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# SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME NOVEL CARBOXAMIDE DERIVATIVES OF PYRAZOLE

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## ABSTRACT

A series of 1-(6-(benzylamino)pyrimidin-4-yl)-5-methyl-1Hpyrazole-4-carboxamides derivatives have been synthesised by using different acids. All steps were synthesised by green procedure with excellent yield. Product obtained were characterised by means of the NMR, IR and Mass spectral analysis. The synthesized compounds were evaluated for their in-vitro antimicrobial activity against different bacterial and fungal strains using Mueller- Hinton Broth dilution method and also invitro antitubercular activity was performed.

**KEYWORDS**: pyrimidin, pyrazole, carboxamides derivatives, biological activities.

## INTRODUCTION

The study of heterocyclic compounds is of great interest both from the theoretical as well as practical point of view. Organic chemistry is largely made up of heterocyclic chemistry. The heterocyclic compounds have a cyclic structure contain two or more heteroatoms in the rings. The number of possible heterocyclic systems is almost illimitable, a massive number of heterocyclic compounds are known and this number is increasing very fast. Heterocyclic rings bearing nitrogen atom are most copious in nature than those containing oxygen or sulphur. Heterocyclic compounds are very broad assigned in nature and are crucial to life in different ways, most of sugars and their derivatives including vitamins and some members of vitamin B group have heterocyclic rings with nitrogen atom. The countless plant alkaloids are aware example of complex nitrogenous ring compounds. They have been utilized for

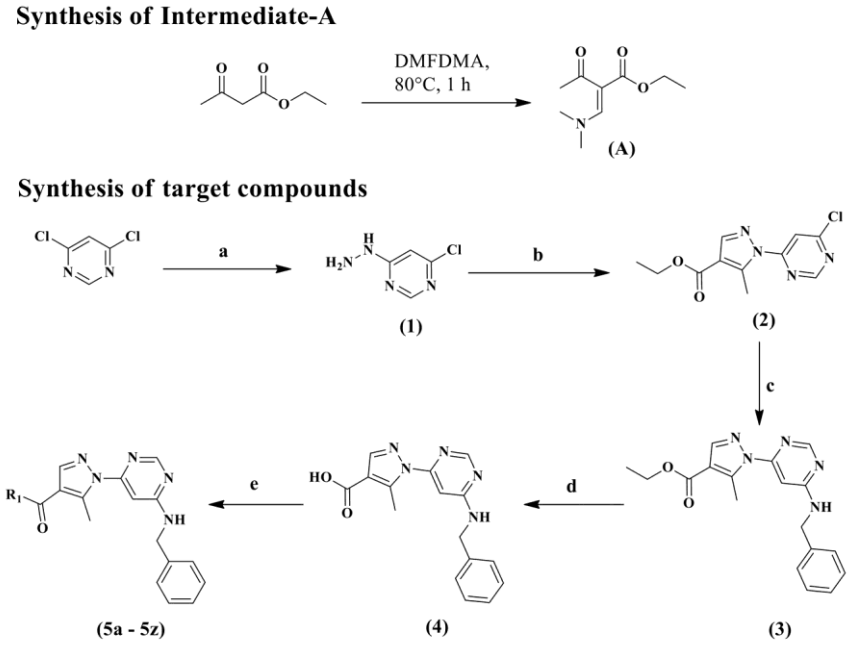
thousands of years in diverse religious, cultural and medicinal applications. Heterocyclic compounds are important components of side chain of the amino acid, histidine. It is found at the region active site of several enzymes, which involved in proton transfer reactions. The invention of penicillin and its marvelous bactericidal properties and the urge to fulfill its synthesis, to promoted intensive research in the area of heterocyclic chemistry.

