Antibacterials and Antimycotics: Part 1: Synthesis and Activity of 2-Pyrazoline Derivatives

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A series of 3-styryl-1,5-diphenyl and 5-styryl-1,3-diphenyl 2-pyrazolines of different substitutions has been synthesized by condensation of substituted α,β-unsaturated ketones with phenylhydrazine hydrochloride in presence of catalytic amount of concentrated HCl. Compounds in the 3-styryl series had OMe, NMe_2, NO_2, OH and isopropyl substituents and those in the 5-styryl series had OMe, NMe_2 and NO_s. The 3-styryl-1,5-diphenyl compounds showed little variation in antibacterial activity towards gram-positive and gram-negative bacteria in terms of geometric mean minimum inhibitory concentrations (MIC). The 4′,4″-NMe_2, 4′,4″-NO_2 and 4′,4″-OMe compounds were found to possess the highest activity in the series. The 5-styryl-1,3-diphenyl series showed lower activities than the 3-styryl series. The *in vitro* antimycotic activity of the 4′,4″-OH and 2′,2″-OH substituted compounds showed good activity than the other molecules in the two series.

Key words antibacterial; antimycotic; geometric mean MIC; 2-pyrazoline

Bacterial resistance[1,2] to antibacterials/antibiotics is a serious problem, and one approach to overcoming it is to explore structural variations of existing molecules. In this connection, electron-rich nitrogen heterocyclics play an important role in diverse biological activities. Introducing a pyrazolidinone[3,4] ring in place of the β-lactum ring (in penicillins and cephalosporins[5]) results in enhanced activity. A second nitrogen[6-10] in the five-membered ring also influences the antibacterial or pharmacokinetic properties. However, there have been only a few reports[11-14] of analogue studies and no clear structure–activity relationships (SAR) are yet apparent. In order to further delineate the SAR for this new type of antibacterial and fungal agents, we have extended our studies to encompass a wider range of substituted 3-styryl 1,5-diphenyl and 5-styryl 1,3-diphenyl 2-pyrazoline derivatives and the results are presented in this paper. In an attempt to evaluate systematically the substituent effects on activity towards bacteria (gram positive, gram negative), fungi and yeast, various 2-pyrazoline derivatives were synthesised.

Chemistry α,β-Unsaturated ketones were reported[15,16] to react with phenyl hydrazine in AcOH/EtOH or pyridine at elevated temperatures producing 2-pyrazolines. But, at high temperature, the pyrazoline moiety decompose to yield isomeric cyclopropane derivatives[17,18] with the expulsion of nitrogen. These methods also require tedious separating techniques to purify the final product. In order to avoid these difficulties, the experimental procedure was modified.

Chart 1 illustrates the synthetic route to several substituted 3-styryl 1,5-diphenyl 2-pyrazoline analogues. The intermediate 2 was synthesized in two steps, *i.e.*, reaction of benzaldehyde and acetone in aqueous NaOH to form monobenzylideneacetone (MBA) and the reaction of 4-methoxybenzaldehyde with MBA to form 4′-methoxydibenzylideneacetone. Other dibenzylideneacetone derivatives were synthesised in a single step. Chart 2 shows the synthetic route to compounds **33**—**36**. The intermediate compounds (**29**—**32**) were synthesized in two steps.

The substituted α,β-unsaturated ketones (**1**—**12**, **29**—**32**) and phenyl hydrazine hydrochloride (used instead of phenyl hydrazine to avoid excessive solubility in EtOH and the possibility of formation of pyrazole as a minor product) were allowed to react in $CHCl_3$/EtOH. A catalytic amount of conc. HCl was used and chloroform was employed for selective solubilization of substituted 2-pyrazoline. The reaction mixture was stirred for two hours at room temperature. The ^1H NMR spectrum of the 3-styryl 1,5-diphenyl 2-pyrazoline moiety showed characteristic peaks at 2.98 (dd, H_A), 3.64 (dd, H_M) and 5.20 (dd, H_X); the H_X value was 5.35 (m) in the case of 5-styryl 1,3-diphenyl 2-pyrazolines (Fig. 1). The IR spectrum showed bands at 1620 of C=N and 2600 (–CH stretching of aromatic ring). The medium intense bands at 1000 and 1590 could be attributed to –CH=CH– and –N-phenyl groups respectively.

