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Intestinal Targeting of Drugs: Rational Design Approaches and Challenges

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Abstract: Targeting drugs to the gastrointestinal tract has been and continues to be an active area of research. Gut-targeting is an effective means of increasing the local concentration of active substance at the desired site of action while minimizing concentrations elsewhere in the body that could lead to unwanted side-effects. Several approaches to intestinal targeting exist. Physicochemical property manipulation can drive molecules to large, polar, low absorption space or alternatively to lipophilic, high clearance space in order to minimize systemic exposure. Design of compounds that are substrates for transporters within the gastrointestinal tract, either uptake or efflux, or at the hepato-biliary interface, may help to increase intestinal concentration. Prodrug strategies have been shown to be effective particularly for colon targeting, and several different technology formulation approaches are currently being researched. This review provides examples of various approaches to intestinal targeting, and discusses challenges and areas in need of future scientific advances.

Keywords: Drug, Gut, Intestine, Non-systemic, Prodrug, Soft drug, Targeting, Transporter.

1. INTRODUCTION

Tissue targeting has become an increasingly important area of research in drug discovery. Tissue targeting refers to having an increased local concentration of drug in a particular tissue or region of the body that is the site of action for that therapeutic target. It is usually done for safety purposes – i.e. the concentration of drug needed at the site of the desired therapeutic target for efficacy would lead to some undesired effect in another area of the body. This undesired effect can be from off-target activity in another tissue, such as the well-documented hERG liability of several drugs that occurs from interaction of the drug substance with a potassium ion channel in the heart [1-4], or can result from on-target activity in an undesired tissue. For instance, a common side effect of statin therapy for hypercholesterolemia is myalgia, and a less common but more serious adverse event is rhabdomyolysis. These are thought to occur from statin inhibition of HMG-CoA reductase in the muscle, the same target that, when inhibited in the liver, leads to the desired effect of lipid lowering [5]. In either case, by targeting the specific tissue of desired therapeutic action, the concentration of drug at the site of undesired action can be minimized, resulting in an increased therapeutic index.

The concept of tissue targeting has long been embraced for certain tissues and therapeutic areas. Skin [6-7], eye [8], ear [9], and lung [10-12] therapies often increase the local concentration of drug using targeted delivery methods (e.g. topical administration, inhalers), usually combined with physicochemical properties of the molecule to prevent significant distribution into the body. Central Nervous System (CNS) agents have not been traditionally considered tissue targeted since the highest measured brain to plasma ratios are most often ≤2 [13]; however, this is currently an active area of research [14-15]. Kidney targeting strategies, reviewed previously [16-17], have made use of amino acid-linked prodrugs (e.g. *N*-acetyl-γ-glutamyl sulfamethoxazole) that are cleaved by kidney-specific enzymes that recognize this motif [18], lysozyme-tethered small molecules, polymeric carriers, and liposomes conjugated to antibodies. Among oral drugs, liver targeting appears to be among the most scientifically advanced. Steraroyl-CoA desaturase (SCD) inhibitors [19-22], glucokinase activators [23], and next generation HMG-CoA reductase inhibitors [5, 24] are recent examples of liver-targeted strategies whereby organic ion transporting polypeptides (OATPs), highly expressed in the liver, actively transport the small molecules of interest into the liver. This is complemented by low passive permeability to restrict both passive exit out of the hepatocyte and diffusion into systemic tissues, thereby increasing the relative concentration of the active drug in the liver versus undesired peripheral tissue (e.g. skin and eye for SCD inhibitors, pancreas for glucokinase activators, and muscle for HMG-CoA reductase inhibitors).

Modulation of targets within the gastrointestinal (GI) tract can impact a number of different therapeutic areas including obesity, diabetes, infectious diseases, and inflammatory disorders, such as ulcerative colitis and Crohn's disease. Recent results from type 2 diabetic patients that have undergone gastric bypass surgery suggest that a significant number of diabetics go into remission within days after surgery [25-26]. Also, emerging data supports the importance of the gut microbiome on health and disease [27-31]. These recent developments suggest the potential for an increased emergence

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of disease-modifying targets within the GI tract. It is therefore important to ensure that rational design approaches to pharmaceutically active compounds are available in order to safely modulate these targets. As with other tissue-targeting approaches, increasing the intestinal concentration of drug versus anti-tissue should increase the safety margin [32-33]. The degree of gut specificity required is highly target dependent and ranges from requiring no systemic absorption (specific) to having some degree of uptake into the GI tract (selective). In addition, intestinal targets can be luminal – within the intestinal lumen or on the luminal side of the apical membrane of the epithelium – and therefore no absorption is needed; or intracellular – within the enterocyte – or in the intestinal tissue on the basolateral side of the enterocyte, in which case some transport across the apical gut wall is necessary. An intestinal-targeted drug can also be thought of as a non-systemic drug, that is, an oral drug with limited systemic bioavailability.

This review will provide design approaches for selectively targeting the gut with an oral drug based on known drugs and discovery programs, as well as potential strategies for future efforts. Approaches include the rational design of active compounds or prodrugs that have specific physicochemical properties for limited absorption or high clearance, identification of substrates for transporters within the gastrointestinal tract or hepato-biliary interface, and the use of formulation technologies. The specific strategy chosen depends on the location of both the intestinal target and anti-tissue, the nature of the chemical substrate, and desired pharmacokinetics and dynamics. An introduction to absorption and metabolism of orally dosed drugs will first be summarized to provide context for the various intestinal-targeting approaches. Gut-targeted inorganic or polymeric drugs, such as lanthanum carbonate [34-36], calcium acetate, sevelamer hydrochloride, sevelamer carbonate [34, 37], and calcium polycarbophil [38-39] are beyond the scope of this review.

2. GUT PHYSIOLOGY AND DRUG PHARMACOKINETICS

In humans, the gastrointestinal tract consists mainly of the stomach, small intestine (duodenum, jejunum, and ileum), and large intestine (cecum, colon, and rectum). The human GI tract is ~8.4 m long and the relative size of the small intestine to the total length of the GI tract is 81% in humans. The small intestine has a large surface area (200 m^2) that is provided by three anatomical modifications. The folds of Kerckring are grossly observable folds of mucosa that increase the surface area by 3-fold [40]. From the plicae circularis project microscopic finger-like pieces of tissue called villi that increase the surface area by 10-fold for humans. Each villus is covered in microvilli, which increase the surface area by 20-fold (Fig. (**1**)). As a result, the small intestine is considered the main site of absorption for nutrients and

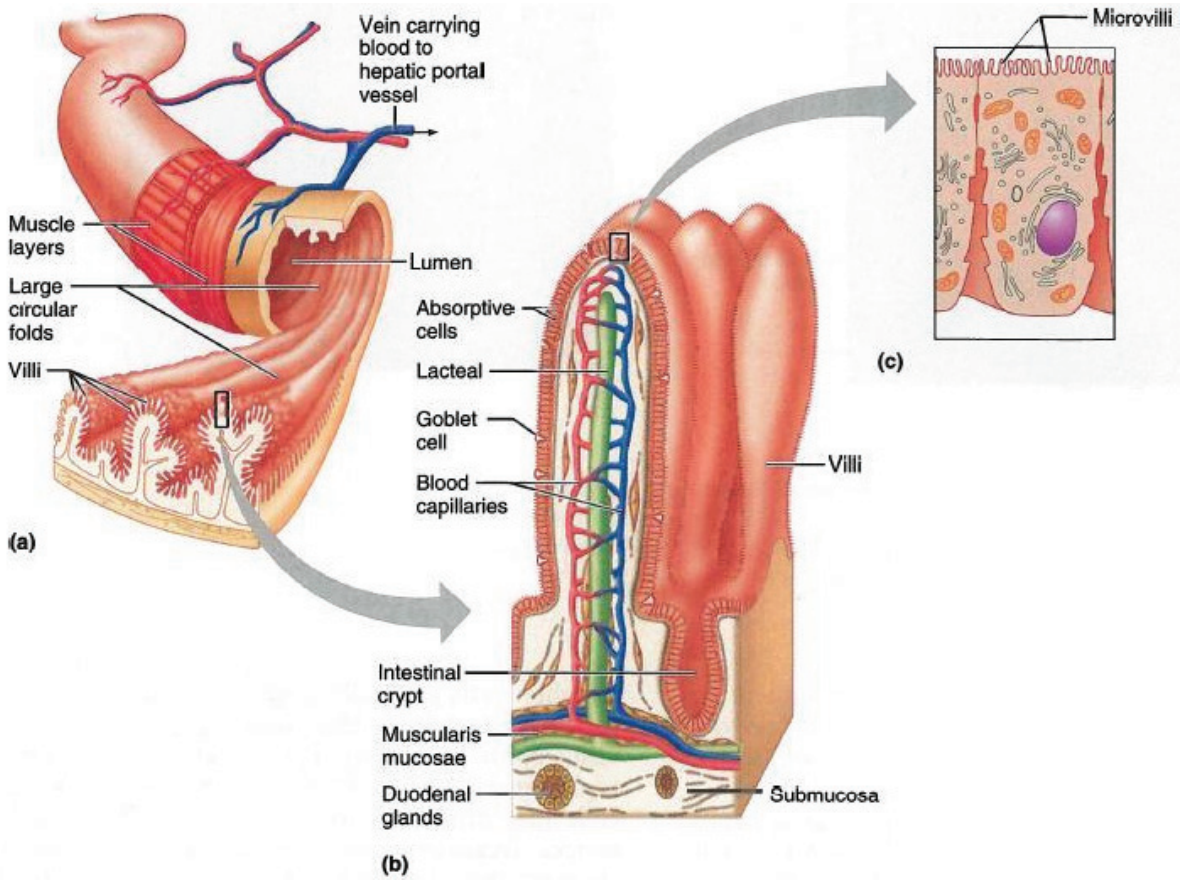


Fig. (1). Anatomy of intestinal tissue. (a) Intestinal section showing folds of Kerckring. (b) Structure of villus. (c) Enterocyte. *MARIEB, ELAINE N., HUMAN ANATOMY & PHYSIOLOGY, 6th,©2004*. Printed and Electronically reproduced by permission of Pearson Education, Inc., Upper Saddle River, New Jersey [42].

xenobiotics. There are several layers of tissue that comprise the intestines and display discrete functions. Enterocytes, a subtype of epithelial cells, are the dominant cell type at the luminal surface and comprise the innermost layer of the mucosa [41].

Unlike the small intestine, the large intestine does not contain villi and is divided into geographical areas by transverse furrows. In addition, the large intestine enterocytes differ somewhat from those of the small intestine; for instance, the large intestine microvilli are less densely packed. Overall, this leads to a much smaller surface area in the large intestine and therefore lower levels of absorption.

Following oral dosing and dissolution, drug molecules can cross the luminal membrane by passive diffusion or active transport (Fig. (**2**)). Passive diffusion is comprised of two pathways: (a) the paracellular pathway in which a drug diffuses through the aqueous pores at the tight junctions between the intestinal enterocytes; and (b) the transcellular (lipophilic) pathway, which requires drug diffusion across the lipid cell membrane of the enterocyte. Active transport is mediated by membrane transporters and is functionally divided into active drug influx and efflux. The relevance of each route to the absorption of a compound is determined by the compound's physicochemical properties and its affinity for various transport proteins [40].

The extent of ionization plays a pivotal role in determining a drug's dissolution rate and passive permeability across the GI tract. Therefore, the pH at the absorption site is a critical factor in facilitating or inhibiting the dissolution and absorption of various ionizable drug molecules. In humans, the pH of chyme is more acidic and can be as low as 1.5. When the chyme arrives in the duodenum, it is quickly neutralized by the secretion of the pancreatic bicarbonate and bile. The pH values of chyme become progressively more alkaline in the distal portion of the small intestine (pH ~7). However, the pH of chyme in the large intestine is generally more acidic (pH ~6) than the pH observed in the small intestine, possibly due to the fermentation mediated by the microbial flora.

2.1. Passive Diffusion

Passive diffusion across the gut wall can occur either through paracellular or transcellular diffusion. In the paracellular pathway, drug molecules are absorbed by diffusion and convective volume flow through the water-filled intercellular space. In general, drugs that are absorbed through this pathway are small molecules (e.g., molecular weight (MW) < 250 Da) and are hydrophilic in nature (Log*P* < 0) [43]. Because the junctional complex has a net negative charge, positively charged molecules pass more readily, whereas negatively charged molecules are repelled [44]. Furthermore, the paracellular pathway offers a limited window for absorption since it accounts for <0.01% of the total surface area of intestinal membrane. In addition, the tight junctions between cells become tighter traveling from the jejunum towards the colon. As a result, traditional sustained release formulations targeting colonic absorption are not appropriate for paracellularly absorbed molecules (e.g. metformin) [45].

The transcellular pathway is the major route of absorption for most drug molecules. In general, the rate of passive transcellular absorption is mainly determined by the rate of transport across the apical cell membrane, which is controlled by the physicochemical properties of the absorbed compound [43]. For good passive transcellular absorption, a compound needs the right balance of solubility in the GI tract and permeability into the gut wall. Unlike the paracellular pathway, compounds that are well absorbed through the transcellular pathway are generally non-ionised, with Log*P* > 0 and MW 300-500. In addition, the hydrogen-bonding capacity as determined by the number of hydrogen bond acceptors (HBA) and hydrogen bond donors (HBD) is generally <10 and <5, respectively [40, 46]. The luminal pH in the GI segments plays a key role in the transcellular membrane permeability of ionizable drugs. For instance, due to lower extent of ionization, basic drugs usually possess relatively higher permeability in the lower GI, where the luminal pH is ~7.4 [47].

2.2. Active Transport

Transporters play an important role in the tissue distribution of xenobiotics and play a significant role in the intestinal absorption of a large number of drugs [48]. Targeting intestinal uptake transporters to increase oral exposure of compounds with low passive permeability has been shown to be an effective strategy to increase bioavailability [49].

