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Dolastatins and their analogues present a compelling landscape of potential natural and synthetic anticancer drug candidates

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ABSTRACT

Human cancer remains a leading cause of global mortality. Traditional treatment methods, while effective are often associated with substantial side effects, high technical requirements, and considerable expenses. Recently, anticancer peptides, such as dolastatin-type peptides naturally found in marine mollusc *Dolabella auricularia*, have gained attention due to their enhanced characteristics and specific targeting of cancer cells with minimal toxicity to normal cells. This review aims to provide a comprehensive summary of the anticancer activities of natural dolastatins and synthetic analogues over the past 35 years, focusing on their utilization in advancing cancer treatment strategies. This updated review encompasses a detailed analysis of numerous studies demonstrating the cytotoxic effects of dolastatins and their synthetic analogues on various human tumour cell lines. The analysis includes investigations into their ability to activate apoptosis pathways, inhibit cell cycle progression, and indirectly limit inflammation and angiogenesis in tumours. Both natural dolastatins and synthetic analogues have demonstrated significant anticancer properties through a variety of mechanisms *in vitro* and *in vivo* pharmacological studies. Some have even advanced to clinical trials, either alone or in combination with other agents,

Abbreviations: **ΔΨm**, mitochondrial membrane potential; **μg**, microgram; **μM**, Micromole; **A549**, human non-small cell lung cancer; **A-549**, adenocarcinoma human alveolar basal epithelial cells; **Ahp**, 3-amino-6-hydroxy-2-piperidone; **AKT**, Protein kinase B; **Ala**, Alanine; **Auc**, The area under the curve; **B16**, Murine melanoma cell line; **BAD**, BCL2 associated agonist of cell death; **Bcl2**, B -cell lymphoma 2; **Chk2**, Checkpoint kinase 2; **COLO 205**, colon carcinoma cell line; **Dap**, (2R,3R,4S)-dolaproine; **Dil**, (3R,4S,5S)-dolaisoleucine; **DL**, Dolastatin; **DLD-1**, colorectal adenocarcinoma cell lin; **DLT**, Dose -limiting toxicty; **DU1452, PC3**, human prostate cancer cell line; **Egr-1**, Early growth response protein 1; **G2**, Gap 2 phase; **GSk-3β**, Glycogen synthase kinase-3 beta; **h**, hours; **H460**, large cell lung cancer xenograft tumour model; **HCT116, KM20L2, LoVo**, Human colon carcinoma cell line; **HeLa**, Human cervix carcinoma cell line; **HeLa S3**, A clonal derivative of the HeLa cell line; **HIV**, human immunodeficiency virus; **HL-60**, human myeloid leukaemia cell line; **HT-29, WiDr**, human colorectal adenocarcinoma cell line; **HUVEC**, human umbilical vein endothelial cells; **Ibu**, (S)-4-amino-2,2-dimethyl-3-oxopentanoic acid; **IC_{50}**, half maximal inhibitory concentration; **IL-1β**, Interleukin-1 beta; **IM9, RPMI8226, U266**, Human multiple myeloma cell line; **KB**, human epithelial carcinoma cell line; **L1210**, mouse lymphocytic leukaemia cell line; **LX-1**, Human lung carcinoma cell line; **M**, Mitosis phase; **m2**, body surface area-based; **Map**, (2S,3R)-2-methyl-3-aminopentanoic acid; **MCF-7, R-27**, human breast cancer cell line; **ME180**, human cervical cancer cell line; **MEL-28, SK-Mel5**, human melanoma cell line; **min**, minutes; **MKN45**, human gastric cancer cell line; **mL**, milliliter; **MTD**, maximum tolerated dose; **MX-1**, Human breast adenocarcinoma cell line; **NCI-H40X**, Human large lung carcinoma cell line; **ng**, nanogram; **nM**, nanomolar; **P388**, Mouse lymphoma cell Line, murine leukaemia cells; **PI3K**, phosphatidylinositol 3-kinase; **PTEN**, Phosphatase and tensin homolog on chromosome 10; **ROS**, Radical oxygen species; **SBC-3**, Human small cell lung cancer cell line; **SCID**, Sever combined immnuodeficiency; **SCLC**, Small Cell Lung Cancer; **SF-295**, Human glioblastoma cell line; **SKOV-3**, Human ovarian cancer xenograft; **VEGF**, Vascular Endothelial Growth Factor; **WHCO1, WHCO6**, oesophageal cancer cell line.

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and have shown promising outcomes. The biological activities of dolastatins and their synthetic analogues offer a promising path in the development of more effective and sustainable anticancer drugs. Their specific action on cancer cells and relative non-toxicity to normal cells highlight their potential as superior cancer therapeutic agents. The current study provides a platform for the most recent preclinical and clinical research on dolastatins and their analogues. Further research into these marine peptides may contribute to the development of sustainable and efficient treatment models for cancer, filling a significant gap in the current cancer therapeutic portfolio.

Introduction

The human genome is equipped with several fences to prevent the formation of tumourous growth or cancer (Ginsburg et al., 2021). The establishment of a tumour microenvironment that may withstand a localised antitumour immune response is another characteristic of tumour progression; these tumour microenvironments maintain unique circumstances that facilitate the induction, progression, and migration of cancerous cells (Maimela et al., 2019). For the treatment of cancer, several methods involving surgery, immunotherapy, hormone therapy, radiology, chemotherapy and even injections of different kinds of ribonucleic acid (RNA) molecules have been used (Chabner and Longo, 2006, Bringmann et al., 2010, Drăgănescu and Carmocan, 2017). Unfortunately, the majority of these available therapies are most often incapable of eliminating all cancer cells, and they may reappear within a short time. Among various treatment modalities, chemotherapy remains a commonly utilized therapeutic approach. However, it is important to note that its efficacy can be limited in advanced stages of cancer, such as those complicated by metastasis, where resistance to chemotherapy is often observed (Farghadani and Naidu, 2022, Zlatian et al., 2015).

Natural compounds have played a crucial role in the field of chemotherapy against cancer for a long time (Castañeda et al., 2022). Increasing chemoresistance in cancer cells is also a significant cause for worry (Abdullah and Chow, 2013); therefore, the developing world anticipates innovative natural therapeutic agents with highly selective cytotoxic capabilities against cancer cells. In the realm of oncology, natural compounds have made a significant impact, serving as the basis for a variety of clinically approved drugs. For instance, paclitaxel and docetaxel, originally isolated from the bark of the Pacific yew tree, are widely used in treating ovarian, breast, and lung cancers(Škubník et al., 2021). Vincristine and vinblastine, both derived from the Madagascar periwinkle, are integral in leukaemia and lymphoma treatment protocols(Banyal et al., 2023). Camptothecin, isolated from the Chinese 'Happy Tree,' has led to the development of topotecan and irinotecan, which are used in treating ovarian and colorectal cancers, respectively (Khaiwa et al., 2021). The oceans contain a large amount of the world's chemical variety; the enormous diversity of chemical entities exclusive to marine organisms is a reflection of their great genetic diversity, which evolved to allow them to adapt to a wide array of nautical environmental situations (Florean et al., 2022). As evidenced by the utilisation of marine invertebrates to treat several illnesses in Ancient Greece, the use of the sea for medicinal purposes started during the Age of Antiquity (Voultsiadou, 2010). In recent decades, marine chemistry has gained significant momentum in the discovery of a plethora of novel biomolecules and compounds of marine origin, such as ziconotide and cytarabine (Ara-C)(Cappello and Nieri, 2021), along with synthetic agents like brentuximab vedotin (SGN-35), trabectedin (ET-743), and eribulin mesylate (E7389), have been successfully utilized in cancer treatments for an extended period (Gerwick and Moore, 2012, Nigam et al., 2019). There are wide arrays of polypeptides with strong anticancer properties that were identified in diverse marine creatures. The structures of these polypeptides differ from those of peptides found in terrestrial plants (Carroll et al., 2022, Nalli et al., 2017), where the presence of hydroxy acids, thiophenols, and D- and L-amino acids is abundant. Some of these peptides are characterized by having ethylenic and acetylenic linkages and hence increase the bioavailability and stability. In comparison to other conventional inhibitors, marine peptides have high activity and effective targeting, while exhibiting lower toxicity. Besides inhibiting cancer cells, other numerous biological actions have been reported, including anticancer, anti-inflammatory, and anti-microbial activity (Gao et al., 2021). Increasing numbers of prospective medications generated from marine peptides are either in clinical development or have been licensed for sale (Negi et al., 2017). Dolastatin-type peptides were first discovered in the Indian Ocean mollusc *Dolabella auricularia*. Since then, 18 variants of dolastatin have been identified namely dolastatin 1–18, containing several unusual amino acids (Zheng et al., 2018). Several other dolastatin-type compounds and dolastatins 10–12, 16 were noted to be extracted from marine benthic cyanobacteria, suggesting cyanobacteria could be the probable producers of these compounds (Luesch et al., 2002, Davies-Coleman et al., 2003, Salvador et al., 2011). Dolastatin 10 and 15 have excitedly received a lot of interest in the area of anticancer research for their compelling cytotoxic and tubulin dynamics targeting activity (Gao et al., 2021). Dolastatin 10 of its fairly simple chemical structure, considered the most robust bioactive agent against several types of cancer, excites chemists to synthesise it in a substantial quantity laying the groundwork for its continued research (Pettit et al., 1989b). Dolastatin 10, 15 and several analogues have set foot in clinical trials as potent anticancer compounds but mostly did not progress beyond phase II (Ratnayake et al., 2020). Numerous research publications have reported on dolastatins and their derivatives up to this point, but as far as we know, few studies have been thoroughly summed up. This study summarizes the contemporary research on dolastatin and its acceptability as a chemotherapeutic drug, especially against ovarian, leukaemia, and non-small cell lung cancer. The present review also mentioned the overview and sources of dolastatins and analogues, both natural and synthetic. Structural synthesis of dolastatin derivatives was also added in this article to boost a finer comprehension for the future production of pharmacologically superior drugs that enable influence the therapeutic modulation of carcinogenesis.

