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Repurposing anti-parasite benzimidazole drugs as selective anti-cancer chemotherapeutics

Fatemeh Movahedi [a], Li Li *[a], Zhi Ping Xu *[a,b]

[a] *Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, St Lucia, QLD 4072, Australia*
[b] *Institute of Biomedical Health Technology and Engineering and Institute of Systems and Physical Biology, Shenzhen Bay Laboratory, Shenzhen 518107, China*

ABSTRACT

Cancer chemotherapy is generally associated with many severe adverse effects. Many cancer studies are currently focused on repurposing conventional non-toxic anti-parasite drugs for cancer treatment. Since cancer cells and parasites have many features in common, some anti-parasite drugs such as benzimidazoles have been recently found to possess the anti-cancer activity. Benzimidazoles act against cancer by inhibiting tubulin polymerization, inducing cancer cell apoptosis, arresting cell cycle and over-generating reactive oxygen specimen. In this review, we summarize the anticancer features of these drugs in recent investigations, lead to reconsideration of benzimidazoles as a family of anti-cancer chemotherapeutics with non-toxicity or low toxicity to the normal cells and tissues. We particularly highlight the recent progresses using nanoformulations for enhanced cancer therapy and provide our prospects in the future research.

KEYWORDS

Anti-parasite; Benzimidazoles; Anti-cancer activity; Inhibition of tubulin polymerization; Selective chemoth erapeutics

*Corresponding authors: ZhiPing Xu, Li Li
E-mail addresses: gordonxu@uq.edu.au; l.li2@uq.edu.au

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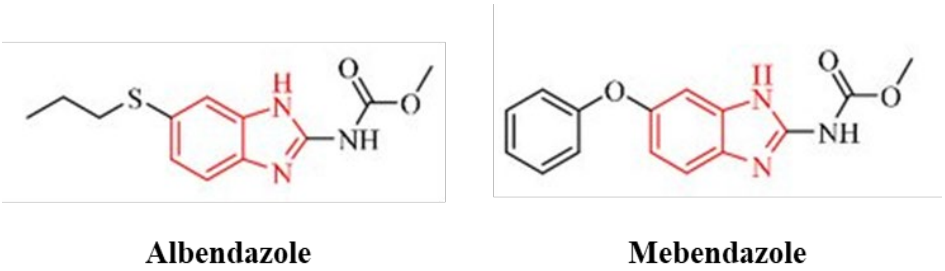
1. Introduction

Conventional chemotherapeutics are normally non-selective. These therapeutic agents not only kill cancer cells but also damage healthy tissues and cells, causing adverse effects [1] and influencing patients' quality of life [2]. For this reason, there is a great motivation to repurpose conventional anti-pathogen drugs with a long-term safety history for cancer chemotherapy. The benzimidazole family is one of such safe drugs.

Benzimidazoles are heterocyclic organic compounds, consisting of imidazole and benzene (as illustrated in **Figure 1**) with a high anti-helminth efficacy and a low level of toxicity to healthy cells [3, 4]. Apart from the high anti-helminth activity, benzimidazoles have been also found to possess anti-fungal [4], anti-bacterial [5], anti-viral [6] and anti-inflammatory activity [7]. Due to extensive similarities between parasites and cancer cells [8], benzimidazoles have also shown some anti-cancer activity, as confirmed in many studies [9]. Moreover, benzimidazole drugs are reported to enhance the efficacy in combination with other treatments such as conventional chemotherapy [10] and radiation therapy [11]. Interestingly, some benzimidazole drugs induce apoptosis of drug-resistant cancer cell lines [12] [13]. More importantly, benzimidazole drugs kill cancer cells in a selective way [14], i.e. *via* targeting tubulin polymerization dominantly in rapidly dividing cells such as parasites and cancer cells [15], leaving healthy cells being not much affected.



Albendazole **Mebendazole**

Figure 1. Chemical structure of benzimidazole drugs (albendazole and mebendazole) as heterocyclic organic compounds, consisting of imidazole and benzene, as well as other functional groups [126].

Although benzimidazole drugs have shown promise for cancer therapy, their low water solubility presents a significant challenge for their clinical application and therapeutic outcomes [16]. To overcome this limitation, nanoformulating benzimidazole drugs has emerged as a viable approach to increase their solubility and improve their efficacy in cancer therapy.

In this review, we aim to summarize the anti-parasite and anticancer features of benzimidazole drugs and their mechanisms of action. We further highlight the superior potentials of benzimidazoles such as safety and effectiveness on resistant cancer cells along with the limitation to their clinical applications, and then present the recent progresses using their nanoformulations for enhanced cancer therapy in combination with other drugs or treatment modes.

2. Anti-parasite performance of safe benzimidazole drugs

Benzimidazole drugs have widely been used as anti-helminth agents in both human and livestock since the 1960s [17]. These drugs have rapidly become more popular than previous medications due to superiority in terms of efficacy, toxicity and application [18]. For instance, benzimidazoles have high anti-parasitic effect on some protozoa, cestodes (tapeworms), trematodes (flukes) and nematodes (roundworms) [19], as summarized in **Table 1**. Interestingly, benzimidazole derivatives, triclabendazole and praziquantel, are considered as the main treatments for trematoda [20]. However, praziquantel had no efficacy while triclabendazole was highly effective for some

trematoda such as *fasciolias* [21].

