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Synthesis and evaluation of antibacterial and antimycobacterial activities of some new pyrazole derivatives

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ABSTRACT: Pyrazoles and sulfonamides are ubiquitously classified as structural fragments in antimicrobial and antimycobacterial agents. In this present study, a series of 14 pyrazole based sulfonamide derivatives (**7-20**) were designed, synthesized regioisomeric structures were elucidated by spectroscopic methods. All target compounds were evaluated for their *in vitro* antibacterial potential against selected Gram (+) and Gram (-) bacterial strains as well as for their antimycobacterial activity. The bioactivity results demonstrated that all compounds showed selective antibacterial activity against *B. subtilis* with MIC values of 1-125 µg/mL. Especially, 1-[(2,4-dichlorophenyl)methyl]-N-(4-methoxybenzenesulfonyl)-3-methyl-1*H*-pyrazole-5-carboxamide **(9)**, 1-[(2,4-Dichlorophenyl)methyl]-N-(4-fluorobenzenesulfonyl)-3-methyl-1H-pyrazole-5-carboxamide **(10)**, N-(3,5-Dichlorobenzenesulfonyl)-1-[(2,4-dichlorophenyl)methyl]-3-methyl-1H-pyrazole-5-carboxamide **(11)** and 1-[(2,4-dichlorophenyl)methyl]-N-(4-fluorobenzenesulfonyl)-5-methyl-1H-pyrazole-3-carboxamide **(17)** displayed the highest antibacterial activity against *B. subtilis* with MIC values of 1 µg/mL, which were more effective compared to the reference chloramphenicol. As a conclusion, the snythesized new pyrazole sulfonamides stand out as promising antimicrobial agents for further development.

KEYWORDS: Pyrazole; sulfonamide; regioisomer; antibacterial activity; antimycobacterial activity.

1. INTRODUCTION

The treatment of bacterial infections is still challenging since the antibacterial drug resistance has been gradually increasing worldwide, which causes tremendous public health burden [1]. Although studies on new antibiotic classes have been growing, obtaining highly safe and potent compounds with acceptable pharmacokinetic properties remains elusive [2]. Therefore, there is still a significant need for development of newer antimicrobial agents.

Nitrogen containing heterocyclic scaffolds, which are privileged structures in medicinal chemistry, exhibit a wide range of biological activities. Among these, pyrazole derivatives are commonly studied structures, owing to their great variety of biological activities including anti-inflammatory [3], antibacterial [4], antiviral [5-7], antitumor [8, 9], fungicidal activities [10] as well as analgesic [11, 12] and anti-diabetic properties [13, 14].

Sulfonamides, namely sulfa drugs, show antimicrobial and antifungal properties [15-17]. Antimicrobial sulfonamides are considered as a competitive inhibitor to PABA (*p*-aminobenzoic acid), inhibit the biosynthesis of DHPA (dihydrofolic acid) play crucial role which is essential for nucleic acid formation and life survival for the bacterial cell [18]. Some of the pyrazole based sulfonamide derivatives have also been reported as antimicrobial [19], COX-2/5-LOX inhibitors [20], PPAR-γ agonists and COX-2 selective inhibitors [14].

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Inspired by the therapeutic potential of pyrazoles and sulfonamides, we designed and synthesized 14 new pyrazole based aryl sulfonamides with the aim of obtaining potent antimicrobial agents. We herein report the synthesis and their *in vitro* antibacterial and antimycobacterial activities.

2. RESULTS AND DISCUSSION

2.1. Chemistry

The synthetic route of intermediates **1-6** and new pyrazole derivatives **7-20** were presented in Figure 1. Condensation of commercially available acetone with dimethyloxalate afforded diketo ester derivative (**1**), which was further reacted with aqueous hydrazine hydrate to give pyrazole ester derivate **(2)**. Subsequently, **2** was alkylated with 2,4-dichlorobenzylbromide to afford two regioisomers (**3** and **4**), which were further reacted with LiOH in THF:H_2O to produce corresponding carboxylic acids. These carboxylic acid intermediates **(5-6**) were finally reacted with appropriate sulfonamide derivatives in the presence of EDC, DMAP in DCM (**7, 10, 14-20**) or DBU, CDI in THF (**9, 11-13**) to yield the title *N*-acylsulfonamide derivatives (**7-20**), respectively. Compound **8** was obtained via acyl chloride derivative of **5** using 4-methylphenylsulfonamide, DIEA in DCM at room temperature.

