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RESEARCH ARTICLE

Synthesis of a New Bicyclicgamma Lactam and Evaluation of their Anticancer Activity

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Abstract

Highly diastereoselective synthesis of γ-lactams 5(a-b) based on the reaction of imines 3(a-b) with phenylsuccinic anhydride is described in this study in moderate yields. The synthesized compounds were tested in vitro for their antioxidant activity. The prepared compounds showed an antioxidant activity. Anticancer activity of the compounds 3(a-b) were tested against breast cancer cells (MCF7), which showed high efficiancy against MCF7 cell lines . The compounds were determined on the basis of the spectral studies using IR, ^{1}H-NMR and^{13}C-NMR.

Keywords: *γ-lactams, Antioxidant, Anticancer, MCF7.*

Introduction

γ – Lactam

Five-membered ring lactams, which are known as γ-lactams or 2-oxopyrrolidines (Figure1), is very important structural motifs in biologically active natural products that is also found in medicinal leads and approved drugs [1]. γ-lactams is commonly known as Heterocyclic compounds. Heterocyclic organic compounds have a structure that contains in its cyclic structure one or more heteroatom in addition to carbon atoms. The importance of heterocyclic compounds is apparent from the wealth and variety of such compounds that occur naturally or are prepared on a commercial scale by the dye and drug industries. Many of these compounds have important physiological functions in plants and animals.[2]

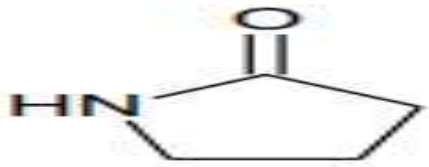


Fig.1

The γ-lactams acquired importance after the introduction of Heliotropamide[3,4] and bis avenanthramide[5,6] are examples of ferrulic acid amides that undergo biosynthetic dimerization to produce γ-lactams, whereas lactacystin[7] and salinosporamide[8] emanate from more complex biosyntheses. Although these compounds share the γ-lactam core, an important difference emerges in the substitution at nitrogen, in that the latter examples are sometimes described as "N-H lactams." Among drug compounds that are approved or in development, N-H lactams are represented significantly more often as shown by rolapitant, UCB-2892 and salinosporamide, in which only UCB-2892 is substituted. There are other lactam compounds that can also induce apoptosis. Watabe et al. found that gamma-lactams, which contain a five-membered ring, are capable of inducing apoptosis in HL-60 cells[8].

MT-21, the synthetic gamma-lactam activates caspase-9 followed by the subsequent activation of caspase-3. Unlike our findings with beta-lactams, caspase-8

was not found to participate in the apoptosis signaling cascade after gamma-lactam treatment [8].

Lactacystin, a gamma-lactam possessing a thio ester moiety and originally isolated from actinomycetes[9] has been found to be a potent inhibitor of chymotryptic- and tryptic-like catalytic activities of the proteasome through covalent bonding to the N-terminal threonine of the beta-subunits[10]. Proteasome inhibition leads to an accumulation of p27[11], IκB-α 78, and Bax79, which can cause G1 cell cycle arrest and apoptosis[12, 13]. For those reasons many believe that proteasome inhibitors are good candidates for anticancer chemotherapeutic drugs [14-15].

Materials and Methods

Instruments and Reagents

All solvents and all reagents were purchased from Fluka, Sigma–Aldrich and Merck. Human cancer cell lines; MCF-7 cell line were obtained from the Iraq biotech Cell Bank Unit. ^{1}HNMR and ^{13}CNMR spectra were recorded on Bruker AVANCE 500 MHz (500 MHz for proton, 125 MHz for carbon) In the Department of Chemistry, University of Tehran, Iran. Spectrometer with tetramethylsilane (TMS) as the internal reference using (DMSO-d6) as a solvent.

General Method for the Preparation of Imines 3(a-b) [16]

Schiff bases (bis imines) were prepared by mixing aldehyde with amine in (25 mL) of ethanol and (2-3) drops of glacial acetic acid. The mixture heated in water bath at (70-80˚C) for 30 min-1h. The reaction was followed by TLC. After the completion of the reaction, the solvent was evaporated, and the product was recrystallized using benzene. Methods of the preparation of the Schiff bases (bis imines) are listed below:

N, N'-bis [(E)-(4-N, N-dimethylphenylamino) methylidene] benzene-1, 4-diamine.3a

The above compound was prepared by reacting p-phenylenediamine (0.005 mol, 0.54g) with 4-(dimethyl amino) benzaldehyde (0.01 mol, 1.49 g). Yield = 87%, m.p. = 113-114^{o}C. IR ($\bar{\upsilon}$, cm^{-1}, KBr disk): 1616 (C=N).

4, 4'-{benzene-1, 4-diylbis [(E) methylylidenenitrilo]} diphenol.3b

This compound was prepared by reacting terephthaldehyde (0.005 mol, 0.67g) with 4-hydroxyaniline (0.01 mol, 1.09 g, 0.96 mL). Yield = 74%, m.p. = 189-191^{0}C. IR ($\bar{\upsilon}$, cm^{-1}, KBr disk): 1593 (C=N).

Preparation of Bis γ-lactams.5 (a-b) [17]

In general the γ-lactam was prepared by the reaction of a mixture of imines with phenylsuccinic in 20 mL of chloroform. The mixture was heated in a water bath at (55-60˚C), the mixture was refluxed for 12-48 h with stirring. The progress of the reaction was followed by TLC. After the completion, the solvent was evaporated and then the product was recrystallized from ethanol.

