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

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ABSTRACT

An attempt is made to design, synthesize and pharmacologically evaluate novel bis-intercalators as possible anticancer agents. According to a literature study on bis-intercalators, 4-substituted bis-benzamides are widely recognized for their medicinal uses and may be effective anticancer and antioxidant agents. Synthetic studies revealed that the yield of Chain linkers L1-L14, 4-substituted bis benzamides, and L-proline derivatives were in the 50-70% range. The 4-substituted bis benzamides compounds have been evaluated for anticancer and antioxidant activity studies. Among the 4-substituted bis benzamides compounds, Compound IIIc, IIIf, and IIIg have revealed promising anticancer and antioxidant activity. Compounds III_7 and III_{12} have good cytotoxicity and antioxidant activity among L-proline derivatives. As a result, the current study emphasizes the significance of 4-substituted bis benzamides and L-proline derivatives with various heterocyclic moiety features responsible for cytotoxicity and antioxidant activity. It might be a starting point for further alterations to create new entities with therapeutically useful properties.

Keywords: Bisbenzamides, L-Proline Derivatives, Cytotoxicity, Antioxidant Activity, Bis-Intercalators.

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INTRODUCTION

Cancer is one of the most threatening illnesses because the cells are aggressive (they grow and divide without regard for usual limitations), invasive (they enter and destroy neighboring tissues), and metastatic (they spread to other areas of the body). These three malignant characteristics, aggressive, invasive, and metastatic malignancies, distinguish them from benign tumors, which develop slowly and do not invade or metastasize (even if certain benign tumor forms may develop into cancer). Cancer may afflict individuals of all ages, including fetuses, although the risk of the more prevalent cancer rises with age. Cancer is caused by external causes (chemicals, radiation, tobacco, and pathogenic organisms) and internal causes (hormones, inherited mutations, mutations from metabolism, and immune conditions). These random factors may work concurrently or sequentially to begin or accelerate cancer development.[1-2]Excessive sun exposure and indoor tanning might prevent more than three million skin cancer occurrences yearly. Cancer may affect animals and plants as well as people.[3]Significant progress has been made in developing targeted treatment medications that operate selectively on observable molecular abnormalities in some malignancies while causing little harm to normal cells.[4]Because of the high likelihood of developing new materials with intriguing, valued functions and desired features, rational design and synthesis of innovative bis-intercalators have developed. Among the bis-intercalators, 4-substituted bis benzamides and L-proline derivatives with symmetric linker chains and asymmetric linker chains are selected for the study.[5]Benzamides are carbonic acid amides of benzoic acid, and it is a natural alkaloids found in the herbs of Berberis pruinosa.[6]Benzamides are shown to possess anticonvulsants, anti-inflammatory, analgesic, antimicrobial, antidepressant, and antitumor activities. The benzamide derivatives were synthesized using various methods and assessed as physiologically active agents. Substituted benzamides are bioactive molecules that may be considered promising chemicals.[7]

L-proline is a non-essential amino acid with a pyrrolidine ring. L-proline, a heterocyclic molecule, is used as a starting material in research to synthesize bigger, often bioactive compounds. It is very stable due to

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its aromaticity, but being a heterocycle, it possesses a reactive side that allows for functionalization.[8]L-proline is a well-known heterocyclic compound in organic and medicinal chemistry. Other biological activities than cytotoxicity have recently been found in compounds containing proline rings, comprising antifungal, antibacterial, and antioxidant activity.[9]L-proline plays a vital role in medicinal chemistry. Proline derivatives are also well known for versatile pharmacological activity. This investigation of these compound's chemistry and biology continues to appeal to synthetic and medicinal organic chemistry.[9,10]

EXPERIMENTAL

General Compound Synthesis Procedure

Step-1

Symmetric Linker Chains (L_1toL_5)

L_1: Urea; L_2: Ethylene Diamine; L_3: Malonamide

These are procured directly (L_1, L_2 and L_3)

Symmetric Linker Chains (L_4 to L_5)

Ia: L_4 (N-(Aminoacetyl)glycinamide)

Pure Chloroacetyl Chloride (0.2 M, 22.4 g, 16.2 ml) was added drop by drop into the mixture of ammonia (0.1M, 1.7g, 2 ml) in 20 ml of dry alcohol with constant stirring. The mixture was stirred in a moisture-free environment for 30 minutes, maintaining a temperature below 20^0. Then a molar excess of ammonia was added, stirring the mixture for an additional hour. The reaction mixture is then filtered, and concentrated under a vacuum to get the solid residue as L_4, which is purified by recrystallization and characterized by spectral and chemical studies.

