International Journal for Pharmaceutical Research Scholars (IJPRS)

V-7, I-1, 2018 ISSN: 2277 - 7873

REVIEW ARTICLE

Benzimidazoles as Anti-VEGFRs: A Review

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Manuscript No: IJPRS/V7/I1/00013, Received On: 23/02/2018, Accepted On: 28/02/2018

ABSTRACT

Angiogenesis can be regarded as a hallmark to deal with different type of cancers as they help tumors proliferate and metastasize. A major angiogenic inducer released by many tumors, vascular endothelial growth factor (VEGF) predominantly dimerizes with the VEGFR2 receptor. The same can be used both as a surrogate biomarker for biological drug activity and a promising target for inhibitors. But, inhibitors are quickly hijacked by tumor cells in more invasive and aggressive forms resulting in resistance and hence poor drug delivery towards tumors. Understanding the current problem there is an urge to design a multi-targeted molecule with appropriate pharmacophoric features to enhance potency along with reduced toxicity and resistance. The present review focuses on synthesis, characterization and biological activity of novel benzimidazole derivatives based on generated Pharmacophore model. These drugs will serve our purpose of discovering novel anticancer agents against the VEGFR that are overexpressed and their capability to decrease the resistance.

KEYWORDS

Benzimidazole, VEGFR, VEGF, Angiogenesis, HIF, Anti-VEGFRs

INTRODUCTION

Angiogenesis, the formation of new blood vessels from pre-existing microvasculature, is a complicated process that usually occurs during wound healing, organ regeneration, and the female reproductive cycle[1]. It can also happen in cancer, whereby the newly created capillaries supply growing tumors with nutrients and allow waste removal[2,3]. Primary tumors, as well as metastatic foci, require oxygen and nutrient transport necessitating sustained angiogenesis. Capillaries must be within a distance of 1-2mm to ensure that cells receive appropriate oxygen and nutrients.[4]

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In malignant angiogenesis, continuous stimulation by the tumor and stromal cells is required to support new growth[5]

If new vessel growth cannot keep up with the rapid growth rate of the tumor, hypoxia ensues, and the tumor can become necrotic. This condition of oxygen deprivation is an essential trigger for tumor vessel growth. There are more than dozen endogenous proteins that can act as positive regulators or activators of tumor angiogenesis. A hypoxic environment stabilizes hypoxia-inducible factor-1(HIF-[1 6] HIF-1 is the primary transcriptional regulator of VEGF. HIF-1 also regulates other target genes: pro-angiogenic cytokines such as fibroblast growth factor-3 (FGF-3) and hepatocyte growth factor (HGF), transcription factors such as annexin V, insulin-like growth factor binding proteins 1,2

and 3 and heat shock factor, and various integrins and matrix metalloproteinases.[7] Angiogenesis can be promoted both by pro-angiogenic cytokines such as VEGF secreted from neoplastic and inflammatory cells as well as upregulation of pro-angiogenic receptors such as VEGFR. Additional important regulators of angiogenesis include tumor necrosis factor α (TNF-α), basic fibroblast growth factor (bFGF), angiopoietin-1 and angiopoietin-2, interleukin-8 (IL-8), and platelet-derived growth factor β (PDGF-β)[6,8,9]. Pro-angiogenic factors are balanced by the significant anti-angiogenic/inhibitory factors including angiostatin, endostatin, interferon-α, and interferon-β[10] Candidate ABT-869, **2**, is currently in phase II trials as VEGFR inhibitors[13].

Vascular endothelial growth factor (VEGF) is a critical pro-angiogenic cytokine released by many tumors, and the angiogenic activity of the VEGF family of proteins is mediated by three VEGFR receptors (VEGFR-1, VEGFR-2, and VEGFR-3). The VEGFR- 2 receptors, the principal kinase involved in multiple processes of angiogenesis has, therefore, became an attractive cancer target for which many small molecules have been developed. Compound BMS-540215, **1**, which emerged from potent indole-based VEGFR-2 kinase inhibitors have shown excellent enzymatic potency against VEGFR-2, a good kinase selectivity profile, an acceptable safety profile, and robust preclinical in vivo activity against a variety of human tumor xenograft models[11,12].

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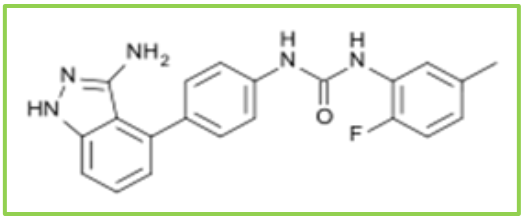

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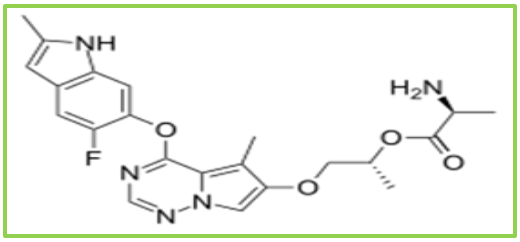


For new vessels to develop, vasodilatation and increased vascular permeability must occur. Endothelial cells become activated and migrate through the basement membrane. The milieu of activated cytokines promotes signaling and survival of these endothelial cells. The cells invade the extracellular matrix (ECM) and begin to form tubular structures. Subsequent re-organization of the ECM helps to support the newly formed tubular structures[9]. The single most important factor in angiogenesis is **vascular endothelial growth factor (VEGF-A/VEGF)**. VEGF, a selective mitogen for endothelial cells, increases microvascular permeability and leakage. Although necessary for healthy development and wound healing, VEGF over-expression has been demonstrated in tumors across species and is associated with malignant angiogenesis. VEGF also increases malignant cell trans-endothelial migration *in vitro*[7]. Serum VEGF levels are elevated in dogs with osteosarcoma and pretreatment elevation is correlated with a disease-free interval. In people, over-expression of VEGF is linked to disease progression and poor prognosis in various carcinomas as well as osteosarcoma. Thus, VEGF may be useful both a surrogate biomarker of drug biologic activity as well as a target for therapy. Another potential surrogate biomarker of anti-angiogenic therapy is tumor hypoxia. Direct tumor hypoxia can be measured by the use of a probe to measure oxygen concentration within the tumor, or in a surrogate manner using endogenous markers such as HIF-1 or exogenous probes (pimonidazole and EF5). In human cancer patients, tumor hypoxia may have prognostic and predictive significance[14].

