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NOVEL THIADIAZOLE DERIVATIVES: SYNTHESIS, CHARACTERIZATION AND ITS ANTIBACTERIAL ACTIVITY

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ABSTRACT
A variety of thiadiazole derivatives were synthesized in the laboratory with the aim of new antibacterial drugs development. There is a mounting requirement to manufacture novel antimicrobial drugs attributable to the increasing resistance towards traditional antibiotic drugs. In specific, compounds embodying 1, 3, 4-thiadiazole moiety are acknowledged to possess exceptional antibacterial activities. All the twelve novel thiadiazole synthesized derivatives are tested for their physiochemical properties. They were obtained in good yields and are characterized by different I.R, N.M.R, and Mass techniques. Compounds EEE_1, EEE_2, EEE_3, EEE_4 show good antibacterial activity against gram-negative and positive bacteria.
Keyword: Thiadiazole, Antimicrobial, Thiosemicarbazide, Agar Diffusion, Heterocyclic Compound
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INTRODUCTION

This research article provides state-of-the-art information regarding the developments, synthetic strategies, exploration of novel methods, and techniques utilized to synthesize novel thiadiazoles and their antibacterial activity. Among the diverse thiadiazoles; extra information concerning the applications and synthesis of 1,3,4-thiadiazoles is accessible in the literature, comparatively fewer concerning 1,2,5-thiadiazoles.[1,2,3,4] However, there is insufficient information regarding 1,2,4-thiadiazoles[5,6,7] and 1,2,3-thiadiazoles.[8,9,10] There is a huge problem associated with resistance in response to the drugs available in the market, which is becoming a global problem.[11,12] The necessity to design novel compounds to contract with this type of resistance has to turn out to be one of the driving forces for this research.

EXPERIMENTAL

Material and Methods

The melting point of derivatives was examined with the aid of capillaries. Recording of IR spectra was aided by FTIR spectrophotometer of company SHIMADZU in range of 4000-400 cm-1. The electrospray mass spectra with the THERMO LCQ mass spectrometer were recorded with the benefit of the max ion trap. ^1H NMR was recorded with the aid of Bruker spectrometer having model DRX-300 MHz in $CDCl_3$ utilizing internal standard as TMS, with ^1H resonance frequency exactly of 300 MHz values are utilized and chemical shift values are denoted in δ ppm.

The Procedure Adopted for Titled Compound Synthesis

Step-1

Different benzaldehydes, substituted differently, were mixed with phosphorous oxychloride and thiosemicarbazide taken in equimolar quantity. The obtained mixture was refluxed for about 2-4 hrs. The obtained reaction mixture was then added to the flask after it was cooled by employing 100ml of ice water, which was once again refluxed for a time period of roughly 4 hrs. and was then subsequently filtered out. The obtained solution was then made neutral with the aid of ammonia solution. After that, precipitate collected was then filtered after that, it was washed employing water, dried and recrystallization was done

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with aid from two solvents ethanol and water. Analysis of purity of the synthesized compounds was carried with the aid of TLC utilizing benzene and acetone in a ratio of 9:1 as mobile phase.

Step-2

Chloroacetylchloride in the concentration of 20 mmol and 10ml dry benzene was added to a mixture of a suitably substituted compound in a concentration of 10 mmol and 2ml dry pyridine with unvarying stirring in a dropwise mode, with the reaction mixture, remained at room temperature. After complete addition, the obtained reaction mixture was allowed refluxed for 6– 8 hours period and then poured in a container having crushed ice. The precipitate obtained was then filtered and washing was done with the aid of water, dried, and recrystallization was done with the aid of water and dioxane to give compounds E_1-E_{12}. The purity of the synthesized compounds was then analyzed with the aid of TLC utilizing benzene and acetone in the proportion of 9:1 as mobile phase.

Step-3

The mixed anhydride technique was utilized for the synthesis of novel cephalosporin's as explained in Scheme 1. Both anhydrous solution and trimethylamine in the concentration of 11.4 mmol and 30ml THF were allowed to cool to -10 °C. Then ethyl chloroformate in the concentration of 11.4 mmol was added dropwise employing uninterrupted stirring. Then the resultant obtained mixture was kept for 30 minutes at 0°C. Then trimethylamine and 10ml 7-aminocephalosporanic acid aqueous solution both in the concentration of 11.4 mmol was poured into the mixture. For 2 hours at room temperature, the mixture was stirred energetically and dilution was done with 30ml distilled water and diethyl ether was utilized for extraction, aqueous phase was made acidic utilizing dilute HCl to nearly pH 3, and ethyl acetate was utilized for extraction. All the obtained extracts mixed simultaneously, dried, and under reduced pressure were evaporated for dryness. The obtained residue was then triturated with the aid of petroleum ether, crystallization is carried out with aid of ethyl alcohol and collected to give different compounds.[13]

