CODEN [USA]: IAJPBB SSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.831662

Available online at: http://www.iajps.com Research Article

SYNTHESIS, CHARACTERISATION AND INVITRO ANTIBACTERIAL SCREENING OF NOVEL THIAZOLE ANALOGUES

JishaMol. V*, Binsalma K.S, Vivek M, Rubeena V. K

Department of Pharmaceutical Chemistry, KTN College of Pharmacy, Kerala University of Health Sciences, Kerala, India.

Abstract:

Thiazole containing N=C-S moiety exhibit broad spectrum of biological activities like fungicidal, antimicrobial, antitubercular activities and thiazole possess biological activities like bactericidal, antifungal, analgesic, antiinflammatory, diuretic, CNS depressant and anticancer activity. Recent literature reports explore the biological importance of thiazole analogues as antibacterial agent. The aim and objective of the present investigation is to develop novel thiazole analogues. In this study 3 novel thiazole analogues were synthesised by Schiff's reaction of 2-amino-4-phenylthiazole with substituted aromatic aldehydes. The puriy of newly synthesized compounds was ascertained by consistency in the TLC as well as melting point determination and were characterised by means of IR spectral analysis. Antibacterial screening was carried out using pour plate agar diffusion method and was tested against Bacillus subtilis, Staphylococcus aureus, E.coli, Pseudomonas aureginosa using Ciprofloxacin (100 µg/ml) as standard drug. Compound T1 exhibited significant activity towards gram positive organism and T2 exhibited significant activity towards gram negative organism when compared to standard drug Ciprofloxacin and others show moderate activity. Finally it was concluded that novel thiazole analogues can be considered as the future lead molecule for drug discovery process.

Key words: *Thiazole analogues, Schiff's reaction, IR spectral analysis, Anti bacterial screening, Pour plate agar diffusion method.*

Corresponding Author:

Jisha Mol.V,

Department of pharmaceutical chemistry,
KTN College of Pharmacy, Kerala University of Health Sciences,
Palakkad, Kerala, India.
Ph.No.8606448744,
Email id- jishanandan09@gmail.com

QR code

Please cite this article in press as Jisha Mol.V et al, ***Synthesis, Characterisation and Invitro Antibacterial Screening of Novel Thiazole Analogues****, Indo Am. J. P. Sci, 2017; 4(07).*

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 anti-microbial [3], analgesic [4], anti-inflammatory [5], anti-cancer [6], anti-tubercular [7], anthelmintic [8] and diuretic [9] activities. Anti-microbial activities of some substituted thiazoles are well established because it posses (S-C=N) toxophoric unit. Thiazoles have enhanced lipid solubility with hydrophilicity. Thiazoles are easily metabolized by routine biochemical reactions and are non-carcinogenic in nature. Promoted by the above observations, it was aimed to synthesize novel thiazole analogues by Schiff" s reaction. The proposed lead molecule of novel thiazole analogue was N-[(4-substituted phenyl)methylidene]-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}. The reactions were monitored by thin-layer chromatography over precoated, preactivated glass plates with solvent system- Chloroform: methanol (9:1).

Synthetic procedure:

a. 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%.[10]

b. Step 2: Preparation of N-[(4-substituted phenyl)methylidene]-4-phenyl-1,3-thiazole-2-amine [T1-T3]

To a stirred solution of a compound (1) (0.02mole) IN ethanol(50ml) containing glacial acetic acid(2ml) was added appropriate aromatic aldehyde (0.02mole) and the mixture is refluxed for 8 hours on a water bath. On removal of the solvent a solid product was obtained. It was dried and recrystallized from ethanol to give the coresponding compounds T1-T3.[11]

Procedure for the synthesis of compound T1

To a stirred solution of a compound (1) (0.02mole) in ethanol (50ml) containing glacial acetic acid (2ml) was added appropriate salicylaldehyde (0.02mole) and the mixture is refluxed for 8 hours on a water bath. On removal of the solvent a solid product was obtained. It was dried and recrystallized from ethanol to give the corresponding compound T1.

