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Synthesis, characterization and anticancer activity of new sulphamoyl isatin derivatives

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ABSTRACT

A new series of Schiff bases of 5-sulphamoyl isatin (V) were synthesized by reacting various substituted aromatic aldehydes with 3-hydrazino-5-sulphamoylisatin (IV). The 3-hydrazino-5-sulphamoylisatin was synthesized by reacting 5-sulphamoylisatin with hydrazine hydrate. All the synthesized compounds were characterized by means of their IR, ^{1}HNMR and Mass spectroscopic data. The designed compounds were screened for anticancer activity against Ehrlich Ascites Carcinoma (EAC) in Swiss Albino mice. Antitumor effect was determined by evaluating tumor volume, viable tumor cells count, non-viable tumor cells count and mean survival time. The standard antitumor drug used was 5-Fluorouracil. The results suggest that the compounds Ve, Vf, Vi and Vj exhibited significant antitumor activity.

Key words: 5-Sulphamoyl isatin, Ehrlich ascites carcinoma, tumor volume, viable cell count, non-viable cell count.

INTRODUCTION

Cancer is a class disease in which a cell or a group of cells display uncontrolled growth, invasion and sometimes metastasis. According to WHO estimates, globally 10 millions new cancer cases are diagnosed each year. It caused about 13% of all human deaths in 2007. It is estimated that by the year 2020, there will be 20 million new cancer cases with 12 million deaths. Although chemotherapy is the mainstay of cancer therapy, the use of available chemotherapeutics is often limited due to undesirable side effects as well as the increasing incidence of drug resistance to cancer chemotherapeutic agents, which is a serious medical problem [1]. Therefore, there is an urgent need to develop new classes of chemotherapeutic agents to treat cancer. Isatin (1H-indole-2, 3-dione) is one of the most promising heterocyclic molecules that have interesting active profiles, and importantly, it is well tolerated by humans [2,3]. Numerous publications have been reported that the isatin scaffold showed anticancer activity against various human tumor cell lines [4-7]. The objective of the present study was to synthesize and evaluate the antitumor effect of synthesized compounds against Ehrlich Ascites Carcinoma (EAC) in Swiss albino mice.

MATERIALS AND METHODS

All the chemicals used to synthesize the title compounds were of analytical grade and purchased from Merck Chemicals Private Limited, Hyderabad. All the reactions were monitored using thin layer chromatography on Silica Gel precoated plates. IR spectra were recorded in KBr on FTIR Brucker spectrophotometer and frequencies are expressed in cm^{-1}. The ^{1}HNMR spectra were recorded on 400MHz Brucker DPX using $CDCl_3$ and DMSO as solvent. Chemical shift values are reported as values in ppm relative to TMS as internal standard. Mass spectra were recorded on VG AUTOSPEC using EI-Ms model.

Synthesis of 5-Sulphamoyl-1H-indole--2,3-dione(III):

a) Synthesis of 5-Sulphamoyl Isonitrosoacetanilide(II):

Taken 5g of Sulphanilamide(I) and added 30 ml of distilled water and 5ml of concentrated hydrochloric acid. It formed clear solution in a beaker (solution I). In another beaker 9g of Chloral hydrate was taken and added 30 ml of water. Made up the solution up to 150ml (solution II). Mixed the solution I and II. To the mixed solution added anhydrous Sodium Sulphate. Stirred the solution until precipitation produced. Taken 12g of Hydroxylamine Hydrochloride and added 150ml of water (Solution III). Then mixed the above the solutions and heated the solution on a water bath for 45 min and kept side for 12-24 h. Formation of crystals of 5-sulphamoyl isonitrosoacetanilide were collected by filtration, washed with water and then dried.

b) Synthesis of 5-Sulphamoyl-1H-Indole-2,3-dione(III):

From the above crystals taken one 1g in dry beaker and added 4ml of concentrated Sulphuric acid with stirring and maintained the temperature at 60ºC. Then kept aside for 12h. The solution was poured onto the crushed ice produced coloured precipitation of 5-Sulphamoyl-1H-Indole-2, 3-dione. The product was filtered, washed with water and dried.