Methodology

A systematic and comprehensive search of the literature was carried out to collate information about Dolastatins and their analogues as potential natural and synthetic anticancer drug candidates. The databases searched for this review were PubMed/MedLine, Scopus, Baidu, TRIP database, Google Scholar, Web of Science and ScienceDirect. The search strategy involved the use of the following keywords and their combinations: "Dolastatins," "Synthetic Analogues," "Anticancer Activity," "Marine-derived Compounds," "Drug Candidates," "Apoptosis Pathways," "Cell Cycle Inhibition," "Angiogenesis," "Clinical Trials," "Cytotoxicity," "Molecular Mechanisms," "Signaling Pathways," and "Cancer Therapy." Both MeSH terms and free-text words were used in the search strategy to ensure a comprehensive search.

Inclusion Criteria: Studies were included if they: (1) investigated the anticancer mechanisms of Dolastatins and their synthetic analogues; (2) discussed their *in vitro* and *in vivo* efficacy; (3) reported on their toxicity profiles; and (4) involved clinical trials assessing the safety and efficacy of these compounds.

Exclusion Criteria: Studies were excluded if they: (1) did not focus on Dolastatins or their analogues; (2) were not related to cancer or

anticancer activities; and (3) were duplicate studies, commentaries, letters to the editor, or opinion pieces.

Titles and abstracts of articles identified from the initial search were screened for relevance. Full-text articles were then assessed based on the inclusion and exclusion criteria. Relevant data were extracted from the included studies, such as the type of study, sample size, type of cancer, anticancer mechanisms of Dolastatins and their analogues, and outcomes of interest. The extracted data were organized and synthesized in tables and figures to provide a comprehensive overview of the potential anticancer activity of dolastatins and analogues. Key findings, mechanisms of action, and signaling pathways were analyzed and presented coherently. The chemical formulas have been validated using PubChem, ChemSpider and the scientific names of the plants according to the World Flora Online (PubChem, 2022, WFO, 2021).

Dolastatin: historical overview, chemical data and analogues

The dolastatin family includes linear and cyclic peptides that were initially isolated from D. auricularia (Pettit et al., 1989b). Among them, dolastatin 10 and dolastatin 15 have shown the highest effective cytotoxic effects on tumourous cells (Pettit et al., 1995, Pettit et al., 1998). The origin of dolastatin, specifically whether it was microbial or not was a subject of constant debate. A very low yield of dolastatins isolated from *D. auricularia* suggests that this slug is not the true precursor of these compounds. Studies exhibit that this organism *sequesters* metabolites from macroalgae and cyanobacteria and might obtain these metabolites through its diet (Pennings and Paul, 1993). For example, dolastatin 10 was originally isolated from *D. auricularia* but later it was isolated from the marine cyanobacterium *Symploca* sp. VP 642 (Luesch et al., 2001). Its analogue symplostatin 1 was obtained from the marine cyanobacterium *Symploca* hynoides (Harrigan et al., 1998a). Another derivative symplostatin 3 was isolated from marine cyanobacterium *Symploca* sp. VP452 (Luesch et al., 2002). Dolastatin 15 was first extracted from *D. auricularia* but it was also isolated from marine cyanobacteria VPG14-73 and VPG16-8 (Pettit et al., 1989a). Some derivatives of dolastatin were also isolated from the extract of Lyngbya majuscula/*Schizothrix calcicola* assemblage and *L. majuscula* strain (Harrigan et al., 1998a, Luesch et al., 1999). Thus, evidence from various studies confirms that the isolation of dolastatin or its derivatives from sea hare feeding of the cyanobacteria are metabolites that have a cyanobacterial origin (Luesch et al., 1999, Newman and Cragg, 2010). Cyanobacteria produce a variety of substances that resemble dolastatins (as an example, Table 1).

Table 1
Isolation of dolastatins and derivatives/analogues from Cyanobacteria.

D. auricularia isolate (Dolastatin group)	Cyanobacterial sources	Cyanobacterial derivatives	Reference
Dolastatin 3	*Lyngbya majuscula*	Homodolastatin 3	(Pettit et al., 1987, Mitchell et al., 2000)
Dolastatin 10	*Symploca hydnoides*	Symplostatin 1	(Harrigan et al., 1998a, Luesch et al., 1999)
Dolastatin 11	*Lyngbya majuscula*	Majusculamide C	(Carter et al., 1984, Pettit et al., 1989b)
Dolastatin 12	*Lyngbya majuscula/ Schizothrix calcicola*	Epidolastatin 12	(Pettit et al., 1989a, Harrigan et al., 1998b)
Dolastatin 13	*Symploca hydnoides*	Symplostatin 2	(Harrigan et al., 1999)
Dolastatin 15	*Dolabella auricularia*	–	(Pettit et al., 1989b)
Dolastatin G	*Lyngbya majuscula*	Lyngbyastatin 2	(Luesch et al., 1999)
Dolastatin H	*Symploca* sp.	Symplostatin 3	(Luesch et al., 2002)

Chemical structure of dolastatins

Dolastatin-type peptides exhibited various chemical structures, and this group consists of unusual amino acids such as (3R,4S,5S)-dolaisoleucine (Dil), (2R,3R,4S)-dolaproine (Dap), or 3-methoxy-5-methyl-4-(methylamino) hexanoic acid. While a few of them are the metabolites of peptide-polyketide biogenesis, others show thiazole (Thz) or thiazolidine ringed structures (Ciavatta et al., 2017). Depending on the chemical structures and origin of isolation, different types of dolastatins have been reported in the literature such as dolastatin 1 to 19, C, D, E, G, H, and I (Gao et al., 2021, Casalme et al., 2022, Poncet, 1999). However, 10 and 15 showed significant cytotoxicity against the cancer cells among this vast range of dolastatins.

The structure of the dolastatin 3 was deduced as cyclo-[L-Val-L-Leu-L-Pro-(R)- and (S)(Gln)Thz-(Gly)Thz]. Further, it was found that dolastatin 10 could be classified as a thiazole-containing cyclic peptide; however, its thiazole ring lacked substitution at the fourth and fifth positions. It consists of subunits of (S)-dolavaline, (S)-valine, (3R,4S,5S)-dolaisoleuine, (S)-dolaphenine and (2R,3R,4S)-dolaproine (Gao et al., 2021). Dolastatin 11 constitutes of two glycine, two alanine units and one unit each of N-methylvaline (MeVal), O,N-dimethyltyrosine (O,N-diMeTyr), and N-methylleucine (MeLeu). Dolastatin 12 is quite similartoits 11 counterparts. Dolastatin 13 on the other hand, is made of threonine, N-methyl-phenylalanine, and two units of valine. Dolastatin 15 was characterized to have two proline units and a 2-hydroxyisovaleric acid (Hiva) unit. Dolastatin 16 constitutes two unique amino acids dolaphenvaline and dolamethylleuine (Pettit et al., 2011a). The depsipeptide dolastatin 15 contains one unique amino acid namely (S)-dolapyrrolidone (Dpy). Interestingly, depsipeptide dolastatin C is part of the early biosynthesis of dolastatin 10. Another cytotoxic depsipeptide, dolastatin D, possessed the novel β-amino acid (2R,3R)-3-amino-2-methylbutanoic acid (Sone et al., 1993). Sone et al. (1996) (Sone et al., 1996) demonstrated the structure of dolastatin H as having 3-phenylpropane-1,2-diol attached through the ester linkage to the C-terminus. Dolastatin E and I are cyclic hexapeptides and consist of three types of five-membered heterocycles (oxazole, thiazole, and oxazoline) (Sone et al., 1997, Ojika et al., 1995).

Some of the important dolastatin structures are shown in Fig. 1 and their derivatives in Fig. 2. Some of the biologically important dolastatins are compiled in Table 2.

Natural analogue

Among the isolated dolastatins, some of them showed remarkable analogy, e.g, dolastatin 10 and H and dolastatin 14 and G. Whereas dolastatin I and E showed close resemblance with other compounds such as cyclooxazoline, westiellamide isolated from severalof organisms such as *Lissoclinum bistratum*, *Prochloron* sp., and *Westiallopsis prolifeca*. Symplostatin is a natural analogue of dolastatins, which was isolated from marine cyanobacterium *Symploca* sp. Furthermore, dolastatin 10 and symplostatin from the marine cyanobacterium *L. majuscula* and *S. calcicole* showed high similarity in structures. Symplostatin 2, a dolastatin 13 analogue, containing unique L-methionine sulfoxide residue was isolated from *S. hydnoides* (Harrigan et al., 1999). Symplostatin 3 is an analogue of dolastatin 10, which was found in a Hawaiian variety of the marine cyanobacterium *Symploca* sp VP452. This compound is characterized by a replacement of the dolaphenine unit of dolastatin 10 by a 3-phenyllactic acid residue. Also, from *Symploca* sp., symplostatin 4 was isolated. In this case, this compound is considered a natural hybrid analogue of Dolastatin 10 and 15 (Taori et al., 2009). Lyngbyastatin is another natural dolastatin analogue group, isolated from *Lyngbya* sp. Lyngbyastatin 1, isolated from *Lyngbya majuscula/Schizothrix calcicola* assemblage is a natural analogue of dolastatin 11 and 12 (Harrigan et al., 1998b). Lyngbyastatin 2, a natural analogue of dolastatin G, was

