Versatile mechanisms of 2-substituted benzimidazoles in targeted cancer therapy

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Abstract
Background: The aim of this review is to provide an overview on diverse anticancer activities of 2-substituted benzimidazole derivatives.

Main body: This review provides a correlation between the various mechanisms of action of benzimidazoles as anticancer and the substitution pattern around the nucleus.

Conclusion: The linker group and substitution at N-1, C-2, C-5, and C-6 positions have been found to be the most contributory factors for anticancer activity. This will help in the further design to afford more selective, potent, and multi-target anticancer of 2-substituted benzimidazole-based compounds.

Keywords: Heterocyclic, Benzimidazole, Anticancer, Cytotoxic effect

Background
Cancer, the uncontrolled, rapid, and pathological proliferations of abnormal cells, is one of the most formidable afflictions in the world jeopardizing human life and health [1–3]. It is the second most life-threatening disease after the cardiovascular disorder according to statistics from the American Cancer Society (ACS) [4, 5]. Cancer can occur in any living cell at any stage of life; the morbidity and mortality associated with disease is on rise. Millions of people worldwide are suffering from this dreaded disease which not only affects the health of the patient but also puts significant socioeconomic, mental, and physical burden on the family members [6–8].

Heterocyclic compounds occupy a central position in medicinal chemistry and are of particular interest and significant importance in the search for new bioactive molecules in the pharmaceutical industry [9]. More interest in the area of benzimidazole-based chemistry was developed in the 1950s, when 5,6-dimethyl-1-(α-D-ribofuranosyl) benzimidazole was found as an integral part of the structure of vitamin B_{12} [10]. Benzimidazole being an isostere of purine-based nucleic acid found to be a privileged lead nucleus widely used in the design of many biologically active molecules [11]. Benzimidazoles exhibit a wide range of biological activities including antibacterial [12], antifungal [13], analgesic [14], and cardiovascular [15] in addition to anticancer activities [16–18].

Main text
Chemistry of benzimidazoles
Benzimidazole is one of the oldest known nitrogen heterocycles and was first synthesized by Hoebrecker [19] then later by Ladenburg [20]. Benzimidazole ring contains two nitrogen atoms with amphoteric nature, possessing both acidic and basic characteristics (Fig. 1). It exists in two equivalent tautomeric forms, in which the hydrogen atom can be located on either of the two nitrogen atoms [16, 18].

Synthesis of 2-substituted benzimidazoles
The synthetic strategy adopted for the preparation of benzimidazoles relies on building up the imidazole ring on a suitable substituted benzenic core.

The first benzimidazole was prepared by Hoebrecker [19], who obtained 2,5-dimethylbenzimidazole by the
reduction and dehydration of 2-nitro-4-methylacetanilide (Scheme 1) [21, 22].

In the majority of cases, \( o \)-phenylenediamines or suitably substituted derivative was used as the starting compound where it was reacted with different one carbon delivering reagents to give the benzimidazole derivatives (Scheme 2). The following reactions were classified according to the reagent used through different pathways from A to R [21–24].

Anticancer activity of 2-substituted benzimidazoles

Recently, the major side effect associated with the traditional anticancer agents is cytotoxicity towards normal cells due to lack of selectivity for the abnormal cells is well noticeable. With the rapid development of cell biology and molecular biology, the strategy of anti-cancer drug research has shifted to new and more selective targets for cancer cell proliferation, such as cancer cell signal transduction pathways, growth factors and their receptors, and apoptosis pathway. Therefore, the search for anticancer agents has been in continuum for many years.

Nevertheless, targeted therapy has some limitations; the chief among them is the potential for the cells to develop resistance. Unfortunately, in most cases, another targeted therapy that could overcome this resistance is not available, so it is advantageous to use targeted therapy in combination, either with other targeted therapy or with traditional therapy [25, 26].

In this review, a literature survey on the anticancer activity of benzimidazole was illustrated. Various anticancer agents (also referred to as antitumor, antiproliferative, and antineoplastics) reported for treatment of varied kinds of cancers act through different mechanisms. Benzimidazole exerted their antitumor activity through versatile mechanisms of action such as DNA alkylation, DNA binding, disturbing tubulin polymerization or depolymerization, enzyme inhibition, antiangiogenic, and signal transduction inhibitors.

2-Substituted benzimidazoles as alkylating agents

Alkylating agents are highly electrophilic compounds that react with nucleophilic groups on DNA, in particular the N-7 of G base, to form a strong covalent bond disrupting replication or transcription.

1,2,5-Trisubstituted benzimidazoles were found to act as alkylating agents, for example, bendamustine 1 (TREANDA\(^*\)), which has been approved by the FDA for the treatment of leukemia, multiple myeloma, and non-Hodgkin’s lymphoma [27, 28].

In addition, the pyrrolo[1,2-a]benzimidazoles-4,7-diones 2a–c are one of the early classes of anticancer agents designed as new DNA cross-linkers acting through cleavages of G and A bases which possess IC\(_{50}\) ranging from 10 to 6000 nM values against various human ovarian and colon cancer cell lines [29].

Benzimidazoles as DNA-binding agents

This class of compounds is known broadly as antimetabolites. These drugs inhibit the enzymes involved in the synthesis of nucleotide building blocks of DNA, resulting in the inhibition of DNA function which may lead to apoptosis [11].

Gao et al. synthesized 2-substituted novel benzimidazole acridine subsidiaries keeping in setting that acridines can intercalate into DNA and benzimidazoles can tie in the DNA minor groove. MTT assay showed that most of the designed synthesized compounds revealed

![Scheme 1 Intramolecular cyclocondensation](image-url)
great antiproliferative action, among which compound 3 exhibited high potency against both chronic myelogenous leukemia K562 and liver HepG2 cells [30].

2,6-Disubstituted benzimidazole-oxindole conjugate derivatives were synthesized by Nayak et al. Furthermore, they investigated the apoptotic system initiated by these conjugates against human breast cancer cell line, MCF-7. Following evaluation, compounds 4a and 4b showed 43.7% and 43.6% and 64.8% and 62.7% apoptosis, respectively, at 1 and 2 μM concentrations [31].

On the other hand, the 5-substituted bisbenzimidazoles Hoechst 33258 (5a) and Hoechst 33342 (5b) were designed as DNA minor groove-binding agents. They displayed in vitro antitumor activities and nonspecifically inhibit the catalytic proliferation of DNA enzymes [32].

Derivatives of 1,2,5-trisubstitued 1-(4-methoxyphenethyl)-1H-benzimidazole-5-carboxylic acid were prepared and assessed as potential chemotherapeutic agents by Gowda et al. Among synthesized analogs methyl 1-(4-methoxyphenethyl)-2-(4-fluoro-3-nitrophenyl)-1H-benzimidazole-5-carboxylate 6 provoked cell death in leukemic cells with an IC50 of 3 μM. Additionally, it stimulates S/G2 cell cycle arrest, and downregulation of cyclin B1, CDK2, and PCNA. Moreover, the replacement of fluorine and nitro with amine, chlorine, or bromine reduced the activity as shown by the SAR study [33].

Hranjec et al. designed and synthesized benzimidazo[1,2-a]quinolones candidates that have the capacity to be incorporated into the space between the DNA base pairs resulting in DNA cleavage. All compounds exerted pronounced antiproliferative activity on five tumor cell lines, whereby compound 7 exerted the highest activity on all cell lines (IC50 = 0.7–25 μM) and showed a special selectivity towards HeLa cells [34].

