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REVIEW

Metabolite Profiling in Anticancer Drug Development: A Systematic Review

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Abstract: Drug metabolism is one of the most important pharmacokinetic processes and plays an important role during the stage of drug development. The metabolite profile investigation is important as the metabolites generated could be beneficial for therapy or leading to serious toxicity. This systematic review aims to summarize the research articles relating to the metabolite profile investigation of conventional drugs and herb-derived compounds for cancer chemotherapy, to examine factors influencing metabolite profiling of these drugs/compounds, and to determine the relationship between therapeutic efficacy and toxicity of their metabolites. The literature search was performed through PubMed and ScienceDirect databases up to January 2019. Out of 830 published articles, 78 articles were included in the analysis based on pre-defined inclusion and exclusion criteria. Both phase I and II enzymes metabolize the anticancer agents/herb-derived compounds . The major phase I reactions include oxidation/hydroxylation and hydrolysis, while the major phase II reactions are glucuronidation, methylation, and sulfation. Four main factors were found to influence metabolite formation, including species, gender, and route and dose of drug administration. Some metabolites were identified as active or toxic metabolites. This information is critical for cancer chemotherapy and anticancer drug development.

Keywords: metabolism, metabolite profile, anticancer, herbal medicine, cancer

Introduction

Cancer remains the major cause of death globally. In 2018, approximately 18 million new cases and 9 million deaths from cancer were estimated to occur worldwide.[1] Several chemotherapeutic agents have been developed for treatment and prevention of cancer, either chemically synthetic drugs or herb-derived compounds.[2–6] As herb-derived anticancer drugs are considered to be less toxic compared with synthetic drugs, attentions to developing new drugs originating from herbal products have substantially been paid worldwide. These include leelamine, the natural active compound from the bark of pine tree,[7] atractylodin and β-eudesmol, the natural active compounds from rhizomes of *Atractylodes lancea* (Thunb) DC,[8] and alantolactone, an active sesquiterpene from *Inula helenium* L.[9]

Drug metabolism and pharmacokinetic (DMPK) studies play an important role in all steps of drug discovery and development, including anticancer drugs.[10] Metabolism is the process of which xenobiotics or endogenous substances in the body are biotransformed to the metabolic products that facilitate their elimination.[11] Drug metabolism involves two main phases, i.e., phase I and phase II metabolism. The primary enzyme system involved in phase I metabolism is cytochrome P450 (CYP), and the

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major enzymes involved in phase II are UDP-glucuronosyltransferase (UGT), sulfotransferase (SULT), glutathione-S-transferase (GST), *N*-acetyltransferase (NAT), and methyltransferase (MT).[12] Drug metabolism plays a crucial role in determining the efficiency and safety of drugs. Drugs undergo metabolism to form numerous stable metabolites, most of which are pharmacologically inactive. On the other hand, in some cases, metabolism may lead to reactive metabolites that can induce adverse effects. Various types of drug metabolism studies have been incorporated during the process of drug discovery and development to generate new chemical entities (NCE) with acceptable safety profiles. This is particularly important for cancer chemotherapeutic drugs with a narrow therapeutic window. Metabolite profiling and identification studies of these compounds and currently used drugs are therefore essential. The main aim of metabolic profiling studies is to identify metabolic pathways and metabolites generated from the biotransformation process. The information obtained from the studies would help to optimize lead compounds for optimal pharmacokinetic and pharmacodynamic properties. Besides, it will help to identify new chemical entities based on the metabolites generated to minimize potential safety liabilities due to the formation of reactive or toxic metabolites. Comparison of information obtained from preclinical studies in animals and humans would also ensure potential adequate coverage of human metabolites in animals and for supporting human prediction.

This systematic review aimed to summarize the research articles relating to the metabolite profiling studies of anticancer drugs (conventional chemical synthetic drugs and targeted small-molecules) and candidate compounds from herbal sources. Factors influencing metabolite profiles (metabolic pathways and types of metabolites generated) and their relationship with anticancer activity and toxicity in vitro, in vivo (animals), and human were also investigated.

Materials and Methods

Study Selection and Inclusion and Exclusion Criteria

This systematic review was performed through the search from PubMed (via Endnote) and ScienceDirect databases up to January 2019. The following keywords were used: "anticancer drug", "anticancer agent", "chemotherapy", "chemotherapeutic drug", "traditional medicine", herbal medicine, "natural compound", "metabolism", "metabolite profile", "metabolite identification", "metabolite characterization", and "cancer". No other search conditions were applied. All articles obtained from the two databases were checked for duplication. The remaining articles were initially screened as per the inclusion criteria based on the content of the abstract section. The inclusion criteria for article selection were 1) articles in full-texts and written in English; 2) articles with the investigation (in vitro, in vivo, or clinical studies) of metabolite profiles of conventional chemotherapeutic drugs, small molecules targeted therapy, candidate synthetic anticancer agents, natural products-derived anticancer compounds or drug candidates. The duplicates, review articles, short communications, case reports, articles with the investigation of other types of drug metabolism studies, or those with insufficient information of metabolite(s) or metabolic pathway(s) were excluded from data analysis.

Data Extraction and Collection

Two independent researchers performed data extraction from all articles. When conflicting opinions arose, the decision was sought from higher professional level personnel, and the decision was considered final. The title and abstract of each article search from PubMed (via Endnote) and ScienceDirect databases using the keywords mentioned above were initially screened for relevant original articles based on the inclusion and exclusion criteria. The full-text articles were carefully examined to confirm their compliance with the defined eligibility criteria. The studies of metabolite profile of chemotherapeutic drugs, targeted small molecules, candidate synthesized anticancer agents, traditional or herbal medicines, and natural compounds for cancer were classify according to types of anticancer agents. The information extracted included: name of anticancer drug or compound/herb, type of studies (in vitro, in vivo, and clinical studies), gender and species of animals used, route and dose of administration of the investigated drugs/compounds, biochemical tools used (liver, prostate, intestine, or kidney microsomes, subcellular fractions, hepatocytes, recombinant enzymes, and whole blood), type of biological samples (plasma, urine, bile, feces, and tumor), and study conclusion.

Results

Study Description

Three hundred and twenty-one out of 830 articles were duplicated or review articles and were initially excluded

from the analysis. The title and abstract were further screened based on eligible criteria, and 411 articles were further excluded from the analysis. Finally, 78 out of 98 articles were included in the analysis; 20 excluded articles were case reports, short communications, and articles with insufficient information. The flow diagram of the search process is presented in Figure 1. Information on metabolite profiles including biochemical tools/biological samples used in the studies of conventional anticancer drugs, synthetic anticancer candidates, small molecules targeted therapy, herb-derived compounds with anticancer activities are summarized in Tables 1–3, respectively and the anticancer activities of each compound are presented in Table 4.

Out of 78 articles included in the analysis (42 in vitro, 42 in vivo, and 16 clinical studies), 47 (60.3%), 14 (17.9%) and 17 (21.8%) articles respectively, investigated metabolite profiles of conventional anticancer drugs/synthetic anticancer candidates, small-molecules targeted therapy, and herb-derived compounds with anticancer activities. These studies involved a total of 57 (57.0%) conventional anticancer drugs/synthetic anticancer candidates,[6,13–58] 22 (22.0%) studies for small-molecules targeted therapy,[4,5,59–70] and 21 (21.0%) studies for herb-derived compounds with anticancer activities[2,3,7,9,71–83] (Tables 1–3). Anticancer drugs or candidate compounds are metabolized by either Phase I, or phase II metabolizing enzyme alone, or both phase I and phase II metabolizing enzymes. The major metabolic pathways of phase I reaction include oxidation/hydroxylation and hydrolysis. The major metabolic pathways of phase II reaction are glucuronidation, methylation, and sulfation (Tables 1–3).

Several factors were found to influence the metabolite profiles of these drugs/compounds, including species, gender, and route and dose of administration. Relationship between the generated metabolites and anticancer activity and/or toxicity were reported in 10 (17.5%) studies for conventional anticancer drugs/synthetic anticancer candidates,[6,13,22,31,32,36,45,50] 1 (4.5%) studies for small-molecules targeted therapy,[65] and 4 (19.0%) studies for herb-derived compounds with anticancer activities.[75,76,78,79]

Discussion

The goals of conducting drug metabolism studies are to identify and characterize all major metabolites of the investigated drugs and specific enzymes responsible for their metabolism; to evaluate the impacts of the metabolites on safety and efficacy of the drug, and to utilize the drug metabolism information to maximize their intellectual property. The identity of metabolites present in any matrix of animal or human provides essential information about the biotransformation pathways involved in the clearance of a drug. Technological advances during the past decade have greatly improved analytical capabilities to detect, identify, and characterize metabolites at previously unattainable levels. Chromatography and electrophoresis are usually methods of choice. Electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) liquid chromatography-mass spectrometry (LC/MS) have become ideal and widely used methods in the identification, structure characterization, and quantitative analysis of drug metabolites. This is due to its superior specificity, sensitivity, and

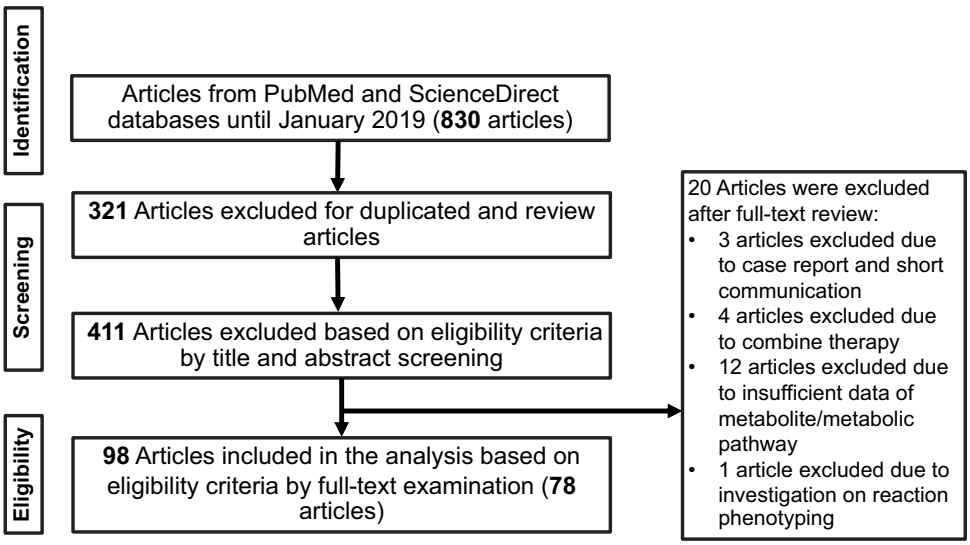

Figure 1 Flow diagram summarizing steps for exclusion and inclusion of the research articles included in the analysis.

