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Microwave Assisted Five Steps Synthesis of Substituted Pyrazines Under Solvent Free Conditions and Its Bioloical Activity

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KEYWORDS

Pyrazine, Ethanediamine dihydrochloride, Antibacterial Activity and Antifungal Activity.

ABSTRACT:

A series of Pyrazine derivatives were Synthesized from reaction of Benzil and substituted ethanediamine dihydrochloride and sodium acetate trihydrate without solvent under the irradiation of Microwave is described. These compounds have been characterized by 1H and13C NMR spectra and Mass spectra. In vitro antibacterial and antifungal activity have been studied for the synthesized compounds. This study reveals that compounds exhibit excellent antibacterial and antifungal activity against all the tested organisms.

I. INTRODUCTION

The biological and physical roles of pyrazines such as DNA cleavage [1], growth inhibition of *Escherichia coli* [2], Cyclooxygenase inhibitory activity [3] and NPY antagonists [4] are well documented. Pyrazines 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 Pyrazines 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 needof 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 Pyrazine derivatives and evaluated their biological activity **65-76.** 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.

2. EXPERIMENTAL

2.1. Synthesis of 5-Aryl-2, 3-Diphenylpyrazines (65-76)

By adopting the literature precedent [12] 2,6-diarylpiperidin-4-ones **27-38** were prepared by the condensation of the appropriate ketones, aldehydes and ammonium acetate in a 1:2:1 ratio. Formation of homopiperazine-5-one **39-50** were prepared by the following literature method [13]. By adopting the literature precedent [14], 1-alkyl-2-arylethanediamine dihydrochloride **51-62** were prepared.

In a conical flask Benzil (5 mmol), 1-alkyl-2arylethanediamine dihydrochloride (5 mmol) and sodium acetate trihydrate (15 mmol) were mixed and irradiated at 160W under microwave condition for a specified time. After completion of the reaction (vide TLC), reaction mixture was cooled to room temperature, the solid crude product was slowly

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precipitated out of the reaction mixture. The crude product was recrystallized from ethanol to get pure compound. The obtained compounds were characterized by melting point, ^{1}H NMR and ^{13}C NMR and Mass Spectroscopy.

2.2. Characterization And Spectral Data For Some Selected Compounds

2.2.1. 2,3,5-triphenylpyrazine(65)

^{1}H NMR (δ, ppm): 7.32 – 7.35, 7.48 – 7.57, 8.16,9.02; ^{13}C NMR (δ, ppm): 151.4, 150.4, 149.6, 138.7, 136.2, 129.8, 128.9, 128.6, 128.5, 128.3, 128.2, 126.8; Mass Spectrum (M+1) Peak: 309

2.2.2. 5 – (4-methoxyphenyl) - 2,3-triphenylpyrazine(67)

^{1}H NMR (δ, ppm):8.61, 7.49, 7.36, 7.32, 7.22, 6.84, 3.75; ^{13}C NMR (δ, ppm): 160.2, 151.9,155.6, 150.1, 141.6, 133.2, 129.8, 128.9, 128.6, 127.6, 125.6, 114.3, 55.9; Mass Spectrum (M+1) Peak: 339.2.

2.2.3. 5 – (2-nitrophenyl) - 2,3-triphenylpyrazine(76)

^{1}H NMR (δ, ppm):8.69, 8.26, 7.79,7.73, 7.52, 7.49, 7.35, 7.28; ^{13}C NMR (δ, ppm): 155.8, 152.0, 151.3, 150.1, 141.9, 134.9, 134.7, 133.8, 131.4, 130.1, 129.4, 128.2, 122.8, 121.9; Mass Spectrum (M+1) Peak: 354.3.

2.3. *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 1.8ml 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 and28^{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.

3. RESULTS AND DISCUSSION

3.1. Chemistry

Target molecules 2,3-diphenylpyrazines **65-76** 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 **27-38** is as follows: A mixture of a ketone **1** structurally diverse aromatic aldehydes **2-13** and ammonium acetate **14** 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 **15-26,** which upon neutralization with aqueous ammonia gave the respective 3-alkyl-2,6-diaryl-piperidin-4-ones **27-38.**

It undergoes Schmidt reaction to yield homopiperazines **39-50** upon treatment with sodium azide and concentrated sulphuric acid. The homopiperazines **39-50** were subjected to hydrolysis reaction by using 6N hydrochloric acid, resulted 1-alkyl-2-arylethanediamine dihydrochlorides **51-62.** It was reacted with benzil **63**, sodium acetate trihydrate **64** to afford respective 5-aryl-2,3-diphenylpyrazines **65-76.** The schematic representation and the physical data for the synthesized compounds **65-76** are given in **Scheme 1**, **Scheme 2** and **Table 1**, respectively.

