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Synthesis and pharmacological screening of some new derivatives of (Z)-[2-(2-substituted benzalamino-4-yl) hydrazono]-indolin-2-ones

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ABSTRACT

Five different (Z)-3-[2-(2- aminooxazol-4-yl) hydrazono]-indolin-2-ones were synthesized from respective 3-[N-(chloroacetyl) hydrazono] indolin-2-one on reaction with urea. Each of that compound has refluxed with different aromatic aldehydes to get the respective (Z)-3-[2-(2-substituted benzalamino oxazol-4-yl)hydrazono]-indolin-2-ones. All the new derivatives of (Z)-3-[2-(2-substituted benzalaminooxazol-4-yl) hydrazono]-indolin-2-ones were assayed for their antibacterial, antifungal, antioxidant and cytotoxic activities by standard methods.

Key words: (Z)-3-[2-(2-substituted benzalaminooxazol-4-yl] hydrazono]-indolin-2-ones, substituted benzaldehydes, Antibacterial activity, Antifungal activity, Antioxidant activity and Cytotoxic activity

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INTRODUCTION

Recent literature survey revels that several oxazole derivatives and reported to possess and pharmacological activities[1-4]. Owing to the diverse biological property of indole derivatives reported from our laboratory[5]. Some of the indole derivatives also have pharmacological activities like antibacterial[6,7],antitubercular[8].We have now undertaken the synthesis and pharmacological evaluation of some new derivatives of (Z)-3-[2-(2-substituted benzalamino oxazol-4-yl] hydrazono-indolin-2-ones.

MATERIALS AND METHODS

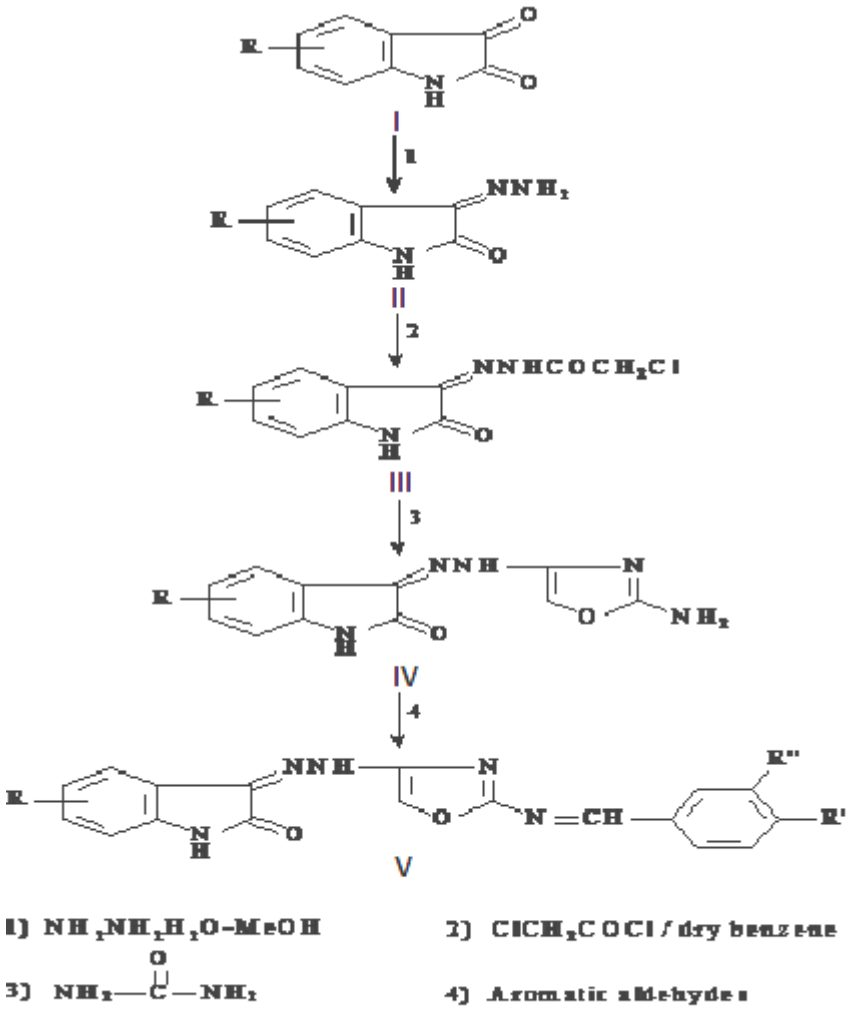
I) Synthesis of 3-hydrazono indolin-2-ones (II)

