Chapter

Contribution of Gut Microbiome to Human Health and the Metabolism or Toxicity of Drugs and Natural Products

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Abstract

Trillions of microorganisms with a complex and diverse community are in the human gastrointestinal tract. Gut microbial genomes have much more genes than human genome, thus having a variety of enzymes for many metabolic activities; therefore, gut microbiota is recognized as an "organ" that has essential functions to human health. There are interactions between host and gut microbiome, and there are correlations between gut microbiome in the healthy state and in certain disease states, such as cancer, liver diseases, diabetes, and obesity. Gut microbiota can produce metabolites from nutrients of dietary sources and from drug metabolisms; these metabolites, for example, short-chain fatty acids (SCFAs), have substantial effects on human health. Drug-microbiome interactions play a crucial role in therapeutic efficiency. Some drugs are able to change compositions of gut microbiota, which can lead to either enhance or reduce therapeutic efficiency. This chapter provides an overview of roles of gut microbiota in human health and diseases and recent research studies on the metabolism or toxicity of drugs and natural products. Since gut bacteria considerably contribute to drug metabolism, research on the influence of gut microbiome on drug candidates (or natural products) should be part of the drug development processes.

Keywords: gut microbiota, gut microbiome, drug-microbiome interactions, drug-microbiota interactions, natural products-microbiome interactions, drug metabolism, drug toxicity, biotransformation, bioconversion

1. Introduction

The human gastrointestinal tract has various microorganisms, and "gut microbiota" has received attentions recently because the microbe population living in human intestine has significant effects to human health. Gut microbiota plays important roles in human, involving in many activities in a host body, for example, metabolism of xenobiotic compounds, immune system, nutrition, inflammation, and behavior. The delivery of prebiotics and probiotics to the human gastrointestinal tract, via dietary products or supplements, is one of the tools for management of microbiota in order to improve host health [1]. Moreover, gut microbiome has interactions with drugs and natural products, producing metabolites, which give

effects on efficacy, metabolism, and toxicity of drugs. Gut microbiota plays a role in the metabolism of drugs and natural products, as well as nutrients in diet or food. The conversion of a dietary soybean isoflavone, daidzein (**1**) or genistein (**2**), to a bioactive compound, *S*-equol (**3**) (**Figure 1**) [2, 3], is a good example for the role of gut bacteria in the production of pharmacologically active agent in human because *S*-equol (**3**) is a potent ligand for estrogen receptor *β* [4]. Daidzein (**1**) is also derived from its corresponding isoflavone glycoside, daidzin (**4**), by *Bifidobacterium*, a representative of major bacterial species of human origin; this bacterium could transform daidzin (**4**) to daidzein (**1**) by cell-associated *β*-glucosidases (**Figure 1**) [5]. Moreover, *O*-desmethylangolensin (**5**) is also found as an intestinal bacterial metabolite of daidzein (**1**) [6, 7].

The transformation of achiral molecule daidzein (**1**) to a chiral molecule equol, which has one chiral center in its molecule, should provide two possible enantiomers of *S*-equol (**3**) and *R*-equol (**3R**) (**Figure 2**). However, gut bacteria selectively gives only *S*-equol (**3**), not *R*-equol (**3R**); this is interesting because only *S*-equol (**3**) has a high affinity to bind with estrogen receptor *β*, while *R*-equol (**3R**) has much less activity [4]. Therefore, *S*-equol (**3**), but not *R*-equol (**3R**), has high affinity for estrogen receptor *β* in human, and *S*-equol (**3**) has more potent estrogenic activity than estradiol [4]. In animal model, although a mixture of the two enantiomers of equol have the ability to inhibit bone loss in ovariectomized mice [8], *S*-equol (**3**) has better inhibitory effects on bone fragility than the racemic mixture containing both *S*-equol (**3**) and *R*-equol (**3R**) [9].

The ability of gut bacteria to selectively produce the correct bioactive isomer of *S*-equol (**3**) needed for human is intriguing. Shimada and co-workers identified enzymes involved in the bioconversion of daidzein (**1**) to *S*-equol (**3**) by the bacterium *Lactococcus* sp. strain 20–92, which was isolated from feces of healthy human [10]. The enzyme daidzein reductase catalyzes the transformation of daidzein (**1**) to (*R*)-dihydrodaidzein (**6**), which is in turn converted to (*S*)-dihydrodaidzein (**7**) by the enzyme dihydrodaidzein racemase (**Figure 2**). The enzyme dihydrodaidzein reductase catalyzes the conversion of (*S*)-dihydrodaidzein (**7**) to *trans*-tetrahydrodaidzein (**8**), which is converted to *S*-equol (**3**) by the enzyme tetrahydrodaidzein reductase [10]. The bioconversion of daidzein (**1**) selectively to *S*-equol (**3**), not *R*-equol (**3R**), by gut bacteria provides human the correct enantiomer for binding with estrogen receptor *β*; this may be host-bacterial mutualism in human intestine. An isoflavone daidzein (**1**) is found in leguminous plants such as soybeans and other

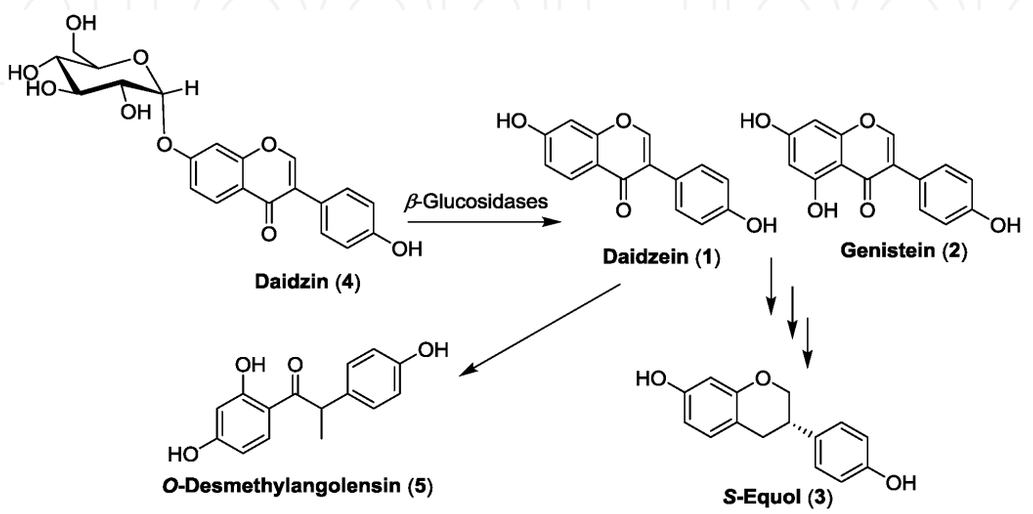


Figure 1.
*Bioconversion of soybean isoflavones, daidzein (**1**), genistein (**2**), and daidzin (**4**), to* S*-equol (**3**) and* O*-desmethylangolensin (**5**) by intestinal bacteria.*

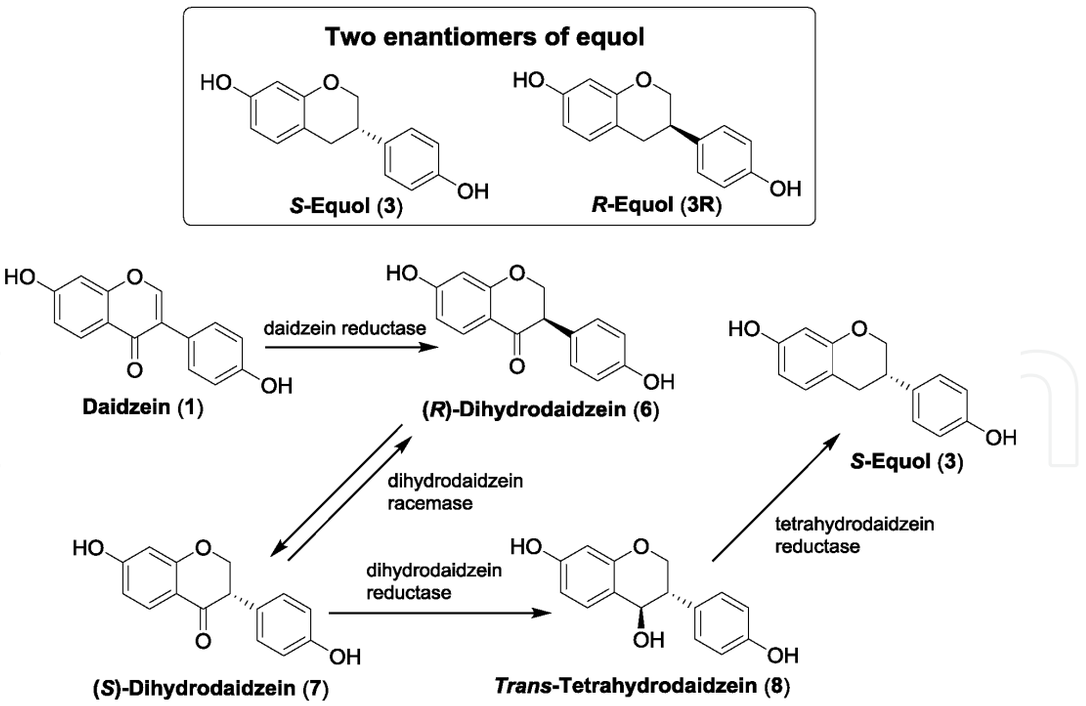


Figure 2.
Structures of two enantiomers of S*-equol (3) and* R*-equol (3R) and the bioconversion of daidzein (1) to* S*-equol (3) by the bacterium* Lactococcus *sp. through the metabolites* (R)*-dihydrodaidzein (6),* (S)*-dihydrodaidzein (7), and* trans*-tetrahydrodaidzein (8).*

legumes, which have been used as food for human since ancient times. Therefore, it is possible that gut bacteria have experienced with daidzein (**1**) long time ago, and their enzymatic evolutions lead to the selective bioconversion of daidzein (**1**) to *S*-equol (**3**), which has biological activity for human. Interestingly, many studies revealed that there is the intestinal microbiota-to-host relationship, i.e., a cross talk, between gut microbiota and human host and interactions between gene products from the microbiome with metabolic systems of human diseases such as obesity and diabetes [11].

The conversion of a dietary soybean isoflavone, daidzein (**1**) or genistein (**2**), to *S*-equol (**3**), by gut bacteria has been known for many years; however, scientists might not be aware of the importance of gut microorganisms in the past. Recently, a number of studies have revealed many essential roles of gut microbiota in human health and diseases. Gut microbiome can transform nutrients and dietary fibers to produce bioactive metabolites, for example, short-chain fatty acids (SCFAs) and nicotinamide, which have a significant impact on human health and diseases. There have been reports on interactions of gut microbiome and compounds, e.g., drugs and natural products, after humans take these compounds as drugs for the treatment of diseases. The metabolites obtained from the metabolism of drugs/natural products by the activities of gut microbiome have either positive or negative effects on therapeutic efficiency. This chapter provides the information of recent studies on the influence of the metabolites produced by gut microbiome on human health and diseases and on the interactions of microbiome and drugs/natural products.

2. Contributions of metabolites produced by gut microbiome to human health and diseases

The human gastrointestinal tract has trillions of microorganisms with a complex and diverse community. Gut microbiome is recognized as an "organ" because gut

microorganisms have metabolic activities similar to an organ and have several essential functions to human health [12]. It is estimated that microbial cells in the human body are 10 times more than human cells and that gut microbiome has 150 times more genes than human genome [13]. Perturbation of gut microbial communities leads to the imbalance of gut microorganisms, by either reducing or increasing particular microbial species or altering the relative abundance of certain microorganisms; this is collectively known as "dysbiosis." Microbial dysbiosis can cause certain diseases such as irritable bowel syndrome, diabetes, cancer, inflammatory bowel diseases, and obesity [14–16]. Gut microorganisms are able to produce many metabolites, which give substantial contributions to human health because they are involved in various physiological processes, i.e., host immunity, cell-to-cell communication, and energy metabolism [17, 18]. The metabolites produced by gut microbiome are linked with human diseases, for example, colorectal cancer [19], depression [20], inflammation and cancer [21], and cardiovascular and metabolic diseases [22, 23]. Among the metabolites produced by gut microbiome, SCFAs considerably play critical roles in human health. Gut microbiome produces acetate (**9**), propionate (**10**), and butyrate (**11**) (**Figure 3**), the respective conjugate bases of acetic acid, propionic acid, and butyric acid; these SCFAs are from saccharolytic fermentation of dietary fibers by gut microorganisms [24]. Butyrate (**11**) from the metabolism of gut microbiome could induce differentiation of colonic regulatory T cells in mice, suggesting that gut microorganisms are substantially involved in immunological homeostasis in the gastrointestinal tract of human [25]. SCFAs produced by gut microbiota are significantly linked with hypertension and kidney diseases [26]. SCFAs are vital fuels for intestinal epithelial cells and can maintain intestinal homeostasis; they are involved in the regulation of gut epithelial cells and immunity that is relevant to inflammatory bowel diseases [27, 28]. SCFAs are able to activate G-protein-coupled receptor, for example, GPR43, which has a role in intestinal inflammatory diseases, i.e., inflammatory bowel diseases [29]. Moreover, SCFAs produced by gut microbiome are energy source for colonocytes and can inhibit histone deacetylases, the enzymes catalyzing the removal of acetyl groups from the lysine residue of histone [30]. Recent study revealed that butyrate (**11**) from the metabolism of gut microbiome could promote histone crotonylation in colon epithelial cells and that the reduction of the gut microbiota leads to many changes in histone crotonylation in the colon [31].

