Journal of Applied Pharmaceutical Science Vol. 13(05), pp 073-080, May, 2023
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2023.119430
ISSN 2231-3354

Antimicrobial and antitubercular activity of novel pyrazole-4-carboxamide derivatives: Synthesis and characterization

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ARTICLE INFO

Received on: 06/10/2022
Accepted on: 29/01/2023
Available Online: 04/05/2023

Key words:
Pyrazole, carboxamide, antibacterial, antifungal, antitubercular.

ABSTRACT

The current research aims to identify the newest class of antifungal, antibacterial, and antitubercular lead compounds. Through the use of a carboxamide linkage, recent research has designed and synthesized a unique class of pyrazole-based molecular hybrids of aryl amines. Using a multistep method, the desired pyrazole carboxamide derivative was prepared. Compounds were characterized using ^{1}HNMR, C^{13} NMR, and MASS spectral techniques. These substances were tested for their ability as antibacterial, antifungal, and antitubercular agents. All the compounds tested against Gram-positive and Gram-negative pathogens and fungal strains showed good antibacterial activity. Against Gram-positive pathogens, compound 5i showed potent activity, compound 5k demonstrated potent activity against Gram-negative strains, and compounds 5a, 5i, and 5j established potent activity against fungal strains and the *Mycobacterium tuberculosis* H37Rv strain.

INTRODUCTION

Over the past two decades, microbial infections have become a major cause of morbidity and a frequent immune system suppressor. Microbes cause numerous poisonous syndromes and common epidemics in human civilizations. In recent years, microbial diseases including tuberculosis (TB), pneumonia, cholera, typhoid, diphtheria, and plague have claimed a heavy toll on humanity. Because they are bacteriostatic and not bactericides, many of the readily available antimicrobial medications today are toxic and cause disease recurrence. Due to the prolonged administration periods, they may also cause resistance to increase (Bhatt and Sharma, 2013).

Of all the infectious diseases, TB causes the highest number of deaths each year. The number of people who have an *Mycobacterium tuberculosis* (MTB) infection that is latent is also estimated to be around 1.7 billion. These people have no symptoms and are not contagious, but they are at risk of developing an infection at some point in their life. A 2-month intense phase of pyrazinamide, ethambutol, rifampicin, and isoniazid, the administration is followed by a 4-month continuation phase of rifampicin, and isoniazid administration is the current TB therapy regimen. The formation of drug-resistant (DR-) strains is influenced by several variables, including the administration of suboptimal drug concentration, inadequate tolerability, and poor patient compliance. It is believed that 3.5% of cases of TB that have just been identified and 18% of TB that have already been treated have MDR-TB, characterized by resistance to both RIF and INH. 8.5% of MDR-TB cases are classified as extensively DR TB (XDR-TB), which is defined as having resistance to RIF and INH in addition to at least one fluoroquinolone and a second-line injectable medication. Drug-susceptible TB, XDR-TB, and MDR-TB patients have relative cure rates of 82%, 34%, and 55%. Therefore, it is essential to create shorter, better-tolerated medication regimes to eradicate DS- and DR-TB completely (Arora *et al*., 2020).

Structure-based drug design is a technique that makes use of computational chemistry techniques to find or create new chemical compounds that potentially bind to a target and inhibit that target protein. Molecular docking is the term used to describe

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the computational process of making molecules "fit" the binding site. This determines how a compound will conform and orient at the desired binding site (SBDD, 2022).

Recently, heterocycles containing nitrogen atoms in their structural motif, particularly pyrazoles and their derivatives, have attracted the attention of researchers. These agents are effective against numerous microbial infections and resistance developed by microorganisms. Pyrazoles have numerous biological activities such as antioxidant (Ambethkar *et al*., 2015; Gressler *et al*., 2010; Mardiana *et al*., 2017), anticancer (Inceler *et al*., 2013; Nitulescu *et al*., 2010; Prasad *et al*., 2013; Rai *et al*., 2015), antimicrobial (Chandna *et al*., 2014; Thumar and Patel, 2012; Vijesh *et al*., 2011), cyclin-dependent kinase inhibitor (Sun *et al*., 2013), tissue non-specific alkaline phosphatase inhibitor (Sidique *et al*., 2009), antiproliferative (Huang *et al*., 2012), antihepatotoxicity (Khalilullah *et al*., 2011; Khan *et al*., 2006), antileishmanial (Dardari *et al*., 2006; Dos Santos *et al*., 2011a, 2011b), antiinflammatory (Kendre *et al*., 2013; Malladi *et al*., 2012; Tewari *et al*., 2014), monoamine oxidase inhibitor (Chimenti *et al*., 2010; Peyssonnaux and Eychène, 2001), antitubercular (Khunt *et al*., 2012; Pathak *et al*., 2012), anticonvulsant (Abdel-Aziz *et al*., 2009; Kaushik *et al*., 2010), and analgesic (Vijesh *et al*., 2013).

Given the above-mentioned pharmacological importance, we have designed target compounds. Several species of bacteria, including two Gram-positive, two Gram-negative, and one mycobacterium as well as one fungus, were screened against the newly synthesized hetero cyclic derivatives.

