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Review

Carrier-Mediated Delivery of Low-Molecular-Weight *N*-Containing Drugs across the Blood–Brain Barrier or the Blood–Retinal Barrier Using the Proton-Coupled Organic Cation Antiporter

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Abstract: While it is true that pharmacotherapy has achieved desired health outcomes, significant unmet medical needs persist in the field of central nervous system (CNS) drugs, particularly for neurodegenerative diseases such as Alzheimer's disease, as well as ocular diseases such as diabetic retinopathy and age-related macular degeneration. Drugs cannot enter the brain from the bloodstream due to the presence of the blood–brain barrier (BBB). Similarly, they cannot enter the eyes from the bloodstream due to the blood–retina barrier (BRB), which is composed of the endothelium or the epithelium. Thus, innovative drug delivery systems that can overcome these barriers based on efflux transporters, hydrophobic lipid bilayer membranes, and tight junctions should be developed using patient-friendly techniques distinct from craniotomy procedures or intravitreal injections. Brain-penetrating CNS drugs and antihistamine drugs commonly share *N*-containing groups. These findings suggest that certain types of cation transporters are involved in their transportation across the cell membrane. Indeed, the proton-coupled organic cation (H^+/OC) antiporter, whose specific characteristics remain unidentified, is responsible for transporting compounds with *N*-containing groups, such as clonidine and pyrilamine, at the BBB, and likely at the BRB as well. Therefore, well-designed low-molecular-weight drugs containing *N*-containing groups as transporter recognition units can enter the brain or the eyes through carrier-mediated transport. In this perspective review, I introduce the implementation and potential of H^+/OC antiporter-mediated transport across the endothelium at the BBB or the BRB using drugs consciously designed with *N*-containing groups as their substrates.

Keywords: the blood–brain barrier; the blood–retina barrier; drug delivery system; transmembrane drug delivery; the proton-coupled organic cation antiporter; carrier-mediated transport

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1. Introduction

In drug discovery and development, cell membrane impermeability poses a significant challenge. Central nervous system (CNS) drugs face difficulties in entering the brain from the circulating blood due to the blood–brain barrier (BBB). Indeed, clinical trials for CNS drugs targeting Alzheimer's disease (AD) have frequently resulted in failure [1]. Moreover, eye drugs administered orally or intravenously face challenges in entering the retina from the circulating blood due to the blood–retina barrier (BRB). Therefore, drugs for treating retinopathy in diabetes or age-related macular degeneration encounter difficulties in reaching the retina due to the presence of the BRB [2]. While drug administration through craniotomy procedures or intravitreal injections is technically feasible, it can cause significant stress and discomfort for patients. Hence, alternative approaches need to be developed. In general, drugs are categorized into low-molecular compounds (molecular weight (MW) < approx. 500), high-molecular compounds (MW > approx. 3000), and middle-molecular compounds (MW approx. 500–approx. 3000) [3]. Carrier-mediated transport

can serve as a solution for enabling low-molecular-weight drugs to traverse the barriers, because a variety of transporters with substrate specificity are expressed at the BBB or the BRB [Figure 1] [4–6]. However, high-molecular-weight drugs, such as monoclonal antibody drugs, cannot penetrate the narrow pores of transporters due to their size. The delivery of high-molecular-weight drugs into cells must utilize other strategies, such as receptor-mediated endocytosis, macropinocytosis, or membrane disruption [3,7–10]. Receptor-mediated transcytosis, using antibodies that target receptors such as the transferrin receptor or insulin receptor on the surface of capillary endothelial cells, is a relatively common approach for delivering drugs into the brain across the BBB. Antibody-drug conjugates can exhibit highly selective delivery into the brain. Nonetheless, low-molecular-weight drugs offer the advantage of being easy to manufacture, handle, and preserve. Regarding biomedicines such as antibody-drug conjugates, reproducing the exact same product is difficult due to post-translational modifications such as sugar chains and conjugation sites linked to drugs. These modifications are biologically produced and introduced in a probabilistic manner. Biosimilars have entered the market as identical copies of the original biomedicines. As the name suggests, biosimilars are not identical to their original biopharmaceuticals, such as antibody drugs, but they are highly comparable to them. Furthermore, they are sometimes not identical to each other between different batches [11]. Moreover, antibody drugs must be stored at a low temperature when preserved for more than one week until they are used. In these aspects, low-molecular-weight drugs are superior. In this perspective review, I introduce the delivery of low-molecular-weight *N*-containing drugs across the BBB or the BRB through carrier-mediated transport, utilizing the proton-coupled organic cation (H^+/OC) antiporter [Figure 2].

