Research Article

Synthesis of Mannich Bases of Thiosemicarbazide as Mutual Prodrug and *In-Vitro* Screening for Anti-Infective Activity

Sachin A Pishawikar*, Harinath N More
Department of Pharmaceutical Chemistry, Bharti Vidyapeeth College of Pharmacy, Near Chitranagari Kolhapur, Maharashtra, India.
***Corresponding author's E-mail:** sachin_pishawikar@rediffmail.com

Accepted on: 02-04-2013; **Finalized on:** 31-05-2013.

ABSTRACT

Mannich bases and thiosemicarbazide individually show varied pharmacological activities like anticancer, antimicrobial, antifungal, anticonvulsant, antimalarial, analgesic and anti-inflammatory. By using mutual prodrug concept, first mannich bases were synthesized using aldehyde, ketones and secondary amines with aliphatic, aromatic, cyclic and heterocyclic nature using mannich reaction and then condensed with thiosemicarbazide to form mannich bases of thiosemicarbazide as mutual prodrug. Structural characterization of synthesized compounds was done using IR and H-NMR. Complexicity in the structure of reactants lead to change in reaction time, temperature and % yield of final product. The compounds were screened for anti- microbial activity using *Escherichia Coli (8739) Staphylococcus aureus (25923),* anti-fungal activity using *Aspergillus niger (16404), Candida albicans (10231)* using BHI (brain heart infusion) broth dilution method and anti tubercular activity by micro plate Alamar Blue assay (MABA).

Keywords: Prodrug, antifungal, antimicrobial, brain heart infusion, mannich bases, thiosemicarbazide

INTRODUCTION

Infectious diseases are caused by bacteria, viruses, fungi, protozoa, parasites, or prions affect millions of people worldwide. Several high-profile reports have examined infectious diseases and development of resistance and emphasized measures such as surveillance, infection control, and better stewardship of existing anti-infectives through appropriate use. Systematic programs to discover and develop new antibiotics and antifungals have been driven to a considerable extent by the development of resistance by these organisms to the existing drugs used against them. The advent of HIV has also created a pool of patients who are susceptible to both serious invasive and superficial infections. The new agents may provide additional options for the treatment and they may help to overcome the limitations of current treatments.[1]

The structural diversities present in microorganism's plays a significant role as targets in development of new drugs. The fungal cell wall is composed of a complex network of proteins and polycarbohydrates that varies in composition depending on the fungal species. On the other hand bacterial cell wall differs by the presence of peptidoglycan (poly-*N*-acetylglucosamine and *N*-acetylmuramic acid), which is located immediately outside of the cytoplasmic membrane. Gram positive bacteria have thick layer of peptidoglycan over inner cytoplasmic membrane, but lack lipopolysaccharides(LPS) while in Gram negative bacteria the peptidoglycan layer is thinner and is located between space of the outer and inner cytoplasmic membrane and contains LPS which make them virulent. During screening of synthesized compounds, depending upon structural features in the compounds variation in antiinfective activity is seen. [1-4]

Mutual prodrug is a type of carrier-linked drug consisting of two pharmacologically active agents coupled together so that each acts as a promoiety for the other agent and *vice versa*.[5]

As mannich bases and thiosemicarbazide individually show varied pharmacological activities like anticancer, antimicrobial, antifungal, anticonvulsant, antimarial, analgesic and anti-inflammatory, in present work it was thought to link these to gather to form mutual prodrug.[6-7]

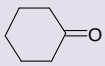
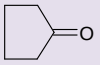
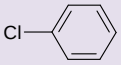
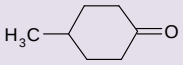
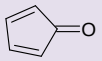
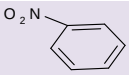
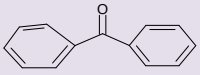
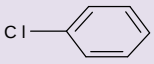
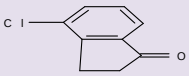
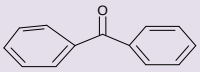
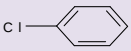
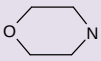
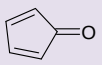
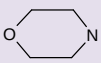
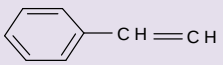
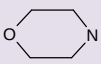
For synthesis of mannich bases use of mannich reaction is done which is amino alkylation of an acidic proton placed next to a carbonyl functional group with formaldehyde and ammonia or any primary or secondary amine. Synthesized mannich bases were condensed with thiosemicarbazide to form mannich bases of thiosemicarbazide.[9-12]

