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NATURAL PRODUCTS: FROM CHEMISTRY TO PHARMACOLOGY (C-T HO, SECTION EDITOR)

# Therapeutic Connection Between Black Tea Theaflavins and Their Benzotropolone Core Structure

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## Abstract

**Purpose of Review** The discrepancy between strong bioactivity and health promoting effects of black tea polyphenols despite their poor bioavailability is an elusive phenomenon in tea research. We discuss the theaflavin core benzotropolone and its derivatives as appealing entities filling the missing link.

**Recent Findings** Health promoting and therapeutic effects of black tea are well documented. Theaflavines are considered to be the major bioactives showing strong anti-oxidative and anti-inflammatory effects. All three isoforms of theaflavins (e.g. TF-1, TF-2 and TF-3) and some higher complex thearubigins contain the benzotropolone (BZ) skeleton as their core structure. Natural occurring BZs such as purpurogallin and others are speculated as potential, but unconfirmed, secondary metabolites of theaflavins by means of biotransformation through gut microbiota. Strong anti-inflammatory bioactivities of BZs corresponding to therapeutic effects had been described.

**Summary** Benzotropolone derivatives as core structure of theaflavins and other high molecular black tea polyphenols potentially generated by bioconversion might be a missing link explaining the "tea mystery". Future studies exploiting the molecular mechanisms of biotransformation, particularly microbial treatment and bioactivity will be needed to further consolidate the role of benzotropolones as promising candidates for therapeutic applications.

**Keywords** Black tea · Benzotropolones · Theaflavins · Thearubigins · Biotransformation

## Therapeutic Effects of Black Tea

Tea derived from leaves of *Camellia sinensis* is the most consumed beverage in the world but only second to water. Although a variety of different teas are produced, usually they are divided in three major groups such as green tea, oolong tea, and black tea which differ in the degree of fermentation and consumption [1–6]. Whereas green tea is unfermented with a consumption of approximately 20%, leaves of oolong tea are semi-fermented and consumed around 2%. Black tea with the highest degree of fermentation rate has the highest consumption worldwide (78%). Accordingly, these different types of tea contain a distinct composition of diverse polyphenols. In green tea, the most abundant polyphenols are catechins, mainly epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG). During the fermentation process, catechins are converted to polymeric polyphenols mainly into theaflavins and thearubigens which appear in oolong tea and in higher amounts in black tea. The formation of black tea polyphenols during fermentation involves two steps: oxidation and polymerization. In the first reaction, green tea catechins, mainly EGC and EGCG (see a and b in Fig. 1) are partially oxidized to quinones under the enzymatic catalysis of polyphenol oxidase (PPO) and peroxidase (POD) both naturally existing enzymes in fresh tea leaves. The second step involves

