Synthesis, Structural Elucidation and Anti Microbial Screening of \(N\)-(4-aryl amine)-2-\{4-phenyl-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-yl\}sulfanyl\}acetamide Derivatives

KEYWORDS
1,2,4 triazole, Acetamide, Antifungal activity, Anti bacterial activity

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ABSTRACT A new series of 2-\{4-phenyl-5-(pyridine-4-phenyl)-4H-\}1,2,4-triazole-3\text-y1 sulfanyl\}N-aryl-acetamide have been synthesized by the condensation of 4-phenyl-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol and 2-Chloro-N-(aryl)-acetamide in presence of anhydrous potassium Carbonate. The Structure of Synthesized compound was assigned by H\text-NMR, MASS Spectra, IR Spectra and Elemental Analysis. All the compound were Screened for their in-vitro Antibacterial, Antifungal and anti tuberculosis activity.

Introduction
The synthesis of compound containing 1,2,4 triazole ring system has attracted attention because of its wide range of pharmaceutical activity. A variety of biological compound such as anti-inflammatory, analgesic, antibacterial, antifungal, anti tubercular, antiviral, antitumor, anticonvulsant, and anti depressant have been reported for Mercapto and thione substituted 1,2,4 triazole ring system\textsuperscript{1,2,7}.

In last few decades the chemistry of 1,2,4-triazole and their fused heterocyclic derivatives has received important because of there effective biological importance. A wide number of drugs containing 1,2,4-triazole ring system are incorporated because of there widely therapeutically interests including anti inflammatory, CNS stimulants, sedatives, anti anxiety, such as fluconazole, intraconazole, Voriconazole, Also there known drug containing the 1,2,4-triazole group eg. Triazolam, Alprazolam, Etizolam and Furaclylin\textsuperscript{2,6,8}.

Moreover sulphur containing heterocycles represents important group of sulphur compound that are promising for use in practical application\textsuperscript{1}. Among these heterocycles the Mercapto and Thione substituted 1,2,4-triazole ring system have been well studied\textsuperscript{4,9}.

Acetanilide derivatives are reported to exhibit a number of biological activities including anesthetic, antipyretic, anti inflammatory, and anti bacterial effects substitution including alkyl thio and alkenylthio derivatives have been carried out primarily at the third position of the 1,2,4-triazole ring as potential antimicrobial agents\textsuperscript{5,1}.

In continuation of our interest on chemistry of functionalized chloroacetamide derivatives because of the high mobility of chlorine atom and reactive N-H group compound containing chloroacetamide.

Materials and Methods
All melting points were determined using open capillary tubes on electronic apparatus and were uncorrected. The IR spectra (4000-400 cm\textsuperscript{-1}) of synthesized compounds were recorded on Shimadzu 8400-s FTIR spectrometer with KBr pellets. To monitor the reactions, establish the identity, purity of reactants and products, thin layer chromatography performed on TLC coated with silica gel using appropriate mobile phase system and spots visualized under UV radiation. Nuclear magnetic resonance spectra was recorded using Bruker 400 MHz model spectrometer using DMSO as a solvent and TMS as internal standard (Chemical shifts in d ppm). All new compounds subjected to elemental analysis and the results obtained were in acceptable range.
The mixture of 4-phenyl-5-(pyridine-4-yl)-4H-1,2,4-triazol-3-yl[sulfanyl]acetamide

**Step 1**
Pyridine-4-carboxylic acid (0.1 mole) in 200 ml methanol and 6.0 ml concentrated H$_2$SO$_4$ was refluxed 12 hours and poured into the cold ice. The obtained product was filtered and washed with cold water. Recrystallized from alcohol. The progress of the reaction was monitored by TLC using toluene:acetone (8:2) as eluent.

**Step 2**
Methyl pyridine-4-carboxylate (0.1 mole) and hydrazine hydrate (0.2 mole) in methanol was refluxed for 15 hours and poured into the ice. The obtained product is filtered and washed with cold water. Recrystallized from ethyl alcohol. The progress of the reaction was monitored by TLC using toluene:acetone (8:2) as eluent.

**Step 3**
The mixture of pyridine-4-carboxydrazide (0.1 mole) and phenyl isothiocyanate (0.1 mole) was refluxed in ethanol (220 ml) for 3 hours. After cooling the formed product was collected by filtration and recrystallized from ethanol. The progress of the reaction was monitored by TLC using toluene:acetone (8:2) as eluent.

