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Synthesis Characterization and Biological Evaluation of Some Novel Amino Pyrazole Derivatives

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Amino pyrazole and their derivatives for their biological activities. Present work describes the synthesis of novel amino pyrazole derivatives and evaluation of their medicinal value. The reaction of 3,5-*bis*(trifluoromethyl) aniline with cyano acetic acid in presence of ethylene dichloride-hydrochloride produced N-[3,5-*bis*(trifluoromethyl)phenyl]-2-cyanoacetamide **(1)**; this intermediate, upon further reaction with dimethyl sulfide in presence of K_2CO_3 followed by reaction with methyl iodide was converted to N-[3,5-*bis*(trifluoromethyl)phenyl]-2-cyano-3,3-*bis*(methylthio)acrylamide **(2)**. The cyclization of **2** with hydrazine in isopropyl alcohol resulted in 3-amino-N-[3,5-*bis*(trifluoromethyl)phenyl]-5-(methylthio)-1*H*-pyrazole-4-carboxamide **(3)** as main scaffold. This isolated scaffold finally reacted with various derivatives of aldehyde followed by reduction with sodium borohydride to isolate target compounds **AP-1** to **AP-17**. The structures of the synthesized compounds were confirmed by mass, FT-IR and ^{1}H NMR analysis. All the compounds were tested for potential antifungal and antimicrobial activity. Out of 17 compounds **AP-1, 3, 4, 7, 9, 10** and **11** compounds having good activity in antimicrobial agent.

Keywords: Pyrazole, Amine derivatives, Carboxamide derivatives, Antimicrobial, Antifungal activity.

INTRODUCTION

Originally, the name of pyrazole was identified as a class of compounds by German chemist Ludwig in 1883. Classical method for the synthesis of pyrrazole was developed by German chemist Hans von Pechmann in 1898, involves reaction of acetylene and diazomethane.

Pyrazole shows special characteristics, including high efficiency, low toxicity and unique reaction mechanism. Owing to these properties, pyrazole derivatives found many applications in the field of biology and chemistry, has attracted increasing interest, occupied a unique place in field of medicinal chemistry due to their wide spectrum of biological activities.

In the recent years, pyrazole derivatives have attracted the attention of medicinal chemists due to their important biological and chemotherapeutic importance. They are known to exhibit a wide range of biological activities and act as CSRC kinase inhibitors involved with ischemic brain pathology [1]. The recently achievement of pyrazole as cyclooxygenase-2 inhibitor (COX-2) has further highlight the importance of these heterocyclic rings in medicinal chemistry. Pyrazole containing pharmaco active agent acts as an important role in medicinal chemistry [2]. In literature, several pyrazole derivatives are acknowledged to possess antibacterial [3-6], antidiabetic [7], anti-inflammatory [8,9], antifungal [10-13], antitubercular [14-16] and antiviral [17-19].

Literature search revealed that the variation of the substituent on 3-amino position of the pyrazole could impressively encouragement the biological activities [20-26]. It was hypothesized that inclusion of various moieties possessing fungicidal and bacterial properties into the backbone of a pyrazole ring might enhance the antibacterial and fungicidal activities of pyrazole analogues. Based on this hypothesis, 17 novel substituted pyrazole derivatives were designed synthesized and evaluated their antimicrobial potential.

EXPERIMENTAL

Chemicals used in the present research work were procured from Spectrochem, Sigma-Aldrich, CDH and Alfa-Aesar. The purification of solvents was carried out as per pro-

cedures reported in literature. Melting points of the synthesized compounds were determined by applying open capillary method using an electrothermal apparatus. The progress of reactions and the purity of the products formed were checked by thin layer chromatography (TLC) using silica gel plates and examined under ultraviolet lamp. The structures of synthesized compounds were corroborated by using different spectral techniques *i.e.*, FT-IR, ^{1}H NMR and mass spectra. The FT-IR spectra were scanned on Shimazdu IR Affinity-I FT-IR spectrophotometer in range of 4000-400 cm^{-1} using potassium bromide (KBr) powder. The NMR spectra were recorded at 400 (^{1}H NMR), on a commercial Bruker Advance II instrument, in deuterated dimethylsulphoxide-d_6 and $CDCl_3$ as solvent. The chemical shift values were noted as δ ppm using tetramethylsilane as an internal standard.

