DESIGN, SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF NOVEL BIS-INTERCALATORS AS POSSIBLE ANTICANCER AGENTS

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ABSTRACT:

The present study is aimed towards designing novel bis-intercalators as possible anticancer agents. The bis-intercalators designed are 4-substituted bisbenzamides and L-proline derivatives. In the present work, 56 bisbenzamide derivatives and 14 L-proline derivatives were synthesized based on docking studies. All the synthesized compounds were evaluated for their antioxidant activity and followed by anticancer activity using standard procedures. The antioxidant activity of synthesized compounds reveals that compound IIbL_6 was found to be potent with IC50 value of 54.51 when compared with the standard BHT. The antioxidant activity of L-Proline derivatives, compound IVL6 is found to be potent to exhibit antioxidant activity with IC50 value of 54.52 compared to standard BHT. The in-vitro cytotoxic activity of 4-substituted bisbenzamides reveals that two of the compounds, IIbL6 and IdL4, are particularly effective against the cancer cell line, with respective IC50 values of 55.5684 and 54.3489. The in-vitro cytotoxic results of L-Proline derivatives against two cell lines of cancer reveals that only two compounds, IVL_6 and $IIIL_4$ could inhibit the cell lines with IC_{50} values of 55.8236 and 48.0132, respectively. Hence, the results of the study could reveal that the synthesized 4-substituted bisbenzamides and L-proline derivatives can be potentially act as anticancer agents.

Key words: *4-substituted bisbenzamide, anticancer agents, L-proline derivatives, cytotoxic activity and cancer cell line.*

INTRODUCTION

Bisbenzamides are the compounds that have two benzamide groups. Bisbenzamides when linked with symmetric and asymmetric linker chains, the resulted compounds were shown to possess cytotoxic activity[1]. L-proline is a non-essential compound containing a pyrrolidine ring. L-proline being a heterocyclic compound finds use in research as a starting material for the synthesis of larger, usually bioactive structure. Its aromaticity makes it relatively stable although as a heterocycle, it has a reactive side which allows for functionalization[2]. L-proline are well known heterocyclic compounds among the organic and medicinal chemistry. Recently some other types of biological activity besides the Cytotoxicity activity have been reported in compounds containing proline ring which include antifungal, antibacterial, antioxidant activity[3, 4].

Cancer known medically as a malignant neoplasm, is a broad group of various diseases, all involving unregulated cell growth. In cancer, cells divide and grow uncontrollably, forming malignant tumors, and invade nearby parts of the body. The cancer may also spread to more distant parts of the body through the lymphatic system or bloodstream. Not all tumors are cancerous. Benign tumors do not grow uncontrollably, do not invade neighbouring tissues, and do not spread throughout the body. There are over 200 different known cancers that afflict humans[5, 6]. The chances of surviving the disease vary greatly by the type and location of the cancer and the extent of disease at the start of treatment. While cancer can affect people of all ages, and a few types of cancer are more common in children, the risk of developing cancer generally increases with age[7]. In chemistry, intercalation is the reversible inclusion of a molecule (or group) between two other molecules (or groups). Examples include DNA intercalation and graphite intercalation compounds[6]. There are several ways molecules (in this case, also known as ligands) can interact with DNA. Ligands may interact with DNA by covalently binding, electrostatically binding, or intercalating.[10] Intercalation occurs when ligands of an appropriate size and chemical nature fit themselves in between base pairs of DNA[8, 9]. These ligands are mostly polycyclic, aromatic, and planar, and therefore often make good nucleic acid stains. Intensively studied DNA intercalators include berberine, Ethidium bromide, proflavine, daunomycin, doxorubicin, and thalidomide. DNA intercalators are used in chemotherapeutic treatment to inhibit DNA replication in rapidly growing cancer cells[10, 11].

MATERIALS AND METHODS

Scheme-I

SYMMETRIC LINKER CHAINS (L_1 to L_5)