(E, Z) 1-1'-(phenyl-1, 4-diyl) bis [2-(4-N, N-dimethylaminophenyl)-5-oxo-3-phenylpyrrolidine-3-carboxylicacid]. (5a)

The compound was prepared by reacting (1.42 mmol, 0.52g) N,N'-bis[(E)-(4-N,N-di methylphenylamino) methylidene]benzene-1,4-diamine (3a) and phenyl succinic anhydride (2.84 mmol ,0.5 g). Yield = 70 %, m.p. = 253-254 ^{0}C. IR ($\bar{\upsilon}$, cm^{-1}, KBr disk): 1712 (–N–C=O), 1666 (HO–C=O). ^{1}H-NMR (500 MHz, DMSO-d$_6$, δ, ppm): 2.655 (m, 2H, C$_4$-H), 3.081 (m, 2H, C$_4$-H), 4.017 (m, 2H, C$_2$-H), 2.99 (s, 12H, -N-CH$_3$) 6.76-8.41 (m, 22H, Ar-H) and 9.89-9.98 (s, 2H, COOH).

(E, Z) 1-1'-(phenyl-1, 4-diyl) bis[2-(2-hydroxy-3-methoxy phenyl)-5-oxo-3-phenylpyrrolidine-3-carboxylicacid]. (5b)

The compound was prepared by reacting 1.42 mmol(0.448 g) 4,4'-{benzene-1,4-diylbis[(E) methyl ylide nenitrilo]}diphenol (3b) and phenyl succinic anhydride (2.84 mmol ,0.5 g). Yield = 80 %, m.p. = 270-271 ^{0}C. IR ($\bar{\upsilon}$, cm^{-1}, KBr disk): 1712 (–N–C=O), 1651(HO–C=O). For syn isomer (Z-isomer) as a major, ^{1}H-NMR (500 MHz, DMSO-d$_6$, δ, ppm): 2.655 (dd, J= 5, 15 Hz,1H, C$_4$-H), 3.074 (m, 4H, C$_4$-H), 4.036 (dd, J= 5.5, 10 Hz, 1H, C$_2$-H), 7.25-7.55, (m, 24H, Ar-H) and 9.94 (s, 1H, COOH). ^{13}C-NMR (125 MHz, DMSO-d$_6$, δ, ppm): 40.27 (C$_4$-H), 47.28 (C$_2$-H), 119.71-140.11 (Ar-C), 169.19 (-COOH) and 174.65 (-N-C=O).

For anti isomer (E-isomer) as a minor, ^{1}H-NMR (500 MHz, DMSO-d$_6$, δ, ppm): 2.564 (dd, J= 4, 15 Hz,1H , C$_4$-H), 3.074 (m, 4H, C$_4$-H), 4.09 (dd, J= 5, 10 Hz, 1H, C$_2$-H), 7.25-7.55, (m, 24H, Ar-H) and 10.12 (s, 1H, COOH). ^{13}C-NMR (125 MHz, DMSO-d$_6$, δ,

ppm): 37.79 (C_4-H), 48329 (C_2-H), 119.71-140.11 (Ar-C), 170.94 (-COOH) and 173.25 (-N-C=O).

Antioxidant Activity[18]

DPPH Radical Scavenging Activity

Methanolic solutions of 1,000 ppm concentration ofγ-lactms5 (a-b) were prepared. Varying amounts (5, 10, 15, 20 and 25 µL) of each methanolic solution of γ-lactms5 (a-b) were taken in separate test tubes containing5 ml of 0.004% methanolic solution of DPPH.

All the test solutions were prepared in triplicate. The mixtures were shaken vigorously and placed in dark for 2 h, or until stable values were obtained. The absorbance of the samples was measured at 517 nm. The percent DPPH radical scavenging activity of each sample and standard was calculated using the following equation:

% DPPH radical scavenging activity = [1 - (A_t / A_o)] x 100

Where, A_t is the absorbance of the sample, and A_o is the absorbance of the control. Mean values from three independent samples were calculated for each compound and ascorbic acid were used as a standard.

Reducing Power Activity

Different amounts of methanolic solutions of γ-lactms5(a-b)i.e., (0.1, 0.2, 0.3, 0.4 and 0.5) mg/ml were mixed with 2.5 ml of the phosphate buffer (200 mmol and pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixtures were incubated at 50 °C. After the incubation, 2.5 ml of 10% trichloroacetic acid was added to the mixtures, followed by centrifugation at 650 rpm for 10 min. The upper layer was separated, and 5 ml of it was mixed with 5 ml of distilled water and 1 ml of 0.1% ferric chloride. The absorbance of the resultant solutions was measured at 700 nm.

Anticancer Activity

Maintenance of Cell Cultures[19, 20]

MCF-7 cell line was obtained from the Iraq biotech Cell Bank Unit This human cell line was maintained in RPMI-1640 supplemented with 10% Fetal bovine, 100 units /mL penicillin, and 100 µg/mL streptomycin. Cells were pass aged using Trypsin-EDTA reseeded at 50% confluence twice a week, and incubated at 37 °C.

Cytotoxicity Assays (MTT assay)[21]

To determine the cytotoxic effect, the MTT cell viability assay was conducted on 96-well plates. Cell lines were seeded at 1 × 10^4cells/well. After 24 hrs. Or a confluent monolayer was achieved, cells were treated with tested compounds 5(a-b). Cell viability was measured after 72 hrs of treatment by removing the medium, adding 28 µL of 2 mg/mL solution of MTT (and incubating the cells for 1.5 h at 37 °C.

