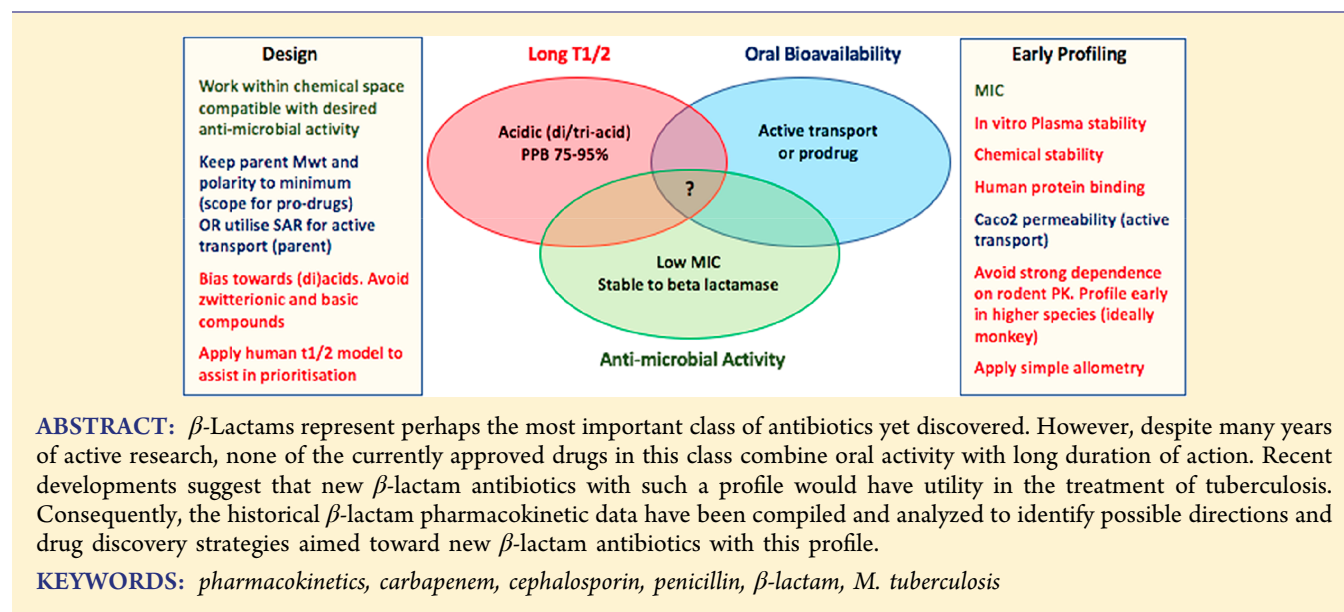# Pharmacokinetics of β-Lactam Antibiotics: Clues from the Past To Help Discover Long-Acting Oral Drugs in the Future

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Ⓢ Supporting Information

ABSTRACT: β-Lactams represent perhaps the most important class of antibiotics yet discovered. However, despite many years of active research, none of the currently approved drugs in this class combine oral activity with long duration of action. Recent developments suggest that new β-lactam antibiotics with such a profile would have utility in the treatment of tuberculosis. Consequently, the historical β-lactam pharmacokinetic data have been compiled and analyzed to identify possible directions and drug discovery strategies aimed toward new β-lactam antibiotics with this profile.

KEYWORDS: pharmacokinetics, carbapenem, cephalosporin, penicillin, β-lactam, M. tuberculosis

The discovery of Penicillin in 1928 and subsequent identification of its reactive β-lactam ring-containing structure heralded perhaps the most successful class of medicines yet discovered.[1,2] Decades of research building on this scientific landmark have led to the discovery of more than 100 marketed antibiotics using only a handful of chemical templates (Figure 1). The impact of this work on human medicine cannot be overstated.[3]

Despite this unprecedented success, new β-lactam antibiotics continue to be identified and developed.[4] Furthermore, after recent years of decline, interest in the field is re-emerging.[5,6] β-Lactams disrupt bacterial cell wall synthesis by inhibiting transpeptidases, involved in cross-linking peptides to form peptidoglycan. Over time, bacteria have evolved resistance mechanisms which have rendered many early drugs ineffective and obsolete prompting a continuing need for new research.[7−11]

Our interest in these antibiotics is linked with the ambition to find new drugs to kill *Mycobacterium tuberculosis* (*M.tb*), the pathogen responsible for tuberculosis (TB). There is huge unmet medical need in TB. Millions of people contract and die from this appalling disease every year.[12] Existing treatment regimens are complex (requiring combinations of several drugs), and long (typically 6 months or more).[13]

In common with other bacteria, *M.tb* utilizes transpeptidases for peptidoglycan cross-linking essential for cell wall synthesis.[14−16] Historically, early β-lactams showed poor anti-TB activity and were not pursued. For a long time this was erroneously attributed to poor drug penetration through the complex, lipophilic outer wall of *M.tb*.[17,18] Recent research has demonstrated that permeability is not generally a problem and that the lack of activity is mainly due to drug inactivation by BlaC, a chromosomally encoded extended spectrum class A β-lactamase produced by *M.tb*.[19] Co-administration of class A β-lactamase inhibitors, such as clavulanic acid[20] or avibactam,[21] enhance the anti-TB activity of some older penicillins and cephalosporins, while newer drugs with stability to class A β-lactamases, such as meropenem and other carbapenems, display promising anti-*M.tb* activity *in vitro* both alone and in combination with β-lactamase inhibitors.[20,22] *M.tb* mutants, in which BlaC is deleted, also show increased susceptibility to β-lactams[23] and studies have demonstrated that carbapenems and cephalosporins inactivate essential *M.tb* transpeptidases.[24−29] Based on these results, new medicinal chemistry
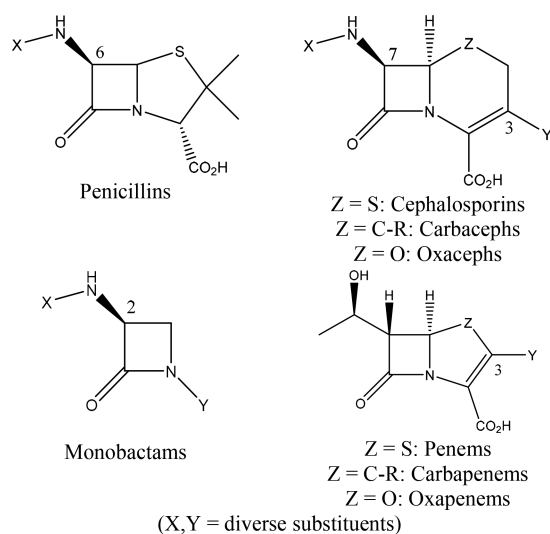
Figure 1. Sub-families of β-lactam antibiotics.

is emerging directed toward novel BlaC stable β-lactams able to inactivate *M.tb* transpeptidases.[30,31]

Further encouragement for the potential of β-lactams in TB treatment is also emerging from early clinical studies with amoxicillin-clavulanic acid and meropenem + amoxicillin-clavulanic acid.[32−36] Carbapenems have been successfully used to treat drug resistant patients[37] and more trials with existing TB-active β-lactams are in progress or planned.[38−41] Thus, one or more of the current β-lactam antibiotics could become part of future TB regimens.[42−44] Currently, however, it is not clear whether one of these drugs will be suitable for general use in all TB patients where infrequent oral dosing will be a requirement (ideally once daily with other combination drugs). A further relevant recent study using ertapenem/clavulanate in an *in vitro* hollow fiber model of TB demonstrated that, similar to β-lactam drugs in other bacteria,[45] time of drug exposure above MIC drives pathogen killing.[46] Thus, based on all the current evidence, orally bioavailable β-lactam antibiotics with long duration of action and potent activity vs *M.tb* would be attractive candidate drugs to investigate for universal TB treatment and, if they maintained broad spectrum antibacterial activity, could readily find application in other bacterial infections.[47]

A cursory inspection of the β-lactam pharmacopeia reveals both oral (dosed as parent or ester prodrug) and parenteral drugs. A small subset of the parenteral drugs have moderate elimination half-life (7 have human $t_{1/2} > 4$ h, see Figure S1), but most drugs in the class are rapidly eliminated ($t_{1/2} < 3$ h). Currently, no available drug is orally bioavailable with long duration of action. While sustained release formulations of oral β-lactams[48,49] and/or pro-drugging existing *M.tb* active parenteral drugs[50] may be productive, drugs with extended half-life should be efficacious at lower doses with longer dosing intervals.[51,52] It is therefore worthwhile to consider the possibility to discover new long-acting oral drugs from the class. In this context, a significant body of clinical and pre-clinical intravenous (i.v.) and oral β-lactam pharmacokinetic (PK) data has been published over many years. We have created an up to date compilation of PK parameters[53,54] and studied them to seek insights that might prove useful for future discovery work in the field. Specifically we investigated whether any structural features/properties could be identified that associated with long elimination half-life and/or oral bioavailability (human

PK data) and also for the class in general, we assessed how effectively human PK can be predicted from pre-clinical studies. (The PK data set compilation, literature references, and more detailed description of the methodology used are provided as Supporting Information.)

