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SELECTIVE ARYLATION OF PHENOL PROTECTED PROPARGYL BROMIDE VIA PD-CATALYSED SUZUKI COUPLING REACTION: SYNTHESIS, MECHANISTIC STUDIES BY DFT CALCULATIONS AND THEIR PHARMACOLOGICAL ASPECTS

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Abstract: Biaryls are the potential source of synthetic drugs. The present study describes the synthesis of a series of functionalized biphenyl derivatives **(3a-3g)** using Pd-catalyzed Suzuki coupling reaction. The experimental results revealed the facile synthesis of biphenyl derivatives **(3a-3g)** with notably high yield (80-88%). Density functional theory (DFT) studies were performed by Gaussian 09 software in order to rationalize the selectivity of coupling at C-Br bond instead of C-Cl bond. In addition of synthesis, the biological activities (biofilm inhibition, hemolytic and anti-thrombolytic) of these novel compounds were investigated. These results exhibited good biofilm inhibition (5.86-65.8%), hemolytic (1.32-30.1%) and anti-thrombolytic activities (9.64-42.5%), indicating the potential use of these compounds for pharmaceutical applications.

Keywords: Suzuki coupling, DFT, phenol, organoboron, biaryl, biofilm

Suzuki reaction of aryl halides with organoboron offers a facile synthetic route to synthesize biaryls (1-2). This cross-coupling reaction is typically attractive and beneficial for the construction of C-C bonds due to number of reasons such as high functional group tolerance, favorable reaction kinetics and easy handling of non-hazardous by-products (1-5). The Biaryl motifs are important substructures in pharmaceuticals as anti-arthritic, cholesterol inhibition, anti-leukemic activities. These are also potentially useful in agrochemicals, energy and functional materials (6-7). Therefore, the synthesis of novel biaryls with the unique therapeutic features is gaining significant attention from the scientific community working in the development of new drugs. In view of the medicinal importance of biaryls, the present research was carried out to synthesize the various functionalized biaryl derivatives *via* Palladium (Pd) catalyzed Suzuki coupling reaction. In addition to the synthesis, we have also investigated the potential pharmaceutical applications of the newly synthesized biaryls to combat various ailments.

MATERIALS AND METHODS

General

Melting point apparatus (B-540) was used for determining the melting points of synthesized solids. ^{1}H-NMR was measured using $CDCl_3$ (Bruker

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ARX at 300, 400, 500, 600 MHz). JMS-HX-110 spectrometer with a data system was used for EI-MS spectra. For column chromatography, silica gel (70–230 and 230–400 mesh) was used. TLC was used for monitoring the completion of reaction, (Merck silica gel 60 PF_{254} cards) and the visualization of compounds was done by UV lamp (254-365 nm).

General procedure for the synthesis of intermediate compound 4-bromo-2-chloro-1-(prop-2-yn-1-yloxy)benzene (2)

4-Bromo-2-chlorophenol (1.0 g, 4.83 mmol), propargyl bromide (0.688 g, 5.78 mmol), potassium carbonate (K_2CO_3) (2.33 g, 16.8 mmol) and 15 mL of dry acetone was added into Schlenk flask. Then it was refluxed at 80°C for 5 h. After that Schlenk flask cooled at room temperature. Completion of the reaction was monitored by TLC followed by the addition of distilled H_2O (10 mL) and dichloromethane (10 mL DCM). The organic layer was separated and the remaining moisture from organic layer was removed by using Sodium sulphate (Na_2SO_4). After filtration and subsequent evaporation, the product was purified by flash chromatography. Mixture of *n*-hexane and ethyl acetate was used as eluent (8).

General procedure for the synthesis of biaryl compounds (3a-g)

A mixture containing Phenol **(2)** (0.250 mg, 1.09 mmol), tetrakis-triphenylphosphine palladium (0.027 g, 2.5 mol%) as catalyst and 1,4-dioxane (2.5 mL) was taken in the Schlenk flask, followed by stirring at room temperature for 20 min in presence of inert atmosphere. To this mixture, respective arylboronic acid (1.54 mmol) together with a known amount of potassium phosphate (K_3PO_4) and water (0.625 mL) were added to a reaction flask. This reaction mixture was again stirred at 80-90°C for 15 h. After cooling the reaction mixture, it was further diluted with ethyl-acetate and distilled water. The organic layer was separated using a separating funnel and sodium sulfate was used to remove the remaining moisture content. The mixture was further concentrated and dried by using rota-vapour. The obtained residue was purified by the column chromatography using a mixture of ethyl acetate and *n*-hexane as an eluent.