The occurrence of fungal and bacterial infections has increased dramatically since past few years. The infection has spread among human and also animals. The widely use of antifungal and antibacterial drugs and their resistance against fungal and bacterial infections has urge to chronic health issue. The resistance of broad spectrum antifungal and antibacterial agents has initiated discovery and modification of the modern antifungal and antibacterial drugs. Thus, these antifungal and antibacterial class of drugs is the vast contribution of the $20^{th}$ century to Medicinal chemistry. Pyrazole are well famous and important nitrogen containing five membered heterocyclic atomic compound. It has chemical compexicity in heterocyclic ring. Pyrazole which has two Nitrogen and aromatic character provides diverse functionality. They are Building blocks of life due to its wide range of biological activity like anti-bacterial, analgesic, anti-microbial, anti-inflammatory and Anti-cancer.

**EXPERIMENTAL**

All reactions were performed with commercially available reagents. The solvents were used of analytical grade. They were used without further decontamination. All reactions were monitored by thin-layer chromatography (TLC) on aluminium plates coated with silica gel, 0.25mm thickness (Merck). Mass spectra were recorded on Shimadzu LCMS 2010 spectrometer (Shimadzu, Tokyo, Japan). The IR spectra were confirmed by FTIR MB 3000 spectrophotometer (ABB Bomem Inc., Canada/Agaram Industries, Chennai) using Zn-Se Optics (490-8500 $cm^{-1}$).[1] H and $^{13}C$ Nuclear Magnetic Resonance spectra were checked in DMSO-*d6* on a Bruker Avance 400F (MHz) spectrometer (Bruker Scientific Corporation Ltd., Switzerland) using residual solvent signal as an internal standard at 400 MHz and 100 MHz respectively.

**Reaction scheme**




**Reagents (a) NH2NH2.H2O, EtOH, 1.5 h, rt;**

*(b)* Intermediate-A, EtOH, 1 h, reflux;

*(c)* Benzylamine, TEA, EtOH, 3 h, reflux;

*(d)* LiOH, MeOH, H2O, 4.5 h, reflux;

*(e)* amines, EDC.HCl, , DMF, 1 h, reflux.

- **Synthetic protocol for Ethyl 2-((dimethyl amino) methylene)-3- oxobutanoate (Intermediate-A)**

Ethyl acetoacetate (5) and DMF-DMA (5) were charged in 100 mL three-necked flask and heated for 1 h at 80°C. After complete consumption of ethyl acetoacetate on TLC plate, the reaction mixture was cooled and quenched with *n*-pentane to remove unreacted DMF- DMA. Formation of product was further confirmed by boiling point analysis. Obtained Reaction mixture was directly used for the next step without further purification to afford ethyl 2-((dimethyl amino) methylene)-3-oxobutanoate (Intermediate-**A**) as dark brown liquid. Yield 90%; B.P. 180°C.

- **Synthetic protocol for 4-chloro-6-hydrazinylpyrimidine (1)**

4,6-Dichloropyrimidine (5 g) in ethanol was charged in 250 ml three-necked flask and was cooled to 5°C. Hydrazine hydrate (4.7 ml) was charged to the above flask dropwise. After complete addition of hydrazine hydrate, reaction mixture was stirred at room temperature for

90 minutes. The reaction mixture was filtered and washed with water to afford crude product which was recrystallized in ethanol to obtain 4-chloro-6- hydrazinylpyrimidine (**1**) as pale yellow solid. Yield 95%; Rf = 0.5 (hexane:ethyl acetate, 4:1); m.p. 164°C; $^1$H NMR 8.83 (s, 1H), 8.17 (s, 1H), 6.76 (s, 1H), 4.50 (s, 2H); EI-MS (m/z): 145.02 (M+1).

