International Journal of Enhanced Research in Medicines & Dental Care (IJERMDC),
ISSN: 2349-1590, Vol. 10 Issue 9, September 2023, Impact Factor: 7.125

Synthesis, Characterization, Antioxidant and Molecular Docking Studies of Novel Mannich Base Derivatives of Isatins for Anti-Cancer Activity

Shaik Riyaz*[1], Gade Sammaiah[2], CH. Ragunath[3]

[1]*Assistant Professor, Prathap Narender Reddy College of Pharmacy, Peddashapur, Shamshabad -509325, Telangana, India
[2]Professor, Department of Pharmaceutical Chemistry, University College of Pharmaceutical Sciences, Kakatiya University, Warangal, Telangana-506009, India
[3]Assistant Professor, Swami Vivekananda Institute of Pharmaceutical Sciences, Vangapally, Yadagirigutta, Yadadri-Bhongir- 508286, Telangana, India

***Correspondence to Author:** Mr. Shaik Riyaz, Assistant Professor, Department of Pharmaceutical chemistry, Prathap Narender Reddy College of Pharmacy Peddashapur, Shamshabad -509325, Telangana, India. Email: sriyaz022@gmail.com

ABSTRACT

A series of novel indoline derivatives with an efficient and easy approach has been designed and 1-((bis(2-chloroethyl)amino)methyl)indoline-2,3-dione derivatives have been synthesized. The synthesized compounds were characterized by elemental analysis of IR, NMR, and Mass spectroscopy. *In-vitro* antioxidant activity of newly synthesized compounds was determined by the α, α-diphenyl-β-picrylhydrazyl (DPPH) free radical scavenging method by using Ascorbic acid as standard drug. Among all the compound VIj (R=5-F) showed efficient antioxidant activity. Molecular docking studies are performed on Cancer main protease (pdb: 5OTF). Among all the compounds, VIp(R=7-COOH) and VIm(R=7COOCH3) derivative compounds on Cancer main protease proved to possess highest binding energy of -7.3 k.cal/mol.

Keywords: Isatin, Antioxidant, Protease, Docking

INTRODUCTION

Indoles are heterocyclic compounds with a various applications in pharmaceutical activities such as cancer, microbial and viral infections, inflammation, depression, migraine, emesis, hypertension[1].

An antioxidant is a molecule that prevents the oxidation of molecules inside a cell. It is a well-known chemical process that allows the removal of electrons or hydrogen from a substance[2]. Free radicals are produced during the biological oxidation reaction. Because the radicals are reactive, they start the chain reaction simultaneously. This can lead to the damage or even the death of a cell[3]. Antioxidant agents are capable of terminating a chain reaction by eliminating free radical intermediates. Hence, they are also called as free radical scavengers[4]. Antioxidants are free radical scavengers which neutralize reactive oxygen species (ROS) produced during aerobic cellular metabolism: superoxide (O^{2-}), hydrogen peroxide (H_2O_2) and peroxynitrite ($OONO-$)[5]. Also, antioxidants exert protective effects on cells against the deleterious effects of ROS on cell membranes, mitochondria, DNA, lipids or proteins. Endogenous antioxidants (catalase, superoxide dismutase, peroxidase and glutathione) exert their activity by scavenging oxygen free radicals and thereby are important in preventing oxidative stress[6].

Molecular docking is one of the most frequently used methods in structure-based drug design, due to its ability to predict the binding-conformation of small molecule ligands to the appropriate target binding site[7]. Characterization of the binding behavior plays an important role in rational design of drugs as well as to elucidate fundamental biochemical processes. It is a method which predicts the preferred orientation of one molecule to a second when a ligand and a target are bound to each other to form a stable complex[8]. Here Insilico studies were performed on Cancer Protease (5OTF).

MATERIALS AND METHOD

All the chemicals and solvents used were obtained from Merck and Himedia. The synthesized compounds were preliminarily confirmed by thin layer chromatography (TLC) plates and UV chamber. Melting points were determined by using capillary tubes and digital melting point apparatus. The final derivatives were characterized by spectral methods such as Fourier transform infrared (FTIR), 1H-NMR and Mass spectroscopy.

Softwares- Several protein–ligand docking software applications that calculate the site, geometry and energy of small molecules or peptides interacting with proteins are available, such as PYRX, AutoDock and AutoDock Vina, rDock, FlexAID, Molecular Operating Environment, Glide and DockThor.

CHEMISTRY

Step-1: Synthesis of Indole-2,3-diones (Isatins):

a) Isonitrosoacetanilide – General Procedure: In a 5 lit. R.B. flask were placed chloral hydrate (0.54 mol) and 1200 ml of water. To this solution, were then added crystallized sodium sulphate (1300gm) followed by a solution of an appropriate aromatic amine in 300ml of water and concentrated hydrochloric acid (0.52mol)[9]. Finally, a solution of hydroxylamine HCl (1.58 mol) in 500 ml of water was added. The contents of the flask were heated over a wire-gauge by a Mecker burner so that vigorous boiling begins in about 45 minutes. After 1 to 2 minutes of vigorous boiling the reaction was completed[10]. During the heating period itself the crystals of isonitrosoacetanilide started separating out. On cooling under the current of water, the entire product was solidified. It was filtered under suction, air dried and purified by recrystallization from suitable solvent[11].

b) Indole-2,3-diones – General Procedure: Sulphuric acid (600g, d:1.84, 326 ml) was warmed at 50^{o}C in a one litre RB flask fitted with an efficient mechanical stirrer and to this, finely powdered appropriate isonitrosoacetanilide (0.46 mol) was added at such a rate so as to maintain the temperature between 60^{o}C to 70^{o}C but not higher[12]. After the addition of isonitroso compound was completed the temperature of the solution was raised to 80^{o}C and maintained at that temperature for 10 minutes, to complete the reaction[13]. Then the reaction mixture was cooled to room temperature and poured onto crushed ice (2.5 kg) while stirring. After standing for about half-an-hour, the product separated was filtered, washed several times with small portions of cold water and dried[14]. Purification of the compound was effected by the recrystallization from methanol. Various derivatives of indole-2,3-diones were prepared by using different aromatic amines and were confirmed by TLC.

