

Synthesis of (*E*)-(4-(Aryl)phenyl)(3-(2-(1-phenyl-3-(thiophen-2-yl)-1*H*-pyrazol-4-yl)vinyl)benzofuran-2-yl)methanones by Claisen Schmidt-Suzuki Cross Coupling and their Antibacterial and Antifungal Activity

Dongamanti Ashok,^{1,*} Mohammad Ziauddin,^{1,2} Bommidi Vijaya Lakshmi¹ and Madderla Sarasija¹

¹ Green and Medicinal Chemistry laboratory, Department of Chemistry, Osmania University, Hyderabad-500 007, India; e-mail: ashokdou@gmail.com

² Department of pharmaceutical Sciences, University College of technology, Osmania University, Hyderabad-500 007, India

Received October 31st, 2016; Accepted February 21st, 2017.

Abstract. A series of ten novel (*E*)-(4-(aryl)phenyl)(3-(2-(1-phenyl-3-(thiophen-2-yl)-1*H*-pyrazol-4-yl)vinyl)benzofuran-2-yl)methanones (**7a-j**) derivatives were prepared by traditional Claisen-Schmidt and Suzuki cross-coupling reaction under conventional and microwave irradiation conditions. The structures of all the newly synthesized compounds were established on the basis of FTIR, ¹H NMR, ¹³C NMR, mass spectral data and elemental analysis. The products were assayed for their *in vitro* antibacterial activity against different types of bacterial strains. Further the antifungal activity was examined by inhibitory action against three fungal strains such as *Aspergillus niger*, *Aspergillus flavus* and *Fusarium oxysporum*.

Keywords: Aromatic boronic acid; Antimicrobial activity; Microwave irradiation; Claisen-Schmidt; Suzuki cross-coupling reaction.

Resumen. Una serie de diez nuevos derivados de (*E*)-(4-(aril)fenil)(3-(2-(1-fenil-3-(tiofen-2-il)-1*H*-pirazol-4-il)vinil)benzofuran-2-il) metanonas (**7a-j**) se prepararon mediante la reacción tradicional de Claisen-Schmidt y el acoplamiento cruzado de Suzuki bajo condiciones convencionales e irradiación de microondas. Las estructuras de todos los nuevos compuestos sintetizados fueron establecidas de acuerdo a FTIR, ¹H NMR, ¹³C NMR, espectrometría de masas y análisis elemental. La actividad antibacterial *in vitro* de los productos fue determinada frente a diferentes tipos de cepas bacterianas. Además, se examinó la actividad antifúngica por acción inhibitoria frente a tres cepas de hongos tales como *Aspergillus niger*, *Aspergillus flavus* y *Fusarium oxysporum*.

Palabras clave: Ácido borónico aromático; Actividad antimicrobiana; Irradiación de microondas; Reacción de Claisen-Schmidt; Reacción de acoplamiento cruzado de Suzuki.

Introduction

Synthesis of heterocyclic compounds has emerged as a powerful technique for generating new molecules useful for drug discovery [1]. Heterocyclic compounds containing nitrogen and oxygen plays important role in agrochemical and pharmaceuticals. Benzofuran derivatives possess a wide range of biological activities such as antimicrobial [2], antitumor [3], anti-inflammatory [4], antidepressant [5], analgesic [6] and hypoglycaemic [7] activities. Benzobromarone [8], a uricosuric agent and 5-methyl-3-p-toluoyl-2[4-(3-diethylaminopropoxy) phenyl] benzofuran [9], which is a β -amyloid aggregation inhibitor are the examples of pharmacologically active benzofuran derivatives (Fig. 1).

Pyrazole, a five-membered aromatic nitrogen heterocycle forms a part of the structure of several natural products and drugs. Pyrazole derivatives are known to possess antimicrobial [10], antitumor [11-12], antihypertensive [13], antidepressant [14], insecticidal [15], antifungal [16], 5 α -reductase-inhibitory [17], antiproliferative [18], antiparasitic [19], herbicidal [20], anti-inflammatory [21-22], antiprotozoal [23], analgesic [22] and androgen receptor modulatory [24] activities. The pyrazole ring is present as the core in a variety of leading drugs such as lonazolac [25] and rimonabant [26] (Fig. 1). Thiophene

structure can be found in certain natural products and is also incorporated in several pharmacologically active compounds. In medicinal chemistry, thiophene derivatives have been very well known for their therapeutic applications *e.g.* tienilic acid, a loop diuretic drug [27] and 4-hydrazino-2-methylthio-5-ethyl-6-methylthieno[2,3-*d*]pyrimidine, an antimicrobial agent [28] (Fig. 1).

Aryl-aryl and aryl-vinyl bond formation is very important in organic synthesis and has a wide range of applications of industrial interest, including the synthesis of pharmaceuticals, polymers, herbicides and other materials [29]. Some natural biaryl derivatives can be used as drugs and an example of this is mastigophorene A (Fig 1) [30]. The palladium catalysed Suzuki cross coupling between organoboron compounds and organic halides leads to the formation of carbon-carbon bond, was first reported by Suzuki and Miyaura and it was a revolution in organic couplings [31]. For synthesis of biaryl compounds, palladium-catalyzed Suzuki-Miyaura coupling is the most attractive method [32]. Pd(Ph₃)₄, one of the most suitable catalyst for Suzuki-Miyaura coupling because of the functional-group tolerance, and low sensitivity towards water and air so that reactions can take place even in aqueous medium [33].

Nowadays, organic synthesis in aqueous phase or including water and water/organic mixture as a solvent has been received

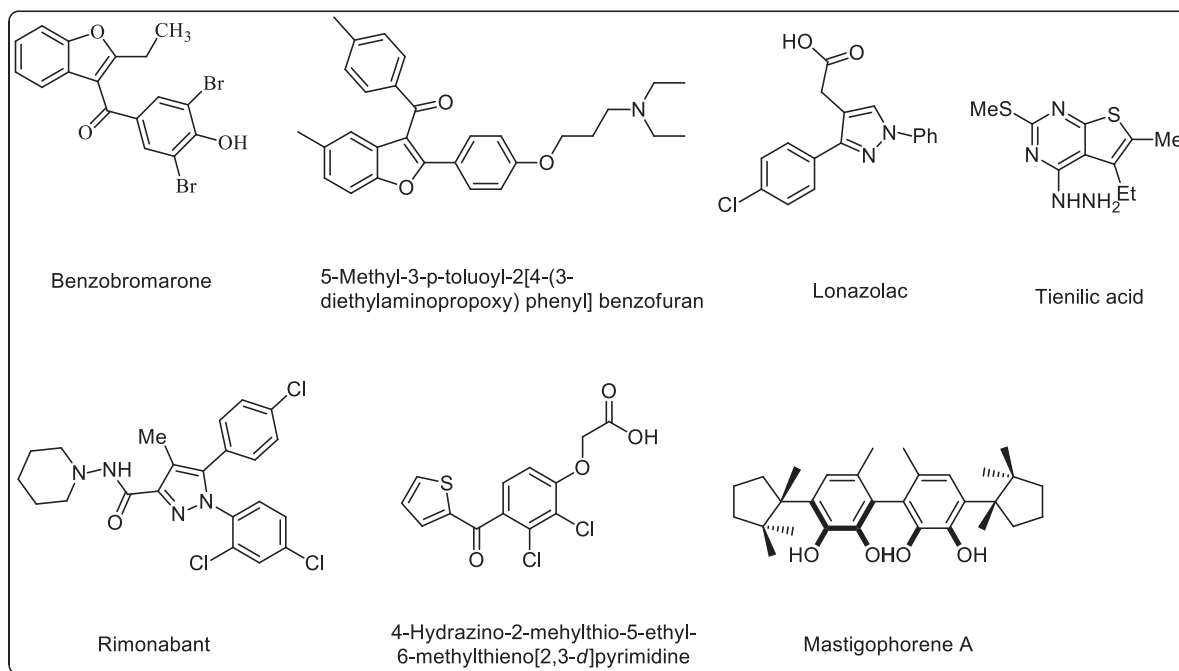


Fig. 1. Structures of some biologically active pyrazoles, thiophenes, biaryls and benzofurans