Procedure for the synthesis of compound T2

To a stirred solution of a compound (1) (0.02mole) in ethanol (50ml) containing glacial acetic acid (2ml) was added appropriate cinnamaldehyde (0.02mole) and the mixture is refluxed for 8 hours on a water bath. On removal of the solvent a solid product was obtained. It was dried and recrystallized from ethanol to give the corresponding compound T2.

Procedure for the synthesis of compound T3

To a stirred solution of a compound (1) (0.02mole) in ethanol (50ml) containing glacial acetic acid (2ml) was added appropriate p-chloro benzaldehyde(0.02mole) and the mixture is refluxed for 8 hours on a water bath. On removal of the solvent a solid product was obtained. It was dried and recrystallized from ethanol to give the corresponding compound T3

Antibacterial screening

Antibacterial activities were carried out in the microbiological laboratory of KTN College of Pharmacy, Chalavara.

a.Test micro-organisms

The organisms used were gram +ve (Bacillus subtilis and Staphylococcus aureus) and gram –ve (Escherichia coli and Pseudomonas aureginosa). The organisms were obtained from the Department of Pharmaceutics, KTN College of Pharmacy, Chalavara.

b. Culture Media
Nutrient agar was used for culturing the bacteria.

Table 1: 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 sub cultured 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 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 Petridish. Numbers were marked on the bottom of petridish to identify each cup. Using sterile syringe injected 0.1ml solution of test, standard and control into the cups. After injection the petridishes were kept at room temperature for 2hrs for uniform diffusion of the agent to occur in seeded agar medium. The petridishes were incubated at 37 ± 0.5^{o}C for 24 hrs. The presence of a definite zone of inhibition of any size observed was compared with standard drug Ciprofloxacin.

Synthetic scheme

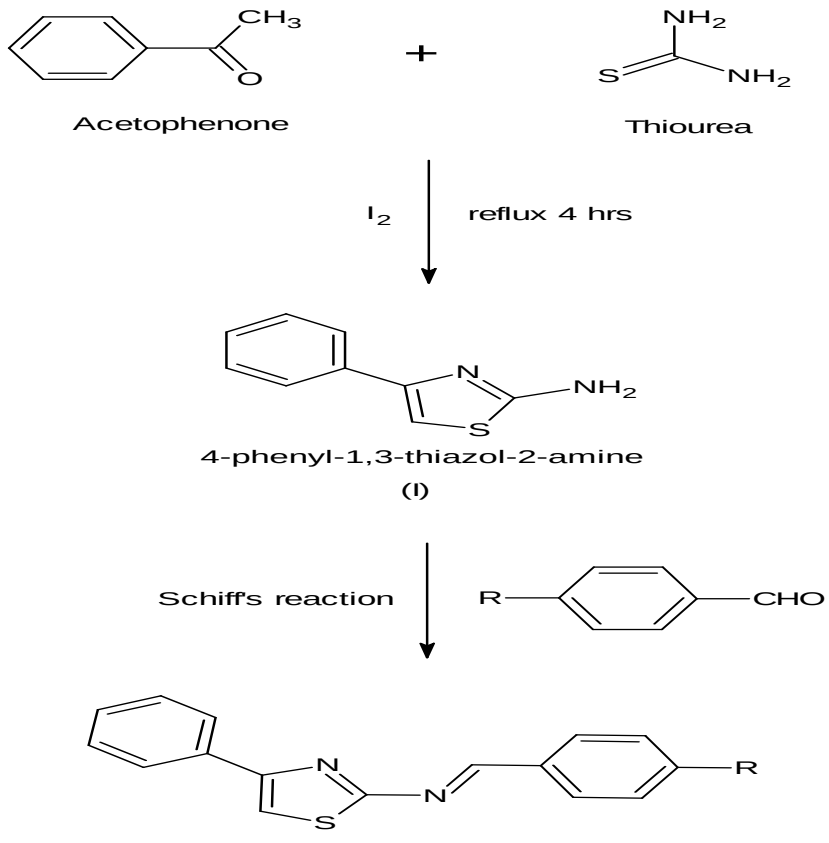
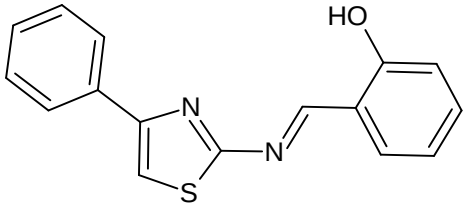
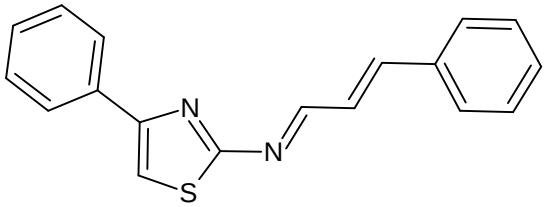
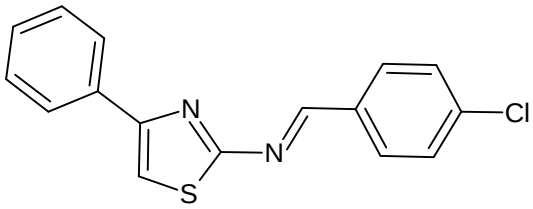