An appropriate indolin-2, 3-dione (**I**, 0.01 mol) was dissolved in alcohol (20 ml) and added hydrazine hydrate (99%, 0.015 mol) while shaking. The reaction mixture was stirred well, warmed on a water-bath for 10 min and left in the refrigerator for 3 hrs. The resultant yellow crystalline solid was filtered, washed repeatedly with small portions of cold water. The product was dried and purified by recrystallisation from chloroform.

The compounds thus obtained were characterized by comparison with their physical constants reported in the literature[9,10].

II) Synthesis of 3-[N-(chloroacetyl)] hydrazono] indolin-2-ones (III)

An appropriate 3-hydrazonoindolin-2-one (II, 0.01 mol) was heated under reflux with chloroacetyl chloride (0.01 mol) in dry benzene under anhydrous conditions using calcium chloride guard-tube for 2 hrs. The product thus formed was filtered and washed and purified by recrystallisation from suitable solvent(s).



SCHEME - 1

III) Synthesis of (Z)-3-[2-(2-aminooxazol-4-yl) hydrazono]-indolin-2-ones (IV)

A solution of 3-[N-(chloroacetyl) hydrazono]-indolin-2-one (**III**, 0.01 mol) in absolute ethanol (50 ml) was treated with a solution of urea (0.01 mol). The reaction mixture was refluxed for 12 hrs. The separated solid was filtered, washed dried and recrystallized from ethanol and water mixture.

Adopting the above procedure five compounds were prepared and the physical data is presented in **Table-1**

Table-1. Physical data of (Z)-3-[2-(-aminooxazol-4-yl) hydrazono] Indolin-2ones (IV)

S.No.	Compound	R	Mol. formula	m.p.(^oC)	Yield(%)
1	IV a	H	$C_{11}H_9N_5O_2$	220	82
2	IV b	5- CH_3	$C_{12}H_{11}N_5O_2$	215	84
3	IV c	5-Cl	$C_{11}H_8N_5O_2Cl$	235	81
4	IV d	5-NO_2	$C_{11}H_8N_6O_4$	203	87
5	IV e	7- CH_3	$C_{12}H_{11}N_5O_2$	213	78

Table. II. Physical data of (Z)-3-[2-(2-substituted benzaliminooxazol-4-yl) hydrazono]-Indolin-2-ones (V)

