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Development of New (N'^{1}, N'^{4})-N'^{1}, N'^{4}-Bis (2-Oxoindolin-3-Ylidene) Succinohydrazides For Anticancer Activity

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Abstract
In the present study a series of fifteen new (N'^{1}, N'^{4})-N'^{1}, N'^{4}-bis(2-oxoindolin-3-ylidene) succinohydrazide derivatives **(IV)** were synthesized by condensation of different indole-2,3-diones (**I**) with succinic acid hydrazide **(III)** in methanol for 12 hrs the compounds were purified by various chromatographic techniques and characterized by spectral data (IR, ^{1}H NMR and Mass Spectral analysis). The compounds were screened for DNA binding studies by HPLC method and cytotoxic studies by Tryphan blue dye exclusion method against A549(Lung cancer) cell lines. All the compounds exhibited DNA binding activity and cytotoxic activity against the cell lines employed. Compounds with chloro and fluoro substitutions on indole group exhibited good DNA binding (52 percent) and cytotoxicity with IC_{50} value 9.3uM against the cell lines.

Keywords
Indole-2,3-dione derivatives, succinic acid hydrazide, DNA binding and cytoxic activity

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INTRODUCTION:
Isatin or 1H-indole-2,3-dione is an indole derivative, the compound was first obtained by Erdman and Laurent in 1841 as a product from the oxidation of indigo dye by nitric acid and chromic acids. The compound is found in many plants. In recent years, indole derivatives have acquired conspicuous significance due to their wide spectrum of biological activities. Isatin is an endogenous compound present in mammalian tissues, including the brain and body fluids. The highest concentration was found in the hippocampus. Administration of isatin to mammals causes a number of behavioral reactions. In vitro isatin is a potent inhibitor of monoamine oxidase B and of atrial natriuretic peptide (ANP) receptor binding. It is a potent inhibitor of both atrial natriuretic peptide (ANP)-stimulated membrane bound guanylate cyclase) and nitric oxide-stimulated soluble guanyl cyclase. The distribution of isatin-specific binding is highest in the cortex, followed by the cerebellum, hypothalamus, hippocampus, brain stem, thalamus and striatum. It is evident from literature, that isatin derivatives are known to be associated with broad spectrum of biological activity like antibacterial, anticonvulsant, analgesic, antiviral, antifungal, antitubercular and antidepressant. Isatin hydrazones have been reported to possess anti-inflammatory activity also. In recent years, there has been a growing interest in the pharmacology of indole derivatives, we have synthesized and reported series of novel (N'^{1}, N'^{4})-N'^{1}, N'^{4}-bis(2-oxoindolin-3-ylidene) succinohydrazide derivatives **(IVa-IVo)** as the targeted compounds as per the scheme and evaluated for their DNA binding and cytotoxic activity.

MATERIALS AND METHODS:

All the reagents and solvents used were of laboratory grade. The melting point of synthesized compounds were determined by open capillary method and were uncorrected. The purity of the compounds was checked by thin layer chromatography (TLC). IR spectra were obtained on a Fourier transform Infrared (FTIR) Perkin Elmer (Spectrum RXI) spectrometer (μ in cm^{-1}) using KBr discs. 1H-NMR spectra were recorded in MeOD with tetramethylsilane (TMS) as the internal standard at 500MHz on a Bruker DMX-500MHz spectrometer. The chemical shifts are reported in parts per million.

A. Isonitrosoacetanilides:

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 (1300 gm) followed by a solution of an appropriate Aniline (I) in 300 ml of water and concentrated Hydrochloric acid (0.52 mol). 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-guage by a mecker burner so that vigorous boiling began in about 45 minutes. After 1 to 2 minutes of vigorous boiling the reaction was completed. During the heating period itself the crystals of isonitrosoacetanilides 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(s).

