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SYNTHESIS, CYTOTOXIC EVALUATION AND MOLECULAR PROPERTIES STUDIES OF NOVEL BENZIMIDAZOLE DERIVATIVES WITH DITHIOCARBAMATE SIDE CHAIN

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ABSTRACT

A series of new benzimidazole dithiocarbamates were synthesized and evaluated for antitumor activity against three cancer cell lines (A-549, MDA-MB and HT-29). The chemical structures of the synthesized compounds were established on the basis of spectral data and elemental analyses. They were further subjected to the molecular properties studies using different softwares viz., Mol inspiration, Mol soft, and ALOPGPS 2.1 program. Toxicity parameters were calculated using Osiris Software. All compounds are nontoxic; fulfil the solubility requirements and passing oral bioavailability criteria. Among the series, compound 4p with benzylamino side chain with 5-methyl group exhibited potent in-vitro antitumor activity with IC_{50} values of 3.38±1.9 µg/ml when compared to Cisplatin with IC_{50} of 10.7±1.5 µg/ml against MDA-MB cell lines.

Keywords: Benzimidazole, Dithiocarbamate, Cytotoxicity, Molinspiration, Molsoft, Osiris.

INTRODUCTION

Heterocyclic compounds, in general, are a very important class of organic compounds with various bioactivities ranging from antibacterial to anticancer. Over the past years benzimidazole derivatives are one of the most extensively studied classes of heterocyclic compounds because of the diverse biological activities associated with this class.[1] Moreover benzimidazole derivatives are structural isoesteres of naturally occurring nucleotides, which allows them to interact easily with the biopolymers of the living systems.[2] Therefore, numerous biological activities and functions have been described: anthelmintic,[3] antifungal,[4] antimicrobial,[5] antiviral,[6] anti-allergic,[7] and antitumor activity.[8] A steadily increasing number of studies have been carried out on benzimidazoles and their antitumor activity. Recent reports indicate that benzimidazole linked to pyrrollo-benzodiazepine conjugates is associated with potent in vitro antitumor activity against a number of human cancer cell lines.[9] Benzimidazole derivatives bearing amidino substituents exhibited noticeable selectivity toward (MCF-7) breast cancer cell lines.[10] A member of benzimidazole carbamates (Carbendazim) exhibited in vitro antitumor activity against both the murine B16 melanoma (IC_{50} = 8.5 µM) and human HT-29 colon carcinoma (IC_{50} = 9.5 µM) cell lines [11] and currently undergoing clinical trials of adult patients with advanced malignancies.[12,13] The mechanism of antitumor activity of FB642 and

other benzimidazoles has yet to be fully elucidated. There is some evidence to suggest that the biological effect of these compounds involve interactions with microtubules, uncoupling of G2/M phase and apoptosis.[14,15] On the other hand, dithiocarbamates are a common class of organic molecules, they form mono and bi-dentate co-ordination to transition metal centers. Transition metal complexes of dithiocarbamates present a wide range of biological activities[16] and have found recent application in the treatment of cancer.[17] Since brassinin (figure 1), a phytoalexin first isolated from cabbage had cancer preventive activity, structural modification of this compound led to the synthesis of isobrassinin[18] and Spiro-brassinin[19] and a series of dithiocarbamates, some of which were found to possess significant in vitro and in vivo antitumor activity.[20] In view of the above and as there is no literature report on dithiocarbamate substituted benzimidazoles, we planned to synthesize such molecules, which possibly combine favorable structural properties of both dithiocarbamate and benzimidazole moieties. Herein we present the synthesis of a series of molecules containing both benzimidazole nucleus and dithiocarbamate chain possessing different substituents on the nitrogen linked through methylene group at 2nd position of the benzimidazole and their evaluation for anti-proliferative effects against human cell lines. A major challenge for the development of a drug is the evaluation of its ADME-Tox properties in humans. About 30% of oral drugs fail in development due to poor pharmacokinetics.[21,22] Among the pharmacokinetic parameters, a low and a highly variable bioavailability is indeed the main reason for discontinuation of further drug development process. Thus, predictions of bioavailability and bioavailability–related properties such as solubility, lipophilicity are important before actual synthesis in order to avoid unnecessary spending. Three good examples of free computational tools which were used to predict pharmacokinetic properties of drug candidates are Molinspiration, Molsoft and Osiris.

Molinspiration, Molsoft and Osiris Calculations[23-26]