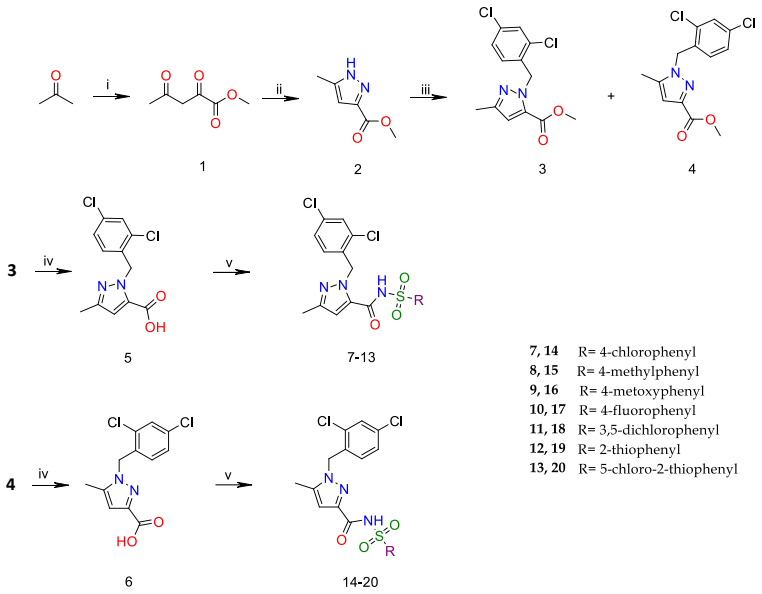

Figure 1. Synthetic route of pyrazole derivatives. Reagents and conditions: **(i)** dimethyloxalate, Na, MeOH, rt, overnight; **(ii)** $NH_2NH_2.H_2O$, MeOH, rt, 1 h; **(iii)** 2,4-dichlorobenzylchloride, K_2CO_3, DMF, 80 ºC, 4 h; **(iv)** LiOH.H_2O, THF:H_2O, 70 ºC, 3 h; **(v)** appropriate sulfonamide, EDC, DMAP, DCM, rt, overnight for **7**, **10**, **14-20**; appropriate sulfonamide, CDI, DBU, THF, 60 ºC, 2.5 h for **9**, **11-13**; acylchloride of **5**, 4-methylphenylsulfonamide, DIEA, DCM, rt, overnight for **8**.

The structure elucidation of the final compounds were performed by means of ^{1}H-NMR, ^{13}C-NMR and HRMS. In the ^{1}H-NMR spectra of the compounds, the methly signal was observed as a singlet at about 2.25 ppm. The pyrazole H4 proton resonated as a singlet at about 6.60 ppm. H6, H5 and H3 protons of the 2, 4-dichlorobenzyl moiety were appeared at about 6.50 as doublets, 7.20 as doublet of doublets and 7.45 ppm and doublets, respectively. The signals of different aryl groups of sulfonamide portion were observed at the aromatic field in compatible with the structures. Moereover, the chemical shift values of methylene protons for final compounds were used as an indication to distinguish between both regioisomers as described previously [11]. Hence, as a result of comparing the ^{1}H-NMR spectra of both isomers, we observed that while the two methylene protons in 1-benzyl-3-methyl isomers appeared at relatively downfield between 5.53 and 5.76 ppm, the same protons in 1-benzyl-5-methyl isomers were observed at relatively upfield ranging from 5.24 to 5.34 ppm. In the ^{13}C-NMR spectra, methyl carbons were observed at about 13.5 ppm and carbon signals of aryl groups were observed at about 110-150 ppm. Carbonyl groups were observed at about 158 ppm. However, the chemical shift values of methylene carbons in ^{13}C-NMR for 1-benzyl-3-methyl isomers were appeared at slightly downfield at about 51.5 ppm, while the same carbons in 1-benzyl-5-methyl isomers were appeared at about 50.5 ppm. The structural characterization of obtained regioisomers (**4** and **19**) were also confirmed using X-ray crystallography as reported previously [21, 22].

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2.2. *In vitro* antimicrobial activity

Antibacterial activity of compounds **7–20** was tested against gram positive bacterial strains including *Staphylococcus aureus, Bacillus subtilis* and *S. epidermidis* and gram-negative bacterial strains including *Escherichia coli* and *Pseudomonas aeruginosa*. Activity results were reported as the minimal inhibitory concentration (MIC) values by microdilution method (Table 1). The results revealed that target compounds showed antibacterial effect against only gram-positive bacteria, particularly on the *B. subtilis* strain.

Table 1. *In vitro* antibacterial activity results of pyrazole derivatives **7-20**.

Compound	MIC (µg/ml)				
	Gram positive bacteria			Gram negative bacteria	
	S. aureus	*B. subtilis*	*S. epidermidis*	*P. aeruginosa*	*E. coli*
7	125	8	500	-	-
8	500	125	250	-	-
9	250	**1**	125	-	-
10	250	**1**	125	-	-
11	500	**1**	250	-	-
12	250	4	250	-	-
13	250	4	250	-	-
14	**62**	31	125	-	-
15	125	16	500	-	-
16	**62**	16	500	-	-
17	125	**1**	250	-	-
18	**31**	4	125	-	-
19	250	62	**16**	-	-
20	125	16	125	-	-
Chloramphenicol	4	4	2	64<	8

- : > 500 µg/ml

Strain types: Staphylococcus aureus ATCC 6538, Bacillus subtilis NRRL B-4378, S. epidermidis ATCC 12228, Pseudomonas aeruginosa ATCC27853, Eschericia coli NRRL B-3008.

When we compared the regioisomeric couples, i.e., **9-16** and **11-18**, regioisomers **9** and **11** having 5-carboxamide side chain (MIC: 1 µg/ml) were preferred over their regioisomeric counterparts **16** and **18** with 3-carboxamide side chain (MIC values of 16 and 4 µg/ml, respectively) against *B. subtilis* NRRL B-4378. Interestingly, regioisomers **10-17** with 4-fluorophenylsulfonamide was found equipotent (MIC: 1 µg/ml). In addition, compounds **12**, **13** and **18** were found to have potency comparable to the reference chloramphenicol, all showing MIC values of 4 µg/ml. However, the title compounds have shown slight effect against *E. coli* and *S. epidermidis* when compared to their activity against *B. subtilis*.

