

Novel synthesis, characterization and antimicrobial activity of some nitrogen containing heterocyclic moiety

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ABSTRACT

A series of 3-[(2,4-Dinitro phenyl)-(substituted amino)-1-ylmethyl-amino]-2-substituted phenyl-5-(3,4,5-trimethoxy benzylidene)-3,5-dihydro-imidazol-4-one derivatives were synthesized by the condensation of 2-substituted phenyl-4- (3,4,5-trimethoxy benzylidene)- 4H-oxazol-5-one and 2,4-Dinitro phenyl hydrazine in Pyridine. Which was further carried out by Mannich reaction using different secondary amines to afford title compounds. The synthesized compounds have been characterized on the basis of elemental analysis and spectral studies like IR, ¹H- NMR, etc. Further they were assayed for their Antimicrobial activity against *E.coli*, *B.subtilis* bacterial species and *A.niger* fungal microorganism.

Key words: Oxazolone, Imidazolone, IR, ¹H- NMR, C, H, and N analysis, Antimicrobial activity.

INTRODUCTION

The chemistry of oxazolone has received important attraction due to their uses as intermediate for the synthesis of some heterocyclic synthesis¹. Imidazolidinones have been reported to possess potent CNS depressant activity^{2,3}. Anticonvulsant activity has also been shown with some imidazole derivatives^{4,5}. 1,2,4-trisubstituted-5-imidazolones have been reported to possess monoamine oxidase (MAO) inhibitory and anticonvulsant activity⁶.

The increasing popularity of Mannich reaction has been fueled by the ubiquitous nature of nitrogen in drugs, natural products and biologically active compounds as well as by its multicomponent reaction to generate diversity⁷. Nitrogen containing N-Mannich bases which have been evaluated for their pharmacological action^{8,9}.

A Chemical substance produced by chemical synthesis, which inhibited the growth of organisms and hence act as antimicrobial agents. These chemical substances interfere the life cycle of organisms with specific processes that are essential for growth and/or division of the cell and many of them are widely used for chemotherapy. Most of the pathogenic bacteria are highly sensitive and susceptible to a new antibiotic or chemical, and thus microorganisms acquire chemical substance/ drug resistance. In view of the advantage offered by applications of Mannich bases in the field of medicine and biology¹⁰, we made an attempt to synthesized novel heterocyclic N-Mannich bases of imidazol-5-one and evaluation of their antimicrobial activity against bacterial and fungal microorganisms. Hence, it was thought interesting to undertake the synthesis, characterization and bactericidal activity of trisubstituted imidazol-5-one moiety. The whole work is represented in Scheme 1.

EXPERIMENTAL

Materials

All the chemicals used were of analytical grade and were further purified by recrystallization and redistilled before used. The solvent used were of laboratory grade.

Synthesis of 2-substituted phenyl-4-(3,4,5-trimethoxy benzylidene)-4H-oxazol-5-one

This was prepared by the well known Erlenmeyer- Plochl Azalactone synthesis method [11]. It is of bright yellow colour compound having M.P. 135°C and yield is 65 %. The structure of Oxazolone compound (III) is shown in Scheme-I. It was confirmed by an elemental analysis and spectral studies. Molecular formula, $C_{19}H_{17}NO_5$: IR (KBr): 1680 cm^{-1} (-C= O), 1610 cm^{-1} (-C= C- Phenyl ring vibration), 1620 cm^{-1} (-C = N-), 1120 cm^{-1} (-C- O-C-). 1H -NMR ($CDCl_3$): 6.5- 8.0 δ (m, 8H; Ar-H and -CH= C- merged), 3.7 δ (S, 3H; Ar- OCH_3).

Synthesis of 3-(2,4-Dinitro phenylamino)-2-substituted phenyl)-5-(3,4,5- trimethoxy benzylidene)-3, 5-dihydro-imidazol-4-one

A mixture of Oxazolone (3.39 gm, 0.01mole) and 2,4-Dinitro phenyl hydrazine (2.97 gm, 0.015 mole) in dry pyridine was heated under reflux for 10 hrs under anhydrous condition. The excess pyridine is distilled off and then reaction mass is cooled and subsequently the reaction mixture was poured into ice-cold water containing conc. HCl. A solid started to separate out was allowed to settle down for sometimes. It was filtered off and wash successively with water, dried and recrystallized from ethanol to give (IV). M.P. is 232°C and Yield is 79 %.

