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Spectral and Antimicrobial Studies of Some r(2), *c*(6)-diarylpiperidin-4- one cyanoaceticacid hydrazones

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Abstract

A novel synthesis of *t*(3)-Methyl-*r*(2),*c*(6)-diarylpiperidin-4-onecyanoaceticacidhydrazones 1–3were synthesized and Characterized by IR, ^{1}H and ^{13}C NMR spectral studies. The structures were investigated for antibacterial and antifungal activity. The observed vicinal proton-proton coupling constants suggest that 1-3 adopts chair conformation with the equatorial orientations of all substituent's.

Keywords: piperidin-4-onecyanoaceticacidhydrazone, Spectral, antibacterial, antifungal activity.

Contents

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1. Introduction

Nuclear magnetic resonance spectroscopy has been widely used in the study of organic compounds [1–5] and reviews also have appeared on this subject. Hydrazones are azomethines, characterized by the presence of the triatomic grouping > C=N-N<. Many of the physiologically active compounds find application[1] in the treatment of several diseases such as tuberculosis, leprosy and mental disorder. On the other hand, aroylhydrazones are reported to possess anti-tuberculor activity.[2,3]. This is attributed to the formation of stable chelates with transition metals present in the cell. Hydrazones act as herbicides, insecticides, nematocides, rodenticides and plant growth regulators. They show spasmolytic activity, hypotensive action and activity against leukaemia, sarcomas and other malignant neoplasms. The structural aspects of these compounds have been reviewed.[1].

In analytical chemistry hydrazones find application in detection, determination and isolation of compounds containing the carbonyl group and they have been extensively used in detection and determination of several metals. Many other analytical potentialities of these compounds have also been explored. The synthesis and antioxidant properties syringaldehyde hydrazones reported by Belkheiri et al [4]. The anticancer activity of some1,4-dihydro-3-(3-

hydroxy-2-naphthyl)-4-substituted-5H-1,2,4-triazoline-5-thiones derived from 3-hydroxy-2-naphthoic hydrazide (3-NAH) studied by Dogan et al [5-7] and Duran et al[8]. The studies of heterocyclic compounds are of much interest due to their biological importance. The high pharmacological concern about piperidin-4-ones is due to their important role as intermediates in the synthesis of many drugs. Piperidine-4-ones and their derivatives have been reported to possess antimicrobial activity (Mobio et al., 1989).

The earlier reports indicate that the biological activities of piperidin-4-ones are associated with substitutions at 2, 3 and 6 positions (Perumal et al., 2001; Bochringer and Shochne, 1961). Hydrazides and hydrazones have interesting ligation properties due to presence of several coordination sites. Furthermore, a number of hydrazide–hydrazone derivatives have been claimed to possess interesting antibacterial and antifungal (Loncle et al., 2004; Garoufalias et al., 2002; Vicini et al., 2002) activities. The derivatives of 3-hydroxy-2-naphthoic acid hydrazide (3-NAH) have been found to exhibit antimicrobial (Dogan et al., 1998, 2002, 2005) and anticancer activities (Duran et al., 2002). In the present study we have reported the synthesis, NMR spectral study and antimicrobial activity of some r(2),c(6 diarylpiperidin-4-onecyanoaceticacidhydrazones**[1-3]**.

2. Experimental

Materials and Methods

Materials:

Cyanoaceticacid hydrazide was purchased from Sigma-Aldrich and was used as such. All the reagents and solvents were of laboratory grade.

Methods

NMR spectra: ^{1}H NMR spectra were recorded on a Bruker DRX-500 NMR spectrometer operating at 500.03 MHz for ^{1}H with the following spectral parameters: acquisition time = around 3.0 s, number of scans = 100, number of data points = 32 K and spectral width = 10330 Hz. Proton decoupled ^{13}C NMR spectra were recorded on a Bruker DRX-500 NMR spectrometer operating at 125.77 MHz for ^{13}C with the following spectral parameters: acquisition time = around 0.5 s; number of scans = 1000; number of data points = 32 K; spectral width = 30000 Hz. All NMR measurements were made in 5 mm NMR tubes. The solutions were prepared by dissolving about 10 mg of the compound in 0.5 mL of DMSO-d6.

