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## **REVIEW**

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# Benzimidazole chemistry in oncology: recent developments in synthesis, activity, and SAR analysis

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A six-membered benzene ring is fused with a five-membered imidazole ring at positions four and five, generating benzimidazole, the benzo derivative of imidazole and a bicyclic aromatic chemical compound. Benzimidazole is a significant pharmacophore in a variety of physiologically active heterocyclic compounds due to its distinctive characteristics and structural framework. Because benzimidazole is both aromatic and heterocyclic, it interacts with a range of biological targets via metal ion interactions,  $\pi-\pi$  stacking, and hydrogen bonding. Its broad range of medicinal chemistry applications, such as anti-inflammatory, antiviral, antifungal, and anticancer therapies, is based on these interactions. Its significance in the development of potentially novel therapeutic pharmaceuticals is highlighted by the fact that its structural flexibility permits the synthesis of derivatives with targeted bioactivity. Derivatives of benzimidazole have garnered significant research interest as potential anticancer medications. These heterocyclic compounds exhibit a wide range of biological activities, such as DNA interaction, enzyme inhibition, and modulation of cellular pathways crucial to cancer development. Thus, to optimize their therapeutic potential, recent studies have focused on evaluating the structure–activity relationships (SAR) of benzimidazole derivatives. The main topics of this review are the current developments in the synthesis, anticancer activity, and SAR studies of benzimidazole derivatives, which will shed light on the increasing role they play in cancer therapies.

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### 1 Introduction

According to WHO, deaths due to cancer are predicted to reach 13 million worldwide by 2030.<sup>1,2</sup> Most malignancies are characterized by the uncontrolled growth of undifferentiated cells. Current estimates indicate that one in every five people will develop cancer by the age of 75.<sup>3</sup> Cancer primarily results from the deregulation of key enzymes and proteins that regulate cell division and proliferation.<sup>4,5</sup> However, despite significant advancements in cancer diagnosis and treatment options, many patients do not respond well to therapy, and others relapse after an initial encouraging response. Although chemotherapy is still a crucial component of cancer treatment, the emergence of drug resistance significantly reduces its effectiveness.<sup>6</sup> To combat drug resistance, higher dosages of chemotherapeutic drugs are

sometimes necessary, which exacerbates drug-induced toxicities.<sup>7,8</sup> Therefore, there is a critical need to create and develop novel cancer therapies with potent activity while maintaining a high therapeutic index.<sup>9</sup>

Recently, O- and S-based heterocycles have been attracting increasing attention in the discovery of innovative anticancer drugs, following the extensive investigation of nitrogen-based heterocycles as anticancer agents. While heterocyclic compounds with a sulfur atom account for the bulk of FDA-approved drugs, heterocyclic compounds with a nitrogen atom are regarded as the most common type of chemical material utilized in medicinal chemistry. Benzimidazole and its derivatives are a family of bioactive compounds with important uses in the pharmaceutical sector. Imidazole, also known as imidazoline, is a heterocyclic molecule.



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Science, Islamic University of Madinah, Saudi Arabia. Between 2000 and March 2025, Prof. Gomha published 296 original research articles and 13 books, focusing on heterocyclic synthesis for medicinal, energy, environmental, and industrial applications. His research integrates green chemistry techniques such as microwave irradiation, ultrasound, mechanochemistry, and the use of ionic liquids. As of March 2025, his work has been cited approximately 8150 times, with an h-index of 52. He has supervised 18 MSc and 9 PhD theses, served on 43 journal editorial boards, reviewed for 176 journals, and contributed to faculty promotions and thesis evaluations. Prof. Gomha has received several national and international honors. He was awarded the Best Paper Prize from the Egyptian Heterocyclic Chemistry Society in 2015, and his work was recognized among the most downloaded by the Journal of Heterocyclic Chemistry in 2018–2019. From 2019 to 2024, he was listed among the World's Top 2% Scientists by Stanford University. The AD Scientific Index ranked him 2nd in Organic Chemistry at Cairo University (2020-2023) and 1st in 2024, as well as 1st at the Islamic University of Madinah. ScholarGPS ranked him 3rd globally in heterocyclic compounds and 112th in chemical synthesis. Cairo University honored him annually for research excellence (2008-2024) and named him a Top 10 Publisher (2015-2024). In 2024, he received the Best Researcher in Chemotherapy Award from Pencis for his contributions to sustainable chemotherapy research.

In 1878, the parent benzimidazole (2) was produced by heating (1) with formic acid

Modified

the synthesis of 2-alkylbenzimidazoles (3, R = alkyl) was developed by Phillips

Scheme 1 Primary method for the synthesis of benzimidazole nucleus 2 and its derivative 3.

NH<sub>2</sub> HO 
$$\frac{1}{4}$$
 HO  $\frac{1}{4}$  HO  $\frac{1}{4}$  HO  $\frac{1}{4}$  HO H  $\frac$ 

### **Key observation:**

- 1) SAR indicated that chalcone moiety may have a significant role in the inhibition of antiproliferative action of these compounds.
- 2) The impact of diversification at the R position on the inhibitory activity was found to be inconclusive, as a methyl group or a fluorine atom was moderately tolerated.

Scheme 2 Synthetic route for benzimidazole-chalcone hybrids 10a and b.

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an azapyrrole, with two nitrogen atoms separated by a single carbon atom. Historically, this molecule was known as glyoxalin when it was initially synthesized in 1858 by German scientist Heinrich Debus using glyoxal, formaldehyde, and ammonia. A six-membered benzene ring is fused with a five-membered imidazole ring at positions four and five to form benzimidazole, the benzo-fused imidazole derivative and a bicyclic aromatic molecule. These substances have antiviral, antifungal, antiparasitic, analgesic, anticancer, and antihistaminic properties, among other medicinal applications. Moreover, benzimidazole derivatives have demonstrated potential in the management of numerous conditions associated with neurology, endocrinology, ophthalmology, and cardiovascular disease. 17

The benzimidazole nucleus has emerged as a key pharmacophore in cancer research due to its broad cytotoxic potential, adaptive tumor inhibitory mechanisms, and facile synthesis methods for producing a wide range of derivatives. Many bioactive chemicals and anticancer medicines have a benzimidazole motif. The benzimidazole scaffold is crucial in the creation of anticancer medications such as bendamustine, carbendazim, nocodazole, and veliparp. It functions in a number of ways. Benzimidazoles, a structurally distinct class of Topo I poisons that function as DNA minor groove binders, including Hoechst 33258 and Hoechst 33342. December 2012.

#### 1.1 Techniques that yield benzimidazoles

The synthesis and chemistry of benzimidazoles have been well reviewed in the literature. In general, the majority of key ingredients mentioned previously may be utilized to develop benzimidazoles, as follows:

- (1) Phenylenediamines
- (2) Acidic compounds
- (3) o-(N-arylamino)arylamine
- (4) o-Nitro arylamines or o-dinitro arenes
- (5) o-Substituted-N-substituted
- (6) Imines
- (7) Amidines
- (8) Heterocyclic scaffolds
- (9) Four components

**1.1.1. Phenylenediamines.** In 1878, the parent benzimidazole (2) was produced by heating (1) with formic acid. <sup>24</sup> Since then, aliphatic acids have been used to produce a wide variety of benzimidazoles (2 and 3). Phillips established the most effective technique for producing 2-alkylbenzimidazoles (3, R = alkyl), which involves refluxing equimolar amounts of diamine and aliphatic carboxylic acid in 4 N hydrochloric acid for three to four hours (Scheme 1).<sup>24</sup>

As shown in Scheme 2, several benzimidazole–chalcone hybrids **10a** and **b** have been produced.<sup>25</sup> Refluxing *o*-phenylenediamine (1) with glycolic acid (4) in HCl produced (1*H*-benzo

Scheme 3 Synthesis of a new class of BCHs 15 and 16.

[d]imidazol-2-yl)methanol (5). 5 and suitable benzyl bromides 6 were used to produce the substituted (1-benzyl-1H-benzo[d] imidazol-2-yl)methanol (7) in the presence of  $K_2CO_3$ . Subsequently, the corresponding primary alcohols 7 were oxidized using Dess–Martin reagent to provide substituted-1-benzyl-1H-benzo[d]imidazole-2-carbaldehydes 8. Using the Claisen–Schmidt reaction with appropriate acetophenones 9, target compounds 10a and b were subsequently generated from 8.

Zhou et al.25 established a novel class of benzimidazolechalcone hybrids or BCHs. These BCHs demonstrated antiproliferative effects in tumor cell lines and significant inhibitory effects in the Topo II-mediated DNA relaxation assay. One of the main enzymes involved in DNA replication, recombination, and repair is type II topoisomerase (Topo II).26,27 Condensation and cyclization of o-phenylenediamine and glycolic acid produced compound 11 and the synthesis pathway for substances 16. The oxidation of the C2-hydroxymethyl of 11 with manganese dioxide produced aldehyde product 12. Intermediate 13 was prepared via the Wittig-Horner reaction of aldehydic compound 12 with methyl diethyl phosphonoacetate. Key intermediate 14 was developed by alkylation of 13 with 3bromopropionic acid. The appropriate amides were produced by reacting carboxylic acid 14 with amines, and then the amides were treated with lithium hydroxide to produce 16. With an IC<sub>50</sub>

value of 0.64  $\mu$ M, compound **16** with a 4-Br substituent had the highest Pin1 inhibitory activity, indicating that the bromine atom is preferred, most likely to react with the residues of amino acid to establish an H-halogen link in this way (Scheme 3).

Scheme 4 presents the synthesis of 23. Potassium permanganate oxidizes 17 to yield 1H-benzo[d]imidazole-2-carboxylic acid (18). The carboxylic acid group of 18 was changed to a carbamoyl group by creating the acyl chloride first, and then reacting it with ammonium hydroxide to produce compound 19. Using Lawesson's reagent, the carbonyl in chemical 19 was thiolated to produce compound 20. Compound 20 was condensed and cyclized with ethyl 3-bromo-2-oxopropanoate to produce an intermediate, 2-(1H-benzo[d]imidazol-2-yl)thiazole 21. The use of 3-bromopropionic acid to alkylate 21 produced the desired product 22. Carboxylic acid 22 was combined with amines to form the corresponding amides, which were then reacted with lithium hydroxide to produce the final product 23 (Scheme 4). Wang et al.28 produced derivatives of 1H-benzimidazole and assessed their anticancer potential against the human prostate cancer cell line PC-3. With IC<sub>50</sub> values of 0.64 μM and 0.37 μM, respectively, compounds 16 and 23 showed the greatest inhibitory effects. According to the SAR investigations, compounds 23 with thiazole rings as linkers exhibited

The SAR studies revealed that the compounds with the thiazole rings as linkers 23 showed better inhibition than the compounds bearing a double bond between terminal carboxyl group and 1*H*-benzimidazole ring 16.

Scheme 4 Synthesis of derivatives of benzimidazole thiazole 22 and 23.

**Key observation:** 

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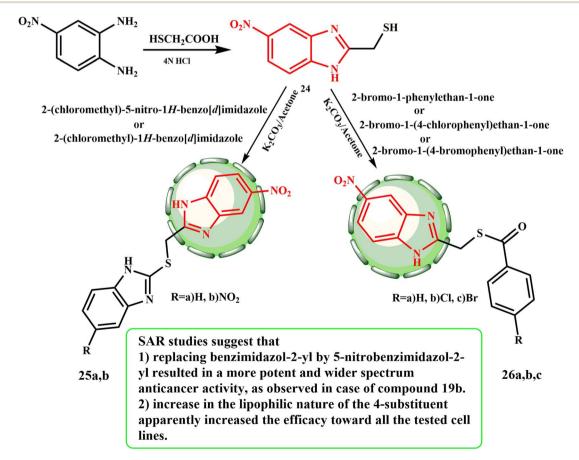
greater inhibition than 16 with a double bond between the 1Hbenzimidazole ring and the terminal carboxyl group.

An effective method for creating novel benzimidazoles 25a and b and 26a-c was accomplished, which began with 2-mercaptomethyl-5-nitro-1*H*-benzimidazole (24)<sup>29</sup> (Scheme 5). El-Gohary et al.30 produced new 1H-benzimidazole compounds and tested them against human cancer cell lines, including HepG-2 (liver), HCT-116 (colon), and MCF-7 (breast), to determine their anticancer potential. The most promising analogues were determined to be compounds 25b, 26b, and 26c. According to the SAR evaluations, compound 25b exhibited a more powerful and broad-spectrum anticancer action when 5nitrobenzimidazol-2-yl was substituted for benzimidazol-2-yl. Furthermore, the improved lipophilicity of the 4-substituent appeared to enhance its effectiveness against all the examined cell lines. Compound 25b, the 5-nitrobenzimidazol-2-yl homologue, had strong and comprehensive anticancer action. For compounds 26b and c to have anticancer action, the substituent at the 4-position of the phenacyl moiety must be lipophilic. The compounds appeared to be more active against the three examined cell lines as the lipophilicity of the 4-substituent was increased, which the activity following the order of 4-bromophenacyl analog 26c > 4-chlorophenacyl analog 26b.