Drug likeness may be defined as a complex balance of various molecular properties and structure features which determine whether a particular molecule is similar to the known drugs. Molecular formula, Molecular weight, Number of Hydrogen Bond Acceptors, Number of Hydrogen Bond Donors, MolLogP, MollogS, Molecular Polar Surface Area, Molecular Volume, and Computed drug-likeness scores were calculated using Molinspiration and Mol soft software programs and compared them with the values obtained for standard drugs Cisplatin, Streptomycin, fluconazole. Molecular Polar Surface Area (MPSA) is calculated based on the methodology published by Ertl *et al.*[27] The lowest degree of lipophilicity is an indication for good water solubility. Activity of all compounds and standard drugs were rigorously analyzed under four criteria of known successful drug activity in the areas of GPCR ligand activity, ion channel modulation, kinase inhibition activity, and nuclear receptor ligand activity. Structure based design is now fairly routine, but many potential drugs fail to reach the clinic because of ADME-Tox liabilities. Toxicity risks (mutagenicity, tumorogenicity, irritation, reproduction) and physico-chemical properties (mi log P, solubility, drug likeness and drug score) of the compounds are calculated by the methodology developed by Osiris. The ALOGPS method is part of the ALOGPS 2.1 program used to predict lipophilicity and aqueous solubility of compounds. The Log Kow (KOW-WIN) program estimates the log octanol/ water partition coefficient. The XLOGP2 is an atomic additive method applying corrections, the XLOGP2, KOW-WIN both are the best supporters for most of the compounds on the basis of lipophilicity (≤5) to consider an oral drug/lead. All the synthesized compounds were found to fulfill the solubility requirements (ALOGPS).

MATERIALS AND METHODS

All the prepared compounds were characterized by ^{1}H-NMR, IR spectroscopy, elemental analysis, and melting point. Melting points were determined in one-end-open capillary tubes on Shital scientific melting point apparatus and are uncorrected. Infrared spectra recorded on Schimadzu infrared

spectrophotometer in KBr pellets. ^{1}H NMR spectra were determined on a Bruker AVANCE-300 spectrometer. All NMR spectra were measured in $CDCl_3$ or DMSO-d_6 solvents using tetra methylsilane (TMS) as an internal standard and ^{1}H chemical shifts are reported as ppm. Mass spectra were recorded by using electrospray ionization technique (ESI) on the VG170708H mass spectrometer. The CHN analyses were conducted using the Perkin Elmer 240B analyzer. Solvents were dried by conventional methods. Silica gel 60-120 mesh (Merck) was used as an adsorbent for column chromatography. TLC was performed on 5-10 cm aluminum plates coated with silica gel 60F-254 (Merck) in an appropriate solvent.

General Procedure for the Preparation of Benzimidazole Dithiocarbamates

The general experimental procedure for the preparation of compounds shown in figure 1 was as follows: An equimolar mixture of appropriate amine 3 and anhydrous potassium phosphate in dimethyl formamide was stirred at room temperature for 5 min, and then carbon disulfide (3 equiv) was added. The reaction mixture was stirred for additional 20 min, and then appropriate 2-chloromethylbenzimidazole 2, (1 equiv) was added. Stirring was continued at room temperature until the reaction was completed as monitored by TLC. The mixture was poured into cold water, then extracted with ethyl acetate (3x30 mL), the organic phase was washed one time with water and dried with sodium sulfate and filtered. The solvent was evaporated under reduced pressure and the resultant residue was purified by chromatography over silica gel using a mixture of petroleum ether and ethyl acetate as solvent to give the desired compounds 4a-4p.

Anticancer Activity on Human Cancer Cell Lines

Culture media

All the cells were grown in plastic T-25 culture flask in Dulbecco's Modified Eagle's Medium.

Cell lines used

A549 (Lung cancer), HT-29 (Colon cancer) and MDA-MB (Breast cancer) cell lines were obtained from the National Center for Cell Science (NCCS) (University of Pune Campus, Pune). Cisplatin was used as the standard drug. IC_{-50} values were determined from the plot: % inhibition versus concentration.

Procedure

These isolates were tested for their susceptibility to the prepared dithiocarbamates substituted benzimidazole derivatives as follows:

The Dulbecco's Modified Eagle's Medium was supplemented with 10% fetal bovine serum (FBS), 2 mm L-glutamine, 100 µg/mL penicillin, 200 µg/mL streptomycin in a humidified atmosphere with 5% CO_2 at 37^{o}C.

Screening the cytotoxic activity

The test compounds were dissolved in DMSO, in a final concentration never exceeding 0.1%, which have no substantial effect on cell growth. 1x10^4 cells (counted by Trypan blue exclusion dye method) in 96-well plates were incubated with test compounds for 48 h. Then the above media were replaced with 90 µL of fresh serum free media and 10 µL of 3-(4,5-dimethlthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) reagent (5 mg/mL) and plates were incubated at 37^{o}C for 4 h, thereafter the above media was replaced with 200 µL of Dimethyl Sulfoxide (DMSO) and incubated at 37^o C for 10 min. Finally, the absorbance at 570 nm was measured on a spectrophotometer (spectra max, Molecular devices). The assay was performed in triplicate for three independent determinations.

Characterization

1H-benzimidazol-2-ylmethyl N, N-diethyldithio-carbamate (4a)

Yield 91%, white solid. mp 118-120^{0}C, IR: $^{\nu}$max cm^{-1} 3269.38 (-NH), 2924.90 (aliphatic -CH), 1231.82 (C=S).^{1}H-NMR (200 MHz, DMSO-d6)δ: 1.17 (m, 6H, $(CH_3)_2$), 3.76 (q, 2H, ethyl-CH_2), 3.94 (q, 2H, ethyl-CH_2), 4.71 (s, 2H, CH_2S), 7.09-7.50 (m, 4H, Ph-H). MS (ESI) m/z: 280 (M+1, 100%). Anal. Calcd for $C_{13}H_{17}N_3S_2$: C 56.08, H 5.79, N 15.09, S 23.03. Found: C 56.10, H 5.75, N 15.12, S 23.02.