■ OBSERVATIONS FROM THE β-LACTAM PK DATA SET

Compounds and PK Data Retrieved. A total of 122 marketed β-lactam antibiotics and development compounds were identified and classified according to their sub-family and ionization class (Table 1). Unsurprisingly, penicillins and

Table 1. Dataset Content and Classification[a]

	di-/triacid	monoacid	neutral/basic	Z/A	zwitterionic	total
carbaceph				1		1
carbapenem		2	6 (3[b])		7	15
cephalosporin	9 (1[b])	28	6 (6[b])	10	9	62
monobactam	2					2
oxaceph	1					1
oxapenem		1				1
penem		4	1 (1[b])			5
penicillin	4	22	3 (3[b])	3	3	35
total	16	57	16	14	19	**122**

Records for 108 Parent Compounds and 14 Prodrugs						
PK parameters compiled[c]						
	V_d	Cl	fu	MRT	i.v. $t_{1/2}$	oral F	oral $t_{1/2}$
human	82	80	93	51	102	78	24
rat	37	40	39	16	53	19	9
dog	43	43	36	15	56	16	11
monkey	26	29	24	16	34	8	6
mouse	23	29	28	−	34	10	5

[a]The ionization classification used reflects the predominant species predicted in aqueous solution at pH 7.4. Di-/triacid predominant species charge < −1; monoacid predominant species charge = −1; neutral/basic predominant species is either neutral (uncharged) or net positively charged (includes 13 oral prodrug esters); Z/A indicates two major species predicted to be present (mixture of monoanion and zwitterion); zwitterionic major species is ionized but overall charge is neutral. [b]Prodrugs. [c]See Abbreviations for definitions.

cephalosporins make up the bulk of the set. Many drugs are monoacids due to the presence of the ubiquitous carboxylate critical to penicillin binding protein (PBP) inhibition. In some drugs these acids are modified to prodrug esters, and across the class an extensive range of additional basic, quaternary, and acidic groups have been appended (X,Y in Figure 1) populating the other ionization classes.

Searches of the primary literature were conducted by drug name and structure to locate references with *in vivo* PK data, focusing specifically on i.v. and oral studies in human and relevant pre-clinical species (rat, mouse, dog and monkey). (Table 1 and Tables S1 and S2). *In silico* physicochemical properties were calculated on all the drugs utilizing commercial software (Table S3).[55]

Human i.v. Data: Factors and Properties Associated with Long Half-Life Drugs. Except for a very small subset of neutral pro-drugs, all the β-lactam antibiotics possess functional groups which are ionized at physiological pH. The majority are

	All Compounds			Zwitterionic			All Acids			Di/triacids		
hFub (%)	n	$t_{1/2}$ (h) mean	Range	n	$t_{1/2}$ (h) mean	Range	n	$t_{1/2}$ (h) mean	Range	n	$t_{1/2}$ (h) mean	Range
≤5	6	2.7	0.9-4.5	0	-	-	6	2.7	0.9-4.5	1	4	-
6-25	20	2.3	0.6-8.6	1	2.2	-	19	2.3	0.6-8.6	4	5.3	4.0-8.6
26-50	17	1.3	0.4-3.2	1	0.8	-	15	1.3	0.4-3.2	5	2.1	1.3-3.2
>50	45	1.3	0.7-3.3	14	1.4	0.8-3.3	19	1.3	0.7-2.1	3	1.2	1.1-1.3

(d)
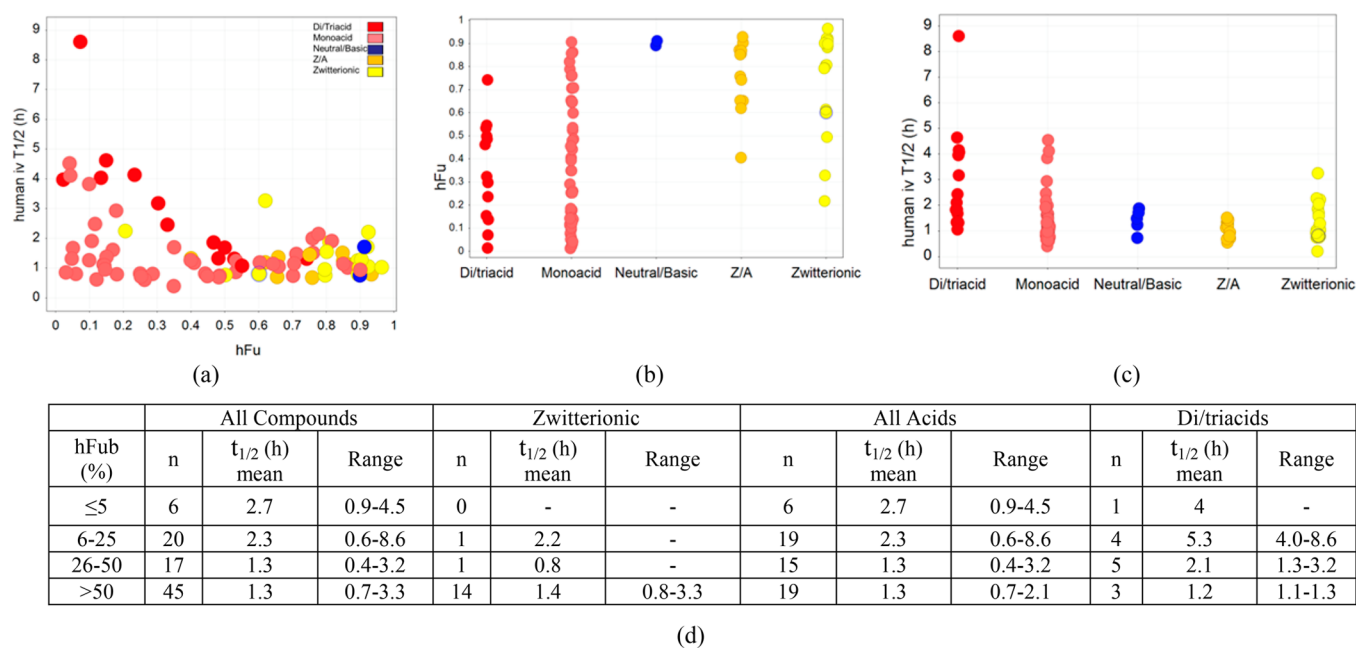
Figure 2. Impact of protein binding and ionization on human elimination half-life. Long-acting drugs tend to be di-/triacids with high plasma protein binding.

negatively charged or zwitterionic (Table 1). The influence of ionizable groups on PK properties has been well documented.[56,57] In general, anionic groups improve aqueous solubility, but simultaneously reduce passive membrane permeability. Acidic drugs generally have low volumes of distribution (typically driven by their poor permeability and/or high plasma protein binding to serum albumin). Renal clearance of unchanged drug also frequently predominates over hepatic metabolism (β-lactam clearance data in Tables S1 and S2). Since $t_{1/2} = 0.693\ V_d/CL$ it follows that most of the β-lactams (which generally have low V_d) would be expected to be short-acting. Additionally, since many of the drugs are largely excreted unchanged in urine, increasing plasma protein binding would also be anticipated to reduce renal clearance and thus extend the elimination half-life. In line with this, all of the longer acting β-lactams do have higher human plasma protein binding (Figure 2a). Mono- and diacidic drugs generally show the highest plasma protein binding (Figure 2b). By contrast, few zwitterionic drugs are highly bound, and almost all have short human half-life (Figure 2c). Across the data set there is a trend toward longer $t_{1/2}$ for di-/triacids (mean human $t_{1/2}$ for di-/triacids 3.0 h, all other classes 1.4 h).