MATERIALS AND METHODS

General

All laboratory-grade chemicals and reagents were procured from commercial suppliers and used without additional purification. Remi Electrothermal capillary melting point apparatus employed for the melting point determination. Mass spectra were recorded on a Shimadzu MS-QP2010 Ultra apparatus. ^{1}H and ^{13}CNMR spectra were acquired from the Bruker spectrophotometer model ultra-shield.

Chemistry

The scheme for the synthesis of the designed pyrazole-4-carboxamide derivative was depicted in Figure 1. The detailed synthetic procedure was enumerated below:

Step-1: Synthesis of ethyl 3-hydroxy-1-phenyl-1H-pyrazole-4-carboxylate (3)

Phenylhydrazine (0.242 g, 2.24 mmol) was added drop by drop to an ethanolic solution of sodium ethoxide (0.61 g, 4.5 mmol), diethyl ethoxy methylene malonate (0.486 g, 2.25 mmol), and they were all cooled in an ice-cold water bath for 10 minutes. The mixture was agitated for 1 hour at 0°C and 2 hours at room temperature. The resultant mixture was added to a solution of 1 N hydrochloric acid. The precipitated solid was filtered out, separated by washing with water and hexane, then thoroughly dried to produce ethyl 3-hydroxy-1-phenyl-1H-pyrazole-4-carboxylate (Huang *et al*., 2017).

Step-2: 3-hydroxy-1-phenyl-1H-pyrazole-4-carbonyl chloride (4)

A mixture of **3** (0.3 g, 1.3 mmol), PCl_3 (0.35 g, 1.3 mmol), I_2 (0.03 g, 0.13 mmol), and DMF were combined in 2.0 ml of DCE and agitated at 100°C for 12 hours while in the open air. To obtain the pure chemical **4**, the contents of the RBF were evaporated, worked up with water, extracted with hexane, and purified via column (Li *et al*., 2021).

Step-3: 1H-pyrazole-4-carboxamide (5a-5m) derivatives

In the presence of an acidic environment, 1 mmol of intermediate **4** was dissolved in ethanol (10 ml), and refluxed with a variety of substituted aromatic amines (a–m) (1 mmol, 1 equivalent). Reaction progress was monitored by TLC and after complete reaction, the crude mass was evaporated under vacuum and worked up with sodium bicarbonate solution followed by brine solution. Further, the pure compound was isolated by column chromatography with a 10% ethyl acetate–hexane solvent system to produce the 1*H*-pyrazole-4-carboxamide (5a–5m) derivatives.

Biological activity

Antibacterial activity

All of the bacterial strains utilized in this experiment were obtained from Osmania University's Department of Microbiology and kept at 4°C. The designed hybrids (5a–5m) were tested for antimicrobial activity against Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) using the disc-diffusion method. The reference antibiotic was Neomycin sulfate (10 µg/ml) in DMSO. The microorganisms were produced by inoculating 0.5 ml of spore suspension (108 spores/ml) culture broth into nutrient agar medium in pre-sterilized Petri dishes. DMSO was used to prepare a stock solution for each of the synthesized compounds (5a–5m). The Petri dishes were seeded with nutrient agar medium, the disc (6 mm in diameter) was filled with 60 µg/ml of each test solution, and the Petri dishes were incubated at 37°C for 24 hours. At the equal preceding concentration, DMF alone was utilized as the control. Each compound's zone of inhibition was measured in millimeters. There were three duplicates of the experiment (Ericsson and Sherris, 1971; Jorgensen *et al*., 1999).

Antifungal activity

The Osmania University's Department of Microbiology provided the fungal strains, which were stored at 4°C. The disc-diffusion method was used to assess the synthesized derivatives' antifungal efficacy against fungus strains (*Candid albicans* and *Aspergillus niger*). The reference antibiotic used was Nystatin (10 µg/ml in DMSO). In pre-sterilized Petri plates, potato dextrose agar medium was placed, and microorganisms were cultured by inoculating the standard suspension of culture broth. DMSO was used to prepare a stock solution for each of the produced compounds (5a–5m). The Petri dishes were incubated at 28°C for 48 hours with the discs (6 mm in diameter) packed with 60 µg/ml of each test solution. The discs were then placed on the seeded potato dextrose agar medium. At the equal preceding concentration, DMF alone was utilized as the control. Each compound's zone of inhibition was measured in millimeters. There were three duplicates of the experiment (NCCLS, 1992).

Antitubercular assay

Test organisms

MTB H 37Rv (ATCC 27294) strains, which are susceptible to isoniazid, were used to assess the antitubercular activity of the synthesized compounds.