**Step 4**
The mixture of N-phenyl-2-(pyridine-4-carbonyl)hydrazinecarbothioamide (0.05 mole) and 80 ml of 2N NaOH was refluxed for 4 hours. The resulting solution was cooled and poured into the ice and neutralized with 2N HCl. The precipitate was filtered and washed with cold water. Dried and recrystallized from ethanol. The progress of the reaction was monitored by TLC using toluene:acetone (8:2) as eluent.

**Step 5**
0.02 mole of chloroacetyl chloride and 2-4 drops of triethylamine was added in the 30 ml of benzene. This mixture was stirred in ice bath. The solution of aryl amine (0.02 mole) and 2-4 drops of triethylamine was added in the 30 ml of benzene. This mixture was refluxed for 5 hours. The resulting ppt. upon cooling was filtered and washed with benzene. Recrystallized from ethanol. The progress of the reaction was monitored by TLC using toluene:acetone (8:2) as eluent.

**Step 6**
The mixture of 4-phenyl-5-(pyridine-4-yl)-4H-1,2,4-triazole-3-thiol (0.01 mole) and 2-chloro-N-(aryl)-acetamide (0.01 mole) in 50 ml dry acetonitrile and anhydrous K$_2$CO$_3$ (0.02 mole) was stirred for 4 hours at room temp. and poured into ice. The product was filtered and washed with cold water. Recrystallized from alcohol.

N-(4-methylphenyl)-2-[(4-phenyl-5-(pyridine-4-yl)-4H-1,2,4-triazol-3-yl)sulfanyl]acetamide

**FTIR** (KBr cm$^{-1}$): 1513 cm$^{-1}$ (C=N in triazole), 3387 cm$^{-1}$ (NH Stretching in Amide), 1633 cm$^{-1}$ (C=O in amide), 1441 cm$^{-1}$ (C=C in aromatic), 1601 cm$^{-1}$ (C=S in thioether linkage), 1641 cm$^{-1}$ (C=C in aromatic), 1522 cm$^{-1}$ (C=N in triazole), 3357 cm$^{-1}$ (-NH), 1601 cm$^{-1}$ (C=C in aromatic), 1641 cm$^{-1}$ (C=S in thioether linkage), 1633 cm$^{-1}$ (C=O in amide), 1441 cm$^{-1}$ (C=C in aromatic), 1523 cm$^{-1}$ (C=N in triazole), 3373 cm$^{-1}$ (NH Stretching in Amide), 1657 cm$^{-1}$ (C=O in amide), 1447 cm$^{-1}$ (C=C in aromatic), 1623 cm$^{-1}$ (C=S in thioether linkage), m/z: 467.7 (M$^+$)

N-(4-chloro-3-fluorophenyl)-2-[(4-phenyl-5-(pyridine-4-yl)-4H-1,2,4-triazol-3-yl)sulfanyl]acetamide

**FTIR** (KBr cm$^{-1}$): 1516 cm$^{-1}$ (C=N in triazole), 3384 cm$^{-1}$ (NH Stretching in Amide), 1635 cm$^{-1}$ (C=O in amide), 1444 cm$^{-1}$ (C=C in aromatic), 1611 cm$^{-1}$ (C=S in thioether linkage), m/z: 406.2 (M$^+$)

N-(4-methoxyphenyl)-2-[(4-phenyl-5-(pyridine-4-yl)-4H-1,2,4-triazol-3-yl)sulfanyl]acetamide
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sulfanylacetamide

FTIR (KBr, cm⁻¹): 1511 cm⁻¹ (C=N in triazole), 3376 cm⁻¹ (-NH Stretching in Amide), 1645 cm⁻¹ (C=O in amide), 1461 cm⁻¹ (C=C in aromatic), 1620 cm⁻¹ (C=O in thioether linkage), 1'H NMR (DMSO-d₆, ppm): 4.28(s, 2H, CH₂), 2.37-2.45 (d, J = 2H, Ar-H), 7.57-7.59 (d, J = 8H, Ar-H), 8.53-8.55 (d, J = 2H, Ar-H), 7.19-7.20 (d, J = 8H, Ar-H), 7.41-7.47 (m, 5H, Ar-H), 10.21 (s, 1H, -NH), Mass spectra (m/z): 488.3(M⁺)

