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Synthesis and Evaluation of New Isatin Derivatives for Cytotoxic and Antioxidant Activities

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ABSTRACT

In the present study N-(2-oxosubstitutedindolin-3-ylidine)-2-phenylacetohydrazides (IV a-h) have been synthesized in good yields and characterized by IR, PMR and mass spectral analyses. Compounds were evaluated for their preliminary *in vitro* cytotoxic activity against HeLa cancer cell lines by standard MTT assay and also were screened for antioxidant activity by DPPH method. Our results shown that two of the analogues IVf and IVc are potent antioxidant agents and IVb and IVg are potent cytotoxic agents.

Keywords: Isatin, antioxidant, cytotoxic.

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INTRODUCTION

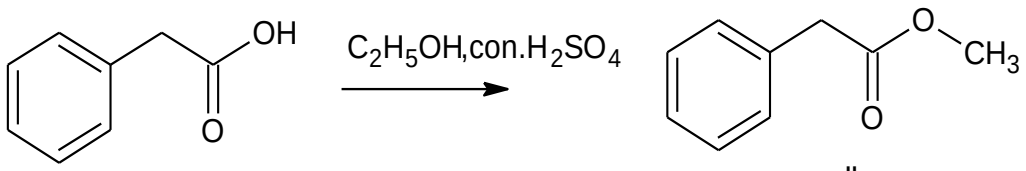
Isatin is a unique molecule possessing both amide and ketocarbonyl groups. Apart from this, it has an active hydrogen atom attached to nitrogen (or oxygen) and an aromatic ring which should substitute at 5- and 7- positions.

It is interesting to note from the literature that the various derivatives with isatin moiety have got CNS activities like anticonvulsant, antipsychotic and other activites like antioxidant, cytotoxic, antihypertensive, antimicrobial, antitubercular etc. In continuation of such investigations and in a search for less toxic pharmacologically and more potential derivatives, we have taken up the synthesis and pharmacological evaluation of some new isatin derivatives.

MATERIALS AND METHOD

The chemicals and solvents are purchased (SD fine) was purchased from local wendors of ALM chemicals, Hanamkonda. Melting points of all synthesized compounds were determined by open capillary tubes using Toshniwal & Cintex melting point apparatus. Expressed in ºC and are uncorrected. The IR spectra KBr pellets were recorded on Bruker spectrometer for the compounds. PMR spectra were recorded for compounds on AV 300MHz NMR Spectrometer, using DMSO as solvent and TMS as an internal standard. The Mass spectra were recorded on ESI-MS ion Mass spectrometer. The purity of the compounds were checked by Thin Layer Chromatography(TLC) on Merck Silica gel 60 F254 pre coated sheet using Petroleum Ether and Ethyl acetate in 1:1 %v/v.

Synthesis of N-(2-oxosubstitutedindolin-3-ylidine)-2-phenylacetohydrazides (IV a-h):



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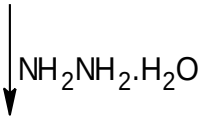

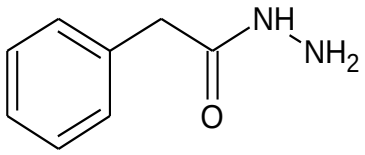

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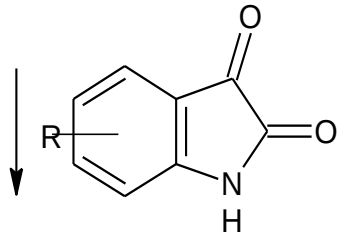
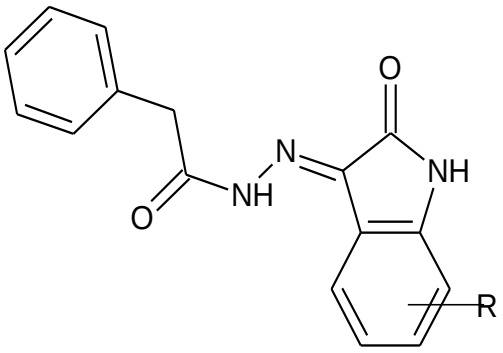
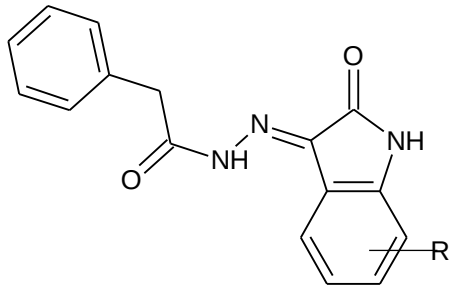




