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Research Article

ANTIBACTERIAL, ANTIOXIDANT AND ANTIPROLIFERATIVE ACTIVITIES OF 2-PIPERAZINOETHYLAMINE DERIVATIVES

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ABSTRACT

A series of new 2-piperazinoethylamines **3(a-f)** were subjected to the *in vitro* biological activity. All compounds were evaluated for their *in vitro* antibacterial activity against clinically isolated strains i.e., *E. Coli, P. Fluorescence, M. Luteus* and *B. Subtilis.* 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 using the MTT assay method against two human cancer cell lines (MCF7 and U373) and one astrocytoma brain tumor (C6 rat glioma) cell line. Compounds **3f**, **3d** and **3e** exhibited good antibacterial activity when compared with other compounds in the series against tested pathogenic bacterial strains. All the compounds showed antioxidant activity, where compound **3b** was the best free radical scavenger and Fe^{2+} ion scavenger. Among the series, compounds **3c** and **3e** showed good activity on all cell lines, whereas the other compounds in the series exhibited moderate activity. This study provided information about the interaction between 2-piperazinoethylamine and *in vitro* biological activity.

Keywords: 2-Piperazinoethylamine, Azo coupling agents, Antibacterial, Antioxidant, Antiproliferative.

1. INTRODUCTION

The analysis of biological activity is mainly carried out by means of UV-Visible spectrophotometer. Piperazine derivatives possess high biological activity for multidrug resistance in cancer and malaria[1, 2]. Pharmaceutical importance of azo compounds is well known for their use as antineoplastics, antidiabetics, antiseptics, anti-inflammatory and other useful chemotherapeutic agents[3]. Majority of these compounds are derived from the coupling of diazotized hetereocyclic amines with aromatic amino and hydroxyl compounds. The medicinal properties of azo compounds particularly synthesized from acetyl salicylic acid, thymol, aldimine and β-napthol etc have been frequently reported [4]. Developing antimicrobial drugs and maintaining their potency, in opposite on to resistance by different classes of microorganisms as well as a broad spectrum of antibacterial activity are some of the major concern of research in this area. Synthesis and antimicrobial activity of azo compound using imatinib intermediate and naphthalene-2-ol has been reported[5].

Generally, free radical attacks the nearest stable molecule, stealing its electron. When the attacked molecule loses its electron, it becomes a free radical itself, beginning a chain reaction cascade resulting in disruption of a living cell [6, 7]. There are two basic categories of antioxidants, namely, synthetic and natural. In general,

synthetic antioxidants are compounds with phenolic structures of various degrees of alkyl substitution, whereas natural antioxidants can be phenolic compounds, nitrogen compounds as well as ascorbic acid[8, 9]. The primary antioxidants comprise essentially sterically hindered phenols and secondary aromatic amines[10]. These antioxidants act usually both through chain transfer and chain termination. The first step of the reactive radical's termination by this type of antioxidants is hydrogen atom transfer from the antioxidant molecule to the reactive radical intermediate[11]. In general, water-soluble antioxidants react with oxidants in the cell cytosol and the blood plasma while lipid-soluble antioxidants protect cell membranes from lipid peroxidation[12]. These compounds may be synthesized in the body or obtained from the diet[13].

A significant part of drug discovery in the past few years has been focused on agents to prevent or treat cancer. This is not surprising because, in most developed countries and, to an increasing extent, cancer is among the three most common causes of death and morbidity. Cancer treatments may involve surgery, radiotherapy and chemotherapy and often a combination of two or all three is employed. Cancer treatment has been a major endeavour of research and development in academia and pharmaceutical industry for the last many years as it is one of the leading causes of death[14]. Still cancer remains the leading cause of death in developing and developed countries. Many of the available anticancer agents exhibit undesirable side effects such as reduced bioavailability, toxicity and drug-resistance[15-17]. Therefore, the search for novel and selective anticancer agents is urgently required due to problems associated with currently available anticancer drugs. Many biological activities are easy to be determined by spectrophotometric methods based on the changes in absorbance. The present paper reporting the biological activity of 2-piperazinoethylamines **3(a-f)** containing piperazine which was studied by UV-Visible spectrophotometer.

