Chapter

Recent Developments of Target-Based Benzimidazole Derivatives as Potential Anticancer Agents

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Abstract

Cancer is one of the major life burdens and around 18.1 million new cancer cases and 9.6 million deaths have been estimated in 2018 globally. Recent reports of the World Health Organization (WHO) stated that about one in six death cases globally is mainly due to cancer. Hence, the development of efficacious drugs with novel mechanisms is necessary for various cancer types. The chemotherapy drug resistance and non-selectivity toward targets have turned the current cancer research on to the highly emerging selective targets for the development of potential anticancer agents. Benzimidazole is regarded as an essential pharmacophore of the cancer research because of wide anticancer potentials with versatile mechanisms to inhibit the tumor progression and also facile synthetic strategies for an easy synthesis of various benzimidazole derivatives. The selective anticancer potentials also depend on the substitution of the benzimidazole nucleus. Therefore, it would lead to providing a path for the development of novel target-specific and highly effective benzimidazole-based anticancer agents.

Keywords: benzimidazole, cancer, specific targets, synthetic strategies

1. Introduction to cancer

Cancer is one of the dreadful diseases in the world and mainly characterized by uncontrolled cell proliferation. Worldwide, one in six women and one in five men develop cancer during their lifetime, and one in eleven women and one in eight men die from the disease. Global data clearly show that nearly half of the new cases and more than half of the cancer deaths worldwide in 2018 are estimated to occur in Asian countries because the region has nearly 60% of the global population and it is estimated to have a rise of over 21.4 million new cases per year, with 13.2 million cancer deaths, by 2030. The top three cancer types *viz.* breast, lung, and colorectal are responsible for one-third of the cancer incidence and mortality burden worldwide [1, 2]. Behavioral risk factors such as tobacco usage and smoking; physical risk factors such as exposure to ionizing radiations and asbestos; and genetic predominant factors are the main contributors to cancer. Even though utmost care has been taken, the disease still causes the death of millions of people globally [3].

Although scientific advances have focused on knowing the exact pathophysiology of the disease and tremendous efforts have been made on early diagnosis of cancer, the overall mortality rate has not subsided. Moreover, the cancer survival rate tends to be extremely low in some developing countries. This is due to the combination of both late-stage detection and limited access to time and qualitative treatment [4].

Radiotherapy, surgery, and chemotherapy are the usual cancer treatment strategies [5]. Among these, chemotherapy is considered as one of the efficient and first-line strategies in suppressing tumor prognosis and eradication. Most of the chemotherapeutic drugs target the key cellular mechanisms and inhibit the cell division and thereby prevent cancer cell multiplication. Current clinical anticancer drugs usually act on metabolically effective or fast replicating cells and show drawbacks such as poor selectivity between cancer cells and healthy cells [6]. Cancer cells generally disturb the cell signaling pathways and tissue morphogenesis for the neoplastic propagation of tumors. Therefore, targeting these cell pathways by cytotoxic agents has been a proven therapeutic approach to subside tumor growth and disease progression. Unfortunately, most of the cytotoxic drugs cause side effects due to the poor selectivity and specificity toward cancer cells. However, the higher toxic profiles and poor tolerance of the present chemotherapeutic drugs are major obstacles to the effective treatment of cancer [7, 8]. Therefore, it is highly pertinent to design and synthesize new anticancer agents with improved efficiency and reduced side effects to complement the present

DNA interacting agents	
Alkylating agents	**Alkylation of DNA bases** **Procarbazine, dacarbazine, and temozolomide**
DNA cleaving agents	Cause strand scission at the binding site-Bleomycin
Cross-linking agents	Binding to DNA results in intra- and inter-strand cross-linking Platinum complexes-carboplatin, cisplatin, oxaliplatin Nitrogen mustards-cyclophosphamide, ifosfamide
Intercalating agents	Stacking between DNA base pairs Anthracyclines-doxorubicin, epirubicin Mitoxantrone and actinomycin-D
Topoisomerase inhibitors	Topoisomerase I-camptothecins Topoisomerase II-Anthracyclines, etoposide
Anti-metabolites	
Purine analogues	Mercaptopurine
Pyrimidine analogues	5-Fluorouracil
DHFR inhibitors	Methotrexate
Antitubulin agents	
Taxol	Paclitaxel, Docetaxel
Vinca alkaloids	Vincristine, Vinblastine, Vinorelbine
Tyrosine kinase inhibitors	
Small molecule	Imatinib (Gleevec): inhibits ABL, c-Kit kinase, PDGFR Gefitinib (Iressa): inhibits EGFR
Monoclonal antibody	Trastuzumab: inhibits EGFR2, HER2
Angiogenesis/ Metastasis inhibitors	
Monoclonal antibody	Bevacizumab (Avastin): targets VEGF

Table 1.
Common anticancer drugs along with their mechanisms of action.

chemotherapeutic approaches. Identifying new drugs and drug combinations for cancer treatment is essential to combat this lethal disease. Hence, further research that emphasizes mainly on the development of efficient chemotherapeutic agents is an emerging area of research in the field of medicinal chemistry. The list of various available chemotherapeutic agents has been shown in **Table 1** [9].

