Development of Benzimidazole Compounds for Cancer Therapy

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Abstract

A fact that is largely unknown in the lay press and even the scientific community is that today cancer kills more people worldwide than tuberculosis (TB), malaria, and human immunodeficiency virus (HIV) combined. Benzimidazole is a heterocyclic aromatic organic compound considered to be a useful pharmacophore in a variety of impactful drugs. The purpose of this review is to highlight the benzimidazole-containing agents that are currently in clinical use or in clinical development as anticancer drugs. It is hoped that this review would function as comprehensive working reference of research accomplishment in the field of discovery and development of benzimidazole-based anticancer drugs.

Keywords: benzimidazole derivatives, privileged pharmacophore, anticancer drugs/agents

1. Introduction

Benzimidazole (**1**) (**Figure 1**) is used as the major scaffold or as a moiety on other scaffolds for the development of a variety of drugs [1–4]. The wide range of pharmacological activities of benzimidazole-containing agents are attributed to the unique fused benzene and imidazole rings, which can interact in a noncovalent manner with a range of biological targets due to the presence of an electron-rich aromatic system and the two hetero-nitrogen atoms [5, 6]. Because of the ability of benzimidazole derivative to interact with a variety of unrelated molecular targets, the term "privileged substructure/moiety" is ascribed to this unique azole agent. It is believed that the interest in benzimidazole chemistry and as a scaffold/moiety in the discovery and development of drugs arose from the discovery of the rare and most prominent benzimidazole compound in nature, *N*-ribosyl-dimethylbenzimidazole (**2**) (**Figure 1**), which serves as an axial ligand for cobalt in vitamin B12 [7].

Although several benzimidazole derivatives have been approved for clinical use, including antiparasitic, antiulcer, antihypertensive, antihistaminic, and antiemetic drugs [1–4], only one anticancer drug, bendamustine (**3**) (**Figure 2**), has received FDA approval [8–10]. Two prominent benzimidazole agents, selumetinib (**4**) (**Figure 2**) [1, 6] and galeterone (**5**) (**Figure 2**) [11], that advanced to phase III clinical trials, but are yet to be approved as anticancer drugs, will also be discussed.
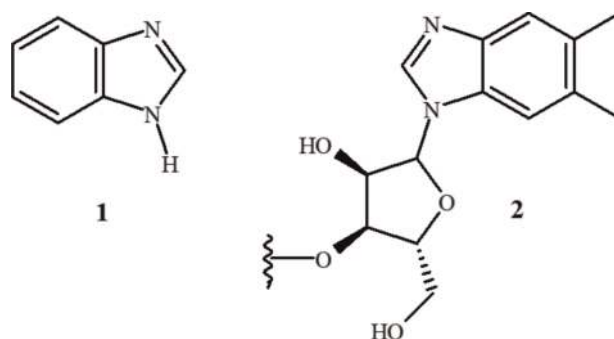
Figure 1.
*Chemical structures of benzimidazole (1) and N-ribosyl-dimethylbenzimidazole (2).*
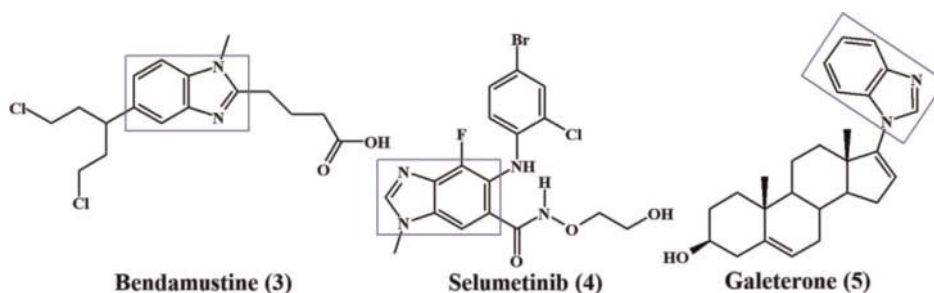
Figure 2.
Chemical structures of bendamustine (3), selumetinib (4), and galeterone (5).

