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Antimicrobial Evaluation of New Synthesized Aza Heterocycles

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Abstract

The Pd-precatalyst was found to exhibit high catalytic activity for C-C bond formation in Suzuki-Miyaura cross-coupling reactions of differentheteroaryl (5-bromo-2-bromoacetyl) thiophene,4- (5-bromothiophen-2-yl)-methyl-1,3-thiazole,isoquinolinium bromide salt) with either aryl or heteroarylboronic acids. All reactions in this study were conducted in water under thermal heating as well as microwave irradiation conditions. The antimicrobial activities of synthesized compounds were evaluated against eight different strains of microorganisms (four fungal strains, two Gram-positive bacteria and two Gram-negative bacteria) using a cup plate diffusion method. The results showed that, most of the 20 tested compounds exhibited varying degrees of antimicrobial activities. However, 6 compounds recorded high antifungal activity (≤ 20 mm zone of inhibition), 3 were antibacterial (against Gram-positive) and 4 showed high broad-spectrum antibacterial efficacy (against Gram-positive and Gram-negative). These bioactive compounds are recommended for further pharmacological and toxicological investigations for possible formulation as wide spectrum antibacterial and antifungal agents.

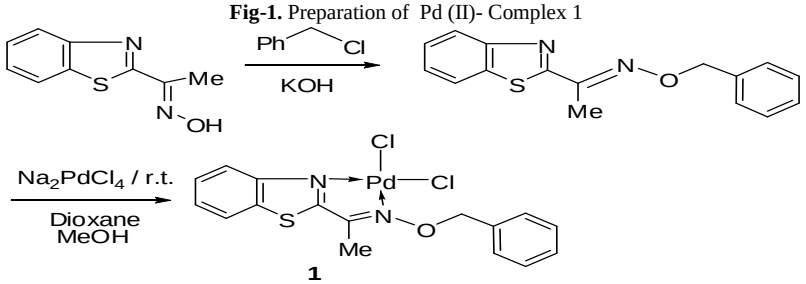
Keywords: Antimicrobial; Synthesized Aza Heterocycles.

1. Introduction

Antibiotics resistance phenomenon is an increasing international public health threat, the numbers of resistant pathogens are growing dramatically, which lead to double the duration of hospital stay, double mortality, morbidity, and making a great burden on the global economy [1]. Accordingly, there is an intrinsic need for new antimicrobial agents with a various mode of actions on microorganisms. The mode of action of the antimicrobial agents includes inhibition of the cell wall synthesis, inhibition of cell membrane function, inhibition of nucleic acid synthesis, inhibition of ribosome function and inhibition of foliate metabolism [2].

The Suzuki-Miyaura cross-coupling reaction has become one of the most efficient methods for the construction of biaryl or substituted aromatic moieties. Compounds that contain these sub-structures constitute important building blocks of polymers, ligands, and a wide range of natural products [3], such as alkaloids, and numerous biologically active pharmaceuticals [4]. Thiophene derivatives are important class of heterocyclic compounds that are widely involved in many agrochemicals and pharmaceuticals [5]. They are employed in drug synthesis, for example gabitril is an antiepilepsy drug [6] and canagliflozin is a drug for the treatment of type 2 diabetes [7]. In addition, thiophene-based molecules have shown numerous biological activities such as antitumor, analgesic, anti-inflammatory and antibacterial activity [7]. Microwave irradiation methodology assists in achieving rapid incorporation of organic synthesis into broad industrial diversities [8]. Moreover, organic reactions that can proceed well in aqueous media offer advantages over those occurring in organic solvents. Transition metal-mediated cross-coupling reactions, particularly those based on palladium as shown in Figure. 1, have become key transformations in organic synthesis. Therefore, developing new catalytic systems as catalysts for Suzuki-Miyaura cross-coupling reaction for the synthesis of biaryls and heterobiaryls will be of great importance [9].

Fig-1. Preparation of Pd (II)- Complex 1



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*Corresponding Author

2. Experimental

2.1. Procedure

Melting points were determined in open glass capillaries with a Gallenkamp apparatus and are uncorrected. The infrared spectra were recorded in potassium bromide disks on a Pye-Unicam SP 3–300 and Shimadzu FTIR 8101 PC infraredspectrophotometer. NMR spectra were recorded with a Varian Mercury VXR-300 NMR spectrometer at 300 MHz (1HNMR) and at 75.46 MHz (13C NMR) using deuterated chloroform (CDCl3) or dimethylsulfoxide (DMSO-d6). Chemical shifts are quoted in d and were related to that of the solvents. Mass spectra (EI) were obtained at 70 eV with a type Shimadzu GCMQP 1000 EX spectrometer. Microwave experiments were carried out using a CEM Discover Labmate_ microwave apparatus (300W with Chem Driver_ Software). Synthesis of Pd(II)-complexes 1 [10, 11] 2-acetyl-5-bromothiophene (2), Fig. 2, 5-bromo-2-(bromoacetyl) thiophene (3) [12]. 4-(5-bromothiophen-2-yl)-2-methyl-1,3 -thiazole (4), **Fig. 3**. 2-(2-(5-bromothiophen-2-yl)-2-oxoethyl)isoquinolinium bromide salt (17), of ethyl 3-(5-bromothiophen-2-ylcarbonyl)pyrrolo[2,1-a] isoquinoline-2-carbonitrile (18), Fig.7, 3- [5-(4-Chlorophenyl) thiophen-2-ylcarbonyl]pyrrolo[2,1-a] isoquinoline-2-carbonitrile (19), Fig. 8, ethyl 3-(5-bromothiophen-2-ylcarbonyl pyrrolo) [2,1-a]isoquinoline-2-carboxylate (20), Fig. 9, Ethyl 3-(5-(4-chlorophenyl) thiophen-2-ylcarbonyl)pyrrolo[2,1-a]-isoquinoline-2-carboxylate (21), Fig. 10, were carried out following the procedures reported in literature [13].