Table 1 Metabolism Studies (Metabolite Profiling) of Conventional Synthetic Anticancer Drugs

Anticancer Drug	Type of Study	Biochemical Tool/Animal/ Human (Route: Dose)	Type of Sample	Analytical Technique	Outcome			Ref.
					Metabolite	Metabolic Pathway	Efficacy/Toxicity of Metabolite	
AM6−36 (3-amino-6-(3′-aminopropyl)-5H-indeno[1,2-c] isoquinoline-5,11-(6H) dione)	In vitro	HLM, human hepatocyte	–	HPLC-QTOF-MS, HPLC-QqQ-MS	1 Metabolite for HLM (4); 7 Metabolites for hepatocyte (4–10)	Reduction (4); Monoacetylation (5); Conversion of amino acid group to an alcohol (7); Reduction of the ketone to an alcohol +conversion of amino acid group to an alcohol (8); Reduction + acetylation (6); Monoacetylation + conversion of amino acid group to an alcohol (9); Glucuronidation (10)	Metabolites 5: lower retinoid X receptor (RXR) homodimers bind to DNA response elements (RXRE) induction activity than parent compound AM6-36 Metabolites 8: exhibited lower activity to inhibit NFκB than AM6-36	[13]
	In vivo	Female Sprague −Dawley rats (PO: 40 mg/kg)	Serum, liver, mammary tissues		1 Metabolites for serum (5); 5 Metabolites for liver tissue (4, 5, and 7–9); No metabolite in mammary tissue			
ARQ 501 (synthetic β-lapachone)	In vitro	Mouse, rat, dog, monkey, human whole blood	–	UPLC-QTOF-MS, HPLC-MS-ARC	4 Metabolites for mouse and rat (M1, M2, M3, M5); 5 Metabolites for dog (M1, M2, M3, M5, unidentified metabolite); 5 Metabolites for monkey (M1, M2, M3, M5, M6); 6 Metabolites for human (M1, M2, M3, M4, M5, M6); ARQ 501 disappeared after 60 min of incubation in human whole blood	Oxidation (M1); Adding 2 oxygen and 2 hydrogen atoms (M2); Adding 1 oxygen and 2 hydrogen atoms (M3); Loss of 1 carbon atom (M4 and M6); Loss of CO group (M5)	Not evaluated	[14]
ARQ 501 (synthetic β-lapachone)	In vivo	Mice (IP: 40 mg/kg), rats (IV: 40 mg/kg)	Plasma	LC-QTOF-MS, HPLC-QqQ-MS	3 Metabolites for all species	Glucosylation + sulfation; Glucuronidation; Sulfation	Not evaluated	[15]
	Clinical trial	Cancer patients (140 mg/m^2)			3 Metabolites			

Cabazitaxel	Phase I clinical trial	Advanced solid tumor patients (IV: 25 mg/m^2 of [^{14}C]-cabazitaxel (50 muCi, 1.85 MBq of [^{14}C]-cabazitaxel))	Plasma, urine, feces	HPLC-RD-MS	6 Metabolites in plasma (Major: RPR123142); 10 Metabolites in urine (Major: M09b); 13 Metabolites in feces (Major: RPR104943, RPR104952, RPR111026, RPR111059)	7-*O*-demethylation + 10-*O*-demethylation + hydroxylation (RPR104952); 10-*O*-demethylation (RPR123142); 7-*O*-demethylation + 10-*O*-demethylation + di-hydroxylation (M09b); 7-*O*-demethylation + di-hydroxylation + 10-*O*-demethylation (RPR111026, RPR111059); 7-*O*-demethylation + di-hydroxylation + 10-*O*-demethylation + hydroxylation (RPR104943); Cleavage is minor pathway plasma and urine	Not evaluated	[16]
Capecitabine	In vivo	Male Wistar rats (PO: 80 mg/kg)	Bile, urine, liver, kidneys	HPLC-TIS-MS/MS, ^{19}F-NMR and ^{1}H-NMR	3 Metabolites for liver (5′-deoxy-5-fluorocytidine, α-Fluoro-β-alanine, 2′-(β-D-glucuronic acid)-5′-deoxy-5-fluorocytidine); 2 Metabolites for bile (5′-deoxy-5-fluorocytidine, 2′-(β-D-glucuronic acid)–5′-deoxy-5-fluorocytidine); 2 Metabolites for kidney (5′-deoxy-5-fluorocytidine, α-Fluoro-β-alanine); 4 Metabolites for urine (5′-deoxy-5-fluorouridine, 5.6-Dihydro-5-fluorouracil, α-Fluoro-β-alanine, 5′-deoxy-5-fluorocytidine)	Glucuronidation (2′-(β-D-glucuronic acid)-5′-deoxy-5-fluorocytidine); Not identified (5′-deoxy-5-fluorocytidine, α-fluoro-β-alanine, 5′-deoxy-5-fluorouridine, 5.6-Dihydro-5-fluorouracil)	Not evaluated	[17]

(*Continued*)

Table 1 (Continued).

Anticancer Drug	Type of Study	Biochemical Tool/Animal/ Human (Route: Dose)	Type of Sample	Analytical Technique	Outcome			Ref.
					Metabolite	Metabolic Pathway	Efficacy/Toxicity of Metabolite	
Carbendazim (2-benzimidazolecarbamic acid methyl ester)	In vitro	RLM, HLM	–	HPLC-ESI-MS	1 Metabolite (5(6)- or 4(7)-hydroxyl carbendazim)	Oxidation	Not evaluated	[18]
	In vivo	Rats (PO: 1000 mg/kg)	Serum		1 Metabolite (2-aminobenzimidazole)	Hydrolysis		
CI941 (Isoxantrone)	In vitro	Rat hepatocyte, human hepatoblastoma (HepG2) cell lines	–	HPLC-UV, HPLC-QqQ-MS, ^{1}H-NMR	12 Metabolites (M2-M13) for rat hepatocyte; No metabolite in HepG2	Hydroxylation (M2, M3); Hydroxylation + oxidation (M8); Hydroxylation + oxidation + glutathione conjugation (M10); Hydroxylation+ glucuronidation (M4, M9); Hydroxylation + sulfation (M5-M7); Oxidation (M11-M13)	Not evaluated	[19]

Cisplatin	In vivo	Male Sprague-Dawley rats (IV: 3mg/kg)	Kidney tissue	HPLC-ESI-QTOF-MS	31 Metabolites (M1-M31)	Hydration (M1); Hydroxylation (M4); Dihydroxylation (M3); Methionine conjugation (M5); Di-methionine conjugation (M15, M29); Cysteine conjugation (M7, M8); Di-cysteine conjugation (M16, M31); Chlorination (M24, M26); Acetylcysteine conjugation (M9, M10); Di-acetylcysteine conjugation (M17); Glutathione conjugation (M11); Thioether conjugation (M13); Hydration+ hydroxylation (M2); Methionine conjugation + hydroxylation (M6); Methionine conjugation + chelation (M18, M20, M25); Methionine conjugation + chelation + hydroxylation (M19); Cysteine conjugation+ chelation (M21, M23, M27, M28, M30); Cysteine conjugation+ chelation + hydroxylation (M22); Glutathione conjugation +hydroxylation (M12); Thioether conjugation + hydroxylation (M14)	Not evaluated	[20]

(*Continued*)

Table 1 (Continued).

Anticancer Drug	Type of Study	Biochemical Tool/Animal/ Human (Route: Dose)	Type of Sample	Analytical Technique	Outcome			Ref.
					Metabolite	Metabolic Pathway	Efficacy/Toxicity of Metabolite	
CP-31398 (N'-[2-[2-(4-methoxyphenyl) ethenyl)-4-quinazolinyl]-N,N-dimethyl-1,3-propanediamine dihydrochloride)	In vivo	Male and Female CD rats (PO: 0, 240, 480, or 960 mg/m^2/day) and beagle dogs (0, 200, 400, or 800 mg/m^2/day)	Plasma	HPLC-QTOF-MS	4 Metabolites for rat (II: hydroxy CP-31398, III: *O*-demethyl CP-31398, IV: *N*-demethyl CP-31398, V: hydroxy *O*-demethyl CP-31398); 4 Metabolites for dog (II: hydroxy CP-31398, III: *O*-demethyl CP-31398, IV: *N*-demethyl CP-31398, VI: di-demethyl CP-31398)	Hydroxylation (hydroxy CP-31398); *O*-demethylation (*O*-demethyl CP-31398); *N*-demethylation (*N*-demethyl CP-31398); Hydroxylation + *O*-demethylation (hydroxy *O*-demethyl CP-31398); Di-demethylation (di-demethyl CP-31398)	Not evaluated	[21]
CPA (Cyproterone acetate)	In vivo	Female Wistar-Han rats (PO: 50 mg/kg [^{3}H] CPA (0.42 MBq/mg))	Bile	HPLC-MS, GC-MS, ^{1}H-NMR	2 Metabolites (M1: 3α-OH-CPA and M2)	Sulfation and/or Glucuronidation	3α-OH-CPA covalently bound to guanine which might be the major adduct formed of CPA	[22]
Crisnatol	In vitro	Human hepatoma (Hep G2) cells, HLM	–	GC-MS, ^{1}H-NMR	3 Metabolites for Hep G2 (M1 and M2: isomeric crisnatol dihydrodiol and M3: crisnatol 1,2-dihydrodiol); 2 Metabolites for HLM (M4: crisnatol 1,2-dihydrodiol and M5: 1-hydroxycrisnatol)	Di-hydroxylation + di-hydrogenation (M3, M4); Hydroxylation (M5)	Not evaluated	[23]
Cyclophosphamide	Clinical trial	Cancer patients (60 mg/kg)	Plasma, urine	GC-MS	1 Active metabolite for both samples (N, N-bis(2-chloroethyl) phosphorodiamidic acid)	Not identified	Not evaluated	[24]

DFS (Trans-2,6-difluoro-4′-(*N, N*)-dimethylamino) stilbene: Stilbene analog)	In vitro	Pooled MLM, RLM, HLM	–	HPLC-ESI-MS/MS	10 Metabolites for MLM (M1-10); 7 Metabolites for RLM (M1, M3-6, M9-10); 6 Metabolites for HLM (M1-3, M5, M7, M10)	Demethylation (M1); Oxidation (M2-M4); Demethylation + Oxidation (M5); Oxidation + Defluorination (M6, M7); Bisoxidation (M8, M9); Isomerization (M10)	Not evaluated	[25]
5′-dFUR (5′-deoxy-5-fluorouridine)	In vivo	Cancer patients (IV: 4 g/m^2)	Plasma	GC-MS	3 Metabolites (N^3-Methylated-5′-dFUR, 5-fluorouracil, and 5,6-dihydro-5-fluorouracil)	Methylation (N^3-Methylated-5′-dFUR); Not identified (5-fluorouracil, and 5,6-dihydro-5-fluorouracil)	Not evaluated	[27]
5′-dFUrd (5′-deoxy-5-fluorouridine)	Clinical trial	Cancer patients (IV: 10 g/m^2)	Blood, plasma, urine	^{19}F-NMR	4 Metabolites in blood and plasma (5-fluorouracil, α-fluoro-β-ureidopropionic acid, α-fluoro-β-alanine, 5,6-dihydrofluorouracil); 3 Metabolites in urine (5-fluorouracil, α-fluoro-β-ureidopropionic acid, α-fiuoro-β-alanine)	Not identified	Not evaluated	[26]
EAPB0203	In vitro	Pooled RLM, DLM	–	HPLC-MS/MS (QqQ and QTOF), ^{1}H-NMR, ^{13}C-NMR	4 Metabolites for DLM (EAPB0202, M7, M2, and M1); 6 Metabolites for RLM (EAPB0202, M7, M6, M3, M2, M1)	Hydroxylation (M1-M3, M6, M7); Demethylation (EAPB0202)	Not evaluated	[28]

(*Continued*)

Table 1 (Continued).

Anticancer Drug	Type of Study	Biochemical Tool/Animal/ Human (Route: Dose)	Type of Sample	Analytical Technique	Outcome			Ref.
					Metabolite	Metabolic Pathway	Efficacy/Toxicity of Metabolite	
EAPB0503 (imiquimod analogs) and EAPB0502 (M' 7) and EAPB0603 (M'5) (active metabolites of EAPB0503)	In vitro	MLM, RLM, DLM, HLM; Rat, dog, and human hepatocyte	–	LC-ESI-MS/MS	EAPB0503: 6 Metabolites for MLM (M'2-M'7); 4 Metabolites for RLM (M'2, M'4, M'5, M'7); 7 Metabolites for DLM (M'1-M'7); 4 Metabolites for HLM (M'2, M'4, M'5, M'7); 4 Metabolites for rat hepatocyte (M'2, M'4, M'5, and M'7); 5 Metabolites for dog hepatocyte (M'1, M'2, M'4, M'6, M'7); 4 Metabolites for human hepatocyte (M'2, M'4, M'5, M'7) EAPB0502: 2 Metabolites for MLM (M'2 and M'3); 2 Metabolites for RLM (M'2 and M'3); 2 Metabolites for DLM (M'2 and M'3); 1 Metabolite for HLM (M'2); 1 Metabolite for rat hepatocyte (M'3) EAPB0603: 2 Metabolites for MLM (M'1, M'2); 1 Metabolite for RLM (M'2); 2 Metabolites for DLM (M'1, M'2); 1 Metabolite for HLM (M'2); 1 Metabolite for dog hepatocyte (M'1)	*O*-demethylation (M'5); *N*-demethylation (M'7); Hydroxylation (M'4, M'6); *O*- and *N*-demethylation (M'2); *N*-demethylation + hydroxylation (M'3); Hydroxylation + *O*-demethylation (M'1)	Not evaluated	[29]

E-DE-BPH (E-3,4-bis (4-Ethylphenyl) hex-3-ene: stilbene derivatives)	In vitro	RLM (Aroclor 1254-treated), PLM	–	HPLC-UV, GC-MS, GC-IR	5 Metabolites for Aroclor 1254-treated RLM (M1: 3,4-bis(4-(1-hydroxyethyl) phenyl) hex-3-ene, M2: E-3-(4-acetylphenyl)-4-(4-(1-hydroxyethyl) phenyl) hex-3-ene, M3: E-3,4-bis(4-acetylphenyl) hex-3-ene, M4: E-3-(4-(1-hydroxyethyl) phenyl)-4-(4-ethylphenyl) hex-3-ene, M5: E-3-(4-acetylphenyl)-4- (4-ethylphenyl) hex-3-ene); 4 Metabolites for PLM (M1, M2, M4, M5)	Hydroxylation (M4); Hydroxylation + oxidation (M5); Di-hydroxylation (M1); Hydroxylation + oxidation + hydroxylation or di-hydroxylation + oxidation (M2); Hydroxylation + oxidation + hydroxylation + oxidation or di-hydroxylation + di-oxidation (M3)	Not evaluated	[30]
2-F-araA (9-β-D-arabinofuranosyl-2-fluoroadenine)	In vivo	Male BDF_1 mice (IV: 400 mg/m^2), female beagle dog (IV: 400 mg/m^2), female rhesus monkey (IV: 400 mg/m^2)	Urine, blood	HPLC-MS	1 Metabolite detected in dog blood (9-β-D-arabinofuranosyl hypoxanthine: 2-F-araH); 2 Metabolites for urine of all species (2-fluoroadenine: 2-F-ad, 2-F-araH)	Not identified	2-F-araH: no antitumor activity, no toxicity in BDF_1 mice bearing L1210 leukemia cells at 200 mg/kg (IP) for 9 days	[31]

(*Continued*)

Table 1 (Continued).

