Acta Pharmaceutica Sinica B 2019;9(2):258–278

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

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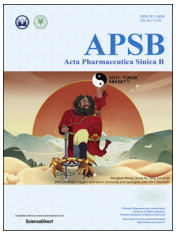


REVIEW

Recent progress and challenges in screening and characterization of UGT1A1 inhibitors

Xia Lv[a,b,†], Yangliu Xia[c,†], Moshe Finel[d], Jingjing Wu[c], Guangbo Ge[a,c,*], Ling Yang[a]

[a]*Institute of Interdisciplinary Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China*
[b]*Key Laboratory of Biotechnology and Bioresources Utilization, Ministry of Education, College of Life Science, Dalian Minzu University, Dalian 116600, China*
[c]*Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, China*
[d]*Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmacy, University of Helsinki, Finland*

Received 8 June 2018; received in revised form 16 August 2018; accepted 27 August 2018

KEY WORDS

UGT1A1 inhibitors;
Drug/herbdrug interactions;
Probe substrates;
High-throughput screening

Abstract Uridine-diphosphate glucuronosyltransferase 1A1 (UGT1A1) is an important conjugative enzyme in mammals that is responsible for the conjugation and detoxification of both endogenous and xenobiotic compounds. Strong inhibition of UGT1A1 may trigger adverse drug/herb–drug interactions, or result in metabolic disorders of endobiotic metabolism. Therefore, both the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have recommended assaying the inhibitory potential of drugs under development on the human UGT1A1 prior to approval. This review focuses on the significance, progress and challenges in discovery and characterization of UGT1A1 inhibitors. Recent advances in the development of UGT1A1 probes and their application for screening UGT1A1 inhibitors are summarized and discussed in this review for the first time. Furthermore, a long list of UGT1A1 inhibitors, including information on their inhibition potency, inhibition mode, and affinity, has been prepared and analyzed. Challenges and future directions in this field are highlighted in the final section. The information and knowledge that are presented in this review provide guidance for rational use of drugs/herbs in order to avoid the occurrence of adverse effects *via* UGT1A1 inhibition, as well as presenting methods for rapid screening and characterization of UGT1A1 inhibitors and for facilitating investigations on UGT1A1–ligand interactions.

*Corresponding author at: Institute of Interdisciplinary Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China.
E-mail address: geguangbo@shutcm.edu.cn (Guangbo Ge).

†These authors made equal contribution to this work.

Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.

https://doi.org/10.1016/j.apsb.2018.09.005

1. Introduction

Uridine-diphosphate (UDP) glucuronosyltransferases (UGT) are a superfamily of phase II conjugating enzymes (EC 2.4.1.17) that catalyse the covalent addition of glucuronic acid from the high energy donor uridine-diphosphate glucuronic acid (UDPGA) to a wide range of lipophilic chemicals that contain a suitable acceptor functional group (Fig. 1)[1]. In mammals, UGTs play predominant roles in the detoxification of many exogenous and endogenous compounds by generating more polar and water-soluble glucuronides. The glucuronide metabolites are mostly biologically inactive and readily excreted from the cell by efflux transporters and, eventually, from the body *via* bile or urine[1]. Mammalian UGTs could be divided, based on evolutionary divergence, into two main families, UGT1 and UGT2, which can be further divided into three subfamilies UGT1A, UGT2A and UGT2B[2]. Another family of UDP-sugar transferases that was reported to conjugate xenobiotics is UGT3. This family contains two members and uses other sugar donor than UDPGA[3], but it will not be further discussed in this review.

Mammalian UGTs are membrane-bound enzymes that are localized in the endoplasmic reticulum (ER) and expressed in a tissue-specific way. Many UGTs are highly expressed in the liver, the most important tissue for xenobiotics metabolism, but some are also expressed in extrahepatic tissues, including intestine, kidney, stomach and lung. In human, ten different UGTs are significantly expressed in the liver at the protein level, namely UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A9, UGT2B4, UGT2B7, UGT2B10, UGT2B15 and UGT2B17, of which UGT2B7 is expressed to the highest level, followed in order of expression level by UGT1A1, UGT2B4, UGT2B15, UGT1A4, UGT2B10, UGT1A9, UGT2B17, UGT1A6, and UGT1A3. Six UGTs were detected in the human intestine, UGT1A1, UGT1A10, UGT2B7, UGT2B17 and very low levels of UGT1A3 and UGT1A4, whereas in the human kidney only three UGTs were detected at the protein level, UGT1A9, UGT2B7 and UGT1A6[4].

Among the human UGTs, UGT1A1 is of particular clinical significance due to its unique activity in the conjugative detoxification of bilirubin, the endogenous by-product of heme metabolism (Fig. 2)[5]. Alongside its essential role in bilirubin metabolism, contribution and involvement in the glucuronidation of few other endobiotics[6], UGT1A1 also participates in the metabolism and detoxification of clinical drugs, such as etoposide, SN-38 (the active metabolite of CPT-11) and other xenobiotics, including environmental toxicants and chemical carcinogens[7]. Importantly, many studies have clearly demonstrated that genetics and environmental factors, could affect the expression or the function of UGT1A1, eventually

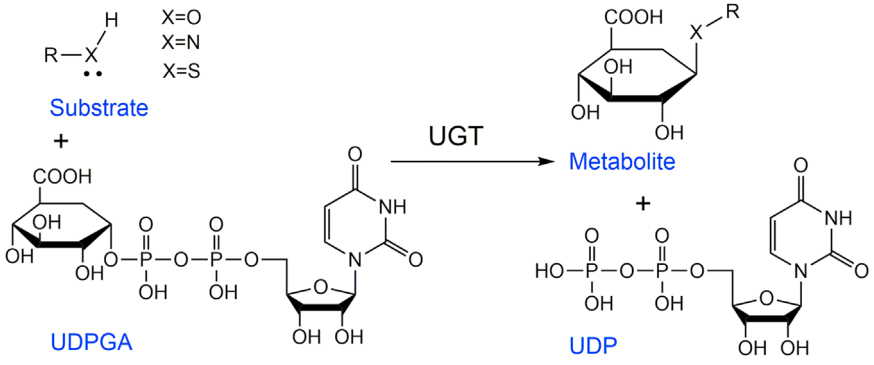


Figure 1 A schematic presentation of UGT-catalysed glucuronidation reactions. Glucuronidation is a bi-substrate reaction that requires an aglycone (for example, a phenol) and a glucuronic acid donor (UDPGA).

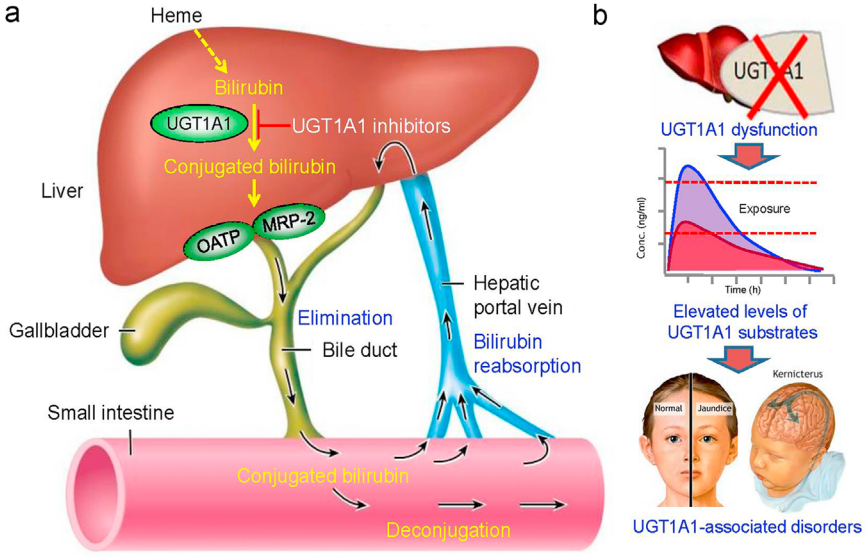


Figure 2 The elimination pathways of bilirubin (a), and disorders associated with UGT1A1 deficiency or absence, including the elevated plasma concentrations and exposure of UGT1A1 substrates, hyperbilirubinemia, and kernicterus (b).

leading to reduced protein level or activity of UGT1A1[8]. Notably, UGT1A1 is a highly polymorphic enzyme with more than one hundred variants, some of which are within the promoter region, like the rather common variant UGT1A1*28. Commonly, the polymorphic variants of UGT1A1 result in lower expression level, lower activity of the enzyme or even complete activity loss[9]. As shown in Fig. 2, the reduced expression/activity of UGT1A1 may increase the plasma concentrations of unconjugated bilirubin, leading to hyperbilirubinemia[10] from the mild Gilbert's syndrome up to kernicterus and the potentially fatal Crigler-Najjar syndrome type I[11–14]. In addition, polymorphic variants of UGT1A1 may lead to drug-induced liver injury. It has been reported that patients possessing the UGT1A1*28 genotype are at a greater risk for irinotecan-induced toxicities, such as severe diarrhea and grades 4 neutropenia[15].

Genetic polymorphisms are important, but are not the only factor and sometimes not the main one, from the general population point of view. Small-molecule inhibitors of UGT1A1 may profoundly influence the catalytic activity of UGT1A1, thereby triggering undesirable effects, like drug/herb–drug interactions (D/HDI) or drug/herb–endobiotic interactions[16]. It has been reported that some therapeutic drugs (*e.g.*, indinavir, nilotinib and sorafenib) or herbal extracts (such as milk thistle, green tea and psoralea corylifolia), as well as several natural compounds in herbs or foods (such as amentoflavone, licochalcone A and emodin) are potent UGT1A1 inhibitors, which could significantly inhibit their activities and lead to undesirable effects[17–25]. Notably, subjects with UGT1A1 polymorphic variants that already possess lower activity due to lower expression level of the enzyme, such as homozygous carriers of the UGT1A1*28 genotype, might be expected to manifest higher susceptibility to adverse effects when co-administrated with potent UGT1A1 inhibitors[26,27]. Thus, the major regulatory agencies, the Food and Drug Administration (FDA) of US and European Medicines Agency (EMA), have recommended to study the inhibition potentials of investigational new drugs on human UGT1A1 before the drug is approved[28,29].

Considering that UGT1A1 plays a pivotal role in endobiotic homeostasis, in addition to its contribution to xenobiotic disposition and detoxification, it was highly necessary to develop practical methods for precise measurements of UGT1A1 activity in complex biological samples. Probe substrates are often used for sensing the real activities of a target enzyme in complex biological systems under physiological conditions. An ideal probe substrate for a target enzyme is expected to be highly specific and reliable, as well as highly sensitive and capable for high-throughput detection. Unfortunately, currently commercially available substrates for UGT1A1 (such as bilirubin, estradiol, and etoposide) have different limitations, such as poor selectivity, poor chemical stability or their use for high-throughput screening (HTS) is unfeasible[30,31]. Thus, it was necessary to develop one or a few more practical probe substrates for this key conjugative enzyme.

