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Synthesis, Characterization and Antimicrobial Activity of Azol Substituted Derivatives

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ABSTRACT
A series of dichlorides **(Ia-h)** followed by 1, 3, 4-Thiadiazol-2-Amine (II a-h) were synthesized by using various dicarboxylic acids(a-h), the further reaction of **(I)** was carried out with thiosemicarbazide in presence of sulphuric acid to converted into corresponding 1, 3, 4-Thiadiazol-2-Amine (II a-h) The structures of these compounds were confirmed by IR, NMR and Mass spectral analysis. The newly synthesized compounds were evaluated for the antibacterial and antifungal activity. The results show that compound (**IIa), (IIe), (IIf) and (IIh)** exhibited moderate to good antibacterial and antifungal activity at 5-100 mcg/ml.

Keywords: Antimicrobial activity, Thionyl chloride, Gresiofluvin, thiosemicarbazide.

INTRODUCTION

Antimicrobials are one of a very important category of drug; these classes of drugs are prescribed right from a simple infection to the serious diseases like cancer and also in life threatening infections like meningitis. So it is quite clear from the spectrum of use that these categories of drugs are very important from medical point of view. But microbial resistance towards the drug creates a very serious problem; because of this development of resistance many drugs are now useless which were very effective before. Moreover, the toxic effects produced by these antibiotics are also reducing their significance. So the need for new antimicrobial is always be their.

It is known that many 1, 3, 4-thiadiazole and 1, 2, 4-triazole derivatives have biological activity, with their antibacterial [1-3], antimycobacterial [4-5], antimycotic [6], antifungal [7-8], antidepressive [9], and cardiotonic [10] action being notable. Recent research has also established for these heterocycles an analgesic [11] and anti-inflammatory [12-13] activities. Meanwhile, N-acylated amino acids are known for their hepatoprotective [14], antimicrobial [15-16] and antitumor action. [17-18]

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Taking these data into account, in the present study, we describe herein the synthesis of some triazole, thiadiazole derivatives and evaluation of their antimicrobial activities.

The present work comprises of synthesis of new antimicrobial agent, in which dicarboxylic acids are used as a starting material, to which the heterocyclic rings i.e. thiadiazole and triazole are substituted (it is reported that the presence of heterocyclic ring in lead structure increases the probability of that compound to be a good antimicrobial) so due to the presence of above features in new compounds we can say that the drug should exhibit good antimicrobial activity.

MATERIAL AND METHOD

Chemistry

The synthesis of dichloride **(Ia-h) (I)** was carried out by the reaction of various dicarboxylic acids and thionyl chloride; it is well established. [23] The various substituted 1, 3, 4-Thiadiazol-2-Amine **(II a-h)** were prepared by reacting with thiosemicarbazide (2 mol) in sulphuric acid derivatives as shown in **Scheme I.** The reaction and purity of compounds were monitored by TLC using precoated silica gel. Structures of the compounds were confirmed by spectral studies.

Biological Activity

The synthesized compounds were screened for the antibacterial and antifungal activity by using the agar-cup

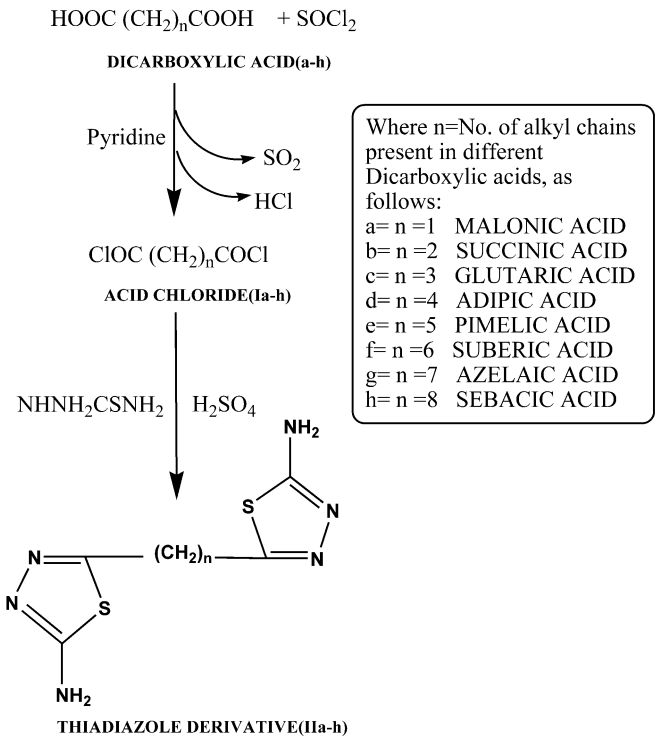
technique in nutrients agar and potato dextrose agar media respectively [19-30] Ciprofloxacin and Gresiofluvin were used as standard drug for the antibacterial and antifungal activity respectively and zone of inhibition of all newly synthesized compounds (**IIIa-j)** and (**IVa-j)** was measured against these standard drug **(Table I** and **II)**. The novel synthesized compounds have shown moderate activity against bacterial strain compared to standard drug. The title compounds have showed the better antibacterial activity and antifungal activity compared with the standard drug. Microbial strains- Staphylococcus aureus **NCIM 2602**; Bacillus subtilis **NCIM 2613**; Escherichia coil **NCIM 2666**; Pseudomonas *aeruginosa* **NCIM 5225**; *Saccharomyces cerevisiae* **NCIM 3220*;*** *Candida albicans* **NCIM 3471*;*** Aspergillus niger **NCIM 813**.

