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Synthesis, Cytotoxic, Antioxidant and Anti-Inflammatory Activity of 3-((3-((6-Benzoyl-1H-Benz [D]Imidazol-2-YL) Amino)-5-Mercapto-4H-1,2, 4-Triazol-4-YL) Imino)Substituted Indol-2-Ones

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ABSTRACT

In the present study novel series of 3-((3-((6-benzoyl-1H-benz[d]imidazol-2-yl) amino)-5-mercapto-4H-1, 2, 4-triazol-4-yl)imino)substituted indolin-2-ones have been synthesized in good yields and characterized by IR, NMR and Mass spectral analyses. Compounds were evaluated for their preliminary *in vitro* cytotoxic activity against HCT-116 (colon), MCF-7 (breast), HeLa (cervical) and HepG2 (hepatocellular) cancer cell lines by standard MTT assay method, antioxidant activity by standard DPPH assay method and also were screened for *in vitro* anti-inflammatory activity.

Keywords: Benzimidazole, Isatin, Cytotoxic, Anti-Inflammatory, Antioxidant.

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INTRODUCTION

Numerous compounds bearing benzimidazole moiety as known as to possess important pharmacological activities such as antioxidant [1], anti-inflammatory [2], antihistaminic [3], cytotoxic [4], antimicrobial [5,6,7] anthelmintic [8] activities.

This manuscript reports the synthesis and evaluation of benzimidazole derivatives for their cytotoxic, antioxidant, anti-inflammatory activities.

MATERIALS AND METHOD

The chemicals and solvents are purchased (SD fine) was purchased from local wonders of ALM chemicals, Hanamkonda. The IR spectra were recorded by KBr pellet method (%transmittance, wave number in cm^{-1}) on Bruker FT-IR in the region 4000-500cm^{-1}. The proton nuclear magnetic resonance (^1H NMR) spectra were measured on a Bruker Avance II 400 NMR (400 MHz) spectrometer using tetramethylsilane (TMS) as internal standard. The solvent used to be DMSO-d_6, unless otherwise indicated. Mass spectra (MS) were measured on an ABI Perkin–Elmer Sciex API-150 mass spectrometer with electrospray ionization, and the relative intensity of each ion peak is presented as a percent (%). Melting points were measured with Thomas Hoover melting point apparatus without correction. Thin-layer chromatography (TLC) on silica gel 60 F_{254} plates was used to monitor reactions. All the chemicals used were of AR grade (Sigma-Aldrich, Hi-media).

Synthesis:

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

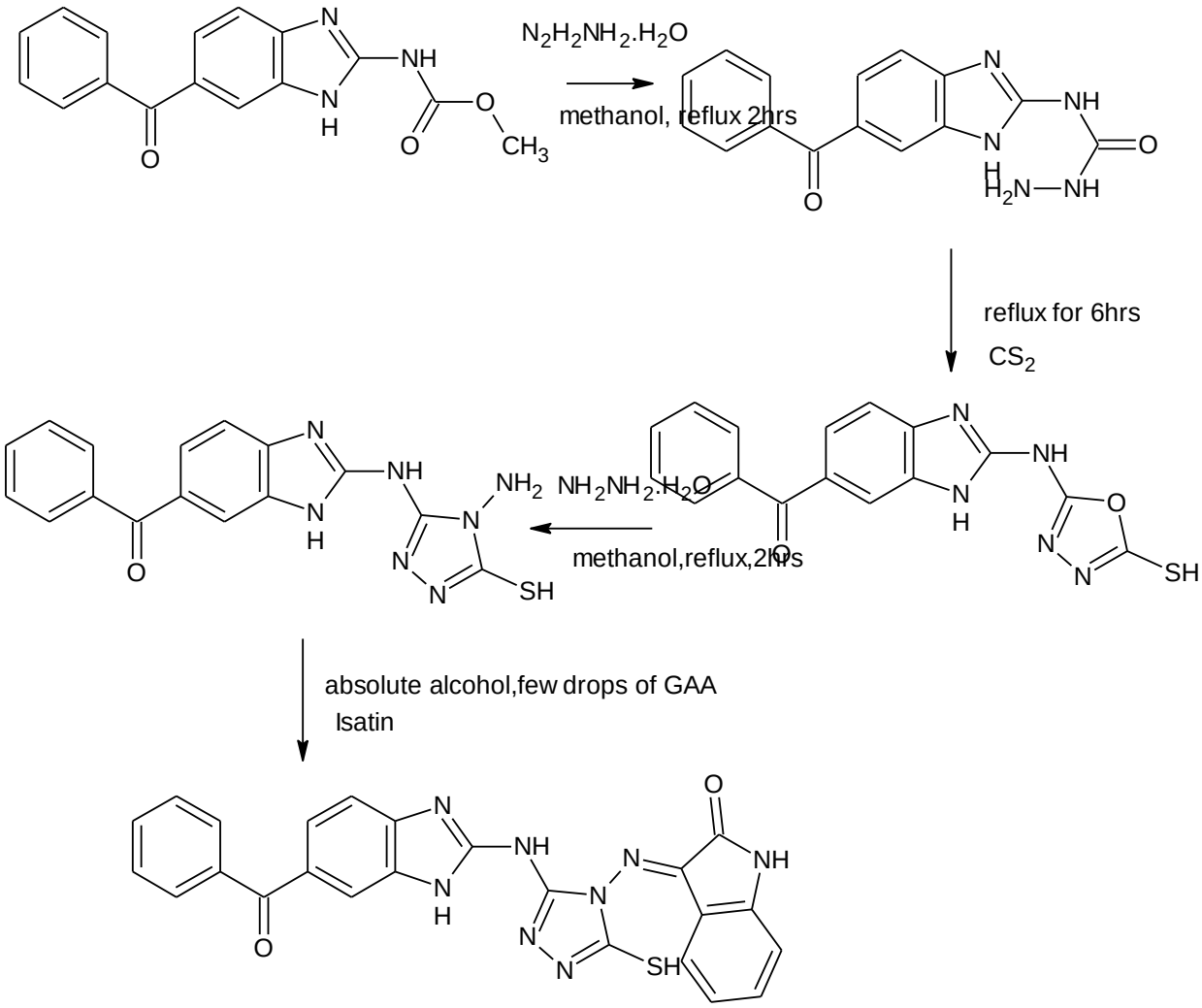
A mixture of methyl (6-benzoyl-1H-benz[d]imidazol-2-yl) carbamate (I) (0.01mole) and 0.01mole of hydrazine hydrate (99%) were taken in 20ml of methanol, heated under reflux on a water bath for 2hrs.The alcohol was reduced to half of its volume and cooled. The product separated and filtered and washed with small portions of cold alcohol first and then with cold water repeatedly and dried. The product was purified by recrystallization from methanol has resulted white solid.

II. Synthesis of (2-((5-mercapto-1, 3, 4-oxadiazol-2-yl) amino)-1H-benz[d]imidazol-6-yl)(phenyl) methanone (IV):

To compound N-(6-benzoyl-1H-benz[d]imidazol-2-yl) hydrazine carboxamide (II)(0.01 mol) carbon disulphide (0.06 mol) in alcoholic Potassium hydroxide (10ml) was added and refluxed for 6 hrs. The contents were concentrated by distillation and acidified with dilute HCl (10%) to obtain a colorless product. The resulting product was filtered, dried and recryastllized from methanol to yield yellow crystalline solid.

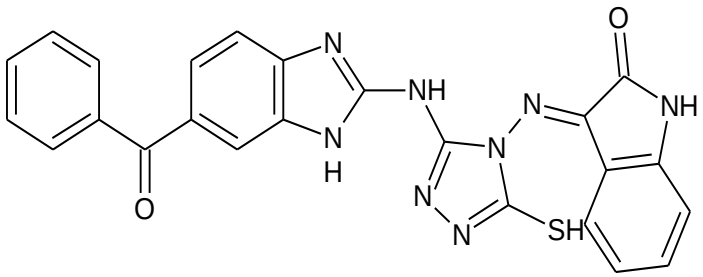
Scheme:

Synthesis of 3-((3-((6-benzoyl-1H-benz[d]imidazol-2-yl) amino)-5-mercapto-4H-1,2 ,4-triazol-4-yl)imino)substituted indolin-2-one(VI a-m)



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

Table 1: Physical data of 3-((3-((6-benzoyl-1H-benz[d]imidazol-2-yl) amino)-5-mercapto-4H-1,2 ,4-triazol-4-yl)imino)indolin-2-one(VI a-m)



