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Stereoselective synthesis, spectral and antimicrobial studies of some cyanoacetyl hydrazones of 3-alkyl-2,6-diarylpiperidin-4-ones

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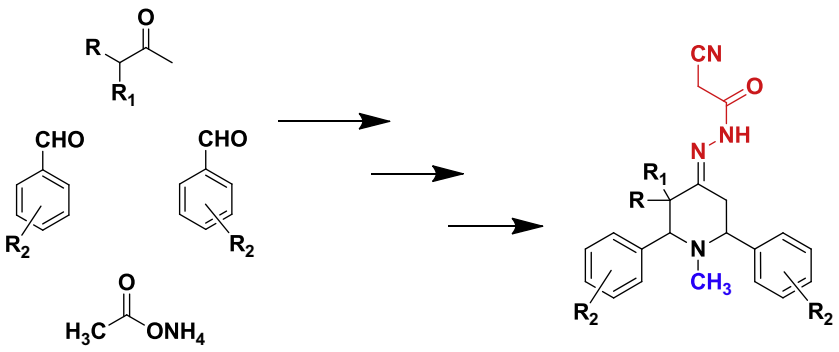
HIGHLIGHTS

- Stereoselective synthesis of some cyanoacetyl hydrazones.
- Characterization through IR, Mass, ^{1}H NMR, ^{13}C NMR and 2D NMR spectral techniques.
- Establishment of stereochemistry through NMR spectra.
- *In-vitro* evaluation of antibacterial and antifungal potencies.

GRAPHICAL ABSTRACT



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ABSTRACT

A series of novel cyanoacetyl hydrazones of 3-alkyl-2,6-diarylpiperidin-4-ones were synthesized stereoselectively and characterized by IR, Mass, ^{1}H NMR, ^{13}C NMR, ^{1}H–^{1}H COSY and ^{1}H–^{13}C COSY spectra. The stereochemistry of the synthesized compounds was established using NMR spectra. Antimicrobial screening of the synthesized compounds revealed their antibacterial and antifungal potencies. Growth inhibition of *Enterobacter Aerogenes* by compound **15** was found to be superior to the standard drug.

Introduction

The piperidine ring is a common structural feature of many alkaloids, natural products and drugs. In the past decades there have been many piperidine derivatives [1] used in clinical and preclinical trials. Piperidin-4-one is an active intermediate in the manufacture of fentanyl analogues [2–4], which are synthetic primary μ-opioid agonists and potent narcotic analgesics. Besides, multisubstituted piperidines are being building units [5–7] for the synthesis of human GABA-A receptor agonists, farnesyl protein transferase inhibitors and optically active indole alkaloids. The blocking of α-positions with respect to the nitrogen atom in the piperidine ring by alkyl groups would improve the biological activity [8]. 3/5-Alkyl-2,6-diarylpiperidin-4-ones possess certain most desirable biological properties such as anti-hypertensive [9], antimicrobial [10], analgesic [11], and antitumor [12]. Several 3-substituted-2,6-diarylpiperidin-4-ones [13] have been evaluated for their antimicrobial efficiency by several researchers.

Hydrazones [14] are important molecules containing highly reactive azomethine group (—CO—NH—N=CH—) and are useful in drug development. Hydrazides [15] and their derivatives show a

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wide range of biological activities such as antibacterial and tuberculostatic properties. Moreover, hydrazine and acyl hydrazine moieties [16] are also found to be the most important pharmacophore in many anti-inflammatory, anti-nociceptive and anti-platelet drug molecules. Cyanoacetyl hydrazones have been reported to exhibit antitumor activity [17–19] against several cell lines. The molecules containing piperidine as well as nitrile moieties [20] such as Pericyazine, Alogliptin, Levocabastine, Piritramide, Diphenozylate are known for their excellent biological potencies.

As the reactivity as well the pharmacological properties of the drugs depend mostly on their stereochemistry, trials are being continued to develop new synthetic procedures that lead to stereoselectivity [21,22]. Among the several spectral techniques available, NMR spectroscopy is a well established method for configurational and conformational studies since the stereoisomers differ considerably in their NMR spectra [23]. Hence, in the present work the stereoselective synthesis of such hybrid molecules *viz.* cyanoacetyl hydrazone derivatives of piperidin-4-ones, NMR spectral studies along with others to establish the stereochemistry and evaluation of their antibacterial and antifungal potencies against some selected micro-organisms have been focused.

Experimental

Materials and methods

All the solvents used for recrystallization and thin layer chromatography were of analytical grade and used without further purification. The progress as well as the completion of reactions was monitored by thin layer chromatography on silica gel precoated aluminum sheets (Type 60 GF254, Merck). All the final compounds were purified by repeated crystallization from suitable solvents. IR spectra were recorded on an AVATAR-330 FT-IR spectrometer (Thermo Nicolet) using KBr (pellet form). The mass spectra of the synthesized compounds were recorded on a Varian Saturn 2000 GC–MS/MS spectrometer using electron impact technique. ^{1}H and ^{13}C NMR spectra for all the compounds were recorded at 400 MHz and 100 MHz, in a Bruker instrument, using $CDCl_3$ as solvent. Tetramethylsilane (TMS) was used as an internal reference for all NMR spectra, with chemical shifts reported in δ units (parts per million) relative to the standard. ^{1}H NMR splitting patterns are designated as singlet (s), broad singlet (bs), doublet (d), doublet of doublets (dd), triplet (t) and multiplet (m). Coupling constants are expressed in Hertz (Hz).