Recent study revealed that SCFAs produced by gut microbiome had relationships with metabolic diseases [32]. The level increase of butyrate (**11**) (**Figure 3**) by gut microbiome can improve insulin response after an oral glucose tolerance test; moreover, the defects in the production or absorption of propionate (**10**) led to an increased risk of type 2 diabetes [32]. Previous study also demonstrated that type 2 diabetes is linked with changes in the composition of gut microbiome because the profile of gut microorganisms in human with type 2 diabetes is different from that without type 2 diabetes (a control group) [33]. SCFAs are known to have a significant impact on the energy homeostasis, i.e., controlling the energy metabolism; therefore, modulation of SCFAs could be a nutritional target to prevent diseases associated with metabolism disorders, for example, type 2 diabetes and obesity [34]. Gut microbiome is also linked with food allergy in human, and changes in the

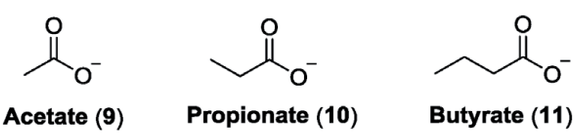


Figure 3.
*Structures of SCFAs including acetate (**9**), propionate (**10**), and butyrate (**11**).*

population and composition of gut microbiota might cause food allergy [35], and the use of gut microbiome is a potential innovative strategy to prevent food allergy in human [36]. In an animal model, certain gut bacteria, e.g., *Clostridia* species, might be useful for prevention or therapy of food allergy [37]. Recent investigation led by Nagler showed that butyrate (**11**) (**Figure 3**) produced by the gut bacterium, *Anaerostipes caccae*, could contribute to the prevention of milk allergy in children [38]. Germ-free mice colonized with bacteria in feces of healthy infants can protect mice against milk allergy, while those colonized with bacteria in feces of milk allergic infants could not protect mice from milk allergy; this result indicates that gut microbiotas are involved in milk allergy. Detailed analysis revealed that compositions of gut bacteria in healthy infants were different from milk allergic infants, and the gut bacterium, *A. caccae*, was the key agent to protect against an allergic response to food [38]. *A. caccae* is a saccharolytic intestinal bacterium producing butyrate (**11**) [39]. It is known that butyrate (**11**) is a key energy source for colonic epithelial cells, regulating energy metabolism and autophagy in the mammalian colon [40]. Therefore, butyrate (**11**) is likely to be the key metabolite responsible for the protection of milk allergy [38]. An independent study revealed that a dietary supplemented with the bacterium *Lactobacillus rhamnosus* could promote tolerance in infants with cow's milk allergy by enrichment of butyrate-producing bacterial strains [41]. The increased levels of butyrate (**11**) in feces of infants who received the supplement with *L. rhamnosus* were observed in the most tolerant infants against milk allergy [41].

Nicotinamide (**12**) is an amide derivative of vitamin B3 or niacin or nicotinic acid (**13**) (**Figure 4**) and is a substrate for nicotinamide adenine dinucleotide (NAD), a coenzyme in many important enzymatic oxidation–reduction reactions, for example, electron transport chain, citric acid cycle, and glycolysis. Nicotinamide (**12**) is known to have a role in neuronal systems in the central nervous system, thus implicating in neuronal death and neuroprotection [42]. Recent study led by Elinav revealed that nicotinamide (**12**) produced by the gut bacterium, *Akkermansia muciniphila*, significantly protected the progression of the neurodegenerative disease, amyotrophic lateral sclerosis (ALS) [43]. The experiment demonstrated that removal of gut microorganisms by treating mice with antibiotics could promote the ALS symptoms in mice, indicating that gut microbiome modulated the progress of ALS disease [43]. The study showed that the species of gut bacteria in healthy human were different from that in ALS patients; *A. muciniphila* was abundant in healthy people, while *Ruminococcus torques* and *Parabacteroides distasonis* were relatively abundant in ALS patients. Remarkably, transplantation of gut bacteria from human gut to germ-free mice revealed that the gut bacterium *A. muciniphila* improved the ALS symptoms, while the gut bacteria *R. torques* and *P. distasonis* worsened the ALS symptoms [43]. Detailed analysis found that the gut bacterium *A. muciniphila* provided nicotinamide (**12**) as a bioactive metabolite that improves the ALS symptoms. Indeed, a direct injection of nicotinamide (**12**) into mice with ALS could improve a motor-neuron function. The study in 37 patients

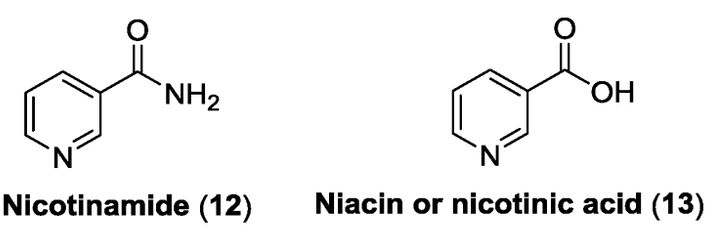


Figure 4.
*Structures of nicotinamide (**12**) and niacin or nicotinic acid (**13**).*

with ALS revealed that the levels of nicotinamide (**12**) in cerebrospinal fluid of ALS patients were lower than that in people without ALS. Moreover, analysis of microbial genes involved in nicotinamide synthesis in feces of ALS patients revealed that people with ALS had less number of the genes for nicotinamide synthesis; these genes were mainly from the gut bacterium *A. muciniphila*. Therefore, it is likely that ALS patients might have less abundance of *A. muciniphila* in their gastrointestinal tract [43]. This work suggests that gut microbiome has a significant link with human disease pathophysiology and that there is an opportunity to use microbial therapeutic targets for certain diseases. Indeed, a clinical trial on human using the gut bacterium, *A. muciniphila*, in overweight and obese insulin-resistant volunteers demonstrated that the gut bacterium could reduce insulin resistance indices and could lower the levels of circulating insulin and blood cholesterol, thus improving the profile of blood lipid and insulin sensitivity [44]. This microbial therapeutic approach is safe and may be applied for the treatment of overweight or obese insulin-resistant people.

It is known that gut microbiota is significantly associated with autism spectrum disorder, a form of mental disorder with difficulties in social communication and interaction [45]. Intriguingly, a recent study led by Sharon and Mazmanian revealed that gut microbiota could produce neuroactive metabolites which contribute to the pathophysiology of autism spectrum disorder, thus regulating behaviors in mice [46]. The experiment showed that germ-free mice receiving gut microbiota from human donors with autism spectrum disorder could induce autistic behaviors in mice. The metabolites produced by gut bacteria, 5-aminovaleric acid (**14**) and taurine (**15**) (**Figure 5**), were found to modulate behaviors related to autism spectrum disorder. Both 5-aminovaleric acid (**14**) and taurine (**15**) are $GABA_A$ receptor agonists [47, 48]. Levels of 5-aminovaleric acid (**14**) in mice with autism spectrum disorder were significantly lower than that in the control mice, while levels of taurine (**15**) in mice with autism spectrum disorder were ca. 50% less than the control group [46]. Administration of 5-aminovaleric acid (**14**) and taurine (**15**) to mice with autism spectrum disorder could improve repetitive and social behaviors, i.e., modulating neuronal excitability in mice brain and improving behavioral abnormalities [46]. This finding suggests that autism spectrum disorder is also related to the influence of gut microbiota; therefore microbiome interventions using fecal microbiota transplantation, as well as supplementation with metabolites produced by gut microorganisms or with probiotics, may improve the quality of life for people with autism spectrum disorder.

Gut microbiome substantially contributes to human health and diseases, and the metabolites produced by gut microbiome mentioned earlier underscore the importance of gut microorganisms in health and certain diseases in human. Health and diseases of individuals partly rely on the conditions of gut microbiome whether they have healthy gut microbiota or unhealthy ones. Gut microbiota is therefore considered as a "hidden" or "forgotten" human organ [12], involving in pathology of Alzheimer's disease [49], endocrine organ involving metabolic diseases [50], and

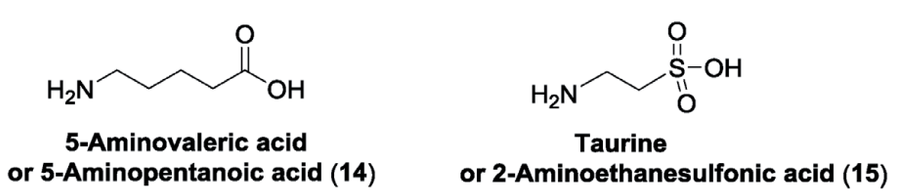


Figure 5.
*Structures of 5-aminovaleric acid or 5-aminopentanoic acid (**14**) and taurine or 2-aminoethanesulfonic acid (**15**).*

chronic gastrointestinal disease [51]. Moreover, gut microbiota is also considered as an "invisible" organ that controls and manipulates the function of drugs [52]. The imbalance of gut microbiota, or known as dysbiosis, leads to unhealthy conditions for human or even causes certain diseases. Therefore, the use of gut microbiota as a therapeutic target for treatments of human diseases is an emerging approach for many diseases, for example, Parkinson's disease [53], cardiovascular disease [54], metabolic disorders [55], hepatocellular carcinoma [56], nonalcoholic fatty liver disease [57], food allergy [58], and heart failure [59]. Supplementation with probiotics or with health-promoting bacteria is a possible therapeutic method and may widely be used in the near future. Fecal microbiota transplantation or supplementation with metabolites from gut microorganism needs more clinical studies; the two approaches will be a challenging research on gut microbiota in the near future.

3. Interactions of gut microbiome and drugs and/or natural products

It is estimated that a total mass of bacteria in the human body is around 0.2 kg (for people with a weight of 70 kg) and that the densities of commensal microorganisms in the human gastrointestinal tract ranged from 10^8 to 10^{11} bacterial cells/g [60]. Oral administration of drugs delivers drugs to the gastrointestinal tract that contains high densities of gut microorganisms, which could encode 150-fold more genes than those of the human genome [61]; therefore, gut microbes are able to encode many enzymes with drug-metabolizing potential [62]. Gut microbiota is recognized as an "invisible organ" responsible for controlling drug functions and modulation of drug metabolism processes [52]. Normally, antibiotic drugs give direct effects toward microorganisms in the human gastrointestinal tract, providing either negative or positive (beneficial) effects to the composition of gut microbiota [63]. However, intestinal microbiota have many important roles in maintenance of human health; therefore, perturbation of gut microbiome by antibiotics could give negative impact to human, for example, loss of colonization resistance that can prevent invading microorganisms colonizing in the human gastrointestinal tract [64]. In addition to antibiotic drugs, a recent study led by Typas demonstrated that nonantibiotic drugs also gave extensive impact on human gut bacteria because around 24% of 1197 drugs showed antibacterial activity toward at least one strain of gut bacteria [65]. This is considered as "antibiotic-like side effects" of nonantibiotic drugs, which could potentially promote antibiotic resistance that is one of the major public health problems worldwide. This finding provides essential information for drug discovery research, i.e., addressing a potential new side effect of drugs and repurposing of nonantibiotic drugs as antibacterial agents.

The next sections will highlight the interactions of gut microbiome, especially the chemistry of the drug metabolites produced by gut microorganisms, toward certain drugs and natural products. The metabolism of drugs or natural products by gut microbiome could lead to the production of bioactive metabolites, which have either beneficial effects or negative properties (i.e., reducing efficacy of drugs or natural products). The study on the interactions of gut microbiota and drugs or natural products as part of drug development process is discussed in the next sections.

3.1 Interactions of gut microbiome and commonly used drugs

Once drugs enter the human gastrointestinal tract, they encounter trillions of microorganisms, which are able to encode 150-fold more genes than human genome [61]. A number of enzymes encoded by gut microbial genes catalyze the

biotransformation of drugs, producing bioactive metabolites, which have effects on human health [60]. Advances in liquid chromatography-mass spectrometry (LC–MS) technology allow the identification of the metabolites produced by gut microbiome, as well as detailed study of pharmacokinetics of drugs and their metabolites, while genome sequencing substantially assists the identification of genes encoding enzymes in gut microorganisms. Zimmermann and co-workers investigated the drug metabolism of an antiviral nucleoside drug, brivudine (**16**), which is used for the treatment of herpes zoster virus; the study was performed using mice inoculated with mutant microbiota [66]. It was found that the bioconversion of brivudine (**16**) to bromovinyluracil (**18**) (or 5-(*E*)-(2-bromovinyl)uracil) was achieved by enzymes from both mammalian cells and gut microbial communities isolated from mice, suggesting that both host and microbiota are capable of such biotransformation (**Figure 6**). Previously, intestinal anaerobic bacteria were found to convert another antiviral drug, sorivudine (**17**), to bromovinyluracil (**18**) (**Figure 6**) [67].

Gut bacteria, *Bacteroides thetaiotaomicron* and *B. ovatus*, were the major species having the highest metabolic activity to convert brivudine (**16**) to bromovinyluracil (**18**) [66]. Comparison of serum kinetics of brivudine (**16**) and bromovinyluracil (**18**) in conventional (a control with bacteria) and germ-free mice after feeding with the drug brivudine (**16**) suggested that intestinal bacteria contributed to the amount of bromovinyluracil (**18**) in serum because the level of bromovinyluracil (**18**) in conventional mice serum was five times higher than that of germ-free mice [66]. The gene, *bt4554*, encoding the enzyme purine nucleoside phosphorylase necessary for the metabolism of brivudine (**16**) is present in *B. ovatus* and conserved in the bacterial phylum *Bacteroidetes*; the expression of the gene *bt4554* is a rate-limiting step [66]. The gut bacterium, *B. thetaiotaomicron*, was found to completely metabolize the drug brivudine (**16**) to bromovinyluracil (**18**), which is absorbed from both the cecum and colon. This study was also able to predict the levels in serum and sources of the metabolite bromovinyluracil (**18**) derived from a drug sorivudine (**17**) (**Figure 6**) [66].

Zimmermann and co-workers also used clonazepam (**19**) (**Figure 7**), an anticonvulsant and antianxiety drug, as a model [66]; the metabolism of this drug in rats gave metabolic products through nitroreduction, oxidation, glucuronidation, and enterohepatic cycling [68]. After an oral administration of a drug clonazepam (**19**) to mice, 7-NH_2-clonazepam (**20**) and 7-NH_2-3-OH-clonazepam (**21**) were found as major metabolites in serum of the conventional mice (**Figure 7**). The host-microbiome pharmacokinetic model revealed that 7-NH_2-clonazepam (**20**) in serum was substantially from a microbial contribution. Experiments also revealed that intestinal microbes could convert glucuronyl-3-OH-clonazepam (**23**) to 3-OH-clonazepam (**22**), which in turn transformed to 7-NH_2-3-OH-clonazepam (**21**) by microbial reduction (**Figure 7**) [66]. The study established a pharmacokinetic model that can predict microbiome or host (human) contributions to drug

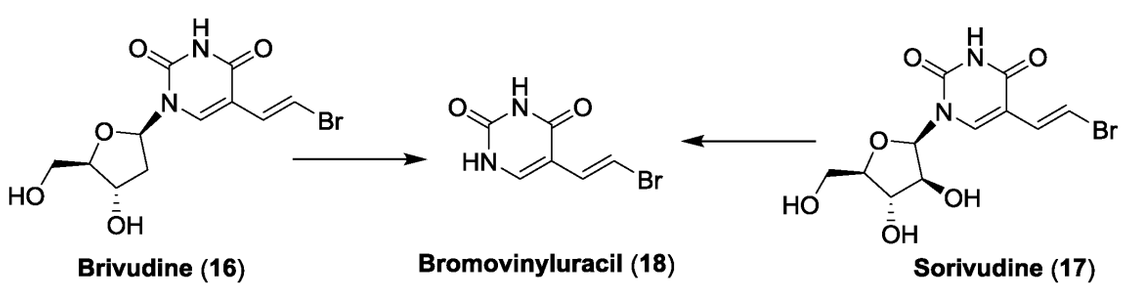


Figure 6.
*Biotransformation of antiviral drugs brivudine (**16**) and sorivudine (**17**) to bromovinyluracil (**18**) by gut bacteria.*

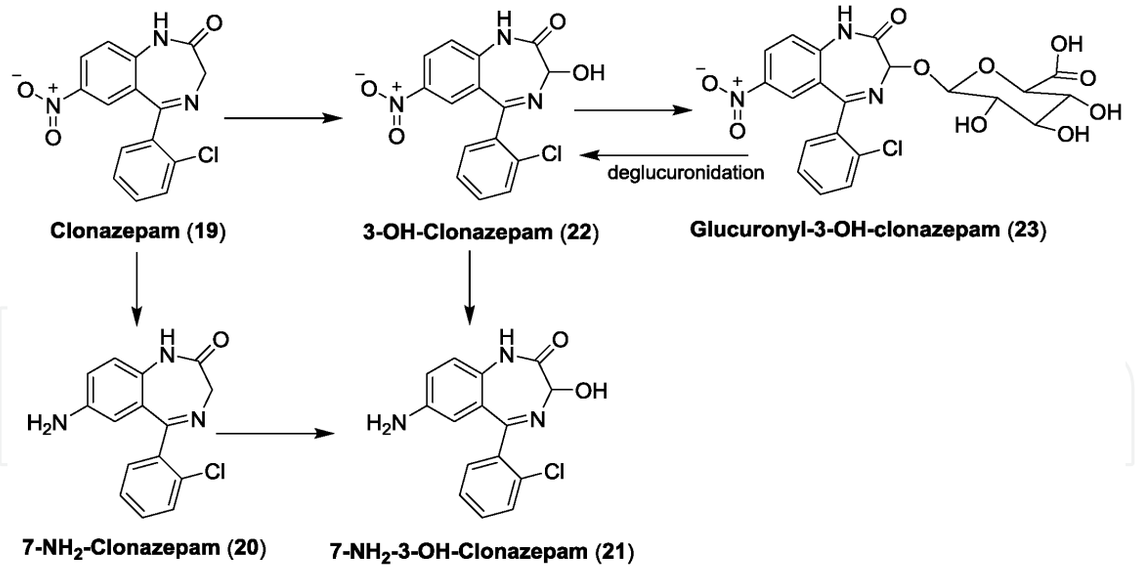


Figure 7.
*Biotransformation of clonazepam (**19**) to the metabolites **20–23** by human intestinal microbes.*

metabolism, e.g., the ability to distinguish drug-metabolizing activity by human or gut bacteria [66]. This research model is particularly useful for the study on drug metabolism in an animal model.