The compounds prepared, yields and physical constants (melting point, HPLC purity, ^1H NMR spectral data, UV absorption, fluorescence emission, CHN analysis) are listed in Table 1. The experimental procedures are described in detail in the experimental section.

Results and Discussion

Antibacterial Activity Pyrazolines[20] and different isomeric forms of di-nitrogen five-membered rings (pyrazoles,[21,22] imidazoles[23] and phenidones[24]) have been reported to exhibit a wide variety of biological activities. Even the precursor α,β-unsaturated ketones (chalcones) have been reported to display anti-bacterial,[25] antiviral[26-28] and other pharmacological activities. Interestingly, pyrazoline derivatives showed activity against bacteria (gram positive and gram negative), fungi and yeast.

Table 2 summarises the biological data gathered for the compounds prepared as part of this study. The values are shown as the geometric means for the minimum inhibitory concentrations over six gram-positive bacterial strains, three gram-negative strains, three fungi and three yeast organisms.[29] This was done because a mean will tend to minimize the effects of the usual experimental uncertainty of the MIC data and also because consolidation of the data of MICs of individual compounds on different

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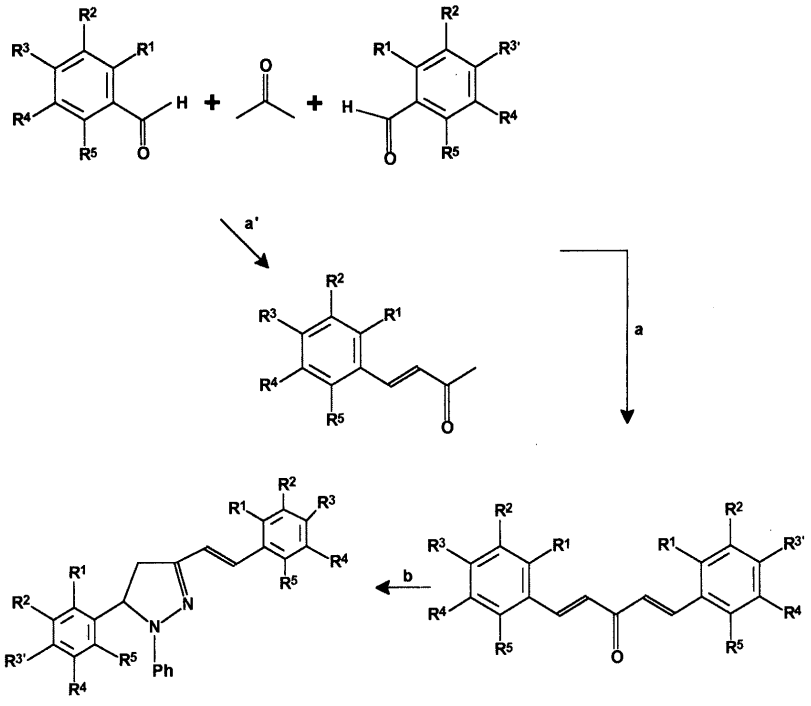
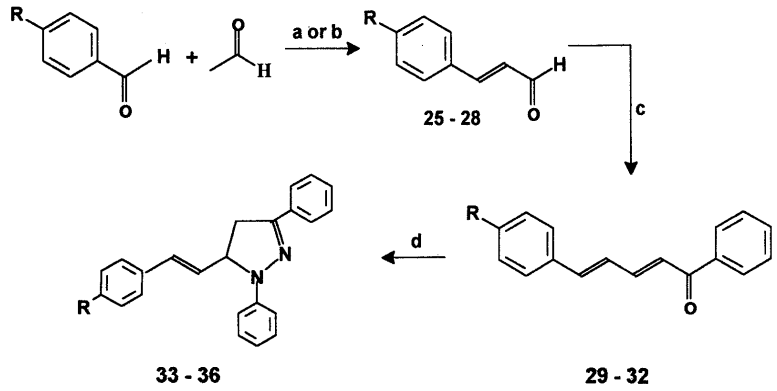


13 - 24 1 - 12

Reagents : a = NaOH, EtOH, 20°C ; a' = aqueous NaOH; b = $C_6H_5NHNH_2$.HCl, $CHCl_3$ /EtOH, HCl

