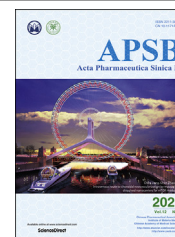




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ORIGINAL ARTICLE

Structure–tissue exposure/selectivity relationship (STR) correlates with clinical efficacy/safety



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Abstract Drug optimization, which improves drug potency/specificity by structure–activity relationship (SAR) and drug-like properties, is rigorously performed to select drug candidates for clinical trials. However, the current drug optimization may overlook the structure–tissue exposure/selectivity-relationship (STR) in disease-targeted tissues vs. normal tissues, which may mislead the drug candidate selection and impact the balance of clinical efficacy/toxicity. In this study, we investigated the STR in correlation with observed clinical efficacy/toxicity using seven selective estrogen receptor modulators (SERMs) that have similar structures, same molecular target, and similar/different pharmacokinetics. The results showed that drug's plasma exposure was not correlated with drug's exposures in the target tissues (tumor, fat pad, bone, uterus), while tissue exposure/selectivity of SERMs was correlated with clinical efficacy/safety. Slight structure modifications of four SERMs did not change drug's plasma exposure but altered drug's tissue exposure/selectivity. Seven SERMs with high protein binding showed higher accumulation in tumors compared to surrounding normal tissues, which is likely due to tumor EPR effect of protein-bound drugs. These suggest that STR alters drug's tissue exposure/selectivity in disease-targeted tissues vs. normal tissues impacting clinical efficacy/toxicity. Drug optimization needs to balance the SAR and STR in selecting drug candidate for clinical trial to improve success of clinical drug development.

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1. Introduction

In the past few decades, despite of significant effort to optimize the drug discovery and development process, 90% of clinical drug development fails in clinical phase I/II/III trial and drug approval^{1,2}. The analysis of the clinical trial data from 2010 to 2017 found four possible reasons for these clinical drug development failures, which included insufficient efficacy (40%–50%), unmanageable toxicity (~30%), poor drug-like properties (10%–15%), and lack of commercial needs and poor strategic realignment (10%)^{1,3,4}. Tremendous effort has been spent and many successful strategies have been implemented in each step of drug development process to improve the success rate⁴. Despite these efforts, the overall success rate of clinical drug development remained at 10% in the past several decades. This persistent high failure rate raises a question if any other aspects of drug discovery/development are overlooked? Two major factors may contribute the high failure rate of clinical drug development⁴: (A) True target validation, which confirms the molecular target is the cause of human disease and drug's intended target, is still challenging for the success of clinical drug development; (B) the current drug optimization may have overemphasized one aspect but overlooked others that may mislead drug candidate selection and unbalance clinical dose/efficacy/toxicity.

In current drug optimization process, two important aspects of drug candidates are rigorously optimized⁴: (1) to achieve high specificity and potency to inhibit the intended molecular target, as measured by low K_i or IC_{50} at pmol/L or nmol/L, where the structure–activity-relationship (SAR) has been well studied to select lead compound for clinical studies^{5,6}, (2) to have better drug-like properties as measured by pharmacokinetics and biopharmaceutics, where pharmacokinetic and biopharmaceutic parameters with certain cut off values are usually used as acceptable compound selection criteria, such as solubility, permeability, stability, protein binding, bioavailability F , absorption rate K_a , drug exposure in plasma (AUC), C_{max} , $T_{1/2}$, clearance CL and volume distribution V ^{7–9}.

However, the current drug optimization process did not fully consider drug exposure/selectivity in disease-targeted organs vs. normal organs, which may have misled drug lead drug candidate selection^{4,10}. Rather, the drug-like property optimization is primarily based on the drug exposure in the plasma, in which drug candidates with better plasma PK parameters are often selected to advance to clinical studies, while drug candidates with low exposure in the plasma are often eliminated without further development^{4,7,11}. The drug exposure in the plasma is often used as a surrogate of therapeutic exposure in disease-targeted tissue based on the well accepted “free drug hypothesis.” This hypothesis believes that only free unbound drug from plasma (not plasma protein bound drug) can distribute to disease-targeted tissues to interact with its molecular target; while free drug concentration in the plasma and in the disease-targeted tissues would be similar at steady state^{12,13}, and thus drug exposure in the plasma can be used as surrogate to predict the pharmacodynamic effect of the drug

candidate. However, this “free drug hypothesis” may only apply to a limited class of drug candidates but not applicable to many other compounds since many factors can cause an asymmetric free drug distribution between plasma and tissue^{4,10,13–34}. This hypothesis also completely ignores the active transport of plasma proteins themselves from systemic circulations to all tissues that also contributes to drug exposure in the tissues^{4,16,17}. Therefore, drug exposure in the plasma, without knowing the exposure in disease-targeted tissue/normal tissues, may mislead the selection of drug candidates for clinical trials^{4,10,22,23,35}. For instance, during drug optimization, drug candidates with high exposure in the plasma are often selected. But some of these selected compounds may have low exposure in the diseased-targeted tissues, which may result low clinical efficacy. In contrast, drug with low plasma exposure is often eliminated; but many of these compounds may have high exposure in the disease-targeted tissues, which may have better clinical efficacy but mistakenly terminated in drug optimization process⁴.

During drug optimization process, structure modification of lead compounds not only changes PK properties in plasma, but also alter drug exposure/selectivity in disease-targeted tissues vs. normal organs⁴. Many clinical failures of drug candidates, either due to lack of efficacy or unmanageable toxicity, may be resulted from inadequate drug exposure in disease targeted tissues, or unexpected drug accumulation in the healthy vital organs. However, the structure–tissue exposure/selectivity relationship (STR) is rarely optimized in drug optimization process, which may impact the lead drug candidate selection and the balance among clinical dose, efficacy and toxicity. In addition, drug candidates, which have similar structures but slight modifications, similar IC_{50} to inhibit the molecular target, and similar plasma PK profile, may have distinct exposure/selectivity in disease targeted-tissues vs. normal organs. Therefore, in addition to SAR, understanding the STR is critical for lead drug candidate selection and the balance of clinical efficacy/toxicity.

In this study, we aimed to investigate the STR, which is correlated with observed clinical efficacy/toxicity profiles, by using a series of drug candidates that have similar or different structures, same molecular target, similar or different PK profiles in plasma. We chose seven selective estrogen receptor modulators (SERMs) in this study³⁶, since there have been large number of SERMs with similar or different structures studied in over 600 clinical trials (<https://clinicaltrials.gov/>) for various indications, including breast cancer, osteoporosis, and menopausal symptoms. Eleven of which have been approved while many others were failed in the clinical trials^{37,38}. We have investigated the discrepancy among the specificity/potency (such as K_i or IC_{50}), plasma pharmacokinetics, and distinct clinical efficacy/toxicity of these SERMs (Supporting Information Table S1). In addition, we studied tissue exposure/selectivity of these SERMs in transgenic mice bearing spontaneous breast cancer, which were associated with their distinct clinical efficacy/toxicity as observed in clinical trials. Furthermore, we also investigated distinct STR for drugs with similar plasma PK profiles and slight structure modifications.

Moreover, we studied an enhanced accumulation of these SERMs in breast tumors compared to surrounding normal tissue. Finally, we also used principal component analysis (PCA) and ordinary least squares (OLS) model to analyze the STR. These data highlight the importance of STR, in addition to SAR, in drug optimization process, which may correlate with clinical efficacy and toxicity.

2. Materials and methods

2.1. Chemicals and reagents

Tamoxifen, toremifene and afimoxifene were purchased from Sigma–Aldrich (St. Louis, MO, USA). Droloxifene, lasofoxifene and nafoxidine were purchased from Santa Cruz Biotechnology (Dallas, TX, USA). Acetonitrile of LC–MS grade was purchased from Sigma–Aldrich. Ultrapure deionized water was obtained from a Milli-Q water system from Millipore (Bedford, MA, USA).

2.2. Animal experiments

Animal study were performed on female MMTV-PyMT mice (FVB/NJ background)³⁹ established by crossing FVB/NJ females (Stock No. 001800) with hemizygous FVB/N-Tg (MMTV-PyMT) 634 Mul/J males (Stock No: 002374) purchased from the Jackson Laboratory (Bar Harbor, ME, USA). Female MMTV-PyMT mice, 8–12 weeks old with tumor sizes of 150–500 mm³, were dosing with tamoxifen, toremifene, afimoxifene, droloxifene, lasofoxifene or nafoxidine at 5 mg/kg by oral (*p.o.*) or 2.5 mg/kg by *i.v.* injection, respectively. At each time point post dosing (0.08, 0.5, 1, 2, 4, and 7 h), samples of blood, plasma, bone, tumor, brain, fat, fatpad, heart, skin, uterus, intestine, kidney, liver, lung, muscle, pancreas, spleen, and stomach were collected from each mouse to measure the drug concentration by LC–MS/MS. All animal experiments were performed in accordance with University of Michigan guidelines covering the humane care and use of animals in research. All animal procedures used in this study were approved by the University Committee on Use and Care Animals at the University of Michigan (Ann Arbor, MI, USA).

2.3. Tissue samples preparation

Aliquot of plasma or blood sample (40 μ L) was dispensed into a Fisher Scientific 96-well plate (Hampton, NH, USA), and then mixed with 40 μ L of ice-cold acetonitrile (100%) and 120 μ L of internal standard solution (25 nmol/L CE302 in ACN). After being vortexed for 10 min, the plate was centrifuged at 3500 rpm for 10 min at 4 °C in a Sorvall Legend X1R centrifuge (Thermo scientific, Waltham, MA, US) to precipitate the protein, and the supernatant was taken for analyze. Other tissue samples were weighed and suspended in 20% acetonitrile (80% water; 1:5 *w/v*), and then homogenized 4 times for 20 s each time at 6500 rpm in a Precellys Evolution system (Montigny-le-Bretonneux, France). Aliquot of the extraction from tissue samples (40 μ L) were mixed with 40 μ L of ice-cold acetonitrile (100%) and 120 μ L of internal standard solution for analysis in the same manner as plasma samples.