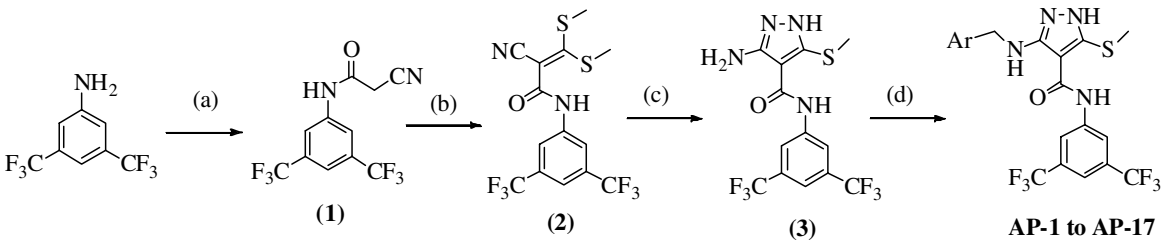
In the present work, novel amino pyrazole derivatives were prepared by following general reaction (**Scheme-I**). The N-[3,5-*bis*(trifluoromethyl)phenyl]-2-cyanoacetamide was prepared by reaction of 3,5-*bis*(trifluoromethyl)aniline and cyanoacetic acid in presence of EDC·HCl in DMF solvent to afford good yield of compound **1**. The compound **1** on reaction with dimethyl sulfide in presence of K_2CO_3 followed by reaction with methyl iodide in DMF solvent, was converted to N-[3,5-*bis*(trifluoromethyl)phenyl]-2-cyano-3,3-*bis*(methylthio)-acrylamide **(2)**. The cyclization of **2** using hydrazine in IPA solvent resulted in 3-amino-N-(3,5-*bis*(trifluoromethyl)-phenyl)-5-(methylthio)-1*H*-pyrazole-4-carboxamide **(3)** as main scaffold. Reaction of **3** with various substituted aromatic aldehyde followed by reduction with $NaBH_4$ produced various derivatives of amino pyrazole AP**(1-17)**. All the synthesized compounds were tested for antifungal and antibacterial activity.

Preparation of N-[3,5-*bis*(trifluoromethyl)phenyl]-2-cyanoacetamide (1): Cyanoacetic acid (16.51 g, 192.11 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) (40.67 g, 261.97 mmol) are dissolved in DMF (170 mL) at 25-35 °C, 3,5-*bis*(trifluoromethyl)aniline (40 g, 174.64 mmol) was added in different lots within 60 min. The resulted reaction mixture was stirred for 120 min. The progress of the reaction was monitored by TLC by using ethyl acetate:cyclohexane (5:5) as a mobile phase. After completion of the reaction, the reaction mixture was dump in water. Precipitated crude material was filtrated out and washed with water. This crude product was purified using diethyl ether to get pure intermediate N-(3,5-*bis*(trifluoromethyl)phenyl)-2-cyanoacetamide **(1)** (41.38 g, 80 % yield) as a off white colour solid.

Preparation of N-[3,5-*bis*(trifluoromethyl)phenyl]-2-cyano-3,3-*bis*(methylthio)acrylamide (2): To a stirred solution of N-[3,5-*bis*(trifluoromethyl)phenyl]-2-cyanoacetamide (**1**) (41 g, 178.9 mmol) in DMF (150 mL), anhydrous potassium carbonate (30.86 g, 223.67 mmol) was added and the reaction mixture was stirred for 120 min at 25-35 °C. After 120 min carbon disulfide (27.42 g, 357.8 mmol) was added in the reaction mass and stirred for another 120 min. Then the reaction mass was gradually cooled to 0-5 °C and methyl iodide (50.79 g, 357.8 mmol) was added within 30 min, the reaction mass warm to 25-35 °C and stirred it at same temperature for 180 min. The reaction progress was monitored by TLC by using ethyl acetate:cyclohexane (5:5) as a mobile phase. After completion of reaction, the mixture was poured into water (700 mL) and stirred for 30 min. The precipitated solid material was filtered, washed with water and dried to afford N-[3,5-*bis*-(trifluoromethyl)phenyl]-2-cyano-3,3-*bis*(methylthio)-acrylamide **(2)** (50.14 g, 70 % yield) as off-white to yellow coloured solid.