IR spectra

FT-IR spectra were recorded on a NICOLET-AVATAR-330 FT-IR spectrophotometer in KBr pellets.

Melting points

Melting points were taken in open capillaries and are uncorrected.

Antibacterial studies

The following gram positive and gram negative bacterial strains have been used for the study.

a. *Staphylococcus aureus*
b. *Streptococcus aureus*
c. *Salmonella typhi*
d. *Klebsiella pneumoniae*
e. *Escherichia coli*

Preparation of test inoculums

Sub-culture (preparation of seeded broth)

The strains of *Staphylococcus aureus, Streptococcus aureus, Salmonella typhi, Klebsiella pneumoniae and Escherichia coli* were inoculated in conical flasks containing 100 mL of sterile nutrient broth. These conical flasks were incubated at 37 ± 1 °C for 24 hours. This was referred to as seeded broth.

Standardization of seeded broth (viable count)

Dilutions

One mL of 24 hours seeded broth of each strain was diluted with 99 mL of sterile normal saline containing 0.05 % tween 80 (8 drops of tween 80 in 1000 mL of normal saline). From that, 1 mL is further diluted to 10 mL with sterile normal saline. This is continued to 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} until 10^{-10} mL of the dilution of seeded broth was obtained.

Incubation of nutrient agar petridishes

The dilutions were studied by inoculating 0.2 mL of each dilution on to the solidified nutrient agar medium by spread plate method after incubation at 37 ± 1 °C for 24 hours. The numbers of well-formed colonies on the plates were counted. The seeded broth was then suitably diluted to have between 10^{5}-10^{7} microorganisms per milliliter or cfu / mL. This was designated as the working stock and used for the antibacterial studies.

Preparation of solution of test compounds

The solution of test compound was prepared by dissolving the compound in dimethyl sulfoxide (DMSO) in specific gravity bottle and stored in refrigerator. The solution was removed from the refrigerator 1 hour prior to its use and allowed to attain room temperature. The solutions of the test compounds were prepared at a concentration of 200

μg/mL for finding the minimum inhibitory concentration. Solvent control of DMSO was also maintained throughout the experiment simultaneously.

Preparation of culture media

Nutrient agar medium and nutrient broth medium were used for the growth of bacteria and the media were sterilized by autoclaving at 151bf/sq inch pressures at 121 °C for 20 min.

(i) Nutrient agar medium (Hi-media)

The nutrient agar medium was prepared by dissolving 28 g of nutrient agar (procured from Hi-Media, Mumbai) in 1000 mL of distilled water.

Formula

Peptone : 1%
Sodium chloride : 0.5 %
Beef extract : 1 %
pH : 7.4 ± 0.2

(ii) Nutrient broth medium (Hi-Media)

The nutrient broth medium was prepared by dissolving 13 g of nutrient broth (procured from Hi-media, Mumbai) in 1000 mL of distilled water.

Fromula

Peptone : 1%
Sodium chloride : 0.5 %
Beef extract : 1 %
pH : 7.4 ± 0.2

Determination of Minimum Inhibition Concentration (MIC) of test compounds using Two-Fold serial dilution method

Testing was done in the seeded broth (10^{-6} to 10^{-7}cfu/mL). The test compounds were taken at different concentrations ranging from 200, 100 and 50 μg/mL for finding minimum inhibitory concentration by using seeded broth as diluents. Similarly, the standard solution of ciprofloxacin drug was prepared at the concentration of 200, 100 and 50 μg/mL with sterile distilled water and DMSO were maintained throughout the experiment simultaneously as control. The study involves a series of three assay tubes for the test compounds against each strain. In the first assay tube, 1.6 mL of seeded broth was transferred and 0.4 mL of the test solution was added followed by mixing thoroughly to obtain a concentration of 200 μg/mL. To the remaining 2 assay tubes, 1 mL of seeded broth was transferred, then from the first assay tube 1 mL of the content was pipetted out and added into second assay tube followed by mixing thoroughly. This type of dilution was repeated for the next assay tube. The same procedure was followed for the standard drug too. Duplicates were also maintained. These were done under aseptic conditions.