In contrast to non-fluorescent probes, fluorescent substrates for target enzyme(s) have inherent advantages, such as high sensitivity and applicability to HTS[32–43]. Recently, significant breakthroughs have been made in the development of fluorescent probes for UGT1A1, and several fluorescent probes that are highly selective for UGT1A1 activities in complex biological samples have been successfully developed (Fig. 3[44]). These fluorescent substrates provide novel tools for HTS and characterization of UGT1A1 inhibitors using fluorescence-based assays[36,41,44]. Furthermore, recent investigations on UGT1A1–ligand interactions have demonstrated that UGT1A1 has multiple ligand-binding sites[45,46]. Thus, developing site-specific probes, *i.e.*, different one for each ligand binding site within UGT1A1, would be highly beneficial for deciphering the interactions between UGT1A1 and its different ligands.

Considering the crucial role of UGT1A1 in bilirubin metabolism, strong inhibition of this conjugative enzyme by xenobiotics,

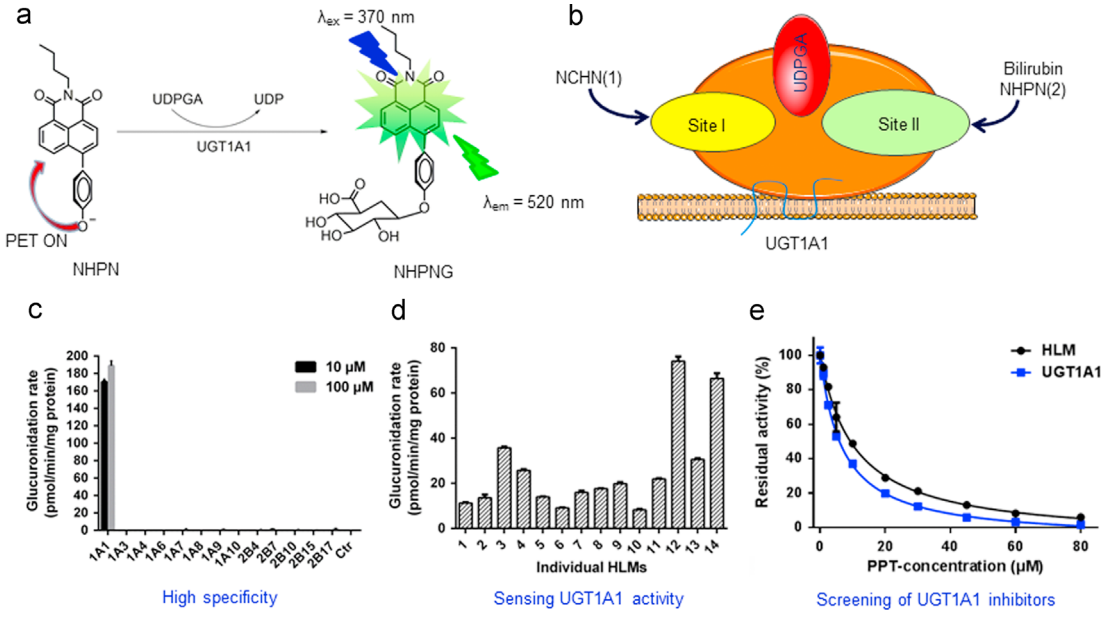


Figure 3 Recent progresses in the development of fluorescent probe substrates for UGT1A1. (a) The structure of *N*-butyl-4-(4-hydroxyphenyl)-1,8-naphthalimide (NHPN) and its proposed mechanism for sensing UGT1A1 activity. (b) A schematic suggestion for the ligand-binding sites of NPHN and *N*-(3-carboxypropyl)-4-hydroxy-1,8-naphthalimide (NCHN) on UGT1A1. (c) The formation rates of NHPN-*O*-glucuronide in various human UGT isoforms. (d) NHPN-*O*-glucuronidation rate (*i.e.*, UGT1A1 activity) in 14 individual human liver microsomes (HLMs). (e) Dose-dependent inhibition curve of NHPN-*O*-glucuronidation by 20(*S*)-protopanaxatriol (PPT), using both HLM and recombinant UGT1A1 as the enzyme sources. Adapted with permission from the Ref. 44. © 2017 American Chemical Society.

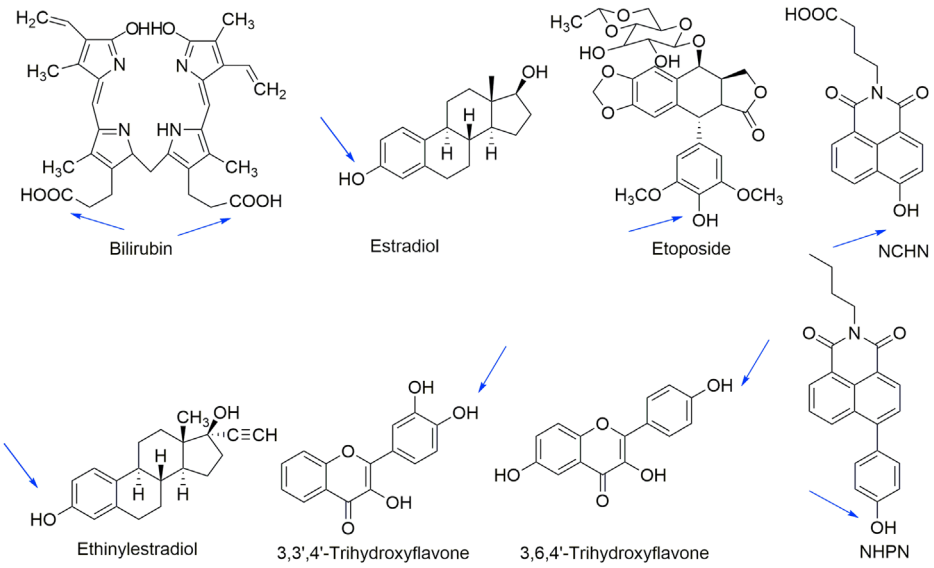


Figure 4 Probe substrates for UGT1A1. The blue arrows indicate conjugation sites by UGT1A1.

such as drugs, food/herbal constitutes, and environmental toxins, may trigger hyperbilirubinemia. Furthermore, inhibition of UGT1A1 may also lead to clinically significant DDI/HDI due to the key roles of UGT1A1 in the detoxification of several drugs with narrow therapeutic windows, such as SN-38 and etoposide[30,47]. Over the past twenty years, a variety of probe substrates for UGT1A1 have been reported, making the screening and evaluation of UGT1A1 inhibitors more convenient and efficient.

This review focuses on the significance, progress and challenges in the discovery and characterization of UGT1A1 inhibitors from therapeutic drugs, environmental toxins, herbal extracts and natural compounds. Recent advances in the development of UGT1A1 probe substrates and their applications for screening and characterization of UGT1A1 inhibitors are discussed and summarized for the first time. Furthermore, a long list of UGT1A1 inhibitors, including information on their inhibition potency, inhibition mode, and affinity, has been analysed and summarized. In addition, challenges and future directions in this field are highlighted in the final section. The information and knowledge that are presented in this review provide a good guidance for rational use of clinical drugs or herbal medicines, in order to avoid the occurrence of adverse side effects *via* UGT1A1 inhibition. The accumulated knowledge is also expected to facilitate investigations on UGT1A1–ligand interactions.

2. Recent progress in the development of UGT1A1 probe substrates

A successful screening for inhibitors of a specific target enzyme(s), and their subsequent and characterization, requires suitable probe substrates. Unfortunately, the number of probe substrates for the human UGT1A1, particularly good ones, was previously very limited since most of them were either not specific enough for use in tissue or cell preparations (such as human liver microsomes, HLM), not sufficiently sensitive, chemically unstable, or too difficult to work with for practical reasons such as poor solubility. The probe substrates that are presented in Fig. 4 and Table 1

Table 1 Probe substrates for human UGT1A1.

Probe substrate	Reaction product	Reaction detection	Feature
4-MU	4-MU-7-*O*-glucuronide	UV	Nonspecific, only recombinant UGT1A1 could be as the enzyme source
Bilirubin	Bilirubin-8-*O*-glucuronide Bilirubin-12-*O*-glucuronide	LC–MS/MS	Highly specific, difficult to work with, reaction yields both mono- and di-glucuronides
17β-Estradiol	β-Estradiol-3-*O*-glucuronide	LC–MS/MS	Conjugated at the 17-OH by UGT2B7 and UGT2B17 in HLM
Ethinylestradiol	Ethinylestradiol-3-*O*-glucuronide	LC–MS	Reaction yields another 17-*O*-glucuronide
Etoposide	Etoposide-4′-*O*-glucuronide	LC–MS	Highly specific in HLM, reaction yields another two alcoholic glucuronides in HLM and human intestine microsomes (HIM)
3,3′,4′-Trihydroxyflavone	3,3′,4′-Trihydroxyflavone-4′-*O*-glucuronide	UV	Highly specific. Conjugated at both the 3- and 3′-OH in HLM
3,6,4′-Trihydroxyflavone	3,6,4′-Trihydroxyflavone-4′-*O*-glucuronide	UV	Highly specific. Conjugated at both the 3- and 6-OH in HLM
NCHN	NCHN-4-*O*-glucuronide	Fluorescence	Highly specific in HLM, the specificity in HIM is currently unclear
NHPN	NHPN-19-*O*-glucuronide	Fluorescence	Highly specific in HLM, only one conjugation site

Table 2 Kinetic parameters of probe substrates for human UGT1A1[30,36,41,44,48–53].

