Synthesis, Characterization and Biological Screening of Novel 1, 2, 4 Triazole Derivatives

Ajay Bagherwal, Dr. Mrunal K. Shirsat
Pacific Academy of Higher education & research University, Udaipur Rajasthan, India

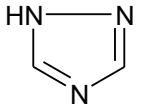
Corresponding author: **Ajay Bagherwal**

Abstracts: *In the present study a series of novel triazole derivative were synthesized and evaluated for antimicrobial and anticonvulsant activity. The entire synthesized compound* ***NJ-01, NJ-02, NJ-03, NJ-04, NJ-05, NJ-06, NJ-07, NJ-08, NJ-09, NJ-10, NJ-11 and NJ-12*** *were characterize by IR, [1]HNMR, and MASS spectral. Newly synthesized compounds were screened for their antibacterial (****Staphylococus aureus, Bacillus sublilis*** *(Gram postive bacteria)* ***Escherichia coli, Psedomonas aeruginosa*** *(Gram negative bacteria) and antifungal (****Candida albicans****) activity. The anticonvulsant activity determined by maximal ectro shock (MES) induced method. The result revealed that, compound* ***NJ-01, NJ-05 and NJ-09*** *showed maximum growth inhibition activity against bacteria and fungi, and show seizure protection and showing quick onset of action.*

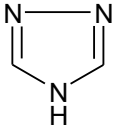
Keywords: *Triazole Derivatives, Benzoic Acid, Anticonvulsant activity, Antimicribial Activity, Pseudomonas aurugiosa and Candida albicans*

Introduction :- Disease caused by bacteria, virus, fungi and other parasites and major caused of death,Disability, social and economical disruption for million of people.[1] Infection deasese raise awrensee of our global vulnerabilit the need for strong health care system and potentially broad abd borderless impactof disease.[2] According to world heath statics 2008 report published by world helath organisation (WHO), The induction will be one of the most serious problem in 2030.[3] Thus developementof novel antimicrobial drug still in demand.

Five member aromatic ring with three atom are called 1,2,4 Triazole is also pyrodiazole.



1H-1,2,4-triazole



4H-1,2,4-triazole

S-Triazole or 1, 2, 4-Triazole

Thus Copound carring Triazole compounds gained importance in medicinal and pharmacetyical field due to a broad range of buiological activity. such as antifungal[4] , anticonvulsant[5], antitubercular[6], antiinflamatory[7] and antimicribial[8] activity.

Although many drugs are available, no drugs has fullfilled all the expectation in term of toxicity, resistance and cost factores. Thera are two types of approches to get new drugs for microbial infection treatments.

A. Synthesis of analogues, modifiaction of exicting compounds for improving microbial infection treatment.

B. Searching novel structure,which pathogen organism never seen before fore the treatment of bacterail and fungus infection.

Various 1, 2, 4-triazole derivatives were synthesized by reacting aromatic acid esters with hydrazine hydrate followed by reaction with alcoholic potassium hydroxide and carbon disulphide. The obtained potassium dithiocarbazinates were cyclized with hydrazine to yield 1, 2, 4-triazole. And further reacted with chloro acetyl chloride and then with amines to give title compound.

All the synthesized compounds were recrystallized and their purity was checked by performing thin layer chromatography. The structure of the compounds was confirmed on the basis of IR, NMR & ^{1}H NMR spectral data.

All newly synthesized triazole derivatives were screened for their activity against fungi **Candida albicans** and compound were evaluated for antibacterial activity against **staphylococcus aureus bacillus subtitis Escherichia coli and Pseudomonas aurugiosa.**

Material and Methodology:

Melting points was determining by open capillary method and are uncorrected. The IR spectra (in KBr pellets) were recorded on IR spectrophotometer. ^{1}HNMR spectra were recorded (DMSO-d6) on MHz spectrophotometer in $CDCl_3$ using TMS as an internal standard. Chemical shift (δ) are expressed in part per million (ppm). Chemical shift values are given in d scales. Mass spectra were recorded by mass spectrophotometer by using electron ionization detector. The progress of reaction was monitored by thin layer chromatography using plate coated silica gel G of 0.25 mm thickness. Eluents used were hexane and ethyl acetate (6:4) as a solvent system and iodine vapors was used as a detecting agent. Spot were visualized through iodine chamber. Solubility of newly synthesized triazole derivative was determined in various organic solvents at room temperature. The reaction pathway summarized in figure 01.

Syntheses of compounds were carried out as per following scheme

Step-I: Synthesis of esters of aromatic acid

A mixture of substituted benzoic acid (0.3 mol), 130 mL of absolute alcohol and 3.3 mL of conc. H_2SO_4 was refluxed for 2 h on water bath. After completion of reaction, excess of ethanol was distilled off and content was transferred into separating funnel containing 310 mL distilled water. Carbon-tetrachloride (20 mL) was added, aqueous layer and ester layer were separated. Ester layer (lower layer) was taken in another separating funnel and shaked it with a strong solution of sodium bicarbonate until all free acid was removed and no further evolution of carbon dioxide occur. Washed once with water and dried by pouring into a small conical flask containing 7.5g magnesium sulphate. Cork the flask, shacked for 2 minutes then carbon tetrachloride was distilled off under reduced pressure. The resulting colourless liquid was collected and the completion of reaction was checked by TLC using hexane and ethyl acetate (6:4) and iodine vapour as a detecting reagent.

