Cancer Medicine Journal

Clinical Research | Vol 9 Issue 1

Nimbolide as a Multi-Target Anticancer Agent: From Mechanisms to Therapeutics

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Received: January 19, 2026; Accepted: February 21, 2026; Published: March 3, 2026

ABSTRACT

BACKGROUND

Nimbolide (NBL), a naturally occurring tetranortriterpenoid compound belonging to the Meliaceae family, possesses wide-ranging pharmacological action, including anticancer activities.

METHODS

This study summarizes the potential of NBL against various cancer cell lines and targeting molecular pathways. This assessment was conducted using PubMed, ScienceDirect, Google Scholar, and the online library.

RESULTS

This study indicates that NBL exerts anticancer effects against bladder, breast, colon, colorectal, gastric, liver, lung, prostate, and renal cancers by inducing apoptosis, cell cycle arrest, autophagy, and cytotoxicity, while suppressing proliferation, invasion, and migration. It also targets key signaling pathways, including nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), phosphoinositide 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/AKT/mTOR), and janus kinase/signal transducer and activator of transcription (JAK/STAT). Additionally, NBL exhibits several favorable pharmacokinetic properties, including 100% human intestinal absorption, and shows no inhibition of key cytochrome P450 enzymes (CYP1A2, CYP2C19, CYP2C9, CYP2D6) or P-glycoprotein. Besides exhibiting anticancer properties, NBL also shows pharmacological relevance, including anti-inflammatory, anti-arthritic, and anti-obesity effects, mediated through

Citation: Mst. Ismatara Khatun, Nimbolide as a Multi-Target Anticancer Agent: From Mechanisms to Therapeutics. Cancer Med J 8(1): 1-24.

pathways such as NF-κB, MAPK, and mTOR. Furthermore, toxicological studies indicate that NBL shows a promising safety profile with low toxicity risks in preclinical models.

CONCLUSION

While clinical evidence on NBL remains confined, the data analysis throughout this study suggests that NBL has potential properties to be considered a viable candidate in the treatment of cancer. Consequently, more clinical trials are suggested to evaluate its efficacy, safety, and right human dosage.

KEYWORDS

Nimbolide; Anticancer; Tetranortriterpenoid; Cytotoxicity; Apoptosis

INRODUCTION

A condition known as cancer occurs when some body cells proliferate out of control and spread to other bodily organs [1]. Cancer is a complex illness brought on by a confluence of environmental and hereditary variables. Diet, alcohol use, smoking, lifestyle, and infectious agents are examples of non-genetic environmental risk factors [2]. According to the latest world health organization (WHO) report, there are around 1.5 million cancer patients in Bangladesh, with 150,000 dying each year [3]. The most common types of cancer are breast, lung, colon, rectal, and prostate. When cancer cells are present, several risk factors have been found that encourage their growth or worsen the condition. These risk factors are mostly associated with bad lifestyle practices, such as physical inactivity [4]. Other causes for the development of cancer in patients are Down's syndrome, Parkinson's disease, schizophrenia, diabetes, anorexia nervosa, Alzheimer's disease, allergy-related diseases, and multiple sclerosis [5]. If any chromosomal aberration affects a protein that plays a crucial role in the cell cycle, quantitatively or qualitatively, it may result in cancer [6].

Treating cancer has been a highly complex process. Cancer treatment methods vary by type and grade [7]. There are different conventional treatment modalities that are available to treat and manage cancer. However, new cancer treatment options are being explored continuously, as over 60% of all current experimental trials worldwide are focusing on tumor cures. Surgery, radiation-based surgical knives, chemotherapy, and radiotherapy are some of the traditional and most widely used treatment methods. Some of the modern modalities include hormone-based therapy, anti-angiogenic modalities, stem cell therapies, immunotherapy, and dendritic cell-based immunotherapy [6]. Even though chemotherapy and radiotherapy are the mainstays of cancer treatment, their efficacies and applications are often hampered by their severe side effects, including cardio-cytotoxicity, nephrotoxicity, myelosuppression, neurotoxicity, hepatotoxicity, gastrointestinal toxicity, mucositis, and alopecia, which directly reduce the quality of life of patients [8]. Fatigue, nausea and vomiting, sleep disturbances, changes in bowel function, and an altered sense of taste are common problems [9]. In addition, physical consequences of specific treatments to identify include cardiac dysfunction, metabolic syndrome, lymphedema, peripheral neuropathy, and osteoporosis [10].

Natural products (NPDs), broadly defined as chemicals produced by living organisms including microbes, marine organisms, animals, fungi, and plants, are widely used as therapeutic agents for treating diseases and maintaining health and "wellness" [11,12]. NPDs provide a sustainable source with considerable efficacy to treat and overcome several disorders and fatal diseases, including cancer [13-15]. In addition, antihypertensive and antimigraine medications have benefited greatly from NPDs [16]. Some plant-derived compounds such as vincristine [17], irinotecan [18], etoposide [19], paclitaxel [20], actinomycin D, mitomycin C, bleomycin, doxorubicin, and L-asparaginase were established drugs that came from microbial sources, and citarabine is the first drug originating from a marine source. In addition, curcumin is one of the most studied chemopreventive agents that allows suppression, retardation, or inversion of carcinogenesis [21]. Several bioactive compound classes, including flavonoids, polyphenols, alkaloids, terpenes, and saponins, have shown significant anti-tumor properties [22].

Nimbolide (NBL) ($C_{27}H_{30}O_7$) is a tetranortriterpenoid compound that was isolated from the leaves and flowers of Azadirachta indica (A. indica) [23]. It is also known as limonoids with a δ lactone ring and an α, β unsaturated ketone [24], which is a traditional medicinal plant of the Meliaceae family [25] and widely distributed in Asia, Africa, and other tropical parts of the world [26]. Recent studies have shown that NBL has various pharmacological activities, including anti-inflammatory [27], antipyretic [26], antihistamine [28,29], antioxidant [23], and anticancer effects [27]. NBL induces apoptosis and inhibits cell proliferation and metastasis in a variety of cancer cells, including prostate, breast cancer, and choriocarcinoma cell lines [25]. In addition, NBL inhibits the expression of Bcl-2, Akt, pAkt, and NF-κB and suppresses the IκBα phosphorylation and the inhibition of p65 nuclear translocation [30,31]. However, there is no review on the anticancer activity of NBL with the botanical sources, molecular mechanisms, pharmacological relevance, pharmacokinetics, toxicological and clinical profile.

So, this review explores the anti-cancer activity of NBL with the botanical sources, molecular mechanisms, pharmacological relevance, pharmacokinetics, toxicological and clinical profiles based on the previous studies.

METHODOLOGY

Literature Search Strategy

An extensive literature search was carried out as of August 02, 2025, using a variety of scholarly databases, such as Web of Science, Springer Link, Wiley Online, ScienceDirect, PubMed, Google Scholar, and the online library. The literature search was conducted using the phrase “Nimbolide” along with several other keywords to locate relevant papers. The following search terms were used to find anticancer activity: “neoplasm,” “malignancy,” “anti-oncogenic effects,” “cellular cytotoxicity,” “tumor suppression,” “programmed cell death,” “mitotic arrest,” “PI3K-AKT-mTOR signalling,” “growth inhibition,” “in vitro cancer studies,” “in vivo cancer studies,” and “preclinical cancer models.” Other pharmacological effects were examined using the following terms: “antiviral activity,” “cardioprotective,” “antioxidant,” “anti-inflammatory,” “neuroprotective,” “anti-cancer,” “antibacterial,” “antimicrobial,” “anti-fibrotic,” “anti-allergic,” and “antinociceptive.” Terms including “plant sources,” “natural origins,” and “extraction methods” were included in searches pertaining to the botanical origins of “Nimbolide.” The toxicological evaluation was carried out with the use of search terms like “toxicity” and “acute toxicity.” Finally, the utilization of “efficacy,” “human studies,” and “clinical trials” yielded clinical evidence.

Inclusion Criteria

The selection of studies was based on the following criteria:

1. Research that identified natural plant sources of NBL, along with studies focusing on its phytochemical characterization, was considered.
2. Studies on the anticancer effects of NBL from a variety of botanical sources.
3. Studies carried out in vitro, in vivo, or ex vivo, whether or not experimental animals are used.
4. Research that either provides or withholds information about the possible anticancer mechanism of action.

5. Studies examining the pharmacokinetics, absorption, distribution, metabolism, and bioavailability of NBL were also included.

Exclusion Criteria

1. Titles and/or abstracts that didn't follow the guideline for requirements of inclusion and duplication of data.
2. Other studies have not found that NBL solves the current issue.
3. Duplicate publications, abstracts, or datasets were excluded.
4. Literature published in languages other than English was not considered.
5. Research that is not available in full text.
6. Studies on anticancer activity, during which the current study's focus is ambiguous by other investigations.

RESULTS AND DISCUSSION

Botanical Sources of Nimbolide

Traditional medicine is still recognized as the preferred primary health care system in many communities, with over 60% of the world's population and about 80% in developing countries [32]. It has been estimated that these medicines derived from plants constitute about 25 percent of modern pharmacopeia [33]. The era of using plant material as anticancer agents started with the isolation of two alkaloids, vinblastine and vincristine, from the Madagascar periwinkle [34]. The most common herbal medicinal component identified from *T. chinensis* is paclitaxel, a well-known first-line chemotherapy treatment/therapy for cancer diseases such as ovarian and breast cancer [35]. Camptothecin (CPT) is a natural product first isolated from the bark of the Chinese tree Camptotheca acuminate (Nyssaceae), from which the semisynthetic analogues topotecan and irinotecan are derived. These compounds are potent anticancer agents widely used clinically throughout the world [36]. The bioactive compounds of Zingiber officinale roscoe (rhizome) are phenolic and terpene, used in colon cancer, ovarian cancer, and breast cancer, and in *Curcuma longa L.* (C. longa), the main active ingredient is curcumin, a polyphenol, which has displayed potent anticancer effects [35]. By using the root of the plant of Peganum harmala, the main bioactive phytochemical harmine was used in breast cancer, and another bioactive compound, kaempferol galactoside, from Bauhinia variegata, was used in the treatment of breast, lung, and liver cancer [37]. However, NBL is a potent bioactive compound primarily found in the plant of the *A. indica* family. It is present in the leaves and flowers of *A. indica L* [26], leaf [38], and seed [39]. NBL has also been isolated from aqueous extract of *A. indica var. Siamensis* [40]. Moreover, different botanical sources of NBL are provided in Table 1.

Table 1: The botanical sources and plant parts of nimbolide.

Plant name	Plant's part	References
A. Indica L	Leaves and flowers	Bodduluru et al., 2014 [26]
		Elumalai et al., 2014 [28]
		Siddiqui et al., 1942 [41]
		Ekong et al., 1967 [42]
		Subramani et al., 2016 [43]
		Ram et al., 2020 [25]
		Lin et al., 2017 [44]
		Pooladanda et al., 2018 [45]
		Jaiswara et al., 2021 [46]
	Tree	Rajendran et al., 2024 [27]
		Wylie et al., 2022 [47]
		Priyadarsini et al., 2010 [48]
		Patra et al., 2019 [49]
	Leaf	Wang et al., 2016 [38]
		Shaheen et al., 2024 [50]

		Chitta et al., 2014 [51]
	Aqueous extract	Rochanakij et al., 1985 [40]
	Plant	Katola et al., 2023 [52]
	Flowers	Roy et al., 2007 [53]
		Cui et al., 2019 [54]
	Seed	Sengupta et al., 2017 [55]
		Cohen et al., 1996 [39]
	Seeds and leaves	Mahmoud et al., 2022 [56]
		Priyadarsini et al., 2009 [57]

Anticancer Activity of Nimbolide against Several Cancers: Underlying Mechanisms

Bladder cancer

Bladder cancer is a prevalent malignancy with considerable morbidity, mortality, and economic burden [58], primarily affecting the bladder urothelium [59]. The WHO reported around 573,278 new global cases in 2020, a number projected to double by 2040, underscoring its growing health impact [60]. Over 90% of urothelial tumors arise in the bladder, while 8% occur in the renal pelvis and 2% in the ureter and urethra [61].