Preparation of 3-amino-N-[3,5-*bis*(trifluoromethyl)-phenyl]-5-(methylthio)-1*H*-pyrazole-4-carboxamide (3): To a stirred solution of N-[3,5-*bis*(trifluoromethyl)phenyl]-2-cyano-3,3-*bis*(methylthio)acrylamide (**2**) (50.0 g, 124.99 mmol) in IPA (200 mL), hydrazine hydrate (12.51 g, 249.99 mmol) was added and reaction mass was heated to reflux temperature for 240 min. The progress of the reaction was monitored by TLC by using methanol:MDC (0.5:9.5) as a mobile phase. After completion of reaction, the reaction mixture was cooled to 25-35 °C and water (400 mL) was added. The reaction mixture was stirred for 60 min at same temperature. The solid precipitated was filtered, washed with water. the wet material dried and crystallization in IPA to afford 3-amino-N-[3,5-*bis*(trifluoromethyl)phenyl]-5-(methylthio)-1*H*-pyrazole-4-carboxamide (**3**) (28.22 g, 60 % yield) as a off-white to yellow coloured solid.

Preparation of 3-(aryl amino)-N-[3,5-*bis*(trifluoromethyl)-phenyl]-5-(methylthio)-1*H*-pyrazole-4-carboxamide (AP-1 to AP-17): The mixture of 3-amino-N-[3,5-*bis*-(trifluoromethyl)phenyl]-5-(methylthio)-1*H*-pyrazole-4-carboxamide **(3)** (1.30 mmol), substituted aromatic aldehyde (1.42 mmol) and catalytic amount of CH_3COOH in IPA (10 mL) was refluxed for 120-180 min. The reaction progress was monitored by TLC by using methanol:MDC (0.5:9.5) as a mobile phase. After completion of the reaction, sodium borohydride (1.42 mmol) was added in lot wise manner into the reaction mixture within 30 min at 25-35 °C. The reaction



a) Cyanoaceticacid, EDC·HCl, DCM, RT 1 h; (b) i. CS_2, K_2CO_3, DMF, 25-35 °C: ii. CH_3I, 0-10 °C to 25-35 °C; (c) NH_2NH_2, IPA, Reflux 240 min (d) i ArCHO, cat. acetic acid, IPA, reflux 120-180 min: ii sodium borohydride, IPA, RT 60 min.

Scheme-I: General synthesis

mixture was then stirred for additionally 60 min at 25-35 °C. The progress of the reduction was monitored by TLC by using methanol:MDC (0.5:9.5) as a mobile phase. After completion of the reaction, the mass was neutralized with CH_3COOH and solvent was distilled out under reduced pressure. The crude material was dissolved in dichloromethane and washed with water. The solvent was distilled out under reduced pressure and the product was crystallized from IPA to give pure 3-(aryl-amino)-N-[3,5-*bis*(trifluoromethyl)phenyl]-5-(methylthio)-1*H*-pyrazole-4-carboxamide (**AP-1** to **AP-17**) as a white to off white coloured compound.

Spectral data

3-(Benzylamino)-N-[3,5-*bis*(trifluoromethyl)phenyl]-5-(methylthio)-1*H*-pyrazole-4-carboxamide (AP-1): Yield: 89.11 %; m.f. $C_{20}H_{16}N_4OSF_6$; m.p. 217-221 ºC; m.w. 474.09. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 2.57 (s, 3H), 4.49-4.47 (d, *J* = 7.2 Hz, 2H), 7.25-7.37 (m, 6H), 7.52 (s, 1H), 8.18-8.20 (m, 2H), 9.62 (s, 1H), 12.51 (s, 1H) ppm; MS: *m/z* 474.8 $(M+H)^+$. IR (KBr, ν_{max}, cm^{-1}): 3344.57 (N-H amine), 1645.28 (-CONH-), 1284.59 (C-N amine), 1124.5 (C-F). Elemental analysis (%) Found (calcd.): C 50.62 (50.63), H 3.40 (3.40), N 11.82 (11.81).

***N*-[3,5-*Bis*(trifluoromethyl)phenyl]-3-(3,4-dimethoxybenzylamino)-5-(methylthio)-1*H*-pyrazole-4-carboxamide (AP-2):** Yield: 68.31 %, m.f. $C_{22}H_{20}N_4O_3SF_6$; m.p. 205-208 ºC; m.w. 534.12. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 2.57 (s, 3H), 3.86 (s, 3H), 3.88 (s, 3H), 4.40-4.42 (d, *J* = 6.8 Hz, 2H), 6.84-6.94 (m, 4H), 7.26-7.33 (m, 1H), 7.58 (s, 1H), 8.10 (s, 1H), 9.46 (s, 1H), 12.51 (s, 1H) ppm; MS: *m/z* 534.9 (M+H). IR (KBr, ν_{max}, cm^{-1}): 3356.14 (N-H amine), 1658.78 (-CONH-), 1278.81 (C-N amine), 1246.02 (-C-O-, ether), 1128.36 (C-F). Elemental analysis (%) Found (calcd.): C 49.44 (49.44), H 3.77 (3.77), 10.47 (10.48), O 8.98 (8.98).

