SYNTHESIS AND EVALUATION OF IN-VITRO ANTICANCER ACTIVITY OF (Z)-N'-(1-((BIS(2-CHLOROETHYL) AMINO) METHYL)-2-OXOINDOLIN-3-YLIDENE)-4-(2,5-DIMETHYL-1H-PYRROL-1-YL)BENZOHYDRAZIDE

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Abstract:
A novel synthetic methodology for preparation of (Z)-N'-(1-((bis(2-chloroethyl)amino)methyl) -2-oxoindolin-3-ylidene)-4-(2,5-dimethyl-1H-pyrrol-1-yl)benzohydrazide derivatives **VIII(a-j)** were prepared by Paal-knorr, Mannich base and Schiff's base reactions. The newly synthesized compounds structures was confirmed by FT-IR, ^{1}H-NMR, ^{13}C-NMR and MASS spectrometry. The percentage yield of the newly synthesized compounds ranged between 75-91%. All the compounds were evaluated for in vitro anticancer activity by MTT assay method against MCF-7, A549, HEPG2 cell lines and antioxidant activates by DPPH method. Compounds **VIII-b, VIII-h,** and **VIII-I** exhibited the highest activities against three cancer cell lines with IC_{50}ranging from **149.16µg/ml to 229.86µg/ml**(MCF-7), **95.31µg/ml** to **298.29µg/ml** (A549), and **115.8µg/ml** to **177.83µg/ml** (HepG2), while compound VIII-h was found to be most potent against A549 cell line. From the results of antioxidant study, compounds **VIII-c, VIII-e, VIII-f,** and **VIII-h** possess much higher radical scavenging activity in DPPH parameter compared to standard used.

Keywords: Ethyl 4-amino benzoate**,** Substituted Isatins, Antioxidant and Anticancer activities, DPPH, MTT assay, MCF-7, A549, HepG2 Cell lines.

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INTRODUCTION.

Due to the increase in cancer incidence, scientific research on cancer morbidity and mortality and improvement in the quality of life of cancer patients is increasing rapidly. Cancer is a serious health concern in all societies, regardless of wealth or social status. In 2022, 21.4 million people worldwide suffered from cancer and 10.4 million patients died from the disease. By 2040, these figures will almost double, and the largest increase will be noticed in low- and middle-income countries where more than two-thirds of world cancer cases occur. Over the last two decades, Schiff's and Mannich bases derivatives have been known as one of the most important groups in medicinal chemistry and they are present in a large number of compounds due to their diverse biological properties such as anti-inflammatory, antimicrobial, analgesic, anti-HIV, anticonvulsant, anti-leishmanial, antimalarial, antitumor, and anti-tubercular activities. Furthermore, Schiff's and Mannich bases are increasingly considered to be a valuable core in medicinal chemistry [1-2].

Isatin (1H-indole-2,3-dione) (1), an oxidized derivative of indole, was first discovered by Erdmann and Laurent in 1840 as a product arising from the oxidation of indigo (2) using nitric and chromic acids [3-4]. The compound was considered synthetic for almost 140 years until it was foundto be present in plants from the Isatis genus, in fruits of the cannon ball tree, Couroupita guianensis Aubl and in secretions from the parotid gland of the Bufo frog Various substituted isatins have also been identified in plants, fungi, symbiotic bacteria and marine molluscs, where they are postulated to play a defensive role against pathogenic organisms (Fig. (1).

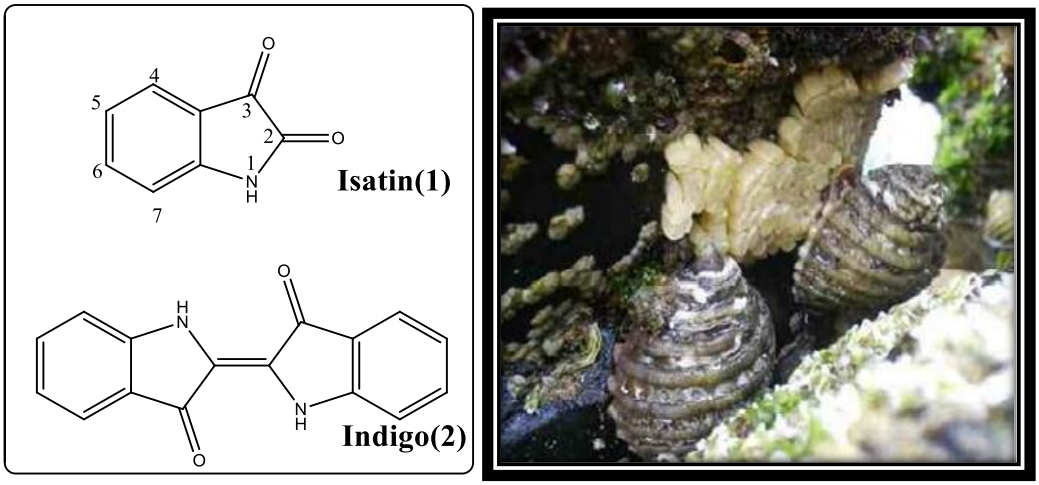

Fig. (1). The chemical structures of isatin (**1**) and indigo (**2**). The photograph shows adult Australian Muricid molluscs (*Dicathaisorbita*) amongst freshly laid egg capsules, a rich source of the cytotoxic isatin derivative

Isatin has a wide variety of biological and pharmacological activities such as antifungal and antibacterial properties and potent caspase inhibitory and anticancer activities [5-6].In humans and other mammals, isatin is found as an endogenous molecule. Although the metabolic pathways of isatin have not yet been fully elucidated, it has been proposed that it is synthesized in vivo from tryptophan-rich foods such as meat, dairy and whole grains. In this pathway, tryptophan is converted to indole by bacteria from the gastrointestinal tract and then transported to the liver where it is oxidized.Comprehensive literature survey carried out on Schiff bases are used as substrates in the preparation of a number of industrial and biologically active compounds via ring closure, cycloaddition and replacement reactions. Moreover, Schiff bases derived from various heterocycles have been reported to possess cytotoxic, antimicrobial, antiproliferative, anticancer, anticonvulsant and antifungal activities [7-10].

In an effort to discover new and effective cytotoxic agents of isatin derivatives for the treatment of cancer and antioxidant activities. The present investigation was undertaken to develop an efficient method for synthesizing isatin derivatives having pyrrole, Schiff's and Mannich baes moieties can be potent anticancer and antioxidant agents.

EXPERIMENTAL SECTION.

