EXECUTIVE SUMMARY OF UGC MINOR RESEARCH PROJECT TITLED:

"STUDY OF STRUCTURE-ACTIVITY RELATIONSHIP OF ACYCLIC AZINES"

UNIVERSITY GRANTS COMMISSION-MINOR RESEARCH PROJECT

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Azines are the condensation products of aldehydes/ketones having the general molecular formula R_1R_2 C=N-N=CR_1R_2 which may be either symmetrical or unsymmetrical. A large number of these azines have been synthesized and some of them, albeit in a random fashion, have been studied for any potential biological activity, in particular, antimicrobial. This study was therefore designed to investigate any possible relationship which may exist that can effectively correlate the structure of these azines with their biological activity, if any.

The first step therefore was to deeply probe the available literature on these azines. However, no such structured study was found available.

The azines were then taken up for syntheses by condensing a wide variety of carbonyl compounds (both aldehydes and ketones) with hydrazine sulphate and/or hydrazine hydrate and their yields compared with the known literature protocol. Variations were introduced in the synthetic methodology to conform to greener techniques such as conservation of energy resources by attempting to carry out these syntheses by stirring at room temperatures as opposed to the known protocol of heating under reflux, sometimes for extremely long durations, even up to 72 hours. Syntheses of aliphatic azines did not meet with success and the focus was then shifted to aromatic carbonyl compounds, aldehydes in particular. A series of such azines were synthesized having ring activating as well as ring deactivating substituents so as to study the effect of both types of substituents on the biological activity of the molecule. Focus was concentrated particularly on the para substituted azines as the ortho substitution introduces other parameters such as steric, etc which may alter drug receptor binding and therefore, the biological activity of the molecule.

Following is the list of some of the key compounds which have been successfully synthesized during this period:

1. Benzalazine (used as the standard parameter for a comparative study)
2. p-Hydroxybenzalzine (ring-activating substituent)
3. Salicylaldazine (ring-activating substituent)
4. p-(N,N-Dimethylamino) benzalazine (ring-activating substituent)
5. p-Nitrobenzalazine (ring-deactivating substituent)
6. p-Anisaldazine (ring-activating substituent)
7. p-Tolualdazine (ring-activating substituent)
8. p-Chlorobenzalazine (ring-deactivating substituent)
9. p-Carboxybenzalazine (ring-deactivating substituent)
10. Vanillin azine

Almost all the compounds synthesized above have been recrystallized using suitable solvents and have been checked for their purity by TLC using silica gel. The melting points were recorded with +/- 0.1^{O}C accuracy and corroborated with reported literature values wherever available. Spectral analyses of the synthesised compounds have been recorded, such as FTIR, $_1$HNMR, C^{13}NMR, Mass and elemental analyses.

The recrystallized azines were then investigated for their biological activity against *E. coli*, *S. Aureus*, *S. Cerevisiae* and *Candida sp.* and a few of them have shown promising results. The

p-hydroxy derivative in particular, has shown significantly higher activity against S. *cerevisiae* as opposed to the standard parameter, benzalazine.

In general however, it was the methyl substituent which showed a significant degree of activity irrespective of whether it was at the ortho or at the para position.

A notable problem which was witnessed during the course of this study was the extremely poor solubility of these azines which may be a significant contributing factor to their degree of biological activity. Solubility of drugs especially for those which are administered through the oral route is of prime importance as it directly affects their absorption and distribution to the site of action. Keeping this in mind, an attempt was made to prepare the salts of the azines. The hydrogen sulphate salts of benzalzine, p-chlorobenzalazine and salicylaldazine were prepared. All new salts of azines, hitherto unreported have been documented with their melting point reports as well as detailed spectral analyses. Some of the salts which have been established through these elemental analyses studies have been tested for antimicrobial activity against S. *aureus* and S. *cerevisiae.* All compounds tested gave promising results with MIC between 0.2-0.4 mg/ml concentration.

In conclusion therefore, it is the methyl substituent which is the leading pharmacophore in these azines, irrespective of whether ketaines or aldazines. The position of the methyl substituent holds no significance however, as substituents (methyl) at both ortho and para positions showed comparable activity.

The azines as their salts were more potent antifungal and antimicrobial agents as opposed to their counterparts. This was largely due to the increase in bioavailability of the potential drug as the solubility of the salts in an aqueous medium is unquestionably higher and therefore plays a significant role in oral administration.

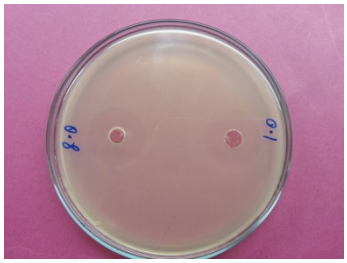

Figure:1

Figure:2

Figure 1: The antimicrobial acitivity of 4-methylbenzalazine at different concentrations of 0.8 mg/ml and 1.0 mg/ml.
Figure 2: The positive control with standard drug erythromycin disc and negative control of DMSO for *E.coli*.