Table 1. The antihelminth effect of benzimidazole drugs

Helminth type	Helminth sub-type	Benzimidazole drug	Outcome	Ref.
Protozoa	*Giardia lamblia*, *Trichomonas vaginalis* and microsporidia in AIDS patients	Fenbendazole, flubendazole, mebendazole	Half inhibitory concentration (IC_{50}) of 0.005 to 0.16 µg/ml	[26, 28]
	Tritrichomonas foetus	Albendazole, mebendazole	IC_{50} of 2.3-9.4 µM	[17]
	Giardia duodenalis	Albendazole, mebendazole and fenbendazole	IC_{50} of 0.19-0.3 µM	[27]
Cestodes	*Moniezia benedeni*	Fenbendazole	Effective in concentration of 1-15 µM	[23]
		Mebendazole	Diminished ATP synthesis after 30 minutes exposure	[24]
Trematoda	Lung flukes (*Paragonimus*)	Triclabendazole	100% cure rate with two doses of 50-75 mg/kg	[127]
	Opisthorchiasis	Mebendazole	100% egg reduction rate and 94% cure rate by 30 mg/kg/day	[128]
	Liver flukes (*Fasciola*)	Triclabendazole	100% egg reduction rate and 86% cure rate by 5 mg/kg	[129]
Nematodes	*Ascaridia galli*	Fenbendazole, parabendazole, mebendazole, oxfenbendazole, thiabendazole	IC50 of 4.5-8 µM	[25]
	Oesophagostomum dentatum	Fenbendazole	99.9% cure rate by 3×3 mg/kg fenbendazole	[130]
	Oesophagostomumquadrispinulatum		100% cure rate by 3×3 mg/kg fenbendazole	
	Ascaris sum		92.4% cure rate by 3×3 mg/kg fenbendazole	
	Trichuris suis		66% cure rate by 3×3 mg/kg fenbendazole	
	Hyostrongylus rubidus		99.9% cure rate by 3×3 mg/kg fenbendazole	

Benzimidazole drugs also have some activity against cestodes [22]. For example, fenbendazole proved efficacy for *Moniezia benedeni* cestode in the concentration of 1-15 μM [23] and mebendazole inhibited ATP synthesis in *Moniezia expansa* after 30 min exposure [24]. In general, many members of benzimidazole family drugs, including fenbendazole, parabendazole, mebendazole, oxfenbendazole and thiabendazole, have demonstrated effectiveness against nematodes with the IC_{50} value of 4.5-8 μM [25].

In addition to intestinal helminths, protozoa such as *iardia lamblia* , *Trichomonas vaginalis* and even microsporidia in AIDS patients, proved high susceptibility to benzimidazole derivatives such as fenbendazole, flubendazole and mebendazole, with the IC50 value being 0.005-0.16 μg/ml [26] (nearly 0.02-0.6 μM). Similarly, *Tritrichomonas foetus* protozoa were affected by albendazole and mebendazole with the IC_{50} of 2.3-9.4 μM [17]. Albendazole, mebendazole and fenbendazole demonstrated the highest activity against protozoa among benzimidazole drugs [27], being 30-50 times more effective on *iardia lamblia* than the non-benzimidazole drug, metronidazole [28].

Benzimidazole drugs are considered as non-toxic anti-helminth agents in human and livestock [29, 30]. Acute toxicities are rarely reported for these drugs [29, 31]. Neither chronic adverse effects in dogs and rats treated with very high dosages, nor irritation, carcinogenicity or teratogenicity in treated rats and rabbits have been observed [32]. The reported adverse effects mainly include abdominal pain or other gastrointestinal symptoms in 6-12% treated patients, headache in 2-3%, hair loss in 2%, and symptoms such as vertigo, thrombocytopenia, sleepiness, fever and fatigue in less than 1% treated patients [33, 34]. The safety of benzimidazole drugs mainly comes from the selective mode of actions on rapidly proliferating cells [35] through specifically targeting tubulin polymerization [36], which enables benzimidazole drugs to possess anti-cancer activity in a very safe way as well.

3. Common features of cancer cells and parasites suit actions of benzimidazole drugs

Parasites and cancer cells have many common features, as summarized in **Table 2**. In general, they are both resistant to apoptosis and capable of unlimited proliferation in human and livestock [37]. Both are capable of changing the expression of antigens exposed to the immune system of the host, masking the membrane components to survive in adverse conditions and secreting enzymes such as protease for the facilitated invasion to the host tissue [8]. Finally, both parasites and cancer cells are able to guide many innate immune cells such as monocytes to form a proper microenvironment, allowing them to survive and proliferate well, evading other tissues and escaping from the immune system [38].

Table 2. Common properties of cancer cell and parasite

Common features of parasites and cancer cells	Ref.
Resistance to apoptosis	[36]
The capability of unlimited proliferation in the host	[36]
Changing the expression of antigens exposed to the host immune system	[7]
Masking components on the cell membrane for survival in adverse conditions	[7]
Secretion of enzymes such as protease to facilitate the invasion	[7]
uiding innate immune cells such as monocytes to allow them to evade and escape from the immune system	[37]

Due to such similar functions, many anti-cancer drugs such as ivermectin [39], artemisinin [40] and histone deacetylasehave inhibitors [41] have been utilized for parasite treatment. On the other hand, some anti-parasite drugs such as niclosamide [42], mefloquine [43] and manzamine A [44] have been employed for cancer chemotherapy. Considering the safety and efficacy of benzimidazole drugs for parasite treatment for many years, these drugs are also good candidates for being repurposed as anticancer drugs.

The mechanisms of biological activity of benzimidazole drugs against parasites and cancer cells are schematically summarized in **Figure 2**, including two major mechanical actions. The first action is their anti-mitotic activity, stemming from the inhibition of tubulin polymerization through binding to tubulin sites of rapidly dividing cells [35]. Suppressing tubulin polymerization results in the disruption of mitotic spindle formation [45] and arrests cell cycle in the mitotic stage [46]. Thus, the disassembly of microtubules reduced the expression of vascular endothelial growth factor receptor-2 (VEFR2) [47] and the suppression of VEFR2 leads to the inhibition of angiogenesis, cell migration and invasion [48]. The alteration of the microtubule network has also affected the activity of hypoxia-inducible factor-1α protein [49] and shifted the balance of anti-apoptotic proteins such as Bcl-2 and apoptosis induction as a result [50].

