Enhancing the Oral Absorption of Kinase Inhibitors Using Lipophilic Salts and Lipid-Based Formulations

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Supporting Information

ABSTRACT: The absolute bioavailability of many small molecule kinase inhibitors (smKIs) is low. The reasons for low bioavailability are multifaceted and include constraints due to first pass metabolism and poor absorption. For smKIs where absorption limits oral bioavailability, low aqueous solubility and high lipophilicity, often in combination with high-dose requirements have been implicated in low and variable absorption, food-effects, and absorption-related drug–drug interactions. The current study has evaluated whether preparation of smKIs as lipophilic salts/ionic liquids in combination with coadministration with lipid-based formulations is able to enhance absorption for examples of this compound class. Lipophilic (docusate) salt forms of erlotinib, gefitinib, ceritinib, and cabozantinib (as example smKIs demonstrating low aqueous solubility and high lipophilicity) were prepared and isolated as workable powder solids. In each case, the lipophilic salt exhibited high and significantly enhanced solubility in lipidic excipients (>100 mg/g) when compared to the free base or commercial salt form. Isolation as the lipophilic salt facilitated smKI loading in model lipid-based formulations at high concentration, increased solubilization at gastric and intestinal pH and in some cases increased oral absorption (~2-fold for cabozantinib formulations in rats). Application of a lipophilic salt approach can therefore facilitate the use of lipid-based formulations for examples of the smKI compound class where low solubility limits absorption and is a risk factor for increased variability due to food-effects.

KEYWORDS: kinase inhibitors, lipophilic salts, lipid-based formulations, drug delivery, poorly water-soluble drug, ionic liquids, drug absorption, SEDDS, Biopharmaceutical Classification System

INTRODUCTION

Small molecule kinase inhibitors (smKIs) are a class of drugs commonly used to target specific kinase-mediated signaling pathways that are involved with, and perturbed in, a number of disease states. In cancer therapy, smKIs have been used for over 15 years either alone or in combination with other therapies to inhibit kinase targets including epidermal growth factor receptor (EGFR), janus kinase (JAK), vascular endothelial growth factor receptor 2 (VEGFR-2), and anaplastic lymphoma kinase (ALK). smKIs have also been developed to treat other conditions in diseases such as rheumatoid arthritis (e.g., tofacitinib (Xeljanz)) and myelofibrosis (ruxolitinib (Jakavi)). In clinical studies, smKIs have demonstrated positive outcomes in autoimmune diseases such as psoriasis, diabetes, and inflammatory bowel disease.

Since the launch of imatinib (Gleevec) in 2001, a total of 41 smKIs have been approved by the FDA, with annual sales now exceeding US$ 30 billion. Ongoing development activity in this area is evidenced by the seven new smKIs that were FDA approved last year for oral delivery (~15% of all 2017 novel drug approvals), namely acalabrutinib (Calquence), abemaciclib (Verzenio), copanlisib (Aliqopa), ribociclib (Kisqali), and...
neratinib (Nerlynx), brigatinib (Alunbrig), and enasidenib (Idhifa), with many more undergoing clinical development.

Despite wide application, many smKIs on the market and in development show low bioavailability after oral administration.\textsuperscript{10−13} This may reflect high first pass metabolism or low absorption, or both. Within the compound class, highly lipophilic and poorly water-soluble molecules are over-represented and the potential for solubility limited absorption\textsuperscript{10} and altered pharmacokinetics when administered with food\textsuperscript{14−20} has been well described. As the majority of smKIs are weakly basic with significant pH-dependent solubility, coadministration of smKI with agents that reduce gastric pH, including antacids, H\textsubscript{2} antagonists, and proton pump inhibitors (which are commonly prescribed in cancer therapy), can also interfere with smKI absorption.\textsuperscript{21−25} These factors illustrate the biopharmaceutical complexity of this compound class and the potential risk of adverse effects or poorer therapeutic outcomes when drug absorption is significantly affected by food or coadministration with other medicines.

Members of the smKI class are therefore good candidates for solubility enhancing formulation approaches that have the potential to reduce the problems associated with low drug absorption.\textsuperscript{26−28} However, smKIs are also a good example of a compound class that provides several physicochemical and product challenges to effective formulation including high melting temperatures (i.e., above 200 °C), high dose (i.e., above 100 mg), and low aqueous solubility (i.e., < 10 μg/mL). In combination, these properties may restrict the ability to apply common enabling approaches to increase oral absorption. For example, a high melting point can increase the propensity for drug recrystallization from amorphous solid dispersions, and for lipid-based formulations, the combination of a high melting point and high dose magnifies the challenge to deliver the dose in one or two dosage units.

In addition, nearly all approved smKIs were granted FDA Fast Track, Breakthrough Therapy, or Accelerated Approval status; this commonly reduces drug development timelines from 10−15 years to less than 5 years.\textsuperscript{29} While obviously beneficial to patients, accelerated product development timelines place a strain on formulators to produce finished formulations that meet stability and manufacturability requirements. This, and the fact that in oncology, patients are used as early as Phase I for clinical evaluation, can also translate to significantly attenuated development times. Compromises in the biopharmaceutical properties of the formulation, and limitations to the number of formulation options that can be explored, may therefore be necessary to ensure that a drug can rapidly enter and progress through clinical evaluation.\textsuperscript{30}

Against this backdrop, there is ongoing interest in the identification of alternate delivery technology solutions for compound classes such as smKIs, where high lipophilicity and high melting points often limit solubility.

We and others have recently shown that converting drugs to lipophilic salt forms can enhance drug solubility (and loading) in lipid-based formulations, in some cases by over 10-fold. This facilitates the application of a proven formulation approach that can otherwise be limited by low drug loading in the formulation. The combination of lipophilic salts with lipid-based formulations has also been shown previously to support significant increases in in vivo drug exposure.\textsuperscript{31−33} The increase in drug loading also minimizes the total quantity of formulation required.

For lipophilic salts, the enhancement in lipid solubility stems primarily from a reduction in intermolecular crystalline forces in the lipophilic salt complex, with the potential to isolate salts as viscous oils, amorphous solids, or low melting point (e.g., < 150 °C) crystalline solids depending on the lipophilic counterion and the intrinsic drug properties. On the basis of this increase in lipid solubility, it becomes increasingly possible to achieve high drug loadings in lipid-based formulations, for example, above 100 mg/g (10% wt.) and higher.

The current study describes the preparation of new lipophilic salts of smKIs. smKIs were chosen as an example of a class of compounds with low solubility, high lipophilicity, and high melting point, and therefore the potential for low and solubility-limited absorption (realizing that not all smKIs show these properties). The overall objective of this approach was to convert the drug form into one that is more amenable to delivery in lipid-based formulations. In turn, transformation into a lipid-based formulation was expected to enhance
absorption after oral administration for molecules where solubility or dissolution rate are the primary limitations to absorption. Four exemplar smKIs were investigated and are shown in Figure 1. These include erlotinib, gefitinib, cabozantinib, and ceritinib. These four marketed smKIs were chosen to provide a range of pKw, melting point, and log P values to exemplify the utility of the approach for both marketed compounds and potentially for similar smKIs that are currently under development. Greater focus was placed on erlotinib and cabozantinib since their respective commercial products (Tarceva and Cometriq) exhibit increased exposure when taken with food or stomach acid reducing drugs, increasing the likelihood that drug absorption may be limited by low drug solubility. Docusate (dioctyl sulfosuccinate) was utilized as the salt counterion based on low toxicity and precedence in foods and pharmaceutical products.

Lipophilic salt forms of all four smKIs demonstrated significant enhancements in lipid solubility in a number of different lipidic excipients and lipid-based formulations, which resulted in substantial increases in drug loading in concept formulations. Subsequent in vivo studies exploring lipid-based formulations of erlotinib and cabozantinib lipophilic salts indicated that the combined use of lipophilic salts and lipid-based formulations has the potential to lead to increased oral absorption.

### EXPERIMENTAL SECTION

**Materials.** Erlotinib free base and hydrochloride salt and gefitinib free base were purchased from Scientific (Cheltenham, Victoria, Australia). Cabozantinib and ceritinib free base were purchased from Shanghai PI Chemicals Ltd. (Shanghai, China). Propylene glycol monocaprylate (Capryol 90), propylene glycol monolaurate (Lauroglycol 90), propylene glycol dicaprate (Labrafac PG), glyceryl monolinoleate (Maisine 35–1), and PEG-8 caprylic/capric glycerides (Labrasol) were donated by Gattefosse (Saint-Priest, France). Long-chain triglyceride (super refined corn oil) was a sample from Croda (Edison, NJ, USA). Medium-chain triglyceride (Miglyol 812) and glycerol monolaurate (Immitor 308) were kindly supplied by Cremer (Hamburg, Germany). Polyoxy-35 castor oil (Kolliphor EL) and PEG-15 hydroxystearate (Kolliphor HS-15) were donated by BASF (Ludwigshafen, Germany). Dioctyl sulfosuccinate sodium salt (sodium docusate, 98%), sodium lauryl sulfate (>99%), sodium taurodeoxycholate (>95%), propylene glycol, sodium carboxymethylcellulose, porcine pancreatin extract (P7545, 8 × USP specifications activity), polysorbate 80 (Tween 80), and 4-bromophenylboronic acid were purchased from Sigma–Aldrich (Castle Hill, New South Wales, Australia). Phosphatidylcholine (PC) (Lipoid E PC S, approximately 99.2% pure, from egg yolk) was obtained from Lipoid (Lipoid GmbH, Ludwigshafen, Germany). Acetonitrile, ethanol, petroleum spirit, chloroform, dichloromethane, methanol, ethyl acetate, and diethyl ether were from Merck (Bayswater, Victoria, Australia) and used without any pretreatment. All other chemicals and solvents were of analytical purity or high performance liquid chromatography (HPLC) grade.

