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Antibacterial and Antifungal Evaluation of Potent N-Hydroxy-2,6-diaryl-3,5-dimethylpiperidin-4-one Oximes

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N-hydroxy-2,6-diaryl-3,5-dimethylpiperidin-4-one oximes [**3a-f**] obtained by the reaction between the N-hydroxy-2,6-diaryl-3,5-dimethylpiperidin-4-ones [**2a-f**] and hydroxyl amine hydrochloride are screened against selected bacteria (*Vibro cholerae, Salmonella typii, Shigella flexneri, Escherichia coli, Staphylococous aureus, Klebsiella pneummonia,* β-*Heamolytic streptococcus* and *Pseudomonas*) and fungi (*Aspergillus flavus, Mucor, Microsporum gypseum* and *Rhizopus*). Disc diffusion method is employed to determine the *in vitro* antibiotic effect. The inhibitory effect of the compounds is very close and identical in magnitude and is comparable with that of the standard antibiotic used.

Key Words: Antibacterial, Antifungal, N-Hydroxy-2,6-diaryl-3,5-dimethylpiperidin-4-one oximes.

INTRODUCTION

Some hydroxylamines and ketoximes have been reported as effective antibacterial, antifungal and antileukemic agents. For example, N-hydroxy urea, one of the effective antineoplasmic agents and cicloproxol-amine has broad spectrum antibacterial[1-4] and antifungal[5,6] activities. Therefore, it has become attractive to synthesize N-hydroxyheterocycles and oximes to determine *in vitro* potency against test bacteria and fungi. In this paper, the synthesis and bactericidal, fungicidal ability of the N-hydroxy-2,6-diaryl-3,5-dimethylpiperidin-4-one oximes (**3a-f**) are reported. The structure of the compounds are established using ^{1}H NMR (Table-1) and ^{13}C NMR studies (Table-2).

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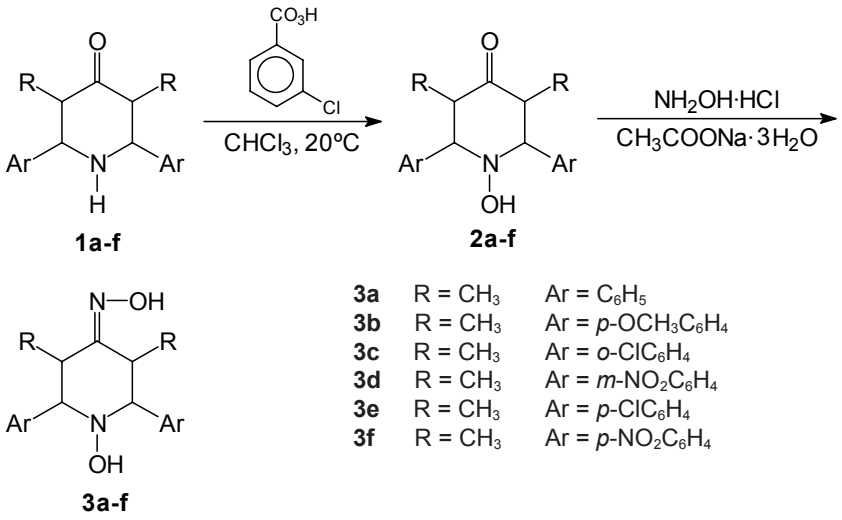
EXPERIMENTAL

Preparation of 2,6-diaryl-3, 5-dimethylpiperidin-4-one [1a-f]: The 3,5-dimethyl-2,6-diarylpiperidones [**1a-f**] were prepared following the procedure adopted by Noller and Baliah[7]. Ammonium acetate (100 m mol), respective substituted benzaldehyde (200 m mol) and appropriate ketone (20 m mol) were dissolved in 95 % alcohol (80 mL) and the solution was heated on a hot plate with gentle swirling until the colour of the mixture changed to orange. The mixture was cooled and poured into ether (100 mL) and concentrated hydrochloric acid (14 mL) was added. The precipitated hydrochloride was collected by filtration and recrystallized from ethanol-ether. The hydrochloride was dispersed in acetone and concentrated aqueous ammonia was added drop wise until a clear solution was obtained. The clear solution was poured into cold water and the solid precipitated was collected and crystallized from ethanol. The observed melting points are in excellent agreement with those of the reported ones.

