous leukemia*) and CCRF-CEM (*Human acute lymphocytic leukemia*) with an IC_{50} value of 9.5 μM. as well as compound **8** showed greatest inhibition to K562 (*Chronic myelogenous leukemia*) with an IC_{50} value of 8.5 μM.

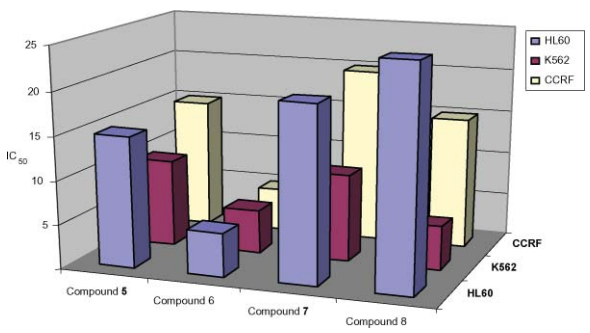


Fig. 2 IC_{50} (μg mL^{-1}) of palladacycles (**5–8**) evaluated in three human leukemic cell lines.

Table 3 Annexin V positive in human promyelocytic leukemia cells

S. No.	Treatment	% of Annexin V + Cells
1	Control	1.29
2	DMSO	0.72
3	Compound **8**	9.25
4	Compound **6**	24

Membrane permeability study and apoptosis study

To evaluate the necrotic cell death, the chosen cell lines were cultured till it attains 50 to 60 percent confluence, then the cells HL60, K562 and CCRF were harvested and suspended in plain RPMI 1640 medium. The samples were maintained at room temperature before the treatment with compounds. The cells were incubated with compound **6** and **8** for 24 h then stained with propidium iodide and analyzed in the Fluorescence Activated Cell Sorter (FACS). In the FACS propidium iodide was excited with 488 nm laser light and the emission (fluorescence) signals were collected and recorded using the appropriate optical filters (585/45 band pass filter) and computer aided software Cell Quest® software. The result suggested that the cells positive to propidium iodide were membrane permeabilized cells and the necrotic cell death occurred over a period of 24 h (for Figure see the ESI†). In the first 6 h, the cells undergo early an apoptotic event like expression of phosphatidylserine monitored by Annexin V. The cells positive to Annexin V are represented in Table 3.

Conclusions

In summary, we have developed a new class of dimeric benzothiophene-based palladacycles, which act as precursor for the preparation of monomeric bis(phosphine) palladium complexes, in good yields under mild reaction conditions. These complexes are efficient catalysts for the synthesis of hindered biaryls from aryl chlorides and boronic acids. Palladacycles **6** and **8** show excellent anticancer activity (highest inhibitory against HL60, K562 and CCRF leukaemia cells with an IC_{50} values 9.5 μM and 8.5 μM, respectively). The membrane permeability and apoptotic studies suggested that the mechanism of the anticancer activity is mediated through apoptosis followed by membrane permeability.

Experimental

All reactions were carried out in oven dried glassware under an atmosphere of dry nitrogen. Chemicals were purchased from Aldrich and used as received unless mentioned otherwise. All solvents used were dried before use. Product purification by column chromatography was accomplished using silica gel 60–120 mesh. Technical grade solvents were used for chromatography and distilled prior to use. NMR spectra were recorded in Fourier transform mode. The ^{1}H NMR, ^{31}P NMR and ^{19}F NMR spectra were recorded on a Bruker-Avance (300 MHz) and Varian-Inova (400 MHz) spectrometer using $CDCl_3$ and C_6D_6 solvents, and TMS as the internal standard. ^{31}P and ^{19}F are referenced to an external standard 85% H_3PO_4 and $CFCl_3$ respectively. Multiplicities in the ^{1}H NMR spectra are described as: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad; coupling constants are reported in Hz. Elemental (CHN) analyse were performed in Vario MICRO cube. The benzothiophene-based palladacycles were prepared according to a published procedure.[12]

General method for the synthesis of palladium complexes

In a Schlenk tube under an atmosphere of nitrogen were placed the palladacycle **5** (0.25 mmol) and benzene (1 mL). The appropriate phosphine (1 mmol, 4 equiv) was added and the resultant mixture was then stirred at room temperature for 2 h. The solvent was removed from the reaction mixture under reduced pressure. The crude product was then crystallized from a mixture of hexane–EtOAc (50 : 50) to give analytically pure palladium phosphine adduct.

Spectral data for 9a complex

Yield: 454 mg (91%). Yellow solid, ^{1}H NMR (300 MHz, $CDCl_3$): 7.96-7.91 (m, 1H), 7.47-7.34 (m, 13H), 7.30–7.23 (m, 6H), 7.18–7.10 (m, 12H), 7.07–7.00 (m, 1H), 6.86-6.76 (m, 3H), 6.52 (d, J = 9.0 Hz, 2H), 3.88 (s, 3H). ^{19}F NMR (376 MHz, $CDCl_3$): –58.47 (s, 3F). ^{31}P NMR (162 MHz $CDCl_3$): 22.29 Anal. Calcd. for $C_{53}H_{41}ClF_3NOP_2PdS{\cdot}CHCl_3{\cdot}CCl_4$: C 51.85, H 3.32, N 1.10. Found: C 51.80, H 3.33, N 1.15.