Probe substrate	Enzyme source	Kinetic parameter		Kinetic behavior	Ref.
		K_m *or* S_{50} (μmol/L)	V_{max} (pmol/min/mg)		
4-MU	UGT1A1	113	308	Michaelis-Menten	48
Bilirubin	UGT1A1	0.1	70	Michaelis-Menten	49
	HLM	0.3	210	Michaelis-Menten	49
17β-Estradiol	UGT1A1	13	1300	Hill	50
	HLM	11	820	Hill	50
Ethinylestradiol	UGT1A1	9.7	600	Hill	51
	HLM	13	1200	Hill	51
Etoposide	UGT1A1	285	124	Michaelis-Menten	30
	HLM	530	110	Michaelis-Menten	30
3,3′,4′-Trihydroxyflavone	UGT1A1	1.53	1920	Substrate Inhibition	53
	HLM	1.75	1990	Substrate Inhibition	53
3,6,4′-Trihydroxyflavone	UGT1A1	0.76	340	Substrate Inhibition	53
	HLM	0.83	340	Substrate Inhibition	53
2-Me-4-OMe TG	UGT1A1	2.7	16.1[a]	Michaelis-Menten	41
NCHN	UGT1A1	126.7	1303	Substrate Inhibition	36
	HLM	364.6	1556	Substrate Inhibition	36
NHPN	UGT1A1	0.7	561	Hill	44
	HLM	4.3	557	Hill	

[a]nmol/L/s.

include both "classical" substrates, such as bilirubin and ethinylestradiol, as well as the new generation of fluorescent substrates probes, namely *N*-(3-carboxypropyl)-4-hydroxy-1,8-naphthalimide (NCHN) and *N*-butyl-4-(4-hydroxyphenyl)-1,8-naphthalimide (NHPN). The kinetic properties of these and the other probes in Fig. 4 and Table 1 are listed in Table 2[30,36,41,44,48–53]. As can be seen from Fig. 4, there is a large variability in chemical structure among the UGT1A1 probe substrates, as it might be expected from an enzyme, like UGT1A1, that has a wide spectrum of substrates. The human UGT1A subfamily members share high amino acid sequence homology (>65%), and their substrate specificity frequently overlap, even if at variable kinetic constants. In fact, many UGT1A1 substrates could be glucuronidated by multiple UGT enzymes, a good example for which is the C-7 phenolic coumarin derivatives, such as 4-methyl-umbelliferone (4-MU)[54]. It is also noteworthy that no high-resolution crystal structure of the substrate binding domain of UGT1A1, or any other mammalian UGT, has been reported yet. Some UGT homology models[55], were derived from structures of microbial UGTs, but they are not sufficient for the simulation of ligands–UGT1A1 interactions or the design of specific substrates for a given human UGT enzyme. Thus, the rational design and development of highly specific probe substrates for UGT1A1, or other UGT enzymes, are very challenging undertakings. This is further true when the goal is to design optical probe substrates with high specificity and sensitivity, which are suitable for high throughput detection or screening.

Several commercial small molecules that are known to be UGT1A1 substrates, such as bilirubin, estradiol, and ethinylestradiol, have been used and might still be in use for testing its activity, but mostly some disadvantages are associated with them[31,56]. The specificity of UGT1A1 for the formation of estradiol-3-*O*-glucuronide or ethinyloestradiol-3-*O*-glucuronide is poor[57]. Moreover, when it is tested in a complex system, such as HLM, and the substrate is estradiol, other UGTs conjugate the same "probe" at the 17-OH more efficiently[58]. Although bilirubin is a UGT1A1 specific substrate that is hardly conjugated by any other human UGT, it is difficult to use bilirubin as a probe for screening and characterization of UGT1A1 inhibitors due to its high light-sensitivity and the formation of both mono- and di-glucuronides[59]. Furthermore, the above-mentioned probe substrates for UGT1A1 are non-fluorescent, making them less suitable for HTS. The glucuronidation rates of these commonly used probes were routinely detected by liquid chromatography combined with UV or mass spectrometry detectors, requiring relatively long time for sample preparation and analysis.

In contrast to these commonly used "old generation" probe substrates for UGT1A1, a new generation of fluorescent probes for UGT1A1 has been emerging in recent years, despite great challenges in the design and development of practical fluorescent probes for any given UGT enzyme. Notably, the fluorescence properties of many fluorophores are often "turned-off" or "blue shifted" following *O*-glucuronidation at the hydroxyl group, which is not highly beneficial for precise measurement of the target enzyme in complex biological samples, due to the background signals from the biological matrix. Five years ago, Terai and coworkers[41] have developed the first "turn-on" fluorescent probes for UGT1A1 with high sensitivity, which was a landmark achievement in this field, even if the specificity of these probes toward UGT1A1 of that compound was only partial. This type of fluorescent probes is intensity-based compounds, designed to work by a mechanism of donor-excited photoinduced electron transfer (d-PET), using TokyoGreen as the fluorophore. More recently, a ratiometric fluorescent probe (NCHN) for UGT1A1 has been developed by us, providing a novel tool for rapid screening of UGT1A1 modulators using microplate reader-based assays[36]. In HLM, NCHN was highly specific for UGT1A1, even if its affinity for this conjugative enzyme was low[36]. This was probably the reason that the binding site of NCHN on UGT1A1 was not highly consistent with that of bilirubin[60]. Recent investigations on inhibition kinetics of UGT1A1 clearly demonstrated that the binding site of NCHN on UGT1A1 was distinct from that of the high affinity binding site of bilirubin, the most important

physiologically relevant substrate for UGT1A1[60]. Taking into account that UGT1A1 may have multiple ligand binding sites, a second practical and, this time high affinity fluorescent probe for UGT1A1 (NHPN) was subsequently designed and well-characterized by us. Unlike the previous substrate NCHN, the new fluorescent probe, NHPN, could serve as a good surrogate for bilirubin to investigate UGT1A1–ligand interactions[44]. Now both fluorescent probes, NCHN and NHPN, were successfully applied for selectively sensing UGT1A1 activities and for HTS, including characterization of UGT1A1 modulators in complex biological samples. Nevertheless, this is not the end of the work since the situation in the small intestine, where UGT1A10 is expressed, might be somewhat different[61]. Hence, further investigations on the rational design and development of fluorescent probes for UGT1A1 (specific in both liver and intestine) with high specificity, good practicability and excellent optical properties (such as long wavelength probes) are still desirable.

Another way to try and learn about the binding sites of the fluorescence probes NCHN and NHPN was by docking them into a homology model of the human UGT1A1. Such a model was previously constructed using the crystal structure of UDP-glucosyltransferases from other sources as the template[55], and it might help to explore the potential interactions between UGT1A1 and ligands, especially potential ligand binding sites of UGT1A1. Hence, a homology model of the human UGT1A1 was constructed using the crystal structure of UDP-glucosyltransferase GtfB (PDB code: 1IIR) as the template, followed by molecular docking simulations in which either bilirubin, NCHN or NHPN was allowed to interact with UGT1A1[44]. The results demonstrated that NCHN and NHPN could bind on UGT1A1, but at two different ligand-binding sites, whereas the ligand-binding site of NHPN on UGT1A1 was identical to that of bilirubin (Fig. 5)[44]. Nevertheless, it is important to remember that the "useful resolution" of such models for substrates design may be limited.

Another approach is the design and synthesis of a series of derivatives with various hydrophobic, acidic and basic groups. The already available UGT1A1 specific probes are very useful in this respect since they give both some starting points for the synthesis, as well as easy ways to screen the interactions of the products with UGT1A1. In any case, since UGT1A1 was reported to have more than one ligand-binding site[31,46], it is advantageous to use multiple probe substrates for screening and characterization of UGT1A1 inhibitors (or activators) for deeper understanding of UGT1A1-mediated D/HDI or drug/herb–endobiotic interactions.

3. UGT1A1 inhibitors

With the development of small molecular probes for UGT1A1, an increasing number of compounds, from different sources and with diverse structures have been discovered and identified as inhibitors of UGT1A1. Due to potent inhibition of the human UGT1A1 by drugs or herb ingredients, several adverse D/HDIs and metabolic disorders that are related to therapeutic drugs and herbs, have been reported. In the present review, we have focused on identified UGT1A1 inhibitors from xenobiotics and their inhibition potency. Thus, many drugs, environmental toxins, herb extracts and natural compounds displaying potent inhibition of UGT1A1 are summarized and discussed.

3.1. *Therapeutic drugs as UGT1A1 inhibitors*

3.1.1. *Protease inhibitors*

Protease inhibitors that were reported to inhibit UGT1A1 are listed in Table 3[24,62–66], along with the available inhibition properties. Several human immunodeficiency virus (HIV) protease inhibitors, including atazanavir, and indinavir, are associated with unconjugated hyperbilirubinemia[24,67]. The study by Zucker and coworkers[24] discovered that the underlying mechanism of indinavir-induced hyperbilirubinemia in patients was a strong competitive inhibition of UGT1A1, including inhibition of bilirubin-*O*-glucuronidation, with a K_i value of 183 μmol/L. Subsequently, Zhang and coworkers[62] reported that six different HIV protease inhibitors, namely atazanavir, indinavir, lopinavir, nelfinavir, ritonavir, and saquinavir, inhibited UGT1A1, at IC_{50} values ranging from 2.3 to 87 μmol/L. A mixed-type inhibition kinetic mechanism was observed for UGT1A1 inhibition by atazanavir and indinavir, with K_i values of 1.9 and 47.9 μmol/L, respectively. In addition, *in vitro–in vivo* extrapolation (IVIVE) results indicated that atazanavir and indinavir are likely to induce hyperbilirubinemia[62]. Efavirenz, a non-nucleoside reverse transcriptase inhibitor that is used for the treatment of HIV type 1 infection, was demonstrated to moderately inhibit UGT1A1-mediated estradiol-3-*O*-glucuronidation in HLM in a noncompetitive manner, with a K_i value of 40.3 μmol/L[63]. An $[I]/K_i$ ratio of 0.32 suggested that the UGT1A1-

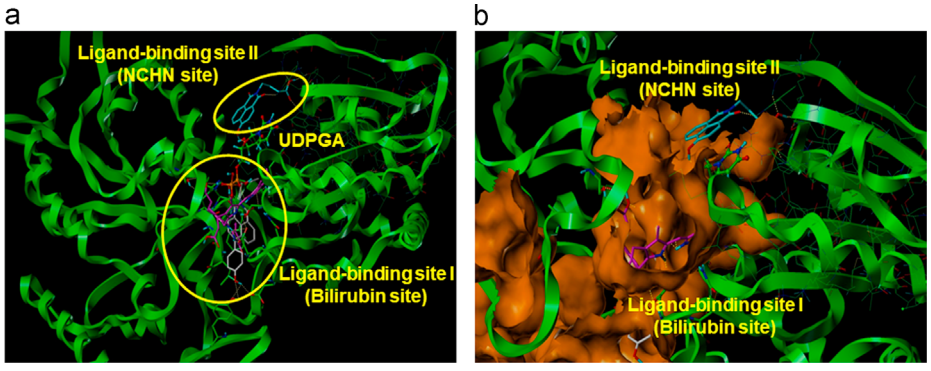


Figure 5 A structural model showing the proposed binding site of bilirubin, NCHN and NHPN in human UGT1A1. Carbon atoms in the three molecules are colored in magenta, cyan and white, respectively. UDPGA is shown in ball and stick type. (a) A stereo view and (b) a detailed view of the crystal structure of modelling UGT1A1 and the stereo diagram of bilirubin, NCHN and NHPN aligned in its corresponding ligand-binding site. Adapted with permission from the Ref. 44. © 2017 American Chemical Society.

Table 3 The inhibitory effects of protease inhibitors on UGT1A1[24,62–66].