Step 2: Synthesis of 1-{[bis(2-chloroethy) amino] methyl}-1H-indole-2,3-dione: The mannich condensation was done by the following procedure. A mixture ofequimolar concentration of Isatin (0.010 moles; 1.47g), Formaldehyde (0.010moles; 0.3g, 1 mL), Bis(2-chloroethyl) amine hydrochloride (0.010 moles; 1.42g)was refluxed in ethanol (50 mL) for 8hrs at 80" C. After filtering the filtrate was concentrated to one third its volume, dried over sodium sulfate. The residue was recrystallized from ethylacetate-petroleum ether giving pure material

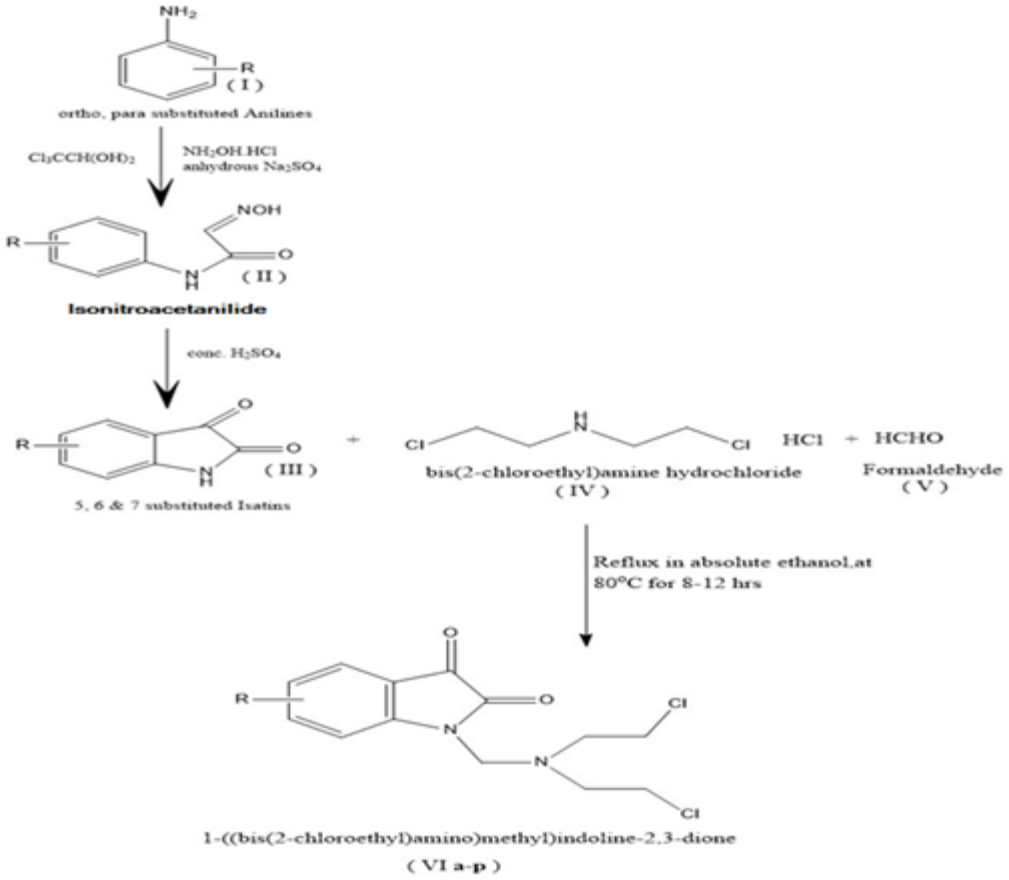

Figure 1: Synthesis of 1-((Bis(2-chloroethyl)amino)methyl)indoline-2,3-dione.(VIa-p)

Table 1: Physical data of 1-((Bis(2-chloroethyl)amino)methyl)indoline-2,3-dione.(VIa-p)

