

## REVIEW ARTICLE

# Advancements in intestinally restricted drugs: Applications, targets, and design strategies

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## Abstract

Drug safety is of paramount importance in the pharmaceutical industry, as it often hinders the successful introduction of new drug candidates into the market. Intestinally restricted drugs, also known as non-systemic drugs, are orally administered compounds that exert therapeutic effects specifically within the intestinal lumen, thereby circumventing side effects associated with systemic exposure. These drugs offer innovative solutions for unmet medical needs in drug development while minimizing toxicity risks. This review provides an overview of the applications of intestinally restricted drugs for inflammatory diseases, irritable bowel syndrome, colorectal cancer, and other digestive system ailments. Based on design strategies, these drugs can be classified into five categories, including high molecular weight compounds, highly polar or positively charged compounds, highly lipophilic and low water solubility compounds, intestinal soft drugs, and intestinal prodrugs. Specifically, it focuses on the design strategies of these drugs and presents an analysis of these strategies through representative drugs from each category. Due to the complex physiological environment of the gastrointestinal tract and the need for precise drug targeting, developing orally administered intestinally restricted drugs remains a significant challenge for scientists. This review enhances understanding of intestinally restricted drugs and offers a valuable reference for advancing their development.

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## 1. Introduction

In the pharmaceutical industry, drug toxicity and safety are critical concerns that often impede the commercialization of novel drug candidates. Safety assessments for new pharmaceuticals are increasingly conducted through a safety window, also referred to as the therapeutic index.<sup>1</sup> Enhancing organ or tissue selectivity and drug target selectivity is pivotal for elevating the therapeutic index. There are numerous studies on non-systemic drugs for the lung,<sup>2-5</sup> skin,<sup>6,7</sup> eye,<sup>8-11</sup> and ear,<sup>12</sup> which achieve higher local concentrations while reducing systemic levels to minimize potential side effects. Intestinally restricted drugs, also known as non-systemic drugs, are orally administered compounds that

exert therapeutic effects specifically within the intestinal lumen, thereby circumventing side effects associated with systemic exposure. The effectiveness of intestinally restricted medications has been demonstrated in the treatment of colorectal cancer (CRC) and inflammatory bowel disease (IBD).<sup>13,14</sup> Moreover, these drugs have demonstrated encouraging results in the treatment of various ailments, including diabetes,<sup>15,16</sup> obesity,<sup>17</sup> immune system dysfunction, and IBDs.<sup>18-20</sup>

Since most intestinally restricted pharmaceuticals are designed for oral administration, their plasma exposure is limited by restricted systemic absorption.<sup>21</sup> These drug molecules predominantly accumulate in the gastrointestinal tract, enabling them to demonstrate their therapeutic effects at the site of administration, thereby reducing adverse effects associated with systemic exposure.<sup>22,23</sup> At present, intestinally restricted drugs can be classified into six distinct groups according to their structural attributes: high molecular weight (MW) molecules; high lipophilicity and low water solubility; highly polar or positively charged compounds; soft drugs; prodrugs selective for the large intestine; and gut-targeting drugs facilitated by efflux transporters.

Despite significant advancements in this field, challenges persist, particularly in optimizing drug delivery methods, ensuring absorption stability, and addressing variability in plasma concentrations. Present research has provided insights into specific design principles and therapeutic targets, such as bile acid (BA) receptors and gut-expressed G-protein-coupled receptors (GPCRs), which have demonstrated promising results in preclinical and clinical studies. However, critical gaps remain in understanding the pharmacokinetics, systemic toxicity, and long-term safety of these therapies. This review aims to address these gaps by synthesizing recent developments in the field, highlighting the molecular mechanisms underlying drug selectivity and their therapeutic implications. In addition, emerging strategies for enhancing safety profiles and delivery efficiency were discussed, paving the way for the next generation of intestinally restricted drugs tailored for localized therapeutic action.

## 2. Therapeutic application of intestinal restrictive drugs

### 2.1. IBD

IBD is a chronic condition that predominantly affects the digestive tract and consists of two major disorders, including ulcerative colitis (UC) and Crohn's disease (CD).<sup>24</sup> Patients with IBD are often treated with pharmacotherapy, which includes the prescription of anti-inflammatory and immunosuppressive medications.<sup>25</sup>

The typical oral delivery route is governed by intestinal factors, such as absorption, metabolism, first-pass effects, and known adverse effects. As a result, medications may scatter throughout the intestine before reaching the colon, reducing their efficacy in the target region. Treatment options often incorporate anti-inflammatory cytokines, such as tumor necrosis factor-alpha, into established therapies. Although these treatments may relieve the early symptoms of the disease, they may worsen the symptoms in the long term.<sup>26</sup> To reduce medication toxicity, ensure precise drug delivery to the target site, and avoid systemic exposure, recent efforts have focused on intestinally restricted drug therapy,<sup>25</sup> which typically target areas within the gastrointestinal tract.

The target locations in the gut for IBD treatment include (1) the intestinal flora of the human gastrointestinal tract and colonic mucosa.<sup>27</sup> (2) the Aryl hydrocarbon receptor (AHR), which is highly prevalent in the human intestinal epithelium and its activation may increase vulnerability to IBD.<sup>28</sup> The activation of AHR can also affect immunosuppression, cause apoptosis, and inhibit the NOD-like receptor family, pyrin domain containing 3 (NLRP3) inflammasome. Present research suggests that drugs, such as Norisoboldine and Cardamonin can act as AHR ligands to treat IBD by suppressing NLRP3 inflammasome activation.<sup>29</sup> (3) Sphingosine-1-phosphate (S1P) receptor, is highly expressed in endothelial cells and can be efficiently targeted by innovative small compounds for oral administration, such as Ozanimod and Etrasimod. These compounds inhibit S1P expression and reduce S1P levels, which are useful for treating IBD.<sup>30</sup> (4) Takeda G-protein-coupled receptor 5 (TGR5) is mostly present in the intestinal lumen and gallbladder. The TGR5-dipeptidyl peptidase 4 (DPP4) bifunctional molecule operates exclusively within the intestinal tract, providing therapeutic benefits for UC while avoiding unwanted systemic side effects.<sup>31</sup> (5) Studies have revealed that Janus kinase 1 (JAK1)/tyrosine kinase 2 (TYK2) dual inhibitors are more effective and safer for treating IBD, although their specific mechanism still requires further investigation.<sup>32</sup>

### 2.2. Irritable bowel syndrome (IBS)

IBS is a chronic and debilitating functional gastrointestinal disorder that affects a significant portion of the global population, with a prevalence ranging from 9% to 23%. Symptoms of IBS include abdominal pain, abdominal distension, and changes in defecation patterns, which typically manifest as diarrhea, constipation, or a combination of both. These symptoms cannot be ascribed to structural or biochemical irregularities and the exact mechanisms underlying IBS development and its physiology remain unclear.<sup>33</sup> Due to

the complex and varied characteristics of IBS symptoms, treatment is often complicated, and misdiagnoses are common. Present treatments for IBS primarily target symptom management without providing therapeutic advantages, and certain medications may also elicit significant adverse effects.<sup>34</sup> To develop IBS treatments that are more efficacious while minimizing systemic adverse effects, researchers have focused on intestinally restricted medications that target specific sites within the gastrointestinal tract.

These precise target locations throughout the intestines include (1) Guanylyl cyclase C (GC-C), which is highly expressed in the digestive tract.<sup>35</sup> At present, the most prevalent GC-C receptor agonists are linaclotide<sup>36</sup> and plecanatide, with plecanatide demonstrating superior efficacy in lowering the severity of chronic constipation, alleviating tension, improving fecal consistency, increasing the frequency of defecation, and enhancing quality of life.<sup>37</sup> (2) Mechanosensitive ion channels: This category includes a variety of ion channels, including transient receptor potential (Trp) channels, piezoelectric channels, two-pore domain potassium (K2p) channels, voltage-gated ion channels, large conductance  $\text{Ca}^{2+}$ -activated K (BKCA) channels, and cystic fibrosis transmembrane conductance regulators (CFTR).<sup>38</sup> Inhibitors of mechanosensitive ion channels can reduce stomach pain and visceral hypersensitivity while regulating intestinal motility. Furthermore, because these ion channels are primarily located in the gastrointestinal tract, drugs targeting them can exert their effects locally, reducing systemic adverse effects. Crofelemer, a US Food and Drug Administration (FDA)-approved CFTR inhibitor, is used for the symptomatic treatment of non-infectious diarrhea in adult human immunodeficiency virus/acquired immunodeficiency syndrome patients undergoing antiretroviral therapy.<sup>39</sup>

### 2.3. Diabetes mellitus (DM)

DM is a metabolic condition primarily caused by impaired insulin activity or secretion. According to the American Diabetes Association, DM is classified into four types: Type I, type II, special types, and gestational DM. Among them, type II diabetes is the most common, accounting for 95% of all diabetes cases. Type II diabetes is primarily treated with oral hypoglycemic drugs, such as insulin secretagogues, biguanides, insulin sensitizers,  $\alpha$ -glucosidase inhibitors, incretin mimetics, islet amyloid antagonists, and sodium-glucose cotransporter-2 (SGLT2) inhibitors.<sup>40</sup> Nonetheless, because of their low bioavailability and short half-life, conventional medications require frequent administration, which raises considerable safety concerns. In response, attention is shifting toward

intestinally restricted medication therapy, which often target specific locations within the gut.

