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# Ultrasound Assisted Synthesis, Molecular Docking Studies and in Vitro Biological Activity of Azine Derivatives as Potential Antifungal Agents

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**Abstract:- There is a need for synthesis of new & potent antifungals due to a rise in systemic fungal infections, resistance as well as toxicity to the existing azole antifungals. The specific role of azines [>C=N-N=C<] as binding molecules or modulators of biological receptors makes them suitable candidates for drug development.[1] In-silico docking of substituted azine ligands on lanosterol-14-α-demethylase [5TZ1.pdb] of C.albicans using Autodock vina (pyrx) suggested that substituted azines with hydrophobic groups are likely to exhibit superior antifungal activities owing to non-bonded interactions with the CYP51 protein. Synthesis of a few of the screened compounds and determination of biological activities was undertaken to validate this docking model.**

**An ultrasound assisted green synthetic protocol has been developed for synthesis of substituted benzalazines using sonicator probe in aqueous medium. Several conventional synthetic methods are known for Bis-Schiff bases, they however suffer from low yields, long reaction duration, harsh conditions & use of organic solvent medium like alcohol, DCM etc.[2] The ultrasound route offered superior yields & shorter reaction times as compared to conventional methods & went smoothly in aqueous medium as against use of organic solvents. Most of the reactions went to completion with greater than 90% yields within 1 minute as indicated by TLC. 4-chloro, 4-methyl, 4-hydroxy & 1-naphthyl derivatives were prepared to establish the docking model. The synthesized compounds were characterized & then tested for their antifungal activity by the agar well diffusion method against *C.albicans* & *S.cerevisiae*. The results of biological activity were as predicted by the docking model, indicating the significance of non-bonded interactions towards mode of action of azines as antifungal agents. The largest zone of inhibition was observed for 1-naphthylbenzalazine making it a potential candidate for hit to lead approach for development of potent antifungal agents.**

***Keywords:-*** *Azine, Antifungal, Docking Studies, Lanosterol Demethylase***.**

## I. INTRODUCTION

There are many synthetic protocols reported in literature for synthesis of azines from carbonyl compounds and hydrazine ranging from stirring at rt, to reflux to thermal condensation [3]. Ultrasound has become a popular alternative to conventional methods of organic synthesis & offers advantages of faster reactions with higher yields. One such report on ultrasound assisted synthesis of azines was found in literature[4] using a bath sonicator of an alcoholic solution of aldehyde and hydrazine hydrate in the presence of catalytic amount of $BiCl_3$. We have developed a new ultrasound assisted synthetic protocol to access azines using probe sonicator in aqueous medium. Substituted aromatic aldehydes reacted smoothly with hydrazine hydrate in the absence of catalyst to give azines with high yields within a minute of ultrasonication. The reaction was clean and did not require purification.

There are isolated records of moderate to good biological activities of azines,[5] however, to the best of our knowledge, there exists no structured study of the antifungal activities of these compounds to transform them as useful leads to treat fungal infections. The azines synthesized were selected based on docking model on the antifungal molecular target Lanosterol-14-α-demethylase. Docking studies predicted good binding affinity of ligands with hydrophobic groups as compared to polar side chains. The synthesized compounds were tested for in-vitro antifungal activities & the results were found to be consistent with the docking studies.

## II. MATERIALS & METHODS

The docking studies were done using Autodock vina (PyRx) software on the molecular target Lanosterol-14-α-demethylase of pathogenic *C. albicans*. The crystal stcuture of CYP51 protein target, 5TZ1 was obtained from the protein data bank and a number of substituted azines were screened in-silico for their binding affinity. The pose values increased in the following order: Benzalazine, p-hydroxybenzalazine, p-chlorobenzalazine, p-tolualdazine & 1-naphthaldazine. The order was consistent with many other substituents in that non-polar side chains resulted in higher binding affinities to the molecular target, indicating that the non-bonded hydrophobic interactions were more important of the binging modes.