Preparation of N-hydroxy-2, 6-diaryl-3, 5-dimethylpiperidin-4-one [2a-f]: The respective piperidones [**1a-f**] and *m*-chloroperbenzoic acid (1:1) were mixed in 20 mL of chloroform at 0 °C. The mixture was extracted with chloroform and washed with 10 % sodium bicarbonate solution. The chloroform layer was dried with anhydrous sodium sulphate and evaporated. The separated solid was subjected to column chromatography. The column was packed with silica gel (100-200 mesh) in hexane. The eluting solvents were benzene, benzene-pet-ether (40:60) (8:2). The compounds were found to be separated in benzene-pet-ether (8:2).

Proton NMR spectra were recorded on a Bruker AMX-400 spectrometer operating at 400 MHz. Samples were prepared by dissolving about 10 mg of compound in 0.5 mL of chloroform-d, containing 1 % TMS. All the chemical shifts are in reference to TMS. ^{13}C NMR spectra were recorded on a Bruker AMX-400 spectrometer operating at 400 MHz and using 10 mm sample tubes. Solution for the measurement of spectra were prepared by dissolving 0.5 g of the compound in 2.5 mL of chloroform-d containing 1 % TMS. All the chemical shifts are in reference to TMS.

Preparation of N-hydroxy-2,6-diaryl-3,5-dimethylpiperidin-4-one oximes [3a-f]: The appropriate N-hydroxy-2,6-diaryl-3,5-dimethylpiperidin-4-ones **2a-f** (0.05 mol) and sodium acetate trihydrate (0.15 mol) were dissolved in boiling ethanol and hydroxylamine hydrochloride (0.06 mol) was added. The mixture was heated under reflux for 15 min and poured into water. The separated solid was filtered off and recrystallized from ethanol.



Scheme-I

Preparation of media: Nutrient broth was used to cultivate bacteria. Agar media was prepared by adding 24 % w/v agar in the nutrient broth for making agar slants. Bacteria were sub-cultured on the nutrient agar slants. The inoculum was prepared by transferring loop full of the corresponding organism from the stock culture into the sterile broth and incubated at 37 °C for bacterial stains. 20 mL of sterile nutrient agar media was added to each petri dish and 2 mL of 24 h broth culture of bacteria was then added to the respective plates and mixed thoroughly by rotatory motion of the plates. The respective oximes hydrochloride was dissolved in water in the concentration of 10 mg/mL. The solution was maintained as a stock solution. The different concentrations (100, 200 and 500 ppm) were prepared from the stock solution. Sterile paper disc of 5 mm diameter was saturated with the three different concentrations and such discs were placed in each seeded agar plates. The petri plates were incubated at 37 °C and zones of inhibitions were measured excluding the diameter of the paper disc (5 mm). Control discs were performed with sterile water.

For the antifungal activity assay, the *in vitro* disc diffusion method is adopted. Sabouraud's dextrose agar is used to culture the fungi. Peptone water (1 %) is used for fresh culture of all the fungi and are maintained by periodic sub culturing in fresh Sabouraud's dextrose medium. Plates for Sabouraud's dextrose medium are prepared with the inocula by adding 1 mL of dilute culture of the test organism. The respective oxime hydrochlorides are dissolved in water in the concentration of 10 mg/mL. The solution was maintained as a stock solution. The different concentrations (100, 200 and 300 ppm) are prepared from the stock solution. Sterile paper disc of 5 mm

diameter is saturated with the three different concentrations and such discs are placed in each seeded agar plates. The petri plates are incubated at 30 °C for 70 h. The inhibition zones are measured excluding the diameter of the paper disc (5 mm). At 500 mg/mL concentration the conventional standard antifungal drug ketoconazole exhibited 20 ± 0.5 mm zone of inhibition against all the test fungi.