Characterization data

4-Bromo-2-chloro-1-(prop-2-yn-1-yloxy)benzene (2)

Obtained as solid, mp = 516°C. ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 7.51 (d, $J_{1,3}$ = 2.4 Hz, 1H), 7.33-7.18 (m, 1H), 6.95 (d, $J_{1,2}$ = 8.4 Hz, 1H), 4.74 (d, $J_{1,3}$ = 2.4 Hz, 2H), 2.53-2.52 (m, 1H). ^{13}C NMR ($CDCl_3$, 150 MHz, δ, ppm): 150.1, 130.0 (m), 121.2, 118.0, 116.1, 78.7, 75.1, 54.1. EI/MS (m/z, + ion mode): $[M^+]^+$ = 246 $[M+2]^+$ = 248, $[M+4]^+$ = 250.

3-Chloro-3,5'-dimethyl-4-(prop-2-yn-1-yloxy)-1,1'-biphenyl (3a)

Obtained as solid, mp = 484°C. ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 7.51 (d, $J_{1,3}$ = 1.8 Hz, 1H), 7.37 (dd, $J_{1,2}$ = 8.4 Hz, $J_{1,3}$ = 2.4 Hz, 1H), 7.11 (br.s, 1H), 7.04 (d, $J_{1,2}$ = 8.4 Hz, 2H), 6.95 (br.s, 1H), 3.47 (s, 2H), 2.40 (br.s, 1H), 2.34 (s, 6H), ^{13}C NMR ($CDCl_3$, 150 MHz, δ, ppm): 21.3, 22.0, 54.2, 76.1, 78.9, 115.3, 122.6, 125.0, 126.3, 128.4, 129.2, 133.4, 138.2, 138.9, 142.9, 153.6. EI/MS (m/z, + ion mode): $[M^+]^+$ = 271, $[M+2]^+$ = 273.

3-Chloro-3,5'-diflouro-4-(prop-2-yn-1-yloxy)-1,1'-biphenyl (3b)

Obtained as solid, mp = 460°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.67-7.62 (m, 2H), 7.33 (dd, $J_{1,2}$ = 8.4, $J_{1,3}$ = 2.0 Hz, 1H), 7.08 (d, $J_{1,2}$ = 8.4 Hz, 1H), 7.01 (d, $J_{1,2}$ = 6.4 Hz, 1H), 6.74 (t, $J_{2(1,3)}$ = 8.8 Hz, 1H), 4.43 (s, 2H), 3.58 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz, δ, ppm): 58.2, 76.2, 79.7, 119.0 (m), 101.2, 116.9, 122.0, 125.6, 130.9, 134.9, 140.1, 152.0, 165.8 (m) 167.0 (m). EI/MS (m/z, + ion mode): $[M^+]^+$ = 279.2, $[M+2]^+$ = 281.

3-Chloro-4'-methyl-[1,1'-biphenyl]-4-ol (3c)

Obtained as solid, mp = 454°C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.52 (d, $J_{1,3}$ = 3.0 Hz, 1H), 7.40-7.36 (m, 3H), 7.22-7.20 (m, 2H), 7.05 (d, $J_{1,2}$ = 9.0 Hz, 1H), 2.36 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz, δ, ppm): 22.0, 116.2, 123.1, 126.2, 127.8, 129.0, 129.8, 130.1, 130.8, 132.1, 139.0, 152.9. EI/MS (m/z, + ion mode): $[M]^+$ = 218.0, $[M+2]^+$ = 220.

3-Chloro-4'-methoxy-[1,1'-biphenyl]-4-o l(3d)

Obtained as solid, mp = 475°C. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 7.48 (d, $J_{1,2}$ = 2.4 Hz, 1H), 7.43-7.40 (m, 2H), 7.34 (dd, $J_{1,2}$ = 8.4, $J_{1,3}$ = 2.4 Hz, 1H), 7.04 (d, $J_{1,2}$ = 8.4 Hz, 1H), 6.95-6.93 (m, 2H), 3.82 (s, 3H). ^{13}C NMR ($CDCl_3$, 150 MHz, δ, ppm): 53.2, 112.3, 114.2, 118.2, 124.3, 128.9, 131.0, 132.9, 133.2, 135.6, 156.2, 159.2. EI/MS (m/z, + ion mode): $[M^+]^+$ = 234.0, $[M+2]^+$ = 236.