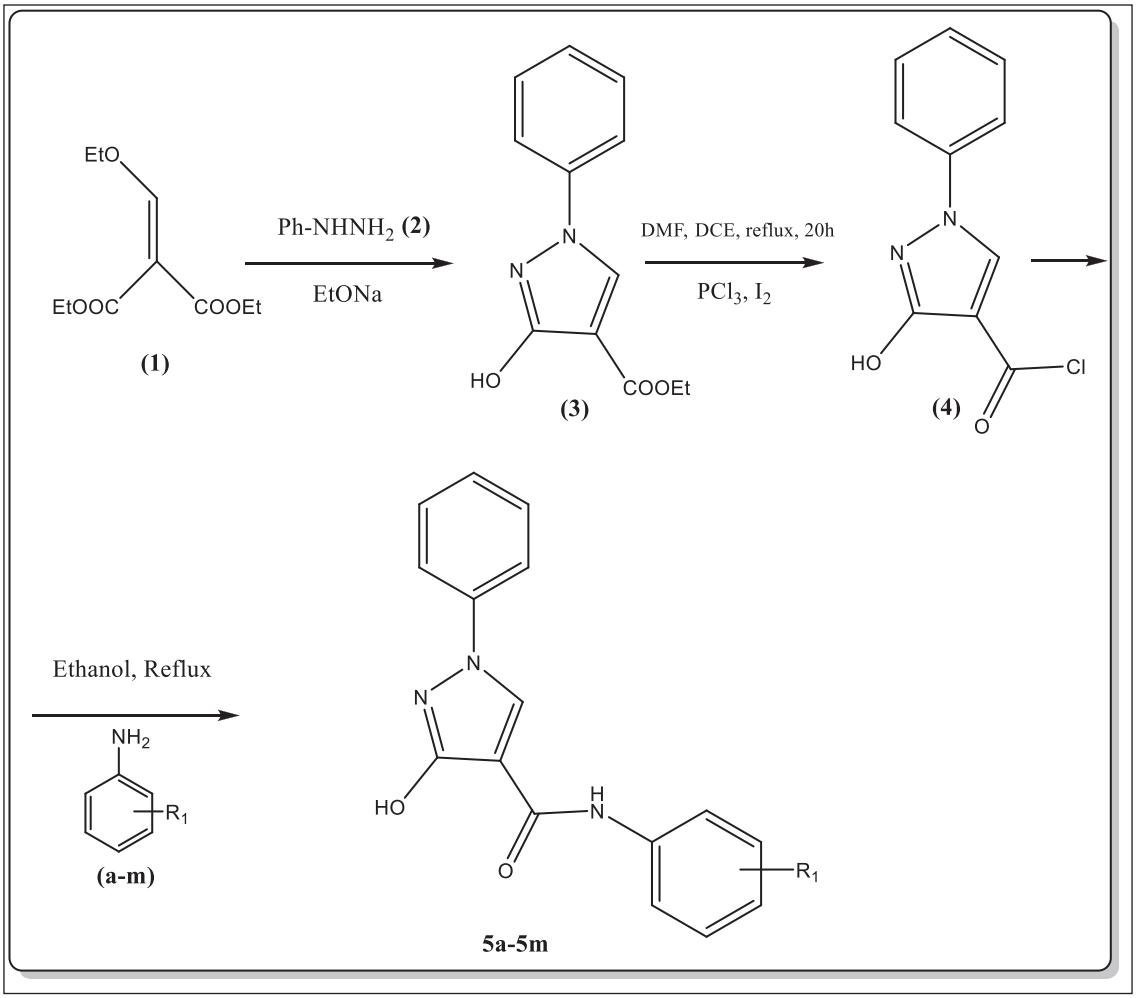

Figure 1. Synthesis of pyrazole-4-carboxamide derivatives.

Preparation of inoculum

To have a fresh batch for the study, the bacterial strains were subcultured and given Muller Hinton broth for 2 weeks at 37°C. By dilution with normal saline solution, bacterial suspensions with 0.5 McFarland standard turbidity, equivalent to 108 CFU, were prepared. In a glass vessel, the liquid was vortexed for 30 seconds, and the particles were allowed to settle [46]. For the inoculation, 100 µl of the microbial suspension was employed.

Preparation of test samples

Stock solutions of the synthesized compounds at a concentration of 100 µg/ml were prepared in DMSO. Title compounds were serially diluted from their corresponding stock solutions to determine the minimal inhibitory concentration for each compound (50, 25, 12.5, 6.25, 3.12, 1.6, and 0.8 µg/ml).

Preparation of growth medium and screening of antitubercular activity screening

After being sterilized using an autoclave at 121°C for 15 minutes, the mycobacterium was grown on Middlebrook 7H11 agar medium with Oleic Albumin Dextrose Catalase. The medium was then diluted with various concentrations of synthesized (5a–o) compounds at different strengths (50, 25, 12.5, 6.25, 3.12, 1.6, and 0.8 µg /ml). Allowed solidify under laminar airflow with the lids slightly open, 5 ml of middle brook 7H11 agar medium was poured into each of the designated quadrants of sterile quad-plates using an aseptic technique.

After solidification, bacterial suspension from the culture broth was inoculated aseptically through a loop (3 mm internal diameter) and cultured for 21 days at 37°C. By counting the colonies that formed on the medium and comparing them with the controls, the minimum inhibitory concentration (MIC) was determined. As negative and positive controls, respectively, DMSO, isoniazid, and pyrazinamide were used (Alqahtani and Asaad, 2014).

RESULTS AND DISCUSSION

Chemistry

All the pyrazole-4-carboxamide derivatives were synthesized in moderate yields from the designed scheme of synthesis. The chemical shift values of each compound determined the structure of the compounds in ^{1}H NMR and ^{13}C NMR. The characteristic pyrazole ring hydrogen chemical shift values appeared around 8.3–8.4 ppm in all compounds. The carboxamide bond linkage C13 chemical shifts were observed in the region of 160–170 ppm in all the synthesized compounds. Further, the

mass spectra of each compound agree with the respective m/z values confirming the formation of the pyrazole-4-carboxamide derivatives. The structural details, percentage yield, and melting points of the synthesized pyrazole-4-carboxamide derivatives were enumerated in Table 1.