Several transporters belonging to the adenosine triphosphate (ATP) binding cassette (ABC) and the solute carrier (SLC) superfamilies are localized on the apical and basolateral membranes of enterocytes. ABC transporters utilize ATP to drive the transport and are called primary active transporters. SLC transporters primarily use the ion gradients (H^+ and Na^+ gradients) created across the membrane by primary active carriers (example: Na^+/K^+-ATPase, Na^+/H^+-ATPase) [50]. ABC transporters expressed in the intestine include P-glycoprotein (Pgp, *ABCB1*), breast cancer resistance protein (BCRP, *ABCG2*), and multidrug resistance proteins (MRP1-6; *ABCC1-6*). These efflux transporters functionally limit the enterocytic levels of their substrates by reducing uptake and facilitating efflux. SLC transporters suggested as relevant at the intestinal apical surface of enterocytes include peptide transporter (PEPT1, *SLC15A1*), organic anion polypeptide transporter (OATP2B1, *SLCO-2B1*), monocarboxylate transporter (MCT1, *SLC16A1*), sodium-multivitamin transporter (SMVT, *SLC5A6*) and organic cation/zwitterion transporters (OCTN1, *SLC22A4;* OCTN2, *SLC22A5*). Several other SLC transporters including organic anion or cation transporters (OATs or OCTs, *SLC22*) have also been identified in the intestine [51-53].

Limited information is available on the transporters that are expressed on the basolateral membrane of the enterocytes. For example, MRP1, MRP3, and MRP4 are efflux transporters expressed on the basolateral membrane. SLC transporters such as OCT1 and basolateral peptide transporters are expressed on the basolateral membrane as well. As the science evolves to understand basolateral transporters further, a clearer picture will emerge as to whether opportunities exist for intestinal targeting via this approach.

Drug-drug or drug-food interactions associated with the inhibition of intestinal transports could result in significant changes in the drug exposure both at the gut and the systemic circulation [49]. The direction and magnitude of exposure changes depend on the transporter involved and the potency of the inhibitory drug. For example, Pgp inhibition results in an increase in the systemic exposure and tissue distribution of drugs, while inhibition of uptake transporters such as OATP2B1 may reduce the systemic and tissue exposure. Apart from several drugs that are known to inhibit intestinal transporters, food compositions such as grapefruit juice and orange juice are also shown to cause inhibition of both uptake and efflux transporters [49].

2.3. Metabolism and Biliary Clearance

The liver is the main metabolizing organ, and many phase-I and phase-II metabolizing enzymes reside in hepatocytes. Cytochrome P450 isoenzymes (CYPs) are responsible for the phase-I metabolism of a large number of endogenous compounds as well as xenobiotics, playing a pivotal role in drug clearance. The superfamily consists of isoenzymes that are highly selective for endogenous substrates as well as isoenzymes that are less selective and metabolize exogenous substrates including drugs. For example, CYP3A4 shows affinity for a wide range of substrates. CYP3A4 attacks lipophilic drugs in positions largely determined by their chemical liability; that is, the ease of hydrogen or electron abstraction. The most important phase II conjugation reactions are catalyzed by the UDP-glucuronyl transferases (UGTs). Glucuronidation involves the transfer of D-glucuronic acid from UDP-glucuronic acid to an acceptor compound. Similarly, sulphotransferases (SULTs) activate or inactivate compounds by transferring a sulfonate group from 3′-phosphoadenosine-5′-phosphosulfate to a hydroxyl or amine group [54]. Conjugation via phase II reactions generally renders the acceptor compound more water-soluble and results in increased excretion in either urine or bile. On the other hand, glutathione-*S*-transferases (GSTs) are the most important family of enzymes involved in the metabolism of alkylating compounds and their metabolites. They are a major defense system in deactivating toxic materials within the body. Apart from these, hepatocytes also lodge several nonspecific and membrane-bound esterases, which are of particular interest in prodrug activation.

Although the small intestine is regarded as an absorptive organ and may act as a rate-limiting barrier, it also has the ability to metabolize drugs by several pathways involving both phase I and phase II reactions and may lead to limited systemic exposure. In our recent analysis of 309 drugs, we noted that about 30% of the drugs in the data set show more than 20% intestinal extraction, underscoring the importance of the intestine as an elimination organ [48]. CYPs are predominantly present in liver but also found in the intestine, kidneys, and lungs. CYP3A4, the most abundant CYP present in human hepatocytes, is also the major CYP present in intestinal enterocytes and is implicated in the metabolic elimination of many drugs [55-56]. The next common metabolic elimination pathways are due to glucuronidation and ester hydrolysis. Furthermore, intestinal hydrolysis is an important reaction in the bioconversion of prodrugs. The UGTs and sulphotransferases are present in the intestine as well as

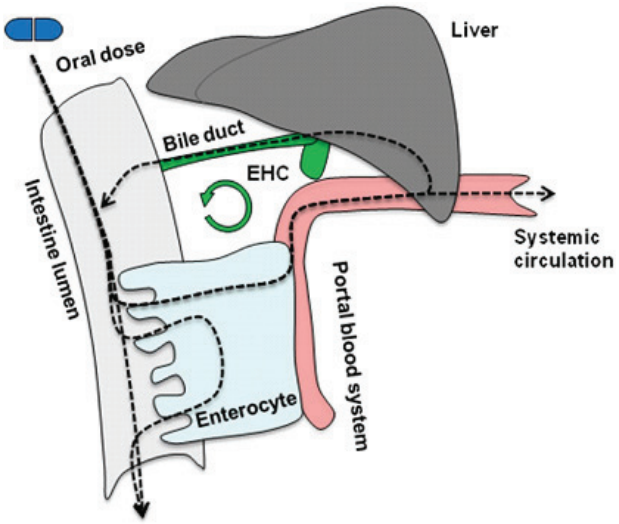


Fig. (2). Schematic of processes occurring during oral drug absorption. Following oral administration, the drug is dissolved in the intestinal lumen and permeates across the enterocytes to reach the portal blood system. Certain chemicals or drugs are restricted from entering the enterocytes owing to the low permeability. Due to the efflux transporters on the apical membrane or possible rate-limiting permeability across the basolateral membrane, drug entering the enterocyte may be effluxed back into the lumen. Also, drugs reaching the portal blood system may be extracted by the liver, and may be secreted into the intestinal lumen via biliary excretion resulting in enterohepatic circulation (EHC).

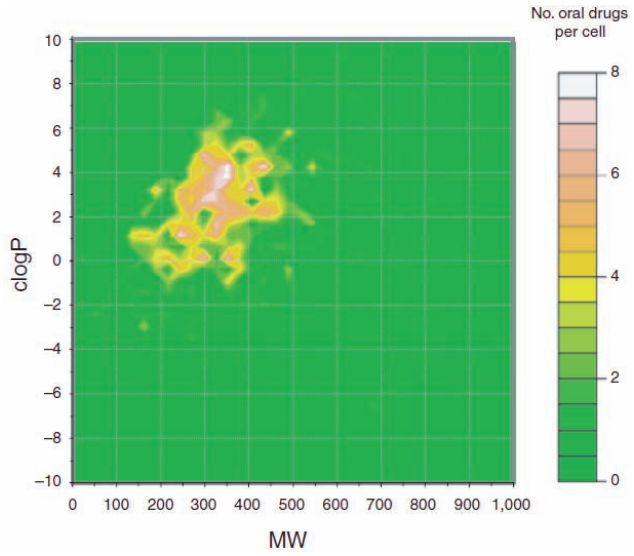


Fig. (3). Molecular property space of 615 oral drugs. Reprinted by permission from Macmillan Publishers Ltd: Nature Biotechnology [71], copyright 2006.

the liver and catalyze the metabolism of many phenol- or catechol-containing drugs (e.g. morphine) during their passage through the gut. The predominant human intestinal carboxylesterase, CES2, preferentially hydrolyzes prodrugs in which the alcohol group of a pharmacologically active molecule has been modified by the addition of a small acyl group

[57]. Several other non-specific esterases and amidases are also localized in the enterocytes and mediate the metabolism of drugs.

Biliary excretion is a major elimination pathway for many drugs and/or their metabolites, and has significant impact on systemic exposure, pharmacological, and toxicological effects of drugs [58-59]. Furthermore, a drug excreted in its unchanged form into the bile has the potential to be reabsorbed from the gut. This process, also called entero-hepatic recirculation, may lead to prolonged drug exposure to the gut and liver. A physicochemical trend analysis suggests that higher values in MW, polar surface area (PSA) and hydrogen bonding ability are clearly overrepresented among compounds with significant biliary excretion, and acidic compounds are predominantly excreted in bile [60]. Biliary excretion is predominantly driven by an active transport mechanism involving uptake and efflux pumps expressed on the hepatic sinusoidal and canalicular membranes, respectively. Multidrug resistance-associated protein 2 (MRP2), multidrug resistance 1 (MDR1) and breast cancer resistance protein (BCRP) are expressed on the hepatic canalicular membrane, and are thought to be responsible for the biliary excretion of drugs and metabolites [61-62]. Organic anion transporting polypeptide (OATP1B1, OATP1B3, OATP2B1) and organic cation transporter 1 (OCT1) on the sinusoidal membrane are involved in the hepatic uptake of several drugs [63-65]. Among these, OATP1B1 and OATP1B3 are selectively expressed in the human liver and exhibit reasonably broad substrate specificities, suggesting the importance of these transporters in the hepatic uptake of many clinically important anionic drugs.

3. GUT-TARGETING STRATEGIES

One role of the medicinal chemist is to design compounds that balance potency with physicochemical properties to achieve exposure at the desired site of action. Typically, the medicinal chemist works to design oral drugs with high systemic exposure. To achieve this profile, a compound must not only be absorbed but must also be stable to the metabolizing enzymes present in both the GI tract and the liver. Beginning with the seminal 'rule-of-five' paper by Lipinski [66], followed by others such as those by Veber [67], Wenlock [68], and Leeson [69-70], the general physicochemical attributes to favor good oral bioavailability (F) and absorption have been clarified based on retrospective analyses (MW ≤ 500, Log*P* ≤ 5, HBD ≤ 5, HBA ≤ 10, rotatable bonds (RB) ≤ 10, PSA ≤ 140) (Fig. (**3**)). It therefore stands to reason that, by careful control of properties outside ideal oral drug space, the medicinal chemist can minimize oral bioavailability. A more recent analysis [48] of >300 drugs examined the relationship between physiochemical properties and the components that contribute to oral bioavailability including fraction absorbed (Fa), fraction escaping gut metabolism (Fg), and fraction escaping hepatic metabolism (Fh). The analysis suggests that drugs with low oral exposure (F < 20%, n of 49, mean F = 7%) have on average higher molecular weight (444), higher total polar surface area (130 Å^2), lower Log*D* (c$\text{Log}D_{\text{pH 6.5}}$ = –0.13), and higher numbers of rotatable bonds (6.8), hydrogen bond acceptors (6.2) and hydrogen bond donors (3.7) as compared to drugs with F ≥ 80% (averages: MW = 317, c$\text{Log}D_{\text{pH 6.5}}$ = 0.29, RB = 4.16, HBA = 3.8, and HBD = 2.0). Furthermore, about 20% of compounds in the less bioavailable cohort fail the rule-of-five criteria while only 1% fail in the F ≥ 80% cohort.

Since an intestinal targeting approach demands low systemic bioavailability and since F = Fa x Fg x Fh, several strategies exist for the medicinal chemist to consider. Fraction absorbed can be minimized by reducing permeability and/or solubility, or by increasing intestinal apical efflux. Fg can be minimized through high intestinal metabolism, while Fh can be minimized through either high hepatic metabolism or biliary clearance.

4. PHYSICOCHEMICAL PROPERTY APPROACH

4.1. Low Fraction Absorbed

Luminal targets may offer the most straightforward path for achieving gut selective therapies since drug absorption can be driven down to very low levels by physicochemical property manipulation. Design of high molecular weight, polar compounds should lead to very low permeability and drug sequestering in the gut lumen. High molecular weight also correlates with low solubility, thereby further reducing absorption. Obviously, the biological target must be able to accommodate a ligand of this type. In addition, care must be taken to ensure that the compound of interest is not a substrate for apical uptake transporters. Examples of drugs that fall into this category and their properties are described.

A number of oral, non-absorbable antibacterial agents are available for the treatment of microbe-caused diseases. Rifaximin (Fig. (**4**)) is an antibiotic used clinically to treat traveler's diarrhea and hepatic encephalopathy and has recently been shown to be effective as a treatment for irritable bowel syndrome [72]. It binds to the beta subunit of bacterial DNA-dependent RNA polymerase, which suppresses the initiation of RNA synthesis chain formation [73-74]. Rifaximin is a derivative of rifamycin with the addition of a fused imidazopyridine [75]. The molecule is non-rule-of-five compliant (MW 786, HBA = 11, PSA = 198 Å^2) and is partially zwitterionic due to a charged resonance structure. In addition, a possible intramolecular hydrogen bond between the relatively basic imidazole nitrogen and adjacent carbonyl may alter the overall charge of the molecule from neutral to cationic. Rifaximin has poor absorption (Fa = 0.4%), due to poor permeability [76] and low aqueous solubility [77-78]. Its solubility in water and pH 6.8 tris phosphate buffer is ≤0.01 mg/mL [79]. Greater than 99% of the administered dose of rifaximin is recovered in the feces [73]. The compound is, however, permeable across the bacterial cell wall, leading to its efficacy. A balance of polarity is needed since compounds that are too polar cannot cross the bacterial cell wall. For example, the more polar pyridinium species **1**, although quite active against isolated DNA-dependent RNA polymerase, has poor broad-spectrum antibiotic activity, presumably due to its inability to cross the bacterial cell wall [75]. Rifaximin shows low rates of adverse events due to its low bioavailability [72]. In general, antibacterials are very large and polar relative to rule-of-five thresholds, due to the different bacterial cell wall composition compared to eukaryotic lipoidal cell membranes. An analysis of the physicochemical properties of several classes of antibacterials has appeared [80].