Step-II: Synthesis of hydrazide of synthesized ester

Produced aromatic esters (0.1 mol) and 80 % hydrazine hydrate (0.1 mol) was refluxed on a water bath for 15 min. enough absolute ethanol was added to obtain a clear solution. Again contents were refluxed for 2 h. Excess alcohol was evaporated and solution was cool down. The solid obtained was separated and recrystallised from ethanol to get the needle shaped crystals.

Step-III: Synthesis of potassium dithiocarbazinate

Substituted aromatic hydrazides (0.02 mol), KOH (0.012 mol) and CS_2 (0.015 mol) in absolute ethanol (350 mL) were stirred for 10 h. After the completion of reaction ether (200 mL) was added. The obtained precipitate was filtered, washed and dried. The synthesized dithiocarbazinate was used for the next step without further purification.

Step-IV: Synthesis of 5-aryl-4-amino-3-mercapto-1,2,4-triazole

Substituted produced dithocarbazinate (0.1 mol), hydrazine hydrate (0.3 mol) and water (30 mL) was refluxed for 3 h, H_2S was evolved during the reaction and clear solution resulted, enough cold water was added and cooled to 5^0c. Acidified the cooled solution with dil. HCl. Obtained precipitate was filtered, washed and recrystallized from 95% ethanol.

Step-V: Synthesis of 5-aryl-4-(chloroacetylamino)-3-mercapto-1,2,4- triazole:

In a two necked flask fitted with reflux condenser containing 100 mL benzene and obtained compound and separating funnel contained chloro acetyl chloride in 30

mL benzene. The mixture was refluxed and chloro acetyl chloride was added in small portions. After addition of chloro acetyl chloride, solution was again refluxed for 5-6 h, cooled and contents were poured on crushed ice. The obtained precipitate was filtered, washed and recrystallized from absolute ethanol.

Step-VI: Synthesis of amino derivative of 5-aryl-4-(chloroacetylamino) -3-mercapto-1, 2, 4- triazole:

Synthesized substituted 5-aryl-4-(chloroacetylamino)-3-mercapto-1, 2, 4-triazole (0.03 mol), respective amines (0.03 mol) and 75 mL benzene was taken in round bottom flask. The contents were refluxed for 5-6 h and cooled. Filtered the precipitate and washed with distilled water several times to remove traces of hydrochloride. Product obtained was recrystallized from appropriate solvent.

Characterization of synthesized compounds:

01.2-(Dimethylamino)-N-(3-mercapto-5-phenyl-4H-1,2,4-triazol-4-yl)-acetamide (NJ-01)

IR (KBr,cm^{-1}):3310 (N-H stretching),3116.0 (aromatic C-H stereching), 2962.6 (C-H streching of methyl group), 2928.8 & 2858.7 (C-H streching of methylene group),2584.5 (S-H streching),1660.6 (C=O streching),1614.8(C=N streching),1580.0 (C=C streching), 760.8 (C-H out of plane bending), 670.0 (C-S streching)

MS(m/s): M^+ Calculated – 277

^{1}H-NMR (400 MHz,DMSO-d_6) δ 8.0(s, 1H, -NH), 7.2-7.6(m, 5H, -C_6H_5), 3.25(s, 2H, -CH_2), 3.1(s, 1H, -SH), 2.3(s, 6H, -CH_3)

02. 2-(Diethyl amino)-N-(3-mercapto-5-phenyl-4H-1,2,4-triazol-4-yl)-acetamide (NJ-02)

IR (KBr,cm^{-1}):3349.1 (N-H stretching),3116.0 (aromatic C-H stereching),2968.0 (C-H streching of methyl group), 2928.8 & 2848.9 (C-H streching of methylene group),2556.4 (S-H streching),1579.9 (C=N streching), 1664.0 (C=O streching),1579.9 (C=C streching), 786.7 (C-H out of plane bending), 661.5,623.1 (C-S streching)

MS(m/s): M^+ Calculated – 305

^{1}H-NMR (400 MHz,DMSO-d_6) δ 8.0(s, 1H, -NH), 7.2-7.6(m, 5H, -C_6H_5), 3.25(s, 2H, -CH_2), 3.1(s, 1H, -SH), 2.5(q, 6H, -CH_3), 1.2(t,2H,CH_2)

03. 2-(Dipropyl amino)-N-(3-mercapto-5-phenyl-4H-1,2,4-triazol-4-yl)-acetamide (NJ-03)

IR (KBr,cm^{-1}):3355.1 (N-H stretching),3116.2 (aromatic C-H stereching),2968.0 (C-H streching of methyl group), 2928.8 & 2848.9 (C-H streching of methylene

group),2556.4 (S-H streching),1416.1 (C=N streching),1662.0 (C=O streching),1579.9 (C=C streching), 823.0 (C-H out of plane bending), 661.5,623.1 (C-S streching)

MS(m/s): M^+ Calculated – 333

^{1}H-NMR (400 MHz,DMSO-d_6) δ 8.0(s, 1H, -NH), 7.2-7.6(m, 5H, -C_6H_5), 3.25(s, 2H, -CH_2), 3.1(s, 1H, -SH), 2.9(t,2H,-CH_2), 1.7 (m,5H,-CH_2CH_3),1.1 (t,2H,-CH_2)

