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Beer constituents as potential cancer chemopreventive agents

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Abstract

Beer is a complex alcoholic beverage made from barley (malt), hop, water and yeast. Phenolic constituents of beer are derived from malt (70–80%) and hop (20–30%). Structural classes include simple phenols, benzoic- and cinnamic acid derivatives, coumarins, catechins, di-, tri- and oligomeric proanthocyanidins, (prenylated) chalcones and flavonoids as well as alpha- and *iso*-alpha-acids derived from hop. Compounds belonging to different structural classes have distinct profiles of biological activity in *in vitro* test systems, and in combination might lead to enhanced effects. Scientific evidence has accumulated over the past 10 years pointing to the cancer preventive potential of selected hop-derived beer constituents, *i.e.*, prenylflavonoids including xanthohumol and isoxanthohumol, and hop bitter acids. Chemopreventive activities observed with these compounds relevant to inhibition of carcinogenesis at the initiation, promotion and progression phases, as well as results from *in vivo* studies on metabolism, bioavailability and efficacy are summarised in this review.
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Keywords: Beer; Hop (*Humulus lupulus* L.); Cancer chemoprevention; Xanthohumol; Isoxanthohumol; 8-Prenylnaringenin; Humulone; *iso*-Alpha-acids; Malt; Epidemiology; Metabolism; Antioxidants

1. Introduction

Beer is one of the most commonly consumed alcoholic beverages. It is rich in nutrient as well as non-nutrient components including carbohydrates, amino acids, minerals, vitamins, and phenolic compounds [1]. According to the Beer Purity Law from 1516, traditional German beer is made only from barley, hop, and water, with the addition of yeast. Various parameters during brewing, *i.e.*, the variety of barley and the malting process, temperature and pH during mashing, sparging, boiling, the variety of hops added during wort-boiling, and yeast fermentation, influence the type and quality of beer.

Hop (*Humulus lupulus* L.) is the raw material of beer that serves as an important source of phenolic compounds. The dried hop cones contain 4–14% polyphenols, mainly phenolic acids, prenylated chalcones, flavonoids, catechins and proanthocyanidins [2–4]. In addition, hop provides a resin containing monoacyl phloroglucides, which are converted to hop bitter acids during the brewing process [5,6]. About 20–30% of beer polyphenols originate from hop, whereas 70–80% are malt-derived [5,7]. Barley polyphenols undergo changes during the malting and brewing process and are less well characterised than phenolic compounds from hop. However, specific proanthocyanidins from barley and malt have been separated and quantified [8–10].

This review summarises the present knowledge on potential cancer preventive effects of beer constituents. It first briefly describes the current evidence on the link between beer consumption and cancer occurrence based on epidemiological studies. Subsequently, investigations with beer in animal models of colon carcinogenesis are presented, followed by a comprehensive compilation of

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known beer constituents which have been isolated, and analysed in a series of *in vitro* test systems indicative of cancer preventive potential. In the second part, the article focuses on results from investigations on cancer preventive mechanisms of prenylflavonoids and bitter acids derived from hop. Biological activities including antioxidant effects, modulation of carcinogen metabolism, anti-inflammatory potential, estrogen ant/agonistic properties, anti-proliferative mechanisms by induction of apoptosis and cell differentiation as well as anti-angiogenic and anti-invasive effects are discussed. Some novel information on metabolism studies, bioavailability and cancer preventive efficacy of selected beer components is also provided.

2. Beer consumption and cancer incidence: epidemiological evidence

Beer drinking is almost invariably related to alcohol consumption, since alcohol-free beers currently account for only 2–3% of the beer market. Low alcohol consumption (10–15 g alcohol/day, equivalent to about on glass (0.3 l) of beer per day), has been found to reduce annual mortality for all causes of death in comparison with non-drinking and heavy drinking [11,12]. This U-shaped pattern was also described in relation to risk of cardiovascular disease and cardiovascular mortality, especially for wine drinking [13–15].

Although this review will focus on potential cancer preventive effects of beer constituents, the possible relation between beer drinking and cancer development should not be underestimated. There is convincing evidence that high alcohol intake is related to carcinogenesis, especially to cancers of the mouth, pharynx, larynx, esophagus, and liver, and probable evidence for an association between heavy drinking and colorectal cancer in men, and breast cancer in women [16]. The risks are essentially due to the ethanol content of alcoholic beverages and increase with the amount of alcohol drunk. Interpretation of data is complicated by confounding factors such as smoking, diet, hormone-replacement therapy and family history in the case of breast cancer.

Several epidemiological studies have investigated the potential role of beer as a cause of cancer. The evaluation of these studies is further complicated by the detection of volatile nitrosamines in beer in the late 1970s [17,18]. As these carcinogenic compounds were generated through the malting process by drying with hot air heated by direct firing techniques, maltsters altered the process to indirect firing techniques, thereby reducing the volatile nitrosamine content in beer to very low levels. Since most epidemiological studies refer to long-term alcohol consumption, these studies cover, in part, periods of time before changes were made in the malting process.

Studies that evaluated the alcohol effect on lung cancer reported more frequently a correlation with beer and liquor than with wine [19]. A similar trend was observed for death from all types of cancer in a pool of Danish cohort studies [20], for alcoholic beverage consumption in relation to risk for colorectal cancer [21–23] and for the association of alcohol intake and small bowel adenocarcinoma in a European multicentre case-control study [24]. On the other hand, beer consumption ($\geqslant$10 g alcohol/day) was reported to significantly decrease the risk of prostate cancer (odds ratio = 0.68 adjusted for age and total energy intake; 95% confidence interval (CI) = 0.49–0.94) in comparison with non-drinking of beer in a Canadian study including 1253 subjects (617 cases and 636 controls) [25]. Also, after a 12 year follow up in a large prospective cohort study of Finish male smokers (alpha-tocopherol, beta-carotene cancer prevention study) recently a decreased relative risk (RR) for renal cell cancer was reported for both the highest alcohol intake category (>25.7 g alcohol/day, RR = 0.53; 95% CI = 0.34–0.83) and for the highest beer intake category (>7.5 g ethanol/day, RR = 0.55; 95% CI = 0.36–0.85) [26]. Due to the obvious positive association between alcohol consumption and other types of cancer, these data should be interpreted with caution.