Tumors may also promote angiogenesis by up-regulating VEGF receptors, specifically VEGFR-1 (flt-1), VEGFR-2 (flk-1/KDR), and less importantly heparin sulfate proteoglycans, and neuropilins[15]. The VEGF receptors are structurally similar, having an extracellular ligand-binding region comprised of seven immunoglobulin-like loop domains attached with a short trans-membrane helix to a cytoplasmic catalytic domain. VEGFR-1 is expressed on both endothelial cells and

monocytes while VEGFR-2 is limited to the endothelium [17]. VEGF is secreted by nearly all solid tumors in response to hypoxia. Upregulation of VEGF has been clearly demonstrated in human cancers[16,18].

VEGFR- Inhibitors

Many anti-angiogenic and anti-vasculogenic drugs exist. Some inhibit endothelial cells directly while others inhibit the signaling cascade or impair the ability of endothelial cells to break down the ECM.

The specific targeting of VEGF involves prevention of ligand-receptor interaction through ligand sequestration with a VEGF antibody, competitive inhibition of kinase activity or blocking the binding of VEGF-A with a monoclonal antibody to the receptor [19]. One of the few FDA approved and best known VEGF inhibitors used in human medicine is bevacizumab (Avastin). Bevacizumab is a humanized monoclonal antibody that targets VEGF-A in the treatment of various cancers, including colorectal, lung, breast, kidney, and glioblastoma.[17,20] Antibody therapy in veterinary medicine lacks due to the cost of development and functionality. However tyrosine kinase inhibitors (TKIs) have gained significant popularity over the last decade. TKIs work by blocking (reversibly or irreversibly) the ATP binding site of a kinase. If ATP cannot bind, the kinase cannot phosphorylate itself or initiate downstream signaling[20]. VEGF, PDGRF, and FGFR are members of the receptor tyrosine kinase family, and their blockade prevents both auto-phosphorylation and signal transduction thereby stopping the angiogenic signal. The advantage of a TKI is twofold in that it is effective against cancers that have upregulation of VEGF and/or VEGF receptors. The TKIs as small molecule inhibitors have been very successful as anti-cancer therapies both alone and in combination with tradition cytotoxic therapies. Some BZs also exhibit tyrosine kinase inhibition, and this may be another mechanism of therapeutic activity in addition to MTI. ABZ appears to inhibit VEGF in malignant ascites formation[21,22], and more recently, a series of novel BZ derivatives have been shown to inhibit various growth factor receptors (EGFR, VEGFR-2, and PDGFR).[23] The molecular mechanism of these actions has not been well described. Further investigation into optimal anti-cancer BZ drug/dose as well as elucidation of the mechanism by which they inhibit tumor growth may open the door to novel adjunctive therapy.

Benzimidazoles

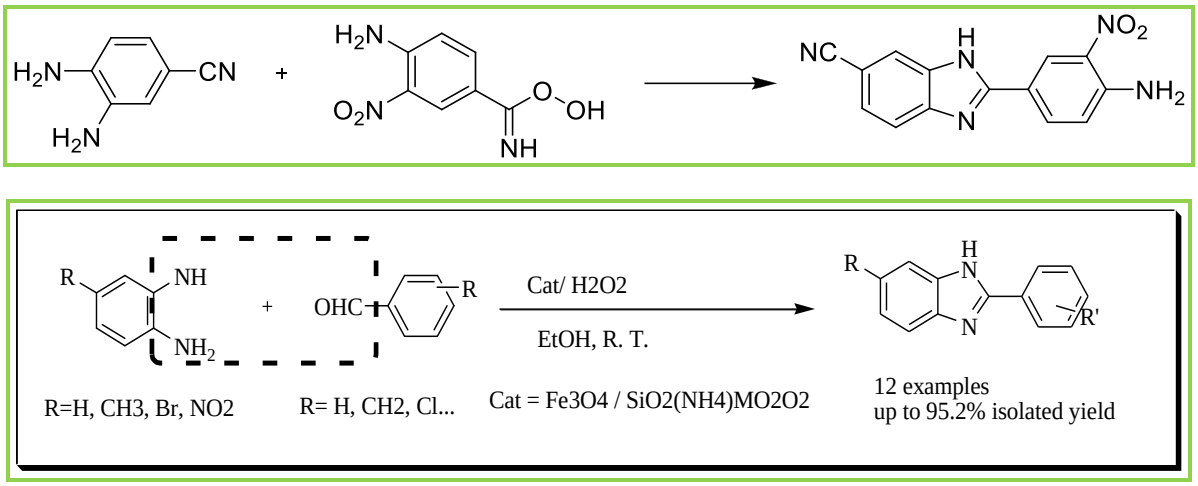
Benzimidazole, because of their diverse biological activity and clinical applications this ring system was proved to be very important as it is involved in numerous antiparasitic, antitumor and antiviral drugs. It is also well known that these molecules are present in a variety of antioxidant and anti-allergic agents. Many derivatives of benzimidazole show antiparasitic and antiprotozoal activities. In recent years, benzimidazole derivatives have attracted particular interest due to their anticancer activity. Benzimidazole showed anticancer activity against DNA Topoisomerase I and colon cancer cell lines. The need for anticancer agents that selectively kill or inhibit the growth of neoplastic cells without affecting non- cancerous host tissues is high and persistent.

There are two general methods for the synthesis of 2-substituted benzimidazole.

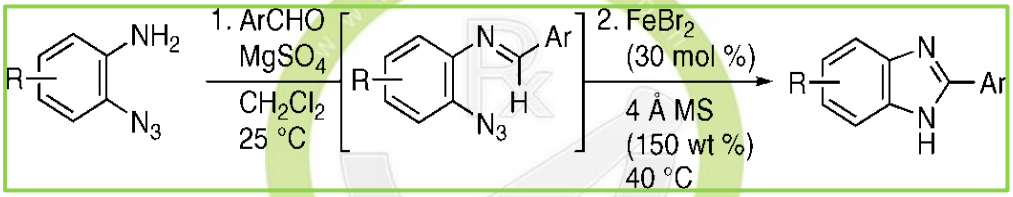
Synthetic Pathways

One is a coupling of *o*-phenylenediamines and carboxylic acids[24] or their derivatives (nitriles, imitates, or orthoesters)[25], which often require strong acidic conditions, and sometimes combined with very high temperature or the use of microwave irradiation[26].

The other way involves a two-step procedure that is oxidative cyclodehydrogenation of aniline Schiff's bases, which are often generated in situ from the condensation of o-phenylenediamines and aldehydes.



The identity of the ortho-substituent of an aryl azide influences its reactivity toward transition metals. Substitution of a vinyl group with an imine disables rhodium (II)-mediated Hamination triggers a Lewis acid mechanism catalyzed by iron(II) bromide to facilitate benzimidazole formation[27].