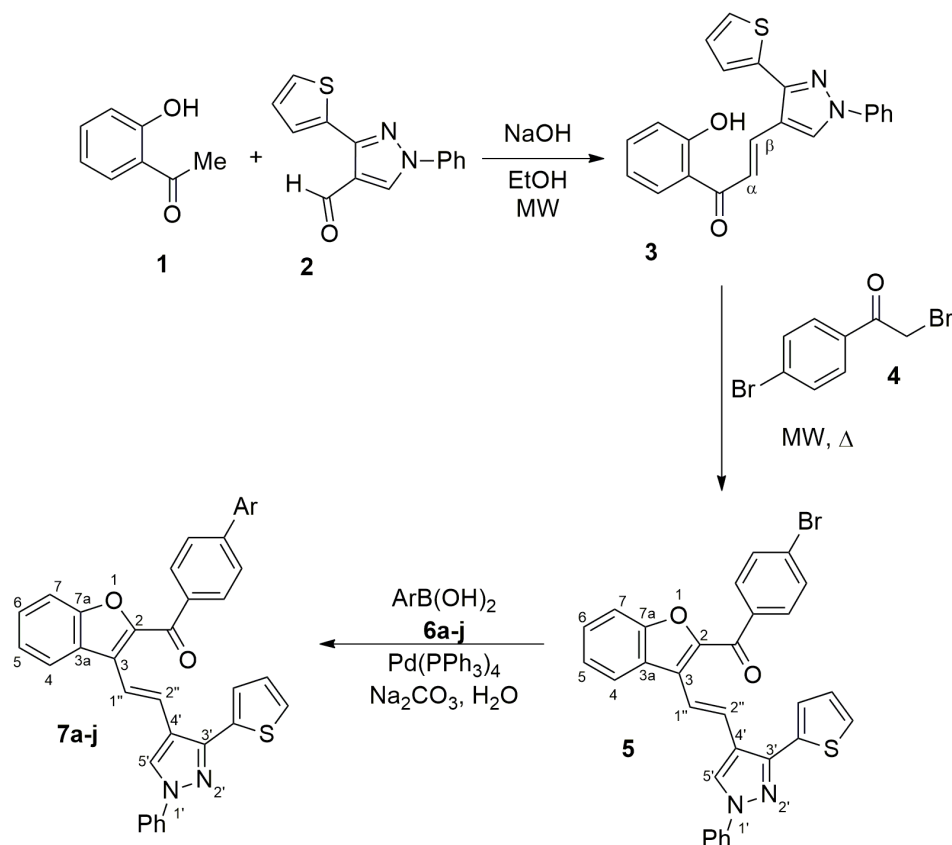
great attention in the field of organic chemistry. Also, it is familiar that microwave assisted organic synthesis (MAOS) is environmental friendly and allows simple separation and catalyst recycling [34]. So, by the microwave assisted synthesis using water as a solvent, one can easily achieve the purpose of green chemistry. In view of the potential bioactivity of benzofuran, biaryl and pyrazole moieties, we have taken up the synthesis of some new benzofuran-pyrazole-biaryl derivatives using Suzuki coupling by the combination of two prominent green chemistry principles, namely microwave assisted synthesis and aqueous phase reactions. We have taken up the synthesis by microwave as well as conventional methods. All the synthesized compounds have tested *in vitro* for their antibacterial and antifungal activities.

Results and Discussion

The synthetic route to compounds **7a-j** is presented in Scheme 1. The condensation of 2-hydroxy acetophenone (**1**) with 1-phenyl-3-(thiophen-2-yl)-1*H*-pyrazole-4-carboxaldehyde [35] (**2**) in the presence of alkali under microwave irradiation [36] gave (*E*)-1-(2-hydroxyphenyl)-3-(1-phenyl-3-(thiophen-2-yl)-1*H*-pyrazol-4-yl)prop-2-en-1-one [37] (**3**) in 95% yield. In the MS of **3** the base peak appeared at 551 corresponding to ($M+H$)⁺. Subsequently, this chalcone (**3**) on reaction with 2-bromo-1-(4-bromophenyl)ethanone (**4**) in presence of potassium carbonate, yielded new benzofuran derivative,

(*E*)-(4-bromophenyl)(3-(2-(1-phenyl-3-(thiophen-2-yl)-1*H*-pyrazol-4-yl)vinyl)benzofuran-2-yl)methanone (**5**). Compound **5** was characterized as (*E*)-(4-bromophenyl)(3-(2-(1-phenyl-3-(thiophen-2-yl)-1*H*-pyrazol-4-yl)vinyl)benzofuran-2-yl)methanone. In the IR spectrum of **5**, a peaks at 825, 1216, 1740 cm⁻¹ confirms the presence of C-Br, C-O-C and carbonyl groups respectively. In ¹H NMR spectrum of **5** showed doublet at δ 7.81 ppm corresponding to two aromatic protons which are ortho to carbonyl group. In ¹³C NMR the carbonyl peak is observed at δ 185.7 ppm.

Compound **5** on Suzuki coupling with different aryl boronic acids (**6a-j**) yielded the target molecules (*E*)-(4-(aryl)phenyl)(3-(2-(1-phenyl-3-(thiophen-2-yl)-1*H*-pyrazol-4-yl)vinyl)benzofuran-2-yl)methanones (**7a-j**). Synthesis of compounds **7a-j** was performed by conventional and microwave irradiation techniques [38] (Table 2). Based on the literature precedence [39] we started our initial studies for the Suzuki coupling reaction with Na₂CO₃ as a base and Pd(PPh₃)₄ as a catalyst. Catalyst loading screening studies tells that increasing the amount of Pd(PPh₃)₄ helping for the good yields (Table 1). Finally we arrived, 15 mol % of Pd(PPh₃)₄ as a optimal condition where we obtained 80% of sole product (**7a**). Compound **7a** was characterized as (*E*)-[1,1'-biphenyl]-4-yl(3-(2-(1-phenyl-3-(thiophen-2-yl)-1*H*-pyrazol-4-yl)vinyl)benzofuran-2-yl)methanone. All peaks in ¹H NMR spectrum of **7a** support the formation of product and in ¹³C NMR of **7a** the carbonyl peak is observed at δ 185.3 ppm. In the MS of **7a** the appearance of base peak at 548 corresponding to ($M+H$)⁺ confirms the formation of Suzuki coupled product.



Scheme 1. Synthetic route for the preparation of titled compounds.