Inhibitor	Substrate	Enzyme source	IC_{50} (μmol/L)	K_i (μmol/L)	Inhibition type	Ref.
Atazanavir	Bilirubin	UGT1A1	2.3	1.9	Mixed	24, 62
		HLM	2.5	1.3	–	
Indinavir	Bilirubin	UGT1A1	87	47.9	Mixed	
		HLM	68	34	–	
		UGT1A1	–	183	Competitive	
Saquinavir	Bilirubin	UGT1A1	13	360	Competitive	
		HLM	5.0	2.5	–	
Lopinavir	Bilirubin	UGT1A1	8.6	–	–	
Ritonavir	Bilirubin	UGT1A1	19	–	–	
Nelfinavir	Bilirubin	UGT1A1	11	–	–	
		HLM	2.7	1.4	–	
Paritaprevir	Ethinylestradiol	HLM	14.5	20	Competitive	64
Efavirenz	*β*-Estradiol	HLM	45.9	40.3	Non-competitive	63
	β-Estradiol	UGT1A1	33.8	–	–	
Faldaprevir	*β*-Estradiol	HLM	0.45	–	–	66
Rifabutin	*β*-Estradiol	HLM	35[a]	–	–	65
Rifampicin	*β*-Estradiol	HLM	70[a]	–	–	

–Not determined.
[a]Incubations with preincubation.

mediated DDI is possible when the high doses of efavirenz are used[63]. A number of protease inhibitors that are available for hepatitis C virus (HCV) treatment, including faldaprevir, telaprevir, simeprevir, daclatasvir, and asunaprevir, could also trigger unconjugated hyperbilirubinemia in the clinic. Faldaprevir strongly inhibited UGT1A1-mediated estradiol-3-*O*-glucuronidation, and since the IC_{50} value was 0.45 μmol/L, that inhibition is likely to contribute significantly to the hyperbilirubinemia that was observed in patients that had been treated with faldaprevir ($I/IC_{50} > 1$)[66]. Paritaprevir was reported to competitively inhibit UGT1A1, at a K_i value of 20 μmol/L[64]. However, due to the low plasma concentration of paritaprevir, it was unlikely to trigger unconjugated hyperbilirubinemia. Rifabutin and rifampicin, two anti-tuberculosis drugs, exhibited moderate inhibitory effects on UGT1A1, with IC_{50} values of 35 and 70 μmol/L, respectively[65]. However, considerable inhibitory effects of rifabutin and rifampicin on UGT1A1 are unlikely since the IC_{50} values of the drugs were much higher than their maximum plasma concentration (C_{max}).

3.1.2. *Tyrosine kinase inhibitors (TKIs)*

Tyrosine kinase inhibitors (TKIs) that exhibit strong to moderate inhibitory effects on UGT1A1 are listed in Table 4[68–74]. Most of the TKIs, including nilotinib, regorafenib, sorafenib, pazopanib, lapatinib, erlotinib, gefitinib and icotinib, have been implicated in the development of hyperbilirubinemia, for which UGT1A1 inhibition is the most likely cause. Lapatinib, pazopanib, regorafenib and sorafenib were reported to strongly inhibit UGT1A1-mediated bilirubin glucuronidation, with IC_{50} values ranging between 0.034 and 3.734 μmol/L[68]. Furthermore, regorafenib and sorafenib displayed competitive inhibition of estradiol-3-*O*-glucuronidation by UGT1A1, and the K_i values in these cases were 0.02 and 0.033 μmol/L, respectively[68]. IVIVE results indicated that UGT1A1 inhibition by regorafenib and sorafenib, but not lapatinib and pazopanib, probably contributed significantly to the hyperbilirubinemia that was observed in the patients. Liu and coworkers[74] have reported that erlotinib and gefitinib competitively inhibit UGT1A1-mediated 4-MU glucuronidation, at K_i values of 0.64 and 2.42 μmol/L, respectively. However, when bilirubin was the substrate, erlotinib exerted a mixed inhibition pattern, with a K_i value of 2.97 μmol/L. In addition, IVIVE results indicated that coadministration of erlotinib at clinical doses with another drug that is predominantly cleared by UGT1A1 may trigger a significant increase in the areas under the plasma drug concentration–time curve (AUC) of the other drug, namely DDI[74]. Nilotinib was demonstrated to inhibit UGT1A1 at K_i values ranging between 0.079 and 0.53 μmol/L. Importantly, the inhibition of UGT1A1 by nilotinib could result in a significant increase in the AUC of SN-38, as well as an increased hyperbilirubinemia at high rate[23,69]. The increased AUC of SN-38 due to nilotinib coadministration may serve as a good example for DDI. Recently, the inhibition of UGT1A1 by icotinib and erlotinib (two compounds with similar chemical structure and physico-chemical properties) were compared and investigated[72]. Both icotinib and erlotinib inhibited NCHN-*O*-Glucuronidation by UGT1A1 noncompetitively. In this case the K_i value displayed by icotinib, 10.04 μmol/L, was clearly higher than the corresponding value of erlotinib, 1.72 μmol/L[72]. IVIVE results indicated that erlotinib had a much higher DDI potential, while icotinib is unlikely to cause a significant DDI *via* UGT1A1 inhibition[72]. Both imatinib and lapatinib were reported to be competitive inhibitors of UGT1A1-mediated 4-MU glucuronidation, with K_i values of 19.1 and 0.5 μmol/L, respectively[70]. In addition, IVIVE results indicated that coadministration of imatinib or lapatinib at clinical doses might trigger a significant increase in the AUC of drugs that are predominantly cleared by UGT1A1. It was also found that dasatinib and imatinib inhibited UGT1A1-mediated paracetamol glucuronidation, at IC_{50} values of 2 and 29 μmol/L, respectively[73]. The $[I]/K_i$ ratio results indicated that at clinical relevant doses, imatinib could result in a 22% increase in the AUC of coadministrated paracetamol *via* UGT1A1 inhibition, while dasatinib could cause only a slight increase in the AUC (6%)[73].

3.1.3. *Other drugs*

Other drugs that display inhibitory effects on UGT1A1 are listed in Table 5[60,75–83]. Tolcapone and entacapone, catechol-*O*-methyltransferase inhibitors that are used as adjunct in the treatment of Parkinson's disease, have similar skeleton, but the use of tolcapone

Table 4 The inhibitory effects of tyrosine kinase inhibitors on UGT1A1[68–74].

Inhibitor	Substrate	Enzyme source	IC_{50} (μmol/L)	K_i (μmol/L)	Inhibition type	Ref.
Nilotinib	4-MU	UGT1A1	–	0.17	Competitive	
	β-Estradiol	HLM	–	0.53	Non-competitive	
	β-Estradiol	UGT1A1	–	0.14	Non-competitive	
	SN-38	HLM		0.286	Non-competitive	69
	SN-38	UGT1A1		0.079	Non-competitive	
Regorafenib	β-Estradiol	HLM	–	0.020	Competitive	68,70,71
	4-MU	UGT1A1	0.045	–	–	
	NCHN	HLM	0.26	0.48	Non-competitive	
	NCHN	UGT1A1	0.25	0.33	Non-competitive	
	NPHN	HLM	0.40	0.048	Competitive	
	NPHN	UGT1A1	0.17	0.027	Competitive	
	Bilirubin	UGT1A1	0.034	–	–	
Sorafenib	β-Estradiol	HLM	–	0.033	Competitive	
	4-MU	UGT1A1	0.066	–	–	
	Bilirubin	UGT1A1	0.048	–	–	
Pazopanib	β-Estradiol	HLM	–	2.34	Competitive	
	4-MU	UGT1A1	1.1	–	–	
	Bilirubin	UGT1A1	37.34	–	–	
Lapatinib	β-Estradiol	HLM	–	0.567	Competitive	
	4-MU	UGT1A1	0.536	–	–	
	4-MU	UGT1A1	0.5	0.5	Competitive	
	Bilirubin	UGT1A1	0.467	–	–	
Erlotinib	4-MU	UGT1A1	–	0.64	Competitive	72,74
	Bilirubin	HLM	4.19	2.97	Mixed	
	NCHN	HLM	0.68	1.23	Non-competitive	
	NCHN	UGT1A1	0.69	1.72	Non-competitive	
Gefitinib	4-MU	UGT1A1	–	2.42	–	
	Bilirubin	HLM	>100	–	–	
Icotinib	NCHN	HLM	5.15	8.55	Non-competitive	72
	NCHN	UGT1A1	8.76	10.04	Non-competitive	
Axitinib	4-MU	UGT1A1	–	–	–	70,73
Imatinib	4-MU	UGT1A1	11.0	19.1	Competitive	
	Paracetamol	UGT1A1	29	–	–	
Vandetanib	4-MU	UGT1A1	–	–	–	
Dasatinib	Paracetamol	UGT1A1	2	–	–	73

–Not determined.

was suspended due to its higher toxicity, and it was replaced by entacapone[84,85]. We have reported that tolcapone (also) displayed relative stronger inhibitory effects on UGT1A1 in comparison with entacapone, and that the use of tolcapone may result in hyperbilirubinemia or significant increase in AUC of drugs that are primarily metabolized by UGT1A1[60]. Levothyroxine, which is used for the treatment of thyroid hormone deficiency, functions as a competitive inhibitor of UGT1A1-mediated 4-MU glucuronidation, at a K_i value of 1.0 μmol/L[75]. Ketoconazole, an antifungal agent, was demonstrated to inhibit UGT1A1 mediated SN-38 glucuronidation in a competitive manner, with a K_i value of 3.3 μmol/L[76]. The increased exposure of SN-38 and reduced formation of SN-38G might be primarily due to UGT1A1 inhibition by ketoconazole, when ketoconazole and irinotecan were coadministered[76]. Vitamin A (retinol) is an important micronutrient in both vegetal and animal diets. A recent study has revealed that vitamin A also exerts competitive inhibition of UGT1A1-mediated 4-MU-glucuronidation, with a K_i value of 31.1 μmol/L[77]. The $[I]/K_i$ value of 3.1 suggested that inhibition of UGT1A1 by vitamin A may exist *in vivo*[77]. It was previously found that among the seven non-steroidal antiinflammatory drugs (NSAID) that were investigated, acetaminophen, diclofenac, diflunisal, indomethacin, ketoprofen, naproxen and niflumic acid, it was niflumic acid that exhibited the highest inhibitory effects on UGT1A1-catalyzed estradiol-3-*O*-glucuronidation in HLM, with an IC_{50} value of 22.2 μmol/L[78]. More recently, everolimus, an inhibitor of the mammalian target of rapamycin (mTOR) that is utilized to prevent rejection of organ transplants, was characterized as a UGT1A1 inhibitor, with a K_i value of 2.3 μmol/L[79]. The calculated $[I]/K_i$ value in this case was 0.004, indicating that everolimus is unlikely to cause clinically significant DDI *via* UGT1A1 inhibition *in vivo*[79]. Diethylstilbestrol, a widely used toxic synthetic estrogen, is a strong competitive inhibitor of UGT1A1-catalyzed estradiol-3-*O*-glucuronidation in HLM, with a K_i value of 2.1 μmol/L[80], and of the UGT1A1-mediated 4-MU-glucuronidation, also competitively, with a K_i value of 3.7 μmol/L[81]. Based on the inhibition constant K_i and plasma concentrations values that were reported for diethylstilbestrol in the literature (C_{max} 24 μmol/L), it is likely to inhibit UGT1A1 *in vivo,* if given intravenously. Zafirlukast, a selective peptide leukotriene receptor antagonist, was found to be a broad specificity UGT inhibitor, with strong inhibiting properties toward UGT1A1 with the K_i value of 1.2 μmol/L[82]. Nonetheless, no DDI *via* UGT1A1 inhibition was predicted for zafirlukast, if the unbound inhibitor concentration was used[82]. Canagliflozin (CNF) and dapagliflozin (DPF), the first sodium glucose cotransporter 2 inhibitors, were reported to inhibit UGT1A1, but DPF was less potent than CNF in this respect[83]. IVIVE results suggested that CNF could result in a

Table 5 The inhibitory effects of other drugs on UGT1A1[60,75–83].