04.2-(Diisopropyl amino)-N-(3-mercapto-5-phenyl-4H-1,2,4-triazol-4-yl)-acetamide (NJ-04)

IR (KBr,cm^{-1}):3325.5 (N-H stretching),3102.5,3095.5 (aromatic C-H stereching),2580.1 (S-H streching),1662.7 (C=O streching), 1429.3 (C=N streching), 1575.6 (C=C streching),1380.8,1365.5 (C-H bendin due to isoprpyl group),819.1 (C-H out of plane bending), 684.0, 630.1 (C-S streching)

MS(m/s): M^+ Calculated – 333

^{1}H-NMR (400 MHz,DMSO-d_6) δ 8.0(s, 1H, -NH), 7.2-7.6(m, 5H, -C_6H_5), 3.25(s, 2H, -CH_2), 3.1(s, 1H, -SH), 2.3(s, 6H, -CH_3),1.1(d,1H,-CH)

05. 2-(Dimethyl amino)-N-(3-mercapto-5-p-tolyl-4H-1, 2, 4-triazol-4-yl)-acetamide (NJ-05)

IR (KBr,cm^{-1}):3226.6 (N-H stretching),3106.0 (aromatic C-H stereching),2958.0 (C-H streching of methyl group), 2928.8 & 2848.9 (C-H streching of methylene group),2564.4 (S-H streching),1662.6 (C=O streching),1611.4 (C=N streching), 787.8 (C-H out of plane bending). **MS(m/s):** M^+ Calculated – 291

^{1}H-NMR (400 MHz,DMSO-d_6) δ 8.0(s, 1H, -NH), 7.0-7.2(m, 4H, -C_6H_5), 3.25(s, 2H, -CH_2), 3.1(s, 1H, -SH), 2.3(s, 6H, -CH_3),2.1 (s,3H,CH_3)

06. 2-(Diethyl amino)-N-(3-mercapto-5-p-tolyl-4H-1,2,4-triazol-4-yl)-acetamide (NJ-06)

IR (KBr,cm^{-1}):3266.6 (N-H stretching),3106.0 (aromatic C-H stereching),2958.0 (C-H streching of methyl group), 2928.8 & 2848.9 (C-H streching of methylene group),2556.4 (S-H streching),1662.6 (C=O streching),1573.8 (C=C streching), 684.9,631.3 (C-S streching)

MS(m/s): M^+ Calculated – 319

^{1}H-NMR (400 MHz,DMSO-d_6) δ 8.0(s, 1H, -NH), 7.2-7.6(m, 5H, -C_6H_5), 3.25(s, 2H, -CH_2), 3.1(s, 1H, -SH),2.5 (q,3H,-CH_3,) 1.1 (t, 2H, -CH_2).

07. 2-(Dipropyl amino)-N-(3-mercapto-5-p-tolyl-4H-1,2,4-triazol-4-yl)-acetamide (NJ-07)

IR (KBr,cm^{-1}):3325.1 (N-H stretching),3116.2 (aromatic C-H stereching),2968.0 (C-H streching of methyl group), 2928.8 & 2848.9 (C-H streching of methylene

group),2556.4 (S-H streching),1626.6 (C=O streching),1579.9 (C=C streching), 1416.1 (C=N streching), 823.0 (C-H out of plane bending), 661.5,623.1 (C-S streching)

MS(m/s): M^+ Calculated – 347

^{1}H-NMR (400 MHz,DMSO-d_6) δ 8.0(s, 1H, -NH), 7.0-7.2(m, 4H, -C_6H_4), 3.25(s, 2H, -CH_2), 3.1(s, 1H, -SH),2.9 (t,3H,-CH_2),2.3 (s,3H,-CH_3),1.7 (m,5H,-CH_2CH_3), 1.1 (t, 2H, -CH_2).

08. 2-(Diisopropyl amino)-N-(3-mercapto-5-p-tolyl-4H-1,2,4-triazol-4-yl)-acetamide (NJ-08)

IR (KBr,cm^{-1}):3292.5 (N-H stretching),3102.5,3045.5 (aromatic C-H stereching), 2582.0 (S-H streching),1429.3 (C=N streching),1575.6 (C=C streching),1662.7(C=O streching), 1380.8,1365.5 (C-H bending due to isopropyl group), 921.6 (C-H bending, rocking), 819.1 (C-H out of plane bending), 674.0,630.1 (C-S streching)

MS(m/s): M^+ Calculated – 247

^{1}H-NMR (400 MHz,DMSO-d_6) δ 8.0(s, 1H, -NH), 7.0-7.2(m, 4H, -C_6H_4), 3.25(s, 2H, -CH_2), 3.1(s, 1H, -SH), 2.9(m, 6H, -CH_3), 2.3 (s, 3H, -CH_3), 1.1(d, 1H, -CH).