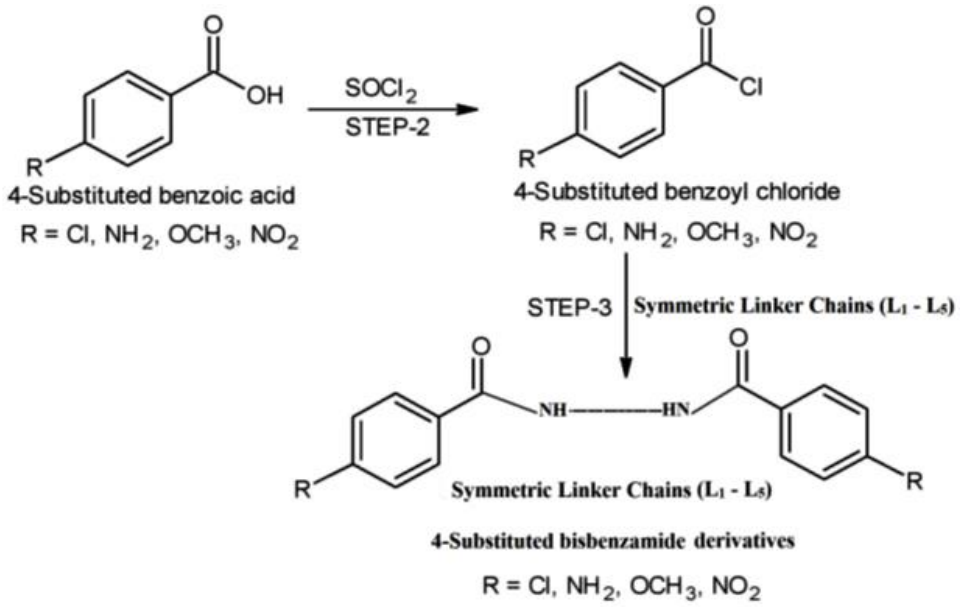
L_1 = Urea

L_2 = Ethylenediamine

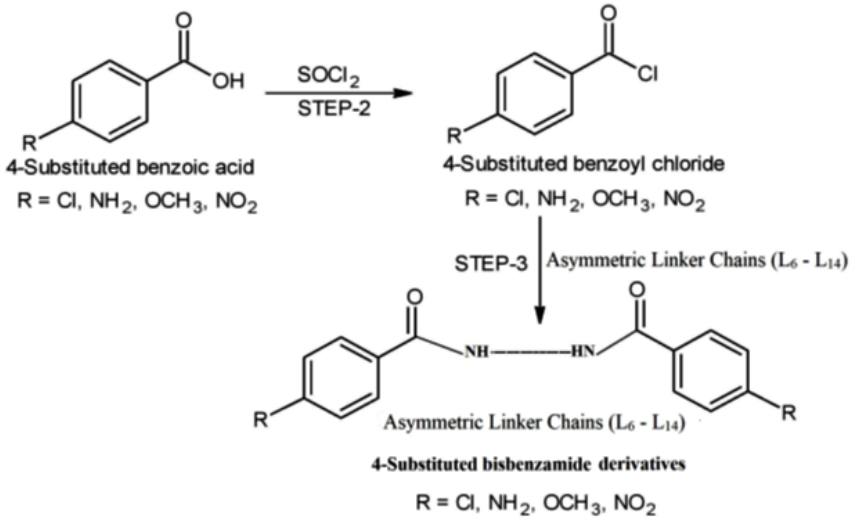
L_3 = Malonamide

L_4 = *N*-(Aminoacetyl)glycinamide

L_5 = *N, N'*-Bis-(2-aminoacetyl)ethylenediamine



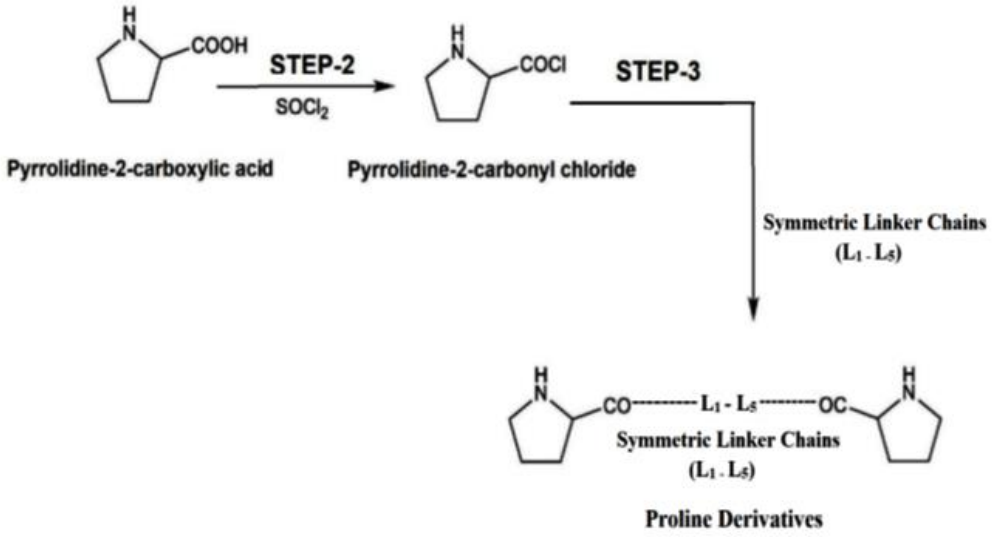
Scheme-II



ASYMMETRIC LINKER CHAINS (L_6 to L_{14})

L_6 = Glycinamide
L_7 = 2-(*N*-Ureido)acetamide
L_8 = *N*$_1$-(2-Acetamido)glycinamide
L_9 = *N*$_1$-(2-Aminoethyl)glycinamide
L_{10} = Malamide
L_{11} = *N*$_1$, *N'*-Bis(2-aminoethyl)malamide
L_{12} = 4-Aminobenzamide
L_{13} = 4-Amino-*N*-(2-aminoethyl)benzamide
L_{14} = 4-Amino-*N*-(2-acetamido)benzamides

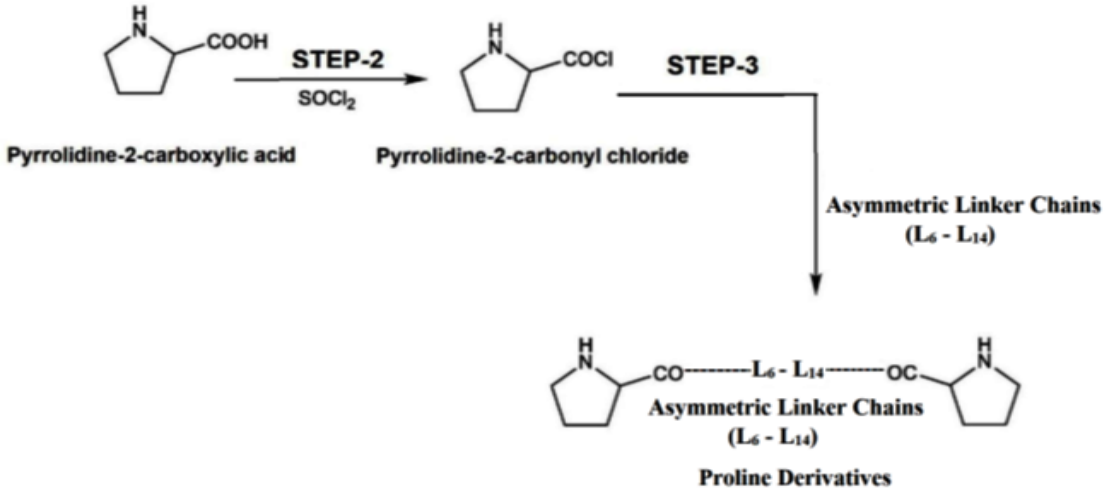
Scheme-III



SYMMETRIC LINKER CHAINS (L_1 to L_5)

L_1 = Urea
L_2 = Ethylenediamine
L_3 = Malonamide
L_4 = *N*-(Aminoacetyl)glycinamide
L_5 = *N, N'*-Bis-(2-aminoacetyl)ethylenediamine

Scheme-IV



ASYMMETRIC LINKER CHAINS (L_6 to L_{14})

L_6	=	Glycinamide
L_7	=	2-(*N*-Ureido)acetamide
L_8	=	N_1-(2-Acetamido)glycinamide
L_9	=	N_1-(2-Aminoethyl)glycinamide
L_{10}	=	Malamide
L_{11}	=	N_1, N'-Bis(2-aminoethyl)malamide
L_{12}	=	4-Aminobenzamide
L_{13}	=	4-Amino-*N*-(2-aminoethyl)benzamide
L_{14}	=	4-Amino-*N*-(2-acetamido)benzamides

General procedure for synthesis of compounds:

Synthesis of 4-substituted bisbenzamides:

Chlorination of 4-substituted benzoic acids:

Treatment with pure redistilled $SOCl_2$ (7.8 ml of 0.12M) in dry ether of 25 ml is reacted with 18.5 g of 4-substituted benzoic acid. It was refluxed for 30 minutes in a dry environment using a water bath. Distillation in a vacuum removes extra SOCl2 and solvent. The residue of the acid chloride is cleaned three times with dry ether (10 ml). This intermediate thus formed is hygroscopic and unstable.

Synthesis of 4-substituted bisbenzamides:

Ammonia and pure chloroacetyl chloride were combined in equal parts and stirred steadily for 30 minutes in 20 ml of 0.1 M, 11.2 g dry methanol (8.1 ml). The aforementioned combination is put in a separate beaker with Linker chain (L1-L14) (0.1 M, g suspended in five ml alcohol), and the two mixtures are then combined and refluxed for about an hour in a water bath. The mixture was concentrated in a hoover and left in a cool place all night. By recrystallizing the solid crystalline product from methanol, the end result was made purer.

Synthesis of L-Proline derivatives:

Chlorination of L-Proline:

L-Proline (0.1M, 18.5 g) is treated with pure redistilled $SOCl_2$ (0.12M, 14.28g, 7.8ml) in dry ether (25 ml). It is refluxed for 30 minutes in a water bath in a moisture-free environment. Excess $SOCl_2$ and solvent were removed by distillation under vacuum. The acid chloride residue was washed with 10 ml dry ether (3 times). This intermediate was hygroscopic and unstable.

Coupling reactions:

Coupling of Pyrrolidine-2-carbonyl chloride with linker chains (L_1-L_{14}):

The pyrrolidin-2-carbonyl chloride was hygroscopic and unstable; hence it was treated immediately with appropriate linker chains (L_1-L_{14}, 0.05M, 3g) in 25 ml of dry alcohol and was stirred for about 30 minutes in the cold. The mixture was concentrated and kept overnight for complete precipitation. The resultant solid was filtered, dried, and purified by recrystallization from an appropriate solvent.

