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# SYNTHESIS, CHARACTERISATION OF ETHYL 7-METHYL-2-(4-NITROPHENYL)-5- PHENYL-5H-[1, 3, 4] THIADIAZOLO [3, 2-A] PYRIMIDINE-6-CARBOXYLATE PROMOTED BY BRONSTD ACID

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## ABSTRACT

This investigation is an efficient and versatile synthesis of ethyl 7-methyl-2-(4-nitrophenyl)-5- phenyl-5H-[1, 3, 4] thiadiazolo [3,2-a] pyrimidine-6-carboxylate promoted by Bronstd acid. These derivatives can be obtained from 5-(4-nitrophenyl)-1, 3, 4-thiadiazol-2-amine with substituted aromatic aldehyde and ethyacetoacetae in the presence of Bronstd acid such as Methanesulphonic acid at reflux. The compound 5-(4-nitrophenyl)-1, 3, 4-thiadiazol-2-amine prepared by the mixture 4-nitrobenzoic acid with thiosemicarbazide in protic acid in DMF at $70^0$C.The titled derivatives can be evaluated by spectroscopic data such as IR, $^1$HNMR, $^{13}$CNMR and LCMS and also the structure of the compounds determined by elemental analysis. In addition to the study of the biological properties.

**KEYWORDS:** 4-nitrobenzoicacid, thiosemicarbazide, 5-(4-nitrophenyl)-1,3,4-thiadiazol-2-amine, aryl aldehyde, MSA, ethyl 7-methyl-2-(4-nitrophenyl)-5- phenyl-5H-[1, 3, 4] thiadiazolo [3,2-a] pyrimidine-6-carboxylate.

## INTRODUCTION

Thiadiazolo Pyrimidines, analogous structure compounds display an important role in pharmacological chemistry. The nucleus and a number of its substituted products exhibit interesting biological and pharmacological properties. Today, Pyrimidines have interested organic chemists because they possess various biological profiles, including antibacterial,

antiviral, anti-inflammatory, antioxidant, and antitumor activity.[1–3] On the other hand, thiadiazolo and their derivatives have been particularly fruitful for their variety of biological effects including antimicrobial,4antituberculosis[5] anticonvulsants[6] and even pesticide[7,8] especially 1,3,4-thiadiazoles which have been studied more with respect to its other structural isomers, have various pharmaceutical activities includingantibacterial,6 ant tuberculosis[9] fungicidal[10], antitumor[11], herbicidal[12], analgesic[13] antiviral[14] and pesticides activity[15], Combining these active substrates with other structures with some sort of biological activity often leads to a new class of activity-improved compounds that may be used as highly biologically active substrates for many purposes.

Today, within the framework of green synthetic organic chemistry, the design and development of safe, clean, and environmentally friendly protocols for synthesizing various molecules, especially pharmaceutical active and industry-leading compounds still remain significant in organic and medicinal chemistry[16] Green approach has some remarkable advantages, including the removal of hazardous materials, the use of fewer solvents, high atom economies and simple workup, and purification. Among these methods, solvent-free synthesis has become a powerful tool for clean and rapid access to diverse compounds.[17] These solvent-less reactions have attracted much attention from chemists for their high efficiency and environmentally friendly route characteristics due to the need for no solvent[18] Additionally, ionic liquids(ILs) have been widely developed as eco-friendly green solvents or media in various fields of science and technology due to their special properties, including control of product distribution, high thermal stability, high activity, non-flammability, and easy recyclability.[19,20] Various organic reactions have been successfully carried out in the presence of ILs.[21,22]

However, most of the existing synthetic methodologies for the synthesis of thiadiazolo [3,2-a]pyrimidines involve low yields, long reaction times, harsh reaction conditions, and generation of by-products. Therefore, the development of more efficient and greener approaches to preparing the functionalized thiadiazolo [3,2-a] pyrimidines is still significant. In continuation of our previous study on the development of green procedures for the synthesis of various biological compounds (Scheme 1).