Compound No.	R^1	R^2	R^3	$R^{3'}$	R^4	R^5
1,13	H	H	H	H	H	H
2,14	H	H	H	OMe	H	H
3,15	H	H	OMe	OMe	H	H
4,16	H	OMe	OMe	OMe	H	H
5,17	OMe	H	OMe	OMe	H	H
6,18	H	OMe	OMe	OMe	OMe	H
7,19	OMe	H	OMe	OMe	H	OMe
8,20	H	H	NMe_2	NMe_2	H	H
9,21	H	H	NO_2	NO_2	H	H
10,22	OH	H	H	H	H	H
11,23	H	H	OH	OH	H	H
12,24	H	H	Isopropyl	Isopropyl	H	H

Chart 1



25 - 28 29 - 32 33 - 36

Reagents: a = KOH, Ac_2O, MeOH; b = H_2SO_4 (**27**); c = C_6H_5COMe, NaOH, EtOH; d = $C_6H_5NHNH_2 \cdot HCl$, $CHCl_3$/EtOH, HCl

Compound No.	R
25, **29**, **33**	H
26, **30**, **34**	OMe
27, **31**, **35**	NMe_2
28, **32**, **36**	NO_2

Chart 2

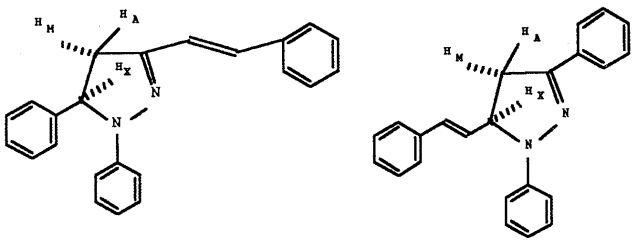
Table 1

Compd. No.	Molecular formula	Morphology mp (°C)	Overall Yield %	HPLC (min) R_t	Purity %	PMR $CDCl_3$, δ, TMS (J in Hz)	Abs. (nm) in EtOH λ_{max}	Fluo. emi. (nm) in EtOH λ_{max}	Elemental analysis (%) Found C	H	N	Calcd. C	H	N
13	$C_{23}H_{20}N_2$	Greenish yellow solid, 126—128	72	4.369	99.8	a, b, 6.9—7.25 (m, 15H, aromatic protons)	375.7	468	85.23,	6.21,	8.63	85.19,	6.17,	8.64
14	$C_{24}H_{22}N_2O$	Greenish yellow solid, 130—131	75	5.569	99.7	a, b, 3.75 (s, 3H, OMe), 6.9—7.25 (m, 14H, aromatic protons)	381.3	465	81.42,	6.17,	7.95	81.36,	6.22,	7.91
15	$C_{25}H_{24}N_2O_2$	Greemish yellow solid, 140—141	77	4.458	100	a, b, 3.78 (s, 3H, aryl methoxy protons), 3.84 (s, 3H, styryl methoxy protons), 6.9—7.25 (m, 13H, aromatic protons)	381.3	467	78.2,	6.21,	7.21	78.13,	6.25,	7.25
16	$C_{27}H_{28}N_2O_4$	Yellow crystals, 137—139	77	5.215	100	a, b, 3.78 (s, 6H, aryl methoxy protons, 3.84 (s, 6H, styryl methoxy protons), 6.9—7.25 (m, 11H, aromatic protons)	378.5	471	73.01,	6.28,	6.30	72.97,	6.31,	6.31
17	$C_{27}H_{28}N_2O_4$	Yellow crystals, 137—138	77	5.275	99.6	a, b, 3.78 (s, 6H, aryl methoxy protons), 3.84 (s, 6H, styryl) methoxy protons), 6.9—7.25 (m, 11H, aromatic protons)	378.5	471	73.05,	6.30,	6.28	72.97,	6.31,	6.31
18	$C_{29}H_{32}N_2O_6$	Yellow crystals, 154—155	70	4.658	100	a, b, 3.78 (s, 9H, aryl methoxy protons), 3.84 (s, 9H, styryl methoxy protons), 6.9—7.25 (m, 9H, aromatic protons)	380.5	472	69.08,	6.34,	5.54	69.05,	6.35,	5.56
