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Review Article

Major Bioactive Prenylated Flavonoids from *Humulus lupulus* L., Their Applications in Human Diseases and Structure-Activity Relationships (SAR) – A Review

Ferah Comert Onder^{1*}, Sevil Kalin², Nebahat Sahin², Gulce Davutlar², Khaled A.N. Abusharkh^{3,4}, Ozlem Maraba², Rabia Selina Hal⁵, Mehmet Ay^{3*}, Lutfun Nahar^{6*}, Satyajit D. Sarker⁷

¹Department of Medical Biology, Çanakkale Onsekiz Mart University, Faculty of Medicine, 17020, Çanakkale, Türkiye.

²Department of Medical System Biology, Çanakkale Onsekiz Mart University, School of Graduate Students, 17020, Çanakkale, Türkiye.

³Department of Chemistry, Natural Products and Drug Research Laboratory, Çanakkale Onsekiz Mart University, Faculty of Science, 17020, Çanakkale, Türkiye.

⁴Department of Chemistry and Chemical Technology, Al-Quds University, Faculty of Science and Technology, East Jerusalem, Palestine.

⁵Çanakkale Onsekiz Mart University, Faculty of Medicine, 17020, Çanakkale, Türkiye.

⁶Laboratory of Growth Regulators, Palacký University and Institute of Experimental Botany, The Czech Academy of Sciences, Šlechtitelů 27, 78371 Olomouc, Czech Republic.

⁷Centre for Natural Products Discovery, School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, James Parsons Building, Byrom Street, Liverpool L3 3AF, United Kingdom.

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Abstract

In recent years, the incidence of cancers, inflammatory diseases, Alzheimer's disease, glucose metabolism disorder and diabetes has increased alarmingly which demands more research into the discovery of new drug candidates to treat these human diseases. Main phytochemicals from *Humulus lupulus* L. (hops) have been demonstrated to have positive impacts on human health, and prenylated flavonoids are one of the major groups of bioactive phytochemicals found in this plant. Thus, this review summarizes the role of major prenylated components in hops in human diseases including cancer, inflammation and viral infections. *In silico* studies of prenylated bioactive compounds against various drug targets such as histone deacetylases (HDACs), sirtuins (SIRT6), and acetylcholinesterase (AChE), and the molecular molecular interactions between protein and ligand have also been included. Furthermore, the structure-activity relationships (SAR) studies on these compounds are highlighted. This review concludes that the prenylated phytochemicals from *H. lupulus* L., including xanthohumol (XN), isoxanthohumol (IXN), 8-prenylnaringenin (8-PN) and 6-prenylnaringenin (6-PN), have promising roles in human health and may contribute to new drug discovery and development.

Introduction

The prenylated flavonoids possess prenyl group(s) on the core structure of a flavonoid. Because of prenylation, the lipophilicity of flavonoids increases, and thus, the interaction with the target proteins increases as well. The net result is the enhancement of biological activity. Bioactive prenylated flavonoids have various activities such as estrogenic, immunomodulatory, and anticancer activities.¹ The Cannabaceae is one of the families that produce a large number of prenylated flavonoids. *H. lupulus* of the Cannabaceae has important prenylated flavonoids such as prenylated chalcones² and naringenin, and phloroglucinol derivatives such as bitter acids contributing to health benefits.³

Humulus lupulus L. (hop) is one of the most significant

raw ingredients used in the making of beer and is widely used in many countries.⁴ This plant has still been investigated extensively by researchers.⁵⁻⁷ Before the seventh century CE (common era), historical evidence has indicated that *H. lupulus* L. has been predominantly utilized for medical purposes rather than for the brewing of beer. Therefore, hops or hop cones are well-known in traditional herbal medicine for their therapeutic properties. There has been an increasing interest in the usage of hops in the pharmaceutical industry.⁸⁻⁹ Figure 1 shows the collected hop cones from Türkiye.

This review focuses on the studies of *H. lupulus* L. prenylated flavonoids. The publications published between 2015 and 2023 in PubMed have been reviewed. The main prenylated flavonoids of *H. lupulus* L. have been indicated

*Corresponding Authors: Ferah Comert Onder, E-mail: ferahcomertonder@comu.edu.tr & Mehmet Ay, E-mail: mehmetay06@comu.edu.tr & Lutfun Nahar, E-mail: nahar@ueb.cas.cz. ©2024 The Author(s). This is an open access article and applies the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>). Non-commercial uses of the work are permitted, provided the original work is properly cited.

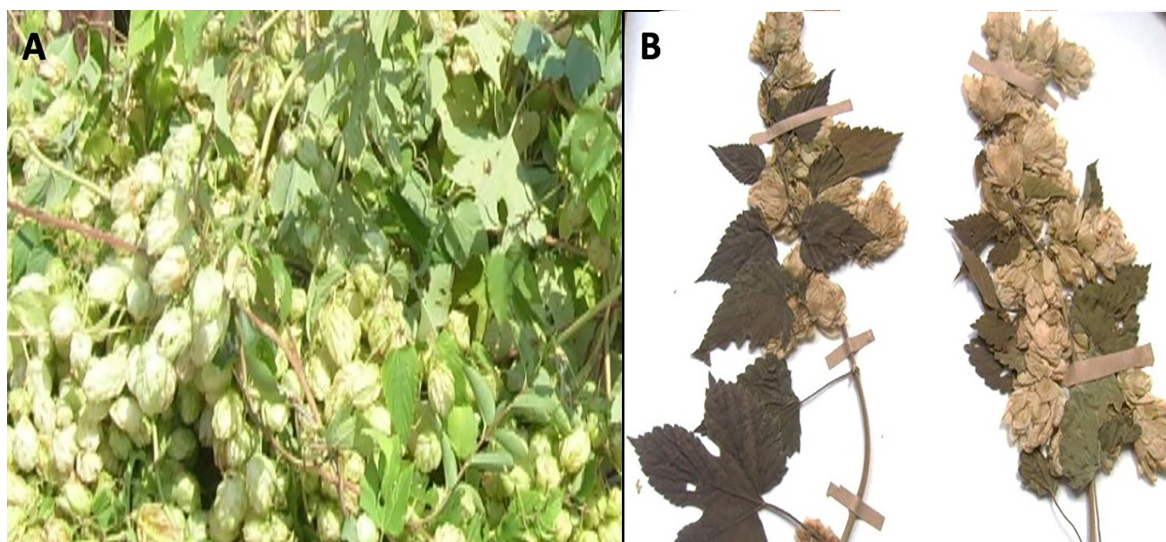


Figure 1. *Humulus lupulus* L. cones. A) collected from Türkiye (Photo by FCO). B) Herbarium image (Photo by Hulusi Malyer).

with their therapeutic values in biological activities such as anticancer, anti-inflammatory, antiviral, antifungal, antimicrobial and etc. *In silico* studies of hop components against various drug targets have also been reviewed. In addition, the prenylated components with the contribution of functional groups have been shown in this review.

Major Components of Hops

Both clinical and epidemiological investigation on the hop plant has become more prevalent due to the health-promoting elements that can greatly lessen the worldwide socioeconomic burden brought on by cancer and cardiovascular diseases and contribute to our understanding of the disease processes.¹⁰ We investigated hop extracts for their biological activities in our previously reported studies.¹¹⁻¹² The investigations have afforded the isolation and identification of pharmacologically significant substances like flavonoids, flavanones, chalcones, and derivatives of phloroglucinol.¹³ The hop flowers have received a lot of interest as a potential source of beneficial small molecules for human health including humulone, lupulone, isohumulone, and xanthohumol (XN) that are known to have antibacterial, anti-inflammatory, anticancer, and antioxidant effects.¹⁴ Hop bitter acids such as phloroglucinol compounds have anticancer properties, and depending on their chemical composition may be divided primarily into two categories: the α -acids (humulone, cohumulone, adhumulone etc..) and β -acids (lupulone, colupulone, adlupulone etc..). The level of bitter acids in hops is directly associated with its resistance to diseases including hop stunt virus, downy mildew, verticillium wilt, and hop mosaic virus. Additionally, it has been reported that hop extracts containing bitter acids have been used for antibacterial therapies.¹⁵ The hop plant also contains several prenylated flavonoids including isoxanthohumol (IXN), desmethylxanthohumol (DMX), 6-geranylneringenin (6-GN), 8-prenylneringenin (8-PN), and 6-prenylneringenin (6-PN). XN is the most significant

hop flavonoid, which has been used in the treatment and prevention of several diseases, including cancer.^{14,16-17} The structures of hop components are given in Figure 2.

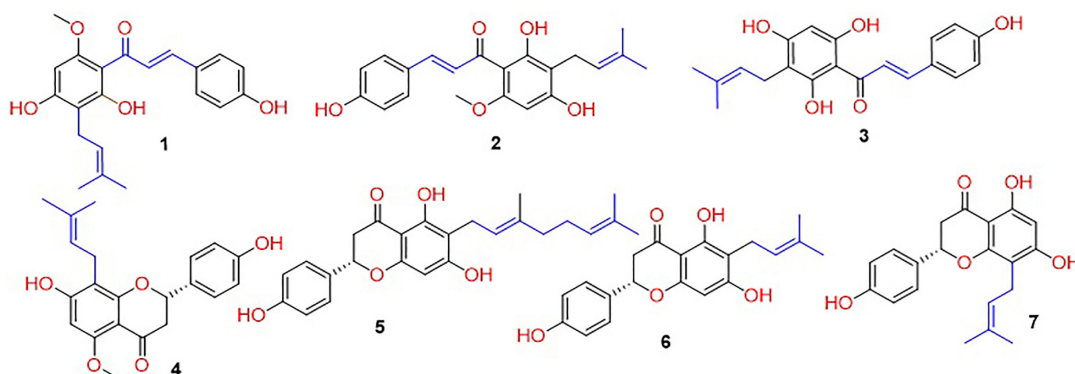
Biological Activities of the Main Prenylated Flavonoids of Hops

In recent years, the biological functions of hops have been the subject of multiple investigations. This section will cover the biological activities of hops including anti-inflammatory, anticancer, anti-Alzheimer, antiviral, antidiabetic, antimicrobial, and antifungal activities. Figure 3 exhibits an illustration of the treatment of the related human diseases with hop bioactive components.

Anticancer activity

Numerous studies reported that XN induced cancer cell death and inhibited tumour growth *in vitro* and *in vivo*.³ The effect of XN was shown in various human cancers such as estrogen receptor-positive breast cancer cells (MCF-7),¹⁸⁻²⁰ ovarian (A2780), colon (HT-29), cervical cancer,^{21,22} lung,^{22,23} melanoma and hepatocellular,^{24,25} and prostate (DU145, PC-3).²⁶⁻²⁸ The function of tumor necrosis factor-associated apoptosis-inducing ligand (TRAIL) in human prostate adenocarcinoma cells (LNCaP) cells was induced and resulted in the expression of caspases-3, -8, -9, Bid and Bax.²⁷ XN inhibits the phosphorylation of the signal transducer and activator of transcription3 (STAT3) and the expression of its downstream target genes cyclinD1, surviving, and Bcl. It induced apoptosis in various PC cells such as Panc1 and BxPC3. As a result, XN can be used as a promising therapeutic agent.^{27,29} Angiogenesis was inhibited by blocking NF- κ B activation in PC cells in *in vitro* and *in vivo* studies by XN treatment. As a result, XN suppressed vascular endothelial growth factor (VEGF) expression and IL-8 in PC cell lines.²⁸ XN was investigated for its potential anticancer activity and mechanism in LSCC (laryngeal squamous cell carcinoma) for the first time.^{30,31} XN affected cell viability in RK33 and RK45

Prenylated components from hops



Bitter acids

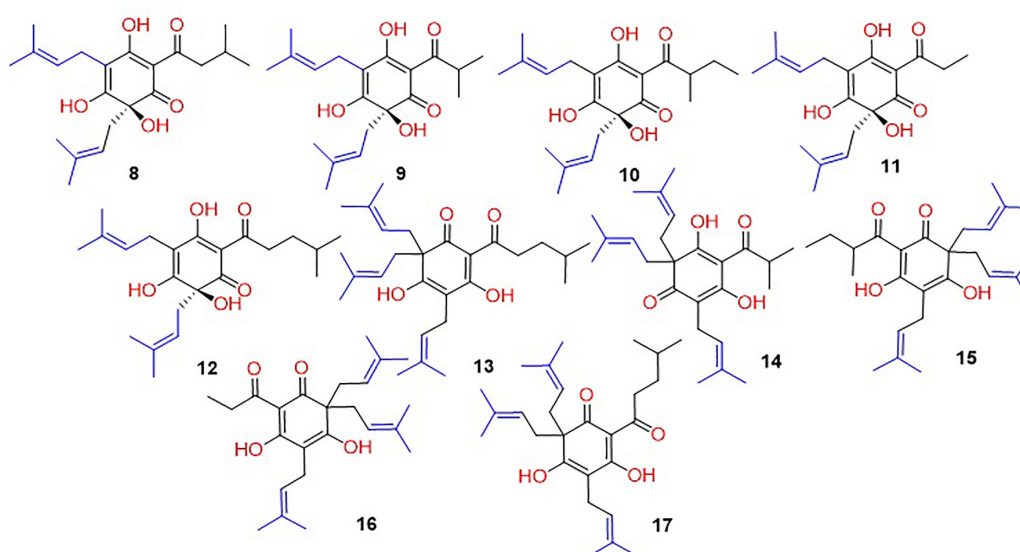


Figure 2. The chemical structures of main prenylated flavonoids of hops. (1) Xanthohumol (XN) (2) Naringenin (3) Desmethylxanthohumol (DMX) (4) Isoxanthohumol (IXN) (5) 6-Geranylnaringenin (6-GN) (6) 8-Prenylnaringenin (8-PN) (7) 6-Prenylnaringenin (6-PN) (8) Humulone (9) Cohumulone (10) Adhumulone (11) Posthumulone (12) Prehumulone (13) Lupulone (14) Colupulone (15) Adlupulone (16) Postlupulone (17) Prelupulone.¹⁴⁻¹⁷

laryngeal cancer cells and normal cells (OLN-93).³⁰ Treatment of A549 and H1563 lung cancer cells with XN caused up-regulated tumour suppressor proteins (p53 and p21) and down-regulated cyclin D1. Thus, it was suggested that XN could play a role as a promising anticancer drug candidate against lung carcinomas.³⁰ Furthermore, the cell viability was dramatically decreased in HT-29 cell line by XN with IC_{50} of 10 μ M.³² According to cell cycle analysis in HT29 cell line, XN was arrested in G2/M phase, and it suppressed cyclin B1 and Ras/MEK/ERK pathway.³² It was reported that cell proliferation in HL-60 leukaemia cells was significantly inhibited by XN, however, it did not cause cleavage or apoptosis of the caspase-3.³³ It was indicated that treating U87 MG cells with XN inhibited the IGFBP2/Akt/Bcl2 that was mediated by activation of miR-204-3p and decreased the cell viability.³⁴ Guo *et al.*³⁵ in their study, reported that treated SGC-7901 cells with 10 μ g/mL of XN

for 72 h contributed to apoptosis approximately 40%. In addition, XN (1 mg/kg) administered intraperitoneally for 3 weeks significantly reduced the growth of tumour volume in BALB/c mice. Furthermore, XN down-regulated the anti-apoptotic proteins Bcl-XL and Bcl-2, and up-regulated the proapoptotic proteins Bax, Bid, PARP and caspase-3. The phosphorylation of PI3K/Akt/mTOR in SGC7901 cells was induced by XN.³⁵ The ERK1/2-Fra1-cyclin D1 pathway was affected in XN-treated cells such as lung cancer cells (HCC827, H1975 and H23).³² In addition, XN reduced the tumor growth in HCC827 xenograft model.³⁶ It has been reported that XN sensitizes colorectal cancer cells to inhibit cell proliferation and DNA damage and apoptosis in CRC cells for chemotherapy.^{37,38} The effect of XN, a polymethoxyflavone, and nobiletin in colorectal cancer stem cells was proposed for its adjuvant potency in cancer therapies. Then, it is determined that