Anticancer Drug	Type of Study	Biochemical Tool/Animal/ Human (Route: Dose)	Type of Sample	Analytical Technique	Outcome			Ref.
					Metabolite	Metabolic Pathway	Efficacy/Toxicity of Metabolite	
Flutamide	In vitro	Male human, male dog, and male rat liver microsomes Male pig liver and prostate microsomes	–	LC-ESI-QTOF-MS	Liver microsomes: 1 Metabolites for human (OH-Flu); 4 Metabolites for dog (Flu-1, OH-Flu (2 isoforms), M1, M3); 4 Metabolites for rat (Flu-1, OH-Flu (3 isoforms), M1, M3 (2 isoforms)); 4 Metabolites for pig (Flu-1, OH-Flu (2 isoforms), M1, M3) Prostate microsomes: 2 Metabolites for pig (OH-Flu, Flu-5)	Monohydroxylation (OH-Flu); Di-hydroxylation (M1); Tri-hydroxylation (M3); Sulfation (Flu-3 sulfate); Glucuronidation (Flu-3 glucuronide); Hydroxylation + mercapturic acid conjugation (hydroxyflutamide mercapturic acid); Hydroxylation + glucuronidation (hydroxyflutamide glucuronide); Di-hydroxylation + glucuronidation (M1 glucuronide)	Formation of a mercapturic acid conjugate from flutamide in human urine, suggesting that the reported liver effects of flutamide are due to the molecular mechanism caused by a reactive intermediate	[32]
	In vivo	Prostate cancer patients) (Oral: 250 mg BID)	Urine		10 Metabolites (Flu-1, Flu-3, Flu-3 sulfate, Flu-3 glucuronide, OH-Flu, M1, M3, hydroxyflutamide mercapturic acid, hydroxyflutamide glucuronide, M1 glucuronide)			
4-Hydroxyanisole	In vivo	Malignant melanoma patients (IA: 80 g)	Urine	GC-MS	4 Metabolites (1: hydroquinone, 3: 3.4-dihydroxyanisole, 4: 3-methoxy-4-hydroxyanisol, and 5: 4-hydroxy-3-methoxyanisole)	Methylated metabolites might be conjugated with glucuronic acid or sulphates	Not evaluated	[33]

Irinotecan	In vitro	HLM, recombinant CYP enzymes (1A1, 1A2, 2C8, 2C9, 3A4, 3A5, and 3A7)	–	HPLC-Spectro-fluorometry, HPLC-MS	4 Metabolites for cells expressing CYP3A4 (M1, NPC, M2, APC); 5 Metabolites for microsomes containing CYP3A4 and CYP3A5 (M1, NPC, M2, APC, M4); 1 Metabolite for cells expressing CYP3A5 (M4); No metabolite for cells expressing CYP2C8, CYP2C9, CYP1A1, CYP1A2, and CYP3A7	Mono-hydroxylation (M1, M2); Loss of terminal piperidine (NPC); Di-oxidation (APC); De-ethylation (M4)	Not evaluated	[34]
	Phase I and II clinical trials	Cavum carcinoma patient (IV: 300 mg/m^2), brain tumor patient (600 mg/m^2), glioblastoma patients (350 mg/m^2)	Urine		7 Metabolites (SN-38-G, M1, NPC, M2, M3, APC, SN-38)			
Ixabepilone	Clinical trial	Cancer patients (IV: 70 mg; 80 nCi)	Plasma, urine, feces	HPLC-AMS, LC-MS	3 Degradants and 4 metabolites for plasma and urine (Deg-1–Deg-3, M8, M16, M19, M41); 3 Degradants and 3 metabolites for feces (Deg-1–Deg-3, M8, M19, M41)	Dehydration (Deg-1-Deg-3); Dehydration + oxygenation (M41); Hydroxylation (M8); Oxygenation + dehydrogenation (M16); Oxygenation + dehydration (M19)	Not evaluated	[35]

(*Continued*)

Table 1 (Continued).

Anticancer Drug	Type of Study	Biochemical Tool/Animal/ Human (Route: Dose)	Type of Sample	Analytical Technique	Outcome			Ref.
					Metabolite	Metabolic Pathway	Efficacy/Toxicity of Metabolite	
JM216 (bis (acetato) ammine-dichloro (cyclohexylamine) Platinum (IV))	Phase I clinical trial	Cancer patients (PO: 120, 200, 300, 420, 540 mg/ m^2)	Plasma	HPLC-MS	6 Platinum metabolites (A, JM383, JM559, D, JM118, F)	Hydrolysis + declorination (JM518, JM559); Di-hydrolysis + di-declorination (JM383); Hydrolysis + declorination +reduction + clorination (JM118); Hydrolysis + declorination + reduction + glutathione conjugation + methylation (A)	JM118: higher cytotoxic activity against 8 human ovarian carcinoma cell lines (HX 62, SKOV-3, CH1, CH1 CISR, 41M, 41M CISR, A2780 A2780 CISR) compared to JM216 and cisplatin JM518 and JM383: similar and less cytotoxic activity to JM216	[36]
	In vitro	Human plasma	Plasma		5 Platinum metabolites (A, JM383, JM559, JM518, JM118)			
K02 (Morpholine-urea-Phe-Hphe-vinylsulfone)	In vitro	HLM, cDNA-expressed CYP3A4	–	HPLC-UV, LC-MS	3 Major metabolites for both (M12, M19, M20)	Hydroxylation	Not evaluated	[37]
Laromustine	In vitro	RLM, DLM, HLM, MLM	–	HPLC-RD	7 Similar metabolites for all species but in different quantity (C1-C7: C4 = major metabolite)	Demethylsufoxidation.; Hydrolysis; Oxidation	Not evaluated	[38]
2-Methoxyestradiol	In vitro	Pooled HLM	–	HPLC, HPLC-QqQ -MS	4 Phase I metabolites (A, B, C, D); 2 Phase II metabolites (G1, G2)	Hydroxylation (A, B, C, D); Glucuronidation (G1, G2)	Not evaluated	[39]
	In vivo	Cancer patients (PO: 800 mg or 2200 mg BID)	Urine		2 Phase II metabolites (G1, G2)	Glucuronidation (G1, G2)		

Mitomycin C	In vivo	Murine adenocarcinomas of the colon, MAC 16 (high DT-diaphorase activity) and MAC 26 (low DT-diaphorase activity) bearing mice (Intra tumor: 500 µg)	Tumor	HPLC-UV	4 Metabolites (*trans*-hydro, *cis*-hydro, 2,7-diaminomitosene (2,7-DM), decarbamoyl 2,7-DM)	Hydrolysis (*trans*-hydro, *cis*-hydro); Reduction (2,7-DM); Reduction + decarbamoyl (decarbamoyl 2,7-DM)	Not evaluated	[40]
NB-506 (6-*N*-formylamino-12,13-dihydro-1,11-dihydroxy-13-(b-D-glucopyranosil) 5*H*-indolo [2,3-*a*] pyrrolo [3,4-*c*] carbazole-5,7 (6*H*)-dione)	In vivo	Male Sprague-Dawley rat (IV: 187.5 mg/m^2 of [^{14}C] NB-506 (1.85 MBq/kg))	Bile	HPLC-RD-MS, ^{1}H NMR, ^{13}C NMR	3 Metabolites (RBM-1, RBM-2, ED501)	Deformyl metabolite (ED501); *O*-glucuronidation (RBM-1); ED501 + glucuronidation (RBM-2)	Not evaluated	[41]
9NC (9-nitro-20(*S*)-camptothecin)	In vivo	Wistar rats (IV: 8 mg/kg)	Bile, urine, feces	LC-IT-MS, LC-QTOF-MS (M2, M5 and M7), ^{1}H NMR	5 Metabolites for bile (M1, M2, M3: 9-AC, M4: 9-AA-CPT, M5); 3 Metabolites for urine (M1, M3, M6); 3 Metabolites for feces (M4, M6, M7: 9-OH-CPT)	Glucuronidation of 9-OH-CPT (M1); Loss of glutamic acid from M5 (M2); Reduction (M3); Acetylation (M4); Glutathione conjugate of 10-OH-CPT (M5); Mercapturic acid conjugate of 10-OH-CPT (M6); Aglycone of M1 (M7)	Not evaluated	[42]
NSC 141549 (4′-(9-Acridinylamino) methanesulfon-m-a nisidide (*m* -AMSA))	In vivo	Male Sprague-Dawley rats (IV: 60 mg/kg and IP: 40 mg/kg)	Plasma (IV), bile (IP)	HPLC-MS	1 Major metabolite for plasma (4-amino-3-methoxymethanesulfonanilide); 1 Major metabolite for bile (9-acridinyl thioether of glutathione)	Methoxylation (4-amino-3-methoxy methanesulfonanilide); Glutathione conjugation (9-acridinyl thioether of glutathione)	Not evaluated	[43]

(*Continued*)

Table 1 (Continued).