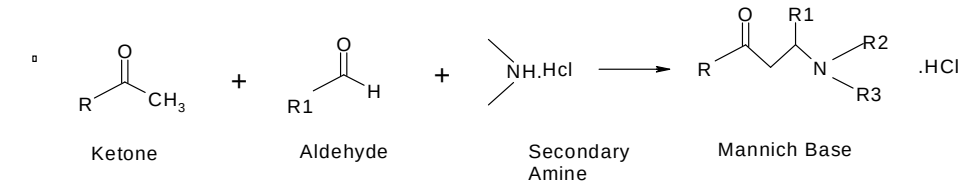
A minimum inhibitory concentration (MIC) is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism. Because a lower MIC value indicates that less of the drug is required in order to inhibit growth of the organism, drugs with lower MIC scores are more effective antimicrobial agents. With intention to estimate MIC the compounds were screened for anti- microbial activity using *Escherichia Coli (8739) Staphylococcus aureus (25923),* for anti-fungal activity using *Aspergillus niger (16404), Candida albicans (10231)* using BHI (brain heart infusion) broth dilution method and anti tubercular activity by micro plate Alamar Blue assay (MABA).[13-21]

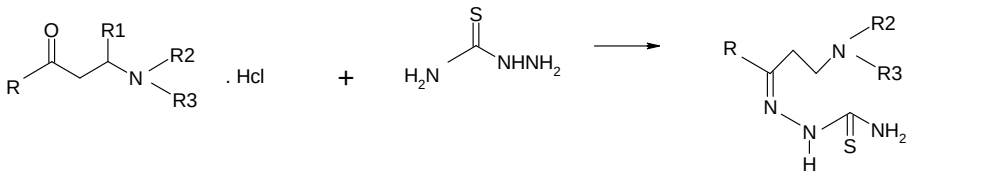
MATERIALS AND METHODS

Synthesis of mannich bases was done using three reactants like aldehyde, ketones and amines having aliphatic, aromatic, cyclic and heterocyclic nature in step–1. Condensation of synthesized mannich bases was done with thiosemicarbazide to form mannich bases of thiosemicarbazide as pro-drugs in step-2. [9-12]

Table 1: List of Synthesized Compounds

S. 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	$CH_3CH_2CH_2$		$CH_3CH_2CH_2CH_2$	$CH_3CH_2CH_2CH_2$
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		





Scheme of Synthesis

Step-I: Synthesis of Mannich base

Procedure: 1.05-1.10 molecular equivalent of amine was taken in flat bottom flask and converted to hydrochloride salt using concentrated hydrochloric acid, formation of salt was confirmed by use of Congo red paper. To this was added 1.00 molecular equivalent of carbonyl compound i.e. ketone and 1.5-2.0 molecular equivalence of aldehyde.

Optimization of reaction conditions with respect to time and temperature had to be done for each individual reaction. Time required was found to vary from 30 minutes to 12-14 hours, with temperature conditions varying from room temperature with mechanical stirring, to heating on water bath at temperature between 80-100^{o}C depending upon complex nature of reactants.

Step-II: Synthesis of Mannich bases of Thiosemicarbazide

It is a simple condensation reaction, where mannich bases synthesized in step-1 were treated with one mole quantity of thiosemicarbazide in presence of alcohol as solvent and refluxing reaction mixture on water bath for around half an hour. Synthesized compounds are shown in table no 1.

Characterization:

Progress and Completion of reaction was identified by determination of R_f value by TLC analysis. Structural characterization was done by using IR and H-NMR. The results for some of the proto type of compounds in with respect to use of aliphatic, aromatic, cyclic and heterocyclic nature of reactant are as follows:

Sample Code

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 streatching 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 streatching 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]

Estimation of anti microbial, antifungal and antitubercular activity [13-21]

Estimation of antimicrobial and antifungal activity was done using BHI (brain heart infusion) broth dilution method using *Escherichia Coli (8739) Staphylococcus aureus (25923),* for anti-microbial activity and *Aspergillus niger (16404), Candida albicans (10231)* for antifungal activity. [13-19]

For estimation of antitubercular activity use of micro plate Alamar Blue assay (MABA) method was done. [20-21]

The results for antimicrobial and antifungal activity are shown in table no. 2 and 3 respectively and for antitubercular activity in table no.4

RESULTS

Depending upon the complexity of structure of three reactants, optimization of reaction conditions with respect to time and temperature had to been done on individual basis. Time required varied from 30 minutes to

12-14 hours. Optimum temperature condition varied from room temperature with mechanical stirring to temperature between 80-100°C. Percentage yield varies from 24% to 73 %.