Gut microbiome has potential ability to metabolite many drugs, thus affecting the therapeutic efficacy due to lower concentrations of drugs. The study on the drug metabolism of 271 commonly used drugs by gut bacteria revealed that, after incubation of drugs with gut bacteria, the levels of 176 drugs (accounting for two thirds of 271 drugs) were significantly reduced, indicating that these drugs were metabolized by gut bacteria [62]. Intriguingly, each bacterial strain (from 76 human gut bacterial strains) could metabolize up to 11–95 drugs [62]. This result suggests that, when designing the drug molecules, the drug metabolism by gut microbes should be seriously considered, particularly the drugs delivered by an oral administration. Therefore, the action of gut microbiome toward individual drug candidates should also be evaluated during the drug development process. Untargeted metabolomics analysis is used to identify products derived from drug metabolism by gut bacteria, and it could properly identify the metabolites from microbial metabolism of drugs [62]. Some drugs, for example, paliperidone, sulfasalazine, and pantoprazole, were previously investigated for their metabolism by gut microbes [69]. Detailed analysis by high-resolution mass spectrometry (HRMS) revealed that drugs with an acetyl ester or an alkene functional group, such as norethisterone acetate (**24**), drospirenone (**25**), and roxatidine acetate (**26**), were metabolized through either deacetylation (removing C_2H_2O) or hydrogenation (adding H_2) by gut bacteria (**Figure 8**) [62]. Gut bacteria metabolized drugs with aliphatic hydroxyl or amine functional group such as dasatinib (**27**), fluphenazine (**28**), and primaquine (**29**) through propionylation (adding C_3H_4O), giving their corresponding *O*- or *N*-propionyl products **30**, **31**, and **32**, respectively (**Figure 8**). The HRMS data clearly indicated the mass difference of 56.026 unit of a propionyl group between the drug and its corresponding derivative [62].

Zimmermann and co-workers investigated the metabolism of drug in mice model and in human gut microbial communities using a corticosteroid drug, dexamethasone (**33**), as a model (**Figure 9**) [62]. It is known that this class of drug is metabolized by the bacterium *Clostridium scindens* through the side-chain cleavage, known as the desmolytic activity, to produce the active androgen form of the drug, dexamethasone-desmo (**34**) (**Figure 9**) [70, 71]. Levels of dexamethasone-desmo

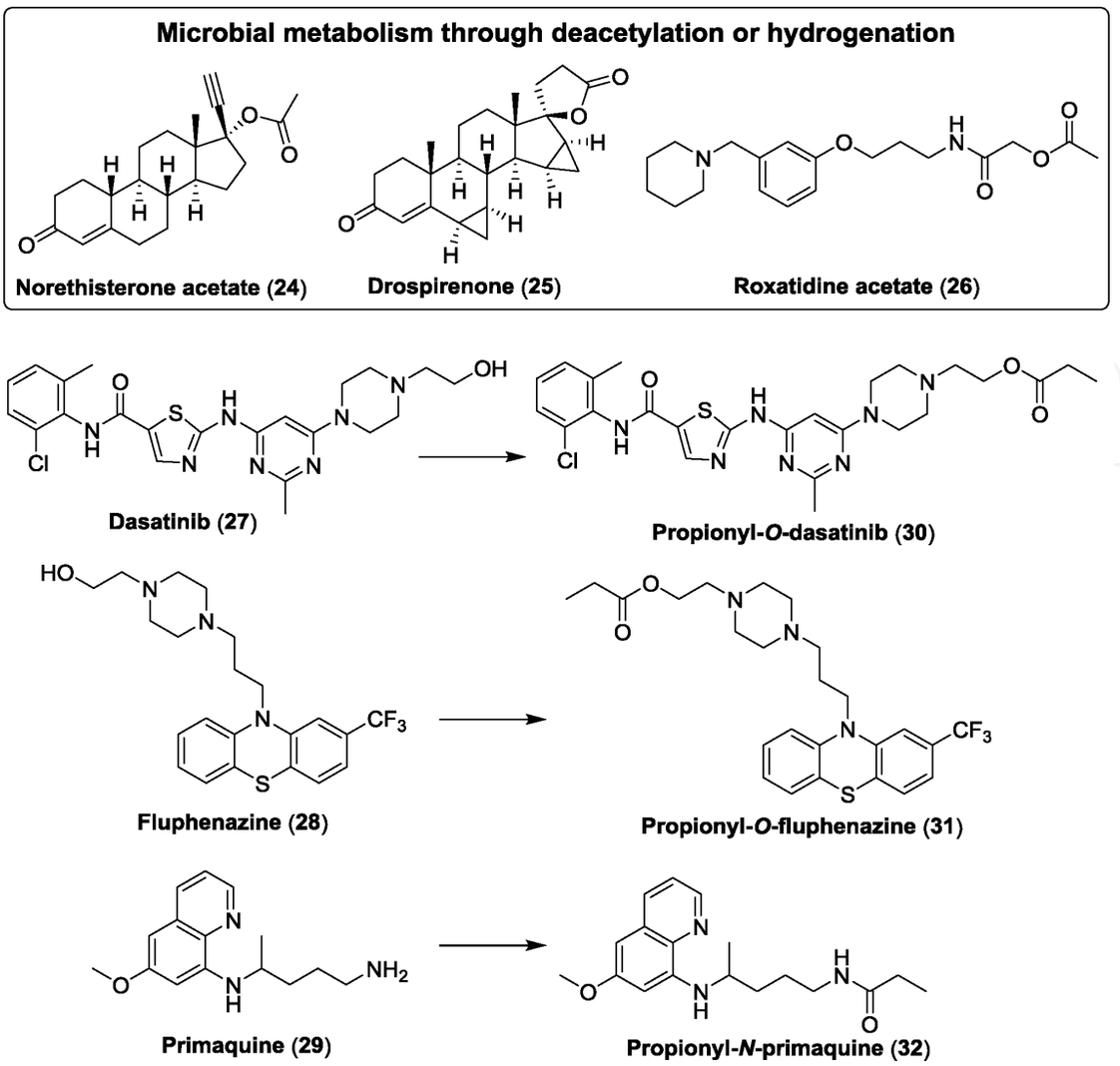


Figure 8.
*Structures of the drugs, norethisterone acetate (**24**), drospirenone (**25**), and roxatidine acetate (**26**) and biotransformation of dasatinib (**27**), fluphenazine (**28**), and primaquine (**29**) to their corresponding products **30**, **31**, and **32**, respectively, by gut bacteria.*

(**34**) were measured after an oral administration of dexamethasone (**33**) to germ-free mice and to mice that have only one bacterial species of *C. scindens*, technically known as gnotobiotic mice ($GN^{C.\ scindens}$). Although dexamethasone (**33**) was found in the cecum of germ-free mice and gnotobiotic mice, the levels of the drug were significantly reduced in gnotobiotic mice, suggesting that the bacterium *C. scindens* associated in these mice is involved in the drug metabolism. Accordingly, levels of the androgen form of the drug, dexamethasone-desmo (**34**), which are derived from the metabolism of dexamethasone (**33**), were higher in both serum and cecum of gnotobiotic mice than that of germ-free mice [62]. Moreover, similar corticosteroid drugs, i.e., prednisone (**35**), prednisolone (**36**), cortisone (**37**), and cortisol (**38**), were also metabolized by the intestinal bacterium *C. scindens* through the desmolytic activity, giving the metabolite products of prednisone-desmo (**39**), prednisolone-desmo (**40**), cortisone-desmo (**41**), and cortisol-desmo (**42**), respectively (**Figure 9**). However, when incubating the drug dexamethasone (**33**) with gut bacterial community isolated from 28 healthy human participants under anaerobic condition, the drug-metabolizing activity had considerable interpersonal variation as suggested by level variations of the drug metabolite, dexamethasone-desmo (**34**) [62]. This result implies that dexamethasone (**33**) is also metabolized by other gut bacterial species, not only *C. scindens*.

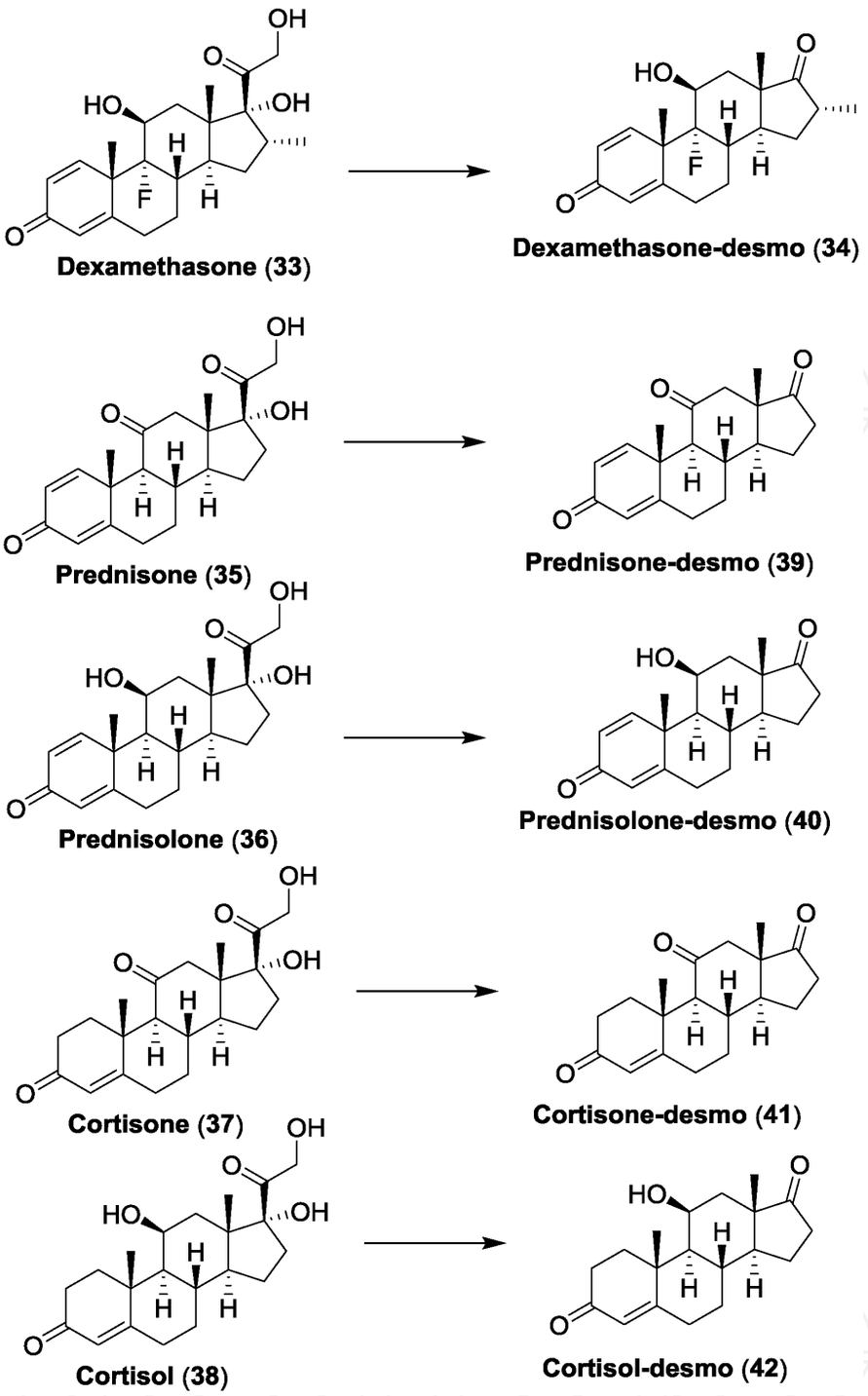


Figure 9.
Gut bacterial metabolism of corticosteroid drugs, dexamethasone (33), prednisone (35), prednisolone (36), cortisone (37), and cortisol (38), to their respective products 34, 39, 40, 41, and 42 via the desmolytic activity.