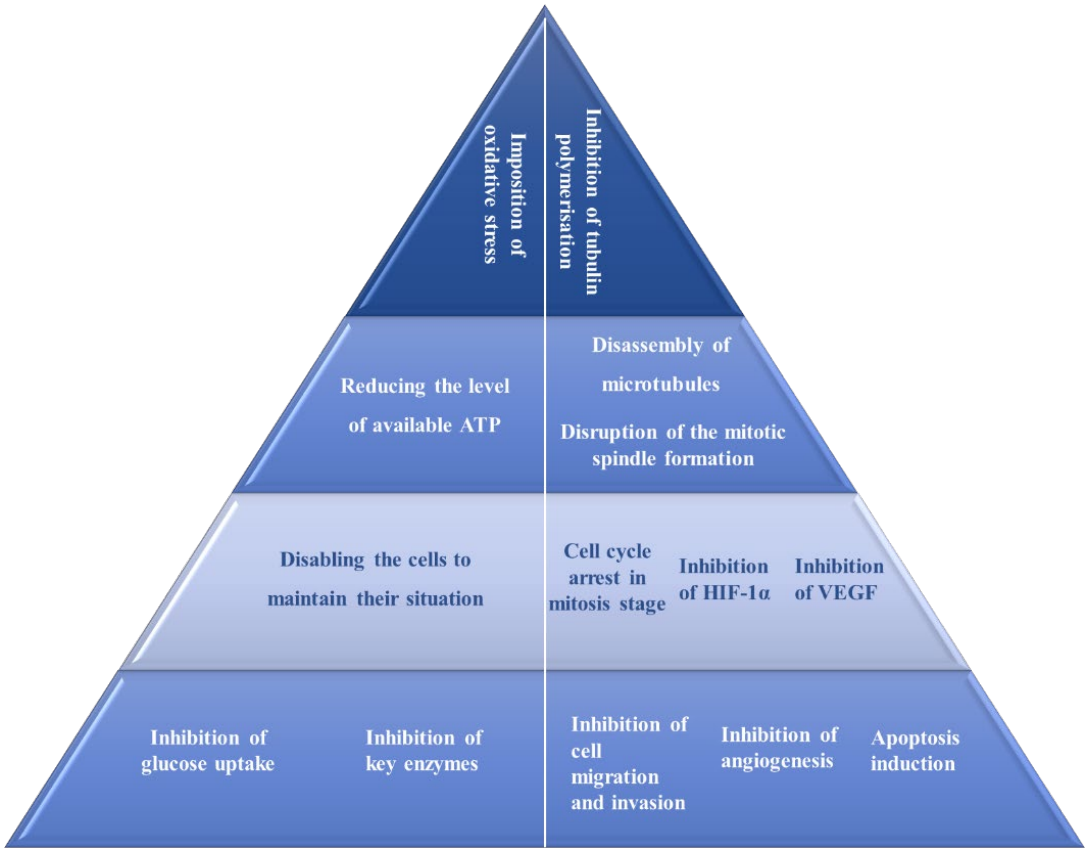


Figure 2. The schematic mechanisms of actions of benzimidazole drugs.

Secondly, benzimidazole drugs are also able to disrupt cell metabolic processes by inducing the oxidative stress [51] and reducing the level of available adenosine triphosphate (ATP) [52]. Such a metabolic disruption may result in the inhibition of glucose uptake [53] and activity of key enzymes such as cytoplasmic and mitochondria malate dehydrogenase [54], phosphoenolpyruvate [55], fumarate reductase [56], protein kinase [57] and cysteine protease [58].

Therefore, the specific mechanisms of action of benzimidazole drugs selectively induce apoptosis of rapidly proliferating parasites and cancer cells, which largely reduces the negative influences on the healthy cells. This

feature identifies these drugs as selective safe anti-cancer chemotherapeutics.

4. Selective anti-cancer effect of benzimidazole family drugs

As listed in Table 3, many benzimidazole family drugs such as albendazole, fenbendazole, mebendazole, flubendazole, pantoprazole, oxibendazole, oxfendazole, rabeprazole, MBIC and IODVA1, have shown anti-cancer effect on a wide range of cancer cells.

Table 3. A summary of reported anticancer activities of benzimidazole drugs

Cancer type	Drug	Cell line	Dosage	Outcome	Ref.
Breast cancer	IODVA1 (guanidino benzimidazole derivative)	MDA-MB-231	(IP) injection of 3.5 mg/kg -three times per week	Diminishing ≥ 50% of tumour volume-significant increase in apoptosis induction	[131]
	Flubendazole	MDA-MB-231, Hs578T BT-549 and 4T1-Luc	10 mg/kg/day, every other day	Anti-metastatic effects through STAT3 inhibition	[88]
	Albendazole	MCF-7	1-100 μM	Increasing oxidative biomarkers, SH depletion, triggering apoptosis, enhancing the expression of Bax/Bcl-xL, p53 and Bax	[59]
Hepatocellular carcinoma	MBIC	Hep2 and Huh7	Three times of 25 mg/kg intraperitoneal injections per week for 4 consecutive weeks	Apoptosis induction through the activation of caspase-3, reduced cell migration and invasion, ROS generation and activation of c-Jun-N-terminal kinase (JNK)	[64]
astric cancer	Pantoprazole	HC27	orally administered daily by gavage at a dose of 75 mg/kg-4 weeks	Repression of telomerase reverse transcriptase (TERT) expression, metastasis inhibition	[89]
	Rabeprazole	AS, KATO III, MKN-28 and MKN-45	-	Inhibiting the phosphorylation of ERK ½	[61]
	Mebendazole	ACP-02, ACP-03 and AP -01	0.15–20 μM	Significant reduction of invasion, migration and MMP-2 activity	[62]