The spectral data of the synthesized pyrazole-4-carboxamide derivatives were given below:

Compound 5a: 3-hydroxy-N,1-diphenyl-1H-pyrazole-4-carboxamide

Pale yellow color solid,**^{1}H NMR(500 MHz, Chloroform-d):** δ 7.07 (1H, tt, *J* = 7.8, 1.2 Hz), 7.18-7.34 (3H, 7.24 (tt, *J* = 7.4, 1.2 Hz), 7.27 (dddd, *J* = 8.2, 7.8, 1.4, 0.5 Hz)), 7.40-7.55 (4H, 7.47 (dddd, *J* = 8.1, 7.4, 1.5, 0.5 Hz), 7.48 (dddd, *J* = 8.2, 1.5, 1.2, 0.5 Hz)), 7.88 (2H, dtd, *J* = 8.1, 1.1, 0.5 Hz), 8.41 (1H, s).**^{13}C NMR:** δ 119.9 (2C, s), 122.8 (2C, s), 127.3 (1C, s), 127.5 (1C, s), 127.8-127.8 (2C, 127.8 (s), 127.8 (s)), 128.1-128.3 (4C, 128.2 (s), 128.2 (s)), 137.4 (1C, s), 139.7 (1C, s), 158.3 (1C, s), 163.2 (1C, s). **ESI-MS:** m/z Anal. Calcd. For $C_{16}H_{13}N_3O_2$ ([M + H]$^+$): 279.30, found 280.25.

Compound 5b: N-(4-chlorophenyl)-3-hydroxy-1-phenyl-1H-pyrazole-4-carboxamide

Pale yellow color solid, **^{1}H NMR(500 MHz, Chloroform-d):** δ 7.24 (1H, tt, *J* = 7.4, 1.2 Hz), 7.35-7.54 (4H, 7.42 (ddd, *J* = 8.1, 1.6, 0.5 Hz), 7.47 (dddd, *J* = 8.1, 7.4, 1.5, 0.5 Hz)), 7.75 (2H, ddd, *J* = 8.1, 1.5, 0.5 Hz), 7.88 (2H, dtd, *J* = 8.1, 1.1, 0.5 Hz), 8.41 (1H, s).**^{13}C NMR:** δ 120.5 (2C, s), 122.8 (2C, s), 127.3 (1C, s), 127.5 (1C, s), 127.8 (1C, s), 128.2 (2C, s), 128.9 (2C, s), 133.7 (1C, s), 137.4 (1C, s), 139.7 (1C, s), 158.3 (1C, s), 163.2 (1C, s). **ESI-MS:** m/z Anal. Calcd. For $C_{16}H_{12}ClN_3O_2$ ([M + H]$^+$): 313.74, found 314.65.

Compound 5c: N-(3-chlorophenyl)-3-hydroxy-1-phenyl-1H-pyrazole-4-carboxamide

Pale yellow color solid, **^{1}H NMR(500 MHz, Chloroform-d):** δ 7.09-7.65 (6H, 7.15 (dt, *J* = 8.1, 1.7 Hz), 7.24 (tt, *J* = 7.4, 1.2 Hz), 7.35 (td, *J* = 8.1, 0.5 Hz), 7.47 (dddd, *J* = 8.1, 7.4, 1.5, 0.5 Hz), 7.59 (dt, *J* = 8.2, 1.7 Hz)), 7.73-7.95 (3H, 7.78 (td, *J* = 1.7, 0.5 Hz), 7.88 (dtd, *J* = 8.1, 1.1, 0.5 Hz)), 8.41 (1H, s).**^{13}C NMR:** δ 119.9 (1C, s), 120.2 (1C, s), 122.8 (2C, s), 127.0 (1C, s), 127.3 (1C, s), 127.5 (1C, s), 127.8 (1C, s), 128.2 (2C, s), 130.0 (1C, s), 132.3 (1C, s), 138.2 (1C, s), 139.7 (1C, s), 158.3 (1C, s), 163.2 (1C, s). **ESI-MS:** m/z Anal. Calcd. For $C_{16}H_{12}ClN_3O_2$ ([M + H]$^+$): 313.74, found 314.65.

Table 1. Structural and physical data of pyrazole-4-carboxamide derivatives.

Compound*	R_1	Melting point (°C)	Yield
5a	-H	184–185	64
5b	-4-Cl	221–223	65
5c	-3-Cl	208–209	69
5d	-2-Cl	199–200	69
5e	-4-NO_2	239–241	70
5f	-3-NO_2	227–228	73
5g	-4-CH_3	253–254	63
5h	-3-CH_3	258–259	61
5i	-3,5- dimethyl	266–268	54
5j	-4-OCH_3	234–235	70
5k	-3-OCH_3	243–244	67
5l	-3,5- dimethoxy	259–260	57
5m	-4-ethyl	267–268	68

