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Design Synthesis and Screening of Mannich Bases of Alliin as Anti - Infective Agents

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Abstract: ***Objective: Alliin is active constituent present in garlic having variouspharmacological activities like antimicrobial, antimalerial, antifungal, anticonvulgent, analgesics and anti - inflammatory type of activity. In alliin form physically it has less stability. A novel attempt has been made in present work to synthesize mannich base derivatives of alliin as stable analogs and screen for anti - infective activity. Method: Aliphatic, aromatic and heterocyclic aldehyde, Ketone, and amines were used for synthesis of mannich bases and were condensed with alliin to form mannich bases of alliin. Synthesized analogs were screened for in vitro bioactivity against gram positive Bacillus subtilis, gram negative Pseudomonas aeruginosa species and determination of zone of inhibition was done. Results: It is observed that all analogs have shown better activity than standard.1000µg/ml concentration solution of analogs was used for antimicrobial activity from results it is found that compound 3a shows maximum activity compared to standard drug. Conclusion: Mannich base derivatives of analogs containing all reactants having aliphatic nature have shown least activity. The analogs with aromatic structural feature have shown moderate to good activity, while analogs containing aromatic and heterocyclic structural features have shown highest anti - microbial activity. All synthesized analogs have shown better activity than standad which is in line with our clam that mannich base combined with alliin should show sinergestic activity.***

Keywords: Anti infective, Mannich bases, Alliin, thiosemicarbazide, anti - microbial

Highlights

- Carry out isolation of alliin from *Allium sativum* L (garlic).
- As alliin is unstable it has to be converted into stable synthetic compound, hence design scheme for synthesis of mannich base derivatives or analogs of alliin as stable compounds.
- Carry out comparative study of anti - infective activity of alliin and its semisynthetic analogs and find out whether the semisynthetic analogs show better activity as expected

1. Introduction

Infectious disease are also known as communicable diseasecaused due to invesion of microorganisms like viruses, bacteria, parasites, prions, protozoa or fungi. According to World Health Organization (WHO) survey millions of deathsoccure every year due to infectious diseases. Some of the diseases are new and caused by resistant strains of microorganisms and hence have no specific treatment available. According to the report some major diseases, such as malaria, cholera and tuberculosis are causing death in the world. As indescrimate use of available drugs is done, their effect has reduced, which has added to the difficulties in treating disease. Hence there is urgent need of new anti - infective agents having diverse mechanism of action with lesser side effects [1]. Hence majority of pharmaceutical companies focused their research on drug discovery through high through put screening to generate and identify new drug candidates. However, the efforts have not resulted in a satisfactory return. Hence most of the researchers have focused on medicinal plant resources as the lead compounds source. Mannich base consisting of aldehyde, Ketone, and amino acids show antimicrobial activity [6 - 7]. The novelty of work consists of condensation reaction between mannich bases and alliin which show better activity than individual [9 - 12].

Computer aided drug design method has became important method for studying biological activities of molecules. The key methodology involves molecular docking study which involves design of drug molecules and studying their interaction with protein binding sites. In present work also the docking of molecules was carried out by using DNA Pol II - normal DNA - dTTP ternary complex (PDB ID 3k58) [22]

Finding out zone of inhibition is required for determination of antimicrobial activity of newly synthesized compounds. Low value of MIC indicates that compound is very active at low concentration. In present work newly synthesized mannich base derivatives of alliin were screening for anti - microbial activity using Bacillus subtilis and Pseudomonas aeruginosa bycup plate method [13 - 21].

2. Materials and method [9 - 12]

Experimental work was carried out by using following steps,

2.1 Extraction of alliin from garlic

The extraction of alliin from allium sativum. Lwas carried out by using Methanol, Chloroform, Water in ratio of 12: 5: 3 as solvent system.

2.2 Synthesis of mannich bases

Accurately weighed quantity equivalent to 1.05 - 1.10 mol. of amine was added to roundbottom flask. Use of concentrated HCL was done to convert it intohydrochloride salt which was confirmed by using congo red paper. To this 1 - 1.5 mol. eq. of aldehyde and 1.00 molecular equivalent of carbonyl compound i. e. ketonewas added. The mixture was refluxon water bath. Optimization of reaction condition and time had to be done on individual basis till the formation of mannich base wascomplete.