***N*-[3,5-*bis*(Trifluoromethyl)phenyl]-3-(4-chlorobenzylamino)-5-(methylthio)-1*H*-pyrazole-4-carboxamide (AP-3):** Yield: 51.40 %, m.f. $C_{20}H_{15}N_4OSF_6Cl$; m.p. 199-203 ºC; m.w. 508.06. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 2.57 (s, 3H), 4.49-4.47 (d, *J* = 7.2 Hz, 2H), 7.29-7.37 (m, 4H), 7.54 (s, 1H), 7.95 (s, 1H), 8.22 (m, 2H), 9.61 (s, 1H), 12.51 (s, 1H) ppm; MS: *m/z* 508.8 $(M+H)^+$. IR (KBr, ν_{max}, cm^{-1}): 3329.14 (N-H amine), 1660.71 (-CONH-), 1276.88 (C-N amine), 1128.36 (C-F), 812.03 (C-Cl). Elemental analysis (%) Found (calcd.): C 47.21 (47.21), H 2.97 (2.97), N 11.01 (11.01), O 3.14 (3.14).

***N*-[3,5-*bis*(Trifluoromethyl)phenyl]-3-(2-bromo-6-fluorobenzylamino)-5-(methylthio)-1*H*-pyrazole-4-carboxamide (AP-4):** Yield: 60.64 %, m.f. $C_{20}H_{14}N_4OSF_7Br$; m.p. 225-229 ºC; m.w. 570.00. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 2.56 (s, 3H), 4.59-4.60 (d, *J* = 4.8 Hz, 2H), 6.69 (s, 1H), 7.16-7.20 (m, 1H), 7.29-7.30 (m, 1H), 7.45-7.47 (d, *J* = 7.8 Hz, 1H), 7.54 (s, 1H), 8.18 (s, 1H), 9.63 (s, 1H), 12.82 (s, 1H) ppm; MS: *m/z* 570.8 $(M+H)^+$. IR (KBr, ν_{max}, cm^{-1}): 3570.24 (N-H amine), 1653 (-CONH-), 1278.81 (C-N amine), 1130.29 (C-F), 678.94 (C-Br). Elemental analysis (%) Found (calcd.): C 42.05 (42.05), H 2.46 (2.47), N 9.81 (9.81), O 2.80 (2.80).

N-[3,5-*bis*(Trifluoromethyl)phenyl]-3-(4-hydroxybenzylamino)-5-(methylthio)-1*H*-pyrazole-4-carboxamide (AP-5): Yield: 70.53 %, m.f. $C_{20}H_{16}N_4O_2SF_6$; m.p. 235-238 ºC; m.w. 490.09. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 2.53 (s, 3H), 4.31-4.32 (d, *J* = 7.2 Hz, 2H), 6.71-6.74 (m, 3H), 7.15-7.18 (m, 2H), 7.29-7.30 (m, 1H), 7.56 (s, 1H), 8.16 (s, 1H), 9.19 (s, 1H), 9.58 (s, 1H), 12.22 (s, 1H) ppm; MS: *m/z* 490.9 $(M+H)^+$. IR (KBr, ν_{max}, cm^{-1}): 3292.49 (N-H amine), 1664.57 (-CONH-), 1377.17 (-O-H), 1282.66 (C-N amine), 1124.5 (C-F). Elemental analysis (%) Found (calcd.): C 48.98 (48.98), H 3.28 (3.29), N 11.41 (11.42), O 6.51 (6.52).