Inhibitor	Substrate	Enzyme source	IC_{50} (μmol/L)	K_i (μmol/L)	Inhibition type	Ref.
Entacapone	4-MU	UGT1A1	9.10	10.48	Competitive	60
	Bilirubin	HLM	34.97	30.82	Mixed	
	NCHN	HLM	16.92	14.65	Non-competitive	
	NCHN	UGT1A1	12.24	15.59	Non-competitive	
Tolcapone	4-MU	UGT1A1	2.38	1.77	Competitive	
	Bilirubin	HLM	1.24	0.68	Mixed	
	NCHN	HLM	2.07	1.03	Non-competitive	
	NCHN	UGT1A1	1.30	2.39	Non-competitive	
Levothyroxine	4-MU	UGT1A1	–	1.0	Competitive	75
Ketoconazole	SN-38	UGT1A1	–	3.3	Competitive	76
	Bilirubin	HLM	53	–	–	
Vitamin A	4-MU	UGT1A1		31.1	Competitive	77
Diclofenac	β-Estradiol	HLM	60.9	112	Non-competitive	78
	4-MU	UGT1A1	57.5	–	–	
Diflunisal	β-Estradiol	HLM	37.8	–	–	
Indomethacin	β-Estradiol	HLM	51.5	–	–	
Niflumic acid	β-Estradiol	HLM	22.2	–	–	
Everolimus	4-MU	UGT1A1	–	2.3	Competitive	79
Diethylstilbestrol	β-Estradiol	HLM	–	2.1	Competitive	80
	4-MU	UGT1A1	–	3.7	Competitive	81
Zafirlukast	4-MU	UGT1A1	0.7	–	–	82
	SN-38	HLM	–	1.2	Non-competitive	
Canagliflozin	β-Estradiol	UGT1A1	–	7.2	Competitive	83
	β-Estradiol	HLM	–	9.1	Competitive	
Dapagliflozin	β-Estradiol	HLM	–	81	Competitive	
	β-Estradiol	HLM	–	81	Competitive	

–Not determined.

significant increase in the AUC for exclusive UGT1A1 substrates *via* UGT1A1 inhibition, while DNF could not inhibit UGTA1 *in vivo*[83].

3.2. *Environmental toxins as UGT1A1 inhibitors*

Environmental toxins that display inhibitory effects on UGT1A1 are listed in Table 6[86,87]. Gossypol is a polyphenolic compound that is found in cotton seeds and was used as a male anti-fertility drug for a long time. However, the clinical utilization of gossypol has always been strongly limited due to its toxicity, including hepatotoxicity, pathological changes in rat and human testes, abnormal sperm and enzyme inhibition. In an HLM study, gossypol was found to moderately inhibit estradiol-3-*O*-glucuronidation, with an IC_{50} value of 23.5 μmol/L. The inhibition kinetic was noncompetitive inhibition and the K_i value was 34.2 μmol/L[86]. The $[I]/K_i$ ratio of 0.56 suggested that the use of gossypol could cause DDI through inhibition of UGT1A1[86]. Four uremic toxins, benzyl alcohol, *p*-cresol, indoxyl sulfate, hippuric acid, and a combination of these four uremic toxins, were found to inhibit UGT1A1 to variable extents. Among the four uremic toxins, *p*-cresol was the most potent inhibitor of UGT1A1-mediated estradiol-3-*O*-glucuronidation in HLM, with a K_i value of 43 μmol/L[87].

3.3. *Herbal extracts that inhibit UGT1A1*

Many studies have investigated the inhibitory effects of herb extracts on UGT1A1 activity (Table 7[20,21,88–93]). For example, inhibitors were tested among the following eight commonly used herbal extracts, milk thistle, saw palmetto, echinacea, green tea epigallocatechingallate, garlic, ginseng, black cohosh, and valerian, of which milk thistle, saw palmetto, echinacea, and epigallocatechingallate exhibited the highest inhibition potency of UGT1A1-mediated estradiol-3-*O*-glucuronidation, with IC_{50} values between 7.8 and 211.7 μg/mL[21]. A volume per dose index (VDI) values suggested that inhibition of intestinal UGT1A1 by epigallocatechingallate and milk thistle, and to a lesser extent by saw palmetto and echinacea, may be clinically relevant[21]. *Andrographis paniculata* and *Orthosiphon stamineus* extracts displayed inhibitory effects on UGT1A1-mediated 4-MU-glucuronidation, with IC_{50} values of 5.0 and 24.65 μg/mL, respectively[88]. *Polygonum multiflorum* extracts exhibited strong inhibitory effects on UGT1A1-mediated bilirubin glucuronidation in HLM and in rat liver microsomes (RLM), with K_i values of 1.6 and 0.3 μmol/L, respectively[89]. Herbal extract of Daio, Kanzo, Keihi, and Ogon strongly inhibited UGT1A1-mediated estradiol-3-*O*-

Table 6 The inhibitory effects of environmental toxins on UGT1A1[86,87].

Inhibitor	Substrate	Enzyme source	IC_{50}(μmol/L)	K_i (μmol/L)	Inhibition type	Ref.
Gossypol	β-Estradiol	HLM	23.5	34.2	Non-competitive	86
P-Cresol	β-Estradiol	HLM	–	43	Competitive	87

–Not determined.

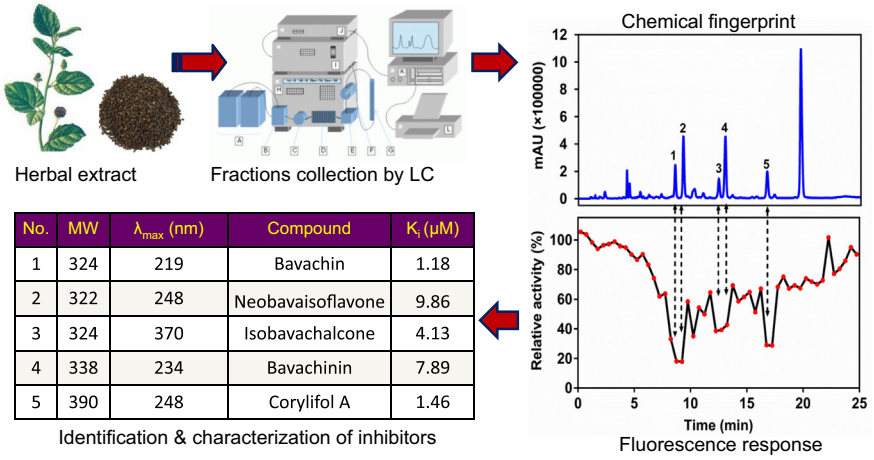
Table 7 Inhibitory effects of herbal extracts on UGT1A1[20,21,88–93].

Inhibitor	Substrate	Enzyme source	IC_{50} (μg/mL)	K_i (μg/mL)	Inhibition type	Ref.
Milk thistle	*β*-Estradiol	HLM	30.4	–	–	21
Saw palmetto	*β*-Estradiol	HLM	55.2	–	–	
Echinacea	*β*-Estradiol	HLM	211.7	–	–	
Epigallocatechingallate	*β*-Estradiol	HLM	7.8	–	–	
Ginseng	*β*-Estradiol	HLM	603[a]	–	–	
Black cohosh	*β*-Estradiol	HLM	299[a]	–	–	
Valerian	*β*-Estradiol	HLM	562[a]	–	–	
Psoraleae Fructus	NCHN	HLM	12.5	–	–	20
Andrographis paniculata	4-MU	UGT1A1	5.0	–	–	88
Orthosiphon stamineus	4-MU	UGT1A1	24.65	–	–	
Polygonum multiflorum	Bilirubin	HLM	–	1.6[b]	Competitive	89
		RLM	–	0.3[b]	Competitive	
Rhei Rhizoma (Daio)	*β*-Estradiol	HLM	–	30	Mixed	90
	SN-38	HLM	–	68	Mixed	
Glycyrrhizae Radix (Kanzo)	*β*-Estradiol	HLM	–	27	Mixed	
	SN-38	HLM	–	95	Mixed	
Cinnamomi Cortex (Keihi)	*β*-Estradiol	HLM	–	33	Competitive	
	SN-38	HLM	–	105	Mixed	
Scutellariae Radix (Ogon)	*β*-Estradiol	HLM	–	23	Competitive	
	SN-38	HLM	–	80	Mixed	
Blueberry	*β*-Estradiol	HLM	62.4	53.1	Competitive	91
Dioscorea nipponica	*β*-Estradiol	HLM	302.4	–	–	92
Ginseng	*β*-Estradiol	HLM	14.5	–	–	93

–Not determined.
[a]Estimated IC_{50}.
[b]Unit in μmol/L.

glucuronidation and UGT1A1-mediated SN38-*O*-glucuronidation in HLM, with K_i values between 23 and 105 μg/mL[90]. Blueberry, a commonly consumed berry, weakly and competitively inhibited UGT1A1 at a K_i value of 53.1 μg/mL[91]. IVIVE results suggested that blueberry is unlikely to cause HDI *via* UGT1A1 inhibition *in vivo*. *Dioscorea nipponica* extract very weakly inhibited UGT1A1 activity with an IC_{50} value of 302.4 μg/mL[92], whereas ginseng extract inhibited UGT1A1-mediated estradiol-3-*O*-glucuronidation in HLM, with an IC_{50} value of 14.5 μg/mL[93]. Based on their VDI values, it was suggested that both *Dioscorea nipponica* extract and ginseng extract were unlikely to cause clinically significant HDI *via* UGT1A1 inhibition *in vivo*[92,93].

It was also found that the crude extract of *Fructus psoraleae* (FP, also named Bu-gu-zhi in Chinese) could inhibit UGT1A1 at an IC_{50} value of 12.51 μg/mL[20]. Subsequently, the LC–UV fingerprinting analysis combined with UGT1A1 inhibition profile was successfully used to identity and characterize the naturally occurring inhibitors of UGT1A1 in FP (Fig. 6)[20]. The LC fractions of the FP extract were collected and assayed by a fluorescence-based high-throughput screening method for the discovery of UGT1A1 inhibitors, using NCHN as the specific fluorescent probe substrate for UGT1A1 and HLM as enzyme sources. Five major constitutes from FP, namely bavachin, corylifol A, neobavaisoflavone, isobavachalcone, and bavachinin, were identified as inhibitors of UGT1A1. This study was a good case study



No.	MW	λ_{max} (nm)	Compound	K_i (μM)
1	324	219	Bavachin	1.18
2	322	248	Neobavaisoflavone	9.86
3	324	370	Isobavachalcone	4.13
4	338	234	Bavachinin	7.89
5	390	248	Corylifol A	1.46

Figure 6 The strategy that was employed for the discovery of natural UGT1A1 inhibitors, guided by chemical fingerprint combined with UGT1A1 enzymatic inhibition profile. Note that the K_i values of the five identified compounds were determined using NCHN as the probe substrate and HLM as the enzyme source. Adapted from the Ref. 20 with permission. © 2015 Elsevier B.V.