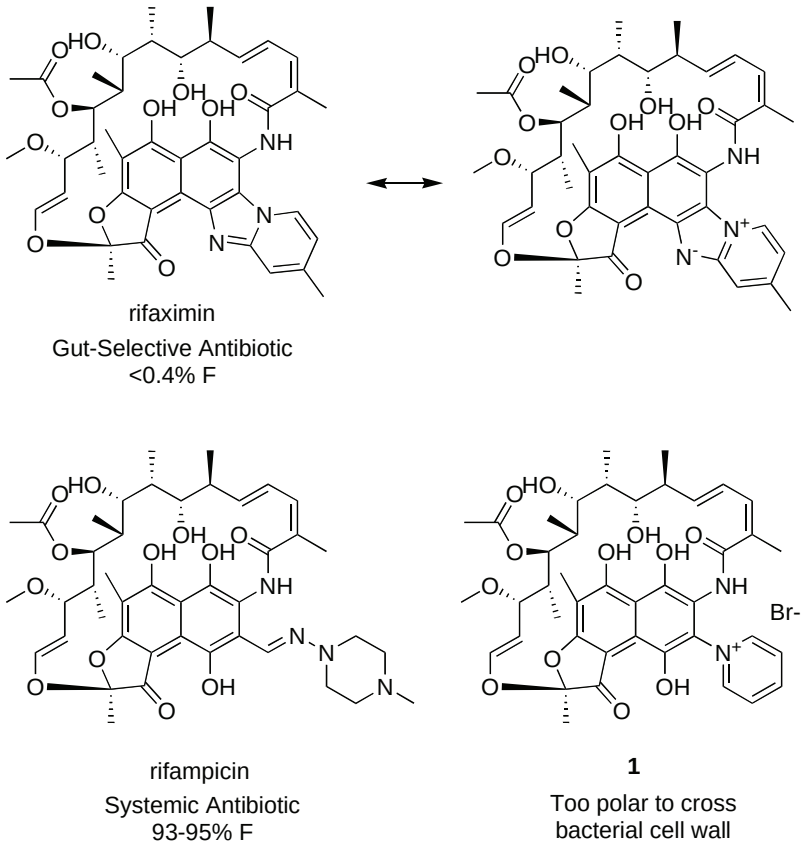


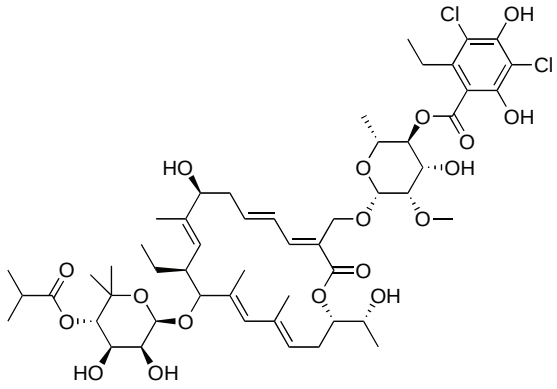
Fig. (4). Rifamycin derivatives with varying permeability range.

Rifaximin can be contrasted with rifampicin, which has improved solubility and permeability, leading to high oral bioavailablity (93-95% F) [81], and therefore is used to treat systemic infections [82]. Its solubility increases significantly in the acidic environment of the stomach (11.4 mg/mL @ pH 2; 1.25 mg/mL @ pH 4.5), presumably due to ionization, while conversely, its permeability is much higher in the duodenum versus stomach (P_{eff} $0.62x10^{-4}$ cm/s duodenum vs. $0.02x10^{-4}$ cm/s stomach). It is estimated that 45% of dose is absorbed in the stomach and the remaining in the small intestine. Absorption is not thought to be enhanced by transporters [81]. Recent work has begun to elucidate the properties that allow some non-rule-of-five compounds to be well absorbed [83].

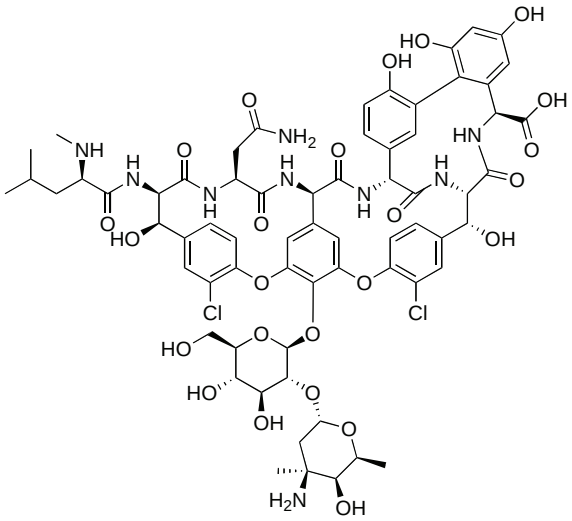
There are several other non-absorbable antibacterials (Fig. (**5**)), some of which are typically administered intravenously or intramuscularly. However, these are also used orally as gut-selective therapies for the treatment of *Clostridium difficile* infections, pseudomembranous colitis, hepatic encephalopathy, and parasitic infections [84-88]. These molecules are large and polar with many hydrogen bond donors that lead to low permeability. Vancomycin (MW 1449, HBD = 21, HBA = 24, PSA = 530 Å^2, RB = 13) has high aqueous solubility (83 mg/mL) but very low permeability which leads to exposure below the detectable limit (<640 ng/mL) after a 250 mg dose [89]. Oral administration of fidaxomicin (MW 1058, HBD = 7, HBA = 15, PSA = 267 Å^2, RB = 15) provides plasma concentrations of parent and its main metabolite of ≤50 ng/mL due to poor solubility. Both drugs are Pgp substrates which also contributes to their low plasma levels [90]. Ramoplanin (MW 2254, HBD = 40, HBA = 41, PSA = 1000 Å^2, RB = 35) is a glycolipodepsipeptide antibacterial currently under investigation for intestinal infections. It is not detected in plasma after oral administration [91]. Other examples include teicoplanin, bacitracin, paromomycin, and neomycin. A review on the pharmacokinetics of antibacterials including chemical structural modifications to enhance oral bioavailability has appeared [92].

Similar approaches have been used in other drug classes. Nystatin is a polyene antifungal agent that is most often used topically on the skin; however, it can also be used orally to prevent systemic candidosis, which occurs when a yeast such as *Candida albicans* travels from the GI lumen into different tissues of the host body [93]. Nystatin binds to the cell membrane of yeast species such as *Candida* within the GI lumen and leads to fungal cell death, preventing systemic infection. Nystatin has shown toxic effects (vein sclerosis, fever, chills, shaking, malaise) when administered systemically, and so minimal absorption is key to its safety profile [94]. Its low absorption is due to low permeability caused by its large size and polarity (MW 926, HBD = 13, HBA = 17, cLog*P* = –3.3, PSA = 320 Å^2).

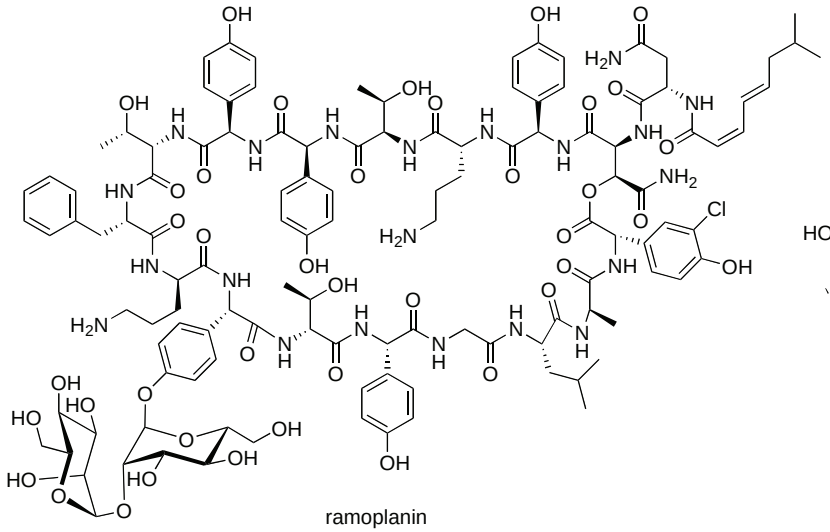
Examples of drugs with low absorption in conjunction with high luminal metabolism to further reduce absorption are provided. Acarbose (MW 646, cLog*P* = –6.6, HBD = 14, HBA = 19, PSA = 321 Å^2) (Fig. (**6**)) is an inhibitor of both pancreatic α-amylase, which functions normally to hydrolyze ingested complex starches to oligosaccharides within the lumen; and α-glycosidase, which is a luminal enterocytic



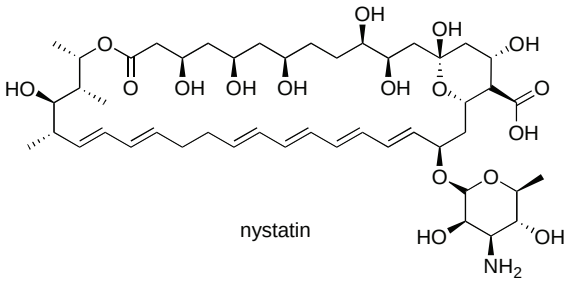
fidaxomicin



vancomycin

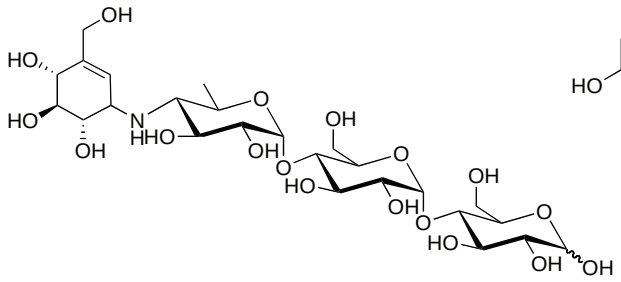


ramoplanin

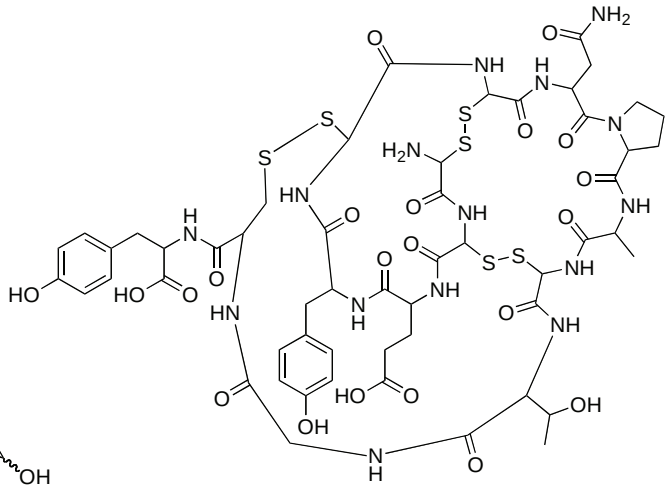


nystatin

Fig. (5). Subset of low-absorption anti-infectives.



acarbose



linaclotide

Fig. (6). Acarbose and linaclotide.

membrane-bound enzyme that then hydrolyzes the oligosaccharides to glucose [95]. Taken with a meal, acarbose essentially reduces absorbed glucose, leading to HbA_{1c} lowering of 0.7% in diabetics [96]. The fraction absorbed of this large, polar drug is 1-2% in humans [97], and 35% of total radioactivity is absorbed after oral administration of radiolabeled drug, largely as degradation products [97]. Acarbose is highly water soluble (1.40 g/mL @ 20 ^{o}C) [98] and so its low absorption is largely due to its low permeability, driven by its large size and high polarity. An experiment performed in germ-free rats demonstrated that metabolism of acarbose is largely via microflora enzymes, although some metabolism does occur from host species luminal hydrolases as well. The small amount of parent that is absorbed is cleared renally, at a rate close to the glomerular filtration rate [97], without systemic metabolism.

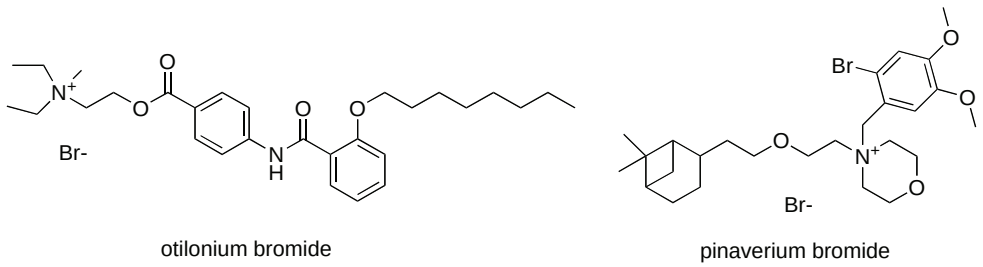
Linaclotide (MW 1456, cLog*P* = –3.4, HBD = 21, HBA = 22, PSA = 725 Å^2, RB = 13) is a 14 amino acid cyclic peptide that is currently approved for irritable bowel syndrome related constipation [99]. Linaclotide is an agonist of the luminally expressed guanylate cyclase type-C (GC-C) receptor [100]. This synthetic peptide with three disulfide bonds is a variant of two endogenous GC-C receptor ligands, guanylin and uroguanylin. Binding of linaclotide to the receptor stimulates the eventual increase in the secretion of chloride and bicarbonate ions leading to increased water secretion into the lumen to maintain isotonic equilibrium. The permeability of linaclotide is very low which contributes to its low oral bioavailability in rats (F = 0.1%). In humans, no detectable plasma drug concentration was observed after doses up to 3 mg [101-102]. In addition, intestinal metabolism of linaclotide is high. Carboxypeptidase A, in the gut, removes the terminal tyrosine residue to produce an active metabolite. In addition, the disulfide bonds of parent and metabolite are reduced in the GI tract leading to inactive linear peptides that are effectively metabolized by proteases. Despite short half-lives in the intestine of both parent and its active metabolite, gut exposure sufficient to provide efficacy is achieved, presumably due to the disulfide bonds delaying access by peptidases.