2. MATERIALS AND METHODS

2.1. Chemistry

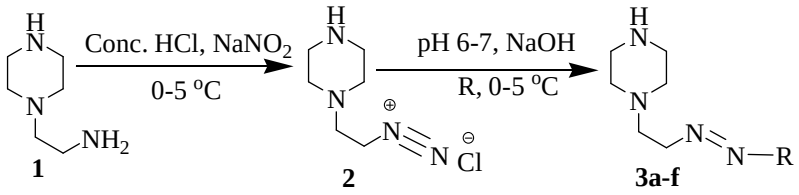
All solvents and reagents were purchased from Sigma Aldrich Chemicals Pvt Ltd. Melting range was determined by Veego Melting Point VMP III apparatus. The UV-Visible spectra were recorded on UV-1800 SHIMADZU UV spectrometer with quartz cell of 1.0 cm path length. The FT-IR spectra were recorded using KBr discs on FT-IR Jasco 4100 infrared spectrophotometer and were quoted in cm^{-1}. ^1H NMR spectra was recorded on Bruker DMX 300 spectrometer using DMSO-d_6 as solvent and TMS as an internal standard. Silica gel column chromatography was performed using Merck 7734 silica gel (60–120 mesh) and Merck-made TLC plates. The purity of compounds was checked by TLC.

2.1.1. General procedure for the synthesis of diazotization of 2-piperazinoethylamine (2)

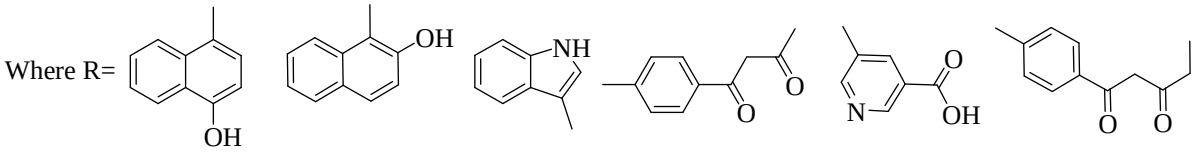
The amine **(1)** (0.721 mmol) was dissolved in 6 N HCl (25-30 mmol). The mixture was cooled by means of an ice-water bath till it attain 0-5 $^{\circ}C$ and an aqueous solution of $NaNO_2$ (0.901 mmol, 10 ml) was added drop wise within 15 min with continuous stirring. Finally the excess of HNO_2 was destroyed by adding solid urea (0.5 g). The intermediate diazonium compound (**2**) was obtained.

2.1.2. General procedure for the synthesis of diazotized derivatives of 2-piperazinoethylamine compounds (3a-f)

Compounds **3(a-f)** was synthesized by the reaction of diazonium compound (**2**) and different coupling agents[18]. During the procedure, the pH value was maintained within 6-7 by 6N NaOH and the temperature at 0-5 $^{\circ}C$. The mixture was stirred for 6-8 hrs. The precipitated crude compound was collected by filtration at vacuum and washed with water. The obtained compounds (**Scheme 1**) were recrystallized from the chloroform.



Scheme 1



3a **3b** **3c** **3d** **3e** **3f**

2.1.2.1. 4-(2-2-(Piperazin-1-yl)ethyldiazenyl) naphthalene-1-ol (3a)

Off white solid. Yield: 65%. FT-IR (KBr, cm^{-1}): 3500 (O-H), 3350 (N-H), 3010 (Ar-H), 1600 (N=N). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.41 (t, 2H, CH_2), 2.08 (s, 1H, NH pip), 2.40 (t, 2H, CH_2), 2.52 (m, 4H, $2CH_2$), 2.70 (m, 4H, $2CH_2$), 5.24 (d, 1H, OH), 6.54 (d, 1H, Ar-H), 7.16 (d, 1H, Ar-H), 7.65 - 8.07 (m, 4H, Ar-H).

2.1.2.2. 1-(Naphthalene-5-yl)-2-(2-piperazin-1-yl)ethyldiazene (3b)

Off brown solid. Yield: 72%. FT-IR (KBr, cm^{-1}): 3450 (O-H), 3400 (N-H), 3010 (Ar-H), 1590 (N=N). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.40 (t, 2H, CH_2), 2.11 (s, 1H, NH pip), 2.40 (t, 2H, CH_2), 2.53 (m, 4H, $2CH_2$), 2.74 (m, 4H, $2CH_2$), 5.25 (d, 1H, OH), 6.53 (d, 1H, Ar-H), 7.16 (d, 1H, Ar-H), 7.63 - 8.04 (m, 4H, Ar-H).