## METHODS AND MATERIALS

### Experimental

All chemical compounds, solvents, reagents here used were analytical grade and they were procured from Merck and Aldrich Company. Melting points of all newly synthesized derivatives were determined in open capillary tubes on an electro Agarwal thermal apparatus and are uncorrected. The purity of the compounds was examined by thin layer chromatography on silica gel coated aluminum plate chromatography (TLC) using n-hexane / EtOAc (2:1) as an eluent. Infrared spectra (FT-IR) of products were recorded in potassium bromide (KBr) pellets using shimidzo 400 spectrometer.1H NMR and 13CNMR spectra of compounds were recorded on a Brucker AMX 400 MHz spectrometer in CDCl3 as a solvent using tetra methyl silane (TMS) as an internal standard. Chemical shifts and coupling constants are reported in δ and Hz respectively.

### 1.5-(4-nitrophenyl)-1, 3, 4-thiadiazol-2-amine (3)

The mixture of 4-nitrobenzoic acid (0.1 mol) and thiosemicarbazide (0.1 mol) in phosphorous oxychloride (30 mL) were refluxed gently for 60 min and cooled followed by careful addition of water (70 mL). The separated solid was filtered and suspended in water and basified with aqueous potassium hydroxide followed by filtration, drying, and crystallization from mixture of DMF and ethanol (8 :2) to obtain colourless solid.

Paleredcompound,Yeild-95%,M.P-179$^0$C,IR[(KBr):3059(aromaticC–H),1615(C=N),cm−1; $^1$HNMR(400MHz,$CDCl_3$)δppm :8.258-2.078(m,4H, Ar-H) , 6.587(s,2H,NH2);$^{13}$CNMR (100MHz, $CDCl_3$) δppm: 173.28, 160.08, 146.09, 138.29, 128.86, 125.04;LCMS (m/z): 223.45 (M+H) ; Molecular formulae of the compound: $C_8$ $H_6$ $N_4$ $O_2$ S ; Analysis of elements: Calculated: C-43.24 , H-2.72 , N- 25.21; Obtained: C-43.18 , H-2.71, N- 25.28.

### The general procedure of Ethyl 7-methyl-2-(4-nitrophenyl) -5-phenyl-5H -[1,3,4] thiadiazolo[3,2-a]pyrimidine-6-carboxylate

The mixture of 5-(4-nitrophenyl)-1, 3, 4-thiadiazol-2-amine and substituted aromatic aldehyde dissolved and taken in 50mL RBF and gradually addition of methane sulfonic acid. The reaction is continuously maintained for three hours and progress of the reaction was found with help of TLC (4:6 = EtOAc: n-hexane) .After completion of the reaction, cooled at room temperature and poured into crushed ice and also extracted ethylacetae. The crude is neutralised with aqueous $NaHCO_3$ and separated the organic layer and also washed with water. Finally, the organic layer was undergoing vacuum distillation and get desired product.

**Ethyl7-methyl-2-(4-nitrophenyl)-5-phenyl-5H-[1,3,4]thiadiazolo[3,2-a]pyrimidine-6-carboxylate**

Paleredcompound, Yeild-87%, M.P-197-199$^0$C, $^1$HNMR (400MHz, $CDCl_3$) δ ppm: 8.256-8.045 (m, 4H, Ar-H), 7.347 (m, 4H,Ar-H), 4.124 (s,1H,-CH-), 3.946-3.657(m,2H,-$CH_2$-), 2.578 (s,3H,-$CH_3$), 1.124 (t, J=8.0Hz,3H, CH3); $^{13}$CNMR (100MHz, $CDCl_3$) δppm: 165.62, 158.39, 152.37, 146.44, 142.09, 137.35, 134.11, 130.04, 128.49, 127.74, 126.18, 123.98, 121.96, 64.09, 60.67, 20.07, 13.74;LCMS (m/z): 420.09 (M+); Molecular formulae of the compound: C $_{21}H_{18}$ $N_4$ $O_4$ S; Analysis of elements: Calculated: C- 59.71, H- 4.29, N- 13.26; Obtained: C- 59.65, H- 4.28, N- 13.35.