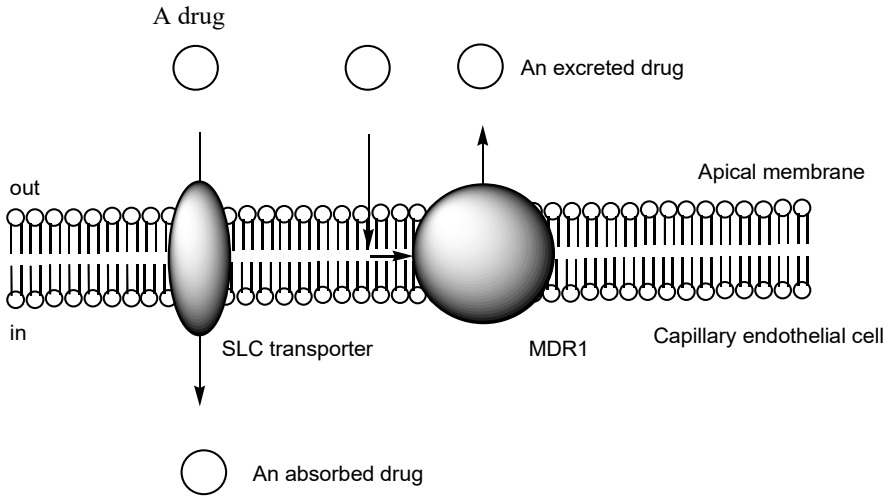

Figure 1. The passage of drugs absorbed by SLC transporters or excreted by MDR1 commonly at the blood–brain barrier (BBB) in the brain and the inner blood–retina barrier (BRB) in the eyes. Additionally, they interact with SLC transporters or MDR1 at the epithelial cell membrane in the small intestine and cancer cell membranes. MDR1 captures drugs that are in the process of passing through the lipid membrane via passive diffusion and expels them to the outside. SLC transporters are expressed in a tissue-specific manner.

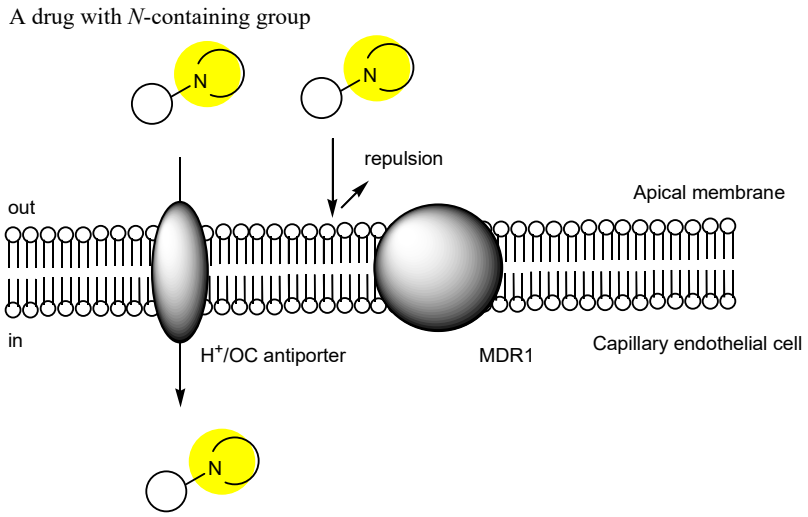

Figure 2. The absorptive passage of drugs with *N*-containing groups as the transporter recognition unit is mediated by the H^+/OC antiporter. In general, amines such as memantine carry a positive charge under physiological pH conditions in the bloodstream. Charged compounds face difficulty in penetrating the lipid membrane through passive diffusion.

