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Synthesis and antimicrobial activity study of some synthesized triazole-pyrazole derivatives

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Abstract: Some Novel 2-(4-((5-amino-4- cyano-3-(methylthio)-1H-pyrazol-1-yl)methyl)-1H-1,2,3-triazol-1- yl)-N-phenylacetamide derivatives have been Synthesized from 5-amino-3-(methylthio)-1H-pyrazole-4-carbonitrile derivatives by multistep process. The structural elucidation of synthesized compound is established by different spectroscopic techniques such as 1H- Nuclear Magnetic resonance, infra-red and Mass.AfterConfirmation of Structure, Further the antimicrobial activity of these Triazole-Pyrazole derivatives were tested against some selected bacterial and fungal strains in two different organic solvents such as N,N-dimethyl formamide and dimethyl sulphoxide.

Keywords: Triazole-pyrazole derivatives, antibacterial activity, multistep reaction, etc

Introduction:

In Organic chemistry, heterocyclic compounds containing nitrogen as heteroatom plays an important role in Pharmaceuticals and Agrochemical [1]. Especially, Nitrogen based five member and six member heterocyclic compounds are found in number of naturally occurring as well as synthesized compound in laboratories for pharmaceutical purpose [2-4]. Triazole is one of the most important five member Heterocyclic compound which possess different biological activities such as antibacterial [5, 6], antioxidant [7, 8], anticancer [9-11], antifungal [12] etc. many drug present in the market possessestriazole as scaffold.

Further, five member heterocyclic compounds having two nitrogen means that pyrazole also shows marvelous activity in medical field [13, 14]. Pyrazole containing compounds have vast application in pharmaceutical as well as biological activity [15-17].

Literature survey shows that when two different heterocycles present in the same molecule, it enhance the biological activity [18, 19]. It is observed that

when the molecules have more than one heterocyclic Scaffold present shows vast biological and pharmaceutical activities [20, 21].

Present work involves some new heterocyclic compounds having Triazole-pyrazole scaffold synthesized from 5-amino-3-(methylthio)-1H-pyrazole-4-carbonitrile derivatives by multistep process. The structural elucidation has been made by different spectroscopic techniques such as proton Nuclear magnetic resonance, FT-IR and mass spectrometry. Further the antimicrobial activity of synthesized compounds were tested against some selected bacterial and fungal strain using two different solvents namely *N,N*-dimethyl formamide and dimethyl sulphoxide.

Material & Methods:

Chemicals and reagents:

The chemicals used for the synthesis such as different substituted anilines,chloro acetyl chloride,malonitrilewere purchased from Spectrochem Pvt. Ltd. Different solvents used in reaction as well as in anti-microbial activity were purchased from Sigma Aldrich Pvt. Ltd and were used directly without any further purification.

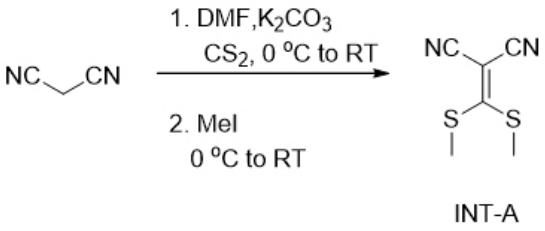
Experiments

Spectroscopy study

For the structure confirmation of all the synthesized compounds, different analytical spectroscopic techniques such as infra-red (IR), Proton nuclear magnetic resonance (1H-NMR), and mass spectrometry were used. For the Infra red spectrum, furrier transport infrared spectrophotometer (IR affinity-1S SHIMADZU) was used. The IR spectrum was done in moisture free atmosphere. The proton spectrum of all the synthesized compounds was recorded on a Bruker AVANCE III (at 400 MHz frequencies). For the ^{1}H NMR spectrum, solution of compounds was prepared in deuterated dimethyl sulphoxide (DMSO-d6) solvent and tetra methylsilane was used as reference material. Mass spectra were determined using direct inlet probe on a SHIMADZUGC-MS (Model-QP2010) mass spectrometer.

Synthesis of 2-(bis(methylthio) methelene)Malanonitrile (INT-A).