2. Synthesis of 5-Sulphamoyl isatin hydrazone(IV):

5-Sulphamoyl isatin (0.01 mol) was dissolved in 20ml of alcohol and added hydrazinhydrate(0.01 mol) with stirring. The reaction mixture was stirred well, reflexed for 3 h. The resultant crystalline solid was filtered, washed repeatedly with small portions of cold water and finally with a small portion of alcohol. The product was dried.

3. Synthesis of 3-[substituted benzylidenehydrazono]-2-oxo-indoline-5-sulphonamide (V a-j):

Added 15 ml of methanol and few drops of glacial acetic acid to equimolar quantities of various substituted aromatic benzaldehyde and the compound (IV). Refluxed the mixture for about 2 h. Then the reaction mixture was cooled to room temperature and poured onto crushed ice with stirring. After standing for 2 h, the product separated was filtered, washed several times with small portions of cold water and dried.

Spectral data of compound(Va)

FT-IR (cm^{-1}): 3425 (N-H Str), 3051 (Aromatic –CH Str), 1377 & 1284 (SO_2 Str), 1659 (C=N Str), 1567(Aromatic C=C str). **^{1}H NMR ($CDCl_3$):** δ 2.5(s, 2H, SO_2NH_2), δ 7-9(m, 8H, Aromatic), δ 9.9(s, 1H, NH), δ 8.1(s, 1H, N=CH). **MS m/z:** 329(M+1).

Spectral data of compound (Vb)

FT-IR (cm^{-1}): 3433 (N-H Str), 3023 (Aromatic –CH Str), 1379 & 1282 (SO_2 Str), 1655 (C=N Str), 1563(Aromatic C=C str). **^{1}H NMR ($CDCl_3$):** δ 2.5(s, 2H, SO_2NH_2), δ 7-9(m, 7H, Aromatic), δ 8.1(s, 1H, N=CH). **MS m/z:** 319(M+1) **.**

Spectral data of compound (Vc)

FT-IR (cm^{-1}): 3412 (N-H Str), 3016 (Aromatic –CH Str), 1375 & 1281 (SO_2 Str), 1652 (C=N Str), 1560(Aromatic C=C str), 1256 &1042 (C-O –C Str). **^{1}H NMR ($CDCl_3$):** δ 2.5(s, 2H, SO_2NH_2), δ 4(s, 3H, OCH_3), δ6.5-9(m, 8H, Aromatic), δ 8.1(s, 1H, N=CH). **MS m/z:** 358(M^+)**.**

Spectral data of compound (Vd)

FT-IR (cm^{-1}): 3543 (OH Str), 3398 (N-H Str), 3029 (Aromatic –CH Str), 1373 & 1288 (SO_2 Str), 1652 (C=N Str), 1560(Aromatic C=C str), 1218 (C-O Str). **^{1}H NMR $CDCl_3$):** δ 2.5(s, 2H, SO_2NH_2), δ 4.5 (s, 1H, OH), δ 7-9(m, 8H, Aromatic), δ 8.1(s, 1H, N=CH). **MS m/z:** 344(M^+) **.**

Spectral data of compound (Ve)