TABLE-1
ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY, MINIMUM INHIBITORY CONCENTRATION (μg/mL)

Code No.	Ar	Antibacterial activity				Antifungal activity		
		E. coli MTCC 443	*P. aeruginosa* MTCC 1688	*S. aureus* MTCC 96	*S. pyogenus* MTCC 442	*C. albicans* MTCC 227	*A. niger* MTCC 282	*A. clavatus* MTCC 1323
AP-1	Phenyl	100	50	125	125	> 1000	> 1000	> 1000
AP-2	3,4-Dimethoxy phenyl	125	250	125	100	> 1000	500	500
AP-3	4-Chloro phenyl	100	250	62.5	250	250	500	1000
AP-4	2-Fluoro-6-bromo phenyl	62.5	100	250	500	1000	1000	1000
AP-5	4-Hydroxy phenyl	100	250	500	500	1000	> 1000	> 1000
AP-6	4-N,N Dimethyl phenyl	250	500	100	250	500	250	250
AP-7	4-Pyridine	200	100	100	250	250	500	500
AP-8	4-Methyl phenyl	250	250	125	250	250	> 1000	> 1000
AP-9	2-Bromo phenyl	62.5	125	500	250	500	1000	1000
AP-10	4-Bromo phenyl	100	32.5	100	125	1000	> 1000	> 1000
AP-11	2-Methyl phenyl	125	100	62.5	100	250	> 1000	1000
AP-12	4-Methoxy phenyl	250	125	250	250	500	500	500
AP-13	2-Chloro phenyl	100	125	250	100	500	1000	1000
AP-14	4-Fluoro phenyl	250	100	250	250	500	250	250
AP-15	3,4-Fluoro phenyl	125	250	500	500	1000	500	500
AP-16	Thiophene	250	250	250	500	1000	500	500
AP-17	2-Hydroxy phenyl	100	125	250	100	> 1000	> 1000	> 1000
Gentamycine		0.05	1	0.25	0.5	–	–	–
Ampicillin		100	100	250	100	–	–	–
Chloramphenicol		50	50	50	50	–	–	–
Ciprofloxacin		25	25	50	50	–	–	–
Norfloxacin		10	10	10	10	–	–	–
Nystatin		–	–	–	–	100	100	100
Greseofulvin		–	–	–	–	500	100	100

***N*-[3,5-*bis*(Trifluoromethyl)phenyl]-3-(4-(dimethylamino)benzylamino)-5-(methylthio)-1*H*-pyrazole-4-carboxamide (AP-6):** Yield: 74.26 %, m.f. $C_{22}H_{21}N_5OSF_6$; m.p. 215-220 ºC; m.w. 517.14. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 2.53 (s,3H), 3.09 (s, 6H), 4.33-4.34 (d, *J* = 6.8 Hz,2H), 6.71-6.74 (m, 2H), 7.05-7.10 (m, 2H), 7.65 (s,1H), 8.15 (s,1H), 9.15 (s,1H), 9.58 (s,1H), 12.22 (s,1H) ppm; MS: *m/z* 490.9 $(M+H)^+$. IR (KBr, ν_{max}, cm^{-1}): 3295.56 (N-H amine), 1665.32 (-CONH-), 1377.17 (C-N amine), 1124.5 (C-F). Elemental analysis (%) Found (calcd.): C 51.06 (51.06), H 4.09 (4.09), N 13.53 (13.53), O 3.09 (3.09).

N-[3,5-*bis*(Trifluoromethyl)phenyl]-5-(methylthio)-3-(pyridin-4-ylmethylamino)-1*H*-pyrazole-4-carboxamide (AP-7): Yield: 67.90 %, m.f. $C_{19}H_{15}N_5OSF_6$; m.p. 226-229 ºC; m.w. 475.09. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 2.58 (s, 3H), 4.51-4.53 (d, *J* = 7.2 Hz, 2H), 7.35-7.39 (m, 2H), 7.81 (s, 1H), 8.22-8.34 (m, 2H), 8.52-8.55 (m, 2H), 9.75 (s, 1H), 12.52 (s, 1H); MS: *m/z* 475.9 $(M+H)^+$. IR (KBr, ν_{max}, cm^{-1}): 3332.25 (N-H amine), 1665.75 (-CONH-), 1278.88 (C-N amine), 1123.42 (C-F). Elemental analysis (%) Found (calcd.): C 48.01 (48.00), H 3.18 (3.18), N 14.73 (14.73), O 3.37 (3.37).