Recently, more 1,2-fused planar benzimidazole derivatives have been reported to exhibit potent cytotoxicity, for example, (pyrimidobenzimidazolone) 8 with in vitro NSCLC growth % from −2.92 to 38.33 and GI50 ranges from −6.13 to −4.82 μM. Compound 9 (1,3-diarylpyrazino benzimidazole) exhibited in vitro % inhibition of lipid peroxidation (AAPH); interaction % with DPPH (2,2-diphenyl-1-picrylhydrazyl) and in vitro % inhibition of soybean lipoxygenase (LOX % inh) was found to be 84, 5, 19 %, respectively [35, 36].

Zhou et al. developed and synthesized a new Cu(II) complex of benzimidazoles 10 possessed antitumor activity. The results suggested that the complex could electrostatically bind to the phosphate group of DNA backbone and partially intercalate into the double helix of DNA because of the bulky structure of the complex and the planarity of the benzimidazole rings [37].
2-Substituted benzimidazoles as tubulin inhibitors

Microtubules are the key components of the cytoskeleton of eukaryotic cells composed of α/β-tubulin heterodimers. They are involved in intracellular migration and transport. Tubulin inhibitors thus act by interfering with the dynamics of microtubules, i.e., growing (polymerization) and shortening (depolymerization). Either way, it leads to mitotic arrest and cell death [38–40].

Lu et al. outlined the synthesis and assessment of a series of tubulin inhibitors. Structure-activity relationships of these compounds prompted recognizable proof of new 2,4-disubstituted benzimidazole and imidazo (4,5-c)pyridine-fused ring templates, represented by compounds 11 and 12, respectively.

Compound 11 enhanced the metabolic stability in liver microsomes and was the most active of the series with IC50 0.022 ± 0.009 μM and 0.025 ± 0.004 against prostate tumor cell PC-3 and melanoma cancer cell A375, respectively [41].

Kamal et al. identified a series of 2,6-disubstituted pyrazolyl benzimidazole analogs as microtubule-destabilizing agents where they showed potent growth-inhibitory activity against most of the sixty cancer cell line panels of NCI ranging from 0.3 to 13 μM. A549 cells treated with 13a–c-arrested cells at the G2/M phase apart from activating cyclin-B1 protein levels and disrupting the microtubule network. Moreover, these conjugates adequately hindered tubulin polymerization with IC50 values of 1.3–3.8 μM. Compound 13b with a methoxy substituent showed obvious cytotoxic potential and incited activity right around 3-fold higher than CA-4 [42].

2,5-Disubstituted novel benzimidazole carbamates linked with indole moieties by sulfur or selenium atoms were synthesized and examined for their inhibitory action against some human cancer cell lines. Among these, compound 14 was identified as the most active antitumor against HT-1080, A-549, and SGC-7901 human cancer cell lines with IC50 values ranging from 0.098 to 0.15 μM. In vitro tubulin polymerization assay revealed that 14 significantly detains tubulin polymerization and upsets tubulin microtubule. Also, a molecular modeling study exhibited that compound 14 bonds with a coupling mode similar to that of nocodazole [43].

Analogs of 2,5-disubstituted benzimidazoles were developed and assessed for their tubulin inhibitory activity. Compound 15 strongly restrained the proliferation of a panel human cancer cells, with IC50 values ranging from 0.006 to 1.774 μM; also it prompted cell cycle arrest at the G2/M phase [44].

2,4/5-Disubstituted class of terphenyl benzimidazoles 16a,b were designed and synthesized as tubulin polymerization inhibitors. 16a,b demonstrated significant anticancer activity with GI50 ranges from < 0.1 to 2.11 μM. These compounds exhibit G2/M phase arrest; besides the analysis of tubulin by western blot, experiments were carried out revealing a concomitant decrease in the polymerized tubulin [45].

Wang et al. designed some 1,2-disubstituted benzimidazole derivatives as potential tubulin polymerization inhibitors and anthropic cancer cell line cytotoxic agents. Compound 17 was established as the most potent tubulin polymerization inhibitory agent with IC50 of 1.5 μM and exhibited antiproliferative activity against several human cancer cell lines [46].
2-Substituted benzimidazoles as enzyme inhibitors

2-Substituted benzimidazoles as sirtuin inhibitors

Sirtuins are class III histone deacetylases (HDACs) that catalyze the deacetylation of proteins. They focus on an extensive variety of cell proteins in the nucleus, cytoplasm, and mitochondria [47, 48]. It has been reported that SIRT1 and SIRT2 are upregulated in numerous tumor sorts and they are able to inactivate some tumor suppressor proteins [49]. Therefore, the development of novel sirtuin inhibitors has been proposed as another line in the treatment of cancer [50, 51].

Yoon et al. developed novel 1,2,5-trisubstituted benzimidazole derivatives as SIRT1 and SIRT2 inhibitors. Compound 18 showed the best inhibitory activity for SIRT1 (IC$_{50}$ = 54.21 μM) and for SIRT2 (IC$_{50}$ = 26.85 μM). Cell proliferation assay demonstrated that compound 18 had pronounced antitumor activity against three different types of cancer cells (breast MDA-MB-468, colon HCT-116, and blood-leukemia CCRF-CEM). The SAR study verified that the replacement of the phenyl group at position N1 led to a loss of activity [52].

2-Substituted benzimidazoles as poly (ADP-ribose) polymerase inhibitors

Poly (ADP-ribose) polymerase-1 (PARP-1) is a key DNA damage-sensing enzyme that facilitates the repair of DNA. PARP-1 contributes to the resistance that often develops after cancer therapy. Consequently, it is assumed that in vivo inhibition of PARP will block DNA repair and increase the maximum therapeutic benefit of chemotherapy [53].

Some 2,4-disubstituted benzimidazoles were demonstrated to have PARP inhibitory activity. Penning et al. reported the design of a new series of 2-substituted-1H-benzimidazole-4-carboxamides. Especially, compound 19 Veliparib (ABT-888) displayed great adequacy against both the PARP-1 and PARP-2 enzymes with a $K_{i}$ (inhibitory constant and reflective of the binding affinity) of 5 nM and in a C41 whole-cell assay with an EC$_{50}$ of 2 nM and exhibited potentiation of two cytotoxic agents in particular temozolomide and cisplatin in both mouse melanoma and breast cancer demonstrate [54].

Abdullah et al. prepared different 2,4,6-trisubstituted benzimidazole derivatives and studied their activity as dual Poly (ADP-ribose) polymerases (PARPs) and Dihydroorotate dehydrogenase (DHODH) inhibitors. Analogs 20a-d were proven to be the most potent compounds of the series with IC$_{50}$ ranges from 0.013–55 to 0.061–44 μM against DHODH and PARPs, respectively [55].

A series of novel 2,4,5-trisubstituted benzimidazole analogs were identified and evaluated for their PARP-1 inhibitory activity by Wang et al. All target compounds demonstrated high intrinsic PARP-1 inhibitory activity and have been assessed for in vitro cellular assays to evaluate the potentiation effect of cytotoxic agents against cancer cell lines. Compound 21 showed strong inhibition against the PARP-1 enzyme with an IC$_{50}$ of 43.7 nM, excellent cell inhibitory activity in HCT116 cells (IC$_{50}$ = 7.4 μM), and potentiation of temozolomide cytotoxicity in the cancer cell line A549 (PF$_{50}$ = 1.6) [56].

Zhou et al. synthesized several derivatives of 2-substituted-3,4-fused benzimidazole carboxamide as PARP-1 inhibitors, which can be 18F-labeled easily for positron emission tomographic (PET) imaging. Of the compounds synthesized, 22 had the highest inhibition potency for PARP-1 with IC$_{50}$ 6.3 nM [57].