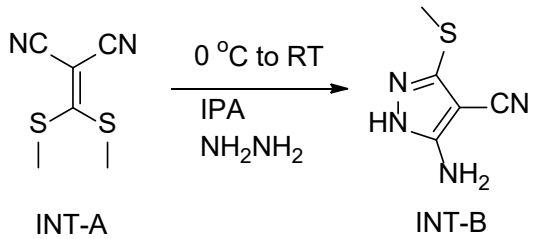
In a 100 ml conical flask equipped with magnetic stirrer and septum was charged with a solution of malononitrile in 10 ml *N,N*-dimethyl formamide. To this dry K_2CO_3 (10 mmol) was added and the mixture was stirred at room temperature (RT) for 2 hours. After completion of 2 hours,carbon disulphide(30 mmol) was added and the mixture was stirred for an additional 2 hours at room temperature. Cool the reaction mixture in ice bath and methyl iodide (20 mmol) was added at 0-5 ^{o}Ctemperature. The resultant mixture was further stirred for 4 h at room temperature. The progress of the reaction was monitored by preparative thin layer chromatographyusing mixture of n-hexane: ethyl acetate (3:7 % v/v) as mobile phase. After completion of the reaction the reaction mixture was poured into cold water with constant stirring. The precipitated crude product was purified by filtration followed by crystallization from ethanol.



Scheme-1

Synthesis of 5-amino-3-(Methylthio)-1H-pyrrole-4-Carbonitrile (INT-B).

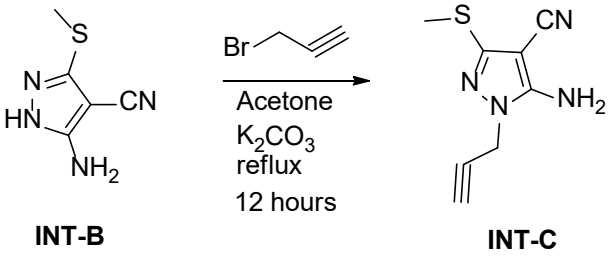
In conical flask equipped with magnetic stirrer and septum was charged with 2-(bis(methylthio)methelene) Malanonitrile(INT-A) (0.1mmol) and 20 ml isopropyl alcohol.The reaction mixture was cooled at 0°Cin ice bath and hydrazine hydrate (0.1mmol) was added. The reaction mixture was stirred atroom temperature (RT) for 2 hours. After completion of the reaction, it was poured into cold water. Thecrude product was precipitated and filtered by filtration under vacuumed. The filtered crude product was crystallized from ethanol.



Scheme-2

Synthesis of 5-amino-3-(methylthio)-1-(prop-2-yn-1-yl)-1H-pyrazole-4-carbonitrile (INT-C)

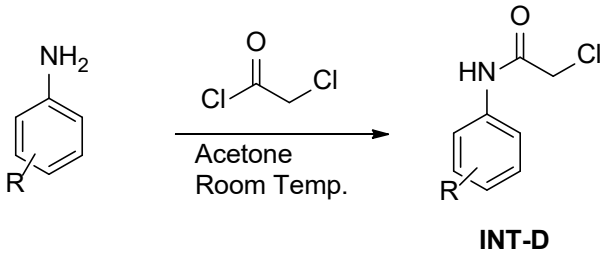
In a Round bottom flask, 5-amino-3-(Methylthio)-1H-pyrrole-4-Carbonitrile (INT-B, 50mmol) was taken in acetone (50ml) and to this anhydrous K_2CO_3(100mmol) was added with constant stirring.After stirring of 5 minutes, propargyl bromide (55 mmol) was added drop wise in reaction mixture. After completion of addition, the reaction mixture was reflux for 12 hours with continuous stirring. The reaction was monitored on thin layer chromatography by using n-hexane: ethyl acetate (4:6 % v/v) as Mobile phase. After completion of the reaction, the reaction mixture was poured into the crushed ice. The separated product was filtered, washed with water and dried under vacuum to afford final compound.