2.1. Synthesis of (E)-1-(5-bromothiophen-2-yl)-3-(dimethylamino) prop-2-en-1- one (5)

A mixture of 2-acetyl-5-bromothiophene (3) (10 g, 50 mmol) and dimethylformamide-dimethylacetal (DMF-DMA) (7.0 mL, 50 mmol) were taken in dry benzene (30 mL) and the mixture was refluxed for 6 hrs, then left to cool at room temperature. The yellow precipitated product was filtered off, washed with petroleum ether (60/80) and dried. Recrystallization from benzene afforded 9.0 g of (E)-1-(5-bromothiophen-2-yl)-3-(dimethylamino) prop-2-en-1-one (5) (94% yield), mp. 110-112 ˚C; IR (KBr) υ 2900, 2804, 1632, 1519, 1434, 1407, 1203 cm-1; 1H NMR (CDCl3) δ 2.92 (s, 3H, CH3), 3.12 (s, 3H, CH3), 5.47 (d, 1H, J = 12 Hz), 7.02 (d, 1H, J = 3.9 Hz), 7.27 (d, 1H, J = 3.9 Hz), 7.75 (d, 1H, J = 12 Hz); MS m/z (%) 263 (M++2, 1.7), 261 (M+, 65.6), 259 (55.2), 244 (51.8), 180 (100), 147 (93.4), 98 (99.1), 82 (77.7). Anal. Calcd for C9H10NOS: C, 61.50; H, 6.71; N, 7.17; S, 16.42. Found: C, 61.37; H, 6.95; N, 7.32; S, 16.10%.

2.2. Synthesis of 3-(5-bromothiophene-2-yl)-1H-pyrazole (6)

To a solution of (E)-1-(5-bromothiophen-2-yl)-3-(dimethylamino) prop-2-en-1-one (5) (3.0 g, 10 mmol) dissolved in absolute ethanol, hydrazine hydrate (0.6 mL, 10 mmol) was added and the reaction mixture was heated at reflux for 4 hrs. The solvent was then the evaporated by rotary evaporator. The yellow precipitate was recrystallized from ethanol giving 2.4 g of yellow crystals of 3-(5-bromothiophene-2-yl)-1H-pyrazole (6) (92% yield); mp. 178-180 ˚C; IR (KBr) υ 3336, 3228, 3024, 1442, 1029 cm-1; 1H NMR (DMSO-d6) δ 6.41 (s, 1H), 6.89 (d, 1H, J = 3.9 Hz), 7.06 (d, 1H, J = 3.9 Hz), 7.16-7.20 (m, 1H), 12.86 (br.s, 1H, NH); 13C NMR (DMSO-d6) δ 104.9, 109.2, 120.3, 130.5, 131.6, 134.0, 144.9; MS m/z (%) 230 (M+, 100), 227 (83.6), 203 (27.8) 161 (18.1), 148 (42.4), 120 (50.5). Anal. Calcd for C7H5BrN2S: C, 36.70; H, 2.20; Br, 34.88; N, 12.23; S, 14.00. Found: C, 36.53; H, 2.07; Br, 35.02; N, 12.10; S, 14.28%.

2.3. Suzuki-Miyaura coupling of 2-acetyl-5-bromothiophene (2) with arylboronic acids using Pd-complex 1 in water under thermal heating

A mixture of 2-acetyl-5-bromothiophene (2) (205 mg, 1 mmol) and the appropriate arylboronic acids 7a-d (1.2 mmol), tetrabutylammonium bromide (194 mg, 0.6 mmol), palladium complex 1 (1.14 mg, 0.25 mol%), KOH (112 mg, 2 mmol), and water (3 mL) were heated under reflux for the appropriate reaction times as listed in Table 1 (monitored by TLC). The cross-coupled products were then extracted with EtOAc (3x20 mL). The combined organic extracts were dried over anhydrous MgSO4 then filtered and the solvent was evaporated under reduced pressure. The residue was then subjected to separation via flash column chromatography with n-hexane/EtOAc (4:1) as an eluent to give the corresponding pure cross-coupled products, Figure. 2. [10, 12-15].

2.4. Suzuki –Miyaura coupling of 2-acetyl-5-bromothiophene (2) with arylboronic acids using Pd-complex 1 in water under microwave irradiation

2-Acetyl-5-bromothiophene (2) (205 mg, 1 mmol) and arylboronic acids 7a-d (1.2 mol) tetrabutylammonium bromide (194 mg, 0.6 mmol) palladium complex 1 (1.2 mg, 0.25 mol %), KOH (112 mg, 2 mmol), and distilled water (3 mL) were mixed in a process glass vial. The vial was capped properly, and thereafter the mixture was heated under microwave irradiating conditions at 160˚C and 250 Watt for the appropriate reaction time, as listed in Table 1. After the reaction was almost complete, the cross-coupled products were then extracted with EtOAc (3x20 mL). The combined organic extracts were dried over anhydrous MgSO4 then filtered and the solvent was evaporated under reduced pressure. The residue was then subjected to separation via flash column chromatography with n-hexane/EtOAc (4:1) as an eluent to give the corresponding pure cross-coupled products (8-11). [15].

2.5. Suzuki-Miyaura coupling of 4-(5-bromothiophen-2-yl)-2-methyl-1, 3-thiazole (4) with arylboronic acids under thermal heating

A mixture of 4-(5-bromothiophen-2-yl)-2-methyl-1, 3-thiazole (4) (266 mg, 1 mmol) and the appropriate arylboronic acids 7a-d (1.2 mol), TBAB (194 mg, 0.6 mmol), palladium complex 1 (1 mol %) and Cs2CO3 (0.652 mg, 2 mmol), in DMF (3 mL) were refluxed at 100 oC for 40 hours. In all cases, the starting bromide 4-(5-bromothiophen-2-yl)-2-methyl-1, 3-thiazole (4) was completely [recovered [10].