The present study used 2-chloromethyl-1H-benzimidazole 27 (ref. 31) as the starting materials to produce novel

benzimidazoles 28 employing a straightforward, effective, and repeatable approach with a simple work-up procedure (Scheme 6). In the following way, the appropriate 27 reacts with 1-(4-fluorophenyl)piperazine in DMF when triethylamine is present to produce 2-(aryl amino)methyl-benzimidazole 28. El-Gohary et al.32 developed and evaluated a variety of 1H-benzimidazole derivatives for their anticancer characteristics. The MTT assay was used to screen the derivatives against the HepG-2 (liver), HCT-116 (colon), and MCF-7 (breast) cancer cell lines. Compound 28 exhibited the strongest activity against all the cell lines, in accordance with the results. The cytotoxic tests of the compounds revealed that they were less active than the reference medication, 5-fluorouracil. According to SAR investigations, the anticancer activity increased with the length of the contact between the aromatic moiety and 5-nitro-1H-benzimidazole.32

The proposed mechanistic route for the formation of benzimidazoles by reacting a derivative of o-phenylene diamine with an organic acid has already been studied.24,33 Furthermore, the role of hydrochloric acid in the reaction has also been investigated. 24,34 The catalytic action of hydrochloric acid is explained by the protonation of oxygen, which activates the carboxyl group. The reaction intermediate is an addition product, which is produced when the carbonyl group of the protonated acid is attacked by the shared electron pair of a nitrogen. Nevertheless, the researchers concluded that the generation of the monoacyl



Scheme 5 Synthesis of novel benzimidazoles 25a and b and 26a-c

1-(4-fluorophenyl)piperazine CICH<sub>2</sub>COOH 4N HCl TEA/DMF 27 SAR studies revealed that 1) increasing the length between 5-nitro-1H-benzimidazole and the aromatic moiety increases the antitumor activity. 2) benzothiazol-2-yl, 6-nitroquinolin-5-yl, thiadiazol-2-yl, 3methyl-5-oxopyrazol-1-yl, and 2,3-dihydronaphthalen-4ylidene, 6-nitroquinolin-5-yl, 2,3-dimethyl-5-oxo-1-28 phenylpyrazol-4-yl into an unsubstituted nucleus of R=NO<sub>2</sub> benzimidazole increases the efficacy of the compounds toward all tested cancer cell lines

Scheme 6 Synthesis of target 2-(aryl amino)methyl-benzimidazole 28.

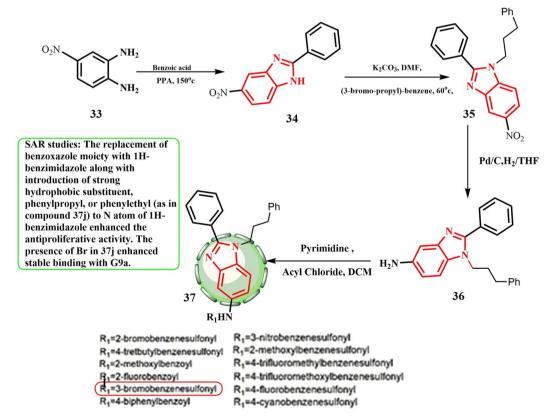
Scheme 7 Synthetic route for the formation of 2-arylbenzimidazole 29.

derivative was a necessary step in the benzimidazole ring building process.  $^{24,34}$  It has been reported that heating aromatic carboxylic acids with o-phenylene diamine in a sealed tube at 180–190 °C produces good yields of 2-arylbenzimidazoles (29, R = Ar). An improved method for producing 2-arylbenzimidazoles 29 from aromatic carboxylic acid uses polyphosphate ester (PPE) or polyphosphoric acid (PPA) as a dehydrator. As an alternative, phosphorus pentoxide has also been documented to function as a dehydrator in the process of producing a derivative of 2-arylbenzimidazole (Scheme 7).  $^{34}$ 

Scheme 8 shows the preparation of certain N'-(substituted-benzylidene)-4-(5-methyl-1H-benzimidazol-2-yl)benzohydrazide derivatives  $\bf 32a$  and  $\bf b$  for the analysis. Three steps were used to get the targeted synthetic substances. Firstly, methyl-4-formylbenzoate and 5-methyl-1,2 phenylenediamine were mixed equimolarly in DMF with  $\rm Na_2S_2O_5$  to yield  $\bf 30$ . Compound  $\bf 31$  was produced by refluxing a mixture of ethanolic solution of hydrazine hydrate and  $\bf 30$ , which occurred in approximately seven hours. The desired compounds  $\bf 32a$  and  $\bf b$  were obtained via condensation of hydrazide with different substituted

Scheme 8 Preparation of certain N'-(substituted-benzylidene)-4-(5-methyl-1H-benzimidazol-2-yl)benzohydrazide derivatives 32a and b.

compound.



Scheme 9 Synthesis of benzimidazole derivatives 37.

benzaldehydes. Compounds 32a and b were discovered to exhibit significant effectiveness against a number of cancer cell lines, preventing their proliferation by 50-84%.35 SAR studies revealed that the antiproliferative action of compound 32b was enhanced by the presence of tri-methoxy as an electrondonating group on hydrazone, as opposed to the electrondrawing di-chloro substitution in 32a.

Scheme 9 shows that commercially produced 4nitrobenzene-1,2-diamine (33) was employed as the starting material for the synthesis of benzimidazole derivatives 37. In

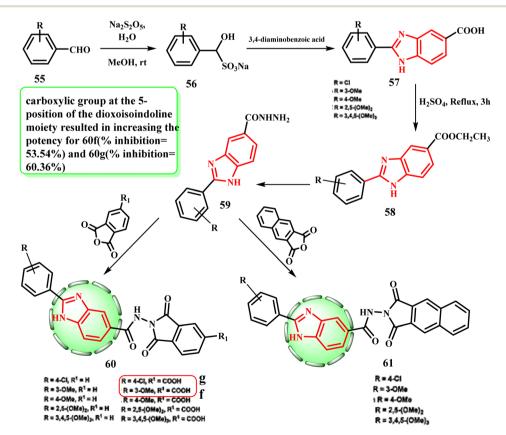
polyphosphoric acid (PPA), 33 first interacted with benzoic acid to produce 34, which then combined with (3-bromo-propyl)benzene to produce intermediate 35 in the presence of K<sub>2</sub>CO<sub>3</sub>. Amino intermediate 36 was produced by reducing intermediate 35 with Pd/C in THF. Essential compounds 37 were obtained by treating amine 36 with suitable acyl chlorides or sulfonyl chlorides and triethylamine in dichloromethane. After various screening rounds, 37i was shown to be an effective G9a antagonist ( $IC_{50} = 1.32 \mu M$ ), which caused the MCF-7 cancer cell line to undergo autophagy (IC<sub>50</sub> =  $5.73 \mu M$ ), where increased

Scheme 10 Approach for the synthesis of benzimidazole-thiazolidinediones 46a-c.

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Scheme 11 Synthesis of compounds 54a and b.

concentrations caused the MCF-7 cells to undergo apoptosis.36 The antiproliferative action was increased by the addition of moiety containing 1H-benzimidazole and a potent hydrophobic substituent, phenylpropyl (found in compound 37j), to the N atom of 1H-benzimidazole. Stable binding with G9a was improved by the presence of Br in 37j.



Scheme 12 Synthesis of N-(1,3-dioxo-isoindol-2-yl)-2-phenyl-1H-benzo[d] imidazole derivatives 60 and 61.

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Scheme 13 Synthesis of benzimidazole analogues 68 and 69

Scheme 14 Procedure for the preparation of 2-arylbenzoimidazole derivatives 74a-d.

Scheme 10 describes the approach for the synthesis of benzimidazole-thiazolidinediones **46a-c**. The Knoevenagel condensation reaction was used to produce the final products **46a-c** in a convergent manner. In the presence of sodium

metabisulfite, 3,4-diaminobenzoic acid (38) was natively altered to its methyl ester 39, which subsequently interacted with various substituted benzaldehydes 40 to yield 1H-benzo[d] imidazole-5-carboxylates 41. Lithium aluminum hydride was

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used to decrease the ester functionalities in 41, resulting in alcohols 42, which were subsequently oxidized to their respective aldehydes 43. Compounds 46a-c were created by employing the Knoevenagel reaction to react different thiazolidinediones with 2-phenyl-1H-benzimidazole-5-carbaldehydes 43 in dry toluene containing piperidine. Both conventional and microwave-assisted synthesis were used to carry out the Knoevenagel condensation reaction for the synthesis of compounds 46a-c. Compounds 46a-c exhibited satisfactory cytotoxicity to prostate, cervical, bone, and lung cell lines ( $IC_{50} = 0.096-0.63$ μM). 37 According to SAR evaluations, with the exception of a few that demonstrated selectivity against A549 cells, compounds with IC<sub>50</sub> values less than 1.0 μM were generated when heterocyclic rings such as morpholine, pyrrolidine, and piperidine were present via an oxo-ethyl linkage. On the HeLa, A549, and HT1080 cell lines, more effective compounds were generated at 1.0 µM when the oxo-ethyl group was substituted out for a benzyl substituent. On almost all the screened cancer cell lines, derivatives such as 46b that had 3,4,5-trimethoxybenzyl substitution at the tail produced higher active molecules at concentrations below 1.0 µM. By contrast, derivative 46a, which included a 3-methyl benzyl moiety on the thiazolidinedione tail, was more effective against all cancer cell lines at 1.78 μM. The most powerful derivative, 46c, has benzimidazole replaced with 4-isobutoxy-3-methoxy.

Scheme 11 displays the procedure used to synthesize compounds 54a and b. Using sodium bisulphite in DMF, 4substituted benzaldehyde 48 reacted with 3,4-diaminobenzoic acid (38) to produce 2-(4-substituted-phenyl)-1*H*-benzo[*d*] imidazole-6-carboxylic acid (49) derivatives in the first step. A simple esterification procedure transformed molecule 49 into methyl ester 50, which was then reacted with hydratedhydrazine to yield 2-(4-substituted-phenyl-6-carbohydrazide)-1H-benzo[d]imidazole (51). Hydrazide derivative 51 was converted to 2-(4-substituted-phenyl)-6-(5-mercapto-1,3,4-oxadiazol-2-yl-1H-benzo[d]imidazole derivative (52) by reacting with carbon disulfide in boiling basic medium of ethanol and NaOH. Compound 52 and acetylated piperazine derivative 53 reacted in acetone at the last reaction step to yield 2-((5-(2-(4-substitutedphenyl)-1*H*-benzo[*d*]imidazol-6-yl)-1,3,4-oxadiazol-2-yl)thio)-1-(4-substituted-piperazin-1-yl) derivatives of ethane-1-on (54a and b). Çevik et al.38 produced a variety of 1H-benzimidazoleoxadiazole compounds and tested them against HeLa, MCF-7, A549, HepG-2, and C6 human cancer cell lines in vitro to determine their anticancer properties. By inhibiting topoisomerase I, compounds 54a (IC $_{50}$  = 0.224  $\pm$  0.011  $\mu$ M) and 54b (IC $_{50}$ =  $0.205 \pm 0.010 \mu M$ ) demonstrated the strongest antiproliferative action against the HeLa cancer cell line when doxorubicin (14.280 mM) was used as the standard medication. However, the majority of the derivatives demonstrated effective antiproliferative activity. The above-mentioned MTT experiment results supported our hypothesis that compounds 54a and **b** would be more potent than Hoechst 33342 (0.306 mM) due to their potential for effective activity and reduced toxicity.

Several aryl aldehyde sodium bisulfates adduct **56** were reacted with the benzoic acid derivative to produce the initial document 2-substituted-benzimidzole-5-carboxylic acids **57**.

Then, benzimidazole derivatives 57 were converted into the ester congeners 58 by a reaction with ethanol in the presence of sulphuric acid. Hydrazide derivatives 59 were generated when 58 reacted with hydrazine hydrate. Lastly, the target hybrids 60 were generated by allowing hydrazides 59 and acid anhydrides to react in acetic acid (Scheme 12).<sup>39</sup> Series 60 was produced by adding a carboxylic group to the dioxoisoindoline moiety at position 5, which increased the potency of 60f (% inhibition = 53.54%) and 60g (% inhibition = 60.36%).

Derivatives of benzimidazoles were synthesized and reported by Huang *et al.* Salicylic acid (62) was first esterified by diazomethane to produce methyl salicylate 63, which allowed the

Scheme 15 Method for the preparation of 5-methyl benzimidazole (76).

Scheme 16 Synthesis of -2-methylbenzimidazole (78).

Scheme 17 Synthesis of BZ 80.

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production of the desired derivatives. After being treated with benzyl bromide and potassium carbonate, the hydroxyl group of 63 was protected with benzyl ether to yield  $64.^{40}$  The saponification reaction then changed component 64 into carboxylic acid 65. After treating compound 65 with dicyclohexylcarbodiimide, amide 67 was produced, which was further transformed into benzimidazole 68 through cyclization–dehydration by refluxing in acetic acid. Lastly, 5 M NaOH was used to saponify compound 68, yielding compound 69 (Scheme 13). Benzoimidazole-4-metylacetate, 68 (A549, IC $_{50}$  70  $\mu$ M), was found to be more effective than benzoimidazole-4-carboxylic acid, 69 (A549, IC $_{50}$  87  $\mu$ M) in the anticancer screening tests. Lastly is a compound 10 to 10 t

Scheme 14 shows the main procedure for the preparation of 2-arylbenzoimidazole derivatives. The readily available starting material 33 was reacted with substituted benzoic acid 70 in polyphosphoric acid (PPA) (71) at 150 °C for 10–15 h to yield the 2-arylbenzoimidazole intermediate 72.<sup>44</sup> The nitro group was reduced to generate an amino intermediate, which was then reacted with substituted aromatic carboxylic acids 73 at 20 °C for 2–4 h with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI) and hydroxy benzotriazole (HOBt) as a condensing agent and triethanolamine<sup>45</sup> as a base to obtain 74a–d. According to the CAM experiment, compound 74d demonstrated a great level of angiogenesis inhibition (79% inhibition per 10 nM per egg),

#### **Key observation:**

Target engagement with electron-withdrawing substituents chloro and bromo on the phenyl ring substituent of the 1*H*-benzimidazole scaffold may be responsible for its anticancer activity.

Scheme 18 Synthesis of substituted 1H-benzimidazole derivatives 83a and b.

Compound	$\mathbf{X}$	$\mathbf{R_1}$	$\mathbf{R}_2$	$\mathbb{R}_3$	$\mathbb{R}_4$
86a	Η	H	OH	H	Η
86b	Η	Н	Н	OH	Н
86c	Η	$OCH_3$	Η	H	$OCH_3$
86e	Η	Н	$OCH_3$	$OCH_3$	$OCH_3$
86 <b>f</b>	Η	Н	H	$N(CH_3)_2$	Н
86g	Η	$NO_2$	H	H	Н
86h	Η	Н	$OCH_3$	OH	Н
86i	Η	Н	Н	$-OCH_2-C_6H_5$	Н

Scheme 19 Synthesis of 2-(substituted-phenyl) benzoimidazole derivatives 86.