2. Introduction to Benzimidazole

Benzimidazole heterocyclic nucleus can be termed as "Master Key" due to its overwhelming biological profile and synthetic applications in medicinal chemistry. It is among the top five most common five-membered aromatic nitrogen heterocycles in U.S. FDA-approved pharmaceutical drugs [10]. Benzimidazoles are structural isosteres of nucleobases due to the fused nitrogen nuclei and they readily interact with biomolecular targets and elicit many biological activities such as anticancer [11], anti-inflammatory [12], antiulcer [13], anti-hypertensive [14], and anthelmintic [15]. Akhtar et al. in his recent review described the therapeutic evolution of benzimidazole scaffolds during the last quinquennial period [16]. This nitrogen-containing heterocycle was present in a number of well-established clinical drugs with diverse therapeutic activities. For instance, drugs like rabeprazole (**1**) and omeprazole (**2**) are benzimidazole-containing drugs, act as proton pump inhibitors, and are, therefore, used in the treatment of stomach ulcers [17]. Albendazole (**3**) and thiabendazole (**4**) are anthelmintic drugs that act by the inhibition of tubulin polymerization and impair the uptake of glucose, eventually leading to the death of the parasites [18]. Nocodazole (**5**) is a well-recognized antineoplastic agent that mainly acts by tubulin polymerization inhibition. Candesartan (**6**) is a benzimidazole-based orally active potent angiotensin II receptor antagonist that is used for the treatment of hypertension [19]. Bendamustine (**7**) is nitrogen mustard which belongs to alkylating agents, a class of chemotherapeutic agent and used in the treatment of chronic lymphomas [20]. Dovotininb (**8**) is the orally active benzimidazole quinolinone compound with potential antineoplastic activity (**Figure 1**). It strongly binds to the fibroblast growth receptor 3 (FGFR3) and inhibits its phosphorylation and induces tumor cell death [21].

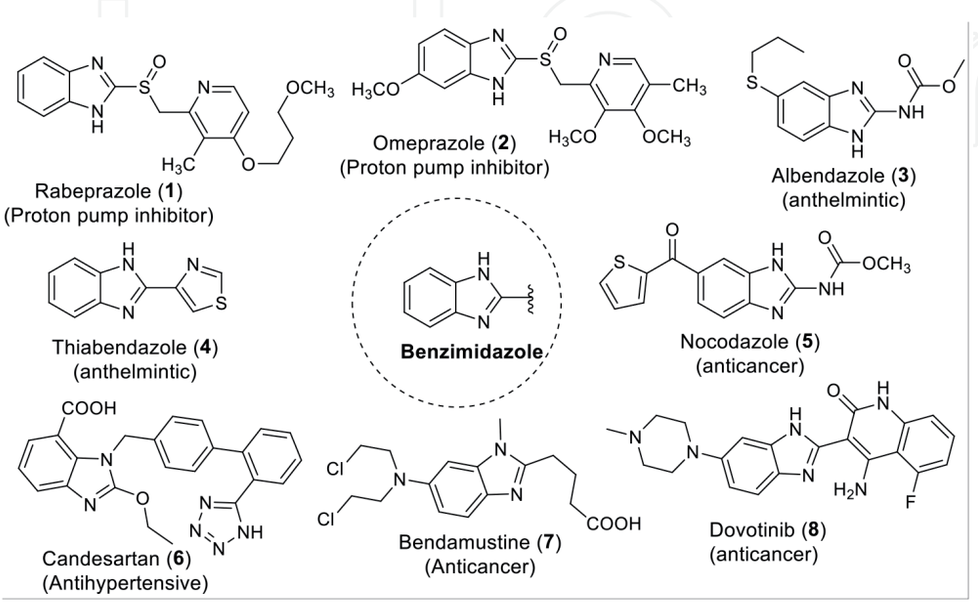


Figure 1.
Examples of drugs and other bioactive molecules containing benzimidazole motif.

In 1954, Tamm, Folkers, and co-workers first reported the synthesis and antiviral activities of halogenated benzimidazole nucleosides [22]. They found that 5,6-dichloro-1-β-D-ribofuranosyl benzimidazole (DRB) has multiple biological activities including activity against RNA and DNA viruses. DRB inhibits cellular RNA polymerase II, thus affecting the multiple cellular processes so that it is more cytotoxic than antiviral. Slayden et al. found that albendazole (**3**) and thiabendazole (**4**) known tubulin inhibitors interfered and delayed the *Mtb* cell division processes [23]. Later, Kumar et al. proposed that the benzimidazole core would be a novel FtsZ inhibitor, which will have activity against both drug-sensitive and drug-resistant *Mtb* [24]. This molecular framework displays numerous biological properties and is usually present in various drug compositions. Benzimidazoles tethered to various bioactive pharmacophores have also displayed potent antitumor activities.

Benzimidazoles have revolutionized the drug discovery process by their diverse range of biological activities, which make this scaffold an indispensable anchor for the innovation of novel therapeutic agents. Thus, the therapeutic potential of the benzimidazole and related drugs has attracted researchers to design and synthesize more potent derivatives with a wide range of pharmacological activities. Owing to the immense synthetic value and extended bioactivities exhibited by benzimidazoles and their derivatives, efforts have been made from time to time to create libraries of these compounds.

3. Target-based benzimidazole derivatives

3.1 Galectin-1 inhibitors

Galectin-1 (Gal-1) is expressed in various normal and pathological conditions and has multiple functions with a wide range of biological activity. Gal-1, a human homodimeric lectin protein of 14KDa, is implicated in many signaling pathways, immune responses associated with cancer progression, neurological conditions, and immune disorders [25]. Gal-1 has a carbohydrate recognition domain (CRD), which is selective toward β-galactosides in the body. Inhibition of human Gal-1 has been regarded as one of the potential therapeutic approaches for the treatment of cancer, as it plays a major role in tumor development and metastasis by modulating various biological functions viz. angiogenesis, apoptosis, migration, and cell immune escape [26]. The overexpression of Gal-1 has been reported in many cancer types like the brain, breast, osteosarcoma, lung, prostate, melanoma, etc. [27]. Gal-1 can mediate neoplastic transformation by interacting with oncogenes, such as H-Ras and promote Ras-mediated signal transduction involving RAF1 and extracellular signal-regulated kinase (ERK). Gal-1 multivalently mediates tumor cell-ECM adhesion at the primary site by cross-linking cell surface glycoproteins, such as integrins, and glycosylated proteins in the ECM, such as laminin and fibronectin [28]. Hence, Gal-1 is regarded as a promising molecular target for the development of new therapeutic drugs for cancer.

