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**RESEARCH ARTICLE**

# SYNTHESIS AND CHARACTERIZATION OF CYTOTOXIC ACTIVITY OF CERTAIN3-((5-((6-BENZOYL)-1H- BENZO] IMIDAZOL -2-YL) AMINO)-1, 3, 4-OXADIAZOL-2-Y L)IMINO)INDOLIN-2-ONES

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***ABSTRACT***

***A*** *series of 3-((5-((6-benzoyl)-1H-benzo] imidazol -2-yl) amino)-1, 3, 4-oxadiazol-2-yl) imino) indolin-2-ones were synthesized .The novel compounds were characterized on the basis of spectral (FT-IR, $^1$H NMR, Mass) analysis. All the synthesized derivatives were screened for anticancer activity against different cell lines like Hela, MCF-7, HCT-116 and HepG2 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 18.011-78.021. The potency of ($IC_{50}$ values) of cytotoxicity of compounds was compared with that of known cytotoxic agent, Cisplatin. Among all the synthesized novel compounds 7-cl showed the most potent activity against against different cancer cell lines like Hela, MCF-7, HCT-116 and, $HepG_2$ Cell lines using MTT assay.*

***Key words:*** *Isatin, anticancer activity, MTT assay.*

A dose of cytotoxic drug sufficient to kill tumor cells, isoften toxic to the normal tissues and leads to many side effects, which in turn limits its treatment efficacy. In this study, we have synthesized thirteen compounds and were evaluated for their cytotoxicity against different cell lines like Hela, MCF-7, HCT-116, and HepG Cancer cell lines, using MTT assay.

Isatins are an important group of heterocyclic compounds which are biologically active and of significant importance in medicinal chemistry. A literature survey identified several isatin derivatives in the development phase as potential new drugs. A variety of biological and pharmacological activities are associated with isatins including analgesic[2], anticonvulsant[3], antidepressant[4], anti-inflammatory[5] and antimicrobial activities[6]. It also shows significant effect on the central nervous system[7].

Isatins are capable of crossing the blood–brain-barrier[8]. Isatin, a heterocyclic compound was identified in animals as a major component of the endogenous monoamine oxidase inhibitor. Isatins with various substituents at 3rd position viz. Substituted phenyl ring moieties, heterocyclic rings and aliphatic systems were reported. Isatin (1H-Indole-2, 3-dione) is one of the most promising new class of heterocyclic molecules having many interesting activity profiles and was found to be well-tolerated in human subjects.

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. Pre coated 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.

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## 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.

**b) Indole-2,3-diones – General Procedure:**
Sulphuric acid (600g, d:1.84, 326 ml) was warmed at 50$^{o}$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$^{o}$C to 70$^{o}$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$^{o}$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

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

A mixture of methyl (6-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 2hrs.The alcohol was reduced to half of its volume and cooled. The product separated was filtered and filtered and washed with 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.

II. Synthesis of (2-((5-amino-1, 2, 4-oxadiazolyl0amino)-1H-benzo[d]imidazol-6y (phenyl) methanone

- N-(6-benzoyl-1H-benzo[d]imidazol-2-yl) hydrazine carboxamide (II) (III, 0.1mol) was dissolved in 25ml of methanol and cooled the solution by adding chopped ice. A cold suspension of cyanogen bromide (CNBr) (0.12mol) in 75 ml of water was added over a period of 5min with rapid stirring. The reaction mixture was stirred for 0.75hrs at room temperature, solid sodium bicarbonate (0.1 mol) was added in small portions to bring the pH 6.5 -7.0. Stirring was continued for another 1hour. The solid separated was filtered, washed with cold water and dried.

III. Synthesis of 3-((5-((6-benzoyl)-1H- benzo] imidazol -2-yl) amino)-1, 3,4-oxadiazol-2-yl) imino) indolin-2-one

- To a warm solution of (2-((5-amino-1,2,4-oxadiazolyl0amino)-1H-benzo[d]imidazol-6yl(phenyl) methanone (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 8-12 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 was characterized by physical data, TLC, melting point, IR spectra, Mass and NMR spectra. Melting points were determined in open capillary tubes on a Thomas Hoover melting point apparatus and are uncorrected.