RESULTS AND DISCUSSION

The structure of the compounds is established using ^{1}H NMR (Table-1) and ^{13}C NMR studies (Table-2). The oximation of ketone makes a considerable shift in proton absorptions. Due to the introduction of oxime group, the syn α and syn β hydrogens are shielded. The α and β hydrogens anti to the oxime group are deshielded. The signals are assigned by comparing with respective ketones. The elemental analysis, melting points and yields are given in Table-3.

TABLE-1
^{1}H NMR CHEMICAL SHIFTS (δ ppm) OF COMPOUNDS **3a-f**

Proton number	**3a**	**3b**	**3c**	**3d**	**3e**	**3f**
H_{2a}	3.78	4.17	4.55	4.03	3.79	4.01
H_{6a}	3.69	4.13	4.38	3.92	3.69	3.90
H_{3a}	3.38-3.42	3.52	3.48	2.82-2.87	2.78	3.42-3.49
H_{5a}	2.80-2.83	2.88	2.76	2.70-2.79	2.64	2.81-2.90
CH_3(5)	1.21	1.34	1.29	1.36	1.22	1.27
CH_3(3)	1.30	1.38	1.55	1.39	1.31	1.39
N-OH	4.37	4.25	4.77	4.32	4.63	4.71
C=N-OH	7.42	7.31	7.40	7.80	7.87	7.82
Aromatic	7.26-7.37	7.21-7.29	7.22-7.43	7.57-7.60	7.29-7.35	7.29-7.42
p-OCH_3(6)	-	3.95	-	-	-	-
p-OCH_3(2)	-	3.95	-	-	-	-

Antibacterial activity: The N-hydroxy-2,6-diaryl-3,5-dimethylpiperidin-4-one oximes [**3a-f**] are screened for their bactericidal activity. The method followed for the present investigation is disc diffusion method suggested by Maruzella *et al.*[8]. The bacterial stains used are *Vibro cholerae, Salmonella typii, Shigella flexneri, Escherichia coli, Staphylococous aureus, Klebsiella pneummonia, β-Heamolytic streptococcus* and *Pseudomonas*. Each value is an average of three determinations. It is apparent from Table-4 that all the six compounds (**3a-f**) are active against the test bacteria. However the compound **3b** is inactive against *Klebsiella pneummonia, Escherichia coli* and *Shigella flexneri*. The compound **3f** is inactive against

TABLE-2
^{13}C NMR CHEMICAL SHIFT VALUES (δ ppm) OF COMPOUNDS **3a-f**

Carbon number	**3a**	**3b**	**3c**	**3d**	**3e**	**3f**
C-2	75.56	75.52	74.56	74.92	74.86	74.72
C-3	43.02	42.19	30.16	42.11	30.90	43.94
C-4	161.95	161.90	162.12	168.01	161.07	168.82
C-5	30.93	31.10	30.89	30.62	30.90	30.93
C-6	75.56	75.62	75.50	74.52	74.86	74.10
CH_3(3)	20.73	15.71	20.50	15.52	15.59	15.54
CH_3(5)	15.76	20.70	15.20	20.90	20.72	20.80
Ipso-C-6	141.96	141.35	141.30	141.65	141.74	141.35
Ipso-C-2	143.65	141.35	141.30	141.65	141.74	141.35
Aromatic	127.38-128.47	127.25-128.40	128.32-128.90	127.39-128.04	128.61-129.41	128.33-128.85
p-OCH_3	-	55.10	-	-	-	-