Recently, a new series of 1-benzyl-1H-benzimidazole derivatives have been synthesized as Gal-1-mediated anticancer agents. The target compound (**9**) showed significant growth inhibition against breast cancer (MCF-7) cells with an IC_{50} value of 7.01 ± 0.20 μM. The target compound also showed good cytotoxicity in the range of 10.69–14.04 μM against colorectal cancer (HCT-116), breast cancer (MDA-MB-231), prostate cancer (DU-145), and lung cancer (A-549). In addition, *in-vitro* Gal-1 expression in cell supernatant of MCF-7 cells with compound (**9**) was measured in enzymatic GAL-1 ELISA studies and found to show dose-dependent reduction from 10 to 300 μM. The target compound showed Gal-1-mediated

apoptosis, which was confirmed by morphological changes in MCF-7-treated cells like blebbing, cell wall deformation, and cell shrinkage, based on the apoptosis studies such as Acridine Orange/Ethidium Bromide (AO/EB) staining, DAPI nucleic acid staining, mitochondrial membrane potential, annexin V/propidium iodide dual staining assay, and dichlorofluorescein (DCF) fluorescence studies. In cell cycle analysis, the target compound selectively arrested MCF-7 cell growth at the G2/M phase and S phase. Further, the binding specificity of target compound toward Gal-1 was confirmed by surface plasmon resonance and fluorescence spectroscopy studies and the specific binding constant value (K_a) of 1.2×10^4 M^{-1} was observed in fluorescence spectroscopy studies, whereas the equilibrium constant (KD) value of 5.76×10^{-4} M was observed in surface plasmon resonance studies. The binding of the target compound to Gal-1 was also confirmed by RP-HPLC studies and found to show 85.44% of binding to Gal-1. The molecular docking studies were also supported based on the strong amino acid interactions such as ARG48, TRP68, and ASP125 with the target compound [29, 30].

Tsung-Chieh Shih et al. reported a novel Gal-1 inhibitor named LLS2 (**10**), which was discovered through the One-Bead-Two-Compound library. The interaction of target gal-1 with LLS2 was confirmed by LC-MS/MS analytical and pull-down assay. The binding complex of LLS2 with Gal-1 selectively decreases membrane-specific H-Ras, and K-Ras pathways, lead to involve in the apoptosis process. The LLS2 exhibited a synergistic effect in combination with paclitaxel against many of the human cancer cell lines such as pancreatic cancer, ovarian cancer, and breast cancer cells *in vitro*. The combination of paclitaxel with LLS2 efficiently reduces the growth of ovarian cancer xenografts in athymic mice *in vivo* (**Figure 2**).

The same group recently published a more potent Gal-1 inhibitor LLS3 (**11**), it impairs castration-resistant prostate cancer progression and invasion. LLS3 targets Gal-1 as an allosteric inhibitor, and reduces Gal-1 binding affinity toward its binding partners and also causes suppression of Akt, and AR signaling pathways. LLS3 showed *in vivo* efficacy in both androgen receptor-positive and negative xenograft models. In addition to potentiating the anticancer effect of docetaxel to cause suppression of tumors, it also efficiently suppresses the progression of prostate cancer cells *in vivo* [31, 32].

3.2 Tubulin protein inhibitors

Tubulin is one of the members of a small family of globular proteins. Several isoforms are present out of which α- and β-tubulins are the most common members of tubulin. The cellular protein tubulin is an important protein for replication.

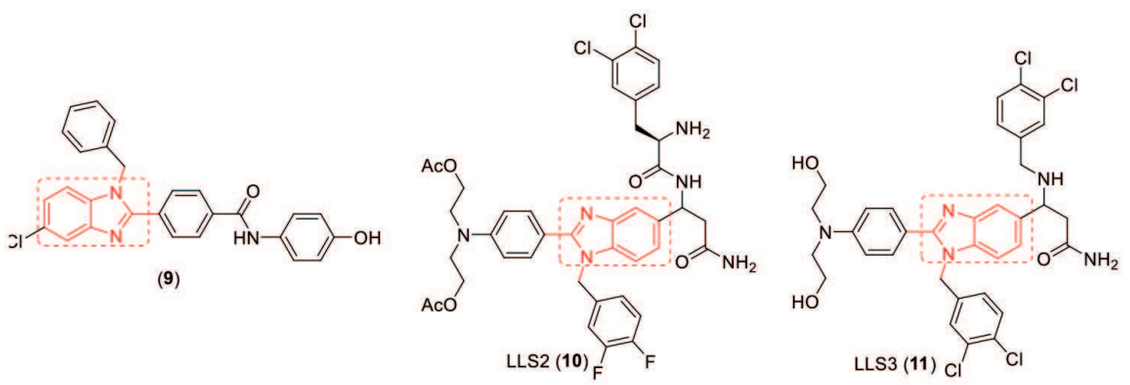


Figure 2.
The novel benzimidazole derivatives as Gal-1 mediated anticancer agents.

Microtubules are hallowing filaments and composed of head and tail polar fashion arrangements of α- and β-tubulins as the constituent subunits. Microtubules contain 13 active protofilaments aligned parallel with the whole axis of the microtubule cylinder. This may provide continuous transport of cellular materials by motor proteins (dynein and kinesin) over distant places. Microtubules also form an integral part of the cytoskeleton and are responsible for the maintenance of cell shape, and motility and intracellular transport of the vesicles, mitochondria, and other components [33, 34]. Moreover, cell division involves the duplication of DNA and the segregation of the replicated chromosomes into two daughter nuclei. The segregation of these chromosomes is mitotic phase is brought by the microtubules. In the formation of the microtubule, the plus (+) end is terminated by β-tubulin whereas the minus (−) end is terminated by α-tubulin. They are always either in a state of polymerization or depolymerization. Microtubules have the ability to shorten or lengthen in a scholastic fashion through loss or addition of α/β-tubulin heterodimers from ends of microtubules. This property is referred to as "dynamic instability" [35, 36]. Microtubules are blessed with a property to grow continuously as long as the free tubulin amount is above a critical level. The critical concentration at the minus end is somewhat higher than at the plus end and the minus end tends to stop growing first. Even above the critical tubulin concentration, its end may suddenly stop growing and begin to shrink. The change from growth to shrinkage has been termed as "catastrophe." After some time, a shrinking microtubule end may "pause" and/or begin to grow again; the latter process is known as "rescue." During mitotic cell division, the chromosomes are segregated by the mitotic spindle, which is formed from tubulin microtubules. Therefore, tubulin dynamics have a distinct role in cell division. Some of the drugs affect the microtubulin dynamics and thus cause either polymerization or depolymerization and thereby alter cellular replication. So at the mechanistic level, tubulin is one of the most attractive and challenging approaches for designing new anticancer compounds.

