SUMMARY

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Nitrogen containing heterocycles constitute the largest portion of chemical entities which are part of many natural products, fine chemicals and biologically active pharmaceuticals vital for enhancing the quality of life. Amongst different heterocyclic systems, the chemistry of nitrogen and sulfur containing compounds has gained importance due their pronounced bioactive nature. One such type of compounds are 1,3,4-oxadiazole, 1,3,4-thiadiazole, 1,2,4-triazole, pyrimidine and their derivatives. In this perspective, the present work **"Synthesis and Bioassay of New Class of Heterocyclic Compounds"** has been taken up. The presentation of the thesis is as shown below.

INTRODUCTION

A brief introduction on the importance of five and six membered heterocycles and the methods of syntheses pertaining to 1,3,4-oxadiazoles, 1,3,4-thiadiazoles, 1,2,4-triazoles and pyrimidines are described. Besides, the actual scope and objectives of the work are also mentioned.

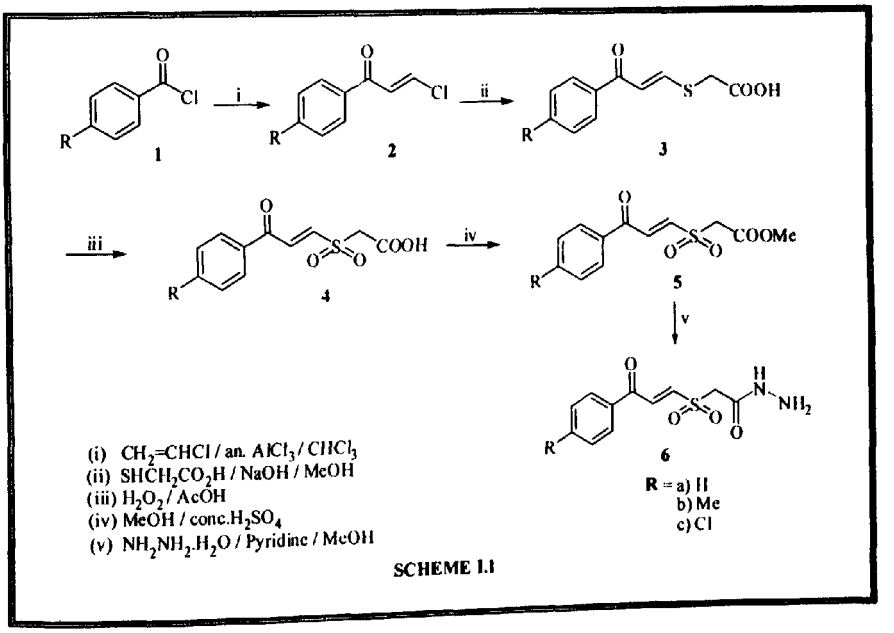
PRESENT WORK

The five and six membered heterocycles *viz.*, 1,3,4-oxadiazoles, 1,3,4-thiadiazoles, 1,2,4-triazoles and pyrimidines represent a class of prominent compounds in heterocyclic arena. The exploitation of simple molecules with different functionalities for the synthesis of these compounds adopting simple, facile and elegant synthetic methodologies is a worthwhile contribution in the field of heterocyclic chemistry. In this perspective, the author did considerable work on the development of mono and bis heterocycles from simple substrates. The results are presented in three chapters.

CHAPTER-I

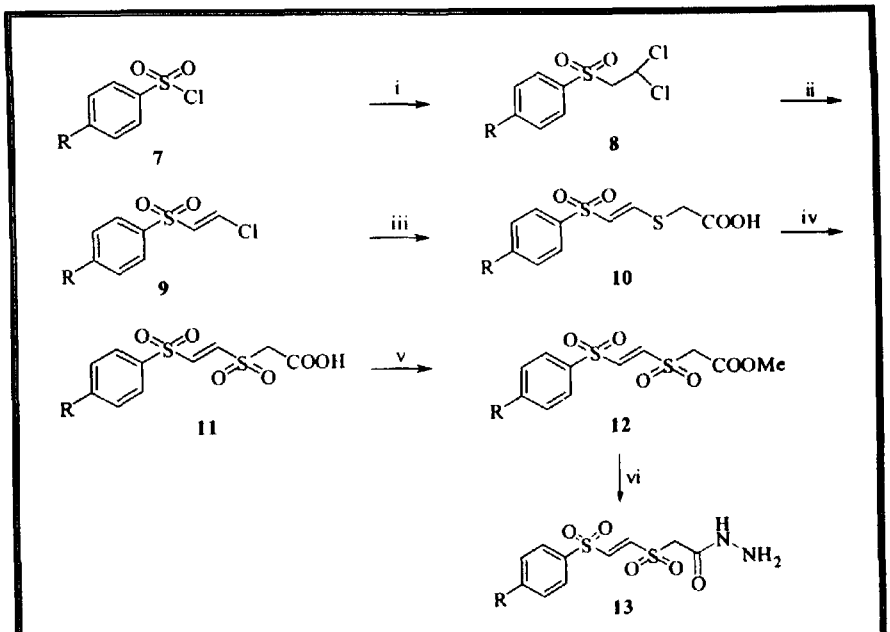
Synthesis and antimicrobial activity of 2-(E-aroylethenesulfonylmethyl)-5-styryl-1,3,4-oxadiazole, 2-(E-aroylethenesulfonylmethyl)-5-styryl-1,3,4-thiadiazole, 3-(E-aroylethenesulfonylmethyl)-4-amino-5-styryl-1,2,4-triazole, 2-(E-arylsulfonyl-ethenesulfonylmethyl)-5-styryl-1,3,4-oxadiazole, 2-(E-arylsulfonylethenesulfonyl-methyl)-5-styryl-1,3,4-thiadiazole and 3-(E-arylsulfonylethenesulfonylmethyl)-4-amino-5-styryl-1,2,4-triazole.

The synthetic intermediates *E*-aroylethenesulfonylacetic acid hydrazide (**6**) and *E*-arylsulfonylethenesulfonylacetic acid hydrazide (**13**) were utilized for the synthesis of the title compounds. The compound **6** was prepared as follows. The vinyl chloride gas was passed into aroyl chloride (**1**) in the presence of anhydrous aluminium chloride under Friedel-Craft's conditions to get 1-aroyl-2-chloroethene (**2**). This on reaction with mercaptoacetic acid in the presence of sodium hydroxide in methanol produced aroylethenemercaptoacetic acid (**3**). The compound **3** was subjected to oxidation to afford aroylethenesulfonylacetic acid (**4**) which on esterification with methanol in the presence of concentrated sulfuric acid furnished *E*-aroylethenesulfonylacetic acid methyl ester (**5**). The treatment of **5** with hydrazine hydrate in the presence of pyridine resulted in the acid hydrazide **6** (Scheme **I.1**).



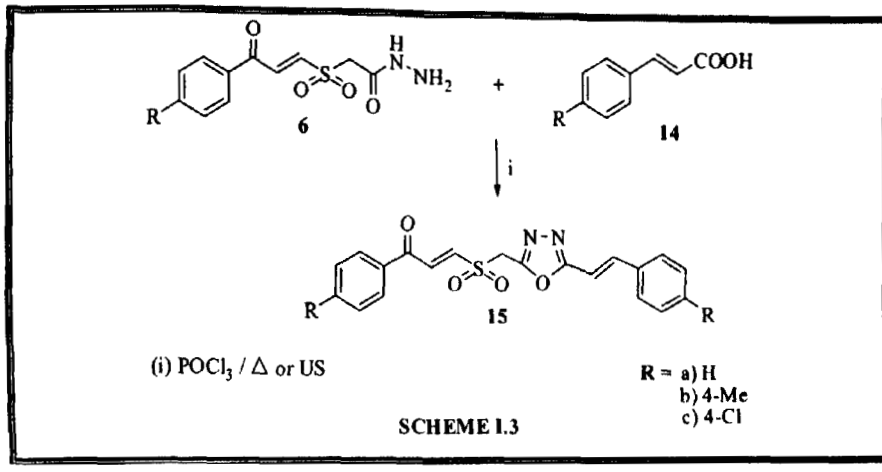
SCHEME I.1

The *E*-arylsulfonylethenesulfonylacetic acid hydrazide (**13**) was synthesized by the condensation of *E*-arylsulfonylethenesulfonylacetic acid ester (**12**) with hydrazine hydrate in the presence of pyridine. The compound **12** was inturn prepared as follows. The vinyl chloride gas was passed into arylsulfonyl chloride (**7**) under Friedel-Craft's conditions to obtain 1-arylsulfonyl-2,2-dichloroethane (**8**). The latter on dehydrohalogenation with triethylamine produced 1-arylsulfonyl-2-chloroethene (**9**). The reaction of **9** with mercaptoacetic acid gave arylsulfonylethenemercaptoacetic acid (**10**) which on oxidation with hydrogen peroxide provided arylsulfonylethenesulfonyl acetic acid (**11**). The esterification of compound **11** led to the formation of corresponding methyl ester **12** (Scheme **I.2**).



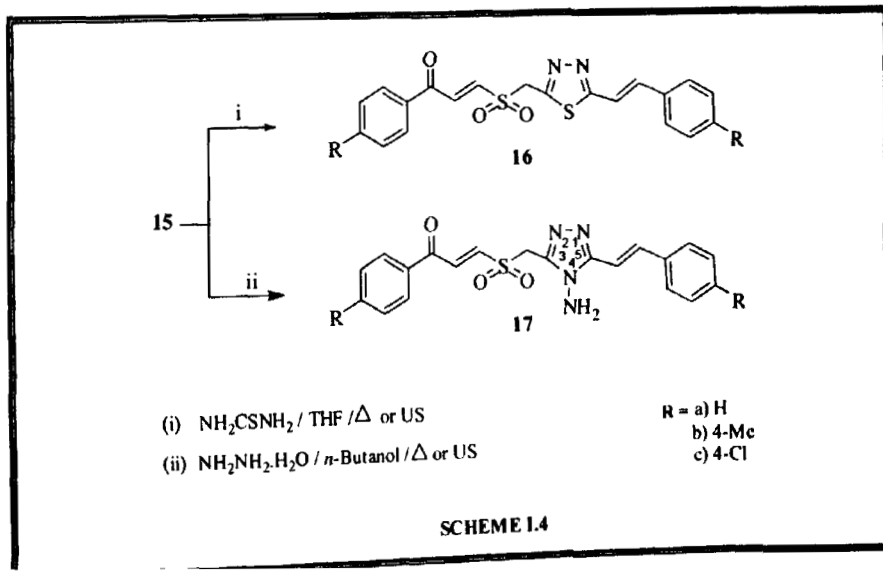
SCHEME I.2

The cyclocondensation of *E*-aroylethenesulfonylacetic acid hydrazide (**6**) with *E*-cinnamic acid (**14**) in the presence of *phosphorus oxychloride* resulted in 2-(*E*-aroylethenesulfonylmethyl)-5-styryl-1,3,4-oxadiazole (**15**) under conventional and ultrasonication methods (Scheme **I.3**).