## PHARMACOLOGICAL SCREENING OF NOVEL COMPOUNDS

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 Compony, USa. Cisplatin was bprocured 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, HeLa, MCF-7,HCT-116 and $HePG_2$ were procured from [NCCS], Pune, India center for cell cultures.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] 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]

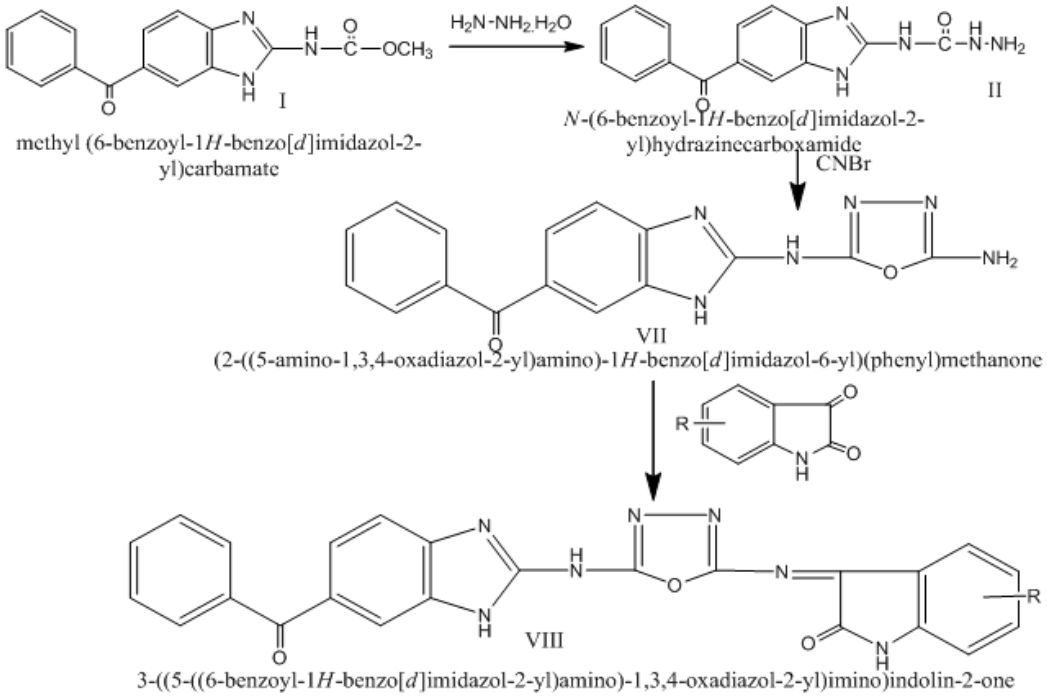
**In vitro cytotoxic activity:**

In-vitro cytotoxic activity against were determined using 96 well tissue culture plates. The cell suspension of $1\times10^5$ 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 ($37^0$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 and 80µl 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.[2] % cytotoxicity=1-[(O.D.in sample well)]/O.D. in control well)] ×100

Physicai data of 3-((5-((6-benzoyl)-1H- benzo] imidazol -2-yl) amino)-1, 3, 4-oxadiazol-2-yl) imino) indolin-2-one (VIII a-m)