A study showed that NBL inhibited bladder cancer EJ and 5637 cells by inducing G2/M cell cycle arrest and JNK phosphorylation while reducing p38, MAPK, AKT phosphorylation, MMP-9 activity, migration, and invasion [62]. These findings highlight NBL's potential as a therapeutic agent for bladder cancer; however, further in-depth studies are needed to validate its efficacy and elucidate its mechanisms in bladder cancer treatment.

Breast cancer

Breast cancer, mainly arising from milk ducts or lobules, is the most common cancer in women [63,64]. In 2020, it caused over 2.3 million cases and 685,000 deaths, with numbers expected to rise to 3 million cases and 1 million deaths by 2040. High oestrogen exposure and BRCA1/BRCA2 gene mutations significantly increase the risk of breast cancer [65,66]. Current treatments of breast cancer, such as endocrine, anti-HER2 therapy, chemotherapy, and radiation, often lead to resistance and serious side effects [67,68]. However, emerging targeted and combination therapies, along with natural compounds that trigger apoptosis through various mechanisms, show promise in managing especially metastatic cases [69,70].

Several studies suggest that NBL has significant anti-breast cancer activity. Elumalai and his teammates demonstrated that NBL induces apoptosis in human breast cancer cells via activation of both extrinsic (death receptor-mediated) and intrinsic (mitochondrial) pathways, involving caspase activation, mitochondrial membrane depolarisation, and cytochrome c release [28,29]. Further, in 2014, they found that NBL inhibits invasion and migration of breast cancer cells by downregulating uPAR and chemokine gene expression, thereby suppressing metastatic potential in both MCF-7 and MDA-MB-231 cell lines [28,29]. In another study, our data demonstrate that NBL inhibits TNBC by altering the integrin and FAK signalling pathway and significantly reduced the metastatic colony formation [71]. NBL also significantly reduced the protein expression of IKKα, IKKβ, and NFκB in breast cancer cell lines, indicating a clear downregulation of the nuclear factor kappa B signalling pathway [72]. Another finding implies that NBL induced autophagy signalling by increasing Beclin 1 and LC3B along with decreased p62 and mTOR protein expression in breast cancer with epigenetic modifications [45]. NBL (0-100 µM) inhibits AR and blocks IGF-1/PI3K/Akt and HIF-1α/VEGF signalling, downregulating DNMT-1, HDAC-6, miR-21, HOTAIR, and H19, while upregulating miR-148a/miR-152, with IC_{50} values of 10.6, 5, 5, and 7 µM, respectively [73]. In another study, in vivo results demonstrated that NBL showed enhanced anti-tumour and anti-metastatic effects and inhibited BCSCs by epigenetic reprogramming of the DNMTs-SFRP1-Wnt/β-catenin signalling [74]. In addition, NBL enhanced cytotoxicity in breast cancer [49]. NBL is a crucial therapeutic strategy for breast cancer treatment, as it inhibits invasion and migration, and it targets critical pathways like IGF-1/PI3K/Akt and HIF-1α/VEGF.

Overall, these findings highlight NBL's strong potential as a multi-targeted therapeutic agent against breast cancer, capable of inducing apoptosis, autophagy, and epigenetic modulation while suppressing proliferation, invasion, and metastasis. Its ability to act on diverse signalling pathways suggests promise for overcoming resistance and improving breast cancer management.

Colon cancer

Colon cancer, primarily an adenocarcinoma, can progress to malignancy if untreated. It ranks as the third most common cancer in men and the fifth leading cause of cancer-related deaths globally [75,76]. Dysregulated Wnt/β-catenin signalling and PPAR involvement contribute to its development [77]. Colon cancer often begins as benign polyps and is managed with surgery, chemo, radiation, and targeted therapies, though these can cause side effects, resistance, and relapse [78,79]. Emerging approaches like immunotherapy, precision medicine, and traditional remedies offer safer, more effective alternatives [80].

Our findings demonstrate that NBL (10 μM) enhances TNF-α and increases cell apoptosis of HT-29 cells by up-regulation of DR5 expression via the JNK pathway [81]. Another study suggests that NBL induces G0/G1 cell cycle arrest by upregulating p21, cyclin D2, and Chk2, and downregulating cyclin A, cyclin E, Cdk2, and Rad17 [82]. These studies indicate that NBL holds significant potential against colon cancer by promoting apoptosis through DR5-JNK signalling and inducing G0/G1 cell cycle arrest via modulation of key cell cycle regulators, suggesting its promise as a targeted therapeutic candidate.

Colorectal cancer

Colorectal cancer is the third most common cancer and the fourth leading cause of cancer-related deaths worldwide. Key signalling pathways like Wnt/β-catenin, EGFR/Ras, and p53 are often disrupted in colorectal cancer [83]. In addition, cytokines and growth factors, including EGFR, IGF-1R, and VEGF-A, are frequently overexpressed, promoting CRC cell growth, survival, and invasion [84]. Recent advances in immunotherapy show potential, particularly in pMMR-MSI-L colorectal cancer cases. Various natural compounds inhibit tumour growth by inducing cell cycle arrest or triggering apoptosis in CRC cells [85].

A study revealed that in colorectal cancer cell lines (HCT-116, HT-29, Caco-2), 10 μmol/L of the compound increased apoptosis and IκBα/p65 levels, while reducing NF-κB activity, proliferation markers, metastasis, angiogenesis factors, and STAT3 phosphorylation [30]. Another study suggests that NBL induced apoptosis and suppressed pro-inflammatory pathways, STAT3, NF-κB, proliferation, COX-1, and IL-6 in HCT116 and HT29 colorectal cancer cell lines in vitro [86]. These insights highlight that NBL has strong anticancer potential in colorectal cancer by inducing apoptosis, inhibiting proliferation, and targeting key pathways like NF-κB and STAT3.

Gastric cancer

Gastric cancer (GC) remains one of the leading causes of cancer-related mortality globally, primarily driven by early genetic alterations such as microsatellite or chromosomal instability [87,88]. Its treatment typically requires a multidisciplinary approach, with chemotherapy being the mainstay. However, while chemotherapy is effective, it often leads to considerable toxicity [89,90]. In recent years, natural compounds like curcumin and resveratrol have shown significant anti-cancer potential in GC, as they inhibit tumor growth and metastasis with fewer side effects, suggesting their value as safer therapeutic alternatives [91-93].

A study result showed that NBL administered orally (10-40 mg/kg) in male Swiss albino Wistar rats significantly reduced gastric tumor weight by enhancing apoptosis and antioxidant enzymes and suppressing inflammatory markers and liver enzymes [94]. NBL may serve as a promising therapeutic candidate for gastric cancer by reducing tumor burden through apoptosis induction, antioxidant enhancement, and suppression of inflammation, offering potential benefits with fewer side effects.

Hepatic cancer/Liver cancer

Hepatocellular carcinoma (HCC) accounts for 7% of all the cancers worldwide and is the most common primary liver malignancy [95] and ranks third in the annual cancer mortality rate with an overall 6-20 months of median survival after diagnosis [25]. The receptor tyrosine kinase pathways, Ras mitogen-activated protein kinase (Ras/Raf/MAPK), phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR), Wnt/β-catenin signaling pathway, ubiquitin/proteasome degradation, and the hedgehog signaling pathway are responsible for initiating and promoting HCC [95].

We found that NBL (6 mg/kg) treatment to HCC mice reduced hepatic tumor size and tumor volume and delayed tumor growth. In addition, NBL increased tight junction proteins and reduced ZO-1-associated nucleic acid binding protein expression and inhibited cell proliferation and suppressed cell cycle progression, cyclin-dependent kinase, and cyclin D1 expression. Furthermore, NBL treatment reduced NF-κB, interleukin 1 beta, and TNF-α expression and abrogated oxidative stress [25]. In another study, treatment with NBL (6 mg/kg) significantly increased hepatic expression of miR-21a-3p, miR-21a-5p, miR-221-5p, and miR-221-3p while decreasing miR-145a-5p. NBL treatment substantially reduced the miR-21a-5p and miR-221-3p and improved miR-145a-5p gene expression, and hepatic tight junction (TJ)-associated proteins such as claudins 1 & 5 mRNA and protein were increased, whilst hepatic claudin 2 mRNA was significantly decreased. Moreover, NBL also regulates cadherins, ROCK 1, MMP 9, cyclin D1, CDK4, NF κB, and TNFα mRNA expression in HCC mice [96]. These findings demonstrate that NBL exerts strong anti-HCC effects by reducing tumor size and growth, enhancing tight junction integrity, modulating key miRNAs, and suppressing proliferation, inflammation, and oxidative stress. Its multi-targeted regulation of cell cycle, adhesion, and signaling pathways underscores its potential as a therapeutic candidate for HCC.

Lung cancer

Lung cancer, a leading cause of cancer-related deaths globally, accounts for approximately 2.2 million new cases and 1.79 million deaths annually [97]. It primarily includes small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), with smoking being a major risk factor [98]. Its development involves dysregulation of multiple signaling pathways related to apoptosis, tumor suppression, and growth promotion [99]. Drug discovery has always benefited greatly from the use of NPDs [100]. Paclitaxel, a plant-derived anticancer drug, acts as an antimicrotubular agent by stabilizing microtubules, promoting their assembly, and preventing disassembly, thereby inhibiting cell division [101].

A study suggests that NBL (3.1-100 μg/ml) induced apoptosis and suppressed proliferation in lung cancer cells by upregulating TNF-α, DR4, and DR5, while downregulating IκB [98]; similarly, another study revealed NBL increased ROS, ER stress, DNA damage, and apoptosis in A549 cells [102]. Furthermore, an in vivo study also demonstrates that NBL triggered apoptosis via caspase activation and reduced proliferation in lung and prostate cancer xenograft models [24]. Studies indicate that NBL induces apoptosis and inhibits proliferation in lung cancer cells by upregulating TNF-α, DR4, and DR5, and downregulating IκB, while also increasing ROS, ER stress, and DNA damage. Additionally, in vivo research shows NBL triggers caspase-mediated apoptosis and suppresses tumor growth in lung cancer models, highlighting its therapeutic potential.

Oral cancer

Oral cancer, a subtype of head and neck cancers, arises within the mouth, affecting areas such as the tongue, gingiva, lips, cheeks, and hard palate [103]. Oral squamous cell carcinoma (OSCC) is the sixth most common cancer globally, with over 300,000 new cases annually, and is increasingly affecting younger populations [104]. Genetic factors, including mutations in tumor suppressor genes (APC, p53), oncogenes (Myc, Ras), and disruptions in cell-cycle regulation, DNA repair, and signaling pathways, contribute to its development [105]. According to "Oral Oncology Reports," pharmacologically active substances that are isolated from microbial and plant sources have outstanding anticancer properties against oral cancer [106].