2.6. Suzuki-Miyaura cross coupling of 4-(5-bromothiophen-2-yl)-2-methyl-1, 3-thiazole (4) with arylboronic acids under microwave irradiation

4-(5-Bromothiophen-2-yl)-2-methyl-1, 3-thiazole (4) (266 mg, 1 mmol) and the appropriate arylboronic acids 7b, e (1.2 mol), tetrabutylammonium bromide (194 mg, 0.6 mmol), palladium complex 1 (1.2 mg, 0.25 mol %), Cs2CO3 (652 mg, 2 mmol), and distilled water (3 mL) were mixed in a process glass vial. The vial was capped properly, and thereafter the mixture was heated under microwave irradiating conditions at 160 ˚C and 250 Watt for appropriate reaction time as listed in Table 2. After the reaction was almost completed the cross-coupled products were then extracted with EtOAc (3x20 mL). The combined organic extracts were dried over anhydrous MgSO4 then filtered and the solvent was evaporated under reduced pressure. The residue was then subjected to separation via flash column chromatography with n-hexane/EtOAc (4:1) as an eluent to give the corresponding pure cross-coupled products (12, 13), Figure. 4 [15].

2.7. Suzuki-Miyaura cross-coupling of 3-(5-bromothiophen-2-yl)-1H-pyrazole (6) with arylboronic acids under microwave irradiation

A mixture of 3-(5-bromothiophen-2-yl)-1H-pyrazole (6) (229 mg, 1 mmol) and arylboronic acids 7a, b,e(1.2 mmol) tetrabutylammonium bromide (194 mg, 0.6 mmol) palladium complex 1 (1.2 mg, 0.25 mol %), KOH (112 mg, 2 mmol), DMF / H2O (2:1) (3 mL) were mixed in a process glass vial. The vial was capped properly, and thereafter the mixture was heated under microwave irradiating conditions at 160˚C and 250 Watt for appropriate reaction time as listed in Table 3. After the reaction was almost completed the cross-coupled products were then extracted with EtOAc (3 x 20 mL). The combined organic extracts were dried over anhydrous MgSO4 then filtered and the solvent was evaporated under reduced pressure. The residue was then subjected to separation via flash column chromatography with n-hexane / EtOAc (4:1) as an eluent to give the corresponding pure cross-coupled products (14-16), Figure. 6 [11].

3-(5-Phenylthiophen-2-yl)-1H-pyrazole (14): Pale yellow powder; mp. 109-110 ˚C; IR (KBr) υ 3236, 1581, 1446, 1346, 1180, 763 cm-1; 1H NMR (DMSO-d6) δ 6.58 (d, 2H, J = 7.8 Hz), 7.16 (d, 1H, J = 3.9 Hz), 7.20 (d, 1H, J = 3.9 Hz), 7.31 (d, 2H, J = 7.8 Hz), 7.39-7.41 (m, 2H, ArH), 7.89 (s, 1H), 12.89 (s, 1H, NH); MS m/z (%) 226 (M+, 31.6), 149 (39.6), 82 (34.3), 77 (22.9), 67 (59.4). Anal. Calcd for C13H10N2S: C, 69.00; H, 4.45; N, 12.38; S, 14.17. Found: C, 68.70; H, 4.24; N, 12.03; S, 14.39%.

3-(5-(4-Chlorophenyl) thiophen-2-yl)-1H-pyrazole (15): Yellow powder; mp. 113-115 ˚C; IR (KBr) υ 3327, 2917, 1032, 785 cm-1; 1H NMR (CDCl3) δ 4.48 (br.s, 1H, NH), 6.72 (d, 1H, J = 2.4 Hz), 6.85 (d, 2H, J = 6.9 Hz), 7.09 (d, 1H, J = 3.6 Hz), 7.32 (d, 1H, J = 3.6 Hz), 7.46 (d, 2H, J = 6.9 Hz), 7.66 (d, 1H, J = 2.4 Hz); Ms m/z (%) 262 (M++2, 32), 260 (M+, 62), 250 (100), 194 (96), 184 (51), 148 (62), 114 (45). Anal. Calcd for C13H9ClN2S: C, 59.88; H, 3.48; N, 10.74; S, 12.30. Found: C, 59.88; H, 3.41; N, 10.65; S, 12.23%.

3-(5-(4-Methoxyphenyl) thiophen-2-yl)-1H-pyrazole (16): Yellow powder; mp. 117-119 ˚C; IR (KBr) υ 3367, 2912, 1029, 786 cm-1; 1H NMR (CDCl3) δ 3.85 (s, 3H, OCH3), 4.16 (br.s, 1H, NH), 6.59 (d, 1H, J = 2.4 Hz), 6.91 (d, 2H, J = 6.9 Hz), 7.16 (d, 1H, J = 3.6 Hz), 7.40 (d, 1H, J = 3. 6 Hz), 7.54 (d, 2H, J = 6.9 Hz), 7.68 (d, 1H, J = 2.4 Hz); Ms m/z (%) 256 (M+,100), 250 (14), 241 (59), 213 (212), 184 (12), 128 (10). Anal. Calcd for C14H12N2OS: C, 65.60; H, 4.72; N, 10.93; S, 12.51. Found: C, 65.64; H, 4.67; N, 11.02; S, 12.32%.

2.8. Antifungal Activity

Tested samples were screened separately in vitro for their antifungal activity against various fungi, namely, Aspergillus fumigatus (RCMB 002003), Geotrichumcandidum (RCMB 002006), Candida albicans (RCMB 005002) and Syncephalastrumracemosum (RCMB 005003) on Sabourad dextrose agar plates. The culture of fungi was purified by single spore isolation technique. The antifungal activity was carried out by agar well diffusion method as mentioned in literature [16]. Briefly, Sabouraud dextrose agar was prepared as the manufacturer's instructions, poured in sterile Petri dishes, left to solidify, and kept upside-down for 15 minutes in an incubator to remove the moisture. Fungal culture (0.1mL) was spread out uniformly on the sabouraud dextrose agar plates and left for 5-10 minutes so that culture is properly adsorbed. Small well of size 6mm were cut into the plates with the help of well cutter. 100 µl of the tested samples (concentration 10mg/mL) were loaded into the well of the plates. All compounds were prepared in dimethyl sulfoxide (DMSO), and the solvent was loaded as control. The plates were kept for an incubation at 30˚C for 3-4 days and then the plates were examined for the formation of zone of inhibition. Each inhibition zone was measured three times to get an average value. Clotrimazole was used as antifungal standard drug.