2. Discussion

2.1. Transporter-Conscious Drug Design

Transporters [12] are membrane transport proteins that absorb or excrete materials across the cell membrane through homeostatic mechanisms. They play a pharmacokinetic role in absorption, distribution, metabolism, and excretion (ADME). Efflux transporters, such as the ATP-binding cassette (ABC) transporters, eliminate waste or hydrophobic toxic materials from cells [13]. On the other hand, transporters that mediate facilitated diffusion, such as the solute carrier (SLC) transporters [Table 1] [14], absorb water-soluble nutritive materials into cells or transport them out of cells to the tissues in need. Representative SLC transporters include peptide transporters, amino acid transporters, organic anion transporters (OCTs), and glucose transporters, reflecting their respective substrates. In general, peptide transporters carry peptides as substrates, while amino acid transporters carry amino acids as substrates. It is true that some substrates may be recognized by multiple transporters simultaneously. However, transporters named after their substrates generally do not transport compounds other than those substrates. Accordingly, SLC transporters recognize the structures of their substrates during transportation, although they may demonstrate relaxed substrate specificity in some cases. Thus, compounds that mimic the structure of various transporter substrates can also be transported by those respective transporters. Transporter-conscious drug design is a promising strategy for drug delivery [3,4]. Mechanically, SLC transporters are categorized into uniporters, symporters, and antiporters based on their material transport mechanisms. The transport mechanisms of SLC transporters, driven by concentration gradient energy, have not been completely elucidated yet, primarily due to the challenging analysis of temporal dynamic changes in microstructure. However, X-ray crystal structures of SLC transporter protein-substrate complexes have revealed binding modes at a molecular level, both in an inward-open state [15] and in an outward-open state [16], consistent with an alternating access mechanism [17]. These findings regarding the interaction between the binding sites of various transporters and the transporter recognition units of corresponding substrates are beneficial for transporter-conscious drug design. Computational calculations can suggest transport mechanisms. The structures of designed drugs need to be refined and optimized through iterative transport experiments conducted in vitro and in vivo.

Table 1. Representative solute carrier (SLC) transporters.

#	Categories	Transporters/Subtypes	Substrates
(1)	Amine transporters	The proton-coupled organic cation (H^+/OC) antiporter, organic cation transporter novel type 1 (OCTN1), OCTN2, OCTN3, multidrug and toxin extrusion protein 1 (MATE1), MATE2, MATE3, plasma membrane monoamine transporter (PMAT)	Cationic amine compounds
(2)	Peptide transporters	Peptide transporter 1 (PEPT1), PEPT2	Peptides
(3)	Amino acid transporters	L-type amino acid transporter 1 (LAT1), LAT2, LAT3, LAT4	Amino acids
(4)	Organic cation transporters (OCTs)	OCT1, OCT2, OCT3, OCT4	Cationic compounds
(5)	Organic anion transporters (OATs)	OAT1, OAT2, OAT3, OAT4, OAT5, organic anion transporting peptides (OATP1A2), OATP1B1, OATP1B3, OATP1C1 OATP2A1, OATP2B1, OATP3A1, OATP4A1, OATP4C1, OATP5A1, OATP6A1	Anionic compounds
(6)	Glucose transporters	Glucose transporter1 (GLUT1), GLUT2, GLUT3, GLUT4, GLUT5, GLUT6, GLUT7	Glucose