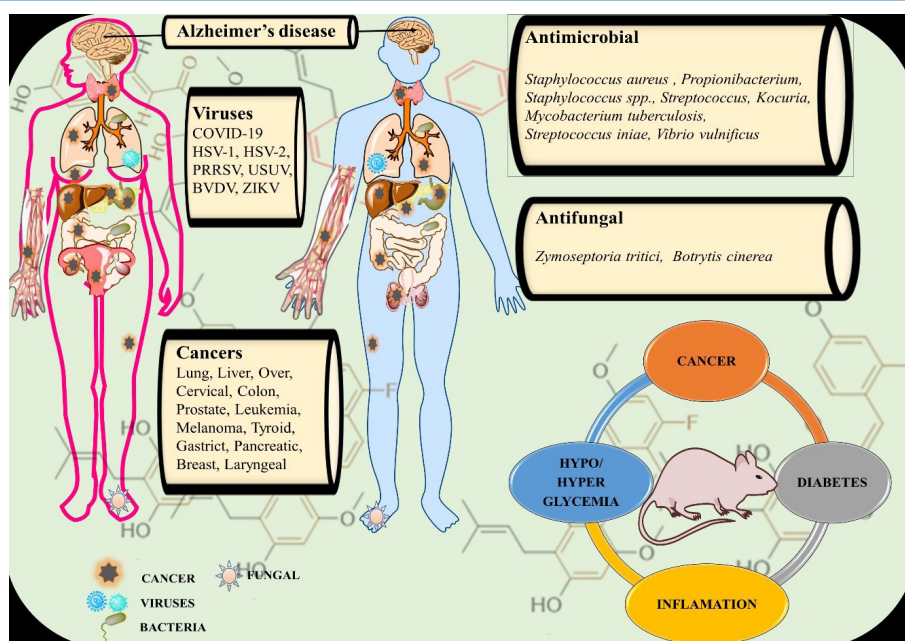


Figure 3. A schematic overview of the components in the related human diseases.

Coronavirus (COVID 19); Porcine reproductive and respiratory syndrome viruses (PRRSV); Usutu virus (USUV); Zika virus (ZIKV); Bovine viral diarrhea virus (BVDV); Herpes viruses (HSV1 and HSV2).

this mixture can suppress the migration of cancer stem cells, and reduce CD44v6 expression.³⁹ It was also reported that XN containing nobiletin sensitized colorectal cancer stem cells to 5-fluorouracil and oxaliplatin.³⁹ In a study, the treatment with XN (50 μM) for 24 hours on U87 glioma cells caused a decrease in cell proliferation ($62.2\% \pm 3.7$), and this resulted in a decrease in cell death ($33.7\% \pm 0.9$). It also reported that XN induced autophagy and thus, inhibited the Akt/mTOR/S6K pathway, and promoted the formation of LC3-II and p62 degradation.⁴⁰ STIM1 signaling inhibited through miR-4725-3p in glioma cells.⁴¹ Furthermore, XN induces the expression levels of miR-204-3p to transcriptionally regulate the ERK/c-Fos signalling pathway.³⁴

XN could inhibit cell viability and induce apoptosis as dose-dependent, and it affected the cell cycle of MCF-7/ADR cells. As a result, XN was reported as a promising compound for doxorubicin-resistant breast cancer cells.¹⁹ It was mentioned for the first time, XN had the ability to sensitize colon cancer cells SN38 as an anticancer agent.³⁷ It was reported that XN inhibits cell proliferation, induces apoptosis, and suppresses metastasis of AGS cells.⁴² It was shown that XN reduced cell proliferation in TPC-1 cancer cells with a concentration of 10 μM . At a concentration of 100 μM , a decrease in cell viability was determined by inducing apoptosis. Therefore, it is believed that XN is a high potential candidate for the treatment of thyroid cancer.⁴³ In a reported study, XN has been investigated in mouse models and *in vitro* against various cancer cells including H520 and H358 cells.⁴⁴ It was shown that XN reduced the expression of GATA3 in mice, whereas it activated STAT4 and T-bet in Th cells.⁴⁵ Two enzymes of the AKR superfamily members are important pharmacological targets for cancer (AKR1B10)

and diabetic complications (AKR1B1) treatments. The inhibitory potentials of the AKR superfamily (AKR1A1, AKR1B1 and AKR1B10) were investigated. According to these findings, XN, IXN, and 8-PN are potent inhibitors of AKR1B1 and AKR1B10.⁴⁶ IXN was investigated *in vitro* in melanoma cell line B16-F10 and *in vivo* in a metastatic model. According to the results, It was observed that XN derivative, IXN decreased the cell viability in melanoma cells dependent on a dose.⁴⁷

Although 8-PN induced the levels of P450 1A1/B1 mRNA in MCF7 cells, 6-PN inhibited P450 1A1 levels at 0.6 μM and for 1B1 at 0.2 μM . XN at 0.28 μM showed the 1A1 inhibitor activity than IXN at 1.6 μM .^{48,49} More importantly, XN has proved to exhibit cytoprotecting due to its ability to induce endogenous antioxidant defence molecules. Overall, the biological results concluded that XN had an excellent inhibitor potential in most cancer types. It is also given in Table 1. Interestingly, it is shown in Figure 4 that XN modulates these multiple molecular pathways leading to the inhibition of cancer features such as inflammation, cell cycle arrest, angiogenesis, apoptosis, proliferation, invasion and migration in various cancers. As a result, due to its antitumor potency and nontoxic properties, XN could be a promising drug candidate for further cancer therapy studies.

Anti-inflammatory activity

Inflammation is a process in the human body that involves inflammatory regulators such as tumour necrosis factor- α (TNF- α), interleukin (IL)-1 β , IL-6, nitric oxide (NO), and reactive oxygen species (ROS). XN was determined with its inhibition activity of TNF- α , IL-1 β , and NO, and activation of NF- κB signaling.⁵⁰ It is thought that the effect of XN on the regulation of NRF2 signalling could be a promising

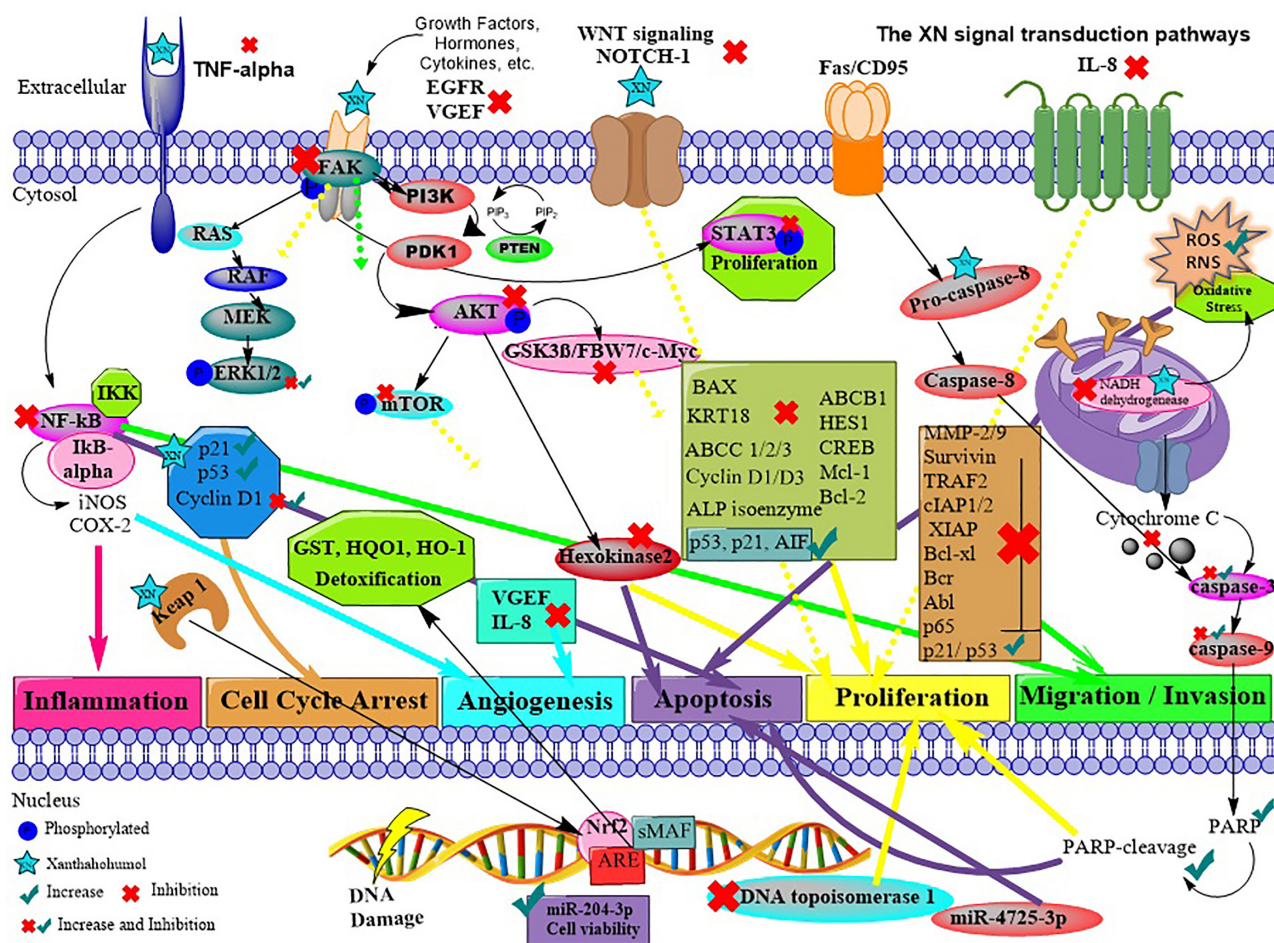


Figure 4. The metabolic pathway of XN (Redrawn).

XN: Xanthohumol; Signal transducer and activator of transcription 3 (STAT3); Epidermal growth factor receptor (EGFR); Focal adhesion kinase (FAK); Glycogen synthase kinase-3 beta, (GSK-3 β); Tumor necrosis factor alpha (TNF-alpha); Glutathione S-transferases (GST); Nuclear factor erythroid 2-related factor 2 (Nrf2); antioxidant response element (ARE); Heme oxygenase 1 (HO-1); NAD(P)H quinone oxidoreductase (HQO-1); small musculoaponeurotic fibrosarcoma oncogene homologue (sMAF); Inhibitor of nuclear factor kappa-B kinase subunit (IKK); Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (I κ B α); Mammalian target of rapamycin (mTOR); Phosphoinositide 3-kinases (PI3K); Pyruvate dehydrogenase lipoamide kinase isozyme 1 (PDK1); Phosphatase and tensin homolog (PTEN); Protein kinase B (AKT); F-box and WD repeat domain-containing 7 (FBW7); Neurogenic locus notch homolog protein 1 (Notch1); Bcl-2 Associated X-protein (BAX); Keratin 18 (KRT18); Induced myeloid leukemia cell differentiation protein (Mcl-1); cAMP response element-binding protein (CREB); B-cell lymphoma 2 (Bcl-2); ATP-binding cassette sub-family B member 1 (ABCB1); Matrix metalloproteinase (MMP); TNF receptor associated factor2 (TRAF2); Cellular inhibitor of apoptosis protein 1 (cIAP1); Apoptosis Inducing Factor (AIF); B-cell lymphoma-extra-large (Bcl-xL); Cluster region protein (BCR); Tyrosine-protein kinase (ABL); Mitogen-activated protein kinase kinase kinase (MEK); Extracellular signal-regulated kinase (ERK); Inducible nitric oxide synthase (iNOS); Cyclooxygenase 2 (COX-2); X-linked inhibitor of apoptosis protein (XIAP); Poly (ADP-ribose) polymerase (PARP); Nuclear factor kappa light chain enhancer of activated B cells (NF-kB); Vascular Endothelial Growth Factor (VEGF); Interleukin-8 (IL-8); hes family bHLH transcription factor 1 (HES1); Cluster of differentiation 95 (CD95); Kelch-like ECH-associated protein 1 (Keap 1); Reactive oxygen species (ROS); Reactive nitrogen species (RNS); Nicotinamide adenine dinucleotide hydrid (NADH).

Table 1. XN and IXN related doses and their actions in different cancer types.

Cancer Type	Cell Line/Animal Model	Action	Dose	Ref
Breast	BALB/c mouse tumor model	Suppression of tumor growth, $\downarrow$ tumor weight $\downarrow$ survival $\uparrow$ caspase cleavage $\downarrow$ Notch-1 $\downarrow$ Ki-67		20
	Adriamycin-resistant MCF-7	$\downarrow$ Cell viability $\uparrow$ radio and chemosensitivity $\uparrow$ Apoptosis $\uparrow$ γ -H2AX $\downarrow$ STAT3 $\downarrow$ MDR1 $\downarrow$ EGFR	10 μ M	19
Colorectal	Xenograft mouse model FHC, SW620, LOVO, CCD841, SW480, CoN, HT29, HCT116, CDD-18Co	$\downarrow$ tumor cell proliferation $\uparrow$ Apoptosis $\uparrow$ cyt.c release $\downarrow$ cell viability $\downarrow$ cell cycle arrest $\uparrow$ Apoptosis $\uparrow$ caspase-3 $\uparrow$ caspase-9 $\downarrow$ cyclin B1 $\downarrow$ MEK/ERK	10 and 100 μ M	32,37
	Male Sprague Dawley rats by using SW480 CRC cells	$\downarrow$ tumor cell proliferation $\uparrow$ Apoptosis $\downarrow$ Wnt/ β -catenin signaling $\downarrow$ Bax $\downarrow$ Bcl-2 $\downarrow$ caspase-3 $\downarrow$ iNOS $\downarrow$ COX-2		38

Table 1. Continued.

