



Synthesis, Characterization and Biological Activities Of N-(3-Methyl-2,6-Diphenyl-Piperidin-4-Ylidine)-N'-Phenyl-Hydrazine

P. Rajesh^{1*}, M. Dinesh Kumar¹, R. Sathish Kumar² and R. Jamunarani¹

¹ Department of Chemistry, Government Arts College (Autonomous), Coimbatore, TN, India.

² Department of Botany, PSG College of Arts and Science, Coimbatore, TN, India

Received:12.06.2014 Accepted: 08.08.2014

Abstract

In the present study a new 2,6-diphenyl-3-methyl-4-piperidone was prepared by Mannich reaction (condensation) of ethyl methyl ketone, aromatic aldehyde and ammonium acetate. N-(3-Methyl-2,6-diphenyl-piperidin-4-ylidene)-N'-phenyl-hydrazine was synthesized by reaction with sodium acetate in water and phenyl hydrazine. The chemical structures were confirmed by means of IR, ¹H & ¹³C NMR spectral data. The compound was screened for acute anticancer, cytotoxicity, antibacterial and antifungal activities and their derivatives also having the same potent activities. The compound exhibited potent antibacterial activity against *Proteus vulgaris* and the better antifungal activity against *Aspergillus flavus*.

Keywords: Piperidin-4-one; phenyl hydrazine; antifungal activity

1. INTRODUCTION

Many natural compounds and drugs contain the piperidine ring system as a structural element. As this class of compounds exhibits pharmacological properties (Garrafo et al. 1993). Indeed 2,6-disubstituted-4-piperidones are the constituents of a number of alkaloids which possess broad spectrum of biological activity (Rubiralta et al. 1991). Piperidones were reported to possess analgesic (Richardo et al. 1979), anti-inflammatory (Jerom and Spencer, 1988), central nervous system (Perumal et al., 2001), Local analgesic (Ganellin and Spickett, 1965), anticancer (Ilena et al. 1985) and antibacterial activity (Mobio et al. 1989) The earlier reports indicated that the biological activities of piperidones were associated with substitutions at 2, 3 and 6 positions (Boschringer et al. 1961). The biological activity was found to be significant in compound possessing aromatic substituents in 2 and/or 6 positions (Noller and Baliah, 1948). The presence of alkyl substitution in 2 or 3 position (Nikolov et al., 1974; Mobio et al., 1985) was also attributed to biological activities. Therefore, it was envisaged that a new series of 2, 6-diphenyl-piperidin-4-ones and their corresponding phenyl hydrazine derivative would result in compounds of potent biological activities. In the present study a new 2,6-diphenyl-3-methyl-4-piperidone was prepared by Mannich reaction (condensation) of ethyl methyl

ketone, aromatic aldehyde and ammonium acetate. N-(3-Methyl-2, 6-diphenyl-piperidin-4-ylidene)-N'-phenyl-hydrazine was synthesized by reaction with sodium acetate in water and phenyl hydrazine. The chemical structures were confirmed by means of IR, ¹H & ¹³C NMR spectral data. The compound was screened for acute anticancer, cytotoxicity, antibacterial and antifungal activities and their derivatives also having the same potent activities.

2. EXPERIMENTAL METHODS

The melting points were taken in open capillary tube and the values are related to literature. The IR spectra of the compounds were recorded on Bruker-Alpha FT-IR spectrometer with KBr pellets in the region 4000 – 400 cm⁻¹. ¹H & ¹³C NMR were recorded using CDCl₃ as a solvent. The chemical shifts are reported as parts per million downfield from TMS. The spectral data (IR, ¹H & ¹³C NMR) are represented in Table 1-3.

