Synthesis and *In Vitro* Biological Activity of [1-(Substituted-benzoyl)-piperidin-4-yl]-(2,4-difluoro-phenyl)-methanone Oximes

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***Abstract*—** A series of new [1-(substituted-benzoyl)-piperidin-4-yl]-(2,4-difluoro-phenyl)-methanone oximes, **3(a-e)** were synthesized by the reaction of 2,4-difluorophenyl(piperidin-4-yl)methanone oxime with various acid chlorides. The synthesized compounds were characterized by FT-IR, ^{1}H NMR and LCMS spectral studies. All compounds were evaluated for their *in vitro* antibacterial activity against clinically isolated strains. These compounds were screened for their antioxidant activity by 2,2-diphenyl-1-picryl-hydrazyl (DPPH$^•$) and ferrous ion chelating assay (Fe^{2+}) methods. Antiproliferative effects was evaluated using the MTT assay method against two human cancer cell lines (MCF7 and U373) and one astrocytoma brain tumor (C6 rat glioma) cell line. All the compounds exhibited moderate antibacterial activity when compared with standard drug. All the compounds showed antioxidant activity, where compound **3b** was the best radical scavenger and Fe^{2+} ion scavenger. Among the series, compounds **3a** and **3d** showed good activity on all cell lines, whereas the other compounds in the series exhibited moderate activity.

***Keywords*-** Piperidine, Antibacterial, Antioxidant, Antiproliferative.

I. Introduction

NITROGEN containing organic compounds, such as amines and heterocycles as inhibitors for protecting metallic surfaces from corrosion in various aggressive environments [1]. Several thousand piperidines have been mentioned in clinical and preclinical studies [2]. Poly-substituted piperidine rings are present in many natural products; they act as anti-hypertensive, anti-bacterial, anti-convulsant, anti-inflammatory, anti-HIV and anticancer drugs [3].

Piperidine fragment was substituted via variety of synthetic reactions to develop more improved moieties with enhanced activity and to suppress the side effects when taken as medicine for different ailments [4, 5]. Specifically, piperidine based chemical entities with aryl substituents have been documented as potent microbial agents [6, 7]. It is important group of heterocyclic compounds in the field of medicinal chemistry due to their biological activities, including cytotoxic and anticancer properties [8].

2,4-Difluorophenyl(piperidin-4-yl)methanone oxime (**1**) is an intermediate for the preparation of risperidone. Risperidone contains the functional groups of benzisoxazole and piperidine as part of its molecular structure. The use of risperidone is considered to be important in the treatment of psychotic disorders and schizophrenia helping to manage schizophrenia's "positive symptoms" such as auditory hallucinations and visual, delusions and thought disturbances. It is believed that its atypical activity profile may be due to its effect on an interaction between the serotonin and dopamine system [9]. The risperidone has combined serotonin and dopamine receptor and plays an important role in the treatment of schizophrenia. This may recur even if the patient has switched to a different antipsychotic [10]. In the present paper, we are reporting a simple, cheap and convenient method for the synthesis of new [1-(substituted-benzoyl)-piperidin-4-yl]-(2,4-difluoro-phenyl)-methanone oximes, **3(a-e)** and their biological activity was determined.

II. Results and discussion

A. Chemistry

In the present work, a series of five new compounds were synthesized. Structure of the synthesized compounds was established on the basis of spectral studies. The chemical structure and physical data of new compounds are given in Table 1. The elemental analyses data showed good agreement between the experimentally determined values and the theoretically calculated values within ± 0.4 %.

The FT-IR spectra of **3(a-e)** were recorded using KBr pellets in the range of 4000-400 cm^{-1}. The absorption bands at 3073-3051 cm^{-1} are assigned to the aromatic-H stretch. The absorption bands at 1655-1640 cm^{-1} are due to the presence of C=O stretch. The absorption band at 3368 cm^{-1} is due to the N-H stretch in compound **1**. The absence of N-H absorption bands in **3a-e** confirmed the synthesized compounds. The strong band at 720 cm^{-1} is assigned to the C-Cl stretch in **3d**. The absorption at 1536 cm^{-1} corresponding to NO_2 (asym. stretch) in **3a**.