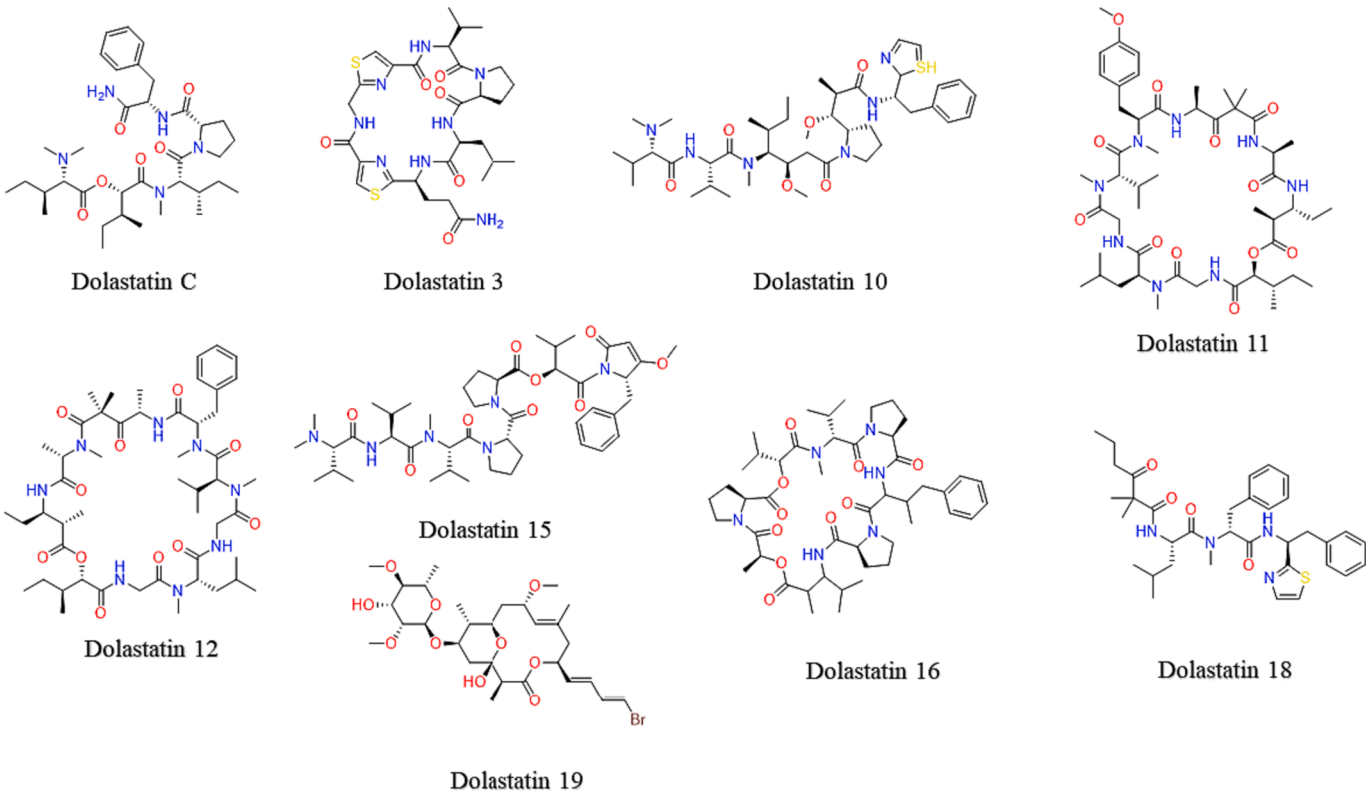


Fig. 1. Chemical structures of some important dolastatins.

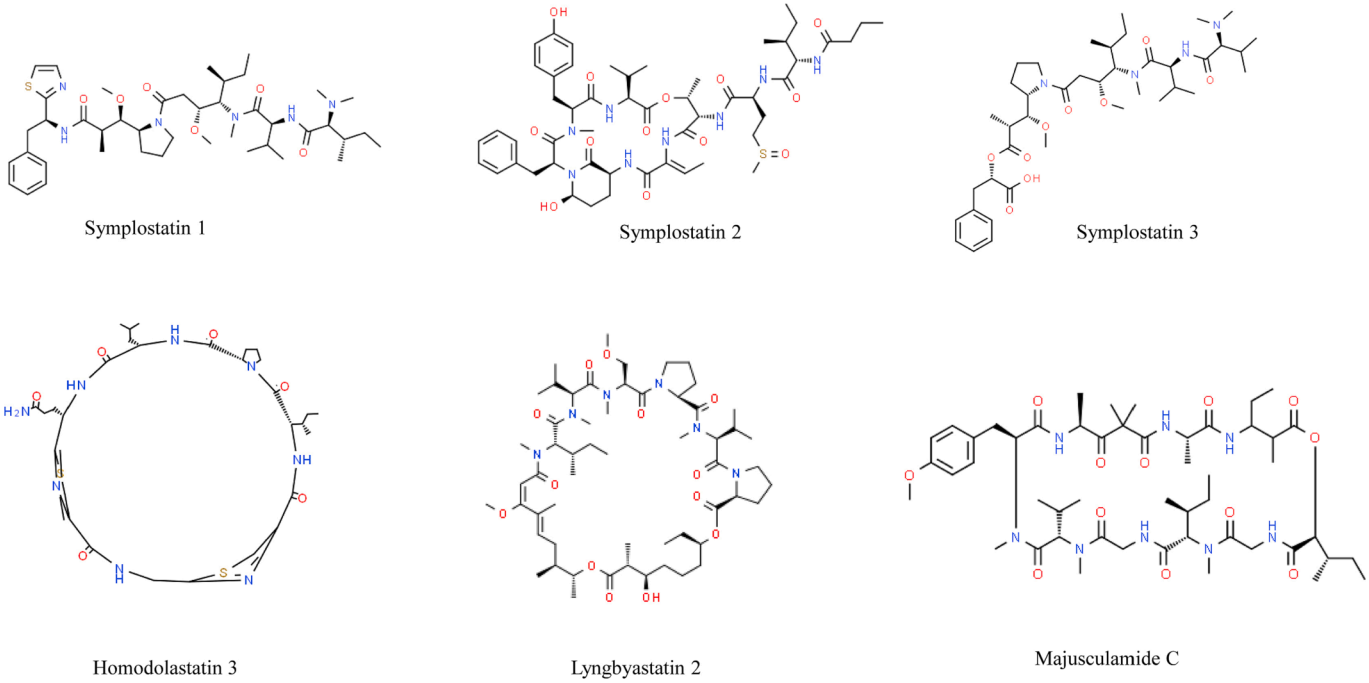


Fig. 2. Chemical structures of Dolastatins derivatives.

isolated from a Guamanian variety of *L. majuscula* (Luesch et al., 1999). Matthew et al. (2007) (Matthew et al., 2007), isolated a new depsipeptide lyngbyastatin 4 from a cyanobacterium *Lyngbya confervoides*. Multiple analogues of dolastatin 10 were isolated by Miyazaki et al. (1995) namely 31c, 35c, 38b, and 50c (Miyazaki et al., 1995). Nogle et al. (2001) (Nogle et al., 2001) also isolated the natural analogues somamides A and B usingq1a < the cyanobacterial assemblage of *L. majuscula* and *Schizothrix* spp. from Fiji. Pitiprolamide, a novel cyclic depsipeptide was isolated from the marine cyanobacterium *Lyngbya majuscula* collected from Piti Bomb Holes, Guam and later found to have similarity with the proline-rich Dolastatin 16 (Montaser et al., 2011). Sone et al. (1993) (Sone et al., 1993) isolated dolastatin H analogue

Table 2
List of important biologically active dolastatins.

Name of the compound	Chemical class	Molecular Weight	Molecular Formula	Synthetic Analogues	Natural Analogues
Dolastatin 3	Linear peptide (Oscillapeptin)	660.8	$C_{29}H_{40}N_8O_6S_2$	NA	NA
Dolastatin 10	Linear peptide	785.1	$C_{42}H_{68}N_6O_6S$	TZT-1027: Soblidotin (Horti et al., 2008, Schöffski et al., 2004) Auristatins (Fennell et al., 2003) Dolastatinol (Gutman et al., 2021) PF-06380101 (Maderna et al., 2014) Eight analogues (D1-D8) (Gajula et al., 2013)	Symplostatin 1 (Luesch et al., 2001, Mooberry et al., 2003)
Dolastatin 11	Depsipeptide	985.2	$C_{50}H_{80}N_8O_{12}$	Ibu-epilyngbyastatin 1 (Bai et al., 2002)	Lyngbyastatin 1 (Harrigan et al., 1998b)
Dolastatin 12	Depsipeptide	969.2	$C_{50}H_{80}N_8O_{11}$		Lyngbyastatin 1 (Harrigan et al., 1998b)
Dolastatin 15	Depsipeptide	837.1	$C_{45}H_{68}N_6O_9$	LU103793 (Villalona-Calero et al., 1998)	Caldoramide (Gunasekera et al., 2016)

NA - non-available.

isodolastatin H from the Japanese sea hare *D. auricularia*. More recently, caldoramide was isolatd from the marine cyanobacterium *Caldora penicillata* from Florida, having notable similarities with dolastatin 10 and 15 (Gunasekera et al., 2016) isolated a modified pentapeptide,. Overall, it is notable that cyanobacteria are an interesting source of peptides resembling dolastatins, becoming new resources for targeting the search for new anticancer compounds.

Synthetic analogues of dolastatins

Since the discovery of dolastatins, researchers developed multiple bioactive synthetic analogues of these natural secondary metabolites.