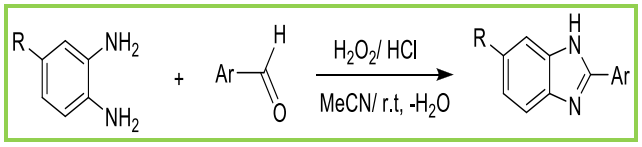


Other Methods : Synthetic Approach

Benzimidazoles are generally prepared by the condensation of *o*-phenylenediamine with organic acids employing hydrochloric acid,[28] polyphosphoric acid,[29] boric acid[30] or *p*-toluene sulphonic acid[31] as a catalyst. In addition, the title compounds also can be obtained either by reduction of *o*-nitroaniline derivatives or by reaction of organic acids derivatives with *o*-phenylenediamine[32]. Yet these reactions are often carried out under high pressure and require for long reaction times. Therefore, the discovery of mild and practical routes for the synthesis of benzimidazoles continues to attract the attention of researchers.

Several improved procedures for the preparation of benzimidazoles have been reported, either by fusion of 2-aminobenzamide and organic acid,[33] or by reaction of *o*-phenylenediamine and organic acid using $FeCl_3/O_2$ as a catalytic Fe_3O/Fe_2O redox cycling approach[34]. Recently, Villemin *et al.*[35] and Loupy *et al.*[36] reported a fast and convenient synthesis of benzimidazoles by condensation of orthoesters with *o*-phenylenediamine in dry medium and using KSF clay under mono-mode microwave irradiation. Guillermo Penieres *et al.*[37] also obtained 2-alkyl benzimidazoles using natural clay under infrared radiation in solvent-free conditions. In addition, the development of novel, but more complicated solid phase routes to benzimidazoles had been advanced[38]. Microwave irradiation of organic reactions has rapidly gained in popularity as it accelerates a variety of synthetic transformations[39]. The application of microwave irradiation with the use of catalysts or mineral supported reagents, under solvent-free conditions, provides unique chemical processes with special attributes such as enhanced reaction rates, higher yields, greater selectivity and ease of manipulation. Therefore, microwave irradiation in organic

synthesis has been the focus of considerable attention in recent years and is becoming an increasingly popular technology[40]. Reactions in ''dry media'' or under solvent-free conditions are especially appealing as they provide an opportunity to work with open vessels, thus avoiding the risk of high-pressure development and with the possibility of upscaling the reactions to more significant scale. Solventless procedures without the use of supporting reagents are particularly eco-friendly[41].



H_2O_2/HCl solvent system was examined here for the synthesis of 2-substituted benzimidazoles in acetonitrile at room temperature. A ratio of 1:1:7:3.5 of 1,2-phenylenediamine/aryl aldehyde/H_2O_2/ HCl was found to be optimum for the coupling of aryl aldehydes and phenylenediamines.

Mechanism of Action, Toxicity, Metabolism, and Efficacy

As an antiparasitic, a BZ (Benzimidazole) acts by binding to the tubulin within the parasite resulting inhibition of cell division and polymerization of microtubules via disruption of the mitotic spindle. *In vitro*, BZs prevent polymerization of tubulin into microtubules via suppression of the mitotic spindle and disruption of the microtubule-kinetochore interaction. The loss of tension leads to poor chromosomal alignment[42]. This causes the cells to arrest in G_2/M, and the cell cannot enter mitosis. Cell death ensues via apoptosis. BZs can also uncouple oxidative phosphorylation in mitochondria. This disruption causes altered metabolism and inhibition of cellular transport in the cell.[43,44] In a time-dependent manner, energy reserves become depleted, and waste excretion becomes inhibited leading to cell death.[45] Possible additional mechanisms of action have been reported with other BZs. The BZ carbendazim appears to target DNA directly; it can induce oxidative stress and epigenetic regulation through hypomethylation. Global changes in methylation patterns are a characteristic trait of many tumors. Benzimidazole derivatives have also been used as anti-cancer inhibitors of PGP (a drug efflux pump important in chemo-resistance) and topoisomerase I (an enzyme that relaxes DNA supercoiling during replication and transcription).[46] It has also been proposed that BZs can inhibit histone deacetylase resulting in hyperacetylation of histones and subsequently affect gene expression[47]. Mebendazole (MBZ) has been shown to induce apoptosis through inactivation of Bcl-2, a protein which suppresses apoptosis by preventing the activation of the caspase pathway. In addition, some BZ derivatives have demonstrated inhibition of EGFR, VEGFR-2 and PDGFR kinase activity, which are commonly upregulated in many cancers[46,48]. Thus, although microtubule inhibition resulting in cell cycle arrest and apoptosis is likely to be the primary anti-cancer mechanism of BZs, other intracellular mechanisms may play a role.

Benzimidazoles as Anti-cancer Agents

In the early 1950s, the anti-cancer potential of the BZs was first discovered when they were added to other compounds such as nitrogen mustard and showed inhibition of carcinoma, mammary adenocarcinoma, and sarcoma in mice.[49] In the 1980s further, work with various benzimidazole alkylating agents in combination with nitrogen mustard derivatives and benzothiazole alkylating agents showed efficacy against lymphocytic leukemias[50,51]. Over the next 30 years, BZs were more thoroughly evaluated for their anticancer effects. Multiple BZ analogs have been evaluated in people and rodent tumor models. The results are incredibly variable, dependent not only on species and tumor type but also dose rate and time. Early work with seven BZ analogs revealed significant inhibition of growth to normal lymphocytes via mitotic arrest by MBZ, parabendazole, cambendazole, and FBZ (Fenbendazole). Other analogs, thiabendazole and oxfendazole (OFZ) showed no activity[52]. Limited information regarding the use of FBZ in both people and animals as a specific anticancer therapy exists. It was noted

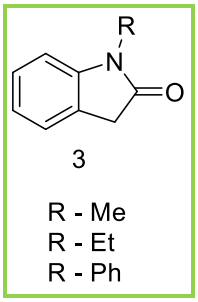
incidentally that FBZ routinely administered in rat food inhibited tumor growth of human xenograft lymphoma when combined with dietary vitamin supplementation (vitamin B, D, K, E, and A)[31].

