DESIGN, SYNTHESIS AND MOLECULAR DOCKING STUDIES OF NOVEL 4-SUBSTITUTED BIS-INTERCALATORS AS POSSIBLE ANTICANCER AGENTS

Saarangi Ramesh[1], T. Parthasarathy[2]*, NJP Subhasini [3]

Abstract:
In the present study, 4-substituted bisbenzamide derivatives were designed, synthesized and then characterized. In the present study, by using docking studies with the help of well characterized and structurally linear ligands as molecular probes, the London dG scoring simulations were used to evaluate most interactive binding sites. In the present work 56 4-substituted bisbenzamide derivative ligands are docked in the Human DNA Topoisomerase I enzyme active site. Docking analysis reveal that most active compounds IbL_6, IdL_4, IcL_9, and IaL_7 interacted with receptor through H-bond. The careful examination of the binding site shown that, compound IbL_6 exhibits H-bond interactions with Human DNA Topoisomerase I active site residues such as Glu356, TGP11, DA113, DG112, ASN352, DT10, Lys425, and Lys374. The compounds IbL_6 and IdL_4 of the 4-substituted bisbenzamide derivatives are found to be Topo I potent inhibitor and thus potentially considered as anti-cancer agents.

Key words: Human DNA Topoisomerase I enzyme, docking analysis, 4-substituted bisbenzamide, anticancer agents and London dG scoring.

[1]Department of Pharmacy, University College of Technology, Osmania University, Hyderabad,Telangana, India.
[2*,3]Department of Chemistry, University College of Science, Osmania University, Hyderabad, Telangana, India.

***Corresponding Author:** T. Parthasarathy
*Department of Chemistry, University College of Science, Osmania University, Hyderabad,Telangana, India.

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INTRODUCTION

Cancer is one of the most dangerous diseases since cancer cells are aggressive (they develop and multiply without considering usual restrictions), invasive (they enter and harm neighbouring tissues), and metastatic (they spread to other parts of the body). Even while some benign tumour types have the potential to develop into cancer, these three malignant characteristics that include aggressive, invasive, and metastatic malignancies that distinguishes them from benign tumours, which grow slowly and do not invade or spread[1].

Docking Studies:

Selection of PDB Structure

The protein data bank (PDB) is a collection of crystal structures for proteins with bound ligands and coactivators. The X-ray crystal structure of the human DNA topoisomerase (70 Kda) in complex with the camptothecin and covalent complex with A 22 base pair DNA duplex (PDB ID: 1T8I) was retrieved from the protein data bank based on excellent resolution and Ramachandran's plot analysis[2, 3]. The structure was picked because, when compared to other options, it had a high resolution of 3.0. The Human DNA topoisomerase (1T8I) has 87.3% of its residues in the quadrangle's most favourable zone, according to the Ramachandran's plot study, and there isn't a single residue in the quadrangle's least favourable region[4, 5].

Ligand Generation and Optimization

ACD/ ChemSketch (12.0) software was used to create sketches of the chemically synthesised 4-substituted benzamide derivatives, which were then saved in mol file format[6, 7]. A methodical conformer search, geometry optimisation, and energy minimization of the lowest energy structure using the Merck Molecular Force Field (MMFF94) were then used to optimize the stored ligands once they were imported into MOE[8, 9]. Hence for further binding studies, the various compounds are stored in mol file format.

Docking Algorithms

Here, we discuss the use of MOE-Dock at Chemical Computing Group Inc., a versatile docking tool that also combines with a GUI (Graphical User Interface) and additional modules including analysis, molecular dynamics and molecular mechanics. Macromolecular crystallographic data, when accessible, can be a valuable source of information for determining active ligands[10, 11]. There are MOE applications available for seeing and understanding the characteristics of receptor active regions and receptor-ligand interactions. These programmes are used to suggest ligand improvements or look up potential binders in ligand databases. MOE-Dock employs a Monte Carlo simulated annealing process to dock a substrate onto a macromolecule's active site[12].

Docking Simulations

London Dg Dock

By default, the MOE London utilizes dG scoring to identify the precise confirmation and configuration of the ligand so as to determine the ideal candidate with the least amount of binding energy, which may be utilised later for the creation of new pharmaceuticals to treat the disorder. The London dG scoring mechanism estimates the unconstrained energy G of interaction of the ligand from a specific position[13, 14].

Active Site Detection and Visualization

The quick geometric technique used to find potential protein-ligand and protein-protein binding sites is based on Edelsbrunner's alpha shapes. A macromolecular structure's sites are rated based on how accessible its hydrophobic contact surfaces are[15]. De novo ligand design attempts that use docking simulations depict particular sites or replace them with "dummy atoms"[16]. The anticonvulsant, anti-inflammatory, analgesic, antibacterial, antidepressant, and anticancer properties of benzamides have been demonstrated. The benzamide derivatives were created utilising a variety of techniques, and they are being evaluated as a range of physiologically active compounds. It is possible to view substituted benzamides as attractive compounds because they are bioactive molecules[17-18].

