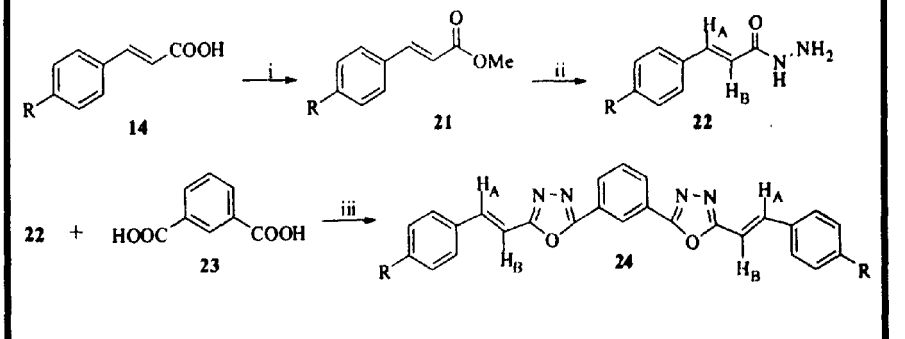
CHAPTER-II

Synthesis and antimicrobial activity of 1,3- / 1,4-bis(styryl-1,3,4-oxadiazolyl / 1,3,4-thiadiazolyl / 1,2,4-triazolyl)benzenes.

CHAPTER-II

The chapter-II deals with 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.

To achieve the above mentioned bis (azolyl) benzenes, *E*-cinnamohydrazide (**22**), isophthalic acid (**23**), terephthalic acid (**27**) were used as synthons. 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** & Table **II.1**).



i) MeOH / Conc. H_2SO_4
ii) $N_2H_4.H_2O$ / Pyridine / MeOH
iii) $POCl_3$ / US

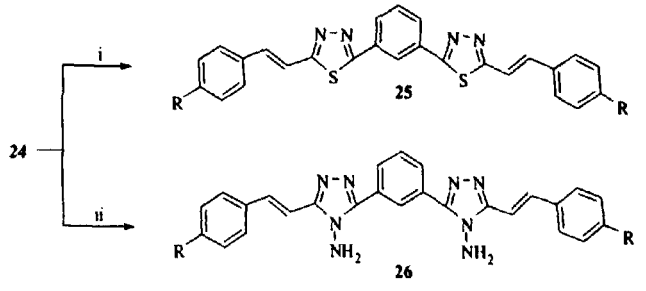
R = a) H
b) Me
c) Cl

SCHEME II.1

The IR spectra of compound **22** displayed absorption bands in the regions 3331-3342 & 3434-3450 (NH_2), 3238-3248 (NH), 1649-1660 (CO) and 1610-1625 cm^{-1} (C=C). The ^1H NMR spectrum of **22a** exhibited a doublet at δ 6.81 due to olefin proton H_B and two broad singlets at 9.65 and 3.98 ppm for NH and NH_2 which disappeared on deuteration. Another doublet due to other olefin proton H_A appeared at much downfield region and merged with aromatic protons. The coupling constant value J = 15.0 Hz indicated that they possess *trans* geometry. The ^{13}C NMR spectrum of **22a** showed signals at δ 120.4, 142.2 and 162.5 ppm due to C-H_B, C-H_A and CO, respectively. The HRMS spectrum of **22a** presented M+Na peak at m/z 185.1824 corresponding to the chemical composition, $C_9H_{10}N_2O$+Na. The IR spectra of **24** showed absorption bands in the regions 1610-1620 (C=C) and 1564-1579 cm^{-1} (C=N) (Table **II.2**). The ^1H NMR spectrum of **24a** (Fig. **II.1**) exhibited a doublet at δ 7.05 ppm due to olefin proton H_B. Another doublet due to olefin proton H_A appeared at much downfield region and merged with aromatic protons. The coupling constant value J = 15.4 Hz indicated that they possess *trans* geometry (Table **II.3**). The ^{13}C NMR spectrum of **24a** (Fig. **II.2**) displayed signals at δ 123.4, 137.8, 156.8 and 159.5 ppm due to C-H_B, C-H_A, C-2 and C-5 in addition to the signals of aromatic carbons (Table **II.3**). The HRMS spectrum of **24a** (Fig. **II.3**) presented M+Na peak at m/z 441.4361 corresponding to the chemical composition, $C_{26}H_{18}N_4O_2$+Na.

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** & Table **II.1**).

The IR spectra of compounds **25** and **26** exhibited absorption bands in the regions 1621-1630 and 1557-1585 cm^{-1} due to C=C and C=N, respectively. Apart from these, in **26** broad absorption bands appeared in the regions 3475-3486 & 3360-3375 cm^{-1} were assigned to NH_2 (Table **II.2**). The ^1H NMR spectra of **25a** and **26a** (Fig. **II.4**) presented a



(i) NH_2CSNH_2 / THF / US

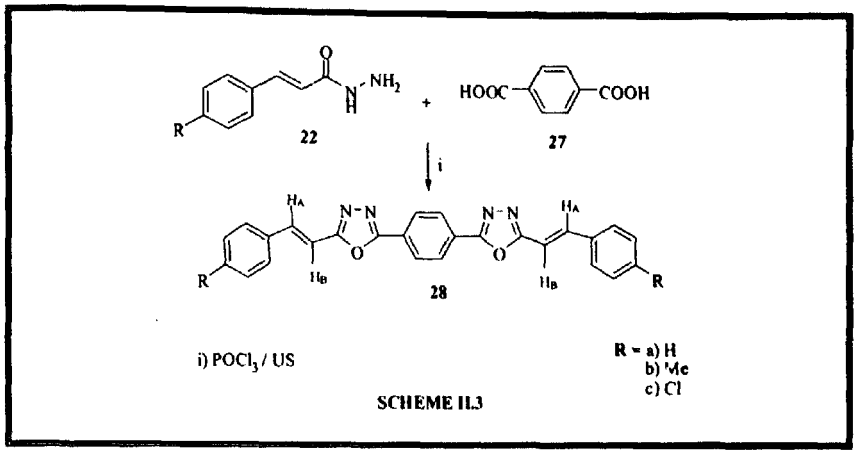
(ii) $NH_2NH_2.H_2O$ / *n*-Butanol / US

R = a) H
b) Me
c) Cl

SCHEME II.2

doublet at δ 7.15 and 7.00 due to olefin proton H_B. The other olefin proton, H_A adjacent to aryl group displayed signal at downfield region and merged with aromatic protons. The coupling constant values J = 15.6 and 15.2 Hz indicated that they possess *trans* geometry. Besides, a broad singlet observed at 5.75 ppm in **26a** was accounted to NH_2 which disappeared on deuteration (Table **II.3**). The ^{13}C NMR spectra of **25a** showed signals at δ 125.5 (C-H_B), 139.5 (C-H_A), 157.2 (C-2), 160.7 (C-5) and **26a** (Fig. **II.5**) at 122.2 (C-H_B), 139.3 (C-H_A), 155.3 (C-3), 158.2 ppm (C-5) (Table **II.3**). The HRMS spectra of **25a** and **26a** (Fig. **II.6**) displayed M+Na peaks at *m/z* 473.5685 and 469.4969 which are in accordance with the molecular formulae, $C_{26}H_{18}N_4S_2$+Na and $C_{26}H_{22}N_8$+Na.

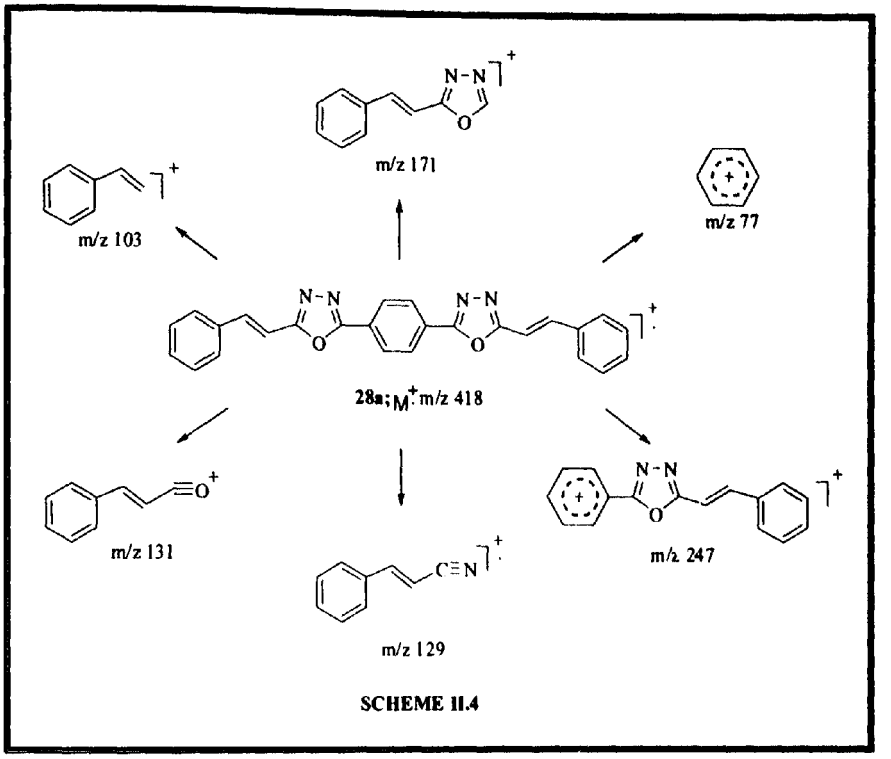
Adopting similar methodology, the reaction of 2 moles of compound **22** with 1 mole of terephthalic acid (**27**) in the presence of phosphorus oxychloride furnished 1,4-(bis(*E*-2-styryl-1,3,4-oxadiazol-5-yl))benzene (**28**) (Scheme **II.3** & Table **II.1**).