Materials and Methods

In this Investigation chemicals were purchased from local dealer with S.D fine and Sigma-Aldrich was used. The synthesized compounds were synthesized by conventional method screened for

anti-cancer and antioxidant activities. Melting points were determined in an open capillary .The IUPAC nomenclature of synthetic compounds VIII-(a-j) was given by using Chem draw Ultra-12 version. The IR spectra were recorded on Bruker FTIR-8400S. ^{1}H-NMR spectra were recorded in DMSO-$d_6$andchemicalshifts(δ)on Bruker DRX-300 MHz. spectrometer using TMS as internal reference with their values expressed in δ ppm. Purity of all the synthesized compounds were routinely checked by TLC on silica gel G in the solvent system [(chloroform: ethyl acetate (7:3)].

General Procedure:

Step-I: Synthesis of ethyl-4-(2,5-dimethyl-1H-pyrrol-1-yl) benzoate (II): In 250 ml round bottom flask take equimolar quantity of ethyl-4-aminobenzoate and hexane-2,5-dione with minimum amount of ethyl acetate and refluxed at 60ºC for 12hrs. The progress of the reaction was monitored by TLC. The reaction mixture was evaporated to get a brown coloured compound with good yield. The compound obtained was recrystallized by ethyl acetate [11].

Step-II: Synthesis of ethyl-4-(2,5-dimethyl-1H-pyrrol-1-yl) benzo hydrazide (III): To the compound II, hydrazine hydrate (99%) was added in 1:5 ratio and was refluxed for about 6 hours in methanol as a solvent. The solvent was evaporated and left for drying. Compound III was characterized by melting point, TLC. Melting point was determined in open capillary tubes on a Thomas Hoover melting point apparatus and was uncorrected [12].

Step-III: Synthesis of Isonitrosoacetanilides (V): Isonitrosoacetanilides are obtained from different substituted anilines. 5g/5ml of anilines were taken into a conical flask along with 30ml of water. To this 5ml of conc. hydrochloric acid was added. In another beaker 9ml of chloral hydrate was added. Further 12g of hydroxylamine hydrochloride was added along with 30ml of water. The mixture was stirred well and anhydrous sodium sulphate was added until a precipitate appeared. The contents of the beaker were then mixed with those in conical flask and heated on a water bath for about 45min and left aside for 24hours. After 24 hours, the mixture was filtered and washed with water until acid free and dried completely [13-15].

Step-IV: Synthesis of substituted Isatin derivatives (VI-(a-j): The isonitrosoacetanilides thus obtained in Step-III were further cyclized with Conc.H_2SO_4 which was heated earlier to about 80°C by adding the isonitrosoacetanilide in smaller portions until it dissolves completely and the mixture was left overnight. After 24 hours, crushed ice was added to the mixture and the product thus obtained was filtered and washed with water until the red litmus turned blue. All isatin derivatives were prepared similarly and dried completely. 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 [16].

Step-V: Synthesis of 1-{[bis(2-chloroethy) amino] methyl}-1H-indole-2,3-dione VII(a-j): The mannich condensation was done by the following procedure. A mixture of equimolar concentration of Isatin (0.010 moles; 1.47g), Formaldehyde (0.010 moles; 0.3g, 1 mL), Bis(2-chloroethyl) amine hydrochloride (0.010 moles; 1.42g) was refluxed in ethanol (50 mL) for 8hrs at 80º C. After filtering the filtrate was dried completely. The residue was recrystallized from ethyl acetate-petroleum ether giving pure compound [17].

Step-VI: Synthesis of (1-((bis(2-chloroethyl)amino)methyl)-2-oxoindolin-3-ylidene)-4-(2,5-dimethyl-1H-pyrrol-1-yl)benzohydrazideVIII-(a-j):1-{[bis(2-chloroethyl)amino]methyl}-1H-indole-2,3-dione (0.0005 moles;0.150g) was condensedd with d 4-(2,5-dimethyl-1H-pyrrol-1-yl)benzo hydrazide(0.0005 moles) in methanol(20mL) and trace amount of glacial acetic acid for about 8 hrs at 80ºC to get .the reaction was monitored by TLC. After completed of reaction the mixture was evaporated to get respective1-{[bis(2-chloroethyl) amino] methyl}-3-(phenylimino)1,3-dihydro-2H-indole-2-one. The compound was recrystallized with methanol solvent [18].

