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Synthesis, Characterization & Biological Evaluation of Novel Mannich Bases of Quinoline Derivatives

Gurdeep Singh[1*], Sandeep Jain[1], P.D. Gokulan[1], PawanTiwari [2], N.K. Jain[2]

[1]Department of Pharmaceutical Chemistry, Shri Ramnath Singh Institute of Pharmaceutical Science & Technology,Sitholi, Gwalior-475001, M.P., India

[2]Department of Pharmaceutical Chemistry, Pranav Institute of Pharmaceutical Science & Research, Sitholi, Gwalior-475001, M.P., India

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ABSTRACT

Series of Mannich bases of Quinoline derivatives have been synthesized and evaluated for their antimicrobial activity. The titled compounds 3a-e were prepared by the condensation of resorcinol and ethyl acetoacetate in the presence of concentrated H_2SO_4 to 7-hydroxy-4- methyl coumarin (1a) which on reaction with thiosemicarbazide gives 7-hydroxy-4-methyl quinolinyl mercaptotriazole (2a). Further condensation of mercaptotriazole, formaldehyde and substituted secondary amines gave mannich bases of Quinoline derivatives (3a-e). The structure of newly synthesized quinoline derivatives have been established on the basis of spectral (IR, ^{1}HNMR, Mass) data. These compounds were screened for antibacterial and antifungal activity against various Gram positive and Gram negative strains. All the compounds show significant antibacterial and antifungal activity.

Key words: Quinoline derivatives, Antibacterial, Antifungal, Mannich bases, Mercaptotriazole.

INTRODUCTION

Most of the therapeutically active compounds contain heterocyclic ring as their basic moiety, which in turn means that the hetero cyclic ring is the active moiety of the particular compound. Quinolines (1-azanapthalene), is an aromatic nitrogen compound characterized by a double - ring structure contains a benzene ring fused to pyridine ring at two adjacent carbon atoms[1]. Literature surveys indicate that quinoline derivatives possess diverse pharmacological activities including antimicrobial (2), antimalarial (3), antiviral (4), antitumor (5), immunomodulatory (6), caspase-3 inhibition (7), antileishmanial (8), local anesthetic (9), antiarrhythmatic (9) and anti-inflammatory activities (10), antitubercular (11), anti-HSV (12), anticonvulsant (13) activities. Also, thiosemicarbazone constitute one of the most versatile classes of compounds possessing a wide spectrum of activities. It has been reported that thiosemicarbazone derivatives possess antimicrobial (1, 14), antimalarial (15), antiamoebic (16) and antitumor (17) activities. They have been in the focus of interest of medicinal chemists in the past decade because of the outstanding biological activities exhibited by several derivatives incorporating the heterocyclic moiety. Similarly, it is well documented that quinoline derivatives possesses various biological activities.

Prompted by literature observations and as a part of our search for bioactive quinoline derivatives, the present work is aimed to design and synthesis of novel series of Mannich bases of 7-hydroxy-4-methyl quinolinyl Mercaptotriazole (3a-e) derived using substituted secondary amines by condensation and their chemical structure were confirmed by IR, ^{1}HNMR & Mass Spectra. All the synthesized compounds were screened for their antibacterial and antifungal activities against various Gram positive and Gram negative strains.

MATERIAL AND METHODS:

Chemistry:

In the present study titled compounds 3a-e were prepared by the condensation of resorcinol and ethyl acetoacetate in the presence of concentrated H_2SO_4 to 7-hydroxy-4- methyl coumarin (1a) which on reaction with thiosemicarbazide gives 7-hydroxy-4-methyl quinolinyl mercaptotriazole (2a). Further condensation of mercaptotriazole, formaldehyde and substituted secondary amines gave mannich bases of Quinoline derivatives (3a-e).

***Corresponding author.**
Gurdeep Singh
[1]Department of Pharmaceutical Chemistry,
Shri Ramnath Singh Institute of Pharmaceutical Science & Technology,
Sitholi,Gwalior-475001, M.P., India
***Tel.:** +91-8602185940,+91-8602185940*
Fax- +91751-235475
***E-mail:**gurdeep06@gmail.com*

Synthesis of 7-hydroxy-4-methyl coumarin (1a):