Table 8 The inhibitory effects of fatty acids on UGT1A1[94].

Inhibitor	Substrate	Enzyme source	IC_{50} (μmol/L)	K_i (μmol/L)	Inhibition type	Ref.
Oleic acid	β-Estradiol	UGT1A1	31.6	23.4	Non-competitive	94
		HLM	–	29.3	Non-competitive	
Linoleic acid	β-Estradiol	UGT1A1	33.1	22.1	Non-competitive	
		HLM	–	24.0	Non-competitive	
Palmitoleic acid	β-Estradiol	UGT1A1	37.1	–	–	
α-Linolenic acid	β-Estradiol	UGT1A1	26.1	–	–	
Arachidonic acid	β-Estradiol	UGT1A1	22.7	–	–	
DHA	β-Estradiol	UGT1A1	11.6	1.8	Non-competitive	
		HLM	–	4.3	Non-competitive	
		HIM	–	5.2	Non-competitive	
EPA	β-Estradiol	UGT1A1	19.9	–	–	
Stearic acid	β-Estradiol	UGT1A1	>50	–	–	
Decanoic acid	β-Estradiol	UGT1A1	>50	–	–	

– Not determined.

for the discovery of UGT1A1 inhibitors from medicinal plants, which would be very helpful for future investigations on UGT1A1-mediated herb–drug interactions.

3.4. *Natural products as UGT1A1 inhibitors*

3.4.1. *Fatty acids*

Fatty acids, an important class of natural products, are carboxylic acids with long straight-aliphatic chains, either saturated or unsaturated, ranging from 4 to 28 carbon atoms in length. The inhibitory effects of 15 saturated and unsaturated fatty acids on UGT1A1-catalyzed estradiol-3-*O*-glucuronidation were investigated. Among the 15 tested fatty acids, 7 displayed strong inhibition, including oleic acid, linoleic acid, docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), palmitoleic acid, arachidonic acid, and α-linolenic acid, with IC_{50} values between 11.6 and 37.1 μmol/L (Table 8)[94]. In addition, oleic acid, linoleic acid, and DHA noncompetitively inhibited estradiol-3-*O*-glucuronidation mediated by both recombinant UGT1A1 and HLM, with K_i values between 1.8 and 29.3 μmol/L. Unlike oleic acid and linoleic acid, however, DHA has a potency to noncompetitively inhibit intestinal estradiol-3-*O*-glucuronidation, with a K_i value of 5.2 μmol/L, probably indicating inhibition of UGT1A10[61]. Interestingly, unsaturated fatty acids exerted strong inhibition against UGT1A1 activity, whereas saturated fatty acid only poorly inhibited UGT1A1 activity. An *in vivo* study demonstrated that low concentrations of DHA result in a significant increase in serum bilirubin *via* UGT1A1 inhibition, while high concentrations of oleic acid, linoleic acid, and DHA cause a decrease in serum bilirubin *via* UGT1A1 induction[94].

3.4.2. *Flavonoids*

Flavonoids that display inhibitory effects on UGT1A1 are listed in Table 9[18,20,25,95–105]. Flavonoids are a class of polyphenolic compounds that are widely distributed in nature and have been developed into drugs, cosmetics and health food due to various pharmacological properties[106]. Five major flavonoids components of FP, bavachin, corylifol A, neobavaisoflavone, isobavachalcone, and bavachinin (see Fig. 5 for their analysis), exhibited strong to moderate inhibitory effects on UGT1A1-mediated NCHN-*O*-glucuronidation in HLM, with K_i values ranging between 1.18 and 9.86 μmol/L[20]. The $[I]/K_i$ value of bavachinin was calculated to be greater than 0.1, indicating that inhibition of UGT1A1 *in vivo* seems likely. However, the $[I]/K_i$ values of the other four compounds that were identified from FP could not be estimated due to the lack of their plasma concentrations[20]. In addition, bavachalcone and corylin, two other major bioactive flavonoids from FP, were examined for UGT1A1 inhibition. Bavachalcone inhibited the 4-MU glucuronidation activity of the enzyme noncompetitively, with a K_i value of 5.41 μmol/L, while corylin did not inhibit UGT1A1[95]. Recent studies have demonstrated that some important flavonoid ingredients of licorice, including licochalcone A (LCA), isoliquiritigenin, and liquiritigenin, inhibit UGT1A1 with K_i values below 10 μmol/L[18,96,107]. Furthermore, LCA, isoliquiritigenin, and liquiritigenin inhibited the UGT1A1-mediated 4-MU glucuronidation competitively, whereas LCA inhibited it noncompetitively, at least when the substrate was NCHN[18,96,107]. In addition, IVIVE results indicated that LCA could increase the AUC of UGT1A1 substrates by 71%–341% *via* UGT1A1 inhibition, while isoliquiritigenin was unlikely to inhibit UGT1A1 *in vivo*[18,98]. Several other flavonoids, including wogonin, scutellarein, baicalein, alpinetin, genkwanin, apigenin, hesperetin, and naringenin were reported to strongly inhibit UGT1A1, with K_i values between 0.02 and 16.47 μmol/L. Kinetics analyses of these inhibitions demonstrate that wogonin[97], scutellarein[98], baicalein[99], and alpinetin[102] are competitive inhibitors of UGT1A1-mediated 4-MU glucuronidation, while hesperetin, and naringenin[100] are noncompetitive inhibitors. Genkwanin and apigenin are competitive inhibitors of billirubin glucuronidation in HLM[103]. IVIVE results indicated that scutellarein was highly likely to cause clinically significant HDI *via* UGT1A1 inhibition *in vivo*, while hesperetin and naringenin might not[98,100].

Some diet-derived constituents including kaempferol, and epigallocatechin gallate (EGCG) also inhibited UGT1A1, but none of them was predicted to inhibit UGT1A1 *in vivo*[104]. It is worth noting that deglycosylation of liquiritin into liquiritigenin, of scutellarein into scutellarin, and of baicalein into baicalin was shown to significantly increase their inhibitory effects towards UGT1A1. The inhibition profiles of several other flavonoids, including daidzein, genistein, biochanin A, chrysin, apigenin and naringenin against UGT1A1-mediated SN38-*O*-glucuronidation were examined in UGT1A1-overexpressing Hela cells, resulting in a range of IC_{50} values between 0.37 and 5.85 μmol/L[101]. Recent studies demonstrated that amentoflavone and sciadopitysin, two natural biflavonoid distributed in many medicinal plants, are strong

Table 9 The inhibitory effects of flavonoids on UGT1A1[18,20,25,95–105].

Inhibitor	Substrate	Enzyme source	IC_{50} (μmol/L)	K_i (μmol/L)	Inhibition type	Ref.
Bavachin	4-MU	UGT1A1	1.79	1.08	Competitive	20
	NCHN	HLM	1.85	1.18	Non-competitive	
	NCHN	UGT1A1	0.75	0.04	Non-competitive	
Neobavaisoflavone	4-MU	UGT1A1	1.80	11.96	Competitive	
	NCHN	HLM	2.42	9.86	Non-competitive	
	NCHN	UGT1A1	2.25	3.95	Non-competitive	
Isobavachalcone	4-MU	UGT1A1	13.04	10.93	Competitive	
	NCHN	HLM	4.43	4.13	Non-competitive	
	NCHN	UGT1A1	3.40	4.09	Non-competitive	
Bavachinin	4-MU	UGT1A1	1.99	2.22	Competitive	
	NCHN	HLM	4.16	7.89	Non-competitive	
	NCHN	UGT1A1	1.27	4.09	Non-competitive	
Corylifol A	4-MU	UGT1A1	1.48	0.47	Competitive	
	NCHN	HLM	1.48	1.46	Non-competitive	
	NCHN	UGT1A1	0.65	0.79	Non-competitive	
Licochalcone A	4-MU	UGT1A1	0.97	0.78	Competitive	18
	NCHN	HLM	0.84	0.54	Non-competitive	
	NCHN	UGT1A1	0.13	0.23	Non-competitive	
Bavachalcone	4-MU	UGT1A1	11.3	5.41	Competitive	95
Corylin	4-MU	UGT1A1	–	-	–	
Liquiritigenin	4-MU	UGT1A1	–	9.1	Competitive	107
Liquiritin	4-MU	UGT1A1	–[a]	–	–	
Isoliquiritigenin	4-MU	UGT1A1	–	0.7	Competitive	96
Wogonin	4-MU	UGT1A1	–	1.40	Competitive	97
Scutellarein	4-MU	UGT1A1	–	0.02	Competitive	98
Scutellarin	4-MU	UGT1A1	>100	–	–	
Baicalein	4-MU	UGT1A1	–	1.2	Competitive	99
Baicalin	4-MU	UGT1A1	>100	–	–	
Hesperetin	4-MU	UGT1A1	4.75	9.62	Non-competitive	100
Naringenin	4-MU	UGT1A1	8.58	7.61	Non-competitive	
	β-Estradiol	UGT1A1	4.89	–	–	101
		Hela1A1	4.24	–	–	
	SN-38	UGT1A1	1.58	–	–	
		Hela1A1	2.63	–	–	
Alpinetin	4-MU	UGT1A1	–	3.0	Competitive	102
Genkwanin	Bilirubin	HLM	23.21	16.47	Competitive	103
Apigenin	Bilirubin	HLM	12.40	4.08	Competitive	
	β-Estradiol	UGT1A1	0.47	–	–	101
		Hela1A1	0.33	–	–	

Table 9 (*continued*)

Inhibitor	Substrate	Enzyme source	IC_{50} (μmol/L)	K_i (μmol/L)	Inhibition type	Ref.
	SN-38	UGT1A1	0.72	–	–	
		Hela1A1	0.48	–	–	
Naringin	4-MU	UGT1A1	14.8	–	–	104
Kaempferol	4-MU	UGT1A1	7.9	–	–	
EGCG	4-MU	UGT1A1	26.2	–	–	
Daidzein	β-Estradiol	UGT1A1	52.1	–	–	101
		Hela1A1	67.1	–	–	
	SN-38	UGT1A1	5.01	–	–	
		Hela1A1	5.85	–	–	
Genistein	β-Estradiol	UGT1A1	1.83	–	–	
		Hela1A1	0.94	–	–	
	SN-38	UGT1A1	0.98	–	–	
		Hela1A1	1.43	–	–	
Biochanin A	β-Estradiol	UGT1A1	1.58	–	–	
		Hela1A1	0.84	–	–	
	SN-38	UGT1A1	0.42	–	–	
		Hela1A1	0.37	–	–	
Chrysin	β-Estradiol	UGT1A1	2.02	–	–	
		Hela1A1	0.98	–	–	
	SN-38	UGT1A1	1.16	–	–	
		Hela1A1	1.26	–	–	
Phloretin	β-Estradiol	UGT1A1	2.17	–	–	
		Hela1A1	1.66	–	–	
	SN-38	UGT1A1	1.96	–	–	
		Hela1A1	2.84	–	–	
Amentoflavone	4-MU	UGT1A1	0.78	2.21	Competitive	25
	NCHN	HLM	0.21	0.24	Non-competitive	
	NCHN	UGT1A1	0.14	0.27	Non-competitive	
Sciadopitysin	4-MU	UGT1A1	0.65	0.54	Competitive	105
	NCHN	HLM	0.35	0.41	Non-competitive	
	NCHN	UGT1A1	0.31	0.45	Non-competitive	

– Not determined.

competitive inhibitors of UGT1A1-mediated 4-MU glucuronidation, but function as noncompetitive inhibitors in both UGT1A1 and HLM when the substrate was NCHN[25,105]. IVIVE results suggested that the use of sciadopitysin could result in a significant increase in the AUC of UGT1A1 substrates *via* UGT1A1 inhibition[107].