Zhang et al. have synthesized a series of 1,2-diarylbenzimidazole derivatives and reported as potential anticancer agents. Among all, the target molecule (**12**) has been found to show significant cytotoxicity against human cancer cells such as A549, HepG2, HeLa, and MCF-7 cells in the range of GI_{50} = 0.71–2.41 μM and also found to show normal cytotoxicity toward normal cells. The apoptosis process by the target compound was confirmed by morphological changes on HepG2 and HeLa-treated cells like cell wall deformation, blebbing, and cell shrinkage, based on apoptosis studies such as mitochondrial membrane potential, annexin V/propidium iodide dual staining assay, and dichlorofluorescein (DCF) fluorescence studies. In cell cycle analysis, the target compound selectively arrested tumor growth at the G2/M phase. Further, the target compound showed significant inhibition of microtubule polymerization with an IC_{50} value of 8.47 μM. The molecular docking simulation studies were performed to confirm the binding of the target compound with microtubule protein and found that the target compound has made strong interactions with protein [37].

Miao et al. reported a novel series of 2-aryl-benzimidazole-based dehydroabietic acid derivatives as potential cytotoxic agents via targeting tubulin polymerization. The synthesized molecules were characterized by elemental and analytical techniques. The target compound (**13**) showed significant growth inhibition against hepatocarcinoma cancer (SMMC-7721) cells with an IC_{50} value of 0.08 ± 0.01 μM. The target compound also showed good cytotoxicity in the range of 0.04–0.07 μM against breast cancer (MDA-MB-231), cervical cancer (HeLa), and colon cancer (CT-26). The apoptosis studies such as ROS levels measurements, loss of mitochondrial membrane potential, and cell cycle analysis were performed to confirm the induction of apoptosis in hepatocarcinoma cancer (SMMC-7721)

cells. In cell cycle analysis, the target compound selectively arrested tumor growth at the G2/M phase. Further, the target compound showed significant inhibition of microtubule polymerization with an IC_{50} of 5 μM. The molecular docking studies supported the selectivity of the target compound to tubulin protein based on strong electronic interactions between the target compounds and tubulin [38].

Wang et al. reported a new series of benzimidazole containing benzsulfamide-pyrazole ring derivatives as potential tubulin polymerization inhibitors. The target compound (**14**) showed significant growth inhibition against lung cancer (A549) cells with an IC_{50} value of 0.15 ± 0.05 μM and also showed good growth inhibition against Hela, HepG2, and MCF-7 cell lines in the range of 0.17–0.33 μM concentration. Further, the target compound showed significant inhibition of microtubule polymerization with an IC_{50} value of 1.52 μM. In cell cycle analysis, the target compound selectively arrested A549 cell growth at the G2/M phase. The target compound showed A549 cell apoptosis based on the studies of annexin V/propidium iodide dual staining assay and cell cycle analysis. The molecular docking studies were also supported based on the strong amino acid interactions such as LYS 352, LYS 254, ASN 258, and CYS 241 with the target compound [39] (**Figure 3**).

Baig et al. have reported a series of imidazo [2,1-b] thiazole-benzimidazole derivatives as antiproliferative agents via tubulin polymerization inhibition. The target molecule (**15**) has shown significant cytotoxicity against human lung (A549) cancer with an IC_{50} value of 1.08 μM. It also showed good cytotoxicity toward DU-145 (prostate), MCF-7 (breast cancer), A549 (lung cancer), and HeLa (cervical cancer) in the range of 1.65–7.55 μM. In cell cycle analysis, the target compound selectively arrested A549 cell growth at the G2/M phase. The target compound showed apoptosis, which was confirmed by morphological changes in A549-treated cells like blebbing, cell wall deformation, and cell shrinkage, based on the apoptosis studies such as Hoechst staining, mitochondrial membrane potential, annexin V/propidium iodide dual staining assay. Further, the target compound exhibits a significant inhibition of microtubule assembly with an IC_{50} of 1.68 μM. The computational studies revealed that the target compound can easily be occupied in the colchicine binding site of the protein [40].

3.3 Carbonic anhydrase inhibitors

The human carbonic anhydrases (hCAs) are an α-family of carbonic anhydrases class and exist in 16 different isoforms [41]. Based on their location in the body,

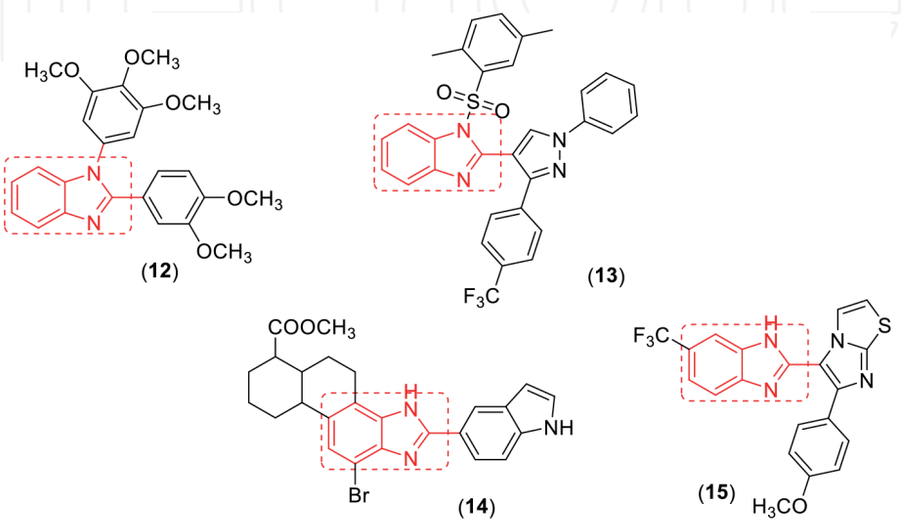


Figure 3.
The target benzimidazole derivatives as selective anticancer agents via targeting tubulin polymerization.

