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SYNTHESIS AND CHARACTERIZATION OF CYTOTOXIC ACTIVITY OF CERTAIN N-(6-BENZOYL-1H-BENZO [d] IMIDAZOL-2-YL)-2-(2-OXOINDOLIN-3-YLIDENE) HYDRAZINE CARBOXAMIDES

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ABSTRACT

A series of N-(6-benzoyl-1H-benzo[d]imidazol-2-yl)-2-(2-oxoindolin-3-ylidene) hydrazine carboxamides were synthesized by treating (5-benzoyl-1H-benzo[d]imidazol-2-yl) carbamates with different substituted isatins. The novel compounds were characterized on the basis of spectral (FT-IR, 1H NMR, Mass) analysis. All the synthesized derivatives were screened for anticancer activity against Hela using MTT assay. All the synthesized compounds produced a dose dependent inhibition of growth of the cells. The IC_{50} values of all the synthetic test compounds were found between 16.03 to 41.75. The potency of (IC_{50} values) of cytotoxicity of compounds was compared with that of known cytotoxic agent,Cisplatin. Among all the synthesized novelcompounds 5-clshowed the most potent activity against MCF-7 cell lines.

KEY WORDS

Isatin, cytotoxicity, MTT assay.

INTRODUCTION

A dose of anticancer drug sufficient to kill tumor cells is often toxic to the normal tissues and leads to many side effects, which in turn limits its treatment efficacy. This is the major disadvantage of many effective Cytotoxic drugs. In this study we have synthesized thirteen compounds and were evaluated for their cytotoxicity against MCF-7(Breast) cancer cell lines.

CHEMISTRY

All the chemicals and solvents (M/s Sigma Aldrich/S.D. Fine chemical/Loba) were purchased from local vendors and solvents were purified before being used. Precoated silica gel F_{254} (Merck)were employed to check the TLC for the reaction progress and purity.Melting points were recorded in open glass capillaries using Thomas Hoover melting point apparatus and are uncorrected.Infrared spectra were recorded on Shimadzu FT-IR spectrophotometer in KBr pellet. Mass spectra were obtained on VG-7070H mass spectrometer and ^{1}HNMR spectra were recorded at 300MHz on Bruker Advance NM spectrometer in CDCl3(7.26)or DMSO-d6(2.49).Chemical shifts are expressed in (ppm) relative to TMS an internal standard.

EXPERIMENTAL METHOD

Materials & Methods:

I. Synthesis of Indole-2, 3-diones (Isatins, III):

a) Isonitrosoacetanilide – General Procedure:

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 (1300gm) followed by a solution of an appropriate aromatic amine in 300ml of water

and concentrated hydrochloric acid (0.52mol). 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-gauge by a Mecker burner so that vigorous boiling begins in about 45 minutes. After 1 to 2 minutes of vigorous boiling the reaction was completed. During the heating period itself the crystals of isonitrosoacetanilide 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 – General Procedure :

Sulphuric acid (600g, d:1.84, 326 ml) was warmed at 50°C in a one litre RB 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 onto crushed ice (2.5 kg) while stirring.

II. Synthesis of* N-*(6-benzoyl-1H-benzo[d]imidazol-2-yl) hydrazine carboxamide

A mixture of methyl (5-benzoyl-1H-benzo[d]imidazol-2-yl) carbamate add 0.01mole of hydrazine hydrate (99%) were taken in 20ml of methanol, heated under reflux on a water bath for 2 hrs. The alcohol was reduced to half of its volume and cooled. The product separated was filtered and small portions of cold alcohol first and then with cold water repeatedly and dried. The product purified by recrystallization from methanol has resulted white solid.

III. Synthesis of N-(6-benzoyl-1H-benzo[d]imidazol-2-yl)-2-(2-oxoindolin-3-ylidene) hydrazine carboxamide (III a-i)

The N-(6-benzoyl-1H-benzo[d]imidazol-2-yl) hydrazine carboxamide (0.01mol) and appropriate isatin (0.01mol) in methanol (20ml) 2 drops of glacial acetic acid heated under reflux on water bath for 8-12 hrs. The product thus obtained was filtered, washed with water.