Ib: L_5 (N,N'-Bis-(2-Aminoacetyl)ethylenediamine)

Pure Chloroacetyl Chloride (0.2 M, 22.4 g, 16.2 ml) is slowly added to the mixture of ethylene diamine (0.1M, 6.2g, 7.4ml) in 20 ml of dry alcohol with constant stirring. The mixture was stirred in a moisture-free environment for 30 minutes, maintaining a temperature below 20^0. Then a molar excess of ammonia was added, stirring the mixture for 1 hour. Then the filtration of the reaction mixture is carried out to concentrate the mixture to obtain the residue in the solid state as L_4, which is purified by recrystallization and characterized by spectral and chemical studies.

Asymmetric Linker Chains (L_6toL_{14})

Ic: L_6(Glycinamide)

Pure chloroacetyl chloride (0.2 M, 22.4 g, and 16.2 ml) is mixed with excess ammonia in dry methanol (20 ml) to the extent of 20 minutes. The mixture is then refluxed for about one hour in anhydrous conditions. Distillation under a vacuum is used to remove excess ammonia and solvent. The resulting solid residue is refined by recrystallization from water.

Id: L_7, 2-(N-Ureido)acetamide

Pure Chloroacetyl Chloride (0.1 M, 11.2 g, 8.1 ml) is mixed with the quantity in an equimolar proportion of ammonia in dry methanol (20 ml) with constant stirring for 30 minutes. In a separate beaker, urea (0.1 M, 6. 2 g in minimum quantity of water) is mixed with the above solution and refluxed in a water bath for about one hour. The mixture was concentrated under a vacuum and was kept overnight in cool. The solid crystalline product, thus obtained, was purified by recrystallization from chloroform.

Ie: L_9, N_1-(2-Aminoethyl)glycinamide

Pure Chloroacetyl Chloride (0.2 M, 22.4 g, 16.2 ml) is stirred with an excess of ethylene diamine in 20 ml of the dry methanol for 20 minutes. The mixture is then refluxed for about one hour in anhydrous conditions. Excess solvents are eliminated using vacuum distillation. The resulting solid residue is refined by recrystallization from water.

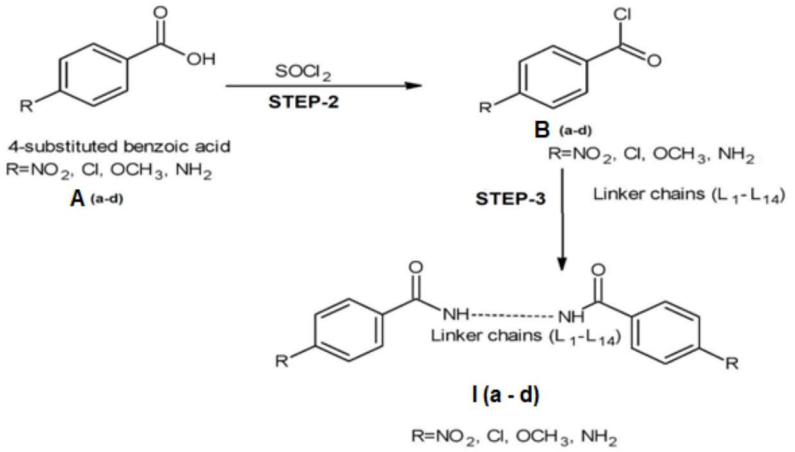
If: L_{13}, 4-Amino-N-(2-aminoethyl)benzamide