In vitro and *In vivo* Evidence of Anti-cancer Effects

Many BZ derivatives have been evaluated *in vitro* and *in vivo* over the last 20 years. A BZ has previously been shown to have strong antiproliferative *in vivo* and *in vitro* effects against colorectal cancer and hepatocellular carcinoma (HT-29) in people.14,[53,54] MBZ induces mitotic arrest in non-small cell lung cancer in mice xenografts.[55] In another study, MBZ had antitumor effects on human lung cancer cell lines *in vitro* and *in vivo*. Flubendazole has demonstrated clinical activity against leukemia and myeloma xenografts especially in combination with vinca alkaloids[56]. A larger study demonstrated cytotoxic effects of carbendazim and benomyl on immortalized human cell lines and primary cell cultures from cancer patients, including leukemia, myeloma, lymphoma, small cell lung cancer, renal and cervical adenocarcinoma[47]. Benomyl appears more potent than the metabolite carbendazim (noted with many drugs) and is more effective against hematologic malignancies while the metabolite carbendazim is more effective against solid tumors[47]. Carbendazim also demonstrated potent antitumor activity against murine B16 melanoma and human HT-29 colon carcinoma cell lines[57].

More recently, a new generation of synthesized BZ-based agents has also shown anticancer effects against murine melanoma models via PARP-1 (Poly (ADP-ribose) polymerase-1), which has a role in repair of single-stranded DNA (ssDNA) breaks[58]. The addition of BZ ligands to existing drugs such as cisplatin analogs has *in vitro* anti-proliferative effects on human MCF-7 breast and HeLa cervical cancer cell lines[59]. Derived compounds, such as 2-substituted BZs also have anticancer effects. Examples of this include bis-bezimidazole derivatives that interfere with DNA topoisomerase I and are cytotoxic against breast adenocarcinoma and skin epidermoid carcinoma. Another example is methyl-2-benzimidazole carbamate; this agent induces apoptosis in cancer cells[54]. A novel derivative, MPTB, can also induce apoptosis in human chondrosarcoma cells[60]. Various derivatives of 2-mercapto benzimidazoles in one study showed antiproliferative activity with notable activity in G_2/M phase arrest with time-dependent induction of apoptosis. Thiazolo-benzimidazole derivatives also activate apoptosis[61]. Thus, many BZ drugs exhibit anticancer properties, with demonstrated efficacy against a broad spectrum of tumor types and several potential mechanisms of action. Other recent discoveries consisting of anti-cancerous activity includes:-

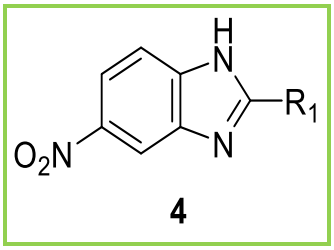
Zienab M. Nofal *et al.*, Compounds had a promising anticancer activity against cell lines PC12. Thus, with further testing, many of the synthesized compounds especially compound **3** have the potential to be developed into potent anticancer agents against HepG2[62].



3

R - Me
R - Et
R - Ph

Blaszczak-swiatkiewicz K., *et al.*, synthesized heterocyclic compounds; belong to a new group of chemical bandings with potential anticancer properties. These compounds belong to the group of drugs with the bioreductive mechanism of action Moreover, derivatives containing benzimidazole ring 4 are active as human DNA Topoisomerase I inhibitors[63].



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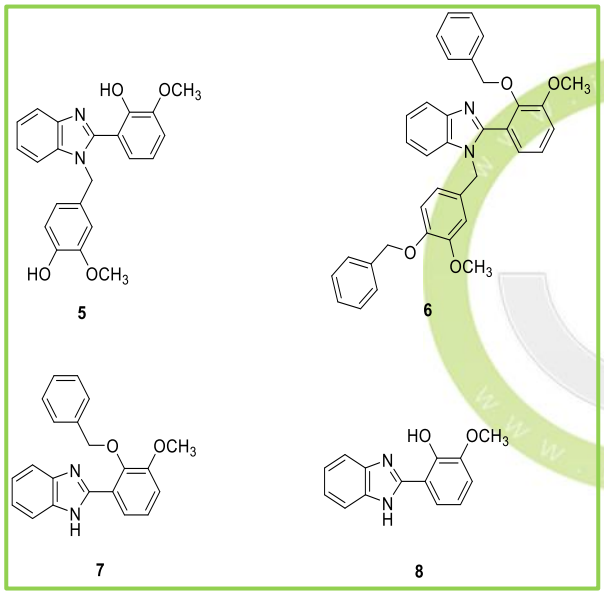
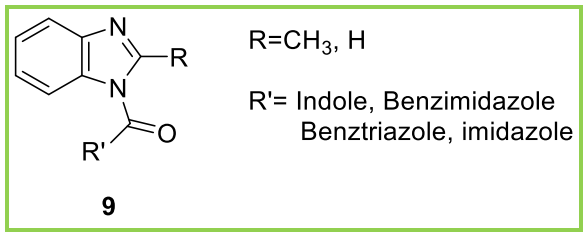
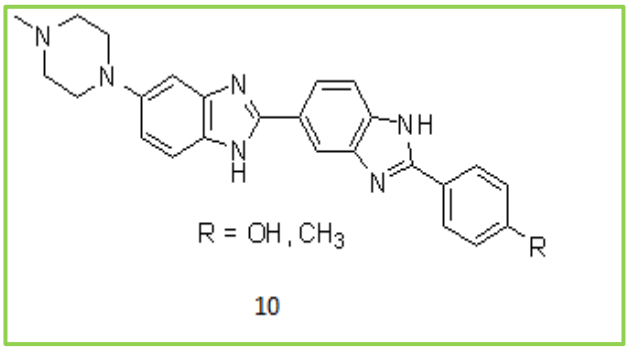
Mohammed Hadi Al–Douh *et al.*, synthesized some new benzimidazole derivatives**,** showed high cytotoxic activity against MCF-7 cell lines and moderate cytotoxic activity against HCT-116 cell lines. Both benzimidazoles **5** and **6** showed moderate cytotoxic activity against MCF-7 cell lines, while the benzimidazole **7** showed no cytotoxic effect with both MCF-7 and HCT-116 cell lines. This preliminary study, benzimidazoles **6** and **8** 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 **6** and **7** are suitable candidates for the development of new anti-cancer drugs[64].



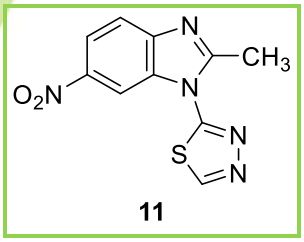
O. B. Patel, *et al.*, The 2-(aryl)-1-(1H-benzo[d] imidazole-1-yl) ethanone and 2-(aryl)-1 (2-methyl- 1Hbenzo[d]imidazol-1-yl) ethanone compounds were synthesised. Compound 9 was screened for cytotoxic activity by XTT based cell viability assay method by using two human cell line VERO and NCI[65].



Sabiha Alper, *et al.*, synthesized some new bi- and tert-benzimidazole derivatives inhibitor of Topoisomerase II. The compound 10 exhibit potent antiproliferative activity against a range of ovarian cell lines and to inhibit transcription in an *in vitro* setting[66].