2.1 Synthesis of N-(3-methyl-2, 6-diphenyl-piperidin-4-ylidene)-N'-phenyl-hydrazine

A mixture of ethylmethyl ketone (0.1 mol), benzaldehyde (0.1 mol) and dried ammonium acetate (0.1 mol) were dissolved in ethanol (50 ml). These contents were heated until the at yellow color turns into orange. This mixture was kept in room temperature for

* P. Rajesh Tel.: +919790025960

E-mail: p_rajesh124@yahoo.co.in

14 hrs. Concentrated ammonia (30 ml) was added followed by Conc. HCl (30 ml) and then it was cooled in ice water. The precipitated hydrochloride was neutralized with dry ether (30 ml). Finally, the neutralized product was filtered and recrystallized from absolute ethanol. Yield: 90% Melting Point: 96°C (Lit M.Pt: 96 - 97°C)

reaction of 3-methyl-2, 6-diphenyl-piperidin-4-one (0.01 mol) with Phenyl hydrazine (0.01 mol) in ethanol (30 ml) was heated with continuous stirring for 3 hrs. The contents were kept undisturbed for 24 hrs. The precipitate was filtered, washed with ether and recrystallized from absolute ethanol. Yield: 56.5% Melting Point: 118°C

Phenyl hydrazine derivative of 3-methyl-2, 6-diphenyl-piperidin-4-one was synthesized by the

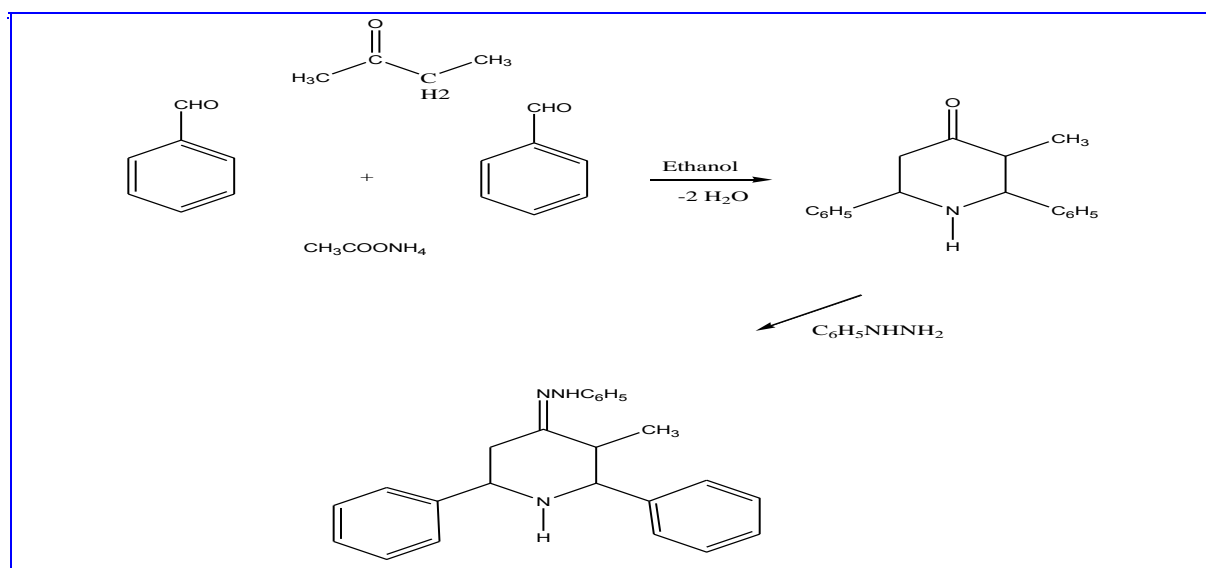


Fig. 1: Synthetic scheme of N-(3-methyl-2, 6-diphenyl-piperidin-4-ylidene)-N'-phenyl-hydrazine

Table 1. Assignment of IR Spectral data

S.No	IR frequency (cm ⁻¹)	Assignment
1	3339 & 3309	N-H Stretching
2	3034	C-H Aromatic
3	2977 - 2896	Sym C-H Stretching in aliphatic
4	2856	Asym C-H Stretching in CH ₃

5	1596	C=N Stretching
6	1494	C=C Stretching
7	1446	C-C Stretching
8	1159	N-N Stretching
9	1085	C-N Stretching
10	698	Ar-C-H Stretching

Table 2. ¹H NMR Spectral data of N-(3-methyl-2,6-diphenyl-piperidin-4-ylidene)-N'-phenyl-hydrazine