3. Beer and cancer prevention: *in vivo* investigations

Several experimental animal studies have examined the effect of alcohol and beer on colorectal tumourigenesis. Nelson and colleagues [27] treated male Sprague–Dawley rats with tap water, 5% ethanol solution or beer as their sole liquid source. Rats were given 10 weekly injections of dimethylhydrazine (DMH) as a colon carcinogen (20 mg/kg body weight (b.w.)) and were killed 14 weeks after the last injection. Whereas 5% ethanol did not influence DMH-induced colonic tumour development, beer significantly reduced the incidence of DMH-related tumours by 33% ($P = 0.028$) and the number of gastrointestinal tumours per rat from 2.91 ± 0.52 in the water/DMH-group to 1.33 ± 0.43 in the beer/DMH group ($P = 0.043$) [27]. Hamilton [28] incorporated high or low amounts of beer (23% or 12% of calories as alcohol in beer) into a liquid diet and induced colonic carcinogenesis in male Fischer 344 rats by 10 weekly subcutaneous injections of azoxymethane (AOM, 7 mg/kg b.w.), a metabolite of DMH. After a further 16 weeks, the high beer group showed a significantly reduced incidence of tumours in the right colon (42% *vs.* 81% in control, $P < 0.01$), but had no effect on left colonic tumourigenesis. However, the low beer group demonstrated a trend towards an increased incidence (42% *vs.* 15% in control, $P = 0.06$) and proportion (28% *vs.* 11% in control, $P = 0.08$) of tumours in the left colon with no effect on right colonic tumourigenesis [28].

The effect of high or low amounts of alcohol (18% or 9% of calories as alcohol) was very similar to that observed with beer, suggesting a predominant role of alcohol rather than of other beer constituents. Recently, Nozawa [29] provided further evidence for the colon cancer preventive potential of beer. DNA damage of colonocytes induced by a single AOM injection (15 mg/kg b.w.) was significantly reduced in male Fischer 344 rats fed beer or a malt extract for 2 weeks. Further, beer and freeze-dried beer, but not 5% ethanol solution, reduced the formation of aberrant crypt foci (ACF) in colonic mucosa induced by AOM (15 mg/kg b.w., two weekly injections), suggesting that non-volatile components of beer are responsible for the reduction. Beer was effective when given only before initiation by carcinogen treatment, during the whole experimental period, or only after initiation with AOM. Significant suppression of ACF formation was also observed by treatment with hot water extracts of malt (5% w/v), especially with extracts of coloured malts, but not of hops (1% w/v). In a long-term experiment, intake of beer for 42 weeks decreased tumour incidence from 86% in the AOM group to 64% in the AOM + beer group and lowered the number of neoplastic lesions, including adenocarcinomas and adenomas, by 44% from 1.95 ± 1.5 in the AOM group to 1.09 ± 1.15 in the AOM + beer group ($P < 0.005$) [29].

In 1996, beer was first reported to possess antimutagenic effects and was later shown to inhibit DNA-adduct formation by various carcinogens [30,31]. Betaine glycine was identified as an active principle against 2-chloro-4-methylthiobutanoic acid, the sanmafish mutagen [32]. Recent evidence suggests that beer also influences promutagenic effects of heterocyclic amines that are formed in the diet during the process of cooking. Freeze-dried and then reconstituted beer solutions significantly reduced the formation of heterocyclic amine-induced DNA adducts in liver and lungs of mice when given either in drinking water or incorporated into the diet [33]. Protective effects were observed both with lager- and stout-type samples.

In addition to these cancer-associated studies, beer was investigated in an experimental model for atherosclerosis [34]. A dark and a lager beer were given at two concentrations (half-diluted beer or 1/10-diluted) for 10 weeks to cholesterol-fed hamsters. At the high dose, both lager and dark beer significantly inhibited atherosclerosis, measured by the percentage of aortal coverage with foam cells, by 62% and 71% compared to a 2% alcohol control ($P < 0.01$). At the low dose, only the lager beer significantly decreased atherosclerosis by 21% compared to the 0.4% alcohol control ($P < 0.01$). This effect was speculated to result from a higher bioavailability of the polyphenols in lager as compared to dark beer. At the high dose, lager also significantly decreased cholesterol and triglycerides, and both beers acted as *in vivo* antioxidants by decreasing the potential of lower density lipoproteins to be oxidised. In a human study, ingestion of 500 ml of beer resulted in a significant temporal increase of plasma antioxidant activity, determined by the total radical-trapping antioxidant parameter (TRAP) assay [35].

4. Beer constituents with cancer chemopreventive potential

Beer is an extremely complex beverage, and several hundreds of constituents have been identified hitherto. A total phenol content of 500–1000 mg/l was measured [36], but part of this may be in fact derived from Maillard products [37]. Apparently, a vast bulk of flavonoid polyphenols in most beers are bound to polypeptides as soluble complexes, while simple free catechins collectively amount to less than 10 mg/l [9].

During storage of beer, haze may be formed by interaction of proteins and polyphenols. In order to prevent haze formation and thus extend the shelf live of beer, polyphenols are partly removed from beer by treatment with polyvinylpolypyrrolidon resin (PVPP). In the late 1990s, we initialised a project to analyse the composition of a PVPP retentate removed from the resin by sodium hydroxide solution. Since alkaline treatment resulted in degradation of the PVPP-bound beer components, initial results were not very encouraging, and we extended the project to unstabilised beer. Activity-guided fractionation of 300 l German-style pilsener led to the isolation of more than 30 compounds, four of which were identified as novel compounds [38]. A comprehensive overview of beer constituents isolated in this study as well as of beer components described in the literature, including reported quantities in beers is given in Table 1.

Most of the compounds isolated in our study were tested for their potential cancer chemopreventive activities in various enzyme- and cell culture tests. These test systems measured (i) antioxidant effects, *e.g.*, scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals, (ii) modulation of carcinogen metabolism by reduction of activating phase 1 cytochrome P450 1A (Cyp1A) enzyme activity and induction of detoxifying phase 2 NAD(P)H:quinone reductase (QR) enzyme activity, and (iii) anti-inflammatory mechanisms through inhibition of inducible nitric oxide synthase (iNOS) induction and inhibition of cyclooxygenase 1 (Cox-1) activity, as described in [39]. Results have been published previously, but a summary is given in Table 2 (modified from [38]). Interestingly, various structural classes of compounds had quite distinct profiles of activity. Radical-scavenging activity was provided by benzoic- and cinnamic acid derivatives (agents **9**, **11**, **12**, **18**, **19**, **20** from Table 1), catechins (agents **24**–**26** from Table 1), di- and tri-meric proanthocyanidins (agents **34**, **39**, **40**, **42** from Table 1), and flavones (agents **54**–**56** from Table 1). The

Table 1
Summary of known phenolic constituents of beer (from [8,9,38,110–116])

