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Mini review

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Drug Metabolites and their Effects on the Development of Adverse Reactions: Revisiting Lipinski's Rule of Five

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Abstract

Many studies have shown that toxicities of anticancer drugs and their adverse effects are related to their chemical structure and high molecular weight that may result in a number of metabolites interacting with drug off-target networks. These factors require further attention for advancing cancer treatment and decreasing toxicities caused by the molecular complexity of antineoplastic agents. Providing more target-selective and tolerable cancer therapy with fewer side effects would not only improve patients' compliance, but also would decrease cancer-remission rates. This review presents several antineoplastic agents and their metabolites with molecular weights greater than 500 g/mol, which reportedly cause more than fifteen types of adverse reactions during breast cancer therapy.

Keywords

Anticancer drug, metabolites, adverse drug reactions, toxicity and ADME

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Introduction

Adverse Drug Reactions in Breast Cancer

Adverse drug reactions (ADRs) are one of the main concerns in the pharmaceutical research and drug development, as they may result in treatment failures and removal of drugs from the market. ADRs can be caused by factors related to either drug, or individual patient characteristics. In respect with drugs, these reactions can be triggered by a variety of factors, such as the dosage, drug formulation, route of administration, drug-drug interactions (DDI), drug-food interactions, drug metabolism, and allergic or hypersensitivity reactions that affect the immunologic system (Cho and Uetrecht, 2017; Fliri et al., 2005; Park et al., 1998).

Drug transporters and their role in DDI during chemotherapy regimens display a significant role in the development of ADRs (Glaeser, 2011; Mealey and Fidel, 2015; Segal et al., 2014). P-glycoprotein is a drug transporter belonging to the ABC transporter family. This family of transmembrane proteins promotes the removal of drugs from the cell. A wide variety of drugs are considered to be substrates of P-gp, as P-gp possesses the ability to target a great number of drugs despite their molecular size and hydrophobic character. P-gp transporters are expressed in many different tissues, including hepatic, renal, intestinal, and the blood-brain barrier (Lin and Yamazaki, 2003). Due to their lack of drug specificity and high expression throughout the body, P-gp transporters are likely to compromise the absorption and metabolism of many drugs. This in turn is likely to influence the bioavailability and toxicity of drugs. Moreover, P-gp transporters are also involved in many DDI. When drugs that are substrates, inhibitors, or inducers of P-gp are taken concurrently, DDI are likely to occur. DDI are often observed during cancer therapy as patients take multiple drugs to alleviate the ADRs caused by this type of treatment (Lin and Yamazaki, 2003). When DDI occur during chemotherapy, the bioavailability of an anticancer drug that is substrate of P-gp can decrease if taken along with an inducer of P-gp. This can likely compromise the effectiveness of the treatment. On the other hand, if an inhibitor of P-gp is taken, the bioavailability of the anticancer drug increases, thus enhancing the likelihood of drug toxicity (Glaeser, 2011). ADRs may also be considered as a patient-related factor, that depend on the patient's age, sex, genetic variability, physical conditions, and other similar factors (Fliri et al., 2005; Nicolson et al., 2010). Therefore, ADRs are related to both the physicochemical properties of the drug (PPD) and the patient's individual characteristics. Furthermore, PPD including the complexity of the molecular

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drug structures, their molecular weight (MW), and their hydrophobicity can fairly predict the pharmacokinetics (PK) and pharmacodynamics (PD) process and account for the development of ADRs.

It is well known that drugs, especially anticancer drugs, do not only affect cancerous cells, but also attack healthy cells. Their metabolism can cause many off-target events during and post-chemotherapy (Dobbelstein and Moll, 2014). The most common short-term ADRs include nausea, vomiting, diarrhea, alopecia, fatigue, anemia, neutropenia, and neuropathy (Flynn et al., 2017; Krukiewicz and Zak, 2016; Society, 2016; Tao et al., 2015). The long-term chronic effects of chemotherapy are usually more complicated and life-threatening. These commonly include cardiomyopathy, neurocognitive dysfunction, posterior cancers, and psychological disorders (Society, 2016; Tao et al., 2015).

A recent study showed that breast cancer (BC) was the most common type of cancer in the United States in 2016 (Smith et al., 2016). Despite a high survival rate of 89% (Society, 2016), it is the second most common cause of death from cancer among American women (Smith et al., 2016). There are multiple factors involved with choosing the appropriate drug treatment for BC, such as the presence of progesterone or estrogen receptors, the size of the tumor, and the number of lymph nodes involved (Society, 2016). Regardless of the stage of the disease, the majority of patients diagnosed with BC undergo chemotherapy treatment (Society, 2016). Adjuvant therapy is often used to treat BC, a process in which the chemotherapy is administered in concomitance with another anticancer drug to increase the efficacy of the treatment. Regardless of the drug(s) chosen or the duration of treatment, chemotherapy is very likely to cause numerous unpleasant ADRs.

Physicochemical properties of drugs

ADRs are strongly related to the physicochemical properties of drugs (PPD). The differences in the PPD can regulate the mechanism of drugs absorption, distribution, efficacy, metabolism and excretion (ADME) of these compounds inside the body. Moreover, these differences are also often correlated with drug promiscuity (DP) (Haupt et al., 2013; Tarcsay and Keseru, 2013), and the "Rule of Five" (RO5) developed by Lipinski (Lipinski et al., 2001). Thus, DP is usually related with compounds with high hydrophobicity, molecular weight and structural flexibility (i.e. high number of rotatable bonds), as well as low target selectivity. In addition, compounds that are positively

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charged, with high basicity containing several nitrogen atoms are also correlated with drug promiscuity (DP) (Haupt et al., 2013; Tarcsay and Keseru, 2013; Vieth et al., 2004). These undesired properties contribute to the many causes of ADRs.

An ideal drug molecule would comply with the physicochemical property guidelines of Lipinski's RO5. It predicts the drug-likeness of a chemical compound with a certain biological activity designed for oral route of administration (Lipinski et al., 2001). According to the RO5, a drug-like compound should have a molecular weight of less than 500 g/mol, a log P value of less than 5 representing its hydrophobicity, no more than 5 hydrogen bond donors (HBD), and no more than 10 hydrogen bond acceptor (HBA) sites (Doak et al., 2014). Further research has added two more conditions: a polar surface area (PSA) of less than or equal to 140 Å and less than 10 rotatable bonds (RB) (Veber et al., 2002), which are correlated with drug permeability and flexibility, respectively. (**Figure 1**)

In compliance with this set of rules, a chemical compound would act as an orally active drug-like compound on the desired target. However, despite the existence of such guidelines to closely predict the ADME of orally administered drugs, many drugs are still far off the limited criteria. Furthermore, a high number of anticancer drugs are given intravenously (IV) rather than orally. This can lead to countless ADRs because drugs administered by IV route have a faster and more systemic effect on the body than the oral route (Hann and Keserü, 2012). Finally, the majority of cancer drugs do not meet the RO5 standard simple because they were developed before the RO5 was published.

Cancer is a complex disease with multiple mechanisms involved. The antineoplastic drugs available on the market today usually lack target specificity and can be metabolized in the body to ADR-inducing metabolites (Flynn et al., 2017). There are limited efforts to overcome drug toxicity by modifying the chemical structure of anticancer drugs (Alisaraie and Tuszynski, 2011; Flynn et al., 2017) calling for further in-depth and precise research in the area.

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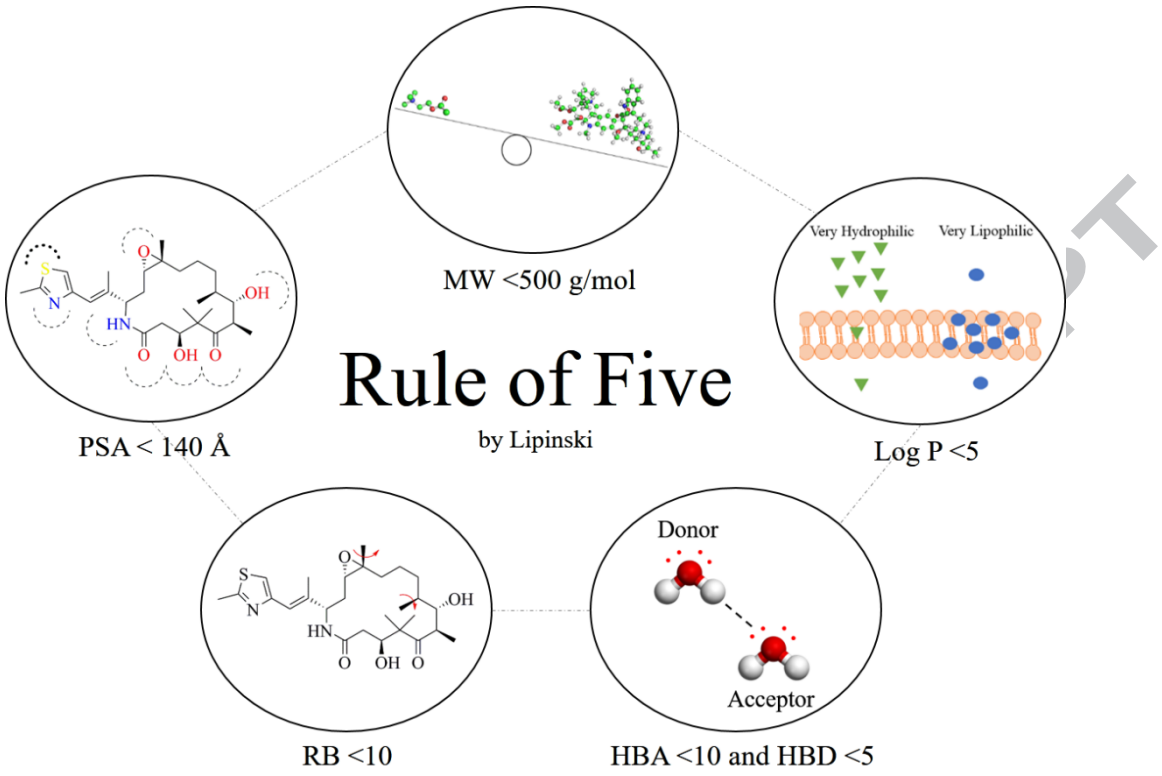

Figure 1: Visual scheme of the Rule of Five criteria: Molecular weight (MW), polar surface area (PSA), rotatable bonds (RB), hydrogen bond acceptors (HBA), hydrogen bond donors (HBD), log P.

Drug Promiscuity

Drug Promiscuity (DP) is correlated with the onset of ADRs by off-target binding, which is due to the lack of drug specificity toward the desired target. Studies have demonstrated that there are physicochemical properties that contribute to a drug promiscuity such as high MW and log P that are most commonly discussed (Haupt et al., 2013; Tarcsay and Keseru, 2013). Even though DP is sometimes desired for the treatment of complex diseases (Haupt et al., 2013) or for an increase of synergy among two different compounds (Yilancioglu et al., 2014), it is preferred to be avoided as it can cause many off-target bindings. This is one of the main challenges faced by medicinal chemists attempting to develop a novel drug or repurpose anticancer drugs with high specificity and little to no ADRs. However, to overcome this problem, there is a great debate as to whether the high MW and lipophilicity of drugs play significant roles in ADRs development.

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Lipophilicity

Lipophilic compounds tend to have higher binding affinity for proteins than hydrophilic drugs, as the majority of the binding targets have more hydrophobic elements s in nature. Therefore, as the majority of drug targets in the body are proteins, hydrophobic drugs are more likely to cause cell toxicity (Tarcsay and Keseru, 2013).

Pharmaceutical companies have been developing drugs with a log P value higher than the proposed limit by the RO5 (Haupt et al., 2013; Tarcsay and Keseru, 2013; Yilancioglu et al., 2014). Haupt *et al.* and Tarcsay *et al.* have both shown such tendency of pharmaceutical companies towards design and development of lipophilic compounds and DP (Haupt et al., 2013; Tarcsay and Keseru, 2013) which could be partly due tothe effect of drugs hydrophobicity on increasing the promiscuity of hydrophilic drugs (Ekins and Williams, 2011; Tarcsay and Keseru, 2013; Yilancioglu et al., 2014). However, the effect of drug lipophilicity may be regulated depending on its route of administration. Interestingly, Vieth *et al.* have demonstrated that the majority of IV drugs have lower log P values than oral drugs (Vieth *et al.*, 2004), probably due to the necessity for high drug solubility for IV formulations.

Molecular Weight

The molecular weight of a compound is defined as the addition of the weight of each atom of its chemical structure. However, this does not take into account the complexity, size or shape of a molecule. Two drugs may have a similar MW, but one may contain a few heavy atoms in a simple structure and the other may contain many atoms in a more complex structure.

Despite the established relationship between drug lipophilicity and DP, that of MW and DP remains inconclusive. There is a deep debate whether or not high MW contributes to DP. The positive relationship among drugs with high MW (>500 g/mol) and their likelihood to cause ADR also remains undetermined (Leeson and Springthorpe, 2007).

Research in pharmaceutical companies have demonstrated that the MW of many developed drugs (Doak et al., 2014; Ekins and Williams, 2011; Leeson and Springthorpe, 2007; Tarcsay and Keseru, 2013; Vieth et al., 2004; Zuegg and Cooper, 2012) is higher than the 500 g/mol limit (set by Lipinski's RO5 (Lipinski et al., 2001)), but have also shown that it is not always related to DP. For instance, Tarcsay *et al.* have demonstrated that among six pharmaceutical companies that have

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reported high MW as a dependant factor of DP, only three of them showed a correlation (Tarcsay and Keseru, 2013). Moreover, Haupt and colleagues reviewed eight research companies and found that only two of them have reported a high probability of promiscuity in drugs with a high MW. Three of these pharmaceutical companies instead, suggested that high MW has a low contribution to DP and the three companies stated that MW was an independent factor (Haupt et al., 2013). Haupt *et al.* have also analyzed 164 ligands that bind to three or more target proteins and have found no relationship between high MW or high hydrophobicity of anticancer drugs and DP (Haupt et al., 2013).