The structural diversities present in microorganisms plays a significant role as far as antimicrobial, antifungal and anti T.B. activity is concerned. It can be seen from activity results shown in table no.2, 3 and 4.

Table 2: Anti-bacterial Activity of Synthesized compounds using *Ciprofloxacin* as standard drug

S. 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

DISCUSSION

Complexity of aldehyde, ketones and amines plays a significant role in deciding time, temperature and % yield of synthesized products.

The structural diversities present in microorganisms plays a significant role in showing activity. On the basis of results of activities carried out following conclusions are drawn,

(1) Alkyl derivatives of Thiosemicarbazide are found to be more active against *Staphylococcus aureus* than *Escherichia Coli.*

(2) Alkyl derivatives of Thiosemicarbazide are found to be more active against *C. albicans*.

Table 3: Anti-fungal Activity of Synthesized compounds using Fluconazloe as standard drug

S. No	Product Code	Activity on *C. albicans* MIC (µg/ml)	Activity on *Aspergillus niger* MIC (µg/ml)
01	K_1	31.25	31.25
02	K_2	31.25	31.25
03	K_3	31.25	31.25
04	K_4	31.25	31.25
05	K_5	31.25	62.5
06	K_6	31.25	62.5
07	K_7	31.25	62.5
08	K_8	16.6	62.5
09	K_9	62.5	62.5
10	K_{10}	31.25	62.5
11	K_{11}	250	31.25
12	K_{12}	62.5	250
13	K_{13}	16.6	62.5
14	K_{14}	62.5	62.5
15	K_{15}	16.6	62.5
16	K_{16}	250	62.5
17	K_{17}	4.0	31.25
18	K_{18}	4.0	16.6
19	K_{19}	16.6	8.3
20	K_{20}	4.0	16.6
21	K_{21}	31.25	62.5
22	K_{22}	16.6	16.6
23	K_{23}	16.6	16.6
24	K_{24}	16.6	16.6
25	K_{25}	16.6	16.6

Table 4: Anti-tubercular Activity of Synthesized compounds using M. tuberculosis using Pyrazinamide & Streptomycin as standard drugs

S. No	Product Code	Activity on *M. tuberculosis* MIC (µg/ml)
01	K_1	6.250
02	K_2	6.250
03	K_3	6.250
04	K_4	6.250
05	K_5	6.250
06	K_6	3.125
07	K_7	3.125
08	K_8	6.250
09	K_9	6.250
10	K_{10}	6.250
11	K_{11}	3.125
12	K_{12}	3.125
13	K_{13}	6.250
14	K_{14}	3.125
15	K_{15}	6.250
16	K_{16}	6.250
17	K_{17}	3.125
18	K_{18}	3.125
19	K_{19}	3.125
20	K_{20}	1.600
21	K_{21}	1.600
22	K_{22}	6.250
23	K_{23}	3.125
24	K_{24}	3.125
25	K_{25}	3.125

Compared to *Apergillus niger*.

(3) Acyl derivatives are found to be partly active.

(4) Mannich bases of Thiosemicarbazides formed from aliphatic carbonyl compounds has shown good anti microbial and antifungal activity against *Staphylococcus aureus* and *C. albicans*.

(5) Use of Unsubistituted aromatic components in the synthesis of thiosemicarbazide gives active derivatives, but thiosemicarbazides with 3 and 4-substituted aromatic ring gives less active compounds.

(6) Highest activity was shown by mannich bases of thiosemcarbazides which have morpholine as amine with one of the other component having aromatic nature.

(7) Thiosemicarbazide derived from aromatic ketones are active although somewhat less active than the thiosemicarbazone from the corresponding aldehyde.

(8) Thiosemicarbazide derived from heterocyclic aldehydes also show comparable activity to alkyl derivatives.

(9) Highest anti T. B activity is shown by mannich bases of thiosemcarbazides which have morpholine as amine with one of the other component having aromatic nature.

(10) Manniche bases with only aliphatic components show moderate activity.

CONCLUSION

Synthesized compounds inhibit the growth of microorganism by inhibiting cell wall synthesis. Hence depending upon the composition of microbial cell wall it is found that different compounds with different structural features show varied activity as discussed. Structural complexicity plays significant role in time required, temperature conditions and % yield of compounds synthesized.