**Lipophilic Salt Preparation: Erlotinib Docusate.** Erlotinib hydrochloride (10.0 g, 23.3 mmol) and sodium docusate (10.4 g, 23.3 mmol) were dissolved in a biphasic solution of dichloromethane (250 mL) and distilled water (250 mL) and vigorously stirred overnight at ambient temperature. The aqueous layer was collected and washed with dichloromethane (2 × 200 mL), and the combined organics were backwashed with 50 mL portions of cold distilled water until negative to a silver nitrate precipitate test. The organic solution was dried (Na2SO4), filtered, and concentrated in vacuo over several hours at 700 mbar. The material crystallized on cooling to give a white powder (18.63 g, yield = 98%).

**Lipophilic Salt Preparation: Gefitinib Docusate.** Gefitinib docusate was formed using the standard metathesis procedure using preformed HCl salt and sodium docusate (Method A). In addition, a second method (Method B) was employed to determine if it was possible to form the lipophilic salt directly from the commercially available free base form using in situ HCl salt formation followed by metathesis. This was successful, and no difference was observed in the material formed from the free base or preformed HCl salt.

**Method A.** Gefitinib hydrochloride (206 mg, 0.43 mmol) and sodium docusate (189 mg, 0.43 mmol) were dissolved in a biphasic solution of dichloromethane (10 mL) and distilled water (10 mL) and stirred overnight at ambient temperature. The aqueous layer was collected and washed with dichloromethane (2 × 10 mL) and the combined organics were washed with 5 mL portions of cold distilled water until a negative silver nitrate (0.02 M) precipitate test was obtained. The organic solution was dried (Na2SO4), filtered, and concentrated in vacuo to give a residue which was crystallized from ether to give a white powder (360 mg, yield = 96%).

**Lipophilic Salt Preparation: Cabozantinib Docusate.** Cabozantinib hydrochloride (3.03 g, 5.62 mmol) and sodium docusate (2.50 g, 5.62 mmol) were dissolved in a biphasic solution of ethyl acetate (100 mL) distilled water (50 mL) and stirred overnight at ambient temperature. The aqueous layer was collected and washed with dichloromethane (2 × 100 mL), and the combined organics were washed with 25 mL portions of cold distilled water until a negative silver nitrate (0.02 M) precipitate test was obtained. The organic solution was dried (Na2SO4), filtered, and concentrated in vacuo. The crude residue was triturated with ether to give the desired product as a white powder (6.75 g, yield = 97%).

**Lipophilic Salt Preparation: Ceritinib Docusate.** Ceritinib hydrochloride (1.00 g, 1.68 mmol) and sodium docusate (0.75 g, 1.68 mmol) were dissolved in a biphasic solution of ethyl acetate (60 mL) and distilled water (30 mL) and stirred for 4 h. The aqueous solution was collected and washed with ethyl acetate (2 × 50 mL) and the combined organics were washed with cold portions of distilled water (30 mL) until a negative silver nitrate (0.02 M) precipitate test was obtained. The resulting solution was dried (Na2SO4), filtered, and concentrated in vacuo to give a white solid that was recrystallized from ethyl acetate/petroleum spirits (vapor diffusion) to give the desired product as a yellow powder (1.58 g, yield = 96%).

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Nuclear Magnetic Resonance (NMR) Spectroscopy. NMR spectra were obtained using a Bruker Avance III Nanobay 400 MHz NMR spectrometer at 400.13 and 100.62 MHz, respectively, coupled to the BACS 60 automatic sample changer. The spectrometer was equipped with a 5 mm PABBO BB-1H/D Z- GRD probe. The $^1$H and $^{13}$C NMR spectra of the purified products were recorded in MeOH-d$_4$ (Aldrich, 99.8% D) or DMSO-d$_6$ (Cambridge Isotope Laboratories Inc., 99.9% D).

Liquid Chromatography—Mass Spectrometry (LC—MS). LC—MS chromatograms were obtained using an Agilent 1200 Series HPLC/Agilent 6100 Series Single Quad LC—MS (pump, 1200 Series G1311A quaternary pump; autosampler, 1200 Series G1329A thermostated autosampler; detector, 1200 Series G1314B variable wavelength detector; software, LC—MSD ChemStation Rev.B.04.01 SP1 with Easy Access Software) and a Luna C8(2) 5 μm 50 × 4.6 mm$^2$ 100 Å column at 30 °C (injection volume, 5 μL; solvent A, water 0.1% formic acid; solvent B, acetonitrile 0.1% formic acid; gradient, 5—100% B over 10 min) with detection at 254 or 214 nm. The mass spectrometer was operated in quadrupole ion mode (Multimode-ES Drying gas temp, 300 °C; vaporizer temperature, 200 °C; capillary voltage (V), 2000 (positive); capillary voltage (V), 4000 (negative); scan range, 100–1000; step size, 0.1 s) with acquisition time of 10 min.

High-Resolution Mass Spectrometry. All analyses were performed on an Agilent 6224 TOF LC—MS Mass Spectrometer coupled to an Agilent 1290 Infinity HPLC (Agilent, Palo Alto, CA) fitted with an Agilent Zorbax SB-C18 Rapid Resolution HT 2.1 × 50 mm$^2$, 1.8 μm column (Agilent Technologies, Palo Alto, CA) using an acetonitrile (A)/water containing 0.1% (w/v) formic acid (B) gradient (5—100% (v/v) B) over 3.5 min at 0.5 mL/min. All data were acquired and reference mass corrected via a dual-spray electrospray ionization (ESI) source. Each scan or data point on the Total Ion Chromatogram (TIC) is an average of 13700 transients, producing a spectrum every second. Mass spectra were created by averaging the scans across each peak and background subtracted against the first 10 s of the TIC. Acquisition was performed using the Agilent Mass Hunter Data Acquisition software (v.B.05.00, Build 5.0.5042.2), and analysis was performed using Mass Hunter Qualitative Analysis (v.B.05.00 Build 5.0.519.13). The mass spectrometer was operated in ionization mode (electrospray ionization drying gas flow, 11 L/min; nebulizer, 45 psi; drying gas temperature, 325 °C; capillary voltage (Vcap), 4000 V; fragmentor, 160 V; skimmer, 65 V; OCT RFV, 750 V; scan range acquired, 100—1500 m/z; internal reference ions, positive Ion Mode = m/z = 121.050873 and 922.009798).

Elemental Analysis. Elemental analysis performed by the Macquarie University Chemical Analysis Facility — EA Service utilizing an Elemental Analyzer, Model PE2400 CHNS/O (PerkinElmer, Shelton, CT, USA).

Full characterization of all the novel lipophilic salts is provided in the Supporting Information.

Polarized Light Microscopy. Samples were mounted on glass microscope slides with a small drop of silicone or mineral oil where it was necessary to facilitate dispersion of powders. Samples were analyzed using a Zeiss AxioLab microscope (Carl Zeiss AG, Oberkochen, Germany) equipped with crossed polarizing filters and digital camera (Canon PowerShot A70, Tokyo, Japan). Images were collected at 10X, 20X, or 32X magnification using remote capture software (Canon Utilities v.2.7.2.16).

X-ray Powder Diffraction (XRPD). XRPD was undertaken using a Shimadzu XRD—7000 diffractometer (Shimadzu Scientific Instruments, Japan) with Cu Kα radiation. The applied voltage and current were 40 kV and 35 mA, respectively. Samples were mounted on stainless steel sample holders and were scanned in continuous mode between 2–40° (2θ), with a step size of 0.02°, a scanning speed of 2°/min. A divergent slit width of 1° was employed for the beam source, and a scattering slit width of 1° and receiving slit width of 0.3 mm were used for the detector.

FT-Infrared Spectroscopy. Spectroscopy measurements were performed using a Fourier Transform Infrared (FTIR) spectrophotometer (Frontier, PerkinElmer, Waltham, MA, USA) equipped with a diamond zinc selenide attenuated total reflection (ATR) unit. FTIR spectra were obtained in transmission mode from between 650 and 4000 cm$^{-1}$ at a 2 cm$^{-1}$ resolution and with an averaged 16 scans for background and each sample.

