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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 from garlic extract. It shows various biological activities such as antimicrobial, antimalerial, antifungal, anticonvulgent, analgesics and anti-inflammatory type of activity but it has less stability in individual form. 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.

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 [2,3]. The novelty of work consists of condensation reaction between mannich bases and alliin. Which show better activity than individual [4-7]. 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) [8]. 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 by cup plate method [9-17].

Materials and Method [4-7]

Experimental work was carried out by using following steps,

Extraction of Alliin from Garlic

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

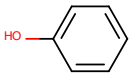
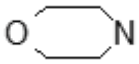
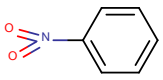
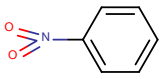
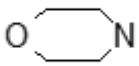
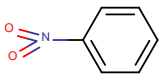
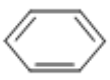
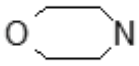
Synthesis of Mannich Bases

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 into hydrochloride 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. ketone was 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 was complete.

Synthesis of Mannich Base Derivatives of Alliin by 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 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				

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 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)

Table 2: Physicochemical properties of compounds.

Compounds	Appearance (Color)	Melting Point	Rf 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

Docking Study

To get the clue regarding antimicrobial activity of compounds, the docking was carriedon Crystal 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. 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 2.

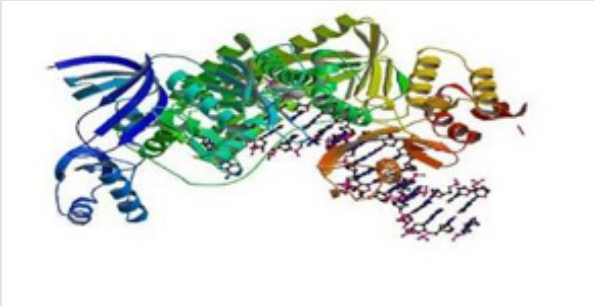


Figure 1: Crystal structure of DNA Pol II-normal DNA- dTTP ternary complex (PDB ID 3k58).

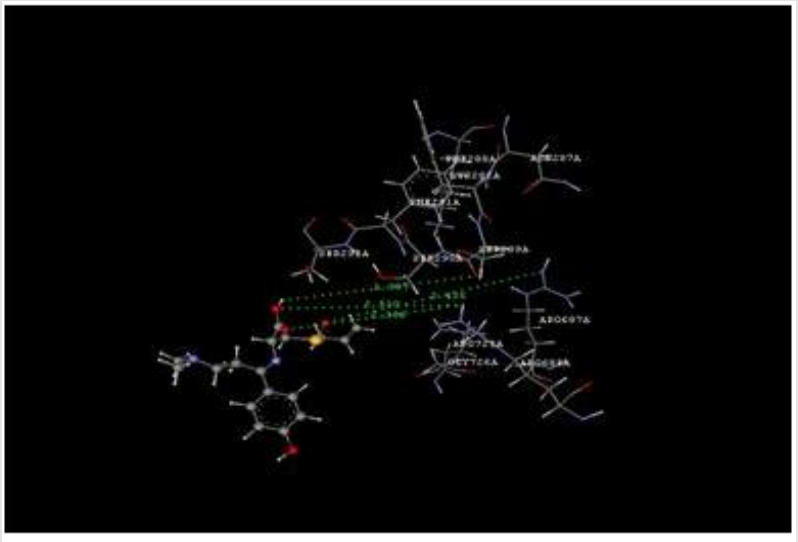


Figure 2: Interactions shown by 3a molecule with receptor.

Screening of Mannich Base Derivatives of Alliin for Anti Microbial [9-17]

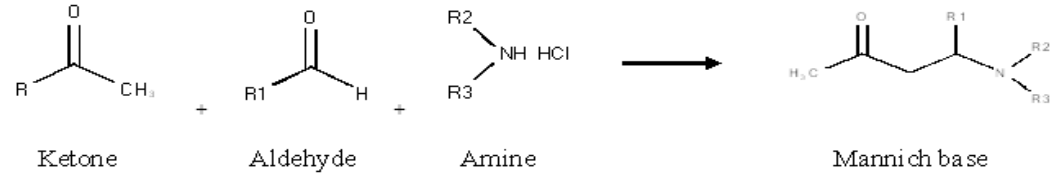
Determination of MIC (Minnimum Inhibitory Concentration) of synthesized mannich base derivatives of alliin was done using Bacillus subtilis and Pseudomonas aeruginosa. Results of MIC are shown in Table 3 under result section.