Fig 1: Synthetic Scheme

Table 2: List of Derivatives

Compound Code	Name of the Compound	Structure of the Compound
T1	N-[(2-hydroxy phenyl)methylidene]-4-phenyl-1,3-thiazole-2-amine	
T2	N-[(4-substituted phenyl)methylidene]-4-phenyl-1,3-thiazole-2-amine	
T3	N-[(4-chloro phenyl)methylidene]-4-phenyl-1,3-thiazole-2-amine	

RESULTS:

Table 3: Preliminary characterization of newly synthesized compounds

Compound code	Molecular formula	Molecular weight	Melting point (^{0}c)	Percentage Yield (%)	R_f value
T1	$C_{16}H_{12}N_2O_5$	122.12	132	46	0.325
T2	$C_{18}H_{14}N_2S$	132.16	150	39	0.25
T3	$C_{16}H_{11}ClN_2S$	140.57	142	40	0.175

IR spectral analysis

IR spectra of the synthesized compounds were recorded using FTIR in the range of 3500-500 cm^{-1} on Perkin Elmer FT-IR Spectrophotometer and the functional groups of the compounds were determined from the IR spectra.

Table 4: IR spectral analysis

Compound code	IR peaks (cm^{-1})
1	Ar-CH Str (3108), C=C Str (1593), C-N Str (1330), C=N Str (1593), NH_2 Str (3429), C-S Str (657)
T1	Ar-CH Str (3251), C=C Str (1598), C-N Str (1276), C=N Str (1598), OH Str (3743), C-S Str (693)
T2	Ar-CH Str (3188), C=C Str (1577), C-N Str (1180), C=N Str (1577), C-H Str (3030)
T3	Ar-CH Str (3053), C=C Str (1591), C-N Str (1299), C=N Str (1591), C-Cl Str (770)

