Zonulin Pathway as a Therapeutic Target

Subjects: Medicine, Research & Experimental

Contributor: Apor Veres-Székely , Csenge Szász , Domonkos Pap , Beáta Szebeni , Péter Bokrossy , Ádám Vannay

The integrity and thus the function of blood–brain barrier (BBB) TJs play a crucial role in the pathomechanism of neuroinflammatory and neurodegenerative diseases. Previously, it has been suggested that targeting different elements of the zonulin pathway, including actin filaments, TJs, or NF-κB, have potential therapeutic effects on CNS diseases. Indeed, encouraging results are accumulating from a recent preclinical study, using myosin light chain kinase (MLCK) inhibitor ML-7, which attenuates BBB disruption by preventing the disintegration of actin cytoskeletal microfilaments. Similarly, blocking the cleavage of TJ proteins by matrix metalloproteases (MMP) inhibitors, using either direct (broad-spectrum or selective MMP-2 and MMP-9) or indirect inhibitors (COX) has been shown to protect BBB. Peroxisome proliferator-activated receptor-γ (PPAR-γ) agonists, such as rosiglitazone, pioglitazone, or D-allose, also prevented BBB integrity by inhibiting NF-κB activation. Therefore, the use of zonulin inhibitors seems to be justified in the treatment of CNS diseases.

zonulin zonula occludens 1 microbiota gut brain dysbiosis

1. Human Studies with Larazotide Acetate

Over the past decade, larazotide acetate (also known as AT-1001), a pharmacological inhibitor of the zonulin pathway, has received increasing attention. Firstly, Wang et al. published a synthetic oligopeptide (GGVLVQPG) in 2000, representing an N'-terminal sequence of zonulin, which had a strong inhibitory effect on receptor binding of zonulin [1]. Since then, a large amount of knowledge has accumulated on this competitive zonulin inhibitor, demonstrating its strong effect on the regulation of TJs and making it one of the most promising therapeutic candidates for celiac disease [2]. Several interventional human studies have demonstrated good tolerability and beneficial effects of larazotide acetate on intestinal permeability (**Table1**).

Table 1. Human clinical studies investigating the therapeutic applicability of larazotide acetate.

Condition	Results	Study (Enrollment)	Clinical Trials Identifier	Ref.
Healthy	good tolerability	Phase I (24)	NCT00386490	[3]
Celiac disease, gluten-free diet	good tolerability	Phase Ib (21)	NCT00386165	[4]

Condition	Results	Study (Enrollment)	Clinical Trials Identifier	Ref.
Celiac disease, gluten challenge	improvement in GI symptoms, good tolerability	Phase IIa (80)	NCT00362856	[5][6] [7][8]
Celiac disease, gluten challenge	improvement in histological scores, good tolerability	Phase IIb (105)	NCT00620451	[9] [10]
Celiac disease, gluten challenge	improvement in GI symptoms, decreased level of anti-tTG IgA	Phase IIb (171)	NCT00492960	[11] [12]
Celiac disease, persistent symptoms with gluten-free diet	improvement in GI and extra-GI symptoms, good tolerability	Phase IIb (342)	NCT01396213	[13] [14]
Celiac disease, gluten-free diet	(terminated based on interim analysis)	Phase III (307)	NCT03569007	[15] [16]
COVID19—MIS-C	improvement in clinical symptoms, decreased level of inflammatory markers and SARS-CoV-2 nucleocapsid (N) protein	case report (1)		[17]
COVID19—MIS-C	improvement in GI symptoms, decreased level of SARS-CoV-2 Spike (S) protein	case series (4)		[18]
COVID19—MIS-C	(not completed)	Phase IIa (20)	NCT05022303	[19]

Abbreviations: Ref.: reference, GI: gastrointestinal, MIS-C: Multisystem Inflammatory Syndrome in Children.

aised the echanism umber of of MIS-C by reducing gastrointestinal symptoms and the severity of systemic inflammation (**Table 1**) [17][18]. Now, its efficacy is under investigation in a phase II, randomized, double-blind, placebo-controlled clinical trial in patients with MIS-C [19]. In addition, the potential use of larazotide acetate in the treatment of metabolic diseases, including insulin resistance, diabetes mellitus, or non-alcoholic fatty liver disease (NAFLD), as well as to improve glucose and lipid metabolism of patients, has been hypothesized [20].

