EXPERT OPINION

Targeted prodrugs in oral drug delivery: the modern molecular biopharmaceutical approach

Arik Dahan†, Mustafa Khamis, Riad Agbaria & Rafik Karaman
†*Department of Clinical Pharmacology, School of Pharmacy, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel*

Introduction: The molecular revolution greatly impacted the field of drug design and delivery in general, and the utilization of the prodrug approach in particular. The increasing understanding of membrane transporters has promoted a novel 'targeted-prodrug' approach utilizing carrier-mediated transport to increase intestinal permeability, as well as specific enzymes to promote activation to the parent drug.

Areas covered: This article provides the reader with a concise overview of this modern approach to prodrug design. Targeting the oligopeptide transporter PEPT1 for absorption and the serine hydrolase valacyclovirase for activation will be presented as examples for the successful utilization of this approach. Additionally, the use of computational approaches, such as DFT and *ab initio* molecular orbital methods, in modern prodrugs design will be discussed.

Expert opinion: Overall, in the coming years, more and more information will undoubtedly become available regarding intestinal transporters and potential enzymes that may be exploited for the targeted modern prodrug approach. Hence, the concept of prodrug design can no longer be viewed as merely a chemical modification to solve problems associated with parent compounds. Rather, it opens promising opportunities for precise and efficient drug delivery, as well as enhancement of treatment options and therapeutic efficacy.

Keywords: DFT and *ab initio* calculations, membrane transporters, molecular biopharmaceutics, passive/active intestinal permeability, prodrug activation, targeted prodrug approach

Expert Opin. Drug Deliv. (2012) 9(8):1001-1013

1. Introduction

Prodrugs are bioreversible derivatives of drug molecules designed to overcome pharmaceutical, pharmacokinetic or pharmacodynamic barriers such as low oral absorption, lack of site specificity, insufficient chemical stability, poor solubility, toxicity, unacceptable taste/odor, etc. The prodrug approach becomes more and more popular and successful; to date, around 10% of all world's marketed medications are prodrugs, 20% of all small molecular medicines approved between 2000 and 2008 were prodrugs and when focusing on 2008 approved drugs, it emerges that over 30% of them were prodrugs [1,2].

During the past decade, the pharmaceutical sciences have undergone a molecular revolution; no longer relying on empirical fitting based on plasma levels, the modern ADME (absorption, distribution, metabolism and excretion) research considers molecular/cellular factors, for example, membrane influx/efflux transporters and cellular protein expression and distribution. This molecular revolution brought a great impact on the field of drug design and delivery in general, and the utilization of the prodrug approach in particular, as will be highlighted in this paper.

10.1517/17425247.2012.697055 © 2012 Informa UK, Ltd. ISSN 1742-5247

Article highlights.

- While the traditional prodrug approach was focused on altering various physiochemical parameters, e.g., lipophilicity and charge state, the modern approach considers molecular/cellular factors such as membrane influx/efflux transporters and cellular protein expression and distribution.
- Targeting PEPT1 for enhanced intestinal absorption is an example for successful utilization of this modern approach to prodrug design; significant enhanced absorption was demonstrated for amino acid, dipeptide and tripeptide prodrugs of various drugs.
- Prodrugs designed to target MCT1, ASBT and other intestinal transporters have also been shown useful in enhancing the absorption of parent drugs with unfavorable drug-like properties.
- The activation step (i.e. the liberation of the parent drug) is essentially the unique and one of the most critical processes for a prodrug to exert therapeutic effect; rational activating-enzyme targeted design is a significant advantage of the modern approach to prodrug design.
- Targeting the serine hydrolase valacyclovirase for activation is an example for successful utilization of this modern approach to prodrug design.
- The 'double-targeted' approach, in which both transporters for permeability and enzymes for activation are accounted for, has been shown to be a promising direction to exploit the molecular revolution in oral drug delivery.
- Computational approach using different molecular orbital methods such as DFT and ab initio could provide a stepping stone for the modern design of prodrugs.
- In the coming years, as more and more information becomes available regarding intestinal transporters and potential activating enzymes, exploiting these for the targeted modern prodrug approach represents an important future direction that will significantly advance the field.

This box summarizes key points contained in the article.

Increasing the intestinal absorption following oral administration is the most frequent rational for prodrug design. To improve oral absorption, a classic prodrug approach can be adopted to mask charged/polar moieties and enhance drug lipophilicity and passive diffusion. This may be achieved by various carboxylic acid esters, which release the active carboxylic acid after hydrolysis [3]. In recent years, the understanding of membrane transporters has promoted a novel 'targeted-prodrug' approach utilizing carrier-mediated transport to increase intestinal permeability. Certainly, this type of approach requires substantial knowledge of the molecular and function characteristics of these membrane transporters.

Prodrugs must be converted to the active parent drug to exert the therapeutic effect. This activation process is not necessary to be specific; however, a good understanding of the possible activating enzymes will help the rational design of successful prodrugs. By considering enzyme-substrate specificity, it is possible to overcome poor site specificity, leading to the desired higher efficacy accompanied by lower toxicity.

Additional modern approach in prodrug design that will be discussed in this paper is the design of prodrugs based on intramolecular processes utilizing molecular orbital methods and correlations between experimental and calculated values. In this report, the authors will present the concepts of modern (vs traditional) biopharmaceutical approach to orally administered prodrugs. They will discuss the new opportunities that the continuous advancement in related fields, for example, molecular and computational biology, brings to the field of oral drug delivery via prodrug.

2. Targeting transporters in prodrug design

The classical approach for prodrug design uses the non-specific strategy of covalently modifying the drug of interest by attaching hydrophilic functionalities (e.g., phosphate) to increase the solubility in the aqueous gastrointestinal (GI) milieu [4-6], or lipophilic moieties (e.g., ester) to increase the passive permeability through the GI wall [7-9]. On the other hand, the molecular revolution enabled more sophisticated strategies to prodrug design to emerge, in which pro-moieties are covalently attached to the molecule of interest to selectively target certain membrane transporters and enzymes. This modern strategy offers a remarkable potential for improving drug bioavailability and selectivity of poorly absorbed drug molecules [10,11].

The recent advances in biochemistry and molecular biology have delivered a lot of information on the function and expression of transporters and enzymes. Many transporters are expressed on the intestinal enterocytes that may be selectively targeted. These include the organic anion transporter (OAT) family [12], organic cation transporter (OCT) family [13,14], sodium-dependent bile acid transporter (ASBT) family [15], sodium-dependent glucose transporter (SGLT) family [16], monocarboxylate transporter (MCT) family [17,18], amino acid transporter PAT1 [19,20], amino acid transporter $ATB^{0,+}$ [21,22], folate transporter (PCFT) [23,24] and oligopeptide transporter (PEPT1) [25-28]. These transporters have been extensively characterized, and have been shown to play important roles in the absorption of certain nutrients and drugs [29-32]. Since significant information is available on the substrate specificity of these transporters, drug moieties could be chemically modified to enhance their oral absorption via targeting intestinal transporters. Of all the intestinal transporters, PEPT1 has captured the greatest attention as a drug transport pathway, mainly due to wide distribution throughout the entire small intestine, broad substrate specificity and high capacity. PEPT1 is characterized as a high-capacity low-affinity transporter, predominantly expressed in the small intestine, and accepts dipeptides, tripeptides and peptidomimetic drugs such as β-lactam antibiotics and angiotensin-converting enzyme (ACE) inhibitors [27,33]. Thus, PEPT1-targeted prodrugs may be a most promising strategy for oral drug delivery.