2.9 Antibacterial Activity

Agar well diffusion method was used in the screening of the antibacterial potential of the synthesized compounds as reported in literature [16]. Shortly, bacterial strains were sub-cultured overnight prior of the

experiment, these strains were *Staphylococcus aureus* (RCMB 000106) and *Bacillis subtillis* (RCMB 000107) as Gram positive bacteria, in addition to *Pseudomonas aeruginosa* (RCMB 000102) and *Escheirchia coli* (RCMB 000103) as Gram negative bacteria. In aseptic conditions, plates containing Nutrient agar were prepared and bacterial strains were spread on the solidified agar. Wells (6mm) were created using a sterile metallic bores and 100 µl of the tested compounds (concentration 10 mg/Ml) were loaded into the wells. All compounds were prepared in dimethyl sulfoxide (DMSO), DMSO was loaded as control. The plates were kept for incubation at 37˚C for 24 h and then the plates were examined for the formation of zone of inhibition. Each inhibition zone was measured three times to get an average value. Sterptomycinwas used as antibacterial standard drug.

3. Results and Discussion

The Suzuki coupling reactions of 2-acetyl-5-bromothiophene (2) with activated and deactivated (4-chlorophenyl, 4- methoxy, thiophene, 3,4-methylenedioxyphenyl)boronic acids 7a-d were carried out under thermal heating as well as microwave irradiation conditions resulting in the formation of a library of the corresponding 5-aryl(heteroaryl)-2-acetylthiophenes 8-11 in full conversions with excellent isolated yields Table 1. The reaction components molar ratios were typically; 1 mmol 2-acetyl-5-bromothiophene (2), 1.2 mmolarylboronic acids 7a-d, 0.6 mmol TBAB, 2 mmoles of KOH using 0.25 mol% of the precatalyst1 in water (3 mL). Table 1: Suzuki coupling of 2-acetyl-5-bromothiophene (2) with arylboronic acids using Pd–complex 1, under thermal heating and microwave irradiation, Figure. 2.

Fig-2. Suzuki coupling of 2-acetyl-5-bromothiophene (2) with arylboronic acids using Pd–complex 1 under thermal heating and microwave irradiation

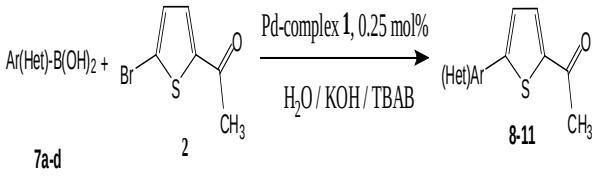
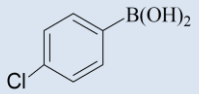
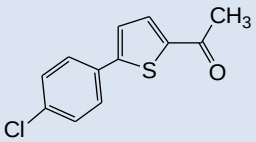
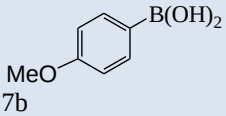
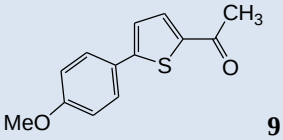
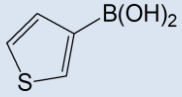
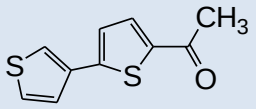
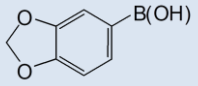
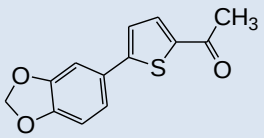
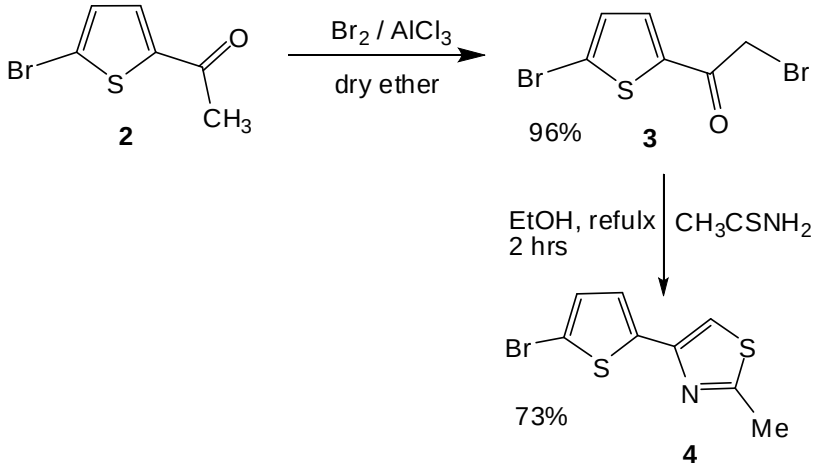


Table-1. Comparison of Suzuki coupling of 2-acetyl-5-bromothiophene (2) with arylboronic acids using Pd–complex 1 under thermal heating and microwave irradiation

Entry	Ar(Het)-B(OH)$_2$	Product	Thermal heating Time, h	yield, %	MW heating time, min	yield, %
1.	 7a	 **8**	5	97	7	92
2.	 7b	 **9**	5	90	7	98
3.	 7c	 **10**	10	91	10	95
4.	 7d	 **11**	15	90	15	90

The heterocyclic bromide namely: 4-(5-bromothiophen-2-yl)-2-methyl-1, 3-thiazole (4) was prepared as shown in Figure. 3, Compound 4 was prepared using Hantzsch method involving the reaction of α −halocarbonyl compounds with thiourea or thioamides.

Fig-3. Preparation of 4-(5-bromothiophen-2-yl)-2-methyl-1, 3-thiazole (4)



The Suzuki-Miyaura cross-coupling reaction of 4-(5-bromothiophen-2-yl)-2-methyl-1, 3-thiazole (4) with (phenyl, methoxyphenyl)boronic acids 7b,eunder microwave irradiation was examined. The reaction components molar ratios were typically; 1 mmol of bromide 3, 1.2 mmolarylboronic acids 7b, e, 0.6 mmol TBAB, 2 mmoles of Cs2CO3 using 1 mol% of the complex 7 in DMF (3 mL). Therefore, towarylboronic acids 7b,e were accordingly coupled with the bromide (4) under microwave irradiation to give the corresponding cross-coupled products 12,13 in good isolated yields as outlined in Table 2.