After removing the MTT solution, the crystals remaining in the wells was solubilized by the addition of 130 µL of DMSO (Dimethyl Sulphoxide) followed by 37 °C incubation for 15 min with shaking. The absorbency was determined on a micro plate reader at 492 nm (test wavelength); the assay was performed in triplicate. The inhibition rate of cell growth (the percentage of cytotoxicity) was calculated as the following equation:

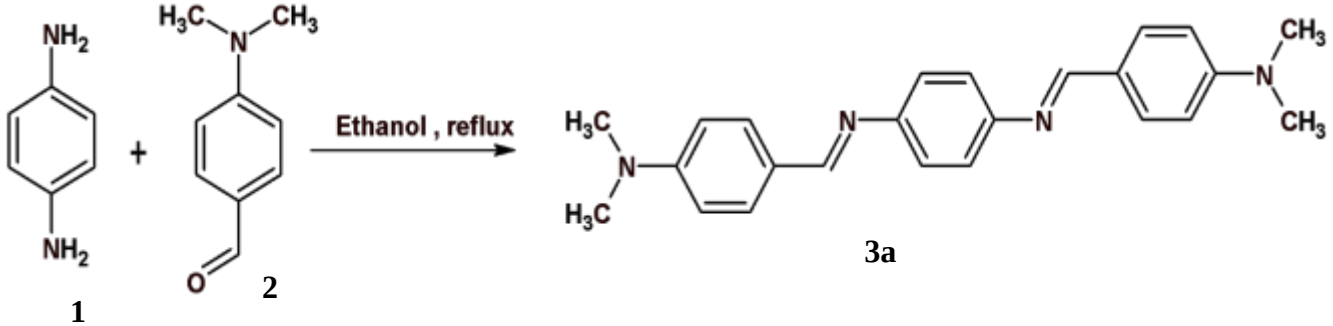
Inhibition rate = A-B/A

Where A and B are the optical density of control and the optical density of test, respectively.

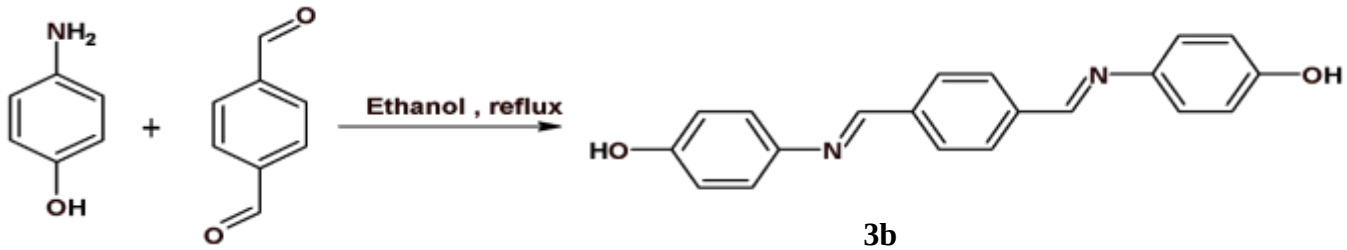
Results and discussion

Chemical

Initially, the preparation of imines 3(a-b) from reacting of 1 or 2 molar aromatic amines 1 with 1or 2 mole of aromatic aldehydes 2 in refluxing ethanol is shown in Scheme 1 and Table 1.



1 2 3a



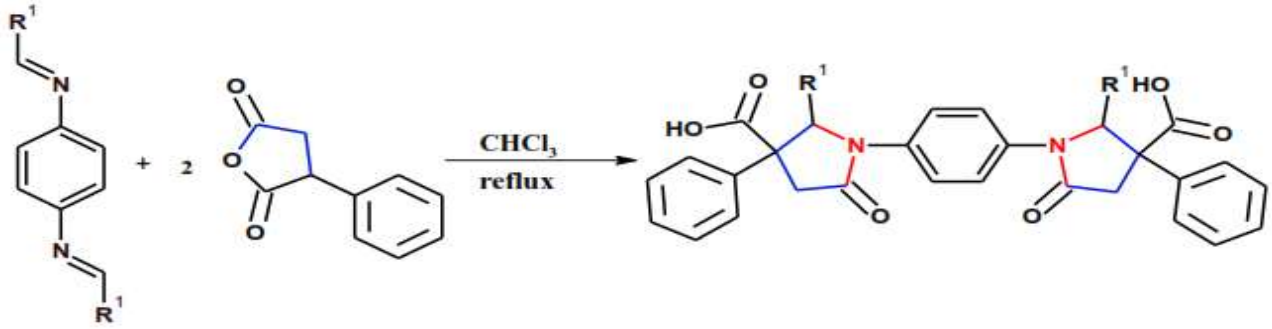
1 2 3b

Scheme 1:

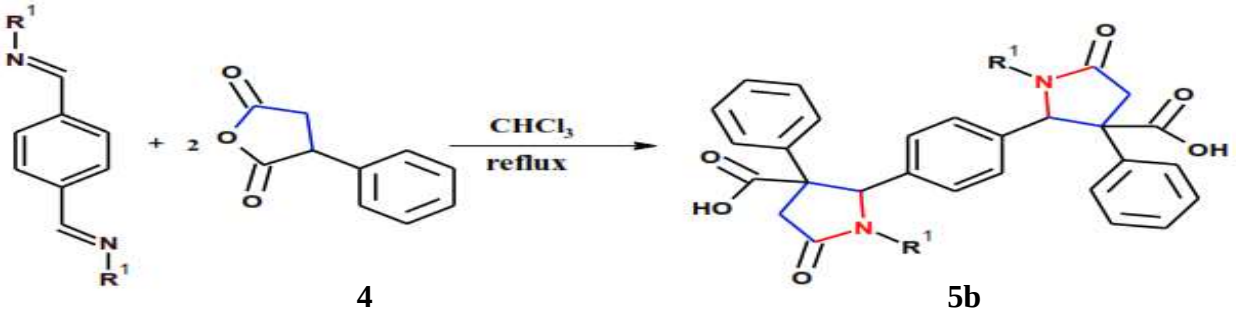
Table 1: Compounds of imine 3(a-b)

Comp. 3(a-b)	C°m.p	% Yield	Time	Solvent of recrystalization
3a	113-114	87	30 min	Benzene
3b	189-191	74	1 h	Benzene
Comp. 3(a-b)	C°m.p	% Yield	Time	Solvent of recrystalization
3a	113-114	87	30 min	Benzene
3b	189-191	74	1 h	Benzene

For synthesis of gamma lactams, the imine-anhydride reaction of imines 3(a-b) and phenylsuccinic anhydride 4 in 20 ml chloroform. The reaction produced 5(a-b) in 70-80 %yield. As shown in Scheme 2 and Table 2.



3a 4 5a

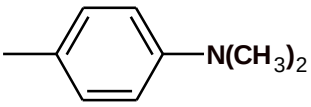
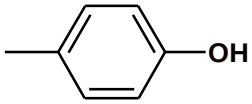


4 5b

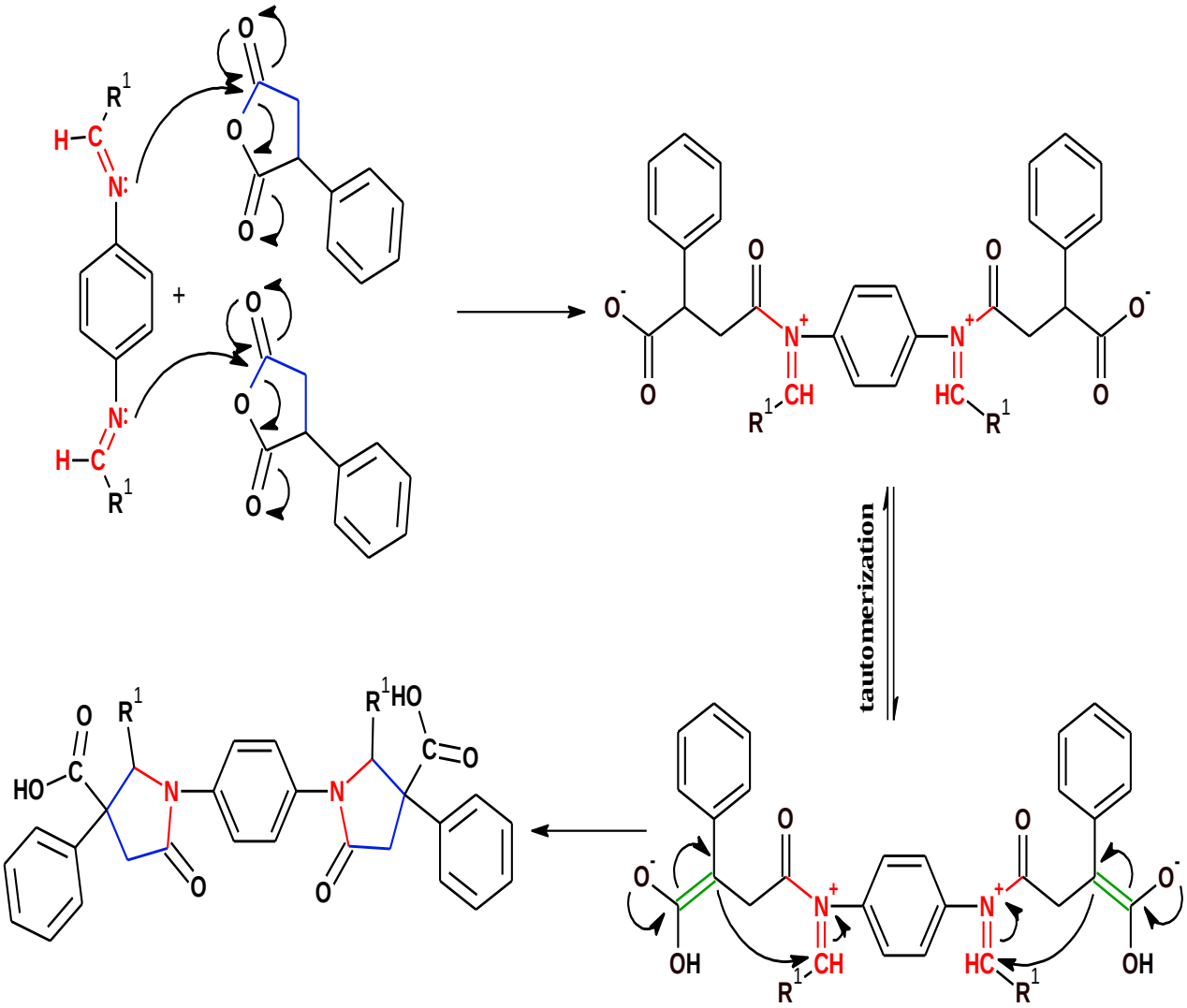
3b

Scheme 2:

Table 2: Compounds of γ-lactams 5(a-b)

Comp.	R^1	m.p	% Yield	Time
5a		253-254	70	12
5b		270-271	80	48

The suggested mechanism of γ- lactams 5(a-b) is outlined in Scheme 3. It is a reaction between phenylsuccinic anhydride with imines 3(a-b) in chloroform to yield γ-lactams 5(a-b).



