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Brief Article

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Optimizing Solubility and Permeability of a Biopharmaceutics Classification System (BCS) Class 4 Antibiotic Drug Using Lipophilic Fragments Disturbing the Crystal Lattice

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Supporting Information

ABSTRACT: Esterification was used to simultaneously increase solubility and permeability of ciprofloxacin, a biopharmaceutics classification system (BCS) class 4 drug (low solubility/low permeability) with solid-state limited solubility. Molecular flexibility was increased to disturb the crystal lattice, lower the melting point, and thereby improve the solubility, whereas lipophilicity was increased to enhance the intestinal permeability. These structural changes resulted in BCS class 1 analogues (high solubility/high permeability) emphasizing that simple medicinal chemistry may improve both these properties.

INTRODUCTION

Poor aqueous solubility is a main hurdle to overcome in current discovery programs with 40−70% of all new hits estimated to have too low solubility to allow complete absorption from the GI tract.[1] We have investigated molecular features of importance for poor solubility[2] and identified structural differences between compounds that are poorly soluble because of strong crystal lattice and those that are poorly hydrated in the aqueous environment. The molecules that are solid state limited in their solubility, are smaller, rigid, and often flat molecules.[2c] These structural features allow the molecules to pack densely in the crystal and provide strong intermolecular bonds through van der Waals interaction, $\pi-\pi$ stacking, and hydrogen bond formation. In comparison, the solvation limited compounds are larger, flexible, and lipophilic.[2a] In addition there are intermediate compounds that have both a strong crystal lattice and a poor solvation. Typically these are highly lipophilic, small, and rigid molecules with a strong hydrogen bond capacity within the crystal lattice.[3] The underlying molecular reason(s) for poor solubility can hence inform the medicinal chemists upon strategies to improve the aqueous solubility.

Solubility together with permeability form the basis for the biopharmaceutics classification system (BCS).[4] The BCS sorts compounds into four classes based on high/low permeability and solubility, in which BCS class 1 represents highly permeable and highly soluble compounds that will be well-absorbed after oral administration. In contrast, class 4 compounds have both low solubility and low permeability. In this study we investigated whether classical medicinal chemistry using esterification could be used to simultaneously improve solubility and permeability for ciprofloxacin, a rare model compound reflecting BCS class 4 drugs with solid-state limited solubility. Our hypothesis was that the solubility and permeability of this compound could be improved through esterification producing derivatives with less strong crystal lattice and improved lipophilicity and that these structural features possibly position the ester derivatives in BCS class 1.

RESULTS

Compounds. Seven ciprofloxacin analogues (Figure 1, **5a**−**g**) were synthesized by esterification of ciprofloxacin. By elongation of the alkyl chains in a stepwise manner (Me, Et, ^{i}Pr, Pr, Bu, Hex, and Bn as an example of a more rigid ester; see Figure 1), a systematic study of the effect of esterification and molecular flexibility on the lipophilicity, melting point, solubility, and permeability could be undertaken. These

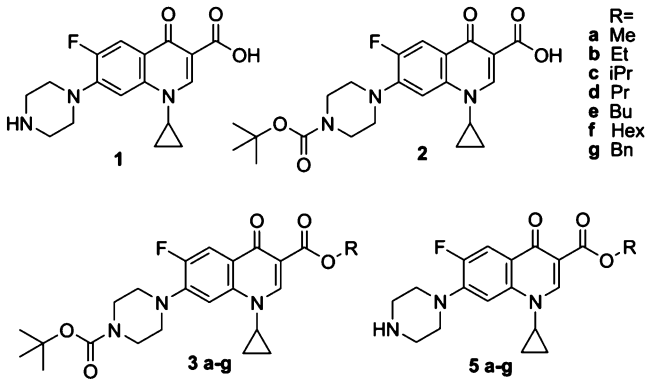

Figure 1. Stepwise scheme of the synthesis process with alkyl chains used given as **5a**−**g**.

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structural modifications of ciprofloxacin produced derivatives with increased flexibility and a lipophilicity range of almost 100 000-fold (Table S1). Further, the esterification eliminated the zwitterionic nature of ciprofloxacin, resulting in basic derivatives with a single pK_a of ~8.6 and reduced hydrogen bond capacity.

