RESEARCH ARTICLE

Anti-Proliferation Effects of Benzimidazole Derivatives on HCT-116 Colon Cancer and MCF-7 Breast Cancer Cell Lines

Mohammed Hadi Al-Douh[1]* Hayder B Sahib[2], Hasnah Osman[3], Shafida Abd Hamid[4], Salizawati M Salhimi[5]

Abstract

Benzimidazoles 1-4 were obtained using modified synthesis methods and studied for their ability to inhibit cell proliferation of colon cancer cell HCT-116 and breast cancer cell MCF-7 using MTT assays. In the HCT-116 cell line, benzimidazole 2 was found to have an IC_{50} value of 16.2±3.85 μg/mL and benzimidazole 1 a value of 28.5±2.91 μg/mL, while that for benzimidazole 4 was 24.08±0.31 μg/mL. In the MCF-7 cell line, benzimidazole 4 had an IC_{50} value of 8.86±1.10 μg/mL, benzimidazole 2 a value of 30.29±6.39 μg/mL, and benzimidazole 1 a value of 31.2±4.49 μg/mL. Benzimidazole 3 exerted no cytotoxity in either of the cell lines, with IC_{50} values >50 μg/mL. The results suggest that benzimidazoles derivatives may have chemotherapeutic potential for treatment of both colon and breast cancers.

Keywords: Benzimidazole - hydroxyl group - benzyloxy group - anti-proliferation - HCT-116 - MCF-7 - MTT assay

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Introduction

Many benzimidazoles are pharmaceutical agents and are used widely in biological system applications (Townsend and Revankav, 1970; Trivedi et al., 2006). Some derivatives of benzimidazoles were reported and used as antiviral agents (Gudmundsson et al., 2000; Cheng et al., 2005), topoisomerase I inhibitors (Kim et al., 1996: 1997; Rangarajan et al., 2000; Mekapati and Hansch, 2001) and as antiproliferative agent (Hong et al., 2004). Some of 4,5,6,7-tetrahalo-1H-benzimidazoles were synthesised and showed antiprotozoal activity of *Acanthamoeba castellanii* (Kopanska et al., 2004), antimycobacterial activity against *Mycobacterioum* strains (Kazimierczuk et al., 2005).

In other studies, they also can act as antibacterial agent (Nezhad et al., 2005; Ozden et al., 2005), and showed anthelmintics activity of *Trichinella spiralis* (Mavrova et al., 2005), anti-inflammatory and analgesic activities (Sondhi et al., 2006) and as inhibitors for the hepatitis B (Li et al., 2006) and C viruses (Beaulieu et al., 2004). Some benzimidazoles were also tested as anti-HIV (Roth et al., 1997; Smith et al., 2003), anticancer agents (Craigo et al., 1999; Rida et al., 2006) and antioxidants (Kus et al., 2008). Recent publication also reported the use of phenolic and anisolic benzimidazole derivatives in vasodilator and antihypertensive studies (Soto et al., 2006), while other alkyloxyaryl benzimidazole derivatives have been tested for the spasmolytic activity (Vazquez et al., 2006).

Most benzimidazoles have functional groups at the positions 4, 5, 6 or 7 of the benzimidazole ring, which increase the activity of the compounds against the tumor cells (Kim et al., 1996: 1997; Garuti et al., 2000). Garuti et al. (2000) reported that benzimidazole **5** is a cytotoxic compound against MCF-7 and the IC_{50} was 26.4 μM (Figure 1).

On the other hand, Kumar et al. (2002) evaluated both benzimidazoles **6** and **7** against MCF-7 and HT-29 (colon cancer cell line) with IC_{50}>100 μM, while the IC_{50} of benzimidazole **6** with leukemic cell line (leukemia) HL-60 was 70 μM. Benzimidazoles **6** and **7** have two substituted functional groups at positions 2 and 4, while benzimidazole **8** has three substituted functional groups at positions 1, 2 and 5 in benzimidazole ring (see Figure 2). It has been shown that benzimidazole **8** inhibited the proliferation of MDA-MB-231 (human breast cancer cells) by 26.1% (Timmegowda et al., 2008).