IV (a-h)

R=H, 5-Br,5-CH_3,5-F,5-Cl,7-Cl,7-F,7-NO_2

Table 1: Physical data of N-(2-oxosubstitutedindolin-3-ylidine)-2-phenylacetohydrazides (IV a-h)



IV (a-h)

S.No.	Compounds	Substituents	Mol. Formula	m.p.(^{O}C)	%yield	Mol. Wt
1	IV a	H	$C_{16}H_{13}N_3O_2$	204-206	42	279
2	IV b	5-Br	$C_{16}H_{12}N_3O_2Br$	273-281	86	357
3	IVc	5-CH_3	$C_{17}H_{15}N_3O_2$	238-240	89	293
4	IV d	5-F	$C_{16}H_{12}N_3O_2F$	230-232	74	297
5	IVe	5-Cl	$C_{16}H_{12}N_3O_2Cl$	246-249	63	329
6	IV f	7-Cl	$C_{16}H_{12}N_3O_2Cl$	254-260	69	329
7	IV g	7-F	$C_{16}H_{12}N_3O_2F$	312-314	75	297
8	IV h	7-NO_2	$C_{16}H_{12}N_4O_4$	321-324	62	324

Table 2: Cytotoxic activity data of N-(2-oxosubstitutedindolin-3-ylidine)-2-phenylacetohydrazides (IV a-h)

S.No.	Compounds	Substituents	IC_{50}(µg/ml)
1	IV a	H	126.22
2	IV b	5-Br	52.99
3	IV c	5-CH_3	93.90
4	IV d	5-F	57.01
5	IV e	5-Cl	67.20
6	IV f	7-Cl	71.33
7	IV g	7-F	52.12
8	IV h	7-NO_2	106.28
9	Standard	Cisplatin	14.23

Synthesis of ethyl 2-phenylacetate:

Phenyl acetic acid was refluxed with ethanol for about 2 hours. Catalytic amount of Sulphuric acid was added to progress the reaction. Ethyl 2- phenyl acetate was formed and the reaction was known as esterification. As esterification was a reversible reaction ethanol was taken double the quantity of phenyl acetic acid.

Synthesis of 2-Phenylacetohydrazide (III):

To the compound II, hydrazine hydrate was added in 1:5 ratio and was refluxed for about 2 hours in methanol as a solvent. The solvent was evaporated; the product thus obtained was filtered and

washed with water and dried.

Synthesis of N-(2-oxosubstitutedindolin-3-ylidine)-2-phenylacetohydrazides (IV a-h)

To a warm solution of 2 -Phenylacetohydrazide (0.01mol) in absolute ethanol (15ml) appropriate indole-2, 3-dione (0.01mol) was added in the presence of glacial acetic acid (3 drops) and the reaction mixture was refluxed for 2-3 hr, then allowed to cool to room temperature. The solid separated was filtered, thoroughly washed with cold water, dried and recryastllized from ethanol. Compound IV (a-h) were characterized by physical data, TLC, melting point, IR spectra, Mass and PMR spectra. Melting points were determined in open capillary tubes on a Thomas Hoover melting point apparatus and were uncorrected.

CHARACTERIZATION:

N-(2-oxosubstitutedindolin-3-ylidine)-2-phenylacetohydrazide IVa: IR (cm^{-1}): 3232.69(-NH str), 1561.23 (-NH bn), 1689.90 (C=O), 1727.55 (-NH), 1618.67 (-C=N),1008.48 (N-N).^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.9(2H,s),6.8-7.2(9H,m),8.5 (1H,NH), 9.5 (1H,NH). MS m/z (%): 280.

N-(5-methyl-2-oxosubstitutedindolin-3-ylidine)-2-phenylacetohydrazideIVc:IR(cm^{-1}): 3291.49(-NH str), 1560.06 (-NH ben), 1692.17 (C=O), 1729.97 (-NH), 1620.13 (-C=N),1060.13 (N-N).^{1}H NMR (DMSO-d_6, 400 MHz) δ : 1.9(2H,s),2.3 (3H,s), 6.7-7.2(8H,m), 8.5 (1H,NH), 9.5 (1H,NH). MS m/z (%): 294.