Non-small cell lung cancer	Oxfendazole	A549, H460, H1299, H1650 and H1975	5-20 µM	Decreased expression of c-Src, upregulation of p53 and p21	[63]
	Mebendazole	H460, A549, H1299, and WI-38	1 mg oral	Induction of abnormal spindle formation, enhanced tubulin depolymerisation, strong antitumour effect, reducing lung colonies	[68]
Ovarian cancer	Albendazole	SKOV3 and OVCAR3	0.5-10 mg/kg	Suppression of tumour growth, ascite formation, VEF and SPARC expression	[71]
Prostate cancer	Oxibendazole	22Rv1 and PC-3	25 mg/kg/day	50% tumour size reduction, increased level of microRNA-204 and p53	[66]
Fibrosarcoma	Mebendazole	BHK-21/C13	460 mg/kg via gastric tube	Enhanced tumour necrosis, diminished vasculature penetration and tumour mitosis	[69]
colorectal cancer	Flubendazole	HCT116, RKO, SW480	10 mg/kg or 30 mg/kg by intraperitoneal (i.p.) injection every other day	Inhibition of STAT3 genes transcription, P-mTOR, P62, BCL2 and upregulation of LC3-I/II and Beclin1 genes	[64]
Neuroblastoma	Flubendazole	primary neuroblastoma cells	50-800 nM	P53-mediated apoptosis induction	[30]
Hepatocellular Carcinoma	Albendazole	Hep2, Hep3B and SKHEP-1	300 mg/kg per day for 20 days - Oral	Cell cycle arrest at 0 -1 or 2 -M depending on drug dosage, tumour growth suppression	[67]
Non-small cell lung cancer	Mebendazole	A549 and WI38	200-800 µg/day-Oral administration	75% tumour size reduction, reduced number and size of colonies in lung, reduced vessel densities	[70]
	Fenbendazole	A549	1 mg - Oral	Mitochondrial translocation of p53, inhibition of glucose uptake and glycolytic enzymes, tumour size reduction	[60]
Brain cancer	Mebendazole	L261	50-100 mg/kg - Oral	Significant enhancement of survival time even compared with vincristine	[132]

In a nutshell, these drugs effectively inhibit tumor proliferation and growth through (1) reducing SH [59], glycolytic enzymes, and glucose uptake [60]; (2) decreasing phosphorylation of ERK1/2 [61]; (3) mitigating the activity of matrix metalloproteinases 2 (MMP-2) [62] and expression of c-Src [63], P-mTOR, P62, Bcl-2 and STAT3 gene transcription [64]; (4) increasing the level of Bax/Bcl-xL [59], p21[63], p53 [59], caspase 3 [65], LC3-I/II and Beclin1 genes [64], microRNA-204 [66], oxidative biomarkers and ROS; (5) activating c-Jun-N-terminal kinase (JNK) [64]; and (6) arresting cell cycle in 0 -1 or 2 -M phase [67] and triggering apoptosis.

These benzimidazole drugs have also proved high efficacy in inducing abnormal spindle formation, increasing tubulin depolymerization [68], inhibiting vasculature penetration [69] and diminishing vessels in tumor tissues [70], suppressing VEF and SPARC expression [71], as well as decreasing colony [70] and ascites formation [71].

A recent study has examined the impact of a wide range of benzimidazole drugs, including flubendazole, parbendazole, oxibendazole, mebendazole, albendazole, and fenbendazole, on tumor cells derived from paraganglioma, pancreatic, and colorectal cancer [72]. Many of these drugs demonstrated IC50 values within the low micromolar or nanomolar range. *In silico* analysis indicated no interaction between those drugs and P-gp permeability glycoprotein that plays a pivotal role in drug efflux in tumors. The results also confirmed moderate to good oral bioavailability. Significantly, target prediction analysis of benzimidazole drugs revealed some cancer-related molecular targets for fenbendazole and mebendazole with high probability scores.

In efforts to achieve higher efficacy with low drug dosages, many new benzimidazole derivatives have been developed for the treatment of cancers such as colon cancer [73], breast cancer [74], lung cancer [75], chondrosarcoma [76] and leukemia [77]. Many new derivatives are screened and effective against a very broad range of cancers [78, 79]. Furthermore, new benzimidazole derivatives have demonstrated high capability for overcoming drug resistance. For instance, a benzimidazole derivative with a pyrrolidine chain significantly reduced the proliferation and migration of sorafenib-resistant hepatocellular carcinoma cells [80]. Similarly, benzoxazole-based zinc and copper complexes showed remarkably increased apoptosis induction in multidrug resistant L5178Y mouse T-lymphoma compared to non-complex ones [81].

Benzimidazole drugs' side effects are rare and mild. Recent studies have further confirmed the selective mode of actions of benzimidazole drugs against cancer cells. For instance, albendazole inhibited ovarian tumor growth but showed no toxicity to HOSE normal ovary cells [71]. Mebendazole did not show any toxicity to HUVEC cells while it was highly toxic to lung cancer cells [70]. Recently, our group has also shown that 4 benzimidazole drugs induced significant anti-cancer efficacy on B16F0 melanoma cells, but no obvious toxicity to HEK293T healthy cells [82]. Such a distinct mode of action comes from targeting tubulin polymerization overexpressed in cancer cells [83]. Furthermore, cancer cells are more susceptible to oxidative stress [84] and benzimidazole drugs are recognized as ROS generators [85, 86].

5. Inhibition of metastatic cancer cells, cancer stem cells, and drug-resistant cancer cells

Interestingly, benzimidazole drugs exhibit anti-metastatic effect through inhibiting cell migration and invasion [65]. As an example, mebendazole suppressed metastatic potential of anaplastic 8505c cells and prevented lung metastasis in advanced thyroid cancer mouse model [87]. Such inhibitions stems from reducing the activity of matrix metalloproteinases 2 (MMP-2) [62] and repression of signal transducer and activator of transcription 3 (STAT3) [88], which is considered as the key regulator of cancer metastasis by transducing the signals from cell surface receptors to the nucleus. Benzimidazole drugs also suppress telomerase reverse transcriptase (TERT) expression [89], whose activation is associated with metastasis [90].

It is well known that most of chemotherapeutics kill bulk cancer cells but not cancer stem cells, where cancer stem cells are responsible for tumor recurrence [91]. Benzimidazole drugs are potent in targeting cancer stem cells

and preventing tumor recurrence. For instance, mebendazole significantly suppressed tubulin polymerization in temozolomide-resistant stem-like glioblastoma cells [92]. It is also reported that mebendazole depleted triple-negative breast cancer stem cells [93].