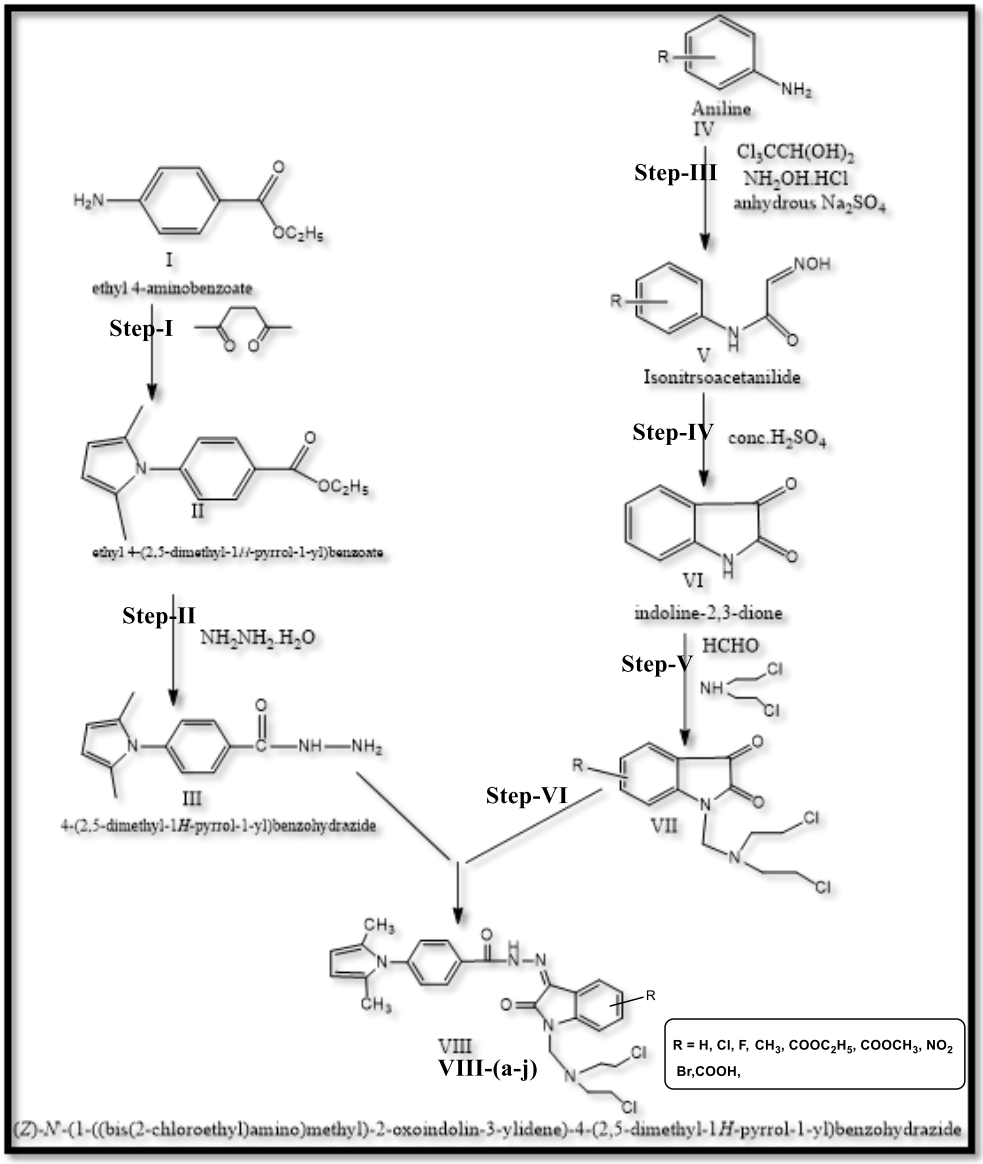

Figure 2. Scheme for the synthesis novel (1-((bis(2-chloroethyl)amino)methyl)-2-oxoindolin-3-ylidene)-4-(2,5-dimethyl-1H-pyrrol-1-yl)benzohydrazideVIII-(a-j)

Compound. VIII-a: (1-((bis(2-chloroethyl) amino) methyl)-2-oxoindolin-3-ylidene)-4-(2,5-dimethyl-1H-pyrrol-1-yl) benzo hydrazide. IR(KBr)cm^{-1}: 3340.99(-N-H*Str*, -CONH- group), 3058.56(-C-H*Str*, Aromatic),2976.58, 2925.71(-CH *Str* in Aliphatic group), 1742.84(-CO *Str* in Indole), 1660.28(-CO *Str* in Amide group), 1504.22(-C=C *Str* in Aromatic), 813.12, 777.26(-C-Cl Str in -CH_2-Cl). ^{1}H-NMR($CDCl_3$-D)δppm: 1.62(s, 6H, -CH_3 protons on Pyrrole ring), 3.00-2.97(t, 4H,protons in –CH_2-Cl),3.57-3.55(t, 4H,protons in –-N-CH_2),5.24(s, 2H, -N-CH_2 Mannich base protons), 5.64(d,2H, Ar-H in pyrrole), 7.42-7.35(t, 2H, Ar-H), 7.46-7.45(d, 2H, A-H),7.63-7.61(d, 2H, Ar-H), 7.97-7.95(t, 2H, Ar-H on indole ring), 8.25(s,1H,-NH proton in Amide).^{13}CNMR(CDCl3-D): 168.65, 161.15, 142.08, 132.54, 131.08, 129.35, 129.09, 128.56,128.14, 126.88, 125.27, 124.48,114.02, 57.20, 48.56, 42.48, 28.56, 22.48. Mass (EI-MS): m/z 512.30(M), 514.30(M + 2, 100%), 516.00(M + 4, 30%).

Compound. VIII-b: (1-((bis(2-chloroethyl) amino) methyl)-2-(5-chloro)oxoindolin-3-ylidene)-4-(2,5-dimethyl-1H-pyrrol-1-yl) benzo hydrazide. IR(KBr)cm^{-1}: 3389.33(-N-H*Str*, -CONH- group), 3071.65(-C-H*Str*, Aromatic), 2998.33, 2966.12, 2809.33(-CH *Str* in Aliphatic group), 1719.02(-CO *Str* in Indole), 1690.32(-CO *Str* in Amide group), 1528.32(-C=C *Str* in Aromatic), 823.43(-C-Cl *Str*, Ar-Cl), 812.12, 787.33(-C-Cl Str in -CH_2-Cl). ^{1}H-NMR($CDCl_3$-D)δppm: 1.98(s, 6H, -CH_3 protons on Pyrrole ring), 3.32-2.87(t, 4H,protons in –CH_2-Cl),3.86-3.45(t, 4H,protons in -N-CH_2),5.76(s, 2H, -N-

CH_2Mannich base protons), 5.98(d,2H, Ar-H in pyrrole), 7.35(s, 1H, Ar-H), 7.65-7.87(d, 2H, A-H),7.98-7.86(d, 2H, Ar-H), 8.03(d, 2H, Ar-H on indole ring), 9.36(s,1H,-NH proton in Amide).^{13}CNMR(CDCl3-D): 170.32, 167.67, 165.32, 158.21, 154.23, 148.33, 140.21, 138.02, 137.33, 132.12, 128.09, 128.22, 126.43, 124.87, 123.23, 119.32, 116.87, 115.32, 110.32, 47.02, 45.12, 29.21, 26.32. Mass (EI-MS): m/z 545.12(M), 547.03(M + 2, 100%), 549.01(M + 4, 30%).

Compound. VIII-c: (1-((bis(2-chloroethyl) amino) methyl)-2-(5-fluoro)oxoindolin-3-ylidene) -4-(2,5-dimethyl-1H-pyrrol-1-yl)benzo hydrazide.IR(KBr)cm^{-1}: 3365.32(-N-H*Str*, -CONH- group), 3098.09(-C-H*Str*, Aromatic), 2987.32, 2978.32, 2812.22(-CH *Str* in Aliphatic group), 1721.32(-CO *Str* in Indole), 1699.02(-CO *Str* in Amide group), 1523.76(-C=C *Str* in Aromatic), 817.32(-C-F*Str*, Ar-F), 812.12, 788.32(-C-Cl Str in -CH_2-Cl). ^{1}H-NMR($CDCl_3$-D)δppm: 2.03(s, 6H, -CH_3 protons on Pyrrole ring), 3.43-2.98(t, 4H,protons in –CH_2-Cl),3.78-3.62(t, 4H,protons in -N-CH_2),5.81(s, 2H, -N-CH_2 Mannich base protons), 5.96(d,2H, Ar-H in pyrrole), 7.26(s, 1H, Ar-H), 7.48-7.56(d, 2H, A-H),7.68-7.62(d, 2H, Ar-H), 8.21(d, 2H, Ar-H on indole ring), 9.03(s,1H,-NH proton in Amide).^{13}CNMR(CDCl3-D): 174.12, 166.03, 164.09, 156.43, 157.12, 147.34, 145.92, 142.13, 140.17, 135.37, 131.09, 129.65, 127.33, 125.21, 122.98, 118.42,115.32, 114.87, 112.43, 48.41, 46.23, 27.65, 24.61. Mass (EI-MS): m/z 529.14(M), 531.21(M + 2, 100%), 533.09(M + 4, 30%).