2.2. The BBB

It is a well-known fact that compounds generally cannot penetrate the brain from the circulating blood due to the presence of the BBB [18]. The BBB is composed of (i) a biological barrier based on excretion by MDR1 (P-glycoprotein), a representative ABC transporter, expressed as a transmembrane protein at the apical membrane of the capillary endothelial cells, (ii) a physical barrier based on the hydrophobic lipid bilayer membrane of the capillary endothelial cells, (iii) a physical barrier created by tight junctions between the capillary endothelial cells due to adhesion molecules such as claudin, and (iv) a physical and biological barrier lined with pericytes and astrocytes. Pericytes are situated on the abluminal aspect of the capillary endothelial cells and provide structural support to capillaries from behind. They secrete certain types of cytokines or bioactive substances to the capillary endothelial cells, contributing to the maintenance of BBB function, such as in its tight junctions and vesicle trafficking [19]. The endfeet of astrocytes physically encircle the capillaries, which are made up of capillary endothelial cells. Vascular smooth muscle cells are positioned between the astrocytic endfeet and the capillary endothelial cells. Astrocytes secrete certain types of bioactive substances to the capillary endothelial cells [20]. In effect, hydrophobic low-molecular weight compounds passing through the membrane are captured by MDR1 and excreted into the circulating blood. However, it is well-known that certain pharmaceutical agents, such as CNS drugs and antihistamine drugs, can penetrate the brain through the BBB. Most CNS drugs have structurally incorporated *N*-containing groups in their molecules. Brain-penetrating compounds (**1**–**10**) recently reported in the last year or two also possess *N*-containing groups [Figure 3] [21–30]. This fact suggests that *N*-containing groups act as the transporter recognition unit for a specific type of cation transporter at the BBB. Moreover, although memantine (MEM), a clinically approved AD drug with an *N*-containing group, forms a positively charged salt under physiological pH, it has demonstrated membrane penetration in a concentration-dependent manner and reached saturation in penetration above a specified concentration level at the BBB [31]. Positively charged salts cannot pass through the hydrophobic lipid bilayer membrane via passive diffusion. Accordingly, MEM was internalized across the membrane not through passive diffusion, but through carrier-mediated transport [Figure 2]. Thus, the internalization through SLC transporters into cells can bypass both intramembranous capture by MDR1 and the physical barrier based on the hydrophobic lipid bilayer membrane [Figures 1 and 2]. Transporter-mediated drug delivery across the BBB into the brain can provide a solution to the membrane impermeability of drugs for CNS disorders such as AD, Parkinson's disease (PD), and epilepsy.

