Synthesis and In Vitro Evaluation of Novel 4-methyl-6-(1-methyl-1H-benzo[d]imidazol-2-yl)-2-propyl-1H-benzo[d]imidazole Derivatives as Cytotoxic Agents

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ABSTRACT: In the present study, a novel series of substituted Bis benzimidazole derivatives were synthesized and characterized by means of IR, H-NMR, Mass spectral and physical analysis. The compounds were screened for in vitro cytotoxic activity by XTT based cell viability assay method by using two human cancer cell line PC-3 and VERO. All synthesis compounds exhibited significant activity against cancer cell line after 48 hours has comparable with standard drug doxorubicin. Among all compound screened, one compound bim-4 was found to be most potent.

KEYWORDS: Bisbenzimidazole; XTT assay; PC-3; cancer cell line.

Introduction
Cancer is a term used for diseases in which abnormal cells divide without control and are able to invade other tissues. Cancer cells can spread to other parts of the body through the blood and lymph systems. Cancer is a class of diseases in which a group of cells display uncontrolled growth, invasion, and sometimes metastasis. Most cancers form a tumour but some, like leukemia, do not. The existing anticancer agents are not sufficient enough to solve this problem. Limited activity, rapid development of resistance and the adverse effects over rides their usefulness. Benzimidazole nucleus is the key building block for a variety of compounds that play crucial roles in the function of a number of biologically important molecules.

Several chemical classes of anticancer drugs have been identified through both empirical screening and rational design of new compounds during the past three decades. These include several heterocyclic dimers such as bis-pyrrollobenzodiazepines, bis-alkyl amino phenyl furans and bis-benzimidazoles. Basic moiety of telmisartan also bears a bis benzimidazole dimer and telmisartan reported as cytotoxic agent in prostate cancer cell line.

These heterocyclic dimers, with acyclic and cyclic spacers, target the DNA to exhibit their anticancer activity by intercalation and alkylation mechanism. Using these dimers as “lead” agents, both intercalating and irreversible alkylating dimers have been prepared to identify heterocycles that can induce DNA binding, inter strand cross-links and disrupt cellular processes necessary for cell maintenance and replication in cancer cells.

Result and Discussion
The Synthesis of bisbenzimidazole and its derivatives depicted in scheme: 1 and 2. All compounds were synthesized by conventional methods. In initial step, 7-methyl-2-propyl-3H-benzo[d]imidazol-5-carboxylate (2a) was synthesized from methyl 3, 4-diamino-5-methyl benzoate (1). Then synthesize compound (2a) react with N-methylbenzene-1, 2-diamine in presence of poly phosphoric acid and gave targeted lead molecule (2b).

The structures of synthesized compounds Bim/04, Bim/05, and Bim/06 were confirmed by its analytical and spectral data.

A typical sharp absorption band at 1676 - 1684 cm\textsuperscript{-1} for C=O in synthesized compounds. In \textsuperscript{1}H NMR doublet=CH-CO- protons and \(\delta\) ppm 1.17(CH\textsubscript{2}), 2. 62 (–CH\textsubscript{3}), 3.79 (OCH\textsubscript{3}), 7.19-7.88 –CH of thiophene and phenyl ring. On basis of Spectral data, it was confirmed that all three compound have been synthesised. All compounds were then screened for in vitro anticancer activity by XTT assay.
Scheme 1 Synthetic Pathway Bisbenzimidazole.

Scheme 2 Substituted Bisbenzimidazole Derivatives.

\[ \text{NaOH, MeOH} \]

Poly Phosphoric Acid

\[ \text{NH}_2 \text{NH}_2 \text{CH}_3 \]

\[ \text{H}_3\text{COOC} \]

\[ \text{CH}_3 \]

\[ \text{NH}_2 \]

\[ \text{H}_3\text{COOC} \]

\[ \text{CH}_3 \]

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\[ \text{CH}_3 \]
Table 1 Cytotoxic activity on human PC-3 cell line.

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<td>BIM/06</td>
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Table 2 Cytotoxic activity on VERO cell line.

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Fig. 1 Cytotoxic activity of compounds BIM/04, BIM/05, BIM/06 on PC-3 cell line (Log conc. Vs % inhibition).
Conclusion

In summary, synthesized Compounds BIM/04, BIM/05, and BIM/06 were screened for cytotoxic activity by XTT assay method by using two human cell line VERO and PC-3. Among all the compounds screened compound (BIM/04) and (BIM/05) were found to be the most potent in the series with 73.123 and 71.323 percentages in PC-3 cell line after 48 hours respectively, in contrast to these both of the compound exhibited significant lower cytotoxic effect on VERO cell line which is consist of normal cell.

Experimental

General

Melting points were determined on an Electrothermal AZ 9000 3MK4 apparatus and are uncorrected. The thin layer chromatography (TLC, Rf values) was performed on silica gel 60 plates F254 (Merck, 0.2 mm thickness) using mobile phase ethyl acetate:methanol 9:8:0.2 and visualization was effected with ultraviolet light. IR spectra were recorded on a FT-IR spectrophotometer (Shimadzu) as potassium bromide discs. 1H NMR spectra were obtained in DMSO-d6 on BRUKER Advance-II 400 MHZ instrument and chemical shift were measured as parts per million downfield from tetramethylsilane (TMS) as internal standard. Mass spectra were obtained using 2010EV LCMS Shimadzu instrument.

Synthetic Procedures

The synthetic strategy leading to the target compounds are illustrated in Scheme 1 and 2.

Synthesis of 2-n-Propyl-4-methyl-6-(methoxycarbonyl) benzimidazole10 (2a).

