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Chemical synthesis, spectroscopic analysis, antioxidant, and antimicrobial activities of some hydrazones derived from 4-fluorobenzohydrazide

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ABSTRACT

Variously substituted 2,6-diphenylpiperidin-4-one 4-fluorobenzhydrazides were synthesized by direct condensation of the corresponding 2,6-diphenylpiperdin-4-one with 4-fluorobenzhydrazide. All the compounds are characterized by IR, NMR spectra readings. NMR spectral assignments are made unambiguously by their one-dimensional (H^1 NMR and C^{13} NMR) NMR spectra. All the compounds have screened for their in vitro anti-oxidant and anti-bacterial activity in contradiction of various free radicals, and various bacterial strains respectively. The current study discloses that these compounds could be used as an outline for future development through derivatization to design more potent anti-oxidant and anti-microbial agents.

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1. Introduction

Heterocyclic compounds are organic compounds with a ring structure that contains in the cycle at least one carbon atom and at least another atoms, such as O, N, or S. The most common cycles contain five or six atoms with the stability of these rings being higher than that of three, four, seven or larger rings. However, many heterocyclic compounds with a different number of atoms than five or six are known. Heterocyclic rings can be either non-aromatic or aromatic. Most non-aromatic heterocyclic compounds are similar in their chemical properties to acyclic compounds. Nuclear magnetic resonance spectral studies of heterocyclic compounds have helped in understanding the impact of electronic and conformation effects on chemical shifts and coupling constants on electro-negativities of heteroatoms. H^1 and C^{13}NMR techniques have been extensively applied in deriving stereo-dynamical information about a wide-ranging variety of systems. H^1chemical shifts are prejudiced by the electronic, steric and magnetic antistrophic effects while C^{13}chemical shifts are largely prejudiced by electronic and steric effects only. Vicinal coupling constant values have been used for conformational analysis [1–10]. Such studies have provided information about the dependence of NMR spectral parameters such as chemical shifts and coupling constants on electronegativities of heteroatoms. H^1 and C^{13} NMR methods have been extensively realistic in deriving sterodynamical evidence about a wide variety of schemes. H1 chemical shifts are influenced by electronic, steric and magnetic anisotropic effects whereas C^{13} chemical shifts are chiefly prejudiced by electronic and steric effects only. Vicinal coupling constant values have been used for the conformational analysis as they can give an idea about the positioning of the substituents [11]. Among the wide variety of heterocycles that have been explored for developing pharmaceutically important molecules, piperidin-4-one exhibit various biological activities like central nervous system depressant, analgesic, antiviral, hypotensive, bactericidal, antioxidant and fungicidal activities [12–17]. The current effort was to explore the possibility of anti-oxidant and anti-bacterial activities in a piperdin ring having hydrazone moiety. See (Fig. 1).

Piperidin-4-one derivatives associated with aromatic substituents at 2nd and 6th positions showed significant biological activities [18,19]. Besides the significant biological properties, piperdin-4-ones are useful building blocks in synthetic organic chemistry and is well documented. The substituent dependent