3,4'-Dichloro-[1,1'-biphenyl]-4-ol (3e)

Obtained as solid, mp = 575°C.^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.50 (d, $J_{1,2}$ = 3.0 Hz, 1H), 7.44-7.33 (m, 2H), 7.06 (d, $J_{1,2}$ = 9.0 Hz, 2H), 6.98-6.71 (m, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz, δ, ppm): 116.2,

124.3, 126.7, 128.3, 129.1, 129.6, 129.6, 130.1, 134.2, 136.1, 138.9, 151.0. EI/MS (m/z, + ion mode): $[M^+]^+$ = 238.0, $[M+2]^+$ = 240, $[M+4]^+$ = 242.0.

1-(3'-Chloro-4'-hydroxy-[1,1'-biphenyl]-3-yl)ethanone (3f)

Obtained as solid, mp = 516°C. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 8.04 (d, $J_{1,2}$ = 7.2 Hz, 1H), 7.97 (d, $J_{1,2}$ = 7.2 Hz, 1H), 7.53(t, $J_{2(1,3)}$ = 7.2 Hz, 1H), 7.50-7.43 (m, 3H), 2.62 (s, 3H). ^{13}C NMR ($CDCl_3$, 150 MHz, δ, ppm): 27.0, 116.5,125.6, 127.8, 127.9, 128.9, 129.0, 129.9, 131.2, 134.0, 136.7, 143.3, 156.0, 198.0. EIMS (m/z, + ion mode): $[M^+]^+$ = 246, $[M+2]^+$ = 248.

3-Chloro-4'-(methylthio)-[1,1'-biphenyl]-4-ol (3g)

Obtained as solid, mp = 516°C. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 7.48-7.45 (m, 2H), 7.42 (d, $J_{1,2}$ = 8.4 Hz, 1H), 7.36 (d, $J_{1,2}$ = 8.4, $J_{1,3}$ = 2.4 Hz, 1H), 7.29 (d, $J_{1,2}$ = 7.2 Hz, 1H), 7.05 (d, $J_{1,2}$ = 8.4 Hz, 2H), 2.53 (s, 3H).^{13}C NMR ($CDCl_3$, 150 MHz, δ, ppm): 15.6, 115.6, 123.4, 126.7, 127.3, 128.1, 128.9, 129.2, 129.9, 133.2, 138.2, 139.0, 150.9. EI/MS (m/z, + ion mode): $[M^+]^+$ = 250, $[M+2]^+$ = 252.

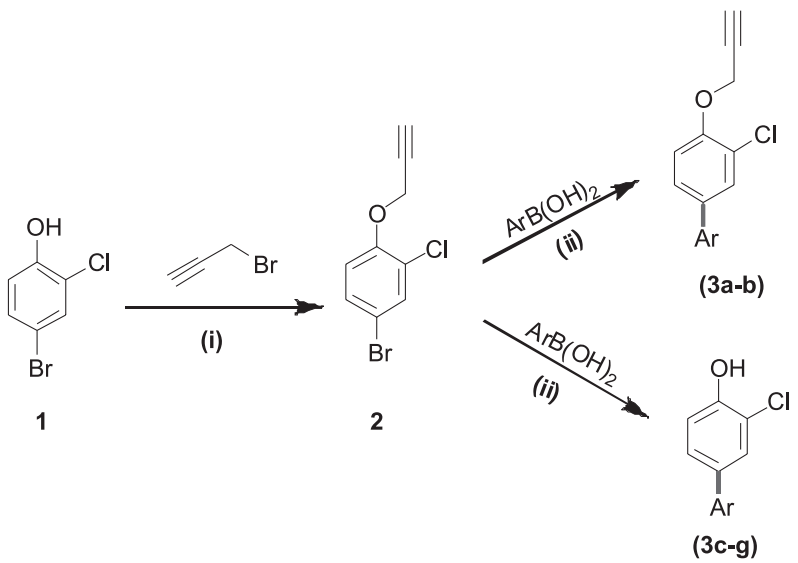
Computational methods

All calculations were performed by using Gaussian 09 software (9) and graphics were analyzed with the help of Gauss view 05 (10). Geometries of the transition states and intermediate products were optimized without any symmetry constraints by implementing a hybrid B3LYP method which provides nice balance between computational cost and accuracy (11-12). For geometries optimization, 6-31G (d) (13, 14) basis set was used for C, H, O, B, Br and Cl atoms and LANL2DZ pseudo potential was used for Pd atom. Every optimized geometry was confirmed by frequency analysis at the same level as a true minimum (no imaginary frequency) or as a transition state (with one imaginary frequency). The reported energies for all mechanistic steps are in kcal mol^{-1}.