The characteristic resonance peaks in ^{1}H NMR for the new compounds were reported using DMSO-d_6. The expected resonances were assigned by their peak multiplicity and integration. The integration of spectra shows good agreement

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with the synthesized compounds. The proton NMR spectral data of NH in **1** show single resonance at δ 9.22 ppm, which is absent in the spectra of **3(a-e)**, indicating the replacement of the acid chloride series. In addition, the resonance appearing in the range of δ 7.84-6.54 ppm as a singlets, doublets, triplets and multiplates is attributed to the aromatic protons. The piperidine protons were resonated at δ 3.61-1.74 ppm. The proton spectral data agree with respect to the number of protons and their chemical shifts with the proposed structures. The synthesized compounds were further confirmed by the appearance of molecular ion peak in mass spectra. Mass spectra of all the newly synthesized compounds showed M^+ fragmentation peak in agreement with their molecular formula. The mass spectra of **3c** showed molecular ion peak at m/z 374.38 which is in agreement with the molecular formula $C_{20}H_{20}F_2N_2O_3$.

TABLE I
CHEMICAL STRUCTURE AND PHYSICAL DATA OF [1-(SUBSTITUTED-BENZOYL)-PIPERIDIN-4-YL]-(2,4-DIFLUORO-PHENYL)-METHANONE OXIMES 3A-E

Compound	R	Structure	Yield (%)	m.p. (°C)
3a			70	170-172
3b			71	148-150
3c			77	145-147
3d			73	176-178
3e			78	135-136

B. Antibacterial activity

The investigation of antibacterial screening data revealed that all tested compounds showed antibacterial activity against four pathogenic bacterial strains. All compounds showed moderate inhibitory activity against tested bacterial strains in comparison to standard drug. Compound 3a and 3d were found to be more potent against gram positive and gram negative bacterial strains (Table 2). The nature of the linkage (substituent on aromatic ring) influences the antibacterial activity. However, the activity of the tested compounds is less than those of standard antibacterial agent used (Fig. 1).

TABLE II
MIC VALUE OF ANTIBACTERIAL ACTIVITY OF **3(A-E)**

Compound	Bacterial strain (mg)			
	E. coli	*P. fluorescence*	*M. luteus*	*B. subtilis*
3a	200	270	205	290
3b	340	360	310	320
3c	325	380	315	305
3d	230	280	220	240
3e	310	375	305	290
Standard	90	90	90	90

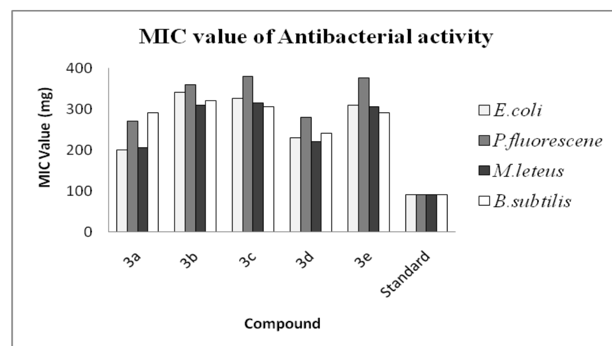


Fig. 1 Comparison of MIC of compounds with standard drug.

C. Antioxidant activity

Compounds **3b** and **3c** showed higher radical inhibition activity due to the presence of hydroxy group (electron donating group) in the aromatic ring. Percentage of DPPH radical scavenging activity was depicted in Table 3. The aromatic ring system with methoxy group in **3b** and **3c** were found to be more active than other compounds in the series. Compounds **3a**, **3d** and **3e** showed moderate antioxidant activity (Fig. 2). The nature of the functional groups is crucial for biological activity. Fe^{2+} ions initiate free radicals through the Fenton and Haber-Weiss reaction. Fenton Weiss reaction is a reaction between ferrous ion and hydrogen peroxide which produces highly reactive hydroxyl radicals implicated in many diseases. Percentage of Fe^{2+} chelating activity values were depicted in Table 4. All the investigated substances were capable of chelating Fe^{2+} ions (Fig. 3). The metal chelating effects of the samples were dependent on concentration and linearly increased with the sample concentration increased.