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demonstrating the highest level of VEGFR-2 kinase inhibitory activity (51.4 nM IC<sub>50</sub>), and effective anti-proliferative potencies against HepG-2 and HUVEC cells (1.47 µM and 2.57 µM, respectively).46 According to the SAR investigations, the phenylacetamide moiety meta- and para-halide substitutes did not increase the kinase inhibitory activity. Furthermore, the imidazole ring and 4-chloroyphenyl together with the 4-methoxyphenylacetamide moiety in compound 74d improved VEGFR-2 kinase binding.

Other benzimidazoles were synthesized similarly. Niementowski started by producing 5-methyl benzimidazole (76, X = 5-methyl) by the condensation of equimolar amounts of 75 and ethyl formate at 225 °C in a sealed tube for three hours to between the reaction 4-methyl-o-phenylenediaminehydrochloride (75) and esters (Scheme 15).24,34

Although a shorter treatment only produced N,N'-diacetylphenylenediamine (Scheme 16), the further treatment of 77 with acetic anhydride produced 2-methyl benzimidazole (78, R

Scheme 20 Synthesis of target compounds 92.

Scheme 21 Synthetic protocol for benzimidazole hybrids 95.

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= CH $_3$ ). Reinhardt found that the 2-methylbenzimidazole yields from 1% and 2% acetic anhydride were increased using diluted hydrochloric acid. <sup>24,34,47</sup>

With the help of the scientific research by Hoebrecker<sup>48</sup> in a six year period, scientists Banerjee<sup>49</sup> set the foundation for existing medical chemistry research by discovering a novel heterocyclic molecule (basic structure and synthesis shown in Scheme 17). BZ is the name of this substance.

The synthesis and cytotoxic potential of 1*H*-benzimidazole scaffolds were examined by Pham *et al.* N,2,6-trisubstituted 1*H*-benzimidazole derivatives **83a** and **b** were prepared from benzene-1,2-diamine derivative 77. There are two steps in the synthetic process (Scheme 18). Firstly, using microwave assistance for the heating requirement, benzene-1,2-diamine derivative 77 was condensed with substituted aromatic aldehyde **81** to produce 2,6-disubstituted 1*H*-benzimidazole derivatives **83a** and **b**. Then, using the non-selective (positive) control involved in paclitaxel (PTX) in the MTT assay, we evaluated the anticancer activity of compounds **83a** and **b** on five cancer cell lines

including hepatocellular carcinoma cell line (HepG-2), human breast cancer cell lines (MDA-MB-231 and MCF-7), the aggressive and highly malignant rhabdomyosarcoma cell line (RMS), and colon carcinoma cell line (C-26). Among the synthesized compounds, compounds 83a (3,4-dichloro) and 83b (5-bromo-2-hydroxy) demonstrated the greatest anticancer activity against all the tested cell lines.<sup>50</sup>

Given that Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub> dissolves readily in an EtOH–H<sub>2</sub>O mixture (9:1 v/v) as the medium for the manufacture of benzimidazole derivatives, the oxidation reaction to yield 2-phenyl benzimidazole derivatives 86 can occur at room temperature, as illustrated in Scheme 19. Huynh *et al.*<sup>51</sup> produced 2-(substituted-phenyl)benzimidazole derivatives by reacting *ortho* phenylenediamines with benzaldehydes and using sodium metabisulphite as an oxidant. Three human cancer cell lines, A549, MDA-MB-231, and PC-3, were used to test these substances for their anticancer activities. The functioning of the phenyl ring system at 2-position, the biochemical properties of the cell lines, and electron-withdrawing and -donating groups

The SAR studies revealed that combining 2-thiobenzimidazole with 2-acetylbenzofuran and 4-aminoacetophenone, resulted in maximum cytotoxic action.

It was observed that benzo fused heterocyclic acetyl derivatives exhibited maximum anticancer activity whereas the addition of 5-5-bromo substituent suppressed its activity.

Scheme 22 Synthesis of benzimidazole-linked 2-acetylbenzofuran derivatives 99a and b

$$R_{3}$$
C
 $NH_{2}$ 
 $R=CH_{3}$ , Ph, H

84

100

101

Scheme 23 Synthesis route to target compounds 101.

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on the benzimidazole scaffold were shown to be associated with variations in the IC50 values. We established additional benzimidazole derivatives with various substituents on the 2-phenyl ring system, such as -OH, -NO2, -CF3, -I, -OMe, -NMe2, and -OCH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>, in parallel to provide more information about the impact of the electron-donating and withdrawing groups on the phenyl ring at the 2-position as well as on the benzimidazole frame. Remarkably, the bioactivities of compounds 86 on the A549 and PC-3 cell lines showed that the methyl group at position 5 is crucial in enhancing their bioactivities (86a > 86b > 86c > 86e > 86f > 86g > 86h > 86i).

Scheme 20 illustrates the three-step synthesis of target compounds 92 used in this investigation. Following the procedure outlined in the literature, the substrates, 1-(4-(5substituted-1*H*-benzo[*d*]imidazol-2-vl)phenvl)ethan-1-ones (89) and 4-(5-substituted-1*H*-benzo[*d*]imidazol-2-yl)benzohydrazides (90), were created. 52 Condensation of the hydrazide with diverse

substituted benzaldehydes 91 (ref. 53) led to the related arylidene hydrazides 92. Cevik et al. 54 produced a variety of 4-(5substituted-1H-benzimidazol-2-yl)-N/-((5-substitutedthiophen/ furan-2-yl)methylene)benzohydrazides (92) and used the MTT assay to evaluate them for their ability to kill A549 and MCF-7 (breast) cancer cell lines, using cisplatin as a (+ve) control. The normal NIH/3T3 cell line was also employed to check the synthesized synthetic substances. Compound 92g exhibited no effect on the normal cell line, while it had the maximum cytotoxic potential in the A549 cell line (IC<sub>50</sub> against A549 = 0.316 $\mu$ M). Compared to cisplatin, compound 92j (IC<sub>50</sub> = 0.0316  $\mu$ M) exhibited the most potent selective cytotoxicity against the MCF-7 cell line. In comparison to 5-fluoro substitution, SAR investigations demonstrated that 5-chloro substitution at the 1Hbenzimidazole ring improved the cytotoxicity against MCF-7 cells.

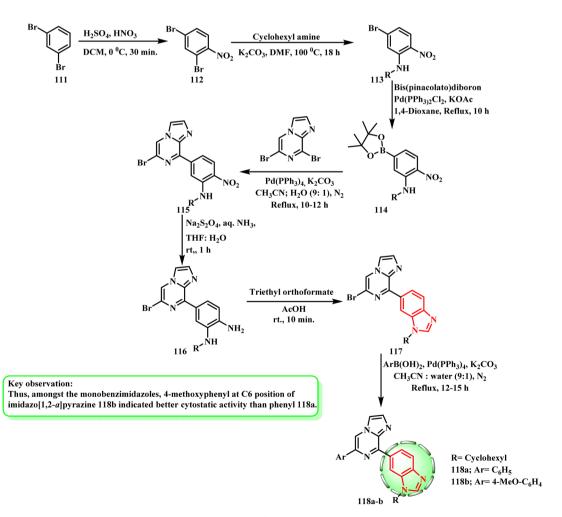
Compound	R	Compound	R
105a	CI—CH <sub>3</sub>	105f	F—
105b	−√Ç−CH₃ ✓ CH₃	105g	OCH <sub>3</sub>
105c	CH <sub>3</sub>	105h	F <sub>3</sub> C
105d	H₃C-CH₃	105i	н₃со-∕
105e	CI	105j	CI

Scheme 24 Synthesis of benzimidazole-urea derivatives 105.

110a, R<sub>1</sub>= 3,4.5-(OCH<sub>3</sub>)<sub>3</sub>, R<sub>2</sub>= 2,4-(OCH<sub>3</sub>)<sub>2</sub> 110b, R<sub>1</sub>= 3,4,5-(OCH<sub>3</sub>)<sub>3</sub>, R<sub>2</sub>= 3,4-(OCH<sub>3</sub>)<sub>2</sub>

 $\begin{array}{c} R_1 \\ H_2O \\ NO_2 \\ Reflux, 6 \text{ h} \end{array}$   $\begin{array}{c} H_{2O} \\ NO_2 \\ Reflux, 6 \text{ h} \end{array}$   $\begin{array}{c} 106 \\ 107 \\ \end{array}$   $\begin{array}{c} 108 \\ Reflux, 3 \text{ h} \\ \end{array}$   $\begin{array}{c} 108 \\ Reflux, 3 \text{ h} \\ \end{array}$   $\begin{array}{c} 108 \\ Reflux, 3 \text{ h} \\ \end{array}$   $\begin{array}{c} R_1 \\ Reflux, 3 \text{ h} \\ \end{array}$   $\begin{array}{c} R_1 \\ Reflux, 3 \text{ h} \\ \end{array}$   $\begin{array}{c} R_1 \\ Reflux, 3 \text{ h} \\ \end{array}$   $\begin{array}{c} R_1 \\ Reflux, 3 \text{ h} \\ \end{array}$   $\begin{array}{c} R_1 \\ Reflux, 3 \text{ h} \\ \end{array}$   $\begin{array}{c} R_1 \\ Reflux, 3 \text{ h} \\ \end{array}$   $\begin{array}{c} R_1 \\ Reflux, 3 \text{ h} \\ \end{array}$ 

Scheme 25 General synthesis of compounds 110a and b.



Scheme 26 Synthesis of mono benzimidazole derivatives 117 and 118a and b.

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The appropriate benzyloxybenzaldehyde 94 was produced by reacting 4-hydroxybenzaldehyde (93) with benzyl chloride to synthesize target compounds 95 (Scheme 21). Abdel-Mohsen  $\it et~al.^{55}$  investigated the effects of a novel class of 1,2-disubstituted 1*H*-benzimidazoles as VEGFR-2 inhibitors on the HepG-2 hepatocellular carcinoma cell line. According to the results, sorafenib (IC $_{50}=10.99~\mu M$ ) had lower cytotoxic activity than one of the created hybrids, 95 (IC $_{50}=1.98~\mu M$ ). According to the SAR evaluations, the elongated side chains of 1*H*-benzimidazole at the one position resulted in activity as a VEGFR-2 inhibitor. It was discovered that the presence of a linker and substituents at positions N-1, C-2, C-5, and C-6 is promising in the anticancer action.

**1.1.2. Acidic compounds.** As shown in Scheme 22, the appropriate 2-acetylbenzofurans (97) were produced by cyclizing salicylaldehyde (96) with chloroacetone in the presence of potassium hydroxide. <sup>56</sup> 2-Substituted thiobenzimidazole sulfate salts (98) were produced when compounds (97) and 2-mercaptobenzothiazole reacted in acidic medium of two equivalents of conc. sulfuric acid using the modified procedure described by Abdel-Aziz *et al.* <sup>57</sup> At room temperature and to provide the appropriate free bases **99**, the free molecules were liberated from the sulfate salts by neutralizing through stirring with an NaHCO<sub>3</sub> solution. With sunitinib serving as the reference medication (IC<sub>50</sub> = 0.18  $\mu$ M), compound **99** 

demonstrated excellent inhibitory efficacy against the A498 human kidney cancer cell line (IC $_{50}=6.97~\mu M$ ). Mixing 2-thiobenzimidazole with 2-acetylbenzofuran and 4-amino-acetophenone had the most cytotoxic effect, according to the SAR investigations. Benzo-fused heterocyclic acetyl derivatives were shown to have the strongest anticancer activity, but the addition of a 5-bromo substituent reduced this activity.

Niementowski heated free base **84** with appropriate amides **100** to produce methyl benzimidazole (**101**,  $R_1 = 5$  (6)1-methyl, R = H,  $CH_3$ , or Ph) (Scheme 23).

Here, we describe how we produced benzimidazole–urea derivatives **105**. The reaction of *O*-phenylene diamine (77) and cyanogen bromide (**102**) in methanol at room temperature produced 2-aminobenzimidazole (**103**) in 88% yield by ammonium hydroxide workup (Scheme 24). Compound **105j** (3-chloro-4-fluorophenyl) was the most active compound against a liver cancer cell lines (HepG-2), with an IC<sub>50</sub> value of 7.5  $\mu$ M. The anticancer activity of the other compounds followed the order of **105d** > **105a** > **105b** > **105e** > **105c** > **105g** > **105f**. The anticancer activity of the other compounds followed the sequence of **105a** > **105b** > **105d** > **105i** > **105f** > **105h** > **105g** with IC<sub>50</sub> values in the range of 2.4–38.5  $\mu$ M, among which compound **105j** was the most effective (IC<sub>50</sub> = **1.9**  $\mu$ M) against non-small lung cancer (A549).<sup>59</sup>

Scheme 27 Schematic of the route for the synthesis of bisbenzimidazole 126.

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1.1.3. o-(N-Arylamino)arylamines. The production and cytotoxic potential of novel 1,2-diarylbenzimidazole analogues were examined by Zhang et al. Following the general procedure indicated in Scheme 25, compounds 110a and b were synthesized by refluxing o-fluoronitrobenzene (106), neutralizing it with NaHCO<sub>3</sub>, and then obtaining analogue 107. After dissolving compound 107 in a mixed solution of ethanol and tetrahydrofuran, the reduction process was initiated by the addition of Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> solution. Following NaHCO<sub>3</sub> alkalization, compounds 108 were produced. Imidazole rings 110a and b were finally formed by refluxing compounds 108 with substituted benzaldehyde 109 after they had been dissolved in anhydrous ethanol. Compound 110b demonstrated minimal toxicity to normal cells and maximum anticancer activity against HeLa, HepG-2, A549, and MCF-7 cells, with IC<sub>50</sub> values of 1.71  $\pm$  0.14, 0.71  $\pm$  0.07,  $2.41\pm0.31$ , and  $1.94\pm0.08~\mu\text{M}$ , respectively. 60 The impact of double substitution on activity was also considered. To develop ortho-para disubstituted compounds and meta-para disubstituted compounds, respectively, -OCH3 with excellent activity was chosen as the substituent, where  $110a < 110b = 2,4-(OCH_3)_2$ < 3,4-(OCH<sub>3</sub>)<sub>2</sub>. As a result, 3,4-(OCH<sub>3</sub>)<sub>2</sub> had the best activity in meta-para disubstitution, which had higher activity than orthopara disubstitution.