These gut-specific target areas include (1) intestinal flora. (2) Peroxisome proliferator-activated receptors (PPARs) are a key target for the treatment of type II diabetes, as it regulates glucose homeostasis, lipid metabolism, lipoprotein metabolism, and insulin sensitivity.<sup>41</sup> PPAR- $\gamma$  agonists, such as thiazolidinediones, can improve insulin sensitivity and glucose utilization while diminishing gluconeogenesis by limiting glucose and fatty acid circulation. These actions are aimed at effectively treating diabetes. Furthermore, PPAR $\gamma$  ligands support mucosal defenses against gastrointestinal candidiasis by polarizing M2a macrophages and modulating blood glucose levels for diabetes management.<sup>42</sup> (3) Specific G protein-coupled receptors (GPCR), such as glucagon-like peptide-1 receptor (GLP-1R), G protein-coupled receptor 119 (GPR119), and G protein-coupled receptor 120 (GPR120), are primarily expressed in the intestines, gallbladder, and pancreatic  $\beta$  cells. GLP-1R can be activated by GLP-1, which is mostly secreted by epithelial L cells in the distal ileum and colon mucosa of the gastrointestinal tract, which stimulates insulin release to lower blood sugar levels.<sup>43,44</sup> Several GLP-1 receptor agonists, including exenatide, liraglutide, lixisenatide, dulaglutide, and semaglutide, are licensed for the treatment of type 2 diabetes.<sup>45</sup> (4) Farnesol X receptor (FXR)<sup>46</sup> and Takeda G-protein-coupled receptor 5 (TGR5)<sup>47</sup> are essential signaling molecules influenced by BAs, which play critical roles in glucose, lipid, and energy metabolism, affecting various organs through nuclear FXR and TGR5. The present study suggests that INT-767, a dual BA receptor agonist targeting both FXR and TGR5, offers significant advantages over single FXR or TGR5 agonists in the treatment of diabetes.<sup>48</sup>

### 2.4. Atherosclerosis

Arteriosclerosis is a chronic inflammatory condition that increases the risk of acute cardiovascular events due to the instability of arteriosclerotic plaques, arterial constriction, or blockage induced by platelet aggregation and thrombosis. In arteriosclerosis, inflammation is mediated by pro-inflammatory cytokines, inflammatory signaling pathways, bioactive lipids, and adhesion molecules.<sup>49</sup> Atherosclerotic cardiovascular disease (CVD) has emerged as a major global health concern. Conventional therapeutic approaches primarily focused on anti-inflammatory therapies and cholesterol reduction. However, many traditional anti-inflammatory medicines are associated with common adverse effects and systemic toxicities. To address these challenges, researchers are investigating a treatment method for arteriosclerosis that modulates lipid absorption in the gut, with a focus on specific locations within the gastrointestinal tract.

These specific target locations in the intestine include the (1) intestinal flora.<sup>50,51</sup> (2) Liver X receptor (LXR) is expressed in macrophages, the small intestine, and the liver, among other organs.<sup>52</sup> As an LXR inverse agonist, SR9243 shows potential as a therapeutic intervention for atherosclerosis and hyperlipidemia.<sup>53</sup> (3) Scavenger receptor class B type I (SR-BI),<sup>54</sup> involved in cholesterol metabolism, has been identified as a promising target. Thus, ITX-5061, a SR-BI inhibitor, is being investigated as a promising therapeutic agent for atherosclerosis.<sup>55</sup>

## 2.5. Cholestasis liver disease

Cholestasis is caused by disruptions in the formation and circulation of bile and is classified as either intrahepatic or extrahepatic. Although both primary and secondary sclerosing cholangitis have the potential to affect bile ducts both within and outside the liver, intrahepatic cholestasis is the predominant manifestation of chronic cholestasis.<sup>56</sup> Pharmacological interventions are often employed to manage common cholestasis, whereas intrahepatic cholestasis during pregnancy often requires liver transplantation to prevent late pregnancy-induced liver injury. Present efforts have transitioned to the utilization of intestinally restricted drug therapy to mitigate early liver damage and minimize systemic adverse effects.

The specific anatomical targets in the gastrointestinal tract for cholestasis treatment include (1) ileal BA transporter (IBAT). IBAT is primarily found on the apical surfaces of cells in the renal tubules, biliary ducts, and on the brush border or parietal membrane of intestinal cells in the ileum.<sup>57</sup> Oral IBAT inhibitors, such as maralixibat, elobixibat, and odeixibat,<sup>58</sup> impede BA transport.<sup>59,60</sup> These drugs predominantly act within the ileum due to the structural properties of IBAT inhibitors and the anatomical localization of IBAT. By remaining undetectable in plasma, the systemic toxicity of the medication is substantially reduced.<sup>61</sup> (2) FXR is highly expressed in intestinal cells of the ileum, with BAs act as endogenous ligands.<sup>62</sup> The development of FXR agonists, notably BAs and their derivatives, has enabled the treatment of cholestasis by regulating BA production, metabolism, and reabsorption in the gastrointestinal tract. This method not only effectively treats diseases but also reduces systemic drug toxicity. FXR agonists, such as obeticholic acid (OCA), tropifexor, and MET409, have been or are currently being studied in clinical trials.<sup>63</sup> (3) Progesterone activates pregnane X receptor (PXR, NR1I2), which are highly expressed in intestinal epithelial cells and hepatocytes. FXR stimulates PXR, and together, these receptors help maintain BA balance. Like FXR, PXR activation suppresses hepatic cholesterol 7 $\alpha$ -hydroxylase (CYP7A1), an enzyme involved in BA production. PXR

activation also increases hepatocyte production of organic anion transporting polypeptide 2 (OATP2), cytochrome P450 3A11 (CYP3A11), and sulfotransferase family 2A member 1 (SULT2A1), which work together to detoxify and excrete BAs, as well as multidrug resistance-associated protein 2 (MRP2) to assist tubular transport.<sup>63</sup> PXR agonist drugs developed using this approach can be designed to act primarily in the gastrointestinal tract, resulting in lower systemic toxicity and fewer adverse effects. This pharmacological class has tremendous potential and requires further research.

## 2.6. CRC

CRC is the third most common cancer worldwide and the second most common cause of cancer-related deaths.<sup>64</sup> CRC is inextricably linked to an individual's lifestyle, living environment, chronic conditions, and genetic predisposition. Traditional treatment typically involves surgical excision followed by adjuvant therapies such as radiotherapy and chemotherapy. Nonetheless, colon cancer is characterized by high malignancy, rapid advancement, strong invasiveness, susceptibility to recurrence, and poor prognosis. Considering these challenges, researchers have investigated intestinally restricted drugs for CRC treatment, focusing on specific locations within the gastrointestinal tract. This approach aims to improve therapeutic effectiveness while minimizing systemic adverse effects.

These specific gut target locations include (1) intestinal flora.<sup>65</sup> and (2) Cyclooxygenase-2 (COX-2) plays a critical role in the development of CRC. JTE-522, a selective COX-2 inhibitor, has been shown to successfully prevent the development of intestinal polyps and lower the incidence of cancer. Clinical investigations have found no serious side effects, such as cardiovascular events or gastrointestinal hemorrhage, confirming its therapeutic potential.<sup>66</sup> (3) Mechanosensitive ion channels are also a key focus in CRC treatment. Tumor stiffness manipulation has been shown to alter cellular activities, promoting tumor development and metastasis. As a result, targeted tumor stiffening has emerged as a novel therapeutic strategy, with mechanosensitive ion channels identified as prospective targets for CRC treatment.<sup>67</sup> Drugs developed using this strategy can be designed to exert their effects primarily within the gastrointestinal tract, reducing systemic toxic side effects and offering great potential for more effective therapies.

## 2.7. Other diseases

In addition to the diseases listed above, tuberculosis and some autoimmune disorders,<sup>68</sup> such as systemic lupus erythematosus and rheumatoid arthritis, have shown potential for treatment through gut flora modulation and



intestinal restriction. Chronic diseases, such as delayed constipation and other ailments, may also benefit from the use of intestinally restricted drugs or delivery routes.<sup>69</sup> Nonetheless, it is important to note that research in this area is still limited, and the mechanisms underlying intestinal restriction have yet to be fully explored. Therefore, further in-depth research is needed to fully understand the complexities of intestinal restriction and its prospective applications.