The racks of assay tubes were placed inside the incubator at 37 ± 1 °C for 24 hours. At the end of 24th hour, assay tubes concentrations were again streaked into nutrient agar plat due to the turbidity of drug-microorganism mixture. The lowest concentration of the test compounds, which caused apparently a complete inhibition of growth of organisms, was taken as minimum inhibitory concentration. The solvent control tube was also observed to find whether there was any inhibitory action. The sterile distilled water and DMSO did not show any inhibition.

Antifungal studies

The following fungal strains have been used for the study.

a. *Candida albicans*
b. *Aspergillus flavus*
c. *Aspergillus niger*
d. *Cryptococcus neoformans*

Sabouraud's dextrose agar (SDA) medium was used for the growth of fungi and testing was done in Sabouraud's dextrose broth (SDB) medium. The subculture and the viable count were carried out by the same procedure as done in antibacterial studies except the temperature which was maintained at 28 ± 1 °C for about 72 hours. Similarly fir disc diffusion method, the petridishes were incubated at 28 ± 1 °C for about 72 hours. the same concentration (in μg/mL) of the test compound, solvent (DMSO) and Miconazole (Standard) were used for the antifungal studies.

Preparation of culture media

(i) SDA medium

Formula

Dextrose : 40 g
Peptone : 10 g
Agar : 15 g

Distilled water : 1000 mL
pH : 5.4

(ii) SDB medium

Formula

Dextrose : 40 g
Peptone : 10 g
Distilled water : 1000 mL
pH : 5.4

The MIC values were obtained in µg/mL. These were converted to µM by using the following formula where 'M' is the molecular weight of the compound.

$$\mathbf{1\ \mu M} = \frac{\mathbf{1\mu g}}{\mathbf{mL}} \div \frac{\mathbf{1000}}{\mathbf{M}.}$$

Preparation of compounds

The parent Piperidin-4-ones of **1–3** were prepared following the procedure of Noller and Baliah [9].

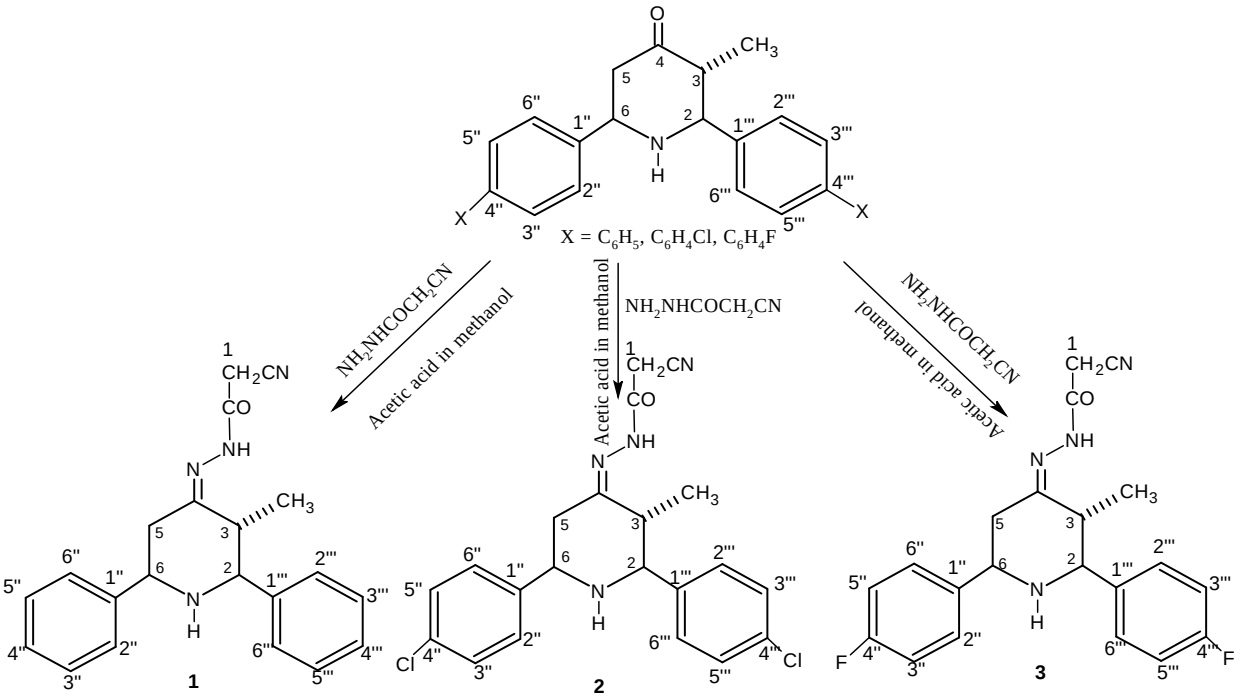
t (3)-alkyl-*r*(2), *c* (6)-Diarylpiperidin-4-onecyanoaceticacidhydrazones (1-3)