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

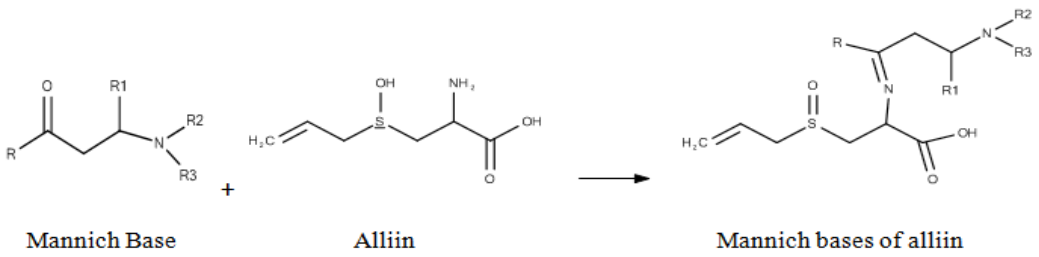
Compound code	Zone of inhibition against gram positive species at 1000 (µg/ml) (cm)	Zone of inhibition against gram negative species at 1000 (µg/ml) (cm)
1a	1.43	1.26
2a	1.57	1.39
3a	2.13	2.24
4a	1.39	1.76
5a	1.47	1.78
6a	1.89	1.72
Std	1.80	1.79

Reaction:

Step I



Step II



Result

Results of Mannich bases of alliin synthesized using various types of aldehyes, ketones, and amines in the form of aliphatic, aromatic and heterocyclic nature are presented in Table 1.

Results of Physicochemical Characterization

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

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 [18-23].

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.

Discussion

As novel 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 7-8 hr, temperature varied from room temperature to heating on water bath at 85 to 100°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 features togeather.

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.

Acknowledgement

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Highlights

1. Carry out isolation of alliin from *Allium sativum L* (garlic).
2. 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.
3. 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.

References

1. Clark FE, Paul EA (1996) Soil Microbiology and Biochemistry. Academic Press Inc San Diego CA, p. 47-49.
2. Pandeya SN, Sriram D, Nath G, De Cleroq E (1999) Eur J Pham 67: 25.
3. Pandeya SN, Lakshmi VS, Pandeya A (2003) Biological Activity of Mannich Bases. Indian J Pharm Sci 65(3): 213-222.
4. March J (1977) In Advanced Organic Chemistry (2nd Edn.)., McGraw Hill International Book Company Oxford University Press Oxford, UK, pp. 820.
5. Waring AJ (1979) In Comprehensive Organic Chemistry. PergamonPress Oxford 1: 1041.
6. Mannic C, Krosche W (1912) The Mannich Reaction revisited. Arch Pharm 250: 647.
7. Thompson BB (1968) The Mannich reaction. Mechanistic and technological considerations. J Pharm Sci 57(5): 715-733.
8. Christfilogiannis P (2001) Current inoculation method in MIC determination. Aquaculture 196(3-4): 297-302.
9. Lengauer T, Rarey M (1996) Computational methods for biomolecular docking. Curr Opin Struct Biol 6(3): 402-406.
10. Jennifer M Andrews (2001) Determination of minimum inhibitory concentrations. Journal of Antimicrobial Chemotherapy 48(1): 5-16.
11. Report of the Working Party on Antibiotic Sensitivity Testing of the British Society of Antimicrobial Chemotherapy.
12. (1997) National Committee for Clinical Laboratory Standards, Specialty Collection: Susceptibility Testing SC21-L. M7-A4. NCCLS, Wayne PA.
13. Winstanley T, Edwards C, Limb D, Megson K, Spencer RJ (1994) Evaluation of a surfactant, Dispersol LN, as an anti-swarming agent in agar dilution susceptibility testing. Journal of Antimicrobial Chemotherapy 33(2): 353-356.
14. Lennette EH, Ballows A, Hausler WJ, Shadomy HJ (1985) Manual of Clinical Microbiology (4th Edn.)., American society for Microbiology, Washington DC, USA.
15. Mac Faddi Jean F (1985) Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Vol I Baltimore MD Williams and Wilkins.
16. (1990) NCCLS, Quality Assurance for Commercially Prepared Microbiological Culture Media. Approved Standard 10(14) NCCLS Document M22-A.
17. Branson E (2001) Clinical relevance of minimum inhibitory concentration Aquaculture 196: 289-296.
18. Popescu A, Doyle RJ (1986) The Gram Stain After More Than a Century. Biotechnic and Histochemistry 71(3): 145-151.
19. Rogers HJ (1986) Cell Walls and Membranes. E and FN SponLtd, pp. 219-221.
20. Bartnicki García S (1968) Cell wall chemistry, morphogenesis, and taxonomy of fungi. Annu Rev Microbiol 22: 87-108.
21. Bhosle D, Bharambe S, Gairola N, Dhaneshwar SS (2006) Mutual prodrug concept: Fundamentals and applications. Indian J PharmSci 68: 286-94.
22. Christfilogiannis P (2001) Current inoculation method in MIC determination. Aquaculture 196(3-4): 297-302.
23. Van de Kamp, Mosettig (1936) The Mannich reaction Chapter No. 10 FF. Blicke J Am Chem Soc 1568: 58.

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