SCHEME II.3

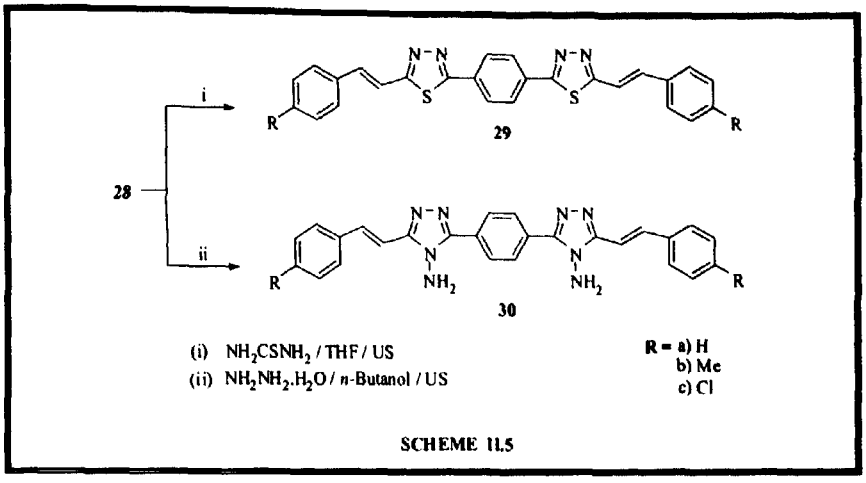
The absorption bands observed in the regions 1619-1630 and 1559-1573 cm^{-1} in the IR spectra of **28** were accounted for C=C and C=N (Table **II.2**). The ^1H NMR spectrum of **28a** (Fig. **II.7**) exhibited a doublet at δ 6.86 ppm due to olefin proton, H_B. Another doublet corresponding to H_A observed at much downfield region and merged with aromatic protons. The coupling constant J_{AB} = 14.4 Hz indicated that they possess *trans* geometry (Table **II.3**). The ^{13}C NMR spectrum of **28a** (Fig. **II.8**) displayed signals at δ 116.1 (C-H_B), 135.6 (C-H_A), 154.2 (C-2), 157.8 ppm (C-5) in addition to the signals of aromatic carbons (Table **II.3**).

The 70 eV mass spectrum of **28a** (Scheme **II.4** & Fig. **II.9**) showed a low intense $M^{+\cdot}$ peak at m/z 418 corresponding to the chemical composition, $C_{26}H_{18}N_4O_2$. Disintegration α to heterocyclic ring of the $M^{+\cdot}$ produced 2-styryl-1,3,4-oxadiazole (m/z 171), styryl (m/z 103) and 2-phenyl-5-styryl-1,3,4-oxadiazole cations (m/z 247). On the other hand, cleavage of heterocyclic ring of the M^+ resulted in the appearance of cinnamonitrile radical cation (m/z 129) and styryl carbonyl cation (m/z 131). The phenyl cation (m/z 77) is the base peak of the spectrum. Thus different daughter ions appeared during the cleavage processes conclusively supports the structure of the compound.



SCHEME II.4

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.5** & Table **II.1**).



SCHEME II.5

The IR spectra of **29** and **30** presented absorption bands in the regions 1618-1627 and 1572-1587 cm^{-1} due to C=C and C=N. Further, **30** displayed broad absorption bands in the regions 3475-3492 & 3365-3376 cm^{-1} for NH_2 (Table **II.2**). The ^1H NMR spectra of **29a** (Fig. **II.10**) and **30a** exhibited a doublet at δ 6.95, 6.80 due to olefin proton H_B. The other olefin proton, H_A adjacent to aryl group appeared as a doublet at downfield region and merged with aromatic protons. The coupling constant values J_{AB} = 14.8 and 14.2 Hz indicated that they possess *trans* geometry. Moreover, a broad singlet observed at 5.69 ppm in compound **30a** was assigned to NH_2 which disappeared on deuteration (Table **II.3**). The ^{13}C NMR spectra of **29a** (Fig. **II.11**) and **30a** presented signals at δ 118.3, 115.2 ($C\text{-}H_B$), 137.3, 133.5 ($C\text{-}H_A$) 155.6, 154.3 (C-2 / C-3) and 157.6, 156.1 (C-5) ppm (Table **II.3**). The HRMS spectra of **29a** and **30a** (Fig. **II.12**) displayed M+Na peaks at m/z 473.5685 and 469.4955 which are in accordance with the molecular formulae, $C_{26}H_{18}N_4S_2$+Na and $C_{26}H_{22}N_8$+Na.

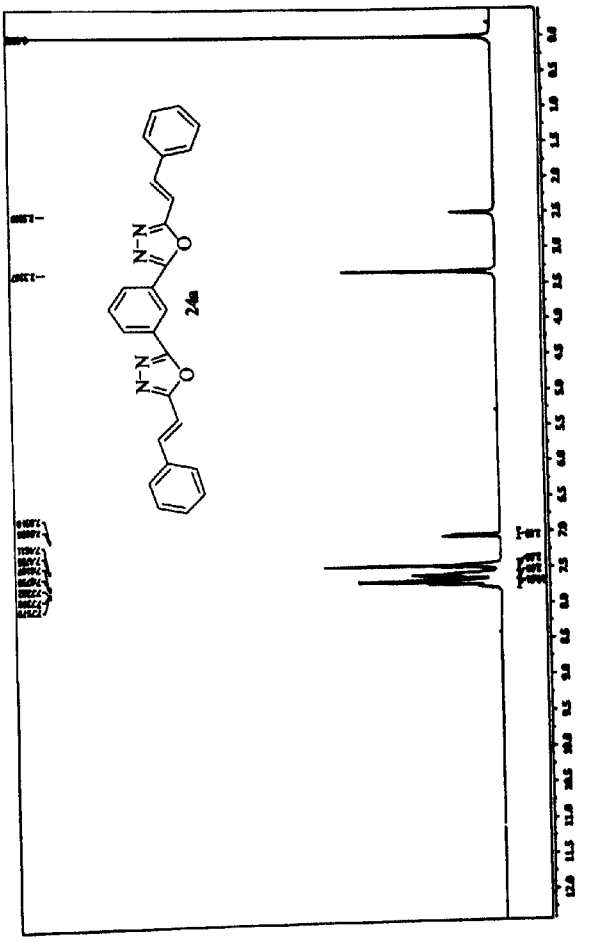
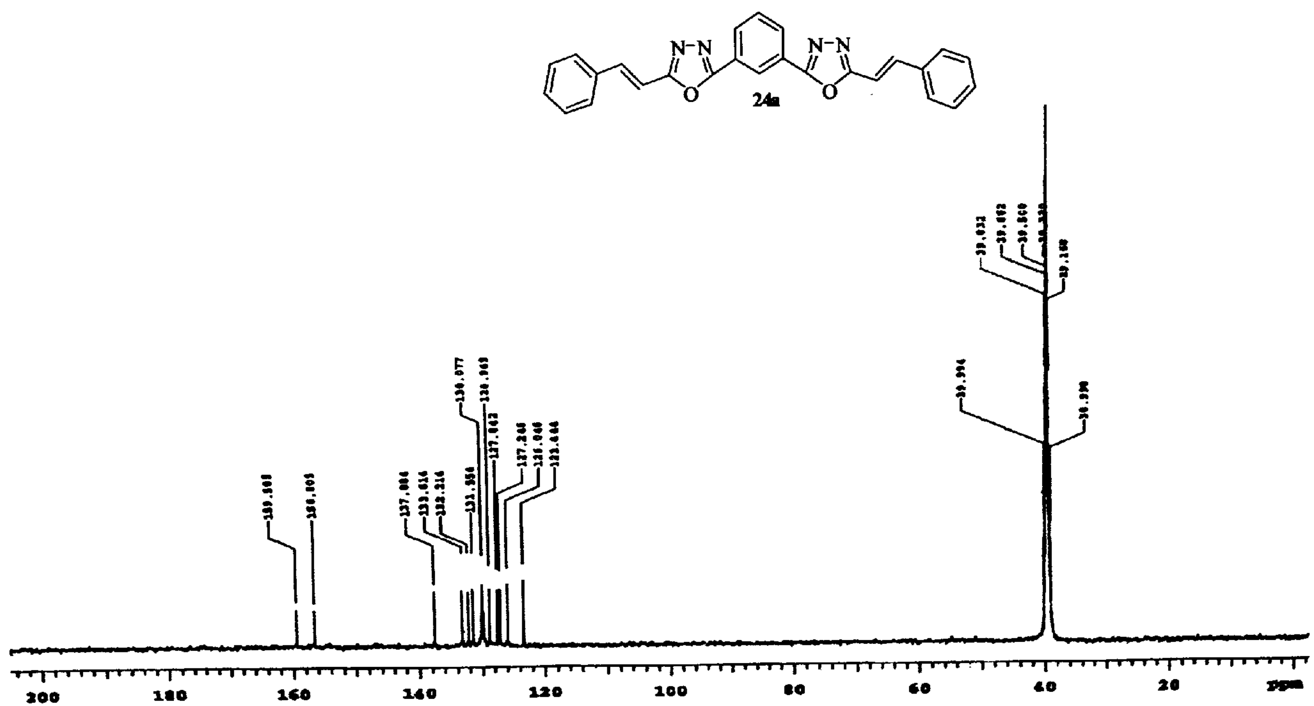


