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Synthesis, Characterization and *In vitro* Antibacterial Screening of Novel Thiazole- 1, 3, 4- Oxadiazole Hybrid Analogues

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ABSTRACT

The aim and objective of the work was to develop novel thiazole-1, 3, 4-oxadizole hybrid analogues and to evaluate theirs *in vitro* antibacterial activity. In this study, 9 novel thiazole-1,3,4-oxadiazole hybrid analogues were synthesized by cyclodehydrogenation reaction of 2-[(4-phenyl-1,3-thiazol-2-yl)amino]acetohydrazide with substituted aliphatic or aromatic acids using phosphorus oxychloride as a dehydrating agent to yield *N*-[(5-substituted-1,3,4-oxadiazol-2-yl)methyl]-4-phenyl-1,3-thiazol-2-amine. The synthesized compounds were then established on the basis of IR, ^{1}H NMR spectral data and screened for *in vitro* antibacterial activity using cup-plate agar diffusion method and were tested against *B. subtilis, S. aureus, E. coli, P. aeruginosa* using Ciprofloxacin (100µg/ml) as standard drug. Compounds APTOM-4e exhibited significant activity towards gram-ve species when compared to standard drug Ciprofloxacin while the others show moderate activity.

INTRODUCTION

Heterocycles are common structural units in marketed drugs and in medicinal chemistry targets in the drug discovery process [1]. Heterocycles with a variety of shapes and electronic and physicochemical properties provide fertile grounds for optimization of drug candidates. Our approach is to take highly halogenated heterocyclic systems and use them as scaffolds for the synthesis of novel compounds by the sequential replacement of halogen atoms with other functionalities. This approach has led to the generation of a number of novel highly substituted heterocyclic species [2]. According to literature survey, thiazoles containing N=C-S moiety were reported to possess antimicrobial [3], analgesic [4], anti-inflammatory [5], anti-cancer [6], anti-tubercular [7], anthelmintic [8] and diuretic activities [9]. In addition 1, 3, 4-oxadiazoles have been reported to have broad biological activities like analgesic [10], anti-inflammatory [11], anti-cancer [12], anti-HIV [13], antiparkinson [14], anti-bacterial [15], antifungal [16] and anti-tubercular agents [17]. The synergism of both the heterocyclic moieties in a single entity may result in the formation of some worthwhile molecules with promising biological activities. Promoted by the above observations, it was aimed to synthesize novel thiazole-1, 3, 4-oxadiazole hybrid analogues. The proposed lead molecule of novel thiazole-1, 3, 4-oxadiazole hybrid analogue was *N*-[(5-substituted-1,3,4-oxadiazol-2-yl)methyl]-4-phenyl-1,3-thiazol-2-amine that envisages a meaningful exploration for newer antibacterial activities with minimum toxicity and high potency.

MATERIALS AND METHODS

Synthesis and characterization

All the chemicals and reagents used in this research work were of the analytical or synthetic grade. Melting points of the synthesized compounds were determined by the open capillary method and are uncorrected. The IR spectra of the synthesized compounds were recorded using Perkin Elmer FT-IR Spectrophotometer in the range of 3500 – 500 cm^{-1}. ^{1}H NMR of the synthesized compounds was recorded in DMSO on Bruker Ultra Shield DPX 500. Chemical shifts were reported in δ (ppm) relative to Tetra Methyl Silane (TMS) as an internal standard. The reactions were monitored by thin-layer chromatography over precoated preactivated glass plates with solvent system Chloroform: methanol (9:1).

Synthetic procedure:

Step 1: Preparation of 4-phenyl-1,3-thiazol-2-amine (1)

A mixture of 0.1 mole of acetophenone, 0.1 mole of iodine and 0.2 mole of thiourea was taken in a 250 ml round bottom flask and heated at 110°C for 4 hours. The reaction mixture was cooled to room temperature and diluted with 100 ml of water and extracted with ether to remove unreacted iodine and acetophenone. Excess of ether was distilled off. This residue then dissolved in boiling water and filtered to remove sulphur. It was allowed to stand for 30 minutes. Make the reaction mixture alkaline (up to pH 8-9) using ammonium hydroxide solution. The solid obtained was filtered and washed successively with water. The separated solid was recrystallized using ethanol. m.p 148°C. Percentage yield was found to be 84% [18].