N-(3-chlorophenyl)-2-[(4-phenyl-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-yl)sulfanyl]acetamide

FTIR (KBr, cm⁻¹): 1515 cm⁻¹ (C=N in triazole), 3447 cm⁻¹ (-NH Stretching in Amide), 1663 cm⁻¹ (C=O in amide), 1456 cm⁻¹ (C=C in aromatic), 1676 cm⁻¹ (S-C=O in thioether linkage), 1'H NMR (DMSO-d₆, ppm): 4.26(s, 2H, CH₂), 7.25-7.27(d, J = 4, 2H, Ar-H), 7.58-7.59(d, J = 8H, Ar-H), 8.55-8.57(d, J = 8H, Ar-H), 7.16-7.18(d, J = 8H, Ar-H), 7.43-7.50(m, 5H, Ar-H), 10.27(s, 1H, -NH), Mass spectra (m/z): 422.8(M⁺)

N-(4-nitrophenyl)-2-[(4-phenyl-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-yl)sulfanyl]acetamide

FTIR (KBr, cm⁻¹): 1545 cm⁻¹ (C=N in triazole), 3457 cm⁻¹ (-NH Stretching in Amide), 1667 cm⁻¹ (C=O in amide), 1453 cm⁻¹ (C=C in aromatic), 1634 cm⁻¹ (S-C=O in thioether linkage), 1'H NMR (DMSO-d₆, ppm): 4.20(s, 2H, CH₂), 7.24-7.25(d, J = 4, 2H, Ar-H), 7.54-7.56(d, J = 8H, Ar-H), 8.57-8.59(d, J = 2H, Ar-H), 7.16-7.18(d, J = 8H, Ar-H), 7.42-7.47(m, 5H, Ar-H), 10.23(s, 1H, -NH), Mass spectra (m/z): 433.4(M⁺)

Result and Discussion:

Results were obtained by reacting The mixture of 4-phenyl-5-(pyridine-4-yl)-4H-1,2,4-triazole-3-thiol and 2-chloro-N-(aryl)-acetamide The IR spectra of the compound showed absorption band at 1500 cm⁻¹ which proves the presence of 1,2,4-triazole ring system, absorption band at 3400 cm⁻¹ proves the –NH stretching, 1600 cm⁻¹ (S-C=O linkage in thioether), 1475(-CH₂ stretching ), 1400 cm⁻¹ (C=C aromatic).

The 1'H NMR of the compound 6(a-j) showed characteristic signal at δ 4.20(s, 2H, CH₂), 7.24-7.27(d, J = 4, 2H, Ar-H), 7.54-7.56(d, J = 8H, Ar-H), 8.57-8.59(d, J = 2H, Ar-H), 7.16-7.18(d, J = 8H, Ar-H), 7.43-7.50(m, 5H, Ar-H), 10.27(s, 1H, -NH). The mass spectrum of all the compound was obtained also in the acceptable range.

Table : 1 Physical Data of Various Synthesized Compound

| Sr. No. | Compound | Molecular Formula | M.W. | M.P. (ºC) | % Yield | % of Carbon Found (Calc.) | % of Hydrogen Found (Calc.) | % of Nitrogen Found (Calc.) |
|---------|----------|------------------|------|-----------|---------|--------------------------|----------------------------|---------------------------|
| 1       | 6a       | C₂₂ H₁₉ N₅ O₅ S | 401.48 | 220-223   | 87      | 65.21                     | 4.77                       | 17.44                     |
| 2       | 6b       | C₂₂ H₁₉ N₅ O₂ S | 417.48 | 230-235   | 89      | 63.29                     | 4.59                       | 16.78                     |
| 3       | 6c       | C₂₁ H₁₆ F₅ N₅ O₂ | 426.8 | 205-208   | 85      | 62.21                     | 3.98                       | 17.27                     |
| 4       | 6d       | C₂₁ H₁₆ N₅ O₂ | 401.4 | 208-212   | 79      | 65.81                     | 4.77                       | 17.44                     |
| 5       | 6e       | C₂₁ H₁₆ Br₅ N₅ O₂ | 466.3 | 215-224   | 75      | 54.08                     | 3.46                       | 15.02                     |
| 6       | 6f       | C₂₁ H₁₆ CI₅ N₅ O₂ | 439.8 | 179-181   | 81      | 57.34                     | 3.44                       | 15.92                     |
| 7       | 6g       | C₂₁ H₁₆ F₅ N₅ O₂ | 405.4 | 211-214   | 78      | 62.21                     | 3.98                       | 17.27                     |
| 8       | 6h       | C₂₁ H₁₆ N₅ O₂ | 387.4 | 225-227   | 75      | 62.10                     | 4.42                       | 18.08                     |
| 9       | 6i       | C₂₁ H₁₆ Cl₅ N₅ O₂ | 421.9 | 224-226   | 80      | 59.78                     | 3.82                       | 16.60                     |
| 10      | 6j       | C₂₁ H₁₆ N₅ O₂ | 432.4 | 177-180   | 74      | 58.32                     | 3.73                       | 19.43                     |