	FHC, CCD841, CoN, HT29, SW480, LOVO, HCT116 and SW620	↓cell proliferation, cell viability, and colony formation ↑Apoptosis ↓HK2 ↓glycolysis ↓EGFR-Akt	25 µM	32
	HCT116 and RKO CRC cell line	↓ CD44v6 ↓S and G2/M ↑cell death-related genes ↓cell viability of CRC stem cells	5–10 and 25 µg/mL	39
Cervical	Ca Ski	↓proliferation ↑Apoptosis ↑caspase-3 ↑caspase-8 ↑caspase-9 ↑cell cycle arrest ↑p53 ↓XIAP	59.96 µM	22
Cholangiocarcinoma	KKU-M139 and KKU-M214	↓cell growth ↓STAT3	20 and 50 µM	29
Laryngeal	RK33 and RK45	↓cell viability ↑Apoptosis ↑caspase-3 ↑caspase-8 ↑caspase-9 ↑p53 ↑p21 ↓cyclin D1 ↓ERK1/2	12.3 and 22.5 µM	30
	SCC4	↓proliferation ↑Apoptosis ↑PARP ↑p53 ↑AIF ↓Bcl-2 ↓Mcl-1	20, 30, 40 µM	31
Thyroid	TPC-1 cell lines	↓TPC-1 cell proliferation. induce cell death, DNA fragmentation and promotes cell cycle arrest in S phase ↑caspase-3,-7 activity, support pro-apoptotic effect	10 µM	43
Glioblastoma	U87 glioblastoma	↓cell viability ↑Apoptosis ↓IGFBP2/Akt/Bcl-2 ↑miR-204-3p ↑ERK/c-Fos	25 µM	34
	Human glioblastoma U87-MG	↑miR-4725-3p ↓cell viability ↓STIM1 signaling	10 and 20 µM	41
Hematological	HL60	Activation of p38 MAPK	1-50 µM	33
Pancreatic	BxPC3, MXPαCa2, and AsPC1	↓ cell proliferation ↓NF-Kb ↓VEGF ↓IL-8 ↓mRNA	0.5–25 µmol/L	28
	PANC1 and BxPC3	↓proliferation, viability, colony formation ↑Apoptosis ↓p-STAT3	5–100 µM	26
	Subcutaneous xenograft mouse model by using BXPc-3 cells	↓tumor growth and angiogenesis ↓NF-κB activation ↓tube formation ↓VEGF ↓IL8	10 mg/kg/week	28
Liver	Huh7, Hep3B, SK-Hep1, and HepG2	↓colony formation, cell viability and confluency ability ↓HES1 ↓Notch1 pathway	5 µM	25
Lung	Xenograft mouse model by using HCC827 cells	↓tumor growth ↓Cyclin D1 ↓ERK1/2-fra1	10mg/kg (i.p.),	36
	A549	Nuclear apoptotic features ↑activity of caspase-3, -8, and -9.	5–50 µM	21
	<i>In vitro</i> and xenograft mouse models using A549, H520 and H358 cell line	Suppressed cell viability, colony formation and induced apoptosis inhibited Akt activity, suppressed NSCLC is xenograft tumor growth ↑PUMA expression in tumor tissues	10 mg/kg (i.p.)	44
Prostate	LNCaP combination with TRAIL	↑caspases-3, -8, and ↑Bax ↓ Bcl-xL	10 µM	27
Melanoma (IXN)	B16-F10 <i>in vivo</i> in a murine metastatic model	↓ Melanoma cell viability suppression of the processes that define metastasis—cell adhesion, invasion, and migration	30 µM	47
Gastric	GC cells (AGS, SGC-7901, MGC-803), GES-1	GC cell viability ↓Bcl-2 activity ↑Bax activity inhibition of NF-κB signaling, ↓ROS overproduction	1–100 µM	42

Xanthohumol (XN); Iso-xanthohumol (IXN); Signal transducer and activator of transcription 3 (STAT3); Multidrug Resistance Mutation (MDR1); Epidermal growth factor receptor (EGFR); Cytochrome c (cyt.c); extracellular signal-regulated kinase (ERK); Inducible nitric oxide synthase (iNOS); Cyclooxygenase 2 (COX-2); X-linked inhibitor of apoptosis protein (XIAP); Poly (ADP-ribose) polymerase (PARP); insulin like growth factor binding protein 2 (IGFBP2); Mitogen-activated protein kinase (MAPK); nuclear factor kappa light chain enhancer of activated B cells (NF-κB); Vascular Endothelial Growth Factor (VEGF); Interleukin-8 (IL-8); hes family bHLH transcription factor 1 (HES1); Non-small cell lung cancer (NSCLC); tumor necrosis factor-related apoptosis-inducing ligand (TRAIL); Reactive oxygen species (ROS); GC (gastric cancer)

strategy for the progression of inflammation-related neurodegenerative diseases.⁵¹

To understand the role of bioactive components isolated from hop extracts, these compounds were tested in various models.⁵² Related to the immune system and the inflammatory process, XN and IXN were identified

as potential for their anti-inflammatory activities.⁵² The *in vivo* anti-inflammatory role of XN was assessed in models of acute and chronic inflammation in different organs. Oxazolone-induced dermatitis was prevented by topical application of XN,⁵³ and also hop components were reported with biomaterial applications such as skin

wound healing and regeneration.⁵⁴ Also, it was reviewed the inhibitor effect of XN on pro-inflammatory enzymes such as COX-1 and COX-2.⁵⁴ When the antiinflammatory activity of hops extracts was characterized in an *in vitro* model of gastroenteritis, identified active compounds that inhibited IL-8 release in human gastric epithelial AGS cells in a dose-dependent manner. Depending on the phytochemical analysis it was revealed that XN-A and XN-D were determined as the main active ingredients.⁵⁵ Hydroalcoholic extract of hops inhibited IL-8 release in a dose-dependent ($IC_{50} = 3.95 \mu\text{g/mL}$). This result indicated that this extract showed a strong inhibitory effect owing to prenylated chalcone content.⁵⁶ According to one of the reported studies on 14 healthy volunteers, XN through diet was observed for its anti-inflammatory effects.⁵⁶ Additionally, it is hypothesized that XN may reduce the immune response in humans to lipoteichoic acid at low doses. Therefore, these effects might be related to the binding or uptake of XN by monocytes.⁵⁶

Consequently, the studies reveal that XN has an anti-inflammatory effect. Its broad-spectrum activity is effective in the treatment of various diseases associated with inflammation.

Anti-Alzheimer activity

The formation of arachidonic acid (AA) in the brain is regulated by monoacylglycerol lipase (MAGL) that terminates the endocannabinoid 2-arachidonoylglycerol (2-AG) signalling.⁵⁷ As a result, monoacylglycerol lipase (MAGL) plays a dual role in controlling brain inflammation by regulating arachidonate and endocannabinoid concentrations.⁵⁸ In this context, the natural product (NP-2) showed an inhibitor activity ($IC_{50} = 9.5 \pm 1.2 \mu\text{M}$) against hMAGL. The study was determined with an inhibitor (NP-2 and/or 8-PN) of hops.⁵⁹ Alzheimer's disease (AD) is characterized by neuroinflammation associated with common neuronal death.⁶⁰ It has been reported that NPs possess inhibition effects of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), which are important enzymes of AD. In a study reported by Orhan *et al.*⁶¹, it was investigated that hop components including XN and acyl phloroglucinol derivatives (compounds 1-14) may have an inhibitor activity to AChE and BChE. As a result, 3-hydroxy-XN with IC_{50} value of $51.25 \pm 0.88 \mu\text{M}$ and XN with IC_{50} value of $71.34 \pm 2.09 \mu\text{M}$ were determined in a moderate potency against AChE. In addition to 3-hydroxy-XN with IC_{50} of $63.07 \pm 3.76 \mu\text{M}$ and XN with IC_{50} of $32.67 \pm 2.82 \mu\text{M}$, 8-PN with IC_{50} of $86.58 \pm 3.74 \mu\text{M}$ also exhibited inhibition activity towards BChE in the micromolar ranges. Thus, XN was identified with its highest inhibition potential compared to reference drug galantamine ($IC_{50} = 46.58 \pm 0.91 \mu\text{M}$). Consequently, these results demonstrate that XN may be a potential candidate for the discovery of new cholinesterase inhibitors for the treatment of AD.

Antiviral activity

Coronavirus (called SARS-COV-2 or COVID 19) has infected millions of people since 2019 worldwide. However, the identified hop stunt viroid (HSVd)⁶², apple fruit crinkle viroid (AFCVd)⁶³, citrus bark cracking viroid (CBCVd)⁶⁴, and hop latent viroid (HLVd)⁶⁴ caused hop diseases.⁶²⁻⁶⁴ For instance, Lin *et al.*⁶⁵ reported with IC_{50} value of $1.53 \mu\text{M}$ of XN as pan-inhibitors of the main protease of COVID-19. Furthermore, XN showed the inhibition activities against several viruses such as porcine reproductive and respiratory syndrome viruses (PRRSV).^{66,67} XN was found as a promising therapeutic agent on $15 \mu\text{M}$ for PRRSV. Liu *et al.*⁶⁷ showed that XN could reduce oxidative stress of PRRSV-induced. 8-PN showed an antiviral effect against influenza virus which provides the inhibition of replication in the changing range ($IC_{50} = 5.5 \pm 0.2$) according to virus types.⁶⁸ Both XN and eeyarestatin I have been reported to suppress replication of Usutu virus (USUV) and Zika virus (ZIKV) but eeyarestatin I has demonstrated a stronger antiviral effect than XN.⁶⁹ In addition, IXN is one of the other prenylflavonoids that affect bovine viral diarrhea virus (BVDV), herpes viruses (HSV1 and HSV2).⁷⁰

Antidiabetic activity

Disruption of glucose metabolism leads to several human diseases such as diabetes mellitus (Type1 and Type2), hyper- and hypoglycemia, and obesity. Although there have been many studies conducted until 2015, the researchers continue to understand the effect potentials of hop components. For instance, one of the studies demonstrated that body weight for obese C57BL/6J mice was lost by XN (0, 30, 60 mg/kg weight/daily amount of XN for 12 weeks).⁷¹ Hop components XN, IXN and 8-PN are potential uncompetitive inhibitors of aldoketo reductase superfamily members of AKR1B1 and AK1B10.⁴⁶ KDT501 is a novel isohumulone, reduces weight, and insulin resistance, and dyslipidemia *in vivo*.⁷² In recent years, 8-PN and naringenin (50 mg/kg) have improved islet dysfunction and glucose homeostasis in streptozotocin (STZ)-induced insulin-deficient diabetic mice with oral administration.⁷³ XN has a protective effect against type-2 diabetes-induced liver fibrosis and steatosis via NRF2/AGE/RAGE/NF- κ B signalling in nonalcoholic fatty liver disease (NAFLD) rat model.⁷⁴ XN was used as a glucose/fructose transport inhibitor agent in a study on glucagon-like peptide.⁷⁵ The IC_{50} values of 8-PN ($45.92 \mu\text{M}$, $>100 \mu\text{M}$, $>50 \mu\text{M}$) and 8-GN ($3.77 \mu\text{M}$, $15.38 \mu\text{M}$, $>50 \mu\text{M}$) were determined against α -glucosidase, α -amylase, and β -glucosidase, respectively.⁷⁶ In a study, the binding affinities of 8-PN and 8-PG have been reported by using the Microscale Thermophoresis analysis as K_d value of $4.1 \pm 1.2 \mu\text{M}$, and $7.4 \pm 3.5 \mu\text{M}$, respectively, against His-tag-labeled hLGP which is associated with type2 diabetes protein.⁷⁷

Antimicrobial activity

The discovery of new drugs with strong antimicrobial

activity, especially against resistant strains, is very important.⁷⁸ For this reason, hop, which has a long history in traditional medicine, has been used as a treatment for many conditions including bacterial infections, as well as for its protective properties.^{78,79} It is known that prenylated flavonoids have been proven to have strong antimicrobial activity.^{80,81} Prenylflavonoids, especially XN was reported in an earlier study with antibacterial activity against Gram-positive bacteria such as *Staphylococcus*, *Propionibacterium*, *Kocuria*, and *Streptococcus*.⁸² XN, chalconaringenin, and naringenin were shown to inhibit the growth of *S. aureus*.⁸³ *Staphylococcus* spp. were reduced by humulone, lupulone and XN. As a result, the strongest effect was obtained by lupulone after XN. Lupulone (~125 µg/mL) and XN (~60 µg/mL) at high concentrations reduced the number of surviving bacterial cells to zero.⁸⁴ It has been reported that lupulone exhibits an inhibitor effect against the growth of methicillin-susceptible *S. aureus* and methicillin-resistant *S. aureus* with MIC values for DMX (0.6-1.2 µg/ml and 19.5-39 µg/mL).⁸⁵ XN and lupulone showed strong antibacterial properties among the studied compounds.⁸⁶ Lou et al.⁸⁷ investigated the combined effect of XN with isoniazid against mice infected with *Mycobacterium tuberculosis*.⁸⁷ In another study, the antimicrobial activities of hops were investigated against *Streptococcus iniae* and *Vibrio vulnificus*. It has been reported that XN shows the strongest antibacterial property against *Streptococcus*.⁷⁸

Antifungal activity

The antifungal activity of prenylflavonoids has been revealed in various studies; for instance, antifungal activity against *Zymoseptoria tritici* was determined by cohumulone and DMX with half-maximum inhibitory concentrations of 0.11 and 0.2 g/L, respectively.⁸⁸ It was demonstrated that IXN significantly inhibited antifungal activity against *B. cinerea* with an EC₅₀ value of 4.32 µg/mL. This study shows that IXN can be used for phytopathogenic fungi.⁸⁹

In Silico Studies

To analyze the effects of inhibitor candidates against drug targets, computer-assisted approaches led to experimental studies.⁹⁰ According to the reported studies, XN, 6-PN, and 8-PN have been evaluated to understand their binding affinities against acyl-protein thioesterase 2.⁹¹ In one of the reported studies, Many flavonoids were investigated against SIRT1 by molecular docking study to understand their interactions to select effective and potent flavonoid candidates. Among these flavonoids, XN was mediated by the activation of SIRT1 and had hepatoprotective effects.⁹² Therefore, among the reported flavonoid compounds, XN displayed the docking score of -5.26 kcal/mol against SIRT1. However, SIRT1 activator STAC showed the lowest docking score -6.20 kcal/mol.⁹² As shown in Table 2, among the *in silico* results of an FDA-approved drug, it was notable that in an earlier reported study, a selective SIRT1 inhibitor, selisistat (EX527) exhibited strong interactions such as hydrogen binding and pi-pi interactions with

Gln345, and His363 and Phe273, respectively.⁹³ The redocking of selisistat and the 2D interactions including structural water molecules and Phe273, Phe297, Ile279, Ile316, Ile411, Ile347, Asp348, Gln345 have been reported in the recent study,⁹⁴ and the docking score was calculated as -7.78 kcal/mol.⁹⁵ Moreover, it was reported that selisistat showed more hydrophobic interactions with SIRT6 including H131, W186, F62, Q111, T213 residues than SIRT1 in the active pocket of the protein.⁹⁶