Structural classes		(mg/l)
Simple phenols		
1	4-Vinyl phenol	Up to 0.15
2	4-Vinyl guaiacol	Up to 0.55
3	4-Ethyl phenol	Up to 0.01
4	Isoeugenol	Up to 0.04
5	4-Hydroxyphenyl ethanol (tyrosol)	Up to 40
6	4-Propyl syringol	Up to 0.2
7	2,3-Dihydroxy-guaiacylpropane-1-one	Up to 0.034
Benzoic acid derivatives		
8	4-Hydroxy benzoic acid	Up to 1.1
9	Protocatechuic acid	Up to 0.3
10	Vanillic acid	Up to 3.6
11	Gallic acid	Up to 0.2
12	Syringic acid	Up to 0.5
13	Salicylaldehyde	n.d.
14	*o*-Vanillin	Up to 1.6
15	Syringic aldehyde	Up to 0.7
Cinnamic acids		
16	Cinnamic acid	n.d.
17	*p*-Coumaric acid	Up to 0.9
18	Caffeic acid	Up to 0.3
19	Ferulic acid	Up to 6.5
20	Sinapic acid	Up to 0.25
Coumarins		
21	Umbelliferon	Up to 1.0
22	Scopoletin	Up to 1.0
23	Daphnetin	Up to 1.0
Flavan-3-ols (Catechins)		
24	(+)-Catechin	Up to 5.4
25	(−)-Epicatechin	Up to 1.1
26	Gallocatechin	n.d.
27	Catechin gallate	5–20
28	Epicatechin gallate	5–20
29	3′-*O*-methylcatechin	n.d.
30	Catechin-7-*O*−β−D-glucopyranosid	n.d.
31	Catechin-7-*O*−β-(6″-*O*-nicotinoyl)-β-D-glucopyranosid	n.d.
Proanthocyanidins		n.d.
32	Procyanidin B 1	n.d.
33	Procyanidin B 2	n.d.
34	Procyanidin B 3	Up to 3.1
35	Procyanidin B 4	n.d.
36	Procyanidin C2	n.d.
37	Prodelphinidin B3	Up to 3.3
38	Prodelphinidin B9	Up to 3.9
39	Prodelphinidin C	n.d.
40	*ent*-Epigallo-catechin-(4α→8, 2α→*O*→7)catechin	n.d.
41	*ent*-Epigallocatechin (4α→6, 2α→*O*→7)catechin	n.d.
42	2,3-*cis*-3,4-*trans*-2-[2,3-*trans*-3,3′,4′,5,7-Pentahydroxyflavan-8-yl]-4-(3,4-dihydroxyphenyl)-3,5,7-trihydroxybenzopyran	n.d.
Chalcones		
43	Xanthohumol	0.002–1.2[a]
44	Desmethylxanthohumol	Traces only
Flavanones		
45	Isoxanthohumol	0.04–3.44[a]
46	Naringenin	n.d.
47	8-Prenylnaringenin	0.001–0.24[a]
48	6-Prenylnaringenin	0.001–0.56[a]
49	6-Geranylnaringenin	0–0.074[a]
50	5-Methyl-6″-dimethyl-4″,5″-dihydropyrano[2″,3″,7,8]naringenin	n.d.
51	Taxifolin (flavanonol)	Up to 1.0
Flavones		
52	Apigenin	n.d.
53	Chrysoeriol	n.d.
54	Tricin	n.d.
55	Apigenin-6-*C*-glucoside (saponaretin)	n.d.
56	Apigenin-6-*C*-glucoside-7-*O*-glucoside (saponarin)	n.d.
57	Vitexin	n.d.
Flavonoles		
58	Kaempferol	16.4
59	Kaempferol-3-rhamnosid	Up to 1.0
60	Quercetin	Up to 10
61	Quercitrin	Up to 2.3
62	Isoquercitrin	Up to 1.0
63	Rutin	Up to 1.8
64	Myricetin	n.d.
65	Myricitrin	n.d.
Alpha-acids		∼1.7
66	Cohumulone	
67	*n*-Humulone	
68	Adhumulone	
iso-Alpha-acids (*cis* and *trans*)		0.6–100[b]
70	*iso*-Cohumulone 20–50%	
71	*iso*-*n*-Humulone 20–50%	
72	*iso*-Adhumulone ∼15%	
Miscellaneous compounds		
73	Indole-3-carboxylic acid	n.d.
74	Indole-3-ethanol (tryptophol)	Up to 0.37
75	1-Methyl-1,3,4,9-tetrahydropyrano [3,4b]indol	n.d.
76	4-Ketopinoresinol (lignan)	n.d.
77	Syringaresinol (lignan)	n.d.
78	3-Acetoxy-propan-1,2-diol	n.d.

n.d., not determined.
[a] Highest levels are found in stout and porter beers [113,116].
[b] In very bitter English ales [5].

catechins (agents **24**–**26**, **29** from Table 1) were additionally identified as good Cox-1 inhibitors, but had only marginal effects on Cyp1A and QR activity. On the other hand, (prenylated) flavanones (agents **45**–**49** from Table 1) were identified as very potent inhibitors of Cyp1A activity at nanomolar concentrations. Concomitantly, they enhanced QR activity as an indication for

Table 2
Summary of potential chemopreventive activities of isolated beer constituents[a]

No.[b]	Compounds	DPPH scavenging	Cyp1A inhibition	QR induction		Inhibition of iNOS induction	Cox-1 inhibition
		SC_{50} (μM)[c]	IC_{50} (μM)[c]	CD (μM)[d]	IC_{50} (μM)[e]	IC_{50} (μM)	IC_{50} (μM)
24	(+)-Catechin	16.9	>5 (0)	>50 (1.0)[e]	>50	>50 (3)[d]	5.2
25	(−)-Epicatechin	13.7	>5 (0)	>50 (0.9)	>50	>50 (16)	7.5
26	Gallocatechin	15.4	>5 (8)	>50 (1.2)	>50	>50 (17)	45.3
29	3′-*O*-methylcatechin	>250	>5 (40)	>50 (1.4)	>50	>50 (0)	24.8
30	catechin-7-*O*−β−D-glucopyranosid	>250	>5 (0)	>50 (1.1)	>50	>50 (0)	>100 (8)[d]
43	Xanthohumol (XN)	>250	0.02	1.7	7.4	12.9	16.6
45	Isoxanthohumol (IX)	>250	0.3	7.5	35.1	21.9	>100 (36)
46	Naringenin	>250	0.2	>50 (1.5)	>50	>50 (10)	164.6
47	8-Prenylnaringenin (8-PN)	>250	0.07	15.5	>50	>50 (15)	27.1
48	6-Prenylnaringenin	>250	0.09	15.4	>50	>50 (33)	113.7
49	5-Methyl-6″-dimethyl-4″,5″-dihydropyrano[2″,3″,7,8]naringenin	>250	1.6	19.6	>50	>50 (24)	>100 (0)
52	Apigenin	>250	0.02	>38.6 (0.9)	38.6	17.5	>100 (22)
54	Tricin	260.0	0.13	>50 (1.9)	>50	>50 (7)	>100 (38)
55	Apigenin-6-C-glucoside (saponaretin)	313.4	>5 (31)	>50 (1.5)	>50	>50 (0)	>100 (10)
56	Apigenin-6-C-glucoside-7-*O*-glucoside (saponarin)	114.8	>5 (1)	>50 (1.6)	>50	>50 (0)	>100 (9)
75	1-Methyl-1,3,4,9-tetrahydropyrano[3,4b]indol	>250	1.5	33.9	>50	>50 (20)	>100 (5)
76	4-Ketopinoresinol (lignan)	191.0	2.2	6.0	34.7	33	>100 (15)