On the contrary, Vieth and colleagues have demonstrated that the MW of drugs can change the administration route employed (Vieth et al., 2004). The authors reported that IV drugs usually have a higher MW than oral drugs and low log P, which suggests that these structures are more likely to have a higher number of polar groups and rotatable bonds. These characteristics cause the drug molecule to be much less structurally rigid. Vieth *et al.* have not related DP with high MW in IV drugs, but it is known that flexible chemical structures are more likely to be promiscuous, thus leading to more off-target events (Haupt et al., 2013).

Active Metabolites

Administration of drugs by IV route achieves a higher bioavailability than oral administration. Drugs administered by IV route are directly and rapidly delivered to the bloodstream, thus obtaining the maximum bioavailability rate (Terwogt et al., 1999a). On the other hand, orally administered drugs first need to be released from their formulation to reach the GIT and then be absorbed by the bloodstream. During this lengthy process, the oral drug can also meet off-targets such as P-gp and metabolic CYP450 enzymes (Mazzaferro et al., 2013; Terwogt et al., 1999a). Drugs that are administered by IV route can also eventually be metabolized by CYP450 enzymes or transported by P-gp, but this is more likely to occur after absorption and distribution to the desired target. Thus, it is evident why most of anticancer drugs are administered by IV route: to assure a high desired bioavailability within a safe dosage and destroy cancer cells more rapidly. To be administered by IV route, the drug must display adequate aqueous solubility. Unfortunately, most anticancer drugs are usually classified as classes II or IV of the Biopharmaceuticals Classification System (BCS), indicating low aqueous solubility (for both classes), and high (class II) and low (class IV) intestinal permeability (FDA, 2017; Sawicki et al., 2016). Thus, modifications to improve the stability and

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solubility of the drugs must be addressed in the IV formulations. Although necessary for effective IV administration, these improvements include additional chemicals that can affect the drug metabolism and cause toxicity.

After distribution and absorption, drugs must be excreted from the body in one of three mechanisms. The unchanged parent compound is excreted by the biliary and renal systems. The third mechanism is through the metabolization of the drug, creating new metabolites of the original drug structure to be excreted (Obach, 2013). Drugs which undergo metabolic reactions can encounter two phases until complete excretion. Phase I, also known as functionalization, makes drugs more polar through oxidative, reductive, and hydrolytic biotransformation reactions. This increase in polarity helps compounds become more water-soluble and thus more easily excreted by renal route. However, these formed metabolites also can become reactive and bind to off-target sites before their excretion. Phase II, also known as the conjugation phase, helps increase the polarity of the drug as well. Endogenous compounds such as glucuronic acid, glycine, and sulfate may be added to further polarize the chemical structure, aiding in the excretion from the body or turning them into reactive metabolites (John H Block, 2011). If reactive metabolites are formed, they have the potential to bind to off-target areas throughout the body and generate ADRs.

Although the cause and relation between drug metabolites and ADRs is difficult to detect, there is a strong likelihood that reactive metabolites formed during drug metabolism promote undesired binding to off-target sites in the body (Stachulski et al., 2013). Some studies have demonstrated that drug metabolites can cause ADRs such as nausea (Flynn et al., 2017), cardiotoxicity (Hanna et al., 2014; Hrynchak et al., 2017), psychotic and dissociative symptoms (Zarate et al., 2012), and neurotoxicity (Barbosa et al., 2014; Leskela et al., 2011). Some studies have shown that the active metabolites are much more likely to cause ADR than their parent drug due to their stronger binding affinities to the off-target proteins than the former. The metabolites of a drug may also cause idiosyncratic events that are not predicted such as the drug's pharmacokinetic or pharmacodynamics properties, thus some negative effects on the immunological system may be experienced (Cho and Uetrecht, 2017; Naisbitt et al., 2000).

Breast Cancer Drugs with High Molecular Weight

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This review presents the most commonly used Breast Cancer (BC) drugs with MW greater than 500 g/mol. The most recent research reports on these drugs were explored to understand their interactions with the desired target receptors and how the metabolism of drugs possessing larger structures may be linked to their ADRs.

There are nearly 490 ADRs of the BC drugs that occur during chemotherapy (Dobbelstein and Moll, 2014; Flynn et al., 2017; Krukiewicz and Zak, 2016). The main focus of this study is on the BC drugs with more than 500 g/mol of MW that are often involved in the occurrence of highly reported ADRs. We will discuss molecular aspects of BC drugs including their MW, chemical complexity, lipophilicity, and how these physicochemical properties might lead to a high metabolic rate. ADRs are complex events that must be considered as a whole in regard to the likelihood of drug-drug interactions (DDIs), the role of drug transporters and CYP450 complex involved in the PK of anticancer drugs. The discussion over the relationship of drug transporters (e.g. P-pg and DDI of antineoplastic drugs) in respect to the occurrence of ADRs is out of the capacity and scope of this review and will be addressed elsewhere. Here, we aim to look into how the structural complexity of BC drugs with MW of greater than 500 g/mol can influence the likelihood of their metabolites formation and raise attention to the common ADRs observed during chemotherapy. (Table 1 and Table 2)

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ADRs	# Drugs Causing ADR	# Drugs MW > 500 g/mol
Abdominal Pain	13	6
Alopecia	16	7
Anemia	15	9
Anorexia	17	8
Arthralgia	12	6
Asthenia	11	5
Constipation	12	4
Cough	13	7
Diarrhea	21	10
Dizziness	13	5
Dyspnea	17	7
Fatigue	16	7
Fever	13	8
Headache	16	6
Hot Flashes	10	5
Insomnia	12	5
Leukopenia	11	9
Myalgia	13	5
Nausea	22	10

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Neutropenia	11	7
Rash	19	9
Stomatitis	12	6
Thrombocytopenia	13	6
Vomiting	21	9

Table 1: ADRs reported at least 10 times for all BC drugs studied, the number of drugs causing the ADR, and from those drugs, the number of drugs with a MW greater than 500 g/mol.

ADRs reported more than 15 times were considered to be the most predominant. Nausea is the most common ADR of all the anticancer drugs studied (Flynn et al., 2017). In terms of abundancy, this is followed by diarrhea, vomiting, anorexia, dyspnea, and alopecia (Flynn et al., 2017). It is noteworthy that the three most predominant ADRs affect the gastrointestinal tract (GIT). Mucositis is another common ADR that affects the GIT during chemotherapy (Curra et al., 2018), however it is reported less than ten times among BC drugs (Flynn et al., 2017). **(Table 1)**

Not only nausea is the most common ADR caused by anticancer drugs, it is also the most common reaction caused by 651 out of 1045 drugs (Fliri et al., 2005). Chemotherapy-induced nausea is the most common ADR reported among patients (Arslan and Ozdemir, 2015).For instance it occurs in approximately 80% of patients receiving doxorubicin (DOX) (Lua et al., 2015). We have thoroughly investigated BC drugs with a MW greater than 500 g/mol in terms of their main administration route, desired target, metabolic pathway metabolites and their reactivity. **(Table 2)**

Drug	Main Route Of Adm	Main Target	Metabolism	Main Metabolites	Activity of Metabolites
Paclitaxel	IV (Yang et al., 2015)	β subunit of microtubules (Abal et al., 2001; Oberlies and	CYP2C8 CYP3A4 (Yamaguchi et al., 2013)	6α-hydroxypaclitaxel p-3'-hydroxypaclitaxel (Yamaguchi et al., 2013)	Active (Zhang et al., 2011)

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		Kroll, 2004; Sharma et al., 2013)			
Doxorubicin	IV (Terwogt et al., 1999b)	TOPII (Rao, 2013) CREB3L1 (Denard et al., 2012)	CYP450 (Skarka et al., 2011)	doxorubicinol, 7-deoxydoxorubicinone doxorubicinone, doxorubicinolone 7-deoxydoxorubicinolone (Joerger et al., 2005)	Active (Minotti et al., 1998)
Everolimus	Oral (Deenen et al., 2012; Pawaskar et al., 2013)	mTOR (complex FKBP12) (Kirchner et al., 2004)	CYP3A4 CYP3A5 CYP2C8 (Kirchner et al., 2004)	hydroxyl-everolimus dimethyl-everolimus dihydroxy-everolimus ring-opened-ev (Kirchner et al., 2004)	Cross-reactivity present (Strom et al., 2007a)
Docetaxel	IV (Nieuweboer et al., 2015)	Microtubules (Nieuweboer et al., 2015)	CYP3A4 CYP3A5 (Nieuweboer et al., 2015)	M1, M2, M3, M4 (Nieuweboer et al., 2015) 5 unnamed (Shou et al., 1998)	M2 is active (Hendrikx et al., 2013)
Epirubicin	IV (Tariq et al., 2015)	TOPII (Tariq et al., 2015)	CYP450 (Sasu et al., 2015)	epirubicinol (Dobbs and Twelves, 1991; Ormrod et al., 1999;	Active (Zaya et al., 2006)

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				Zaya et al., 2006) Aglycone, Glucoronide (Ormrod et al., 1999; Zaya et al., 2006)	
Fulvestrant	IM (Ciruelos et al., 2014; Robertson and Harrison, 2004)	Estrogen receptor (Ciruelos et al., 2014; Johnston and Cheung, 2010)	CYP3A4, sulphate conj (Robertson and Harrison, 2004)	17-ketone, sulphone analogue and conjugates (Robertson and Harrison, 2004)	17-ketone is active (Robertson and Harrison, 2004)
Goserelin	SC (Cockshott, 2000)	GnRH analog (Rody et al., 2005)	Hydrolysis at C-terminal and renal (Cockshott, 2000)	hexapeptide (1-7) and 1-8,1-9, 5-7, 5-9, 5-10 (Cockshott, 2000)	Inactive (Cockshott, 2000)
Ixabepilone	IV (Comezoglu et al., 2009; Fumoleau et al., 2007)	Microtubules (Denduluri and Swain, 2011)	CYP3A4 (Comezoglu et al., 2009)	Deg-1, Deg-2, Deg-3, M8, M16, M41, M19 (Comezoglu et al., 2009)	Active (Comezoglu et al., 2009)

Table 2: BC drugs with a MW greater than 500 g/mol, their administration routes, target, enzymatic metabolism, main metabolites, and reactivity of metabolites. Abbreviations: intravenous (IV), intramuscular (IM) and subcutaneous (SC).

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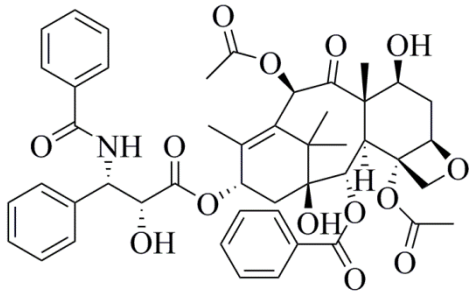
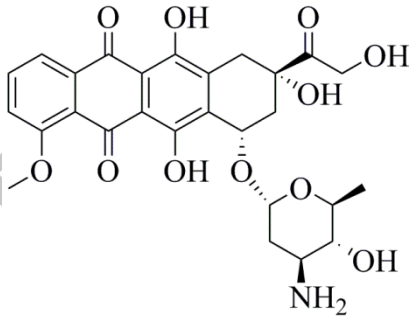
We have also investigated the physicochemical properties of the BC drugs used in this study, including their complex molecular structure, RO5 parameters, likelihood of being a P-gp substrate and classification on the BCS. (**Tables 3** and Table **4**)

BC drugs with MW > 500 g/mol
Paclitaxel 
Doxorubicin 
Everolimus

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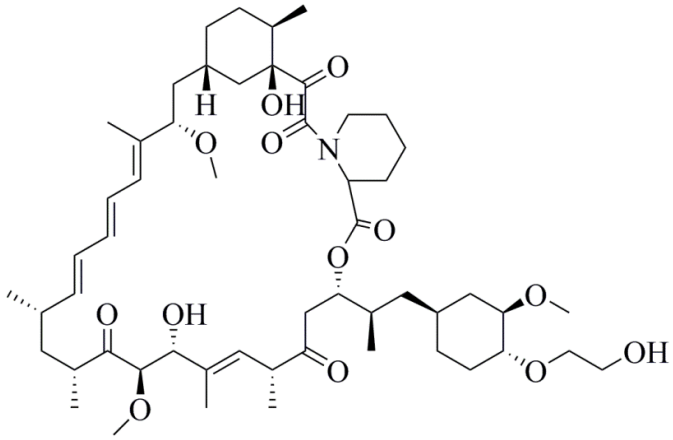
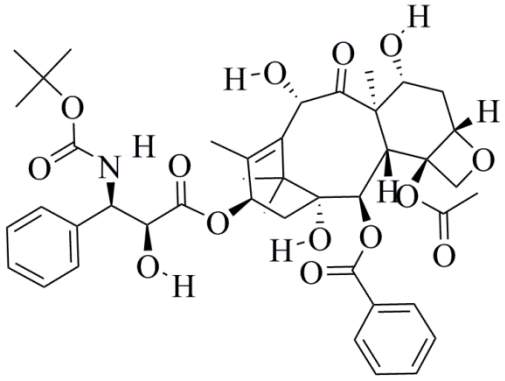
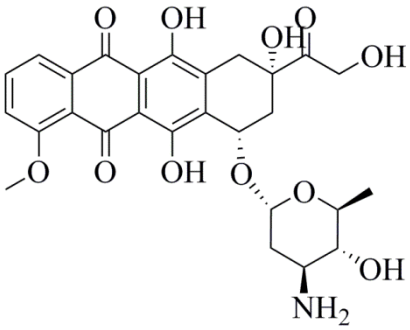


Docetaxel



Epirubicin



Fulvestrant

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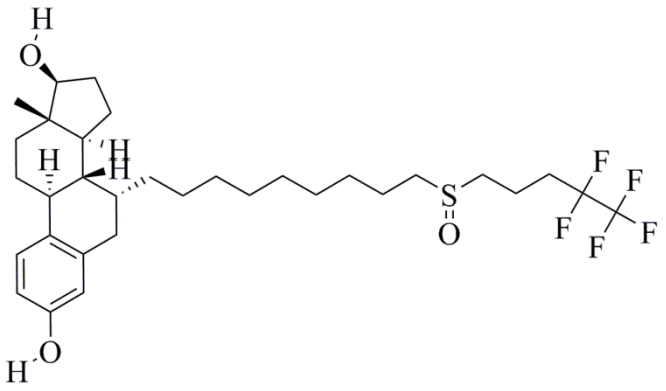
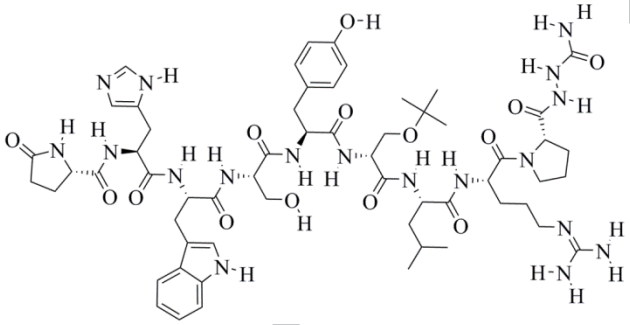


Goserelin



Glu[1]-His[2]-Trp[3]-Ser[4]-Tyr[5]-D[6]-Ser(But)[7]-Leu[8]-Arg[9]-Pro[10]-Azgly-NH_2

Ixabepilone

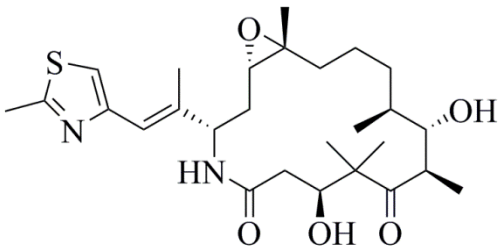


Table 3: Molecular structures of BC drugs with a MW greater than 500 g/mol used in this study.