Starcevic et al. (2007) have tested several

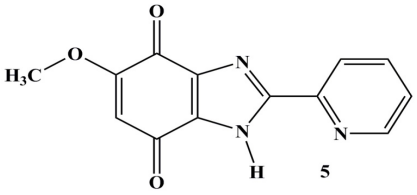

Figure 1. The Chemical Structure of Benzimidazole 5.

[1]*Chemistry Department, Faculty of Science, Hadhramout University of Science and Technology (HUST), Mukalla, Hadhramout, Yemen,* [2]*Pharmacology Department, College of Pharmacy, Karbala University, Iraq,* [3]*School of Chemical Sciences,* [5]*School of Pharmaceutical Sciences, Universiti Sains Malaysia (USM), Pulau Pinang,* [4]*Kulliyyah of Science, International Islamic University Malaysia (IIUM), Pahang, Malaysia *For correspondence: mhd_douh@yahoo.com*

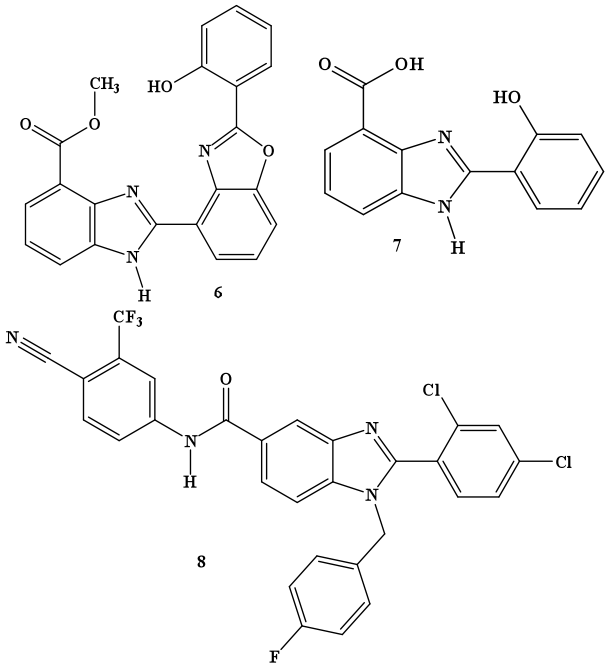

Figure 2. The Chemical Structures of Benzimidazoles 6-8

benzimidazoles which inhibit the growth of MCF-7 cells, the substitution functional groups of those benzimidazoles are at the positions 2 and 5 of the benzimidazole ring.

In view of its importance, we report here the study of the ability of the synthesized benzimidazoles **1**-**4** (see Figure 3) to inhibit cell proliferation for breast cancer cell MCF-7 and colon cancer cell HCT-116 using MTT assay.

Materials and Methods

Routine cell culture

The cell used was adherent and of human origin. Cell was grown on tissue culture flasks (Nunc, Denmark) at 37°C in a humidified incubator with the CO_2 level maintained at 5% (Freshney, 2000). The MCF-7 breast cancer cell line was cultured in DMEM growth medium (Gibco, UK), while the HCT-116 colon cancer cell line was cultured in McCoy's growth medium (Gibco, UK). The media was supplemented with 10% heat inactivated fetal calf serum (HIFCS, Gibco, UK) and 1% penicillin/ streptomycin (Sigma-Aldrich, Germany). The cells used for the experiments were between passage 4 and 7. The cell line was obtained from American Type Culture Collection (ATCC, Rockville, MD, USA).

Cytotoxicity evaluation

Tetrazolium dye, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used as a measurement of cell proliferation. The MTT assay requires cellular metabolic activity to convert the colourless tetrazolium to the purple-coloured formazon product. Therefore, this assay measure only viable cells. MTT viability assay was performed with slight modification as described by Mosmann (Mosmann, 1983).