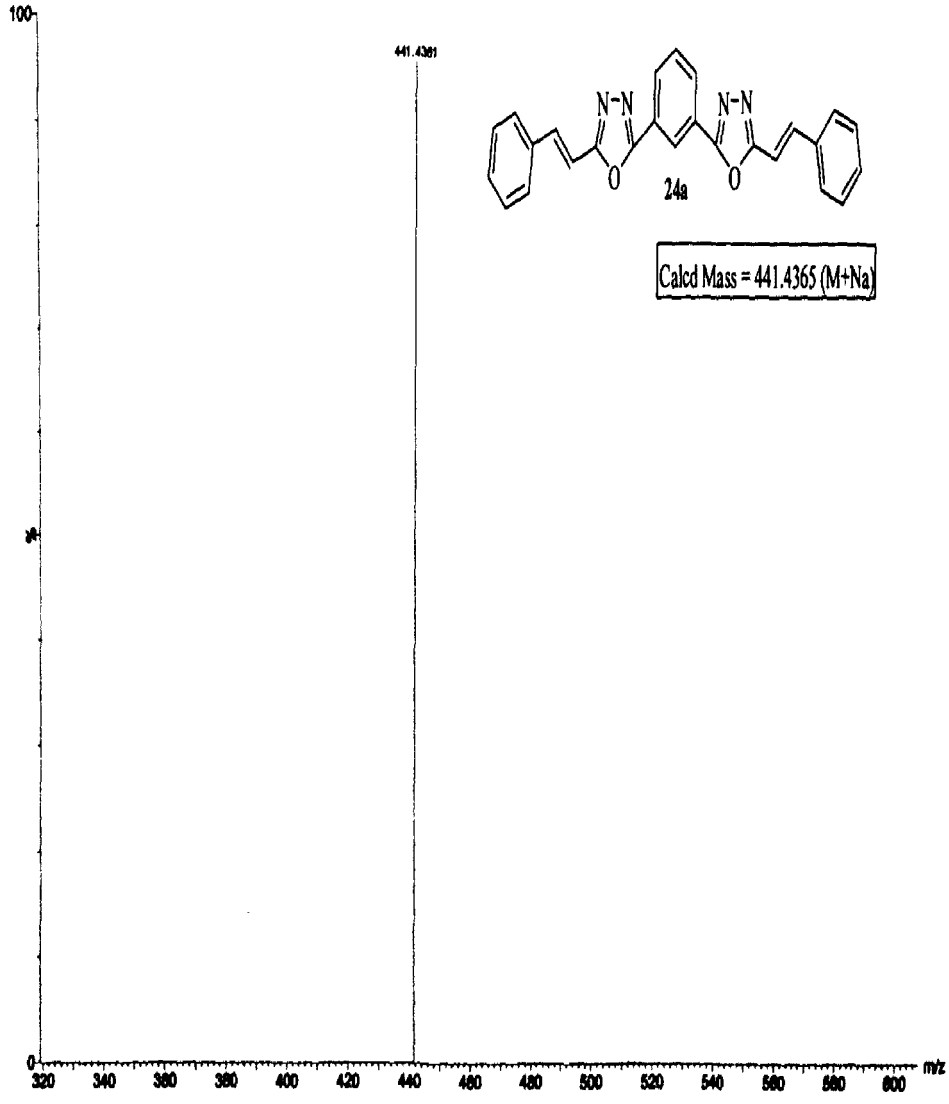
Fig. II.1

Fig. II.2



24a

Fig. II.3


100
%
0
441.4361
N-N
N-N
O
O
24a
Calcd Mass = 441.4365 (M+Na)
320
340
360
380
400
420
440
460
480
500
520
540
560
580
600
m/z

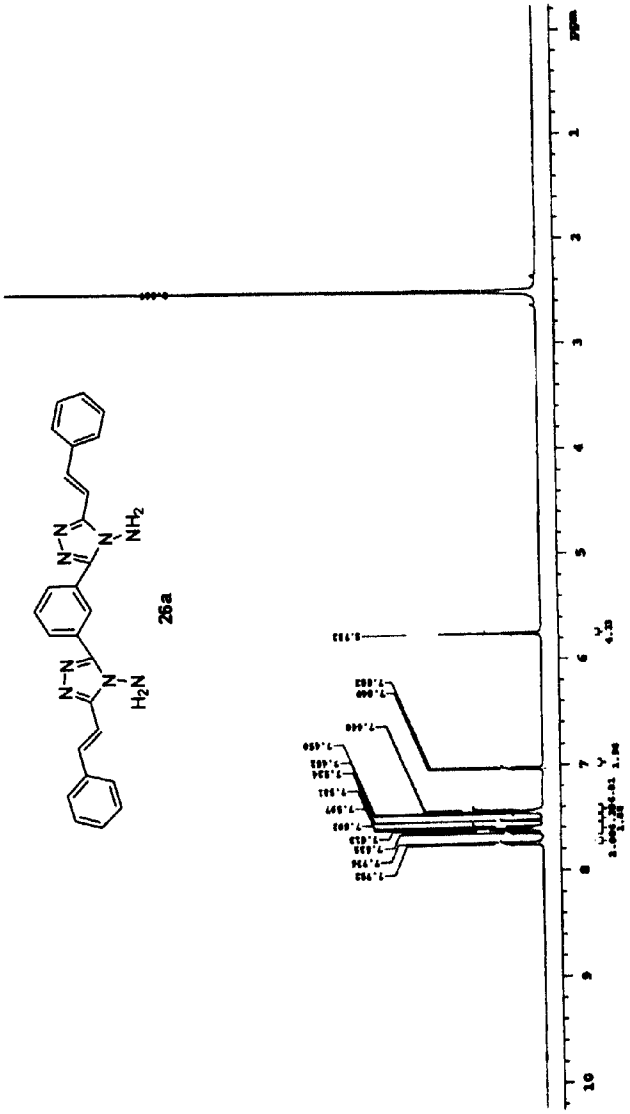
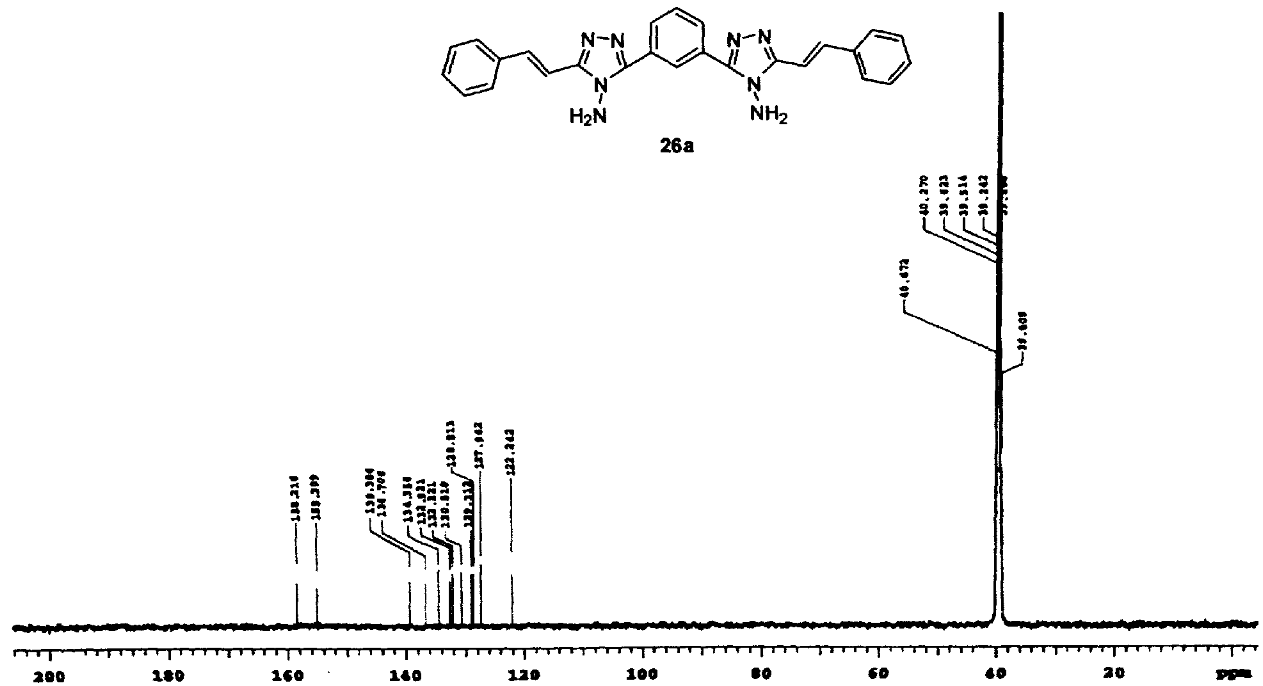