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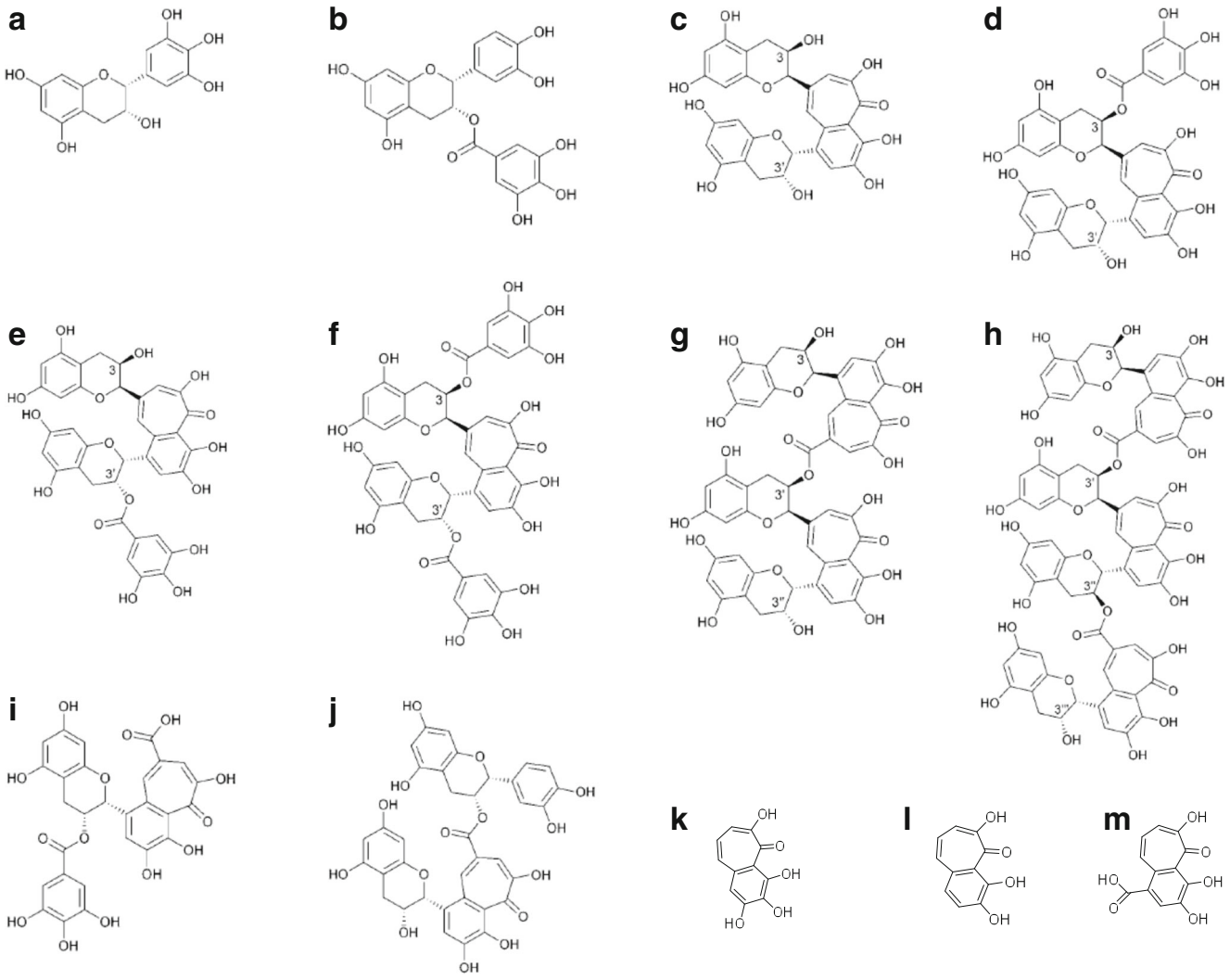


**Fig. 1** Molecular structures of major tea metabolites. **a** Epigallocatechin (EGC). **b** Epigallocatechin gallate (EGCG). **c** Theaflavin-1 (TF-1). **d** Theaflavin-3-*O*-gallate (TF-2a). **e** Theaflavin-3′-*O*-gallate (TF-2b). **f** Theaflavin-3,3′-*O*-*O*-digallate (TF-3). **g** Theadibenzotropolone A. **h** Theatribenzotropolone A. **i** Epitheaflavic acid-3-gallate. **j** Theaflavate B. **k** Purpurogallin (PPG). **l** (3, 4, 6-trihydroxy-5*H*-benzo[7]-annulen-5-one). **m** (1-carboxy-3, 4, 6-trihydroxy-5*H*-benzo[7]-annulen-5-one)

polymerization which consists of a nucleophilic addition reaction of the resulting gallocatechin quinones to catechin quinones, followed by further oxidation by oxygen or hydrogen peroxide. Elimination of carbon dioxide and rearrangement then complete the synthesis of the benzotropolone structure. All theaflavin isoforms contain the benzotropolone core structure from the reaction of a catechol and pyrogallol during the polymerization reaction. In addition, several thearubigens contain one or more benzotropolone moieties as core structures in their molecules [4, 5, 7, 8]. It is the benzotropolone moiety which results in dark orange to dark brown color characteristic for black tea [4, 6, 9]. It is interesting to note that black tea contains also significant amounts of catechins due to their incomplete conversion during the fermentation process [5].