Systematic identification of drug-metabolizing genes encoded by gut bacteria provides the mechanistic insights into drug metabolism in human [62]. Genes of the gut bacterium *Bacteroides thetaiotaomicron* were cloned into *Escherichia coli*, leading to the identification of new 16 gene products, which were able to metabolite 18 drugs to 41 different metabolites [62]. Certain gene products have specificity and cross-activity, and gene deletion and complementation techniques revealed the mechanisms of individual gene products. For instance, the *bt2068* gene encodes the enzyme that could reduce (adding H_2) norethisterone acetate (**24**) (**Figure 8**), as well as other similar steroid drugs such as levonorgestrel and progesterone, while the *bt2367* gene encodes acyltransferase that converts

the drug pericyazine (**43**) to both acetyl- and propionyl-pericyazine products, e.g., acetyl-*O*-pericyazine (**44**) and propionyl-*O*-pericyazine (**45**), respectively (**Figure 10**) [62]. It is known that the metabolism products of a drug diltiazem (**46**) are *N*-desmethyldiltiazem (**47**), *N*,*N*-didesmethyldiltiazem (**48**), *O*-desmethyldiltiazem (**49**), *N*,*O*-didesmethyldiltiazem (**50**), desacetyldiltiazem (**51**), desacetyl-*N*-desmethyldiltiazem (**52**), desacetyl-*N*,*N*-didesmethyldiltiazem (**53**), desacetyl-*O*-desmethyldiltiazem (**54**), and desacetyl-*N*,*O*-didesmethyldiltiazem (**55**) (**Figure 10**) [72]. The gene *bt4096* in gut bacteria is responsible for the deacetylation of diltiazem (**46**) and its metabolites **47**–**50** to give their corresponding deacetylated products **51**–**55**, respectively (**Figure 10**) [62]. This study suggests that gut bacteria substantially contribute to drug metabolism in the human gastrointestinal tract, and the metabolism of drug candidates by gut microbiome should be studied as a part of the drug development processes.

The drug metabolism by gut microbiome can give negative effects to drug efficacy, thus leading to the decrease in efficiency and potency of certain drugs. L-dopa or levodopa (**56**) (**Figure 11**) is the first-line drug for the treatment of Parkinson's disease; the metabolism of this drug by gut microbiome provides negative effects for Parkinson's patients. The drug L-dopa (**56**) can cross the blood–brain barrier, entering the central nervous system and then transforming to a neurotransmitter,

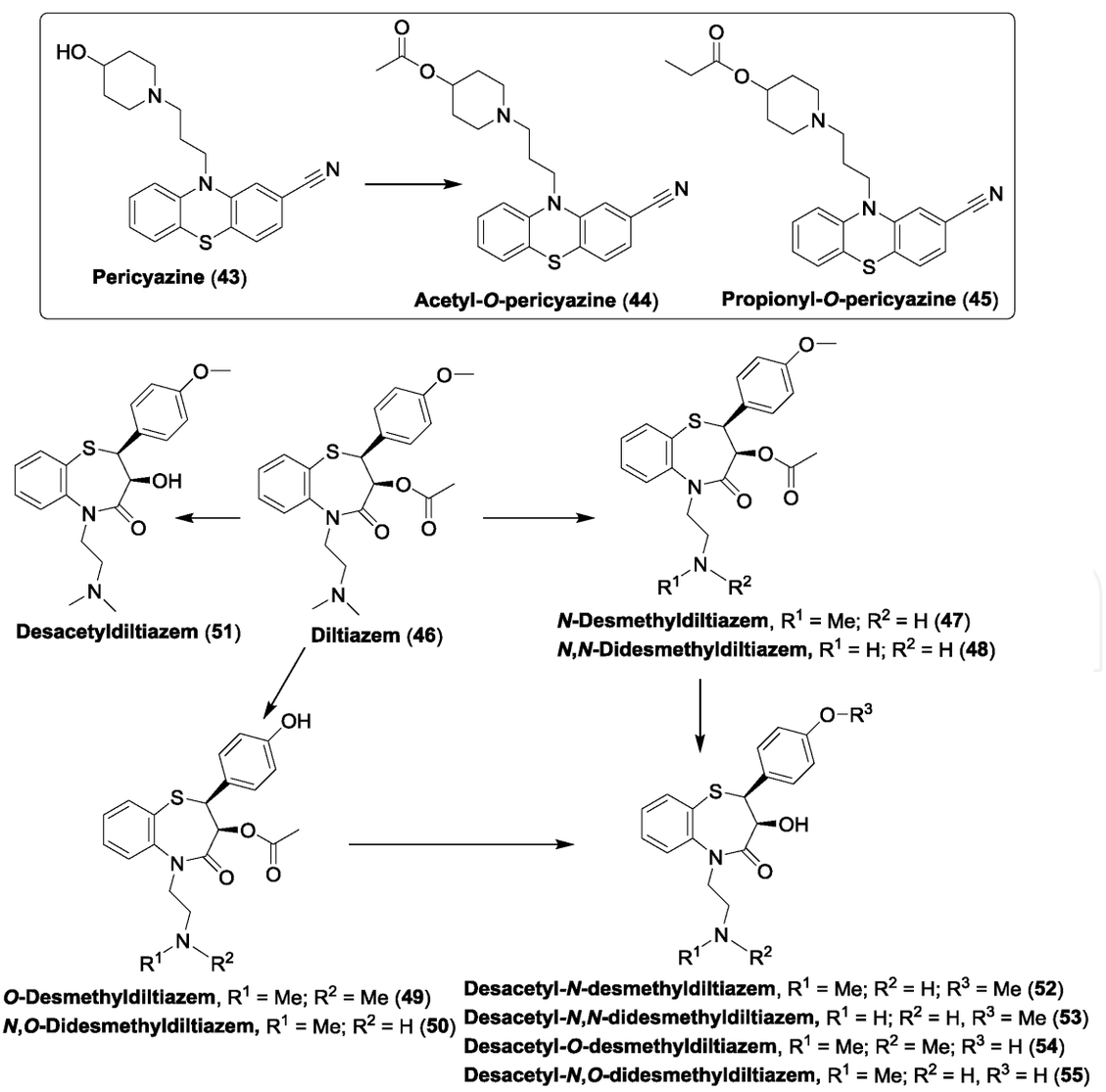


Figure 10.
*Metabolism of pericyazine (**43**) to acetyl-O-pericyazine (**44**) and propionyl-O-pericyazine (**45**) and metabolism of diltiazem (**46**) to the metabolite products **47**–**55** by gut bacteria.*

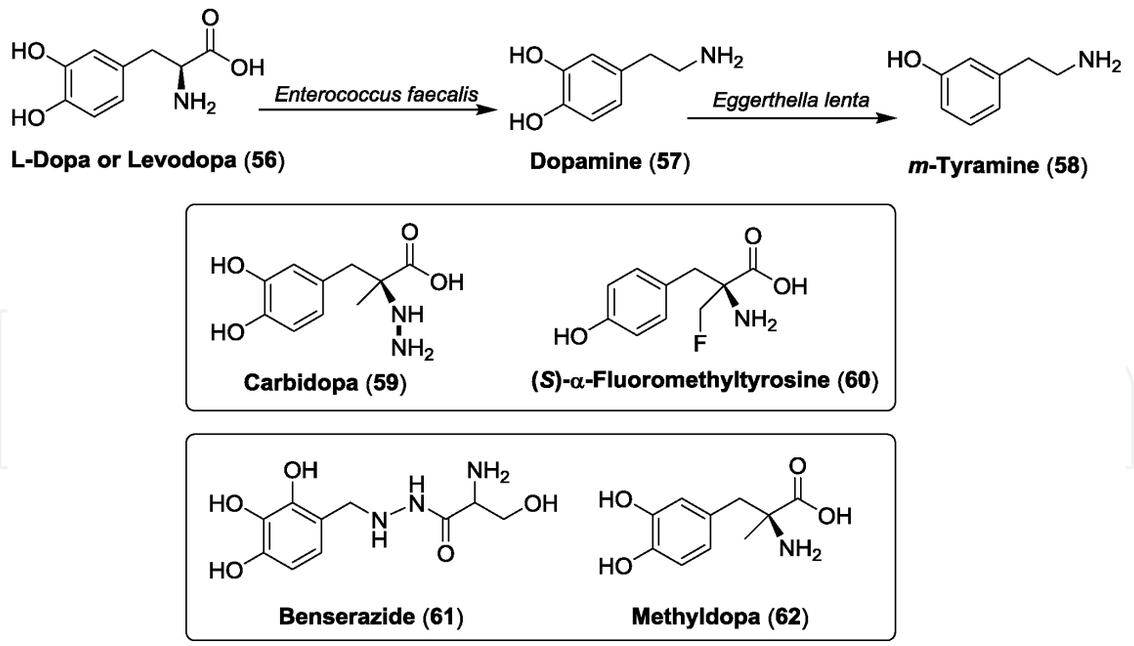


Figure 11.
*Bioconversion of L-dopa (**56**) to dopamine (**57**) and* m*-tyramine (**58**) by gut bacteria and structures of inhibitors of amino acid decarboxylases, carbidopa (**59**),* (S)*-α-fluoromethyltyrosine (**60**), benserazide (**61**), and methyldopa (**62**).*

dopamine (**57**), by the enzyme pyridoxal phosphate L-amino acid decarboxylase (**Figure 11**). It is known that intestinal microflora (gut microbiome) can metabolite L-dopa (**56**) [73] and that the formation of *m*-tyramine (**58**) from L-dopa (**56**) is a side effect of this drug for parkinsonism (**Figure 11**) [74]. A neurotransmitter, dopamine (**57**), is the only active agent needed for the treatment of parkinsonism, and it should be formed from L-dopa (**56**) at the central nervous system after L-dopa (**56**) crossing the blood–brain barrier. However, the generation of dopamine (**57**) from the drug L-dopa (**56**) can occur at the human gastrointestinal tract (known as peripheral metabolism), not at the central nervous system, thus giving undesirable side effects. To prevent this peripheral metabolism, an inhibitor of pyridoxal phosphate L-amino acid decarboxylase, carbidopa (**59**) (**Figure 11**), is coadministered with the drug L-dopa (**56**). It is known for many years that microorganisms can decarboxylate L-dopa (**56**) to dopamine (**57**), which in turn undergoes the dehydroxylation reaction to give *m*-tyramine (**58**) [75]. The treatment of L-dopa (**56**) is improved when patients receive broad-spectrum antibiotics, which suppress the growth of gut bacteria, indicating that gut bacteria are involved in the decrease of therapeutic efficiency of L-dopa (**56**) [76]. Therefore, gut microbiome can potentially reduce the drug efficacy of L-dopa (**56**) through their metabolic activities toward the drug.

Recent study led by Prof. Balskus revealed that the gut bacterium *Enterococcus faecalis* has the *tyrDC* gene encoding the enzyme tyrosine decarboxylase that is able to decarboxylate both L-dopa (**56**) and an amino acid, tyrosine [77]. Moreover, the gut bacterium *Eggerthella lenta* has the *dadh* gene encoding a molybdenum cofactor-dependent dopamine dehydroxylase, which is the enzyme responsible for the dehydroxylation of dopamine (**57**) to *m*-tyramine (**58**) (**Figure 11**). The metabolism of L-dopa (**56**) and dopamine (**57**) in complex gut microbiotas of Parkinson's patients is dependent on the *tyrDC* and *dadh* genes [77]. The study demonstrated that carbidopa (**59**), an inhibitor of pyridoxal phosphate L-amino acid decarboxylase, failed to prevent L-dopa (**56**) metabolism in complex gut microbiotas of Parkinson's patients [77]. However, another inhibitor, (*S*)-α-fluoromethyltyrosine

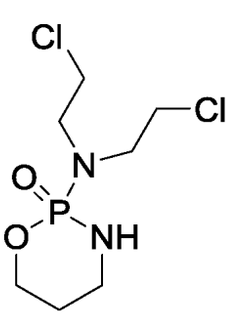
(**60**) (**Figure 11**), could prevent the decarboxylation of L-dopa (**56**) that is from the metabolic activities of both the bacterium *E. faecalis* and complex gut microbiotas of Parkinson's patients [77]. In a mouse model, levels of the drug L-dopa (**56**) increased in serum when coadministered (*S*)-α-fluoromethyltyrosine (**60**) with L-dopa (**56**) to mice colonized with the gut bacterium *E. faecalis* [77]. An independent study led by Prof. Aidy also identified the *tdc* gene responsible for tyrosine decarboxylases in the gut bacterium *E. faecium*; bacterial tyrosine decarboxylases efficiently convert the drug L-dopa (**56**) to dopamine (**57**) [78]. Aidy and co-workers also found that carbidopa (**59**) in L-dopa (**56**) combination therapy did not inhibit the activities of decarboxylase enzymes in gut bacteria, *E. faecalis* and *E. faecium* [78]. Moreover, other decarboxylase inhibitors, benserazide (**61**) and methyldopa (**62**) (**Figure 11**), also failed to inhibit the decarboxylase activity of gut bacteria toward the drug L-dopa (**56**). These studies demonstrated gut microbiota significantly reduced the levels of the drug L-dopa (**56**) in a body, thus contributing to the higher dosages required for the Parkinson's patients that have gut microbiome with high metabolism toward the drug L-dopa (**56**). Variations in gut microbiota among Parkinson's patients might contribute to the different responses, i.e., harmful side effects and decreased efficacy, to the drug L-dopa (**56**). Therefore, gut microbiota plays a critical role in the drug metabolism and considerably contributes to treatment outcomes of this drug.

Gut microbiota can improve therapeutic efficiency of certain drugs for particular treatments. Cancer immunotherapy is relatively new for cancer treatment using human immune system to control and eradicate cancer cells, and it is more precise and personalized, thus providing more effectiveness with fewer side effects than other cancer treatments. Gut microbiota was found to play a role in cancer immunotherapy targeting CTLA-4, a protein receptor downregulating the immune system, because anticancer effects of CTLA-4 blockade were found to depend on gut bacteria of *Bacteroides* species, e.g., *Bacteroides thetaiotaomicron* or *B. fragilis* [79]. The study demonstrated that germ-free mice did not show the response to CTLA blockade, thus defecting an anticancer property of the drug. Indeed, this drug deficiency could be improved by gavage with the gut bacterium *B. fragilis* through immunization with the bacterium polysaccharides or by adoptive transfer of *B. fragilis*-specific T cells. Therefore, this research study demonstrates that the gut bacterium could help patients treated with a monoclonal antibody drug for the treatment of cancer targeting CTLA-4 [79].

