Recent progress and challenges in screening and characterization of UGT1A1 inhibitors

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**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.

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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). 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. 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. 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, 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.

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). Alongside its essential role in bilirubin metabolism, contribution and involvement in the glucuronidation of few other endobiotics, 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. Importantly, many studies have clearly demonstrated that genetics and environmental factors, could affect the expression or the function of UGT1A1, eventually

![Figure 1](image1.png)

**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](image2.png)

**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\(^11\)-\(^12\). 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, licorichalcone A and emodin) are potent UGT1A1 inhibitors, which could significantly inhibit their activities and lead to undesirable effects\(^7\)-\(^25\). 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,
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\(^\text{30,47}\). 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

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

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**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, Ethinylestradiol-4'-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 |
include both “classical” substrates, such as bilirubin and ethynylestradiol, as well as the new generation of fluorescent probes, namely N-(3-carboxypropyl)-4-hydroxy-1,8-naphthalimide (NHPN) and N-buty1-4-(4-hydroxyphenyl)-1,8-naphthalimide (NCHN). 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 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-umbelliflorone (4-MU)\textsuperscript{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\textsuperscript{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 ethynylestradiol, have been used and might still be in use for testing its activity, but mostly some disadvantages are associated with them.\textsuperscript{11,56} The specificity of UGT1A1 for the formation of estradiol-3-O-glucuronide or ethynylestradiol-3-O-glucuronide is poor\textsuperscript{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.\textsuperscript{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\textsuperscript{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\textsuperscript{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.\textsuperscript{46} In HLM, NCHN was highly specific for UGT1A1, even if its affinity for this conjugative enzyme was low.\textsuperscript{46} This was probably the reason that the binding site of NCHN on UGT1A1 was not highly consistent with that of bilirubin.\textsuperscript{40} 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

### Table 2: Kinetic parameters of probe substrates for human UGT1A1\textsuperscript{30,36,41,44,48–53}

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

\*nmol/L/s.
physiologically relevant substrate for UGT1A1\textsuperscript{61}. 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\textsuperscript{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, is followed by molecular docking simulations in which either UGT1A1 modulators or the known substrates were constructed using the crystal structure of UDP-glucuronosyltransferase 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\textsuperscript{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)\textsuperscript{44}. Nevertheless, it is important to remember that the “useful resolution” of such models for substrates design may be limited.

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\textsuperscript{38}, 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-glucuronosyltransferase 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\textsuperscript{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)\textsuperscript{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\textsuperscript{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\textsuperscript{24,42–66}, along with the available inhibition properties. Several human immunodeficiency virus (HIV) protease inhibitors, including atazanavir, and indinavir, are associated with unconjugated hyperbilirubinemia\textsuperscript{44,67}. The study by Zucker and coworkers\textsuperscript{24} discovered that the underlying mechanism of indinavir-induced hyperbilirubinemia in patients was a strong competitive inhibition of UGT1A1, including inhibition of bilirubin-\textit{O}-glucuronidation, with a \( K_i \) value of 183 \( \mu \text{mol/L} \). Subsequently, Zhang and coworkers\textsuperscript{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 \( \mu \text{mol/L} \). 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 \( \mu \text{mol/L} \), respectively. In addition, in vitro–in vivo extrapolation (IVIVE) results indicated that atazanavir and indinavir are likely to induce hyperbilirubinemia\textsuperscript{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-\textit{O}-glucuronidation in HLM in a noncompetitive manner, with a \( K_i \) value of 40.3 \( \mu \text{mol/L} \)\textsuperscript{61}. An [I]/\( K_i \) ratio of 0.32 suggested that the UGT1A1-