S. No.	Compd.	R	R'	R"	Mol. formula	m.p. (^oC)	Yield (%)
1	V a	H	H	H	$C_{18}H_{13}N_5O_2$	250	73
2	V b	H	Cl	H	$C_{18}H_{12}N_5O_2C1$	240	63
3	V c	H	-$N(CH_3)_2$	H	$C_{20}H_{18}N_6O_2$	207	58
4	V d	H	OH	OCH_3	$C_{19}H_{15}N_5O_4$	220	60
5	V e	5-CH_3	H	H	$C_{19}H_{15}N_5O_2$	240	60
6	V f	5-CH_3	Cl	H	$C_{19}H_{14}N_5O_2Cl$	251	65
7	V g	5-CH_3	-$N(CH_3)_2$	H	$C_{21}H_{20}N_6O_2$	222	58
8	V h	5-CH_3	OH	OCH_3	$C_{20}H_{17}N_5O_4$	227	63
9	V i	5-Cl	H	H	$C_{18}H_{12}N_5O_2Cl$	257	62
10	V j	5-Cl	Cl	H	$C_{18}H_{11}N_5O_2Cl_2$	270	60
11	V k	5-Cl	-$N(CH_3)_2$	H	$C_{20}H_{17}N_6O_2Cl$	250	66
12	V l	5-Cl	OH	OCH_3	$C_{19}H_{14}N_5O_4Cl$	240	61
13	V m	5-NO_2	H	H	$C_{18}H_{12}N_6O_4$	217	70
14	V n	5-NO_2	Cl	H	$C_{18}H_{11}N_6O_4Cl$	229	66
15	V o	5-NO_2	-N($CH_3)_2$	H	$C_{20}H_{17}N_7O_4$	224	58
16	V p	5-NO_2	OH	O CH_3	$C_{19}H_{14}N_6O_6$	213	59
17	V q	7-CH_3	H	H	$C_{19}H_{15}N_5O_2$	245	68
18	V r	7-CH_3	Cl	H	$C_{19}H_{14}N_5O_2Cl$	270	63
19,20	V s	7-CH_3	-$N(CH_3)_2$	H	$C_{21}H_{20}N_6O_2$	223	58

IV) Synthesis of (Z)-3-(2-substituted benzalamino oxazol-4-yl) hydrazono-indolin-2-ones (V)

A solution of an appropriate (Z)-3-[2-(2-amino oxazol-4-yl)hydrazono-indolin-2-one (I**V**, 0.01 mol) in ethanol (60 ml) and 2-3 drops of glacial acetic acid was refluxed with four different aromatic aldehydes (0.01 mol) for 10 hrs. The solvent was distilled off, the residue was washed with petroleum ether 40-60^oC and recrystallized from appropriate solvent(s).

Twenty compounds were prepared by following the above procedure. The physical and analytical data of compounds are given in **Table-II.**

Purification of compounds has been effected by recrystallisation from appropriate solvents viz.,%Yield:73%;Charecterization of a representative compound:Va:IR; in cm^{-1}at 3180(NH),1728(C=O, lactam),16809(C=C,aromatic),1545(C=N),^{1}HNMR(DMSO-d_6 at δppm**)**

1094(s,1H,lactam),8.67(s,1H,NH),7.91-8.07(m,9H,Ar-),7.47(s,1H,N=CH),6.97(s,1H,oxadiazole-H)

Biological and pharmacological assays

1. Antibacterial activity by cup plate method[11]

The antibacterial activity of synthesized compounds was conducted against two gram positive bacteria viz., *Bacillus subtilis* and *Staphylococcus aureus* and two gram negative bacteria viz., *Escherichia coli* and *Proteus vulgaris* by using cup plate method. Ampicillin sodium was employed as standard to compare the results. The test organisms were subcultured using nutrient agar medium. The tubes containing sterilized medium were inoculated with respective bacterial strain. After incubation at 37^{o}C $\pm$ 1^{o}C for 24 hours, they were stored in refrigerator. The stock cultures were maintained. Bacteria inoculum was prepared by transferring a loopful of stock culture to nutrient broth (100 ml) in conical flasks (250 ml). The flasks were incubated at 37^{o}C $\pm$ 1^{o}C for 48 hours before the experimentation. Solution of the test compounds were prepared by dissolving 10 mg each in dimethylformamide (10 ml, AnalaR grade). A reference standard for both gram positive and gram negative bacteria was made by dissolving accurately weighed quantity of ampicillin sodium in sterile distilled water, separately.