Step 2: Preparation of ethyl [(4-phenyl-1,3-thiazol-2-yl)amino]acetate (2)

A mixture of compound 1 (0.01 mole) and ethyl chloroacetate (0.01 mole) with potassium carbonate (0.015 mole) in methanol (25ml) was refluxed on a water bath for about 12 hours. The reaction mixture was then cooled, filtered and the solvent was then distilled off under reduced pressure. The solid thus obtained was recrystallized using methanol. m.p 159^0c. Percentage yield was found to be 74% [19].

Step 3: Preparation of 2-[(4-phenyl-1,3-thiazol-2-yl)amino]acetohydrazide (3)

A mixture of compound 2 (0.01 mole) and hydrazine hydrate (0.1 mole) was taken in 250 ml round bottom flask attached to a reflux condenser and refluxed with 50 ml of 95% ethanol for 15 hrs. After 15 hrs the reaction mixture was poured onto the crushed ice to separate the product. The solid thus obtained was recrystallized using ethanol. m.p 133^0c. Percentage yield was found to be 61% [20].

Step 3: N-[(5-substituted-1,3,4-oxadiazolyl-2yl)methyl-4-phenyl-1,3-thiazol-2-amine (4)

To a mixture of compound 3 (0.01mole), substituted acid (0.1 mole), phosphorous oxychloride (6 ml) was added dropwise. The mixture was then refluxed for 4-5 hrs at 120^0c. The reaction mixture was cooled, poured into crushed ice and made alkaline by 20% sodium hydroxide solution. The solid separated was filtered and recrystallized from ethanol [21].

Antibacterial screening

Antibacterial activities were carried out in the microbiological laboratory of Devaki Amma Memorial College of Pharmacy, Chelembra.

a. Test micro-organisms

The organisms used were gram +ve (Bacillus subtilis and Staphylococcus aureus) and gram – ve (Escherichia coli and Pseudomonas aeruginosa). The organisms were obtained from the Department of Pharmaceutics, Devaki Amma Memorial College of Pharmacy, Chelembra.

b. Culture Media

Nutrient agar was used for culturing the bacteria.

Table 01 Composition of culture media

Ingredients	Quantity (g/l)
Peptone	5
Sodium chloride	5
Beef extract	1.5
Yeast extract	1.5
Agar	1.5
Final pH	7.4 ± 25 ^{0}C

28 g of the above culture medium was suspended in 1000 ml of distilled water and boiled to dissolve the media completely. The solution was sterilized by autoclaving at 121^{0}C for 20 minutes.

c. Inoculation

All the bacteria were subcultured on sterile nutrient agar slants and incubated at 37 ± 0.5 ^{o}C for 24 hours. Inoculated 5 ml each of sterile nutrient broth with a loop full of each organism and was added to the sterilized nutrient agar medium. The sterile inoculated media were poured into previously sterilized petri dishes and marked to distinguish the organism and allowed to settle. All these stages were done under aseptic conditions.

d. Preparation of the standard solution

Standard drug solution of Ciprofloxacin (100µg/ml) was prepared in distilled water.

e. Preparation of the test solution

The sample solutions were prepared in DMSO. The concentrations used for antibacterial screening were 100 µg/ml.

f. Incubation

Using a sterile cork borer of about 5 mm diameters, wells were made in each Petri dish. Numbers were marked on the bottom of the petri dish to identify each cup. Using sterile syringe injected 0.1 ml solution of test, standard, and control into the cups. After injection, the petri dishes were kept at room temperature for 2 hrs for uniform diffusion of the agent to occur in seeded agar medium. The petri dishes were incubated at 37 ± 0.5°C for 24 hrs. The presence of a definite zone of inhibition of any size observed was compared with standard drug Ciprofloxacin.