*Procedure for the synthesis of compounds **15-28***

The parent compounds (**1–14**) were synthesized by Mannich condensation of aromatic aldehydes, ketones and ammonium acetate in ethanol. Compounds **1–3** were methylated by methyl iodide in the presence of K_2CO_3 and acetone at refluxing conditions. The title compounds **15–28** were prepared as follows: A mixture of 3-alkyl-2,6-diphenylpiperidin-4-ones (2 mmol) and cyanoacetic hydrazide (2 mmol) in ethanol (10 mL) was refluxed for 3–6 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the solid product separated on cooling was collected by filtration and washed well with water. Pure sample was obtained by recrystallization from 1:1 mixture of ethanol and ethyl acetate.

Procedure for antimicrobial assay

The antimicrobial activities of the synthesized compounds against different pathogens were determined by Agar Well diffusion method or cork borer method.

Using a sterile inoculation loop, 20 pure colonies of the test organism were transferred to 5 ml of sterile nutrient broth and incubated at 37 °C for 18 h. The modified agar well diffusion method of Perez et al. [27] was employed. Each selective medium was inoculated with the microorganism suspended in sterile water. Once the agar was solidified, it was punched with 6 mm diametre wells and filled with 25 μL of the sample and blank (ethanol and antibiotic). The test was carried out by triplicate. The plates were incubated at 35 ± 2 °C for 24 h. The antimicrobial activity was calculated by applying the expression in mm.

Results and discussion

A series of new cyanoacetyl hydrazones of 3-alkyl-2,6-diarylpiperidin-4-ones were prepared (Scheme 1) in good to excellent yields by the condensation of cyanoacetic hydrazide with piperidin-4-ones. A higher degree of diastereoselectivity (selective formation of *E* isomer) was achieved owing to the presence of alkyl substituent adjacent to the carbonyl group which decides the orientation of the hydrazone moiety (—NH—N=) as *anti* with respect to it. Therefore the stereoselectivity of the reaction is ascertained as substrate controlled. The numbering of carbon atoms in the compounds is shown in Fig. 1 and the protons are numbered accordingly. The newly synthesized hydrazones were characterized by IR, Mass, ^{1}H NMR, ^{13}C NMR, ^{1}H–^{1}H COSY and ^{1}H–^{13}C COSY spectral studies. The signals in the ^{1}H NMR spectra were assigned based on their chemical shift values, splitting pattern, coupling constants and correlations in the 2-D NMR spectra for compound **26** and for other compounds the signals were assigned by comparing chemical shift values of them with the former. The ^{1}H–^{1}H COSY and ^{1}H–^{13}C COSY unraveled the problems during assignment of the signals in ^{1}H and ^{13}C NMR spectra.

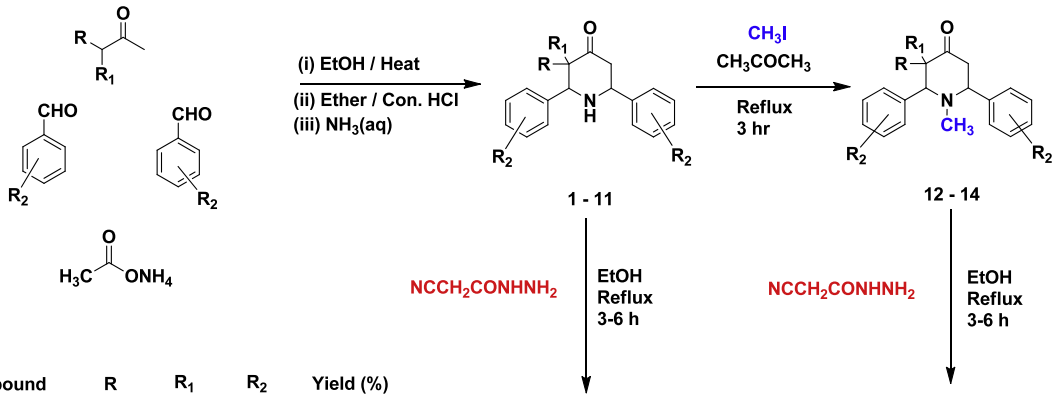
Analysis of IR and mass spectra

Infrared spectra of the synthesized compounds exhibited significant absorptions pertaining to the functional groups present such as imino(C=N), carbonyl(C=O),cyano(CN) and the absorption frequencies (in cm^{-1}) are presented in Table 1. Mass spectra of recorded compounds have shown $(M + H)^{+}$ values and the values are included in Table 1.

Analysis of ^{1}H NMR spectra

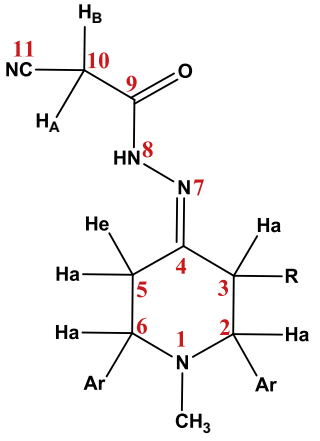
The ^{1}H chemical shifts of all the synthesized compounds are tabulated in Table 2. The ^{1}H NMR spectrum of compound **26** is shown in Fig. 2. In the ^{1}H NMR spectrum of compound **26**, a sharp singlet found in the downfield region at 9.21 ppm corresponding to one proton is ascribable to the —CONH— hydrogen of the hydrazone moiety. All the protons of the phenyl rings at C2 and C6 carbons resonate from 7.47 ppm to 7.29 ppm as multiplets corresponding to 10 hydrogens. The methyl protons at nitrogen (N1) and C3 are observed as sharp singlet and doublet respectively in the upfield region at 1.73 ppm and 0.81 ppm corresponding to three protons each.