2. Preclinical Studies with Larazotide Acetate

Recently, human and basic research studies have revealed that high zonulin levels may affect the permeability of not only the intestine but also of other organs. Therefore, numerous preclinical studies have aimed to investigate the efficacy of larazotide acetate in experimental animal models of various diseases. Briefly, treatment with larazotide acetate has been shown to improve epithelial barrier function, thereby attenuating the severity of the investigated disorders, including colitis, vasculitis, fibrosis, arthritis, and respiratory or liver diseases (**Table 2**).

Table 2. Preclinical animal studies investigating the therapeutic applicability of larazotide acetate.

Model	Species	Administration	Daily Dose	Results	Ref.
celiac disease	gliadin-sensitized HLA-	p.o. gavage	0.25 mg	reduced intestinal permeability and	[21]

Model	Species	Administration	Daily Dose	Results	Ref.
	HCD4/DQ8 transgenic mouse			macrophage infiltration	
		p.o. gavage	0.3 mg	reduced intestinal permeability	[22]
intestinal permeability	*Il10*$^{-/-}$ mouse	p.o. gavage	5 mg	reduced intestinal permeability and inflammation	[23]
spontaneous colitis		p.o. in drinking water	0.1 or 1 mg/mL	reduced intestinal permeability and inflammation	[24]
DSS induced colitis	zonulin transgenic mouse	p.o. in drinking water	1 mg/mL	reduced intestinal permeability	[25]
radiation-induced enteropathy	mouse	i.p.	0.25 mg	improved clinical state and histological scores, inhibited bacterial translocation, elevated TJ protein levels	[26]
healthy (pharmacokinetics)	pig	p.o. capsule	0.05 mg/kg	determining pharmacokinetics of larazotide acetate in the small intestine	[27]
Ruminococcus blautia gnavus colonization	germ-free mouse	p.o. in drinking water	0.15 mg/mL	reduced intestinal permeability	[28]
spontaneous T1D	BB diabetic-prone rat	p.o. in drinking water	0.01 mg/mL	inhibited development of diabetes	[29]
rheumatoid arthritis	mouse	p.o. in drinking water	0.15 mg/mL	attenuated arthritis	[30]
	Il10ra$^{-/-}$ mouse, *Cldn8*$^{-/-}$ mouse	p.o. gavage	2 × 0.05 mg	reduced intestinal permeability, inflammation, and joint swelling	[31]
vasculitis	mouse	i.p.	0.5 mg	reduced intestinal permeability and LPS translocation, prevented cardiovascular lesions	[32]
LPS-induced acute lung injury		i.t.	0.05 mg	reduced severity, decreased inflammatory markers	[33]

Model	Species	Administration	Daily Dose	Results	Ref.
		i.v.	0.01 or 0.025 or 0.05 mg		
influenza		i.v.	0.15 mg	reduced severity of acute lung injury	[34]
salivary gland fibrosis		i.p.	5 mg/kg	improved epithelial barrier function, ameliorated fibrosis	[35]
NAFLD		p.o. in drinking water	0.1 or 1 mg/mL	reduced intestinal permeability	[36]
		p.o. gavage	2 × 0.03 or 2 × 0.3 mg		
acute liver failure	rat	p.o. in drinking water	0.01 mg/mL	decreased intestinal damage	[37]
		p.o. gavage	2 × 0.03 mg		

originally created [2] e primary [38] hallenging intestinal e phase II below the quantification limit (0.5 ng/mL) even after 7 or 14 days of daily treatment [5]. Therefore, no systemic effect should be expected after the per os treatment with larazotide acetate. At the same time, as shown in **Table 2**, summarizing the different methods of administration, intratracheal, intravenous, or intraperitoneal administration of larazotide acetate produced beneficial effects.