![Figure 5](image-url)  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.
mediated DDI is possible when the high doses of efavirenz are used\(^6^3\). 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-β-glucuronidation, and since the \(IC_{50}\) value was 0.45 \(\mu\)mol/L, that inhibition is likely to contribute significantly to the hyperbilirubinemia that was observed in patients that had been treated with faldaprevir (\(IC_{50}>1\))\(^6^6\). Paritaprevir was reported to competitively inhibit UGT1A1, at a \(K_i\) value of 20 \(\mu\)mol/L\(^6^4\). 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 ranging between 0.034 and 0.53 \(\mu\)mol/L. Importantly, the inhibition of UGT1A1 by rifabutin could result in a significant increase in the AUC of SN-38, as well as an increased hyperbilirubinemia at high rate\(^2^3,6^9\). 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\(^7^2\). Both icotinib and erlotinib inhibited NCHN-O-Glucuronidation by UGT1A1 noncompetitively. In this case the \(K_i\) value displayed by icotinib, 10.04 \(\mu\)mol/L, was clearly higher than the corresponding value of erlotinib, 1.72 \(\mu\)mol/L. IVIVE results indicated that erlotinib had a much higher DDI potential, while icotinib is unlikely to cause a significant DDI via UGT1A1 inhibition\(^7^2\). Both imaﬁnib and lapatinib were reported to be competitive inhibitors of UGT1A1-mediated 4-MU glucuronidation, with \(K_i\) values of 19.1 and 0.5 \(\mu\)mol/L, respectively\(^7^0\). In addition, IVIVE results indicated that coadministration of imaﬁnib 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 imaﬁnib inhibited UGT1A1-mediated paracetamol glucuronidation, at \(IC_{50}\) values of 2 and 29 \(\mu\)mol/L, respectively\(^7^3\). The \(I/I/K_i\) ratio results indicated that at clinical relevant doses, imaﬁnib could result in a 22% increase in the AUC of coadministered paracetamol via UGT1A1 inhibition, while dasatinib could cause only a slight increase in the AUC (6%)\(^7^3\).
was suspended due to its higher toxicity, and it was replaced by entacapone. 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. Levothyroxine, which is used for the treatment of thyroid hormone deficiency, functions as a competitive inhibitor of UGT1A1-mediated 4-MU-glucuronidation, with an IC50 value of 1.0 μmol/L. Ketoconazole, an antifungal agent, was demonstrated to inhibit UGT1A1 mediated SN-38 glucuronidation in HLM, with an IC50 value of 0.53 μmol/L. The calculated [I]/Ki value of 3.3 suggested that inhibition of UGT1A1 by vitamin A may exist in vivo. 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 IC50 value of 22.2 μmol/L. 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 Ki value of 2.3 μmol/L. The calculated [I]/Ki value in this case was 0.004, indicating that everolimus is unlikely to cause clinically significant DDI via UGT1A1 inhibition in vivo. Diethylstilbestrol, a widely used toxic synthetic estrogen, is a strong competitive inhibitor of UGT1A1-catalyzed estradiol-3-O-glucuronidation in HLM, with a Ki value of 2.1 μmol/L, and of the UGT1A1-mediated 4-MU-glucoconidation, also competitively, with a Ki value of 3.7 μmol/L. Based on the inhibition constant Ki values of 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. IVIVE results suggested that CNF could result in a

| Inhibitor | Substrate | Enzyme source | IC50 (μmol/L) | Ki (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 |     |
|           | SN-38     | UGT1A1        | –            | 0.079       | Non-competitive |     |
| Regorafenib | β-Estradiol | HLM           | –            | 0.020       | Competitive     |     |
|           | 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     |     |
|           | Bilirubin | HLM           | 4.19         | 2.97        | Mixed          |     |
|           | NCHN      | UGT1A1        | 0.68         | 1.23        | Non-competitive |     |
| Gefitinib | 4-MU      | UGT1A1        | –            | 2.42        | –              |     |
| Icotinib | NCHN      | HLM           | 5.15         | 8.55        | Non-competitive |     |
|           | NCHN      | UGT1A1        | 8.76         | 10.04       | Non-competitive |     |
| Axitinib | 4-MU      | UGT1A1        | –            | –           | –              |     |
| Imatinib | 4-MU      | UGT1A1        | 11.0         | 19.1        | Competitive     |     |
| Vandetanib | 4-MU      | UGT1A1        | –            | –           | –              |     |
| Dasatinib | 4-MU      | UGT1A1        | 2            | –           | –              |     |

–Not determined.
significant increase in the AUC for exclusive UGT1A1 substrates via UGT1A1 inhibition, while DNF could not inhibit UGT1A1 in vivo.33.