Anticancer Drug	Type of Study	Biochemical Tool/Animal/ Human (Route: Dose)	Type of Sample	Analytical Technique	Outcome			Ref.
					Metabolite	Metabolic Pathway	Efficacy/Toxicity of Metabolite	
NSC 652287 (2,5-bis(5-hydroxymethyl-2-thienyl) furan)	In vitro	Dog liver S9 fraction	–	GC-MS	1 Major metabolite	Methylation or oxidation	Not evaluated	[44]
NSC 674495 (2-(4-Amino-3-methylphenyl) benzothiazole: *DF* 203)	In vitro	Breast cancer cell lines (MCF-7, T-47D, and MDA-MB-435) and renal cancer cell lines (TK-10, A498, and CAKI-1)	–	HPLC-spectro-fluorometry, ^{1}H NMR	1 Major metabolite for sensitive cell lines (T-47D, MDA-MB-435): (6c: 2-(4-amino-3-methylphenyl)-6 hydroxybenzothiazole)	Hydroxylation	6c: low anti-cancer activity against MCF-7 cell lines (IC_{50}>100 M)	[45]
PAC-1 (4-benzyl-piperazin-1-yl)-acetic acid (3-allyl-2-Hydroxy-benzylidene)-hydrazine	In vivo	Male Wistar rats (PO: 50 mg/kg)	Urine, feces, bile	HPLC-QTOF-MS, HPLC-IT-MS	14 Metabolites for urine (M1-M7, M9-M11, M13-M16); 3 Metabolites for feces (M1, M6, M13); 4 Metabolites for bile (M4, M13, M14, M16)	Debenzylation (M1); Hydroxylation (M2-M6); Dihydroxylation (M8–M12); Carboxylation (M7); Dihydrodiol formation (M13); Glucuronidation (M14); Hydroxylation + glucuronidation (M15-M16)	Not evaluated	[46]
	In vitro	RLM	–		12 metabolites (M1-M6, M8-M13)			
Paclitaxel	In vivo	Ovarian carcinoma patients (IV: 135 mg/m^2)	Plasma, urine	HPLC-MS	2 Metabolites for plasma (P1: 6α-hydroxypaclitaxel, P3: 7-epipaclitaxel); 3 Metabolites for urine (U1: 6α-hydroxypaclitaxel, U2: 10-deacetylpaclitaxel, U4: 7-epipaclitaxel)	Not identified	Not evaluated	[47]

Paclitaxel	In vitro	Liver microsomes from: Sprague-Dawley & hairless rats (males, females), BALB/c & OF1 mice (males, females), male guinea-pigs, male rabbits, male dogs, male monkeys, and humans	–	HPLC-UV	1 Metabolite for male & female SD and hairless RLM, male & female BALB/c MLM (3′*p*-hydroxypaclitaxel); 2 Metabolites for male & female OF1 mice & male rabbits (6α hydroxypaclitaxel and 3′*p*-hydroxypaclitaxel); 2 Metabolites for male dog (6α-hydroxypaclitaxel, 6α,3′*p*-dihydroxypaclitaxel) liver microsomes; 3 Metabolites in male guinea-pigs, male monkey, human liver microsomes (6α-hydroxypaclitaxel, 3′*p*-hydroxypaclitaxel, 6α,3′*p*-dihydroxypaclitaxel)	Mono-hydroxylation (3′*p*-hydroxypaclitaxel, 6α-hydroxypaclitaxel); Di-hydroxylation (6α,3′*p*-dihydroxypaclitaxel)	Not evaluated	[48]
	In vivo	Male Dunkin Hartley albino guinea-pigs (IV: 6 mg/kg)	Bile		2 metabolites (M1: 6α Hydroxypaclitaxel, M2: 3'*p*-Hydroxypaclitaxel)			
Phenylbutyrate	Clinical trial	Human (PO: 0.36 mmol/kg (5 g/75 kg))	Plasma, urine	GC-MS	3 Metabolites for both samples (phenylacetate, phenylacetylglutamine, phenylbutyrylglutamine)	Not identified	Not evaluated	[49]
Phyllanthoside	In vivo	Male CDF_1 mice	Plasma	HPLC-UV	1 Metabolite (M)	Aglycone	M: anticancer activity against human rhabdomyosarcoma cell lines (A204 cell lines) lower than parent compound with IC_{50} of 24 μM and 0.47 nM	[50]

(*Continued*)

Table 1 (Continued).

Anticancer Drug	Type of Study	Biochemical Tool/Animal/ Human (Route: Dose)	Type of Sample	Analytical Technique	Outcome			Ref.
					Metabolite	Metabolic Pathway	Efficacy/Toxicity of Metabolite	
Sepin-1	In vitro	MLM, RLM, HLM	–	UHPLC-QTOF-MS	7 Metabolites for all species (M1-M7)	Reduction (M1, M2); Dimer formation (M3-M5); Cysteine conjugation (M6); Glutathione conjugation (M7)	Not evaluated	[51]
SN30000 (3-(3-Morpholinopropyl)-7,8-dihydro-6H-indeno[5,6-e] [1,2,4] triazine 1,4-dioxide: tirapazamine analog)	In vivo	Female NIH-III mice (IP: 186 mg/kg)	Plasma, liver, HT29 tumor	LC-MS/MS	19 Metabolites for plasma (M1-M19); 16 Metabolites for liver (M3-M7, M9, M10-M19); 19 Metabolites for tumor (M1-M19)	Reduction; Oxidation; Glucuronidation	M14: acute toxicity (hypothermia) similarly to SN30000	[6]
Tamoxifen	In vivo	Athymic mice (PO and SC: 200 mg/kg/day, 50 mg/kg/day, and 12.5 mg/kg/day), Breast cancer patients (10 mg BID)	Serum	HPLC-UV	2 Metabolites for mouse serum after PO and SC of 200 mg/kg/day and 50 mg/kg/day and patient serum (4-Hydroxytamoxifen and *N*-desmethyl tamoxifen); Higher level of metabolites with higher doses	Hydroxylation (4-hydroxytamoxifen); *N*-demethylation (*N*-desmethyltamoxifen)	Not evaluated	[52]
Tamoxifen	In vitro	Human liver homogenate, human hepatoblastoma (HepG2) cell lines	–	LC-ESI-MS	6 Metabolites for human liver homogenate (M1-M5, M8); 4 Metabolites for HepG2 (M2-M5)	*N*-Di-desmethylation (M1); *N*-Desmethylation (M4); Hydroxylation (M2 and M3); *N*-oxidation (M5); *N*-Desmethylation + hydroxylation (M6, M7); *N*-oxidation + hydroxylation (M8)	Not evaluated	[53]
	In vivo	Breast cancer patients (PO: 60 mg OD or 30 mg OD > 6 months)	Plasma		8 Metabolites (M1-M8; M8 only in patients who received long term > 6 months)			

Tamoxifen	In vivo	Female Rowett athymic nude rats (PO: 5 mg/kg/day or SC: 50 mg)	Serum, liver, lung, retroperitoneal adipose tissue, kidneys, brain, muscle, heart, spleen, stomach, small intestine, uterus tissues		2 Metabolites for all samples except adipose tissue (4-Hydroxytamoxifen, *N*-Desmethyltamoxifen) after 21 days PO; 4-Hydroxytamoxifen for all samples except adipose tissue and uterus after 33 days PO; *N*-Desmethyltamoxifen for all samples except adipose tissue after 33 days PO; 2 Metabolites for liver, lung, brain, heart, and kidney (4-Hydroxy tamoxifen and *N*-Desmethyltamoxifen) after 33 days SC; Level of metabolites detected in SC lower than PO	Hydroxylation (4-Hydroxytamoxifen); Demethylation (*N*-Desmethyltamoxifen)	Not evaluated	[54]

(*Continued*)

Table 1 (Continued).

Anticancer Drug	Type of Study	Biochemical Tool/Animal/ Human (Route: Dose)	Type of Sample	Analytical Technique	Outcome			Ref.
					Metabolite	Metabolic Pathway	Efficacy/Toxicity of Metabolite	
Tamoxifen	Clinical trial	Breast cancer patients (PO: 20–40 mg/day)	Plasma	UHPLC-QqQ-Quantum-MS and UHPLC-Exactive Plus Orbitap-MS	40 Metabolites (1–23, 25–27, 29–36, 38, 39, 42–45)	Demethylation + hydroxylation + glucuronidation (1, 9, 13, 19); Hydroxylation + glucuronidation (2, 11, 15, 18); Carboxylation + glucuronidation (3); Di-hydroxylation + oxidation + glucuronidation (4, 21); Di-methylation + tetra-hydroxylation + glucosylation (5); Demethylation + carboxylation (6); Di-hydrodiol (7); Carboxylation (8); Demethylation + hydroxylation (10, 25, 27.32, 44); Hydroxylation (12, 29, 30, 33); Hydroxylation + carboxylation (14); Di-hydroxylation (16, 17, 31); Hydroxylation + sulfation (20); Di-demethylation + tri-hydroxylation + glucoside (22,26); Di-demethylation + hydroxylation (23); E-tamoxifen (34); Di-demethylation (35); Demethylation + desaturation (36); N-demethylation (38); Desaturation (39); Hydroxylation + desaturation (42, 45); Adding NO molecule (43)	Not evaluated	[55]

TAS-102 (tipiracil hydrochloride (TPI) + trifluridine (FTD))	Clinical trial	Advanced solid tumor patients (PO: 1000 nCi of [^{14}C]-TPI)	Plasma, urine, feces	HPLC-QqQ-MS	1 Major metabolite for plasma, urine, and feces (6-hydroxymethyluracil (6-HMU))	Not identified	Not evaluated	[56]
		Advanced solid tumor patients (PO: 200 nCi of [^{14}C]-FTD)			1 Major metabolites for plasma (Trifluoromethyluracil (FTY)); 3 Major metabolites for urine (FTY, FTD Glu U3, FTD Glu U4)	Glucuronidation (FTD Glu U3 and FTD Glu U4); Unidentified (FTY)		
TNP-470 (O-(chloroacetyl-carbamoyl) fumagillol)	In vitro	Human hepatocyte, human liver, intestinal, kidney microsomes	–	HPLC-QqQ-MS	6 Metabolites for cultured human hepatocytes (M1-M6: M2 and M5 = predominant metabolites of extracellular and intracellular, respectively); 3 Metabolites for HLM (M1, M2, M4); 2 Metabolites for intestine and kidney microsomes (M2, M4)	Esterase (M4); Esterase + Epoxide hydrolysis (M2)	Not evaluated	[57]
TW01003 ((3E,6E)-3-Benzylidene-6-[(5-hydroxypyridin-2-yl) methylene] piperazine-2,5-dione)	In vivo	Pig (IV) and male Wistar rats (IV: 7 mg/kg and PO: 36 mg/kg)	Plasma (rat) and urine (pig)	HPLC	1 Metabolite for pig urine (TW-01003 sulfate); 2 Metabolites for rat plasma after IV (TW-01003 sulfate (major), TW-01003 glucuronide); 2 Metabolites after PO (TW-01003 sulfate, TW-01003 glucuronide (major))	Sulfation (TW-01003 sulfate); Glucuronidation (TW-01003 glucuronide)	Not evaluated	[58]

Abbreviations: AMS, Accelerator Mass Spectrometry; ARC, Accurate Radioisotope Counting; DLM, Dog Liver Microsome; ESI, Electrospray Ionization; GC, Gas Chromatography; HLM, Human Liver Microsome; HPLC, High Performance Liquid Chromatography; IR, Infrared spectroscopy; IP, Intraperitoneal; IT, Ion trap; IV, Intravenous; LC, Liquid Chromatography; MLM, Monkey Liver Microsome; MS, Mass Spectrometry; NMR, Nuclear Magnetic Resonance spectroscopy; PLM, Pig Liver Microsome; PO, Per oral; QqQ, Triple quadrupole; QTOF, Quadrupole Time-of-flight; RD, Radio Detection; RLM, Rat Liver Microsome; SC, Subcutaneous; TIS, Turbo Ion Spray; UHPLC, Ultra-High Performance Liquid Chromatography; UPLC, Ultra Performance Liquid Chromatography; UV, Ultraviolet detection.

Table 2 Metabolism Studies (Metabolite Profiling) of Small Molecules-Targeted Anticancer Drugs

Anticancer Drug	Type of Study	Biochemical Tool/Animal/ Human (Route: Dose)	Type of Sample	Analytical Technique	Outcome			Ref.
					Metabolite	Metabolic Pathway	Efficacy/ Toxicity of Metabolite	
AZD8055	In vitro	RLM	–	UHPLC-IT-MS	5 Metabolites (M1-M5)	Demethylation (M1); Hydroxylation (M2); Oxidation (M3 and M4); Di-oxidation (M4); Morpholine ring opened AZD8055 (M5); Demethylation + glucuronidation (M6); Hydroxylation + glucuronidation (M7); Glucuronidation (M8)	Not evaluated	[59]
	In vivo	Male Sprague-Dawley rat (PO: 20 mg/kg)	Plasma, urine, feces		8 Metabolites in all samples (M1-M8)			
Brivanib	In vitro	Pooled HLM and cytosol, human cDNA-expressed CYP, and human recombinant SULTs	-	HPLC-LTQ-IT-MS	6 Metabolites (M7, M19, M25, M26, M31 M33)	Hydroxylation (M7); Oxidation (M26, M31); Sulfation (M25); Oxidation + sulfation (M19, M33)	Not evaluated	[60]

EPZ-5676 (Pinometostat)	In vitro	RLM, DLM, HLM, and rat, dog, and human hepatocyte	-	HPLC-RD-IT-TOF-MS	6 Metabolites for RLM (EPZ007769, EPZ007309, M3, M4, M6, M8); 1 Metabolite for DLM (EPZ007769); 5 Metabolites for HLM (EPZ007769, EPZ007309, M3, M4, M6); 7 Metabolites for rat hepatocyte (EPZ007769, EPZ007309, M1a/b, M2, M3, M6, M8); 4 Metabolites for dog hepatocyte (EPZ007769, EPZ007309, M1a/b, M2); 5 Metabolites for human hepatocyte (EPZ007769, EPZ007309, M1a/b, M2, M6)	Mono-hydroxylation (EPZ007769); Mono-hydroxylation + oxidation (EPZ026194); Dealkylation of *N*-isopropyl (EPZ007309); *N*-dealkylation + tri-oxidation (M1a/b); *N*-dealkylation + di-oxidation (M2); *N*-dealkylation + mono-oxidation (M3); *N*-dealkylation + keto-oxidation (M4); Mono-oxidation + dehydrogenation (M5); Mono-oxidation (M6 and M7); *N*-dealkylation + hydroxylation (M8); *N*-dealkylation (M9, M10 and M11); Oxidative cleavage of ribose ring (M12); *N*-dealkylation + oxidation (M13)	Not evaluated	[61]
	In vivo	Sprague– Dawley rats (IV: 30 mg/kg/day (100 µCi/kg)) and Beagle dogs (IV: 10 mg/kg/day (200 µCi/animal))	Plasma, urine, feces, bile (rat and dog)		2 Metabolites for rat plasma (EPZ007769, EPZ026194); 3 Metabolites for rat urine (EPZ007769, EPZ026194, M5); 2 Metabolites for dog urine (EPZ007769, EPZ026194); 4 Metabolites for rat feces (EPZ007769, EPZ026194, M7, M9); 4 Metabolites for dog feces (EPZ007769, EPZ026194, M7, M8); 4 Metabolites for rat bile (EPZ007769, EPZ026194, M5, M6); 2 Metabolites for dog bile (EPZ007769, EPZ026194)			
	Phase I clinical trial	Cancer patient (IV: 36 mg/m^2/day)	Plasma, urine		2 Metabolites for plasma (EPZ007769, EPZ007309); 10 Metabolites for urine (EPZ007769, EPZ007309, M1a/b, M2, M8-M13)			

(*Continued*)

Table 2 (Continued).