Table 1. Screening Studies for the Catalyst Loading

Pd(PPh ₃) ₄ mol %	Conventional heating		MW heating	
	Time, h	Yield, %	Time, min	Yield, %
1	5	10	20	18
4	5	12	20	16
8	5	20	20	28
12	5	25	20	40
15	5	67	20	80

Antibacterial activity


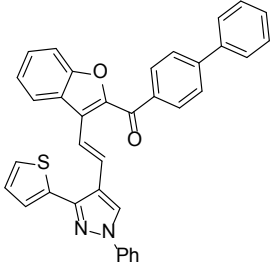
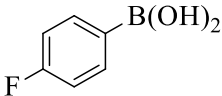
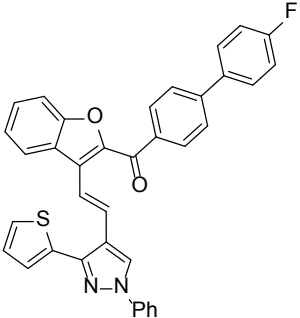
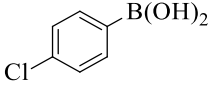
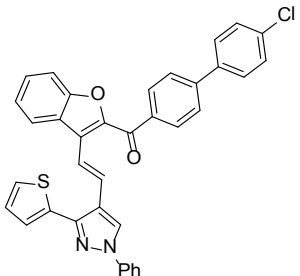
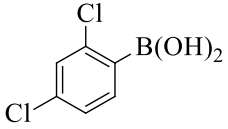
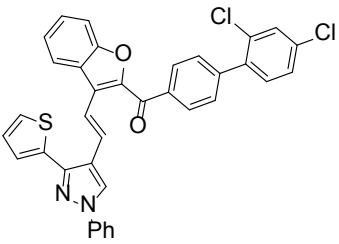
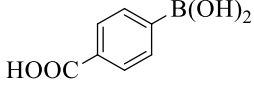
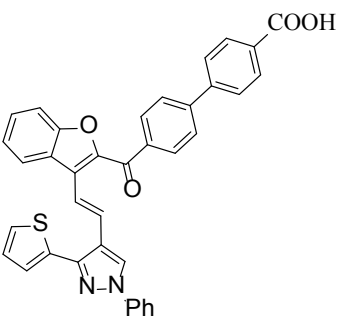
The synthesized novel compounds **5** and **7a-j** were screened for their antibacterial activity against different types of bacterial strains, they are Gram positive bacterial strains of *Bacillus subtilis* and *Staphylococcus aureus*, Gram negative bacterial strains of *Klebsiella pneumoniae* and *Escherichia coli* at a concentration of 10 µg/mL and 20 µg/mL (Fig. 2). Some of the synthesized compounds shown high activity and some shown moderate activity compared to standard drug *Gatifloxacin* at a concentration of 10 µg/mL and 20 µg/mL. The antibacterial

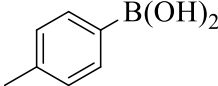
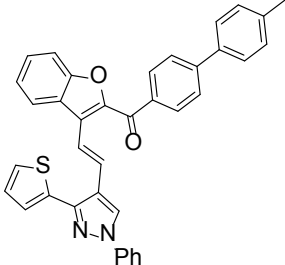
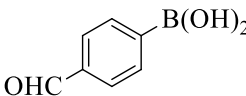
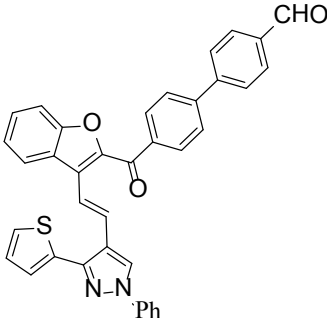
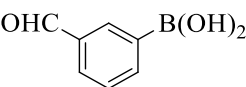
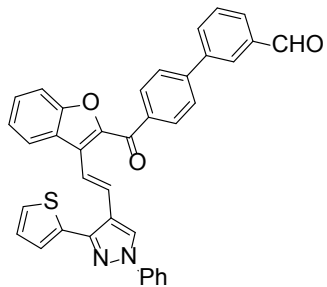
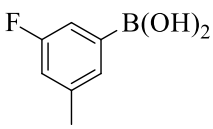
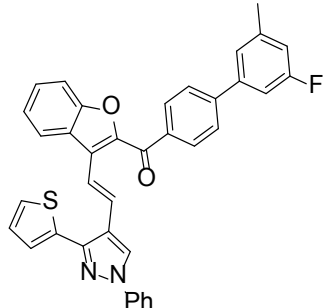
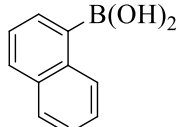
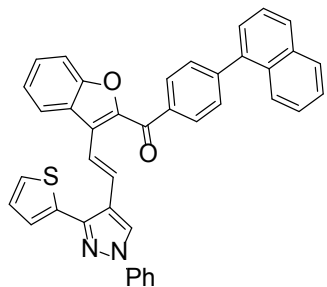
activity of compound **7e** (Ar= 4-carboxyphenyl), **7g** (Ar= 4-formylphenyl) and **7b** (Ar=4-fluorophenyl) shown high zone of inhibition against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Klebsiella pneumoniae* compared to the standard drug at a concentration of 10 µg/mL and 20 µg/mL. Whereas the compounds **7c** (Ar= 4- chlorophenyl) and **7d** (Ar=2,4-dichlorophenyl) were almost equal to the standard drug at a concentration of 10 µg/mL and 20 µg/mL.

Antifungal activity

The antifungal activity of synthesized compounds **5** and **7a-j** were tested against three pathogenic fungi, namely *Fusarium oxysporum*, *Aspergillus nigerzeae*, and *Aspergillus flavus*, by the poison plate technique at a concentration of 100 µg/mL (Fig. 3). Some of the synthesized compounds shown moderate to high antifungal activity compared to standard drug clotrimazole at a concentration of 100 µg/mL. The antifungal activity of compounds **7b** (Ar= 4-fluorophenyl), **7d** (Ar= 2,4- dichlorophenyl) and **7h** (Ar=3-formyl) shown high zone of inhibition against *Aspergillus flavus* and *Fusarium oxysporum* compared to the standard drug. Compounds **7c** (Ar= 4-chlorophenyl), **7e** (Ar= 4-carboxyphenyl) shown high zone of inhibition against

Table 2. Comparison of yield of synthesized compounds (**7a-j**)

Compound	ArB(OH) ₂	Product	Conventional method		MWI	
			Time (h)	Yield (%)	Time (min)	Yield (%)
7a			5	72	20	80
7b			5.5	70	25	84
7c			5.5	71	20	83
7d			6	73	20	80
7e			6.5	72	20	82

Compound	ArB(OH) ₂	Product	Conventional method		MWI	
			Time (h)	Yield (%)	Time (min)	Yield (%)
7f			6	71	25	80
7g			7	65	25	81
7h			6.5	65	25	80
7i			6	72	20	83
7j			5.5	71	20	82

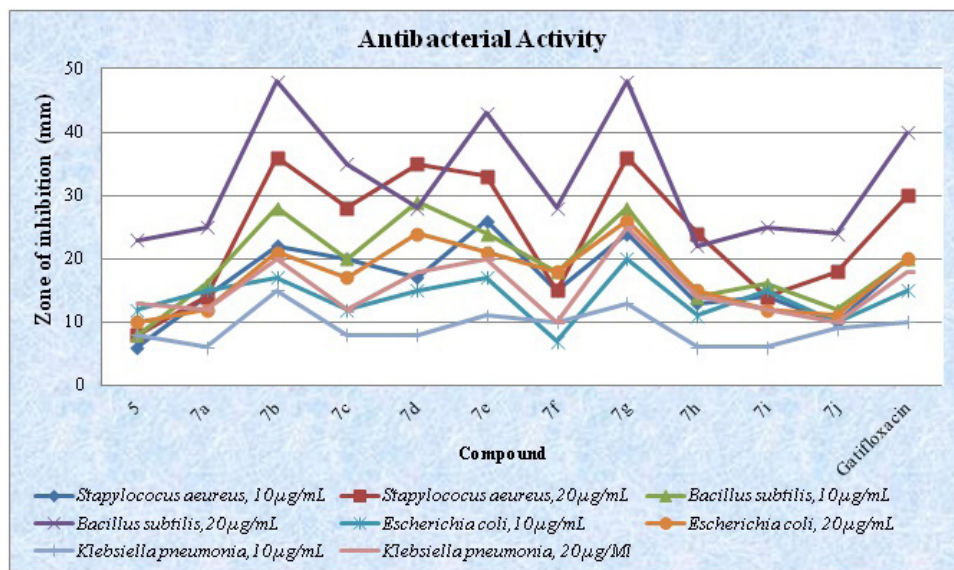


Fig. 2. Antibacterial activity of compounds 5 and 7a-j.