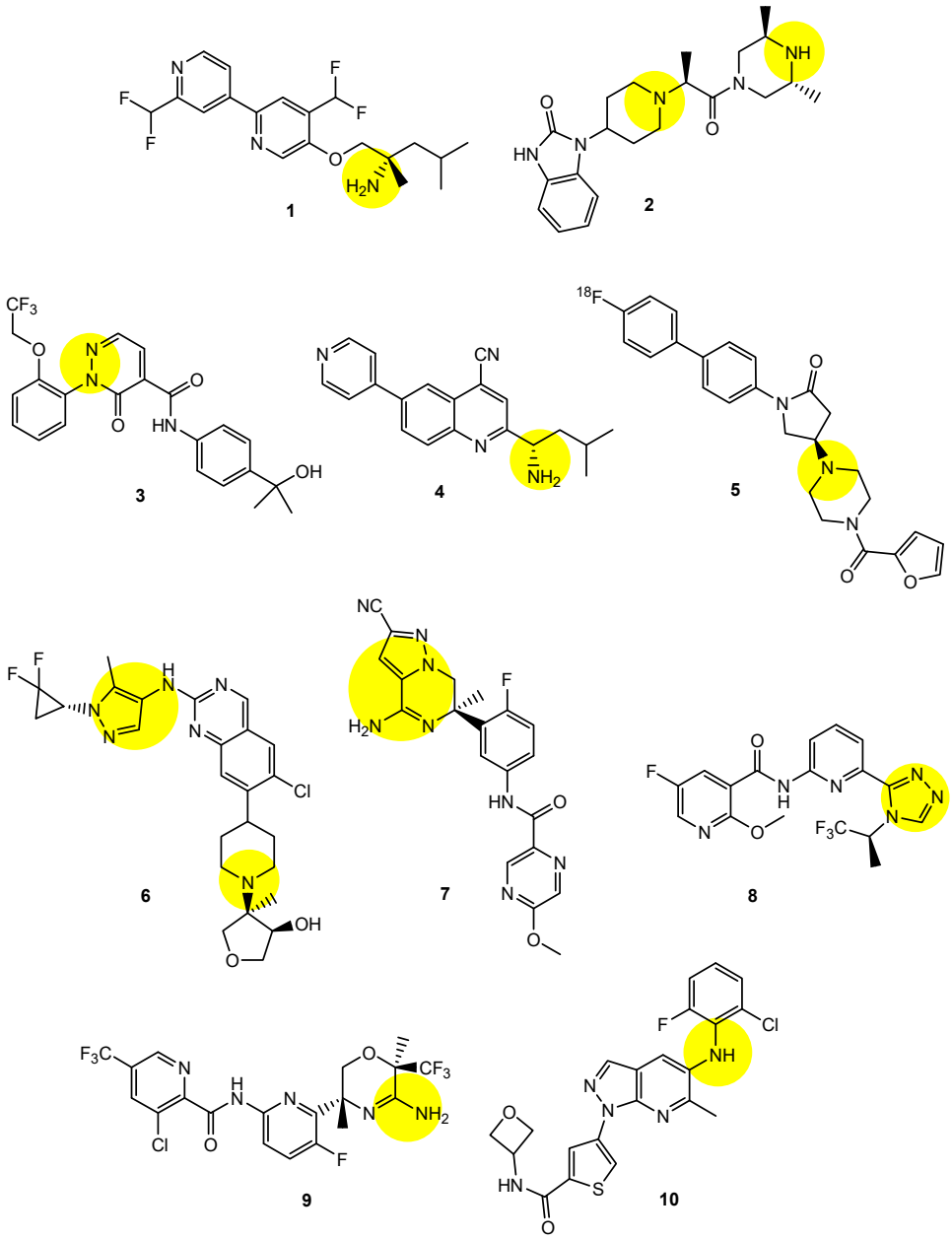

Figure 3. Recently reported brain-penetrant compounds (**1**–**10**) possessing *N*-containing groups.

2.3. The Proton-Coupled Organic Cation (H^+/OC) Antiporter at the BBB

OCT1, OCT2, OCT3, organic cation transporter novel type 1 (OCTN1), OCTN2, multidrug and toxin extrusion protein 1 (MATE1), MATE2, and plasma membrane monoamine transporter (PMAT) are well-known as typical transporters for organic cations. However, which transporters facilitate the uptake of CNS drugs with *N*-containing groups at the BBB remains unknown. It has been suggested that the H^+/OC antiporter might facilitate their transport, although its amino acid sequence and topology have not been biologically identified yet [32]. Clonidine, quinidine, *p*-chloroamphetamine, cocaine, codeine, diacetylmorphine, diphenhydramine, MDMA (3,4-methylenedioxymethamphetamine), methadone, morphine, nalbuphine, nicotine, oxycodone, and tramadol [Figure 4] have been shown to be transported by the mouse H^+/OC antiporter in apical membrane of the capillary endothelial cells across the BBB in an in situ mouse brain perfusion assay [33]. Moreover, pyrilamine, oxycodone, quinidine, amantadine, diphenhydramine, MEM, and aconitine [Figure 5] have been shown to be transported by the human H^+/OC antiporter