Antimycobacterial activity of compounds **7–20** were also investigated against *Mycobacterium smegmatis, M. fortuitum* subs. *fortuitum* and *M. avium*. As seen in Table 2, all compounds resulted in very slight antimycobacterial activity. The highest antimycobacterial activity was obtained by compound **14** and **16** (MIC= 62 µg/ml) within the series against *M. smegmatis* strain.

3. CONCLUSION

In the current work, we described the synthesis of a series of new regioisomeric pyrazole derivatives, which were investigated for their antimycobacterial and antibacterial activities. Although none of the obtained compounds showed any activity against gram-negative organisms, they were mostly active against the gram-positive bacteria. Especially, compounds **12**, **13** and **18** were found comparable to the reference chloramphenicol, and compounds **9-11**, **17** had the highest and more potent antibacterial activity against *B. subtilis* with MIC values of 1 µg/ml. Based on our preliminary results, **9-11** and **17** can be considered as lead antibacterial compounds for further optimization of the aryl sulfonamide side chain in the active regioisomer of the pyrazole core.

Table 2. *In vitro* antimycobacterial activitiy results of pyrazole derivatives.

Compound	MIC (µg/ml)		
	M. smegmatis	*M. fortuitum*	*M. avium*
7	125	-	-
8	-	-	-
9	250	500	-
10	500	-	-
11	125	500	-
12	500	-	-
13	500	-	-
14	**62**	500	-
15	250	500	250
16	**62**	-	250
17	250	500	500
18	500	-	-
19	250	-	-
20	125	-	-
Streptomycin	1	16	16
Amikacin	0.25	1	1

-: > 500 µg/ml
Strain types: Mycobacterium smegmatis ATCC 14468, M. fortuitum subsp. fortuitum ATCC 6841, M. avium clinical strain.

4. MATERIALS AND METHODS

4.1. Chemistry

Starting materials were purchased from Merck, Fluka and used without further purification. The reactions were monitored by thin layer chromatography (TLC) on silica plates (F-254), visualizing under UV light. ^{1}H and ^{13}C NMR spectra were performed on a Bruker digital 300 MHz FT-NMR spectrometer (Bruker Bioscience, MA, USA) using tetramethylsilane as the internal standard. Chromatographic purification of the compounds were performed with Interchim Puriflash 4250 (Montluçon, France) using Interchim silica gel columns. According to the UPLC-MS method which using (A) water + 0.1 % Formic Acid and (B) acetonitrile + 0.1 % Formic Acid; flow rate = 0.3 mL/min, Column: Aquity BEH C18 column (2.1 x 100 mm, 1.7 mm), purity for all final compounds was >95%. Melting point of the titled compounds acquired on an MP90 digital melting point apparatus (Mettler Toledo, Columbus, OH, USA) and were uncorrected. Compounds **1-3** were synthesized in the same manner as reported in the literature [11, 23-24].

Methyl 2,4-dioxopentanoate (1). Na (1.2 g, 52 mmol, 1.2 equiv) was dissolved in MeOH in ice-cold bath to give sodium methoxide, acetone (3.2 ml, 43.4 mmol, 1 equiv) was added onto that solution subsequently and the reaction was left to stir for 20 minutes. After that, dimethyloxalate (5.12 g, 43.4 mmol, 1 equiv) was added onto it, and left to stir overnight at rt. Next day, the precipitated sodium salts of the product was filtered over gooch funnel. The filtered product was poured onto water and acidified with conc. HCl solution and then extracted with diethylether. The collected organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated to give the crude which was used without further purification for next step (1307 mg, 20.9%). Mp 60.4-61.5 ºC [23], 61.4-62.1 ºC.

Methyl 5-methyl-1H-pyrazole-3-carboxylate (2). Hydrazine hydrate was added dropwise in 30 minutes to a solution of **1** (700 mg, 4.85 mmol, 1 equiv) in MeOH on an ice-coldbath. After adding hydrazine hydrate, the mixture was taken to rt and stirred for another 30 minutes. The reaction mixture was concentrated in vacuo, partitioned between diethyl eter and $NaHCO_3$ solution. The collected organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated in vacuo. The crude which was used for next step. Mp 77-79 ºC [24], 81-82 ºC. ^{1}H-NMR (DMSO-d_6): δ 2.49 (3H, s), 3.76 (3H, s), 6.47 (1H, s), 13.2 (1H, s). HRMS *m/z* calculated for $C_6H_9N_2O_2S$ $[M+H]^+$ 141.0664; found: 141.0661.

