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SYNTHESIS OF MANNISH BASES OF THIOSEMICARBAZIDE AS DNA POLYMERASE INHIBITORS AND NOVEL ANTIBACTERIAL AGENTS

SACHIN A PISHAWIKAR* AND HARINATH N MORE

Department of Pharmaceutical Chemistry Bharti Vidyapeeth College of Pharmacy
Near Chitranagari Kolhapur, Maharashtra – 416 013. (INDIA)

ABSTRACT

Synthesis of new antimicrobial compounds is need of the hour due to prevalence of resistance to antibacterial agents. Different pharmacological activities like, anticancer, antimicrobial, antifungal, anticonvulsant, antimalarial, analgesic and anti-inflammatory are shown by mannich bases and thiosemicarbazide individually. Synthesis of mannich bases was done using aldehyde, ketones and amines having aliphatic, aromatic, cyclic and heterocyclic nature. Synthesized mannich bases were condensed with thiosemicarbazide to form noval mannich bases of thiosemicarbazide as mutual pro-drugs. Spectral analysis was done using IR and H-NMR. Docking analysis performed on the DNA polymerase enzyme (PDB ID 2v4q) showed strong hydrophobic interaction between carbon atom of ketone and amino acids phenylalanine, tyrosine and threonine, charged interactions with aspartine and Vanderwall's interactions with phenylalanine, aspartine, lysine alanine and threonine, In vitro testing was done using BHI(brain heart infusion) broth dilution method against S. aureus (ATCC- 10231) and E. Coli (ATCC-16404).

Analogs with aromatic and substituted aromatic aldehyde showed least activity, analogs with aliphatic aldehyde, ketones and amines showed greater activity in Staphylococcus aureus compared to Escherichia Coli. Analogs having morpholine as amine showed comparable activity in both. Compounds $K_{17,}$ $K_{18,}$ $K_{19,}$ K_{20} have shown comparable highest activity.

KEYWARDS : Thiosemicarbazide, mannich bases, brain heart infusion broth, docking, DNA polymerase

SACHIN A PISHAWIKAR
Department of Pharmaceutical Chemistry Bharti Vidyapeeth College of Pharmacy
Near Chitranagari Kolhapur, Maharashtra – 416 013. (INDIA)

Corresponding author

INTRODUCTION

A mutual pro-drug consists of two pharmacologically active agents coupled together so that each acts as a pro-moiety for the other agent and *vice versa.* Individually mannich bases and thiosemicarbazide show different types of Pharmacological activities like anticancer, antimicrobial, antifungal, anticonvulsant, antimarial, analgesic and anti-inflammatory. Hence coupling of mannich base and thiosemicarbazide should be mutual prodrug and should act as promoiety for each other. In present work successful attempt has been made to link separately synthesized mannich bases and thiosemicarbazide to form mutual prodrug with a hypothesis that the synthesized compound should act synergistically, and show better activities. [1-4]
The Mannich reaction which is named after Chemist Carl Mannich is multi-component condensation of a nonenolizable aldehyde, a primary or secondary amine and an enolizable carbonyl compound that is ketoneleading to formation of final product the β-amino-carbonyl compound also known as a Mannich base.[5,6,7]
Computational methodologies have become a crucial component of many drug discovery programmes, from hit identification to lead optimization One key methodology is docking of small molecules to protein binding sites, pioneered during the early 1980s. The docking process involves the prediction of ligand conformation and orientation (or posing) within a targeted binding site. In general, there are two aims of docking studies: accurate structural modeling and correct prediction of activity. Docking is important in the study of protein ligand interaction properties such as binding energy, geometry complimentarily, electron distribution, hydrogen bond donor acceptor, hydrophobicity and polarizability. [8,9,]
A Minimum inhibitory concentration (MIC) is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against specific organism. It is the lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism after overnight incubation. MIC values can be determined by a number of standard test procedures. The most commonly employed methods are the tube dilution method and agar dilution methods. Serial dilutions are made of the products in bacterial growth media. The test organisms are then added to the dilutions of the products, incubated, and scored for growth. [11-15]
Synthesized analogs were evaluated for antibacterial activity using BHI(brain heart infusion) broth dilution method against Staphylococcus aureus (ATCC- 10231) and Escherichia Coli (ATCC-16404) using Ciprofloxacin as standard drug. [16-18]

EXPERIMENTAL

Chemistry

General procedure for synthesis of compounds:

Step one

The three components used in synthesis of mannich bases were used in proportions of 1.00 molecular equivalent of Carbonyl compound (Ketone), 1.05-1.10 molecular equivalent of Amine and 1.5-2.0 molecular equivalence of aldehyde respectively. Amine was first converted into hydrochloride salt using concentrated hydrochloric acid, checked by Congo red paper. Addition of accurately weighed molecular quantities of Ketone and aldehyde was done. Optimization of reaction conditions in relation to time and temperature had to be done on individually basis.