2.1.2.3. 2-(2-(2-Piperazine)-1-yl)ethyldiazonyl) -1H-indole-3-carbaxyaldehyde (3c)

Off yellow solid.Yield: 85 %. FT-IR (KBr, cm^{-1}): 3450 (N-H), 3300 (N-H indole), 3000 (Ar-H), 1590 (N=N). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.40 (t, 2H, CH_2), 2.13 (s, 1H, NH pip), 2.45 (t, 2H, CH_2), 2.48 (m, 4H, $2CH_2$), 2.64 (m, 4H, $2CH_2$), 7.15 (s, 1H, Ar-H), 7.45 (m, 4H, Ar-H), 10.05 (s, 1H, indole-NH).

2.1.2.4. 1-(4-(2-(Piperazin-1-yl)ethyl)diazenyl) phenyl)butane-1,3-dione) (3d)

Off white solid. Yield: 88 %. FT-IR (KBr, cm^{-1}): 3450 (N-H), 3025 (Ar-H), 1750 (C=O), 1590 (N=N). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.41 (t, 2H, CH_2), 1.50-1.55 (m, 4H, $2CH_2$), 2.08 (s, 3H, CH_3), 2.39 (m, 4H, $2CH_2$), 2.50 (t, 2H, CH_2), 3.33 (s, 2H, CH_2), 5.73 (s, 1H, NH pip), 7.42 (d, 2H, Ar-H), 7.82 (d, 2H, Ar-H).

2.1.2.5. 5-((2-Piperazin-1-yl)ethyl)diazenyl) nicotinic acid (3e)

Off brown solid. Yield: 85 %. FT-IR (KBr, cm^{-1}): 3400 (N-H), 3020 (Ar-H), 1700 (COOH), 1600 (N=N). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.42 (t, 2H, CH_2), 2.14 (s, 1H, NH pip), 2.40 (t, 2H, CH_2), 2.45 (m, 4H, $2CH_2$), 2.68 (m, 4H, $2CH_2$), 8.23 (s, 1H, Ar-H), 8.80 (s, 1H, Ar-H), 8.98 (s, 1H, Ar-H), 10.85 (s, 1H, COOH).

2.1.2.6. 1-(4-((2-Piperazin-1-yl)ethyl) diazeneyl)phenyl)pentane-1,3-dione (3f)

Off white solid. Yield: 85 %. FT-IR (KBr, cm^{-1}): 1600 (N=N), 1725 (C=O), 3005 (Ar-H), 3400 (NH). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.15 (t, 3H, CH_3), 1.45 (t, 2H, CH_2), 2.13 (s, 1H, NH pip), 2.40 (t, 2H, CH_2), 2.48 (q, 2H, CH_2), 2.43 (m, 4H, $2CH_2$), 2.64 (m, 4H, $2CH_2$), 3.65 (s, 2H, CH_2), 7.25 (d, 2H, Ar-H), 7.85 (d, 2H, Ar-H).

2.2. Pharmacology

2.2.1. Antibacterial activity

Broth dilution assay was carried out according to the method developed by malgorza *et al.*, 2007; Hwang *et al.,* 2010; Jae *et al.*, 2009 in a microtitre plate (96 well plate) with slight modifications[19-21]. 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 aluminium 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).

2.2.2. Antioxidant activity

DPPH radical scavenging assay

The free radical scavenging activity was measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay[22-24] according to the method described earlier by Brand-Williams *et al.*, 1995; Bursal *et al.,* 2011; Naima Saeed *et al.*, 2012. 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. A dose responsive curve was plotted to determine the IC_{50} values. IC_{50} is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity [25]. All the tests were run in triplicate and averaged.

2.2.3. Ferrous ion chelating assay

The chelating activity of the Schiff base derivatives for ferrous ions (Fe^{2+}) was measured according to the method[26] of Dinis *et al.,* 1994. 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. A dose responsive curve was plotted to determine the IC_{50} values. IC_{50} is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity. 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.

2.2.4. In vitro 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

Human breast cancer (MCF7), human glioma (U373) and astrocytoma brain tumor (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 % CO_2 at 37 °C. The cells were subcultured twice each week, seeding at a density of about 2×10^3 cells/ml.

In vitro cell viability assay-MTT assay

The potential effects on cell viability were investigated by using the MTT assay[26]. 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 (100 µM, 200 µM 400 µM expressed as final concentration) 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.