*compound refers to the chemical structures mentioned in figure 1

Compound 5d: N-(2-chlorophenyl)-3-hydroxy-1-phenyl-1H-pyrazole-4-carboxamide

Pale yellow color solid, **^{1}H NMR(500 MHz, Chloroform-d):** δ 7.14-7.36 (3H, 7.22 (ddd, *J* = 8.0, 7.7, 1.4 Hz), 7.24 (tt, *J* = 7.4, 1.2 Hz), 7.29 (ddd, *J* = 8.0, 7.7, 1.6 Hz)), 7.39-7.54 (3H, 7.46 (ddd, *J* = 8.0, 1.6, 0.5 Hz), 7.47 (dddd, *J* = 8.1, 7.4, 1.5, 0.5 Hz)), 7.72-7.95 (3H, 7.78 (ddd, *J* = 8.0, 1.4, 0.5 Hz), 7.88 (dtd, *J* = 8.1, 1.1, 0.5 Hz)), 8.42 (1H, s).**^{13}C NMR:** δ 121.8 (1C, s), 122.7-122.8 (3C, 122.8 (s), 122.8 (s)), 127.3 (1C, s), 127.5 (1C, s), 127.8 (1C, s), 128.1-128.3 (4C, 128.2 (s), 128.2 (s), 128.3 (s)), 129.2 (1C, s), 134.6 (1C, s), 139.7 (1C, s), 158.3 (1C, s), 163.2 (1C, s). **ESI-MS:** m/z Anal. Calcd. For $C_{16}H_{12}ClN_3O_2$ ([M + H]$^+$): 313.74, found 314.60.

Compound 5e: 3-hydroxy-N-(4-nitrophenyl)-1-phenyl-1H-pyrazole-4-carboxamide

Light brown color solid, **^{1}H NMR(500 MHz, Chloroform-d):** δ 7.18-7.54 (5H, 7.24 (tt, *J* = 7.4, 1.2 Hz), 7.35 (ddd, *J* = 8.7, 2.3, 0.4 Hz), 7.47 (dddd, *J* = 8.1, 7.4, 1.5, 0.5 Hz)), 7.88 (2H, dtd, *J* = 8.1, 1.1, 0.5 Hz), 8.13 (2H, ddd, *J* = 8.7, 1.8, 0.4 Hz), 8.43 (1H, s).**^{13}C NMR:** δ 116.6 (2C, s), 122.8 (2C, s), 125.0 (2C, s), 127.3 (1C, s), 127.5 (1C, s), 127.8 (1C, s), 128.2 (2C, s), 137.4 (1C, s), 139.7 (1C, s), 147.3 (1C, s), 158.3 (1C, s), 163.2 (1C, s). **ESI-MS:** m/z Anal. Calcd. For $C_{16}H_{12}N_4O_4$ ([M + H]$^+$): 324.30, found 325.20.

Compound 5f: 3-hydroxy-N-(3-nitrophenyl)-1-phenyl-1H-pyrazole-4-carboxamide

Light browncolor solid, **^{1}H NMR(500 MHz, Chloroform-d):** δ 7.24 (1H, tt, *J* = 7.4, 1.2 Hz), 7.35-7.54 (4H, 7.42 (dt, *J* = 8.2, 1.5 Hz), 7.45 (ddd, *J* = 8.4, 8.2, 0.5 Hz), 7.47 (dddd, *J* = 8.1, 7.4, 1.5, 0.5 Hz)), 7.61 (1H, ddd, *J* = 8.4, 1.7, 1.6 Hz), 7.81-7.95 (3H, 7.87 (ddd, *J* = 1.7, 1.5, 0.5 Hz), 7.88 (dtd, *J* = 8.1, 1.1, 0.5 Hz)), 8.41 (1H, s).**^{13}C NMR:** δ 112.0 (1C, s), 119.9 (1C, s), 122.8 (2C, s), 123.3 (1C, s), 127.3 (1C, s), 127.5 (1C, s), 127.8 (1C, s), 128.2 (2C, s), 129.6 (1C, s), 137.5 (1C, s), 139.7 (1C, s), 143.9 (1C, s), 158.3 (1C, s), 163.2 (1C, s). **ESI-MS:** m/z Anal. Calcd. For $C_{16}H_{12}N_4O_4$ ([M + H]$^+$): 324.30, found 325.25.

Compound 5g: 3-hydroxy-1-phenyl-N-(p-tolyl)-1H-pyrazole-4-carboxamide

Pale yellow color solid, **^{1}H NMR(500 MHz, Chloroform-d):** δ 2.21 (3H, s), 7.02-7.16 (4H, 7.08 (ddd, *J* = 8.1, 1.6, 0.5 Hz), 7.09 (ddd, *J* = 8.1, 1.4, 0.5 Hz)), 7.24 (1H, tt, *J* = 7.4, 1.2 Hz), 7.47 (2H, dddd, *J* = 8.1, 7.4, 1.5, 0.5 Hz), 7.88 (2H, dtd, *J* = 8.1, 1.1, 0.5 Hz), 8.41 (1H, s).**^{13}C NMR:** δ 21.3 (1C, s), 117.9 (2C, s), 122.8 (2C, s), 127.3 (1C, s), 127.5 (1C, s), 127.8 (1C, s), 128.2 (2C, s), 129.6 (2C, s), 137.4 (1C, s), 139.7 (1C, s), 141.5 (1C, s), 158.3 (1C, s), 163.2 (1C, s). **ESI-MS:** m/z Anal. Calcd. For $C_{17}H_{15}N_3O_2$ ([M + H]$^+$): 293.33, found 294.25.