In addition to ionization, the impact of other global physicochemical properties on half-life was also assessed. A cursory inspection confirmed all the β-lactam drugs with longest $t_{1/2}$ to be highly polar (log D < −1, PSA > 150) with high numbers of rotatable bonds, H-bond donors and acceptors. To probe more deeply, in silico molecular descriptors were calculated and a principal component analysis (PCA-XY) undertaken to look for possible correlations with the experimentally determined human half-life (see Tables S3−S5).[58]

The PCA scores showed that the set of drugs defines a homogeneously populated molecular property space (see Figure 3, below). Compounds at the boundaries include small drugs such as sulbactam and clavulanic acid (lower right quadrant) and drugs with high molecular weight (upper and lower left quadrants). Interestingly, the 10 drugs with the longest human $t_{1/2}$ (>3 h) cluster in a specific area of the PCA score plot suggesting that the set of calculated molecular properties used could provide a way to capture the molecular property profile associated with extended half-life. The PCA loadings (Figure S3) highlighted how the experimentally derived human in vivo PK parameters correlated with the calculated molecular descriptors. In the analysis we were interested in identifying properties that showed the strongest correlation with human $t_{1/2}$ and, for each descriptor, optimal values that could be used to inform medicinal chemistry optimization (Table 2 and Figure S4 for linear correlation scatter plots). Compound flexibility (expressed as number of rotatable bonds or fraction of rotatable bonds) showed a strong correlation with human half-life with more than 95% of the compounds with $t_{1/2} > 2$ h displaying 5−10 rotatable bonds or a fraction of rotatable bonds between 0.17 and 0.29. Similarly, compounds with $t_{1/2} > 2$ h are characterized by a total number of oxygen and nitrogen atoms ranging between 9 and 15 and a large polar surface area. The partition coefficient cyclohexane/water also correlates well (most favorable compounds value < −4).

The set of 101 compounds for which human half-life data were available was used to develop a statistical quantitative structure−activity relationship model for predicting the human half-life of new compounds. Initially the collection was randomly divided so that a training set (80% of the total) could be used for model learning to predict the half-life for the test set (20% of the total).

To assess the robustness and utility of the consensus model as a predictive tool, compounds in the test set were evaluated and a goodness of fit of $R^2 = 0.64$ was obtained between predicted and experimental values (Figure 4, Table S6). Although built with a relatively small data set, the model correctly classified 85% of compounds with very short half-life (11 out of 13 compounds with $t_{1/2} < 1.5$ h) and 78% of compounds with $t_{1/2} > 1.5$h (7 out 9 compounds). An expanded computational model for predicting human $t_{1/2}$ has been built
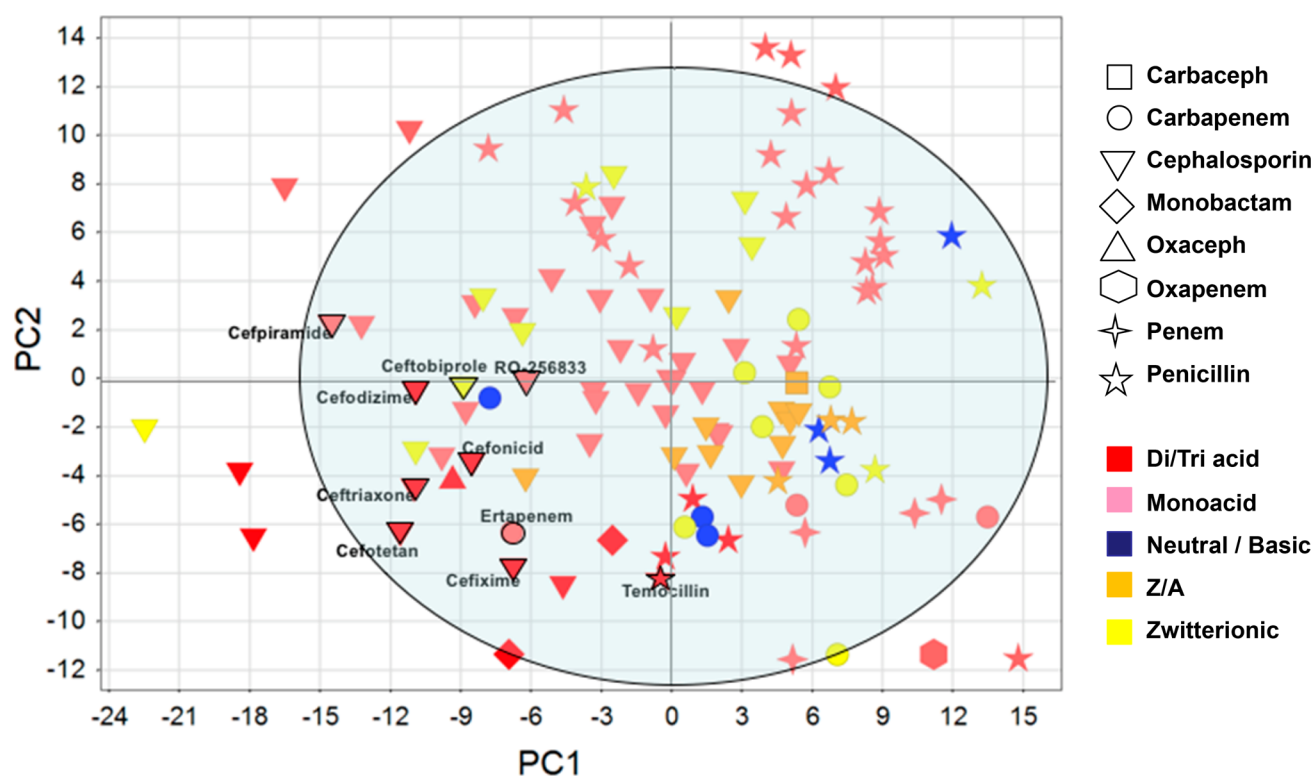
Figure 3. PCA scores defining the molecular property space of the β-lactam data set. The drugs named on the plot have the longest human $t_{1/2}$ (>3 h).

Table 2. Favorable Molecular Descriptor Ranges Capturing >95% of the β-Lactam Drugs with Human $t_{1/2}$ > 2 h

molecular descriptor	favorable range
no. of rotatable bonds	5−10
fraction of rotatable bonds	0.17−0.29
molecular polar surface area	>190 A^2
O and N atoms count	9−15
fraction of sp^3 carbons	<0.5
no. of sp^3 carbons	4−10
cyclohexane/water partition coefficient	< −4
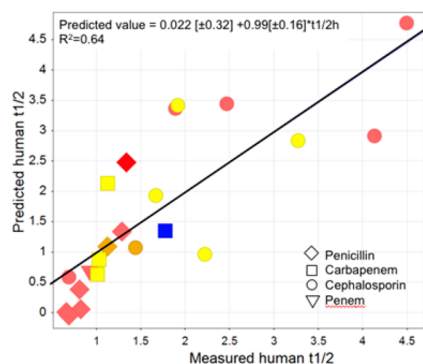
**Figure 4.** Correlation between calculated and experimental human $t_{1/2}$.

utilizing the full set of available human half-life data as training set (101 $t_{1/2}$ values) and is available on request.[59]

Human Oral Data: Factors Impacting Oral Bioavailability. As highlighted above, the physicochemical properties of parent β-lactam drugs generally lead to poor membrane permeability and many consequently have low human oral bioavailability. There are, however, exceptions to this (Figure 5a). Orally bioavailable β-lactam parent drugs utilize active transport mechanisms for absorption.[60] Drugs are initially sequestered into epithelial cells lining the intestinal lumen, frequently through the proton coupled peptide transporter PepT1 (SLC15A1) in the apical membrane.[61−64] PepT1 is a prominent member of an emerging group of transporters of clinical importance.[65] The structural similarity of the β-lactam cores to the natural substrate tripeptides is believed to be critical for PepT1 recognition.[66] However, since not all β-lactam drugs are transporter substrates, it is clear that the nature of the pendant substituents attached to the β-lactam ring is also important for binding and uptake. For cephalosporins and penicillins, many of the orally bioavailable drugs possess a basic amine in the 7- or 6-amide side chains, respectively. Furthermore, most of the orally bioavailable cephalosporin parent drugs possess a small 3-substituent (≤3 heavy atoms) suggesting that overall size may be important (Figure 5b, parent drugs).