N-[3,5-*bis*(Trifluoromethyl)phenyl]-3-(4-methylbenzylamino)-5-(methylthio)-1*H*-pyrazole-4-carboxamide (AP-8): Yield: 64.52 %, m.f. $C_{21}H_{18}N_4OSF_6$; m.p. 245-249 ºC; m.w. 488.11. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 2.35 (s, 3H), 2.52 (s, 3H), 4.34-4.35 (d, *J* = 6.8 Hz, 2H), 7.12-7.16 (m, 4H), 7.78 (s, 1H), 8.12-8.15 (m, 2H), 9.59 (s, 1H), 12.20 (s, 1H). MS: *m/z* 489.9 $(M+H)^+$. IR (KBr, ν_{max}, cm^{-1}): 3290.29 (N-H amine), 1663.51 (-CONH-), 1281.65 (C-N amine), 1123.5 (C-F). Elemental analysis (%) Found (calcd.): C 51.64 (51.64), H 3.71 (3.71), N 23.33 (23.34), O 3.28 (3.28).

N-[3,5-*bis*(Trifluoromethyl)phenyl]-3-(2-bromobenzylamino)-5-(methylthio)-1*H*-pyrazole-4-carboxamide (AP-9): Yield: 55.66 %, m.f. $C_{20}H_{15}N_4OSBrF_6$; m.p. 281-284 ºC; m.w. 552.01. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 2.53 (s, 3H), 4.32-4.34 (d, *J* = 7.2 Hz, 2H), 7.12-7.15 (m, 2H), 7.19-7.22 (m, 2H), 7.75 (s, 1H), 8.11-8.13 (m, 2H), 9.56 (s, 1H), 12.19 (s, 1H). MS: *m/z* 452.9 $(M+H)^+$. IR (KBr, ν_{max}, cm^{-1}): 3571.24 (N-H amine), 16532.52 (-CONH-), 1277.81 (C-N amine), 1131.19 (C-F), 679.91 (C-Br). Elemental analysis (%) Found (calcd.): C 43.41 (43.41), H 2.73 (2.73), N 10.13 (10.13), O 2.88 (2.89).

N-[3,5-*bis*(Trifluoromethyl)phenyl]-3-(4-bromobenzylamino)-5-(methylthio)-1*H*-pyrazole-4-carboxamide (AP-10): Yield: 64.70 %, m.f. $C_{20}H_{15}N_4OSBrF_6$; m.p. 271-274 ºC; m.w. 552.01. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 2.56 (s, 3H), 4.74.49 (d, *J* = 7.2 Hz, 2H), 7.30-7.36 (m, 4H), 7.53 (s, 1H), 7.94 (s, 1H), 8.22 (m, 2H), 9.62 (s, 1H), 12.51 (s, 1H). MS: *m/z* 452.9 $(M+H)^+$. IR (KBr, ν_{max}, cm^{-1}): 3329.14 (N-H amine), 1661.12 (-CONH-), 1276.88 (C-N amine), 1127.86 (C-F), 678.15 (C-Br). Elemental analysis (%) Found (calcd.): C 43.41 (43.41), H 2.72 (2.73), N 10.13 (10.13), O 2.88 (2.89).

N-[3,5-*bis*(Trifluoromethyl)phenyl]-3-(2-methylbenzylamino)-5-(methylthio)-1*H*-pyrazole-4-carboxamide (AP-11): Yield: 65.31 %, m.f. $C_{21}H_{18}N_4OSF_6$; m.p. 251-253 ºC; m.w. 488.11. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 2.34 (s, 3H), 2.52 (s, 3H), 4.33-4.35 (d, *J* = 6.8 Hz, 2H), 7.12-7.16 (m, 4H), 7.79 (s, 1H), 8.11-8.13 (m, 2H), 9.59 (s, 1H), 12.20 (s, 1H). MS: *m/z* 489.9 $(M+H)^+$. IR (KBr, ν_{max}, cm^{-1}): 3290.29 (N-H amine), 1662.81 (-CONH-), 1281.65 (C-N amine), 1123.5 (C-F). Elemental analysis (%) Found (calcd.): C 51.64 (51.64), H 3.70 (3.71), N 23.33 (23.34), O 3.28 (3.28).

N-[3,5-*bis*(Trifluoromethyl)phenyl]-3-(4-methoxybenzylamino)-5-(methylthio)-1*H*-pyrazole-4-carboxamide (AP-12): Yield: 73.14 %, m.f. $C_{21}H_{18}N_4O_2SF_6$; m.p. 210-214 ºC; m.w. 504.11. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 2.35 (s, 3H), 3.82 (s, 3H), 2.52 (s, 3H), 4.34-4.35 (d, *J* = 6.8 Hz, 2H), 6.85-6.87 (d, *J* = 7.2 Hz, 2H), 7.25-7.27 (d, *J* = 7.2 Hz, 2H), 7.75 (s, 1H), 8.12-8.15 (m, 2H), 9.58 (s, 1H), 12.20 (s, 1H). MS: *m/z* 504.9 $(M+H)^+$. IR (KBr, ν_{max}, cm^{-1}): 3291.12 (N-H amine), 1663.45 (-CONH-), 1281.75, (C-N amine), 1123.5 (C-F). Elemental analysis (%) Found (calcd.): C 50.01 (50.00), H 3.60 (3.60), N 11.11 (11.11), O 6.34 (6.34).