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	Rule of Five Criteria						BSC	P-gp	Ref.
Drugs	MW (g/mol)	Log P	HBA	HBD	RB	PSA (Å)	Class	Subst.	
Paclitaxel	853.92	3.96	15	4	15	221.29	IV	Yes	(Ghadi and Dand, 2017; Harding et al., 2018; Shugarts and Benet, 2009; Surapaneni et al., 2012)
Doxorubicin	543.52	-1.67	9	6	5	206.07	III	Yes	(Benival and Devarajan, 2012; Gallois et al., 1998; Harding et al., 2018)
Everolimus	958.24	5.9	15	3	9	204.66	IV	Yes	(FDA, 2017; Harding et al., 2018; Pawaskar et al., 2013; Sawicki et al., 2016; Yokomasu et al., 2009)
Docetaxel	807.89	4.6	15	5	14	224.45	IV	Yes	(Agency, 2010; Ghadi and Dand, 2017; Harding et al., 2018; Nieuweboer et al., 2015)
Epirubicin	543.53	1.85	12	6	5	222.89	II or	Yes	(Benet et al., 2011;

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							IV		Cho and Jung, 2015; Jamieson et al., 2014; PubChem; Sottani et al., 2008)
Fulvestrant	606.78	7.92	2	2	15	76.74	IV	Yes	(Benet et al., 2011; Harding et al., 2018; Huang et al., 2017; Zaragoza Dörwald, 2012b)
Goserelin	1269.43	-0.26	18	18	32	537.10	II or IV		(Benet et al., 2011; PubChem; Zaragoza Dörwald, 2012a)
Ixabepilone	506.70	1.77	7	3	2	140.29	II	Yes	(Dorwald, 2012; Harding et al., 2018; Li et al., 2016a; Shen et al., 2011)
Drugs violating RO5	8	2	5	3	4	7			

Table 4: Parameters of RO5 criteria and number of drugs violating it, their Biopharmaceuticals Classification System (BCS) and likelihood of P-gp substrate for each BC drug with a MW greater than 500 g/mol investigated in this review.

Paclitaxel

Paclitaxel (PTX) has a MW of ~ 854 g/mol and log P of 3.96. (**Table 4**)

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PTX possesses a large and complex molecular structure. It was first isolated from the bark of a yew tree called *Taxus brevifolia* from the Pacific Northwest in North America in 1968. However, due to the slow growth rate of the tree and the low concentration of paclitaxel present, the supply for clinical use is now created through semi-synthetic methods from a precursor called 10-deacetylbaccatin III. PTX is indicated for the treatment of a variety of cancers such as breast, ovarian, and lung cancer (Fernandez-Peralbo et al., 2014). The drug is mainly administered by IV route (Yang et al., 2015). Since paclitaxel is hydrophobic (log P equal to 3.96); it works best when formulated into a formulation vehicle to reach the target tissue effectively. There are currently two formulations approved in the US, Cremophor EL® (CrEL) (Zhang et al., 2013) and nanoparticle albumin-bound PTX (nab–paclitaxel) (Li et al., 2015). CrEL has a formulation vehicle of polyoxyethylated castor oil and dehydrated alcohol known to cause a variety of side effects, including neurotoxicity (Leskela et al., 2011) and hypersensitivity reactions. (Li et al., 2015; Zhang et al., 2013)

The main cytotoxic binding site of the PTX can be found in the centrosomes and the spindle microtubules (MTs) formed during cell division. Centrosomes are responsible for organizing MT in the organelle to use for cellular functions (Nogales et al., 1998). The drug is recognized by the centrosomes, cell division is blocked and therefore the cells die (Abal et al., 2001). The α-and β-tubulins are the main building block subunits of MT (Nogales et al., 1998), that bind in a head to tail layout to create each MT protofilament (Lowe et al., 2001). The drug binding site is one of the three domains present in the multifunctional monomer structure (Nogales et al., 1998). PTX is an MT stabilizing agent (Tuszynski et al., 2012) that binds to the β subunit of tubulin near the N- terminus. The drug's binding promotes the polymerization process and stops cell division as the stabilized MT cannot continue to function, thus blocking mitosis and inducing apoptosis (Fernandez-Peralbo et al., 2014; Nogales et al., 1995). The binding site of paclitaxel is between the β-tubulin (Nogales et al., 1995) core helix H7 (Oberlies and Kroll, 2004) and the M loop, binding to residues 1-31, 217-231 and Arg382 (Abal et al., 2001). When bonded correctly, the drug creates strong contact between the MT protofilaments by locking the tubulin into an active straight conformation (Sharma et al., 2013).

A study with molecular dynamics simulations was carried out by Sharma *et al.* on the importance of 2′-OH group. It was noted that a hydrogen bond is consistently formed between 2′-OH group of PTX and the β-tubulin at the D26 residue. Located on the C-3 side chain, the 2′-OH-group is responsible for the majority of the binding free energy (~ 80%) of the side chain and also

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responsible for the hydrogen bonding interaction, which is vital for the antitumor activity of paclitaxel.

After IV administration, PTX is extensively distributed, despite extensive binding to plasma proteins, presumably albumin (Aronson, 2010). This binding occurs spontaneously through hydrophobic interactions. This bond type is weak, and is therefore useful for the eventual release of the drug to the target cells. This effect is where the idea of nanoparticle serum albumin-bound paclitaxel delivery comes from (Yang et al., 2013). The elimination and distribution of PTX is accomplished by ABCB1 proteins, also known as P-glycoprotein (P-gp) or MDR1 (Sissung et al., 2006). P-gp is an energy-dependent efflux transporter that gains its energy through ATP hydrolysis (Lin and Yamazaki, 2003), which can be found in many tissues, notably the blood–brain barrier and haematopoietic precursor cells (Sissung et al., 2006).

MTs are involved with molecular transport within neurons. Since PTX targets MT, severe neuropathies can be a side effect when the wrong cells are caught in the crossfire (Garber, 2005). Besides nausea, neutropenia and cumulative peripheral neuropathy are also observed as side effects from the use of the drug during chemotherapy. Neutropenia and neurotoxicity side effects have been associated with P-gp and with polymorphisms of isoforms CYP2C8 and CYP3A5 that correspond to the metabolism of PTX (Leskela et al., 2011; Meilke, 2007; Sissung et al., 2006). The carrier CrEL has also been known to cause side effects. These include hypersensitivity reactions, nephrotoxicity, cardiotoxicity and neurotoxicity. These are fairly common possibilities as CrEL is widely used in paclitaxel delivery (Zhang et al., 2013).

The IV formulation of paclitaxel is created with the solubilizing agent CrEL due to the poor solubility of the drug. The use of CrEL is not ideal because it can cause hypersensitivity reactions, worsening the side effects of taking PTX alone. Administering the drug orally would be more convenient for patients and pose less risk of these reactions. However, the low bioavailability of this drug makes oral administration difficult. PTX has a high affinity for the efflux protein P-gp, which causes unwanted intestinal absorption by the mucosa of the GIT (de Jonge et al., 2005).

The co-administration of oral paclitaxel with cyclosporin A (CsA) has been shown to increase the bioavailability of paclitaxel. CsA inhibits P-gp and CYP3A4, two proteins known to interact negatively with the anticancer drug, by causing drug efflux or metabolism, respectively. This PK means that CsA can be administered before an oral dosage of paclitaxel to ensure the drug is active. CrEL is still given through IV to patients on an oral paclitaxel and CsA treatment plan. Diluted with water, CrEL does not seem to affect the PK of oral paclitaxel negatively. Its important

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role in the treatment regime is to encapsulate the drug in micelles in the intestine, thereby decreasing unwanted GIT absorption of the drug (de Jonge et al., 2005).

PTX can also be administered by an injectable suspension with Abraxane™ which may be an alternative to CrEL, but many adverse effects can still occur. Ideally, oral administration of paclitaxel would eliminate the side effects experienced by either of these vehicles. Oral administration of paclitaxel would be the preferable method of administration because it would improve patient compliance as it is likely to cause fewer side effects. Without the need to use IV dosing, the need for hospitalization and medical assistance would be eliminated. Furthermore, administering the drug orally would be more cost effective and would more easily facilitate long-term treatment regimens. The drug is a substrate of P-gp , and this limits the bioavailability of its oral administration because P-gp can control the transporting the drug from the intestinal lumen after its hepatobiliary excretion (Pandita et al., 2011). (**Table 4**)

The metabolism of paclitaxel is performed by cytochrome P450 enzymes in liver. Cytochrome 450 represents a group of enzymes found in both the smooth endoplasmic reticulum of hepatocytes and the epithelial cells of the small intestine. These enzymes are responsible for the phase I metabolism of 80% of drugs currently in use, including chemotherapy drugs (Mittal et al., 2015). A stepwise catalysis using CYP2C8 and CYP3A4 is performed to metabolize the drug first to the two major metabolites, then to further metabolize to other minor metabolites (Vaclavikova et al., 2004). (**Figure 2**)

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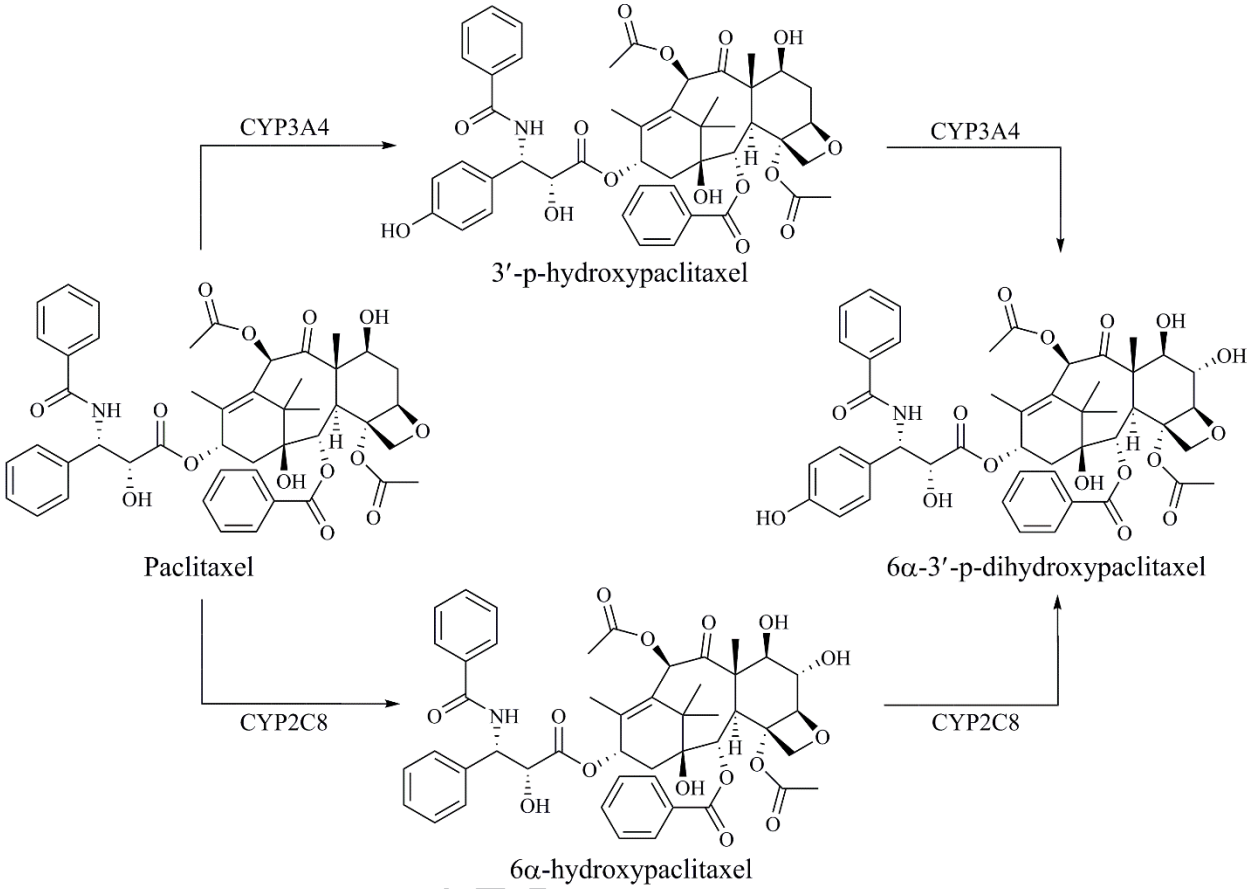

Figure 2: The major metabolites of paclitaxel.