Spectral analysis:

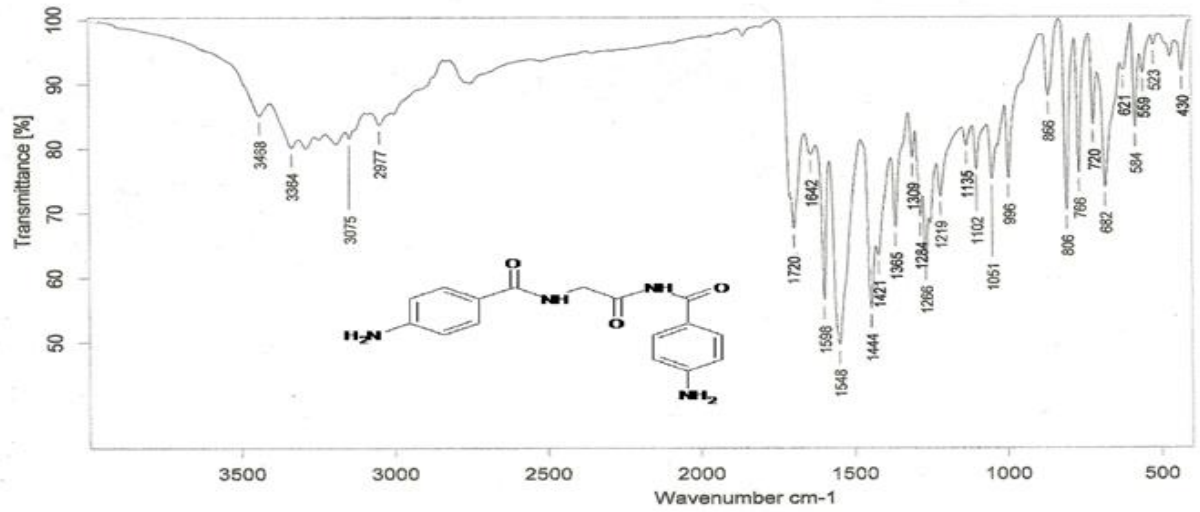


Figure 1. IR Spectra of *N,N'*-(1-oxoethane-1,2-diyl)bis(4-aminobenzamide) (IIbL6)

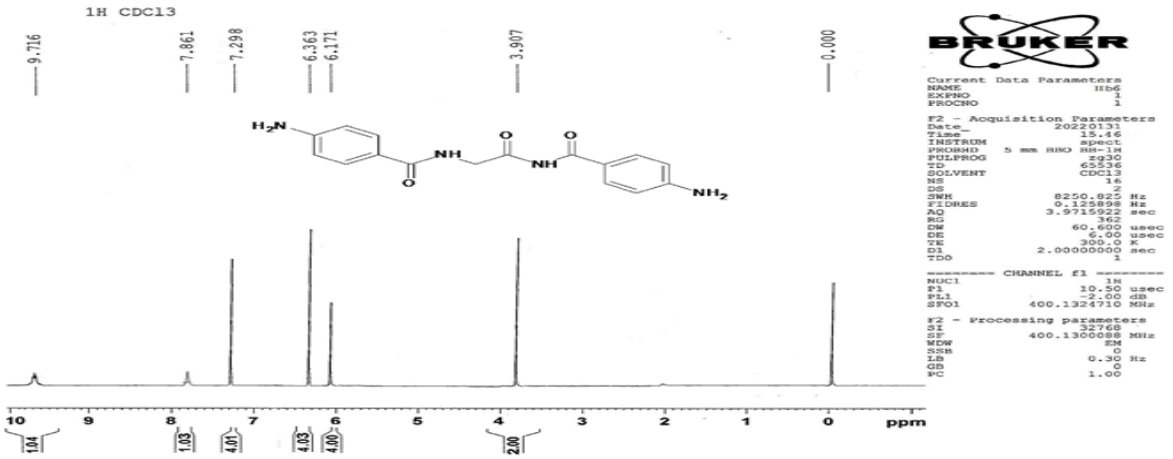


Figure 2. NMR Spectra of *N,N'*-(1-oxoethane-1,2-diyl)bis(4-aminobenzamide) (IIbL6)

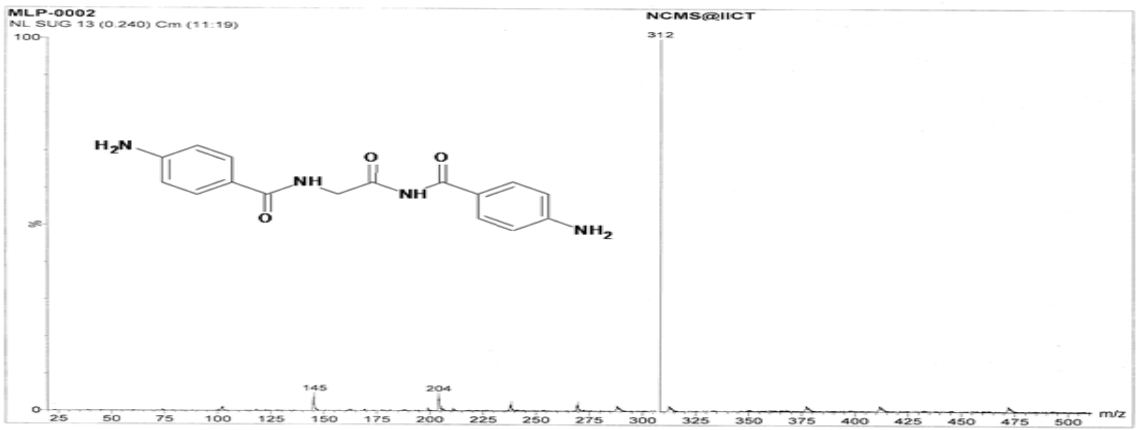


Figure 3. Mass Spectra of *N,N'*-(1-oxoethane-1,2-diyl)bis(4-aminobenzamide) (IIbL6)

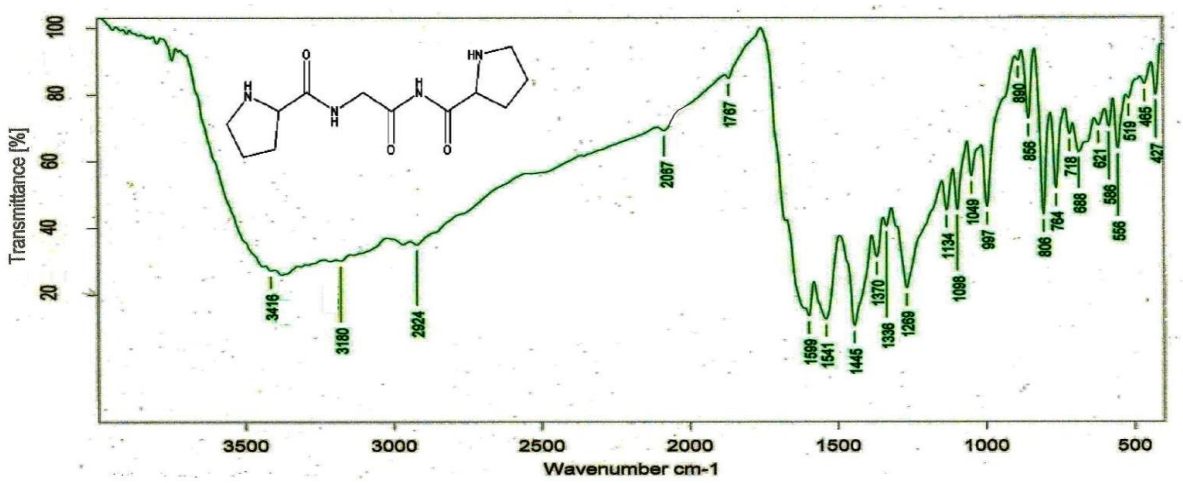


Figure 4. IR Spectra of *N*, *N*'-(1-oxoethane-1,2diyl)dipyrrolidine-carboxamide (IVL_6)