in apical membrane in an in vitro assay using human CMEC/D3 cells [34–36] as the BBB model [37]. All of these substrates possess *N*-containing groups. Therefore, the H^+/OC antiporter may be a strong candidate for transendothelial transport of compounds with *N*-containing groups at the BBB. Transporter-conscious drug design targeting H^+/OC antiporter is an effective method for drug delivery across the BBB [32,38,39]. The transport of compounds with *N*-containing groups through an H^+/OC antiporter has been shown to be not inhibited competitively by tetraethyl ammonium (TEA) (OCT1-3, OCTN1, and OCTN2 substrate) or serotonin (PMAT substrate) [4]. The rank order of expressed mRNA level in human CMEC/D3 cells was OCTN2 >> OCTN1 > PMAT >> OCT3 > OCT1 [34]. The mRNA level of the H^+/OC antiporter was undetectable due to its unknown amino acid sequence. OCTN2 transported L-carnitine, acetyl-L-carnitine, acetylcholine, dopamine, norepinephrine, thiamine, quinidine, verapamil, TEA, 1-methyl-4-phenylpyridinium, pyrilamine, diphenhydramine, procainamide, and lidocaine in an in vitro assay using human embryonic kidney (HEK) 293 cells stably expressing mouse OCTN2 [40]. Interestingly, the H^+/OC antiporter and OCTN2 share the same substrates, such as quinidine, pyrilamine, and diphenhydramine, due to relaxed substrate specificity. Pharmaceutical scientists should pay close attention to the analysis and consideration of experimental results.