The remaining orally active β-lactam drugs are ester prodrugs. Bioavailability data were retrieved for only a small number of these (Figure 5b, prodrugs). Interestingly, however, the examples with the highest human oral bioavailability fall within physicochemical property space where good oral bioavailability is frequently observed (Figure 5: log D = 0−2, MW < 550, HBD = 0−2).[67]

Pre-clinical Data. We analyzed the historical pre-clinical i.v. PK data to look for possible retrospective insights for predicting human PK that might inform future pre-clinical profiling strategies. Simple plots comparing animal and human PK parameters across the entire data set (Figure S5) suggested only weak correlations between human and monkey PK parameters (CL and $t_{1/2}$), with no correlations apparent in

a)

b)
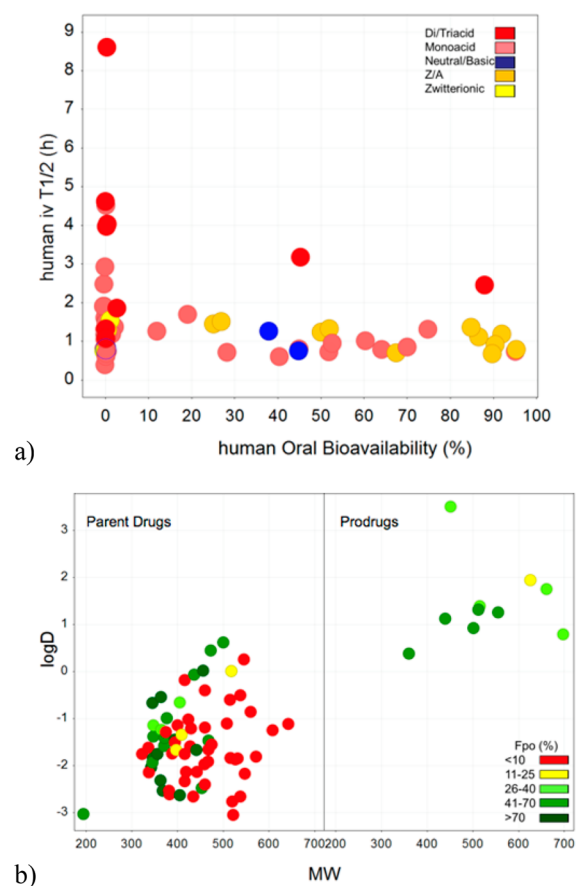
Figure 5. Orally bioavailable β-lactams. (a) Oral bioavailability vs $t_{1/2}$ showing ionization classification of the oral drugs. (b) Size and lipophilicity of actively transported parent drugs with good oral bioavailability are similar. Prodrugs with highest oral bioavailability tend to be smaller and less lipophilic.

other species. Several historical publications have detailed successful application of allometry to predict human PK for specific β-lactams.[68−71] We therefore assessed the general applicability of this approach to the class. The xenologic open-access allometry PK tool was utilized in this work.[72] A total of 43 compounds in the data set were available with PK parameters for human and at least one other preclinical species.

Utilizing this software, extrapolations based on monkey data delivered the best human predictions (Table 3 and Table S7. > 80% of compounds predicted within the 2-fold cut off, irrespective of parameter predicted). The next best species in accuracy of prediction was dog (77−79% of Clearance and half-life predictions were within 2-fold of experimental values, slightly less accurate for V_d ss (64%)). Human predictions based on mouse or rat PK were generally less accurate, a trend noted by other researchers with a more diverse set of drugs.[73,74] Similar accuracy of prediction (80−84%) was also obtained using a multi-species allometry approach with variable exponents on compounds for which PK data were available in at least three species.[75]

POTENTIAL DIRECTIONS FOR FUTURE RESEARCH AND PROSPECTS FOR FINDING NEW LONG-ACTING ORAL B-LACTAM ANTIBIOTICS

Compilation of the historical β-lactams and analysis of their PK data confirmed the ubiquitous highly polar and ionized nature of these drugs. It also highlighted ionization class and protein

Table 3. Allometric Scaling Predictions of Human PK Parameters from Pre-clinical Experimental Data, Accurate to within 2-fold

	Human Clearance Prediction				
	monkey	rat	mouse	dog	multi-species allometry
n	25	34	23	35	25
no. within 2-fold	20	20	15	27	21
% accuracy	80	59	65	77	84
% under-predicted	16	9	17	6	16
% over-predicted	4	32	17	17	−
	Human $t_{1/2}$ Prediction				
n	23	34	20	33	23
no. within 2-fold	22	17	14	26	19
% accuracy	96	50	70	79	83
% under-predicted	4	38	20	18	−
% over-predicted	−	9	10	3	17
	Human V_d ss Prediction				
n	23	33	20	33	23
no. within 2-fold	20	21	15	21	22
% accuracy	87	64	75	64	96
% under-predicted	9	15	20	18	−
% over-predicted	4	21	5	18	4

binding as key parameters to consider in future efforts targeting new long-acting drugs. Existing drugs from the class with low clearance and the longest human half-life are polar (di)acids with high plasma protein binding. By contrast, drugs with low-to-moderate human plasma protein binding (<75%) are generally shorter acting with higher clearance (Figure 2 and Figure S2).

The observed PK profile of β-lactams arises from the interplay of many *in vivo* processes (see Figure S7). In addition to normal drug discovery considerations stability of the β-lactam ring to hydrolysis needs to be taken into account.[76] To minimize clearance, future long-acting drugs will require excellent plasma stability (and to avoid susceptibility to other hydrolytic enzymes such as DHP-I[77,78]). Based on our analysis, synthetic efforts aimed at finding new plasma stable (di/tri) acidic analogues, targeting and prioritizing compounds with higher human plasma protein binding for *in vivo* evaluation would be expected to maximize the likelihood of delivering drug candidates with low clearance and extended half-life. The computational model we have developed could also be utilized. Although built from a limited data set, it reliably identifies compounds characterized by a very low $t_{1/2}$. Hence potential new targets for synthesis predicted in that range could be deprioritized (or redesigned). Extending the duration of action by increasing protein binding to slow the elimination of unchanged parent drug clearly needs to be balanced with the requirement to deliver and sustain drug levels in target tissues sufficient for anti-bacterial activity[79−81] (Figure S7). While our analysis takes no account of possible metabolism or non-linearity in protein binding (seen with some β-lactam drugs),[82] or consideration of specific mechanisms potentially involved in drug elimination,[78] the historical data suggest that a reasonable starting point for balancing these considerations would be to prioritize new compounds for *in vivo* evaluation with human fub in the range 6−25% (Figure 2d and Figure S2).

An alternative approach to finding new β-lactams with extended half-life would be to design and synthesize derivatives

with the explicit aim of reducing renal clearance (maintaining low hepatic clearance and good plasma stability). This could be envisaged, for example, via the judicious introduction of metabolically stable lipophilic groups, compatible with anti-bacterial activity, onto the β-lactam cores. Although this approach would clearly not be without significant challenges (e.g., given the inherent highly polar nature of the β-lactams it may be necessary to increase molecular weight significantly and other favorable properties of these drugs such as their excellent aqueous solubility could be compromised) it would represent a departure from the past where drug discovery in this field was mainly driven by consideration of anti-bacterial profile (many of the drugs were discovered in an era where pre-clinical PK evaluation was not routinely performed). Applying the tools of modern drug discovery this approach warrants serious consideration.

Whichever approach is employed, conducting early pre-clinical i.v. PK studies will be important to gain understanding around the hepatic/renal clearance routes of new compounds and inform the ensuing medicinal chemistry.