Additional complexity arises when the biological target of interest is found within the enterocyte or within the intestinal tissue. In these cases, sufficient absorption is required to modulate the target without achieving high systemic drug concentrations. This can be achieved using the same general approach as exemplified above – driving towards polar drug space – with attenuation of properties to allow for a low level of absorption. For instance, if permeability is low, higher solubility may be required to achieve modest absorption. A key challenge associated with an intracellular target is the ability to have sufficient residency time at the site of action to achieve the desired efficacy. If properties are balanced, high luminal concentrations of drug may provide reasonable flux across the apical membrane. Once inside the enterocyte, lower drug concentrations coupled with low passive permeability may enable longer residency times within the gut wall.

Otilonium bromide (cation: MW 484, cLog*P* = 4.0, PSA = 65 Å^2, RB = 17) and pinaverium bromide (cation: MW 512, cLog*P* = 5.8, PSA = 37 Å^2, RB = 10) (Fig. (**7**)) are quaternary ammonium antispasmodics used for the treatment of gastrointestinal disorders such as irritable bowel syndrome (IBS) [103]. In general, these compounds effect smooth muscle relaxation by blocking calcium channels and, in the case of otilonium bromide, inhibiting muscarinic receptors. Importantly, the ability to limit these compounds to the GI tract avoids calcium channel activity on cardiac tissue as well as the adverse side effects associated with systemic anticholinergics, such as tachycardia, nausea, dry mouth and confusion. The presence of the positively charged amine in both compounds results in poor absorption into the systemic circulation as well as limited exposure to the CNS. Both compounds are poorly absorbed in rats (otilonium bromide Fa < 3 %; pinaverium bromide Fa = 9%) and any drug that is absorbed is rapidly excreted into the bile, further limiting systemic exposure. For example, oral administration of ^{14}C-otilonium bromide to rats indicated that the compound is almost exclusively limited to the GI tract with weak radioactivity in the liver, lending support to the observation that excretion to the bile acts as a second mechanism to limit oral exposure of this compound [104]. Otilonium bromide is virtually undetected in humans after oral administration of a pharmacologically active dose with no anti-cholinergic side-effects [105]. These data support a design approach leading to incorporation of a quaternary ammonium group into a molecule of interest in order to limit systemic exposure.

4.2. High Metabolism

An alternative approach to the design of polar, beyond rule-of-five molecules for reduced permeability, is the design of large lipophilic, beyond rule-of-five compounds. Molecules of this type usually will have significant levels of metabolism-driven clearance and so Fg, the fraction escaping gut metabolism, or Fh, the fraction escaping hepatic metabolism, can drive the required poor systemic exposure. In addition, compounds in this space tend to have low aqueous

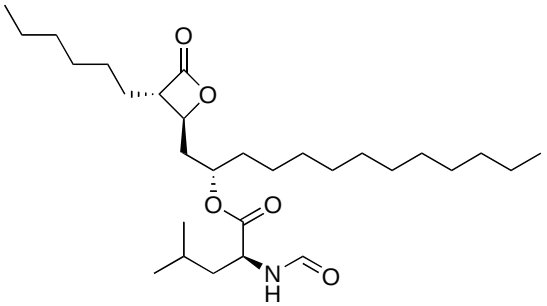


otilonium bromide pinaverium bromide

Fig. (7). Otilonium bromide and pinaverium bromide.

solubility, while permeability can vary greatly depending on molecular size, leading to diverse degrees of absorption. Highly lipophilic compounds may enrich in specific hydrophobic regions within a cell or within the GI tract, thereby enhancing duration of action at the target (See Section 4.3).

Orlistat (Fig. (**8**)) is a small molecule pancreatic and gastric lipase inhibitor that is approved for the treatment of obesity [106-108]. Orlistat's site of action is in both the stomach and small intestine [108] where it covalently binds to these serine proteases with the catalytic serine opening orlistat's β-lactone. Because the lipase active site is blocked, it cannot function normally to hydrolyze dietary triglycerides to absorbable monoglycerides and free fatty acids. With drug treatment, the triglycerides that remain are not effectively absorbed and are eliminated in the feces, thereby lowering the caloric intake and leading to weight loss. Orlistat also inhibits lipoprotein and hepatic lipases *in vitro*, which are expressed in the vascular endothelium. Inhibition of these lipases could lead to elevated plasma cholesterol and triglyceride levels [109] and so sequestration of the drug to the GI tract is important to achieve a therapeutic window. Orlistat is a very lipophilic molecule (cLog*P* = 8.6) and has very low aqueous solubility (<0.01 mg/mL) driving its low bioavailability (F < 1%) [106]. The majority of the drug (83%) is eliminated unchanged in the feces and the remaining is thought to pass into the gut where it is readily metabolized [110] to two main inactive metabolites resulting from ester cleavage [111-112]. Extremely low plasma concentrations of parent drug were detected in clinical trials (<10 ng/mL, <1% F) [109].



orlistat

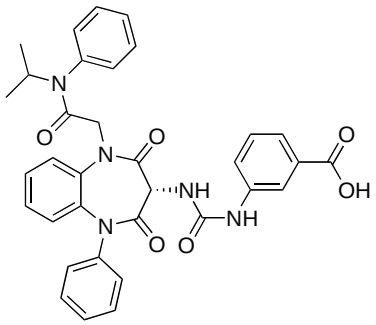
Fig. (8). Orlistat.

Cholecystokinin (CCK) is a gastrointestinal hormone that is released from duodenal endocrine I-cells in response to a meal. CCK activates the CCK1 receptor (CCK1R) in the GI tract and modulates a number of physiological responses that enable digestion and promote satiety [113-114]; therefore, CCK1R agonists may be useful for the treatment of obesity [115-116]. Activation of only the CCK1R located on the vagal afferents within the GI tract [117-118] was deemed sufficient as robust decreases in food intake were achieved in rodent models with poorly absorbed agonists [119-120]. For example, GI181771X [119, 121-122] (MW 606, cLog*P* = 5.1, cLog$D_{pH\ 6.5}$ = 4.1, PSA = 139 Å^2) and **2** [120] (MW 561, cLog*P* = 7.4, PSA = 88 Å^2) are lipophilic, acidic CCK1R agonists with limited systemic exposure despite demonstrating robust food intake effects in rodent models (Fig. (**9**)). GI181771X demonstrated low oral bioavailability (0.4%) in rats which was believed to be driven by poor absorption. Total intravenous clearance was moderate (22 mL/min/kg) and primarily driven by excretion of parent into the bile (67% of dose excreted in bile over 24 h) as the compound was metabolically stable in liver microsomes. Similarly, **2** demonstrated low total clearance (5.3 mL/min/kg) and low oral bioavailability (F = 7%) in mouse.

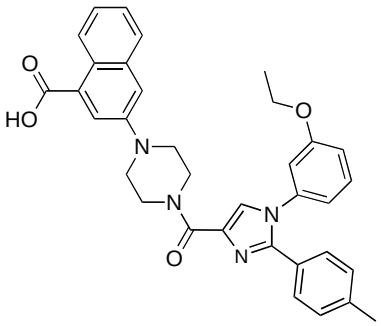
Researchers at Pfizer deliberately pursued a gut-targeted approach in the design of CCK1R agonists CE-326597 and **3** [123-124]. Because CCK1R agonists cause gallbladder contraction, reduced exposure to the gallbladder to avoid sustained contraction was desired to allow for the normal physiology of gallbladder emptying and filling that occurs in response to food. In order to avoid exposure to the gallbladder, not only was low systemic exposure desired but also low biliary clearance. This was achieved by avoiding lipophilic acids, which are often substrates for efflux transporters on hepatocytes. Compounds were designed to be lipophilic, high molecular weight, and neutral in order to achieve enough absorption into the gut wall to elicit a pharmacological response along with high hepatic metabolism to ensure low systemic exposure, and low biliary clearance to avoid sustained gallbladder contraction. This approach led to the discovery of a prototype CCK1R agonist CE-326597 (MW 595, cLog*P* = 6.3, Log$D_{pH\ 7.4}$ = 4.9) which had poor systemic exposure in rats (F = 1%) primarily due to its low fraction absorbed (9%) and moderate hepatic clearance (42 mL/min/kg). A more lipophilic backup compound, **3** (MW 649, cLog*P* = 7.7, Log$D_{pH\ 7.4}$ = 5.6), was also discovered which had more limited absorption (Fa = 0.56%) and enhanced clearance (Cl = 176 mL/min/kg) leading to significant reductions in oral exposure (F = 0.13%). Both compounds demonstrated low biliary clearance in mice at pharmacologically active doses. Drug concentrations in gut tissue and portal blood were measured and corrected for non-specific binding and demonstrated high gut:portal ratios (Fig. (**9**)). The higher lipophilicity of **3** likely contributed to its enhanced gut:portal ratio as compared to CE-326597. One challenge when working in more lipophilic space is the impact on solubility. In the CCK example above, CE-326597 and **3** had very poor solubility in their crystalline forms, which led to little to no absorption, significantly impacting preclinical efficacy. In order to achieve sufficient absorption for efficacy, a solubilising spray-dried dispersion (SDD) formulation was identified that improved the dissolution rate of the compounds and provided sufficient absorption to achieve efficacy [125-127].

4.3. High Absorption and High Metabolism: Soft Drugs

A soft drug is a pharmacologically active molecule that is purposefully designed to undergo a facile metabolic transformation into an inactive metabolite, which effectively 'shuts off' the activity after a sufficient time to achieve efficacy or after leaving the desired site of action [128-129]. This strategy may be particularly well suited if the mechanism requires only a brief period of action (e.g. agonism), the compound has a slow off rate from the receptor or relies upon covalent modification for activity, or if the target allows for a highly potent lipophilic molecule that provides an increased residency time in the target tissue.



GI181771X



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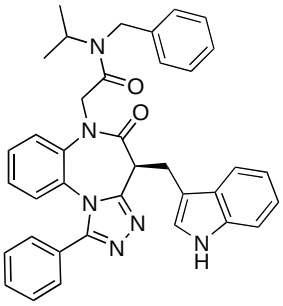
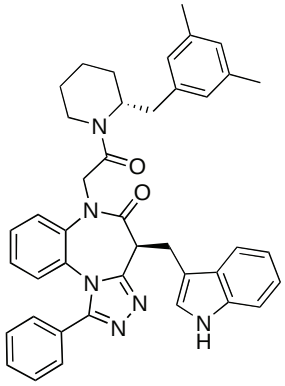


CE-326597

Gut:portal (free): 190 (2 h); 65 (4 h)



3

Gut:portal (free): 494 (2 h); 371 (4 h); 461 (8 h)

Fig. (9). CCK agonists.

While the soft drug strategy has been most often employed for dermal and respiratory targets where the drug can be delivered directly to the site of action, it can also be employed to target enterocytes through oral dosing. The particular challenges for applying this strategy in the gut is the need to balance the standard hurdles encountered with oral delivery involving solubilization and stability in the gut with retention and distribution within enterocytes and rapid clearance from plasma. A potent lipophilic agent can operate within this paradigm if sufficient solubility is provided by formulation and the molecule possesses sufficient membrane permeability to efficiently enter the enterocyte. Once in the enterocyte, these properties should allow the agent to distribute and be retained longer within the cell given its lower unbound fraction.

Selectivity is determined by differential stability in the enterocyte versus hepatocyte or other site of metabolism. Consideration must also be given to relative unbound fractions between *in vitro* assays and *in vivo*. The advantage afforded by high binding in enterocyte retention can be undone if the high free intrinsic clearance by hepatocytes is slowed due to a low free fraction in plasma.

Metabolites and their relative activities to the parent compound must be given consideration. While lipophilic molecules may be rapidly metabolized by CYP P450s, a single hydroxylation on a larger lipophilic molecule may not afford complete ablation of the parent's activity. A pathway involving glucuronidation or enzymatic hydrolysis of an amide or ester may provide a greater change in the physical properties and thus activities for the metabolites versus the parent compound. The key challenge in this approach is to tune the series to be stable in the intestine while rapidly cleared in the liver or other sites of metabolism.

An example where a soft-drug approach has been employed for a gut-targeted mechanism is in the inhibition of microsomal triglyceride transport protein (MTP). MTP is present in the enterocytes of the upper GI tract, where it is required for the absorption of dietary lipids and the assembly of chylomicrons. MTP is also expressed in the liver, where it is required for the formation and secretion of cholesterol- and triglyceride-containing very low-density lipoproteins (VLDL) particles. While inhibiting either of these sites of activity might provide a therapeutic benefit, early non-selective inhibitors (e.g. lomitapide) had their therapeutic potential limited by plasma liver enzyme elevation [130] attributed to liver fat accumulation as a consequence of hepatic MTP inhibition (Fig. (**10**)). While lomitapide (MW 694, cLog*P* = 7.0) is still being advanced for patients whose elevated cholesterol levels cannot be controlled with existing therapies, others have pursued a soft drug strategy to provide agents that selectively inhibit gut-MTP within the enterocyte.