An in vivo study on male Syrian hamsters showed that NBL induced apoptosis by upregulating GSK-3β, miR-126, let-7, and pro-apoptotic proteins, while inhibiting cell cycle progression (S and G2/M phases) and suppressing PI3K/Akt signaling and proliferation [107]. Similarly, another study revealed that in vitro studies using SCC-4 and SCC-9 cell lines demonstrated that NBL enhanced DNA damage response (DDR) and apoptosis via activation of p-p53S15 and p-ATM, while reducing markers of oxidative DNA damage and tumor proliferation [108]. Further studies showed that treatment with NBL increased ROS, apoptosis, endoplasmic reticulum stress, caspase activation, and mitochondrial dysfunction while decreasing cell proliferation [109]. These insights suggest that NBL shows strong potential as a natural therapeutic agent against oral cancer due to its ability to induce apoptosis, modulate key signaling pathways like PI3K/Akt, and suppress tumor growth both in vitro and in vivo, which makes it a novel, promising candidate for investigation in the future.

Pancreatic cancer

Pancreatic cancer (PC) remains a major cause of cancer-related mortality globally, characterized by challenges in early diagnosis and poor outcomes despite surgical and chemotherapeutic interventions [110]. PDAC originates from exocrine cells and is influenced by genetic factors, as 10-15% of cases carry mutations in genes like BRCA1/2, CDKN2A, and PALB2. Major risk factors include smoking, diabetes, chronic pancreatitis, and obesity. The K-Ras mutation plays a key role in the progression of precursor lesions to PDAC. Additionally, the tumor microenvironment promotes immune evasion, posing challenges to effective therapy [111]. In pancreatic cancer research, naturally occurring small molecule compounds have long been emphasized as possible treatments to stop the spread of the disease and make chemo resistant tumors more sensitive [112].

Several studies of NBL have shown significant anticancer potential against pancreatic cancer in various in vitro studies. NBL (0.78-12.5 µM) showed anticancer activity in AsPC-1 cells with an IC50 value of 2.30 µM and induced cytotoxicity [49]. Treatment with NBL, ranging from 1 to 50 µM concentrations, significantly reduced the expression levels of the phosphorylated forms of AKT, PI3K, ERK, mTOR, and p70S6 kinase with the IC50 values of 5, 3, and 5 µM, respectively. In addition, NBL suppresses migration, invasion, EMT, and anchorage-independent growth of pancreatic cancer cells and induces reactive oxygen species generation, autophagy, and apoptosis [43]. Another study highlights that the anticancer efficacy of NBL is associated with reduced mutant p53 as well as increased mitochondrial activity in the form of increased mitochondrial reactive oxygen species (ROS) and mitochondrial mass. NBL induces higher levels of caspase activation. Moreover, the CD44+

population of MIA PaCa-2 cells decreased with NBL exposure [113]. Treatment with NBL effectively induced anticancer activity, reduced the growth of PDACs, and induced high levels of ROS generation with an IC50 value of 5 μM. Finally, NBL (5 mg/kg) treatment to SOD2-overexpressing PDAC xenografts resulted in significant inhibition of tumor growth and metastasis [114]. NBL demonstrates strong anticancer potential against pancreatic cancer by inhibiting key signaling pathways such as AKT, PI3K, ERK, and mTOR, while suppressing migration, invasion, and EMT. It also induces ROS generation, autophagy, and apoptosis and reduces mutant p53 levels, effectively inhibiting tumor growth and metastasis in both in vitro and in vivo models.

Placental cancer

Placental cancer involves tumor invasion into placental tissue, affecting its vital role in foetal support [115,116].

NBL inhibited BeWo cell growth (IC_{50}: 2.01 and 1.19 μM), increased ROS, Apaf-1, caspase-3, and PARP cleavage, and reduced PCNA and the Bcl-2/Bax ratio [117]. Another study found that NBL showed potent cytotoxicity against placental cancer with IC_{50} values of 2.01 and 1.19 μM and increased glutathione, ROS, Apaf-1, caspase-3, and ADP-ribose, while reducing proliferation and the Bcl-2/Bax ratio, indicating strong pro-apoptotic effects [117]. This study highlights NBL's promising natural therapeutic potential against placental cancer by promoting apoptosis and oxidative stress.

Prostate cancer

Prostate cancer originates in the gland cells of the prostate, and it stands second in terms of occurrence in men [92,93]. PCa development and progression are dependent on androgen receptor signaling [56]. Age, ethnicity, family history, obesity, Lynch syndrome, and mutation in genes (BRCA1 or BRCA2) are the prominent risk factors associated with the initiation of prostate cancer [92,93].

Several studies showed that NBL has shown potent anticancer activity against prostate cancer across various in vitro and in vivo models. Studies using cell lines such as PC-3, DU145, LNCaP, U2OS, and PC-3 D12 have demonstrated that NBL induces significant apoptosis and inhibits proliferation, metastasis, and cell survival through modulation of key molecular pathways. Specifically, it activates pro-apoptotic markers including Fas ligand, FADD, Bax, Bad, caspases (3, 8, 9, 10), IGFBP3, and cytochrome c, while downregulating the PI3K/Akt/mTOR, NF-κB, IGF1/IGF1R, XIAP, Bcl-2, cleaved PARP, and MAPK (B-RAF/p.ERK) signaling cascades [56,72,92,93,118,119]. Concentration-dependent cytotoxicity has been observed with IC_{50} values ranging from 0.5 to 6 μM, indicating its strong therapeutic potential. Kashif et al. (2017) [120] further confirmed NBL's efficacy in DU-145, PC-3, and A-549 cells with increased apoptosis and reduced viability. Additionally, in vivo experiments on male SCID mice demonstrated its safety and tumor-reducing effects. Collectively, these findings suggest NBL as a promising multitargeted agent for prostate cancer therapy. These studies highlight that NBL is a promising natural compound with strong anticancer potential against prostate cancer through its multi-pathway targeting, like PI3K/Akt/mTOR, NF-κB, IGF1/IGF1R, and pro-apoptotic effects.

Nasopharyngeal cancer

Nasopharyngeal carcinoma (NPC), a tumor arising from epithelial cells that cover the surface and line the nasopharynx, is a rare malignancy worldwide [121]. On a global scale, NPC is a rare form of cancer, with a typical incidence rate of less than one case per 100,000 person-years, and causes over 65,000 deaths annually worldwide [122]. JNK and p38 MAPK activities are found upregulated in nasopharyngeal carcinoma [123]. Several genomic alterations in NPC, such as mutations in NF-κB-negative regulators, loss of CDKN2A/CDKN2B, amplification of CCND1, mutations in TP53, and aberrations in the PI3K/MAPK pathways [122].

A study revealed that NBL induces cell cycle arrest and apoptosis in epithelial cancer HONE-1 and NPC-039 cells in vitro (0-8 μM) by activating caspase-3, -8, -9, PARP, Bik, Bax, and t-Bid, leading to decreased cell viability [121]. NBL effectively induces cell cycle arrest and apoptosis in nasopharyngeal carcinoma cells by activating caspases and pro-apoptotic proteins, resulting in reduced cell viability. These findings highlight its potential as a therapeutic agent against NPC.

Renal cancer

Renal cell carcinoma (RCC) is among the most fatal genitourinary cancers, with the highest mortality rate [124]. In 2020, it accounted for approximately 431,000 new cases and 179,000 deaths worldwide. RCC progression is mainly driven by alterations in key signaling pathways, including VHL/HIF, PI3K/AKT/mTOR, p53, cAMP, and TGF-β. Common genetic mutations in RCC involve genes like SETD2, PBRM1, BAP1, MTOR, and KDM5C [125]. NPDs show promise in RCC by inducing apoptosis and autophagy and inhibiting angiogenesis, tumor metabolism, and cell motility via multiple pathways [126].

A study explored that NBL exhibits potent anticancer effects against renal cancer by inducing G2/M cell cycle arrest, upregulating p53 and caspases, and downregulating Bcl-2, Mcl-1, and pro-caspase-8 in 786-O and A-498 cells [23]. NBL can be a strong anticancer agent, as it effectively induces apoptosis, arrests the cell cycle, and suppresses Bcl-2 and Mcl-1 in renal cancer.

In conclusion, NBL shows broad anticancer potential by targeting multiple cancer types through mechanisms like apoptosis induction, cell cycle arrest, autophagy, and inhibition of proliferation, invasion, and metastasis. It modulates critical signaling pathways such as NF-κB, PI3K/Akt/mTOR, and JAK/STAT, and also affects epigenetic regulation and oxidative stress. While preclinical studies highlight its therapeutic promise, further research is needed to clarify its pharmacokinetics, optimize delivery methods, and assess safety. Future directions include exploring NBL's synergy with existing treatments and conducting clinical trials to confirm its efficacy. Overall, NBL represents a promising natural compound for developing novel, multi-targeted anticancer therapies. The anticancer mechanisms of NBL are depicted in Table 2 and the anticancer mechanism of NBL is shown in Figure 1.

Table 2: Mechanism of anticancer activity of nimbolide.

Cancer Type	Experimental Model/Cell Lines	Tested Concentrations, (R/A)/ Dose	IC50	Mechanisms/ Results	References
Bladder Cancer	EJ and 5637 cell lines, in vitro	0–12μM	3 μM	↑G2/M, cell cycle arrest, JNK phosphorylation, ↓p38MAPK and AKT phosphorylation, MMP-9 activity, migration, invasion	Shin et al., 2019 [62]
Breast Cancer	MCF-7, MDA-MB-231 cell line, in vitro	2–6 μM/ml	4 and 6 μM/ml	↑Apoptosis, proapoptotic proteins Bax, Bad, Fas-L, TRAIL, FADDR, cytochrome c, Cleavage of pro-caspase-8, pro-caspase-3 and PARP ↓Anti-apoptotic proteins Bcl-2, Bcl-xL, Mcl-1 and XIAP-1, growth of MCF-7 and MDA-MB-231 cells, cell proliferation, viability	Elumalai et al., 2012 [64]

