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VIOLACEIN: IT'S ANTICANCER PROPERTIES

Aktivitas Antikanker Violacein

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ABSTRACT
Violacein is a bacterial secondary product with various bioactivities, including anticancer activities. This narrative review aimed to evaluate anticancer potentials based on its modes of action, either at cellular, subcellular, or molecular levels or in tumour microenvironment. At cellular level, violacein can inhibit cancer cell proliferation, arrest cell cycle, induce apoptosis, autophagy, and cell differentiation. At subcellular level, violacein can modulate processes in mitochondria. At molecular level, violacein can generate reactive oxygen species, attenuate inflammation, repair oncogenes, upregulate suppression genes, inhibit or activate several cancer vital enzymes, and control various signalling pathways. Violacein indirectly influences communication between cancer cells and their tumour microenvironment by inducing apoptosis and autophagy and inhibiting metalloproteinases and angiogenesis. Violacein inactivates several signalling pathways, including MAPK, Akt/NF-kB, JAK2/STAT3, and TGFβ, which are essential for cancer cell development. Violacein is a promising anticancer drug candidate with broad coverage of various cancer diseases and diverse modes of action.

Keywords: *Apoptosis, Autophagy, Bacterial secondary product, Microenvironment, Signalling pathway*

ABSTRAK
Violacein merupakan produk sekunder bakteri yang penting karena memiliki berbagai bioaktivitas, termasuk aktivitas antikanker. Tinjauan naratif ini bertujuan untuk mengevaluasi potensi antikanker violacein yang ditinjau berdasarkan mekanisme aksi violacein, baik pada tingkat seluler, subseluler, molekuler, atau aksi dalam lingkungan mikro tumor. Pada tingkat seluler, violacein dapat menghambat proliferasi sel kanker, menghentikan siklus sel, serta menginduksi apoptosis, autofagi, dan diferensiasi sel. Pada tingkat subseluler, violacein dapat memodulasi berbagai proses dalam mitokondria. Pada tingkat molekuler, violacein dapat menghasilkan spesies oksigen reaktif, mengurangi inflamasi, memperbaiki onkogen, meningkatkan regulasi gen supresi, menghambat atau mengaktifkan enzim-enzim vital kanker, serta mengendalikan berbagai jalur pensinyalan. Violacein secara tidak langsung memengaruhi komunikasi antara sel kanker dan lingkungan mikro tumornya dengan jalan menginduksi apoptosis dan autofagi serta menghambat metaloproteinase dan angiogenesis. Violacein menonaktifkan beberapa jalur pensinyalan, termasuk MAPK, Akt/NF-kB, JAK2/STAT3, dan TGFβ, yang dibutuhkan untuk perkembangan sel kanker. Violacein merupakan kandidat obat antikanker yang menjanjikan dengan berbagai mekanisme aksi.

Kata Kunci: *Apoptosis, Autofagi, Jalur persinyalan, Lingkungan mikro tumor, Produk sekunder bakteri.*

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INTRODUCTION

Cancer remains a major public health concern and is one of the leading causes of death worldwide. According to the International Agency for Research on Cancer for the year 2022 report, approximately 20 million new cancer cases were diagnosed, and 9.7 million cancer-related deaths occurred (Bray et al. 2024). Roughly one in nine men and one in twelve women die from cancer (Bray et al. 2024). These cancer statistics underscores the urgent need for the development of novel and effective anticancer therapies. In recent years, considerable attention has been directed toward the exploration of microbial secondary metabolites as potential anticancer agents. Secondary metabolites are bioactive compounds produced by microorganisms as part of their metabolic activities. These metabolites are not directly involved in the bacterial growth, development, or reproduction (Mohan et al. 2022). Instead, these molecules were often produced as stress response, to enhance environmental adaptability and to facilitate interactions to other organisms (Ruiz et al. 2010). Bacteria, in particular, have proven to be invaluable source of pharmacologically active secondary metabolites, such as streptomycin (*Streptomyces griseus*, antibacterial activity), bile salt hydrolase (*Lactobacillus acidophilus*, cholesterol lowering activity), tacrolimus (*Streptomyces tsukubaensis*, immunosuppressant activity) (Vaishnav and Demain 2011).

Noteworthy, many bacterial secondary metabolites have been successfully developed into anticancer drugs, such as actinomycin D, bleomycins, doxorubicin, and pentostatin (Mohan et al. 2022). However, the clinical use of existing chemotherapeutic agents is increasingly compromised by the emergence of drug resistance and significant dose-limiting toxicities (Al-malky, Al Harthi, & Osman 2020; Chorawala, Oza, & Shah 2012). Consequently, there is a need to explore new bacterial secondary metabolites with anticancer properties. Among the bacterial metabolites with significant potential is violacein (VIOL), a violet pigment predominantly synthesized by *Chromobacterium violaceum* and *Janthinobacterium lividum* (Dahlem et al. 2022; Durán et al. 2021a). VIOL is synthesized by bacteria through sequential actions of five enzymes encoded by the vioA to vioE genes, through which condensation of two tryptophan molecules occur (Choi et al. 2015). VIOL is produced under stress conditions, resulting in prolonged survival bacteria (Yogini, Waman, & Rajashree 2022). VIOL is also suggested as respiratory pigment. VIOL can be released into the surrounding environment and act as a defensive mechanisms by inducing cell death of competitiors or pathogens (Yogini et al. 2022). VIOL has demonstrated a wide range of bioactivities, including antimicrobial, antiparasitic, and anticancer effects (Durán et al. 2021b). Despite these promising properties, its potential as an anticancer agent remains undervalued.

This study evaluates updated research results on the biological activities of violacein against cancer. The current review aims to briefly overview violacein, its anticancer spectrum, and its modes of action at cellular, subcellular (mitochondria), molecular, and microenvironment levels. This review provides new insight into VIOL as an anticancer agent that may introduce new alternatives for anticancer therapy.

METHODS

The existing articles were searched from PubMed, ScienceDirect, and Google Scholar using the terms violacein and cancer up to 10th October 2024. Among 468 articles, 99 were relevant to the objectives of this review. The review critically analysed relevant articles. The research findings concerning violacein's roles were possible via intense extraction of collected data or information on cellular, subcellular, molecular, and microenvironment levels of cancer cells' behaviour. It synthesised information and knowledge on modes of action of violacein to delineate the complexity of carcinogenesis.

RESULTS AND DISCUSSION

A short overview of violacein and its anticancer spectrum

Several bacterial strains can produce VIOL as their secondary metabolite. Two

major producing strains are *Chromobacetrium violaceum* and *Janthinobacterium lividum*. VIOL is a deep violet-coloured indole derivate (Dahlem et al. 2022; Kim et al. 2021). It is an insoluble compound that prevents its potential therapeutic uses. In addition to violacein, the strain may also produce deoxyviolacein (Figure 1). Both have potential anticancer activity (Menezes et al. 2013).

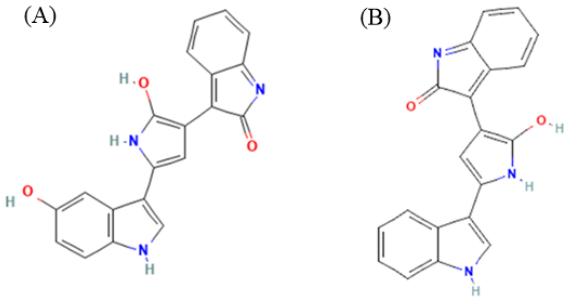


Figure 1. Chemical structure of violacein (A) and deoxyviolacein (B)

Besides its bioactivities, like antibacterial, antifungal, antiviral, antioxidant, and antiparasitic (Neroni et al. 2022; Rivero Berti et al. 2020), VIOL also exhibits anticancer activity (Table 1) (Dahlem et al. 2022; Masuelli et al. 2015). Interestingly, in vivo studies showed that VIOL occurs without toxicity to main organs (Bromberg et al. 2010).