Abbreviations: Ref.: reference; p.o.: per os; i.p.: intraperitoneal; i.v. intravenous; DSS: dextran sulphate sodium; TJ: tight junction; T1D: type 1 diabetes; LPS: lipopolysaccharide; NAFLD: non-alcoholic fatty liver disease.

The original drug has to undergo further pharmacological development for extraintestinal use. Recent studies have reported that modification of larazotide acetate or its derivates has improved lipophilicity and intestinal absorption [39][40][41]. The resulting compound retained the biological activity of larazotide acetate and was detectable (20–30 ng/mL) in the plasma of mice after a single per os administration [41].

Besides larazotide acetate, there is another synthetic zonulin-related peptide fragment known as AT-1002, which, unlike larazotide acetate (AT-1001), has proved to be an agonist of zonulin receptors. Indeed, treatment of epithelial or endothelial cells with AT-1002 led to increased permeability by reversible opening of TJs [42][43]. Since its discovery, AT-1002 has become an important permeability-modulating component in drug development that can be used to increase the absorption and distribution of other drugs [42][44]. Several studies showed that AT-1002 can be used to increase intestinal, intranasal, intratracheal, or transdermal penetration of various compounds improving their bioavailability [45].

These preclinical data suggest that larazotide acetate or other zonulin receptor modulators (by choosing the appropriate route of administration) may prevent BBB integrity and should be investigated in CNS-related diseases, as well.

4. Other Receptor Modulators

Although binding to zonulin receptors, including PAR_2 and EGFR, leads to the disruption of TJs, literary data on modulation of PAR_2 and EGFR by inhibitors other than larazotide acetate are confusing (**Table 3**).

Recently, it has been shown that, in contrast to larazotide acetate, peptidic antagonists of PAR_2, including FSLLRY-NH_2 or SLIGRL-NH_2, decreased the expression of ZO-1 and claudin-1 and destroyed the barrier function of nasal epithelial cells [46]. Similarly, a small molecule antagonist, GB83, exerted harmful effects on colon epithelial cells by decreasing the expression of autophagy- and TJ-related factors and increased permeability [47]. In contrast, inhibition of the PAR_2 pathway by GB88 in lung epithelial cells [48] or using I-191 in arterial endothelial cells [49] moderated actin rearrangement and TJ disruption and reduced the permeability of the cellular monolayers. Moreover, a non-peptidic PAR_2 ligand, the full agonist AC-55541, ameliorated the IL-17-induced loss of epithelial resistance in brain microvascular endothelial cells [50].

The EGFR tyrosine kinase inhibitor AG1478 also prevented TJ disassembly and epithelial resistance impairment in microvascular endothelial cells modeling BBB [51], in lung epithelial-like cells [52], and in oral epithelial tumour cells [53]. In contrast, decreased expression of TJs, barrier dysfunction, and increased permeability were induced by other EGFR tyrosine kinase inhibitors, such as erlotinib [54], gefitinib, icotinib [55], or dacomitinib [56][57] in intestinal epithelial cells. Similar effects were found in other cell types after treatment with lapatinib [58] or vandetanib [59]. These studies suggest that these compounds have a significant impact on the complex signaling pathway of EGFR, triggering stress responses, and finally leading to cell death [55]. This phenomenon may be the underlying molecular mechanism of diarrhea, which is one of the most frequent side effects of second-generation EGFR inhibitors [60].

All these data together suggest that PAR_2 or EGFR modulators could be used to regulate epithelial or endothelial barrier function, considering that the applied drug should affect the PPI-DAG-PKC pathway, which plays a central role in zonulin-induced TJ disruption, but not ERK, JNK, or Akt signaling, which are essential for the physiological regulation of basic cellular processes, including cell growth, survival, proliferation, and apoptosis [61].

Table 3. Effect of PAR_2 and EGFR modulators on TJ integrity and/or transcellular permeability of epithelial or endothelial cells based on literary data.