2-Substituted benzimidazoles as methionine synthase inhibitors (MetSIs)

Enzyme methionine synthase catalyzes the transfer of a methyl group from 5-methyltetrahydrofolate to homocysteine, to obtain methionine and tetrahydrofolate; thus, inhibition of methionine synthase (MetS) would settle DNA and RNA [58].

One of the most potent inhibitors of MetS was 2,5-disubstituted benzimidazole 23 which showed IC$_{50}$ of 50 ± 5 μM with a score of the lowest free energy of binding – 1610.42 kJ/mol [59].

Other 2,5-disubstituted benzimidazole derivatives were designed and evaluated for their inhibitory activity against purified rat liver methionine synthase using a radiometric enzyme assay. Compounds 24a,b were proven to be the most powerful compounds, with
IC\textsubscript{50} values of 20 µM and 18 µM, respectively. Modeling and docking studies determine the molecular requirements of the activity of this class of compounds [60].

2-Substituted benzimidazoles as DHFR inhibitors
Dihydrofolate reductase (DHFR) is a critical enzyme in folate metabolism. It converts dihydrofolate (DHF) to tetrahydrofolate (THF), which is essential for purine and thymidine (TMP) synthesis in cell proliferation. Depressing DHFR activity results in THF deficiency and disruption of subsequent DNA replication and resulting in cell death.

A new series of 1,2,6-trisubstituted triazine-benzimidazole hybrid was developed as a potent DHFRI. Compound 25 was found to be the most active DHFRI with IC\textsubscript{50} of 1.05 µM. These findings are the outcome of their inhibitory activities tested over 60 human tumor cell lines, whereas 25 revealed a broad spectrum of antitumor activities with a GI\textsubscript{50} value of 9.79 µM [11, 61].

Recently, Singla et al designed and synthesized novel series of 1,2,6-trisubstituted quinazolin-4-one-benzimidazoles. As per the results of dihydrofolate reductase enzyme immunoassay, compound 26 exhibited comparable activity with IC\textsubscript{50} value of 0.011 µM in contrast to methotrexate (IC\textsubscript{50} = 0.02 µM). The preparatory interactive examinations with calf thymus DNA by UV–visible and fluorescence spectroscopy disclosed that compound 26 was viably intercalated with ct-DNA [62].

2-Substituted benzimidazoles as topoisomerase I and II inhibitors
Topoisomerase enzymes are a family of enzymes which catalyze the cleavage, annealing, and supercoiling of DNA and considered a major target for antineoplastic agents used in the treatment of breast, lung, and prostate cancer, sarcomas, and hematological malignancies. All topoisomerase II-directed agents are able to interfere with at least one step of the catalytic cycle [63, 64].

2-Substituted benzimidazole derivatives 27a,b were synthesized and screened against topoisomerase I with inhibition percent 95.4%, 90.2%, respectively. Meanwhile, the cytotoxic activity against HeLa (cervix adenocarcinoma), MCF7 (breast adenocarcinoma), and A431 (skin epidermoid carcinoma) cells were measured, the IC\textsubscript{50} were found to range from 6.04 to > 30 µM [65].

A series of new pyrazolo[3,4-d]pyrimidine possessing 4-((1H-benzimidazol-2-yl)-phenylamine moiety has been designed and synthesized by single et al. Compounds 28, 29, 30, and 31 turned out to be the most efficacious candidates in this series, with mean GI\textsubscript{50} values of 1.30 µM, 1.43 µM, 2.38 µM, and 2.18 µM, respectively, against several cancer cell lines. Moreover, these compounds induced apoptosis and suppressed human topoisomerase (Topo) IIa. These discoveries established a framework for the sound outline of powerful agents for probing proteins, which are relied upon to give vital knowledge into the field of DNA and protein connections [66].

In a study of some novel fused heterocyclic compounds as eukaryotic topoisomerase II inhibitors. It was discovered that 5-methylcarboxylate-2-phenylthiobenzimidazole, 32, with IC\textsubscript{50} 17 µM was more active than the reference drug etoposide (IC\textsubscript{50} = 21.8 µM) [67].

Singh et al. worked on the synthesis of a novel class of topoisomerase I inhibitor as 2-aryl-5-substituted-2,5-bisbenzimidazole derivatives. Analogs 33a,b were found to be with the highest ability to induce DNA cleavage with IC\textsubscript{50} in the micromolar range (0.6–5.5) against U87, MCF7, and HeLa human tumor cells. The SAR study revealed that the introduction of halogen groups at the phenyl ring increased the binding affinity to ct-DNA particularly fluoro and chloro groups which showed significant cytotoxic activity to human tumor cell lines [68].

Jin et al. prepared a series of heterocyclic derivatives of 5-phenyl-bibenzimidazoles, 34, and assessed them for their topoisomerase I poisoning activity and cytotoxicity. It was concluded that topoisomerase I poisoning activity was related to analogs that had a hydrogen atom capable of hydrogen bond formation, thus influencing the activity [69].

A new Cu\textsuperscript{2+} complex of 2-pyridinylbenzimidazole-5-carboxylic acid analogs were designed and prepared by Galal et al. Among all, compound 35 was found to be the most potent candidate inhibiting the topoisomerase II on the DNA-relaxing activity of P388 topoisomerase II with IC\textsubscript{50} 2.5 µM. Meanwhile, the growth-inhibitory effects of the tested compounds on 21 human solid tumor cell lines (8 lung, 7 colon, and 6 gastric cancer cell lines) with mean GI\textsubscript{50} = 0.091 µM [70].
2-Substituted benzimidazoles as androgen receptor activity antagonists

Testosterone and dihydrotestosterone control protein anabolism and influence basal metabolism through the upregulation of the androgen receptor. Although androgens have numerous valuable impacts and are critical for sexual orientation and male advancement, endogenous androgens, for example, testosterone fortify hyperplasia of the prostate and intensify androgen-subordinate prostate disease [71].

Elancheran et al. designed and synthesized a series of 1,3,5-tri-substituted 2-oxobenzimidazoles derivatives and investigated them as androgen receptor antagonistic activity. It can be concluded that compound 36 is the most active compound from the series against prostatic PC-3 and LNCaP cancer cell lines [72].

Ng et al. have investigated some 1,2,5,6-tetrasubstituted benzimidazoles as androgen receptor antagonists for their utilization in prostate malignancy. The SAR studies have prompted the 1-(4-bromobenzyl) derivative 37 as the most intense androgen receptor opponent which has an ID50 of 0.13 mg/day [73].

2-Substituted benzimidazoles as α-glucosidase inhibitors

α-Glucosidase inhibitors have wide biological significance as chemotherapeutic agents for the treatment of carbohydrate mediated diseases such as diabetes and cancer [74]. Consequently, 2,5,6-trisubstituted benzimidazoles bearing thiourea moiety have been synthesized and assessed for α-glucosidase inhibition using Baker’s yeast α-glucosidase enzyme. All tested compounds exhibited variable α-glucosidase inhibitory activity, while compound 38 showed significant inhibitory effects with an IC50 value of 35.83 ± 0.66 μM, which was more potent than the standard acarbose (IC50 = 774.5 ± 1.94 μM) [75].

2-Substituted benzimidazoles as the G9a histone methyltransferase inhibitors

G9a is initially identified as a H3K9 methyltransferase that specifically mono- and dimethylates "Lys-9" of histone H3 (H3K9me1 and H3K9me2, respectively) in euchromatin. Histone lysine methylation plays a central epigenetic role in the organization of chromatin domains and the regulation of gene expression [76]. Mutation and amplification of HMTs are frequently observed in human cancers and employed as a promising target in cancer therapy [77].