MATERIALS AND METHODS

Synthesis of 4-Substituted Bisbenzamide Derivatives with Symmetric Linker Chains

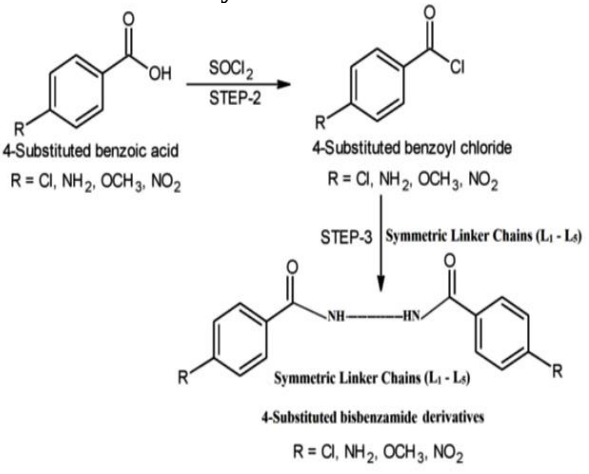

Figure.1: Scheme-I

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

Synthesis of 4-Substituted Bisbenzamide Derivatives with Asymmetric Linker Chains

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

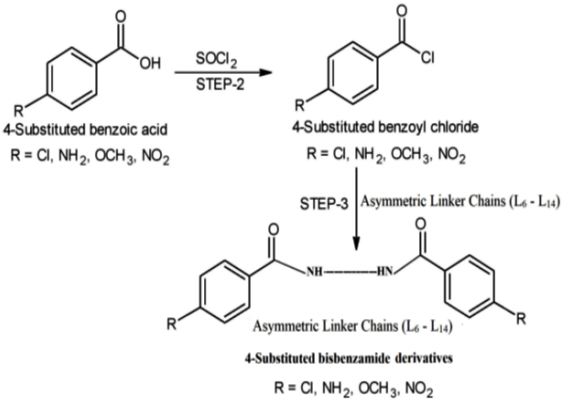

Figure.2: Scheme-II

ASYMMETRIC LINKER CHAINS (L_6 to L_{14})

Physical data of Scheme-I and Scheme-II compounds:

Table 1. Physical data of Scheme-I compounds

Compounds	R	Linker Chain	Molecular formula	Molecular weight	IUPAC name
IaL1	Cl	-NHCONH-	$C_{15}H_{10}Cl_2N_2O_3$	337.13	*N,N'*-carbonylbis(4-chlorobenzamide)
IaL2	Cl	-NHCH$_2$CH$_2$NH-	$C_{16}H_{14}Cl_2N_2O_2$	331.78	*N,N'*-ethane-1,2-diylbis(4-chlorobenzamide)
IaL3	Cl	-NHCOCH$_2$CONH-	$C_{17}H_{12}Cl_2N_2O_4$	315.32	*N,N'*-bis (4-chlorobenzoyl)propanediamide
IaL4	Cl	-NHCH$_2$CONHCOCH$_2$NH-	$C_{18}H_{15}Cl_2N_3O_4$	313.33	*N,N'*-[Iminobis(2-oxoethane-2,1-diyl)]bis(4-chlorobenzamide)
IaL5	Cl	-NHCH$_2$CONHCH$_2$CH$_2$NHCOCH$_2$NH-	$C_{20}H_{20}Cl_2N_2O_4$	376.23	*N,N'*-((ethane-1,2-diylbis(azanediyl))bis(2-oxoethane-2,1-diyl))bis(4-chlorobenzamide)
IbL1	NH$_2$	-NHCONH-	$C_{15}H_{14}N_4O_3$	298.28	4-amino-N-{[(4-aminophenyl)carbonyl]carbamoyl}benzamide
IbL2	NH$_2$	-NHCH2CH$_2$NH-	$C_{16}H_{18}N_4O_2$	298.35	*N,N'*-ethane-1,2-diylbis(4-aminobenzamide)
IbL3	NH$_2$	-NHCOCH$_2$CONH-	$C_{17}H_{16}N_4O_4$	340.70	*N,N'*-bis[(4-aminophenyl)carbonyl]propanediamide
IbL4	NH$_2$	-NHCH$_2$CONHCOCH$_2$NH-	$C_{18}H_{19}N_5O_4$	369.24	*N,N'-[*iminobis(2-oxoethane-2,1-diyl)]bis(4-aminobenzamide)
IbL5	NH$_2$	-NHCH$_2$CONHCH$_2$CH$_2$NHCOCH$_2$NH-	$C_{20}H_{24}N_6O_4$	412.27	*N,N'-((*ethane-1,2-diylbis(azanediyl)) bis(2-oxoethane-2,1-diyl))bis (4-aminobenzamide)
IcL1	OCH$_3$	-NHCONH-	$C_{17}H_{16}N_2O_5$	328.25	4-methoxy-*N*-{[(4-methoxyphenyl)carbonyl] carbamoyl}benzamide