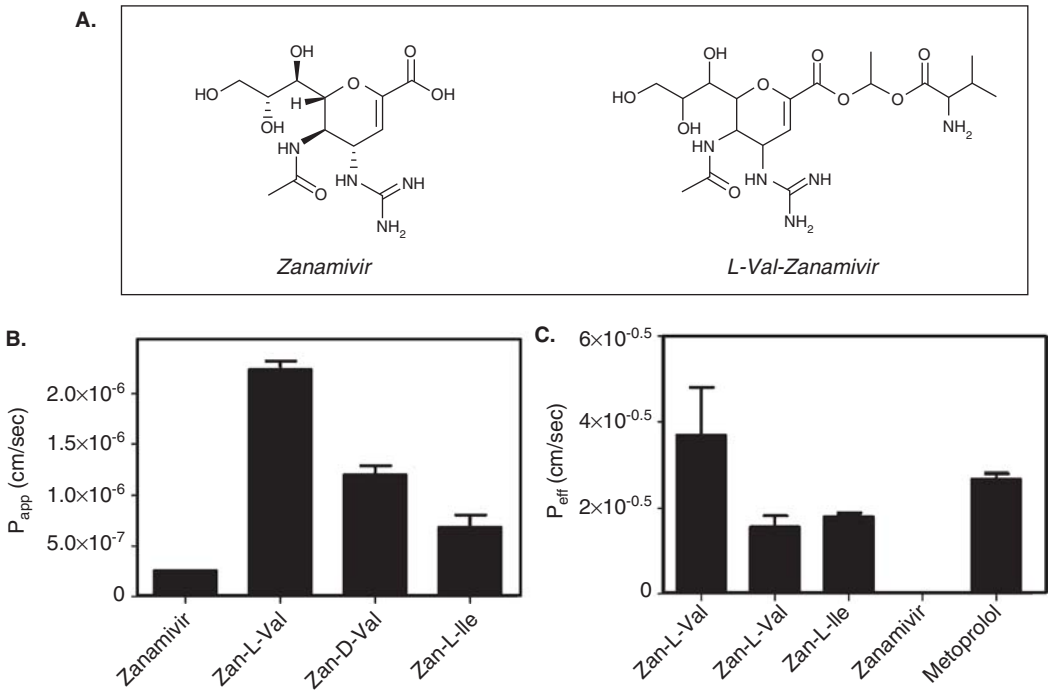


Figure 1. Molecular structure of zanamivir and its L-valyl prodrug (A), and the permeability of zanamivir and its amino acids prodrug across Caco-2 monolayers (B), and in the single-pass rat jejunal perfusion method (C).
Reproduced from [37] with permission. Copyright (2003) American Chemical Society.

Zanamivir and oseltamivir are the two Food and Drug Administration (FDA) approved neuraminidase inhibitors for the treatment of influenza infection. Oseltamivir (Tamiflu®) is a carboxylic acid ester that may represent a successful example for the 'traditional' approach for prodrug design. The very low oral bioavailability (> 5%) of oseltamivir carboxylate increased to approximately 80% for oseltamivir in humans [34]. While incidences of resistance against oseltamivir have been reported in the literature [35], there are no such reports with zanamivir to date. However, the polar nature of zanamivir results in very low oral bioavailability (~ 2%) for this clinically important compound [36]. A modern prodrug approach was taken in trying to improve zanamivir's absorption following oral administration, targeting PEPT1 for carrier-mediated transport [37]. For this purpose, a series of acyloxy ester prodrugs of zanamivir conjugated with amino acids were synthesized and characterized for chemical stability, membrane transport and enzymatic activation. In comparison with zanamivir, the L-valyl prodrug of zanamivir showed threefold higher uptake by PEPT1 overexpressing cells, indicating recognition between the prodrug and the transporter. Subsequent intestinal permeability studies of zanamivir's L-valyl and other amino acid prodrugs compared with the parent drug in Caco-2 transepithelial experiments and in the rat jejunal perfusion model confirmed that this mechanistic targeted prodrug strategy significantly improves the intestinal epithelial cell permeability of zanamivir (**Figure 1**) [37]. Since no oral delivery route of zanamivir currently exists [38], despite the high clinical importance of this drug in fighting the common seasonal flu as well as the recent H1N1 global pandemic [39], this work demonstrates that the modern approach for prodrug design has the potential to provide the high oral bioavailability necessary for oral zanamivir therapy, and hence to enable new treatment options.

Since amino acid ester prodrugs have been found to be substrates for PEPT1, a variety of amino acid, dipeptide and tripeptide prodrugs have been investigated for their suitability as substrates for PEPT1. These include the anticancer agent floxuridine [40-42], the antiviral agents acyclovir, gancyclovir and zidovudine [43,44], the anticancer agents melphalan [45] and gemcitabine [46], the antihypotensive agent midodrine [47], the antiosteoporotic agent alendronate [48] and others. These studies have assured that mono amino acid and dipeptide ester prodrugs generally provide enhanced PEPT1-mediated transport, and as a result, improved oral absorption and bioavailability.

Targeting of monocarboxylate transporter type 1 (MCT1) has also been shown to result in enhanced intestinal prodrug/drug absorption. MCT1, a low-affinity high-capacity transporter that transports unbranched aliphatic monocarboxylates, is widely expressed along the entire intestinal tract [49]. XP13512 is a carbamate prodrug of the GABA analog gabapentin; since gabapentin suffers from many poor pharmacokinetic properties including high variability, saturable absorption and lack of dose proportionality, an efficient prodrug may allow significantly better drug-like properties. It has been shown that following oral administration of XP13512 the systemic bioavailability of gabapentin was

dramatically increased in preclinical [50] and clinical [51] studies, including dose proportionality, while MCT1 plays a significant role in this improvement [50,52].

Additional transporter that was exploited as a potential target for improved absorption of prodrugs is the ASBT [15], and significantly improved absorption of the antiviral agent acyclovir [53] and the GABA analog gabapentin [54] have been reported. Other transporters have also been targeted, as noted above. Overall, this modern approach for oral prodrug design allows a more mechanistic and intelligent strategy to enable increased intestinal absorption of poorly permeable compounds. Since intestinal permeability is, alongside the drug solubility, the most important factors governing drug absorption following oral administration [55-58], the improved permeability achieved by this approach may enhance drug-like properties and 'developability' [59,60], thereby allowing new orally administered treatment options.

3. Targeting enzymes in prodrug design

After absorption, a subsequent essential step in effective prodrug therapy is the activation (hydrolysis) of the prodrug to the active therapeutic agent. The most important enzymes involved in the bioconversion of ester-based prodrugs include paraoxonase, carboxylesterase, acetylcholinesterase and cholinesterase [61-63]. However, in the classical/traditional approach for prodrug design, the activation mechanism was often overlooked, and as long as the parent drug could be regenerated, the activation was considered successful, and the enzymes responsible for this activation did not capture much further attention. As noted above, the activation process is not necessary to be specific, yet a good knowledge of the activating enzyme/s will help to rationally design successful prodrugs. Hence, the identification of enzymes responsible for activating different classes of prodrugs, particularly in humans, can provide important new targets for the design of more effective therapeutic agents.

Valacyclovir is the 5′-valyl ester prodrug of the antiviral drug acyclovir. Valacyclovir increased the oral bioavailability of its parent drug acyclovir by three- to fivefold [64]. This improved absorption provided by the prodrug has been shown to be attributable to carrier-mediated intestinal transport of the prodrug via hPEPT1 [43,65]. As such, valacyclovir represents a successful example for the modern transporter-targeted prodrug approach. However, the efficiency of valacyclovir as an antiviral drug relies also on the rapid *in vivo* conversion of valacyclovir to acyclovir. Given the importance of nucleoside analogs in pharmacotherapy, the identification of the activation mechanism/s of valacyclovir was of high significance. At first, it has been shown in several studies that enzymatic (rather than chemical) hydrolysis of valacyclovir is the predominant *in vivo* activation mechanism in rats, primates and humans [66-68]. Moreover, valacyclovir was found to be relatively stable in the GI milieu, accompanied by high susceptibility to intracellular enzymatic hydrolysis [69]. Also, Burnette *et al*. purified and sequenced several peptide fragments of the major polypeptide hydrolyzing valacyclovir from a purified preparation of a putative novel protein from rat liver [70]. However, in 2003, Kim *et al.* succeeded to purify, identify and characterize the human enzyme that activates valacyclovir to acyclovir, named valacyclovirase, a serine hydrolase containing a catalytic triad S122-H255-D227 [71,72]. Further characterization studies have found that valacyclovirase is one of the primary enzymes activating amino acid ester prodrug, with high and specific preference for amino acid esters as substrates that is attributed to the critical residue D123 forming electrostatic interaction with the α-amino group of substrates. Valacyclovirase contains a large leaving group accommodating groove, which accommodates various leaving groups including nucleoside analogs, as well as simple alcohols such as methanol, ethanol and benzyl alcohol [61,73-76].