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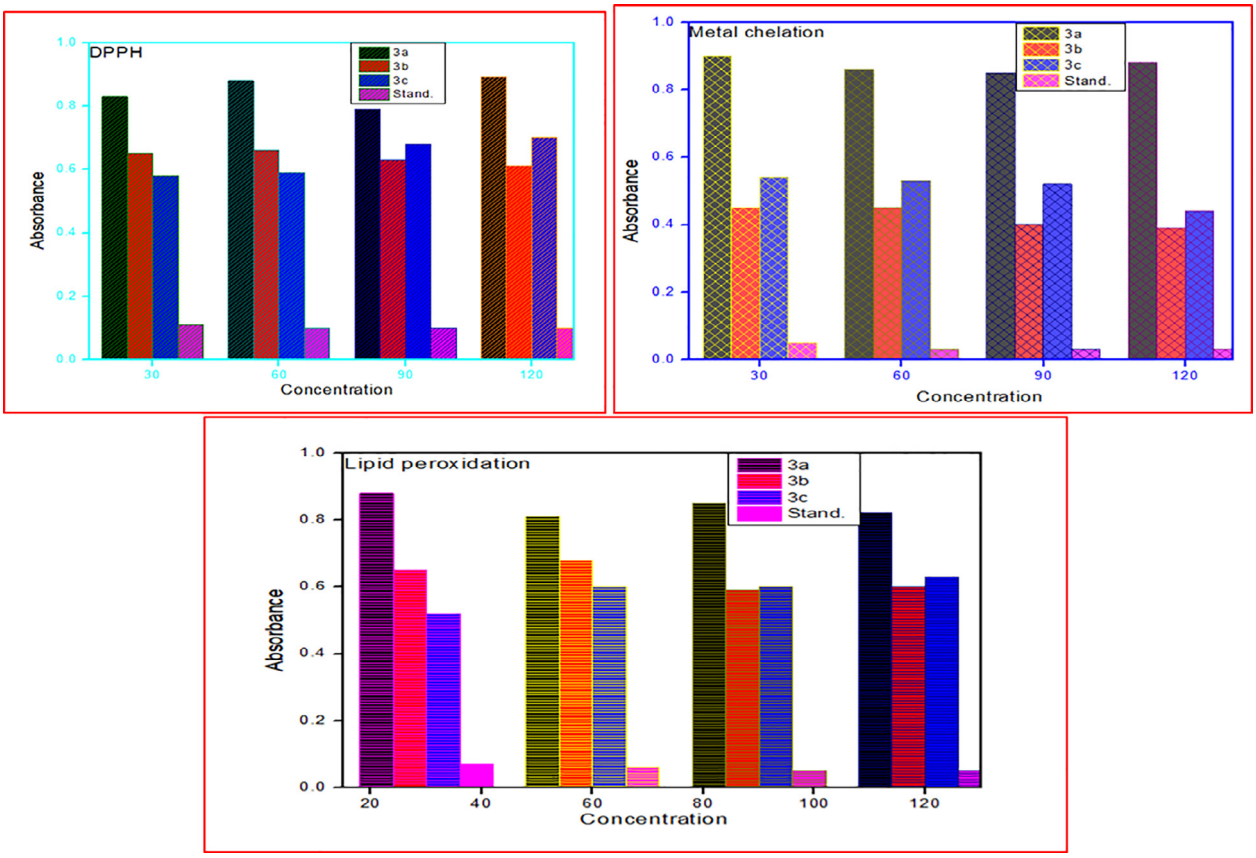


Fig. 1. Anti-oxidant potential of synthesized compounds (a) DPPH (b) Lipid peroxidation (c) Metal chelation. α – Tocopherol, EDTA, and Citric acid signify butylated hydroxytoluene. Data with altered superscripts are significantly different at $p \leq 0.05$.

conformation of the central heterocyclic ring and the presence of supramolecular synthons makes the solid-state structural chemistry of piperdin-4-one derivatives an interesting area to explore.

In the current study, a new series of 4-fluoro-N′-(substituted piperidines) benzohydrazides 3**(a-c**) were amalgamated using a reaction of piperidin-4-one with 4-fluorobenzohydrazide in presence of acetic acid in methanol. The structures were confirmed using FT-IR, H^1 NMR, and C^{13} NMR. The newly amalgamated hydrazone derivatives were implemented with potent antioxidants and antibacterial studies.

2. Experimental

2.1. Materials and methods

All the chemicals and reagents applied in this work were supplied from Thermo-Fisher Chemical Scientific Company. The melting point was taken in open glass capillaries on SUNTEX melting point apparatus and is uncorrected.