2.4. LC–MS/MS analysis of drug concentration

The LC–MS/MS analysis was performed on ABI-5500 Qtrap (Sciex, Ontario, Canada) mass spectrometer with electrospray

ionization source, interfaced with a Shimadzu high performance liquid chromatography (HPLC) system. LC system parameters were set with separation performed on a Waters Xbridge C18 column (Milford, MA, USA) (50 mm \times 2.1 mm ID, 3.5 μ m), mobile phase composed by A (100% H₂O with 0.1% formic acid) and B (100% acetonitrile with 0.1% formic acid), and the flow rate set at 0.4 mL/min. The mass spectrometer was operated in a positive mode with multiple reaction monitoring (MRM) for analysis. The MRM transitions (Q1/Q3) were monitored with 372.2/71.5 for tamoxifen, 406.2/72 for toremifene, 388.2/71.8 for afimoxifene, 388.2/71.8 for droloxifene, 414.2/98.2 for lasofoxifene, 426.2/98 for nafoxidine or 474.2/269.2 for raloxifene. The acquisition and processing of data were conducted by Analyst Software version 1.6 from Applied Biosystems (MDS SCIEX; Carlsbad, CA, USA). The analytical assay was validated according to U.S. Food and Drug Administration (FDA) guidance for linearity (2–5000 ng/mL), matrix effect, recovery, low detection limit, quality control (QC) in different biological matrix, including plasma, blood, tumor, and each different organ homogenates.

2.5. Principal component analysis (PCA) and ordinary least squares (OLS) model

Molecule descriptors of different SERMs include autocorrelation descriptors, charge descriptors, log P descriptors, refractivity descriptors, compositional descriptors, topological descriptors, connectivity descriptors, composite descriptors from VOE and Kappa shape indices were collected from the open RDkit API. Descriptors were normalized and processed by PCA analysis through scikit-learn API (module PCA with component numbers set as 3). The OLS model for the correlation between components or specific descriptors (univariate feature analysis) and drug's tissue partition coefficient K_p was processed using Linear Regression module from scikit-learn API.

2.6. Pharmacokinetic

Non-compartment model was used to calculate plasma or tissue AUC for each drug with Phoenix/WinNonlin software (version 6.4; Pharsight, Mountain View, CA, USA). AUC_{total} (AUC_{last/72 h} + AUC_{inf}) was used for the comparison among different drugs and calculation of K_p .

2.7. Statistical analysis

Statistical analysis was conducted using GraphPad Prism 8.0 software. Statistical significance between two treatment groups was analyzed by Student's *t*-test with two-tailed *P* values. A *P*-value < 0.05 was considered significant. Data are presented as mean \pm SEM.

3. Results

3.1. Drug exposure in tissue but not in plasma was associated with their clinical efficacy/toxicity

3.1.1. No correlation between drug exposure in plasma and in disease-targeted tissues in most cases

In drug development process, drug exposure in plasma (drug concentration and area under the curve, AUC) is often used as a surrogate of drug exposure in the disease-targeted tissue (such as

tumor). Drug candidate with high plasma drug exposure is often selected for further clinical studies³⁵. To verify if drug exposure in plasma was correlated with drug exposure in disease-targeted tissues, we measured the drug concentration and AUC in plasma and target tissues (tumor, fatpad, bone) after oral administration of 7 SERMs (Fig. 1A) in transgenic mice with spontaneous breast cancer (MMTV-PyMT). As shown in Fig. 1B–D, plasma AUC values of these seven SERMs were not correlated with AUCs in the disease target tissues (tumor, fatpad, bone). Three different scenarios were observed among the seven drugs: (1) high plasma drug concentration can be used to predict high tumor concentration. Nafoxidine had higher drug concentration in both the plasma and tumors than raloxifene (Fig. 1E); (2) similar plasma drug concentrations did not predict higher tumor drug concentration. Tamoxifen and raloxifene had similar plasma drug concentration, whereas tumor concentration of tamoxifen was 4-fold higher than raloxifene (Fig. 1F); (3) low plasma drug concentration did not predict higher tumor concentration. Toremifene had 1.5- to 2-fold lower plasma drug concentration than raloxifene, but 1.5-fold higher drug concentration in the tumor with raloxifene (Fig. 1G).

3.1.2. Correlation of drug exposure in the disease-targeted tissues but not in the plasma with drug clinical efficacy/safety

To study if drug exposure in the disease-targeted tissue was better than plasma exposure to correlate with drug clinical efficacy/safety, we compared two FDA approved SERMs (tamoxifen and raloxifene) with similar plasma drug exposure and well-documented distinct efficacy/toxicity profiles^{40,41}. Although tamoxifen and raloxifene had similar drug exposure in the plasma (Fig. 1E), tamoxifen exposure in the tumor and fatpad was 4- and 9-fold higher than raloxifene, respectively (Fig. 2A). Clinically, tamoxifen was widely used in breast cancer treatment⁴⁰, while raloxifene showed modest or no response in a therapeutic clinical study of breast cancer^{42,43}, and was only approved for breast cancer prevention in clinic (Table S1)⁴¹. A long-term observation with 81-month follow-up, a higher dose of raloxifene (60 mg/day) only retained 76% and 78% of the effectiveness of a lower dose of tamoxifen (20 mg/day) in preventing invasive disease and noninvasive disease (STAR trial, Fig. 2B and Supporting Information Table S2)^{41,44}. Clearly, the SAR alone of these SERMs could not fully explain their distinct clinical efficacy (Table S1). For instance, raloxifene ($K_i \sim 0.4$ nmol/L)⁴⁵ has 20-fold higher binding affinity with the molecular target (human ER α) and 100-fold higher potency in inhibiting breast cancer cells (MCF-7) than tamoxifen ($K_i \sim 10$ nmol/L)^{46–48}. One explanation of tamoxifen's efficacy is from its active metabolite 4-hydroxytamoxifen. However, the 4-hydroxytamoxifen and raloxifene demonstrated similar IC₅₀ in MCF-7 cell line^{47,49,50}. The IC₅₀ alone cannot fully explain its superior efficacy than raloxifene. Interestingly, raloxifene (60 mg/day) has lower risk of uterine cancer and other toxicity related to uterus (Table 2C). It was hypothesized that the decreased uterus toxicity of raloxifene was due to its estrogen antagonist properties in uterus, while tamoxifen was partial estrogen agonist³⁷. However, raloxifene was not a complete ER antagonist in uterus since it still increased uterine weight in the preclinical studies^{51,52}. Our data found that raloxifene has less drug accumulation in the uterus, which may partially explain the decreased side effect in uterus (Fig. 2C)⁴¹. Furthermore, raloxifene has less side effects compared to tamoxifen in skin, stomach, lung and brain⁴¹ (Fig. 2C), which is in consistent with less drug accumulation in those organs (Fig. 2A).

3.1.3. Relationship of drug exposure in the tissues and in the plasma

Drug exposure in the tissue is determined by drug exposure in the plasma and tissue/plasma partition coefficient (K_p)⁵³, as shown in Eq. (1):

$$\text{Drug exposure in the tissue} = \text{Drug exposure in the plasma} \times K_p \quad (1)$$

where drug exposure in the plasma and tissue can be calculated by drug concentration vs. time curve, and K_p values can be calculated by $C_{\text{tissue}}/C_{\text{plasma}}$ or $\text{AUC}_{\text{tissue}}/\text{AUC}_{\text{plasma}}$ ⁵³. As shown in Table 1, Supporting Information Tables S2 and S3, K_p ($\text{AUC}_{\text{tissue}}/\text{AUC}_{\text{plasma}}$ ratio) of seven different SERMs in tumor, fatpad and bone were significantly different although some drugs had similar plasma AUCs. Therefore, drug exposure in the plasma exposure as a surrogate of drug exposure in the disease-targeted tissue was not appropriate since it neglected the differences of K_p values in various tissues for different drug candidates. For instance, although tamoxifen and raloxifene had similar drug exposure in the plasma (Fig. 1E), the K_p of tamoxifen in tumor and fatpad were 4- and 10-fold higher than that of raloxifene (Table 1), which determined its significantly higher exposure in both organs. It is worth noting that K_p can be calculated by the ratio of free fraction of drug in plasma (f_u) vs. free fraction of drugs in tissues (f_{ut}) at steady state (f_u/f_{ut}). However, this calculation is based on the “free drug hypothesis”, which assumes that free drug concentration in tissue is similar to that in plasma^{12,13}, which may not be applicable here, since many drugs may have an asymmetric free drug distribution between plasma and tissue even at steady state^{13–19}.

3.2. Drug tissue selectivity may impact the balance of efficacy/toxicity, which is often overlooked in drug optimization process

Dose escalation is always performed in clinical phase I/II trials where MTD is normally used for cancer treatment. MTD is often associated with the drug exposure in certain toxicity related organs⁵⁴. Dose escalation could certainly achieve adequate drug exposure in the disease-targeted tissue, but it may also increase drug exposure in the healthy vital organs or blood cells causing adverse effects. Drug tissue selectivity is an important parameter that determines the balance between efficacy and toxicity as shown in Eq. (2):

$$\text{Drug tissue selectivity} = C_{\text{tissue}} / \sum C_{\text{tissue}} \text{ or } \text{AUC}_{\text{tissue}} / \sum \text{AUC}_{\text{tissue}} \quad (2)$$

where sum of C_{tissue} or $\text{AUC}_{\text{tissue}}$ is total drug concentration or AUC in different tissues.