Solid State Characterization. Differential scanning calorimetry (DSC) thermograms showed that only one polymorph was obtained of each of the derivatives synthesized. No indications of salt formations or solvent residues were obtained, and the final product obtained was the free base. The melting point of the derivatives ranged from 255 °C (**5a**, Me ester) to 186 °C (**5f**, Hex ester) in comparison to the melting point of 266 °C for ciprofloxacin. The melting temperatures of the unbranched alkyl chain esters, e.g., all derivatives except ^{i}Pr and Bn esters, decreased with increased chain length (Figure 2).

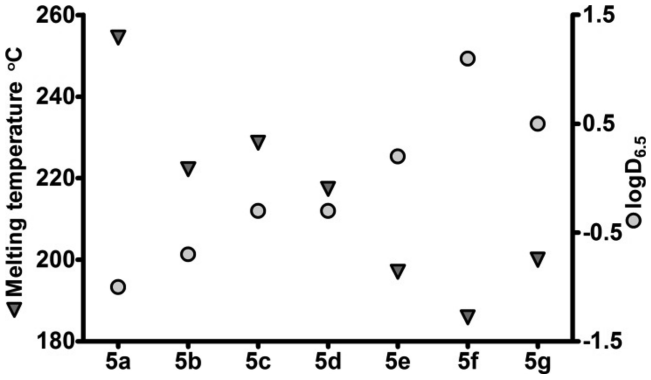

Figure 2. Calculated lipophilicity (log $D_{pH\ 6.5}$ from ADMET Predictor, circles) and melting point (triangles) for derivatives. Standard deviation of melting point is <2 °C for all derivatives and hidden by the symbol.

Apparent Solubility. The chemical modifications led to higher solubility for three of the derivatives of ciprofloxacin (Figure 3). Derivative **5a** showed 48-, 66-, and 111-fold higher solubility at pH 7.4 ($PhB_{7.4}$), at pH 6.5 ($PhB_{6.5}$), and in the fasted state simulated intestinal fluid (FaSSIF, pH 6.5). **5b** showed 2- to 3-fold higher solubility in the three media, whereas **5e** had 4-, 27-, and 10-fold higher solubility compared to ciprofloxacin in $PhB_{7.4}$, $PhB_{6.5}$, and FaSSIF, respectively. Compounds **5c** and **5d** displayed solubilities of 0.5−1 of that of ciprofloxacin. For the more lipophilic derivatives (**5f** and **5g**) the solubility was significantly reduced compared to ciprofloxacin, with the compounds having 10- to 30-fold lower solubility in all media (Figure 3). A significant solubilization in lipid aggregates naturally available in the intestine, as simulated with FaSSIF, was only seen for the most lipophilic compound (**5f**), i.e., the hexyl derivative with a log *P* of 3.3 (log $D_{pH6.5}$ of 1.1; see Figure 2). This is in agreement with our previous finding that for compounds with a log *P* of 3 or more, significantly increased apparent solubility can be expected in intestinal fluids compared to pure water or buffers.[2a]

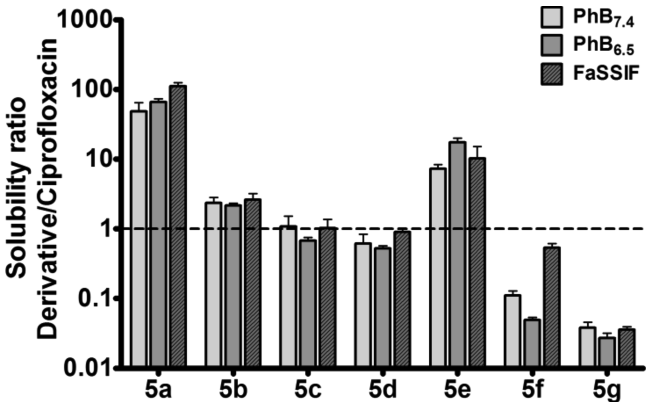

Figure 3. Solubility ratio with standard error (solubility of derivative/solubility of ciprofloxacin) in phosphate buffers pH 7.4 ($PhB_{7.4}$) and pH 6.5 ($PhB_{6.5}$), and fasted state simulated intestinal fluid (FaSSIF).