IR spectra were recorded on an AVATAR 330 FT-IR spectrometer in the KBr pellets. H^1 and C^{13} NMR spectra were recorded on a Bruker Advance FT-NMR spectrometer operating at 400.23 MHZ prepared by dissolving approximately 10 mg and 50 mg of the compounds respectively, in 0.5 ml $CdCl_3$-d6 / DMSO d_6. All the nuclear magnetic resonance measurements were made using 5mn tubes.

2.2. General procedure

The 3,3-dimethyl-2r,6c-diphenyl piperidin-4-one 3(A-C) were prepared by the condensation of appropriate ketones, aldehydes and ammonium acetate in a 1:2:1 ratio, according to the method described by Noller and Baliah [20]. A reaction mixture of 3,3-dimethyl-2,6-diphenyl piperidin-4-one (1 mmol) and 4-fluorobenzhydrazide (1.5 mmol) was dissolved in the solvent of methanol (30 ml) and acetic acid (2 ml) was added as catalyst. The reaction mixture was refluxed for 4–5 h. After completion of the reaction the product and were recrystallized with ethanol. The pure compounds 3(a-c) were obtained.

2.2.1. 4-fluoro-N′-(3,3-dimethyl-2,6-diptolylpiperidin-4-ylidene) benzohydrazide (3a)

Molecular formula: $C_{28}H_{30}FN_3O$, Exact mass: 443.21, Molecular weight: 443.56, Elemental analysis: C = 75.82, H = 6.82, N = 9.47. IR (KBr, Vmax Cm^{-1}): 1638.7 (C = N), 1492.0 (C = O), 3402.6 (N–H st piperdin), 3314.0 (N–H st N–H amide), 2975.7, 2863.6, 2811.6 (C–H st). ^{1}H NMR (400 MHz, $CdCl_3$-d6, δ):1.08(d, 6H, $2CH_3$ at the piperdin ring), 1.17(s, 1H, NH at piperdin ring), 2.02 (s, 6H, CH_3 at phenyl ring), 2.27 (dd, 1H, C5-1Ha), 3.89 (d, 1H, C5-1He), 3.92 (d, 1H, C2-1Ha), 7.00–7.09 (m, 8H, Ar-H), 9.99 (s, 1H, NH, amide N–H). C^{13} NMR (400 MHz, $CdCl_3$-d6, δ): 20.05 and 23.07($2CH_3$ at piperdin ring), 39.18 (CH_3 at phenyl ring), 47.31(C-5), 50.00 (C-3), 61.37 (C-6), 69.33(C-2), 126.48–130.87 (Ar-C), 137.27–140.26 (ipso carbons), 163.92 (C-4), 165.85(NHCO) and 166.50 (C-4 at hydrazide ring).

2.2.2. 4-fluoro-N′-(2,6-bis (4-chlorophenyl)-3,3-dimethyl piperidin-4-ylidene) benzohydrazide (3b)

Molecular formula: $C_{26}H_{24}Cl_2FN_3O$, Exact mass: 483.13, Molecular weight: 484.39, Elemental analysis: C = 64.47, H = 4.99, N = 8.67. IR (KBr, Vmax Cm^{-1}): 1638.2 (C = N), 1490.2 (C = O), 3415.7 (N–H st piperidin), 3311.8(N–H st amide), 3048.8, 2977.4,

2806.1 (C–H st). H^1 NMR (400 MHz, $CdCl_3$-d6, δ): 1.17(d, 6H, $2CH_3$ at piperidin ring), 2.60(s, 1H, NH at piperidin ring), 2.37(t, 1H, C5-1Ha), 3.64(d, 1H, C5-1He), 3.81(d, 1H, C2-1Ha), 3.92(dd, 1H, C6-1Ha), 7.18–7.33(m, 8H, Ar-H), 9.99(s, 1H, N–H, amide, N–H). C^{13} NMR (400 MHz, $CdCl_3$-d6, δ):20.36 and 22.9 ($2CH_3$ at piperidin ring), 29.7(C-5), 47.1(C-3), 60.5(C-6), 68.8 (C-2), 127.9–130.3(Ar-C), 133.5–141.4(ipso carbons), 163.9(NHCO), 167.6(C-4 at hydrazide ring).