S.No	¹ H NMR(CDCl ₃) δ values(ppm)	Assignment
1	7.05 – 7.51 (m, 15H)	Ar-H
2	6.80 – 6.85 (d, 1H)	-NH at C4-H
3	3.89 - 3.93 (dd, 1H)	-NH at C1-H
4	3.53 – 3.55 (d, 1H)	C3-H
5	2.92 – 2.97 (t, 1H)	C6-H
6	2.56 – 2.62 (dd, 1H)	C2-H
7	2.09 – 2.52 (d, 2H)	C5-H
8	1.03 (d, 3H)	CH ₃ at C3-H

Table 3. ¹³C NMR Spectral data of N-(3-methyl-2,6-diphenyl-piperidin-4-ylidene)-N'-phenyl-hydrazine

S.No	¹³ C NMR(CDCl ₃) δ values(ppm)	Assignment
1	143.0, 143.6 & 146.0	Ipsocarbons
2	126.1 – 129.1	Ar carbons
3	76.5 & 77.4	C-4
4	69.26	C-2
5	60.84	C-6
6	44.40	C-3
7	34.54	C-5
8	12.32	CH ₃ at C-3

3. BIOLOGICAL ACTIVITIES

The synthesized compound was evaluated for antibacterial and antifungal studies. The bacterial strains were obtained from Department of Microbiology, Karpagam University, Coimbatore, Tamilnadu. Bacterial and fungal (Six) cultures were maintained in the Nutrient broth.

3.1 Antibacterial activity

The antibacterial activity was determined by Well diffusion method. Bacterial cultures namely *Proteus vulgaris*, *Pseudomonas putida* and *Streptococcus faecalis* were maintained in the Nutrient broth.

Methodology

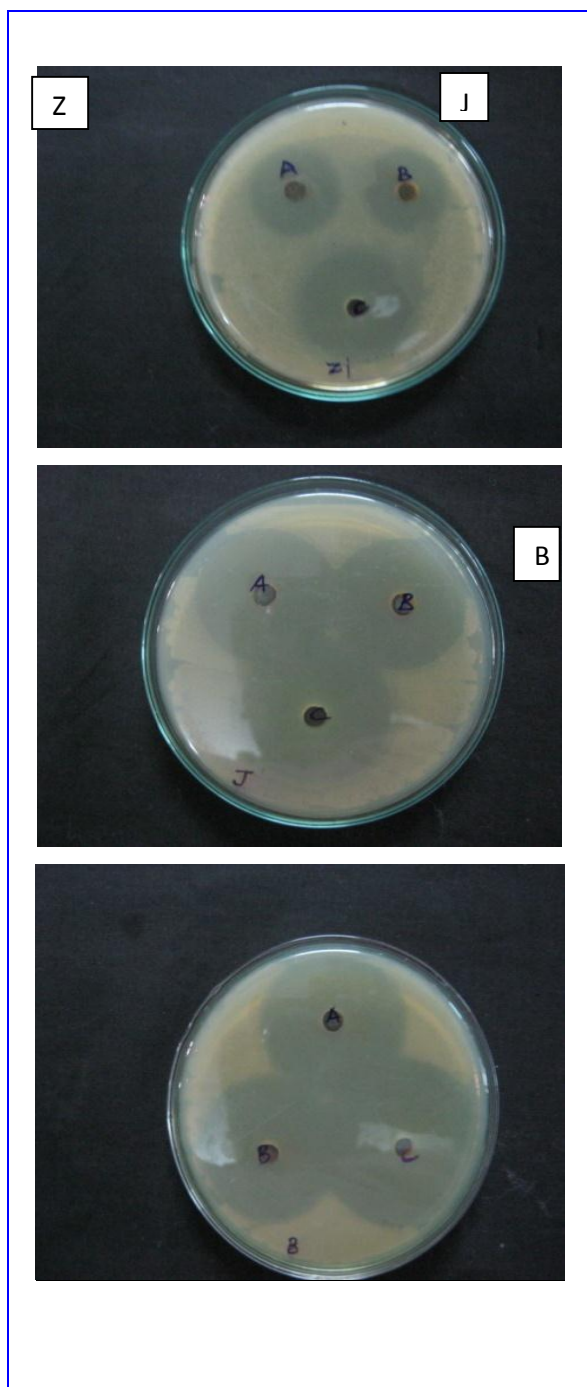
Antibacterial activities of the synthesized compounds were investigated by using pathogens by Kirby Bauer disc diffusion method (modified) (Bauer et al. 1966). A 100 µL of microbial culture broth was spread using sterile L-rod on Muller Hinton agar plates. The spread plates were allowed to stand for 10 min. The wells (5 mm size) were created on spread plates using sterile gel core. Two different concentrations (25 µg/ml and 50 µg/ml) of compounds and positive control (50 µg/ml) (tetracycline) were prepared. A 100 µL of the two different compounds and positive control were poured into wells on all plates with the help of micropipette. All the plates were kept for incubation at 37°C for 24 hours. After incubation, the zone of inhibition was measured. Each screening test was conducted with three replicates and the data are presented as mean ± SE (standard error of the mean).