Spectral data for 9b complex

Yield: 400 mg (77%). Yellow solid, ^{1}H NMR (400 MHz, C_6D_6): 8.90-8.85 (m, 1H), 7.57-7.54 (m, 1H), 7.33 (d, J = 8.8 Hz, 2H), 7.28-7.23 (m, 1H), 7.14-7.09 (m, 1H), 6.92 (d, J = 8.8 Hz, 2H), 3.29 (s, 3H), 2.30-2.21 (m, 6H), 2.13–2.02 (m, 12H), 1.78–1.48 (m, 30H), 1.18–0.93 (m, 18H). ^{19}F NMR (376 MHz, $CDCl_3$): –57.50 (s, 3F). ^{31}P NMR (162 MHz $CDCl_3$): 22.49 Anal. Calcd. for $C_{53}H_{77}F_3NOP_2SPdCl$: C 61.38, H 7.48, N 1.35. Found: C 61.40, H 7.55, N 1.29.

General method for the Suzuki coupling of aryl halides and aryl boronic acids

In an oven dried reaction vessel, aryl halide (1 mmol), aryl boronic acid (1.5 mmol), Cs_2CO_3 (3 mmol), catalyst **9a** or **9b**, were charged with 1,4-dioxane (3 mL). The mixture was stirred at 100 °C under a N_2 atmosphere and the progress of the reaction was monitored by GC. After completion of the reaction, water was added and the reaction mixture was extracted with EtOAc (3 × 20 mL). The combined organic layers were dried over anhydrous $MgSO_4$ and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography (silica gel, hexane) to obtain pure biaryl.

Spectral data

2,2′-Dimethyl-biphenyl 10a. Colorless liquid; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.23-7.13 (m, 6H), 7.08-7.03 (m, 2H), 2.05 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 19.81, 125.50, 127.11, 129.24, 129.77, 135.78, 141.56. EI-MS (m/z): 182 (M^+). Anal. Calcd. for $C_{14}H_{14}$: C 92.26, H 7.74. Found: C 92.21, H 7.77.

2′-Methylbiphenyl-2-carbaldehyde 10b. Yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.72 (s, 1H), 8.03-7.98 (m, 1H), 7.64-7.57

(m, 1H), 7.51-7.45 (m, 1H), 7.32-7.14 (m, 5H), 2.10 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.23, 125.59, 126.97, 127.73, 128.20, 129.99, 130.09, 130.66, 133.63, 133.72, 136.06, 137.38, 145.56, 192.13. EI-MS (*m/z*): 196 (M^+). Anal. Calcd. for $C_{14}H_{12}O$: C 85.68, H 6.16. Found: C 85.61, H 6.21.

4-(2′,6′-Dimethyl-biphenyl-4-yl)-morpholine 10c. White solid: mp 82-84 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.07-6.97(m, 5H), 6.93-6.87 (m, 2H), 3.88-3.82 (m, 4H), 3.20-3.14 (m, 4H), 2.02 (s, 6H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.91, 49.36, 66.91, 115.47, 126.78, 127.18, 129.82, 132.73, 136.53, 141.53, 160.04. ESI-MS (*m/z*): 268 ($M+H^+$) HRMS: *m/z* calcd for $C_{18}H_{21}NO$ ($M+H^+$) 268.1701, found 268.1703. Anal. Calcd. for $C_{18}H_{21}NO$: C 80.86, H 7.92, N 5.24. Found: C 80.82, H 7.95, N 5.27.

4′-Fluoro-2,6-dimethyl-biphenyl 10d. Colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.13-6.99 (m, 7H), 2.0 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.80, 115.35 (d, Jc = 21.41 Hz), 127.21, 127.33, 128.54 (d, Jc = 8.23 Hz), 130.55 (d, Jc = 7.68 Hz), 136.20, 136.84 (d, Jc = 3.84 Hz), 140.78, 161.72 (d, Jc = 245.35 Hz). EI-MS (*m/z*): 200 (M^+). Anal. Calcd. for $C_{18}H_{21}NO$: C 80.86, H 7.92, N 5.24. Found: C 80.82, H 7.95, N 5.27.

2,6,2′-Trimethyl-biphenyl 10e. Colorless liquid; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.27-7.17 (m, 3H), 7.15-6.95 (m, 4H), 1.97 (s, 3H), 1.94 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 19.55, 20.50, 126.17, 127.02, 127.10, 127.35, 128.90, 130.05, 135.47, 135.63, 140.66, 141.04. EI-MS (*m/z*): 196 (M^+). Anal. Calcd. for C18H21NO: C 80.86, H 7.92, N 5.24. Found: C 80.82, H 7.95, N 5.27.