3. RESULTS AND DISCUSSION

3.1. Chemistry

Formation of diazotized derivatives of 2-piperazinoethylamine was confirmed by recording their ^{1}H NMR, FT-IR and UV-visible spectra. The synthesis employs readily available starting materials and simple procedures making this method very attractive and convenient for the synthesis of various azo compounds.

The absorptions around 3000 cm^{-1} in synthesized compounds confirm the aromatic stretching vibrations and the appearance of a medium to strong absorption bands above 1600 cm^{-1} due to a stretching vibration of the N=N bond formation in synthesized compounds. The characterization of new compounds was based upon a careful comparison of ^{1}H NMR spectra. An important characteristic feature in the ^{1}H NMR spectra of **1** showed NH_2 proton in 5.42 ppm, which was absent in the spectra of **3a-f**. The ^{1}H NMR spectra of new compounds showed multiplet (piperazine ring) in the region of δ, 2.40 - 2.74. Similarly a doublet appeared at δ, 6.53 - 8.98 are due to the protons of the aromatic group. The ^{1}H NMR spectra of **3a-f** showed NH group in the region of δ, 2.08-2.14.

3.2. Biology

3.2.1. In vitro antibacterial activity

The investigation of antibacterial screening data revealed that all tested compounds showed antibacterial activity against four pathogenic bacterial strains. Among the series **3a-f**, compounds **3f**, **3d** and **3e** exhibited a significant antibacterial activity against Gram positive and Gram negative bacteria. Compounds **3c**, **3b** and **3a** showed moderate inhibitory activity against tested bacterial strains in comparison to standard drug. Compound **3f** and **3d** was found to be more potent against gram positive and gram negative bacterial strains with the 92-99 % (at 500 µg) zone of inhibition (Table 1). The nature of the linkage (substituent on aromatic ring) influences the antibacterial activity. Compounds **3a-f** showed antibacterial activity in the order: **3f** > **3d** > **3e** > **3c** > **3b > 3a** against tested bacterial strains. However, the activities of the tested compounds are less than those of standard antibacterial agents used.

3.2.2. In vitro antioxidant activity

The compound **3b** and **3a** showed higher radical inhibition activity due to the presence of hydroxy group (electron donating group) in the aromatic ring[27]. Percentage of DPPH radical scavenging activity and IC_{50} values (Fig. 1) were depicted in Table 2. The aromatic ring system with hydroxyl group in **3b** and **3a** were found to be more active than other compounds in the series. Compounds **3c**-**3f** showed moderate antioxidant activity. The nature of the functional groups is crucial for biological activity. All the investigated substances were capable of chelating Fe^{2+} ions. 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[28]. The metal chelating effects of the samples were dependent on concentration and linearly increased with the sample concentration increased (Fig. 2). The affinity of **3a-f** for ferrous ions was relatively low comparison to EDTA. However the activity of **3b** was nearer to standard (Table 3).

3.2.3. In vitro antiproliferative activity

The antiproliferative action of the synthesized compounds **3a–f** was 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 4. The results were expressed as percentage of cell proliferation compared with cells in control (cells treated with vehicle, 0.1% DMSO). Compounds **3c** containing indole group and **3e** containing pyridine group are more potent antiproliferative activity (Fig. 3). Ortho substitution gave better results compared to para substitution in **3b** and **3a**, respectively. Antiproliferative activity of **3d** and **3f** in the series showed moderate activity.

4. CONCLUSION

In conclusion, Compounds **3f**, **3d** and **3e** exhibited a significant antibacterial activity against Gram positive and Gram negative bacteria. The compound **3b** showed higher radical inhibition and Fe^{2+} chelating activity. Compounds **3c** and **3e** 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*.

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Table 1: Antibacterial activity of the compounds 3(a-f)