The two main metabolites of PTX are 6α-hydroxypaclitaxel (6α-OHP), metabolized by CYP2C8 and 3′-p-hydroxypaclitaxel (3′p-OHP), metabolized by CYP3A4, both of which are structural isomers (Lee et al., 2016). 6α-hydroxypaclitaxel and 3′-p-hydroxypaclitaxel are further metabolized to 6α-,3′-p-dihydroxypaclitaxel (Yamaguchi et al., 2013). This is also performed by the CYP2C8 and CYP3A4 enzymes, respectively (Green, 2008). (**Figure 2**)

The minor metabolites are not as important in the metabolism process or in their anticancer activities due to their much smaller concentrations. An *in vivo* experiment showed the existence of the two main metabolites and seven minor metabolites, detected in the bile of patients. This included the identification of 10-deacetylpaclitaxel, 10-deacetylbaccatin III and baccatin III.

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Moreover, metabolite C2-hydroxypaclitaxel is also formed during PTX metabolism (Vaclavikova et al., 2003).

The same MT stabilization effects of paclitaxel are also shown by the main metabolites 6α-OHP and 3′-p-OHP (Vainchtein et al., 2006). However, the metabolites are both less active and thus less cytotoxic due to the structural changes they undergo during their metabolism (Zhang et al., 2011). All metabolites of the parent compound are less active and less cytotoxic. The major metabolite 6α-OHP has been shown to be about 30-fold less active than its parent drug. Metabolites 3′-p-OHP, 6α-OHP, and baccatin III have all displayed a lowered cytotoxicity as well (Vaclavikova et al., 2004). CrEL is used to inhibit the metabolism of paclitaxel into 6α-hydroxypaclitaxel (Jamis-Dow et al., 1995).

Many attempts have been made to reduce the ADRs of PTX, including inhibiting its metabolism into one of its main metabolites and adapting different formulations for a better bioavailability of the drug into the desired target. However, these modifications have not been able to lessen the unpleasantness of the many chemotherapy side effects patients must endure. PTX is considered to be a large and complex molecule, meaning it has many possible sites for metabolic reactions to take place and the rate of excretion to increase. This is evidenced by the high number of metabolites created during PTX metabolism, which could play a significant role in off-target binding and cause ADRs.

Therefore, it is important to attempt chemical modifications on the structure of PTX to decrease its size and consequently the number of possible sites of metabolic reactions on the drug. This would in turn decrease the chance of ADRs occurrence, as many are likely the product of metabolites binding to off-target sites. This is still challenging as chemotherapeutic drugs might possess pharmacophores that are also toxicophores, as it is the case of PTX. Thus it would limit choices of structural modifications on the chemical structure of PTX. It is imperative that the exact mechanism of binding affinity between the drug and target be researched because the drug could then be modified to include only the required functional groups to interact with the proper target residues. This would increase the target specificity of the drug, decrease metabolite formation, and thus reduce its side effects.

Doxorubicin

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Doxorubicin (DOX), a member of the anthracycline drug class (Menna et al., 2007), has a MW of approximately 544 g/mol and a log P value of -1.67 (Gallois et al., 1998). (Table 4)

It is extremely hydrophilic, thus it should be administered mainly by IV route to guarantee its bioavailability throughout the circulatory system. If administered orally, the drug would not cross the cell membrane due to its low membrane permeability and would instead be rapidly excreted. Its MW is not very high compared to the other drugs discussed here, but it has a complex molecular structure due to the daunosamine group attached at its C-7 position.

Two anticancer compounds, doxorubicin and daunorubicin, were first isolated from the bacteria *Streptomyces peucetius* in the 1960s (Minotti et al., 2004). The only difference in their similar structures is in their side chains, which greatly affect their anti-cancer activities (Minotti et al., 2004). A primary alcohol terminates the side chain of DOX, meaning it is primarily used to treat solid tumors such as breast cancer, soft tissue sarcomas, and numerous types of lymphomas. The side chain of daunorubicin terminates with a methyl group, meaning it is better suited to treat acute lymphoblastic or myeloblastic leukemias (Minotti et al., 2004).

Topoisomerase II (TOPII), an enzyme essential in DNA replication, is the main target of DOX (Rao, 2013). This drug is a topoisomerase poison, which breaks doubled stranded DNA by increasing levels of covalently bound topoisomerase to create lesions that cause the DNA to break down (Rao, 2013). Cancer cells usually have high levels of both topoisomerase I and II present because they proliferate so quickly. This can help predict how well the cancer will respond to treatment from a topoisomerase poison (Rao, 2013). More specifically, a high level of TOPII, usually found in solid tumors, would indicate that an anthracycline like DOX would be an effective type of treatment (Rao, 2013). DOX localizes in the nucleus of the cancer cell to reach its target (Heibein et al., 2012). However, this does not always occur. When DOX is converted to its metabolite doxorubicinol, it is no longer capable of entering the nucleus and attacking the cancer cell DNA (Heibein et al., 2012).

Anthracyclines like DOX also show iron chelation activity. This is important because iron is involved in cell proliferation, DNA synthesis, mitochondrial electron transport, and oxygen sensing. Cancer cells proliferate quickly, meaning they require higher levels of iron to function and uptake iron at a higher rate. By chelating iron, DOX is less available for the use of cancer cells and slows their rate of growth. However, it also creates iron mediated reactive oxygen species through this process, which can cause cardiomyopathy. Because DOX both poisons topoisomerase and chelates iron, this drug is considered a dual inhibitor (Rao, 2013).

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Other research has shown another way doxorubicin inhibits cell proliferation. CREB3L1 is a membrane protein that acts as a transcription factor (Denard et al., 2012). DOX stimulates proteolytic cleavage of CREB3L1 through its creation of ceramides, lipid molecules which trigger the cleavage (Patel and Kaufmann, 2012). Cleavage of this transcription factor causes suppression of the cell cycle as the NH_2-terminal leaves the cytosol after cleavage and enters the nucleus (Denard et al., 2012). The molecule post cleavage is a messenger that can induce apoptosis of cancer cells as well as other functions (Patel and Kaufmann, 2012). This mechanism is referred to as regulated intramembrane proteolysis (Denard et al., 2012). Therefore, it can be said that CREB3L1 is another main target of doxorubicin.

The main administration route of DOX is by IV route due to its known safety and effectiveness. It can be administered through numerous other routes as well, including intra-arterially, intravesically for treatment of bladder cancer, and intraperitoneally (Terwogt et al., 1999a). There are also numerous ways to alter the drug delivery to improve the drug's function or reduce side effects. Nanoparticles have been used effectively as a drug delivery system (Li et al., 2017; Li et al., 2016b) through both intraperitoneal and IV injections (Reddy and Murthy, 2004).

Liposomes in particular have shown success and safety in DOX delivery (Liu et al., 2017). Doxil is an example of a nano-drug used to deliver the drug through the use of liposomes (Barenholz, 2012). DOX administration through slow infusions reduces the patient's chance of developing cardiotoxicity (Singal et al., 1997), however, rapid infusions are more likely to cause it. The metabolites of DOX that have been found experimentally are doxorubicinol (Hanna et al., 2014), 7-deoxydoxorubicinone, doxorubicinone, doxorubicinolone, and 7-deoxydoxorubicinolone (Joerger et al., 2005). (Figure 3)

The main metabolite seems to be doxorubicinol, a metabolite 60 to 160 times less active than its parent compound and with a half-life of 30 hours (Dubbelboer et al., 2017). It is both ineffective and harmful when DOX is converted to doxorubicinol (Kassner et al., 2008). This reaction is catalyzed by DOX-reducing enzymes CBR1 and CBR3 which are found in highest concentration in the liver (Joerger et al., 2005; Skarka et al., 2011). A two electron reduction occurs on the ketone on carbon 13 to form doxorubicinol, a secondary alcohol metabolite (Joerger et al., 2005; Skarka et al., 2011). The liver plays the largest role in this metabolism, along with the kidneys and the GIT (Joerger et al., 2005; Kassner et al., 2008).

When doxorubicin accumulates in the heart, some aldo-keto reductase (AKR) enzymes (AKR1A1, AKR1B1, AKR1B10, AKR1C3 and AKR1C4) reduce the drug to doxorubicinol

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(Kassner et al., 2008; Skarka et al., 2011). This accumulation and metabolism can cause both acute reversible toxicity and chronic irreversible toxicity after strong and prolonged dosage, which therefore limits the usage of the DOX (Hanna et al., 2014; Licata et al., 2000). There may be other enzymes involved in the metabolism, as more study is needed (Kassner et al., 2008). The metabolite doxorubicinol can no longer effectively enter the nucleus to induce apoptosis in the cancer cell, so this metabolism renders the drug much less effective in cancer therapy (Heibein et al., 2012).

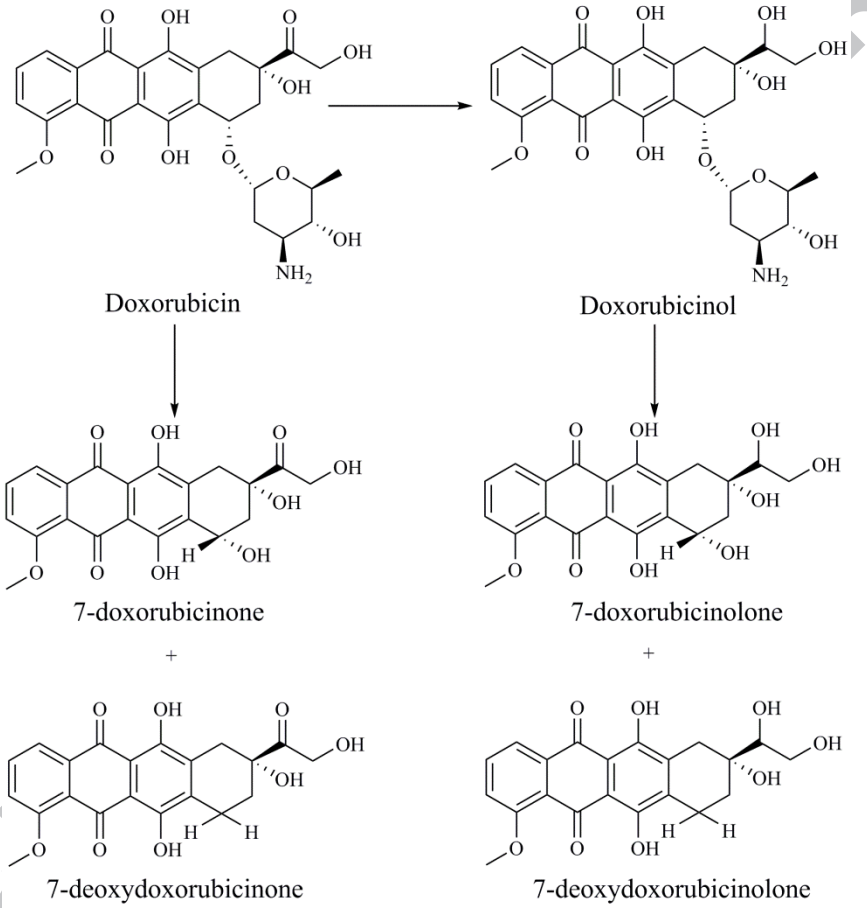

Figure 3: The main metabolites of Doxorubicin.

Metabolite 7-deoxydoxorubicinone is produced by the metabolism of DOX, and 7-deoxydoxorubicinolone is made from the metabolism of doxorubicinol. (**Figure 3**)

In both of these conversions, the C7-linked daunosamine sugar group is removed reductively to create an aglycone radical via a semi-quinone intermediate. Protonation of this radical creates the metabolites 7-deoxydoxorubicinone or 7-deoxyrubicinolone depending on the starting material.

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Doxorubicinone (from DOX metabolism) and doxorubicinolone (from doxorubicinol metabolism) lose their sugar moiety by acid-catalysed hydrolysis (Joerger et al., 2005; Skarka et al., 2011). This hydrolysis is likely catalyzed by cytochrome P450 reductase; however, the detailed mechanism of microsomal reductase response yet requires further studies (Joerger et al., 2005; Skarka et al., 2011).

There is research being performed to reduce the side effects of DOX by creating new drug delivery formulations. However, more studies are needed to evaluate exactly how DOX is bound to the desired target, as this remains unclear. After acquiring the knowledge of the interaction affinity between the drug and its target, one can suggest modifications in the structure of DOX to avoid off-target events.

Everolimus

A derivative of the antibiotic sirolimus, everolimus (EVE) is an immunosuppressive macrolide which has a stable 2-hydroxyethyl chain substitution at position 40 on the parent drug (Kirchner et al., 2004). Due to its greater stability and solubility, EVE is more potent in its antiproliferative and immunosuppressive effects than sirolimus (Kirchner et al., 2004). This drug is a large molecule with a MW of 958.24 g/mol and a complex chemical structure. EVE is the only drug discussed in this review whose main administration route is oral. (**Table 2**)

This is despite its high log P value of 5.9, which normally indicates that the drug is too lipophilic to be orally administered. However, EVE also exhibits polarity in its molecular structure, thus improving its bioavailability enough to make oral administration a possibility. Therefore, EVE is expected to achieve a steady-state more easily because it has a shorter half-life, as its concentration peak is seen in 1.3-1.8 hour after administration (Kirchner et al., 2004).

EVE is a mammalian target of rapamycin (mTOR) inhibitor, which stops cell proliferation (from G1-phase to S-phase), angiogenesis (inhibition of hypoxia-inducible factor 1α (HIF1α) expression (Kirchner et al., 2004)) and consequently cell survival (Deenen et al., 2012). It is also approved by the FDA for the treatment of patients with advanced renal cell carcinoma (Atkins et al., 2009; Pawaskar et al., 2013) after failure of treatment with sunitinib or sorafenib (Atkins et al., 2009). The PK properties of EVE are variable. The drug is quickly absorbed by the hepatic and intestinal cytochrome P4503A4 enzymes (Pawaskar et al., 2013; Yokomasu et al., 2009) and the drug efflux pump P-gp (Yokomasu et al., 2009), undergoing oxidative metabolism (Pawaskar et al., 2013). The enzymes CYP3A5 and CYP2C8 also seem to be involved in the drug's metabolism. Due to the GIT

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interactions aforementioned, patients are recommended to take their dosage either with or without food, but in a consistent manner to avoid drug expose fluctuations (Kirchner et al., 2004). EVE inhibits mTOR kinase activity by binding to FKBP12. The complex formed minimizes the activity of downstream effectors S6 ribosomal protein kinase (S6K1) and eukaryotic elongation factor 4E-binding protein (4EBP), involved in the phatidylinositol 3-kinase (P13K)-AKT pathway (Atkins et al., 2009).