IcL2	OCH_3	-NHCH2CH_2NH-	$C_{18}H_{20}N_2O_4$	328.32	*N*,*N*'-ethane-1,2-diylbis(4-methoxybenzamide)
IcL3	OCH_3	-NHCOCH_2CONH-	$C_{19}H_{18}N_2O_6$	370.28	*N*,*N*'-bis[(4-methoxyphenyl)carbonyl] propanediamide
IcL4	OCH_3	-NHCH_2CONHCOCH_2NH-	$C_{20}H_{21}N_3O_6$	399.29	*N*,*N*'-[iminobis(2-oxoethane-2,1-diyl)]bis(4-methoxybenzamide)
IcL5	OCH_3	-NHCH_2CONHCH_2CH_2NHCOCH_2NH-	$C_{22}H_{26}N_4O_6$	442.26	*N*,*N*'-((ethane-1,2-diylbis(azanediyl)) bis(2-oxoethane-2,1-diyl)) bis(4-methoxybenzamide)
IdL1	NO_2	-NHCONH-	$C_{15}H_{10}N_4O_7$	358.71	*N*,*N*'-bis (4-nitrobenzoyl)propanediamide
IdL2	NO_2	-NHCH2CH_2NH-	$C_{16}H_{14}N_4O_6$	358.25	*N*,*N*'-[Iminobis(2-oxoethane-2,1-diyl)]bis(4-nitrobenzamide)
IdL3	NO_2	-NHCOCH_2CONH-	$C_{17}H_{12}N_4O_8$	400.16	*N*,*N*'-((ethane-1,2-diylbis(azanediyl))bis(2-oxoethane-2,1-diyl))bis(4-nitrobenzamide)
IdL4	NO_2	-NHCH_2CONHCOCH_2NH-	$C_{19}H_{16}N_4O_8$	428.29	*N*,*N*'-[Iminobis(2-oxoethane-2,1-diyl)]bis(4-nitrobenzamide)
IdL5	NO_2	-NHCH_2CONHCH_2CH_2NHCOCH_2NH-	$C_{20}H_{20}N_6O_8$	472.29	*N*,*N*'-((ethane-1,2-diylbis(azanediyl)) bis(2-oxoethane-2,1-diyl))bis (4-nitrobenzamide)

Table 2. Physical data of Scheme-II compounds

Compounds	**R**	**Linker Chain**	**Molecular formula**	**Molecular weight**	**IUPAC name**
IIaL6	Cl	-NHCH_2CONH-	$C_{17}H_{14}Cl_2N_2O_3$	365.14	*N*,*N*'-(1-oxoethane-1,2-diyl)bis(4-chlorobenzamide)
IIaL7	Cl	-NHCH_2CONHCH_2CONH-	$C_{18}H_{14}Cl_2N_3O_3$	408.78	4-chloro-N-{[({[(4-chlorophenyl)carbonyl]amino} acetyl)amino] acetyl} benzamide
IIaL8	Cl	-NHCONHCH_2CONH-	$C_{17}H_{13}Cl_2N_3O_4$	394.32	4-chloro-*N*-[(2-{[(4-chlorophenyl)carbonyl]amino}-2-oxoethyl) carbamoyl] benzamide
IIaL9	Cl	-NHCH_2CONHC H$_2$CH$_2$NH-	$C_{18}H_{17}Cl_2N_3O_3$	394.23	4-chloro-*N*-{2-[({[(4-chlorophenyl) carbonyl] amino} acetyl) amino] ethyl} benzamide
IIaL10	Cl	- NHCO$(CH_2)_2$OHCONH-	$C_{18}H_{14}Cl_2N_2O_5$	409.23	*N*,*N*'-bis[(4-chlorophenyl)carbonyl]-2-hydroxybutanediamide
IIaL11	Cl	-NH$(CH_2)_2$NHCOCHOHCH_2CONH(C H$_2)_2$NH-	$C_{22}H_{24}Cl_2N_4O_5$	495.28	2-hydroxy-N1,N4-bis(2-(4-chlorobenzamido)ethyl)succinamide
IIaL12	Cl	-NHArCONH-	$C_{21}H_{14}Cl_2N_2O_3$	413.25	(4-chloro-*N*-[(4-{[(4-chlorophenyl) carbonyl] amino} phenyl) carbonyl] benzamide
IIaL13	Cl	-NHArCONH$(CH_2)_2$NH-	$C_{23}H_{19}Cl_2N_3O_3$	456.30	4-chloro-*N*-{4-[(2-{[(4-chlorophenyl) carbonyl]amino} ethyl)carbamoyl] phenyl}benzamide
IIaL14	Cl	-NHArCONHCH_2CONH-	$C_{23}H_{17}Cl_2N_3O_4$	470.24	4-chloro-*N*-{4-[(2-{[(4-chlorophenyl)carbonyl]amino}-2-oxoethyl) carbamoyl]phenyl}benzamide
IIbL6	NH2	-NHCH_2CONH-	$C_{16}H_{16}N_4O_3$	312.27	*N*,*N*'-(1-oxoethane-1,2-diyl)bis(4-aminobenzamide)