Fig-4. Suzuki-Miyaura coupling of 4-(5-bromothiophen-2-yl)-2-methyl-1, 3-thiazole (4) with arylboronic acids

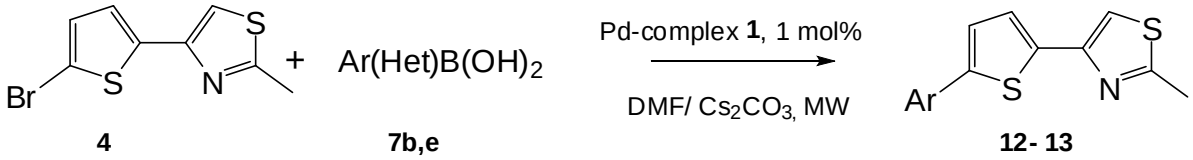
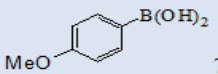
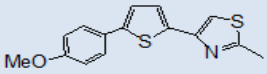
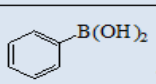
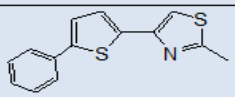
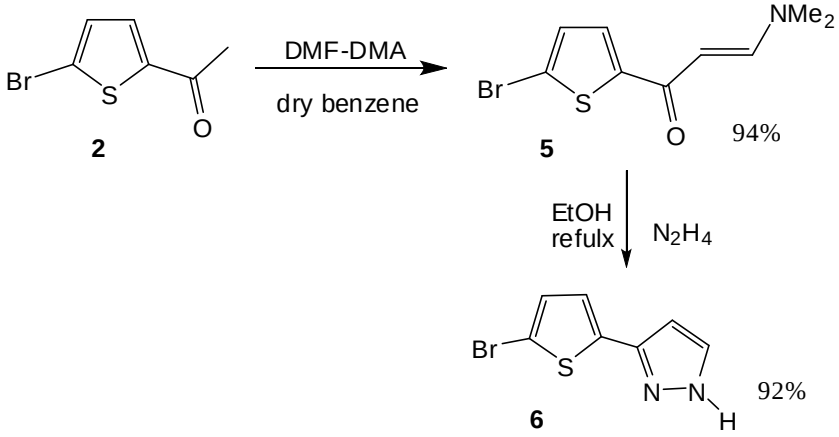


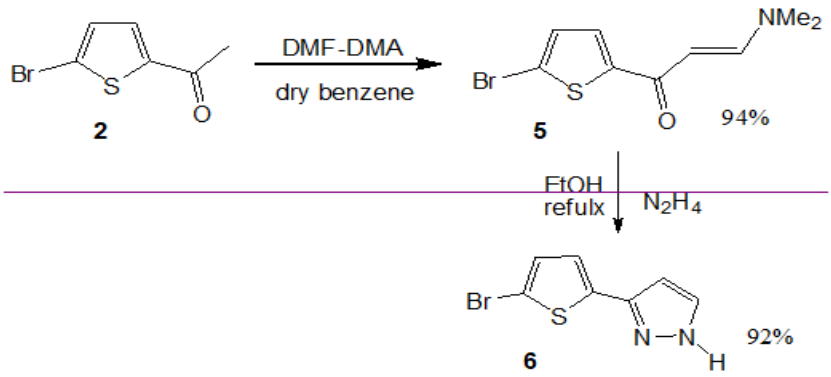
Table-2. Suzuki coupling of 4-(5-bromothiophen-2-yl)-2-methyl-1, 3-thiazole (**4**) with arylboronic acids **7b,e** under microwave irradiation

Entry	Ar (Het) B (OH)$_2$	Product	MW heating time, min	yield, %
1	 7b	 12	40	76
2	 7e	 13	30	80

The synthesis of 3-(5-bromothiophen-2-yl)-1H-pyrazole 6bytreatment of 2-acetyl-5-bromothiophene (2) with dimethylformamide-dimethylacetal (DMF-DMA) in dry benzene at reflux temperature afforded a single product that was identified as (E)-1-(5-bromothiophen-2-yl)-3-(dimethylamino)prop-2-en-1-one (5) as shown in Figure. 5. Heating a mixture of compound 5 with hydrazine hydrate in refluxing ethanol resulted in the formation of 3-(5-bromothiophen-2-yl)-1H-pyrazole (6).

Fig-5. Synthesis of 3-(5-bromothiophen-2-yl)-1H-pyrazole





The application of the precatalyst1 in the synthesis of 3-(5-arylthiophen-2-yl)-1H-pyrazoles (14-16) via Suzuki-coupling reactions was investigated as described in Table 3. Thus, Suzuki coupling reactions of 3-(5-bromothiophen-2-yl)-1H-pyrazole (6) with arylboronic acids 7a,b,e were carried out under microwave irradiation conditions resulting in the formation of the corresponding new 3-(5-arylthiophen-2-yl)-1H-pyrazole(14-16) in reasonable yields. In all cases, the reaction components molar ratios were typically; 1 mmol 3-(5-bromothiophen-2-yl)-1H-pyrazole (6), 1.2 mmolarylboronic acids 7a,b,e 0.6 mmol TBAB, 2 mmoles of KOH using 0.5 mol% of the precatalyst1 in DMF (3 mL). The identity of the coupling products was confirmed by 1H NMR, MS, IR and elemental analyses. The 1H NMR spectrum of 3-(5-(4- methoxyphenyl) thiophen-2-yl)-1H-pyrazole 16, taken as a typical example of the series prepared, revealed a singlet signal at δ 3.85 due to 4-methoxy proton in addition to the aromatic protons. The mass spectrum of compound 15 showed a molecular ion peak (M+) at m/z 256.