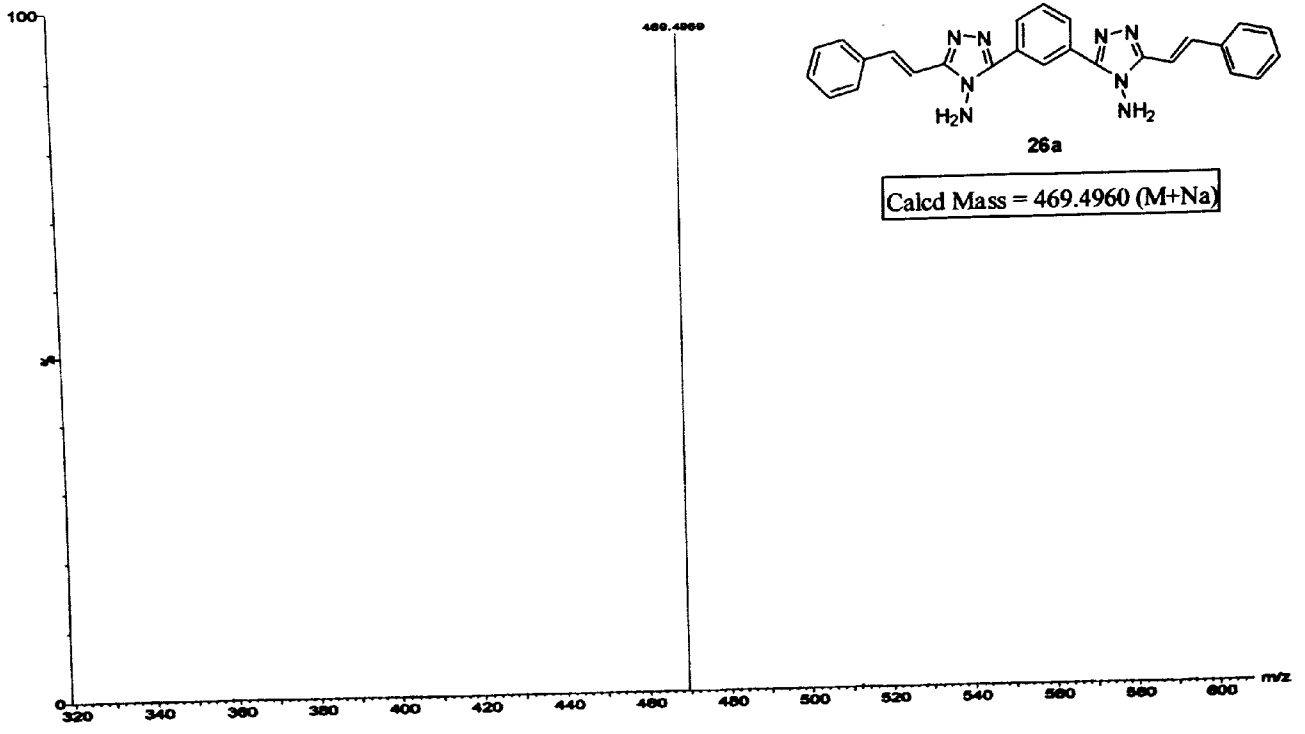
Fig. II.4

Fig. II.5



26a

Fig. II.6



26a

Calcd Mass = 469.4960 (M+Na)

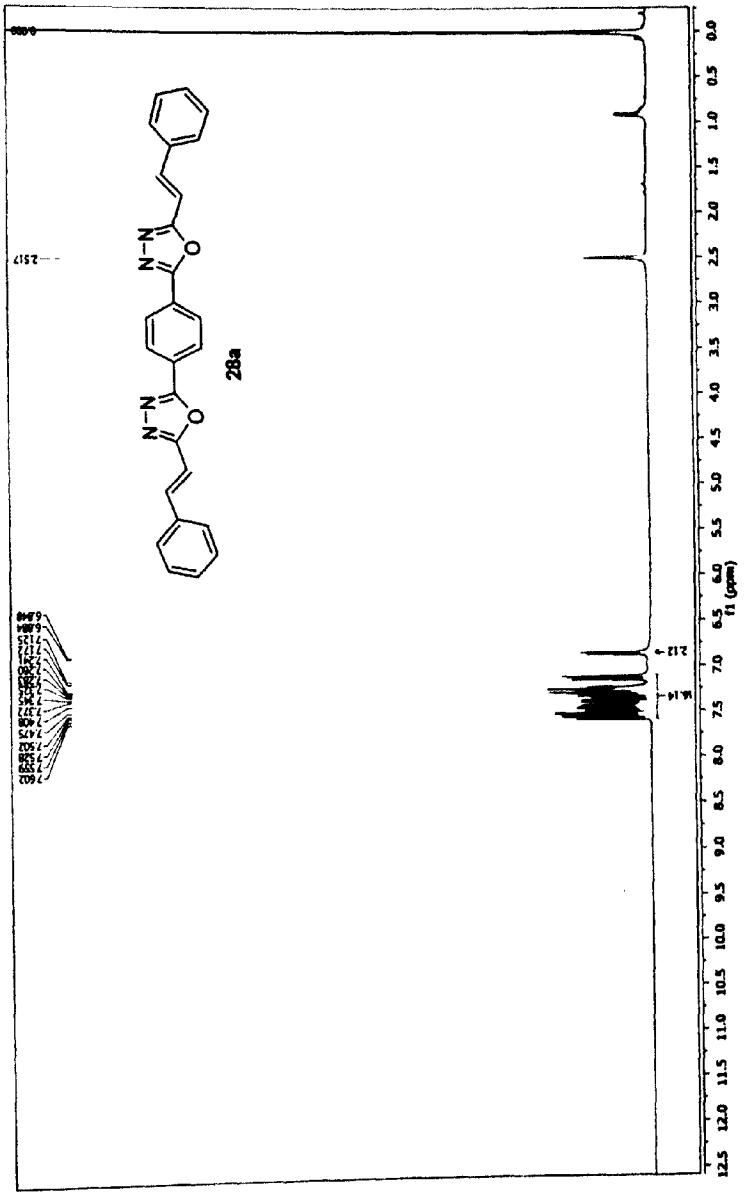
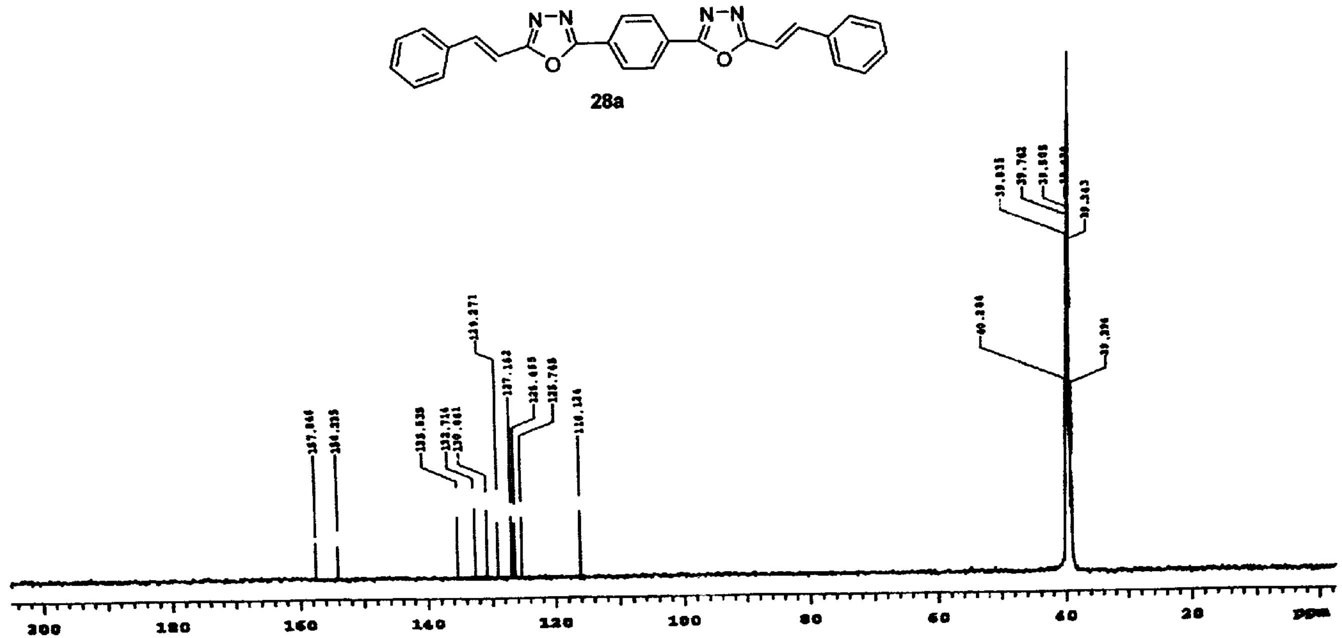