### 3. Design strategies for intestinal restrictive drugs

Presently, well-established criteria govern the design of oral drugs, encompassing attributes, such as low MW < 500 Da, an appropriate lipid-water partition coefficient ( $\log P < 5$ ), ample water solubility (>100  $\mu\text{M}$ ), high cell membrane permeability (Caco-2 apparent permeability coefficient,  $P_{app} > 1 \times 10^{-6} \text{ cm/s}$ ), low human liver microparticle clearance (intrinsic clearance <25  $\mu\text{L/min/mg}$  protein), and minimal glycoprotein transport ( $P_{app}$  [basolateral to basal apex] and  $P_{app}$  [apical to basolateral] < 3).<sup>70</sup> However, these criteria do not directly apply to the development of intestinally restricted drugs, which differ from conventional oral medications and require distinct design strategies. A comprehensive analysis of the strategies employed for developing these drugs has led to their categorization into six primary types:

- (A) High MW drugs: These drugs tend to stay within the intestine for an extended period, thereby increasing the likelihood of intestinal interactions.<sup>71</sup>
- (B) Highly polar or positively charged drugs: Drugs with a positive charge or high polarity can engage in electrostatic interactions with the intestinal cells, capitalizing on the negatively charged surface of the intestinal cells, resulting in enhanced retention within the intestine.
- (C) Drugs with high lipophilicity and low water solubility: Lipophilic and poorly water-soluble drugs are more likely to interact with lipids within the gastrointestinal tract, which can influence drug absorption.<sup>72</sup>
- (D) Intestinal soft drugs: These drugs are metabolized into biologically inactive compounds once entering the systemic circulation. This process can effectively reduce both systemic exposure and potential toxicity.<sup>73</sup>
- (E) Intestinal prodrugs: These drugs lack inherent biological activity but undergo enzymatic conversion in the intestine to become active forms.<sup>74</sup>
- (F) Compounds that serve as substrates for ABC transporters: These drugs can be efficiently removed from cells by ABC transporters within the gastrointestinal tract, which helps prevent their intracellular accumulation.<sup>75</sup>

Representative drugs have been selected for detailed discussion, including insights into their targets, effects, and synthesis.

#### 3.1. High MW compounds

High MW compounds aim to decrease the water solubility and cell permeability of drugs by increasing their MW. The objective of this strategy is to restrict the absorption of medications in the gut, thereby attaining intestinal restriction.<sup>69,71</sup> This strategy has been widely applied in the design and development of intestinally restricted drugs (Figure 1).

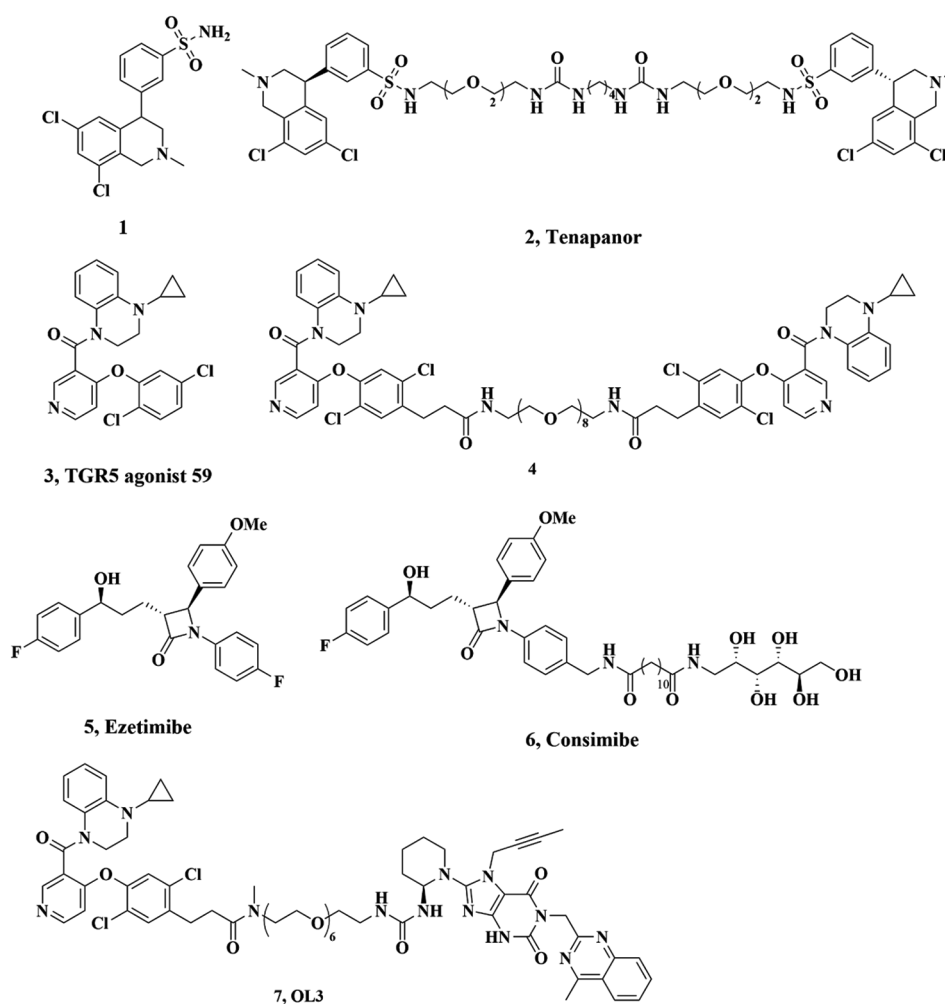
##### 3.1.1. Tenapanor

Tenapanor (compound 2; Figure 1) (MW = 1145 Da, Rb = 29, TPSA = 217  $\text{\AA}^2$ ) has been approved by the FDA for the treatment of IBS complicated by constipation (IBS-C) in adult patients.<sup>76</sup> It functions by inhibiting sodium-hydrogen exchanger 3 (NHE3), thereby enhancing sodium ( $\text{Na}^+$ ) levels in the intestines, stimulating gastrointestinal peristalsis, and alleviating constipation in the gastrointestinal tract. Furthermore, it increases the transepithelial electrical resistance of small intestinal cells, lowers phosphate ion permeability, and inhibits radioactive phosphate uptake in the colon.<sup>77</sup> The major target of tenapanor, NHE3, is predominantly expressed on the intestinal cavity surface but can also be detected in nephron absorptive cells. NHE3 is essential for promoting the absorption of  $\text{Na}^+$  and water from the duodenum into the left colon. Therefore, blocking NHE3 is a viable new therapeutic approach in managing IBS-C.<sup>78</sup>

The NHE3 inhibitory effects of compound 1 (Figure 1) have been observed to be moderate in *in vitro* studies. In contrast, its elevated biological activity and high systemic exposure are attributed to its modest MW (370 Da). To mitigate systemic exposure, tenapanor was developed by augmenting the number of hydrogen bond donors, acceptors, and rotatable bonds within the solvent-exposed region of the pharmacophore.<sup>79,80</sup> Compared to placebo, tenapanor reduced body weight, increased defecation frequency, and dose-dependent softening of fecal consistency. Moreover, the plasma concentration of tenapanor remained below the quantification limit (0.5 ng/mL) in 95% of the samples collected throughout the assays. This indicates minimal systemic absorption of the drug within the human body.<sup>81</sup>

##### 3.1.2. TGR5 agonists

The TGR5 agonist (compound 4; Figure 1) (MW = 1401 Da) exhibits low permeability in small intestine Caco-2 cells, thereby diminishing drug absorption in the small intestine



**Figure 1.** The chemical structure of intestinal restrictive drugs with high molecular weight  
 Abbreviations: TGR5: Takeda G-protein-coupled receptor 5.

and limiting systemic exposure.<sup>72,82</sup> TGR5 activation induces the production and release of incretins, including GLP-1 and GLP-2, in the intestine.<sup>83,84</sup> GLP-1 plays a vital role in regulating glucose homeostasis, whereas GLP-2 is essential for gastrointestinal health. Compound 4 (Figure 1) demonstrated *in vivo* hypoglycemic effects and mitigated gallbladder-related adverse effects.

A novel low-absorption TGR5 agonist, OL3, was developed by combining a novel TGR5 agonist (compound 3; Figure 1) with linagliptin, a DPP4 inhibitor.<sup>85</sup> OL3 exhibits glucose-lowering properties through the activation of TGR5 and inhibition of DPP4. In the Caco-2 model, OL3 exhibited low permeability ( $P_{app} = 0.03 \times 10^{-6} \text{ cm/s}$ ), and ICR mice demonstrated a low level of systemic exposure. Furthermore, the absence of gallbladder filling was attributed to the minimal systemic absorption of OL3.

### 3.1.3. Vancomycin

Vancomycin (MW = 1449 Da) is a complex tricyclic glycopeptide with strong antibacterial activity. This is accomplished by inhibiting the synthesis of peptidoglycan, an essential component of the bacterial cell walls. It is particularly effective against staphylococci and has limited activity against enterococcal streptococci. Despite its high water solubility, the low permeability of vancomycin restricts gastrointestinal absorption, thereby minimizing systemic exposure. When taken orally, its bioavailability is <10%, making it ideal for treating intestinal infections but unsuitable for systemic infections.<sup>86</sup> For most patients, independent of their intestinal or renal status, oral administration of vancomycin results in limited intestinal absorption, with the medication being predominantly excreted in feces following its therapeutic effects.