15ml of conc.H_2SO_4 was stirred mechanically in a wide necked flask with external ice water cooling until the temperature of the acid is about 5°C. Meanwhile, 3.7g of powdered resorcinol was added to 4.5g (4.4 ml) of ethyl acetoacetate, the mixture was stirred until complete solution was obtained. Now, this solution was slowly added to sulphuric acid, so that the temperature of the mixture does not rise above 10°C. Then, the stirring was continued for 30 minutes. The mixture was poured into 100g of crushed ice. The solid 7-hydroxy-4- methyl coumarin separated out was filtered off It was purified by dissolving in cold 10% aqueous NaOH and re-precipitated by the addition of dil. hydrochloric acid. It was recrystallized from methylated spirit. [Yield: 78 %, m.p. 126 °C, IR (KBr cm^{-1}) 1590 (ArC=C), 1726 (C=0), 1104 (C-O), 2987 (CH)]

Synthesis of 7-hydroxy-4-methyl-quinoIinyl [l,5-c] mercaptotriazole (2a):

A mixture of 7-hydroxy-4-methyl coumarin 35.2 g (0.2mol) and thiosemicarbazide 18.2 g (0.2mol) in anhydrous pyridine (50ml) was heated under reflux for 2 hrs. Subsequently, the reaction mixture was poured into crushed ice containing concentrated hydrochloric acid (10ml). A dark brown solid separated out and was allowed to settle down for 1hr. It was filtered off, washed, dried in vacuum and re-crystallized from methanol as a brownish yellow crystalline mass. [Yield: 78 %, m.p. 133 °C, IR (KBr cm^{-1}) 3407 (OH), 1587 (ArC=C), 2278 (C=N), 1212 (C-N), 2289 (S-H), 2929 (CH), 1146 (C-S)]

Synthesis of mannich bases of 7-hydroxy-4-methyl quinolinyl mercaptotriazole (3a-e):

A mixture of (2a), a secondary amine (0.05mol) and formaldehyde (1.25mol) in ethanol (95.5%, 50ml)) were heated under reflux for 10 hrs. Subsequently, the solvents were removed by distillation, and the solid mass obtained was washed with water. It was dried in vacuum and re-crystallized from methanol. Similarly other members' 3a-e was prepared and their physical and analytical data were recorded. All the synthesized compounds were screened for their antibacterial and antifungal activities against various Gram positive and Gram negative strains.

SPECTRAL STUDIES:

All melting points were recorded in Digital melting point apparatus and are uncorrected. The IR spectra were recorded on Perkin Elmer FTIR spectrometer using KBr pellets. ^{1}HNMR spectra were recorded on Bruker Avance II 400 MHz NMR (d in ppm) relative to TMS as internal standard. The purity of compounds were checked by TLC using precoated silica gel G plate method using ethyl acetate: glacial acetic acid: water and the spots were examined by I_2 Vapor or under UV lamp and R_f value has been reported in Table-I

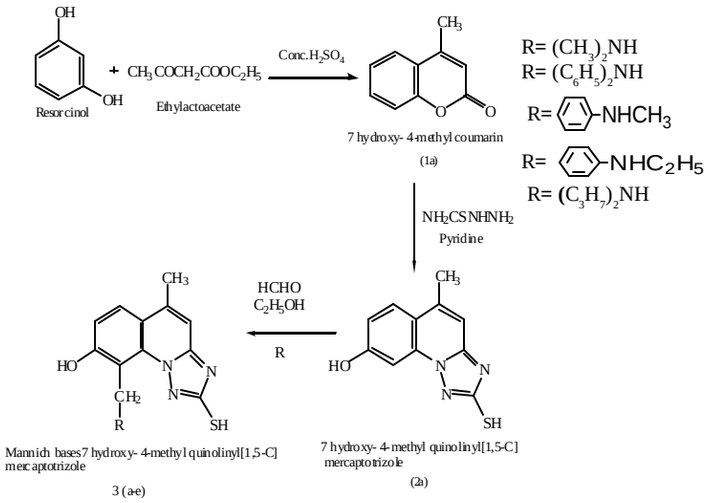
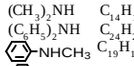
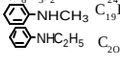


Table- I. Characterization data of compounds 3a-e

Compound Code	R	Mol. Formula	Mol. Weight	M.P.(°C)	%Yield	RfValue
3a	$(CH_3)_2NH$	$C_{14}H_{16}N_4O_5$	288.37	168	68.02	0.65
3b	$(C_6H_5)_2NH$	$C_{24}H_{20}N_4O_5$	412.51	172	72.47	0.71
3c		$C_{19}H_{18}N_4O_5$	350.44	188	69.81	0.77
3d		$C_{20}H_{20}N_4O_5$	364.46	152	57.83	0.72
3e	$(C_3H_7)_2NH$	$C_{18}H_{24}N_4O_5$	344.48	170	72.83	0.81