The overall polar and charged properties of most β-lactams, including all the long-acting parenteral drugs, are generally associated with poor oral bioavailability. Despite this, both active uptake and pro-drug strategies have historically delivered oral drugs. Considering the active transport approach, expanding SAR for PepT1 transport in an appropriate *in vitro* assay could assist in finding new orally bioavailable β-lactam parent drugs.[83] *In silico* models for PepT1 recognition have been developed and structural studies are now emerging which will potentially facilitate the rational design of further substrates.[84–90] However, since PepT1 is only one of several transporters utilized by β-lactams and only mediates the first step in absorption, such data may not correlate well with oral bioavailability. (Published data indicate that some β-lactam drugs are accumulated intracellularly and metabolized, suggesting that SAR for the basolateral membrane transporters and PepT1 may not be identical.) In light of this, utilizing *in vitro* permeability/flux assays in cell lines expressing relevant transporters offers a more pragmatic way forward. A study with 23 β-lactam drugs demonstrated a good correlation between the overall net flux of intact β-lactams through a Caco-2 monolayer and human oral bioavailability.[91] Permeation across rat jejunum also correlated well with human bioavailability for a small set of β-lactams.[92] In future discovery work, early profiling of new molecules in a suitable cell permeability assay should rapidly identify actively transported parent drugs with potential to show good oral bioavailability.

Within the di-/triacid group (highlighted with highest potential for long $t_{1/2}$) only cefixime and ceftibuten are orally active (via active transport).[93,94] Multiple transporters have been implicated in the uptake of these two drugs so finding future oral di-/triacids utilizing similar mechanisms is likely to rely on serendipity. Alternatively, synthesis of orally bioavailable prodrugs of long-acting (di-/triacid) β-lactams unable to utilize active transport mechanisms can also be envisaged. Since historic prodrugs with the highest human oral bioavailability fall within property space where orally bioavailable drugs are frequently found, it follows that carefully controlling physicochemical properties should produce prodrug candidates with good passive permeability. Thus, minimizing the size and polarity of the parent β-lactam pendant substituents (X,Y in Figure 1) as far as possible (within the constraints required for anti-bacterial activity and moderate-high plasma protein binding) should maximize space to introduce pro-drugging ester substituents

and, by implication, the likelihood of finding orally bioavailable prodrugs.[95,96]

Prioritizing active compounds for *in vivo* profiling and generating data to build confidence early in discovery that the desired human PK profile will ultimately be attained is critical in drug discovery. Our analysis emphasizes a further challenge in the β-lactam field which is that rodent and human PK frequently do not correlate well. In general, i.v. PK studies in monkey appear to offer reasonable predictions for human, but it is not feasible to conduct such studies routinely at an early stage of drug discovery. Based on the historical data, a pragmatic approach would be to apply the simple allometry from mouse i.v. PK data (65–75% of predictions within 2-fold of the actual human values) but interpret the results with caution and rapidly advance the most promising compounds into a second PK species to increase confidence.

In summary, new orally bioavailable β-lactam antibiotics with long elimination half-life have the potential for widespread use in the treatment of TB and other bacterial diseases. Historical β-lactam PK data have been compiled and used to suggest pragmatic drug discovery strategies which could be pursued in future research efforts. Based on our analysis, and given the lack of current drugs with this profile, it is clear that combining together the features needed for long half-life and good oral bioavailability along with the desired anti-bacterial profile is very challenging, and it remains to be seen if this goal is ultimately achievable. However, since much of the early medicinal chemistry in this field was empirically driven, it is possible that utilizing future strategies with more emphasis on PK properties could yet deliver new antibiotics with the potential to be transformational in TB and other bacterial infections.

■ ASSOCIATED CONTENT

ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsinfecdis.8b00160.

> Notes on data compilation selection criteria; Table S1: Compilation of PK parameter values extracted from literature; Table S2: Mean PK parameter values; Table S3: Compound classification and calculated properties; Table S4: Smiles Strings; Figure S1: Structures of long-acting drugs; Figure S2: Human clearance and impact of plasma protein binding; Table S5: PCA scores; Table S6: Experimental and calculated human $t_{1/2}$ values; Table S7: Human PK predictions from allometry; additional details of the analysis of molecular descriptors and development of the computational human half-life prediction model; Figure S4: Linear correlation scatter plots summarized in the main article; methodology used in the allometry calculations; Figure S5: Simple plots of human vs animal PK parameters; Figure S6: Simple plots of in silico properties vs human $t_{1/2}$; Figure S7: Generalized scheme highlighting key processes relevant to the PK profile and antibacterial efficacy of β-lactam antibiotics; and additional references (PDF)

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

CL, plasma or total clearance (mL/min/kg); fu, fraction unbound in plasma; i.v. $t_{1/2}$, elimination half-life after an intravenous dose (h); MIC, minimum inhibitory concentration; MRT, mean residence time (h); M.tb, *Mycobacterium tuberculosis*; TB, tuberculosis; oral F, bioavailability after oral dosing (%); oral $t_{1/2}$, elimination half-life after oral dosing; PCA, principal component analysis; PK, pharmacokinetics; PSA, polar surface area; V_d, Volume of distribution (L/kg)

■ NOTES

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■ REFERENCES

(1) Fleming, A. (1929) On the antibacterial action of cultures of a penicillium, with special reference to their use in the isolation of B. influenzæ. *Br. J. Exp. Pathol.* 10 (31), 226–236.

(2) Crowfoot, D., Bunn, C. W., Rogers-Low, B. W., and Turner-Jones, A. (1949). X-ray crystallographic investigation of the structure of Penicillin, in *Chemistry of Penicillin* (Clarke, H. T., Johnson, J. R., and Robinson, R., Eds.), pp 310–367, Princeton University Press.

(3) Kardos, N., and Demain, A. L. (2011) Penicillin: the medicine with the greatest impact on therapeutic outcomes. *Appl. Microbiol. Biotechnol.* 92 (4), 677–87.

(4) Butler, M. S., Blaskovich, M. A., and Cooper, M. A. (2017) Antibiotics in the clinical pipeline at the end of 2015. *J. Antibiot.* 70 (1), 3–24.

(5) Qin, W., Panunzio, M., and Biondi, S. (2014) Beta lactam antibiotics renaissance. *Antibiotics (Basel, Switz.)* 3 (2), 193–215.

(6) Kong, K.-F., Schneper, L., and Mathee, K. (2010) Beta-lactam Antibiotics: From Antibiosis to Resistance and Bacteriology. *APMIS* 118 (1), 1–36.

(7) Eze, U. A., Eze, N. M., and Mustapha, A. (2015) Enzymatic inactivation of Penicillins: an emerging threat to global public health. *Int. J. Pharm. Sci. Res.* 6 (8), 3151–3160.

(8) Sun, J., Deng, Z., and Yan, A. (2014) Bacterial multidrug efflux pumps: Mechanisms, physiology and pharmacological exploitations. *Biochem. Biophys. Res. Commun.* 453 (2014), 254–267.

(9) Talbot, G. H. (2013) β-Lactam antimicrobials: what have you done for me lately? *Ann. N. Y. Acad. Sci.* 1277 (1), 76–83.

(10) Heesemann, J. (1993) Mechanisms of resistance to beta lactam antibiotics. *Infection* 21 (Suppl 1), S4–S9.

(11) King, D. T., Sobhanifar, S., and Strynadka, N. C. (2016) One ring to rule them all: Current trends in combating bacterial resistance to the beta lactams. *Protein Sci.* 25 (4), 787–803.

(12) WHO, Global tuberculosis report 2017, http://www.who.int/tb/publications/global_report/en/.

(13) D'Ambrosio, L., Centis, R., Sotgiu, G., Pontali, E., Spanevello, A., and Migliori, G. B. (2015) New anti-tuberculosis drugs and regimens: 2015 update. *ERJ. Open Res. 1*, 00010-2015.

(14) Sahare, P., and Moon, A. (2014) Penicillin binding proteins: An insight into novel antibacterial drug target. *Int. J. Sci. Eng. Res.* 5 (8), 13–23.