The nutrient agar medium was sterilized by autoclaving at 121^{o}C (15 lb/sq. inch) for 15 min. The petriplates, tube and flasks plugged with cotton were sterilized in hot-air oven at 160^o, for an hour. Into each sterilized petriplate (10 cm diameter), about 27 ml of molten nutrient agar medium was poured and inoculated with the respective strain of bacteria (6 ml of inoculum to 300 ml of nutrient agar medium) was transferred asceptically. The plates were left at room temperature to allow the solidification. In each plate, three cups of 6 mm diameter were made with sterile borer. Then 0.1 ml of the test solution was added to the respective cups asceptically and labeled, accordingly. The plates were kept undisturbed for atleast 2 hours in refrigerator to allow diffusion of the solution properly into nutrient agar medium. After incubation of the plates at 37^o $\pm$ 1^{o}C for 24 hours, the diameter of zone of inhibition surrounding each of the cups was measured with the help of an antibiotic zone reader. All the experiments were carried out in triplicate. Simultaneously, controls were maintained employing 0.1 ml of dimethyl formamide to observe the solvent effects.

2) Antifungal activity[12]: All those compounds screened for antibacterial activity were also tested for their antifungal activity. The fungi employed for screening were : *Aspergillus niger, Aspergillus flavus, Fusarium oxysporum* and *Cunninghamella verticulata.*

The test organisms were sub-cultured using potato-dextrose-agar medium. The tubes containing sterilized medium were inoculated with test fungi and after incubation at 25^o for 48 hours, they were stored at 4^o in refrigerator.The inoculum was prepared by taking a loopful of stock culture to about 100 ml of nutrient broth, in 250 ml conical flasks. The flasks were incubated at 25^o for 24 hours before use.The solutions of test compounds were prepared by a similar procedure described under the antibacterial activity. A reference standard (1 mg/ml conc.) was prepared by dissolving 10 mg of Clotrimazole in 10 ml of dimethylformamide (AnalaR grade). Further, the dilution was made with dimethylformamide itself to obtain a solution of 100 µg/ml concentration.

The potato-dextrose-agar medium was sterilized by autoclaving at 121^o (15 lb/sq. inch) for 15 minutes. The petriplates, tubes and flasks with cotton plugs were sterilized in hot-air oven at 150^o, for an hour. In each sterilized petriplate, about 27 ml of molten potato-dextrose-agar medium inoculated with respective fungus (6 ml of inoculum in 300 ml of potato-dextrose

medium) was added, aseptically. After solidification of the medium at room temperature three discs of 6 mm diameter were made in each plate with a sterile borer. Accurately 0.1 ml (100 µg/disc) of test solution was transferred to the discs aseptically and labelled, accordingly. The reference standard, 0.1ml (10 µg/disc) was also added to the discs in each plate. The plates were kept undisturbed at room temperature for 2 hours, atleast to allow the solution to diffuse properly into the potato-dextrose-agar medium. Then the plates were incubated at 25^{o}C for 48 hours. The diameter of the zone of inhibition was read with the help of an antibiotic zone reader. The experiments were performed in triplicate in order to minimize the errors

3) Antioxidant Activity[13]

Preparation of standard solutions of Ascorbic acid : Required amount of ascorbic acid was accurately weighed and dissolved in distilled water to prepare 1 mM state solution. Solutions of different concentrations of ascorbic acid 10 nM, 30 nM, 100 nM, 300 nM, 1 µM, 3 µM, 100 µM, 300 µM, 1 mM were prepared from stock solution.

Preparation of DPPH solution : 0.05 mM of DPPH was prepared by dissolving 19.71 mg of DPPH in 100 ml of methanol. The solution was protected from sunlight to prevent the oxidation of DPPH.

Preparation of Test compounds : Required amount of test compound was dissolved in methanol and 1 mM stock solution was prepared. Solutions of concentrations ranging from 100 nM to 1 mM were prepared from the stock solution.

Standard graph of Ascorbic acid : 0.2 ml of DPPH solution was added to 2.8 ml of ascorbic acid solution in a test tube wrapped with aluminium foil and its absorbance was read out at 517 nm using UV-visible double beam spectrophotometer. The results were plotted on a graph and IC_{50} value was determined.

Test compounds : The IC_{50} values of the test compounds were determined by a procedure similar to the ascorbic acid determination.