[a] Table modified from [38]. The following *in vitro* test systems were utilised: DPPH, scavenging of the stable free radical diphenylpicrylhydrazyl; Cyp1A, inhibition of the enzyme activity of cytochrome P450 1A; QR, induction of the specific activity of NAD(P)H:quinone oxidoreductase (QR) in Hepa1c1c7 murine hepatoma cells; iNOS, inhibition of lipopolysaccharide-mediated induction of inducible nitric oxide synthase (iNOS) in murine macrophages, measured *via* nitrite levels in cell culture supernatants; Cox-1, inhibition of sheep-seminal vesicle-derived cyclooxygenase-1 enzymatic activity.

[b] Compounds **9**, **11**, **12**, **18**, **19**, **20**, **34**, **39**, **40**, **42** were identified as potent DPPH radical scavengers with halfmaximal scavenging concentrations (SC_{50}) of 44.8, 8.5, 44.2, 20.5, 47.5, 31.2, 7.6, 9.7, 16.0, and 11.0 μM, respectively, but were not active in any other assay at the highest concentrations tested, *i.e.*, 5 μM for Cyp1A inhibition, 50 μM for QR induction and inhibition of iNOS induction, respectively, and 100 μM for Cox-1 inhibition. Compounds **8**, **10**, **16**, **17**, and **73** were not active at the highest concentrations tested including DPPH radical scavenging at 250 μM. Compounds **5**, **31**, **41**, **53**, **77**, and **78** were not tested.

[c] SC_{50}: halfmaximal scavenging concentration; IC_{50}: halfmaximal inhibitory concentration. Values in parentheses indicate the percentage of inhibition at the indicated concentrations.

[d] CD: concentration required to double the specific activity of QR. Values in parentheses indicate the fold induction at the indicated concentrations.

[e] IC_{50}: concentration that results in 50% inhibition of cell viability.

elevated detoxification, and partly had anti-inflammatory potential by inhibition of iNOS induction and Cox-1 activity. Although these data are derived from a subset of known beer constituents that are present in beer in only small quantities, the results suggest that the combination of compounds with different activity profiles might enhance a potential biological effect.

4.1. Hop-derived prenylflavonoids: xanthohumol, isoxanthohumol, 8-prenylnaringenin

As a major outcome of our activity-guided fractionation of beer, we isolated the prenylated chalcone xanthohumol (agent **43** Table 1, XN) as an interesting constituent that demonstrated activity in most screening assays (Table 2). It is well known that prenylflavonoids in beer are derived from hop [40]. Prenylated hop flavonoids are secreted along with bitter acids and essential oils by lupulin glands of the female hop inflorescences (hop cones). Since lupulin glands lack the enzyme necessary for the conversion of chalcones to flavanones, they produce (exclusively) flavonoids of the chalcone type with XN as the most abundant (82–89%) of the total amount of prenylated flavonoids in European hop varieties [41]. During the brewing process, XN is converted into the corresponding isomeric prenylflavanone isoxanthohumol (agent **45** Table 1, IX), which represents the major prenylflavonoid in beer [42]. It is assumed that 8-prenylnaringenin (agent **47** Table 1, 8-PN) is formed non-enzymatically during drying, storage, and extraction of hop, and levels in beer are generally very low [2]. Chemical structures of these compounds are given in Fig. 1.

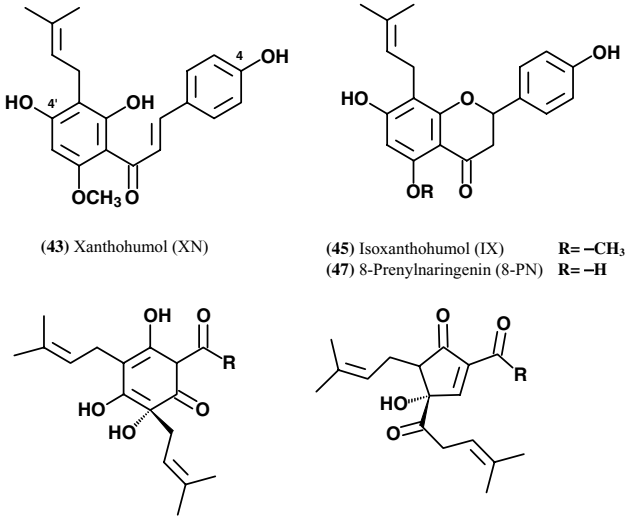

Fig. 1. Chemical structures of selected beer constituents.

To better characterise the chemopreventive potential of XN, the compound was tested in additional cell- and enzyme-based *in vitro* bioassays with markers relevant for measuring inhibition of carcinogenesis during the initiation, promotion and progression stage. Results were compared with those of the well known chemopreventive agent resveratrol, a tri-hydroxy stilbene found in grapes and red wine [43,44]. Importantly, in the mouse mammary gland organ culture (MMOC) model, XN prevented preneoplastic mammary lesion formation induced by dimethyl-benz[*a*]antracene at 200-fold lower concentrations than resveratrol, with an IC_{50} value of 0.02 μM [45].