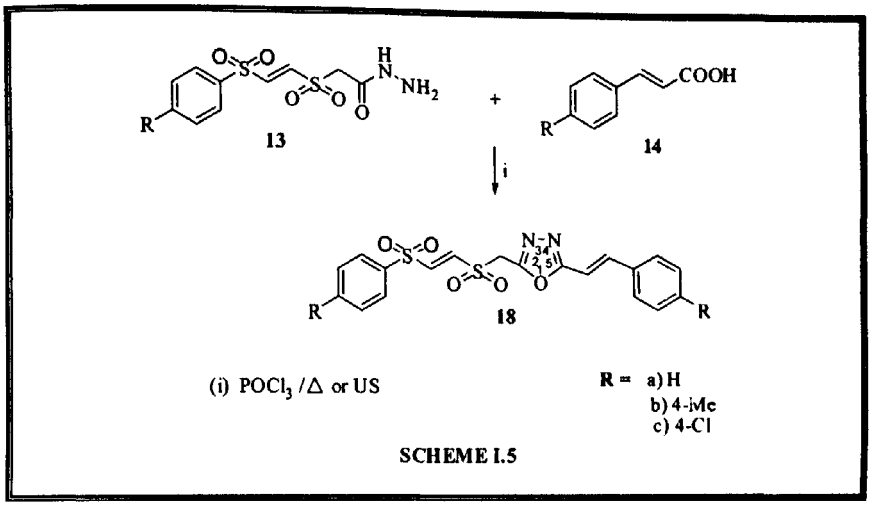
SCHEME I.3

Interconversion of oxadiazole to thiadiazole was effected by treating **15** with thiourea in tetrahydrofuran to obtain 2-(*E*-aroylethenesulfonylmethyl)-5-styryl-1,3,4-thiadiazole (**16**). Besides, the reaction of **15** with hydrazine hydrate in *n*-butanol furnished 3-(*E*-aroylethenesulfonylmethyl)-4-amino-5-styryl-1,2,4-triazole (**17**). The compounds **16** and **17** were also prepared under ultrasonication (Scheme **I.4**).



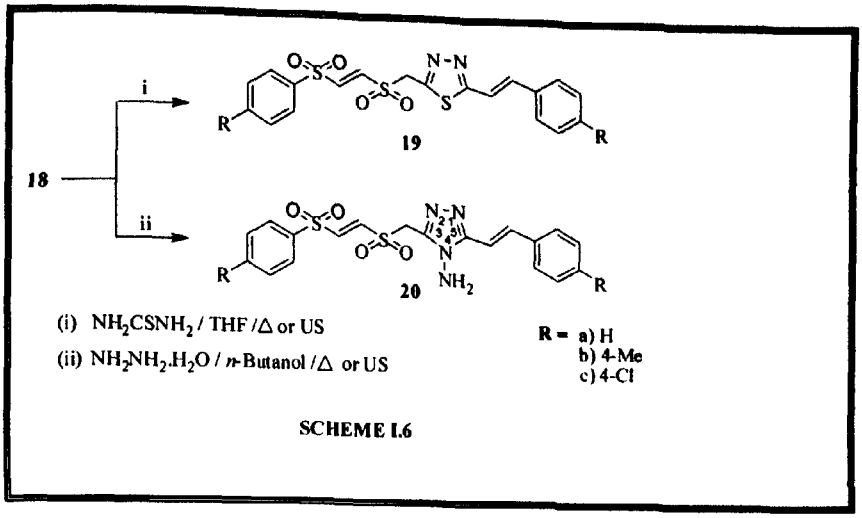
SCHEME I.4

On the other hand, the reaction between compounds **13** and **14** in the presence of phosphorus oxychloride yielded 2-(*E*-arylsulfonylethenesulfonylmethyl)-5-styryl-1,3,4-oxadiazole (**18**). The compound **18** was also synthesized adopting ultrasonication methodology (Scheme **I.5**).



SCHEME I.5

Moreover, 2-(*E*-arylsulfonylethenesulfonylmethyl)-5-styryl-1,3,4-thiadiazole (**19**) was synthesized by the reaction of compound **18** with thiourea in tetrahydrofuran. Similarly interconversion of oxadiazole to triazole was performed by the treatment of **18** with hydrazine hydrate in *n*-butanol to get 3-(*E*-arylsulfonylethenesulfonylmethyl)-4-amino-5-styryl-1,2,4-triazole (**20**). Furthermore, the compounds **19** and **20** were prepared under ultrasonication (Scheme **I.6**). The structures of all the new compounds were established by IR, ^{1}H NMR, ^{13}C NMR, mass and elemental analyses.



SCHEME I.6

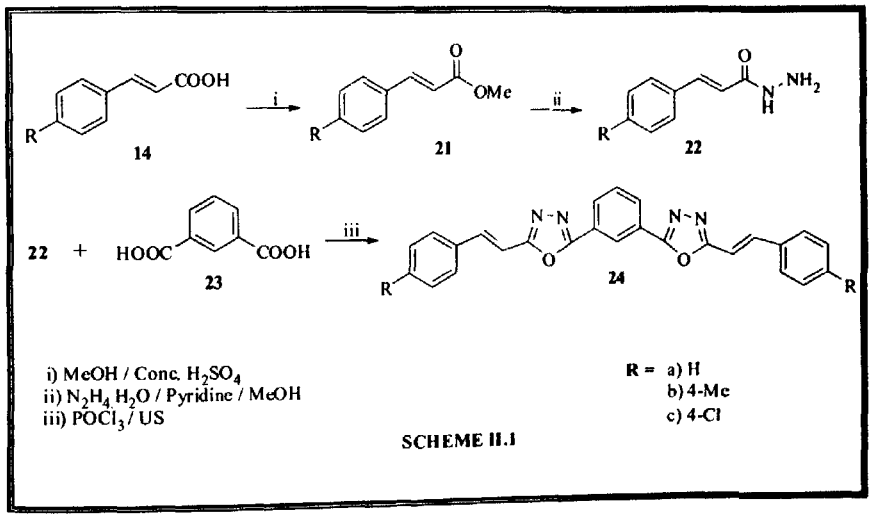
Antimicrobial activity

The compounds **15-20** were screened for antimicrobial activity at four different concentrations 12.5, 25, 50 and 100 μg/well. The antibacterial activity was carried out against *S. aureus*, *B. subtilis* (Gram-positive bacteria) and *P. aeruginosa*, *K. pneumoniae* (Gram-negative bacteria) using Chloramphenicol as reference drug. The compounds were also evaluated for antifungal activity against *P.chrysogenum* and *A. niger* using Ketoconazole as standard drug. The results indicated that Gram-positive bacteria were more susceptible towards the tested compounds than Gram-negative ones. In fact, the compound **20c** exhibited excellent antibacterial activity (41 mm at 100 μg/well) particularly on *B. subtilis* when compared with the standard drug Chloramphenicol (38 mm at 100 μg / welll). All the tested compounds inhibited the spore germination against tested fungi. The compounds **20a** and **20c** showed excellent antifungal activity particularly against *P. chrysogenum* when compared with the standard drug Ketoconazole at all tested concentrations. It was observed that arylsulfonylethenesulfonylmethyl azoles **18-20** displayed higher antimicrobial activity than the aroylethenesulfonylmethyl azoles **15-17**. The presence of electron withdrawing chloro substituent on the aromatic ring enhanced the activity.

CHAPTER-II

Synthesis and antimicrobial activity of 1,3-(bis-(E-2-styryl-1,3,4-oxadiazol-5-yl))benzene, 1,3-(bis(E-2-styryl-1,3,4-thiadiazol-5-yl))benzene, 1,3-bis-(E-3-styryl-4-amino-1,2,4-triazol-5-yl))benzene, 1,4-(bis(E-2-styryl-1,3,4-oxadiazol-5-yl))benzene, 1,4-(bis(E-2-styryl-1,3,4-thiadiazol-5-yl))benzene and 1,4-(bis(E-3-styryl-4-amino-1,2,4-triazol-5-yl))benzene.

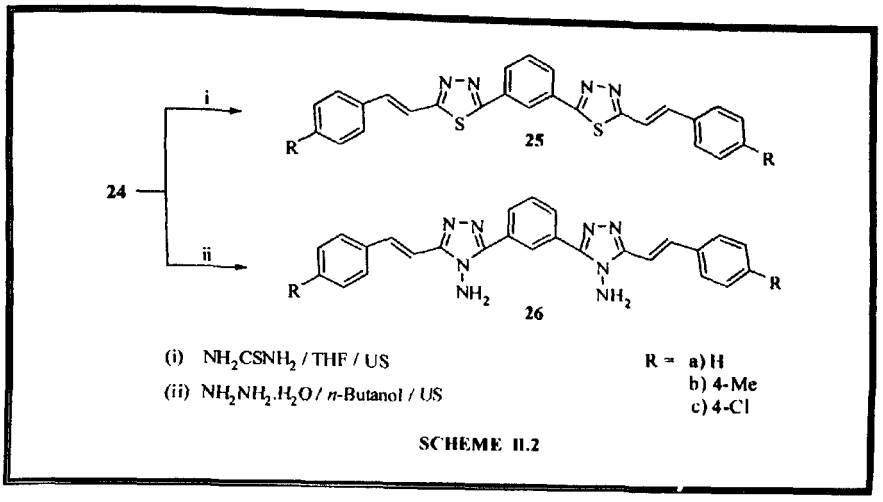
Encouraged by the results of our approach towards the synthesis of a new class of aryl/aroylethenesulfonylmethyl azoles, *E*-cinnamohydrazide (**22**), isophthalic acid (**23**), terepthalic acid (**27**) were used as synthons to prepare symmetrical bis(azoles). The compound **22** was prepared as follows. The reaction of *E*-cinnamic acid (**14**) with methanol in the presence of concentrated sulfuric acid produced methyl cinnamate (**21**) which on treatment with hydrazine hydrate in methanol in the presence of pyridine yielded **22**. The cyclocondensation of 2 moles of compound **22** with 1 mole of isophthalic acid (**23**) in the presence of phosphorus oxychloride under ultrasonication gave 1,3-(bis(*E*-2-styryl-1,3,4-oxadiazol-5-yl))benzene (**24**) (Scheme **II.1**).



SCHEME II.1

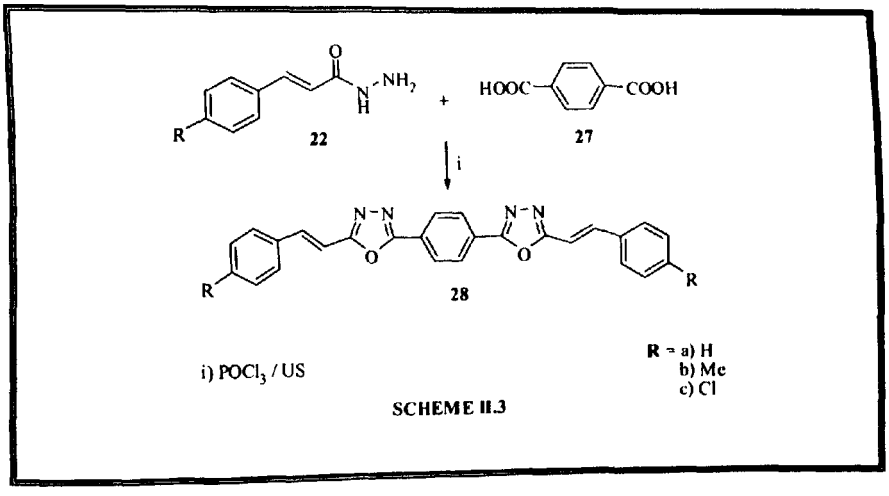
The thiadiazole and triazole rings were developed by the interconversion of oxadiazole with appropriate nucleophiles. Thus 1,3-(bis(*E*-2-styryl-1,3,4-thiadiazol-5-yl))benzene (**25**) was prepared by the reaction of **24** with thiourea in tetrahydrofuran under ultrasonication. Likewise, 1,3-(bis(*E*-3-styryl-4-amino-1,2,4-triazol-5-

yl))benzene (**26**) was obtained by the treatment of **24** with hydrazine hydrate in *n*-butanol (Scheme **II.2**).