| S.no. | Compound Name | R | Molecular formula | Molecular weight | Melting range(°C) | Percentage yield |
|---|---|---|---|---|---|---|
| 1 | VIIIa | H | $C_{24}H_{15}N_7O_3$ | 449 | 309-310 | 89 |
| 2 | VIIIb | 5-CH3 | $C_{25}H_{17}N_7O_3$ | 463 | 301-302 | 81 |
| 3 | VIIIc | 7-CH3 | $C_{25}H_{17}N_7O_3$ | 463 | 290-292 | 82 |
| 4 | VIIId | 5-F | $C_{24}H_{14}N_7O_3F$ | 457 | 294-295 | 87 |
| 5 | VIIIe | 5-COOCH3 | $C_{26}H_{17}N_7O_5$ | 507 | 298-300 | 83 |
| 6 | VIIIf | 5-CL | $C_{24}H_{14}N_7O_3Cl$ | 363 | 292-293 | 81 |
| 7 | VIIIg | 7-CL | $C_{24}H_{14}N_7O_3Cl$ | 363 | 296-298 | 82 |
| 8 | VIIIh | 5-Br | $C_{24}H_{14}N_7O_3Br$ | 528 | 298-299 | 83 |
| 9 | VIIIi | 6-Br | $C_{24}H_{14}N_7O_3Br$ | 528 | 306-308 | 81 |
| 10 | VIIIj | 5-No2 | $C_{24}H_{14}N_8O_5$ | 494 | 302-304 | 84 |
| 11 | VIIIk | 7-No2 | $C_{24}H_{14}N_8O_5$ | 494 | 304-305 | 82 |
| 12 | VIIIl | 5-COOH | $C_{25}H_{15}N_7O_5$ | 493 | 306-307 | 79 |
| 13 | VIIIm | 7-COOCH3 | $C_{26}H_{17}N_7O_5$ | 507 | 308-309 | 82 |

Synthesis of 3-((5-((6-benzoyl)-1H- benzo] imidazol -2-yl) amino)-1, 3, 4-oxadiazol-2-yl) imino) indolin-2-one




R=H,5-$CH_3$,5-Cl,5-Br,5-$NO_2$,7-$NO_2$,5-COOH,5-$COOCH_3$,7-$COOCH_3$

- In vitro cytotoxic activity of 3-((5-((6-benzoyl)-1H- benzo] imidazol -2-yl) amino)-1, 3,4-oxadiazol-2-yl) imino) indolin-2-one VIII (a-m)

| S. No. | COMPOUND NAME | R | $IC_{50}$(µg/ml.) | | | |
|---|---|---|---|---|---|---|
| | | | MCF-7 | HeLa | HCT-116 | HEPG-2 |
| 1 | VIIIa | H | 72.56659 | 43.1245 | 65.321 | 41.5214156 |
| 2 | VIIIb | 5-$CH_3$ | 59.16667 | 65.43424 | 44.5706 | 60.5415646 |
| 3 | VIII c | 7-$CH_3$ | 57.951 | 63.60759 | 84.127 | 90.6415616 |
| 4 | VIII d | 5-F | 18.3251 | 25.69549 | 30.5953 | 95.654656 |
| 5 | VIII e | 5-$COOCH_3$ | 15.84642 | 29.73643 | 69.6078 | 58.5211456 |
| 6 | VIII f | 5-Cl | 19.74026 | 26.37478 | 78.6617 | 47.7214581 |
| 7 | VIII g | 7-Cl | 18.01136 | 23.57664 | 73.511 | 37.7214581 |
| 8 | VIII h | 5-Br | 82.91375 | 86.17737 | 76.977 | 42.5214172 |
| 9 | VIII i | 6-Br | 62.09901 | 66.34799 | 65.432 | 54.5214161 |
| 10 | VIII j | 5-$NO_2$ | 38.70238 | 39.95147 | 61.783 | 61.421171 |
| 11 | VIII k | 7-$NO_2$ | 28.16923 | 31.87683 | 88.1735 | 70.5214612 |
| 12 | VIII l | 5-COOH | 19.67051 | 23.62637 | 76.453 | 67.1412171 |
| 13 | VIII m | 7-$COOCH_3$ | 21.2314 | 17.34 | 117.897 | 25.2121521 |
| 14 | Standard | Cisplatin | 11.67 | 14.975 | 8.1 | 7.56 |

## RESULTS AND DISCUSSION

In present study, the compounds of 3-((5-((6-benzoyl)-1H-benzo] imidazol-2-yl) amino)-1, 3, 4-oxadiazol-2-yl) imino) indolin-2-ones were synthesized as depicted in the Scheme. The thirteen different novel c were prepared. The physical data of the all synthesized compounds ompounds were purified by column chromatography. The thirteen compounds were tested for their invitro cytotoxic activity against, HeLa, MCF-7, HCT-116, and HePG2, cancer cell lines by using MTT assay method. The results were satisfactory. All the compounds were effective against all the cell lines. The compounds depicted results are significant for all the four cell lines.

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