The observed adverse effects of EVE include hypertriglyceridemia, hypercholesterolemia, opportunistic infections (Kirchner et al., 2004; Pawaskar et al., 2013), thrombocytopenia, leukocytopenia (Kirchner et al., 2004), anemia, rash, fatigue, diarrhea and nausea (Pawaskar et al., 2013). Patients with hepatic impairment should have the drug dose reduced by half (Kirchner et al., 2004).

EVE is metabolized by CYP3A4 enzymes undergoing to mainly hydroxylation and demethylation metabolic pathways (Kirchner et al., 2004). The main metabolites of EVE are 34-hydroxy-everolimus and 16-O-demethyl, corresponding to a mass increase of 16 units for a single hydroxylation and 14 units for one demethylation (Lhoest et al., 2000). Moreover, the further metabolites of 34-hydroxy-everolimus were already elucidated and the reaction mechanism is shown in a study by Hallensleben and co-workers (Hallensleben et al., 2000). **(Figure 4)**

Minor metabolites of EVE, dihydroxy-everolimus and dimethyl-everolimus, as well as the open-ring form of EVE are found in the blood. All the major and minor metabolites are formed within two hours of administration. Eleven metabolites were elucidated by the hydroxylation and methylation pathways *in vitro*. The structures currently identified are 12-hydroxy-everolimus, 34-hydroxy-everolimus, and 39-O-, 27-O-, 16-O-desmethyl-everolimus and 17, 18, 19, 20, 21, 22-tris-epoxide-everolimus. **(Figure 5)**

Moreover, metabolites 11-, 24-, 25-, 46-, 49-hydroxy-everolimus and 40-O-dehydroxyethyl-everolimus were also already elucidated (Hallensleben et al., 2000; Kirchner et al., 2004; Lhoest et al., 2001; Lhoest et al., 2000; Vidal et al., 1998). In bile, 98% of the drug is excreted in the form of metabolites, and the other 2% is excreted in urine. The excretion process takes around 18-35 hours. Since EVE undergoes rapid clearance due to its high intensive metabolism rate, it requires twice daily administration (Kirchner et al., 2004).

The majority of the previously metabolites have also shown cross reactivity during adjuvant chemotherapy and with immunosuppressant treatments (Strom et al., 2007a; Strom et al., 2007b). In

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addition, an *in vitro* proposed fragmentation of EVE in pig liver microsomes is also elucidated (Hallensleben et al., 2000; Vidal et al., 1998). (Figure 6)

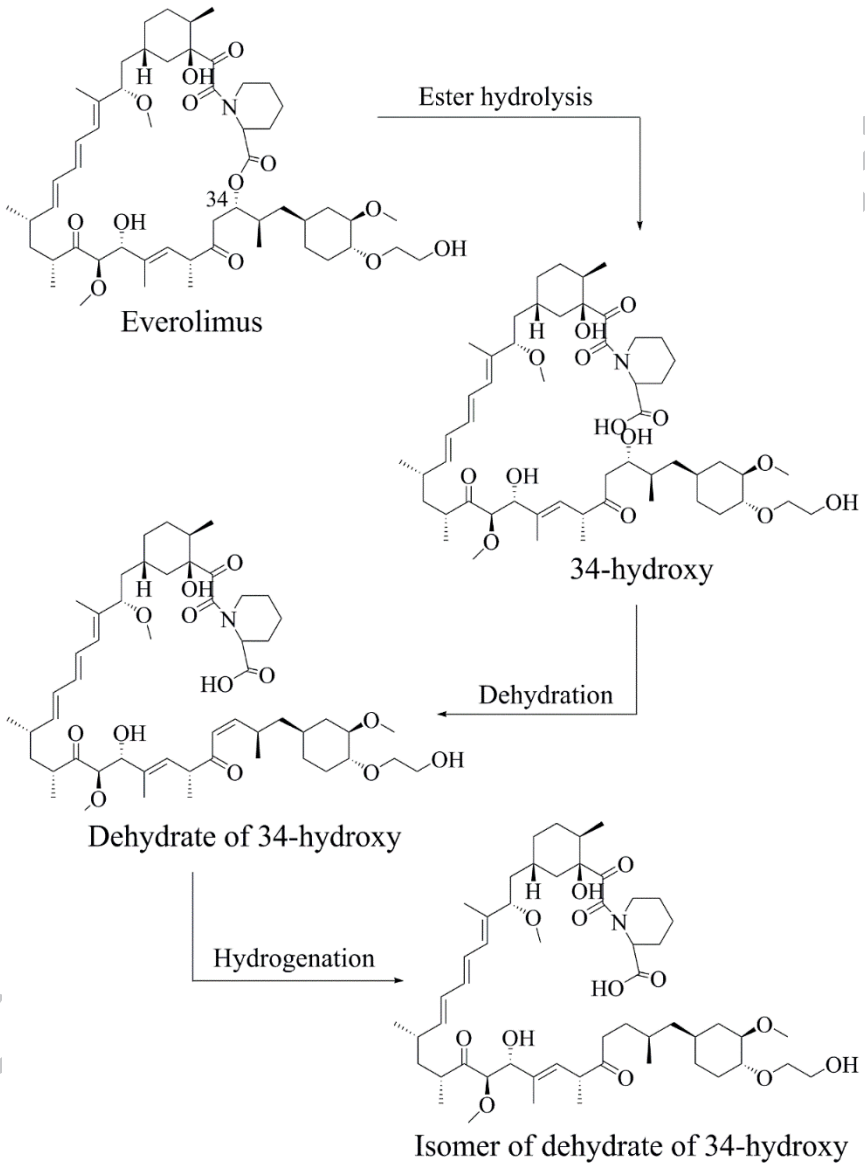

Figure 4: The major metabolites of everolimus.

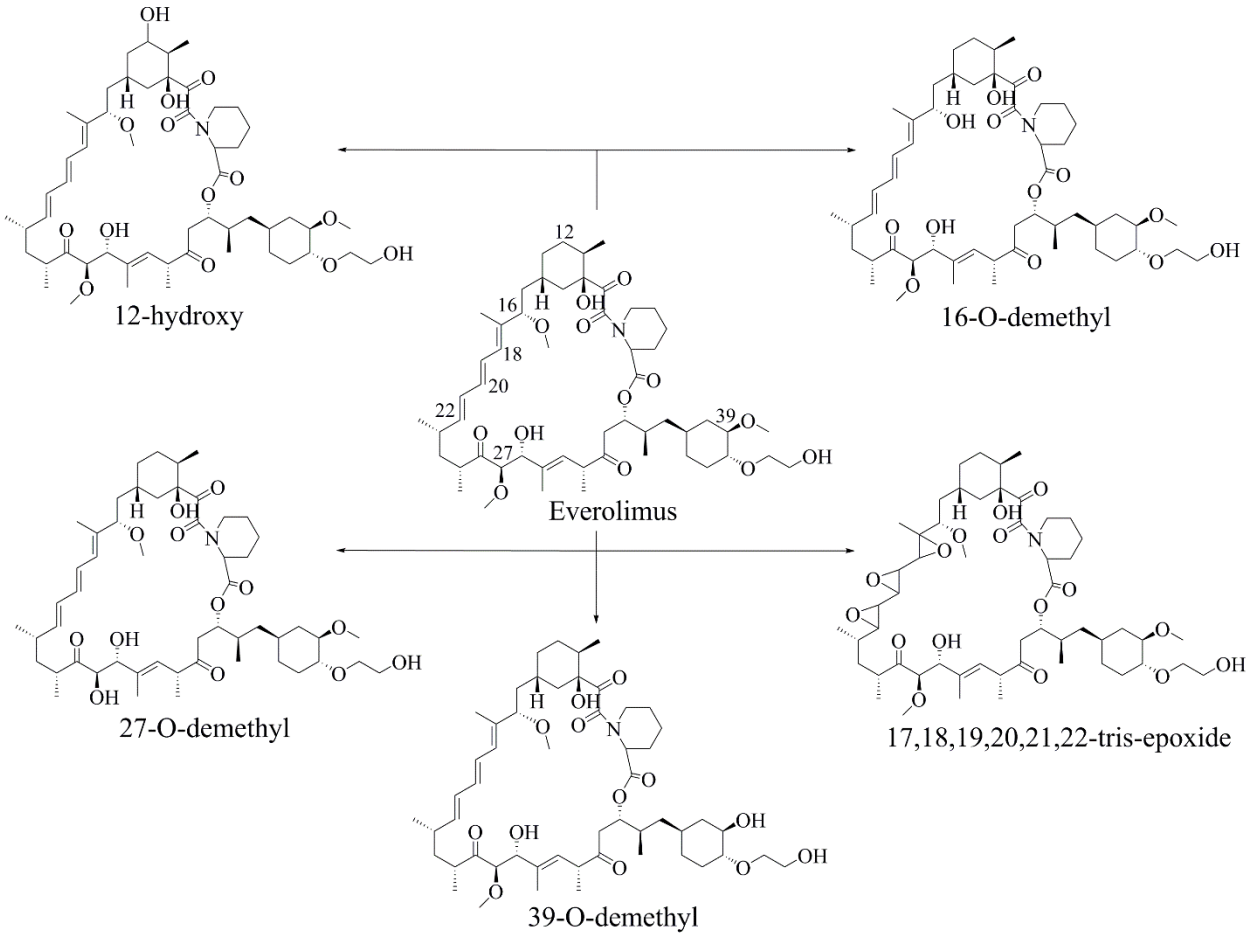

Figure 5: Minor metabolites of everolimus.

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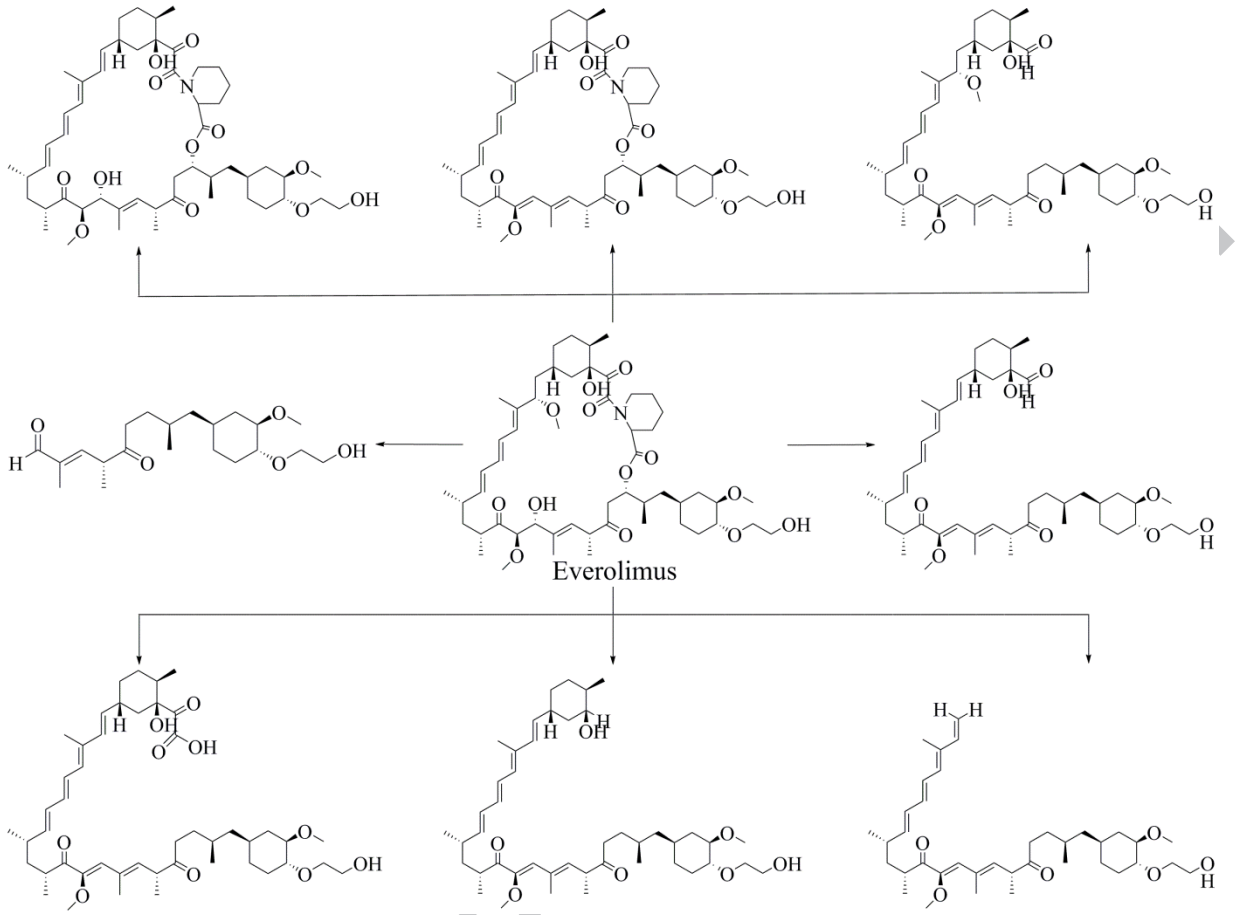

Figure 6: Proposed fragmentation of metabolites of everolimus.

