Facile Synthesis of Some 2-Alkyl-3-Aryl-5, 6-Diphenyl-2, 3-Dihydro-Pyrazines and their Biological Activity

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Abstract* - The reaction of benzil, 1-alkyl-2-arylethanediamine dihydrochloride and sodium acetate trihydrate gave the target molecule dihydropyrazine derivatives 30-34. The compounds have been characterized on the basis of their FT-IR, ^{1}H NMR and ^{13}C NMR spectroscopies. All the compounds are screened for their antibacterial activity against *Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi* and antifungal activity against *Candida albicans, Aspergillus flavus, Rhizopus and Mucor.* Ciprofloxacin is used for the standard for antibacterial and amphotericin B is used for the standard for antifungal studies. Structure Activity Relationship (SAR) led to the conclusion that compounds 32 and 34 exhibited excellent in vitro antibacterial activity against *Klebsiella pneumoniae and Pseudomonas aeruginosa.* Whereas the same set of compounds exerted potent *in vitro* antifungal activity against *Aspergillus flavus* and *Mucor.

Keywords: **Benzil, Tethanediamine Dihydrochloride, Dihydropyrazines, Antibacterial Activity, Antifungal Activity**

I. Introduction

The biological and physical roles of dihydropyrazines (DHPs) such as DNA cleavage [1], growth inhibition of *Escherichia coli* [2], Cyclooxygenase inhibitory activity [3] and NPY antagonists [4] are well documented. Dihydropyrazines are universal in the human body [5] however, there is little reported concerning the biological and physiological roles of DHPs. Yamaguchi *et al.* [1] reported generation of free radicals from dihydropyrazines with DNA Strand-Breakage activity. Takechi *et al.* [6] reported the growth inhibition and mutagenesis induced in Escherichia coli by dihydropyrazines with DNA strand-cleaving activity. 2-cyanopyrazine derivatives show anticancer antiinflammatory and analgesic activities [7]. Pyrazine derivatives exhit a tuberculostatic activity [8]. It also exhit a antimicrobial [9] and biological [10] activities. Alkyl substituted pyrazines are found in the growth medium of the polymyxin-producing bacterium *Paenibacillus polymyxa*[11].

These observations places new emphasis on the need of as well as search for alternative new and more effective antimicrobial agents with a broad spectrum.

In the course of broad programme in developing biologically active molecules, we have recently reported the synthesis of 2,3-dihydropyrazine derivatives and evaluated their biological activity **30-34.** In order to extend our knowledge in structure-activity relationship, all the synthesized compounds are tested for their *in vitro* antibacterial and antifungal activities and the influence of some structural variations by varying the substituents at the phenyl ring in the synthesized compounds towards their biological activities is evaluated.

II. Experimental

A. Chemistry

The course of reaction and the purity were ascertained by performing TLC. Melting points were determined in open capillaries and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX 400 spectrometer operating at 400.13 MHz for ^{1}H and 100.62 MHz for ^{13}C in $CDCl_3$. IR spectra were recorded in KBr discs on an Avatar (300 FT-IR) Thermo Nicolet spectrometer.

By adopting the literature precedent [12] 2,6-diarylpiperidin-4-ones **13-17** were prepared by the condensation of the appropriate ketones, aldehydes and ammonium acetate in a 1:2:1 ratio. Formation of homopiperazine-5-one **18-22** were prepared by the following literature method [13]. By adopting the literature precedent [14], 1-alkyl-2-arylethanediamine dihydrochloride **23-27** were prepared.

B. Synthesis of 2-Alkyl-3-Aryl-5, 6-Diphenyl-2, 3-Dihydro-pyrazines (30-34)

A homogeneous solution of benzil (5 mmol), 1-alkyl-2arylethanediamine dihydrochloride (5 mmol) in ethanol, sodium acetate trihydrate (15 mmol) was added. The precipitated sodium chloride was filtered off and the filterate

was refluxed for 2 hours. On completion of the reaction as indicated by TLC, the reaction mixture was poured into crushed ice and the resulting solid was filtered under solution and purified by column chromatography on silica gel. Elution with benzene : petether 60-80^{0}C (4:1) gave the product in the pure form.

2-Methyl-3,5,6-triphenyl-2,3-dihydropyrazine **30**: IR (KBr) (cm^{-1}): 3055, 2972, 2924, 1628; ^{1}H NMR (d ppm): 1.33,3.353.43 , 4.08, 7.273.46; ^{13}C NMR (d ppm): 19.7, 57.0, 66.4 , 127.8, 129.4 137.4 , 141.3.

2-Methyl-3-(4-methylphenyl)-5,6-diphenyl-2,3-dihydro- pyrazine **31**: IR (KBr) (cm^{-1}): 3054, 2970, 2923, 1620; ^{1}H NMR (d ppm): 1.33, 2.36, 3.343.42, 4.04, 7.187.46; ^{13}C NMR (d ppm): 19.8, 21.1, 57.1, 66.2,127.9, 129.1, 136.9, 137.6, 138.5, 159.2, 159.4.