Raman Spectroscopy. Analysis employed a Raman microscope (Raman Micro 200, PerkinElmer, Waltham, MA, USA) operating at a 785 nm excitation wavelength, coupled with a cool charged coupled device (CCD) detector set at −50 °C and an Olympus BX51 microscope equipped with a 5× objective lens. Samples were mounted on a 76 × 25 mm$^2$ quartz slide (ProSciTech, Kirwan, Queensland, Australia) and positioned on a motorized stage controller capable of moving along x, y, and z-axes. Preliminary experiments indicated that the best Raman spectra were obtained using an exposure time between 10–15 s and 10 scans. The scan range was 500–3000 cm$^{-1}$, and the resolution was 4 cm$^{-1}$. Raw Raman spectra were analyzed using the Spectrum software (v.6.3, PerkinElmer). Baseline corrections were performed when needed.

Hot-Stage Microscopy. Samples were mounted between two glass-coverslips on a Linkam HFS 91 hot-stage connected to a TP93 temperature controller (Linkam Scientific Instruments, Tadworth, United Kingdom). Samples were heated at a rate of 10 °C/min and monitored continuously using a Zeiss AxioLab microscope (Carl Zeiss, Oberkochen, Germany) equipped with crossed polarizing filters and digital camera (Canon PowerShot A70, Tokyo, Japan). Images were collected on sample heating at 10X, 20X, or 32X magnification using remote capture software (Canon Utilities v.2.7.2.16). Complete melting was defined as the lowest temperature at which the sample was completely free of birefringence or, in the case of amorphous samples, the lowest temperature at which no solid structures were evident.

Differential Scanning Calorimetry (DSC). Approximately 1–5 mg of sample was directly weighed into aluminum pans that were hermetically sealed. DSC analysis was performed using a PerkinElmer DSC 8500 (Waltham, MA, USA) with Intracooler III integrated cooler. During the analysis, sample chambers were continually flushed under nitrogen (20 mL/min) and an empty aluminum pan was used to establish the baseline and as a reference control. Samples were analyzed in one heating cycle from −20 °C up to 275 °C at a heating rate of 10 °C/min. Glass transition temperatures (Tgs), if present, were calculated using the Pyris software (v.11.1.1.0497, PerkinElmer) and the half-height technique.

Lipid Excipient Kinetic Solubility Screening of smKIs. The kinetic solubility of free base, commercial salt, and lipophilic salt forms of the four smKIs were determined in
selected lipidic excipients. Known concentrations of the drug/salt in lipidic excipients were ultrasonicated (Elmasonic TI-H, Singen, Germany) at a 45 kHz frequency at 30 °C for 15 min intervals followed by analysis by polarized light microscopy to confirm whether the compound had completely dissolved.

**Lipid-Based Formulations.** Archetypical long-chain and medium-chain self-emulsifying drug delivery systems (SEDDS) were utilized in this study. The long-chain formulation (LC–SEDDS) consisted of 30% (w/w) long-chain triglyceride (corn oil), 30% glyceryl monolinoleate, 30% polyoxy 35 castor oil, and 10% ethanol. The medium-chain formulation (MC–SEDDS) consisted of 20% glyceryl monocaprylate, 40% propylene glycol monocaprylate, and 40% polyoxy 35 castor oil.

**smKI Lipophilic Salt Kinetic Solubility and Incorporation into Lipid-Based Formulations.** Placebo lipid-based formulation was first prepared by weighing the appropriate amount of each of the excipients into a glass vial, with prior excipient melting, if needed. Excipients were then mixed thoroughly to generate an isotropic solution.

The kinetic solubility of smKI and respective salts was assessed in these formulations in the same manner as described for performing the kinetic solubility tests in single lipidic excipients.

To prepare final formulations for *in vitro* and *in vivo* tests, the target mass of lipophilic salt was added to a glass vial followed by the placebo formulation and mixing at 30–40 °C until the drug was completely dissolved. Formulations were allowed to equilibrate at room temperature for at least 24 h prior to analysis.

**smKI Solubilization during *in Vitro* Performance Testing.** *In vitro* digestion experiments were performed as described previously by the LFCS Consortium but with some modifications to incorporate an initial dispersion step at acidic pH to capture the likely gastric solubilization of weakly basic drugs. In brief, the experimental setup consisted of a pH-stat apparatus (Metrohm AG, Herisau, Switzerland), comprising a Titrando 802 propeller stirrer/804 Ti Stand combination, a glass pH electrode (Biotrode), and dosing units coupled to 10 mL autoburets (800 Dosino). The apparatus was connected to a PC and operated using Tiamo v.2.0 software.

In the first step, the lipid based formulation (0.36 g) was dispersed in 6.5 mL of a 0.01 N HCl solution (pH 2) within a thermostat-jacketed glass reaction vessel (Metrohm) using an overhead propeller stirrer 15 mm in diameter rotating at ~450 min⁻¹. Samples (0.5 mL) were periodically removed and centrifuged (Heraeus Fresco 21, Thermo Scientific, Langen selbold, Germany) at 21 000g (37 °C) for 10 min. After centrifugation, an aliquot of the aqueous phase was removed and diluted with solvent and mobile phase prior to analysis by HPLC. Maximal solubility values were typically reached within the first 5 h of the solubility study since longer equilibration times resulted in some colloidal instability.

After the 24 h incubation, the pH (Biotrode, Metrohm) of the remaining aqueous medium was within the pH range of 6.5 ± 0.25 (not shown). Polarized light microscopy and Raman microscopy of the remaining solid phase indicated that there was no chemical modification of either erlotinib or cabozantinib free base during the 24 h equilibration period. In the case of the docusealates of erlotinib and cabozantinib, Raman analysis of crystalline precipitates indicated that this was predominantly the free base form of the drug.

**HPLC Drug Quantification.** All HPLC analysis were performed on an Alliance 2695 separation module and 486 tunable UV absorbance detector (Waters Instruments, Milford, MA, USA). Details of the respective methods can be found in Table S1 in the Supporting Information.

**Oral Bioavailability Studies.** All animal experiments were approved by the Monash Institute of Pharmaceutical Sciences Animal Ethics Committee and were conducted in accordance with the Australian and New Zealand Council for the Care of Animals in Research and Teaching guidelines. The exposure of erlotinib and cabozantinib after oral administration of the
formulations was examined in conscious male Sprague–Dawley rats (280 ± 30 g) with a CX-2012S catheter (BASi, West Lafayette, IN, USA) surgically inserted into the carotid artery. The rats were kept in Ramborn metabolic cages and blood sampling was conducted by a Culex automated blood sampler (ABS) (BASi, West Lafayette, IN, USA). Where animals received drug intravenously, cannulas were also inserted into the jugular vein as described previously.

Oral treatments were administered via oral gavage. Control formulations of erlotinib hydrochloride (commercial salt form) and cabozantinib (crystalline free base) were administered as suspensions in a standard aqueous vehicle consisting of 0.5% (w/v) sodium carboxymethylcellulose, 0.9% (w/v) sodium chloride and 0.4% (w/v) polysorbate 80. These suspensions were prepared via vortex-mixing the formulations (at room temperature) until a homogeneous dispersion of suspended drug was formed. Lipophilic salt forms of erlotinib and cabozantinib were administered as lipid-based formulations (between 31.25–250 mg of formulation (per rat) depending on the dose administered) that were dispersed in water immediately prior to administration. In the erlotinib study, rats received a dosing volume of 0.5 mL (of aqueous suspension or dispersed lipid-based formulation) immediately followed by further 0.5 mL of water. In the cabozantinib study, rats received a dosing volume of 1.0 mL as a single dose.

Intravenous administration of cabozantinib (for the purpose of calculating absolute oral bioavailability) was achieved via a 10 min constant rate infusion into an indwelling jugular vein cannula. Compound was formulated at 1.4 mg/mL (as free base equivalents) in a vehicle composed of 10% (v/v) DMSO, 40% (v/v) propylene glycol, and 1% (v/v) Kolliphor HS-15 in saline, giving a colorless solution of apparent pH 6.0. The formulation was filtered through a 0.22 μm sterile filter prior to dosing, and the concentration of cabozantinib in the filtered solution was determined via UPLC–MS/MS.

Blood samples (100 μL; collected into chilled borosilicate vials containing heparin as anticoagulant) were taken from the carotid artery cannula up to 24 h (for erlotinib) or 48–72 h (for cabozantinib) postdosing and centrifuged at 2250g for 5 min to separate plasma. During the blood collection period, the rats had free access to water at all times but remained fasted for a further 4–8 h following drug administration. At the end of the experiments, rats were euthanized by intracardiac administration of 0.5 mL of pentobarbital solution (100 mg/mL).

Plasma exposure parameters were determined via standard noncompartmental analysis, with areas under the plasma concentration–time curve from zero to designated time intervals (AUC_{0–t}) calculated using either the linear or linear up-log down trapezoidal method.