Scheme-3

Synthesis of 2-chloro-N-phenylacetamide derivatives (INT-D)

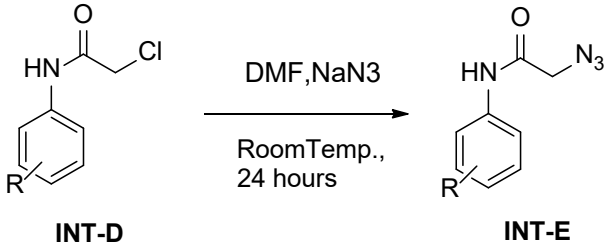
In a round bottom flask, solution of various substituted Aniline (1mmol) in acetone has been taken and thenchloroacetyl chloride (1mmol) was added drop wise. The resulting mixture was stirred for 15 minutes at room temperature.After completion of reaction, the reaction mixture was poured into crushed ice with constant stirring. The obtained solid product was filtered under vacuum and give wash with cold water. It was dried under vacuum and used in next step without further purification.



Scheme-4

Synthesis of 2-azido-N-phenylacetamide derivatives (INT-E)

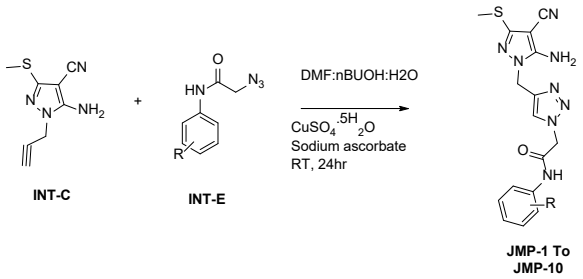
Prepared a solution of 2-chloro-N-phenylacetamide derivatives INT-D (1 mmol) using dry DMF solvent into the round bottom flask. To this add, Sodium Azide (NaN_3, 3 mmol) was added slowly. The resulting mixture was stirred at Room Temperature for 24 hours. After completion of the reaction, the reaction mixture was poured in to crushed ice. The resultant product was Filtered dried under vacuum.



Scheme-5

General synthesis of Final Compound

In a round bottomflask, *N,N*-dimethyl formamide:H_2O:n-butanol (1:1:1) portion was taken. In the resultant mixture,previously prepared INT-C (1 mmol) and INT-E(1mmol) was added at room temperature.To this mixture, catalytic amount of sodium ascorbate and coppersulphatepentahydrate was added.The resulting solution was stirred at room temperature for 24hours. The reaction progress was monitored by thin layer chromatography by taking n-hexane: ethyl acetate (2:7 % v/v) as mobile phase.After the completion of the reaction, mixture was poured into the crushed ice. The separated product was filtered and washed with dilute ammonia solution and dried it.



Scheme-6

Table 1: Physical data of all the synthesized compounds

Sr. No.	Compound code	Substitution (R)	Molecular formula	Molecular weight
1	JMP-1	-H	$C_{16}H_{16}N_8OS$	368.42
2	JMP-2	2-Cl	$C_{16}H_{15}N_8OSCl$	402.86
3	JMP-3	4-Cl	$C_{16}H_{15}N_8OSCl$	402.86
4	JMP-4	2-F	$C_{16}H_{15}N_8OSF$	386.41
5	JMP-5	4-F	$C_{16}H_{15}N_8OSF$	386.41
6	JMP-6	2-CH_3	$C_{17}H_{18}N_8OS$	382.44
7	JMP-7	4-CH_3	$C_{17}H_{18}N_8OS$	382.44
8	JMP-8	2-OMe	$C_{17}H_{18}N_8O_2S$	398.44
9	JMP-9	4-OMe	$C_{17}H_{18}N_8O_2S$	398.44
10	JMP-10	4-NO_2	$C_{16}H_{15}N_9O_3S$	413.41