VIOL is a hydrophobic bisindole that is present in encapsulated extracellular membrane vesicles. Although it is insoluble in water, it can remain in the aqueous phase when it is present within membrane vesicles (Choi et al. 2020). Therefore, the membrane-vesicle-enclosed VIOL can be transported to the aqueous environment (Venkatramanan and Nalini 2024).

Table 1. Anticancer activities of violacein at a cellular level

Type of cancer, cell lines	Anticancer activity at a cellular level
Brain tumor	Inhibit metastasis, Induce apoptosis (Malla et al., 2021)
Breast cancer, MCF-7	Inhibit cell proliferation (Hashimi et al., 2015) and metastasis (Platt et al., 2014), and induce apoptosis and necrosis (Alshatwi et al., 2016).
Cervix cancer, HeLa	Inhibit cell proliferation (Alem et al., 2020; Dahlem et al., 2022) and metastasis (Dahlem et al., 2022)
Colon cancer, HT29, and HCT116	Inhibit cell proliferation (Hashimi et al., 2015) and induce apoptosis (de Carvalho et al., 2006)
Colorectal cancer, HT29	Inhibit cell proliferation and metastasis (de Souza Oliveira et al., 2022; Durán et al., 2021; Faria et al., 2022), and induce apoptosis (Durán et al., 2021).
Head and neck carcinoma (HNC)	Inhibits cell proliferation (Hashimi et al., 2015) and induce apoptosis and autophagy (Masuelli et al., 2015)
Hepatocellular carcinoma (HCC), Huh7 and Hep3B	Inhibit cell proliferation and arrest cell cycle, and induce apoptosis (Kim et al., 2021)
Leukemia HL60, TF1	Inhibit cell proliferation (Paredes-Gamero et al., 2013; Queiroz et al., 2012), and induce apoptosis (Ferreira et al., 2004)
Lung cancer, A549	Inhibit cell proliferation (Rivero Berti et al., 2020) and induce apoptosis (Hashimi et al., 2015)
Osteosarcoma rhabdomyosarcoma	Inhibit cell proliferation and metastasis, and induce apoptosis (Milosevic et al., 2023)
Skin cancer, 2237, B16F10, C50, NIH3T3	Inhibit cell proliferation and induce apoptosis (Mojib et al., 2011)
Urinary bladder, HTB4 (T24), HTB9 (5637)	Inhibit cell proliferation and arrest cell cycle, and induce apoptosis (Neroni et al., 2022)

1. Effect of violacein on cancer progression at the cellular level

VIOL's anticancer modes of action cover four levels: cellular, subcellular, molecular, and cancer microenvironment. The cellular responses against VIOL include cell proliferation, cell cycle, apoptosis, autophagy, necrosis, and cell differentiation.

1.A. Effect of violacein on cancer cell proliferation

VIOL is a steadfast medicine capable of devastating at least three cancer features: proliferation inhibition, cell death resistance, and metastasis inhibition (Durán et al. 2021a). It exhibits antiproliferative activity in many cancer cell lines (Tables 1 and 2). Its various modes of action determine the therapeutic potential of VIOL in effectively suppressing many cancer cell lines. However, it cannot inhibit the proliferation of any cell lines (Ferreira et al. 2004). As shown in Table 2, we can divide the cell lines into two groups based on their sensitivity (IC_{50}) against VIOL. We can use IC_{50} =1 μM as an arbitrary cutoff. Several cell lines are susceptible to VIOL because the $IC_{50} \leq 1$ μM and the other are not very sensitive because the $IC_{50} > 1$ μM. Several cell lines are sensitive against VIOL, like breast, bladder, colon, cervix, HNC (Head and neck cancer), leukemia, lung, osteosarcoma, prostate, rhabdomyosarcoma, and skin cancers.

Table 2. IC_{50} of violacein on the cell proliferation of various cancer lines

Types of cancer	Cell lines	IC_{50} (μM)	Ref.
Breast cancer	MCF-7	4.500 in 24 h 1.700 in 48 h 0.510 in 72 h	(Alshatwi et al. 2016)
		0.106 in 24h	(Hashimi et al. 2015)
		1.890 in 48h	(Dahlem et al. 2022)
Bladder cancer	HTB4 (T24)	0.114 in 24h	(Neroni et al. 2022)
	HTB9 (5637)	0.113 in 24h	(Neroni et al. 2022)
Colon cancer	HT29	0.045 in 24h	(Hashimi et al. 2015)
	HCT116	0.284 in 24h	(Hashimi et al. 2015)
		1.200 in 48h	(Mojib et al. 2011)
Cervix	HeLa	0.352 in 24h	(Hashimi et al. 2015)
		1.000 in 48h	(Dahlem et al. 2022)
Human uveal melanoma	92.1	2.780 in 24h	(Saraiva et al. 2004)
	OCM-1	3.690 in 24h	(Saraiva et al. 2004)
HCC	Huh7	7.970 in 24h 6.710 in 48h 6.100 in 72h	(Kim et al. 2021)
		1.880 in 48h	(Dahlem et al. 2022)
	Hep3B	8.010 in 24h 8.410 in 48h 8.230 in 72h	(Kim et al. 2021)
	HepG2	9.860 in 48h	(Dahlem et al. 2022)
HNC	FaDu	5.790 in 24h 5.290 in 48h 5.940 in 72h	(Masuelli et al. 2015)
	CAL-27	6.720 in 24h 4.130 in 48h 4.150 in 72h	(Masuelli et al. 2015)
	SCC-15	8.070 in 24h 7.060 in 48h 4.650 in 72h	(Masuelli et al. 2015)
	SALTO	6.880 in 24h	(Masuelli et al. 2015)

Types of cancer	Cell lines	IC_{50} (µM)	Ref.
		2.320 in 48h	
		2.480 in 72h	
	HN5	0.268 in 24h	(Hashimi et al. 2015)
Leukemia	HL60	0.700 in 24h	(Ferreira et al. 2004)
Lung	A549	0.286 in 24h	(Hashimi et al. 2015)
		0.660 in 48h	(Dahlem et al. 2022)
Osteosarcoma	OS	0.350 to 0.880	(Milosevic et al. 2023)
Prostate	PC3	0.269 in 24h	(Hashimi et al. 2015)
Rhabdomyosarcoma	RMS	0.350 to 0.880	(Milosevic et al. 2023)
Skin cancer	A431	0.288 in 24h	(Hashimi et al. 2015)
	2237	0.500 in 48h	(Mojib et al. 2011)
	SK-MEL5	0.390 in 48h	(Dahlem et al. 2022)
	SW620	0.620 in 48h	(Dahlem et al. 2022)
	PANC-1	1.440 in 48h	(Dahlem et al. 2022)
	CC-SW-1	2.890 in 48h	(Dahlem et al. 2022)

1.B. Effect of violacein on arresting cancer cell cycle

VIOL can arrest the cell cycle at the sub-G1 phase (Kim et al. 2021) and G2/M and induce G0/G1 (Kido et al. 2021). It can inhibit cell proliferation associated with sub-G1 phase and G2/M phase arrest (Mojib et al. 2011). As described in Table 3, VIOL can significantly increase the population of cells in the sub-G1 phase and decrease the population of cells in the G0/G1, S, and G2/M phases (Kim et al. 2021).