1-(2,6-Dimethyl-phenyl)-naphthalene 10f. White solid: mp: 74-75 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.89-7.78 (m, 2H), 7.55-7.39 (m, 2H). 7.34-7.29 (m, 2H), 7.25-7.09 (m,4H), 1.90 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.38, 125.34, 125.68, 125.74, 126.03, 126.41, 127.17, 127.24, 127.29, 128.26, 131.71, 133.72, 136.97, 138.72, 139.61. EI-MS (*m/z*): 232 (M^+). Anal. Calcd. for $C_{18}H_{16}$: C 93.06, H 6.94. Found: C 93.10, H 6.99.

1-(2-Methoxyphenyl)naphthalene 10g. White solid: mp: 98-99 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.85-7.76 (m, 2H), 7.56-7.18 (m, 7H), 7.05-6.94 (m, 2H), 3.66 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.53, 110.97, 120.55, 125.37, 125.56, 125.63, 126.42, 127.29, 127.64, 128.12, 128.99, 129.50, 131.93, 132.15, 133.45, 136.93, 157.26. EI-MS (*m/z*): 232 (M^+). Anal. Calcd. for $C_{17}H_{14}O$: C 87.15, H 6.02. Found: C 87.12, H 5.99.

3′-Methoxy-2-methyl-biphenyl 10h. Colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.31-7.13 (m, 5H), 6.88-6.78 (m, 3H), 3.81 (s, 3H), 2,26 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.44, 55.21, 112.24, 114.80, 121.65, 125.68, 127.27, 129.00, 129.61, 130.26, 135.30, 141.77, 143.34, 159.24. EI-MS (*m/z*): 198 (M^+). Anal. Calcd. for $C_{14}H_{14}O$: C 84.81, H 7.12. Found: C 84.79, H 7.15.

2,4,4′-Trimethoxy-biphenyl 10i. Yellow viscous liquid; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.40-7.34 (m, 2H), 7.19-7.14 (m, 1H), 6.92-6.86 (m, 2H), 6.52-6.48 (m, 2H), 3.83 (s, 3H), 3.79 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.10, 55.25, 55.36, 98.83, 104.41, 113.34, 123.09, 130.33, 130.65, 130.86, 157.24, 158.20, 159.86. ESI-MS (*m/z*): 245 $(M+H)^+$. HRMS: *m/z* calcd for $C_{15}H_{16}O_3$ $(M+H)^+$ 245.1177, found 245.1186. Anal. Calcd. for $C_{15}H_{16}O_3$: C 73.75, H 6.60. Found: C 73.71, H 6.66.

2-Methoxy-2′-methyl-biphenyl 10j. Colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.33-7.04 (m, 6H), 6.99-6.87 (m, 2H), 3.74 (s, 3H), 2.12 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 19.90, 55.36, 110.61, 120.40, 125.40, 127.27, 128.52, 129.54, 129.96, 130.81, 130.97, 136.78, 138.60, 156.56. EI-MS (*m/z*): 198 (M^+). Anal. Calcd. for $C_{14}H_{14}O$: C 84.81, H 7.12. Found: C 84.85, H 7.16.

Biological Methods

Tissue culture. All the experiments pertaining anticancer activity were done with the HL60, K562 and CCRF cell lines. The cell lines were stored in the liquid nitrogen for long-term preservation. These cells were maintained in RPMI 1640 (Gibco Life Technologies, USA) containing 10% fetal calf serum (Sigma Chemicals Company, St. Louis, USA) with 1% $NaHCO_3$, penicillin streptomycin and Gentamycin. The cultures were grown in 5% CO_2 in air at 37 °C and the cells were used within the first 5–7 passages after being revived from liquid nitrogen.

Flow cytometry. The flow cytometric studies were done in the FACS Calibur flow cytometer. The membrane permeability and apoptosis studies were analyzed in FACS. The cells were grown up to sub confluence in a 75 Sq cm cell culture flask. The cell membrane permeability was evaluated upon cyclopalladated compound treatment. The cells were harvested, washed and counted. One million cells were taken in a sample tube then treated with cyclopalladated compound **6** and **8** for 6 h then the cells were stained with propidium iodide for 30 min the stained samples were applied in the flow cytometer and analyzed. The propidium iodide was excited with an argon-ion laser 488 nm. The emission light was collected at 585/45-band pass filter. The scatter and the fluorescence data were collected from the FACS machine. The data was analyzed using Becton Dickinson Cell Quest® software.

Apoptosis study. In order to investigate the apoptotic property for cyclopalladated compounds, HL60 (Human Promyelocytic leukemia cells) cells were treated with the compound **6** and **8** for 6 h and stained the cells with Annexin V FITC. The resultant cells were analyzed in the FACS (fluorescence activated cell sorter).

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