In a study with GOLD algorithm, 6-PN and 8-PN showed inhibitory activity against HDAC enzymes calculated as HDAC2 (62.8 kcal/mol), HDAC4 (66.5 kcal/mol) HDAC7 (56.9 kcal/mol), HDAC8 (59.5 kcal/mol) for 6-PN and HDAC2 (37 kcal/mol), HDAC4 (57.6 kcal/mol), HDAC7 (56.7 kcal/mol), HDAC8 (64.8 kcal/mol) for 8-PN.⁹⁷ Taken together, based on the *in silico* data both 6-PN and 8-PN were suggested to exhibit inhibitory activity against HDAC enzymes of class I and II comparable to standard HDACi such as naturally identified and biologically active TSA (trichostatin A) and FDA-approved drug SAHA (Vorinostat).⁹⁷ Among the *in silico* results of an FDA-approved drug, HDAC2 inhibitor, SAHA has the LibDock score and binding energy as -126.37 kcal/mol and -33.25 kcal/mol, respectively. It was shown that the hydrogen bond interactions with Arg39, His183, Gly305, Gly154 amino acids and pi-pi interaction with Phe155.^{98,99} The affinity score of SAHA was compared with similar analogue.¹⁰⁰ SAHA was reported for four with His18, Gly27, Lys31 and Lys331 and five hydrogen bonds with Asp104, His145, His146, Asp181 and Tyr208 to HDAC1 and HDAC2, respectively. For HDAC4 and HDAC6, SAHA showed hydrogen bond interactions with His802, Asp840 and His842, and Gly582 and His614, respectively.¹⁰¹ The binding energies of SAHA for HDAC2 (-20.884 kcal/mol and -8.287 kcal/mol) and HDAC6 (-20.231 kcal/mol and -10.664 kcal/mol) were determined. Also, it was found as -17.547 kcal/mol and -11.064 kcal/mol for TSA drug for HDAC6.¹⁰² In one of the reported studies, the estimated binding free energy of SAHA for HDAC8 was calculated as -4.35 kcal/mol.¹⁰³ Docking score of SAHA against HDAC2 was found as -7.068 kcal/mol by MOE software.¹⁰⁴ Furthermore, in one of the reported studies, SAHA was compared with naturally occurring components including apigenin, flavone, and luteolin against HDAC1 and HDAC2.¹⁰⁵ Molecular docking simulation results showed that the studied components exhibited lower binding energy with focused docking compared to reference molecule SAHA-HDAC1 (-8.46 kcal/mol) and HDAC2 (-8.45 kcal/mol).¹⁰⁵ On the contrary of focused docking, the free energy of interaction results for blind docking were reported as -7.23 kcal/mol and -7.45 kcal/mol for HDAC1 and HDAC2, respectively. Thus, the best interaction energy was found for flavone and apigenin with the specific residues.¹⁰⁵ In another study, the docking score of TSA-HDAC6 by the Glide method was determined as -8.782 kcal/mol, and the interaction for TSA was reported with Phe643 in an earlier study.^{106,107} The docking scores and interactions are given

in Table 2.

IFD and GOLD molecular docking protocols were applied to confirm the catalytic binding energies of XN against cholinesterases, AChE and BChE.⁶¹ According to docking results, 8-PN showed a binding energy of -8.86 kcal/mol with BChE. H-bond and π - π interactions were observed between 8-PN and specific amino acid residues including Gly117, His438, and Ser198 of BChE. It was also

supported by *in vitro* analysis ($IC_{50} = 86.58 \pm 3.74 \mu M$). Whereas, 8-PN showed no significant inhibition of AChE, but moderately inhibited plasma cholinesterase ($IC_{50} = 86.6 \mu M$).¹⁰⁸ Natural product dihydroquercetin was studied against AChE and BChE.¹⁰⁹ FDA-approved inhibitors such as donepezil, galantamine, and rivastigmine are used in the treatment of Alzheimer's disease. Donepezil is used to inhibit the hydrolysis of AChE, and is a noncompetitive

Table 2. The docking scores and molecular interactions of XN, 8-PN, and the inhibitors of drug targets.

Compounds	Docking score, binding free energy (kcal/mol) against AChE	Target	Specific amino acid residues	Interactions	Ref.
XN	-5.26	SIRT1	-	-	92
STAC (activator)	-6.20	SIRT1			92
Selisistat (EX527)	-7.78	SIRT1	Gln345, His363 Phe273 Phe297, Ile279, Ile316, Ile411, Ile347, Asp348,	H-bonds, pi-pi	93-96
8-PN	HDAC2 (62.8), HDAC4 (66.5) HDAC7 (56.9), HDAC8 (59.5) for 6-PN and HDAC2 (37), HDAC4 (57.6), HDAC7 (56.7), HDAC8 (64.8) for 8-PN (GOLD program)	HDACs			97
SAHA	126.37 (LibDock) -33.25, -8.46, -7.23	HDAC1	Arg39, His183, Gly305, Gly154, Phe155, His18, Gly27, Lys31 and Lys331	H-bonds, pi-pi	98-100, 105
SAHA	-20.884 and -8.287 -7.068, -8.45, -7.45	HDAC2	Asp104, His145, His146, Asp181, Tyr208, Tyr308, His33, Pro34	H-bonds	98-100, 104, 105
SAHA	For HDAC6 -20.231 and -10.664 For HDAC8 -4.35	HDAC4	His802, Asp840, His842, Gly582, His614, His610, His611, His651, Tyr782	H-bonds	101, 102
TSA	-17.547 and -11.064, -8.782	HDAC6	His610, His651, Tyr782, Pro501, Phe620, Phe680, Phe643	H-bonds, stacking	102, 106, 107
Donepezil	-19.71, -17.57, -17.567, -17.257, -15.23, -13.603, -13.2, -12.3, -12.2, -11.9, -11.5, -10.567, -10.5, -10.46, -10.41, -10.30, -9.68, -9.17, -8.5, -8.4, -11.93*, -9.81*	AChE	Tyr122, Phe293, Arg294, Leu287, Trp284, Trp84, Ser291, Val292, Tyr335, Trp88, Trp279, Phe288, Phe290, Phe330, Tyr334, Arg289, Leu282, Phe331, Tyr121, Trp86, Glu202, Trp286, Phe295, Phe388, Tyr337, His447, Trp439, Tyr449, Val340, Gly82, Thr83, Gly121, Val132, Trp286, Tyr124, Phe338, Val294, Tyr341, Tyr72, Tyr465, Ser203, Asp74, Ser125	H-bonds, pi-alkyl bonds, pi-pi stacking, cation-pi, carbon-H-bonds, hydrophobicity-drophilic	111-157
Rivastigmine	-7.64, -7.0	AChE	Tyr449, Trp439, Tyr337, Val240, Gly482, Thr83, Val132, Trp86, Gly121, Trp286, Tyr124, Tyr337, Tyr449, Val340		110, 111, 129, 137
Neostigmine	12.20 and -9.54	AChE			130
Galantamine	-10.562, -8.16, -7.1	AChE	Gly118, Gly119, Tyr121, Ala201, Glu199, Asp74, Trp86, Phe338, Tyr337, Glu202, Trp439, Gly82, Thr83, Tyr124, Gly121, Val132		133, 136, 137

*is indicated as kJ/mol. Abbreviations. Xanthohumol (XN); 8-prenylflavonin (8-PN); Acetylcholinesterase (AChE); Histone deacetylases (HDAC); Sirtuin 1 (SIRT1).

and irreversible inhibitor.¹¹⁰ The binding energies of FDA-approved drugs donepezil, galantamine and rivastigmine were reported.¹¹¹ According to the recently reported studies, among the *in silico* results of FDA-approved drugs, the binding energies of donepezil were reported between -19.71 kcal/mol and -8.4 kcal/mol calculated by using various algorithms such as AutoDock Vina, Glide/SP/XP, and MOE etc.¹¹²⁻¹²⁸ Furthermore, docking scores were given as -10.46 kcal/mol and -7.64 kcal/mol for donepezil and rivastigmine, respectively, by using Glide/SP.¹²⁹ In our previously reported study, we calculated induced fit docking (IFD) and quantum polarized ligand docking (QPLD) scores as -12.20 kcal/mol and -9.54 kcal/mol for known inhibitor neostigmine, respectively, against AChE. In addition, it was found as -9.67 kcal/mol and -5.77 kcal/mol against BChE.¹³⁰ Recently, a flavonol glucuronide querciturone was reported for its higher docking score (-13.43 kJ/mol) than the other naturally occurring compounds that had a strong binding affinity than donepezil (-9.81 kJ/mol).¹³¹ The binding energies of donepezil were reported as -11.93 kJ/mol and -9.73 kJ/mol for AChE and BChE, respectively.¹³² In another study, the binding affinities against AChE were determined as -10.567 kcal/mol and -10.562 kcal/mol for donepezil and galantamine, respectively.¹³³ It was reported that the binding free energy of donepezil was -10.30 kcal/mol.¹³⁴ Whereas, the binding free energy for donepezil was given as -8.5 kcal/mol for AChE.¹³⁵ The binding energies of donepezil and galantamine were found as -10.41 kcal/mol and -8.16 kcal/mol, respectively.¹³⁶ The interactions and docking scores were reported for donepezil, galantamine and rivastigmine in detail.¹³⁷ The galantamine interactions were Asp74, Trp86, Phe338, Tyr337 and Glu202.¹³³ The active site residues of AChE such as Phe295, Trp86, Trp286, Tyr337, Tyr465, Tyr124, His447, Ser203 etc. with donepezil have been reported in various recently reported studies.¹³⁸⁻¹⁵⁵ The interactions between the target protein and drug was reported as H bonds with Tyr122, Phe293, Arg294, alkyl bonds with Leu287, Trp284, and pi-pi stacking with Trp84, and carbon-hydrogen bond with Ser291, Val292, Tyr335.¹⁵⁶

The main interactions for donepezil were reported for H bonds, hydrophobic and pi-interactions with Trp88, Trp279, Phe288, Phe290, Phe330, Tyr334, Arg289. For galantamine, the interactions with residues including Gly118, Gly119, Tyr121, Ala201, Glu199 were different than donepezil active site residues.¹³⁶ We reported in our previous study that the interactions were observed with Tyr337, Trp86, Phe338, Tyr341, Asp74, Gly120 for coumarin-based compounds.¹³⁰ In another coumarin-based study, some interactions for donepezil were mentioned as Leu282, Phe288, Phe290, and Phe331 for hydrophobic and Tyr121 for hydrophilic.¹⁵⁷ In one of the reported studies, donepezil had the highest score than protoberberine alkaloids against AChE and the interactions were reported.¹⁵⁸

It was reported that XN inhibits Mpro and is a potent

pan-inhibitor against various coronaviruses. A molecular docking approach was used to estimate the potent inhibitory activities of XN against various coronaviruses. Binding scores ranged from -6 kcal/mol to -8 kcal/mol. A hydrogen bond has been determined between XN and Cys-145 residue of SARS-CoV-2 Mpro.⁶⁵ The molecular docking study was performed against LXR α with XN. The docking scores were calculated as -7.51 and -8.64 kcal/mol for related ligands and XN, respectively. These data indicated that XN has a stronger binding affinity.¹⁵⁹ It was demonstrated that XN and its derivative TXN (tetrahydro-XN) could bind to the ligand binding site of PPAR γ (peroxisome proliferator-activated receptor gamma) with molecular docking simulations.¹⁶⁰ It has been estimated that XN and dapsone drugs might affect tumour progression via abnormal expression of LCAT and NAT2, respectively. For this purpose, a molecular docking study was carried out with NAT2, XN and LCAT (PDB ID 4X96). The binding score of XN was calculated as -7.1 kcal/mol. The interactions between XN and LCAT have been observed with related amino acids such as Asp56, Thr54, Lys53, and Tyr51. These results have indicated that tumour progression of high-risk COPD patients may be altered by XN and a known drug.⁴⁴ Four prenylflavonoids XN, IXN, 8-PN and a semisynthetic prenylflavonoid derivative, tetrahydroxanthohumol (TXN) were tested with *in silico* studies to understand binding potency against human farnesoid X receptor (FXR). XN binds to the ligand binding domain of FXR388. Furthermore, a molecular docking study suggests that three key functional groups in prenylflavonoids including 4-hydroxyphenyl, 2-hydroxy and carbonyl group contribute to form of H-bond with related amino acid residues of FXR-LBD.¹⁶¹ Molecular docking study was performed with XN and MD2. It was reported that XN could bind to MD2 and the 4'-hydroxyphenyl of XN formed an H-bond with Arg90 at the active pocket of MD2.¹⁶² To evaluate the binding affinity of XN, a molecular docking study was performed. XN was well localized to IKK β . The free binding energy for XN was determined as -7.61 kcal/mol.¹⁶³ The other study focused on identifying a direct target for XN and reported that AKT kinase activity was analyzed by a molecular docking study. According to the obtained results, XN displayed H-bond interaction with Ala230, Glu228, Lys158, and Glu234 in the backbone of AKT1. In addition, the interactions with Glu236, Thr213 and Lys181 were observed for AKT2. Besides, it was indicated that XN could bind with PDK1 and p70S6K.¹⁶⁴ Hop-derived nine compounds against α -glucosidase were evaluated by *in silico* approaches. Molecular docking results showed that allosteric and catalytic sites of α -glucosidase interacted with IXN and lupulone, respectively. As a result of this study, it is thought that a diet rich in hops may be beneficial for hyperglycemia.¹⁶⁵ Prenylated flavonoids including XN, and prenylnaringenin of hops act as positive modulators of GABA-induced responses at GABA receptors. *H. lupulus* L. contains neuroactive compounds that are useful in

traditional medicine. Humulone (α -acid) and 6-PN are the most active neuroactive compounds of hops. Both humulone and 6-PN act mainly on the γ -aminobutyric acid (GABA) domain. The interactions were evaluated between humulone and other reported hop compounds active at GABA-receptors.¹⁶⁶ Compounds 8-PN and 6-PN were performed by molecular docking studies for type 2 diabetes-associated proteins.⁷⁶ In one of the reported studies, to investigate the binding affinities between 23 compounds including 8PN and 8-PG flavonoids and glycogen phosphorylase, MM-PBSA and MM-GBSA were calculated as $(2.1 \pm 0.4 \text{ kcal/mol})$ and $(0.4 \pm 0.4 \text{ kcal/mol})$ for 8PN, and $(-4.5 \pm 0.33 \text{ kcal/mol})$ and $(-5.9 \pm 0.4 \text{ kcal/mol})$ for 8-PG, respectively.⁷⁷ The reactions of XN, IXN, 6-PN and 8-PN with AFB1 and AFBO were investigated by *in silico* analysis with free energy calculation.¹⁶⁷

In the highlight of this information, hop prenylated flavonoids could be determined by *in silico* approaches including molecular docking and simulation studies against various drug targets to identify their potential inhibitor candidate.