2.2.3. 4-fluoro-N′-(2,6-bis (3-bromophenyl)-3,3-dimethyl piperidin-4-ylidene) benzohydrazide (3c)

Molecular formula: $C_{26}H_{24}Br_2FN_3O$, Exact mass: 571.03, Molecular weight: 573.29, Elemental analysis: C = 54.47, H = 4.22, N = 7.33. IR (KBr, Vmax Cm^{-1}): 1638.3(C = N), 1501.1(C = O), 3221.4(N–H st amide), 3031.2, 2926.5(C–H st). H^1 NMR (400 MHz, DMSO d_6, δ): 1.90(d, 6H, $2CH_3$ at piperidin ring), 2.15 (s, 1H, NH at piperidin ring), 2.50(s, 1H, C5-1Ha), 4.0(d, 1H, C6-1Ha), 7.25–7.38 (m, 8H, Ar-H), 10.03(s, 1H, NH amide, NH). C^{13} NMR (400 MHz, DMSO d_6, δ): 20.98 and 21.68 (CH_3 at piperidin ring), 23.45(C-5), 115.78–130.72 (Ar-C), 132.22 and 137.98(ipso carbons), 163.01(C-4), 163.39(NHCO), and 169.43(C-4 at hydrazone ring)

2.3. Sample preparation for anti-oxidation activity

Different sample concentrations (w/v) were made by dissolving 30, 60, 90, and 120 mg of starch in 1 ml of double-distilled water followed by sonication for 21 min to confirm better mixing. The mixture obtained was utilised for determining different anti-oxidant assays.

2.3.1. DPPH radical scavenging activity

The 1, 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity of samples was determined by the method proposed by Ashwar et al., [21] with some alterations. 5 ml of each sample of changing concentrations (30, 60, 90 and 120 mg/ml) was mixed with a 60 mm solution of DPPH in methanol (5 ml). The mixture was vortexed for about a minute and kept at 25 ℃ for 35 min in a dark room. The absorbance of the resulting mixture was determined at 517 nm using a UV–visible spectrophotometer against the blank, α-Tocopherol was taken as + ve control dissolved in double-distilled water. 1, 1-diphenyl-2-picrylhydrazyl scavenging ability was measured with the following formulae

$$DPPH\ scavenging\ activity(\%) = (1 - As)/Ac * 100$$

where Ać is the absorbance of the control, and Aś is the absorbance of the sample.

2.3.2. Metal chelating activity

Metal chelating activity was assessed according to the method Shah et al., [22]. The absorbance of the resulting mixture was dignified at room temperature against blank at 650 nm and compared to citric acid (standard). The ferrous ion chelation ability of each sample was calculated as per cent chelating effect by the following formulae

$$Chelating\ effect(\%) = (Ac - As)/Ac * 100$$

whereas Ać is the absorbance of the control and Aś is the absorbance.

2.3.3. Inhibition of lipid peroxidation

This test was calculated by the following method of Shah et al., [22]. Starch suspension (1 ml) of varying concentrations was added to linoleic acid (1 ml, 0.1% w/v), hydrogen peroxide (0.2 ml, 30 mm), ascorbic acid (0.2 ml, 100 mm), and ferric nitrate (0.2 ml, 20 mm). The mixture was allowed to nurture at 37 ℃ for an hour. Afterwards, the termination of the reaction was carried out by adding tri-chloroacetic acid (1.0 ml, 10% m/v), and thiobarbituric acid (1.0 ml, 1% m/v). Reaction mixture tubes were kept in a boiling water bath for 25 min followed by centrifugation at 6000 rpm for 5 min. The supernatant was collected and its absorbance was determined at 535 nm against blank and compared to EDTA (standard).