Breast Cancer	MDA-MB-231 and MCF-7 cells, in vitro	1–5 µM	1.97 and 5.04 µM	↑Autophagy, Beclin 1, LC3B, apoptotic cell death, caspase 3 and 9, H3K27Ac, ↓cell Proliferation, Bcl-2, HDAC-2, p62, mTOR	Pooladanda et al., 2018 [45]
Breast Cancer	MCF-7 cells, MDA-MB-231, EAhy926 cell lines, in vitro	0–100µM	10.6, 5, 5, and 7µM	↑ miR-148a/miR-152 ↓AR, IGF-1/PI3K/Akt and /HIF-1alpha/VEGF, DNMT-1, HDAC-6, miR-21, HOTAIR, and H19, miR-148a/miR-152	Nivetha et al., 2022 [73]
	Athymic nude mice, in-vivo	10 mg/kg	-		
Breast Cancer	MCF-7, MDA-MB-231 cell lines, in vitro	2–6µM	4.0 and 2.7, 6.0 µM and 3.2 µM	↑TIMP-2 ↓Invasion, migration, uPAR chemokine, pEGFR, VEGFR, IKKa, IKKb, NF- κB	Elumalai et al., 2014 [28,29]
Breast Cancer	MDA-MB-231 and MDA-MB-468 cells, in vitro	2.5–10 µM	7.5 µM and 8.5 µM		
	Mice, in vivo	20 mg/ kg	-	↑Cyclin E, γH2AX and 14-3-3σ levels, ↓Cell proliferation, migratory, and invasive, αV and β3, ILK, FAK, and PAK levels, filopodial structures, pPAK, S phase, CDK2 phosphorylation, AKT and mTOR	Arumugam et al., 2021 [71]
Breast Cancer	MCF 7 and MDA MB 231 cell lines, in vitro	2–6 µM	2.7 µM, 6.0 µM and 3.2 µM, respectively	↓PI3K, Akt phosphorylation, proliferation and survival, IKKα, IKKβ, and NFκB	Arunakaran et al., 2013 [72]
Breast Cancer	MDA-MB-231 cell line, in vitro	5–20 µM	16.28 and 9.32 µM, 10.64 and 5.68 µM		
	BALB/c mice, in vivo	20 mg/kg	-	↑Hypomethylation of SFRP1, Apoptosis ↓Sphere formation, drug resistance, EMT, and metastasis, DNMTs, Wnt/β-catenin signaling	Mohapatra et al., 2023 [74]
Pancreatic and Breast Cancer	MCF-7, MDA-MB-231 and AsPC-1 cells, in vitro	0.39–12.5 µM	4.02, 2.24, and 2.30 µM respectively	↑Cytotoxicity	Patra et al., 2019 [49]
Colon Adenocarcinoma	HT-29 cell line, in vitro	10 µm	-	↑JNK phosphorylation, Bid, DR5 level, TNF-α, apoptosis, ↓Proliferation	Boonyarat et al., 2016 [81]
Colon Cancer	HT-29 cell line, in vitro	2.5 –10 µM	-	↑p21, cyclin D2, Chk2 ↓cyclin A, cyclin E, Cdk2, Rad17.cell cycle arrest G0/G1	Roy et al., 2006 [82]
Colorectal Cancer	HCT116 and HT29 cell lines, in vitro	-	-	↑Apoptosis ↓pro-inflammatory pathways, STAT3 and NF-Kb, proliferation, COX1, IL-6	Patel et al., 2018 [86]
Colorectal Cancer	HCT-116, HT-29, and Caco-2 cell lines, in vitro	10 µmol/L	-	↑Apoptosis, IκBα, p65 ↓NF-κB, proliferation, Bcl-2, Bcl-xL, c-IAP-1, and surviving cyclin D1 and c-Myc MMP-9, ICAM-1, CXCR4, metastasis, angiogenesis, VEGF, STAT3, phosphorylation	Gupta et al., 2013 [30]
	Nude mice model, n=4, in vivo	5–20 mg/kg i.p.	-		
Gastric Cancer	Male Swiss albino Wistar rats, in vivo (n=6)	10–40 mg/kg, orally	-	↑Apoptosis, SOD, GPx, CAT, MDA, TNF-α, (IL)-1β, IL-2, IL-6, COX-2, PGE2, VEGF, NF-Kb ↓Tumor weight, level of phase I enzymes, LDH, HDAC, AST, ALP, ALT	Gu et al., 2025 [94]
Hepatic Cancer	Swiss albino mice, in vivo (n = 48)	6 mg/kg b. wt. orally	-	↑miR-21a-3p, miR-21a-5p, miR-221-5p and miR-221-3p, claudins 1&5 mRNA and protein ↓ miR-21a-5p and miR-221-3p, claudin 2 mRNA, hepatic claudin 2 mRNA and protein expression tumor growth, cadherins, ROCK 1, MMP 9, cyclin D1, CDK4, NF κB and TNFα mRNA expression	Vairappan et al., 2025 [96]
Liver Cancer	Male Swiss albino mice (CD-1 strain), in vivo	6 mg/kg, i.p.	-	↑ZO-1 protein, occludin expression, TJ ↓Cell proliferation, cell cycle progression, NF-κB, interleukin 1 beta and TNF-α, tumor growth, cyclin dependent kinase, CyclinD1, DEN/NMOR, AFP levels, plasma levels of AST, ALT, and ALP, PCNA-positive nuclei, IL-1β	Ram et al., 2020 [25]
Lung Cancer	A549 cell line, in vitro	3.1–100 µg/ml	31.55µg/ml	↑TNF-α, DR5 and DR4, apoptosis ↓Proliferation, IκB	Kumar et al., 2017 [98]
Lung Cancer	A549 cell line, in vitro	-	-	↑ROS, Apoptosis, ER stress, DNA damage ↓Colony formation	Chen et al., 2024 [102]
Lung Cancer, Prostate Cancer	Xenograft mice model, in vivo	200–400 mg/kg	-	↑Apoptosis, caspase 3, 8, and 9 ↓Proliferation	Kashif et al., 2019 [24]

Oral Cancer	SCC-4, SCC-9 cell lines, in vitro	-	-	↑p-p53S15, DDR and apoptosis, p-ATM ↓8-oxodG levels, MRN, ATMS1891, and γ-H2AX, KU-55933, tumor formation, cell proliferation	Arvindh et al., 2025 [108]
Nasopharyngeal Cancer	HONE-1 and NPC-039 cells, in vitro	0–8 μM	-	↑Cell cycle arrest, caspase-3, −8, and −9 and poly (ADP-ribose) polymerase, Bik, Bax, and t-Bid, apoptosis, ↓Cell viability	Chien et al., 2017 [121]
Oral Cancer	Male Syrian hamsters, in vivo	0–1000μM	-	↑ GSK-3β, miR-126 and let-7, p-cyclin D1Thr286 and pro-apoptotic proteins, subG0/G1, apoptosis, ↓S and G2/M, PI3K/Akt, proliferation	Sophia et al., 2016 [107]
Oral Squamous Cell Carcinoma	-	-	-	↑ROS, apoptosis, ER stress ↓Tumor growth	Peng et al., 2025 [109]
Pancreatic Cancer	HPAC, MIAPaCa-2 and PANC-1 cells, in vitro	1–50 μM	5, 3 and 5 μM respectively	↑Apoptosis, autophagy ↓Cancer growth, metastasis, proliferation, invasion, migration, ↓AKT, PI3K, ERK, mTOR and p70S6 kinase, ↑ROS	Subramani et al., 2016 [43]
Pancreatic Cancer	MIA PaCa-2, BX-PC3 cell lines, in vitro	-	-	↑ Apoptosis, mitochondrial activity ↓ p53, sphere formation, CD44+	Kumar et al., 2018 [113]
Pancreatic Cancer	hTERT-HPNE, HPAC, AsPC-1, Capan-1, Capan-2, PANC-1, MIAPaCa-2, and BxPC-3 cell lines, in vitro	-	5 μM	↑ ROS, apoptosis ↓Metastasis of PDACs, SOD2, epithelial-to-mesenchymal transition, invasion, migration, and colony-forming, pPI3K, pAkt, pERK, pp70s6k, and pmTOR	Mehmetoglu-Gurbuz et al., 2023 [114]
Placental Cancer	BeWo cells line, in vitro	-	2.01 and 1.19 μM	↑ Glutathione, ROS, apoptosis, Apaf-1 and caspase-3, ADP-ribose, ↓Proliferation, Bcl-2/Bax	Harish Kumar et al., 2009 [117]
Prostate Cancer	U2OS cells and DU145 cells, in vitro	-	1.26 μM, 4.00 Mm	↑Apoptosis, PI3K/Akt/GSK-3, mTOR and p62, E-cadherin, HDAC6 protein ↓NF-κB, cells migration, B-RAF/p.ERK	Mahmoud et al., 2022 [56]
Prostate Cancer and Lung Cancer	Du-145, PC-3, A-549 cells, in vitro	0.625–10 μM	6.86 and 4.97 μM, 8.01 and 5.83 μM, 11.16 and 7.59 μM respectively	↑Cytotoxicity, apoptosis ↓Stained cancer cells	Kashif et al., 2017 [120]
	Male SCID mice, in vivo	10 μL			
Prostate Cancer	PC-3 cells, in vitro	2μM	-	↑Apoptosis, mTOR, altered the PI3K-Akt ↓Proliferation, cell survival	Raja Singh et al., 2017 [119]
Prostate Cancer	PC-3 cell line, in vitro	0.5–2 μM	2 μM	↑Apoptosis, mRNA of Fas ligand, FADDR, Bax, Bad and IGF binding protein 3, caspases 8, 3, 10, 9, Bax and cytochrome c ↓PI3K, Akt, IGF1 and IGF1R, XIAP, Bcl2, cleaved PARP, p-Akt and IGF1R PI3K/Akt, proliferation	Raja Singh et al., 2014 [118]
Prostate Cancer	LNCaP and PC-3, PC-3 D12 cell lines, in vitro	0.5 μM	-	↑Apoptosis, cytotoxicity ↓Metastases, NF-κB, proliferation	Zhang et al., 2022 [92,93]
Prostate Cancer	PC3 cell lines, in vitro	2–6 μM	4.0 μM	↓PI3K, Akt phosphorylation, proliferation and survival, IKKα, IKKβ, and NFκB	Arunakaran et al., 2013 [72]
Renal Cancer	786-O and A-498 RCC cell line, in vitro	1–4 μM	-	↑cdc2, cdc25c, caspase-3, -9, poly ADP-ribose polymerase (PARP), p53, cdc2, cdc25c ↓Pro-caspase-8 in 786-O and A-498 cells, Bcl-2 and Mcl-1, cell cycle growth, G2/M arrest, cyclin A, cyclin B, cdc2, and cdc25c	Hsieh et al., 2015 [23]

↑ : Increase /activation /upregulation/stimulation, ↓ :Decrease /inhibition /downregulation, MOMP: Mitochondrial outer membrane permeability, ROS: Reactive oxygen species, PI3K: Phosphatidyl Inositol-3 Kinase, GSK-3 β : Glycogen Synthase Kinase-3 β , MMP-9: Matrix metalloproteinase-9, JNK: c-Jun N-terminal kinase, Bid: BH3 interacting-domain death agonist, DR5: Death receptor 5, TNF-α: Tumor necrosis factor, pPAK :Phosphorylated p21 activated kinase SOD2: Superoxide dismutase 2, VEGF: Vascular endothelial growth factor; s.c. :subcutaneously, OSCC: Human oral squamous cell carcinoma,), PARP:Poly (ADP-ribose) Polymerase, EMT:Epithelial to Mesenchymal Transition, DNMTs :DNA Methyltransferases, HDAC :Histone Deacetylase, ICAM-1 :Intercellular Adhesion Molecule-1, CXCR4 :C-X-C Chemokine Receptor Type 4, IL-6 : Interleukin 6, COX-2 : Cyclooxygenase-2, i.p. : intraperitoneal, GPx : Glutathione Peroxidase, CAT:Catalase, AST : Aspartate Aminotransferase), ALT :Alanine Aminotransferase, ALP : Alkaline Phosphatase, PCNA : Proliferating Cell Nuclear Antigen, AFP : Alpha-Fetoprotein, FAK : Focal Adhesion Kinase, PGE2 : Prostaglandin E2, DDR : DNA Damage Response, ATM : Ataxia Telangiectasia Mutated Kinase, ZO-1 :Zonula Occludens-1, IGF :Insulin-like Growth Factor, and CDK : Cyclin-Dependent Kinase