Figure 1:^{1}H NMR spectrum of JMP-3

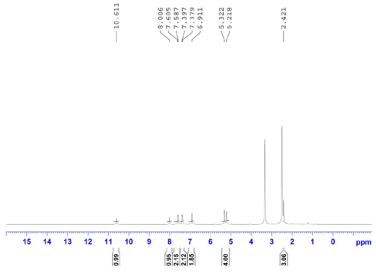


Figure 2: IR spectrum of JMP-3

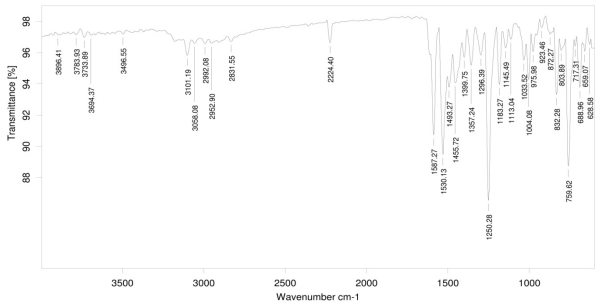
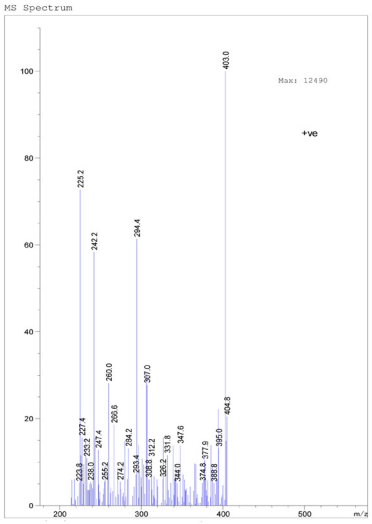


Figure 3: Mass spectrum of JMP-3



Spectral data

JMP-1:^{1}HNMR (DMSO-d_6, δ ppm):

2.423 (3.00, -CH_3), 5.211 (2.25, -CH_2-, Open chain), 5.323 (2.00, -NH_2), 7.062 (1.82, Ar-H), 7.306 (1.14, Ar-H), 7.345 (2.18, Ar-H), 7.584 (2.13,-CH_2-, Open chain), 8.014 (0.85,Ar-H), 10.519 (1.02,-NH-)

IR (cm^{-1}):3413.94 (N-H symmetric and asymmetric –NH_2 group), 3191.33 (=C-H alkene stretching), 2974.66, 2922.39, 2850.37 (C-H stretching), 2200.66 (-CN group stretching), 1624.10, 1538.10, (N-H bending primary amines), 1447.27 (alkanes C-H bending), 1378.52, 1345.95 (alkanes C-H rocking), 1249.901166.20 (C-N stretching aliphatic amines), 1015.67 (=C-H bending alkane)
Mass (m/z): 369.0

JMP-2:^{1}HNMR (DMSO-d_6, δ ppm):2.457 (3.02, -CH_3), 5.218 (2.19, -CH_2-, Open chain), 5.423 (2.00, -NH_2), 7.056 (1.11, Ar-H), 7.315(1.16, Ar-H), 7.348(1.18, Ar-H), 7.446 (1.09,Ar-H), 7.610 (2.09,-CH_2-, Open chain), 8.019 (0.96,Ar-H), 10.528 (1.07,-NH-)

IR (cm^{-1}):3489.28. 3472.95 (N-H symmetric and asymmetric –NH_2 group), 3115.29, 3045.29 (=C-H alkene stretching), 3001.29, 2951.38, 2937.28, 2832.85 (C-H stretching), 2231.37 (-CN group stretching), 1586.29 (N-H bending primary amines), 1451.26 (alkanes C-H bending), 1408.83, 1392.48. 1375.23 (alkanes C-H rocking), 1294.29 (C-N stretching aromatic amine), 1255.92, 1186.12, 1155.28, 1124.28 (C-N stretching aliphatic amines), 1010.32 (=C-H bending alkane)
Mass (m/z):403.0

JMP-3:^{1}HNMR (DMSO-d_6, δ ppm):2.421(3.06,-CH_3), 5.218(2.00,-CH_2-, open chain), 5.322(2.00,-NH_2), 6.911(1.85,-CH_2-, open chain),7.397 (2.12,Ar-H), 7.605(2.15,Ar-H), 8.006 (0.95,Ar-H), 10.611(0.99,-NH-)

IR (cm^{-1}):3496.37 (N-H symmetric and asymmetric –NH_2 group), 3101.19, 3058.08 (=C-H alkene stretching), 2992.08, 2952.90, 2831.55 (C-H stretching), 2224.40 (-CN group stretching), 1587.27 (N-H bending primary amines), 1455.72 (alkanes C-H bending), 1399.75, 1375.24 (alkanes

C-H rocking), 1296.39 (C-N stretching aromatic amine), 1250.28, 1183.27, 1145.49, 1113.04 (C-N stretching aliphatic amines), 1004.08 (=C-H bending alkane)
Mass (m/z):403.0

JMP-4: ^{1}H NMR (DMSO-d_6, δ ppm):