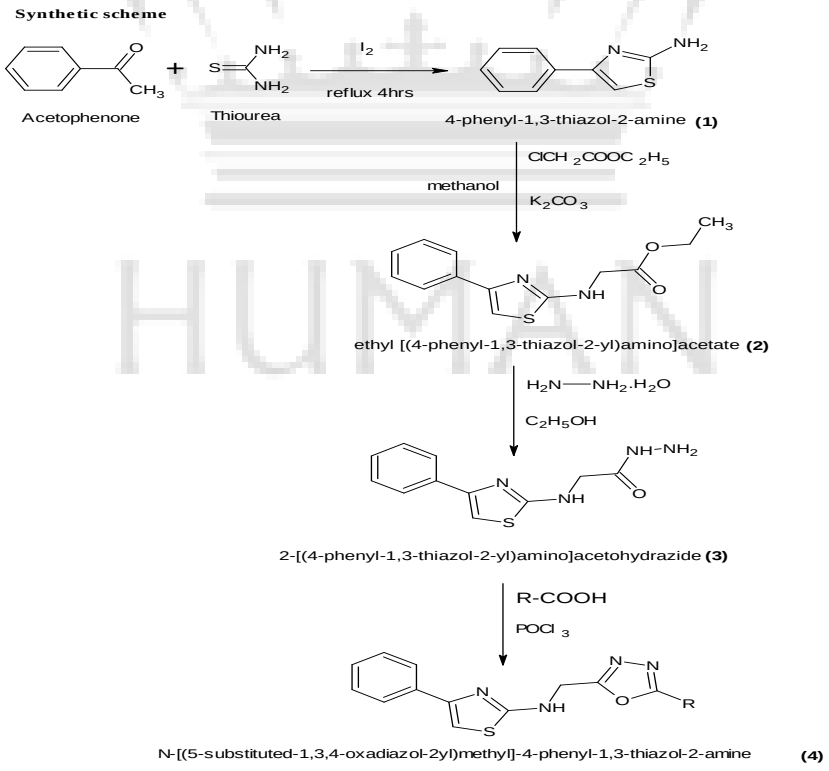
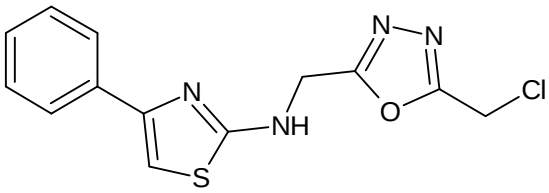
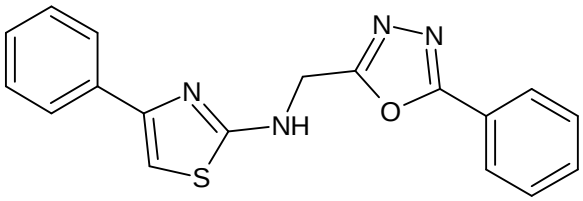
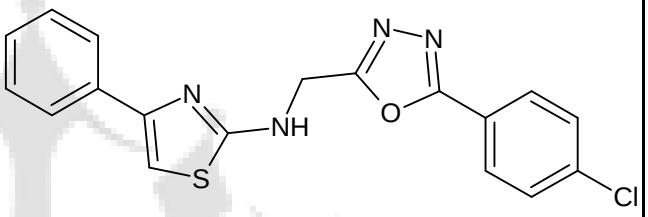
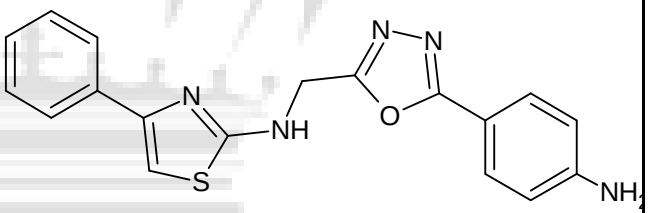
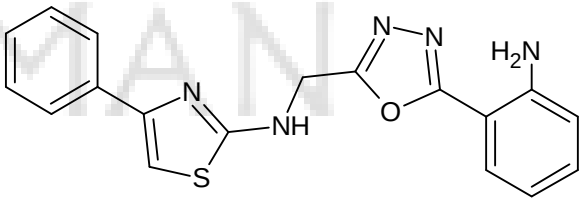
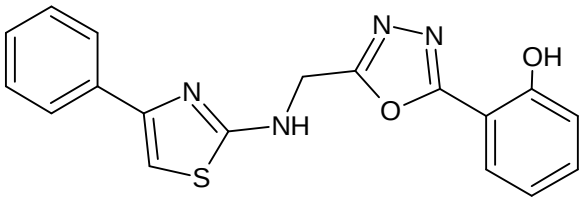