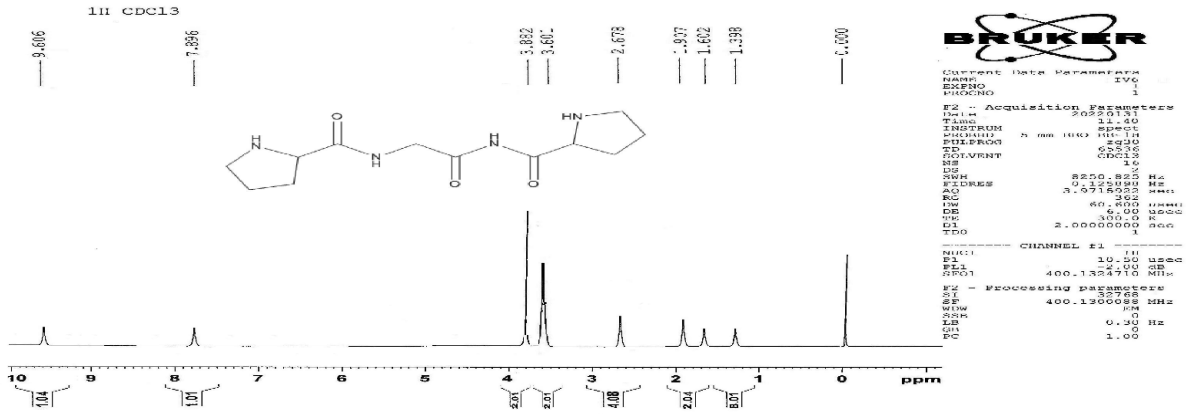


Figure 5. NMR Spectra of *N*, *N*'-(1-oxoethane-1,2diyl)dipyrrolidine-carboxamide (IVL_6)

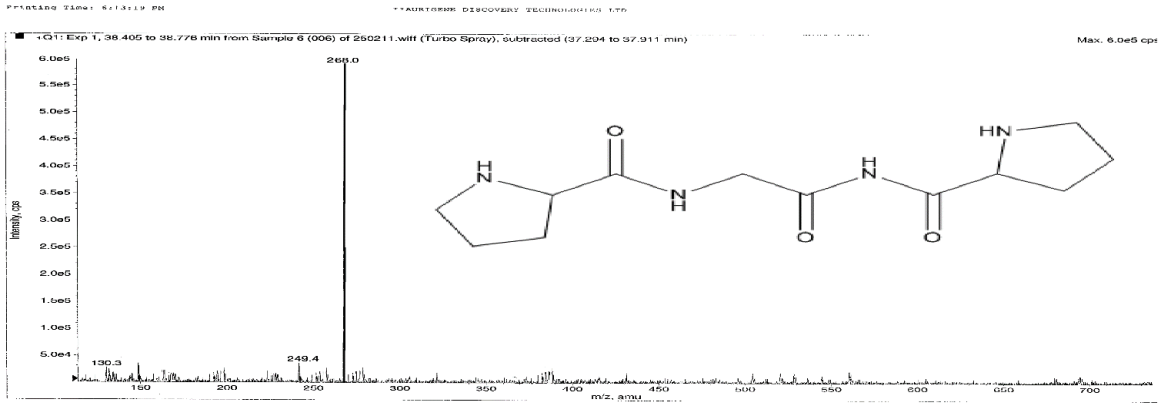


Figure 6. Mass Spectra of *N*, *N*'-(1-oxoethane-1,2diyl)dipyrrolidine-carboxamide (IVL_6)

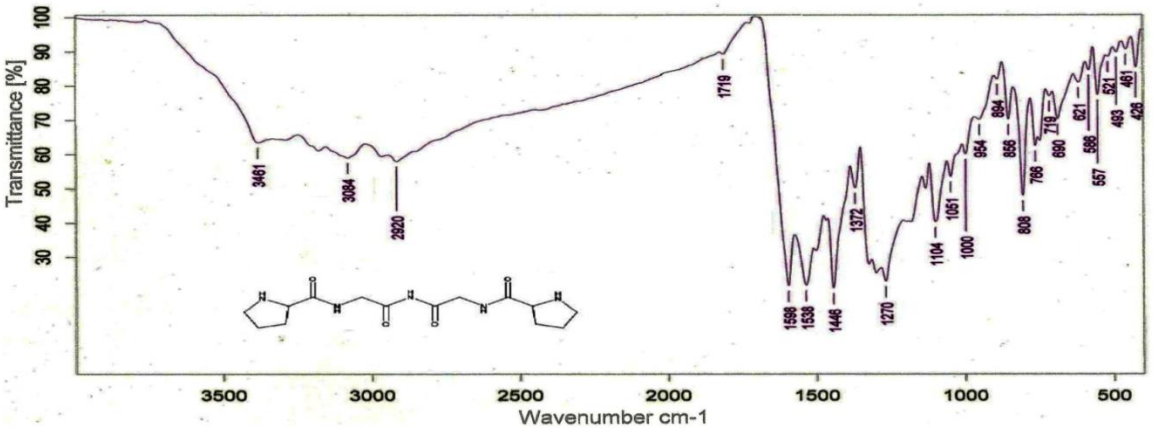


Figure 7. IR Spectra of *N*, *N*'-[iminobis(2-oxoethane-2,1-diyl)]dipyrrolidine-2-carboxamide

(IIIL4)

Figure 8. NMR Spectra of *N*, *N*'-[iminobis(2-oxoethane-2, 1-diyl)]dipyrrolidine-2-carboxamide

(IIIL4)

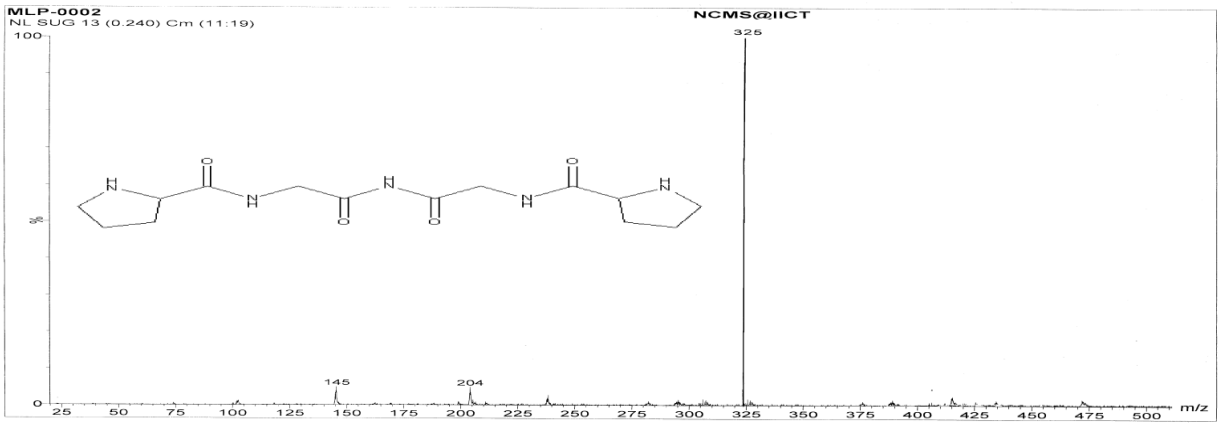


Figure 9. Mass Spectra of *N*, *N*'-[iminobis (2-oxoethane-2,1-diyl)] dipyrrolidine-2-carboxamide

(IIIL4)

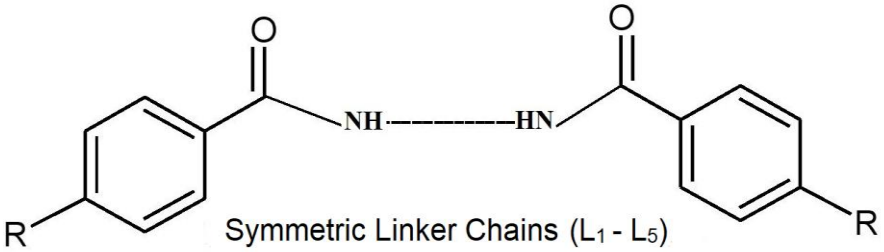
RESULTS:

Antioxidant activity:

All the synthesized compounds of Scheme-I and Scheme-II were evaluated for their antioxidant activity by following in vitro methods, i. e., DPPH assay and Hydrogen peroxide (H_2O_2) method.