Two closely spaced doublets forming an AB quartet around 3.78 ppm correspond to two protons are assigned as the methylene protons of C10 based on their spin–spin coupling constant values. The actual chemical shift values of these diastereotopic geminal protons exhibiting AB spin system of coupling was found out using second order spectral analysis [24]. Further, a multiplet centered at 2.69 ppm with one proton integral is assignable to H3 proton. In addition, a doublet at 3.22 ppm with a large vicinal coupling constant ^{3}J = 11.2 Hz showing cross peak in the ^{1}H–^{1}H COSY spectrum



Compound	R	R_1	R_2	Yield (%)
15	CH_3	H	H	82
16	CH_3CH_2	H	H	85
17	CH_3	CH_3	H	78
18	CH_3	H	*p*-CH_3	81
19	CH_3	H	*p*-OCH_3	83
20	CH_3	H	*o*-Cl	77
21	CH_3	H	*m*-NO_2	75
22	CH_3	H	*p*-F	78
23	CH_3	H	*p*-Cl	82
24	CH_3	H	*p*-Br	80
25	CH_3	H	*o*-,*m*-,*p*-OCH_3	84
26	CH_3	H	H	87
27	CH_3CH_2	H	H	86
28	CH_3	CH_3	H	82

Scheme 1. Synthetic scheme of cyanoacetyl hydrazones **15–28.**



Ha - Axial hydrogens;
He - Equatorial hydrogens;
H_A and H_B - Diastereotopic hydrogens

Fig. 1. Numbering pattern followed for compounds **15–28** to explain NMR spectra.

Table 1
Significant IR absorption frequencies (in cm^{-1}) and $(M + H)^+$ values of compounds **15–28.**

Compound	—NH—	—CN	>C=O	>C=N—	Mass (observed) $(M + H)^+$
15	3208	2262	1699	–	–
16	3063	2257	1711	–	–
17	3187	2266	1675	–	–
18	3194	2255	1705	–	375.70
19	3189	2263	1685	1610	407.70
20	3179	2266	1681	–	–
21	3194	2266	1683	1526	–
22	3192	2264	1684	–	–
23	–	2260	1697	1594	–
24	–	2263	1693	1593	503.10
25	3197	2257	1687	–	527.70
26	3166	2261	1681	1600	361.40
27	3170	2266	1679	1633	375.50
28	3176	2258	1686	1600	–

(Fig. 4) with the proton at 2.36 ppm is attributable to the H6 proton and the latter is perceivable as the H5a proton. The large vicinal coupling (>10 Hz) observed between them discloses their antiperi-planar disposition. The remaining two signals at 2.88 and 2.83 ppm are conspicuously assigned for H2 and H5e protons respectively based on their coupling constants [$^3J_{(H2a,\ H3a)}$ = 10 Hz and $^2J_{(H5e,\ H5a)}$ = 14.4 Hz] as well as their correlations in the ^1H–^1H COSY spectrum.

Table 2
^{1}H chemical shifts, splitting pattern and spin–spin coupling constant values of compounds **15–28.**