Pure PABA (0.1 M, 13.71 g) is treated with pure redistilled $SOCl_2$ (0.1M, 11.9 g, 7.2 ml) in 25 ml dry ether. The mixture is stirred for 30 minutes in cold conditions, and the excess solvent is removed by distillation. Then, it was agitated for an hour while treated with ammonia (0.1M, 1.7 g, and 2 ml) in 25 ml

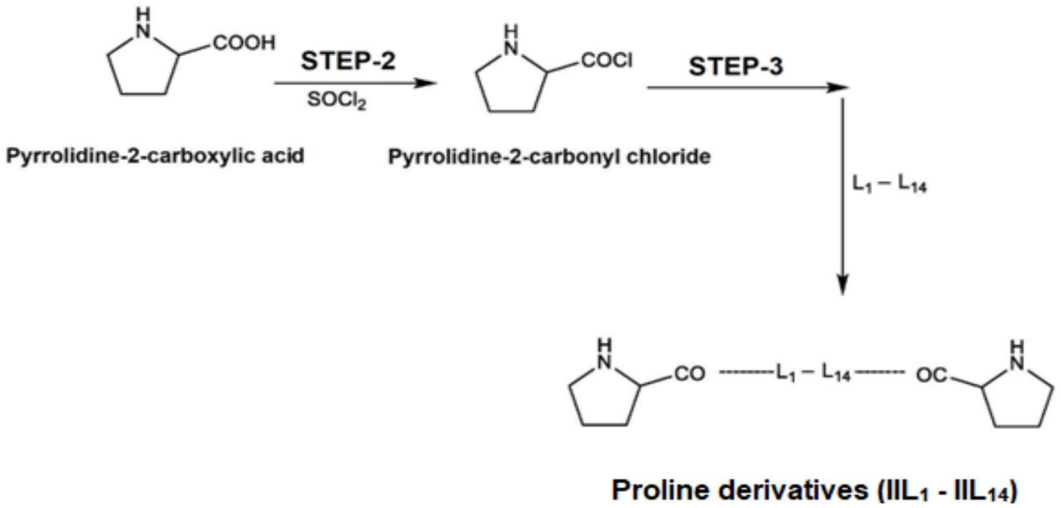
of dry alcohol. The mixture was then concentrated under a vacuum and kept overnight. The crystalline solid thus obtained was purified by recrystallization from methanol.

Ig: L_{14}, 4-Amino-N-(2-aminoacetamido)benzamide

Pure PABA (0.1 M, 13.71 g) is treated with pure redistilled $SOCl_2$ (0.1M, 11.9 g, 7.2 ml) in 25 ml dry ether. The mixture is then stirred for 30 minutes in cold conditions, and the excess solvent is removed by distillation. Then, it was agitated for an hour while being treated with glycinamide (0.1M, 7.4 g) in 25 ml of dry alcohol. The mixture was then concentrated under a vacuum and kept overnight. The crystalline solid thus obtained was purified by recrystallization from methanol.



Scheme-1



Scheme-2

Symmetric Linker Chains (L_1 to L_5)

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

Asymmetric Linker Chains (L_5 to L_{14})

L_6 = Glycinamide
L_7 = 2-(N-Ureido) acetamide
L_8 = N1-(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) benzamide

Step-2: Chlorination of 4-Substituted Benzoic Acids

Procedure

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

Step-3: Synthesis of 4-Substituted Bis Benzamides

An equimolar amount of ammonia and pure chloroacetyl chloride (0.1 M, 11.2 g, 8.1 ml) were combined in 20 ml dry methanol with steady stirring for 30 minutes. The above mixture is added to a separate beaker along with the Linker chain (L1-L14) (0.1 M, g suspended in five ml alcohol), and the combined mixture is then refluxed in a water bath for around an hour. The mixture was concentrated under a vacuum and was kept overnight in a cool place. The solid crystalline product, thus obtained, was purified by recrystallization from methanol.

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.

Cytotoxic Activity

The MTT Cell Proliferation Assay (Scheme-I and Scheme-II) quantifies the rate of cell proliferation as well as the decrease in cell viability that results from metabolic processes that cause apoptosis or necrosis.