S. No.	Compound	Substituents (-R)	Molecular formula	M. Wt	M.R. (ºC)	Yield (%)
1	VI a	H	$C_{24}H_{16}N_8SO_2$	480	291-292	84
2	VI b	5-CH_3	$C_{25}H_{18}N_8SO_2$	494	>300	83
3	VI c	7-CH_3	$C_{25}H_{18}N_8SO_2$	494	297-299	82
4	VI d	5-F	$C_{24}H_{15}N_8SO_2F$	498	298-299	75
5	VI e	5-$COOCH_3$	$C_{26}H_{18}N_8SO_4$	538	297-298	79
6	VI f	5-Cl	$C_{24}H_{15}N_8SO_2Cl$	514	296-298	72
7	VI g	7-Cl	$C_{24}H_{15}N_8S0_2Cl$	514	287-288	73
8	VI h	5-Br	$C_{24}H_{15}N_8SO_2Br$	559	286-289	81
9	VI i	6-Br	$C_{24}H_{15}N_8SO_2Br$	559	284-286	76
10	VI j	5-NO_2	$C_{24}H_{15}N_9SO_4$	525	285-287	71
11	VI k	7-NO_2	$C_{24}H_{15}N_9SO_4$	525	284-287	77
12	VI l	5-COOH	$C_{25}H_{16}N_8SO_4$	524	281-284	77
13	VI m	7-$COOCH_3$	$C_{26}H_{18}N_8SO_4$	538	283-285	82

III. Synthesis of (2-((4-amino-5-mercapto-4H-1, 2, 4-triazol-3-yl) amino)-1H-benz [d]imidazol-6-yl) (phenyl) methanone (V):

(2-((5-mercapto-1, 3, 4-oxadiazol-2-yl) amino)-1H-benz [d]imidazol-6-yl) (phenyl) methanone (III) was taken in a round bottom flask dissolved in methanol. To that 0.01 mole of hydrazine hydrate (99%) was added to that and refluxed for 2hrs. The alcohol was reduced to half of its volume and cooled. The product separated was 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**.**

IV. Synthesis of 3-((3-((6-benzoyl-1H-benz[d]imidazol-2-yl) amino)-5-mercapto-4H-1, 2 ,4-triazol-4-yl) imino)substituted indolin-2-ones (VI a-m):

The(2-((4-amino-5-mercapto-4H-1,2,4-triazol-3-yl)amino)-1H-benz[d]imidazol-6-yl)(phenyl) methanone (0.01mol), appropriate isatin (0.01mol) in methanol (20ml) and 2 drops of glacial acetic acid were heated under reflux on water bath for 8-12 hrs. The product thus obtained was filtered, washed with water.

Spectral data of 3-((3-((6-benzoyl-1H-benz[d]imidazol-2-yl) amino)-5-mercapto-4H-1, 2,4-triazol-4-yl)imino) substituted indolin-2-one(VI a-m):

IR spectra of 3-((3-((6-benzoyl-1H-benz[d]imidazol-2-yl) amino)-5-mercapto-4H-1,2 ,4-triazol-4-yl)imino)indolin-2-one VI a :

A band at 3370.55 cm-1 indicates the N-H stretching. A band at 2879.03 cm^{-1} indicates the presence of aliphatic C-H stretching. A band at 1638.54 cm^{-1} indicates C=O stretch of amide. A band at 1731.56 cm^{-1} indicates C=O stretch of ketone. A band at 1263 cm^{-1} indicates C-N stretch.

MASS spectrum of 3-((3-((6-benzoyl-1H-benz[d]imidazol-2-yl) amino)-5-mercapto-4H-1,2 ,4-triazol-4- yl)imino)indolin-2-one VI a:

ESI-MS spectrum of VIa has shown a molecular ion $[M+H]^+$ at m/z 481.From this data, the molecular weight of the compound was determined to be 480.

NMR spectrum of3-((3-((6-benzoyl-1H-benzo[d]imidazol-2-yl) amino)-5-mercapto-4H-1,2 ,4-triazol-4-yl)imino)indolin-2-oneVIa:3.2,(SH,1H(s),6.9

7.7(12H(m)4.3,NH(s),11.2(NH),13.5(NH),

BIOLOGICAL DATA

In vitro cytotoxic activity

The cell cultures HCT-116 (colon), HepG2 (hepatocellular), HeLa (cervical) and MCF-7 (breast) cancer 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 MCF-7, HCT-116 and HepG2 cancer cell lines was determined using 96 well tissue culture plates. The method followed in the evaluation was standard MTT assay method[9]. The cell suspension of 1×105 cells/ml was prepared in complete growth medium. The drug solution was serially diluted at concentration of 10µg/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 (37^0C, 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.

Evaluation of antioxidant activity:

α,α-Diphenylpicrylhydrazyl (DPPH 1ml of 0.135mM in methanol), a stable free radical was used for the evaluation of the antioxidant activity of the test compounds.[10] 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.)