Fig. II.7

Fig. II.8



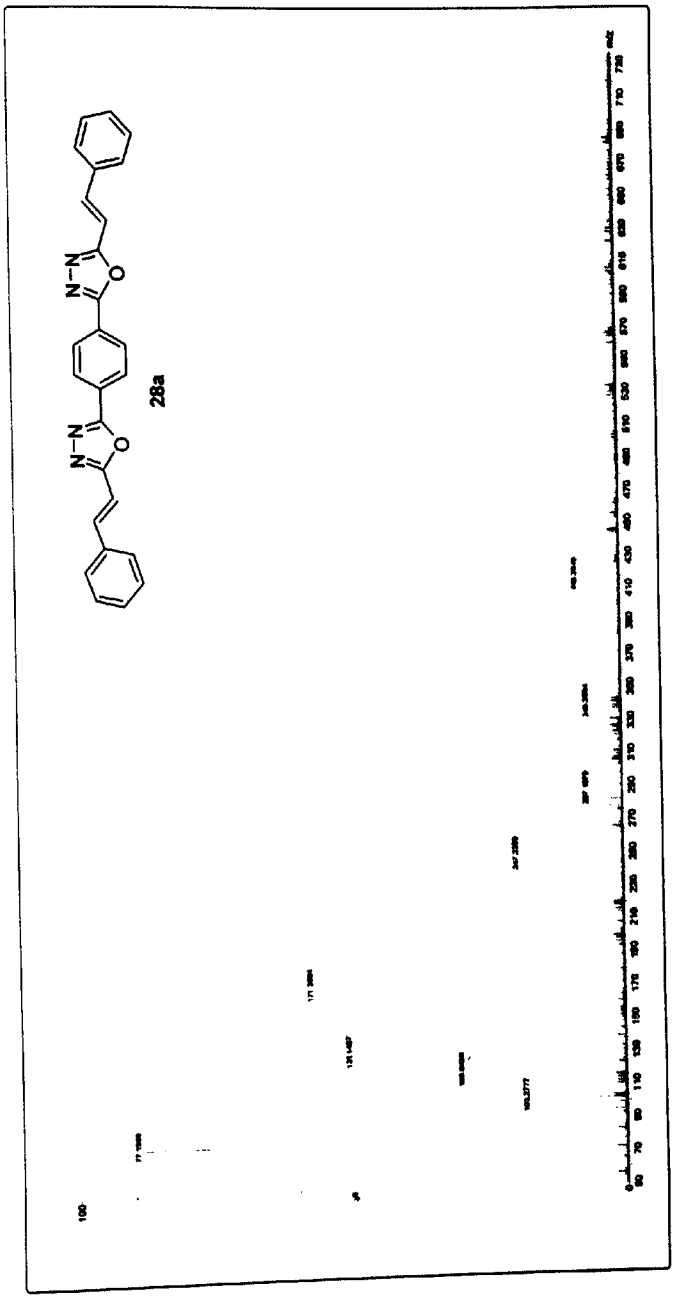
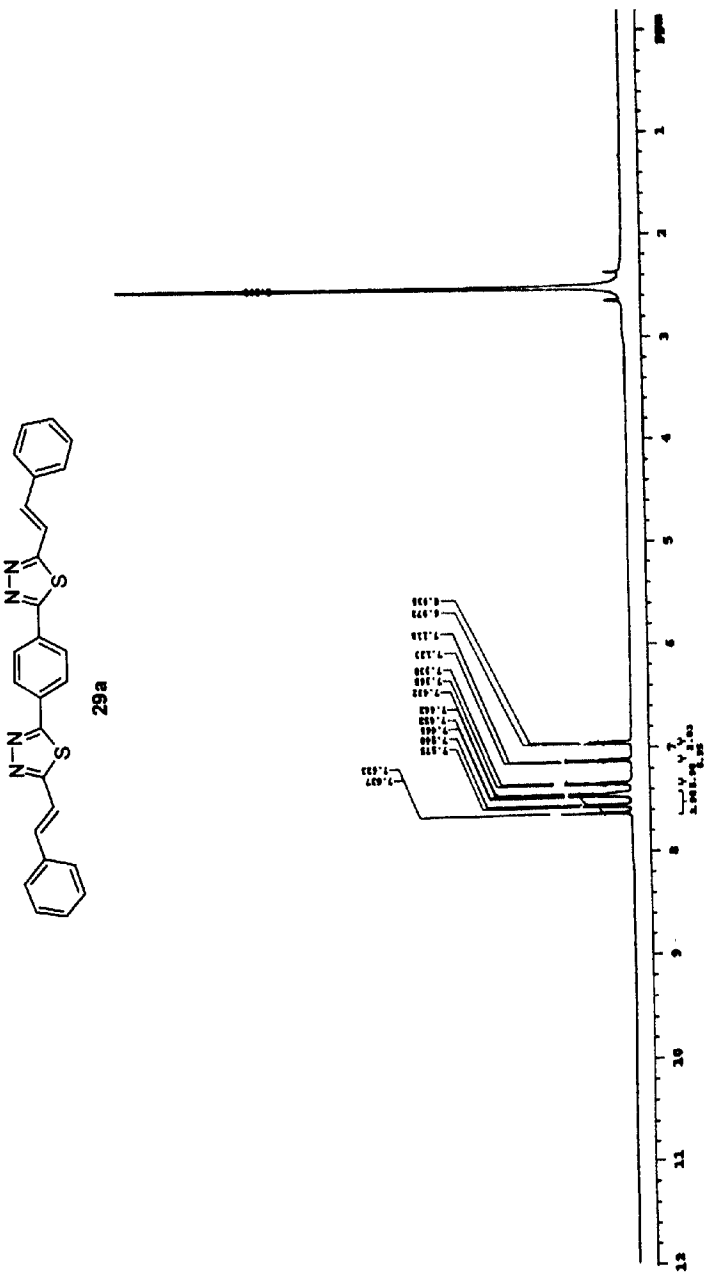


Fig. II.9



29a

Fig. II.10

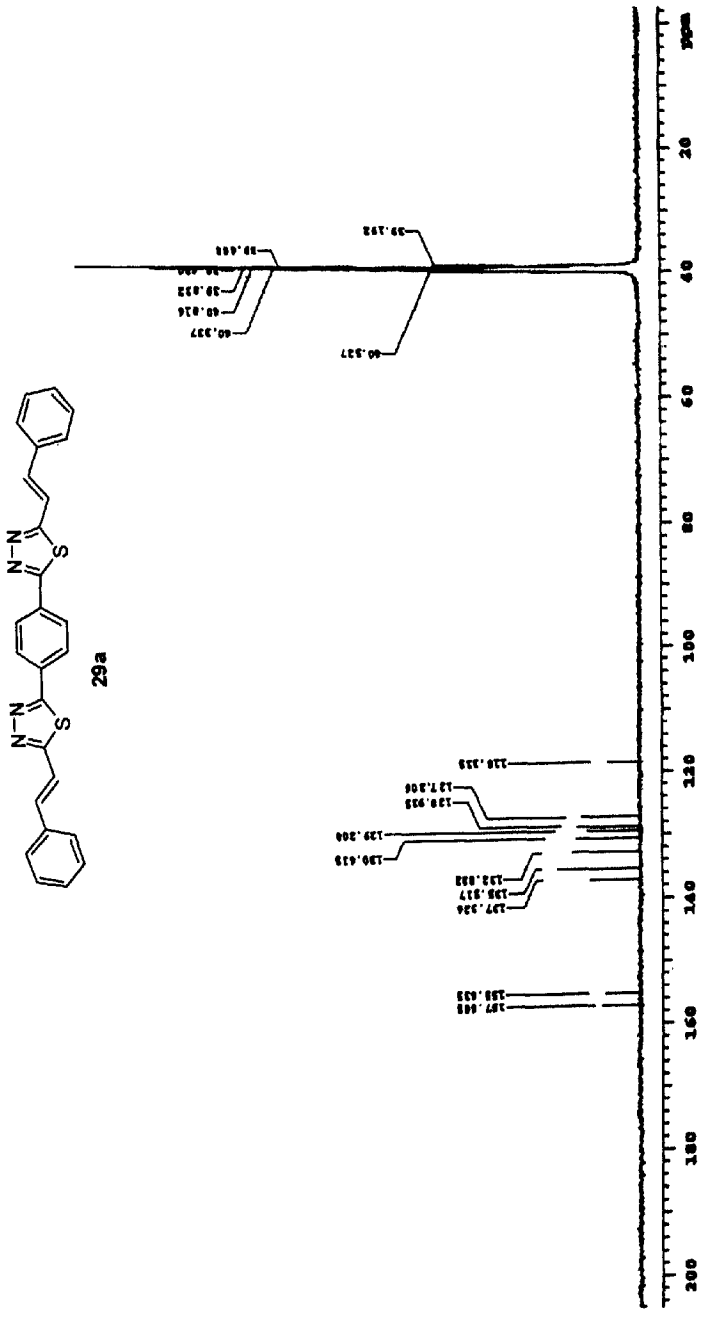
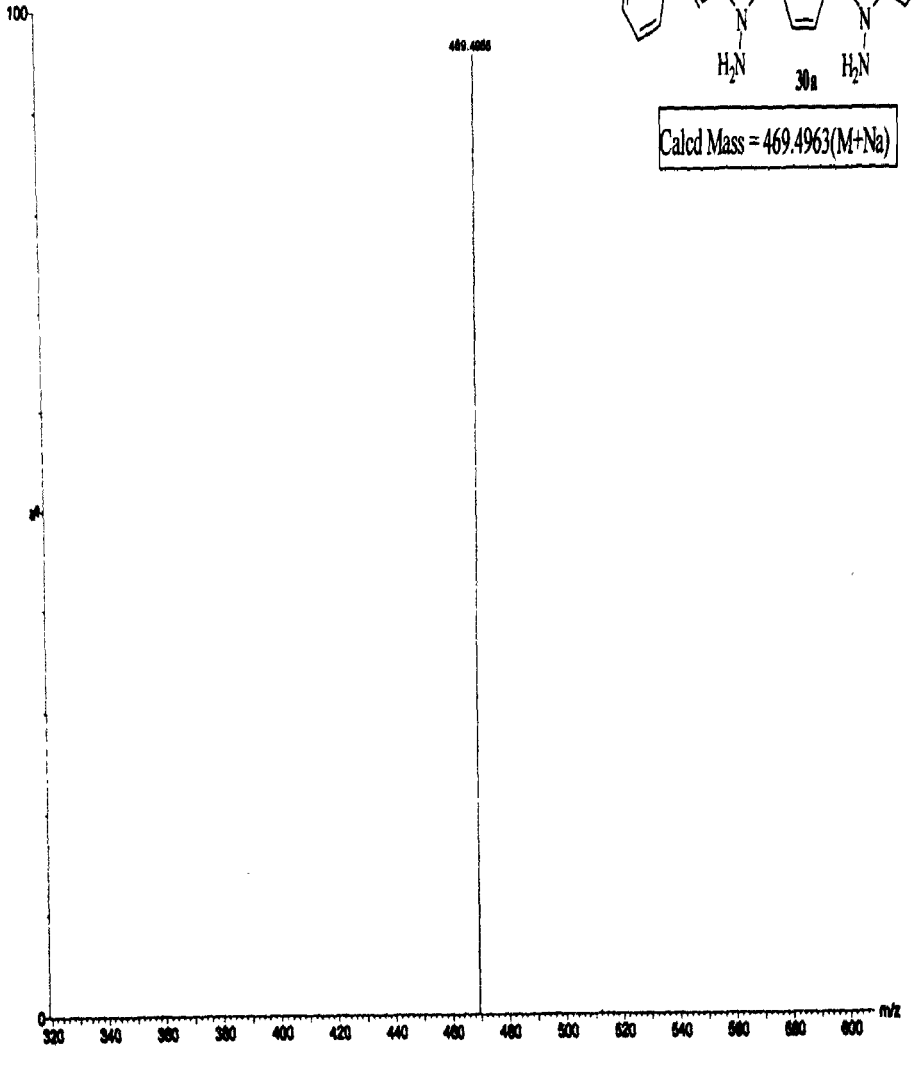
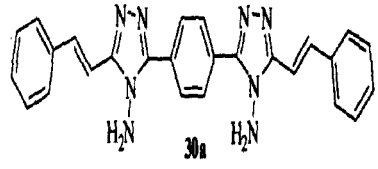