Preparation of Plasma Samples for HPLC–MS/MS Analysis. To determine the plasma concentrations of erlotinib, a 10 μL aliquot of plasma was spiked with 10 μL of an internal standard (IS) solution (1 μg/mL gefitinib in 50% methanol) and vortexed for 0.5 min. Plasma protein precipitation was completed by addition of 1 mL of methanol and vortex-mixing for 1 min. After centrifugation at 4500g for 5 min, 150 μL of the supernatant was subsequently transferred to vials for HPLC–MS/MS analysis.

For cabozantinib, a 50 μL aliquot of plasma was spiked with 10 μL of an internal standard (IS) solution (5 μg/mL diazepam in 50% acetonitrile). Plasma protein precipitation was completed by addition of 120 μL of acetonitrile and vortex-mixing for 20 s. After centrifugation at 4500g for 3 min, 150 μL of the supernatant was subsequently transferred to vials for HPLC–MS/MS analysis.

HPLC–MS/MS Analysis of Erlotinib in Plasma. A Shimadzu LC–MS 8050 system (Shimadzu Scientific Instruments, Kyoto, Japan) was used for analysis and consisted of a CBM-20A system controller, a DGU-20A, solvent degasser, two LC-30AD pumps, a SIL-30AC autosampler, a CT-20A column oven (held at 40 °C), and a triple quadrupole mass spectrometer with an electrospray ionization interface (ESI). The desolvation line (DL), the interface and the heat block were maintained at 250, 300, and 400 °C, respectively. The flow rate of the nebulizing gas, heating gas and drying gas were 3 L/min, 10 L/min, and 10 L/min, respectively. For each injection, 1.5 μL of sample was injected onto a C18 Ascentis Express column (50 mm × 2.1 mm; 2.7 μm, Supelco, Bellefonte, PA). The tray temperature in the autosampler was maintained at 15 °C. The mobile phase was a mixture of solvent A and B with a flow rate of 0.3 mL/min. Solvent A was milli-Q water with 0.1% formic acid, and solvent B was 100% (v/v) methanol with 0.1% formic acid. The gradient started with 30% of solvent B, linearly increased to 90% over 2 min, remained at 90% for 0.7 min, before returning to 30% over 0.5 min and equilibrated for 1.3 min until the next injection. The total run time was 4.5 min. Erlotinib was detected by ion transition m/z positive 394.20 to 278.10.

HPLC–MS/MS analysis of Cabozantinib in Plasma. A Waters Xevo TQS micro triple quadrupole mass spectrometer equipped with a Waters Acquity UPLC–MS (Waters Corporation, Milford, MA, USA) was used for analysis. Mass spectrometry was conducted in positive electrospray ionization mode (ESI) using source block and desolvation temperature of 120 and 650 °C, respectively, and desolvation and cone gas flow rate of 1000 L/h and 50 L/h, respectively. For each injection, 1.5 μL of sample was injected onto an Ascentis Express RP-Amide column (50 mm × 2.1 mm; 2.7 μm, Supelco, Bellefonte, PA, USA). The autosampler and column compartment was maintained at 10 and 40 °C, respectively. The mobile phase was a mixture of solvent A and B with a flow rate of 0.4 mL/min. Solvent A was milli-Q water with 0.05% formic acid, and solvent B was acetonitrile. The gradient started with 2% of solvent B, linearly increased to 10% from 0.2 to 0.3 min, 10 to 80% of solvent B from 0.3 to 2.7 min, and 80 to 95% of solvent B from 2.7 to 2.8 min, remained at 95% solvent B for 0.5 min, before returning to 2% solvent B over 0.20 min and equilibrated for 1 min until the next injection. The total run time was 5 min. Cabozantinib was detected by ion transition m/z positive 502.26 to 347.19 at a cone voltage and collision energy of 20 and 35 V, respectively.

RESULTS

smKl Lipophilic Salt Chemical Characterization. Lipophilic salts of the four selected smKIs were successfully prepared via salt metathesis reaction as confirmed by 1H NMR and 13C NMR spectroscopy, with salt purity and molecular weight verified by elemental analysis and high resolution mass spectrometry, respectively (see Supporting Information). The 1H NMR spectrum of the lipophilic docusate salt form was compared with both the free base and hydrochloride/(S)-malate commercial salt forms to confirm protonation. The ratios of signal integrations from the drug and counterion confirmed salt purity. Lipophilic salt purity determined by
LC−MS was >99% for all of the lipophilic salts (see Supporting Information).

FTIR spectroscopy was performed on the free base and docusate salt forms of the four drugs (see Figure S2 of the Supporting Information) to support 1H NMR spectroscopic analysis confirming the formation of docusate salts. Specifically, FTIR confirmed the presence of a distinctive C=O ester stretch in all salt forms (erlotinib docusate, 1739 cm−1; gefitinib docusate, 1737 cm−1; cabozantinib docusate, 1731 cm−1; ceritinib docusate, 1732 cm−1), characteristic of docusate esters (1734 cm−1 in docusate free acid).

**smKI Lipophilic Salt Physical Characterization: Polarized Light Microscopy.** Free base and traditional salt forms (hydrochloride or (S)-malate) were characterized by well-defined crystalline particles (Figure 2). Erlotinib docusate and gefitinib docusate were both crystalline white powders. Under polarized light, these powders consisted of birefringent, irregular shaped particles. Cabozantinib docusate was isolated as a white powder that was nonbirefringent and glass-like. Attempts to isolate a crystalline cabozantinib docusate were unsuccessful though a full physical form screen was not performed. Ceritinib docusate was a crystalline yellow powder, characterized by birefringent needle-shaped crystals.

**Lipophilic Salt Physical Characterization: XRPD.** XRPD was performed to further probe the physical characterization of lipophilic salts prepared in this work and to verify that working batches of the erlotinib, gefitinib, cabozantinib, and ceritinib free base were consistent with the most stable crystalline forms based described in the literature. XRPD patterns are presented in Figure 3.

Erlotinib free base was substantially crystalline, exhibiting significant diffraction peaks at 7.4, 24.6, and 27.5° 2θ, consistent with a previous description of a stable hydrated form of this drug.48 Erlotinib hydrochloride exhibited significant diffraction peaks at 6.3, 9.6, 20.2, 21.1, and 22.4° 2θ, consistent with “Form B” previously characterized.39 Erlotinib docusate demonstrated evidence of crystallinity in the XRPD, with two notable diffraction peaks at 4.6 and 8.5° 2θ. These peaks were absent in the erlotinib free base and hydrochloride salt diffractogram but occur in a similar 2θ range as a principal diffraction peak in the free docusate acid diffractogram (4.3° 2θ; see Figure S3 of the Supporting Information).

The gefitinib free base diffractogram contained significant diffraction peaks at 15.8, 19.3, 22.4, 24.0, 24.2, and 26.3° 2θ, consistent with Thorat et al.40 Gefitinib docusate showed similar diffraction patterns to erlotinib docusate in that it demonstrated a notable diffraction peak at low 2θ values (3.3° 2θ).
Cabozantinib free base showed significant diffraction peaks at 14.0, 18.4, 22.0, and 24.0° 2θ. Several crystalline forms of cabozantinib free base have been described previously. The crystalline pattern of the working batch used in this work was consistent with “Form M2” previously described. The (S)-malate salt had significant diffraction peaks at 12.0, 12.7, 18.2, and 23.6° 2θ, which is consistent with the form (“Form N2”) used in the commercial product. Consistent with the polarized light microscopy image (Figure 2), cabozantinib docusate was amorphous, showing no evidence of crystallinity in its diffractogram.

Ceritinib free base had significant diffraction peaks 7.2, 13.3, and 18.3° 2θ, consistent with “Form A” previously characterized. The diffractogram for ceritinib docusate contained diffraction peaks at 4.7, 7.1, 17.8, and 18.6° 2θ. All crystalline docusate salts demonstrated significant diffraction peaks between 3−5° 2θ.

smKI Lipophilic Salt Physical Characterization: Hot-Stage Microscopy and DSC. Hot-stage microscopy and DSC evaluation provided complementary insights into the thermal properties of lipophilic salts. In high-stage experiments, free base forms of the drug all displayed a single melting event on heating (Figure 4). Measured melting points by DSC of the free base and applicable commercial salts (Figure 5) were consistent with described literature values. Erlotinib free base showed a sharp endotherm at 157.2 °C, consistent with previous work. Erlotinib hydrochloride showed an endotherm at 243.8 °C (peak, T\textsubscript{onset} at 239 °C) consistent with Tien et al. Gefitinib free base showed a sharp endotherm at 196.8 °C, consistent with Thorat et al. Cabozantinib free base showed an endotherm at 220.5 °C, consistent with “Form M2” previously described. Cabozantinib (S)-malate showed a sharp endotherm at 179.6 °C, consistent with “Form N2” of this salt. Ceritinib free base
showed a sharp endotherm at 179.6 °C, also consistent with previous work.43

Docusate salt forms of erlotinib, gefitinib, and ceritinib exhibited a direct solid-to-liquid transition during the heating cycle (Figure 4). Notably, these transitions occurred at significantly lower temperatures than the free base melting point, as evidenced by hot-stage microscopy (Figure 4) and DSC (Figure 5). The docusate salt-free base melting point

Figure 4. Hot-stage microscopy of free base and docusate salt forms of (a) erlotinib, (b) gefitinib, (c) cabozantinib, and (d) ceritinib demonstrating the significant reduction in melting point in the case of docusate salts. Images were captured at 10× or 20× magnification.