Compound. VIII-d: (1-((bis(2-chloroethyl) amino) methyl)-2-(5-bromo)oxoindolin-3-ylidene)-4-(2, 5-dimethyl-1H-pyrrol-1-yl)benzo hydrazide.IR(KBr)cm^{-1}: 3383.15(-N-H*Str*, -CONH- group), 3087.43(-C-H*Str*, Aromatic), 2987.34, 2954.04(-CH *Str* in Aliphatic group), 1721(-CO *Str* in Indole), 1684.87(-CO *Str* in Amide group), 1546.50(-C=C *Str* in Aromatic), 817.86, 772.71(-C-Cl Str in -CH_2-Cl). ^{1}H-NMR($CDCl_3$-D)δppm: 1.56(s, 6H, -CH_3 protons on Pyrrole ring), 3.00-2.97(t, 4H,protons in –CH_2-Cl),3.57-3.55(t, 4H,protons in -N-CH_2),5.24(s, 2H, -N-CH_2 Mannich base protons), 5.64(d,2H, Ar-H in pyrrole), 7.46(s, 1H, Ar-H), 7.48-7.47(d, 2H, A-H),7.97-7.96(d, 2H, Ar-H), 7.95(d, 2H, Ar-H on indole ring), 9.12(s,1H,-NH proton in Amide).^{13}CNMR(CDCl3-D): 173.78, 168.34, 163.19, 152.64, 148.55,143.23, 137.64, 134.37, 136.53, 126.44, 124.77, 124.68, 123.09, 122.19, 121.06, 116.47, 112.57, 112.41, 100.65, 48.56, 42.48, 27.56, 23.48. Mass (EI-MS): m/z 591.06(M), 593.12(M + 2, 100%), 595.40(M + 4, 30%).

Compound. VIII-e: (1-((bis(2-chloroethyl) amino) methyl)-2-(5-nitro)oxoindolin-3-ylidene) -4-(2,5-dimethyl-1H-pyrrol-1-yl)benzo hydrazide.IR(KBr)cm^{-1}: 3402.32(-N-H*Str*, -CONH- group), 3087.32(-C-H*Str*, Aromatic), 2987.31, 2932.65, 2832.18(-CH *Str* in Aliphatic group), 1708.32(-CO *Str* in Indole), 1693.31(-CO *Str* in Amide group), 1623.41(-NO_2*Str* in Ar-NO_2), 1519.03(-C=C *Str* in Aromatic), 818.32(-C-Cl *Str*, Ar-Cl), 799.41(-C-Cl Str in -CH_2-Cl). ^{1}H-NMR($CDCl_3$-D)δppm: 2.04(s, 6H, -CH_3 protons on Pyrrole ring), 3.24-2.85(t, 4H,protons in –CH_2-Cl),3.73-3.38(t, 4H,protons in -N-CH_2),5.43(s, 2H, -N-CH_2 Mannich base protons), 5.83(d,2H, Ar-H in pyrrole), 7.29(s, 1H, Ar-H), 7.55-7.86(d, 2H, A-H),7.94-7.99(d, 2H, Ar-H), 8.12(d, 2H, Ar-H on indole ring), 9.19(s,1H,-NH proton in Amide).^{13}CNMR(CDCl3-D): 178.43, 166.01, 160.32, 155.18, 153.43, 146.09, 139.32, 137.23, 136.32, 129.03, 126.12, 125.22, 123.90, 120.15, 119.23, 117.12, 114.02, 112.32, 111.21, 49.22, 44.43, 28.43, 23.23. Mass (EI-MS): m/z 556.14(M), 558.03(M + 2, 100%), 560.01(M + 4, 30%).

Compound. VIII-f: (1-((bis(2-chloroethyl) amino) methyl)-2-(5-fluoro,7-chloro) oxoin dolin-3-ylidene)-4-(2,5-dimethyl-1H-pyrrol-1-yl) benzo hydrazide.IR(KBr)cm^{-1}: 3401.32(-N-H*Str*, -CONH- group), 3087.32(-C-H*Str*, Aromatic), 2978.32, 2932.43, 2812.41(-CH *Str* in Aliphatic group), 1723.31(-CO *Str* in Indole), 1687.31(-CO *Str* in Amide group), 1510.23(-C=C *Str* in Aromatic), 824.21(-C-F*Str*, Ar-F), 801.32, 798.32(-C-Cl Str in -CH_2-Cl). ^{1}H-NMR($CDCl_3$-D)δppm: 1.95(s, 6H, -CH_3 protons on Pyrrole ring), 3.29-2.78(t, 4H,protons in –CH_2-Cl),3.78-3.98(t, 4H,protons in -N-CH_2),5.48(s, 2H, -N-CH_2 Mannich base protons),5.87(d,2H, Ar-H in pyrrole), 7.54(s, 1H, Ar-H), 7.78-7.82(d, 2H, A-H),7.97-7.91(d, 2H, Ar-H), 8.21(d, 2H, Ar-H on indole ring), 9.12(s,1H,-NH proton in Amide).^{13}CNMR(CDCl3-D): 173.43, 166.34, 165.32, 156.33, 152.54, 147.22, 139.54, 136.65, 134.21, 130.87, 129.21, 127.34, 125.76, 122.32, 120.33, 118.43, 115.98, 113.76, 113.21, 49.21, 46.33, 25.24, 24.22. Mass (EI-MS): m/z 563.11(M), 564.21(M + 2, 100%), 567.09(M + 4, 30%).

Table 1. Physical data of novel novel(1-((bis(2-chloroethyl)amino)methyl)-2-oxoindolin-3-ylidene)-4-(2,5-dimethyl-1H-pyrrol-1-yl)benzohydrazide: VIII-(a-j).