Mostafa M. Ramla, *et al.*, Different substituent were introduced in position 1 of 2-methyl-5(6)-nitro-1H- benzimidazole in order to obtain different side chains having different heterocyclic compounds, for example, thiadiazoles, tetrazoles, triazoles, thiazoles, triazines, and imidazoles. The antitumor effect of the compound 11 was studied against breast cancer (MCF-7) and compound 11 [IC_{50} = 4.52 l g] was found to be active[67].



Benzimidazole as a Possible Therapy for Resistant Tumors

Acquired tumor resistance to chemotherapy necessitates novel therapies for successful cancer treatment. The drugs most often associated with acquired resistance are paclitaxel, docetaxel, vinca alkaloids, doxorubicin, daunorubicin, epirubicin, etoposide, dactinomycin, and mitomycin C[68]. Many mechanisms of tumor resistance exist. A frequently encountered mechanism involves cells with the MDR (Multi-Drug Resistant) phenotype that utilizes alterations in the Golgi apparatus, lysosomes and other organelles that affect post-translation pathways. The Golgi

apparatus is central to maintaining growth and survival of cancer cells and may be an additional therapeutic target site. A BZ based chemical compound, 2-(substituted phenyl)-benzimidazole can displace the resident Golgi proteins by inhibiting its ability to recycle these proteins leading to inhibition of cell proliferation[69.] Another resistance mechanism involves PARP-1 (Poly (ADP-ribose) polymerase-1). PARP-1 is activated by DNA damage, causing it to cleave NAD^+ and transfer ADP-ribose units which aid DNA repair, allowing cancer cells to evade apoptosis following DNA damage. Some cancer cells overexpress PARP-1, making it an attractive target. Various formulation and chemical alterations of BZ cores such the addition of a piperidinyl or pyrrolidinyl +/- an alkyl group on the nitrogen at the 2 position demonstrate positive enzymatic and cellular assay results for PARP-1 inhibition. Continued modifications demonstrate improved pharmacokinetic properties and potent oral efficacy, making BZs yet more attractive as novel anticancer agents[58].

CONCLUSION

Angiogenesis is necessary for continued primary tumor growth as well as establishing distant metastases. Regulation of angiogenesis is primarily by a vascular endothelial growth factor (VEGF). Given that sustained angiogenesis is necessary for tumor growth, therapeutic strategies which reduce or block the effects of tumor-associated VEGF are currently being investigated for the treatment of various cancers and have improved survival in many cancers and may help to delay progression of micro-metastasis.

Since the original finding of elevated $VEGF_S$ concentrations of in patients with cancer , many studies have reported similar findings in patients with breast cancer and many other types of cancer, with higher levels often found in metastatic disease than in localized disease or in progressive disease during treatment[70] . Correlations with prognosis have also been reported for several cancers, *e.g.*, ovarian cancer.

It has been testified that VEGF also plays an important role in the growth of sinusoidal endothelial cells and hepatocyte, or termed liver regeneration and in the development of primary and secondary liver carcinoma and benign liver pathological changes such as hemangioma, focal hyperplasia, and hepatitis, and hepatitis by promoting neovascularization[71]. VEGF is expressed mainly in HCC cells (Hepato Carcinoma Cells), sinusoidal endothelial cells, Kupffer cells, epatic macrophages etc. These results demonstrate that VEGF signaling is both important for SEC (sinusoidal endothelial cells) development and required for lipoprotein uptake in the liver. The study provides evidence for VEGFR2 role in liver metastasis and hence can be used as a target to study against liver cancer[72].

Various clinical trials have validated the clinical importance of anti-VEGF or anti-VEGF receptor (VEGFR) therapy. Currently, the humanized monoclonal antibody bevacizumab (blocks VEGF-A), and the tyrosine kinase inhibitors sunitinib and sorafenib (inhibit VEGFRs) are approved for patients with various malignancies and several others are expected in the coming years. Unfortunately, anti-VEGF/VEGFR treatment is not void of side effects. An array of unexpected side effects is now seen in clinical practice[73].

It is, therefore, worthwhile to synthesize oxindole bearing benzimidazole moiety which may have less toxicity and more potentiality for VEGFR.

REFERENCES

1. Hoeben, A. N. N., Landuyt, B., Highley, M. S., Wildiers, H., Van Oosterom, A. T., & De Bruijn, E. A. (2004). Vascular endothelial growth factor and angiogenesis. *Pharmacological reviews*, *56*(4), 549-580.

2. Bäckman, U., & Christofferson, R. (2005). The selective class III/V receptor tyrosine kinase inhibitor SU11657 inhibits tumor growth and angiogenesis in experimental neuroblastomas grown in mice. *Pediatric research*, *57*(5 Part 1), 690-695.

3. Carmeliet, P., & Jain, R. K. (2000). Angiogenesis in cancer and other diseases. *nature*, *407*(6801), 249.

4. Folkman, J. (1971). Tumor angiogenesis: therapeutic implications. *New england journal of medicine*, *285*(21), 1182-1186.

5. O'Reilly, M. S. (2003). Therapeutic strategies using inhibitors of angiogenesis. In *Tumor Suppressor Genes* (pp. 599-633). Humana Press.

6. Mizukami, Y., Kohgo, Y., & Chung, D. C. (2007). Hypoxia inducible factor-1–independent pathways in tumor angiogenesis. *Clinical cancer research*, *13*(19), 5670-5674.

7. Prager G. W., Poettler. M. (2011). Angiogenesis in cancer: Basic mechanism and therapeutic advances. Hämostaseologie. 32

8. Ferrara, N., Gerber, H. P., & LeCouter, J. (2003). The biology of VEGF and its receptors. *Nature medicine*, 9(6), 669-676.

9. Eichhorn, M. E., Kleespies, A., Angele, M. K., Jauch, K. W., & Bruns, C. J. (2007). Angiogenesis in cancer: molecular mechanisms, clinical impact. *Langenbeck's archives of surgery*, 392(3), 371-379.

10. Losiewicz, M. D., Carlson, B. A., Kaur, G., Sausville, E. A., & Worland, P. J. (1994). Potent inhibition of CDC2 kinase activity by the flavonoid L86-8275. *Biochemical and biophysical research communications*, 201(2), 589-595.

11. Bhide, R. S., Cai, Z. W., Zhang, Y. Z., Qian, L., Wei, D., Barbosa, S., ... & Barrish, J. C. (2006). Discovery and preclinical studies of (R)-1-(4-(4-Fluoro-2-methyl-1 H-indol-5-yloxy)-5-methylpyrrolo [2, 1-f][1, 2, 4] triazin-6-yloxy) propan-2-ol (BMS-540215), an in vivo active potent VEGFR-2 inhibitor. *Journal of medicinal chemistry*, *49*(7), 2143-2146.