Inhibition was calculated as per cent by the following equation

$$\%\ inhibition = Ac - - As/Ac * 100$$

whereas 'Ac' is the absorbance of the control and 'As' is the absorbance of the sample.

2.4. Anti-bacterial activity

Anti-bacterial activity of synthesized compounds 3(**a-c**) was carried out using the two-fold serial dilution method [23]. For this streptomycin have been used as standard. Stock solutions of 3(a-c) were made in DMSO (1gm/ml). Compounds were verified in the concentration of 200, 100, 50, 25, 12.5, 6.25, and 3.12 μl^{-1} (Two-fold serial dilution).

Bacterial strains such as Bacillus subtilis, Klebsiella pneumonia, Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus obtained from the faculty of Medicine, Annamalai University, Annamalai Nagar 608002, Tamil Nadu India were used to screen the antibacterial activity of the newly synthesized compounds 3(**a-c**). The bacterial strains were cultured in Sabouraud dextrose broth (SDB) at pH 7.4 ± 0.2 (HI-media, Mumbai) and Nutrient broth (NB) at pH 5.6 respectively.

3. Results and discussions

3.1. Chemistry

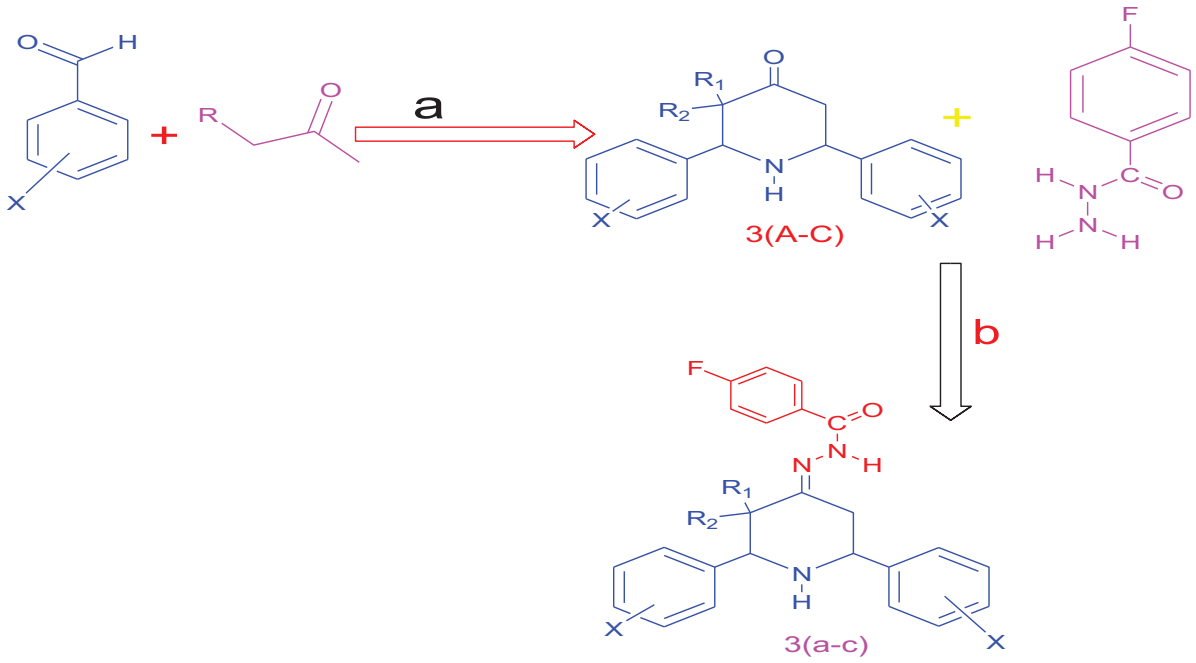
3.1.1. Synthesis of (E)-4-fluoro-N′-(substituted piperidines) benzohydrazides 3(a-c)