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 IC50 value of 23.5 μmol/L. The inhibition kinetic was noncompetitive inhibition and the Ki value was 34.2 μmol/L.86 The [I]/Ki ratio of 0.56 suggested that the use of gossypol could cause DDI through inhibition of UGT1A186. 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 Ki value of 43 μmol/L.86

| Inhibitor      | Substrate | Enzyme source | IC50(μmol/L) | Ki (μmol/L) | Inhibition type | Ref. |
|----------------|-----------|---------------|--------------|-------------|----------------|------|
| Entacapone     | 4-MU      | UGT1A1        | 9.10         | 10.48       | Competitive    | 60.75–83 |
|                | 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|      |
| Levotheroxyline| 4-MU      | UGT1A1        | –            | 1.0         | Competitive    | 75.76 |
| Ketocnazole    | SN-38     | UGT1A1        | –            | 3.3         | Competitive    |      |
|                | Bilirubin | HLM           | 53           | –           | –              |      |
| Vitamin A      | 4-MU      | UGT1A1        | –            | 31.1        | Competitive    | 77   |
| Dichlofenac    | 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         | –           | –              |      |
| Nitric 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     | UGT1A1        | –            | 1.2         | Non-competitive|      |
| Canagliflozin  | β-Estradiol | UGT1A1 | –            | 7.2         | Competitive    | 83   |
| Dapagliflozin  | β-Estradiol | HLM     | –            | 9.1         | Competitive    |      |
|                | β-Estradiol | HLM     | –            | 81          | Competitive    |      |
|                | β-Estradiol | HLM     | –            | 81          | Competitive    |      |

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 IC50 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 relevant21. Andrographis paniculata and Orthosiphon stamineus extracts displayed inhibitory effects on UGT1A1-mediated 4-MU-glucuronidation, with IC50 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 Ki 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-glucuronidation in HLM.

| Inhibitor       | Substrate | Enzyme source | IC50(μmol/L) | Ki (μmol/L) | Inhibition type | Ref. |
|-----------------|-----------|---------------|--------------|-------------|----------------|------|
| Gossypol        | β-Estradiol | HLM     | 23.5         | 34.2        | Non-competitive| 86.75 |
| P-Cresol        | β-Estradiol | HLM     | –            | 43          | Competitive    | 87   |

~Not determined.
glucuronidation and UGT1A1-mediated SN38-O-glucuronidation in HLM, with $K_i$ values between 23 and 105 μg/mL. Blueberry, a commonly consumed berry, weakly and competitively inhibited UGT1A1 at a $K_i$ value of 53.1 μg/mL. 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 IC50 value of 302.4 μg/mL, whereas ginseng extract inhibited UGT1A1-mediated estradiol-3-O-glucuronidation in HLM, with an IC50 value of 14.5 μg/mL. 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.

It was also found that the crude extract of Fructus psoraleae (FP, also named Bu-gu-zhi in Chinese) could inhibit UGT1A1 at an IC50 value of 12.5 μg/mL. Subsequently, the LC–UV fingerprinting analysis combined with UGT1A1 inhibition profiling was successfully used to identify and characterize the naturally occurring inhibitors of UGT1A1. Five major constituents from FP, namely bavachin, corylifol A, neobavaisoflavone, isobavachalcone, and bavachinin, were identified as inhibitors of UGT1A1. This study was a good case study.

| Inhibitor Substrate Enzyme source | IC50 (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          | –             | –              |     |
| Black cohosh β-Estradiol HLM     | 299          | –             | –              |     |
| Valerian β-Estradiol HLM         | 562          | –             | –              |     |
| 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          | Competitive    | 89  |
| Rhei Rhizoma (Daio) β-Estradiol HLM | –       | 30            | Mixed          | 90  |
| Glycyrrhiza Radix (Kanzo) SN-38 HLM | –       | 68            | Mixed          |     |
| Cinnamomum Cortex (Keihi) SN-38 HLM | –       | 95            | Mixed          |     |
| Scutellariae Radix (Ogon) SN-38 HLM | –       | 105           | 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.
*Estimated IC50.
Unit in μmol/L.