Anticancer Drug	Type of Study	Biochemical Tool/Animal/ Human (Route: Dose)	Type of Sample	Analytical Technique	Outcome			Ref.
					Metabolite	Metabolic Pathway	Efficacy/ Toxicity of Metabolite	
HM781-36B (1-[4-[4-(3,4-dichloro-2-fluorophenylamino)-7-methoxyquinazolin-6-yloxy]-piperidin-1-yl] prop-2-en-1-one hydrochloride)	In vivo	Male beagle dogs (PO: 8 mg/kg)	Urine, feces, plasma	HPLC-UV, LC-ESI-IT-MS, LC-QTOF-MS/MS	4 Metabolites for plasma (M1, M2, M8, M10); 3 Metabolites for urine and feces (M1, M2, M10)	Di-hydroxylation (M1); Demethylation (M2); *N*-Oxidation (M3, M9); Defluorination + hydroxylation (M4); Dechlorination + hydroxylation (M5); Demethylation + di-hydroxylation (M6); *N*-oxidation + di-hydroxylation (M7); Hydroxylation (M8); Deacryloylpiperideine (M10)	Not evaluated	[5]
	In vitro	DLM, HLM, Recombinant CYP enzymes (3A4, 2D6, 1A2, 2C9, 2C19, and 2E1)	–		10 Metabolites for HLM (M1-M10); 4 Major metabolites for DLM (M1, M2, M8, M10); 10 Metabolite for rCYP3A4 (M1-M10); 1 Metabolite for rCYP2D6 (M2)			
Imatinib	In vitro	Insect-cell microsomes that contained cDNA expressing human CYP isozymes (CYP1A1, 1A2, 1B1, 4F2, 4F3A/B, 3A4)	–	HPLC-LTQ-MS	6 Metabolites (*N*-demethyl-, Unk#3, Unk#5, Unk#6, Unk#7, Unk#8) for CYP3A4; 4 Metabolites for CYP1A1 (*N*-demethyl-, Unk#3, Unk#5, Unk#7); 1 Metabolite for 1A2 (7); 2 Metabolites for 1B1 (5 and 7); 2 Metabolites for 4F2 (*N*-demethyl- and 5); 2 Metabolites for 4F3A and 4F3B (*N*-demethyl- and 5)	*N*-demethylation (*N*-demethyl-); Hydroxylation (Unk#3 and Unk#5); *N*-oxidation (Unk#6, Unk#7, and Unk#8)	Not evaluated	[62]
Imatinib	In vitro	HLM	–	HPLC-ESI-QqQ-MS	28 Metabolites (1–17, 19–24, 27–29, 32, 33)	Hydroxylation/*N*-oxidation (3, 8, 9,11–14, 18, 19, 22–24, 32); Hydroxylation/*N*-oxidation + *N*-demethylation (7, 10, 16, 20); Glucuronidation (27–29, 33); Other (2, 6, 31)	Not evaluated	[63]
	Clinical trial	Cancer patients (PO: 400-800 mg)	Plasma		31 Metabolites (1–14, 16, 18–20, 22–33)			

Irosustat	In vitro	RLM, DLM, MLM, HLM, Rats and dogs hepatocytes	–	HPLC-UV, HPLC-MS and ^{1}H NMR	RLM: 12 Metabolites for male (M7-M11, P-24, M13-M16, M18, 667-coumarin) & female (M7-M11, M13-M16, M18, P36, 667-coumarin); DLM: 14 Metabolites for male & female (P-14, M7-M10, P-23, P-24, M13-M16, M18, P-36, 667-coumarin); MLM: 9 Metabolites for male (P-14, M7-M10, P-24, M13, M14, M16); 8 Metabolites for female (P-14, M7-M10, P-24, M13, M16); HLM: 14 Metabolites for male & female (M7-M11, P-23, P-24, M13-M16, M18, P-36, 667-coumarin); Hepatocytes: 6 Metabolites for male rat (M2, M3, M7, M12, M17, 667-courmarin); 2 Metabolites for male dog (M12, M17); 7 Metabolites for female human (M1, M2, M12, M16, M17, 667-coumarin)	Hydroxylation (M7, M9, M13, M14, M18); Desulfamoylation (667-coumarin); Hydroxylation + desulfamoylation (M8, M15, M16, and P-36); Hydroxylation + desulfamoylation + glucuronidation (M1, M2, and M12); Hydroxylation + desulfamoylation + sulfation (M17); Not identified (M11)	Not evaluated	[64]

(*Continued*)

Table 2 (Continued).

Anticancer Drug	Type of Study	Biochemical Tool/Animal/ Human (Route: Dose)	Type of Sample	Analytical Technique	Outcome			Ref.
					Metabolite	Metabolic Pathway	Efficacy/ Toxicity of Metabolite	
JNJ-38877605	In vitro	Rat, rabbit, dog, monkey, and human hepatocytes and liver subcellular fractions	–	LC-MS	12 Metabolites for all species hepatocyte and liver subcellular fraction (M1-M12)	Hydroxylation (M1, M3); *N*-demethylation (M2); Oxidation (M4); *N*-demethylation + hydroxylation (M5-M7); Hydroxylation + glucuronidation (M8); *N*-demethylation + hydroxylation + glucuronidation (M9 and M11); *N*-demethylation + glucuronidation (M10); *N*-demethylation + oxidation + glutathione conjugation (M12)	M1-M5: crystal formation in kidney; M10: renal toxic metabolites in rabbit and human (species-specific toxic metabolite)	[65]
	In vivo	Rat (PO: 2.5 mg/kg), dog (PO: 2.5 mg/kg), and rabbit (PO: 100 mg/kg)	Plasma, urine, feces		6 Metabolites for rat plasma, urine, feces (M1-M6); 4 Metabolites for dog plasma, urine, feces (M1-M4); 7 Metabolites for rabbit plasma, urine, feces (M2-M7, M10)			
	Phase I clinical trial	Cancer patients (PO: 60 mg)	Plasma, urine		4 Metabolites for human plasma and urine (M2, M3, M5, and M10)			

Lenvatinib	In vivo	Male Sprague Dawley rats (PO: 30 mg (12.685 MBq)/kg), Cynomolgus monkey (PO: 30 mg (12.685 MBq)/kg), and Solid tumor or lymphoma patients (PO: 24 mg (100 μCi))	Plasma, urine, feces and bile	HPLC-LTQ-IT-MS, HPLC-LTQ-Orbitrap-MS	27 Metabolites for rat (M1-M3, M5, MET5, MET6-MET8, MET10, MET12, MET15, MET17, MET18, MET19a, MET19b, MET20.1, MET21-MET24, MET24.1, MET27, MET29, MET29.1, MET30, MET32, MET34, MET35); 24 Metabolites for monkey (M1-M3, M2′, M3′, M5, MET3, MET4, MET9, MET10, MET13, MET16, MET18, MET20, MET20.1, MET22, MET23, MET24.1, MET26, MET27, MET29.1, MET30, MET32, MET34); 18 Metabolites for human (M1-M3, M2′, M3′, M5, MET3, MET4, MET20.1, MET22, MET24, MET27, MET29.1, MET30-MET32, MET34 MET36)	Oxidation/hydroxylation (M3′, MET34, MET35); Di-oxidation/Di-hydroxylation (MET36); *N*-oxidation (M3); Dealkylation (M1, M2); Hydrolysis (M5, MET30, MET32); Hydrolysis + oxidation/hydroxylation (MET29); Dealkylation + oxidation/hydroxylation (M2′); Oxidation/hydroxylation + glucuronidation (MET31); Dealkylation + glucuronidation (MET27); Dealkylation + glucuronidation + hydrolysis (MET24); Hydrolysis/*O*-dearylation (MET29.1); Hydrolysis/*O*-dearylation + other conjugation (MET20.1, MET24.1); Glutathione/cysteine conjugation and subsequence biodegradation (MET7, MET8, MET10, MET12, MET13, MET15, MET18, MET19b, MET26); Glutathione/cysteine conjugation and subsequence biodegradation + intramolecular arrangement (MET19a and MET21); Glutathione/cysteine conjugation and subsequence biodegradation + intramolecular arrangement + dimerization (MET16, MET17, MET20, MET22, MET23); Glutathione/cysteine conjugation and subsequence biodegradation + intramolecular arrangement + glutathione/cysteine conjugation and subsequence biodegradation (MET3, MET4, MET6, MET9)	Not evaluated	[66]

(*Continued*)

Table 2 (Continued).

Anticancer Drug	Type of Study	Biochemical Tool/Animal/ Human (Route: Dose)	Type of Sample	Analytical Technique	Outcome			Ref.
					Metabolite	Metabolic Pathway	Efficacy/ Toxicity of Metabolite	
Neratinib	In vitro	Rat hepatocytes	-	UHPLC-DAD, UHPLC-Quadrupole-Orbitrap-MS	12 Metabolites (M1-M12)	*O*-Dealkylation + glutathione conjugation (M1, M2); *O*-Dealkylation (M3); Oxygenation + glutathione conjugation (M4, M5); Glutathione conjugation (M6, M7); Oxygenation (M8); *N*-Acetylcysteine conjugation (M9, M11); *N*-demethylation (M10); *N*-oxygenation (M12)	Not evaluated	[67]
	In vivo	Male Sprague–Dawley rats (PO: 20 mg/kg)	Bile, urine		12 Metabolites for bile (M1-M12); 6 Metabolites for urine (M3 and M8-M12)			
ON 013100 ((E)-2,4,6-trimethoxystyryl-3-hydroxy-4-methoxybenzyl sulfone	In vitro	Colon cancer cell lines (drug resistant, colo-205, and drug sensitive, colo-320	–	HPLC-MS, HPLC-MS/MS	E-form and Z-form of glucuronidation of ON 013100 were detected in colo-205 incubation system but not colo-320	Glucuronidation	Not evaluated	[68]
Osimertinib	In vitro	HLM, MLM	–	UHPLC-MS/MS	7 Metabolites for HLM (DM-1: AZ5104, DM-2:AZ7550, OH-1, OH-2, OH-3, OH-4, OH-5); 4 Metabolites for MLM (DM-1, DM-2, OH-2, OH-4)	*N*-Demethylation (DM1 and DM-2); Oxidation (OH-1, OH-5); *N*-oxidation (OH-2, OH-4)	Not evaluated	[69]
Pimasertib	Clinical trail	Cancer patients (PO: 60 mg)	Plasma, urine, and feces	UPLC-MS	2 Metabolites (M445, M554)	Oxidation (M445); Phosphoethanolamine conjugation (M554)	Not evaluated	[70]