4-fluorobenzhydrazide (0.001 m) and suitable piperidine (0.001 m) were mixed in methanol (30 ml) along with acetic acid 2 ml. The reaction mixture was taken in an RB flask and refluxed for about 4- hours [Scheme 1]. The progress of the reaction was monitored by TLC (thin layer chromatography) after the completion of methanol was removed by slow evaporation. The obtained (E)-4-fluoro-N′-(substituted piperidenes) benzohydrazides 3(**a-c**) were recrystallized from ethanol. The melting points of the recrystallized products were determined in open capillaries. The investigation of IR, H^1 NMR and C^{13} NMR spectral data was performed to detect and establish the newly synthesized compounds.

Structural elucidation of compound **3a**

In H^1 NMR spectra for compound **3a**, a broad and more downfield D_2O transferrable singlet at 9.994 ppm was characteristic of the NH of the amide group. Another, broad singlet signal resonated at 2.33 ppm was assigned for the NH proton for the piperidin-4-one ring. Signal broadening is due to the faster exchange of the NH proton with the solvent moisture than the resonance time scale. Two doublets were observed in the region of 1.17 and 3.89 ppm due to H-2a and the methyl group of C-3 at piperdin ring. Three doublets were observed in the region of 3.92, 2.25 and 2.82 ppm due to H-5e, H-5a, and H-6a. Multiple signals appeared at 2.85 ppm corresponding to one proton integral due to H-3a.

In C^{13} NMR of compound 3a, two downfield resonances at 165.8 and 163.9 ppm were assigned to C = N (C-9) and C = O (=N-NH-CO-) carbons, respectively. The carbon resonances observed around 140.2 and 137.5 ppm were due to ipso carbons. However, there were four signals around 69.3, 50.0, 47.3 and 39.1 ppm which were conveniently assigned to the C-2, C-3, C-5 and C-6 carbons respec-



Reagents and conditions: (a) CH_3COONH_4, C_2H_5OH (b) CH_3OH, CH_3COOH, 4-hours, 60° c

Sample code		R_1	R_2	X
A	a	$-CH_3$	$-CH_3$	P $-CH_3$
B	b	$-CH_3$	$-CH_3$	P-Cl
C	c	-CH3	-CH3	m-Br

Scheme 1. Demonstration of the synthesis of 4-fluoro-N′-(substituted piperidines) benzohydrazides 3(a-c).

tively. The C^{13} chemical shift values of the methyl carbons (C-3 at the piperdin ring and C-4 at the phenyl ring) were observed at 18.4 and 20.0 ppm. The signals at 166.3 ppm and 136.3 ppm were assigned to C-4 and C-5 of the hydrazide ring.

3.2. Biological activity

Piperidin-4-one pharmacophores are found to have a wide range of pharmacological properties. We have developed the system that combines piperidin-4-one pharmacophore and hydrazide moieties to produce the corresponding hydrazones 3(a-c) with the anticipation of several encouraging anti-oxidant, anticancer, antimicrobial agents emerging. The present work also aimed to investigate the structure–activity relationship for anti-oxidant and antimicrobial activities of hybrid molecules containing the piperidin-4-one pharmacophore and hydrazones.

3.2.1. Anti-oxidant potential

Three different hydrazone derivatives were synthesized and evaluated for their in vitro free radical scavenging activity against various free radicals. Our result provides evidence that synthetic compound 3(a-c) showed a concentration-dependent antiradical activity. IC50 values for free radical scavenging of various synthetic compounds 3(a-c) are shown in Table 1. It is well known that an increase in anti-oxidant activity is observed with the spare of alkyl

Table 1
IC50 values for free radical scavenging activity (mg/ml).