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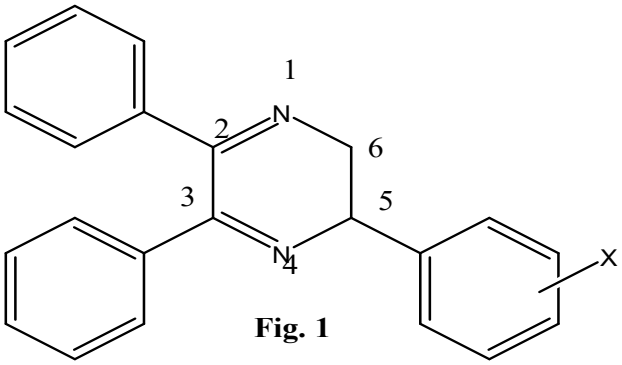
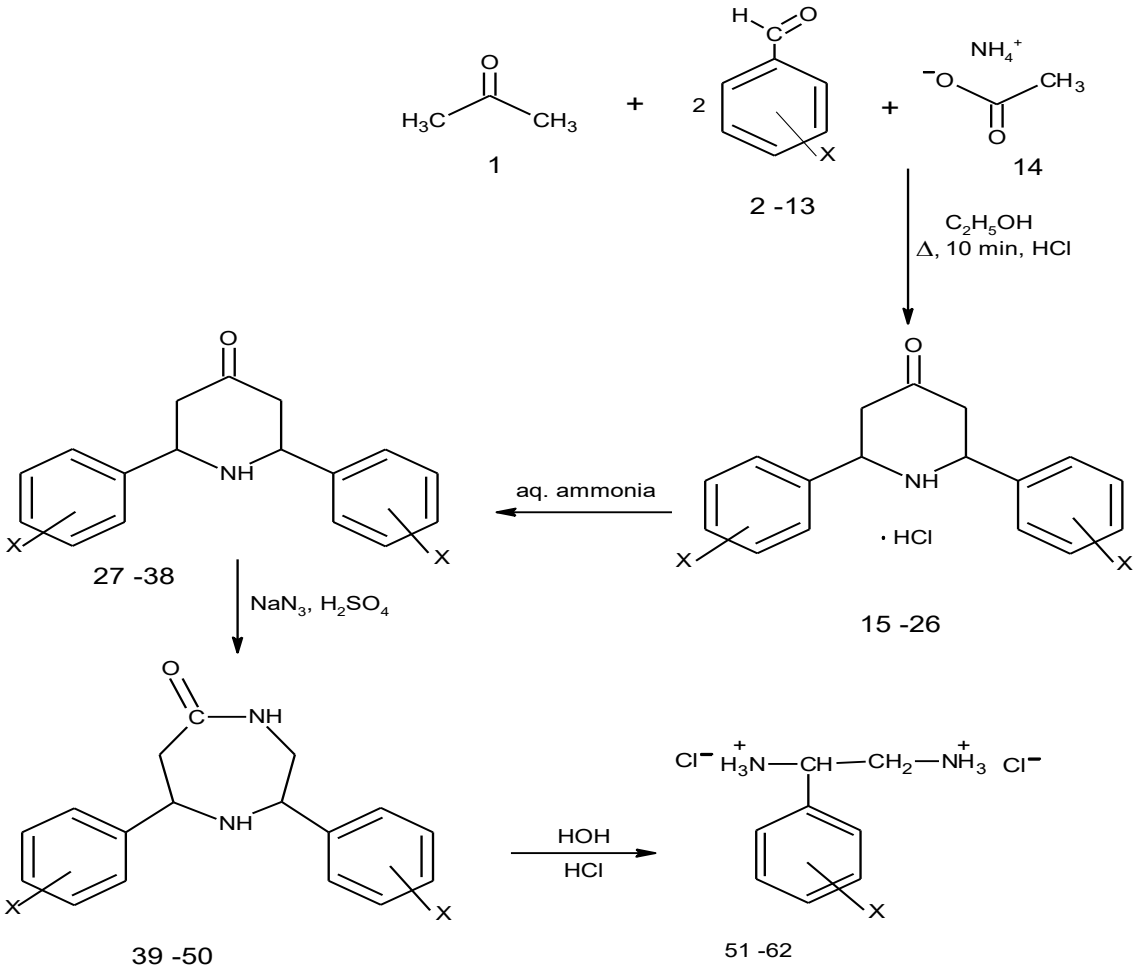