- **Synthetic protocol for Ethyl 1-(6-chloropyrimidin-4-yl)-5-methyl-1*H*-pyrazole- 4-carboxylate (2)**

To a solution of **1** (5 g) in ethanol (100 mL) was added Intermediate-**A** (10mL) and the reaction mixture was stirred for 45 minutes. After that the reaction mixture was refluxed for 1 h. The reaction mixture was cooled and poured on crushed ice to obtain white solid as crude product which was filtered, dried and recrystallized from ethanol to obtain ethyl 1-(6-chloropyrimidin-4-yl)-5-methyl-1*H*-pyrazole-4-carboxylate (**2**) as white crystals. Yield 75%; Rf = 0.8 (hexane:ethyl acetate, 4:1); m.p. 180°C; $^1$H NMR 9.936 (s, 1H), 8.492 (s, 1H), 8.238 (s, 1H), 4.216 (q, 2H), 2.782 (s, 3H), 1.312 (t, *J*=7.2 Hz, 3H). EI-MS (m/z):268.07 (M+2).

- **Synthetic protocol for Ethyl 1-(6-(benzylamino)pyrimidin-4-yl)-5-methyl-1*H*-pyrazole-4-carboxylate (3)**

To a solution of **2** (5g) in ethanol (100 mL) was added triethylamine (10 mL) and benzyl amine (3.8mL) and the mixture was stirred at 80$^0$C for 3 h. After completation of reaction mixture was poured on ice-cold water. white precipitates were filtered off, dried and recrystallized with ethanol to obtained Ethyl 1-(6-(benzylamino)pyrimidin-4-yl)-5-methyl-1*H*-pyrazole-4-carboxylate (**3**) as white solid. Yield 95%; Rf = 0.35 (hexane:ethyl acetate, 5:5); m.p. 188-192°C; $^1$H NMR: 9.912(s, 1H), 8.432 (s, 1H), 8.212 (s, 1H), 7.32-7.22 (m, 5H), 4.204 (s, 2H), 4.106 (q, *J*=7.2 Hz, 2H), 2.122 (s, 3H), 1.312 (t, *J*=7.2 Hz, 3H); EI-MS (m/ z): 338.47 (M+1).

- **Synthetic protocol for 1-(6-(benzylamino)pyrimidin-4-yl)-5-methyl-1*H*- pyrazole-4-carboxylic acid (4)**

To a previously cooled solution of **3** (5g) in methanol:water (2:1) at 0-5$^0$C, lithium hydroxide (2g) was added portion wise. After completion of addition, the mixture was stirred at room temperature for 4.5 h. After completion of reaction, reaction mixture was poured on ice-cold water and acidified with 1N HCl. Precipitates formed were filtered, washed with water and dried to afford 1-(6-(benzylamino)pyrimidin-4-yl)-5- methyl-1*H*-pyrazole-4- carboxylic acid (**4**) as white solid. Yield 96%; Rf = 0.30 (hexane:ethyl acetate, 3:7); m.p. 182°C; $^1$H NMR 10.824 (s,1H),

9.932 (s, 1H), 8.422 (s, 1H), 8.12 (s, 1H),7.32-7.22 (m,5H), 4.211 (s, 2H), 2.102 (s, 3H); EI-MS (m/z): 310.04 (M+1).

- **Synthetic protocol for 1-(6-(benzylamino)pyrimidin-4-yl)-5-methyl-1H- pyrazole-4-carboxamides (5a-5g)**

To a stirred solution of **4** (1mmol) in DMF was added HOBT (0.7 mmol) under anhydrous conditions at room temperature. EDC.HCl (1.5 mmol) was added to the above reaction mixture and stirred for 15 minutes. Triethylamine (3.0 mmol) was added and to the reaction mixture and it was further stirred for 5 minutes. After this, the corresponding amine (1.0 mmol) was added and reaction mixture was allowed to stir at room temperature for 1 h. Reaction was monitored on TLC. After completion of reaction, the reaction mixture was poured in cold-water and recrystallized from ethanol to obtain title compounds (**5a-5g**).