Table 02: List of synthesized compounds

Compound Code	Name of the Compound	Structure of the Compound
APTOM-4a	*N*-{[5-(chloromethyl)-1,3,4-oxadiazol-2-yl]methyl}-4-phenyl-1,3-thiazol-2-amine	
APTOM-4b	4-phenyl-*N*-[(5-phenyl-1,3,4-oxadiazol-2-yl)methyl]-1,3-thiazol-2-amine	
APTOM-4c	*N*-{[5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl]methyl}-4-phenyl-1,3-thiazol-2-amine	
APTOM-4d	*N*-{[5-(4-aminophenyl)-1,3,4-oxadiazol-2-yl]methyl}-4-phenyl-1,3-thiazol-2-amine	
APTOM-4e	*N*-{[5-(2-aminophenyl)-1,3,4-oxadiazol-2-yl]methyl}-4-phenyl-1,3-thiazol-2-amine	
APTOM-4f	2-(5-{[(4-phenyl-1,3-thiazol-2-yl)amino]methyl}-1,3,4-oxadiazol-2-yl)phenol	

RESULTS AND DISCUSSION

Table 03: Preliminary characterization of newly synthesized compounds

Compound code	Molecular formula	Molecular weight	Melting point (^{0}c)	Percentage yield (%)	R_f value
APTOM-4a	$C_{13}H_{11}ClN_4OS$	306.77064	90	64	0.57
APTOM-4b	$C_{18}H_{14}N_4OS$	334.9496	130	52	0.6
APTOM-4c	$C_{18}H_{13}ClN_4OS$	368.84002	134	47	0.62
APTOM-4d	$C_{18}H_{15}N_5OS$	349.4096	196	92	0.73
APTOM-4e	$C_{18}H_{15}N_5OS$	349.4096	154	71	0.71
APTOM-4f	$C_{18}H_{14}N_4O_2S$	350.39436	168	57	0.615

Spectral data of synthesized compounds

The synthesized compounds were confirmed by IR and ^{1}H NMR spectra.

Table 04: IR spectral analysis

Compound code	IR peaks (cm^{-1})
APTOM-4d	Ar–CH Str (3222), C=C Str (1594), C-N Str (1278), C=N Str (1594), C-S Str (702), NH Str (3337), CH_2 Str (2975), C-O-C (1022), NH_2 Str (3422)
APTOM-4f	Ar–CH Str (3111), C=C Str (1594), C-N Str (1331), C=N Str (1594), C-S Str (708), NH Str (3250), CH_2 Str (2919), C-O-C (1027), Phenolic OH(3430)

Table 05: ^{1}H NMR spectral analysis

Compound code	^{1}H NMR (DMSO) δ(ppm)
APTOM-4d	1.26-1.28 (d, 2H, CH_2 of $NHCH_2$), 3.37 (s, 2H, NH_2, D_2O exchangeable), 4.17 (s, 1H, NH, D_2O exchangeable), 5.96 (s, 1H, CH of thiazole), 6.55-7.06 (m, 4H, ArH of aniline), 7.25-7.80 (m, 5H, ArH).
APTOM-4f	1.26-1.28 (d, 2H, CH_2 of $NHCH_2$), 3.35 (s, 1H, NH, D_2O exchangeable), 5.96 (s, 1H, CH of thiazole), 7.00 (s, 1H, phenolic OH), 7.06-7.27(m, 4H, ArH of phenol), 7.34-7.80 (m, 5H, ArH).