2-Methyl-3-(2-chlorophenyl)-5,6-diphenyl-2,3-dihydro pyrazine **32**: IR (KBr) (cm^{-1}): 3062, 2966, 2924, 1618; ^{1}H NMR (d ppm): 1.40 , 3.403.48 , 4.82 ,7.237.48 ;^{13}C NMR (d ppm): 18.86, 57.76, 62.2, 127.1, 129.9, 134.0, 137.5, 139.3, 159.4, 159.7.

2-Ethyl-3,5,6-triphenyl-2,3-dihydropyrazine **33**: IR (KBr) (cm^{-1}): 3059, 2966, 2929, 1605; H NMR (d ppm): 0.98,1.40147, 3.023.11, 4.04, 7.107.37 ; 13C NMR (d ppm): 10.9, 26.1, 63.0, 65.5, 127.8, 129.0, 130.1, 138.1 ,142.1,159.9.

2-Ethyl-3-(4-fluorophenyl)-5,6-diphenyl-2,3-dihydro pyrazine **34**: IR (KBr) (cm^{-1}): 3059, 2969, 2922, 1605; ^{1}H NMR (d ppm): 1.03 ,1.461.57, 3.01-3.08 ,4.06 , 7.02□7.41 ;

^{13}C NMR (d ppm): 10.8, 26.0, 63.1, 64.9, 115.8, 128.5, 130.2, 138.0, 159.9, 161.7, 163.9.

C. Microbiology

In vitro antibacterial and antifungal activity. The *in vitro* antimicrobial activities of the compounds were tested in Sabouraud's dextrose broth (SDB, Hi-media, Mumbai) for fungi and nutrient broth (NB, Hi-media, Mumbai) for bacteria by the twofold serial dilution method [15]. The test compounds were dissolved in dimethyl sulfoxide (DMSO) to obtain 1 mg/ml stock solutions. Seeded broth (broth containing microbial spores) was prepared in NB from 24 hrs old bacterial cultures on nutrient agar (Hi-media, Mumbai) at 37 + 1 ^{0}C while fungal spores from 24 hrs to 7-day-old Sabouraud's agar slant cultures were suspended in SDB. The colony forming units (cfu) of the seeded broth were determined by the plating technique and adjusted in the range of 10^4-10^5 cfu/ml. The final inoculum size was 105 cfu/ml for the antibacterial assay and 1.1-1.5 x 10^2 cfu/ml for the antifungal assay. Testing was performed at 7.4 x 0.2. Exactly 0.2 ml of the solution of test compound was added to 1.8 ml of seeded broth to form the first dilution. One ml of this was diluted with a further 1 ml of the seeded broth to give the second dilution and so on until six such dilutions were obtained. A set of assay tubes containing only seeded broth was kept as control and likewise solvent controls were also run simultaneously. The tubes were incubated in biochemical oxygen demand (BOD) incubators at 37^{0}1 ^{0}C for bacteria and 28^0 1 ^{0}C for fungi. The minimum inhibitory concentrations (MICs) were recorded by visual observations after 24 hrs (for bacteria) and 72-96 hrs (for fungi) of incubation. Ciprofloxacin was used as a standard for the bacterial study while Amphotericin B was used as a standard for the fungal study.

III. Results And Discussion

A. Chemistry

Target molecules 2,3-dihydropyrazines **30-34** were synthesized as a result of a five step synthetic strategy. One of the direct synthetic route for the formation of 2,6-diaryl-piperidin-4-ones 13-17 is as follows: A mixture of appropriate ketones **1-2** structurally diverse aromatic aldehydes 3-6 and ammonium acetate 7 in the ratio of 1:2:1, was warmed for 10 min and hydrochloric acid was added to afford 3-alkyl-2,6-diaryl-piperidin-4-ones hydrochlorides **8-12,** which upon neutralization with aqueous ammonia gave the respective 3-alkyl-2,6-diaryl-piperidin-4-ones **13-17.** It undergoes Schmidt reaction to yield homopiperazines 18-22 upon treatment with sodium azide and concentrated sulphuric acid. The homopiperazines **18-22** were subjected to hydrolysis reaction by using 6N hydrochloric acid, resulted 1-alkyl-2-arylethanediamine dihydrochlorides **23-27.** It was reacted with benzil 28, sodium acetate trihydrate **29** to afford respective 2-alkyl-3-aryl-5,6-diphenyl-2,3-dihydropyrazines **30-34.** The schematic representation and the physical data for the synthesized compounds **30-34** are given in Scheme 1 and Table 1, respectively.