IIbL7	NH2	-NHCH$_2$CONHCH$_2$CONH-	$C_{23}H_{21}N_5O_4$	431.25	4-chloro-N-{[({[(4-aminophenyl)carbonyl]amino} acetyl)amino] acetyl} benzamide
IIbL8	NH2	-NHCONHCH$_2$CONH-	$C_{18}H_{21}N_5O_3$	355.32	4-chloro-*N*-[(2-{[(4-aminophenyl)carbonyl]amino}-2-oxoethyl) carbamoyl] benzamide
IIbL9	NH2	-NHCH$_2$CONHC H$_2$CH$_2$NH-	$C_{18}H_{21}N_5O_3$	355.28	4-chloro-*N*-{2-[({[(4-aminophenyl) carbonyl] amino} acetyl) amino] ethyl} benzamide
IIbL10	NH2	- NHCO(CH$_2$)$_2$OHCONH-	$C_{18}H_{18}N_4O_5$	370.29	*N*,*N*'-bis[(4-aminophenyl)carbonyl]-2-hydroxybutanediamide
IIbL11	NH2	- NH(CH$_2$)$_2$NHCOCHOHCH$_2$CONH(CH$_2$)$_2$NH-	$C_{22}H_{28}N_6O_5$	456.26	2-hydroxy-N1,N4-bis(2-(4-aminobenzamido)ethyl)succinamide
IIbL12	NH2	-NHArCONH-	$C_{21}H_{18}N_4O_3$	374.25	(4-amino-*N*-[(4-{[(4-aminophenyl) carbonyl] amino} phenyl) carbonyl] benzamide
IIbL13	NH2	-NHArCONH(C H$_2$)$_2$NH-	$C_{23}H_{23}N_5O_3$	417.28	4-amino-*N*-{4-[(2-{[(4-aminophenyl) carbonyl]amino} ethyl)carbamoyl] phenyl}benzamide
IIbL14	NH2	- NHArCONHCH$_2$CONH-	$C_{23}H_{21}N_5O_4$	431.29	4-amino-*N*-{4-[(2-{[(4-aminophenyl)carbonyl]amino}-2-oxoethyl) carbamoyl]phenyl}benzamide
IIcL6	OCH3	-NHCH$_2$CONH-	$C_{18}H_{18}N_2O_5$	342.33	*N*,*N*'-(1-oxoethane-1,2-diyl)bis(4-methoxybenzamide)
IIcL7	OCH3	-NHCH$_2$CONHCH$_2$CONH-	$C_{20}H_{21}N_3O_6$	399.78	4-methoxy-N-{[({[(4-methoxyphenyl)carbonyl]amino} acetyl)amino] acetyl} benzamide
IIcL8	OCH3	-NHCONHCH$_2$CONH-	$C_{19}H_{19}N_3O_6$	385.32	4-methoxy-*N*-[(2-{[(4-methoxyphenyl)carbonyl]amino}-2-oxoethyl) carbamoyl] benzamide
IIcL9	OCH3	-NHCH$_2$CONHC H$_2$CH$_2$NH-	$C_{20}H_{23}N_3O_5$	385.33	4-methoxy-*N*-{2-[({[(4-methoxyphenyl) carbonyl] amino} acetyl) amino] ethyl} benzamide
IIcL10	OCH3	- NHCO(CH$_2$)$_2$OHCONH-	$C_{20}H_{20}N_2O_7$	400.23	*N*,*N*'-bis[(4-methoxyphenyl)carbonyl]-2-hydroxybutanediamide
IIcL11	OCH3	- NH(CH$_2$)$_2$NHCOCHOHCH$_2$CONH(CH$_2$)$_2$NH-	$C_{24}H_{30}N_4O_7$	486.28	2-hydroxy-N1,N4-bis(2-(4-methoxybenzamido)ethyl)succinamide
IIcL12	OCH3	-NHArCONH-	$C_{23}H_{20}N_2O_5$	404.25	(4-methoxy-*N*-[(4-{[(4-methoxyphenyl) carbonyl] amino} phenyl) carbonyl] benzamide
IIcL13	OCH3	-NHArCONH(CH$_2$)$_2$NH-	$C_{25}H_{25}N_3O_5$	447.00	4-methoxy-*N*-{4-[(2-{[(4-methoxyphenyl) carbonyl]amino} ethyl)carbamoyl] phenyl}benzamide
IIcL14	OCH3	-NHArCONHCH$_2$CONH-	$C_{25}H_{23}N_3O_6$	461.24	4-methoxy-*N*-{4-[(2-{[(4-methoxyphenyl)carbonyl]amino}-2-oxoethyl) carbamoyl]phenyl}benzamide
IIdL6	NO2	-NHCH$_2$CONH-	$C_{16}H_{12}N_4O_7$	372.27	*N*,*N*'-(1-oxoethane-1,2-diyl)bis(4-nitrobenzamide)
IIdL7	NO2	-NHCH$_2$CONHCH$_2$CONH-	$C_{18}H_{15}N_5O_6$	429.25	4-nitro-N-{[({[(4-nitrophenyl)carbonyl]amino} acetyl)amino] acetyl} benzamide
IIdL8	NO2	-NHCONHCH$_2$CONH-	$C_{17}H_{13}N_5O_8$	415.32	4-nitro-*N*-[(2-{[(4-

					nitrophenyl)carbonyl]amino}-2-oxoethyl) carbamoyl] benzamide
IIdL9	NO2	-NHCH$_2$CONHC H$_2$CH$_2$NH-	$C_{18}H_{17}N_5O_7$	415.38	4-nitro-*N*-{2-[({[(4-nitrophenyl) carbonyl] amino} acetyl) amino] ethyl} benzamide
IIdL10	NO2	- NHCO(CH$_2$)$_2$OHCONH-	$C_{18}H_{14}N_4O_9$	430.29	*N,N'*-bis[(4-nitrophenyl)carbonyl]-2-hydroxybutanediamide
IIdL11	NO2	-NH(CH$_2$)$_2$NHCOCHOHCH$_2$CONH(CH$_2$)$_2$NH-	$C_{22}H_{24}N_6O_9$	516.46	2-hydroxy-N1,N4-bis(2-(4-nitrobenzamido)ethyl)succinamide
IIdL12	NO2	-NHArCONH-	$C_{21}H_{14}N_4O_7$	434.36	(4-nitro-*N*-[(4-{[(4-nitrophenyl) carbonyl] amino} phenyl) carbonyl] benzamide
IIdL13	NO2	-NHArCONH(C H$_2$)$_2$NH-	$C_{23}H_{19}N_5O_7$	477.48	4-nitro-*N*-{4-[(2-{[(4-nitrophenyl) carbonyl]amino} ethyl)carbamoyl] phenyl}benzamide
IIdL14	NO2	- NHArCONHCH$_2$CONH-	$C_{23}H_{17}N_5O_8$	491.49	4-nitro-*N*-{4-[(2-{[(4-nitrophenyl)carbonyl]amino}-2-oxoethyl) carbamoyl]phenyl}benzamide

General procedure for synthesis of compounds:

STEP-I: 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.