Compound 5h: 3-hydroxy-1-phenyl-N-(m-tolyl)-1H-pyrazole-4-carboxamide

Pale yellow color solid, **^{1}H NMR(500 MHz, Chloroform-d):** δ 2.30 (3H, s), 6.90 (1H, ddd, *J* = 8.0, 1.8, 1.6 Hz), 7.15-7.30 (3H, 7.22 (ddd, *J* = 8.2, 8.0, 0.5 Hz), 7.22 (ddd, *J* = 1.6, 1.4, 0.5 Hz), 7.24 (tt, *J* = 7.4, 1.2 Hz)), 7.40-7.59 (3H, 7.47 (dddd, *J* = 8.1, 7.4, 1.5, 0.5 Hz), 7.53 (ddd, *J* = 8.2, 1.8, 1.4 Hz)), 7.88 (2H, dtd, *J* = 8.1, 1.1, 0.5 Hz), 8.42 (1H, s).**^{13}C NMR:** δ 21.3 (1C, s), 118.6 (1C, s), 119.9 (1C, s), 122.8 (2C, s), 127.3 (1C, s), 127.5 (1C, s), 127.8 (1C, s), 128.1-128.3 (3C, 128.1 (s), 128.2 (s)), 129.0 (1C, s), 133.7 (1C, s), 138.4 (1C, s), 139.7 (1C, s), 158.3 (1C, s), 163.2 (1C, s). **ESI-MS:** m/z Anal. Calcd. For $C_{17}H_{15}N_3O_2$ ([M + H]$^+$): 293.33, found 294.25.

Compound 5i: N-(3,5-dimethylphenyl)-3-hydroxy-1-phenyl-1H-pyrazole-4-carboxamide

Pale yellow color solid, **^{1}H NMR(500 MHz, Chloroform-d):** δ 2.31 (6H, s), 6.74-6.89 (3H, 6.80 (dd, *J* = 2.6, 1.3 Hz), 6.84 (t, *J* = 2.6 Hz)), 7.24 (1H, tt, *J* = 7.4, 1.2 Hz), 7.47 (2H, dddd, *J* = 8.1, 7.4, 1.5, 0.5 Hz), 7.88 (2H, dtd, *J* = 8.1, 1.1, 0.5 Hz), 8.42 (1H, s).**^{13}C NMR:** δ 21.3 (2C, s), 118.6 (2C, s), 122.8 (2C, s), 127.3 (1C, s), 127.5 (1C, s), 127.7-127.8 (2C, 127.7 (s), 127.8 (s)), 128.2 (2C, s), 138.3-138.5 (3C, 138.4 (s), 138.5 (s)), 139.7 (1C, s), 158.3 (1C, s), 163.2 (1C, s). **ESI-MS:** m/z Anal. Calcd. For $C_{18}H_{17}N_3O_2$ ([M + H]$^+$): 307.35, found 308.25.

Compound 5j: 3-hydroxy-N-(4-methoxyphenyl)-1-phenyl-1H-pyrazole-4-carboxamide

Pale yellow color solid, **^{1}H NMR(500 MHz, Chloroform-d):** δ 3.76 (3H, s), 6.64 (2H, ddd, *J* = 8.8, 2.7, 0.5 Hz), 7.18-7.39 (3H, 7.24 (tt, *J* = 7.4, 1.2 Hz), 7.33 (ddd, *J* = 8.8, 1.7, 0.5 Hz)), 7.47 (2H, dddd, *J* = 8.1, 7.4, 1.5, 0.5 Hz), 7.88 (2H, dtd, *J* = 8.1, 1.1, 0.5 Hz), 8.41 (1H, s).**^{13}C NMR:** δ 56.0 (1C, s), 114.5 (2C, s), 120.5 (2C, s), 122.8 (2C, s), 127.3 (1C, s), 127.5 (1C, s), 127.8 (1C, s), 128.2 (2C, s), 137.4 (1C, s), 139.7 (1C, s), 158.3 (1C, s), 159.8 (1C, s), 163.2 (1C, s). **ESI-MS:** m/z Anal. Calcd. For $C_{17}H_{15}N_3O_3$ ([M + H]$^+$): 309.33, found 310.15.

Compound 5k: 3-hydroxy-N-(3-methoxyphenyl)-1-phenyl-1H-pyrazole-4-carboxamide

Pale yellow color solid, **^{1}H NMR(500 MHz, Chloroform-d):** δ 3.72 (3H, s), 6.72 (1H, ddd, *J* = 8.3, 1.5, 1.3 Hz), 7.16-7.30 (2H, 7.22 (td, *J* = 8.2, 0.5 Hz), 7.24 (tt, *J* = 7.4, 1.2 Hz)), 7.33-7.54 (4H, 7.38 (td, *J* = 1.4, 0.5 Hz), 7.47 (dddd, *J* = 8.1, 7.4, 1.5, 0.5 Hz), 7.46 (dt, *J* = 8.2, 1.4 Hz)), 7.88 (2H, dtd, *J* = 8.1, 1.1, 0.5 Hz), 8.41 (1H, s).**^{13}C NMR:** δ 56.0 (1C, s), 106.4 (1C, s), 116.7 (1C, s), 119.9 (1C, s), 122.8 (2C, s), 127.3 (1C, s), 127.5 (1C, s), 127.8 (1C, s), 128.2 (2C, s), 129.7 (1C, s), 139.3 (1C, s), 139.7 (1C, s), 158.1 (1C, s), 158.3 (1C, s), 163.2 (1C, s). **ESI-MS:** m/z Anal. Calcd. For $C_{17}H_{15}N_3O_3$ ([M + H]$^+$): 309.33, found 310.20.