A mixture of Corresponding 3t-alkyl-2r,6c-diarylpiperidin-4-one (25 mmol), Cyanoaceticacid hydrazide (25 mmol) and acetic acid (0.5 mL) in methanol (10 mL) was refluxed for one hour. A solid mass was formed. The separated solid was collected on a Buchner funnel and washed with ice-cold water. Then it was recrystallized from methanol

3. Results and Discussion

t (3)-alkyl-*r*(2), *c* (6)-Diarylpiperidin-4-onecyanoaceticacidhydrazones **[1-3]**

In 1948 Noller and Baliah[9] reported a very convenient and non-laborious one-pot synthesis of 3*t*-alkyl-2*r*,6*c*-diarylpiperidin-4-ones by the condensation of methyl ketones, aromatic aldehydes and ammonium acetate in 1:2:1 molar. This method has been used successfully for the synthesis of variously substituted pipridin-4-ones.[10,11] Manimekalai and co-workers have synthesized several substituted 3*t*-alkyl-2*r*,6*c*-difuranylpiperidin-4-ones and 3*t*-benzyl-2*r*,6*c*-diarylpiperidin-4-ones by adopting this method. These piperidin-4-ones have been converted to hydrazones, semicarbazones [16], thiosemicarbazones [17] and phenylhydrazone [18] by the reaction of the carbonyl group with suitable reagents. Three t(3)-alkyl-*r* (2), *c* (6)-Diarylpiperidin-4-onecyanoaceticacidhydrazones **[1-3]** (**Scheme 1**) were synthesized in the present study. The antimicrobial activity and their conformational behavior was analyzed using ^{1}H and ^{13}C NMR spectroscopy. The physical data of **1-3** are given in **Table 1.**



Scheme 1

Table 1. Physical data of compounds 1-3

Compound	Yield (%)	mp°C
1	150-152	92
2	170-172	85
3	177–179	86

For all these compounds IR, ^{1}H NMR and ^{13}C NMR spectra have been recorded. The important IR stretching frequencies are given in **Table 2**.

Table 2. Characteristic IR stretching frequencies (cm^{-1}) of compounds 1-3

Assignments	Compounds		
	1	**2**	**3**
ν_{C-H}	2981.32	2924.85	2962. 23
$\nu_{C=O}$	1688.78	1686.39	1654.26
$\nu_{C=C}$	1516.94 1446.88	1491.22 1454.62	1524.65 1432.76
$\nu_{C=N}$	1602..23	1571.65	1596.. 32
$\nu_{C\equiv N}$	1172.66	1170.51	1164.34
ν_{N-H} (pip)	3350.86	3441.10	3306.26
ν_{N-H} (Amide)	3309.25	3270.12	3270.06