B. Indole-2, 3-diones (I):

Sulphuric acid (600 g, d, 1.84, 326 ml) was warmed at 50^0C in a one-liter R.B. 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°C to 70°C but not higher. External cooling was applied at this stage so that the reaction could be carried out more rapidly. After the addition of isonitroso compound was completed the temperature of the solution was raised to 80°C and maintained at that temperature for 10 minutes to complete the reaction. Then the reaction mixture was cooled to room temperature and poured on to 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. Purification of the compound was affected by the recrystallization from methanol.

C Synthesis of succinic acid hydrazide (III):

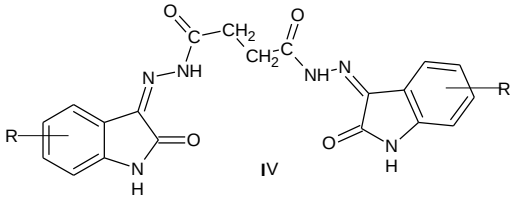
Diethylsuccinate (II, 0.1 mole) in alcohol was refluxed with hydrazine hydrate (99.9%, 0.02 mole) for 15 minutes. The resulting compound was cooled, and the solvent was removed by distillation. The product thus obtained was recrystallized from ethanol. m.p. 155-157 °C.

D. (N'[1], N'[4])-N'[1], N'[4]-bis(2-oxoindolin-3-ylidene) succinohydrazide (IV):

A mixture of an appropriate indole 2, 3-dione (I,0.02 mol) and Diethylsuccinic acid hydrazide (III, 0.01 mol) in methanol (50 ml) was refluxed for 12 hours. The solvent was removed by distillation and resulting white solid was dried and recrystallized from methanol, purified by column chromatography.

Physical Data of all newly synthesized compounds (N'[1], N'[4])-N'[1], N'[4]-bis(2-oxoindolin-3-ylidene) succinohydrazide derivatives were given in Table-1.

Table: I Physical data of (N'[1], N'[4])-N'[1], N'[4]-bis(2-oxoindolin-3-ylidene) succinohydrazides. (IVa-IVo)



IV

S.No.	Comp.d	Substituent R	Mol formula	M.P. (°C)	Yield (%)	Mol.wt.
1	IV a	H	$C_{20}H_{16}N_6O_4$	275-276	60	404
2	IVb	5-CH_3	$C_{22}H_{20}N_6O_4$	279-280	70	432
3	IVc	5-Cl	$C_{20}H_{14}Cl_2N_6O_4$	290	55	473
4	IVd	5-Br	$C_{20}H_{14}Br_2N_6O_4$	308	50	562
5	IVe	5-F	$C_{20}H_{14}F_2N_6O_4$	290	50	440
6	IVf	5-COOH	$C_{22}H_{12}N_6O_4$	305-308	60	492
7	IVg	5-NO_2	$C_{20}H_{14}N_8O_8$	310	40	494
8	IVh	5-$COOCH_3$	$C_{24}H_{20}N_6O_8$	270-272	40	520
9	IVi	6-Br	$C_{20}H_{14}Br_2N_6O_4$	265-268	40	562
10	IVj	4-Cl, 5-F	$C_{20}H_{12}F_2Cl_2N_6O_4$	280-282	45	509

11	IVk	7-CH_3	$C_{22}H_{20}N_6O_4$	285-286	50	432
12	IVl	7-NO_2	$C_{20}H_{14}N_8O_8$	295-297	50	494
13	IVm	7-COOH	$C_{22}H_{12}N_6O_4$	300-302	50	492
14	IVn	7-Cl	$C_{20}H_{14}Cl_2N_6O_4$	290	50	473
15	IVo	7-$COOCH_3$	$C_{24}H_{20}N_6O_{84}$	265	50	520

Spectral Data of Newly synthsised compounds (IVa-IVo)

IVa: (N'[1], N'[4])-N'[1], N'[4]-bis(2-oxoindolin-3-ylidene) succinohydrazide:

IR(KBr) cm^{-1}: 3364(NH), 3152 (NH), 2976 (C-H), 1765 (C=O), 1683(C=O), 1615(C=O), 1442(C=N), 1389(C=N).