As opposed to the traditional low attention the activation step used to receive, it is essentially the unique and one of the most critical processes for a prodrug to exert therapeutic effect. If the activating enzyme/s are identified, it will be possible to design prodrugs to target these enzymes, which will significantly increase the chances for the effective production of the active parent drug. Although the amino acid ester prodrug strategy has been applied to many nucleoside analogs and was successful in improving oral absorption by targeting PEPT1, the activation step of these prodrugs was not well studied and was considered to be non-specific until the identification of valacyclovirase. This was further exploited as will be presented hereinafter.

The positively charged (in physiological pH) guanidino functionality is well known for its important biological roles *in vivo*; many biologically active receptors show affinity for the L-arginine residue attributable to strong electrostatic interaction between the positively charged guanidino group and the negatively charged carboxylate in the active site of the receptor. Hence, when rationally designing an inhibitor, the guanidine functionality is frequently selected to mimic the arginine residue of the endogenous substrate and secure the affinity of the drug to the target [77]. Guanidino-containing drug molecules include the antidiabetic drug metformin, the H_2-receptor antagonist famotidine, the aminoglycoside antibiotic drug streptomycin, the anticoagulant thrombin inhibitor argatroban and others. However, guanidino-containing compounds are frequently associated with low oral bioavailability; inherent polarity and positive charge in the GI tract make it difficult for them to be orally absorbed by passive diffusion. For instance, while the classical approach for prodrug design was successful in enhancing the oral absorption of oseltamivir carboxylate by making the simple ethyl ester, this strategy failed when applied for its guanidine analog, guanidino oseltamivir carboxylate [78]. Several other attempts to mask the guanidino group by classical prodrugs can be found in the literature, with varying degrees of success [79-82]. The modern prodrug approach, targeting PEPT1 for intestinal transport, may

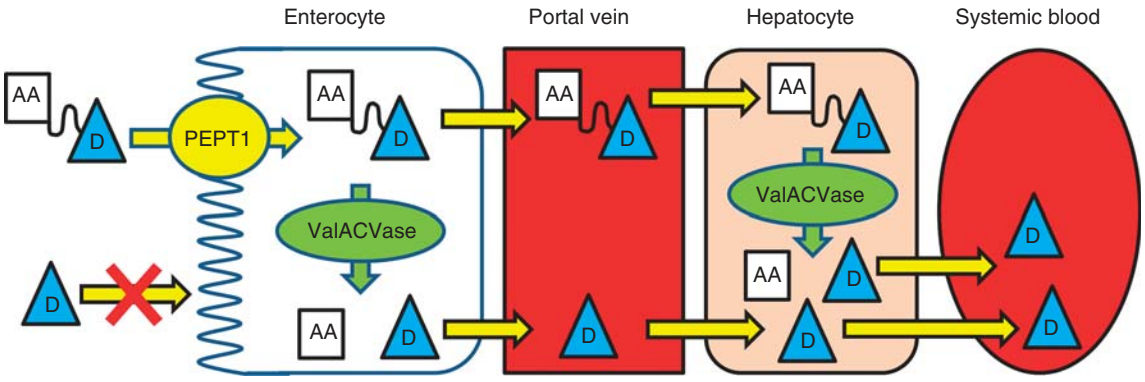
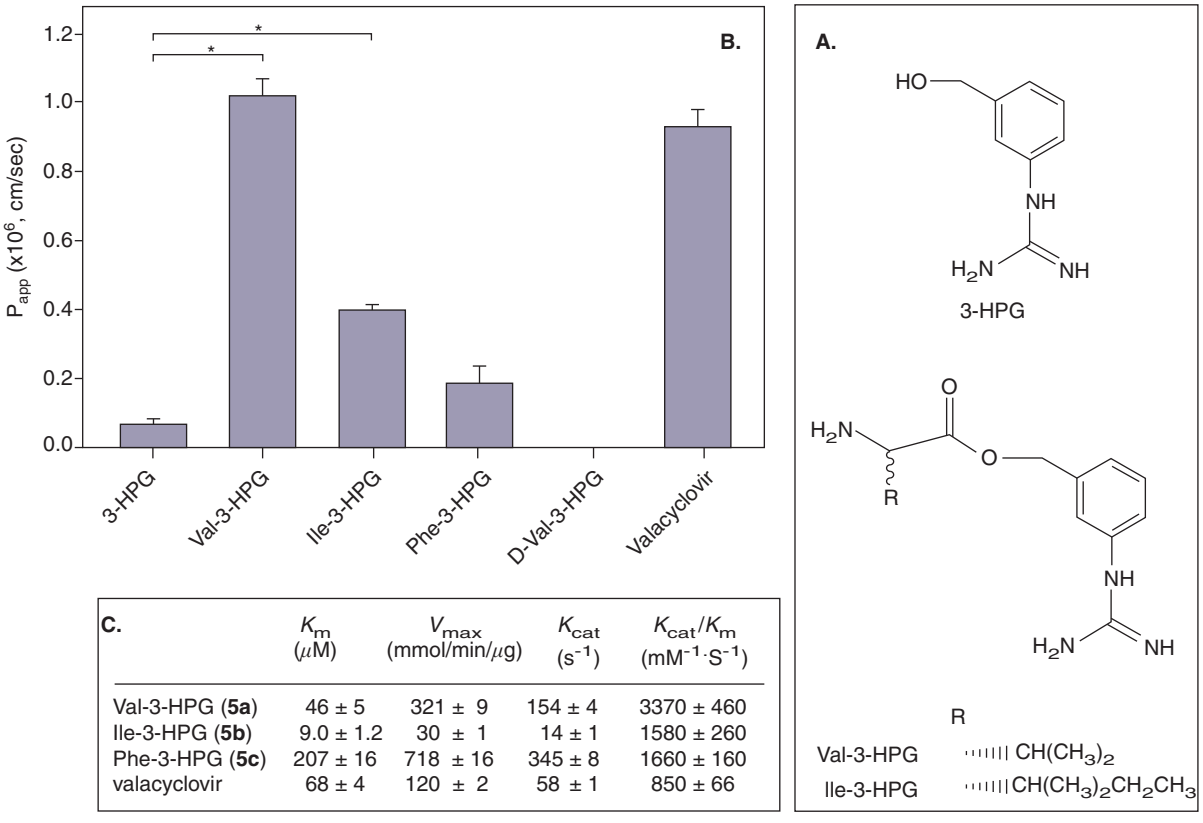


Figure 2. Illustration of the 'double-targeted' prodrug approach, accounting for both transport via PEPT1 and activation via valacyclovirase.



C.

	K_m (μM)	V_{max} (mmol/min/μg)	K_{cat} (s^{-1})	K_{cat}/K_m ($mM^{-1}\cdot S^{-1}$)
Val-3-HPG (**5a**)	46 ± 5	321 ± 9	154 ± 4	3370 ± 460
Ile-3-HPG (**5b**)	9.0 ± 1.2	30 ± 1	14 ± 1	1580 ± 260
Phe-3-HPG (**5c**)	207 ± 16	718 ± 16	345 ± 8	1660 ± 160
valacyclovir	68 ± 4	120 ± 2	58 ± 1	850 ± 66

Figure 3. Molecular structure of zanamivir and its L-valyl prodrug (A), and the permeability of zanamivir and its amino acids prodrug across Caco-2 monolayers (B), and in the single-pass rat jejunal perfusion method (C).

Reproduced from [83] with permission. Copyright (2008) American Chemical Society.

be a promising strategy to increase the oral absorption of guanidino-containing molecules.