Compound	Molecular Formula	R	Molecular Weight(gm)	Melting Point(°C)	% Yield
VIII-a	$C_{26}H_{27}Cl_2N_5O_2$	H	512.43	165-167	76
VIII-b	$C_{26}H_{26}Cl_3N_5O_2$	5-Cl	546.87	195-197	87
VIII-c	$C_{26}H_{26}Cl_3FBN_5O_2$	5-F	530.42	196-198	75
VIII-d	$C_{26}H_{26}Cl_2BrN_5O_2$	5-Br	591.32	180-182	92
VIII-e	$C_{26}H_{26}Cl_2N_6O_4$	5-NO_2	557.42	260-264	68
VIII-f	$C_{26}H_{25}Cl_3FN_5O_2$	5F6Cl	564.86	200-202	78
VIII-g	$C_{27}H_{29}Cl_2N_5O_2$	7-CH_3	526.45	284-286	85
VIII-h	$C_{26}H_{26}Cl_2FN_5O_2$	7-F	530.42	202-204	65
VIII-i	$C_{29}H_{31}Cl_2N_5O_4$	5-$COOC_2H_5$	584.49	282-284	68
VIII-j	$C_{28}H_{29}Cl_2N_5O_4$	7-CO0CH_3	570.49	280-282	80

Compound. VIII-g: Compound. VIII-f: (1-((bis(2-chloroethyl) amino) methyl)-2-(5-methyl)oxoindolin-3-ylidene)-4-(2,5-dimethyl-1H-pyrrol-1-yl)benzohydrazide: IR(KBr)cm^{-1}: 3438.01(-N-H*Str*, -CONH- group), 3058.98(-C-H*Str*, Aromatic), 2974.62, 2918.82(-CH *Str* in Aliphatic group), 1723.06(-CO *Str* in Indole), 1687.02(-CO *Str* in Amide group), 1447.13(-C=C *Str* in Aromatic), 826.31, 757.55(-C-Cl Str in -CH_2-Cl). ^{1}H-NMR($CDCl_3$-D)δppm: 1.67(s, 6H, -CH_3 protons on Pyrrole ring), 2.31(s, 3H, Ar-CH_3 in indole ring), 3.00-2.99(t, 4H,protons in –CH_2-Cl),3.57-3.56(t, 4H,protons in –-N-CH_2),5.24(s, 2H, -N-CH_2 Mannich base protons), 5.64(d,2H, Ar-H in pyrrole), 7.29-7.27(d, 2H, Ar-H), 7.48-7.41(d, 2H, A-H),7.86(s, 1H, Ar-H), 7.97-7.95(d, 2H, Ar-H on indole ring), 9.03(s,1H,-NH proton in Amide).^{13}CNMR(CDCl3-D): 172.03, 168.32, 148.32, 138.12,134.32, 132.09, 130.21, 129.21, 128.09, 127.32, 124.34, 122.65,118.32, 59.23, 50.12, 48.09, 30.23, 27.33. Mass (EI-MS): m/z 526.45(M), 527.03(M + 2, 100%), 529.04(M + 4, 30%).

Compound. VIII-h: (1-((bis(2-chloroethyl) amino) methyl)-2-(7-fluoro) oxoindolin-3-ylidene)-4-(2,5-dimethyl-1H-pyrrol-1-yl) benzo hydrazide. IR(KBr)cm^{-1}: 3368.32(-N-H*Str*, -CONH- group), 3098.43(-C-H*Str*, Aromatic), 2984.32, 2998.21, 2812.02(-CH *Str* in Aliphatic group), 1728.32(-CO *Str* in Indole), 1696.23(-CO *Str* in Amide group), 1528.32(-C=C *Str* in Aromatic), 823.12(-C-F *Str*, Ar-F), 812.16, 786.33(-C-Cl Str in -CH_2-Cl). ^{1}H-NMR($CDCl_3$-D)δppm: 2.23(s, 6H, -CH_3 protons on Pyrrole ring), 3.48-2.93(t, 4H,protons in –CH_2-Cl),3.62-3.72(t, 4H,protons in -N-CH_2),5.88(s, 2H, -N-CH_2 Mannich base protons), 5.78(d,2H, Ar-H in pyrrole), 7.54(s, 1H, Ar-H), 7.67-7.79(d, 2H, A-H),7.82-7.88(d, 2H, Ar-H), 8.09(d, 2H, Ar-H on indole ring), 9.23(s,1H,-NH proton in Amide).^{13}CNMR(CDCl3-D): 173.09, 166.23, 165.23, 156.43, 157.12, 147.34, 146.22, 142.13, 140.17, 139.32, 135.23, 129.65, 127.22, 125.09, 124.98, 123.41, 118.43, 114.87, 112.65, 48.12, 40, 29.43, 25.54. Mass (EI-MS): m/z 530.14(M), 532.13(M + 2, 100%), 534.21(M + 4, 30%).

Compound. VIII-i: (1-((bis(2-chloroethyl) amino) methyl)-2-(5-ethylaceto) oxoindolin-3-ylidene)-4-(2,5-dimethyl-1H-pyrrol-1-yl) benzo hydrazide.IR(KBr)cm^{-1}: 3332.54(-N-H*Str*, -CONH- group), 3087.32(-C-H*Str*, Aromatic), 2986.54, 2921.43, 2865.32(-CH *Str* in Aliphatic group), 1732.43(-CO *Str* in Indole), 1687.21(-CO *Str* in Amide group), 1521.32(-C=C *Str* in Aromatic), 818.13, 798.32(-C-Cl Str in -CH_2-Cl). ^{1}H-NMR($CDCl_3$-D)δppm: 1.99(s, 6H, -CH_3 protons on Pyrrole ring), 2.87-3.23(t, 4H,protons in –CH_2-Cl), 3.10-30(t, 2H, $COOCH_2$-),3.46-3.34(t, 4H,protons in -N-CH_2),4.10-4.02(t, 3H in acetyl protons), 5.65(s, 2H, -N-CH_2 Mannich base protons), 5.72(d,2H, Ar-H in pyrrole), 7.34(s, 1H, Ar-H), 7.65-7.69(d, 2H, A-H),7.74-7.78(d, 2H, Ar-H), 8.21(d, 2H, Ar-H on indole ring), 9.25(s,1H,-NH proton in Amide).^{13}CNMR(CDCl3-D): 176.32, 168.32, 162.43, 158.54, 155.23, 146.23, 144.98, 140.13, 139.43, 135.23, 133.21, 128.56, 126.45, 125.09, 122.23, 1120.43, 119.34, 113.43, 110.12, 49.65, 48.34, 29.67, 25.56. Mass (EI-MS): m/z 583.18(M), 585.02(M + 2, 100%), 587.40(M + 4, 30%).