**(1-(6-(benzylamino)pyrimidin-4-yl)-5-methyl-1*H*-pyrazol-4-yl(morpholino) methanone (5a)**

White solid; $^{1}$H-NMR (DMSO-d6, 400 MHz) δ (ppm): 8.428 (s, 1H), 8.260 (s, 1H), 7.837 (s, 1H), 7.344-7.253 (m, 5H), 6.994 (s, 1H), 4.618 (s, 2H), 3.599(broad s, 4H), 3.530(broad s, 4H), 2.647 (s, 3H); $^{13}$C-NMR (DMSO, 100 MHz) δ (ppm): 163.60, 163.10, 157.78, 141.05, 140.19, 128.38, 127.24, 126.89, 125.49, 117.47, 94.37, 66.15, 43.45, 39.45, 13.41; EI-MS (m/z): 379.1 (M+1).

**1-(6-(benzylamino)pyrimidin-4-yl)-5-methyl-N-phenyl-1*H*-pyrazole-4- carboxamide (5b)**

White solid; $^{1}$H-NMR (DMSO-d6, 400 MHz) δ (ppm): 9.936 (s, 1H), 8.463 (s, 1H), 8.330 (s,1H), 8.286 (s, 1H), 7.707-7.738 (m, 10H), 7.018 (s, 1H), 4.636 (s, 2H), 2.90 (s, 3H); $^{13}$C-NMR (DMSO, 100 MHz) δ (ppm): 163.61, 161.23, 157.79, 143.90, 140.60, 139.24, 139.01, 128.56,128.39, 127.24, 126.90, 123.41, 120.14, 117.54, 95.30, 39.45, 13.16; EI-MS (m/z): 385.5 (M+1).

**1-(6-(benzylamino)pyrimidin-4-yl)-N-(4-fluorophenyl)-5-methyl-1*H*-pyrazole-4carboxamide (5c)**

Off white solid; 1H-NMR (DMSO-d6, 400 MHz) δ (ppm): 10.006 (s, 1H), 8.463 (s, 1H), 8.317 (s, 2H), 7.741 (s, 2H), 7.349-7.173 (m, 7H), 7.016 (s, 1H), 4.638 (s, 2H), 2.898 (s, 3H); 13C-NMR (DMSO, 100 MHz) δ (ppm): 163.62, 161.16, 159.31, 157.38, 143.94, 140.55, 139.27, 135.35, 127.50, 121.94, 117.35, 115.11, 95.33, 56.84, 43.47, 39.43,

13.15; EI-MS (m/z): 403.5 (M+1).

**1-(6-(benzylamino)pyrimidin-4-yl)-5-methyl-N-(4-methylbenzyl)-1*H*-pyrazole-4-carboxamide (5d)**

White solid; 1H-NMR (DMSO-d6, 400 MHz) δ (ppm): 8.742 (s, 1H), 8.447 (s, 1H), 8.272 (s, 1H), 8.207 (s, 1H), 7.339-7.256 (m, 10H), 6.983 (s, 1H), 4.629 (s, 2H), 4.452 (s, 2H), 2.885 (s, 3H); 13C-NMR (DMSO, 100 MHz) δ (ppm): 163.40, 162.42, 157.75, 143.39, 140.31, 139.73, 139.28, 128.38, 128.26, 127.18, 126.89, 126.69, 117.05, 95.19, 41.90, 39.45, 13.09; EI-MS (m/z): 412.2 (M+1).

**1-(6-(benzylamino)pyrimidin-4-yl)-N-(2,3-dichlorophenyl)-5-methyl-1*H*-pyrazole- 4-carboxamide (5e)**

White solid; ; 1H-NMR (DMSO-d6, 400 MHz) δ (ppm): 9.181 (s, 1H), 8.460 (s, 1H), 8.332 (s, 2H), 7.741 (d, J= 7.6 Hz, 1H), 7.357 – 7.261 (m, 4H), 7.158 (d, J= 7.2 Hz, 1H), 7.092–6.938 (m, 4H), 4.635 (s, 2H), 3.839 (s, 3H), 2.897 (s, 3H); 13C-NMR (DMSO, 100 MHz) δ (ppm): 163.94, 161.05, 157.77, 151.33, 143.77, 140.60, 139.29, 128.38, 126.57, 120.10, 117.40, 111.28, 95.39, 55.63, 39.58, 13.12; EI-MS (m/z): 415.6 (M+1).