### 3.1.4. Derivative of Ezetimibe

Ezetimibe (compound 5; [Figure 1](#)) is often used in combination with statin therapy to improve hypolipidemic efficacy and minimize the risk of CVD in individuals with atherosclerosis or diabetic hyperlipidemia.<sup>87-89</sup> However, scientists have discovered that ezetimibe causes a high systemic exposure, as it circulates through the body through both intestinal and hepatic pathways after exerting its effects. Jaehne *et al.*<sup>90</sup> developed canosimibe (compound 6; [Figure 1](#)) to reduce systemic drug exposure by increasing the number of rotatable bonds and the MW of Ezetimibe. Canosimibe has a MW of 810 g/mol and 26 rotatable bonds. Following oral administration, the remaining dose is excreted in feces after intestinal absorption, with no detectable drug residues or metabolites in serum tests. Despite its experimental promise, canosimibe is unsuitable for clinical use due to its poor therapeutic impact. Nonetheless, this serves as an important study avenue, suggesting that restricting the enterohepatic circulation of ezetimibe in the colon by incorporating additional rotatable bonds and polymer groups may reduce the systemic exposure rate and minimize drug-related adverse effects.<sup>72</sup>

## 3.2. Highly polar or positively charged compounds

The cell membrane is a crucial structural composed of a phospholipid bilayer. Fat-soluble, non-polar molecules are found in all cells and diffuse freely across the membrane. Since permeability and solubility are important factors in drug absorption from the gut, increasing a drug's polarity or charge can reduce its absorption and improve gut retention. This approach achieves low permeability by adhering to the principles of similar-phase solubility. These highly polar molecules usually contain polar groups or positive charges, such as hydroxyl, amino, carboxylic, and sulfonic acids.<sup>91</sup> As a result; a common method in drug design involves incorporating polar groups or positive charges into molecules to lower their permeability, making them ideal for intestinal restriction ([Figure 2](#)).

### 3.2.1. Acarbose

Acarbose (compound 8; [Figure 2](#)), an inhibitor of both human and bacterial  $\alpha$ -glucosidase, reduces blood glucose levels by slowing carbohydrate hydrolysis and is extensively used to treat type 2 diabetes. Industrially, acarbose is produced utilizing motile actinomycetes.<sup>92</sup> The incorporation of two mannose units in acarbose increased its polarity and extraordinary hydrophilicity while maintaining a low lipophilicity (cLogP = -9). Consequently, its oral bioavailability is <2% in healthy volunteers.<sup>93</sup>

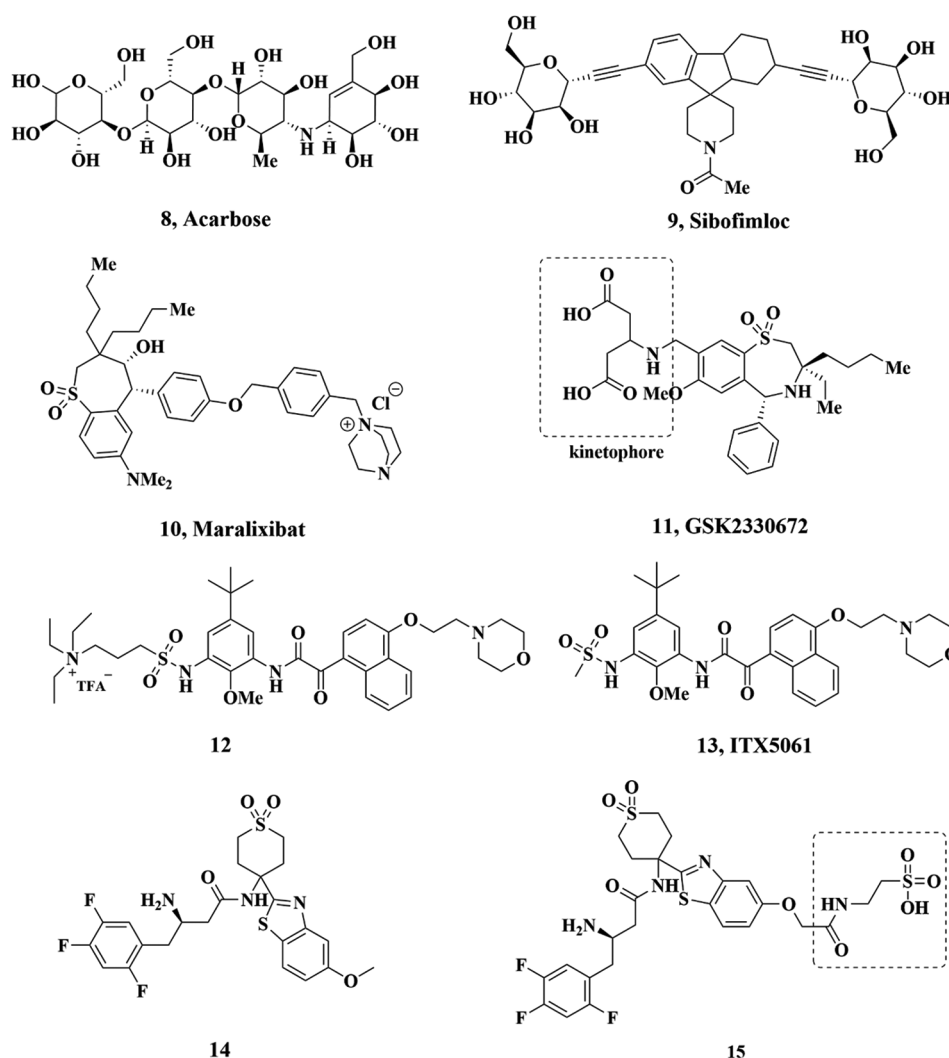
### 3.2.2. Sibofimloc

In experiments, sibofimloc (compound 9; [Figure 2](#)), an oral small molecule and FimH blocker, showed low systemic absorption. Its principal site of action is the gastrointestinal lumen and mucus layer, where it targets FimH-expressing bacteria and protects them from digestion by the gut microbiota. By blocking FimH on the bacterial surface, sibofimloc prevents bacteria from interacting with intestinal epithelial cells, thereby reducing the local production of pro-inflammatory cytokines. Furthermore, sibofimloc has the unique capacity to aggregate FimH adhesins on multiple bacterial cells simultaneously, enabling them to agglomerate and facilitate their removal from the intestinal lumen.<sup>94</sup> In an open-label, multicenter phase 1b study, sibofimloc showed minimal systemic exposure, was well-tolerated in patients with active celiac disease, and reduced specific inflammatory biomarkers.<sup>13</sup> Originally developed by Vertex Pharmaceuticals, sibofimloc has advanced to a phase II clinical trial (NCT03943446) for CD. Due to two mannose units (clogP = 0.3, HBD = 8, HBA = 12, TPSA = 200 Å<sup>2</sup>), its strong polarity is a key factor contributing to its low systemic exposure in both healthy individuals and CD patients.<sup>69</sup>

### 3.2.3. Maralixibat (Livmarli) and GSK2330672

Maralixibat (compound 10; [Figure 2](#)) is an oral small-molecule ileal BA transporter (IBAT) inhibitor developed by Mirum Pharmaceuticals. It is specifically indicated for the treatment of rare cholestatic liver diseases, such as progressive familial intrahepatic cholestasis (PFIC), biliary atresia, and Alagille syndrome (ALGS).<sup>95,96</sup> Patients with ALGS who underwent surgical intervention to interrupt enterohepatic circulation experienced relief from pruritus.<sup>60</sup> As an alternative, pharmacological disruption of BA transport through IBAT targeting has emerged as a promising approach for managing pruritus. Given its critical function in BA reabsorption, IBAT is regarded as an optimal target for pharmacological interventions targeting BA transport.<sup>97</sup> On September 29, 2021, the FDA granted Maralixibat its initial approval for treating cholestatic pruritus in patients with ALGS aged 1 year and older.<sup>98</sup>

The structural design of maralixibat involves incorporating a charged kinetochore into the pharmacophore of benzothiophene-class IBAT inhibitors. A rigid 1,4-disubstituted benzene linker is essential to preserve an equilibrium among crystallinity, hygroscopicity, potency, and water solubility.<sup>69</sup> In rat studies, peripheral and portal vein plasma levels of the drug were undetectable following oral administration, suggesting inadequate intestinal absorption.





highest *in vitro* activity ( $pIC_{50} = 6.8$ ), excellent tolerance, low permeability, and good gastrointestinal stability *in vitro*. The Madin-Darby canine kidney (MDCK) cell monolayer exhibited low permeability ( $<0.1 \times 10^{-6} \text{ cm}^2/\text{s}$ ). In *in vivo* DMPK studies involving rats, the compound demonstrated low systemic exposure and a high fecal drug recovery rate of  $83 \pm 3\%$ .

### 3.2.5. $\beta$ -Homophenylalanine derivatives

Shen *et al.*<sup>100</sup> used a  $\beta$ -homophenylalanine derivative (compound 14; Figure 2) as a starting point in their discovery program for non-systemic intestine-targeted (NSIT) DPP4 inhibitors. They concluded that the sixth position of the benzothiazole moiety may be the best option for introducing highly polar kinetochores. Relevant studies have shown that this location can tolerate rather big substituents, such as 2-morpholine ethyl.<sup>101</sup> They synthesized a variety of drugs by incorporating highly polar moieties, such as kinase carriers containing sulfonic acids, quaternary ammonium salts, and carbohydrate fragments, into systemic DPP4 inhibitors. Finally, Shen *et al.*<sup>100</sup> discovered the NSIT DPP4 inhibitor (compound 15; Figure 2) with a negative  $cLogP$  value, high hydrophilicity, and a highly polar segment linked by two carbon atoms. In the studies, this compound demonstrated potent DPP4 inhibition and water solubility. After oral administration of up to 30 mg/kg, the inhibitor specifically targeted intestinal DPP4 activity, leaving plasma DPP4 activity unaffected. Importantly, the NSIT compounds showed minimal toxicity due to their restricted systemic exposure.