Acknowledgement: Authors wish to acknowledge Dr. Kishore G. Bhat, HOD. Of Microbiology from Maratha Mandal's Nathajirao G. Halgekar Institute of Dental Science and Research Centre Belgaum for their kind cooperation in allowing 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.

REFERENCES

1. Clark, F.E. and Paul, E.A. Soil Microbiology and Biochemistry Academic Press, Inc., San Diego, CA. 1996, 47-49.
2. Popescu, A., Doyle, R.J. The Gram Stain After More Than a Century Biotechnic & Histochemistry, 71(3), 1986, 145.
3. Rogers, H.J. Cell Walls and Membranes E. & F.N. Spon Ltd., London, England. 1968, 219-221.
4. Bartnicki-García, S. Cell wall chemistry, morphogenesis, and taxonomy of fungi. *Annu. Rev. Microbiol.* 22, 1968, 87-108.
5. Bhosle D, Bharambe S, Gairola N, Dhaneshwar S S. Mutual prodrug concept: Fundamentals and applications, Indian J Pharm Sci, 68, 2006, 286-94.
6. Pandeya S. N., Sriram, D., Nath, G. and De Cleroq, E., Eur. J. Pham., 67, 1999, 25.
7. Pandeya S. N., Lakshmi V. S. and Pandeya A.., Biological Activity Of Mannich Bases, Indan J. Pharm. Sci., 65(3), 2003, 213-222.
8. Van de Kamp and Mosettig, The Mannich reaction Chapter No. 10 F. F. Blicke J. Am. Chem. Soc., 1568, 1936, 58.
9. March, J., In; Advanced Organic Chemistry, 2nd Edn., McGraw Hill International Book Company, Oxford University Press, Oxford, 1977, 820.
10. Waring, A.J., In; Comprehensive Organic Chemistry, Vol. 1. Pergamon Press, Oxford, 1979, 1041.
11. Mannich, C, and Krosche, W., The Mannich Reaction revisited, Arch, Pharm., 250, 1912, 647.
12. Thompson, B.B., The Mannich reaction. Mechanistic and technological considerations J. Pharm Sci., 57, 1968, 715.
13. Jennifer M. Andrews, Determination of minimum inhibitory concentrations, Journal of Antimicrobial Chemotherapy, 48, 2001, 5-16.
14. Report of the Working Party on Antibiotic Sensitivity Testing of the British Society of Antimicrobial Chemotherapy. A guide to sensitivity testing. *Journal of Antimicrobial Chemotherapy,* 27, 1991, 1-50.
15. National Committee for Clinical Laboratory Standards. (1997). *Specialty Collection*: *Susceptibility Testing*. SC21-L. M7-A4. NCCLS, Wayne, PA.
16. Winstanley, T., Edwards, C., Limb, D., Megson, K. and Spencer, R. J. Evaluation of a surfactant, Dispersol LN, as an anti-swarming agent in agar dilution susceptibility testing. *Journal of Antimicrobial Chemotherapy,* 33, 1994, 353-6.
17. Lennette, E.H., Ballows, A., Hausler, W.J.Jr., and Shadomy, H.J. Manual of Clinical Microbiology. 4th ed. 1985 Washington D.C.: American society for Microbiology.
18. Mac Faddin, Jean F. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Vol. I, 1985, Baltimore, MD.: Williams & Wilkins.
19. N.C.C.L.S. Quality Assurance for Commercially Prepared Microbiological Culture Media. Approved Standard, 10(14), 1990, NCCLS Document M22-A.
20. Branson, E. Clinical relevance of minimum inhibitory concentration Aquaculture, 196, 2001, 289-296.
21. Christfilogiannis, P. Current inoculation method in MIC determination. Aquaculture 196, 2001, 297-302.
22. Palomino JC, Portaels F. Simple procedure for drug susceptibility testing of Mycobacterium tuberculosis using a commercial colorimetric assay. Eur J Clin Microbiol Infect Dis; 18, 1999, 380-3.
23. Maria C. S. Lourenco, Marcus V. N deSouza, Alessandra C Pinheiro, Marcelle de L. Ferreira, Rasnisb B, Goncalves, Thais Cristina M Nogneira, Monica A Peralta. Evaluation of anti-Tubercular activity of nicotinic and isoniazid analogues. ARKIVOC- 2007(XV), 181-191.

Source of Support: Nil, **Conflict of Interest:** None.