TABLE III
DPPH RADICAL SCAVENGING ACTIVITY OF THE TESTED COMPOUNDS

Compound	Scavenging activity (%)				
	Concentration (µg/mL)				
	20	40	60	80	100
3a	22	32	45	54	63
3b	41	58	61	65	68
3c	40	56	60	62	61
3d	20	31	42	52	60
3e	20	30	43	52	58
Standard	59	61	64	69	74

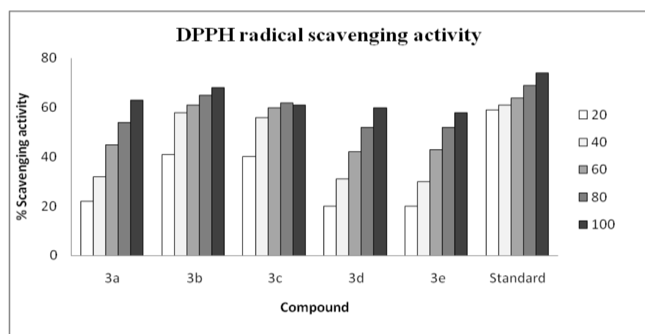


Fig. 2 Concentration verses % activity of **3(a-e)** using DPPH Method.

TABLE IV
FERROUS ION CHELATING ACTIVITY OF THE TESTED COMPOUNDS

Compound	% of Ferrous ion chelating activity				
	Concentration (μg/mL)				
	20	40	60	80	100
3a	23	34	48	56	64
3b	29	75	79	82	85
3c	25	73	72	80	81
3d	21	32	45	57	62
3e	18	26	36	45	57
Standard	72	79	81	83	88

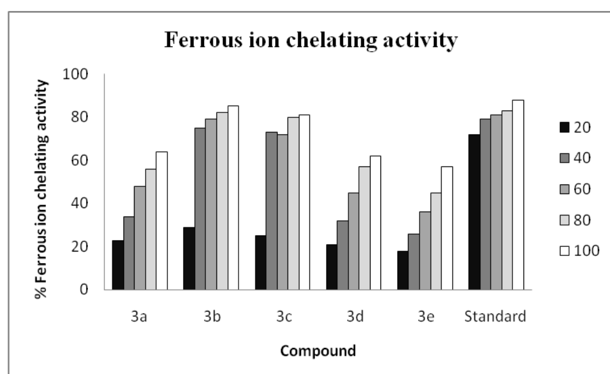


Fig. 3 Concentration verses % activity of **3a-e** using Fe^{2+} chelating Method.

D.Antiproliferative activity

The antiproliferative action of the synthesized compounds **3(a–e)** were tested against three different cell lines. The activity was evaluated by measuring the levels of surviving cells after incubation for 24 h with the test samples using the MTT colorimetric assay based on the ability of metabolically active cells to convert the pale yellow MTT to a blue formazan product which is quantifiable spectrophotometrically. The percentage cell survival for tested compounds against human cancer cells (MCF7 and U373) and astrocytoma brain tumor (C6 rat glioma) cells are tabulated in Table 5. The results were expressed as percentage of cell proliferation compared with cells in control (cells treated with vehicle, 0.1% DMSO). Compound **3a** containing nitro group and **3d** containing chloro groups are more potent antiproliferative activity (Fig. 4). Antiproliferative activity of other compounds in the series showed moderate activity.

TABLE V
ANTIPROLIFERATIVE ACTIVITY OF **3(A-E)** AGAINST CANCER CELLS DETERMINED BY MTT TEST (μM)

Compound	% Cell survival					
	MCF7		U373		C6	
	200	400	200	400	200	400
3a	53.26	71.83	91.03	93.46	87.51	87.94
3b	32.15	43.02	74.43	84.11	71.21	81.30
3c	28.23	36.00	63.40	71.32	61.34	73.28
3d	33.53	46.29	94.62	94.92	88.34	88.91
3e	27.15	35.37	63.21	71.11	61.20	72.21
Control	100.0	100.0	100.0	100.0	100.0	100.0