A unique approach was taken to evaluate the feasibility of this strategy, the double-targeted prodrug approach. In this approach, both transport and activation processes are accounted for, already at the initial prodrug design stage (**Figure 2**). A series of amino acid esters of a model guanidine-containing compound, [3-(hydroxymethyl)phenyl]guanidine (3-HPG), was synthesized and evaluated for both transport and activation [76,83]. Compared with the parent molecule, the valine and the isoleucine esters exhibited a significant increased Caco-2 and rat intestinal permeability that was shown to be mediated by PEPT1 (**Figure 3**). Remarkably, these L-amino acid prodrugs of 3-HPG were shown to

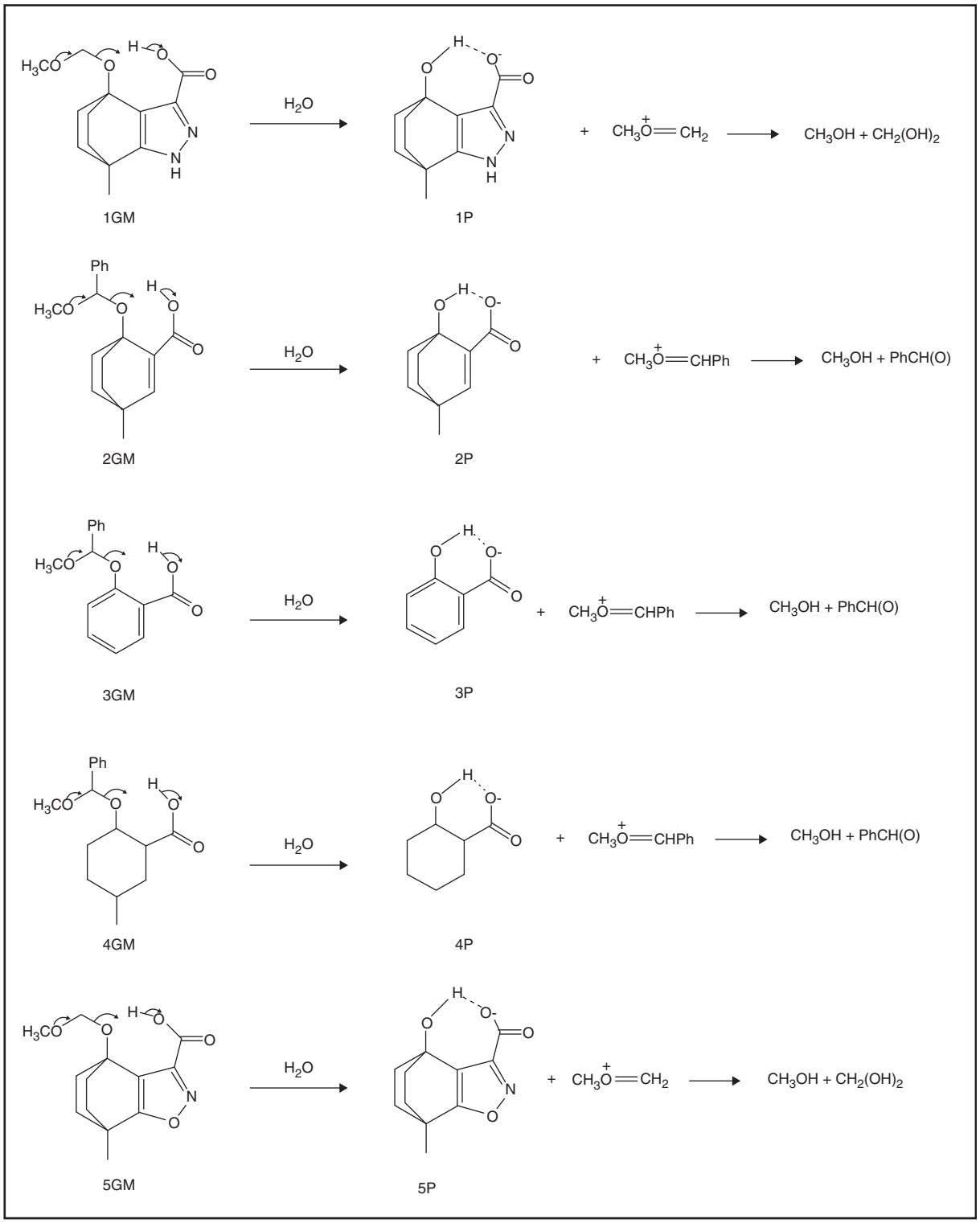


Figure 4. Proton transfer reaction in Kirby's acetals 1 – 5, where GM and P represent the reactant and the product, respectively.

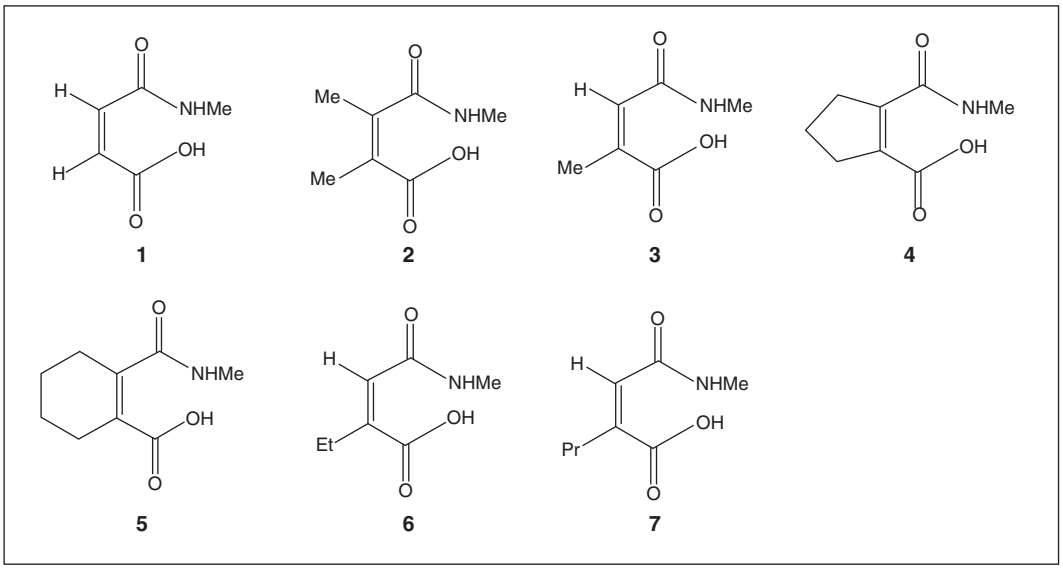


Figure 5. Chemical structures for seven of Kirby's maleamic acid amides 1 – 7.

be effectively activated by valacyclovirase, with K_m values in the range of the positive control valacyclovir, thereby liberating the parent moiety [83]. At the same time, the possibility that other enzymes can also contribute to the activation of amino acid ester prodrugs cannot be ruled out. These novel studies, in which both transport and activation processes are taken into consideration already at the earliest stages, represent the next step in the modern approach to drug delivery using prodrugs, which may greatly minimize the empirical elements of the development and hence may result in better products with more predictable performance in comparison with the current practice.

In an additional example, the authors have designed a novel type of prodrugs, substituting the *sn-2* positioned fatty acid of a phospholipid by the non-steroidal anti-inflammatory drug indomethacin, through a linker [84-88]. Physiologically, the *sn-2* positioned fatty acid is cleaved by the enzyme phospholipase A_2 (PLA_2), liberating a free fatty acid and a lysophospholipid as the lipolysis products. The substitution of the *sn-2* positioned fatty acid by a drug moiety, hence, was designed to target PLA_2 as the activating enzyme for this class of prodrugs. Although it was reported that PLA_2 strictly requires a fatty acid at the *sn-2* positioned [89], it was found that, depending on the number of carbons in the linker, PLA_2 was able to recognize and activate the phospholipidic prodrugs of indomethacin: *in vitro* incubation of the prodrug with the 5-carbon linker with the enzyme showed 60% activation, while shorter linkers were significantly less susceptible for activation by PLA_2 [84]. Subsequent *in vivo* investigations in rats revealed that following oral administration there was no absorption of the intact prodrugs, however, the prodrug with the 5-carbon linker was continuously activated by PLA_2 throughout the intestinal tract, resulting in a controlled release profile of the liberated free indomethacin in the systemic circulation [84]. This research shows the advantage of rational activating-enzyme targeted design of prodrugs, which minimizes the empirical elements of the activation process, and allows to better control the liberation of the free active drug moiety from the intact prodrug.