Fig. 1



Scheme 1

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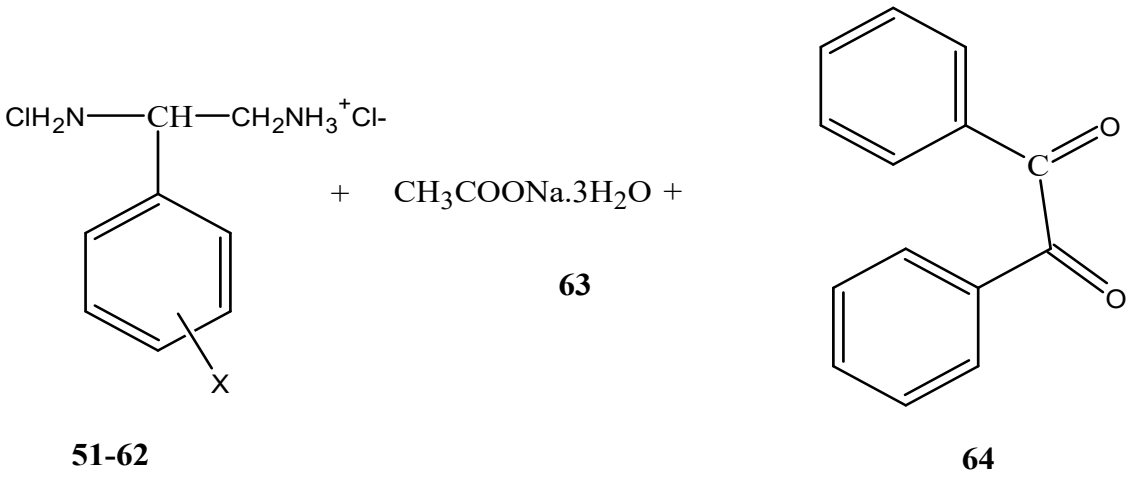
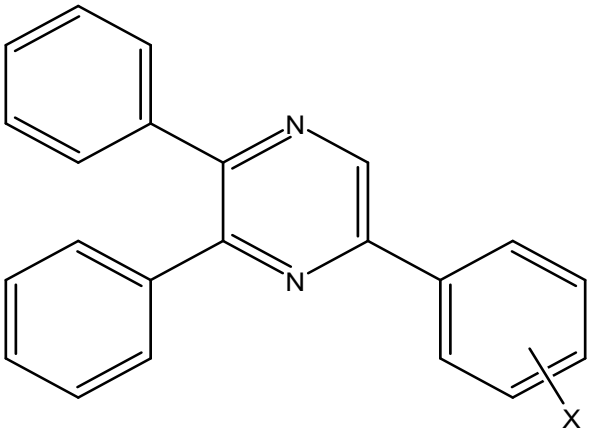


51-62 **64**

Microwave irradiation, 160W



65 -76

Scheme 2

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 **65-76** was confirmed by melting points, one dimensional NMR (^{1}H and ^{13}C) spectroscopic data and Mass Spectroscopic data.

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Table 1 Physical data for Compounds 65 – 76

Compound	X	Yield (%)	m.p. (°c)
65	H	86	167
66	4-CH_3	82	145
67	4-OCH_3	84	143
68	4-Cl	82	136
69	4-F	78	153
70	4-NO_2	80	214
71	4-OH	82	263
72	2-OCH_3	82	148
73	2-CH_3	78	147
74	3-OCH_3	84	146
75	3-CH_3	80	149
76	2-NO_2	78	202

3.2. Antibacterial Activity

The synthesized 2,3-dihydropyrazines **65-76** 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 **65** without any substituent at the para position of the aryl moiety at C-5 position of the six membered heterocyclic ring exhibited antibacterial activity in vitro at 200 µg/ml against all the tested organisms except *S. typhi.* They inhibit at a MIC of100 µg/ml.