Pharmacology

Biofilm inhibition assay by microtitre plate method

The novel biaryl derivatives were examined for their biofilm inhibition activity (15), where Ethylacetate was used for preparing solutions of test samples. In brief, the sample solution (100 µL) (**3a–g**), nutrient broth (100 µL) and the 20 µL of bacterial suspension were inoculated in 96-well plates. Rifampicin and nutrient broth were used as positive and negative controls, respectively. The incubation of well plates was done at 37°C for 24 h. Each plate was washed thrice by Phosphate-buffer solution (PBS) (220 µL), followed by shaking to remove adherent bacteria. The 95% methanolic solution (220 µL) was used to fix remaining bacteria from well plates, followed by oven drying.



Scheme 1. Synthesis of intermediate compound 4-bromo-2-chloro-1-(prop-2-yn-1-yloxy)benzene **(2)** and Suzuki cross coupling of **(2)** with various aryl boronic acids. Conditions: **(i)**, **1** (1 g, 4.83 mmol), propargylbromide (0.68 g, 5.78 mmol), potassium carbonate (K_2CO_3) (2.33 g, 16.8 mmol), 80ºC, dry acetone, reflux 5 h. **(ii), 2** (0.1 g, 1 eq, 0.38 mmol), $ArB(OH)_2$ (1.1 eq, 0.71 mmol), K_3PO_4 (0.21 g, 1.02 mmol), $Pd(PPh_3)_4$ (0.011 g, 2.5 mol%), 1,4-dioxane: H_2O (4 : 1), reflux 18 h, 90°C

For staining the plates, crystal violet (220 µL) was used (50% concentration) for 5 min. Excess stain was removed by tap water from these plates. For removing the remaining dye attached with cells, glacial acetic acid (220 µL) (33% concentration) was used. OD value of each well was examined with the help of the microplate reader (BioTek, Winooski, VT, USA) at 630 nm.

The Biofilm inhibition was quantified by measuring OD_{540} using the following expression:

$$\% \text{ Biofilm inhibition} = \frac{\text{OD growth control} - \text{OD sample control}}{\text{OD growth control}} \times 100$$

Assay of hemolytic activity

The cytotoxicity of novel biaryl compounds **(3a-g)** was also tested using the previously reported method (16). In a typical procedure, a known amount of human blood (heparinized, 3 mL) was collected from the volunteers, which was centrifuged at 1000 rpm for 5 min. After discarding the plasma, chilled 5 mL (4°C) Phosphate-buffer saline (PBS) (pH ~7.4) was used to wash the cells thrice. For each assay, erythrocytes were maintained (108 cells/ mL). After that, the synthesized compounds (100 µL) were mixed with blood cells separately and then the incubation of samples was done at 37°C for 35 min followed by agitation for 10 min. After incubation (for 5 min), the samples were further kept on ice, followed by centrifuge process (100 rpm for 5 min). The chilled buffer solution (4°C) was used for 10 times dilution of supernatants from each tube. As a positive control, the Triton X-100 (0.1% v/v) and as a negative control, the phosphate buffer saline (PBS) was used. Later on, the absorbance of the test solution was measured at 576 nm by using µ Quant (Biotek, USA). The percentage (%) lysis of RBCs of every sample was calculated accordingly.

Analysis of Anti-Thrombolytic activity

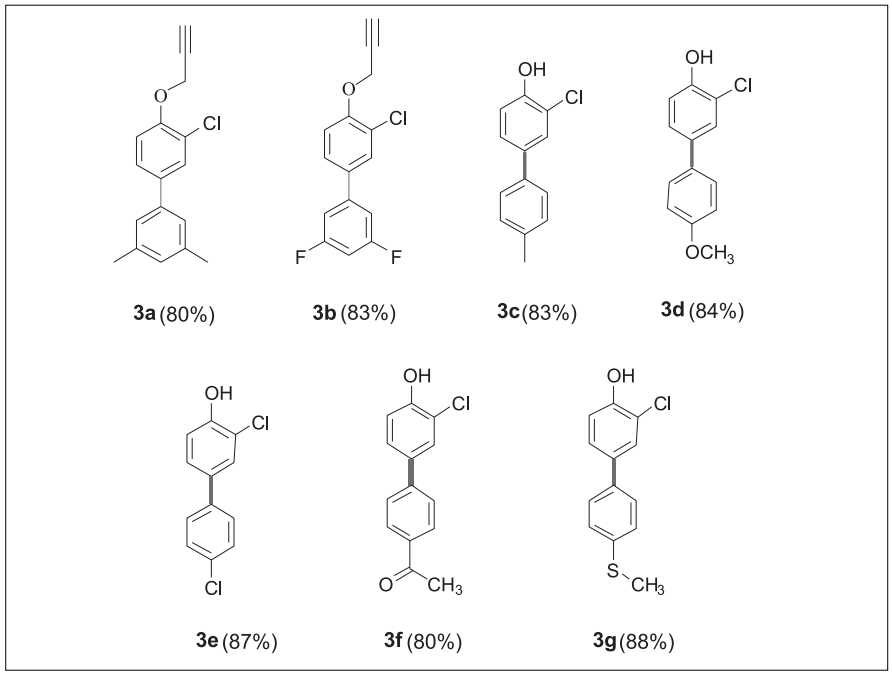
After the consent of healthy volunteers, the blood samples were collected accordingly. In the weighed micro-centrifuge, the blood (100 mL) was added to form the clots. Then in micro-tubes, the solution (100 µL) of the novel synthesized compounds with particular a concentration (1 mg/mL) was added, and incubation was done (37°C for 45 min). Streptokinase and deionized water were used as positive and negative controls respectively (16).

