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Synthesis, cytotoxic and antioxidant evaluation of some new N-{5-[(4-fluorophenyl) carbonyl]-1H-benzimidazol-2-yl} -2-(3-oxo-2, 3-dihydro-1H-substitutedisoindol-1-yl) hydrazine carboxamides

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Abstract: *The present study involves synthesis of new heterocyclic moieties and screening them for cytotoxic and antioxidant activities. The titled compounds were synthesized by condensation reaction with different substituted isatin derivatives. The compounds were evaluated for their cytotoxic activity by MTT assay method and antioxidant activity by DPPH method. Among the compounds para chloro derivative was active as cytotoxic and ortho fluoro derivative was active as an antioxidant.*

Key words: *MTTassay, DPPHassay, cytotoxic.*

I. Introduction

Benzimidazole carbamates are popularly known as anthelminthic drugs like fenbendazole, albendazole, thiabendazole, mebendazole etc. Benzimidazole carbamates exert their toxic action on helminthes by binding to the parasite ß-tubulin microtubules.[1]Inhibition of microtubule polymerization and impaired glucose uptake and also inhibits fumarase reductase and glucose transport, which is associated with adenosine triphosphate (ATP) synthesis.[2] Due to point mutations in parasite ß-tubulin confer resistance to the pharmacological activity of these agents, this being the major limitation to their use. Experimental evidence is available to show that albendazole not only kills the adult stages of gut-dwelling helminthes, but it also kills or sterilizes eggs[3] and larvae[4].

In view of biological significance of benzimidazole carbamate moiety, it is planned to synthesize some new derivatives of benzimidazole carbamate containing isatin or oxadiazole moieties to get more potent compounds and evaluate them for some biological activities.

II. Experimental Work

II.1: Synthesis of N-{5-[(4-fluorophenyl) carbonyl]-1H-benzimidazol-2-yl} hydrazine carboxamide (II):
Methyl-{5-[(4-fluorophenyl) carbonyl]-1H-benzimidazol-2-yl} carbamate (I)(0.01mol) was refluxed with hydrazine hydrate 99% (0.2mole) in 20 ml of methanol for 2 hrs. The solvent was evaporated and poured into ice cold water and collected the product by filtration dried and purified with methanol. ESI-MS $[m/z]^+$ 315.

II.2: Synthesis of N-{5-[(4-fluorophenyl) carbonyl]-1H-benzimidazol-2-yl} -2-(3-oxo-2, 3-dihydro-1H-substitutedisoindol-1-yl) hydrazine carboxamides (III a-m):
To a warm solution of N-{5-[(4-fluorophenyl)carbonyl]-1H-benzimidazol-2-yl} hydrazine carboxamide (II) (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 III (a-m) were characterized by physical data, TLC, melting point, IR, Mass and PMR spectra. Melting points were determined in open capillary tubes on a Thomas Hoover melting point apparatus and were uncorrected.

III. Characterization

III.1: N-{5-[(4-fluorophenyl)carbonyl]-1H-benzimidazol-2-yl}-2-(3-oxo-2,3-dihydro-1H-isoindol-1-yl) hydrazine carboxamide (III a): % yield: 65. m.p:250-253. IR (KBr $cm^{-}1$): 3375.43 (N-H st), 1727.82 (C=O st), 1639.70 (C=O st), 1269.72(C-N st), 1114.00(C-F st)**.**PMR:Ar-7.1(t,1H),7.38-7.41(t,2H),7.5-7.6(t,2H),7.8-7.9(t,3H),8.1(d,2H),8.35(d,1H),11.45(s,1NH),12.0(s,2H),14.0(s,NH). ESI-MS: (m/z) 445$[M]^+$

III.2: N-{5-[(4-fluorophenyl) carbonyl]-1H-benzimidazol-2-yl} -2-(5-methyl-3-oxo-2,3-dihydro-1H-isoindol-1-yl) hydrazine carboxamide (III b): % yield: 62,m.p:253-255, IR(KBr $cm^{-}1$): 3373.43 (N-H st), 1726.82 (C=O st), 1639.69 (C=O st),1269.70(C-N st),1114.00(C-F st)**.**PMR:1.12(s,3H),Ar-7.09(t,1H),7.38-7.41(d,1H),7.51-7.61(t,2H),7.8-7.9(t,3H),8.1(d,2H),8.35(d,1H),11.45(s,1NH),12.0(s,2H),14.0(s,NH).ESI-MS: (m/z) 459$[M]^+$