1H-Benzimidazol-2-ylmethyl N, N-diisopropyl-dithiocarbamate (4b)

Yield 89%, white solid, mp 136-138°C, IR: ^{v}max cm^{-1} 3310.27 (NH), 2935.12 (-CH aliphatic), 1242.34 (C=S).^{1}H NMR (300 MHz, $CDCl_3$)δ: 1.5 (m, 14H, $(CH_3)_4$ and $(CH)_2$ of isopropyl), 4.91 (s, 2H, -CH_2S), 7.21-7.70 (m, 4H, Ph-H), 10.55 (bs, 1H, NH). MS (ESI) m/z: 308 (M+1). Anal. Calcd for $C_{15}H_{21}N_3S_2$: C 58.79, H 6.58, N 13.71, S 20.93. Found: C 58.76, H 6.55, N 13.74, S 20.96.

1H-Benzimidazol-2-ylmethy N-piperidinodithiocarbamate (4c)

Yield 86%, white solid, mp 168-170°C, IR: ^{v}max cm^{-1} 3290.09 (NH), 2945.69 (-CH aliphatic), 1267.14 (C=S). ^{1}H NMR (200 MHz, DMSO-d6) δ: 1.60 (s, 6H, piperidine $(CH_2)_3$), 3.89 (bs, 2H, -NCH_2), 4.20 (bs, 2H, -NCH_2), 4.73 (s, 2H, -CH_2S), 7.12-7.47 (m, 4H, Ph-H), 12.35 (bs, 1H, NH). MS (ESI) m/z: 292 (M+1). Anal. Calcd for $C_{14}H_{17}N_3S_2$ C 54.87, H 5.92, N 18.28, S 20.93. Found: C 54.90, H 5.94, N 18.24, S 20.92.

1H-Benzimidazol-2-ylmethyl N-ethyl piperazinodithiocarbamate (4d)

Yield 78%, light brown solid, mp 164-166°C, IR: ^{v}max cm^{-1} 3260.93 (NH), 2931.75(-CH aliphatic), 1253.44 (C=S). ^{1}H NMR (200 MHz, DMSO-d6) δ: 0.99 (t, 3H, CH_3), 2.36 (q, 2H, -CH_2 ethyl), 2.47 (m, 4H, -$N(CH_3)_2$), 3.91 (bs, 2H, -NCH_2), 4.21 (bs, 2H, -NCH_2), 4.74 (s, 2H, -CH_2S), 7.10-7.54 (m, 4H, Ph-H), 12.36 (bs, 1H, NH). MS (ESI) m/z: 321 (M+1). Anal. Calcd for $C_{15}H_{20}N_4S_2$ C 53.70, H 6.31, N 20.87, S 19.12. Found: C 53.68, H 6.29, N 20.90, S 19.13.

1H-Benzimidazol-2-ylmethyl N-morpholinodithiocarbamate (4e)

Yield 96%, White solid. mp 186-188°C, IR: ^{v}max cm^{-1} 3289.32 (NH), 2960.05 (-CH aliphatic), 1260.24 (C=S). ^{1}H NMR (200 MHz, DMSO-d6) δ: 3.66 (m, 4H, -$O(CH_3)_2$), 3.98 (bs, 2H, -NCH_2), 4.18 (bs, 2H, -NCH_2), 4.76 (s, 2H, -CH_2S), 7.12-7.47 (m, 4H, Ph-H), 12.37 (bs, 1H, NH). MS (ESI) m/z: 294 (M+1). Anal. Calcd for. $C_{13}H_{15}N_4OS_2$ C, 50.63; H, 5.23; N, 18.17, O 5.19, S 20.79. Found: C 50.64, H 5.25, N 18.13, O 5.23, S 20.76.

1H-Benzimidazol-2-ylmethyl N-benzylaminodithiocarbamate (4f)

Yield 90%, light brown solid, mp 200-202°C, IR: ^{v}max cm^{-1} 3296.00 (NH), 2972.56 (-CH aliphatic), 1261.12 (C=S). ^{1}H NMR (200 MHz, DMSO-d6) δ: 4.80 (s, 2H, -CH_2), 4.85 (d, 2H, -CH_2S), 7.29-8.38 (m, 8H, Ph-H), 10.69 (bs, 1H, NH). MS (ESI) m/z: 329.45 (M+1). Anal. Calcd for: $C_{16}H_{16}N_4S_2$: C 58.51, H 4.91, N 17.06, S 19.52. Found: C 58.54, H 4.95, N 17.00, S 19.51.