Compound No	R	Molecular Formula	Molecular Weight	Rf-Value	Melting Point ^{0}C	% Yeild
VIa	H	$C_{13}H_{14}Cl_2N_2O_2$	301	0.73	100-102	89%
Vib	5-CH_3	$C_{14}H_{16}Cl_2N_2O_2$	315.19	0.75	220-225	87%
Vic	7-CH_3	$C_{14}H_{16}Cl_2N_2O_2$	315.19	0.65	285-295	90%
Vid	5-Cl	$C_{13}H_{13}Cl_3N_2O_2$	335.61	0.70	140-150	87%
Vie	7-Cl	$C_{13}H_{13}Cl_3N_2O_2$	335.61	0.79	140-150	94%
Vif	5-NO_2	$C_{13}H_{13}Cl_2N_3O_2$	346.16	0.61	174-179	92%
VIg	7-NO_2	$C_{13}H_{13}Cl_2N_3O_2$	346.16	0.66	174-177	89%
VIh	5-Br	$C_{13}H_{13}BrCl_2N_2O_2$	380.06	0.78	138-145	93%
VIi	7-Br	$C_{13}H_{13}BrCl_2N_2O_2$	380	0.80	144-148	92%
Vij	5-F	$C_{13}H_{13}FCl_2N_2O_2$	319.16	0.75	109-112	85%
Vik	7-F	$C_{13}H_{13}FCl_2N_2O_2$	319.16	0.72	78-85	84%
Vil	5-$COOC_2H_5$	$C_{16}H_{18}Cl_2N_2O_4$	373.23	0.66	160-165	93%
Vim	7-$COOCH_3$	$C_{15}H_{16}Cl_2N_2O_4$	359.20	0.68	285-295	90%
Vin	6-Cl 5-F	$C_{13}H_{12}FCl_3N_2O_2$	353.60	0.76	125-135	82%
VIo	5-Cl 7-F	$C_{13}H_{12}FCl_3N_2O_2$	353.60	0.78	79-88	94%
VIp	5-COOH	$C_{14}H_{14}Cl_2N_2O_4$	345.18	0.43	75-79	84%

SPECTRA DATA:

1-((bis(2-chloroethyl)amino)methyl)indoli ne-2,3-dione (VIa): IR (KBr) cm^{-1}: 2980(CH-Ar), 1682(NH-C=0), 1609(C-N), 1343(C-N), 1510(Ar C=C), 791(Al C- H), 747(C-Cl). **^{1}H-NMR (CDCl3 - D) δppm** : 2.8(t,4H,N-CH_2), 3.6(t,4H,CH_2-Cl), 4.6(s,2H, N-CH_2-N), 7.23(t,1H, Ar-H), 7.69(d,1H,Ar-H), 7.9(t,1H,Ar-H), 8.1(d,1H,Ar-H). **Mass Spectrum (EI-MS) : (** M+1) Peak was observed at 301 m/z and (M+4) Peak was observed at 305 m/z.

1-((bis(2-chloroethyl)amino)methyl)-5- methylindoline-2,3-dione (VIb): IR (KBr) cm^{-1}: 2983(CH-Ar), 1686(NH-C=0), 1607(C=N), 1340(C-N), 1513(Ar C=C), 786(Al C- H), 741(C-Cl). **^{1}H-NMR (CDCl3 - D) δppm** : 2.8(t,4H,N-CH_2), 3.6(t,4H,CH_2-Cl), 4.6(s,2H,N-CH_2-N), 7.7(s,1H,Ar-H), 7.92(d,1H,Ar-H), 8.1(d,1H,Ar-H). **Mass Spectrum (EI-MS) : (** M+1) Peak was observed at 315 m/z and (M+4) Peak was observed at 319 m/z

1-((bis(2-chloroethyl)amino)methyl)-7- methylindoline-2,3-dione(VIc): IR (KBr) cm^{-1}: 2997(CH-Ar), 1673(NH-C=0), 1609(C=N), 1337(C-N), 1511(Ar C=C), 791(Al C-H), 742(C-Cl). **^{1}H-NMR (CDCl3 - D) δppm** : 2.9(t,4H,CH_2-Cl), 3.5(t,4H,N-CH_2), 4.8(s,2H)N-CH_2-N), 7.96(d,1H,Ar), 8.55(t,1H,Ar-H), 8.76(d,1H,Ar-H). **Mass Spectrum (EI-MS) : (** M+1) Peak was observed at 315 m/z and (M+4) Peak was observed at 319 m/z

1-((bis(2-chloroethyl)amino)methyl)-5- chloroindoline-2,3-dione (VId): IR (KBr) cm^{-1}: 2979(CH-Ar), 1683(NH-C=0), 1601(C=N), 1342(C-N), 1507(Ar C=C), 789(Al C- H), 750(C-Cl). **^{1}H-NMR (CDCl3 - D) δppm** : 2.9(t,4H,N-CH_2), 3.6(t,4H,CH_2-Cl), 4.5(s,2H,N-CH_2-N), 7.72(s,1H,Ar-H), 7.9(d,1H,Ar-H), 8.2(d,1H,Ar-H). **Mass Spectrum (EI-MS) : (** M+1) Peak was observed at 335 m/z and (M+4) Peak was observed at 341 m/z

1-((bis(2-chloroethyl)amino)methyl)-7- chloroindoline-2,3-dione (VIe): IR (KBr) cm^{-1}: 2992(CH-Ar), 1680(NH-C=O), 1602(C=N), 1339(C-N), 1504(Ar C=C),792(Al C-H),752(C-Cl). **^{1}H-NMR (CDCl3 - D) δppm** : 2.9(t,4H,CH_2-Cl), 3.5(t,4H,N-CH_2), 4.7(s,2H)N-CH_2-N), 7.8(d,1H,Ar), 8.6(t,1H,Ar-H), 8.8(d,1H,Ar-H). **Mass Spectrum (EI-MS) : (** M+1) Peak was observed at 335 m/z and (M+4) Peak was observed at 341 m/z