Compound	IC50 values for free radical scavenging		
	DPPH	Lipid peroxidation	Metal chelation
3a	**4.99**	**5.1381**	**5.1926**
3b	4.4159	4.4159	3.6742
3c	4.1713	3.9497	4.0249
Standard used	1.8166(α-topocherol)	1.4491(EDTA)	1.2247(citric acid)

chains such as methyl, ethyl to phenyl rings due to the electronic resonance outcome of the phenyl group [24]. The results of the current study show the presence of methyl group substitution in position 3rd of piperdin-4-one compounds exerts an abundant inhibitory effect against various free radicals. Compound owning electron-donating methyl (3a) substitutions at the *para*-position of the phenyl ring attached to the C-2 and C-6 carbon of the piperdin moiety showed excellent free radical scavenging effects compared to the standard anti-oxidant, a known antioxidant used as the positive control. Compound with electron-donating methyl substitution at the para position of the phenyl ring attached to the C-2 and C-6 carbons and methyl substitution at the 3rd position of piperidin-4-one compounds exposed extraordinary activities. These results confirm reports by other workers on in vitro- free radical scavenging effects of organic molecules incorporating an electron-donating group

Table 2
In vitro anti-bacterial (MIC μm/ml) of compounds 3(a-c) by 2-fold serial dilution method.

Compounds	MIC(μm/ml)				
	Bacillus subtilis	Klebsiella pneumonia	Escherichia coli	Pseudomonas aeruginosa	Staphylococcus aureus
3a	200	>200	>200	100	100
3b	12.5	50	50	25	25
3c	12.5	25	50	50	25
Streptomycin	25	25	12.5	12.5	12.5

(methyl) at the para position of the phenyl ring [25–27]. Compounds owning electron-withdrawing Bromo (3c), and chloro (3b) substitutions at the Meta and para position of the piperidine moiety showed venerable in vitro free radical scavenging effects against various free radicals. These less or venerable free radical scavenging effects of compounds with Bromo and chloro may be due to the electron removing the inductive effect of halogens. Underneath mentioned results are in contour with other findings [25,26,28]. The radical scavenging effects are measured by alteration in colour from purple to light yellow and analysis of the absorbance at 517 nm [29]. The scavenging capability of synthetic compounds 3(a-c) is depicted graphically in figure A.

3.2.2. Anti-bacterial potential

In vitro anti-bacterial activity of the amalgamated hydrazones was carried out against B. subtilis, S.aureus, K. pneumonia, P. aeruginosa and E.coli by the two-fold serial dilution method using streptomycin as the standard. The MIC values are presented in Table 2. Analysis of in vitro antibacterial effects of all the 4-fluoro-N′-(substituted piperdines) benzohydrazides 3(a-c) revealed a diverse range of inhibitory activity (6.25–200 μg/ml) against all pathogens. Compound (3a) owning electron-donating methyl at the para position of phenyl ring attached to C-2 and C-6 carbons of piperidine moiety show good antibacterial activity against all the tested bacterial strains in the sort of 25–200 μg/ml among the three compounds. Substitution of electron removing Bromo (3c) and chloro (3b) respectively, at Meta and para position of phenyl rings show temperate anti-bacterial activity against all the tested bacterial strains at MIC values of 12.5–100 μg/ml.

4. Conclusion

We report three new synthesized (E)-4-fluoro-N′-(substituted piperidines) benzohydrazides 3(a-c). The IR, NMR (H^1 and C^{13}) spectral characterization deep-rooted the hydrazone derivatives formation. The consequence of anti-bacterial and anti-oxidant activities of compounds 3(a-c) presented that the compounds having electron-donating group had good anti-bacterial as well as anti-oxidation activities.

CRediT authorship contribution statement

Tanzeer Ahmad Dar: Conceptualization, Methodology, Validation, Formal analysis, Writing – original draft. **Balasankar Thirunavukkarasu:** Conceptualization, Writing – review & editing, Supervision. **A. Ganapathi:** . **J. Winfred Jebaraj:** .

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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