The apparent solubility was used to calculate the dose number (Do).[4] Completely dissolved compounds have Do < 1. Compound **5a** resulted in complete dissolution of the maximum dose in all media, and so did **5e** when measured in $PhB_{6.5}$. In comparison, ciprofloxacin obtained Do > 7 in all three media. Hence, the esterification using methyl and butyl alkyl chains transform ciprofloxacin to become soluble under physiologically relevant conditions. The resulting different apparent solubility measurements were not considered a result of different particle size, since the particle size distribution of derivatives was similar to that of ciprofloxacin.

Permeability. All derivatives showed higher permeability than ciprofloxacin (Table 1) and were predicted to be >90% absorbed (providing absorption is not solubility-limited) based on our in-house correlation between Caco-2 permeability and fraction absorbed.[5] Several of the compounds showed significantly higher permeability in the basolateral to apical (b−a) direction than in the apical to basolateral (a−b) direction, e.g., derivative **5d** displayed 146-fold higher permeability in the b−a direction. This large ratio cannot solely be explained by the ion trapping effect caused by the pH gradient used in the experiment[6] and hence suggests that active efflux transporters are involved in the cellular permeation. This ratio was significantly reduced when the derivatives were coadministered with the P-gp inhibitor GF120918. Hence, it was concluded that P-gp contributed to the large b−a/a−b ratio observed.

BCS. BCS of the derivatives is shown in Figure 4. At the pH of the intestinal fluid (pH 6.5) the **5a** and **5e** esters transform ciprofloxacin from BCS class 4 to BCS class 1. All other derivatives were BCS class 2 compounds for which appropriate formulation may result in a BCS 1 performance in vivo.

■ DISCUSSION AND CONCLUSION

Ciprofloxacin is a compound with solid-state limited solubility, and hence, we decided to improve the aqueous solubility by introducing bulky, flexible side chains and reducing the hydrogen bond capacity of the molecule to disrupt the crystal lattice. Intermolecular interactions can be lowered by this approach. The long, flexible alkyl chains will perturb the crystal lattice, and the reduced hydrogen bond capacity, as a result of esterification of the carboxylic acid function, will result in less ordered intermolecular hydrogen bond patterns.[7] A similar approach in which the flat, planar molecular structure of phosphodiesterase-4 inhibitors was disrupted through introduction of bulky substituents based on cyclohexyl was recently published.[8] The authors speculated that the higher solubility of the derivatives was an effect of a less ordered crystal lattice; however, the solid state was not characterized to prove this. For the ciprofloxacin derivatives herein melting point was used to investigate how the crystal lattice was affected by the alkyl chains. Indeed, melting point was shown to decrease with

Table 1. Permeability, Solubility, and BCS Classification of Ciprofloxacin and Synthesized Derivatives[a]

substance	P_{app}(a−b) ($\times 10^6$ cm/s)	P_{app}(b−a) ($\times 10^6$ cm/s)	ratio b/a	P_{app}(a−b + 1 μM GF) ($\times 10^6$ cm/s)	P_{app}(b−a + 1 μM GF) ($\times 10^6$ cm/s)	ratio b/a + 1 μM GF	BCS P_{app}	BCS $S_{pH6.5}$	BCS class
ciprofloxacin	0.19 ± 0.06	0.92 ± 0.03	5	nd	nd	nd	low	low	4
5a	1.01 ± 0.27	19.66 ± 0.60	19	22.01 ± 1.90	8.29 ± 1.32	0.4	high	high	1
5b	3.45 ± 0.53	24.31 ± 0.70	7	4.12 ± 0.21	3.69 ± 0.61	0.9	high	low	2
5c	4.69 ± 0.24	35.18 ± 4.51	7	4.56 ± 1.02	11.96 ± 0.76	2.8	high	low	2
5d	0.43 ± 0.04	62.75 ± 5.03	146	62.95 ± 1.93	26.93 ± 1.44	0.4	high	low	2
5e	4.95 ± 3.04	65.89 ± 0.65	13	21.56 ± 2.37	59.65 ± 2.42	2.6	high	high	1
5f	13.88 ± 3.40	263.55 ± 40.97	19	15.46 ± 1.59	7.76 ± 0.84	0.5	high	low	2
5g	6.42 ± 0.76	116.08 ± 9.24	18	21.54 ± 3.41	53.14 ± 3.50	2.5	high	low	2