Target	Type	Compound	Cell Line	Effect on TJs and/or Transcellular Permeability	Ref.
PAR_2	peptidic antagonist	FSLLRY-NH_2	pHNECs	harmful	[46]
		SLIGRL-NH_2			
	non-peptidic full agonist	AC-55541	hBMECs	protective	[50]
	small molecule antagonist	GB88	A549		[48]

Target	Type	Compound	Cell Line	Effect on TJs and/or Transcellular Permeability	Ref.
			hECs		[49]
		GB83	Caco2	harmful	[47]
			hCMEC/D3		[51]
		AG1478	Calu-3	protective	[52]
			HSC-3		[53]
		erlotinib			[54]
EGFR	tyrosine kinase inhibitor	gefitinib	IEC-6		[55]
		icotinib		harmful	
		dacomitinib	T84		[57]
		lapatinib	HBCCs		[58]
		vandetanib	Calu-6		[59]

Abbreviations: pHNECs: primary human nasal epithelial cells; hBMECs: human brain microvascular endothelial cells; hECS: primary human arterial endothelial cells; hCMEC: primary human cardiac microvascular endothelial cells; HBBCs: primary human breast cancer epithelial cells.

1. Wang, W.; Uzzau, S.; Goldblum, S.E.; Fasano, A. Human zonulin, a potential modulator of intestinal tight junctions. J. Cell Sci. 2000, 113, 4435–4440.
2. Slifer, Z.M.; Krishnan, B.R.; Madan, J.; Blikslager, A.T. Larazotide acetate: A pharmacological peptide approach to tight junction regulation. Am. J. Physiol. Liver Physiol. 2021, 320, G983–G989.
3. Safety of Larazotide Acetate in Healthy Volunteers. Available online: https://clinicaltrials.gov/ct2/show/NCT00386490 (accessed on 17 March 2023).
4. Safety Study of Larazotide Acetate to Treat Celiac Disease. Available online: https://clinicaltrials.gov/ct2/show/NCT00386165f (accessed on 17 March 2023).
5. A Leffler, D.; Kelly, C.P.; Abdallah, H.Z.; Colatrella, A.M.; A Harris, L.; Leon, F.; A Arterburn, L.; Paterson, B.M.; Lan, Z.H.; Murray, J. A Randomized, Double-Blind Study of Larazotide Acetate to Prevent the Activation of Celiac Disease During Gluten Challenge. Am. J. Gastroenterol. 2012, 107, 1554–1562.
6. Paterson, B.M.; Lammers, K.M.; Arrieta, M.C.; Fasano, A.; Meddings, J.B. The safety, tolerance, pharmacokinetic and pharmacodynamic effects of single doses of AT-1001 in coeliac disease subjects: A proof of concept study. Aliment. Pharmacol. Ther. 2007, 26, 757–766.
7. Fasano, A.; Paterson, B. Materials and Methods for the Treatment of Celiac Disease. U.S. Patent 8034776B2, 11 November 2011.
8. Safety and Tolerability Study of Larazotide Acetate in Celiac Disease Subjects. Available online: https://clinicaltrials.gov/ct2/show/NCT00362856 (accessed on 17 March 2023).