A Perspective of Hops Structures

Prior core structures are widely used to discover drugs in medicinal chemistry. Chalcones include α,β -unsaturated carbonyl group, and are abundantly naturally occurring

compounds in plants. It is reported that these natural compounds participate in various biological activities.¹⁶⁸ XN has an *E*-isomer of chalcone group on the structure that increases the biological activity. These reactive carbonyl compounds are associated with broad-spectrum pharmacological properties like cancer, anti-inflammatory and immunosuppressive activities.¹⁶⁹ Most biologically important compounds have benzopyran (chromen) scaffolds as therapeutic agents for various diseases.¹⁷⁰ 8- and 6-PNs as the most known phytoestrogens include the substitution of benzopyran-4-one core structure with prenyl and free hydroxy groups. IXN contributes to higher biological activity similar to XN than 6-PN due to having a methoxy group at 5-position on the benzopyran ring. 8-PN has the strongest activity than 6-PN because of the localization of the prenyl group on the benzopyran ring. Phloroglucinol type-compounds as humulone and lupulone play a role in biological applications with their 2- and 3-prenyl groups, respectively, as well as free hydroxyl groups and the acidity of enol moiety. Figure 5 shows the structures of effective functional groups.

Conclusion

Natural products play an important role in the treatment and prevention of various human diseases. The importance of using plants as anticancer agents in modern medicine

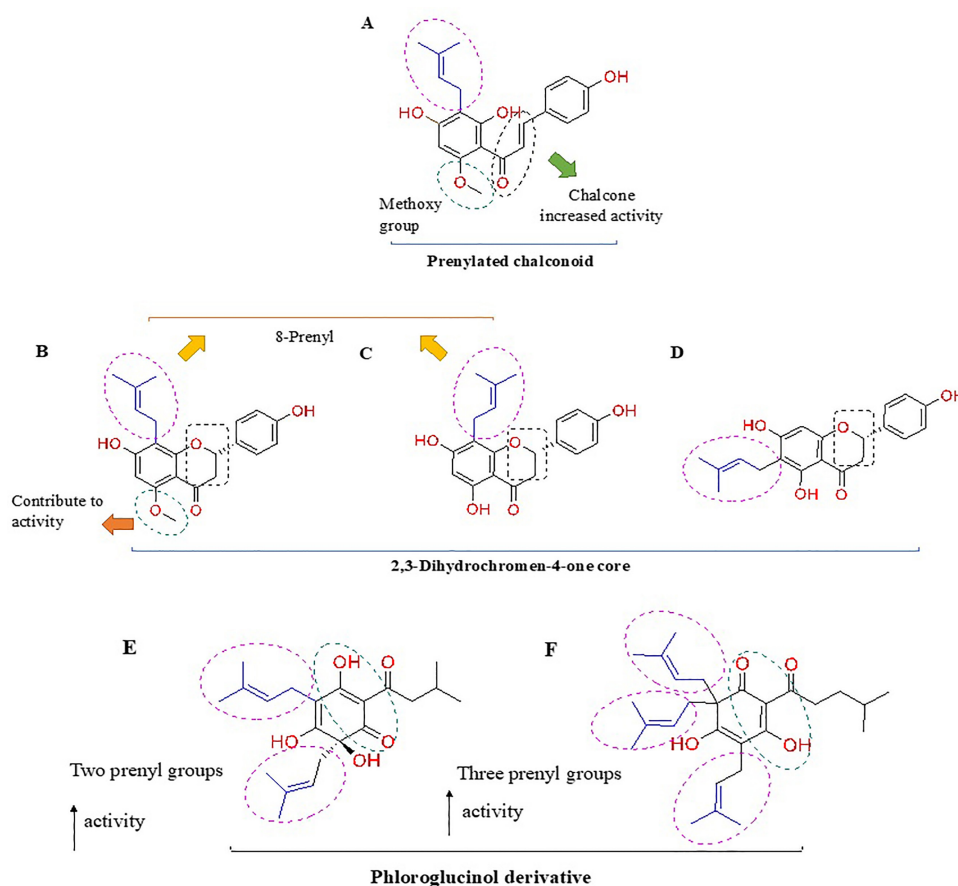


Figure 5. An overview of hops structures.

is well documented. Therefore, new sources of natural products or new structural features showing anticancer activity may contribute to the identification of promising phytotherapeutics. As a result, prenylated flavonoids including XN, IXN, 8-PN and 6-PN from hops play a role with their strong anticancer activity in many solid tumours such as glioma, breast, pancreas, prostate etc.. XN was involved in multiple signaling pathways, especially significant cancer-related pathways such as MAPK/ERK, Akt/mTOR/S6K, and was indicated as a promising candidate for further studies. miRNAs are important regulatory agents and have a role in many cancers, thus, miRNAs are promising potential targets for miRNA-targeted therapy.¹⁷¹ XN regulate transcriptionally ERK/c-Fos signalling pathway by the inducing of miR-204-3p expression. It can be thought that XN or its analogues may be potential small molecule inhibitors of miRNA-targeted therapy in the future.

Recent studies showed that XN and its analogues were dramatically associated with significant drug targets such as HDACs, SIRT6, and AChE in computational analysis. We hope that hop prenylated compounds may be used for clinical translation.

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Author Contributions

Ferah Comert Onder: Conceptualization, Writing - Original Draft, Writing - Review & Editing. Sevil Kalin: Writing - Original Draft. Nebahat Sahin: Writing - Original Draft. Gulce Davutlar: Writing - Original Draft. Khaled A.N. Abusharkh: Writing - Original Draft. Ozlem Maraba: Writing - Original Draft. Rabia Selina Hal: Writing - Original Draft. Mehmet Ay: Conceptualization, Writing - Original Draft, Writing - Review & Editing. Lutfun Nahar: Conceptualization, Writing - Review & Editing. Satyajit D. Sarker: Conceptualization, Writing - Review & Editing.

Conflict of Interest

The authors report no conflicts of interest.

References

- Yang X, Jiang Y, Yang J, He J, Sun J, Chen F, et al. Prenylated flavonoids, promising nutraceuticals with impressive biological activities. *Trends Food Sci Technol*. 2015;44(1):93-104. doi:10.1016/j.tifs.2015.03.007
- Liu M, Hansen PE, Wang G, Qiu L, Dong J, Yin H, et al. Pharmacological profile of xanthohumol, a prenylated flavonoid from hops (*Humulus lupulus*). *Molecules*. 2015;20(1):754-79. doi:10.3390/molecules20010754
- Lin M, Xiang D, Chen X, Huo H. Role of characteristic components of *Humulus lupulus* in promoting human health. *J Agric Food Chem*. 2019;67(30):8291-302. doi:10.1021/acs.jafc.9b03780
- Caffrey A, Ebeler SE. The occurrence of glycosylated aroma precursors in *Vitis vinifera* fruit and *Humulus lupulus* hop cones and their roles in wine and beer volatile aroma production. *Foods*. 2021;10(5):935. doi:10.3390/foods10050935
- Tatasciore S, Santarelli V, Neri L, González Ortega R, Faieta M, Di Mattia CD, et al. Freeze-drying microencapsulation of Hop extract: Effect of carrier composition on physical, techno-functional, and stability properties. *Antioxidants (Basel)*. 2023;12(2):442. doi:10.3390/antiox12020442
- Xia T, Zhang J, Guo Y, Jiang Y, Qiao F, Li K, et al. *Humulus lupulus* L. extract protects against senior osteoporosis through inhibiting amyloid β deposition and oxidative stress in APP/PS1 mutated transgenic mice and osteoblasts. *Molecules*. 2023;28(2):583. doi:10.3390/molecules28020583
- Xia TS, Lin LY, Zhang QY, Jiang YP, Li CH, Liu XY, et al. *Humulus lupulus* L. extract prevents ovariectomy-induced osteoporosis in mice and regulates activities of osteoblasts and osteoclasts. *Chin J Integr Med*. 2021;27(1):31-8. doi:10.1007/s11655-019-2700-z
- Rossini F, Virga G, Loreti P, Iacuzzi N, Ruggeri R, Provenzano ME. Hops (*Humulus lupulus* L.) as a novel multipurpose crop for the Mediterranean region of Europe: Challenges and opportunities of their cultivation. *Agriculture*. 2021;11(6):484. doi:10.3390/agriculture11060484
- Alonso-Esteban JL, Pinela J, Barros L, Ćirić A, Soković M, Calhella RC, et al. Phenolic composition and antioxidant, antimicrobial and cytotoxic properties of hop (*Humulus lupulus* L.) Seeds. *Ind Crops Prod*. 2019;134:154-9. doi:10.1016/j.indcrop.2019.04.001
- Girisa S, Saikia Q, Bordoloi D, Banik K, Monisha J, Daimary UD, et al. Xanthohumol from Hop: Hope for cancer prevention and treatment. *Int Union Biochem Mol Biol*. 2021;73:1016-44. doi:10.1002/iub.2522
- Comert Onder F, Ay M, Aydoğan Türkoğlu S, Tura Köçkar F, Çelik A. Antiproliferative activity of *Humulus lupulus* extracts on human hepatoma (Hep3B), colon (HT-29) cancer cells and proteases, tyrosinase, β -lactamase enzyme inhibition studies. *J Enzyme Inhib Med Chem*. 2016;31(1):90-8. doi:10.3109/14756366.2015.1004060
- Comert Onder F, Ay M, Sarker SD. Comparative study of antioxidant properties and total phenolic content of the extracts of *Humulus lupulus* L. and quantification of bioactive components by LC-MS/MS and GC-MS. *J Agric Food Chem*. 2013;61(44):10498-506. doi:10.1021/jf4031508
- Ponticelli M, Russo D, Faraone I, Sinisgalli C, Labanca F, Lela L, et al. The promising ability of *Humulus*

- lupulus* L. iso- α -acids vs. diabetes, inflammation, and metabolic syndrome: A systematic review. *Molecules*. 2021;26(4):954. doi:10.3390/molecules26040954
14. Lin M, Zhang J, Chen X. Bioactive flavonoids in *Moringa oleifera*, and their health-promoting properties. *J Funct Foods*. 2018;47:469-79. doi:10.1016/j.jff.2018.06.011
 15. Blaxland J. The antibacterial activity of *Humulus lupulus* against mycobacteria. [dissertation]. Cardiff: Cardiff University; 2015.
 16. Aggarwal D, Upadhyay SK, Singh R, Tuli HS. Recent patents on therapeutic activities of xanthohumol: a prenylated chalconoid from hops (*Humulus lupulus* L.). *Pharm Pat Anal*. 2021;10(1):37-49. doi:10.4155/ppa-2020-0026
 17. Jiang CH, Sun TL, Xiang DX, Wei SS, Li WQ. Anticancer activity and mechanism of xanthohumol: a prenylated flavonoid from hops (*Humulus lupulus* L.). *Front Pharmacol*. 2018;22:530. doi:10.3389/fphar.2018.00530
 18. Karabín M, Hudcová T, Jelínek L, Dostálek P. Biologically active compounds from hops and prospects for their use. *Compr Rev Food Sci Food Saf*. 2016;15(3):542-67. doi:10.1111/1541-4337.12201
 19. Liu M, Yin H, Qian X, Dong J, Qian Z, Miao J. Xanthohumol, a prenylated chalcone from Hops, inhibits the viability and stemness of doxorubicin-resistant MCF-7/ADR cells. *Molecules*. 2016;22(1):36. doi:10.3390/molecules22010036
 20. Sun Z, Zhou C, Liu F, Zhang W, Chen J, Pan Y, Ma L, Liu Q, Du Y, Yang J, et al. Inhibition of breast cancer cell survival by Xanthohumol via modulation of the Notch signaling pathway *in vivo* and *in vitro*. *Oncol Lett*. 2018;15(1):908-16. doi:10.3892/ol.2017.7434
 21. Abd Malek SN, Yong WK., Ho YF. Xanthohumol induces apoptosis and S phase cell cycle arrest in A549 non-small cell lung cancer cells. *Pharmacogn Mag*. 2015;11(44):275. doi:10.4103/0973-1296.166069
 22. Yong WK, Abd Malek SN. Xanthohumol induces growth inhibition and apoptosis in ca ski human cervical cancer cells. *Evid Based Complement Alternat Med*. 2015;2015:921306. doi:10.1155/2015/921306
 23. Sławińska-Brych A, Mizerska-Kowalska M, Król SK, Stepulak A, Zdzińska B. Xanthohumol impairs the PMA-driven invasive behaviour of lung cancer cell line A549 and exerts anti-EMT action. *Cells*. 2021;10(6):1484. doi:10.3390/cells10061484
 24. Seitz T, Hackl C, Freese K, Dietrich P, Mahli A, Thasler RM, et al. Xanthohumol, a prenylated chalcone derived from hops, inhibits growth and metastasis of melanoma cells. *Cancers*. 2021;13(3):511. doi:10.3390/cancers13030511
 25. Kunnimalaiyaan S, Sokolowski KM, Balamurugan M, Gamblin TC, Kunnimalaiyaan M. Xanthohumol inhibits notch signaling and induces apoptosis in hepatocellular carcinoma. *Calvisi D, editor. PLoS One*. 2015;10(5):e0127464. doi:10.1371/journal.pone.0127464
 26. Jiang W, Zhao S, Xu L, Lu Y, Lu Z, Chen C, et al. The inhibitory effects of xanthohumol, a prenylated chalcone derived from hops, on cell growth and tumorigenesis in human pancreatic cancer. *Biomed Pharmacother*. 2015;73:40-7. doi:10.1016/j.biopha.2015.05.020
 27. Kłósek M, Mertas A, Król W, Jaworska D, Szymaszal J, Szliszka E. Tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis in prostate cancer cells after treatment with xanthohumol-a natural compound present in *Humulus lupulus* L. *Int J Mol Sci*. 2016;17(6):837. doi:10.3390/ijms17060837
 28. Saito K, Matsuo Y, Imafuji H, Okubo T, Maeda Y, Sato T, et al. Xanthohumol inhibits angiogenesis by suppressing nuclear factor- κ B activation in pancreatic cancer. *Cancer Sci*. 2018;109(1):132-40. doi:10.1111/cas.13441
 29. Dokduang H, Yongvanit P, Namwat N, Pairojkul C, Sangkhamanon S, Yageta MS, et al. Xanthohumol inhibits STAT3 activation pathway leading to growth suppression and apoptosis induction in human cholangiocarcinoma cells. *Oncol Rep*. 2016;35(4):2065-72. doi:10.3892/or.2016.4584
 30. Sławińska-Brych A, Król SK, Dmoszyńska-Graniczka M, Zdzińska B, Stepulak A, Gagoś M. Xanthohumol inhibits cell cycle progression and proliferation of larynx cancer cells *in vitro*. *Chem Biol Interact*. 2015;240:110-8. doi:10.1016/j.cbi.2015.08.008
 31. Li Y, Wang K, Yin S, Zheng H, Min D. Xanthohumol inhibits proliferation of laryngeal squamous cell carcinoma. *Oncology Lett*. 2016;12(6):5289-94. doi:10.3892/ol.2016.5313
 32. Liu X, An LJ, Li Y, Wang Y, Zhao L, Lv X, et al. Xanthohumol chalcone acts as a powerful inhibitor of carcinogenesis in drug-resistant human colon carcinoma and these effects are mediated via G2/M phase cell cycle arrest, activation of apoptotic pathways, caspase activation and targeting Ras/MEK/ERK pathway. *J BUON*. 2019;24(6):2442-7.
 33. Mi X, Wang C, Sun C, Chen X, Huo X, Zhang Y, et al. Xanthohumol induces paraptosis of leukemia cells through p38 mitogen activated protein kinase signaling pathway. *Oncotarget*. 2017;8(19):31297-304. doi:10.18632/oncotarget.16185
 34. Chen PH, Chang CK, Shih CM, Cheng CH, Lin CW, Lee CC, et al. The MIR-204-3p-targeted IGFBP2 pathway is involved in xanthohumol-induced glioma cell apoptotic death. *Neuropharmacology*. 2016;110:362-75. doi:10.1016/j.neuropharm.2016.07.038
 35. Guo D, Zhang B, Liu S, Jin M. Xanthohumol induces apoptosis via caspase activation, regulation of BCL-2, and inhibition of PI3K/AKT/Mtor-kinase in human gastric cancer cells. *Biomed Pharmacother*. 2018;106:1300-6. doi: 10.1016/j.biopha.2018.06.166
 36. Gao F, Li M, Zhou L, Liu W, Zuo H, Li W. Xanthohumol targets the ERK1/2FRA1 signaling axis to reduce cyclin D1 expression and inhibit non-small cell lung