**2. Ethyl5-(4-hydroxyphenyl)-7-methyl-2-(4-nitrophenyl)-5H-[1,3,4]thiadiazolo[3,2-a]pyrimidine-6-carboxylate**

Pale yellow compound, Yeild-89%, M.P-197-214 -216$^0$C, $^1$HNMR (400MHz, $CDCl_3$) δ ppm: 9.124 (s,1H,-OH),8.246-7.894 (m, 4H, Ar-H), 6.942-6.722 (m, 4H,Ar-H), 4.236 (s,1H,-CH-), 3.846-3.745(m,2H,-$CH_2$-), 2.536 (s,3H,-$CH_3$), 1.084 (t, J=7.6Hz,3H, $CH_3$); $^{13}$CNMR (100MHz, $CDCl_3$) δppm: 165.62, 160.03, 152.76, 150.12, 149.32, 142.09, 137.21, 132.62, 130.24, 127.63, 123.02, 120.74, 64.02, 60.75, 20.07, 13.22;LCMS (m/z): 439.23 (M+); Molecular formulae of the compound: C $_{21}H_{18}$ $N_4$ $O_5$S; Analysis of elements: Calculated: C- 57.53, H- 4.14, N- 12.73; Obtained: C- 57.47, H- 4.13, N- 12.79.

**3. ethyl5-(4-hydroxy-3-methoxyphenyl)-7-methyl-2-(4-nitrophenyl)-5H-[1,3,4]thiadiazolo [3,2-a]pyrimidine-6-carboxylate**

White compound, Yeild-91%, M.P-225-227$^0$C, $^1$HNMR (400MHz, $CDCl_3$) δ ppm: 9.576 (s,1H,-OH), 8.294-8.146 (m, 4H, Ar-H), 7.174-6.946(m, 3H,Ar-H), 4.042 (s,1H,-CH-), 3.947-3.762(m,2H,-$CH_2$-), 2.462 (s,3H,-$CH_3$), 1.072 (t, J=8.0Hz,3H, $CH_3$); $^{13}$CNMR (100MHz, $CDCl_3$) δppm:164.66, 158.34, 151.67, 148.34, 144.09, 142.74, 140.13, 137.04, 132.56, 130.68, 128.67, 123.96, 120.65, 65.74, 60.48, 57.62, 20.64, 14.02**;**LCMS (m/z): 469.39 (M+); Molecular formulae of the compound: C 22H20 N4 O6S; Analysis of elements: Calculated: C- 56.40, H- 4.30, N- 11.96; Obtained: C- 56.35, H- 4.28, N- 12.03.

**4. Ethyl5-(4-chlorophenyl)-7-methyl-2-(4-nitrophenyl)-5H-[1,3,4]thiadiazolo[3,2-a] pyrimidine-6-carboxylate**

Paleyellowcompound, Yeild-90%, M.P-192-194$^0$C, $^1$HNMR (400MHz, $CDCl_3$) δ ppm: 8.272-8.042 (m, 4H, Ar-H), 7.354-7.294(m,4H,Ar-H), 4.246 (s,1H,-CH-), 3.942-3.726(m,2H,-$CH_2$-), 2.524 (s,3H,-$CH_3$), 1.114 (t, J=8.0Hz,3H, $CH_3$); $^{13}$CNMR (100MHz,

$CDCl_3$) δppm: 167.22, 159.30, 151.03, 147.38, 142.88, 136.25, 132.01, 130.85, 128.28, 128.69, 126.55, 123.47, 120.87, 60.84, 21.07, and 13.66;LCMS (m/z): 458.64 (M+); Molecular formulae of the compound: $C_{21}H_{17}Cl\ N_4\ O_4\ S$; Analysis of elements: Calculated: C- 55.20, H- 3.75, N- 12.26 ; Obtained: C- 55.15, H- 3.73, N- 12.33.