Compound	—CONH—	$10H_A$, $10H_B$	H6a	H2a	H5e	H3a	H5a	N–CH_3/NH	C3–Me/Others	Aromatic
15	9.86(s)	H_A 3.81 (d, J = 18.4 Hz) H_B 3.75(d, J = 18.4 Hz)	3.91 (d, J = 11.2 Hz)	3.52 (d, J = 9.6 Hz)	3.02 (d, J = 12.4 Hz)	2.57 (m)	2.20 (t, J = 12.8 Hz)	2.04 (bs)	0.88 (d, J = 6 Hz)	7.51–7.25 (m)
16	9.93(s)	H_A 3.84(d, J = 18.4 Hz) H_B 3.74(d, J = 18.4 Hz)	3.91 (d, J = 11.2 Hz)	3.64 (d, J = 10 Hz)	3.03 (d, J = 13.6 Hz)	2.46 (t, J = 8.4 Hz)	2.19 (t, J = 12.4 Hz)	1.97 (s)	C3–Me 0.83 (t, J = 6 Hz) CHH 1.60 (m) CHH 1.27 (m)	7.51–7.27 (m)
17	9.41(s)	Signal merged with H6a and H5e 3.89–3.74	3.87 (d, J = 11.6 Hz)	Merged with CH_2 signal 3.78–3.74	2.80 (d, J = 13.6 Hz)	–	2.39 (t, J = 12.4 Hz)	1.93 (s)	CH_3 1.19(s) CH_3′1.04(s)	7.58–7.31 (m)
18	9.75(bs)	H_A 3.80(d, J = 18 Hz) H_B 3.75 (d, J = 18 Hz)	3.86 (d, J = 12.8 Hz)	3.47 (d, J = 9.6 Hz)	2.97 (d)	2.54 (m)	2.17 (t, J = 12.8 Hz)	1.74 (bs)	0.87 (d, J = 6 Hz) $ArCH_3$ 2.34 (s), 2.33 (s)	7.37 (d, J = 7.2 Hz), 7.32 (d, J = 7.2 Hz), 7.16 (d, J = 7.2 Hz)
19	9.61(s)	Merged with —OCH_3 signal 3.85–3.79	Merged with —OCH_3 signal 3.85–3.79	3.46 (d, J = 10 Hz)	2.93 (d, J = 13.6 Hz)	2.51 (m)	2.17 (t, J = 12.8 Hz)	1.92 (bs)	0.87 (d, J = 6 Hz)	7.41 (d, J = 8 Hz), 7.35 (d, J = 8 Hz), 6.88 (d, J = 8 Hz)
20	9.10(s)	H_A 4.35(s) H_B 4.30(s)	Merged with H5e signal 3.83	Overlapped with H6a signal 3.83	3.30 (s)	2.63 (s)	Merged with NH signal	2.10 (s)	0.97(s)	7.77–7.26 (m)
21	11.14(s)	Unresolved multiplet 3.86(m)	4.07 (d, J = 11.6 Hz)	3.69 (d, J = 9.6 Hz)	3.48 (d, J = 13.6 Hz)	3.18 (m)	Merged with NH signal 2.09	2.04 (s)	0.89 (d, J = 5.6 Hz)	8.43 (s), 8.38 (s), 8.18 (d, J = 8 Hz), 8.13 (d, J = 8 Hz), 7.97 (d, J = 6.4 Hz), 7.86 (bs),7.59 (m)
22	10.02(s)	H_A 3.83(d, J = 18.8 Hz) H_B 3.75(d, J = 18 Hz)	3.91 (d, J = 11.2 Hz)	3.51(d, J = 9.6 Hz)	3.04 (d, J = 13.6 Hz)	2.51 (m)	2.15 (t, J = 12.4 Hz)	2.01 (bs)	0.87 (d, J = 4.8 Hz)	7.50 (s), 7.42 (s), 7.05 (t, J = 7.2 Hz)
23	10.08(s)	H_A 3.84(d, J = 18.8 Hz) H_B 3.74(d, J = 18.4 Hz)	3.91 (d, J = 11.2 Hz)	3.50 (d, J = 10 Hz)	3.05 (d, J = 13.6 Hz)	2.50 (m)	2.13 (t, J = 12.8 Hz)	2.00 (bs)	0.87 (d, J = 6 Hz)	7.47 (d, J = 7.6 Hz), 7.39 (d, J = 7.2 Hz), 7.34 (d, J = 7.2 Hz)
24	9.36(bs)	Merged with H6a signal 3.86–3.73	3.87 (d, J = 8.4 Hz)	3.49 (d, J = 10 Hz)	2.90 (d, J = 14 Hz)	2.51 (bs)	2.15 (t, J = 12.8 Hz)	Merged with H5a signal	0.88 (d, J = 5.6 Hz)	7.49 (d, J = 7.2 Hz), 7.38 (d, J = 7.6 Hz), 7.33 (d, J = 7.2 Hz)
25	9.24(s)	Merged with OCH3 signal 3.89–3.78	4.11 (d, J = 6.4 Hz)	3.43(d, J = 9.6 Hz)	2.88 (d, J = 13.2 Hz)	2.55 (m)	2.22 (t, J = 12.4 Hz)	2.04 (s)	0.93 (d, J = 5.2 Hz)	6.67 (d, J = 6 Hz)
26	9.21(s)	H_A 3.80(d, J = 18.4 Hz) H_B 3.75(d, J = 18.4 Hz)	3.22 (d, J = 11.2 Hz)	2.88 (d, J = 10 Hz)	2.83 (d, J = 14.4 Hz)	2.69 (m)	2.36 (t, J = 13.2 Hz)	1.73 (s)	0.81 (d, J = 6 Hz)	7.47–7.29 (m)
27	9.84(s)	H_A 3.18(d, J = 18.4 Hz) H_B 3.70(d, J = 18.4 Hz)	3.28 (d, J = 11.2 Hz)	3.01 (d, J = 10 Hz)	2.94(d, J = 14 Hz)	2.54 (m)	2.34 (t, J = 12.8 Hz)	1.71 (s)	0.79 (t, J = 7.2 Hz) CH 1.54 (m), CH 1.14(m)	7.49–7.25 (m)
28	9.68(s)	Unresolved signal 3.78–3.75	3.17 (bs)	3.06 (bs)	2.78 (s)	–	2.51 (bs)	1.76 (s)	3 Me 1.26 (s) 3 Me′ 0.93 (s)	7.52–7.31 (m)

Analysis of ^{13}C NMR spectra

The ^{13}C NMR spectral data of compounds **15–28** are tabulated in Table 3. The ^{13}C NMR spectrum of compound **26** is shown in Fig. 3. The ^{13}C NMR spectrum of compound **26** shows two less intense signals at 164.8 and 157.0 ppm respectively and they have been assigned to the amido carbon (C9) and C4 carbon of the piperidine ring. The *ipso* carbon atoms of the phenyl rings resonate at 143.3 and 142.5 ppm respectively as less intense signals. All the aromatic carbon atoms collectively resonate between 128.9 and 127.0 ppm. The C11 of cyano group shows its resonance as a less intense signal at 114.0 ppm. The N—CH_3 carbon is found at 41.4 ppm as evidenced by its correlation with respective protons in the ^{1}H–^{13}C COSY spectrum (Fig. 5). Similarly the resonance of C10 carbon