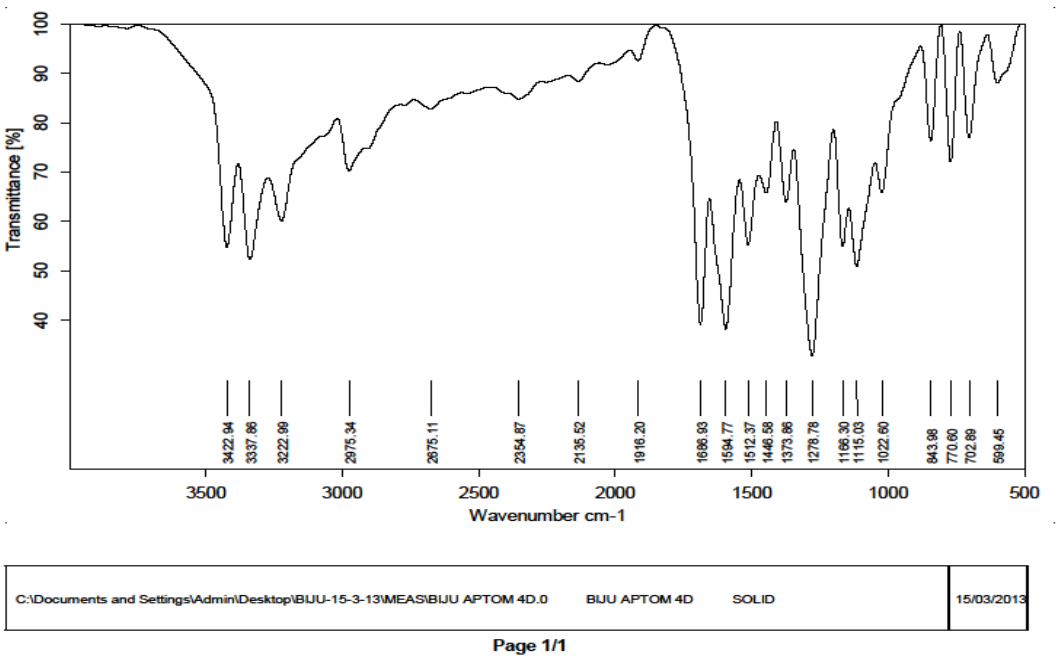


Fig 01: IR spectrum of *N*-{[5-(4-aminophenyl)-1,3,4-oxadiazol-2-yl]methyl}-4-phenyl-1,3-thiazol-2-amine [APTOM-4d]

Antibacterial screening

The synthesized analogues were screened for antibacterial activity against gram (+) bacteria (*Bacillus subtilis, Staphylococcus aureus,)* and gram (-) bacteria (*Escherichia coli, Pseudomonas aeruginosa)* using cup-plate agar diffusion method and comparison against standard antibacterial drug Ciprofloxacin. The inhibition zones produced by the standard, control, and the samples are tabulated.

Table 06: Zone of inhibition for antibacterial activity

Sample (µg/ml)	Zone of inhibition (mm)			
	E. coli **(ATCC 25922)**	***B. subtilis*** **(ATCC 6633)**	***P. aeruginosa*** **(ATCC 27853)**	***S. aureus*** **(ATCC 25923)**
Control	0	0	0	0
Standard	20	21	20	21
APTOM-4b	17	15	18	14
APTOM-4c	16	14	16	13
APTOM-4e	18	15	19	15
APTOM-4g	15	14	15	13
APTOM-4h	15	12	14	11
APTOM-4s	13	11	13	12