they are classified into cytosolic hCAs such as CA I, CA II, CA III, CA VII, and CA XIII; transmembrane hCAs such as CA IV, CA IX, CA XII, CA XIV, and CA XV; mitochondrial-bound hCAs such as CA Va and Vb; secretory hCAs such as CA VI; and catalytically inactive isoforms like CA VIII, CA X, and CA XI, which are considered as CA-related proteins (CARPs) [42]. Among all, the hCA isoforms IX and XII are overexpressed in many of cancer types as these are tumor-associated transmembrane bound enzymes, mainly hypoxic tumors, which are regarded as emerging potential targets for various tumor types [43]. The overexpression of hCA isoforms IX and XII further contributes to the tumor progression, angiogenesis, metastasis, and proliferation of a variety of tumor cells [44]. In order to exhibit potential cytotoxicity without adverse effects, an anticancer agent should selectively inhibit tumor-associated hCAs IX and XII over other hCAs. Therefore, current cancer research focuses on the development of various heterocycles that selectively target tumor-linked hCA isoforms IX and XII for effective treatment strategies in cancer therapy [45]. Another hCA isoform II is also found to overexpress in some forms of cancer and other conditions like edema, glaucoma, and epilepsy.

Recently, a new series of 2-substituted-benzimidazole-6-sulfonamides have been reported as anticancer potentials by testing against four physiologically relevant hCAs such as CA I, CA II, CA IX, and CA XII. The analysis of hCA inhibition results showed that the new series of benzimidazole-based sulfonamide derivatives exhibited selective inhibition toward tumor-associated isoforms such as CA IX and CA XII. The target molecule (**16**) of this series had shown a promising inhibition at low μM range against hCA IX and XII isoform, with an inhibitory constant (K_i) value of 2.2 and 22.3 μM. Another potent compound **(17)** also exhibited good inhibition at low μM range against hCA IX and XII, with an inhibitory constant (K_i) value of 5.9 and 7.9 μM respectively. Hence, it is concluded that these benzimidazole derivatives might be potential anticancer agents exhibiting a novel mechanism through inhibition of hCA isoforms IX and XII [46]. Asta Zubriene et al. have reported a series of novel benzenesulfonamides with benzimidazole derivatives as selective human carbonic anhydrase I, II, VII, XII, and XIII inhibitors. The target molecules were synthesized from the precursor benzimidazole derivative with different phenacyl bromides. The target molecules **(18, 19)** were evaluated against five physiological relevant hCA isoforms (hCA, EC 4.2.1.1) CA I, CA II, CA VII, CA XII, and CA XIII. The target compound exhibited a promising inhibitory action at a lower nanomolar level against selected hCAs with an inhibitory constant (Ki) value range of 1.67–66.7 μM. Another target molecule has shown significant inhibition at lower nanomolar level against selected hCAs with an inhibitory constant (Ki) value range of 2.86–62.5 μM [47] (**Figure 4**).

3.4 Epidermal growth factor receptor (EGFR) inhibitors

The Epidermal Growth Factor Receptor is a subfamily transmembrane glycoprotein (ErbB-1) of ErbB class of tyrosine kinase receptors and, other subfamilies include HER2/neu (ErbB-2), Her 3 (ErbB-3) and, Her 4 (ErbB-4) [48]. The internal ligands like EGF and TGFα facilitate the growth-promoting signal to cells by interacting with EGFR receptors and regulate epithelial tissue development and homeostasis [49, 50]. In cancer, especially epithelial malignancies, due to overproduction of EGFR ligands in the tumor micro environment causes continual activation (or) mutations of EGFR receptors, result in enhances epithelial tumor growth, metastasis and invasion [51, 52].

In a recent study, a new series of benzimidazole-based triazole and thiadiazole derivatives were synthesized and evaluated as selective EGFR inhibitors. The single-crystal X-ray crystallographic analysis has been performed to confirm the molecular