TABLE-3
MELTING POINT, YIELD AND ELEMENTAL ANALYSIS DATA OF COMPOUNDS **3a-f**

Compd.	m.p. (ºC)	Yield (%)	m.f.	Elemental analysis: Found (Calcd.) %		
				C	H	N
3a	142	60	$C_{19}H_{22}N_2O_2$	74.35 (73.55)	7.08 (7.10)	9.06 (9.03)
3b	165	85	$C_{19}H_{26}N_2O_4$	69.07 (68.11)	7.05 (7.03)	7.33 (7.57)
3c	149	70	$C_{19}H_{20}N_2O_2Cl_2$	60.11 (60.16)	5.30 (5.28)	8.36 (7.38)
3d	167	66	$C_{19}H_{20}N_4O_6$	57.19 (57.00)	5.45 (5.00)	14.19 (14.00)
3e	153	73	$C_{19}H_{20}N_2O_2Cl_2$	61.33 (60.16)	5.39 (5.28)	8.14 (8.44)
3f	172	58	$C_{19}H_{20}N_4O_6$	57.36 (57.00)	5.54 (5.00)	14.76 (14.00)

Escherichia coli. The *in vitro* inhibition profiles of the compounds are given in Table-4. The inhibitory effects of the compounds are very close and identical in magnitude and are comparable with that of the standard antibiotic used. In this study, the conventional standard antibacterial drug chloramphenicol at 500 µg/mL concentration exhibited 30 ± 0.5 mm zone of inhibition against all the test bacteria.

TABLE-4
in vitro INHIBITION PROFILE OF THE COMPOUNDS **3a-f** AGAINST TEST BACTERIA

Bacteria	**3a** (ppm)			**3b** (ppm)			**3c** (ppm)			**3d** (ppm)			**3e** (ppm)			**3f** (ppm)		
	100	200	500	100	200	500	100	200	500	100	200	500	100	200	500	100	200	500
S. aureus	10	12	17	6	8	9	8	9	13	12	16	19	10	12	17	7	11	19
Pseudomonas	15	17	20	13	15	17	12	16	20	16	19	25	14	16	21	11	16	19
Klebsiella	13	16	21	–	–	–	12	15	19	7	9	17	7	9	13	9	13	20
E. coli	8	9	13	–	–	–	11	17	21	11	14	19	10	14	20	–	–	–
β-H. streptococcus	13	15	18	12	15	19	12	17	21	21	24	29	30	34	38	9	13	16
S. typii	13	15	19	11	14	19	18	20	24	28	31	36	27	31	36	30	34	39
V. cholerae	20	24	29	13	17	21	17	21	26	19	21	24	25	28	31	15	19	26
S. flexneri	6	10	14	–	–	–	7	11	15	8	13	17	10	14	19	14	16	21

All values are in millimeter (mm), representing the diameter of the zone of inhibition.

TABLE-5
in vitro INHIBITION PROFILE OF THE COMPOUNDS **3a-f** AGAINST TEST FUNGI

Fungi	**3a** (ppm)			**3b** (ppm)			**3c** (ppm)			**3d** (ppm)			**3e** (ppm)			**3f** (ppm)		
	100	200	500	100	200	500	100	200	500	100	200	500	100	200	500	100	200	500
M. gypseum	19	21	25	25	27	31	15	17	21	17	21	25	16	19	21	14	15	20
A. flavus	7	9	10	13	14	17	14	17	21	16	20	24	16	20	25	10	14	20
Rhizopus	–	–	–	12	14	19	12	15	19	15	19	25	–	–	–	–	–	–
Mucor	20	21	26	–	–	–	15	19	21	10	12	15	25	29	31	20	25	30

All values are in millimeter (mm), representing the diameter of the zone of inhibition.

Antifungal activity: The inhibitory effect of the products (**3a-f**) against select fungi is studied in detail. The method used for this study is disc diffusion method. The fungal stains used in the study *viz.*, *Aspergillus flavus, Mucor, Microsporum gypseum* and *Rhizopus*. The fungal response is tabulated in Table-5. Each value is an average of three determinations. From the Table-5 it is known that compounds **3a**, **3e** and **3f** are inactive against *Rhizopus*, wheres compound **3b** is inactive against *Mucor*. Otherwise all the compounds are active against all the test fungi. The inhibitory effects of the compounds are very close and identical in magnitude and are comparable with that of the standard antibiotic used. In this study, the conventional standard antifungal drug ketoconazole at 500 µg/mL concentration exhibited 20 ± 0.5 mm zone of inhibition against all the test fungi.

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