The compound (IV) is shown in scheme-I. It was confirmed by an elemental analysis and spectral studies. Molecular formula, $C_{25}H_{21}N_5O_8$: IR (KBr): 1708 cm^{-1} (-C = O), 1096 cm^{-1} (-C = C-Phenyl ring vibration), 1619 cm^{-1} (-C = N-), 1586(S) cm^{-1} (-CH =C-), 1300(W) cm^{-1} (-C-N- Stretching), 3300-3000 cm^{-1} (-N-H-). 1H -NMR ($CDCl_3$): 6.7- 8.2 δ (m, 13H; Ar-H and -CH= C- merged); 8.85 δ (S, 1H; Ar-NH- N-), 3.81 δ (S, 3H; Ar- OCH_3).

Synthesis of 3-[(2,4-Dinitro phenyl)-(substituted amino)-1-ylmethyl-amino]-2- substituted phenyl-

5-(3,4,5- trimethoxy benzylidene)-3,5-dihydro-imidazol-4-one

A mixture of compound (IV) (0.01 mole) and formaldehyde (0.02 mole) was refluxed in methanol (25 ml) for 1hr. A secondary amine (0.01mole) was added and reaction mixture further refluxed for 3 hrs. Methanol was distilled off and the product was recrystallized from suitable solvent to give (V).

Similarly other compounds of this series were obtained by above method. 14 compounds have been prepared which are listed in Table 1.

The Synthetic protocol for the synthesis of N- Mannich bases in general is furnished in the Scheme 1.

Measurments

C, H, and N content of the entire sample were estimated by Perkin-Elmer 2400 Series II, C, H, N, and S Elemental Analyzer, Italy. The IR spectra of the entire sample were scanned in KBr pellets on a NICOLET- 400 D FTIR spectrophotometer. The 1H -NMR spectral studies were carried out on 400-MHz FT-NMR instrument in $CDCl_3$ as a solvent. Melting points were uncorrected and determined in open capillary. Purity of the compounds was checked by TLC on silica gel and was purified using column chromatography.

Spectral data of N- Mannich bases

IR (ν , cm^{-1})

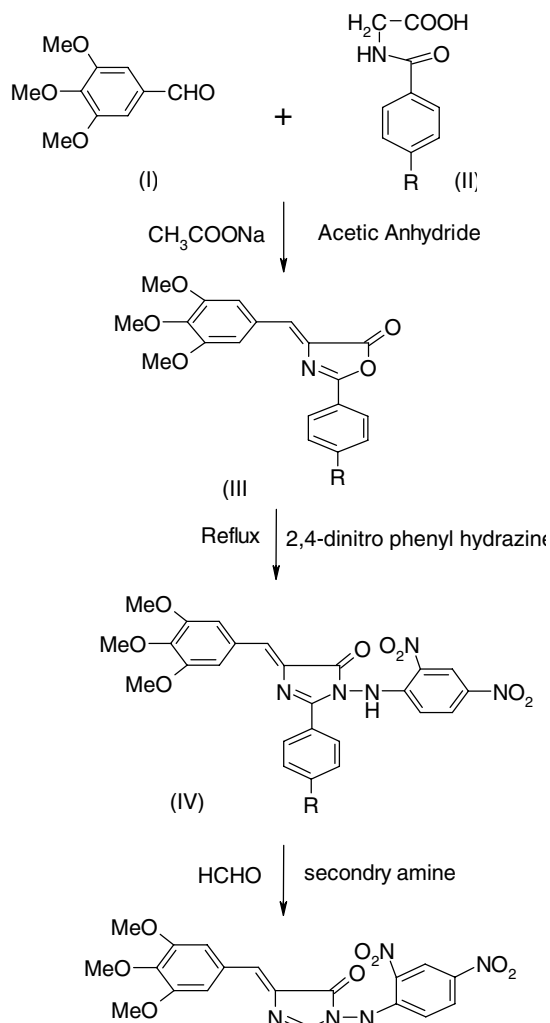
1700- 1770 (-C = O stretching of Imidazolone), 1620- 1680 (-C = N- stretching), 1600- 1650 (-C = C stretching), 1200- 1240 (-C-O- stretching of Ar- O-), 700- 750 (-C- Cl stretching), 2830- 2940(-CH stretching of methylene bridge), 1440- 1470 & 1350- 1380 (-CH stretching of alkane), 3017 (-CH stretching of Benzene ring).