Gut microbiome also improves therapeutic effect of a cancer immunotherapy targeting immune checkpoint inhibitor via the PD-1/PD-L1 pathway [59]. Antibiotics are found to give negative effects for patients treated with cancer immunotherapies as they inhibit the efficacy of immune checkpoint inhibitor drug that targets the programmed cell death receptor of the PD-1/PD-L1 pathway [59]. Moreover, suppression of growth of gut bacteria by antibiotic drugs leads to the decrease of drug efficacy, suggesting that gut microorganisms are important for this cancer therapy. The study demonstrated that gut microbiota provided significant effects on cancer immunotherapies targeting the PD-1/PD-L1 interaction because there was substantial association between commensal microorganisms and therapeutic response of anticancer drug that inhibits the activity of PD-1 and PD-L1 immune checkpoint proteins [80]. Gut bacteria including *Collinsella aerofaciens*, *Enterococcus faecium*, and *Bifidobacterium longum* were found to be associated with the improvement of this cancer immunotherapy. Intriguingly, reconstitution of germ-free mice with fecal samples from patients with good drug response could help to control tumor, leading to better efficacy of anti-PD-L1 cancer therapy [80]. An independent study revealed that the gut bacterium *Akkermansia muciniphila*

assists cancer immunotherapy targeting the PD-1/PD-L1 interaction toward epithelial tumors [81]. The study on fecal microbiota transplantation demonstrated that germ-free or antibiotic-treated mice receiving gut bacteria from patients with good response to cancer immunotherapy have significant therapeutic improvement, while those receiving the samples from nonresponding patients do not have such improvement for cancer immunotherapy [81]. Restoration of the drug efficacy in germ-free mice receiving the samples from nonresponding patients was simply achieved by oral supplementation with the gut bacterium *A. muciniphila*, indicating the benefit of gut microbiota for this cancer immunotherapy. Another independent research also found similar benefits of gut microbiota on anti-PD-1 immunotherapy in melanoma patients; this study investigated microbiome samples from 112 patients with metastatic melanoma and found that there were substantial differences in the composition and diversity of gut microbiome obtained from patients with good drug response and from nonresponding patients [82]. Patients with good response to immunotherapeutic PD-1 blockade have abundance of gut bacteria of the family *Ruminococcaceae* and *Faecalibacterium*, while patients with poor response to immunotherapeutic PD-1 blockade tend to have relative abundance of *Bacteroidales*. It is possible that patients with a favorable gut microbiome, e.g., *Ruminococcaceae* and *Faecalibacterium*, toward the immunotherapeutic PD-1 blockade therapy have improved systemic and immune responses mediated by certain factors such as improvement of effector T cell function in the periphery, increase of antigen production, and improvement of the tumor microenvironment [82].

Gut microbiota also has an important role in chemotherapy for cancer treatment because they can modulate drug efficacy, for example, eliminating the anticancer properties of the drug or mediating toxicity [83]. Cyclophosphamide (**63**) (**Figure 12**) is a drug used in cancer chemotherapy for many types of cancers, as well as for autoimmune diseases, and its mechanism is through the stimulation of anticancer immune responses. In a mouse model, the composition of gut microbiota is changed after administration of cyclophosphamide (**63**), and this drug induces the translocation of certain Gram-positive bacteria into secondary lymphoid organs. Gut bacteria could stimulate certain immune responses beneficial to cancer therapy. Germ-free mice carrying tumor treated with antibiotics to kill Gram-positive bacteria had less therapeutic response, and their tumors exhibited resistance to the drug cyclophosphamide (**63**), suggesting that gut microbiota improves anticancer immune response [84]. Gut bacteria, *Enterococcus hirae* and *Barnesiella intestinihominis*, were found to help cyclophosphamide-induced therapeutic immunomodulatory response, thus improving the efficacy of this alkylating immunomodulatory drug [85].



Cyclophosphamide (63)

Figure 12.
*Structure of an anticancer drug, cyclophosphamide (**63**).*

The research studies mentioned earlier demonstrate the interactions of drugs and gut microbiome that provide beneficial effects on cancer therapy. However, interactions of gut microbiome and drugs can also give negative influence in cancer treatment, for example, the treatment of an anticancer drug, gemcitabine (**64**) or 2′,2′-difluorodeoxycytidine (**Figure 13**), which is a derivative of cytidine nucleoside base. A research led by Straussman showed that the bacterium *Mycoplasma hyorhinis* was found to be the cause of gemcitabine (**64**) resistance in colon carcinoma models [86]. In a colon cancer mouse model, *M. hyorhinis* could metabolize gemcitabine (**64**) to the corresponding deaminated derivative, 2′,2′-difluorodeoxyuridine (**65**), that does not have anticancer activity (**Figure 13**). The conversion of gemcitabine (**64**) to 2′,2′-difluorodeoxyuridine (**65**) was previously reported [87], and the nucleoside-catabolizing enzymes, i.e., cytidine deaminase, in the bacterium, *M. hyorhinis*, were also identified [88]. Straussman and co-workers analyzed genes and genomes of 2674 bacterial species and found that most of the *Gammaproteobacteria* class had the gene coding for the enzyme cytidine deaminase, thus potentially mediating gemcitabine resistance [86]. In a mouse model of colon carcinoma, mice receiving an antibiotic, ciprofloxacin, showed a good response to the anticancer drug gemcitabine (**64**), indicating that suppression of the growth of certain bacteria led to the improvement of the drug efficacy. Investigation of human pancreatic ductal adenocarcinoma collected from pancreatic cancer surgery revealed that there were intratumor bacteria, mainly belonging to the class *Gammaproteobacteria* such as *Enterobacteriaceae* and *Pseudomonadaceae* families in these samples; the intratumor bacteria can mediate resistance to chemotherapy of the drug gemcitabine (**64**) [86]. Therefore, the metabolism of the drug gemcitabine (**64**) to 2′,2′-difluorodeoxyuridine (**65**) by gut microbiota provides the negative effects for cancer treatment. This study underscores the importance of the research on drug metabolism by gut microbiome, which should be investigated for the new drug candidates during the drug development processes.

Additional example for the negative effects of gut microbiota for cancer chemotherapy is the treatment of colorectal cancer with the drugs, oxaliplatin (**66**) and fluorouracil or 5-FU (**67**) (**Figure 13**); the gut bacterium *Fusobacterium*

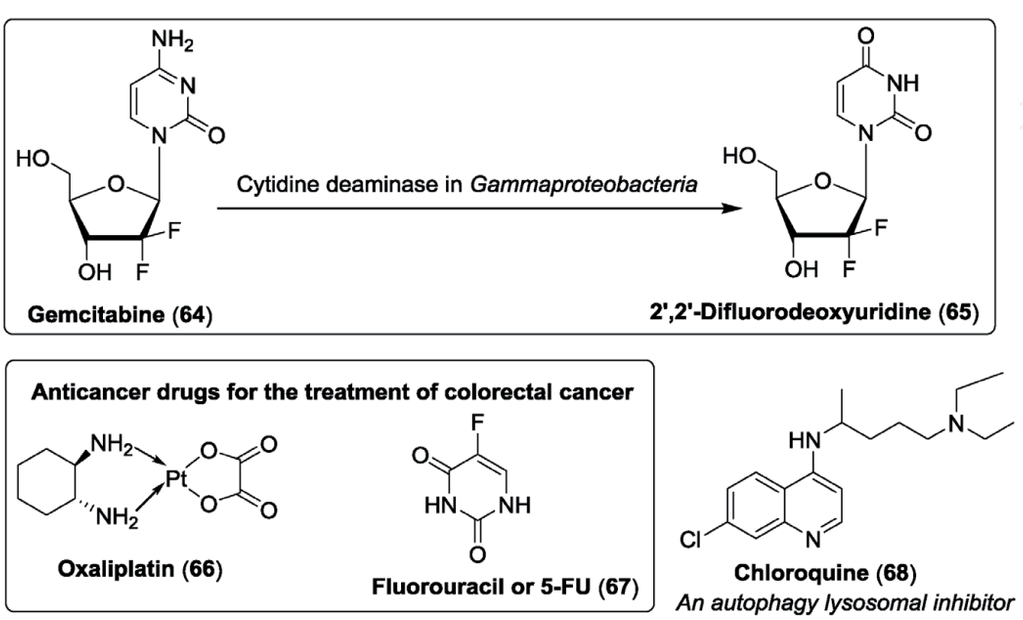


Figure 13.
*Biotransformation of gemcitabine (**64**) to its metabolite 2′,2′-difluorodeoxyuridine (**65**); structures of anticancer drugs, oxaliplatin (**66**) and fluorouracil or 5-FU (**67**), and autophagy lysosomal inhibitor, chloroquine (**68**).*

nucleatum was found to promote resistance to chemotherapy for colorectal cancer [89]. Analysis of colorectal cancer tissues collected from patients with recurrence or without recurrence of cancer revealed that the bacterium *F. nucleatum* is associated with the recurrence of colorectal cancer, which is derived from chemoresistance toward the drugs [89]. Cultivation of colorectal cancer cells co-cultured with *F. nucleatum* revealed that the bacterium potentially activated an autophagy pathway in colorectal cancer cells. An addition of a known autophagy lysosomal inhibitor, chloroquine (**68**) (**Figure 13**), could inhibit autophagic flux in the *F. nucleatum*-cultured cells, confirming the autophagy activation induced by the gut bacterium *F. nucleatum* [89]. Moreover, this bacterium reduced cell apoptosis of colorectal cancer cells, indicating that it specifically induced resistance toward the drugs oxaliplatin (**66**) and fluorouracil (**67**). Co-cultured cancer cells with the bacterium *F. nucleatum* and treated cancer cells with the drugs oxaliplatin (**66**) and fluorouracil (**67**) in the presence of autophagy lysosomal inhibitor, chloroquine (**68**), could eradicate chemoresistant effect, strongly confirming that the bacterium *F. nucleatum* induced chemoresistance through the autophagy pathway [89]. Detailed mechanistic study revealed that the bacterium *F. nucleatum* mediated chemoresistance through the TLR4 and MYD88 signaling pathway [89]. An independent study showed that the gut bacterium *F. nucleatum* is a diagnostic marker of colorectal cancer because patients with this cancer generally have high density of this bacterium in tumor cells [90]. Several studies have shown the prevalence of the bacterium *F. nucleatum* in colorectal tissues and fecal samples of patients, and those with high density of this bacterium tend to have lower rate of survival [91]. Therefore, manipulation of the bacterial population of *F. nucleatum* might be useful for the treatment of colorectal cancer, and this bacterium is potentially a diagnostic and/or prognostic marker for colorectal cancer.

In addition to drug metabolism, gut microbiota is also involved in drug–drug interactions when patients take two drugs at the same time, particularly when using antibiotics together with other drugs. Several studies have demonstrated the effects of antibiotic drugs on the metabolic activities of gut microbiota toward drugs and phytochemicals [92]. An example of a drug–drug interaction is the contribution of an antibiotic drug, amoxicillin (**69**), to a nonsteroidal anti-inflammatory drug aspirin (**70**) (**Figure 14**) [93]. It is worth mentioning that aspirin (**70**) is used not only for a pain reliever but also for primary prevention of cardiovascular disease [94] and cancer chemoprevention [95]. Recent study showed that amoxicillin (**69**) potentially affected the composition of gut microbiota by reducing number and species of intestinal bacteria in rats; the abundance of the gut bacteria, *Prevotella copri* and *Helicobacter pylori*, was reduced significantly after rats receiving amoxicillin (**69**) [93]. Gut microorganisms in rats could metabolite aspirin (**70**) to salicylate or salicylic acid (**71**) (**Figure 14**). Salicylate is a conjugate base of salicylic acid (**71**). It is known that the drug aspirin (**70**) is not responsible for a pain relief, but its metabolite, salicylic acid (**71**), is the active metabolite responsible for a pain relief with anti-inflammatory effect [96]. Therefore, gut microbiota plays an important role in the biotransformation of the drug aspirin (**70**) into the active metabolite, salicylic acid (**71**). After an oral administration of an antibiotic drug amoxicillin (**69**) to rats, the reduction of the metabolism of aspirin (**70**) into salicylic acid (**71**) was observed, suggesting the decrease of gut microbiota by amoxicillin (**69**) led to the reduction of the biotransformation of aspirin (**70**) into salicylic acid (**71**). Further study on the pharmacokinetics of aspirin (**70**) in rats revealed that amoxicillin (**69**) significantly affected the pharmacokinetic properties of aspirin (**70**) [93]. This study indicates that changes of the composition of gut microbiome by antibiotic drugs could substantially disturb the therapeutic effect of other drugs.

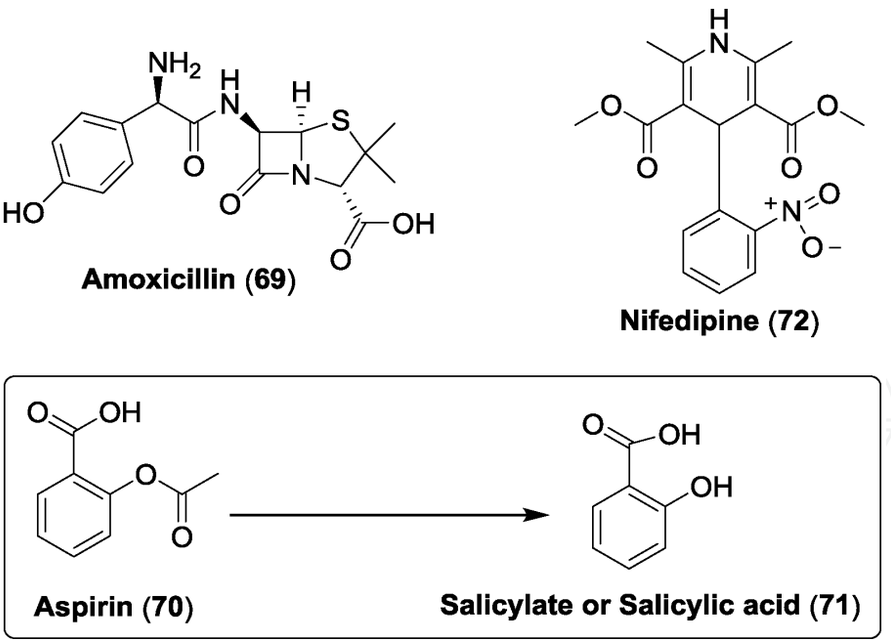


Figure 14.
*Structures of amoxicillin (**69**), aspirin (**70**) and its metabolite, salicylate or salicylic acid (**71**), and nifedipine (**72**).*

Previous study also showed that antibiotics substantially reduced the metabolic activity of gut microbiota toward aspirin (**70**), leading to the reduction of an antithrombotic effect of aspirin (**70**) [97]. Moreover, environmental changes, e.g., high-altitude hypoxia, also give effects on the pharmacokinetics and pharmacodynamics of aspirin (**70**) because of the changes in gut microbiota [98]. In an animal model, the plateau hypoxic environment affected the composition of gut microbiome because it increased the bacterial species of *Bacteroides* in rat feces but reduced numbers of the bacteria of the genus *Prevotella*, *Coprococcus*, and *Corynebacterium*. Changes in gut microbiome affected the metabolism of aspirin (**70**), thus altering the bioavailability of aspirin (**70**) in patients [98]. Plateau hypoxic environment also has the effects on the drug nifedipine (**72**), which could be metabolized by gut microorganisms (**Figure 14**) [99]. Nifedipine (**72**) is a drug for the treatment of hypertension, precordial angina, and certain vascular diseases. Plateau hypoxic environment was found to alter the composition of gut microbiota in an animal model, thus affecting the bioavailability of nifedipine (**72**) [99].