19	$C_{29}H_{32}N_2O_6$	Yellow crystals, 170—172	70	4.263	100	a, b, 3.78 (s, 9H, aryl methoxy protons), 3.84 (s, 9H, styryl methoxy protons), 6.9—7.25 (m, 9H, aromatic protons)	365	515	69.09,	6.35,	5.54	69.05,	6.35,	5.56
20	$C_{27}H_{30}N_4$	Brownish yellow crystals, 180—82	72	5.506	99.9	a, b, 2.5 (s, 12H, *N*-methyl protons), 6.9—7.25 (m, 13H, aromatic protons)	419	589	78.98,	7.36,	13.66	79.02,	7.32,	13.66
21	$C_{23}H_{18}N_4O_4$	Brownish yellow crystals, 190—192	72	5.858	100	a, 6.72 (d, 1H, $J=16$ Hz, ciinamoyl proton), 7.54 (d, 1H, $J=8$Hz, ciinamoyl proton), 7.02—7.40 (m, 13H, aromatic protons)	412.5	492	66.7,	4.33,	13.51	66.67,	4.35,	13.53
22	$C_{23}H_{20}N_2O_2$	Greenish yellow solid, 133—135	75	6.581	99.5	a, b, 6.9—7.25 (m, 13H, aromatic protons), 11.89 (br s, 1H, ArOH)	378.3	478	77.57,	5.59,	7.85	77.53,	5.62,	7.87
23	$C_{23}H_{20}N_2O_2$	Greenish yellow solid, 128—129	70	6.317	99.8	a, b, 11.39 (br s, 1H, ArOH), 6.9—7.25 (m, 13H, aromatic protons)	378.5	478	77.56,	5.60,	7.87	77.53,	5.62,	7.87
24	$C_{23}H_{32}N_2$	Pale yellow semi solid	72	4.371	100	a′, b, 1.2 (s, 3H, CH_3 protons), 1.3 (s, 3H, CH_3 protons), 2.9 (m, 1H), 6.9—7.25 (m, 1H, aromatic protons)	340.7	453	85.31,	7.81,	6.85	85.29,	7.84,	6.86
33	$C_{23}H_{20}N_2$	Pale yellow crystals, 120—121	72	3.085	99.7	a′, b′, 6.9—7.25 (m, 14H, aromatic protons)	363	455	85.22,	6.20,	8.58	85.19,	6.17,	8.64
34	$C_{24}H_{22}N_2O$	Pale yellow crystals, 120—122	73	2.805	100	a′, b′, 3.78 (s, 3H, aryl methoxy protons), 6.9—7.25 (m, 14H, aromatic protons)	365	463	81.38,	6.21,	7.92	81.36,	6.22,	7.91
35	$C_{25}H_{25}N_3$	Brownish yellow crystals, 180—182	70	4.063	100	a′, b′, 2.5 (s, 6H, *N*-methyl protons), 6.9—7.25 (m, 14H, aromatic protons)	409	450	81.77,	6.79,	11.44	81.74,	6.82,	11.64
36	$C_{23}H_{19}N_3O_2$	Brownish yellow crystals, 173—174	70	4.425	100	a′, b′, 6.9—7.25 (m, 14H, aromatic protons)	375	490	78.36,	6.51,	7.08	78.39,	6.53,	7.04

a = 2.98 (dd, H_A, $J_{AM}=7.6$, $J_{MX}=12$), 3.64 (dd, H_M, $J_{AM}=7.6$, $J_{MX}=12$), 5.20 (dd, H_X, $J_{AX}=12$, $J_{MX}=12$); b = 6.50 (d, 1H, $J=16$), 7.40 (d, 1H, $J=8$); a′ = 2.98 (dd, H_M, $J_{AM}=7.6$, $J_{MX}=12$), 3.64 (dd, H_M, $J_{AM}=7.6$, $J_{MX}=12$), 5.35 (m, 1H, H_x); b′ = 6.50 (m, 1H, cinnamoyl proton), 7.40 (d, 1H, cinnamoyl proton).