SCHEME II.2

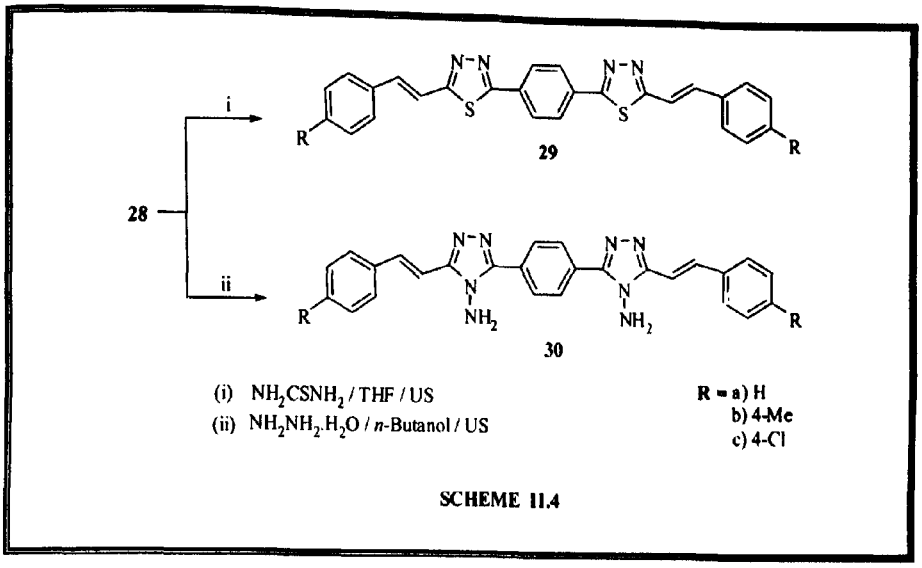
Adopting similar methodology, the reaction of 2 moles of compound **22** with 1 mole of terephthalic acid (**27**) in the presence of phosphorus oxychloride furnished 1,4-(bis(*E*-2-styryl-1,3,4-oxadiazol-5-yl))benzene (**28**) (Scheme **II.3**).



SCHEME II.3

In addition to these, the reaction of compound **28** with thiourea in tetrahydrofuran under ultrasonication gave 1,4-(bis(*E*-2-styryl-1,3,4-thiadiazol-5-yl))benzene (**29**). In a similar way 1,4-(bis(*E*-3-styryl-4-amino-1,2,4-triazol-5-yl))benzene (**30**) was prepared by the treatment of **28** with hydrazine hydrate in

n-butanol (Scheme **II.4**). The structures of all the new compounds were confirmed by spectral data and microanalyses.



SCHEME II.4

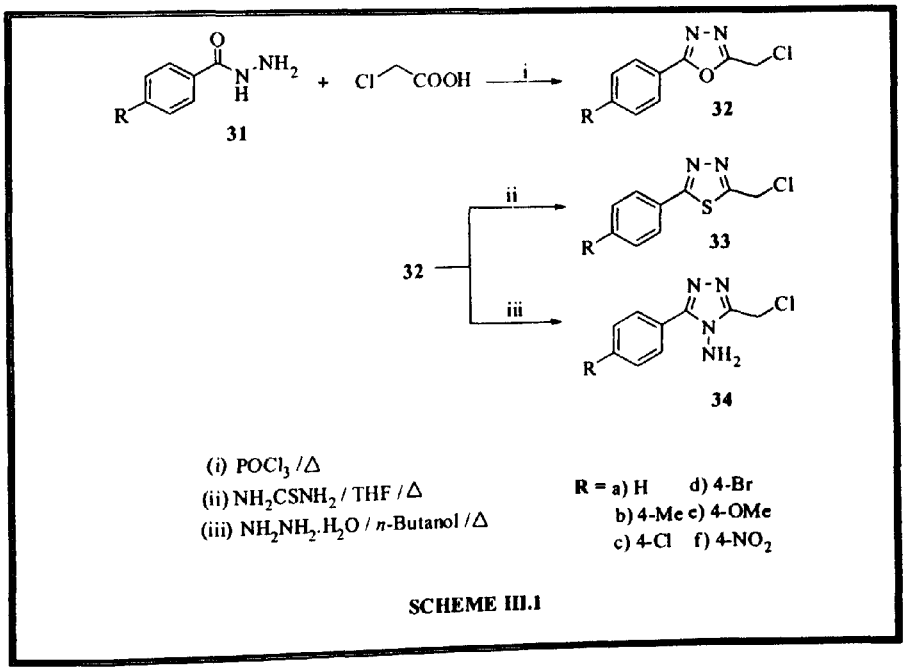
Antimicrobial activity

The compounds **24-26** & **28-30** were evaluated for antimicrobial activity at four concentrations 12.5, 25, 50 and 100 μg/well. The results of antibacterial activity revealed that amongst all the tested compounds **29c**, **30a** and **30c** displayed greater antibacterial activity when compared with the standard drug Chloramphenicol particularly against *S. aureus*. All the tested compounds inhibited the spore germination against tested fungi. The compounds **29c**, **30a** and **30c** exhibited excellent antifungal activity when compared with the standard drug Ketoconazole at all tested concentrations. Further it was noticed that 1,4-phenylene bis(azoles) **28, 29** and **30** showed higher antimicrobial activity when compared with the respective 1,3-phenylene bis(azoles) **24, 25** and **26**.

CHAPTER-III

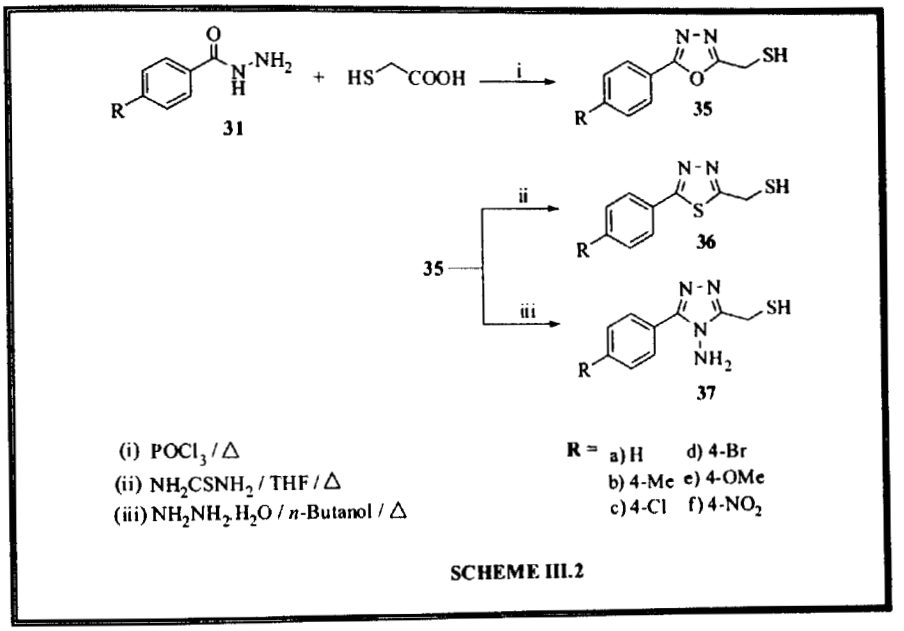
Synthesis and antimicrobial activity of 2-(5-phenyl-1,3,4-oxadiazol-2-yl)methylthio)-4-chloro-6-methylpyrimidine, 2-(5-phenyl-1,3,4-thiadiazol-2-yl)methylthio)-4-chloro-6-methylpyrimidine, 2-(5-phenyl-4-amino-1,2,4-triazol-3-yl)methylthio)-4-chloro-6-methylpyrimidine, 2,4-bis((5-phenyl-1,3,4-oxadiazol-2-yl)methylthio)-6-methylpyrimidine, 2,4-bis((5-phenyl-1,3,4-thiadiazol-2-yl)methylthio)-6-methylpyrimidine and 2,4-bis((5-phenyl-4-amino-1,2,4-triazol-3-yl)methylthio)-6-methylpyrimidine.

To achieve the above mentioned heterocycles 2-chloromethyl azoles, 2-methanethiol azoles and substituted pyrimidine were utilized as reactive intermediates. The 2-(chloromethyl)-5-phenyl-1,3,4-oxadiazole (**32**) was obtained by the reaction of aryl acid hydrazide (**31**) with chloroacetic acid in the presence of phosphorus oxychloride. The reaction of **32** with thiourea in tetrahydrofuran provided 2-(chloromethyl)-5-phenyl-1,3,4-thiadiazole (**33**). Likewise, 3-(chloromethyl)-5-phenyl-4*H*-1,2,4-triazol-4-amine (**34**) was prepared by the treatment of **32** with hydrazine hydrate in *n*-butanol (Scheme **III.1**).



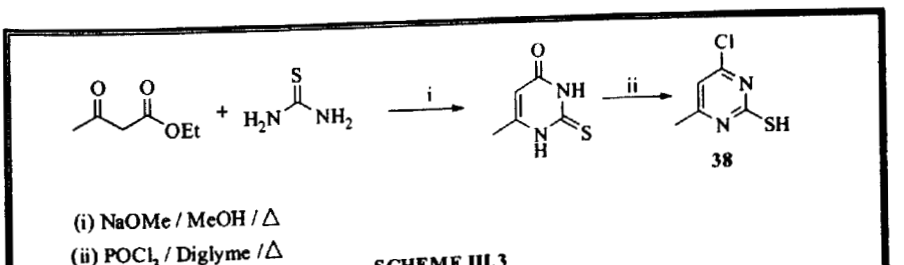
SCHEME III.1

On the other hand, (5-phenyl-1,3,4-oxadiazol-2-yl)methanethiol **(35)** was prepared by the cyclocondensation of compound **31** with mercaptoacetic acid in the presence of phosphorus oxychloride. Interconversion of oxadiazole to thiadiazole was effected by treating **35** with thiourea in tetrahydrofuran to get (5-phenyl-1,3,4-thiadiazol-2-yl)methanethiol **(36)**. Furthermore, the reaction of **35** with hydrazine hydrate in *n*-butanol furnished (4-amino-5-phenyl-4*H*-1,2,4-triazol-3-yl)methanethiol **(37)** (Scheme **III.2**).