Spectral data: Compound IIIA: IR (KBr, cm^{-1}): 3376.99(NH str), 1728.13(C=O str.), 2972.18(Aliphatic C-H). ^1H NMR (DMSO-d_6 &$CDCl_3$ 300 MHz) δ =7-8 (m, 12H,Ar),10.7(s, 1H, NH)11.50(s, 1H, NH), and 13.5 (s, 1H, NH).ES-MS(m/z):424.

Chemicals

Fetal bovine serum (FBS), Dulbecco's modified eagle's medium(DMEM), pencillin, amphotericin B and streptomycin were purchased from Himedia (Mumbai,India) MTT (3-(4,5-dimethylthiazol-2yl)-2,5diphenyltetrazolium bromide) was purchased from Sigma Aldrich Company, USA. Cisplatin was procured from local market with trade name as cytoplatin 50mg/50ml marketed by Cipla Pvt. Ltd, Ahmedabad , India, Himedia, Mumbai, India.

Cell culture

The cell cultures like MCF-7 (Breast), center for cell sciences [NCCS], pune, India. These cells 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%penciline-streptomycin-amphotericin-B-antibiotic solution. Cells were seeded in $25cm^2$ tissues culture flasks [Tarsons, Mumbai, INDIA] at250,000 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* cytotoxic activity**

In-vitro cytotoxic activities against MCF-7 were determined using 96 well tissue culture plates.

The cell suspension of 1×10^5 cells/mL was prepared in complete growth medium. The drug solution were serially diluted at concentration of 10µg/ml to100µg/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 CO_2 incubator (37°C, 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 cell suspension. After 48hrs of treatment with different concentrations of test drug solution, the cells were incubated with 20µl of MTT (2.5mg/mL) for 2 hrs. After 24 hrs medium was removed and80µl of lysis buffer was added to each well the plate was wrapped in aluminum foil to prevent the oxidation of the dye and the plate was placed on a shaker for overnight.7. The absorbencies were recorded on the ELISA reader at 562nm wavelength. The absorbance of the test was compared with that of DMSO control to get the % inhibition. The cytotoxic effects of the compounds were calculated as percentage inhibition in cell growth as per the formula.[1] % cytotoxicity=1-[(O.D.in sample well)]/O.D. in control well)] ×100.