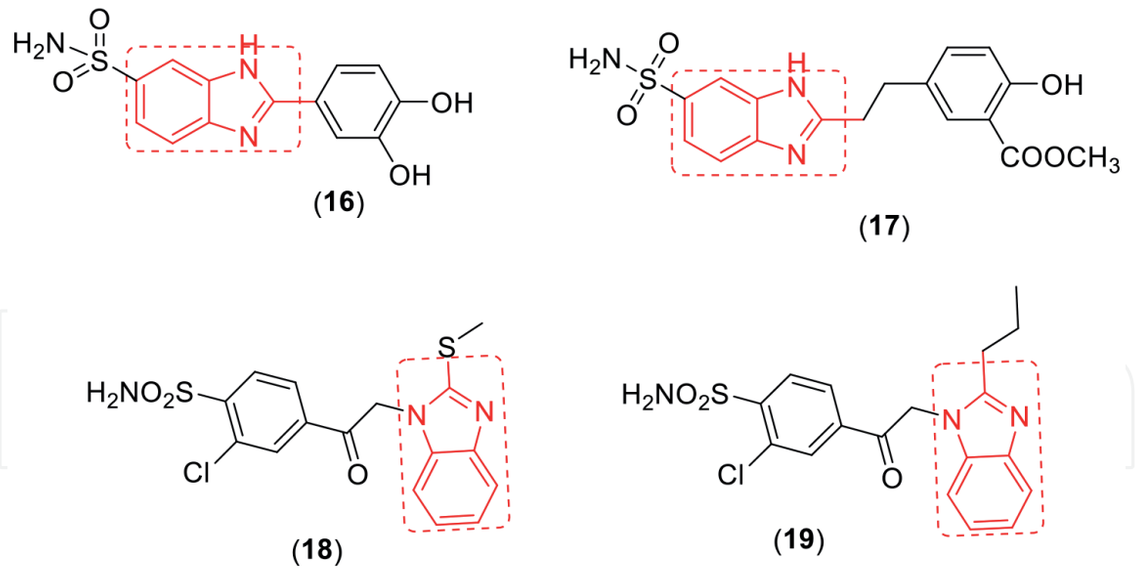


Figure 4.
The benzimidazole derivatives as human carbonic anhydrase enzyme mediated anticancer agents.

structure of the target compound. The synthesized compounds were evaluated for their EGFR kinase inhibitory potencies with erlotinib as the reference standard and, most of the compounds showed promising activities. The cell inhibition studies were also performed and the target compound (**20**) exhibited a significant inhibition and exhibited EGFR kinase inhibitory activity (over ≥30%) against MCF7 cells. The molecular docking studies indicated that the target compound showed two-hydrogen bonding interactions with residues of LYS721 and THR830 at the binding site of EGFR tyrosine kinase [53]. Akhtar et al. reported the benzimidazole-oxadiazole hybrids as selective EGFR and erbB2 receptor inhibitors. In *in vitro* cell inhibition studies, the target compound (**21**) exhibited a significant inhibition with an IC_{50} of 5.0 μM against breast cancer (MCF-7) cells. The target compound was found to show significant inhibition of EGFR and erbB2 receptor at 0.081 and 0.098 μM respectively. Most of the synthesized compounds exhibited a good cytotoxic activity against selected human cancer cell lines. In cell cycle analysis, the target compound selectively arrested MCF-7 cell growth at the G2/M phase. The computational and 3D-QSAR studies indicated that the target compound exhibited strong interactions with Asp831, Met769, and Thr830 of the EGFR enzyme [54].

Akhtar et al. have synthesized benzimidazole-based pyrazole derivatives through a one-pot multicomponent reaction and evaluated them for their potential anticancer activities. The synthesized compounds were screened against selected human cancer cell lines such as MCF-7, MDA-MB231, A549, HepG2, and HaCaT. The evaluation of EGFR inhibitory activities was performed for all the synthesized compounds. The target compound (**22**) exhibited promising cytotoxicity against the lung (A549) cancer cell lines with an IC_{50} value of 2.2 mM and the EGFR receptor inhibition value with an IC_{50} of 0.97 mM. In cell cycle analysis, the target compound selectively arrested A549 cell growth at the G2/M phase. In addition, it suppressed the growth of lung cancer cells by inducing apoptosis. In molecular docking studies, the target compound showed strong electronic interactions with Met769, Thr830, Lys721, and Phe699 of the active pocket of the EGFR receptor [55]. Yuan et al. have synthesized a library of 6-amide-2-aryl benzoxazole/benzimidazole derivatives and evaluated them for their selective inhibitory activities against VEGFR-2. The library of compounds exhibited selective anticancer activity against the liver hepatocellular carcinoma (HepG2), and human umbilical vein endothelial cells (HUVECs) over the lung cancer (A549) and breast (MDA-MB-231) cancer

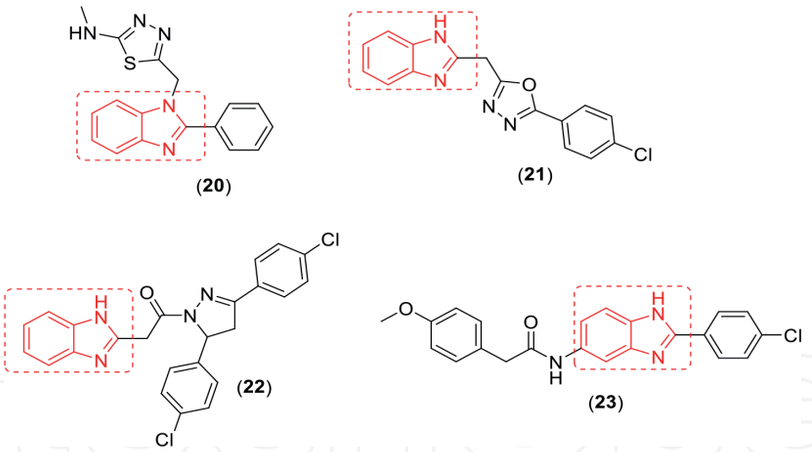


Figure 5.
The benzimidazole derivatives as selective anticancer agents via targeting EGFR.

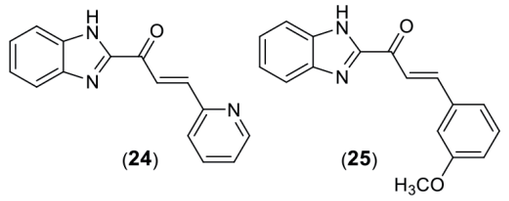
cell lines. The target compound exhibited a significant growth inhibition against HepG2 and HUVEC with IC_{50} values of 1.47 and 2.57 mM, respectively. The target compound (**23**) showed anti-angiogenesis ability (79% inhibition at 10 nM/eggs) by chick chorioallantoic membrane (CAM) assay and exhibited excellent VEGFR-2 kinase inhibition with an IC_{50} of 0.051 mM. The computational analysis showed that the target compound made strong interactions with the active site of VEGFR-2 kinase. It is concluded that the 6-amide-2-arylbenzoxazole/benzimidazole derivatives are essential inhibitors of VEGFR-2 kinase for the treatment of anti-angiogenesis [56] (**Figure 5**).