Black tea has well-documented health beneficial and therapeutic effects against a variety of degenerative diseases. It is generally believed that the health-promoting effects of black tea are based on their antioxidant and anti-inflammatory activities as their main underlying mechanisms [1–5, 10]. Chronic inflammation is widely recognized as a major underlying cause of various degenerative diseases. The accumulation of free radicals such as reactive oxygen species (ROS) or reactive nitrogen species (RNS) and inflammatory mediators (e.g., NO, prostaglandins, leukotrienes, thromboxanes) generated by immunocompetent leukocytes (e.g., macrophages and eosinophiles) are responsible for damaging effects on cells and organs [11–17]. In accordance with its anti-inflammatory effects, several studies demonstrated effects of black tea against diseases related to chronic inflammation such as cardiovascular, gastrointestinal, neurological and immunological disorders, diabetes, rheumatoid arthritis, and different cancers [1–5, 10].

## Bioactivity of Theaflavins

Although accounting for only for 3–6% of the dry weight, health beneficial effects of black tea are mainly attributed to the group of theaflavins. Strong anti-oxidative effects of the three theaflavin isoforms have been described, reflected by prevention of pro-carcinogenic lipid peroxidation, lipoprotein oxidation, and DNA damage and mutation [1, 3, 18–23]. According to their anti-oxidative activity prominent anti-inflammatory effects for the four isoforms such as theaflavin (TF-1), theaflavin-3-*O*-gallate (TF-2a), theaflavin-3′-*O*-gallate (TF-2b), and theaflavin-3,3′-*O*-*O*-digallate (TF-3) (see c, d, e, and f in Fig. 1) have been described [1–5, 10, 24]. Previously, we observed strong anti-inflammatory activities of TF-2 (e.g., a mixture of TF-2a and TF-2b) as demonstrated by inhibition of edema formation which corresponded to attenuation of key inflammatory cascade genes by decreased NF κB and AP-1 signaling [20]. Also, TF-1 and TF-3 have been shown to target the NF κB signaling pathway [1, 18]. Accordingly, a downregulation of a variety of inflammatory genes by the three theaflavin isoforms have been described [1, 18, 19, 21, 22]. Chemopreventive and anti-cancer effects have been observed for each of the theaflavin isoforms: TF-1 [25], TF-2 [1, 19, 25, 26], and TF-3 [1, 18, 26]. These effects include cell cycle arrest and suppression of inflammation but also induction of apoptosis [1–3, 23, 24]. The induction of apoptosis has been described for TF-1, TF-2, and TF-3 [1, 19, 20, 25–28]. Pro-apoptotic activity of TF-2 were induced by the mitochondrial signaling pathway [20]. Noteworthy, theaflavins induced cell cycle arrest and apoptotic cell death selectively only in tumor cells but not in their normal cell counterparts [19, 29]. These results emphasize the role of theaflavins as major bioactives in black tea. Based on these observations, we developed a theaflavin-enriched black tea extract using a natural process of fermentation which showed strong anti-inflammatory effects [30]. Noteworthy, inflammatory surrogate genes for degenerative diseases which showed a close correlation throughout cell-based, animal, and clinical settings were prominently attenuated.