TZT-1027 (soblidotin) was a novel synthetic dolastatin 10 derivative, developed by Schöffski et al. (Schöffski et al., 2004). Monomethyl auristatin E was another dolastatin 10 analogue and later it was linked with monoclonal antibodies, leading to the development of brentuximab vedotin (Adcetris) and polatuzumab vedotin (Polivy), which are used in chemotherapy (Gao et al., 2021, Newman and Cragg, 2014, Maderna et al., 2014, Maderna and Leverett, 2015). PF-06380101 is a new auristatin analogue with N-terminal modifications that include amino acids with α,α-disubstituted carbon atoms (Pathak et al., 1994). Synthadotin (ILX651) emerged as a promising synthetic derivative of dolastatin 15, targeted for locally advanced or metastatic melanoma (Hammond et al., 2004, Newman and Cragg, 2014). A dolastatin 15 analogue LU 103793 was reported to inhibit tubulin polymerisation (Kerbrat et al., 2003). Bai et al. (2009) (Bai et al., 2009), synthesized an oncolytic synthetic analogue of dolastatin 15, tasidotin where the carboxyl-terminal ester group of dolastatin 15 was replaced by carboxy-terminal *tert*-butyl amide. Gajula et al. (2013) (Gajula et al., 2013) synthesized eight analogues (D1-D8) of dolastatin 10. Bai et al. (2002) synthesized dolastatin 11 analogue lyngbyastatin 1-Ibu-epilyngbyastatin 1 mixture, consisting of ibu ((S)-4-amino-2,2-dimethyl-3-oxopentanoic acid) epimer.

The interest in the synthesis route of dolastatin analogues continues and recent examples of publications include the studies of various authors.

Dolastatinol, a methylene hydroxyl dolastatin 10 analogue was developed by Gutman et al. (2021). It preserved the anticancer effect of the original compound, but facilitated the synthesis approach (Gutman et al., 2021). Casalme et al. (2022) (Casalme et al., 2022) performed second-generation synthesis of dolastatin 16 derivatives by modification of its amino acids dolaphenvaline and dolamethylleuine. Nonetheless, the work was addressed to look for antifouling activity against the cypris larvae *Amphibalanus amphitrite*, other directions can be explored concerning their cytotoxic activities.

Table 3 summarizes the most representative dolastatin analogues, their chemical structures, and detailed synthetic routes.

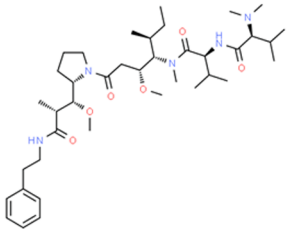
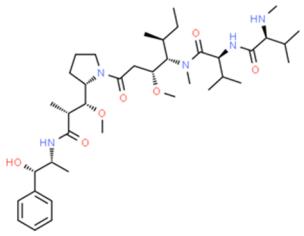
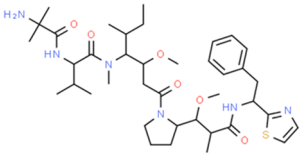
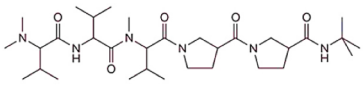
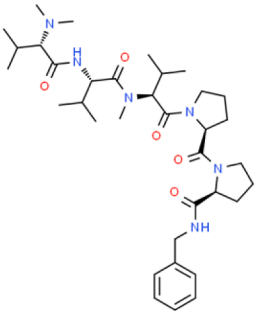
Bioavailability and pharmacokinetics

Bioavailability and pharmacokinetics are essential factors to consider when evaluating the potential of drug candidates, including dolastatins and their analogues, as anticancer agents. Dolastatin 10 has shown higher potentiality in terms of bioavailability and it is one of the 18 different types of dolastatins derived from *D. Auricularia* (Gao et al., 2021). The structural uniqueness of dolastatin-type peptides, including the presence of thiophenols, hydroxy acids, and the formation of acetylenic and ethylenic bonds, contributes to their stability and bioavailability (Zheng et al., 2018, Gao et al., 2021).

Dolastatin analogue Synthadotin (SYN-D), is a dolastatin analogue that has been chemically modified to improve its bioavailability and anticancer pharmacological properties. The modifications have also enhanced its oral bioavailability compared to previous generations of dolastatins (Hammond et al., 2004). This compound has been chemically refined to enhance its pharmacological characteristics, making it a promising candidate in oncology; while retaining the potent anticancer efficacy of its predecessor dolastatin 10, Synthadotin aims to bring down the level of toxicity, which is often a concern in cancer therapeutics (Ebbinghaus et al., 2004, Ebbinghaus et al., 2005). In its clinical evaluations, Synthadotin has progressed to Phase II trials specifically for cases of hormone-resistant prostate cancer, indicating a step forward in understanding its therapeutic potential(Ebbinghaus et al., 2004). The bioavailability of Synthadotin is a critical aspect that needs to be explored to better comprehend its pharmacokinetics and eventual clinical efficacy; however, the precise data regarding its bioavailability hasn't been provided here, and would require a thorough review of clinical trial results or pharmacokinetic studies.

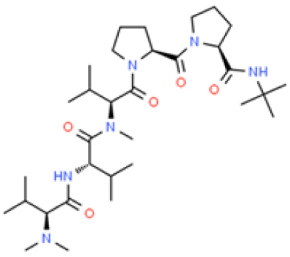
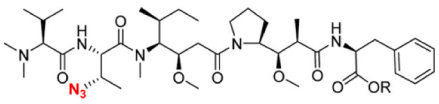
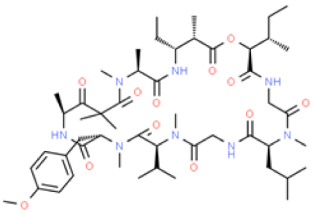
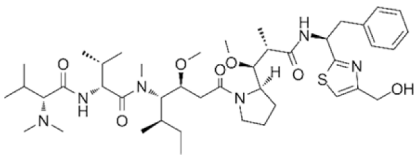
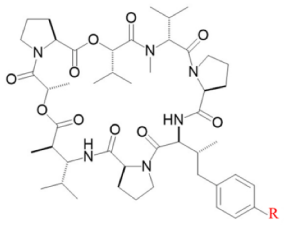
Auristatin TP Sodium Salt, a novel derivative of Dolastatin 10, inherits its anticancer lineage from Dolastatin 10 while embracing a distinct modification known as tyramide phosphate. This modification significantly augments the bioavailability and anticancer efficacy of Auristatin TP Sodium Salt, as compared to its parent compound. The anticancer activity of Auristatin TP Sodium Salt has been evaluated through ED50 values, which range from < 1.2 to 54.6 nm, showcasing a spectrum of enhanced efficacy across different cancer cell lines (Pettit

Table 3
Dolastatin derivatives, their structures, and detailed synthetic routes.

Compound	Structure	Synthetic Route Description	References
TZT-1027 (soblidotin)		Synthesized via peptide coupling of modified amino acid precursors, followed by cyclization to form the macrocyclic structure characteristic of dolastatin 10 derivatives.	Schöffski et al., 2004
Monomethyl auristatin E (MMAE)		Developed by selective methylation of dolastatin 10′s amino groups, followed by the attachment of a maleimidocaproyl (mc) linker for antibody conjugation.	Gao et al., 2021; Newman and Cragg, 2014
PF-06380101		Features N-terminal modifications where the naturally occurring amino acids are replaced with synthetic analogs containing α,α-disubstituted carbon atoms, achieved through solid-phase peptide synthesis.	Pathak et al., 1994
Synthadotin (ILX651)		Obtained by synthetic modification of the dolastatin 15 peptide backbone, optimizing side chain functionality for better tumour targeting and increased antitumour efficacy.	Hammond et al., 2004; Newman and Cragg, 2014
LU 103,793		Synthesized by modifying the peptide sequence of dolastatin 15 to include non-natural amino acids that enhance tubulin binding, using solution-phase peptide synthesis techniques.	Kerbrat et al., 2003

(*continued on next page*)

Table 3 (*continued*)

Compound	Structure	Synthetic Route Description	References
Tasidotin		The carboxyl-terminal ester group of dolastatin 15 is replaced with a carboxy-terminal tert-butyl amide using an amidation reaction to improve pharmacokinetic properties.	Bai et al., 2009
Dolastatin 10 Analogues (D1-D8)		A series of dolastatin 10 analogs synthesized by varying the side chains and terminal groups to analyze the impact on cytotoxic activity, using iterative peptide synthesis methods.	Gajula et al., 2013
Lyngbyastatin 1 Ibu-epilyngbyastatin 1 mixture		Created by incorporating the ibu epimer into the dolastatin 11 framework via selective enzymatic epimerization, followed by esterification reactions.	Bai et al., 2002
Dolastatinol		Engineered by introducing a methylene hydroxyl group into the dolastatin 10 molecule, employing a modified total synthesis pathway that streamlines production.	Gutman et al., 2021
Second-generation dolastatin 16 derivatives		These derivatives are synthesized through a multi-step process involving the selective functionalization of amino acid precursors dolaphenvaline and dolamethylleuine, followed by peptide coupling and macrolactamization.	Casalme et al., 2022

et al., 2011b). The mechanism of action primarily involves the disruption of microtubule dynamics, a hallmark of dolastatin analogues, leading to cell cycle arrest and apoptosis in cancer cells. The bioavailability of Auristatin TP Sodium Salt has been significantly enhanced due to the tyramide phosphate modification, which improves the pharmacokinetic profile of the drug, allowing for better absorption and systemic availability (Ebbinghaus et al., 2005). While exact bioavailability percentages are under further investigation, preliminary studies indicate a favorable profile that potentially translates to improved clinical efficacy.