An ideal drug candidate is expected to have high tissue selectivity and exposure in the disease-targeted organ for better efficacy (such as tumors) but low tissue selectivity and exposure in vital healthy organs to reduce toxicity. In contrast, if a drug candidate has high selectivity and high exposure in healthy vital organs but low in disease-targeted ones, it may not be able to reach its therapeutic concentration. Further, if a drug has low selectivity and exposure in both disease-targeted and healthy vital organs, it may be safe even with high drug exposure in the plasma in phase I studies, but it may achieve in inadequate efficacy in phase II/III studies. This dilemma should be avoided during drug optimization in lead compound selection.

As shown in Fig. 3A and B, the tissue exposure and selectivity were compared between tamoxifen and raloxifene. Although tamoxifen and raloxifene have similar plasma exposure, tamoxifen

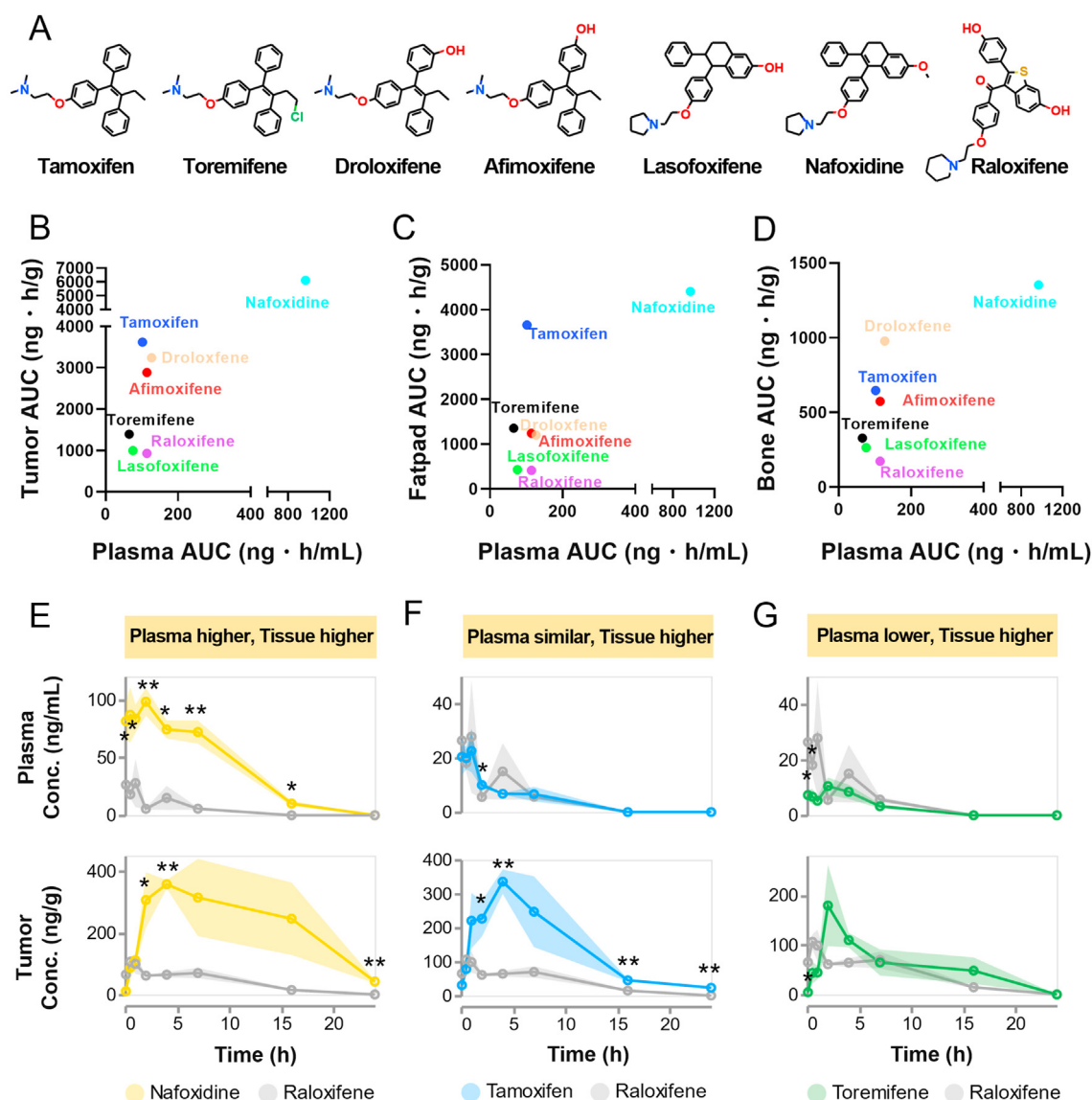


Figure 1 Drug exposure in the plasma was not correlated with drug exposure in the disease targeted tissue among seven SERMs with different or similar chemical structures. (A) Structures of seven SERMs. (B–D) Plasma AUC vs. tissue AUC of tumor (B), fatpad (C) and bone (D). (E) Concentration–time curve of nafoxidine vs. raloxifene, in which nafoxidine had higher drug concentration in the plasma and tumor. (F) Concentration–time curve of tamoxifen vs. raloxifene, in which two drugs had similar plasma concentration, but tamoxifen had much higher tumor concentration. (G) Concentration–time curve of toremifene vs raloxifene, in which toremifene had lower plasma concentration, but higher drug concentration in the tumor with raloxifene. MMTV-PyMT transgenic mice with spontaneous breast cancer were orally administered with tamoxifen, toremifene, afimoxifene, droloxifene, lasofoxifene, nafoxidine and raloxifene (5.0 mg/kg). Three mice were sacrificed at each time point to collect plasma and other tissues. The drug concentration in all samples and their calculated AUC by non-compartment model were compared among different compounds (Supporting Information Figs. S1 and S2). Data in E, F and G were represented as mean \pm SEM ($n = 3$ per time point, two-tailed t -test). Asterisks indicate the following P -value: * $P < 0.05$, ** $P < 0.01$.

has 3- to 6-fold higher exposure than raloxifene in tumor, fatpad, and bone. In addition, tamoxifen tissue selectivity in fatpad and tumor was 3- and 1.5-fold higher than that of raloxifene, whereas both drugs had similar bone selectivity (Fig. 3B). Due to the different tissue selectivity, higher dose of raloxifene (60 mg QD) would only achieve 34% and 77% of exposure in fatpad and tumor compared to lower dose of tamoxifen (20 mg QD), although they could achieve similar drug exposure in the bone (Supporting Information Table S5). This may at least partially explain why raloxifene (60 mg QD) was less effective than tamoxifen (20 mg

QD) in treatment or prevention of breast cancer although raloxifene showed higher specificity/potency against estrogen receptor (ER) from enzymatic assay or cell based assay (lower K_i or IC_{50})^{45,47}, while both drugs were effective in treating osteoporosis³⁷.

When tamoxifen was compared to toremifene that both were approved for treatment of breast cancer^{40,55}, the data showed that tamoxifen had higher tumor and fatpad tissue exposure than toremifene (Fig. 3C), but similar selectivity in the two tissues (Fig. 3D) and similar cytotoxicity against breast cancer cell line MCF-7 and MDA-231^{49,56}. Therefore, higher dose of toremifene