4.1.1. Modulation of carcinogen metabolism

Indicative of chemopreventive potential in the initiation phase, XN was found to potently inhibit phase 1 Cyp1A activity, using microsomes from β-naphthoflavone-induced rat hepatoma cells as an enzyme source and 3-cyano-7-ethoxycoumarin as a substrate. Kinetic experiments indicated a mixed competitive mechanism, with a halfmaximal inhibitory concentration (IC_{50}) of 0.02 μM. Resveratrol was about tenfold less active. XN was identified as a monofunctional inducer of NAD(P)H:quinone reductase (QR) activity, with a CD value (concentration required to double the specific activity of QR) of 1.7 μM. These results were in line with previous reports. Henderson [46] described *in vitro* effects of prenylflavonoids including XN, IX, and 8-PN on cDNA-expressed human Cyp1A1, Cyp1B1, Cyp1A2, Cyp3A4 and Cyp2E1. XN noncompetitively inhibited the 7-ethoxyresorufin *O*-deethylase (EROD) activity of human Cyp1A1 with a K_i of 0.05 μM, whereas the EROD activity of Cyp1B1 was inhibited in a competitive manner. The flavanones IX and 8-PN were more effective inhibitors of Cyp1A2 acetanilide 4-hydroxylase activity than XN, and also potently inhibited the Cyp1A2-mediated metabolism of aflatoxin B1 and of the heterocyclic amine 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) [47]. XN, IX and 8-PN were poor inhibitors of Cyp2E1 and Cyp3A4, as measured by their effect on chlorzoxazone hydroxylase and nifedipine oxidase activities. Gross-Steinnmeyer [48] investigated the potential of IX and 8-PN to induce mRNA levels of Cyp1A1, Cyp1A2, QR and glutathione *S*-transferase (GST) A1 in human primary hepatocytes. Cyp1A2 transcription was significantly activated by both compounds at 25 and 50 μM concentrations, whereas mRNA levels of Phase 2 enzymes were not induced. In contrast, consistent with findings of Miranda [45,49], both compounds in our experiments moderately induced QR activity in Hepa1c1c7 mouse hepatoma cells (Table 2). In this cell line, IX also induced Cyp1A activity, indicating an aryl hydrocarbon receptor-mediated bi-functional mechanism of enzyme induction [45,49].

4.1.2. Antioxidant potential

As an additional indication of anti-initiating potential, we investigated the antioxidant capacity of XN. At a concentration of 1 μM, XN was 8.9-fold and 2.9-fold more potent than the reference compound Trolox, a water-soluble Vit. E analog, in scavenging $OH^{\cdot}$– and $ROO^{\cdot}$ radicals in the oxygen radical absorbance capacity (ORAC) assay. In addition, XN dose-dependently inhibited superoxide anion radical production in the xanthine/xanthine oxidase (X/XO) system and by TPA-stimulated HL-60 leukaemia cells differentiated to granulocytes, whereas resveratrol was inactive in both systems up to a concentration of 100 μM. In previous studies, XN at 5 and 25 μM inhibited Cu^{2+}-induced *in vitro* oxidation of human low-density lipoprotein (LDL) and lipid peroxidation in rat liver microsomes induced by Fe^{2+}-ascorbate or *tert*-butyl hydroperoxide [50,51]. Prenylated flavanones including IX and 8-PN were less effective than prenylated chalcones. In contrast, the nonprenylated chalcone chalconaringenin and the nonprenylated flavanone naringenin demonstrated pro-oxidant effects on LDL oxidation. When the peroxynitrite generator 3-morpholinosydnonimine (SIN-1) was utilised to stimulate LDL oxidation, an aurone and an endoperoxy derivative of XN were identified as the main oxidation products. This points to an important role of the α,β-unsaturated keto functionality as a novel antioxidant pharmacophore [52].

4.1.3. Anti-inflammatory activities

With respect to mechanisms involved in the prevention of tumour promotion, XN demonstrated anti-inflammatory potential by inhibition of Cox-1 and -2 activity with IC_{50} values of 16.6 and 41.5 μM. Resveratrol was significantly more potent in inhibiting Cox-1 activity with an IC_{50} of 1.6 μM. XN also prevented nitric oxide release by LPS-stimulated Raw 264.7 murine macrophages. Similar effects were described for additional hop components [53]. Apparently, the inhibition is mediated at the protein level by suppression of LPS-induced iNOS protein expression [54].

4.1.4. Antiestrogenic potential of xanthohumol

Hormones like 17β-estradiol are regarded as endogenous tumour promoters, stimulate cell growth *via* interaction with estrogen receptors and increase the risk for breast and uterine cancer [55]. Hop has repeatedly been reported to possess estrogenic properties. Therefore, we investigated pro- and antiestrogenic properties of XN and IX in Ishikawa cell culture. This human endometrial cancer cell line responds to estrogens with elevated alkaline phosphatase activity. Concomitant treatment with estrogens and test compounds allows the identification of antiestrogens. XN efficiently inhibited estrogen-mediated induction of alkaline phosphatase without possessing intrinsic estrogenic potential, whereas IX was identified as a weak estrogen agonist [45]. Resveratrol demonstrated activities consistent with mixed estrogen/antiestrogen properties [56]. The antiestrogenic effect of XN was confirmed *in vivo*. In juvenile female Sprague–Dawley rats, XN (at a daily dose of 100 mg/kg by mouth (p.o.) for three days) reduced the 17α-ethinylestradiol (EE, 1 μg/kg; subcutaneous (s.c.))-induced gain in uterine wet weight by 31%, without any stimulatory effect in the absence of EE (unpublished data). XN and IX showed very low affinity for both ERs in competitive receptor binding assays (relative binding affinity >0.0001) [57]. We could recently demonstrate that XN inhibits human recombinant aromatase (Cyp19) activity and might therefore reduce endogenous estrogen levels.

4.1.5. Estrogenic properties of 8-prenylnaringenin

8-PN has been identified as one of the most potent phytoestrogens, based on several *in vitro* investigations. These include estrogen receptor binding studies, screens with recombinant yeast expressing estrogen receptor constructs, stimulation of estrogen-responsive MCF-7 cell proliferation, induction of alkaline phosphatase activity in the Ishikawa cell line, and in the estrogen inducible MVLN luciferase assay [57–63]. The effect of 8-PN was described as almost pure estrogen agonistic activity with a relative binding affinity of about 0.1 (in comparison to 17β-estradiol) for both estrogen receptors (ER) α and β. In ovariectomised rats, 8-PN (at 30 mg/kg/day s.c. for 2 weeks) suppressed ovariectomy-mediated bone density- and uterine weight loss [64]. When given at a dose of 100 μg/ml in drinking water (approximately 15.8 mg/kg b.w./day for 3 days), 8-PN induced a characteristic estrogenic mitotic response in the vaginal epithelium of ovariectomised mice [65]. In that study, 8-PN did not increase uterine mass, but short-term treatment for 4 h significantly induced uterine vascular permeability. In a recent investigation with ovariectomised rats, a single dose of 10 mg/kg b.w. of 8-PN significantly stimulated uterine wet weight, uterine epithelial height as well as vaginal epithelial height [66]. Expression of estrogen-regulated genes in the uteri of ovariectomised rats was assessed at the transcriptional levels by quantitative real time PCR. ERα and clusterin gene expression were downregulated by estradiol, whereas the expression of complement C3 was up-regulated. 8-PN treatment resulted in a similar expression pattern, although less pronounced. Overall, these data provide conclusive evidence that 8-PN has to be regarded as an estrogen agonist in female reproductive tract organs [66]. In contrast, 8-PN was shown to possess antiandrogenic activity in an androgen inducible yeast receptor assay and by androgen receptor binding studies [67].