7- Hydroxy-4-methyl-8[N-methyldimethyl] quinolinyl [1,5-C] 2" mercaptotriazole (3a):
Yellow crystal; IR (KBr, cm^{-1}) 3343 (OH), 1590 (ArC=C), 2232 (C=N), 1246 (C-N), 2300 (S-H), 2929 (CH), 1178 (C-S); ^{1}HNMR ($CDCl_3$) d 2.3 (s, 9H, $-CH_3$), 3.0 (s, 1H, SH), 3.6 (s, 2H, CH_2), 5.0 (s, 1H, OH), 6.9-7.4 (m, 3H, ArH); Mass m/z 288M^+, 206 B^+

7-Hydroxy-4-methyl-8[N- methyldiphenyl] quinolinyl [1,5-C]2" mercaptotriazole (3b):
Pale yellow crystal; IR (KBr, cm^{-1}) 3245 (OH), 1510 (ArC=C), 2205 (C=N), 1259 (C-N), 2246 (S-H), 2985 (CH), 1178 (C-S); ^{1}HNMR ($CDCl_3$) d 2.3 (s, 3H, $-CH_3$), 3.0 (s, 1H, SH), 4.3 (s, 2H, CH_2), 5.1 (s, 1H, OH), 6.4-7.7 (m, 15H, ArH); Mass m/z 412M^+, 232 B^+

7-Hydroxy-4-methyl-8[N- methyl N- phenyl aniline] quinolinyl [1,5-C]2" mercaptotriazole (3c):
Yellowish white crystal; IR (KBr, cm^{-1}) 3384 (OH), 1598 (ArC=C), 2135 (C=N), 1259 (C-N), 2408 (S-H), 2916 (CH), 1155 (C-S); ^{1}HNMR ($CDCl_3$) d 2.2-2.8 (s, 6H, $-CH_3$), 3.2 (s, 1H, SH), 4.7 (s, 2H, CH_2), 5.3 (s, 1H, OH), 6.6-7.8 (m, 9H, ArH); Mass m/z 348M^+, 212 B^+

7-hydroxy-4-methyl-8[N- methyl N-ethyl aniline] quinolinyl [1,5-c]2" mercaptotriazole (3d):
Creamish white crystal; IR (KBr, cm^{-1}) 3346 (OH), 1614 (ArC=C), 2087 (C=N), 1188(C-N), 2207(S-H), 2975 (CH), 1151(C-S); ^{1}HNMR ($CDCl_3$) d 2.1-2.7 (s, 6H, $-CH_3$), 2.9(s, 2H, CH_2), 3.1 (s, 1H, SH), 4.6 (s, 2H, CH_2), 5.2 (s, 1H, OH), 6.6-7.8 (m, 8H, ArH); Mass m/z 362M^+, 229 B^+

7-hydroxy-4-methyl-8[N- methyl N-dipropyl] quinolinyl [1,5-c]2" mercaptotriazole (3e):
Yellowish Bright Crystal; IR (KBr, cm^{-1}) 3424 (OH), 1588 (ArC=C), 2143 (C=N), 12018(C-N), 2259(S-H), 2964 (CH), 1164(C-S); ^{1}HNMR ($CDCl_3$) d 1.0 (s, 6H, $-CH_3$), 1.4(s, 4H, CH_2), 2.3 (s, 4H, CH_2), 2.6 (s, 3H, CH_3), 3.2 (s, 1H, SH), 5.2 (s, 1H, OH), 6.9-7.6 (m, 5H, ArH); Mass m/z 344M^+, 226 B^+

ANTIMICROBIAL ACTIVITY:

Antibacterial activity:
A novel prepared series of mannich bases of quinoline derivatives were screened for their antibacterial activity in vitro in comparison with ampicillin as a reference drug using the standard agar disc diffusion method against four bacterial species: *Staphylococcus aureus* (AUMC B71), *Bacillus Cereus* (AUMC B70) represented by *Escherichia coli* (AUMC B69) and *Pseudomonas aeruginosa* (AUMC B72).