Support for this gut-targeting strategy has been obtained in Phase 2 clinical trials by Johnson & Johnson (usistapide; MW 573, cLog*P* = 7.4) [131] and Japan Tobacco (granotapide) [132] with their ester-containing MTP inhibitors. The gut-targeting success of these researchers relied upon the differential stability of the ester functionality towards intestinal versus liver esterases. While granotapide (MW 719,

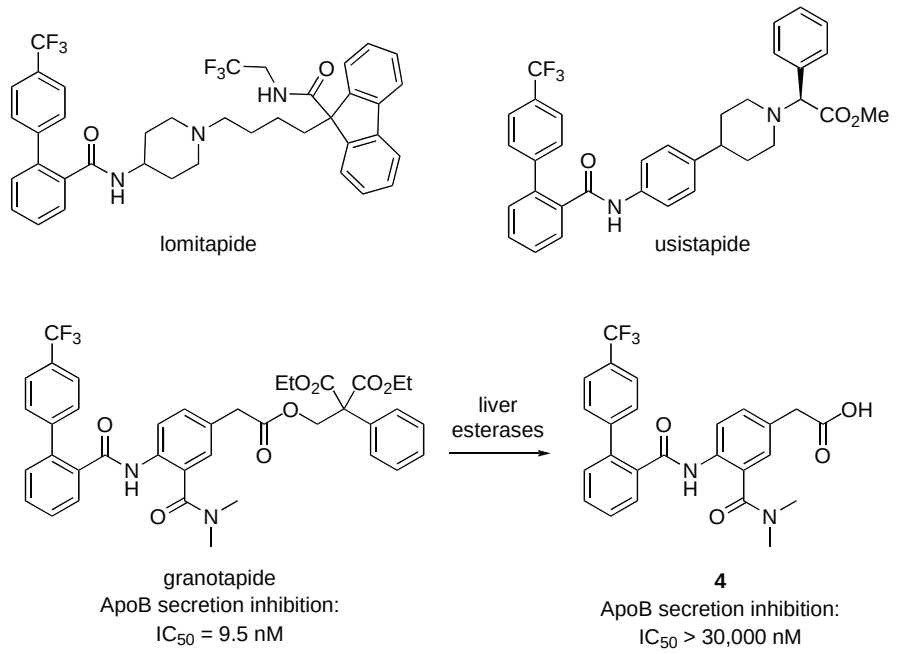


Fig. (10). MTP inhibitors.

cLog*P* = 6.0) is stable in enterocytes where it acts to inhibit intestinal MTP, once inside hepatocytes it is rapidly hydrolyzed to the inactive acid **4** (Fig. (**10**), MW 470, cLog*P* = 3.2), minimizing the intra-hepatic concentration of active to reduce hepatic MTP inhibition. The efficacy and selectivity of these agents is dependent on their duration of action within the enterocytes, which is dependent on their physicochemical properties. Although the relative concentrations of the compound between the intestine and liver were not reported, presumably due to the difficulty in such measures for lipophilic gut targeted compounds, the researchers provide evidence of a >1000-fold differential in site-specific activity.

Lubiprostone (Fig. (**11**)) (MW 390, cLog*P* = 3.8, RB = 11), an example of a small lipophilic drug with high gut metabolism, is prescribed for the treatment of constipation among irritable bowel syndrome patients. There is still active debate concerning the exact mechanism of action [133-134]. The initially reported molecular target was the voltage-gated ClC-2 chloride channel, which is a transmembrane ion channel located on the apical membrane of stomach, small intestine, and colonic epithelial cells, along with other tissues, notably lung [133, 135-137]. Lubiprostone binds to the ClC-2 channel on the enterocyte, which induces efflux of a chloride ion from the cytoplasm into the intestinal lumen. This leads to increased Na^+ and water efflux into the lumen, softening stool and relieving constipation. Lubiprostone is not appreciably absorbed and human plasma levels were below the quantifiable limit (<0.01 ng/mL) after an efficacious 24 μg b.i.d. dose [138]. Lubiprostone achieves its high gut-selectivity from extensive gut metabolism. Metabolism is thought to take place within the gut wall of the stomach and jejunum by microsomal carbonyl reductase. One active metabolite is known which makes up <10% of radiolabeled dose of parent [1]. This metabolite is absorbed into the plasma and has a half life of 0.9–1.4 h. Both parent and metabolite are detected in minute amounts in the feces.

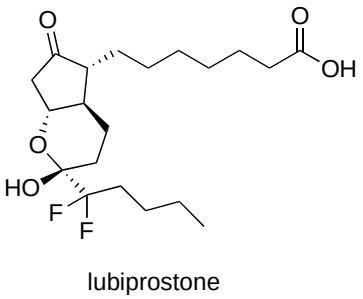


Fig. (11). Lubiprostone.

4.4. Case Study - Opioid Antagonists

Opioid receptors lie on enteric neurons found within the myenteric plexus. Endogenous opioid peptides are locally released to act on these receptors and regulate gastric motility and secretory processes. One common side effect of opioid analgesics such as morphine is constipation due to inhibition of peristalsis. Opioid antagonists are anticipated to relieve opioid-induced bowel dysfunction (OBD); however, limited exposure to the CNS is important to prevent counteracting the analgesic effects of opioid agonists [139]. Naloxone (MW 327; cLog*P* = 0.2, cLog$D_{pH\ 6.5}$ = 1.0) (Fig. (**12**)) is an example of an opioid antagonist with low systemic exposure despite having high absorption (Fa = 90%). The structure of naloxone notably contains a number of metabolic handles to ensure high clearance. Indeed, naloxone undergoes extensive hepatic metabolism to a glucuronide metabolite which limits its oral bioavailability (F = 2%) [140]. Naloxone modulates the three opioid receptor subtypes (mu, delta, gamma) and has demonstrated the ability to alleviate OBD. However, it has a narrow therapeutic index due to its ability to cross the blood brain barrier and therefore its use is limited.

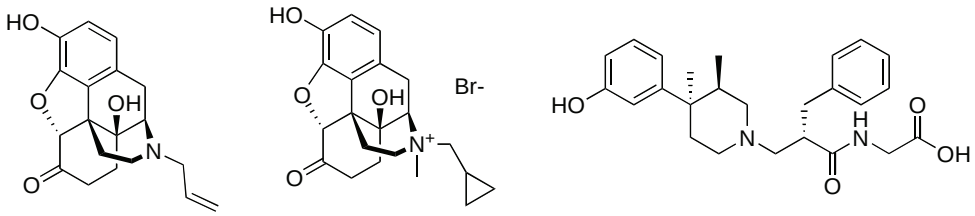


Fig. (12). Opioid Antagonists.

A second generation μ-selective drug, methylnaltrexone (MW 356, cLog*P* = –2.6, clog$D_{pH\ 6.5}$ = –3.6), was approved for the treatment of opioid-induced constipation and has an improved therapeutic index [141]. Because of its charged nature, methylnaltrexone has reduced oral exposure (F < 1%) due to limited permeability and it does not cross the blood brain barrier. Interestingly, methylnaltrexone's tissue selectivity is species dependent. *N*-demethylation to CNS-penetrant naltrexone is observed in preclinical rodent models but not in humans [142-143]. This result should be considered when pursuing quaternary ammonium salts as a design approach to limit systemic exposure.

Subsequent design of a third generation compound with restricted CNS exposure led to the discovery of alvimopan, a potent, μ-selective antagonist with poor systemic exposure (human F = 6%) approved for the treatment of post-operative ileus patients [144]. The compound was designed to avoid the use of a charged quaternary center for improved tissue selectivity and therapeutic index and modulate potency at the μ receptor by increasing the size and the polarity of the substituent on the piperidine nitrogen [145]. Alvimopan is a high molecular weight zwitterion (MW 425, cLog*P* = 2.2, cLog$D_{pH\ 6.5}$ = 0.9) and these characteristics help to limit absorption, likely through limited solubility as well as permeability. In addition, metabolism by human gut microflora further reduces systemic exposure, but leads to a peripherally exposed active acid metabolite due to amide hydrolysis (rat F = 53%) [144]. This example highlights another important challenge associated with gut targeting. Understanding the activity and exposure of key metabolites should be considered as part of a gut targeting strategy.

5. TRANSPORTER-MEDIATED APPROACHES

5.1. Uptake Transporters

Understanding the transport mechanisms and required structural activity relationships (SAR) involved in both the influx and efflux of drug molecules could allow medicinal chemists to use rational drug design for gut-targeting. Theoretically, with a poorly permeable compound, active apical uptake into the enterocyte could enable a gut-selective strategy. The chemical matter of interest may itself be a transporter substrate or, in certain cases, a transporter recognition moiety could be added to a core pharmacophore. Also, dose is an important consideration when pursuing a transporter strategy. A low-dose compound may be needed to avoid saturating the transporter. The following describes key uptake transporters that could be targeted with a low permeability compound to enable sufficient absorption for efficacy.

PEPT1 (*SLC15A1*) is a low-affinity (K_m of 200 μM to 15 mM) high-capacity transporter, localized on the apical membrane and mediates transport of di- and tri-peptides. The 3D structure of the substrate-binding site of PEPT1 is not yet known, but its template has been proposed by its large variety of substrates [146-147]. Quantitative structure-activity relationships suggest high electron densities at the first and third side chains, as well as the presence of hydrophobic side chains, significantly contribute to overall binding affinity [148]. PEPT1 is the most studied intestinal uptake transporter for its utility in absorption enhancement via prodrug approaches. For example, valacyclovir, a L-valine ester prodrug of acyclovir was effectively designed to increase the oral absorption and plasma exposure of acyclovir [149]. Similarly, the limited oral bioavailability of the potent and selective group II metabotropic glutamate (mGlu) 2/3 receptor agonist, eglumegad, was shown to be improved by its peptidyl prodrug, LY544344 (Fig. (**13**) [150-151]. Other successful examples include levodopa or L-3,4-dihydroxyphenylalanine (L-dopa), azidothymidine or zidovudine (ZT), and various amino acid ester prodrugs [152-153]. It is believed that these prodrugs and/or the parent drugs are highly concentrated in enterocytes due to lack of an efficient transport system on the basolateral membrane or due to the release of active parent in the enterocytes [150-

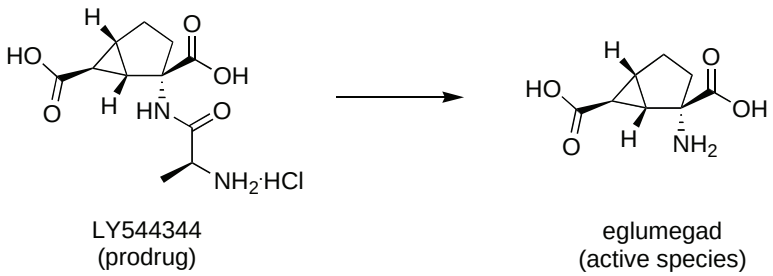


Fig. (13). Lilly mGlu 2/3 receptor agonist and prodrug.

151]. This is exemplified by the high levels of the active compound eglumegad in the intestinal cells following PEPT1-mediated uptake of the prodrug LY544344 [150-151].

OATPs are Na^+-independent solute carriers of a wide range of organic anion compounds across the plasma membrane. Of 39 OATPs identified in the human body, OATP2B1 is primarily expressed in the human small intestine [154-156]. Similar to PEPT1, an acidic environment at the brush-boarder membrane may promote transport activity of OATP2B1, which could provide high enterocytic accumulation of its substrates [157]. At pH 7.4, OATP2B1 transports the sulfate-conjugated steroids estrone-3-sulfate and dehydroepiandrosterone sulfate (DHEAS) [154, 156], and at lower pH (consistent with acidic microenvironment of intestinal mucosa) broadens its specificity to include taurocholate [158], suggesting that OATP2B1 may play a role in the enterohepatic circulation of both bile acids and estrogen [155]. OATP2B1 plays a pivotal role in oral absorption as it can transport the 3-hydroxy-3-methylglutaryl-Coenzyme A (HMG-CoA) reductase inhibitors rosuvastatin, pravastatin, and atorvastatin, and the anti-diabetic glibenclamide [157-161] (Fig. (**14**)).

MCT1 is well characterized and is known to play a role in intestinal drug absorption [163-164]. Prototypical substrates of MCT1 generally consist of weak organic acids with the carboxyl group attached to a relatively small R group containing lipophilic or hydrophilic moieties [165]. MCT1 is a low affinity, high capacity transporter that has been shown to transport unbranched aliphatic monocarboxylates such as acetate and proprionate and the substituted monocarboxylates pyruvate, lactate, acetoacetate and β-hydroxybutyrate [166]. MCT1 is also thought to be responsible for the intestinal absorption of the β-lactam antibiotics such as carbenicillin indanyl sodium as well as phenethicillin and propicillin [167]. Targeting of MCT1 by pharmacologically active drugs has been shown to result in enhanced intestinal drug uptake. For example, XP13512 is rapidly absorbed along the length of the intestine via MCT1 (as well as the SMVT). XP13512 is an anionic compound produced by the reversible modification of the amine group of gabapentin (which has limited oral absorption), with an acyloxyalkyl-carbamate promoiety [168].

Several of the intestinal uptake transporters are proton-driven (ex. PEPT1, OATP2B1, MCT1), suggesting high transport across the apical membrane due to the existence of pH-gradient across this membrane. MCT1 catalyses the facilitative diffusion of substrate across the plasma membrane, coupled with the translocation of a proton. The driving force for transport is provided by both the substrate- and proton-gradients, with the pH gradient determining the extent of transport activity. At the apical membrane of enterocytes, an inward proton gradient is generated through the activity of an electroneutral proton/cation exchanger, Na^+/H^+ antiporter [169]. Such a physiological arrangement essentially suggests high enterocytic accumulation of these transporter substrates.

5.2. Efflux Transporters

ABC transporters like Pgp and BCRP, localized on the apical membrane, are involved in the efflux of a wide variety of drugs and chemicals. These efflux pumps may be utilized to restrict the systemic exposure while providing adequate gut luminal or enterocytic exposure.