**1-(6-(benzylamino)pyrimidin-4-yl)-5-methyl-N-(p-tolyl)-1*H*-pyrazole-4- carboxamide (5f)**

Pale yellow solid; 1H-NMR (DMSO-d6, 400 MHz) δ (ppm): 9.181 (s, 1H), 8.520 (s, 1H), 8.233 (s, 2H), 7.754 (d, J= 7.6 Hz, 1H), 7.351–7.254 (m, 4H), 7.152 (d, J= 7.2 Hz, 1H), 6.925 (m, 4H), 4.637 (s, 2H), 2.897 (s, 3H), 2.839 (s, 3H); 13C-NMR (DMSO, 100 MHz) δ (ppm): 163.94, 161.05, 157.77, 151.33, 143.77, 140.60, 139.29, 128.38, 126.57, 120.10, 117.40, 111.28, 95.39, 41.63, 39.58, 13.12; EI-MS (m/z): 399.6 (M+1).

**1-(6-(benzylamino)pyrimidin-4-yl)-N-(4-methoxyphenyl)-5-methyl-1*H*-pyrazole-4-carboxamide (5g)**

$^{1}$H-NMR (DMSO-d6, 400 MHz) δ (ppm): 9.936 (s, 1H), 8.463 (s, 1H), 8.330 (s,1H), 8.286 (s, 1H), 7.707-7.738 (m, 10H), 7.018 (s, 1H), 4.636 (s, 2H), 2.90 (s, 3H); 13C-NMR (DMSO, 100 MHz) δ (ppm): 164.61, 160.23, 157.79, 143.90, 140.60, 139.24, 139.01, 128.56,128.39, 127.24, 126.90, 123.41, 120.14, 117.54, 40.45, 19.16; EI-MS (m/z): 414.1 (M+1).Table-1 Physical data of the product 5(a-g)

| SR.NO. | COMPOUNDS | M.P. | % YIELD | Rf |
|---|---|---|---|---|
| 1 | 5a | 204-206 | 92 | 0.4 |
| 2 | 5b | 174-176 | 90 | 0.4 |
| 3 | 5c | 200-202 | 89 | 0.3 |
| 4 | 5d | 204-206 | 86 | 0.5 |
| 5 | 5e | 178-180 | 87 | 0.2 |
| 6 | 5f | 206-208 | 86 | 0.3 |
| 7 | 5g | 214-216 | 91 | 0.6 |

**Antimicrobial activity**

The analysis of antimicrobial activity data **table 2**, we clinch that some compounds showed good to remarkable antibacterial and antifungal activity against the illustrative specie compared to standard drugs.Against Gram-positive bacteria *B. subtilis*, compound **5b** (MIC=100 µg/mL) were shown potent as compare to ampicillin (MIC=100 µg/mL). Against *C. tetani*, compound **5b** (MIC=50 µg/mL) prompted outstanding activity as compare to ampicillin (MIC=250 µg/mL), ciprofloxacin (MIC=100 µg/mL) and comparable activity to that norfloxacin. Against *S. aureus*, compound **5g** (MIC=100 µg/mL) has shown supplementary potent as compare to ampicillin (MIC=100 µg/mL). Against Gram-negative bacteria *E. coli*, compound **5d** (MIC=100 µg/mL) have shown equal activity as compare to ampicillin (MIC=100 µg/mL). Against *S. typhi*, compounds **5g** (MIC=100 µg/mL) were found equipotent to ampicillin (MIC=100 µg/mL). It has remained observed that against *C. albicans*, compounds **5g** (MIC=250 µg/mL) found very good activity as compare to griseofulvin (MIC=500 µg/mL). **Table 2 *In vitro* antimicrobial activities of 5(a-g) MICs (µg/mL).**