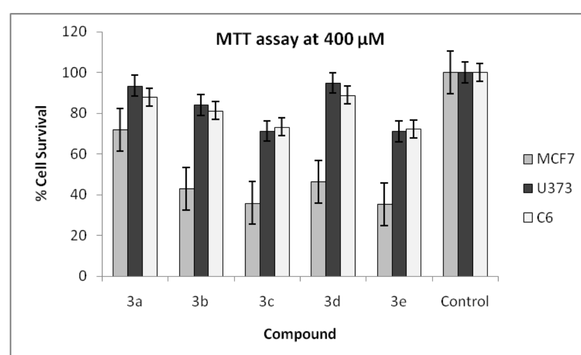


Fig. 4 MTT assay for 3(a-e) at 400 μM

III. EXPERIMENTAL

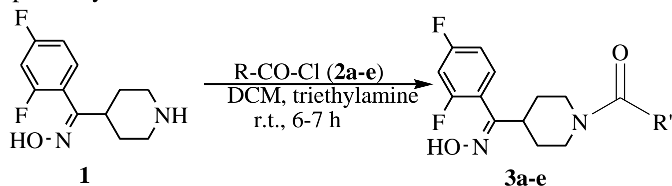
Chemistry

All solvents and reagents were purchased from Sigma Aldrich Chemicals. Melting points were determined on an electrically heated VMP-III melting point apparatus (Veego, India). The elemental analyses of the compounds were performed on a Perkin Elmer 2400 Elemental Analyser. The FT-IR spectra was recorded using KBr discs on FT-IR Jasco 4100 infrared spectrophotometer. The NMR spectra were recorded using Bruker DPX-400 spectrometer at 400 MHz for ^{1}H NMR with tetramethylsilane as the internal standard. Mass spectral data were obtained by LC-MSD Trap XCT. Silica gel column chromatography was performed using Merck 7734 silica gel (60–120 mesh) and Merck-made TLC plates.

*General procedure for the [1-(substituted-benzoyl)-piperidin-4-yl]-(2,4-difluoro-phenyl)-methanone Oximes, **3(a-e)***

A solution of 2,4-difluorophenyl(piperidin-4-yl)methanone oxime (**1**) (1.0 eq) in DCM was taken and cooled to 0-5 °C in an ice bath. Triethylamine (3.0 eq) was added to the cold reaction mixture and stirred for 10 min, then different acid chlorides (**2a–e**) (1.0 eq) were added. The reaction mixture was allowed to stir at room temperature for 6-7 h. The progress of the reaction was monitored by TLC. Upon completion, the solvent was removed under reduced pressure and residue was taken in water and extracted with ethyl acetate. The organic layer was washed with 10 % ammonium chloride solution and finally water wash was given to organic layer and dried with anhydrous sodium sulphate. The solvent

was evaporated to get crude product which was purified by column chromatography over silica gel (60–120 mesh) using hexane: ethyl acetate (8:2) as an eluent. New compounds were prepared by the method summarized in Scheme 1.



Scheme 1

*(2,4-Difluoro-phenyl)-[1-(4-nitro-benzoyl)-piperidin-4-yl]-methanone oxime **(3a)***

FT-IR (KBr, cm^{-1}): 3298 (O-H), 3053 (Ar-H), 1648 (C=O), 1536 (NO_2), 1159 (C-N), 1117 (C-F). ^1H NMR (DMSO-d_6) δ ppm: 10.91 (s, 1H, OH), 7.42 (d, 2H, Ar-H), 7.29 (d, 2H, Ar-H), 7.12 (d, 1H, Ar-H), 7.01 (d, 1H, Ar-H), 6.91 (s, 1H, Ar-H), 3.53 (t, 4H, pip-H), 2.48 (d, 4H, pip-H), 1.96 (m, 1H, pip-H), MS (ESI) m/z: 389.35. Anal. Calcd. for $C_{19}H_{17}F_2N_3O_4$: C, 58.61; H, 4.40; N, 10.79; Found: C, 58.64; H, 4.43; N, 10.75 %.

*(2,4-Difluoro-phenyl)-[1-(3,4,5-trimethoxy-benzoyl)-piperidin-4-yl]-methanone oxime **(3b)***

FT-IR (KBr, cm^{-1}): 3318 (O-H), 3055 (Ar-H), 1640 (C=O), 1171 (C-N), 1091 (C-F). ^1H NMR (DMSO-d_6) δ ppm: 9.98 (s, 1H, OH), 7.78 (s, 2H, Ar-H), 7.15 (d, 1H, Ar-H), 7.09 (d, 1H, Ar-H), 6.91 (s, 1H, Ar-H), 3.81 (s, 9H, OCH_3), 3.58 (t, 4H, pip-H), 2.30 (m, 4H, pip-H), 1.74 (m, 1H, pip-H). MS (ESI) m/z: 434.43. Anal. Calcd. for $C_{22}H_{24}F_2N_2O_5$ (in %): C, 60.82; H, 5.57; N, 6.45. Found: C, 60.86; H, 5.54; N, 6.41.