09.2-(Dimethylamino)-N-(3-mercapto-5-(4-methoxyphenyl)-4H-1,2,4-triazol-4-yl)-acetamide (NJ-09)

IR (KBr,cm^{-1}):3310 (N-H stretching), 2952.6 (C-H streching of methyl group), 2928.8 & 2858.7 (C-H streching of methylene group),2584.5 (S-H streching),1660.6 (C=O streching), 1614.8 (C=N streching),1585.0 (C=C streching), 773.4 (C-H out of plane bending), 614.5 (C-Sstreching)

MS(m/s): M^+ Calculated – 307

^{1}H-NMR (400 MHz,DMSO-d_6) δ 8.0(s, 1H, -NH), 6.7-6.9 (m, 4H, -C_6H_4), 3.7(s, 3H, -OCH_2), 3.25(s, 2H, -CH_2), 3.1(s, 1H, -SH), 2.3(s, 5H, -CH_3)

10.2-(Diethylamino)-N-(3-mercapto-5-(4-methoxyphenyl)-4H-1,2,4-triazol-4-yl)-acetamide (NJ-10)

IR (KBr,cm^{-1}):3319.1 (N-H stretching),3116.2 (aromatic C-H stereching),2958.0 (C-H streching of methyl group), 2925.8 & 2848.9 (C-H streching of methylene group),2566.4 (S-H streching),1644.6 (C=O streching),1579.9 (C=C streching), 786.7 (C-H out of plane bending), 671.5,623.1 (C-Sstreching)

MS(m/s): M^+ Calculated – 335

^{1}H-NMR (400 MHz,DMSO-d_6) δ 8.0(s, 1H, -NH), 6.7-6.9 (m, 4H, -C_6H_4), 3.7(s, 2H, -OCH_3), 3.25(s, 2H, -CH_2), 3.1(s, 1H, -SH), 2.5(q, 3H, -CH_3), 1.1(s, 3H, -CH_3),

11.2-(Dipropylamino)-N-(3-mercapto-5-(4-methoxyphenyl)-4H-1,2,4-triazol-4-yl)acetamide (NJ-11)

IR (KBr,cm^{-1}):3310.9 (N-H stretching),3116.9,3032.1 (aromatic C-H stereching),2928.7 (C-H streching of methylene group),2562.8.5 (S-H streching),1660.0 (C=O streching),1562.9 (C=C streching), 1313.6 (C=N streching), 822.6 (C-H out of plane bending), 670.0,615.0 (C-S streching)

MS(m/s): M^+ Calculated – 363

^{1}H-NMR (400 MHz,DMSO-d_6) δ 8.0(s, 1H, -NH), 6.7-6.9(m, 4H, -C_6H_4),3.7(s, 3H, -OCH_3) 3.25(s, 2H, -CH_2), 3.1(s, 1H, -SH), 2.9(s, 2H, -CH_2), 1.7(m, 5H, -CH_2CH_3),1.1(t, 2H, -CH_2).

12.2-(Diisopropylamino)-N-(3-mercapto-5-(4-methoxyphenyl)-4H-1,2,4-triazol-4-yl)acetamide (NJ-12)

IR (KBr,cm^{-1}): 3310 (N-H stretching),3116.9,3032.1 (aromatic C-H stereching), 2928.7 & 2851.3(C-H streching of methylene group),2562.8 (S-H streching),1660.0 (C=O streching),1562.9 (C=C streching), 1520.0 (C=N streching),1384.7,1373.7 (C-H Bending due to isopropyl group), 922.6 (C-H bending,rocking) 822.6 (C-H out of plane bending), 670.9,615.0 (C-S streching)

MS(m/s): M^+ Calculated – 363

^{1}H-NMR (400 MHz,DMSO-d_6) δ 8.0(s, 1H, -NH), 6.7-6.9(m, 4H, -C_6H_4),3.7(s, 3H, -OCH_3) 3.25(s, 2H, -CH_2), 3.1(s, 1H, -SH), 2.9(m, 6H, -CH_3), 1.1(d, 1H, -CH).

Antimicrobial studies: All the test compounds were evaluated for the antibacterial activity against Staphylococcus aurous, Bacillus subtilis (gram positive) Escherichia coli and Pseudomonas aeruginosa (gram negative) and antifungal activity against Candida albicans. Nutrient broth was used for the preparation of inoculums of bacteria and Mueller Hinton's agar was used for the screening method. The test bacteria and fungi were sub cultured using Mueller Hinton's agar and dextrose medium respectively. The tube containing sterilized medium were inoculated with respective bacterial strain. After incubation at 37±1°C (bacteria) and 25±1°C (fungi) for 24 hours. They are stocked in refrigerator. The stock cultures were maintained. bacterial inoculums was prepared by transferring a loop full of stick culture to nutrient broth 100ml in conical flask the flask were incubated at 37±1°C (bacteria) and 25±1°C (fungi) for 24 hours before the experimentation.

Preparation of Antimicrobial Test solution: Test solution of the compound were prepared by dissolving 10 mg of compound in 10 ml of giving solution with concentration of 1000 µg/ml. Standard solution of antibiotics and antifungal agent were prepared by dissolving 10 mg of compound in 10 ml of solution, for each tested

strain, the growth condition and the stability of the medium were checked in negative controls.

Culture Medium: Nutrient broth was used for the preparation of inoculums of the bacteria and Mueller Hinton agar was used for the screening method. The test bacteria and fungi were sub culture using Mueller Hinton agar and dextrose agar medium. The tube containing sterilized medium were inoculated with respective bacterial strain. After incubation at 37±1°C (bacteria) and 25±1°C (fungi) for 24 hours. They are stocked in refrigerator. The stock cultures were maintained. bacterial inoculums was prepared by transferring a loop full of stick culture to nutrient broth 100ml in conical flask the flask were incubated at 37±1°C (bacteria) and 25±1°C (fungi) for 24 hours before the experimentation.