Compound	Concentration	Bacterial strain			
		E. coli	*P. fluorescence*	*M. luteus*	*B. subtilis*
3a	1µg	10.78	2.03	5.56	14.07
	10µg	24.62	15.34	16.50	31.30
	50µg	38.97	31.82	39.80	51.39
	100µg	61.39	66.37	74.34	69.60
	200µg	65.37	67.22	75.24	74.56
	300µg	66.39	69.26	76.28	75.44
	400µg	75.59	71.77	77.57	76.94
	500µg	77.22	74.48	78.74	77.30
3b	1µg	13.85	10.12	16.36	15.96
	10µg	23.56	27.75	20.35	23.85
	50µg	30.79	65.16	28.04	30.83
	100µg	47.54	68.58	45.82	46.26
	200µg	54.59	69.94	64.32	59.22
	300µg	63.86	72.88	71.12	62.32
	400µg	71.17	77.99	75.76	73.74
	500µg	79.90	82.21	79.13	78.13
3c	1µg	20.78	12.03	25.56	14.07
	10µg	34.62	25.34	36.50	29.30
	50µg	48.97	31.82	49.80	41.39
	100µg	51.39	46.37	54.34	59.60
	200µg	65.37	57.22	65.24	74.56
	300µg	76.39	69.26	71.28	79.44
	400µg	82.59	79.77	78.57	81.94
	500µg	86.22	84.48	85.74	87.30
3d	1µg	50.89	19.40	12.03	35.49
	10µg	52.28	26.99	20.09	45.12
	50µg	63.59	48.33	44.66	56.36
	100µg	69.76	72.72	62.73	83.96
	200µg	80.81	79.02	77.40	87.67
	300µg	82.81	84.73	85.31	88.06
	400µg	87.59	86.83	87.62	91.98
	500µg	92.40	96.43	95.92	94.50
3e	1µg	39.83	37.72	39.48	34.34
	10µg	48.92	49.92	52.03	45.19
	50µg	55.08	54.30	62.57	57.23
	100µg	61.41	66.36	69.24	69.58
	200µg	67.00	70.77	73.50	75.61
	300µg	79.93	78.12	80.24	84.17
	400µg	85.25	88.31	88.33	87.43
	500µg	90.48	93.01	94.38	93.92
3f	1µg	20.15	44.22	52.59	46.90
	10µg	33.92	54.97	53.01	50.70
	50µg	47.47	69.29	60.76	55.87
	100µg	58.40	70.17	68.18	56.70
	200µg	66.19	72.78	72.55	57.64
	300µg	70.42	81.52	79.18	73.88
	400µg	73.89	90.68	90.02	84.87
	500µg	94.19	99.18	96.81	96.84
Standard	1µg	28.50	40.28	20.08	23.07
	10µg	39.81	43.41	41.03	35.50
	50µg	54.08	61.26	53.66	48.80
	100µg	78.06	83.19	82.65	87.61
	200µg	86.08	95.27	89.41	91.90
	300µg	95.27	96.25	94.41	98.86
	400µg	96.38	98.38	96.38	99.32
	500µg	99.51	99.51	99.10	99.69

Table 2: DPPH radical scavenging activity of the tested compounds

Compounds	% of Scavenging activity Concentrations (µg)					IC_{50} (µg/ml)
	20	40	60	80	100	
3a	56	59	63	67	75	17.85
3b	57	62	78	85	91	17.24
3c	24	30	38	41	48	104.16
3d	12	15	45	48	50	100.00
3e	46	51	54	57	61	38.46
3f	24	33	41	48	57	84.72
Standard	59	61	64	69	74	16.94

Table 3: Ferrous Ion Chelating activity of the tested compounds

Compounds	% of Ferrous ion chelating activity Concentrations (µg)					IC_{50} (µg/ml)
	20	40	60	80	100	
3a	59	73	82	89	95	16.24
3b	65	75	88	99	99	15.15
3c	33	42	49	58	69	61.22
3d	40	54	65	79	82	39.21
3e	61	78	85	90	96	16.12
3f	36	77	80	84	88	25.97
Standard	72	79	81	83	88	13.88

Table 4: Antiproliferative activity of 3(a-f) against cancer cells determined by MTT test (µM)

Compound	% Cell survival								
	MCF7			U373			C6		
	100	200	400	100	200	400	100	200	400
3a	22.78	35.79	48.91	27.12	39.01	50.24	35.86	41.37	51.09
3b	26.01	39.79	54.57	31.77	42.47	53.99	45.99	50.12	58.56
3c	50.78	63.76	80.73	45.14	64.38	79.99	60.78	75.57	85.89
3d	45.19	57.88	73.26	42.21	63.49	71.05	58.21	73.08	78.96
3e	54.48	69.12	82.16	48.91	69.68	81.37	68.27	79.46	88.46
3f	35.58	47.79	61.57	32.87	43.49	60.98	47.61	52.84	66.66
Control	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