It is important to note that up to 70% of the dry weight of black tea accounts to thearubigins. The heterogeneous group of thearubigins consist of thousands of structurally distinct higher order polyphenols which includes theadi- and tribenzotropolones, theanaphthoquinones, bistheaflavins, theasinensins, theacitrins among others [4, 5, 7, 8]. Several thearubigens contain one (epitheaflavic acid-3-gallate or theaflavate B) or more benzotropolone moieties as core structures such as theadi - and theatribenzotropolone A (see g, h, i, and j in Fig. 1). The enormous structural complexity and diversity of thearubigins is based on the oligomerization of different building block such as catechins but also polyhydroxylated theaflavins and theacitrins in oxidative cascade reactions during the second step in the fermentation process [4, 5, 7, 8]. Since the chemistry of thearubigins is largely still unknown, there have been only limited reports on their biological activities although some biological effects have been described [4, 5, 7, 8]. For large molecular thearubigins, it had been hypothesized that they are probably not absorbed and might be converted to other metabolites during the intestinal passage [4, 7, 8, 31].

A mystery in tea research has been the discrepancy between the well-established bioactivity of high molecular weight polyphenols (e.g., theaflavins) on the one hand and poor bioavailability on the other [1, 2, 4, 10, 32]. This is evident either by the failure or by the detection of only minute amounts of theaflavins in blood or urine as demonstrated by in vivo animal and clinical settings [1, 2, 4, 10]. In blood, urine or feces analysis in rodents, theaflavins were either not detectable [33] or found only in very limited amounts either modified or non-modified [34, 35]. In the context of limited information on bioavailability and biotransformation by theaflavins, it is interesting to note that a rapid degradation of theaflavins at physiological pH values around 7.4 have been demonstrated [4, 5]. Sang and coworkers could only find small amounts of TF-1, TF-2a, TF-2b, glucuronidated or sulfated TF-3, and gallic acid after TF-3 application indicating biotransformation in mice [4, 35]. Interestingly, a recent study suggests that the benzotropolone moiety plays a major role in reduced permeability of cell membranes induced by theaflavins through AMPK signaling pathways [36]. Due to the recent understanding of the important role of microbiota in nutrition biotransformation of higher molecular weight, tea polyphenols metabolized by gut microbiota are discussed to play an important role in their health benefits. For black tea polyphenols, surprisingly, little research on absorption and microbial metabolism had been conducted. Therefore, the impact of microbiota on generation of theaflavin and thearubigin-derived metabolites still remains unclear [37–39]. Studies by measures of healthy humans, mouse models, or human fecal preparations demonstrated a microbial bioconversion of mono- and di-gallate theaflavins to TF-1, derivatives of gallic acid and pyrogallol as well as different phenolic acids such as 4-hydroxyphenylacetic acid, 3-hydroxyphenylacetic acid, 3,4-dihydroxyphenylacetic acid, 3-(4′-hydroxyphenyl) propionic acid, and hippuric acid among others [40–44]. The role of microbiota appear to be even more complex as indicated by studies showing differential modulatory effects of tea polyphenols on intestinal bacterial populations leading to distinct individual metabolome profiles in rodents and humans [43, 45–49].

## Core Moiety of Theaflavins—Benzotropolones and Potential Therapeutic Applications

The exclusive characteristic of theaflavins is the benzotropolone skeleton, i.e., the core moiety of theaflavins. The enzymatically catalyzed oxidation of catechins generates the benzotropolone

core attached with unchanged side chains. An example of naturally occurring benzotropolone containing compounds is purpurogallin. The biological and pharmacological activities of purpurogallin (PPG), a natural occurring benzotropolone-containing compound found in the nut gall of *Quercus* spp. are well known (see k in Fig. 1). Cytoprotective effects against free radical-induced liver damage were first demonstrated over 25 years ago [50]. Later, strong anti-oxidative and anti-inflammatory effects of PPG have been demonstrated in numerous studies [51–57]. Strong anti-inflammatory activities of purpurogallin have been demonstrated by downregulation of a variety of inflammatory genes (*COX*-2, *TNF*-$\alpha$, *iNOS*, *IL-1*$\beta$, or *IL-6*) thus decreasing free radical-induced tissue damage [55–57]. In correspondence, we observed a prominent downregulation of these and other inflammatory genes by PPG [58]. Previously, a suppression of the translocation of the p65 NFκB subunit into the nucleus and the degradation of IκB had been shown to be the molecular mechanisms underlying the purpurogallin-mediated attenuation of inflammation in BV2 cells [56]. The anti-inflammatory properties are in correspondence to effects of purpurogallin against cancer [59–61] and also to cardio- and hepatoprotective effects [50, 53, 62]. In addition, proapoptotic effects for purpurogallin as another underlying pathway leading to anti-proliferative and anti-cancer effects have been reported [63].