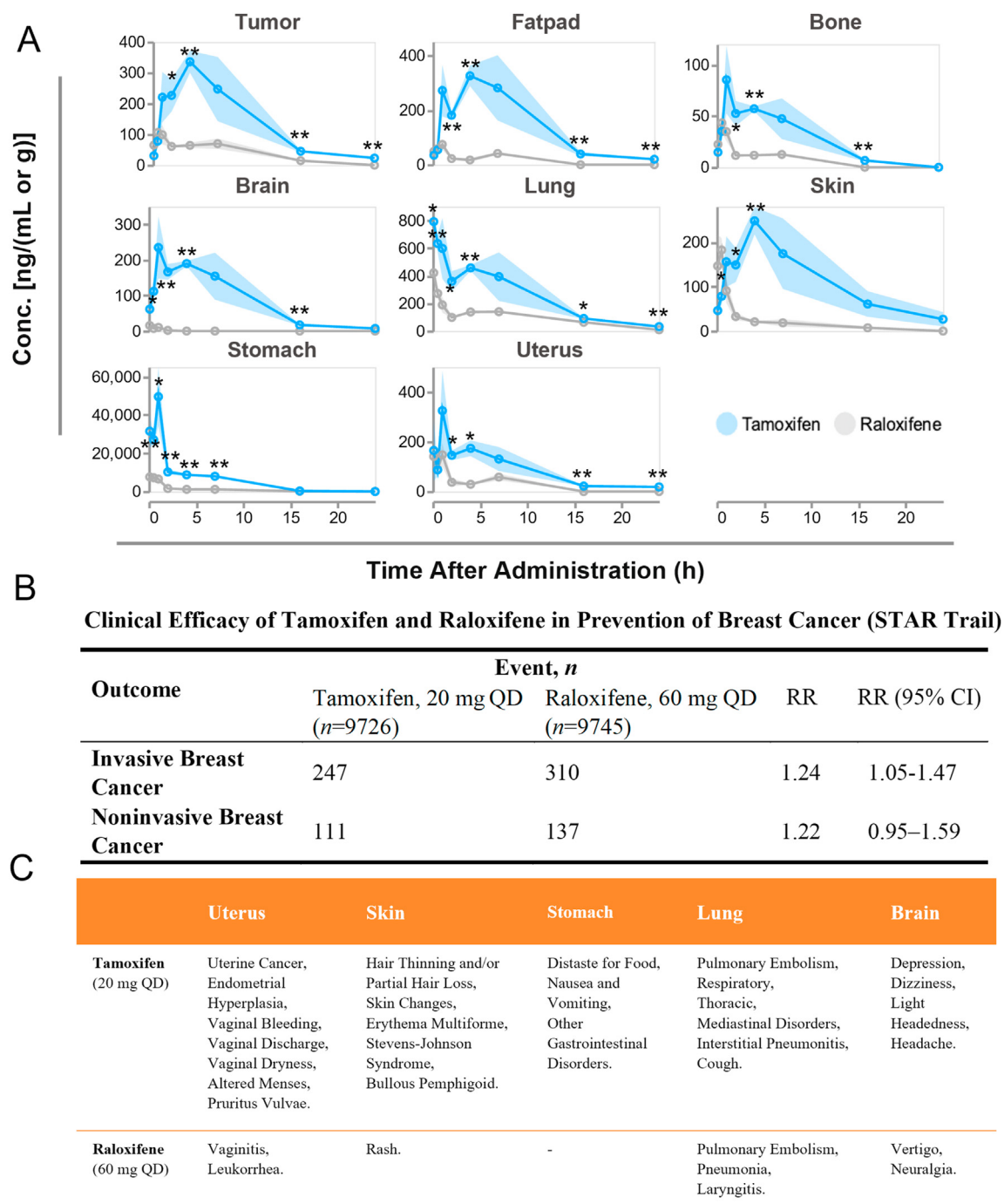


Figure 2 Drug exposure in the tissue, not in the plasma, was correlated with drug clinical efficacy/safety. (A) Concentration–time curve of tamoxifen vs. raloxifene, in which two drugs had similar plasma concentration, but tamoxifen had much higher concentration in tumor, fatpad, bone, uterus, skin, stomach, lung and brain. (B) Clinical efficacy of tamoxifen (20 mg/d) vs. raloxifene (60 mg/d). (C) Comparison of clinical adverse effects associated with different organs. Data of (C) and (D) were summarized from FDA labels of both drugs^{41,42}. Data were represented as mean \pm SEM ($n = 3$ per time point, two-tailed *t*-test). Asterisks indicate the following *P*-value: **P* < 0.05, ***P* < 0.01.

(60 mg QD) could achieve similar drug exposure levels as compared to lower dose of tamoxifen (20 mg QD, Supporting Information Table S6), which also demonstrated similar clinical anticancer efficacy. However, lung selectivity of toremifene were 2-fold higher than that of tamoxifen (Fig. 3D, with same dose), and thus, the high dose of toremifene (60 mg QD) may lead to a 2-fold increase of drug exposure in the lung to tamoxifen (20 mg

QD, Table S6). This is consistent with the clinical observation that toremifene (60 mg QD) increased incidence of pulmonary embolism compared with tamoxifen (20 mg QD)⁵⁵.

Dose selection without considering tissue selectivity and exposure may lead to the failure in balancing clinical efficacy/toxicity. Typically, MTD is used in clinical trials especially in cancer patients. However, dose escalation based on MTD without

Table 1 Summary of plasma and target tissues distribution kinetic parameters. K_p values were calculated by $AUC_{\text{tissue}}/AUC_{\text{plasma}}$.

Parameter	Toremifene	Lasofloxifene	Afimoxifene	Tamoxifen	Raloxifene	Droloxifene	Nafoxidine
AUC_{plasma}	64.9	75.4	114	101.8	114.2	126.7	969
AUC_{tumor}	1393	996	2886.5	3621.3	929.4	3238.8	6118.7
AUC_{fatpad}	1356.2	429.8	1242.8	3660.1	416.2	1201.5	4404.8
AUC_{bone}	327.6	264.6	573.9	646.4	172.8	978.5	1353.1
K_p_{tumor}	21.5	13.2	25.3	35.6	8.1	25.6	6.3
K_p_{fatpad}	20.9	5.7	10.9	36	3.6	9.5	4.5
K_p_{bone}	5.1	3.5	5	6.3	1.5	7.7	1.4

considering drug exposure/selectivity in tissues may increase the failure rate of clinical trials. For instance, nafoxidine and tamoxifen have similar binding affinity to ER⁵⁷. However, nafoxidine was terminated due to its dermatologic toxicity in most patients, such as ichthyosis, cutaneous photosensitivity, and cutaneous erythema after 4–8 weeks of treatment^{58,59}. It is interesting that high dose of nafoxidine (180–270 mg daily) was used in these clinical trials to achieve clinical anticancer efficacy, while such high dose also caused chronic side effects in skin^{59,60}. In contrast, tamoxifen which was efficacious to treat breast cancer with 20–40 mg daily dose, caused similar but manageable skin toxicity⁴⁰. By analyzing the tissue exposure and selectivity of these two drugs (Fig. 3E and F), we found that nafoxidine has similar or higher exposure and selectivity in tumor and fatpad, and similar exposure and selectivity in skin, which may partially explain the clinical observations. It is worth noting that the active metabolite of tamoxifen (4-hydroxy tamoxifen) may also contribute these differences in efficacy/toxicity, which suggest both SAR and STR are important in drug optimization.

3.3. Slight structure modification altered drug exposure and selectivity in various tissues despite similar drug exposure in the plasma

Slight structure modifications are often performed to optimize lead compounds since these small structure changes may impact binding affinity to the molecular target and pharmacokinetics. However, it is not known if these small changes in structure may alter drug exposure/selectivity in the tissues, which may impact their clinical efficacy/toxicity. Therefore, we compared the exposure and tissue selectivity in various tissues of four SERMs with very similar chemical structure: tamoxifen, toremifene, afimoxifene and droloxifene (Fig. 4). Only one substitution was altered in toremifene (–Cl), afimoxifene (–OH) and droloxifene (–OH) compared to tamoxifen (Fig. 4A), while afimoxifene (–OH) and droloxifene (–OH) are isomers with same –OH substitution in two different positions. The structure similarity of these four drugs were further quantitated by the euclidean distance between these molecule descriptors (Fig. S3).

First, we compared plasma and tissue concentrations after oral administration of the four drugs. The data showed that slight structure modification drastically altered oral bioavailability, plasma exposure, tissue exposure and selectivity (Fig. S4). It is not surprising that different bioavailability (F) of four drugs may change drug exposure in the plasma and in the tissues (tamoxifen F 39.4%; toremifene 18.5%; droloxifene 45.3%; afimoxifene F 32.4%). However, it is surprising that tamoxifen and droloxifene had similar bioavailability and exposure in the plasma, but they

have very distinct tissue exposure and selectivity in the lung, fatpad, fat, and tumor.

In order to avoid the impact of different bioavailability of drugs on tissue exposure, we further compared the tissue exposure/selectivity after the i.v. administration of these four drugs with similar structure. As shown in Fig. 4B and C after IV injection, all four drugs had similar drug exposure in the plasma or blood, but distinct tissue exposure in most organs, such as brain, fat, fatpad, heart, intestine, kidney, liver, lung, muscle, pancreas, skin, spleen, stomach, tumor and uterus (Fig. 4B). Tamoxifen and toremifene have 2-fold exposure difference in the fat, intestine and skin. The exposure of tamoxifen is 26- and 4-fold higher in fat and fatpad than that of afimoxifene (Fig. 4C). In addition, two isomers afimoxifene and droloxifene showed 4.8- and 6.8-fold exposure difference in the kidney and liver (Fig. 4C).

Moreover, tissue selectivity of these four drugs were also different as shown in Fig. 4D. When compared tamoxifen and toremifene, tamoxifen had higher selectivity in fat, skin and intestine, whereas toremifene had higher selectivity in uterus, spleen, kidney and lung. Two isomers afimoxifene and droloxifene also showed different tissue selectivity, where droloxifene had higher selectivity in heart, uterus, spleen and tumor but lower selectivity in the liver, kidney, stomach and brain than afimoxifene. Both afimoxifene and droloxifene showed higher lung but lower pancreas selectivity compared to tamoxifen and toremifene. These data clearly suggest that slight structure modifications might drastically alter drug exposure and selectivity in different tissues despite similar exposure in the plasma. However, these phenomena are often overlooked in lead compound selection in drug optimization process.

However, it is worth noting that slight structure modification may also completely alter exposure and selectivity in plasma and various tissues regardless oral or IV administration. For instance, lasofloxifene (–OH) and nafoxidine (–OMe) have slightly different substitutions at the same position (Fig. 5A). Nafoxidine achieved a higher drug exposure in the plasma and in most of the tissues than lasofloxifene after oral administration since nafoxidine (83.4%) has 3-fold higher oral bioavailability than lasofloxifene (F 26.9%) (Fig. S5B). However, IV administration of these two drugs also showed that slight structure modification may also alter drug exposure in both plasma and tissues. As shown in Fig. 5, nafoxidine had a 2- to 3-fold higher exposure in the plasma and 6- and 8-fold higher exposure in the spleen and fat than that of lasofloxifene. In addition, two drugs also showed different tissue selectivity after i.v and p.o. administration (Fig. 5D and Fig. S5). Nafoxidine had higher selectivity in stomach, liver, fat, fatpad, spleen, kidney, but lower selectivity in heart, tumor and pancreas compared to lasofloxifene.