### 3.3. High lipophilic and low water solubility compounds

Water solubility determines the rate of disintegration, dissolution, and diffusion of solid dosage forms or suspensions. Consequently, compounds with low water solubility are more likely to exhibit low systemic absorption. There was a robust correlation between low water solubility and high lipophilicity. As pharmaceuticals necessitate dissolution for absorption, an additional approach to attain gut specificity is to concentrate on regions with high lipophilicity and low solubility.<sup>102</sup> It is customary to estimate the lipophilicity of a compound using the  $\log P$  value, which is the logarithm of the octyl/water partition coefficient.<sup>91</sup> As water solubility decreases with increasing  $\log P$ , some researchers have initiated investigations into lipophilic drugs to impede absorption in the small intestine, thus retaining the compound within the gastrointestinal tract.<sup>103</sup> Several key structural characteristics, including molecular volume, dipole polarity, and the number of hydrogen bond donors and acceptors, influence the  $\log P$  value. Increasing  $\log P$  values, eliminating polar moieties,

and employing ring fusion to create a rigid, planar skeleton are strategies used by medicinal chemists to develop gut-targeting compounds with low water solubility and reduced intestinal absorption (Figure 3).

#### 3.3.1. Fexaramine and its derivatives

Fexaramine (compound 19; Figure 3), an FXR agonist, exhibits poor systemic absorption due to its low ionization capacity and three lipophilic groups: a biphenyl group, a styrene group, and a hexane ring, apart from which it is composed. Therefore, fexaramine exhibits exceptionally high lipid solubility but negligible water solubility. FXR, encoded by the nuclear receptor Nr1h4, functions as a ligand-activated transcription factor and is present in numerous tissues, including white and brown adipose tissue, adrenal glands, kidneys, stomach, duodenum, jejunum, ileum, colon, gallbladder, and liver.<sup>104</sup> FXR activation in the liver contributes to liver regeneration,<sup>105</sup> inhibits hepatic BA synthesis, and regulates glucose, lipid, and cholesterol homeostasis.<sup>106,107</sup> By ameliorating the metabolic profile of obesity induced by a high-fat diet, oral fexaramine reduces inflammation, increases insulin sensitization, decreases weight gain, and promotes the browning of white adipose tissue.<sup>17</sup>

Furthermore, Wang *et al.*<sup>108</sup> successfully designed and synthesized a novel intestinally restricted FXR agonist, fexaramine-3 (compound 20; Figure 3), by replacing the cyclohexanamide group with the more lipophilic 6-(methylamino) naphthamide. Compared with fexaramine, fexaramine-3 exhibited higher agonist activity and better selectivity in the ileum. Intestinally restricted FXR activation offers advantages in reducing obesity and insulin resistance, along with providing other systemic benefits.

#### 3.3.2. Betulinic acid and its derivatives

Betulinic acid (BA; compound 16; Figure 3) is a triterpenoid extracted from the leaves of *Betula platyphylla*. It has been reported to be a selective TGR5 agonist with moderate potency ( $EC_{50} = 2.25 \mu\text{M}$ ) and antihyperglycemic effects.<sup>109</sup> TGR5 is a G-protein-coupled receptor expressed in intestinal L-cells, and its activation promotes GLP-1 and peptide YY secretion.<sup>110</sup> TGR5 is a novel target for the treatment of metabolic disorders. Furthermore, BA has exhibited the capacity to modulate the production of numerous molecules implicated in the pathogenesis of neuroinflammatory diseases.<sup>111</sup>

Due to its pentacyclic structure, which is both extremely rigid and lipophilic, BA is poorly soluble in water and has limited intestinal absorption. Nan *et al.*<sup>109</sup> experimentally investigated the introduction of a minor lipophilic group

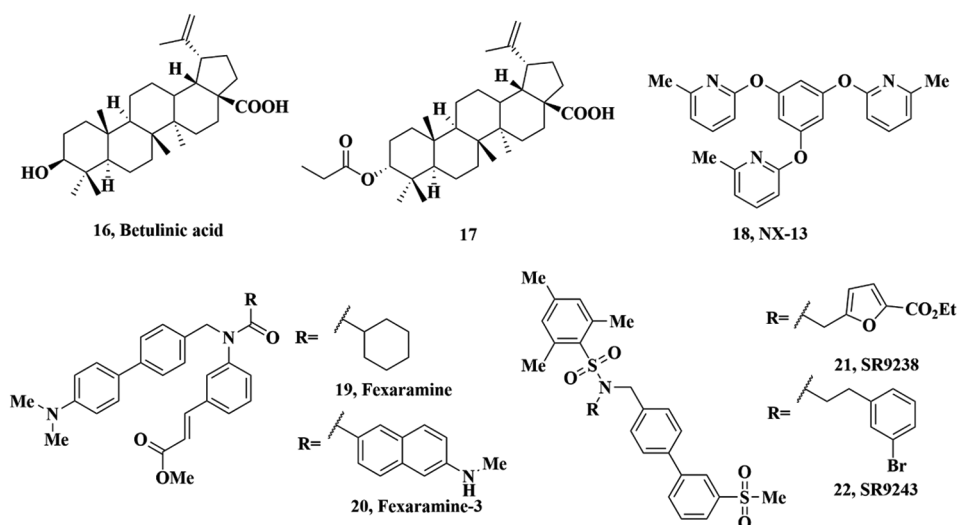


Figure 3. The chemical structure of intestinal restrictive drugs with high lipophilic and low water solubility

at position C-3 to design and synthesize a sequence of TGR5 agonists derived from BA. By employing structure-activity relationship analysis, they successfully identified compound 17 (Figure 3) as having the greatest *in vitro* activity ( $EC_{50} = 313$  nM). Compound 17 exhibited higher lipophilicity ( $cLogP = 6.85$ ) than that of BA ( $cLogP = 4.93$ ). Oral administration of compound 17 to rats at a dose of 10 mg/kg resulted in BA concentrations in plasma and urine falling below 5 ng/mL and 1 ng/mL, respectively. The recovery of approximately 85% of the BA derivative in the feces suggests that most derivatives persisted in the intestine, thereby leading to minimal systemic exposure.

### 3.3.3. NX-13

NX-13 (compound 18; Figure 3), initially reported by researchers at Landos Biopharma, is a novel, intestinally restricted, orally active small-molecule drug candidate designed for treating IBD and UC. Nucleotide-binding oligomerization domain, Leucine-rich repeat-containing X1 (NLRX1), a distinctive nucleotide-binding domain leucine-rich repeat-containing receptor (NLR) with notable regulatory and anti-inflammatory properties, has recently gained recognition as a potential target for immune metabolism. NX-13 selectively and locally activates the NLRX1 pathway within the gastrointestinal tract due to its high binding affinity for NLRX1.<sup>112</sup> This activation increases oxidative phosphorylation in immune cells, decreases the differentiation of effector CD4-positive T cells, and reduces the production of inflammatory cytokines in the gastrointestinal tract.

NX-13 demonstrates considerable lipophilicity ( $cLogP = 6.5$ ); however, precise information regarding its permeability and solubility remains inaccessible. Initial

safety investigations were carried out in rodents to evaluate the safety profile of NX-13 when administered orally at a dose of 1000 mg/kg. Its pharmacokinetics, characterized by restriction to the intestine, partly contribute to its favorable safety profile by producing local colon concentrations over a thousand times higher than plasma concentrations.<sup>113</sup> As a result, the substance has a low systemic exposure. To further explore and refine NX-13, this promising candidate will be advanced to a phase II clinical trial (NCT05785715) encompassing patients with UC.<sup>114</sup>

### 3.3.4. SR9238 and SR9243

Burris *et al.*<sup>115, 116</sup> identified two structurally related liver X receptor (LXR) inverse agonists, SR9238 (compound 21; Figure 3) and SR9243 (compound 22; Figure 3), which were discovered and used to evaluate the inverse agonism of LXR. As a sterol-responsive nuclear receptor, LXR plays a critical role in maintaining cholesterol homeostasis by promoting cholesterol efflux and limiting low-density lipoprotein (LDL) uptake.<sup>117</sup> Furthermore, nuclear LXR directly affects the expression of glycolytic and lipogenic genes, making it a key transcriptional regulators of cholesterol metabolism. Burris *et al.*<sup>53</sup> found that oral administration of gut-specific LXR inverse agonists greatly reduced intestinal sterol O-acyltransferase 2 (SOAT2) expression. This, in turn, lowered circulating LDL cholesterol and triglyceride levels without affecting peripheral LXR target genes involved in intestinal cholesterol reabsorption. This discovery could lead to more effective treatments for hyperlipidemia and atherosclerosis.

Burris *et al.*<sup>118</sup> meticulously developed these drugs by leveraging the recently described chemical framework of tertiary sulfonamide LXR antagonists, designed to

attract corepressor proteins in biochemical experiments. Among the compounds designed, SR9238 displayed some selectivity for LXR- $\beta$ , with an inhibitory concentration of 214 nM for LXR- $\alpha$  and 43 nM for LXR- $\beta$ . This careful selection process underscores the scientific rigor behind the discovery, ensuring the effectiveness and safety of the drugs.