Nutrient agar media of the requisite composition viz., peptone (2.5g), beef extract (0.5g), agar-agar (10g) and distilled water (500 mL) was prepared and pH of the medium was adjusted to 6.6. For the preparation of media all the above ingredients (except agar-agar) were weighed and dissolved in distilled water (250 mL) by application of gentle heating. After dissolving the ingredients completely, more distilled water and weighed agar-agar added. Then, it was filtered through cotton to obtain a clear solution. The mixture was autoclaved for 30 min at a pressure of 1.5 kg/cm^2. All the glass wares were cleaned with chromic acid and then sterilized by keeping in oven. Medium was cooled to 37.1 °C and homogenous suspension was prepared by transferring aseptically a loopful of all the corresponding microorganism from fresh subculture into agar medium followed by vigorous shaking.20 mL of this medium was poured into ach sterilized Petri-dish under aseptic conditions and allowed to set.Sterile 5-mm filter paper disc was saturated with 10 µL of the solution of test compound and Ampicillin as a reference drug. In addition an other disc was impregnated with the solvent DMSO and served as a negative control. The discs were then dried for 1h and placed in the each plate. The seeded plates were incubated at 35±2 °C for 24-48 h. The radii of inhibition zones (in mm) of triplicate sets were measured and results are given in Table-II.

Table- II. *In-vitro* Anti-bacterial and Anti-fungal activity of newly synthesized compounds 3a-e

Inhibition Zones (in mm)							
		Gram-Positive		Gram-Negative		Pathogens	
S. No.	R	*S. aureus*	*B. Cereus*	*E.Coli*	*P. aeruginosa*	*C. albicans*	*A. niger*
3a	$(CH_3)_2NH$	14	17	-	17	-	15
3b	$(C_6H_5)_2$ NH	21	20	21	19	-	13
3c		-	14	19	-	14	-
3d		10	13	26	28	17	16
3e	$(C_3H_7)_2NH$	12	16	20	15	27	22
	Ampicillin	24	26	29	33	-	-
	Fluconazole	-	-	-	-	30	24

— indicates no activity

as representative of Gram positive strains while Gram negative strains were

Antifungal activity:
The synthesized compounds 3a-e were tested for their antifungal activity in vitro in comparison with Fluconazole as a reference drug using the standard agar disc diffusion method against two pathogens namely *Candida albicans* and *Aspergillus niger*.

Spore suspension in sterile distilled water was prepared from a 2-5 days old culture of the test fungi grown on Sabourarud agar media. The final spore concentration was nearly 5×10^4 spore mL^{-1}. About 15 mL of growth medium was added to sterilized Petri dishes of 9 cm diameter and inoculated with 1 mL of spore suspension. Plates were shaken gently to homogenize the inocula. Sterile 5-mm filter paper disc was saturated with 10 µL of test compound solution and fluconazole (40 µ mol mL^{-1} in DMSO). In addition, another disc was impregnated with DMSO and served as a negative control. The discs were dried for 1 h and placed in the centre of each plate. The seeded plates were incubated at 28±2 °C for 7 days. The radii of inhibition zones (in mm) of triplicate sets were measured at successive intervals during the incubation period and the results are given in Table-II.

RESULT AND CONLCUSION:
A number of mannich bases of 7-hydroxy-4-methyl quinolinyl mercaptotriazole (3a-e) derivatives were prepared and tested for their antibacterial and antifungal activity. The synthesis is based on the condensation of resorcinol and ethyl acetoacetate in the presence of concentrated H_2SO_4 to give 7-hydroxy-4-methyl coumarin (1a) which on reaction with thiosemicarbazide gives 7-hydroxy-4-methyl quinolinyl mercaptotriazole (2a). Further the mercaptotriazole undergoes mannich reaction in the presence of formaldehyde and substituted secondary amines to give mannich bases of Quinoline derivatives (3a-e). All the above reactions are observed summarized in scheme. All synthesized compounds 3a-e were recrystallized with methanol and identified by TLC using ethyl acetate: glacial acetic acid: water. Spots were visualized through the iodine chamber or under UV lamp and the R_f value was calculated and were found to be in the range of 0.65-0.81 cm.

The structures of various compounds were assigned on the basis of their R_f values, melting points, IR, ^{1}HNMR spectral data. The compounds were evaluated for antibacterial activity using the standard agar disc diffusion method. They showed moderate activity against most of the tested bacterial strains. Among them compound 3b and 3d showed maximum activity than the other compounds

against both Gram positive and Gram negative bacteria compared with standard drug Ampicillin. The compounds also evaluated for antifungal activity standard agar disc diffusion method against two pathogens namely *Candida albicans* and *Aspergillus niger*. The compound 3e shows highly significant activity compared with standard drug fluconazole.

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