Requirements

Breast cancer cell lines MCF7 and MDAMB231 were gifts from Dr. Radha at the CCMB in Hyderabad, India. We bought 96 healthy tissue culture plates with flat bottom surfaces (Tarson) and fetal bovine serum from Gibco, United States. DMEM, MTT, Trypsin, and EDTA are supplied by Sigma Chemicals, while the 4,5-Dimethylthiazol-2-yl-2,5-diphenyltetrazoliumbromide is provided by Gibco (USA) provided.

Maintenance of Cell Line

As an adherent culture, breast cancer cell line MCF7, MDAMB231 was grown in DMEM medium in the presence of the fetal bovine serum (10%), penicillin (100g/ml), streptomycin (200g/ml) and L-glutamine (2mM). The culture is then kept undisturbed in a moist environment with CO_2 (5%).

Preparation of Test Compound

The 10mg/ml stock solutions of DMSO are diluted with sterile PBS to achieve the desired concentration.

MTT Assay Procedure

The cytotoxicity of several drugs was evaluated using the MTT assay, which employs the reduction of the dye MTT tetrazolium to form the formazan product (blue). One thousand one hundred four cells are

counted using the Trypan blue per and are cultured in a 96-well plate in DMEM with FBS (10%) for 2 days at a temperature of 370°C with various chemical concentrations. After replacing the media mentioned above with 200 l of DMSO and incubating the plates at 370C for 10 min, the plates were then incubated at 370C for 4 hours. Using a spectrometer, the absorbance is measured at a wavelength of 570nm. The concentration versus percentage of cell viability plot was used to determine the IC-50 values.

RESULTS AND DISCUSSION

Cytotoxic Activity

Table-1: Anticancer Activity of 4-Substituted Bis Benzamides(Scheme-I)

Compound	Concentration (µg/ml)				% Inhibition
	200	100	50	25	IC50
Test	1.2926	1.9275	1.8401	1.5654	99.9502
IbL_7	0.81255	1.1546	1.1387	1.1965	42.7297
IaL_7	0.7827	1.2665	1.2466	1.1254	44.8336
IcL_5	0.9594	1.4125	1.2903	1.0256	32.3759
IdL_4	0.5374	0.7372	0.991	0.8956	54.3489
IcL_9	0.6101	0.7949	1.0158	0.9856	48.1785
IdL_5	0.6723	0.8723	1.1395	1.0208	42.8947
IbL_6	0.6304	1.1571	1.500	0.13403	55.5684

Table-2: Anticancer Activity of L-Proline Derivatives (Scheme-II)

Compound	Concentration (µg/ml)				% Inhibition
	200	100	50	25	IC50
Test	1.2926	1.9275	1.8401	1.5654	99.9551
IIL_4	0.6124	0.7391	0.9395	0.9159	48.0132
IIL_9	0.7034	0.8272	1.1126	1.306	40.2847
IIL_3	0.867	1.041	1.108	1.122	40.1354
IIL_7	0.6488	0.7253	1.004	1.188	44.9195
IIL_5	0.6304	0.9162	1.008	1.002	46.4464
IIL_6	0.5204	0.9285	0.8531	0.9779	55.8236
IIL_8	0.6798	0.7497	1.163	1.233	42.2871

CONCLUSION

Table-1 contains information on the in vitro cytotoxic testing of fresh 4-substituted bis benzamides (Scheme-I). The cancer cell line is used to screen the test chemicals. We used doxorubicin as the benchmark. Looking at Table-1 reveals that two of the compounds, IbL6 and IdL4, are particularly effective against the cancer cell line, with respective IC50 values of 55.5684 and 54.3489. At the same time, compound IcL_9 is slightly active toward the cancer cell line with an IC_{50} value of 48.1785.The in vitro cytotoxic results of Scheme-II (L-Proline derivatives) against two cell lines of cancer are shown in the second Table. Among the compounds synthesized, only two compounds, IIL_6 and IIL_4 could inhibit the cell line with IC_{50} values of 55.8236 and 48.0132, respectively. In comparison, compound IIL_5 is slightly active toward the cancer cell line with an IC_{50} value of 46.4464.These substances have similar action to that of standard doxorubicin.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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