III.3: N-{5-[(4-fluorophenyl) carbonyl]-1H-benzimidazol-2-yl} -2-(7-methyl-3-oxo-2, 3-dihydro-1H-isoindol-1-yl) hydrazine carboxamide (III c): % yield: 63, m.p: 252-254, IR(KBr $cm^{-}1$): 3375.43 (N-H st), 1727.82 (C=O st), 1639.70 (C=O st), 1269.72(C-N st),1114.00(C-Fst)**.**PMR:1.13(s,3H),Ar-7.1(t,1H),7.38-

7.41(d,1H),7.5-7.6(t,2H),7.8-7.9(t,3H),8.1(d,2H),8.35(d,1H),11.45(s,1NH),12.0(s,2H),14.0(s,NH)..ESI-MS: (m/z) 459[M]$^+$

III.4: N-{5-[(4-fluorophenyl) carbonyl]-1H-benzimidazol-2-yl} -2-(5-fluoro-3-oxo-2, 3-dihydro-1H-isoindol-1-yl) hydrazine carboxamide (III d): % yield: 65, m.p:240-242, IR(KBr cm^{-}1): 3375.43 (N-H st), 1727.92 (C=O st), 1639.80 (C=O st), 1269.92(C-N st),1114.80(C-Fst)**.**PMR:Ar-7.14(t,1H),7.38-7.45(t,1H),7.52-7.61(t,2H),7.8-7.9(t,3H),8.1(d,2H),8.35(d,1H),11.45(s,1NH),12.1(s,2H),14.1(s,NH).ESI-MS: (m/z) 463[M]$^+$

III.5: N-{5-[(4-fluorophenyl) carbonyl]-1H-benzimidazol-2-yl} -2-(5-carbomethoxy-3-oxo-2, 3-dihydro-1H- isoindol-1-yl) hydrazine carboxamide (III e): % yield: 68, m.p:252-255, IR(KBr cm^{-}1): 3375.23 (N-H st), 1727.62 (C=O st), 1639.70 (C=O st), 1269.22(C-N st),1114.00(C-Fst)**.**PMR:Ar-7.11(t,1H),7.38-7.4(t,1H),7.49-7.59(t,2H),7.8-7.9(t,3H),8.1(d,2H),8.35(d,1H),11.41(s,1NH),12.0(s,2H),14.0(s,NH).ESI-MS: (m/z) 503[M]$^+$

III.6: N-{5-[(4-fluorophenyl) carbonyl]-1H-benzimidazol-2-yl} -2-(5-chloro-3-oxo-2, 3-dihydro-1H-isoindol-1-yl) hydrazine carboxamide (III f): % yield: 62, m.p:240-242. IR(KBr cm^{-}1): 3438.26(N-H st), 1734.62 (C=O st), 1642.66 (C=O st), 1271.67 (C-N st), 1225.72 (C-F st), 1093.96(C-Clst).PMR:Ar-7.12(t,1H), 7.38-7.42(t,1H), 7.5-7.6(t,2H),7.87.9(t,3H),8.1(d,2H),8.35(d,1H),11.45(s,1NH),12.0(s,2H),14.1(s,NH). ESI-MS: (m/z) 479[M]$^+$

III.7: N-{5-[(4-fluorophenyl) carbonyl]-1H-benzimidazol-2-yl} -2-(7-chloro-3-oxo-2, 3-dihydro-1H-isoindol-1-yl) hydrazine carboxamide (III g): % yield: 61, m.p:239-242, IR(KBr cm^{-}1): 3377.43 (N-H st), 1729.82 (C=O st), 1639.70 (C=O st), 1269.72(C-N st),1114.00(C-F)**.**PMR:Ar-7.11(t,1H),7.38-7.41(t,1H),7.51-7.62(t,2H),7.8-7.9(t,3H),8.1(d,2H),8.35(d,1H),11.45(s,1NH),12.0(s,2H),14.0(s,NH). ESI-MS: (m/z) 459[M]$^+$