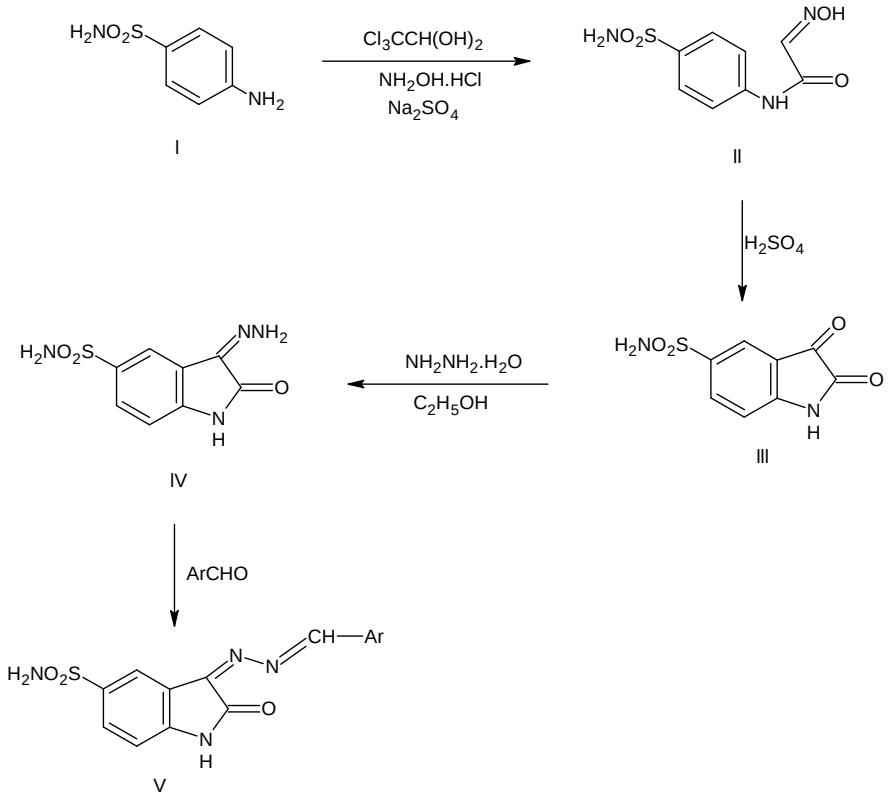
FT-IR (cm^{-1}): 3402 (N-H Str), 3009 (Aromatic –CH Str), 1370 & 1289 (SO_2 Str), 1651 (C=N Str), 1567(Aromatic C=C str). **^{1}H NMR ($CDCl_3$):** δ 2.5(s, 2H, SO_2NH_2), δ 2.8(s,6H, -$N(CH_3)_2$, δ 6.5-9(m, 8H, Aromatic), δ 8.1(s, 1H, N=CH). **MS m/z:** 371(M^+)**.**

Spectral data of compound (Vh)

FT-IR (cm^{-1}): 3420 (N-H Str), 3022 (Aromatic –CH Str), 1373 & 1290 (SO_2 Str), 1656 (C=N Str), 1566(Aromatic C=C str), 1525 &1350(Aromatic NO_2 Str). **^{1}H NMR ($CDCl_3$):** δ 2.5(s, 2H, SO_2NH_2), δ 6.5-9(m, 8H, Aromatic), δ 8.1(s, 1H, N=CH). **MS m/z:** 373(M+1).

Spectral data of compound (Vj)
FT-IR (cm^{-1}): 3406 (N-H Str), 3010 (Aromatic –CH Str), 1376 & 1280 (SO_2 Str), 1654 (C=N Str), 1568(Aromatic C=C str). **^{1}H NMR ($CDCl_3$):** δ 2.5(s, 2H, SO_2NH_2), δ 4(s, 2H, NH_2), δ 7-9(m, 8H, Aromatic), δ 8.1(s, 1H, N=CH). **MS m/z:** 343(M^+) **.**

Figure 1: Synthesis of 3-[substituted benzylidene hydrazono]-2-oxo-indoline-5-sulphonamide(V)



Statistical Analysis
Values are expressed as mean ± SEM. Statistical analysis was carried out by one way ANOVA. The values were considered to be significant at $p<0.05$.

Experimental animals
Swiss albino mice, weighing (23 – 25 g), were used in this study. They were maintained in a well-ventilated room at a temperature of 25 ± 1° C with 12/12 h light/dark cycle in polypropylene cages. Standard pellet feed and tap water were provided *ad libitum* throughout the experimentation period. The animals were acclimatized to laboratory conditions for two weeks prior to initiation of experiments. The project proposal was approved by the Institutional Animal Ethical Committee (ref.no:1694/PO/a/13/CPCSEA).

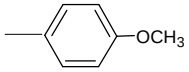
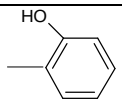
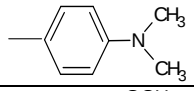
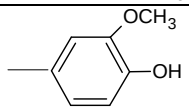
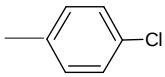
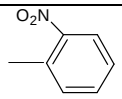
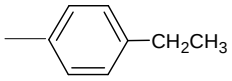
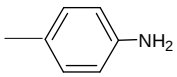
Acute oral toxicity study
Acute oral toxicity test was performed by following OECD guideline – 420. Increasing doses of the extracts, up to 2000 mg/kg body wt, were administered to the mice.