Evaluation of anti-inflammatory activity:

***In vitro* Anti-inflammatory activity:**

The synthesized compounds were evaluated for their *in vitro* anti-inflammatory activity by TMPD assay method.[11].This assay is based on chromogenic assay based on oxidation of N,N,N',N,-tetra methyl-p-phenylenediamine (TMPD) during the reduction of prostaglandinH_2 by COX-2 enzyme. This measures the peroxides component of cyclooxygenases. The peroxide activity is assayed calorimetrically by monitoring the appearance of oxidized N,N,N',N,-tetramathyl-p-phenylenediamine (TMPD) at 590nm.The final volume of the assay was 220µl I All the wells Background wells contains 160µl of assay buffer and 10µl of heme and 10µl of enzyme. The inhibitor wells contain150µl of assay buffer and 10µl of heme, 10µl of enzyme and 10µl of inhibitor. The plate was shaken for a few seconds and incubated for five minutes at 25ºC.Then 20µl of colorimetric substance, 20µl of arachidonic acid were added. The plate was again shaken for a few seconds and incubated for five minutes at 25ºC.Then the absorbance was noted at 590nm using plate reader.

RESULTS AND DISCUSSION

In vitro anti oxidant activity data of 3-((3-((6-benzoyl-1H-benzo[d]imidazol-2-yl) amino)-5-mercapto-4H-1,2 ,4-triazol-4-yl)imino)substitutedindolin-2-ones (VI a-m)IC_{50} values were presented in table 2 and are compared with the IC_{50} values of standard ascorbic acid with the value of 5.83µg/ml. Most significant of them was the compound VI b(R= 5- CH_3) with an IC_{50} value of 41.01 µg/ml, which is comparatively potent. Compounds VI m (R=7- COO CH_3), VI a (R=H) VI c(R=7- CH_3) have been found to be next in order of antioxidant activity with IC_{50} values of 41.72 µg/ml; 45.21 µg/ml; 48.99 µg/ml respectively. The IC_{50} values of rest of the compounds are in the range of 41.01µg/ml to 59.04µg/ml.

Table 2: Pharmacological activity data of 3-((5-((6-benzoyl)-1H- benzo[d] imidazol -2-yl) amino)-1, 3, 4-oxadiazol-2-yl) imino) substitutedindolin-2-ones (VI a-m)

S.No.	Compound	R	Antioxidant activity IC_{50}(µg/ml)	Cytotoxic activity MCF-7 IC_{50}(µg/ml)	Cytotoxic activity HCT-116 IC_{50}(µg/ml)	Cytotoxic activity HepG2 IC_{50}(µg/ml)	COX-1 IC_{50}(µg/ml)[a]	COX-2 IC_{50}(µg/ml)[a]
1	VI a	H	45.21	41.75	43.12	55.50	51.32	2.81	69.65
2	VI b	5-CH_3	41.01	30.62	65.43	37.47	65.21	3.091	70.43
3	VI c	7-CH_3	48.99	20.36	63.60	40.37	76.42	2.92	73.45
4	VI d	5-F	50.02	17.17	25.69	34.318	87.51	1.72	71.42
5	VI e	5-$COOCH_3$	51.09	16.50	29.73	51.08	48.34	3.00	72.31
6	VI f	5-Cl	52.01	16.04	26.37	65.34	56.43	2.31	71.41
7	VI g	7-Cl	50.09	16.95	23.57	35.00	71.32	2.09	70.12
8	VI h	5-Br	58.07	16.38	86.17	26.85	76.21	2.81	72.43
9	VI i	6-Br	59.04	16.03	66.34	65.32	65.32	3.07	71.32
10	VI j	5-NO_2	54.03	32.98	39.95	39.50	54.43	3.21	70.21
11	VI k	7-NO_2	55.91	40.63	31.87	54.03	61.21	3.23	79.93
12	VI l	5-COOH	49.89	15.86	23.62	54.03	59.32	3.51	62.32
13	VI m	7-$COOCH_3$	41.72	32.32	17.34	36.75	54.32	3.21	72.43
14	standard	Ascorbic acid	5.83	---	---	---	---	---	---
15	standard	Indomethacin	---	---	---	---	---	0.71	0.93
16	standard	Cisplatin	---	11.67	14.97	12.19	7.54	---	---