Pharmacological Screening

Antimicrobial Activity

Antimicrobial actions of the novel synthesized cephalexin and cephalosporins were examined with the aid of the agar diffusion method utilizing standard strains of Gram-positive and negative bacteria utilizing agar media having soya. Dimethyl sulfoxide was utilized as a solvent for test derivatives and minimum inhibitory concentrations (MIC values) were also determined of these cephalosporins utilizing the standard dilution technique.

Agar Diffusion Method

Utilizing this technique, by pouring method Petri dishes of agar are prepared. Agar is inoculated with microorganisms. During the agar dilution method, dissimilar concentrations of antibiotics are assimilated in the agar medium, likewise for anaerobes and aerobes. The Plates are allowed to incubate for 24 hours at a temperature maintained at 37°C. A clear zone of inhibition is acquired when antimicrobial material diffuses throughout the agar. This zone diameter is then capable of being measured and then the estimation of the degree of antimicrobial substance activity can be examined.[14]

Material and Methods

The novel synthesized compounds microbiological testing was done with the aid of the agar well diffusion method.

Standard Drug

Cephalexin.

Media

The nutrient agar media was utilized for the purpose, which embodies different constituents as prescribed.

Experimental Procedures

For the estimation of the first round agar-diffusion method was made use, with the aid of nutrient agar medium, agar well diffusion test was executed following the procedure given by Magaldi et al. 2004 and

autoclaving of the medium was executed for 15 minutes at 15 lbs pressure which then was straight away cooled to about 50-55°C with the aid of water bath after taking it away from the autoclave. The cooled medium obtained was then poured into Petri plates sterilized previously to a homogeneous depth of 4mm, corresponding to just about 40ml in a 90mm plate. Just after the medium had been solidified, the obtained culture was then inoculated in the medium. Laminar flow was utilized for the operation and a sterile cotton swab was dipped in the time limit of 15minutes after adjusting the density of the inoculums into the bacterial suspension, which is standardized or inoculated with the aid of 1ml of the organism suspension. To make sure that the distribution of the inoculums is uniform throughout, a sterile swab was utilized on the surface of the nutrient agar medium. To allow excess moisture to be absorbed plates were untouched for 3 to 5 minutes. Sterilized cork borer was utilized for the formation of wells of agar and into each of the wells test compound in diluted form in the concentrations of the 600 and 500 µg/ml were placed and for control, DMSO was utilized.

RESULTS AND DISCUSSION

Twelve novel derivatives were synthesized by the scheme described in Fig.-1 to Fig.-3. Table-1 represents the substituents in place of R in final synthesized compounds. The physiochemical data acquired for step 1 are presented in Table-2.