Burris *et al.*<sup>116</sup> developed SR9243, an LXR inverse agonist with significant potential for cancer treatment. SR9243 significantly suppressed the Warburg effect and lipogenesis in cancer cells by downregulating the expression of glycolysis and lipogenesis genes, leading to tumor cell death while avoiding side effects such as weight loss, hepatotoxicity, or inflammation. Notably, intravenous administration of SR9243 resulted in systemic exposure, whereas oral treatment resulted in considerable intestinal exposure rather than plasma exposure. The high lipophilicity (cLogP = 7.6) and poor water solubility of the molecule further underscore its unique pharmacokinetic properties. These distinctive features of SR9243 highlight its potential as a versatile and effective cancer treatment.

### 3.4. Intestinal soft drugs

The concept of “intestinal soft drugs” was introduced in 1977 and has recently gained attention with significant research and development efforts in enhancing their therapeutic efficacy.<sup>119</sup> The phrase “soft drugs” refers to therapeutic molecules that are intentionally designed to be converted by the body into inactive and non-toxic substances, thereby reducing systemic exposure and minimizing toxicity.<sup>120</sup> The fundamental part of soft drug design techniques involves managing and predicting metabolic processes by incorporating specific fragments within the original molecule. These fragments allow the molecule to act as a substrate for metabolic enzymes while remaining active against its original target.

Soft drugs are often confused with prodrugs, as both involve planned metabolic processes and may depend on enzymatic hydrolysis. Soft drugs are active molecules that are deactivated by metabolic events, whereas prodrugs are initially inactive and must undergo activation to exert their therapeutic effects. While soft drugs have been widely used in localized applications targeting the eyes, skin, lungs, and other local locations, their use in the gastrointestinal system poses additional challenges due to fluctuated pH values and the influence of intestinal bacteria.<sup>121</sup> Nonetheless, certain drugs have been successfully developed as intestinally restricted drugs using the soft drug strategy (Figure 4).

#### 3.4.1. PF-02575799

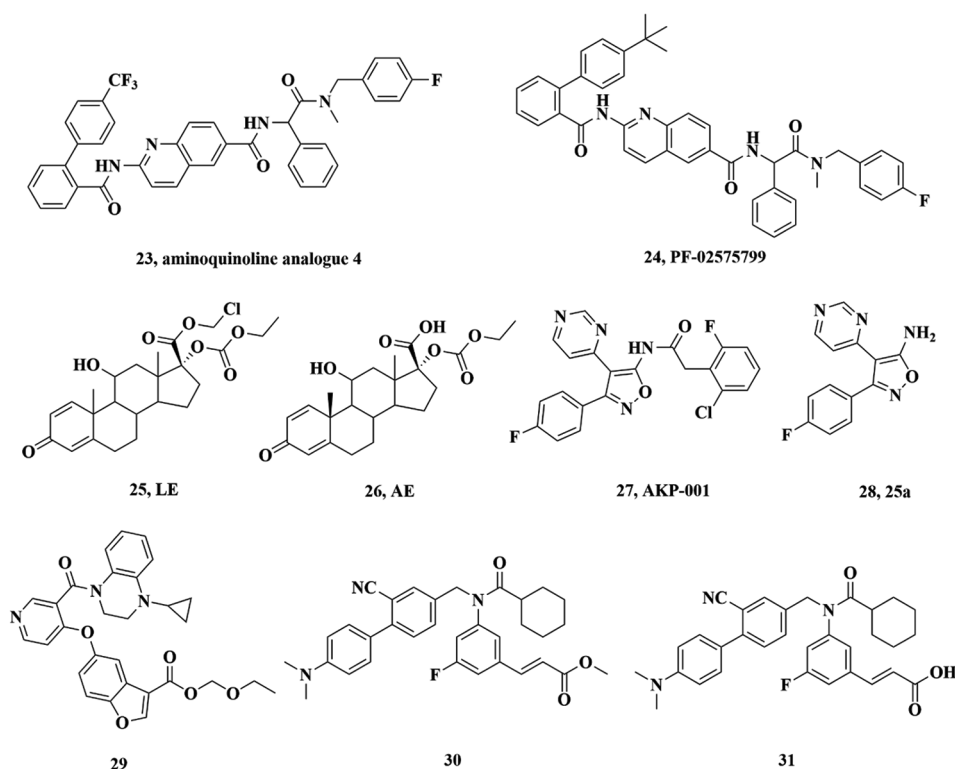
PF-02575799 (compound 24; Figure 4), developed by Robinson *et al.*,<sup>122</sup> incorporates an additional metabolic

site into a microsomal triglyceride transfer protein (MTP) inhibitor (aminoquinoline analog 23) by replacing 4'-trifluoromethyl with t-Bu. Compound 23 (Figure 4) plays a crucial role in lipid metabolism as an MTP by facilitating the conversion of triglycerides into triglyceride-rich lipoproteins, such as chylomicrons in the gastrointestinal tract and very LDL in hepatocytes.<sup>123</sup> It inhibits fat absorption in the gastrointestinal tract by preventing chylomicron aggregation within intestinal epithelial cells.<sup>124</sup> However, compound 23 showed no evidence of gut selectivity in a definitive study. PF-02575799 offers advantages over compound 23 in terms of robust efficacy in rats (minimum effective dose, MED = 3 mg/kg) and specific targeting of the intestines.

This compound undergoes CYP-mediated dealkylation, generating metabolites both *in vivo* and *in vitro*. PF-02575799 exhibited minimal systemic exposure (bioavailability <3%) in both rats and dogs, indicating its potential as an intestinal soft drug. Moreover, the metabolite profile of this compound was similar in rats and humans.

#### 3.4.2. Loteprednol Etabonate (LE)

LE (compound 25; Figure 4) is a non-toxic and locally active corticosteroid.<sup>125</sup> The effectiveness of LE in treating gastrointestinal inflammation in rodents was examined by Bodor *et al.*<sup>126</sup> through oral and rectal administration. Although the drug was efficiently transported to the upper gastrointestinal tract through oral administration of the LE solution, its distribution to the colon was restricted because of absorption or degradation mechanisms. Conversely, oral administration of LE suspension resulted in comprehensive permeation across most of the gastrointestinal tract within 8 h, with negligible infiltration into the rectum. The results of rectal administration indicated that LE maintained its integrity for more than 5 h within the rectal loop and exhibited some dispersion across the rectal membrane. Throughout the experiment, plasma concentrations of LE and its inactive metabolite,  $\Delta 1$ -cortienic acid etabonate (AE), remained consistently below the detection limit of 0.1  $\mu\text{g/ml}$ . *In vitro* studies suggested that LE was stable in the stomach, but it was hydrolyzed by the cecal microbiota in the cecum. LE was efficiently distributed within the mucosa at concentrations of approximately 2.5 – 4.0  $\mu\text{g/g}$  tissue when dissolved. LE undergoes a rapid two-step metabolic conversion, first converting into the inactive metabolite AE (compound 26; Figure 4) and subsequently into the active compound  $\Delta 1$ -cortienic acid *in vivo*. Thus, despite its potent local anti-inflammatory activity, LE exhibits fewer systemic adverse effects than other corticosteroids.



**Figure 4.** The chemical structure of intestinal soft drugs  
Abbreviations: LE: Loteprednol etabonate; AE: Acid etabonate.

These findings indicate that local administration of LE, either orally or through the gastrointestinal tract, could be a viable strategy for managing IBD. Moreover, the mechanism of action of LE aligns with that of soft drugs, as it is deactivated in the systemic circulation after exerting its therapeutic effect in the intestine, thereby reducing the likelihood of systemic adverse effects on the body.

### 3.4.3. AKP-001

Isoxazoles exhibit significant inhibitory activity against p38 mitogen-activated protein (MAP) kinase,<sup>127</sup> but they are susceptible to hydrolysis in the liver, producing isoxazole-5-amines. To address this concern, scientists appended an amide group to the isoxazole moiety to produce AKP-001.<sup>128</sup> This inhibitor has shown potential as a therapeutic intervention for chronic inflammation, owing to its ability to inhibit cytokine secretion and circumvent hepatotoxicity commonly associated with specific p38 MAP kinase inhibitors.<sup>129</sup>

The notable suitability of AKP-001 (compound 27; Figure 4) as a soft drug is primarily attributed to the negligible activity exhibited by its expected metabolite (compound 28; Figure 4). When administered orally at a dosage of 30 mg/kg to male rats, AKP-001 undergoes a

swift metabolic process, leading to a relatively low plasma concentration ( $C_{\max} = 0.88$  ng/ml) and comparatively high concentrations ( $C_{\max} = 136$  ng/mL) of its metabolites. It has been established that AKP-001 has an oral bioavailability of less than 1%. Despite the initial classification as an antidrug, additional analysis revealed that AKP-001 is an intestinally restricted soft drug with therapeutic advantages in the treatment of IBD. Therefore, it is now categorized as a soft drug with intestinal restrictions.