organisms could be useful to detect meaningful trends. The geometric mean MICs of synthesized compounds were compared with those of ciprofloxan (**37**, Table 2) for the antibacterial study and naftifine (**38**, Table 2) for the antifungal study. The 4′,4″-$N(Me)_2$, 4′,4″-NO_2, and 4′,4″-OMe (**20**, **21** and **15**) substituents on the phenyl and/or styryl ring were found to result in the highest activity among the series against a broad range of gram-positive and gram-negative bacteria (Table 2). Interestingly, 3-styryl-1,5-diphenyl 2-pyrazoline (**13**, with only 3-styryl and 5-phenyl substitution) showed activity against gram-positive but not gram-negative bacteria. This often happens when a molecule is lipophilic[30] drastic increase in activity against bacteria of the compounds with substituents -$N(Me)_2$, -NO_2 and -OMe could be attributable to the strong electronic-driving nature of the molecules (either electron-donating or electron-withdrawing). Higher potency of the NMe_2-substituted molecule (**20**) than the methoxy (**15**) substituted one may be due to a more lipophilic nature ($\pi = 0.18$ for $N(Me)_2$ compared to -0.02 for methoxy in octanol/water[31]). The 2′,4′-, 2″,4″- and 3′,4′,3″,4″-dimethoxy derivatives (**16**, **17**) showed reduced activity and 2′,4′,6′-, 2″,4″,6″- and 3′,4′,5′-, 3″,4″,5″-trimethoxy substituents (**18**, **19**) had even lower activity. In general, poor activity of highly substituted molecules may reflect spatial crowding which may disfavor entry into bacteria by passive diffusion, but rather may require a carrier.[32] It has been suggested that low substitution on an aromatic ring aids passive diffusion[33] and this consistent with the MIC data. Thus, steric factors and excessive electronic nature of the susbstituents on the aromatic ring (core) would probably diminish the activity. Hydroxy (phenolic) substitution at the 2′,2″- or 4′,4″-positions (**22**, **23**) resulted in compounds less active than the $N(Me)_2$, NO_2 and OMe derivatives. The Isopropyl group at the 4′,4″-positions on the aromatic ring also afforded poor activity. This suggests that compounds with an alkyl chain is inactive on the aryl ring, are likely to show weak activity.

5-Styryl systems showed similar activity features to those of 3-styryl systems. The parent molecule (**33**) showed activity against gram-positive bacteria (>100 μg/ml) and no activity against gram-negative ones. 5-(*p*-Methoxystyryl) group on the 2-pyrazoline ring (**34**) did not improve the activity. This observation suggested that the position



1,5-diphenyl-3-styryl 2-pyrazoline 1,3-diphenyl-5-styryl 2-pyrazoline

Fig. 1

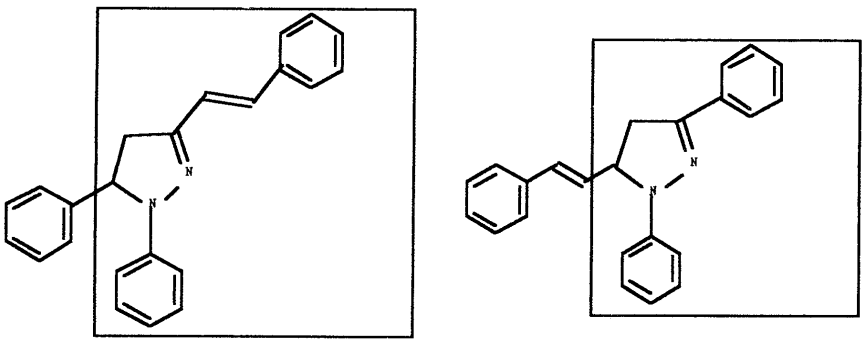
Table 2. Geometric Mean MIC (μg/ml) Values

Compound No.	Gram positive bacteria	Gram negative bacteria	Fungi	Yeast
13	>100	NA	11.3	13.6
14	52.05	63.4	14.23	14.29
15	6.56	6.27	15.92	14.62
16	11.51	11.98	21.63	17.36
17	11.01	11.66	11.89	19.1
18	24.14	25.27	21.94	24.85
19	23.78	25.64	23.16	24.27
20	3.55	6.0	50.79	47.05
21	5.16	3.3	47.45	46.68
22	23.56	23.37	5.28	3.66
23	23.42	22.87	3.63	3.48
24	>200	>200	>200	>200
33	>100	>100	14.8	12.16
34	50.7	50.49	11.60	12.15
35	8.48	8.57	22.82	28.48
36	11.03	11.89	13.57	13.26
37[a]	0.023	0.033	—	—
38[b]	—	—	0.011	0.028

NA = No activity. *a*) Ciprofloxan. *b*) Naftifine.