BIOLOGICAL ACTIVITY:

In vitro cytotoxic activity*:* [1,2]

The cell cultures HeLa (cervical) cancer cell lines were procured from National Center for Cell Sciences [NCCS], Pune, India. These cell lines were grown in culture and maintained using suitable media (DMEM) and were grown in culture medium supplemented with 10% fetal bovine serum, 1% L-glutamate and 1% penicillin-streptomycin-amphotericin-B-antibiotic solution. Cells were seeded in 25cm^2tissue culture flasks [Tarsons, Mumbai, INDIA] at 250,000 cells\flask in a total volume of 9Ml. When confluent, all the cells were trypsinized and seeded in 96-well tissue culture plates [Tarsons, Mumbai, INDIA].

In vitro anticancer activity against HeLa cancer cell line was determined using 96 well tissue culture plates. The method followed in the evaluation was standard MTT assay method.(Alley,1998,Scuderio,1998) The cell suspension of 1×105 cells/ml was prepared in complete growth medium. The drug solution was serially diluted at concentration of 10µg/ml to 100µg/ml with complete growth medium containing 1µg/ml, 3µg/ml, 10µg/ml, 30µg/ml and 100µg/ml concentrations (<2%DMSO solution).The 100µl of cell suspension was added to each

well of 96-well tissue culture plates. The cells were allowed to grow in a CO_2 incubator (37^0C,5% CO_2, 90% relative humidity) for 24 hrs. The test drug solutions in complete growth medium (100µl) were added after 24hrs incubation to the wells containing a cell suspension. After 48hrs of treatment with different concentrations of test drug solutions, the cells were incubated with 20µl of MTT (2.5mg/ml) for 2 hrs. After 24 hrs medium was removed and 80µl of lysis buffer was added to each well the plate was wrapped in aluminum.

Evaluation of antioxidant activity: [3]

α,α-Diphenyl picrylhydrazyl (DPPH 1ml of 0.135mM in methanol), a stable free radical was used for the evaluation of the antioxidant activity of the test compounds (Liyana-Pathiana and Shahidi, 2005). To 1ml of the test compound (at different concentrations), 1ml of DPPH solution were added, mixed thoroughly and absorbance (optical density) read at 517nm against blank. The percentage reduction of free radical Concentration (OD) with different concentrations of test compounds was calculated and compared with standard, ascorbic acid. Results were expressed as IC_{50} values (concentration of test required to scavenge 50 % free radicals.)

RESULTS AND DISCUSSION

Phenyl acetic acid was taken as a starting material for the synthesis of the titled compounds. Phenyl acetic acid was esterified and that was treated with hydrazine hydrazine to get its hydrazone. That was treated with different isatin derivatives to get title compounds.

The *in vitro* cytotoxic activity of N-(2-oxosubstitutedindolin-3-ylidine)-2-phenylacetohydrazides (IVa-IVh) was represented in table 2 and figure 1. The IC_{50} values are in between 52.17 to 126.22. Among them IV b and IV g are active having IC_{50}values 52.99 and 52.12. Among them IV h is least active showing IC_{50} value 106.28.

The *in vitro* antioxidant activity of N-(2-oxosubstitutedindolin-3-ylidine)-2-phenylacetohydrazides (IVa-IVh) was represented in table 3 and figure 2. The IC_{50} values are in between 45.64 to 72.24. Among them IV f and IV e are active having IC_{50}values 51.62 and 53.84. Among them IV a is least active showing IC_{50} value 72.24.

The present study results indicate the cytotoxic and antioxidant activity data of series of N-(2-oxosubstitutedindolin-3-ylidine)-2-phenylacetohydrazides (IVa-IVj).The results of the study revealed that the compounds to possess good spectrum of cytotoxic and antioxidant activity.