MTT assay

Cell was grown overnight in 96-well microtiter plate at 50,000 cells/well. A dilution of **1**-**4** were prepared by dissolving the samples in DMSO and were diluted further with cell culture medium to the required concentrations. Cell was treated with different concentrations of the benzimidazoles **1**-**4** (1-50 µg/mL) for 48 hr. The stock solution was used to make the final concentration of 50, 30, 20, 10, 5 and 1 µg/mL. The final DMSO concentration in the medium was 1%. The untreated cells received only DMSO as a negative control, while

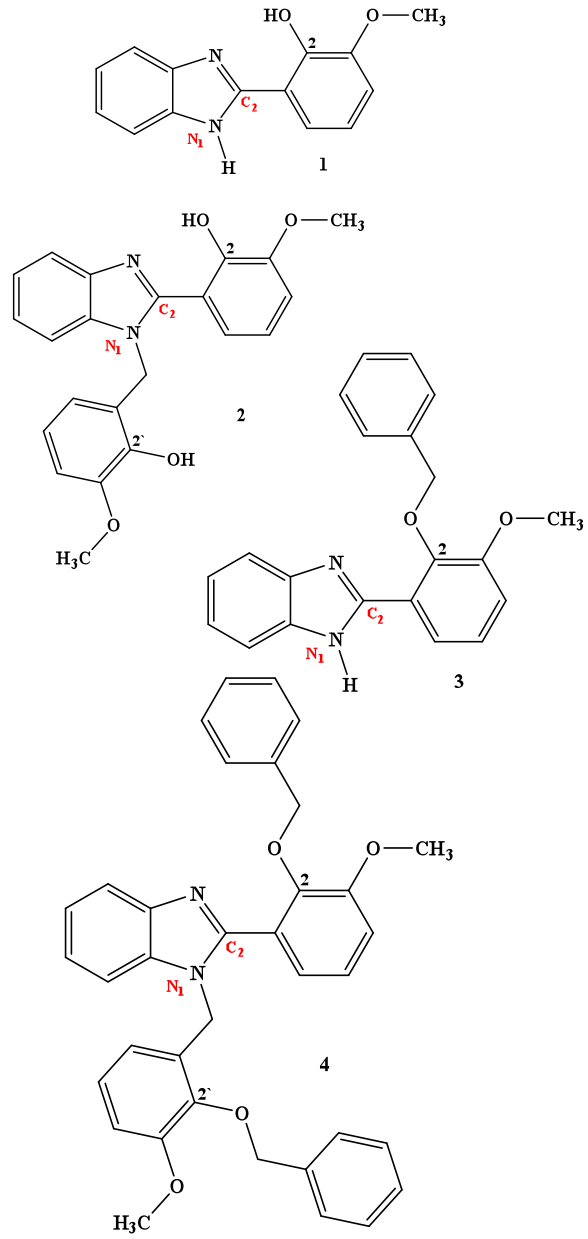

Figure 3. The Chemical Structures of the Evaluated Benzimidazoles 1-4

Table 1. The IC_{50} Values of the Benzimidazoles 1-4 Against HCT-116 and MCF-7 Cells

Benzimidazole	HCT-116 (IC_{50} mg/mL)	MCF-7 (IC_{50} mg/mL)
1	28.5±2.91	31.2±4.49
2	16.2±3.85	29.3±6.39
3	not detected*	not detected*
4	24.1±0.31	8.86±1.10

*higher than 50 mg/mL

22-oxovincaleukoblastine sulfate salt or vincristine sulfate salt was used as a positive control with a concentration of 40 ng/mL.

MTT was first prepared as a stock solution in 5 mg/mL of phosphate buffer saline PBS. At the end of the treatment period (48 hr), 20 µL of MTT solution was added to each well. The plates were incubated at 37ºC, 5% CO_2 for 5 hr. The medium was removed and 200 µL of DMSO was added to each well to dissolve the formazon crystals. The plates were shaken vigorously for one minute at room temperature.

The optical density reading (OD) was recorded using a micro plate reader (Hitachi U-2000, Japan) at 570 nm for absorbance and 650 nm as reference filter. Percentage of cell growth inhibition is determined by mean±SD, using the following equation: Percentage of cell growth inhibition=1-[(OD_{Sample}-OD_{Blank})/ ($OD_{Control}$-OD_{Blank})]

IC_{50} is the concentration that inhibits 50% from the cell growth. Cells proliferation (IC_{50}) was analysed and calculated for the benzimidazoles **1**-**4** by linear regression equation: **y=*m*x+b**

Where y is the percentage of inhibition and it set to be 50%, *m* is the slope of the standard curve, x is the concentration of compound tested in µg/mL, and b is the y-intercept of the line of standard curve.