Anticancer studies of dolastatins and their analogues

In vitro studies

The bioactive antitumour properties of the dolastatin-type peptides were first investigated by Pettit and his co-workers (Pettit et al., 1995). In the dolastatins group, dolastatin 10, 15 and its synthetic analogues had been proven to be potent anti-proliferative active compounds with sub-micromolar activity toward numerous cancer cell lines. Both

preclinical and clinical studies were conducted with dolastatins and their derivatives which were discussed in detail in the following sections. Table 4 and Fig. 3 depict the most representative evaluation of the pharmacological activity of dolastatins and their analogues. In this regard, dolastatin 10 showed *in vitro* effectiveness against the lymphoblastic leukaemia P388 cell lines better than rhizoxin, vinblastine, and phomopsin A (Bai et al., 1990a). Preliminary studies also indicated that the peptide may inhibit microtubule assembly and tubulin-dependent GTP hydrolysis (Bai et al., 1990b). Yamada et al. (2000) (Yamada et al., 2000) executed a bioassay-directed examination of cytotoxic constituents of the Japanese specimens of *D. auricularia* to isolate several cytotoxic peptides including dolastatins C, D, E, G, and H, isodolastatin H, and dolastatin I. Among them, dolastatin H, isodolastatin H, and doliculide showed strong cytotoxic properties (Table 3). An isodolastatin H analogue, malevamide D, isolated from the marine cyanobacterium *S. hydnoides* demonstrated toxicity against several cell lines including P388, A-549, HT-29, and MEL-28 cell lines at the subnanomolar level (Horgen et al., 2002). In another study, the dolastatin 10 analogue symplostatin 3 exhibited cytotoxicity where the activity might be through disruption of microtubules (Luesch et al., 2002). Similar to that, another dolastatin 10 analogue, symplostatin 1, was isolated from the latter marine cyanobacterium and exhibited cytotoxicity and induced 80 % microtubules loss at 1 ng/mL (Harrigan et al., 1998a). Majusculamide C showed cytotoxic potentials against different human cancer cells with a noted stimulation of actin polymerization. Results portrayed that analogues lacking the 30-membered ring were inactive (Ali et al., 2005). Homodolastatin 16, a higher homologue of the potential anticancer agent dolastatin 16, exerted moderate activity against oesophageal and cervical cancer cell lines with an IC_{50} of 4.3 to 10.1 μg/mL (Davies-Coleman et al., 2003); whereas compound kulokekahilide-1, a homodolastatin 16 analogue isolated from the mollusc *Philinopsis speciosa*, showed cytotoxicity against P388 (IC_{50} of 2.1 μg/mL) (Tan, 2007).

Sato et al. (2007) (Sato et al., 2007) demonstrated that dolastatin 15 inhibited the growth of human myeloma RPMI8226, U266, and IM9 cell lines with IC_{50} of(0.5–1 nM). Moreover, the exposure of RPMI8226 cells to this compound at nM concentrations for 24 h led to a typical morphological appearance of apoptosis with condensed chromatin and fragmented nuclei, the presence of apoptotic bodies, and induced G2/M cell cycle arrest. Further, dolastatin 15 activated chk2 kinase, caused loss of $\Delta\Psi m$, activated caspase-3, −9, −8, and induced apoptosis, indicating microtubule assembly hindrance. Lopus (2013) (Lopus, 2013) investigated the antimitotic mechanism of action of dolastatin 15. Results showed that this compound inhibited HeLa cellular proliferation, halted the cells in the mitotic phase, interrupted microtubule assembly, and hindered free tubulin reassembly to microtubules. Further exploration of the mode of action revealed that dolasatin 15 reduced the interpolar distance, and activated a loss of tension across kinetochore pairs in mitotic HeLa cells, ensuing in the accumulation of BubR1 checkpoint protein, which is an inducer of mitotic arrest and thereby, inhibition of cell proliferation.

TZT-1027 (soblidotin) is another synthesized derivative of dolastatin 10, which was observed to exhibit potent anticancer properties (Pettit et al., 1987). Natsume et al. (2000) (Natsume et al., 2000) investigated the ability of TZT-1027 to interact with tubulin as microtubule proteins are important for mitosis. Results showed that the analogue inhibited the polymerization process in a dose-dependent manner with an IC_{50} value of 2.2 μM and the effect was better than that of vinblastine. Besides, TZT-1027 and Dolastatin 10 prevented monosodium glutamate-induced tubulin polymerization with an IC_{50} value of 1.2 μM and 2.8 μM, respectively. Alongside, TZT-1027 and dolastatin 10 hindered the hydrolysis of GTP on tubulin and the binding of GTP to tubulin. Thus, TZT-1027 and dolastatin 10 could be regarded as microtubule-disrupting agents portraying their anticancer effects. In another study, TZT-1027 triggered significant cell contraction with membrane blebbing in cultured HUVEC within 30 min to 1 h. Interestingly, the *in vitro* activities of TZT-1027 were found to be around 10 times greater than those of vincristine (Otani et al., 2000). TZT-1027 has also exhibited cytotoxic activity against various human cancer cell lines including leukaemia, gastric, breast, prostate, and colon where the outcome indicated that the compound is a more potent inhibitor than vincristine, cisplatin, and 5-fluorouracil. Mechanistic analysis revealed that TZT-1027 induced apoptosis evident by the typical morphological changes, DNA fragmentation, and growth arrest at the G2/M phase of the cell cycle in HL-60, MCF-7 and DU145 cells (Watanabe et al., 2000). A similar kind of observation has also been reported by Hashiguchi et al. (2004) (Hashiguchi et al., 2004) showing a concentration-dependent cytotoxicity of TZT-1027 against MCF-7 cells after 48 and 72 h of incubation; while another breast cancer cell line R-27 was found resistant to TZT-1027. Akashi et al. (2007)(Akashi et al., 2007) explored the effect of TZT-1027 on cell cycle progression using tsFT210 mouse mammary carcinoma cells that express a temperature-sensitive mutant of Cdc2 cells. Results portrayed that the compound arrested cells in the M phase rather than in G2. Furthermore, TZT-1027 increased the *in vitro* effect of radiation on human cancer cells.

In vivo studies

The dolastatins and their analogues were well reported for inhibition of the proliferation of diverse types of cancerous cells *in vitro* culture. Additionally, dolastatins and analogues have also been observed to portray their anticancer effect *in vivo* models, which will be illustrated in this section. Table 3 summarizes the most representative studies.

The anti-tumour effect of dolastatin 10 was explored in *in vivo* using advanced-stage human ovarian carcinoma xenograft which exhibited a greater activity in comparison to dolastatin 15. Dolastatin 10 has potently inhibited tumour proliferation and induced tumour growth delay by 6.1 days (Aherne et al., 1996). Dolastatin 10 induced Bcl-2 phosphorylation leading to apoptosis in small cell lung cancer cell lines and xenografts. The *in vivo* activity of dolastatin 10 on SCID mice and metastatic SCLC xenoplantation models has been also evaluated. The studies revealed that intravenous administration of Dolastatin 10 had an acute effect on inhibition and delay of tumour growth. Moreover, it persuaded measurable shrinkage of tumours and possessed better survival of tumour bearing animals (Kalemkerian et al., 1999). The efficacy of Dolastatin 10 was observed in the mice bearing DLD-1 and COLO 205 tumour models and upon intravenous administration they possessed negligible toxicity but potently caused tumour growth inhibition (Shnyder et al., 2007).

TZT-1027 (soblidotin), a dolastatin 10 derivative, is considered a newly synthesized antitumour compound. This drug has demonstrated remarkable antitumour activity against various tested murine tumours and human xenografts, which led to its Phase I clinical trials in Japan (Kobayashi et al., 1997). TZT-1027 was found to be efficacious against P388 leukaemia when administered both intraperitoneally as well as intravenously. Although intravenous administration of TZT-1027 was observed to be quite effective for murine solid tumours its intraperitoneal injection seemed to be ineffective against all the tested tumours except colon 26 adenocarcinoma. In experiments with drug-resistant P388 leukaemia, TZT-1027 exhibited better effectivity against cisplatin-resistant P388 and slight activity against 5-fluorouracil and vincristine-resistant P388, but negligible activity was noted against adriamycin-resistant P388. Moreover, TZT-1027 showed strong activity against human xenografts where tumours regression was significantly noticed in mice bearing MX-1 breast and LX-1 lung carcinomas. The action of TZT-1027 on the assembly of microtubules was also examined and results depicted that at a dose of 10 μM of TZT-1027, the assembly of microtubules of porcine brain was completely inhibited. Hence, the mechanism of antitumour action of TZT-1027 was attributed to microtubule assembly inhibition (Kobayashi et al., 1997).

TZT-1027 has also been demonstrated to exhibit an influential antitumour effect in human tumours xenografted in nude mice from

Table 4
Therapeutic use and evaluation of dolastatins and their analogues in various anticancer research both *in vitro* and *in vivo*, providing the IC_{50} value and the administered dose, respectively.