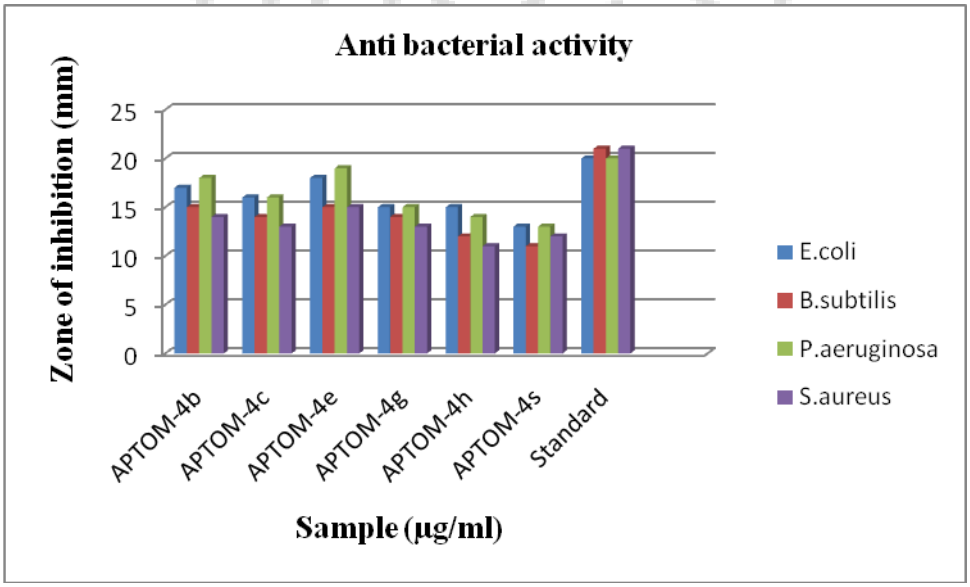


Fig 02: Zone of inhibition for antibacterial activity

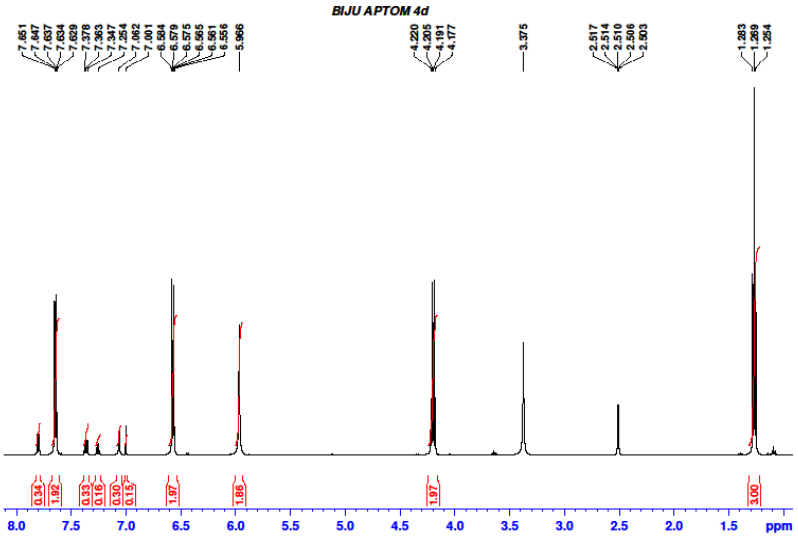


Fig 03: ^{1}H NMR spectrum of *N*-{[5-(4-aminophenyl)-1,3,4-oxadiazol-2-yl]methyl}-4-phenyl-1,3-thiazol-2-amine [APTOM-4d]

CONCLUSION

The present work describes the synthesis of novel thiazole-1, 3, 4-oxadizole hybrid analogues and their antibacterial activity. Compound APTOM-4e exhibited significant activity towards gram-ve species when compared to standard drug Ciprofloxacin while the others show moderate activity. The purity of the compounds and the completion of reaction thus synthesized were ascertained by consistency in melting point and by TLC and the structures of the synthesized compounds were assigned on the basis of the spectral data.

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