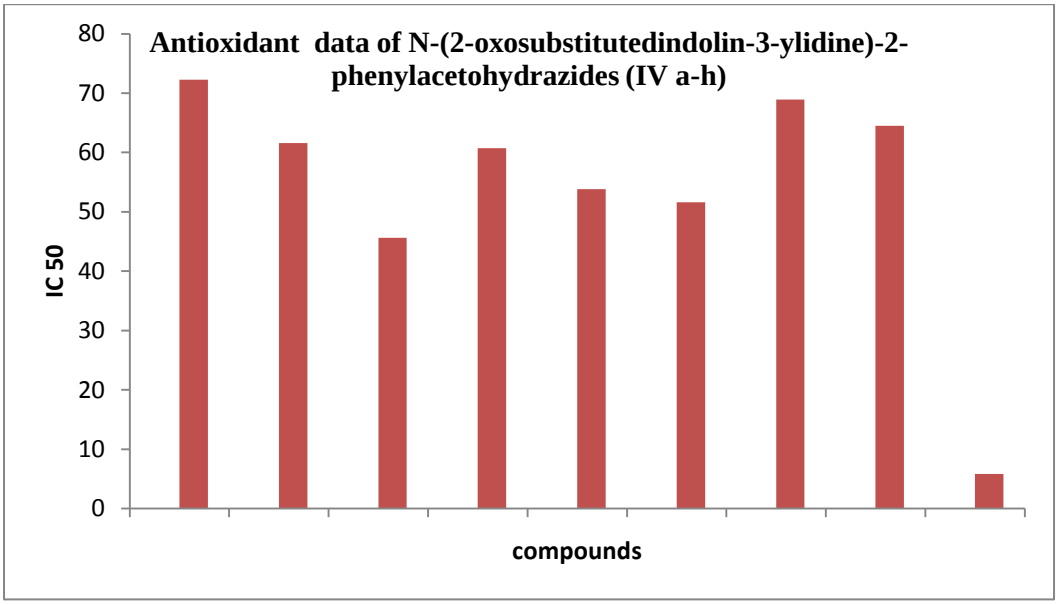


Figure 1: Graphical representation of antioxidant activity data of N-(2-oxosubstitutedindolin-3-ylidine)-2-phenylacetohydrazides (IV a-h)

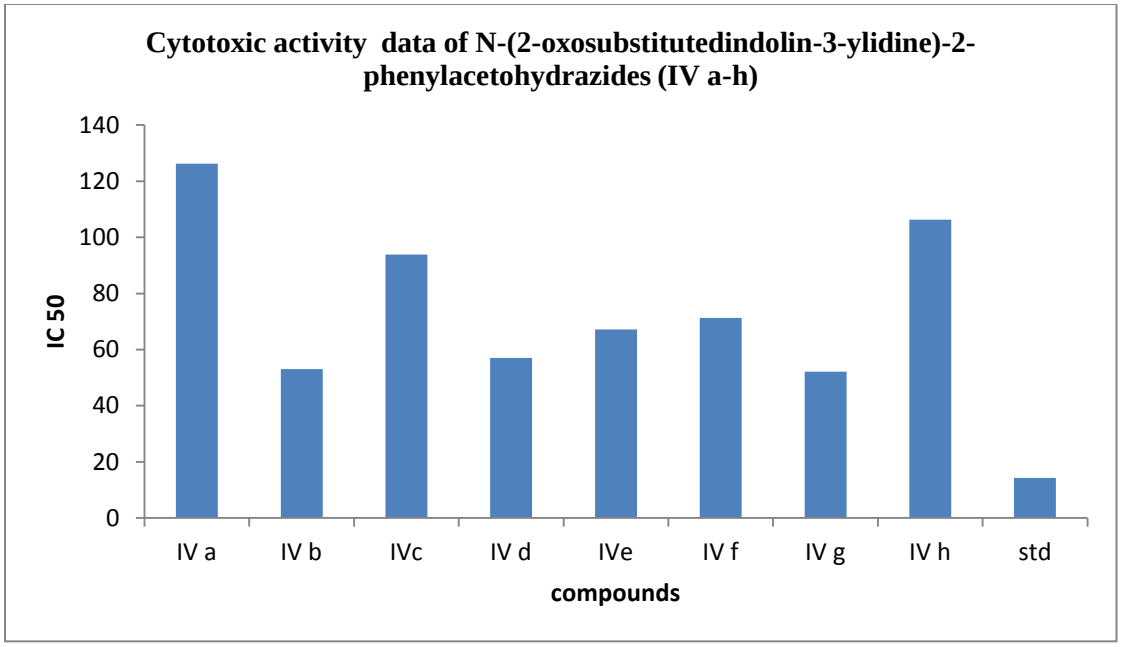


Figure 2: Graphical representation of Cytotoxic activity data of N-(2-oxosubstitutedindolin-3-ylidine)-2-phenylacetohydrazides (IV a-h)

Table 3: Antioxidant data of N-(2-oxosubstitutedindolin-3-ylidine)-2-phenylacetohydrazides (IV a-h)

S.No.	Compounds	Substituents	IC_{50}(µg/ml)
1	IV a	H	72.24
2	IV b	5-Br	61.57
3	IVc	5-CH_3	45.64
4	IV d	5-F	60.75

5	IVe	5-Cl	53.84
6	IV f	7-Cl	51.62
7	IV g	7-F	68.91
8	IV h	7-NO_2	64.48
9	Standard	Ascorbic acid	5.84

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