Table 3. Effect of violacein on cancer cell cycle (%)

Cell line	Violacein (µM)	Sub-G1	G0/G1	S	G2/M
T24*	0.0	6.90	65.60	13.50	14.00
	1.0	9.40	46.60	31.40	12.60
5637*	0.0	7.90	24.80	39.20	28.10
	1.0	8.70	48.60	27.70	15.00
CAL-27**	0.0	3.98	74.53	4.88	16.34
	5.0	10.98	10.01	34.11	42.83
	10.0	74.43	6.73	5.71	12.44
SCC-15**	0.0	4.58	66.90	8.84	19.55
	5.0	14.62	37.51	17.64	30.16
	10.0	78.26	7.74	7.52	6.41
FADU**	0.0	4.34	59.29	9.06	27.81
	5.0	13,23	54.05	9.46	2.78
	10.0	85.54	8.020	2.49	4,11
SALTO**	0.0	9.45	57.88	13.32	19.87
	5.0	33.13	55.24	7.66	4.45
	10.0	67.97	27.86	2.34	1.94
MCF-10A**	0.0	0.35	82.83	5.96	11.06
	5.0	0.65	81.92	6.58	11.11
	10.0	0.80	79.87	6.41	13.21
2237***	0.0	Nd	44.52	48,44	7.04
	0.1	Nd	46.52	46.45	7.03
	0.2	Nd	47.63	43.26	9.11
	0.5	Nd	50.43	39.42	10.16
	1.0	Nd	52.77	23.91	23.32
Huh7****	0.0	0.00	49.60	17.90	32.60
	5.0	1.10	48.70	17.20	33.00

Cell line	Violacein (µM)	Sub-G1	G0/G1	S	G2/M
	10.0	12.30	49.10	14.50	24.10
	20.0	35.90	37.00	11.20	15.90

note: * (Neroni et al. 2022); **(Masuelli et al. 2015); *** (Mojib et al. 2011); **** (Kim et al. 2021) ; Nd: not determined

1.C. Effect of violacein on inducing apoptosis

Cancer is closely related to the altered mechanism of cell death, which falls into three modes: apoptosis, autophagy, and necroptosis (necrosis) (Benvenuto et al. 2020). VIOL can induce apoptosis, resulting in the antiproliferative effect on several cancer cell lines (Table 4) (de Carvalho et al. 2006; Kim et al. 2021; Masuelli et al. 2015). However, in the case of leukaemia, the antiproliferative effects of VIOL are not mediated by apoptosis and autophagy (Queiroz et al. 2012).

VIOL can cause nuclear condensation that results in the loss of MMP (Mitochondrial membrane potential) and subsequently induces early apoptosis. Both can inhibit cancer cell proliferation (Kim et al. 2021). It can prevent the growth of various cancer cells by inducing apoptosis, autophagy, and necrosis. However, it is ineffective in leukemia cells, normal lymphocytes, and monocytes (Ferreira et al. 2004; Masuelli et al. 2015; Melo et al. 2003).

Table 4. The effect of violacein on apoptotic cell death

Cell line	Violacein (µM)	Apoptotic cell (%)	Ref.
Hub7	0.00 at 24h	4.15	(Kim et al. 2021)
	5.00 at 24h	7.30	
	10.00 at 24h	43.50	
	20.00 at 24h	77.05	
2237	0.00 at 48h	17.50	(Mojib et al. 2011)
	0.10 at 48h	26.60	
	0.20 at 48h	49.60	
	0.50 at 48h	60.60	
	1.00 at 48h	78.00	
MCF7	0.45 at 48h	78.00	(Alshatwi et al. 2016)
	0.45 at 72h	61.00	
CACO-2	0.00 at 24-72h	2.00	(de Carvalho et al. 2006)
	5.00 at 24h	11.00	
	5.00 at 48h	45.00	
	5.00 at 72h	68.00	
HT29	0.00 at 24-72h	2.00	(de Carvalho et al. 2006)
	5.00 at 24h	8.00	
	5.00 at 48h	40.00	
	5.00 at 72h	38.00	
HL60	0.5 at 72h	35.00	(Melo et al. 2003)
EAT	0.0 at 72h	5.0	(Bromberg et al. 2010)
	2.0 at 72h	6.0	
	4.0 at 72h	12.5	
	5.0 at 72h	28.5	

1.D. Effect of violacein on inducing cell cancer autophagy

Autophagy (self-eating) is an intracellular degradation process that supports nutrient recycling. This process is like a recycling process that removes or recycles unnecessary molecules for other purposes (Amaravadi, Kimmelman, & Debnath 2019).

It is an essential regulatory process in cancer. However, autophagy has controversial roles in cancer. It can act either by protecting against the start of cancer or by promoting cancer growth (Amaravadi et al. 2019; Ascenzi et al. 2021). It can degrade oncogenic proteins that then can suppress cancer. Nevertheless, cancer cells can activate autophagy for survival in cellular stress conditions (Benvenuto et al. 2020). VIOL can also modulate autophagy. It can induce the autophagy of several cancer cell lines. Therefore, treatment with VIOL can induce autophagy (Gonçalves et al. 2016; Masuelli et al. 2015).

1.E. Effect of violacein on increasing necrosis

Necrosis or necroptosis is a passive cell death. Poor nutrient supply disrupts energy-dependent and membrane-mediated ion channels and can trigger necrosis. Necrotic cells have increased cell volume and loss of membrane integrity. As a result, cell lysis or damage occurs, increasing the release of lysosomal lytic enzymes such as proteases and nucleases. VIOL can also induce necroptosis and increase necroptotic cells. Its ability to increase necroptosis is probably due to its ability to generate intracellular ROS (Table 5) (Alshatwi, Subash-Babu, & Antonisamy 2016).

Table 5. Influence of violacein on the ROS production

Cell line	Violacein (μM)	% of control	Ref
CACO-2	2.5 at 4h	140	(de Carvalho et al. 2006)
	5.0 at 4h	160	
	7.5 at 4h	150	
	10.0 at 4h	140	
HT-29	2.5 at 4h	105	(de Carvalho et al. 2006)
	5.0 at 4h	110	
	7.5 at 4h	105	
	10 at 4h	100	
CAL-27	5.0 at 05h	100	(Masuelli et al. 2015)
	10.0 at 0.5h	100	
	20.0 at 0.5h	108	
SCC-15	5.0 at 0.5h	102	(Masuelli et al. 2015)
	10.0 at 0.5h	102	
	20.0 at 0.5h	119	
FaDu	5.0 at 0.5h	102	(Masuelli et al. 2015)
	10.0 at 0.5h	118	
	20.0 at 0.5h	120	
EAT	5 at 8h	137	(Bromberg et al. 2010)
	4.0 at 12h	138	
	5.0 at 24h	120	
Huh7	0.5 at 6h	150	(Dahlem et al. 2022)
	1.0 at 6h	160	
	1.0 at 11h	160	
	2.5 at 6h	120	
A549	1.0 at 6h	110	(Dahlem et al. 2022)
	1.0 at 11h	170	
	2.5 at 11h	170	
MCF7	0.25 at 24h	175	(Alshatwi et al. 2016)
	0.45 at 24h	200	
	4.50 at 24h	210	

1.F. Effect of violacein on modulating cell differentiation

Cancer is principally undifferentiated and inadequately differentiated cell populations (Fulghieri, Stivala, & Sottile 2021; Solé and Aguadé-Gorgorió 2021). The disturbances in differentiation are significant in leukemias (Motofei 2022; Paredes-Gamero et al. 2013). In the case of leukemia, morphological studies showed that VIOL can induce terminal differentiation and partly inhibit leukemic cell growth by inducing cell differentiation (Melo et al. 2009; Melo et al. 2003; Paredes-Gamero et al. 2013).