Benzimidazole drugs are also found to prevent the radiation-induced transformation of cancer cells into radiation-resistant cells, and furthermore sensitize some drug-resistant cells. Many studies have reported the susceptibility of taxane-resistant cancer cells to benzimidazole drugs, especially albendazole [13, 94]. The superiority of albendazole to taxane drugs comes from targeting tubulin polymerization [95]. Albendazole also demonstrated higher efficacy in reducing the level of Bcl-2 antiapoptotic protein, whose expression is elevated in drug-resistant cells [94]. Flubendazole showed inhibitory activity on vinblastine-resistant leukemia cells in spite of glycoprotein overexpression [12]. Temozolomide-resistant glioblastoma cells also showed susceptibility to mebendazole [92]. Therefore, benzimidazole drugs own great potentials for the treatment of drug-resistant cancer cells. Note that benzimidazole treatment may also result in tubulin mutation and subsequent resistance [92].

Due to the successful outcomes in *in vitro* and *in vivo* studies, clinical trials are ongoing for cancer therapy with benzimidazole drugs. For example, clinical study of mebendazole as adjuvant treatment for colon cancer is in Phase 3 (NCT03925662) and mebendazole in combination with other antiprotozoal agents including albendazole for neoplasm therapy is in Phase 2 (NCT02366884). Three Phase 1 clinical trials are also ongoing for brain tumors (NCT02644291, NCT01729260, NCT0183787862). Letrozole and omeprazole are other benzimidazole drugs in Phase 2 clinical trials before the surgery treatment (to stop the growth of tumor cells and then surgery to remove the tumor) or adjuvant chemotherapy for breast cancer (NCT03774472 and NCT02595372).

6. Nanoformulation for enhanced anti-cancer effect

In spite of the broad spectrum of applications in cancer therapy, the low water solubility of benzimidazole drugs impedes their clinical applications and therapeutic outcomes [16]. Chemical modification of benzimidazoles generates many new derivatives with higher water solubility [16, 96]. More promisingly, nanoformulating benzimidazole drugs that are safely used for many years has emerged as a feasible approach to increase the bioavailability with extra advantages, including:

i. Extending the lifetime of benzimidazole drugs in blood circulation: Kupffer cells are specialized macrophages that play a crucial role in defending against foreign particles, bacteria, and other debris that enter the bloodstream [97]. However, the unique physicochemical properties of nanoparticles present a challenge to these cells due to their small size. Smaller nanoparticles, particularly those with a neutral or slightly negative charge, are less likely to be recognized and phagocytozed by Kupffer cells and cleared by the immune system [98], resulting in a longer circulation time in the bloodstream. This extended circulation time allows nanoparticles to accumulate at the tumor site with greater specificity and in higher quantity. This "stealth effect" of nanoparticles is dependent on several factors, such as their size [99], shape [100], and surface chemistry [101].

ii. Enhanced permeability and retention (EPR) at the tumor site: EPR is a phenomenon observed in solid tumors where nanoparticles can passively accumulate at the tumor site. The leaky blood vessels and impaired lymphatic drainage within the tumor microenvironment allow nanoparticles to accumulate

selectively at the tumor site [102]. This accumulation can increase the efficacy of anti-cancer drugs while reduce systemic exposure to healthy tissues, thus improving the therapeutic index of drugs [103].

iii. Potential stimuli-responsive release: Stimuli-responsive nanoparticles can be designed to release drugs in response to specific stimulators within the tumor microenvironment, allowing for the targeted delivery of drugs to cancer cells while minimizing the exposure of healthy tissues to high drug concentrations [104]. For instance, lipid-coated calcium phosphate (LCP) nanoparticles released albendazole at pH 6-6.5, which is supposed to be similar to that in the tumor microenvironment while keeping the cargo intact at pH 7.4 [105].

iv. Potential target delivery: Nanoparticles can have targeting capability by functionalizing their surface with specific molecules that can selectively bind to cancer cells. Nanoparticles can circulate until they encounter the target cancer cells, where they can bind to the receptors on the cell surface and enter the cells via endocytosis. This allows the nanoparticles to deliver their therapeutic payload directly to the cancer cells, while minimize exposure to healthy tissues and reduce systemic toxicity. Folic acid has widely been used as a targeting moiety for the targeted delivery of benzimidazole drugs to cancer cells due to overexpression of the folate receptors on cancer cells [106]. As an example, the use of folic acid-targeted chitosan nanoparticles for the delivery of mebendazole in the treatment of murine triple-negative breast cancer has been shown to be particularly effective by significantly reducing tumor size and extending the survival time of mice with triple-negative breast cancers [107]. Similarly, utilizing folate-conjugated bovine serum albumin (BSA) for co-delivery of albendazole and nanosilver simultaneously inhibited the energy metabolism of tumor cells, demonstrating superior anti-tumor efficacy compared to other nanoparticles lacking folic acid modification [108].

Numerous nanoformulations of benzimidazole drugs have been developed in light of the aforementioned benefits. Some of the formulations, such as liposome for fenbendazole [109] and compritol [110], silver nanoparticles [111] for mebendazole, graphite-diamond nanoparticles for thiabendazole [112], polybutycyanocrylate [113], solid lipid nanoparticles for albendazole [114], and methoxy polyethylene glycol-polycaprolactone nanoparticles for flubendazole [115], have already been developed for parasite therapy. Of course, these similar nanoformulations can also be explored for cancer therapy. An example is incorporating albendazole into chitosan-coated PLA nanoparticles (260 –480 nm), which elevated albendazole release to 200-fold compared to untreated albendazole, resulting in superior mucoadhesion and cytotoxicity [116]. Some nanoformulations of benzimidazole drugs have also been specifically studied for cancer therapy. As illustrated in **Figure 3**, albumin nanoparticles in the size of 7-10 nm were promising carriers of albendazole, reducing tumor size at a very low drug dosage while 200 nm albumin nanoparticles were less effective [117]. In addition to confirming improved anti-cancer efficacy of albendazole in nanoparticle forms, these results confirm the importance of the nanoparticle size to the treatment efficiency. The uptake of nanoparticles plays a pivotal role to increasing the treatment efficiency and smaller nanoparticles are more likely to be taken up by tumor cells [117].