2.513 (3.07, -CH_3), 5.224 (2.06, -CH_2-, Open chain), 5.399 (2.12, -NH_2), 7.033 (1.17, Ar-H), 7.302 (1.03, Ar-H), 7.342 (0.98, Ar-H), 7.437 (1.01,Ar-H), 7.591 (2.15,-CH_2-, Open chain), 8.019 (0.96,Ar-H), 10.334 (1.12,-NH-)

IR (cm^{-1}):3491.47 (N-H symmetric and asymmetric –NH_2 group), 3112.83, 3047.38 (=C-H alkene stretching), 2998.81, 2945.93, 2935.28, 2838.35 (C-H stretching), 2225.98 (-CN group stretching), 1583.23 (N-H bending primary amines), 1448.28 (alkanes C-H bending), 1403.20, 1397.03. 1372.38 (alkanes C-H rocking), 1291.38 (C-N stretching aromatic amine), 1254.29, 1181.38, 1149.23, 1121.37 (C-N stretching aliphatic amines), 1005.39 (=C-H bending alkane)
Mass (m/z):387.2

JMP-5:^{1}H NMR (DMSO-d_6, δ ppm):

2.488 (3.01,-CH_3), 5.202(2.08,-CH_2-, open chain), 5.306(2.06,-NH_2), 6.867 (1.97,-CH_2-, open chain), 7.421 (2.18,Ar-H), 7.587(2.03,Ar-H), 8.021 (0.99,Ar-H), 10.577 (1.07,-NH-)

IR (cm^{-1}):3495.28. 3476.92 (N-H symmetric and asymmetric –NH_2 group), 3121.38, 3047.92 (=C-H alkene stretching), 3004.72, 2955.28, 2941.38, 2837.45 (C-H stretching), 2232.39 (-CN group stretching), 1589.18 (N-H bending primary amines), 1458.56 (alkanes C-H bending), 1411.39, 1391.32. 1379.27 (alkanes C-H rocking), 1298.21 (C-N stretching aromatic amine), 1256.39, 1187.29, 1158.53, 1125.29 (C-N stretching aliphatic amines), 1007.92 (=C-H bending alkane)
Mass (m/z):387.2

JMP-6: ^{1}H NMR (DMSO-d_6, δ ppm):

2.214(2.89,-CH_3), 2.451 (3.09, -CH_3), 5.203(2.27, -CH_2-, Open chain), 5.507 (2.08, -NH_2), 7.067 (1.19, Ar-H), 7.330(1.21, Ar-H), 7.367(1.12, Ar-H), 7.434 (1.03,Ar-H), 7.592 (2.02,-CH_2-, Open chain), 8.134 (0.99,Ar-H), 10.325 (1.01,-NH-)

IR (cm^{-1}):3487.39. 3472.83 (N-H symmetric and asymmetric –NH_2 group), 3118.20, 3056.67 (=C-H alkene stretching), 3009.27, 2954.29, 2949.27, 2833.77 (C-H stretching), 2238.26 (-CN group stretching), 1585.72 (N-H bending primary amines), 1465.29 (alkanes C-H bending), 1419.29, 1405.28. 1372.38 (alkanes C-H rocking), 1294.29 (C-N stretching aromatic amine), 1253.20, 1184.28, 1153.56, 1122.49 (C-N stretching aliphatic amines), 1001.52 (=C-H bending alkane)
Mass (m/z):383.1

JMP-7: ^{1}H NMR (DMSO-d_6, δ ppm):2.189(3.09,-CH_3), 2.402 (2.93,-CH_3), 5.192(2.02,-CH_2-, open chain), 5.288 (2.03,-NH_2), 6.776 (1.99,-CH_2-, open chain), 7.263 (2.15,Ar-H), 7.369 (2.07,Ar-H), 7.989 (0.92,Ar-H), 10.563 (1.04,-NH-)

IR (cm^{-1}):3472.31. 3477.27 (N-H symmetric and asymmetric –NH_2 group), 3113.39, 3098.72, 3052.48 (=C-H alkene

stretching), 3011.38, 2958.24, 2955.89, 2835.24 (C-H stretching), 2241.92 (-CN group stretching), 1582.93 (N-H bending primary amines), 1469.37 (alkanes C-H bending), 1427.92, 1413.23, 1402.58 (alkanes C-H rocking), 1298.94 (C-N stretching aromatic amine), 1257.29, 1186.02, 1154.83, 1124.18 (C-N stretching aliphatic amines), 1002.85 (=C-H bending alkane)
Mass (m/z):383.1