2. Effect of violacein on cancer progression at the subcellular level

Cancer disorders are strictly associated with mitochondrial dysfunctions causative to cell proliferation, metabolic reprogramming, apoptosis resistance, invasion/metastasis, and angiogenesis induction. These dysfunctions also affect the function of immune cells in the Tumor microenvironment (TME) (Bachmann, Pontarin, & Szabo 2019). Mitochondria have two interlinked roles in regulating apoptosis and producing ATP (Burke 2017). In cancer cells, mitochondria can face two significant dysfunctions. First, they resist the induction of MMP, which is the limiting step of apoptosis. Second, they reveal reduced oxidative phosphorylation, which results in less ATP generation (Rustin and Kroemer 2007). Normally differentiated cells trust mainly oxidative phosphorylation for the energy needed for cellular processes, but most cancer cells rely on aerobic glycolysis instead, a phenomenon termed "the Warburg effect" (Vander Heiden, Cantley, & Thompson 2009).

VIOL administration can increase the MMP. and dissipate the MMP disruption. By increasing the MMP, mitochondrial outer membrane permeabilization (MOMP) may allow VIOL to trigger apoptosis (Kim et al. 2021; Leal et al. 2015). Therefore, VIOL increase of the MOMP exhibits antiproliferation effects against several cancer cell lines (Mojib et al. 2011).

Mitochondria can contact and communicate with the endoplasmic reticulum (ER). The ER-mitochondrial contact site contains many proteins and enzymes that regulate fundamental cellular processes, including autophagy and ER stress. In addition, this site is engaged by oncogenes and suppressor genes. Changes in this contact site and ER stress can affect mitochondrial (dys)regulation, thereby influencing the progression of cancer cells (Yang et al. 2023). VIOL can stimulate apoptosis due to ER stress. Through ER stress, VIOL can induce the death of leukemia cells (Queiroz et al. 2012).

3. Effect of violacein on oxidative stress and inflammation

VIOL can affect cancer progression at molecular levels (Table 6). It interferes with survival transduction signaling pathways in various cell lines, such as activating caspase, transcription of NFκB target genes, and p38 (mitogen-activated protein kinase) MAPK, TNFα (Tumor necrosis factor-alpha) signal transduction (Ferreira et al. 2004). It may also induce kinome reprogramming (Queiroz et al. 2012).

Table 6. Influence of violacein on Caspase-3 and Caspase-9 activity (%)

Cell line	Violacein µM	Caspase-3	Caspase-9	Ref.
CACO-2	0	100		(de Carvalho et al. 2006)
	5 at 24h	300		
	5 at 48h	350		
	5 at 72h	325		
HT29	0	100		(de Carvalho et al. 2006)
	5 at 24h	150		
	5 at 48h	175		
	5 at 72h	140		
	0.6 at 24h	0		(Hashimi et al. 2015)
2237	0 at 48h	100	100	(Mojib et al. 2011)
	0.1 at 48h	120	111	

Cell line	Violacein µM	Caspase-3	Caspase-9	Ref.
	0.2 at 48h	120	156	
	0.5 at 48h	320	400	
	1.0 at 48h	520	600	
Hub7	0	100	0	(Kim et al. 2021)
	5 at 24h	100	100	
	10 at 24h	100	2,200	
	20 at 24h	750	7,900	
	10 at 8h	240		(Dahlem et al. 2022)
MCF7	0	100	100	(Alshatwi et al. 2016)
	0.45	183	167	
	4.5	200	167	
	10 at 8h	115		(Dahlem et al. 2022)
EAT	0	100	100	(Bromberg et al. 2010)
	3 at 72h	250	240	
	4 at 72h	265	260	
	5 at 72h	550	600	
HCT116	10 at 8h	125		(Dahlem et al. 2022)
A549	0.6 at 24h	180		(Hashimi et al. 2015)

Note: Caspase activity was calculated as a percentage of caspase activity in nontreated cells, defined as 100%

3.A. Effect of violacein on the generation of ROS

The generation of ROS is related to redox metabolism or oxidative phosphorylation. The dysfunctions in redox metabolism of cancer cells can induce abnormal accumulation of ROS. Cancer progression is related either to ROS-dependent malignant transformation or oxidative stress-apoptosis (Wang et al. 2021). ROS plays contradictory roles in cancer proliferation, metastasis, and apoptosis. Particularly, it plays a central role in the induction of apoptosis (Kim et al. 2021).

VIOL can increase the generation and accumulation of ROS, which can then induce apoptosis. even not occur in any cell line (Table 5) (Alshatwi et al. 2016; Bromberg et al. 2010; de Carvalho et al. 2006; Jędruszczak et al. 2019; Masuelli et al. 2015). Additionally, as a potent antioxidant, VIOL can also protect the membranes against ROS-induced peroxidation. It is vital in protecting membranes against accumulated ROS due to oxidative stress (Cao et al. 2007; Konzen et al. 2006).

3.B. Effect of violacein on attenuating cancer-related inflammation

The inflammation process is critical to control the growth of cancer cells. Both acute and chronic inflammation can create harmful effects on the cell. Cancer progress is also an inflammatory disease (Verinaud et al. 2015). As a bacterial bioactive compound, VIOL does not induce inflammation. Interestingly, it can reduce acute and chronic inflammation. It can suppress the production of cytokine (in acute inflammation) and stimulate regulatory T cells (in chronic inflammation) (Verinaud et al. 2015).

3.C. Effect of violacein on regulating oncogenes and tumor suppressor genes (TSGs)

Genetic changes such as activated oncogenes or dysfunctional TSGs often occur within cancer cells (Bonomi et al. 2014).

Effect of violacein on regulating oncogenes

When mutated, oncogenes, such as RAS and RAF, are crucial for cancerogenesis, particularly in inflammation and angiogenesis (Borrello, Degl'Innocenti, & Pierotti 2008). VIOL can diminish the sustainability of RAS- and RAF-mutated cancer cells. As a result, VIOL can significantly induce apoptosis, modulate autophagy and reduce the invasion capacity (Gonçalves et al. 2016).

Effect of violacein on regulating cancer-suppressor genes (TSGs)

TSGs can regulate cell cycle stages, proliferation, DNA repair, and apoptosis. Their dysfunctions can stimulate cancer cell growth and metastasis (Joyce, Rayi, & Kasi 2024). As metastatic suppressors, TSGs are essential in inhibiting DNA synthesis, activating DNA repair, arresting the cell cycle, and inducing apoptosis, autophagy, and ferroptosis (Megino-Luque and Bravo-Cordero 2023; Rusin 2024; Szewczyk-Roszczenko and Barlev 2023).