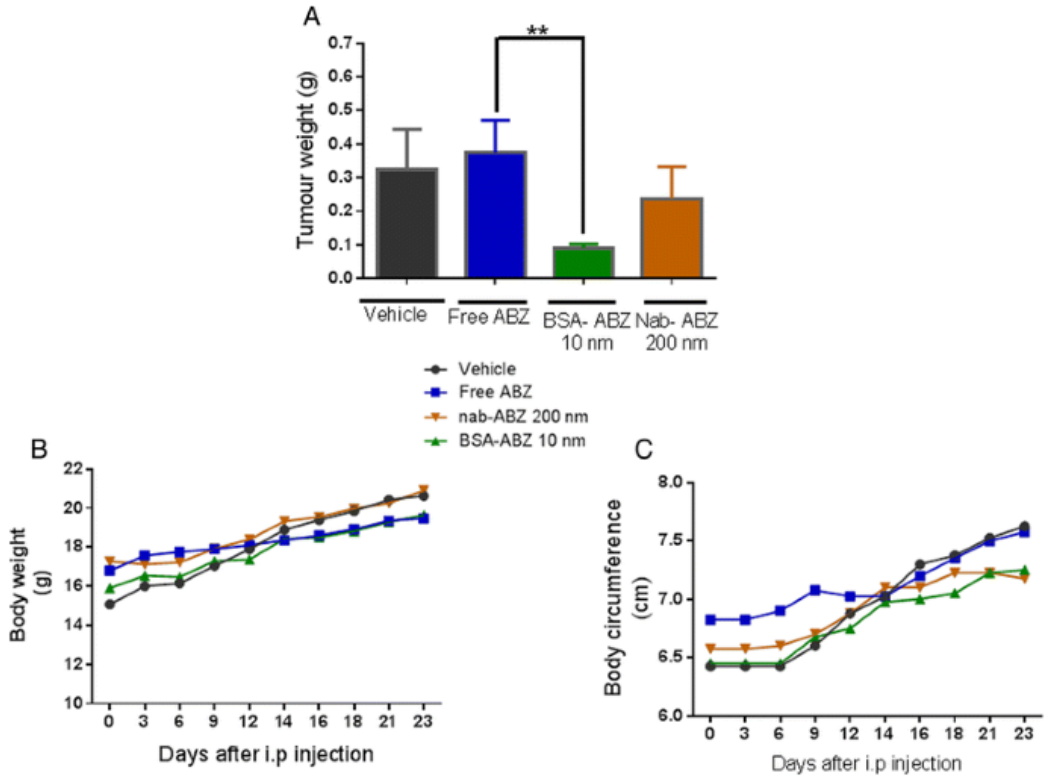


Figure 3. The average tumor weight **(A)**, average body weight **(B)**, and body circumference **(C)** in OVCAR3-bearing mice treated with free albendazole or albumin nanoparticle formulation of albendazole in 10 or 200 nm nanoparticles [117].

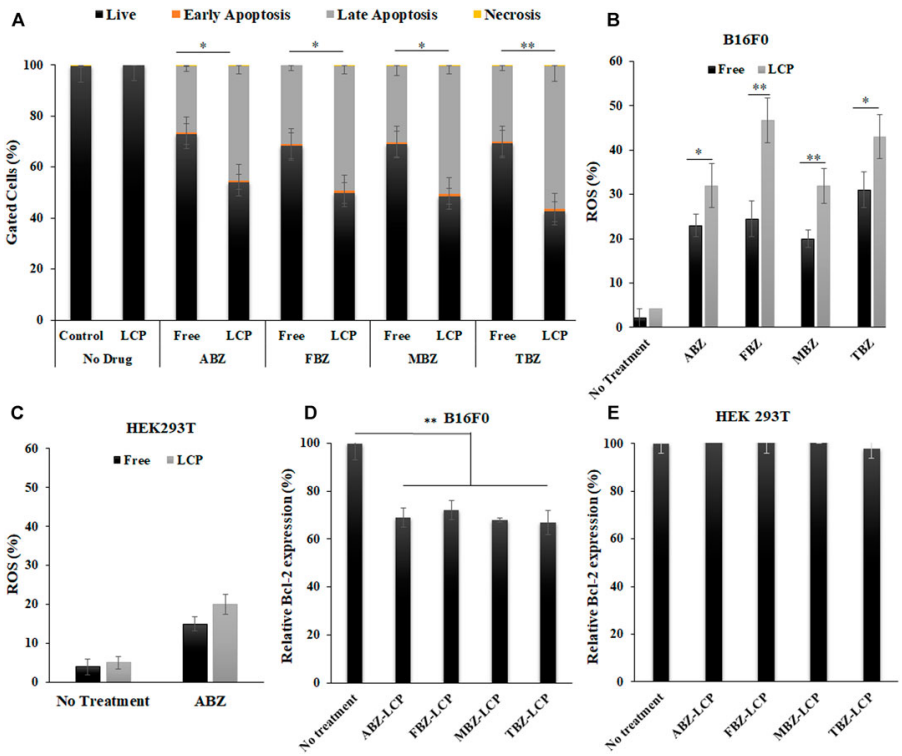


Figure 4. Apoptosis induction in B16F0 cells **(A)**, ROS generation in B16F0 cells **(B)**, ROS generation in HEK293T cells **(C)**, relative Bcl-2 expression in B16F0 cells **(D)**, and relative Bcl-2 expression in HEK293T cells **(E)** treated with free or LCP formulation of benzimidazole drugs (2.5 µg/ml) for 24 h [82].

Recently, our group formulated benzimidazole drugs into 50 nm LCP nanoparticles. These drug-loaded LCP nanoparticles increased the solubility in PBS by 100-200% and significantly enhanced the apoptosis-induced anti-cancer efficacy in the treatment of B16F0 melanoma cells via generating more reactive oxygen species (ROS) and inhibiting Bcl-2 expression, as demonstrated in Figure 4. Very obviously, these drug-loaded LCP nanoparticles did not show any obvious toxicity and Bcl-2 inhibition in HEK293T healthy cells [82, 105].