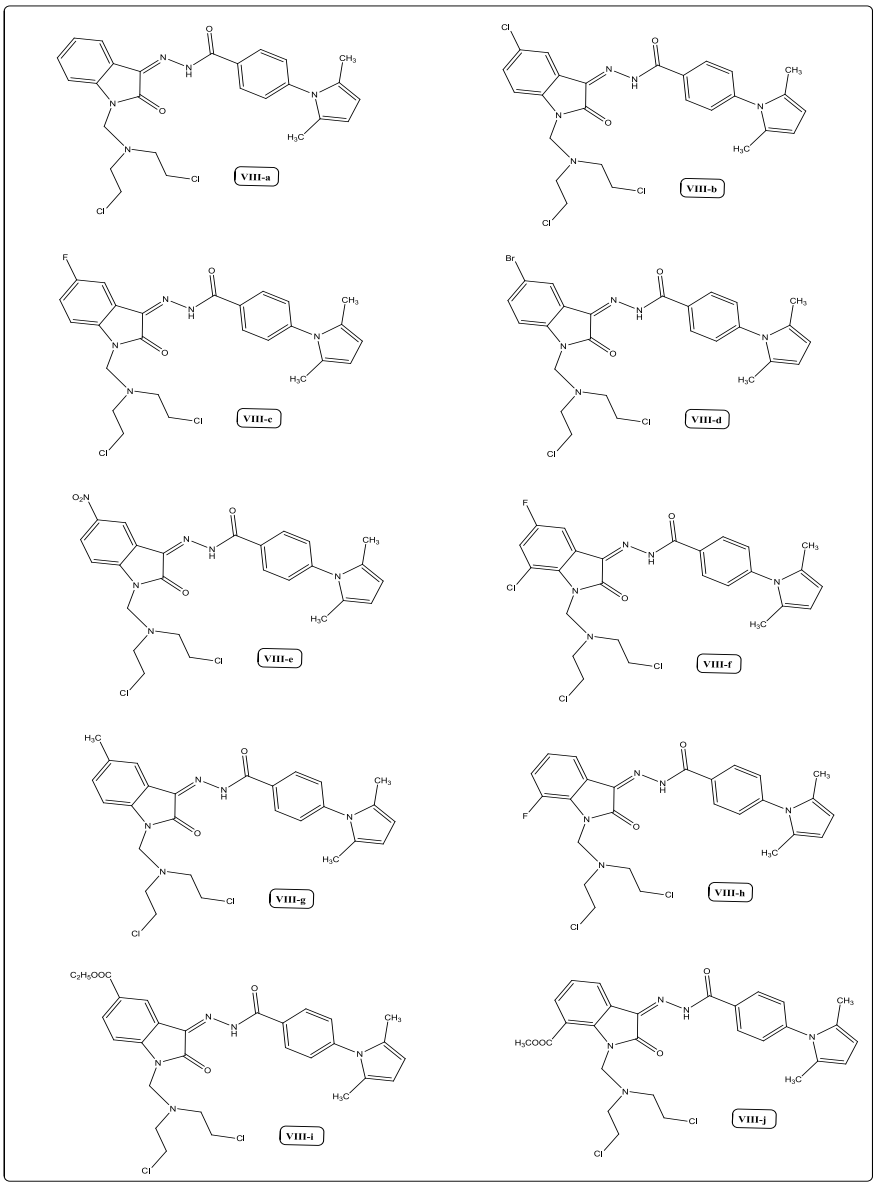

Figure 3. Novel synthesized compounds VIII-(a-j)

Compound. VIII-j: (1-((bis(2-chloroethyl) amino) methyl)-2-(7-methylaceto) oxoindolin-3-ylidene)-4-(2,5-dimethyl-1H-pyrrol-1-yl) benzo hydrazide. IR(KBr)cm^{-1}: 3386.90(-N-H*Str*, -CONH- group), 3076.32(-C-H*Str*, Aromatic), 2956.32, 2965.54, 2898.32(-CH *Str* in Aliphatic group), 1728.09(-CO *Str* in Indole), 1698.34(-CO *Str* in Amide group), 1523.21(-C=C *Str* in Aromatic), 823.15, 789.32(-C-Cl Str in -CH_2-Cl). ^{1}H-NMR($CDCl_3$-D)δppm: 1.89(s, 6H, -CH_3 protons on Pyrrole ring), 2.34-3.20(t, 4H,protons in –CH_2-Cl), 3.32(t, 2H, $COOCH_2$-),3.52-3.65(t, 4H,protons in -N-CH_2),4.32-4.43(t, 3H in acetyl protons), 5.48(s, 2H, -N-CH_2 Mannich base protons), 5.81(d,2H, Ar-H in pyrrole), 7.32(s, 1H, Ar-H), 7.45-7.53(d, 2H, A-H),7.83-7.92(d, 2H, Ar-H), 8.09(d, 2H, Ar-H on indole ring), 9.17(s,1H,-NH proton in Amide).^{13}CNMR(CDCl3-D): 173.98, 169.23, 165.23, 160.21, 157.34, 147.56, 143.56, 140.87, 139.23, 137.12, 134.21, 129.32, 124.21, 123.28, 121.82, 121.23, 118.43, 115.44, 113.15, 48.54, 46.334, 27.45, 24.59. Mass (EI-MS): m/z 569.16(M), 571.03(M + 2, 100%), 573.04(M + 4, 30%).

Pharmacological Activity:

Antioxidant activity: The synthesized novel (1-((bis(2-chloroethyl) amino) methyl)-2-oxoindolin-3-ylidene)-4-(2,5-dimethyl-1H-pyrrol-1-yl)benzohydrazideVIII-(a-j)wereevaluated for antioxidant activity by employing Ascorbic acid as the standard (reference antioxidant molecule) using the DPPH (2,2-diphenyl -1-picryl-hydrazyl-hydrate) radical scavenging assay. DPPH in ethanol shows a strong absorption band at 517 nm (independent of pH from 5.0 to 6.5), and the solution appears to be deep violet in color. 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 (10µM, 20µM, 40µM, 60µM, 80µM, 100µM) 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 (OD) was read at 517 nm against blank. The % reduction of free radical concentration (OD) with different concentration of test compounds was calculated and was compared with standard, ascorbic acid[19].

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

The results were expressed as IC_{50} values (the concentration of test required to scavenge 50% free radicals).