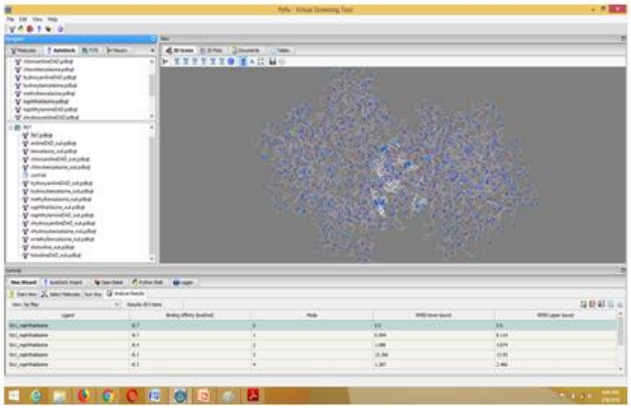


Fig 1

Ultrasound assisted synthesis:

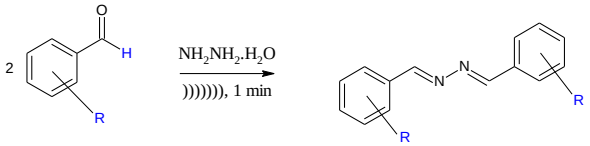
The synthesis of azines was done using a probe sonicator operating at 22 kHz frequency with the following settings: tON = 1.0 s; tOFF = 15 s; INT = 12 min; Tem = 25 deg.

General protocol for synthesis of azines: To 10 mmol of aldehyde was added 5 mmol of hydrazine hydrate and sonicated in aqueous medium till the reaction was complete, as monitored by TLC. The product was filtered, dried and its IR spectrum was recorded.

Each of the synthesized compounds gave a characteristic sharp C=N stretch in IR at 1623-1593 $cm^{-1}$. The disappearance of aldehydic C=O stretch at1735-1700 $cm^{-1}$ indicates conversion to the diimine bond.

Benzalazine: 1623.3 $cm^{-1}$; 4-chlorobenzalazine: 1593.4 $cm^{-1}$; 4-hydroxybenzalazine: 1608.3 $cm^{-1}$; 4-tolualdazine: 1619.5 $cm^{-1}$; 1-naphthylazine: 1612.4 $cm^{-1}$.

The yields of each of the compounds synthesized was quantitative & the reaction times were much shorter than conventional reaction conditions.



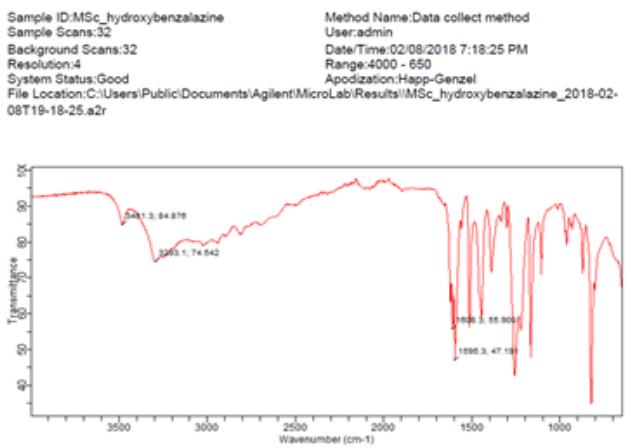




Fig 2

## III. RESULTS

➢ *Docking Studies*

In-silico screening of azine derivatives showed an increased binding pose value for naphthalene derivatives possibly due to certain hydrophobic and charge transfer interactions with key residue of the macromolecule lanosterol demethylase. This lead us to predict a superior activity of these compounds as compared to unsubstituted azines.

| **Compound** | **Binding pose** | **M.W** |
|---|---|---|
| Benzalazine | -7.2 | 208 |
| p-hydroxybenzalazine | -7.2 | 240 |
| p-tolualdazine | -8.1 | 236 |
| p-chlorobenzalazine | -7.7 | 277 |
| 1-naphthaldazine | -10.2 | 308 |

Table 1

The antimicrobial studies were done on two pathogenic strains of fungus namely C. albicans & C. tropicalis.

The compounds were dissolved in dmso and an Antibiotic Sensitivity Test was performed using the agar diffusion method. The compounds were used at a concentration of 6.25 mg/mL & the zones of inhibition after 1 day of incubation were measured for both microorganisms.

| ***C.albicans*** | |
|---|---|
| Compound | Zone of Inhibition (mm) |
| DMSO | 0 |
| Benzalazine | 0 |
| 4-hydroxy | 0 |
| 4-chloro | 10 |
| 4-methyl | 11 |
| 1-nap | 13 |