2. Benzimidazole agents in the clinic and in clinical development

2.1 Bendamustine (3)

Bendamustine (**3**) (**Figure 2**) was discovered in a structure-activity relationship (SAR) campaign directed to obtain more effective and safer water-soluble analogs of chlorambucil (**6**) (**Figure 3**), a nitrogen mustard, which is used clinically against chronic lymphatic leukemia, lymphomas, and advanced ovarian and breast carcinomas [12]. The strategy was replacement of the benzene ring in compound **6** with purine-like *N*-methylbenzimidazole moiety in the hope of obtaining an anticancer agent with antimetabolite and DNA-alkylating activities. Although bendamustine was first synthesized in the early 1960s [13], it was approved under the trade name Treanda® by the US Food and Drug Administration (FDA) in 2008 for the treatment of chronic lymphocytic leukemia, multiple myeloma, and non-Hodgkin's lymphoma [10, 14–16].
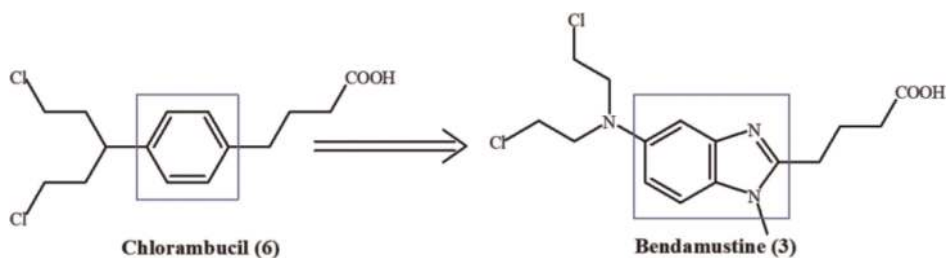
Figure 3.
Replacement of aromatic benzene ring of chlorambucil (6) to produce bendamustine (3).

2.1.1 Chemistry

Bendamustine (**3**) 4-{5-[bis(2-chloroethyl)amino]-1-methylbenzimidazol-2-yl} butanoic acid was first synthesized via eight synthetic steps with an overall yield of 12% [13, 17]. However, Chen and colleagues have developed a new, more efficient, and cost-effective route focused on the use of sustainable chemistry for the synthesis of bendamustine hydrochloride, with the overall yield improved from 12 to 45% as outlined in **Figure 4** [18]. This new synthesis is currently used for the commercial production of **3**.

2.1.2 Summary of bendamustine's preclinical and clinical pharmacology

Even though bendamustine is an alkylating agent, due to its ability to cause intra-strand and inter-strand cross-links between DNA bases, it has been reported that the DNA breaks induced by bendamustine are more extensive/durable than those induced by other alkylating agents, such as chlorambucil, cyclophosphamide, or carmustine [19–21]. In addition, the drug was shown to exhibit partial cross-resistance to other alkylating agents. These data suggested that bendamustine may possess additional mechanisms of action. Indeed, a comprehensive study by Leoni and colleagues clearly demonstrated that bendamustine exhibits a distinct pattern of activities unrelated to other alkylating drugs. Using a variety of lymphoid cancer cell lines, the study concluded that mechanisms of action include induction of mitotic catastrophe, inhibition of mitotic checkpoints, and activation of DNA-damage stress response and apoptosis. Compared to other alkylating agents, bendamustine was shown to activate the base excision DNA repair pathway rather than the alkyl transferase DNA repair mechanism [20].