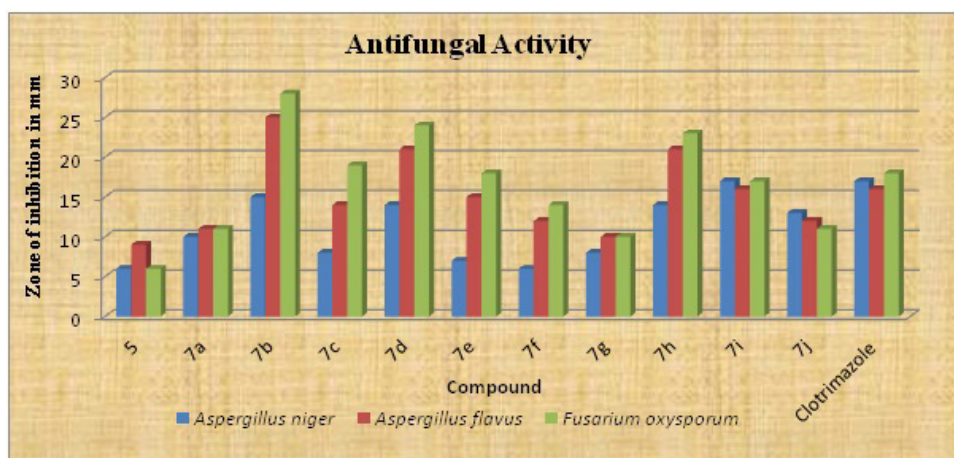


Fig. 3. Antifungal activity of compounds 5 and 7a-j.

Fusarium oxysporum compared to the standard drug. The compound **7i** (Ar= 3-fluoro-5-methylphenyl) shown zone of inhibition against tested fungi were almost equal to the standard drug. Rest of the compounds shown moderate activity against pathogenic fungi compared to standard.

Conclusions

A series of some new (*E*)-(4-(aryl)phenyl)(3-(2-(1-phenyl-3-(thiophen-2-yl)-1*H*-pyrazol-4-yl)vinyl)benzofuran-2-yl) methanones (**7a-j**) derivatives were prepared by traditional Claisen-Schmidt and Suzuki cross-coupling reactions under conventional and microwave irradiation methods. All final compounds were investigated for their *in vitro* antimicrobial

activity. Some of the compounds show better antimicrobial activity against selected microorganisms compared with the reference drugs. Thus, the above compounds can be considered as lead compounds for further development of more potent antimicrobial agents.

EXPERIMENTAL

General

Stuart SMP30 apparatus is used to determine melting points. Purity of the compounds was checked by TLC on silica gel 60 F₂₅₄ (Merck). All the microwave irradiation experiments were performed in a CEM Discover microwave system and reaction

temperatures were monitored by an equipped IR sensor. ^1H NMR and ^{13}C NMR spectra were recorded on Bruker Avance II 400 spectrometer using TMS as an internal standard. IR spectra were recorded in KBr on a Shimadzu FTIR 8400S spectrophotometer. Mass spectra were recorded on a Shimadzu GCMS-QP 1000 mass spectrometer. The elemental analysis was carried out on a Vario-11CHN analyzer.

Procedure for the synthesis of compound (*E*)-1-(2-hydroxyphenyl)-3-(1-phenyl-3-(thiophen-2-yl)-1*H*-pyrazol-4-yl)prop-2-en-1-one (3):

A mixture of 2-hydroxyacetophenone (**1**) (240 mg, 2 mmol), 1-phenyl-3-(thiophen-2-yl)-1*H*-pyrazole-4-carboxaldehyde (**2**) (508 mg, 2 mmol) in ethanol (2 mL) and NaOH (200 mg, 5 mmol) was introduced into CEM discover microwave reaction vessel equipped with a magnetic stirrer. The vessel was sealed and then placed into the microwave cavity. Initial microwave irradiation of 100 W was used, the temperature being ramped from room temperature to the desired 80 °C temperature. Progress of the reaction was monitored by TLC. After completion of the reaction (10 min) it was diluted with cold water and acidified with diluted HCl, the precipitate formed was filtered, dried and recrystallized from ethanol to afford pure chalcone (**3**) (Yield: 95%).

A yellowish solid: mp 202-204 °C; IR (KBr) ν_{max} 3416 (OH), 1681 (C=O), 1620 (C=CH) cm^{-1} . ^1H NMR (CDCl_3 , 400 MHz) δ 6.92-6.96 (m, 1H, Ar-H), 7.03 (d, J = 9.0 Hz, 1H, Ar-H), 7.18-7.20 (m, 1H, Ar-H), 7.35-7.56 (m, 7H, Ar-H, H_α), 7.80 (d, J = 7.7 Hz, 2H, Ar-H), 7.86 (d, J = 7.78 Hz, 1H, Ar-H), 8.14 (d, J = 15.3 Hz, 1H, H_β), 8.38 (s, 1H, pyrazole-H), 12.90 (s, 1H, OH); ^{13}C NMR (CDCl_3 , 100 MHz) δ 110.3, 117.9, 118.9, 119.4, 120.6, 121.2, 126.9, 127.0, 127.3, 127.6, 127.9, 129.6, 131.6, 133.5, 136.5, 138.7, 139.0, 148.3, 162.5, 192.2; Anal. Calcd for $\text{C}_{22}\text{H}_{16}\text{N}_2\text{O}_2\text{S}$: C, 70.95; H, 4.33; N, 7.52. Found: C, 70.90; H, 4.29; N, 7.46.

Procedure for the synthesis of compound (*E*)-(4-bromophenyl)(3-(2-(1-phenyl-3-(thiophen-2-yl)-1*H*-pyrazol-4-yl)vinyl)benzofuran-2-yl)methanone (5)

Conventional method: A mixture of (*E*)-1-(2-hydroxyphenyl)-3-(1-phenyl-3-(thiophen-2-yl)-1*H*-pyrazol-4-yl)prop-2-en-1-one (**3**) (372 mg, 1 mmol) and 2-bromo-1-(4-bromophenyl)ethanone (**4**) (276 mg, 1 mmol), 5 g of anhydrous K_2CO_3 and acetone (15 mL) was taken in 100 mL round bottomed flask and it was refluxed for 4 h. After completion of reaction as followed by the TLC examination, cold water was added. The solid separated was filtered and washed with water and extracted with hot 5% NaOH solution. The alkaline solution was cooled to 0-5 °C and neutralized with diluted HCl. The precipitated solid was filtered, washed with cold water and dried. The crude product was purified by column chromatography on silica gel using hexane:ethyl acetate (7:3 v/v) as eluent and further recrystallized from methanol to afford the desired product **5** (Yield 75%).