VIOL can upregulate several TSGs, namely p53, p21(Cyclin-dependent kinase inhibitor-1), p27, p62 (Sequestosome-1), Bax, and Bcl2 (Table 6). It can also affect Beclin-1, a crucial regulator of autophagy and the communicator between autophagy and apoptosis (Prerna and Dubey 2022). As an autophagy protein, Beclin-1 can interact with a Bcl-2-interacting protein. The interaction between Beclin-1 and Bcl-2 can regulate autophagy and be involved in cancer diseases (Xu and Qin 2019). Moreover, VIOL can influence the formation of both Bax (pro-apoptotic protein) and Bcl-2 (anti-apoptotic protein). The increased Bax/Bcl-2 ratio means the increase of Bax and the decrease of Bcl-2, which are related to the growth of cancer cells. VIOL can decrease the formation of Bcl-2 and simultaneously increase the formation of Bax. This condition is inducing apoptosis (Luo et al. 2022; Masuelli et al. 2015; Mojib et al. 2011).

Table 7. Effect of violacein on Bax/Bcl-2 ratio

Cell line	Violacein µM	ratio	Ref
2237 fibrosarcoma	0,00	1.00	(Mojib et al. 2011)
	0.10	1.10	
	0.20	2.00	
	0.50	5.00	
	1.00	11.00	
MCF7	0.00	1.20	(Alshatwi et al. 2016)
	0.45	2.50	
	4.50	2.60	
FaDu	0.00	1.69	(Masuelli et al. 2015)
	5-7.5	2.36	
CAL-27	0.00	1.72	
	5-7.5	2.70	
SCC-15	0.00	0.91	
	5-7.5	1.52	
SALTO	0.00	0.52	
	5-7.5	1.49	

4. Effect of violacein on cancer-key enzymes

4.A. Effect of violacein on caspases

The apoptosis induction is concurrent with increased activities of caspase-2, -3, and -9 (Bromberg et al. 2010). VIOL can increase the expression of caspases in various cell lines (Alshatwi et al. 2016; Bromberg et al. 2010; Ferreira et al. 2004; Melo et al. 2003; Mojib et al. 2011); however, it can also increase the expression of caspases, as in the case of A549 cell line (Hashimi, Xu, & Wei 2015).

4.B. Effect of violacein on matrix metalloproteinases (MMP-2 and -9)

As a promising anticancer agent, VIOL can influence the activation of MMP-2 and MMP-9, which have an essential role in the metastatic process. Active MMP-2 and MMP-9 are required to produce inflammatory chemokine, CXCL12, that can interact with the chemokine receptor, CXCR4. CXCR4 has crucial implications in metastasis. MMP-2 and -9 can initiate its expression. VIOL can knock down the activities of MMP-2 and -9, and as a result, the

progression marker CXCR4 is also knockdown. VIOL can efficiently inhibit the inflammatory injury-induced MMP-2/-9 mediated CXCL-12 chemokine and the specific metastatic ligand CXCR4 expression. Therefore, VIOL is known for its antimetastatic effect. VIOL can directly inhibit the secretion of CXCL-12. Indeed, it has a unique anticancer mechanism that reduces CXCL12/CXCR4, chemokine-receptor-ligand interaction. In the MCF cell line, its inhibition against MMP-2 activation is mediated by the cytokine (TNFα and TGFβ) (Platt et al. 2014).

4.C. Effect of violacein on protein kinases

Distinctive serine/threonine and tyrosine kinases are essential receptors of various signalling pathways (Yan et al. 2006). Any dysregulation of kinase function may impact cancer progression (Pillai et al. 2015). VIOL can inhibit Akt (Protein kinase B) phosphorylation with the succeeding activation of apoptotic pathways and downregulation of NFκB signalling (Kodach et al. 2006). It can also induce kinome reprogramming to overcome the death-signalling dysfunctions of resistant leukaemia cells. Kinome profiling analysis for protein kinase shows that apoptosis and autophagy do not mediate the death-induced leukemic progenitor cells (Queiroz et al. 2012).

4.D. Effect of violacein on poly-ADP-ribose-polymerase (PARP)

PARPs have the potential to repair the DNA single-strand breaks. PARP can produce PAR (Poly(ADP-ribosyl)-ation, or PARylation) during DNA damage. PARP interacts with various oncogenic proteins (Alemasova and Lavrik 2019; Rajawat, Shukla, & Mishra 2017). Therefore, the inhibition of PARP using PARP inhibitors (PARPi) is a promising anticancer strategy (Rajawat et al. 2017). During apoptosis, PARP cleavage happens. An increase in apoptosis is associated with an increased PARP cleavage (Alshatwi et al. 2016; Mojib et al. 2011). VIOL treatment can increase PARP cleavage (Masuelli et al. 2015), which indicates that VIOL can promote apoptosis (Mehta et al. 2015).

4.E. Effect of violacein on fatty acid synthase (FASN)

Lipids can supply the building blocks needed for the sustainability of cancer growth. They can also serve as an alternative fuel source to generate ATP. In lipid metabolism, fatty acid synthase (FASN) is critical to cancer growth and survival. It can rewire cancer cells to attain high energy needs. Moreover, FASN has functional roles in glycolysis and amino acid metabolism (Fhu and Ali 2020). Cellular FA biosynthesis requires FASN (Günenc et al. 2022). By downregulating FASN, VIOL can induce apoptosis (Aires-Lopes et al. 2024).

4.F. Effect of violacein on the low molecular weight protein tyrosine phosphatase (LMWPTP)

LMWPTPs regulate cell growth and proliferation by dephosphorylating/inactivating RTKs (Receptor tyrosine kinase). Disturbances in the regulation of protein tyrosine phosphorylation can trigger cancers (He et al. 2014). The overexpressed LMWPTP can potentiate cell-cell contact stability, supporting cancer aggressiveness (Chiarugi et al. 2004; Raugei, Ramponi, & Chiarugi 2002). LMWPTP is a positive regulator of cancer progression and metastasis (Faria et al. 2021). Its expression significantly increases in colorectal cancer (CRC). LMWPTP inhibition can reduce CRC growth and metastasis (Hoekstra et al. 2015). The decrease in LMWPTP level can also stimulate the autophagy process (Faria et al. 2020). VIOL can repair energetic metabolism by decreasing LMWPTP activity and shifting glycolysis to oxidative metabolism. This shift is associated with altering mitochondrial efficiency. VIOL can inhibit LMWPTP, which stimulates invasive migration (Faria et al. 2022).

5. Effects of violacein on several signaling pathways in cancer

Signaling pathways are fundamental regulators of cellular communication, both in normal and cancer cells. Changes in these pathways can influence cell performance and have consequences for emerging diseases, notably cancer (Radak and Fallahi 2024). Several signaling pathways can reg-

ulate metabolic pathways and have implications for cell proliferation. They incorporate nutrients into biomass. Specific mutations enable cancer cells to consume and metabolise nutrients needed for their proliferation (biosynthesis) rather than produce ATP efficiently (bioenergy). An appropriate understanding of the links between cellular metabolism and growth control may lead to a better approach to treating cancer (Vander Heiden et al. 2009).

There are many pathways involved in cancer. All these pathways can increase cancerogenesis, decrease apoptosis, and tumour suppressor genes. The inability of cells to regulate signaling pathways can lead to cancer. Cells can terminate the signal pathways by using the activity of GTPase of G protein, phosphatase to reserve the effect of protein kinase and inactivate the receptor of the pathway.