Tested Dolastatin/Derivatives	Experimental study		Concentration used		Main results	Reference
	In vitro using cell lines	*In vivo* on animal model	IC_{50}	Dose		
Dolastatin 10	SKOV-3, CH1, A2780, HX/62, 41 M human ovarian carcinoma SW620, SW480, LoVo, MAWI, BE, HT29 colorectal carcinoma cells	BALB/C mice bearing tumour cells	0,035–0,061 nM	450 μg/kg	↓tumour growth	(Aherne et al., 1996)
	NCI-H69, NCI-H82, NCIH446, NCI-H510 small lung carcinoma cells	CB-17 SCID mice with tumour xenografts	0.032–1,3 nM	450 μg/kg	↑apoptosis ↑Bcl-2 ↑cell cycle arrest ↑G2/M phase	(Kalemkerian et al., 1999)
Dolastatin C Dolastatin D Dolastatin E Dolastatin G Dolastatin H (18) Isodolastatin H (19) Dolastatin I	HeLa S3 human cervix carcinoma cells	–	17 ng/mL 2,2 ng/mL 22 ng/mL 1 ng/mL 2.2 ng/mL 1 ng/mL 12 ng/mL	–	↑cytotoxicity	(Yamada et al., 2000)
TZT-1027 (derivative of dolastatin 10)	human umbilical vascular endothelial cells (HUVEC) C26 colorectalcancer cells	BALB/c and CDF1 mice bearing	10–7 g/mL	1–4 mg/kg	↓tumour vascularization ↑apoptosis ↑microtubule cytoskeleton disruption ↑cell cycle arrest ↑Bcl-2 ↑caspase 3 ↑DNA damage	(Watanabe et al., 2006a)
	HL-60 leukaemia cells MCF-7 breast cancer cells	–	<0.001 μg/mL 0.036 μg/mL	–	↓cell growth ↑morphological changes	(Watanabe et al., 2000)
	MKN45 stomach cancer cells		5.9 μg/mL		↑DNA fragmentation ↑cell cycle arrest	
	PC-3, DU145 prostate cancer cells		5.9 μg/mL		↑G2/M phase ↑apoptosis	
	WiDr colon cancer cells		0.0014 μg/mL			
	P388 leukaemia cells Colon 26 adenocarcinoma cells B16 melanoma cells M5076 sarcoma cells	DBA/2,BALBc, C577BL, BALB/c nu/nu mice	1–10 μM	0,5–3 mg/kg	antitumour effect ↑cytotoxicity	(Kobayashi et al., 1997)
	MX-1 breast cancer cells LX-1 lung cancer cells	cellsBALB/C nude mice	–			
	SBC-3 human small lung cancer cells	BALB/c-*nu/nu* nude mice and CDF1 mice	–	2 mg/kg	↓tumour size VEGF interaction magnifies damage to the tumour vasculature	(Natsume et al., 2003)
	murine fibrosarcoma cells	BALB/C mice	–	1—2 mg/kg	↓tumour growth without significant body weight loss in mice	(Watanabe et al., 2006b)
	murine colon cancer cells	BALB/c and CDF1 mice	–	1–4 mg/kg	↑ apoptosis ↑vascular permeability and marked extravascular leakage of erythrocytes	(Watanabe et al., 2006a)
Combinatorial treatment of TZT-1027 with cisplatin gemcitabine, irinotecan hydrochloride, fluorouracil, paclitaxel & docetaxel	murine leukaemia cancer non-small cell lung cancer	Female CDF1 mice and BALB/cnu/ nu nude mice	–	–	↓ tumour growth ↑lifespan	(Natsume et al., 2006)
TZT-1027 with radiation	H460, A549 lung cancer cells	Athymic nude mice	–	5 mg/kg	↓tumour growth ↑damage to the vascular endothelium ↑microtubule disruption ↑cell cycle arrest	(Akashi et al., 2007)
Auristatin PYE (a synthetic derivative of dolastatin 10)	DLD-1, COLO 205 colon adeno-carcinoma	CD1-Foxnl nude immunodeficient mice	For DLD-1 4.4 nM For COLO 205: 1.2 nM	10 mg/bw	↑microtubule disruption ↓tumour cells growth ↑cell cycle arrest G2/M phase ↑apoptosis ↓tumour volume	(Shnyder et al., 2007)
Dolastatin 15	cervical cancer cells	HeLa	2.8 nM	–	↑mitotic arrest disrupted cellular microtubules	(Lopus, 2013)

(*continued on next page*)

Table 4 (*continued*)

Tested Dolastatin/Derivatives	Experimental study		Concentration used		Main results	Reference
	In vitro using cell lines	*In vivo* on animal model	IC_{50}	Dose		
					↓re-polymerization of cellular microtubules	
Dolastatin 15 derivatives conjugated with trastuzumab	SK-OV-3 ovarian cancer cells MDA-MB-231, MCF-7, SK-BR-3 breast cancer cells	SCID mice bearing	1,5 nM 2,6 nM	20 mg/kg	↓ cancer cells growth ↓tumour volume	(Gianolio et al., 2012)
Dolastatin 15 along with celecoxib		Sprague Dawley rats with colon cancer	–	5 μg/rat	↑apoptosis PI3-K ↓, Akt ↓, GSk-3β ↓, IL-1β↓, Bad↑ Egr-1↑ PTEN↑, ROS↓ ↑Bcl-2 ↑mitochondrial damage	(Piplani et al., 2013)
Homodolastatin 16	ME180 cervical cancer cells WHCO1 WHCO6 esophageal cancer cell	–	8,3 μg/mL 4,3 μg/mL 1,01 μg/mL	–	↑ cytotoxicity	(Davies-Coleman et al., 2003)
Symplostatin 1 (Dolastatin 10 analogue)	KB eprdermoid carcinoma		0.3 ng/mL		↑ cytotoxicity	(Harrigan et al., 1998a)
Dolastatin 16	NCI-H460 lung cancer cells	–	0,0012 μg/mL	–	↑ cytotoxicity	(Davies-Coleman et al., 2003)
	KM20L2 colorectal cancer cells		1.2 μg/mL			
	SF-295 brain cancer cells		0,0052 μg/mL			
	SK-MEL5 melanoma cells		3.3 μg/mL			

Abbreviations and symbols: ↓decrease, ↑increase, BAD (BCL2 Associated Agonist Of Cell Death) (Bad); Early growth response protein 1 (Egr-1); Glycogen synthase kinase-3 beta (GSk-3β); Interleukin-1 beta (IL-1β); Mitochondrial membrane potential (ΔΨm); Phosphatase and tensin homolog on chromosome 10 (PTEN); Phosphatidylinositol 3-kinases (PI3-K); Protein kinase B (Akt); Radical oxygen species (ROS); Vascular Endothelial Growth Factor (VEGF).

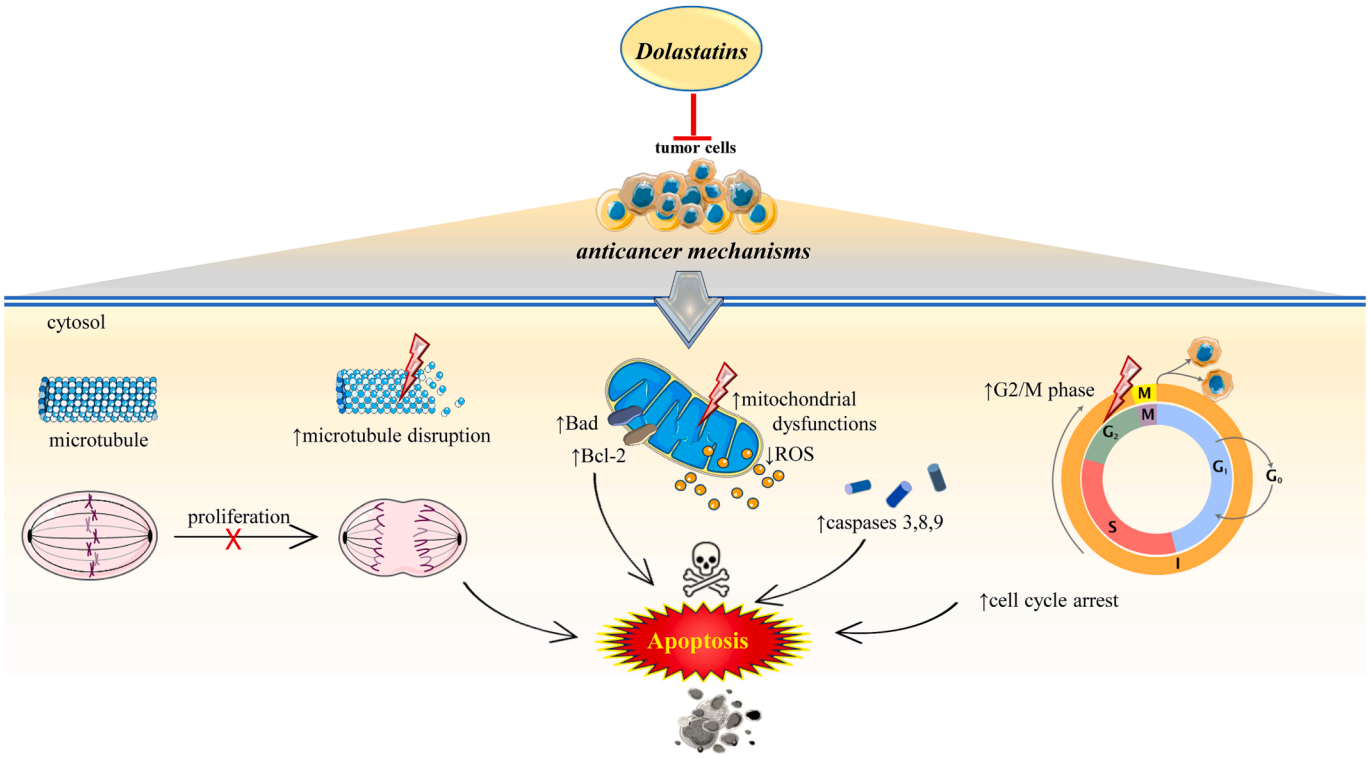


Fig. 3. Illustrative scheme regarding the most important potential anticancer mechanisms of dolastatins and their analogues. Dolastatins disrupt microtubules, inhibiting cell proliferation. Concurrently, they induce mitochondrial dysfunctions, modulating reactive oxygen species (ROS) levels and the expression of pro- and anti-apoptotic factors such as Bad and Bcl-2. This activation cascades to the stimulation of caspases 3, 8, and 9, ultimately promoting apoptosis and causing cell cycle arrest at the G2/M phase. Symbols: ↑increase, ↓decrease, X inhibition.

different types of cancer including gastric, colon, lung, breast, liver, ovarian, and renal cancer lines (Fujita et al., 2000). The *in vivo* anti-tumour activity of TZT-1027 was evaluated against Meth A solid tumours which was implanted subcutaneously into mice. Results depicted that TZT-1027 imparted neither toxic deaths nor induced severe body weight loss in mice. The antitumour activity of the drug was

found to be associated with ischemic necrosis of the tumours which was likely connected to the induction of local circulatory disturbances. When the *in vivo* antitumour activity of TZT-1027 was compared with that of vincristine (an alkaloid derived from vinca (*Catharanthus roseus*)) and docetaxel (taxanes) it significantly demonstrated a better effectiveness in the tumour model (Watanabe et al., 2006a). A similar group of researchers also wanted to explore the antivascular effect of TZT-1027 along with its antitumour effect in mice bearing Colon26 tumours. Studies exhibited that a significant tumour reduction and enhancement of the survival period of mice without toxic death in advanced-stage Colon26 tumours occurred upon a single administration of TZT-1027 intravenously. Simultaneously, TZT-1027 caused tumour necrosis, and extravascular leakage of erythrocytes followed by thrombus formation with deposition of fibrin after 3 and 24 h of administration. It suggests that TZT-1027 not only had a cytotoxic effect but also exerted indirect antivascular properties (Watanabe et al., 2006b).