III.8: N-{5-[(4-fluorophenyl) carbonyl]-1H-benzimidazol-2-yl} -2-(5-bromo-3-oxo-2,3-dihydro-1H-isoindol-1-yl) hydrazine carboxamide (III h): % yield:66 m.p :236-238, IR(KBr cm^{-}1): 3375.93 (N-H st), 1727.92 (C=O st), 1639.70 (C=O st), 1269.92(C-N st),1114.10(C-F st)**.** PMR:Ar-7.12(t,1H),7.39-7.42(t,1H),7.5-7.6(t,2H),7.8-7.9(t,3H),8.1(d,2H),8.35(d,1H),11.45 (s,1NH),12.1(s,2H),14.0(s,NH).ESI-MS: (m/z) 524[M]$^+$

III.9: N-{5-[(4-fluorophenyl) carbonyl]-1H-benzimidazol-2-yl} -2-(7-fluoro-3-oxo-2, 3-dihydro-1H-isoindol-1-yl) hydrazine carboxamide (III i): % yield: 65,m.p:241-243, IR(KBr cm^{-}1): 3375.93 (N-H st), 1727.82 (C=O st), 1639.70 (C=O st), 1269.92(C-N st),1114.20(C-F st)**.** PMR:Ar-7.16(t,1H),7.38-7.44(t,2H),7.5-7.62(t,2H),7.8-7.9(t,3H),8.1(d,2H),8.35(d,1H),11.46(s,1NH),12.0(s,2H),14.0(s,NH).ESI-MS: (m/z) 463[M]$^+$

III.10:N-{5-[(4-fluorophenyl) carbonyl]-1H-benzimidazol-2-yl} -2-(5-nitro-3-oxo-2, 3-dihydro-1H-isoindol-1-yl) hydrazine carboxamide (III j): % yield: 58, m.p:254-256. IR (KBr cm^{-}1): 3593.44 (N-H st), 1737.68 (C=O st), 1644.76 (C=O st), 1270.14 (C-N st), 1224.10 (C-F st), 1092.36(C-Cl st).PMR:Ar-7.09(t,1H),7.33-7.41(t,1H),7.48-7.59(t,2H),7.8-7.9(t,3H),8.1(d,2H),8.35(d,1H),11.45s,1NH),12.0(s,2H), 14.0(s,NH). ESI-MS: (m/z) 490[M]$^+$

III.11: N-{5-[(4-fluorophenyl) carbonyl]-1H-benzimidazol-2-yl}-2-(7-nitro-3-oxo-2, 3-dihydro-1H-isoindol-1-yl) hydrazine carboxamide (III k): % yield: 57, m.p:255-257. IR (KBr cm^{-}1): 3375.43 (N-H st), 1727.82 (C=O st), 1639.50 (C=O st), 1269.52(C-N st), 1114.00(C-Fst)**.**PMR:Ar-7.01(t,1H),7.38-7.31(t,1H),7.5-7.6(t,2H),7.8-7.9(t,3H),8.1(d,2H),8.35(d,1H),11.45 (s,1NH),12.0(s,2H),14.0(s,NH).ESI-MS: (m/z) 490[M]$^+$

III.12: N-{5-[(4-fluorophenyl) carbonyl]-1H-benzimidazol-2-yl}-2-(5-carboxy-3-oxo-2, 3-dihydro-1H-isoindol-1-yl) hydrazine carboxamide (III l): % yield: 58, m.p:248-250. IR(KBr cm^{-}1): 3375.43 (N-H st), 1727.82 (C=O st), 1639.56 (C=O st), 1269.72(C-N st),1114.00(C-F st)**.** PMR:Ar-7.11(t,1H),7.38-7.41(t,1H),7.5-7.6(t,2H),7.8-7.9(t,3H),8.1(d,2H),8.35(d,1H),11.45 (s,1NH),12.0(s,2H),14.0(s,NH).ESI-MS: (m/z) 489[M]$^+$