Figure 5. DSC thermograms of free base and salt forms of (a) erlotinib, (b) gefitinib, (c) cabozantinib, and (d) ceritinib. Peak melting points are labeled in the traces, and confirm the significant reduction in melting point when using docusate salt forms.
difference ($\Delta T_m$) followed the following rank order: gefitinib docusate ($\Delta T_m - 100.2 \, ^\circ C$) > erlotinib docusate ($\Delta T_m - 85.9 \, ^\circ C$) > ceritinib docusate ($\Delta T_m - 65.9 \, ^\circ C$). In the case of amorphous cabozantinib docusate, the glass transition temperature was measured by DSC at $\sim 52 \, ^\circ C$.

**Lipid Excipient Kinetic Solubility Screening of smKI Lipophilic Salts.** The solubility of free base, lipophilic salt, and commercial salt forms (where applicable) of erlotinib, gefitinib, cabozantinib, and ceritinib was measured in a range of chemically diverse lipidic excipients, and model medium-chain (MC–SEDDS) or long-chain (LC–SEDDS) lipid based formulations. These solubility values are reported in Figure 6. Values are expressed as milligrams of the equivalent free base concentration per gram of excipient (mg/g).

Erlotinib free base solubility was <20 mg/g in seven of the nine excipients, and only achieved a solubility above 50 mg/g in PEG 400. The solubility of erlotinib hydrochloride did not reach 5 mg/g in any of the tested excipients. Similar low excipient solubility was confirmed for gefitinib, cabozantinib, and ceritinib (generally <20–25 mg/g), with an occasional exception in, for example, PEG 400. In contrast, the docusate salt forms were all substantially more lipid-soluble. For example, erlotinib docusate achieved at least 50 mg/g in six lipidic excipients, and $\geq 150$ mg/g was achieved in glyceryl monocaprylate, propylene glycol monolaurate, and PEG–8 caprylic/capric glycerides. The docusate salt form of erlotinib offered no significant solubility benefit in triglycerides and was less soluble in PEG 400 (<50 mg/g) than the free base form (50–100 mg/g). Gefitinib docusate, cabozantinib docusate, and ceritinib docusate achieved concentrations that were generally at least five-fold higher than the solubility values obtained using the free base form. As demonstrated in Figure 6, the higher solubility in lipidic excipients of the docusate salt forms translated to increased solubility in model SEDDS formulations, with each lipophilic salt form achieving a loading of 100 mg/g in at least one formulation.

Lipid solubility studies were linked with downstream physical stability assessment to determine if the high dissolved drug concentrations could be maintained on storage ($25 \, ^\circ C/60\% \text{ RH}$ for 3 months). Stability follow-ups were performed
for selected excipients, namely a minimum of three excipients per compound, and at least one excipient in which a solubility of 100 mg/g or greater was achieved. Erlotinib docusate was physically stable at 3 months in propylene glycol monocaprylate (150 mg/g), glyceryl monocaprylate (150 mg/g), and glyceryl monolinoleate (100 mg/g); gefitinib docusate physically stable at 3 months in propylene glycol monocaprylate (100 mg/g), glyceryl monocaprylate (150 mg/g), PEG-8 caprylic/capric glycerides (50 mg/g), and PEG 400 (25 mg/g); cabozantinib docusate physically stable at 3 months in propylene glycol monocaprylate (100 mg/g), glyceryl monocaprylate (50 mg/g), PEG-8 caprylic/capric glycerides (100 mg/g), and polyoxyl 35 castor oil (25 mg/g); ceritinib docusate physically stable at 3 months in propylene glycol monocaprylate (100 mg/g), glyceryl monocaprylate (50 mg/g), and PEG-8 caprylic/capric glycerides (50 mg/g).

In addition, in the case of erlotinib docusate, initial batches yielded an amorphous solid but with some refinement to the preparation method, we were able to isolate the crystalline salt form. Small-scale trials to determine possible solubility differences between amorphous and crystalline forms were therefore performed. Results confirmed high lipid solubility of both physical forms, for example, proving it was possible to achieve erlotinib concentrations >100 mg/g in propylene glycol monocaprylate when using amorphous or crystalline docusate salt forms.

**In Vitro Evaluation of smKI Lipophilic Salt Lipid-Based Formulations of Erlotinib and Cabozantinib.** In vitro biorelevant testing was performed for SEDDS containing lipophilic salts of erlotinib and cabozantinib. These tests were conducted to determine whether smKI lipophilic salt-containing lipid based formulations were effective in generating a reservoir of solubilized drug, and whether the solubilized concentrations were higher than those attained using the free base or commercial salt forms.

Experiments were performed using a new method that combined an initial dispersion phase at gastric pH (pH 2) followed by an intestinal digestion phase (at pH 6.5). Maximal theoretical drug concentrations during the (acidic) dispersion and (≈neutral) intestinal digestion phase were ~5500 μg/mL and ~2500 μg/mL, respectively, with the 50% drop in maximal attainable concentration on moving to intestinal conditions reflecting the dilution on addition of the intestinal buffer (see methods section for more details). For experiments conducted with free base or the commercial salt, drug alone (crystalline powder) was evaluated for consistency with the control suspension formulations administered in the in vivo studies. For the lipophilic salts where lipid solubility was higher and therefore lipid-based formulations possible, the drug was introduced predissolved in the SEDDS formulations at 100 mg/g free base concentrations (204 mg/g erlotinib docusate or 184 mg/g cabozantinib docusate). The results are presented in Figure 7.

Under simulated gastric conditions, dissolution of suspensions of erlotinib free base or hydrochloride salt achieved drug concentrations of 606.3 ± 148.7 μg/mL and 780.4 ± 103.4 μg/mL in the gastric buffer, respectively (Figure 7a). However, after only 5 min at pH 6.5 (the 35 min time point in Figure 7a), drug concentrations decreased to <50 μg/mL and were <30 μg/mL by the end of the test. The data suggest significant precipitation of dissolved drug on moving to the higher intestinal pH. In contrast, SEDDS formulations containing erlotinib docusate fared better under both gastric and intestinal conditions. At pH 2, the LC–SEDDS provided for complete solubilization of erlotinib (5429 ± 301.9 μg/mL at 30 min). The MC–SEDDS resulted in slightly lower than maximal concentrations (4640 ± 475.6 μg/mL at 30 min) due to a small amount of oil that was collected in the pellet phase after centrifugation. Polarized light microscopy (not-shown) confirmed that this oil pellet was nonbirefringent.

Five minutes after initiation of lipid digestion at pH 6.5, the SEDDS maintained relatively high solubilized concentrations of erlotinib (911.6 ± 232.2 μg/mL for the LC–SEDDS and 1464.2 ± 314.0 μg/mL for the MC–SEDDS), although these concentrations were submaximal due to precipitation. After 60 min of digestion, solubilized erlotinib concentrations were 289.2 ± 93.4 μg/mL for the LC–SEDDS and 368.1 ± 81.1 μg/mL for the MC–SEDDS. These were ~10-fold higher than concentrations achieved by the free base and hydrochloride salt forms at the same point of the test.

Similar solubilization trends were evident on testing of cabozantinib free base and cabozantinib docusate containing SEDDS formulations (Figure 7b). At pH 2, concentrations of cabozantinib free base were initially ~400 μg/mL but decreased over time to 161.3 ± 126.7 μg/mL, possibly as a result of in situ formation of the HCl salt. On moving to pH
6.5, the solubilized concentration decreased further to 31.6 ± 126.7 μg/mL by the end of the test. The MC–SEDDS formulation containing cabozantinib docusate resulted in higher solubilization at pH 2 (2857 ± 146.4 μg/mL), although some pelleted oily phase dictated that maximal concentrations were not achieved. The LC–SEDDS formulation resulted in higher concentrations (439.7 ± 98.0 μg/mL) than that attained for the free base, but was less effective than the MC-SEDDS. Under intestinal conditions at pH 6.5, solubilized drug concentrations were 604.0 ± 8.4 μg/mL after 5 min for the MC–SEDDS and 255.8 ± 143.0 μg/mL for the LC–SEDDS. This was substantially higher than the concentrations provided by dissolution of the free base. Concentrations had decreased to ~20–40 μg/mL by the end of the test.

The physical and chemical form of precipitated drug generated during in vitro testing was examined to probe for possible dissociation of the lipophilic salts on exposure to biorelevant media and to support interpretation of the solubilization data during digestion (Figure 7). As an initial evaluation of physical and chemical properties, selected precipitates were analyzed by polarized-light microscopy and Raman spectroscopy. For hot-stage microscopy and 1H NMR spectroscopic analysis, it was necessary to first wash the precipitates with cold 10% (v/v) chloroform in diethyl ether to

Figure 8. Characterization of the precipitate obtained following in vitro digestion of erlotinib docusate containing MC–SEDDS at pH 6.5. (a) Polarized light microscopy image reveals a crystalline precipitate, but a lack of distinct crystals. (b) Raman spectroscopy and (c) 1H NMR spectroscopy confirm that the precipitate is predominantly erlotinib free base.