Recently, 1,2,5-trisubstituted benzimidazole scaffold was discovered as G9a histone methyltransferase inhibitor by Zhang et al. Based on structure optimization, 24 structural analogs are designed and synthesized. The kinase inhibition assay showed that compound 39 potently inhibits G9a with an IC50 of 1.32 μM. Besides, the MTT assay revealed that MCF-7 is most sensitive to 39 among five different breast cancer cells with IC50 = 5.73 ± 0.95. In addition, compound 39 induces obvious autophagy in MCF7 cells by fluorescence microscope assays and western blot analysis [78].

2-Substituted benzimidazoles as pyruvate kinase inhibitors

Recently, the role of the M2 isoform of pyruvate kinase in the change in cellular metabolism to aerobic glycolysis has been proposed [79]. Tumor cells entirely express the
embryonic M2 isoform of pyruvate kinase (PKM2); the dimeric form assists cell growth by increasing glycolytic intermediates for biosynthetic processes, but when energy levels decrease, the enzyme shifts to the tetrameric form and makes oxidative phosphorylation easy [80].

Consequently, the discovery of 1,2-disubstituted benzimidazole series was described as potent and selective PKM2 activators by Guo et al.; Compound 40 was reported to have appreciable inhibitory activity against PKM2 cancer cells with a lactate dehydrogenase (LDH) IC$_{50}$ value of 3.5 μM [81].

2-Substituted bisbenzimidazoles as telomerase inhibitors
Maji et al. reported a new series of 2,6-disubstituted bisbenzimidazole-carbazoles and was evaluated for their antiproliferative activity as telomerase inhibitors where compound 41 was recognized as the most potent derivative with an IC$_{50}$ value of 0.6 ± 0.01 μM [82].

Similarly, 2-substituted tribenzimidazole derivatives were synthesized and evaluated for their telomerase inhibitory properties. Compound 42 was found to be the most active compound of the series [83].

In addition, compounds 43a-c are derivatives of 2-substituted-5,6-fused benzimidazole that exhibited in vitro inhibition of hTERT expression, telomerase inhibition, and suppression of prostatic cancer cell growth with IC$_{50}$ values 5.1 to 27.9 μM [84].

2-Substituted benzimidazoles as antiangiogenic agents and signal transduction inhibitors
Tumor growth and development of distant metastasis are supported through angiogenesis where new blood vessels extend from pre-existing ones. This process is mediated via the vascular endothelial growth factor (VEGF) which links to its receptor tyrosine kinase (RTK) to advance the proliferation and survival of endothelial cells. Consequently, using kinase inhibitors to adjust VEGFR signaling is considered as a powerful method for the inhibition of tumor angiogenesis [85, 86].

Protein kinases regulate most aspects of normal cellular function, especially signals transduction from the cell membrane into the interior of the cell [87]. Signal transduction relates to the methods by which regulatory molecules that control essential processes of cell growth and differentiation, convey within the cell. Most malignancies have abnormal or overexpressed signal transduction factors which depend on the kinase enzymes thus recommending them as targets for therapeutic progress [88, 89].

A large number of benzimidazoles were reported to possess protein kinases inhibitory activity [90].

2-Substituted benzimidazoles as VEGFR inhibitors
Keeping this in mind, 2-substituted benzimidazole analogs were prepared and assessed for their in vivo antitumor and antiangiogenic activities. Compounds 44 and 45 showed cytotoxic effects on MDA-MB-31 cells and HUVEC with IC$_{50}$ value ranges from 0.40 to 6 μM and from 0.05 to 1.5, respectively, and effectively antagonized VEGF-A165/NRP-1 binding [91].

In addition, a series of 2,5/6-disubstituted benzimidazole derivatives were developed as potent VEGFR-2 (KDR) kinase inhibitors. Among them, compound 46 showed the most potent VEGFR-2 inhibitory activity with an IC$_{50}$ value of 0.03 μM and it also demonstrated strong anticancer activity against the tested cancer cell lines [85].

Temirak et al. identified 1-aryl,2-furyl benzimidazoles and tested their anti-cancer activity where compound 47 showed the most potential antiangiogenic effect against VEGFR2 kinase with an IC$_{50}$ value of 6.98 μg/mL [92].

A novel series of 1,2-disubstituted benzimidazol-furan hybrids were designed, synthesized, and evaluated for their in vitro cytotoxic activity against breast (MCF-7) and hepatocellular (HepG2) carcinoma cell lines 48a,b, 49-51. Two of the synthesized conjugates, 48b and 49, showed potent antiproliferative properties against the MCF-7 cell line (IC$_{50}$ = 21.25 and 21.35 μM, respectively). Additionally, compounds 48a,b, 50, and 51 showed promising potency (IC$_{50}$ = 25.95, 22.58, 26.94, and 31.06 μM, respectively) against the liver carcinoma cell line HepG2. Meanwhile, the in vitro evaluation on
VEGFR-2 in the MCF-7 cell line showed their potent inhibitory activity ranges from 92–96%, compound 48a was found to have promising VEGFR-2 inhibitory activity (IC\textsubscript{50} = 0.64 \mu M) [93].

Novel conjugates of 2,5,6-trisubstituted benzimidazoles were synthesized and examined against VEGFR-1 and VEGFR-2. Compound 52 displayed VEGFR-2 inhibitory activity with a 50% inhibition concentration value as low as 0.02 ± 0.03 \mu M. VEGFR-2 active compounds display good activity against VEGFR-1 up to 91% inhibition at 10 \mu M concentration. The compounds likewise give a chance of establishing the framework for promising molecules of anticancer activity [94].

2-Substituted benzimidazoles as EGFR inhibitors

The EGFR (also known as erbB1 or HER1) and the related human epidermal growth factor receptor HER2 (also known as erbB2) is an encouraging aim for anticancer drug design because of its value in tumor growth, metastasis, and angiogenesis [95–98].

Akhtar et al. identified the synthesis of oxadiazole attached to benzimidazole at position C\textsubscript{2} as potential EGFR and erbB2 receptor inhibitors and assessed their cytotoxic activity. The most active compounds against breast cancer cell lines constitute para-substituted chloro/methoxy phenyl at the fifth position of oxadiazole 53\textsubscript{a,b}, while, derivative 53\textsubscript{a} specifically affected cell cycle arrest at G\textsubscript{2}/M phase with an increase in apoptosis. Also, it inhibited EGFR and erbB2 receptor at 0.081 and 0.098 \mu M concentration [99]. A series of 1,2,7-trisubstituted benzimidazole derivatives were developed and evaluated for mutant non-small-cell lung cancer activity and epidermal growth factor receptor (EGFR) by Lelais et al. Compound 54 was proven to be the most powerful compound of the series against lung cancer cell lines [100].

2-Substituted benzimidazoles as FGFR1 inhibitors

Fibroblast growth factor receptor (FGFR) represents an attractive oncology target for cancer therapy in perspective of its basic part in advancing tumor formation and progression, and additionally making resistance-affirmed treatments [101].

Yan et al. synthesized a series of 2,5-disubstituted benzimidazole and evaluated them for effective inhibition effect of the fibroblast growth factor receptor. Compound 55 was recognized as a potent pan-FGFR inhibitor and exhibited outstanding in vitro inhibitory activity against a panel of FGFR-amplified cell lines with IC\textsubscript{50} < 0.1 nM. Also, 55 gave almost total control of tumor development (96.9 % TGI) in NCI-H1581 (FGFR1-amplified) xenograft mice model [102].