RESULTS AND DISCUSSION

Chemistry

The protection of aromatic amino or hydroxyl groups in the form of amine derivatives or ethers

Table 1. Substrate scope of Suzuki coupling of 4-bromo-2-chloro-1-(prop-2-yn-1-yloxy)benzene with variety of arylboronic acids.



3a (80%) **3b** (83%) **3c** (83%) **3d** (84%)

3e (87%) **3f** (80%) **3g** (88%)

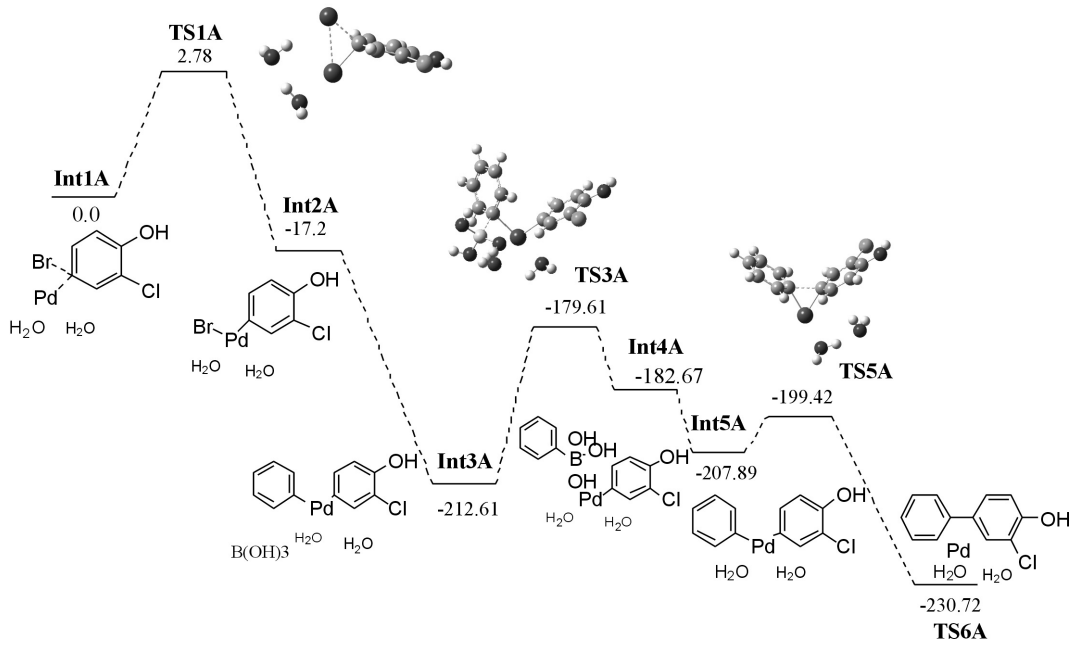


Figure 1. Energy profile for three major steps of the Suzuki coupling at Br position starting from **Int1A** (all values are in kcal mol^{-1})