JMP-8: ^{1}H NMR (DMSO-d_6, δ ppm):

2.426 (3.03, -CH_3), 3.769 (3.12,-OCH_3), 5.187 (2.16, -CH_2-, Open chain), 5.487 (2.05, -NH_2), 7.051 (1.13, Ar-H), 7.319(1.13, Ar-H), 7.353(1.07, Ar-H), 7.392 (1.11,Ar-H), 7.496 (2.09,-CH_2-, Open chain), 8.064 (1.16,Ar-H), 10.396 (1.08,-NH-)

IR (cm^{-1}):3487.25. 3479.19 (N-H symmetric and asymmetric –NH_2 group), 3110.37, 3092.48, 3065.49 (=C-H alkene stretching), 3018.28, 2961.38, 2951.38, 2832.49 (C-H stretching), 2235.28 (-CN group stretching), 1585.82 (N-H bending primary amines), 1473.49 (alkanes C-H bending), 1431.29, 1415.62, 1405.28 (alkanes C-H rocking), 1293.76 (C-N stretching aromatic amine), 1261.83, 1180.25, 1150.29 (C-N stretching aliphatic amines), 1005.29 (=C-H bending alkane)
Mass (m/z):399.0

JMP-9: ^{1}H NMR (DMSO-d_6, δ ppm):

2.458 (2.98,-CH_3), 3.657 (3.06,-OCH_3), 5.224(2.09,-CH_2-, open chain), 5.309 (2.08,-NH_2), 6.804 (2.05,-CH_2-, open chain), 7.462 (2.12,Ar-H), 7.526 (2.03,Ar-H), 8.057 (0.98,Ar-H), 10.546 (1.09,-NH-)

IR (cm^{-1}):3479.92.31. 3471.39 (N-H symmetric and asymmetric –NH_2 group), 3111.39, 3096.32, 3059.37 (=C-H alkene stretching), 3013.29, 2957.23, 2959.37, 2831.33 (C-H stretching), 2248.35 (-CN group stretching), 1589.23 (N-H bending primary amines), 1478.36 (alkanes C-H bending), 1431.53, 1417.83, 1407.22 (alkanes C-H rocking), 1309.38 (C-N stretching aromatic amine), 1255.28, 1176.39, 1156.22, 1121.39 (C-N stretching aliphatic amines), 1003.29 (=C-H bending alkane)
Mass (m/z): 399.0

JMP-10:^{1}H NMR (DMSO-d_6, δ ppm):

2.458 (2.98,-CH_3), 5.224(2.09,-CH_2-, open chain), 5.309 (2.08,-NH_2), 6.804 (2.05,-CH_2-, open chain), 7.528 (2.06,Ar-H), 7.687(2.13,Ar-H), 8.027 (1.04,Ar-H), 10.563 (1.06,-NH-)

IR (cm^{-1}):3472.31. 3477.27 (N-H symmetric and asymmetric –NH_2 group), 3113.39, 3098.72, 3052.48 (=C-H alkene stretching), 3011.38, 2958.24, 2955.89, 2835.24 (C-H stretching), 2241.92 (-CN group stretching), 1582.93 (N-H bending primary amines), 1469.37 (alkanes C-H bending), 1427.92, 1413.23, 1402.58 (alkanes C-H rocking), 1298.94 (C-N stretching aromatic amine), 1257.29, 1186.02, 1154.83, 1124.18 (C-N stretching aliphatic amines), 1002.85 (=C-H bending alkane)
Mass (m/z): 414.2

Antimicrobial activity

Microorganisms tested:

The antimicrobial activity of all the triazole derivatives was tested against

some selected gram positive and gram negative bacterial strains in two different solvents, *N,N*-dimethyl formamide and dimethyl sulphoxide. For the antimicrobial activity, agar well diffusion method was used. In this method, first of all colonies of bacteria is developed into petriplates under controlled condition. The microorganisms were maintained on nutrient agar and MGYP medium. TheGram positive bacteria studied were*Bacilluscereus* (BC), *Corynebacteriumrubrum* (CR), *Bacillussubtilis* (BS) and *Staphylococcusaureus* (SA). Gram negative bacteria were *Klebsiellapneumoniae*(KP), *Staphylococcustyphimurium*(ST), *Escherichia coli* (EC),*Pseudomonas aeruginosa*(PA).