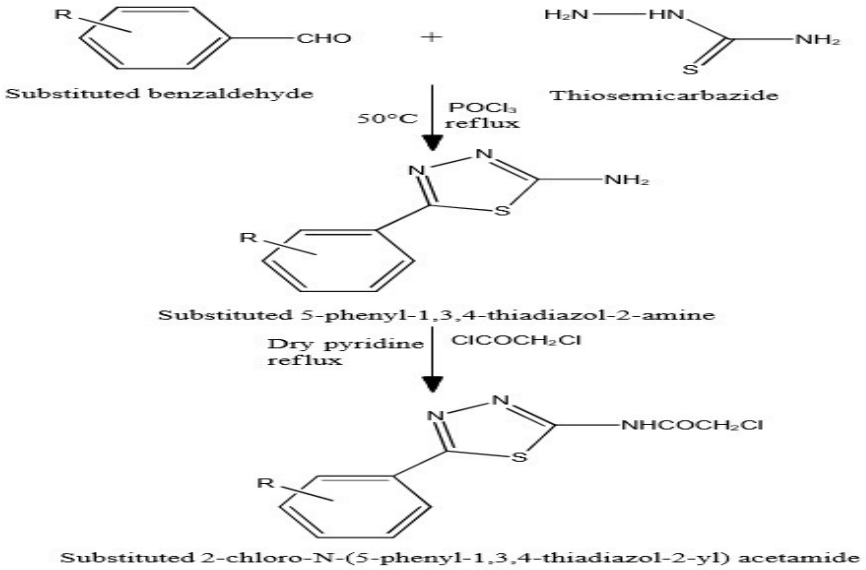

Fig.-1: Step 1

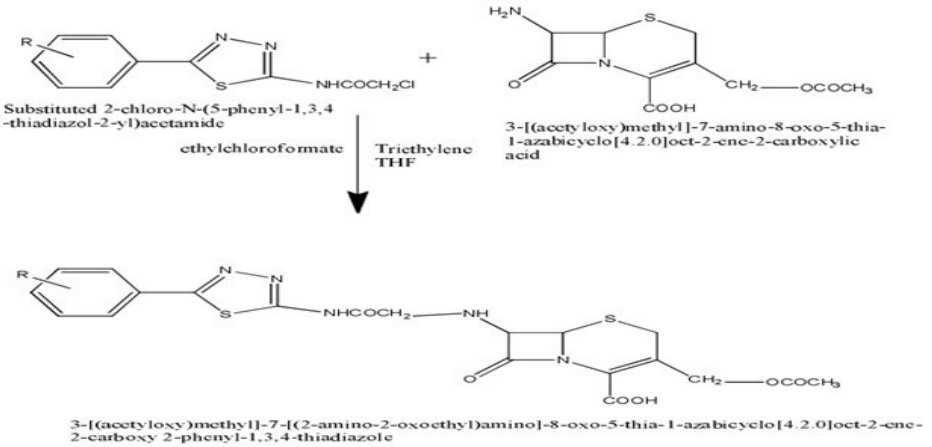

Fig.-2: Step 2

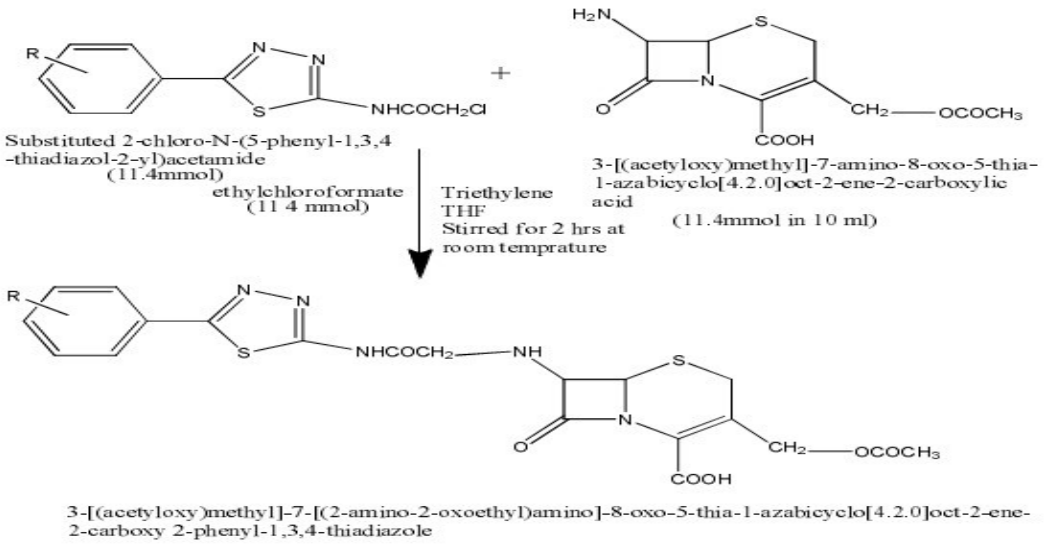

Fig.-3: Step 3

Table-1: Substitution of R with Different Groups of Final Synthesized Derivatives

S.No	Compound	R
1.	EE_1	H
2.	EE_2	*o*-CH_3
3.	EE_3	*p*-CH_3
4.	EE_4	*o*-Cl
5.	EE_5	*p*-Cl
6.	EE_6	*o*-Br
7.	EE_7	*p*-Br
8.	EE_8	*m*-NO_2
9.	EE_9	*p*-NO_2
10.	EE_{10}	2,4-Cl
11.	EE_{11}	OH
12.	EE_{12}	OCH_3