DPPH method:

Table 1. Antioxidant activity (% Inhibition, IC_{50} values) of 4-Substituted bisbenzamide derivatives (Scheme-I) with Symmetric Linker Chains ($L_1 – L_5$)



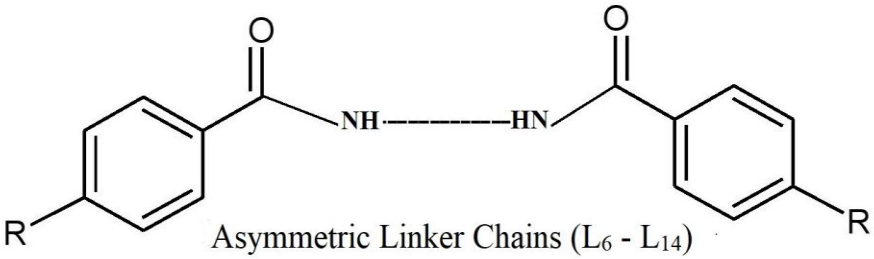
4-Substituted bisbenzamide derivatives

R = Cl, NH_2, OCH_3, NO_2

Code	R	Linker Chain	% Inhibition					IC_{50} µg/ml
			20	40	60	80	100	
IaL_1	-Cl	-NHCONH-	24.48	28.42	31.67	42.85	48.95	112.95
IaL_2	-Cl	-NHCH2CH_2NH-	28.62	35.84	49.66	55.84	62.68	105.48
IaL_3	-Cl	-NHCOCH_2CONH-	14.51	24.98	35.38	38.98	44.82	122.84
IaL_4	-Cl	-NHCH_2CONHCOCH_2NH-	25.62	31.56	35.84	41.78	47.95	109.64
IaL_5	-Cl	-NHCH_2CONHCH_2CH_2NHCO CH_2NH-	29.58	36.94	41.85	48.66	51.23	112.45
IbL_1	-NH_2	-NHCONH-	36.75	38.14	41.78	49.84	52.63	105.62
IbL_2	-NH_2	-NHCH2CH_2NH-	24.50	27.84	30.52	32.41	36.85	130.74
IbL_3	-NH_2	-NHCOCH_2CONH-	51.42	55.84	65.85	72.86	84.68	60.28
IbL_4	-NH_2	-NHCH_2CONHCOCH_2NH-	38.55	42.51	48.87	50.12	54.65	102.52
IbL_5	-NH_2	-NHCH_2CONHCH_2CH_2NH COCH$_2$NH-	35.42	39.84	42.56	44.51	50.12	110.84
IcL_1	-OCH_3	-NHCONH-	25.42	29.84	32.25	37.66	42.42	102.55
IcL_2	-OCH_3	-NHCH2CH_2NH-	35.42	38.52	40.25	45.75	49.75	121.62
IcL_3	-OCH_3	-NHCOCH_2CONH-	39.52	40.75	45.64	51.84	55.85	111.42
IcL_4	-OCH_3	-NHCH_2CONHCOCH_2NH-	42.15	45.85	51.45	57.65	63.42	104.35
IcL_5	-OCH_3	-NHCH_2CONHCH_2CH_2NH COCH$_2$NH-	42.15	49.62	55.42	58.84	64.32	94.35
IdL_1	-NO_2	-NHCONH-	52.61	65.38	70.12	73.63	80.25	102.66
IdL_2	-NO_2	-NHCH2CH_2NH-	28.66	36.42	40.66	45.11	52.24	101.62
IdL_3	-NO_2	-NHCOCH_2CONH-	32.62	36.45	39.84	43.51	48.62	112.23
IdL_4	-NO_2	-NHCH_2CONHCOCH_2NH-	59.46	64.98	69.82	72.55	75.63	**59.89**

IdL_5	$-NO_2$	$-NHCH_2CONHCH_2CH_2NHCOCH_2NH-$	21.42	27.65	30.53	32.56	36.84	130.12
Std. (BHT)			**69.84**	**73.02**	**76.19**	**77.78**	**80.95**	**49.44**

Table 2. Antioxidant activity (% Inhibition, IC_{50} values) of 4-Substituted bisbenzamide derivatives (Scheme-II) with Asymmetric Linker Chains (L_6 – L_{14})



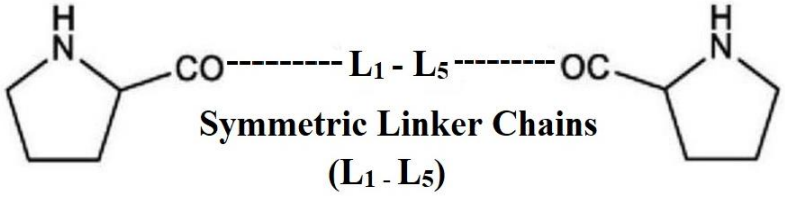
4-Substituted bisbenzamide derivatives

R = Cl, NH_2, OCH_3, NO_2

Code	R	Linker Chain	% Inhibition					IC_{50} µg/ml
			20	40	60	80	100	
$IIaL_6$	-Cl	$-NHCH_2CONH-$	16.85	28.75	36.66	39.87	45.78	122.84
$IIaL_7$	-Cl	$-NHCH_2CONHCH_2CONH-$	48.80	55.42	68.75	79.62	81.32	**67.78**
$IIaL_8$	-Cl	$-NHCONHCH_2CONH-$	26.42	29.44	34.51	41.74	45.95	112.95
$IIaL_9$	-Cl	$-NHCH_2CONHCH_2CH_2NH-$	31.55	36.44	51.41	55.88	61.49	105.48
$IIaL_{10}$	-Cl	$-NHCO(CH_2)_2OHCONH-$	13.91	23.62	29.88	32.86	45.97	122.84
$IIaL_{11}$	-Cl	$-NH(CH_2)_2NHCOCHOHCH_2CONH(CH_2)_2NH-$	21.52	27.88	31.52	34.52	49.62	109.49
$IIaL_{12}$	-Cl	-NHArCONH-	41.35	44.78	47.45	51.26	54.95	119.88
$IIaL_{13}$	-Cl	$-NHArCONH(CH_2)_2NH-$	45.82	47.98	50.22	53.64	59.62	105.65
$IIaL_{14}$	-Cl	$-NHArCONHCH_2CONH-$	51.42	54.68	57.85	61.62	65.66	101.82
$IIbL_6$	$-NH_2$	$-NHCH_2CONH-$	58.24	65.42	68.55	71.21	73.54	**54.51**
$IIbL_7$	$-NH_2$	$-NHCH_2CONHCH_2CONH-$	14.32	17.84	21.42	25.49	30.56	129.52
$IIbL_8$	$-NH_2$	$-NHCONHCH_2CONH-$	28.52	34.65	39.87	41.42	47.55	109.32
$IIbL_9$	$-NH_2$	$-NHCH_2CONHCH_2CH_2NH-$	33.44	37.78	41.52	46.66	49.88	99.52
$IIbL_{10}$	$-NH_2$	$-NHCO(CH_2)_2OHCONH-$	32.21	36.66	39.51	42.55	45.78	110.52
$IIbL_{11}$	$-NH_2$	$-NH(CH_2)_2NHCOCHOHCH_2CONH(CH_2)_2NH-$	42.68	47.78	51.32	61.56	72.95	105.98
$IIbL_{12}$	$-NH_2$	-NHArCONH-	13.48	21.54	24.68	29.84	31.52	139.23
$IIbL_{13}$	$-NH_2$	$-NHArCONH(CH_2)_2NH-$	25.54	29.42	32.62	35.65	38.94	125.65
$IIbL_{14}$	$-NH_2$	$-NHArCONHCH_2CONH-$	26.45	29.65	34.55	38.78	41.21	103.65
$IIcL_6$	$-OCH_3$	$-NHCH_2CONH-$	13.54	15.28	21.68	24.84	29.15	128.52
$IIcL_7$	$-OCH_3$	$-NHCH_2CONHCH_2CONH-$	29.84	32.33	35.85	40.12	43.75	105.42
$IIcL_8$	$-OCH_3$	$-NHCONHCH_2CONH-$	29.98	32.52	36.47	39.25	40.85	101.25
$IIcL_9$	$-OCH_3$	$-NHCH_2CONHCH_2CH_2NH-$	54.52	65.84	68.92	71.43	73.51	**61.23**
$IIcL_{10}$	$-OCH_3$	$-NHCO(CH_2)_2OHCONH-$	32.51	37.06	41.52	45.95	57.62	95.88