It was also found that the crude extract of Fructus psoraleae (FP, also named Bu-gu-zhi in Chinese) could inhibit UGT1A1 at an IC50 value of 12.5 μg/mL. Subsequently, the LC–UV fingerprinting analysis combined with UGT1A1 inhibition profile was successfully used to identify and characterize the naturally occurring inhibitors of UGT1A1 in FP (Fig. 6). 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 constituents from FP, namely bavachin, corylifol A, neobavaisoflavone, isobavachalcone, and bavachinin, were identified as inhibitors of UGT1A1. This study was a good case study.

**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.
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 ω3-linolenic acid, with IC50 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 Ki 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 Ki value of 5.2 μmol/L, probably indicating inhibition of UGT1A105. Interest-ingly, 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 induction18.

3.4.2. Flavonoids

Flavonoids that display inhibitory effects on UGT1A1 are listed in Table 918,20,25,95–105. 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 properties106. Five major flavonoids components of FP, bavachin, corylifol A, neobavaisoflavone, isobavachalcone, and bavachin (see Fig. 5 for their analysis), exhibited strong to moderate inhibitory effects on UGT1A1-mediated NCHN-O-glucuronidation in HLM, with Ki values ranging between 1.18 and 9.86 μmol/L.20. The [I]/Ki value of bavachinin was calculated to be greater than 0.1, indicating that inhibition of UGT1A1 in vivo seems likely. However, the [I]/Ki values of the other four compounds that were identified from FP could not be estimated due to the lack of their plasma concentrations20. In addition, bavachalcone and corylin, two other major bioactive flavonoids from FP, were examined for UGT1A1 inhibition. Buvachalcone inhibited the 4-MU glucuronidation activity of the enzyme noncompetitively, with a Ki value of 5.41 μmol/L while corylin did not inhibit UGT1A195. Recent studies have demonstrated that some important flavonoid ingredients of licorice, including licochalcone A (LCA), isoliquiritigenin, and liquiritigenin, inhibit UGT1A1 with Ki values below 10 μmol/L18,96,107. Furthermore, LCA, isoliquiritigenin, and liquiritigenin inhibited the UGT1A1-mediated 4-MU glucuronidation competitively, whereas LCA inhibited it noncompetitively, at least when the substrate was NCHN18,96,107. 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 vivo18,96. Several other flavonoids, including wogonin, scutellarein, baicalein, alpinetin, genkwanin, apigenin, hesperetin, and naringenin were reported to strongly inhibit UGT1A1, with Ki values between 0.02 and 16.47 μmol/L. Kinetics analyses of these inhibitions demonstrate that wogonin, scutellarein, baicalein, and apigenin are competitive inhibitors of UGT1A1-mediated 4-MU glucuronidation, while hesperetin, and naringenin are noncompetitive inhibitors. Genkwanin and apigenin are competitive inhibitors of bilirubin glucuronidation in HLM103. IVIVE results indicated that scutellarein was highly likely to cause clinically significant HDI via UGT1A1 inhibition in vivo, while hesperetin and naringenin might not98,100.

Some diet-derived constituents including kaempferol, and epigallocatechin gallate (EGCG) also inhibited UGT1A1, but none of them was predicted to inhibit UGT1A1 in vivo104. It is worth noting that deglycosylation of liquiritigenin 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 IC50 values between 0.37 and 5.85 μmol/L102. Recent studies demonstrated that amentoflavone and scidadopisynin, two natural biflavonoid distributed in many medicinal plants, are strong