5-Nitro-1H-benzimidazol-2-ylmethyl N, N-diethyldithiocarbamate (4g)

Yield 86%, brown solid, mp 126-128°C, IR: ^{v}max cm^{-1}3282.73 (NH), 2931.13 (-CH aliphatic), 1243.16 (C=S). ^{1}H NMR (200 MHz, DMSO-d6) δ: 1.20 (m, 6H, $(CH_3)_2$), 3.75 (q, 2H, ethyl-CH_2), 3.96 (q, 2H, ethyl-CH_2), 4.81 (s, 2H, -CH_2S), 7.67-8.41 (m, 3H, Ph-H). 13.01 (bs, 1H, NH). MS (ESI) m/z: 325 (M+1). Anal. Calcd for $C_{13}H_{16}N_4O_2S_2$: C, 48.13; H, 4.97; N, 17.27, S 19.77. Found: C, 48.14; H, 4.93; N, 17.30, S 19.73.

5-Nitro-1H-benzimidazol-2-ylmethyl N-piperidinodithiocarbamate (4h)

Yield 82%, light brown solid, mp 162-164°C, IR: ^{v}max cm^{-1} 3299.04 (NH), 2935.45 (-CH aliphatic), 1233.42 (C=S); ^{1}H NMR (200 MHz, DMSO): δ 1.61 (s, 6H, piperidine-$(CH_2)_3$), 3.91 (bs, 2H, -NCH_2), 4.19 (bs, 2H, -NCH_2), 4.83 (s, 2H, -CH_2S), 7.64-8.40 (m, 3H, Ph-H), 13.00 (bs, 1H, NH). MS (ESI) m/z: 337 (M+1). Anal. Calcd $C_{14}H_{16}N_5O_2S_2$: C 47.84, H 4.88, N 19.93, O 9.11, S 18.25. Found: C 47.80, H 4.88, N 19.90, O 9.10, S 18.29.

5-Nitro-1H-benzimidazol-2-ylmethyl N-ethyl piperazinodithiocarbamate (4i)

Yield 80%, light yellow solid, mp 156-158°C, IR: ^{v}max cm^{-1} 3298.12 (NH), 2930.46 (-CH aliphatic), 1243.74 (C=S). ^{1}H NMR (200 MHz, DMSO-d6) δ: 0.99 (t, 3H, CH_3), 2.41 (q, 2H, ethyl- CH_2), 3.30 (s, 4H, -$N(CH_2)_2$), 3.92 (bs, 2H, -NCH_2), 4.20 (bs, 2H, -NCH_2), 4.86 (s, 2H, -CH_2S), 7.64-8.40 (m, 3H, Ph-H), 13.02 (bs, 1H, NH). MS (ESI) m/z: 367 (M+1). Anal. Calcd $C_{15}H_{19}N_6O_2S_2$: C 47.35, H 5.30, N 22.09, O 8.41, S 16.85. Found: C 47.38, H 5.25, N 22.07, O 8.46, S 16.84.

5-Nitro-1H-benzimidazol-2-ylmethyl N-morpholinodithiocarbamate (4j)

Yield 91%, light yellow solid, mp 190-194°C, IR:

^{v}max cm^{-1} 3271.12 (NH), 2922.18 (-CH aliphatic), 1263.09 (C=S).^{1}H NMR (200 MHz, DMSO-d6) δ: 3.66 (m, 4H, -O(CH_2)$_2$), 3.97-4.17 (BS, 4H, -N(CH_2)$_2$), 4.86 (s, 2H, -CH_2S), 7.64-8.40 (m, 3H, Ph-H), 13.04 (bs, 1H, NH). MS (ESI) m/z: 339 (M+1). Anal. Calcd for: $C_{13}H_{15}N_5O_3S_2$: C 44.18, H 4.28, N 19.82, S 13.58. Found: C 44.22, H 4.25, N 19.80, S 13.59.

5-Nitro-1H-benzimidazol-2-ylmethyl N-benzylaminodithiocarbamate (4k)

Yield 90%, light brown solid, mp 202-204^{o}C, IR: ^{v}max cm^{-1} 3298.00 (NH), 2982.56 (-CH aliphatic), 1260.12 (C=S). ^{1}H NMR (200 MHz, DMSO-d6) δ: 4.80 (s, 2H, -CH_2), 4.85 (d, 2H, -CH_2S), 7.29-8.38 (m, 8H, Ph-H), 10.69 (bs, 1H, NH). MS (ESI) m/z: 359 (M+1). Anal. Calcd for: $C_{16}H_{15}N_5O_2S_2$: C 51.46, H 4.05, N 18.75, O 8.57, S 17.17. Found: C 51.41, H 4.00, N 18.79, O 8.60, S 17.20.

5-Methyl-1H-benzimidazol-2-ylmethyl N, N-diethyldithiocarbamate (4l)

Yield 92%, yellow solid, mp 128-130^{o}C, IR: ^{v}max cm^{-1} 3292.09 (NH), 2935.25 (-CH aliphatic), 1260.19 (C=S). ^{1}H NMR (200 MHz, DMSO-d6): δ 1.17 (m, 6H, ethyl-(CH_3)$_2$), 2.36 (s, 3H, CH_3), 3.72 (q, 2H, ethyl-CH_2), 3.94 (q, 2H, ethyl-CH_2), 4.67 (s, 2H, -CH_2S), 6.93-7.37 (m, 3H, Ph-H). 12.25 (bs, 1H, NH). MS (ESI) m/z: 294 (M+1). Anal. Calcd for: $C_{14}H_{19}N_3S_2$: C 57.30, H 6.53, N 14.32, S 21.85. Found: C 57.33, H 6.48, N, 14.32, S 21.87.