Microwave irradiation method: A mixture of (*E*)-1-(2-hydroxyphenyl)-3-(1-phenyl-3-(thiophen-2-yl)-1*H*-pyrazol-4-yl)

prop-2-en-1-one (**3**) (372 mg, 1 mmol) and 2-bromo-1-(4-bromophenyl)ethanone (**4**) (276 mg, 1 mmol) was dissolved in minimum quantity of ethanol and adsorbed by 5 g of anhydrous K_2CO_3 . This was introduced into CEM discover microwave reaction vessel equipped with a magnetic stirrer. The vessel was sealed and then placed into the microwave cavity. Initial microwave irradiation of 300 W was used, the temperature being ramped from room temperature to 120 °C temperature. Once this was reached, the reaction mixture was heated at this temperature for 6 min. After completion of reaction as followed by the TLC examination, cold water was added to. The solid separated was filtered and washed with water and extracted with hot 5% NaOH solution. The alkaline solution was cooled to 0-5 °C and neutralized with diluted HCl. The precipitated solid was filtered, washed with cold water and dried. The crude product was purified by column chromatography on silica gel using hexane:ethyl acetate (7:3 v/v) as eluent and further recrystallized from methanol to afford the desired product **5** (Yield: 88%)

A pale yellow solid: mp 232-234 °C; IR (KBr) ν_{max} 1740 (C=O), 1602 (C=CH), 1216 (C-O-C), 825 (C-Br) cm^{-1} . ^1H NMR (CDCl_3 , 400 MHz) δ 7.21-7.23 (m, 1H, Ar-H), 7.32-7.36 (m, 1H, Ar-H), 7.45-7.57 (m, 7H, Ar-H, H-1''), 7.63-7.67 (m, 3H, Ar-H), 7.81 (d, J = 7.5 Hz, 2H, Ar-H), 7.89 (d, J = 16.8 Hz, 1H, H-2''), 8.07-8.09 (m, 2H, Ar-H), 8.29 (d, J = 1.7 Hz, 1H, Ar-H), 8.33 (s, 1H, pyrazole-H); ^{13}C NMR (CDCl_3 , 100 MHz) δ 113.7, 119.2, 119.6, 119.9, 122.7, 124.9, 125.3, 125.4, 126.1, 126.6, 126.9, 127.1, 127.7, 128.4, 128.7, 129.5, 129.8, 129.9, 131.3, 134.7, 137.6, 139.5, 146.4, 148.6, 153.0, 185.7; ESI MS m/z (rel int): 551 ($\text{M}+\text{H}$) $^+$ (100). Anal. Calcd for $\text{C}_{30}\text{H}_{19}\text{BrN}_2\text{O}_2\text{S}$: C, 65.34; H, 3.47; N, 5.08. Found: C, 65.31; H, 3.44; N, 5.02.

General procedure for the synthesis of compounds (*E*)-(4-(aryl)phenyl)(3-(2-(1-phenyl-3-(thiophen-2-yl)-1*H*-pyrazol-4-yl)vinyl)benzofuran-2-yl)methanones (7a-j):

Conventional heating method:

To a round bottomed flask was added (*E*)-(4-bromophenyl)(3-(2-(1-phenyl-3-(thiophen-2-yl)-1*H*-pyrazol-4-yl)vinyl)benzofuran-2-yl)methanone (**5**) (550 mg, 1 mmol), $\text{Pd}(\text{PPh}_3)_4$ (0.15 mmol), arylboronic acid (1.30 mmol) (**6a-j**), Na_2CO_3 (3.00 mmol) and water (20 mL) sequentially. The mixture was degassed with nitrogen over 15 min. Then, the reaction mixture was stirred at 80 °C for 5-7 h (Table 2) under nitrogen atmosphere. After cooled to room temperature, the mixture was diluted with EtOAc (15 mL) and washed with H_2O and brine solution. The organic layer was washed with 10 mL of water and it was dried over anhydrous magnesium sulphate. The solvent was evaporated and the residue was purified by using silica gel column chromatography (1:3 v/v ethylacetate: hexane) to afford pure products (**7a-j**).

Microwave irradiation method: A degassed mixture of (*E*)-(4-bromophenyl)(3-(2-(1-phenyl-3-(thiophen-2-yl)-1*H*-pyrazol-4-yl)vinyl)benzofuran-2-yl)methanone (**5**) (550 mg, 1 mmol), $\text{Pd}(\text{PPh}_3)_4$ (0.15 mmol), arylboronic acid (1.30 mmol) (**6a-j**), Na_2CO_3 (3.00 mmol) and water (5 mL) was introduced into CEM discover microwave reaction vessel equipped with a

magnetic stirrer. The vessel was sealed and then placed into the microwave cavity. Initial microwave irradiation of 180 W was used, the temperature being ramped from room temperature to the desired 80 °C temperature. Once this was reached, the reaction mixture was heated at this temperature for appropriate time as shown in Table 2. The reaction mixture was stirred continuously during the reaction. After cooled to room temperature, the mixture was diluted with EtOAc and washed with H₂O and brine solution. The organic layer was washed with 10 mL of water and it was dried over anhydrous magnesium sulphate. The solvent was evaporated and the residue was purified by using silica gel column chromatography (1:3 v/v ethylacetate: hexane) to afford pure products (**7a-j**).

(E)-[1,1'-biphenyl]-4-yl(3-(2-(1-phenyl-3-(thiophen-2-yl)-1H-pyrazol-4-yl)vinyl)benzofuran-2-yl)methanone (7a). A pale yellow solid: mp 225-227 °C; IR (KBr) ν_{\max} 1739 (C=O), 1598 (C=CH), 1216 (C-O-C) cm⁻¹. ¹H NMR (CDCl₃, 400 MHz) δ 7.19-7.21 (m, 1H, Ar-H), 7.30-7.34 (m, 1H, Ar-H), 7.41-7.53 (m, 8H, Ar-H), 7.54-7.58 (m, 1H, Ar-H), 7.63 (d, J = 7.5 Hz, 1H, Ar-H), 7.68-7.81 (m, 7H, Ar-H, H-1''), 8.00 (d, J = 16.5 Hz, 1H, H-2''), 8.15 (d, J = 8.0 Hz, 1H, Ar-H), 8.21 (d, J = 8.7 Hz, 2H, Ar-H), 8.33 (s, 1H, pyrazole-H); ¹³C NMR (CDCl₃, 100 MHz) δ 112.7, 119.1, 120.2, 120.4, 123.1, 124.2, 124.8, 124.9, 125.9, 126.0, 126.1, 126.8, 127.0, 127.2, 127.3, 127.7, 128.2, 128.3, 129.0, 129.4, 130.5, 134.9, 136.6, 139.5, 139.9, 145.4, 146.3, 147.9, 154.8, 185.3; ESI MS m/z (rel int): 549 (M+H)⁺(100). Anal. Calcd for C₃₆H₂₄N₂O₂S: C, 78.81; H, 4.41; N, 5.11. Found: C, 78.75; H, 4.36; N, 5.07.

(E)-(4'-fluoro-[1,1'-biphenyl]-4-yl)(3-(2-(1-phenyl-3-(thiophen-2-yl)-1H-pyrazol-4-yl)vinyl)benzofuran-2-yl)methanone (7b). A pale yellow solid: mp 242-244 °C; IR (KBr) ν_{\max} 1741 (C=O), 1599 (C=CH), 1235 (C-O-C), 696 (C-F) cm⁻¹. ¹H NMR (CDCl₃, 400 MHz) δ 7.15-7.21 (m, 3H, Ar-H), 7.31-7.34 (m, 1H, Ar-H), 7.41-7.66 (m, 9H, Ar-H, H-1''), 7.72-7.80 (m, 5H, Ar-H), 7.98 (d, J = 16.8 Hz, 1H, H-2''), 8.15 (d, J = 8.0 Hz, 1H, Ar-H), 8.20 (d, J = 8.5 Hz, 2H, Ar-H), 8.33 (s, 1H, pyrazole-H); ¹³C NMR (CDCl₃, 100 MHz) δ 113.7, 114.1, 117.3, 119.2, 119.5, 119.6, 119.9, 122.7, 124.9, 125.3, 125.4, 125.8, 126.1, 126.2, 126.4, 126.6, 126.9, 127.7, 128.4, 128.7, 129.5, 129.8, 129.9, 131.3, 132.9, 134.6, 137.6, 139.5, 146.5, 148.6 (J = 244), 153.3, 185.7; ESI MS m/z (rel int): 567 (M+H)⁺(100). Anal. Calcd for C₃₆H₂₃FN₂O₂S: C, 76.31; H, 4.09; N, 4.94. Found: C, 76.27; H, 4.03; N, 4.91.