After being nitrated with sulfuric and nitric acids, the commercially available 1,3-dibromobenzene (111) was converted to 112, which was then selectively replaced with

cyclohexylamine in DMF to produce 113. Bis(pinacolato) diboron was used to boronate 113 in the presence of Pd(PPh<sub>3</sub>)<sub>2</sub>Cl<sub>2</sub> and KOAc, producing 114. Suzuki-Miyaura crosscoupling of 114 with dibromo-imidazo[1,2-a]pyrazine61,62 and Pd(PPh<sub>3</sub>)<sub>4</sub> afforded compound 115 together with traces of disubstituted products. Amines 116 were obtained by reducing the derivatives with Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> in ammonia. Then, triethyl orthoformate was cyclized in acetic acid to produce intermediate 117. Using Pd(PPh<sub>3</sub>)<sub>4</sub> and K<sub>2</sub>CO<sub>3</sub>, Suzuki reactions of the intermediates with unsubstituted and substituted phenylboronated were performed in CH<sub>3</sub>CN: H<sub>2</sub>O, yielding 118a and b (ref. 63) (Scheme 26). Mono benzimidazole derivatives 118a and b showed specific effectiveness against subpanels of melanoma, colon, leukemia, and central nervous system (GI<sub>50</sub> = 0.31-0.39 µM). Therefore, 4-methoxyphenyl at the C6 position of imidazo[1,2-a]pyrazine 118b showed superior cytostatic activity compared to phenyl 118a among the mono benzimidazoles.

The commercially available 1,3-dibromobenzene (111) was nitrated to produce 112, which was then replaced with cyclohexylamine in DMF to produce 113. After reducing 113 with Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> in ammonia to yield 119, the corresponding benzimidazole 121 was obtained by cyclization with triethyl orthoformate (120) in acetic acid. Bis(pinacolato)diboron (122) was used to boronate the derivative in the presence of Pd(PPh<sub>3</sub>)<sub>2</sub>Cl<sub>2</sub> (123) and KOAc, yielding 124. Using Pd(PPh<sub>3</sub>)<sub>4</sub> and K<sub>2</sub>CO<sub>3</sub>, Suzuki-Miyaura cross-coupling of benzimidazole boronated

Scheme 28 Synthesis of benzimidazole-tethered pyrazole 132.

Scheme 29 Synthesis of benzimidazole derivatives 140a-g.

Scheme 30 Synthesis of (1H-benzo[d]imidazol-2-yl)carbamate analogue 145.

125 with intermediate 124 was carried out to yield 126 (Scheme 27). All the screened cell lines showed the cytotoxicity of compounds 118b and 126, which also displayed significant growth inhibitory concentrations of 2.10 and 2.23  $\mu$ M,

respectively.<sup>63</sup> The colon cancer cell line HCC-2998 was discovered to be extremely sensitive to derivative **126** among these tumor-sensitive cell lines, exhibiting a negative growth percentage value (lethal impact). The cancer cell line from the

central nervous system (SF-539) was found to be highly susceptible to derivative 118b. Ct-DNA intercalates with imidazo [1,2-a]pyrazine-benzimidazoles 118a and bisbenzimidazole 126,64 exhibiting superior activity than bisbenzimidazole, as a key interaction for basic physiologically noteworthy effects. With cytostatic effects on the cell line, mono benzimidazole derivative 118a was superior to bisbenzimidazole 126; nevertheless, compound 126 subsequently revealed a cytotoxic influence on various cancer cell lines.

Scheme 28 shows the synthesis of benzimidazole-tethered pyrazole 132. Arylhydrazine and suitable aralkyl ketone 128 are first condensed in glacial acetic acid to produce pyrazolebased carbaldehyde 131. Hydrazone intermediate 129 is then cyclized utilizing Vilsmeier-Haack reaction. Compound 132 had the least toxicity and most promising effects, with IC<sub>50</sub> values of 30.9, 32.8, and 80 μM against MRC5 cells, AsPC1, and SW1990.65 Additionally, SAR investigations showed that a 4fluorophenyl moiety increased its efficacy against all the evaluated cell lines.

**1.1.4.** *o*-Nitro arylamines or *o*-dinitro arenes. In their study aimed at selectively targeting cancer cells, Wu et al.66 established a novel category of chemicals called benzimidazoles. Scheme 29 shows how various amines reacted with commercial substance 133 to produce amine-substituted nitrobenzene intermediates 134. The corresponding anilines 135 and 138 were obtained by reducing the nitro groups in 134. Subsequently, the final products 140a-g were obtained by coupling with 2-(4-(ethylsulfonyl)phenyl)acetic acid (Scheme 29). The SAR revealed that the ethyl sulfone fragment was necessary for the high activity, while trifluoromethyl at the C-6 position of the benzimidazole moiety and fluoro on the phenyl ring were crucial for the activity. Moreover, hybrids 140a and b showed encouraging efficacy against the AR-positive LNCaP, 22Rv1, and C4-2B prostate cancer cell lines (IC<sub>50</sub>: 6.3-8.3 µM and 4.6-8.1

μM, respectively). When the size was increased to isopropyl formamide (140c), the activity was reduced by almost nine-fold. These findings showed that the best methyl group substituent for RORγ transcriptional activity was located at the R<sub>1</sub> position. Potency losses of 73 and 3.7 times were seen in comparable compounds 140d and 140e when the methyl or methoxy group at the R<sub>1</sub> position was fused to the R<sub>2</sub> position, respectively. However, the resultant compound 140f had somewhat increased efficacy when the isopropyl formamide moiety at the  $R_1$  position was fused to the  $R_2$  location.

Methyl(5-(4-(methyl(oxetan-3-yl)amino)benzoyl)-1H-benzo[d] imidazol-2-yl)carbamate (145) was obtained via the microwave<sup>67</sup> condensation cyclization of 1,3-bis(methoxycarbonyl)-2-methyl-2-thiopseudourea and intermediate 143 (Scheme 30). Compound 145 with oxetanyl substitution demonstrated significant cytotoxic activity against prostate (PC-3 and PC3MLN4 cell lines), lung (A549 cell lines), and ovarian cancers with considerable activity toward profoundly aggressive carcinogenic cell lines (IC<sub>50</sub> = 0.9-3.8  $\mu$ M).<sup>68</sup> The growth of existing tumors was greatly suppressed by compound 145 (30 mg kg<sup>-1</sup>) without causing any detectable toxicity (T/C: 0.36). Additionally, as seen in compound 145, SAR studies showed that the addition of the para to ketone group in mebendazole and the oxetane group in the phenyl ring on the left side of the compound increased the anticancer activity. A methyl carbamate moiety on the right side of the 1H-benzimidazole ring also increased the cytotoxic activity of the compound.

The synthetic route for benzimidazole-quinoline<sup>69</sup> hybrid 151 is presented in Scheme 31. Firstly, key intermediate 150,70 obtained initially from core nucleus benzimidazole-5carboxylate 149, was effectively produced by a 'one pot' nitro reductive cyclization process between ethyl 3-nitro-4-(substituted amino)benzoate 148 and 5-bromothiophene-2carbaldehyde using Na2S2O4 in DMSO.71 When tested against

Scheme 31 Synthetic route for benzimidazole-quinoline hydride 151

two distinct cell lines, all other drugs showed moderate to good anticancer efficacy. Using cisplatin as a reference medication, compound 151 demonstrated activity against the A37 (IC $_{50}=34.7\pm0.9~\mu g~mL^{-1}$ ) and MDA-MB-231 (IC $_{50}=20.4\pm1.1~\mu g~mL^{-1}$ ) cell lines using the MTT test. However, the other substances did not show preference for these specific cell lines. Chemical substance 151 also showed cytotoxic properties. The IC $_{50}$  value of compound 151 (A375) was 34.7  $\pm$  0.9  $\mu g~mL^{-1}.^{42}$  Compound 151 presented the highest % inhibition and lowest IC $_{50}$  value of 604.8  $\mu g~mL^{-1}$ .

**1.1.5.** *o*-Substituted-*N*-substituted. The production and cytotoxic potential of *N*-substituted benzimidazole acrylonitrile hybrids were examined by Perin *et al.* Unsubstituted or cyano substituted *N*-isobutyl **152** was produced by uncatalyzed microwave-assisted amination beginning with the corresponding *o*-chloronitrobenzenes. Then, compounds **152** were reduced with  $SnCl_2 \times 2H_2O$  in MeOH, which served as the primary precursor for the production of target molecules **156a** and **b**. The cyclocondensation reaction of amino derivative **153** with 2-

cyanoacetamide (154) at elevated temperatures produced Nsubstituted-2-cyanomethylbenzimidazoles 155. The condensation reaction of systems 155 with a chosen aromatic aldehyde in pure ethanol, followed by the addition of a few drops of piperidine as a weak base yielded the equivalent N-substituted-2benzimidazolyl acrylonitrile 156a and b, as demonstrated in Scheme 32. As lead compounds, N-substituted benzimidazole acrylonitrile, which has N-isobutyl and cyano substituents on the benzimidazole nuclei (156a and b), demonstrated potent antiproliferative action, while being noticeably less hazardous than the reference systems staurosporine and docetaxel.<sup>72</sup> According to SAR investigations, the affinity of the phenyl moiety significantly increased when an electron-donating parasubstituted NMe2 group was added. This tendency for access to optimum binding with Cys241 was reduced by substituting the electron-withdrawing cyano group (156b). The chance of favorable positioning was increased by the inclusion of the significant N-isobutyl group.

$$R_{1} = H, CN$$

$$R_{1} = H, CN$$

$$R_{1} = H, CN$$

$$R_{1} = H, R_{2} = H, R_{2}$$

### **Key observations:**

- 1) Both cyano as well as isobutyl substituents attached to the benzimidazole nuclei electron-donating para-substituted NMe<sub>2</sub> group on the phenyl moiety showed most promising anticancer activity.
- 2) Substitution of electron-withdrawing {cyano} group (156b) decreased the potential to take part in optimal binding with Cys241.

Scheme 32 Synthesis of N-substituted-2-benzimidazolyl acrylonitrile derivatives 156a and b

Scheme 33 Synthesis of various 1-benzyl-1*H*-benzimidazole analogues **164a** and **b**.

Kommi et al. investigated the production and cytotoxic potential of benzimidazole derivatives. Scheme 33 depicts the synthetic method utilized to prepare 1-benzyl-1H-benzimidazole analogues 164a and b. 4-Formyl-N-phenylbenzamide 163 was utilized to condense  $N^1$ -benzyl-4-chlorobenzene-1,2diamine (160). Using benzyl bromide (158) and the nitro group of compound 159, the commercially available 4-chloro-2nitro aniline (157) was first converted to N-benzyl-4-chloro-2nitroaniline (159). Then, it was further reduced to an amino functional group using stannous chloride dihydrate, which produced N<sup>1</sup>-benzyl-4-chlorobenzene-1,2-diamine  $(160).^{73}$ Alternatively, the commercially existing 4-formyl benzoic acid (161) and aniline derivative (162) were used to create 4-formyl-Nphenylbenzamide (163).74 Lastly, 4-formyl-N-phenylbenzamide (163) and  $N^1$ -benzyl-4-chloro orthophenylene diamine (160) were refluxed in ethanol in the presence of Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub>, yielding target compounds **164a** and **b**.<sup>75</sup> Among the compounds, with an IC<sub>50</sub> value of 7.01  $\pm$  0.20  $\mu$ M, **164b** (4-(1-benzyl-5-chloro-1*H*-benzo[*d*]imidazol-2-yl)-*N*-(4-hydroxyphenyl)benzamide) demonstrated the highest efficacy and stopped the MCF-7 cell cycle in the G2/M phase and S-phase.<sup>76</sup> The SAR investigations revealed that larger substituents linked to the benzene ring (**164a**) reduced the cytotoxic activity of the examined compounds.

**1.1.6. Imines.** Oxibendazole with *O-n*-propyl was chosen for further investigation. In this phenotypic screening assay, *O-n*-propyl was determined to be the ideal size for hydrophobic interaction with tubulin binding, while O-ethyl and *O-n*-butyl derivatives were less active than oxibendazole. Thus, as a default benzimidazole C6 tail, oxibendazole with *O-n*-propyl was used (Scheme 34).<sup>77</sup>

**1.1.7. Amidines.** Target compound 2-(2-pyrimidinylamino) benzimidazole **172** (ref. 78) was produced, as indicated in

HO F 
$$n$$
-PrI  $K_2CO_3$ , Acetone  $NO_2$   $NHCH_3$   $NHCH_$ 

Scheme 34 General synthesis of compound 169.

**Key observation:** 

SAR studies displayed that in compound 172, as the number of methoxy groups is increased on the phenyl ring attached at position 6 of pyrimidine scaffold, the anticancer action is also increased. This may be because of the electron-releasing effect.

Scheme 35 Synthesis of 2-(2-pyrimidinylamino)benzimidazole 172

Scheme 35, where initially, 2-guanidinobenzimidazole (170)<sup>79</sup> was combined with olefinic derivatives arylidenes (171) under general mild reaction conditions. The amino group at position C2 of olefinic molecule 170 was attacked nucleophilically, and then an oxidative cyclization process took place. Compound (172) was obtained by the reaction<sup>80</sup> of compound 170 with arylidene malononitrile (171) in ethanol under reflux using piperidine as a catalyst. Ismail *et al.*<sup>81</sup> produced unique 2-amino(substituted pyrimidin-2-yl)benzimidazole hybrid 172 and tested it against a range of human cancer cell lines *in vitro* to determine its anti-cancer properties. Five-dose testing revealed that compound 172 had the most potential anti-cancer activity.

SAR studies revealed that the more methoxy groups on the phenyl ring attached at position 6 of the pyrimidine scaffold, the stronger the anticancer activity of compound **172**. This can be ascribed to the electron-releasing effect.