Figure 9. Characterization of the precipitate isolated following in vitro testing of cabozantinib docusate containing lipid-based formulations. (a) Polarized light microscopy images reveal evidence of needle shape crystals on digestion of the MC–SEDDS formulation, but a lack of distinct crystalline particles in the case of the (b) LC–SEDDS formulation. (c) 1H NMR spectroscopy of the precipitate from the MC–SEDDS formulation confirms that the precipitate contains predominantly cabozantinib free base.
remove residual lipids. This was followed by a 20 h drying step to remove any solvents.

As shown in Figure 8a, the precipitate that formed during in vitro digestion of MC−SEDDS containing erlotinib docusate was crystalline but lacked evidence of ordered crystals. Raman spectral analysis shown in Figure 8b (described in more detail in Figure S4 of the Supporting Information) indicated that the precipitate consisted of erlotinib free base. 1H NMR spectroscopic analysis of the solvent-washed precipitate also confirmed the presence of erlotinib free base (Figure 8c). However, this precipitate melted before reaching 100 °C (Figure S5 of the Supporting Information), which is notably lower than the melting temperature of the most stable crystalline form of erlotinib free base (157.2 °C, Figure 5).

The LC−SEDDS containing cabozantinib docusate was the only lipophilic salt SEDDS formulation to result in significant drug precipitation during the acidic gastric dispersion phase. The precipitate formed (Figure 7b) was confirmed as cabozantinib docusate salt by Raman and 1H NMR spectroscopy (Figure S6 of the Supporting Information). The precipitate did not contain distinct drug crystals and in fact exhibited crystalline patterns that are more characteristic of liquid crystalline phases (Figure S6 of the Supporting Information).

During the in vitro digestion phase, the precipitate from the MC−SEDDS formulation containing cabozantinib docusate consisted of thin, needle-shaped crystals while there were no distinct crystals in the case of the LC−SEDDS precipitate (Figure 9a,b). 1H NMR spectroscopic analysis confirmed that the precipitate formed on digestion of the MC−SEDDS formulation contained cabozantinib free base (Figure 9c). On heating, this precipitate exhibited some restructuring at below 115 °C and underwent a complete solid to liquid transition by 200 °C (see Figure S7 of the Supporting Information). The melting behavior of the precipitated cabozantinib free base was notably below the melting point of the most stable crystalline form of cabozantinib free base (220.5 °C, Figure 5). 1H NMR also confirmed that the precipitate formed on digestion of the LC−SEDDS formulation was cabozantinib free base (see Figure S8 of the Supporting Information).

**Solubility and Supersaturation Evaluation of smKIs.** The aqueous solubility of free base and docusate salt forms of erlotinib and cabozantinib was measured in simulated gastric fluid (pH 2) and intestinal digestion buffer (pH 6.5) with or without digested SEDDS (Table 1). The solubility values of all four smKIs and the respective salts at pH 2 and 6.5 are also summarized in Table S2 of the Supporting Information.

Consistent with many drugs in the smKI class,10 the solubility of erlotinib and cabozantinib free base was higher at pH 2 than at pH 6.5 (Table 1). Docusate lipophilic salt forms were substantially less soluble at pH 2 than the free base. At pH 6.5, erlotinib and cabozantinib free base solubility was very low (<10 μg/mL), presumably reflecting the high log P values of these molecules. The docusate salts were more soluble (2−3-fold) than free base at pH 6.5, although absolute solubility was still low likely reflecting decreased drug ionization, reduced association with the docusate counterion and conversion to the free base. In the digested MC−SEDDS and LC−SEDDS (at pH 6.5), the solubility of docusate salts ranged from 100−200 μg/mL. These values are ~2-fold higher than the free base under the same conditions, and ~5−10-fold higher than the saturated solubility of either the free base or the docusate salt in the absence of digested lipid. The solubility of erlotinib docusate in dispersed LC−SEDDS at pH 6.5 was 111.3 ± 17.0 μg/mL and therefore similar to the solubility in the presence of digested lipid-based formulation.

The solubility values for erlotinib docusate and cabozantinib docusate in digested lipid-based formulations (Table 1) were also used to estimate the level of supersaturation generated in the in vitro tests described in Figure 7. The degree of supersaturation reflects changes to thermodynamic activity, and in the absence of an absorption membrane, the likelihood of drug precipitation.46 To capture supersaturation in this study, the maximum supersaturation ratio (SR46) during the digestion phase was calculated using eq 1:

$$SR^M = \frac{drug \text{ dose}/intestinal \text{ phase \ volume}}{drug \text{ solubility \ in \ digested \ lipid \ formulation}}$$  

At the 100 mg/g drug loading (free base equivalents), the calculated SR46 values for erlotinib docusate containing MC−SEDDS and LC−SEDDS formulations were 14.5 and 18.0, respectively. Since values greater than 1 denote the presence of supersaturation, these SR46 values are indicative of very high thermodynamic activity and a significant driving force toward precipitation in an in vitro test (but conversely the potential for a driving force toward absorption where an absorption sink is present in vivo). SR46 values for cabozantinib docusate containing MC−SEDDS and LC−SEDDS were also very high, SR46 = 25.1 and 18.6 for MC−SEDDS and LC−SEDDS, respectively.

**Effect of Intestinal pH on in Vitro Solubilization of Cabozantinib Lipophilic Salts.** The evidence of significant drug precipitation during in vitro testing of model lipid-based formulations (Figure 7) prompted further experiments to determine whether intestinal solubilization of smKIs lipophilic
salts would be significantly higher under slightly more acidic conditions such as those expected in the upper reaches of the duodenum. Using the MC–SEDDS formulation containing cabozantinib docusate as an exemplar, in vitro digestion experiments were repeated at pH 5.5 and in the presence and absence of pancreatic lipase. This confirmed the possibility of significantly higher (5.6-fold) solubilization at slightly more acidic conditions (Figure 10). Notably, this effect was also independent of digestion, which suggested that the pH-shift was the primary driver of the generation of supersaturation and precipitation.

**In Vivo Evaluation of smKI Lipophilic Salt Lipid-Based Formulations.** Model SEDDS formulations containing 100 mg/g (free base equivalent) of erlotinib docusate or cabozantinib docusate were progressed into in vivo pharmacokinetic studies in rats. These smKI were selected for in vivo evaluation based on their low measured solubility at pH 6.5 (<20 μg/mL) and thus greater likelihood of suffering from solubility-limited absorption.

For erlotinib docusate, the MC–SEDDS formulation was used. An aqueous suspension of the crystalline hydrochloride salt (the commercially used salt form of erlotinib) was used as the reference control. Data obtained after administration of 12.5, 25, and 100 mg/kg dose levels (obtained by dosing larger quantities of the same formulation) are shown in Figure 11 and summarized in Table 2. At the lowest dose (12.5 mg/kg), AUC, C_{\text{max}}, and T_{\text{max}} values for the MC–SEDDS lipophilic salt formulation or the HCl salt (as an aqueous suspension) formulation were generally consistent. At 25 mg/kg, erlotinib AUC increased proportionally with dose (i.e., ~2.0-fold) for both lipophilic salt and HCl treatments. Closer analysis at this dose level suggested slightly higher exposure (AUC and C_{\text{max}}) after administration of the lipophilic salt formulation, although this effect was small and not statistically significant. Differences in AUC and C_{\text{max}} were more pronounced at the 100 mg/kg dose, with the data sets revealing a trend toward greater proportionality between dose and exposure using the lipophilic salt formulation (Figure 11). Variability in exposure was largely consistent across the various doses for the lipophilic salt MC–SEDDS but increased with dose for the HCl aqueous suspension. Realizing the potential for GI side effects for smKIs and the use of a nontraditional salt counterion (docusate) in the current studies, postdosing histological evaluation of the rat GI mucosa (glandular stomach, nonglandular stomach, and small intestine) was conducted to assess for potential GI effects. No evidence of irritancy on acute dosing in rats was apparent (see Table S3 of the Supporting Information).

![Figure 10](image1.png)  
**Figure 10.** In vitro intestinal solubilization of cabozantinib during dispersion/digestion of MC–SEDDS containing cabozantinib docusate, described by the area under the intestinal solubilization curve. Experiments were conducted in the presence (digesting) and absence (nondigesting) of pancreatic lipase. Values are means (n ≥ 2) ± SD.

