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Exploring Novel Pyrazole-Fused 1H-tetrazol-5-yl: Synthesis, SAR and Antimicrobial Efficacy

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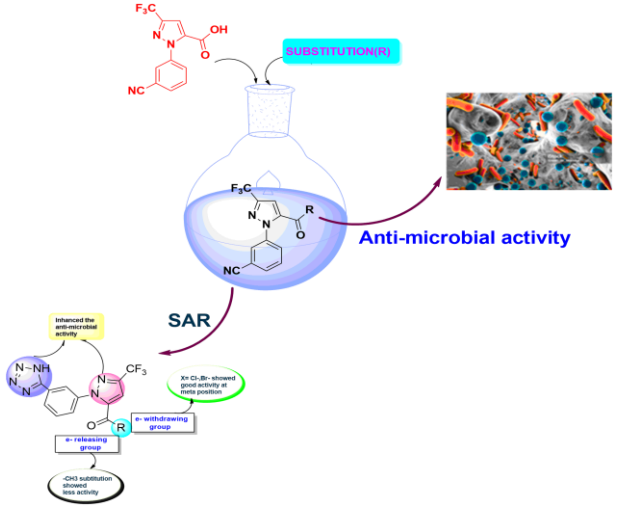
ABSTRACT

In this study, we present the synthesis, and biological activity of pyrazole-linked tetrazole derivatives (6A-6Q). All derivatives were screened for their antimicrobial activity against both gram-positive bacteria (*Staphylococcus aureus, Bacillus subtilis, Bacillus megaterium*) and gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Shigella spp.*). The compounds were synthesized through a five-step reaction process and further characterized using ^{1}H NMR and LCMS spectral techniques. The synthetic compounds 6A–6Q were evaluated for their experimental antimicrobial activity, with ampicillin used as a positive control. According to the in vitro assays, compounds 6D and 6E exhibited higher activity against all tested strains due to the presence of halogens at the meta position. Molecules 6A, 6C, 6J, 6K, and 6E demonstrated good to moderate antibacterial activity based on their structure-activity relationship (SAR) studies. Conversely, compounds 6G, 6H, 6I, and 6P showed lower inhibition due to the presence of electron-donating groups in their structures towards the tested bacteria.

Graphical Absract



INTRODUCTION

The threat human health posed by the spread of drug-resistant microorganisms is growing [1], [2]. By 2050, it is predicted that drug-resistant diseases will kill 10 million people annually if nothing is done [3]An effective tool for treating bacterial infections is an antimic robial agent [4], [5].

Due to the extraordinary capacity of the resultant compounds to copy the structure of the moieties [6] and alter their binding affinities to proteins, heterocyclic chemistry is one of the most crucial techniques for creating novel compounds with diverse biological activity [7], [8]. Numerous natural products, including nucleic acids, protines, hormones, antibiotics, terpenoids, vitamins, and alkaloids, as well as agrochemicals, dyestuffs, medicinal compounds, etc., contain heterocycle subunits [9]. An important method for finding bioactive substances with new or better pharmacological characteristics recently has been the synthesis of heterocycles [10], [11]. Particularly interesting and receiving a lot of attention from medicinal chemists are heterocyclic molecules that include nitrogen [12], [13].

Tetrazole scaffolds is an important in heterocyclic chemistry due to it's vast uses like in pharmaceutical, natural products, paints as explosives [14], [15] attracted a lot of interest as an exclusive core structure for the development of therapeutic candidates. Tetrazole are regarded as carboxylic acid group due to their same planer system and pka values [16], [17]. In medicinal chemistry Tetrazole derivative play an significant role as anti-covid [18], anti-cancer [19], [20], anti-tubercular [21], [22], anti-microbial [23], [24], anti-malarial [25], anti-diabetic [26], anti-oxidant [27], [28], anti-hypertensive [29], anti-cancer[30], [31] enzyme inhibitor [32], [33] and some other biological activity also.