7. Combination therapy with benzimidazole drugs

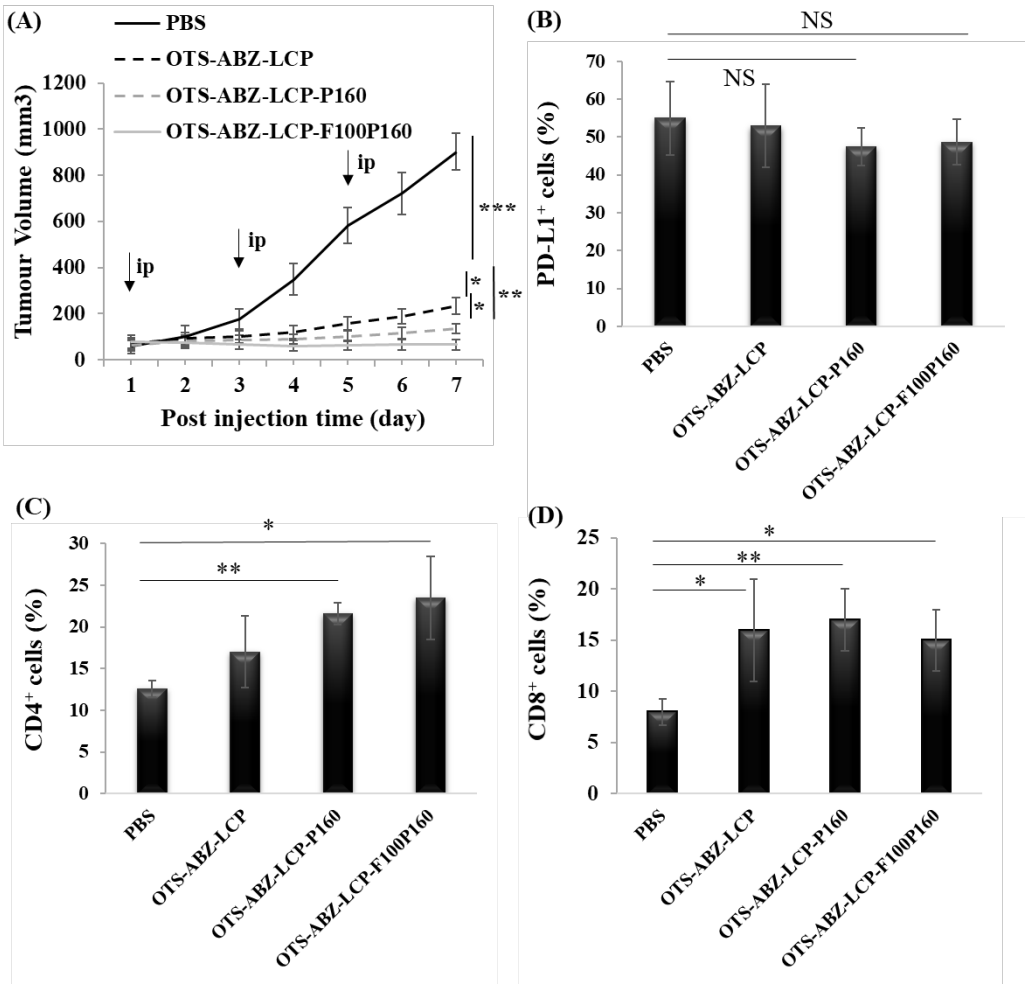


Figure 5. Improved chemotherapeutic outcomes by dual ligand targeting. **(A)** Tumor growth profile reflecting *in vivo* anti-tumor efficacy of OTS-ABZ-LCP, OTS-ABZ-LCP-P160 and OTS-ABZ-LCP-F100P160 intraperitoneally injected 3 times every two days in the B16F0-bearing mouse model compared to that of PBS injection; **(B)** PD-L1 expression by excised tumour cells; **(C)** $CD4^+$ and **(D)** $CD8^+$ immune cell populations in tumor tissues [131].

More frequently, benzimidazole drugs are combined with other chemotherapeutics, such as paclitaxel [10], trametinib [118], gemcitabine [119] and methoxyestradiol [120], to enhance the anti-cancer treatment efficacy. Flubendazole and albendazole at the low dosage were found to significantly potentiate the anti-proliferative effect of paclitaxel on colon cancers [10]. 2-Methoxyestradiol was the other microtubule-binding agent, exhibiting synergistic anti-cancer effect in combination with albendazole [120]. Mebendazole in combination with the methyl ethyl ketone (MEK) inhibitor, trametinib, demonstrated encouraging results for melanoma treatment by rapidly phosphorylating MEK and extracellular signal-regulated kinases (ERKs), and increasing apoptosis markers such as cleaved caspase-3 and poly (ADP-ribose) polymerase (PARP) [118]. The therapeutic effects of gemcitabine was also

promoted in combination with mebendazole [119] and parbendazole [121]. Interestingly, fenbendazole showed excellent anti-tumourigenic effect in combination with supplementary vitamins [122]. In addition to vitamins, fenbendazole in combination with rapamycin demonstrated synergistic anti-cancer effect against A549 cancer cells [123]. We also developed lipid-coated calcium phosphate (LCP) nanoparticles combining albendazole (ABZ) and a TOPK inhibitor, OTS964 for synergistic treatment of skin cancer. The dual-targeting capacity of the LCP nanoparticles to the programmed death ligand-1 (PD-L1) and folate receptor overexpressed on the surface of skin cancer cells enabled the combination therapy to completely suppress the skin tumour growth (**Figure 5A and B**) [124]. Furthermore, such combination treatment induced a certain level of local anti-cancer immunity by recruiting more CD4+ and CD8+ T cells into the tumor tissues (**Figure 5C and D**).

Moreover, benzimidazole drugs have also sensitized tumor cells to radiation therapy [93]. For example, as shown in **Figure 6**, mebendazole enhanced survival time and reduced colony formation of malignant meningioma [11], and also prevented radiation-induced conversion of triple-negative cancer cells into drug-resistant breast cancer-initiating cells and elevated the sensitivity of cancer cells to radiation [93]. Similarly, albendazole sensitized small cell lung cancer and metastatic melanoma cells to radiation therapy [125]. Albendazole not only induced DNA damage in those cells, but also arrested cell cycle at 2/M phase where cells are more sensitive to ionizing radiation. Therefore, combination of this antihelminthic drug with radiation therapy led to a synergistic cancer inhibition.