![Figure 11](image2.png)  
**Figure 11.** In vivo exposure in rats of erlotinib (ERL) after oral administration of a MC-SEDDS formulation containing erlotinib docusate (ERL_DoS) in comparison to an aqueous suspension of erlotinib hydrochloride (ERL-HCl). Data are expressed as mean (n ≥ 3) ± SD. Total ERL exposure, expressed as AUC_{0–24h}, is also shown with respect to the dose level (as ERL free base, equivalents).
For cabozantinib in vivo studies (in Figure 12 and summarized in Table 3), oral treatments were all administered at 25 mg/kg and included the docusate lipophilic salt in MC–SEDDS and LC–SEDDS formulations containing cabozantinib docusate (CBZ.DoS) in comparison to an aqueous suspension and LC–SEDDS suspension of cabozantinib free base. Data are expressed as mean (n ≥ 3) ± SD. Total CBZ exposure, expressed as absolute bioavailability (BA), is also shown with respect to the formulation type.

at 25 mg/kg and included the docusate lipophilic salt in MC–SEDDS and LC–SEDDS formulations, and the free base form either as an aqueous suspension or as a suspension in the LC–SEDDS formulation. An intravenous dose was also administered at 5 mg/kg using cabozantinib free base to allow calculation of absolute bioavailability (BA).

Lipophilic salt containing MC–SEDDS and LC–SEDDS formulations generated higher (1.5- and 1.8-fold, respectively) absolute BA values in comparison to the free base aqueous suspension. The absolute BA from the LC–SEDDS formulation containing suspended cabozantinib free base was similar to the same formulation containing dissolved cabozantinib docusate (83.4% vs 80.8%, Table 3), although as expected, closer analysis of the data reveals a trend toward an increased T_{max} in the lipid suspension group and higher variability in exposure.

**DISCUSSION**

Low aqueous solubility at intestinal pH, high lipophilicity, and high melting point are common features of the small molecule kinase inhibitor (smKI) compound class.10 These properties can lead to poor oral absorption, food-effects, and absorption-related drug–drug interactions,” all of which have the potential to hamper clinical effectiveness. Given the prevalence of positive food-effects and high compound lipophilicity among smKIs,” lipid-based formulations provide one potential option to increase oral exposure and reduce variability in instances where low aqueous solubility limits bioavailability. Another common feature of drugs in this class, however, is high dose. Under these circumstances, unless the drug exhibits high solubility in commonly used lipid vehicles, pill burden issues commonly arise when pursuing a lipid-based formulation approach.

Recent studies have shown that transforming an acidic or basic drug into a lipophilic salt form can (i) dramatically enhance drug loading in lipid based formulations31–33,50 and (ii) increase oral exposure when paired with an appropriate lipid based formulation.31,32 The current study therefore explored the utility of lipophilic salt forms as a means to unlock the absorption enhancing potential of lipid-based formulations for exemplar smKIs.

**Lipophilic Salt Forms of smKIs Significantly Increase Drug Solubility in Lipid-Based Formulations.** Lipophilic salts of smKIs were successfully prepared by salt metathesis reactions using the lipophilic counterion, docusate (or dioctyl sulfosuccinate). As the sodium salt, docusate has been used previously in pharmaceutical formulations as a disintegrant, surfactant, or coating agent.33 It is also used clinically in adults and in children as a stool softener (Dulcolax, Colace etc.) and is approved by the FDA in doses of up to 300 mg per day. Docusate is also commonly used at varying concentrations in food products as a wetting agent, solubilizer, emulsion stabilizer, or dispersing aid, for example, at 25 ppm levels in cocoa-containing beverages.34 The LD_{50} level for sodium docusate in rats after oral administration is 1.9 g/kg.34 It is also Generally Regarded as Safe (GRAS) for oral administration.

Docusate shows potential for the formation of effective lipophilic pharmaceutical salts due to its strong acidic center (calculated pK_{a} of ~0 at 25 °C, using ACD Laboratories Software, v11.02) and branched, flexible backbone that hinders the formation of a tightly packed crystal lattice. This utility was confirmed in the current study where docusate salts of erlotinib, gefitinib, cabozantinib, and ceritinib were isolated as workable powders comprising either amorphous or low melting (T_{m} < 125 °C) crystalline particles.

As expected, due to the high lipophilicity of the docusate counterion (cLog P ≈ 4), docusate salts of smKIs resulted in decreases in aqueous solubility in acidic buffers (Table 1). Under more basic intestinal conditions (pH 6.5), the aqueous solubility of the docusate salts was significantly higher than the free base; however, absolute solubility was low in both cases. In contrast, the net result of a depressed melting point and increased lipophilicity led in most cases to marked increases in solubility in lipidic excipients as illustrated in Figure 6.

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**Table 3. Pharmacokinetic Summary Following in Vivo Administration of Cabozantinib Formulations to Rats**

<table>
<thead>
<tr>
<th>treatment (25 mg/kg)</th>
<th>AUC_{0–inf} (µg × h/mL)</th>
<th>C_{max} (µg/mL)</th>
<th>T_{max} (h)</th>
<th>BA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cabozantinib free base aqueous suspension</td>
<td>295.9 ± 60.1</td>
<td>12.9 ± 3.2</td>
<td>6.3 ± 2.9</td>
<td>47.2 ± 10.9</td>
</tr>
<tr>
<td>cabozantinib docusate in MC–SEDDS</td>
<td>435.6 ± 50.9</td>
<td>29.1 ± 4.3</td>
<td>3.3 ± 0.9</td>
<td>69.3 ± 9.8</td>
</tr>
<tr>
<td>cabozantinib docusate in LC–SEDDS</td>
<td>524.0 ± 28.8</td>
<td>28.2 ± 3.2</td>
<td>3.6 ± 0.8</td>
<td>83.4 ± 3.3</td>
</tr>
<tr>
<td>cabozantinib free base in LC–SEDDS</td>
<td>500.8 ± 64.4</td>
<td>25.6 ± 6.9</td>
<td>6.5 ± 2.7</td>
<td>80.8 ± 10.1</td>
</tr>
</tbody>
</table>

*Values are expressed as mean values (n = 4 unless otherwise specified) ± SD. The absolute bioavailability (BA) was calculated from an IV treatment arm utilizing cabozantinib free base. BA, bioavailability. Suspension.*
Notably, lipophilic salt forms of smKIs regularly achieved solubility values in excess of 100 mg/g (free base equivalents) in several excipients. This is a 5–10-fold increase in solubility over the equivalent free base forms.

To capture the impact of this increase in lipid solubility on the development of smKI lipid-based formulations, we introduce here a “Lipid Based Formulation Dose Number” (LBFD0) as described by eq 2:

$$LBFD_0 = \frac{D/W}{S \times X}$$

(2)

Where D is the highest target dose per dosage unit, W is the formulation mass per dosage unit, S is the solubility in the lipid based formulation, and X is the drug saturation level in the formulation. LBFD0 captures the relationship between the highest “required” drug concentration in the formulation, if the daily dose were to be delivered in a single dosage unit, and drug solubility in the formulation. LBFD0 values below or close to 1 are desirable to lipid based formulation development as they point to the possibility of delivering the target dose in a single dosage unit. Equally, increasing LBFD0 corresponds to an increase in daily pill burden.

The LBFD0 values shown in Table 4 were calculated using the recommended daily dose of each smKI, a formulation mass

Table 4. Calculated “Lipid Based Formulation Dose Number” for Four smKIs Investigated in This Study

<table>
<thead>
<tr>
<th>smKI and daily dose</th>
<th>lipid based formulation dose number (LBFD0)</th>
</tr>
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<tbody>
<tr>
<td>free base</td>
<td>commercial salt</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>erlotinib, 150 mg</td>
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<tr>
<td>gefitinib, 250 mg</td>
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<tr>
<td>cabozantinib, 140 mg</td>
<td>7.8</td>
</tr>
<tr>
<td>ceritinib, 750 mg</td>
<td>125</td>
</tr>
</tbody>
</table>

“Recommended maximum daily dose according to FDA product labels. ^LBFD0 calculated from eq 2. na, Not applicable.

of 0.75 g (a conservative capsule fill amount), and an 80% saturation level for solubility measured in MC–SEDDS (using values in Figure 6). As seen from Table 4, the lipophilic salt form affords a dramatic reduction in the LBFD0 value for all smKI. For example, in the case of erlotinib, the high solubility of the lipophilic salt form is such that LBFD0 for erlotinib doxusate is close to 1 (= 1.7), suggesting the possibility of delivering the dose in 1 or 2 capsules. In contrast, because of low lipid solubility, the corresponding LBFD0 values for the free base and hydrochloride salt forms are much higher (>10 or >50, respectively). Similarly, for cabozantinib, the low lipid solubility of the free base or (S)-malate salt form precludes the possibility of delivering the maximum dose in a single capsule (high LBFD0 values) whereas the value for the doxusate salt is 1.6. When compared to existing free base or commercial salts, lipophilic salts of the smKIs discussed here are therefore more suitable candidates for lipid-based formulation development.

In Vitro and in Vivo Performance Synergy of Lipophilic Salt—Lipid Based Formulation Combinations.

Lipid-based formulations enhance the oral absorption of poorly water-soluble drugs by avoiding dissolution, boosting the solubilization capacity of the GI tract and by generating a reservoir of solubilized drug in the colloids that efficiently shuttle drug to the site of absorption.12 By facilitating formulation in lipid based formulations, lipophilic salt forms of smKIs may therefore also indirectly promote oral absorption, by facilitating the use of an absorption enhancing formulation strategy, with the possibility that this leads to a dose reduction and reduced likelihood of food-effects.