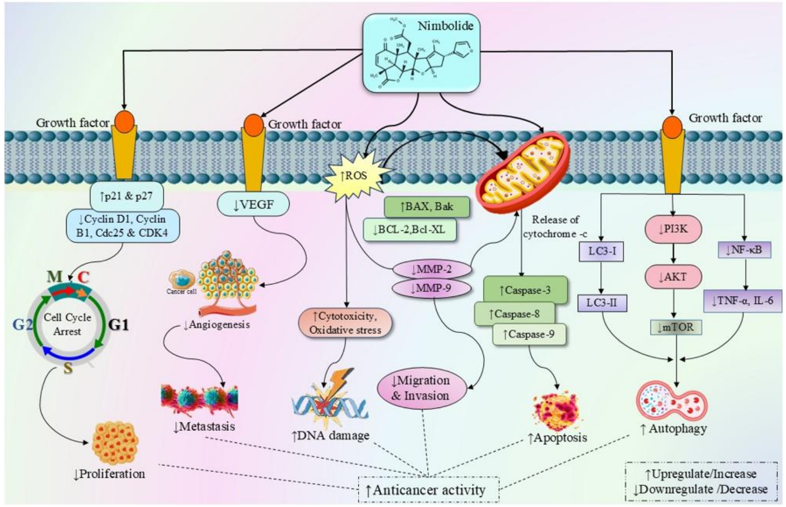

Figure 1: The anticancer mechanism of nimbolide. [Nimbolide (NBL) exerts anticancer activity by inducing cell cycle arrest through upregulation of p21 and p27 and downregulation of cyclins and CDKs, leading to reduced proliferation. It inhibits angiogenesis and metastasis by decreasing VEGF and matrix metalloproteinases (MMP-2 and MMP-9), thereby reducing migration and invasion. NBL also increases reactive oxygen species (ROS), causing DNA damage and oxidative stress, which trigger apoptosis via mitochondrial cytochrome c release and activation of caspases. Additionally, it promotes autophagy by inhibiting the PI3K/AKT/mTOR and NF-κB signaling pathways].

Biopharmaceutical's Profile

The word "biopharmaceuticals" has long been used to refer to drugs made from biological materials, such as tissues, cells, and whole organisms [127]. Biopharmaceuticals have many advantages. For instance, they rarely cause the side effects associated with conventional small-molecule drugs and solely target certain molecules [128]. Pharmacokinetics (PK) play a vital role in drug discovery as they aid in assessing and refining a compound's ADME (absorption, distribution, metabolism, and excretion) characteristics, facilitating the selection of a clinical candidate that ensures optimal safety and efficacy. NBL ($C27H30O7$) is a limonoid-type natural compound with a molecular mass of 466.52 g/mol, and it's a water-soluble compound.

In our computational ADME prediction, NBL exhibited several favorable ADME properties, including compliance with Lipinski's Rule of 5, a molar refractivity of 120.99, moderate lipophilicity (log Po/w of 2.17), and water solubility (Log S of -3.94), indicating it is soluble. NBL exhibits 100% human intestinal absorption and shows no inhibition of key cytochrome P450 enzymes (CYP1A2, CYP2C19, CYP2C9, CYP2D6) or P-glycoprotein. However, it acts as a CYP3A4 inhibitor, which suggests a potential for drug-drug interactions involving CYP3A4 substrates. NBL also showed low to moderate total clearance (log ml/min/kg = 0.249) and is not a substrate for the renal OCT2 transporter, suggesting limited renal excretion via this pathway. Based on Lipinski's Rule and the overall ADME profile, NBL demonstrates strong potential as an orally bioavailable therapeutic candidate. However, parameters such as skin permeability, blood-brain barrier penetration, CNS accessibility, and volume of distribution were less favorable, which may limit its use in certain delivery routes or CNS-targeted applications, as detailed in Table 3.

Table 3: Various pharmacokinetic parameters of nimbolide, including its ADME profile and related characteristics.

Properties	**Factors**	**Nimbolide**
Physicochemical Properties	Formula	C27H30O7
	Molecular weight (g/mol)	466.52
	Number of heavy atoms	34
	Number of aromatic heavy atoms	5
	Number of H-bond donors	o
	Number of H-bond acceptors	7

	Molar refractivity	120.99
Lipophilicity	Log Po/w (XLOGP3)	2.17
Drug-likeness	Lipinski	Yes; 0 violation
	Bioavailability score	0.55
Water Solubility	Log S (ESOL)	-3.94
	Class	Soluble
	Caco2 permeability (log Papp in 10-6 cm/s)	0.92
Absorption	Intestinal absorption (human) numeric (% Absorbed)	100
	Skin permeability (log Kp cm/h)	−3.599
	P-glycoprotein I inhibitor	Yes
	P-glycoprotein II inhibitor	Yes
Distribution	BBB permeability (log BB)	−0.675
	CNS permeability (log PS)	−2.658
	VDss (human) (log L/kg)	0.028
Metabolism	CYP1A2 inhibitor	No
	CYP2C19 inhibitor	No
	CYP2C9 inhibitor	No
	CYP2D6 inhibitor	No
	CYP3A4 inhibitor	Yes
Excretion	Total clearance (log ml/min/kg)	0.249
	Renal OCT2 substrate	No

Pharmacological Relevance

Pharmacology plays a vital role in the discovery and application of drugs as therapeutic tools, aiming to benefit individuals by alleviating symptoms, minimizing impairments, enhancing outcomes, prolonging life, or preventing illness [129]. Recent studies have shown that NBL has a variety of pharmacological qualities, including anti-inflammatory [27], antipyretic [26], antihistamine [28], antioxidants [23], and anticancer actions [27]. NBL shows strong anticancer potential by inducing apoptosis, arresting the cell cycle, and inhibiting proliferation, inflammation, and metastasis. It acts through key pathways such as PI3K/Akt/mTOR, NF-κB, and STAT3, while modulating apoptotic proteins, cyclins, and microRNAs. NBL also enhances oxidative stress and epigenetic regulation, contributing to tumor suppression. In vivo studies support its efficacy with minimal toxicity, highlighting its promise as a natural anticancer agent.

NBL is a promising natural anticancer agent due to its multi-targeted action and low toxicity. NBL at 125-500 nM showed a significant reduction in the levels of TNFα, IL-6, IFNγ, NO/iNOS, and PGE2/COX-2 in LPS-activated BV-2 cells and increased expression of phospho-p65 and phospho-IκBα [52]. In an in vivo study, treatment with NBL 1 mg/kg and 3 mg/kg, respectively, decreases LOXL2, TNF-α, IL-1β, pNF-κB, TGF-β1, α-SMA, N-cadherin, and extracellular matrix [130]. In another investigation, NBL 0.5-10 µM and 3 mg/kg were used both in vitro and in vivo, and the results showed that NBL increased GSH, Nrf-2, SOD-1, and HO-1 protein expression and concomitantly abrogated the LPS-triggered TNF-α, p38 MAPK, mTOR, and GSK-3β protein expression, and TNF-α-regulated NF-κB and HDAC-3 crosstalk was ameliorated by NBL with promising anti-nitrosative, antioxidant, and anti-inflammatory properties in LPS-induced ARDS [131]. NBL suppressed the body weight and significantly reduced the hepatic parameters and altered the antioxidant parameters and also decreased the level of inflammatory cytokines. Additionally, NBL suppressed the mRNA expression of glucose-6-phosphatase HO-1 and nuclear factor erythroid-2-related factor-2 (Nrf2) [92,93]. Another investigation demonstrated that NBL (0.5-2 mM) elicits depletion of the antioxidant defense system in sperm and induces oxidative stress in the epididymal sperm of rats [132]. Treatment with NBL the formation of bioflim was greatly reduced [133]. Treatment of cells with 0.5-5.0 µm concentrations of NBL resulted in moderate to very strong growth inhibition in U937, HL-60, THP1, and B16 cell lines and decreased the number of cells in the G0/G1 phase, with initial increases in S and G2/M phases, enhancing the effectiveness of cell cycle disruption [53]. NBL (20 mg/kg) was reduced in arthritic score, organ indices, volume of paw, and edema formation, along with substantial enhancement in body weight, and also showed that there was a significant reduction in destruction of joints and inflammation and a markedly enhanced health and reduced inflammation via lessening the pro-inflammatory cytokine expression in arthritic rats [54]. NBL has a broad pharmacological application in various diseases and has ability to target various key signaling pathways, which makes it a promising candidate for further study. The pharmacological activities of NBL are shown in Table 4.

Table 4: The pharmacological activities of nimbolide.

Diseases/ Effect Name	Dose/ Concentration	Mechanism	References
Neuroinflammation	125–500 nM, in vitro	↑Phospho-p65, phospho-IκBα ↓TNFα, IL-6, IFNγ, iNOS, COX-2, p38, ROS, Nrf2	Katola and Olajide., 2023 [52]
Anti-Inflammatory	1 mg/kg and 3 mg/kg (i.p.), in vivo	↓Extracellular matrix, LOXL2, TNF-α, IL-1β, pNF-κB, TGF-β1, α-SMA, N-cadherin	Diddi et al., 2019 [130]
Acute Respiratory Distress Syndrome (ARDS)	0.5–10 µM, in vitro 3 mg/kg (i.p.), in vivo	↑GSH, Nrf-2, SOD-1, HO-1 ↓Nitrosative-oxidative stress, iNOS, myeloperoxidase, NF-κB, HDAC-3, TNF-α, p38 MAPK, mTOR, GSK-3β	Pooladanda et al., 2019 [131]
Obesity	5, 10, 15 mg/kg, in vivo	↑LDL, AST, ALT, ALP, MDA ↓IL-1β, IL-6, TNF-α, HO-1, Nrf2, HDL, HFABP, CAT, SOD, GPx, GSH, TAC	Zhang et al., 2022 [92,93]
Antioxidant	0.5-2 mM, in vitro	↓SOD, GR, GPx, H2O2, LPO, α-Glucosidase	Kumbar et al., 2012 [132]
Antibacterial	2 and 4 µg ml–1, in vitro	↑PI uptake, ↓biofilm formation, cell number, intracellular activity	Sarkar et al., 2016 [133]
Antiproliferative	0.5-5.0 µM, in vitro	↑G2/M phases, number of cells, apoptosis ↓G0/G1 phase, cell growth, cell proliferation ↓G0/G1 phase, cell growth, cell proliferation	Roy et al., 2007 [53]
Antiarthritic	20 mg/kg, in vivo	↓Arthritic score, organ indices, volume of paw, edema formation, inflammation, TNF-a, IL-6, IL-1b, IL-10, iNOS, P-IkBa, Nf-kb	Cui et al., 2019 [54]

↑: Increased / Upregulated; ↓: Decreased / Downregulated; p65: Subunit of NF-κB (Nuclear factor kappa-light-chain-enhancer of activated B cells); IκBα: Inhibitor of kappa B alpha; TNF-α: Tumor Necrosis Factor-alpha; IL-1β: Interleukin-1 beta; IL-6: Interleukin-6; IFNγ: Interferon-gamma; iNOS: Inducible Nitric Oxide Synthase; COX-2: Cyclooxygenase-2; LOXL2: Lysyl oxidase-like 2; TGF-β1: Transforming Growth Factor beta 1; α-SMA: Alpha-Smooth Muscle Actin; N-cadherin: Neural cadherin; GSH: Glutathione; Nrf2: Nuclear factor erythroid 2–related factor 2; SOD-1: Superoxide Dismutase 1; HO-1: Heme oxygenase 1; HDAC-3: Histone Deacetylase 3; mTOR: Mammalian Target of Rapamycin; GSK-3β: Glycogen Synthase Kinase 3 beta; LDL: Low-Density Lipoprotein; HDL: High-Density Lipoprotein; AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; ALP: Alkaline Phosphatase; MDA: Malondialdehyde; HFABP: Heart Fatty Acid-Binding Protein; CAT: Catalase; SOD: Superoxide Dismutase; GPx: Glutathione Peroxidase; GR: Glutathione Reductase; TAC: Total Antioxidant Capacity; H_2O_2: Hydrogen Peroxide; LPO: Lipid Peroxidation; JNK: c-Jun N-terminal Kinase; Bid: BH3-interacting domain death agonist; DR5: Death Receptor 5; PI3K: Phosphoinositide 3-kinase; Akt: Protein Kinase B; IKKα: IκB kinase alpha; IKKβ: IκB kinase beta; PI: Propidium Iodide; NF-κB: Nuclear Factor kappa-light-chain-enhancer of activated B cells; P-IkBα: Phosphorylated inhibitor of NF-κB alpha; i.p.: Intraperitoneal; G0/G1 phase: Resting and initial growth phase of the cell cycle; G2/M phase: Pre-mitotic and mitotic phase of the cell cycle