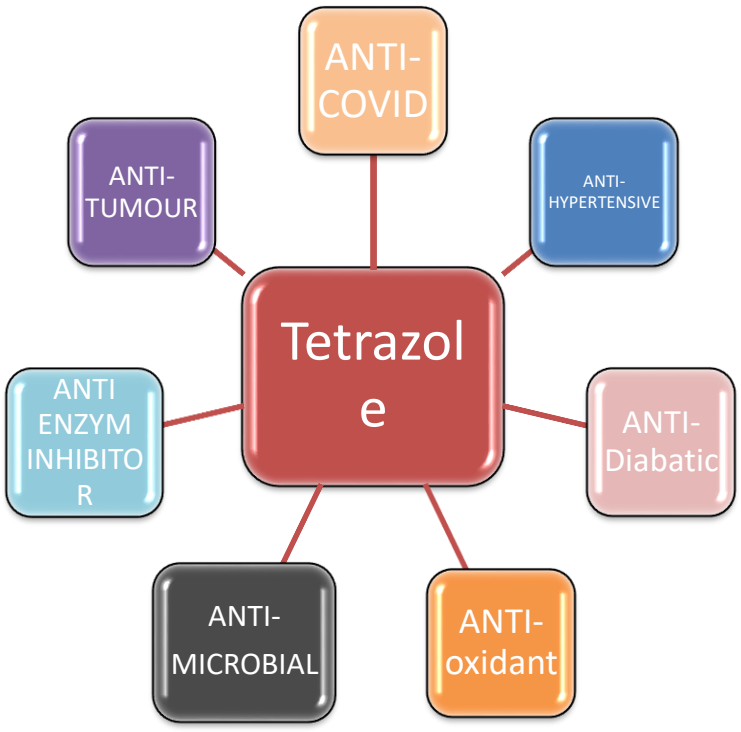


Figure: 1 Medicinal Activity for tetrazole

The last ten years have seen a dramatic increase in interest in pyrazole chemistry, mostly because of the intriguing features that have been found to be displayed by different pyrazole derivatives[34]. Mounir Cherfi and team developed pyrazole and tetrazole sub unit as antibacterial drugs in 2022 [35]. Tukaram matreand colleagues developedpyrazol containing tetrazole derivative that act as antimicrobial agents in 2024 [36].In this current work, we synthesized a pyrazole linked tetrazole derivative and allthe compounds were evaluated for their antimicrobialactivity against *Staphylococcus aureus* (Gram+), *Bacillus Subtills*(Gram+), *Bacillus megaterium*(Gram+), *Escherichia coli*(Gram-), *Pseudonymous spp.*(Gram-), *Shigella sp.* (Gram-).Our goal is to support ongoing research in creating innovative strategies for discovering and developing effective antimicrobial agents.

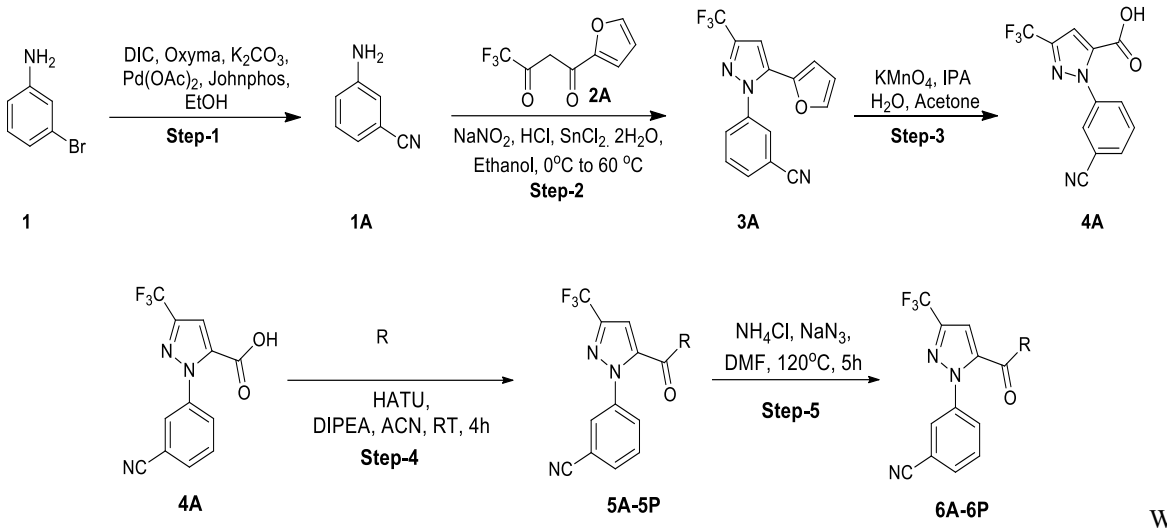
Methods and Materials

All compounds and solvents were purchased from Sigma-Aldrich and used without further purification. A gradient of MeOH in MDC was created using the elutes and employed for thin-layer chromatography (TLC) on silica gel plates (60F254, 0.2 mm thick, Merck). In this study, we used a Bruker Avance II 400 NMR spectrometer with tetra methyl silane (TMS) as the internal standard to perform 1H NMR spectra in CDCl3 solution at 400 MHz. The chemical shift values in 1H NMR are reported in parts per million (ppm).

For the LCMS equipment, which recorded data using WATERS, a mobile phase consisting of 0.15% formic acid in acetonitrile was used.

Chemistry

The intended anti-microbial active component was produced by the five-step synthesis method shown in **Scheme 1**.Commercially available 3 bromoaniline (**1**) was taken to formation of CN bond by cross coupling reaction via Buchwald-Hartwig amination reaction to prepare **1A.**Reaction between **1A** and 4,4,4-trifluoro-1-(furan-2-yl)butane-1,3-dione **(2A)** outlines the production process that yields compound **3A** via diazotization using 3-aminobenzonitrile followed by reduction, and a coupling reaction[37]. Compound **4A** is produced by Oxidation reaction of compound **3A** using $KMnO_4$[38].1-(3-cyanophenyl)-3-(trifluoromethyl)-1H-pyrazole-5-carboxylic acid (4A) treated with HATU and various aldehydes to create **5A-5Q** derivatives in the presence of DIPEA and acetonitrile via coupling reaction[39]. The required derivative (6A-6Q) was obtained by performing an Azidation process. Compound 5a-5Q was therefore administered in DMF and given NaN3 treatment.All the properties of desired derivatives shown in **Table 1.**