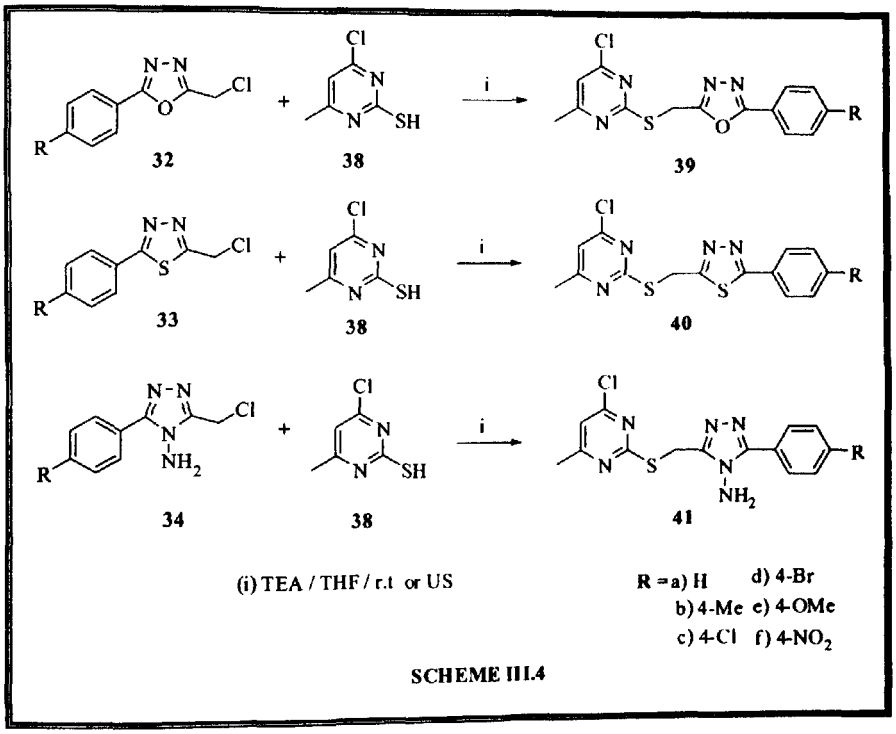
SCHEME III.2

The reaction of ethyl acetoacetate with thiourea in the presence of sodium methoxide produced 6-methyl-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one. This on treatment with phosphorus oxychloride resulted in 4-chloro-6-methylpyrimidine-2-thiol **(38)** (Scheme **III.3**).



SCHEME III.3

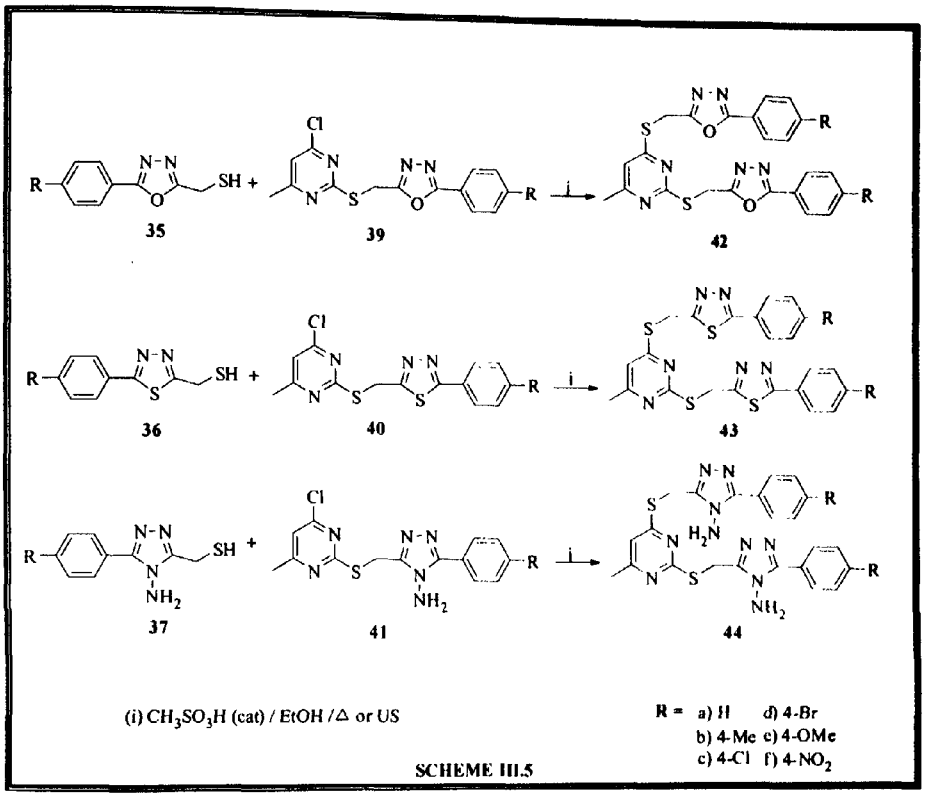
The bis heterocyclic compounds methylthio linked bis(oxadiazolyl)pyrimidines, bis(thiadiazolyl)pyrimidines and bis(triazolyl)pyrimidines were prepared as follows. The reaction between compounds **32** and **38** in the presence of triethylamine in tetrahydrofuran afforded 2-(5-phenyl-1,3,4-oxadiazol-2-ylmethylthio)-4-chloro-6-methylpyrimidine (**39**). In a similar way 2-(5-phenyl-1,3,4-thiadiazol-2-ylmethylthio)-4-chloro-6-methylpyrimidine (**40**) was obtained by the treatment of **33** with **38**. Likewise 2-(5-phenyl-4-amino-1,2,4-triazol-3-ylmethylthio)-4-chloro-6-methylpyrimidine (**41**) was synthesized by the reaction of **34** with **38**. The compounds **39**, **40**, and **41** were also prepared under ultrasonication in an ultrasonic bath operating at a frequency of 35 kHz (Scheme **III.4**).



SCHEME III.4

Apart from these, 2,4-bis((5-phenyl-1,3,4-oxadiazol-2-yl)methylthio)-6-methylpyrimidine (**42**) was prepared by the reaction of **35** with **39** in the presence of a catalytic amount of methanesulfonic acid. Similarly 2,4-bis((5-phenyl-1,3,4-thiadiazol-2-yl)methylthio)-6-methylpyrimidine (**43**) and 2,4-bis((5-phenyl-4-amino-1,2,4-triazol-3-yl)methylthio)-6-methylpyrimidine (**44**) were also synthesized by the reaction of **36** with **40** and **37** with **41**. The compounds **42**, **43**, and **44** were also obtained under

ultrasonication (Scheme **III.5**). higher yields were obtained in shorter reaction times under ultrasonication spectral and analytical tools were utilized to ascertain the structures of the new compounds.



SCHEME III.5

Antimicrobial Activity

The compounds **42-44** were tested for antimicrobial activity at four concentrations 12.5, 25, 50, and 100 μg/well. The results of antibacterial activity indicated that all the tested compounds exhibited more antibacterial activity towards Gram-negative bacteria than Gram-positive bacteria. The **43c** and **43f** were effective particularly against *P. aeruginosa.* All the tested compounds inhibited the spore germination against the tested fungi. The compounds **44c** and **44f** displayed excellent antifungal activity when compared with the standard drug Ketoconazole at all the tested concentrations. The compounds having chloro, bromo and nitro substituents on the phenyl ring showed higher antimicrobial activity than those with methyl and methoxy substituents.

ABOUT THE AUTHOR

The author *Mr. M. Madhu Sekhar* was born on 28th March, 1987 at Krisnapuram village, Kurnool District, Andhra Pradesh, India. After his initial schooling in Vempenta, he obtained **B.Sc.,** degree in 2008 and **M.Sc.,** degree in 2010 from Sri Krishnadevaraya University, Tirupati. He has qualified for **JRF** in june 2011. Later he joined for doctoral programme in 2012 under the supervision of *Prof. A. Padmaja,* Department of Chemistry, S.V.U. College of Sciences, S.V. University, Tirupati. He has been working as UGC-JRF research fellow since 2012.

LIST OF PUBLICATIONS

1. Synthesis and antimicrobial activity of 1,3- / 1,4-phenylene linked bis (azoles)
   M. Madhu Sekhar, G. Sravya, V. Padmavathi, A. Padmaja, R. Usha and P. Supraja
   Res. Chem. Intermed., **42**, 7947 (2016).

2. Ultrasound promoted synthesis and bioassay of a new class of methylthio linked bis(azolyl) pyrimidines
   M. Madhu Sekhar, U. Nagarjuna, V. Padmavathi, A. Padmaja, M.V, Reddy and T.Vijaya
   Chinese Chem. Lett., (In press).

3. Ultrasound promoted synthesis of a new class of *E*-aroylethenesulfonylmethyl / *E*-arylsulfonylethenesulfonylmethylstyrylazoles
   M. Madhu Sekhar, G. Yamini, K. Divya, V. Padmavathi and A. Padmaja,
   Arkivoc (Communicated)

4. Synthesis, antioxidant and cytotoxic activities of bisamidomethane styryl, pyrrolyl and pyrazolyl benzazoles
   S. Durgamma, M. Madhu Sekhar, P. Ramachandra Reddy, G. Dinneswara Reddy, V. Padmavathi and A. Padmaja
   J. Chem. Sci (Communicated)

5. A mild and versatile synthesis of bis(indolyl)methanes catalyzed by benzene-sulfonic acid as potential antioxidants
   S. Pulla Reddy, M. Madhu Sekhar, K. Divya, V. Padmavathi and A. Padmaja,
   J Heterocyclic Chem. (Accepted)

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Synthesis and antimicrobial activity of 1,3-/1,4-phenylene linked bis(azoles)

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Abstract A new class of 1,3- and 1,4-phenylene linked bis(azoles) were prepared from *E*-cinnamohydrazide, isophthalic/terephthalic acids adopting ultrasonication methodology and tested for antimicrobial activity. Amongst all the tested compounds **7c**, **9a**, and **9c** are potential antimicrobial agents against *S. aureus* and *P. chrysogenum*.

Keywords Bis(oxadiazoles) · Bis(thiadiazoles) · Bis(triazoles) · Cyclocondensation · Antimicrobial activity

Introduction

The π-conjugated heterocycles comprise prominent class as they find applications in the fields of materials science and pharmaceutical chemistry. One such type of compounds include oxadiazoles, thiadiazoles, and triazoles. In fact, during the last two decades, the chemistry of azoles and their derivatives have received considerable attention owing to their synthetic and effective biological importance. 2,5-Disubstituted 1,3,4-oxadiazole derivatives possess a broad spectrum of pharmacological applications such as analgesic [1], antimicrobial [2], antiviral [3], anticonvulsant [4], and antihypertensive [5]. Moreover compounds having

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oxadiazole bonded with aromatic and heteroaromatic rings are reported as materials for highly durable organic EL devices [6]. 1,3,4-Thiadiazoles exhibit diverse biological activities due to the presence of =N–C–S moiety [7]. Also, thiadiazole is the most significant heterocycle core present in acetazolamide, the carbonic anhydrase inhibitor, being used in the treatment of glaucoma [8], epileptic seizures [9], hemiplegic migraine [10], etc. 1,2,4-Triazole and its derivatives display anti-inflammatory [11], anticancer [12], antidepressant [13], antibacterial [14], antifungal [15], and anticonvulsant properties [16]. In fact, triazole is the constituent of several pharmaceuticals such as triazolam, alprazolam, estazolam, and ribavirin. Furthermore, emergence of multidrug resistant strains of bacteria is a problem of ever increasing significance. Consequently, the development of new antimicrobial agents will remain the challenging task for medicinal chemists. On the basis of this background and our continued search for the synthesis of biologically potent heterocycles [17–20] the present work synthesis of a new class of bis heterocycles 1,3- and 1,4-phenylene linked bis(azoles) and investigation of antimicrobial activity has been taken up.