Antibacterial Testing: The Mueller Hinton agar medium, the Petri plates and flask plugged with cotton was sterilized was sterilized by autoclaving at 121^0C. In each sterilized Petri plate (10 ml in diameter) about 30ml of molten nutrient agar medium inoculated with the respective strain of bacteria was transfer aseptically. The plate was left at room temperature to allow the solidification. In each plate, were placed on the medium in which solution of test compound were added and labeled accordingly. The plate was kept undisturbed for at least 20 min in refrigerator to allow diffusion of the solution properly in the nutrient agar medium. The plates were incubated at 37±1°C for 24 hours.

Antifungal Testing: The Dextrose agar medium, the petri-plate and flask unplugged with cotton was sterilized by autoclaving at 121^0C In each sterilized Petri plate (10 ml in diameter) about 30ml of molten nutrient agar medium inoculated with the respective strain of fungi was transfer aseptically. The plate was left at room temperature to allow the solidification. In each plate, were placed on the medium in which solution of test compound were added and labeled accordingly. The plate was kept undisturbed for at least 20 min in refrigerator to allow diffusion of the solution properly in the nutrient agar medium. The plates were incubated at 25±1°C for 24 hours.

Minimum Inhibitory Concentration Determination:

The solution of newly synthesized compounds and standard drug was compared at 1000, 500, 250, 125, 62.5 and 31.5 µg/ml concentration in well of micro plate by diluting in liquid standard nutrient broth. The bacteria suspensions used for inoculation were prepared 10^6cells/ml by diluting fresh culture. The lowest

concentration of the compound that inhibits macroscopic growth was determined and minimum inhibitory concentrations {MICs} were reported.

Anticonvulsant Activity Study:

Anticonvulsant activity was determined by Maximal Electro Shock (MES) induced method. Albino rats of either sex weighing 150-200 g were divided into different group for different synthesized compound, control and standard. The animals of all groups were treated with 100 mg/kg, 150mg/kg in suitable solvent by i.p. route. Except control group which received only solvent. Standard group received (Phenytoin) 25 mg/kg body weight by i.p. route. The effect of drug was observed after 30 min and 4h of the drug treatment.

Seizures were produced in rats by 'Techno' convulsometer by delivering a current of 150 mA through the corneal electrodes for a period of 0.2 seconds. The animal was placed on the table and its head was fixed. The electrodes were dipped in normal saline and placed gently on the cornea. The shock was delivered by putting on the switch of the instrument. The animals were observed for the following parameters.

(a) Tonic phases - Flexor phase - Extensor phase
(b) Clonic phase (intermediate jerking of the limbs).
(c) Stupor (unconsciousness).
(d) Recovery/Death.

Time for each phase was noted by stop watch. Drug treated animals, were observed for presence or absence of extensor and flexor component of tonic phase during seizures.

Result and discussion:

All the Physiochemical properties such as their Melting point determination, TLC (determination of R_F value) and percentage yield data of compounds summarized in **Table No.01**.

Anticonvulsant screening of synthesized compounds NJ1, NJ5 and NJ9 showed seizure protection at both 100 and 150 mg/kg dose after 30 min and 4 h showing quick onset of action. The synthesized compound NJ2, NJ6 and NJ10 were somewhat less active than NJ1, NJ5 and NJ9 reveals that their high concentration is required to cross blood brain barrier. Remaining compound (NJ3, NJ7, NJ11, NJ4, NJ8, and NJ12) was inactive as shown in **Table No.02**

Comparison of anticonvulsant activity:

1. Effect of substituents on benzene ring

NJ05>NJ01>NJ09

From present study it was found that methyl substituted ring was most active and unsubstituted ring was least active. Methoxy substituted compound was intermittent in activity. Study revealed that electron releasing\donating group generally exhibiting activity.

2. Effect of chain length

NJ01>NJ02>NJ03>NJ04>NJ05>NJ06>NJ07>NJ08>NJ09>NJ10>NJ11>NJ12

Study revealed that as chain length of compounds is increased from methyl to propyl or isopropyl, the activity of compounds decreases. It may be due to steric hindrance. Electron donating group of benzene ring exhibited better anticonvulsant activity as compared to unsubstitution.

Antimicrobial screening of synthesized compounds NJ1, NJ5 and NJ9 showed maximum growth inhibition activity against bacteria and fungi. The synthesized compound NJ2, NJ6, NJ7, NJ10 and NJ11 were somewhat less active than NJ1, NJ5 and NJ9. Remaining compounds (NJ3, NJ4, NJ8 and NJ12) were near to inactive, as shown in **Table No.03 and 04**.

Comparison of antimicrobial activity:

1. Effect of substituents on benzene ring

NJ09>NJ05>NJ1

From present study it was found that methoxy substituted ring was most active and unsubstituted ring was least active. Methyl substituted compound was intermittent in activity. Study revealed that electron releasing/electron donating group generally exhibited activity.