New 2,6-disubstituted benzimidazoles were synthesized by Gryshchenko et al. and assessed for inhibition of FGFR1 kinase activity. Compounds 56\textsubscript{a,b} displayed the most potent activity with an IC\textsubscript{50} value of 0.63 \mu M and 0.32 \mu M respectively. The SAR study showed that the presence of the hydroxyl group at the meta position of the benzene ring of 56\textsubscript{a,b} caused rapid augmentation of inhibition towards FGFR1 [103].

2-Substituted benzimidazole Tie-2 receptor tyrosine kinase inhibitors

Tie-2 is a receptor tyrosine kinase that is essential for the formation of the embryonic vasculature and is strongly implicated in tumor angiogenesis [104].

Novel 2,5-disubstituted benzimidazolyl-4-aminopyrrolopyrimidine analogs 57\textsubscript{a,b} were identified as potent inhibitors of the Tie-2 kinase. Compound 57\textsubscript{a} with 2,6-di-fluoro-phenylsulfonyl group was discovered to have Tie-2 kinase and cellular (Caco-2BA/AB) IC\textsubscript{50} of 568 and 518 nM, respectively. The corresponding urea analog compound 57\textsubscript{b} was more potent inhibitor against...
Tie-2 kinase cellular assays with IC\textsubscript{50} of 241 and 112 nM, respectively [87].

2-Substituted benzimidazoles as heparanase inhibitors
Heparanase is an endo $\beta$-D-glucuronidase that cleaves heparan sulfate polymers in the extracellular matrix and regulates the release of many growth factors that are involved in tumor invasion. The finding that heparanase is elevated in a variety of tumors and is subsequently linked to the development of pathological processes makes the inhibition of this enzyme a target for anticancer development programs [105].

Pan et al. incorporated a 2,5,6-trisubstituted bisbenzimidazole with urea derivatives and assessed their potential to suppress heparanase enzyme. Compound 58 revealed high heparanase inhibitory activity with IC\textsubscript{50} ranging from 0.075–0.27 $\mu$M [106].

2-Substituted benzimidazoles as COX-1 and COX-2 inhibitors
In the aim of developing novel technique for cancer treatment, cyclooxygenase enzymes inhibitors was introduced [107]. Cyclooxygenase proteins (COX) are the principle organizers of prostaglandin (PG) biosynthesis from arachidonic acid. COX enzymes are classified principally into two isoforms: a constitutive form (COX-1) and an inducible form (COX-2). The COX-2 isoform was found to be excessively expressed in tough human malignancies, for example, breast cancer, bladder, prostate, and colon. The COX-2 enzyme plays a part in apoptosis control, by increasing angiogenesis [108].

In this context, new series of 2-substituted benzimidazole pyrazoles were prepared and evaluated for their antiproliferative activity against breast carcinoma (MCF-7) and non-small cell lung cancer (A549) cell lines. Compound 59 was the most active against both A549 and MCF-7 cell lines with IC\textsubscript{50} = 8.46 and 6.42 $\mu$M, respectively. Also, 59 is the most COX-2 selective compound among all synthesized derivatives; it showed inhibitory activity against COX-2 enzymes with IC\textsubscript{50} = 0.10 Mm, S.I. = 104.67) compared with celecoxib (COX-2 IC\textsubscript{50} = 1.11 $\mu$M, S.I. = 13.33) [109].

2-Substituted benzimidazoles as Aurora A/B kinase inhibitors
Aurora kinases are serine-threonine kinases that play a critical role in the mitotic events of cell division [110]. They are essential to secure the correct progress of cell cycle during mitosis or meiosis [111]. Different benzimidazoles have been developed as Aurora kinase inhibitors for cancer treatment.

Novel 2,6-disubstituted benzimidazole derivatives have been designed and synthesized as Aurora kinase inhibitors. The entire target compounds were determined against cancer cell lines U937, K562, A549, LoVo, and HT29 and were screened for Aurora A/B kinase inhibitory activity in vitro. Compound 60 demonstrated selective cancer cell line inhibitory activity towards U937, LoVo, and HT29 with IC\textsubscript{50} values 3.821, 1.422, and 1.299 $\mu$M, respectively. In addition, 60 revealed high Aurora A/B kinase inhibitory activities (IC\textsubscript{50} 124.9 and 191.0 nM) [112].

Compound 61, a 2-aminobenzimidazole derivative was identified and proven to act as potent Aurora kinase inhibitor with IC\textsubscript{50} against Aurora A/B 17 and 5 nM, respectively. 2-Aminobenzimidazole acts as a bioisostere of the biaryl urea moiety of SNS-314, a potent Aurora kinase inhibitor, thus entered into the clinical study. This series of compounds present more aqueous solubility while keeping comparable potency in in vitro assays; compared with SNS-314, 61, in particular, has also exhibited a comparable profile to SNS-314 [113].

A novel 2,5-disubstituted benzimidazole (AT-9283) was described by Howard et al. as a multi-targeted kinase inhibitor, with special activity towards Aurora kinases A and B. Compound 62 (AT-9283) inhibited HCT116 cell line (IC\textsubscript{50} = 0.03 $\mu$M) and demonstrated the polyploid cellular phenotype characteristically related to Aurora B kinase inhibition (IC\textsubscript{50} of approximately 3 nM). Compound 62 demonstrated in vivo efficacy in mouse xenograft models and is currently under evaluation in phase I clinical trials [114].

In an effort to identify novel compounds targeting Aurora kinase enzyme, Sharma et al. developed 1,2,5-trisubstituted benzimidazole derivatives. Among the
several prepared compounds, 63 proved to be 1.25-fold more active than the positive control 5-FU, with a GI\textsubscript{50} value of 18.12 \(\mu\text{M}\) (MG-MID). Moreover, interaction of the compound 48 with Aurora A enzyme showed selective inhibition of Aurora A kinase with IC\textsubscript{50} value of 0.01 \(\mu\text{M}\) [115].

2-Substituted benzimidazoles as checkpoint kinase (Chk1 and Chk2) inhibitors

Checkpoint kinase is a serine-threonine protein kinase that coordinates the DNA damage response and is activated by phosphorylation prompting cellular response such as cell cycle regulation, DNA repair, or apoptosis. Chk proteins are invariably more abundant in tumors as compared with normal tissues [116]. Inhibition of Chk2 has been proposed to be a significant argument of current cancer therapies [117, 118].

A series of 2/2,5-disubstituted benzimidazole derivatives was designed and synthesized by Song et al. as inhibitors of checkpoint kinase 1 (Chk1). Most of them exhibited moderate to good Chk1 inhibitory activities. Among them, compounds 64a–c showed significant Chk1 inhibitory activities with IC\textsubscript{50} values of 4.05, 6.23, and 2.33 nM, respectively [119].

Some 2-substituted benzimidazoles were reported by Ni et al. as a novel class of small molecule ChK-1 inhibitors. Compound 65 has emerged as a potent and selective compound with IC\textsubscript{50} value 0.32 nM [120].

Neff et al. have celebrated a new series of 2,5-disubstituted benzimidazole compounds containing pendant alcohol and amine moieties were found to be active against Chk2. Compound 66 was found to have the best inhibitory activity with IC\textsubscript{50} value 14 ± 8 nM [121].

Galal et al. have applied a structure-based design to synthesize a new series of 2,5-di substituted benzimidazole compounds containing pendant alcohol and amine moieties were found to be active against Chk2. The activities of the conjugates as checkpoint kinase inhibitors and as an antitumor were evaluated. The results indicated that compounds 67 and 68 inhibited Chk2 activity with high potency IC\textsubscript{50} ranges from 5.5 to 52.8 nM [122].