^{1}HNMR (δ): 10.5(s, 2H,NH), 10.0(s, 2H,NH), 7.8(d, 2H, ArH), 7.7(d,2H, ArH), 7.5(t, 2H, ArH), 7.2(t, 2H, ArH), 2.5(s, 4H, 2(CH_2)).

IVb: (N'[1],N'[4])-N'[1],N'[4]-bis(5-methyl-2-oxoindolin-3-ylidene)succinohydrazide:

IR(KBr) cm^{-1}: 3362(NH), 3142 (NH), 2976 (C-H), 1764 (C=O), 1685(C=O), 1616(C=O), 1444(C=N), 1392(C=N).

^{1}HNMR (δ): 10.6(s, 2H,NH), 10.0(s, 2H,NH), 8.2(s, 2H, ArH), 7.1(d, 2H, ArH), 6.6(d, 2H, ArH), 2.5(s, 4H, 2(CH_2)), 2.3(s, 4H, 2(CH_3)).

IVc: (N'[1],N'[4])-N'[1],N'[4]-bis(5-chloro-2-oxoindolin-3-ylidene)succinohydrazide:

IR(KBr) cm^{-1}: 3363(NH), 3143 (NH), 2973 (C-H), 1763 (C=O), 1683(C=O), 1613(C=O), 1443(C=N), 1393(C=N).

^{1}HNMR (δ): 10.6(s, 2H,NH), 10.1(s, 2H,NH), 7.9(s, 2H, ArH), 7.8(d, 2H, ArH), 7.5(d, 2H, ArH), 2.4(s, 4H, 2(CH_2)).

IVd: (N'[1],N'[4])-N'[1],N'[4]-bis(5-bromo-2-oxoindolin-3-ylidene)succinohydrazide:

IR(KBr) cm^{-1}: 3363(NH), 3143 (NH), 2973 (C-H), 1763 (C=O), 1683(C=O), 1613(C=O), 1443(C=N), 1393(C=N).

^{1}HNMR (δ): 10.6(s, 2H,NH), 10.1(s, 2H,NH), 8.1(s, 2H, ArH), 7.7 (d, 2H, ArH), 7.4(d, 2H, ArH), 2.4(s, 4H, 2(CH_2)).

IVe: (N'[1],N'[4])-N'[1],N'[4]-bis(5-fluoro-2-oxoindolin-3-ylidene)succinohydrazide:

IR(KBr) cm^{-1}: 3353(NH), 3133 (NH), 2963 (C-H), 1753 (C=O), 1693(C=O), 1623(C=O), 1453(C=N), 1383(C=N).

^{1}HNMR (δ): 10.6(s, 2H,NH), 10.0(s, 2H,NH), 7.8 (d, 2H, ArH), 7.7(s, 2H, ArH), 7.3(d, 2H, ArH), 2.5(s, 4H, 2(CH_2)).

IVf:(N'[1],N'[4])-N'[1],N'[4]-bis(5-carboxy-2-oxoindolin-3-ylidene)succinohydrazide:

IR(KBr) cm^{-1}: 3361 (NH), 3142 (NH), 2973 (C-H), 1764 (C=O), 1683(C=O), 1634(C=O), 1464(C=N), 1373(C=N).

^{1}HNMR (δ): 12.7 (s,2H, OH), 10.6(s, 2H,NH), 10.0(s, 2H,NH), 8.5 (s, 2H, ArH), 8.3(d, 2H, ArH), 8.0(d, 2H, ArH), 2.6(s, 4H, 2(CH_2)).

IVg:(N'[1], N'[4])-N'[1], N'[4]-bis(5-nitro-2-oxoindolin-3-ylidene)succinohydrazide:

IR(KBr) cm^{-1}: 3363 (NH), 3144 (NH), 2975 (C-H), 1766 (C=O), 1685(C=O), 1635(C=O), 1466(C=N), 1375(C=N).

^{1}HNMR (δ): 10.6(s, 2H,NH), 10.0(s, 2H,NH), 8.5 (s, 2H, ArH), 8.1(d, 4H, ArH), 2.6(s, 4H, 2(CH_2)).