9. Pérez, L.C.; León, F. Clinical trial data provides hope for attenuation of mucosal injury in coeliac disease. Eur. J. Intern. Med. 2012, 23, e77.
10. Randomized, Double-Blind, Placebo-Controlled Study of Larazotide Acetate in Subjects with Active Celiac Disease. Available online: https://clinicaltrials.gov/ct2/show/NCT00620451 (accessed on 17 March 2023).
11. Kelly, C.P.; Green, P.H.R.; Murray, J.A.; Dimarino, A.; Colatrella, A.; Leffler, D.A.; Alexander, T.; Arsenescu, R.; Leon, F.; Jiang, J.G.; et al. Larazotide acetate in patients with coeliac disease undergoing a gluten challenge: A randomised placebo-controlled study. Aliment. Pharmacol. Ther. 2013, 37, 252–262.
12. Study to Assess the Efficacy of Larazotide Acetate for the Treatment of Celiac Disease. Available online: https://www.clinicaltrials.gov/ct2/show/NCT00492960 (accessed on 17 March 2023).
13. Leffler, D.A.; Kelly, C.P.; Green, P.H.; Fedorak, R.; DiMarino, A.; Perrow, W.; Rasmussen, H.; Wang, C.; Bercik, P.; Bachir, N.M.; et al. Larazotide Acetate for Persistent Symptoms of Celiac Disease Despite a Gluten-Free Diet: A Randomized Controlled Trial. Gastroenterology 2015, 148, 1311–1319.e6.
14. A Double-blind Placebo-controlled Study to Evaluate Larazotide Acetate for the Treatment of Celiac Disease. Available online: https://www.clinicaltrials.gov/ct2/show/NCT01396213 (accessed on 17 March 2023).
15. Study to Evaluate the Efficacy and Safety of Larazotide Acetate for the Relief of CeD Symptoms. Available online: https://clinicaltrials.gov/ct2/show/NCT03569007 (accessed on 17 March 2023).
16. Machado, M.V. New Developments in Celiac Disease Treatment. Int. J. Mol. Sci. 2023, 24, 945.
17. Yonker, L.M.; Gilboa, T.; Ogata, A.F.; Senussi, Y.; Lazarovits, R.; Boribong, B.P.; Bartsch, Y.C.; Loiselle, M.; Rivas, M.N.; Porritt, R.A.; et al. Multisystem inflammatory syndrome in children is driven by zonulin-dependent loss of gut mucosal barrier. J. Clin. Investig. 2021, 131, e149633.
18. Yonker, L.M.; Swank, Z.; Gilboa, T.; Senussi, Y.M.; Kenyon, V.B.; Papadakis, L.B.; Boribong, B.P.; Carroll, R.W.M.; Walt, D.R.; Fasano, A. Zonulin Antagonist, Larazotide (AT1001), As an Adjuvant Treatment for Multisystem Inflammatory Syndrome in Children: A Case Series. Crit. Care Explor. 2022, 10, e0641.
19. AT1001 for the Treatment of COVID-19 Related MIS-C. Available online: https://clinicaltrials.gov/ct2/show/NCT05022303 (accessed on 17 March 2023).
20. Al Refaei, A. Larazotide acetate as a preventive and therapeutic pharmacotherapy in obesity and metabolic syndrome. Med. Hypotheses 2022, 167, 110940.
21. Gopalakrishnan, S.; Durai, M.; Kitchens, K.; Tamiz, A.P.; Somerville, R.; Ginski, M.; Paterson, B.M.; Murray, J.A.; Verdu, E.F.; Alkan, S.S.; et al. Larazotide acetate regulates epithelial tight

junctions in vitro and in vivo. Peptides 2012, 35, 86–94.