Experimental

Apparatus and analysis

Melting points were determined in open capillaries on a Mel-Temp apparatus and are uncorrected. The homogeneity of the compounds was checked by TLC (silica gel H, BDH, hexane/ethyl acetate, 3:1). The IR spectra were recorded on a Thermo Nicolet IR 200 FT-IR spectrometer as KBr pellets and the wave numbers were given in cm^{-1}. The ^1H NMR spectra were recorded in $CDCl_3$/DMSO-d_6 on a Jeol JNM λ-400 MHz spectrometer. The ^{13}C NMR spectra were recorded in $CDCl_3$/DMSO-d_6 on a Jeol JNM spectrometer operating at λ-100 MHz. High-resolution mass spectra were recorded on Micromass Q-TOF micromass spectrometer using electrospray ionization. All chemical shifts were reported in δ (ppm) using TMS as an internal standard. The microanalyses were performed on a Perkin-Elmer 240C elemental analyzer. The temperature was measured by flexible probe throughout the reaction. Ultrasonication was performed in a Bandelin Sonorex RK 102H ultrasonic bath operating at frequency of 35 kHz. The starting compound *E*-cinnamohydrazide was prepared as per the literature precedent [21].

General procedure for the synthesis of 1,3-(bis(*E*-2-styryl-1,3,4-oxadiazol-5-yl)) benzenes (4a–c)/1,4-(bis(*E*-2-styryl-1,3,4-oxadiazol-5-yl))benzenes (5a–c)

The *E*-cinnamohydrazide (1) (2 mmol), isophthalic acid (2)/terephthalic acid (3) (1 mmol), and $POCl_3$ (5 mL) were subjected to ultrasound irradiation for 50–70 min at room temperature. After completion of the reaction (monitored by TLC) the excess $POCl_3$ was removed under vacuum, and the residue was poured onto crushed ice. The separated solid was collected by filtration and washed with

saturated sodium bicarbonate solution followed by water. It was dried and recrystallized from 2-propanol.

Spectral data for compounds (4a–c/5a–c)

1,3-(Bis(E-2-styryl-1,3,4-oxadiazol-5-yl))benzene (4a)

M.p. 150–152 °C. IR (KBr) (cm^{-1}): 1615 (C=C), 1571 (C=N). ^1H NMR (400 MHz, DMSO-d_6): δ 7.05 (d, 2H, C–H_B, J = 15.4 Hz), 7.46–7.75 (m, 16H, H_A and Ar–H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 123.4 (C–H_B), 137.8 (C–H_A), 156.8 (C-2), 159.5 (C-5), 126.0, 127.2, 127.8, 128.9, 130.0, 131.5, 132.2, 133.6 ppm (aromatic carbons). HRMS (*m/z*): 441.4361 [M+ Na]; Anal. calcd. for $C_{26}H_{18}N_4O_2$: C, 74.63; H, 4.34; N, 13.39 %. Found: C, 74.76; H, 4.38; N, 13.55 %.

1,3-(Bis(E-2-(4-methylstyryl)-1,3,4-oxadiazol-5-yl))benzene (4b)

M.p. 165–167 °C. IR (KBr) (cm^{-1}): 1610 (C=C), 1564 (C=N). ^1H NMR (400 MHz, DMSO-d_6): δ 2.29 (s, 6H, Ar–CH_3), 7.09 (d, 2H, C–H_B, J = 15.2 Hz), 7.41–7.69 (m, 14H, H_A and Ar–H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 24.2 (Ar–CH_3), 122.9 (C–H_B), 135.2 (C–H_A), 157.1 (C-2), 158.6 (C-5), 125.7, 126.3, 126.9, 127.4, 128.6, 129.5, 131.3, 134.6 ppm (aromatic carbons). HRMS (*m/z*): 469.4902 [M+ Na]; Anal. calcd. for $C_{28}H_{22}N_4O_2$: calcd. C, 75.32; H, 4.97; N, 12.55 %. Found: C, 75.43; H, 5.00; N, 12.70 %.

1,3-(Bis(E-2-(4-chlorostyryl)-1,3,4-oxadiazol-5-yl))benzene (4c)

M.p. 182–184 °C. IR (KBr) (cm^{-1}): 1620 (C=C), 1579 (C=N). ^1H NMR (400 MHz, DMSO-d_6): δ 7.12 (d, 2H, C–H_B, J = 15.8 Hz), 7.71–7.93 (m, 14H, H_A and Ar–H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 124.7 (C–H_B), 136.5 (C–H_A), 157.5 (C-2), 159.2 (C-5), 127.8, 128.2, 130.1, 131.2, 131.9, 132.8, 134.6, 137.9 ppm (aromatic carbons). HRMS (*m/z*): 510.3260 [M+ Na]. Anal. calcd. for $C_{26}H_{16}Cl_2N_4O_2$: C, 64.08; H, 3.31; N, 11.50 %. Found: C, 64.17; H, 3.33; N, 11.69 %.

1,4-(Bis(E-2-styryl-1,3,4-oxadiazol-5-yl))benzene (5a)

M.p. 155–157 °C. IR (KBr) (cm^{-1}): 1623 (C=C), 1562 (C=N); ^1H NMR (400 MHz, DMSO-d_6): δ 6.86 (d, 2H, C–H_B, J = 14.4 Hz), 7.12–7.60 (m, 16H, H_A and Ar–H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 116.1 (C–H_B), 135.6 (C–H_A), 154.2 (C-2), 157.8 (C-5), 125.7, 126.4, 127.1, 129.2, 130.8, 132.7 ppm (aromatic carbons). HRMS (*m/z*): 441.4370 [M+ Na]. Anal. calcd. for $C_{26}H_{18}N_4O_2$: C, 74.63, H, 4.34, N, 13.39 %. Found: C, 74.75; H, 4.37; N, 13.55 %.

1,4-(Bis(E-2-(4-methylstyryl)-1,3,4-oxadiazol-5-yl))benzene (5b)

M.p. 168–170 °C. IR (KBr) (cm^{-1}): 1619 (C=C), 1559 (C=N); ^1H NMR (400 MHz, DMSO-d_6): δ 2.25 (s, 6H, Ar–CH_3), 6.90 (d, 2H, C–H_B, J = 14.2 Hz), 7.09–7.54

(m, 14H, H_A and Ar–H). ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 23.8 (Ar–CH_3), 115.0 (C–H_B), 132.6 (C–H_A), 152.9 (C-2),156.6 (C-5), 124.2, 125.9, 126.9, 127.5, 128.1, 133.9 ppm (aromatic carbons). HRMS (*m/z*): 469.4908 [M+ Na]; Anal. calcd. for $C_{28}H_{22}N_4O_2$: C, 75.32; H, 4.97; N, 12.55 %. Found: C, 75.42; H, 4.99; N, 12.69 %.

1,4-(Bis(E-2-(4-chlorostyryl)-1,3,4-oxadiazol-5-yl))benzene (5c)

M.p. 183–185 °C. IR (KBr) (cm^{-1}): 1630 (C=C), 1573 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.97 (d, 2H, C–H_B, J = 14.5 Hz), 7.16–7.68 (m, 14H, H_A and Ar H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 116.9 (C H_B), 137.6 (C–H_A), 154.5 (C-2), 157.9 (C-5), 126.7, 128.4, 130.4, 131.6, 132.8, 134.4 ppm (aromatic carbons). HRMS (*m/z*): 510.3258 [M+ Na]; Anal. calcd. for $C_{26}H_{16}Cl_2N_4O_2$: C 64.08; H, 3.31; N, 11.50 %. Found: C, 64.15; H, 3.30; N, 11.71 %.

General procedure for the synthesis of 1,3-(bis(*E*-2-styryl-1,3,4-thiadiazol-5-yl))benzenes (6a–c)/1,4-(bis(*E*-2-styryl-1,3,4-thiadiazol-5-yl))benzenes (7a–c)

A mixture of **4/5** (1 mmol), thiourea (4 mmol), and tetrahydrofuran (8 mL) was put into a sealed tube and heated at reflux conditions under ultrasonication for 90–120 min. The progress of the reaction was monitored by TLC. After completion of the reaction, the contents of the flask were extracted with dichloromethane. The dichloromethane layer was washed with water, brine solution and dried over anhydrous Na_2SO_4. The solvent was removed on a rotary evaporator and the resultant residue was purified by column chromatography (silica gel, 60–120 mesh) using ethyl acetate–hexane (1:3) as eluent.

Spectral data for compounds (6a–c/7a–c)

1,3-(Bis(E-2-styryl-1,3,4-thiadiazol-5-yl))benzene (6a)

M.p. 166–168 °C. IR (KBr) (cm^{-1}): 1625 (C=C), 1580 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.15 (d, 2H, C–H_B, J = 15.6 Hz), 7.50–7.85 (m, 16H, H_A and Ar–*H*) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 125.5 (C H_B), 139.5 (C H_A), 157.2 (C-2), 160.7 (*C*-5), 128.3, 129.1, 130.1, 130.8, 131.4, 133.3, 134.2, 136.2 ppm (aromatic carbons). HRMS (*m/z*): 473.5685 [M+ Na]; Anal. calcd for $C_{26}H_{18}N_4S_2$: (450.58) C, 69.31; H, 4.03; N, 12.43 %. Found: C, 69.24; H, 4.04; N, 12.56 %.

1,3-(Bis(E-2-(4-methylstyryl)-1,3,4-thiadiazol-5-yl))benzene (6b)

M.p. 173–175 °C. IR (KBr) (cm^{-1}): 1563 (C=N), 1622 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.32 (s, 6H, Ar–CH_3), 7.11 (d, 2H, C–H_B, J = 15.5 Hz), 7.48–7.72 (m, 14H, H_A and Ar–H) ppm, ^{13}C NMR (DMSO-d_6): δ 24.7 (Ar–CH_3), 123.0 (C–H_B), 137.8 (C–H_A), 155.4 (C-2), 159.3 (C-5), 128.2, 128.7, 129.3, 130.4, 131.1, 132.0, 132.8, 134.9 ppm (aromatic carbons). HRMS (*m/z*): 501.6212 [M+ Na];

Anal. calcd. for $C_{28}H_{22}N_4S_2$: C, 70.26; H, 4.63; N, 11.71 %. Found: C, 70.38; H, 4.67; N, 11.94 %.