1H NMR (δ , ppm):

(R= H)

MDPI

6.9- 8.7 δ (11H, m, Ar- H and -CH=C- merged), 4.25 δ (2H, s, CH_2 of methylene bridge), 2.37 δ (2H, t, CH_2 adjacent to N of piperidine ring), 1.50 δ (2H, m, CH_2 of piperidine ring), 3.96 δ (3H, s, Ar- OCH_3).



Where R=C,Cl

R'= Pyrrolidine	→	MPPY
Piperidine	→	MPPI
Morpholine	→	MPMO
N-methyl piperazine	→	MPNMP
N-ethyl aniline	→	MPENA
Indol	→	MPIN
Ethyleneimine	→	MPEI

Scheme 1: Synthetic protocol of N-Mannich bases

MDMO

6.9- 8.75 δ (11H, m, Ar- H and $-\text{CH}=\text{C}$ -merged), 4.31 δ (2H, s, CH_2 of methylene bridge), 2.48 δ (2H, t, CH_2 adjacent to N of morpholine ring), 3.57 δ (2H, t, CH_2 adjacent to O of morpholine ring), 3.96 δ (3H, s, Ar-OCH_3).

MDNMP

7.0- 8.75 δ (11H, m, Ar- H and $-\text{CH}=\text{C}$ -merged), 4.33 δ (2H, s, CH_2 of methylene bridge), 2.37 δ (2H, s, CH_2 of piperazine ring), 2.24 δ (3H, s, CH_3 of $-\text{N}-\text{CH}_3$ of piperazine ring), 3.96 δ (3H, s, Ar-OCH_3).

MDPY

6.85- 8.7 δ (11H, m, Ar- H and $-\text{CH}=\text{C}$ -merged), 4.29 δ (2H, s, CH_2 of methylene bridge), 4.38 δ (2H, t, CH_2 adjacent to N of pyrrolidine ring), 1.54 δ (2H, t , CH_2 of pyrrolidine ring), 3.96 δ (3H, s, Ar-OCH_3).

MDNEA

6.8- 8.75 δ (17H, m, Ar- H and $-\text{CH}=\text{C}$ -merged), 4.90 δ (2H, s, CH_2 of methylene bridge), 3.50 δ (2H, q , CH_2 of $-\text{N}---\text{CH}_2\text{CH}_3$ of N- ethyl aniline), 1.11 δ 3H, t, CH_3 of $-\text{N}---\text{CH}_2\text{CH}_3$ of N- ethyl aniline), 3.96 δ (3H, s, Ar-OCH_3).

MDEI

6.85- 8.70 δ (11H, m, Ar- H and $-\text{CH}=\text{C}$ -merged), 4.41 δ (2H, s, CH_2 of methylene bridge), 1.97 δ (2H, t, CH_2 of ethyleneimine ring), 3.96 δ (3H, s, Ar-OCH_3).

MDIN

6.75- 8.7 δ (17H, m, Ar- H and $-\text{CH}=\text{C}$ -merged), 5.08 δ (2H, s, CH_2 of methylene bridge), 3.96 δ (3H, s, Ar-OCH_3).

(R= Cl)

MDPI

6.9- 8.78 δ (10 m, Ar- H and $-\text{CH}=\text{C}$ -merged), 4.20 δ (2H, s, CH_2 of methylene bridge), 1.27 δ (2H, t, CH_2 adjacent to N of piperidine ring), 1.42 δ (2H, m, CH_2 of piperidine ring), 3.93 δ (3H, s, Ar-OCH_3).