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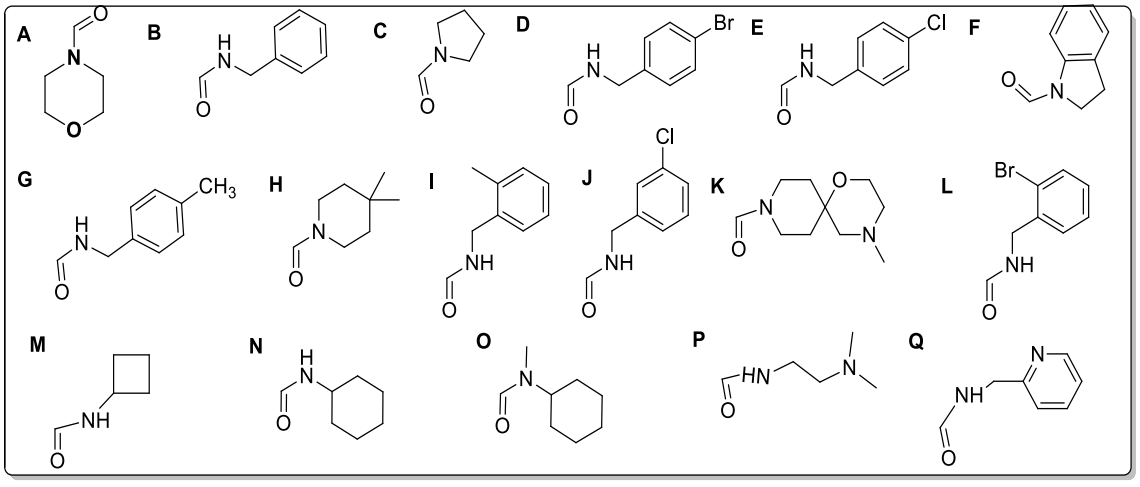
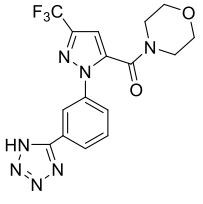
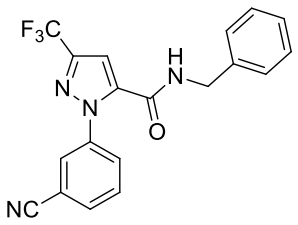
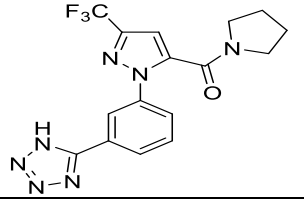
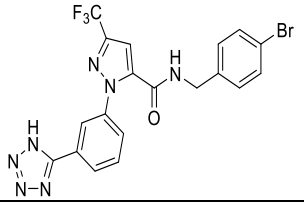
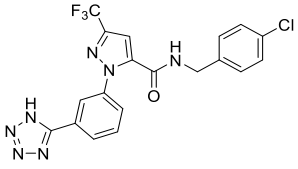
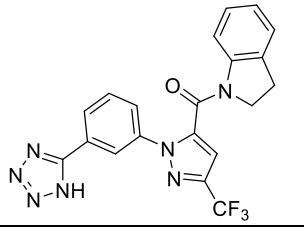
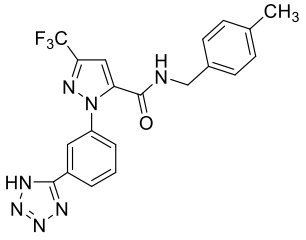
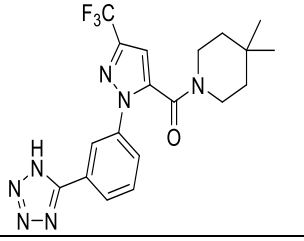
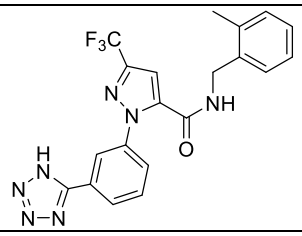
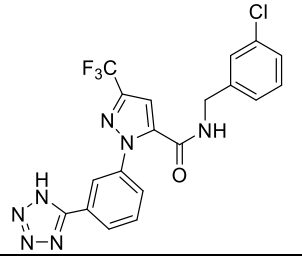
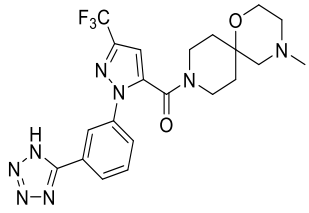
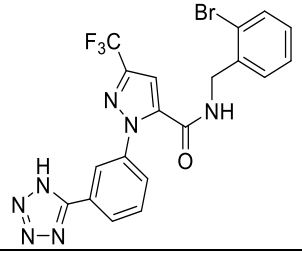
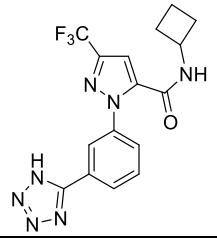
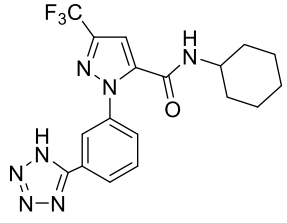
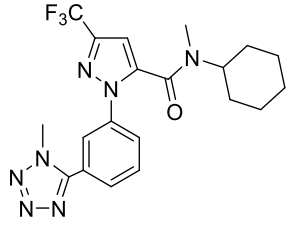


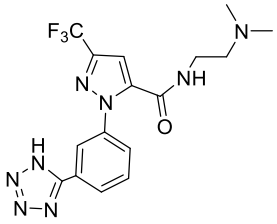
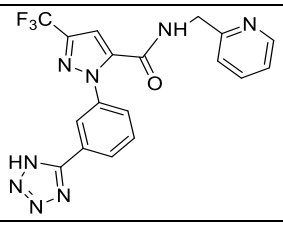
Scheme 1: **Synthetic route of pyrazole linked tetrazole derivative.**

Table 1: Properties of compounds

Compound Number	Name	Structure	Yield	M.W.
6A	(1-(3-(1H-tetrazol-5-yl)phenyl)-3-(trifluoromethyl)-1H-pyrazol-5-yl)(morpholino)methanone		58.76%.	393.33