**1.1.8. Heterocyclic scaffolds.** Ethyl-5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylate (173) was synthesized from the commercially available *tert*-butyl acetoacetate by adopting a previously reported literature procedure<sup>82</sup> and route outlined in Scheme 36. Target compound 175 was prepared *via* the hydrolysis of compound 174 using KOH as a base in a watermethanol mixture under reflux conditions. Subsequently, amide derivative 177 was synthesized by coupling acid 175 with

Scheme 36 Synthetic scheme for target compound 177.

Me NO<sub>2</sub>

N NO<sub>2</sub>

H-O 179

KOH, DMF, Ph<sub>3</sub>P, Cul 70 °C, 2 h

R 178a-b

a, R= H

b, R= CN

Key observation:

SAR studies revealed substituting the phenyl group of pyrazole ring at position 4 with a polar cyano- group enhanced its activity, thus 
$$182b > 182a$$
.

Scheme 37 Synthetic route from pyrazoles 178a and b to pyrazole-benzimidazole conjugates 182a and b, respectively.

Scheme 38 Synthesis of imidazo[2,1-b]thiazole-benzimidazole conjugate 188.

Scheme 39 Synthesis of designed compound 193.

various amines **176** using the TBTU coupling reagent at ambient temperature in DMF solvent. Rasal *et al.*<sup>83</sup> produced a unique array of 2,4-dimethyl-1*H*-pyrrole-3-carboxamide hybrids with a 1*H*-benzimidazole moiety using the molecular hybridization

approach. At a dose of 10  $\mu$ M, they were tested for their anticancer activity against a variety of human cancer cell lines. Even at low concentrations, some of them demonstrated strong antiproliferative effects by functioning as VGEF inhibitors, RSC Advances Review

Scheme 40 Reaction of substituted benzoyl chloride with 2-aminobenzimidazole.

whereas compound 177, or 5-(1H-benzo[d]imidazol-2-yl)-N-(1-cyclohexylethyl)-2,4-dimethyl-1<math>Hpyrrole-3-carboxamide, showed notable anticancer activity. According to SAR evaluations, the amide linkage had an impact on the anticancer efficacy of the compounds.

4-(5-(4-Formylphenoxy)-3-methyl-4-nitro-1*H*-pyrazol-1-yl) benzonitrile (**180b**) was obtained by reacting 4-(5-chloro-3-methyl-4-nitro-1*H*-pyrazol-1-yl)benzonitrile (**178b**) with 4-hydroxybenzaldehyde (**179**) in the presence of potassium hydroxide, DMF, and a catalytic amount of copper(i)iodide and triphenylphosphine, respectively. 4-(3-Methyl-4-nitro-5-(4-(5-nitro-1*H*-benzo[*d*]imidazol-2-yl)phenoxy)-1*H*-pyrazol-1-yl) benzonitrile (**182b**) was produced by the cyclocondensation

reaction of compound **180a** and **b** with 4-nitro-1,2-phenylenediamine (**181**) in the presence of sodium thio-sulphate and DMF (Scheme 37). Combining compound (**182b**) with doxorubicin was shown to greatly boost the anticancer effect of the drug and limit MCF-7 cell cycle growth. <sup>84</sup> According to SAR investigations, the anticancer activity of the 1*H*-benz-imidazole moiety decreased when carboxylic or nitro groups were present at position 5, but it increased when a polar cyano-group was substituted for the phenyl group in the pyrazole ring at position 4.

The synthesis and cytotoxic potential of imidazo[2,1-*b*] thiazole-benzimidazole conjugate **188** were examined by Baig *et al.* As illustrated in Scheme 38, imidazothiazole-

2) N-methylene phenyl substitutions revealed more potent cytotoxic activity than the compounds

2) N-methylene phenyl substitutions revealed more potent cytotoxic activity than the compounds with smaller chain lengths and nonaromatic substitutions.

Scheme 41 Reaction representation of 1H-benzimidazole with propyl chloride.

**Key observation:** 

Compound 202a showed better, albeit low, activity against the cell lines studied than compound 202b. The different substituents on the benzyl ring (electron donating or withdrawing) may be responsible for these differences

Scheme 42 Reaction of 2-methyl-1*H*-benzo[*d*]imidazole with alkyl halide.

Scheme 43 Synthesis of benzimidazole derivatives 205 and 207.

benzimidazole conjugate 188 was produced by oxidatively cyclizing imidazo[2,1-b]thiazole-5-carbaldehyde (186) and substituting o-phenylenediamine (187) with sodium metabisulphite in ethanol. By using the Vilsmeier-Haack reaction with the equivalent imidazo[2,1-b]thiazole (185), which was derived from suitable 2-bromo-1-arylethanone 184 and 2-

aminothiazole (183), imidazo[2,1-b]thiazole-5-carbaldehyde (186) was produced. Compound 188 (IC<sub>50</sub> = 1.08  $\mu$ M) showed significant cytotoxicity against the A549 cell line.85 The cytotoxicity of conjugates containing electron-withdrawing groups as substituents, such as p-trifluoromethyl and p-methoxy

#### **Key observations:**

SAR studies suggested that the presence of 4-fluorobenzyl at 1H-benzimidazole ring and an electronwithdrawing group at indole ring showed relatively higher anticancer effects 210a-b.

The compound 210b containing p-fluorobenzyl at 1H-benzimidazole ring and -Br at R position was one of the prominent compounds against MCF-7 cells.

Scheme 44 Synthetic route for the preparation of benzimidazoles 210a and b.

human gastric cancer cell lines.

3) All these modifications and the inhibitory results revealed that the sulfonyl groups were important for their inhibitory activity which can maintain or enhance the antiproliferative activity against

three tested human cancer cells.

4) The correlation between halogen substitution and anticancer activity was found to be 4-Br

4) The correlation between halogen substitution and anticancer activity was found to be 4-Bi (214b) > 4-F (214a) > 2-Cl (214c).

Scheme 45 Synthesis of tertiary sulfonamide derivatives containing a benzimidazole moiety 214a-d.

substituents, against A549 cells was demonstrated by SAR evaluations

The synthesis and cytotoxic potential of 2-(aminomethyl) benzimidazole derivatives were examined by Al-Sultan *et al.* Scheme 39 states that all synthesized compounds began with amine derivative **189** and an equivalent quantity of TEA

(primary amines did not use TEA). After being dissolved in 25 mL of diethyl ether and allowed to cool to 5 °C, chloroacetyl chloride (190) was gradually added to the mixture until the fumes stopped.<sup>86</sup> The antiproliferative features of the developed compounds on human breast cancer (T47D) and human alveolar cell carcinoma (A549) cells were evaluated *in vitro* using

## Key observations:

- 1) Complex **216(a-b)** showed better anticancer activity than the standard drug docetaxel at a concentration of 0.1 µM against the A-2780 cell line.
- 2) The well-known zinc metal structural were found to be favorable than cobalt metal ligand with the benzimidazole skeleton for the activity against A-2780 cell line.

Scheme 46 Synthesis of benzimidazole metal complexes 216a-d.

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## **Key observation:**

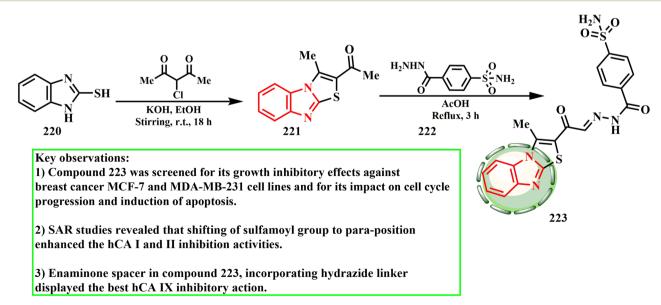
compound 219 bearing a terminal phenyl ring substituted with two electron-donating groups proved to be the most potent against both cell lines; HT-29 and MDA-MB-468.

Scheme 47 Synthesis of compound phenylthiazolo[3,2-a]benzimidazole 219

Vero cells (from the kidney of an African green monkey) as a standard control. The morphology of T47D with an inhibitory concentration of the cytotoxic chemicals, gefitinib, and the control was explained by additional research to confirm the antiproliferative effects of the extremely toxic substances (193) on T47D. Lastly, compounds 193 displayed IC<sub>50</sub> values that were nearly identical to that of the positive control.

In THF and TEA, we used a normal reaction between substituted benzoyl chloride 195 (1.1 mmol) and 2-aminobenzimidazole (194) (1 mmol) (Scheme 40). N-(Benzimidazol-2yl)-2-substituted benzamide structures 196 were synthesized efficiently in a single step. Interestingly, compound 196 showed the strongest anti-proliferative action against MCF-7 cancer cells (IC<sub>50</sub> =  $3.84 \pm 0.62 \mu M$ ).87

3,3'-(1,3-phenylene(methylene))(1-alkylseries of benzimidazolium)salts (200) was produced and characterized using the stated approach (Scheme 41).88 We previously employed propyl chloride (197) as the electrophile for 1,2-(bromomethylene)benzene.89 The method adopted for the synthesis of the title compounds was selective for the 1-position



Scheme 48 Synthesis of 3-methylthiazolo[3,2-a]benzimidazole-benzene sulfonamide conjugate 223.

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Scheme 49 Synthesis of benzimidazole derivative 228.

heterocycle. Indeed, upon treating alkyl benzimidazole 198 with m-xylyl dibromide (1,3-(bromomethylene)benzene) (199), the respective bisbenzimidazolium salt was observed to give 200. ul Huda et al. 90 developed and produced unique 1,10-(1,3-phenylenebis(methylene))bis(3-alkyl/aryl-1H-benzimidazol-3-ium) salt hybrids, and using the SRB assay, evaluated their anticancer potential against the MCF-7 and HCT-116 (CRC) human breast cancer cell lines. Compound 200b was shown to be the most effective anticancer drug overall, inhibiting the growth of HCT-116 cells with an  $IC_{50}$  of 0.1 µg mL<sup>-1</sup>. According to the SAR evaluations, the notable pharmacological effect of compound 200 was caused by its lengthy N-substituted alkyl chain, which presented it as extremely lipophilic. Compounds with shorter chain lengths, such as isopropyl chain (200a) and nonaromatic replacements, exhibited less cytotoxic action than N-methylene phenyl (200b) substitutions.

The compounds were prepared in accordance with the literature 91,92 (Scheme 42). Because of its efficiency in supplying

high quantities of hydroxide ions, KOH is a characteristically strong base utilized in a range of laboratory and chemical synthesis applications. For 12 h, the reaction was carried out at 80 °C. According to the results, **202a** had relatively antiproliferative efficacy against the A549, DLD-1, and L929 cell lines compared to cisplatin; however, **202b** was essentially effective against the A549 cell line. Compound **202b** has substituents with the methyl group at the 2-, 3-, 5-, and 6-positions, whereas compound **202a** has a chloro group on the benzyl ring at the 3-position. Compared to compound **202b**, compound **202a** had more advanced, albeit weaker, activity against the cell lines under investigation. The various substituents on the benzyl ring, which may either donate or withdraw electrons, could be the cause of these variations.<sup>93</sup>

Ren *et al.* researched the production of benzimidazole derivatives and their potential for cytotoxicity. Scheme 43 shows the preparation of compound **207**. Benzimidazole intermediates **205** were produced by cyclizing 3-bromo-1,2-

Scheme 50 Synthesis of benzimidazole derivative 232.

238a; R= 2-Cl 238b; R= 4-Cl 238c; R= 2,4-diCl

238d; R= 3,4,5-triOMe

**Key observations:** 

1) Among the synthesized molecules, compound 238b showed the most balanced cytotoxic effect against lung (A-549) and breast (MCF-7) cancer cells with  $IC_{50}$  values of 4.6 and 11.0  $\mu$ M, respectively.

2) A chlorine is preferred either at para position of the N-benzyl ring along with para fluoro in the piperazine-phenyl part (i.e. 238b), affording potent antiproliferative activity in tested tumor cells in the low micromolar range.

3) Additionally, the presence of bulky groups like 2,4-dichloro (238c) and 3,4,5-trimethoxy on the *N*-benzyl ring (238d) decreased the activity.

Scheme 51 Synthesis of benzimidazole derivatives 238a-d.

benzenediamine (203) with various aldehydes 204. Subsequently, compound 207 was produced by the Suzuki reaction between compound 205 and 3,4,5-trimethoxyphenylboronic acid (206). Compound 207, which is slightly more potent than colchicine, showed the strongest inhibitory effects on the growth of cancer cells ( $IC_{50} = 50 \text{ nM}$ ). In melanoma tumors, compound 207 showed a 78.70% inhibition rate. Stronger hydrogen bonds and hydrophobic interactions may be the cause of the significant activity of 207, according to the SAR evaluations.

Until the starting materials were exhausted (as determined by TLC), a combination of the proper o-phenylenediamine (1 mmol) (208), related indole derivative (1 mmol) (209), and Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub> (40%) (2 mL) in EtOH (4 mL) was refluxed<sup>95,96</sup> (Scheme 44). Especially compound 210a and b could alter the ER target gene expression, and an integrated stress response

was induced in a dose-related manner.<sup>97</sup> The MCF-7 transcriptome was shown to be significantly influenced by compounds **210a** and **b**, which resulted in the upregulation and downregulation of an appropriate number of genes. According to the SAR investigations, the presence of an electron-withdrawing group at the indole ring and 4-fluorobenzyl at the 1*H*-benzimidazole ring demonstrated comparatively stronger anticancer effects. Furthermore, the lipophilic characteristic of the indole moiety was improved by the presence of – Br, which facilitated effective binding.

The synthesis and cytotoxic potential of tertiary sulfonamide derivatives with a benzimidazole moiety were examined by Gao *et al.* Benzyl chloride **212** and 3,4,5-trimethoxyaniline (**211**) interacted in the presence of  $K_2CO_3$  in acetone to give secondary amine **213**, which reacted with different benzene sulfonyl chloride derivatives **37** to obtain tertiary sulfonamide

**Key observations:** 

1)Furthermore, the cyclopentyl 241a derivative was less likely to inhibit the viability of the cancer cells than erlotinib.

2) SAR studies suggested that when 4-nitrophenyl 241b was replaced with cyclopentyl (as in compound 241a) there was a significant reduction in the potency. It was also observed that reduction in the polarity of molecules and increase in the bulkiness of the substituent decreased the effectiveness of the tested compound i.e.241a>241b.