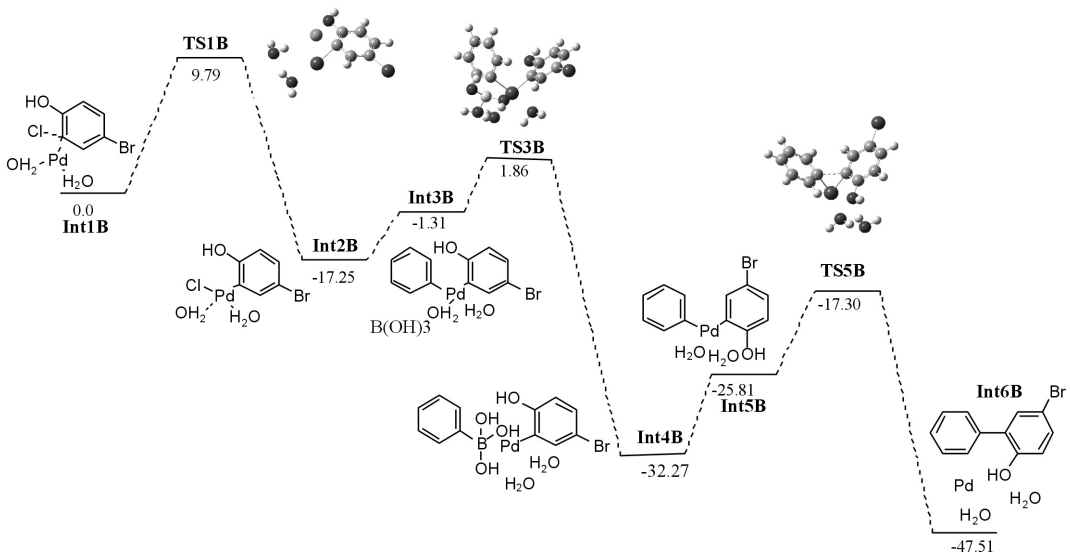


Figure 2. Energy profile for three major steps of the Suzuki coupling at Cl position starting from **Int1B** (all values are in kcal mol^{-1})

and their subsequent cleavage constitute a useful chemical transformation in organic synthesis. The presence of two orthogonal π-bonds in propargyl group further makes it attractive, which perhaps help in its facile cleavage in the presence of transition metal complexes (17-18).

In the present research work, we investigated the Suzuki reaction of 4-bromo-2-chloro-1-(prop-2-yn-1-yloxy)benzene **(2)** with different aryl boronic acids under optimal conditions. According to our survey, to date no such study has been reported on the synthesis and biological activities of various derivatives **(3a-g)** of 4-bromo-2-chloro-1-(prop-2-yn-1-yloxy)benzene. As presented in the Scheme 1, the first step is the synthesis of intermediate compound 4-bromo-2-chloro-1-(prop-2-yn-1-yloxy)

benzene **(2)**, which was obtained in 90% yield from the reaction between 4-bromo-2-chlorophenol **(1)** and propargyl bromide (Scheme 1). In general, the aryl halides which are substituted with electron-withdrawing groups are appropriate substrates for the cross-coupling reaction (19). Since the hydroxyl group attached with the phenyl ring has electron donating ability, we first protected the -OH group before carrying out the Suzuki cross-coupling reaction.

In the second step, Suzuki coupling of **(2)** with various arylboronic acids was conducted that eventually led to the synthesis of various biaryl derivatives **(3a-g)** in high yields (80-88%) (Table 1). The Biaryl Compounds **3a** and **3b** were obtained with protected ether group; while compounds **3c-3g** were de-propargylated to corresponding phenol in the reaction mixture.

In past, the Palladium catalyzed depropargylation (cleavage of C-O propargyl bond) of aryl propargyl ethers cleaved to the corresponding phenol has already been reported (17, 20). Various substituents on the phenyl ring of the starting ether **(2)** and aryl-boronic acids were well tolerated during the reaction. The structures of these novel synthesized compounds were characterized by Mass spectrometry, ^{1}H-NMR, and ^{13}C-NMR spectra.

DFT study

Oxidative addition

Recently, Huang and co-workers (21) proved that B3LYP/6-31G (d) with pseudopotential on Pd (LANL2DZ) is suitable to model the Suzuki coupling reaction. In order to confirm the selectivity of coupling at C-Br position over C-Cl, we started to form two complexes **Int1A** (Br) and **Int1B** (Cl).

Table 2. Biofilm inhibition (%) of Suzuki coupled compounds.

Entry	Compounds	Biofilm Inhibition%
1	3a	32.1 ± 0.25
2	3b	65.8 ± 0.34
3	3c	5.86 ± 0.05
4	3d	25.8 ± 0.12
5	3e	41.8 ± 0.21
6	3f	54.6 ± 0.25
7	3g	43.3 ± 0.43
8	Rifampicin	96.5 ± 0.65