Anticancer activity:

Evaluation of in vitro anticancer activity against MCF-7, A549 & HepG2 cancer cell lines: In vitro anticancer activity against MCF-7(Human Breast cancer cell line) A549(Human Lung cancer cell line) & HepG2(Human Liver cancer cell line)cancer cell lines was determined using 96-well tissue culture plates. The cell suspension of $1x10^5$ cells/ml was prepared in complete growth medium. Stock solutions of ten compounds were prepared in DMSO. The stock solutions were serially diluted with complete growth medium containing 50 mg/ml of gentamycin to obtain working test solution of required concentrations (having1<100% DMSO). The 100 µL of cell suspension was added to each well of the 96-well tissue culture plates. The cells were allowed to grow in CO_2 incubator (37^0C, 5% CO_2, 90% relative humidity) for 24 h. The test materials in complete growth medium (100 µL) were added after 24 h incubation to the wells containing cell suspension. After 48 h of treatment with different concentrations of test compounds, the cells were incubated with MTT (2.5 mg/ml) for 2 h. The medium was then removed and 100µL of DMSO were added into each well to dissolve formazan crystals, the metabolite of MTT. After thoroughly mixing, read the absorption on a spectrophotometer or an ELISA reader at 570nm wavelength [20].

The IC_{50}value was determined using below equation.

linear regression equation i.e.

Y=Mx+C.

Here, Y=50, M and C values were derived from the viability graph.

RESULTS AND DISCUSSIONS.

Synthesis: In the present study, the compounds were synthesized as depicted in Scheme-I. Totally ten novel (1-((bis(2-chloroethyl) amino)methyl)-2-oxoindolin-3-ylidene)-4-(2,5-dimethyl-1H-pyrrol-1-yl)benzo hydrazide VIII-(a-j) were prepared by treatment substituted Isatin mannich bases [{[bis(2-chloroethyl)amino]methyl}-1H-indole-2,3-dione, VII-(a-j)] was condensedd with 4-(2,5-dimethyl-1H-pyrrol-1-yl)benzo hydrazide. The preparation of the title compounds is outlined in Scheme-I. The physical data of the all synthesized compounds were shown in Table 1. Al l the synthesized compounds were purified by column chromatography using chloroform and ethyl acetate as solvent and the reactions were monitored by TLC. The synthesized compounds (figure 3) were confirmed by means of their IR, ^{1}HNMR, ^{13}CNMR and MASS spectral analysis.

Spectral data: All of the FT-IR spectra of the compound VIII-(a-j) revealed the appearance of C=O Stretching band which is at between 1680-1742$cm^{-1}$and aromatic –CH stretching at around 3000-3120cm^{-1}. All the compounds are showed various adsorption band starting from specific band at 3320-3486cm^{-1} related to amide –N-H group, at 2757-2999$cm^{-1}$for aliphatic -C-H stretching. ^{1}H-NMR spectra showed the following chemical shift starting from singlet at δ1.89-2.43 related to methyl(-CH_3) proton, triplet at δ3.00-3.85 related to methylene(-CH_2-) proton, singlet at δ4.54-5.54 related to mannich (-CH_2-) proton, singlet, doublet and triplet at δ6.98-8.12 related to aromatic protons, singlet at δ8.52-9.32 related to Amide (-CONH-) proton. While the ^{13}C-NMR spectrum of the synthesized compounds was found in equivalent with its assigned structure and showed these showed these signals in δppm: 168.65, 161.15, 142.08, 132.54, 131.08, 129.35, 129.09, 128.56, 128.14, 126.88, 125.27, 124.48,114.02, 57.20, 48.56, 42.48, 28.56, 22.48.

Antioxidant activity: The antioxidant activity of all the synthesized compounds performed using DPPH method and the results given in Table 2. The values are expressed in IC_{50} that is, ability of the

test compound required to decrease the concentration of test free radical by 50%. All the synthetic compounds produced a concentrations dependent scavenging of free radical. The IC_{50} values of all the synthetic test compounds were found ranges between **10.62 to 2.67μM**. Among all the test compounds, compounds **VIII-c (IC_{50}, 10.62μM), VIII-e (IC_{50}, 15.94μM), VIII-f (IC_{50}, 10.62μM),** and **VIII-h (IC_{50}, 15.58μM)** had more potent antioxidant activity against DPPH free radicals. It is proposed that DPPH may be scavenged by an antioxidant through donation of hydrogen (H·) to form a stable DPPH-H molecule which does not absorb at 517 nm. Thus the results show that synthesized compound **VIII-F (IC_{50}, 10.62μM)**shown highest percentage of free radical scavenging activity. It was observed that the test compounds with electron withdrawing groups (**5-F, 7-Cl**) on the Indole ring favors anti-oxidant activity.

Table 2. Antioxidant activity of Novel synthesized compounds VIII-(a-j)-IC50Values.

S. No	Compounds	R	IC50Valuesμ M
1	VIII-a	H	20.67
2	VIII-b	5-Cl	16.83
3	**VIII-c**	**5-F**	**14.63***
4	VIII-d	5-Br	24.67
5	**VIII-e**	**7-NO_2**	**15.94***
6	**VIII-f**	**5-F6Cl**	**10.62***
7	VIII-g	5-CH_3	19.35
8	**VIII-h**	**7-F**	**15.58***
9	VIII-i	5-$COOC_2H_5$	18.28
10	VIII-j	7-$COOCH_3$	17.82
11	Ascorbicacid	-	5.87

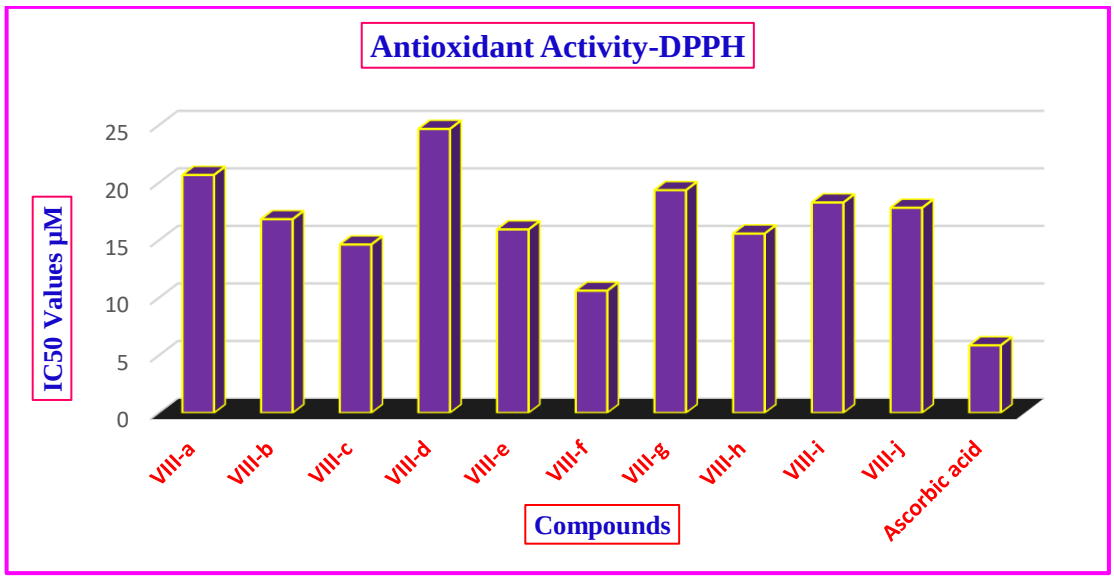

Figure 4. Graphical representation Antioxidant activity of Novel synthesized compounds VIII-(a-j)-IC50Values.