STEP-II: 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.

Spectral analysis:

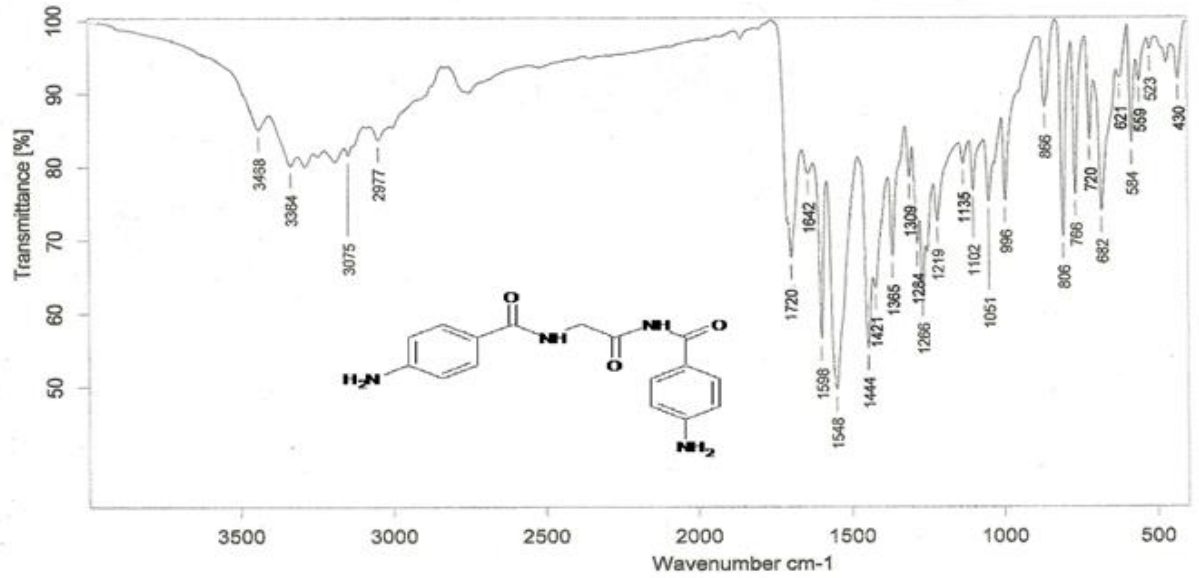

Figure 3. IR Spectra of *N,N'*-(1-oxoethane-1,2-diyl)bis(4-aminobenzamide) (IIbL6)

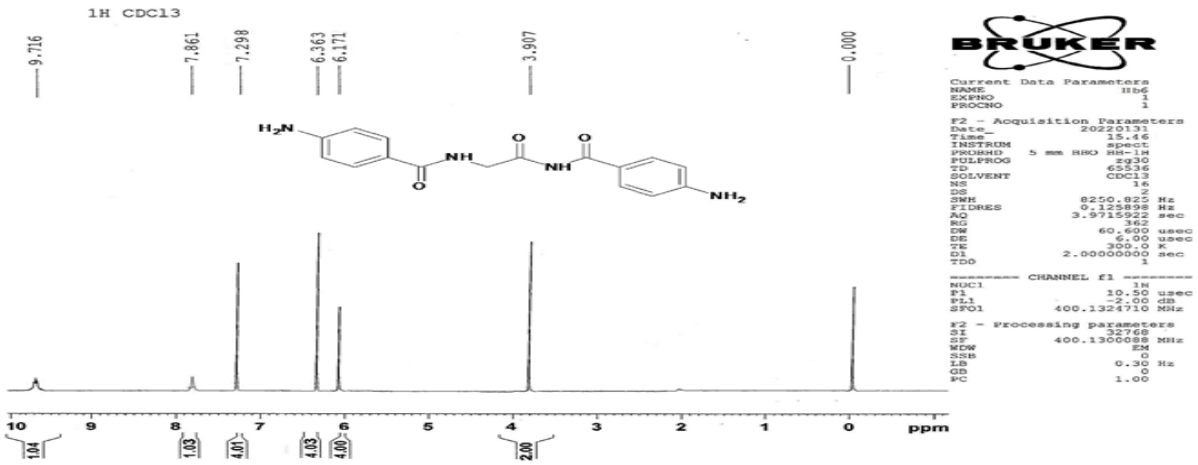

Figure 4. NMR Spectra of *N,N'*-(1-oxoethane-1,2-diyl)bis(4-aminobenzamide) (IIbL6)