Step Two

The synthesized mannich bases were condensed with thiosemicarbazide to form mannich bases of thiosemicarbazide.

Scheme of Synthesis:-

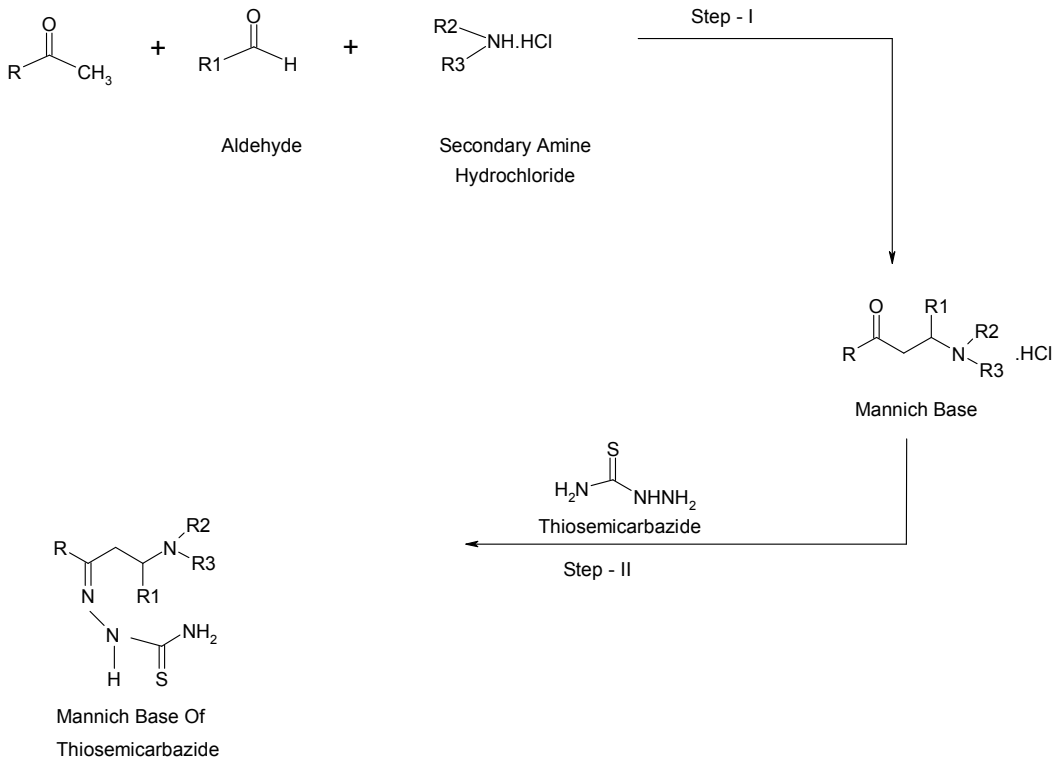
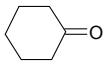
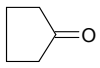
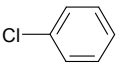
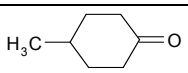
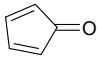
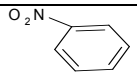
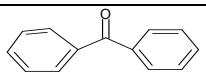
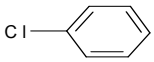
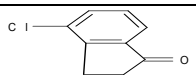
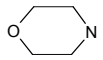
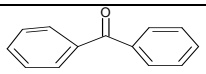
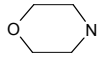
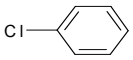
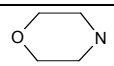
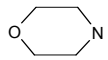
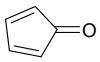
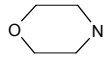
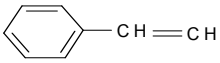
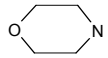