Methyl 1-[(2,4-dichlorophenyl)methyl]-3-methyl-1H-pyrazole-5-carboxylate (3) *and* ***Methyl 1-[(2,4-dichlorophenyl)methyl]-5-methyl-1H-pyrazole-3-carboxylate (4).*** 2,4-dichlorobenzylchloride (185 µl, 1.33 mmol, 1.1 equiv) and K_2CO_3 (393 mg, 3.64 mmol, 3 equiv) were added to to a solution of **2** (170 mg, 1.21 mmol, 1 equiv) in DMF. The resulting mixture was heated at 80ºC for 4 hours. After cooling it to rt, it was poured onto water, extracted with ethyl acetate. The collected organic layer was dried over anhydrous Na_2SO_4, filtered, concentrated in vacuo to give the crude which was purified by automated-flash chromatography on silica gel (12 g), eluting with a gradient of 0–50% ethyl acetate in hexane to give two regioisomers (104 mg,

28.6% for **3**; 140 mg, 38.5% for **4**). Spectral data for **3** as follows: Mp 90.1- 91.0 ºC, ^{1}H-NMR ($CDCl_3$): δ 2.33 (3H, s), 3.82 (3H, s), 5.77 (2H, s), 6.44 (1H, d, *J* = 8.4 Hz), 6.72 (1H, s), 7.11 (1H, dd, *J* = 8.4, 2.1 Hz), 7.39 (1H, d, *J* = 2.1 Hz). HRMS *m/z* calculated for $C_{13}H_{13}N_2O_2Cl_2$ $[M+H]^+$ 299.0354; found: 299.0359. Spectral data for **4** as follows: Mp 90.1- 90.6 ºC, ^{1}H-NMR (DMSO-d_6): *δ* 2.27 (3H, s), 3.76 (3H, s), 5.43 (2H, s), 6.63 (1H, s), 6.77 (1H, d, *J* = 8.4 Hz), 7.41 (1H, dd, *J* = 8.4, 2.1 Hz), 7.68 (1H, d, *J* = 2.1 Hz). HRMS *m/z* calculated for $C_{13}H_{13}N_2O_2Cl_2$ $[M+H]^+$ 299.0354; found: 299.0351.

***1-[(2,4-Dichlorophenyl)methyl]-3-methyl-1H-pyrazole-5-carboxylic acid* (5).** LiOH.H_2O (42.5 mg, 1.01 mmol, 3 equiv) was added to a solution of **3** (104 mg, 0.33 mmol, 1 equiv) in THF:water (4 ml:2 ml). The resulting mixture was heated at 70 ºC for 3 hours. The reaction mixture was cooled to rt, concentrated in vacuo, diluted with water and neutralized with a solution of conc. HCl. The resulting solid was filtered and recrystallized from EtOH and H_2O to give **5** (87 mg, 89.6%). Mp 192.3-193.0 °C. ^{1}H-NMR (DMSO-d_6): *δ* 2.19 (3H, s), 5.70 (2H, s), 6.58 (1H, d, *J* = 8.4 Hz), 6.73 (1H, s), 7.35 (1H, dd, *J* = 8.4, 2.1 Hz), 7.63 (1H, d, *J* = 2.1 Hz), 13.4 (1H, s); ^{13}C-NMR (DMSO-d_6): *δ* 13.5, 51.5, 111.4, 128.1, 129.2, 129.6, 132.7, 133.1, 134.4, 135.2, 147.8, 160.9. HRMS *m/z* calculated for $C_{12}H_{11}N_2O_2Cl_2$ $[M+H]^+$ 285.0198; found: 285.0190.

***1-[(2,4-Dichlorophenyl)methyl]-5-methyl-1H-pyrazole-3-carboxylic acid* (6).** Prepared from **4** (91 mg, 0.3mmol, 1 equiv) under the same manner as compound **5** (75.5 mg, 87.1%). Mp 198.8-199.3 °C. ^{1}H-NMR (DMSO-d_6): *δ* 2.24 (3H, s), 5.39 (2H, s), 6.52 (1H, s), 6.73 (1H, d, *J* = 1.8 Hz), 7.40 (1H, dd, *J* = 8.1, 1.8 Hz), 7.67 (1H, d, *J* = 1.8 Hz); ^{13}C-NMR (DMSO-d_6): δ 11.0, 50.1, 108.4, 128.3, 129.3, 130.6, 133.0, 133.6, 133.9, 141.0, 145.1, 164.1. HRMS *m/z* calculated for $C_{12}H_{11}N_2O_2Cl_2$ $[M+H]^+$ 285.0198; found: 285.0188.

N-(4-Chlorobenzenesulfonyl)-1-[(2,4-dichlorophenyl)methyl]-3-methyl-1H-pyrazole-5-carboxamide (7). In a solution of **5** (150 mg, 0.53 mmol, 1 equiv) in DCM (dichloromethane) (10 ml) were added EDC (113.2 mg, 0.59 mmol, 1.1 equiv), DMAP (13.5 mg, 0.11 mmol, 0.2 equiv) under Ar atmosphere. The reaction mixture was stirred for 10 minutes at rt. After that, 4-chlorobenzensulfonamide (113.1 mg, 0.59 mmol, 1 equiv) was added and left to stir overnight at rt. Upon completion of the reaction, the mixture was partitioned between DCM and aq. $NaHCO_3$ solution. The collected organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated to give the crude which was purified by automated-flash chromatography on silica gel (12 g), eluting with a gradient of 0–10% DCM in MeOH (103.2 mg, 42.4%). Mp 198.7-199.6 °C. ^{1}H-NMR (DMSO-d_6): *δ* 2.14 (3H, s), 5.61 (2H, s), 6.47 (1H, d, *J* = 8.4 Hz), 6.71 (1H, s), 7.27 (1H, dd, *J* = 8.4, 2.1 Hz), 7.53 (2H, d, *J* = 8.7 Hz), 7.54 (1H, d, *J* = 2.1 Hz), 7.80 (2H, d, *J* = 8.7 Hz) ; ^{13}C-NMR (DMSO-d_6): *δ* 13.7, 51.2, 109.9, 127.8, 128.9, 129.5, 129.8, 132.6, 132.9, 135.5, 137.1, 147.1. HRMS *m/z* calculated for $C_{18}H_{15}N_3O_3SCl_3$ $[M+H]^+$ 457.9900; found: 457.9906.