It seen that many pharmacologically relevant substitution patterns on the aromatic ring could be introduced with high efficiency. It was observed that aromatic aldehydes carrying either electron releasing or electron withdrawing substituents in the ortho and para positions afford high yields of products. The numbering of the target compound is done in Fig. 1. The

structure of the synthesized compounds **30-34** was confirmed by melting points, FT-IR, one dimensional NMR (^{1}H and ^{13}C) spectroscopic data.

B.Antibacterial Activity

The synthesized 2,3-dihydropyrazines **30-34** were tested for their antibacterial activity in vitro against Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa and Salmonella typhi. Ciprofloxacin was used as standard drug whose minimum inhibitory concentration (MIC) values were provided in Table 2.

Compound **30** and **33** without any substituent at the para position of the aryl moiety at C-3 position of the six membered heterocyclic ring exhibited antibacterial activity in vitro at 200 mg/ml against all the tested organisms except *K. pneumoniae and P. aeruginosa.* They inhibit at a MIC of 100 mg/ml. It is obvious from the Table 2 that substitution of ethyl group for methyl at C-2 position in **30** (compound **33**) did not change the activity.

Introduction of methyl group at the para position of the aryl moiety at C-3 position in 30 (compound **31**) results increase in activity against all the tested organisms. Replacement of hydrogen present at the ortho position of the aryl moiety at C-3 position of **30** (compound **32**) by chloro function showed the activity in the range of 6.25 to 25 mg/ml against all the tested organisms.

Due to the replacement of hydrogen by fluoro function at the para position of the aryl moiety at C-3 position of **33** (compound **34**) exhibited excellent antibacterial activity against all the tested organisms. A comparative studies of minimum inhibitory concentration for compounds **30-34** using standard. Ciprofloxacin versus bacterial strains given in Fig. 2.

C. Antifungal Activity

The in vitro antifungal activity of the synthesized compounds **30-34** was studied against the fungal strains viz., *Candida albicans, Aspergillus flavus, Rhizopus and Mucor.* Amphotericin B was used as a standard drug whose MIC values are provided in Table 3.

Generally all the synthesized compounds exerted a wide range of modest in vitro antifungal activity against all the tested organisms except **30** which failed to show activity against *Candida albican*s and Rhizopus and **33** against *Candida albicans* even at a high concentration of 200 mg/ml.

The compound **30** without any substituent at the para position of the aryl group present at C-3 position of the six membered heterocyclic moiety did not show in vitro antifungal activity even at a maximum concentration of 200 mg/ml against *Candida albicans* and *Rhizopus* while against Aspergillus flavus and Mucor registered activity at a MIC of 100 mg/ml respectively.

Introduction of ethyl group at C-2 in **30** (compound **33**) did not enhance activity against all the tested fungal strains except Rhizopus which the inhibition is observed only at 200 mg/ml. This compound also failed to show antifungal activity against Candida albicans even at 200 mg/ml. By the introduction of methyl group at the para position of the aryl moiety at C-3 position of **30** (compound **31**) results the activity was increased against all the tested organisms.

Due to the replacement of hydrogen by chloro function at the ortho position of the aryl moiety at C-3 position of **30** (compound **32**) showed good antifungal activity against all the tested organisms. Substitution of fluoro group in place of hydrogen function in **33** led to compound **34** showed excellent antifungal activity against all the tested organisms.

Minimum inhibitory concentration of compounds **30-34** was compared with standard Amphotericin B against fungal strains shown in Fig. 3.

IV. Conclusion

A close examination of the in vitro antibacterial and antifungal activity profile in differently substituted 2,3-dihydropyrazines **30-34** against the tested bacterial strains viz., *S. aureus, K. pneumoniae, P. aeruginosa and S. typhi* and the fungal strains viz., *C. albicans, A. flavus, Rhizopus and Mucor* respectively, provides a better structure activity relationship correlation. This may be summarized as follows: the results of this study show that the presence of both electron-donating substituent (methyl) and electron-withdrawing substituent (chloro, fluoro) at ortho, para positions on the phenyl ring in compounds **30-34** are responsible for the activity against all the tested organisms.

These observations may promote a development of our research in this field. Further development of this group of compounds may lead to compounds with better pharmacological profile than standard drugs and serve as templates for the construction of better drugs to combat bacterial and fungal infection.