The compound were tested using agar cup method for anti-microbial and anti fungal activity using E.Coli, P.Aeruginosa, S.Aureus, S.Pyogenus (bacteria) C.Albicans, A.Niger and A.Claycus (fungi) are listed in below tables respectively. The table shows the anti microbial activity against gram positive, gram negative bacteria and fungi. Comparison of antimicrobial activity produced by compounds with that of standard antimicrobial drug reveals that the produced compounds shows moderate to good activity against all species of bacterial and fungal strains under study.

Table 3: Belowe Table Shows Antifungal Activity of Standard Drugs

| Drug | E.Coli | P. Aeruginosa | S.Aureus | S.Pyogenus |
|------|--------|---------------|----------|------------|
|      | MTCC443| MTCC1688      | MTCC96   | MTCC442    |
| µg/ml|        |               |          |            |
| Gentamycin | 0.05 | 1            | 0.25     | 0.5        |
| Ampicillin | 100   | 100          | 250      | 100        |
| Chloramphenicol | 50 | 50           | 50       | 50         |
| Ciprofloxacin | 25   | 25           | 50       | 50         |
| Norfloxacin | 10    | 10           | 10       | 10         |
Table 4: Shows Antibacterial Activity

| Sr No. | Code No. | E.Coli  | P. Aeruginosa | S. Aureus | S. Pyogenes |
|--------|----------|---------|---------------|-----------|-------------|
| 1      | 6a       | 125     | 500           | 500       | 1000        |
| 2      | 6b       | 200     | 500           | 100       | 125         |
| 3      | 6c       | 125     | 250           | 250       | 1000        |
| 4      | 6d       | 500     | 1000          | 500       | 500         |
| 5      | 6e       | 250     | 200           | 125       | 500         |
| 6      | 6f       | 500     | 200           | 100       | 500         |
| 7      | 6g       | 1000    | 250           | 500       | 250         |
| 8      | 6h       | 250     | 500           | 250       | 250         |
| 9      | 6i       | 500     | 1000          | 100       | 250         |
| 10     | 6j       | 500     | 200           | 500       | 100         |

Table 5: Shows Antifungal Activity

| Sr No. | Code No. | C. Albi-cans | A. -Niger | A. Clav-antus |
|--------|----------|--------------|-----------|---------------|
| 1      | 6a       | 1000         | 1000      | 1000          |
| 2      | 6b       | 500          | 1000      | 1000          |
| 3      | 6c       | 1000         | 1000      | 1000          |
| 4      | 6d       | 500          | 1000      | 1000          |
| 5      | 6e       | 1000         | 1000      | 1000          |
| 6      | 6f       | 500          | 1000      | 1000          |
| 7      | 6g       | 1000         | 1000      | 500           |
| 8      | 6h       | 1000         | 500       | 1000          |
| 9      | 6i       | 1000         | 1000      | 500           |
| 10     | 6j       | 1000         | 500       | 1000          |

Conclusion:
A series of 10 compound of \(N\)-(4-aryl amine)-2-[(4-phenyl-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-yl)sulfanyl]acetamide derivatives was synthesized and the structure of the compounds were well supported by the IR, 1H NMR, and mass spectra. The anti bacterial and Anti fungal activity of the compounds was studied, which shows that the compounds had well to moderate activity against bacteria and fungi. Table 2: Belowe Table Shows Antibacterial Activity of Standard Drugs

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