[a]nd: not determined. Values are presented as the mean ± standard deviation based on triplicates.

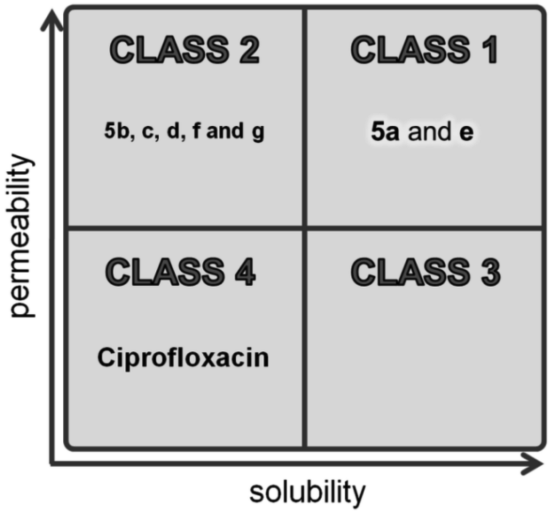

Figure 4. BCS classification of ciprofloxacin and synthesized derivatives.

increased chain length, and the reduction was 80 °C for the derivative with the most flexible substituent (**5f**) compared to the parent compound. **5a** reduced the melting point with 11 °C, although this substituent does not significantly increase the number of conformations formed. However, the methyl ester of **5a** blocks the intermolecular hydrogen bond formed between the hydroxyl function in the carboxylic acid and the carbonyl of the quinolone which is likely to affect the molecular packing and the hydrogen bond structure within the crystal lattice.[9] The less ordered crystal lattice resulted in equal or increased aqueous solubility for **5a**−**e**, whereas **5f** and **5g** as a consequence of increased lipophilicity showed 10- to 30-fold lower water solubility compared to ciprofloxacin. The results from the solubility and permeability studies were combined to sort the compounds into the BCS. All analogues were high permeability compounds, hence pushing the BCS 4 compound into class 1 or 2. **5a** showed high solubility in all media studied and hence was sorted as a BCS 1. **5e** was sorted as BCS 2 at pH 7.4 and as BCS 1 at pH 6.5. However, all other derivatives were BCS class 2 analogues and hence may be possible to transform to BCS 1 behaving compounds through formulations. Of the BCS 2 derivatives produced in this work, **5b** would be the most interesting to undertake such efforts for because of its low Do (3 and 5 at pH 7.4 and 6.5, respectively) and comparably low efflux ratio.

This work was undertaken to prove the impact of small molecular changes on the crystal lattice and hence the aqueous solubility and to simultaneously design these molecular fragments so that improved permeability is obtained. Therefore, little consideration was given to the biological effect and/or toxicity of the structural modifications and the derivatives were not tested for toxicity, potency, tissue distribution, or elimination. In vitro metabolism experiments performed showed that the derivatives were relatively stable with 0.5% (**5a**) to 2.4% (**5f**) ciprofloxacin formed after 1 h of incubation in hepatocytes. The compounds were also stable during transport and solubility studies.

To conclude, classical medicinal chemistry increased molecular flexibility and lipophilicity, resulting in improved solubility and permeability of a poorly absorbed model compound. These findings can guide medicinal chemists in optimizing poorly soluble, solid-state limited structures with regard to absorption through synthesis of compounds with less ordered crystal lattice using flexible, lipophilic side chains.