A recent example of a gut-targeted strategy combined the use of physical properties in analog design in conjunction with the use of efflux transporters to further limit plasma exposure. Inhibition of diacylglycerol acyltransferase 1

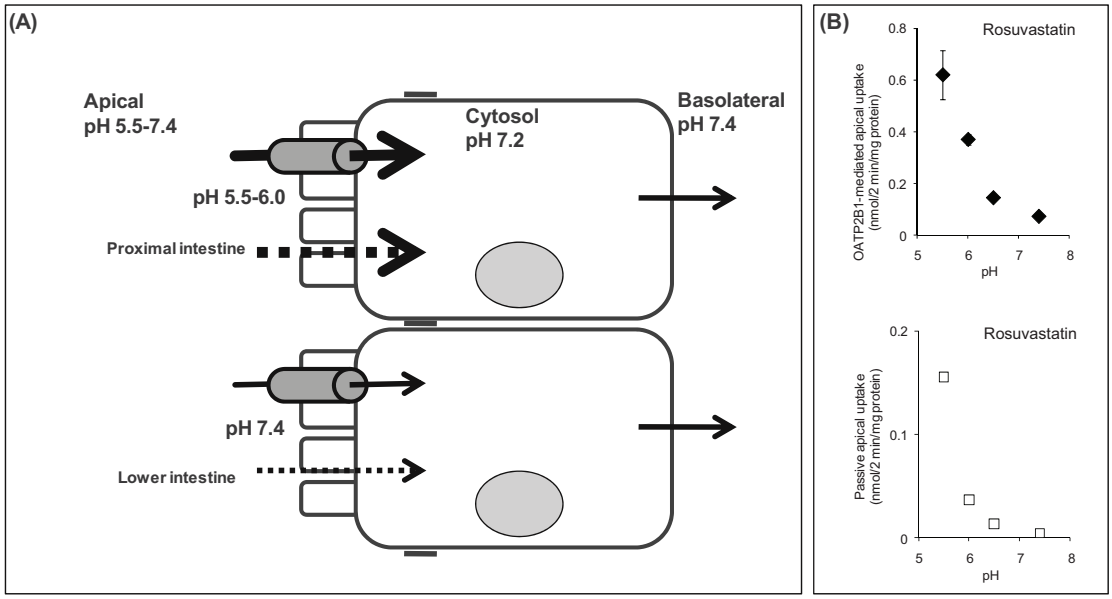


Fig. (14). pH-dependent active and passive transport across apical membrane of enterocytes. (**A**) Schematic representation of enterocytes and local pH. Due to relatively higher pH gradient across the apical membrane compared to basolateral membrane, especially at the upper small intestine, anionic drugs may accumulate in the enterocytes. In addition, transporters like OATP2B1, PEPT1 and MCT1 show high activity at low pH and therefore facilitate cellular accumulations of their substrates. (**B**) pH-dependent OATP2B1-mediated active and passive apical uptake of rosuvastatin in OATP2B1-transfected (top panel) and wild-type HEK293 cells (bottom panel) [162].

(DGAT1) in the enterocyte is hypothesized to alter handling of nutrients and therefore may be a useful approach for the management of obesity. Avoiding inhibition of DGAT1 in skin and sebaceous glands was desired and therefore a tissue targeted program was pursued by researchers at Novartis [170]. Successful design of a lipophilic acid, **5**, (Fig. (**15**), MW 470, cLog*P* = 6.7, Log$D_{pH\ 6.8}$ = 2.7) led to a potent DGAT1 inhibitor with little absorption in rat as measured by portal vein exposure. The compound demonstrated low plasma clearance and its low systemic exposure was therefore driven primarily through limited oral absorption. Sustained high gut to portal levels of **5**, as measured by total drug concentration in regions of the GI tract as compared to portal plasma, were observed and was hypothesized to be due in part to its high lipophilicity which could help to enrich the compound within the cell (Fig. (**15**)). In addition, the compound is a Pgp substrate as measured by its efflux potential in an over-expressed cell-line. This effect of allowing continual absorption and efflux may contribute to its ability to maintain high local concentrations within the enterocyte. A concentration-effect relationship based on gut and portal exposures suggests that the observed efficacy (triglyceride lowering) was driven by exposure of the compound within the gut wall.

Loperamide hydrochloride is an opioid receptor agonist widely used for the treatment of diarrhea. The drug works through activation of opioid receptors located in the myenteric plexus to reduce peristalsis. At a pharmacological dose of 2 mg, loperamide has limited systemic exposure (F = 0.3%) due to extensive first pass metabolism leading to *N*-desmethyl-loperamide as the major metabolite. In addition, loperamide has poor passive permeability and is a substrate for the Pgp transporter found in the GI tract and on the blood-brain barrier. Efflux provides an additional mechanism for achieving residency time within the GI tract as well as limiting exposure to opioid receptors within the CNS. Indeed, no evidence of opioid related effects within the CNS was observed at pharmacological doses.

5.3. Biliary Excretion

Another tactic for achieving gut selectivity involves the design of compounds that are primarily cleared through the bile. If the anti-tissue is not the liver or the gallbladder, this strategy can be an effective means of limiting systemic exposure.

Ezetimibe (Fig. (**16**)) is a cholesterol absorption inhibitor that inhibits the protein Niemann-Pick C1-like 1 (NPC1L1) [171]. This transporter is located on the apical surface of enterocytes and functions to transport cholesterol from the lumen into the enterocyte cytoplasm where it eventually enters the circulation. Ezetimibe inhibits the NPC1L1 transporter, thereby lowering the amount of dietary and biliary cholesterol that is absorbed. Ezetimibe is rapidly absorbed into the enterocyte where it is partially glucuronidated by uridine 5-diphosphate (UDP)-glucuronosyl-transferase 1A1, 1A3, and 2B15 [172]. Both ezetimibe and glucuronidated ezetimibe pass into the liver where further glucuronidation occurs. Glucuronidated ezetimibe and remaining parent are excreted into the bile and pass back into the intestine. The glucuronidated ezetimibe is a more potent NPC1L1 inhibitor than the parent and this process of enterohepatic recirculation enables sustained inhibition of the transporter. Parent and metabolite have a plasma half life of ~22 h in humans

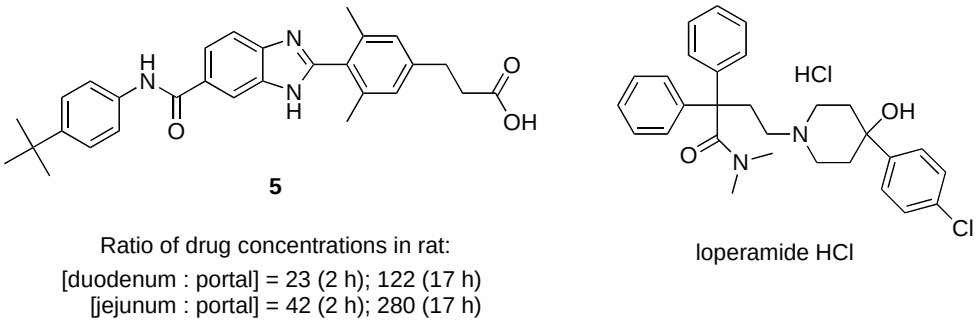


Fig. (15). Novartis investigational DGAT-1 inhibitor, **5**, and loperamide HCl.

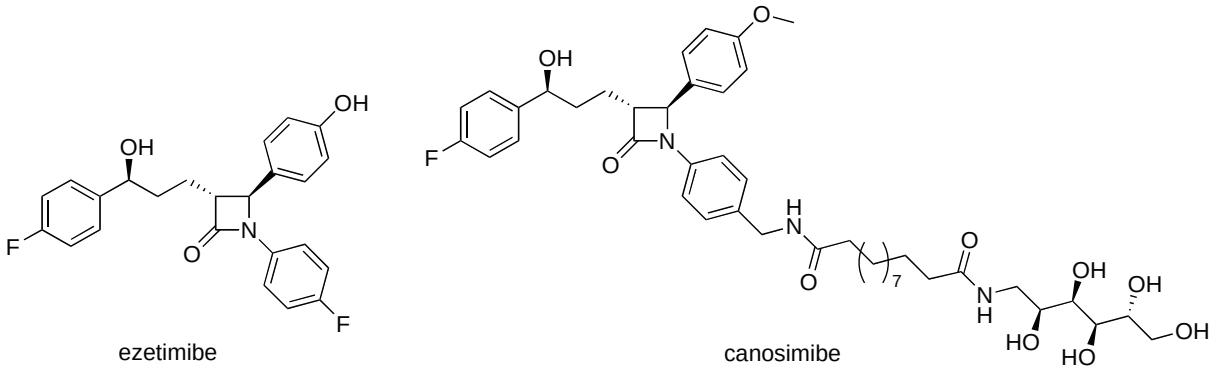


Fig. (16). Ezetimibe and canosimibe.

and 90% is excreted in the feces [171]. The absolute bioavailability in humans has not been determined due to its very low solubility in aqueous media suitable for intravenous injection, however a C_{max} of 3.4–5.5 ng/mL for parent and 70.6–73.6 ng/mL for parent plus metabolite was observed after a 10 mg oral dose [172]. Recent data suggest that ezetimibe and its active metabolite may also exert an inhibitory effect on NPC1L1 expressed on hepatocytes [173] to reduce reabsorption of biliary cholesterol. Further studies are necessary to fully elucidate the mechanism of this drug and understand whether this is truly a gut-selective therapy.

Researchers have pursued the design of non-absorbable ezetimibe derivatives to further reduce hepatic and systemic drug concentrations and therefore increase its therapeutic window [174-175]. Sanofi's strategy culminated in the discontinued Phase 3 candidate canosimibe (MW 810, cLog*P* = 4.9, HBD = 8, PSA = 209 $Å^2$, RB = 26) [176-177]. Their aim was to append the ezetimibe template with low absorption groups such as polyols, permanent cations, and permanent anions through a linker that would allow binding of the compound to the target NPC1L1 protein. Compounds were optimized for efficacy and low systemic exposure in pre-clinical *in vivo* models. After a 200 mg dose of canosimibe in humans, exposure levels were below the quantifiable limit (<1.0 ng/mL) and 92% of drug was excreted unchanged in feces [178]. Unfortunately, efficacy was disappointingly low in Phase 3 studies, leading to discontinuation of the drug candidate. These data suggest that the efficacy of ezetimibe may be due to its inhibition of the transporter in both the intestine and liver.

6. PRODRUG APPROACHES

Prodrugs are derivatives of pharmacologically active agents that must undergo an enzymatic cleavage and/or chemical reaction *in vivo* to release the active parent drug [179]. Prodrugs may be classified based on the specific site of conversion, namely extracellular activation in digestive fluids or the systemic circulation, or intracellular activation (e.g. proton pump inhibitors and antiviral nucleoside analogs) [180]. The deliberate design of the appropriate prodrug may facilitate targeted delivery of pharmacologically active drugs to the intestine or colon [181-182]. In fact, site-selective chemical conversion or bioactivation of a drug is a strategy employed by a number of researchers to target inflammatory bowel diseases (IBD) such as ulcerative colitis or Crohn's disease [183], duodenal ulcers [184], colon cancer [185], intestinal infections or infestation [186-187], and diarrhea [188].

In addition to the soft drug approach (high clearance) discussed in section 4.3, an alternative approach is to deliver a poorly absorbed prodrug that relies upon gradual conversion to the active species within the gut or even delaying release of the pharmacologically active moiety until the latter portions of the intestine by capitalizing on the reductive environment in the colon. Active drugs are generally liberated from a prodrug in the GI tract by the action of bacterial enzymes [189] such as azo-reductases [190], glucosidases [191], or glucuronidases [192-194]. In addition to GI tract-targeted prodrugs, other prodrug strategies have focused on site-selective activation to active drug to target the liver or kidneys [195-196]. These approaches are clearly distinct from the traditional use of prodrugs utilized to enhance oral absorption of drugs used to treat systemic disease [197]. Accordingly, the focus of this prodrug review is on rational drug design aimed at specifically delivering a drug to a portion of the GI tract that would not be exposed to pharmacologically active substance under normal absorptive processes, i.e., approaches aimed at improving site-specific intestinal drug exposure.

6.1. Azo-Linked Prodrugs

Azo-linked prodrugs have the potential to act as drug carriers that either selectively release therapeutic agents in the colon or alter the absorption characteristics of drugs that require colon-specific delivery [198]. Mesalamine (5-aminosalicyclic acid or 5-ASA) is used as first-line anti-inflammatory therapy for mild to moderate ulcerative colitis and with limited utility in maintenance therapy of Crohn's disease [183]. Although 5-ASA could be administered topically as an enema or rectal suppository formulation [199-201], it is more generally administered orally as a prodrug in the form of sulfasalazine, which consists of 5-ASA and sulfapyridine linked by an azo bond (Fig. (**17**)). Sulfapyridine and 5-ASA are readily absorbed in the upper intestinal tract when administered individually, while the azo linkage prevents absorption in the stomach and small intestine [201-202]. The absorption of sulfasalazine in the small intestine is low (Fa < 20%) and absorbed sulfasalazine is primarily excreted in urine (~5%) or bile (~3%), resulting in generally greater than 80% of an oral sulfasalazine dose in the colon [202-204]. The active drug (5-ASA) and the pharmacologically inactive sulfapyridine are liberated from sulfasalazine, apparently mainly due to anaerobic bacterial action in the colon involving azo-redutases [190] but other mechanisms have been postulated [198, 205]. Although 5-ASA is a salicylate, the pharmacological mechanism is unknown, but is likely not related to inhibition of cyclooxygenases, since nonsteroidal anti-inflammatory drugs may exacerbate IBD. Studies in humans indicate that 5-ASA is poorly absorbed (25%) from the colon resulting in very high intraluminal 5-ASA concentrations (up to 10 mM following an oral dose of 3 g/day) and is mainly excreted unchanged in the feces [203, 206-207]. In addition, 5-ASA has been formulated as pH-sensitive release tablets targeting the terminal ileum and colon or as delayed-release capsules, which is released throughout the small intestine and colon [208].