Experimental Section

Melting points were determined in Thermonik melting point apparatus and are uncorrected. IR spectrum was recorded on Thermonicolet FTIR 200 spectrophotometer by using KBr pellet values are expressed in cm^{-1}. NMR spectra were recorded in DMSO-d_6 using varian 300Mz mercury plus and chemical shift are reported in δ (ppm). Mass spectra were recorded on GCMS-QP 2010 Shimaduzu and mass values are reported in m/z.

Microbial strains- *Staphylococcus aureus* **NCIM 2602**; *Bacillus subtillis* **NCIM 2613**; *Escherichia coil* **NCIM** 2666; *Pseudomonas Aeruginosa* **NCIM 5225**; *Aspergillus niger* **NCIM 813;** *Saccharomyces cerevisiae* **NCIM 3220;** *Candida albicans* **NCIM 3471**. All the chemicals used are of reagent grade and substituted aromatic amines and other chemicals used during synthesis are of SD fine grade.

SCHEME:I



Method of Synthesis for Thiadiazole Derivatives

STEP-1

Synthesis of Acid Chloride [31] **(I a-h)**

Dicarboxylic acid (1mol) and thionyl chloride (2 mol) were taken in round bottom flask and this reaction mixture was heated in water bath with occasional stirring for 20-30 min till no more hydrogen chloride evolved. Subsequently the solution was refluxed for two hrs, and distilled off. The acid chloride thus obtained, was used as such for further reaction without any purification.

Conversion of a carboxylic acid to an acid chloride cannot be done with aqueous HCl (the more stable acid is favored), but must be done with anhydrous $SOCl_2$ or PCl_3.

STEP-2

Synthesis of 1, 3, 4-Thiadiazol-2-Amine [32] **(II a-h)**

A mixture of acid chloride (1 mol) and thiosemicarbazide (2 mol) in sulphuric acid 10 ml were heated on a boiling water bath for 5hrs. Ethanol was added to the mixture, and heated under reflux for 10 h. The reaction mixture was cooled to room temperature and poured into ice cold water. The precipitated solid was filtered, washed with water and dried product was recrystallised from suitable solvents. [15]