Toxicological Profile

While preclinical safety and toxicity testing is crucial for the development of new medications, particularly anticancer treatments, since efficacy does not equate to non-toxicity, PK is an essential component of drug development [38]. The primary aim of toxicological assessments during drug development is to ensure the safety profile of potential therapeutic agents [134]. Since toxicity and clinical safety significantly influence the success of drug development, integrating toxicology studies early in the research and development process helps eliminate candidates with unfavorable safety profiles before entering clinical trials [135]. NBL, a limonoid from A. indica, exhibits a favorable toxicological profile in preclinical studies. It shows low acute toxicity with an intraperitoneal LD50 of ~225 mg/kg in mice, while oral and subcutaneous routes are better tolerated [131]. Genotoxicity studies, including the Ames assay, indicate no mutagenic potential [38]. Oral administration up to 2000 µg/kg caused no mortality but minor hematological changes. Interestingly, NBL also demonstrated hepatoprotective effects in liver injury models. Despite promising safety indicators, more comprehensive toxicological evaluations, including chronic and reproductive studies, are needed. NBL shows a promising safety profile with low toxicity risks, though further long-term studies are essential to confirm its clinical safety.

Clinical Evidence

Clinical studies, often known as studies or trials, are an essential component of the research process used to develop a novel medication [136]. As developers plan the clinical study, they will define their objectives for each phase of clinical research and initiate the Investigational New Drug (IND) process, which is a required step before starting clinical trials [137]. NPDs have traditionally been used since ancient times and in folk medicine for treating various diseases and health conditions [138]. NBL currently lacks clinical studies in humans and remains supported solely by preclinical evidence. Several animal studies have demonstrated the anticancer and cancer-preventive properties of NBL, offering substantial support for its continued development as a potential cancer treatment [76]. NBL exhibits significant potential as an anticancer and chemopreventive agent; however, comprehensive preclinical studies on its pharmacokinetics and toxicity are still required [50]. NBL shows strong anticancer effects through apoptosis and cell cycle arrest in various cancers. It holds great potential for future clinical application, though more human studies are needed.

CONCLUSION

In conclusion, NBL demonstrates remarkable anticancer potential by modulating diverse molecular mechanisms, including the induction of apoptosis, cell cycle arrest, autophagy, and suppression of tumor cell proliferation, invasion, and metastasis. This study and numerous others highlight NBL's ability to target and regulate critical signaling pathways such as PI3K/Akt/mTOR, NF-κB, STAT3, JAK/STAT, and Wnt/β-catenin, which play pivotal roles in cancer progression and resistance. Toxicological studies reveal a relatively safe profile, with an LD_{50} of approximately 225 mg/kg in mice, suggesting low acute toxicity and good tolerability in preclinical models. Despite these promising findings, clinical translation is hindered by the lack of comprehensive pharmacokinetic data, detailed toxicity profiling, and, importantly, human clinical trials to establish efficacy and safety in patients. Moreover, challenges such as bioavailability, optimal dosing, and delivery methods remain to be addressed. Future research should focus on thorough preclinical evaluations, including chronic toxicity and pharmacodynamics; the development of novel drug delivery systems to improve bioavailability; and well-designed clinical trials to confirm therapeutic benefits. Additionally, exploring synergistic effects of NBL with existing chemotherapeutics and targeted therapies may enhance its anticancer efficacy and help overcome drug resistance. Collectively, NBL represents a promising natural compound with multifaceted anticancer properties, warranting further investigation to translate its preclinical success into effective clinical applications.

CONFLICT OF INTEREST

Not applicable.

FUNDING

University Higher Education Fund number CL/CO/A/8.

ACKNOWLEDGMENT

Authors extend their appreciation to the University Higher Education Fund for funding this research work under the Research Support Program for Central Labs at King Khalid University through the project number CL/CO/A/8.

AUTHORS' CONTRIBUTIONS

All authors have substantially contributed to this work, including the conception and design of the study, execution, data acquisition, analysis, **Background** and interpretation, as well as revising or critically reviewing the manuscript. All authors have approved the final version of the manuscript for publication, agreed on the selected journal for submission, and take full responsibility for all aspects of the work. All authors have read and agreed to the published version of the manuscript.

REFERENCES

1. Brown JS, Amend SR, Austin RH, et al. (2023) Updating the definition of cancer. Molecular Cancer Research 21(11): 1142-1147.
2. Rahman MS, Suresh S, Waly MI (2018) Risk factors for cancer: Genetic and environment. In Bioactive Components, Diet and Medical Treatment in Cancer Prevention. Cham: Springer International Publishing: 1-23.
3. Wasim Bin H, Mohammad Al-Masum M (2024) State of cancer care in Bangladesh. The Daily Star.
4. Franco-García JM, Castillo-Paredes A, Rodríguez-Redondo Y, et al. (2024) Greater physical activity levels are associated with lower prevalence of tumors and risk of cancer in Spanish population: A cross-sectional study. Heliyon 10(7).
5. Tabarés-Seisdedos R, Dumont N, Baudot A, et al. (2011) No paradox, no progress: Inverse cancer comorbidity in people with other complex diseases. The Lancet Oncology 12(6): 604-608.
6. Abbas Z and Rehman S (2018) An overview of cancer treatment modalities. Neoplasm 1: 139-157.
7. Debela DT, Muzazu SG, Heraro KD, et al. (2021) New approaches and procedures for cancer treatment: Current perspectives. SAGE Open Medicine 9: 20503121211034366.
8. Mayer A, Ladwa R, Porceddu SV (2024) Radiation and chemotherapy. Head and Neck Cancer: Treatment, Rehabilitation, and Outcomes 1: 87.
9. Williams SA (2004) The effect of education in managing side effects in women receiving chemotherapy for treatment of breast cancer. Number 1/2004 31(1): e16-e23.
10. Emery J, Butow P, Lai-Kwon J, et al. (2022) Management of common clinical problems experienced by survivors of cancer. The Lancet 399(10334): 1537-1550.
11. Bontempo P, De Masi L, Rigano D (2023) Functional properties of natural products and human health. Nutrients 15(13): 2961.
12. Rakib IH, Khan MS, Ayub A, et al. (2025) Salvianolic acid A enhances sedative effect of diazepam through the GABAergic pathway: In vivo, molecular docking, and pharmacokinetics approaches. Neurotoxicology and Teratology: 107517.
13. Asma ST, Acaroz U, Imre K, et al. (2022) Natural products/bioactive compounds as a source of anticancer drugs. Cancers 14(24): 6203.
14. Sharma KK, Al Hasan MS, Rouf R, et al. (2025) Assessment of antiemetic and modulatory activity of dihydrocoumarin on copper sulfate induced emetic chicks: An in vivo investigation. Food Chemistry Advances 6: 100930.

15. Sharma R, Kumar S, Kaushik N, et al. (2025) Decoding the mystery of blood cancer: Cause, diagnosis, and management. Current Cancer Therapy Reviews 21(1): 40-53.
16. Yuan H, Ma Q, Ye L, et al. (2016) The traditional medicine and modern medicine from natural products. Molecules 21(5): 559.
17. Škubník J, Pavlíčková VS, Ruml T, et al. (2021) Vincristine in combination therapy of cancer: Emerging Trends in Clinics. Biology 10(9): 849.
18. Chai Y, Liu JL, Zhang S, et al. (2024) The effective combination therapies with irinotecan for colorectal cancer. Frontiers in Pharmacology 15: 1356708.
19. Jang JY, Kim D, Im E, et al. (2025) Etoposide as a key therapeutic agent in lung cancer: Mechanisms, efficacy, and emerging strategies. International Journal of Molecular Sciences 26(2): 796.
20. Chen Q, Xu S, Liu S, et al. (2022) Emerging nanomedicines of paclitaxel for cancer treatment. Journal of Controlled Release 342: 280-294.
21. Nobili S, Lippi D, Witort E, et al. (2009) Natural compounds for cancer treatment and prevention. Pharmacological Research 59(6): 365-378.
22. Zhang J, Wu Y, Li Y, et al. (2024) Natural products and derivatives for breast cancer treatment: From drug discovery to molecular mechanism. Phytomedicine 129: 155600.
23. Hsieh YH, Lee CH, Chen HY, et al. (2015) Induction of cell cycle arrest, DNA damage, and apoptosis by nimbolide in human renal cell carcinoma cells. Tumor Biology 36(10): 7539-7547.
24. Kashif M, Hwang Y, Kim WJ, et al. (2019) In-vitro morphological assessment of apoptosis induced by nimbolide; A limonoid from Azadirachta indica (Neem Tree). Iranian Journal of Pharmaceutical Research: IJPR 18(2): 846.
25. Ram AK, Vairappan B, Srinivas BH (2020) Nimbolide inhibits tumor growth by restoring hepatic tight junction protein expression and reduced inflammation in an experimental hepatocarcinogenesis. World Journal of Gastroenterology 26(45): 7131.
26. Bodduluru LN, Kasala ER, Thota N, et al. (2014) Chemopreventive and therapeutic effects of nimbolide in cancer: The underlying mechanisms. Toxicology in Vitro 28(5): 1026-1035.
27. Rajendran P, Renu K, Abdallah BM, et al. (2024) Nimbolide: Promising agent for prevention and treatment of chronic diseases (recent update). Food & Nutrition Research 68: 10-29219.
28. Elumalai P, Arunakaran J (2014) Review on molecular and chemopreventive potential of nimbolide in cancer. Genomics & Informatics 12(4): 156.
29. Elumalai P, Brindha Mercy A, Arunkamar R, et al. (2014) Nimbolide inhibits invasion and migration, and down-regulates uPAR chemokine gene expression, in two breast cancer cell lines. Cell Proliferation 47(6): 540-552.
30. Gupta SC, Prasad S, Sethumadhavan DR, et al. (2013) Nimbolide, a limonoid triterpene, inhibits growth of human colorectal cancer xenografts by suppressing the proinflammatory microenvironment. Clinical Cancer Research 19(16): 4465-4476.
31. Ansari MO, Shadab GGHA, Siddique HR (2017) Targeting cancer signaling pathways by nimbolide: A review on chemoprevention and therapeutic studies. Cancer Therapy & Oncology International Journal 8(4), pp.66-70.
32. Mintah SO, Asafo-Agyei T, Archer MA, et al. (2019) Medicinal plants for treatment of prevalent diseases. Pharmacognosy-Medicinal Plants 17: 1-9.