6B	1-(3-(1H-tetrazol-5-yl)phenyl)-N-benzyl-3-(trifluoromethyl)-1H-pyrazole-5-carboxamide		21.87%.	413.36
6C	(1-(3-(1H-tetrazol-5-yl)phenyl)-3-(trifluoromethyl)-1H-pyrazol-5-yl)(pyrrolidin-1-yl)methanone		90.14%.	377.33
6D	1-(3-(1H-tetrazol-5-yl)phenyl)-N-(4-bromobenzyl)-3-(trifluoromethyl)-1H-pyrazole-5-carboxamide		71.13%.	492.26
6E	1-(3-(1H-tetrazol-5-yl)phenyl)-N-(4-chlorobenzyl)-3-(trifluoromethyl)-1H-pyrazole-5-carboxamide		93.02%.	447.81
6F	(1-(3-(1H-tetrazol-5-yl)phenyl)-3-(trifluoromethyl)-1H-pyrazol-5-yl)(indolin-1-yl)methanone		64.20%.	425.38
6G	1-(3-(1H-tetrazol-5-yl)phenyl)-N-(4-methylbenzyl)-3-(trifluoromethyl)-1H-pyrazole-5-carboxamide		89.93%.	427.39
6H	(1-(3-(1H-tetrazol-5-yl)phenyl)-3-(trifluoromethyl)-1H-pyrazol-5-yl)(4,4-dimethylpiperidin-1-yl)methanone		89.74%.	419.41

6I	1-(3-(1H-tetrazol-5-yl)phenyl)-N-(2-methylbenzyl)-3-(trifluoromethyl)-1H-pyrazole-5-carboxamide		31.73%	427.39
6J	1-(3-(1H-tetrazol-5-yl)phenyl)-N-(3-chlorobenzyl)-3-(trifluoromethyl)-1H-pyrazole-5-carboxamide		73.35%	447.81
6K	(1-(3-(1H-tetrazol-5-yl)phenyl)-3-(trifluoromethyl)-1H-pyrazol-5-yl)(4-methyl-1-oxa-4,9-diazaspiro[5.5]undecan-9-yl)methanone		31.84%.	476.46
6L	1-(3-(1H-tetrazol-5-yl)phenyl)-N-(2-bromobenzyl)-3-(trifluoromethyl)-1H-pyrazole-5-carboxamide		45.25%.	492.26
6M	1-(3-(1H-tetrazol-5-yl)phenyl)-N-cyclobutyl-3-(trifluoromethyl)-1H-pyrazole-5-carboxamide		82.27%.	377.33
6N	1-(3-(1H-tetrazol-5-yl)phenyl)-N-cyclohexyl-3-(trifluoromethyl)-1H-pyrazole-5-carboxamide		48.62%.	405.39
6O	N-cyclohexyl-N-methyl-1-(3-(1-methyl-1H-tetrazol-5-yl)phenyl)-3-(trifluoromethyl)-1H-pyrazole-5-carboxamide		12.46%.	433.44

6P	1-(3-(1H-tetrazol-5-yl)phenyl)-N-(2-(dimethylamino)ethyl)-3-(trifluoromethyl)-1H-pyrazole-5-carboxamide		12.84%.	394.36
6Q	1-(3-(1H-tetrazol-5-yl)phenyl)-N-(pyridin-2-ylmethyl)-3-(trifluoromethyl)-1H-pyrazole-5-carboxamide		41.36%.	414.35

In Vitro Assay

A widely used technique for examining in vitro anti-microbial efficacy is the cup plate method [40], [41]To investigate in-vitro anti-microbial activity, gram-positive (Staphylococcus aureus, Bacillus Subtills, Bacillus megaterium) and gram-negative (Escherichia coli, Pseudonymous, Shigella sp.) bacteria were combined with Staphylococcus aureus (Gram+). All of the bacterial cultures that were used in this method were first maintained in nutrient broth and then incubated at 37 °C for the entire night. The molten agar was incubated for one full hour to solidify and set before being transferred to the disinfected petri dishes. Using a cotton swab, the bacterial culture was uniformly swabbed onto the sterile plates.Using sterilized cork, 6mm wide bores were created on agar media. Using a sterile tip and micropipette, the test chemical solution (concentration measured at 1000 µg/ml) was poured into each bore. For the positive control ampicillin, which is used as standard, a plate was similarly produced. The prepared plates were kept at 370C for a full day of incubation. Following the incubation period, a clean zone around each bore, indicating the anti-microbial activity of the piperidine derivative under test that was related to 1,4-diazepane. The activity was reported in millimeters based on the average diameter of these obvious zones of inhibition that emerged. By applying the liquid dilution approach, a value for the MIC (minimum inhibitory concentration) was determined[42], The MIC values of all derivatives were determined for the Gram-positive Staphylococcus aureus bacteria. The compounds under investigation were diluted at a 50 mg/ml concentration in an appropriate solvent. This is comparable to a standard ampicillin solution with a 50 mg/ml concentration. The preparation of the bacterial culture inoculum was done. One milliliter of the test material at the designated concentration and 0.2 milliliters of the inoculum are added to a series of test tubes. Moreover, 3.8 milliliters of sterile water were supplied to each test tube. To find out if there was any turbidity, all of these test tubes were kept under observation and incubated for a day. A comparable procedure was employed to screen ampicillin, a drug that is often taken. The MIC values are those at which there was no discernible bacterial growth.