4.1.6. Antiproliferative and differentiation-inducing mechanisms

Miranda and colleagues [68] investigated antiproliferative and cytotoxic effects of prenylflavonoids in human cancer cell lines MCF-7 (ER-positive breast cancer), HT-29 (colon cancer) and A-2780 (ovarian cancer). After treatment for 4 days, IC_{50} values for XN were determined as 3.5, >10 and 5.2 μM, respectively. IX was less effective, with IC_{50} values of 4.7, >10 and 25.7 μM. Both compounds inhibited DNA synthesis in MCF-7 cells, measured by [^{3}H]-thymidine incorporation into DNA, but did not induce apoptosis within 24 h at concentrations up to 100 μM. Interestingly, primary hepatocytes were not affected by XN and IX at 10 μM concentrations and treatment for 24 or 48 h [68]. We could demonstrate that similar to resveratrol, XN at 10 and 20 μM concentrations led to a cell cycle arrest of cultured ER-negative MDA-MB-435 breast cancer cells in S-phase. Induction of apoptosis in 14.9% of attached cells after incubation with 25 μM XN for 48 h was observed by flow cytometry [45]. In addition, XN significantly reduced proliferation of the HCT-116-derived colon cancer cell line 40–16. IC_{50} values decreased from 5.2 μM after 24 h treatment to 3.6 and 2.3 μM after 48 and 72 h incubation, respectively. Induction of apoptosis as a mechanism of cell death was detected by Western Blotting experiments. Incubation with 15 μM XN for 48 h and with 5 μM for 72 h resulted in activation of the death receptor pathway, indicated by activation of caspase 8, as well as of the mitochondrial pathway *via* caspase 9 activation. Consequently, also the executioner caspases 3 and 7 were activated and led to cleavage of poly (ADP-ribose) polymerase (unpublished data).

Most tumour cells in solid tumours adapt to low oxygen supply and grow under chronic hypoxic conditions. Goto [69] recently discovered that synthesis of triglycerides and cholesterol esters was more than twofold increased when the highly invasive human fibrosarcoma cell line HT-1080 was grown under hypoxia. XN at a concentration of 3 μM was able to reduce the amount of triglycerides and to inhibit lipid droplet formation induced by hypoxia. Also, XN was significantly more effective in inhibiting the proliferation and motility of HT-1080 under hypoxia than under normoxia. Since XN has been described as an inhibitor of diacylglycerol acyltransferase (DGAT) activity and DGAT-1 mRNA expression [70,71], a causal link between these effects and a selectivity of XN for hypoxic cancer cells was postulated.

As an additional mechanism to control hyperproliferation of cancer cells, XN induced terminal cell differentiation in HL-60 human promyelocytic leukaemia cell culture [45]. We could demonstrate that HL-60 cells underwent growth arrest after treatment with XN. As an indication of differentiation along the monocytic-macrophagic lineage, expression of non-specific acid esterase was detected in 42.4% and 44.8% of viable cells when HL-60 cells were treated with 3.1 and 6.25 μM of XN for 3 days. Similarly, HL-60 cells were arrested in G1 phase of the cell cycle when treated with 8-PN at 50 μM for 2 and 4 days [72].

4.1.7. Antiangiogenic activity of prenylflavonoids

Angiogenesis – the formation of new blood vessels from pre-existing microvasculature – is recognised as a key mechanism in tumour development and formation of metastases. Without adequate blood supply, a tumour cannot grow beyond a critical size due to a lack of nutrients and oxygen [73]. To investigate antiangiogenic properties of XN and IX, we developed a human *in vitro* antiangiogenic test system based on the principle of wound healing [74]. Fragments of placental vessels were cultured in the presence or absence of the compounds for 3 weeks in fibrin gels. With XN, we observed a dose-dependent reduction of newly formed capillary growth at a concentration range of 0.5–10 μM and determined an IC_{50} value of 2.2 μM. IX was 63.1% and 86.5% inhibitory at 1 and 10 μM concentrations, respectively. Further mechanistic investigations were performed with HMEC-1, an immortalised human microvascular endothelial cell line. XN and IX effectively inhibited migration of HMEC-1 cells after wounding in a wound closure assay, with IC_{50} values of 0.03 and 0.6 μM, respectively. Also, XN effectively inhibited microcapillary formation of HMEC-1 cells on basement membrane matrix at 1, 5 and 10 μM, respectively, whereas at 0.1 μM, some tubes started to form within the incubation period of 6 h. After treatment with IX at a 10 μM concentration, we observed shortened tubes. These effects were only partly due to inhibition of HMEC-1 proliferation [75].

We used intravital microscopy as an *in vivo* model to characterise the effect of XN on tumour angiogenesis and tumour growth. ER-negative human MX-1 breast tumour xenografts were implanted in dorsal skinfold chamber preparations in female severe combined immuno-deficient (SCID) mice. Starting from day 15 after tumour implantation, animals were treated with XN (1000 mg/kg b.w./day in DMSO s.c., $n = 7$) or DMSO as control ($n = 7$), respectively, for 7 and 14 days. Two-dimensional tumour surface was documented with digital photography using bright field microscopy. Functional vessel density as an indicator of angiogenesis was quantified using intravital fluorescence video microscopy after injection of FITC-labelled dextran as a plasma marker. In this animal model, s.c. application of XN for 7 and 14 days inhibited the growth of the breast tumour xenografts by 46% and 83%, respectively, in comparison with the untreated control group, and reduced the size of established tumours by 30% and 56%, respectively. Concomitantly, XN-treatment for 14 days reduced tumour-induced neovascularisation by 33% in comparison with the untreated control group [76].