Recent study led by Kittakoop revealed that valproic acid or valproate (**73**) (**Figure 15**), an anticonvulsive drug used for treatments of epilepsy and bipolar disorder, had effects on the biosynthesis of fatty acids in microorganisms including representative gut microbiome [100]. Valproic acid (**73**) is also an epigenetic modulator, acting as an inhibitor of histone deacetylase [101]. Initially, Kittakoop and co-workers employed "One strain many compound" (OSMAC) approach using the marine fungus *Trichoderma reesei* treated with an epigenetic modulator, valproic acid (**73**), aiming to modulate the fungus *T. reesei* to produce new natural products, which are secondary metabolites. However, valproic acid (**73**) was found to have the effects on the biosynthesis of fatty acids, which are primary metabolites, instead of natural products that are secondary metabolites [100]. The study revealed that valproic acid (**73**) at a concentration of 100 μM could either inhibit or induce the biosynthesis of certain fatty acids in fungi, yeast, and bacteria. Valproic acid (**73**) inhibited the biosynthesis of palmitoleic acid (C16:1), α-linolenic acid (C18:3), arachidic acid (C20:0), and lignoceric acid (C24:0) in the fungus *Fusarium oxysporum*, while it induced the production of α-linolenic acid (C18:3) in the fungus *Aspergillus*

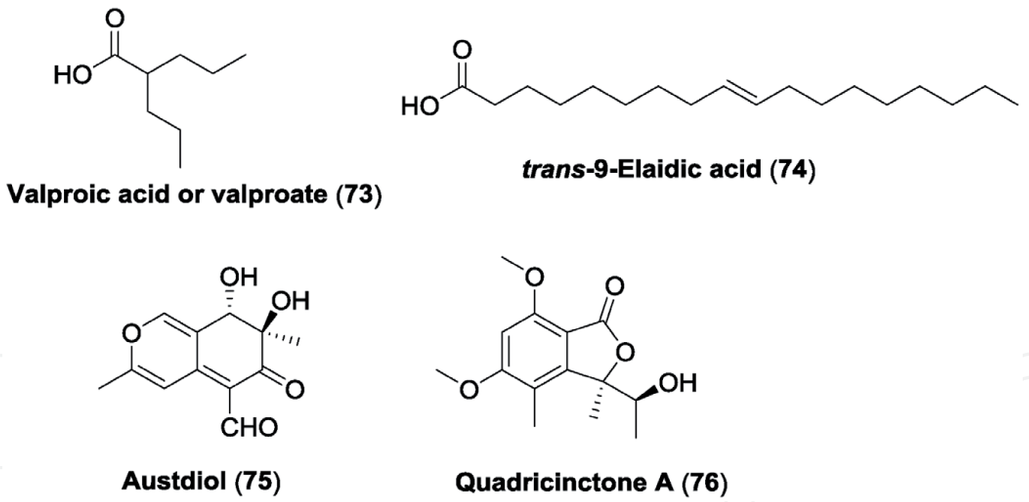


Figure 15.
Structures of valproic acid or valproate (73), trans*-9-elaidic acid (74), austdiol (75), and quadricinctone A (76).*

aculeatus [100]. The bacterium of the genus *Pediococcus* is commonly found as gut microbiome in humans and animals [102]; valproic acid (**73**) was found to inhibit the production of lignoceric acid (C24:0) in the bacterium, *Pediococcus acidilactici* [100]. The yeast *Candida utilis* was found as gut microbiome in pediatric patients with inflammatory bowel disease [103]; valproic acid (**73**) inhibited the biosynthesis of palmitoleic acid (C16:1) and α-linolenic acid (C18:3) in *C. utilis* [100]. The yeast *Saccharomyces cerevisiae* was previously found as a prevalent gut microbiome in human [104], and the drug valproic acid (**73**) was found to inhibit the production of *α*-linolenic acid (C18:3) in the yeast *S. cerevisiae* [100]. Interestingly, valproic acid (**73**) could induce the biosynthesis of *trans*-9-elaidic acid (**74**) (**Figure 15**) in the yeast *Saccharomyces ludwigii* [100]. In human, *trans*-9-elaidic acid (**74**) could increase intracellular Zn^{2+} in macrophages and inhibit *β*-oxidation in peripheral blood macrophages [105, 106]; this suggests that the production of *trans*-9-elaidic acid (**74**) in gut microorganisms induced by the drug valproic acid (**73**) may indirectly give the effects to human. Valproic acid (**73**) also had effects on the biosynthesis of polyketides because it substantially reduced the production of austdiol (**75**) (90% reduction) and quadricinctone A (**76**) (50% reduction) (**Figure 15**), which are the polyketides of the fungus *Dothideomycetes* sp. [100]. The biosynthesis of fatty acids is considerably similar to that of polyketides, i.e., sharing the same catalytic roles and biosynthetic precursors [107]. Therefore, the drug valproic acid (**73**) possibly gives effects on the biosynthetic pathways of both fatty acids and polyketides because of their biosynthetic similarities. Gut microbes have biosynthetic gene clusters involving in the biosynthesis of many bioactive natural products including polyketides [108]; some natural products produced by gut microbiome have biological activities. This study suggests that commonly used drugs could potentially give the effects on the biosynthesis of secondary metabolites (natural products) of gut microbiome.

3.2 Interactions of gut microbiome and natural products

Traditional medicine and natural products have significant interactions with gut microbiome. Many studies revealed that dietary natural products modulating gut microbiota are useful for prevention and management of diabetes mellitus [109]. Recent study revealed that a traditional Chinese herbal formula and an antidiabetic drug, metformin (77) (**Figure 16**), could improve the treatment of

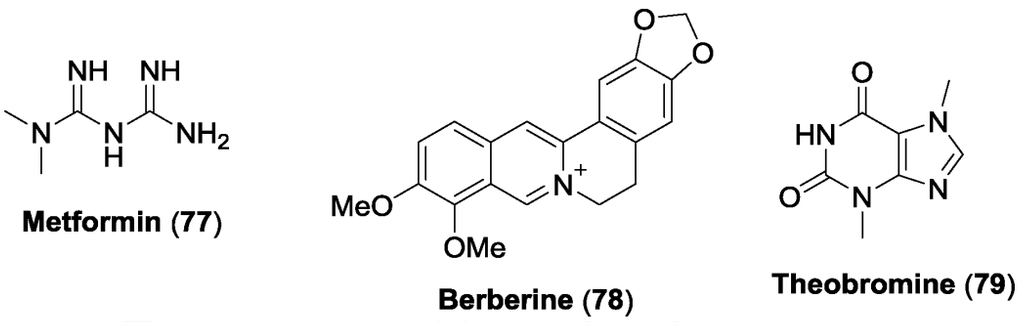


Figure 16.
Structures of metformin (77), berberine (78), and theobromine (79).

type 2 diabetes with hyperlipidemia by enriching certain beneficial species of gut bacteria, for example, *Faecalibacterium* sp. and *Blautia* [110]. The study was carried out in 450 patients with type 2 diabetes and hyperlipidemia, and the profiles of gut microbiota were analyzed using fecal samples in patients treated with metformin and a traditional Chinese herbal formula. An antidiabetic drug metformin (**77**) and herbal medicine significantly changed the gut microbiota profile that led to the enhancement of therapeutic effects of the drugs [110]. The traditional Chinese herbal formula used in the study contains the plants including *Coptis chinensis*, *Momordica charantia*, *Rhizoma anemarrhenae*, and *Aloe vera*, as well as red yeast rice from the fermentation; this herbal recipe is practically used in clinical application [110]. Among the plants used in this formula, *Coptis chinensis* contains an alkaloid berberine (**78**) (**Figure 16**). An independent study revealed that berberine (**78**) could significantly change the composition of gut microbiota in high-fat diet-fed rats [111]. An alkaloid berberine (**78**) was able to enrich selectively short-chain fatty acid-producing bacteria such as the genus *Blautia* and *Allobaculum* [111]. Another independent study also revealed that both metformin (**77**) and berberine (**78**) could change profiles of gut microbiota in high-fat diet-induced obesity in rats [112]. Substantial reduction of the diversity of gut microbiota was observed by both metformin (**77**) and berberine (**78**) because 60 out of the 134 operational taxonomic units were decreased after treatment with both drugs. However, there were considerable increases in short-chain fatty acid-producing bacteria, e.g., the genus *Butyricicoccus*, *Blautia*, *Allobaculum*, *Phascolarctobacterium*, and *Bacteroides*, after treatment with both metformin (**77**) and berberine (**78**) [112]. Therefore, in addition to the direct benefit toward the treatment of diabetes and obesity, the drugs, metformin (**77**) and berberine (**78**), could also improve gut microbiota profile by increasing short-chain fatty acid-producing bacteria and thus mediating their useful effects on the host [112]. As mentioned earlier in Section 2, gut microbiomes that produce short-chain fatty acids provide many beneficial effects on human health [24, 25].

Recent study revealed that berberine (**78**) could prevent ulcerative colitis by modifying gut microbiota and regulating T regulatory cell and T helper 17 cell in a dextran sulfate sodium-induced ulcerative colitis mouse model [113]. The diversity of gut microbiota was reduced by berberine (**78**), which markedly interfered the abundance of certain bacterial genus such as *Bacteroides*, *Desulfovibrio*, and *Eubacterium*. Therefore, the mechanisms of berberine (**78**) for the prevention of ulcerative colitis are by regulating the balance of T regulatory cell and T helper 17 cell, as well as by modifying gut microbiota [113]. Theobromine (**79**) (**Figure 16**) is a xanthine alkaloid of cocoa beans and found in chocolate, and its structure is closely related to caffeine. A cocoa-enriched diet containing theobromine (**79**) could decrease the intestinal immunoglobulin A secretion and immunoglobulin

A-coating bacteria, i.e., the genus of *Bacteroides*, *Staphylococcus*, and *Clostridium* [114]. A cocoa-enriched diet had effects on a differential toll-like receptor pattern, which led to changes in the intestinal immune system [114]. Moreover, further experiments in rats revealed that a diet containing 10% cocoa and a diet supplemented with 0.25% theobromine (**79**) could reduce the gut bacterium *Escherichia coli*, while a diet with 0.25% theobromine (**79**) reduced the gut bacterial community of *Clostridium histolyticum*, *C. perfringens*, *Streptococcus* sp., and *Bifidobacterium* sp. [115]. The amounts of short-chain fatty acids increased after feeding rats with a diet containing 10% cocoa and that supplemented with 0.25% theobromine (**79**), while both diets decreased the abundance of immunoglobulin A (IgA)-coated bacteria. It is worth mentioning that gut IgA-coated bacteria could potentially cause intestinal disease such as inflammatory bowel disease, and eradication of these bacteria may prevent or reduce intestinal disease development [116]. Therefore, the active natural product theobromine (**79**) in cocoa able to reduce the amounts of immunoglobulin A-coated bacteria, and to modify the profile of gut microbiota, provides beneficial effects on human health [115].

It is known that berberine (**78**) has poor solubility; however, it can show effectiveness for the treatment of certain diseases; therefore, there might be a specific mechanism to deliver berberine (**78**) to an organ system. In an animal model, berberine (**78**) was found to convert to dihydroberberine (**80**) in an intestinal ecosystem of rats (**Figure 17**); the metabolite dihydroberberine (**80**) exhibited much better absorption rate than its parent drug, berberine (**78**) [117]. Incubation of berberine (**78**) with human gut bacteria isolated from gastrointestinal human specimens also produced dihydroberberine (**80**), and the amounts of dihydroberberine (**80**) obtained from the biotransformation of gut bacteria were higher than that obtained from other bacteria, which were not gut bacteria and used as the control. This experiment confirmed that gut microbiota could convert berberine (**78**) into its absorbable form, dihydroberberine (**80**); therefore intestinal microbiota is considered to be a "tissue" or an "organ" that is able to transform berberine (**78**)

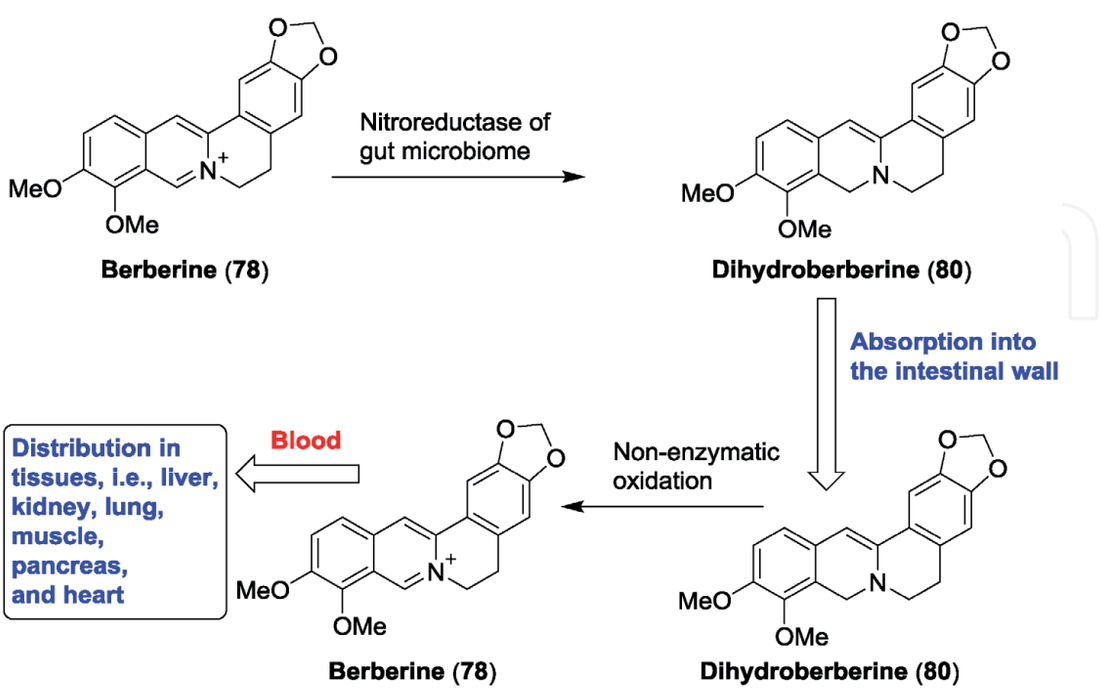


Figure 17.
Bioconversion of berberine (78) into an absorbable form, dihydroberberine (80), by gut bacteria; absorption of dihydroberberine (80) into the intestine wall and nonenzymatic conversion of dihydroberberine (80) to the active form berberine (78).

into an absorbable form, dihydroberberine (**80**) [117]. Mechanistic study revealed that gut microbiome uses the enzyme nitroreductases to catalyze the conversion of berberine (**78**) to dihydroberberine (**80**) (**Figure 17**). Dihydroberberine (**80**) was found to be absorbed in intestinal epithelia, but it was reverted to the active form berberine (**78**) soon after entering tissues of the intestinal wall. Detailed analysis showed that the conversion of dihydroberberine (**80**) back to berberine (**78**) was by a nonenzymatic oxidation through multi-faceted factors, for example, superoxide anion and metal ions, which occurred in intestinal epithelial tissues (**Figure 17**) [117]. Previous report demonstrated that dihydroberberine (**80**) in its sulfate form, e.g., dihydroberberine sulfate, also showed better absorption in the intestine than its parent drug, berberine (**78**) [118]. Recent independent studies revealed that dihydroberberine (**80**) has interesting biological activities, for example, anti-inflammatory activity through dual modulation of NF-κB and MAPK signaling pathways [119], synergistic effects with an anticancer drug sunitinib on human non-small cell lung cancer cell lines by inflammatory mediators and repressing MAP kinase pathways [120], and inhibition of ether-a-go-go-related gene (hERG) channels expressed in human embryonic kidney 293 (HEK293) cells [121]. It is worth mentioning that the metabolite products from gut biomicrobiota, i.e., dihydroberberine (**80**), have different biological activity from its parent drug, berberine (**78**). Therefore, the drug development process should include a research study on the metabolism of natural products (as drug candidates) by gut microbiota.