(15) Schoonmaker, M., Bishai, W., and Lamichhane, G. (2014) Nonclasssical transpeptidases of mycobacterium tuberculosis alter cell size, morphology, the cytosolic matrix, protein localization, virulence and resistance to beta lactams. *J. Bacteriol.* 196 (7), 1394–1402.

(16) Erdemli, S. B., Gupta, R., Bishai, W. R., Lamichhane, G., Amzel, L. M., and Bianchet, M. A. (2012) Targeting the cell wall of Mycobacterium tuberculosis: Structure and mechanism of L,D-transpeptidase 2. *Structure* 20 (12), 2103–2115.

(17) Jankute, M., Cox, J. A., Harrison, J., and Besra, G. S. (2015) Assembly of the Mycobacterial Cell Wall. *Annu. Rev. Microbiol.* 69, 405–23.

(18) Jarlier, V., Gutmann, L., and Nikaido, H. (1991) Interplay of cell wall barrier and beta-lactamase activity determines high resistance to beta-lactam antibiotics in Mycobacterium chelonae. *Antimicrob. Agents Chemother.* 35, 1937–1939.

(19) Wang, F., Cassidy, C., and Sacchettini, J. (2006) Crystal Structure and Activity Studies of the Mycobacterium tuberculosis β-Lactamase Reveal Its Critical Role in Resistance to β-Lactam Antibiotics. *Antimicrob. Agents Chemother.* 50 (8), 2762–2771.

(20) Hugonnet, J. E., Tremblay, L. W., Boshoff, H. I., Barry, C. E., III, and Blanchard, J. S. (2009) Meropenem-clavulanate is effective against extensively drug-resistant Mycobacterium tuberculosis. *Science* 323, 1215–1218.

(21) Deshpande, D., Srivastava, S., Chapagain, M., Magombedze, G., Martin, K. R., Cirrincione, K. N., Lee, P. S., Koeuth, T., Dheda, K., and Gumbo, T. (2017) Ceftazidime-avibactam has potent sterilizing activity against highly drug resistant tuberculosis. *Sci. Adv. 3*, e1701102.

(22) Kaushik, A., Makkar, N., Pandey, P., Parrish, N., Singh, U., and Lamichhane, G. (2015) Carbapenems and Rifampicin Exhibit synergy against Mycobacterium tuberculosis and Mycobacterium acscessus. *Antimicrob. Agents Chemother.* 59, 6561–6567.

(23) Flores, A. R., Parsons, L. M., and Pavelka, M. S., Jr. (2005) Genetic analysis of the β-lactamases of mycobacterium tuberculosis and mycobacterium smegmatis and susceptibility to beta β-lactam antibiotics. *Microbiology* 151, 521–532.

(24) Wivagg, C. N., Bhattacharyya, R. P., and Hung, D. T. (2014) Mechanisms of β-lactam killing and resistance in the context of Mycobacterium tuberculosis. *J. Antibiot.* 67, 645–654.

(25) Cordillot, M., Dubee, V., Triboulet, S., Dubost, L., Marie, A., Hugonnet, J.-E., Arthur, M., and Mainardi, J.-L. (2013) In vitro cross-linking of Mycobacterium peptidoglycan by L,D transpeptidases and inactivation of these enzymes by carbapenems. *Antimicrob. Agents Chemother.* 57 (12), 5940–5945.

(26) Li, W. J., Li, D. F., Hu, Y. L., Zhang, Z. E., Bi, L. J., and Wang, D. C. (2013) Crystal structure of L,D-transpeptidase LdtMt2 in complex with Meropenem reveals the mechanism of carbapenem against Mycobacterium tuberculosis. *Cell Res.* 23, 728–731.

(27) Dubee, V., Triboulet, S., Mainardi, J. L., Etheve-Quelquejeu, M., Gutmann, L., Marie, A., Dubost, L., Hugonnet, J. E., and Arthur, M. (2012) Inactivation of mycobacterium tuberculosis L,D trans-peptidase LdtMt1 by carbapenems and Cephalosporins. *Antimicrob. Agents Chemother.* 56 (8), 4189–4195.

(28) Dhar, N., Dubée, V., Ballell, L., Cuinet, G., Hugonnet, J. E., Signorino-Gelo, F., Barros, D., Arthur, M., and McKinney, J. D. (2015) Rapid Cytolysis of Mycobacterium tuberculosis by Faropenem, an Orally Bioavailable β-Lactam Antibiotic. *Antimicrob. Agents Chemother.* 59 (2), 1308–1319.

(29) Jaganath, D., Lamichhane, G., and Shah, M. (2016) Carbapenems against Mycobacterium tuberculosis: a review of the evidence. *Int. J. Tuberc Lung Dis* 20 (11), 1436–1447.

(30) Iannazzo, L., Soroka, D., Triboulet, S., Fonvielle, M., Compain, F., Dubee, V., Mainardi, J. L., Hugonnet, J. E., Braud, E., Arthur, M.,

and Etheve-Quelquejeu, M. (2016) Routes of Synthesis of Carbapenems for Optimizing Both the Inactivation of l,d-Transpeptidase LdtMt1 of Mycobacterium tuberculosis and the Stability toward Hydrolysis by β-Lactamase BlaC. *J. Med. Chem.* 59 (7), 3427–3438.

(31) Kumar, P., Kaushik, A., Lloyd, E. P., Li, S.-G., Mattoo, R., Ammerman, N. C., Bell, D. T., Perryman, A. L., Zandi, T. A., Ekins, S., Ginell, S. L., Townsend, C. A., Freundlich, J. S., and Lamichhane, G. (2017) *Nat. Chem. Biol.* 13 (1), 54–61.

(32) Diacon, A. H., van der Merwe, L., Barnard, M., von Groote-Bidlingmaier, F., Lange, C., Garcia-Basteiro, A. L., Sevene, E., Ballell, L., and Barros-Aguirre, D. (2016) Beta lactams against tuberculosis − New trick for an old dog? *N. Engl. J. Med.* 375 (4), 393–394.

(33) Chambers, H. F., Kocagoz, T., Sipit, T., Turner, J., and Hopewell, P. C. (1998) Activity of amoxicillin/clavulanate in patients with tuberculosis. *Clin. Infect. Dis.* 26, 874–877.

(34) Donald, P. R., Sirgel, F. A., Venter, A., et al. (2001) Early bactericidal activity of amoxicillin in combination with clavulanic acid in patients with sputum smear positive pulmonary tuberculosis. *Scand. J. Infect. Dis.* 33, 466–469.

(35) Nadler, J. P., Berger, J., Nord, J. A., Cofsky, R., and Saxena, M. (1991) Amoxicillin-Clavulanic Acid for Treating Drug-Resistant Mycobacterium tuberculosis. *Chest* 99 (4), 1025–1026.

(36) Keener, A. B. (2014) Oldie but goodie: Repurposing penicillin for tuberculosis. *Nat. Med.* 20 (9), 976–978.

(37) Sotgiu, G., D'Ambrosio, L. D., Centis, R., Tiberi, S., Esposito, S., Dore, S., Spanevello, A., and Migliori, G. B. (2016) Carbapenems to treat Multidrug and extensively drug resistant Tuberculosis: A systematic review. *Int. J. Mol. Sci.* 17, 373–382.

(38) ClinicalTrials.gov NCT01730664, PK/PD of Ertapenem In Patients With TB.

(39) ClinicalTrials.gov NCT03174184, Early Bactericidal Activity of Rifampin + Meropenem + Amoxicillin/Clavulanate in Adults With Pulmonary TB.

(40) ClinicalTrials.gov NCT02381470, Early Bactericidal Activity Study of Faropenem (With Amoxicillin/Clavulanic Acid) in Patients With Pulmonary Tuberculosis.

(41) van Rijn, S. P., van Altena, R., Akkerman, O. W., van Soolingen, D., van der Laan, T., de Lange, W. C., Kosterink, J. G., van der Werf, T. S., and Alffenaar, J. W. (2016) Pharmacokinetics of ertapenem in patients with multidrug-resistant tuberculosis. *Eur. Respir. J.* 47, 1229–1234.