(E)-(4'-chloro-[1,1'-biphenyl]-4-yl)(3-(2-(1-phenyl-3-(thiophen-2-yl)-1H-pyrazol-4-yl)vinyl)benzofuran-2-yl)methanone (7c). A pale yellow solid: mp 232-234 °C; IR (KBr) ν_{\max} 1738 (C=O), 1586 (C=CH), 1230 (C-O-C), 744 (C-Cl) cm⁻¹. ¹H NMR (CDCl₃, 400 MHz) δ 7.19-7.21 (m, 1H, Ar-H), 7.31-7.35 (m, 1H, Ar-H), 7.40-7.62 (m, 10H, Ar-H, H-1''), 7.70-7.81 (m, 6H, Ar-H), 7.99 (d, J = 16.8 Hz, 1H, H-2''), 8.14 (d, J = 8.0 Hz, 1H, Ar-H), 8.20 (d, J = 8.2 Hz, 2H, Ar-H), 8.35 (s, 1H, pyrazole-H); ¹³C NMR (CDCl₃, 100 MHz) δ 112.2, 119.2, 120.2, 120.3, 124.3, 124.9, 125.2, 125.8, 125.9, 126.0, 126.1, 126.9,

127.0, 127.6, 127.7, 128.0, 128.5, 129.5, 130.6, 134.9, 135.5, 137.7, 139.5, 142.4, 142.9, 147.7, 154.8, 186.7; ESI MS m/z (rel int): 583 (M+H)⁺(100). Anal. Calcd for C₃₆H₂₃ClN₂O₂S: C, 74.15; H, 3.98; N, 4.80. Found: C, 74.11; H, 3.93; N, 4.76.

(E)-(2',4'-dichloro-[1,1'-biphenyl]-4-yl)(3-(2-(1-phenyl-3-(thiophen-2-yl)-1H-pyrazol-4-yl)vinyl)benzofuran-2-yl)methanone (7d). A pale yellow solid: mp 236-238 °C; IR (KBr) ν_{\max} 1740 (C=O), 1598 (C=CH), 1233 (C-O-C), 742 (C-Cl) cm⁻¹. ¹H NMR (CDCl₃, 400 MHz) δ 7.18-7.20 (m, 1H, Ar-H), 7.31-7.60 (m, 10H, Ar-H, H-1''), 7.68-7.82 (m, 6H, Ar-H), 7.95-8.00 (m, 3H, Ar-H, H-2''), 8.13 (d, J = 8.0 Hz, 1H, Ar-H), 8.34 (s, 1H, pyrazole-H); ¹³C NMR (CDCl₃, 100 MHz) δ 112.1, 119.4, 121.2, 123.3, 124.3, 124.8, 125.2, 125.8, 125.6, 126.0, 126.1, 126.9, 127.0, 127.6, 127.7, 128.0, 128.5, 129.5, 130.6, 134.9, 135.5, 137.7, 139.5, 142.4, 142.9, 147.3, 154.5, 186.5; ESI MS m/z (rel int): 617 (M+H)⁺(100). Anal. Calcd for C₃₆H₂₂Cl₂N₂O₂S: C, 70.02; H, 3.59; N, 4.54. Found: C, 69.97; H, 3.54; N, 4.51.

(E)-4'-(3-(2-(1-phenyl-3-(thiophen-2-yl)-1H-pyrazol-4-yl)vinyl)benzofuran-2-carbonyl)-[1,1'-biphenyl]-4-carboxylic acid (7e). A pale yellow solid: mp 248-250 °C; IR (KBr) ν_{\max} 1739 (C=O), 1598 (C=CH), 1216 (C-O-C) cm⁻¹. ¹H NMR (CDCl₃, 400 MHz) δ 7.18-7.20 (m, 1H, Ar-H), 7.31-7.34 (m, 1H, Ar-H), 7.45-7.84 (m, 14H, Ar-H, H-1''), 7.98-8.00 (m, 3H, Ar-H, H-2''), 8.13 (d, J = 8.0 Hz, 1H, Ar-H), 8.25 (d, J = 8.5 Hz, 2H, Ar-H), 8.33 (s, 1H, pyrazole-H), 13.09 (s, 1H, -COOH); ¹³C NMR (CDCl₃, 100 MHz) δ 112.6, 119.2, 120.1, 123.2, 124.3, 125.0, 125.3, 125.8, 126.0, 126.1, 126.9, 127.7, 127.8, 128.6, 129.5, 131.3, 131.6, 134.9, 136.6, 139.5, 146.3, 147.4, 154.8, 180.5, 187.3; ESI MS m/z (rel int): 593 (M+H)⁺(100). Anal. Calcd for C₃₇H₂₄N₂O₄S: C, 74.98; H, 4.08; N, 4.73. Found: C, 74.92; H, 4.04; N, 4.69.

(E)-(4'-methyl-[1,1'-biphenyl]-4-yl)(3-(2-(1-phenyl-3-(thiophen-2-yl)-1H-pyrazol-4-yl)vinyl)benzofuran-2-yl)methanone (7f). A pale yellow solid: mp 212-214 °C; IR (KBr) ν_{\max} 1739 (C=O), 1598 (C=CH), 1216 (C-O-C) cm⁻¹. ¹H NMR (CDCl₃, 400 MHz) δ 2.42 (s, 3H, Me-H), 7.18-7.20 (m, 1H, Ar-H), 7.33-7.40 (m, 3H, Ar-H), 7.42-7.63 (m, 9H, Ar-H, H-1''), 7.70-7.79 (m, 5H, Ar-H), 7.96 (d, J = 8.0 Hz, 1H, Ar-H), 8.13-8.19 (m, 3H, Ar-H, H-2''), 8.31 (s, 1H, pyrazole-H); ¹³C NMR (CDCl₃, 100 MHz) δ 21.1, 112.7, 119.1, 120.2, 120.4, 123.1, 124.2, 124.7, 124.9, 125.9, 126.1, 126.0, 126.7, 126.8, 127.1, 127.7, 128.3, 129.4, 129.7, 130.5, 135.0, 136.4, 137.0, 138.2, 139.5, 145.4, 146.3, 148.0, 154.8, 185.3; ESI MS m/z (rel int): 563 (M+H)⁺(100). Anal. Calcd for C₃₇H₂₆N₂O₂S: C, 78.98; H, 4.66; N, 4.98. Found: C, 78.92; H, 4.62; N, 4.94.