Table- 2: Physicochemical Data acquired for Step-1

S. No.	Molecular Formula	Molecular Weight	TLC(Rf)	M.P.	% Yield
1.	C9H9N3S*(p)*	191.25	0.48	215-216^{0}C	79%
2.	C8H7N3S	177.23	0.29	224-225^{0}C	71%
3.	C9H9N3SO	207.25	0.52	209-210^{0}C	78%
4.	C8H7N3SBr(*p)*	256.12	0.61	247-248^{0}C	61%
5.	C9H9N3S	191.25	0.34	210-211^{0}C	73%
6.	C8H7N3SCl2	246.12	0.62	240-242^{0}C	82%
7.	C8H7N3SCl	211.67	0.21	204-206^{0}C	67%
8.	C8H8N3SO	193.23	0.57	184-185^{0}C	73%
9.	C8H7N3SCl*(p)*	211.67	0.34	230-232^{0}C	77%
10.	C8H7N3SBr	256.12	0.39	192-194^{0}C	62%
11.	C8H7N2SO2*(p)*	222.22	0.58	227-229^{0}C	60%
12.	C8H7N2SO2	222.22	0.38	236-238^{0}C	66%

The IR spectrum of final compounds (EEE_1-EEE_4) shows the characteristic bands in the region 780nm, 1350nm,17-45nm,1660-1730nm,3100-3150nm,1700-1750nm,1630nm,1595-1605nm,870nm, 870nm, and 1780-1885nm for C-NO_2, C=N, Carboxylate C=O, Amide C=O, N-H, C=O, C=C, -NO_2, C-Cl, C-Br groups,

and β-lactam C=O. The ^{H}NMR spectrum of final compounds (EEE_1-EEE_4) showed multiplet in the region δ7.52-8.33 ppm due to Ar-H, showed doublet in the region δ5.56-5.75 ppm due to C_7 –H, double doublet in the region δ5.10 ppm due to C_6-H, showed singlet in the region δ8.31-8.36 ppm due to –CH=N, showed doublet in the region δ4.70-4.75 ppm due to $–CH_2$-O-C=O, showed multiplet in the region δ3.09-3.16 ppm due to C_4-H, showed singlet in the region due to δ-CH_3. Different spectra of important derivatives are presented from figure 4 to figure 6.