5-Methyl-1H-Benzimidazol-2-ylmethyl N-piperidinodithiocarbamate (4m)

Yield 89%, white solid, mp 136-138^{o}C, IR: ^{v}max cm^{-1} 3299.13 (NH), 2930.09 (-CH aliphatic), 1257.98 (C=S). ^{1}H NMR (200 MHz, DMSO-d6) δ: 1.60 (s, 6H, piperidine-(CH_2)$_3$), 2.36 (s, 3H, CH_3), 3.88 (bs, 2H, -NCH_2), 4.20 (bs, 2H, -NCH_2),4.70 (s, 2H, -CH_2S), 6.93-7.37 (m, 3H, Ph-H), 12.25 (bs, 1H, NH). MS (ESI) m/z: 306 (M+1). Anal. Calcd for: $C_{15}H_{20}N_4S_2$: C 56.22, H 6.29, N 17.48, S 20.01. Found: C 56.25, H 6.30, N 17.45, S 20.04 .

5-Methyl-1H-benzimidazol-2-ylmethyl N-ethylpiperazinodithiocarbamate (4n)

Yield 83%, white solid, mp 170-172^{o}C, IR: ^{v}max cm^{-1} 3287.46 (NH), 2929.90 (-CH aliphatic), 1257.39 (C=S).^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.20 (t, 3H, ethyl- CH_3), 2.49 (s, 3H, CH_3), 2.41 (q, 4H, ethyl -CH_2), 2.56 (m, 4H, -N(CH_2)$_2$), 3.95 (bs, 2H, -NCH_2), 4.45 (bs, 2H, -NCH_2), 7.00-7.40 (m, 3H, Ph-H). MS (ESI) m/z: 335 (M+1). Anal. Calcd for $C_{16}H_{23}N_5S_2$: C 54.98, H 6.63, N 20.04, S 18.35. Found: C 54.95, H 6.65, N 20.03, S 18.36.

5-Methyl-1H-benzimidazol-2-ylmethyl N-morpholinodithiocarbamate (4o)

Yield 94%, white solid, mp 190-192^{o}C, IR: ^{v}max cm^{-1} 3279.61 (NH), 2930.90 (-CH aliphatic), 1257.19 (C=S).^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.45 (s, 3H, CH_3), 3.75 (m, 4H, -O(CH_2)$_2$), 3.92 - 4.40 (bs, 4H, -NCH_2), 4.91 (s, 2H, -CH_2S), 7.00-7.60 (m, 3H, Ph-H), 10.20(bs, 1H, NH). MS (ESI) m/z: 308 (M+1). Anal. Calcd for: C_{14}H18N_4OS_2: C 52.15, H 5.63, N 17.38, O 4.96, S 19.89. Found: C 52.17, H 5.65, N, 17.33, O 4.99, S 19.87.

5-Methyl-1H-benzimidazol-2-ylmethyl N-benzyldithiocarbamate (4p)

Yield 92%, white solid, mp 188-190^{o}C, IR: ^{v}max cm^{-1} 3269.11 (NH), 2939.05 (-CH aliphatic), 1259.19 (C=S).^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.45 (s, 3H, CH_3), 4.65 (s, 2H, -CH_2), 4.94 (s, 2H, -CH_2S), 7.00-7.45 (m, 8H, Ph-H), 8.50 (bs, 1H, NH). MS (ESI) m/z: 328 (M+1). Anal. Calcd for: $C_{17}H_{18}N_4S_2$: C 59.62, H 5.30, N 16.36, S 8.73. Found: C 59.63, H 5.36, N 16.30, S 8.74.

RESULTS AND DISCUSSION

Chemistry

2-Chloromethyl benzimidazoles 2, the key intermediates for the synthesis of the title compounds 4a-4p (figure-1) were prepared by the addition of chloro acetic acid to different substituted o-phynylenediamines in 4N Hydrochloric acid as reported earlier.[28] The dithiocarbamates (4a-p) were synthesized from various amines and carbon disulfide, using dimethyl formamide as solvent and anhydrous potassium phosphate as base, followed by treatment with 2-chloromethyl benzimidazole. Completion of the reaction was monitored by TLC. The title compounds 4a-4p were obtained in good yields (78-96%). The structures of the compounds are inconsistent with NMR spectra. The mass spectra of the compounds revealed the