12. Cai, Z. W., Zhang, Y., Borzilleri, R. M., Qian, L., Barbosa, S., Wei, D., ... & Wautlet, B. S. (2008). Discovery of brivanib alaninate ((S)-((R)-1-(4-(4-fluoro-2-methyl-1 H-indol-5-yloxy)-5-methylpyrrolo [2, 1-f][1, 2, 4] triazin-6-yloxy) propan-2-yl) 2-aminopropanoate), a novel prodrug of dual vascular endothelial growth factor receptor-2 and fibroblast growth factor receptor-1 kinase inhibitor (BMS-540215). *Journal of medicinal chemistry*, *51*(6), 1976-1980.

13. Albert, D. H., Tapang, P., Magoc, T. J., Pease, L. J., Reuter, D. R., Wei, R. Q., ... & Wang, B. (2006). Preclinical activity of ABT-869, a multitargeted receptor tyrosine kinase inhibitor. *Molecular cancer therapeutics*, *5*(4), 995-1006.

14. Wilson, W. R., & Hay, M. P. (2011). Targeting hypoxia in cancer therapy. *Nature Reviews Cancer*, *11*(6), 393-410.

15. Roy, H., Bhardwaj, S., & Ylä-Herttuala, S. (2006). Biology of vascular endothelial growth factors. *FEBS letters*, 580(12), 2879-2887.

16. Scheidegger, P., Weiglhofer, W., Suarez, S., Kaser-Hotz, B., Steiner, R., Ballmer-Hofer, K., & Jaussi, R. (1999). Vascular endothelial growth factor (VEGF) and its receptors in tumor-bearing dogs. *Biological chemistry*, 380(12), 1449-1454.

17. Sousa Moreira, I., Alexandrino Fernandes, P., & Joao Ramos, M. (2007). Vascular endothelial growth factor (VEGF) inhibition-a critical review. *Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents)*, 7(2), 223-245.

18. Millanta, F., Silvestri, G., Vaselli, C., Citi, S., Pisani, G., Lorenzi, D., & Poli, A. (2006). The role of vascular endothelial growth factor and its receptor Flk-1/KDR in promoting tumour angiogenesis in feline and canine mammary carcinomas: a preliminary study of autocrine and paracrine loops. *Research in Veterinary Science*, *81*(3), 350-357.

19. Wedge. S. R., Jurgensmeier. J. M. (2008). Vascular endothelials growth factor receptor

tyrosine kinase inhibitors for the treatment of cancer. In: Dieter Marme NF, 395-423.

20. Mitchell, P. (2011). A systematic review of the efficacy and safety outcomes of anti-VEGF agents used for treating neovascular age-related macular degeneration: comparison of ranibizumab and bevacizumab. *Current medical research and opinion*, *27*(7), 1465-1475.

21. Potashman, M. H., Bready, J., Coxon, A., DeMelfi, T. M., DiPietro, L., Doerr, N., ... & Gu, Y. (2007). Design, synthesis, and evaluation of orally active benzimidazoles and benzoxazoles as vascular endothelial growth factor-2 receptor tyrosine kinase inhibitors. *Journal of medicinal chemistry*, *50*(18), 4351-4373.

22. Cachoux, F., Isarno, T., Wartmann, M., & Altmann, K. H. (2006). Total Synthesis and Biological Assessment of Benzimidazole-Based Analogues of Epothilone A: Ambivalent Effects on Cancer Cell Growth Inhibition. *ChemBioChem*, 7(1), 54-57.

23. Li, Y., Tan, C., Gao, C., Zhang, C., Luan, X., Chen, X., ... & Jiang, Y. (2011). Discovery of benzimidazole derivatives as novel multi-target EGFR, VEGFR-2 and PDGFR kinase inhibitors. *Bioorganic & medicinal chemistry*, 19(15), 4529-4535.

24. Katritzky, A. R., & Lagowski, J. M. (1984). Reactivity of five-membered rings with two or more heteroatoms.

25. Czarny, A., Wilson, W. D., & Boykin, D. W. (1996). Synthesis of mono-cationic and dicationic analogs of hoechst 33258. *Journal of heterocyclic chemistry*, 33(4), 1393-1397.

26. Bourgin, K., Loupy, A., & Soufiaoui, M. (1998). 'Three new ways to synthesize 1, 3-azole derivatives by microwaves, Trois nouvelles voies de synthese des derives 1, 3-azoliques sous micro-ondes. *Tetrahedron*, *54*, 8055-8064.

27. Shen, M., & Driver, T. G. (2008). Iron (II) bromide-catalyzed synthesis of benzimidazoles from aryl azides. *Organic letters*, *10*(15), 3367-3370.

28. Hein, D. W., Alheim, R. J., & Leavitt, J. J. (1957). The use of polyphosphoric acid in the synthesis of 2-aryl-and 2-alkyl-substituted benzimidazoles, benzoxazoles and benzothiazoles1. *Journal of the American Chemical Society*, *79*(2), 427-429.

29. Pushkina, L. N., Mazalov, S. A., & Poatovskii, I. Y. (1962). Synthesis of Benzimidazoles for the Study of their Scintillating Properties. *Zh. Obshch. Khim*, *32*, 2624-2633.

30. Yoshiyuki, T., & Kazuaki, Y. (1980). The modified synthetic reaction of 2-alkylbenzimidazoles: condensation of o-phenylenediamine with fatty acids in p-TSA. Hokkaido Daigaku Koagakubu Kenkyu Hokoku, *Chem. Abstr*, 93, 204-537.

31. Ramana, D. V., & Kantharaj, E. (1994). Mass spectrometer as a probe in the synthesis of 2-substituted benzimidazoles. *Tetrahedron*, 50(8), 2485-2496.

    a. Preston, P. N. (1974). Synthesis, reactions, and spectroscopic properties of benzimidazoles. *Chemical Reviews*, 74(3), 279-314.

32. Wright, J. B. (1951). The chemistry of the benzimidazoles. *Chemical Reviews*, 48(3), 397-541.

33. Reddy. V. P., Prasunamba. P. L., Reddy. P. S. N., Ratnam. C. V. (1983). Synthesis of Quinazolin-4-ones & Benzimidazoles: Fusion of 2-Aminobenzamide & 1,2-Diaminobenzene with Organic Acids. Indian Journal of Chemistry. 22, 917–918.

34. Singh, M. P., Sasmal, S., Lu, W., & Chatterjee, M. N. (2000). Synthetic utility of catalytic Fe (III)/Fe (II) redox cycling towards fused heterocycles: a facile access to substituted benzimidazole, bisbenzimidazole and imidazopyridine derivatives. *Synthesis*, *2000*(10), 1380-1390.