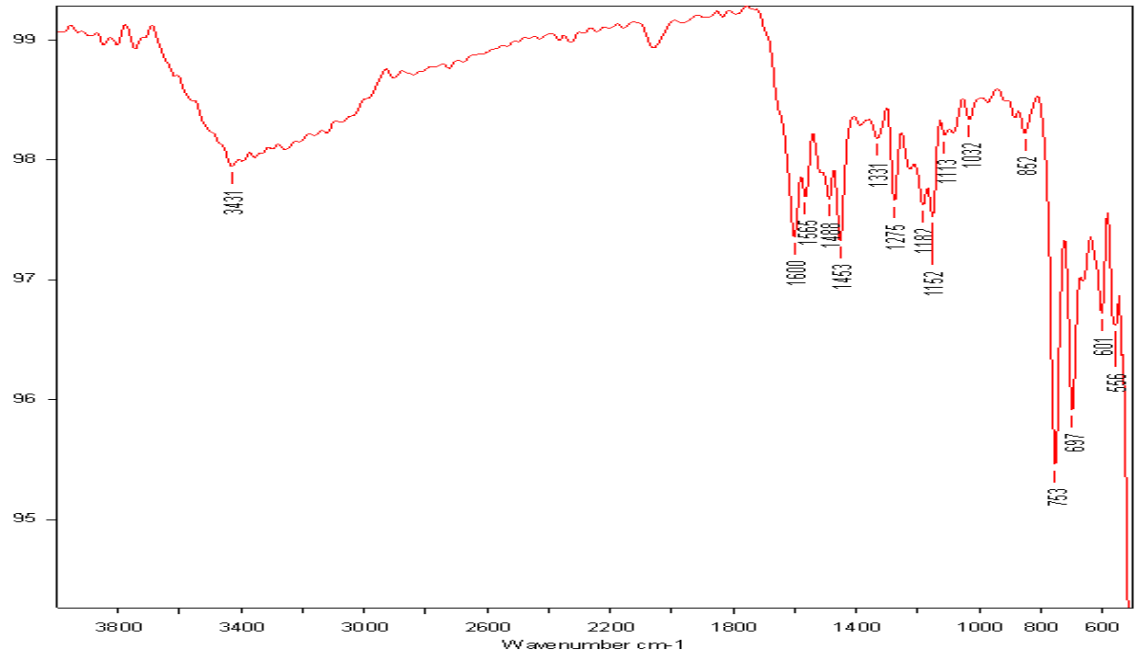


Fig 2: IR Spectrum of N-[(2-hydroxy phenyl)methylidene]-4-phenyl-1,3-thiazole-2-amine [T1]

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 pour-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 5: 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	23	22	22	23
T1	20	24	15	12
T2	24	13	12	10
T3	20	21	19	20