***1-[(2,4-Dichlorophenyl)methyl]-3-methyl-N-(4-methylbenzenesulfonyl)-1H-pyrazole-5-carboxamide* (8).** In a solution of **5** (149.6 mg, 0.52 mmol, 1 equiv) in DCM (10 ml) were added $SOCl_2$ (95.2 µl, 1.31 mmol, 2.5 equiv) and catalytic amount of DMF. The resulting mixture was refluxed for 2.5 hours. After cooling to rt, the mixture was evaporated in vacuo and the obtained crude were added DIEA (136.8 µl, 0.79 mmol, 1.5 equiv), p-toluenesulfonamide (89.7 mg, 0.52 mmol, 1 equiv), left to stir under Ar atmosphere overnight at rt. The reaction mixture was partitioned between DCM and $NaHCO_3$. The collected organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated to give the crude which was purified by automated-flash chromatography on silica gel (12 g), eluting with a gradient of 0–10% DCM in MeOH (83.7 mg, 36.7%). Mp 135.8-137.0 °C. ^{1}H-NMR ($CDCl_3$): *δ* 2.25 (3H, s), 2.47 (3H, s), 5.64 (2H, s), 6.46 (1H, d, *J* = 8.1 Hz), 6.61 (1H, s), 7.03 (1H, dd, *J* = 8.4, 2.1 Hz), 7.27-7.35 (3H, m), 7.84 (1H, d, *J* = 8.4 Hz), 7.95 (2H, d, *J* = 8.4 Hz); ^{13}C-NMR ($CDCl_3$): *δ* 13.7, 21.7, 51.8, 108.7, 126.4, 127.1, 128.5, 128.8, 129.1, 129.6, 129.7, 133.2, 133.6, 135.2, 145.5, 148.5, 156.2. HRMS *m/z* calculated for $C_{19}H_{18}N_3O_3SCl_2$ $[M+H]^+$ 438.0446; found: 438.0456.

***1-[(2,4-Dichlorophenyl)methyl]-N-(4-methoxybenzenesulfonyl)-3-methyl-1H-pyrazole-5-carboxamide* (9).** In a solution of **5** (60 mg, 0.21 mmol, 1 equiv) in THF (tetrahydrofuran) (10 ml) was added CDI (68.2 mg, 0.42 mmol, 2 equiv), and refluxed for 1.5 hours. After that, DBU (78 µl, 0.52 mmol, 2.5 equiv) and 4-methoxybenzenesulfonamide (39.7 mg, 0.21 mmol, 1.01 equiv) onto that mixture and heated at 60 ºC for an hour. After cooling it to rt, it was evaporated to dryness, partitioned between EtOAc and aq. $NaHCO_3$. The collected organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated to give the crude which was purified by automated-flash chromatography on silica gel (12 g), eluting with a gradient of 0–10% DCM (49 mg, 51.2%). Mp 179.1-180 °C. ^{1}H NMR (DMSO-d_6): *δ* 2.17 (3H, s), 3.85 (3H, s), 5.53 (2H, s), 6.56 (1H, d, *J* = 8.4 Hz), 7.00 (1H, s), 7.11 (2H, d, *J* = 8.7 Hz), 7.27 (1H, dd, *J* = 8.4, 2.1 Hz), 7.57 (1H, d, *J* = 2.1 Hz), 7.85 (2H, d, *J* = 8.7 Hz); ^{13}C NMR (DMSO-d_6): *δ* 13.6, 51.5, 56.2, 110.5, 114.7, 127.9, 128.7, 129.1, 130.1, 130.4, 131.0, 133.2, 134.2, 134.7, 147.6, 158.0, 163.6. HRMS *m/z* $[M+H]^+$ calculated for $C_{19}H_{18}Cl_2N_3O_4S$ 454.0395; found 454.0416.

1-[(2,4-Dichlorophenyl)methyl]-N-(4-fluorobenzenesulfonyl)-3-methyl-1H-pyrazole-5-carboxamide (10). Prepared from **5** (200 mg, 0.7 mmol, 1 equiv) and 4-fluorobenzenesulfonamide (135 mg, 0.77 mmol, 1.1 equiv) as the same manner as compound **7**. The crude was purified by automated-flash chromatography on silica gel (12 g), eluting with a gradient of 0–10% DCM in MeOH (99.2 mg, 32%). Mp 293.2-294.1°C. ^{1}H NMR (DMSO-d_6): δ 2.10 (3H, s), 5.72 (2H, s), 6.38 (1H, s), 6.40 (1H, d, J = 8.4 Hz), 7.14 (2H, t, J = 9.0 Hz), 7.27 (1H, dd, J = 8.4, 2.1 Hz), 7.57 (1H, d, J = 2.1 Hz), 7.72 (2H, dd, J = 9.0, 5.4 Hz); ^{13}C NMR (DMSO-d_6): δ 13.7, 50.9, 108.9 ($^4J_{C\text{-}F}$ = 2.25 Hz), 114.8 ($^2J_{C\text{-}F}$ = 21.75 Hz), 127.8, 128.8, 129.6 ($^3J_{C\text{-}F}$ = 7.5 Hz), 129.8, 132.4, 132.5, 136.5, 139.5, 142.7, 146.5, 163.2 ($^1J_{C\text{-}F}$ = 245.25 Hz), 163.8. HRMS *m/z* [M+H]$^+$ calculated for $C_{18}H_{15}Cl_2N_3O_3FS$ 442.0195; found 442.0207.