Table 4. Antibacterial activity of N-(3-methyl-2,6-diphenyl-piperidin-4-ylidene)-N'-phenyl-hydrazine

S.no	Photos named as	Organism name	Zone of inhibition (mm in diameter)		
			A	B	C
1	J	<i>Proteus vulgaris</i>	28 ± 1.0	24 ± 1.0	41
2	B	<i>Pseudomonas putida</i>	20 ± 1.0	15 ± 1.0	48
3	Z	<i>Streptococcus faecalis</i>	24 ± 1.0	16 ± 1.0	35

- A - 100 µg/ml
- B - 50 µg/ml
- C - 30 µg/ml positive control,
(tetracycline)

Well size - 5 mm



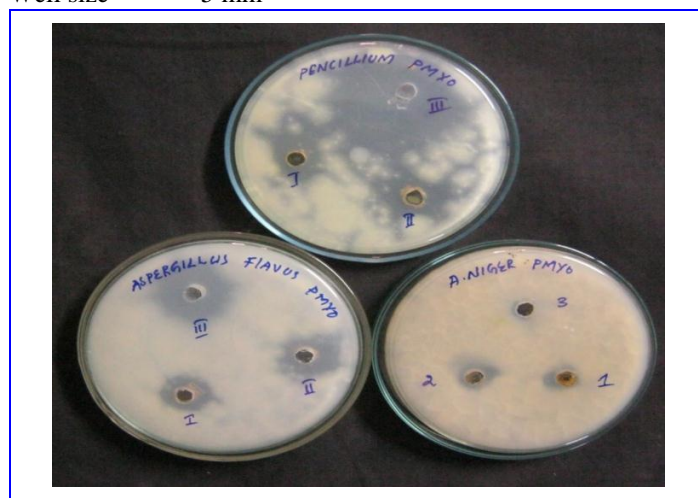
3.2 Antifungal activity

The antifungal activity of the compound was tested against fungal cultures namely *Aspergillus nigar*, *Aspergillus flavus* and *Pencillium* using sabour and dextrose agar medium. The sterilized medium (40-50°C) was inoculated with the suspension of the microorganism (matched to Mcfarland barium sulphate standard) and poured into a petridish to give a depth 5mm. the paper impregnated with the test compound was on placed the solidified medium. The plates were preincubated at room temperature (37°C) for 48 hrs for antifungal activity. Amphotericin (30 µg/ml) was used as standard. The observed zone of inhibition is presented in Table 5.

Table 5. Antifungal activity of N-(3-methyl-2, 6-diphenyl-piperidin-4-ylidene)-N'-phenyl-hydrazine

S.no	Organism name	Zone of inhibition (mm in diameter)		
		1	2	3
1	<i>Aspergillus nigar</i>	6 ± 1.0	12 ± 1.0	9 ± 1.0
2	<i>Aspergillus flavus</i>	12 ± 1.0	21 ± 1.0	19 ± 1.0
3	<i>Pencillium</i>	10 ± 1.0	19 ± 1.0	32 ± 1.0

- 1 - 100 µg/ml
 - 2 - 50 µg/ml
 - 3 - 30 µg/ml positive control,
(Amphotericin)
- Well size - 5 mm