- cancer. *Oncol Rep.* 2020;44(4):1365-74. doi:10.3892/or.2020.7697
37. Scagliarini A, Mathey A, Aires V, Delmas D. Xanthohumol, a prenylated flavonoid from hops, induces DNA damages in colorectal cancer cells and sensitizes SW480 cells to the SN38 chemotherapeutic agent. *Cells.* 2020;9(4):932. doi:10.3390/cells9040932
 38. Harish V, Haque E, Śmiech M, Taniguchi H, Jamieson S, Tewari D, et al. Xanthohumol for human malignancies: Chemistry, pharmacokinetics and molecular targets. *Int J Mol Sci.* 2021;22(9):4478. doi:10.3390/ijms22094478
 39. Turdo A, Glaviano A, Pepe G, Calapà F, Raimondo S, Fiori ME, et al. Nobiletin and xanthohumol sensitize colorectal cancer stem cells to standard chemotherapy. *Cancers.* 2021;13(16):3927. doi:10.3390/cancers13163927
 40. Lu WJ, Chang CC, Lien LM, Yen TL, Chiu HC, Huang SY, et al. Xanthohumol from *Humulus Lupulus* L. induces glioma cell autophagy via inhibiting AKT/MTOR/S6K pathway. *J Funct Foods.* 2015;18:538-49. doi: 10.1016/j.jff.2015.08.020
 41. Ho KH., Chang CK., Chen PH., Wang YJ, Chang WC, Chen KC. Mir-4725-3p targeting stromal interacting molecule 1 signaling is involved in xanthohumol inhibition of glioma cell invasion. *J Neurochem.* 2018;146(3):269-88. doi:10.1111/jnc.14459
 42. Wei S, Sun T, Du J, Zhang B, Xiang D, Li W. Xanthohumol, a prenylated flavonoid from Hops, exerts anticancer effects against gastric cancer *in vitro*. *Oncol Rep.* 2018;40(6):3213-22. doi:10.3892/or.2018.6723
 43. Carvalho DO, Freitas J, Nogueira P, Henriques SN, Carmo AM, Castro MA, et al. Xanthohumol inhibits cell proliferation and induces apoptosis in human thyroid cells. *Food Chem Toxicol.* 2018;121:450-7. doi:10.1016/j.fct.2018.09.021
 44. Li X, Jin L, Ma Y, Jiang Z, Tang H, Tong X. Xanthohumol inhibits non-small cell lung cancer by activating PUMA-mediated apoptosis. *Toxicology.* 2022;470:153141. doi:10.1016/j.tox.2022.153141
 45. Zhang W, Pan Y, Gou P, Zhou C, Ma L, Liu Q, et al. Effect of xanthohumol on Th1/Th2 balance in a breast cancer mouse model. *Oncol Rep.* 2018;39(1):280-8. doi:10.3892/or.2017.6094
 46. Seliger JM, Misuri L, Maser E, Hintzpeter J. The hop-derived compounds xanthohumol, isoxanthohumol and 8-prenylnaringenin are tight-binding inhibitors of human aldo-keto reductases 1B1 and 1B10. *J Enzyme Inhib Med Chem.* 2018;33(1):607-14. doi:10.1080/14756366.2018.1437728
 47. Krajnović T, Drača D, Kaluđerović GN, Dunderović D, Mirkov I, Wessjohann LA, et al. The hop-derived prenylflavonoid isoxanthohumol inhibits the formation of lung metastasis in B16-F10 murine melanoma model. *Food Chem Toxicol.* 2019;129:257-68. doi:10.1016/j.fct.2019.04.046
 48. Bolton JL, Dunlap TL, Hajirahimkhan A, Mbachu O, Chen SN, Chadwick L, et al. The multiple biological targets of hops and bioactive compounds. *Chem Res Toxicol.* 2019;32(2):222-33. doi:10.1021/acs.chemrestox.8b00345
 49. Wang S, Dunlap TL, Howell CE, Mbachu OC, Rue EA, Phansalkar R, et al. Hop (*Humulus lupulus* L.) extract and 6-prenylnaringenin induce P450 1A1 catalyzed estrogen 2-hydroxylation. *Chem Res Toxicol.* 2016;29(7):1142-50. doi:10.1021/acs.chemrestox.6b00112
 50. Dabrowski W, Gagos M, Siwicka-Gieroba D, Piechota M, Siwec J, Bielacz M, et al. *Humulus lupulus* extract rich in xanthohumol improves the clinical course in critically ill COVID-19 patients. *Biomed Pharmacother.* 2023;158:114082. doi:10.1016/j.biopha.2022.114082
 51. Xiao-Lei S, Tian-Shuang X, Yi-Ping J, Na-Ni W, Ling-Chuan X, Ting H, et al. *Humulus lupulus* L. extract and its active constituent xanthohumol attenuate oxidative stress and nerve injury induced by iron overload via activating AKT/GSK3 β and Nrf2/NQO1 pathways. *J Nat Med.* 2023;77(1):12-27. doi:10.1007/s11418-022-01642-1
 52. Vazquez-Cervantes GI, Ortega DR, Blanco Ayala T, Pérez de la Cruz V, Esquivel DFG, Salazar A, et al. Redox and anti-inflammatory properties from hop components in beer-related to neuroprotection. *Nutrients.* 2021;13(6):2000. doi:10.3390/nu13062000
 53. Cho YC, You SK, Kim HJ, Cho CW, Lee IS, Kang BY. Xanthohumol inhibits IL-12 production and reduces chronic allergic contact dermatitis. *Int Immunopharmacol.* 2010;10(5):556-61. doi:10.1016/j.intimp.2010.02.002
 54. Nurzynska A, Klimek K, Michalak A, Dos Santos Szweczyk K, Arczewska M, Szalaj U, et al. Do curdlan hydrogels improved with bioactive compounds from hop exhibit beneficial properties for skin wound healing? *Int J Mol Sci.* 2023;24(12):10295. doi:10.3390/ijms241210295
 55. Sangiovanni E, Fumagalli M, Santagostini L, Forino M, Piazza S, Colombo E, et al. A bio-guided assessment of the anti-inflammatory activity of hop extracts (*Humulus lupulus* L. cv. Cascade) in human gastric epithelial cells. *J Funct Foods.* 2019;57:95-102. doi:10.1016/j.jff.2019.03.041
 56. Jung F, Staltner R, Tahir A, Baumann A, Burger K, Halilbasic E, et al. Oral intake of xanthohumol attenuates lipoteichoic acid-induced inflammatory response in human PBMCs. *Eur J Nutr.* 2022;61(8):4155-66. doi: 10.1007/s00394-022-02964-2
 57. Chen C. Inhibiting degradation of 2-arachidonoylglycerol as a therapeutic strategy for neurodegenerative diseases. *Pharmacol Ther.* 2023;244:108394. doi:10.1016/j.pharmthera.2023.108394
 58. Barrie N, Manolios N. The endocannabinoid system

- in pain and inflammation: Its relevance to rheumatic disease. *Eur J Rheumatol.* 2017;4(3):210-18. doi:10.5152/eurjrheum.2017.17025
59. Tung MC, Fung KM, Hsu HM, Tseng TS. Discovery of 8-prenylnaringenin from hop (*Humulus lupulus* L.) as a potent monoacylglycerol lipase inhibitor for treatments of neuroinflammation and Alzheimer's disease. *RSC Adv.* 2021;11(49):31062-72. doi:10.1039/d1ra05311f. Erratum in: *RSC Adv.* 2022;12(31):20217. doi:10.1039/d1ra05311f
 60. Cummings JL, Morstorf T, Zhong K. Alzheimer's disease drug-development pipeline: few candidates, frequent failures. *Alzheimer's Res Ther.* 2014;6(4):37. doi:10.1186/alzrt269
 61. Orhan IE, Jedrejek D, Senol FS, Salmas RE, Durdagi S, Kowalska I, et al. Molecular modeling and in vitro approaches towards cholinesterase inhibitory effect of some natural xanthohumol, naringenin, and acyl phloroglucinol derivatives. *Phytomedicine.* 2018;42:25-33. doi:10.1016/j.phymed.2018.03.009
 62. Sasaki M, Shikata E. On some properties of hop stunt disease agent, a viroid. *Proc Jpn Acad B.* 1977;53(3):109-12. doi:10.2183/pjab.53.109
 63. Sano T, Isono S, Matsuki K, Kawaguchi-Ito Y, Tanaka K, Kondo K, et al. Vegetative propagation and its possible role as a genetic bottleneck in the shaping of the apple fruit crinkle viroid populations in apple and hop plants. *Virus Genes.* 2008;37(3):298-303. doi:10.1007/s11262-008-0270-9
 64. Jakse J, Radisek S, Pokorn T, Matousek J, Javornik B. Deep-sequencing revealed Citrus bark cracking viroid (CBCV d) as a highly aggressive pathogen on hop. *Plant.* 2015;64:831-42. doi:10.1111/ppa.12325
 65. Lin Y, Zang R, Ma Y, Wang Z, Li L, Ding S, et al. Xanthohumol is a potent pan-inhibitor of coronaviruses targeting main protease. *Int J Mol Sci.* 2021;22(22):12134. doi:10.3390/ijms222212134
 66. Liu X, Bai J, Jiang C, Song Z, Zhao Y, Nauwynck H, et al. Therapeutic effect of xanthohumol against highly pathogenic porcine reproductive and respiratory syndrome viruses. *Vet Microbiol.* 2019;238:108431. doi:10.1016/j.vetmic.2019.108431
 67. Liu X, Song Z, Bai J, Nauwynck H, Zhao Y, Jiang P. Xanthohumol inhibits PRRSV proliferation and alleviates oxidative stress induced by PRRSV via the Nrf2-HMOX1 axis. *Vet Res.* 2019;50:61. doi:10.1186/s13567-019-0679-2
 68. Hanada A, Morimoto R, Horio Y, Shichiri M, Nakashima A, Ogawa T, et al. Influenza virus entry and replication inhibited by 8-prenylnaringenin from *Citrullus lanatus* var. *citroides* (wild watermelon). *Food Sci Nutr.* 2022;10(3):926-35. doi:10.1002/fsn3.2725
 69. Rodrigo I, Ballesta C, Nunes EB, Pérez P, García-Arriaza J, Arias A. Eeyarestatin I, an inhibitor of the valosin-containing protein, exhibits potent virucidal activity against the flaviviruses. *Antivir Res.* 2022;207:105416. doi:10.1016/j.antiviral.2022.105416
 70. Żołnierczyk AK, Mączka WK, Grabarczyk M, Wińska K, Woźniak E, Anioł M. Isoxanthohumol—biologically active hop flavonoid. *Fitoterapia.* 2015;103:71-82. doi:10.1016/j.fitote.2015.03.007
 71. Miranda CL, Elias VD, Hay JJ, Choi J, Reed RL, Stevens JF. Xanthohumol improves dysfunctional glucose and lipid metabolism in diet-induced obese C57BL/6J mice. *Arch Biochem Biophys.* 2016;599:22-30. doi:10.1016/j.abb.2016.03.008
 72. Kok BP, Galmozzi A, Littlejohn NK, Albert V, Godio C, Kim W, et al. Intestinal bitter taste receptor activation alters hormone secretion and imparts metabolic benefits. *Mol Metab.* 2018;16:76-87. doi:10.1016/j.molmet.2018.07.013
 73. Park S, Sim KS, Hwangbo Y, Park SJ, Kim YJ, Kim JH. Naringenin and phytoestrogen 8-prenylnaringenin protect against islet dysfunction and inhibit apoptotic signaling in insulin-deficient diabetic mice. *Molecules.* 2022;27(13):4227. doi:10.3390/molecules27134227
 74. Wang W, Chen Z, Zheng T, Zhang M. Xanthohumol alleviates T2DM-induced liver steatosis and fibrosis by mediating the NRF2/RAGE/NF-κB signaling pathway. *Future Med Chem.* 2021;13(23):2069-208. doi:10.4155/fmc-2021-0241.
 75. Hayakawa M, Hira T, Nakamura M, Iida T, Kishimoto Y, Hara H. Secretion of GLP-1 but not GIP is potently stimulated by luminal D-allulose (D-psicose) in rats. *Biochem Biophys Res Commun.* 2018;496(3):898-903. doi:10.1016/j.bbrc.2018.01.128
 76. Sun H, Wang D, Song X, Zhang Y, Ding W, Peng X, et al. Natural prenylchalconaringenins and prenylnaringenins as antidiabetic agents: α-glucosidase and α-amylase inhibition and in vivo antihyperglycemic and antihyperlipidemic effects. *J Agric Food Chem.* 2017;65(8):1574-81. doi:10.1021/acs.jafc.6b05445
 77. Brás NF, Neves RP, Lopes FA, Correia MA, Palma AS, Sousa SE, et al. Combined *in silico* and *in vitro* studies to identify novel antidiabetic flavonoids targeting glycogen phosphorylase. *Bioorg Chem.* 2021;108:104552. doi:10.1016/j.bioorg.2020.104552
 78. Lee M, Lee J, Kim JM, Hong D-H, Chin J, Kim H, et al. Antibacterial activity of prenylated flavonoids isolated from hop against fish pathogens *Streptococcus iniae* and *Vibrio vulnificus*. *Biotechnol Bioprocess Eng.* 2022;27(3):361-9. doi:10.1007/s12257-021-0247-2
 79. Sychrová A, Škovranová G, Čulenová M, Bittner Fialová S. Prenylated flavonoids in topical infections and wound healing. *Molecules.* 2022;27:4491. doi:10.3390/molecules27144491.
 80. Bartmańska A, Wałęcka-Zacharska E, Tronina T, Popłoński J, Sordon S, Brzezowska E, et al. Antimicrobial properties of spent Hops extracts, flavonoids isolated therefrom, and their derivatives. *Molecules.* 2018;23:2059. doi:10.3390/molecules23082059
 81. Araya-Cloutier C, den Besten HMW, Aisyah S, Gruppen H, Vincken J-P. The position of prenylation