A series of compounds comprising 2,5-disubstituted benzimidazole and dimethylpyrazolyl were synthesized. The cytotoxic activity of all compounds was tested against 60 types of human cancer cell lines. The results declared that compound 69 was found to be the most potent molecule against lung and breast cancers [123].

2-Substituted benzimidazoles as protein kinase rhCK2α inhibitors

Protein kinase 2 (CK2) is a constitutively active serine/threonine protein kinase which takes part in a direction of substantial varieties of processes identified with cell survival and multiplication including cell cycle, apoptosis, or angiogenesis [124]. CK2 is a generally appropriated enzyme that phosphorylates various regulatory proteins [125]. CK2 articulation appears to be overexpressed in numerous solid tumors; consequently, inhibition of CK2 activity can reduce the viability of cancer cells [126].

Schneider et al. described the synthesis and CK2 inhibitory activity of 2-substituted-4,5,6,7-tetra halogenated benzimidazole derivatives. It was observed that, compound 70 displayed the highest activity towards CK2 with a half maximal lethal dose (LD\textsubscript{50}) of 4.75 ± 1.02 \(\mu\text{M}\) [127].

Pagano et al. designed and synthesized conjugates of 2-substituted tetrabromo-benzimidazole derivatives and examined against CK2 enzyme activity. Upon evaluation, compound 71 was shown to display the lowest \(K_i\) value as a CK2 inhibitor (40 nM) [128].

In the same context, Andrzejewska et al. prepared 2-substituted polyhalogenobenzimidazoles and assessed the combined derivatives for their CK2 inhibitory activity utilizing CK2 pure from rodent liver cytosol. The most effective hindrance of CK2 was achieved just if the benzene ring of benzimidazole is tetrahalogenated which is obviously confirmed by the \(K_i\) value of 4,5,6,7-tetra-bromo-2-trifluoromethyl benzimidazole, 72 \(K_i = 0.60 \mu\text{M}\) [129].

In addition, 1,2-disubstituted-1-H-benzimidazole was developed by Chojnacka et al. and assessed for protein kinase rhCK2α catalytic subunit inhibition and
cytotoxicity against two cancer cell lines. Compound 73 was identified as the most active with a $K_i$ value $2.42 \pm 0.32 \mu M$ [130].

2-Substituted benzimidazoles as cyclin-dependant kinase inhibitors

Cyclin-dependent kinases (CDKs) constitute a class of serine-threonine protein kinases that play an important role in the regulation of the cell cycle [131].

Lin et al. reported the CDK inhibitory activity of a novel series of 2,4-disubstituted benzimidazolyl-pyrazolo[3,4-b]pyridines. The representative analog 74 was found to be a potent inhibitor of CDK1 with IC$_{50}$ 0.0056 µM and a significant decline of the in vitro cellular proliferation in HeLa, HCT116, and A375 human tumor cell lines with IC$_{50}$ 0.015, 0.010, and 0.010 µM, respectively [132].

2-Substituted benzimidazoles as insulin-like growth factor receptor-1 inhibitors

The insulin-like growth factor receptor-1 (IGF-1R) is a membrane receptor tyrosine kinase. It plays an important role in mutagenesis and cell survival [133, 134]. Overexpression of IGF-1R and IGF-1 was demonstrated in a variety of tumors, including glioma, lung, ovary, breast, carcinomas, sarcomas, and melanoma [135].

Consequently, 2,5,7-trisubstituted benzimidazole (BMS-53692470) 75 was discovered as a novel small inhibitor of IGF-1R IC$_{50}$ 100 nM [136].

2-Substituted benzimidazoles as allosteric mitogen-activated protein kinase (MEK1) and phosphatidylinositol 3-kinase (PI3K) inhibitors

The phosphoinositide 3-kinase (PI3K) pathway is a key signal transduction system that links oncogenes and multiple receptor classes to many essential cellular functions and is perhaps the most commonly activated signaling pathway in human cancer. There are four highly homologous isoforms, assigned PI3Kα, PI3Kβ, PI3Kγ, and PI3Kδ, each having an unmistakable cluster of physiological capacities [137]. Initiating changes in PI3Kα have been found in about a fourth of breast and endometrial tumors, recognizing PI3K as an imperative focus for novel growth therapeutics [138].

Recently, Dort et al. synthesized 1,2-disubstituted benzimidazole derivatives and displayed them as dual MEK/PI3K inhibitory agents by direct coupling of a potent PI3K inhibitor to an allosteric MEK inhibitor using a covalent linker moiety. A prototype dual-acting agent, compound 76 exhibited high in vitro inhibition of both PI3K (IC$_{50}$ = 172 nM) and MEK1 (IC$_{50}$ = 473 nM) [139].

A new series of 1,2-disubstituted benzimidazole pyrimidone derivatives were blended by Certal et al. and assessed them for treatment of phosphatase and TENsin homolog (PTEN)-deficient cancers as PI3Kβ has emerged as the isoform involved in the tumorigenicity of PTEN-deficient tumors. Among all compounds, 77, showed significant activity and selectivity for PI3Kβ (IC$_{50}$ 100 nM), achieved tumor growth delay with IC$_{50}$ ranging from 76 to 188 nM and adequate in vitro pharmacokinetic properties. The SAR study showed that N-methyl benzimidazole compound resulted in a slight improvement of activity, compared with 5-fluoro derivatives [140].

2-Substituted benzimidazoles as a farnesyl-binding pocket of PDEδ inhibitors

K-Ras is one of the most common mutated signal-transducing human oncogenes. Ras signaling activity requires correct cellular localization of the GTPase. The spatial association of K-Ras is controlled by the prenyl-binding protein PDEδ that has a fundamental part in keeping up with the best possible cell dispersion of Ras proteins. Thus inhibition of the Ras-PDEδ cooperation by small molecules hinders Ras signaling [141].