Principle : The method is based on the principle described by Blois *et al.*[13] method. The model of scavenging the stable DPPH (1,1-diphenyl-2-picryl-hydrazil) radical is a widely used method to evaluate antioxidant activities in a relatively shorter time compared with other methods. The effect of antioxidants on DPPH radical scavenging was thought to be their hydrogen donating ability[14].DPPH is a stable free radical and accepts an electron (or) hydrogen radical to become a stable diamagnetic molecule[15]. The reduction capability of DPPH radical was determined by the decrease in its absorbance at 517 nm induced by antioxidants. The absorption maximum of a stable DPPH radical in ethanol was at 517 nm.
The decrease in absorbance of DPPH radical caused by antioxidants, because of the reaction between antioxidant molecules and radical, progresses, which results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a discoloration from purple to yellow. Hence, DPPH is usually used as a substrate to evaluate antioxidative activity of antioxidants[16,17].

The reduction in absorbance is calculated as percentage inhibition as follows :

$$\% \text{ inhibition} = \frac{\text{Absorbance of Blank} - \text{Absorbance of Test}}{\text{Absorbance of Blank}} \times 100$$

4) **Cytotoxic Activity[**18,19**] Microculture tetrazolium (MTT) assay**

Materials RPMI-1640 (Himedia, Mumbai, India), Trypsin 0.25% (Gibcous, USA), FBS (fetal bovine serium) (Gibcous, USA),MTT 4 mg/ml (Himedia),DMSO (Merck, India), Lysis buffer (15% SLS in 1:1 DMF and water). Composition of RPMI; 9.54 GM/LIT, 10% FBS, 2000 mg sodium bicarbonate, 250 µl each of pencillin (60 mg/ml), streptomycin (100mg/ml), amphotericin (200 mg/ml). Composition of RPMI; 9.54 gm/lit, 10% FBS, 2000 mg sodium bicarbonate, 250 µl each of penicillin (60 mg/ml), streptomycin (100 mg/ml), amphotericin (200 mg/ml).

Principle : Microculture tetrazolium assay (MTT) is based on the metabolic reduction of 3-(4,5-dimethylthiazol-2,5-diphenyl)tetrazolium bromide (MTT) to water insoluble formazan crystals with mitochondrial dehydrogenase enzyme, which gives direct correlation of viable cells.

Method : 0.1 ml of the cell suspension (containing 5 x 10^5 cells / 100 µl) and 0.1 ml of the compound solution (10, 20, 50, 100, 150 and 200 µg in DMSO such that the final concentration of DMSO in media is less than 1%) was added to the 96 well plates and kept in carbon dioxide incubator with 5% CO_2 at 37^{o}C for 72 hours. Blank contains only cell suspension and control wells contain 1% DMSO and cell suspension. After 72 hours, 20 µl of MTT was added and kept in carbon dioxide incubator for 2 hours followed by 80 µl of lysis buffer (15% SLS in 1:1 DMF and water). The plate was covered with aluminium foil to protect from light, then the 96 well plate was kept on rotary shaker for 8 hours. After 8 hours the 96 well plates were processed on ELISA reader for absorption at 562 nm. The readings were averaged and viability of the test samples was compared with DMSO control.

RESULTS AND DISCUSSION

Antibacterial activity The antibacterial activity of (Z)-3-[2-(2-substituted benzalaminooxazol-4-yl) hydrazono]-indolin-2-one (**V**) is presented in Table III. It is observed from the table that, all the compounds have a noticeable degree of inhibition specifically against gram positive organisms i.e., *B. subtilis* and *S. aureus*. The compound **Vg** has shown greater inhibitory effect against *B. subtilis* and *S. aureus* with the zone of inhibition of 17 and 16 mm. This has been followed by compound **Vf** with zone of inhibition of 16 and 15 mm.