EXPERIMENTAL SECTION

Synthesis of Derivatives. All reactions were stirred under ambient conditions and monitored by TLC if not otherwise is stated. For detailed synthesis and analysis of intermediate compounds see the Supporting Information. Purity was determined with analytical RPHPLC−MS and elemental analysis. The HPLC system (Gilson) was connected to a Finnigan AQA quadrupole mass spectrometer. Onyxmonolithic C18 column (50 mm × 4.6 mm) and a gradient of CH_3CN/H_2O (0.05% HCOOH) with a flow rate of 4 mL/min were used, and detection was performed with UV (DAD, 214 and 254 nm) and MS (ESI). Elemental analysis was performed at Mikrokemi AB, Uppsala, Sweden. All products were >95% pure according to LC−MS and elemental analysis. Microscopy (Nikon Eclipse TS100, 40× magnification) was used to visually inspect particle size of obtained material. DSC (DSC6200, Seiko, Japan) using 0.5−1.0 mg of each derivative was performed to determine the melting temperatures (T_m) and to analyze possible occurrence of polymorph material or residual traces of organic solvents. Each sample was weighed into an aluminum pan and covered with a pierced lid before being heated to 50 °C above the expected melting temperature at a rate of 10 °C/min while being purged with liquid nitrogen. All experiments were performed in triplicate.

Free Bases of 1-Cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylates 5a−g. An amount of 50−100 mg of **4a**−**g** (see Supporting Information) was dissolved in 4−10 mL of MeOH and applied to a PL-HCO_3MP StratoSphere SPE column (Varian). Solvent was removed under vacuum, and cold diethyl ether was added to precipitate the final products which were obtained after filtration. The white crystals obtained were dried under vacuum and analyzed by ^{1}H and ^{19}F NMR, LC−MS, DSC, XRPD and by elemental analyses. **5a** was prepared from **4a**. ^{1}H NMR (400 MHz, D_2O): δ 1.11−1.20 (m, 2H), 1.34−1.43 (m, 2H), 3.50−3.68 (m, 9H), 3.89 (s, 3H), 7.52 (d, J = 7.6 Hz, 1H), 7.71 (d, J = 13.1 Hz, 1H), 8.67 (s, 1H). $C_{18}H_{20}FN_3O_3$ MW: 345. MS (ESI): m/z 346 [M + H$^+$]. Anal. ($C_{18}H_{20}FN_3O_3 \cdot 0.2H_2O$) C, H, N. Shows diffraction peaks in X-ray powder diffraction (XRPD) studies, indicating crystalline material. T_m:

254.5 ± 0.4 °C. **5b** was prepared from **4b**. ^{1}H NMR (400 MHz, D_2O): δ 1.09−1.21 (m, 2H), 1.33−1.45 (m, 5H), 3.44−3.66 (m, 9H), 4.37 (m, 2H), 7.52 (m, 1H), 7.71 (d, J = 13.1 Hz, 1H), 8.67 (s, 1H). $C_{19}H_{22}FN_3O_3$ MW: 359. MS (ESI): m/z 360 $[M + H]^+$. Anal. ($C_{19}H_{22}FN_3O_3$) C, H, N. Shows diffraction peaks in XRPD studies, indicating crystalline material. T_m: 222.2 ± 1.9 °C. **5c** was prepared from **4c**. ^{1}H NMR (400 MHz, D_2O): δ 1.12−1.21 (m, 2H), 1.34−1.46 (m, 8H), 3.49−3.71 (m, 9H), 5.22 (m, 1H), 7.55 (d, J = 7.5 Hz, 1H), 7.76 (d, J = 13.3 Hz, 1H), 8.68 (s, 1H). $C_{20}H_{24}FN_3O_3$ MW: 373. MS (ESI): m/z 374 $[M + H]^+$. Anal. ($C_{20}H_{24}FN_3O_3 \cdot 0.1H_2O$) C, H, N. Shows broad unresolved diffraction peaks in XRPD studies, indicating low crystalline material or amorphous material. T_m: 228.7 ± 0.7 °C. **5d** was prepared from **4d**. ^{1}H NMR (400 MHz, D_2O): δ 1.03 (t, J = 7.3 Hz, 3H), 1.11−1.19 (m, 2H), 1.34−1.41 (m, 2H), 1.80 (m, 2H), 3.49−3.67 (m, 9H), 4.17 (t, J = 6.7 Hz, 2H), 7.51 (d, J = 7.3 Hz, 1H), 7.69 (d, J = 13.1 Hz, 1H), 8.63 (s, 1H). $C_{20}H_{24}FN_3O_3$ MW: 373. MS (ESI): m/z 374 $[M + H]^+$. Anal. ($C_{20}H_{24}FN_3O_3$) C, H, N. Shows diffraction peaks in XRPD studies, indicating crystalline material. T_m: 217.4 ± 1.2 °C. **5e** was prepared from **4e**. ^{1}H NMR (400 MHz, D_2O): δ 1.03 (t, J = 7.4 Hz, 3H), 1.09−1.19 (m, 2H), 1.33−1.53 (m, 4H), 1.77 (m, 2H), 3.48−3.68 (m, 9H), 4.32 (t, J = 6.7 Hz, 2H), 7.50 (d, J = 7.3 Hz, 1H), 7.66 (d, J = 13.1 Hz, 1H), 8.62 (s, 1H). $C_{21}H_{26}FN_3O_3$: MW 387. MS (ESI): m/z 388 $[M + H]^+$. Anal. ($C_{21}H_{26}FN_3O_3$) C, H, N. Shows diffraction peaks in XRPD studies, indicating crystalline material. T_m: 197.0 ± 0.7 °C. **5f** was prepared from **4f**. ^{1}H NMR (400 MHz, D_2O): δ 1.03 (t, J = 7.4 Hz, 3H), 1.09−1.19 (m, 2H), 1.33−1.70 (m, 8H), 1.77 (m, 2H), 3.48−3.68 (m, 9H), 4.32 (t, J = 6.7 Hz, 2H), 7.50 (d, J = 7.3 Hz, 1H), 7.66 (d, J = 13.1 Hz, 1H), 8.62 (s, 1H). $C_{23}H_{30}FN_3O_3$ MW: 415. MS (ESI): m/z 416 $[M + H]^+$. Anal. ($C_{23}H_{30}FN_3O_3 \cdot 0.2H_2O$) C, H, N. Shows broad unresolved diffraction peaks in XRPD studies, indicating low crystalline material or amorphous material. T_m: 185.9 ± 0.5 °C. **5g** was prepared from **4g**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.07−1.16 (m, 2H), 1.26−1.34 (m, 2H), 3.11 (m, 4H), 3.26 (m, 4H), 3.41 (m, 1H), 5.39 (s, 2H), 7.23−7.55 (m, 6H), 8.05 (d, J = 13.3 Hz, 1H), 8.53 (s, 1H). $C_{24}H_{24}FN_3O_3$ MW: 421. MS (ESI): m/z 422 $[M + H]^+$. Anal. ($C_{24}H_{24}FN_3O_3$) C, H, N. Shows diffraction peaks in XRPD studies, indicating crystalline material. T_m: 200.0 ± 1.4 °C.

Solubility Measurements. The apparent solubility were measured in $PhB_{7.4}$, $PhB_{6.5}$, and FaSSIF using a previously described small shake flask method.[10] $PhB_{7.4}$ was prepared according to USP 25.[11] $PhB_{6.5}$ and FaSSIF were prepared according to the protocol developed by Galia and co-workers.[12] The compounds were weighed in excess into vials (n = 3) with 500 μL of solubility medium and shaken (37 °C, 300 rpm), and after 4 h (selected to reflect the time allowed for dissolution in the small intestine in vivo) the suspensions were centrifuged twice at 10 000g for 10 min to separate solid materials from the solution. The supernatant was determined for concentration with LC−MS/MS analysis. The derivatives were stable during the 4 h in all solvents. Standard error of solubility ratio (SE_{SR}) was calculated from

$$SE_{SR} = SR\sqrt{\frac{\sigma_{S_A}^{\,2}}{S_A^{\,2}} + \frac{\sigma_{S_B}^{\,2}}{S_B^{\,2}}} \quad (1)$$

in which SR is the solubility ratio between mean derivative solubility (S_A) over mean ciprofloxcacin solubility (S_B). σ_{S_A} and σ_{S_B} are the standard errors for the solubility mean values, respectively. The Do was calculated from

$$Do = \frac{M_0}{V_0 C_S} \quad (2)$$

where M_0 is dose, V_0 the volume (set to 250 mL), and C_S the measured apparent solubility.[4] The maximum oral dose given (according to the Swedish Physician Desk Reference) was 2.3 mmol, which was used for the BCS.