1-((bis(2-chloroethyl)amino)methyl)-5-nitroindoline-2,3-dione (Vif):IR (KBr) cm^{-1}: 2982(CH-Ar),1686(NH-C=0),1604(C=N), 1340(C-N), 1508(Ar C=C),794(Al C- H),748(C-Cl). **^{1}H-NMR (CDCl3 - D) δppm** : 2.8(t,4H,N-CH_2), 3.6(t,4H,CH_2-Cl), 4.6(s,2H,N-CH_2-N), 7.7(s,1H,Ar-H), 7.9(d,1H,Ar-H), 8.2(d,1H,Ar-H). **Mass Spectrum (EI-MS) : (** M+1) Peak was observed at 346 m/z and (M+4) Peak was observed at 350 m/z

1-((bis(2-chloroethyl)amino)methyl)-7-nitroindoline-2,3-dione (VIg): IR (KBr) cm^{-1}: 2998(CH-Ar), 1678(NH-C=O), 1611(C=N), 1345(C-N), 1510(Ar C=C), 790(Al C-H), 747(C-Cl). **^{1}H-NMR (CDCl3 - D) δppm** : 2.8(t,4H,CH_2-Cl), 3.5(t,4H,N-CH_2), 4.7(s,2H)N-CH_2-N), 7.8(d,1H,Ar), 8.5(t,1H,Ar-H), 8.7(d,1H,Ar-H). **Mass Spectrum (EI-MS) : (** M+1) Peak was observed at 346 m/z and (M+4) Peak was observed at 350 m/z

1-((bis(2-chloroethyl)amino)methyl)-5-bromoindoline-2,3-dione(VIh): IR (KBr) cm^{-1}: 2992(CH-Ar), 1682(NH-C=0), 1601(C=N), 1347(C-N), 1509(Ar C=C), 788(Al C- H), 743(C-Cl). **^{1}H-NMR (CDCl3 - D) δppm** : 2.9(t,4H,N-CH_2), 3.6(t,4H,CH_2-Cl), 4.7(s,2H,N-CH_2-N), 7.7(s,1H,Ar-H), 7.9(d,1H,Ar-H), 8.4(d,1H,Ar-H). **Mass Spectrum (EI-MS) : (** M+1) Peak was observed at 380 m/z and (M+4) Peak was observed at 386 m/z

1-((bis(2- chloroethyl)amino)methyl)-7- bromoindoline-2,3-dione(VIi): IR (KBr) cm^{-1}: 2990(CH-Ar), 1687(NH-C=0), 1605(C=N), 1337(C-N), 1505(Ar C=C), 791(Al C-H), 741(C-Cl). **^{1}H-NMR (CDCl3 - D) δppm** 2.9(t,4H,CH_2-Cl), 3.6(t,4H,N-CH_2), 4.8(s,2H)N-CH_2-N), 7.9(d,1H,Ar), 8.6(t,1H,Ar-H), 8.7(d,1H,Ar-H). **Mass Spectrum (EI-MS) : (** M+1) Peak was observed at 380 m/z and (M+4) Peak was observed at 386 m/z

1-((bis(2-chloroethyl)amino)methyl)-5-fluoroindoline-2,3-dione (Vij): IR (KBr) cm^{-1} – 2997(CH-Ar), 1689(NH-C=O), 1605(C=N),1339(C-N), 1504(Ar C=C), 797(Al C-H), 752(C-Cl). **^{1}H-NMR(CDCl3-D)δppm**: 3.0(t, 4H, N-(CH_2)2), 3.6(t, 4H, CH_2Cl), 4.8(s, 2H, N-CH_2-N), 7.9(d,1H, Ar-H). 8.5(d,1H, Ar-H), 8.7(s,1H, Ar-H). **Mass Spectrum (EI-MS):** M^+ Peak was observed at 319 m/z and M+ 6 Peak was observed at 325 m/z

1-((bis(2-chloroethyl)amino)methyl)-7- fluoroindoline-2,3-dione (VIk): IR (KBr) cm^{-1}: 2981(CH-Ar), 1676(NH-C=O), 1608(C=N), 1351(C-N), 1517(Ar C=C), 791(Al C-H), 756(C-Cl). **^{1}H-NMR (CDCl3 - D) δppm** : 2.9(t,4H,CH_2-

Cl), 3.9(t,4H,N-CH_2), 4.8(s,2H)N-CH_2-N), 7.9(d,1H,Ar), 8.5(t,1H,Ar-H), 8.77(d,1H,Ar-H). **Mass Spectrum (EI-MS) : (** M+1) Peak was observed at 319 m/z and (M+4) Peak was observed at 325 m/z

1-((bis(2-chloroethyl)amino)methyl)-5- ethylindoline-2,3-dione (VIl): IR (KBr) cm^{-1}: 2990(CH-Ar), 1679(NH-C=0), 1611(C=N), 1346(C-N), 1509(Ar C=C), 797(Al C-H), 751(C-Cl). **^{1}H-NMR (CDCl3 - D) δppm** : 1.25(t,3H,Ethyl CH3), 4.2(q,2H,Ar-H), 2.9(t,4H,N-CH_2), 3.5(t,4H,CH_2-Cl), 4.8(s,2H)N-CH_2-N), 7.96(d,1H,Ar), 8.55(d,1H,Ar-H), 8.76(s,1H,Ar-H). **Mass Spectrum (EI-MS) : (** M+1) Peak was observed at 373 m/z and (M+4) Peak was observed at 377 m/z

methyl 1-((bis(2-chloroethyl)amino)methyl)-2,3-dioxoindoline-7-carboxylate (VIm): IR (KBr) cm^{-1}: 2985(-CH), 1680(NH-C=0), 1619(C=N),1352(C- N),1721(C=O), 1517(Ar C=C), 784(Al C-H), 738(C-Cl). **^{1}H-NMR (CDCl3 - D) δppm** :3.0(t,4H,N-CH_2), 3.5(t,4H,CH2-Cl), 3.8(s,3H,$COOCH_3$), 4.7(s,2H, N-CH_2- N), 7.5(t,1H)Ar, 8.0(d,1H)Ar, 8.2(d,1H)Ar. **Mass Spectrum (EI-MS) : (** M+1) Peak was observed at 359 m/z and (M+4) Peak was observed at 363 m/z.