The relationship between the antitumour and anti-vascular effect of the antimicrotubule agent TZT-1027 was also elucidated in both early and advanced-stage SBC-3/Neo and SBC-3/VEGF tumour models. Results enumerated that TZT-1027 exhibited significant and potent antitumour efficacy against P388 leukaemia in CDF1 mice at a very low dose as compared with vincristine, whereas CA-4-P showed no antitumour effect in the aforementioned *in vivo* model. Results explained that there was marked necrosis with subsequent disappearance of the tumour with scar formation and a significant presence of erythrocytes in the tumour vasculature, followed by scattering and spillage of these erythrocytes. tumour Interestingly vincristine and docetaxel were capable of only exhibiting antitumour activity against early-stage SBC-3/Neo and SBC-3/VEGF tumours, and advanced-stage SBC-3/Neo tumours (Natsume et al., 2003). Two separate *in vivo* antitumour models comprising of murine P388 leukaemia ascites tumour model and the A549 solid tumour model, were used to examine the combinatorial effect of TZT-1027 with docetaxel, gemcitabine, paclitaxel, cisplatin, irinotecan hydrochloride and fluorouracil. The schedule of administration was selected as first TZT-1027 alone then combined drugs were administered after 24 h. Data revealed that in the P388 cell ascites tumour model, there was a prominent increase in the lifespan of mice when administered TZT-1027 combined with irinotecan hydrochloride, and a marked reduction in tumour growth when TZT-1027 was combined with gemcitabine, docetaxel, and cisplatin in the A549 solid tumour model. Sequential administration, in particular when the combined drug was administered first, displayed better antitumour efficacy (Natsume et al., 2006).

Radio sensitisation through TZT-1027 was examined *in vivo* in human lung adenocarcinoma cells. Results depicted that treatment with TZT-1027 alone resulted in proportionately lesser tumour growth inhibition whereas combinatorial treatment of both TZT-1027 and radiation imparted a distinctly pronounced inhibitory effect. Though no prominent tissue damage or toxicities *viz.*, weight loss or diarrhoea were found in mice treated with TZT-1027. Researchers also tried to examine the effect of TZT-1027 on tumour vasculature and its responsibility for the antitumour activity of this drug *in vivo*. Immunostaining images revealed loss of endothelial cells, capillaries of tumour looked congested with thrombus formation and extensive necrosis at the tumour core which is an indication of a distinctive anti-vascular effect of TZT-1027 in the H460 tumour model (Akashi et al., 2007). It demonstrated a potent anticancer effect in human colon adenocarcinoma cell lines both *in vitro* as well as *in vivo* in the colon tumours, targeting the microtubules (Shnyder et al., 2007).

Dolastatin 15 derivatives (Dol15) were tethered covalently to a representative antibody, trastuzumab and their effective anticancer effects were explored in SKOV-3 human ovarian cancer xenograft. The study demonstrated that trastuzumab-amide-Dol15-C-terminus conjugate was an extremely efficacious therapy for the SKOV-3 tumour succeeding in an initial regression of tumour size along with the delay in the growth and long-term remission of the tumour in xenograft mice (Gianolio et al., 2012). Piplani et al. (2013) (Piplani et al., 2013) demonstrated that dolastatin 15 along with celecoxib, has stimulated anti-cancer effects in colon cancer in a rat model via a mechanism involving the downregulation of PI3K/AKT signalling pathway. The study showed that drugs inhibited colon carcinogenesis by stimulating apoptosis through increased production of reactive oxygen species along with the spillage of intracellular calcium by decreasing the membrane potential of the colon cancer cells. Their study also showed a decreased gene expression of important transcription factors like PI3-K, Akt, GSK-3β and IL-1β, which were involved in the regulation of tumour progression. However, co-administration of celecoxib and dolastatin, increases the expression level of Bad (Bcl-2 family protein), PTEN and Egr-1 which aids in reducing the tumour development thereby intensifying the rate of apoptosis. More recently, Ratnayake et al. (Ratnayake et al., 2020) suggested that this compound can suppress hypoxia-inducible factor 1 (HIF-1) α-mediated cancer cell viability and vascularization.

Clinical studies

Preclinical studies of dolastatin 10 revealed activity against various human and murine tumours in cell cultures and mice models. In this sense, the early clinical trial study by Pitot et al. (1999) (Pitot et al., 1999) was conducted on patients with advanced solid tumours (Table 5). The aim of the Phase I clinical trial of dolastatin 10 was to characterize its biological effects, maximum tolerated dose (MTD), and pharmacokinetics. 30 patients have taken dolastatin 10 intravenous bolus of a total of 94 cycles at a range of doses of 65–455 μg/m^2. Results enumerated that the MTD is 400 μg/m^2 for patients with slight prior treatment and 325 μg/m^2 for heavily pre-treated patients. Later another group of researchers conducted a Phase I trial of dolastatin 10 to study its effectiveness in patients having refractory or relapsed acute leukaemia, chronic myelogenous leukaemia in blast phase, or myelodysplastic syndrome. Results depicted that MTD in patients was noted within 3 weeks of the cycle (ClinicalTrials.gov). Phase I trial was also conducted to study the effectiveness of dolastatin 10 in patients who have chronic myelogenous leukaemia in the blast phase and refractory or relapsed acute leukaemia (ClinicalTrials.gov).

Dolastatin 10 was also practised as the first or second-line chemotherapy for advanced breast cancer. Patients with measurable metastatic breast cancer received Dolastatin 10 at a concentration of 400 μg/m^2 and the results showed that it had no significant activity. Additionally, the drug also showed haematological toxicity in a tolerable range. It was hypothesized that a sizeable dose of Dolastatin 10 might conquer its drug resistance and persuade compelling antitumour activity (Perez et al., 2005). In phase II clinical trial, patients with hormone-refractory prostate cancer received dolastatin 10 at a dose of 400–450 μg/m^2 every 3 weeks. A total of 56 cycles have been conducted and among 16 patients, nine exhibited major toxicities. Overall the compound dolastatin 10 revealed outstanding forbearance, especially among the elderly pretreatment population, but the clinical competency as a lone drug was solemnly inadequate (Vaishampayan et al., 2000). Phase II trials involving patients with advanced non-small-cell lung cancer or melanoma, who had not received prior chemotherapy, administered dolastatin-10 at a dosage of 400 μg/m2 every three weeks. Despite the treatment, which included an average of 2 cycles of dolastatin-10, no objective responses were observed in either patient group(Krug et al., 2000) (Margolin et al., 2001).This suggests that dolastatin 10 is unlikely to exhibit considerable activity in the treatment of these advanced cancers. Further, a phase II study was conducted on patients with advanced colorectal cancer with dolastatin-10 who have no prior chemotherapeutic treatment for metastatic disease. 14 patients have taken doses from 300 to 450 μg/m^2 for every 21 days. Results declared that there were no prime objective responses to the administration of drugs rather toxicity mainly heamatologic was prominent. Dolastatin-10 deficient clinically prominent activity in advanced colorectal cancer while taken at this dose and schedule (Saad et al., 2002). To determine

Table 5
Selected clinical trials of dolastatins and their analogues in different types of cancers.

Bioactive molecules	Identifier	Investigator	Last Updated	Cancer type	Study Phase/ Type	Number of participants	Status	Source
Dolastatin 10	–	H.C. Pitot	1999	Solid tumour	Phase I	30	Completed	(Pitot et al., 1999)
Dolastatin 10	NCT00003693	M.D. Jorge Cortes & M.D. Anderson	2018	Leukaemia	Phase I	30	Completed	ClinicalTrials.gov
	NCT00003693	J. Cortes	2018	Leukaemia, Myelodysplastic syndromes	Phase I	30	Completed	ClinicalTrials.gov
Dolastatin 10	–	U. Vaishampayan	2000	Prostate cancer	Phase II	16	Completed	(Vaishampayan et al., 2000)
		L.M. Krug	2000	Lung cancer	Phase II	16	Completed	(Krug et al., 2000)
		K. Margolin	2001	Melanoma	Phase II	12	Completed	(Margolin et al., 2001)
		E.D. Saad	2002	Colorectal cancer	Phase II	14	Completed	(Saad et al., 2002)
		M.A. Hoffman	2003	Ovarian cancer	Phase II	28	Completed	(Hoffman et al., 2003)
		E.A. Perez	2005	Breast cancer	Phase II	21	Completed	(Perez et al., 2005)
		H.L. Kindler	2005	Pancreaticobiliary cancers	Phase II	28	Completed	(Kindler et al., 2005)
	NCT00003778	M. von Mehren	2013	Soft tissue sarcoma	Phase II	37	Completed	(Von Mehren et al., 2004)
	NCT00003914	H.C. Pitot	2011	Kidney cancer	Phase II	30	Completed	ClinicalTrials.gov
	NCT00005579	S.M. Grunberg	2013	Leukaemia Lymphoma	Phase II	74	Completed	ClinicalTrials.gov
	NCT00003626	M.H.A. Hussain	2013	Prostate cancer	Phase II	15–30	Completed	ClinicalTrials.gov
	NCT00003557	H.L. Kindler	2014	Bile Duct, Gallbladder, Liver Cancer	Phase II	16	Completed	ClinicalTrials.gov
	NCT00003677	H.L. Kindler	2018	Pancreatic cancer	Phase II	9	Completed	ClinicalTrials.gov
Dolastatin 15 analog LU 103793	–	R.S. Marks	2003	Lung cancer	Phase II	17	Completed	(Marks et al., 2003)
		P. Schöffski	2004	Refractary cancer	Phase I	21	Completed	(Schöffski et al., 2004)
TZT-1027 (derivative of Dolastatin 10)		M.J.A. de Jonge	2005	Solid tumour	Phase I	17	Completed	(de Jonge et al., 2005)
	–							
		J Horti	2008	Lung cancer	Phase I	49	Completed	(Horti et al., 2008)
		K. Tamura	2007	Solid tumour	Phase I	18	Completed	(Tamura et al., 2007)
		N. Yamamoto	2009	Solid tumour	Phase I	40	Completed	(Yamamoto et al., 2009)
TZT-1027 and carboplatin	–	A. Greystoke	2006	Solid tumour	Phase I	14	Completed	(Greystoke et al., 2006)
TZT-1027	–	G. J. Riely	2007	Lung cancer	Phase II	32	Completed	(Riely et al., 2007)
SYN-D	–	L.A. Hammond	2004	Solid tumour	Phase I	1–6	Completed	(Hammond et al., 2004)