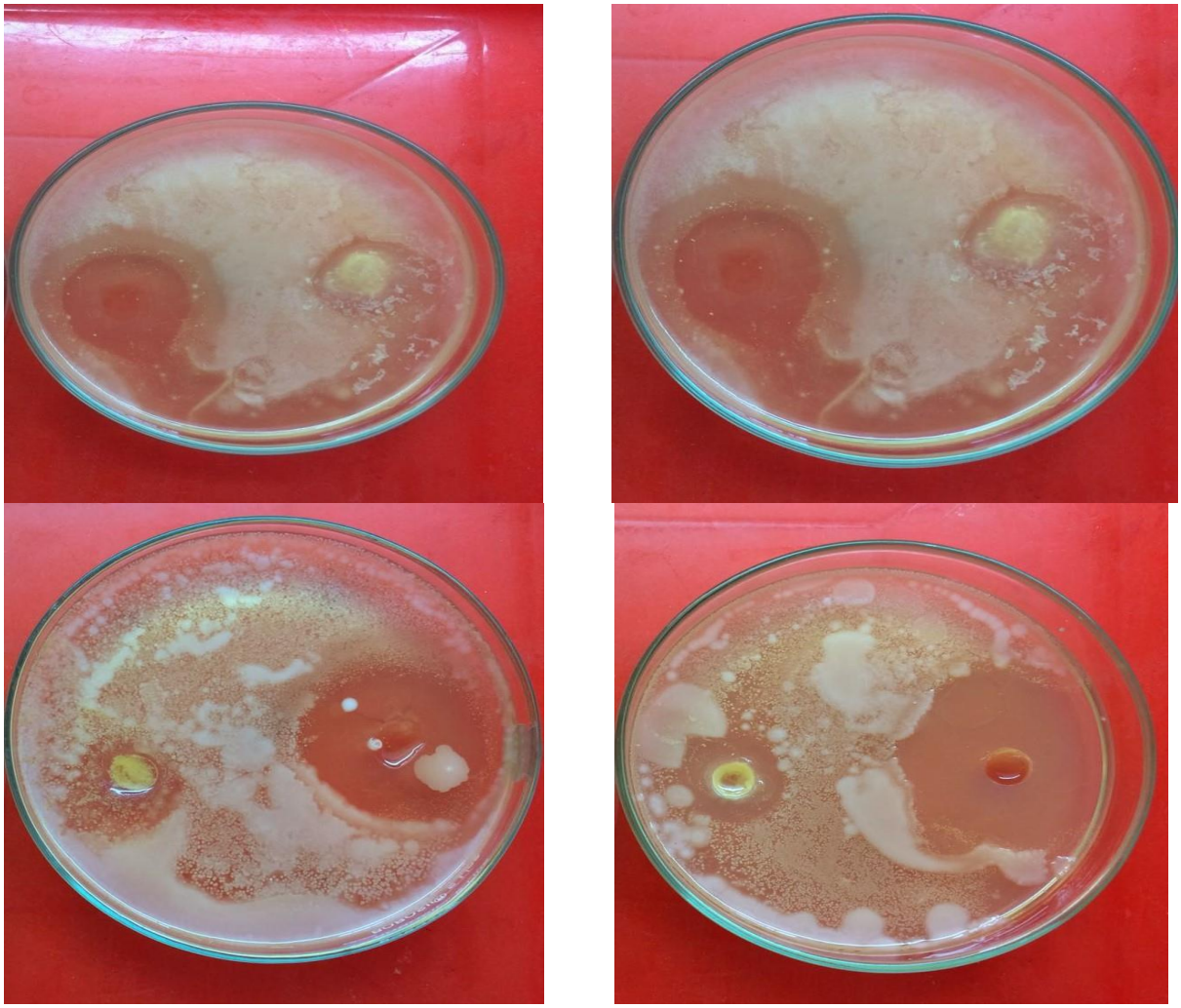


Fig 3: Anti bacterial activity against gram positive and gram negative organisms

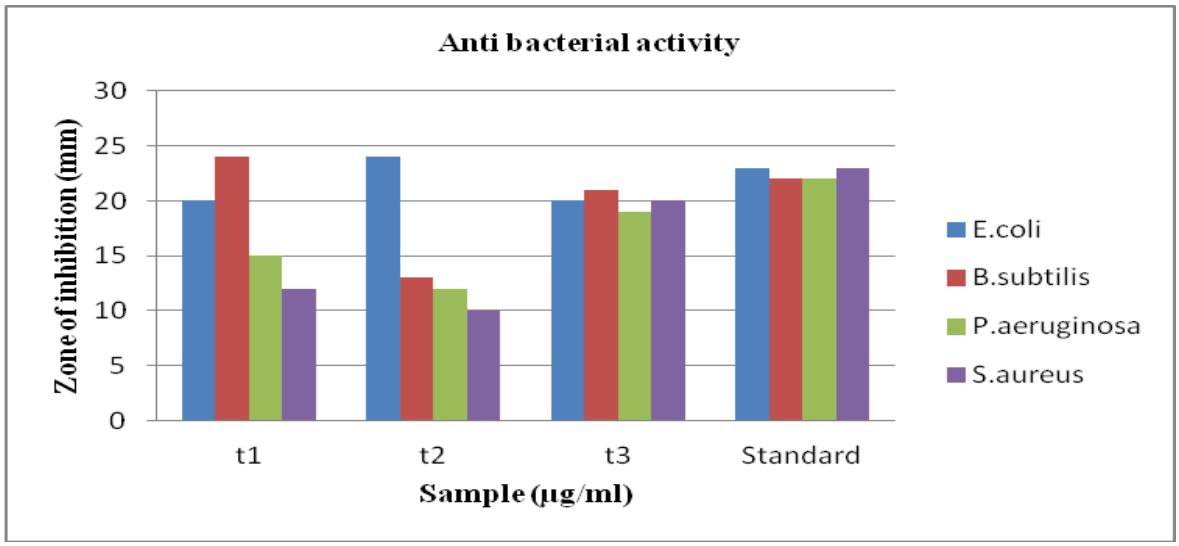


Fig 4: Zone of inhibition for antibacterial activity

DISCUSSION:

Molecular structures were drawn using chemsketch. The synthetic method was preceded in a step wise manner. Acetophenone were condensed with thiourea in presence of iodine as catalyst and yield corresponding 4-phenyl-1,3-thiazole amine. Then Schiff's reaction was carried out in which 2-amino-4-phenyl thiazole was treated with substituted aromatic aldehyde and yield N-[(4-substituted phenyl)methlidene]-4-phenyl-1, 3-thiazole-2-amine. Antibacterial screening of the synthesised compounds were carried out using pour diffusion method and were tested against Bacillus subtilis, Staphylococus aureus, E.coli, Pseudomonas aeruginosa using Ciprofloxacin as standard. Compound T1 exibit significant activity towards gram positive organism and T2 exibit significant activity towards gram negative organism when compared with standard drug Ciprofloxacin and others show moderate activity.

CONCLUSION:

This work was aimed at rational approach in drug design and development of some novel thiazole derivatives which posses antibacterial activity. In the study 3 novel thiazole analouges were synthesised by Schiff's reaction of 2-amino-4-phenyl thiazole with substituted aromatic aldehyde to yield N-[(4-substituted phenyl)methylidene]-4-phenyl-1, 3-thiazol-2-amine. Then the derivatives were characterised by studying IR spectral analysis. Purity of compounds was ascertained by consistency in melting point and R_f value by T.L.C. Antibacterial screening of synthesised compounds were carried out using pour plate agar diffusion method and were tested against Bacillus substillus, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa using ciprofloxacin as standard drug. The compound T2 shows significant activity against gram negative bacteria (E.coli) and compound T1 shows a significant anti-bacterial activity against gram positive bacteria (B.substillus) while comparing with standard drug ciprofloxacin. All together it may be concluded that thiazole hybrid analogues considered as future lead molecule for drug discovery process. This work also emphasise the importance of rational drug design approaches.

ACKNOWLEDGEMENT

I bow **God,** the Almighty for his blessings showering on me, which have brightened my way and strengthened me to tackle all my and strengthened me to tackle all my hurdles. I gratefully thanks for his kindness that enabled me in the timely completion of my thesis work. I'm obliged to record my respectful thanks to **Dr L. Sutharson,** Principal K.T.N College of Pharmacy, Chalavara, for providing me all facilities and encouragement for successful completion of the thesis work. I would like to express sincere thanks to **Mr Premkrishnan P. P,** Managing Director, K.T.N College of Pharmacy, Chalavara. I express my profound gratitude to **Mr Padmaraj C. P,** and **Mrs DivyaT.A** Assistant Professor of Pharmaceutical Chemistry for his timely help, constructive criticism, and valuable suggestions at various stages of work. I'm extremely grateful to **Mrs Devi Swapna P. V,** Assistant Professor of Pharmaceutical Analysis for her generous contribution in carrying out the interpretation of I R Spectrum. I convey my sincere thanks to **Mrs Aswathy B** and **Mrs Remya Ravindran,** Department of Pharmaceutics for their generous help and support. I convey my sincere thanks to **Mr Manikandan** and **Mr Suryanarayanan** for their generous help and support. I extend my heartful thanks to all the Teaching staff of this college who have directly or indirectly contributed to my thesis work. I convey my thanks to **Mrs Sreeja**, **Kalyanikutti,** Librarian and all other non-teaching staff members of this college for their co-operation.

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