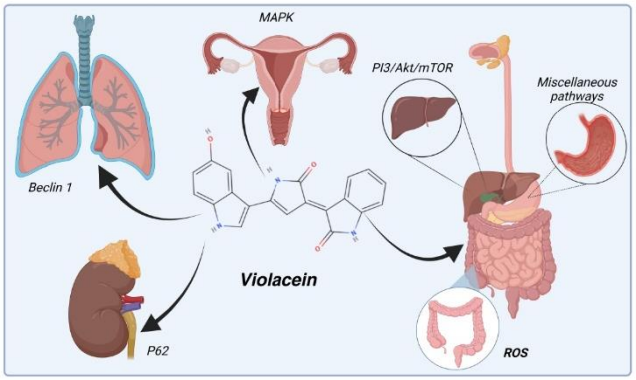


Figure 2. Effects of violacein on various signaling pathways

The anticancer activity of VIOL closely interacts with signalling receptors and pathways. VIOL can regulate apoptosis and autophagy by participating in several signalling pathways, such as the PI3K (Phosphatidylinositol 3-kinase)/Akt/mTOR (Mammalian target of rapamycin, Ser/Thr protein kinase) signalling pathway, the MAPK signalling pathway, Beclin-1, ROS signalling pathway, P62/SQSTM1 (Luo et al. 2022). The anticancer potential of VIOL depends on its ability to inhibit or activate regulatory signaling pathways involved in apoptosis and autophagy. VIOL can strongly affect the apoptosis and autophagy process as an inhibitor or activator (Figure 2).

5.A. Interaction of violacein on mitogen-activated protein kinases pathways (MAPK Pathways)

MAPK signaling pathway is one of the most important transduction pathways that mediate signal transfer from the cell surface to the intracellular. It plays a crucial role in autophagy. MAPK can activate RAS and RAF. Therefore, mutations in the MAPK (Mitogen-activated protein kinase) pathway significantly impact cancer development. Overexpression of MAPK occurs in many types of cancer. Three MAPK pathways can regulate cellular activities, namely the MAPK/ERK1/2 (Extracellular regulated kinase 1/2), MAPK/JNK, and p38/MAPK signaling pathways (Luo et al. 2022).

The MAPK/ERK1/2 (also known as Ras-Raf-MEK (Mitogen-activated protein kinase)-ERK pathway) embraces many proteins, interconnecting by phosphorylating. It acts as an "on" or "off" switch. B-RAF or N-RAS mutations can activate the MAPK/ERK1/2 and AKT signal pathways. If one of the above proteins is mutated, the switch is stuck. This stacking switch is a vital step in cancer growth. The anticancer effect of VIOL is associated with the downregulation of ERK1/2 and AKT signalling (Kim et al. 2019). VIOL can inhibit the ERK1/2 phosphorylation (Masuelli et al. 2015). By inhibiting the phosphorylation of MAPK, VIOL can induce apoptosis and inhibit angiogenesis. Moreover, VIOL can diminish the viability of RAS- and RAF-mutated cancer cells. VIOL can also reduce the invasion capacity of metastatic cells (Gonçalves et al. 2016).

The MAPK/JNK can stimulate autophagy via the Beclin-1-dependent or Beclin-1-independent pathway. p38/MAPK is also extensively involved in autophagy regulation. Meanwhile, the p38/MAPK has a dual role in regulating autophagy as a positive or negative regulator. Meanwhile, the p38/MAPK pathway negatively affects autophagy regulation (Luo et al. 2022), and VIOL can activate p38/MAPK (Ferreira et al. 2004).

5.B. Interaction of violacein on Akt-signaling pathway (The PI3K-PKB/Akt Pathway)

The Akt or PI3K-Akt signaling pathway can promote survival and growth in response to ligands. This pathway has two essential proteins, Akt (protein kinase B) and PI3K (phosphatidylinositol 3-kinase). Akt is pivotal in cell metabolism, growth and division, apoptosis suppression, and angiogenesis. Disruptions in the Akt-signalling pathways are associated with cancer (Nitulescu et al. 2018). Various receptors, such as tyrosine kinases, integrins, B and T cell receptors, cytokine receptors, and G protein-coupled receptor (GPCR), can activate Akt signaling. They can then induce the production of phosphatidylinositol (3,4,5) trisphosphates (PIP3) by PI3K. Several AKT activation pathways exist, such as PI3K/Akt/mTOR, Akt/ERK1/2, and AMPK.

5.C. PI3K/Akt/mTOR signaling pathway

The PI3K/Akt/mTOR signalling pathway can regulate autophagy. It has three signalling molecules: PI3K, Akt, and mTOR (Luo et al. 2022). PI3K can catalyse the phosphorylation of the phosphatidylinositol. mTOR can promote anabolic metabolism and inhibit autophagy induction. The use of mTOR inhibitors is good in inhibiting cancer cell proliferation. Autophagy induction can be beneficial or detrimental depending on the stage of cancer. For example, autophagy may promote cancer progression by providing nutrients to cancer cells. In this case, inhibition of autophagy may sensitise cancer cells to metabolic stress conditions, leading to cell death. However, autophagy can suppress cancer growth. Defects in autophagy may enhance genomic instability and promote cancer progression. Thus, autophagy has potential dual functions in cancer promotion, suppression, and promotion (Kim and Guan 2015).

Like many other indole alkaloids, VIOL can precisely regulate cancer cells' autophagy by targeting the PI3K/Akt/mTOR signalling pathway to exhibit anticancer activity. VIOL can bind and interact with GPCR and RTK receptors of PI3K/Akt/mTOR. This interaction can promote the PI3K/Akt/mTOR signalling pathway (Kim et al. 2019; Luo et al. 2022).

5.D. Effect of violacein on Akt/ERK1/2 pathway

In the case of HCC (Hepatocellular carcinoma), VIOL's ability to exhibit antiproliferative activity, arrest the cell cycle, and induce apoptosis is associated with the downregulation of AKT and ERK1/2 signaling (Kim et al. 2019). VIOL can inhibit the (STAT3)/AKT/ERK pathways. It can be promoted by receptors, G protein-coupled receptor (GPCR) and RTK. However, VIOL can inhibit the (STATs)/Akt/ERK pathway by interacting with GPCR and RTK receptors (Kim et al. 2019). In addition, VIOL can inhibit proteins, like ERK and AKT, in survival signalling pathways triggered by EGFR (Epidermal growth factor receptor) and AXL (Tryrosine-protein kinase receptor UFO) receptors. EGFR and AXL are the most pertinent RTKs in CRC, particularly during CRC invasion. VIOL can decrease EGFR and AXL expression (de Souza Oliveira et al. 2022).

5.E. AMPK signalling pathway

VIOL can regulate autophagy by controlling AKT/mTOR and AMPK signalling pathways.

As an indole alkaloid, VIOL has the potential to regulate autophagy that contributes to the efficacy of VIOL in preventing and treating cancer. VIOL can target the autophagy process associated with cancer, including the PI3K/Akt/mTOR signaling pathway, MAPK signaling pathway, ROS signaling pathway, Beclin-1, and so on. (Luo et al. 2022).

low molecular weight protein tyrosine phosphatase (LMWPTP) favors the glycolytic profile in some tumors. There is a positive expression correlation between LMWPTP and energy metabolism enzymes

such as acetyl-CoA carboxylase (ACC) and fatty acid synthase (FASN). In addition, the potential of violacein to reprogram energetic metabolism and LMWPTP activity.