4. Miscellaneous agents

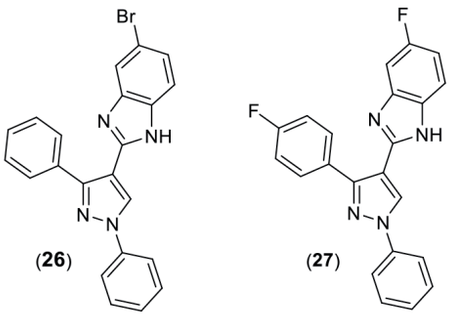
Wu et al. synthesized a series of novel benzimidazole-2-substituted phenyl or pyridine propyl ketene derivatives and two representative compounds (**24**) and (**25**) showed significant inhibitory activity against colorectal (HCT116), breast (MCF-7), and liver (HepG2) cell lines, and effective inhibition of tumor growth in BALB/c mice with colon carcinoma HCT116 cells [57]. Reddy et al. reported a series of pyrazole-containing benzimidazole hybrids and evaluated them for their potential anti-proliferative activity against lung (A549), breast (MCF-7), and cervical (HeLa) cell lines. The compounds (**26**) and (**27**) showed potent growth inhibition against all the cell lines tested, with IC_{50} values in the range of 0.83–1.81 μM [58]. Gowda et al. synthesized a series of novel 1-(4-methoxyphenethyl)-1H-benzimidazole-5-carboxylic acid derivatives and the compound (**28**) induced maximum cell death in leukemic cells (K562 and CEM cell lines), through inducing apoptosis via S/G2 cell cycle arrest; down regulation of CDK2, Cyclin B1 and PCNA; cleavage of PARP; and elevated levels of DNA strand breaks [59]. Akhtar et al. reported a series of benzimidazole-linked oxadizole hybrids and the compounds were screened for their anticancer and *in vitro* EGFR and erbB2 receptor inhibition assay. Two of the compounds (**29**) and (**30**) displayed promising activity. The compound 70a showed EGFR inhibition and induced apoptosis by G2/M cell cycle arrest [54] (**Figure 6**).

5. Synthetic strategies

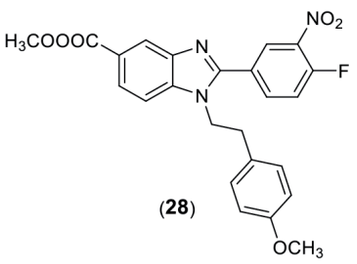
The first benzimidazole (2,5-dimethylbenzimidazole) (**3**) or 2,6-dimethylbenzimidazole (**4**) was prepared in 1872 by Hoebrecker through reduction of



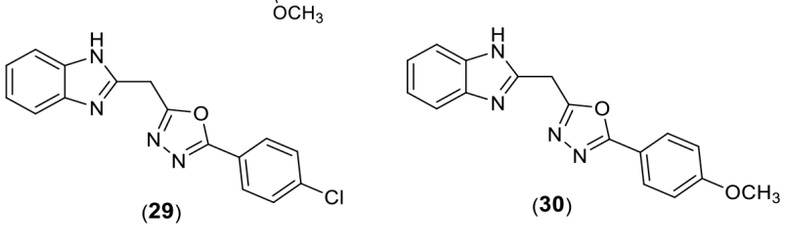
Antiproliferative activity, IC_{50} in μM		
HCT116	MCF7	HepG2
0.062±1.45	3.64 ± 0.39	0.34 ± 0.68
0.03 ± 0.02	9.80± 1.35	0.75 ± 5.41



Antiproliferative activity, IC_{50} in μM		
A549	MCF-7	HeLa
1.81±0.4	0.83±0.3	1.76±0.7
1.13±0.2	0.95±0.3	1.57±0.3



Antiproliferative activity, IC_{50} in μM	
K562	CEM
3	4



Antiproliferative activity, IC_{50} in μM						
EGFR	erbB2	MCF7	HaCaT	MDA-MB231	HepG2	A549
0.081	0.61	5.0	9.51	4.51	2.51	5.2
0.098	0.91	2.5	3.8	0.131	15.6	13.2

Figure 6.
The novel benzimidazole derivatives as potential anticancer agents.

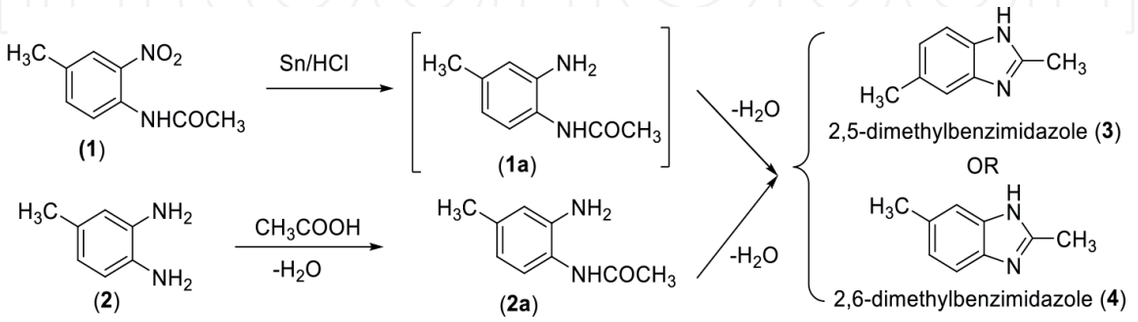


Figure 7.
General syntheses of benzimidazoles from aniline derivatives.

2-nitro-4-methylacetanilide [60] (**1**) (**Figure 7**). Several years later, the synthesis of benzimidazole was reported by refluxing 3,4-diamino toluene (**2**) with acetic acid [61]. Many synthetic ways toward the construction of benzimidazole ring started

from commercially available benzene derivatives containing nitrogen functionalities, especially ortho derivatives. Hence, a number of methods have been reported for the synthesis of bioactive benzimidazoles and their derivatives. The majority of these involve the condensation of *O*-phenylene diamines (**5**) and its derivatives with carboxylic acids (**6**), esters, alcohols, or aldehydes [62].