*(2,4-Difluoro-phenyl)-[1-(4-methoxy-benzoyl)-piperidin-4-yl]-methanone oxime **(3c)***

FT-IR (KBr, cm^{-1}): 3293 (O-H), 3073 (Ar-H), 1645 (C=O), 1159 (C-N), 1117 (C-F). ^1H NMR (DMSO-d_6) δ ppm: 10.91 (s, 1H, OH), 7.84 (d, 2H, Ar-H), 7.69 (d, 1H, Ar-H), 7.12 (d, 1H, Ar-H), 7.10 (d, 1H, Ar-H), 7.05 (d, 2H, Ar-H), 3.73 (s, 3H, CH_3), 3.13 (t, 4H, Pip-H), 2.48 (m, 4H, Pip-H), 1.96 (m, 1H, Pip-H). MS (ESI) m/z: 374.38. Anal. Calcd. for $C_{20}H_{20}F_2N_2O_3$: C, 64.16; H, 5.38; N, 7.48; Found: C, 64.11; H, 5.34; N, 7.43%.

*[1-(2,6-Dichloro-benzoyl)-piperidin-4-yl]-(2,4-difluoro-phenyl)-methanone oxime **(3d)***

FT-IR (KBr, cm^{-1}): 3289 (O-H), 3060 (Ar-H), 1655 (C=O), 1165 (C-N), 1105 (C-F), 720 (C-Cl). ^1H NMR (DMSO-d_6) δ ppm: 10.61 (s, 1H, OH), 7.63-7.42 (m, 3H, Ar-H), 7.05 (d, 1H, Ar-H), 6.85 (d, 1H, Ar-H), 6.54 (s, 1H, Ar-H), 3.25 (t, 4H, pip-H), 2.59 (m, 4H, pip-H), 1.75 (m, 1H, pip-H). MS (ESI) m/z: 413.25. Anal. Calcd. for $C_{19}H_{16}Cl_2F_2N_2O_2$ (in %): C, 55.22; H, 3.90; N, 6.78. Found: C, 55.25; H, 3.93; N, 6.81.

*[1-(4-Methyl-benzoyl)-piperidin-4-yl]-(2,4-difluoro-phenyl)-methanone oxime **(3e)***

FT-IR (KBr, cm^{-1}): 3279 (O-H), 3060 (Ar-H), 1645 (C=O), 1165 (C-N), 1115 (C-F). ^1H NMR (DMSO-d_6) δ ppm: 10.91 (s, 1H, OH), 7.62 (d, 2H, Ar-H), 7.29 (d, 2H, Ar-H), 7.12 (d, 1H, Ar-H), 7.10 (d, 1H, Ar-H), 6.88 (s, 1H, Ar-H), 3.61 (t, 4H, pip-H), 2.51 (d, 4H, pip-H), 2.12 (s, 3H, CH_3), 1.96 (m, 1H, pip-H). MS (ESI) m/z: 358.38. Anal. Calcd. for $C_{20}H_{20}F_2N_2O_2$: C, 67.03; H, 5.62; N, 7.83; Found: C, 67.12; H, 5.57; N, 7.89 %.

Antibacterial activity

Broth dilution assay was carried out according to the method developed [11-13] in a microtitre plate (96 well plate) with slight modifications. In brief over night culture of the above mentioned pathogens were made every time in Muller Hinton's broth and were diluted with the fresh Muller Hinton's broth till the A_{600} reaches 0.05. 100 μL of the each diluted bacterial cultures (A_{600}=0.05) were dispensed to their respective wells (96 well polypropylene micro titer plate) in triplicates. A blank is maintained which contains only sterile Muller Hinton's broth. The plates were covered with sterile aluminum foil to avoid contamination and were incubated at 37 °C for 18 hr in a refrigerated bacteriological incubator. The plate was read in UV-Visible microplate spectrophotometer at 600 nm (photometric) with 10 seconds of shaking; the values obtained for each pathogen and drug of different concentrations were averaged and are negative with the empty broth (Blank).

Minimum Inhibitory Concentration (MIC)

The minimal inhibitory concentration (MIC) was determined by broth micro dilution method. The MIC value was defined as the lowest concentration of compounds whose absorbance was comparable with the negative control wells (broth only, without inoculum). MIC values and comparison with standard antibiotic (Carbenicillin) was tabulated as the mean of three replicates.