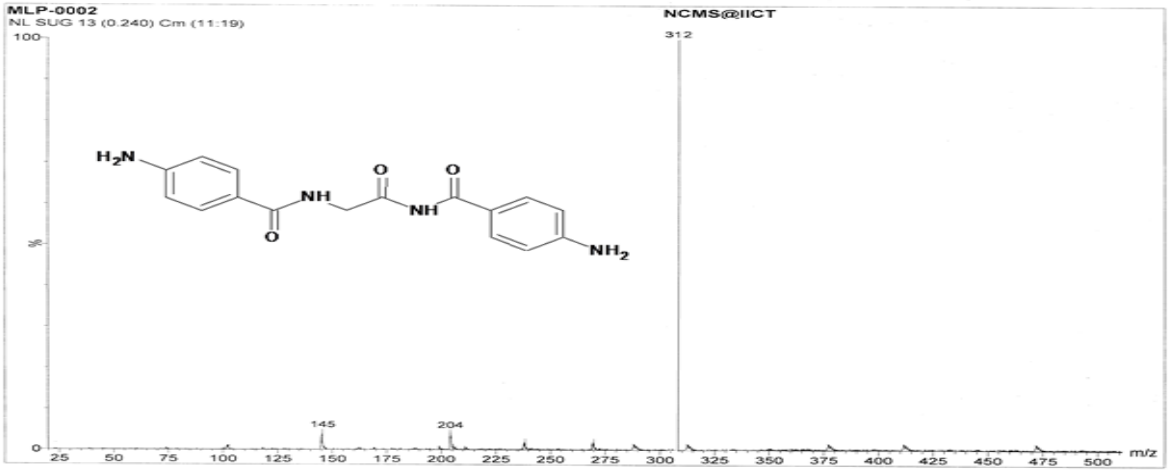

Figure 5. Mass Spectra of *N,N'*-(1-oxoethane-1,2-diyl)bis(4-aminobenzamide) (IIbL6)

RESULTS AND DISCUSSION

Docking Studies:

The protein data bank was used to obtain the X-ray crystal structure of the human DNA topoisomerase (70 Kda) in complex with camptothecin and in covalent complex with a 22 base pair DNA duplex (PDB ID: 1T8I). ACD/ ChemSketch (12.0) software was then used to create diagrams of the chemically synthesised 4-Substituted bisbenzamide derivatives. The analysis of the ligand-protein interaction used the target protein receptor because it had acceptable geometrical properties.

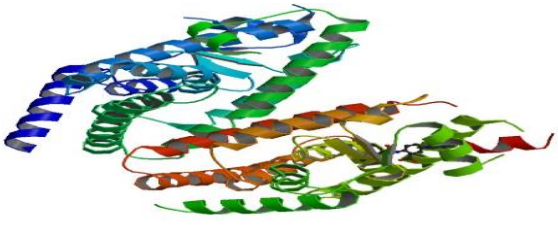

Figure 6. Crystal structure of the human DNA topoisomerase (PDB ID: 1T8I)

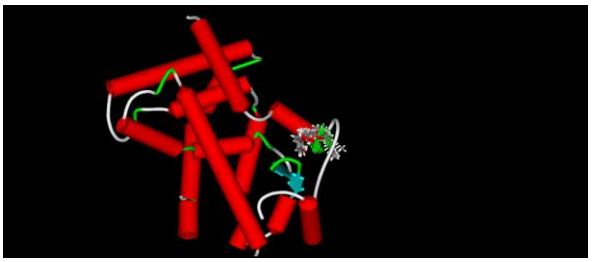

Figure 7. Binding of Ligand Molecule in Active Site Pocket

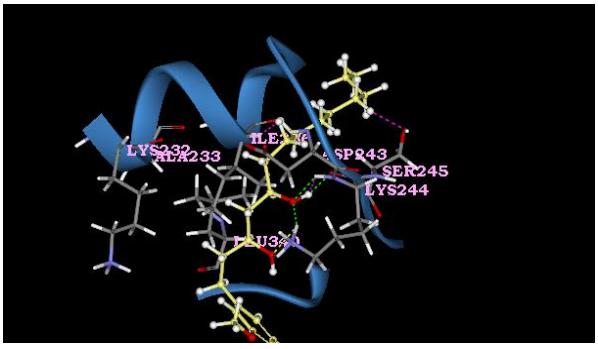

Figure 8. H-bond interactions of potent compound withactive site residues of Human DNA Topo I

Table 3. London Dg scoring of all 4-Substituted Bis-benzamide ligand compounds (Scheme - I) with Symmetric Linker Chains ($L_1 – L_5$)

Code	R	Linker Chain	Name of the Linker Chain	London dG Scoring
IaL_1	-Cl	-NHCONH-	Urea	-7.5358
IaL_2	-Cl	-$NHCH_2CH_2NH$-	Ethylenediamine	-8.4313
IaL_3	-Cl	-$NHCOCH_2CONH$-	Malonamide	-6.6478
IaL_4	-Cl	-$NHCH_2CONHCOCH_2NH$-	N-(Aminoacetyl)glycinamide	-5.4587
IaL_5	-Cl	-$NHCH_2CONHCH_2CH_2NHCOCH_2NH$-	N,N'-Bis-(2aminoacetyl) ethylene Diamine	-8.1088
IbL_1	-NH_2	-NHCONH-	Urea	-8.2173
IbL_2	-NH_2	-$NHCH_2CH_2NH$-	Ethylenediamine	-7.9524
IbL_3	-NH_2	-$NHCOCH_2CONH$-	Malonamide	-8.1054
IbL_4	-NH_2	-$NHCH_2CONHCOCH_2NH$-	N-(Aminoacetyl)glycinamide	-10.5264
IbL_5	-NH_2	-$NHCH_2CONHCH_2CH_2NHCOCH_2NH$-	N,N'-Bis-(2aminoacetyl) ethylene Diamine	-8.2280
IcL_1	-OCH_3	-NHCONH-	Urea	-7.6478
IcL_2	-OCH_3	-$NHCH_2CH_2NH$-	Ethylenediamine	-8.2415
IcL_3	-OCH_3	-$NHCOCH_2CONH$-	Malonamide	-8.1088
IcL_4	-OCH_3	-$NHCH_2CONHCOCH_2NH$-	N-(Aminoacetyl)glycinamide	-9.8547
IcL_5	-OCH_3	-$NHCH_2CONHCH_2CH_2NHCOCH_2NH$-	N,N'-Bis-(2aminoacetyl) ethylene Diamine	-8.3830
IdL_1	-NO_2	-NHCONH-	Urea	-8.1054
IdL_2	-NO_2	-$NHCH_2CH_2NH$-	Ethylenediamine	-9.5287
IdL_3	-NO_2	-$NHCOCH_2CONH$-	Malonamide	-8.2280
IdL_4	-NO_2	-$NHCH_2CONHCOCH_2NH$-	N-(Aminoacetyl)glycinamide	-12.8451
IdL_5	-NO_2	-$NHCH_2CONHCH_2CH_2NHCOCH_2NH$-	N,N'-Bis-(2aminoacetyl) ethylene Diamine	-11.9873