MDMO

6.9-8.8 δ (10H, m, Ar- H and $-\text{CH}=\text{C}$ -merged), 4.27 δ (2H, s, CH_2 of methylene bridge),

2.39 δ (2H, t, CH₂ adjacent to N of morpholine ring), 3.47 δ (2H, t, CH₂ adjacent to O of morpholine ring), 3.93 δ (3H, s, Ar- OCH₃).

MDNMP

7.0- 8.8 δ (10H, m, Ar- H and -CH=C-merged), 4.29 δ (2H, s, CH₂ of methylene bridge), 2.32 δ (2H, s, CH₂ of piperazine ring) 2.17 δ (3H, s, CH₃ of -N- CH₃ of piperazine ring), 3.93 δ (3H, s, Ar- OCH₃).

MDPY

6.9-8.45 δ (10H, m, Ar- H and -CH=C-merged), 4.29 δ (2H, s, CH₂ of methylene bridge), 4.25 δ (2H, t, CH₂ adjacent to N of pyrrolidine ring), 1.52 δ (2H, t, CH₂ of pyrrolidine ring), 3.93 δ (3H, s, Ar- OCH₃).

MDNEA

6.85- 8.75 δ (15H, m, Ar- H and -CH=C-merged), 4.83 δ (2H, s, CH₂ of methylene bridge), 3.45 δ (2H, q, CH₂ of -N---CH₂CH₃ of N- ethyl aniline), 1.04 δ (3H, t, CH₃ of -N---CH₂CH₃ of N-ethyl aniline), 3.93 δ (3H, s, Ar- OCH₃).

MDEI

6.88- 8.72 δ (10H, m, Ar- H and -CH=C-merged), 4.37 δ (2H, s, CH₂ of methylene bridge), 1.9 δ (2H, t, CH₂ of ethyleneimine ring), 3.93 δ (3H, s, Ar- OCH₃).

MDIN

6.79- 8.7 δ (16H, m, Ar- H and -CH=C-merged), 5.01 δ (2H, s, CH₂ of methylene bridge), 3.93 δ (3H, s, Ar- OCH₃).

Bactericidal activity

Bactericidal Study of N-Mannich bases of Imidazol-5-one

Novel synthesized N-Mannich bases of imidazol-5-one were screened for their bactericidal activity by using different bacterial and fungal microorganisms. The test was performed by using the agar cup borer method with some modifications using Streptomycin and Imidazole as standard for bacterial and fungal culture respectively as shown in Table 2.

Anti-microbial assay

Agar cup-plate method¹² was used for the

Table 1: Physical and Analytical data of compounds

Compd. No.	R'	Molecular Formula	M.P. °C	% Yield	Elemental Analysis					
					% C		% H		% N	
					Found	Calcd.	Found	Calcd.	Found	Calcd.
1.	(R= H)	C ₃₁ H ₃₂ N ₆ O ₈	171	65	60.04	60.19	5.12	5.19	13.60	13.63
2.	MDMO	C ₃₀ H ₃₀ N ₆ O ₉	135	69	58.11	58.25	4.77	4.85	13.60	13.59
3.	MDNMP	C ₃₁ H ₃₃ N ₇ O ₈	184	72	58.83	58.95	5.13	5.22	15.48	15.53
4.	MDPY	C ₃₀ H ₃₀ N ₆ O ₈	152	76	59.67	59.80	4.91	4.98	13.89	13.95
5.	MDNEA	C ₃₄ H ₃₂ N ₆ O ₈	212	71	62.62	62.57	4.93	4.90	12.87	12.88
6.	MDEI	C ₂₈ H ₂₆ N ₆ O ₈	117	62	58.41	58.53	4.43	4.52	12.16	12.19
7.	MDIN	C ₃₄ H ₂₈ N ₆ O ₈	197	66	62.99	62.96	4.24	4.32	12.91	12.96
	(R= Cl)									
1.	MDPI	C ₃₁ H ₃₁ N ₆ O ₈ Cl	190	68	57.15	57.18	4.68	4.76	12.87	12.91
2.	MDMO	C ₃₀ H ₂₉ N ₆ O ₉ Cl	162	78	55.04	55.17	4.38	4.44	12.81	12.87
3.	MDNMP	C ₃₁ H ₃₂ N ₇ O ₈ Cl	203	67	55.78	55.89	4.73	4.80	14.69	14.72
4.	MDPY	C ₃₀ H ₂₉ N ₆ O ₈ Cl	186	81	56.46	56.55	4.48	4.55	13.14	13.19
5.	MDNEA	C ₃₄ H ₃₁ N ₆ O ₈ Cl	223	67	59.39	59.43	4.49	4.51	12.21	12.23
6.	MDEI	C ₂₈ H ₂₅ N ₆ O ₈ Cl	129	73	55.13	55.21	4.04	4.11	13.77	13.80
7.	MDIN	C ₃₄ H ₂₇ N ₆ O ₈ Cl	218	76	59.68	59.78	3.87	3.95	12.26	12.30