Scheme 3: The suggested mechanism

Antioxidant Activity

DPPH radical scavenging activity:

The antioxidant activities of compounds (5a&5b) were investigated. The antioxidant activity was determined by using DPPH assay. Due to its simplicity and accuracy, DPPH assay is the most widely used method to assess antioxidant potential of compounds.

Therefore, the antiradical activities of test compounds Table (3) have been determined using DPPH assay.

During this assay, antioxidant is used to reduce the alcoholic solution of DPPH resulting in the formation of the non-radical form DPPH-H in the reaction. And, the dark colored DPPH radical solution in the presence of an antioxidant compound turned yellow-colored diphenylpicrylhydrazine in the presence of antioxidants and thus absorbance of the solution decreases.

The DPPH assay is commonly used to assess free radical scavenging activity of antioxidants. Fig. 2 shows a noteworthy decline in the concentration of DPPH radical in terms of % inhibition Table (4) due to the scavenging ability of test compounds. The change in absorbance was measured at 517 nm.

From DPPH radical scavenging activities of the tested compounds 5(a,b) and standard antioxidant in Figure (2) we found that the compound (5a) have more ability to radical scavenging from (5b), but less than the standard antioxidant compound (Vit. C).

Table 3: Absorption values of the compounds

Comp.	Conc. 1000 ppm																			
	5 µL				10 µL				15 µL				20 µL				25 µL			
	A_1	A_2	A_3	A_a	A_1	A_2	A_3	A_a	A_1	A_2	A_3	A_a	A_1	A_2	A_3	A_a	A_1	A_2	A_3	A_a
5a	0.228	0.229	0.227	0.228	0.173	0.172	0.172	0.172	0.154	0.155	0.157	0.155	0.143	0.142	0.144	0.143	0.128	0.129	0.129	0.128
5b	0.251	0.252	0.253	0.252	0.246	0.246	0.247	0.246	0.222	0.224	0.223	0.223	0.187	0.188	0.188	0.187	0.145	0.147	0.146	0.146
Vit. C	0.059	0.058	0.059	0.058	0.042	0.043	0.043	0.042	0.036	0.035	0.035	0.035	0.034	0.034	0.034	0.034	0.031	0.031	0.032	0.031

Table 4: Values of inhibition of compounds

Compo.	Conc. 1000 ppm														
	5 µL			10 µL			15 µL			20 µL			25 µL		
	A_a	A_o	(I%)	A_a	A_o	(I %)	A_a	A_o	(I %)	A_a	A_o	(I %)	A_a	A_o	(I %)
5a	0.228	0.635	64.09	0.172	0.635	72.86	0.155	0.635	75.53	0.143	0.635	77.48	0.128	0.635	79.73
4b	0.252	0.635	60.31	0.246	0.635	61.20	0.223	0.635	64.88	0.187	0.635	70.44	0.146	0.635	77.00
Vit. C	0.058	0.635	90.76	0.042	0.635	93.28	0.035	0.635	94.43	0.034	0.635	94.64	0.031	0.635	95.06

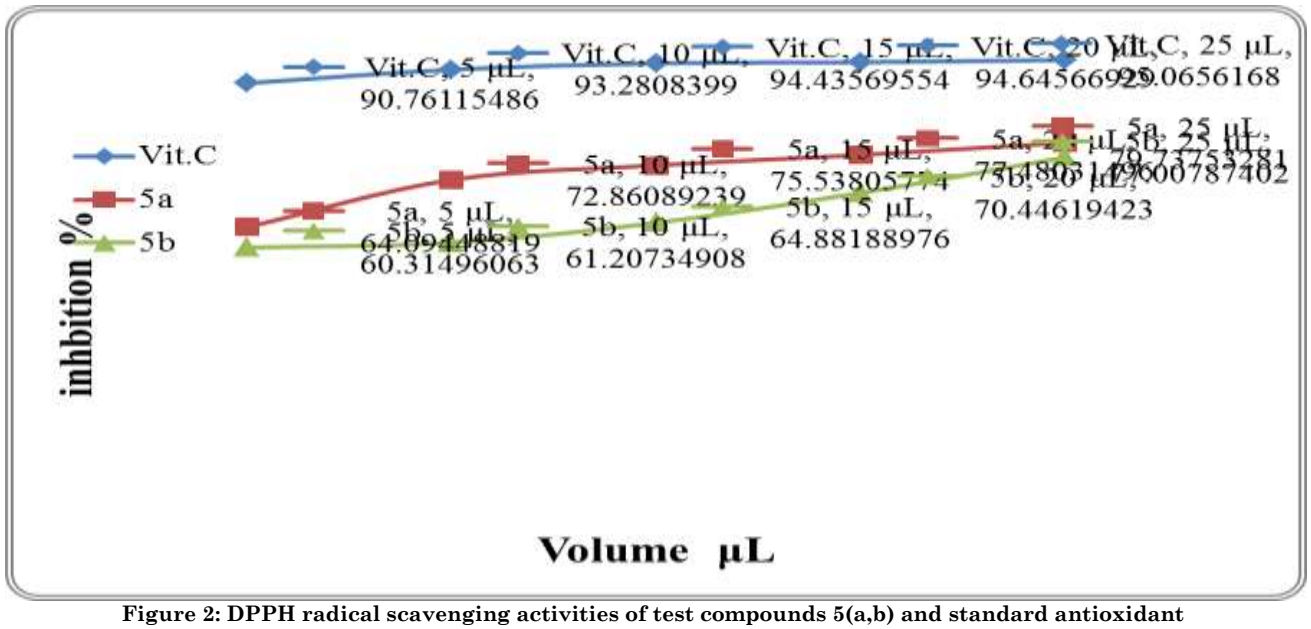


Figure 2: DPPH radical scavenging activities of test compounds 5(a,b) and standard antioxidant