29a
39.393
39.688
39.488
39.033
60.816
60.337
60.537
130.339
137.308
139.933
139.308
139.618
133.088
139.317
137.526
150.633
137.668
20
40
60
80
100
120
140
160
180
200
ppm

Fig. II.11

Fig. II.12





30a

Calcd Mass = 469.4963(M+Na)

TABEL II.1

Compd. No.	m.p. (°C)	Yield (%)	Mol. formula (Mol. wt.)	Found (Calcd.) %		
				C	H	N
24a	150-152	84	$C_{26}H_{18}N_4O_2$	74.76	4.38	13.55
			(418.45)	(74.63)	(4.34)	(13.39)
24b	165-167	82	$C_{28}H_{22}N_4O_2$	75.43	5.00	12.70
			(446.51)	(75.32)	(4.97)	(12.55)
24c	182-184	86	$C_{26}H_{16}Cl_2N_4O_2$	64.17	3.33	11.69
			(487.33)	(64.08)	(3.31)	(11.50)
25a	166-168	85	$C_{26}H_{18}N_4S_2$	69.24	4.04	12.56
			(450.57)	(69.31)	(4.03)	(12.43)
25b	173-175	87	$C_{28}H_{22}N_4S_2$	70.38	4.67	11.94
			(478.63)	(70.26)	(4.63)	(11.71)
25c	195-197	90	$C_{26}H_{16}Cl_2N_4S_2$	60.05	3.12	13.81
			(519.48)	(60.11)	(3.10)	(13.65)
26a	163-165	83	$C_{26}H_{22}N_8$	70.00	4.98	25.30
			(446.50)	(69.94)	(4.97)	(25.10)
26b	169-171	81	$C_{28}H_{26}N_8$	70.97	5.55	23.80
			(474.56)	(70.87)	(5.52)	(23.61)
26c	194-196	88	$C_{26}H_{20}Cl_2N_8$	60.72	3.93	13.83
			(515.39)	(60.59)	(3.91)	(13.76)
28a	155-157	87	$C_{26}H_{18}N_4O_2$	74.75	4.37	13.55
			(418.45)	(74.63)	(4.34)	(13.39)
28b	168-170	85	$C_{28}H_{22}N_4O_2$	75.42	4.99	12.69
			(446.51)	(75.32)	(4.97)	(12.55)
28c	183-185	91	$C_{26}H_{16}Cl_2N_4O_2$	64.15	3.30	11.71
			(487.33)	(64.08)	(3.31)	(11.50)

Contd...

29a	165-167	87	$C_{26}H_{18}N_4S_2$	69.40	4.05	12.56
			(450.57)	(69.31)	(4.03)	(12.43)
29b	172-174	90	$C_{28}H_{22}N_4S_2$	70.20	4.64	11.60
			(478.63)	(70.26)	(4.63)	(11.71)
29c	196-198	95	$C_{26}H_{16}Cl_2N_4S_2$	60.22	3.13	13.84
			(519.48)	(60.11)	(3.10)	(13.65)
30a	162-164	86	$C_{26}H_{22}N_8$	69.75	5.01	25.31
			(446.50)	(69.94)	(4.97)	(25.10)
30b	173-175	89	$C_{28}H_{26}N_8$	70.95	5.51	23.76
			(474.56)	(70.87)	(5.52)	(23.61)
30c	193-195	92	$C_{26}H_{20}Cl_2N_8$	60.69	3.94	13.94
			(515.39)	(60.59)	(3.91)	(13.76)

TABLE II.2

Compd. No.	IR (KBr) cm^{-1}			
	NH_2		C=C	C=N
24a	-	-	1615	1571
24b	-	-	1610	1564
24c	-	-	1620	1579
25a	-	-	1625	1580
25b	-	-	1622	1563
25c	--		1630	1585
26a	3480	3362	1624	1560
26b	3475	3360	1621	1557
26c	3486	3375	1629	1566
28a	-	-	1623	1562
28b	-	-	1619	1559
28c	-	-	1630	1573
29a	-	-	1626	1572
29b	-	-	1620	1584
29c	-	-	1627	1587
30a	3488	3370	1618	1584
30b	3475	3365	1622	1581
30c	3492	3376	1625	1583

TABLE II.3

Compd. No.	^{1}H NMR ($CDCl_3$ / DMSO-d_6) (δ, ppm)	^{13}C NMR ($CDCl_3$ / DMSO-d_6) (δ, ppm)
24a	7.05 (d, 2H, H_B, J =15.4 Hz), 7.46-7.75 (m, 16H, H_A & Ar-H)	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 (aromatic carbons)
24b	2.29 (s, 6H, Ar-CH_3) 7.09 (d, 2H, H_B, J = 15.2 Hz), 7.41-7.69 (m, 14H, H_A & Ar-H)	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 (aromatic carbons)
24c	7.12 (d, 2H, H_B, J = 15.8 Hz), 7.71-7.93 (m, 14H, H_A & Ar-H)	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 (aromatic carbons)
25a	7.15 (d, 2H, H_B, J =15.6 Hz), 7.50-7.85 (m, 16H, H_A & Ar-H)	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 (aromatic carbons)
25b	2.32 (s, 6H, Ar-CH_3) 7.11 (d, 2H, H_B, J = 15.5 Hz), 7.48-7.72 (m, 14H, H_A & Ar-H)	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 (aromatic carbons)
25c	7.20 (d, 2H, H_B, J = 16.0 Hz), 7.75-7.97 (m, 14H, H_A & Ar-H)	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 (aromatic carbons)

Contd....

26a	5.75 (bs, 4H, NH_2), 7.02 (d, 2H, H_B, J = 15.2 Hz), 7.44-7.75 (m, 16H, H_A & Ar-H)	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 (aromatic carbons)
26b	2.26 (s, 6H, Ar-CH_3), 5.68 (bs, 4H, NH_2), 7.05 (d, 2H, H_B, J =15.0 Hz), 7.40-7.73 (m, 14H, H_A & Ar-H)	23.9 (Ar-CH3), 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 (aromatic carbons)
26c	5.81 (bs, 4H, NH_2) 6.98 (d, 2H, H_B, J = 15.5 Hz), 7.68-7.82 (m,14H, H_A & Ar-H)	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 (aromatic carbons)
28a	6.86 (d, 2H, H_B, J =14.4 Hz), 7.12-7.60 (m, 16H, H_A & Ar-H)	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 (aromatic carbons)
28b	2.25 (s, 6H, Ar-CH_3), 6.90 (d, 2H, H_B, J = 14.2 Hz), 7.09-7.54 (m, 14H, H_A & Ar-H)	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 (aromatic carbons)
28c	6.97 (d, 2H, H_B, J = 14.5 Hz), 7.16-7.68 (m, 14H, H_A & Ar-H)	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(aromatic carbons)
29a	6.95 (d, 2H, H_B, J = 14.8 Hz), 7.11-7.63 (m, 16H, H_A & Ar-H)	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 (aromatic carbons)
29b	2.28 (s, 6H, Ar-CH_3) 6.90 (d, 2H, H_B, J = 14.6 Hz), 7.08-7.59 (m, 14H, H_A & Ar-H)	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, 132.5, 133.1 (aromatic carbons)

Contd....

29c	6.98 (d, 2H, H_B, J = 14.8 Hz), 7.24-7.72 (m, 14H, H_A & Ar-H)	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 (aromatic carbons)
30a	5.69 (bs, 4H, NH_2), 6.80 (d, 2H, H_B, J =14.2 Hz), 7.12-7.54 (m, 16H, H_A & Ar-H)	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 (aromatic carbons)
30b	2.21 (s, 6H, Ar-CH_3), 5.75 (bs,4H, NH_2), 6.76 (d, 2H, H_B, J = 14.0 Hz), 7.02-7.44 (m, 14H, H_A & Ar-H)	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 (aromatic carbons)
30c	5.84 (bs, 4H, NH_2), 6.89 (d, 2H, H_B, J = 14.4 Hz), 7.18-7.67 (m, 14H, H_A & Ar-H)	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 (aromatic carbons)

_E_-Cinnamohydrazide (22)

The preparation of this compound involves the following steps.