1,3-(Bis(E-2-(4-chlorostyryl)-1,3,4-thiadiazol-5-yl))benzene (6c)

M.p. 195–197 °C. IR (KBr) (cm^{-1}): 1630 (C=C), 1585 (C=N); ^1H NMR (400 MHz, DMSO-d_6) 7.20 (d, 2H, C–H_B, J = 16.0 Hz), 7.75–7.97 (m, 14H, H_A and Ar-*H*) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 124.9 (C–H_B), 139.4 (C–H_A), 158.1 (C-2), 161.8 (C-5), 128.8, 129.4, 130.3, 130.6, 131.9, 132.4, 134.6, 136.8 ppm (aromatic carbons). HRMS (*m/z*): 542.4575 [M+ Na]; Anal. calcd. for $C_{26}H_{16}Cl_2N_4S_2$: C, 60.11; H, 3.10; N, 13.65 %. Found: C, 60.05; H, 3.12; N, 13.81 %.

1,4-(Bis(E-2-styryl-1,3,4-thiadiazol-5-yl))benzene (7a)

M.p. 165–167 °C. IR (KBr) (cm^{-1}): 1626 (C=C), 1572 (C=N); ^1H NMR (400 MHz, DMSO-d_6): δ = 6.95 (d, 2H, C–H_B, J = 14.8 Hz), 7.11–7.63 (m, 16H, H_A and Ar-H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 118.3 (C–H_B), 137.3 (C–H_A), 155.6 (C-2), 157.6 (C-5), 127.2 128.9, 129.2, 130.4, 132.8, 135.5 ppm (aromatic carbons). HRMS (*m/z*): 473.5685 [M+ Na]; Anal. calcd. for $C_{26}H_{18}N_4S_2$ C, 69.31; H, 4.03; N, 12.43 %. Found: C, 69.40; H, 4.05; N, 12.56 %

1,4-(Bis(E-2-(4-methylstyryl)-1,3,4-thiadiazol-5-yl))benzene (7b)

M.p. 172–174 °C. IR (KBr) (cm^{-1}): 1620 (C=C), 1584 (C=N), ^1H NMR (400 MHz, DMSO-d_6): δ 2.28 (s, 6H, Ar–CH_3), 6.90 (d, 2H, C–H_B, J = 14.6 Hz), 7.08–7.59 (m, 14H, H_A and Ar–H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 24.1 (Ar–CH_3), 117.9 (C–H_B), 135.6 (C–H_A), 154.2 (C-2), 157.1 (C-5), 127.8, 128.2, 129.9, 130.2, 132.7, 133.1 ppm (aromatic carbons).HRMS (*m/z*): 501.6215 [M+ Na]; Anal. calcd. for $C_{28}H_{22}N_4S_2$: C, 70.26; H, 4.63; N, 11.71 % Found: C, 70.20; H, 4.64; N, 11.60 %.

1,4-(Bis(E-2-(4-chlorostyryl)-1,3,4-thiadiazol-5-yl))benzene (7c)

M.p. 196–198 °C. IR (KBr) (cm^{-1}): 1627 (C=C), 1587 (C=N); ^1H NMR (400 MHz, DMSO-d_6) δ 6.98 (d, 2H, C–H_B, J = 14.8 Hz), 7.24–7.72 (m, 14H, H_A and Ar–H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 118.9 (C–H_B), 136.8 (C–H_A), 153.6 (C-2), 158.9 (C-5), 129.1, 129.7, 130.6, 131.3, 133.6, 135.8 ppm (aromatic carbons); HRMS (*m/z*): 542.4570 [M+ Na]; Anal. calcd for $C_{26}H_{16}Cl_2N_4S_2$: C, 60.11; H, 3.10; N, 13.65 % Found: C, 60.18; H, 3.13, N, 13.84 %.

General procedure for the synthesis of 1,3-(bis(*E*-3-styryl-4-amino-1,2,4-triazol-5-yl))benzenes (8a–c)/1,4-(bis(*E*-3-styryl-4-amino-1,2,4-triazol-5-yl))benzenes (9a–c)

A solution of **4**/**5** (1 mmol) and hydrazine hydrate (4 mmol) in *n*-butanol (5 mL) was kept under ultrasonication for 60–80 min. Then KOH (2 mmol) was added to

the contents of the flask and the precipitate formed was filtered. The solid obtained was acidified with conc. HCl to pH ≈ 3 and washed with water. It was dried and purified by column chromatography (silica gel, 160–120 mesh) using ethyl acetate–hexane (1:3) as eluent.

Spectral data for compounds (8a–c/9a–c)

1,3-(Bis(E-3-styryl-4-amino-1,2,4-triazol-5-yl))benzene (8a)

M.p. 163–165 °C. IR (KBr) (cm^{-1}): 3362, 3480 (NH_2), 1624 (C=C), 1560 (C=N); ^1H NMR (400 MHz, DMSO-d_6): δ 5.75 (bs, 4H, NH_2), 7.02 (d, 2H, C–H_B, J = 15.2 Hz), 7.44–7.75 (m,16H, H_A and Ar–*H*), ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 122.2 (C–H_B), 139.3 (C–H_A), 155.3 (C-3), 158.2 (C-5), 127.9, 128.8, 129.3, 130.5, 132.2, 132.9, 134.3, 136.7 ppm (aromatic carbons); HRMS (*m/z*): 469.4969 [M+ Na]; Anal. calcd. for $C_{26}H_{22}N_8$: C, 69.94, H, 4.97; N, 25.10 %. Found: C, 70.00; H, 4.98; N, 25.30 %.

1,3-(Bis(E-3-(4-methylstyryl)-4-amino-1,2,4-triazol-5-yl))benzene (8b)

M.p. 169–171 °C. IR (KBr) (cm^{-1}): 3360, 3475 (NH_2), 1621 (C=C), 1557 (C=N); ^1H NMR (400 MHz, DMSO-d_6): δ 2.26 (s, 6H, Ar CH_3), 5.68 (bs, 4H, NH_2), 7.05 (d, 2H, C–H_B, J = 15.0 Hz), 7.40–7.73 (m,14H, H_A and Ar–H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 23.9 (Ar–CH_3), 121.0 (C–H_B), 139.8 (C–H_A), 152.7 (C-3), 156.9 (C-5), 128.5, 129.6, 129.0, 130.1, 132.1, 133.5, 134.1, 134.5 ppm (aromatic carbons). HRMS (*m/z*): 497.5490 [M + Na]. Anal. calcd. for $C_{30}H_{26}N_8$: C, 70.87; H, 5.52; N, 23.61 %. Found: C, 70.97; H, 5.55; N, 23.80 %.

1,3-(Bis(E-3-(4-chlorostyryl)-4-amino-1,2,4-triazol-5-yl))benzene (8c)

M.p. 194–196 °C. IR (KBr) (cm^{-1}): 3375, 3486 (NH_2), 1629 (C=C), 1566 (C=N); ^1H NMR (400 MHz, DMSO-d_6): δ 5.81 (bs, 4H, NH_2), 6.98 (d, 2H, C–H_B, J = 15.5 Hz) 7.68–7.82 (m,14H, H_A and Ar–H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 121.6 (C–H_B), 137.4 (C–H_A), 157.5 (C-3), 160.1 (C-5), 129.5, 129.3, 130.6, 131.4, 131.9, 132.4, 133.8, 135.9 ppm (aromatic carbons). HRMS (*m/z*): 538.3871 [M+ Na]; Anal. calcd. for $C_{26}H_{20}Cl_2N_8$: C, 60.59; H, 3.91; N, 13.76 %. Found: C, 60.72; H, 3.93; N, 13.83 %.

1,4-(Bis(E-3-styryl-4-amino-1,2,4-triazol-5-yl))benzene (9a)

M.p. 162–164 °C. IR (KBr) (cm^{-1}): 3370, 3488 (NH_2), 1618 (C=C), 1584 (C=N); ^1H NMR (400 MHz, DMSO-d_6): δ 5.69 (bs, 4H, NH_2), 6.80 (d, 2H, C–H_B, J = 14.2 Hz), 7.12–7.54 (m, 16H, H_A and Ar–H), ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 115.2 (C–H_B), 133.5 (C–H_A), 154.3 (C-3), 156.1 (C-5), 126.9, 127.8, 129.6, 131.0, 132.2, 134.1 ppm (aromatic carbons) HRMS (*m/z*): 469.4955 [M+ Na]; Anal. calcd. for $C_{26}H_{22}N_8$: C, 69.94; H, 4.97; N, 25.10 % Found: C, 69.75; H, 5.01; N, 25.31 %.

1,4-(Bis(E-3-(4-methylstyryl)-4-amino-1,2,4-triazol-5-yl))benzene (9b)

M.p 173–175 °C. IR (KBr) (cm^{-1}): 3365, 3475 (NH_2), 1581 (C=N), 1622 (C=C); ^1H NMR (400 MHz, DMSO-d_6): δ = 2.21 (s, 6H, Ar–CH_3), 5.75 (bs, 4H, NH_2), 6.76 (d, 2H, C–H_B, J = 14.0 Hz), 7.02–7.44 (m, 14H, H_A and Ar–H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 23.5 (Ar–CH_3) 116.7 (C–H_B), 131.6 (C–H_A), 150.9 (C-3), 154.4 (C-5), 127.2, 128.7, 129.3, 131.4, 132.5, 133.6 ppm (aromatic carbons); HRMS (*m/z*): 497.5491 [M+ Na]; Anal. calcd. for $C_{28}H_{26}N_8$: C, 70.87; H, 5.52; N, 23.61 %. Found: C, 70.95; H, 5.51; N, 23.76 %.

1,4-(Bis(E-3-(4-chlorostyryl)-4-amino-1,2,4-triazol-5-yl))benzene (9c)

M.p 193–195 °C. IR (KBr) (cm^{-1}): 3376, 3492 (NH_2), 1583 (C=N), 1625 (C=C); ^1H NMR (400 MHz, DMSO-d_6): δ 5.84 (bs, 4H, NH_2), 6.89 (d, 2H, C H_B, J = 14.4 Hz) 7.18–7.67 (m,14H, H_A and Ar–H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 114.6 (C–H_B), 133.9 (C–H_A), 152.8 (C-3), 157.5 (C-5), 128.4, 129.5, 130.7, 132.8, 133.9, 135.6 ppm (aromatic carbons). HRMS (*m/z*): 538.3869 (M+ Na]; Anal calcd for $C_{26}H_{20}Cl_2N_8$: C, 60.59; H, 3.91; N, 13.76 % Found: C, 60.69; H, 3.94; N, 13.94 %.