Compound 5l: N-(3,5-dimethoxyphenyl)-3-hydroxy-1-phenyl-1H-pyrazole-4-carboxamide

Pale yellow color solid, **^{1}H NMR(500 MHz, Chloroform-d):** δ 3.73 (6H, s), 6.16 (1H, t, *J* = 1.8 Hz), 6.41 (2H, dd, *J* = 2.1, 1.8 Hz), 7.24 (1H, tt, *J* = 7.4, 1.2 Hz), 7.47 (2H, dddd, *J* = 8.1, 7.4, 1.5, 0.5 Hz), 7.88 (2H, dtd, *J* = 8.1, 1.1, 0.5 Hz), 8.41 (1H, s).**^{13}C NMR:** δ 56.0 (2C, s), 101.7 (1C, s), 106.4 (2C, s), 122.8 (2C, s), 127.3 (1C, s), 127.5 (1C, s), 127.8 (1C, s), 128.2 (2C, s), 138.7 (1C, s), 139.7 (1C, s), 158.3 (1C, s), 161.6 (2C, s), 163.2 (1C, s). **ESI-MS:** m/z Anal. Calcd. For $C_{18}H_{17}N_3O_4$ ([M + H]$^+$): 339.35, found 340.30.

Compound 5m: N-(4-ethylphenyl)-3-hydroxy-1-phenyl-1H-pyrazole-4-carboxamide

Pale yellow color solid, **^{1}H NMR(500 MHz, Chloroform-d):** δ 1.07 (3H, t, *J* = 7.5 Hz), 2.56 (2H, q, *J* = 7.5 Hz), 7.03-7.30 (5H, 7.09 (ddd, *J* = 8.1, 1.3, 0.6 Hz), 7.17 (ddd, *J* = 8.1, 1.4, 0.6 Hz), 7.24 (tt, *J* = 7.4, 1.2 Hz)), 7.47 (2H, dddd, *J* = 8.1, 7.4, 1.5, 0.5 Hz), 7.88 (2H, dtd, *J* = 8.1, 1.1, 0.5 Hz), 8.42 (1H, s).**^{13}C NMR:** δ 14.6 (1C, s), 28.7 (1C, s), 117.9 (2C, s), 122.8 (2C, s), 127.3 (1C, s), 127.5 (1C, s), 127.8 (1C, s), 128.2 (2C, s), 129.9 (2C, s), 137.4 (1C, s), 139.7 (1C, s), 144.2 (1C, s), 158.3 (1C, s), 163.2 (1C, s). **ESI-MS:** m/z Anal. Calcd. For $C_{18}H_{17}N_3O_2$ ([M + H]$^+$): 307.35, found 308.25.

Antimicrobial activity

Antibacterial activity

The antibacterial potential of 13 synthesized novel pyrazole carboxamide derivatives (5a–5m) against 2 Gram-positive (*S. aureus* and *B. subtilis*) and 2 Gram-negative organisms (*P. aeruginosa* and *E. coli*) was examined in this study. The results of the antibacterial activity were listed in Table 2 and were expressed as the zone of inhibition (mm) of all compounds tested against Gram-negative and Gram-positive microorganisms. The antibacterial potential of all the title compounds was discovered using an *in vitro* antibacterial assay. The standard drug (Neomycin at 10 µg/ml) zone of inhibition was used to compare all compounds' potential to inhibit microbial growth.

At a concentration of 60 µg/ml, the compounds 5e, 5i, 5j, and 5k showed considerably higher inhibition of Gram-positive bacteria growth than the other compounds. All the compounds under study demonstrated greater inhibitory power against *B. subtilis* than *S. aureus* among the two Gram-positive bacterial strains. The most potent compound is 5i, which showed nearly similar antibacterial activity at a dose of 60 µg/ml when compared to the standard neomycin at a dose of 10 µg/ml.

At a concentration of 60 µg/ml the compounds 5d, 5e, 5i, 5j, and 5k showed relatively higher inhibition of Gram-negative bacteria growth than the other compounds. All the compounds under study established greater inhibitory potential against *E. coli* than *P. aeruginosa* among the two Gram-negative bacterial strains. The most potent compound is compound 5k, and when compared with standard neomycin activity at a dose of 10 µg/ml, compound 5k showed equivalent antibacterial potency at a level of 60 µg/ml.

It is clear from the findings of the *in vitro* antibacterial assay that the substituent groups on the phenyl ring system have an impact on the basic pyrazole carboxamide nucleus's ability to distinguish between Gram-positive and Gram-negative bacteria. The compounds that have electron-donating groups, like 5i (3,5-dimethyl), 5j (4-OCH_3), 5k (4-OCH_3), and compounds with strong electron-withdrawing groups, such as 5e (4-NO_2), 5d, and (2-Cl) are active against both Gram-negative and Gram-positive bacterial strains. Relatively the pyrazole-4-carboxamide derivative displayed a good tendency toward the inhibition of

Table 2. Zone of inhibition (mm) of the compounds against Gram positive and Gram-negative bacterial strains.