Antitumor studies
Ehrlich ascites carcinoma (EAC) cells were obtained from the National Centre for Cell Science, Pune, India and maintained *in vivo* in Swiss albino mice, by intraperitoneal (i.p.) transplantation of 1×10^6 cells/mouse every 10 days. The mice were divided into various groups of 8 animals each. EAC cells were collected from the donor mice and suspended in sterile isotonic saline (0.9 % NaCl). The viable EAC cells were counted (with the aid of trypan blue staining) under the microscope and were adjusted to 10^6cells/mL. An amount (0.1 mL) of these cells per 10 g body weight of the animal was injected (i.p.) into the animals on day 0. One day of incubation was allowed for multiplication of the cells.

Control group mice were administered 0.9 % NaCl solution for 16 days. Test groups EAC mice were administered test compounds (50 & 100mg/kg, i.p.). Standard group animals were receiving 5-fluorouracil (20 mg/kg, i.p.). The

treatment continued daily for 14 days. On day 21, four animals from each group were sacrificed and the remaining animals were kept for further observation for 3 weeks to determine host life span.

Table-1: Physical characterization of 3-[substituted benzylidene hydrazono]-2-oxo-indoline-5-sulphonamide (Va-j)

Comp. no	Ar	Molecular formula	Molecular weight	Percentage yield	Melting Point °C	R_f Value
Va		$C_{15}H_{12}N_4O_3S$	328	78%	88-90°C	0.72
Vb		$C_{13}H_{10}N_4O_4S$	318	71%	80-83°C	0.73
Vc		$C_{16}H_{14}N_4O_4S$	358	85%	85-87°C	0.68
Vd		$C_{15}H_{12}N_4O_4S$	344	67%	87-90°C	0.69
Ve		$C_{17}H_{17}N_5O_3S$	371	76%	84-87°C	0.70
Vf		$C_{16}H_{14}N_4O_5S$	374	80%	94-96°C	0.62
Vg		$C_{15}H_{11}ClN_4O_3S$	362	72%	90-92°C	0.67
Vh		$C_{15}H_{11}N_5O_5S$	373	69%	87-89°C	0.59
Vi		$C_{17}H_{16}N_4O_3S$	356	74%	89-91°C	0.64
Vj		$C_{15}H_{13}N_5O_3S$	343	77%	87-89°C	0.66

Determination of antitumor parameters

Tumor volume*:* The mice were dissected and the ascitic fluid was collected from the peritoneal cavity. The fluid volume was measured in a graduated centrifuge tube and packed cell volume was determined after centrifuging (REMI, R4C and REMI Group) at 1000 rpm for 5 min.

Viable/non-viable tumor cell count: The cells from the preceding test were stained with trypan blue (0.4 % in normal saline) dye. The cells that took up the dye were non-viable while those that did not were viable. Viable and nonviable cells were counted and cell count computed as in Eq 1.

Cell count = C/(A x T) …….. (1)

Where C is the number of cells (viable or nonviable) multiplied by the dilution factor, A is the area occupied by the liquid film and T is the thickness of the liquid film.

Increase in life span*:* The effect of synthesized compounds on tumor growth was monitored by recording mice mortality daily for a period of 3 weeks from the starting day and the percent increase in life span (ILS) was calculated for each animal group with respect to the control [8-12].