Antiangiogenic effects of 8-PN were discovered in an *in vitro* model of angiogenesis based on capillary-like tube formation on a three-dimensional collagen gel. Bovine microvascular endothelial cells derived from the adrenal cortex (BME cells) and endothelial cells from the bovine thoracic aorta (BAE cells) were stimulated with various pro-angiogenic factors alone and in combination. After treatment with 8-PN, in comparison with the known anti-angiogenic agent genistein, both compounds inhibited endothelial cell invasion and tube formation with equal potency, and IC_{50} values between 3 and 10 μM were determined for 8-PN. Also, 8-PN reduced blood vessel growth *in vivo* in the early chicken chorioallantoic membrane (CAM) assay, measured *via* vessel lengths and vein diameters, with similar qualitative and quantitative changes as observed with genistein [77]. The effects of 8-PN on cell aggregation, growth and invasion were investigated in an invasive MCF-7/6 variant of the human MCF-7 breast cancer cell line. Due to a functional defect in the E-cadherin/catenin invasion suppressor complex, these cells have acquired invasive potential. 8-PN was found to stimulate E-cadherin-dependent aggregation and growth of MCF-7/6 cells in suspension. However, 8-PN did not affect MCF-7/6 cells invasion in chick heart fragments *in vitro*. All these effects were similar to those observed with β-estradiol [78].

4.1.8. Antiviral effects

XN and further hop constituents were shown to possess antiviral activity against a series of DNA and RNA viruses [79]. XN was able to inhibit HIV-1 induced cytopathic effects, the production of viral p24 antigen and reverse transcriptase in C8166 lymphocytes, with EC_{50} values of 2.3, 3.6 and 1.4 μM, respectively. XN also inhibited HIV-1 replication in peripheral blood mononuclear cells with an EC_{50} value of 58.5 μM. However, the activity of recombinant HIV-1 reverse transcriptase and the HIV-1 entry into cells were not inhibited by XN [80].

4.1.9. Metabolism and bioavailability of prenylflavonoids

There is increasing interest in the metabolism, bioavailability and distribution of prenylated flavonoids and chalcones from hop, due to the potential chemopreventive activities of XN and the potent estrogenicity of 8-PN. Incubation of 8-PN with human liver microsomes led to the isolation of 12 metabolites, and the two most abundant metabolites were estrogenic *in vitro* [81]. *In vitro* metabolism studies with XN using rat and human liver microsomes yielded four metabolites, including XN with an additional hydroxyl group and dehydro-cycloXN, generated by a proposed mixed enzymatic and chemical biotransformation pathway [82]. Glucuronidation of XN *in vitro* provided two monoglucuronides of XN. The major metabolite (approximately 89% of the total glucuronides generated in the incubations) was formed by connection of the glucuronide residue to the hydroxyl at C-4′, whereas in the minor metabolite (~10%), the glucuronide residue was connected to the oxygen at C-4 [83]. Microbial transformation of XN with different strains of microorganisms was attempted to produce XN metabolites for further biological investigations. XN and three metabolites showed antimalarial activity against *Plasmodium falciparum* [84,85].

After a single oral application of XN (1000 mg/kg b.w.) to female Sprague–Dawley rats, fecal samples collected after 24 and 48 h contained 89% unmetabolised XN. Sixteen novel metabolites were isolated in addition to six previously known metabolites. Biotransformation resulted in the formation of cyclic prenyl moieties that included the oxygen at C-4′ or at C-2′ as well as of metabolites with non-cyclic oxygenated prenyl substituents. Further, XN-4′-*O*-glucuronide, XN-4-*O*-methyl ether, XN-4-*O*-acetate, IX and an IX metabolite were identified [86]. In a recent study, the metabolism of XN and IX with human liver microsomes was reinvestigated. Six metabolites of XN and 13 metabolites of IX were identified [87]. The authors described hydroxylation of a prenyl methyl group as the primary route of oxidative metabolism of both compounds. Importantly, IX was partly demethylated to form 8-PN. This might be relevant for the reported estrogenicity of hop extracts. To mimic reactions that could take place in the stomach, XN was incubated with diluted hydrochloric acid, and the cyclisation to form the isomeric flavanone IX was monitored. The reaction followed first order kinetics with a half life of 37 min [87].

We investigated the oral bioavailability of XN in a kinetic experiment. XN (suspended in 4% corn starch solution) was applied by gavage to female Sprague–Dawley rats at a single dose of 1000 mg/kg b.w. At 1, 2, 4, 8, 24 and 48 h after application, plasma was collected, and XN and metabolites were extracted and analysed by reversed-phase high performance liquid chromatography (RP-HPLC). The XN-4′-*O*-glucuronide was identified as the major metabolite with a T_{max} at 4 h and a C_{max} of 3.1 μM. Unmetabolised XN was detectable with a C_{max} of 0.34 μM (equal to 0.12 μg/ml) 4 h postadministration [88]. Only minor quantities of IX were detected within 48 h, suggesting that the *in vitro* incubation experiment to convert XN to IX [87] might overestimate the rate of IX formation from XN *in vivo*. In the rat uterotrophy assay described above, we detected an overall antiestrogenic effect after three daily applications of 100 mg XN/kg b.w. Based on the observed biological response, these results also contradict the proposed formation of IX and subsequent demethylation to the potent estrogen 8-PN, at least in juvenile female rats.

Avula and co-workers [89] determined the pharmacokinetics of XN in male Wistar rats. In a first experiment, rats received either oral or intravenous (i.v.)

administration of a low dose (20 mg/kg b.w.) of XN. In a second experiment, rats received a single oral administration of up to 500 mg XN/kg b.w. Plasma, urine, and faeces were collected at varying time points and assayed for their XN content by HPLC. Consistent with our report [86], XN and its metabolites were excreted mainly in faeces within 24 h of administration. Plasma levels of XN fell rapidly within 60 min after i.v. administration; in contrast to our study, no XN was detected in plasma after oral administration in either experiment.

4.2. Hop bitter acids: alpha-acids and iso-alpha acids

From a brewers point of view, hop acids, divided into alpha-acids (humulones; agents **66**–**68** Table 1, Fig. 1) and beta-acids (lupulones) are the most important class of hop compounds [3,5,90]. They are present in hop cones at up to 25% dry weight and have pronounced bacteriostatic activity, especially *vs.* gram-positive bacteria [91]. Beta-acids are extremely sensitive to oxidation and do not survive the brewing process. During wort-boiling, the poorly water-soluble alpha-acids go into solution and are isomerised to *iso*-alpha-acids (isohumulones; agents **70**–**72** Table 1); this process is involved in the generation of the bitter flavour of beer. Overall, *iso*-alpha-acids represent one of the most abundant classes of polyphenolics in beer (Table 1); concentrations of up to 100 mg/l have been reported in very bitter English ales [5].