22. Silva, M.A.; Jury, J.; Sanz, Y.; Wiepjes, M.; Huang, X.; Murray, J.; David, C.S.; Fasano, A.; Verdú, E.F. Increased Bacterial Translocation in Gluten-Sensitive Mice Is Independent of Small Intestinal Paracellular Permeability Defect. Dig. Dis. Sci. 2012, 57, 38–47.
23. Liu, Z.; Shen, T.; Chen, H.; Zhou, Y.; Zhang, P.; Ma, Y.; Moyer, M.P.; Zhang, M.; Chu, Z.; Qin, H. Functional characterization of MIMP for its adhesion to the intestinal epithelium. Front. Biosci. 2011, 16, 2106–2127.
24. Arrieta, M.C.; Madsen, K.; Doyle, J.; Meddings, J. Reducing small intestinal permeability attenuates colitis in the IL10 gene-deficient mouse. Gut 2008, 58, 41–48.
25. Sturgeon, C.; Lan, J.; Fasano, A. Zonulin transgenic mice show altered gut permeability and increased morbidity/mortality in the DSS colitis model. Ann. N. Y. Acad. Sci. 2017, 1397, 130–142.
26. Kwak, S.Y.; Jang, W.I.; Park, S.; Cho, S.S.; Lee, S.B.; Kim, M.-J.; Park, S.; Shim, S.; Jang, H. Metallothionein 2 activation by pravastatin reinforces epithelial integrity and ameliorates radiation-induced enteropathy. Ebiomedicine 2021, 73, 103641.
27. Enomoto, H.; Yeatts, J.; Carbajal, L.; Krishnan, B.R.; Madan, J.P.; Laumas, S.; Blikslager, A.T.; Messenger, K.M. In vivo assessment of a delayed release formulation of larazotide acetate indicated for celiac disease using a porcine model. PLoS ONE 2021, 16, e0249179.
28. Deng, J.; Azzouz, D.F.; Ferstler, N.; Silverman, G.J.J.b. Sex-dependent Lupus Ruminococcus blautia gnavus strain induction of zonulin-mediated intestinal permeability and autoimmunity. Front. Immunol. 2022, 13, 897971.
29. Watts, T.; Berti, I.; Sapone, A.; Gerarduzzi, T.; Not, T.; Zielke, R.; Fasano, A. Role of the intestinal tight junction modulator zonulin in the pathogenesis of type I diabetes in BB diabetic-prone rats. Proc. Natl. Acad. Sci. USA 2005, 102, 2916–2921.
30. Tajik, N.; Frech, M.; Schulz, O.; Schälter, F.; Lucas, S.; Azizov, V.; Dürholz, K.; Steffen, F.; Omata, Y.; Rings, A.; et al. Targeting zonulin and intestinal epithelial barrier function to prevent onset of arthritis. Nat. Commun. 2020, 11, 1995.
31. Matei, D.E.; Menon, M.; Alber, D.G.; Smith, A.M.; Nedjat-Shokouhi, B.; Fasano, A.; Magill, L.; Duhlin, A.; Bitoun, S.; Gleizes, A.; et al. Intestinal barrier dysfunction plays an integral role in arthritis pathology and can be targeted to ameliorate disease. Med 2021, 2, 864–883.e9.
32. Rivas, M.N.; Wakita, D.; Franklin, M.K.; Carvalho, T.T.; Abolhesn, A.; Gomez, A.C.; Fishbein, M.C.; Chen, S.; Lehman, T.J.; Sato, K.; et al. Intestinal Permeability and IgA Provoke Immune Vasculitis Linked to Cardiovascular Inflammation. Immunity 2019, 51, 508–521.e6.
33. Rittirsch, D.; Flierl, M.A.; Nadeau, B.A.; Day, D.E.; Huber-Lang, M.S.; Grailer, J.J.; Zetoune, F.S.; Andjelkovic, A.V.; Fasano, A.; Ward, P.A. Zonulin as prehaptoglobin2 regulates lung permeability

and activates the complement system. Am. J. Physiol. Cell Mol. Physiol. 2013, 304, L863–L872.