Reducing Power Assay

Figures (3) and Table (5) show the reducing power of 5(a-b) as a function of their concentration. In this assay, the yellow colour of the test solution changes to various shades of green and blue, depending on the reducing power of each compound. The presence of reducers (i.e. antioxidants) causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. Therefore, measuring the formation of Perl's Prussian blue at 700 nm can monitor the Fe^{2+} concentration. The reducing power of the compounds increased with concentration.

The reducing power of 5a and 5b were excellent Figurs (3); at 0.5 mg/ml of the reducing power of 5a and 5b were higher than 0.514 and 0.495 respectively. At 0.1 mg/ml of the reducing powers of 5a and 5b were 0.283 and 0.265 respectively, and at 0.3 mg/ ml were 0.398 and 0.375 respectively. Reducing power of Vit.C at 0.5 mg/ml was 0.841.

Table 5: Absorption values of the compounds

Comp.	Concentration (mg/ml)																			
	0.1				0.2				0.3				0.4				0.5			
	A_1	A_2	A_3	A_a	A_1	A_2	A_3	A_a	A_1	A_2	A_3	A_a	A_1	A_2	A_3	A_a	A_1	A_2	A_3	A_a
5a	0.286	0.281	0.284	0.283	0.341	0.348	0.345	0.344	0.398	0.399	0.397	0.398	0.457	0.464	0.465	0.462	0.51	0.515	0.517	0.514
5b	0.265	0.269	0.263	0.265	0.331	0.338	0.332	0.333	0.374	0.372	0.38	0.375	0.441	0.446	0.443	0.443	0.497	0.491	0.497	0.495
Vit. C	0.328	0.324	0.329	0.327	0.451	0.448	0.453	0.451	0.573	0.579	0.571	0.574	0.698	0.689	0.7	0.696	0.844	0.838	0.841	0.841

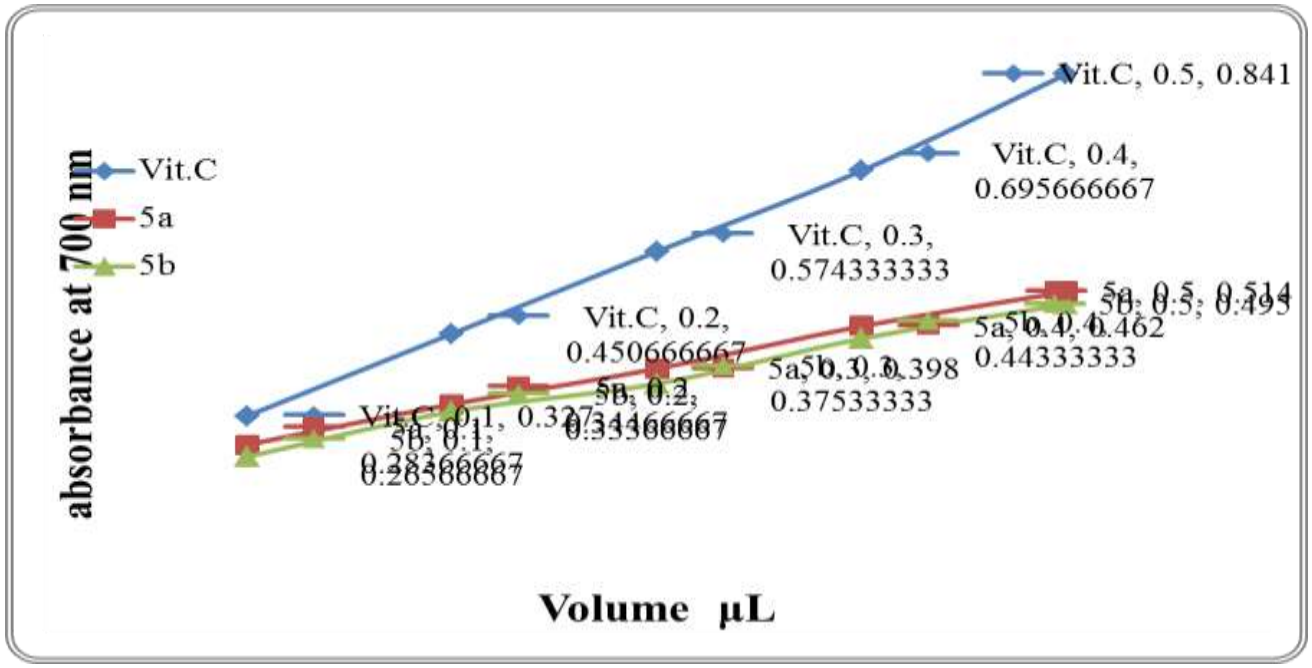


Figure 3: Reducing power of 5(a-b)