3.4.3. *Quinones*

Quinones are widely distributed in plant species and have multiple pharmacological activities[108,109]. Quinones that display inhibitory effects on UGT1A1 are listed in Table 10[19,101,110,111]. It was recently demonstrated that 10 major quinone constituents of *Polygonum multiforum*, namely *cis*-emodindianthrones, *trans*-emodindianthrones, emodin-8-*O*-glc, polygonumnolide C2, emodin, polygonumnolide C3, citreorosein, polygonumnolide C4, physcion, and rhein, are naturally occurring potent inhibitors of UGT1A1, with K_i values between 0.863 to 127.3 μmol/L[19]. The inhibition of UGT1A1 activity by these quinones might be one of the reasons for *P. multiforum*-associated adverse effects, particularly elevated bilirubin levels and liver injury[19]. Another study demonstrated that emodin competitively inhibited UGT1A1 activity in three model systems, HLM, RLM and recombinant UGT1A1, with K_i values of 5.40, 10.02 and 4.85 μmol/L, respectively[110]. In addition, emodin displayed strong inhibitory effects in the UGT1A1/Hela1A1 system when estradiol-3-*O*-glucuronidation and SN-38-*O*-glucuronidation activities were examined ($IC_{50} < 2$ μmol/L)[101]. It should be added, however, that emodin is probably a broad specificity inhibitor and it was shown to inhibit UGT1A10 and/or UGT1A8[112], and perhaps other UGTs as well. Tanshinone I, tanshinone IIA, cryptotanshinone, and dihydrotanshinone I are major quinone constituents of Danshen that displayed moderate inhibitory effects on UGT1A1 ($IC_{50} > 40$ μmol/L)[111]. Furthermore, since the C_{max} values of cryptotanshinone and dihydrotanshinone I were much lower than their IC_{50} values, it was concluded that both of them could not inhibit UGT1A1 *in vivo*[111].

3.4.4. *Lignans*

Lignans that were shown to display inhibitory effects on UGT1A1 are listed in Table 11[101,104,113–116]. Lignans are a large group of natural products that are widely spread in the plant kingdom[117]. Milk thistle flavonolignans (silybin A, silybin B, isosilybin B, isosilychristin, and silydianin) were demonstrated to inhibit UGT1A1-mediated 4-MU glucuronidation, at IC_{50} values ranging between 5.3 and 53.5 μmol/L. This resulted in a prediction that none of them was likely to inhibit UGT1A1 *in vivo*[104]. It was previously reported that silybin inhibits the UGT1A1-mediated 7-hydroxy-4-trifluoromethylcoumarin glucuronidation, at an IC_{50} value of 1.4 μmol/L[114], whereas the metabolite, silibinin-glucuronide was found in another study to inhibit rat UGT1A1 in RLM, with a K_i value of 16 μg/mL[113]. Podophyllotoxin, an important lignan that is found in multiple plants, was demonstrated to inhibit the UGT1A1-mediated 4-MU-glucuronidation competitively, with a K_i value of 4.0 μmol/L[115]. However, 1, 10 and 100 μmol/L of podophyllotoxin did not really inhibited HLM-catalyzed SN-38 glucuronidation, since the residual activities were 109.7%, 103.8%, and 64.1% of the negative control, respectively. At the same concentrations, podophyllotoxin also had barely a minor effect on HLM-catalyzed estradiol-3-*O*-glucuronidation, with residual activities being 95.1%, 89.1%, and 84.1% of the negative control, respectively[115]. Thus, inhibition of UGT1A1 by podophyllotoxin is substrate-dependent and mostly mild, namely medium and weak inhibition towards HLM-mediated estradiol-3-*O*-glucuronidation and SN-38-*O*-glucuronidation, respectively[115]. Honokiol, another plant lignan, was found to slightly inhibit UGT1A1-mediated estradiol-3-*O*-glucuronidation, with an IC_{50} value of 50.5 μmol/L[116]. Magnolol and macelignan, two lignans that are found in multiple plants, exhibited similar inhibitory effects on estradiol-3-*O*-glucuronidation and SN-38-*O*-glucuronidation, regardless of whether recombinant UGT1A1, or over-expressing Hela cells were the enzyme source[101].

Table 10 The inhibitory effects of quinones on UGT1A1[19,101,110,111].

Inhibitor	Substrate	Enzyme source	IC_{50} (μmol/L)	K_i (μmol/L)	Inhibition type	Ref.
cis-Emodin dianthrones	Bilirubin	RLM	–	0.8630	Competitive	19
trans-Emodin dianthrones	Bilirubin	RLM	–	1.083	Competitive	
Emodin-8-*O*-glc	Bilirubin	RLM		3.425	Competitive	
Polygonumnolide C2	Bilirubin	RLM	–	4.291	Non-competitive	
Polygonumnolide C3	Bilirubin	RLM	–	12.89	Non-competitive	
Polygonumnolide C4	Bilirubin	RLM	–	77.42	Un-competitive	
Physcion	Bilirubin	RLM	–	94.75	Non-competitive	
Emodin	Bilirubin	RLM	–	10.01	Competitive	
Rhein	Bilirubin	RLM	–	127.3	Mixed	
Citreorosein	Bilirubin	RLM	–	18.56	Mixed	
Emodin	Bilirubin	UGT1A1	–	4.85	Competitive	110
	Bilirubin	HLM	–	5.40	Competitive	
	Bilirubin	RLM	–	10.02	Competitive	
	β-Estradiol	UGT1A1	1.27	–	–	101
		Hela1A1	0.77	–	–	
	SN-38	UGT1A1	0.96	–	–	
		Hela1A1	0.63	–	–	
Tanshinone I	4-MU	UGT1A1	77.2	–	–	111
Tanshinone IIA	4-MU	UGT1A1	69.8	–	–	
Cryptotanshinone	4-MU	UGT1A1	43.5	–	–	
Dihydrotanshinone I	4-MU	UGT1A1	67.3	–	–	

–Not determined.

Table 11 The inhibitory effects of lignans on UGT1A1[101,104,113–116].

Inhibitor	Substrate	Enzyme source	IC_{50} (μmol/L)	K_i (μmol/L)	Inhibition type	Ref.
Silibinin-glucuronide	Bilirubin	RLM	–	16[a]	Competitive	113
Silybin A	4-MU	HIM	64.8			104
		UGT1A1	28.8			
Silybin B	4-MU	HLM	87.3			
		HIM	46.9			
		UGT1A1	27.5			
Isosilybin B	4-MU	HLM	–	–	–	
		HIM	187	–	–	
		UGT1A1	51.1	–	–	
Isosilychristin	4-MU	HLM	–	–	–	
		HIM	–	–	–	
		UGT1A1	53.5	–	–	
Silydianin	4-MU	HLM	97.7	–	–	
		HIM	–	–	–	
		UGT1A1	5.3	–	–	
Silybin	7-Hydroxy-4-trifluoromethylcoumarin	UGT1A1	1.4	–	–	114
Podophyllotoxin	4-MU	UGT1A1		4.0	Competitive	115
Honokiol	*β*-Estradiol	HLM	50.5	–	–	116
Magnolol	*β*-Estradiol	UGT1A1	36.8	–	–	101
		Hela1A1	22.6	–	–	
	SN-38	UGT1A1	13.2	–	–	
		Hela1A1	16.4	–	–	
Macelignan	*β*-Estradiol	UGT1A1	7.40	–	–	
		Hela1A1	5.33	–	–	
	SN-38	UGT1A1	4.73	–	–	
		Hela1A1	2.71	–	–	

–Not determined.
[a]Unit in μg/mL.

3.4.5. Other natural compounds

Alongside the above listed, widely occurring plant compounds, there are other plant compounds that are frequently found, such as polyphenolic acids, polyphenolics, terpenoids, coumarins and alkaloids, that were reported to inhibit UGT1A1 (Table 12[90,93,101,118–123]). Salvianolic acids A and B, two major polyphenolic acids ingredients in Danshen, strongly inhibited UGT1A1-catalyed bilirubin glucuronidation, *via* mixed type inhibition kinetics, with K_i values of 0.22 and 4.50 μmol/L, respectively[118]. Demethylzeylasteral, a triterpenoid that is isolated from *Tripterygium wilfordii* Hook F, functions as a non-competitive inhibitor of the UGT1A1-mediated 4-MU-glcuronidation, with a K_i value of 21.7 μmol/L[119], and PPT, a triterpenoid component that is isolated from Ginseng, exerted strong noncompetitive inhibition towards UGT1A1, with a K_i value of 8.8 μmol/L[121]. The $[I]/K_i$ value of 0.2 that was calculated for PPT inhibiting UGT1A1, suggests that PPT might also inhibit the enzyme *in vivo*. Another study found that 20(*S*)-ginsenoside Rg3 and 20(*S*)-ginsenoside Rh2, two triterpenoid components that are isolated from Ginseng, exerted potent inhibitory effects on UGT1A1-mediated estradiol-3-*O*-glucuronidation in HLM[93]. Notably, among the five major xanthophylls that were investigated, astaxanthin, zeaxanthin, *β*-cryptoxanthin, canthaxanthin and lutein, the strongest UGT1A1 inhibition was exhibited by *β*-cryptoxanthin, with a K_i value of 12.2 μmol/L[120]. Its calculated $[I]/K_i$ value for UGT1A1 was 0.012, indicating that *β*-cryptoxanthin was, however, unlikely to cause a clinically significant DDI *via* UGT1A1 inhibition[120]. Glycyrrhetinic acid, a triterpenoid component from liquorice, exhibited moderate inhibitory effects on UGT1A1-mediated estradiol-3-*O*-glucuronidation and SN-38 glucuronidation in HLM, with K_i values of 28.8 and 25.4 μmol/L, respectively[90]. Brachyantheraoside A2, a triterpenoid saponin from Stauntonia brachyanthera, competitively inhibited UGT1A1-catalyzed 4-MU glucuronidation, with a K_i value of 9.3 μmol/L[122]. The inhibition profiles of several natural compounds, including gingerol (6-shogaol, 6-, 8-, and 10-gingerol), stilbenoid (resveratrol), capsaicinoid (capsaicin), and coumestan (psoralidin) toward UGT1A1 were tested in UGT1A1-overexpressing Hela cells, revealing IC_{50} values ranging between 0.86 and 122 μmol/L[101]. Corydaline, a bioactive isoquinoline alkaloid from *Corydalis tubers*, was found to moderately inhibit UGT1A1-mediated estradiol-3-*O*-glucuronidation, at a K_i value of 57.6 μmol/L[123].