N-(3,5-Dichlorobenzenesulfonyl)-1-[(2,4-dichlorophenyl)methyl]-3-methyl-1H-pyrazole-5-carboxamide (11). Prepared from **5** (120 mg, 0.42 mmol, 1 equiv) and 3,5-dichlorobenzenesulfonamide (69.2 mg, 0.425 mmol, 1.01 eq) as the same manner as compound **9**. The crude was purified by automated-flash chromatography on silica gel (12 g), eluting with a gradient of 0–10% DCM in MeOH (66.3 mg, 31.9%). Mp 190.8-192.1 °C. ^{1}H NMR ($CDCl_3$): δ 2.17 (3H, s), 5.59 (2H, s), 6.49 (1H, d, J = 8.4 Hz), 6.83 (1H, s), 7.25 (1H, dd, J = 8.4, 2.1 Hz), 7.56 (1H, d, J = 2.1 Hz), 7.75 (1H, d, J = 1.8 Hz), 7.90 (2H, t, J = 1.8 Hz); ^{13}C NMR (DMSO-d_6): δ 13.6, 51.6, 110.7, 126.3, 127.9, 129.0, 129.8, 132.7, 133.0, 133.1, 134.9, 135.5, 144.0, 147.4, 159.5. HRMS *m/z* [M+H]$^+$ calculated for $C_{18}H_{14}Cl_4N_3O_3S$ 491.9510; found 491.9509.

1-[(2,4-Dichlorophenyl)methyl]-3-methyl-N-(thiophene-2-sulfonyl)-1H-pyrazole-5-carboxamide (12). Prepared from **5** (200 mg, 0.7 mmol, 1 equiv) and 2-thiophenesulfonamide (118 mg, 0.71 mmol, 1.01 equiv) as the same manner as compound **9**. The crude was purified by automated-flash chromatography on silica gel (25 g), eluting with a gradient of 0–10% DCM in MeOH (144 mg, 47.7%). Mp 205.4-205.7 °C. ^{1}H NMR (DMSO-d_6): δ 2.18 (3H, s), 5.6 (2H, s), 6.61 (1H, d, J = 8.4 Hz), 6.99 (1H, s), 7.17 (1H, dd, J = 5.1, 3.6 Hz), 7.29 (1H, dd, J = 8.4, 2.1 Hz), 7.59 (1H, d, J = 2.1 Hz), 7.77 (1H, dd, J = 3.6, 1.5 Hz), 8.02 (1H, dd, J = 5.1, 1.5 Hz); ^{13}C NMR (DMSO-d_6): δ 13.6, 51.6, 110.6, 127.9, 128.0, 129.2, 130.2, 132.9, 133.3, 134.2, 134.5, 134.7, 135.2, 139.9, 147.6, 158.2. HRMS *m/z* [M+H]$^+$ calculated for $C_{16}H_{14}Cl_2N_3O_3S_2$ 429.9854; found 429.9859.

N-[(5-Chlorothiophene-2-yl)sulphonyl]-1-[(2,4-dichlorophenyl)methyl]-3-methyl-1H-pyrazole-5-carboxamide (13). Prepared from **5** (118 mg, 0.41 mmol, 1 equiv) and 5-chlorothiophene-2-sulfonamide (82.5 mg, 0.42 mmol, 1.01 equiv) as the same manner as **9**. The crude was purified by automated-flash chromatography on silica gel (25 g), eluting with a gradient of 0–10% DCM in MeOH (89.8 mg, 46.7%). Mp 265.0-265.4 °C. ^{1}H NMR (DMSO-d_6): δ 2.12 (3H, s), 5.76 (2H, s), 6.41 (1H, s), 6.45 (1H, d, J = 8.4 Hz), 6.95 (1H, d, J = 3.9 Hz), 7.21 (1H, d, J = 3.9 Hz), 7.28 (1H, dd, J = 8.4, 2.1 Hz), 7.58 (1H, d, J = 2.1 Hz); ^{13}C NMR (DMSO-d_6): δ 13.7, 50.9, 109.1, 122.2, 126.2, 127.8, 128.6, 128.8, 129.6, 132.4, 132.6, 136.4, 142.1, 146.6, 152.9, 164.3. HRMS *m/z* [M+H]$^+$ calculated for $C_{16}H_{13}Cl_3N_3O_3S_2$ 463.9464; found 463.9478.