34. Shirey, K.; Lai, W.; Patel, M.; Pletneva, L.; Pang, C.; Kurt-Jones, E.; Lipsky, M.; Roger, T.; Calandra, T.; Tracey, K.; et al. Novel strategies for targeting innate immune responses to influenza. Mucosal Immunol. 2016, 9, 1173–1182.
35. Mao, X.; Min, S.; Zhu, M.; He, L.; Zhang, Y.; Li, J.; Tian, Y.; Yu, G.; Wu, L.; Cong, X. The Role of Endothelial Barrier Function in the Fibrosis of Salivary Gland. J. Dent. Res. 2022, 102, 82–92.
36. Caffrey, R.; Marioneaux, J.; Bhat, M.; Prior, C.; Madan, J.; Laumas, S.; Sanyal, A. FRI-267-Serial measurement of serum dextran absorption by novel competition ELISA demonstrates larazotide acetate significantly improves “leaky gut” in a Western diet murine model of metabolic liver disease. J. Hepatol. 2019, 70, e511–e512.
37. Caliskan, A.R.; Gul, M.; Yılmaz, I.; Otlu, B.; Uremis, N.; Uremis, M.M.; Kilicaslan, I.; Gul, S.; Tikici, D.; Saglam, O.; et al. Effects of larazotide acetate, a tight junction regulator, on the liver and intestinal damage in acute liver failure in rats. Hum. Exp. Toxicol. 2021, 40, S693–S701.
38. Haddadzadegan, S.; Dorkoosh, F.; Bernkop-Schnürch, A. Oral delivery of therapeutic peptides and proteins: Technology landscape of lipid-based nanocarriers. Adv. Drug Deliv. Rev. 2022, 182, 114097.
39. Di Micco, S.; Musella, S.; Sala, M.; Scala, M.C.; Andrei, G.; Snoeck, R.; Bifulco, G.; Campiglia, P.; Fasano, A. Peptide derivatives of the zonulin inhibitor larazotide (AT1001) as potential anti SARS-CoV-2: Molecular modelling, synthesis and bioactivity evaluation. Int. J. Mol. Sci. 2021, 22, 9427.
40. Di Micco, S.; Musella, S.; Scala, M.C.; Sala, M.; Campiglia, P.; Bifulco, G.; Fasano, A. In silico analysis revealed potential anti-SARS-CoV-2 main protease activity by the zonulin inhibitor larazotide acetate. Front. Chem. 2021, 8, 628609.
41. Di Micco, S.; Rahimova, R.; Sala, M.; Scala, M.C.; Vivenzio, G.; Musella, S.; Andrei, G.; Remans, K.; Mammri, L.; Snoeck, R. Rational design of the zonulin inhibitor AT1001 derivatives as potential anti SARS-CoV-2. Eur. J. Med. Chem. 2022, 244, 114857.
42. Motlekar, N.A.; Fasano, A.; Wachtel, M.S.; Youan, B.-B.C. Zonula occludens toxin synthetic peptide derivative AT1002 enhances in vitro and in vivo intestinal absorption of low molecular weight heparin. J. Drug Target. 2006, 14, 321.
43. Li, M.; Oliver, E.; Kitchens, K.M.; Vere, J.; Alkan, S.S.; Tamiz, A.P. Structure–activity relationship studies of permeability modulating peptide AT-1002. Bioorganic Med. Chem. Lett. 2008, 18, 4584–4586.
44. Ding, R.; Zhao, Z.; He, J.; Tao, Y.; Zhang, H.; Yuan, R.; Sun, K.; Shi, Y. Preparation, Drug Distribution, and In Vivo Evaluation of the Safety of Protein Corona Liposomes for Liraglutide Delivery. Nanomaterials 2023, 13, 540.