Violacein treatment induced a shift of glycolytic to oxidative metabolism associated with alteration in mitochondrial efficiency, as indicated by higher oxygen consumption rate. Particularly, violacein treated cells displayed higher proton leak and ATP-linked oxygen consumption rate (OCR) as an indicator of the OXPHOS preference. Notably, violacein is able to bind and inhibit LMWPTP. Since the LMWPTP acts as a hub of signaling pathways that offer tumor cells invasive advantages, such as survival and the ability to migrate, our findings highlight an unexplored potential of violacein in circumventing the metabolic plasticity of tumor cells (Faria et al. 2022).

5.F. Janus kinase 2: JAK2/STAT3 pathway

JAK2 (Janus kinase 2) can link cytokine and growth factor-initiated signal transduction to p27 regulation. JAK2 becomes activated by binding cytokines and growth factors. Then, the receptor-associated tyrosine kinase JAK2 can phosphorylate p27. VIOL is an activator of the JAK2/STAT3 pathway that transduces signals from the extracellular to the intracellular (nucleus) upon binding cytokines and growth factors to RTK (Jäkel et al. 2012). Moreover, the capacity of VIOL to induce cell death in cancer cells involves STAT3. It is hypothesised that VIOL acts as an antagonist that can bind 5-HT2C receptors that can activate the JAK/STAT pathway. 5-HT(2C) receptor activation does not affect JAK2 phosphorylation; instead, VIOL can block STAT3 phosphorylation (Fears et al. 2019).

5.G. Effect of violacein on transforming growth factor-β (TGFβ) signaling

TGFβ signaling suppresses cancer progression by inducing apoptosis, arresting the cell cycle, inhibiting proliferation, and promoting cell differentiation. TGFβ is also an oncogene in radical tumours, wherein it creates immune-suppressive tumour microenvironments and induces cell proliferation, invasion, angiogenesis, and metastasis. Therefore, suppressing TGFβ signals is a potential approach for inhibiting cancerogenesis and metastasis (Ali et al. 2023). TGFβ preserves typical cellular homeostasis by controlling the cell cycle and apoptosis. Elevated TGFβ levels correlate occasionally with cancer progression. TGFβ is a main regulator of the EMT (Epithelial-mesenchymal transition), a critical step of metastasis. (Singh, Gouri, & Samant 2023)

VIOL can potentially decrease the EMT marker (N-cadherin) even under the elevated with TGFβ (de Souza Oliveira et al. 2022). Notably, TGFβ-stimulated EMT can promote invasion and metastasis and reduce E-cadherin expression. As resveratrol, VIOL may inhibit EMT through TGF-β signalling pathway-mediated Snail/E-cadherin expression, which may be VIOL's potential mechanism for inhibiting invasion and metastases (Ji et al. 2015). Elevated TGF-β levels in cancer cells can avoid immune surveillance. TGF-β blockade has become a promising target in cancer healing (Singh et al. 2023).

5.H. Effect of violacein on Toll-Like Receptor (TLR) signaling pathway

TLRs have a crucial role in activating innate immunity. They recognize distinct molecular patterns following immune responses in identifying various components. They also can lead to the formation of NF-κB and AP-1 (Activated protein-1), responsible for inflammatory responses. Therefore, TLR signaling can regulate the host defense. In humans, members of the TLR family are ten (TLR1–TLR10). They exist in a variety of cancers. Cancer cells' damage-associated molecular patterns (DAMPs) can stimulate TLR activation in immune cells in the TME. Accordingly, TLR agonists have shown promising therapeutic benefits as anticancer agents capable of stopping cancer progression (Kawasaki and Kawai 2014).

VIOL may contribute to future immune strategies for cancer therapy. It can activate TLR signaling pathways that initiate and influence the immune and inflammatory response. Specifically, VIOL can interact with hTLR8 bound as an agonist. The anti-inflammatory effect of VIOL can induce negative feedback on TLR signaling and apoptosis (Venegas et al. 2019).

5.I. Effect of violacein on transcription of nuclear factor kappa B (TNFκB) signaling

TNFκB (TNFα) is a member of the TNF family of cytokines. It is a kind of cytokine secreted by adipose tissue. TNF signalling occurs through two receptors: TNFR1 and TNFR2. TNFR1 signalling tends to be proinflammatory and apoptotic, whereas TNFR2 signalling is anti-inflammatory and promotes cell proliferation. TNFα human is a potent activator for NF-kB pathway activator. It is a proinflammatory cytokine that induces apoptosis and necrosis. It stimulates the NF-κB pathway via TNFR2 and promotes cancer growth and metastasis.

TNFκB is a main cytokine involved in inflammation, immunity, cellular homeostasis, and cancer progression. It is an antitumor cytokine engaged in the innate and adaptive immune system. It is obliged for the proper proliferation and function of NK cells, T cells, B cells, macrophages, and dendritic cells. It is also an essential effector molecule in the cell-mediated killing certain cancers. However, TNFκB is either a primary mediator of cancer-related inflammation or a tumor-promoting factor. This of TNFκB has a paradoxical effect on cancers. High doses of TNFκB can stimulate hemorrhagic tumor necrosis. By contrast, low-dose TNFκB can activate angiogenesis and promote cancer progression.

Anticancer activity of VIOL in HL60 cells is related to activating TNFκB target genes. VIOL effects resemble TNFκB signal transduction. It can directly activate TNFR1-signaling. Hence, VIOL represents the anticancer drugs mediating apoptosis through the specific activation of TNFR1 (Ferreira et al. 2004). Apoptosis of HL60 cells mediated by VIOL occurs by specific activation of TNFR1 (Leal et al. 2015).

5.J. Effect of violacein on the nuclear factor kappa-B (NFκB) pathway

Many different stimuli or inducers can regulate NFκB. Cytokines such as IL-1β and TNFα are potent inducers of NFkB. NFkB can contribute to the resolution of inflammation, which disrupts the function of TNFkB (Nuclear factor kappa B). NFkB transcription factors involve cell proliferation and death, protecting the cell against TNFκB-induced apoptosis. These two functions are related to cancer. Cancer cells usually express NFkB activation, followed by unregulated proliferation or insensitivity to cell death (Zinatizadeh et al. 2021). The downstream NFκB can upregulate a set of genes, including the p50 and p65 subunits of NFκB and TRAF2. There is a causal link between the activation of NFκB and the initiation and development of cancer (Assenat et al. 2006).

VIOL can increase the production of TNFα, which is usually in concurrence with the activation of NFκB. The transcription of NFκB target genes leads VIOL's anticancer activity in addition to the activation of caspase and p38 MAPK. VIOL-mediated NFκB activation is specific to HL60 cells, as evident by the lack of TNFα production (Assenat et al. 2006; Ferreira et al. 2004).

5.K. Effect of violacein on the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway

Nrf2 is a transcription regulator that regulates the expression of various genes, which can protect cells from oxidative stress. Activation of Nrf2 may reduce cancer risk and prevent cancer. Elevated Nrf2 characterises cancers. However, the higher levels of Nrf2 may also activate cancer development. Activation of Nrf2 can induce antioxidant response element (ARE)-dependent expression of detoxifying and antioxidant defence proteins, preventing genome instability. Overexpression of Nrf2 leads to cancer progression. The activated Nrf2 can enhance the expression of antioxidant enzymes that have a role in reducing oxidative stress and chronic inflammation. The number of antioxidant compounds can be improved by supplementation, but increasing the levels of antioxidant enzymes requires the activation of Nrf2 by ROS-dependent and independent mechanisms (Lee et al. 2013; Prasad 2016).