EVE interacts with atorvastatin, pravastatin, rifampicin, azithromycin (Kirchner et al., 2004), itraconazole, and ketoconazole. The interactions with itraconazole and ketoconazole are both CYP3A-mediated metabolism, administered either orally or intravenously (Yokomasu et al., 2009). There is a 74% reduction of EVE clearance when receiving itraconazole. Interestingly, co-administration of itraconazole and everolimus intraintestinally increases the blood concentration of EVE, however, this increase was not observed when itraconazole was administered by IV route. On the other hand, when EVE was administered by IV, and itraconazole by the intestinal route, the intestinal concentration of EVE decreased, however the blood EVE concentration was not affected. This might be due to inhibition of the intestinal first-pass metabolism of EVE (Yokomasu et al., 2009). The large MW of EVE and the complexity of its structure are likely the main contributing factors to the high number of metabolic reactions.

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Docetaxel

Docetaxel (DOCE) was first isolated in the 1980s from the leaves of *Taxus baccata*, a type of yew tree that grows in Europe. A semisynthetic derivative of PTX, (Kenmotsu and Tanigawara, 2015), DOCE is also a member of the taxane class (Baker et al., 2006). DOCE was granted FDA approval for treatment of locally advanced metastatic BC that has resisted other forms of treatment (Kenmotsu and Tanigawara, 2015). Today, this anticancer drug is used against many types of cancers, such as breast, gastric, ovary, head and neck, non-small cell lung, esophageal, uterus, and metastatic castration resistant prostate cancer (Kenmotsu and Tanigawara, 2015; Nieuweboer et al., 2015). With a high MW of 807.89 g/mol (Kenmotsu and Tanigawara, 2015) and low bioavailability (Baker et al., 2006), this drug is administered only through IV route (Nieuweboer et al., 2015). Research to improve oral administration bioavailability is ongoing, but the bioavailability of DOCE is currently below 10% (Baker et al., 2006).

DOCE is an MT stabilizer that disrupts its dynamic behaviour, thereby stops cell proliferation. It is extensively metabolized by CYP3A isoenzymes, resulting in many inactive pharmacological products (Baker et al., 2006). To be eliminated, DOCE requires drug transporters such as P-gp, ABCC2, and ABCC10 to excrete the drug and its metabolites into the bile and feces (Nieuweboer et al., 2015). The majority of drug (~ 75%) is eliminated in the feces, with a smaller amount of ~ 6% excreted in the urine (Kenmotsu and Tanigawara, 2015). There are drug interactions of DOCE observed with PTX, DOX, and EVE (Nieuweboer et al., 2015). The main ADR, which occurs during cancer treatment with docetaxel is neutropenia (93%) due to the lack of drug specificity, which also affects normal cells such as monocytes, therefore leading to myelosuppression (Baker et al., 2006; Ho and Mackey, 2014; Kenmotsu and Tanigawara, 2015; McKeage, 2012; Nieuweboer et al., 2015; Wu et al., 2015).

The metabolism of DOCE occurs by first pass effect in the liver by involvement of CYP3A4 and CYP3A5 isoenzymes (Baker et al., 2006; Nieuweboer et al., 2015). P-gp excretes DOCE into the intestinal lumen or bile, restricting the possible GIT absorption (Nieuweboer et al., 2015). There are four metabolites formed in the metabolism of DOCE. Nieuweboer *et al.* firstly introduced them as M1 through M4 (Nieuweboer et al., 2015) (**Figure 7**).

M2 is a primary alcohol created by oxidation of a methyl group on the parent compound. M1 and M3 are unstable diastereoisomer metabolites formed by further oxidation of M2. The further oxidation of M2 also forms M4, a ketone metabolite. Research showed no evidence that

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phase II metabolism of DOCE occurs (Guitton et al., 2005; Nieuweboer et al., 2015). There was little anti-tumour activity demonstrated by the majority of the metabolites. This lack of activity suggests that the inactivation of the drug in the body is likely due to its metabolism (Baker et al., 2006; Nieuweboer et al., 2015). During the first 48 hours, the major metabolite and three minor inactive metabolites are responsible for the fecal elimination of 80% of the drug radioactivity (McKeage, 2012). Metabolite M2 was the only one to show activity (Hendrikx et al., 2013).

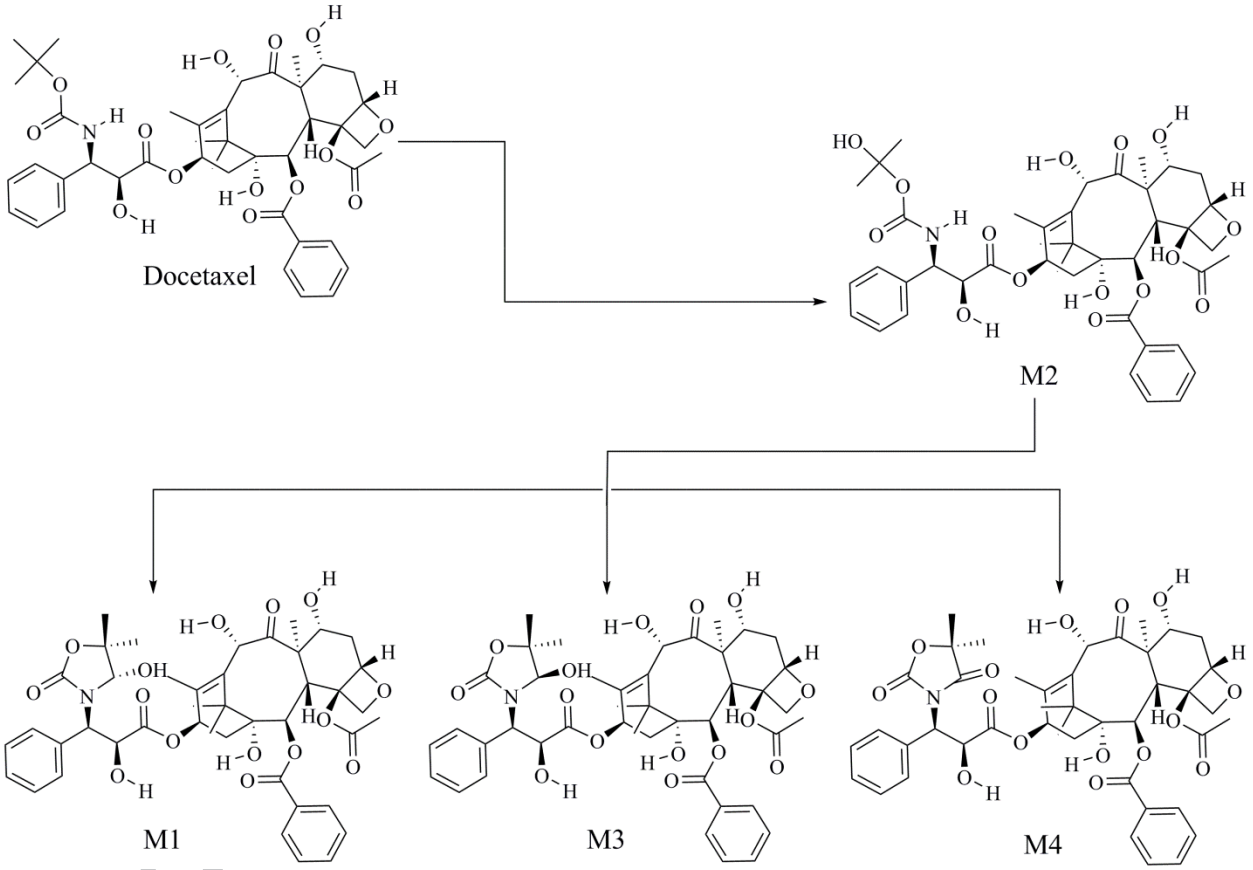

Figure 7: The main metabolites of docetaxel.

These metabolites have been studied and named differently in other papers as well. The research study by Shou *et al.* (Shou et al., 1998) shows that CYP3A4 and CYP3A5 are responsible for the oxidation formation of the primary metabolite RPR104952, and that further oxidation of RPR104952 forms two more metabolites, RPR111059 and RPR111026. An epimer of DOCE, RP70617, forms metabolite XII by the same enzymes (Shou et al., 1998). The metabolite names are

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different, however, it concurs with the aforementioned study by Nieuweboer *et al.* (Nieuweboer et al., 2015).

Complementary alternative medicines (CAMs) are used by an estimated 40% of cancer patients alleviate chemotherapy side effects. *Echinacea purpurea*, St. John's wort, and garlic are popular CAMs used by patients during DOCE treatment (Nieuweboer et al., 2015). Alternative medicines can potentially influence toxicity and therapeutic effects by changing drug transporters and metabolic pathways.

DOCE has a log P value of 4.6, which indicates a high affinity for adipose tissue. Highly lipophilic drugs have increased volumes of distribution in obese patients. While the clearance of DOCE remains the same, obese patients showed an increase in the volume of distribution and the elimination half-life of the terminal phase when compared to non-obese patients (Wu et al., 2015). This indicates that special attention is required when calculating the drug dosage for obese patients.

A study by Kenmotsu and Tanigawara showed that DOCE toxicity occurs more frequently in Japanese patients than those of American or European descent (Kenmotsu and Tanigawara, 2015). Even with a lower dose administered, there is a greater relationship between DOCE concentration and hematological toxicities in Japanese patients than any others. Currently, the standard dose of DOCE is 75 mg/m^2 in both Japanese and global trials (Kenmotsu and Tanigawara, 2015).

Even though semi-synthetic drugs are developed to enhance drug selectivity and decrease ADRs, DOCE and PTX both have complex structures with many available sites for enzymatic metabolic reactions. This is still true for DOCE, despite its lighter structure, due to its significantly higher log P value (4.6 vs 3.96, respectively). (**Table 4**)

As previously mentioned, drugs with a high lipophilicity are more promiscuous, which can lead to a higher number of ADRs.

Epirubicin

Epirubicin (EPI) is an anthracycline similar to DOX, but it is generally considered to be a safer alternative because its cardiotoxic effects are much less severe (Jamieson et al., 2014; Tariq et al., 2015). EPI is used for the treatment of breast and ovarian cancer, gastric cancer, lung cancer and lymphomas (Shin, 2013). Similar to DOX, EPI also has a lighter MW (543.53 g/mol) compared to

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the other drugs presented here. The main differences between these similar drugs are their PK properties.

These drugs can be differentiated only by the orientation of their 4-hydroxy group (Zaya et al., 2006). Although not subject to the same degree of reduction by aldo-keto reductase (AKR) enzymes as DOX, EPI can be conjugated to form glucuronides through phase II metabolic reactions, and catalyzed by uridine glucuronosyl transferase enzymes (UGTs) (Dobbs and Twelves, 1991; Ormrod et al., 1999). EPI detoxification is performed through use of the UGT2B7-dependant glucuronidation pathway (Zaya et al., 2006). It is transported by the ABC and SLC families of membrane proteins, leading to both the uptake of the drug in the liver and to the eventual biliary excretion (Jamieson et al., 2014).

EPI is administered only by IV route. Its low log P value of 1.85 gives the drug a low bioavailability and makes its oral administration ineffective (Tariq et al., 2015). (**Table 4**)

EPI halts cell division by targeting intercalating DNA strands. This method causes DNA to take on an inconvenient complex formation, thus inhibiting DNA and RNA synthesis (Shin, 2013). Similarly to DOX, the drug also binds to TOPII, causing intracellular mechanisms that ultimately cause cell death (Tariq et al., 2015).

The main metabolite formed during EPI metabolism is epirubicinol (Dobbs and Twelves, 1991; Jamieson et al., 2014; Ormrod et al., 1999). The liver metabolizes EPI to epirubicinol, aglycone, and glucuronide metabolites (Ormrod et al., 1999), followed by the biliary excretion of the glucuronide conjugate (Zaya et al., 2006). Although epirubicinol is considered to be active, it may not reach sufficiently high concentrations *in vivo* to be considered cytotoxic. The aglycone and glucuronide metabolites also seem to lack cytotoxic and cardiotoxic effects (Ormrod et al., 1999). According to the FDA, EPI is metabolized to 13-dihydroxyepirubicinol by the AKRs. Both undergo glucoronidation by glucuronosyl transferase and hydrolysis process, followed by reduction to 7-deoxy aglycone.

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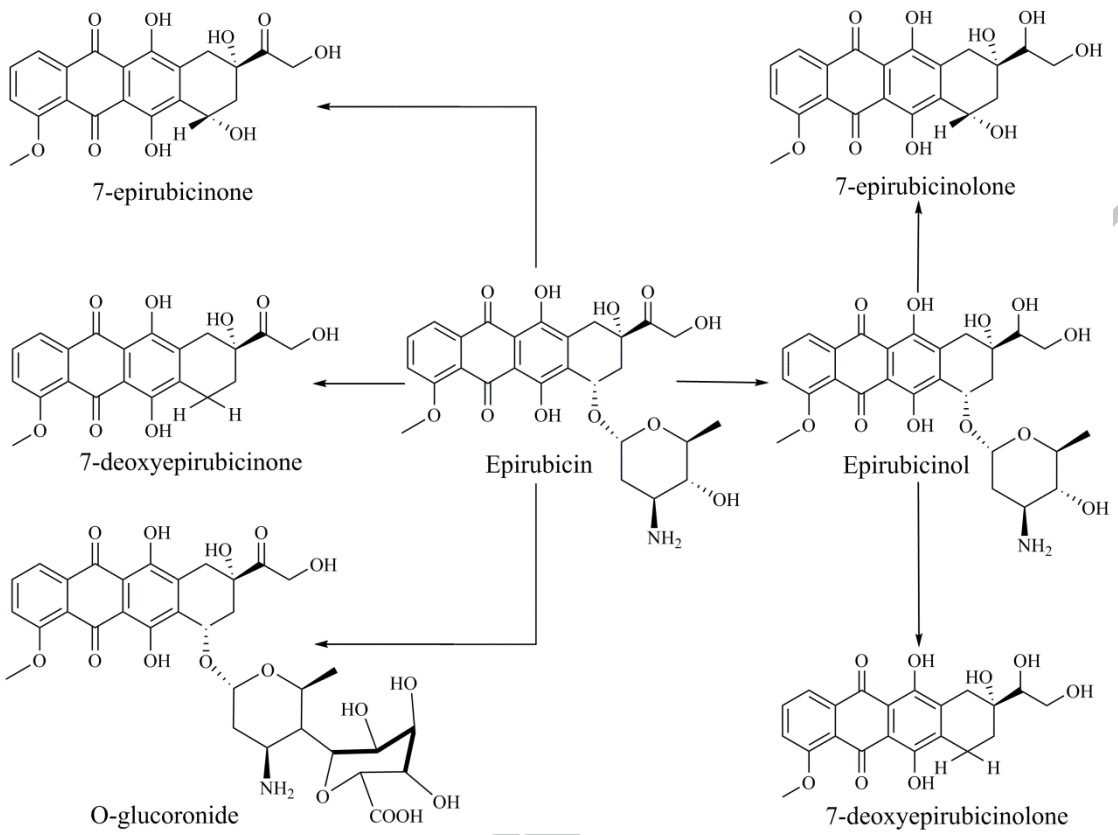

Figure 8: The major metabolites of epirubicin.