2. Effect of chain length

NJ09>NJ10>NJ05>NJ06>NJ01>NJ02>NJ11>NJ12>NJ07>NJ08>NJ03>NJ04

Study revealed that as chain length of compounds is increased from methyl to propyl or isopropyl, the activity of compounds decreases. It may be due to steric hindrance. Electron donating group of benzene ring exhibited better antimicrobial activity as compared to unsubstitution.

Conclusion: The synthesized compounds were then evaluated for their anticonvulsant studies. The anticonvulsant studies were performed on albino rats by Maximal Electro Shock (MES) induced method using Phenytoin as the reference drug. It was found that the compound NJ-1, NJ-5, NJ-9 showed better activity while

compounds NJ-2, NJ-6, NJ-10 showed good activity and the remaining other compounds were inactive.

Electron donating group of benzene ring exhibited better anticonvulsant activity as compared to unsubstitution. Further work may be undertaken on this nucleus for the search of better anticonvulsant agents with less toxicity.

The synthesized compounds were then again subjected to biological evaluation. The activity was greatly influenced by ring substituents. Ampicillin and Griseofulvin were used as reference drugs, Ampicillin as antibacterial and Griseofulvin as antifungal drug.

It was found that the compound NJ-1, NJ -5, NJ -9 showed better activity while compounds NJ-2, NJ-6, NJ-7, NJ-10 and NJ-11 showed good activity and the remaining other compounds were near to inactive.

Electron donating group of benzene ring exhibited better antimicrobial activity as compared to unsubstitution. Further work may be undertaken on this nucleus for the search of better antimicrobial agents with less toxicity.

Reference:

01. *Breman, Alilion, Mills A. conquering the intolerable burden of malaria: what'new, what's needed; a summary? Ame. jour. of Trop. medi 71:1-15,2004.*
02. *1. Tsukuda T, Shiratori Y, Watanabe M, Ontsuka H, Hattori K, Shirai M, et al. Modeling, synthesis and biological activity of novel antifungal agents (1) Bioorg Med Chem Lett. 1998; 8:1819–24. [PubMed] [Google Scholar]*
03. *2. Küçükgüzel I, Güniz Küçükgüzel S, Rollas S, Otük-Sanis G, Ozdemir O, Bayrak I, et al. Synthesis of some 3-(arylalkylthio)-4-alkyl/aryl-5-(4-aminophenyl)-4H-1,2,4-triazole derivatives and their anticonvulsant activity. Farmaco. 2004; 59:893–901. [PubMed] [Google Scholar]*
04. *3. Husain MI, Amir M. Synthesis of some new substituted thiosemicarbazides and triazoles as possible anticonvulsants. J Indian Chem Soc. 1986; 63:317–9. [Google Scholar]*
05. *4. Valentina P, Ilango K, Deepthi M, Harusha P, Pavani G, Sindhura KL, et al. Antioxidant activity of some substituted 1,2,4-triazo-5-thione Schiff base. J Pharm Sci Res. 2009; 1:74–7. [Google Scholar]*
06. *5. Sahin G, Palaska E, Kelicen P, Demirdamar R, Altinok G. Synthesis of some new 1-acylthiosemicarbazides, 1,3,4-oxadiazoles, 1,3,4-thiadiazoles and 1,2,4-triazole-3-thiones and their anti-inflammatory activities. Arzneimittelforschung. 2001; 51:478–84. [PubMed] [Google Scholar]*
07. *Siddiqui, A.; Islam, U.; Siddiqui, N., Asian J. Chem., 2004, 16, 534.*

08. *Bahel, S.C.; Srivastava, S.K.; Pathak, R.B., Indian. J. Pharm. Sci., 1989, 28, 254.*
09. *Srivastava, S.K.; Srivastava S.; Srivastava, S.D., Ind. J. Chem. 2002, 41B, , 1937.*
10. *Zamini, K.; Faghihi K.; Tofighi, T.; Shariatzadeh, M.R., Turk J. Chem., 2004, 28, 95-100.*
11. *Udupi, R.H.; Kulkarni, V.M.; Purushottamchar, P.; Srinivasalu, N., J. Ind. Chem. Soc., 2002, 79, 381-382.*
12. *Amir, M.; Khan, M.S.Y.; Zaman, M.S., Indian. J. Chem., 2004, 43B, 2189.*
13. *Mehboob, R.; Ramalingom, T.; Satur, P.B., Indian. J. Chem., 1989, 28B, 695.*
14. *Kucukguzel, I., Kucukguzel, G.S., Rollas, S., Otuk-Sanis, G., Ozdemir, O., Bayrak, I., Altug, T., and Stables, J.P., "Synthesis of some 3-(Arylalkylthio)-4-alkyl/aryl-5-(4-aminophenyl)-4H-1, 2, 4-triazole derivatives and their anticonvulsant activity", IL Farmaco, 2004, 59, 893-901.*
15. *Almasirad, A., Tabatabai, A.S., Faizi, M., Kebriaeezadeh, A., Mehrabi N., Dalvandi, A. and Shafiee A., Bioorganic & Medicinal Chemistry Letters, 2004, 14, 6057-6059.*