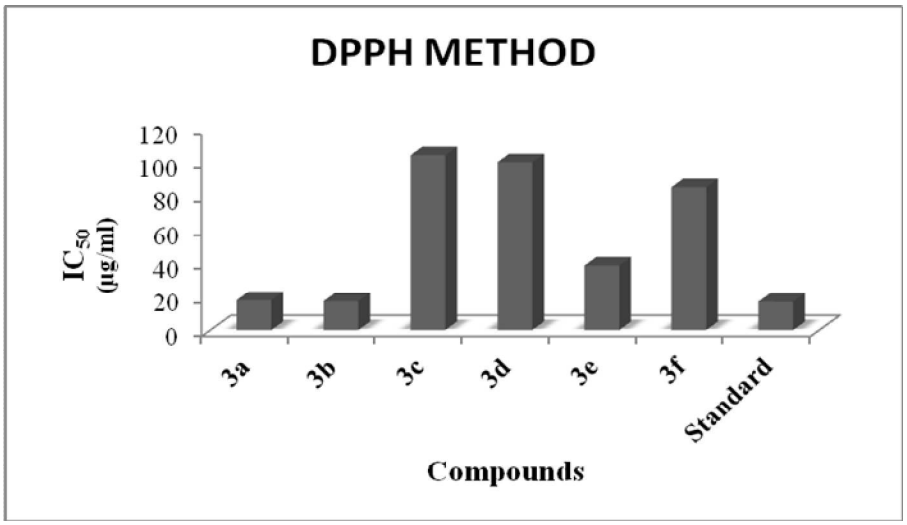

Fig. 1: IC_{50} values of 3a-f using DPPH Method

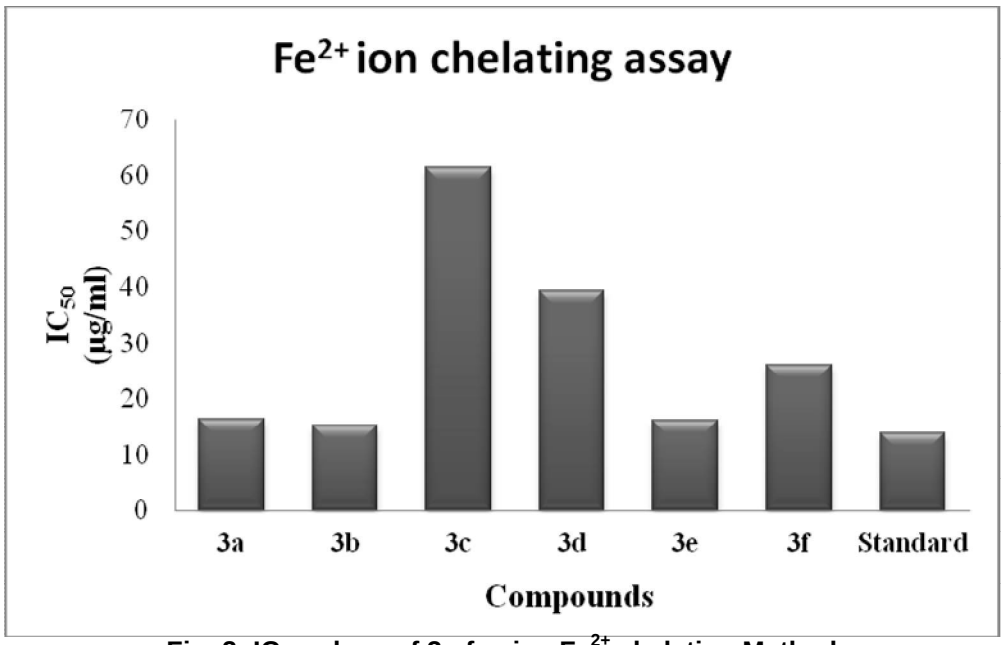

Fig. 2: IC_{50} values of 3a-f using Fe^{2+} chelating Method

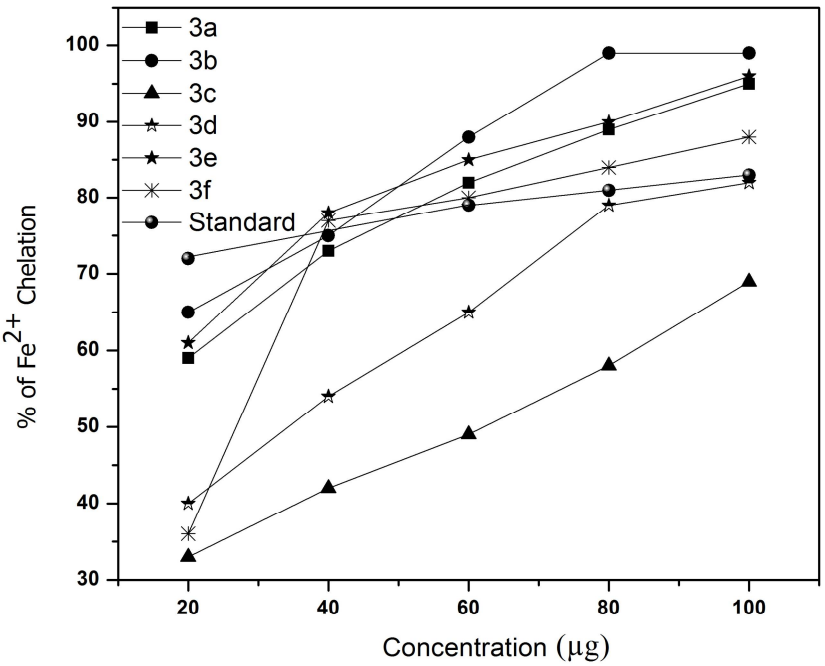

Fig. 3: Concentration verses % activity of 3a-f using Fe^{2+} chelating Method

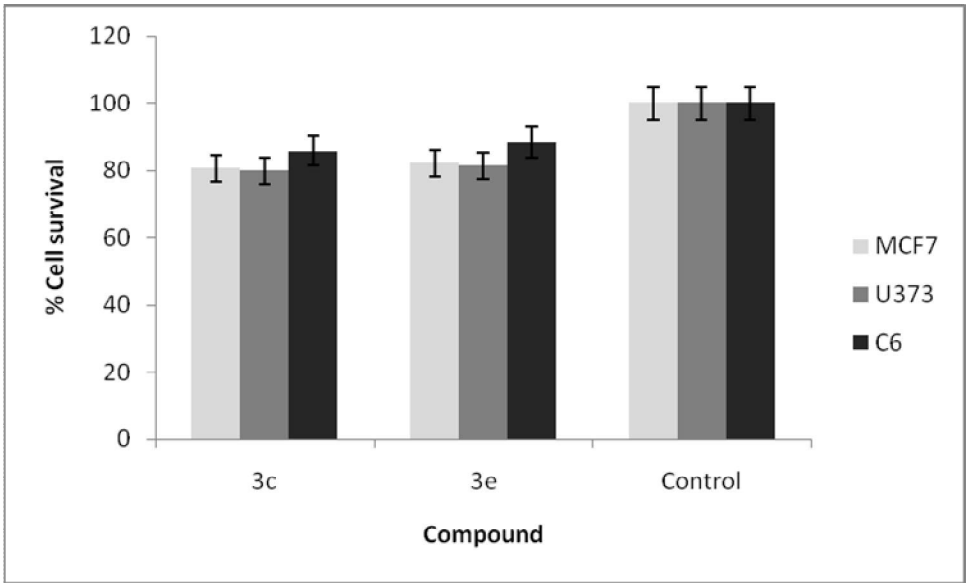

Fig. 4: MTT assay for 3c and 3e at 400 µM

REFERENCES

1. Gan LL, Fang B and Zhou CH. Synthesis of azole-containing piperazine derivatives and evaluation of their antibacterial, antifungal and cytotoxic activities. Bull Korean Chem Soc. 2010;31:3684-3692.
2. Osa Y, Kobayashi S, Sato Y, Suzuki Y, Takino K, Takeuchi T, Miyata Y, Sakaguchi M and Takayanagi H. Structural properties of dibenzosuberanylpiperazine derivatives for efficient reversal of chloroquine resistance in Plasmodium chabaudi. J Med Chem. 2003;46:1948-1956.
3. Sanjay FT, Dinesh MP, Manish PP and Ranjan GP. Synthesis and antibacterial activity of novel pyrazolo[3, 4-b]quinoline based heterocyclic azo compounds and their dyeing performance. Saudi Pharm J. 2007;15:48-54.
4. Awad IMA. Synthesis and application of novel sulpha drugs based on quinoxaline□2□one and/or quinoxaline□2□thione. J Chem Tech Biotech. 1992; 53:227-236.
5. Mallesha L, Karthik CS, Nithin KS and Mallu P. Synthesis and In Vitro biological activity of (E)-1-((4-Methyl-3-(4-(pyridin-3-yl)amino)phenyl)diazenyl) naphthalein-2-ol. Chem Sci Review Lett. 2013:2; 2013.
6. Cheeseman KH and Slater TF. Free radical in medicine. Churchill Livingstone, New York, 1993.
7. Mitchell RN and Cotran RS. Basic Pathology. 7th edn. Harcourt (India) Pvt Ltd, New Delhi, 2003;3-33.