33. Jain S, Dwivedi J, Jain PK, et al. (2016) Medicinal plants for treatment of cancer: A brief review. Pharmacognosy Journal 8(2).
34. Khazir J, Mir BA, Pilcher L, et al. (2014) Role of plants in anticancer drug discovery. Phytochemistry Letters 7: 173-181.
35. Siddiqui AJ, Jahan S, Singh R, et al. (2022) Plants in anticancer drug discovery: From molecular mechanism to chemoprevention. BioMed Research International 2022(1): 5425485.
36. Moraes DFC, de Mesquita LSS, do Amaral FMM, et al. (2017) Anticancer drugs from plants. In Biotechnology and production of anti-cancer compounds. Cham: Springer International Publishing: 121-142.
37. Iqbal J, Abbasi BA, Mahmood T, et al. (2017) Plant-derived anticancer agents: A green anticancer approach. Asian Pacific Journal of Tropical Biomedicine 7(12): 1129-1150.
38. Wang L, Zhang J, Ong PS, et al. (2016) Anticancer properties of nimbolide and pharmacokinetic considerations to accelerate its development. Oncotarget 7(28): 44790.
39. Cohen E, Quistad GB, Casida JE (1996) Cytotoxicity of nimbolide, epoxyazadiradione and other limonoids from neem insecticide. Life Sciences 58(13): 1075-1081.
40. Rochanakij S, Thebtaranonth Y, Yenjai C, et al. (1985) Nimbolide, a constituent of azadirachta indica, inhibits plasmodium falciparum in culture. The Southeast Asian Journal of Tropical Medicine and Public Health 16(1): 66-72.
41. Siddiqui S (1942) A note on the isolation of three new bitter principles from the nim oil. Current Science 11(7): 278-279.
42. Ekong DEU (1967) Chemistry of the meliacins (limonoids). The structure of nimbolide, a new meliacin from Azadirachta indica. Chemical Communications (London) (16): 808a-808a.
43. Subramani R, Gonzalez E, Arumugam A, et al. (2016) Nimbolide inhibits pancreatic cancer growth and metastasis through ROS-mediated apoptosis and inhibition of epithelial-to-mesenchymal transition. Scientific Reports 6(1):19819.
44. Lin H, Qiu S, Xie L, et al. (2017) Nimbolide suppresses non-small cell lung cancer cell invasion and migration via manipulation of DUSP4 expression and ERK1/2 signaling. Biomedicine & Pharmacotherapy 92: 340-346.
45. Pooladanda V, Bandi S, Mondi SR, et al. (2018) Nimbolide epigenetically regulates autophagy and apoptosis in breast cancer. Toxicology In Vitro 51: 114-128.
46. Jaiswara PK, Gupta VK, Sonker P, et al. (2021) Nimbolide induces cell death in T lymphoma cells: Implication of altered apoptosis and glucose metabolism. Environmental Toxicology 36(4): 628-641.
47. Wylie MR, Windham IH, Blum FC, et al. (2022) In vitro antibacterial activity of nimbolide against Helicobacter pylori. Journal of Ethnopharmacology 285: 114828.
48. Priyadarsini RV, Murugan RS, Sripriya P, et al. (2010) The neem limonoids azadirachtin and nimbolide induce cell cycle arrest and mitochondria-mediated apoptosis in human cervical cancer (HeLa) cells. Free Radical Research 44(6): 624-634.
49. Patra A, Satpathy S, Hussain MD (2019) Nanodelivery and anticancer effect of a limonoid, nimbolide, in breast and pancreatic cancer cells. International Journal of Nanomedicine: 8095-8104.
50. Shaheen S, Khalid S, Aaliya K, et al. (2024) Insights into Nimbolide molecular crosstalk and its anticancer properties. Medical Oncology 41(6): 158.
51. Chitta K, Paulus A, Caulfield TR, et al. (2014) Nimbolide targets BCL2 and induces apoptosis in preclinical models of Waldenströms macroglobulinemia. Blood Cancer Journal 4(11): e260-e260.

52. Katola FO, Olajide OA (2023) Nimbolide targets multiple signalling pathways to reduce neuroinflammation in BV-2 microglia. Molecular Neurobiology 60(9): 5450-5467.
53. Roy MK, Kobori M, Takenaka M, et al. (2007) Antiproliferative effect on human cancer cell lines after treatment with nimbolide extracted from an edible part of the neem tree (*Azadirachta indica*). Phytotherapy Research 21(3): 245-250.
54. Cui X, Wang R, Bian P, et al. (2019) Evaluation of antiarthritic activity of nimbolide against Freund's adjuvant induced arthritis in rats. Artificial Cells, Nanomedicine, and Biotechnology 47(1): 3391-3398.
55. Sengupta P, Raman S, Chowdhury R, et al. (2017) Evaluation of apoptosis and autophagy inducing potential of Berberis aristata, Azadirachta indica, and their synergistic combinations in parental and resistant human osteosarcoma cells. Frontiers in Oncology 7: 296.
56. Mahmoud N, Dawood M, Huang Q, et al. (2022) Nimbolide inhibits 2D and 3D prostate cancer cells migration, affects microtubules and angiogenesis and suppresses B-RAF/p. ERK-mediated in vivo tumor growth. Phytomedicine 94: 153826.
57. Priyadarsini RV, Manikandan P, Kumar GH, et al. (2009) The neem limonoids azadirachtin and nimbolide inhibit hamster cheek pouch carcinogenesis by modulating xenobiotic-metabolizing enzymes, DNA damage, antioxidants, invasion and angiogenesis. Free Radical Research 43(5): 492-504.
58. Sanli O, Dobruch J, Knowles MA, et al. (2017) Bladder cancer. Nature Reviews Disease Primers 3(1): 1-19.
59. Mushtaq J, Thurairaja R, Nair R (2019) Bladder cancer. Surgery (Oxford) 37(9): 529-537.
60. Dyrskjøt L, Hansel DE, Efstathiou JA, et al. (2023) Bladder cancer. Nature Reviews Disease Primers 9(1): 58.
61. Clark PE, Agarwal N, Biagioli MC, et al. (2013) Bladder cancer. Journal of the National Comprehensive Cancer Network 11(4): 446-475.
62. Shin SS, Hwang B, Muhammad K, et al. (2019) Nimbolide represses the proliferation, migration, and invasion of bladder carcinoma cells via Chk2-mediated G2/M phase cell cycle arrest, altered signaling pathways, and reduced transcription factors-associated mmp-9 expression. Evidence-Based Complementary and Alternative Medicine 2019(1): 3753587.
63. Sharma GN, Dave R, Sanadya J, et al. (2010) Various types and management of breast cancer: An overview. Journal of Advanced Pharmaceutical Technology & Research 1(2): 109-126.
64. Elumalai P, Gunadharini DN, Senthilkumar K, et al. (2012) Induction of apoptosis in human breast cancer cells by nimbolide through extrinsic and intrinsic pathway. Toxicology Letters 215(2): 131-142.
65. Fernandez SV, Russo J (2010) Estrogen and xenoestrogens in breast cancer. Toxicologic Pathology 38(1): 110-122.
66. Kori S (2018) An overview: Several causes of breast cancer. International Journal of Epidemiology 2(1): 01-17.
67. Burguin A, Diorio C, Durocher F (2021) Breast cancer treatments: Updates and new challenges. Journal of Personalized Medicine 11(8): 808.
68. Noel B, Singh SK, Lillard Jr JW, et al. (2020) Role of natural compounds in preventing and treating breast cancer. Frontiers in Bioscience (Scholar Edition) 12: 137.
69. Harbeck N, Gnant M (2017) Interpretation of the evidence for the efficacy and safety of statin therapy. Lancet 389(10074): 1134-1150.
70. Yuan L, Cai Y, Zhang L, et al. (2022) Promoting apoptosis, a promising way to treat breast cancer with natural products: A comprehensive review. Frontiers in Pharmacology 12: 801662.

71. Arumugam A, Subramani R, Lakshmanaswamy R (2021) Involvement of actin cytoskeletal modifications in the inhibition of triple-negative breast cancer growth and metastasis by nimbolide. Molecular Therapy-Oncolytics 20: 596-606.
72. Arunakaran J, Arunkumar R, Elumalai P, et al. (2013) Impact of quercetin, diallyl disulfide and nimbolide on the regulation of nuclear factor kappa B expression in prostate and breast cancer cell lines. Natural Products Chemistry and Research 1(10.4172): 2329-6836.
73. Nivetha R, Arvindh S, Baba AB, et al. (2022) Nimbolide, a neem limonoid, inhibits angiogenesis in breast cancer by abrogating aldose reductase mediated IGF-1/PI3K/Akt signalling. Anti-Cancer Agents in Medicinal Chemistry-Anti-Cancer Agents) 22(14): 2619-2636.
74. Mohapatra P, Madhulika S, Behera S, et al. (2023) Nimbolide-based nanomedicine inhibits breast cancer stem-like cells by epigenetic reprogramming of DNMTs-SFRP1-Wnt/β-catenin signaling axis. Molecular Therapy Nucleic Acids 34.
75. Khan FA, Albalawi R, Pottoo FH (2022) Trends in targeted delivery of nanomaterials in colon cancer diagnosis and treatment. Medicinal Research Reviews 42(1): 227-258.
76. Wang T, Zhang Y, Taaffe DR, et al. (2022) Protective effects of physical activity in colon cancer and underlying mechanisms: A review of epidemiological and biological evidence. Critical Reviews in Oncology/Hematology 170: 103578.
77. Jansson EÅ, Are A, Greicius G, et al. (2005) The Wnt/β-catenin signaling pathway targets PPARγ activity in colon cancer cells. Proceedings of the National Academy of Sciences 102(5): 1460-1465.
78. Alrushaid N, Khan FA, Al-Suhaimi E et al. (2023) Progress and perspectives in colon cancer pathology, diagnosis, and treatments. Diseases 11(4): 148.
79. Dekker E, Tanis PJ, Vleugels JL, et al. (2019) Pure-amc (Colorectal cancer). Lancet 394(10207): 1467-1480.
80. Esmeeta A, Adhikary S, Dharshnaa V, et al. (2022) Plant-derived bioactive compounds in colon cancer treatment: An updated review. Biomedicine & Pharmacotherapy 153: 113384.
81. Boonyarat C, Yenjai C, Reubroycharoen P, et al. (2016) Combination of nimbolide and TNF-α-increases human colon adenocarcinoma cell death through JNK-mediated DR5 up-regulation. Asian Pacific Journal of Cancer Prevention 17(5): 2637-2641.
82. Roy MK, Kobori M, Takenaka M, et al. (2006) Inhibition of colon cancer (HT-29) cell proliferation by a triterpenoid isolated from Azadirachta indica is accompanied by cell cycle arrest and up-regulation of p21. Planta Medica 72(10): 917-923.
83. Wu WK, Wang XJ, Cheng AS, et al. (2013) Dysregulation and crosstalk of cellular signaling pathways in colon carcinogenesis. Critical Reviews in Oncology/Hematology 86(3): 251-277.
84. Stefani C, Miricescu D, Stanescu-Spinu II, et al. (2021) Growth factors, PI3K/AKT/mTOR and MAPK signaling pathways in colorectal cancer pathogenesis: Where are we now?. International Journal of Molecular Sciences 22(19): 10260.
85. Rejhová A, Opattová A, Čumová A, et al. (2018) Natural compounds and combination therapy in colorectal cancer treatment. European Journal of Medicinal Chemistry 144: 582-594.
86. Patel MJ, Tripathy S, Mukhopadhyay KD, et al. (2018) A supercritical CO2 extract of neem leaf (*A. indica*) and its bioactive liminoid, nimbolide, suppresses colon cancer in preclinical models by modulating pro-inflammatory pathways. Molecular Carcinogenesis 57(9): 1156-1165.