Table 2

| ***C.tropicalis*** | |
|---|---|
| Compound | Zone of Inhibition (mm) |
| DMSO | 0 |
| Benzalazine | 0 |
| 4-hydroxy | 0 |
| 4-chloro | 10 |
| 4-methyl | 11 |
| 1-nap | 13 |

Table 3

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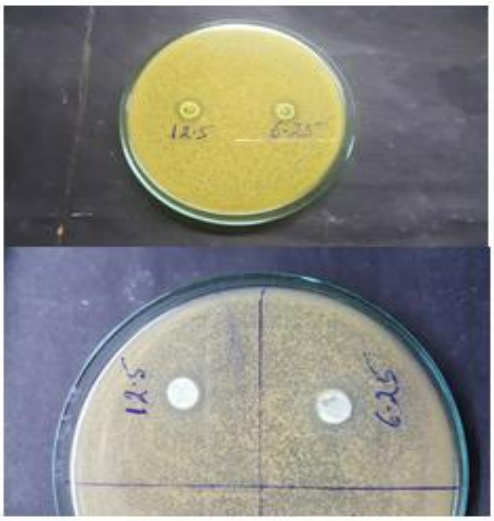


Fig 3

The MIC (Minimum inhibitory concentration) of the compounds were also determined using the broth microdilution method followed by the resazurin test for growth of microorganism at that concentration of the bioactive compound.

| ***C.albicans*** | | | | | |
|---|---|---|---|---|---|
| **Conc. (mg/mL)** | **Benzalazine** | **4-hydroxy** | **4-chloro** | **4-methyl** | **1-nap** |
| 12.5 | - | + | + | - | - |
| 6.25 | + | + | + | - | - |
| 3.125 | + | + | + | + | + |
| 1.5625 | + | + | + | + | + |

Table 4

| ***C.tropicalis*** | | | | | |
|---|---|---|---|---|---|
| **Conc. (mg/mL)** | **Benzalazine** | **4-hydroxy** | **4-chloro** | **4-methyl** | **1-nap** |
| 12.5 | - | + | + | - | - |
| 6.25 | + | + | + | - | - |
| 3.125 | + | + | + | + | + |
| 1.5625 | + | + | + | + | + |

Table 5

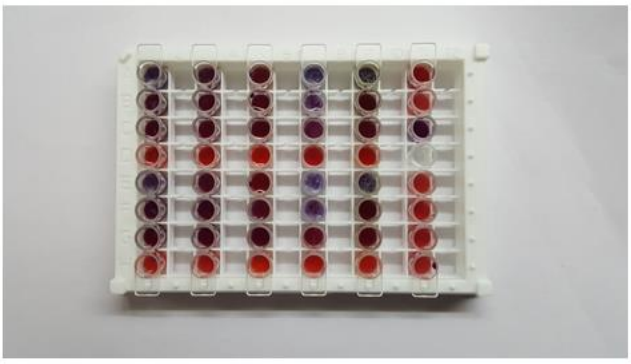

Fig 4

## IV. CONCLUSION

Thus, a rapid & convenient ultrasound assisted route was developed to synthesize aldazines by condensation of substituted aromatic aldehydes with hydrazine in an aqueous medium & absence of any catalyst. The reaction was clean & rapid as indicated by the conversion on the TLC plates. The recorded IR spectrums indicate conversion of the carbonyl compound to its diimine. Hence a green synthetic route has been developed to synthesize azine derivatives. The synthesized compounds were tested for their antifungal activity against pathogenic fungal strains. The results of the antifungal tests were in sync with the docking studies which indicated that hydrophobic groups in general lead to higher biological activity as compared to polar functional groups.

4-tolualdazine & 1-naphthaldazine were found to be most biologically active with the maximum zones of inhibition in the antibiotic sensitivity tests as well as lowest inhibitory concentration.

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## REFERENCES

[1]. Safari, J., Gandomi-Ravandi, S., *RSC Adv*, 4, **2014**, 46224-46249
[2]. Khalid M.K., Waqas J., Nida A., Muhammad T., Shahnaz P., Guillermo A.M., *Ultrason. sonochem.*, 21, 2014, 1200-1205.
[3]. K. Veena, M. Ramaiah, G,J. Vanita, T.S. Avinash, V.P. Vaidya, *E Journ. of Chem. Hindawi*, 2011 **8**(1), 354-360
[4]. Kurteva V., Simeonov S.P., Stoilova-Disheva M., *Pharm & Pharmacy*, **2011,** 2, 1-9.