Assignments of NMR signals

A. ^{1}H NMR Spectra

The high resolution ^{1}H NMR spectra of t(3)-methyl-r(2), *c*(6)-diphenylpiperidin-4-onecyano aceticacid hydrazone (**1**), t(3)-methyl-r(2),*c*(6)-bis(*p*-chlorophenyl) piperidin-4-onecyanoacetic acid hydrazone (**2**) and t(3)-methyl-r(2),*c*(6)-bis(*p*-fluorophenyl)piperidin-4-onecyanoacetic acid hydrazone (**3**) have been recorded in DMSO-d_6 and analyzed. The signals were assigned based on their positions, integrals and multiplicities. The numbering of carbon atoms in **1–3** is shown in **Scheme 1**. Protons are numbered accordingly. Thus, the proton at C-2 is denoted as H-2 and that at C-5is denoted as H-5. **Tables 3** and **4** report the chemical shifts and coupling constants observed for **1-3**.

Table 3. ^{1}H NMR data (ppm) of t(3)-methyl-r(2),*c*(6)-diarylpiperidin-4-onecyanoaceticacidhydrazone 1-3.

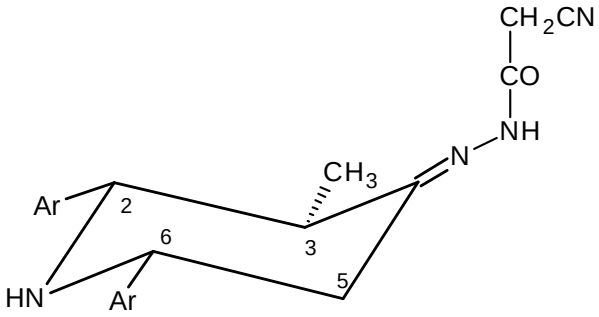
Proton	Compound		
	1	**2**	**3**
H–2a	3.19	3.17	3.17
H–3a	2.64	2.67	2.67
H–5e	3.34	3.38	3.37
H–5a	2.04	2.03	2.03
H–6a	3.28	3.26	3.26
H–2″ and H–6″	7.53	7.52	7.51
H–2‴ and H–6‴	7.51	7.50	7.50
H–3″ and H–5″	7.36	7.34	7.33
H–3‴ and H–5‴	7.38	7.38	7.38
H–4″ and H–4‴	7.26	-	-
H^{1}	3.82	3.81	3.81
3-Alkyl protons	0.88	0.86	0.86
NH(pip)	2.63	2.62	2.62
NH	11.34	11.32	11.32

Table 4. *Vicinal* coupling constants (Hz) of 1-3.

Compound	$J_{2a,3a}$	$J_{6a,5a}$	$J_{5e,5a}$	$J_{6a,5e}$	$J_{CH,CH3}$
1	10.0	11.5	14.0	2.5	6.5
2	9.5	11.5	12.0	a	6.0
3	9.5	11.5	12.0	a	6.0

[a]H-6 and H-5e were observed only as a doublets due to poor resolution.

The observed vicinal proton-proton coupling constants suggest that **1-3** adopts chair conformation with the equatorial orientations of all substituent's as shown in **Scheme 2**.



1. Ar =C_6H_5, **2**. Ar = p-ClC_6H_4 **3**. Ar = p-FC_6H_4

Scheme 2

B. ^{13}C NMR SPECTRA

3-methyl-r(2),*c*(6)-diarylpiperidin-4-onecyanoaceticacidhydrazones **[1-3]**

^{13}C NMR spectra have been recorded in DMSO-d6 for the diarylpiperidin-4-onecyanoaceticacidhydrazone **1-3**. The aromatic carbons could easily be distinguished by their characteristic absorption around120 ppm. The ipso carbons should absorb at higher frequency compared to other aromatic carbons. The signals around 60-70 ppm are assigned to benzylic carbons C(2) and C(6) in **1-3**. Among these signals the one at higher frequency is assigned to C(2) based on the known deshielding effect of isopropyl group at C(3). The signals around 162 ppm are due to C=O carbons. The low frequency (upfield) signals around 21 and 13 ppm are due to methine carbons and methyl carbon of CH_2CN and CH_3 at C(3). The C(3) and C(5) carbons absorb in the region ≈45 and ≈36 ppm respectively. **Table 5** report the ^{13}Cchemical shifts observed for **1-3**.