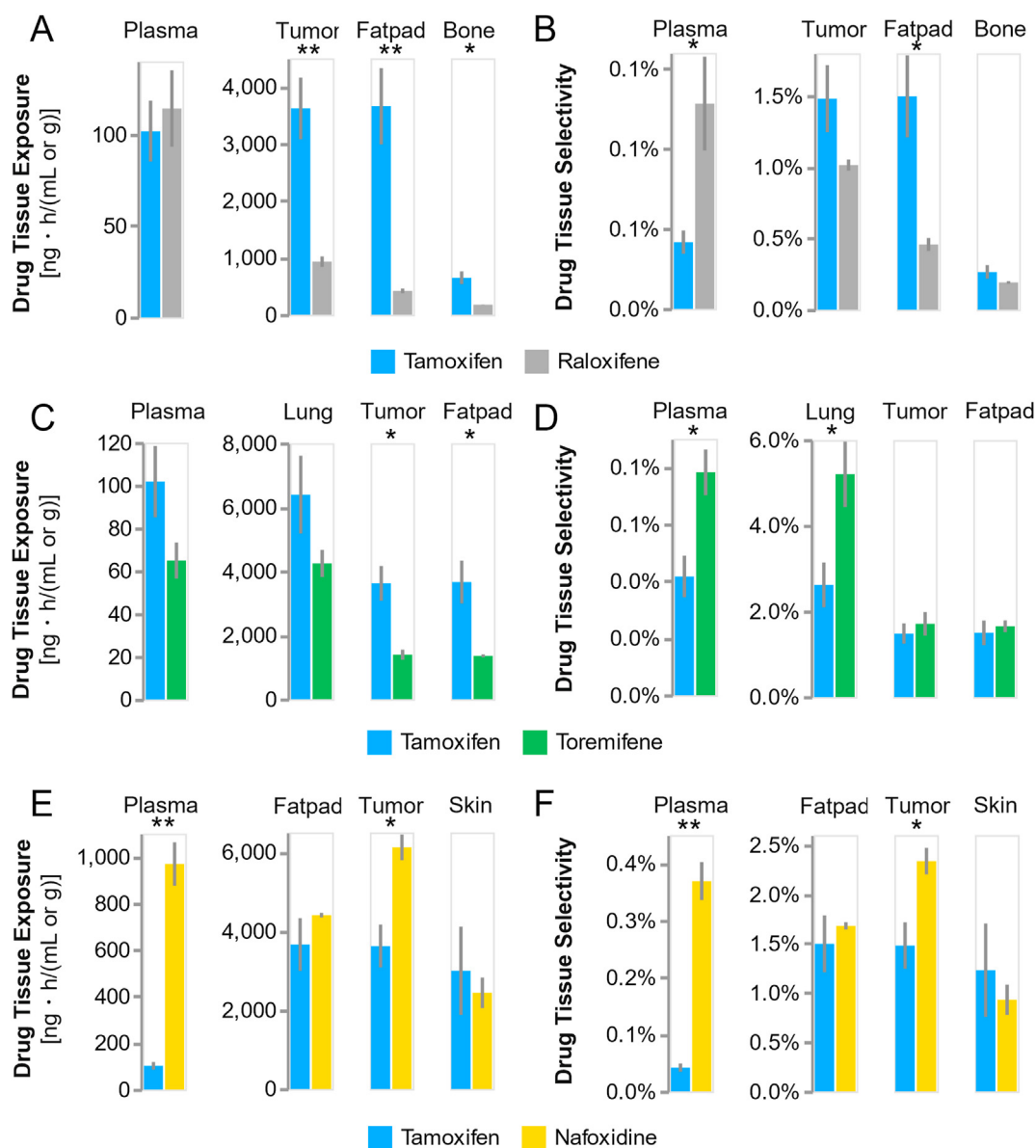


Figure 3 Drug tissue selectivity is a critical parameter that tips the balance of efficacy/toxicity. (A and B) Comparison of drug exposure (A) and selectivity (B) in tissues like tumor, fatpad and bone between tamoxifen and raloxifene after oral administration (5 mg/kg). (C and D) Comparison of drug exposure (C) and selectivity (D) in tissues like lung, tumor and fatpad between tamoxifen and toremifene after oral administration (5 mg/kg). (E and F) Comparison of drug exposure (E) and selectivity (F) in tissues like fatpad, tumor and skin between tamoxifen and nafoxidine after oral administration (5 mg/kg) in MMTV-PyMT transgenic mice with spontaneous breast cancer. Data were represented as mean \pm SEM ($n = 3$, two-tailed t -test). Asterisks indicate the following P -value: * $P < 0.05$, ** $P < 0.01$.

3.4. An enhanced accumulation of SERMs in tumors compared to normal tissue

Drug exposure in tumors is often different from that in plasma or normal adjacent tissues. Two possible reasons might be contributed to the unique drug exposure in tumors: one possibility is that drug have some unique binding capacity to tumor tissues; another reason is that tumors have enhanced permeability retention (EPR) effect to trap protein-bound drug molecules^{4,28–33}. In such case, the

“free drug” hypothesis is incorrect to assume that only free drug (but not protein bound drugs) can distribute to tumor tissues^{4,33}. Very interestingly, we observed that all seven SERMs has higher drug accumulations in spontaneous breast cancer tissues compared to surrounding normal tissues (fatpad) after i.v. injection (Fig. 6A–G). In particular, the AUC of afimoxifene, droloxifene, lasofoxifene, raloxifene is 2.2-, 3.1-, 2.8- and 3.1-fold higher in tumor tissues than in the normal fatpad (Supporting Information Table S7). Thus, the enhanced tumor accumulation of these seven SERMs is likely due to

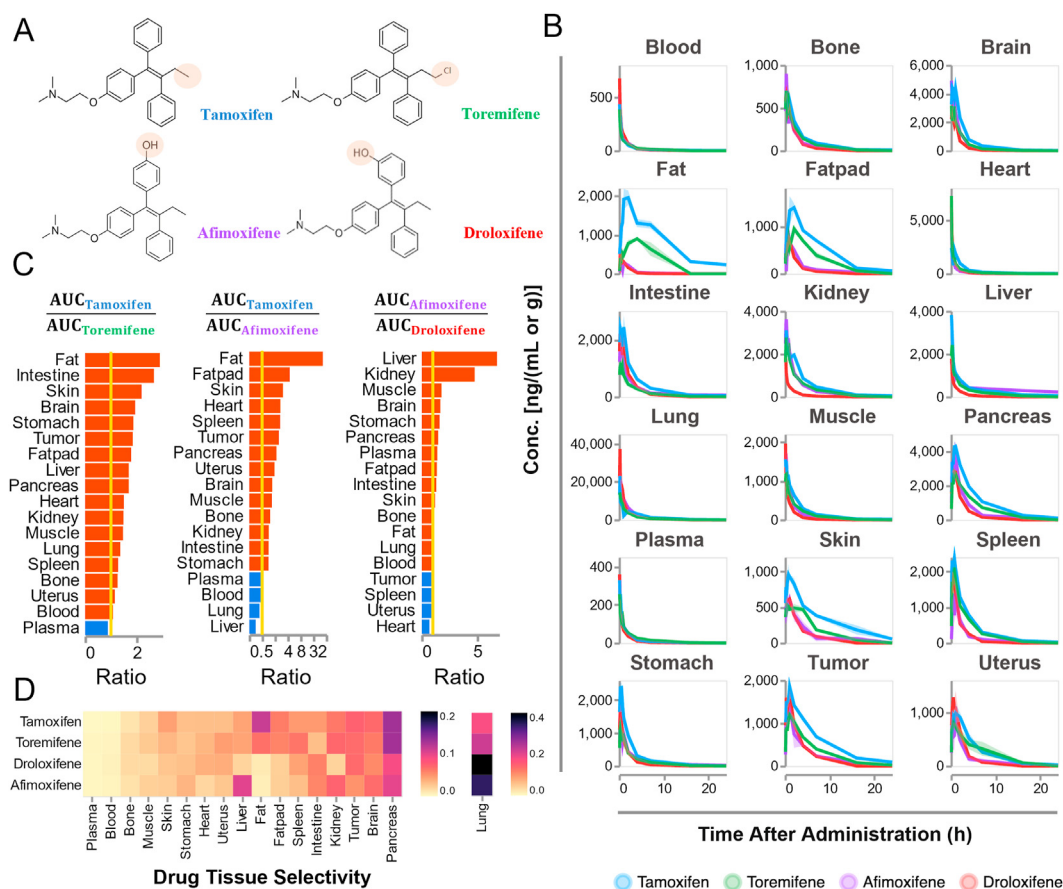


Figure 4 Slight structure modification altered drug exposure and selectivity in tissues despite similar exposure in the plasma. (A) The chemical structure of afimoxifene, droloxifene, tamoxifen and toremifene. (B) Concentration–time curve after i.v. administration of afimoxifene, droloxifene, tamoxifen and toremifene (i.v. 2.5 mg/kg) on MMTV-PyMT transgenic mice with spontaneous breast cancer ($n = 3$ at each time point). Data were represented as mean \pm SEM. (C) AUC ratio of tamoxifen vs. toremifene, tamoxifen vs. afimoxifene and afimoxifene vs. droloxifene. Average AUC in each tissue was used for the ratio calculation. (D) Drug tissue selectivity calculated by AUC_{tissue}/AUC_{total} using data collected in (B).

the accumulation of protein/drug complex (>90% plasma protein binding) through EPR effect in tumors.

3.5. Principal component analysis (PCA) and ordinary least squares (OLS) analysis of STR

Understanding STR may provide guidance for drug optimization. However, current knowledge for such relationship is very limited. It has been hypothesized that drug physicochemical properties such as lipophilicity ($\log P$), solubility, ionization (pK_a), polarity (such as polar surface area, PSA), plasma protein or tissue binding, and molecular weight (MW) may influence drug exposure in the tissues^{61–63}. These concepts have been confirmed by numbers of compounds with very different structures and distinct physicochemical properties⁶¹. However, the six SERMs in our study had very similar physicochemical properties (Table 2). For instance, droloxifene and afimoxifene had very similar solubility, TPSA, $\log P$, plasma protein binding and molecular weight, but distinct drug exposure in various tissues. Likewise, lasofoxifene and nafoxidine had similar $\log P$, $\log S$, protein binding, and pK_a , but they exhibit as large as 5-fold difference in fat and spleen

accumulation. Thus, the commonly used physicochemical properties may not explain the difference in the drug exposure and selectivity in tissues.