IVh:(N'[1],N'[4])-N'[1],N'[4]-bis(5-carbomethoxy-2-oxoindolin-3-ylidene)succinohydrazide:

IR(KBr) cm^{-1}: 3363 (NH), 3144 (NH), 2975 (C-H), 1766 (C=O), 1685(C=O), 1635(C=O), 1466(C=N), 1375(C=N).

^{1}HNMR (δ): 10.6(s, 2H,NH), 10.0(s, 2H,NH), 8.5 (s, 2H, ArH), 8.1(d, 2H,ArH), 7.9(d, 4H, ArH), 3.9(s, 6H, 2($(CH_3)_2$), 2.6(s, 4H, 2(CH_2)).

IVk: (N'[1],N'[4])-N'[1],N'[4]-bis(7-methyl-2-oxoindolin-3-ylidene)succinohydrazide:

IR(KBr) cm^{-1}: 3365 (NH), 3147 (NH), 2980 (C-H), 1772 (C=O), 1692(C=O), 1643(C=O), 1475(C=N), 1385(C=N).

^{1}HNMR (δ): 11.6(s, 2H,NH), 10.6(s, 2H,NH), 7.6(d, 2H, ArH), 7.3(d, 2H,ArH), 7.1(t, 2H, ArH), 2.5(s, 4H, 2(CH_2)), 2.1(s, 6H, 2($(CH_3)_2$).

IVl: (N'[1],N'[4])-N'[1],N'[4]-bis(7-nitro-2-oxoindolin-3-ylidene)succinohydrazide:

IR(KBr) cm^{-1}: 3361 (NH), 3144 (NH), 2978 (C-H), 1771 (C=O), 1692(C=O), 1644(C=O), 1477(C=N), 1388(C=N).

^{1}HNMR (δ): 11.6(s, 2H,NH), 10.6(s, 2H,NH), 8.3(d, 2H, ArH), 8.2(d, 2H,ArH), 7.5(t, 2H, ArH), 2.5(s, 4H, 2(CH_2)).

IVm: (N'[1],N'[4])-N'[1],N'[4]-bis(7-carboxy-2-oxoindolin-3-ylidene)succinohydrazide:

IR(KBr) cm^{-1}: 3362 (NH), 3148 (NH), 2984 (C-H), 1781 (C=O), 1684(C=O), 1656(C=O), 1490(C=N), 1392(C=N).

^{1}HNMR (δ): 12.7(s, 2H, OH),11.6(s, 2H,NH), 10.6(s, 2H,NH), 8.3(d, 2H, ArH), 8.1(d, 2H,ArH), 7.4(t, 2H, ArH), 2.5(s, 4H, 2(CH_2)).

IVn: (N'[1],N'[4])-N'[1],N'[4]-bis(7-chloro-2-oxoindolin-3-ylidene)succinohydrazide:

IR(KBr) cm^{-1}: 3358 (NH), 3135 (NH), 2978 (C-H), 1774 (C=O), 1678(C=O), 1645(C=O), 1480(C=N), 1381(C=N).

^{1}HNMR (δ): 11.8(s, 2H,NH), 10.4(s, 2H,NH), 7.7(d, 2H, ArH), 7.5(d, 2H,ArH), 7.2(t, 2H, ArH), 2.3(s, 4H, 2(CH_2)).

IVo: (N'^{1},N'^{4})-N'^{1},N'^{4}-bis(7-carbomethoxy-2-oxoindolin-3-ylidene)succinohydrazide:

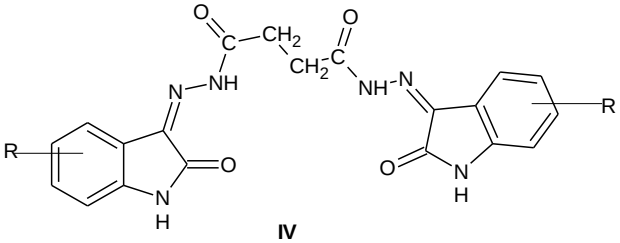
IR(KBr) cm^{-1}: 3364 (NH), 3139 (NH), 2989 (C-H), 1782 (C=O), 1685(C=O), 1652(C=O), 1485(C=N), 1385(C=N).