The results are average ± S.D of three separate experiments $p < 0.05$

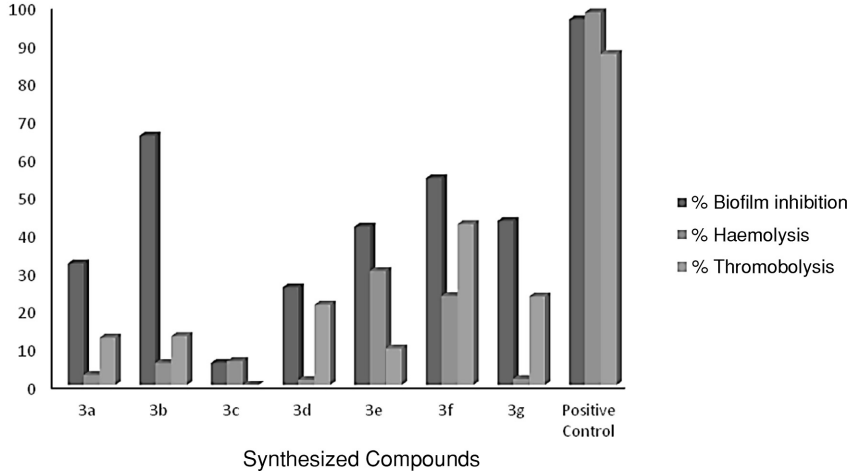


Figure 3. Biofilm inhibition, Hemolytic and Anti-thrombolytic activities of novel biaryls **(3a-g)**

Oxidative addition of palladium in the C–Br bond of **Int1A** is much more favorable than in case of **Int1B** (C-Cl). The oxidative addition transition state of **TS1A** is located at a barrier of 2.78 kcal mol^{-1} from **Int1A**, whereas the analogous transition state **TS1B** of C-Cl coupling from **Int1B** lies at a much more higher barrier of 9.79 kcal mol^{-1} as compared to **TS1A** (Fig. 1). This is in complete agreement with the experimental observation, where the products are dominantly obtained as a result of oxidative addition at C-Br bond. It is believed that the very low activation barrier for **TS1A** is due to the high reactivity of C-Br bond over the C-Cl bond. The C-Cl (2.07^{-}) and C-Pd (1.98^{-}) bond lengths in **TS1B** are shorter and stronger as compared to the C-Br (2.14^{-}) and C-Pd (1.99^{-}) bonds in case of **TS1A**. The oxidative addition step in both cases is exothermic by 17.2 and 17.25 kcal mol^{-1} from **Int1A** (Fig. 1) and **Int1B** (Fig. 2), respectively. **Int2A** and **2B** (product of oxidative addition) have almost equal stability.

Transmetalation

Coordination of boronic acid with **Int2A** (C-Br) and **Int2B** (C-Cl) generates **Int3A** and **Int3B**, respectively. The intermediate **Int3A**, generated from the cleavage of C-Br bond is highly exothermic by 212.63 kcal mol^{-1}, however, the cleavage of the Pd–Cl bond is very less exothermic by -1.31 kcal mol^{-1} and Intermediate **Int3B**, generated from cleavage of the Pd–Cl bond, is almost on the similar energy level compared to **Int1B**. The transmetalation transition state, translated form **Int3A** is exothermic by -179.61 kcal mol^{-1} as compared to the starting complex **Int1A**, whereas the analogous transition state from the **Int3B** is located at activation barrier of 1.86 kcal mol^{-1}. Again the higher activation barrier of Pd-Cl transmetalation step is in accordance with the experimental results, that the formation of a product as a result of C-Cl bond coupling is negligible. The transmetalation product **Int4A** from **TS3A** is exothermic by -182.67 kcal mol^{-1} and **Int4B** from **TS3B** is exothermic by -32.27 kcal mol^{-1}.

Reductive elimination

Very surprisingly, the reductive elimination step in both cases turned out to be the one with the very low activation barrier in the catalytic cycle for both coupling products (rate limiting step). The transition state **TS5A** from **Int4A** is exothermic and located at a barrier of -199.22 kcal mol^{-1} as compared to the **Int1A**. Similarly, the **TS5B** from **Int4B** is also exothermic and located at -17.30 kcal mol^{-1}. From the overall computational results, it is concluded that the observed selectivity of C-Br coupling over C-Cl coupling may be attributed to the low activation barrier of the oxidative addition step of **Int1A** over **Int1B**, and to the associated with a very low activation barrier of the reductive elimination step and is complete agreement with the experimental products.