$IIcL_{11}$	$-OCH_3$	$-NH(CH_2)_2NHCOCHOHCH_2CONH(CH_2)_2NH-$	19.42	23.56	29.42	34.65	39.78	126.52
$IIcL_{12}$	$-OCH_3$	-NHArCONH-	32.28	45.12	51.48	61.25	65.48	105.48
$IIcL_{13}$	$-OCH_3$	$-NHArCONH(CH_2)_2NH-$	14.62	21.28	32.64	37.45	41.62	120.45
$IIcL_{14}$	$-OCH_3$	$-NHArCONHCH_2CONH-$	22.32	25.46	29.85	32.65	49.51	109.45
$IIdL_6$	$-NO_2$	$-NHCH_2CONH-$	38.29	46.65	49.28	52.45	55.25	91.42
$IIdL_7$	$-NO_2$	$-NHCH_2CONHCH_2CONH-$	43.62	47.46	51.25	55.36	59.42	78.56
$IIdL_8$	$-NO_2$	$-NHCONHCH_2CONH-$	45.12	48.62	53.44	57.86	62.45	85.42
$IIdL_9$	$-NO_2$	$-NHCH_2CONHCH_2CH_2NH-$	41.64	45.15	53.68	55.65	61.32	89.66
$IIdL_{10}$	$-NO_2$	$-NHCO(CH_2)_2OHCONH-$	48.63	54.87	68.95	72.82	78.65	95.42
$IIdL_{11}$	$-NO_2$	$-NH(CH_2)_2NHCOCHOHCH_2CONH(CH_2)_2NH-$	37.52	45.87	48.96	51.42	60.24	98.54
$IIdL_{12}$	$-NO_2$	-NHArCONH-	38.90	41.35	44.78	47.65	47.48	119.44
$IIdL_{13}$	$-NO_2$	$-NHArCONH(CH_2)_2NH-$	43.27	45.62	48.62	50.47	53.63	101.65
$IIdL_{14}$	$-NO_2$	$-NHArCONHCH_2CONH-$	45.62	48.66	51.26	53.45	54.66	95.78
Std. (BHT)			**69.84**	**73.02**	**76.19**	**77.78**	**80.95**	**49.44**

Table 3. Antioxidant activity (% Inhibition, IC_{50} values) of Proline derivatives (Scheme-III) with Symmetric Linker Chains ($L_1 – L_5$)



Proline Derivatives

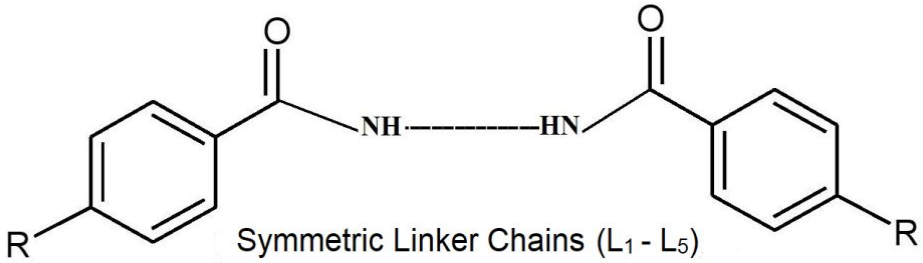
Code	Linker Chain	% Inhibition					IC_{50} µg/ml
		20	40	60	80	100	
$IIIL_1$	-NHCONH-	23.81	26.63	30.16	31.75	34.92	117.10
$IIIL_2$	-NHCH2CH2NH-	47.62	49.21	52.38	55.56	57.14	85.74
$IIIL_3$	$-NHCOCH_2CONH-$	53.97	57.14	60.32	61.90	63.49	94.76
$IIIL_4$	$-NHCH_2CONHCOCH_2NH-$	49.21	50.79	53.97	57.14	58.73	98.68
$IIIL_5$	$-NHCH_2CONHCH_2CH_2NHCOCH_2NH-$	53.42	55.56	58.73	60.32	61.90	88.55
Std. (BHT)		**69.84**	**73.12**	**76.19**	**77.78**	**80.95**	**47.44**

Table 4. Antioxidant activity (% Inhibition, IC_{50} values) of Proline derivatives (Scheme-IV) with Asymmetric Linker Chains (L_6 – L_{14})

Code	Linker Chain	% Inhibition					IC_{50} µg/ml
		20	40	60	80	100	
IVL_6	-$NHCH_2CONH$-	48.95	62.42	68.74	71.45	75.89	**54.52**
IVL_7	-$NHCH_2CONHCH_2CONH$-	31.75	33.33	36.51	38.10	41.27	80.29
IVL_8	-$NHCONHCH_2CONH$-	31.75	33.33	36.51	38.10	41.27	79.39
IVL_9	-$NHCH_2CONHCH_2CH_2NH$-	40.52	43.65	49.88	52.42	55.63	95.87
IVL_{10}	-$NHCO(CH_2)_2OHCONH$-	41.31	51.69	52.87	56.24	59.63	99.84
IVL_{11}	-$NH(CH_2)_2NHCOCHOHCH_2$ $CONH(CH_2)_2NH$-	54.87	56.66	59.63	61.42	60.80	100.22
IVL_{12}	-NHArCONH-	43.54	48.72	51.69	52.87	56.66	96.77
IVL_{13}	-$NHArCONH(CH_2)_2NH$-	35.42	32.55	37.48	39.65	42.66	110.32
IVL_{14}	-$NHArCONHCH_2CONH$-	32.65	34.23	38.61	37.20	43.27	108.74
Std. (BHT)		**69.84**	**73.12**	**76.19**	**77.78**	**80.95**	**47.44**

Hydrogen peroxide (H_2O_2) method:

Table 5. Antioxidant activity (% Inhibition, IC_{50} values) of 4-Substituted bisbenzamide derivatives (Scheme-I) with Symmetric Linker Chains (L_1 – L_5)