Table 01: Physical parameters of triazole derivatives synthesized compounds

S. No.	R	R_1 & R_2	Mol. Form.	Rf Value	Mol.Wt.	%Yield	M.P. (°C)
NJ-1	H	Methyl	$C_{12}H_{15}O_1N_5S_1$	0.56	277	56	170-172
NJ-2	H	Ethyl	$C_{14}H_{19}O_1N_5S_1$	0.63	305	63	153-155
NJ-3	H	Propyl	$C_{16}H_{23}O_1N_5S_1$	0.45	333	46	174-176
NJ-4	H	Isopropyl	$C_{16}H_{23}O_1N_5S_1$	0.66	333	52	108-110
NJ-5	CH_3	Methyl	$C_{13}H_{18}O_1N_5S_1$	0.48	291	48	158-160
NJ-6	CH_3	Ethyl	$C_{15}H_{22}O_1N_5S_1$	0.52	319	42	128-130
NJ-7	CH_3	Propyl	$C_{17}H_{26}O_1N_5S_1$	0.74	347	54	184-186
NJ-8	CH_3	Isopropyl	$C_{17}H_{26}O_1N_5S_1$	0.61	347	46	202-204
NJ-9	OCH_3	Methyl	$C_{13}H_{18}O_2N_5S_1$	0.59	307	55	212-214
NJ-10	OCH_3	Ethyl	$C_{15}H_{22}O_2N_5S_1$	0.60	335	49	166-168
NJ-11	OCH_3	Propyl	$C_{17}H_{26}O_2N_5S_1$	0.68	363	61	196-198
NJ-12	OCH_3	Isopropyl	$C_{17}H_{26}O_2N_5S_1$	0.78	363	58	182-184

Table 02. Effect of Synthesized Compounds on Maximal Electro Shock Convulsion in albino rats. (At 150 mg/kg dose after 4 h)

Code No.	Time(sec) in various phase of convulsion				
	Flexion (mean±SE)	Extensor (mean±SE)	Clonic (mean±SE)	Stupor (mean±SE)	Recovery /Death
NJ1	0.9±0.1	5.9±0.3	2.0±0.2	99±0.2	Recovery
NJ2	1.6±0.2	6.9±0.3	3.1±0.2	112±0.2	Recovery
NJ3	2.8±0.2	11.5± 0.6	6.9±0.3	129±0.2	Recovery
NJ4	3.9±0.3	11.8±0.6	6.9±0.3	128±0.2	Recovery
NJ5	0.6±0.2	4.9±0.7	0.8±0.3	90±0.2	Recovery
NJ6	1.5±0.2	6.5±0.7	3.1±0.2	106±0.3	Recovery
NJ7	2.8±0.3	11.3±0.6	6.8±0.2	125±0.3	Recovery
NJ8	3.8±0.3	13.8±0.6	6.8±0.3	133±0.2	Recovery
NJ9	1.0±0.2	7.1±0.7	3.4±0.2	111±0.2	Recovery
NJ10	2.1±0.2	7.5±0.7	3.9±0.3	121±0.3	Recovery
NJ11	2.6.± 0.2	12.8±0.5	5.9±0.4	121±0.2	Recovery
NJ12	3.9±0.4	13.4±0.4	7.3±0.2	132±0.2	Recovery
C	4.0±0.3	13.8±0.5	7.8±0.3	133±0.4	Recovery
SD	-	4±0.7	0.8±0.3	86±1.8	Recovery

*** C: Control, * SD: standard (Phenytoin), * - : No Activity**

Table 03: Data of antimicrobial activity of synthesized 1,2,4-Triazole Derivatives

S. No.	Compound	Diameter of zone of inhibition (mm)				
		B. subtilis	S. aureus	E. coli	P. aeruginosa	C. albicans
1	NJ1	14	16	15	14	13
2	NJ2	13	14	15	13	11
3	NJ3	11	14	13	12	11
4	NJ4	10	13	13	11	10
5	NJ5	15	18	16	15	14

6	NJ6	14	17	16	14	13
7	NJ7	14	15	14	12	13
8	NJ8	11	14	13	10	12
9	NJ9	16	19	17	15	15
10	NJ10	15	18	17	14	15
11	NJ11	13	18	15	14	13
12	NJ12	12	15	14	12	13
Ampicillin		16	20	18	15	-
Griseofulvin		-		-	-	16

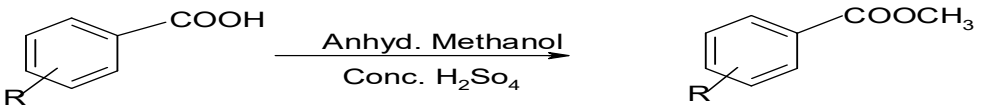
Table 04. Data of antibacterial and antifungal activity of synthesized 1,2,4-Triazole Derivatives

S. No.	**Comp.**	**B. subtilis**				**S. aureus**				**E. coli**				**P. aeruginosa**				**C. albicans**			
Dilution		**I**	**II**	**III**	**IV-V**	**I**	**II**	**III**	**IV-V**	**I**	**II**	**III**	**IV-V**	**I**	**II**	**III**	**IV-V**	**I**	**II**	**III**	**IV-V**
1	NJ1	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+
2	NJ2	-	-	-	+	-	-	+	+	-	-	+	+	-	-	-	+	-	-	+	+
3	NJ3	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
4	NJ4	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
5	NJ5	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+
6	NJ6	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+
7	NJ7	-	-	+	+	-	-	-	+	-	-	+	+	-	-	-	+	-	-	+	+
8	NJ8	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
9	NJ9	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+
10	NJ10	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+
11	NJ11	-	-	-	+	-	-	+	+	-	-	+	+	-	-	-	+	-	-	+	+
12	NJ12	-	-	+	+	-	-	+	+	-	-	+	+	-	-	-	+	-	-	+	+
Ampicillin		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
Griseofulvin																		-	-	-	-