*uncorrected

evaluation of antimicrobial agents. A test tube containing sterile melted top agar (2 %) previously cooled to 50° C and with 0.2 ml suspension of the test culture, mixed thoroughly and poured in the Petri dish containing sterile base agar medium (Nutrient agar) and allowed it to solidify. The cup borer was sterilized by dipping into absolute ethanol and flaming it and then allowed to cool it down, with the help of sterile cup-borer; three cups in the agar-plate were marked and were injected with 0.1 ml of test solution, 0.1 ml of standard drug streptomycin in DMSO (Dimethyl sulfoxide) solvent and 0.1 ml of DMSO solvent respectively. Then the plates were allowed to diffuse for 20 min in refrigerator at 4-5°C. The plates were incubated in upright position at 37°C for 24 hrs and on the next day the zone of inhibition of surrounding each cup was observed.

RESULTS AND DISCUSSION

The N-Mannich bases vary considerably in their range of effectiveness. Some are effective against a limited variety of microorganisms while some are broad spectrum. When chemical substance is added in agar cup, the radial diffusion through the agar produces a concentration gradient. Test organism is inhibited at the minimum inhibitory concentration, giving rise to a clear zone of inhibition.

Of these different N-Mannich bases, MPPY and MPMO were found to have good activity against *E.coli* and *B.subtilis* respectively. While MPPI, MPNMP and MPNEA were found to be moderately active against *E.coli*. Where as MPPI

Table 2: Bactericidal activity of compounds

Compd. No.	N-Mannich bases	Diameter of Zone of inhibition (in mm)		
		<i>E.coli</i>	<i>B.subtilis</i>	<i>A.niger</i>
(R= H)				
1	MPPI	++	++	+
2	MPMO	+++	+++	++
3	MPNMP	++	+	+
4	MPPY	+++	+++	++
5	MPNEA	++	+	+
6	MPEI	+	-	-
7	MPIN	+	++	+
(R= Cl)				
1	MPPI	++	++	+
2	MPMO	+++	+++	++
3	MPNMP	++	+	+
4	MPPY	+++	+++	++
5	MPNEA	++	+	+
6	MPEI	+	-	-
7	MPIN	+	++	+
Standard drugs				
	Streptomycin	26	24	-
	Imidazole	-	-	22
Highly active	=	+++ (inhibition zone >15 mm)		
Moderately active	=	++ (inhibition zone 9- 14 mm)		
Slightly active	=	+ (inhibition zone 1- 8 mm)		
Inactive	=	- (inhibition zone <1 mm)		

and MPIN were found to be moderately active against *B.subtilis*. And the other compounds had less or negligible activity against these microbial species respectively. MPPY, MPMO was found to be moderately active against *A.niger* as a fungal species while other compounds had less or no activity.

CONCLUSION

Thus on the basis of present study, it was concluded that the synthetic compounds were found

to have good application against the different bacterial as well as fungal species and provide significant role for drug designing and other pharmaceuticals approaches. Determination of effectiveness of chemical substance against a specific pathogen is essential to proper therapy.

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