TKI258 (Dovitinib)	Cohort	Cancer patients (PO: 500 mg)	Plasma, Urine, and Feces	HPLC, LTQ-Orbitrap-MS	3 Metabolites for plasma (M8, M9, M26); 6 Metabolites for urine (M4, M5, M8, M9, M22, M26); 8 Metabolites for feces (M2, M5, M6, M8, M10, M26, M32, M33)	Fluorobenzyl ring hydroxylation (M6, M10); *N*-demethylation (M8); Piperazine *N*-oxide (M9); Glucuronidation (M26); Piperazine *N*-dealkylation (M30); 2 (+O, −2H) on piperazine (M32); (+O, −2H) on piperazine (M33); Fluorobenzyl ring hydroxylation + sulfation (M2 and M5); Fluorobenzyl ring hydroxylation + glucuronidation (M4); *N*-demethylation + hydroxylation + sulfation (M23)	Not evaluated	[4]

Abbreviations: CYP, Cytochrome P450; DAD, Diode Array Detection; DLM, Dog Liver Microsome; ESI, Electrospray Ionization; HLM, Human Liver Microsome; HPLC, High Performance Liquid Chromatography; IT, Ion trap; IV, Intravenous; LC, Liquid Chromatography; LTQ, Linear Trap Quadrupole; MS, Mass Spectrometry; MLM, Monkey Liver Microsome; NMR, Nuclear Magnetic Resonance spectroscopy; PO, Per oral; QqQ, Triple quadrupole; QTOF, Quadrupole Time-of-flight; RLM, Rat Liver Microsome; TOF, Time-of-Flight; UHPLC, Ultra-high performance liquid chromatography; UPLC, Ultra Performance Liquid Chromatography; UV, Ultraviolet detection.

efficiency over other methods such as radioimmunoassay (RIA), gas chromatography/mass spectrometry (GC/MS),[44] and liquid chromatography (LC) with ultraviolet detection (UV),[19,76] fluorescence,[34,45] radioactivity[38,81] and mass spectrometry (MS)[13,18,64] detection. Most of the works in metabolite analysis were carried out using triple-quadrupole mass spectrometers.[28,39,55,57,63,82] The main advantages include its superior quantitative capabilities in the multiple reaction monitoring (MRM) mode and the fact that a family of metabolites can easily be identified using neutral-loss and precursor ion scans.

Factors Influencing Metabolite Formation

Several factors were shown to influence metabolite formation of the parent drugs/compounds with anticancer activities. These included species, gender, and route and dose of administration of the parent drugs/compounds.

Species

Metabolic pathways and metabolite profiles of anticancer drugs/candidate compounds varied with animal species investigated. This is explained by the difference in the expression of metabolizing enzymes. For example, CYP1A1 and 1A2 enzymes are presented in mouse, rat, dog, monkey, and human, whereas CYP2C9, 2C19, 2D6, and 3A4 enzymes are only presented in human.[99] In a study of flutamide (nonsteroidal antiandrogen used primarily for prostate cancer) metabolism, only one metabolite (designated OH-flu) was detected in human after incubation with human liver microsomes. On the other hand, four metabolites (Flu-1, OH-flu, M1, and M3) were detected after incubation of the compound with liver microsomes from rat, dog, and pig.[32] Moreover, similar metabolite (OH-flu) was detected in rat and pig, but this metabolite was detected as 3 and 2 isoforms in rat and pig, respectively.[32] For the synthetic β-lapachone, ARQ 501, similar phase I metabolites (M1-M3, and M5) were detected after incubation with whole blood of mouse, rat, dog, monkey, and human. On the other hand, only one phase I metabolite M4 was detected in human and M6 metabolite was detected in both human and monkey.[14]

Gender

Gender is another factor that influences the metabolite profiles of anticancer drugs/candidate compounds due to difference in the expression levels of metabolizing enzymes between males and females. For example, in human, the levels of CYP2E1 and 1A2 are found to be higher in males than females, while the level of CYP3A4 is higher in

Table 3 Metabolism Studies (Metabolite Profiling) of Herb-Derived Compounds with Anticancer Activities

Anticancer Drug	Type of Study	Biochemical Tool/Animal/ Human (Route: Dose)	Type of Sample	Analytical Technique	Outcome			Ref.
					Metabolite	Metabolic Pathway	Efficacy/Toxicity of Metabolite	
Alantolactone (*Inula helenium* L.)	In vivo	Male Sprague–Dawley rats (PO: 100 mg/kg)	Urine, feces, bile	UPLC-TOF-MS	11 Metabolites for urine (M1-M5, M10-M11, M14-M16, M18); 10 Metabolites for bile (M1-M4, M8, M12, M25, M33, M43-M44); 38 Metabolites for feces (M1-M13, M17-M25, M27-M31, M33, M35-M44)	Oxidation (M1-M9); Di-oxidation (M10-M11); Demethylation (M12-M13); Demethylation to carboxylic acid (M14-M15); Hydration (M16); Hydrogenation (M17-M18); Addition of H_2S (M19-M20); Addition of H_2S + oxidation (M21-M23); AL_2-S (M24-M25: one sulfur-containing dimer metabolites of alantolactone); AL_2-S + oxidation (M27-M31); AL_2-SS (M33: two sulfur-containing dimer metabolites of alantolactone); AL_2-SS + oxidation (M35-M39); AL_2-SSS (M40: three sulfur-containing dimer metabolites of alantolactone); AL_2-SSSS (M41: four sulfur-containing dimer metabolites of alantolactone); AL_2-SSSSS (M42: five sulfur-containing dimer metabolites of alantolactone); Cysteine conjugation (M43); *N*-acetylcysteine conjugation (M44)	Not evaluated	[9]
Astragali Radix water extract (containing calycosin-7-β-glucoside, formononetin, calycosin, ononin, astragaloside IV)	In vivo	Male Sprague–Dawley rats (PO: 4 g/kg and 16 g/kg *Astragali Radix* water extract)	Plasma	UHPLC-MS/MS	4 Metabolites (calycosin-7-β-glucoside-3′-glucuronide for calycosin-7-β-glucoside, formononetin-3′-glucuronide for formononetin; calycosin-3′-glucuronide for calycosin, daidzein-3′-glucuronide); No metabolites for ononin and astragaloside	Glucuronidation	Not evaluated	[71]

Calphostin C (*Cladosporium cladosporoides*)	In vitro	CD-1 MLM	–	LC-MS	1 Metabolite (after incubating liver microsomes with porcine esterase)	Breaking the ester bond	Not evaluated	[72]
	In vivo	Female CD-1 mice (IP: 40 mg/kg)	Plasma		1 Metabolite			
CAT (3,6,7-trimethoxyphenanthroindolizidine (isolated from *Tylophora atrofolliculata*))	In vivo	Male Wistar rats (PO: 6 mg/kg)	Urine	RRLC-ESI-QTOF-MS	21 metabolites (M1-M21)	Di-demethylation + di-glucuronidation (M1-M3); Di-demethylation + glucuronidation (M4-M9); Demethylation + glucuronidation (M10-M12); Di-demethylation (M13-M15); Demethylation (M16-M18); Oxidation (M19); Hydroxylation (M20-M21)	Not evaluated	[73]
Dimethoxycurcumin (Curcumin analog)	In vitro	MLM (male CD-1), HLM (CYPream)	–	HPLC-QTRAP, HPLC-QqQ-LIT-MS	8 Metabolites for MLM (369, 371, 383, 385, 399, 401, 559, 561); 7 Metabolites for HLM (369, 383, 385, 399, 401, 559, 561)	Di-*O*-demethylation (369); Di-*O*-demethylation + hydrogenation (371); *O*-demethylation (383); *O*-demethylation + hydrogenation (385); Hydrogenation (399); Di-hydrogenation (401); *O*-demethylation + glucuronidation (559); *O*-demethylation + hydrogenation + glucuronidation (561)	Not evaluated	[74]
Fisetin (flavonoid compound)	In vivo	Female C57BL/6J mice (IP: 223 mg/kg)	Plasma	HPLC-MS/MS	3 Metabolites (M1, M2, M3: geraldol)	Glucuronidation (M1, M2); Methoxylation (M3)	M3: 2.5 and 1.1-fold higher cytotoxic effect against LLC cell line (Lewis carcinoma) and EAhy 926 cell line (endothelial cell) compared to fisetin, respectively; M3 exhibited 1.5-fold fold higher cytotoxic effect against NIH 3T3 cell line (normal cell)	[75]

(*Continued*)

Table 3 (Continued).

Anticancer Drug	Type of Study	Biochemical Tool/Animal/ Human (Route: Dose)	Type of Sample	Analytical Technique	Outcome			Ref.
					Metabolite	Metabolic Pathway	Efficacy/Toxicity of Metabolite	
Flavone-8-acetic acid (FAA)	In vitro	HLM, MLM expressing CYP enzyme induced by Aroclor 1245	–	HPLC-UV, HPLC-UV-MS	6 Metabolites for MLM (M1: 3′,4′-dihydrodiol-FAA, M2: 5.6-epoxy-FAA, M3a: 4′-OH-FAA, M3b: 3′-OH-FAA, M3c: 3′,4′-epoxy-FAA, M4: 6-OH-FAA); One human liver microsome (sample 14) produced M3a and another human liver microsome (sample 15) produced 3 metabolites of M1, M3a, M3c: 3′,4′-epoxy-FAA	Epoxide hydrolase reaction (M1); Epoxidation (M2, M3c); Hydroxylation (M3a, M3b, M4)	Interspecies difference metabolism could involve with difference anticancer activity	[76]
Furanodiene (Rhizoma Curcumae)	In vitro	Rat liver S9, RLM	–	HPLC-ESI-MS, HR-ESI-MS, and ^{1}H NMR, ^{13}C NMR, 2D NMR	6 Metabolites for rat liver S9 (M1: 1β,10α,4α,5β-diepoxy-8α-hydroxy-glechoman-8α,12-olide, M2: 2β-hydroxyl-aeruginolactone, M3: 14-hydroxyl-aeruginolactone, M4: 1β,8β-dihydroxyeudesm-4(14),7(11)-dien-8α,12-olide or 1β,8β-dihydroxyeudesm-4,7(11)-dien-8α,12-olide, M5: 1β,8β-dihydroxyeudesm-3,7(11)-dien-8α,12-olide M6: aeruginolactone); 1 Metabolite for RLM (M6)	Oxidation (M6); Oxidation + Hydroxylation (M2, M3); Epoxidation (M1); Epoxidation + new bond formation (M4, M5)	Not evaluated	[77]
	In vivo	Male Sprague-Dawley Rat (PO: 100 mg/kg)	Bile, urine, feces		6 Metabolites for bile and urine (M1-M6); 1 Metabolite for feces (M6)			

(-)-grandisin (extracted from *Piper solmsianum*)	In vitro	HLM	–	GC-MS and LC-MS	4 Metabolites (M1: 4-*O*-demethylgrandisin, M2: 4,4′-di-*O*-demethylgrandisin, M3: 3,4-di-*O*-demethylgrandisin or 3,5-di-*O*-demethylgrandisin, M4: 3-*O*-demethylgrandisin)	Demethylation	Not evaluated	[2]
Irisflorentin (*Belamcanda chinensis*)	In vitro	RLM	–	HPLC-UV	7 Metabolites (M1: 6,7-Dihydroxy-5,3′,4′,5′-tetramethoxy isoflavone, M2: Irigenin, M3: 5,7,4′-Trihydroxy-6,3′,5′ trimethoxy isoflavone, M4: 6,7,4′-Trihydroxy-5,3′,5′-trimethoxy isoflavone, M5: 6,7,5′-Trihydroxy-5,3′,4′-trimethoxy isoflavone; M6 and M7: unidentified	Cleavage of methylene acetyl group (M1); Adding of hydroxyl and methoxyl groups (M2-M5)	M1 & M2: 4.6 and 8.4-fold cytotoxic activities against DU145 and 4- and 8.2-fold against MCF-7 cell lines compared with parent compound.	[78]
Leelamine (bark of pine tree)	In vitro	HLM	–	LC-MS/MS	1 Metabolite in the presence of NADPH generation system	Hydroxylation	Not evaluated	[7]
	In vivo	Male ICR mice (IP: 10 mg/kg)	Urine, feces		1 Metabolite for urine not in feces			

(*Continued*)

Table 3 (Continued).