The solution of the synthesized compounds was prepared into *N,N*-dimethyl formamide and dimethyl sulphoxide solvent of 25 mg/ml concentration. For each compound in each selected solvent for a particular one strain, the experiment was repeated three times. The average of these three values is graphically represented in Figures 4 to 7 along with appropriate uncertainty values.

For the better understanding of antimicrobial activity of all the synthesized compounds, the standard antibiotic compounds were also tested via same method. For the antibacterial study, chloramphenicol and tetracycline are used as reference for comparison with activity of synthesized compounds.

Figure 4: Inhibition of synthesized compounds against selected gram positive bacterial strains in DMF

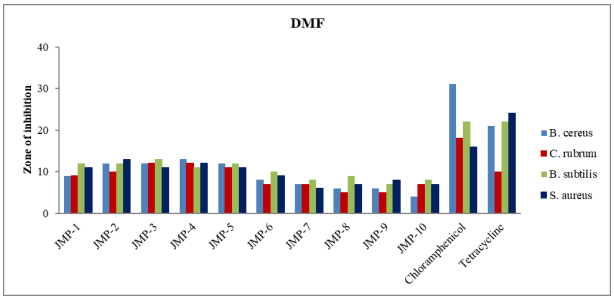


Figure 5: Inhibition of synthesized compounds against selected gram positive bacterial strains in DMSO

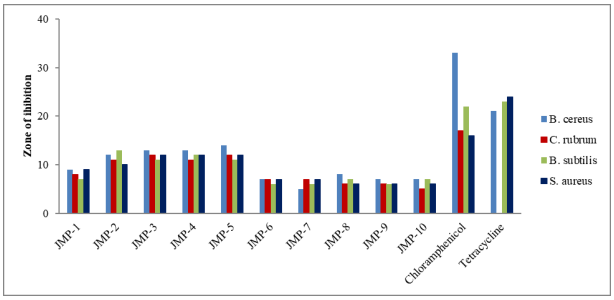


Figure 6: Inhibition of synthesized compounds against selected gram negative bacterial strains in DMF

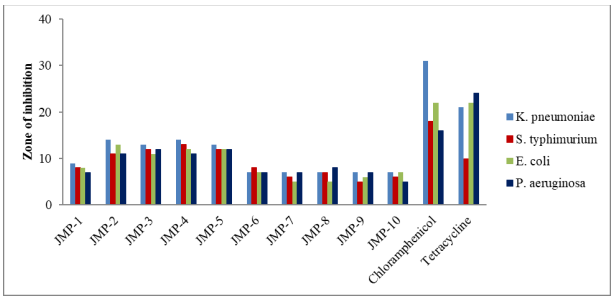
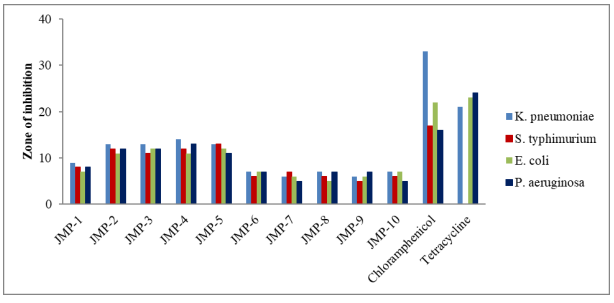


Figure 7: Inhibition of synthesized compounds against selected gram negative bacterial strains in DMSO



Some physical constants such as substitution, molecular formula and molecular weight is given in Table 1.

The structure characterization was carried out by different spectroscopic techniques and the various spectrums of JMP-3 compounds are given as Figure 1 to 3. Figure 1 shows proton nuclear magnetic resonance spectrum of JMP-3 compound in duterated dimethyl sulphoxide solvent. For proton spectra, tetra methyl silane used as internal standard. Infra red and mass spectrum is given as Figure 2 and Figure 3 respectively.

The antibacterial activities of all the synthesized compounds were checked through the agar well diffusion method. The antibacterial activity was checked into two different solvent such as *N,N*-dimethyl formamide and dimethyl sulphoxide.