N-(4-Chlorobenzenesulfonyl)-1-[(2,4-dichlorophenyl)methyl]-5-methyl-1H-pyrazole-3-carboxamide (14). Prepared from **6** (153 mg, 0.54 mmol, 1 equiv) and 4-chlorobenzenesulfonamide (113 mg, 0.59 mmol, 1.1 equiv) as the same manner as compound **7**. The crude was purified by automated-flash chromatography on silica gel (25 g), eluting with a gradient of 0–10% DCM in MeOH (196.5 mg, 79.3%). Mp 170.8-171.4 °C. ^{1}H NMR ($CDCl_3$): δ 2.14 (3H, s), 5.24 (2H, s), 6.49 (1H, s), 6.53 (1H, s), 7.12 (1H, dd, J = 8.4, 2.1 Hz), 7.36 (1H, d, J = 2.1 Hz), 7.43 (2H, d, J = 8.7 Hz), 8.01 (2H, d, J = 8.7 Hz); ^{13}C-NMR ($CDCl_3$): δ 11.1, 50.5, 107.6, 127.8, 129.0, 129.2, 129.6, 130.0, 131.7, 133.0, 134.9, 137.3, 140.6, 142.0, 143.4, 158.5. HRMS *m/z* [M-H]$^-$ calculated for $C_{18}H_{13}Cl_3N_3O_3S$ 455.9743; found 455.9730.

1-[(2,4-Dichlorophenyl)methyl]-5-methyl-N-(4-methylbenzenesulfonyl)-1H-pyrazole-3-carboxamide (15). Prepared from **6** (150 mg, 0.53mmol, 1 equiv) and p-toluenesulfonamide (99 mg, 0.58 mmol, 1.1 equiv) under as the same manner as compound **7**. The crude was purified by automated-flash chromatography on silica gel (12 g), eluting with a gradient of 0–10% DCM in MeOH (77 mg, 33.1%). Mp 165.1-165.7 °C. ^{1}H NMR ($CDCl_3$): δ 2.22 (3H, s), 2.44 (3H, s), 5.32 (2H, s), 6.58-6.61 (2H, m), 7.20 (1H, dd, J = 8.4, 2.1 Hz), 7.34 (2H, d, J = 8.4 Hz), 7.44 (1H, d, J = 2.1 Hz), 8.03 (2H, d, J = 8.4 Hz), 9.2 (1H, s); ^{13}C-NMR ($CDCl_3$): δ 11.1, 21.7, 50.5, 107.6, 127.9, 128.5, 129.1, 129.4, 129.5, 131.8, 132.9, 134.7, 135.9, 141.9, 143.6, 144.9, 158.5. HRMS *m/z* [M+H]$^+$ calculated for $C_{19}H_{18}Cl_2N_3O_3S$ 438.0446; found 438.0440.

1-[(2,4-Dichlorophenyl)methyl]-N-(4-methoxybenzenesulfonyl)-5-methyl-1H-pyrazole-3-carboxamide (16). Prepared from **6** (150 mg, 0.53mmol, 1 equiv) and 4-methoxybenzenesulfonamide (112 mg, 0.58 mmol, 1.1 equiv) as the same manner as compound **7**. The crude was recrystallized from hexane and ethylacetate (103.5 mg, 42.9%). Mp 151.1-151.5 °C. ^{1}H NMR ($CDCl_3$): δ 2.22 (3H, s), 3.88 (3H, s), 5.32 (2H, s), 6.58 (1H, s), 6.60 (1H, d, J = 8.4 Hz), 7.00 (2H, d, J = 9.0 Hz), 7.20 (1H, dd, J = 8.4, 2.1 Hz), 7.44 (1H, d, J = 2.1 Hz), 8.09 (2H, d, J = 9.0 Hz), 9.18 (1H, s); ^{13}C-NMR ($CDCl_3$): δ 11.1, 50.5, 55.6, 107.6, 114.0, 127.9, 129.0, 129.5, 130.3, 130.8, 131.8, 132.9, 134.8, 141.9, 143.7, 158.5, 163.9. HRMS *m/z* [M-H]$^-$ calculated for $C_{19}H_{16}Cl_2N_3O_4S$ 452.0239; found 452.0240.

1-[(2,4-Dichlorophenyl)methyl]-N-(4-fluorobenzenesulfonyl)-5-methyl-1H-pyrazole-3-carboxamide (17). Prepared from **6** (200 mg, 0.7 mmol, 1 equiv) and 4-fluorobenzenesulfonamide (135 mg, 0.77 mmol, 1.1 equiv) as the same manner as compound **7**. The crude was was purified by automated-flash chromatography on silica gel (12 g), eluting with a gradient of 0–5% DCM in MeOH (156 mg, 50.3%). Mp 210.1-210.5 °C. ^{1}H NMR ($CDCl_3$): δ 2.23 (3H, s), 5.33 (2H, s), 5.92-6.62 (2H, m), 7.19-7.27 (3H, m), 7.45 (1H, d, J= 2.1 Hz), 8.17-8.21 (2H, m), 9.21 (1H, s); ^{13}C NMR ($CDCl_3$): δ 11.1, 50.5, 107.6, 116.2 ($^2J_{C-F}$ = 22.5 Hz), 127.9, 129.0, 129.6, 131.5 ($^3J_{C-F}$ = 9.75 Hz), 131.6, 132.9, 134.8, 142.0, 143.4, 158.4, 164.3 ($^1J_{C-F}$ = 245.25 Hz). HRMS *m/z* $[M+H]^+$ calculated for $C_{18}H_{15}Cl_2FN_3O_3S$ 442.0195, found 442.0198.