Scheme 52 Route for constructing the target thiazolo-benzimidazole hybrids 241a and b.

Scheme 53 Synthesis of new fused benzimidazole analogues 246a and b.

derivatives **214a–d** (Scheme 45). MGC-803 cells were more sensitive to compounds **214a–d** than PC-3 and MCF-7 cells. Among the compounds that had notable antiproliferative activity, compound **214b** demonstrated the strongest anticancer

activity against MGC-803 cells (IC $_{50}=2.19~\mu M$ ). The presence of a 3,4,5-trimethoxy group at the phenyl ring was crucial for the anticancer actions according to the SAR evaluations. The most effective compound against human stomach cancer cell lines

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Scheme 54 Synthesis of benzimidazole-bearing pyrazole derivative 251.

was compound **214d**, which had a methyl group and a 1*H*-benzimidazole moiety. 4-Br (**214b**) > 4-F (**214a**) > 2-Cl (**214c**) was the association between halogen substitution and anticancer efficacy. The sulfonyl groups were crucial for their inhibitory

effect, which can maintain or improve the antiproliferative activity against the three tested human cancer cells, according to all the reported changes and inhibitory findings.

$$N_{3}$$
 $N_{N}$ 
 $N_{N$ 

**Key observations:** 

- 1) Compound 254 with a chloro substituent (2-Cl) in the chalcone ring of the hybrid 1*H*-benzimidazole derivative displayed the highest cytotoxicity effects on all cancer cell lines tested.
- 2) Additionally, linkage of the benzyl moiety to the triazole ring further enhanced the antiproliferative property of the compound.

Scheme 55 Reaction between the azide derivative 253 and propargyl molecule 252.

Scheme 56 Synthesis of different fluoroaryl benzimidazole derivatives 257a-c.

The production and cytotoxic potential of new benzimidazole ligands and their cobalt(II) and zinc(II) complexes were examined by Yılmaz *et al.* ZnCl<sub>2</sub>·6H<sub>2</sub>O or CoCl<sub>2</sub> in EtOH was used to produce benzimidazole complexes **216a–d** from benzimidazole ligands (**215a**, **215b**, and **215c**). Ligands of benzimidazoles **215a** (ref. 99) and **215b** (ref. 100) were produced using the methods outlined in the literature. In this work, the Mizoroki–Heck reaction was used to synthesize benzimidazole ligand **215d** for the first time, similar to the literature technique<sup>101,102</sup> (Scheme 46). Compounds **216a–d** (log IC<sub>50</sub> = -0.97, -1.30, 1.13, and  $-0.73~\mu\text{M}$ , respectively) were identified to have higher anticancer potency than the standard docetaxel medication (log IC<sub>50</sub> =  $-0.81~\mu\text{M}$ ) against the A-2780 cell line at 0.1  $\mu\text{M}$  concentration.<sup>99</sup>

Scheme 47 shows the route for the preparation of target compound **219** in this work. Acetophenone and 2-mercaptobenzimidazole **217** reacted in refluxing acetic acid with five equivalents of sulphuric acid to produce sulphate salt **218** in a one-pot, two-component heterocyclization process. Phenylthiazolo[3,2-*a*]benzimidazole **219** was obtained by neutralizing sulphate salt **218** by stirring with an aqueous solution of sodium

bicarbonate. HT-29 (colon) and MDA-MB-468 (breast) cancer cell lines were used to investigate the cytotoxic potential of synthetic compound **219**. CD133 inhibition in cancer stem cells and the cytotoxicity of specific 3-phenylthiazolo[3,2-a]benzimidazoles including their design, direct synthesis, and *in vitro* biology were investigated. Compound **219** decreased the surface expression of CD133 on cells by 50% and showed strong anticancer activity against both cancer cell lines with IC50 values of 9 and 12  $\mu$ M, respectively. According to the SAR investigation, the electron-donating group in the phenyl ring of **219** enhanced the suppression of tumor cells.

NHR

However, employing 3-chloro-2,4-pentanedione, the acetyl thiazolo[3,2-a]benzimidazole derivative (221) was constructed as previously described for the mercaptan alternative 220. The target 223 was obtained, as shown in Scheme 48, by condensing the acetylthiazolo[3,2-a]benzimidazole derivative (221) with 4-(hydrazinecarbonyl)benzene sulfonamide (222) in acetic acid. Compound 223 was found to induce cell cycle arrest and death and exhibit prospective proliferation inhibition against the MCF-7 and MDA-MB231 breast cancer cell lines. 104 According to the SAR investigations, the hCA I and II inhibitory activities

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Scheme 57 Reaction representative of benzimidazole with different reagents.

were improved by moving the sulfamoyl group to the *para*-position. Compound 223, which has a hydrazide linker and an enaminone spacer, exhibited the highest hCA IX inhibitory activity.

The synthesis and cytotoxic potential of benzimidazole derivatives were examined by Atmaca et al. By first reacting the amino group of 224 with ethylbromo acetate to produce ethyl [5,6-dichloro-2-ethyl-1*H*-benzimidazol-1-yl]acetate (225), the intended powerful derivative 228 was synthesized. Subsequently, compound 226 was produced by reacting acetate derivative 225 with hydrazine hydrate in ethanol. Finally, the desired hybrid molecule 228 was produced by the successful condensation of the nucleophilic nitrogen with the substituted aromatic aldehyde 227 (Scheme 49). Compound 228 was found to exhibit a potential cytotoxic effect against the MCF-7 ( $IC_{50}$  =  $17.8 \pm 0.24 \ \mu g \ mL^{-1}$ ), DU-145 (IC<sub>50</sub> =  $10.2 \pm 1.4 \ \mu g \ mL^{-1}$ ), and H69AR ( $IC_{50} = 49.9 \pm 0.22 \, \mu g \, mL^{-1}$ ) cancer cell lines.<sup>105</sup> Compound 228 with a bromo substituent showed the strongest anticancer potential and may be a promising anticancer treatment drug according to the SAR investigations. Furthermore, it was noted that the presence of halogen atoms enhanced its oral absorption and increased its membrane permeability. It was discovered that bromination increased the cytotoxic potential, while also improving the stability and protein binding affinity.

The synthesis of benzimidazole derivatives was reported by Yadav *et al.* <sup>106</sup> By first esterifying mercaptan derivative **220** with ethyl chloroacetate **(229)** to produce acetate **230**, the desired derivative **232** was synthesized. After that, **230** was subjected to hydrazinolysis reaction with hydrazine hydrate to yield **231**. Lastly, the Schiff base reactionof acetohydrazide **231** with aromatic aldehyde gave **232** (Scheme 50). Yadav *et al.* <sup>106</sup> established a novel series of 1*H*-benzimidazole compounds and tested their anti-proliferative properties *in vitro*. When tested against the MCF-7 cell line, compound **232** (IC $_{50} = 0.0013 \, \mu M$ ) was found to be a more effective anti-cancer therapeutic candidate than 5-fluorouracil. According to the SAR investigations, the anti-tumor activity increased when an OH group was present on the phenyl ring and decreased when a di- or tri-substituent was present.

Using the well-known general techniques, compounds 238a-d were produced in accordance with the reaction sequence shown in Scheme 51. Thus, using established techniques, 2-acetyloxymethylbenzimidazole (233), which was produced by acetylating 2-hydroxymethylbenzimidazole, 107 was converted into *N*-benzylated derivatives 234 by treating it with substituted benzyl halides in the presence of a base. *N*-Benzyl-2-hydroxymethylbenzmidazoles 235 were produced by hydrolyzing intermediates 234 in aqueous alkali, and they then

**Key observations:** 

1) SAR studies revealed that a phenyl substitution on the 1*H*-benzimdazole increased the potency in both the ligand complexes and that ruthenium complexes 262 were more effective than their iridium counterparts that is, compound 263.

2) Methyl substitution262b produces no effect on potency compared to the unsubstituted compound 262a in ruthenium series but it loses some potency in iridium complexes 263 for most of cell lines.

Scheme 58 Generalized process to synthesize benzimidazole complex 262a and b and 263

reacted with thionyl chloride to form alkyl chlorides **236**. To obtain the target compounds **238a–d**, intermediate alkyl chlorides **236** were finally treated with the relevant phenylpiperazine derivatives **237**. Özdemir *et al.*<sup>108</sup> developed and produced a unique range of 1*H*-benzimidazole-piperazine hybrids, and then tested them against two human cancer cell lines (MCF-7 and A549) to determine whether they have antiproliferative properties. Compound **238b** demonstrated the most potent cytotoxicity (IC $_{50} = 11.0~\mu\text{M}$  against MCF-7 cells and 4.6  $\mu\text{M}$  against A549 cells). A mono-chloro substituent on the *N*-benzyl ring at the *ortho-* (**238a**) or *para-*positions (**238b**) increased the potency against the A549 cell line, according to the SAR evaluations. Furthermore, the activity was reduced when large groups

such as 3,4,5-trimethoxy and 2,4-dichloro (238c) were present on the *N*-benzyl ring (238d).

Scheme 52 showed the synthesis methods used for constructing the target thiazol-benzimidazole hybrids **241a** and **b**. Thiosemicarbazone **239** (ref. 109–113) with 2-bromo-1-(1-methyl-1H-benzo[d]imidazo-2-yl)ethan-1-one (**240**) in refluxing ethanol<sup>114,115</sup> afforded the corresponding targeted 2-(2-(substituted)hydrazinyl)-4-(1-methyl-1H-benzo [d]imidazol-2-yl) thiazole derivative<sup>116</sup> **241a** and **b**. Compound **241a** revealed strong cytotoxic activity by EGFR TK inhibition<sup>117</sup> (IC<sub>50</sub> = 109.71 nM)<sup>109</sup> as well as an anti-breast cancer agent. <sup>118,119</sup> SAR evaluations of this study revealed a notable decrease in potency when 4-nitrophenyl **241b** was substituted with cyclopentyl (as in

Key observations:

1) Complexes 265a-b showed good anticancer activity.

2) SAR studies revealed that the electron-donating ability of the methyl group in 265a enhances the stacking of the substituted

compound and that the higher binding affinity of complex 265b was due to the hydrophobic nature of methyl-substituted bipyridyl which increased intercalating of the complex.

Scheme 59 Reaction representative of benzimidazole with BIGH ligand.

compound 241a). Additionally, it was found that the efficacy of the tested drug was reduced when the polarity of the molecules decreased and the bulkiness of the substituent increased.

Subsequently, compound **245** was converted into the intended 2-amino-substituted analogues **246a** and **b** in the reaction with an excess of the appropriate amine microwave irradiation utilizing the previously disclosed optimized reaction conditions<sup>120,121</sup> (Scheme 53). Perin *et al.*<sup>122</sup> produced aminosubstituted *N*-methylated-benzimidazo[1,2-*a*]quinolines and tested them against human cancer cell lines *in vitro* to determine if they had any anti-proliferative properties. Two of the strongest substances, **246a** and **246b**, were specifically active against the HCT-116 cancer cell line, causing cell death and a reduction in the proportion of cells in the S phase (IC<sub>50</sub> values of 0.2 and 0.4  $\mu$ M, respectively). Between the acyclic derivatives, compound **246a** with the *N*,*N*-dimethylaminopropyl substituent demonstrated stronger efficacy against the MCF-7 and HCT-116 cell lines, according to the SAR evaluations.

Scheme 54 lists the synthetic pathways for the synthesis of 251. Substituted benzaldehyde 248 and acetophenone 247 were chosen and stirred in a pure form in 30% NaOH to produce chalcone 249. Intermediate compound 250 with compound 249 under reflux in absolute ethanol could be readily converted into the final compound 251. By causing cell cycle arrest in the G2/M phase and apoptosis by binding to active pockets of EGFR (IC $_{50} = 0.97~\mu\text{M}$ ), compound 251 demonstrated encouraging

growth inhibition on the A549 cell line ( $IC_{50}=2.2~\mu M$ ).<sup>124</sup> Additional research revealed that the effectiveness of compound **251** is attributed to its chloro atom at the 4-position of the phenyl substituent connected to C3 and C5 of the pyrazoline ring.

Therefore, the reaction between the pre-synthesized azide derivative 253 and propargyl molecule 252 (ref. 125 and 126) produced a structural alternative of 1,2,3-triazole-benzimidazole-chalcone hybrid 254 (Scheme 55) by using dichloromethane/water (1:1) as the solvent solution, catalyzed by CuSO<sub>4</sub>·5H<sub>2</sub>O and sodium ascorbate at room temperature. On all the chosen cancer cell lines, hybrid 254 with the chlorosubstituent (2-Cl) demonstrated the strongest cytotoxic effect.127 Compared to doxorubicin, the most active compound, 254, was almost fourfold less active in MDA-MB-231 cells, fortyeight times less active in T47-D cells, and fifteen-fold less active in PC-3 cells. The documented chloro-substituted benzimidazole-triazole hybrids were already demonstrated to have good cytotoxic effects against mouse embryonic fibroblast cell lines NIH/3T3 (IC<sub>50</sub> 1.63 µM) according to their structure-activityrelationship (SAR).128 Additionally, the SAR showed that the inclusion of chloro-substituents in the chalcone ring increased the cytotoxic effect of the hybrid 1H-benzimidazole derivative against the PC-3, MDA-MB-231, and T47-D cell lines. Furthermore, the antiproliferative properties of the compound were

#### **Key observations:**

- 1) SAR studies revealed that compound 268 which had methyl substituent at 2-position of 1*H*-benzimidazole ring exhibited better inhibitory activity than those which had it at 5- or 6-position of the ring.
- 2) It was also reported in previous year studies that chrysin derivatives with higher polarity exhibited more potent anticancer activities and that the OH group at position 5 was important.

Scheme 60 General synthesis of compound 268.

- 1) SAR studies revealed that the core structures of the 1*H*-benzimidazole and tetrazole are significant for increasing the neddylation inhibition activities of the derivatives, and changing the substitutions of heterocyclic cycloalyllic and benzoalylic groups decreases the aforementioned activity of the compounds.
- 2) The phenylethylamine led to the superior inhibition of derivatives, such as 272.

Scheme 61 Preparation of derivative 272.