There are four main routes for the extensive metabolism of the drug: i. epirubicinol is formed via a reduction of the C-13 keto-group, ii. both the parent drug and its metabolite epirubicinol can be conjugated with glucuronic acid, iii. the formation of epirubicin and epirubicinol aglycones is facilitated through a hydrolytic process in which the amino sugar moiety is removed and iv. 7-deoxyepirubicin and 7-deoxyepirubicionol aglycones are formed through a redox process in which the amino sugar moiety is removed (Drugbank). (**Figure 8**)

EPI is prone to free radical formation in the body. Due to its quinone structure, the drug is able to act as an electron acceptor in reactions catalyzed by oxidoreductive enzymes. The activity of CYP450, an oxidoreductive enzyme, is instrumental in the conversion mechanism. Since the expression of CYP450 enzymes is high in the GIT tissues, drug detoxification occurs by these enzymes most notably in the small intestine (Sasu et al., 2015).

The lowered cardiotoxicity of EPI (Cortes-Funes and Coronado, 2007) shows that this modification of the DOX structure lessens the danger of cardiotoxic metabolites. However, it is

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evident that many other side effects such as nausea are still caused by the EPI.nausea. The extensive formation of metabolites during EPI clearance in the body is responsible for the likelihood of ADRs.

Fulvestrant

The MW of Fulvestrant (FULV) is approximately 607 g/mol and its log P value is 7.92. (**Table 4**) FULV does not contain as many atoms as the other anticancer drugs discussed in this review, but it still has a similarly high MW due to a few heavy atoms in its structure (i.e. five fluorine atoms and one sulphur atom in the drug's structure total 132 g/mol of the 607 g/mol molecule. Moreover, the complexity of the molecule is greatly increased by its long lipophilic alkyl chain composed of eleven carbon atoms.

FULV is an aromatase inhibitor that blocks production of estrogen through its interactions with the estrogen-producing enzyme aromatase. This has demonstrated increased efficacy compared with the estrogen receptor (ER) antagonist tamoxifen (Flynn et al., 2017), in postmenopausal women as first-line endocrine treatment for advanced BC and as adjuvant therapy for postmenopausal women with early BC (Ciruelos et al., 2014). FULV is part of a third generation of aromatase inhibitors. Its design was based on tamoxifen, a selective ER antagonist that can cause further complications such as endometrial cancer because it mimics the effect of estrogen. This effect causes anti-proliferative activity and induces apoptosis in cells (Johnston and Cheung, 2010).

FULV is used for the treatment of postmenopausal women with estrogen receptor positive locally advanced or metastatic BC (Ciruelos et al., 2014; Johnston and Cheung, 2010). The drug binds to the ER, resulting in degradation of the receptor and downregulation of the signalling pathway. Current FDA approval for this drug states that it can be used as a second line of defense against breast cancer when other anti-estrogen therapies have failed (Al-Mubarak et al., 2013).

The numerous FULV binding mechanisms include impaired dimerization, increased ER turnover, and disrupted nuclear localization. The binding affinity of FULV is 100 times greater than that of tamoxifen, which induces the rapid degradation of estrogen receptor as well as reductions of progesterone levels (Ciruelos et al., 2014). After the drug is bound to its target, it begins working as an antagonist to estrogen by inducing conformational changes to the receptor. This causes the receptor to become less active, inhibits gene transcription, blocks the binding site to estrogen, and increases the receptor turnover (Johnston and Cheung, 2010).

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It has been suggested that FULV is cleared by the liver and extrahepatic metabolism, mostly excreted in the feces during *in vitro* studies. These studies have also shown that FULV and its 17-ketone, sulphur analogues, and sulphur conjugates were the main excretory metabolites. However, *in vivo* studies have also shown that the drug can form other metabolites. It can be converted to a ketone at the 3- or 17- positions of the steroid nucleus, as well as forming sulphate and glucuronide metabolites. At the 9- position, it is also capable of forming a sulphone metabolite. (**Figure 9**) Studies have also shown that the metabolites of FULV are largely inactive. The 17-keto metabolite is the only compound that has demonstrated activity, but it is 4.5 fold less active than the parent compound. This antineoplastic drug is metabolized by CYP3A4 and sulphate conjugation reactions. Research has shown that the sulphate conjugation is the major pathway for FULV metabolism prior to its excretion (Robertson and Harrison, 2004).

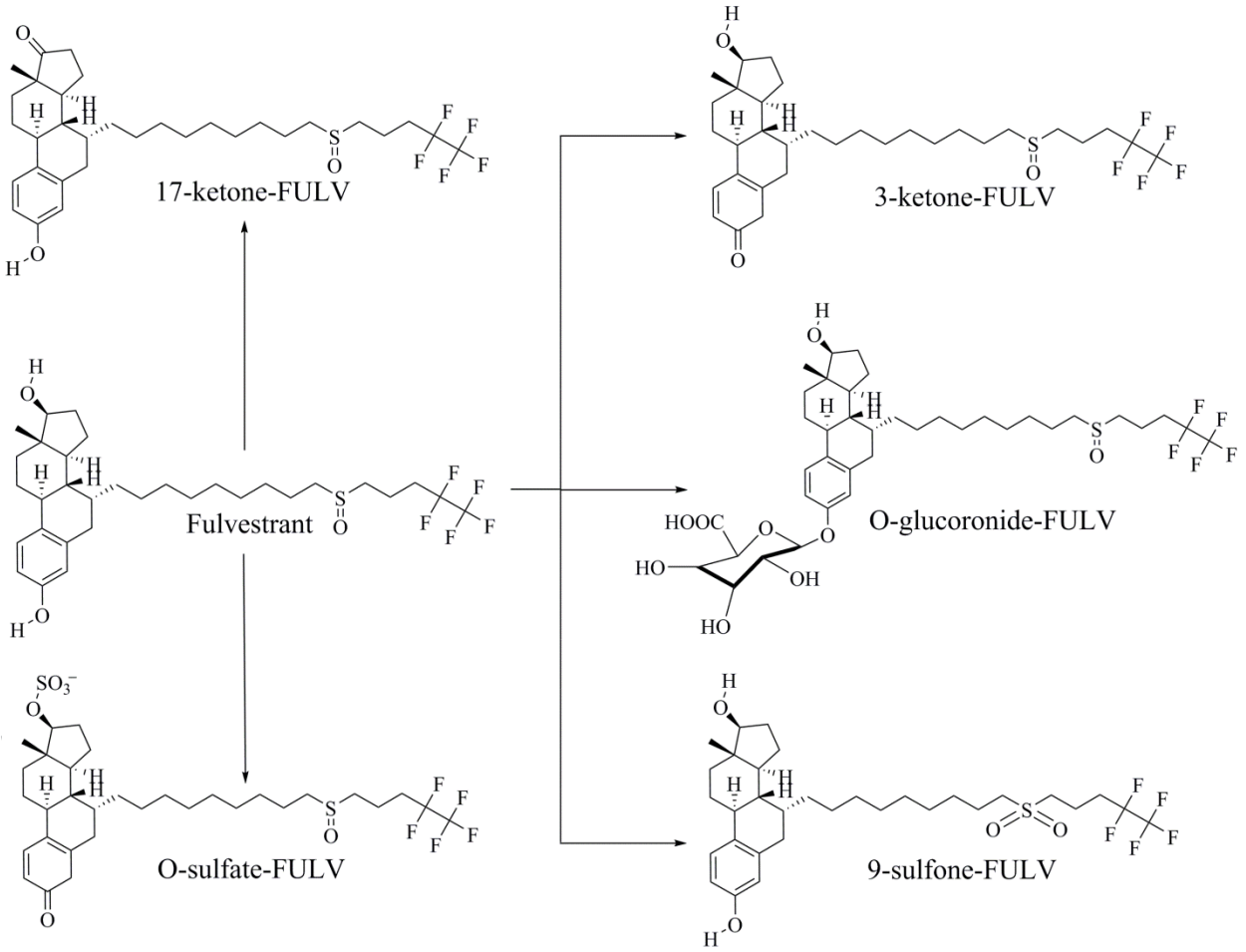

Figure 9: The major metabolites of fulvestrant.

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The parenteral administration of FULV provides better control over endocrine treatment when compared to oral administration. The former is advantageous because it reduces the risk of unwanted oral absorption and interactions with food or other drugs. Breast cancer patients are often using multiple drug therapies at once, thus avoiding drug interactions is crucial. Oral endocrine therapy must be also taken regularly by patients to increase its effectiveness as it has been estimated that 20% of patients do not take their medication regularly (Ciruelos et al., 2014). Recent studies have shown that oral delivery is not an appropriate route of administration for FULV due to its low level of bioavailability and pre-systemic metabolism (Robertson and Harrison, 2004). As a result of its high log P value (7.92), FLUV has a low oral absorption which hinders the drug's absorption from the GIT. (**Table 4**)

To omit these issues, an intramuscular formation of FULV was developed to ensure a slow controlled release of the drug and increase its bioavailability (Robertson and Harrison, 2004), with a current licensed dosage of 250 mg (Johnston and Cheung, 2010).

Goserelin

The drug goserelin (GOSE) with Log P of -0.26 is based on the structure of GnRH (gonadotropin-releasing hormone), but it is modified to become 100 times more powerful. When a drug is designed to mimic a protein, it is very likely to have a high MW. Similarly the design of GOSE resulted in a highly complex structure and a large MW of 1269.43 g/mol. (**Table 4**)

A synthetic decapeptide, GOSE contains different residues at positions 6 and 10 when compared to the endogenous GnRH (pGlu^{1}-His^{2}-Trp^{3}-Ser^{4}-Tyr^{5}-D^{6}-Ser (But)^{7}-Leu^{8}-Arg^{9}-Pro^{10}-Azgly-NH_2). The residue at position 6 is usually a D-amino acid in synthetic GnRH. The use of a D-amino acid is as a bioactive stabilizing moiety with a supportive effect, since it protects GnRH against enzymatic degradation (Rody et al., 2005). The residue Azgly stands for aza-glycine and belongs to the aza-amino acids, is known to have a protective effect against proteolytic degradation (Zhang et al., 2015).

The use of a synthetic GnRH analog usually leads to a potent release of stored luteinizing hormone (LH) and follicle-stimulating hormone (FSH). This effect appears after prolonged exposure due to the desensitization of the gonadotropin cells, which causes the down regulation of GnRH receptors and dysregulation of intracellular signaling. With respect to GnRH receptor

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binding and other PK parameters, synthetic GnRH analogs have a binding affinity that is 100-200 times greater than native GnRH (Rody et al., 2005).

The route of administration of GOSE is by subcutaneous (SC) depot injection into the anterior abdominal wall (Mitchell, 2004). A slow release of up to 28 days occurs from the biodegradable lactide-glycolide copolymer matrix (Cockshott, 2000) helps to avoid rapid metabolic cleavage of the peptide (Mitchell, 2004). Oral and intranasal administration routes are ineffective (Rody et al., 2005) because the GIT hydrolyzes the peptide with proteolytic enzymes (Cockshott, 2000). GOSE has been shown to cause less acute toxicity than cytotoxic chemotherapies when used alone or in combination with another chemotherapy agent (Mitchell, 2004).

The clearance of GOSE is accomplished in majority by the hydrolysis of the C-terminal amino acids. The renal pathway plays a major role in the metabolic clearance of this drug (Cockshott, 2000). During metabolism, the metabolite (1–7 sequence) hexapeptide is detectable in urine, serum (Cockshott, 2000) and bile (Rody et al., 2005). Minor metabolites such as 1-9, 5-10, and 5-7 have also been detected. (Figure 10)

Despite these numerous metabolic pathways, the parent compound GOSE is still the major circulatory metabolite. The composition of major and minor metabolites in the urine depends on the species being tested, but all should show some fragmentation. The parent drug can be found in a 10-30% proportion in animal urine, meaning it is not entirely metabolized. In bile, GOSE is fragmented, with (5-7) tripeptide metabolites found in the highest concentration among other small peptide fragments. These fragments, as well as the parent compound, are eliminated predominantly (~ 90%) by renal excretion (Rody et al., 2005).