III.13: N-{5-[(4-fluorophenyl) carbonyl]-1H-benzimidazol-2-yl}-2-(5-carbomethoxy-3-oxo-2, 3-dihydro-1H-isoindol-1-yl) hydrazine carboxamide (III m):% yield: 59, m.p:251-254.IR (KBr cm^{-}1): 3375.33 (N-H st), 1727.82 (C=O st), 1639.70 7.9(t,3H),8.1(d,2H),8.35(d,1H), (C=O st), 1269.72(C-Nst),1114.00(C-Fst)**.**PMR:Ar-7.05(t,1H),7.38-7.41(t,1H),7.5-7.6(t,2H),7.8-11.45(s,1NH),12.0(s,2H),14.0(s,NH).ESI-MS: (m/z) 503[M]$^+$

IV. Biologicalactivity

IV.1: In vitro cytotoxic activity:

The cell cultures HeLa (cervical) 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^2 tissue 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 cell line was determined using 96 well tissue culture plates. The method followed in the evaluation was standard MTT assay method.[5,6] The cell suspension of 1×105 cells/ml was prepared in complete growth medium. The drug solution was serially diluted at concentration of 1µg/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^{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 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.

IV.2: In vitro antioxidant activity:

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.[7] 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.)

V. Results And Discussion

The title compounds were synthesized by taking Methyl-{5-[(4-fluorophenyl) carbonyl]-1H-benzimidazol-2-yl} carbamates as starting material. This was treated with hydrazine hydrate to get N-{5-[(4-fluorophenyl) carbonyl]-1H-benzimidazol-2-yl} hydrazine carboxamide. This is again treated with substituted isatin derivatives to get title compounds.

The in vitro cytotoxic activity data of N-{5-[(4-fluorophenyl) carbonyl]-1H-benzimidazol-2-yl}-2-(3-oxo-2,3-dihydro-1H-substituted isoindol-1-yl) hydrazine carboxamides (III a-m) on HeLa cell lines and antioxidant activity were presented in table 1. The IC_{50} values of all these synthetic compounds for cytotoxic activity were found between 23.59 and 33.89.Among the series, Compound III f (R=5-Cl) showed better activity with IC_{50} value as 23.59 µg/ml. The Compounds III d (R=5-F) and III a (R=H) are next in order having IC_{50} values as 23.69 µg/ml and 24.23 µg/ml respectively. Compound III j (R=5-NO_2) was least active among them with an IC_{50} value as 33.89 µg/ml. The IC_{50} values of all these synthetic compounds for antioxidant activity were found between 35.36 and 49.36. Among the series, III I (R= 7-F) was active with an IC_{50} value as 35.36 µg/ml. Compound III h (R=5-Br) was next in order with an IC_{50} value as 42.36 µg/ml. Compound III c (R=7-CH_3) was least active among them with an IC_{50} value as 49.36 µg/ml.

VI. Conclusion:

In summary a new series of Benzimidazole carbamate molecules were synthesized, characterized and screened for cytotoxic and antioxidant activities. Among the synthesized compounds 7-chloro substitution increases antioxidant activity and 5-chloro substitution increases cytotoxic activity.