Pharmacology

Biofilm inhibition assay

The formation of biofilm by pathogenic bacteria causes various fatal infections that may lead to death in some worst cases. This film is an aggregation of bacterial cells, covered by a self-produced polymeric material (22). Inhibition of formation by novel biaryls **(3a-g)** was investigated against *Escherichia coli*, where the results revealed the moderate activity of most of the compounds (Fig. 3, Table 2). In particular, the compound **3b** showed higher activity (65.8%) as compared to other compounds against *E. coli*, which might have its origin in the two electron withdrawing fluoro groups attached with the phenyl ring (23). On the other hand, other biaryls exhibited moderate activity, when compared with standard drug rifampicin. The biaryl **3c** displayed the lowest activity 5.86%, which contains electron donating methyl moiety. Overall, the results reveal that all newly synthesized biaryls could be a potential source of biofilm inhibition at high concentrations.

Hemolytic activity

The cytotoxicity of novel biaryls **(3a-g)** was also studied in the present work. The biaryls **3e** and **3f** exhibited high hemolytic activity ~ 30.1 and 23.5%, respectively. Some of the novel biaryls viz. **3a, 3b, 3c, 3d, 3g** exhibited hemolytic activity in a safe range (below 10%) (Table 3, Fig. 3). From the observed variations in the results of percentage lysis of RBCs, it is concluded that the electron withdrawing and electron donating substituents have a pronounced effect on the hemolytic activity of the compounds (24). Moreover, the cytotoxicity of the derivatives *viz*. **3e** and **3f** can be optimized by doing changes in the structure of molecules, for their practice as toxic compounds to inhibit the growth of cancer cells (25, 26).

Anti-thrombolytic Activity

Blood clot formation is a severe problem that hinders the blood circulation in the human body. Thrombus obstructs the blood flow by blocking the blood vessel, therefore hindering the tissues of normal blood flow and oxygen. These consequences led to the necrosis of tissues in that area. Many drugs

such as ticlopidine, heparin, clopidogrel, urokinase, streptokinase and plasminogen activator (t-PA) have been explored as the clot lysis agents. However, for clinical practice, only some were found of potentially beneficial (27-30). In this research work, we investigated the anti-thrombolytic potential of novel biaryl derivatives **(3a-g)**. The results showed that the compound **3f** exhibited appreciable thrombolytic activity (~ 42.5%), while **3g** and **3d** displayed moderate % lysis ~ 23.4 and 21.2%, respectively. The standard drug streptokinase, however, exhibited high % lysis ~ 87.4%. The compounds **3b**, **3a**, and **3e** exhibited reasonable % lysis, while the compound **3c** was found inactive against clot lysis. The biaryls **3f**, **3g**, and **3d** exhibited reasonable % lysis, which can be attributed to -$COCH_3$, -SCH_3 and -OCH_3 substituents attached to the aromatic system. Overall, it can be seen that the anti-thrombolytic activity showed by biaryls **(3a-g)** have a large effect from the nature of attached substituents (23) (Table 4, Fig. 3).

CONCLUSIONS

The present study reports the synthesis of various novel biaryls **(3a-g)** with efficient yields under mild optimized conditions. The density functional theory studies revealed that the oxidative addition and transmetalation steps are the rate controlling steps. Energies of located transitions states of all steps proved that C-Br coupling is preferred over C-Cl coupling. The synthesized novel biaryls exhibited good biofilm inhibition, hemolytic and anti-thrombolytic activities. From the observed variations in the results, it is concluded that the electron withdrawing and donating substituents have a pronounced effect on the biological activities of these newly synthesized biaryl derivatives. Our experimental results revealed that these compounds could be a potential source of pharmaceutical agents at higher concentrations.

Table 3. Hemolytic activity % of Suzuki coupled compounds.

Entry	Compounds	Hemolytic activity%
1	3a	2.64 ± 0.12
2	3b	5.76 ± 0.05
3	3c	6.32 ± 0.07
4	3d	1.32 ± 0.09
5	3e	30.1 ± 0.16
6	3f	23.5 ± 0.13
7	3g	1.59 ± 0.02
8	PBS	1.09 ± 0.01
9	Triton-X-100	98.4 ± 0.72

The results are average ± S.D of three separate experiments $p < 0.05$

Table 4. Anti-Thrombolytic activity % of Suzuki coupled compounds.

Entry	Compounds	% Thrombolysis
1	3a	12.5 ± 0.25
2	3b	12.9 ± 0.23
3	3c	----
4	3d	21.2 ± 0.19
5	3e	9.64 ± 0.09
6	3f	42.5 ± 0.45
7	3g	23.4 ± 0.21
9	Streptokinase	87.4 ± 0.83

The results are average ± S.D of three separate experiments $p < 0.05$

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Author contributions

HF, NR, HI, TM designed and performed the experiments, MTA, KR and KA analysed the data and contributed to the writing of the manuscript. TR, MNASZ and SI contributed in the technical refining of the data.

Conflicts of interest

The authors declare no conflict of interest.

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