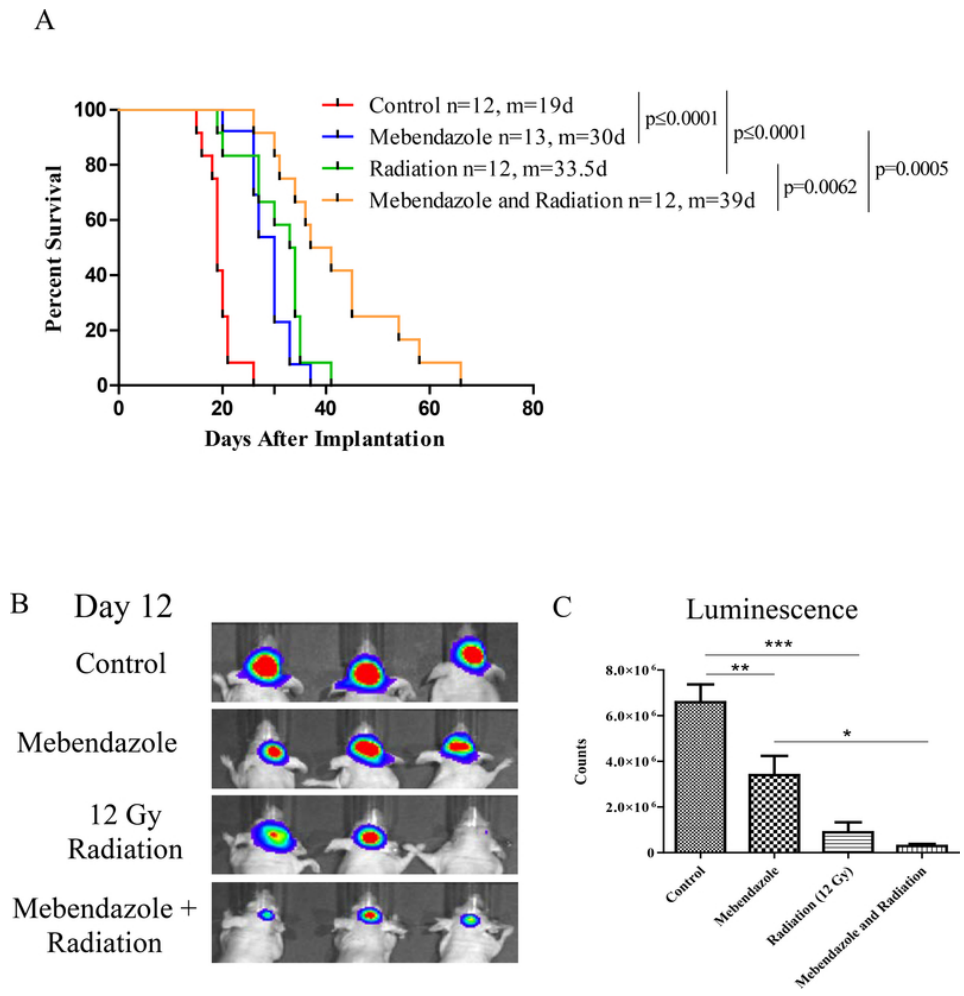


Figure 6. Increased survival rate **(A)**, reduced tumour size obtained by IVIS imaging **(B)**, and quantified average luminescence by IVIS **(C)** with the combination of mebendazole and radiation in KT21M1 -bearing mice [11].

8. Conclusion and future directions

Initially emerged as anti-helminth drugs, benzimidazole drugs have recently been repurposed as anti-cancer agents largely due to their safety in the long term applications. Benzimidazoles prevent cancer cell growth by inhibiting tubulin polymerization and disrupting mitotic spindles, leading to cell cycle arrest and inhibition of angiogenic factors such as HIF-1α and VEF. Moreover, these agents disable cell maintenance by elevating ROS generation and reducing ATP, thus suppressing many key enzymes and inducing apoptosis. A higher level of tubulin polymerization in rapidly dividing cells leads to a selective toxic effect of benzimidazoles on cancerous cells, leaving healthy cells intact. The higher sensitivity of cancer cells to ROS augmentation is the other factor for the selective activity of benzimidazoles. Such a selective effect, along with high efficacy in cancer treatment, represents benzimidazoles as a promising anti-cancer chemotherapeutics with minimum adverse side effects.

Benzimidazoles are also able to synergize other therapeutic approaches such as chemotherapy and radiation therapy. Some members of this family have also shown anti-cancer effect on drug-resistant cells and even cancer stem cells. Benzimidazoles not only enhance the efficacy of radiation therapy but also prevent cells from transformation to be resistant to the radiation treatment. On the other hand, benzimidazole treatment may cause tubulin mutation, and thus the combination with other therapeutics may be considered as the better choice.

Despite the extensive potentials of benzimidazole drugs for cancer treatment, their clinical application is still limited due to low water solubility and bioavailability. Nanoformulation of these drugs is promising for improving the solubility and bioavailability in addition to providing the opportunity of enhancing their circulation and tumor accumulation, targeted delivery and stimuli-responsive release. Until now, there are few studies that have investigated nanoformulations of these drugs for cancer therapy. Therefore, nanoformulation of benzimidazoles represents a promising future direction as it is still at the infant stage of research. Moreover, combination with other therapeutic modules, such as chemotherapeutics, radiation sensitizers, immune checkpoint inhibitors as well as target therapy in nanoformulations would synergize the therapeutic outcomes and bloom the repurpose applications of benzimidazole drugs in the fight with cancers in the near future.

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Conflict of interest

All the authors claim that the manuscript is completely original. The authors also declare no conflict of interest.

Author contributions

Conceptualization: Li Li, ZhiPing Xu; Investigation: Fatemeh Movahedi; Writing–original draft: Fatemeh Movahedi; Writing–review & editing: Li Li, ZhiPing Xu.

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