35. Villemin, D., Hammadi, M., & Martin, B. (1996). Clay catalysis: condensation of orthoesters with O-substituted aminoaromatics into heterocycles. *Synthetic communications*, *26*(15), 2895-2899.

36. Loupy, A., Petit, A., Hamelin, J., Texier-Boullet, F., Jacquault, P., & Mathe, D. (1998). New solvent-free organic synthesis using focused microwaves. *Synthesis*, *1998*(09), 1213-1234.

37. Penieres, C. G., Imelda, A. B., Jose Guadalupe, C. L., Guadalupe José, E. G., & Alvarez, T. C. (2000). Synthesis of benzimidazoles in dry medium. *Synthetic Communications*, *30*(12), 2191-2195.

38. Wu, Z., Rea, P., & Wickham, G. (2000). 'One-pot'nitro reduction–cyclisation solid phase route to benzimidazoles. *Tetrahedron Letters*, 41(50), 9871-9874.

    a. Tumelty, D., Schwarz, M. K., Cao, K., & Needels, M. C. (1999). Solid-phase synthesis of substituted benzimidazoles. *Tetrahedron letters*, 40(34), 6185-6188.

    b. Huang, W., & Scarborough, R. M. (1999). A new "traceless" solid-phase synthesis strategy: Synthesis of a benzimidazole library. *Tetrahedron letters*, 40(14), 2665-2668.

    c. Smith, J. M., Gard, J., Cummings, W., Kanizsai, A., & Krchňák, V. (1999). Necklace-coded polymer-supported combinatorial synthesis of 2-arylaminobenzimidazoles. *Journal of Combinatorial Chemistry*, 1(5), 368-370.

    d. Sun, Q., & Yan, B. (1998). Single bead IR monitoring of a novel benzimidazole synthesis. *Bioorganic & medicinal chemistry letters*, *8*(4), 361-364.

    e. Lee, J., Gauthier, D., & Rivero, R. A. (1998). Solid phase synthesis of 1-alkyl-2-alkylthio-5-carbamoylbenzimidazoles. *Tetrahedron letters*, *39*(3-4), 201-204.

39. Caddick. S. (1995) Microwave Assisted Organic Reactions. Tetrahedron, 51(38), 10403–10432.

40. For recent reviews on microwave-assisted organic reaction, see:

    a. Varma, R. S. (1999). Solvent-free synthesis of heterocyclic compounds using microwaves. *Journal of Heterocyclic Chemistry*, *36*(6), 1565-1571.

    b. Varma. R.S. (1999) Solvent-Free Organic Synthesis. Green Chem., 1(1), 43–55.

    c. Bose, A. K., Banik, B. K., Lavlinskaia, N., Jayaraman, M., & Manhas, M. S. (1997). MORE chemistry in a microwave. *ChemInform*, 28(47), 18-24.

    d. Majetich, G., & Hicks, R. (1995). Applications of microwave-accelerated organic synthesis. *Radiation Physics and Chemistry*, 45(4), 567-579.

    e. Abramovitch, R. A. (1991). Applications of microwave energy in organic chemistry. A review. *Organic preparations and procedures international*, 23(6), 683-711.

41. Efros, L. S. (1953). Synthesis of 4-Aminobenzimidazoles and Comparison of Their Chemical Properties with Those 5-Aminobenzimidazoles. *Zh. Obshch. Khim*, 23, 957-963.

42. Clément, M. J., Rathinasamy, K., Adjadj, E., Toma, F., Curmi, P. A., & Panda, D. (2008). Benomyl and colchicine synergistically inhibit cell proliferation and mitosis: evidence of distinct binding sites for these agents in tubulin. *Biochemistry*, 47(49), 13016-13025.

43. McKellar, Q. A., & Scott, E. W. (1990). The benzimidazole anthelmintic agents-a review. *Journal of veterinary pharmacology and therapeutics*, *13*(3), 223-247.

44. Gary, A. T., Kerl, M. E., Wiedmeyer, C. E., Turnquist, S. E., & Cohn, L. A. (2004). Bone marrow hypoplasia associated with

fenbendazole administration in a dog. *Journal of the American Animal Hospital Association*, *40*(3), 224-229.

45. Kahn, C. M., & Line, S. (2005). The Merck veterinary manual. Whitehouse Station, NJ: Merck. & Co.

46. Li, Y., Tan, C., Gao, C., Zhang, C., Luan, X., Chen, X., ... & Jiang, Y. (2011). Discovery of benzimidazole derivatives as novel multi-target EGFR, VEGFR-2 and PDGFR kinase inhibitors. *Bioorganic & medicinal chemistry*, *19*(15), 4529-4535.

47. Laryea, D., Gullbo, J., Isaksson, A., Larsson, R., & Nygren, P. (2010). Characterization of the cytotoxic properties of the benzimidazole fungicides, benomyl and carbendazim, in human tumour cell lines and primary cultures of patient tumour cells. *Anti-Cancer Drugs*, 21(1), 33-42.

48. Doudican, N., Rodriguez, A., Osman, I., & Orlow, S. J. (2008). Mebendazole induces apoptosis via Bcl-2 inactivation in chemoresistant melanoma cells. *Molecular Cancer Research*, 6(8), 1308-1315.

49. Hirschberg, E., Gellhorn, A., & Gump, W. S. (1957). Laboratory evaluation of a new nitrogen mustard, 2-[di-(2-chloroethyl) aminomethyl] benzimidazole, and of other 2-chloroethyl compounds. *Cancer research*, 17(9), 904-910.

50. Omar, A. M. M. E., Mohsen, M. E., Habib, N. S., & Aboulwafa, O. M. (1982). Potential alkylating agents derived from benzimidazole and benzothiazole. *Journal of pharmaceutical sciences*, 71(9), 991-993.

51. Ibrahim, E. S. A., Omar, A. M. M. E., Mohsen, M. E., & Khalil, M. A. (1980). Novel potential anticancer agents derived from benzimidazole. *Journal of pharmaceutical sciences*, *69*(11), 1348-1350.

52. Holden, H. E., Crider, P. A., & Wahrenburg, M. G. (1980). Mitotic arrest by benzimidazole analogs in human lymphocyte cultures. *Environmental and Molecular Mutagenesis*, *2*(1), 67-73.

53. Pourgholami, M. H., Akhter, J., Wang, L., Lu, Y., & Morris, D. L. (2005). Antitumor activity of albendazole against the human colorectal cancer cell line HT-29: in vitro and in a xenograft model of peritoneal carcinomatosis. *Cancer chemotherapy and pharmacology*, *55*(5), 425-432.