Result and discussion

The relationship between structure and activity was examined by looking at structural changes caused by the attachment of different substitutions and where they were placed on the heterocyclic ring in the produced derivative (Figure **2**). Biological activity was shifting as a result of the heterocyclic ring and aryl group's different locations. The anti-microbial properties of various substituted phenyl rings coupled to pyrazole and pyrazole coupled to 1H-tetrazole were discovered to differ in this investigation. Different replacements exhibit different electrical characteristics, which are reflected in the efficiency of these substitutions against microbes. Anti-microbial activity can differ based on the heterocyclic or aryl ring, according to SAR study. Manufactured compounds' heterocyclic ring replacements may either pull away or contribute electrons. The antimicrobial activity rises with replacements of halogenated groups at different locations on a heterocyclic ring. Table **2** shows that the chloro group is more active at the meta position when phenyl rings are replaced in compounds with different halogenated groups. Similar to compound **6D**, compound **6E** was shown to have greater increased antibacterial activity. The good inhibition against strains was attributed to the bromine group's substitution at the meta position. In contrast, compound **6J** and compound **6L** exhibit moderate activity in the chloro and bromo groups substituted by the para and ortho groups. Compounds **6O** and **6P** have reduced activity against all microorganisms due to the substitution of amine-aryl rings; conversely, compound **6Q**'s pyridin ring with methyl group. Compounds **6O** and **6P** have reduced activity against all microorganisms due to the substitution of amine-aryl rings, while compound **6Q**'s pyridin ring with methyl group has reduced inhibitory activity. Additionally, because compound 6I had an electron-releasing group at the o-position, its activity was decreased. In compounds **6G** and **6H**, it was demonstrated that the methyl group, a weak electron donor, is less active than other groups at position 4 of the phenyl ring. Compounds **6A, 6C, 6F,** and **6K** have higher antibacterial activity when the heterocyclic ring was present as a replacement.Compound **6N**'s cyclo alkyl is the cause of its strong anti-bacterial activity. Additional substituted derivatives, **6B** and compound **6M**, showed only moderate action.

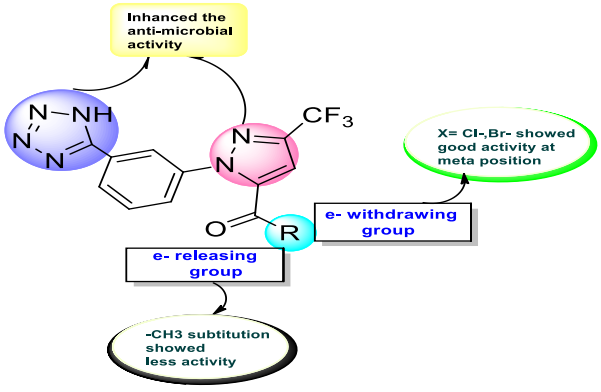


Figure 2: Structure Activity Relationship Study

The results of the investigation indicate that most of the chemicals examined had variable inhibitory effects on the growth of the investigated bacterial strains, as Table **2** illustrates. Compounds **6D** and **6E** demonstrated strong inhibitory activity against the tested microbial strains because they both had electron-withdrawing groups in the para position. In contrast to the reference medication Ampicillin, compound **6J** exhibits modest inhibition when substituted with -Br and compound **6L** exhibits -Cl on its heterocyclic ring at the meta position. Compounds **6H, 6I, 6P**, and **6G**, which contain electron-releasing groups, exhibit moderate to reduced activity against all strains of microorganisms, both Gram-positive and Gram-negative.Compounds **6A, 6C**, and **6F** were substituted with heterocyclic rings, which resulted in compounds with good inhibitory action against Pseudomonas species, while compound 6N had no impact on *Pseudonymous spp*. In comparison to the examined strains of *B. subtilis*, *E. coli*, *Staphylococcus aureus*, and *Pseudomonas spp.*, compounds **6K, 6O**, and **6Q** showed reduced inhibitory effect. The graphical representation of antibacterial activity is shown in Fig. **3**.

Table 2: Antibacterial activity of tested compounds as a zone of inhibition in MIC(µg/mL) of synthesized compound.

Compound Number	*Staphylococcus aureus* (Gram+)	*Bacillus Subtills* (Gram+)	*Bacillus megaterium* (Gram+)	*Escherichia coli* (Gram-)	*Pseudonymous spp.* (Gram-)	*Shigella sp.* (Gram-)
6A	36	32	44	19	25	32
6B	20	28	25	10	12	15
6C	35	32	40	15	22	30
6D	40	33	44	19	26	30
6E	39	33	45	18	-	-
6F	34	30	40	16	25	27
6G	20	26	25	10	19	25
6H	22	18	30	12	15	18
6I	21	19	28	11	-	19
6J	30	28	37	-	18	28
6K	21	18	25	16	12	15
6L	32	31	23	17	17	29
6M	15	16	20	15	12	13
6N	38	30	39	16	-	26
6O	20	26	25	11	13	26
6P	22	24	13	15	16	23
6Q	24	23	29	12	14	20
Ampicillin	40	35	48	21	29	33