Anticancer Drug	Type of Study	Biochemical Tool/Animal/ Human (Route: Dose)	Type of Sample	Analytical Technique	Outcome			Ref.
					Metabolite	Metabolic Pathway	Efficacy/Toxicity of Metabolite	
MPD (Methyl protodioscin: isolated from *Dioscorea collettii* var. *hypoglauca*))	In vivo	Male Sprague-Dawley rats (PO: 80 mg/kg)	Urine	^{1}H NMR, ^{13}C NMR, HRSI-MS	10 Metabolites (M1: Dioscin, M2: pregna-5,16-dien-3β-ol-20-one-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-β-D- glucopyranoside, M3: diosgenin, M4: protobioside, M5: methyl protobioside, M6: 26-*O*-β-D-glucopyrannosyl (25*R*)-furan-5-ene-3β,22α, 26-trihydroxy-3-*O*-α-L-rhamnopy-ranosyl-(1→ 4)-β-D-glucopyranoside, M7: 26-*O*-β-D-glucopyranosyl (25*R*)-furan-5-ene-3β,26-dihydroxy-22-methoxy-3-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranoside, M8: prosapogenin A of dioscin, M9: prosapogenin B of dioscin, M10: diosgenin-3-*O*-β-D-glucopyranoside)	Dealkylation; Dehydration; Oxidation	All metabolites: lower cytotoxic activities against human HepG2, NCI-H460, MCF-7 and HeLa cell lines than parent drug & MPD. However, M1 and M4 exhibited strong anti-cancer activities against HepG2 (M1), NCI-H460 (M1, M4), and HeLa cell lines (M4).	[79]

Oridonin (ORI) (Diterpinoid of *Isodon rubescens*)	In vivo	Male Spraque-Dawley rats (PO: 10 mg/kg)	Bile, urine	UPLC-QqQ TOF-MS	17 Metabolites for bile (M1-M4, M6-M18); 10 Metabolites for urine (M1-M6, M12, M14-M16)	Hydroxylation (M1-M3); Ketone Formation (M4); Hydroxylation + hydration (M5); Dehydroxylation (M6-M7); Di-dehydroxylation (M8-M11); Dehydration (M12); Dehydroxylation + dehydration (M13); Didehydroxylation + oxidized to carboxylic acid (M14); Desaturation + oxidized to carboxylic acid (M15); Hydration (M16); Dehydroxylation + glucuronidation (M17, M18)	Not evaluated	[80]
Quercetin (flavonoid)	In vitro	Human hepatocellular carcinoma cell lines (HepG2)	–	HPLC-RD	1 Metabolite (4)	*O*-methylation	Not evaluated	[81]
Trabectedin (ET-743: isolated compound from *Ecteinascidia turbinata*)	Clinical trial	Cancer patients (IV: 1 mg of trabectedin (2.5 MBq [^{14}C] trabectedin (70 µCi)))	Urine and feces	HPLC-QqQ-MS and LC-LSC	8 Fractions for urine (U1-U8); 10 Fractions for feces (F1-F10) (U4, F3, and F5: ETM-204; U5 and F6: ETM-217; U6 and F7: ET-759A and ET-731; U7 and F9: ET-745, ETM-259; U8 and F10: ET759B)	Dehydroxylation (ET-745); Dehydroxylation +demethylation (ET-731); Oxidation (ET-759A); Breaking up of the molecule to the individual tetrahydroisoquinoline subunits (ETM259 and ETM204); ETM-259 + acetate ester hydrolysis (ETM217)	Not evaluated	[82]
Yuanhuapine (Isolated from *Daphne genkwa*)	In vivo	Male Sprague-Dawley rats (Oral: 5 mg/kg)	Urine	UPLC-QTOF-MS	12 Metabolites (M1-M12)	Hydroxylation (M1-M4); Di-hydroxylation (M5, M6); Tri-hydroxylation (M7); Hydroxylation + glucuronidation (M8); Methylation + di-hydroxylation (M9); (Hydroxylation + methylation) + (hydroxylation + glucuronidation) (M10); Reduction + 2(Cysteine conjugation) (M11); Hydration + (S-Cysteine conjugation) (M12)	Not evaluated	[3]

(*Continued*)

Table 3 (Continued).

Anticancer Drug	Type of Study	Biochemical Tool/Animal/ Human (Route: Dose)	Type of Sample	Analytical Technique	Outcome			Ref.
					Metabolite	Metabolic Pathway	Efficacy/Toxicity of Metabolite	
Ziyuglycoside II (*Sanguisorba officinalis* L.)	In vitro	Rat liver homogenate	–	LC-QTOF-MS	16 metabolites (H-M1-H-M16)	Glucuronidation (H-M1); Glucose conjugation (H-M2-H-M4); Dehydration + glucuronidation (H-M5); Formolation of ziyuglycoside II (adding CO: H-M6); Oxidation + reduction (H-M7); Dehydrogenation (H-M8); Demethylation (H-M9); Dehydration (H-M10, H-M11); Dearabinosylation + oxidation (H-M12, H-M13); Dearabinosylation (H-M14); Dearabinosylation + reduction (H-M15, H-M16)	Not evaluated	[83]
	In vivo	Male Sprague-Dawley rats (Oral: 50 mg/kg)	Urine, feces	UFLC-QTOF-MS	10 Metabolites for urine (U-M1-U-M10); 10 Metabolites for feces (F-M1 and F-M10)	Glucuronidation + glycosylation (U-M1); Glucuronidation + deglycosylation + decarboxylation + dihydroxylation (U-M2); U-M2 + deoxidation + methylation (U-M3); Glucuronidation + loss of C_2H_2O (U-M4); U-M4 + deoxidation + methylation (U-M5); Decarboxylation (U-M7); Glucosylation (U-M6); Dearabinosylation (U-M9 and F-M9); Dearabinosylation + oxidization (U-M8, F-M5, F-M6); Dearabinosylation +dehydrogenation (U-M10); Oxidation (F-M1); F-M1 + dehydrogenation (F-M2); Dehydration (F-M3and F-M4); Dearabinosylation + loss of CO (F-M7); Dearabinosylation + demethylation (F-M8); F-M9 + dehydrogenation (F-M10)		

Abbreviations: ESI, Electrospray Ionization; GC, Gas Chromatography; HLM, Human Liver Microsome; HPLC, High Performance Liquid Chromatography; HR, High Resonance; HRSI, High Resolution Single Ion; IP, Intraperitoneal; IV, Intravenous; LC, Liquid Chromatography; MS, Mass Spectrometry; MLM, Monkey Liver Microsome; NMR, Nuclear Magnetic Resonance spectroscopy; PO, Per oral; QqQ, Triple quadrupole; LIT, Linear Ion Trap; LSC, Liquid Scintillation Counting; QTOF, Quadrupole Time-of-flight; QTRAP, Linear ion trap Triple Quadrupole; RD, Radio Detection; RLM, Rat Liver Microsome; RRLC, Rapid Resonance Liquid Chromatography; TIS, Turbo Ion Spray; TOF, Time-of-Flight; UHPLC, Ultra-high performance liquid chromatography; UPLC, Ultra Performance Liquid Chromatography; UV, Ultraviolet detection.

Table 4 Activities of Conventional Anticancer Drugs, Synthetic Anticancer Candidates, Small Molecules Targeted Therapy, Herb-Derived Compounds

Compounds	References	Activity
Conventional anticancer drugs		
AM6-36 (3-amino-6-(3′-aminopropyl)-5H-indeno[1,2-c] isoquinoline-5,11-(6H) dione)	[13]	Antiproliferation (Breast cancer cell line (MCF-7))
ARQ 501 (synthetic β-lapachone)	[15]	Anticancer (Pancreatic, head and neck cancer, and leiomyosarcoma)
Cabazitaxel	[16]	Anticancer (Prostate cancer)
Capecitabine	[17]	Anticancer (Colorectal cancer)
Carbendazim	[18]	Apoptotic induction, Antitumor (Solid tumor)
CI-941(Iosoxantrone)	[19]	Anticancer (Breast and prostate cancers)
CP-31398 (*N*′-[2-[2-(4-methoxyphenyl) ethenyl)-4-quinazolinyl]-*N, N*-dimethyl-1,3-propanediamine Dihydrochloride)	[84,85]	Antiproliferative (Colon cancer (DLD1) and lung cancer (H460) and intestinal tumor cells)
CPA (Cyproterone acetate)	[22]	Anticancer (Prostate cancer)
Cisplatin	[20]	Anticancer (Testicular, ovarian, bladder, cervical, esophageal, small cell lung, head and neck cancers)
Crisnatol	[23]	Antitumor (Hepatoma)
Cyclophosphamide	[24,86]	Anticancer (Leukemia, lymphoma, breast, lung, prostate, and ovarian cancers)
DFS (Trans-2,6-difluoro-4′-(*N, N*-dimethylamino) stilbene: Stilbene analog)	[25]	Antitumor (Colorectal cancer)
5′-dFUrd and 5′-dFUR (5′-deoxy-5-fluorouridine)	[26,27]	Anticancer (Gastric, colorectal, and breast cancer)
EAPB0203	[28]	Antitumor (Melanoma, T lymphoma, colon, and breast cancers)
EAPB0503	[29]	Antitumor (Melanoma, T lymphoma, and colon cancer)
E-DE-BPH (E-3,4-bis (4-Ethylphenyl) hex-3-ene: stilbene derivatives)	[30]	Anticancer
2-F-araA (9-β-D-arabinofuranosyl-2-fluoroadenine)	[31]	Antitumor (Leukemia)
Flutamide	[32]	Antiandrogen (Prostate cancer)
4-Hydroxyanisole	[33]	Anticancer (Malignant melanoma)
Irinotecan	[34]	Anticancer (Colon and lung cancer)
Ixabepilone	[87]	Anticancer (Metastatic breast cancer)
JM216	[36]	Antitumor (Human ovarian carcinoma cell model)
KO2 (Morpholine-urea-Phe-Hphe-vinylsulfone)	[37]	Inhibit cancer progress by inhibiting potent cysteine protease
Laromustine	[38]	Anticancer (Leukemia)
2-Methoxyestradiol	[39]	Anticancer (Metastatic breast cancer, prostate cancer, and various tumors)
Mitomycin C	[88]	Anticancer (Bladder, colon, and breast cancers)
NB-506 (6-*N*-formylamino-12,13-dihydro-1,11-dihydroxy-13-(b-D-glucopyranosil) 5*H*-indolo [2,3-*a*]pyrrolo [3,4-*c*]carbazole-5,7 (6*H*)-dione)	[41,89]	Antitumor (lung and colon cancers, and metastatic cells)
9NC (9-Nitro-20(*S*)-camptothecin)	[90]	Anticancer (Ovarian, tubal, and peritoneal cancer)
NSC 141549 (4′-(9-Acridinylamino) methanesulfon-rn-anisidide *(rn* -AMSA))	[43]	Antitumor (L1210 leukemia and Lewis lung carcinoma)
NSC 652287 (2,5-bis(5-hydroxymethyl-2-thienyl) furan)	[44,91]	Antiproliferative (renal carcinoma cells)
NSC 674495 (2-(4-Amino-3-methylphenyl) benzothiazole: *DF* 203)	[45]	Antitumor (Breast cancer (MCF-7 and MDA 468) cells models)
PAC-1 (4-benzyl-piperazin-1-yl)-acetic acid (3-allyl-2-Hydroxy-benzylidene)-hydrazine	[46,92]	Antitumor (colon cancer cell models)
Paclitaxel	[47]	Anticancer (Breast, lung, head, neck, and ovarian cancers)

(*Continued*)

Table 4 (Continued).