Although bendamustine is approved for the treatment of a variety of lymphoid cancers, its activity has also been reported in several cancers, including cancers of small cell lung, breast, hepatic, bile duct, and head and neck. The studies by Chow and colleagues using leukemic cell lines in vitro or ex vivo cells from patients with
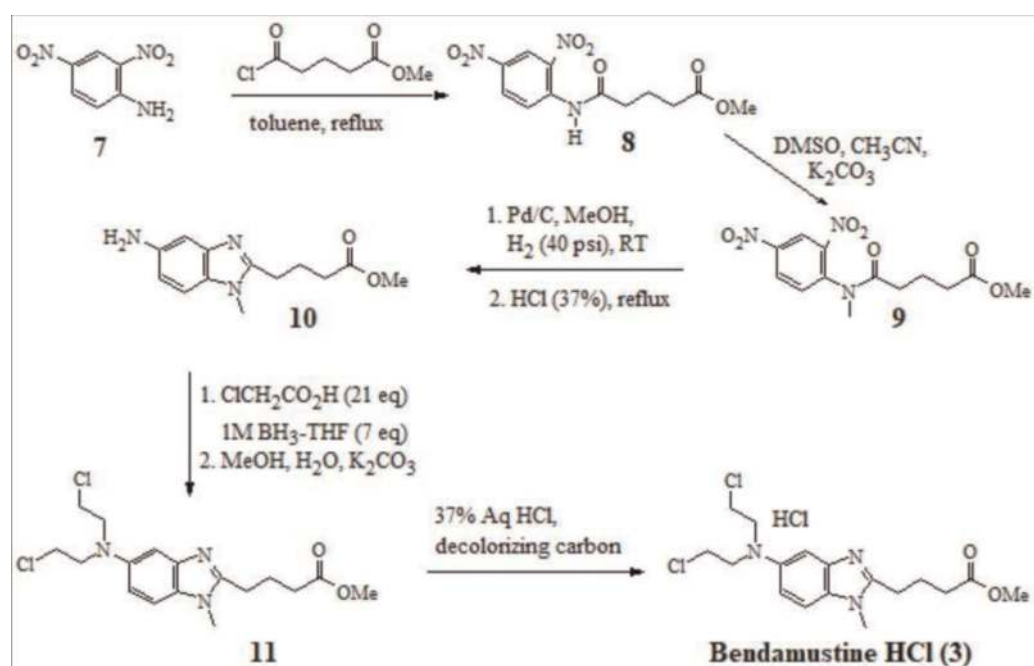
Figure 4.
New optimized synthesis of bendamustine hydrochloride (3).

leukemic progression to clarify interactions between bendamustine and other chemotherapeutic drugs unraveled synergy with cladribine, in contrast to observed antagonism with mitoxantrone or doxorubicin. The observation of synergism between bendamustine and rituximab (an anti-CD20 antibody) in in vitro CD20-positive DOHH-2 and WSU-NHL cell lines and ex vivo B-cell chronic lymphocytic leukemia (CLL) cells [22] and in mice with Daudi xenografts [23] provided the impetus for clinical trials combining these two drugs [24, 25].

Based on the discussion above, it is obvious that bendamustine is an "old drug rediscovered." For over 30 years, bendamustine was used in Eastern Germany as monotherapy for several cancers, including breast cancer, chronic lymphocytic leukemia (CLL), Hodgkin's lymphoma, non-Hodgkin's lymphoma (NHL), and multiple myeloma (MM) [26–37]. However, following the reunification of Germany, other countries initiated clinical trials of bendamustine as a single agent and in combination with other drugs. Bendamustine has achieved worldwide regulatory approval and is a standard-of-care drug for the treatment on many lymphoid malignancies. Several articles that provide comprehensive reviews of the discovery and development of this unique drug are available [8–10].

2.2 Selumetinib (4)

Selumetinib (4) (AZD6244: ARRY-142866) is an orally available, potent, selective inhibitor of mitogen-activated protein kinase (MAPK)/extracellular signal-related kinase (ERK) kinases 1 and 2 (MEK1 and MEK2) [6]. This agent has been extensively studied in many preclinical and clinical studies in several tumor types with mixed results. Here, we will summarize the chemistry, preclinical studies, and clinical studies.

2.2.1 Chemistry

Selumetinib (6-(4-bromo-2-chloroanilino)-7-fluoro-N-(2-hydroxyethoxy)-3-methyl benzimidazole-5-carboxamide) (4) is a diarylamine hydroxamide, containing mono-methylated benzimidazole subunit [38, 39]. It is a second-generation, orally active small molecule that acts as a selective and ATP-uncompetitive inhibitor of MEK1 and MEK2, binding to the allosteric binding site [38, 39]. The synthesis of selumetinib is yet to be reported in the literature.