(42) Ramon-Garcia, S., del Rio, R. G., Villarejo, A. S., Sweet, G. D., Cunningham, F., Barros, D., Ballell, L., Mendoza-Losana, A., Ferrer-Bazaga, S., and Thompson, C. J. (2016) Repurposing clinically approved Cephalosporins for tuberculosis therapy. *Sci. Rep.* 6, 34293.

(43) Rullas, J., Dhar, N., McKinney, J. D., Garcia-Perez, A., Lelievre, J., Diacon, A. H., Hugonnet, J. E., Arthur, M., Angulo-Barturen, I., Barros-Aguirre, D., and Ballell, L. (2015) Combinations of beta lactam antibiotics currently in clinical trials are efficacious in a DHP-I-Deficient Mouse Model of Tuberculosis Infection. *Antimicrob. Agents Chemother.* 59 (8), 4997–4999.

(44) Kurz, S. G., and Bonomo, R. A. (2012) Reappraising the use of beta lactams to treat tuberculosis. *Expert Rev. Anti-Infect. Ther.* 10 (9), 999–1006.

(45) Auckenthaler, R. (2002) Pharmacokinetics and pharmacodynamics of oral beta lactam antibiotics as a two dimensional approach to their efficacy. *J. Antimicrob. Chemother.* 50 (T1), 13–17.

(46) van Rijn, S. P., Srivastava, S., Wessels, M. A., van Soolingen, D., Alffenaar, J. W., and Gumbo, T. (2017) The sterilizing effect of ertapenem-clavulanate in a hollow fiber model of tuberculosis and implications on clinical dosing. *Antimicrob. Agents Chemother.* 61 (9), e02039 DOI: 10.1128/AAC.02039-16.

(47) Potel, G., Chau, N. P., Pangon, B., Fantin, B., Vallois, J. M., Faurisson, F., and Carbon, C. (1991) Single daily dosing of antibiotics: Importance of in vitro killing rate, serum half-life and protein binding. *Antimicrob. Agents Chemother.* 35 (10), 2085–2090.

(48) Hoffman, A., Danenberg, H. D., Katzhendler, I., Shuval, R., Gilhar, D., and Friedman, M. (1998) Pharmacodynamic and pharmacokinetic rationales for the development of an oral controlled-release amoxicillin dosage form. *J. Controlled Release* 54, 29–37.

(49) Extended release formulation of beta-lactam antibiotics, Int. Patent Appl. WO 2005030178.

(50) Teitelbaum, A. M., Meissner, A., Harding, R. A., Wong, C. A., Aldrich, C. C., and Remmel, R. P. (2013) Synthesis, pH-dependent, and plasma stability of Meropenem prodrugs for potential use against drug-resistant tuberculosis. *Bioorg. Med. Chem.* 21 (17), 5605–17.

(51) Gunaydin, H., Altman, M. D., Ellis, J. M., Fuller, P., Johnson, S. A., Lahue, B., and Lapointe, B. (2018) Strategy for Extending Half-life in Drug Design and its Significance. *ACS Med. Chem. Lett.* 9, 528.

(52) Smith, D. A., Beaumont, K., Maurer, T. S., and Di, L. (2018) Relevance of Half-Life in Drug Design. *J. Med. Chem.* 61, 4273.

(53) The present data set was extended from several previous literature compilations: Bergan, T. (1984) Pharmacokinetics of beta-lactam antibiotics. *Scand. J. Infect. Dis.* 42 (Suppl), 83–98. Obach, R. S., Lombardo, F., and Waters, N. J. (2008) Trend Analysis of a Database. *Drug Metab. Disposition* 36 (7), 1385–1405. Kwan, K. C., and Rogers, J. D. (1983) Pharmacokinetics of beta lactam antibiotics, *Antibiotics*, Chap. 15, pp 247–270, Springer. Bergan, T. (1987) Pharmacokinetic properties of the Cephalosporins. *Drugs* 34 (Suppl 2), 89–104.

(54) Additional data without primary literature sources from the Pharmapendium database (https://www.pharmapendium.com) were also included in the compilation along with some unpublished GSK data (for details, see Supporting Information).

(55) The analyses and plots presented used subsets of the 122 compounds where all the relevant experimental data were retrieved. Commercial software: *PipelinePilot*, version 9.1.0.13, BIOVIA (http://accelrys.com/products/collaborative-science/biovia-pipeline-pilot/). *Stardrop*, version 6.0, Optibrium (www.optibrium.com/stardrop/). *VolSurf+*, version 1.0.7.l, Molecular Discovery (www.moldiscovery.com/software/vsplus/). *Simca*, version 14.0.0.1359, Umetrics (http://umetrics.com/products/simca).

(56) Manallack, D. T., Prankerd, R. J., Yuriev, E., Oprea, T. I., and Chalmers, D. K. (2013) The Significance of Acid/Base Properties in Drug Discovery. *Chem. Soc. Rev.* 42 (2), 485–496.

(57) Charifson, P. S., and Walters, W. P. (2014) Acidic and Basic Drugs in Medicinal Chemistry: A Perspective. *J. Med. Chem.* 57 (23), 9701–9717.

(58) Prodrugs were converted into the correspondent drug molecule (see Supporting Information).

(59) The authors can be contacted for a copy of the model. Software licenses required: PipelinePilot, Stardrop, Volsurf (see ref 55).

(60) Sinko, P. J., and Amidon, G. L. (1988) Characterisation of the oral absorption of beta lactam antibiotics I. Cephalosporins: Determination of Intrinsic membrane absorption parameters in the rat. *Pharm. Res.* 5 (10), 645–650.

(61) Herrera-Ruiz, D., Wang, Q., Cook, T. J., Knipp, G. T., Gudmundsson, O. S., Smith, R. L., and Faria, T. N. (2001) Spatial Expression Patterns of Peptide Transporters in the Human and Rat Gastrointestinal Tracts, Caco-2 In vitro cell culture model, and multiple human tissues. *AAPS Pharm. Sci.* 3 (1), 100.

(62) Brandsch, M., Knutter, I., and Bosse-Doenecke, E. (2008) Pharmaceutical and pharmacological importance of peptide transporters. *J. Pharm. Pharmacol.* 60 (5), 543–85.

(63) Brandsch, M. (2013) Drug Transport via the Intestinal peptide transporter PepT1. *Curr. Opin. Pharmacol.* 13, 881–887.

(64) Brandsch, M. (2009) Transport of Drugs by proton-coupled peptide transporters: pearls and pitfalls. *Expert Opin. Drug Metab. Toxicol.* 5 (8), 887–905.

(65) Hillgren, K. M., Keppler, D., Zur, A. A., Giacomini, K. M., Stieger, B., Cass, C. E., and Zhang, L. (2013) Emerging Transporters of Clinical Importance: An Update from the International Transporter Consortium. *Clin. Pharmacol. Ther.* 94 (1), 52–63.

(66) Bailey, P. D., Boyd, C. A. R., Bronk, J. R., Collier, I. D., Meredith, D., Morgan, K. M., and Temple, C. S. (2000) How to Make

Drugs Orally Active: A Substrate Template for Peptide Transporter PepT1. *Angew. Chem., Int. Ed.* 39 (3), 505−508.

(67) Lipinski, C. A., Lombardo, F., Dominy, B. W., and Feeney, P. J. (1997) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Delivery Rev.* 23, 3−25.

(68) Sawada, Y., Hanano, M., Sugiyama, Y., and Iga, T. (1984) Prediction of the disposition of beta lactam antibiotics in humans from pharmacokinetic parameters in animals. *J. Pharmacokinet. Biopharm.* 12 (3), 241−261.

(69) Richter, W. F., Heizmann, P., Meyer, J., Starke, V., and Lave, T. (1998) Animal pharmacokinetics and interspecies scaling of Ro-25−6833 and related (Lactamylvinyl)Cephalosporins. *J. Pharm. Sci.* 87 (4), 496−500.

(70) Wajima, T., Yano, Y., Fukumura, K., and Oguma, T. (2004) Prediction of human pharmacokinetic profile in animal scale up based on normalizing time course profiles. *J. Pharm. Sci.* 93 (7), 1890−1900.

(71) Kurihara, A., Naganuma, H., Hisaoka, M., Tokiwa, H., and Kawahara, Y. (1992) Prediction of human pharmacokinetics of panipenem-betamipron, a new carbapenem, from animal data. *Antimicrob. Agents Chemother.* 36 (9), 1810−1816.