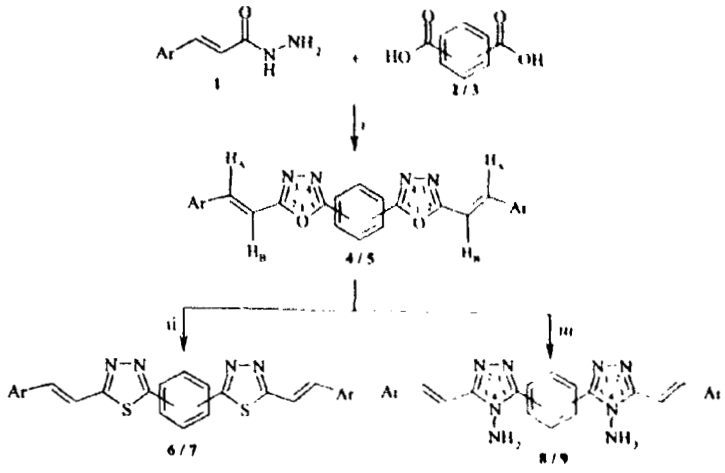
Experimental procedure for antimicrobial activity

The in vitro antimicrobial studies were carried out by agar well diffusion against test organisms [22, 23]. Nutrient broth (NB) plates were swabbed with 24 h old broth culture (100 µL) of test bacteria. Using the sterile cork borer, wells (6 mm) were made into each Petri plate. The compounds were dissolved in DMSO of 5 mg/mL and from this 2.5, 5, 10, and 20 µL (12.5, 25, 50, 100 µg/mL) were added into the wells by using sterile pipettes. Simultaneously, the standard antibiotics, chloramphenicol for antibacterial activity and ketoconazole for antifungal activity (as positive control) were tested against the pathogens. The samples were dissolved in DMSO, which showed that no zone of inhibition acts as negative control. The plates were incubated at 37 °C for 24 h for bacteria and at 28 °C for 48 h for fungi. After appropriate incubation, the diameter of zone of inhibition of each well was measured. Duplicates were maintained and the average values were calculated for eventual antimicrobial activity. Broth dilution test was used to determine minimum inhibitory concentration (MIC) of the above mentioned samples [24, 25]. Freshly prepared nutrient broth was used as diluents. The 24 h old culture of the test bacteria *S. aureus*, *B. subtilis*, *P. aeruginosa*, and *K. pneumoniae* and the test fungi *A. niger* and *P. chrysogenum* were diluted 100-fold in nutrient broth (100 µL bacterial cultures in 10 mL NB). The stock solution of the synthesized compounds was prepared in DMSO by dissolving 5 mg of the compound in 1 mL of DMSO. Increasing concentrations of the test samples (1.25, 2.5, 5, 10, 20, 40 µL of stock solution contains 6.25, 12.5, 25, 50, 100, 200 µg of the compounds) were added to the test tubes containing the bacterial and fungal cultures. All the tubes were incubated at 37 °C for 24 h for bacteria and at 28 °C for 48 h for fungi. The tubes

were examined for visible turbidity and using NB as control. Control without test samples and with solvent was assayed simultaneously. The lowest concentration that inhibited visible growth of the tested organisms was recorded as MIC. To determine the minimum bactericidal concentration (MBC) [26] and minimum fungicidal concentration (MFC) [27] for each set of test tubes in the MIC determination, a loopful of broth was collected from those tubes, which did not show any growth and was inoculated on sterile nutrient broth (for bacteria) and PDA (for fungi) by streaking. Plates inoculated with bacteria and fungi were incubated at 37 °C for 24 h and at 28 °C for 48 h, respectively. After incubation, the lowest concentration was noted as MBC (for bacteria) or MFC (for fungi) at which no visible growth was observed.

Results and discussion

The 1,3- and 1,4-phenylene linked bis(oxadiazoles), bis(thiadiazoles), and bis(triazoles) were synthesized from the simple substrates *E*-cinnamohydrazide (**1**), isophthalic acid (**2**), and terephthalic acid (**3**). The cyclocondensation of 2 mol of compound **1** with 1 mol of compound **2** in the presence of $POCl_3$ under ultrasonication afforded 1,3-(bis(*E*-2-styryl-1,3,4-oxadiazol-5-yl))benzene (**4**). The compound **4** was interconverted to thiadiazole and triazole by treating with appropriate nucleophiles. Thus 1,3-(bis(*E*-2-styryl-1,3,4-thiadiazol-5-yl))benzene (**6**) was prepared by the reaction of **4** with thiourea in THF under ultrasonication. Likewise, 1,3-(bis(*E*-3-styryl-4-amino-1,2,4 triazol-5-yl))benzene (**8**) was obtained by the treatment of **4** with hydrazine hydrate in *n* butanol. The ^{1}H NMR spectra of **4a**, **6a**, and **8a** exhibited a doublet at δ 7.05, 7.15, 7.02 ppm due to olefin proton H_B. The signal due to other olefin proton, H_A adjacent to aryl group appeared as a doublet at much downfield region and merged with aromatic protons. The coupling constant $J_{AB} \approx 15.4$ Hz indicated that they possess *trans* geometry. Apart from these, a broad singlet was observed at δ 5.75 ppm in compound **8a** due to NH_2. The signals of NH_2 disappeared when D_2O was added. Adopting similar methodology, the reaction of 2 mol of compound **1** with 1 mol of compound **3** in the presence of $POCl_3$ furnished 1,4-(bis(*E*-2-styryl-1,3,4-oxadiazol-5-yl))benzene (**5**). Also, the reaction of compound **5** with thiourea in THF under ultrasonication produced 1,4-(bis(*E*-2-styryl-1,3,4-thiadiazol-5-yl))benzene (**7**). In a similar way 1,4-(bis(*E*-3-styryl-4-amino-1,2,4-triazol-5-yl))benzene (**9**) was prepared by the reaction of **5** with hydrazine hydrate in *n*-butanol (Scheme 1; Table 1). The ^{1}H NMR spectra of **5a**, **7a**, and **9a** presented a doublet at δ 6.86, 6.95, 6.80 ppm due to H_B. Another doublet corresponding to H_A observed at much downfield region and merged with aromatic protons. The coupling constant $J_{AB} \approx 14.3$ Hz indicated that they possess *trans* geometry. Further, a broad singlet was appeared at 5.69 ppm due to NH_2 in compound **9a** which disappeared on deuteration. The structures of all the compounds were also established by IR, ^{13}C NMR, mass spectra and microanalyses.



(i) $POCl_3$ /)))
(ii) NH_2CSNH_2 / THF /)))
(iii)$NH_2NH_2.H_2O$ / *n*-Butanol /)))

Ar
a) Ph
b) 4-Me-Ph
c) 4-Cl-Ph

Scheme 1 Synthesis of 1,3-/1,4-phenylene linked bis(azoles) **4**–**9**

Table 1 The reaction time and yield of the compounds **4**–**9**

Isophthalic acid			Terephthalic acid		
Product	Time (min)	Yield (%)	Product	Time (min)	Yield (%)
4a	65	84	**5a**	55	87
4b	70	82	**5b**	65	85
4c	60	86	**5c**	50	91
6a	110	85	**7a**	95	93
6b	120	87	**7b**	100	90
6c	95	90	**7c**	90	95
8a	75	83	**9a**	64	86
8b	80	81	**9b**	66	89
8c	72	88	**9c**	60	92

Antimicrobial activity

The compounds **4**–**9** were dissolved in DMSO at four different concentrations 12.5, 25, 50, and 100 mg L^{-1}. Bacterial strains *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and fungi *Aspergillus niger* and

Table 2 The in vitro antibacterial activity of compounds 4–9

Compound	Zone of inhibition (mm)							
	Gram-positive bacteria							
	S. aureus				*B. subtilis*			
	12.5 µg per well	25 µg per well	50 µg per well	100 µg per well	12.5 µg per well	25 µg per well	50 µg per well	100 µg per well
4a	-	-	-	13 ± 2	-	-	-	10 ± 1
4b	-	-	-	9 ± 2	-	-	-	8 ± 2
4c	08 ± 2	10 ± 3	12 ± 2	15 ± 3	7 ± 1	9 ± 2	11 ± 2	13 ± 1
5a	-	-	13 ± 1	14 ± 2	-	-	9 ± 2	12 ± 2
5b	-	-	-	11 ± 3	-	-	-	9 ± 2
5c	11 ± 3	13 ± 2	16 ± 3	18 ± 2	10 ± 2	12 ± 1	14 ± 2	16 ± 1
6a	16 ± 1	18 ± 2	20 ± 1	23 ± 3	13 ± 1	16 ± 2	18 ± 3	20 ± 2
6b	13 ± 1	15 ± 1	17 ± 2	20 ± 1	10 ± 1	12 ± 2	15 ± 2	17 ± 1
6c	19 ± 1	21 ± 2	23 ± 3	26 ± 2	17 ± 1	19 ± 2	21 ± 3	24 ± 2
7a	27 ± 2	29 ± 3	31 ± 1	34 ± 2	23 ± 2	25 ± 3	27 ± 2	29 ± 4
7b	22 ± 1	24 ± 2	27 ± 2	30 ± 1	20 ± 2	22 ± 2	24 ± 3	26 ± 3
7c	31 ± 3	33 ± 2	36 ± 2	38 ± 4	26 ± 2	28 ± 1	30 ± 3	33 ± 3
8a	21 ± 2	23 ± 3	25 ± 2	27 ± 2	19 ± 2	21 ± 3	23 ± 2	25 ± 2
8b	16 ± 1	18 ± 2	20 ± 3	23 ± 3	14 ± 1	17 ± 2	19 ± 1	21 ± 3
8c	24 ± 3	26 ± 2	29 ± 3	31 ± 2	21 ± 2	23 ± 2	25 ± 2	30 ± 3
9a	30 ± 3	32 ± 1	34 ± 3	39 ± 3	27 ± 2	29 ± 3	32 ± 1	34 ± 2
9b	26 ± 3	29 ± 1	31 ± 2	34 ± 2	23 ± 2	25 ± 2	27 ± 3	31 ± 2
9c	32 ± 2	34 ± 3	37 ± 2	41 ± 4	30 ± 2	32 ± 3	35 ± 2	37 ± 4

Table 2 continued

Compound	Zone of inhibition (mm)							
	Gram-positive bacteria							
	S. aureus				*B. subtilis*			
	12.5 µg per well	25 µg per well	50 µg per well	100 µg per well	12.5 µg per well	25 µg per well	50 µg per well	100 µg per well
Chloram-phenicol	30 ± 1	32 ± 2	35 ± 2	37 ± 2	32 ± 3	34 ± 2	36 ± 2	40 ± 2
Control (DMSO)	-	-	-	-	-	-	-	-

Compound	Zone of inhibition (mm)							
	Gram-negative bacteria							
	P. aeruginosa				*K. pneumoniae*			
	12.5 µg per well	25 µg per well	50 µg per well	100 µg per well	12.5 µg per well	25 µg per well	50 µg per well	100 µg per well
4a	-	-	-	-	-	-	-	-
4b	-	-	-	-	-	-	-	-
4c	-	-	-	-	-	-	-	-
5a	-	-	-	-	-	-	-	-
5b	-	-	-	-	-	-	-	-
5c	-	-	-	-	-	-	-	-
6a	9 ± 2	10 ± 2	13 ± 1	15 ± 2	14 ± 2	17 ± 2	21 ± 1	23 ± 3
6b	7 ± 1	9 ± 2	11 ± 2	13 ± 1	11 ± 3	14 ± 1	17 ± 2	19 ± 2
6c	11 ± 2	13 ± 3	16 ± 2	18 ± 1	17 ± 2	21 ± 2	24 ± 1	26 ± 3
7a	12 ± 2	14 ± 1	17 ± 2	19 ± 2	25 ± 1	24 ± 2	27 ± 3	30 ± 2
7b	10 ± 2	12 ± 3	13 ± 2	16 ± 3	18 ± 2	20 ± 2	23 ± 2	26 ± 3

Table 2 continued

Compound	Zone of inhibition (mm)							
	Gram-negative bacteria							
	P. aeruginosa				*K. pneumoniae*			
	12.5 µg per well	25 µg per well	50 µg per well	100 µg per well	12.5 µg per well	25 µg per well	50 µg per well	100 µg per well
7c	15 ± 1	17 ± 2	20 ± 3	22 ± 1	29 ± 2	30 ± 3	32 ± 2	34 ± 2
8a	11 ± 2	13 ± 3	15 ± 2	17 ± 3	20 ± 2	23 ± 3	25 ± 3	27 ± 2
8b	9 ± 3	11 ± 1	12 ± 2	14 ± 2	15 ± 3	17 ± 3	20 ± 2	24 ± 1
8c	13 ± 2	15 ± 3	17 ± 2	20 ± 4	22 ± 2	25 ± 2	28 ± 3	30 ± 3
9a	17 ± 3	19 ± 2	21 ± 1	23 ± 2	25 ± 3	27 ± 1	30 ± 2	32 ± 2
9b	14 ± 2	16 ± 2	18 ± 2	20 ± 1	21 ± 2	24 ± 2	26 ± 2	29 ± 3
9c	20 ± 2	22 ± 2	24 ± 2	26 ± 2	28 ± 2	30 ± 1	33 ± 2	37 ± 3
Chloram-phenicol	25 ± 1	27 ± 2	29 ± 3	32 ± 1	38 ± 2	40 ± 2	42 ± 3	44 ± 2
Control (DMSO)	-	-	-	-	-	-	-	-

-, no activity, ±, standard deviation

Pencillium chrysogenum were obtained from Department of Biotechnology, Sri Padmavathi Mahila Visvavidyalayam, Tirupati, India.