Compound*	Zone of inhibition of the compounds (60 µg/ml) in mm[a]			
	B. subtilis	*S. aureus*	*E. coli*	*P. aeruginosa*
5a	14	16	11	14
5b	13	14	14	14
5c	15	15	13	12
5d	18	19	17	18
5e	20	21	19	18
5f	19	17	15	17
5g	18	14	15	13
5h	14	13	11	9
5i	23	19	16	15
5j	21	18	14	12
5k	21	19	22	21
5l	14	15	10	11
5m	13	14	09	10
Solvent control (DMSO)	4	3	3	3
Neomycin sulphate (10 µg/ml)	24	23	22	20

*compound refers to the chemical structures mentioned in figure 1

Gram-positive bacteria rather than Gram-negative bacteria. This relative specificity of the pyrazole-4-carboxamides might be attributed to the better interactions of the compounds with the Gram-positive macromolecular network than the more stringent Gram-negative bacteria.

Antifungal activity

The synthesized compounds' antifungal profile was also developed against specific fungus strains (Table 3). Interestingly, compounds 5a, 5i, 5j, 5l, and 5m significantly inhibited the growth of the two tested fungi. Additionally, *A. niger* is found to be more susceptible to synthesized compounds than other strains. The most effective antifungal of all the derivatives, 5a, 5i demonstrated maximal growth inhibition with zones of inhibition of 21 and 19 mm against *A. niger* and *C. albicans* followed by 5j, 5l, and 5m. Compared to *A. niger*, all substances showed decreased sensitivity to *C. albicans* except 5j (zone of inhibition of 22 mm^3 as shown in Table 3). The outcomes show that the pyrazole carboxamide derivatives may have antifungal properties. In comparison to compounds containing electron-withdrawing groups, those containing electron-donating groups are more effective against the tested fungal strains. Furthermore, the nonpolar substituents had greater effectiveness against the fungi than the remaining substituent-containing compounds.

Table 3. Zone of inhibition (mm) of the compounds against fungal strains.

Compound*	Zone of inhibition of the compounds (60 µg/ml) in mm[a]	
	A. niger	*C. albicans*
5a	21	19
5b	13	13
5c	12	13
5d	15	14
5e	16	17
5f	16	14
5g	15	13
5h	14	16
5i	21	19
5j	20	22
5k	15	14
5l	18	16
5m	19	18
Solvent control (DMSO)	4	3
Nystatin (10 µg/ml)	24	21

*compound refers to the chemical structures mentioned in figure 1

Antitubercular activity

Using the middle brook 7H11 medium, 13 synthesized compounds (5a–5m) were tested for antitubercular activity against the MTB H 37Rv (ATCC 27294) strain at a range of doses (100, 50, 25, 12.5, 6.25, 3.12, 1.6, and 0.8 µg/ml). The antitubercular activity was enumerated as MIC and these values were compared to those of the Isoniazid and pyrazinamide reference drugs. The findings showed that just a few of the test substances had reasonably effective antitubercular activity against the MTB H 37Rv (ATCC 27294) strain, which is isoniazid sensitive. The outcomes are shown in Table 4.

Based on the observations, compounds 5e (MIC: 3.12 µg/ml) 5g and 5m (MIC: 6.25 µg/ml), and 5h (MIC: 12.5 µg/ml) exhibit significant antitubercular activity against MTB H 37Rv (ATCC 27294) strain, followed by compounds 5m, 5k, 5b,

Table 4. MIC of synthesized derivatives against MTB strain.

Compound	MIC against *MTB* H37Rv (µg/ml)
5a	50
5b	25
5c	25
5d	NA
5e	3.12
5f	25
5g	6.25
5h	12.5
5i	50
5j	NA
5k	25
5l	50
5m	6.25
Isoniazid	0.36
Pyrazinamide	25

5c, and 5f (MIC: 25 µg/ml), which exhibit moderate antitubercular activity. According to the findings, the new pyrazole carboxamide derivatives are not nearly as effective as the widely used drugs pyrazinamide and isoniazid. Except for 5j, and 5d, all the compounds have some antitubercular activity against the MTB H 37Rv (ATCC 27294) strain.

CONCLUSION

In conclusion, the designed pyrazole-4-carboxamide derivatives were synthesized through the proposed synthetic scheme in moderate to good yields. The spectral analysis of the synthesized compounds by NMR and MASS techniques determined the structure of all compounds. All the synthesized compounds were tested for their antibacterial, antifungal, and antitubercular activities. Among the tested compounds the compounds with electron-donating groups displayed noticeable inhibition of bacterial growth against both Gram-negative and Gram-positive strains as well as tested fungal strains. In the case of antitubercular assay compounds 5e, 5g, and 5m displayed a promising bacterial inhibition. Further studies, regarding the molecular mechanism of antimicrobial activity of this pyrazole-4-carboxamide, are needed, to establish the complete mechanism of action.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

FINANCIAL SUPPORT

There is no funding to report.

CONFLICT OF INTEREST

The authors disclose no conflict of interest.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

PUBLISHER'S NOTE

This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

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How to cite this article:
Asha Deepthi C, Prathyusha J. Antimicrobial and antitubercular activity of novel pyrazole-4-carboxamide derivatives: Synthesis and characterization. J Appl Pharm Sci, 2023; 13(05):073–080.