The antibacterial activity of all the synthesized compounds against selected gram positive bacterial strains in *N,N*-dimethyl formamide was represent as graph in Figure 4. From this Figure, it is observed that that against *Bacillus cereus* in DMF, all the tested compounds showed inhibition and out of them JMP-4 showed maximum and JMP-10 showed minimum inhibition.

The inhibition of any molecule is depending on the structure and functional group present in it. In the present study, core structure of tested molecules was same:triazoleonly side substitutions are different. JMP-4 having 2-fluoro substitution whereas JMP-10 possess 4-nitro substitution. Hence, from the result , it revealed that the molecule have halogen substitution showed good to moderate inhibition as compared to presence of alkyl/nitro substitution in the molecules.

InDMF,against*Corynebacteriumrubrum,* JMP-3 (containing 4-chloro substitution) and JMP-4 (containing 2-fluoro substitution) showed maximum and almost up to same extent of the inhibition. In DMF, against *Bacillus subtilis*, 4-chloro substitution (as JMP-3) showed maximum inhibition. Against *Staphylococcusaureus*, again JMP-2 (possess 2-chloro substitution) showed maximum inhibition. From the result, it is clear that the halogen substituted derivatives are found to be more effective against selected bacterial strains.

Figure 5 showed zone of inhibition of all the synthesized compounds against selected gram positive bacterial strains in dimethyl sulphoxide solvent. From the Figure 5, it is cleared that the all the synthesized compounds showed good to moderate inhibition against selected bacterial strains. Against *Bacilluscereus*, JMP-5 (4-fluoro substitution) showed maximum inhibition whereas 4-methyl substituted compound (as JMP-7) showed minimum inhibition. In DMSO, against *Corynebacteriumrubrum*, 4-chloro substituted compound (as JMP-3) and 4-fluoro substituted compound (JMP-5) found to be more effective whereas 4-nitro substituted compound (as JMP-10) show minimum inhibition. Against *Bacillussubtilis*, all tested compounds showed good inhibition but not any one showed more than standard antibiotics. In DMSO, against *Staphylococcusaureus*, JMP-3, JMP-4 and JMP-5 showed maximum inhibition and almost up tosame extent of inhibition.

Figure 6 and 7showed zone of

inhibition against some selected negative bacterial strains in DMF and DMSO solvents respectively. Against *Klebsiella pneumonia*, JMP-2 and JMP-4 showed maximum inhibition whereas rest of the compounds showed good inhibition but not more than standard antibiotics. JMP-4 having 2-fluoro substitution showed maximum inhibition against*Staphylococcustyphimurium*. Against*Escherichia coli*JMP-2 having 2-chloro substitution found to be more effective in DMF. JMP-3 and JMP-5 compounds showed maximum and almost same extent of the inhibition. From the results it is cleared that the halogen substituted compound found to be more effective against all selected gram negative bacterial strains in DMF solvent.

From the Figure 7, it is observed that the 2-fluoro substituted compound (as JMP-4) possess maximum inhibition against *Klebsiellapneumoniae*, *Staphylococcustyphimurium* and *Pseudomonas aeruginosa* in DMSO solvent. Against *Staphylococcustyphimurium*, JMP-9compound is found to be lower effective and almost up to same extent. In DMSO, no one showed inhibition more than standard antibiotics drug.

Conclusion

In the present work, some new nitrogen based heterocyclic compounds have beensynthesized by multistep process.

The confirmation of the synthesis of 2-(4-((5-amino-4- cyano-3-(methylthio)-1H-pyrazol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)-N-phenylacetamide derivativesby different spectroscopic techniques. The antimicrobial activity of all these synthesized compounds were also tested against some selected bacterial and fungal strains in two different solvents such as N,N-dimethyl formamide and domethylsulphoxide.

From the result; it was revealed that the all synthesized compounds showedgood to moderate biological activity against selected strains. However, some halogen substituted compounds are showed higher inhibition then alkyl or without substituted compounds. Hence form the study, it is clear that the halogen substitution increases antimicrobial activity of 2-(4-((5-amino-4- cyano-3-(methylthio)-1H-pyrazol-1-yl) methyl)-1H-1,2,3-triazol-1- yl)-N-phenylacetamide derivatives.

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