Table 4. London Dg scoring of all 4-substituted Bis-benzamide ligand compounds (Scheme - II) with Asymmetric Linker Chains ($L_6 – L_{14}$)

Code	R	Linker Chain	Name of the Linker Chain	London dG Scoring
$IIaL_6$	-Cl	-$NHCH_2CONH$-	Glycinamide	-7.2546
$IIaL_7$	-Cl	-$NHCH_2CONHCH_2CONH$-	2-(N-Ureido)acetamide	-11.9830
$IIaL_8$	-Cl	-$NHCONHCH_2CONH$-	N_1-(2-Acetamido)glycinamide	-6.5287
$IIaL_9$	-Cl	-$NHCH_2CONHCH_2CH_2NH$-	N_1-(2-Aminoethyl)glycinamide	-8.2154
$IIaL_{10}$	-Cl	-$NHCO(CH_2)_2OHCONH$-	Malamide	-6.8542
$IIaL_{11}$	-Cl	-$NH(CH_2)_2NHCOCHOHCH_2CONH(CH_2)_2NH$-	N_1, N'-Bis(2-aminoethyl) malamide	-8.2192
$IIaL_{12}$	-Cl	-NHArCONH-	4-Aminobenzamide	-9.3245
$IIaL_{13}$	-Cl	-$NHArCONH(CH_2)_2NH$-	4-Amino-N-(2-aminoethyl) benzamide	-8.2192
$IIaL_{14}$	-Cl	-$NHArCONHCH_2CONH$-	4-Amino-N-(2-acetamido) benzamide	-9.5216
$IIbL_6$	-NH_2	-$NHCH_2CONH$-	Glycinamide	-13.5632
$IIbL_7$	-NH_2	-$NHCH_2CONHCH_2CONH$-	2-(N-Ureido)acetamide	-10.4873
$IIbL_8$	-NH_2	-$NHCONHCH_2CONH$-	N_1-(2-Acetamido)glycinamide	-8.4527
$IIbL_9$	-NH_2	-$NHCH_2CONHCH_2CH_2NH$-	N_1-(2-Aminoethyl)glycinamide	-7.6090
$IIbL_{10}$	-NH_2	-$NHCO(CH_2)_2OHCONH$-	Malamide	-8.7546
$IIbL_{11}$	-NH_2	-$NH(CH_2)_2NHCOCHOHCH_2CONH(CH_2)_2NH$-	N_1, N'-Bis(2-aminoethyl) malamide	-9.0268
$IIbL_{12}$	-NH_2	-NHArCONH-	4-Aminobenzamide	-10.5685
$IIbL_{13}$	-NH_2	-$NHArCONH(CH_2)_2NH$-	4-Amino-N-(2-aminoethyl) benzamide	-8.5263
$IIbL_{14}$	-NH_2	-$NHArCONHCH_2CONH$-	4-Amino-N-(2-acetamido) benzamide	-7.8452
$IIcL_6$	-OCH_3	-$NHCH_2CONH$-	Glycinamide	-9.3254
$IIcL_7$	-OCH_3	-$NHCH_2CONHCH_2CONH$-	2-(N-Ureido)acetamide	-8.2154
$IIcL_8$	-OCH_3	-$NHCONHCH_2CONH$-	N_1-(2-Acetamido)glycinamide	-9.2354
$IIcL_9$	-OCH_3	-$NHCH_2CONHCH_2CH_2NH$-	N_1-(2-Aminoethyl)glycinamide	-12.2192
$IIcL_{10}$	-OCH_3	-$NHCO(CH_2)_2OHCONH$-	Malamide	-10.5362
$IIcL_{11}$	-OCH_3	-$NH(CH_2)_2NHCOCHOHCH_2CONH(CH_2)_2NH$-	N_1, N'-Bis(2-aminoethyl) malamide	-8.2192
$IIcL_{12}$	-OCH_3	-NHArCONH-	4-Aminobenzamide	-6.2541
$IIcL_{13}$	-OCH_3	-$NHArCONH(CH_2)_2NH$-	4-Amino-N-(2-aminoethyl) benzamide	-8.2173
$IIcL_{14}$	-OCH_3	-$NHArCONHCH_2CONH$-	4-Amino-N-(2-acetamido) benzamide	-7.3658
$IIdL_6$	-NO_2	-$NHCH_2CONH$-	Glycinamide	-10.8545
$IIdL_7$	-NO_2	-$NHCH_2CONHCH_2CONH$-	2-(N-Ureido)acetamide	-7.6090