Table.5 ^{13}C NMR data (ppm) of 3-methyl-r(2),*c*(6)-diarylpiperidin-4-onecyanoaceticacidhydrazone (1-3).

carbon	Compound		
	1	**2**	**3**
–C=O	162.5	162.5	162.5
C–2	69.29	69.25	69.24
C–3	44.56	44.56	44.56
C–4	162.5	162.5	162.5
C–5	36. 36	36. 35	36. 35
C–6	60.71	60.70	60.70
C–1′	21.5	21.3	21.3
C–1″	143.8	143.6	143.5
C–1‴	143.6	143.4	143.3
C–2″, C–6″	128.5	128.4	128.4
C–2‴, C–6‴	127.6	127.2	127.2
C–3″, C–5″	128.7	127.8	127.7
C–3‴, C–5‴	128.5	128.4	128.4
C–4″, C–4‴	127.5, 127.5	129.2, 131.9	129.2, 131.8
CH_3	12.97	12.96	12.97

Antimicrobial Activity

The preliminary antimicrobial activities of compounds **1-3** were examined using two fold serial dilution methods.

Antibacterial study

The bacterial strains *viz., Staphylococcus aureus, Streptococcus aureus, Salmonella typhi, Klebsiella pneumoniae and Escherichia coli* were used for this study. DMSO was used as control while Cefotaxime is used as standard. The MIC values for antibacterial activities are given in **Table 6**. It is seen that Compounds **2** and **3** is more active than the standard against bacterial strains except *Escherichia coli*. It is also seen that compounds with a halogen atom in the aromatic ring are more active than compounds without an aromatic substituent in the aromatic ring.

Table 6. *In vitro* antibacterial activity of compounds 1-3

Compounds	Minimum inhibitory concentration (MIC) in µM				
	Staphylococcus aureus	*Streptococcus aureus*	*Salmonella typhi*	*Klebsiella pneumoniae*	*Escherichia coli*
1	222.72	222.72	222.72	a	a
2	12.06	12.06	48.27	24.10	193.0
3	12.00	11.03	46.25	24.00	190.0
Cefotaxime	54.89	54.89	109.78	109.78	54.89

'a' no inhibition even at 200 µg/mL

Antifungal activity

The fungal strains *viz., Candida albicans, Aspergillus flavus, Aspergillus niger and Cryptococcus neoformans* were used for this study. DMSO was used as control while Miconazole is used as standard. The MIC values for antibacterial activities are given in **Table 7**.

It is seen that **2** and **3** are more active than the standard against all the tested fungal stains. It is also seen that compounds with a halogen atom in the aromatic ring is found to increase the antifungal activity.

Table 7. *In vitro* antifungal activity of compounds 1-3

Compounds	**Minimum inhibitory concentration (MIC) in µM**			
	Candida albicans	***Aspergillus flavus***	***Aspergillus niger***	***Cryptococcus neoformans***
1	111.40	55.70	111.40	111.40
2	48.30	24.1	24.1	48.3
3	47.15	23.2	23.3	46.5
Miconazole	60.01	120.15	120.15	60.01

4. Conclusion

The observed vicinal proton-proton coupling constants suggest that **1-3** adopts chair conformation. In these conformations the aryl groups are equatorial and the alkyl group at C-3 is equatorial in the 3t-alky compounds. In vitro antibacterial activities have been studied for Compounds **2** and **3** are more active than the standard against bacterial strains except *Escherichia coli.* In vitro antifungal activities have been studied for Compounds **2** and **3** are more active than the standard against all the tested fungal stains. It is also seen that compounds with a halogen atom in the aromatic ring is found to increase the antifungal activity.

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