(E)-4'-(3-(2-(1-phenyl-3-(thiophen-2-yl)-1H-pyrazol-4-yl)vinyl)benzofuran-2-carbonyl)-[1,1'-biphenyl]-4-carbaldehyde (7g). A pale yellow solid: mp 246-248 °C; IR (KBr) ν_{\max} 1740 (C=O), 1670 (C=O of aldehyde), 1602 (C=CH), 1216 (C-O-C) cm⁻¹. ¹H NMR (CDCl₃, 400 MHz) δ 6.96 (d, J = 8.5 Hz, 1H, Ar-H), 7.19-7.35 (m, 2H, Ar-H), 7.44-8.02 (m, 16H,

Ar-H, H-1'', H-2''), 8.15 (d, $J = 7.7$ Hz, 1H, Ar-H), 8.15 (d, $J = 8.2$ Hz, 1H, Ar-H), 8.34 (s, 1H, pyrazole-H); ^{13}C NMR (CDCl_3 , 100 MHz) δ 112.7, 115.9, 119.2, 120.2, 120.3, 123.2, 124.3, 125.0, 125.2, 125.9, 126.0, 126.1, 126.9, 127.3, 127.7, 127.9, 128.5, 129.0, 129.1, 129.5, 130.3, 130.5, 130.6, 131.6, 135.8, 134.9, 137.7, 139.5, 154.8, 185.2, 191.8; ESI MS m/z (rel int): 577 ($\text{M}+\text{H}$) $^+$ (100). Anal. Calcd for $\text{C}_{37}\text{H}_{24}\text{N}_2\text{O}_3\text{S}$: C, 77.06; H, 4.19; N, 4.86. Found: C, 77.02; H, 4.14; N, 4.82.

(*E*)-4'-(3-(2-(1-phenyl-3-(thiophen-2-yl)-1*H*-pyrazol-4-yl)vinyl)benzofuran-2-carbonyl)-[1,1'-biphenyl]-3-carbaldehyde (7h). A pale yellow solid: mp 228-230 °C; IR (KBr) ν_{max} 1740 (C=O), 1695 (C=O of aldehyde), 1602 (C=CH), 1231 (C-O-C) cm^{-1} . ^1H NMR (CDCl_3 , 400 MHz) δ 7.19-7.21 (m, 1H, Ar-H), 7.31-7.35 (m, 1H, Ar-H), 7.44-7.83 (m, 13H, Ar-H, H-1''), 7.92-8.03 (m, 3H, Ar-H, H-2''), 8.14-8.25 (m, 4H, Ar-H), 8.35 (s, 1H, pyrazole-H), 10.12 (s, 1H, -CHO); ^{13}C NMR (CDCl_3 , 100 MHz) δ 112.7, 119.2, 120.2, 120.3, 123.1, 124.3, 124.9, 125.1, 125.9, 126.0, 126.1, 126.9, 127.0, 127.5, 127.7, 128.5, 129.5, 129.5, 129.7, 130.6, 133.1, 134.9, 137.0, 137.3, 139.5, 140.9, 143.7, 146.3, 147.8, 154.8, 185.2, 192.0; ESI MS m/z (rel int): 577 ($\text{M}+\text{H}$) $^+$ (100). Anal. Calcd for $\text{C}_{37}\text{H}_{24}\text{N}_2\text{O}_3\text{S}$: C, 77.06; H, 4.19; N, 4.86. Found: C, 77.02; H, 4.14; N, 4.82.

(*E*)-(3'-fluoro-5'-methyl-[1,1'-biphenyl]-4-yl)(3-(2-(1-phenyl-3-(thiophen-2-yl)-1*H*-pyrazol-4-yl)vinyl)benzofuran-2-yl)methanone (7i). A pale yellow solid: mp 252-254 °C; IR (KBr) ν_{max} 1739 (C=O), 1598 (C=CH), 1216 (C-O-C), 691 (C-F) cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz) δ 2.38 (s, 3H, Me-H), 7.18-7.19 (m, 1H, Ar-H), 7.20-7.58 (m, 10H, Ar-H), 7.62-7.95 (m, 6H, Ar-H, H-1''), 7.98-7.90 (m, 3H, Ar-H, H-2''), 8.13 (d, $J = 8.0$ Hz, 1H, Ar-H), 8.34 (s, 1H, pyrazole-H); ^{13}C NMR (CDCl_3 , 100 MHz) δ 22.7, 112.6, 119.2, 120.1, 123.2, 123.9, 124.3, 124.4, 125.0, 125.3, 125.8, 126.0, 126.0, 126.1, 126.9, 127.7, 127.8, 128.8, 128.6, 129.3, 129.5, 130.0, 130.2, 130.7, 131.3, 131.6, 134.9, 136.6, 138.4, 139.5, 146.3, 147.4, 154.8, 184.6; ESI MS m/z (rel int): 581 ($\text{M}+\text{H}$) $^+$ (100). Anal. Calcd for $\text{C}_{37}\text{H}_{25}\text{FN}_2\text{O}_2\text{S}$: C, 76.53; H, 4.34; N, 4.82. Found: C, 76.49; H, 4.31; N, 4.78.

(*E*)-(4-(naphthalen-1-yl)phenyl)(3-(2-(1-phenyl-3-(thiophen-2-yl)-1*H*-pyrazol-4-yl)vinyl)benzofuran-2-yl)methanone (7j). A pale yellow solid: mp 268-270 °C; IR (KBr) ν_{max} 1741 (C=O), 1600 (C=CH), 1232 (C-O-C) cm^{-1} . ^1H NMR (CDCl_3 , 400 MHz) δ 7.19-7.21 (m, 1H, Ar-H), 7.29-7.34 (m, 2H, Ar-H), 7.41-7.81 (m, 16H, Ar-H, H-1''), 7.90-7.96 (m, 3H, Ar-H), 8.04 (d, $J = 16.5$ Hz, 1H, H-2''), 8.16 (d, $J = 8.0$ Hz, 1H, Ar-H), 8.26 (d, $J = 8.5$ Hz, 2H, Ar-H), 8.37 (s, 1H, pyrazole-H); ^{13}C NMR (CDCl_3 , 100 MHz) δ 112.7, 115.5, 119.2, 119.4, 120.2, 120.4, 123.1, 124.3, 125.0, 125.3, 125.6, 126.0, 126.1, 126.4, 126.9, 127.0, 127.4, 127.7, 128.3, 128.4, 128.8, 129.3, 129.5, 129.5, 129.9, 130.1, 130.1, 131.2, 131.3, 133.8, 139.1, 139.2, 139.5, 145.5, 154.8, 171.7, 185.5; ESI MS m/z (rel int): 599 ($\text{M}+\text{H}$) $^+$ (100). Anal. Calcd for $\text{C}_{40}\text{H}_{26}\text{N}_2\text{O}_2\text{S}$: C, 80.24; H, 4.38; N, 4.68. Found: C, 80.21; H, 4.42; N, 4.64.

Biological Assay

Antibacterial activity

The synthesized novel compounds **5** and **7a-j** were screened for their antibacterial activity against different types of bacterial strains, they are Gram negative bacterial strains of *Pseudomonas aeruginosa* and *Escherichia coli*, Gram positive bacterial strains of *Bacillus subtilis* and *Staphylococcus aureus* at a concentration of 50 $\mu\text{g/mL}$. The cultures were diluted with 5% autoclaved saline and the final volume was made with concentration approximately 10^5 - 10^6 CFU/mL. The synthesized compounds were diluted in acetone for antibacterial biological assays. For agar disc diffusion method [40], the liquid form of test compound was soaked on to the disc and then allowed to air dry, such that the disc gets completely saturated with test compound. The saturated chemical discs were introduced onto the upper layer of the medium evenly loaded with the bacteria. The discs were dipped in different chemical samples, were placed over the evenly spread bacterial nutrient media and incubated at 37 °C for 24 to 48 h for better inhibition of bacteria. The zones of inhibition were measured after 24 to 48 h. All the experiments were carried out in triplicates and the results were expressed as zone of inhibition in mm. The zones of inhibition of synthesized compounds were compared with the zone of inhibition of standard antibiotic concentration (50 $\mu\text{g/mL}$). The antibacterial activity was evaluated and the results are presented in Fig. 2.