Zimmermann et al. described the design of a novel 1, 2-disubstituted benzimidazole derivatives as potent PDEδ supressors. Among the compounds developed,
| Type of substitution | Mechanism of action | References |
|----------------------|---------------------|------------|
| 1-CH₃                | Alkylating agent    | [31, 32]   |
| 2-Aliphatic acid derivative | DNA binding agent | [33]       |
| 5-Nitrogen mustard   | DNA binding agent   | [34]       |
| 1,2-fused            | Alkylating agent    | [35]       |
| 5-Amide              | DNA binding agent   | [36]       |
| 6-CH₃                | DNA binding agent   | [37]       |
| 2-Acridine           | DNA binding agent   | [38]       |
| 6-Oxindole           | DNA binding agent   | [39]       |
| Head to tail bisbenzimidazole | DNA binding agent | [40]       |
| 5-Aromatic/piperazine| DNA binding agent   | [41]       |
| 1-Aromatic           | DNA binding agent   | [42]       |
| 2-Aromatic           | DNA binding agent   | [43]       |
| 5-Ester              | DNA binding agent   | [44]       |
| 1,2-Fused            | DNA binding agent   | [45]       |
| Cu complex with tetra benzimidazoles | DNA binding agent | [46]       |
| 2-Indole             | Tubulin inhibitor   | [47]       |
| 4-Aromatic           | Tubulin inhibitor   | [48]       |
| 2-Pyrazole           | Tubulin inhibitor   | [49]       |
| 6-Aliphatic/halogen  | Tubulin inhibitor   | [50]       |
| 2-Carbamate          | Tubulin inhibitor   | [51]       |
| 5-Se connector/indole| Tubulin inhibitor   | [52]       |
| 2-Urea               | Tubulin inhibitor   | [53]       |
| 5-O-linker-Aromatic  | Tubulin inhibitor   | [54]       |
| 2-Aromatic           | Tubulin inhibitor   | [55]       |
| 4-H/OH/CH₃           | Tubulin inhibitor   | [56]       |
| 1-Aromatic ketone    | Tubulin inhibitor   | [57]       |
| 2-Aromatic           | Tubulin inhibitor   | [58]       |
| 1,2-Aromatic         | Sirtuin 1&2 inhibitor| [59]       |
| 5-Ester              | Sirtuin 1&2 inhibitor| [60]       |
| 2-Piperidine         | Poly (ADP-ribose) polymerase inhibitor | [61]       |
| 4-Carboxamide        | Poly (ADP-ribose) polymerase inhibitor | [62]       |
| 2-Aromatic           | Poly (ADP-ribose) polymerase inhibitor | [63]       |
| 4- Carboxamide       | Poly (ADP-ribose) polymerase inhibitor | [64]       |
| 5/6-Halogen          | Poly (ADP-ribose) polymerase inhibitor | [65]       |
| 2-Aromatic           | Poly (ADP-ribose) polymerase inhibitor | [66]       |
| 3,4-Fused            | Poly (ADP-ribose) polymerase inhibitor | [67]       |
| 2-CH₂-OH             | Methionine synthase inhibitors | [68]       |
| 5-NO₂                | Methionine synthase inhibitors | [69]       |
| 2-Aromatic amide     | Methionine synthase inhibitors | [70]       |
| 5-NO₂                | Methionine synthase inhibitors | [71]       |
| 1-Aromatic           | DHFR inhibitor      | [72]       |
| 2-CH₂                | DHFR inhibitor      | [73]       |
| 6-Triazine           | DHFR inhibitor      | [74]       |
| 1-Aromatic           | DHFR inhibitor      | [75]       |
| 2-CH₂                | DHFR inhibitor      | [76]       |
| 6-Hetero-aromatic    | DHFR inhibitor      | [77]       |
| 2-Aromatic           | Topoisomerase I&II inhibitors | [78]       |
| 4-Ester              | Topoisomerase I&II inhibitors | [79]       |
| Head to tail bisbenzimidazole | Topoisomerase I&II inhibitors | [80]       |
| 2-Aromatic           | Topoisomerase I&II inhibitors | [81]       |
| 5-piperazine         | Topoisomerase I&II inhibitors | [82]       |
Table 1 Correlation between the type of substitution and the exerted mechanism of action *(Continued)*

| Type of substitution | Mechanism of action                        | References |
|----------------------|--------------------------------------------|------------|
| Cu complex           | Topoisomerase &II inhibitors               | [76]       |
| 2-Pyridine           |                                            |            |
| 5-COOH               |                                            |            |
| 1-Aromatic           | Androgen receptor antagonistic activity    | [78]       |
| 2-C=O                |                                            |            |
| 3-Aliphatic          |                                            |            |
| 5-NO₂                |                                            |            |
| 1-Aromatic           | Androgen receptor antagonistic activity    | [79]       |
| 2-Aliphatic          |                                            |            |
| 5, 6-Halogen         |                                            |            |
| 2-Aromatic           | α-glucosidase inhibitors                   | [82]       |
| 5,6-Aliphatic        |                                            |            |
| 1,2-Aromatic         | G9a Histone Methyltransferase inhibitors   | [85]       |
| 5-Amide              |                                            |            |
| 1-Hetero-aromatic    | Pyruvate kinase inhibitors                 | [88]       |
| 2-Aromatic           |                                            |            |
| 5,6-Halogen          | Telomerase inhibitors                      | [89]       |
| 2-Aromatic           | Telomerase inhibitors                      | [90]       |
| 6-Piperazine         |                                            |            |
| Tribenzimidazoles    | Telomerase inhibitors                      | [91]       |
| 2-Aromatic           | Telomerase inhibitors                      |            |
| 6-Piperazine         |                                            |            |
| 2-Aromatic           | Telomerase inhibitors                      | [92]       |
| 5,6-Fused            |                                            |            |
| 2-Cyclic amine/piperazine |                                  | [93]       |
| 2-Aromatic           | VEGFR-2 inhibitor                          | [98]       |
| 2-Aromatic           | VEGFR-2 inhibitor                          | [99]       |
| 6-Quinoline          |                                            |            |
| 1-Aromatic           | VEGFR-2 inhibitor                          | [100]      |
| 2-Furan              |                                            |            |
| 1-Amide/Heteroaromatic | VEGFR-2 inhibitor                  | [101]      |
| 2-Furan              |                                            |            |
| 2-Amide              | VEGFR-2 inhibitor                          | [102]      |
| 5,6-Aliphatic        |                                            |            |
| 2-Oxadiazole         | EGFR inhibitors                            | [107]      |
| 1-Aliphatic amine    | EGFR inhibitors                            | [108]      |
| 2-Amide              |                                            |            |
| 6-Halogen            |                                            |            |
| 2-Indazole           | FGFR1 inhibitors                           | [110]      |
| 5-Piperazine         |                                            |            |
| 2-Aromatic ketone    | FGFR1 inhibitors                           | [111]      |
| 6-Aliphatic          |                                            |            |
| 2-Pyrlopyrimidine    | Tie-2 receptor tyrosine kinase inhibitors   | [95]       |
| 5-Sulfonyl/urea      |                                            |            |
| 2-Aromatic           | Heparanase inhibitors                      | [114]      |
| 5, 6-Aliphatic       |                                            |            |
| 2-Aromatic           | COX1&2 inhibitors                          | [117]      |
| 5-Aliphatic amine    |                                            |            |
| 2-NH-aromatic        | Aurora A/B kinase inhibitors               | [120]      |
| 5-CF₃                |                                            |            |
| 2-Pyrazole           | Aurora A/B kinase inhibitors               | [121]      |
| 5-Morpholine         |                                            |            |
| 1,2-Aliphatic        | Aurora A/B kinase inhibitors               | [122]      |
| 5-Hetero-aromatic    |                                            |            |
compound 78 was reported as the most active compound of the series against the farnesyl-binding pocket of PDEδ with KD 87 ± 35 nanomolar affinity as the introduction of a piperidine moiety acts as a hydrogen-bonding donor [142].

A series of 4-substituted derivatives of the pan class I PI 3-kinase inhibitor 2-(difluoromethyl)-1-[4,6-di-(4-morpholinyl)-1,3,5-triazin-2-yl]-1H-benzimidazole (ZSTK474) were prepared by Rewcastle et al. to synthesize a range of 1,2,4-trisubstituted benzimidazole derivatives with 4-aminoalkoxy substituents. The compounds were evaluated using two human tumor cell lines. Upon evaluation, it was found that compound 79 displayed the best enzyme inhibitor with IC50 ranges from 4.5 to 11 μM and revealed the highest antitumor activity with IC50 0.04 μM. Moreover, 79 showed the best overall activity against the U87MG xenograft model, but less potent than ZSTK474 [143].