2.2.2 Summary of selumetinib's preclinical and clinical pharmacology

Selumetinib inhibits the enzymatic activity of purified constitutively active MEK1 with a half maximal inhibitory concentration (IC_{50}) of 14 nM and was shown to be highly selective for inhibition of these targets compared to other related kinases [39]. Using several human cancer cell lines such as NSCLC, melanoma, and pancreatic and colorectal cell lines, it was shown that selumetinib was a potent antiproliferative agent. Analysis of the data revealed that cell lines with mutant BRAF and RAS were sensitive to selumetinib [40]. Selumetinib had little effect on the growth of Malme-3, the control cell line to the melanoma. Additional studies suggested that the growth inhibitory effects of selumetinib was not due to wide-ranging cytotoxicity [39], and it was also established that selumetinib effectively inhibits the phosphorylation of ERK 1 and ERK 2, which are substrates of MEK1 and MEK2 in the MAP kinase pathway. This mechanism of action was confirmed in tumor xenografts. Additionally, increased markers of apoptosis such as cleaved caspase 3 and decreased cell proliferation were seen in response to treatment with selumetinib in the xenograft models [39, 40].

The promising preclinical in vitro and in vivo data provided the rationale for multiple clinical trials in cancers with activated Raf-MEK-ERK signaling. In preparation for clinical evaluation of selumetinib, it was originally developed as a free base and administered as a liquid suspension, but subsequently a capsule formulation of the hydrogen sulfate salt was found to be more suitable for further development [38]. Several phase I and II clinical trials conducted against solid tumors to test the impact of selumetinib as a monotherapy were unsuccessful [41–44]. This led to the conduct of several clinical trials with selumetinib in combination with other cancer drugs. A notable trial was the randomized phase II study of selumetinib in combination with docetaxel, as a second-line treatment for patients with KRAS-mutant advanced NSCLC which showed very promising results [45]. The median progression-free survival was 5.3 months with selumetinib + docetaxel and 2.1 months with docetaxel alone. The objective response rate was 37% for selumetinib + docetaxel vs. 0% for docetaxel alone ($p < 0.001$), and the median overall survival was 9.4 months for selumetinib + docetaxel vs. 5.2 months for docetaxel alone (HR for death, 0.80 [80% CI, 0.56–1.14]; one-sided $p = 0.21$). Unfortunately, in a multinational 510 randomized patients with previously treated advanced KRAS-mutant NSCLC trial, the combination of selumetinib with docetaxel did not improve progression-free survival compared with docetaxel alone [46]. Clearly, addition clinical studies are required to realize the potential impact of selumetinib alone and in combination with other drugs for the treatment of a variety of cancers [47, 48].

2.3 Galeterone (5)

Galeterone (also called VN/124-1 or TOK-001) is an orally available anticancer agent. It was rationally designed as an inhibitor of androgen biosynthesis via inhibition of 17α-hydroxylase/17,20-lyase (CYP17), the key enzyme which catalyzes the biosynthesis of androgens from the progestins. Through extensive and rigorous preclinical studies, galeterone was shown to modulate two other targets in the androgen/androgen receptor (AR) signaling pathway [11] and shown to inhibit the eukaryotic initiation factor 4E (eIF4E) protein translational machinery [49]. Galeterone advanced successfully through phases I and II clinical trials in prostate cancer patients but was unsuccessful in the pivotal phase III clinical trial in men with castration-resistant prostate cancer (CRPC), harboring AR splice variants (e.g., AR-V7). We present a summary of the chemistry, preclinical studies, and clinical studies [50].

2.3.1 Chemistry

Galeterone, 3β-hydroxy-17-(1H-benzimidazole-1-yl)androsta-5,16-diene (**5**), is one of a series of novel Δ^{16}-17-azolyl steroid, which, unlike previously known 17-heteroaryl steroids, the azole moiety is attached to the steroid nucleus at C-17 via a nitrogen of the azole. The synthesis of galeterone from commercially available 3β-acetoxyandrosta-5-en-17-one (**12**) is presented in **Figure 5** [11, 51], and a facile and large-scale preparation (commercial process) of the compound has been developed but is yet to appear in the literature.