Many dietary phytochemicals have cancer chemopreventive ability over the induction of the Nrf2 pathway. They exert antioxidant and anti-inflammatory functions by activating the Nrf2 pathway. VIOL has been identified as a potent Nrf2 activator (Li et al. 2016; Qin and Hou 2016).

5.L. Interaction of violacein with calcium signaling

The potential of VIOL to induce apoptosis is closely linked to its influence on intracellular Ca2+ signaling mechanisms. The increase in [Ca2+] results from oxidative conditions and can have a detrimental effect. The excessive ROS in cells can lead to elevated calcium levels, potentially impairing mitochondrial function and activating phospholipases, proteases, and endonucleases, ultimately leading to irreversible damage to the membrane, organelles, and cell death.

6. Effect of violacein on the cancer microenvironment

Cancer progression is dependent not only on its intrinsic behavior but also on its microenvironment. The tissue microenvironment can provide the need for cancer growth and metastasis, such as nutrients, oxygen, and growth factors. Cancer develops in a complex microenvironment for its sustained growth. They can also interact with their surrounding non-malignant cells (Yaacoub et al. 2016).

6.A. Effect of violacein on the epithelial-mesenchymal transition (EMT)

EMT is a cellular developing process from epithelial cells to mesenchymal phenotypes. An embryonic gene program awkwardly activated during cancer growth facilitates cancer cell detachment from epithelial tissue and subsequent metastasis. The EMT process in cancer cells can interrupt intracellular tight junctions, damage cell-cell contact, and result in changes in epithelial to mesenchymal cells.

VIOL can induce the EMT process by modulating the downstream of epithelial features. It can decrease the amount of proteins involved in EMT, such as β-catenin, N-cadherin, and Snail, and increase E-cadherin. Therefore, VIOL can disrupt cancer cell migration (de Souza Oliveira et al. 2022)

6.B. Effect of violacein on the extracellular matrix (ECM)

Cancer development depends not only on the changes in cellular, subcellular, and molecular levels but also on the ECM (Extracellular matrix). It creates a specific milieu that supports and nurtures cancer cell development. The matrix and dwelling cells are integrative cell-matrix that act synergistically. Crosstalk exists between cancer cells and ECM (Motofei 2022).

Cancer progression is associated with the dysfunction of apoptosis and the fundamental communication between cancer and reactive cells (Yaacoub et al. 2016). Apoptosis is related to the cellular communication with the microenvironment. Apoptotic cells can promote survival, proliferation, and inflammation and prevent survival and inflammation. Dying cells can communicate by transferring chemical information to their neighbors, resulting in the whole tissue's communication (Riley and Bock 2022). All kinds of programmed cell death (apoptosis, autophagy, and necroptosis) have crucial roles in metastatic progression. Cancer cells must overcome these types of cell death to metastasise (Su et al. 2015).

ECM is often under hypoxic conditions. Hypoxia can significantly increase the anticancer effects of VIOL (Hashimi et al. 2015; Mehta et al. 2015). Under normal or hypoxic conditions, VIOL can exhibit antiproliferative activity in various cancer cell lines. However, hypoxia can significantly increase the anticancer effects of violacein. Hypoxia synergises the effects of violacein (Hashimi et al. 2015).

6.C. Effect of violacein on the inflammatory pro-tumorigenic microenvironment

The autophagy process is associated with the inflammasome activation that may stimulate the pro-tumor or antitumor responses. In the context of tumors, autophagy can interact with the inflammasome pathway via various mechanisms. These mechanisms of the autophagy-inflammasome pathway can favour carcinogenesis. Therefore, combining strategies targeting autophagy and inflammation has potential in anticancer treatment (Chung et al. 2020). Autophagy has essential protective roles against disease.

Nevertheless, autophagy may have opposing roles in cancer. It has different effects in preventing early tumour progression and establishing and metastasising tumours. In addition, autophagy also has roles

in the tumour microenvironment and associated immune cells (Debnath, Gammoh, & Ryan 2023; Yan and Chen 2022).

VIOL has anti-inflammatory effects by decreasing the production of inflammatory cytokines. It is applicable in attenuating acute and chronic inflammation. Its inflammatory effect is *via* TLR8 signaling (de Souza Oliveira et al. 2022).

6.D. The activity of violacein on the role of macrophages

Tumor-associated macrophages (TAMs) are highly present in the TME and vital in cancer-associated inflammation and progression. Though inflammatory M1 macrophages suppress cancer growth, anti-inflammatory M2 macrophages promote cancer growth (de Souza Oliveira et al. 2022). Cancer-associated inflammation may alter ECM, which then supports the cancer growth. Cancer-associated inflammation is associated with cytokines, chemokines, and growth factors. They are vital in inflammation and cancer progression (Bonomi et al. 2014; de Souza Oliveira et al. 2022; Yaacoub et al. 2016).

VIOL can inhibit cell proliferation and migration. Death cells can stimulate the inflammatory activation of macrophages. Macrophages deficient in the inflammasome component Nlrp3 are less sensitive toward VIOL treatment. The immunogenic features of induced cell death make VIOL an exciting candidate as an inducer of immunogenic cell death (Dahlem et al. 2022).

The supernatant of VIOL-killed cells can promote macrophage activation (de Souza Oliveira et al. 2022). VIOL directly affects macrophage activation and can induce the expression of inflammatory cytokines (Tnf, Il6, and IL1b) in M0 and M2 macrophages and reduce Tgfb. In inflammatory M1 macrophages, VIOL can also increase the expression of Tnf and Il6; however, it decreases Il1b and can not alter Tgfb expression.

6.E. Effect of violacein on cancer progression, angiogenesis, and metastasis

Cytokines, chemokines, and growth factors are essential in angiogenesis, a vital component of TME. In cancer, angiogenesis is needed for the vasculature formation necessary for supplying nutrients, oxygen, and growth factors for cancer cells' rapid proliferation and metastatic spreading (Bonomi et al. 2014).

VIOL can down-regulate the expression of chemokine/receptor CXCL12/CXCR4 (chemokine-reseptor-ligant interaction), which is vital for angiogenesis. Meanwhile, cancer development without angiogenesis creates hypoxic conditions. This hypoxic condition can increase the anticancer agent's effectiveness in killing cancer cells (Choi et al. 2021).

CONCLUSIONS AND FUTURE PERSPECTIVES

Many cancer cell lines display disparity in cellular responses and sensitivities to violacein. Violacein has been proven for its anticancer activities at cellular, subcellular, molecular, and microenvironment levels. It has the anticancer potential against many types of human cancer cell lines and metastatic invasion. It has anti-migratory and immunogenic cell death properties. Violacein has been reported to interact with various signalling pathways. However, further studies are needed to confirm the cellular responses as the outcome of the violacein transduction.

The future perspective of violacein for cancer treatment depends on the progress of the clinical trial. It seems that violacein is a promising drug candidate for various stages of cancer. Particular focus should be on its role as an agonist or antagonist of various cancer-signaling pathways. In addition, the VIOL potency in improving immunogenic features of induced cell death is an exciting insight for further studies investigating its role as an inducer of immunogenic cell death. A formula for using violacein and other anticancer agents should be investigated.

Declaration of generative AI and AI-assisted technologies During the preparation of this work

The authors used GRAMMARLY in order to ensure grammar correctness. After using this tool/service, the authors reviewed and edited the content as needed and

take(s) full responsibility for the content of the publication.

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