(i) Esterification of cinnamic acid

(ii) Reaction of methyl cinnamate with hydrazine hydrate

Methyl cinnamate (21)

In a 100 ml round-bottomed flask fitted with a reflux condenser, cinnamic acid (**14**) (0.10 mol), methanol (20 ml) and concentrated sulfuric acid (2 ml) were taken and refluxed on a water bath for 4-5 hrs. The reaction mixture was cooled and poured onto crushed ice. The separated solid was filtered on a Buchner funnel, washed with cold water and dried. The crude ester was recrystallized from methanol to get pure methyl cinnamate (86%), m.p. 34-36°C (lit.[310, 311] 34°C); methyl 4-methylcinnamate (75%), m.p. 55-57°C (lit.[312] 57-58°C); methyl 4-chlorocinnamate (86%), m.p. 76-78°C (lit.[313] 76°C).

_E_-Cinnamohydrazide (22)

A mixture of methyl cinnamate (**21**) (0.01 mol), hydrazine hydrate (0.011 mol), methanol (6 ml) and pyridine (0.3 ml) was taken in a 50 ml round-bottomed flask fitted with a reflux condenser and refluxed on a water bath for 3-5 hrs. The contents of the flask were cooled and poured onto crushed ice. The resultant solid was collected by filtration, dried and recrystallized from methanol to get pure cinnamohydrazide. The compounds prepared adopting these methods are listed in Table **II.4**

TABLE II.4

Compd. No.	R	m.p. (°C)	Yield (%)
22a	H	154-156	79
22c	Cl	174-176	84
22b	Me	146-148	76

1,3-(Bis(*E*-2-styryl-1,3,4-oxadiazol-5-yl))benzene (24)

The *E*-cinnamohydrazide (**22**) (0.02 mol), isophthalic acid (**23**) (0.01 mol) and phosphorus oxychloride (4 ml) were taken in a 100 ml round-bottomed flask fitted with a reflux condenser carrying a calcium chloride guard-tube was subjected to ultrasound irradiation in an ultrasonic bath at a frequency of 35 kHz for 60-70 min at room temperature. After completion of the reaction (monitored by TLC), the excess phosphorus oxychloride was removed under reduced pressure 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. The compounds synthesized adopting similar methodology are listed in Table **II.5**.

TABLE II.5

Compd. No.	R	m.p. (°C)	Yield (%)
24a	H	150-152	84
24b	Me	165-167	82
24c	Cl	182-184	86

1,3-(Bis(*E*-2-styryl-1,3,4-thiadiazol-5-yl))benzene (25)

A mixture of 1,3-(bis(*E*-2-styryl-1,3,4-oxadiazol-5-yl))benzene (**24**) (0.001 mol), thiourea (0.004 mol) and tetrahydrofuran (8 ml) was taken in a sealed tube and heated at reflux conditions under ultrasonication in an ultrasonic bath at a frequency of 35 kHz for 95-120 min at room temperature. The progress of the reaction was monitored by TLC. After completion of the reaction, the contents of the flask were extracted with dichloromethane. The organic 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. The compounds thus prepared are presented in Table **II.6**.

TABLE II.6

Compd. No.	R	m.p. (°C)	Yield (%)
25a	H	166-168	85
25b	Me	173-175	87
25c	Cl	195-197	90

1,3-(Bis(*E*-3-styryl-4-amino-1,2,4-triazol-5-yl))benzene (26)

A solution of 1,3-(bis(*E*-2-styryl-1,3,4-oxadiazol-5-yl))benzene (**24**) (0.001 mol) and hydrazine hydrate (0.004 mol) in *n*-butanol (5 ml) was taken in a 100 ml round-bottomed flask fitted with reflux condenser and kept under ultrasonication at a frequency of 35 kHz for 5-10 min. Then, KOH (0.002 mol) was added to the contents of the flask and continued sonication for a period of 60-65 min. The precipitate formed was filtered and acidified with conc. HCl to pH ≈ 3. It was washed with water, dried and purified by column chromatography (silica gel, 160-120 mesh) using ethyl acetate-hexane (1:3) as eluent. The physical data of the compounds thus obtained is given in Table **II.7**.

TABLE II.7

Compd. No.	R	m.p. (°C)	Yield (%)
26a	H	163-165	83
26b	Me	169-171	81
26c	Cl	194-196	88

1,4-(Bis(*E*-2-styryl-1,3,4-oxadiazol-5-yl))benzene (28)

A mixture of *E*-cinnamohydrazide (**22**) (0.02 mol), terephthalic acid (**27**) (0.01 mol) and phosphorus oxychloride (4 ml) was taken in a 100 ml round-bottomed flask fitted with a reflux condenser carrying a calcium chloride guard-tube. The reaction mixture was subjected to ultrasound irradiation in an ultrasonic bath at a frequency of 35 kHz for 50-65 min at room temperature. After completion of the reaction (monitored by TLC), the excess phosphorus oxychloride was removed under reduced pressure and the resultant residue was poured onto crushed ice. The separated solid was filtered on a Buchner funnel and washed with saturated sodium bicarbonate solution followed by water. It was dried and recrystallized from 2-propanol. The compounds obtained accordingly are presented in Table **II.8**.

TABLE II.8

Compd. No.	R	m.p. (°C)	Yield (%)
28a	H	155-157	87
28b	Me	168-170	85
28c	Cl	183-185	91

1,4-(Bis(*E*-2-styryl-1,3,4-thiadiazol-5-yl))benzene (29)

The 1,4-(bis(*E*-2-styryl-1,3,4-oxadiazol-5-yl))benzene (**28**) (0.001 mol), thiourea (0.004 mol) and tetrahydrofuran (8 ml) were taken in a sealed tube and heated at reflux conditions under ultrasonication for 90-100 min. The progress of the reaction was monitored by TLC. After completion of the reaction, the contents of the flask were extracted with dichloromethane, 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. The compounds prepared in a similar way are shown in Table **II.9**.

TABLE II.9

Compd. No.	R	m.p. (°C)	Yield (%)
29a	H	165-167	93
29b	Me	172-174	90
29c	Cl	196-198	95

1,4-(Bis(*E*-3-styryl-4-amino-1,2,4-triazol-5-yl))benzene (30)

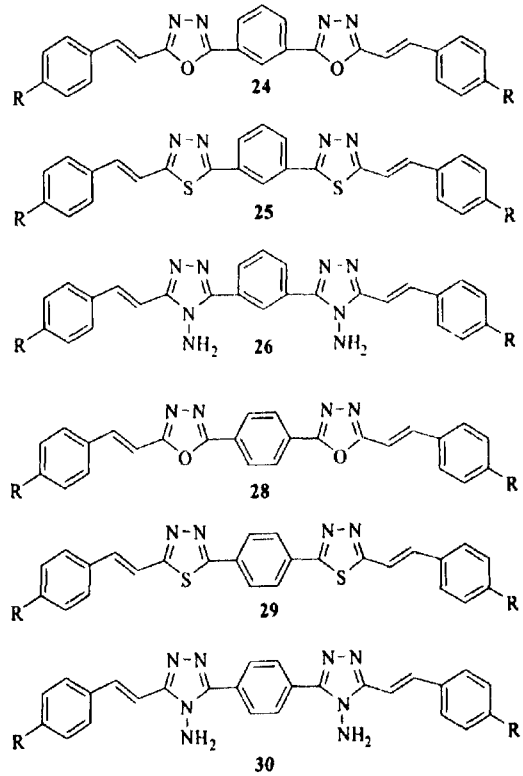
In a 100 ml round-bottomed flask, 1,4-(bis(*E*-2-styryl-1,3,4-oxadiazol-5-yl))-benzene (**28**) (0.001 mol), hydrazine hydrate (0.004 mol) and *n*-butanol (5 ml) were taken and sonicated at frequency of 35 kHz for 5-10 min and continued sonication for period of 60-66 min at room temperature. Then, KOH (0.002 mol) 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. The compounds synthesized by following the same procedure are listed in Table **II.10**.

TABLE II.10

Compd. No.	R	m.p. (°C)	Yield (%)
30a	H	162-164	86
30b	Me	173-175	89
30c	Cl	193-195	92

ANTIMICROBIAL ACTIVITY

The compounds 1,3-(bis-(*E*-2-styryl-1,3,4-oxadiazol-5-yl))benzene (**24**), 1,3-(bis-(*E*-2-styryl-1,3,4-thiadiazol-5-yl))benzene (**25**), 1,3-bis-(*E*-3-styryl-4-amino-1,2,4-triazol-5-yl))benzene (**26**), 1,4-(bis(*E*-2-styryl-1,3,4-oxadiaol-5-))benzene (**28**), 1,4-(bis(*E*-2-styryl-1,3,4-thiadiazol-5-yl))benzene (**29**) and 1,4-(bis(*E*-3-styryl-4-amino-1,2,4-triazol-5-yl))benzene (**30**) (**Chart**) were assayed for antimicrobial activity.



CHART

Methodology

The methodology followed and microorganisms used to study the antimicrobial activity are described in chapter **I**.