Table 1
List of Synthesized Compounds

Sr. No.	Code	R	R1	R2	R3
1	**K_1**	CH_3	H	C_2H_5	C_2H_5
2	**K_2**	CH_3	H	CH_3	CH_3
3	**K_3**	CH_3	CH_3CH_2	$CH_3CH_2CH_2$	$CH_3CH_2CH_2$
4	**K_4**	CH_3CH_2	$CH_3CH_2CH_2$	CH_2CH_2OH	CH_2CH_2OH
5	**K_5**	$CH_3CH_2CH_2$	$CH_3-CH-CH_3$	$CH_3CH_2CH_2CH_2$	$CH_3CH_2CH_2CH_2$
6	**K_6**		CH_3CH_2	$CH_3CH_2CH_2$	$CH_3CH_2CH_2$
7	**K_7**			C_2H_5	C_2H_5
8	**K_8**	CH_3		$CH_3CH_2CH_2$	$CH_3CH_2CH_2$
9	**K_9**	CH_3		$CH_3CH_2CH_2$	$CH_3CH_2CH_2$
10	**K_{10}**	CH_3		$CH_3CH_2CH_2$	$CH_3CH_2CH_2$
11	**K_{11}**		$CH_3CH_2CH_2$	CH_2CH_2OH	CH_2CH_2OH
12	**K_{12}**		CH_3CH_2	C_2H_5	C_2H_5

13	**K_{13}**		H	C_2H_5	C_2H_5
14	**K_{14}**		H	C_2H_5	C_2H_5
15	**K_{15}**		H	C_2H_5	C_2H_5
16	**K_{16}**	CH_3		C_2H_5	C_2H_5
17	**K_{17}**		H	C_2H_5	C_2H_5
18	**K_{18}**	CH_3	H		
19	**K_{19}**	$CH_3CH_2CH_2$	H		
20	**K_{20}**		H		
21	**K_{21}**		H		
22	**K_{22}**	CH_3			
23	**K_{23}**	CH_3			
24	**K_{24}**		H		
25	**K_{25}**		H		

***Characterization:* -** Structurally important features of synthesized compounds were indentified using IR and ^{1}H-NMR to conform success of synthetic scheme.

In IR as per the structural features representative peaks as C-H aliphatic in between 2940-2950 cm^{-1}, C-H aromatic 1380-1390 cm^{-1}, substituted aromatic ring 900-910 cm^{-1}, C – H stretching between 2915- 2930 cm^{-1}, C-N between 1490-1510 cm^{-1}, C = N stretching between 1590-1600 cm^{-1}, C = S stretching between 1225 -1240 cm^{-1}, N – H stretching between 3240-3250 cm^{-1}, CH_2 – CH_2 between 2930-2940 cm^{-1}, C-O-C between 1100-1125 cm^{-1} were observed leading to conformation of synthesis of specific compound.

NMR Data :- ^{1}H-NMR (DMSO-d_6) δ ppm : 2.5-2.844 (m,6H,CH_2), 3.631-3.705 (3H,CH_3), 5.171 (s,1H,NH),7.1-8.031 (m,4H,morpholino proton), MS (m/z): 222(M-), [$C_{10}H_{14}N_3SO$ - 224]

Following are IR and NMR datas for some of the synthesized compounds,

K_2 : - 4 (1- propane 2 one) propane - N – methylamine thiosemicarbazide

IR data for said compound is C – H stretching at 2934.14 cm^{-1}, N – H stretching at 3256.34 cm^{-1}**,** C = S stretching at 1255. 19 cm^{-1}, C = N stretching at 1587.47 cm^{-1}, CH_2 – CH_2 at 2931.93 cm^{-1}

NMR Data :- ^{1}H-NMR (DMSO-d_6) δ ppm : 1.2-1.4 (m,6H,CH_2), 2.2 (3H,CH_3), 2.683 (6H,-N(CH_3)$_2$), 4.939 (s,1H,NH). MS (m/z): 165 (M+), [$C_8H_{16}N_3S$-162]

K_{14} – 4 (1-phenylethanone) Propane- N-ethylamine thiosemicarbazide

IR data for said compound is C – H stretching at 2924.44 cm^{-1}, N – H stretching at 3239.85 cm^{-1} , C = S stretching at 1239. 47 cm^{-1}, C = N stretching at 1597.67 cm^{-1}, CH_2 – CH_2 at 2938.98 cm^{-1}**.**

NMR Data :- ^{1}H-NMR (DMSO-d_6) δ ppm: 1.2-1.6 (m, 6H, CH_2), 2.8-2.977 (10 H, N (C_2H_5)$_2$),

5.173 (s,1H,NH),7.498-8.725 (4 H, m, aromatic) MS (m/z): 295 (M+), [$C_{15}H_{23}N_4S$-291]

K_{17} - 4 (1- propane 2 one) propane - N – tetra hydro- 1-4 oxazinel thiosemicarbazide : C = N stretching at 1577.87 cm^{-1}, C = S stretching at 1235.17 cm^{-1}, N – H stretching at 3246.94 cm^{-1}, $CH_2 – CH_2$ at 2936.93 cm^{-1}.