Antifungal activity

The antifungal activity of synthesized compounds was tested against three pathogenic fungi, namely *Fusarium oxysporum*, *Aspergillus niger*, and *Penicillium italicum*, by the poison plate technique at a concentration of 50 $\mu\text{g/mL}$. Three kinds of fungi were incubated in PDA at 25 ± 1 °C for 5 days to get new mycelium for antifungal assay, then a mycelia as disks of approximately 0.45 cm diameter cut from the culture medium were picked up with a sterilized inoculation needle and inoculated in the center of PDA plate. Test compounds were dissolved in acetone (10 mL) then added to the Potato Dextrose Agar medium (PDA, 90 mL). The final concentration of compounds in the medium was adjusted to 50 $\mu\text{g/mL}$. The inoculated plates were incubated at 25 ± 1 °C for 5 days. Acetone was diluted with sterilized distilled water and used as control, while clotrimazole (50 $\mu\text{g/mL}$) was used as standard control for each treatment three replicates of experiments were carried out. The radial growth of the fungal colonies was measured on the sixth day.

Acknowledgements

The authors are thankful to The Head, Department of Chemistry, Osmania University, Hyderabad for providing laboratory facilities. We also thankful to The Director, CFRD, Osmania University, Hyderabad for providing elemental and spectral analysis facilities.

References

1. Hermakens, P. H.; Ottenheijm, H. C.; Rees, D. C. *Tetrahedron* **1997**, 53, 5643-5678.
2. Manna, K.; Agarwal, Y. K. *Bioorg. Med. Chem. Lett.*, **2009**, 19, 2688-2692.
3. Hayakawa, I.; Shioya, R.; Agatsuma, T.; Furukawa, H.; Naruto, S.; Sugano, Y. *Bioorg. Med. Chem. Lett.* **2004**, 14, 455-458.
4. Santana, L.; Teijeira, M.; Uriarte, E.; Teran, C.; Liñares, B.; Villar, R.; Laguna, R.; Cano, E. *Eur. J. Pharm. Sci.* **1999**, 7, 161-165.
5. Malik, W. U.; Mahesh, V. K.; Raishighani, M. *Indian J. Chem.* **1971**, 9, 655-657.
6. Fry, D. J.; Ficken, E. G.; Burrows, R. W. *Brit.* **1969**, 1, 168495.
7. Brady, B. A.; Kennedy, J. A.; Sullivan, W. I. *Tetrahedron* **1973**, 29, 359-362.
8. Elks, J.; Ganallin, C. R. Dictionary of Drugs, Chapman and Hall, **1990**, 1st edition.
9. Choi, H. D.; Seo, S. P. G.; Son, B. W.; Won, B. W. *Arch. Pharm. Res.* **2004**, 27, 19-24.
10. Primo, F. T.; Fröhlich, P. E. *Acta Farm. Bonaerense* **2005**, 24, 421-425.
11. Riedel, R. *Arzneim. Forsch.* **1981**, 31, 655-663.
12. Vinod, K.; Kamalneet, K.; Girish Kumar, G.; Anil Kumar, S. *Eur. J. Med. Chem.* **2013**, 69, 735-753.
13. Nargund, L. V. G.; Hariprasad, V.; Reddy, G. R. N. *Indian J. Pharm. Sci.* **1993**, 55, 1-5.
14. Sangani, C. B.; Jigar Makawana, A.; Zhang, X.; Teraiya Shashikant, B.; Lin, L.; Zhu, H.-L. *Eur. J. Med. Chem.* **2014**, 76, 549-557.
15. Sankappa Rai, U.; Isloor, A. M.; Shetty, P.; Pai, K. S. R.; Fun, H. K. *Arabian J. Chem.* **2015**, 8, 317-321.
16. Dong, F.; Chen, X.; Liu, X.; Xu, J.; Li, Y.; Shan, W.; Zheng, Y. J. *Chromatogr. A* **2012**, 1262, 98-106.
17. Amr A.-G.; Abdel-Latif, N. A.; Abdalla, M. M. *Acta Pharm.* **2006**, 56, 203-218.
18. Oh, H. C.; Cho, J. H.; El-Gamal, M. KR Pat. 2013010514.
19. Lee, L. H.; Le Hir de Fallois, L. P.; Timmons, P. R.; Cawthorne, W. G.; Perez De Leon, A. WO Pat. 2008005489.
20. Sherman, T. D.; Duke, M. V.; Clark, R. D.; Sanders, E. F.; Matsumoto, H.; Duke, O. S. *Pestic. Biochem. Phys.* **1991**, 40, 236-245.
21. Ragab, A. F.; Abdel Gawad, N. M.; Georgey H. H.; Said, M. F. *Eur. J. Med. Chem.* **2013**, 63, 645-654.
22. Mohy El-Din, M. M.; Senbel, A. M.; Bistawroos, A. A.; El-Mallah, A.; Nour El-Din, N. A.; Bekhit, A. A.; Abd El Razik, H. A. *Basic Clin. Pharmacol. Toxicol.* **2011**, 108, 263273.
23. Hantoon, M. A. *Minn. Med.* **2001**, 84, 102. This reference must be revised.
24. Zhang, X.; Li, X.; Allan, G. F.; Sbriscia, T.; Linton, O.; Lundeen, S. G.; Sui, Z. *J. Med. Chem.* **2007**, 50, 3857-3869.
25. Yang, X.; Jin, Y.; Liu, H.; Jiang, Y.; Fu, H. *RSC Adv.* **2012**, 2, 11061-11066.
26. Fong, T. M.; Heymsfield, S. B. *Int. J. Obes.* **2009**, 33, 947-955.
27. Rademacher, P. M.; Woods, C. M.; Huang, Q.; Szklarz, G. D.; Nelson, S. D. *Chem. Res. Toxicol.* **2012**, 25, 895-903.
28. Bhuiyan M. H.; Khandkar, M. M. *Pak. J. Sci. Ind. Res.* **2009**, 52, 180-185.
29. Suzuki, A. in: *Boronic Acids. Preparation, Applications in Organic Synthesis and Medicine*; Hall, D. G.; Ed.; Wiley-VHC: Weinheim, **2005**, pp. 123.
30. Bringmann, G.; Mortimer, A. J. P.; Keller, P.A.; Gresser, M. J.; Garner, J.; Breuning, M. *Angew. Chem. Int. Ed.*, **2005**, 44, 5384-5427.
31. Miyaoura, N.; Yamada, K.; Suzuki, A. *Tetrahedron Lett.* **1979**, 20, 3437-3440.
32. Suzuki, A. *Pure Appl. Chem.*, **1994**, 66, 213-22.
33. Mondal, M.; Bora, U. *Green Chem.* **2012**, 14, 1873-1876.
34. Anderson, S. C.; Handy, S. T. *Synthesis* **2010**, 2721-2724.
35. Kiyani, H.; Albooyeh, F.; Fallahnezhad, S. *J. Mol. Struct.* **2015**, 1091, 163-169.
36. Ashok, D.; Srinivas G.; Kumar. A. V.; Gandhi, D. M. *Russ. J. Bioorg. Chem.* **2016**, 42, 560-566.
37. Halnor, V. B.; Joshi, N. S.; Karale, B. K.; Gill, C. H. *Heterocycl. Commun.* **2005**, 11, 167-172.
38. Ashok, D.; Lakshmi, B. V.; Sarasija, M. *Lett. Org. Chem.* **2016**, 13, 76-84.
39. Macro, B.; Helena, R.; Cristina, P.; Annamaria, D.; Paolo, V. *Molecules*, **2013**, 18, 1188-1213.
40. H. J. Benson, Microbiological Applications, Boston, MA, U.S.A., W.C. Brown Publications, **1990**, 156-159. This reference must be revised.