Besides their important antibiotic properties, alpha- and *iso*-alpha-acids possess many biological activities relevant for cancer prevention. Humulones and lupulones have radical-scavenging and lipid peroxidation inhibitory activity [92]. These effects might contribute to the potent inhibition of bone resorption. When dentine pieces as bone resorption substrates were incubated *in vitro* with mouse bone cell suspension (osteoclasts), bone resorption (pit formation) was inhibited most potently by humulone with an IC_{50} value of 5.9 nM; XN was also active at about 200-fold higher concentrations. Both compounds were postulated as potential therapeutic drugs for osteoporosis [93].

Humulone (agent **67** Table 1) demonstrated significant anti-inflammatory activity by suppression of Cox-2 gene transcription in murine osteoblastic MC3T3-E1 cells [94]. TNF-α-dependent Cox-2 induction was inhibited with an IC_{50} of about 30 nM, measured by a reduction of secreted prostaglandin E_2, by direct assessment of Cox-2 catalytic activity and by Northern blot analyses. A luciferase reporter assay suggested that humulone blocked Cox-2 expression by interfering with transcription factors NF-κB and NF-IL6, independent of a glucocorticoid receptor mediated response. Sheep seminal vesicle-derived Cox-1 activity was hardly affected at concentrations below 10 μM, but Cox-2 enzymatic activity from TNF-α-stimulated MC3T3-E1 cells was inhibited *in vitro* with an IC_{50} of 1.6 μM. Humulone effectively prevented 12-*O*-tetradecanoylphorbol-13-acetate (TPA)- or arachidonic acid-induced inflammatory ear edema in mice, and significantly suppressed tumour promotion by TPA in the two-stage mouse skin model. Topical application of humulone (1 mg) 30 min before TPA treatment (1 μg twice weekly) very effectively reduced tumour incidence at week 18 by 93% and tumour multiplicity by 99% [95].

Humulone treatment at low concentrations up to 2.5 μM resulted in induction of cell differentiation in human HL-60 promyelocytic leukaemia cells and several other leukaemia cells lines. Interestingly, humulone enhanced the differentiation-inducing capacity of 1α, 25-dihydroxyvitamin D_3. It was speculated that the combination of both drugs might lower the well known side effects of 1α,25-dihydroxyvitamin D_3, *i.e.*, hypercalcemia and dissolution of bone mineral [96].

In addition to the inhibition of cell proliferation by induction of differentiation, humulone or a mixture of hop bitter acids, respectively, induced apoptosis in HL-60 cells at elevated concentrations up to 100 μg/ml. Induction of apoptosis was measured by DNA laddering and the appearance of a sub-G1 DNA peak by flow cytometry [97,98]. Modulation in levels of Bcl-2 family proteins by hop bitter acids, with a decrease in antiapoptotic Bcl-X_L levels and a significant increase in proapoptotic Bax protein resulted in disruption of mitochondrial integrity and cytochrome c release. Subsequent activation of caspase-9 and -3 was accompanied by cleavage of DNA-fragmentation factor DFF-45 and PARP. In addition, hop bitter acids promoted up-regulation of the death receptor Fas and its ligand FasL, activation of pro-caspase-8 and cleavage of Bid. These results indicated a link between receptor- and mitochondria-mediated cell death in HL-60 cells treated with hop bitter acids [98].

Similar to the prenylflavonoids from hop, humulone was also found to inhibit angiogenesis. Humulone significantly prevented *in vivo* angiogenesis in chick embryo chorioallantoic membranes (CAM) in a dose-dependent manner, with an ED_{50} of 1.5 μg/CAM. It inhibited *in vitro* tube formation and proliferation of vascular endothelial cells and suppressed the production of the pro-angiogenic vascular endothelial growth factor (VEGF) from endothelial and tumour cells [99].

Two recent reports suggest that *iso*-alpha-acids (agents **70**–**72** Table 1, Fig. 1) might have beneficial effects for the treatment of diabetic symptoms by inhibition of aldose reductase, activation of peroxisome proliferator-activated receptor (PPAR) α and γ and reduction of insulin resistance [100,101]. These reports are of interest in view of the potential role of PPAR α and ligands in cancer prevention, but also as a causal factor in colon carcinogenesis [102–104].

Little information is available regarding the bioavailability, metabolism and toxicity of alpha- or *iso*-alpha-acids. Prier (1960) reported that hop extracts,

pre-isomerised by hop processors to mainly contain bitter–tasting isohumulones, had an ED_{50} of 1000 mg/kg b.w. in rats when administered as a single dose dissolved in corn oil. When fed to Sprague–Dawley rats for 90 days at levels of 0.01%, 0.1% and 1% in the diet, a reduction in body weight gain was observed at the highest level (due to bitter taste?), but no histopathological changes related to treatment were noted (derived from [105]). I.v. administration of 3 mg/kg humulone to cats caused instantaneous hyperventilation and an increase in oxygen consumption, followed by a gradual rise in body temperature; in rabbits, the effects of humulone were generally less pronounced [106].

5. Conclusions

This review did not set out to explain a potential cancer preventive effect of beer, the aim was rather to summarise the chemopreventive activities of some beer components as far as they are known. Based on the presented evidence, the chemopreventive potential of beer constituents warrants further investigation. Potential preventive effects of hop-derived prenylflavonoids and humulone, mainly derived from *in vitro* experiments, are well established. Future *in vivo* studies on bioavailability, distribution, efficacy and safety in long-term animal models for cancer chemoprevention are however still needed. Since these compounds are present in beer in only minor quantities, they may not be (solely) responsible for the observed preventive effects of beer in animal models, *e.g.*, for colon cancer. On the other hand, biological activities, metabolism and bioavailability of the more abundant *iso*-alpha-acids are essentially unknown so far. Also, malt-derived beer components require further investigation. Studies with melanoidins, *i.e.*, polymeric and coloured final products of the Maillard reaction which are formed non-enzymatically during the roasting of malt, indicate peroxyl radical scavenging potential [107,108]. Melanoidin fractions with relatively high molecular weight (10–>200 kDa) also weakly induced NADPH-cytochrome *c* reductase and size-dependently modulated GST activities in the Caco-2 colon cancer cell line [109].

The information presented here illustrates that beer is an extremely complex mixture of bioactive substances. Therefore, a thorough exploration of the chemopreventive activities of isolated prominent beer components seems to make eminent sense. Nevertheless, future studies should also focus on defined combinations to explore whether the mixture can be more efficacious than single components.

Conflict interest statement

None declared.

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