Cell Culture and Metabolism. Caco-2 cells (American Tissue Collection, Rockville, MD) were maintained in an atmosphere of 90% air and 10% CO_2 as described previously.[13] For transport experiments, monolayers formed from 3.3 × 10^5 cells seeded on polycarbonate filter inserts (12 mm diameter, pore size 0.4 μm; Costar) were used. The transport experiments were performed in a pH gradient of 6.5/7.4 in the apical to basolateral direction at 37 °C and were started by the application of the drug solution (10 μM) to the donor side. The filter inserts (n = 3) were stirred at 500 rpm, and the receiver chambers were sampled continuously for 60 min. The transport experiment was repeated with 1 μM GF120918,[14] to specifically inhibit P-gp. All samples were analyzed with LC−MS/MS directly after termination of the experiment. The derivatives were stable in the transport assay (no detection of ciprofloxacin) and used at nontoxic concentrations. Apparent permeability (P_{app}) was calculated from

$$P_{app} = \frac{\Delta Q}{\Delta t}\frac{1}{AC_0} \quad (3)$$

where $\Delta Q/\Delta t$ is steady-state flux (mol/s), C_0 is the initial concentration in the donor chamber at each time interval (mol/mL), and A is the surface area of the filter (cm^2).

Metabolism of the derivatives was investigated by incubating the derivatives (1 μM) for 60 min in 0.5 mg/mL pooled human liver microsomes (Invitrogen) and in 0.5 × 10^6 cells/mL freshly prepared human hepatocytes at 37 °C isolated according to a previously published protocol.[15] After termination of the experiment with MeCN (50% v/v) the samples were analyzed for concentration of ciprofloxacin and parent derivative with LC−MS/MS. Positive controls for esterase, CYP450, and UGT activity were p-nitrophenol acetate, dextromethorphan, midazolam, diclofenac, and 7OH-coumarin.

BCS. If the maximum oral dose (2.3 mmol, corresponding to 750 mg of ciprofloxacin) was soluble in 250 mL of $PhB_{6.5}$ and $PhB_{7.4}$, the derivatives were defined as highly soluble. Permeability values were corrected for the pH gradient and then superimposed onto our in-house correlation of P_{app} and fraction absorbed to determine high or low permeability.[5]

Analytical Methods. A ThermoFinnigan TSQ Quantum Discovery triple−quadrupole (electrospray ionization) coupled to a Waters Acquity UPLC instrument was used for concentration determination. A gradient was used (1% mobile phase B to 90% over 2 min total run) on a Waters HSS T3 1.8 μm column, 2 mm × 50 mm with mobile phase A consisting of 5% MeCN in water (0.1% formic acid) and mobile phase B 100% MeCN with 0.1% formic acid. The flow rate was 0.5 mL/min. An amount of 5 μL of sample was injected and detected in MRM mode. The limit of quantification (LoQ) was estimated based on the concentration that yielded a signal-to-noise ratio of >10. For the compound with lowest sensitivity (ciprofloxacin) the LoQ was 2 nM.

■ ASSOCIATED CONTENT

Supporting Information

Detailed experimental, analytical, and spectral data for **2**, **3a−g**, and **4a−g** and physicochemical properties of derivatives. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

FaSSIF, fasted state simulated intestinal fluid; $PhB_{6.5}$, phosphate buffer pH 6.5; $PhB_{7.4}$, phosphate buffer pH 7.4; BCS, biopharmaceutics classification system; Do, dose number; P_{app}, apparent permeability; LoQ, limit of quantification; DSC, differential scanning calorimetry; T_m, melting point; log *P*, octanol−water partition coefficient

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