- of isoflavonoids and stilbenoids from Legumes (Fabaceae) modulates the antimicrobial activity against Gram-positive pathogens. *Food Chem.* 2017;226:193-201. doi:10.1016/j.foodchem.2017.01.026
82. Yamaguchi N, Satoh-Yamaguchi K, Ono M. In vitro Evaluation of antibacterial, anticollagenase, and antioxidant activities of hop components (*Humulus lupulus*) addressing acne vulgaris. *Phytomedicine.* 2009;16(4):369-76. doi:10.1016/j.phymed.2008.12.021
 83. Stompor M, Żarowska B. Antimicrobial activity of xanthohumol and its selected structural analogues. *Molecules.* 2016;21:608. doi:10.3390/molecules21050608
 84. Bogdanova K, Röderova M, Kolar M, Langova K, Dusek M, Jost P, et al. Antibiofilm activity of bioactive hop compounds humulone, lupulone and xanthohumol toward susceptible and resistant *Staphylococci*. *Res Microbiol.* 2018;169(3):127-34. doi:10.1016/j.resmic.2017.12.005
 85. Bocquet L, Sahpaz S, Bonneau N, Beaufay C, Mahieux S, Samaillie J, et al. Phenolic compounds from *Humulus lupulus* as natural antimicrobial products: New weapons in the fight against methicillin resistant *Staphylococcus aureus*, *Leishmania mexicana* and *Trypanosoma brucei* strains. *Molecules.* 2019;24:1024. doi:10.3390/molecules24061024
 86. Kobus-Cisowska J, Szymanowska-Powalowska D, Szczepaniak O, Kmiecik D, Przeor M, Gramza-Michałowska A, et al. Composition and in vitro effects of cultivars of *Humulus lupulus* L. Hops on cholinesterase activity and microbial growth. *Nutrients.* 2019;11(6):1377. doi:10.3390/nu11061377
 87. Lou H, Zhang F, Lu L, Ding Y, Hao X. Xanthohumol from *Humulus Lupulus* L. potentiates the killing of *Mycobacterium tuberculosis* and mitigates liver toxicity by the combination of isoniazid in mouse tuberculosis models. *RSC Adv.* 2020;10(22):13223-31. doi:10.1039/c9ra10347c
 88. Bocquet L, Rivière C, Dermont C, Samaillie J, Hilbert JL, Halama P, Siah A, Sahpaz S. Antifungal activity of Hop extracts and compounds against the wheat pathogen *Zymoseptoria tritici*. *Ind Crops Prod.* 2018;122:290-97. doi: 10.1016/j.indcrop.2018.05.061
 89. Yan YF, Wu TL, Du SS, Wu ZR, Hu YM, Zhang ZJ, et al. The antifungal mechanism of isoxanthohumol from *Humulus lupulus* Linn. *Int J Mol Sci.* 2021;22(19):10853. doi:10.3390/ijms221910853
 90. Mavra A, Petrou CC, Vlasiou MC. Ligand and structure-based virtual screening in combination, to evaluate small organic molecules as inhibitors for the XIAP anti-apoptotic protein: The xanthohumol hypothesis. *Molecules.* 2022;27(15):4825. doi:10.3390/molecules27154825
 91. Kores K, Kolenc Z, Furlan V, Bren U. Inverse molecular docking elucidating the anticarcinogenic potential of the hop natural product xanthohumol and its metabolites. *Foods.* 2022;11(9):1253. doi:10.3390/foods11091253.
 92. Sayed AM, Hassanein EHM, Salem SH, Hussein OE, Mahmoud AM. Flavonoids- mediated SIRT1 signaling activation in hepatic disorders. *Life Sci.* 2020;259:118173. doi:10.1016/j.lfs.2020.118173
 93. Peck B, Chen CY, Ho KK, Di Fruscia P, Myatt SS, Coombes RC, et al. SIRT inhibitors induce cell death and p53 acetylation through targeting both SIRT1 and SIRT2. *Mol Cancer Ther.* 2010;9(4):844-55. doi:10.1158/1535-7163.MCT-09-0971
 94. Spinck M, Bischoff M, Lampe P, Meyer-Almes FJ, Sievers S, Neumann H. Discovery of Dihydro-1,4-Benzoxazine Carboxamides as Potent and Highly Selective Inhibitors of Sirtuin-1. *J Med Chem.* 2021;64(9):5838-49. doi:10.1021/acs.jmedchem.1c00017
 95. Purushotham N, Singh M, Paramesha B, Kumar V, Wakode S, Banerjee SK, et al. Design and synthesis of amino acid derivatives of substituted benzimidazoles and pyrazoles as Sirt1 inhibitors. *RSC Adv.* 2022;12(7):3809-3827. doi:10.1039/d1ra06149f
 96. Timucin AC, Basaga H. SIRT6 Is a Positive regulator of aldose reductase expression in U937 and heLa cells under osmotic stress: In vitro and In silico insights. *PLoS One.* 2016;11(8):e0161494. doi:10.1371/journal.pone.0161494
 97. Venturelli S, Niessner H, Sinnberg T, Berger A, Burkard M, Urmann C, et al. 6- and 8-Prenylnaringenin, novel natural histone deacetylase inhibitors found in hops, exert antitumor activity on melanoma cells. *Cell Physiol Biochem.* 2018;51(2):543-56. doi:10.1159/000495275
 98. Alsawalha M, Rao Bolla S, Kandakatla N, Srinivasadesikan V, Veeraraghavan VP, Surapaneni KM. Molecular docking and ADMET analysis of hydroxamicacidsasHDAC2inhibitors. *Bioinformation.* 2019;15(6):380-87. doi:10.6026/97320630015380
 99. Cai J, Wei H, Hong KH, Wu X, Zong X, Cao M, et al. Discovery, bioactivity and docking simulation of Vorinostat analogues containing 1,2,4-oxadiazole moiety as potent histone deacetylase inhibitors and antitumor agents. *Bioorg Med Chem.* 2015;23(13):3457-71. doi:10.1016/j.bmc.2015.04.028
 100. Praseetha S, Bandaru S, Nayariseri A, Sureshkumar S. Pharmacological analysis of vorinostat analogues as potential anti-tumor agents targeting human histone deacetylases: an epigenetic treatment stratagem for cancers. *Asian Pac J Cancer Prev.* 2016;17(3):1571-6. doi:10.7314/apjcp.2016.17.3.1571
 101. Ibrahim TS, Malebari AM, Mohamed MFA. Design, Synthesis, in vitro anticancer evaluation and molecular modelling studies of 3,4,5-trimethoxyphenyl-based derivatives as dual EGFR/HDAC hybrid inhibitors. *Pharmaceutics.* 2021;14(11):1177. doi:10.3390/ph14111177
 102. Anh DT, Hai PT, Dung DTM, Dung PTP, Huong LT, et al. Design, synthesis and evaluation of novel indirubin-based

- N-hydroxybenzamides, N-hydroxypropenamides and N-hydroxyheptanamides as histone deacetylase inhibitors and antitumor agents. *Bioorg Med Chem Lett.* 2020;30(22):127537. doi:10.1016/j.bmcl.2020.127537
103. Zhang S, Huang W, Li X, Yang Z, Feng B. Synthesis, biological evaluation, and computer-aided drug designing of new derivatives of hyperactive suberoylanilide hydroxamic acid histone deacetylase inhibitors. *Chem Biol Drug Des.* 2015;86(4):795-804. doi:10.1111/cbdd.12554.
104. Hieu DT, Anh DT, Tuan NM, Hai PT, Huong LT, Kim J, et al. Design, synthesis and evaluation of novel N-hydroxybenzamides/N-hydroxypropenamides incorporating quinazolin-4(3H)-ones as histone deacetylase inhibitors and antitumor agents. *Bioorg Chem.* 2018;76:258-67. doi:10.1016/j.bioorg.2017.12.007.
105. Scafuri B, Bontempo P, Altucci L, De Masi L, Facchiano A. Molecular docking simulations on histone deacetylases (HDAC)-1 and -2 to investigate the flavone binding. *Biomedicines.* 2020;8(12):568. doi:10.3390/biomedicines8120568
106. Abdelmoniem N, H Abdallah M, M Mukhtar R, Moutasim F, Rafie Ahmed A, Edris A, et al. Identification of novel natural dual HDAC and Hsp90 inhibitors for metastatic TNBC using e-pharmacophore modeling, molecular docking, and molecular dynamics studies. *Molecules.* 2023;28(4):1771. doi:10.3390/molecules28041771
107. Furumai R, Komatsu Y, Nishino N, Khochbin S, Yoshida M, Horinouchi S. Potent histone deacetylase inhibitors built from trichostatin A and cyclic tetrapeptide antibiotics including trapoxin. *Proc Natl Acad Sci U S A.* 2001;98(1):87-92. doi:10.1073/pnas.98.1.87
108. Nouri Z, Fakhri S, El-Senduny FF, Sanadgol N, Abd-ElGhani GE, Farzaei MH, et al. On the neuroprotective effects of naringenin: pharmacological targets, signaling pathways, molecular mechanisms, and clinical perspective. *Biomolecules.* 2019;9(11):690. doi:10.3390/biom9110690
109. Khare N, Maheshwari SK, Jha AK. Screening and identification of secondary metabolites in the bark of *Bauhinia variegata* to treat Alzheimer's disease by using molecular docking and molecular dynamics simulations. *J Biomol Struct Dyn.* 2021;39(16):5988-98. doi:10.1080/07391102.2020.1796798
110. Sepehri S, Saeedi M, Larijani B, Mahdavi M. Recent developments in the design and synthesis of benzylpyridinium salts: Mimicking donepezil hydrochloride in the treatment of Alzheimer's disease. *Front Chem.* 2022;10:936240. doi:10.3389/fchem.2022.936240
111. Ali MR, Sadoqi M, Møller SG, Boutajangout A, Mezei M. Assessing the binding of cholinesterase inhibitors by docking and molecular dynamics studies. *J Mol Graph Model.* 2017;76:36-42. doi:10.1016/j.jmkgm.2017.06.027
112. Khan KI, Kamel MM, Mohamed LW, Doghish AS, Alnajjar R, Al-Karmalawy AA, et al. Design, synthesis, and biological evaluation of thienopyrimidine derivatives as multifunctional agents against Alzheimer's disease. *Drug Dev Res.* 2023;84(5):937-61. doi:10.1002/ddr.22064
113. Asgarshamsi MH, Fassihi A, Dehkordi MM. Design, synthesis, molecular docking, and molecular dynamics simulation studies of novel 3-hydroxypyridine-4-one derivatives as potential acetylcholinesterase inhibitors. *Chem Biodivers.* 2023;20(7):e202300325. doi:10.1002/cbdv.202300325
114. Cömert Önder F. Discovery of donepezil-like compounds as potential acetylcholinesterase inhibitors determined by pharmacophore mapping-based virtual screening and molecular docking. *Med J SDU.* 2023;30(2):143-53.
115. Gupta M, Kumar A, Prasun C, Nair MS, Kini SG, Yadav D, et al. Design, synthesis, extra-precision docking, and molecular dynamics simulation studies of pyrrolidin-2-one derivatives as potential acetylcholinesterase inhibitors. *J Biomol Struct Dyn.* 2023;41(13):6282-94. doi:10.1080/07391102.2022.2106515.
116. Koly HK, Sutradhar K, Rahman MS. Acetylcholinesterase inhibition of Alzheimer's disease: identification of potential phytochemicals and designing more effective derivatives to manage disease condition. *J Biomol Struct Dyn.* 2023. doi:10.1080/07391102.2023.2166992
117. Gyebi GA, Ogunyemi OM, Ibrahim IM, Ogunro OB, Afolabi SO, Ojo RJ, et al. Identification of potential inhibitors of cholinergic and β -secretase enzymes from phytochemicals derived from *Gongronema latifolium* Benth leaf: an integrated computational analysis. *Mol Divers.* 2023. doi:10.1007/s11030-023-10658-y
118. El-Hussieny M, Abd-El-Maksoud MA, Soliman FM, Fouad MA, El-Ashrey MK. Dual-target ligand discovery for Alzheimer's disease: triphenylphosphoranylidene derivatives as inhibitors of acetylcholinesterase and β -amyloid aggregation. *J Enzyme Inhib Med Chem.* 2023;38(1):2166040. doi:10.1080/14756366.2023.2166040
119. Shah-Abadi ME, Ariaei A, Moradi F, Rustamzadeh A, Tanha RR, Sadigh N, et al. In Silico interactions of natural and synthetic compounds with key proteins involved in Alzheimer's disease: prospects for designing new therapeutics compound. *Neurotox Res.* 2023;41(5):408-30. doi:10.1007/s12640-023-00648-1.
120. Madhav H, Abdel-Rahman SA, Hashmi MA, Rahman MA, Rehan M, Pal K, et al. Multicomponent petasis reaction for the identification of pyrazine based multi-target directed anti-Alzheimer's agents: In-silico design, synthesis, and characterization. *Eur J Med Chem.* 2023;254:115354. doi:10.1016/j.ejmech.2023.115354