Sulfasalazine therapy results in the formation of the inactive sulfapyridine, which is almost completely absorbed and extensively metabolized through acetylation, hydroxylation and conjugative pathways [203]. Sulfapyridine is linked to adverse events experienced by patients on sulfasalazine therapy. This has led to the development of second-generation 5-ASA-containing azo prodrugs: olsalazine and balsalazide [198]. Olsalazine is essentially a dimer of 5-ASA linked by an azo bond, designed to limit the release of metabolites with potential adverse pharmacology. Absorption of olsalazine is low (F = 2.3%) [209] and although cleavage results in the release of 2 moles of 5-ASA, olsalazine is considered less active in the treatment of ulcerative colitis, likely due to slower degradation of the azo bond [210]. Balsalazide bioavailability is also low (F < 1%) and is metabolized to

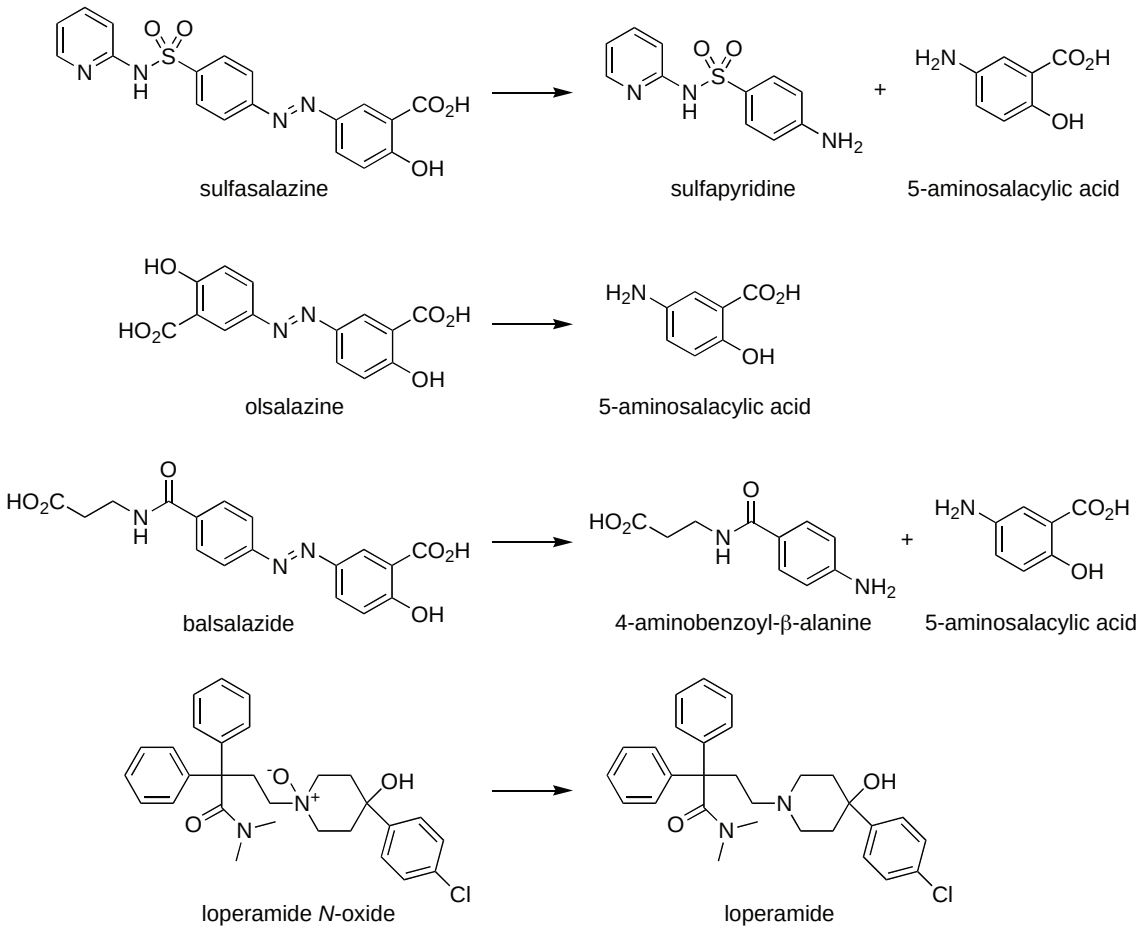


Fig. (17). Prodrugs relying on reductive microbial metabolism.

5-ASA and the inactive 4-aminobenzoyl-β-alanine in the colon [203-204]. A recent meta-analysis, however, indicated similar efficacy for sulfasalazine compared to olsalazine and balsalazide in the treatment of mild to moderate ulcerative colitis, and it is suggested that the second generation agents be reserved for patients experiencing adverse events on sulfasalazine [211].

6.2. Amine Oxide Prodrugs

Loperamide *N*-oxide (Fig. (**17**)) is an investigational agent developed as a prodrug of loperamide with the goal to reduce systemic loperamide absorption and release loperamide to the intestinal lumen in a controlled manner [188]. Loperamide *N*-oxide is 50% less potent as a μ-opioid receptor agonist compared to loperamide. However, loperamide *N*-oxide (1 mg) exhibited equivalent antidiarrheal activity to loperamide (2 mg) in humans with 4-fold lower loperamide plasma concentrations. Pharmacokinetic studies in healthy volunteers and patients with diarrhea indicated that loperamide is gradually formed along the gastrointestinal tract due to reduction of the prodrug by intestinal microflora as well as intestinal epithelia. Loperamide *N*-oxide administration resulted in decreased loperamide plasma levels and delayed absorption compared to dosing loperamide itself, supporting the potential benefit of reduced systemic absorption and directly targeting the distal intestine and colon. Loperamide *N*-oxide administration therefore has the potential to be a more favorable antidiarrheal agent due to targeted and prolonged availability of loperamide at the site of action [212-213].

6.3. Glucuronide and Glucoside Prodrugs

Orally administered drugs are often metabolized to glucuronides in the intestine or liver by UDP-glucuronosyltransferases (UGTs) and subsequently excreted via biliary elimination. These glucuronides could subsequently be metabolized by bacterial β-D-glucuronidases [192], releasing the parent aglycone, which could then undergo reabsorption or enterohepatic circulation [214]. This metabolic process could accordingly result in prolonged exposure for drugs that undergo extensive enterohepatic circulation and also result in extended re-exposure in the intestinal tract. Glucuronides and plant glucosides (e.g. flavonoids, amygdalins, and sennosides) are generally water soluble and poorly absorbed, and would require bacterial enzyme hydrolysis to release the parent aglycone from the glucuronide or glucoside. These reactions are catalyzed by bacterial β-D-glucuronidases or β-D-glucosidase, respectively. Administration of drugs as glucoronides or glucosides could therefore be utilized for colon-specific drug delivery since these conjugates are generally stable in the stomach and small intestine [182]. Further research is needed to fully understand which microbial species cleave prodrugs of this type and how individual patient microflora variability effects drug concentrations.

Dexamethasone-, prednisolone-, hydrocortisone-, fludrocortisone-21-β-glucoside, and several other steroidal glucosides have been synthesized to study their potential as candidates for glucosidase-mediated release and colon delivery (Fig. (**18**)) [215]. Preclinical studies in the rat indicated that oral administration of dexamethasone or prednisolone resulted in a small amount (~1%) of drug reaching the cecum due to high absorption, while a significant proportion of dexamethasone (60%) or prednisolone (15%) reached the cecum when administered as the corresponding glucoside [191]. It was also demonstrated in guinea pig that the oral absorption of dexamethasone-21-β-glucoside is low (<1%), while approximately 20-30% of the oral dose reached the cecum [216].

Dexamethasone-, budesonide-, and menthol-β-D-glucuronides [193-194] have also been synthesized in order to explore colon-specific targeting [193-194]. The budesonide prodrug exhibited improved pharmacology and decreased adrenal suppression [182]. This strategy provides the potential advantage to reduce systemic steroid exposure and associated adverse events employed in the treatment of IBD.

6.4. Amino Acid Prodrugs

5-Aminosalicyl-glycine (Fig. (**19**)) was synthesized as a potential prodrug of 5-aminosalicyclic acid (5-ASA) and *in vivo* studies in the rat indicated selective delivery or release of 5-ASA (~50% of oral dose) in the colon [217]. The intent of amino acid (e.g. alanine, methionine, tyrosine, and glutamic acid) incorporation is to increase polarity and reduce oral absorption in the stomach and small intestine until the prodrug is cleaved by intestinal microflora in the colon, an approach that was also evaluated preclinically to deliver salicylic acid [182]. Since there are several intestinal uptake transporters that use amino acids as recognition motifs, screening for transport activity may be important for this particular prodrug strategy to avoid undesired active uptake.

6.5. Cyclodextrin Prodrugs

Prednisolone α-cyclodextrin (Fig. (**19**)) was evaluated in an attempt to reduce the absorption of prednisolone and limit the systemic adverse events associated with topical or local administration of corticosteroids used in the treatment of ulcerative colitis [218]. Cyclodextrins (CyDs) are polar cyclic oligosaccharides, exhibit low intestinal absorption, and are known to be poorly hydrolyzed by glycosidases. However, they are metabolically degraded by colonic microflora to release their payload. Accordingly, a number of CyD-appended drugs including ketoprofen, prednisolone and 5-fluorouracil were synthesized to investigate specific intestinal delivery [182]. In a rat model of ulcerative colitis, both parent and CyD conjugate improved markers of anti-inflammatory therapeutic effect to a similar extent. Prednisolone, however, showed high levels of systemic adverse events, while the CyD conjugate was similar to control for this measure. *In vitro* and *in vivo* studies indicated that the conjugate maintains colonic levels at low but constant levels for a longer period of time than the parent leading to this beneficial profile.

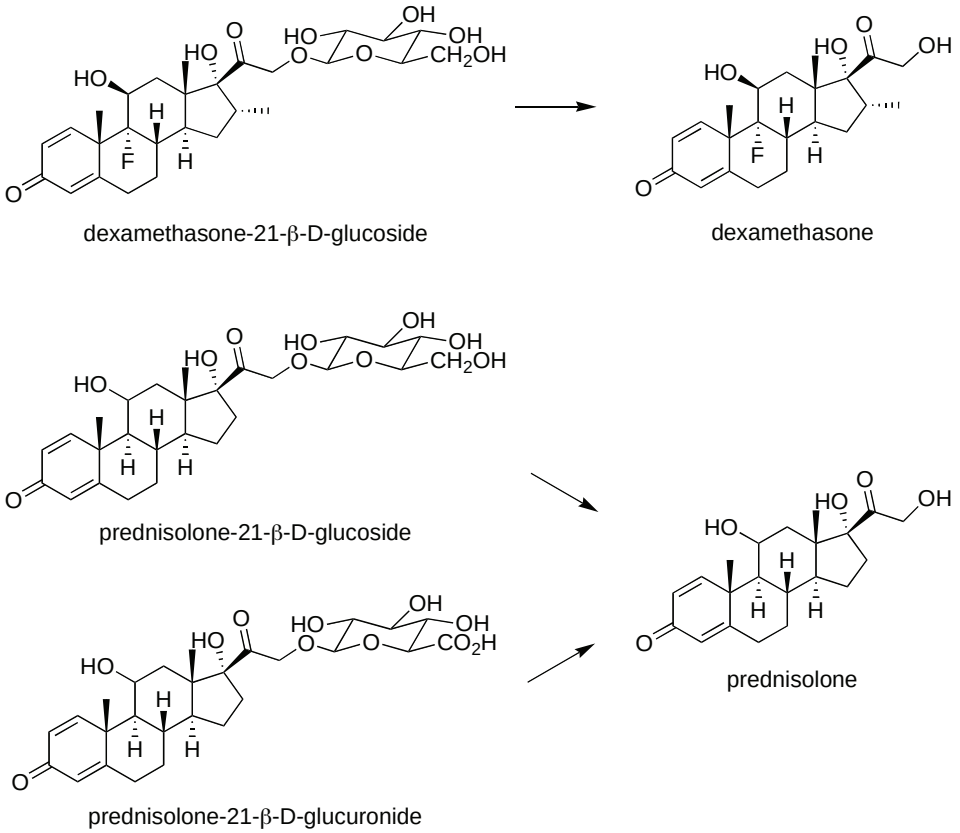


Fig. (18). Glucoside and glucuronide prodrugs.

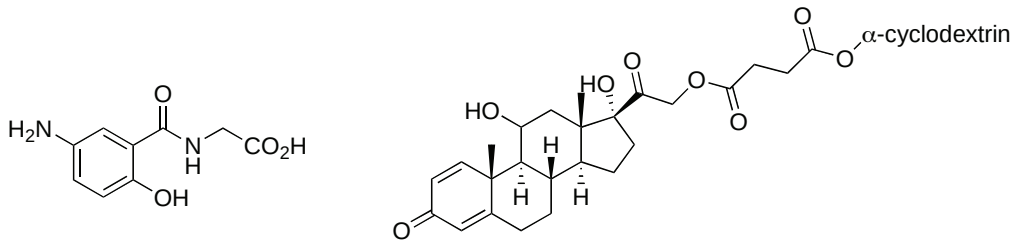


Fig. (19). Poorly permeable prodrugs for colonic targeting.

6.6. Local-Acting Intestinal Prodrugs

The proton pump inhibitor omeprazole (Fig. (**20**)) was not specifically designed as a prodrug, but is an example of a site-activated prodrug used in the treatment of duodenal and gastric ulcers [179, 219]. Omeprazole is a weak base, and weak bases are known to accumulate in acidic environments such as the acidic canaliculi of the parietal cell in the gastric mucosa due to pyridine protonation. In this environment, omeprazole is converted to the active sulfenamide where it irreversibly binds to a cysteine group in (H^+/K^+)-ATPase, resulting in inhibition of gastric acid production [220]. Omeprazole is ampholytic (pK_as of 3.97 and 8.8) with low aqueous solubility. Administration of ^{14}C-omeprazole in humans indicated high absorption and moderate mean oral bioavailability (F = ~50%), with dose-dependent increases in bioavailability (F = 40-97%) [221]. The plasma elimination half-life is short (0.5-3 h) due to high systemic clearance. The pharmacodymamic effect of omeprazole is, however, much longer than expected from limited pharmacokinetic accumulation and is explained by drug accumulation in the acidic intestinal environment [221]. Several other proton pump inhibitors including lansoperazole, pantoperazole, rabeprazole, esomeprazole, and tenatoprazole were introduced to the market with a mechanism of action comparable to omeprazole.