molecular ion as the base peak for almost all compounds. In the ^{1}H NMR spectra, NH proton of benzimidazole appeared at around δ 10.20-13.02, methylene protons of SCH_2 were seen as a sharp singlet between δ 4.70 & 4.94, and aromatic protons of benzimidazole appeared between δ 7.00 & 8.50. Elemental compositions were within ± 0.4% of the calculated values. In the present study the cytotoxic activities of the synthesized compounds were determined by the MTT assay, using the A-549 (Lung cancer), MDA-MB (Breast cancer), and HT-29 (Colon cancer) cell lines. Cisplatin was used for comparison. The assay was performed in triplicate for three independent determinations. The antitumor activity was expressed as IC_{50} (µg/mL), the concentration of the compound that inhibits the proliferation rate of the tumor cells by 50% as compared to the control untreated cells. Compounds with IC_{50} >200 µg/mL were considered to be inactive. The results of the prepared compounds (4a-4p) and the references for preliminary cytotoxic testing are shown in table 1. Results revealed that the majority of the synthesized compounds showed varying degree of inhibition against the tested cell lines. Compound 4p with benzylamino side chain and 5-methyl group exhibited significant activity against MDA-MB and HT-29 cell lines with IC_{50} values 3.38 and 22.00 µg/mL respectively. However the corresponding benzylamino compound 4f, without the methyl group at 5th position showed less potency against these cell lines with IC50 values 28.33 and 56.80 µg/mL respectively. The activity against these two cell lines (MDA-MB and HT- 29) was further decreased with nitro substitution as indicated by the greater IC_{50} values (125.08 and 156.80 µg/mL) exhibited by compound 4k. However, the same 5-nitro substituted compound 4k exhibited greater potency (IC_{50} = 47.80 µg/mL) against A-549 cell lines than the corresponding unsubstituted compound 4f (IC_{50} = 129.16 µg/mL). In contrast to earlier observations, the corresponding methyl derivative 4p showed even less potency against A-549 cell lines than the un-substituted and 5-nitro derivatives. In case of the compounds with alkyl substituted amino dithiocarbamates, the compound 4b, with diisopropyl substitution on the nitrogen showed greater potency than the corresponding diethyl amine compound 4a against A-549 cell lines with IC_{50} values 115.66 and 151.34 µg/mL respectively, indicating the favorable contribution made by the bulky groups. However cyclization of the alkyl substituents to form piperidine and morpholine ring resulted in a greater decrease in potency, as evidenced from the IC_{50} values (>200 µg/mL) of Compounds 4c and 4e. All the synthesized compounds were subjected to molecular properties studies by computational softwares as Molinspiration, Molsoft (Molsoft, 2007), Osiris and ALOGPS 2.1. A good bioavailability can be achieved with an appropriate balance between solubility partitioning properties. The computed physico-chemical properties, Drug likeness scores and Toxicity profiles for all the synthesized compounds are tabulated in the tables 2, 3, and 4.

CONCLUSION

In conclusion a series of benzimidazole linked dithiocarbamates have been synthesized and structures were ascertained based on spectral and elemental analyses. All the compounds were evaluated for antitumor activity against three cancer cell lines (A-549, MDA-MB and HT-29) and studied for their molecular properties using different computational softwares. A few molecules showed significant anti-proliferative activity against different cancer cell lines. More importantly, compound 4p exhibited potential in vitro cytotoxic activity against MDA-MB cancer cell lines, indicating that arylamine side chain with methyl substituent at position 5 of benzimidazole contribute positively to the activity. The compound 4p emerged as an interesting molecule which can be further subjected to in vivo evaluation. A good correlation was observed between experimental and in-silico studies for the synthesized compounds.

CONFLICT OF INTEREST

The authors express no conflict of interest.

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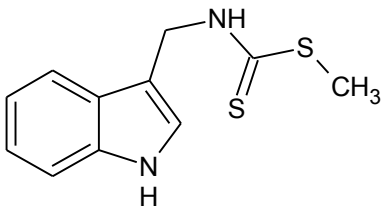