| Inhibitor | Substrate | Enzyme source | IC50 (μmol/L) | Ki (μmol/L) | Inhibition type | Ref. |
|-----------|-----------|--------------|--------------|-------------|----------------|-----|
| Oleic acid | β-Estradiol | UGT1A1 | 31.6 | 23.4 | Non-competitive | 94 |
| Linoleic acid | β-Estradiol | UGT1A1 | 33.1 | 22.1 | Non-competitive | - |
| Palmitoleic acid | β-Estradiol | UGT1A1 | 37.1 | - | Non-competitive | - |
| α-Linolenic acid | β-Estradiol | UGT1A1 | 26.1 | - | Non-competitive | - |
| Arachidonic acid | β-Estradiol | UGT1A1 | 22.7 | - | Non-competitive | - |
| DHA | β-Estradiol | UGT1A1 | 11.6 | 1.8 | Non-competitive | - |
| EPA | β-Estradiol | UGT1A1 | 19.9 | - | Non-competitive | - |
| Stearic acid | β-Estradiol | UGT1A1 | > 50 | - | Non-competitive | - |
| Decanoic acid | β-Estradiol | UGT1A1 | > 50 | - | Non-competitive | - |

– Not determined.
| Inhibitor         | Substrate | Enzyme source | $IC_{50}$ (μmol/L) | $K_i$ (μmol/L) | Inhibition type | Ref. |
|------------------|-----------|---------------|-------------------|---------------|----------------|------|
| Bavachin         | 4-MU      | UGT1A11       | 1.79              | 1.08          | Competitive     | 20   |
|                  | NCHN      | HLM           | 1.85              | 1.18          | Non-competitive |      |
|                  | NCHN      | UGT1A11       | 0.75              | 0.04          | Non-competitive |      |
| Neobavaisoflavone| 4-MU      | UGT1A11       | 1.80              | 11.96         | Competitive     |      |
|                  | NCHN      | HLM           | 2.42              | 9.86          | Non-competitive |      |
|                  | NCHN      | UGT1A11       | 2.25              | 3.95          | Non-competitive |      |
| Isobavachalcone  | 4-MU      | UGT1A11       | 13.04             | 10.93         | Competitive     |      |
|                  | NCHN      | HLM           | 4.43              | 4.13          | Non-competitive |      |
|                  | NCHN      | UGT1A11       | 3.40              | 4.09          | Non-competitive |      |
| Bavachinin       | 4-MU      | UGT1A11       | 1.99              | 2.22          | Competitive     |      |
|                  | NCHN      | HLM           | 4.16              | 7.89          | Non-competitive |      |
| Corylifol A      | 4-MU      | UGT1A11       | 1.27              | 0.47          | Competitive     |      |
|                  | NCHN      | HLM           | 1.48              | 1.46          | Non-competitive |      |
|                  | NCHN      | UGT1A11       | 0.65              | 0.79          | Non-competitive |      |
| Licochalcone A   | 4-MU      | UGT1A11       | 0.97              | 0.78          | Competitive     | 18   |
|                  | NCHN      | HLM           | 0.84              | 0.54          | Non-competitive |      |
|                  | NCHN      | UGT1A11       | 0.13              | 0.23          | Non-competitive |      |
| Bavachalcone     | 4-MU      | UGT1A11       | 11.3              | 5.41          | Competitive     | 95   |
| Corylin          | 4-MU      | UGT1A11       | –                 | –             | –              |      |
| Liquiritigenin   | 4-MU      | UGT1A11       | –                 | 9.1           | Competitive     | 107  |
| Liquiritin       | 4-MU      | UGT1A11       | –                 | –             | –              |      |
| Isoliquiritigenin| 4-MU      | UGT1A11       | –                 | 0.7           | Competitive     | 96   |
| Wogonin          | 4-MU      | UGT1A11       | –                 | 1.40          | Competitive     | 97   |
| Scutellarin      | 4-MU      | UGT1A11       | –                 | 0.02          | Competitive     | 98   |
| Scutellarin      | 4-MU      | UGT1A11       | –                 | >100          | –              |      |
| Baicalein        | 4-MU      | UGT1A11       | –                 | 1.2           | Competitive     | 99   |
| Baicalin         | 4-MU      | UGT1A11       | –                 | >100          | –              |      |
| Hesperetin       | 4-MU      | UGT1A11       | –                 | 4.75          | Non-competitive | 100  |
| Naringenin       | 4-MU      | UGT1A11       | 8.58              | 7.61          | Non-competitive |      |
| \(\beta\)-Estradiol | UGT1A1 | –             | 4.89              | –             | –              | 101  |
| \(\beta\)-Estradiol | SN-38 | UGT1A1       | 4.24              | –             | –              |      |
| Alpinetin        | 4-MU      | UGT1A11       | –                 | 1.58          | –              |      |
| Genkwanin        | Bilirubin | HLM           | 23.21             | 16.47         | Competitive     | 103  |
| Apigenin         | Bilirubin | HLM           | 12.40             | 4.08          | Competitive     | 101  |
| \(\beta\)-Estradiol | UGT1A1 | –             | 0.47              | –             | –              |      |
| Hela1A1          | –         | 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|      |
| Sciadopitysin     | 4-MU      | UGT1A1        | 0.14               | 0.27           | Non-competitive|      |
|                   | NCHN      | HLM           | 0.65               | 0.54           | Competitive    | 105  |
|                   | NCHN      | UGT1A1        | 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 NCHN25,105. IVIVE results suggested that the use of sciadopitysin could result in a significant increase in the AUC of UGT1A1 substrates via UGT1A1 inhibition.