N-[3,5-*bis*(Trifluoromethyl)phenyl]-3-(2-chlorobenzylamino)-5-(methylthio)-1*H*-pyrazole-4-carboxamide (AP-13): Yield: 65.76 %, m.f. $C_{20}H_{15}N_4OSClF_6$; m.p. 202-205 ºC; m.w. 508.06. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 2.53 (s, 3H), 4.32-4.34 (d, *J* = 7.2 Hz, 2H), 7.11-7.15 (m, 2H), 7.19-7.21 (m, 2H), 7.75 (s, 1H), 8.11-8.13 (m, 2H), 9.56 (s, 1H), 12.19 (s, 1H). MS: *m/z* 5089 $(M+H)^+$. IR (KBr, ν_{max}, cm^{-1}): 3571.24 (N-H amine), 16532.52 (-CONH-), 1277.81 (C-N amine), 1131.19 (C-F), 679.91 (C-Br), 812.03 (C-Cl). Elemental analysis (%) Found (calcd.): C 47.21 (47.21), H 2.97 (2.97), N 11.01 (11.01), O 3.15 (3.14).

N-[3,5-*bis*(Trifluoromethyl)phenyl]-3-(4-fluorobenzylamino)-5-(methylthio)-1*H*-pyrazole-4-carboxamide (AP-14): Yield: 60.87 %, m.f. $C_{20}H_{15}N_4OSF_7$; m.p. 244-247 ºC; m.w. 492.09. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 2.56 (s, 3H), 4.49-4.47 (d, *J* = 7.2 Hz, 2H), 7.32-7.41 (m, 4H), 7.55 (s, 1H), 7.95 (s, 1H), 8.12-8.15 (m, 2H), 9.61 (s, 1H), 12.51 (s, 1H). MS: *m/z* 492.8 $(M+H)^+$. IR (KBr, ν_{max}, cm^{-1}): 3329.14 (N-H amine), 1660.71 (-CONH-), 1276.88 (C-N amine), 1130.36 (C-F). Elemental analysis (%) Found (calcd.): C 48.78 (48.78), H 3.07 (3.07), N 11.38 (11.38), O 3.26 (3.25).

N-[3,5-*bis*(Trifluoromethyl)phenyl]-3-(3,4-difluorobenzylamino)-5-(methylthio)-1*H*-pyrazole-4-carboxamide (AP-15): Yield: 59.48 %, m.f. $C_{20}H_{14}N_4OSF_8$; m.p. 239-243 ºC; m.w. 510.08. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 2.56 (s, 3H), 4.49-4.47 (d, *J* = 7.2 Hz, 2H), 6.75 (m, 1H), 7.15-7.21 (m, 2H), 7.55 (s, 1H), 7.75 (s, 1H), 8.11-8.14 (m, 2H), 9.63 (s, 1H), 12.52 (s, 1H). MS: *m/z* 510.8 $(M+H)^+$. IR (KBr, ν_{max}, cm^{-1}): 3329.14 (N-H amine), 1661.52 (-CONH-), 1276.88 (C-N amine), 1131.25 (C-F). Elemental analysis (%) Found (calcd.): C 47.05 (47.06), H 2.77 (2.76), N 10.98 (10.98), O 3.12 (3.13).

N-[3,5-*bis*(Trifluoromethyl)phenyl]-5-(methylthio)-3-(thiophen-3-ylmethylamino)-1*H*-pyrazole-4-carboxamide (AP-16): Yield: 65.60 %, m.f. $C_{18}H_{14}N_4OS_2F_6$; m.p. 210-215 ºC; m.w. 480.05. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 2.34 (s, 3H), 2.53 (s, 3H), 4.34-4.36 (d, *J* = 6.8 Hz, 2H), 6.74-6.76 (m, 2H), 7.59 (s, 1H), 7.75 (s, 1H), 8.12-8.14 (m, 2H), 9.60 (s, 1H), 12.22 (s, 1H). MS: *m/z* 480.9 $(M+H)^+$. IR (KBr, ν_{max}, cm^{-1}): 3291.29 (N-H amine), 1662.81 (-CONH-), 1280.65 (C-N amine), 1123.5 (C-F). Elemental analysis (%) Found (calcd.): C 45.01 (45.00), H 2.94 (2.94), N 11.67 (11.66), O 3.33 (3.33).