2.3.2 Summary of galeterone's preclinical and clinical pharmacology

Using intact CYP17 expressing *Escherichia coli*, galeterone was shown to be a potent inhibitor of the enzyme with an IC_{50} value of 300 nM and was shown to be more potent than abiraterone (IC_{50} value of 800 nM) [52]. Additional studies by
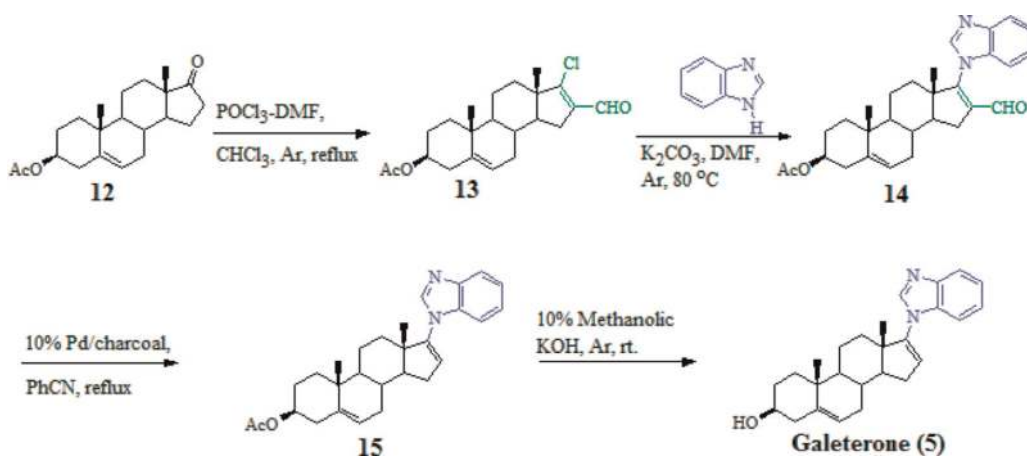
Figure 5.
Synthesis of galeterone.

our group revealed that galeterone could disrupt androgen signaling through multiple targets [51–54].

We strongly believe that the increased efficacy of galeterone in several prostate cancer models both in vitro and in vivo is due to its ability to downregulate the AR and block androgen binding to AR. Using well-established AR-competitive binding assays (against the synthetic androgen [^{3}H]R1881), galeterone was equipotent to Casodex in LNCaP cells but had a slightly higher affinity for the wild-type receptor in PC3-AR cells. In transcriptional activation assays (utilizing a luciferase reporter), galeterone was shown to be a pure AR antagonist of the wild-type AR and the T877A mutation found in LNCaP cells [53]. In prostate cancer cell lines, galeterone inhibited the growth of CRPCs, which had increased AR and were no longer sensitive to Casodex [53] and was also shown to inhibit the growth of AR-negative prostate cancer cells [54]. In addition, galeterone demonstrated superior synergy for growth inhibition in combination with everolimus or gefitinib compared with Casodex [55].

Recent in vitro studies have shown additional activities of galeterone, including proteasomal degradation of AR and its splice variants [56, 57] and inhibition of the eukaryotic initiation factor 4E (eIF4E) protein translational machinery via induction of proteasomal degradation of mitogen-activated protein kinase-interacting kinases 1 and 2 (Mnk1 and Mnk2) [58, 59].

Because of the short half-life ($t_{1/2}$ = ∼40 min) in mice, galeterone was administered twice daily in our antitumor efficacy studies. Galeterone (0.13 mmol/kg twice daily) caused a 93.8% reduction (p = 0.00065) in the mean final LAPC-4 xenograft volume compared with controls, and this efficacy was significantly more effective than castration or our most potent CYP17 inhibitor, VN/85-1 [51]. In another antitumor efficacy study, treatment of galeterone (0.13 mmol twice daily) was very effective in preventing the formation of LAPC4 tumors (6.94 vs. 2410.28 mm^3 in the control group). Galeterone (0.13 mmol/kg twice daily) and VN/124-1 (0.13 mmol/kg twice daily) + castration induced *regression* of LAPC4 tumor xenografts by 26.55 and 60.67%, respectively [53]. Using castration-resistant prostate cancer (CRPC) HP-LNCaP tumor xenografts, we showed that galeterone + everolimus (m-TORC1 inhibitor) acted in concert to reduce tumor growth [60]. Utilizing the androgen-dependent LAPC-4 prostate cancer xenograft model, we have shown galeterone is more efficacious than the blockbuster prostate cancer drug abiraterone (Zytiga®) [61]. We also reported that galeterone potently inhibits the growth of CRPC CWR22Rv1 tumor xenografts [56].