2.3 Synthesis of Mannich base derivatives of Alliinby condensation

Mannich bases synthesized in first step were condensed with alliin using alcohol as solvent. Refluxing was carried out on water bath. The time and temperature had to be optimize on individual basis. The synthesized derivatives are shown in table no.1

2.4 Physicochemical and spectral characterization of synthesized mannich base derivatives of alliin.

Under physicochemical characterization determination of color, apperience, melting point determination and determination of Rf value. was done mentioned in table no.2. The determination of Rf value was carried out by using Thin layer chromatography method by using n - butanol: glacial acetic acid: Distilled water (2: 1: 1 V/V/V)

2.5 Docking study

To get the clue regarding antimicrobial activity of compounds, the docking was carriedonCrystal structure of DNA Pol II - normal DNA - dTTP ternary complex (PDB ID 3k58) using V life MDS softwere. Structure of PDB is shown in figure 1

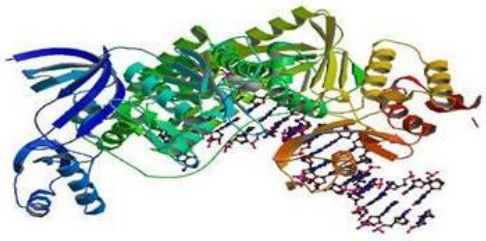


Figure 1: Crystal structure of DNA Pol II - normal DNA - dTTP ternary complex (PDB ID 3k58)

The different types interactions like Hydrogen Bond, Aromatic, Hydrophobic, Charge, Vander wall interactions[23]. As prototype the interactions shown by 3a molecule are shown in figure no.2

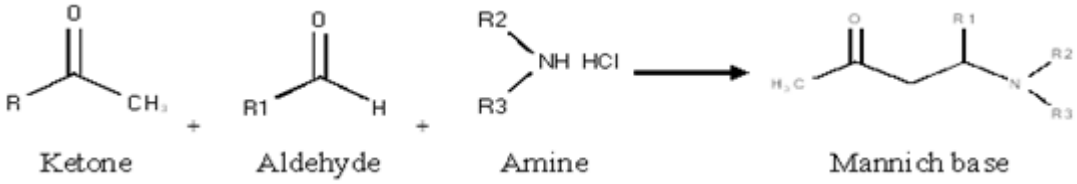
2.6 Screening of mannich base derivatives of alliin for anti microbial [13 - 21]

Determination of MIC (Minnimum Inhibitory Concentration) of synthesized mannich base derivatives of alliin was done usingBacillus subtilisand Pseudomonas aeruginosa. Results of MIC are shown in table no.3 under result section.

Reaction: -

Step I

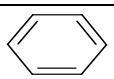
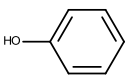
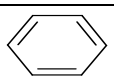
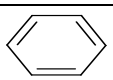
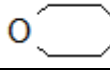
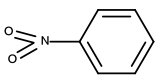
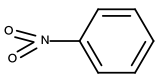
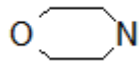
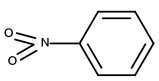
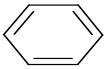
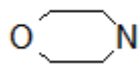
Step II



3. Results

3.1 Results of Mannich bases of alliinsynthesized using various types of aldehyes, ketones, and amines in the form of aliphatic, aromatic and heterocyclic nature are presented in table no.1

Table 1: Synthesized mannich bases of Alliin

Compound	R	R1	R2	R3
1a		H	CH_3	CH_3
2a		H	CH_3	CH_3
3a				
4a		H	CH_3	CH_3
5a		H		
6a				

3.2 Results of Physicochemical characterization

Results of Physicochemical characterization of synthesized mannich base derivatives of alliin are presented in Table 2

Table 2: Physicochemical properties of compounds

Compounds	Appearance (Color)	Melting Point	R_f Value
1a	Yellow	180 - 184	0.35
2a	White	94 - 96	0.47
3a	Brown	143 - 145	0.55
4a	Yellow	130 - 134	0.38
5a	White	182 - 184	0.55
6a	White	70 - 72	0.57
Alliin	White	124 - 128	0.575

3.3 Results of Docking study

Results of Docking interactions of molecule 3a are presented as prototype. It shows strong hydrogen bond interaction with amino acids Arginine (ARG 687A), Lysine (LYS 282A) at distance of 1.840 and 2.269 respectively. The charge interactios by ARG687A at distance of2.719. Vander wall interactions are observed with THR727A, ARG687A, ARG685A, PHE291A, SER290A, SER289A, ASN287A, TRP286A, PHE285A, LYS282A amino acids which show that synthesized molecule to be can bind with target with strong affinity.