Fig-6. Suzuki coupling of 3-(5-bromothiophen-2-yl)-1H-pyrazole (6) with arylboronic acids 7a, b,e

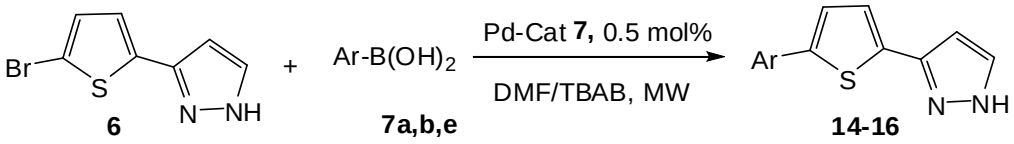
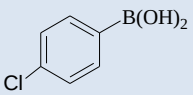
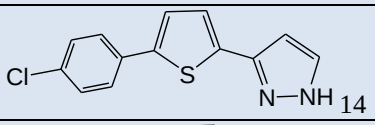
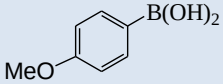
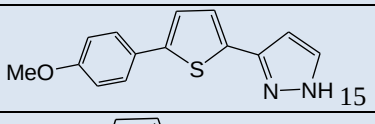
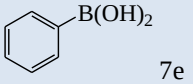
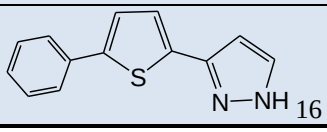
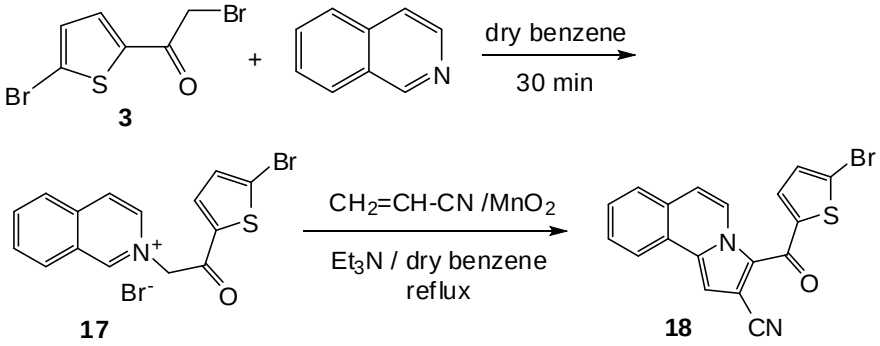


Table-3. Microwave irradiation of Suzuki coupling of 3-(5-bromothiophen-2-yl)-1H-pyrazole (6) with arylboronic acids 7a, b,e

Entry	ArB(OH)2	Product	MW irradiation time/min	Yield%
1	 7a	 14	35	42
2	 7b	 15	35	43
3	 7e	 16	30	45

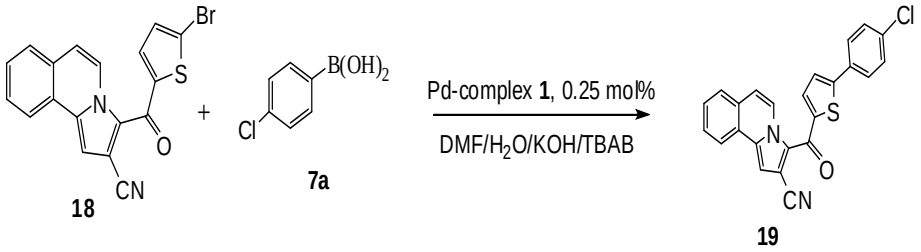
Quinolinium salts were found to be potent inhibitors of lymphocyte apoptosis and protein kinase C. In addition, the use of heteroaromatic N-ylides as 1, 3-dipoles has received increasing interest in the synthesis of new condensed heterocyclic structures via 3+2 cyclo addition. As part of our research interest towards developing new routes for the synthesis of fused heterocyclic systems. The synthesis of starting substrate; 3-[(5-bromothiophen-2-ylcarbonyl pyrrolo] [2,1-a]isoquinoline-2-carbonitrile (18) via [3+2] cyclo addition reaction is reported. Thus treatment of 5-bromo-2-(bromoacetyl) thiophene (3) with quinoline in dry benzene at refluxing temperature gave the corresponding quinolinium bromide salt (17). Reaction of the bromide salt (17) with acrylonitrile in dry benzene at refluxing temperature, in the presence of triethylamine, resulted in the formation of the annulated 3-[(5-bromothiophen-2-ylcarbonyl]pyrrolo[2,1-a]-isoquinoline-2-carbonitrile (18) as shown in Figure. 7. Spectroscopic data as well as elemental analyses of the obtained products were reported in [13].

Fig- 7. Suzuki cross-coupling reaction of 3-[(5-bromothiophen-2-ylcarbonyl] pyrrolo[2,1-a]isoquinoline-2-carbonitrile (18)



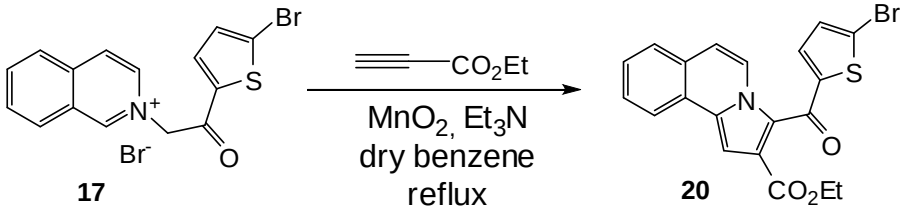
The application of complex 1 (0.25 mol %) in the Suzuki-Miyaura cross-coupling reaction of 3-[(5-bromothiophen-2-ylcarbonyl] pyrrolo[2,1-a]isoquinoline-2-carbonitrile (18) with 4-chlorophenyllboronic acid7a, using DMF/H2O/KOH/TBAB as catalytic system under both microwave irradiation as well as thermal heating, Figure. 8. The cross-coupling was completed (as examined by TLC) within 15 hours of thermal heating and after 10min of microwave irradiation with excellent isolated yields of the corresponding 3-[5-(4-Chlorophenyl) thiophen-2-ylcarbonyl]pyrrolo[2,1-a]isoquinoline-2-carbonitrile (19). Although the structure of 3-[(5-bromothiophen-2-ylcarbonyl] pyrrolo[2,1-a]isoquinoline-2-carbonitrile (18) seems a complicated one, the main reaction site is however an activated bromide attributed to the presence of an electron withdrawing carbonyl function at position 5 of the thiophene ring.