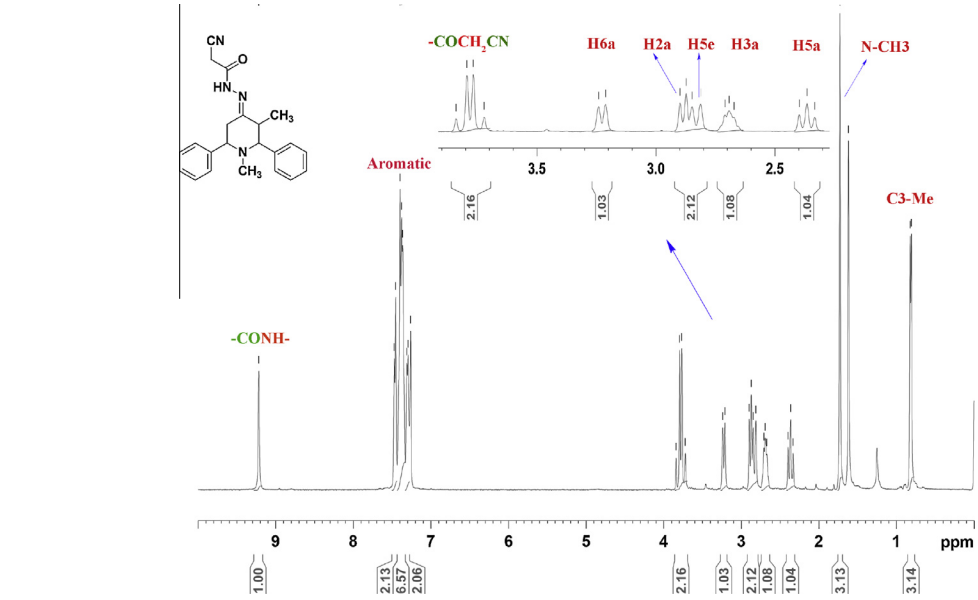


Fig. 2. ^{1}H NMR spectrum of compound **26.**

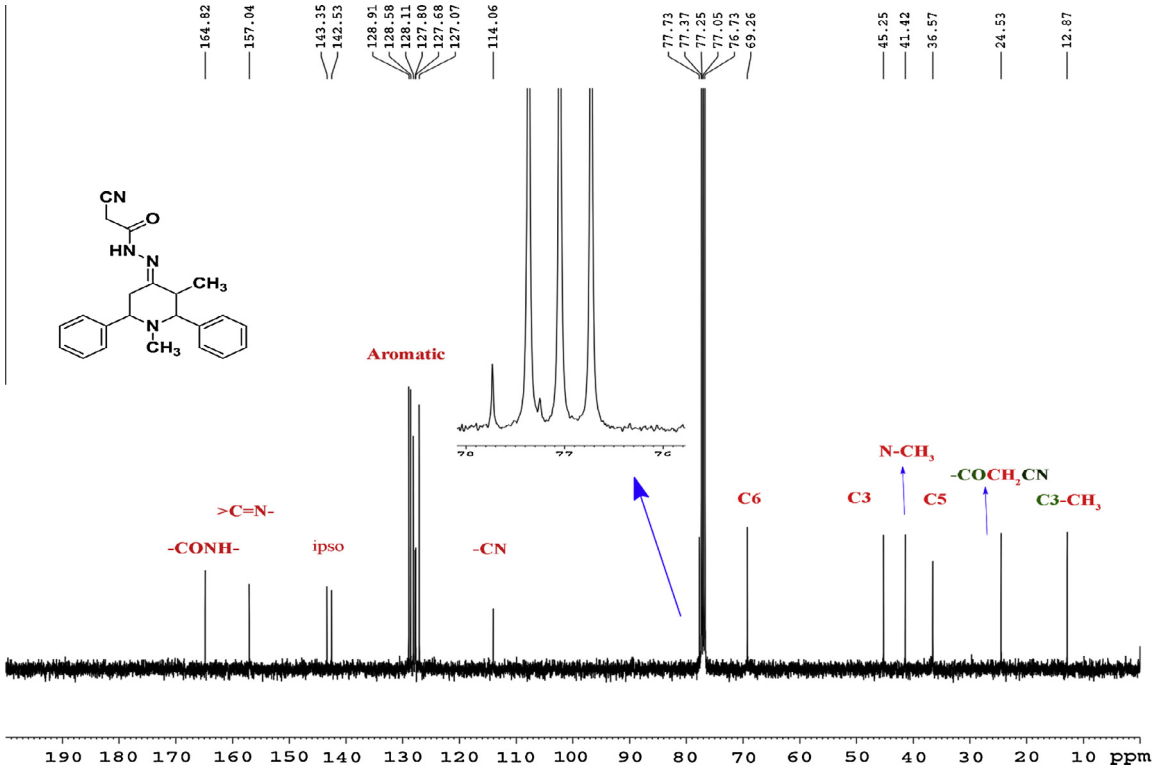


Fig. 3. ^{13}C NMR spectrum of compound **26.**

and C3–Me group are assigned based on their correlations with their protons in the ^{1}H–^{13}C COSY spectrum as 24.5 ppm and 12.8 ppm respectively.

In piperidin-4-ones, it is well known that as a result of hydrazone formation [25], the more electronegative carbonyl group is replaced by a less electronegative C=N (imino group) bond and it causes shielding of C5 & C3 carbons and deshielding of C6 & C2 carbons relatively. Therefore the shielding/deshielding make the chemical shifts of the piperidine ring carbons in the order C2 > C6 > C3 > C5. In this case also the signals are found in the order as per the above expectation. The assignment of signals due to C2 and C6 carbons is done based on the correlations observed in the ^{1}H–^{13}C COSY spectrum. The C2 carbon resonates almost along with the solvent ($CDCl_3$) peak at 77.7 ppm while the signal of C6 carbon is found at 69.2 ppm. The C6 carbon is relatively more shielded than C2 because, it acquires a slight negative charge transferred from C5 carbon as a result of hydrazone formation [25]. The signals of C3 and C5 carbon were differentiated with the help of ^{1}H–^{13}C COSY spectrum as 45.2 ppm and 36.5 ppm. The downfield shift up to 8.6 ppm of C3 carbon is caused by the