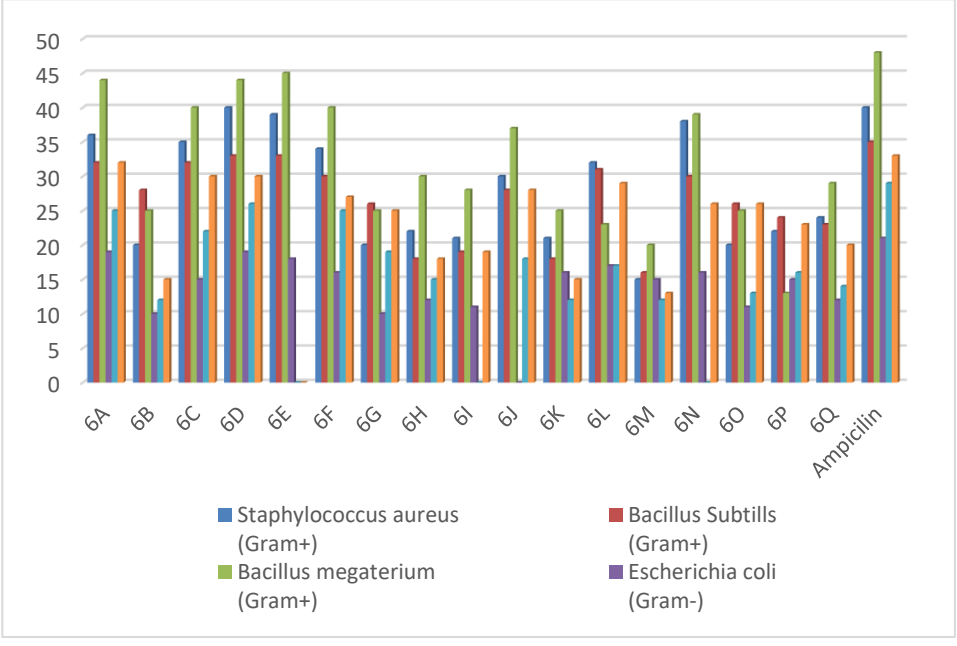


Figure 3: Statistical diagram of antimicrobial activity

Experimental

Preparation of Intermediate-1A

Following the addition of Oxyma (41.30 g, 290.646 mmol), 3-Bromoaniline (25 g, 145.323 mmol), and K2CO3 (40.16 g, 290.646 mmol) at room temperature, a 2.0 L three neck flask holding a suspension of DIC (36.67 g, 290.646 mmol, 2 eq.) in EtOH (1000 ml, 40 V) was filled. N2(g) was used to purge the suspension for fifteen minutes. Johnphos (2.16 g, 7.2661 mmol, 0.05 eq.) and Pd(OAc)2 (0.978 g, 4.3597 mmol, 0.03 eq.) were added, and the reaction mixture was once again purged with N2(g) for 15 minutes before being agitated for an hour at room temperature. The reaction mixture was then heated for an entire night at 80°C. Ethyl acetate:hexane (5:5) was used as the mobile phase in the TLC, which verified the reaction's completion. UV was used to visualize the TLC.

EtOH was extracted from the reaction mixture under vacuum once the reaction was finished. The reaction mixture was passed through a celite bed filter after being diluted with 700 cc of ethyl acetate. The filtrates were extracted using ethyl acetate (2x500 ml) after being diluted with 1000 ml of water. To acquire crude product, organic layers were mixed, dried over Na2SO4, and then concentrated in a vacuum.

Preparation of Intermediate-3A

After cooling to 0 0C, an aqueous solution of sodium nitrite (9.815 g, 142.25 mmol) in water (42 ml) was added to a 1.0 L three neck flask that contained a suspension of 3-aminobenzonitrile in 12 N HCl (35.56 ml). After one hour of stirring the solid suspension, 35.53 milliliters of SnCl2.2H2O solution in 12 N HCl was added, making that the internal temperature did not rise over 5.0C. The reaction mixture was stirred for two hours at 0–5 0C before Intermediate-2A (29.32 g, 142.25 mmol) was added and heated for the entire night at 60 0C. The TLC verified that the reaction had finished.

Following the reaction's conclusion, ethanol was vacuum-evaporated. The aqueous solution was extracted using ethyl acetate (2x500 ml), diluted with water (700 ml), and basified with aqueous NaHCO3 (70 g in 700 ml). To acquire crude product, organic layers were mixed, dried over Na2SO4, and then concentrated in a vacuum.

Preparation of Intermediate-4A

Intermediate-3A (22 g, 72.549 mmol) in acetone (450 ml) at room temperature and an aqueous solution of KMnO4 (80.25 g, 507.84 mmol) in water (450 ml) were added to a 2.0 L three neck flask. After two hours of heating at 60 ^{0}C, the reaction mixture was cooled to room temperature. 450 milliliters of IPA were used to quench the reaction mixture, which was then agitated overnight at room temperature. Using ethyl acetate as the mobile phase, TLC verified the reaction's completion. UV light was used to visualize the TLC,68.62% yield.

The reaction mixture was filtered through Celite bed and solid cake was washed with acetone/water mixture (300 ml; 1: 1) and methanol (300 ml). The filtrate was evaporated under reduced pressure to remove organic solvents. The aqueous layer was basified with 1 *N* NaOH (100 ml) solution and extracted with diethyl ether (200 mL). The aqueous layer was poured on to crushed ice, acidified with 2 *N* HCl (50 ml) under constant stirring. The solid obtained was collected by filtration and washed with n-Hexane. The obtained solid dried under high vacuum to get desire product.

General procedure of Compound (5A-5Q)

HATU (0.507 g, 1.3360 mmol, 1.5 eq.) and DIPEA (0.38 ml, 2.6673 mmol, 3 eq.) were added to a stirred solution of 1-(3-cyanophenyl)-3-(trifluoromethyl)-1H-pyrazole-5-carboxylic acid (0.250 g, 0.889 mmol, 1 eq.) in Acetonitrile (2.5 ml, 10 V). The reaction mixture was then stirred at room temperature for one hour. Following that, different substitution was added to the reaction mixture and stirred overnight at room temperature. TLC (10% MeOH in DCM) was used to track the reaction's advancement.

The reaction mixture was extracted using ethyl acetate (2 X 30 ml) and quenched with sat. NaHCO3 (10 ml). To extract the crude chemical (0.628 g), the mixed organic layers were dried over Na2SO4 and then evaporated under vacuum. The layers were then cleaned with 30 ml of Brine. Without more purification, the crude chemical was utilized for the following stage.

General procedure of Compound (6A-6Q)

NaN3 (0.742 g, 11.4280 mmol, 8 eq.) and NH4Cl (0.534 g, 9.9990 mmol, 7 eq.) were added to a stirred solution of 3-(5-(morpholine-4-carbonyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl)benzonitrile (0.628 g, 1.4280 mmol, 1 eq.) in DMF (6.2 ml, 10 V). The reaction mixture was then heated at 120°C for five hours. TLC (10% MeOH in DCM) was used to track the reaction's advancement.