1. **5-((5′-amino-1′, 3′, 4′-thiadiazol-2′-yl) methyl)-1, 3, 4-thiadiazol-2-amine (Ia).**
   M.P. (ºC): 120-122, % Yield: 68.4, IR-(KBr): (C-S Het str.)711, (C-N Het str.) 1461, (N-N str.) 1625, (Aliphatic C-H str.) 2888, (N-H str.) 3484 cm^{-1}.^{1}H N.M.R (DMSO): 3.596 (s, 2H, CH), 3.954 (s, 4H, NH)
2. **5-(2″-(5′-amino-1′, 3′, 4′-thiadiazol-2′-yl) ethyl)-1, 3, 4-thiadiazol-2-amine (Ib).**
   M.P. (ºC): 132-134, % Yield: 64.6. IR-(KBr): (C-S Het str.) 646, (C-N Het str.) 1531, (N-N str. 1620), (Aliphatic C-H str.) 2962, (N-H str.) 3473 cm^{-1}.^{1}H N.M.R (DMSO) - 3.045 (s, 4H, CH), 3.563 (s, 4H, NH)
3. **5-(3″-(5′-amino-1′, 3′, 4′-thiadiazol-2′yl) propyl)-1, 3, 4-thiadiazol-2-amine (Ic).**
   M.P. (ºC): 106-108, % Yield: 69.1, IR-(KBr): (C-S Het str.) 671, (C-N Het str.) 1560, (N-N str.)1606, (Aliphatic C-H str.) 2893, (N-H str.) 3460 cm^{-1}. ^{1}H N.M.R (DMSO): 1.78-1.86 (p, 2H, CH, *J*=8), 2.71-2.74. (t, 4H, CH, *J*=7.2), 3.817 (s, 4H, NH)
4. **5-(4″-(5′-amino-1′, 3′, 4′-thiadiazol-2′yl) butyl)-1, 3, 4-thiadiazol-2-amine (Id).**
   M.P. (ºC): 104-106, % Yield: 61.5. IR-(KBr): (C-S Het str.) 688,(C-N Het str.) 1452, (N-N str.) 1581,(Aliphatic C-H str.) 2954, (N-H str.) 3454 cm^{-1} .^{1}H N.M.R (DMSO): 1.4-1.7(m, 4H, CH), 2.3-2.8 (m, 4H, CH), 3.726 (s, 4H, CH)
5. **5-(5″-(5′-amino-1′, 3′, 4′-thiadiazol-2′yl) pentyl)-1, 3, 4-thiadiazol-2-amine (Ie).**
   M.P. (ºC): 136-138, % Yield: 63.5. IR-(KBr): (C-S Het str.) 686, (C-N Het str.) 1483, (N-N str.) 1598, (Aliphatic C-H str.) 2887, (N-H str.) 3467 cm^{-1} .^{1}H N.M.R (DMSO): 1.15-1.21 (p, 2H, CH, *J*-6), 1.53-1.61 (p, 4H, CH, *J*=8), 2.50-2.53(t, 4H, CH, *J*=7.2), 3.734 (s, 4H, NH)
6. **5-(6″-(5′-amino-1′, 3′, 4′-thiadiazol-2′yl) hexyl)-1, 3, 4-thiadiazol-2-amine (If).**
   M.P. (ºC): 128-130, % Yield: 66.7. IR-(KBr): (C-S Het str.) 694, (C-N Het str.) 1485, (N-N str.) 1637, (Aliphatic C-H str.) 2873, (N-H str.) 3510 cm^{-1}.^{1}H N.M.R (DMSO): 1.19-1.33(m, 4H, CH), 1.34-1.81 (m, 4H, CH), 1.42-1.47 (t, 4H, CH, *J*=8.8), 3.788(s, 4H, NH)
7. **5-(7″-(5′-amino-1′, 3′, 4′-thiadiazol-2′yl) heptyl)-1, 3, 4-thiadiazol-2-amine (Ig).**
   M.P. (ºC): > 300, % Yield: 69.6. IR-(KBr): (C-S Het str.) 682, (C-N Het str.) 1465, (N-N str.) 1571,

(Aliphatic C-H str.) 2850, (N-H str.) 3452 cm^{-1}.^{1}H N.M.R (DMSO): 1.1-1.4 (m, 6H, CH), 1.6-1.9 (m, 4H, CH), 2.51-2.54 (t, 4H, CH, *J*=6.4), 4.034 (s, 4H, NH)

8. **5-(8″-(5′-amino-1′, 3′, 4′-thiadiazol-2′yl) octyl)-1, 3, 4-thiadiazol-2-amine (Ih).**
M.P. (ºC): 144-146, % Yield: 65.8. IR-(KBr): (C-S Het str.) 690, (C-N Het str.) 1452, (N-N str.) 1579, (Aliphatic C-H str.) 2864, (N-H str.) 3461 cm^{-1}.^{1}H N.M.R (DMSO) - 1.14-1.39 (m, 8H, CH), 1.51-1.92(m, 4H, CH), 2.43-2.49(t, 4H, CH, *J*=12), 3.873 (s, 4H, NH)

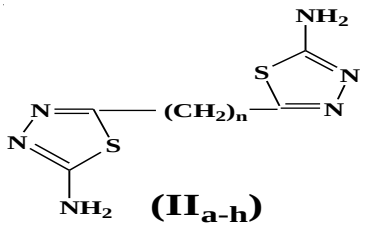
RESULT AND DISCUSSION

We have synthesized a series of eight novel 1, 3, 4-Thiadiazol-2-Amine (IIa-h) derivatives using dichlorides of various dicarboxylic acids. Structures of the synthesized compounds were established on the basis of IR, ^{1}H NMR, Mass and HRMS spectral data in order to substantiate the structures of the compounds.