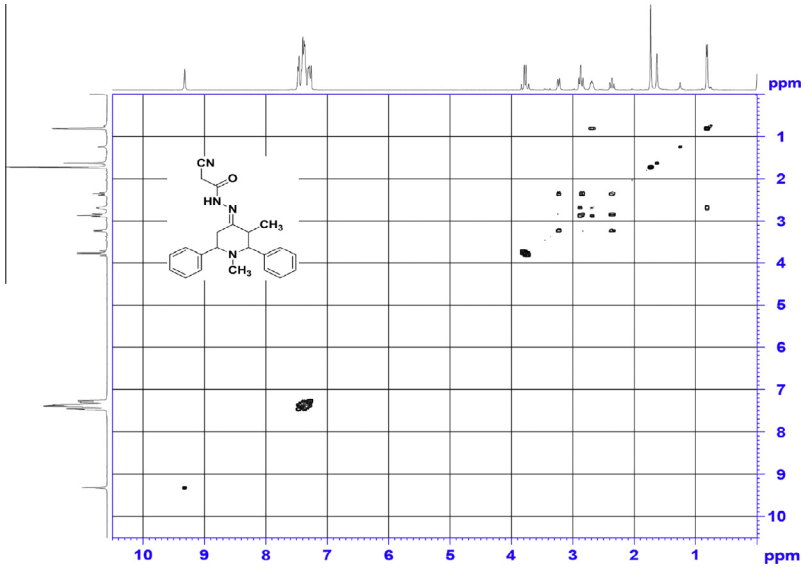


Fig. 4. ^{1}H–^{1}H COSY spectrum of compound **26.**

Table 3
^{13}C chemical shift values of compounds **15–28**.

Compound	—NHCO—	C4	—CN	C2	C6	C3	N–CH_3	C5	C10	C3–Me/others	Aromatic
15	165.1	158.0	114.1	69.2	60.7	45.3	–	36.1	24.5	C3–Me 12.1	*Ipso* 142.7, 142.2 Others 128.7–126.6
16	165.2	157.0	114.1	67.5	60.7	52.0	–	36.6	24.4	$CH_3$12.0, $CH_2$19.0	*Ipso* 142.7, 142.1 Others 128.7–126.6
17	165.2	161.5	114.1	70.4	61.0	43.5	–	32.4	24.6	C3–Me 22.6, C3–Me′ 21.0	*Ipso* 143.0, 139.9 Others 129.1–126.3
18	165.3	158.4	114.2	68.9	60.4	45.3	–	36.2	24.5	C3–Me 12.1 $ArCH_3$ 21.2 $ArCH_3'$ 21.1	*Ipso* 139.9, 139.4 Others 137.6–127.5
19	165.1	158.2	114.0	68.5	60.1	45.4	–	36.3	24.5	C3–Me 12.1 $ArOCH_3$ 55.3 $ArOCH_3$ 55.3	*Ipso* 135.0, 134.5 159.2, 159.2 Others 128.8–127.7
20	164.9	156.3	114.0	63.0	56.5	45.4	–	33.6	24.7	C3–Me 11.5	*Ipso* 134.0, 132.5, 139.5 Others 129.7–127.4
21	164.8	154.9	114.6	67.7	59.0	44.4	–	35.9	24.3	C3–Me 11.7	*Ipso* 147.8, 147.6 144.9, 144.1 Others 121.4–134.1
22	165.6	157.9	114.1	68.3	59.9	45.5	–	36.4	24.5	C3–Me 12.0	*Ipso* 138.5, 138.0 Others 129.3–115.3
23	165.6	157.6	114.1	68.3	59.8	45.3	–	36.2	24.5	C3–Me 12.0	*Ipso* 141.1, 140.6, 133.7, 133.6 Others 129.1–127.9
24	164.9	156.7	114.0	68.4	60.0	45.2	–	35.9	24.6	C3–Me 12.0	*Ipso* 141.5, 141.0 Others 131.9–128.3
25	164.7	157.3	114.1	69.5	56.2	45.3	–	36.0	24.6	C3–Me 12.1–OCH_3 61.1, 60.9, 60.4	*Ipso* 153.4, 153.2, 138.3, 137.7, 137.6 Others 104.6, 103.5
26	164.8	157.0	114.0	77.7	69.2	45.2	41.4	36.5	24.5	C3–Me 13.8	*Ipso* 143.3, 142.5 Others 128.9–127.0
27	165.2	156.5	114.1	76.0	69.2	52.1	41.4	37.0	24.4	CH_2 19.7 CH_3 12.0	*Ipso* 143.5, 142.5 Others 128.8–127.1
28	165.3	161.2	114.1	79.1	69.6	43.2	42.5	32.9	24.5	C3–Me 23.6 C3-Me′ 22.2	*Ipso* 143.9, 139.2 Others 129.9–127.0

replacement of one hydrogen atom with a methyl group and comparatively more upfield shift of C5 carbon is because of the interaction between the nitrogen atom attached to the imino group and C5 carbon resulting in the negative polarization of C5 carbon [25].

Analysis of 2-D NMR spectra

The correlation spectroscopy has its own significance in assigning signals of ^{1}H and ^{13}C NMR spectra unambiguously. The assignment of signals in both ^{1}H and ^{13}C NMR spectra are reassured by