**Key observations:** 

- 1) Compound 275b exhibits potent cytotoxic activity against the UACC-62 and breast cancer BT-549 cancer cells lines due to the impact of the lipophilic trifluoromethyl substitution on the biological activity profile. .
- 2) The well-known 4-methoxy structural setting on the pyrido[1,2-a]benzimidazole skeleton 275a was found to be showed different cytotoxic profiles.

Scheme 62 Synthesis of pyrido benzimidazole derivatives 275a and b

further strengthened by the attachment of a benzyl moiety to the triazole ring.

In the tetrafluoropyridine rings (256), the amines all reacted at the next most electrophilic site, adding to C-2 to create aminopyridine derivatives 257a-c (ref. 129) (Scheme 56), adding to C-2, forming aminopyridine derivatives 257a-c (Scheme 56).

Bhambra *et al.*<sup>45</sup> established a library of fluoroaryl benzimid-azole derivatives **257a–c**, which showed micromolar inhibition against the K-562 and MCF-7 cell lines. The product obtained by adding ethylenediamine **257c** was considerably less active against G361 and HOS, but exhibited good activity against two other cell lines. According to reports, compounds **257a** and **257c** 

activate caspases, which are crucial for the death of cancercausing cells.

An effective intermediary for the subsequent synthesis of various benzimidazole-heterocyclic compounds was thiosemicarbazone analogue 258. Thus, 2,5-dioxopyrrolidine derivative 259 was formed by the condensation of 258 with various acid anhydrides, such as succinic anhydride in acetic acid. Additionally, thiazolidinone derivative 260 was produced by cyclizing 258 with a haloketone, such as ethyl bromoacetate (Scheme 57). Abd El-Meguid et al. 130 established a novel series of 1H-benzimidazole compounds, which were evaluated against the HeLa cell line using doxorubicin as the standard medication. The compounds with the strongest anti-cancer activity against the HeLa cell line were 259 (1.62  $\pm$  0.16  $\mu M)$  and 260  $(1.44 \pm 0.06 \,\mu\text{M})$ . According to the SAR evaluations, the cytotoxic potential was enhanced by an increase in nitrogen atoms and presence of pyrrolidine or thiazole rings.

The generalized process illustrated in Scheme 58 was used to synthesize each of the ruthenium compounds. The analogue of ruthenium metal complex 262a was obtained by treating benzimidazole ligand 261 with para-cymene ruthenium(II) [(p-cymene)RuCl<sub>2</sub>]<sub>2</sub> and sodium acetate in dichloromethane for 24 h at room temperature. Pentamethylcyclopentadienyl chlorido

iridium(III) was the starting point for the preparation of halfsandwich iridium(III) complexes 263. Yellol et al. 131 established a variety of novel iridium(III) 263 and ruthenium(II) 262a and **b** C,N-cyclometalated benzimidazole complexes. The A2780 (ovary), 5637 (bladder), SISO (uterine cervical), and HT29 (rectal) human cancer cell lines were used to investigate their cytotoxicity. In the active hy926 (umbilical vein endothelial) human cell line, certain complexes were anti-angiogenic and triggered apoptosis by enhancing caspase-3 at a concentration of 0.5 µM. In contrast to their respective ligands, the metal complexes exhibited noticeably higher cell growth inhibitory rates. According to the SAR evaluations, ruthenium complex 262a was more potent than its iridium counterpart, compound 263, where a phenyl substitution on the 1H-benzimdazole enhanced the potency in both ligand complexes.

The ligand BIGH was produced using a well-known process<sup>132</sup> (Scheme 59). By using DOX according to the MTT assay, the produced complexes were tested for their pro-apoptotic and anticancer effectiveness against the normal HEK293 (embryonic kidney), HeLa (cervical), MCF-7 (breast), and A549 human cancer cell lines.133 The produced complexes exhibited a moderate level of activity against the A549, HeLa and MCF-7 cancer cell lines. All the evaluated cancer cell lines were successfully inhibited by

Scheme 63 Synthesis of benzimidazole analogous 281a and b.

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complexes 265a (IC<sub>50</sub> HeLa = 36.70  $\pm$  0.073  $\mu$ M; IC<sub>50</sub> MCF-7 =  $43.20 \pm 0.048~\mu\text{M}$ ; and  $IC_{50}~A\text{-}549 = 51.30 \pm 0.018~\mu\text{M})$  and **265b** (IC<sub>50</sub> HeLA =  $45.85 \pm 0.031$   $\mu$ M; IC<sub>50</sub> MCF-7 =  $51.30 \pm 0.083$   $\mu$ M; and  $IC_{50}$  A549 = 32.30  $\pm$  0.052  $\mu$ M), respectively. The SAR studies indicate that the hydrophobic nature of the methyl-substituted bipyridyl contributed to the higher binding affinity of complex 265b by increasing its intercalation, whereas the electrondonating capacity of the methyl group in complex 265a enhanced the stacking of the substituted molecule.

The synthesis and cytotoxic potential of chrysinbenzimidazole derivatives were examined by Wang et al. 134 By first reacting the hydroxy group of 266 with dibromo alkane to yield 267, the most powerful derivatives were synthesized. The essential hybrid molecule 268 was later produced by reacting bromide derivative 267 with benzimidazole in acetone (Scheme 60). Wang et al. 134 established a novel variety of chrysin-1H-benzimidazole compounds and tested them against the MFC cell line to see if they may be used as anticarcinogenic agents. Compound 268 was determined to be the most promising antiproliferative drug among the derivatives (IC<sub>50</sub> = 25.72  $\pm$  3.95  $\mu$ M), causing MFC cells to undergo apoptosis and inhibiting the progression of the cell cycle in the G0/G1 phase in a dose-dependent manner. According to the SAR evaluations, the compounds with a methyl substituent at the 1H-benzimidazole ring 2-position had enhanced inhibitory efficacy compared to those with that at the 5- or 6-position of the ring. Additionally, investigations from the previous year revealed that when the OH group was at position 5 and the chrysin derivatives had higher polarity, they showed stronger anti-cancer effects.

Derivative 272 was prepared according to the route shown in Scheme 61. Trifluoroacetic acid was hydrolyzed to get derivatives 272, and after candesartan and triphenylmethyl bromide reacted to form intermediate 270, which was subsequently condensed with different substituted amines. Compared to candesartan cilexetic, the optimal benzimidazole-derived 272 demonstrated enhanced NAE inhibition, namely CDC (IC50 = 5.51 μM vs. 16.43 μM), together with effective target inhibitory action and selective death of cancerous cells according to research. 135 In an enzyme assay, the optimal benzimidazolederived 272 showed better neddylation inhibition than CDC. It also showed promising target inhibitory action and cancer cell killing selectivity. The results of cellular mechanism investigations and tumor growth suppression in A549 human lung cancer cells in vivo suggest that 272 may be developed as a promising neddylation inhibitor for anticancer treatment. According to SAR investigations, the core structures of tetrazole and 1H-benzimidazole are significant for increasing the neddylation inhibitory activities of the derivatives, whereas changing the substitutions of the heterocyclic cycloalyllic and benzoalylic groups diminished the activity of the compounds.

The production and cytotoxic potential of pyrido[1,2-a] benzimidazole hybrids were examined by Samia et al. 136 The initial reaction of chloro derivative 273 resulted in the development of the most effective derivative that was investigated, 136 and dioxane with appropriate arylamines 274 produced the intended hybrid molecules 275a and b (Scheme 62). The anticancer activity results showed that compound 275b had the most significant inhibition, as indicated by the growth percentage (G%), against the breast cancer BT-549 (19.39%) and

**Key observations:** 

- 1) Of the compounds evaluated, 286b exhibited the most promising anticancer properties.
- 2) Presence of a N-substituted bulky group and a 5-membered ring at carbonyl group of amide side linkage (286a) suppressed the activity of the compound.

Scheme 64 Synthesis of fused benzimidazole-isoguinolinone scaffolds 286a and b.

SAR studies revealed that substitution of a branched aliphatic aldehyde moiety increased the anticancer potential (compound **287**).

Fig. 1 2-Mercapto-1*H*-benzimidazole nucleus with anticancer activity

melanoma UACC-62 (11.90%) cell lines. Among the compounds tested, compound 275b was found to have promising anticancer activity. This might be because of how the biological activity profile is affected by the lipophilic trifluoromethyl substitution. Compound 275a exhibits weak activity against the breast cancer BT-549 (87.45%) and melanoma UACC-62 (86.58%) cell lines.

Scheme 63 describes the synthetic procedures used to establish logically constructed 1-(9*H*-pyrido[3,4-*b*]indol-1-yl) (281a and b) derivatives that are connected to benzimidazole and benzoxazole. Pure 2-bromo-1-(9*H*-pyrido[3,4-*b*]indol-1-yl) ethanone (277) was obtained by reacting the starting compound 1-(9*H*-pyrido[3,4-*b*]indol-1-yl)ethanone (276) with *N*-bromo succinimide (NBS) in dry dichloromethane (DCM) solvent and stirring the reaction mixture for approximately 30 min at room temperature. 1-(2-Methylthiazol-4-yl)-9*H*-pyrido

[3,4-*b*]indole (278) was obtained by refluxing the resultant ethanone intermediate 277 with ethanethioamide in pure EtOH for approximately 12 h. The obtained thiazole 278 (ref. 137) was converted to pure 4-(9*H*-pyrido[3,4-*b*]indol-1-yl)thiazole-2-carbaldehyde (279) by oxidizing it with selenium dioxide in ethanol for 24 h at 100 °C. To produce their respective benzimidazoles/benzoxazoles as pure final compounds 281a and b, aldehyde 279 was finally allowed to react with different substituted 1,2-diamine 280 in the presence of diacetoxyiodobenzene (IBD) in dry 1,4-dioxane by stirring at room temperature for around 15 min. In the study conducted by Sireesha *et al.*, <sup>138</sup> a novel type of 1*H*-benzimidazole-linked  $\beta$ -carboline was produced, and its anticancer activity against a range of human cancer cell lines was evaluated. Compounds 281a (IC<sub>50</sub> = 0.092  $\pm$  0.001  $\mu$ M) and 281b (IC<sub>50</sub> = 0.81  $\pm$  0.062

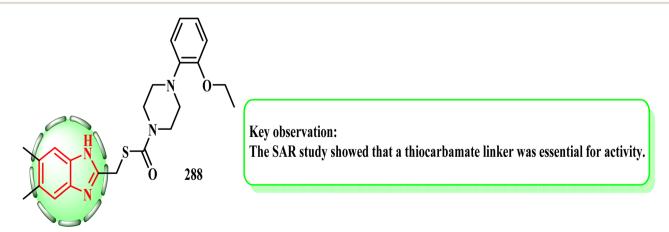


Fig. 2 Benzimidazole moiety with its antiproliferative activity.

The SAR study proved that the electron-donating group smoothed the insertion of the molecule **289** into DNA.

Fig. 3 IH-Benzimidazole scaffold with its in vitro studies.

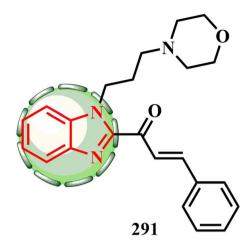
μM) had the greatest anticarcinogenic properties against the MCF-7 cell line, according to the findings. According to the SAR evaluations, compound **281a** was more active than the reference standard due to the presence of 4,5-dimethoxy substitutions (MCF-7 =  $0.092 \pm 0.001$  μM, A549 =  $0.72 \pm 0.042$  μM, Colo-205 =  $0.34 \pm 0.071$  μM, and A-2780 =  $1.23 \pm 0.55$  μM). Alternatively,

compound **281b** showed good action on four cancer cell lines despite having weak electron-donating 4,5-dimethyl substitutions on the benzimidazole moiety (A-2780 = 1.80  $\pm$  0.59  $\mu M$ , MCF-7 = 0.81  $\pm$  0.062  $\mu M$ , A549 = 1.90  $\pm$  0.88  $\mu M$ , and Colo-205 = 0.41  $\pm$  0.12  $\mu M$ ).

## **Key observation:**

SAR studies showed that benzimidazole derivatives containing a 1,3,4-oxadiazole ring (290) had greater anticancer activity than benzimidazoles with other heterocyclic rings.

Fig. 4 Hybrid 290 with its antiproliferative properties.

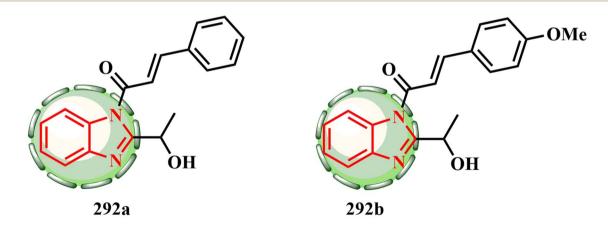


SAR studies showed that the *N*-substitution of benzimidazole with a hydrocarbon spacer linked to nitrogen (291) was essential for activities.

Fig. 5 Benzimidazole-chalcone hybrid 291 with its promising antiproliferative character.

**1.1.9. Four components.** Both the production and cytotoxic potential of fused benzimidazole–isoquinoline scaffolds were examined by He *et al.*<sup>139</sup> They used amine **284**, carboxylic acid **285**, methyl 2-formylbenzoate (**282**), and isonitrile **283** in methanol to perform a Ugi four-component reaction (U-4CR). This was followed by overnight stirring at normal room temperature and 15 min of microwave irradiation with 10% TFA/DCE at 150 °C (Scheme 64).<sup>139</sup> The findings indicated that

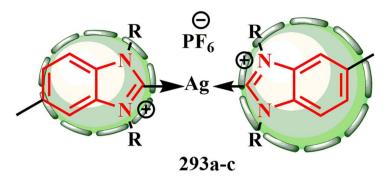
286b exhibited the most promising cytotoxic activity among the compounds ( $GI_{50} = 23.78 \mu M$  [SW620] and  $GI_{50} = 24.13 \mu M$  [HT29]), suppressing growth and causing cell cycle arrest at the G2/M checkpoint because of the weakened signaling *via* CDK1 and cyclin B1 protein and increased p21 and p53 action. <sup>139</sup> SAR evaluations revealed that the activity of this compound was inhibited by the presence of a 5-membered ring and an *N*-



# **Key observation:**

SAR studies have shown the importance of *p*-methoxy phenyl substituents and unsubstituted phenyl rings in chalcones for anticancer activity.