Antioxidant activity

DPPH radical scavenging assay

The free radical scavenging activity was measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay according to the method described earlier [14, 15]. The stock solution was prepared by dissolving 24 mg DPPH with 100 mL methanol and stored at 20 °C until required. The working solution was obtained by diluting DPPH solution with methanol to attain an absorbance of about 0.98±0.02 at 517 nm using the spectrophotometer. A 3 ml aliquot of this solution was mixed with 100 μL of the sample at various concentrations (20 - 100 μg/mL). The reaction mixture was shaken well and incubated in the dark for 15 min at room temperature. Then the absorbance was taken at 517 nm. The control was prepared as above without any sample. Ascorbic acid (Vit-C) was used as positive control. All the experiments were done in triplicates and the values are averaged. All the tests were run in triplicate and averaged.

Scavenging effect (%) = [(control absorbance - sample absorbance) / control absorbance] * 100

Ferrous ion chelating assay

The chelating activity of the Schiff base derivatives for ferrous ions (Fe^{2+}) was measured according to the method of [16]. Briefly, 0.5 mL different concentration of synthesized

compounds was added to a solution of 2 mM $FeCl_2$ (0.05 mL). The reaction was initiated by the addition of 5 mM ferrozine (0.2 mL). The mixture was shaken vigorously and left at room temperature for 10 min. Ferrozine reacted with the divalent iron to form stable magenta complex species that were very soluble in water. Absorbance of the solution was then measured spectrophotometrically at 562 nm. EDTA was used as a positive control. All the experiments were done in triplicates and the values are averaged. All the tests were run in triplicate and averaged. The percentage inhibition of ferrozine-Fe^{2+} complex formation by the compounds was calculated as:

Percentage of inhibition (%) = $[(A_0 - A_1)/A_0] \times 100$

Where A_0 was the absorbance of the control, and A_1 was the absorbance of the test sample.

Antiproliferative activity

Drugs and solutions

The 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was dissolved (5 mg/ml) in phosphate buffer saline pH 7.2 and filtered (0.22 ml) before use. The RPMI 1640 cell culture medium, MTT and fetal bovine serum (FBS) were purchased from Merck chemicals.

Cell lines and culture conditions

MCF7, U373 and C6 rat glioma cell lines were procured from National Center for Cell Sciences, Pune, India. All cells were grown in RPMI-1640 supplemented with 10 % heat inactivated FBS, 100 IU/ml penicillin, 100 mg/ml streptomycin and 2 mM-glutamine. Cultures were maintained in a humidified atmosphere with 5 % CO2 at 37 °C. The cells were subcultured twice each week, seeding at a density of about 2×103 cells/ml.

In vitro cell viability assay-MTT assay

The potential effects on cell viability were investigated by using the MTT assay [17]. It is an indicator of metabolically active cells. A known number of MCF7, U373 and C6 rat glioma cells were transferred into 96 well plates in a volume of 200 µL of culture medium and incubated for 48 h before addition of test compound. Cells were then exposed to known concentrations of the compound to be tested (200 µM and 400 µM) for 24 h at 37 °C. After drug exposure, the culture medium was removed and 20 µl of MTT reagent (diluted in culture medium, 5 mg/mL) was added. After incubating for 4 h, the MTT/medium was removed and DMSO (100 µL) was added to each well and plates were agitated for 1 min. Absorbance of the coloured solution was measured on a multi-well plate reader (Victor3, Perkin Emler) using a test wavelength of 570 nm. Results were evaluated by comparing the absorbance of the wells containing compound treated cells with the absorbance of wells containing 0.1 % DMSO alone (solvent control). Conventionally, cell viability was estimated to be 100 % in the solvent control and assay was performed in triplicate.

IV. Conclusion

A series of new [1-(substituted-benzoyl)-piperidin-4-yl]-(2,4-difluoro-phenyl)-methanone oximes, **3(a-e)** were synthesized and their biological activity have been evaluated. All the compounds produced moderate changes in activity against Gram-positive and Gram-negative bacteria. The compound **3b** showed higher radical inhibition and Fe^{2+} chelating activity. Compounds **3a** and **3d** are shown to be more antiproliferative activity. From this work, we were able to identify a few active molecules which are capable of inhibiting the growth of cancer cell lines *in vitro*. Hence, there is the need for further investigations to clarify the features underlying the biological activity of these new piperidine derivatives.

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