^{1}HNMR (δ): 11.6(s, 2H,NH), 10.5(s, 2H,NH), 8.2(d, 2H, ArH), 8.0(d, 2H,ArH), 7.4(t, 2H, ArH), 3.9 (s, 6H, 2(CH_3))2.4(s, 4H, 2(CH_2)).

CYTOTOXIC ACTIVITY AND DNA BINDING STUDIES:

Tryphan blue is one the several dyes apart from (Erytrosin-B {acid red 51}, nigrocin acid black 2) recommended for use in dye exclusion procedures for viable cell counting. It is based on principle that live (viable cells) actively pump out the dye whereas dead (non- vialbe) cell does not. A549 (lung cancer) cell lines were grown as adherent cells in DMEM media supplemented with 10% fetal bovine serum, 100 ug/ml pencillin, 200ug/ml streptomycin 2mM L-glutamine and the culture was maintained in a humified atmosphere with 5% CO_2 at 37^{0}C for 72 hrs. Drug solutions of the compounds are made in the medium with DMSO which served as control. Each well is added with medium. This method is particularly recommended for assay in suspension cultures. The results of the assay are shown in the Table II.

Table-II: Cytotoxicity of N'^{1}, *N*'^{4}-bis[(3*Z*)-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene] butanedihydrazides (IV) using Tryphan blue dye exclusion method.



IV

S.No.	Compound	Substituent	IC_{50} value (µM)
		R	
1	IVa	H	19.65
2	IVb	5-CH_3	17.00
3	IVc	5-Cl	12.98
4	IVd	5-Br	13.28
5	IVe	5-F	11.72
6	IVf	5-COOH	NA
7	IVg	5-NO_2	13.76
8	IVh	5-$COOCH_3$	NA
9	IVi	6-Br	14.32
10	IVj	4-Cl, 5-F	9.34
11	IVk	7-CH_3	18.65
12	IVl	7-NO_2	13.43
13	IVm	7-COOH	NA
14	IVn	7-Cl	10.34
15	IVo	7-$COOCH_3$	NA
16	Cisplatin)		6.63