Results

The benzimidazoles **1**-**4** are already synthesized and characterised by HRMS, 1D and 2D NMR (Al-Douh et al., 2007: 2012; Al-Douh, 2010). Benzimidazoles **1**, **2** and **3** are obtained as single crystals and their structures were determined and studied by X-ray crystallography (Al-Douh et al., 2006: 2009a,b), while benzimidazole **4** was obtained as a syrup (Al-Douh, 2010; Al-Douh et al., 2011b).

Cytotoxicity effect of benzimidazoles 1-4 on MCF-7 and HCT-116

As seen in Figure 3, the chemical structures of the benzimidazoles studied show the functional groups as a hydroxyl and benzyloxy groups. Benzimidazole **1** has hydroxyl group at the position 2 of the substituted aromatic ring in the position C2 of the benzimidazole ring, while in **3**; the hydroxyl group was replaced with benzyloxy group in the position 2. Both benzimidazoles **1** and **3** have no substituted group in the position N1 of the benzimidazole ring. Benzimidazole **2** has two hydroxyl groups in the positions 2 and 2` of the substituted aromatic rings in the positions N1 and C2 of the benzimidazole ring. For benzimidazole **4**, the hydroxyl groups were replaced with two benzyloxy groups in the positions 2 and 2` of the substituted aromatic rings.

The benzimidazoles **1**-**4** have been evaluated against MCF-7 and HCT-116 cells using Mosmann's method (1983) to show the cytotoxic effect of both hydroxyl and benzyloxy functional groups in the positions N1 and C2 of the benzimidazole ring. Our preliminary results on MCF-7 showed that benzimidazole **4** exhibits the lowest IC_{50} value at 8.86±1.10 µg/mL. Benzimidazoles **1** and **2** showed moderate toxicity, where the IC_{50} for both compounds

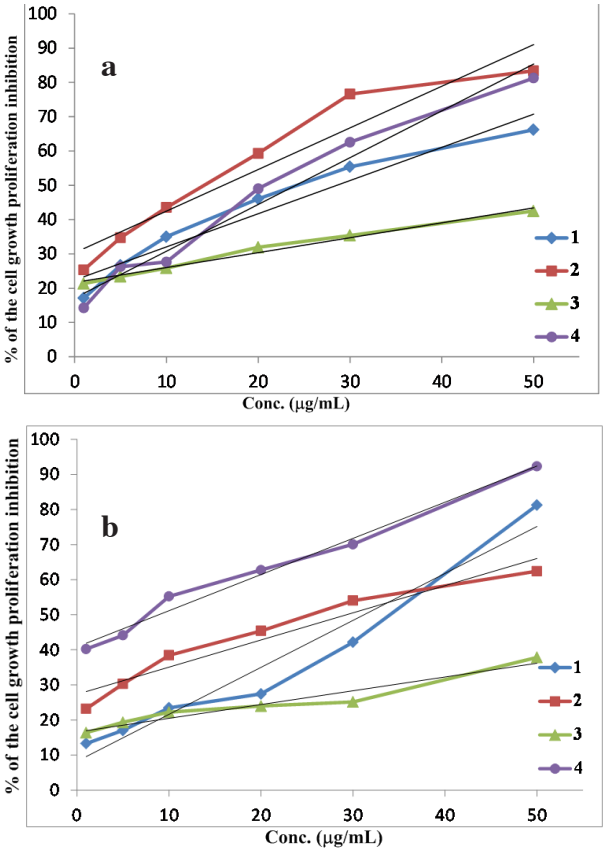

Figure 4. Dose-response Curvesfor the Evaluated Benzimidazoles 1-4 a) HCT 116 cells; MCF-7 cells

were 31.21±4.49 µg/mL and 29.29±6.39 µg/mL (Figure 4), respectively. Benzimidazole **3** was considered not toxic to MCF-7 cells with the IC_{50}>50 µg/mL. The HCT-116 cells showed that benzimidazole **2** exhibited the lowest IC_{50} value at 16.18±3.85 µg/mL. Both **1** and **2** benzimidazoles showed moderate toxicity towards HCT-116 with the IC_{50} values of 28.54±2.91 µg/mL and 24.08±0.31 µg/mL, respectively. Compound 3 was considered not cytotoxic with the IC_{50} value >50 µg/mL. Table 1 summarised all the IC_{50} values for all the compounds tested.