| Compd. | Gram-positive bacteria | | | Gram-negative bacteria | | | Fungi | |
|---|---|---|---|---|---|---|---|---|
| | BS MTC C 441 | CT MTC C 449 | SA MTC C 96 | EC MTC C 443 | ST MTC C 98 | VC MTC C 3906 | CA MTC C 227 | TR MTC C 296 |
| 5a | 250 | 250 | 500 | 200 | 200 | 250 | >1000 | >1000 |
| 5b | **100** | **50** | 500 | 500 | 500 | **100** | 500 | 500 |
| 5c | 250 | 500 | 500 | 250 | 200 | 250 | 1000 | 1000 |
| 5d | 250 | 500 | 250 | **100** | 125 | 250 | 1000 | 250 |
| 5e | 200 | **200** | 500 | 200 | 250 | **200** | 500 | 1000 |
| 5f | 250 | **100** | 250 | 500 | 500 | 250 | 1000 | >1000 |
| 5g | 200 | 250 | **100** | 125 | **100** | 250 | **250** | 1000 |
| A | 100 | 250 | 100 | 100 | 100 | 250 | - | - |
| B | 50 | 50 | 50 | 50 | 50 | 50 | - | - |
| C | 50 | 100 | 25 | 25 | 25 | 50 | - | - |
| D | 10 | 50 | 10 | 10 | 10 | 100 | - | - |
| E | - | - | - | - | - | - | 100 | 100 |
| F | - | - | - | - | - | - | 500 | 100 |

BS: *Bacillus subtilis*; CT: *Clostridium tetani*; SA: *Staphylococcus aureus*; EC: *Escherichia coli*; ST: *Salmonella typhi*; VC: *Vibrio cholerae*; CA: *Candida albicans*; TR: *Trichophyton rubrum*. MTCC: Microbial Type Culture Collection. A: Ampicillin, B: Chloramphenicol, C: Ciprofloxacin, D: Norfloxacin, E: Nystatin, F: Griseofulvin.

**Antituberculosis Activity**

**Table 3: *In vitro* antituberculosis activity (% Inhibition) of 5(a-f) against *M. tuberculosis* H37Rv (at concentration 250 µg/mL).**

| Compounds | % Inhibition |
|---|---|
| 5a | **96** |
| 5b | **95** |
| 5c | 79 |
| 5d | 67 |
| 5e | 88 |
| 5f | 65 |
| Rifampicin | 97 |
| Isoniazid | 99 |

The preliminary showing of the title compounds for their *in vitro* antituberculosis activity against *M. Tuberculosis H37Rv* bacteria. The bioassay results reached for the usefulness of all the synthesized analogues against *M. tuberculosis* H37Rv is précised in **table 3**Compound **5a** have shown highest potency as compare to Rifampicin against *M. tuberculosis* with 96% inhibition. Other compound **5b** showed against *M. tuberculosis* with 9% at 250 µg/mL respectively inhibition.Compounds **5a** (MIC = 100 µg/mL)**, 5b** (MIC = 50 µg/mL)**, 5g** (MIC = 62.5 µg/mL) exhibited moderate potent as compared to rifampicin (MIC = 40 µg/mL) (**table 4**).

**Table 4: *In vitro* antituberculosis activity of 5a, 5b, 5g compounds exhibiting higher % inhibition against *M. tuberculosis* H37Rv (MICs, µg/mL).**

| Compounds | % Inhibition | MIC(µg/mL) |
|---|---|---|
| 5a | 96 | 100 |
| 5b | 95 | 50 |
| 5g | 94 | 62.5 |
| Rifampicin | 97 | 40 |
| Isoniazid | 99 | 0.20 |

**CONCLUSION**

An efficient method for preparing carboxamide derivatives of pyarazole was described and the structure of synthesized targeted molecules determine by IR, $H^1$ NMR and Mass spectra

and evaluate their antimicrobial activity.

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