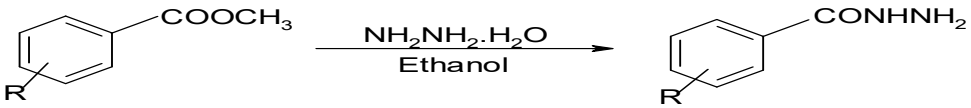
I-1000μg/ml, II-500μg/ml, III-250μg/ml, IV-125μg/ml, V-62.5μg/ml
(-) indicates absence of growth; (+) indicates presence of growth

Figure-01- Scheme / Chemical Reaction of Triazole Derivatives

(Step-I): Esters *of aromatic acid*

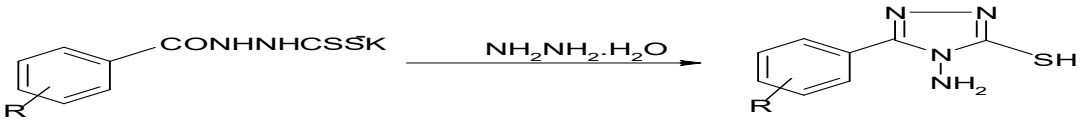


(Step-II): Hydrazide of synthesized ester



(Step-III): Potassium dithiocarbazinate

(Step-IV): 5-aryl-4-amino-3-mercapto-1,2,4-triazole



(Step-V): 5-aryl-4-(chloroacetylamino)-3-mercapto-1,2,4- triazole:

(Step-VI): Amino derivative of 5-aryl-4-(chloroacetylamino) -3-mercapto-1,2,4- triazole:

R	R_1 & R_2 (Amines)
H	Methyl
H	Ethyl
H	Propyl
H	Isopropyl
CH3	Methyl
CH3	Ethyl
CH3	Propyl
CH3	Isopropyl
OCH3	Methyl
OCH3	Ethyl
OCH3	Propyl
OCH3	Isopropyl

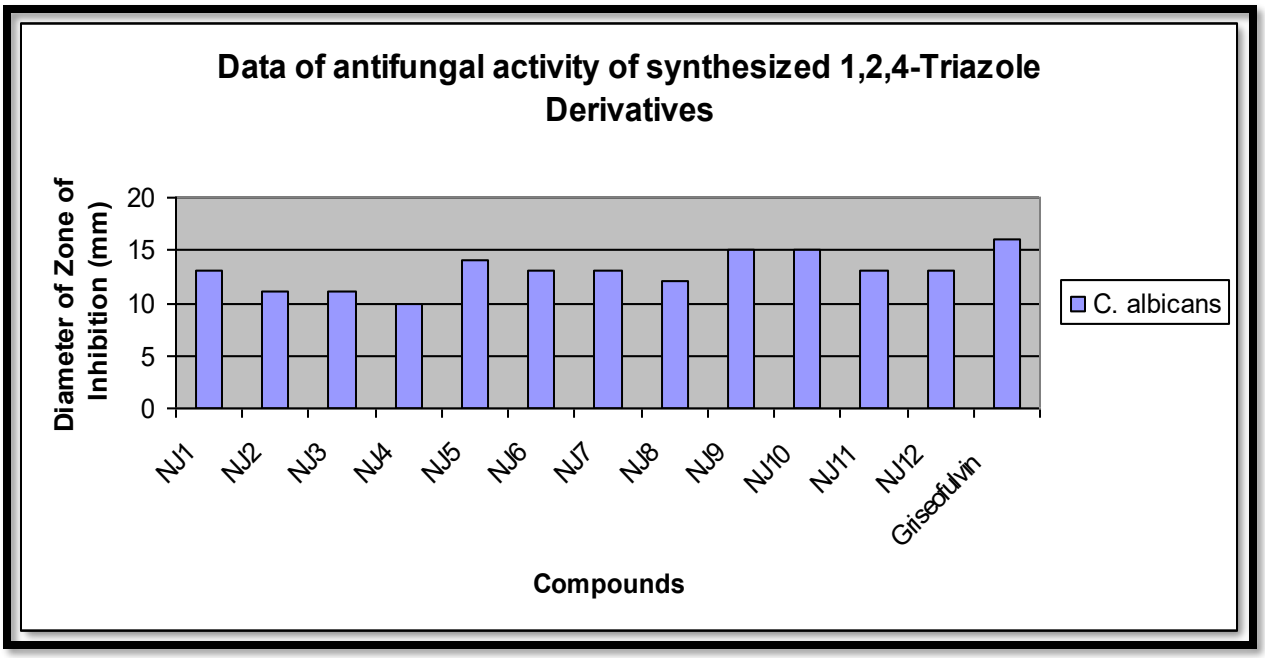


Fig.03 -Antifungal screening indicated that among the compound NJ-01.NJ-05 and NJ-09 reveled that the test compounds showed moderate activity against Candida albicans.