87. Thrift AP, Wenker TN, El-Serag HB (2023) Global burden of gastric cancer: Epidemiological trends, risk factors, screening and prevention. Nature Reviews Clinical oncology 20(5): 338-349.
88. Grabsch HI, Tan P (2013) Gastric cancer pathology and underlying molecular mechanisms. Digestive Surgery 30(2): 150-158.
89. Joshi SS, Badgwell BD (2021) Current treatment and recent progress in gastric cancer. CA: A Cancer Journal for Clinicians 71(3): 264-279.
90. Zhao N, Wang W, Jiang H, et al. (2023) Natural products and gastric cancer: Cellular mechanisms and effects to change cancer progression. Anti-Cancer Agents in Medicinal Chemistry-Anti-Cancer Agents 23(13): 1506-1518.
91. Kumar S, Goel A, Bhat A (2020) Natural compounds in the treatment of gastric cancer: A review. Phytotherapy Research 34(3): 487-498.
92. Zhang J, Jung YY, Mohan CD, et al. (2022) Nimbolide enhances the antitumor effect of docetaxel via abrogation of the NF-κB signaling pathway in prostate cancer preclinical models. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research 1869(12): 119344.
93. Zhang L, Li Y, Sun D, et al. (2022) Protective effect of nimbolide against high fat diet-induced obesity in rats via Nrf2/HO-1 pathway. Journal of Oleo Science 71(5): 709-720.
94. Gu Y, Liu B, Xia X, et al. (2025) Chemoprotective effect of nimbolide against N-methyl-N-nitrosourea induced gastric cancer via alteration of apoptosis and NF-κB signaling pathway. Acta Cirúrgica Brasileira 40: e402125.
95. Dimri M and Satyanarayana A (2020) Molecular signaling pathways and therapeutic targets in hepatocellular carcinoma. Cancers 12(2): 491.
96. Vairappan B, Mukherjee V, Subramanian SB, et al. (2025) Nimbolide attenuates hepatocellular carcinoma by regulating miRNAs 21, 145 and 221 and their target gene expression. Gene 937: 149126.
97. Doghish AS, Ismail A, Elrebehy MA, et al. (2022) A study of miRNAs as cornerstone in lung cancer pathogenesis and therapeutic resistance: A focus on signaling pathways interplay. Pathology-Research and Practice 237: 154053.
98. Kumar B, Bhaskar D, Rajadurai M, et al. (2017) In vitro studies on the effect of Azadirachta indica Linn. in lung cancer A549 cell lines. World Journal of Pharmacy and Pharmaceutical Sciences 6(9): 1627-1640.
99. Choudhary N, Bawari S, Burcher JT, et al. (2023) Targeting cell signaling pathways in lung cancer by bioactive phytocompounds. Cancers 15(15): 3980.
100. Yang Y, Li N, Wang TM, et al. (2021) Natural products with activity against lung cancer: A review focusing on the tumor microenvironment. International Journal of Molecular Sciences 22(19): 10827.
101. Wen T, Song L, Hua S (2021) Perspectives and controversies regarding the use of natural products for the treatment of lung cancer. Cancer Medicine 10(7): 2396-2422.
102. Chen X, Zhang H, Pan Y, et al. (2024) Nimbolide exhibits potent anticancer activity through ROS-mediated ER stress and DNA damage in human non-small cell lung cancer cells. Applied Biochemistry and Biotechnology 196(1): 182-202.
103. Usman S, Jamal A, Teh MT, et al. (2021) Major molecular signaling pathways in oral cancer associated with therapeutic resistance. Frontiers in Oral Health 1: 603160.
104. Sophia J, Kowshik J, Dwivedi A, et al. (2018) Nimbolide, a neem limonoid inhibits cytoprotective autophagy to activate apoptosis via modulation of the PI3K/Akt/GSK-3β signalling pathway in oral cancer. Cell Death & Disease 9(11): 1087.
105. Ali J, Sabiha B, Jan HU et al. (2017 Genetic etiology of oral cancer. Oral Oncology 70: 23-28.

106. Gunasekaran V, Sathishkumar P (2024) Why natural drugs being attracted to treat oral cancer?-Highlights from “Oral oncology reports”. Oral Oncology Reports 10: 100395.
107. Sophia J, Kiran Kishore TK, Kowshik J, et al. (2016) Nimbolide, a neem limonoid inhibits phosphatidyl inositol-3 kinase to activate glycogen synthase kinase-3β in a hamster model of oral oncogenesis. Scientific Reports 6(1): 22192.
108. Arvindh S, Priyadarshini M, Baba AB, et al. (2025) The neem limonoid nimbolide modulates key components of the DNA damage response signalling in cellular and animal models of oral squamous cell carcinoma. Current Pharmaceutical Biotechnology 26(3): 428-442.
109. Peng BY, Wu CY, Lee CJ, et al. (2025) Nimbolide induces cell apoptosis via mediating er stress-regulated apoptotic signaling in human oral squamous cell carcinoma. Environmental Toxicology 40(2): 347-356.
110. He X, Wang N, Zhang Y, et al. (2022) The therapeutic potential of natural products for treating pancreatic cancer. Frontiers in Pharmacology 13: 1051952.
111. Javadrashid D, Baghbanzadeh A, Derakhshani A, et al. (2021) Pancreatic cancer signaling pathways, genetic alterations, and tumor microenvironment: The barriers affecting the method of treatment. Biomedicines 9(4): 373.
112. Lohse I, Wildermuth E, Brothers SP (2018) Naturally occurring compounds as pancreatic cancer therapeutics. Oncotarget 9(83): 35448.
113. Kumar S, Inigo JR, Kumar R, et al. (2018) Nimbolide reduces CD44 positive cell population and induces mitochondrial apoptosis in pancreatic cancer cells. Cancer letters 413: 82-93.
114. Mehmetoglu-Gurbuz T, Lakshmanaswamy R, Perez K, et al. (2023) Nimbolide inhibits SOD2 to control pancreatic ductal adenocarcinoma growth and metastasis. Antioxidants 12(10): 1791.
115. Khazzaka A, Rassy E, Sleiman Z, et al. (2022) Systematic review of fetal and placental metastases among pregnant patients with cancer. Cancer Treatment Reviews 104: 102356.
116. Serrano MA, Macias RIR, Briz O, et al. (2007) Expression in human trophoblast and choriocarcinoma cell lines, BeWo, Jeg-3 and JAr of genes involved in the hepatobiliary-like excretory function of the placenta. Placenta 28(2-3): 107-117.
117. Harish Kumar G, Chandra Mohan KVP, Jagannadha Rao A, et al. (2009) Nimbolide a limonoid from Azadirachta indica inhibits proliferation and induces apoptosis of human choriocarcinoma (BeWo) cells. Investigational New Drugs 27(3): 246-252.
118. Raja Singh P, Arunkumar R, Sivakamasundari V, et al. (2014) Anti-proliferative and apoptosis inducing effect of nimbolide by altering molecules involved in apoptosis and IGF signalling via PI3K/Akt in prostate cancer (PC-3) cell line. Cell Biochemistry and Function 32(3): 217-228.
119. Raja Singh P, Sugantha Priya E, Balakrishnan S, et al. (2017) Inhibition of cell survival and proliferation by nimbolide in human androgen-independent prostate cancer (PC-3) cells: Involvement of the PI3K/Akt pathway. Molecular and Cellular Biochemistry 427(1): 69-79.
120. Kashif M, Hwang Y, Hong G, et al. (2017) In vitro comparative cytotoxic effect of Nimbolide: A limonoid from Azadirachta indica (Neem tree) on cancer cell lines and normal cell lines through MTT assay. Pakistan Journal of Pharmaceutical Sciences 30.
121. Chien SY, Hsu CH, Lin CC, et al. (2017) Nimbolide induces apoptosis in human nasopharyngeal cancer cells. Environmental Toxicology 32(8): 2085-2092.

122. Fang Q, Wang J, Wu T, et al. (2025) Research progress of traditional drug therapies for nasopharyngeal carcinoma by the PI3K/Akt Signaling Pathway. Pharmacognosy Magazine 21(2): 336-347.
123. Pua LJW, Mai CW, Chung FFL, et al. (2022) Functional roles of JNK and p38 MAPK signaling in nasopharyngeal carcinoma. International Journal of Molecular Sciences 23(3): 1108.
124. Murali R, Gopalakrishnan AV (2023) Molecular insight into renal cancer and latest therapeutic approaches to tackle it: An updated review. Medical Oncology 40(12): 355.
125. Jiang A, Li J, He Z, et al. (2024) Renal cancer: Signaling pathways and advances in targeted therapies. MedComm 5(8): e676.
126. Feng C, Lyu Y, Gong L, et al. (2022) Therapeutic potential of natural products in the treatment of renal cell carcinoma: A review. Nutrients 14(11): 2274.
127. Pande AH, Prasad YR, Anakha J (2025) What are biopharmaceuticals?. Exploration of Drug Science 3: 100880.
128. Kesik-Brodacka M (2018) Progress in biopharmaceutical development. Biotechnology and Applied Biochemistry 65(3): 306-322.
129. Rang H (2017) What is pharmacology?.
130. Diddi S, Bale S, Pulivendala G, et al. (2019) Nimbolide ameliorates fibrosis and inflammation in experimental murine model of bleomycin-induced scleroderma. Inflammopharmacology (1): 139-149.
131. Pooladanda V, Thatikonda S, Bale S, et al. (2019) Nimbolide protects against endotoxin-induced acute respiratory distress syndrome by inhibiting TNF-α mediated NF-κB and HDAC-3 nuclear translocation. Cell Death & Disease 10(2): 81.
132. Kumbar SB, Jadaramkunti UC, Aladakatti RH (2012) In-vitro effect of nimbolide, an isoprenoid of neem leaf, on antioxidant system of rat cauda epididymal spermatozoa: A dose dependent study. Journal of Applied Pharmaceutical Science: 84-93.
133. Sarkar P, Acharyya S, Banerjee A, et al. (2016) Intracellular, biofilm-inhibitory and membrane-damaging activities of nimbolide isolated from Azadirachta indica A. Juss (Meliaceae) against meticillin-resistant Staphylococcus aureus. Journal of Medical Microbiology 65(10): 1205-1214.
134. Dorato MA, Buckley LA (2006) Toxicology in the drug discovery and development process. Current Protocols in Pharmacology 32(1): 10-30.
135. Hornberg JJ, Laursen M, Brenden N, et al. (2014) Exploratory toxicology as an integrated part of drug discovery. Part I: Why and how. Drug Discovery Today 19(8): 1131-1136.
136. Bhuia MS, Chowdhury R, Afroz M, et al. (2025) Therapeutic efficacy studies on the Monoterpenoid Hinokitiol in the treatment of different types of cancer. Chemistry & Biodiversity 22(5): e202401904.
137. Akhondzadeh S (2016) The importance of clinical trials in drug development. Avicenna Journal of Medical Biotechnology 8(4): 151.
138. Dias DA, Urban S, Roessner U (2012) A historical overview of natural products in drug discovery. Metabolites 2(2): 303-336.