**5. Ethyl5-(4-cyanophenyl)-7-methyl-2-(4-nitrophenyl)-5H-[1,3,4]thiadiazolo[3,2-a] pyrimidine -6-carboxylate**

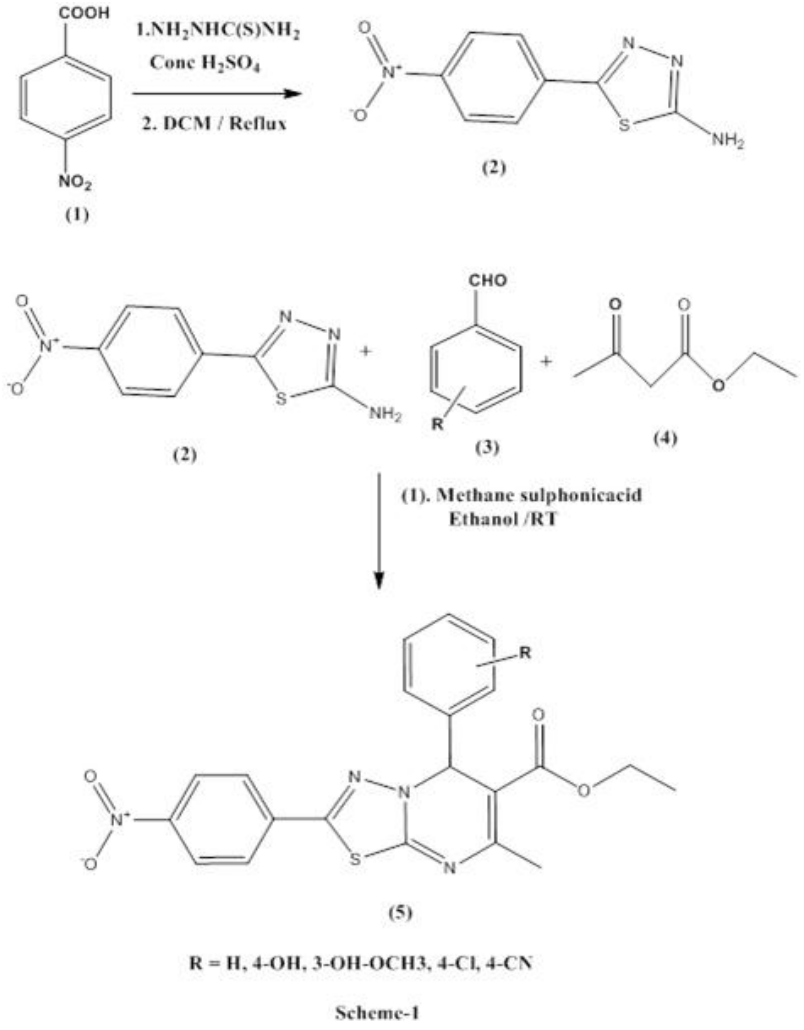
Paleredcompound, Yeild-85%, M.P-187-189$^0$C, $^1$HNMR (400MHz, $CDCl_3$) δ ppm: 8.274-8.074 (m, 4H, Ar-H), 7.658-7.458(m, 4H,Ar-H), 4.247 (s,1H,-CH-), 3.958-3.789(m,2H,-$CH_2$-), 2.614 (s,3H,-$CH_3$), 1.158 (t, J=8.0Hz,3H, $CH_3$) ; $^{13}$CNMR (100MHz, $CDCl_3$) δppm: 167.77, 159.49, 151.56, 145.98, 141.58, 136.35, 135.58, 130.28, 129.68, 128.18, 127.95, 123.56, 120.25, 60.05, 20.57, 13.95;LCMS (m/z): 448.10 (M+) ; Molecular formulae of the compound: $C_{22}H_{17}\ N_5\ O_4\ S$ ; Analysis of elements: Calculated: C- 59.05 , H- 3.83, N- 15.65 ; Obtained: C- 59.00, H- 3.80, N- 15.70.

## 3. RESULTS AND DISCUSSION

The synthetic routes are outlined in Schemes-1

In this investigation, the synthesis of novel deigned and an efficient synthesis of a series of 2-amino thiazolo analogous via benzimidazoles as robust biological agents was CuI2 catalyst reported. There are different analogous can be prepared from titled intermediate such as Ethyl 7-methyl-2-(4-nitrophenyl) -5-phenyl-5H -[1,3,4] thiadiazolo[3,2-a]pyrimidine-6-carboxylate (5a-e) can be obtained from 5-(4-nitrophenyl)-1, 3, 4-thiadiazol-2-amine treated with substituted aromatic aldehyde and ethylacetoactete in the presence of methane sulfonic acid at RT. The intermediate can be synthesised from the 4-nitro benzoic acid with thiosemicarbazide in protic acid as catalyst in MDC as a solvent at reflux.