1-((bis(2- chloroethyl)amino)methyl)-6- chloro-5-fluoroindoline-2,3- dione (VIn): IR (KBr) cm^{-1}: 2997(CH-Ar), 1694(NH-C=O), 1615(C=N), 1349(C-N), 1517(Ar C=C), 783(Al C- H), 737(C-Cl). **^{1}H-NMR (CDCl3 - D) δppm** : 2.9(t,4H,N-CH_2), 3.5(t,4H,CH_2-Cl), 5.2(s,2H)N-CH_2-N), 7.96(s,1H,Ar), 8.55(s,1H)Ar. **Mass Spectrum (EI-MS) : (** M+1) Peak was observed at 353 m/z and (M+4) Peak was observed at 361 m/z

1-((bis(2- chloroethyl)amino)methyl)-5- chloro-7-fluoroindoline-2,3- dione (VIo): IR (KBr) cm^{-1}: 2995(CH-Ar), 1691(NH-C=0), 1609(C=N), 1346(C-N), 1513(Ar C=C), 799(Al C- H), 753(C-Cl). **^{1}H-NMR (CDCl3 - D) δppm** : 2.9(t,4H,N-CH_2), 3.5(t,4H,CH_2-Cl), 5.2(s,2H,N-CH_2-N), 7.8(s,1H,Ar), 8.5(s,1H,Ar-H). **Mass Spectrum (EI-MS) : (** M+1) Peak was observed at 353 m/z and (M+4) Peak was observed at 361 m/z

1-((bis(2- chloroethyl)amino)methyl)-2,3- dioxoindoline-7-carboxylic acid (VIp): IR (KBr) cm^{-1}: 2998(CH-Ar), 1682(NH-C=0), 1607(C=N), 1344(C-N), 1504(Ar C=C), 791(Al C- H), 745(C-Cl). **^{1}H-NMR (CDCl3 - D) δppm** : 2.9(t,2H,CH_2-Cl), 3.5(t,2H,N-CH_2), 5.2(s,2H)N-CH_2-N), 7.96(d,1H,Ar), 8.2(s,1H)Ar, 8.76(d,1H,Ar-H), 12.4(s,1H, Acid H). **Mass Spectrum (EI-MS) : (** M+1) Peak was observed at 345 m/z and (M+4) Peak was observed at 349 m/z

Pharmacological Evaluation:

***In-Vitro* Antioxidant Activity:** The synthesized 1-((bis(2-chloroethyl)amino)methyl)indoline-2,3-dione derivatives were evaluated for antioxidant activity by using Ascorbic acid as the standard drug by performing DPPH (2,2-diphenyl -1-picryl-hydrazyl-hydrate) radical scavenging assay method. DPPH in ethanol shows a strong absorption band at 517 nm and the solution appears to be deep violet in color[15].As the DPPH radical is scavenged by the donated hydrogen from the antioxidant, the absorbance is diminished according to the stoichiometry. 0.5 mL of DPPH solution (0.2 mM) was mixed with 0.1 mL of various concentrations (12.5µM, 25µM, 50µM, 100µM, 200µM, 400µM) of test compounds and 1.5 mL ethanol was added. The mixture was kept at room temperature for 30 min under dark condition, and then the absorbance was read at 517 nm against blank[16]. The % reduction of free radical concentration with different concentration of test compounds was calculated and was compared with standard, ascorbic acid. The reduction in absorbance is calculated as percentage inhibition.

$$\text{DPPH Scavenged (\%)} = \frac{\text{Absorbance of Blank} - \text{Absorbance of Test}}{\text{Absorbance of Blank}} \times 100$$

Table 2: In vitro antioxidant activity data of synthesized 1-((bis(2-chloroethyl) amino)methyl)indoline-2,3-dione derivatives

S.No	Compound No	-R Substitution	IC50 Values
1.	VI-a	H	9.454
2.	VI-b	5-CH_3	10.111
3.	VI-c	7-CH_3	14.118
4.	VI-d	5-Cl	16.125

5.	VI-e	7-Cl	13.145
6.	VI-f	5-NO_2	17.565
7.	VI-g	7-NO_2	10.887
8.	VI-h	5-Br	11.327
9.	VI-i	7-Br	19.482
10.	VI-j	5-F	9.329
11.	VI-k	7-F	13.327
12.	VI-l	5-$COOC_2H_5$	15.349
13.	VI-m	7-$COOCH_3$	13.007
14.	VI-n	6-Cl 5-F	11.384
15.	VI-o	5-Cl 7F	14.485
16.	VI-p	5-COOH	16.387
17.	Ascorbic acid		05.871