Antioxidant Activity

The antioxidant activity data of (Z)-3-[2-(2-substitutedbenzalamino-oxazol-4-yl)hydrazono]-indolin-2-ones (**V**) is presented in Table V. Compounds have shown antioxidant activity with IC_{50} values in the range of 9.62 to 22.32 µm. Among the test compounds, compound **V** (R = 5-Cl, R' = OH and R" = OCH_3) has shown highest percentage of free radical scavenging activity. Compound **Vd** (R = H, R' = OH, R" = OCH_3) with an IC_{50} value of 10.12 µM is to next in the order of antioxidant activity, where as rest of the compounds have shown moderate percentage of free radical scavenging activity.

Table-III. Antibacterial activity of (Z)-3-[2-(2-substituted benzalaminoxazol-4-yl) hydrazono]-indolin-2-ones (V)

S.No	Compound	R	R'	R''	Zone of inhibition (in mm)			
					B. subtilis	***S. aureus***	***E. coli***	***P. vulgaris***
1	V a	H	H	H	9	6	--	--
2	V b	H	Cl	H	13	11	15	13
3	V c	H	$-N(CH_3)_2$	H	9	10	7	--
4	V d	H	OH	OCH_3	12	9	5	5
5	V e	5-CH_3	H	H	15	9	--	--
6	V f	5- CH_3	Cl	H	16	15	17	--
7	V g	5- CH_3	$-N(CH_3)_2$	H	17	16	12	--
8	V h	5- CH_3	OH	OCH_3	11	9	5	5
9	V i	5-Cl	H	H	15	14	14	9
10	V j	5-Cl	Cl	H	15	11	14	9
11	Vk	5-Cl	$-N(CH_3)_2$	H	9	10	5	5
12	V l	5-Cl	OH	OCH_3	10	8	--	--
13	V m	5-NO_2	H	H	13	9	9	
14	V n	5-NO_2	Cl	H	14	12	15	13
15	V o	5-NO_2	$-N(CH_3)_2$	H	10	10	--	--
16	V p	5-NO_2	OH	OCH_3	12	9	--	--
17	V q	7-CH_3	H	H	10	9	--	--
18	V r	7-CH_3	Cl	H	15	12	--	--
19	V s	7-CH_3	$-N(CH_3)_2$	H	7	6	--	--
20	V t	7-CH_3	OH	OCH_3	10	7	6	5
21	Ampicillin(10 mg/cup)				22	20	18	17

Table- IV. Antifungal activity of (Z)-3-[2-(2-substituted benzalaminoxazol-4-yl) hydrazono]-indolin-2-ones (V)

Concentration of the test compound: 100 mg/cup

S.No	Compound	R	R'	R''	Zone of inhibition (in mm)			
					A. niger	***C. verticulata***	***F. oxysporum***	***A. flavus***
1	V a	H	H	H	7	6	--	--
2	V b	H	Cl	H	8	6	--	--
3	V c	H	$-N(CH_3)_2$	H	--	5	--	--
4	V d	H	OH	OCH_3	5	4	--	--
5	V e	5-CH_3	H	H	5	4	--	--
6	V f	5-CH_3	Cl	H	8	5	--	--
7	V g	5-CH_3	$-N(CH_3)_2$	H	5	5	--	--
8	V h	5-CH_3	OH	OCH_3	4	--	--	--
9	V i	5-Cl	H	H	5	--	--	--
10	Vj	5-Cl	Cl	H	8	--	--	--
11	V k	5-Cl	$-N(CH_3)_2$	H	6	4	--	--
12	V l	5-Cl	OH	OCH_3	5	4	--	--
13	V m	5-NO_2	H	H	8	5	--	--
14	V n	5-NO_2	Cl	H	4	5	--	--
15	V o	5-NO_2	$-N(CH_3)_2$	H	5	5	--	--
16	V p	5-NO_2	OH	OCH_3	6	3	--	--
17	V q	7-CH_3	H	H	5	5	--	--
18	V r	7-CH_3	Cl	H	4	3	--	--
19	V s	7-CH_3	$-N(CH_3)_2$	H	5	5	--	--
20	V t	7-CH_3	OH	OCH_3	5	--	--	--
21	Clotrimazole (10 mg/cup)				19	20	22	15