The substitution of hydroxyl group (-OH) with the benzyloxy ring at the position 2 of benzimidazole **1**, appeared to decrease the cytotoxic effect of **3** on both MCF-7 and HCT-116 cell lines. In benzimidazole **1**, position N1 is hydrogen atom, but in **2**, position N1 has trisubstituted aromatic ring, and these differences does not seem to affect the IC_{50} values of both benzimidazoles in MCF-7. However, hydrogen atom in position N1 appeared to contribute to the slight improvement of IC_{50} value of HCT-116.

Compounds **2** and **4** have the same benzimidazole nucleus. Compound **4** has two benzyloxy rings in the positions 2 and 2` of the substituted aromatic ring in the positions N1 and C2 of the benzimidazole ring, making it bulkier than **2**. Benzimidazole **2** has two hydroxyl groups in the positions 2 and 2` of the substituted aromatic ring in the positions N1 and C2 of the benzimidazole ring, making it more stable in aqueous solution. This may contribute to the stronger cytotoxic effect of benzimidazole **2** in HCT-116. In contrast, benzimidazole **4** showed a stronger cytotoxic effect in MCF-7 compared to benzimidazole

2. This contrary observation may be attributed to the sensitivity of different type of cell lines toward different type of compounds.

Discussion

In conclusion, the benzimidazole **4** showed high cytotoxic activity against MCF-7 cell lines with IC_{50} = 8.86±1.10 μg/mL, and moderate cytotoxic activity against HCT-116 cell lines with IC_{50}=24.08±0.31 μg/mL, while benzimidazole **2** showed lowest IC_{50} value at 16.18±3.85 μg/mL against HCT-116 cell lines. Both benzimidazoles 1 and 2 showed moderate cytotoxic activity against MCF-7 cell lines, while the benzimidazole **3** showed no cytotoxic effect with both MCF-7 and HCT-116 cell lines.

From this preliminary study, benzimidazoles **2** and **4** showed more promising results compared to other benzimidazoles. However, it is imperative to expand the study to include other types of cancer cell lines as well as normal cells in order to determine whether these two benzimidazoles **2** and **4** are suitable candidates for the development of new anti-cancer drugs.

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References

Al-Douh MH (2010). Synthesis, Characterization and Anti-Proliferation Study of Some Benzimidazole Derivatives. PhD Thesis. Universiti Sains Malaysia (USM), Malaysia, ISNB: 978-3-8443-3294-0, LAP LAMBERT Academic Publishing GmbH & Co. KG, Dudweiler Landstr, 99, 66123 Saarbrücken, Germany.

Al-Douh MH, Hamid SA, Osman H, Ng SL, Fun HK (2006). 2-(2-Benzyloxy-3-methoxyphenyl)-1*H*-benzimidazole. *Acta Crystallogr*, E, **62**, 3954-6.

Al-Douh MH, Hamid SA, Osman H (2007). 1D and 2D NMR studies of 2-(2-benzyloxy-3-methoxyphenyl)-1*H*-benzimidazole. *Indo J Chem*, **7**, 190-6.

Al-Douh MH, Osman H, Hamid SA, Kia R, Fun HK (2009a). 2-[1-(2-Hydroxy-3-methoxybenzyl)-1*H*-benzimidazol-2-yl]-6-methoxyphenol monohydrate. *Acta Crystallogr E*, **65**, 913-4.

Al-Douh MH, Osman H, Hamid SA, Kia R, Fun HK (2009b). 2-[1-(2-Hydroxy-3-methoxybenzyl)-1*H*-benzimidazol-2-yl]-6-methoxyphenol methanol 1.13-solvate. *Acta Crystallogr E*, **65**, 925-6.