54. Refaat, H. M. (2010). Synthesis and anticancer activity of some novel 2-substituted benzimidazole derivatives. *European journal of medicinal chemistry*, 45(7), 2949-2956.

55. Sasaki, J. I., Ramesh, R., Chada, S., Gomyo, Y., Roth, J. A., & Mukhopadhyay, T. (2002). The Anthelmintic Drug Mebendazole Induces Mitotic Arrest and Apoptosis by Depolymerizing Tubulin in Non-Small Cell Lung Cancer Cells 1 Supported in part by grants from the National Cancer Institute and the NIH Specialized Program of Research Excellence in Lung Cancer P-50-CA70907 and P01 CA78778-01A1 (both to JAR), by gifts to the Division of Surgery and Anesthesiology from Tenneco and Exxon for the Core Laboratory Facility, by The University of Texas MD Anderson Cancer Center Support Core Grant CA16672 .... Molecular Cancer Therapeutics, 1(13), 1201-1209.

56. Spagnuolo, P. A., Hu, J., Hurren, R., Wang, X., Gronda, M., Sukhai, M. A., ... & Fine, N. (2010). The antihelmintic flubendazole inhibits microtubule function through a mechanism distinct from Vinca alkaloids and displays preclinical activity in leukemia and myeloma. *Blood*, 115(23), 4824-4833.

57. Hao, D., Rizzo, J. D., Stringer, S., Moore, R. V., Marty, J., Dexter, D. L., ... & Weitman, S. D. (2002). Preclinical antitumor activity and pharmacokinetics of methyl-2-benzimidazolecarbamate (FB642). *Investigational new drugs*, *20*(3), 261-270.

58. Tong, Y., Bouska, J. J., Ellis, P. A., Johnson, E. F., Leverson, J., Liu, X., ... & Rodriguez, L. E. (2009). Synthesis and evaluation of a new generation of orally

efficacious benzimidazole-based poly (ADP-ribose) polymerase-1 (PARP-1) inhibitors as anticancer agents. *Journal of medicinal chemistry*, *52*(21), 6803-6813.

59. Gümüş, F., Eren, G., Açık, L., Celebi, A., Ozturk, F., Yılmaz, S., ... & Elerman, Y. (2009). Synthesis, cytotoxicity, and DNA interactions of new cisplatin analogues containing substituted benzimidazole ligands. *Journal of medicinal chemistry*, *52*(5), 1345-1357.

60. Li, Y., Tan, C., Gao, C., Zhang, C., Luan, X., Chen, X., ... & Jiang, Y. (2011). Discovery of benzimidazole derivatives as novel multi-target EGFR, VEGFR-2 and PDGFR kinase inhibitors. *Bioorganic & medicinal chemistry*, 19(15), 4529-4535.

61. Sarhan, A. A., Al-Dhfyan, A., Al-Mozaini, M. A., Adra, C. N., & Aboul-Fadl, T. (2010). Cell cycle disruption and apoptotic activity of 3-aminothiazolo [3, 2-a] benzimidazole-2-carbonitrile and its homologues. *European journal of medicinal chemistry*, 45(6), 2689-2694.

62. Nofal, Z. M., Soliman, E. A., Abd El-Karim, S. S., El Zahar, M. I., Srour, A. M., Sethumadhavan, S. H. A. L. I. N. I., & Maher, T. J. (2011). Novel benzimidazole derivatives as expected anticancer agents. *Acta Pol Pharm*, 68, 519-534.

63. Błaszczak S. K., Marek M., Kaplińska K., Kruszyński R., Trzęsowska-K. A. and Olasik E. M. (2012). New benzimidazole derivatives with potential cytotoxic activity. Acta ABP Biochimica Polonica, 59, 279-288.

64. Al-Douh, M. H., Sahib, H. B., Osman, H., Hamid, S. A., & Salhimi, S. M. (2012). Anti-proliferation effects of benzimidazole derivatives on HCT-116 colon cancer and MCF-7 breast cancer cell lines. *Asian Pacific Journal of Cancer Prevention*, *13*(8), 4075-4079.

65. Patel. O. B. (2011). Synthesis and Biological Evaluation of Benzimidazole Derivatives as Anticancer Agents, International Journal of Pharmaceutical and Applied Sciences., 2(1), 15-19.

66. Alper, S., Arpaci, Ö. T., Aki, E. Ş., & Yalçin, I. (2003). Some new bi-and ter-benzimidazole derivatives as topoisomerase I inhibitors. *Il Farmaco*, *58*(7), 497-507.

67. Ramla, M. M., Omar, M. A., El-Khamry, A. M. M., & El-Diwani, H. I. (2006). Synthesis and antitumor activity of 1-substituted-2-methyl-5-nitrobenzimidazoles. *Bioorganic & medicinal chemistry*, 14(21), 7324-7332.

68. Wattel, E., Preudhomme, C., Hecquet, B., Vanrumbeke, M., Quesnel, B., Dervite, I., ... & Fenaux, P. (1994). p53 mutations are associated with resistance to chemotherapy and short survival in hematologic malignancies. *Blood*, 84(9), 3148-3157.

69. Lio, S. C., Johnson, J., Chatterjee, A., Ludwig, J. W., Millis, D., Banie, H., ... & Richards, M. L. (2008). Disruption of Golgi processing by 2-phenyl benzimidazole analogs blocks cell proliferation and slows tumor growth. *Cancer chemotherapy and pharmacology*, 61(6), 1045-1058.

70. Higgins, K. J., Liu, S., Abdelrahim, M., Vanderlaag, K., Liu, X., Porter, W., ... & Safe, S. (2008). Vascular endothelial growth factor receptor-2 expression is down-regulated by 17β-estradiol in MCF-7 breast cancer cells by estrogen receptor α/Sp proteins. *Molecular endocrinology*, 22(2), 388-402.

71. Park, Y. N., Kim, Y. B., Yang, K. M., & Park, C. (2000). Increased expression of vascular endothelial growth factor and angiogenesis in the early stage of multistep hepatocarcinogenesis. *Archives of pathology & laboratory medicine*, *124*(7), 1061-1065.

72. Carpenter, B., Lin, Y., Stoll, S., Raffai, R. L., McCuskey, R., & Wang, R. (2005). VEGF is crucial for the hepatic vascular development required for lipoprotein uptake. *Development*, *132*(14), 3293-3303.

73. Roodhart, J. M., & Voest, E.E. (2008). The molecular basis of class side effects due to treatment with inhibitors of the

1 VEGF/VEGFR pathway. *Current clinical*
2 *pharmacology*, *3*(2), 132-143.