8. Hall CA and Cuppett SL. Structure-activities of natural antioxidants. Antioxidant methodology in vivo and in vitro concepts. 1997;2-29.
9. Hudson BJF, Food Antioxidants, Elsevier Applied Science, London, 1990.
10. Gugumus F. Oxidation Inhibition in Organic Materials. CRC Press, Boca Raton, Florida, USA, 1990.
11. Pospısil J, Horak Z, Pilar J, Billingham NC, Zweifel H and Nespurek S. Influence of testing conditions on the performance and durability of polymer stabilisers in thermal oxidation. Polym Degrad stab, 2003:82;145-162.
12. Sies H. Oxidative stress: oxidants and antioxidants. Exp Physiol. 1997: 82;291-295.
13. Vertuani S, Angusti A and Manfredini S. The antioxidants and pro-antioxidants network: an overview. Current Pharma Design. 1997:10; 1677-1694.
14. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T and Thun MJ. Cancer statistics. Can J Clin, 2008:58;71–96.

15. Altmann KH. Microtubule-stabilizing agents: A growing class of important anticancer drugs. Current Opinion Chem Bio. 2001:5; 424-431.
16. Odwyer ME and Druker BJ. The role of the tyrosine kinase inhibitor STI571 in the treatment of cancer. Current cancer drug targets. 2001:1;49-57.
17. Mallesha L, Mohana KN and Veeresh B. Synthesis and biological activities of Schiff bases of gabapentin with different aldehydes and ketones: a structure–activity relationship study. Med Chem Res. 2012:21;1-9.
18. Karthik CS, Mallesha L, Santhosh MV and Mallu P. In Vitro Antimicrobial Activity of Some New Azo Compounds Synthesized from 2-Aminoethyl Piperazine. Chem Sci Rev Lett. 2014:3;224-230.
19. Hwang , Jae-Sam, Lee J, Hwang B, Sung-Hee N, Eun-Young Y, Seong-Ryul K and Lee D G. Isolation and characterization of psacotheasin, a novel Knottin-type antimicrobial peptide from Psacothea hilaris. J Microbiol Biotechnol. 2010:20;708-711.