Demethyleneberberine (**81**), berberrubine (**82**), jatrorrhizine (**83**), and thalifendine (**84**) were found as major metabolites in rats after an oral administration of berberine (**78**) (**Figure 18**) [122]. Comparison of the levels of these metabolites in conventional rats (a control group) and pseudo germ-free rats revealed that liver and intestinal bacteria were involved in the metabolism and disposition of berberine (**78**) in vivo. It is worth mentioning that some metabolites from this biotransformation exert important biological activities. For example, demethyleneberberine (**81**) inhibits oxidative stress, steatosis, and mitochondrial dysfunction in a mouse

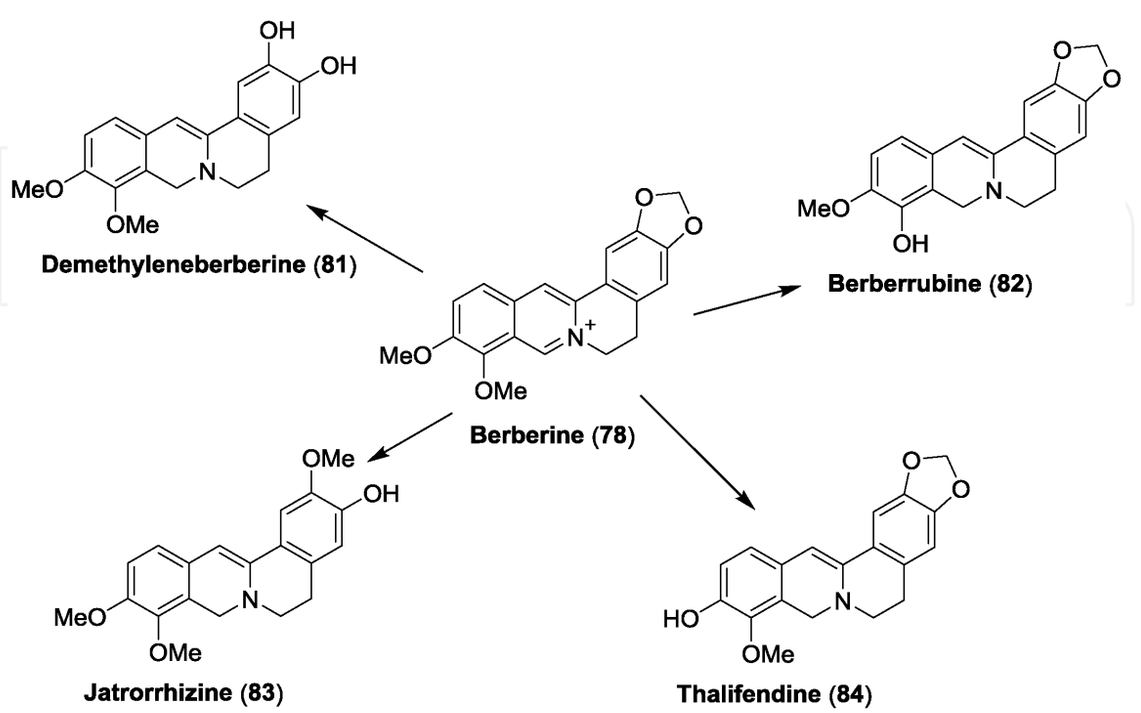


Figure 18.
Biotransformation of berberine (78) to demethyleneberberine (81), berberrubine (82), jatrorrhizine (83), and thalifendine (84) by gut bacteria.

model, which is a potential therapy for alcoholic liver disease [123]. Berberrubine (**82**) was found to reduce inflammation and mucosal lesions in dextran sodium sulfate-induced colitis in mice, which might be useful for the treatment of ulcerative colitis [124]. Jatrorrhizine (**83**) could reduce the uptake of 5-hydroxytryptamine and norepinephrine by the inhibition of uptake-2 transporters, thus exerting antidepressant-like action in mice [125]. Therefore, the biotransformation of berberine (**78**) by gut bacteria leads to the production of bioactive metabolites, which have interesting pharmacological properties; this underscores the impact of gut microbiota in the drug development process for natural products.

Since there are interactions between gut microbiota and natural products, efforts have been made to use natural compounds for the treatment of gut microbiota dysbiosis, which is the imbalance of microorganisms in the human gastrointestinal tract. Dysbiosis of gut microbiota is strongly associated with some diseases such as type 2 diabetes, inflammatory bowel disease, obesity, and nonalcoholic fatty liver disease [16, 126]. Alkaloids of a medicinal plant, *Corydalis saxicola*, were used to prevent gut microbiota dysbiosis in an animal model [127]. Major alkaloids in *Corydalis saxicola* are berberine (**78**), jatrorrhizine (**83**), dehydrocavidine (**85**), palmatine (**86**), and chelerythrine (**87**) (**Figures 18** and **19**). Among these alkaloids, berberine (**78**), palmatine (**86**), and chelerythrine (**87**) are the main active principles for the treatment of antibiotic-induced gut microbiota dysbiosis through the key enzyme, CYP27A1, which is involved in the biosynthesis of bile acid [127]. This study provides insights for the discovery of natural products for the treatment of gut microbiota dysbiosis.

Xanthohumol (**88**) is a prenylflavonoid in hops (*Humulus lupulus*), which is responsible bitter flavor in beer (**Figure 20**). Xanthohumol (**88**) has interesting pharmacological properties, for example, improving cognitive flexibility in young mice [128] and having beneficial effects toward metabolic syndrome-related diseases such as type 2 diabetes and obesity [129]. The comparative study on germ-free and human microbiota-associated rats toward the metabolism of xanthohumol (**88**) revealed that gut bacteria could transform xanthohumol (**88**) to isoxanthohumol (**89**) and 8-prenylnaringenin (**90**), respectively (**Figure 20**) [130]. The metabolism of xanthohumol (**88**) was further studied using human intestinal bacteria, *Eubacterium ramulus* and *E. limosum*. It is worth mentioning that an independent study revealed that the bacteria of the genus *Eubacterium* are normally abundant in the human gastrointestinal tract; their densities in human gut are up to 10^{10} colony-forming units/g of intestinal content [131]. Xanthohumol (**88**) is spontaneously converted to isoxanthohumol (**89**), which is in turn bioconverted to 8-prenylnaringenin (**90**) by the gut bacterium, *E. limosum* (**Figure 20**) [132]. 8-Prenylnaringenin (**90**) is biotransformed to *O*-desmethylxanthohumol (**91**) by the bacterium *E. ramulus*; this bacterium could also convert *O*-desmethylxanthohumol (**91**) to desmethyl-*α*,*β*-dihydroxanthohumol (**92**). Moreover, the bacterium *E. ramulus* was

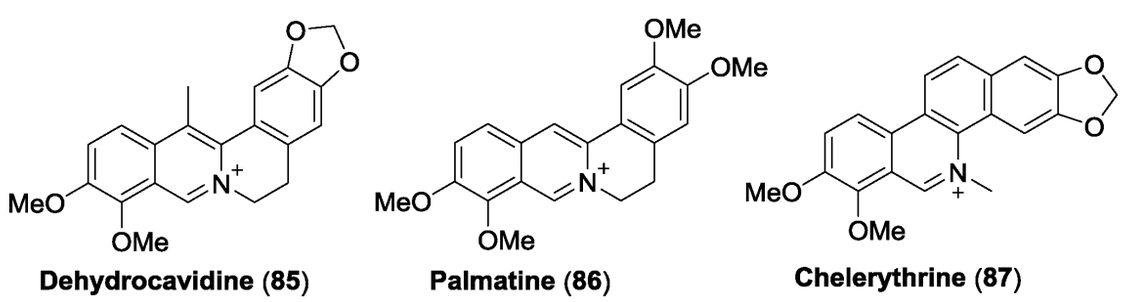


Figure 19.
Structures of dehydrocavidine (85), palmatine (86), and chelerythrine (87).

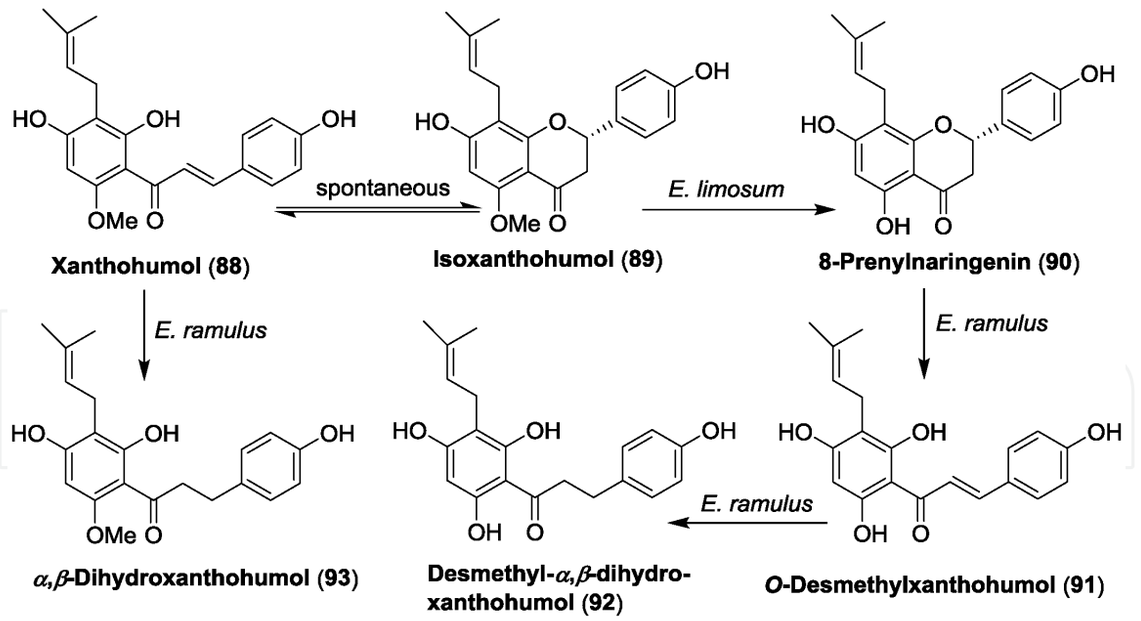


Figure 20.
*Bioconversion of xanthohumol (**88**) to isoxanthohumol (**89**), 8-prenylnaringenin (**90**), O-desmethylxanthohumol (**91**), desmethyl-α,β-dihydroxanthohumol (**92**), and α,β-dihydroxanthohumol (**93**) by human gut bacteria.*

able to transform xanthohumol (**88**) to *α*,*β*-dihydroxanthohumol (**93**) (**Figure 20**) [132]. An independent study in healthy women volunteers revealed that isoxanthohumol (**89**) could be bioconverted to 8-prenylnaringenin (**90**) in human intestine and that the bacterial microbiota isolated from fecal samples of female volunteers could also biotransform isoxanthohumol (**89**) to 8-prenylnaringenin (**90**) [133]. Another study demonstrated that 8-prenylnaringenin (**90**) has potent estrogenic property, and it could relieve climacteric symptoms, i.e., vasomotoric complaints and osteoporosis, and may be useful for the treatment of menopausal complaints [134]. These studies conclusively show that the metabolites produced by gut microbiome, i.e., 8-prenylnaringenin (**90**), are actually bioactive compounds, not the parent natural products, and they have different pharmacological activities from their parent natural products. Gut microbiome is therefore important for in vivo biotransformation of natural products, providing bioactive metabolites responsible for therapeutic effects.

Gut microbiome can biotransform natural products to bioactive metabolite essentially for therapeutic effects, for example, the biotransformation of isoxanthohumol (**89**) to bioactive 8-prenylnaringenin (**90**) [133]. However, gut microbiome can also produce toxic metabolites from the biotransformation of natural products, thus giving negative side effects. Camptothecin (CPT) is a natural alkaloid of a plant, *Camptotheca acuminata*, and has anticancer property with topoisomerase inhibitory activity [135]. Irinotecan or CPT-11 (**94**) is an alkaloid derivative of camptothecin and used as anticancer drug (**Figure 21**). Irinotecan (**94**) is a prodrug, which is transformed in vivo through hydrolysis by carboxylesterase enzymes, giving an active metabolite, SN-38 (**95**) (**Figure 21**) [136]. Uridine diphosphate-glucuronosyltransferase enzymes catalyze the conversion of SN-38 (**95**) to a glucuronidated derivative, SN-38G (**96**) (**Figure 21**). The metabolite SN-38G (**96**) is inactive for cancer cells and is excreted into the gastrointestinal tract [137], where the gut bacteria use *β*-glucuronidase enzymes to convert SN-38G (**96**) to SN-38 (**95**) that causes severe diarrhea in patients (**Figure 21**) [138]. This side effect reflects the significant negative effects of gut bacteria in the drug metabolism. However, the use of antibiotics, e.g., levofloxacin, to reduce