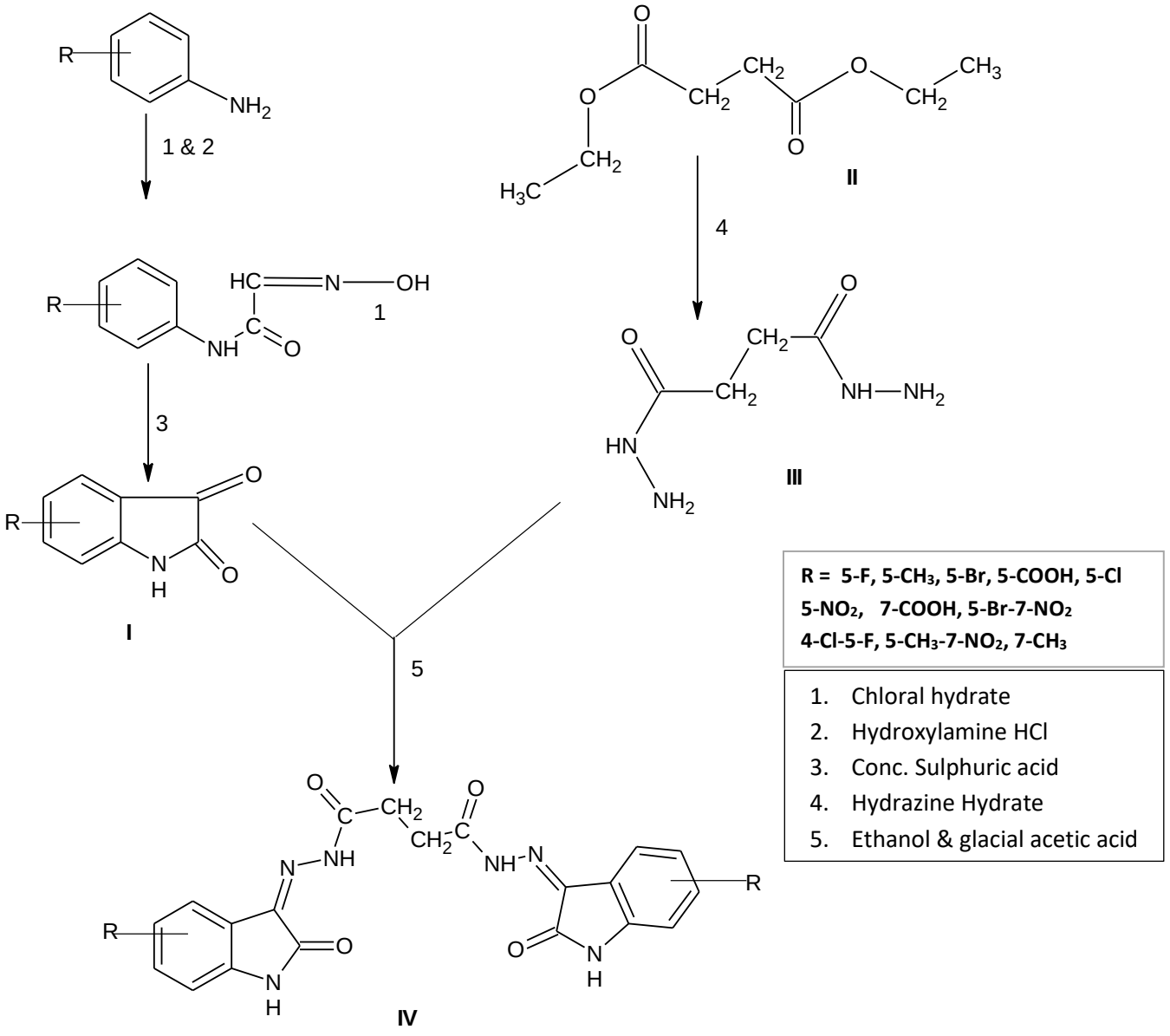
The DNA binding studies using Herring sperm DNA was done on HPLC which is based on the principle that the amount of drug which binds with DNA can be detected with the reduction in the peak area. Initially the binding studies are carried out with varying amount of drug and the percentage binding was calculated. Then the amount of drug that is bound at higher concentrations of DNA was calculated by carrying the study at varying concentrations of DNA. The results of are shown Table III.

Table III: DNA binding study of compounds N'[1], N'[4])-N'[1], N'[4]-bis(2-oxoindolin-3-ylidene) succinohydrazides (IV) by using Herring sperm DNA.

S. No.	Compound	Substituent R	%binding
1	IV b	5-CH_3	48.56
2	IV g	5-NO_2	52.56
3	IVi	6-Br	43.45
4	IVn	7-Cl	43.28
5	Cisplatin		80.90

RESULTS AND DISCUSSION.

The DNA binding studies of **N'[1], N'[4])-N'[1], N'[4]-bis(2-oxoindolin-3-ylidene) succinohydrazides** (IV) by herring sperm DNA is presented in the table II. The compounds IVg showed good binding activity with 52 percent followed by compounds IVb, IVi and IVn and the results were evaluated by taking Cisplatin as the standard drug.The cytotoxic activity of eleven compounds out of fifteen exhibited good activity. The compounds IVj, showed an IC_{50} value of 9.3uM followed by IVn and IVe.



CONCLUSION:

The study reports the successful synthesis of the title compounds in good yields and moderate to good activities of these derivatives containing isatin moiety which is comparable with the standard drug. It is observed that the increased DNA binding and cytotoxic activity was attributed to the presence of pharmacologically active substituent succinohydrazide.

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