Compounds (**IIa-h)** showed absorption bands ranging from 670-715 cm^{-1} for C-S Hetero str. and 3460-3510 cm^{-1} for N-H str. 1575-1630 cm^{-1} for N-N str. 1430-1600 cm^{-1} for C-N Hetero str., 2840-3000 cm^{-1} for Aliphatic C-H str., 1575-1630 cm^{-1} for N-N str., but not showed any bands ranging 1700-1725 for C=O str., 3500-3560 for O-H str., and 1345-1400 for C-O str., which ensures the absence of any acidic group in synthesized compounds. These data gave conformation of formation of thiadiazole ring in their respective spectra.

In particular, it must be pointed out that in ^{1}H NMR the characteristic peaks at δ 3.296 ppm (CH_3 group of aliphatic chain), δ 3.957 ppm (N-H group of amine attached with thiadiazole), indicate the presence of above groups in their respective structure. The compounds (**II a-j)** showed prominent singlet at δ 1.19-1.33, m, 4H. and 1.34-1.81, m, 4H. for subsequent protons of CH_3 groups present in aliphatic chain between two thiadiazole rings.

Table I: Antibacterial and antifungal activity of synthesized novel series of 1, 3, 4-Thiadiazol-2-Amine (II a-h) derivatives by cup-plate (agar cup) method



(II_{a-h})

Compound	*Ec*	*Bs*	*Pa*	*Sa*	*Sc*	*Ca*	*An*
IIa	17	14	20	NA	18	19	10
IIb	NA	17	9	10	8	12	15
IIc	NA	7	NA	NA	8	10	8
IId	NA	12	16	12	7	NA	NA
IIe		20	NA	18	22	NA	17
IIf	12	18	10	12	20	10	NA
IIg	13	14	NA	13	15	14	10
IIh	NA	16	8	16	19	11	NA
Ciprofloxacin	25	18	30	18			
Gresiofluvin					23	17	19

Gram-positive bacterial strains: *B. subtillis -Bacillus Subtillis; S. aureus- Staphylococcus aureus* Gram-negative bacterial strains: *E coli –Escherichia coli*; *P.aeruginosa– Pseudomonas Aeruginosa* Fungal strains: *S. cerevisiae -Saccharomyces Cerevisiae; C. Albicans -Candida Albicans; A. niger- Aspergillus niger.* The concentration of test compounds was 100 µg / ml. The concentration of test compounds was 100 µg / ml. Solvent used DMF. NA = Not active

In ^{13}CNMR the characteristic peaks appeared at 159-162 ppm showed the peaks of aromatic carbon in molecule, peaks at 31.20 conforms the methyl group on aliphatic chain ring, these information conforms the formation of desired compound.

Gram-positive bacterial strains: *B. subtillis -Bacillus Subtillis; S. aureus- Staphylococcus aureus* Gram-negative bacterial strains: *E coli –Escherichia coli*; *P.aeruginosa– Pseudomonas* Fungal strains: *S. cerevisiae -; C. Albicans - Candida Albicans; A. niger- Aspergillus niger.*

The synthesized compounds were evaluated for in vitro antibacterial and antifungal activity against various strains Gram-positive bacterial strains: *Bacillus Subtillis; Staphylococcus aureus*, Gram-negative bacterial strains: *Escherichia coli*; *Pseudomonas aeruginosa*, Fungal strains *Saccharomyces cerevisiae; Aspergillus niger, Candida albicans* using nutrient agar cup plate method. The results are given in **Table-I**.

We studied the effect of thiadiazole amine ring. The results showed that compounds **(IIa), (IIe), (IIf)** and **(IIh)** exhibited comparable antibacterial and antifungal activity with the standard antibiotics ciprofloxin and greseofluvin. It has been observed that compound **(IIa)** show better antibacterial and antifungal activity compare to others compounds **(IIe), (IIe)** and **(IIh)** were shown satisfactory antibacterial and antifungal activity.

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