Table- V. Antioxidant activity of (Z)-[2-(2-substituted benzalamino-4-yl) hydrazono]-Indolin-2-ones (V)

S.No.	Compd.	R	R'	R"	IC50Value(mM)
1	V a	H	H	H	20.93
2	V b	H	Cl	H	21.61
3	V c	H	$-N(CH_3)_2$	H	19.32
4	V d	H	OH	OCH_3	10.12
5	V e	5-CH_3	H	H	20.42
6	V f	5-CH_3	Cl	H	21.62
7	V g	5-CH_3	$-N(CH_3)_2$	H	14.52
8	V h	5-CH_3	OH	OCH_3	11.19
9	V i	5-Cl	H	H	17.12
10	V j	5-Cl	Cl	H	15.49
11	V k	5-Cl	$-N(CH_3)_2$	H	13.42
12	V l	5-Cl	OH	OCH_3	9.62
13	Vm	5-NO_2	H	H	17.46
14	V n	5-NO_2	Cl	H	18.66
15	V o	5-NO_2	$-N(CH_3)_2$	H	17.33
16	V p	5-NO_2	OH	OCH_3	11.52
17	V q	7-CH_3	H	H	21.26
18	V r	7-CH_3	Cl	H	22.32
19	V s	7-CH_3	$-N(CH_3)_2$	H	19.16
20	V t	7-CH_3	OH	OCH_3	11.46
	Ascorbic acid				5.87

Cytotoxic Activity

IC_{50} values of cytotoxic activity of (Z)-3-[2-(2-substituted benzalaminooxazol-4-yl)hydrazono]-indolin-2-ones (**V**) are given in Table VI. They have exhibited moderate activity against HBL-100 and HeLa cell lines. Among the test compounds, compound **Vd** (R = H, R' = OH and R" = OCH_3) has shown more cytotoxic activity with IC_{50} values of 210.53 µm against HBL-100 cell lines whereas compound **V** (R = 5-Cl, R' = OH and R" OCH_3) has shown more activity with IC_{50} value of 251.06 µm against HeLa cell lines. Compounds **Vo** and **Vq** have not shown any activity against both cell lines employed.

Table. VI. Cytotoxic activity of (Z)-[2-(2-substituted benzalamino-4-yl) hydrazono]-indolin-2-ones (V)

S.No.	Compd.	R	R'	R"	HBL-100 cell lines IC_{50} values (mM)	HeLa cell lines IC_{50} values (mM)
1	V a	H	H	H	495.02	432.01
2	V b	H	Cl	H	276.36	240.45
3	V c	H	$-N(CH_3)_2$	H	395.02	--
4	V d	H	OH	OCH_3	210.53	390.12
5	V e	5-CH_3	H	H	425.04	--
6	V f	5-CH_3	Cl	H	298.16	292.13
7	V g	5-CH_3	$-N(CH_3)_2$	H	315.65	--
8	V h	5-CH_3	OH	OCH_3	235.63	--
9	V i	5-Cl	H	H	311.73	256
10	V j	5-Cl	Cl	H	290	311.06
11	V k	5-Cl	$-N(CH_3)_2$	H	285.12	290.66
12	V l	5-Cl	OH	OCH_3	262	251.06
13	V m	5-NO_2	H	H	411.11	395
14	V n	5-NO_2	Cl	H	273	278.11
15	V o	5-NO_2	$-N(CH_3)_2$	H	456.12	413.09
16	V p	5-NO_2	OH	OCH_3	310.26	411.78
17	V q	7-CH_3	H	H	--	--
18	V r	7-CH_3	Cl	H	320	270
19	Vs	7-CH_3	$-N(CH_3)_2$	H	360	432
20	Vt	7-CH_3	OH	OCH_3	-	--

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