The high relative abundance, low toxicity, low cost, eco-friendliness, sustainability, and adaptability of Methanesulphonic acid as a catalyst have all drawn attention to organic processes catalyzed by Bronstd acid. In heterocyclic chemistry, Bronstd acid catalysts have found several uses and are commonly employed in organic synthesis. It recent developments in the synthesis of chemicals that are significant to biology, including nitrogen heterocycles.




Scheme-1

### 3.2. Biological activities Antibacterial and antifungal activities

The titled derivatives were evaluated for their in-vitro antibacterial and antifungal activities following micro broth dilution method. The *invitro* antibacterial activity was examined against gram-positive (Bacillus subtilis and Staphylococcus aureus) and gram-negative (Escherichia coli and P. aeruginosa) microorganisms. The *invitro* antifungal activity was evaluated against Aspergillus Niger and C.albicans microorganisms. The standard drugs used for this study were Streptomycin was used for antibacterial screening. Ketonozole was used for antifungal screening. The standard strains used for screening of antibacterial and antifungal activities were procured from the Culture collection and geneank (MTCC), Chandigarh, India. Mueller Hinton Broth was used as a nutrient medium for bacteria and Sabouraud dextrose Broth for fungal growth. Inoculums size for test strain was adjusted to 108 CFU/mL by comparing the turbidity. The results were recorded in the form of primary and secondary evaluation. The stock solution (2000 µg/mL) of the compounds under investigation and standard drugs were prepared by successive two fold dilution.

**Table I: Antimicrobial activity of compounds (5a-5f).**

| Entry | Antibacterial MIC (µg/mL) | | | | Antifungal MIC (µg/mL) | |
|---|---|---|---|---|---|---|
| **Strains** | **B. subtilis** | **S. aureus** | **P. aeruginosa** | **E. coli** | **A. Niger** | **C. Albicans** |
| 5a | 06 | 05 | 08 | 07 | 05 | 05 |
| 5b | 17 | 16 | 18 | 17 | 13 | 14 |
| 5c | 18 | 16 | 18 | 14 | 13 | 14 |
| 5d | 22 | 21 | 20 | 21 | 16 | 17 |
| 5e | 21 | 20 | 19 | 18 | 16 | 18 |
| 5f | 08 | 07 | 05 | 03 | 09 | 10 |
| Streptomycin | 25 | 25 | 25 | 25 | - | - |
| Ketonozole | - | - | - | - | 22 | 22 |
| DMSO | | | | | | |

In the preliminary examination 500, 250 and 100 µg/mL concentrations of the compounds were used. The compounds found to be active in this primary screening were further examination. In secondary screening, 200, 100, 50 and 25 µg/mL concentrations were used. The inoculated wells were incubated overnight at 37°C in a humid atmosphere. The highest dilution showing complete inhibition was considered as a minimum inhibition concentration (MIC). The MIC values revealed that the synthesized compounds showed moderate to good inhibition. Compounds 5d, 5e exhibited good excellent activities against bacterial strains. The MIC values of antifungal activity shown that compound 5c and 5c exhibited good activity against all fungal strain. Antimicrobial activity of compounds **(5a-5f)** is listed in Table-I.

## 4. CONCLUSION

In the present study, it is reported that the synthesized Ethyl 7-methyl-2-(4-nitrophenyl)-5-phenyl-5H-[1, 3, 4] thiadiazolo [3, 2-a] pyrimidine-6-carboxylate are developed synthesis through simple synthetic approaches to search newer antimicrobial agents. For this, antimicrobial evaluation against various bacterial and fungal strains using disc diffusion method was studied. The antimicrobial compounds (5a-5e) were subjected to assess drug-like properties. The results of this microbiological assay have been further investigated in order to explore the mode of action of these outstanding antimicrobial agents along with toxicity studies. In conclusion, it is possible that auxiliary modifications in these bioactive compounds shall be of great effort to improve the selective antimicrobial agents.

## 5. AKOWNLDEMENT

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