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

For MCF-7 cell lines, All the synthesized compounds have depicted moderate cytotoxic activity when compared to that of the standard cisplatin with an IC_{50} value of 11.67 µg/ml. Compounds VI l (R=5-COOH); VI i(R=Br), VI f (R=5-Cl), VI h(R=5-Br), VI e (5- C $OOCH_3$), VI g (R=7-Cl) have shown moderate cytotoxic activity with IC_{50} values of 15.86 µg/ml, 16.03 µg/ml, 16.04 µg/ml, 16.39 µg/ml, 16.95 µg/ml respectively. Compounds VI e (5- $COOCH_3$), VI g(7-Cl),VI d(5-F) showed the IC_{50} values of 16.50µg/ml, 16.95 µg/ml,17.17 µg/ml which depicted the moderate activity when compared to that of standard. Compounds VI b (R=5-CH_3),VI j (R=5-NO_2), VI m (R=7-$COOCH_3$ have depicted the values of 30.62 µg/ml, 32.98 µg/ml, 32.32 µg/ml respectively. The IC_{50} values of all compounds are ranging from 15.86 µg/ml to 41.75 µg/ml. For HeLa cell lines, All the synthesized compounds have shown very less activity when compared to that of the standard cisplatin with an IC_{50} values of 8.1 µg/ml. Compound VI m (R=7-$COOCH_3$) has shown the comparatively potent cytotoxic activity with IC_{50} value of 17.34µg/ml. Next in the order are the

compounds VIg (R=7-Cl), VI l (R=5-COOH), VI d (R=5-F), VI l (R= 5-Cl) with the IC_{50} values of 23.57 µg/ml, 23.62 µg/ml, 25.69 µg/ml, 23.37 µg/ml respectively. The IC_{50} values of all compounds are ranging from 17.34µg/ml to 86.17µg/ml. For HCT-116 cell lines, All the synthesized compounds have shown very less activity when compared to that of the standard cisplatin with an IC_{50} values of 8.1 µg/ml. Among all the compounds compound VI h (R=5-Br) has shown significant activity with the cytotoxic value of 26.85µg/ml. The compounds next in order are VIg (R=7-Cl), VI m (R=7-$COOCH_3$) ,VIb(R=5-CH_3),VI c (R=7-CH_3)with the IC_{50} values of 35.00 µg/ml, 36.75 µg/ml, 37.47 µg/ml, 40.37 µg/ml respectively. The IC_{50} values of all compounds are ranging from 26.85µg/ml to 55.50µg/ml. For $HepG_2$ Cell lines, among the compounds compound VI e (5-$COOCH_3$) has shown comparatively potent cytotoxic activity of 48.34µg/ml. The compounds next in order are VIa(R=H),VIm(R= 7-$COOCH_3$), VIb(R=5- CH_3), VI c (R=7-CH_3) with the IC_{50} values of 51.32 µg/ml, 54.32µg/ml, 48.34 µg/ml, 56.43 µg/ml respectively. The IC_{50} values of all compounds are ranging from 48.34µg/ml to 76.421µg/ml respectively.

The COX-1 and COX-2 inhibitory activity of thirteen compounds of 3-((3-((6-benzoyl-1H-benzo[d]imidazol-2-yl) amino)-5-mercapto-1, 3, 4-oxadiazol-2-yl)imino)substituted indolin-2-one (VI a-m) is depicted in table- 2. For COX -1 activity the compound VI d(R=5-F) has shown significant activity when compared to that of standard, with the IC_{50} value of 1.72µg/ml. Compounds VI f (5-Cl),VI c (R= 7-CH_3), VI b (R=5-CH_3)), VI a (R=H) have been found to be next in order with IC_{50} values of 2.31 µg/ml, 3.18 µg/ml, 3.192 µg/ml, 3.81 µg/ml respectively. The IC_{50} values of the all compounds are in the ranging from1.72 µg/ml to 4.81µg/ml. Among all the compounds, compound VI a (R=H) showed the inhibition of 89.65µg/ml. Other compounds in the order are VIg (R=7-Cl), VI j (R=5-No_2), VI b (5-CH_3) with the IC_{50} values of 90.12 µg/ml, 90.21µg/ml, 90.43 µg/ml respectively. The IC_{50} values of the compounds are in the range of 89.65µg/ml to 93.45µg/ml.

CONCLUSION

The present study involves synthesis of 3-((3-((6-benzoyl-1H-benz[d]imidazol-2-yl) amino)-5-mercapto--1, 3, 4-oxadiazol-2-yl) imino) substituted indolin-2-ones (VI a-m) and evaluation of their cytotoxic, antioxidant and anti-inflammatory activities. The titled compounds have shown good cytotoxic agents and moderate anti-inflammatory and antioxidant agents.

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