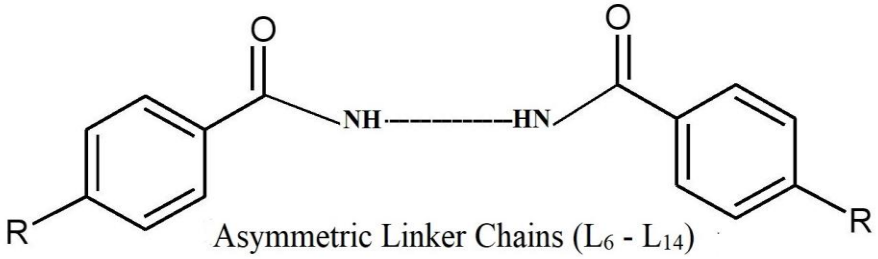
4-Substituted bisbenzamide derivatives

R = Cl, NH_2, OCH_3, NO_2

Code	R	Linker Chain	% Inhibition					IC_{50} µg/ml
			20	40	60	80	100	
IaL_1	-Cl	-NHCONH-	11.52	13.85	20.65	26.42	30.53	124.62
IaL_2	-Cl	-NHCH2CH_2NH-	52.21	65.42	68.48	71.21	73.36	95.58
IaL_3	-Cl	-$NHCOCH_2CONH$-	55.26	58.86	61.62	63.47	66.78	103.58

IaL_4	-Cl	$-NHCH_2CONHCOCH_2NH-$	35.44	42.51	51.88	56.67	58.12	112.23
IaL_5	-Cl	$-NHCH_2CONHCH_2CH_2NHCOCH_2NH-$	37.06	51.32	55.34	59.25	62.65	91.56
IbL_1	$-NH_2$	-NHCONH-	12.85	18.68	23.56	28.64	31.62	121.52
IbL_2	$-NH_2$	-NHCH2CH2NH-	53.21	58.79	64.51	69.42	71.28	91.47
IbL_3	$-NH_2$	$-NHCOCH_2CONH-$	38.52	40.25	45.75	49.75	51.48	118.56
IbL_4	$-NH_2$	$-NHCH_2CONHCOCH_2NH-$	40.75	45.64	47.55	51.84	55.66	109.87
IbL_5	$-NH_2$	$-NHCH_2CONHCH_2CH_2NHCOCH_2NH-$	52.84	59.72	61.32	64.95	66.34	101.48
IcL_1	$-OCH_3$	-NHCONH-	47.65	52.14	54.66	58.95	64.52	92.32
IcL_2	$-OCH_3$	-NHCH2CH2NH-	34.48	37.85	41.43	43.88	46.88	121.56
IcL_3	$-OCH_3$	$-NHCOCH_2CONH-$	34.12	36.65	41.84	49.62	59.42	121.84
IcL_4	$-OCH_3$	$-NHCH_2CONHCOCH_2NH-$	37.95	41.62	47.26	53.62	60.34	115.68
IcL_5	$-OCH_3$	$-NHCH_2CONHCH_2CH_2NHCOCH_2NH-$	38.90	41.35	44.78	47.65	47.48	119.44
IdL_1	$-NO_2$	-NHCONH-	43.27	45.62	48.62	50.47	53.63	101.65
IdL_2	$-NO_2$	-NHCH2CH2NH-	45.62	48.66	51.26	53.45	54.66	95.78
IdL_3	$-NO_2$	$-NHCOCH_2CONH-$	35.48	38.95	41.42	43.85	46.86	121.56
IdL_4	**$-NO_2$**	$-NHCH_2CONHCOCH_2NH-$	65.21	68.45	72.95	76.51	81.83	**56.16**
IdL_5	**$-NO_2$**	$-NHCH_2CONHCH_2CH_2NHCOCH_2NH-$	58.64	63.94	67.29	71.37	75.55	**61.54**
Std. (BHT)			**57.5**	**71.25**	**77.84**	**81.62**	**88.65**	**42.42**

Table 6. Antioxidant activity (% Inhibition, IC_{50} values) of 4-Substituted bisbenzamide derivatives (Scheme-II) with Asymmetric Linker Chains (L_6 – L_{14})



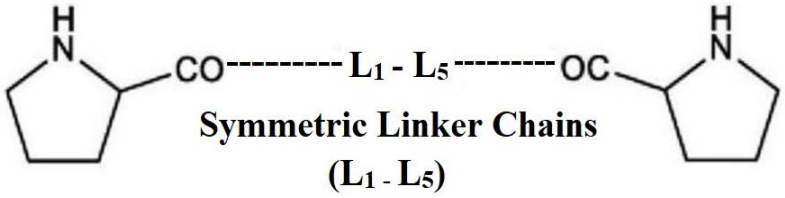
4-Substituted bisbenzamide derivatives

R = Cl, NH_2, OCH_3, NO_2

Code	R	Linker Chain	% Inhibition					IC_{50} µg/ml
			20	40	60	80	100	
$IIaL_6$	-Cl	$-NHCH_2CONH-$	14.62	21.28	32.64	37.45	41.62	120.45
$IIaL_7$	**-Cl**	$-NHCH_2CONHCH_2CONH-$	61.26	67.84	73.52	81.33	85.47	**68.84**
$IIaL_8$	-Cl	$-NHCONHCH_2CONH-$	28.66	36.42	40.66	45.11	52.24	101.62
$IIaL_9$	-Cl	$-NHCH_2CONHCH_2CH_2NH-$	32.62	36.45	39.84	43.51	48.62	112.23
$IIaL_{10}$	-Cl	$-NHCO(CH_2)_2OHCONH-$	16.54	25.32	27.85	30.26	33.62	133.56
$IIaL_{11}$	-Cl	$-NH(CH_2)_2NHCOCHOHCH_2CONH(CH_2)_2NH-$	21.42	27.65	30.53	32.56	36.84	130.12
$IIaL_{12}$	-Cl	-NHArCONH-	38.29	46.65	49.28	52.45	55.25	91.42
$IIaL_{13}$	-Cl	$-NHArCONH(CH_2)_2NH-$	43.62	47.46	51.25	55.36	59.42	78.56
$IIaL_{14}$	-Cl	$-NHArCONHCH_2CONH-$	45.12	48.62	53.44	57.86	62.45	85.42
$IIbL_6$	**$-NH_2$**	$-NHCH_2CONH-$	58.24	65.42	68.55	71.21	73.54	**54.51**
$IIbL_7$	$-NH_2$	$-NHCH_2CONHCH_2CONH-$	35.32	44.25	51.52	53.52	59.51	102.58
$IIbL_8$	$-NH_2$	$-NHCONHCH_2CONH-$	32.51	37.06	41.52	45.95	57.62	95.88
$IIbL_9$	$-NH_2$	$-NHCH_2CONHCH_2CH_2NH-$	13.54	15.28	21.68	24.84	29.15	128.52
$IIbL_{10}$	$-NH_2$	$-NHCO(CH_2)_2OHCONH-$	35.42	38.52	40.25	45.75	49.75	121.62
$IIbL_{11}$	$-NH_2$	$-NH(CH_2)_2NHCOCHOHCH_2CONH(CH_2)_2NH-$	39.52	40.75	45.64	51.84	55.85	111.42
$IIbL_{12}$	$-NH_2$	-NHArCONH-	42.15	45.85	51.45	57.65	63.42	104.35
$IIbL_{13}$	$-NH_2$	$-NHArCONH(CH_2)_2NH-$	42.15	49.62	55.42	58.84	64.32	94.35
$IIbL_{14}$	$-NH_2$	$-NHArCONHCH_2CONH-$	39.62	46.27	53.58	57.65	64.58	110.52
$IIcL_6$	$-OCH_3$	$-NHCH_2CONH-$	42.68	47.78	51.32	61.56	72.95	105.98
$IIcL_7$	$-OCH_3$	$-NHCH_2CONHCH_2CONH-$	41.35	44.78	47.45	51.26	54.95	119.88
$IIcL_8$	$-OCH_3$	$-NHCONHCH_2CONH-$	45.82	47.98	50.22	53.64	59.62	105.65
$IIcL_9$	**$-OCH_3$**	$-NHCH_2CONHCH_2CH_2NH-$	60.62	65.55	71.48	79.97	83.21	**61.23**
$IIcL_{10}$	$-OCH_3$	$-NHCO(CH_2)_2OHCONH-$	51.42	54.68	57.85	61.62	65.66	101.82
$IIcL_{11}$	$-OCH_3$	$-NH(CH_2)_2NHCOCHOHCH_2CONH(CH_2)_2NH-$	36.75	38.14	41.78	49.84	52.63	105.62
$IIcL_{12}$	$-OCH_3$	-NHArCONH-	24.48	28.42	31.67	42.85	48.95	112.95
$IIcL_{13}$	$-OCH_3$	$-NHArCONH(CH_2)_2NH-$	28.62	35.84	49.66	55.84	62.68	105.48
$IIcL_{14}$	$-OCH_3$	$-NHArCONHCH_2CONH-$	14.51	24.98	35.38	38.98	44.82	122.84
$IIdL_6$	$-NO_2$	$-NHCH_2CONH-$	21.52	27.88	31.52	34.52	49.62	109.49
$IIdL_7$	$-NO_2$	$-NHCH_2CONHCH_2CONH-$	38.88	40.68	44.56	59.38	61.35	70.47
$IIdL_8$	$-NO_2$	$-NHCONHCH_2CONH-$	52.54	58.27	65.48	67.84	69.62	65.42
$IIdL_9$	$-NO_2$	$-NHCH_2CONHCH_2CH_2NH-$	22.25	25.67	30.72	36.42	40.85	135.84