N-(3,5-Dichlorobenzenesulfonyl)-1-[(2,4-dichlorophenyl)methyl]-5-methyl-1H-pyrazole-3-carboxamide (18). Prepared from **6** (200 mg, 0.7 mmol, 1 equiv) and 3,5-dichlorobenzenesulfonamide (174 mg, 0.77 mmol, 1.1 equiv) as the same manner as compound **7**. The crude was was purified by automated-flash chromatography on silica gel (12 g), eluting with a gradient of 0–5% DCM in MeOH (168 mg, 48.5%). Mp 162.1-162.6 °C. ^{1}H NMR ($CDCl_3$): δ 2.25 (3H, s), 5.34 (2H, s), 6.62 (1H, d, J = 8.4 Hz), 6.65 (1H, s), 7.22 (1H, dd, J = 8.4, 2.1 Hz), 7.46 (1H, d, J = 2.1 Hz), 7.61 (1H, t, J = 1.8 Hz), 8.04 (2H, d, J = 1.8 Hz); ^{13}C-NMR ($CDCl_3$): δ 11.1, 50.6, 107.8, 124.9, 126.9, 127.9, 129.0, 129.6, 131.6, 132.9, 133.9, 135.8, 141.6, 142.2, 143.1, 158.3. HRMS *m/z* $[M+H]^+$ calculated for $C_{18}H_{14}Cl_4N_3O_3S$ 491.9510; found 491.9520.

1-[(2,4-Dichlorophenyl)methyl]-5-methyl-N-(thiophene-2-sulfonyl)-1H-pyrazole-3-carboxamide (19). Prepared from **6** (200 mg, 0.7 mmol, 1 equiv) and 2-thiophenesulfonamide (126 mg, 0.77 mmol, 1.1 equiv) as the same manner as compound **7**. The crude was recrystallized from methanol and ethyl acetate (120.6 mg, 39.9%). Mp 191.8-192.3 °C. ^{1}H NMR ($CDCl_3$): δ 2.24 (3H, s), 5.33 (2H, s), 6.62 (1H, d, J = 8.4 Hz), 6.65 (1H, s), 7.13 (1H, dd, J = 5.1, 3.9 Hz), 7.21 (1H, dd, J = 8.4, 2.1 Hz), 7.45 (1H, d, J = 2.1 Hz), 7.69 (1H, dd, J = 5.1 Hz, 1.2 Hz), 7.97 (1H, dd, J = 3.9 Hz, 1.2 Hz), 9.29 (1H, bs); ^{13}C-NMR ($CDCl_3$): δ 11.2, 50.5, 107.6, 127.3, 127.9, 129.1, 129.6, 131.7, 132.9, 133.8, 134.8, 135.1, 139.2, 142.0, 143.5, 158.4. HRMS *m/z* $[M+H]^+$ calculated for $C_{16}H_{14}Cl_2N_3O_3S_2$ 429.9854; found 429.9861.

N-[(5-Chlorothiophen-2-yl)sulfonyl]-1-[(2,4-dichlorophenyl)methyl]-5-methyl-1H-pyrazole-3-carboxamide (20). Prepared from **6** (200 mg, 0.7 mmol, 1 equiv) and 5-chlorothiophene-2-sulfonamide (157 mg, 0.77 mmol, 1.1 equiv) as the same manner as compound **7**. The crude was was purified by automated-flash chromatography on silica gel (12 g), eluting with a gradient of 0–5% DCM in MeOH (62.4 mg, 19%). Mp 179.9-180.7 °C. ^{1}H NMR ($CDCl_3$): δ 2.25 (3H, s), 5.34 (2H, s), 6.24 (1H, d, J = 8.4 Hz), 6.66 (1H, s), 6.96 (1H, d, J = 3.9 Hz), 7.21 (1H, dd, J = 8.4, 2.1 Hz), 7.45 (1H, d, J = 2.1 Hz), 7.74 (1H, d, J = 3.9 Hz), 9.27 (1H, s). ^{13}C NMR ($CDCl_3$): δ 11.1, 50.5, 107.7, 126.6, 127.9, 129.0, 129.6, 131.6, 132.9, 134.5, 134.9, 136.8, 139.5, 142.1, 143.3, 158.4. HRMS *m/z* $[M+H]^+$ calculated for $C_{16}H_{13}Cl_3N_3O_3S_2$ 463.9464; found 463.9450.

4.2. *In vitro* antimicrobial activity studies

Compounds **7-20** were evaluated for antibacterial activity as studied previously [25]. *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* NRRL B-4378, *S. epidermidis* ATCC 12228, *Pseudomonas aeruginosa* ATCC27853 and *Eschericia coli* NRRL B-3008 were used in activity studies. Antimicrobial activities of the compounds tested against that bacteria species based on CLSI standards [26]. Chloramphenicol was used as the reference compound.

Evaluation of the antimycobacterial activity was performed according to CLSI standards and previously described literatures [27-29] a broth microdilution method that allows the determination of MIC for a given substance against different mycobacteria subspecies. Targeted compounds were evaluated for their *in vitro* antimycobacterial activity against three types of strain of tuberculous mycobacteria, namely *M. smegmatis* ATCC 14468, *M. fortuitum* subs. *fortuitum* ATCC 6841 and *M. avium* clinical strain. Streptomycin and amikacin were used as reference compounds.

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