Supplementary Data of Table 2: MIC (μg/ml) for Individual Organisms

Compd. No.	Gram-positive						Gram-negative			Fungi			Yeast			
	BM	BS	BP	SA	SL	MF	EC	PA	PV	AN	TV	RO	CL	CA	CU	SC
13	>100	>100	>100	>100	>100	>100	NA	NA	NA	10	13	11	16	12	15	12
14	50	42	65	52	56	70	65	56	15	12	16	13	13	13	15	16
15	5	7	7	6	9	6	6	8	5	16	14	18	16	12	17	14
16	12	14	14	9	11	10	12	13	9	22	20	23	18	16	21	15
17	13	12	10	11	8	13	12	12	11	23	19	18	21	22	18	16
18	27	23	29	20	25	22	32	21	24	20	24	22	21	27	24	28
19	25	21	27	22	20	29	24	27	26	20	23	27	26	29	20	23
20	4	3	6	2	2	7	9	4	6	45	56	52	48	45	54	42
21	3	5	7	4	5	9	6	3	2	42	48	53	46	48	50	43
22	20	23	27	29	19	25	29	20	20	3	7	7	3	5	2	6
23	22	27	24	29	21	19	23	26	20	6	4	2	6	3	2	4
24	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200
33	>100	>100	>100	>100	>100	>100	>100	>100	>100	12	18	15	14	10	13	12
34	56	53	48	54	46	48	52	55	45	10	13	12	15	11	12	11
35	6	9	4	12	13	11	10	7	9	22	18	30	27	29	28	30
36	7	12	15	11	10	13	12	14	10	12	16	13	11	18	12	13
37	0.025	0.5	0.06	0.04	0.01	0.008	0.06	0.03	0.02	—	—	—	—	—	—	—
38	—	—	—	—	—	—	—	—	—	0.08	0.003	0.006	0.025	0.03	0.02	0.04



i ii

i. Extended π-electronic structure

ii. Less extended π-electronic structure as compared with (i)

Fig. 2

of the styryl group has no effect on the activity and safely we can say that electron-driving substituents ($N(Me)_2$, OMe) doubled the anti-bacterial activity and afforded broad-spectrum activity against both gram-positive and gram-negative bacteria. There was no loss of activity with $N(Me)_2$ and NO_2 styryl substituents (**35**, **36**) in comparison with **20** and **21**.

Antifungal/Yeast Activity All these compounds showed broad-spectrum anti-fungal/yeast activity. Unsubstituted 1,5-diphenyl-3-styryl 2-pyrazoline (**13**) and its isomer 1,5-diphenyl-5-styryl 2-pyrazoline showed geometric mean MIC 11.3 and 13.6 μg/ml against the fungi and yeasts tested, respectively. Its isomer 5-styryl-1,3-diphenyl 2-pyrazoline (**33**) showed geometric mean MIC values of 14.8 and 12.16 μg/ml for fungi and yeast, respectively. The activity of these compounds could be well understood since allylamine derivatives,[34] a potent anti-fungal agent bearing a styryl functionality, showed enhanced activity. The lower MIC of the 3-styryl chromophore may be because of its extended electronic structure (Fig. 2).

Monomethoxy (4′-position, **14**), dimethoxy (2′,4′,2″,4″-, 3′,4′,3″,4″-position, **16**, **17**) and trimethoxy (2′,4′,6′-, 2″,4″,6″- and 3′,4′,5′-, 3″,4″,5″- **18**, **19**) showed diminished antifungal activity. -$N(Me)_2$ and NO_2 (**20**, **21**) substituents further decreased the activity and the 4′,4″-isopropyl substituted molecule (**24**) was least active. The hydroxy (**22**, **23**) substituent, irrespective of position (2′- or 4′-) on the aromatic ring produced a 4-fold increment in the activity as compared with that of the parent molecule (**13**, **33**).

Conclusions In summary, the parent molecules **13** and **33** did not show any significant difference in their anti-bacterial and antifungal activities. Compounds **20**, **21** and **15** (4′,4″-NMe_2, 4′,4″-NO_2 and 4′,4″-methoxy) exhibited good anti-bacterial activity among the two series. Compounds **16**, **17**, **18** and **19** (3′,4′,3″,4″-, 2′,4′,2″,4″-3′,4′,5′-, 3″,4″,5″- and 2′,4′,6′-, 2″,4″,6″-methoxy derivatives) were found to be less effective. Phenolic derivatives (**22**, **23**) also showed the same potency as trimethoxy derivatives. Changes in the styryl group did not cause any marked difference in the antibacterial activity and mono-substituted compounds (**34**, **35** and **36**) showed lower activity than the 4′,4″- disubstituted molecules. Compounds **13** and **33** exhibited better anti-fungal/yeast than anti-bacterial activity. Phenolic derivatives (**22**, **23**) were effective anti-fungal/yeast molecules. Other substituents (NMe_2, OMe, NO_2) resulted in compounds less effective even to the parent molecules. The isopropyl-substituted compound (**24**) was the least active against bacteria and fungi/yeast. Though some compounds in this series were apparently much less active than ciprofloxacin (anti-bacterial drug) and naftifine (antifungal drug), this series represents a new class of anti-bacterial and anti-fungal/yeast compounds.