Based on these impressive preclinical data, galeterone was licensed by the University of Maryland, Baltimore, to Tokai Pharmaceuticals, Inc., who initiated Androgen Receptor Modulation Optimized for Response 1 (ARMOR1) phase 1/ phase 2 trials in castrate-resistant prostate cancer patients on November 5, 2009 [11]. The ARMOR phase I and phase II studies conducted with galeterone demonstrated that galeterone is well tolerated with promising clinical activity in patients with CRCP [62, 63]. To determine whether galeterone has clinical activity in patients with C-terminal loss of the androgen receptor, circulating tumor cells were retrospectively tested for C-terminal loss. Of the seven patients identified, six had PSA50 responses. These promising phases I and II studies enabled the selection of galeterone 2500 mg/day dose for the pivotal phase III trial (ARMOR3-SV, NCT02438007). The retrospective data of patients with C-terminal loss of the androgen receptor supported the design of ARMOR3-SV pivotal trial in which patients with AR-V7 were randomized to receive either galeterone or enzalutamide. Regrettably, the trial was discontinued following review by the independent Data Monitoring Committee, though no safety concerns were cited regarding this recommendation [50]. Gratifyingly, Educational and Scientific LLC (ESL), Baltimore, announced (December 17, 2018) that the University of Maryland, Baltimore (UMB), has granted ESL an exclusive license for the development of galeterone for the treatment of patients with CRPC. We eagerly await the initiation of a new phase III clinical study of galeterone in men with prostate cancer.

3. Concluding remarks

Despite the enormous literature on the synthesis and preclinical evaluation of numerous benzimidazole-containing compounds, it is unclear why very few of this class of compounds have entered clinical trials for evaluation as potential anticancer drugs. Given the fact that many benzimidazole-containing drugs have achieved blockbuster status for other diseases, it may be reasonable to suggest that the researchers interested in the development of benzimidazole-contained anticancer drugs should carefully study the process that have resulted in successful non-cancer benzimidazole drugs. We hope that this review will stimulate research activities that would eventually produce new anticancer benzimidazole drugs.

Acknowledgements

Conflict of interest

VCON is the lead inventor of galeterone; the patents and technologies thereof are owned by the University of Maryland, Baltimore. The other authors declare no potential conflict of interest.

Note added in proof

During the review of this manuscript, it was reported that an analog of Selumetinib called Binimetinib (Mektovi) in combination with a BRAF inhibitor

(Encorafenib, Braftovi) was approved by US Food and Drug Administration (FDA) for the treatment of unresectable or metastatic melanoma with BRAF mutations [64].

List of abbreviations

AME	apparent mineralocorticoid excess
AR	androgen receptor
ARMOR	androgen receptor modulation optimized for response
AR-V7	a type of androgen receptor splice variant
BRAF	a human gene that encodes a protein called B-Raf
CI	confidence interval
CLL	chronic lymphocytic leukemia
CRPC	castration-resistant prostate cancer
CWR22Rv1	a type of AR/AR-splice variants positive human prostate cancer cell line
CYP17	cytochrome P450 17α-hydroxylase/17,20-lyase
DNA	deoxyribonucleic acid
eIF4E	eukaryotic initiation factor 4E
ERK1 and ERK2	extracellular signal-regulated kinases 1 and 2
ESL	Educational and Scientific LLC
FDA	Food and Drug Administration
HIV	human immunodeficiency virus
HR	hazard ratio
IC_{50}	is the concentration of inhibitor required to inhibit the enzyme activity by 50%
KRAS	Kirsten retrovirus-associated DNA sequences
LAPC-4	a type of AR-positive human prostate cancer cell line
LNCaP	a type of AR-positive human prostate cancer cell line
MAPK	mitogen-activated protein kinase
MEK1 and MEK2	MAPK/ERK kinase
MM	multiple myeloma
Mnk1 and Mnk2	mitogen-activated protein kinase-interacting kinases 1 and 2
NHL	non-Hodgkin's lymphoma
NSCLC	non-small cell lung carcinoma
PC3-AR	a type of AR-negative prostate cancer cell line transfected with AR
PSA	prostate-specific antigen
RAF	rapidly accelerated fibrosarcoma
RAS	retrovirus-associated DNA sequences
SAR	structure-activity relationship
TB	tuberculosis
TOK-001	another code name of galeterone
UMB	University of Maryland, Baltimore
VN/85-1	code name for a CYP17 inhibitor/AR antagonist/AR degrader
VN/124-1	original code name of galeterone

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