Fig-8. Suzuki-Miyaura cross-coupling reaction of 3-[(5-bromothiophen-2-ylcarbonyl] pyrrolo[2,1-a]isoquinoline-2-carbonitrile (18) with 4-chlorophenyllboronic acid



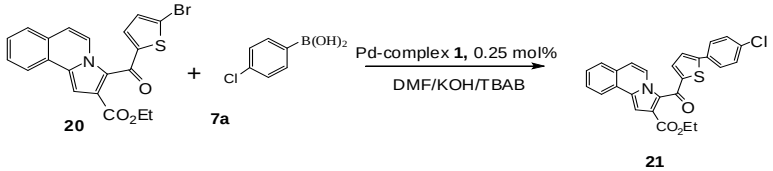
Heating a mixture of the quinolinium bromide (17) with ethyl propiolate as a dipolarophile in refluxing benzene, in the presence of triethylamine and manganese dioxide, furnished the corresponding ethyl 3-(5-bromothiophen-2-ylcarbonyl)pyrrolo[2,1-a]isoquinoline-2-carboxylate (20) as shown in Figure. 9. Structure of compound 20 was deducted from the elemental analyses and spectral data (MS, IR, 1H NMR) of the reaction product [13].

Fig-9. Suzuki cross-coupled reaction of ethyl 3-(5-bromothiophene-2-carbonyl) pyrrolo[2,1-a]isoquinoline-2-carboxylate (20) with 4-chlorophenyllboronic acid 7a



The Suzuki-Miyaura cross-coupling reaction of ethyl 3-(5-bromothiophene-2-ylcarbonyl)pyrrolo[2,1-a]isoquinoline-2-carboxylate (20) with 4-chlorophenylboronic acids 7a, using DMF/KOH/TBAB as catalytic system under both microwave irradiation as well as thermal heating, was generalized as described in Figure. 10. The cross-coupling reaction was achieved within 8 hours of thermal heating and after 10min of microwave irradiation to furnish the corresponding Ethyl 3-(5-(4-chlorophenyl)thiophen-2-ylcarbonyl)pyrrolo[2,1-a]-isoquinoline-2-carboxylate(21) in very high isolated yields.

Fig-10. The Suzuki-Miyaura cross-coupling reaction of ethyl 3-(5-bromothiophene-2-ylcarbonyl)pyrrolo[2,1-a]isoquinoline-2-carboxylate (20) with 4-chlorophenylboronic acids 7a



The results of the antimicrobial testing are presented in (Table 4) and (Table 5), these results of the synthesized compounds were interpreted compared to the standard antifungal and antibacterial drugs. The antifungal activity of the standard antifungal drug the Clotrimazol ranged from 20.8 to 26.3 mm zone of inhibition (ZI). While, the standard antibacterial drug (Streptomycin) ranged from 25.4 to 29.7mm ZI, results equal or above 20 mm ZI were considered as high antimicrobial effect Regarding the antifungal activity of the synthesized compounds, results showed that, most of the compounds exhibited varied degrees of activities. However,6 compounds recorded high antifungal activity, which were the compounds number 5, 14, 15, 16, 17. In addition, some compounds, such as number 9,10 and 20 exhibited no activity against one of the fungal species which is *Syncephalastrum racemosum*, while the compound number 3 exhibited no activity against *Candida albicans* and *Syncephalastrum racemosum*. On the other side, the results of the antibacterial activity showed that, almost all compounds exhibited varied degrees of antibacterial activities against the gram-positive bacteria (*Staphylococcus aureus*and *Bacillissubtilis*). However, the compounds number 2, 3, and 13, were the most active against the gram-positives. Interestingly some compounds were effective against both of the gram negative and the gram-positive bacteria, which were 14, 15, 16 and 17. Differences between the sensitivity of the gram-positive and the gram-negative bacteria is related to the bacterial cell wall thickness, the gram-negative have impenetrable cell wall, because of the presence of an outer membrane covering the cell wall (the peptidoglycan layer) which make it more resistant to drugs [17]. The compounds synthesized in the current investigation are AzaHeterocyles. Heterocyles nucleus is very important in the majority of synthesized drugs including antimicrobial, anti-inflammatory, analgesic, antimalarial, antianxiety, antidepressant, antihistaminic, antioxidant, antitubercular, and many more [18]. Numerous studies were conducted on new antimicrobial drugs. Some researchers had synthesized a series of novelbenzimidazole, benzoxazole and benzothiazole compounds, they found that Benzimidazole and benzoxazole recorded promising results against bacterial strains and benzothiazole against fungal strains [19]. In contrast, El-Shehry et al.[20]. synthesized new nitrogenated heterocyclic compounds such as pyrrolone, pyridazinone, pyrimidine, triazole and oxadiazole derivatives utilizing furan one ring, but they showed moderate, weak and no activity against some bacteria and fungi. Interestingly, based on the findings of our study, some compounds recorded high antibacterial and antifungal effects. These compounds should be carefully tested for possible toxicity, since the fungal cells are eukaryotes and their relative similarity with the animal eukaryotic cells indicates that these compounds could be lethal to the animal cells too. In general, all these the tested compounds should be subjected to integrated studies, including chemical, pharmacological, physiological and toxicological studies as well as antimicrobial investigation in vitro and in vivo. However, the current study provides promising compounds as potent antimicrobial drugs.