Synthesis of benzimidazoles in the presence of various catalysts involves the condensation of *O*-phenylene diamines with *ortho* esters in the presence of Lewis acids like $ZrCl_4$, $SnCl_4$, $TiCl_4$, $HFCl_4$, etc. The most commonly used method for synthesis of benzimidazoles (7) is Phillip's method, which involves the condensation of *O*-phenylene diamines (**5**) with carboxylic acids (**6**) or its derivatives by heating the reagents in the presence of concentrated hydrochloric acid [62] (**Figure 8**).

The benzimidazole derivatives (**14**) were synthesized under mild conditions with inherently low cost by many researchers using (**8**), (**9**), (**10**), (**11**), (**12**), and (**13**), as reactants (**Figure 9**). Suheyla et al. demonstrated the synthesis of benzimidazoles by condensation of O-phenylene diamine with an appropriate aldehyde (**8**) in the presence of sodium metabisulfite. They proposed the reaction that depends on forming the bisulfite adduct of the aryl aldehyde to prepare benzimidazole.

Hanan et al. have reported one-pot conversion of aromatic and heteroaromatic 2-nitroamines (**9**) into bicyclic 2*H*-benzimidazoles employs formic acid, iron

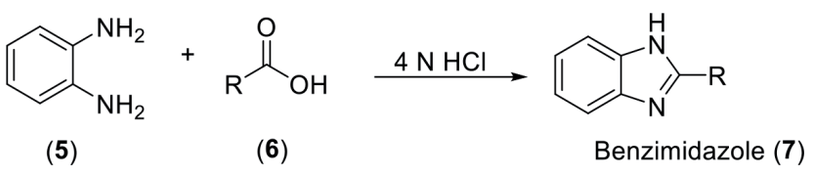


Figure 8.
Phillip's condensation for the synthesis of benzimidazoles.

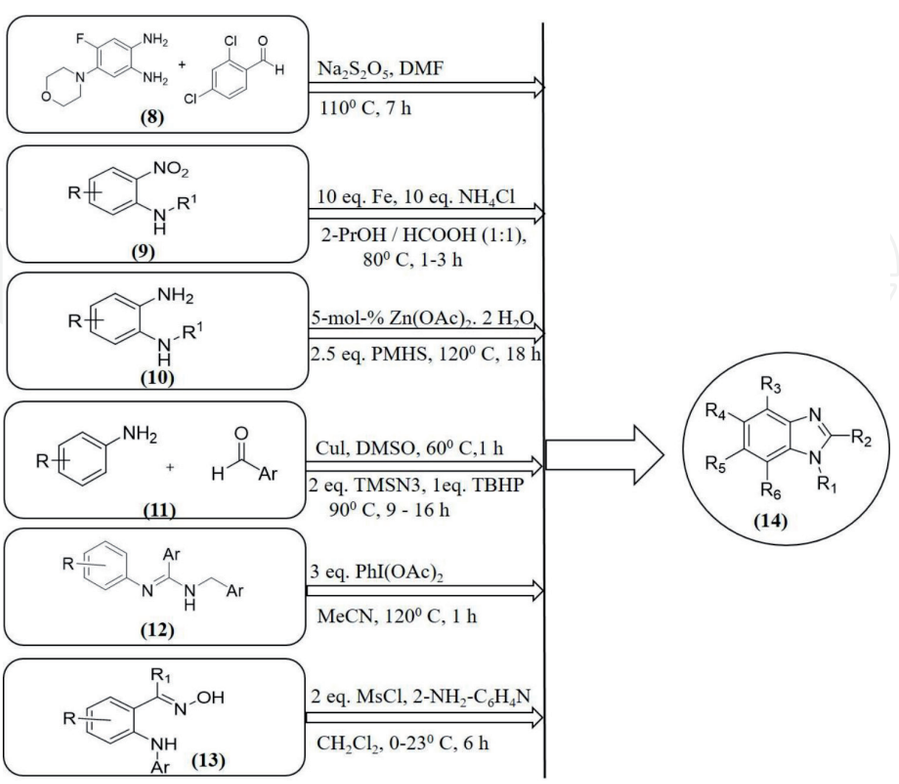


Figure 9.
Synthetic strategies of benzimidazoles.

powder, and NH_4Cl as an additive to reduce the nitro group and effect the imidazole cyclization with high-yields [63]. Nale et al. developed a method for the synthesis of benzimidazole derivatives in the presence of zinc catalysts from N-substituted formamides and various o-phenylenediamines [64] (**10**). Mahesh et al. developed a method of one-pot, multicomponent reaction, which enables the transformation of commercial aryl amines, aldehydes, and azides (**11**) into various benzimidazoles *via* an efficient copper-catalyzed amination of N-aryl imines [64]. Lin et al. developed a method for solvent/oxidant-switchable synthesis of multisubstituted benzimidazoles *via* metal-free selective oxidative annulation of arylamidines [65] (**12**). Wray et al. synthesized various *N*-aryl-1*H*-indazoles and benzimidazoles from common arylamino oximes (**13**) in good to excellent yields [66].

6. Conclusion

There are numerous benzimidazole derivatives for various cancer types involving unique types of mechanism. Although it is a widely used pharmacophore, still very few target-specific benzimidazoles are available. Therefore, researchers across the world need to develop new benzimidazole derivatives that are more target specific and help in the cancer treatment to overcome non-selective toxicity and adverse effects. This chapter mainly focused on target-based benzimidazole derivatives and synthetic strategies. Hence, it would give more ideas to young medicinal researchers to develop target-specific benzimidazole derivatives as potential cytotoxic agents.

Conflict of interest

Authors declare "no conflict of interest."

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