Al-Douh MH, Osman H, Hamid SA (2011a). Synthesis and characterization of *N*-1-(2-hydroxy-3-methoxybenzyl)-2-(2-hydroxy-3-methoxyphenyl)-1*H*-benzimidazole. *Hadh Univ J Nat Appl Sci*, **8**, 11-32.

Al-Douh MH, Osman H, Hamid SA (2011b). Synthesis and characterization of novel benzimidazole: *N*-1-(2-Benzyloxy-3-methoxybenzyl)-2-(2-benzyloxy-3-methoxyphenyl)-1*H*-benzimidazole. *Univ Aden J Nat Appl Sci*, **15**, In Press.

Al-Douh MH, Osman H, Hamid SA (2012). Synthesis and characterization of 2-(2-hydroxy-3-methoxyphenyl)-1*H*-benzimidazole. *Hadh Univ J Nat Appl Sci*, **9**, 49-64.

Beaulieu PL, Bousquet Y, Gauthier J, et al (2004). Non-nucleoside benzimidazole-based allosteric inhibitors of the hepatitis C virus NS5B polymerase: Inhibition of subgenomic hepatitis C virus RNA replicons in Huh-7 Cells. *J Med Chem*, **47**, 6884-92.

Cheng J, Xie J, Luo X (2005). Synthesis and antiviral activity against Coxsackie virus B3 of some novel benzimidazole derivatives. *Bioorg Med Chem Lett*, **15**, 267-9.

Craigo W A, LeSueur B W, Skibo E B (1999). Design of highly active analogues of the pyrrolo[1,2-a]benzimidazole antitumor agents. *J Med Chem*, **42**, 3324-33.

Freshney RI (2000). Culture of animal cells: a manual of basic technique. John Wiley & Sons, Inc., New York, USA, 336.

Garuti L, Roberti M, Malagoli M, Rossi T, Castelli M (2000). Synthesis and antiproliferative activity of some benzimidazole-4,7-dione derivatives. *Bioorg Med Chem Lett*, **10**, 2193-5.

Gudmundsson KS, Tidwell J, Lippa N, et al (2000). Synthesis and antiviral evaluation of halogenated β-D- and -L-erythrofuranosylbenzimidazoles. *J Med Chem*, **43**, 2464-70.

Hong SY, Chung KH, You HJ, et al (2004). Synthesis and biological evaluation of benzimidazole-4,7-diones that inhibit vascular smooth muscle cell proliferation. *Bioorg Med Chem Lett*, **14**, 3563-6.

Kazimierczuk Z, Andrzejewska M, Kaustova J, Klimesova V (2005). Synthesis and antimycobacterial activity of 2-substituted halogenobenzimidazoles. *Eur J Med Chem*, **40**, 203-8.

Kim JS, Gatto B, Yu C, et al (1996). Substituted 2,5`-bi-1H-benzimidazoles: Topoisomerase I inhibition and cytotoxicity. *J Med Chem*, **39**, 992-8.

Kim JS, Gatto B, Yu C, et al (1997). Terbenzimidazoles: Influence of 2``-, 4-, and 5-substituents on cytotoxicity and relative potency as Topoisomerase I poisons. *J Med Chem*, **40**, 2818-24.

Kopanska (n. Zastapilo) K, Najda A, Zebrowska J, et al (2004). Synthesis and activity of 1H-benzimidazole and 1H-benzotriazole derivatives as inhibitors of *Acanthamoeba castellanii*. *Bioorg Med Chem*, **12**, 2617-24.

Kumar D, Jacob MR, Reynolds MB, Kerwin SM (2002). Synthesis and evaluation of anticancer benzoxazoles and benzimidazoles related to UK-1. *Bioorg Med Chem*, **10**, 3997-4004.

Kus C, Kilcigil GA, Ozbey S, et al (2008). Synthesis and antioxidant properties of novel N-methyl-1,3,4-thiadiazol-2-amine and 4-methyl-2H-1,2,4-triazole-3(4H)-thione derivatives of benzimidazole class. *Bioorg Med Chem*, **16**, 4294-303.