(1-(3-(1H-tetrazol-5-yl)phenyl)-3-(trifluoromethyl)-1H-pyrazol-5-yl)(morpholino)methanone(6A)

^{1}H NMR (400 MHz, DMSO-d_6): δ 3.43 (s, 4H), 3.57 (s, 4H), 7.30 (s, 1H), 7.74-7.80 (m, 2H), 8.16 (d, *J* = 7.60 Hz, 1H), 8.24 (s, 1H). **LCMS (Method-H3):** R_T = 2.061 min.; m/z: 394.0 [M^+ + H]

1-(3-(1H-tetrazol-5-yl)phenyl)-N-benzyl-3-(trifluoromethyl)-1H-pyrazole-5-carboxamide(6B)

^{1}H NMR (400 MHz, DMSO-d_6): δ 4.41 (d, *J* = 4 Hz, 2H), 7.24-7.34 (m, 6H), 7.53 (s, 1H), 7.55-7.66 (m, 2H), 8.14-8.17 (m, 1H), 9.47 (t, *J* = 4 Hz, 1H). **LCMS (Method-H3):** R_T = 2.330 min.; m/z: 414.2 [M^+ + H]

3-(5-(pyrrolidine-1-carbonyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl)benzonitrile(6C)

LCMS (Method-J2): R_T = 3.423 min.; m/z: 335.25 [M^+ + H]

(1-(3-(1H-tetrazol-5-yl)phenyl)-3-(trifluoromethyl)-1H-pyrazol-5-yl)(pyrrolidin-1-yl)methanone(6D)

^{1}H NMR (400 MHz, DMSO-d_6): δ 1.83 (s, 4H), 3.40 (s, 4H), 7.34 (s, 1H), 7.39 (d, *J* = 8 Hz, 1H), 7.55 (t, *J* = 6.8 Hz, 1H), 8.05-8.10 (m, 2H). **LCMS (Method-H3):** R_T = 2.199 min.; m/z: 378.2 [M^+ + H]

N-(4-chlorobenzyl)-1-(3-cyanophenyl)-3-(trifluoromethyl)-1H-pyrazole-5-carboxamide (6E)

^{1}H NMR (400 MHz, DMSO-d_6): δ 4.37 (d, *J* = 5.6 Hz, 1H), 7.28-7.36 (m, 4H), 7.47-7.49 (m, 2H), 7.59 (t, *J* = 7.6 Hz, 1H), 8.09-8.12 (m, 2H), 9.48 (t, *J* = 5.6 Hz, 1H). **LCMS (Method-H3):** R_T = 2.514 min.; m/z: 448.0 [M^+ + H]

3-(5-(indoline-1-carbonyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl)benzonitrile(6F)

^{1}H NMR (400 MHz, DMSO-d_6): δ 3.15 (t, *J* = 7.6 Hz, 2H), 4.19 (t, *J* = 8 Hz, 2H), 7.10 (d, *J* = 7.2 Hz, 1H), 7.19 (t, *J* = 7.6 Hz, 1H), 7.30 (d, *J* = 7.2 Hz, 1H), 7.50 (d, *J* = 8 Hz, 1H), 7.55 (s, 2H), 7.97 (d, *J* = 7.6 Hz, 1H), 8.07 (d, *J* = 7.2 Hz, 1H), 8.21 (s, 1H). **LCMS (Method-H3):** R_T = 2.484 min.; m/z: 426.2 [M^+ + H]

1-(3-cyanophenyl)-N-(4-methylbenzyl)-3-(trifluoromethyl)-1H-pyrazole-5-carboxamide(6G)

^{1}H NMR (400 MHz, DMSO-d_6): δ 2.26 (s, 3H), 4.33 (d, *J* = 5.6 Hz, 2H), 7.08-7.15 (m, 4H), 7.43 (m, 2H), 7.55 (t, *J* = 7.6 Hz, 1H), 8.08-8.11 (m, 2H), 9.39 (t, *J* = 5.6 Hz, 1H). **LCMS (Method-H3):** R_T = 2.482 min.; m/z: 428.0 [M^+ + H]

(1-(3-(1H-tetrazol-5-yl)phenyl)-3-(trifluoromethyl)-1H-pyrazol-5-yl)(4,4-dimethylpiperidin-1-yl)methanone(6H)

^{1}H NMR (400 MHz, DMSO-d_6): δ 0.87 (s, 6H), 1.02 (s, 2H), 1.25 (s, 2H), 3.25 (s, 2H), 3.56 (s, 2H) 7.25 (s, 1H), 7.47 (d, *J* = 7.6 Hz, 1H), 7.63 (t, *J* = 8 Hz, 1H), 8.10 (d, *J* = 8 Hz, 1H), 8.15 (s, 1H). **LCMS (Method-H3):** R_T = 2.501 min.; m/z: 420.2 [M^+ + H]

1-(3-(1H-tetrazol-5-yl)phenyl)-N-(2-methylbenzyl)-3-(trifluoromethyl)-1H-pyrazole-5-carboxamide(6I)

^{1}H NMR (400 MHz, DMSO-d_6): δ 32.24 (s, 3H), 4.38 (d, *J* = 5.6 Hz, 2H), 7.12-7.20 (m, 6H), 7.49-7.62 (m, 3H), 8.12 (s, 1H), 9.31 (t, *J* = 5.6 Hz, 1H). **LCMS (Method-H3):** R_T = 2.469 min.; m/z: 428.0 [M^+ + H]

1-(3-(1H-tetrazol-5-yl)phenyl)-N-(3-chlorobenzyl)-3-(trifluoromethyl)-1H-pyrazole-5-carboxamide(6J)