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Table I Physical Data for Compounds 30 -34

Compound	R	R_1	Yield (%)	m.p (mC)
30	CH_3	H	80	187
31	CH_3	4-CH_3	72	177
32	CH_3	2-Cl	76	144
33	C_2H_5	H	84	114
34	C_2H_5	4-F	68	129

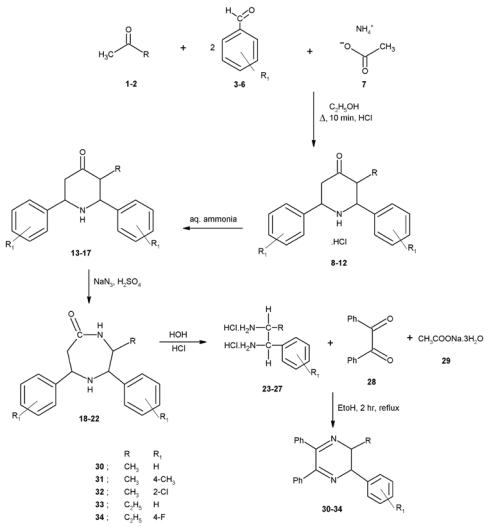
Table II in Vitro Antibacterial Activity of Compounds 30-34

Entry	Minimum Inhibitory Concentration (MIC) in mg/ml			
	S. aureus	*K. pneumoniae*	*P. aeruginosa*	*S. typhi*
30	200	100	100	200
31	50	25	50	100
32	25	6.25	6.25	25
33	200	100	100	200
34	6.25	3.13	3.13	12.5
Ciprofloxacin	25	50	50	12.5

Table III in Vitro Antifungal Activity of Compounds 30-34

Entry	Minimum Inhibitory Concentration (MIC) in mg/ml			
	C. albicans	*A. flavus*	*Rhizopus*	*Mucor*
30	-	100	-	100
31	200	50	100	25
32	50	12.5	50	25
33	-	100	200	100
34	25	3.13	6.25	3.13
Amphotericin B	50	25	25	25

'-' No inhibition even at a higher concentration of 200 mg/ml



Scheme 1 Synthesis of 2,3-dihydropyrazines 30-34

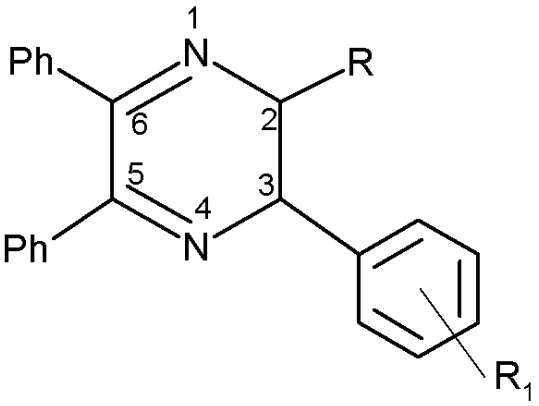
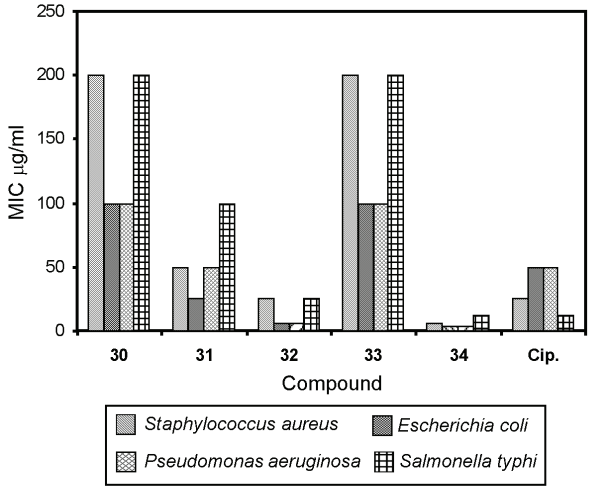
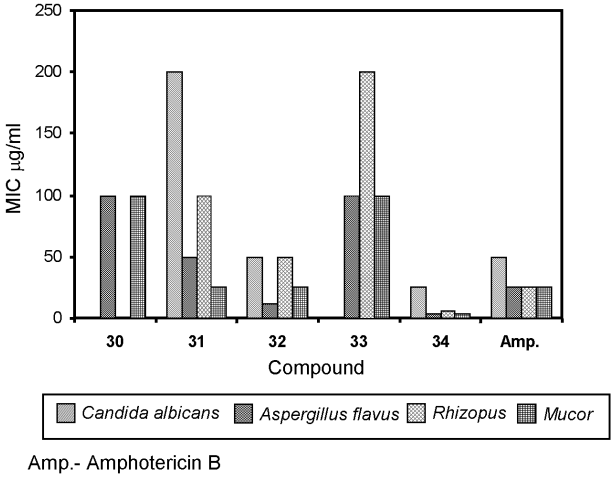


Fig.1 Numbering of 30-34



Cip. – Ciprofloxacin

Fig.2 Comparision of minimum inhibitory concentration of compounds 30-34 with Ciprofloxacin (as standard) against bactrerial strains from serial dilution method



Amp.- Amphotericin B

Fig.3 Comparision of minimum inhibitory concentration of compounds 30-34 with Amphotericin B (as standard) against fungal strains from serial dilution method

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