Antibacterial activity

The results of antibacterial activity presented in Table 2 and Fig. 1 reveal that Gram-positive bacteria were more susceptible towards the tested compounds than Gram-negative bacteria. The compounds **6**, **7**, **8**, and **9** exhibited moderate to good antibacterial activity against Gram-positive bacteria and Gram-negative bacteria. However, compounds **4** and **5** displayed low activity against Gram-positive bacteria and no activity against Gram-negative bacteria. Further, it was noted that 1,4-phenylene bis(azoles) **5**, **7**, and **9** showed higher antibacterial activity when compared with the respective 1,3-phenylene bis(azoles) **4**, **6**, and **8**. Amongst all the tested compounds **7c**, **9a**, and **9c** displayed greater antibacterial activity when compared with the standard drug chloramphenicol particularly against *S. aureus*.

Antifungal activity

All the tested compounds inhibited the spore germination against tested fungi. It was observed that all the compounds exhibited pronounced antifungal activity towards *P. chrysogenum* compared to *A. niger*. The compounds **6**, **9** displayed higher activity whereas **4** and **5** showed least activity. It was observed that compounds **8** and **9** showed greater activity than **6** and **7**. The compounds **7c**, **9a**, and **9c** displayed excellent antifungal activity when compared with the standard drug ketoconazole at all tested concentrations (Table 3; Fig. 2).

MIC, MBC, and MFC of the compounds 7c, 9a, and 9c

The minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) values of the tested

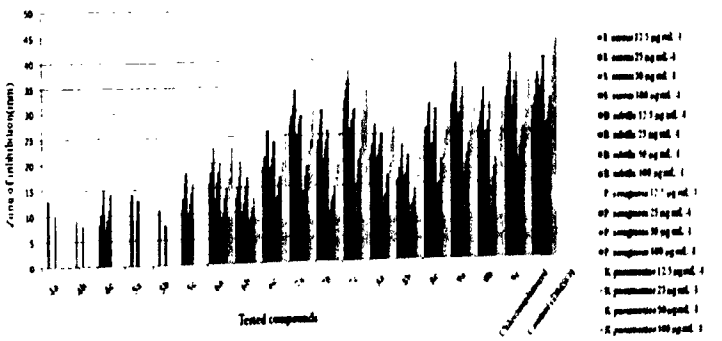


Fig. 1 The in vitro antibacterial activity of compounds **4**–**9**

Table 3 The in vitro antifungal activity of compounds **4–9**

Compound	Zone of inhibition (mm)							
	A. niger				*P. chrysogenum*			
	12.5 µg per well	25 µg per well	50 µg per well	100 µg per well	12.5 µg per well	25 µg per well	50 µg per well	100 µg per well
4a	–		–	10 ± 2			11 ± 1	13 ± 2
4b	–	–	–	7 ± 1				10 ± 1
4c	–	–	–	12 ± 3	8 ± 1	10 ± 2	12 ± 1	15 ± 2
5a	–	8 ± 2	10 ± 1	13 ± 2	11 ± 2	13 ± 3	15 ± 2	17 ± 2
5b	–	–		9 ± 2		8 ± 1	10 ± 3	13 ± 2
5c	–	10 ± 2	13 ± 1	16 ± 2	14 ± 3	16 ± 2	18 ± 2	20 ± 3
6a	13 ± 2	15 ± 2	18 ± 1	21 ± 2	20 ± 3	22 ± 2	24 ± 3	26 ± 1
6b	9 ± 2	11 ± 2	13 ± 1	15 ± 2	17 ± 1	19 ± 2	21 ± 1	23 ± 2
6c	15 ± 1	18 ± 2	21 ± 3	25 ± 2	23 ± 2	25 ± 1	28 ± 1	30 ± 2
7a	19 ± 2	21 ± 2	24 ± 1	26 ± 3	31 ± 1	34 ± 2	36 ± 2	38 ± 2
7b	16 ± 1	18 ± 2	20 ± 2	23 ± 2	27 ± 2	29 ± 2	31 ± 1	33 ± 2
7c	23 ± 1	25 ± 2	27 ± 3	29 ± 2	35 ± 3	37 ± 2	38 ± 2	41 ± 3
8a	17 ± 2	19 ± 3	22 ± 1	24 ± 2	25 ± 2	27 ± 2	29 ± 1	32 ± 1
8b	14 ± 2	16 ± 1	18 ± 2	21 ± 3	22 ± 2	24 ± 1	26 ± 2	28 ± 2
8c	19 ± 1	21 ± 2	23 ± 2	25 ± 3	28 ± 1	30 ± 2	33 ± 3	35 ± 2
9a	24 ± 2	26 ± 2	28 ± 2	32 ± 3	34 ± 2	36 ± 2	39 ± 1	42 ± 2
9b	21 ± 1	23 ± 2	25 ± 3	28 ± 2	31 ± 1	33 ± 2	35 ± 1	37 ± 2
9c	27 ± 1	30 ± 3	33 ± 2	35 ± 2	37 ± 2	39 ± 1	42 ± 2	45 ± 1
Ketoconazole	29 ± 3	31 ± 2	34 ± 2	37 ± 1	34 ± 1	36 ± 2	37 ± 2	39 ± 3
Control (DMSO)	–	–	–	–	–	–	–	–

–, no activity; ±, standard deviation

compounds are shown in Table 4. MIC is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism. (But does not guarantee that the microorganisms are completely killed.) The MBC/MFC is the lowest concentration of antibiotic required to kill a particular bacterium/fungi. The MBC/MFC involves an additional set of steps performed once the minimum inhibitory concentration (MIC) is determined. The antimicrobials are usually regarded as bactericidal/fungicidal if the MBC/MFC is not greater than four times the MIC [28]. The compounds **7c**, **9a**, and **9c** exhibited low MIC values. The MBC and MFC values in **7c**, **9a**, and **9c** are 2 × MIC in the case of *S. aureus* and *P. chrysogenum*. However, the other compounds showed bactericidal and fungicidal effects greater than 2 × MIC. The structure–activity relationship of the synthesized compounds indicated that 1,4-phenylene bis(azoles) **5**, **7**, **9** displayed comparatively higher antimicrobial activity than the corresponding 1,3-phenylene bis(azoles) **4**, **6**, **8**. This may be due to the presence of effective conjugation in former compounds. It was observed that compounds having thiadiazole and triazole moieties (**6–9**)

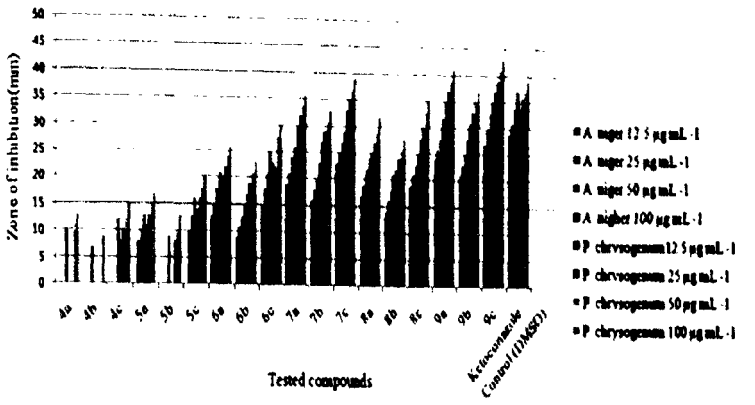


Fig. 2 Thein vitro antifungal activity of compounds **4**–**9**

Table 4 MIC, MBC and MFC of compounds **7c**, **9a**, and **9c**

Compound	Minimum inhibitory concentration MIC (MBC/MFC) μg ml^{-1}					
	S. aureus	*B. subtilis*	*P. aeruginosa*	*K. pneumoniae*	*A. niger*	*P. chrysogenum*
7c	6.25 (12.5)	50 (200)	100 (>200)	100 (>200)	25 (100)	12.5 (25)
9a	6.25 (12.5)	25 (100)	50 (200)	100 (>200)	25 (100)	12.5 (25)
9c	6.25 (12.5)	25 (100)	50 (200)	100 (200)	12.5 (50)	12.5 (25)
Chloramphenicol	6.25	6.25	6.25	12.5		
Ketoconazole	-	-	-		6.25	12.5

–, no activity

exhibited greater antimicrobial activity than compounds with oxadiazole unit (**4**, **5**). Amongst the compounds **6**–**9** those with triazoles (**8**, **9**) displayed slightly higher activity than thiadiazoles (**6**, **7**). Moreover, the compounds with a chloro substituent on the aromatic ring enhanced the activity when compared with methyl and unsubstituted compounds. The compounds **7c**, **9a**, and **9c** are identified as potent antimicrobial agents against *S. aureus* and *P. chrysogenum*

Conclusion

In conclusion a new class of bis heterocycles 1,3-/1,4-(bis(*E*-2-styryl-1,3,4-oxadiazol/thiadiazol-5-yl))benzenes and 1,3-/1,4-(bis(*E*-3-styryl-4-amino-1,2,4-triazol-5-yl))benzenes were prepared from the simple substrates *E*-cinnamohydrazide, isophthalic acid and terephthalic acid adopting ultrasonication methodology and tested for antimicrobial activity. The 1,4-phenylene linked bis(azoles) exhibited higher antimicrobial activity than 1,3-phenylene linked bis(azoles). Moreover,

compounds with triazole and thiadiazole moieties showed greater antimicrobial activity than those having oxadiazole. The compounds **7c**, **9a**, and **9c** are identified as potential antimicrobial agents against *S. aureus* and *P. chrysogenum*. Further, the compounds containing chloro substituent on aromatic ring enhanced the activity.

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