There exist only few studies on the bioactivity of other low molecular benzotropolone derivatives. In 2004, Sang and coworkers performed a structure-activity relationship (SAR) analysis of 18 benzotropolone derivatives including the major theaflavins, theaflavates, theaflavic acids, and PPG among other BZ molecules. They found the benzotropolone moiety to be essential for anti-inflammatory activities and cytotoxic effects against three different cancer cell lines [54]. In a recent study, we looked for relationships between molecular structure and biological activity of different benzotropolone derivatives affecting viability and inflammation. We were intrigued by the fact that the benzotropolone core structure with only one ketone group and three hydroxyl groups (3, 4, 6-trihydroxy-5*H*-benzo[7]-annulen-5-one; see 1 in Fig. 1) showed comparable anti-inflammatory effects to ibuprofen in a mouse carrageenan-induced paw edema model. Anti-proliferative effects against U-937 and Caco-2 cells correlated to a significant downregulation of *COX-2* potentially through inhibition of AP-1 and/or NF κB signaling [58]. Our observations are in line with reports of anti-cancer effects of purpurogallin [59–61, 63] but also some other benzotropolone derivatives [54] against a variety of different cancer cell lines and further emphasize the therapeutic potential of benzotropolones. Noteworthy, as compared to PPG, we found even stronger anti-inflammatory effects of a new described BZ compound (1-carboxy-3, 4, 6-trihydroxy-5*H*-benzo[7]-annulen-5-one; see m in Fig. 1) which prominently inhibited *COX-2*, *TNF*-$\alpha$, *ICAM-1*, *IL-1*$\beta$, and *IL-8* on the one hand and showed only mild toxicity on the other. Information gained by our group and elsewhere suggest that positioning of functional groups around the benzotropolone moiety drastically affects the bioactivity against proliferation and inflammation. Therapeutic effects of benzotropolones appear to be based on specific interaction with cellular targets in inflammatory pathways thus serving as pharmacological targets. The concept of subtle changes in the molecular structure fundamentally affecting the bioactivity due to a specific interaction with cellular targets were previously found for different stilbene analogs to induce apoptosis in cancer cells [64]. Mechanistic studies point to an interaction of BZs either directly or indirectly with NF κB and/or AP-1 as important transcription factors in the regulation of inflammatory genes [4, 54–57] but certainly further studies are needed.

## Conclusion and Future Directions

The discrepancy between strong bioactivity and health-promoting effects of black tea polyphenols despite their poor bioavailability have been a matter of debate in tea research. Until now, the search for putative metabolites has been unsuccessful and their nature remains unknown. Strong bioactivities exhibited by natural purpurogallin and accumulating evidence for other benzotropolone derivatives have confirmed their role as the pharmacophore of theaflavins. Herein, we speculate the possible generation of secondary metabolites containing the benzotropolone structure by means of in vivo biotransformation such as through gut microbiota or other mechanisms. Thus, benzotropolones derivatives as core molecules of theaflavins and some thearubigins are appealing candidates as missing link explaining the "tea mystery." So far, in vivo detection of BZ derivatives in urine or blood is warranted. Further mechanistic studies on bioavailability, bioaccessibility, and bioactivity are needed to consolidate the role of BZs as bioactive metabolites of black tea providing the foundation for potential therapeutic applications.

### Compliance with Ethical Standards

**Human and Animal Rights and Informed Consent** This article does not contain any studies with human or animal subject performed by any of the authors.

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