### 3.4.4. TGR5 agonists

To mitigate the systemic side effects, Han *et al.*<sup>130</sup> initially employed the soft drug strategy in the development of TGR5 agonists. A series of ester-based TGR5 agonists with soft-drug properties were designed and synthesized. Subsequent investigations focused on compound 29 (Figure 4), which exhibited an exceptional decrease in peak glucose levels, as determined by oral glucose tolerance tests. In animal models, its antihyperglycemic effects were comparable to those of systemic TGR5 agonists. More importantly, administering compound 29 for three consecutive days did not result in a statistically significant increase in gallbladder volume. The results highlight the potential of this soft TGR5 agonist to alleviate the negative consequences associated with gallbladder filling. This



implies that soft TGR5 agonists may have clinical utility in addressing systemic side effects.

#### 3.4.5. Derivative of Fexaramine

Shim *et al.*<sup>131</sup> have significantly advanced the development of a gut-specific FXR partial agonist by synthesizing fexaramine analogues. As previously stated, FXR is a prospective pharmaceutical target for treating hepatic diseases related to BA metabolism.<sup>132</sup> Compound 30 (Figure 4), based on fexaramine as the lead compound, incorporates fluorine at the C-5 position of the aniline ring and CN substituent at the C-2 position of the biphenyl ring. Compound 30 was particularly effective in reducing the hepatic fibrogenic zone, liver fibrosis markers, and blood aspartate aminotransferase levels. After oral administration in rats, this agonist was largely absorbed by the gut. Once it enters the portal vein, serum esterase quickly metabolizes it into an inactive molecule (compound 31; Figure 4). This discovery provides significant support for gut-specific FXR agonistic action, further emphasizing its potential therapeutic application.

### 3.5. Intestinal prodrugs

Recently, there has been a significant increase in research and development efforts dedicated to the formulation of intestinal prodrugs. The term “prodrug” was first proposed by Albert in 1958 to denote substances that necessitate chemical transformations within the organism to exhibit their therapeutic properties.<sup>133</sup> Despite its potential to optimize drug therapy, this concept of prodrugs gained international recognition in the early 1970s. Across, prodrug strategies, pharmaceutical compounds can be optimized for enhanced stability, improved targeting precision, and increased bioavailability, thereby augmenting their therapeutic efficacy. Concurrently, these methodologies overcome obstacles, including inadequate oral absorption, restricted water solubility, and drug toxicity.<sup>134</sup> The development of intestinal prodrugs entails transforming inactive drug forms into active forms through enzymatic catalysis or chemical alterations within the intestine. This strategy not only enhances the stability of pharmaceuticals but also reduces adverse effects, thereby facilitating more precise intestinal targeting.

Several well-established methodologies have been thoroughly investigated and implemented to develop intestinal prodrugs (Figure 5). Two methods frequently utilized to develop effective intestinal prodrugs are rational structural modifications of drug molecules and binding them to targeted intestinal peptides. Another commonly employed tactic is to leverage the catalytic properties of the intestinal enzymes. By incorporating readily hydrolyzable chemical bonds or substrates, this approach

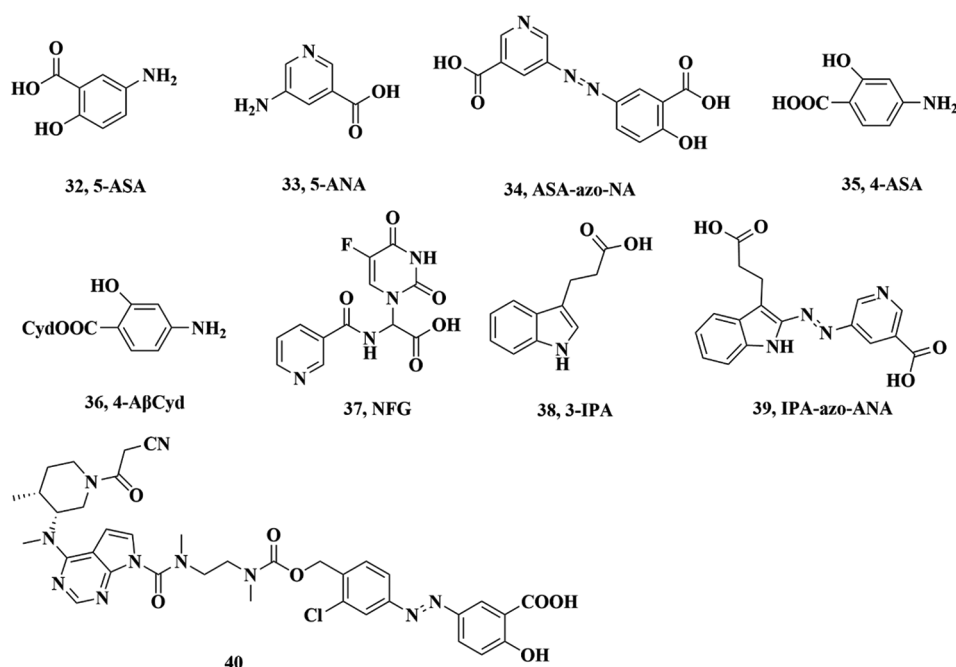
enables prodrugs to undergo enzymatic degradation in the intestine, transforming them into active therapeutic compounds.<sup>135</sup>

#### 3.5.1. The 5-ASA-based intestinally restricted prodrugs

5-Aminosalicylic acid (5-ASA; compound 32; Figure 5), an anti-inflammatory agent, is a well-established therapeutic choice for treating mild to moderate IBD, such as UC and Crohn's disease.<sup>136</sup> The three distinct functional groups of 5-ASA, such as aromatic amines, carboxylic acid, and phenols, have been effectively harnessed for their binding capabilities with pre-cursors. Leveraging these functional groups to engage with various functional moieties exemplifies a strategic and versatile approach to developing intestinally restricted drugs.

Jeong *et al.*<sup>137</sup> developed a 5-ASA azo-coupled with nicotinic acid prodrug (ASA-azo-NA; compound 34; Figure 5) by conjugating 5-ASA with a GPR109A agonist. This prodrug can be activated by microbial azo reductase in the large intestine to generate 5-ASA and 5-ANA (compound 33; Figure 5). 5-ANA can activate GPR109A, which facilitates GPR109A-mediated signaling and modulates various pathophysiological processes, including inflammation and tumorigenesis.<sup>138,139</sup> This study revealed that ASA-azo-NA exhibited negligible blood levels of 5-ANA following oral administration. In contrast, substantial levels of 5-ANA were observed in the circulatory system after the oral ingestion of pure 5-ANA. These findings suggest that ASA-azo-NA demonstrates colonic delivery efficiency comparable to sulfasalazine (SSZ), a colon-targeted 5-ASA prodrug. ASA-azo-NA effectively limits systemic absorption and mitigates side effects associated with systemic exposure, such as skin flushing.<sup>140</sup> Therefore, ASA-azo-NA represents an intestinal prodrug that successfully reduces the risk of cutaneous toxicity by restricting the systemic absorption of 5-ANA.

In May 2018, the FDA approved tofacitinib as the first JAK inhibitor for managing moderately to severely active UC. However, concerns have arisen regarding the escalated systemic exposure associated with increased doses of tofacitinib. To address this concern, Jiaying *et al.*<sup>141</sup> successfully developed 5-ASA-based colon-specific delivery systems, such as 5-ASA-PABA-MAC and 5-ASA-PABA-diamine. Using these delivery systems, they synthesized a series of novel colon-targeted azo prodrugs based on tofacitinib. Specifically, compound 40 (Figure 5) demonstrated the ability to decrease systemic exposure while increasing colon exposure compared to tofacitinib.



**Figure 5.** The chemical structure of intestinal prodrugs

Abbreviations: 5-ASA: 5-aminosalicylic acid; 5-ANA: 5-amino-1-naphthoic acid; 4-ASA: 4-aminosalicylic acid; NFG: N-nicotinyl-2-(5-fluorourac-yl)-D,L-glycine; 3-IPA: 3-indolepropionic acid.

### 3.5.2. 4-AβCyd

Extensive clinical trials have shown that 4-aminosalicylic acid (4-ASA; compound 35; [Figure 5](#)) is effective and safe for the topical treatment of active ulcerative proctitis or left-sided UC, with several advantages over 5-ASA. These advantages include improved stability and effectiveness as well as a lower incidence of severe side effects such as cytopenia.<sup>142</sup> 4-ASA is also associated with a low risk of causing pancreatitis.<sup>143</sup> However, it has the same disadvantage of being extensively and rapidly absorbed in the upper gastrointestinal tract before reaching the colon, which may be ascribed to its weakly acidic nature ( $pK_a = 3 - 4$ ).<sup>144</sup>

To reduce side effects, 4-AβCyd (compound 36; [Figure 5](#)) was created by combining 4-ASA and β-cyclodextrin. Compared with the rectal administration of 4/5-ASA, 4-AβCyd showed moderate improvement in colitis. The cecum and feces of the rats released 68% and 92% of 4-AβCyd, respectively.<sup>145</sup> The pancreas and liver of the prodrug-treated group showed no pathological alterations, indicating the superiority of 4-AβCyd over sulfadiazine and oral 5-ASA. This technique efficiently preserves the therapeutic properties of the original drug while limiting potential health risks.