the therapeutic potency of dolastatin-10 on relapsed platinum-sensitive ovarian cancer, the patients were treated intravenously with a drug at a dose of 400 μg/m^2 every three weeks, and the tumour status was also measured. Results exhibited that under the confined level of toxicity, the drug was competent only for a few patients, which stipulated that dolastatin 10 required further refinements for the treatment of platinum-sensitive metastasis ovarian cancer (Hoffman et al., 2003). Soft tissue sarcomas are unusual malignancies with hardly any therapeutic choices for recurrent or metastatic disease. In the phase II trials of dolastatin 10, it was injected intravenously at a dose of 400 μg/m^2 and replicated every 21 days. During this treatment course, no patient showed apparent improvement, but one passed away from respiratory failure, which pointed to the fact that dolastatin 10 was not vindicated for additional research on the chemotherapy for advanced or recurrent soft tissue sarcoma (Von Mehren et al., 2004).

To characterize the effectiveness and toxicity of dolastatin 10 for patients with advanced hepatobiliary and pancreatic cancers, two parallel phase II trials were performed. Patients with metastatic liver cancer, cholangiocarcinoma, or gallbladder and pancreatic cancer have not received prior chemotherapeutic treatment before administration of the drug showed no obvious therapeutic effect but toxicity was tolerable (Kindler et al., 2005). Kindler also conducted a phase II trial with dolastatin 10 in patients suffering from metastatic adenocarcinoma of the pancreas. The toxic effects of the drug were used to assess its antitumour activity (ClinicalTrials.gov). Apart from these studies, a phase II trial was conducted to determine the effectiveness of dolastatin 10 in leukaemia, lymphoma, prostate, kidney, bile duct, gallbladder, and liver cancer (ClinicalTrials.gov). A phase II study was performed in patients with advanced non-small-cell lung cancer with dolastatin 15 analogue LU 103793. Patients have taken this drug at a concentration of 2.5 mg/m^2 consecutively for 5 days every 3 weeks and a total of 42 treatment cycles were administered with proportionately modest toxicity. No responses were seen. As no effective response was noted in patients with advanced lung cancer, the drug was stated inactive (Marks et al., 2003).

TZT-1027, a derivative of dolastatin 10 and a novel microtubule-interfering agent, showed higher antitumour properties and reduced toxicity in comparison with its parent compound, dolastatin 10 (Miyazaki et al., 1995) by restricting the assembly of microtubule by binding to tubulins (Natsume et al., 2000). The anticancer activity of this drug both *in vitro* and *in vivo* manifests its efficacy in clinical trials. In the current scenario, TZT-1027 was undergoing clinical evaluation, with a reduction in the size of the tumour and disease stabilization, being noticed in a subset of patients in the following paragraphs. Phase I study was conducted in patients with advanced solid tumours to evaluate the

pharmacokinetics and determine the dose-limiting toxicities (DLT) and the MTD of TZT-1027. 17 patients were administered escalating doses of TZT-1027 ranging from 1.35 to 2.7 mg/m^2 on the 1st and 8th days of a 3-week cycle, of which eight patients experienced pain and other side effects. A distinct correlation was also noted between the amount of decrease in several neutrophils and the AUC (area under the curve) of TZT-1027 (de Jonge et al., 2005). Later Phase I study of TZT-1027 was also conducted for non-small cell lung cancer. Results explained that the MTD opted to be 4.8 mg/m^2 for patients. Out of 49 patients, eight patients had dose-limiting toxicity (DLT). The pharmacokinetic parameters tended to amplify linearly with dose (Horti et al., 2008). In advanced refractory cancer, the 1-hour intravenous infusion of TZT-1027 every 3 weeks seems to be quite helpful for the patients. The recommended dose was considered to be 2.7 mg/m^2 in cancer patients (Schöffski et al., 2004).

In Japan, patients with advanced solid tumours were administered TZT-1027 on days 1st and 8th in 3-week courses. Three doses of TZT-1027 namely 1.5, 1.65, and 1.8 mg/m^2 were evaluated, of which 1.5 mg/m^2 was found to be the MTD which was quite a lower dose as compared to European patients (Tamura et al., 2007). In another study, Japanese patients with advanced solid tumours were administered weekly for 3 weeks with TZT-1027. The MTD tumour was 2.1 mg/m^2 where few patients did show some DLT (Yamamoto et al., 2009). Studies also demonstrated the combinatorial effect of TZT-1027 with carboplatin in patients with advanced solid tumour. The Phase I clinical trial was conducted with 14 patients who have taken a total of 55 cycles at three dose levels. Patients were administered 1.6 to 2.0 mg/m^2 doses of TZT-1027 and carboplatin and first DLTs were noted with 1.6 mg/m^2 of TZT-1027 and carboplatin. The most noticeable toxicities observed in patients include constipation, fatigue, neutropenia, infection, anaemia, and vomiting (Greystoke et al., 2006). A phase II trial of TZT-1027 was also conducted in patients with non-small cell lung cancer. The drug was administered on days 1st and 8th of a 21-day cycle and showed no anticancer activity in patients, hence, further development of TZT-1027 was not recommended (Riely et al., 2007). SYN-D a type of dolastatin analogue is a pentapeptide possessing a distinct mode of action that potentially varies from tubulin inhibitors and microtubule stabilizers. SYN-D has been chemically altered to ameliorate its pharmacological properties and made orally bioavailable with a therapeutic window. Three phase I studies were performed to determine the MTD and DLT of SYN-D. Neutropenia, anorexia, fatigue, and diarrhoea were the toxicity observed in patients after the administration of the drug (Hammond et al., 2004).

Challenges and limitations in the clinical development of dolastatins as anticancer agents

Dolastatins and their analogues have shown potential as adjuvants in oncology, but there are several limitations, clinical gaps, and pitfalls that need to be considered before they can be utilized as standard therapeutic options. First, these compounds often suffer from poor bioavailability due to issues like poor solubility, rapid metabolism, and limited tissue penetration. This significantly hampers their therapeutic efficacy, even though nanoparticle-based delivery methods have been explored with limited success.

Second, a narrow therapeutic index makes the management of toxicity particularly challenging, especially considering the severe side effects, including damage to normal healthy cells; balancing efficacy and tolerability to find the right therapeutic window is a considerable challenge. Third, cancer cells often develop resistance to chemotherapy agents, including dolastatins and their analogues; tumour heterogeneity further complicates this, as different cell populations within the same tumour may respond differently to a treatment. Fourth, while combination therapies could enhance treatment outcomes, identifying synergistic combinations and their appropriate sequencing is complex. Issues like drug interactions, overlapping toxicities, and potential antagonistic effects must be carefully considered. Fifth, obtaining regulatory approval for new anticancer agents like dolastatins is often a lengthy and costly process, affecting their accessibility; the complexity of clinical trial design, including patient stratification, appropriate control arms, and endpoints, needs meticulous planning to generate meaningful data. Moreover, demonstrating significant long-term survival benefits and improved quality of life is crucial for their adoption as standard treatment options. Finally, the high costs associated with these novel and complex therapies could limit their accessibility, necessitating consideration of economic factors in their clinical development.

Conclusions and future perspectives

Dolastatins and their analogues present a compelling landscape of potential natural and synthetic anticancer drug candidates. With their origin from marine organisms and their ability to inhibit microtubule assembly, these compounds have exhibited promising anticancer activities in preclinical studies. However, while the prospects are encouraging, several critical considerations should be taken into account. The remarkable cytotoxic potency of dolastatins and analogues makes them appealing candidates for targeting rapidly dividing cancer cells. Nonetheless, their therapeutic use is associated with challenges. Toxicity to healthy cells, the development of resistance, and issues related to specificity and delivery pose substantial hurdles that need to be addressed before they can be translated into effective clinical therapies. The limited translational clinical data available necessitates further comprehensive research through well-designed clinical trials to establish their safety, efficacy, and potential as adjuvants in oncology. Additionally, identifying reliable biomarkers for patient selection will be vital in optimizing treatment outcomes and personalizing therapy. Despite these obstacles, the future holds promise for dolastatins and analogues as anticancer drugs. Their potential in combination therapies, targeted delivery strategies, and synergy with immunotherapies could enhance their efficacy and broaden their application in cancer treatment. Moreover, investment in research, collaborations between different stakeholders, and continued innovation in drug development will play a pivotal role in advancing the understanding and utilization of these compounds. In conclusion, dolastatins and analogues stand as intriguing candidates with the potential in oncology adjuvant treatment. Addressing the current limitations and clinical challenges through scientific exploration and evidence-based approaches will pave the way for these compounds to contribute significantly to the fight against cancer and improve the lives of patients worldwide.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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