Anticancer Profiles

The anticancer potential of the developed compounds was assessed in terms of inhibition rate of cell growth on MCF-7 cancer cell lines Table (6). (E, Z) 1-1'-(phenyl-1,4-diyl) bis[2-(4-N,N-dimethylaminophenyl)-5-oxo-3-phenylpyrrolidine-3-carboxylicacid] (5a) and (E,Z) 1-1'-(phenyl-1,4-diyl) bis[2-(2-hydroxy-3-methoxy phenyl)-5-oxo-3-phenylpyrrolidine-3-carboxylicacid] (5b)on MCF-7 at varying concentrations (6.25, 12.5, 25, 50 and 100 µg/ml were determined and given in Figures (4 and 5) and Picture (1 and 2). From Figure (4), the compound 5a had viability of 24, 33, 43, 67 and 77 % at 6.25, 12.5, 25, 50 and 100 µg/ml respectively. Also, from Figure (5), the compound 5b had viability of 20, 30, 43, 70 and 78 % at 6.25, 12.5, 25, 50 and 100 µg/ml respectively. Thus, MCF-7 cells showed low viability on treatment with 5a which indicated good anticancer activities of this compound. Therefore, it may be concluded that these compounds follow different mechanisms and interact differently with different cellular targets.

Table 6: Inhibition rate of cell growth for (5a) and (5b)

Comp.	Concentration µg/ml	Cytotoxicity %
5a	6.25	24
	12.5	33
	25	43
	50	67
	100	77
5b	6.25	20
	12.5	30
	25	43
	50	70
	100	78

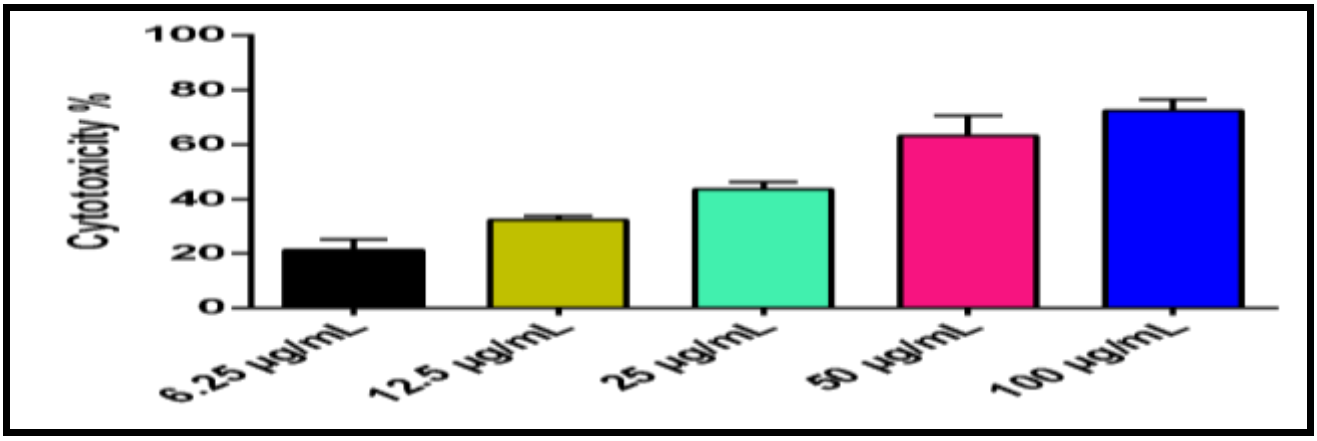
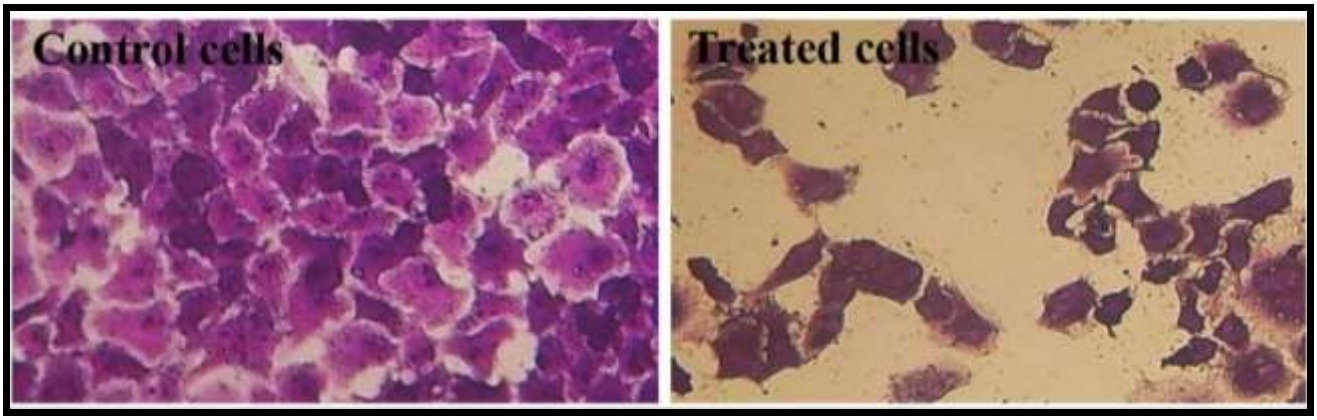