Figure: 1 Brassinin

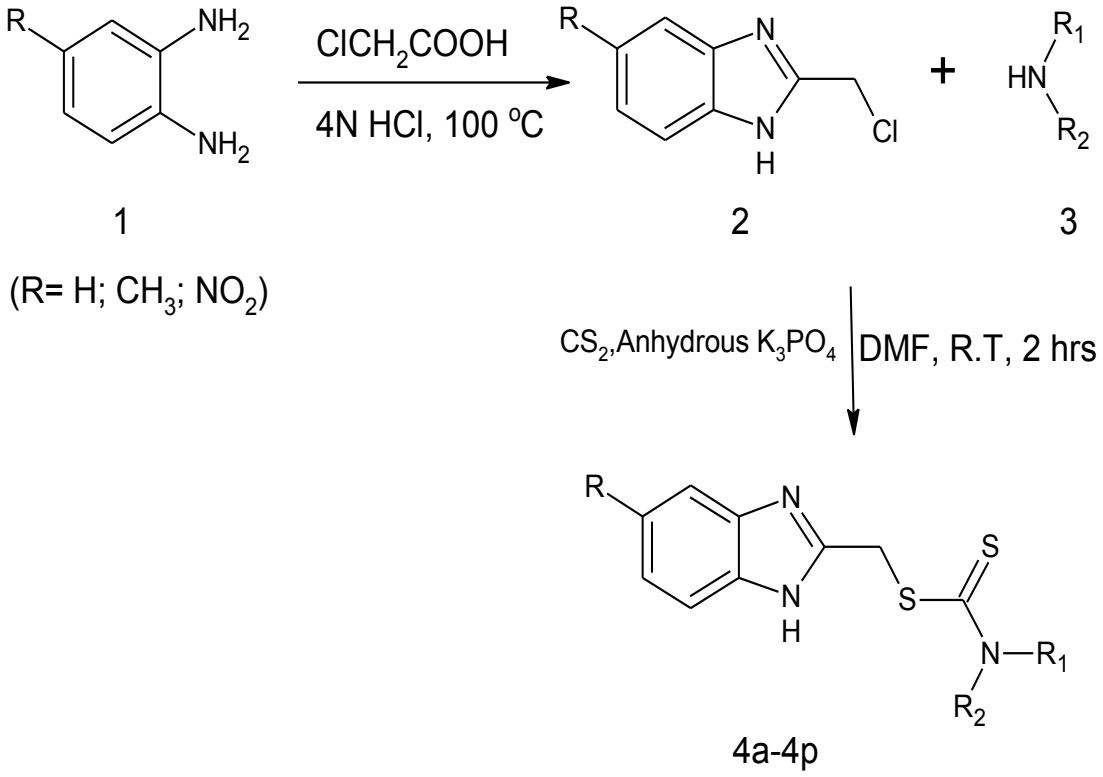
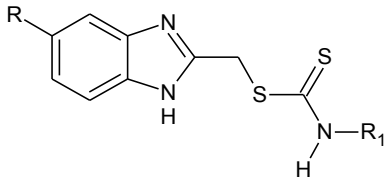
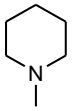
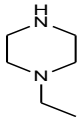
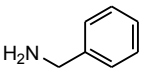
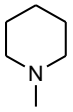
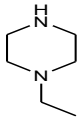
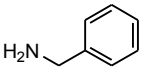
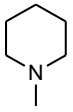
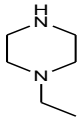
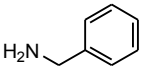


Figure 2: Synthesis of benzimidazole dithiocarbamate derivatives

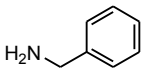
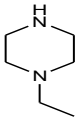
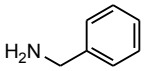
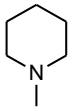
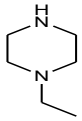
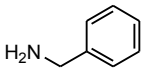
Table1: In-vitro cytotoxicity of compounds 4a-4p against three human cell lines



Compound	R	R_1	A-549	(IC50 µg/mL) MDA-MB	HT-29
4a	H	$-(C_2H_5)_2$	151.34±1.3	NA	NA
4b	H	-di isopropyl	115.66±2.0	NA	NA
4c	H		NA	NA	NA
4d	H		200±1.8	164.30±1.9	NA
4e	H		NA	NA	NA
4f	H		129.16±2.2	28.33±1.9	56.80±0.8
4g	NO_2	$-(C_2H_5)_2$	NA	NA	NA
4h	NO_2		NA	NA	NA
4i	NO_2		137.70±2.1	106.60±0.5	NA
4j	NO_2		192.20±1.9	NA	NA
4k	NO_2		47.8±0.3	125.08±1.8	156.80±2.1
4l	$-CH_3$	$-(C_2H_5)_2$	>200	>200	>200
4m	$-CH_3$		NA	NA	NA
4n	$-CH_3$		NA	NA	NA
4o	$-CH_3$		NA	NA	NA
4p	$-CH_3$		NA	3.38±1.9	22.00±2.5
Cisplatin	-	-	9.2±1.4	10.7±1.5	6.56±0.9

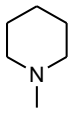
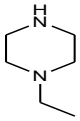
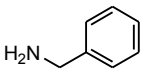
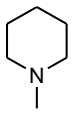
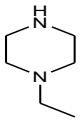
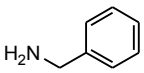
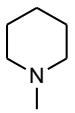
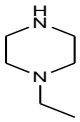
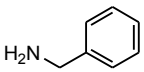
NA- No Activity, i.e., IC_{50} >200 µg/mL

Table 2: Physicochemical properties and Drug likeness scores of Dithiocarbamates substituted benzimidazole derivatives using Molinspiration and Molsoft softwares

Drug likeness												
Compd.	R	R_1	LogP ≤5	TPSA≤ 140Å^2	n ON ≤10	n OHNH ≤5	n atoms	n violations	n rotb ≤ 10	MW <500	MV	DL-S
4a	H	-$(C_2H_5)_2$	2.73	24.32	3	2	18	0	6	279	275.64	0.69
4b	H	-di isopropyl	3.65	24.35	3	1	20	0	6	307	314.8	0.55
4c	H		3.55	25.03	3	1	19	0	4	291	285.37	1.01
4d	H		2.81	29.14	4	1	21	0	5	320	319.87	1.12
4e	H		2.51	32.92	4	1	19	0	4	293	275.62	0.48
4f	H		3.22	32.15	3	2	21	0	6	313	288.53	0.85
4g	NO_2	-$(C_2H_5)_2$	2.76	57.72	5	1	21	0	7	324	300	-0.18
4h	NO_2		3.58	58.44	5	1	22	0	5	336	310.44	-0.07
4i	NO_2		2.04	60.25	6	1	24	0	6	365	344	-0.12
4j	NO_2		2.54	66.34	6	1	22	0	5	338	300.68	-033
4k	NO_2		3.26	65.56	5	2	24	0	7	358	313.60	-0.53
4l	-CH_3	-$(C_2H_5)_2$	3.31	24.32	3	1	19	0	6	293	296.66	0.13
4m	-CH_3		4.13	25.03	3	1	20	0	4	305	306.39	0.54
4n	-CH_3		3.39	29.14	4	1	22	0	5	334	340.88	0.46
4o	-CH_3		3.09	32.92	4	1	20	0	4	307	296.63	0.11
4p	-CH_3		3.80	32.15	3	2	22	0	6	327	309.55	0.18
Cisplatin	-	-	-2.826	52.046	2	4	5	0	0	298	101	