4. Further challenges and future directions

From the physiological function point of view, UGT1A1 is one of the most important mammalian UGTs, due to its essential role in bilirubin metabolism. In most cases, dysfunction or strong inhibition of UGT1A1, either due to inherited mutation(s), or inhibition by drugs or other xenobiotics, could be detected in the clinic based on their effects on the plasma levels of unconjugated bilirubin. The levels of total blood bilirubin and unconjugated bilirubin are often determined in routine clinical testing. Many UGT1A1 inhibitors, such as several flavonoids and pentacyclic triterpenoids, that were identified from *in vitro* assays, turned out to be ineffective *in vivo*. Poor cell permeability and poor metabolic stability of these natural compounds, together leading to poor bioavailability, are probably the major causes of their ineffectiveness *in vivo*. Notably, the majority of the data presented in the review was derived from *in vitro* assays, and often the inhibitor concentration was much higher than the expected plasma concentration. In other words, the inhibitory effects of these compounds on intracellular UGT1A1,

Table 12 The inhibitory effects of other natural compounds on UGT1A1[90,93,101,118–123].

Inhibitor	Substrate	Enzyme source	IC_{50} (μmol/L)	K_i (μmol/L)	Inhibition type	Ref.
Salvianolic Acid A	Bilirubin	UGT1A1	1.13	0.22	Mixed	118
Salvianolic Acid B	Bilirubin	UGT1A1	10.87	4.50	Mixed	
Protocatechuic aldehyde	Bilirubin	UGT1A1	738.01	–	–	
Rosmarinic acid	Bilirubin	UGT1A1	149.53	–	–	
Danshensu	Bilirubin	UGT1A1	340.20	–	–	
Demethylzeylasteral	4-MU	UGT1A1	-	21.70	Non-competitive	119
β-Cryptoxanthin	*β*-Estradiol	HLM	18.8	12.2	Competitive	120
Lutein	*β*-Estradiol	HLM	45.5	–	–	
Canthaxanthin	*β*-Estradiol	HLM	38.5	–	–	
Astaxanthin	*β*-Estradiol	HLM	>50	–	–	
Zeaxanthin	*β*-Estradiol	HLM	>50	–	–	
20(*S*)-Protopanaxatriol	4-MU	UGT1A1	–	8.8	Non-competitive	121
Glycyrrhetinic acid	*β*-Estradiol	HLM	–	28.8	Mixed	90
	SN-38	HLM	–	25.4	Mixed	
Brachyantheraoside A2	4-MU	UGT1A1	–	9.3	Competitive	122
20(*S*)-Ginsenoside Rg3	*β*-Estradiol	HLM	89.0	–	–	93
20(*S*)-Ginsenoside Rh2	*β*-Estradiol	HLM	54.5	–	–	
Psoralidin	*β*-Estradiol	UGT1A1	2.21	–	–	101
		Hela1A1	3.85	–	–	
	SN-38	UGT1A1	0.86	–	–	
		Hela1A1	1.07	–	–	
6-Shogaol	*β*-Estradiol	UGT1A1	8.46	–	–	
		Hela1A1	9.89	–	–	
	SN-38	UGT1A1	1.52	–	–	
		Hela1A1	1.08	–	–	
6-Gingerol	*β*-Estradiol	UGT1A1	135	–	–	
		Hela1A1	80.1	–	–	
	SN-38	UGT1A1	122	–	–	
		Hela1A1	77.3	–	–	
8-Gingerol	*β*-Estradiol	UGT1A1	17.2	–	–	
		Hela1A1	14.2	–	–	
	SN-38	UGT1A1	8.40	–	–	
		Hela1A1	14.1	–	–	
10-Gingerol	*β*-Estradiol	UGT1A1	10.2	–	–	
		Hela1A1	18.5	–	–	
	SN-38	UGT1A1	5.09	–	–	
		Hela1A1	5.76	–	–	
Resveratrol	*β*-Estradiol	UGT1A1	3.42	–	–	
		Hela1A1	1.85	–	–	
	SN-38	UGT1A1	28.3	–	–	
		Hela1A1	19.4	–	–	
Capsaicin	*β*-Estradiol	UGT1A1	51.3	–	–	
		Hela1A1	21.4	–	–	
	SN-38	UGT1A1	23.3	–	–	
		Hela1A1	16.2	–	–	
Corydaline	*β*-Estradiol	HLM	137.1	57.6	Mixed	123

–Not determined.

particularly their *in vivo* potency against UGT1A, have not been well investigated. In order to make in the future such studies more meaningful, it is necessary to develop new or refined test methods. Among the issues to be considered in future inhibition assays are keeping the inhibitor concentration low, which is close to its plasma concentration. Further investigations on the design and development of fluorescent probes for UGT1A1 with high specificity, high sensitivity, good practicability and excellent optical properties (such as long wavelength probes), and their applications in HTS of UGT1A1 inhibitors in complex biological systems are still highly desirable.

In contrast to a wide range of structurally diverse UGT1A1 inhibitors, UGT1A1 inducers or simulators are rarely reported and most studies focus on transcriptional regulation of the UGT1A1 gene[124–126]. Induction of UGT1A1 expression by synthetic or natural compounds in a clinical setting to treat UGT1A1 deficiencies, such as phenobarbital treatment, is common in neonatals. It is less clear, but an interesting idea, whether having an activity stimulating compound for UGT1A will be effective in prevention of CPT-11/SN-38 toxicity, for patients who are homozygous carriers of the polymorphic variant UGT1A1*28[127]. To this end, it is necessary to develop methods for HTS of UGT1A1 inducers or simulators in living systems such as cryo-preserved human hepatocytes. Although the newly developed fluorescent probes for UGT1A1 (NCHN and NHPN) have been used for rapid screening of UGT1A1 inducers at the function level[36,44], the poor cell

permeability of NCHN and the short emission wavelength of their glucuronides make them unsuitable probe substrates for screening UGT1A1 inducers or simulators in hepatocytes culture. Hence, cell-based assays in combination with highly sensitive and practical fluorescence detection for HTS of UGT1A1 inducers or simulators are one of the challenging objectives in both academic research and for drug development.

Besides UGT1A1, other human UGT enzymes also play important roles in the metabolism and detoxification of therapeutic drugs and other xenobiotics. For example, UGT1A4 plays a major role in the metabolism of trifluoperazine[128], a drug that was first used for the treatment of schizophrenia and later, more broadly, epilepsy. UGT1A4 can be specifically inhibited by hecogenin (a compound from sisal plant), which may trigger potential risks of HDI[128]. UGT2B7 is perhaps the most important human UGT enzyme in drug metabolism, since it is involved in the conjugation of many drugs including the HIV/AIDS (acquired immunodeficiency syndrome) drugs (such as zidovudine) and the opioids (such as codeine and morphine)[129,130]. Therefore, attention should be paid to screening and characterization inhibitors of other human UGTs, since UGT-mediated DDI and HDI usually involve multiple UGT enzymes rather than UGT1A1 only[7]. In contrast to UGT1A1, the specific substrates for some other human UGT enzymes are rarely reported, due to the common overlapping substrate specificity of UGTs.

Considering the inherent advantages of fluorescent probe substrates, such as highly sensitivity, and applicability to HTS assay[131], there is a clear advantage in the design and development of practical and highly specific fluorescent probe substrates for a target UGT enzyme. Notably, the design principles are already available and experience in the design and development of specific fluorescent substrates for UGT1A1 will surely assist us and other researchers in developing new specific fluorescent substrates for other UGTs. In fact, several groups have already tried in the past to develop fluorescent substrates for other human UGTs, as well as to construct efficient fluorescence-based assays for HTS of inhibitors toward target enzyme. For instance, 1-naphthol was found to be a good fluorescent substrate for UGT1A6, which can be used for HTS of UGT1A6 inhibitors using recombinant enzyme as an enzyme source[132]. However, 1-naphthol can be conjugated by other human UGTs as well, limiting its applications to the recombinant enzyme, rather than use in more complex system such as HLM. Very recently, a set of fluorescent 7-hydroxycoumarine derivatives have been developed as specific substrates for UGT1A10 (an extrahepatic UGT) and at least two of them (compound **2** and **4**), appear to work well in tissue preparations[133]. All these findings are very helpful for the design and development of fluorescent probes for different human UGTs, and we hope that more practical fluorescent probes for human UGT enzymes will be successfully developed and used in the near future.

5. Concluding remarks

The key roles of UGT1A1 in both endobiotic homeostasis and xenobiotic metabolism have drawn much attention from both academic and drug industry scientists. Now the US FDA and other regulatory agencies have recommended that the inhibition potentials of investigational new drugs on the human UGT1A1 should be evaluated before approval. In this review, the significance, progress and challenges in the discovery and characterization of UGT1A1 inhibitors, as well as recent advances in the development of UGT1A1 probe substrates for screening and characterization of UGT1A1 inhibitors, have been described. The tools for UGT1A1-related investigations, such as probe substrates and specific inhibitors of this key conjugative enzyme, have been summarized for the first time. More importantly, lists of UGT1A1 inhibitors, along with detail information that includes their inhibition potency, mode of inhibition, and affinity (when available), have been prepared and presented. The information and knowledge that are given in this review are expected to provide guidance for rational use of clinical drugs or herbal medicines in order to avoid the occurrence of adverse side effects *via* UGT1A1 inhibition, as well present practical methods for rapid screening and characterization of UGT1A1 inhibitors and for facilitating the investigations on UGT1A1–ligand interactions. We hope that this review will also facilitate the development of more specific and practical tools for other human UGTs in the near future.

Acknowledgments

This work was supported by the NSF of China (81773687, 81703606, 81573501, 81473181), the National Key Research and Development Program of China (2017YFC1700200 and 2017YFC1702000), the Fundamental Research Funds for the Central Universities (wd01185), and the National S&T Major Projects of China (2017ZX09101004), Program of Shanghai Academic/Technology Research Leader (18XD1403600), the Innovative Entrepreneurship Program of High-level Talents in Dalian (2016RQ025 & 2017RQ121), and the Doctoral Scientific Research Foundation of Liaoning Province, China (20170520059).

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