^{1}H NMR (400 MHz, DMSO-d_6): δ 4.41 (d, *J* = 5.6 Hz, 2H), 7.09-7.70 (m, 8H), 8.17 (s, 2H), 9.52 (t, *J* = 5.6 Hz, 1H). **LCMS (Method-H3):** R_T = 2.517 min.; m/z: 448.0 [M^+ + H]

(1-(3-(1H-tetrazol-5-yl)phenyl)-3-(trifluoromethyl)-1H-pyrazol-5-yl)(4-methyl-1-oxa-4,9-diazaspiro[5.5]undecan-9-yl)methanone (6K)

^{1}H NMR (400 MHz, DMSO-d_6): 1.47-1.54 (m, 2H), 1.78-1.92 (m, 2H), 2.18 (s, 3H), 2.38 (s, 2H), 2.97 (t, *J* = 11.2 Hz, 1H), 3.26-3.32 (m, 2H), 3.50-3.52 (m, 2H), 3.65 (s, 2H), 4.15 (d, *J* = 12.8 Hz, 1H), 7.25 (s, 1H), 7.47 (d, *J* = 7.6 Hz, 1H), 7.60 (t, *J* = 8 Hz, 1H), 8.06 (s, 1H), 8.08 (s, 1H). **LCMS (Method-H3):** R_T = 2.255 min.; m/z: 477.2 [M^+ + H]

1-(3-(1H-tetrazol-5-yl)phenyl)-N-(2-bromobenzyl)-3-(trifluoromethyl)-1H-pyrazole-5-carboxamide (6L)

^{1}H NMR (400 MHz, DMSO-d_6): δ 4.42 (d, *J* = 5.2 Hz, 2H), 7.21 (s, 1H), 7.32 (d, J = 6.8 Hz, 2H), 7.52 (s, 2H), 7.59-7.61 (m, 2H), 8.11 (m, 2H), 9.49 (t, *J* = 5.2 Hz, 1H). **LCMS (Method-H3):** R_T = 2.619 min.; m/z: 492.0 [M^+ + H]; 494.0 [M^+ + 2H]

1-(3-(1H-tetrazol-5-yl)phenyl)-N-cyclobutyl-3-(trifluoromethyl)-1H-pyrazole-5-carboxamide(6M)

^{1}H NMR (400 MHz, DMSO-d_6): 1.63-1.65 (m, 1H), 1.87 (s, 3H), 1.99-2.15 (m, 2H), 4.24 (m, 1H), 7.33-7.34 (m, 2H), 7.44-7.54 (m, 2H), 8.01-8.07 (m, 2H), 9.15-9.16 (m, 1H). **LCMS (Method-H3):** R_T = 2.260 min.; m/z: 378.2 [M^+ + H]

1-(3-(1H-tetrazol-5-yl)phenyl)-N-cyclohexyl-3-(trifluoromethyl)-1H-pyrazole-5-carboxamide(6N)

R_T = 2.381 min.; m/z: 406.2 [M^+ + H]

1-(3-(1H-tetrazol-5-yl)phenyl)-N-cyclohexyl-N-methyl-3-(trifluoromethyl)-1H-pyrazole-5-carboxamide(6O)

LCMS (Method-H3): R_T = 2.440 min.; m/z: 420.2 [M^+ + H]

1-(3-(1H-tetrazol-5-yl)phenyl)-N-(2-(dimethylamino)ethyl)-3-(trifluoromethyl)-1H-pyrazole-5-carboxamide(6P)

^{1}H NMR (400 MHz, DMSO-d_6): 2.78 (s, 6H), 3.19 (t, *J* = 14 Hz, 2H), 3.82 (t, *J* = 14 Hz, 2H), 7.35 (s, 1H), 7.47 (d, J = 7.6 Hz, 1H), 7.56 (t, J = 7.6 Hz, 1H), 8.04-8.07 (m, 2H), 9.12 (s, 1H). **LCMS (Method-H3):** R_T = 2.031 min.; m/z: 395.0 [M^+ + H]

1-(3-(1H-tetrazol-5-yl)phenyl)-N-(pyridin-2-ylmethyl)-3-(trifluoromethyl)-1H-pyrazole-5-carboxamide (6Q)

^{1}H NMR (400 MHz, DMSO-d_6): δ 4.48 (d, *J* = 6 Hz, 2H), 7.20-7.32 (m, 2H), 7.51 (s, 1H), 7.63-7.70 (m, 2H), 7.11-7.12 (m, 2H), 8.48 (d, J = 3.6 Hz, 1H), 9.50 (t, *J* = 6 Hz, 1H).**LCMS (Method-H3):** R_T = 2.046 min.; m/z: 415.2 [M^+ + H].

CONCLUSION

Synthesis, characterization, and antimicrobial activity have all been conducted on a number of 1H-tetrazole clubbed pyrazole derivatives in this research study. Gram-positive (*Staphylococcus aureus, Bacillus Subtills, Bacillus megaterium*) and gram-negative (*Escherichia coli, Pseudonymous, Shigella sp.)* bacterial strains were used in an in vitro assay for anti-microbial activity. The results showed that the synthesized compounds had good activity when compared to the standard drug ampicillin. Compounds **6D** and **6E** have the highest levels of efficacy against all microbial strains. The microbial activity of all derivatives varies from moderate to low based on their structure-activity connection.

List of abbreviation

M.P – melting point
MeOH – methanol
TLC- Thin Layer Chromatography
LCMS - liquid chromatography-mass spectrometry.
TEA – Triethanolamine
DCM – Methylene Chloride
DIPEA – N,N-Diisopropylethylamine
DMF – N,N-Dimethylformamide
HATU –Hexafluorophosphate Azabenzotriazole Tetramethyl Uronium
MIC -Minimum inhibition concentration

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