RESULTS AND DISCUSSION

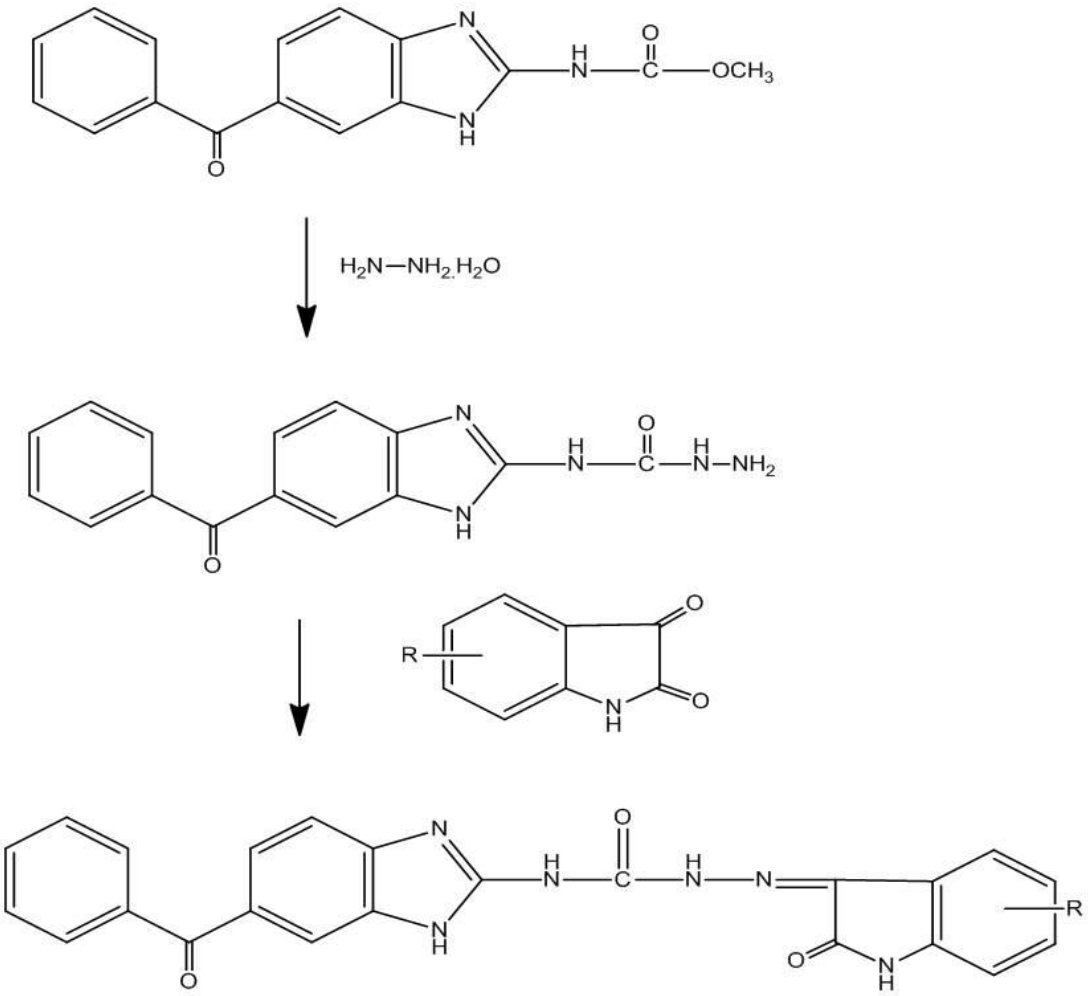
In present study, the compounds were synthesized as depicted in Scheme. The thirteen different novel N-(6-benzoyl-1H-benzo[d]imidazol-2-yl)-2-(2-oxoindolin-3-ylidene) hydrazine carboxamides were prepared. The physical data of the all synthesized compounds were purified by column chromatography. The thirteen compounds were tested for their *in vitro* cytotoxic activity against MCF-7(Breast) cancer cell lines by using MTT assay method. The results were satisfactory. Among all the compounds1I (R=6-Br) was effective against the entire cell lines. The compound I F(R=5-Cl) was next in order for MCF-7 cells.

Table 1: Physical data of N-(6-benzoyl-1H-benzo[d]imidazol-2-yl)-2-(2-oxoindolin-3-ylidene) hydrazine carboxamide (III a-i)

S.No.	Compound	Substituents (R)	m.p (°C)	Yield (%)	Mol.Wt
1	1a	H	265	70	423
2	1b	5-CH_3	270	75	437
3	1c	7-CH_3	272	72	437
4	Id	5-F	260	85	441
5	1e	5-$COOCH_3$	280	69	481
6	1f	5-Cl	285	70	457
7	1g	7-Cl	279	82	457
8	1h	5-Br	284	84	402
9	Ii	5-NO_2	285	76	468
10	1j	7-NO_2	270	81	468
11	1k	5-COOH	272	79	467
12	1i	7-$COOCH_3$	273	80	481

Table 2: ***In vitro*** **cytotoxic activity of** ***N-(6-benzoyl-1H-benzo[d]imidazol-2-yl)-2-(2-oxoindolin-3-ylidene) hydrazine carboxamide (IIIa-i):***

S.No.	Compound	R	IC_{50}(µg/ml)
1	A	H	41.75
2	B	5-CH_3	30.62
3	C	7-CH_3	20.36
4	D	5-F	17.17
5	E	5-$COOCH_3$	16.50
6	F	5-Cl	16.04
7	G	7-Cl	16.95
8	H	5-Br	16.38
9	I	6-Br	16.03
10	J	5-NO_2	32.98
11	K	7-NO_2	40.63
12	L	5-COOH	16.50
13	M	7-$COOCH_3$	19.54
14	Standard	Cisplatin	11.67



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

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