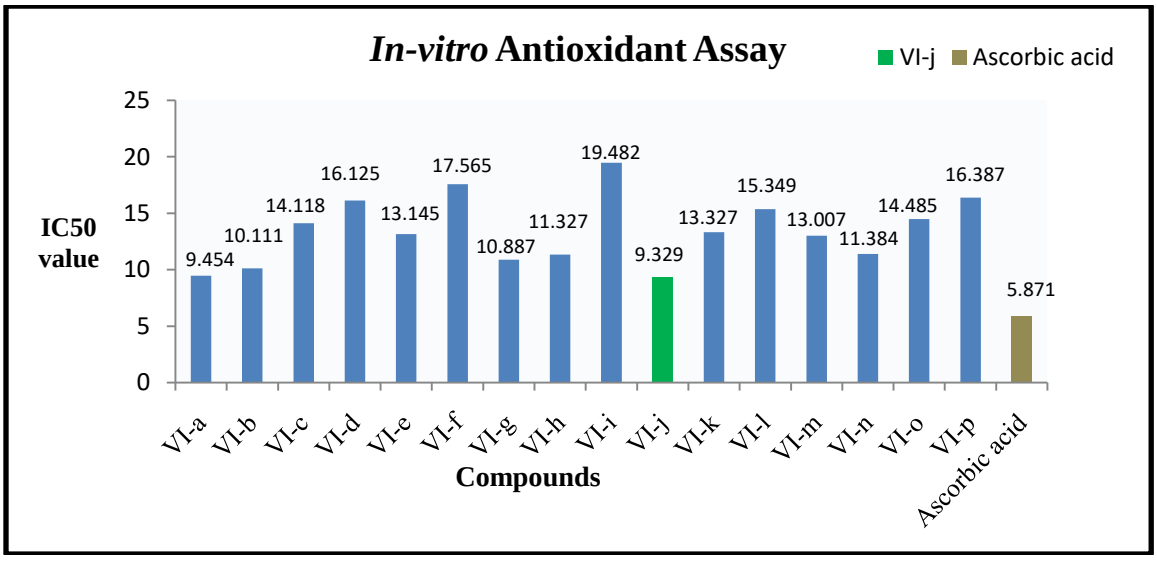

Figure 2: *In-vitro* Antioxidant activity of synthesized derivatives of substituted of 1-((Bis(2-chloroethyl)amino)methyl)indoline-2,3-diones (VIa-VIp)

Molecular docking study:

The docking studies of all the derivatives **VI(a-p)** were performed using molecular modeling software Autodock 4.2 (The Scripps Research Institute, CA, USA) installed on a single machine running on a 3.4-GHz Pentium processor with Windows 11 as the operating system. CANCER main protease enzyme (pdb code 5OTF was taken from the RCSB,used as target protein . Targetprotein pdb was further refined by removal of water molecules and by adding polar hydrogens andKollman charges. For the docking, a grid spacing of 0.450 Å and 120 × 120 × 120 number ofpoints were used. The grid was centered on the active site. The auto grid program generatedseparate grid maps for all atom types of the ligand structures and one for electrostatic interactions.Discovery studio 2021 was used to generate the energy minimized conformations of the ligands inpdb format. Energy minimized conformation of ligands was subjected to calculation of Gasteiger Huckel charges and saved in default format of Autodock. Autodock generated 10 possible bindingconformations, i.e., 10 runs for each docking by using LGA search.

Table 3: Lipinski's Rule of Five of synthesized compounds (VIa-VIp)

S.No.	Compound No	Molecular Weight (g/mol) <500	Lipophilicity (CLogP) <5	HBD <5	HBA <10	Rule Violations <2	Lipinski's Rule
1.	VIa	301	2.26	2	3	0	Yes

2.	VIb	315.19	1.79	1	3	0	Yes
3.	VIc	315.19	3.18	1	2	0	Yes
4.	VId	335.61	2.53	1	3	0	Yes
5.	VIe	335.61	2.46	1	4	0	Yes
6.	VIf	346.16	2.50	1	2	0	Yes
7.	VIg	346.16	1.68	1	4	0	Yes
8.	VIh	380.06	3.21	1	2	0	Yes
9.	VIi	380	2.21	2	5	0	Yes
10.	VIj	319.16	1.45	1	3	0	Yes
11.	VIk	319.16	2.01	1	2	0	Yes
12.	VIl	373.23	1.44	2	4	0	Yes
13.	VIm	359.20	1.77	1	3	0	Yes
14.	VIn	353.60	2.44	1	1	0	Yes
15.	VIo	353.60	1.22	1	5	0	Yes
16.	VIp	345.18	1.05	1	3	0	Yes

Lipinski rule of five parameters like molecular weight, number of hydrogen bond acceptors (HBA), number of hydrogen bond donors (HBD), was studied using online servers Molinspiration (Molinspiration Cheminformatics,Nova Ulica, Slovak Republic) and OSIRIS(Organic Chemistry, Switzerland) property calculator. All the calculated values were given in Table:2.