Methyl 4-amino-3-methylbenzoate (1) (8.25 g, 50 mmol) was acylated with butyryl chloride (5.3 mL, 50 mmol) in chlorobenzene at 100°C. The resulting amide was reacted with fuming nitric acid in sulphuric acid (60%) at 0°C. The resulting methyl 4-(butyrylamino)-3-methyl-5-nitro-benzoate was reduced with hydrogen (5 bars) and palladium (10% on carbon) in methanol. The resulting amino compound was dissolved in glacial acetic acid and heated under reflux for 1.5 hours. After evaporation of the acetic acid water was added and the pH was adjusted to 9 by addition of concentrated ammonia. This solution was extracted with ethyl acetate (3 x 100 ml), and the combined organic layers were washed with aqueous NaHCO3 solution and dried (MgSO4). After addition of charcoal and filtration the solvent was evaporated to give 2a (9.0 g, 78% overall) as oil.

Synthesis of 2-n-Propyl-4-methyl-6-(1-methylbenzimidazol-2-yl)benzimidazole 10,11 (2b).

To a solution of 6.9 g (30 mmol) of 57 in methanol (500 ml) was added a solution of 40 g of NaOH in water (300 ml), and the mixture was heated under reflux for 2 h. After evaporation of methanol, water (700 ml) was added to the residue and the pH was adjusted to 5 by addition of aqueous citric acid (30%). The precipitated solid was washed with ethanol, filtered off, and dried to yield 5.46 g (25 mmol) of 2-n-propyl-4-methyl-6-carboxybenzimidazole. This was dissolved in polyphosphoric acid (65 g) at 150°C, and N-methyl-o-phenylenediamine dihydrochloride (1a) (4.88 g, 25 mmol) was added in small portions. After

Fig. 2. Cytotoxic activity of compound BIM/04, BIM/05, BIM/06 on VERO cell line (log conc. Vs % inhibition).
stirring at 150°C for 20 hours the mixture was allowed to cool and then poured into water (300 ml). The pH was adjusted to 9 by addition of concentrated ammonia (ice cooling). The precipitated solid was filtered off, dried, and boiled in ethyl acetate (300 ml). After cooling, the solid was filtered off, washed with diethyl ether, and dried to give 2b (5.86 g, 60% overall) as a yellowish white solid.

**General Synthesis of 3-(4-methyl-6-(1-methyl-1H-benzo[d]imidazol-2-yl)-2-propyl-1H benzof[d]imidazole-1-yl)-1-arylpropan-1-one**

**Bis-Benzimidazole (2b)** dissolved in DMF and stirred vigorously with potassium carbonate at room temperature for 1 hour. To the resulting suspension, 3-chloro-1-phenylpropan-1-one or 3-chloro-1-(thiophene-3-yl) propan-1-one or 3-chloro-1-(4-methoxyphenyl)propan-1-one, previously dissolved in DMF, was added drop wise with stirring over a period of 1 hours. The reaction was allowed to proceed for further 13 hours and the solvent was removed under vacuum. Residue was treated with dilute HCl and extracted with ethyl acetate. The organic layer was washed with brine, distilled water and dried over anhydrous sodium sulphate. The solvent was evaporated under reduced pressure to afford the product as brownish amorphous solid.

**3-(4-methyl-6-(1-methyl-1H-benzo[d]imidazol-2-yl)-2-propyl-1H-benzo[d]imidazo-l-yl)-1-phenylpropan-1-one (BIM/04):** Brownish amorphous solid of melting point 203°C-205°C, MS (m/z): 337.25 (M+1), 435.1 (M-1), IR (KBr), cm⁻¹: 1685 (C=O). ¹HNMR (DMSO-d₆), δ ppm: 0.926-0.975 (t, 3H, Methyl), 1.721-1.771 (m, 2H, -CH), 2.49-2.61 (s, 3H, Methyl), 2.841-2.89 (t, 2H, -CH), 3.34-3.80 (2, 3H, Methyl), 7.10-7.57 (m, 4H, Phenyl), 7.61 (d,2H,-CH), 7.46-7.71 (m, 5H, Ar-H).

**1-(4-methoxyphenyl)-3-(4-methyl-6-(1-methyl-1H-benzo[d]imidazol-2-yl)-2-propyl-1H-benzo[d]imidazol-1-yl)-1-phenylpropan-1-one (BIM/06):** Yellowish brown solid of melting point 234°C-247°C, MS (m/z): 467.12 (M+1), 435.1 (M-1), IR (KBr), cm⁻¹: 1684cm⁻¹(C=O), 1260cm⁻¹(C-O). ¹HNMR (DMSO-d₆), δ ppm: 0.926-0.975 (t, 3H, Methyl), 1.721-1.771 (m, 2H, -CH), 2.39-2.61 (s, 3H, Methyl), 2.83-2.89 (t, 2H, -CH), 3.34-3.80 (2, 3H, Methyl), 7.10-7.57 (m, 4H, Phenyl), 7.61 (d,2H,-CH), 7.46-7.71 (m, 5H, Ar-H), 3.79 (s, 3H, OCH₃).

**Biological Activity**

**In vitro anticancer activity**

All the synthesized compounds were screened for the in vitro cytotoxic activity by XTT based cell viability assay method, using PC-3 i.e. Prostate cancer line (Table 1), VERO i.e. normal cell line (Table 2) cell line and doxorubicin as standard. [13] Graph depicted in Figure:1 shows % inhibition of cell growth on PC-3 and VERO cell line respectively.

**Aknowledgement**

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**Reference**