To better dissect molecular structure descriptors of these drugs that may be associated with drug exposure and tissue selectivity in various tissues, we used RDKit API to collect more than 300 molecular structure descriptors (Supporting Information Table S8) and decomposed them into 3 components through PCA analysis (Fig. 7A). By using components instead of traditional physicochemical properties, the variance among drugs with similar structure could be better captured. More than 80% of property variance existing among these compounds has been captured by three components (Fig. 7B). To avoid the risk of overfitting the data due to limited number of drugs, a simple ordinary least squares (OLS) model was used to describe the possible correlation between molecule structure properties (represented by components) and partition coefficient in different tissues (represented by $K_p = AUC_{tissue}/AUC_{plasma}$). As shown in Fig. 7C, a good linear correlation existed in several tissues including spleen, uterus, fatpad and skin by using only component 1. Higher r -square scores were observed as more components were fitted into this model, in which

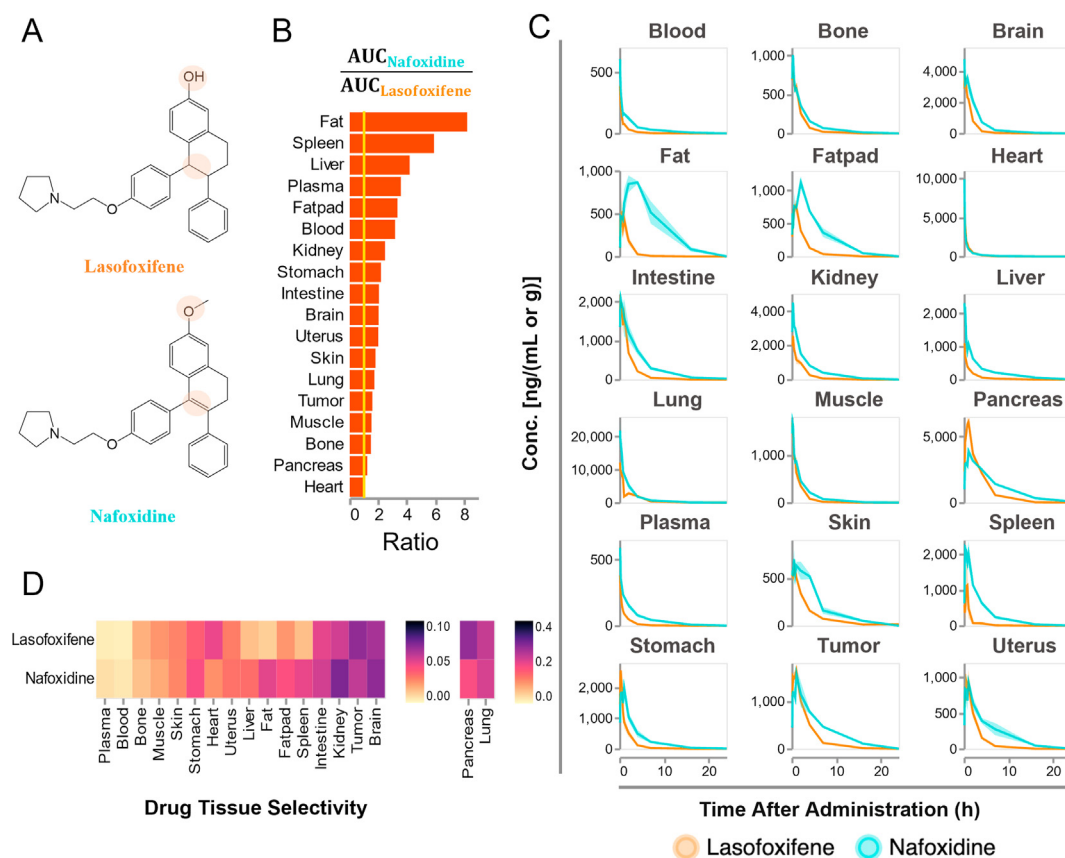


Figure 5 Slight structure modification altered drug exposure and selectivity in both plasma and tissues. (A) The chemical structure of lasofoxifene and nafoxidine. (B) AUC ratio of nafoxidine vs lasofoxifene. Average AUC in each tissue was used for the ratio calculation. (C) Concentration–time curve after i.v. administration of lasofoxifene and nafoxidine (IV 2.5 mg/kg) on MMTV-PyMT transgenic mice with spontaneous breast cancer ($n = 3$ at each time point). Data were represented as mean \pm SEM. (D) Drug tissue selectivity calculated by $AUC_{\text{tissue}}/AUC_{\text{total}}$ using data collected in (B).

11 of 17 tissues showed good linear relation when considering all three components. Similar observations were seen between three principle components (structure descriptors) and the tissue partition coefficient K_p after oral administration of seven SERMs (Fig. 7C). The analysis confirmed good performance of OLS model in correlating molecular structure properties with drug's tissue partition coefficient K_p among the structurally similar drugs.

Univariate feature analysis showed that drug's tissue partition coefficient K_p correlated with different molecular properties in a tissue-dependent manner (Fig. 7D and Fig. S6.) Three representative descriptors were selected to demonstrate such tissue-dependent correlations (Fig. 6E). Autocorr2D descriptors describes molecular geometry by the distribution of atomic properties on the molecule topology with 192 dimensions (6 atomic properties and up to 24 topological distances)⁶⁴. AUTOCORR2D_95 had good linear relation with K_p in fatpad ($R^2 = 0.95$), but not with K_p in the tumor ($R^2 = 0.66$) or bone ($R^2 = 0.23$). AUTOCORR2D_36 was correlated well with K_p in tumor ($R^2 = 0.96$), but not K_p in fatpad or bone. AUTOCORR2D_43 was correlated well with K_p in bone ($R^2 = 0.98$), but not K_p in fatpad or tumors. It is worth noting that these analyses are preliminary due to the limited number of compounds, and more comprehensive descriptors can be inferred from these 2D or 3D descriptors to better predict the relationship between molecule structure descriptors and drug exposure in the tissues using data from more compounds in the future^{65,66}. More studies

need to be performed to dissect these Autocorr2D descriptors so that chemists can use to design new modifications in drug optimization.

4. Discussion

For drug optimization process, an ideal lead drug candidate for advancing to clinical studies should have two properties: (1) it has high specificity and potency against molecular target with low K_i (or low IC_{50}) but without nonspecific binding to other irrelevant molecular targets, where SAR has been well performed in drug optimization process; (2) it has high tissue exposure/selectivity in disease-targeted tissues to exert efficacy, but low tissue exposure/selectivity in healthy tissues to reduce toxicity, where the STR has been overlooked during drug optimization process⁴. However, the drug exposure in the plasma is often used as a surrogate of drug exposure in the disease-targeted tissues for efficacy. Drug candidates with high plasma exposure are often selected for further clinical development while those with low plasma exposure are often terminated in early drug discovery process. These selection strategies may be only applicable for some drug candidates but not for others^{4,8}. In fact, drug exposure in the plasma is determined by both elimination (clearance) and distribution into different organs⁶⁷. As a result, drug candidates with high exposure in the plasma (with good oral bioavailability) may be resulted from either low elimination that is preferred, or low tissue distribution which is not

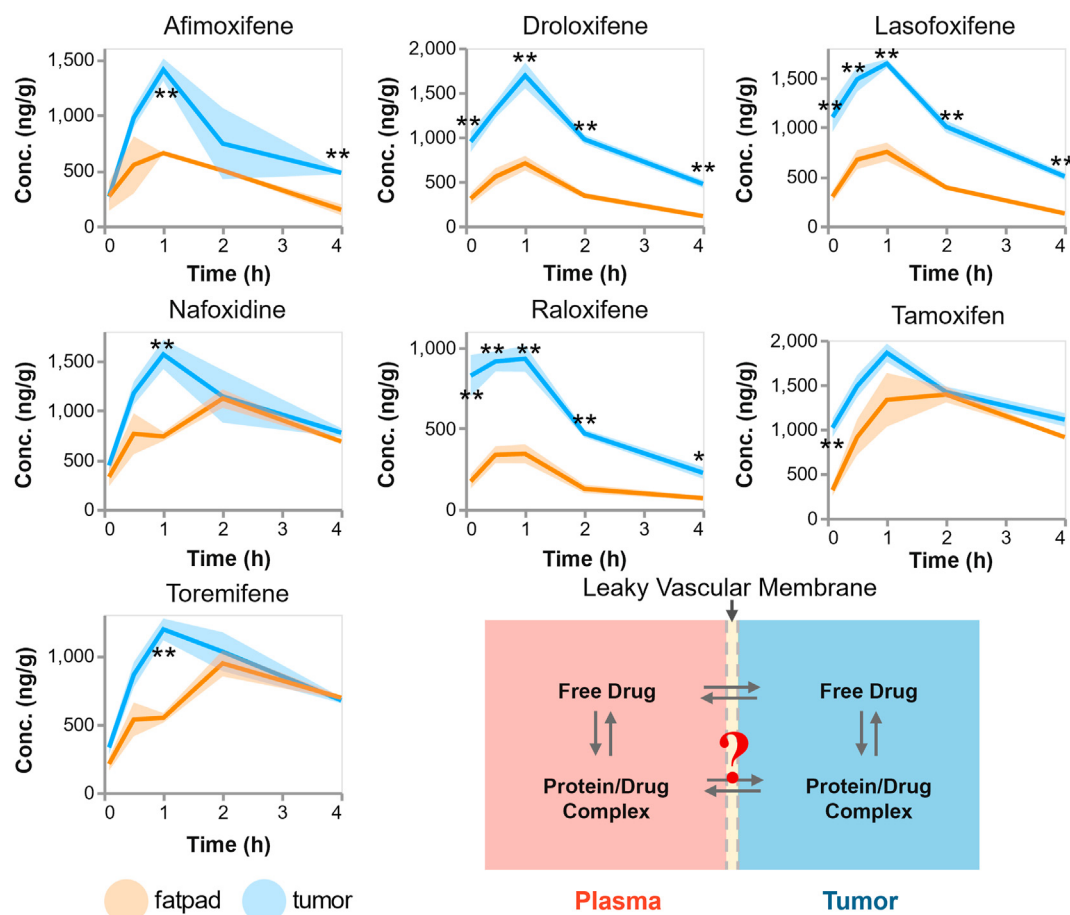


Figure 6 An enhanced accumulation of seven SERMs in tumors compared to normal fat pad tissue. (A–G) Concentration–time curve of tumor and fatpad (normal tissue surrounding tumors) after i.v. administration of seven SERMs (2.5 mg/kg) on MMTV–PyMT transgenic mice with spontaneous breast cancer ($n = 3$ at each time point); (H) An illustration of mechanism of enhanced tumor accumulation. Tumor vascular are abnormal and leaky compared to vascular in normal tissue, which allows more protein/drug complex enters to tumors resulting an enhanced drug accumulation. Data were represented as mean \pm SEM ($n = 3$ per time point, two-tailed t -test). Asterisks indicate the following P -value: * $P < 0.05$, ** $P < 0.01$.

preferred, since it may result in low concentration in the disease target tissues. Those drug candidates with high plasma exposure (good oral bioavailability) but low tissue exposure may have low organ toxicity even with high maximum tolerated dose (MTD), but it may also lack adequate efficacy. In such case, high dose is often required to achieve efficacy in clinical trials, that may lead to more adverse events in the blood cells or some other vital organs⁵⁴. In contrast, low drug exposure in the plasma may be due to either high elimination (and low bioavailability) that is not preferred and often eliminated correctly; or high tissue distribution (with reasonable oral bioavailability) that may be preferred depends on the tissue exposure. If drug candidates have good *in vivo* stability and relatively good bioavailability, their low plasma exposure may be determined by high tissue distribution, which is beneficial to achieve better efficacy at low dose. However, large proportion of these types of compounds are often mistakenly terminated before they could be advanced to clinical trials.