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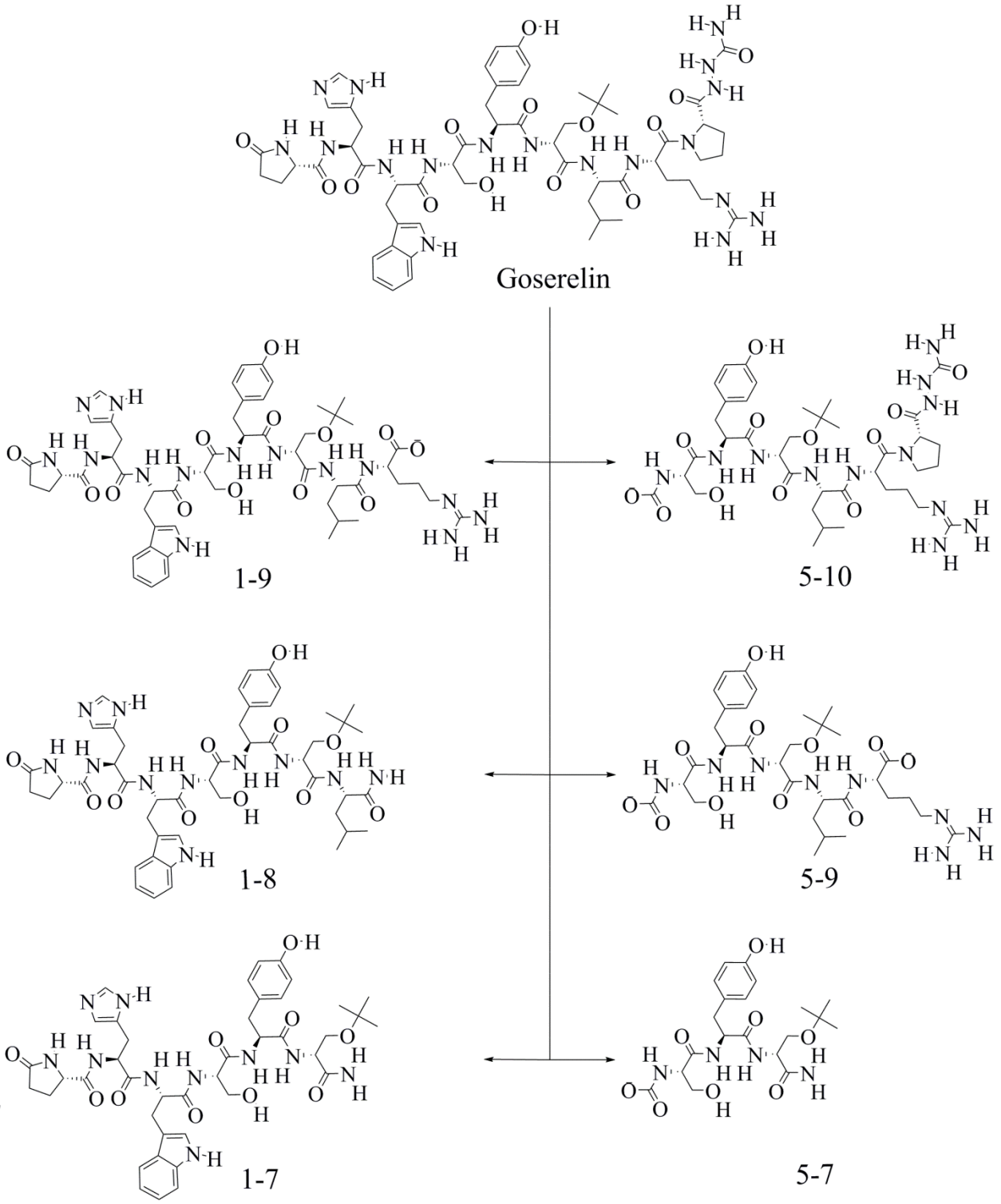

Figure 10: The main fragments of goserelin.

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Research performed by Cockshott showed the activity and proportion of these circulating metabolites (Cockshott, 2000). Fragment 1-7 and the parent compound are the predominant metabolites, with 5-10, 5-9, and 5-7 also detected in small amounts. No activity was detected in synthesized versions of fragments 5-10, 5-9, and 5-7 (Cockshott, 2000). It has also been shown that fragment 1-9 is inactive, which can be used to infer that fragment 1-7 also possesses no activity (Cockshott, 2000). In urine, fragment 5-10 and the parent compound are the predominant metabolites after 48 hours. Minor metabolic fragments 5-7, 5-9, 1-7, 1-8, and 1-9 are also present in the urine in very small quantities (Cockshott, 2000). (**Figure 10**)

The main ADRs of GOSE are similar to the main symptoms of menopause due to the effects of hypoestrogenism. Symptoms can include hot flushes, palpitations, vaginal dryness, sweating, depression, headaches and loss of libido. This treatment does not seem to cause cardiac side effects in premenopausal women, but males treated with this drug for prostate cancer seem to be at a higher risk for angina and myocardial infarction. This risk is even higher when the patients receive combined therapy with doxorubicin (Hydock et al., 2011; Rody et al., 2005). Two years after treatment with GOSE, follow up appointments have shown that the main lasting side effects include hot flushes and vaginal dryness, as well as nausea, vomiting, alopecia and infections to a lesser degree (Cockshott, 2000; Moore et al., 2015; Rody et al., 2005).

Ixabepilone

Ixabeplione (IXA) is the lightest and smallest anticancer drug discussed here with a MW of 506.70 g/mol. Despite its comparatively lighter MW, it is still considered to have a complex molecular structure due to its numerous available fragments for metabolic reactions and its high hydrophilicity (log P of 1.77). (**Table 4**)

IXA is a semisynthetic analog of epothilone B, a drug isolated from the myxobacterium *Sorangium cellulosum*. It was approved by the FDA in 2007 and is currently used in 17 countries (Lee et al., 2008). Its synthesis requires replacing the lactone oxygen with nitrogen to obtain higher plasma stability than epothilones (Fountzilas et al., 2013). This is accomplished by substituting an azide group for the oxygen at position 16 in the macrolide ring, however, these modifications have caused it to become one-fold less cytotoxic than epothilones (Fumoleau et al., 2007). Drugs in the epothilone class induce apoptosis of cancer cells by binding to tubulin and arresting cells in the G2/M phase of mitosis. Similar to taxanes, epothilones bind to microtubules and stabilize them.

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However, they bind to a different site on tubulin and therefore can be used against tumours on which taxane treatment is ineffective (Denduluri and Swain, 2011). Although the semisynthetic drug IXA displays an increased plasma stability and water solubility when compared to other epothilones, the modifications have caused it to be one-fold less cytotoxic (Fumoleau et al., 2007). It is formulated in polyoxyethylated castor oil (Fumoleau et al., 2007), which contains the toxic protein ricin (Pittman et al., 2013). As a result, ADRs such as hypersensitivity events can occur (Fumoleau et al., 2007).

IXA is currently used to treat breast, lung, and colon cancer (Fumoleau et al., 2007). A high activity is demonstrated in either form of administration, IV administration with an ethanol-Cremophor formulation or when given orally to mice (Fumoleau et al., 2007). As with other MT stabilizing agents, IXA is known to cause neuropathy directly after treatment or in the months afterward. The peripheral neuropathy is usually cumulative but reversible through a reduction in drug dosage (Denduluri and Swain, 2011).

The metabolism of IXA is predominantly hepatic oxidation. For patients with hepatic impairment, the drug dosage must be modified accordingly to compensate for their increased risk of toxicity (Denduluri and Swain, 2011). CYP3A4 is the main enzyme responsible for IXA metabolism (Comezoglu et al., 2009; Fountzilas et al., 2013).

IXA as well as metabolites M8, M19, and M41 have been detected in plasma, urine and feces samples. Moreover, metabolite M16 has also been identified in the plasma and urine (Comezoglu et al., 2009). This study has not determined whether the degradants were generated *in vivo, ex vivo* or whether this occurs during storage or extraction. The oxidative products present in the samples are also of unknown origin. They may have either been derived from the degradants of the parent compound or their formation may have been a result of the metabolites degrading in a similar fashion (Comezoglu et al., 2009).

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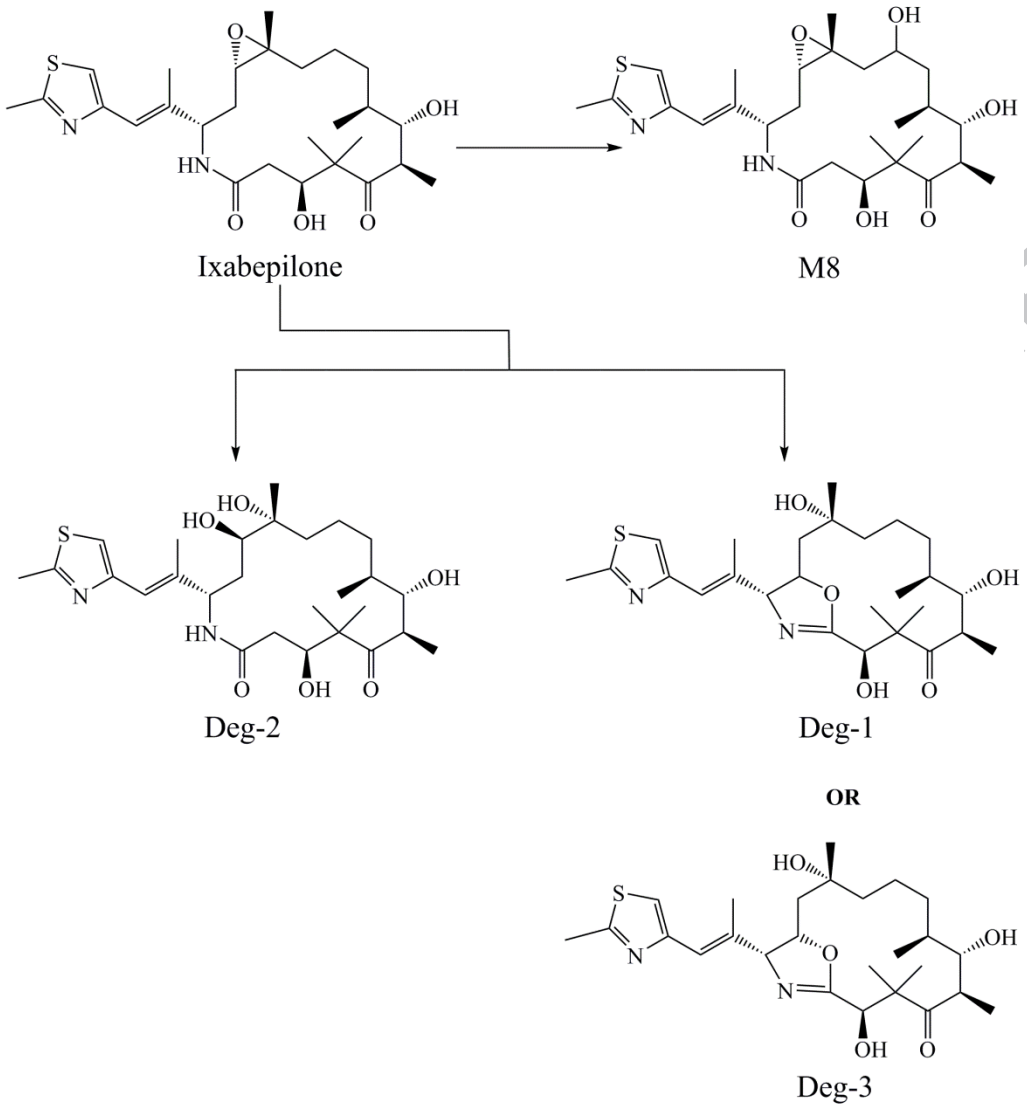

Figure 11: The major metabolites of ixabepilone.

Although IXA displays higher plasma stability than the epothilones, it is still considered a drug of poor stability. This can be attributed to the labile epoxide and lactam rings in its structure, thus causing quick degradation in aqueous environments. Research by Comezoglu *et al.* showed that the degradation process starts with an intramolecular cyclization to create an oxazine (Deg-1), which is then hydrolyzed to a diol derivative of IXA (Deg-2) or an isomeric form (Deg-3). More research is required to determine if the degradants are formed *in vivo* or *ex vivo* (Comezoglu et al., 2009). (**Figure 11**)

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Conclusion

This review looked into eight breast cancer drugs with large and complex chemical structures that also cause common ADRs during chemotherapy. The drugs were chosen due to their MW (greater than 500 g/mol), and structural features that are in disagreement with Lipinski's RO5 criteria (Lipinski et al., 2001). Their MW varied from 506.70 g/mol (IXA) to as high as 1269.43 g/mol (GOSE). It is also known that seven of the anticancer drugs have polar surface areas higher than 140 Å, a limit defined by the RO5 criteria. Only FULV has a PSA within the RO5 limits (76.74 Å). Moreover, two drugs (EVE and FULV) have a log P higher than the RO5 limit of 5 (5.9 and 7.9, respectively); five drugs do not comply with the limited number of HBA groups (PTX, EVE, DOCE, EPI, and GOSE); three drugs have more than the recommended 5 HBD groups (DOX, EPI, and GOSE); and four have more than the 10 rotatable bond (PTX, DOCE, FULV, and GOSE). The GOSE is the drug in least agreement with the RO5, followed by PTX, EVE, and DOCE. Although the RO5 is not very directly related to ADRs, as most of the drugs are administered by IV route due to their low oral bioavailability, they are still useful to predict "drugability" and pharmacokinetic properties of a drug.

The size and complexity of the molecular structures of anticancer drugs play a significant role in drug metabolism. These factors can enhance the chances of metabolite formation and drug promiscuity that can account for off-target binding. Depending on the drug administration route, these events can occur rapidly and compromise the treatment effectiveness. For instance drugs taken by IV route act quickly on the bloodstream, likely spread dangerous metabolites throughout the body within days, hours or even minutes. These metabolites are then able to cause a variety of ADRs throughout the body. Even though many attempts are being made to improve drug delivery systems, there is no anticancer therapy that is completely tolerable and safe. One of the reasons for that is the complexity of such molecular structures, including challenges derived from the physicochemical properties of the drug and patient individual characteristics.

Moreover, patient discomfort caused by regular IV medication can lead to a lack of compliance and/or treatment failure. New and sophisticated oral drug delivery systems are being developed to improve drug bioavailability and provide a more comfortable treatment for cancer patients. However, there are limited efforts being made to revisit existing cancer drugs and modify their chemistry to overcome their toxicity or discomforting effects. Indeed, this is challenging due to the fact that pharmacophores responsible for drug effectiveness might also be toxicophores that

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cause drug toxicity; thus they could limit chemical modifications of the drugs structures. Researchers may develop drug analogues that are potentially capable of stronger interactions towards their desired target and with reduced ADRs.

Another issue that should be taken into consideration is the lack of information available regarding the toxicity caused by the metabolites formed during drug metabolism for most of the antineoplastic drugs discussed here, and thus all of their ADRs are not fully known.

This review aimed to draw more attention towards the importance and necessity of in-depth revisiting the chemistry of anti-cancer drugs to reduce their toxicities. However, the effects of optimization of drugs dosage, their routes of administration and patient medical history, all certainly remain inevitable. The drug discovery research will have to take a long path, but undoubtedly and eventually will bring satisfaction and promising results for better patient care.

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