In this study, two exemplar self-emulsifying lipid based formulations (MC–SEDDS and LC–SEDDS) were utilized to probe for potential benefits to smKI absorption when using lipophilic salt forms. These “off-the-shell” formulations were not purposely developed to maximize the utility of lipophilic salts, but instead were chosen to provide comparison with previous studies.

A gastric/intestinal in vitro performance test was initially employed to probe the solubilization patterns of the free base, commercial salt, and lipophilic salt forms of erlotinib and cabozantinib under biorelevant conditions. The solubility of the free base and the commercial salt forms was high under simulated gastric conditions but as the target concentration for total drug solubilization (~5.5 mg/mL) was significantly higher than the respective solubility values (Table 1), the majority of drug remained undissolved. Solubilized concentrations dropped further at intestinal pH (<10 μg/mL), and as such, less than 50 μg/mL drug remained in solution after only 5 min at intestinal pH (see Figure 7). In contrast, the lipophilic salts of erlotinib and cabozantinib in combination with the SEDDS formulations resulted in substantially higher solubilized concentrations in the gastric phase and this advantage was initially maintained under simulated intestinal conditions. Prolonged contact of the lipophilic salts with intestinal pH in vitro, however, led to evidence of drug precipitation and a drop in solubilization.

Supporting Raman and 1H NMR spectroscopic analysis indicated that the precipitates observed under intestinal conditions comprised smKI free base. Precipitation was therefore likely to be triggered by a decrease in drug ionization at pH 6.5 (given the low pKf for erlotinib and cabozantinib) and, in turn, dissociation of erlotinib and cabozantinib lipophilic salts to the free base form, the solubility of which was significantly lower than that of the lipophilic salt in the dispersed and digested lipid-based formulations (Table 1). Using the model lipid-based formulations here, dissociation at pH 6.5 therefore led to supersaturation, and in the absence of absorption, phase-separation of solid free base within 15 min.

Support for this mechanistic interpretation can be drawn from the significantly decreased in vitro precipitation when analogous tests were performed at pH 5.5, where more of the smKI would be expected to be ionized and available for association with the lipophilic counterion (thereby increasing affinity for the dispersed lipid phases) (see Figure 10). Furthermore, while lipid based formulation digestion has been found to be a trigger for increasing drug thermodynamic activity in the small intestine,46,47 the negligible performance difference observed when conducting experiments in the presence or absence of digestion enzymes indicate that the increase in pH on moving from gastric to intestinal conditions was the primary driver of supersaturation/precipitation.

In light of the increases in drug solubilization seen during in vitro evaluation of the lipophilic salt-containing SEDDS formulations, subsequent studies sought to provide in vivo proof of concept of utility. In general, in vivo evaluations of SEDDS formulations of erlotinib and cabozantinib lipophilic salts in fasted rats resulted in similar or increased oral absorption of the smKIs and a reduction in variability. In the case of erlotinib, the difference in performance between control and lipophilic salt formulations was most evident at the
highest dose level (100 mg/kg—see Figure 11), although increases in exposure were not statistically significant. Realizing the significant increases in in vitro performance of the SEDDS formulations of the lipophilic salts, the relatively moderate increases in in vivo exposure suggest either less effective performance in vivo or better than expected absorption of erlotinib from the crystalline form. For highly lipophilic compounds such as erlotinib, it is possible that high intestinal permeability is sufficient to offset low GI solubility and provide for effective absorptive flux.

In the case of cabozantinib, the absolute oral bioavailability of the crystalline free base was ~47%, and this increased to ~83% when dosed as the docusate salt in the LC−SEDDS formulation. The lipophilic salt formulation also resulted in a decrease in absorption variability, in particular for cabozantinib docusate in LC−SEEDS (Table 3). Interestingly, in vivo, the LC−SEDDS formulation outperformed the MC−SEDDS formulation, which was in contrast to the solubilization results in the in vitro tests (see Figure 7b). Redissolution and absorption of a high-energy cabozantinib precipitate (see Figure 7 of the Supporting Information) may explain the discrepancy between in vitro solubilization and in vivo absorption data. Alternatively, and consistent with the suggestion above for erlotinib, the in vitro digestion test may have overestimated the extent of precipitation in vivo, where supersaturation may have resulted in absorption rather than precipitation. Deeper understanding of the role of solubilization, supersaturation and permeability in driving the absorption of lipophilic salts of smKI from lipid-based formulations may be possible via the use of combined lipid digestion−drug absorption models as previously described.52

Closer analysis of the pharmacokinetic profiles (Figure 12) also reveals very similar plasma concentrations between 0−4 h for MC−SEDDS and LC−SEDDS but a more rapid drop in the plasma drug concentrations between 4−6 h for the MC−SEDDS treatment. The very rapid drop in plasma concentration for the lipid based formulations of the lipophilic salts at 4 h post dose is unusual and coincides with the period when food is reprovided to the animals. The speed of the decrease in plasma concentration is sufficiently rapid that it likely reflects changes to systemic pharmacokinetics rather than a decrease in absorption. This could be either an acute increase in clearance or volume of distribution, potentially stimulated by feeding. These changes warrant further study and may also involve changes to biliary processing since evidence of enterohepatic recycling has been described previously for cabozantinib.53,54

Cabozantinib free base was also administered to rats as a crystalline suspension in the LC−SEDDS formulation to determine the extent to which lipids could boost the exposure of suspended cabozantinib. Absolute bioavailability of this treatment was ~81%, and thus similar to the same formulation containing dissolved cabozantinib docusate. This finding indicates that the presence of dispersed and digested lipids in the GI tract can play a positive role in promoting cabozantinib absorption under either condition. Variability in drug exposure, however, was higher after administration of the lipid suspension formulation (see Table 3) and in general, lipid solution formulations, such as those made possible by the use of the docusate lipophilic salt, are preferred over suspensions to simplify physical stability, characterization and reproducibility.

Lipid-Based Formulation Design Considerations for Lipophilic Salts. To fully harness the high drug loading potential that lipophilic salts provide in lipid-based formulations, effective drug solubilization during gastric dispersion is required followed by solubilization and supersaturation at the absorptive site.55 For smKIs, the present study suggests that high drug concentrations are attained under gastric conditions and that high degrees of supersaturation result on transfer of the dispersed SEDDS formulations from the gastric to the intestinal environment. In vitro this resulted in significant precipitation. The precipitation risk was likely compounded by the relatively low pKa of the drugs employed (erlotinib and cabozantinib) and the stressful nature of the in vitro test (i.e., rigorous mixing, the lack of absorptive sink etc.). In contrast to the precipitation observed in vitro, for cabozantinib, absorption was almost complete after administration of the lipid based formulation, and dose linearity was apparent even up to 100 mg/kg for erlotinib. As such, the significant precipitation seen in vitro may not significantly limit exposure in vivo. Nonetheless, a rational design approach could lead to better performance of lipophilic salt containing formulations of smKIs, for example, through careful selection of excipients, precipitation inhibitors, and the design of the colloidal phase postdispersion and digestion. The combination of lipid-based formulation plus precipitation inhibitors would also provide an effective means of probing the mechanisms of smKI drug absorption in vivo and identifying instances of solubility limited absorption. This is the scope of planned future work.

CONCLUSIONS

In response to the challenge of solubility limited drug absorption, including some orally delivered anticancer drugs,10,30 this work has demonstrated that lipophilic salt forms of weakly basic smKIs significantly enhance the ability to develop effective oral lipid-based formulations. Using docusate as an example counterion, and erlotinib, gefitinib, cabozantinib, and ceritinib as example smKIs, lipophilic salt forms were all significantly more soluble in lipidic excipients in comparison to free base and commercial salt forms. This translated to high (>10% wt.) smKI loadings in exemplar self-emulsifying lipid based formulations. Considering the high dose requirements in this compound class, the use of lipophilic salt forms is therefore an approach that has the potential to "unlock" the application of lipid based formulations in enhancing fasted-state absorption of smKIs. The synergy between smKI lipophilic salts and lipid based formulations was not limited to drug loading benefits; there were also downstream gains in in vitro solubilization and moderate increases (and less variability) in absorption in vivo in preclinical animal models. Whether these results translate to increases in absorption in humans, and whether this provides significant clinical benefit, remains to be determined. Nonetheless, the data reported herein suggest that transformation of candidate smKI into lipophilic salts to facilitate assembly into lipid-based dose forms provides one possible option to overcome solubility limited absorption for poorly water-soluble compounds, particularly when the drug is weakly basic and the dose is high.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.molpharmaceut.8b00858.
Physicochemical characterization of lipophilic salts of kinase inhibitors; histology results following in vivo dosing of formulations to rats; additional synthetic details for select salt intermediates (PDF)

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Notes

The authors declare the following competing financial interest(s): This work describes intellectual property in the use of ionic liquids/lipophilic salts in drug delivery that has been assigned to Lonza.

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