N-[3,5-*bis*(Trifluoromethyl)phenyl]-3-(2-hydroxybenzylamino)-5-(methylthio)-1*H*-pyrazole-4-carboxamide (AP-17): Yield: 66.61 %, m.f. $C_{20}H_{16}N_4O_2SF_6$; m.p. 241-244 ºC; m.w. 490.09. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 2.53 (s, 3H), 4.31-4.32 (d, *J* = 7.2 Hz, 2H), 6.71-6.77 (m, 4H), 77.56

(s, 1H), 8.11-8.13 (m, 2H), 9.58 (s, 1H), 9.89 (s, 1H), 12.22 (s, 1H). MS: *m/z* 490.9 $(M+H)^+$. IR (KBr, ν_{max}, cm^{-1}): 3292.49 (N-H amine), 1664.57 (-CONH-), 1376.89 (-O-H), 1281.56 (C-N amine), 1124.5 (C-F). Elemental analysis (%) Found (calcd.): C 48.98 (48.98), H 3.29 (3.29), N 11.41 (11.42), O 6.52 (6.54).

Biological evaluation: All manufacturing compounds were tested for their *in vitro* antifungal and antibacterial activity. For evaluation of antibacterial activity, the representative strains of Gram-negative bacteria (*P. aeruginosa, E. coli*) and Gram-positive bacteria (*S. pyogenus, S. aureus*) are used. For testing antifungal activity *A. clavatus, A. niger* and *C. albicans*, were used. All standard medicine was procured from Institute of Micro Care Laboratory, Surat, India. For testing antifungal activity greseofulvin & nystatin standard were used while for antibacterial activity The ampicillin, chloramphenicol, gentamycin, ciprofloxacin and norfloxacin standard were used for the comparison. The minimum inhibitory concentration (MIC) was prominent as the minimum concentration of the test element, which completely inhibits the growth of microorganism *i.e.* 100 % transparency.

RESULTS AND DISCUSSION

In the present study, 17 novel amino pyrazole compounds were synthesized reasonably in good yields. The presence of characteristic peaks in FT-IR, NMR and mass spectra confirmed the structure of titled compound derivatives. All the synthesized compounds were tested for antibacterial and antifungal activity.

Antibacterial evaluation: Compounds **AP-1, 3, 5, 10, 13** and **17** were found to be equipotent while **AP-4** and **AP-9** were found to be more potent to ampicillin (MIC = 100 µg/mL) against *E. coli* (MTCC 443). Compound **AP-4, 7, 11** and **14** were found to be equipotent to ampicillin (MIC = 100 µg/mL) while compound **Ap-1** and **Ap-10** were found to be equipotent to chloramphenicol (MIC = 50 µg/mL) against *P. aeruginosa* (MTCC 1688). Compounds **AP-6, 7** and **10** were found to be equipotent while **AP-3 and AP-11** were found to be more potent to ampicillin (MIC = 100 µg/mL) against *S. aureus* (MTCC 96) and compounds **AP-1**, **5**, **8**, **10** and **17** were found good potent against *S. pyogenus* (MTCC 442). Overall, Compound **AP-1, 3, 4, 7, 10** and **11** showed good to moderate potential antibacterial activity (Table-1).

Antifungal evaluation: Compounds **AP-1, 3, 4, 5, 10, 13** and **17** were found to be equipotent and compound **AP-4** and **AP-9** were found to be higher potent to greseofulvin (MIC = 500 µg/mL) against *C. albicans* (MTCC 227). All other manufactured compounds were found to be moderate potent than standard drugs against *A. niger* (MTCC 282) and *A. clavatus* (MTCC 1323) (Table-1).

Conclusion

The novel amino pyrazole compounds were synthesized with good yield using commercial grade raw materials. The structures of all the compounds were confirmed by, ^{1}H NMR mass and FT-IR. The manufactured compounds were tested for potential biological activities. All the compounds were found to possess reasonably good antifungal activity and compounds **AP-1, 3, 4, 7, 9, 10** and **11** were found to be most potent antimicrobial agent.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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