Table 2. In Vitro Antibacterial Activity of Compounds **65-76**

Compound	Minimum Inhibitory Concentration (MIC) in µg/ml			
	S. aureus	***K. pneumoniae***	***P. aeruginosa***	***S.typhi***
65	200	200	200	100
66	100	100	200	50
67	100	25	25	12.5
68	12.5	25	25	12.5
69	6.25	12.5	6.25	3.13
70	3.13	6.25	3.13	3.13

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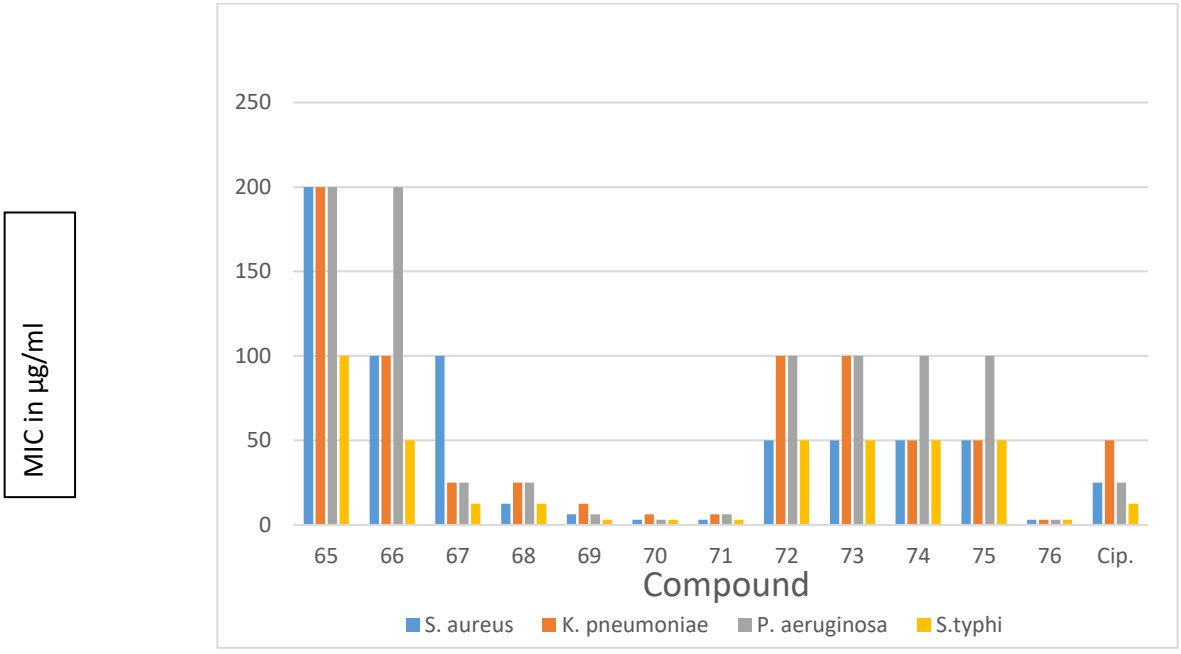
71	3.13	6.25	6.25	3.13
72	50	100	100	50
73	50	100	100	50
74	50	50	100	50
75	50	50	100	50
76	3.13	3.13	3.13	3.13
Ciprofloxacn	25	50	25	12.5

Introduction of methyl and methoxy group at the para position of the aryl moiety at C-5 position in **65 (Compound 66 and 67),** results increase in activity against all the tested organisms. Replacement of hydrogen present at the ortho position and meta position of the aryl moiety at C-5 position of **65** (compound **72, 73, 74 and 75**) by methyl and methoxy function showed the activity in the range of 50 to 100 µg/ml against all the tested organisms.

Due to the replacement of hydrogen by chloro, fluoro, nitro and hydroxyl group at the para position and ortho position of the aryl moiety at C-5 position of **65 (Compound 68, 69, 70,71 and 76),** exhibited excellent antibacterial activity against all the tested organisms. A comparative studies of minimum inhibitory concentration for compounds **65-76** using standard. Ciprofloxacin versus bacterial strains given in **Fig. 2**.



Cip. - Ciprofloxacin

Fig.2. Comparision of minimum inhibitory concentration of compounds **65-76** with Ciprofloxacin (as standard) against bactrerial strains from serial dilution method

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3.3. Antifungal Activity

The in vitro antifungal activity of the synthesized compounds **65-76** 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**.

Table 3. In Vitro Antifungal Activity of Compounds **65-76**

Compound	Minimum Inhibitory Concentration (MIC) in µg/ml			
	C. albicans	***A. flavus***	***Rhizopus***	***Mucor***
65	-	200	-	200
66	200	100	200	100
67	100	100	200	100
68	100	50	100	25
69	50	25	50	25
70	12.5	6.25	25	6.25
71	6.25	3.13	6.25	6.25
72	100	50	100	100
73	100	50	100	100
74	100	50	100	50
75	50	50	100	100
76	6.25	3.13	6.25	3.13
Amphotericin -B	50	25	50	25

Generally, all the synthesized compounds exerted a wide range of modest in vitro antifungal activity against all the tested organisms except **65** which failed to show activity against *Candida albican*s and Rhizopus even at a high concentration of 200 µg/ml.