Fig. 6 Benzimidazole-chalcone hybrids 292a and b with their SAR.



R= Propyl, Butyl, Pentyl, respectively

An SAR study showed that the activity of these complexes was enhanced by increasing the length of the side chain, which in turn increased the lipophilicity of the benzimidazole ring.

Fig. 7 Benzimidazole complexes 293a-c with their SAR.

substituted bulky group at the carbonyl group of the amide side linkage (286a).

Tahlan *et al.*<sup>140</sup> employed ((1*H*-benzo[*d*]imidazol-2-ylthio) acetamido)benzo hydrazide to establish a novel azomethine of 2-mercapto-1*H*-benzimidazole. Using 5-fluorouracil as the standard medication, they were screened *in vitro* using the SRB assay against the HCT-116 (colorectal) human cancer cell line. Compound 287 was found to be the most potent of all the

synthesized derivatives and have strong anticancer activity (IC $_{50}$  = 30  $\mu g$  mL $^{-1}$ ). According to the SAR study findings, the anticancer potential was enhanced by substituting a branched aliphatic aldehyde moiety (compound 287) (Fig. 1).

However, compound 288 had strong antiproliferative activity against MCF-7 cells, with an  $IC_{50}$  of 5.58  $\mu g$  mL<sup>-1</sup>, which was similar to the  $IC_{50}$  of the common therapeutic doxorubicin, which has an  $IC_{50}$  of 4.1  $\mu g$  mL<sup>-1</sup>. The fundamental manner of



## **Key observation:**

Methyl ester is substituted with a hydroxyl group at C-2, whereas a fluoro group at the C5 position in the aryl ring (294) inhibits microtubule and is very active against breast cancer cells

Fig. 8 1H-Benzimidazole-5-carboxylate 294 with its activity on MCF-7 cell line.

action of compound 288 was to induce cell cycle arrest and apoptosis at the G2/M phase. A thiocarbamate linker was necessary for action, according to the SAR investigation, as shown in Fig. 2.<sup>141</sup>

Compound **289** (ref. 142) B-norcholesteryl benzimidazole demonstrated activity when tested against the HeLa, MCF-7, T-47D, and SKOV3 cell lines, with an IC $_{50}$  in the range of 7.90  $\mu$ M to 20.10  $\mu$ M. <sup>143,144</sup> The SAR<sup>145</sup> study demonstrated that the electron-donating group enhanced the insertion of this molecule into DNA<sup>142</sup> (Fig. 3).

Hybrid **290** (ref. 146) exhibited strong antiproliferative properties; its  $GI_{50}$  values against the SNB-75 and COLO 205 cell lines were 0.09  $\mu$ M and 0.35  $\mu$ M, respectively. Additionally, with a selectivity index of 3.66, it demonstrated moderate selectivity towards prostate cancer cell lines. According to SAR research, the benzimidazole derivatives with a 1,3,4-oxadiazole ring exhibited stronger anticancer properties than those with other heterocyclic rings<sup>147,148</sup> (Fig. 4).

Moreover, compound **291** (ref. 149 and 150) had IC<sub>50</sub> values of 8.91  $\mu$ M, 10.93  $\mu$ M, and 10.67  $\mu$ M against the MCF-7, HepG-2, and OVCAR-3 cell lines, respectively, indicating its promising antiproliferative action. Alternatively, cisplatin had IC<sub>50</sub> values of 11.70  $\mu$ M, 3.97  $\mu$ M, and 16.04  $\mu$ M, respectively. <sup>151</sup> According

to the SAR investigations, the activities were attributed to the *N*-substitution of benzimidazole with a nitrogen-linked hydrocarbon spacer<sup>149,152</sup> (Fig. 5).

Compounds **292a** and **b** had  $IC_{50}$  values in the range of 6.83  $\mu$ M to 18.16  $\mu$ M. Both substances exhibited strong affinity for the tyrosine kinase receptor. *P*-Methoxy phenyl substituents and unsubstituted phenyl rings are crucial for the anticancer action of the chalcones, according to the SAR studies<sup>153</sup> (Fig. 6).

When tested against the MDA-MB-231 and HCT-116 cell lines, complexes **293a–c** demonstrated greater anticancer activity than their respective benzimidazole ligands, with IC $_{50}$  values ranging from 4.22  $\mu$ M to 10.3  $\mu$ M. Additionally, their ligands showed reduced activity, with IC $_{50}$  values between 25.51  $\mu$ M and 34.21  $\mu$ M, which were similar to that of the reference pharmaceuticals 5-FU (IC $_{50}=5.5$   $\mu$ M against HCT-116 cells) and tamoxifen (IC $_{50}=8.20$   $\mu$ M against MDA-MB-231 cells). Increasing the side chain length improved the activity of the complexes, according to a SAR investigation, in which it additionally contributed to making the benzimidazole ring more lipophilic 154 (Fig. 7).

Methyl 2-(5-fluoro-2-hydroxyphenyl)-1*H*-benzimidazole-5-carboxylate (MBIC)<sup>155</sup> (**294**) with a methyl ester is substituted with a hydroxyl group at C-2, whereas a fluoro group at the C5

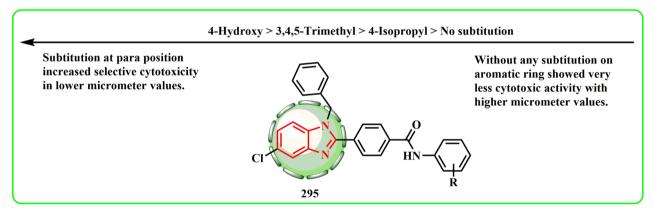


Fig. 9 Representative SAR studies of 1-benzyl-1*H*-benzimidazole analogues as anticancer agents.

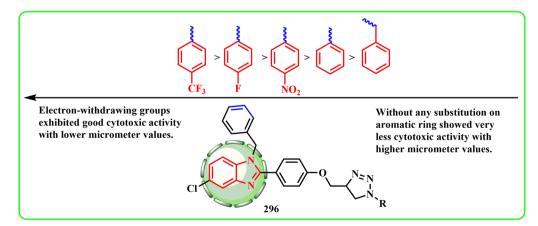


Fig. 10 Representative SAR studies of 1-benzyl-1H-benzimidazole-triazole analogues as anticancer agents.

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position in the aryl ring inhibits microtubule<sup>156</sup> and is very active against breast cancer cells<sup>157,158</sup> (Fig. 8).

With an IC $_{50}$  of 10.69  $\pm$  0.14  $\mu$ M, 13.89  $\pm$  0.74  $\mu$ M, 7.01  $\pm$  0.20  $\mu$ M, 14.04  $\pm$  0.62  $\mu$ M, and 12.91  $\pm$  0.52  $\mu$ M against the A-549, DU-145, MCF-7, MDA-MB-231, and HCT-116 cancer cell lines, the 4-hydroxy compound 295 showed a noticeable decrease in tumor cell proliferation (Fig. 9). The 4-hydroxy arylamide moiety 295 of the 1*H*-benzyl benzimidazole derivative had strong anticancer activity, according to the SAR analysis. MCF-7 development was significantly inhibited when aromatic or heterocyclic rings, such as pyrrole, 3-ethoxy-4-hydroxyphen, and 3,5-dimethoxy-4-hydroxyphen, were connected to benzyl benzimidazole scaffolds. 159

Further investigation of the distinct 1-benzyl-1*H*-benzimid-azole hybrids for selective gal-1 inhibition resulted in the development of a variety of novel 1-benzyl-1*H*-benzimidazole-triazole analogues due to the promoting results of 1-benzyl-1*H*-benzimidazole analogues as possible anticancer agents mediated by gal-1. The 1,3-dipolar cycloaddition of the benzimidazole

intermediate with a terminal alkyne group to various benzyl and phenyl azides in the presence of a copper(ı) catalyst produced 1-benzyl-1*H*-benzimidazole-triazole hybrids. 4-Triflourophenyl compound **296** showed no cytotoxicity against normal embryonic kidney cells, but it displayed cytotoxic function against MCF-7, NCI-H460, MDA-MB-231, A-549, and HaCaT cells with IC $_{50}$  values of 1.3  $\pm$  0.18  $\mu$ M, 0.99  $\pm$  0.01  $\mu$ M, 0.94  $\pm$  0.02  $\mu$ M, 0.63  $\pm$  0.21  $\mu$ M, and 2.99  $\pm$  0.09  $\mu$ M, respectively. The SAR investigations demonstrated that in comparison to electron-donating groups and in the absence of any substitution on the aromatic ring, electron-withdrawing groups on the aromatic ring have remarkably greater cytotoxicity (Fig. 10).  $^{160}$ 

Hagar *et al.*<sup>161</sup> benzimidazole-1,3,4-oxadiazole-chalcone hybrids were established and synthesized (Fig. 11), and their ability to inhibit EGFR for cell destruction was studied. According to cell line tests, compound **297**, which has a paramethoxyphenyl group at the second position of the benzimidazole ring, demonstrated potential potency in inhibiting apoptosis and EGFR inhibition.

Fig. 11 Benzimidazole-containing moiety exhibiting anticancer activity.

#### Key observation;

SAR showed that the replacement of *p*-fluorophenyl **298b** with *p*-chlorophenyl **298c** decreased anticancer activity.

Fig. 12 Indole-benzimidazole derivatives with anticancer properties.

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#### **Key observation;**

SAR, the fluorophenyl derivatives **299b-c** increased the inhibitory activity in comparison to trifluoromethylphenyl derivative **299a** 

Fig. 13 Benzimidazoldiphenyl-2-imino-thiazolidine-4-ol 299a-c derivatives with anticancer properties.

### **Key observation:**

SAR studies have shown the presence of the benzyl and electron donor groups on the compounds **300a-b** revealed a significant impact on Topo II inhibitory activity

Fig. 14 Benzimidazole-rhodanine conjugates 300a and b with anticancer properties.

The anticancer activities of indole–benzimidazole derivatives were reported.  $^{162}$  Among the evaluated compounds, **298a** (ref. 163) (R<sub>1</sub> = methyl and R<sub>2</sub> = Br) had the highest activity, with an IC<sub>50</sub> value of 28.73  $\mu$ M. As a result, compound **298a** may have anticancer properties together with potential modulatory effects on estrogen and the corresponding receptors. The p-fluorophenyl scaffold molecule **298b** exhibited less productive anticancer properties, according to the structural activity relationship (SAR). At this point, the anticancer activity was reduced when p-chlorophenyl **298c** was substituted for p-fluorophenyl (Fig. 12).

Under efficient metal-free conditions, benzimidazoldiphenyl-2-imino-thiazolidine-4-ol derivatives **299a–c** were synthesized. Doxorubicin was administered as a control for evaluating the targeted compounds against four distinct human

cancer cell lines, including breast, colon, prostate, and lung panels. In comparison to doxorubicin (IC<sub>50</sub> = 1.75  $\mu$ M), compounds **299a** (IC<sub>50</sub> = 3.89  $\mu$ M), **299b** (IC<sub>50</sub> = 2.80  $\mu$ M), and **299c** (IC<sub>50</sub> = 3.14  $\mu$ M) had the strongest inhibitory activity against the lung cancer cell line among the published compounds characterized for anticancer activity. Additionally, compared to trifluoromethylphenyl functionalities **299a**, the inhibitory effect was enhanced by the fluorophenyl functions **299b–c** (Fig. 13).

In another study, Li *et al.*<sup>167</sup> showed that a panel of benzimidazole–rhodamine conjugates have potent antiproliferative properties against human cervical, breast, lung, and prostate cancer cells, as well as human lymphoma and acute leukemia. As non-intercalative Topo II inhibitors, compounds **300a** and **b** bind to the Topo II enzyme ATP-binding site to inhibit enzymatic activity. The Topo II inhibitory action was significantly

influenced by the benzyl and electron donor groups in the compounds (Fig. 14).

### 2 Conclusion

The structural analogy of benzimidazole to nucleosides presents it as a potentially effective anticancer agent. The metal complexes or benzimidazole hybrids with antiproliferative properties were presented in this review. The examples included in this overview demonstrated the variety of synthetic preparation techniques used to achieve benzimidazoles and their antiproliferative properties. Additionally, we focused our attention to the SAR of the various molecular templates based on BZ that have been established by researchers globally. To accomplish our objectives, we collected information from an extensive range of publications to provide researchers, medicinal chemists, and drug designers with an excellent foundation for the development of the next generation of safe and effective BZ-based therapy. This review may shed light on the wide range of cancers that benzimidazoles can target, such as MCF-7, HepG2, MGC-803, HeLa, HCT-116, A-549, PC-3, MDA-MB-231, HUVEC, NIH/3T3, RMS, C-26, HT1080, LNCaP, 22Rv1, C4-2B prostate, DU-145, HEK293, MCF12A, H69AR, A2780, SISO, HT29, HCC2998, SF-539, UACC-62, and breast cancers BT-549, SW620, K-562, A37, SNB-75, COLO 205, and OVCAR-3. Their ability to disrupt important cellular processes such as Topo II-mediated DNA, VGEF, hCA IX, cell cycle progression, and mitosis has been demonstrated by investigations. This can lead to novel possibilities for the development of precision medicine-based benzimidazole anticancer drugs. Furthermore, this is the route forward to develop new medicinal compounds and benzimidazole anticancer drugs.

# Data availability

The article describes a study that did not utilize any data.

### **Author contributions**

Basant Farag, Sobhi M. Gomha, and Doaa A. Elsayed contributed to the conceptualization, supervision, and writing – review and editing. Basant Farag and Doaa A. Elsayed performed formal analysis and data curation. Magdi Zaki and Doaa A. Elsayed were responsible for investigation, methodology, and validation. Basant Farag and Doaa A. Elsayed led the writing – original draft preparation. All authors read and approved the final version of the manuscript.

### Conflicts of interest

The authors declare that they have no known financial conflicts of interest or personal relationships that could have influenced the work presented in this study.

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