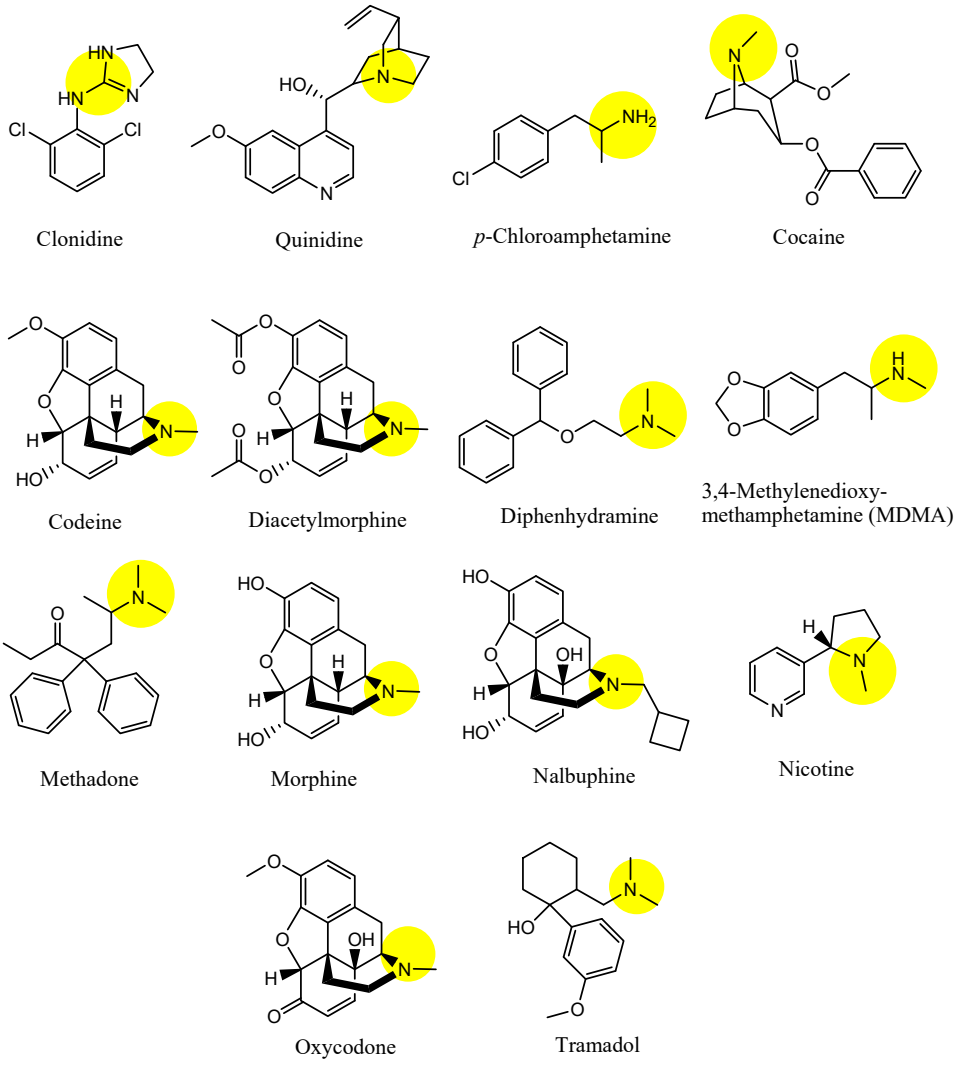

Figure 4. Plausible substrates of mouse unidentified H^+/OC cation antiporter.

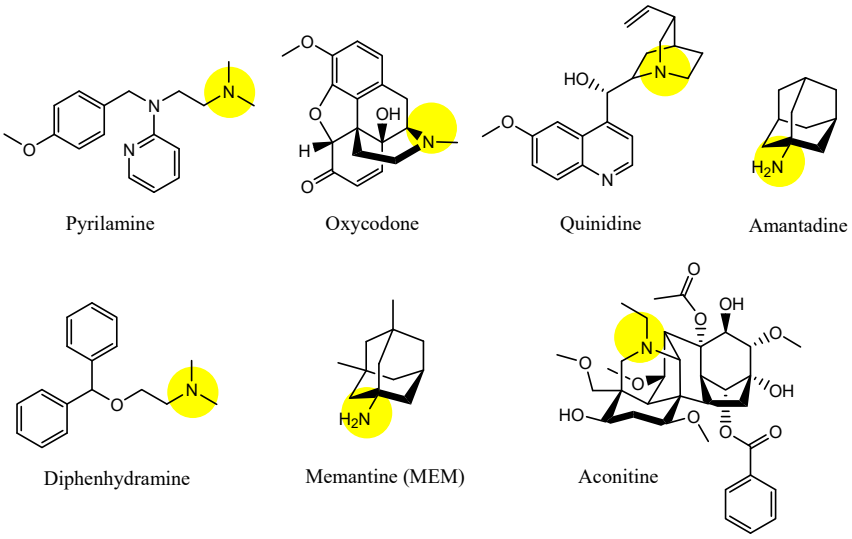

Figure 5. Plausible substrates of human unidentified H^+/OC cation antiporter.

2.4. The BRB

It is also known that compounds generally cannot penetrate the retina from the circulating blood due to the BRB. This barrier system maintains certain physiological homeostatic conditions in the eyes. The BRB is anatomically divided into the inner BRB [41] of the retinal capillary endothelial cells and the outer BRB of the retinal pigment epithelial cells [Figures 6 and 7]. The inner BRB is constructed by (i) a biological barrier based on excretion by MDR1 expressed at the apical membrane of the capillary endothelial cells, (ii) a physical barrier based on the hydrophobic lipid bilayer membrane of the capillary endothelial cells, (iii) a physical barrier based on the tight junctions between the capillary endothelial cells due to adhesion molecules such as claudin, and (iv) a physical and biological barrier lined with pericytes and Müller cells. Similarly, the outer BRB is constructed by (i) a biological barrier based on excretion by MDR1 expressed at the apical membrane of the retinal pigment epithelial cells [42], (ii) a physical barrier based on the hydrophobic lipid bilayer membrane of the retinal pigment epithelial cells, and (iii) a physical barrier based on the tight junctions between the retinal pigment epithelial cells due to adhesion molecules such as claudin. In fact, the inefficiency of drug administration into the retina at the BRB is an issue that needs to be addressed for patients suffering from retinopathy of diabetes or age-related macular degeneration. On the other hand, eye drops are susceptible to drainage into the nose through the nasolacrimal duct due to tears. The external eye barrier structure prevents eye drops from reaching the retina. Furthermore, intraocular administration through injection poses risks of increased ocular pressure, bleeding, and infections. Therefore, a non-invasive drug delivery system across the BRB into the retina should be developed, and, as many types of transporters are expressed at the BRB, carrier-mediated transport across the BRB is suggested as a potential method for this [43,44].