Figure4:Cytotoxic effect of 5aon MCF-7 cell line



Picture 1: Cytotoxic effect of 5a on MCF-7 cell line

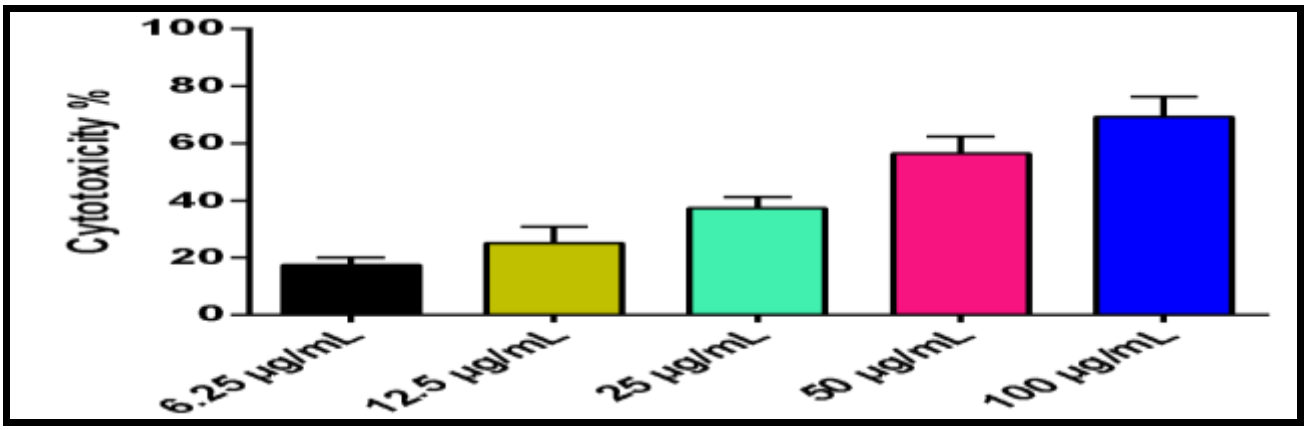
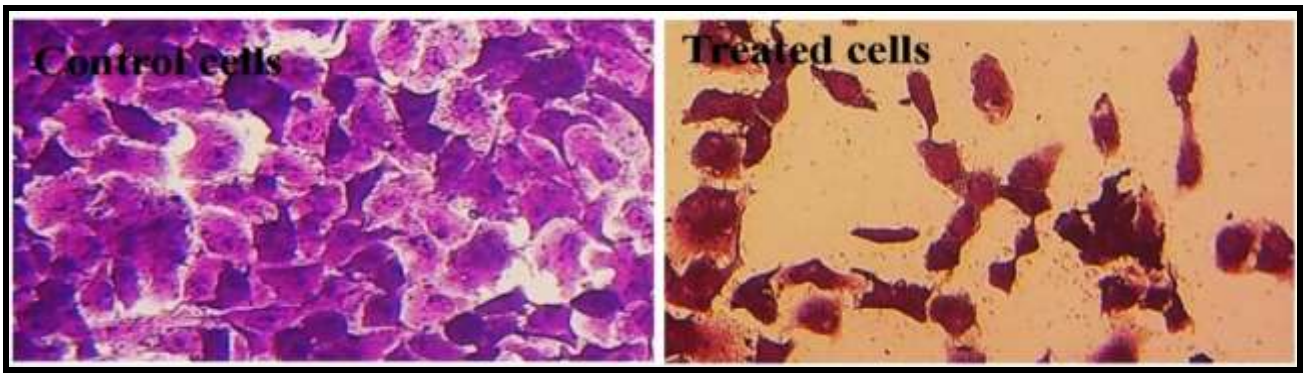


Figure 5: Cytotoxic effect of 5b on MCF-7 cell line



Picture 2: Cytotoxic effect of 5b on MCF-7 cell line

Nitrogen containing heterocyclic organic compounds having extra keto group show interesting chemical property as well as biological activity. Our finding is also concomitant with the earlier work.[22] who found that γ – Lactam can have the power to absorb free radicals and react as antioxidants. From our results we think that

γ – Lactam like beta-lactam has the potential anticancer medications. Many studies suggest that beta-lactam could play a role as anticancer drug. We think that gamma-lactams have tumor cell-killing ability through induction of DNA-damage and subsequent apoptosis, or it have the ability to preferentially induce apoptosis in tumor cells, but not in non transformed or normal cell [23,24]. Watabe et al. found that gamma-lactams are able to inducing apoptosis in HL-60 cells[25].

Gamma-lactam (Lactacystin), a possessing a thio ester moiety and originally isolated from actinomycetes[26], has been found to be a potent inhibitor of chymotryptic- and tryptic-like catalytic activities of the proteasome by covalent bonding to the N-terminal threonine of the beta-subunits[27]. Proteasome inhibition leads to an accumulation of p27 [28], IκB-α [29], and Bax[30], which can cause G1 cell cycle arrest and apoptosis [31]. It is for these reasons many believe that proteasome inhibitors are good candidates for anticancer chemotherapeutic drugs[32].

Conclusion

In the present study, Tetra substituted γ-lactams were synthesized and structurally characterized. The prepared (E, Z) 1-1'-(phenyl-1,4-diyl) bis[2-(4-N,N-dimethylaminophenyl)-5-oxo-3-phenylpyrrolidine-3-carboxylicacid] (5a) and (E,Z) 1-1'-(phenyl-1,4-diyl) bis [2-(2-hydroxy-3-methoxy phenyl)-5-oxo-3-phenylpyrrolidine-3-carboxylicacid] (5b) showed cytotoxic activity against MCF-7 cell lines revealing good activities. As well, the compounds revealed good effectiveness as antioxidant and could be believed as valuable templates for further investigations to get more potent agents.

Acknowledgments

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