Acknowledgement

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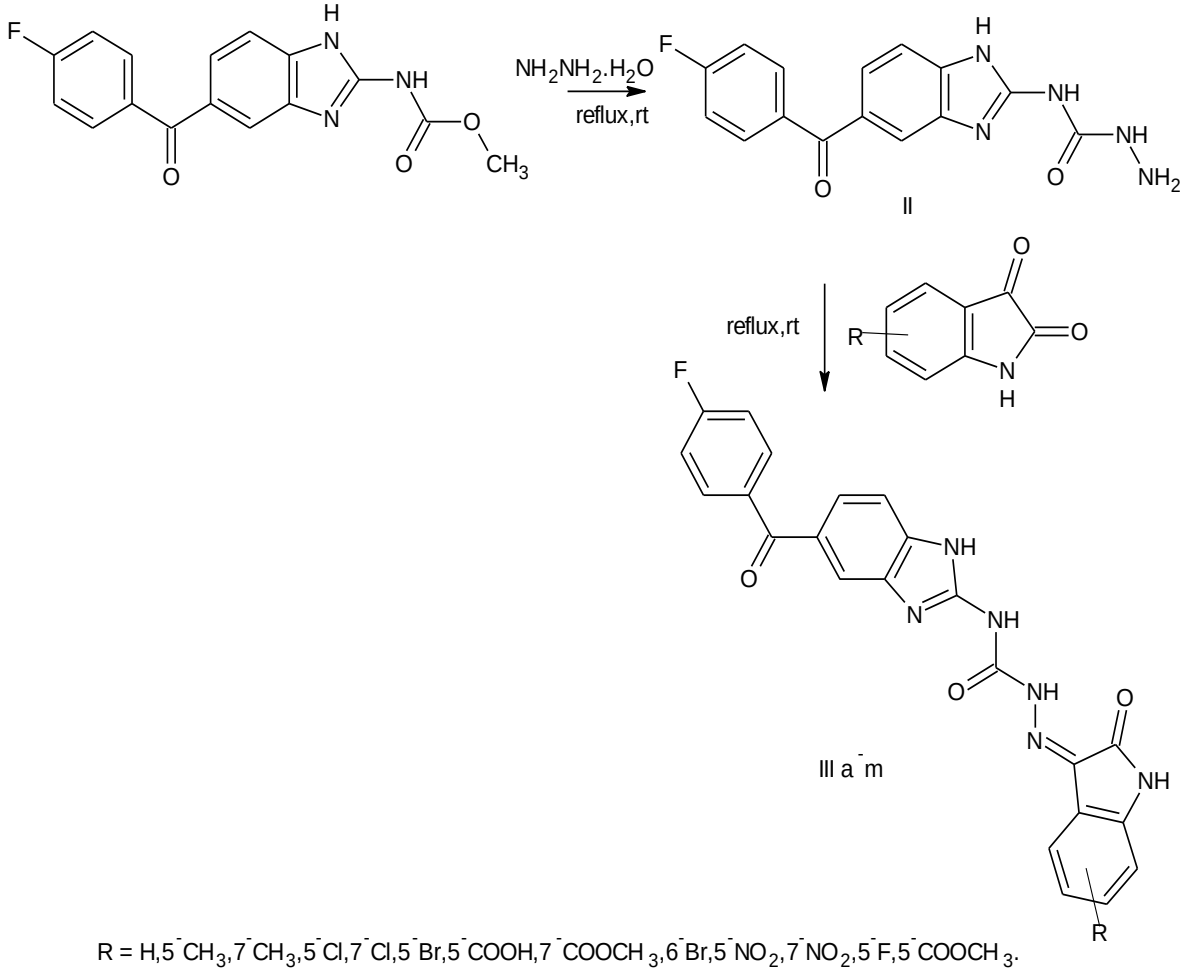
Scheme:

Synthesis of N-{5-[(4-fluorophenyl) carbonyl]-1H-benzimidazol-2-yl} -2-(3-oxo-2, 3-dihydro-1H-substitutedisoindol-1-yl) hydrazine carboxamides (III a-m):



R = H,5-CH_3,7-CH_3,5-Cl,7-Cl,5-Br,5-COOH,7-$COOCH_3$,6-Br,5-NO_2,7-NO_2,5-F,5-$COOCH_3$.

Table 1: In vitro cytotoxic activity data of N-{5-[(4-fluorophenyl) carbonyl]-1H-benzimidazol-2-yl}-2-(3-oxo-2, 3-dihydro-1H- substituted isoindol-1-yl) hydrazine carboxamides (III a-m):

S. No.	Compound Name	R	HeLa IC_{50} (□g/ml)*	Antioxidant IC_{50} (□g/ml)*
1	III a	H	24.23	41.36
2	III b	5-CH3	25.31	48.36
3	III c	7-CH3	26.23	49.36
4	III d	5-F	23.59	48.36
5	III e	5- $COOCH_3$	24.89	39.35
6	III f	5-Cl	23.69	39.23
7	III g	7-Cl	31.23	36.23
8	III h	5-Br	30.52	42.36
9	III i	7-F	28.23	35.36
10	III j	5-NO_2	33.89	39.23
11	III k	7- NO_2	32.51	39.53
12	III l	5-COOH	29.36	40.36
13	III m	7-$COOCH_3$	28.56	39.36
14	Std	Cisplatin	11.66	---
15	Std	Ascorbic acid	---	5.78

*****Values are expresses as means (n=4)