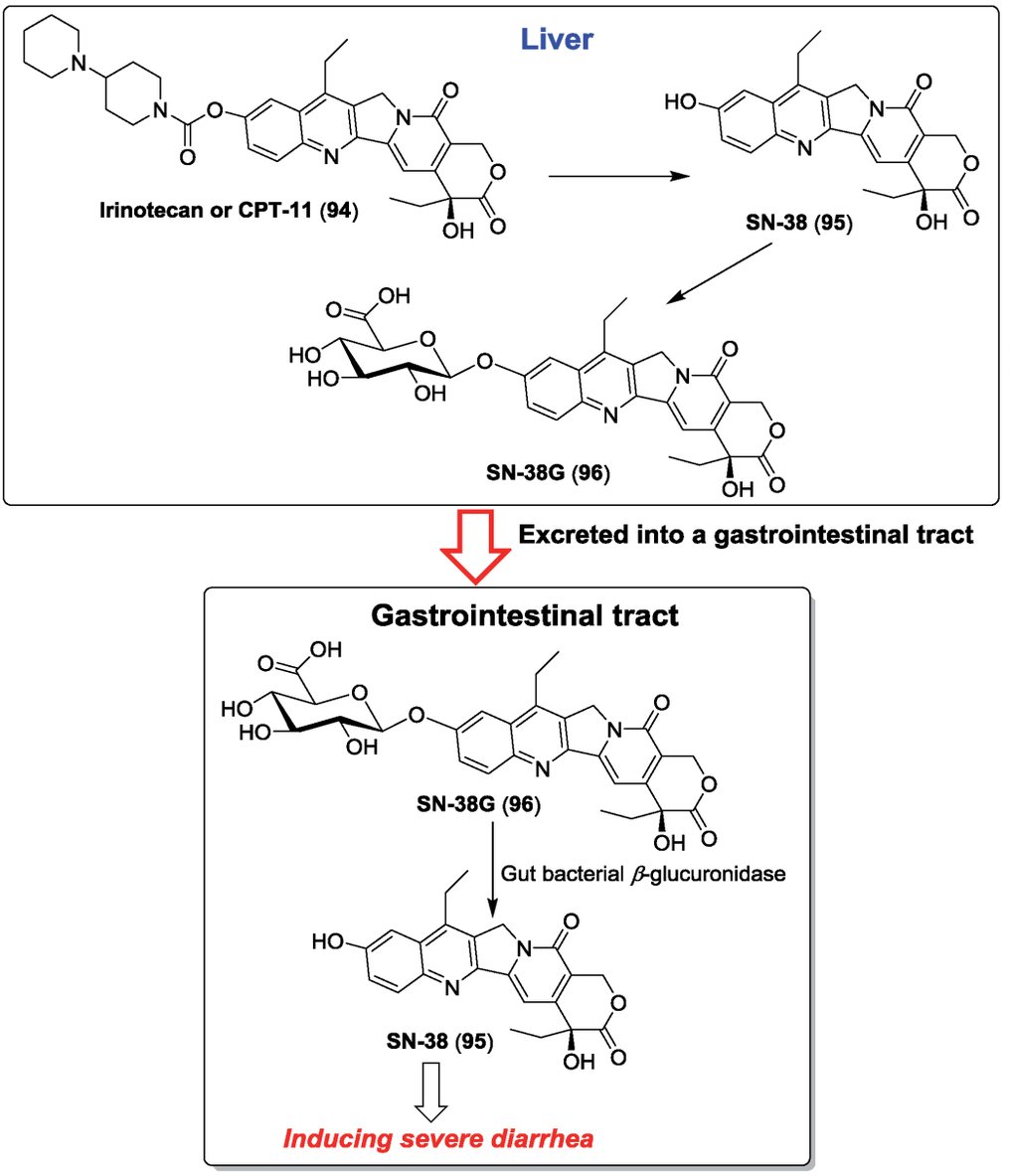


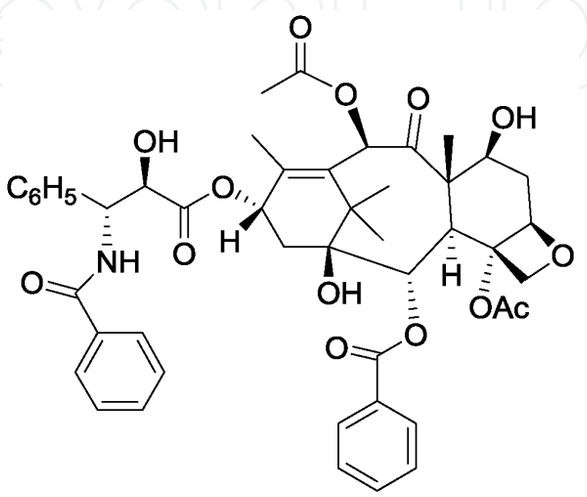
Figure 21.
*Structures of irinotecan or CPT-11 (**94**) and its metabolites, SN-38 (**95**) and SN-38G (**96**), and the bioconversion of SN-38G (**96**) to SN-38 (**95**) by gut bacterial β-glucuronidase.*

the population of gut bacteria in the gastrointestinal tract is not recommended for patients because it has many consequent problems [139]. Gut microbiotas are important for a healthy gastrointestinal tract, and they play many essential roles in dietary metabolisms [140, 141]; the treatment of cancer should not give any effects to gut microorganisms. Therefore, the use of antibiotic drugs, which affect gut microbiota, is not recommended. To reduce the diarrhea side effect without affecting gut microorganisms, a research led by Redinbo employed appropriate inhibitors of gut bacterial *β*-glucuronidase enzymes, in order to prevent the formation of SN-38 (**95**), a causative agent of severe diarrhea in patients [142]. Certain inhibitors exhibited *β*-glucuronidase inhibitory activity in living bacterial cells without disturbing the growth of gut bacteria or giving any damaging effects toward mammalian cells. Indeed, in a mouse model, mice treated with both irinotecan (**94**) and a *β*-glucuronidase inhibitor had less diarrhea and bloody diarrhea than the group receiving only the drug irinotecan (**94**). Therefore, the inhibition of microbial *β*-glucuronidases could prevent the production of toxic metabolite, SN-38 (**95**), during the treatment of anticancer drug, irinotecan (**94**) [142]. This is an example

of a toxic drug metabolite produced by the activity of gut microbiome, and the manipulation of the enzyme activity of gut bacteria could be done by using another drug (an inhibitor of bacterial enzyme).

Some natural products can alter the composition of gut microbiome, and changes in gut microbiome lead to the drug-induced negative side effects on certain treatments. Paclitaxel or Taxol (**97**) is an anticancer drug for the treatment of many types of cancers (**Figure 22**), and it is a natural product isolated from a Pacific yew tree, *Taxus brevifolia*. In a mouse model, paclitaxel (**97**) chemotherapy could change the composition of gut bacterial community and induce negative effects such as sickness behaviors, i.e., fatigue and anorexia, increased central and peripheral inflammation, and impaired cognitive performance [143]. These negative effects might be associated with changes in gut bacteria because paclitaxel (**97**) therapy decreased the abundance of gut bacteria including *Lachnospiraceae* bacteria and butyrate-producing bacteria, which are necessary for human gut health [143]. Therefore, the negative effects of cancer chemotherapy may be attenuated by improving the composition of gut microbiota, for example, the use of prebiotic or probiotic supplements, which has become one of the emerging approaches to change the microbiota composition, thus improving therapeutic outcome for patients treated with anticancer drugs [144].

Antibiotic drugs have significant effects on the metabolism of drugs and phytochemicals because they could suppress enzymatic activities of gut microbiome [92]. Therefore, if patients are treated with an antibiotic drug together with another drug, there are possible drug–drug interactions due to changes of gut microbiota caused by antibiotic drugs. Lovastatin (**98**) (**Figure 23**), a natural polyketide isolated from the fungus *Aspergillus terreus* [145], is a cholesterol-lowering drug, which is a member of the statin family. Lovastatin (**98**) has the interactions with antibiotics through the mediation of gut microbiome [146]. Incubation of lovastatin (**98**) with human and rat fecalase revealed the biotransformation of this drug by gut microbiota, giving four major metabolites including demethylbutyryl-lovastatin (**99**), hydroxylated-lovastatin (**100**), hydroxy acid-lovastatin (**101**), and OH-hydroxy acid-lovastatin (**102**) (**Figure 23**) [146]. These four metabolites were also found in rat plasma, and they might be from gut microbiota-mediated metabolism of the drug lovastatin (**98**). Among the drug metabolites, hydroxy acid-lovastatin (**101**) is the active form, which could effectively inhibit 3-hydroxy-3-methylglutaryl coenzyme-A reductase, the target enzyme of this cholesterol-lowering drug [147].



Paclitaxel or Taxol (97)

Figure 22.
Structure of an anticancer drug paclitaxel or Taxol (97).

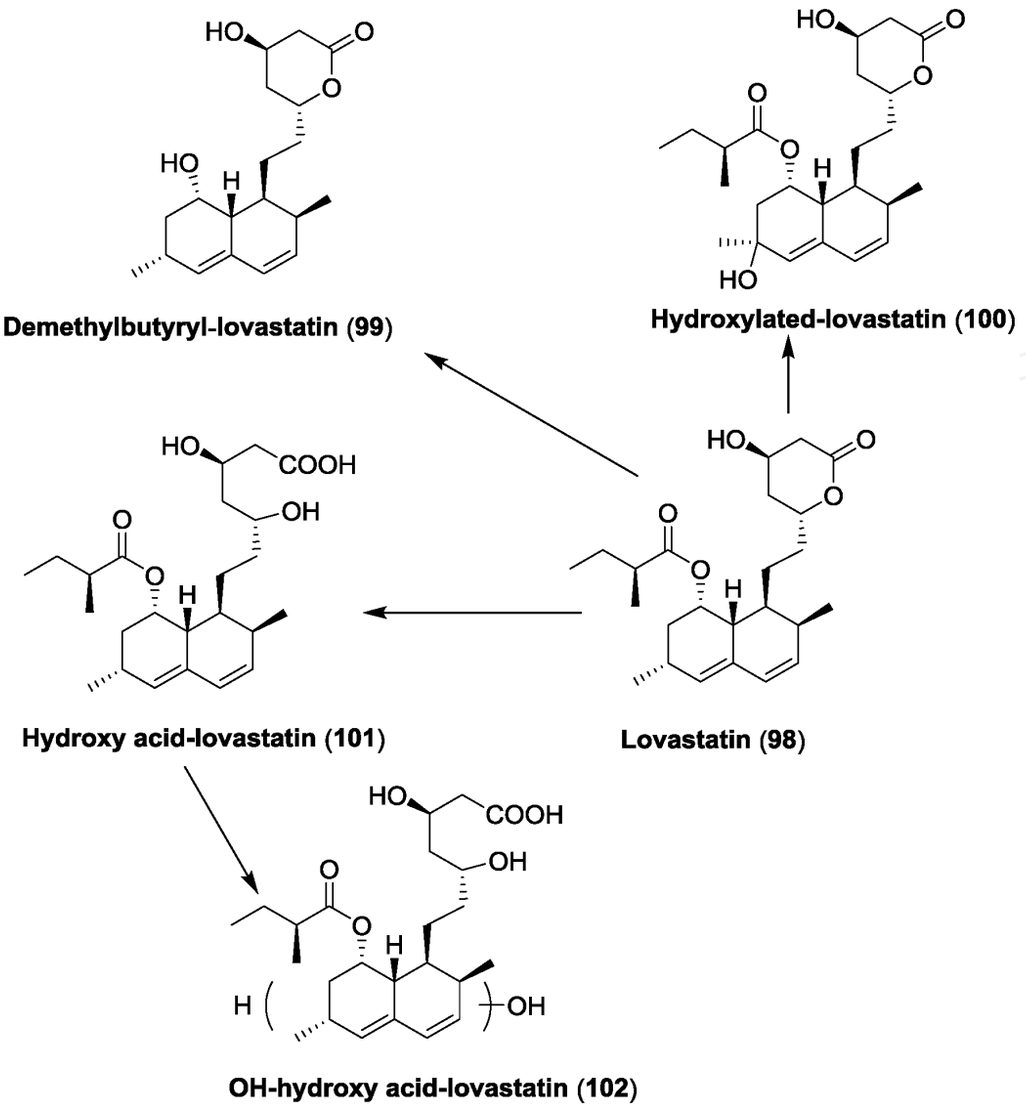


Figure 23.
*Structures of major metabolites of lovastatin (**98**) including demethylbutyryl-lovastatin (**99**), hydroxylated-lovastatin (**100**), hydroxy acid-lovastatin (**101**), and OH-hydroxy acid-lovastatin (**102**).*

In an animal model, rats with an oral administration of lovastatin (**98**) were compared with those treated with lovastatin (**98**) and antibiotics; the pharmacokinetic study revealed that the levels of the active metabolite hydroxy acid-lovastatin (**101**) in antibiotic-treated rats were lower than that without antibiotics. This result indicates that antibiotic drugs reduce the biotransformation of the drug lovastatin (**98**) to its active form, hydroxy acid-lovastatin (**101**), because antibiotics affect gut microbiome. The in vivo metabolism of lovastatin (**98**) to its active form, hydroxy acid-lovastatin (**101**), is important for the therapeutic efficacy of this drug; therefore antibiotic intake of patients treated with lovastatin (**98**) would lead to the decrease of the active form, hydroxy acid-lovastatin (**101**), thus decreasing its therapeutic effects [146]. This study clearly demonstrates the drug–drug interaction mediated by changes of gut microbiome.

4. Conclusions

Intriguingly, gut microbiome is very important for human health and diseases, and it is therefore recognized as an "organ" or a "tissue" in the human body. Gut microorganisms have much more genes encoding enzymes than those of human

genome; therefore, enzymes of these microbes are involved in many biochemical processes, i.e., metabolism of xenobiotics (compounds not produced in human host, e.g., drugs and pollutants) and dietary sources. Metabolites produced by gut microbiome play significant roles in human health and diseases; these metabolites include short-chain fatty acids such as butyrate (**11**), as well as other metabolites, e.g., nicotinamide (**12**), 5-aminovaleric acid (**14**), and taurine (**15**) (see Section 2). Since gut microbiome and its metabolites substantially contribute to human health and diseases, a therapy by intervention strategies using gut microbiota can potentially be useful for some diseases, for example, metabolic disorders, cardiovascular disease, food allergy, and neurological disorders. Supplementation with probiotics or certain gut bacteria, as well as their metabolites, may be a new therapeutic method in the future. Fecal microbiota transplantation, e.g., transferring gut bacteria from healthy individuals into patients, is a challenging research study in the near future.

Gut microbiome can metabolite commonly used drugs and natural products. Drug metabolism by gut microorganisms decreases the levels of drugs in serum, thus disturbing the drug pharmacokinetics, which can lead to alteration of therapeutic efficiency. Moreover, metabolites produced by the drug metabolism of gut microbiome contribute considerably to the drug efficacy. For example, the levels of the drug L-dopa (**56**) are substantially reduced by the metabolic activity of gut microbiome, and this results in the requirement of higher doses for the Parkinson's patients with gut microbiome that has high metabolic activity toward the drug L-dopa (**56**) (see Section 3.1). This example well demonstrates the role of gut microorganisms on treatment outcomes of the commonly used drugs. Gut microbiome could improve many drug therapies, for example, cancer immunotherapy targeting CTLA-4 blockade and immune checkpoint inhibitor via the PD-1/PD-L1 pathway. Moreover, the metabolism of gut microbiome improves drug efficacy because it assists the bioconversion of some drugs into their active forms, for example, a biotransformation of lovastatin (**98**) to its active form, hydroxy acid-lovastatin (**101**), and a bioconversion of aspirin (**70**) to salicylic acid (**71**) that actively reduces pain. Interestingly, gut microbiome involves in a biotransformation of an alkaloid natural product berberine (**78**) to an absorbable form, dihydroberberine (**80**), which is absorbed at the intestine system (see Section 3.2). This result demonstrates that gut microbiome facilitates drug delivery of berberine (**78**) that has poor solubility by a biotransformation to an absorbable form, dihydroberberine (**80**), which is in turn converted to its active form berberine (**78**) in the human body. Since gut microbiome plays many important roles in drugs and natural products, the metabolism of natural products and drug candidates by gut microbiome should therefore be studied, and it should be a part of the drug development process. Gut microbiome can potentially play a crucial role for the improvement of drug safety and efficacy.

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Conflict of interest

The author declares no competing interests or no conflict of interest.

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