Table 2: Anticancer activity of 3-[substituted benzylidene hydrazono]-2-oxo-indoline-5-sulphonamide on EAC cell lines in mice

Treatment	Dose	Viable tumor cells count (10^6cells/mouse)	Non-viable tumor cells count (10^6 cells/mouse)	Tumor volume (ml)	Mean survival time (days)	% ILS
Control	EAC cell bearing mice	2.72±0.28	0.36±0.12	5.34±0.12	21.12±0.18	100
5-Flurouracil	20 mg/kg	0.52± 0.43**	0.96±0.22**	2.13±0.21**	36.23±0.42	171
CP-Va	50mg/kg	2.63±0.13	0.24±0.42	5.12±0.14	20.13±0.14	95
CP-Va	100mg/kg	1.94±0.32	0.58±0.48	3.21±0.16	27.12±0.17	128
CP-Vb	50mg/kg	2.72±0.19	0.43±0.08	4.78±0.25	21.14±0.19	100
CP-Vb	100mg/kg	1.44± 0.38	0.56±0.34	3.25±0.25	28.19±0.13	133
CP-Vc	50mg/kg	2.90± 0.09	0.38±0.10	5.25±0.15	20.13±0.15	95
CP-Vc	100mg/kg	1.92±0.14	0.59±0.48	3.65±0.32	28.14±0.23	133
CP-Vd	50mg/kg	2.78±0.22	0.41±0.54	5.23±0.14	21.12±0.12	100
CP-Vd	100mg/kg	2.06±0.28	0.60±0.24	4.95±0.32	21.14±0.21	100
CP-Ve	50mg/kg	1.27±0.16	0.48±0.32	3.50±0.21	29.12±0.23	137
CP-Ve	100mg/kg	0.68±0.34 **	0.82±0.44 **	2.43±0.18**	34.13±0.24	161
CP-Vf	50mg/kg	1.18±0.28	0.50±0.16	3.45±0.34	29.15±0.16	138
CP-Vf	100mg/kg	0.55±0.32**	0.86±0.48**	2.54±0.25**	34.17±0.18	161
CP-Vg	50mg/kg	2.68±0.23	0.36±0.64	5.26±0.24	22.14±0.12	104
CP-Vg	100mg/kg	2.16±0.26	0.55±0.29	4.89±0.22	21.15±0.21	100
CP-Vh	50mg/kg	2.56±0.18	0.41±0.22	5.54±0.18	20.12±0.15	95
CP-Vh	100mg/kg	1.62±0.52	0.61±0.49	3.18±0.21	26.18±0.24	123
CP-Vi	50mg/kg	1.06±0.29	0.56±0.04	3.65±0.72	30.27±0.19	143
CP-Vi	100mg/kg	0.56±0.38**	0.88±0.18**	2.52±0.41**	34.58±0.35	163
CP-Vj	50mg/kg	1.18±0.58	0.48±0.18	3.60±0.72	29.72±0.14	140
CP-Vj	100mg/kg	0.60±0.16**	0.82±0.27**	2.40±0.18**	35.02±0.22	165

*Values are expressed as Mean ±SEM for 4 animals in each group. **P<0.05 as compared to control group*

RESULTS AND DISCUSSION

A new series of 5-Sulphonamide isatin derivatives (Va-j) have been synthesized and screened for antitumor activity. Physical data, TLC, IR, ^{1}HNMR, Mass spectral data confirmed the structures and purity of the compounds. Antitumor activity was screened by determining different parameters like tumor volume, viable and non-viable tumor cells count, mean survival time, and percentage increase in life span of the animal.

Results of anticancer activity are shown in Table-2. In EAC bearing mice, a regular rapid increase in ascites tumor volume was noted. Ascites fluid is the direct nutritional source for tumor cells and a rapid increase in ascites fluid with tumor growth would be a means to meet the nutritional requirement of tumor cells. From the observations, it was found that the compounds Ve, Vf, Vi, Vj increased life span of the animals, decreased tumor volume & viable tumor cells count and increased non-viable tumor cells count at the dose of 100mg/kg b.wt.. Compounds Va, Vb, Vc,Vh exhibited less anticancer activity when compared to the standard drug. It may be concluded that the test compounds, by decreasing the nutritional fluid volume and arresting the tumor growth, and this could be the reason for the increase life span of EAC- bearing mice.

CONCLUSION

The study reports the successful synthesis of 3-(substituted benzylidene hydrazono)-2-oxo-indoline-5-sulphonamide and evaluating for their anticancer activity. The results revealed that the compounds exhibited anticancer activity. Compounds Ve, Vf, Vi, Vj are more potent in inhibiting tumor growth. The study would be a basic for the development of sulphamoyl isatin for their bioevaluation.

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