NMR Data :- ^{1}H-NMR (DMSO-d_6) δ ppm : 2.5-2.844 (m,6H,CH_2), 3.631-3.705 (3H,CH_3), 5.171 (s,1H,NH),7.1-8.031 (m,4H,morpholino proton), MS (m/z): 222(M-), [$C_{10}H_{14}N_3SO$ - 224]

Docking Study

To conform mechanism of antibacterial activity of synthesized compounds and it interaction potential with decided target, the docking analysis was performed on the DNA polymerase enzyme (PDB ID 2v4q) using Vlife MDS 3.5. Docking studies, showed strong hydrophobic, charged and Vanderwall's interactions. All synthesized molecules were docked into the same binding site.

Docking study showed strong hydrophobic interaction between amino acids Phenyl alanine(PHE93), Tyrosine (TYR1104) and Threonine (THR45) with carbon of ketone at distance 2.998, 4.193 and 4.354 respectively with and with carbon of aldehydic component at distance 4.799.

Van der Walls interactions are observed with PHE11, ALA57, ASP105, LYS159, THR45 and charged interactions with ASP105, which might be playing important role selective binding of compounds with target. Figure no. I, II and III Representing interactions shown by K_{20} with DNA polymerase enzyme (PDB ID 2v4q).

Estimation Of Antibacterial Activity

The MIC(Minimum inhibitory concentrations) of synthesized compounds, for Anti-bacterial activity was carried done using Brain Heart Infusion(BHI) broth dilution method against (ATCC Code 10231), E.coli. (ATCC Code 16404), Using following procedure,

9 dilutions of each drug were done with BHI for MIC.

1. In the initial tube 20 microliter of drug was added into the 380 microliter of BHI broth.
2. For dilutions 200 microliter of BHI broth was added into the next 9 tubes separately.
3. Then from the initial tube 200 microliter was transferred to the first tube containing 200microliter of BHI broth. This was considered as 10^{-1} dilution.
4. From 10^{-1} diluted tube 200microliter was transferred to second tube to make 10^{-2} dilution.
5. The serial dilution was repeated up to 10^{-9} dilution for each drug.
6. From the maintained stock cultures of required organisms, 5microliter was taken and added into 2ml of BHI (brain heart infusion) broth.
7. In each serially diluted tube 200microliter of above culture suspension was added.
8. The tubes were incubated for 24 hours at 37^{o}c and observed for turbidity

Table 2

Anti-bacterial Activity of Synthesized compounds using Ciprofloxacin as standard drug

Sr. No	Product Code	Activity On **S.aureus** MIC (µg/ml)	Activity On **E.coli** MIC (µg/ml)
01	K_1	16.60	500.00
02	K_2	16.60	500.00
03	K_3	16.60	500.00
04	K_4	16.60	500.00
05	K_5	16.60	500.00
06	K_6	31.25	125.00

07	K_7	250.00	125.00
08	K_8	62.50	250.00
09	K_9	62.50	250.00
10	K_{10}	62.50	250.00
11	K_{11}	62.50	62.50
12	K_{12}	62.50	62.50
13	K_{13}	62.50	62.50
14	K_{14}	62.50	62.50
15	K_{15}	250.00	250.00
16	K_{16}	125.00	250.00
17	K_{17}	16.60	31.25
18	K_{18}	16.60	31.25
19	K_{19}	16.60	16.60
20	K_{20}	16.60	16.60
21	K_{21}	16.60	62.50
22	K_{22}	250.00	250.00
23	K_{23}	16.60	16.60
24	K_{24}	62.50	16.60
25	K_{25}	62.50	16.06

Figure 1
Representing interactions shown by K_{20} with DNA polymerase enzyme (PDB ID 2v4q)

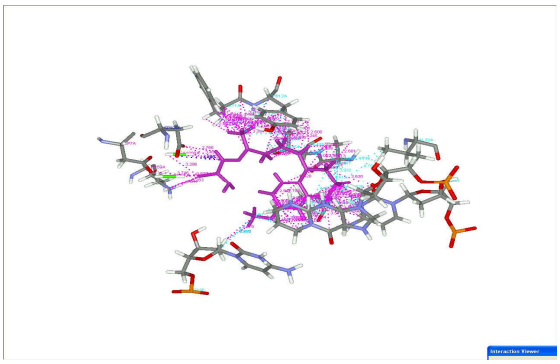


Figure 2
Interactions shown by K_{20} with aminoacid restudies of DNA polymerase enzyme (PDB ID 2v4q):