In this paper, we studied seven SERMs with similar or different structures as an example to show that drug exposure in the plasma was not correlated with drug exposure/selectivity in the disease-targeted tissues (tumor, fatpad and bone) for most compounds (Fig. 1). Although some compounds (such as tamoxifen and raloxifene) had similar exposure in the plasma, their exposure/

selectivity in the tissues were very different that were associated with their clinical efficacy/toxicity profiles (Figs. 2 and 3). Therefore, using drug exposure in plasma to select lead compounds to advance to clinical studies may significantly contribute to the high failure rate (90%) in drug development in addition to the challenges in target validation⁴. For the validated molecular targets, the lack of efficacy in clinical failure may be due to the lack of drug exposure/selectivity in the disease target tissue, while unmanageable toxicity in clinical studies may be due to the high drug exposure/selectivity in the healthy vital organs if the drug candidates have very reasonable potency and specificity to their molecular targets⁴. However, there is no technology to directly investigate tissue exposure/selectivity in clinical patients, while tissue exposure/selectivity in preclinical model is often overlooked due to labor intensive nature of these types of studies.

In this study, we highlighted three important PK parameters for studying STR (drug exposure, partition coefficient K_p , and drug selectivity in tissues), which were closely associated with drug efficacy/toxicity. Drug exposure (AUC_{tissue}) and drug partition coefficient (K_p) in the tissues determined the amount of drug accumulated in certain tissue (Table 1), while drug tissue selectivity may impact the drug's therapeutic window between efficacy and toxicity (Fig. 3).

Table 2 Physicochemical properties among SERMs with similar structures. The source of data comes from Drugbank database (go.drugbank.com).

Parameter	Tamoxifen	Toremifene	Droloxifene	Afimoxifene	Lasofloxifene	Nafoxidine
LogP	5.93	5.65	5.43	5.44	6.36	6.36
LogS	-5.6	-6	-5.1	-5.1	-6	-5.91
pK _a	8.76	8.76	8.49	8.66	8.98	8.95
TPSA	12.47	12.47	32.7	32.7	32.7	21.7
Protein binding	98%	92%	N/A	99%	99%	N/A
MW	371.524	405.969	387.523	387.523	413.561	425.572

N/A: not available.

In addition, we observed that the seven SERMs have higher accumulation in tumors compared to normal surrounding tissues (fatpad)³², which is likely either due to the tumor EPR effect of protein-bound drugs, or drug unique binding to tumor tissues, which requires further investigation (Fig. 6 H)^{28–33}. It is well accepted that protein larger than 40 KD have higher accumulation in tumors by EPR effect than in normal tissues⁶⁸. Serum albumin (68 KD), the major plasma protein that binds to drugs, has strong EPR effect to be preferentially accumulated in tumors as observed with radioactive labeling albumin⁶⁹. The ability of albumin carrying small molecular cargo for long circulation and enhanced tumor accumulation was discovered by injection of a fluorescent dye Evan blue with a high binding affinity to albumin in mice^{69,70}. Based on these finding, various of small molecular albumin-binding prodrugs were designed for binding to serum albumin for better tumor delivery^{71–73} such as an albumin-binding prodrug of doxorubicin, *i.e.*, the (6-maleimido) caproylhydrazone derivative of doxorubicin (DOXO-EMCH)⁷⁴, albumin binding drugs or imaging probes. These small molecules bind to albumin with a low micromolar affinity and have enhanced tumor accumulation by tumor EPR effect compared to normal tissues^{75–78}. Most anticancer small molecular drugs have high protein binding (>90%) with micromolar binding affinity. Indeed, we observed the seven SERMs have enhanced tumor accumulation compared to surrounding normal tissues. This phenomena were also observed for doxorubicin and Evans blue³². Both drug protein binding levels and binding affinity to albumin may affect the tumor accumulation of the drugs. The protein binding levels are determined by percentage of drugs binding to albumin, while the binding affinity of drugs to albumin is determined by binding constant (K). If the compounds have higher K , it may have higher retention in tumor. For instance, the plasma protein binding level of Evan blue is 68%⁷⁹ but with high binding affinity to albumin (K of $0.4\text{--}0.39 \times 10^6$ mol/L)⁸⁰. In comparison, drug molecules may have variable binding levels and binding affinity K . The binding affinity to albumin of tamoxifen and doxorubicin were 1.8×10^4 and 7.50×10^3 mol/L^{81,82}, respectively. The tighter binding of Evans blue to albumin results in higher accumulation in the tumors with much longer retention compared to small molecular drugs⁶⁹. The binding levels (>90%) and binding affinity (1.8×10^4) of seven SERMs would enhance drug accumulation in the tumors. It is also worth noting that drug binding to the know or unknown targets in tumor tissues may also enhance tumor drug accumulation in tumors that required further studies. Clearly, the “free drug hypothesis,” which proposes only free drugs are able to distribute to tissues for their pharmacological effect, may not be accurate for anticancer drugs with EPR effect in tumor tissues.

“Free drug hypothesis” may not be accurate even in the normal organs since it also completely ignored the active transport of

plasma proteins themselves that contribute the drug exposure/selectivity in all tissues. It has been shown that transport of both free unbound drugs and protein-bound drugs are presented in normal tissues and disease-targeted tissues^{10,22,23}. Previous study found that albumin-bound small molecules (tyrosine kinase inhibitors), interacting with albumin-binding proteins on vascular and in tissues, mediate tissue accumulation of these small molecules in normal tissues and are associated with their toxicity¹⁷. In addition, the translational pharmacokinetics and pharmacodynamic (PK/PD) models also suggest that total drug levels were preferred over free drug level in PK/PD relationships of many drugs^{19,34}. It is important to recognize that only free drug can interact with its molecular targets for pharmacological effect and the free drug concentration in the disease targeted tissues is important. However, the current method to estimate free drug fraction in tissue (f_u) using tissue homogenates, which destroys all subcellular structures, does not truly represent free drug concentration at the site of action^{13,23}. The overemphasis of free drug concentration in the plasma may mislead drug optimization process and lead drug candidate selection. We propose to use total drug tissue exposure or K_p (total drug in tissue/plasma ratio) to select drug candidate in drug optimization^{4,24–34}.

Interestingly, drugs inhibiting the same target are often used in different clinical indications. Evaluation of drug exposure in the tissue may provide guidance to select which indications should be studied in clinical trial. For instance, tamoxifen and raloxifene both target to estrogen receptor, but tamoxifen is used for breast cancer treatment⁴⁰, while raloxifene is approved for osteoporosis and breast cancer prevention⁴¹. Raloxifene was initially developed for breast cancer treatment but failed in clinical trial⁸³, and it was later tested and approved to treat osteoporosis⁴¹. Raloxifene showed more potent cytotoxicity against breast cancer cell lines compared to tamoxifen^{47,84} and shared a very similar plasma exposure to tamoxifen, which provided justification for raloxifene to be evaluated to treat breast cancer. However, the raloxifene exposure in the tumor and fatpad is 4- and 9-fold lower than that of tamoxifen, while raloxifene selectivity in the tumor and fatpad is also 3- and 1.5-fold less than that of tamoxifen (Fig. 2A). Although dose of raloxifene was 3-fold higher than tamoxifen, raloxifene did not achieve the similar concentration in tumor and fatpad as compared to tamoxifen. This may at least partially explain the poor efficacy of raloxifene in treating breast cancer despite its high *in vitro* potency. The higher potency of active metabolite (4-hydroxy tamoxifen) of tamoxifen along cannot fully explain the superior efficacy of tamoxifen in breast cancer patients since 4-hydroxy tamoxifen and raloxifene have similar potency. If drug exposure and selectivity in tissues were considered in early development of raloxifene, the clinical failure of raloxifene in breast cancer patients may be avoided. Similarly, lasofloxifene was