3.4.3. Quinones
Quinones are widely distributed in plant species and have multiple pharmacological activities106,109. Quinones that display inhibitory effects on UGT1A1 are listed in Table 11101,110,111. It was recently demonstrated that 10 major quinone constituents of Polygonum multiflorum, namely cis-emodindianthrones, trans-emodindianthrones, emodin-8-O-gl, polygonummonolide C2, emodin, polygonummonolide C3, citreorosein, polygonummonolide C4, physcion, and rhein, are naturally occurring potent inhibitors of UGT1A1, with $K_i$ values ranging 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. multiflorum-associated adverse effects, particularly elevated bilirubin levels and liver injury.15. 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/HelaA1 system when estradiol-3-O-glucuronidation and SN-38-O-glucuronidation activities were examined (IC50 < 2 μmol/L).110. It should be added, however, that emodin is probably a broad specificity inhibitor and it is shown to inhibit UGT1A10 and/or UGT1A8112, 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 (IC50>40 μmol/L).111. Furthermore, since the $C_{max}$ values of cryptotanshinone and dihydrotanshinone I were much lower than their IC50 values, it was concluded that both of them could not inhibit UGT1A1 in vivo.

3.4.4. Lignans
Lignans that were shown to display inhibitory effects on UGT1A1 are listed in Table 11101,104,113-118. Lignans are a large group of natural products that are widely spread in the plant kingdom. Milk thistle flavonolignans (silybin A, silybin B, isosilybin B, isosilychristin, and silydianin) were demonstrated to inhibit UGT1A1-mediated 4-MU glucuronidation, at IC50 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 IC50 value of 1.4 μmol/L, whereas the metabolite, silybinin-glucuronide was found in another study to inhibit rat UGT1A1 in RLM, with a $K_i$ value of 16 μg/mL. 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. 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 IC50 value of 50.5 μmol/L. 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.

| Inhibitor | Substrate | Enzyme source | IC50 (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-gl | Bilirubin | RLM | – | 3.425 | Competitive | |
| Polygonummonolide C2 | Bilirubin | RLM | – | 4.291 | Non-competitive | |
| Polygonummonolide C3 | Bilirubin | RLM | – | 12.89 | Non-competitive | |
| Polygonummonolide 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 |
| Emodin | Bilirubin | HLM | – | 5.40 | Competitive | |
| Emodin | Bilirubin | RLM | – | 10.02 | Competitive | |
| β-Estradiol | UGT1A1 | HelaA1 | 1.27 | – | – | 101 |
| SN-38 | UGT1A1 | HelaA1 | 0.77 | – | – | |
| 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.
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 1290,104,113–116). Salvianolic acids A and B, two major polyphenolic acids ingredients in Danshen, strongly inhibited UGT1A1-catalyzed bilirubin glucuronidation, via mixed type inhibition kinetics, with a $K_i$ value of 0.22 and 4.50 μmol/L, respectively18. Demethylezylasteral, a triterpenoid that is isolated from Tripterygium wilfordii Hook F, functions as a non-competitive inhibitor of the UGT1A1-mediated 4-MU-glucuronidation, with a $K_i$ value of 21.7 μmol/L120, 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/L121. The inhibition profile of these compounds on intracellular 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, may also inhibit the enzyme.