The compound **65** without any substituent at the para position of the aryl group present at C-5 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 200 µg/ml respectively.

By the introduction of methyl or methoxy group at the para position of the aryl moiety at C-5 position of **65** (compound **66 and 67**) results the activity was increased against all the tested organisms.

Due to the replacement of hydrogen by choro, fluoro, nitro and hydroxyl functionat the para or ortho position of the aryl moiety at C-5 position of **65 (Compound 68 and 69),** showed good antifungal activity and **Compound 70,71** and **76** exhibited excellent antifungal activity against all the tested organisms.

Replacement of hydrogen present at the ortho position and meta position of the aryl moiety at C-5 position of **65** (compound **72, 73, 74 and 75**) by methyl and methoxy function showed the activity in the range of 50 to 100 µg/ml against all the tested organisms.

Minimum inhibitory concentration of compounds **65-76**

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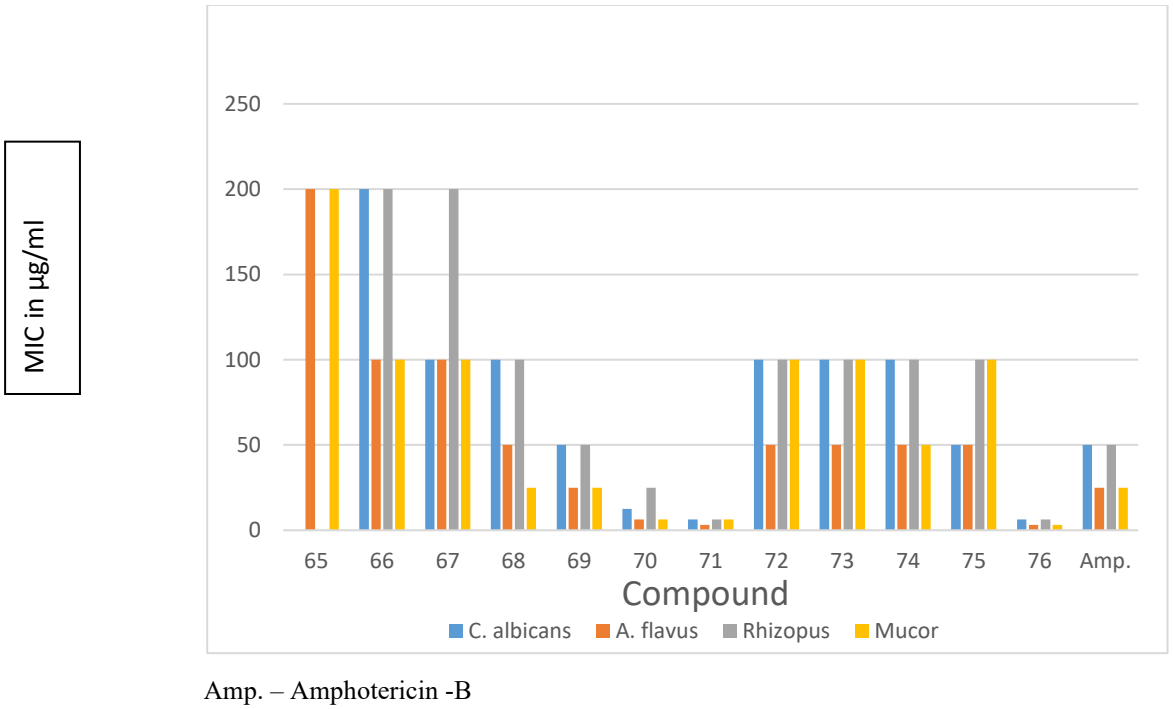
was compared with standard Amphotericin B against fungal strains shown in **Fig. 3**.



Amp. – Amphotericin -B

Fig.3. Comparision of minimum inhibitory concentration of compounds **65-76** with Amphotericin -B (as standard) against fungal strains from serial dilution method

4. CONCLUSION

In conclusion, the solvent free microwave assisted synthesis of Pyrazine derivatives represents a promising and sustainable approach for accessing biologically active compounds with diverse applications in drug discovery and organic synthesis. This methodology offers several advantages like high yield, reduced environmental impact and broad substrate scope, making it an alternative to traditional synthetic approach.

A close examination of the in vitro antibacterial and antifungal activity profile in differently substituted 2,3-dihydropyrazines **65-76** 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 structureactivity 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 **65-76** 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 combatbacterial and fungal infection.

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