4. The use of computational approaches in modern prodrugs design

The use of computational methods for calculating molecular and physicochemical properties has been perennial goal of pharmaceutical, organic and inorganic chemists alike. Energy-based calculations on biological relevance structures that have pharmaceutical interest are a great challenge. Today, quantum mechanics such as *ab initio*, semi-empirical and density functional theory (DFT) and molecular mechanics are accepted widely as an efficient tools to provide structure–energy calculations to be exploited for the prediction of potential important drugs and prodrugs alike [90].

Ab initio quantum mechanics is based on a rigorous treatment of the Schrodinger equation with some number of approximations. The use of *ab initio* methods is restricted to systems having less than 30 atoms due to the extreme cost of computation time [91]. On the other hand, the semi-empirical methods (MINDO, MNDO, MINDO/3, AM1, PM3 and SAM1) that are based on Schrodinger equation with the addition of terms and parameters to fit experimental data have provided a plenty of information for practical application [92]. DFT is also a semi-empirical method widely used to calculate structures and energies for medium-sized systems of biological and pharmaceutical interest, and it is not restricted to the second row of the periodic table [93]. Unlike quantum mechanics, molecular mechanics which is a

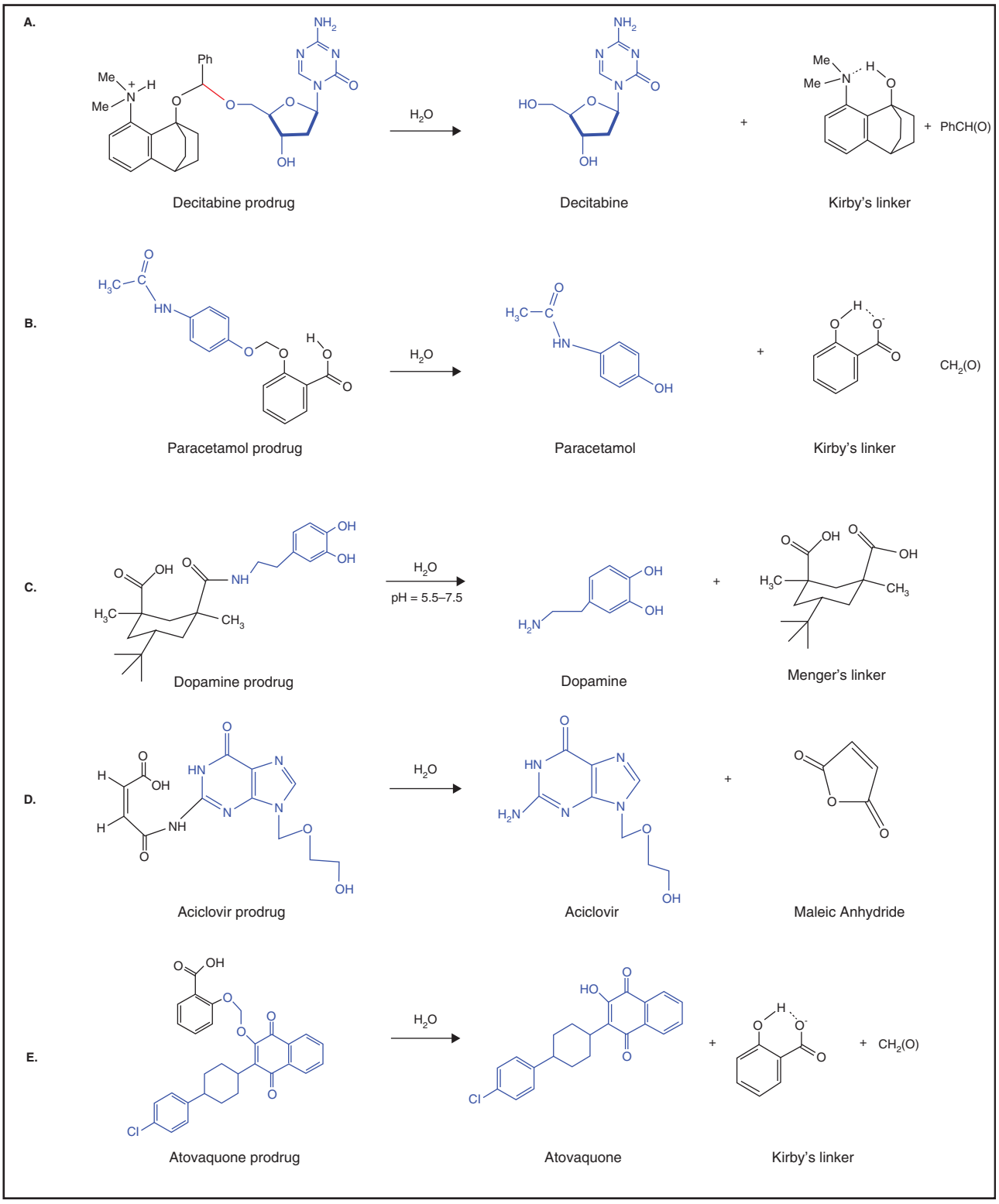


Figure 6. Schematic representation of hydrolytic cleavage at physiologic environments for prodrugs of (A) aza-nucleoside, (B) paracetamol, (C) dopamine, (D) acyclovir and (E) atovaquone.

mathematical approach used for the prediction of structures, energy, dipole moment and other physical properties is able to handle many diverse biological and chemical systems such as proteins, large crystal structures and a relatively large solvated systems, however, this method is limited by the determination of parameters such as the vast number of unique torsion angles present in structurally diverse moieties [94].

These modern computational methods can be used for an intelligent design of innovative prodrugs. For example, mechanisms of some intramolecular processes that have been utilized for better understanding of enzyme catalysis have been recently investigated and exploited for the design of novel prodrug linkers [95-97]. Using molecular mechanics, DFT and *ab initio* methods, various intramolecular processes were studied in order to assign the factors affecting the rate-determining step and playing dominant roles in governing the process rate. These processes include: i) proton transfer between two oxygens in Kirby's acetals (**Figure 4**) [97], and proton transfer between nitrogen and oxygen in Kirby's enzyme models [98]; ii) intramolecular acid-catalyzed hydrolysis in some of Kirby's maleamic acid amide derivatives (**Figure 5**) [99] and iii) proton transfer between two oxygens in rigid systems as investigated by Menger [95]. These studies have revealed the following conclusions: i) rate accelerations in intramolecular processes are a result of both entropy and enthalpy effects in intramolecular cyclization processes where enthalpic effects were predominant, steric effects was the driving force for the accelerations, whereas proximity orientation was the determining factor in the cases of proton transfer reactions; ii) the distance between the two reacting centers is the main factor determining whether the reaction type is intermolecular or intramolecular. In the cases by which the distance exceeded 3 Å, an intermolecular engagement was preferred due to involvement of a water molecule (solvent) whereas, an intramolecular engagement prevailed when the distance between the electrophile and nucleophile was less than 3 Å and iii) the efficiency of proton transfer between two oxygens and between nitrogen and oxygen in Kirby's enzyme models is attributed to a relatively strong hydrogen bonding developed in the products and the transition states leading to them.

It was concluded from the studies on intramolecularity that there is a crucial necessity to investigate the reaction mechanism for assigning the factors determining the reaction rate for a better design of an efficient chemical device to be exploited as prodrug linker and to have the potential to chemically (not enzymatically) liberate the active drug in a programmable (controlled) manner. For example, the mechanism for the proton transfer in Kirby's acetals were explored [97,98] and directed the synthesis of novel prodrugs of aza-nucleosides for the treatment of myelodysplastic syndromes where the prodrug linker is attached to the hydroxyl group of the nucleoside (**Figure 6**) [100]. The prodrugs were designed such that they will undergo cleavage reactions in physiological environments such as stomach at pH 1.5, intestine at pH 6.5 or/and blood circulation at pH 7.4, with rates that are solely dependent on the structural features of the pharmacologically inactive linker. Different linkers were also investigated for the design of large number of prodrugs such as anti-Parkinson (dopamine) [101], anti-viral (acyclovir) [102] and anti-malarial (atovaquone) [98] (**Figure 6**) that might be efficient in releasing the parental drugs in various rates that are dependent on the nature or the structural features of the linkers and provide new novel prodrugs that might have the potential to be with enhanced dissolution and membrane penetration and hence better bioavailability. These examples highlight the great potential and impact that modern computational approaches for prodrug design may have when appropriately exploited.