Compounds	References	Activity
Phenylbutyrate	[93]	Antitumor (Human prostate cancer, hepatocarcinoma and hepatoblastoma models), Anticancer (Acute myelogenous leukemia)
Phyllanthoside	[50]	Antitumor (Bl6 melanoma and P388 leukemia)
Sepin-1	[51]	Antiproliferative (Breast cancer, leukemia, and neuroblastoma), Antitumor (breast carcinoma), Apoptotic induction
SN30000 (3-(3-Morpholinopropyl)-7,8-dihydro-6H-indeno [5,6-e] [1,2,4] triazine 1.4-dioxide: tirapazamine analog)	[6,94]	Antiproliferative (Human colon cancer (HT29) and cervical (SiHa) cancer cells), Antitumor (HT29 and SiHa cancer cells models)
Tamoxifen	[54,55]	Antiestrogen (Breast cancer)
TAS-102 (tipiracil hydrochloride (TPI) + trifluridine (FTD))	[56]	Anticancer (Metastatic colorectal cancer)
TNP-470 (O-(chloroacetyl-carbamoyl) fumagillol)	[57]	Antitumor (B16 BL6 melanoma, M 5076 reticulum cell sarcoma, Lewis lung carcinoma, Walker 256 carcinoma), Anticancer, Antiangiogenesis
TW01003 ((3E,6E)-3-Benzylidene-6-[(5-hydroxypyridin-2-yl) methylene] piperazine-2,5-dione)	[58]	Antitumor, Antiangiogenesis
Small molecules-targeted anticancer drugs		
AZD8055	[59]	Antiproliferation, Apoptotic induction, and Migration reduction (Leukemia, cervical, breast cancer, and laryngeal carcinoma)
Brivanib	[60,95]	Anticancer (Hepatocellular carcinoma)
EPZ-5676 (pinometostat)	[61]	Anticancer (Leukemia)
HM781-36B (1-[4-[4-(3,4-dichloro-2-fluorophenylamino)-7-methoxyquinazolin-6-yloxy]-piperidin-1-yl] prop-2-en-1-one hydrochloride)	[5]	Anticancer (Advanced solid tumors i.e. non-small-cell lung cancer (NSCLC) patients with EGFR mutation (T790M), breast, and gastric cancer)
Imatinib	[62,63]	Anticancer (Chronic myelogenous leukemia (CML) and gastrointestinal stromal tumors (GIST))
Irosustat	[64]	Antitumor (Breast cancer model), Anticancer (Breast cancer)
JNJ-38877605	[65]	Antitumor (Prostate, non-small-cell lung, and gastric cancer and glioblastoma cells models)
Lenvatinib	[66]	Anticancer (Hepatocellular carcinoma, melanoma, renal carcinoma, non-small-cell lung carcinoma, glioblastoma multiforme, ovarian and endometrial carcinoma)
Neratinib	[67]	Anticancer (Breast cancer)
ON 013100 ((E)-2,4,6-trimethoxystyryl-3-hydroxy-4-methoxybenzyl sulfone (Kinase inhibitor))	[68]	Anticancer (Lymphoma and acute lymphoid leukemia)
Osimertinib	[69]	Anticancer (Non–small cell lung cancer)
Pimasertib	[70]	Anticancer (Non-small cell lung, colorectal cancer, and head and neck squamous cell carcinoma)
TKI258 (dovitinib)	[96]	Anticancer (Renal cell carcinoma, advanced breast cancer, relapsed multiple myeloma and urothelial cancer)
Herb-derived compounds		
Alantolactone (Inula helenium L.)	[9]	Antitumor (Liver cancer cells model)
Astragali Radix (water extract)	[71]	Apoptotic induction
Calphostin C (Cladosporium cladosporoides)	[72]	Antitumor (Leukemia model), apoptotic induction
CAT (3,6,7-trimethoxyphenan-throindolizidine (isolated from *Tylophora atrofolliculata*))	[73]	Antitumor
Dimethoxycurcumin (Curcumin analog)	[74]	Antiproliferation and apoptosis induction (Human colon (HCT116) cancer cells model)

(*Continued*)

Table 4 (Continued).

Compounds	References	Activity
Fisetin (flavonoid compound)	[75]	Antiproliferation (Human leukemia (HL60), breast (MCF7), colon (HT29), liver (SK-HEP-1, Caco-2), neuroblastoma (SHEP, WAC-2), prostate (LNCaP, PC3) cells models)
Flavone-8-acetic acid (FAA)	[76]	Antitumor (Solid murine and human tumor cells models)
Furanodiene (Rhizoma Curcumae)	[77]	Antiproliferation (Human cervical (Hela), laryngeal (Hep-2), Leukemia (HL-60), prostate (PC3), and gastric (SGC-7901) cancer and fibrosarcoma (HT-1080) cells models)
(-)-grandisin (extracted from *Piper solmsianum*)	[2,97]	Antitumor (Ehrlich Ascites Tumoral (EAT) models)
Irisflorentin (Belamcanda chinensis)	[78]	Antiproliferation (prostate (DU145) and breast (MCF-7) cancer models)
Leelamine (bark of pine tree)	[7]	Antiproliferation by apoptotic induction (breast cancer model),
MPD (Methyl protodioscin: isolated from *Dioscorea collettii* var. hypoglauca))	[79]	Antiproliferation (Leukemia and solid tumors models)
Oridonin (ORI) (Diterpinoid of *Isodon rubescens*)	[80]	Antiproliferation (Murine and human melanoma cells models)
Quercetin (flavonoid)	[98]	Antiproliferative by apoptotic induction (Colon carcinoma (CT-26), prostate adenocarcinoma (LNCaP), lymphoblastic leukemia MOLT-4 T-cells, and human lymphoid (Raji) cell models), Antitumor (breast (MCF-7) and colon carcinoma (CT-26) models)
Trabectedin (ET-743: isolated compound from *Ecteinascidia turbinata*)	[82]	Anticancer (Soft tissue sarcoma, ovarian and breast cancer)
Yuanhuapine (Isolated from *Daphne genkwa*)	[3]	Antiproliferative (Murine lymphocytic leukemia (P-338), Human lung carcinoma (A-549) cells models)
Ziyuglycoside II (*Sanguisorba officinalis* L.)	[83]	Antiproliferative (Gastric and breast carcinoma)

females.[100] In rat liver, the expression level of CYP2C12 is higher in females than males, while the level of CYP2C11 is higher in males than females.[101] Ventura et al investigated the metabolic profiles of irosustat (inhibitor of steroid sulfatase under development for hormone-sensitive cancers such as breast and prostate cancer) using liver microsomes from male and female rats, dogs, monkeys, and humans. In rats, 11 metabolites (designated M7-M11, M13-M16, M18 and 667-coumarin) were detected in both genders, while 1 different metabolite each was detected in males (P-24) and females (P-36).[64] Eight similar metabolites (P-14, M7-M10, M13, M16, and P-24) were detected in both genders of monkeys, whereas only M14 metabolite was detected in only male monkeys. In dogs and humans, similar 14 metabolites were detected in both genders.[64] These results suggest that the metabolites generated from the parent anticancer drugs/candidate compounds may vary depending on the expression levels of the responsible metabolizing enzymes in each gender.

Route of Administration

Route of administration was found to be one contributing factor that influences the metabolite profiles of anticancer drugs/candidate compounds. Following intravenous administration of TW-01003 (a piperazinedione derivative designed as an antimitotic agent), the major metabolite (TW-01003 sulfate) was detected in rat plasma. Following oral administration, on the other hand, TW-01003 glucuronide became major metabolite.[58] This might be due to the enterohepatic recirculation, which increases the level of glucuronide conjugates in the systemic circulation.[58] In another metabolism study of tamoxifen (selective estrogen-receptor modulator for breast cancer), however, two similar metabolites (4-hydroxytamoxifen and *N*-desmethyltamoxifen) were detected in mouse serum following both oral and subcutaneous routes of administration.[52]

Dose

The dose level of anticancer drugs/candidate compounds was not found to be the major factor that influenced their metabolite profiles, but the metabolite levels. In the study of tamoxifen metabolism in mice, higher levels of metabolites were reported after an oral dose of 200 compared with 50 mg/kg/day.[52] In the study of JM 216 or satraplatin, a platinum drug, the number of metabolites detected was similar among patients receiving different doses of the compound

(120, 200, 300, 420, and 540 mg/m^2). Plasma levels of each metabolite were not significantly different in patients who received different doses of the drug. For example, metabolite A was detected at 20–58.9% for 120 mg/m^2, 10.2–50% for 200 mg/m^2, 8.7–76.2% for 300 mg/m^2, 8.2–85.9% for 420 mg/m^2, and 5.1–44.4% for 540 mg/m^2.[36] Such broad range of metabolite levels in plasma observed in patients receiving the same dose might be due to inter-individual variability metabolizing enzyme(s) responsible for JM216 metabolism in each patient. These results suggest that the metabolite profiles of anticancer drugs/candidate compounds are not completely affected by the dose of administration but may be influenced by the dose level and duration of administration.

Contribution of Metabolites to Anticancer Activity and Toxicity of Anticancer Drugs/Candidate Compounds

The metabolites generated from the parent anticancer drugs/candidate compounds can be both active and inactive metabolites. This information is essential for cancer chemotherapy with a narrow therapeutic window, particularly in individuals with increased or decreased drug-metabolizing enzyme activities influenced by genetic and nongenetic factors. Several studies showed that biotransformation could significantly influence the activity and toxicity of the anticancer drugs/candidate compounds. The metabolites of some active drugs/compounds exhibited more potent anticancer activity than their parent compounds. For example, the cytotoxic activity of the M3 metabolite of fisetin, a plant polyphenol, against Lewis carcinoma (LLC) cell line was shown to be about 2-fold of fisetin itself (IC_{50} or 50% inhibitory concentrations of 24 vs. 59 μM). However, both M3 and fisetin exhibited similar cytotoxic activities against the endothelial EAhy 926 cell line (IC_{50} 72 vs. 76 μM). It was noted however, that the cytotoxic activity of this metabolite against the normal cell line, NIH 3T3, was relatively higher than fisetin (IC_{50} 128 vs. 195 μM).[75] The M1 and M2 metabolites of the synthetic compound irisflorentin were shown to exhibit more potent cytotoxic activity against human prostate cancer (DU145) and breast cancer (MCF-7) cell lines compared with the parent drug (IC_{50} 65.12 vs. 35.71 vs.>300 μM and 74.17 vs. 36.30 vs. >300 μM, respectively).[78] The metabolite of a potent antileukemic compound, phyllanthoside, also exhibited lower cytotoxic activity against human rhabdomyosarcoma A204 cell line compared with the parent compound (IC_{50} of 24 μM and 0.47 nM, respectively).[50] Some metabolites showed low or no cytotoxic activity. For example, The metabolite 6c of NSC 674495, an antitumor 2-(4-Aminophenyl) benzothiazoles, exhibited low cytotoxic activity against MCF-7 cell line (IC_{50}>100 M) and was not shown to be an active metabolite.[45] The anticancer activities of the metabolites of these compounds should be further investigated in animals and humans to confirm their activities in vivo. Some metabolites may be pharmacologically inactive, while some may exhibit toxicity in animal models. For examples, 2-F-araH, the metabolite of 2-F-araA and the antitumor 9-β-D-arabinofuranosyl-2-fluoroadenine exhibited no antitumor activity in BDF_1 mice bearing L1210 leukemia cells following 200 mg/kg intraperitoneal dosing for nine days.[31] The M1 to M5 metabolites of JNJ-38877605 (a potent and selective Met receptor tyrosine kinase inhibitor) were shown to be associated with crystal formation in the kidney. The M10 metabolite, on the other hand, was shown to be the toxic metabolite found in humans (at sub-therapeutic dose 60 mg OD) and rats and was also noted as a species-specific toxic metabolite.[65] Since renal toxicity could be observed even at a subtherapeutic dose, this toxicity is also likely to be observed at standard dose. The M14 metabolite of the tirapazamine analog SN30000 was found to be the cause of acute toxicity (hypothermia) similarly to the parent drug after the administration at the maximum tolerated dose (MTD: 186 mg/kg SN30000).[6] However, hypothermia was also observed at 50% of MTD. Thus, this toxicity can be observed at the therapeutic dosage (75% of MTD).[94]

Conclusion

Drug metabolism plays a critical role in determining the pharmacological and toxicological effects of a drug in human. Metabolite profiling study is essential in determining therapeutic efficacy and toxicity of anticancer drugs/compounds from both chemical synthesis or natural sources. The generated metabolite profiles, either the types and amounts can be significantly influenced by factors such as species, gender, and route and dose of administration. Moreover, the metabolites can be both active and toxic metabolites, and some metabolites are inactive. The information of metabolite profile wills provides beneficial knowledge in anticancer agent development to improve anticancer activity and safety profiles of anticancer drugs or drug candidates.

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Author Contributions

Nadda Muhamad: Conception and design, data analysis and interpretation, drafting the article, final approval, agreement to be accountable for the accuracy or integrity of the work.

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