Experimental

Melting points were taken on a VEB Analytica Dreader HMK equip ped with a hot plate and are uncorrected. IR spectra were taken on a Perkin-Elmer spectrometer in dry chloroform and peaks are given on the cm^{-1} scale. ^1H NMR spectra were recorded on a JEOL JNM EX-90 or Varian XL-200 spectrometer. Chemical shifts were reported on δ units relative to tetramethylsilane and $CDCl_3$ was used as the solvent unless otherwise specified. TLC was performed on Silica gel G (ACME). Solvents used in the synthesis and experimental work were purified as per the standard literature[35] procedures. Elemental analysis (C, H, N) was performed on a Carlo-Erba Model 1106 elemental analyzer. UV absorption spectra and fluorescence emission spectra were recorded on a Perkin-Elmer UV-Vis spectrometer and an LS5B spectrophotometer, respectively. The purity of the synthesized compounds was determined by HPLC, Shimadzu (LC-6A, CLS-ODS, Shim-pack 6.0 m × 5.0 φ; UV-Vis and fluorescence detection; flow rate, 2 ml/min; MeOH : water = 1.5 : 0.5). All the substituted benzaldehydes were purchased from Fluka Chemical Co., Switzerland, except 2- and 4-hydroxy benzaldehydes which were prepared as reported.[35]

4′-Mono methoxy dibenzylideneacetone (**2**) Benzaldehyde (0.4 mol) and acetone (1.1 mol) were added with stirring to aqueous NaOH (10%, 10 ml) over 15 min. The reaction mixture was stirred for 2 h at 20 °C, then neutralized with 2% dil. HCl. Monobenzylideneacetone was extracted with EtOAc and removal of the EtOAc by distillation yielded the product as white flakes. Monobenzylideneacetone (0.02 mol) and 4-methoxy-benzaldehyde (0.02 mol) were dissolved in EtOH (50 ml) and added to aqueous NaOH (40%, 10 ml) under stirring for 30 min. at 20 °C. The reaction mixture was further stirred for one hour and the contents were kept at 0 °C for one day. The pale yellow fluffy precipitate was filtered off under suction and dried in a desiccator.

Dibenzylideneacetone Derivatives (1, 3—8, 11) Differently substituted dibenzylideneacetone derivatives were prepared using the following general procedure. A substituted benzaldehyde (0.02 mol) and acetone (0.01 mol) were dissolved in EtOH (50 ml) with stirring, and aqueous NaOH (30%, 10 ml) was added dropwise over a period of 30 min. The reaction mixture was stirred for one hour at 20 °C, then kept at 0 °C for one day. The yellow fluffy suspended material was filtered off under suction and thoroughly washed with water until the washings were to neutral to litmus. The yellow solid was dried in a desiccator.

Preparation of Compounds 9 and 10 Aqueous NaOH (50%, 20 ml) was added a little at a time to a solution of 2-/4-hydroxybenzaldehyde

(0.02 mol) and acetone (0.01 mol) in EtOH (50 ml) over a period of 30 min. The contents of the flask were stirred for 3 h at 10 °C. The reaction mixture was neutralised with 1% dilute HCl and kept at 0 °C for one day. The greenish yellow solid was filtered off under suction and dried in a desiccator.

Preparation Compounds 25, 26 and 28 A 25% solution of KOH in methanol was added dropwise to a cold mixture (R = H, OMe, NO_2, Chart 2) of (0.38 mol) and un/substituted acetaldehyde (2.65 mol), until the suspension had disappeared. Then, 200 ml of acetic anhydride was added. The excess acetaldehyde was evaporated off and the contents were poured into an excess amount of water containing 90 ml of conc. HCl. A dark yellow crystalline solid was obtained. Recrystallisation from ethanol yielded a pure product.