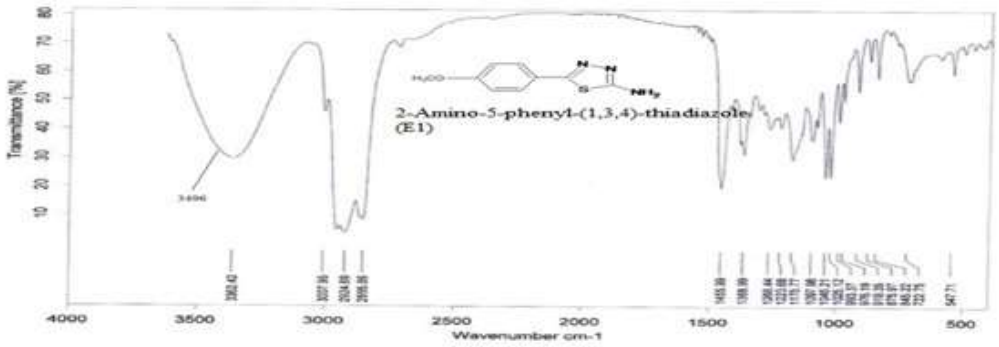

Fig.-4: IR Spectrum acquired for E_1

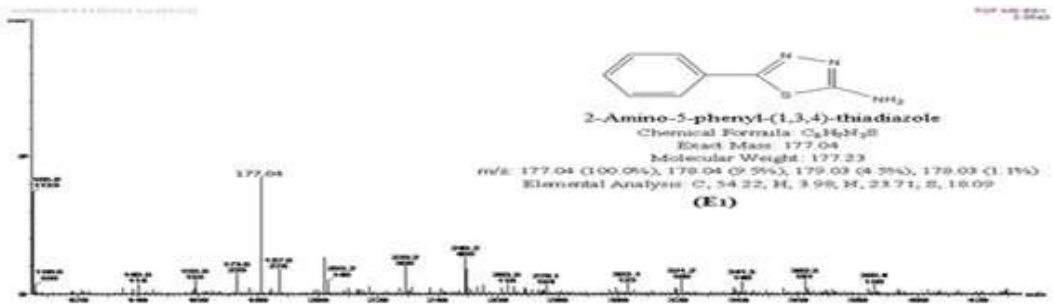

Fig.-5: Mass Spectrum acquired for E_1

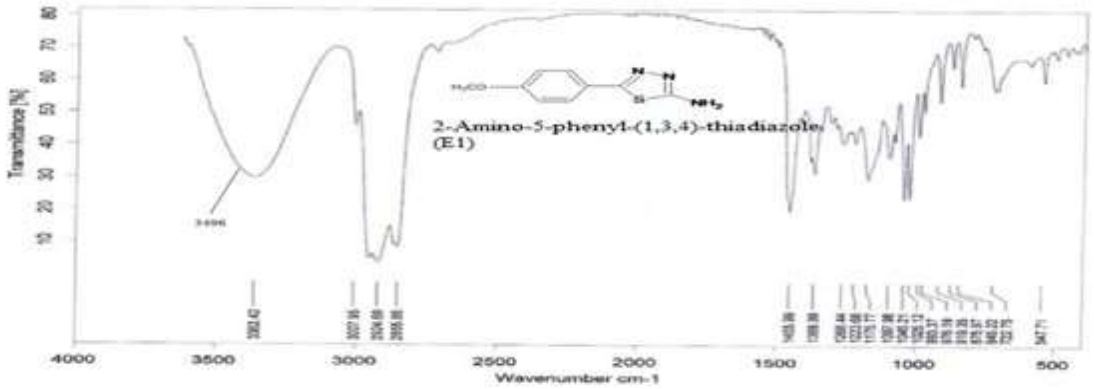

Fig.-6: IR spectrum acquired for E_2

The mass spectrum of final compounds EEE_1 EEE_2, $EEE_3$and EEE_4 shows a molecular ion peak at m/z 523.04,498.08,568.99 and534.06, respectively. From screening results, it has been observed that final compounds EEE_1, EEE_2, EEE_3, and EEE_4 possess significant activity against gram- negative Pseudomonas aeruginosa, Escherichia coli. EEE_1, EEE_2, EEE_3, and EEE_4 possess significant activity against gram-positive Micrococcus luteus, Staphylococcus aureus. Antibacterial activity results are presented in Table-3 and Table-4.

Table -3: Results acquired for Antibacterial Activity in Opposition to Gram-negative Bacteria

Gram-negative Bacteria		*Pseudomonas aeruginosa*				Zone of inhibition(mm)			
S.NO	Compounds Code	Concentration (µg/ml)							
		ZI	% I	ZI	%I	ZI	% I	ZI	% I
1	Std (Cephalexin)	20	19.11	13	13.22	16	15.21	16	25.55
2	EEE1	14	21.33	13	17.33	20	16.96	18	23.33
3	EEE2	16	24.44	14	11.44	21	19.78	19	26.67
4	EEE3	23	20.00	16	13.22	19	16.16	21	23.33
5	EEE4	26	18.00	16	16.33	24	19.64	20	25.56

Table -4: Results acquired for antibacterial activity in opposition to gram-positive bacteria

Gram-positive Bacteria		*Staphylococcus Aureus*				Zone of inhibition a n d % Inhibition			
S.NO	Compounds Code	Concentration (µg/ml)							
		ZI	% I	ZI	%I	ZI	% I	ZI	% I
1	Std (Cephalexin)	22	23.33	21	22.22	24	25.56	26	27.78
2	EEE1	20	21.11	22	23.33	24	25.56	25	26.67
3	EEE2	23	24.44	22	23.33	23	24.44	23	24.44
4	EEE3	22	23.33	23	24.44	24	25.56	24	25.56
5	EEE4	21	22.22	22	23.33	24	25.56	24	25.56

Compounds EEE_1, EEE_2, EEE_3, and EEE_4 showed antibacterial activity. Antibacterial activity against Escherichia coli by EEE_1 is more than the standard drug Cephalexin MIC value (EEE_1) is 36µg/ml is more than that of cephalexin i.e 30 µg/ml. EEE_2 shown more MIC value against staphylococcus aureus i.e 29µg/ml is more compared to that of cephalexin i.e 25µg/ml.

CONCLUSION

Heterocyclic derivatives of thiadiazole were established to encompass significant antibacterial activity. Several derivatives show potential and necessitate further investigation to get better agents. These may act as lead molecules for the future. Structures of novel synthesized thiadiazole derivatives were established after analyzing their IR, H-NMR studies. After the antimicrobial screening, it was examined that all of the derivatives synthesized have proficient activity against the organisms tested. The compounds EEE_1, EEE_2, EEE_3, EEE_4 show good antibacterial activity against gram-negative (Escherichia coli, Pseudomonas aeruginosa), gram-positive bacteria (Bacillus subtilis, Staphylococcus aureus), respectively, whereas other compounds demonstrate reasonable to good activity as compared to reference compound cephalexin. Bactericidal screening data also revealed that compounds of all compounds among them four compounds were productively synthesized and backed up with the physiochemical and spectral data.

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