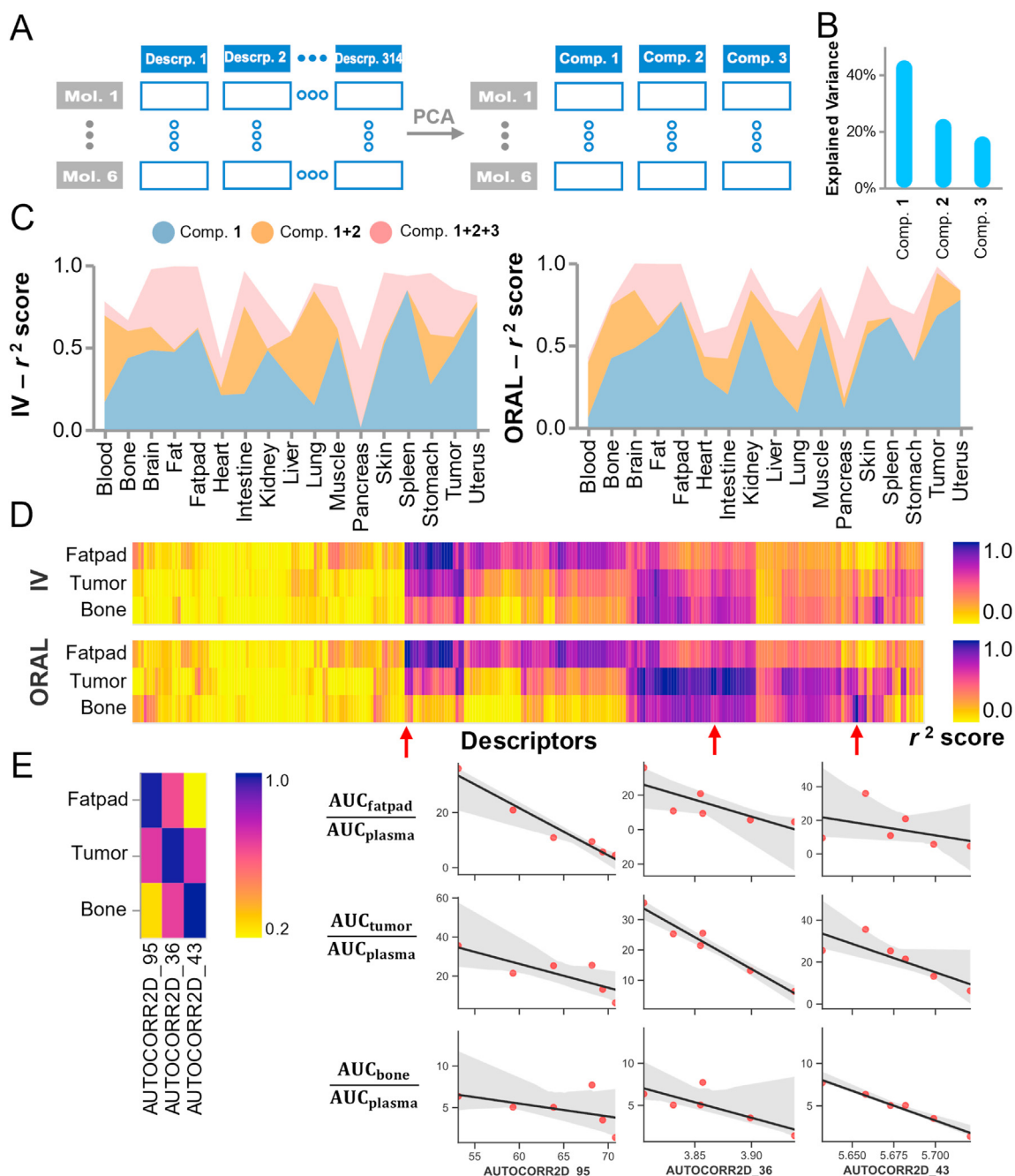


Figure 7 Molecular structure descriptors influence drug exposure in tissues. (A) PCA analysis to decompose molecule structure descriptors to 3 components. (B) Percentage of molecule property variance explained by components. (C) Ordinary least squares analysis of drug's partition coefficient in tissue (represented by $K_p = AUC_{\text{tissue}}/AUC_{\text{plasma}}$) against molecule structure (represented by components) for both *i.v.* and oral data. (D) Univariate feature analysis of collected descriptors (clustered) in target tissues including fatpad, tumor and bone. (E) Representative descriptors selected from (D, oral) to explain the difference of properties correlated to different tissues.

approved in Europe for osteoporosis treatment in postmenopausal women with increased risk of fracture. Lasofoxifene are currently being investigated in a phase II trial for ER^+ , $ESR1$ -mutant metastatic breast cancer and granted fast track designation by FDA⁸⁵. The clinical investigation of lasofoxifene was based on its ability as an antagonist of $ER\alpha$ with Y537S mutations, its long half-life and good bioavailability, as well as better anti-tumor activity in mouse xenograft models of endocrine therapy-resistant breast cancer compared to fulvestrant. However, our data showed that the

exposure of lasofoxifene in tumor and fatpad is 3.6- and 8.5-fold lower than that of tamoxifen despite a similar plasma exposure (Table 1)⁸⁶. Thus, the clinical dose regimen of lasofoxifen should be carefully adjusted to consider the drug exposure in the fatpad/tumor rather than in plasma exposure for clinical efficacy in addition to its unique activity as an ER antagonist. Fortunately, drug exposure in disease-targeted tissues have been used for drug candidate selection in certain diseases areas, such as drugs targeting central nerve system (CNS)^{10,22,23}. The drug level in brain

or in the cerebrospinal fluid (CSF) have always been measured and considered as a selection criterion for CNS drug optimization^{23–27}. Any drug candidate with no ability to reach brain would be terminated for further development⁴. However, drug optimization in other therapeutic areas rarely adapt a criterion to ensure drug exposure in the disease-targeted organs vs. normal organs^{8,10}. For anticancer drug discovery, target engagement is indeed often assessed in xenograft model, or occasionally in human tumor resection^{28–33,87–90}. However, how STR in the tumors vs. normal tissues is rarely assessed. In some clinical trials of anticancer drugs, the target engagement is often investigated in peripheral blood mononuclear cells (PBMC) because of easy access. However, we caution that target engagement in PBMC may be also misleading since target engagement in the PBMC may not reflect the target engagement in the solid tumors^{32,91}.

Given the importance of STR in relationship to efficacy/toxicity, it is ideal to use STR in rational drug design in drug optimization process. However, STR is currently poor understood with limited data available on very few compounds. Understanding how structure properties related to specific tissue accumulation requires more data gathering in tissue accumulation from hundreds and thousands of compounds. As a preliminary testing, we used univariate feature analysis to study STR as a proof of concept. However, due to the limited data number, we were only able to test if K_p is correlated with different molecular properties dependent on tissues. The correlation of structure descriptors (AUTOCORR2D_95, AUTOCORR2D_43, AUTOCORR2D_36) with fatpad, tumor and bone were observed in these seven SERMs, which may be challenging to extend the findings to other scaffolds. These preliminary studies was used to test the method feasibility, while larger data sets are required for future STR studies, so that it can be used in rational drug design in the future^{4,92–95}.

It is worth noting the tissue exposure/selectivity was measured in preclinical mouse models, which was used to correlate clinical efficacy/toxicity. It is possible that tissues exposure/selectivity in animals may be different from human, and thus it should be cautious for extrapolation between STR in animals and clinical efficacy/toxicity in human. Therefore, it is useful if non-invasive imaging technology can be developed in human to evaluate STR using tissue exposure/selectivity in human directly in the future. In addition, it is equally important to optimize both drug potency/specificity in inhibiting its molecular target by SAR and drug's tissue exposure/selectivity in disease targeted tissues vs. normal tissues by STR. The optimal balance of structure–tissue exposure/selectivity–activity relationship (STAR) is required for lead drug candidate selection and drug optimization⁴, to improve success rate of clinical drug development. Further, target validation, which confirms the molecular target is the cause of the disease and drug's intended target, is also critical for any drug discovery program. Improvement of drug optimization using STAR and rigorous target validation will significantly improve the success of clinical drug development⁴.

5. Conclusions

The failure rate of current clinical drug development remains to be high (90%) despite of significant effort to optimize each step of drug development process. The suboptimal target validation or drug optimization may be two major factors that contribute to high failure rate. Although drug potency/specificity (by SAR) in inhibiting its target and drug-like properties are rigorously

optimized to select the best lead drug candidate during drug optimization process, STR is overlooked in drug optimization process, which may mislead the drug candidate selection and impact the balance of clinical efficacy/toxicity.

In this study, we investigated the STR to correlate with observed clinical efficacy/toxicity profiles of a series of clinical approved or tested drug candidates (seven selective estrogen receptor modulators, SERMs). These studied drugs have similar or slightly different structures, same molecular target, similar or different PK profiles in plasma and tissues. The results showed that drug exposure in plasma of seven SERMs was not correlated with drug exposure in the disease-target tissues (tumor, fatpad and bone) in most cases. Drug exposure in the target tissues (tumor, fatpad, bone, and uterus), not in the plasma, was correlated with drug clinical efficacy/toxicity. In addition, slight structure modifications of four SERMs, which did not change plasma drug exposure, significantly altered drug exposure and selectivity in various tissues. Further, seven SERMs showed higher tumor accumulation compared to surrounding normal tissues, which is likely due to tumor EPR effect of protein-bound drugs. Finally, principal component analysis (PCA) and ordinary least squares (OLS) model showed decomposed molecular descriptor components may distinguish STR. These data suggest that STR alters drug's tissue exposure/selectivity in disease-targeted tissues vs. normal tissues impacting clinical efficacy/toxicity. Drug optimization needs to balance the SAR and STR in selecting drug candidate for clinical trial to improve success of clinical drug development.

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Author contributions

Lipeng Dai, Miao He, Hebao Yuan, Huixia Zhang, Jinhui Liao, Bo Wen performed experiments and analyze the data. Hongxiang Hu and Wei Gao analyze the data and write the manuscript. Yan Li, Maria Palmisano, Mohamed Dit Mady Traore revised the idea and manuscript. Wei Gao, Simon Zhou and Duxin Sun conceived the idea and write the manuscript. All of the authors have read and approved the final manuscript.

Conflicts of interest

Yan Li, Maria Palmisano, and Simon Zhou were formerly employee of Celgene Corporation (now it is part of Bristol Myers Squibb). The authors have no conflicts of interest to declare.

Appendix A. Supporting information

Supporting data to this article can be found online at <https://doi.org/10.1016/j.apsb.2022.02.015>.

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