5. Expert opinion

For many years, the prodrug approach in general used to be viewed as a last option strategy, almost an act of desperation after all other possible solutions exhausted; this is no longer the case. In fact, taking the prodrug approach should be considered very early on in the development process. Indeed, as noted above, the prodrug approach becomes more and more popular and successful.

The molecular revolution has significantly changed the pharmaceutical sciences in general, and the way the prodrug approach is used in particular. While the classic prodrug approach was focused on altering various physiochemical parameters, for example, lipophilicity and charge state, the modern approach considers molecular/cellular factors, for example, membrane influx/efflux transporters and cellular protein expression and distribution. An *a priori* mechanistic design that aims to enable absorption by specific transports, as well as activation by specific enzymes, may greatly improve the process efficiency, and allow for novel oral treatment options. Minimizing the empirical elements by taking the targeted prodrug approach promotes an intelligent and powerful process, as the outcomes may be significantly more predictable; knowledge of the prodrug activating enzyme(s) in preclinical animals and their counterpart(s) in human may improve the translation of preclinical data to clinical trial design, potentially speeding the prodrug development process and lowering its cost. In addition, good knowledge of the transporter(s) and enzyme(s) involved in the absorption and activation may allow to predict and recognize potential competition-based drug–drug interactions. A critical aspect that was not covered in this paper is the site-specific targeting potential; a prodrug designed to be activated by a specific enzyme that is overexpressed in the target site may allow to target the free drug to the site of action, resulting in improved efficacy and reduced toxicity.

The computational approach using different molecular orbital methods such as DFT presented in this paper could provide a stepping stone for the design of linkers to be conjugated to drugs with poor bioavailability to furnish pharmaceutical devices with a potential to release the parent drug in slow release manner and to improve their bioavailability. Based on

such results in the field thus far, a few innovative prodrugs were synthesized, that were kinetically studied and their *in vitro* kinetics results promise a breakthrough in this field. Hence, the authors posit that the great potential and impact that modern computational approaches for prodrug design carry will be increasingly recognized and exploited, leading to a significant step forward in the field.

Overall, in the coming years, more and more information will undoubtedly become available regarding intestinal transporters and potential enzymes that may be exploited for the targeted modern prodrug approach. Hence, the concept of prodrug design can no longer be viewed as merely a chemical modification to solve problems associated with parent compounds. Rather, it opens promising opportunities for precise and efficient drug delivery, as well as enhancement of treatment options and therapeutic efficacy. In authors' opinion, the novel 'double-targeted' approach presented above, in which both transporters for intestinal permeability and enzymes for activation are accounted for, represents the direction for significant exploitation of the molecular revolution in oral drug delivery via prodrugs. Considerably, the simultaneous utilization of all three approaches described in this paper, that is, using the computational approaches for rational design that will allow targeting of a prodrug to a certain transporter for enhanced absorption followed by targeting an enzyme for enhanced/predicted activation, represents an important future direction that will significantly advance the field.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Huttunen KM, Raunio H, Rautio J. Prodrugs – from serendipity to rational design. Pharmacol Rev 2011;63:750-71
2. Stella VJ. Prodrugs: some thoughts and current issues. J Pharm Sci 2010;99:4755-65
3. Beaumont K, Webster R, Gardner I, Dack K. Design of ester prodrugs to enhance oral absorption of poorly permeable compounds: challenges to the discovery scientist. Curr Drug Metab 2003;4:461-85
4. Amidon GL, Leesman GD, Elliott RL. Improving intestinal absorption of water-insoluble compounds: a membrane metabolism strategy. J Pharm Sci 1980;69:1363-8
5. Fleisher D, Bong R, Stewart BH. Improved oral drug delivery: solubility limitations overcome by the use of prodrugs. Adv Drug Deliv Rev 1996;19:115-30
6. Stella VJ, Nti-Addae KW. Prodrug strategies to overcome poor water solubility. Adv Drug Deliv Rev 2007;59:677-94
7. Bernard T. Prodrugs: bridging pharmacodynamic/pharmacokinetic gaps. Curr Opin Chem Biol 2009;13:338-44
8. Ettmayer P, Amidon GL, Clement B, Testa B. Lessons learned from marketed and investigational prodrugs. J Med Chem 2004;47:2393-404

•• **Excellent review presenting the fundamentals of the prodrug approach.**

9. Rautio J, Kumpulainen H, Heimbach T, et al. Prodrugs: design and clinical applications. Nat Rev Drug Discov 2008;7:255-70
10. Han HK, Amidon GL. Targeted prodrug design to optimize drug delivery. AAPS PharmSci 2000;2:E6

•• **Excellent overview of the modern targeted approach to prodrug design**

11. Majumdar S, Duvvuri S, Mitra AK. Membrane transporter/receptor-targeted prodrug design: strategies for human and veterinary drug development. Adv Drug Deliv Rev 2004;56:1437-52
12. Tamai I. Oral drug delivery utilizing intestinal OATP transporters. Adv Drug Deliv Rev 2012; In press
13. Jonker JW, Schinkel AH. Pharmacological and physiological functions of the polyspecific organic cation transporters: OCT1, 2, and 3 (SLC22A1-3). J Pharmacol Exp Ther 2004;308:2-9
14. Koepsell H, Lips K, Volk C. Polyspecific organic cation transporters: structure, function, physiological roles, and biopharmaceutical implications. Pharm Res 2007;24:1227-51
15. Balakrishnan A, Polli JE. Apical sodium dependent bile acid transporter (ASBT, SLC10A2): a potential prodrug target. Mol Pharm 2006;3:223-30
16. Cao X, Gibbs S, Fang L, et al. Why is it challenging to predict intestinal drug absorption and oral bioavailability in human using rat model. Pharm Res 2006;23:1675-86
17. Tsuji A. Tissue selective drug delivery utilizing carrier-mediated transport systems. J Control Release 1999;62:239-44
18. Varma M, Ambler C, Ullah M, et al. Targeting intestinal transporters for optimizing oral drug absorption. Curr Drug Metab 2010;11:730-42
19. Anderson CMH, Grenade DS, Boll M, et al. H+/amino acid transporter 1 (PAT1) is the imino acid carrier: an intestinal nutrient/drug transporter in human and rat. Gastroenterology 2004;127:1410-22
20. Thwaites DT, Anderson CMH. The SLC36 family of proton-coupled amino acid transporters and their potential role in drug transport. Br J Pharmacol 2011;164:1802-16
21. Ganapathy ME, Ganapathy V. Amino acid transporter ATB0, + as a delivery system for drugs and prodrugs. Curr Drug Targets Immune Endocr Metab Disord 2005;5:357-64
22. Hatanaka T, Haramura M, Fei Y-J, et al. Transport of amino acid-based prodrugs by the Na+- and Cl– coupled amino acid transporter ATB0, + and expression of the transporter in tissues amenable for drug delivery. J Pharmacol Exp Ther 2004;308:1138-47
23. Qiu A, Jansen M, Sakaris A, et al. Identification of an intestinal folate transporter and the molecular basis for hereditary folate malabsorption. Cell 2006;127:917-28
24. Zhao R, Diop-Bove N, Visentin M, Goldman ID. Mechanisms of membrane

transport of folates into cells and across epithelia. Annu Rev Nutr 2011;31:177-201

25. Bai JPF, Amidon GL. Structural specificity of mucosal-cell transport and metabolism of peptide drugs: implication for oral peptide drug delivery. Pharm Res 1992;9:969-78

26. Brandsch M, Knutter I, Bosse-Doenecke E. Pharmaceutical and pharmacological importance of peptide transporters. J Pharm Pharmacol 2008;60:543-85