20. Jae-Sam, Hwang, Lee J, Yeon-Ju K, Hea-Son B, Eun-Young Y, Seong-Ryul K, Jae-Pil J and Lee D G. Isolation and characterization of a defensin-like peptide (Coprisin) from dung beetle, copris tripartitus. Int J Pept. Article ID 136284, 2009
21. Malgorzata C, Pawel M, Zdybika-Barabas A, Piotr S and Teresa J. Purification and characterization of eight peptides from Galleria mellonella immune hemolymph. Peptides. 2007:28;533-546.
22. Brand-Williams W, Cuvelier ME, and Berset C. Use of free radical method to evaluate antioxidant activity. Lebensmittel-Wissenschaft und-Technol, 1995:28;25–30.
23. Bursal E and Gulcin I. Polyphenol contents and in vitro antioxidant activities of lyophilized aqueous extract of kiwifruit (Actinidia deliciosa). Food Res Int. 2011: 44;1482–1489.
24. Saeed N, Khan MR and Shabbir M. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts torilis leptophylla L. BMC comp alternative med. 2012:12; 221-225.
25. Wojdyło A, Oszmianski J and Czemerys R. Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chem. 2007:105; 940–949.
26. Dinis TC, Madeira VM, and Almeida LM. Action of phenolic derivatives (acetaminophen, salicylate and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. arch biochem biophys. 1994:315;161-169,
27. Mallesha L, Mohana KN, Veeresh B, Alvala R, and Mallika A. Synthesis and in vitro antiproliferative activity of 2-methyl-3-(2-piperazin-1-yl-ethyl)-pyrido [1, 2-a] pyrimidin-4-one derivatives against human cancer cell lines. Arch pharm res. 2012:35; 51-57.
28. Lloyd RV, Hanna PM and Mason RP. The origin of the hydroxyl radical oxygen in the Fenton reaction. Free Radical Bio Med. 1997:22;885-888.