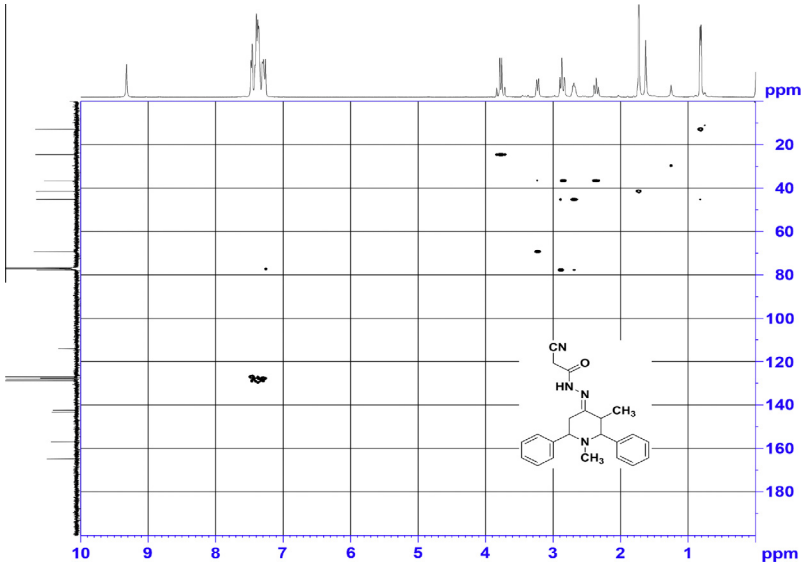


Fig. 5. $^{1}H–^{13}C$ COSY spectrum of compound **26.**

recording $^{1}H–^{1}H$ COSY and $^{1}H–^{13}C$ COSY spectra for the representative example **26** (Figs. 4 and 5) and the correlations are shown in Table 4. The results are interpreted as follows.

There are significant correlations in the $^{1}H–^{1}H$ COSY spectrum between the protons at 3.22 ppm & 2.36 ppm and between the latter one with the signal at 2.83 ppm due to H6a, H5a and H5e protons which constitute an AMX spin system of coupling. The signal at 2.69 ppm correlating with two other signals at 2.88 ppm and 0.81 ppm signifies the H3a and the remaining two as H2a and C3–Me group. The methylene protons of the cyanoacetyl group correlating with each other in the $^{1}H–^{1}H$ COSY spectrum substantiate their second order coupling behavior arising out of AB spin system.

In the $^{1}H–^{13}C$ COSY spectrum, the signal at 36.5 ppm correlated with protons at 2.83 ppm (H5e) and 2.36 ppm (H5a) is accountable for C5 carbon while the signal at 69.2 ppm correlated to H6 (3.22 ppm) is attributable to C6 carbon. In addition, there are significant correlations between ^{13}C and ^{1}H signals of methylene group of cyanoacetyl moiety. Despite other, the presence of correlations due to N—CH_3, C2 and C3 carbons are also obvious from the $^{1}H–^{13}C$ COSY spectrum. These correlations confirm the assignment of ^{1}H and ^{13}C signals in the NMR spectra.

Stereochemistry

The large coupling observed between H6 & H5 and H2 & H3 protons [$^{3}J_{(H6a,\ H5a)}$ = 11.2 Hz and $^{3}J_{(H2a,\ H3a)}$ = 10.0 Hz] supports the *trans* axial orientation of the ring protons and consequently the equatorial orientation of the substituents attached to the heterocyclic ring. This observation strongly advocates to a stable chair conformation for compound **26** and its analogues. Further, it is observed in the ^{13}C NMR spectrum of the compound **26** that the C5 carbon is shielded by 8.6 ppm than C3 which is possible only when the cyanoacetyl moiety is *syn* to C5 and thereby it is clear that the configuration of the molecule is '*E*' as evidenced by the literature [26]. From the ^{13}C NMR spectrum along with the

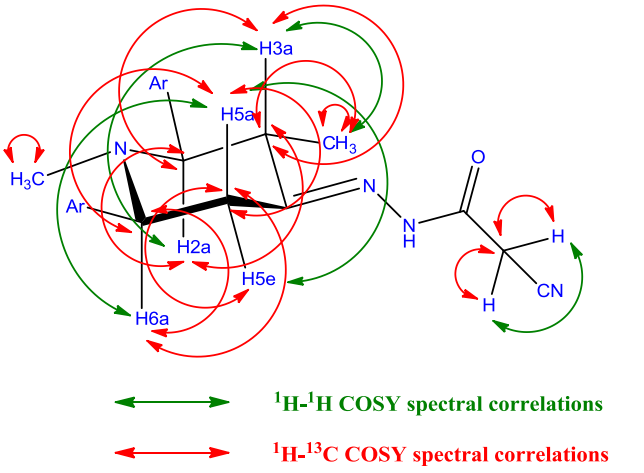


Fig. 6. Selected $^{1}H–H$ COSY, $^{1}H–^{13}C$ COSY spectral correlations and the preferred conformation of compound **26.**

Table 4
$^{1}H–^{1}H$ COSY and $^{1}H–^{13}C$ COSY spectral correlations of compound **26.**

^{1}H chemical shifts in ppm	Correlations in the $^{1}H–^{1}H$ COSY (^{1}H chemical shifts in ppm)	Correlations in the $^{1}H–^{13}C$ COSY (^{13}C chemical shifts in ppm)
7.47–7.29 (Aromatic Hydrogens)	7.47–7.29	128.9–127.0
3.80, 3.75 (H10)	3.75, 3.80	24.5
3.22 (H6a)	2.83, 2.36	69.2, 36.5
2.88 (H2a)	2.69	77.7, 45.2, 36.5
2.83 (H5e)	3.22, 2.36	77.7, 45.2, 36.5
2.69 (H3a)	2.88, 0.81	77.7, 45.2
2.36 (H5a)	3.22, 2.83	69.2, 36.5
0.81 (C3—CH_3)	2.69	45.2, 12.8
1.73 (N—CH_3)	–	41.4

Table 5
Results of anti-microbial screening of compounds **15–28**.