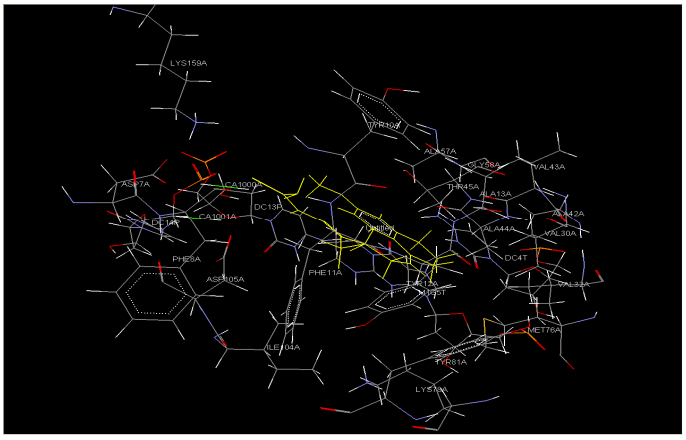
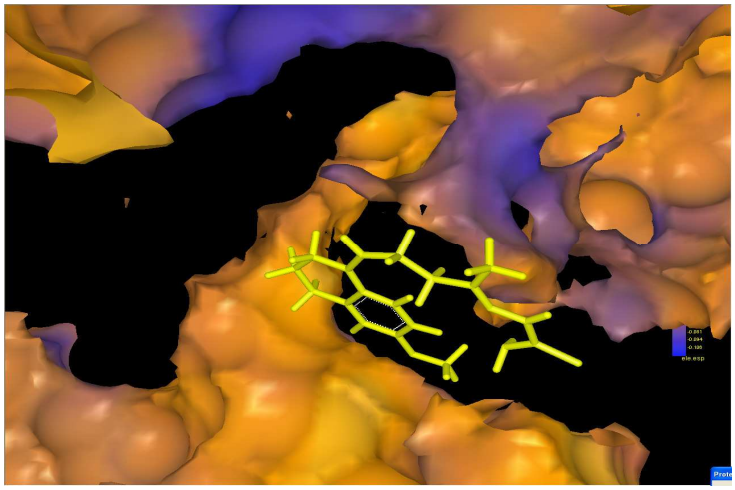


Figure 3
Docking of compound K_{20} on pdb pocket of DNA polymerase enzyme



RESULTS AND DISCUSSION

Chemistry

Due to aliphatic, aromatic, cyclic and heterocyclic nature of three components, optimization of reaction conditions with respect to time and temperature had to be carried out. The time required for completion of synthesis of mannich bases varied from 30 minutes to 12-14 hours. Temperature conditions varied from room temperature with mechanical stirring to temperature between 80-100^{o}C by heating on water bath. Percentage yield of synthesized compounds varied from 24% to 73 %.

Structural Analysis of synthesized compounds:

All compounds were characterized with IR and ^{1}H-NMR. As per the structural features in synthesized compounds, representative peaks like C-H aliphatic in between 2940-2950 cm^{-1}, C-H aromatic 1380-1390 cm^{-1}, substituted aromatic ring 900-910 cm^{-1}, C – H streatching between 2915- 2930 cm^{-1}, C-N between 1490-1510 cm^{-1}, C = N stretching between 1590-1600 cm^{-1}, C = S stretching between 1225 -1240 cm^{-1}, N – H stretching between 3240-3250 cm^{-1}, CH_2 – CH_2 between 2930-2940 cm^{-1}, C-O-C between 1100-1125 cm^{-1} are observed leading to conformation of synthesis of specific compound.

Activity

Gram + ve organism have thick layer of peptidoglycan but lack lipopolysaccharides, while Gram – ve bacteria have thinner peptidoglycan along with additional thick lipopolysaccharide layer in there cell membrane. These features play significant role in the activity shown by synthesized compounds. Compounds with predominant aliphatic structure showed better activity against Gram + ve bacteria, while compounds with aromatic, cyclic and heterocyclic structural features showed better activity against Gram – ve bacteria.

Compounds with morpholine as amine component along with aliphatic or aromatic ketone, showed comparable activity in both. Compounds synthesized using heterocyclic, cyclic, aromatic or substituted aromatic aldehydes showed lesser activity.

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us to carry out desired pharmacological activity. Author is also thankful to Principal, Dr. H. N. More of Bharati Vidyapeeth College of Pharmacy, Kolhapur for providing excellent facilities to carry out synthetic work.

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