$IIdL_{10}$	$-NO_2$	$-NHCO(CH_2)_2OHCONH-$	25.79	28.53	32.17	35.69	39.45	99.42
$IIdL_{11}$	$-NO_2$	$-NH(CH_2)_2NHCOCHOHCH_2CONH(CH_2)_2NH-$	28.59	38.09	46.85	49.29	53.97	70.05
$IIdL_{12}$	$-NO_2$	-NHArCONH-	47.44	55.34	62.95	68.75	70.35	68.72
$IIdL_{13}$	$-NO_2$	$-NHArCONH(CH_2)_2NH-$	48.17	52.84	58.22	61.92	66.69	60.15
$IIdL_{14}$	$-NO_2$	$-NHArCONHCH_2CONH-$	42.64	46.39	53.68	58.28	66.62	61.99
Std. (BHT)			**57.5**	**71.25**	**77.84**	**81.62**	**88.65**	**42.42**

Table 7. Antioxidant activity (% Inhibition, IC_{50} values) of Proline derivatives (Scheme-III) with Symmetric Linker Chains (L_1 – L_5)



Proline Derivatives

Code	Linker Chain	% Inhibition					IC_{50} µg/ml
		20	40	60	80	100	
$IIIL_1$	-NHCONH-	24.48	28.42	31.67	42.85	48.95	112.95
$IIIL_2$	-NHCH2CH2NH-	28.62	35.84	49.66	55.84	62.68	105.48
$IIIL_3$	$-NHCOCH_2CONH-$	14.51	24.98	35.38	38.98	44.82	122.84
$IIIL_4$	$-NHCH_2CONHCOCH_2NH-$	51.42	55.84	65.85	72.86	84.68	**60.28**
$IIIL_5$	$-NHCH_2CONHCH_2CH_2NHCOCH_2NH-$	55.61	64.38	75.42	80.29	85.54	**57.62**
Std. (BHT)		**57.5**	**71.25**	**77.84**	**81.62**	**88.65**	**42.42**

Table 8. Antioxidant activity (% Inhibition, IC_{50} values) of Proline derivatives (Scheme-IV) with Asymmetric Linker Chains (L_6 – L_{14})

Code	Linker Chain	% Inhibition					IC_{50} µg/ml
		20	40	60	80	100	
IVL_6	-$NHCH_2CONH$-	55.62	70.84	76.68	79.98	85.24	**50.44**
IVL_7	-$NHCH_2CONHCH_2CONH$-	64.52	74.85	81.52	84.68	89.88	**46.89**
IVL_8	-$NHCONHCH_2CONH$-	59.21	72.42	79.62	80.81	80.75	**49.62**
IVL_9	-$NHCH_2CONHCH_2CH_2NH$-	38.88	40.68	44.56	59.38	61.35	70.47
IVL_{10}	-$NHCO(CH_2)_2OHCONH$-	52.54	58.27	65.48	67.84	69.62	65.42
IVL_{11}	-$NH(CH_2)_2NHCOCHOHCH_2$ $CONH(CH_2)_2NH$-	22.25	25.67	30.72	36.42	40.85	135.84
IVL_{12}	-NHArCONH-	25.79	28.53	32.17	35.69	39.45	99.42
IVL_{13}	-$NHArCONH(CH_2)_2NH$-	28.59	38.09	46.85	49.29	53.97	70.05
IVL_{14}	-$NHArCONHCH_2CONH$-	47.44	55.34	62.95	68.75	70.35	68.72
Std. (BHT)		**57.5**	**71.25**	**77.84**	**81.62**	**88.65**	**42.42**

Cytotoxic Activity:

Table 9. Anticancer activity of 4-substituted bisbenzamides (Scheme-I and Scheme-II)

Compound	IC_{50} (µm)			
	MCF-7	HT-29	A 549	PC-3
IcL_5	125.9	40.7	49.37	43.2
IdL_4	**12.9**	**10.2**	**21.6**	**8.4**
IdL_5	70.5	35.6	38.4	39.5
$IIbL_6$	**10.5**	**9.2**	**19.5**	**9.6**
$IIaL_7$	80.6	50.4	49.6	53.5
$IIbL_7$	38.4	33.7	50.1	37.6
$IIcL_9$	40.6	35.6	55.7	61.8
Doxorubicin	**8.61**	**6.56**	**14.31**	**4.85**

Table 10. Anticancer activity of L-Proline derivatives (Scheme-III and Scheme-IV)

Compound	IC_{50} (µm)			
	MCF-7	**HT-29**	**A 549**	**PC-3**
$IIIL_3$	58.6	49.7	62.1	58.2
$IIIL_4$	**10.4**	**9.2**	**17.4**	**9.2**
$IIIL_5$	**11.4**	**13.5**	**18.9**	**11.2**
IVL_6	**9.2**	**8.4**	**16.6**	**8.8**
IVL_7	38.4	52.6	71.5	37.3
IVL_8	62.5	71.6	82.7	60.1
IVL_9	49.7	68.5	NA	59.6
Doxorubicin	**8.61**	**6.56**	**14.31**	**4.85**

CONCLUSION

The present study is aimed towards designing novel bis-intercalators as possible anticancer agents. All the synthesized compounds were evaluated for their antioxidant activity and followed by anticancer activity using standard procedures. The antioxidant activity of synthesized compounds reveals that compound $IIbL_6$ was found to be potent with IC50 value of 54.51 when compared with the standard BHT. The antioxidant activity of L-Proline derivatives, compound IVL6 is found to be potent to exhibit antioxidant activity with IC50 value of 54.52 compared to standard BHT. The *in-vitro* cytotoxic activity of 4-substituted bisbenzamides reveals that two of the compounds, IIbL6 and IdL4, are particularly effective against the cancer cell line, with respective IC50 values of 55.5684 and 54.3489. The *in-vitro* cytotoxic results of L-Proline derivatives against two cell lines of cancer reveals that only two compounds, IVL_6 and $IIIL_4$ could inhibit the cell lines with IC_{50} values of 55.8236 and 48.0132, respectively. Hence, the results of the study could reveal that the synthesized 4-substituted bisbenzamides and L-proline derivatives can be potentially act as anticancer agents.

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