Table 4: Binding free energy and predicted inhibitory constant values of the synthesizedcompounds (VIa-VIp)

Compound No	Confirmation No.	Binding free energy(Kcal/mol)	Inhibitory constant (μM)	Best binding pose	No. of Hydrogen bonds	Rank (based on binding energy)
7-F	3	-6.7	0.750	GLY1432.23A^o	2	5
5-CH3	7	-6.7	1.110	THR262.024^o	1	5
5-COOC2H5	4	-6.6	0.552	GLY1432.23A^o	0	6
5-F	3	-7	1.490	GLU1661.73A^o	1	2
5-NO2	6	-7	2.540	GLU1662.08A^o	3	2
5-Br	4	-5.9	5.910	GLY1434.23A^o	1	9
5-Cl	4	-6.4	2.502	LYS123.14A^o	0	7
5-CL-7-F	6	-6.8	7.110	GLY1438.93A^o	0	4
6-CL-5-F	7	-6.8	5.470	GLY1438.99A^o	1	4
6-Br	8	-6	3.421	ASN1513.96A^o	2	8
7-COOCH3	4	-7.3	5.888	THR262.024A^o	3	1
7-Br	3	-6.9	4.222	GLY1432.28A^o	1	3
7-COOH	1	-7.3	7.548	GLU1668.78A^o	1	1
7-Cl	2	-6.7	9.547	GLY1435.99A^o	1	5
7-CH3	4	-6.5	7.528	ASN1519.98A^o	0	7
7-NO2	6	-6.7	9.456	THR263.424A^o	1	5
-H	3	-5.6	3.125	THR261.924A^o	2	10

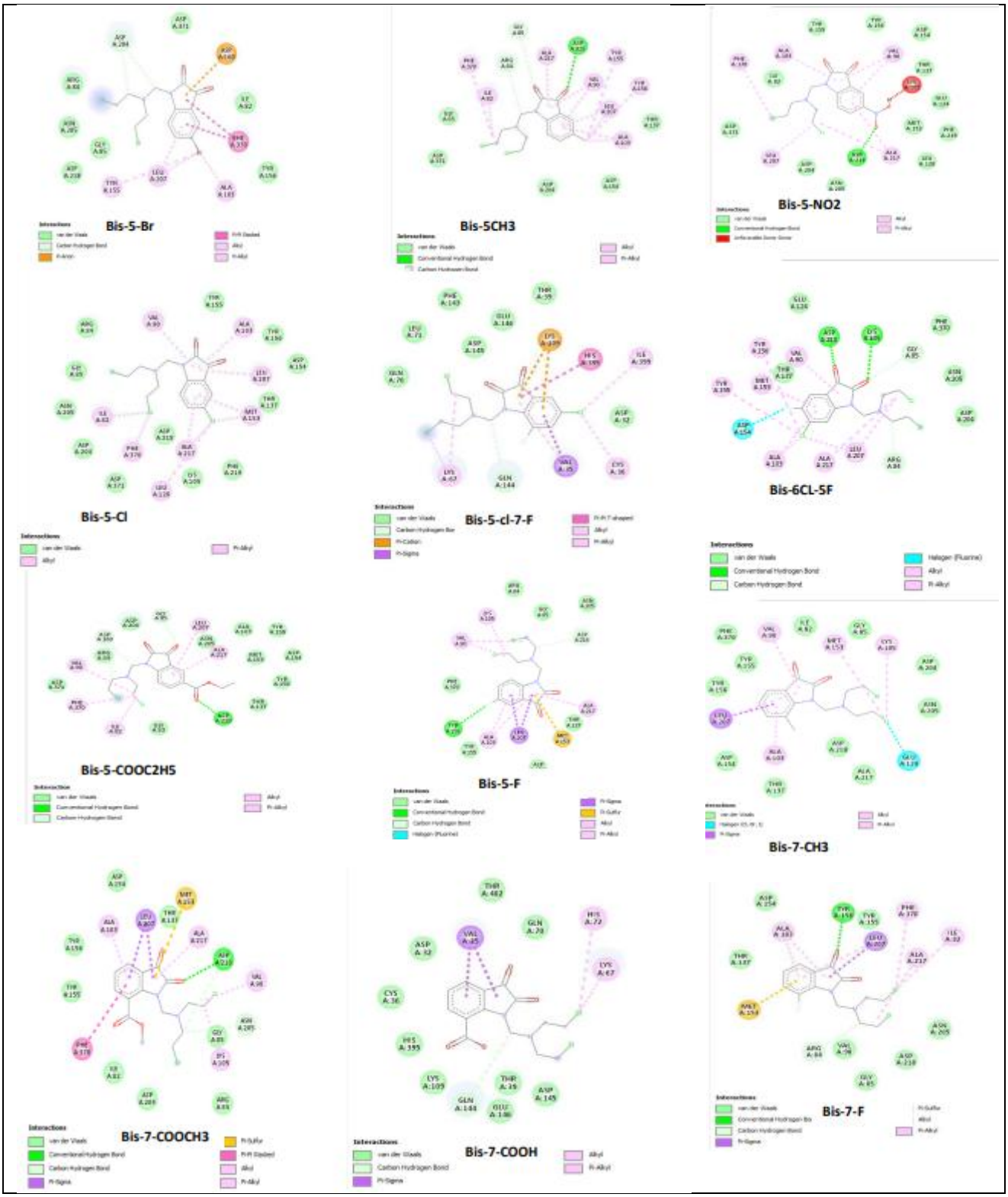

Figure 3: 2D interaction of protein (5OTF) and ligands

RESULTS AND DISCUSSION

A new series of isatin derivatives 1-((bis(2-chloroethyl)amino)methyl)indoline-2,3-dione derivatives were synthesized and Physical data of all the synthesized compounds are shown in Table 1.