Li YF, Wang GF, He PL, et al (2006). Synthesis and anti-Hepatitis B virus activity of novel benzimidazole derivatives. *J Med Chem*, **49**, 4790-4.

Mavrova AT, Anichina KK, Vuchev DI, et al (2005). Synthesis and antitrichinellosis activity of some 2-substituted-[1,3]thiazolo[3,2-a]benzimidazol-3(2H)-ones. *Bioorg Med Chem*, **13**, 5550-9.

Mekapati SB, Hansch C (2001). Comparative QSAR studies on bibenzimidazoles and terbenzimidazoles inhibiting Topoisomerase I. *Bioorg Med Chem*, **9**, 2885-93.

Mosmann TJ (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J Immunol Methods*, **65**, 55-63.

Nezhad A K, Rad M N S, Mohabatkar H, Asrari Z, Hemmateenejad B (2005). Design, synthesis, antibacterial and QSAR studies of benzimidazole and imidazole chloroaryloxyalkyl derivatives. *Bioorg Med Chem*, **13**, 1931-8.

Ozden S, Atabey D, Yildiz S, Goker H (2005). Synthesis and

potent antimicrobial activity of some novel methyl or ethyl 1H-benzimidazole-5-carboxylates derivatives carrying amide or amidine groups. *Bioorg Med Chem*, **13**, 1587-97.

Rangarajan M, Kim J S, Sim S P, et al (2000). Topoisomerase I inhibition and cytotoxicity of 5-bromo- and 5-phenylterbenzimidazoles. *Bioorg Med Chem*, **8**, 2591-600.

Rida SM, El-Hawash SAM, Fahmy HTY, Hazzaa AA, El-Meligy MMM (2006). Synthesis of novel benzofuran and related benzimidazole derivatives for evaluation of in vitro anti-HIV-1, anticancer and antimicrobial activities. *Arch Pharm Res*, **29**, 826-33.

Roth T, Morningstar ML, Boyer PL, et al (1997). Synthesis and biological activity of novel nonnucleoside inhibitors of HIV-1 reverse transcriptase. 2-Aryl-substituted benzimidazoles. *J Med Chem*, **40**, 4199-207.

Smith MBK, Hose BM, Howkins A, et al (2003). Molecular modeling calculations of HIV-1 reverse transcriptase nonnucleoside inhibitors: Correlation of binding energy with biological activity for novel 2-aryl-substituted benzimidazole analogues. *J Med Chem*, **46**, 1940-7.

Sondhi SM, Singh N, Kumar A, Lozach O, Meijer L (2006). Synthesis, anti-inflammatory, analgesic and kinase (CDK-1, CDK-5 and GSK-3) inhibition activity evaluation of benzimidazole/benzoxazole derivatives and some Schiff's bases. *Bioorg Med Chem*, **14**, 3758-65.

Soto SE, Molina RV, Crespo FA, et al (2006). Relaxant activity of 2-(substituted phenyl)-1H-benzimidazoles on isolated rat aortic rings Design and synthesis of 5-nitro derivatives. *Life Science*, **79**, 430-5.

Starcevic K, Kralj M, Ester K, et al (2007). Synthesis, antiviral and antitumor activity of 2-substituted-5-amidino-benzimidazoles. *Bioorg Med Chem*, **15**, 4419-42.

Timmegowda NR, Swamy SN, Kumar CSA, et al (2008). Synthesis, characterization and evaluation of benzimidazole derivative and its precursors as inhibitors of MDA-MB-231 human breast cancer cell proliferation. *Bioorg Med Chem Lett*, **18**, 432-5.

Townsend LB, Revankav GR(1970). Benzimidazole nucleosides, nucleotides, and related derivatives. *Chem Rev*, **70**, 389-438.

Trivedi R, De SK, Gibbs RA (2006). A convenient one-pot synthesis of 2-substituted benzimidazoles. *J Mol Catal A Chem*, **245**, 8-11.

Vazquez GN, Vilchis MMR, Mulia LY, et al (2006). Synthesis and antiprotozoal activity of some 2-(trifluoromethyl)-1H-benzimidazole bioisosteres. *Eur J Med Chem*, **41**, 135-41.