27. Kikuchi A, Tomoyasu T, Tanaka M, et al. Peptide derivation of poorly absorbable drug allows intestinal absorption via peptide transporter. J Pharm Sci 2009;98:1775-87

28. Lee VHL, Chu C, Mahlin ED, et al. Biopharmaceutics of transmucosal peptide and protein drug administration: role of transport mechanisms with a focus on the involvement of PepT1. J Control Release 1999;62:129-40

29. Giacomini K, Huang S, Tweedie D, et al. Membrane transporters in drug development. Nat Rev Drug Discov 2010;9:215-36

•• **Excellent review of transporters in drug delivery and discovery.**

30. Mizuno N, Niwa T, Yotsumoto Y, Sugiyama Y. Impact of drug transporter studies on drug discovery and development. Pharmacol Rev 2003;55:425-61

31. Shugarts S, Benet L. The role of transporters in the pharmacokinetics of orally administered drugs. Pharm Res 2009;26:2039-54

32. Martinez MN, Amidon GL. A mechanistic approach to understanding the factors affecting drug absorption: a review of fundamentals. J Clin Pharmacol 2002;42:620-43

•• **Excellent review presenting the fundamentals of oral drug absorption.**

33. Jappar D, Hu Y, Smith DE. Effect of dose escalation on the in vivo oral absorption and disposition of glycylsarcosine in wild-type and pept1 knockout mice. Drug Metab Dispos 2011;39:2250-7

34. Aoki FY, Doucette KE. Oseltamivir: a clinical and pharmacological perspective. Expert Opin Pharmacother 2001;2:1671-83

35. Le QM, Kiso M, Someya K, et al. Avian flu: isolation of drug-resistant H5N1 virus. Nature 2005;437:1108-8

36. Cass L, Efthymiopoulos C, Bye A. Pharmacokinetics of zanamivir after intravenous, oral, inhaled or intranasal administration to healthy volunteers. Clin Pharmacokinet 1999;36:1-11

37. Varghese Gupta S, Gupta D, Sun J, et al. Enhancing the intestinal membrane permeability of zanamivir: a carrier mediated prodrug approach. Mol Pharm 2011;8:2358-67

• **A research demonstrating PEPT1-targeted prodrug approach, enhancing the intestinal absorption of zanamivir.**

38. Miller JM, Dahan A, Gupta D, et al. Enabling the intestinal absorption of highly polar antiviral agents: ion-pair facilitated membrane permeation of zanamivir heptyl ester and guanidino oseltamivir. Mol Pharm 2010;7:1223-34

39. Dawood F, Jain S, Finelli L, et al. Emergence of a novel swine-origin influenza A (H1N1) virus in humans. N Engl J Med 2009;360:2605-15

40. Landowski CP, Vig BS, Song X, Amidon GL. Targeted delivery to PEPT1-overexpressing cells: acidic, basic, and secondary floxuridine amino acid ester prodrugs. Mol Cancer Ther 2005;4:659-67

41. Tsume Y, Hilfinger J, Amidon G. Potential of amino acid/dipeptide monoester prodrugs of floxuridine in facilitating enhanced delivery of active drug to interior sites of tumors: a two-tier monolayer in vitro study. Pharm Res 2011;28:2575-88

42. Tsume Y, Hilfinger JM, Amidon GL. Enhanced cancer cell growth inhibition by dipeptide prodrugs of floxuridine: increased transporter affinity and metabolic stability. Mol Pharm 2008;5:717-27

43. Han H-K, de Vrueh RLA, Rhie JK, et al. 5′-Amino acid esters of antiviral nucleosides, acyclovir, and AZT are absorbed by the intestinal PEPT1 peptide transporter. Pharm Res 1998;15:1154-9

44. Li F, Maag H, Alfredson T. Prodrugs of nucleoside analogues for improved oral absorption and tissue targeting. J Pharm Sci 2008;97:1109-34

45. Mittal S, Song X, Vig BS, et al. Prolidase, a potential enzyme target for melanoma: design of proline-containing dipeptide-like prodrugs. Mol Pharm 2005;2:37-46

46. Song X, Lorenzi PL, Landowski CP, et al. Amino acid ester prodrugs of the anticancer agent gemcitabine: synthesis, bioconversion, metabolic bioevasion, and hPEPT1-mediated transport. Mol Pharm 2005;2:157-67

47. Tsuda M, Terada T, Irie M, et al. Transport characteristics of a novel peptide transporter 1 substrate, antihypotensive drug midodrine, and its amino acid derivatives. J Pharmacol Exp Ther 2006;318:455-60

48. Ezra A, Hoffman A, Breuer E, et al. A peptide prodrug approach for improving bisphosphonate oral absorption. J Med Chem 2000;43:3641-52

49. Halestrap A, Meredith D. The SLC16 gene family - from monocarboxylate transporters (MCTs) to aromatic amino acid transporters and beyond. Pflugers Arch 2004;447:619-28

50. Cundy KC, Annamalai T, Bu L, et al. XP13512, a novel gabapentin prodrug: II. Improved oral bioavailability, dose proportionality, and colonic absorption compared with gabapentin in rats and monkeys. J Pharmacol Exp Ther 2004;311:324-33

51. Cundy KC, Sastry S, Luo W, et al. Clinical pharmacokinetics of XP13512, a novel transported prodrug of gabapentin. J Clin Pharmacol 2008;48:1378-88

52. Cundy KC, Branch R, Chernov-Rogan T, et al. XP13512, a novel gabapentin prodrug: I. Design, synthesis, enzymatic conversion to gabapentin, and transport by intestinal solute transporters. J Pharmacol Exp Ther 2004;311:315-23

53. Tolle-Sander S, Lentz KA, Maeda DY, et al. Increased acyclovir oral bioavailability via a bile acid conjugate. Mol Pharm 2004;1:40-8

54. Rais R, Fletcher S, Polli JE. Synthesis and in vitro evaluation of gabapentin prodrugs that target the human apical sodium-dependent bile acid transporter (hASBT). J Pharm Sci 2011;100:1184-95

55. Amidon GL, Lennernas H, Shah VP, Crison JR. A theoretical basis for

a biopharmaceutic drug classification: The correlation of in vitro drug product dissolution and in vivo bioavailability. Pharm Res 1995;12:413

•• **Excellent seminal paper developing the fundamental factors dictating oral drug absorption.**

56. Dahan A, Miller JM, Amidon GL. Prediction of solubility and permeability class membership: provisional BCS classification of the world's top oral drugs. AAPS J 2009;11:740-6

57. Dahan A, Miller JM, Hilfinger JM, et al. High-permeability criterion for BCS classification: segmental/pH dependent permeability considerations. Mol Pharm 2010;7:1827-34

58. Lennernas H. Human intestinal permeability. J Pharm Sci 1998;87:403-10

59. Sun D, Yu L, Hussain M, et al. In vitro testing of drug absorption for drug 'developability' assessment: forming an interface between in vitro preclinical data and clinical outcome. Curr Opin Drug Discov Devel 2004;7:75-85

60. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev 2001;46:3-26

61. Landowski CP, Lorenzi PL, Song X, Amidon GL. Nucleoside ester prodrug substrate specificity of liver carboxylesterase. J Pharmacol Exp Ther 2006;316:572-80

62. Liederer BM, Borchardt RT. Enzymes involved in the bioconversion of ester-based prodrugs. J Pharm Sci 2006;95:1177-95

63. Satoh T, Hosokawa M. The mammalian carboxylesterases: from molecules to functions. Annu Rev Pharmacol Toxicol 1998;38:257-88

64. Weller S, Blum M, Doucette M, et al. Pharmacokinetics of the acyclovir pro-drug valacyclovir after escalating single- and multiple-dose administration to normal volunteers. Clin Pharmacol Ther 1993;54:595-605

65. Balimane PV, Tamai I, Guo A, et al. Direct evidence for peptide transporter (PepT1)-mediated uptake of a nonpeptide prodrug, valacyclovir. Biochem Biophys Res Commun 1998;250:246-51

66. Burnette TC, de Miranda P. Metabolic disposition of the acyclovir prodrug valacyclovir in the rat. Drug Metab Dispos 1994;22:60-4