***Invitro* Antioxidant activity-** Compounds of have been evaluated for antioxidant activity by DPPH method. The IC_{50} values of the test compounds are presented in Table 2, Figure-2. Compounds (VIa-p) showed antioxidant activity in the range of 9.32 to 19.48 µM. Compound **VIj(R=5-F)** showed comparatively more antioxidant activity with IC_{50} value of **9.32 µM** when comparable to IC_{50} value of ascorbic acid IC_{50} 5.8 µM.

Molecular docking study- All the synthesized compounds showed satisfactory results and among the all compounds, **(VIp)7-COOH** & **(VIm)7COOCH3** derivative compounds on Cancer main protease (PDB:5OTF) proved to possess highest binding energy of **-7.3 k.cal/mol.**

CONCLUSION

All the desirable compounds are synthesized, characterized by IR, Mass, NMR spectroscopy and antioxidant activity and insilico studies were performed. Compounds (VIa-p) showed antioxidant activity in the range of 9.32 to 19.48 µM. Compound VIj(R=5-F) showed comparatively more antioxidant activity with IC_{50} value of 9.32 µM when comparable to IC_{50} value of ascorbic acid IC_{50} 5.8 µM. In insilico studies all the synthesized compounds had showed satisfactory results and among all the compounds, VIp (7-COOH) & VIm (7COOCH3) derivative compounds on Cancer main protease (PDB:5OTF) proved to possess highest binding energy of -7.3 k.cal/mol.

ACKNOWLEGDEMENT

The authors thank the Department of Pharmaceutical Chemistry University College of Pharmaceutical Sciences, Kakatiya University, Warangal, for providing laboratories and chemicals to complete our project work and for its continuous support.

REFERENCES

[1]. G. Sammaiah et all: Anti -inflammatory and Antinociceptive effects of the alcoholic extract of Indian *Polygala* in experimental animals. East & central African Journal Pharmaceutical Sciences 2007; 10: 39-44.

[2]. Faruk Alam et all: Synthesis, characterization and biological evaluation some novel schiff base and mannich base of isatin and its derivatives with benzimidazole. Innovations in Pharmaceuticals and Pharmacotherapy. 2016; 4 (1), 177-187.

[3]. M. Sarangapani et all: Synthesis and antimicrobial activity of 1-(N,N- disubstitutedamino)methyl-3-imino-(2-phenyl-3,4-dihydro-4-oxo-quinazolin-3-yl)indol- 2-ones. Indian J. Het. Chem. 1994; 3: 257.

[4]. B.Shoba Rani et all: Synthesis and characterization of new isatin derivatives for cytotoxic activity. International Journal of Pharmacy and Biological Sciences 2015; 5(2): 163-166.

[5]. S.K. Sridhar, S.N. Pandeya, S.K. Bajpai, H. Manjula, "Evalution of Antibacterial and Antiviral Activities of Isatin Derivatives. Indian Drugs 1999; 36(6): 412-414

[6]. B.Shoba Rani et all: Synthesis and characterization of cytotoxic activity of certain N-(6-Benzo[d] imidazol-2-yl)-2- (2-oxoindolin -3- ylidene)hydrazide carboxamides. International Journal of Pharmacy and Biological Sciences 2014; 4(4): 29-34.

[7]. Wadhwa P: Molecular docking study of novel covid-19 protease with current clinical management agents. Asian Journal of Pharmaceutical and Clinical Research 2020; 13(9): 37-39.

[8]. Somashekhar M et all: *In-vivo* Anticonvulsant Activity of Novel Derivatives ff 1,3,4-Oxadiazole. Research and Reviews in Computational Chemistry 2018; 1: 10-19.

[9]. K. Mogilaiah et all: Synthesis and antimicrobial evaluation of 3-[3-(p- methoxyphenyl)-1,8-naphthyridine-2-yl hydrazono]-2-indolinone derivatives. Indian J.Het. Chem. 2002, 11: 283.

[10]. K. Karthik et all: Synthesis and evaluation ofnew bis-isatin derivatives for cytotoxic activity. World Journal of Pharmaceutical Research 2014; 3(2): 2429-2433.

[11]. Yengala Ram mohan et all: Synthesis, characterization and evaluation of cytotoxic and antioxidant properties of 4-(2,5- dimethyl-1H-pyrrol-1-ylbenzohydrazide Schiff bases of isatin derivatives. Inventi Rapid: Med Chem. 2016; (2): 49-54.

[12]. Maharaj Pogula et all: Synthesis and characterization of new isatin derivatives for anti -inflammatory activities. International Journal of Pharmacy and Pharmaceutical Sciences 2012; 4(3):155-158

[13]. Olcay Bekircan et all: Synthesis of Schiff and Mannich Bases of Isatin Derivatives with 4-Amino-4,5-Dihydro-

1H-1,2,4-Triazole-5-Ones. Molecules 2008; 13.

[14]. Renukadevi et all: Synthesis and antimicrobial activity of 3-(5'-substituted-3'- phenylindol-2'-ylcarbohydrazido)-2-indolinones and 1-substituted aminomethyl-3-(5'- substituted-3'-phenylindol-2' -ylcarbohydrazido)-2-indolinones. Indian Journal Heterocyclic Chemistry 1999; 9(2): 113-118.

[15]. S. Srivastava: Constituents and cytotoxicity of *Polygala erioptera*. International Journalof Chemical Sciences 2008; 6(2): 643-646.

[16]. S.N. Pandeya et all: Synthesis and antimicrobial activity of Schiff and Mannich bases of isatin and its derivatives with pyrimidine. Il Farmaco 1999; 54(9): 624-628.