Table-4. Antifungal activity for the tested samples

Tested compound	***Aspergillus fumigatus***	***Geotrichumcandidum***	***Candida albicans***	***Syncephalastrum racemosum***
Clotrimazole (30 µg/ml)	26.3 ± 0.08	23.2 ± 0.03	21.4 ±0.05	20.8 ± 0.02
2	19.3 ±0.03	18.4 ± 0.05	17.2 ± 0.04	16.4 ± 0.09
3	19.3 ± 0.03	16.4 ± 0.03	NA	NA
4	16.3 ± 0.07	14.2 ± 0.08	11.3 ± 0.09	17.3 ± 0.02
5	21.4 ± 0.08	19.3 ± 0.05	20.3 ± 0.06	21.3 ± 0.03
6	23.3 ± 0.9	21.3 ± 0.03	20.3 ± 0.02	18.3 ± 0.01
8	17.3 ± 0.08	16.4 ± 0.1	16.2 ± 0.04	14.0 ± 0.09
9	11.8 ± 0.08	10.9 ± 0.08	10.1 ± 0.1	NA
10	10.2± 0.8	9.4 ± 0.05	8.4 ± 0.1	NA
11	15.5 ± 0.05	14.9 ± 0.04	14.6 ± 0.01	13.8 ± 0.05
12	19.3 ± 0.03	17.2 ± 0.04	16.2 ± 0.8	16.8 ± 0.4
13	17.3 ± 0.08	18.4± 0.05	16.2 ± 0.02	15.9 ± 0.08
14	26.3 ± 0.8	23.2 ± 0.3	19.9 ± 0.04	20.9 ± 0.04
15	26.3 ± 0.08	23.2 ±0.03	20.8 ± 0.2	21.4 ± 0.05
16	25.3 ± 0.09	22.4 ± 0.1	20.2 ± 0.07	20.7 ± 0.05
17	25.4 ± 0.05	22.46± 0.09	19.9 ± 0.07	26.9 ± 0.04
18	20.1 ± 0.07	18.3 ± 0.05	15.4 ± 0.03	13.2 ± 0.03
19	17.3 ± 0.07	15.4 ± 0.03	14.2 ± 0.05	18.4 ± 0.05
20	15.6 ± 0.03	12.2 ± 0.07	11.9 ± 0.1	NA
21	18.4 ± 0.09	16.2 ± 0.08	15.2 ± 0.0	17.9± 0.0

Mean zone of inhibition in mm ± standard deviation beyond well diameter (6 mm) produced on a range of environmental and clinically pathogenic microorganisms using 5 mg/mL of the tested samples. NA= no activity.

Table-5. The antibacterial activity for the tested sample

Tested compounds	*Staphylococcus aureus* (G + ve)	*Bacillissubtillis* (G + ve)	*Pseudomonas aeruginosa* (G – ve)	*Escheirchia coli* (G – ve)
Streptomycin (30 µg/ml)	28.1 ±0.07	29.7 ±0.06	25.4 ±0.09	29.7 ±0.07
2	22.9±0.03	24.2±0.09	18.8±0.07	17.9±0.03
3	20.3 ± 0.03	21.7 ± 0.03	11.1 ± 0.01	16.3 ± 0.08
4	13.5 ± 0.01	12.2 ± 0.052	NA	14.2 ± 0.01
5	13.5 ± 0.01	12.2 ± .052	NA	14.2 ± 0.01
6	13.2 ± 0.1	14.3 ± 0.03	NA	15.5 ± 0.04
8	18.9 ± 0.05	20.2 ± 0.07	15.0 ± 0.09	12.8 ± 0.07
9	14.4 ± 0.01	16.7 ± 0.03	NA	10.5 ± 0.08
10	11.2± 0.01	13.3 ± 0.03	NA	9.47± 0.08
11	16.4 ± 0.05	18.6 ± 0.09	13.5 ± 0.01	11.2 ± 0.09
12	21.6 ± 0.09	23.9 ± 0.08	16.9 ± 0.02	18.7 ± 0.03
13	13.5 ± 0.01	12.2 ± 0.052	NA	14.2 ± 0.01
14	22.6 ± 0.03	21.9 ± 0.07	20.3 ± 0.04	24.2 ± 0.07
15	25.9 ± 0.03	27.2 ±0.08	25.3 ± 0.08	20.8 ± 0.09
16	26.9 ± 0.03	28.2 ± 0.04	24.3 ± 0.08	28.8 ± 0.04
17	26.1 ± 0.06	27.6 ± 0.06	24.9 ± 0.08	28.8 ± 0.09
18	20.4 ± 0.03	21.1 ± 0.04	12.2 ± 0.06	15.9 ± 0.09
19	13.4 ± 0.01	14.6 ± 0.05	NA	12.8 ± 0.9
20	18.2 ± 0.04	20.9 ± 0.03	14.2 ± 0.09	17.4 ± 0.04
21	20.1 ± 0.06	22.6 ± 0.05	16.3 ± 0.08	19.1 ± 0.09

Mean zone of inhibition in mm ± standard deviation beyond well diameter (6 mm) produced on a range of environmental and clinically pathogenic microorganisms using 5 mg/mL of the tested samples,NA= no activity.

4. Conclusion

The rapid emergence of antibiotics-resistant pathogens, either bacterial or fungal infections, has accelerate the need for the development of new effective antimicrobial drugs. Herein, 20 new compounds were synthesized by the reaction of different bromides (5-bromo – 2-acetyl thiophene (2), 4-(5-bromothiophen-2-yl)-methyl-1,3-thiazole (4), 3-(5-bromothiophen-2-yl)-1H-pyrazole (6), isoquinolinium bromide salt (17)) with aryl(heteroaryl)boronic acids via Suzuki-Miyaura cross-coupling reaction, Pd(II) complex and microwave irradiation, the coupling of 5-bromo – 2-acetyl thiophene (2) and isoquinolinium bromide (17) salt were more reactive in water under microwave irradiation condition (7-15 min), whereas the two bromides (4,6) take longer time (30-40 min). The 20 cross- couple products were screened for their antimicrobial potential. The results reproduced 6 new antifungal, 3 new antibacterial (against Gram-positive) and 4 wide spectrum antibacterial agents (against gram-positive and gram-negative). These interesting findings could be the base for further future investigations in order to synthesize novel antimicrobial drugs.

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