121. Paolino M, de Candia M, Purgatorio R, Catto M, Saletti M, Tondo AR, et al. Investigation on novel E/Z 2-benzylideneindan-1-one-based photoswitches with AChE and MAO-B dual Inhibitory activity. *Molecules*. 2023;28(15):5857. doi:10.3390/molecules28155857
122. Upadhyay SP, Singh V, Sharma R, Zhou J, Thapa P, Johnson DK, et al. Influence of ligand geometry on cholinesterase enzyme - A comparison of 1-isoidolinone based structural analog with Donepezil. *J Mol Struct*. 2022;1247:131385. doi:10.1016/j.molstruc.2021.131385
123. Carocci A, Barbarossa A, Leuci R, Carrieri A, Brunetti L, Laghezza A, et al. Novel phenothiazine/donepezil-like hybrids endowed with antioxidant activity for a multi-target approach to the therapy of Alzheimer's disease. *Antioxidants*. 2022;11(9):1631. doi:10.3390/antiox11091631
124. Ogunsuyi OB, Omege FB, Olagoke OC, Oboh G, Rocha JBT. Phytochemicals from African eggplants (*Solanum macrocarpon* L) and Black nightshade (*Solanum nigrum* L) leaves as acetylcholinesterase inhibitors: an in-silico study. *J Biomol Struct Dyn*. 2023;41(16):7725-34. doi:10.1080/07391102.2022.2124194
125. Makarian M, Gonzalez M, Salvador SM, Lorzadeh S, Hudson PK, Pecic S. Synthesis, kinetic evaluation and molecular docking studies of donepezil-based acetylcholinesterase inhibitors. *J Mol Struct*. 2022;1247:131425. doi:10.1016/j.molstruc.2021.131425
126. Sari S., Yilmaz M. Acetylcholinesterase inhibition, molecular docking and ADME prediction studies of new dihydrofuran-piperazine hybrid compounds. *Med Chem Res*. 2021;30:2114-26. doi:10.1007/s00044-021-02788-5
127. Khalid S, Zahid MA, Ali H, Kim YS, Khan S. Biaryl scaffold-focused virtual screening for anti-aggregatory and neuroprotective effects in Alzheimer's disease. *BMC Neurosci*. 2018;19(1):74. doi:10.1186/s12868-018-0472-6
128. Eissa KI, Kamel MM, Mohamed LW, Galal MA, Kassab AE. Design, synthesis, and biological evaluation of thienopyrimidine and thienotriazine derivatives as multitarget anti-Alzheimer agents. *Drug Dev Res*. 2022;83(6):1394-07. doi:10.1002/ddr.21968
129. David B, Schneider P, Schäfer P, Pietruszka J, Gohlke H. Discovery of new acetylcholinesterase inhibitors for Alzheimer's disease: virtual screening and in vitro characterisation. *J Enzyme Inhib Med Chem*. 2021;36(1):491-6. doi:10.1080/14756366.2021.1876685
130. Onder FC, Sahin K, Senturk M, Durdagi S, Ay M. Identifying highly effective coumarin-based novel cholinesterase inhibitors by *in silico* and *in vitro* studies. *J Mol Graph Model*. 2022;115:108210. doi:10.1016/j.jmgm.2022.108210
131. Abbas-Mohammadi M, Moridi Farimani M, Salehi P, Ebrahimi SN, Sonboli A, Kelso C, Skropeta D. Molecular networking based dereplication of AChE inhibitory compounds from the medicinal plant *Vincetoxicum funebre* (Boiss. & Kotschy). *J Biomol Struct Dyn*. 2022;40(5):1942-51. doi:10.1080/07391102.2020.1834455
132. Ogunsuyi OB, Omege FB, Ijomone OM, Oboh G, Rocha JBT. Effect of chlorogenic acid plus donepezil on critical neurocortical enzyme activities, inflammatory markers, and synaptophysin immunoreactivity in scopolamine-assaulted rats, supported by multiple ligand simultaneous docking. *J Food Biochem*. 2022;46(11):e14312. doi:10.1111/jfbc.14312
133. Abdalla Ali A, Mhamad SA, Hasan AH, Ahmad I, Abdullah SA, Jamil S, et al. Synthesis, biological evaluation and molecular modeling studies of modulated benzyloxychalcones as potential acetylcholinesterase inhibitors. *J Biomol Struct Dyn*. 2023. doi:10.1080/07391102.2023.2220032
134. Brunetti L, Leuci R, Carrieri A, Catto M, Occhineri S, Vinci G, et al. Structure-based design of novel donepezil-like hybrids for a multi-target approach to the therapy of Alzheimer's disease. *Eur J Med Chem*. 2022;237:114358. doi:10.1016/j.ejmech.2022.114358
135. Orabi MAA, Orabi EA, Abdel-Sattar ES, English AM, Hatano T, Elimam H. Structural determination and anticholinesterase assay of C-glycosidic ellagitannins from *Lawsonia inermis* leaves: A study supported by DFT calculations and molecular docking. *Fitoterapia*. 2023;164:105360. doi:10.1016/j.fitote.2022.105360
136. Şahin S. A single-molecule with multiple investigations: Synthesis, characterization, computational methods, inhibitory activity against Alzheimer's disease, toxicity, and ADME studies. *Comput Biol Med*. 2022;146:105514. doi:10.1016/j.combiomed.2022.105514
137. Patil N, Chandel V, Rana A, Jain M, Kaushik P. Investigation of *Cannabis sativa* phytochemicals as anti-Alzheimer's agents: An in silico study. *Plants (Basel)*. 2023;12(3):510. doi:10.3390/plants12030510
138. Eissa KI, Kamel MM, Mohamed LW, Doghish AS, Alnajjar R, Al-Karmalawy AA, et al. Design, synthesis, and biological evaluation of thienopyrimidine derivatives as multifunctional agents against Alzheimer's disease. *Drug Dev Res*. 2023;84(5):937-61. doi:10.1002/ddr.22064
139. Khan S, Ullah H, Taha M, Rahim F, Sarfraz M, Iqbal R, et al. Synthesis, DFT Studies, molecular docking and biological activity evaluation of thiazole-sulfonamide derivatives as potent Alzheimer's inhibitors. *Molecules*. 2023;28(2):559. doi:10.3390/molecules28020559
140. Kaya G, Noma SAA, Barut Celepci D, Bayıl İ, Taskin-Tok T, Gök Y, et al. Design, synthesis, spectroscopic characterizations, single crystal X-ray analysis, in vitro xanthine oxidase and acetylcholinesterase inhibitory evaluation as well as in silico evaluation of selenium-based N-heterocyclic carbene compounds. *J Biomol*

- Struct Dyn. 2023. doi:10.1080/07391102.2022.2163696
141. Nisar M, Gondal HY, Cheema ZM, Yousaf S, Nadeem H. New azole-derived hemiaminal ethers as promising acetylcholinesterase inhibitors: synthesis, X-ray structures, in vitro and in silico studies. J Biomol Struct Dyn. 2023. doi:10.1080/07391102.2023.2190805
 142. Salem AM, Mostafa NM, Al-Sayed E, Fawzy IM, Singab ANB. Insights into the role of *Erythrina corallodendron* L. in Alzheimer's disease: *in vitro* and in silico approach. Chem Biodivers. 2023;20(7):e202300200. doi:10.1002/cbdv.202300200
 143. Madhav H, Abdel-Rahman SA, Hashmi MA, Rahman MA, Rehan M, Pal K, et al. Multicomponent Petasis reaction for the identification of pyrazine based multi-target directed anti-Alzheimer's agents: In-silico design, synthesis, and characterization. Eur J Med Chem. 2023;254:115354. doi:10.1016/j.ejmech.2023.115354
 144. El-Hussieny M, Abd-El-Maksoud MA, Soliman FM, Fouad MA, El-Ashrey MK. Dual-target ligand discovery for Alzheimer's disease: triphenylphosphoranylidene derivatives as inhibitors of acetylcholinesterase and β -amyloid aggregation. J Enzyme Inhib Med Chem. 2023;38(1):2166040. doi:10.1080/14756366.2023.2166040
 145. Abd El-Aziz NM, Shehata MG, Alsulami T, Badr AN, Elbakatoshy MR, Ali HS, et al. Characterization of orange peel extract and its potential protective effect against aluminum chloride-induced Alzheimer's disease. Pharmaceuticals (Basel). 2022;16(1):12. doi:10.3390/ph16010012
 146. Haji Ali S, Osmaniye D, Sağlık BN, Levent S, Özkay Y, Kaplancıklı ZA. Design, synthesis, and evaluation of novel 2H-benzo[b][1,4]thiazin-3(4H)-one derivatives as new acetylcholinesterase inhibitors. Molecules. 2022;27(7):2121. doi:10.3390/molecules27072121
 147. Tok F, Sağlık BN, Özkay Y, Kaplancıklı ZA, Koçyiğit-Kaymakçioğlu B. N-substituted arylidene-3-(methylsulfonyl)-2-oxoimidazolidine-1-carbohydrazone as cholinesterase inhibitors: Design, synthesis, and molecular docking study. Chem Biodivers. 2022;19(8):e202200265. doi:10.1002/cbdv.202200265
 148. Osmaniye D, Ahmad I, Sağlık BN, Levent S, Patel HM, Ozkay Y, et al. Design, synthesis and molecular docking and ADME studies of novel hydrazone derivatives for AChE inhibitory, BBB permeability and antioxidant effects. J Biomol Struct Dyn. 2023;41(18):9022-38. doi:10.1080/07391102.2022.2139762
 149. Potshangbam AM, Nandeibam A, Amom T, Potshangbam N, Rahaman H, Rathore RS, et al. An *in silico* approach to identify potential medicinal plants for treating Alzheimer disease: a case study with acetylcholinesterase. J Biomol Struct Dyn. 2022;40(4):1521-33. doi:10.1080/07391102.2020.1828170.
 150. Honorio P, Hannongbua S, Saparpakorn P. Roles of hybrid donepezil scaffolds as potent human acetylcholinesterase inhibitors using in silico interaction analysis, drug-likeness, and pharmacokinetics prediction. Chem Biol Interact. 2022;368:110227. doi:10.1016/j.cbi.2022.110227
 151. Pandey S, Singh BK. De-novo Drug Design, molecular docking and in-silico molecular prediction of achei analogues through CADD approaches as anti-alzheimer's agents. Curr Comput Aided Drug Des. 2020;16(1):54-72. doi:10.2174/1573409915666190301124210
 152. Nazir N, Karim N, Abdel-Halim H, Khan I, Wadood SF, Nisar M. Phytochemical analysis, molecular docking and anti-amnesic effects of methanolic extract of *Silybum marianum* (L.) Gaertn seeds in scopolamine induced memory impairment in mice. J Ethnopharmacol. 2018;210:198-208. doi:10.1016/j.jep.2017.08.026
 153. Sahin Z, Ertas M, Bender C, Bülbül EF, Berk B, Biltekin SN, et al. Thiazole-substituted benzoylpiperazine derivatives as acetylcholinesterase inhibitors. Drug Dev Res. 2018;79(8):406-25. doi:10.1002/ddr.21481
 154. Rahman A, Ali MT, Shawan MM, Sarwar MG, Khan MA, Halim MA. Halogen-directed drug design for Alzheimer's disease: a combined density functional and molecular docking study. Springerplus. 2016;5(1):1346. doi:10.1186/s40064-016-2996-5
 155. Meena P, Nemaysh V, Khatri M, Manral A, Luthra PM, Tiwari M. Synthesis, biological evaluation and molecular docking study of novel piperidine and piperazine derivatives as multi-targeted agents to treat Alzheimer's disease. Bioorg Med Chem. 2015;23(5):1135-48. doi:10.1016/j.bmc.2014.12.057
 156. Mirza FJ, Zahid S, Amber S, Sumera, Jabeen H, Asim N, et al. Multitargeted molecular docking and dynamic simulation studies of bioactive compounds from *Rosmarinus officinalis* against Alzheimer's Disease. Molecules. 2022;27(21):7241. doi:10.3390/molecules27217241
 157. Kamel NN, Aly HF, Fouad GI, Abd El-Karim SS, Anwar MM, Syam YM, et al. Anti-Alzheimer activity of new coumarin-based derivatives targeting acetylcholinesterase inhibition. RSC Adv. 2023;13(27):18496-510. doi:10.1039/d3ra02344c
 158. Honorio P, Sainimnuan S, Hannongbua S, Saparpakorn P. Binding interaction of protoberberine alkaloids against acetylcholinesterase (AChE) using molecular dynamics simulations and QM/MM calculations. Chem Biol Interact. 2021;344:109523. doi:10.1016/j.cbi.2021.109523.
 159. Chen S-F, Chen P-Y, Hsu H-J, Wu M-J, Yen J-H. Xanthohumol suppresses MylIP/Idol gene expression and modulates LDLR abundance and activity in HepG2 Cells. J Agric Food Chem. 2017;65(36):7908-18. doi:10.1021/acs.jafc.7b02282
 160. Zhang Y, Bobe G, Miranda CL, Lowry MB, Hsu

- VL, Lohr CV, et al. Tetrahydroxanthohumol, a xanthohumol derivative, attenuates high-fat diet-induced hepatic steatosis by antagonizing PPAR γ . *Elife* 2021;10:e66398. doi:10.7554/eLife.66398
161. Yang L, Broderick D, Campbell Y, Gombart AF, Stevens JF, Jiang Y, et al. Conformational modulation of the farnesoid x receptor by prenylflavonoids: insights from hydrogen deuterium exchange mass spectrometry (HDX-MS), fluorescence titration and molecular docking studies. *Biochim Biophys Acta*. 2016;1864(12):1667-77. doi:10.1016/j.bbapap.2016.08.019
162. Chen G, Xiao B, Chen L, Bai B, Zhang Y, Xu Z, et al. Discovery of new MD2-targeted anti-inflammatory compounds for the treatment of sepsis and acute lung injury. *Eur J Med Chem*. 2017;139:726-40. doi:10.1016/j.ejmech.2017.08.036
163. Cho JM, Yun SM, Choi YH, Heo J, Kim NJ, Kim SH, et al. Xanthohumol prevents dextran sulfate sodium-induced colitis via inhibition of Ikk β /NF-KB signaling in mice. *Oncotarget*. 2017;9(1):866-80. doi:10.18632/oncotarget.23183
164. Liu X, Song M, Wang P, Zhao R, Chen H, Zhang M, et al. Targeted therapy of the AKT kinase inhibits esophageal squamous cell carcinoma growth in vitro and in vivo. *Int J Cancer*. 2019;145(4):1007-19. doi:10.1002/ijc.32285
165. Wang L, Zhang Y, Johnpaul IA, Hong K, Song Y, Yang X, et al. Exploring two types of prenylated bitter compounds from Hop plant (*Humulus lupulus* L.) against α -glucosidase in vitro and in silico. *Food Chem*. 2022;370:130979. doi:10.1016/j.foodchem.2021.130979
166. Benkherouf AY, Logrén N, Somborac T, Kortensniemi M, Soini SL, Yang B, et al. Hops compounds modulatory effects and 6-prenylnaringenin dual mode of action on Gaba receptors. *Eur J Pharmacol*. 2020;873:172962. doi:10.1016/j.ejphar.2020.172962
167. Štern A, Furlan V, Novak M, Štampar M, Kolenc Z, Kores K, et al. Chemoprotective Effects of Xanthohumol against the carcinogenic mycotoxin aflatoxin B1. *Foods*. 2021;10(6):1331. doi:10.3390/foods10061331
168. Zhuang C, Zhang W, Sheng C, Zhang W, Xing C, Miao Z. Chalcone: A privileged structure in medicinal chemistry. *Chem Rev*. 2017;117(12):7762-810. doi:10.1021/acs.chemrev.7b00020
169. Arshad L, Jantan I, Bukhari SN, Haque MA. Immunosuppressive effects of natural α,β -unsaturated carbonyl-based compounds, and their analogs and derivatives, on immune cells: A review. *Front Pharmacol*. 2017;8:22. doi:10.3389/fphar.2017.00022
170. Xiu C, Hua Z, Xiao BS, Tang WJ, Zhou HP, Liu XH. Novel benzopyran derivatives and their therapeutic applications: a patent review (2009-2016). *Expert Opin Ther Pat*. 2017;27(9):1031-45. doi:10.1080/13543776.2017.1338687
171. Baumann V, Winkler J. miRNA-based therapies: strategies and delivery platforms for oligonucleotide and non-oligonucleotide agents. *Future Med Chem*. 2014;6(17):1967-84. doi:10.4155/fmc.14.116