Results and Discussion

Antibaterial activity

The results of antibacterial activity presented in Table **II.11** and Fig. **II.13** revealed that *Staphylococus aureus, Bacillus subtilis,* (Gram-positive bacteria) were more susceptible towards the tested compounds than *Pseudomonas aeruginosa, Klebsiella pneumoniae* (Gram-negative bacteria). The compounds **25**, **26**, **29** and **30** exhibited moderate to good antibacterial activity against Gram-positive bacteria than Gram-negative bacteria. However, compounds **24** and **28** displayed low activity against Gram-positive bacteria and no activity against Gram-negative bacteria. Further it was noticed that 1,4-phenylene bis(azoles) **28**, **29** and **30** showed higher antibacterial activity when compared with the respective 1,3-phenylene bis(azoles) **24**, **25** and **26**. Amongst all the tested compounds **29c**, **30a** and **30c** 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 (*Aspergillus niger & Pencillium chrysogenum).* It was observed that all the compounds exhibited pronounced antifungal activity towards *P. chrysogenum* than on *A.niger.* The compounds **25**, **26**, **29** and **30** displayed higher activity whereas **24** and **28** showed least activity. It was observed that compounds **29** and **30** exhibited greater activity than **25** and **26**. The compounds **29c**, **30a** and **30c** displayed excellent antifungal activity when compared with the standard drug Ketoconazole at all tested concentrations (Table **II.12** and Fig **II.14**).

The minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) values of the tested compounds are shown in Table **II.13**. MIC is the lowest concentration of an antimicrobial that will inhibit

the visible growth of a microorganism. (But it is not sure 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.[309] The compounds **29c**, **30a** and **30c** exhibited low MIC values. The MBC and MFC values in **29c**, **30a** and **30c** are 2×MIC in 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) **28**, **29** and **30** displayed comparatively higher antimicrobial activity than the corresponding 1,3-phenylene bis(azoles) **24**, **25** and **26**. This may be due to the presence of effective conjugation in former compounds. It was observed that compounds having thiadiazole and triazole moieties **25**, **26**, **29** and **30** exhibited greater antimicrobial activity than compounds with oxadiazole unit **24** and **28**. Amongst the compounds **25**, **26**, **29** and **30** those with triazole ring **26**, **30** displayed slightly higher activity than thiadiazoles **25** and **29**. Moreover, the compounds having chloro substituent on aromatic ring enhanced the activity when compared with methyl and unsubstituted compounds. The compounds **29c**, **30a** and **30c** were identified as potent antimicrobial agents against *S. aureus* and *P.chrysogenum*.

TABLE II. 11

The *in vitro* antibacterial activity of compounds **24-26 & 28-30.**

Compd. No.	Zone of inhibition (mm)															
	Gram-positive bacteria								Gram-negative bacteria							
	S. aureus				*B. subtilis*				*P. aeruginosa*				*K. pneumoniae*			
	12.5 μg/ well	25 μg/ well	50 μg/ well	100 μg/ well	12.5 μg/ well	25 μg/ well	50 μg/ well	100 μg/ well	12.5 μg/ well	25 μg/ well	50 μg/ well	100 μg/ well	12.5 μg/ well	25 μg/ well	50 μg/ well	100 μg/ well
24a	-	-	-	13±2	-	-	-	10±1	-	-	-	-	-	-	-	-
24b	-	-	-	9±2	-	-	-	8±2	-	-	-	-	-	-	-	-
24c	08±2	10±3	12±2	15±3	7±1	9±2	11±2	13±1	-	-	-	-	-	-	-	-
25a	16±1	18±2	20±1	23±3	13±1	16±2	18±3	20±2	9±2	10±2	13±1	15±2	14±2	17±2	21±1	23±3
25b	13±1	15±1	17±2	20±1	10±1	12±2	15±2	17±1	7±1	9±2	11±2	13±1	11±3	14±1	17±2	19±2
25c	19±1	21±2	23±3	26±2	17±1	19±2	21±3	24±2	11±2	13±3	16±2	18±1	17±2	21±2	24±1	26±3
26a	21±2	23±3	25±2	27±2	19±2	21±3	23±2	25±2	11±2	13±3	15±2	17±3	20±2	23±3	25±3	27±2
26b	16±1	18±2	20±3	23±3	14±1	17±2	19±1	21±3	9±3	11±1	12±2	14±2	15±3	17±3	20±2	24±1
26c	24±3	26±2	29±3	31±2	21±2	23±2	25±2	30±3	13±2	15±3	17±2	20±4	22±2	25±2	28±3	30±3
28a	-	-	13±1	14±2	-	-	9±2	12±2	-	-	-	-	-	-	-	-
28b	-	-	-	11±3	-	-	-	9±2	-	-	-	-	-	-	-	-
28c	11±3	13±2	16±3	18±2	10±2	12±1	14±2	16±1	-	-	-	-	-	-	-	-
29a	27±2	29±3	31±1	34±2	23±2	25±3	27±2	29±4	12±2	14±1	17±2	19±2	25±1	24±2	27±3	30±2
29b	22±1	24±2	27±2	30±1	20±2	22±2	24±3	26±3	10±2	12±3	13±2	16±3	18±2	20±2	23±2	26±3
29c	31±3	33±2	36±2	38±4	26±2	28±1	30±3	33±3	15±1	17±2	20±3	22±1	29±2	30±3	32±2	34±2

Contd...

30a	30±3	32±1	34±3	39±3	27±2	29±3	32±1	34±2	17±3	19±2	21±1	23±2	25±3	27±1	30±2	32±2
30b	26±3	29±1	31±2	34±2	23±2	25±2	27±3	31±2	14±2	16±2	18±2	20±1	21±2	24±2	26±2	29±3
30c	32±2	34±3	37±2	41±4	30±2	32±3	35±2	37±4	20±2	22±2	24±2	26±2	28±2	30±1	33±2	37±3
Chloram-phenicol	30±1	32±2	35±2	37±2	32±3	34±2	36±2	40±2	25±1	27±2	29±3	32±1	38±2	40±2	42±3	44±2
Control (DMSO)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

(-) No activity, (±) Standard deviation.

TABLE II. 12

The *in vitro* antifungal activity of compounds **24-26 & 28-30.**

Compd. No.	Zone of inhibition (mm)							
	A. niger				*P. chrysogenum*			
	12.5 μg/well	25 μg/well	50 μg/well	100 μg/well	12.5 μg/well	25 μg/well	50 μg/well	100 μg/well
24a	-	-	-	10±2	-	-	11±1	13±2
24b	-	-	-	7±1	-	-	-	10±1
24c	-	-	-	12±3	8±1	10±2	12±1	15±2
25a	13±2	15±2	18±1	21±2	20±3	22±2	24±3	26±1
25b	9±2	11±2	13±1	15±2	17±1	19±2	21±1	23±2
25c	15±1	18±2	21±3	25±2	23±2	25±1	28±1	30±2
26a	17±2	19±3	22±1	24±2	25±2	27±2	29±1	32±1
26b	14±2	16±1	18±2	21±3	22±2	24±1	26±2	28±2
26c	19±1	21±2	23±2	25±3	28±1	30±2	33±3	35±2
28a	-	8±2	10±1	13±2	11±2	13±1	15±2	17±2
28b	-	-	-	9±2	-	8±1	10±3	13±2
28c	-	10±2	13±1	16±2	14±3	16±2	18±2	20±3
29a	19±2	21±2	24±1	26±3	31±1	34±2	36±2	38±2
29b	16±1	18±2	20±2	23±2	27±2	29±2	31±1	33±2
29c	23±1	25±2	27±3	29±2	35±3	37±2	38±2	41±3
30a	24±2	26±2	28±2	32±3	34±2	36±2	39±1	42±2
30b	21±1	23±2	25±3	28±2	31±1	33±2	35±1	37±2
30c	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.

TABLE II. 13

MIC, MBC and MFC of compounds **29c, 30a** and **30c.**

Compd. No.	Minimum inhibitory concentration MIC (MBC / MFC) µg / well					
	S. aureus	*B. subtilis*	*P. aeruginosa*	*K.pneumoniae*	*A. niger*	*P.chrysogenum*
29c	6.25(12.5)	50(200)	100(>200)	100(>200)	25(100)	12.5(25)
30a	6.25(12.5)	25(100)	50(200)	100(>200)	25(100)	12.5(25)
30c	6.25(12.5)	25(100)	50(200)	100(200)	12.5(50)	12.5(25)
Chloram-phenicol	6.25	6.25	6.25	12.5	-	-
Ketoconazole	-	-	-	-	6.25	12.5

(-) No activity.

Fig. II.13.

The *in vitro* antibacterial activity of compounds **24-26 & 28-30.**

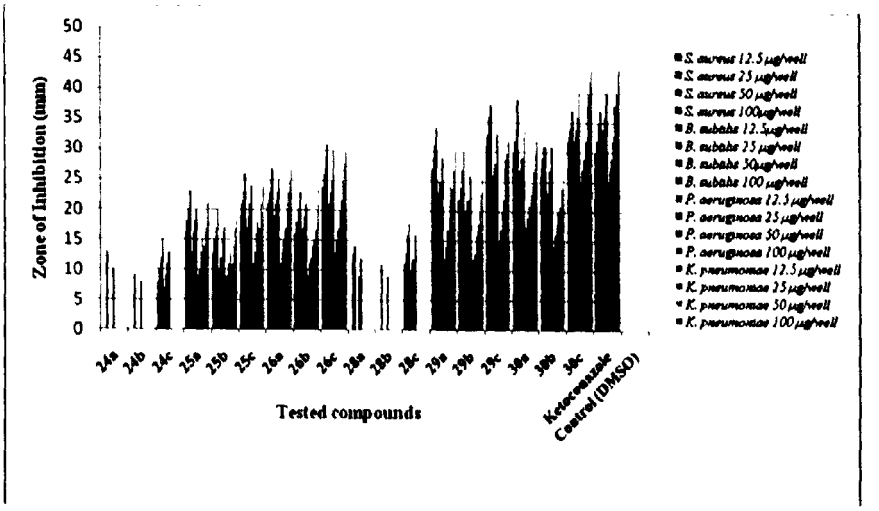


Fig. II.14.

The *in vitro* antifungal activity of compounds **24-26 & 28-30.**