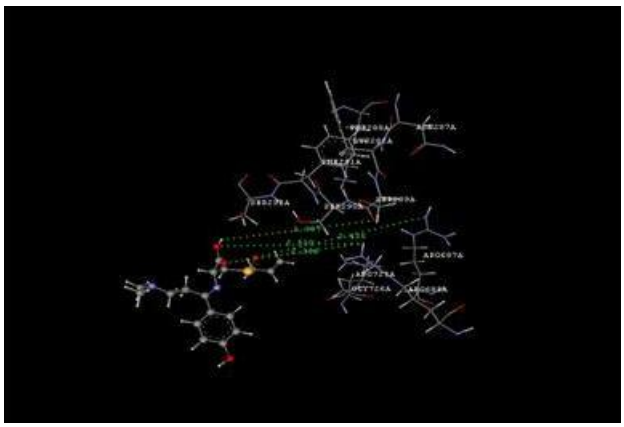


Figure 2: Interactions shown by 3a molecule with receptor

3.4 Estimation of anti - microbial activity

Estimation of anti microbial activity was carried out by using Minimum inhibitory concentration method against Bacillus subtilis and Pseudomonas aeruginosa as gram+ve and gram - ve bacteria respectively. The determination of zone of inhibition was carried our compared with ciprofloxacin marketed formulation.

Table 3: Determination of zone of inhibition of compounds against gram positive Bacillus substilis and gram negative pseudomonas aeroginosa species

Compound code	Zone of inhibition against gram positive species at 1000 (µg/ml) (cm)	Zone of inhibition against gram negative species at 1000 (µg/ml) (cm)
1a	1.43	1.26
2a	1.57	1.39
3a	**2.13**	**2.24**
4a	1.39	1.76
5a	1.47	1.78
6a	1.89	1.72
Std	1.80	1.79

4. Discussion

As noval attempt synthesis of mannich base derivatives of alliin as stable analogs was done. Due to use of different types of aliphatic, aromatic and heterocyclic aldehydes, ketones and amines the time of reaction and conditions have to be optamized. The time and temperature condition varied from 45 minutes to 7 - 8hr, temperature varied from room temperature to heating on water bath at 85 to 100^{o}C. The % yield varied from 45 to 80%.

While sereening for antimicrobial activity, it was found that compounds with all components having aliphatic nature showed very less activity, while compounds with some aromatic feature showed moderate activity. Maximum activity was observed in derivatives with aromatic and heterocyclic featurestogeather.

5. Conclusion

It can be concluded that attempt to synthesize stable mannich base derivative was successful. Docking study helped in indetification of probable mechanism by which synthesized analogs would show pharmacological activity. Screening for antimicrobial activity has shown promicing results. Further toxocilogical study would yield compounds having less side effect with better action.

Declarations

Competing Interests: The authors declare no conflicts of interest.

Ethical Approval: Not required

Acknowledgements
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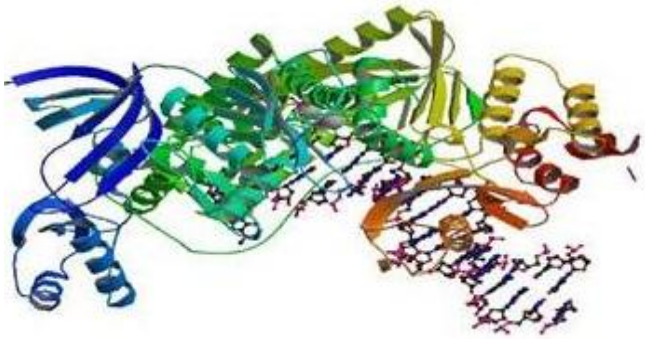

Figure 1: Crystal structure of DNA Pol II - normal DNA - dTTP ternary complex (PDB ID 3k58)

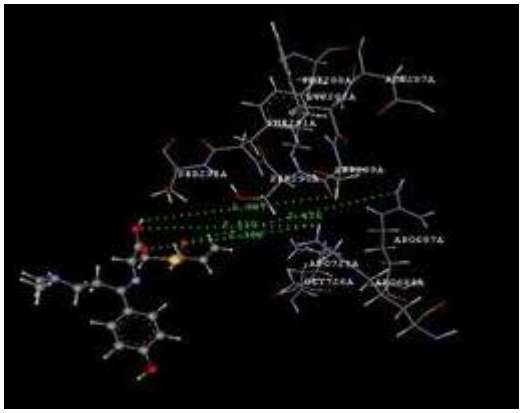

Figure 2: Interactions shown by 3a molecule with receptor

Table 1: Synthesized mannich bases

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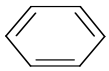
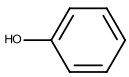
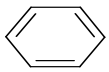
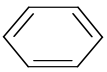
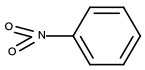
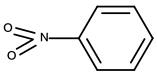
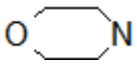
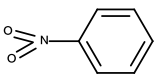
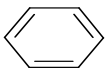
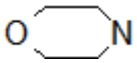
1a		H	CH_3	CH_3
2a		H	CH_3	CH_3
3a				
4a		H	CH_3	CH_3
5a		H		
6a				

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