### Table 1 Correlation between the type of substitution and the exerted mechanism of action (Continued)

| Type of substitution | Mechanism of action | References |
|----------------------|---------------------|------------|
| 2-Hetero-aromatic    | Checkpoint kinase (Chk1&Chk2) inhibitors | [127] |
| 2-Hetero-aromatic    | Checkpoint kinase (Chk1&Chk2) inhibitors | [128] |
| 2-Aromatic 5-Amide   | Checkpoint kinase (Chk1&Chk2) inhibitors | [129] |
| 2-Aromatic 5-COOH/Amide | Checkpoint kinase (Chk1&Chk2) inhibitors | [130] |
| 2-CH3 5-Pyrazole     | Checkpoint kinase (Chk1&Chk2) inhibitors | [131] |
| 2-Aliphatic amines/CF3, 4,5,6,7-Halogen | Protein kinase rhCK2α inhibitor | [135, 136] |
| 2- CF3, 4,5,6,7-Halogen | Protein kinase rhCK2α inhibitor | [137] |
| 2- CH3, 4,5,6,7-Halogen | Protein kinase rhCK2α inhibitor | [138] |
| 2-Pyrazolo-pyridine 4-Aliphatic | Cyclin-dependant kinase (CDK) inhibitors | [140] |
| 2-Hetero-aromatic 4-Morpholine 7-Aliphatic | Insulin-like growth factor receptor-1 (IFG-1R) inhibitors | [144] |
| 1-Triazine 2-Halogen | Allosteric Mitogen-Activated Protein Kinase (MEK1) and Phosphatidylinositol 3-Kinase (PI3K) inhibitors | [147] |
| 2-Hetero-aromatic | Allosteric Mitogen-Activated Protein Kinase (MEK1) and Phosphatidylinositol 3-Kinase (PI3K) inhibitors | [148] |
| 1,2-Aromatic | Farnesyl binding pocket of PDEδ inhibitor | [141] |
| 1-Triazine 2-CHF2 3-O-linker | Farnesyl binding pocket of PDEδ inhibitor | [143] |
| 2-Aromatic 6-Quinazolin | Dual c-Met and VEGFR-2 inhibitors | [145] |
| 2-Hetero-aromatic 6-Piperazine | Multi-target receptor tyrosine kinase inhibitors | [147] |
| 2-Pyrole 6-Amide | Multi-target receptor tyrosine kinase inhibitors | [148] |
| 2-Pyrazine 6-Aliphatic amine | Multi-target receptor tyrosine kinase inhibitors | [149] |
| 1-Aliphatic 2-Aromatic amine | Multi-target receptor tyrosine kinase inhibitors | [149] |

2-Substituted benzimidazoles as dual c-Met and VEGFR-2 inhibitors c-Met (mesenchymal endothelial transition) kinase is a subclass that is important, associated with its ligand, hepatocyte growth factor (HGF), for normal mammalian
growth [144]. Targeting several biochemical pathways of cancer can be accomplished by using several drugs with different modes of action or through a single moiety that could adjust multiple targets of a multi-component disorder [145].

Accordingly, 2,6-disubstituted benzimidazolyl quinazolinamine derivatives were synthesized and evaluated by Shi et al. Compound 80 was found to be the most potent against c-Met and VEGFR-2 with IC₅₀ values of 0.05 μM and 0.02 μM respectively [146].

2-Substituted benzimidazoles as multi-target receptor tyrosine kinase inhibitors

The development of resistance against drugs that act as a single kinase inhibitor makes inhibition of more than one protein kinase an acceptable idea because tumor cells are attacked concomitantly at several relevant targets. Moreover, if a single cancer-related kinase becomes drug-resistant, a multi-targeted drug would still act on the remaining array of target kinases. Some benzimidazoles with 2,6-disubstitution were found to have multi-target receptor tyrosine kinase inhibitory activity. Dovitinib, 81, a substituted 3-benzimidazolyl hydroquinolin-2-one, exhibited dual inhibition of FGFR (fibroblast growth factor receptor) and VEGFRs [147].

Li et al. designed a new series of 2,6-disubstituted benzimidazoles and evaluated their biological activity against HEPG-2 cells and different kinases (EGFR, PDGFR-a, PDGFR-b, VEGFR-2). Compound 82 was found to exhibit high cytotoxicity against HEPG-2 cells with an IC₅₀ value of approximately 2 μM. Further kinase assay study showed that 82 has IC₅₀ 86.9, 18.7, and 16.5 μM against EGFR, VEGFR-2, and PDGFR, respectively [148].

McBride et al. synthesized 2,5-disubstituted benzimidazole indazole hybrids as multi-inhibitors of VEGFR-1, VEGFR-2, PDGFR, FGFR-1, and HUVEC and found that compounds 83 possessed favorable pharmacokinetics and exhibit impressive tumor growth inhibition property against different cell lines tested with IC₅₀ 0.028, 0.078, 0.69, 0.048, and 0.097 μM, respectively [149].

Determann et al. reported the synthesis of 1,2-disubstituted benzimidazole derivatives and evaluated against four cancer-related protein kinases namely Aurora B, PLK1 (polo-like kinase 1), FAK (focal adhesion kinase), and VEGFR-2, where compound 84 demonstrated high inhibitory activity with IC₅₀ values 6.0 ± 0.2, 1.2 ± 0.2, 3.4 ± 0.8, 7.2 ± 0.3 μM, respectively [98].

Conclusion

This review highlights the current status of 2-substituted benzimidazole anticancer molecules. The linker group and substitution at N-1, C-2, C-5, and C-6 positions have been found to be the most contributory factors for anticancer activity. Table 1 of SM is a correlation between the type of substitution and the exerted mechanism of action. We hope this paper will form a comprehensive foundation and reference source that will open up new opportunities for researchers interested in drug designing of benzimidazoles as anticancer. This depends on the specific design of molecules targeting multiple receptors/enzyme/protein, particularly keeping in mind to lessen side effects and toxicity.

Abbreviations

G: Guanine; A: Adenosine; IC₅₀: Half maximal inhibitory concentration; SAR: Structure-activity relationship; CDK2: Cyclin-dependent kinase-2; PCNA: Proliferation cell nuclear antigen; NCI: National Cancer Institute; NSCL: C: Non-small-cell lung cancer; GI₅₀: Half maximal inhibitory growth; Ki: Inhibitory constant and reflective of the binding affinity; PF₅₀: Potentiation effect; HDAC: Histone deacetylases; PARP-1: Poly (ADP-ribose) polymerase-1; MetS: Methionine synthase; DHFR: Dihydrofolate reductase; ct-DNA: Circulating tumor DNA; ID₅₀: Half maximal inhibitory dose; HMTs: Histone methyltransferase; PKM2: M2 isofrom of pyruvate kinase; hTERT: Human telomerase reverse transcriptase; VEGF: Vascular endothelial growth factor; RTK: Receptor tyrosine kinase; VEGFR: Vascular endothelial growth factor receptor; HUVEC: Human umbilical vein endothelial cell; KDR: Kinase domain receptor; EGFR: Epidermal growth factor receptor; FGFRs: Fibroblast growth factor receptors; TgI: Tumor growth inhibition; COX: Cyclooxygenase; S.I.: Selective inhibition; Chk: Checkpoint kinase; PK: Protein kinase; CDK: Cyclin-dependent kinase; P38K: Phosphoinositide 3-kinase; MEK: Mitogen-activated protein kinase; PDEδ: Prenyl-binding protein; c-Met: Cytoplasmic mesenchymal endothelial transition; PDGFR: Platelet-derived growth factor receptor; GI: Growth inhibition; EC₅₀: Median effective concentration required to induce a 50% effect

Plagiarism declaration

I hereby declare that this submission is my own work and to best of my knowledge, it contains no material previously published or written by another person, except where due acknowledgement is made. Furthermore, I believe that no contents of this material have been accepted for the award of any other degree or diploma in any other university or tertiary institution.

Authors’ contributions

HA: data collection and integration, prepared the first draft of the manuscript. HM: supervision and manuscript preparation. All authors have read and approved the manuscript.

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