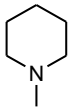
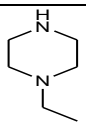
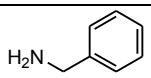
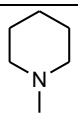
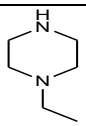
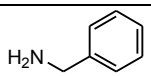
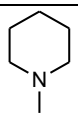
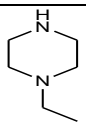
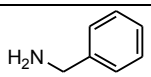
MW, Molecular weight; TPSA, Total Polar Surface Area; nON, no. of Hydrogen bond acceptors; nOHNH, no. of Hydrogen bond donors; nrotb, no. of Rotatable bonds.

Table 3: Toxicological studies and Drug likeness scores of Dithiocarbamates substituted benzimidazole derivatives using Osiris software

S. No.	R	R_1	M	T	I	R. E	CLP	Clog S	MW	DLS	DS
4a	H	$-(C_2H_5)_2$	N	N	N	N	3.28	-2.92	279	1.84	0.79
4b	H	-di isopropyl	N	N	N	N	3.21	-3.36	265	1.18	0.74
4c	H		N	N	N	N	2.44	-3.28	291	0.6	0.66
4d	H		N	N	N	N	2.32	-2.19	320	6.24	0.87
4e	H		N	N	N	N	3.55	-2.39	293	1.31	0.8
4f	H		N	N	N	N	0.5	-4.01	313	0.45	0.6
4g	NO_2	$-(C_2H_5)_2$	N	N	N	N	3.14	-3.38	324	-4.4R	0.41Y
4h	NO_2		N	N	N	N	3.4	-3.74	336	-5.72R	0.38Y
4i	NO_2		N	N	N	N	2.75	-2.75	365	-0.19	0.61LG
4j	NO_2		N	N	N	N	2.19	-2.19	338	-4.91R	0.43Y
4k	NO_2		N	N	N	N	3.68	-3.68	427	-0.56	0.47LGY
4l	$-CH_3$	$-(C_2H_5)_2$	N	N	N	N	3.59	-3.27	293	1.14	0.7
4m	$-CH_3$		N	N	N	N	3.85	-3.62	305	-0.22	0.55
4n	$-CH_3$		N	N	N	N	3.19	-2.54	334	5.34	0.84
4o	$-CH_3$		N	N	N	N	2.64	-2.74	307	0.52	0.71
4p	$-CH_3$		N	N	N	N	3.87	-4.37	327	-0.25	0.49
Cisplatin	-	-	N	N	N	N	0.5	1.93	297	-1.77	0.53

M, Mutagenic; T, Tumorogenic; I, Irritant; R.E, Reproductive Effective; MW, Molecular weight in g/mol; CLP, ClogP; logS, Solubility mol/lit; DL, Drug-Likeness; D-S, Drug-Score.

Table 4: ALOGP calculations of Dithiocarbamates substituted benzimidazole derivatives

S. No.	R	R_1	ALOGPS	KoW-WIN	XLOGP2
4a	H	$-(C_2H_5)_2$	-4.42 (10.69 mg/l)	2.77	2.22
4b	H	-di isopropyl	-4.92 (3.67 mg/l)	3.60	3.14
4c	H		-4.48 (9.57 mg/l)	3.14	2.23
4d	H		-4.27 (17.41 mg/l)	1.86	1.44
4e	H		-4.23 (17.48 mg/l)	1.40	0.97
4f	H		-5.49 (1.03 mg/l)	3.28	2.94
4g	NO_2	$-(C_2H_5)_2$	-4.74 (5.95 mg/l)	2.58	2.11
4h	NO_2		-4.79 (5.47 mg/l)	2.96	2.12
4i	NO_2		-4.26 (20.24 mg/l)	1.68	1.33
4j	NO_2		-4.33 (15.68 mg/l)	1.21	0.86
4k	NO_2		-5.47 (1.22 mg/l)	3.10	2.83
4l	$-CH_3$	$-(C_2H_5)_2$	-4.82 (4.45 mg/l)	3.31	2.65
4m	$-CH_3$		-4.86 (4.23 mg/l)	3.69	2.67
4n	$-CH_3$		-4.37 (14.38 mg/l)	2.41	1.87
4o	$-CH_3$		-4.45 (10.95 mg/l)	1.94	1.41
4p	$-CH_3$		-5.64 (0.76 mg/l)	3.83	3.37
Cisplatin	STD				

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