$IIdL_8$	$-NO_2$	$-NHCONHCH_2CONH-$	N_1-(2-Acetamido)glycinamide	-9.5241
$IIdL_9$	$-NO_2$	$-NHCH_2CONHCH_2CH_2NH-$	N_1-(2-Aminoethyl) glycinamide	-9.0268
$IIdL_{10}$	$-NO_2$	$-NHCO(CH_2)_2OHCONH-$	Malamide	-10.8654
$IIdL_{11}$	$-NO_2$	$-NH(CH_2)_2NHCOCHOHCH_2CONH(CH_2)_2NH-$	N_1, N'-Bis(2-aminoethyl) malamide	-7.6478
$IIdL_{12}$	$-NO_2$	-NHArCONH-	4-Aminobenzamide	-8.1542
$IIdL_{13}$	$-NO_2$	$-NHArCONH(CH_2)_2NH-$	4-Amino-N-(2-aminoethyl) benzamide	-8.1088
$IIdL_{14}$	$-NO_2$	$-NHArCONHCH_2CONH-$	4-Amino-N-(2-acetamido) benzamide	-10.8564

In the current study, docking calculations using structurally linear, well-characterized ligands as molecular probes were used to assess potential binding locations using London dG scoring simulations. In the active site of the Human DNA Topoisomerase I enzyme, all 56 4-substituted bisbenzamide derivative ligands were docked. The Tables contains the docking results for 56 4-substituted bisbenzamide derivatives. The most active compounds, IIbL6, IdL4, IIcL9, and IIaL7, interacted with receptors via H-bonds, according to docking analyses. A more thorough examination of the binding pocket revealed that the chemical IIbL6 has H-bond interactions with Human DNA Topoisomerase I active site residues such as DG112, TGP11, DA113, Glu356, DT10, ASN352 and Lys425.

CONCLUSION

The majority of active molecules, IIbL6, IdL4, IIcL9, and IIaL7, interacted with receptors through H-bonds, according to docking analyses. Therefore, it may be concluded that compound IIbL6 and IdL4 of the 4-substituted bisbenzamide derivatives are the most effective topo I inhibitors and may operate as an anti-cancer target. Thus, the most effective topo I inhibitors were discovered to be compound IIbL6 of the 4-substituted bisbenzamide derivatives which can be potential to be used as anticancer agent.

REFERENCES

1. Jackson BG. Mechanism-based target identification and drug discovery in cancer research. Science. 2000; 287: 1969-1973.
2. Bart L. Staker. Structures of three classes of anticancer agents bound to the Human Topoisomerase I – DNA covalent complex. Journal of Medicinal Chemistry.2015; 48: 2336-2345.
3. Tim R, Blower. Crystal structure and stability of gyrase fluoroquinolone cleaved complexes from Mycobacterium tuberculosis. Proceedings of the National Academy of Sciences. 2016; 113: 1706-1713.
4. Matthew R. Redinbo. Crystal structures of Human Topoisomerase I in Covalent and Noncovalent complexes with DNA. Science. 199; 279: 1504-1513.
5. Morris GM. AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility. Journal of Computational Chemistry. 2009; 30: 2785-2791.
6. Koji Ogata. Lead generation and optimization based on Protein-Ligand Complementarity. Molecules. 2010; 15: 4382-4400.
7. Alexander D, Wade and David J, Huggins. Optimization of Protein-Ligand Electrostatic interactions using an Alchemical Free Energy method. J. Chem. Theory Comput.2019; 15: 6504-6512.
8. Hahnbeom Park. Force Field Optimization guided by small molecule crystal lattice data enables consistent Sub-Angstrom Protein-Ligand Docking. J. Chem. Theory Comput.2021; 17: 2000-2010.
9. Sabine Schultes. Ligand efficiency as a guide in fragment hit selection and optimization. Drug Discovery Today: Technologies. 2010; 7: 157-162.
10. Raquel Dias, Walter Filgueira de Azevedo Jr. Molecular docking algorithms. Curr Drug Targets. 2008; 9: 1040-1047.
11. Pedro HM, Torres. Key Topics in Molecular Docking for Drug Design. International Journal of Molecular Sciences. 2019; 20: 4574-4602.
12. Natasja Brooijmans and Irwin DK. Molecular Recognition and Docking Algorithms. Annual Review of Biophysics and Biomolecular Structure. 2003; 32: 335-373.
13. Saba Munawar. Molecular docking guided Grid-Independent Descriptor analysis to probe the impact of water molecules on Conformational Changes of hERG inhibitors in Drug Trapping Phenomenon. International Journal of Molecular Sciences. 2019; 20: 3385-3412.
14. Kirk E. Hevener. Validation of Molecular Docking Programs for Virtual Screening against Dihydropteroate Synthase. J Chem Inf Model. 2009; 49: 444-460.
15. Sriram Sankararaman. Active site prediction using evolutionary and structural information. Bioinformatics. 2010; 26: 617-624.
16. Lukas Jendele. Prankweb: a web server for ligand binding site prediction and visualization.

Nucleic Acids Research. 2019; 47: 345-349.
17. Mohammad Asif. Pharmacological Potential of Benzamide Analogues and their Uses in Medicinal Chemistry**.** Modern Chemistry and Applications. 2016; 4: 1-10.
18. Bonifacio VDB. Proline Derivatives in Organic Synthesis. Org. Chem. Highlights. 2007.