### 3.5.3. IPA-azo-ANA

3-IPA (compound 38; [Figure 5](#)) acts as a communication medium with potent antioxidant and anti-inflammatory

properties. Furthermore, they linked 5-ANA to an azo bond to obtain IPA-azo-ANA (compound 39; [Figure 5](#)). The colon specificity of these derivatives was confirmed by measuring their partition coefficient ( $\log D = 6.8$ ) in a 1-octanol/isotonic phosphate buffer system at pH 6.8. The chemical stability of these derivatives in the intestine was also verified, and their release was monitored by assessing their presence in the small intestine and cecum of rats. The results demonstrated that these derivatives released 3-IPA in the cecal contents while remaining stable in the small intestinal contents. IPA-azo-ANAs exhibited colon specificity in both *in vitro* and *in vivo* experiments. Oral administration of IPA-azo-ANA showed superior efficacy compared to SSZ in reducing colonic injury and inflammation, suggesting that intestinally restricted 3-IPA ameliorates colitis in rats.<sup>146</sup>

### 3.5.4. N-nicotinyl-2-(5-fluorourac-yl)-D,L-glycine

Lee *et al.*<sup>147</sup> introduced an acetoxy group at the pyrimidine nitrogen (N-1 position) of 5-fluorouracil (5-FU), resulting in NFG (compound 37; [Figure 5](#)) formation. Following this, nicotinamide is hydrolyzed by bacterial enzymes in the large intestine, resulting in its spontaneous degradation and subsequent liberation of 5-FU. In addition to its susceptibility to inactivation by dihydrouracil dehydrogenase, the low bioavailability and inconsistent absorption of 5-FU in the liver and gastrointestinal mucosa impede its effectiveness in treating numerous

malignancies, including CRC.<sup>148</sup> The nitrogen atom within the pyrimidine ring plays a pivotal role in promoting the formation of secondary amides. However, its ability to engage in diazo reactions is restricted. As a result, using an amide bond to conjugate amino acids signifies a promising strategy for developing enter-limiting prodrugs containing 5-FU.

By employing these strategies, pharmaceuticals can attain an intestinally restricted mode of action, which permits targeted conversion or release into the colon and rectum. This improves therapeutic effectiveness while reducing systemic adverse effects. These approaches have considerable potential as innovative treatments for various diseases.

### 3.6. Compounds that act as substrates for ABC transporters

ABC transporters utilize the energy from ATP hydrolysis to facilitate the translocation of their substrates across the biofilm toward the extracellular environment, thereby effectively preventing intracellular accumulation and achieving intestinal restriction. P-glycoprotein (P-gp),<sup>149</sup> breast cancer resistance protein (BCRP), and MRP2 exhibit broad substrate specificity for diverse drugs, toxins, and metabolites.<sup>150</sup> Within the intestine, these three proteins primarily mediate the efflux of drug-like compounds into the intestinal lumen, thus limiting the absorption of exogenous substances. However, limited efforts have been dedicated to the development of intestinally restricted drugs (Figure 6).<sup>75</sup>

#### 3.6.1. DGAT1 inhibitor

The hydrolysis of triglycerides occurs in the gut, followed by their reassembly within intestinal cells, with the latter process facilitated by DGAT1 and DGAT2.<sup>151</sup> Serrano-Wu *et al.*<sup>152</sup> designed an intestinally restricted DGAT1 inhibitor. DGAT1 inhibitors are potential therapeutic agents for managing hyperlipidemia and non-alcoholic fatty liver disease.<sup>153,154</sup> The primary role of DGAT1 is to facilitate triglyceride synthesis by combining free fatty acids and glycerol in the intestine, resulting in triglyceride

formation. Subsequently, these triglycerides are enveloped by cholesteryl ester to form a complex, ultimately leading to the generation of cholesteryl ester-enriched chylomicrons that enter the circulation. DGAT1 inhibitors can inhibit this process. By incorporating a carboxylic acid group into compound 41 (Figure 6), this compound minimized plasma exposure through the action of ABC transporters, including P-gp. The compound demonstrated dose-dependent efficacy in reducing post-prandial triglyceride levels in mice through oral administration, as evidenced by liver tissue sections and related metabolic indicators. It also exhibited a significant inhibitory effect on the elevation of triglyceride levels during normal food intake. Moreover, it effectively suppressed hepatic lipid accumulation without affecting appetite or body weight in mice. Although the plasma concentration of compound 7 (Figure 1) remained lower than its biological (39 nM) and cellular titers (390 nM) throughout the post-prandial period *in vivo*, its pharmacological efficacy persisted for 6 h in dogs. No adverse effects were observed in vital sign monitoring, hematological analysis, or clinical chemistry assessments. These findings suggest that intestinal restriction strategies may enhance the safety profile of DGAT1 inhibitors.

#### 3.6.2. Salazosulfapyridine

Salazosulfapyridine (compound 42; Figure 6), a fundamental component in the treatment of IBD, such as UC, demonstrates restricted oral bioavailability as a result of its diminished intestinal absorption.<sup>155</sup> Dahan *et al.*<sup>156</sup> illustrated that efflux transport facilitated by MRP2/ABCC (ATP-Binding Cassette, Subfamily C) and BCRP decreases the intestinal permeability of sulfasalazine, thereby achieving its intestinal confinement. The impact of P-gp, MRP2, and BCRP inhibitors on the transmembrane permeability of sulfasalazine was investigated using Caco-2 cells. Both the MRP2 inhibitor (MK-571) and the BCRP inhibitor (fumitremorgin C) significantly enhanced the effective intestinal permeability coefficient of sulfasalazine. In contrast, P-gp inhibitors verapamil and quinidine had no effect on sulfasalazine's permeability. The

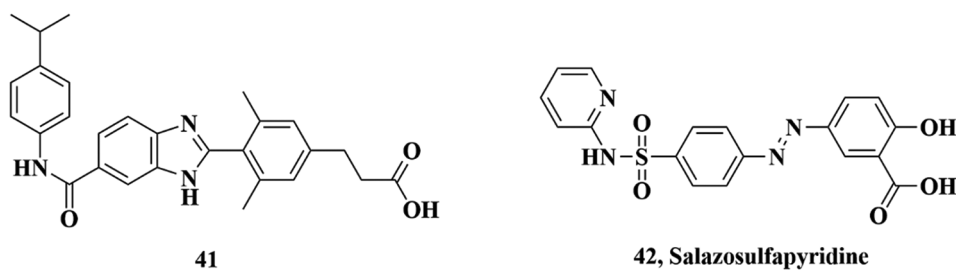


Figure 6. The chemical structure of ABC transporter substrates

findings of these studies suggest that the efflux transport facilitated by MRP2 and BCRP decreases the permeability of sulfasalazine, leading to its intestinal restriction and subsequent therapeutic benefits.

#### 4. Conclusion and future perspectives

At present, intestinally restricted drugs hold a significant place in the pharmacopeia and are widely used in treating a variety of diseases, especially diabetes, IBD, and cholestasis. In the study of these diseases, several receptors for intestinally restricted drugs have been identified. For example, in the treatment of diabetes, G-protein-coupled receptors TGR5 and GPR119 play vital roles. TGR5 restores insulin sensitivity and induces GPL-1 secretion, while GPR119 promotes satiety through various pathways.<sup>158</sup> In cholestasis treatment, FXR and PXR have emerged as essential targets. FXR inhibits BA synthesis, while PXR promotes cellular uptake of BAs.<sup>63,159</sup>

Although intestinally restricted drugs have been used in large numbers in clinical applications, oral formulations of these drugs remain a big challenge for scientists. The primary problem in developing this class of drugs lies in their unstable absorption within the gastrointestinal tract, which leads to highly variable plasma concentrations. In the future, further exploration should focus on the following points: (1) Long-term research should be expanded in developing intestinally restricted drugs and assessing their toxicity in clinical applications. (2) Management of complex intestinal physiology should be addressed, with a clearer understanding of the complex mechanisms of drug transmembrane transport to reduce systemic drug exposure.<sup>160</sup> (3) Safe dosage ranges for these drugs should be further defined, in terms of their concentrations and duration of action, to achieve the desired therapeutic effect while enhancing both efficiency and safety. (4) Methods to detect drug concentrations in the gut and accurately measure drug levels at the site of action should be improved.<sup>69</sup>

To solve the existing problems, scientists are committed to revolutionizing the pharmaceutical technology of intestinally restricted drugs, with the goal of advancing their development. Future advancements in pharmaceutical technology are expected to develop drugs that block the transit between the intestinal tract and the systemic circulation, significantly reducing systemic exposure and thus minimizing adverse side effects caused by systemic exposure during disease treatment. Intestinally restricted drugs are also expected to prevent disease spread to the surrounding area by inhibiting disease-causing factors, such as the receptors or complements, thereby confining the disease to its original site. These drugs are expected to

play an important role in treating and preventing cancer and other intestinal diseases by inhibiting the receptors or complements of disease-causing factors, thus limiting the disease spread. In the future, the integration of additional technologies may allow intestinally restricted drugs to continue providing safe and effective treatments for patients with unmet medical needs.

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#### Conflict of interest

The authors declare they have no competing interests.

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#### Ethics approval and consent to participate

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#### Consent for publication

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#### Availability of data

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