Anticancer activity: The novel (1-((bis(2-chloroethyl) amino)methyl)-2-oxoindolin-3-ylidene)-4-(2,5-dimethyl-1H-pyrrol-1-yl)benzo hydrazide VIII-(a-j) compounds were screened for cytotoxic activity against three cancer cell lines (MCF-7, A549, HepG2 cell lines) by MTT assay method, with doxorubicin as a standard drug. All the results (Table 2) proposed compounds **VIII-b, VIII-h,** and **VIII-I** exhibited the highest activities against three cancer cell lines with IC_{50}ranging from **149.16µg/ml to 229.86µg/ml**(MCF-7), **95.31µg/ml** to **298.29µg/ml** (A549), and **115.8µg/ml** to **177.83µg/ml** (HepG2), while compound VIII-h was found to be most potent against A549 cell line. whereas, remaining of the compounds showed moderate activity against MCF-7 cell lines.

Table.2: Test compounds treated with MCF-7, A549, HepG2 cells showing the IC $_{50}$ values

S. No	SAMPLE NAME	IC_{50} (µg)		
		MCF	A549	HepG2
1	**VIII-a**	229.86	102.41	167.74
2	**VIII-b**	**149.16***	244.62	132.84
3	**VIII-c**	167.74	289.67	164.97
4	**VIII-d**	205.95	205.47	119.86
5	**VIII-e**	213.1	166.14	177.83
6	**VIII-f**	151.52	298.29	135.07
7	**VIII-g**	160.11	208.22	127.34
8	**VIII-h**	188.28	**95.31***	158.45
9	**VIII-i**	157.2	238.32	**115.8***
10	**VIII-j**	202.59	205.7	144.93
11	**Doxorubicin**	4.89	8.32	12.67

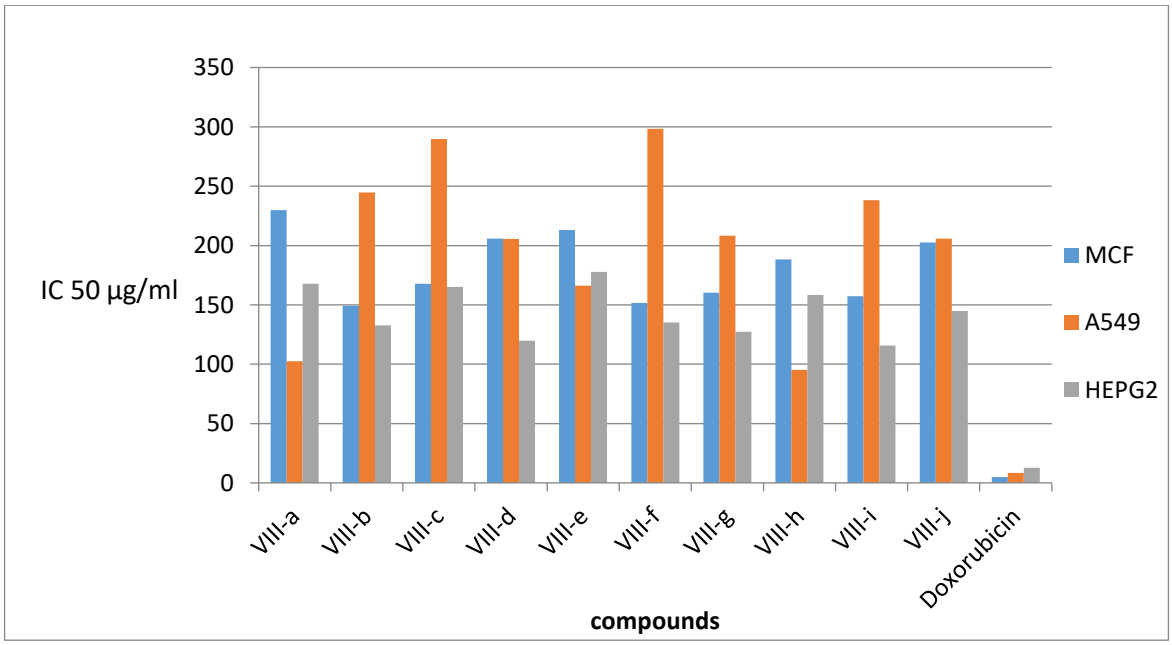

Figure 5: Graphical representation of Anticancer activity of novel synthesized compounds VIII-(a-j)-IC50 Values on MCF-7, A549 and HepG2 Cell lines.

CONCLUSION.

In conclusion, we have described simple and efficient protocol for the synthesis of novel (1-((bis(2-chloroethyl) amino)methyl)-2-oxoindolin-3-ylidene)-4-(2,5-dimethyl-1H-pyrrol-1-yl)benzohydrazideVIII-(a-j) derivatives with good yields. Structures of the compounds were confirmed by spectral analysis (FT-IR, ^{1}H-NMR, ^{13}C-NMR, and MASS). All the synthesized compounds VIII-(a-j) have been investigated for their in vitro antioxidant and anticancer activities. In the newly synthesized compounds, it is clear that the highest antioxidant activity was found in compound VIII-c, VIII-e, VIII-f, and VIII-h, whereas anticancer anticancer activity was observed in compound VIII-b, VIII-h, and VIII-i. To summarize, we found that the novel(1-((bis(2-chloroethyl)amino)methyl)-2-oxoindolin-3-ylidene)-4-(2,5-dimethyl-1H-pyrrol-1-yl)benzo hydrazide VIII-(a-j) has emerged as a valuable lead. Few of the synthesized compounds might be useful antioxidant and anticancer agents in future. These novel synthesized derivatives have proved to be promising candidates for future efficacy evolution.

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CONFLICT OF INTEREST: The authors declare that they have no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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