4-*N*,*N*-Dimethylamino Cinnamaldehyde (27) 4-*N*,*N*-Dimethylamino benzaldehyde (0.2 mol) was added gradually to 150 ml of concentrated sulphuric acid. Then, acetaldehyde (0.6 mol) was added dropwise with stirring over a period of 3 h at 0 °C. The dark-coloured reaction mixture was poured onto an excess of ice. A yellow precipitate was formed when the solution was neutralized with 10% NaOH solution. The precipitate was dried and extracted with petroleum ether and the product was recrystallized from petroleum ether.

Preparation of Compounds 27—30 A solution of **23—26** (0.01 mol) and acetophenone (0.01 mol) in EtOH was added to aqueous NaOH (50%, 20 ml) over a period of one hour under stirring. The reaction mixture was stirred for another 3 h at room temperature. The contents were poured into excess water and yellow precipitate was filtered off under suction.

Preparation of Compounds 13—24 and 33—36 The target molecules (**13—24**, **33—36**) were prepared by using the general procedure described here. Chloroform (5 ml) and a catalytic amount of concentrated HCl was added to an ethanolic solution of α,β-unsaturated ketones (**1—12** and **29—32**, 0.02 mol) and phenylhydrazine hydrochloride (0.01 mol). The reaction mixture was stirred for 3 h at room temperature. The contents of the flask were poured into water and kept at 0 °C for two days (Charts 1 and 2). The yellow crystalline solid was filtered off and stored in an amber colored bottle (for spectral and other data see Table 1).

Microbiological Experimentals The MIC of each compound was determined by diluting the compound serially in 2-fold steps with dimethylsulfoxide and dispensing aliquots (50 μl) of each dilution into the microwells (8 mm diameter) of multiwell plates, plated with HiMedia nutrient agar. Each microwell was then inoculated with 50 μl of a bacterial suspension (10^7 CFU/ml) obtained from a bacterial culture grown to the stationary phase. The plates were incubated at 37 °C and growth was determined after 18 h. MICs were determined with HiMedia Potato Dextrose (2% agar, pH 6.5) for fungal studies and malt extract (2% agar, pH 4.8) for yeasts. The test compounds were dissolved in DMSO and serially diluted in 2-fold steps, and dispensing aliquots (50 μl) of each dilution were dispensed into the microwells (8 mm) of multi well plates. The above experimental procedure was followed and the micro well plates were incubated at 30 °C for 48 h. MIC was defined as the lowest substance concentration of the substance at which no signs of bacterial or fungal growth was detectable macroscopically.

Since the compounds were solubilized in DMSO in order to achieve the desired concentrations in the culture media, control media always included appropriate concentrations of DMSO (1% or 5%). Such concentrations of DMSO were shown in other experiments to have no effect on bacterial and fungal growth or viability.

References and Notes

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29) The geometric mean was calculated by use of the following formula: Geometeric Mean = Antilog$[\Sigma(\log x_1 + \log x_2 + \cdots \log x_n)/n]$. The gram-positive organisms employed were *Bacillus megaterium* (2326), *Bacillus subtilis* (2547), *Bacillus pumilus* (2327), *Staphylococcus aureus* (2079), *Sarcina lutea* (2103), and *Micrococcus flavus* (2376). The gram-negative organisms used were *E. coli* (2574), *Pseudomonas aeruginosa* (2036), *Proteus vulgaris* (2027). The following fungi were used for Antimycotic study: *Aspergillus niger* (1024), *Trichoderma viridae* (1051), *Rhizopus oryzae* (1009), *Candida utilis* (3336), and yeasts employed were *Candida albicans* (3100), *Curvularia lunata* (716) and *Saccharomyces cerevisiae* (3044). All the strains were obtained from NCIB, Pune, India and number in the parenthesis is the culture code. MIC for each organism is available as supplementary data.
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36) 4-Methoxybenzylideneacetone is supposed to exist in several resonance hybrids. In this case, the 4′-methoxy group would confer considerable stabilization, thus favoring a large contribution of structure B to the resonance hybrid B′, at least in the reacting state. Thus, the electron-donating group participates in resonance by means of a mesomeric effect favoring the formation of the specified isomer. Minor impurities separated by taking advantage of their solubility in *n*-hexane–chloroform (9 : 1) and stirring for two minutes. The supernatant solution afforded the desired isomer, which was recrystallized to give pure isomer.