(72) This tool applies fixed exponents for Clearance (0.75) and V_{d}ss (1.0). Development of the software was funded by the Bill and Melinda Gates Foundation for use in neglected diseases and is available as free download from the MMV website or at https://www.dropbox.com/s/6bxicyac5kjils1/PKTool.pdf?dl=0.

(73) Imam, M. T., Venkateshan, M. D., Tandon, M., Saha, N., and Pillai, K. K. (2011) Comparative Evaluation of US Food and Drug Administration and Pharmacologically Guided Approaches to Determine the Maximum Recommended Starting Dose for First-in-Human Clinical Trials in Adult Healthy Men. *J. Clin. Pharmacol.* 51, 1655−1664.

(74) Caldwell, G. W., Masucci, J. A., Yan, Z., and Hageman, W. (2004) Allometric scaling of pharmacokinetic parameters in drug discovery: Can human CL, V_{ss} and $t_{1/2}$ be predicted from in-vivo rat data? *Eur. J. Drug Metab. Pharmacokinet.* 29 (2), 133−143.

(75) Kang, H. E., and Lee, M. G. (2011) Approaches for predicting human pharmacokinetics using interspecies pharmacokinetic scaling. *Arch. Pharmacal Res.* 34, 1779−88.

(76) *β*-Lactam hydrolysis mechanisms reviewed in the following: Deshpande, A. G., Baheti, K. G., and Chatterjee, N. R. (2004) Degradation of beta lactam antibiotics. *Curr. Sci.* 87 (12), 1684−1695.

(77) Drusano, G. L. (1986) An overview of the pharmacology of imipenem/cilastatin. *J. Antimicrob. Chemother.* 18 (Suppl E), 79−92.

(78) Kropp, H., Sundelof, J. G., Hajdu, R., and Kahan, F. M. (1982) Metabolism of Thienamycin and Related Carbapenem Antibiotics by the Renal Dipeptidase, Dehydropeptidase-I. *Antimicrob. Agents Chemother.* 22, 62−70.

(79) Honeybourne, D. (1994) Antibiotic penetration into lung tissues. *Thorax* 49, 104−106.

(80) Muller, M., dela Pena, A., and Derendorf, H. (2004) Issues in Pharmacokinetics and pharmacodynamics of anti-infective agents: Distribution in Tissue. *Antimicrob. Agents Chemother.* 48, 1441−1453.

(81) While *β*-lactam antibiotics work through an irreversible mechanism, in vitro studies with Ertapenem demonstrate that free concentration drives efficacy: Nix, D. E., Matthias, K. R., and Ferguson, E. C. (2004) Effect of Ertapenem Protein Binding on Killing of Bacteria. *Antimicrob. Agents Chemother.* 48, 3419−3424.

(82) Zeitlinger, M. A., Derendorf, H., Mouton, J. W., Cars, O., Craig, W. A., Andes, D., and Theuretzbacher, U. (2011) Protein Binding: Do we ever learn ? *Antimicrob. Agents Chemother.* 55, 3067−3074.

(83) Snyder, N. J., Tabas, L. B., Berry, D. M., Duckworth, D. C., Spry, D. O., and Dantzig, A. H. (1997) Structure-Activity relationship of CarbaCephalosporins and Cephalosporins: Antibacterial Activity and Interaction with the Intestinal Proton-Dependent Dipeptide Transport Carrier of Caco-2 cells. *Antimicrob. Agents Chemother.* 41 (8), 1649−1657.

(84) Foley, D. W., Rajamanickam, J., Bailey, P. D., and Meredith, D. (2010) Bioavailability through PepT1: the role of computer modelling in intelligent drug design. *Curr. Comput.-Aided Drug Des.* 6 (1), 68−78.

(85) Wanchana, S., Yamashita, F., Hara, H., Fujiwara, S. I., Akamatsu, M., and Hashida, M. (2004) Two- and Three-dimensional QSAR of carrier mediated Transport of *β*-Lactam antibiotics in Caco-2 cells. *J. Pharm. Sci.* 93 (12), 3057−3065.

(86) Biegel, A., Gebauer, S., Hartrodt, B., Brandsch, M., Neubert, K., and Thondorf, I. (2005) Three-dimensional Quantitative Structure Activity relationship analyses of *β*-lactam antibiotics and tripeptides as substrates of the mammalian H+/Peptide cotransporter peptide PEPT1. *J. Med. Chem.* 48, 4410−4419.

(87) Samsudin, F., Parker, J. L., Sansom, M. S., Newstead, S., and Fowler, P. W. (2016) Accurate prediction of ligand affinities for a proton dependent oligopeptide transporter. *Cell Chem. Biol.* 23, 299−309.

(88) Beale, J. H., Parker, J. L., Samsudin, F., Barrett, A. L., Senan, A., Bird, L. E., Scott, D., Owens, R. J., Sansom, M. S., Tucker, S. J., Meredith, D., Fowler, P. W., and Newstead, S. (2015) Crystal structure of the extracellular domain from PepT1 and PepT2 provide novel insights into mammalian peptide transport. *Structure* 23, 1889−1899.

(89) Boggavarapu, R., Jeckelmann, J. M., Harder, D., Ucurum, Z., and Fotiadis, D. (2015) Role of electrostatic interactions for ligand recognition and specificity of peptide transporters. *BMC Biol.* 13, 58.

(90) Newstead, S. (2017) Recent advances in understanding proton coupled peptide transport via the POT family. *Curr. Opin. Struct. Biol.* 45, 17−24.

(91) Bretschneider, B., Brandsch, M., and Neubert, R. (1999) Intestinal Transport of *β*-lactam antibiotics: Analysis of the affinity at the H+/Peptide Symporter (PEPT1), the Uptake into Caco-2 Cell Monolayers and the Transepithelial Flux. *Pharm. Res.* 16 (1), 55−61.

(92) Saitoh, H., Aungst, B. J., Tohyama, M., Hatakeyama, Y., Ohwada, K., Kobayashi, M., Fujisaki, H., and Miyazaki, K. (2002) In vitro permeation of beta-lactam antibiotics across rat jejunum and its correlation with oral bioavailability in humans. *Br. J. Clin. Pharmacol.* 54 (4), 445−8.

(93) Sugawara, M., Iseki, K., Miyazaki, K., Shiroto, H., Kondo, Y., and Uchino, J. I. (1991) Transport characteristics of Ceftibuten, cefixime and cephalexin across human jejunal brush-border membrane. *J. Pharm. Pharmacol.* 43 (12), 882−884.

(94) Iseki, K., Sugawara, M., Sato, K., Naasani, I., Hayakawa, T., Kobayashi, M., and Miyazaki, K. (1999) Multiplicity of the H+-Dependent Transport Mechanism of Dipeptide and Anionic *β*-Lactam Antibiotic Ceftibuten in Rat Intestinal Brush-Border Membrane. *J. Pharmacol. Exp. Ther.* 289, 66−71.

(95) Recently published work on Ertapenem prodrugs, which lie outside this optimal property space for good oral bioavailability, also identified very few derivatives with good oral bioavailability in dog: Singh, S. B., Rindgen, D., Bradley, P., Suzuki, T., Wang, N., Wu, H., Zhang, B., Wang, L., Ji, C., Yu, H., Soll, R. M., Olsen, D. B., Meinke, P. T., and Nicoll-Griffith, D. A. (2014) Design, synthesis, structure-function relationship, bioconversion, and pharmacokinetic evaluation of ertapenem prodrugs. *J. Med. Chem.* 57 (20), 8421−44.

(96) Active transport routes can also be utilized by some *β*-lactam prodrugs and has been observed with tebipenem pivoxil: Kato, K., Shirasaka, Y., Kuraoka, E., Kikuchi, A., Iguchi, M., Suzuki, H., Shibasaki, S., Kurosawa, T., and Tamai, I. (2010) Intestinal Absorption mechanism of Tebipenem Pivoxil, a Novel Oral Carbapenem: Involvement of OATP Family in Apical Membrane Transport. *Mol. Pharmaceutics* 7 (5), 1747−1756.