RESULTS & DISCUSSION

The results of antibacterial activity of synthesized against disease causing pathogens are shown in Figure 3. The highest zone of inhibition was observed *Proteus vulgaris* with a zone diameter of 28 ± 1 mm at a concentration of 100 $\mu\text{g/ml}$, it is similar with positive control (i.e 41 ± 1 mm) and which is very similar to previous studies (Jayaseelan et al. 2012). The lowest zone of inhibition was observed *Pseudomonas putida* with a zone diameter of 20 ± 1 mm at a concentration of 100 $\mu\text{g/ml}$, it is more than positive control (i.e 48 ± 1 mm). The antifungal activity of synthesized against disease causing pathogens are shown in Figure 4. The highest zone of inhibition was observed *Aspergillus flavus* with a zone diameter of 21 ± 1 mm at a concentration of 50 $\mu\text{g/ml}$, it is more than positive control (i.e 19 ± 1 mm zone of inhibition). The lowest zone of inhibition was observed *Penicillium* with a zone diameter of 19 ± 1 mm at a concentration of 50 $\mu\text{g/ml}$, it is more than positive control (i.e 32 ± 1 mm).

REFERENCES

- Bauer, A. W., Kirby, W. M., Sherris, J. C. and Turck, M., Antibiotic susceptibility testing by a standardized single disk method, *J. Am. Clin. Pathol.*, 45(4), 493-496 (1966).
- Bochringer, C. F. and Shochne, G. M. B. H., Synthesis and Biological Evaluation of Novel Benzimidazol/Benzoxazolylethoxypiperidone Oximes, *Brit Pat Appl.*, BP 866488 (1961).
- Ganellin, C. R. and Spickett, R. G., Compounds affecting the central nervous system. 4-Piperidones and related compounds, *J Med Chem.*, 8(5), 619-625(1965).
doi:10.1021/jm00329a015
- Garrafo, H. M., Caceres, J., Daly J. W. and Spande, T. F., Alkaloids in Madagascan Frogs (Mantella): Pumiliotoxins, Indolizidines, Quinolizidines, and Pyrrolizidines, *J. Nat. Prod.*, 56(7), 1016-1038 (1993).
doi:10.1021/np50097a005
- Hagenbach, R. E. and Gysin H., Über einige heterozyklische Thiosemicarbazone, *Experientia.*, 8(5), 184-188 (1952).
doi:10.1007/BF02173735
- Ilena, B., Dobre, V. and Niculescu-Duvaz, I., Potential anticancer agents. XXVI. Spin Labelled Nitrosoureas, *J. Prakt. Chem.*, 327(4), 667-674 (1985). doi:10.1002/prac.19853270418
- Jayaseelan, C., Abdul Rahuman, A., Vishnu Karthi, A., Marimuthu, S., Santhosh Kumar, T., Bagavan, A., Gaurav, K., Bhaskara Rao. and Karthik, L., *Spectrochim. Acta. PART A.*, 90, 78-84(2012).
doi:10.1016/j.saa.2012.01.006
- Jerom, B. R. and Spencer, K. H., N-Heterocyclic-N-(4-Piperidinyl) amides, *Eur Pat Appl.*, EP 277794 (1988).
- Mobio, I. G., Soldatenkov, A. T., Fedrov, V. O., Ageev, E. A., Sergeeva, N. D., Lin, S., Stashenku, E. E., Prostakov, N. S. and Andreeva, E. L., Synthesis and physiological activity of 2,3,6-triaryl-4-oxo (hydroxyl, oximino, amino) piperidine. *Khim. Farm.*, 23(4), 421-427(1989).
- Nikolov, M., Stefanora, D. and Chardanov, D., The effect of tempidon on the EEG of cats in acute and chronic experiments, *Acta Nerv. Super.*, 16, 264-267(1974).
- Noller, C. R. and Baliah, V., The preparation of some piperidine derivatives by the Mannich reaction. *J. Am. Chem. Soc.*, 70(11), 3853-3855(1948).
doi:10.1021/ja01191a092
- Perumal, R. V., Adiraj, M. and Shanmugapandian, P., Synthesis, analgesic and anti-inflammatory evaluation of substituted 4-piperidones. *Indian Drugs.*, 38, 156-159(2001).
- Richardo, G., Juan, B. C., Mario, R. A., Roldan, M. and Peinado, C. R., *Fernando Spen.*, 47, 166-172 (1979).
- Rubiralta, M., Giralt, E. and Diel, A., *Piperidone Structure, Preparation and Synthetic Applications of Piperidone and its Derivatives*, Elsevier, Amsterdam (1991).