Compound	Bacterial strains										Fungal strains									
	Gram positive				Gram negative															
	S. Aureus		*B. Subtilis*		*E. Coli*		*E. Aerogenes*		*M. Luteus*		*M. Himaelis*		*P. Chrysogenum*		*C. Albicans*		*A. Flavus*		*A. Tamarii*	
	Z.I.	A.I.	Z.I.	A.I.	Z.I.	A.I.	Z.I.	A.I.	Z.I.	A.I.	Z.I.	A.I.	Z.I.	A.I.	Z.I.	A.I.	Z.I.	A.I.	Z.I.	A.I.
15	–	–	12	0.40	–	–	34	1.21	–	–	12	0.60	12	0.34	9	0.26	–	–	–	–
18	–	–	10	0.33	–	–	–	–	–	–	9	0.45	–	–	6	0.17	–	–	22	0.79
19	–	–	8	0.27	–	–	14	0.50	9	0.41	15	0.75	–	–	19	0.56	15	0.44	15	0.54
20	12	0.38	8	0.27	11	0.39	11	0.39	6	0.27	13	0.65	30	0.86	–	–	–	–	18	0.64
21	–	–	–	–	9	0.32	12	0.43	–	–	14	0.70	–	–	12	0.71	22	0.65	–	–
23	–	–	8	0.27	12	0.43	9	0.32	8	0.36	–	–	30	0.86	–	–	–	–	–	–
24	–	–	9	0.30	14	0.50	11	0.39	–	–	14	0.70	25	0.71	12	0.71	15	0.44	21	0.75
26	16	0.50	14	0.46	–	–	–	–	15	0.66	15	0.75	28	0.80	8	0.23	20	0.59	20	0.71
27	–	–	13	0.43	–	–	–	–	–	–	–	–	23	0.66	7	0.20	25	0.74	19	0.68
28	16	0.50	13	0.43	–	–	–	–	–	–	18	0.90	20.	0.57	28	0.80	–	–	–	–
Standard	32	1	30	1	28	1	28	1	22	1	20	1	35	1	35	1	34	1	28	1

Z.I. – Zone of Growth Inhibition; A.I. – Activity Index (Inhibition Zone of Compound/Inhibition Zone of the Standard Drug).
Standard Drug used: For anti–bacterial activity – Ciprofloxacin; For anti–fungal activity – Amphotericin–B.

correlations, it is concluded that the cyanoacetyl group is orienting *anti* to the C3 carbon and the molecule exists as *E* isomer. Selected $^{1}H–^{1}H$ COSY and $^{1}H–^{13}C$ COSY spectral correlations and preferred conformation of compound **26** is illustrated through Fig. 6.

Antimicrobial evaluation

The synthesized compounds were evaluated for their antimicrobial activity against some selected Gram-positive bacteria such as *Staphylococcus Aureus* and *Bacillus Subtilis* and Gram-negative bacteria *Escherichia Coli*, *Enterobacter Aerogenes*, *Micrococcus Luteus* and fungal strains such as *Mucor Himaelis*, *Penicillium Chrysogenum*, *Candida Albicans*, *Aspergillus Flavus* and *Aspergillus Tamarii*. The result of antimicrobial screening is listed in Table 5**.** From the zone of growth inhibition values obtained, it is observed that the most of the compounds have more antifungal activities and moderate antibacterial activities.

The growth inhibition against *S. Aureus* was found to be poor for compound **20** with *o*-Cl substituent; while compounds **26** and **28** with N–Me group are having satisfactory activity. However, all the remaining compounds failed to show any activity. Most of the compounds except compound **21** with electron withdrawing NO_2 substituent at *m*- position of the phenyl ring showed moderate activity against *B. Subtilis*.

Halogen substituted and NO_2 substituted compounds show moderate activity against *E. Coli* while remaining compounds exhibit no activity. In the case of *E. Aerogenes*, compound **15** exhibited excellent activity and compound **19** is moderately active, halo derivatives and NO_2 derivative showed poor activity. The remaining compounds have no ability to control the growth. The compound **26** has a better potency to control the growth of *M. Luteus* and except compounds **19, 20, 23** and **26**, no other compounds are found to act significantly.

Excellent activity against *M. Himaelis* was shown by compound **28** while better to good activity was shown by compounds **15, 19, 20, 24, 21** and **26**. The compounds **20, 23** and **26** exhibited growth control over *P. Chrysogenum* preceded by compounds **24, 27** and **28**. A significant growth control of *C. Albicans* was achieved by compound **28** with an activity index of 0.80. The compounds **24** and **21** also have shown better activity.

A satisfactory growth control was achieved by compounds **27** and **21** over *A. Flavus*. It is pertinent to note that compound **21** with NO_2 substituent was active better toward fungi than bacteria. Good results were given by compounds **18, 24** and **26** for the growth control of *A. Tamarii*. Moderate activity was observed for compounds **19, 20** and **27**.

From the antimicrobial screening, it can be concluded that the screened compounds show better activity toward fungi than bacteria. The compound **15** shows superior activity against *E. Aerogenes* than the standard.

Conclusion

A series of novel cyanoacetyl hydrazones of 3-alkyl-2,6-diarylpiperidin-4-ones were synthesized stereoselectively and characterized by IR, Mass, ^{1}H NMR, ^{13}C NMR, $^{1}H–^{1}H$ COSY and $^{1}H–^{13}C$ COSY spectra. The configuration of the molecules about the newly formed imino bond and the preferred conformation of the heterocyclic ring were established through NMR spectral studies. Antimicrobial screening of the synthesized compounds has been carried out using disc diffusion method for some selected microorganisms. Compound **15** showed superior activity than the standard against the bacterium *E. Aerogenes* while the remaining compounds exhibited moderate to excellent activity against all the tested organisms.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.molstruc.2014.07.050.

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