### Table 11

| Inhibitor                        | Substrate                  | Enzyme source | $IC_{50}$ (μmol/L) | $K_i$ (μmol/L) | Inhibition type | Ref. |
|----------------------------------|----------------------------|---------------|-------------------|---------------|----------------|------|
| Silibinin-glucuronide            | Bilirubin                  | RLM           | –                 | 16$^a$        | Competitive    | 113  |
| Silibin A                        | 4-MU                       | HIM           | 64.8              |               |                | 104  |
| Silibin B                        | 4-MU                       | UGT1A1        | 28.8              |               |                |      |
|                                  |                            | 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           | –                 | –             | –              |      |
| 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              | –             | –              |      |
|                                  |                            | UGT1A1        | 13.2              | –             | –              |      |
|                                  |                            | Hela1A1       | 16.4              | –             | –              |      |
| Macelignan                       | β-Estradiol                | UGT1A1        | 7.40              | –             | –              |      |
|                                  |                            | Hela1A1       | 5.33              | –             | –              |      |
|                                  |                            | UGT1A1        | 4.73              | –             | –              |      |
|                                  |                            | Hela1A1       | 2.71              | –             | –              |      |

$^a$Unit in μg/mL.

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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,
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. Induction of UGT1A1 expression by synthetic or natural compounds in a clinical setting to treat UGT1A1 deficiencies, such as phenobarbital treatment, is common in neonates. 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. 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, the poor cell

### Table 12: The inhibitory effects of other natural compounds on UGT1A1

| 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            | –            | –              |      |
| Cantaxanthin       | 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   |
| Glycyrrhetinic acid| SN-38      | HLM           | –               | 25.4         | Mixed          |      |
| Brachyantheraose 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  |
| Psoralidin         | SN-38      | UGT1A1        | 0.86            | –            | –              |      |
| SN-38              | Hela1A1    |               | 1.07            | –            | –              |      |
| 6-Shogaol          | β-Estradiol | UGT1A1        | 8.46            | –            | –              |      |
| 6-Shogaol          | SN-38      | UGT1A1        | 1.52            | –            | –              |      |
| 6-Shogaol          | Hela1A1    |               | 1.08            | –            | –              |      |
| 6-Gingerol         | β-Estradiol | UGT1A1        | 135             | –            | –              |      |
| 6-Gingerol         | SN-38      | UGT1A1        | 80.1            | –            | –              |      |
| 6-Gingerol         | Hela1A1    |               | 77.3            | –            | –              |      |
| 8-Gingerol         | β-Estradiol | UGT1A1        | 17.2            | –            | –              |      |
| 8-Gingerol         | SN-38      | UGT1A1        | 14.2            | –            | –              |      |
| 10-Gingerol        | β-Estradiol | UGT1A1        | 10.2            | –            | –              |      |
| 10-Gingerol        | SN-38      | UGT1A1        | 5.09            | –            | –              |      |
| 10-Gingerol        | Hela1A1    |               | 5.76            | –            | –              |      |
| Resveratrol        | β-Estradiol | UGT1A1        | 3.42            | –            | –              |      |
| Resveratrol        | SN-38      | UGT1A1        | 1.85            | –            | –              |      |
| Capsaicin          | β-Estradiol | UGT1A1        | 51.3            | –            | –              |      |
| Capsaicin          | SN-38      | UGT1A1        | 23.3            | –            | –              |      |
| Corydaline         | β-Estradiol | HLM           | 137.1           | 57.6         | Mixed          | 123  |

Not determined.
permeability of NCHN and the short emission wavelength of their glucuronides make them unsuitable probe substrates for screening UGT1A1 inducers or simulators in hepatocyte 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 trifluoperaziné, 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. 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). 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. 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, 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. 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-hydroxyxcoumarine 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. 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.

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