7. FORMULATION AND TECHNOLOGY APPROACHES

Formulation technology offers an additional approach for targeting drug concentrations to specific locations of the gastrointestinal tract. Many different techniques are available depending upon where the target of interest is located. For example, a modified release dosage form such as an osmotic tablet can be used to slowly release drug throughout the length of the GI tract, whereas a floating capsule can focus delivery in the upper portion of the gastrointestinal system and pH responsive coatings can be used to facilitate colonic delivery. Most options center around a more passive delivery approach – i.e. attaining higher concentrations of drug in the correct region of the target of interest, and relying on normal pathways of absorption. A few technologies offer the potential for a more active pathway of delivery. While many of these technologies are currently more exploratory in nature, they offer potential for more specified targeting options in the future.

7.1. Upper GI

Gastric retentive dosage forms offer prolonged release within the stomach, effectively providing a continuous bathing of the upper GI or, alternatively, decreasing exposure to lower GI environments. This can be accomplished through a number of different formulation types, though in general they aim to take advantage of size, density, or bioadhesivity in order to avoid gastric emptying. Regardless of the specific formulation, the dosing regimen (i.e. consideration of fed/fasted effects) is key to the performance of these dosage forms [222].

Given normal physiological conditions, larger non-disintegrating unit doses would be expected to have prolonged residence time in the stomach. However, difficulty in swallowing these larger unit doses is potentially an issue with some patient populations. Consequently, a number of swellable or expandable formulations have been proposed [223]. These formulations use a number of different mechanisms for increasing size or bulk after oral administration. For example, the Accordion Pill (Intec Pharma) contains a series of folded polymeric sheets impregnated with active drug components, compressed within a standard gelatin capsule [224]. After administration, the capsule shell dissolves, the polymeric sheets hydrate and unfold, simultaneously releasing drug and occupying sufficient bulk to interfere with normal transit processes. In another example, metformin has been formulated as an expandable tablet available for commercial use [225-226]. Polymeric excipients are utilized that swell in the presence of gastric fluid, thereby increasing the overall bulk of the tablet. In each case, eventually the dosage

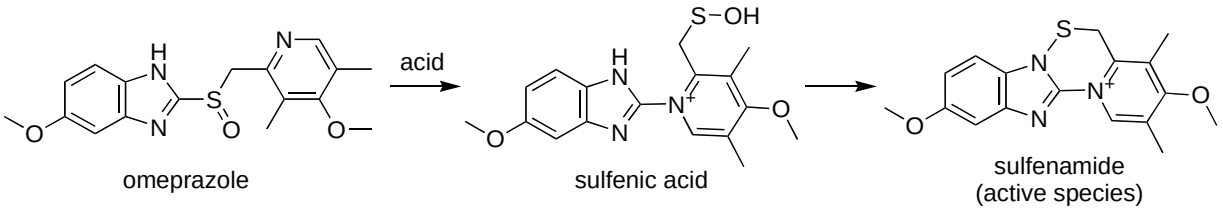


Fig. (20). Pathway for metabolic activation of omeprazole.

form needs to safely pass out of the stomach, and consequently the degradation and emptying process should be carefully considered.

Floating capsules attempt to take advantage of density in order to prolong residency in the stomach. This is contingent upon having material in the stomach to float upon, hence dosing in the fed state is key to this method of delivery. Floating dosage forms may take advantage of low-density materials, gas-generating reactions, or air pockets embedded within the unit dose. Examples of marketed formulations include Valrelease (diazepam floating capsule), Cifran OD (ciprofloxacin gas-generating form), Madopar, Topalkan, Almagate, and liquid Gaviscon (effervescent viscous liquid) [227].

An additional option for gastric retention centers around bioadhesivity. Various polymeric materials are employed that have the potential to interact with the mucosal layer lining the stomach walls, thereby prolonging the contact time of the drug [222]. Rapid turnover of the mucosal layer may decrease prolonged effectiveness of this formulation approach.

7.2. Intestinal Release

For targeting various regions in the intestinal tract, a number of enteric/pH-responsive coatings and excipients are available. These materials are typically polymers that are designed to dissolve or erode at specific pH ranges, selected based upon the region of desired drug release. These materials also function to protect acid labile drugs from gastric fluid or limit exposure in cases where the active ingredient may be irritating to the upper GI.

Common enteric or pH-responsive excipients including cellulose and methacrylic acid based polymers, are shown in (Table **1**). Delivery targeted to the duodenum, jejunum, ileum, or colon may be affected through the use of these materials, which display dissolution under pH conditions ranging from approximately 5.0 to 7 [187, 228].

Table 1. Degradation pH of Several Formulation Coatings

Name	Degradation pH
hydroxypropyl methylcellulose phthalate series	4.5-5.4
Coateric (polyvinyl acetate phthalate)	5.0
cellulose acetate phthalate	5.0
hydroxypropyl methylcellulose acetate succinate series	5.5-6.5
Eudragit series (methacrylic acid–methyl methacrylate copolymers)	5.5-7.0
Marcoat	7.0

7.3. Lower GI

There are four primary methods of release for drug delivery to the lower GI, each taking advantage of a specific property of the colonic region – pH, transit time, pressure, or bacteria. The use of pH-responsive polymers as described above has gained significant headway in delivery of drugs used for the treatment of diseases such as ulcerative colitis, irritable bowel, and Crohn's disease. For example, mesalazine (marketed under trade names Asacol MR, Mesren MR, Ipocol, and Salofalk) has been developed as a series of Eudragit-coated tablets for the treatment of ulcerative colitis. Here, the tablet core is protected through the gastric environment, exposing the core and releasing the active component only once the target pH environment is reached and the Eudragit coating dissolves [229-230]. Budesonide, for treatment of Crohn's disease, also utilizes various polymers in the Eudragit series for the coating on slow-release granules (sold as Entocort and Budenofalk) [229].

Dosage forms can be designed to respond to local flora within the lower GI region, taking advantage of the fact that the colonic region hosts significantly larger populations of a variety of bacterial species as compared to other regions of the GI [229, 231]. One approach involves the use of excipients in film coatings, capsule shells, or tableting components, which are acted upon by the bacterial populations of this region. For example, various saccharides such as cellulose, pectin, chitosan, and xylan, are not digested in the upper regions of the human digestive tract. However, they are known substrates for bacterial degradation in the colon. When these materials are acted upon by the endogenous bacteria, they are degraded into lower molecular weight materials. This leads to the loss of mechanical strength, compromised film or tablet integrity, and subsequent release of the contained active components [232]. Several reviews offer in depth descriptions of individual mono, di, or polysaccharides which have been investigated for colonic drug delivery [187, 230].

One interesting approach to colon-specific delivery attempts to capitalize on the increased pressure of this region, driven by strong peristaltic forces and decreased fluid content present. The goal is to allow a dosage form to transit intact to the lower GI where it could be crushed by local pressure increases, thereby releasing the contents of the capsule. Takaya has proposed the Pressure-controlled Colon Delivery Capsule, or PCDC, utilizing a water insoluble ethylcellulose capsule shell, active ingredient, and a fill which liquefies at body temperature [229, 233-234]. Though still in early phases, the PCDC offers an interesting avenue for further development.

Time-driven approaches have been suggested with numerous platforms. The Pulsincap device was an early attempt to delay release based upon a preset lag time before release of drug. Though a novel concept, this platform demonstrated a high degree of variability and highlighted the variations in individuals' transit times [229]. More recent approaches have pursued combined approaches to help circumvent such differences. For example, the Alza Corporation offers an osmotic capsule designed to deliver drug to the colonic region. Taking advantage of enteric polymer coatings and swellable cores, the OROS-CT system allows for the retention of drug until the capsule reaches the colon, at which time the active substance can be released over a period of 4 to 24 hours [232].

7.4. Trans-GI

Modified release dosage forms are designed to deliver drug at specified release rates during normal passage through the GI. These dosage forms typically deliver drug throughout the system, potentially offering greater exposure at lower points of interest where immediate release formulations may not reach. Traditional matrix or osmotic core tablets are commonly used formulations for delivering continuous release over a specified time profile. Multiparticulate systems using coated beads or microspheres are also employed. Often these types of dosage units are of sufficient size as to achieve gastric retention as well, in which case food effects should be considered. Examples of modified release products include Ambien CR, Allegra D, and Focalin XR [235].

7.5. Remote Release Options

Several companies have proposed electronic capsule systems: Philips Research has developed a technology they refer to as the iPill and the IntelliCap system, and Pharmaceutical Profiles has Enterion. These compact mechanical devices resemble traditional capsules, but contain sensors, processors, and pumps to enable real-time monitoring of parameters such as local pH, temperature, and transit time. Data can be received externally by radio frequency transmission, and either internal or external release mechanisms utilize this information to determine the location of the capsule and correct time of release of the onboard drug payload. It is suggested that burst or metered release as well as multi-location release may be possible with this technology [236-237]. Though these devices offer a range of delivery capabilities, they are primarily used for diagnostics and research purposes rather than long-term treatment at the current time.

7.6. Active Targeting Approaches

As the field of targeted delivery continues to develop, additional active-targeted formulation options are being investigated. Among these are a host of nanoparticulate systems that seek to interact with tissues of interest through receptor-specific surface ligands or nano-carrier composition. For example, it has been demonstrated that M-cell surfaces differ from other enterocytes in carbohydrate composition. Elan has proposed utilizing a coating upon nanoparticulate structures that bind selectively to these specific carbohydrate residues in efforts to direct delivery of the encapsulated compound [238]. As another approach to gut-targeted nanoparticulate delivery, solid-lipid nanoparticles have been used to target gut lymphatic tissue in treatment with antiretroviral drug moieties [239].

An additional technology of interest is that of the cochleate delivery vehicle. Formed of a phospholipid membrane sheet stabilized in a coil structure through the use of calcium ions, the delivery device is predicted to interact with cellular membranes along the GI and to deposit poorly permeable compounds directly into the local enterocytes [240-241]. Through selective design of drug molecules, it would be envisioned that the compound could become trapped and therefore highly localized in these surface cells, effectively limiting systemic exposure. While some promising studies have been presented in the literature, additional maturation of the technology is needed to fully understand its potential.

8. CHALLENGES AND CONCLUSION

Given the number of known and emerging mechanisms for pharmacological intervention within the GI tract that could potentially impact health and disease, we have described a variety of approaches for the rational design of small molecules to selectively and safely modulate these gut targets. These include proper choice of physical properties to avoid high systemic exposure, strategic use of uptake and efflux intestinal transporters to enrich drug concentrations within the GI tract, and taking advantage of the numerous metabolic enzymes within enterocytes and hepatocytes to enable soft drug and prodrug approaches for achieving tissue selectivity. Among the aspects considered are target location within the GI tract, systemic tissues desired to avoid, and the physicochemical properties of the active agent for selecting a strategy that can be used alone or in combination to achieve the desired gut selectivity. Pharmacologically active compounds as well as approved drugs were discussed that demonstrate the utility of these strategies to selectively partition chemical entities into the gastrointestinal tract. Potential formulation technologies to consider have also been discussed.

A number of challenges and pitfalls exist that are endemic to tissue targeting programs. In order to confidently set dose and test a mechanism in the clinic, the target must be modulated by the appropriate concentration of drug for sufficient time in order to achieve the desired endpoint. Plasma exposures will likely not be a relevant surrogate of tissue exposures for compounds with increased tissue-partitioning. Measuring drug exposure at the site of action is potentially achievable preclinically both in the lumen and within the enterocyte; however, these experiments are technically challenging, and enterocyte measurements may be subject to error due to contamination by non-absorbed drug [123]. In addition, accurate translation of these exposure data to the clinic is hampered by challenges associated with measuring drug concentrations in the GI tract, although somewhat invasive methods are available for measuring luminal concentrations [123]. Optimization of mechanistic models, such as the physiology-based pharmacokinetic model, GastroPlus [242-243], for tissue targeting programs to predict both plasma and tissue concentrations may help to guide future efforts. To confidently test the mechanism in the clinic, identification of intestinal-specific proximal mechanism biomarkers is key for determining if the compound of interest is modulating the target at the site of action. A good understanding of the relationship between biomarker and drug concentrations preclinically will enable clinical interpretations.

Intestinally targeted compounds may also have added hurdles associated with demonstrating safety. As with all programs, design of compounds that are selective for the target of interest is important to avoid potential safety liabilities associated with off-target pharmacology. In addition, intestinally targeted compounds will have, by definition, low systemic exposures and therefore achieving sufficient systemic exposure multiples over *in vitro* activities in regulatory safety studies may be a challenge, especially for prodrug and soft drug approaches in rodent species.

In conclusion, the identification of compounds that selectively partition into the gastrointestinal tract remains an area of high interest within the pharmaceutical community given the number of targets localized in this region that can impact important diseases. The rational design of compounds that safely modulate these targets will enable the successful delivery of drugs to these patients.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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ABBREVIATIONS

CNS = Central Nervous System

D = Distribution Coefficient

F = Bioavailability

Fa = Fraction Absorbed

Fg = Fraction Escaping Gut Metabolism

Fh = Fraction Escaping Hepatic Metabolism

GI = Gastrointestinal

HBA = Hydrogen Bond Acceptors

HBD = Hydrogen Bond Donors

hERG = Human Ether-à-go-go-Related Gene

MW = Molecular Weight

RB = Rotatable Bonds

P = Partition Coefficient

P_{eff} = Effective Permeability

PSA = Polar Surface Area

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