45. Brunner, J.; Ragupathy, S.; Borchard, G. Target specific tight junction modulators. Adv. Drug Deliv. Rev. 2021, 171, 266–288.
46. Wang, J.; Kang, X.; Zhi-Qun, H.; Shen, L.; Qing, L.; Meng-Yue, L.; Li-Ping, L.; Jun-Hao, Y.; Mei, H.; Ye, J. Protease-activated receptor-2 decreased zonula occlidens-1 and claudin-1 expression and induced epithelial barrier dysfunction in allergic rhinitis. Am. J. Rhinol. Allergy 2021, 35, 26–35.
47. Kim, Y.; Lee, Y.; Heo, G.; Jeong, S.; Park, S.; Yoo, J.-W.; Jung, Y.; Im, E. Modulation of Intestinal Epithelial Permeability via Protease-Activated Receptor-2-Induced Autophagy. Cells 2022, 11, 878.
48. Wang, Y.-J.; Yu, S.-J.; Tsai, J.-J.; Yu, C.-H.; Liao, E.-C. Antagonism of Protease Activated Receptor-2 by GB88 Reduces Inflammation Triggered by Protease Allergen Tyr-p3. Front. Immunol. 2021, 12, 557433.
49. Ushakumari, C.J.; Zhou, Q.L.; Wang, Y.-H.; Na, S.; Rigor, M.C.; Zhou, C.Y.; Kroll, M.K.; Lin, B.D.; Jiang, Z.Y. Neutrophil Elastase Increases Vascular Permeability and Leukocyte Transmigration in Cultured Endothelial Cells and Obese Mice. Cells 2022, 11, 2288.
50. Xu, B.; Chen, J.; Fu, J.; Yang, R.; Yang, B.; Huo, D.; Tan, C.; Chen, H.; Wang, X. Meningitic Escherichia coli-Induced Interleukin-17A Facilitates Blood–Brain Barrier Disruption via Inhibiting Proteinase 3/Protease-Activated Receptor 2 Axis. Front. Cell Neurosci. 2022, 16, 814867.
51. Liu, W.; Wang, P.; Shang, C.; Chen, L.; Cai, H.; Ma, J.; Yao, Y.; Shang, X.; Xue, Y. Endophilin-1 regulates blood–brain barrier permeability by controlling ZO-1 and occludin expression via the EGFR–ERK1/2 pathway. Brain Res. 2014, 1573, 17–26.
52. Petecchia, L.; Sabatini, F.; Usai, C.; Caci, E.; Varesio, L.; Rossi, G.A. Cytokines induce tight junction disassembly in airway cells via an EGFR-dependent MAPK/ERK1/2-pathway. Lab. Investig. 2012, 92, 1140–1148.
53. Kakei, Y.; Teraoka, S.; Akashi, M.; Hasegawa, T.; Komori, T. Changes in cell junctions induced by inhibition of epidermal growth factor receptor in oral squamous cell carcinoma cells. Exp. Ther. Med. 2017, 14, 953–960.
54. Fan, L.; Hu, L.; Yang, B.; Fang, X.; Gao, Z.; Li, W.; Sun, Y.; Shen, Y.; Wu, X.; Shu, Y.; et al. Erlotinib promotes endoplasmic reticulum stress-mediated injury in the intestinal epithelium. Toxicol. Appl. Pharmacol. 2014, 278, 45–52.
55. Hong, S.; Gu, Y.; Gao, Z.; Guo, L.; Guo, W.; Wu, X.; Shen, Y.; Sun, Y.; Wu, X.; Xu, Q. EGFR inhibitor-driven endoplasmic reticulum stress-mediated injury on intestinal epithelial cells. Life Sci. 2014, 119, 28–33.
56. Van Sebille, Y.Z.; Gibson, R.J.; Wardill, H.R.; Ball, I.A.; Keefe, D.M.; Bowen, J.M. Dacomitinib-induced diarrhea: Targeting chloride secretion with crofelemer. Int. J. Cancer 2018, 142, 369–380.

57. Van Sebille, Y.Z.; Gibson, R.J.; Wardill, H.R.; Secombe, K.R.; Ball, I.A.; Keefe, D.M.; Finnie, J.W.; Bowen, J.M. Dacomitinib-induced diarrhoea is associated with altered gastrointestinal permeability and disruption in ileal histology in rats. Int. J. Cancer 2017, 140, 2820–2829.
58. Leech, A.O.; Vellanki, S.H.; Rutherford, E.J.; Keogh, A.; Jahns, H.; Hudson, L.; O'donovan, N.; Sabri, S.; Abdulkarim, B.; Sheehan, K.M.; et al. Cleavage of the extracellular domain of junctional adhesion molecule-A is associated with resistance to anti-HER2 therapies in breast cancer settings. Breast Cancer Res. 2018, 20, 140.
59. Zhou, Y.; Zhang, Y.; Zou, H.; Cai, N.; Chen, X.; Xu, L.; Kong, X.; Liu, P. The multi-targeted tyrosine kinase inhibitor vandetanib plays a bifunctional role in non-small cell lung cancer cells. Sci. Rep. 2015, 5, 8629.
60. Hirsh, V.; Blais, N.; Burkes, R.; Verma, S.; Croitoru, K. Management of Diarrhea Induced by Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitors. Curr. Oncol. 2014, 21, 329–336.
61. Oda, K.; Matsuoka, Y.; Funahashi, A.; Kitano, H. A comprehensive pathway map of epidermal growth factor receptor signaling. Mol. Syst. Biol. 2005, 1, 2005-0010.