67. de Miranda P, Burnette TC. Metabolic fate and pharmacokinetics of the acyclovir prodrug valacyclovir in cynomolgus monkeys. Drug Metab Dispos 1994;22:55-9

68. Soul-Lawton J, Seaber E, On N, et al. Absolute bioavailability and metabolic disposition of valacyclovir, the L-valyl ester of acyclovir, following oral administration to humans. Antimicrob Agents Chemother 1995;39:2759-64

69. Sinko PJ, Balimane PV. Carrier-mediated intestinal absorption of valacyclovir, the L-valyl ester prodrug of acyclovir. 1. Interactions with peptides, organic anions and organic cations in rats. Biopharm Drug Dispos 1998;19:209-17

70. Burnette TC, Harrington JA, Reardon JE, et al. Purification and characterization of a rat liver enzyme that hydrolyzes valacyclovir, the L-valyl ester prodrug of acyclovir. J Biol Chem 1995;270:15827-31

71. Kim I, Chu X-Y, Kim S, et al. Identification of a human valacyclovirase. J Biol Chem 2003;278:25348-56

• **An excellent research identifying a novel target enzyme for prodrug activation.**

72. Lai L, Xu Z, Zhou J, et al. Molecular basis of prodrug activation by human valacyclovirase, an alpha-amino acid ester hydrolase. J Biol Chem 2008;283:9318-27

73. Gupta D, Gupta SV, Lee K-D, Amidon GL. Chemical and enzymatic stability of amino acid prodrugs containing methoxy, ethoxy and propylene glycol linkers. Mol Pharm 2009;6:1604-11

74. Kim I, Crippen GM, Amidon GL. Structure and specificity of a human valacyclovir activating enzyme: a homology model of BPHL. Mol Pharm 2004;1:434-46

75. Kim I, Song X, Vig BS, et al. A novel nucleoside prodrug-activating enzyme: substrate specificity of biphenyl hydrolase-like protein. Mol Pharm 2004;1:117-27

76. Sun J, Dahan A, Walls ZF, et al. Specificity of a prodrug-activating enzyme hVACVase: the leaving group effect. Mol Pharm 2010;7:2362-8

77. Sun J, Miller JM, Beig A, et al. Mechanistic enhancement of the intestinal absorption of drugs containing the polar guanidino functionality. Expert Opin Drug Metab Toxicol 2011;7:313-23

78. Li W, Escarpe PA, Eisenberg EJ, et al. Identification of GS 4104 as an orally bioavailable prodrug of the influenza virus neuraminidase inhibitor GS 4071. Antimicrob Agents Chemother 1998;42:647-53

79. Clement B, Mau S, Deters S, Havemeyer A. Hepatic, extrahepatic, microsomal, and mitochondrial activation of the N-hydroxylated prodrugs benzamidoxime, guanoxabenz, and Ro 48-3656 ([[1-[(2s)-2-[[4-[(hydroxyamino) iminomethyl]benzoyl]amino]-1-oxopropyl]-4-piperidinyl]oxy]-acetic acid). Drug Metab Dispos 2005;33:1740-7

80. Huttunen KM, Mannila A, Laine K, et al. The first bioreversible prodrug of metformin with improved lipophilicity and enhanced intestinal absorption. J Med Chem 2009;52:4142-8

81. Maryanoff BE, McComsey DF, Costanzo MJ, et al. Exploration of potential prodrugs of RWJ-445167, an oxyguanidine-based dual inhibitor of thrombin and factor Xa. Chem Biol Drug Des 2006;68:29-36

82. Saulnier MG, Frennesson DB, Deshpande MS, et al. An efficient method for the synthesis of guanidino prodrugs. Bioorg Med Chem Lett 1994;4:1985-90

83. Sun J, Dahan A, Amidon GL. Enhancing the intestinal absorption of molecules containing the polar guanidino functionality: a double-targeted prodrug approach. J Med Chem 2010;53:624-32

•• **An excellent research presenting simultaneous targeting of transporter and enzyme for absorption and activation.**

84. Dahan A, Duvdevani R, Dvir E, et al. A novel mechanism for oral controlled release of drugs by continuous degradation of a phospholipid prodrug along the intestine: in-vivo and in-vitro evaluation of an indomethacin–lecithin conjugate. J Control Release 2007;119:86-93

85. Dahan A, Duvdevani R, Shapiro I, et al. The oral absorption of phospholipid prodrugs: in vivo and in vitro mechanistic investigation of trafficking of a lecithin-valproic acid conjugate following oral administration. J Control Release 2008;126:1-9

86. Dahan A, Hoffman A. Mode of administration-dependent brain uptake of indomethacin: sustained systemic input increases brain influx. Drug Metab Dispos 2007;35:321-4

87. Dvir E, Elman A, Simmons D, et al. DP-155, a lecithin derivative of indomethacin, is a novel nonsteroidal antiinflammatory drug for analgesia and Alzheimer's disease therapy. CNS Drug Rev 2007;13:260-77

88. Dvir E, Friedman JE, Lee JY, et al. A novel phospholipid derivative of indomethacin, DP-155, shows superior safety and similar efficacy in reducing brain amyloid beta in an Alzheimer's disease model. J Pharmacol Exp Ther 2006;318:1248-56

89. Kurz M, Scriba GKE. Drug–phospholipid conjugates as potential prodrugs: synthesis, characterization, and degradation by pancreatic phospholipase A2. Chem Phys Lipids 2000;107:143-57

90. Reddy M, Erion M. Free energy calculations in rational drug design. Kluwer/Plenum Press; New York: 2001

91. Schrodinger E. Quantisierung als eigenwertproblem. Ann Phys 1926;79:361-76

92. Dewar MJS, Thiel W. Ground states of molecules. 38. The MNDO method. Approximations and parameters. J Am Chem Soc 1977;99:4899-907

93. Parr R, Yang W. Density functional theory of atoms and molecules. Oxford University Press; Oxford: 1989

94. Burkert U, Allinger NL. Molecular mechanics. In: ACS Monograph 177. American Chemical Society; Washington DC:1982. p. 339

95. Karaman R. Analysis of Menger's 'spatiotemporal hypothesis'. Tetrahedron Lett 2008;49:5998-6002

96. Karaman R. Cleavage of Menger's aliphatic amide: a model for peptidase enzyme solely explained by proximity orientation in intramolecular proton transfer. J Mol Struct 2009;910:27-33

97. Karaman R. The efficiency of proton transfer in Kirby's enzyme model, a computational approach. Tetrahedron Lett 2010;51:2130-5

98. Karaman R, Hallak H. Computer-assisted design of pro-drugs for antimalarial atovaquone. Chem Biol Drug Des 2010;76:350-60

•• **Demonstration of utilizing computational approaches in prodrug design.**

99. Karaman R. Analyzing the efficiency in intramolecular amide hydrolysis of Kirby's N-alkylmaleamic acids – a computational approach. Comput Theor Chem 2011;974:133-42

100. Hejaz H, Karaman R, Khamis M. Computer-assisted design for paracetamol masking bitter taste prodrugs. J Mol Model 2012;18:103-14

101. Karaman R. Computational-aided design for dopamine prodrugs based on novel chemical approach. Chem Biol Drug Des 2011;78:853-63

102. Karaman R, Dajani KK, Qtait A, Khamis M. Prodrugs of acyclovir – a computational approach. Chem Biol Drug Des 2012;79:819-34

•• **Demonstration of utilizing computational approaches in prodrug design.**

Affiliation

Arik Dahan[†1], Mustafa Khamis[2], Riad Agbaria[1] & Rafik Karaman[2]
[†]Author for correspondence
[1]Ben-Gurion University of the Negev, School of Pharmacy, Faculty of Health Sciences, Department of Clinical Pharmacology, P.O. Box 653, Beer-Sheva 84105, Israel
Tel: +972 8 6479483;
Fax: +972 8 6479303;
E-mail: arikd@bgu.ac.il
[2]Al-Quds University, Faculty of Pharmacy, Department of Bioorganic Chemistry, P.O. Box 20002 Jerusalem, Palestine