2.5. H^+/OC Antiporter at the Inner BRB

It has been clarified that compounds with *N*-containing groups are transported into the retina across the inner BRB. Clonidine (a substrate of the H^+/OC antiporter) has been shown to be absorbed at the inner BRB via carrier-mediated transport in a pH-dependent manner, implying H^+/OC antiporter-mediated transport. Moreover, clonidine has been shown to competitively inhibit transport of desipramine, propranolol, pyrilamine, verapamil, imipramine, quinidine, amantadine, and timolol at the inner BRB in an in vitro assay using TR-iBRB2 cells. Furthermore, clonidine did not inhibit TEA and L-carnitine transportation in this assay system [45]. These results suggest that the H^+/OC antiporter was involved in the transport of compounds with *N*-containing groups at the inner BRB. Thus, transporter-

conscious designed drugs with *N*-containing groups can be delivered into the retina across the inner BRB.

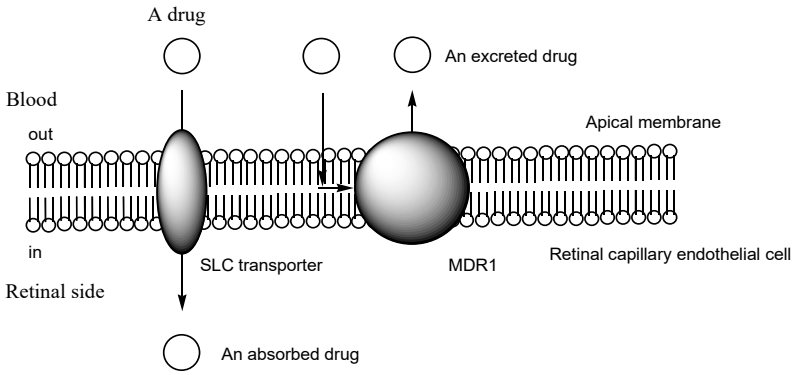

Figure 6. The passage of drugs absorbed by SLC transporter or excreted by MDR1 at the inner BRB.

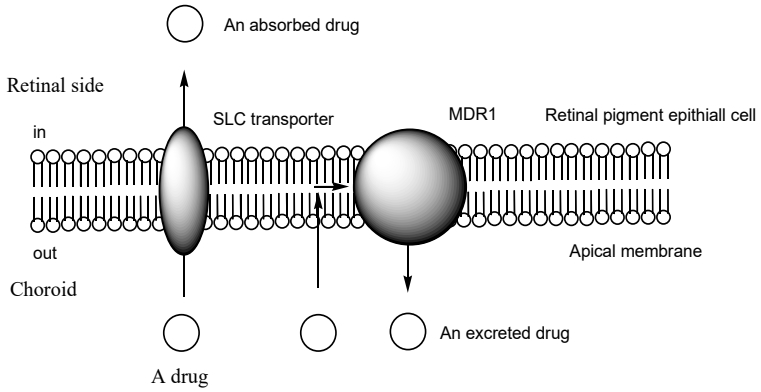

Figure 7. The passage of drugs absorbed by SLC transporter or excreted by MDR1 at the outer BRB.

2.6. Implementation of Transporter-Conscious Drug Design with N-Containing Groups

Transporters recognize the structures of their substrates. Therefore, compounds with transporter recognition units can be transported across the membrane through the pores of their corresponding transporters. There are two approaches to transporter-conscious drug design: (i) drug compounds that possess *N*-containing groups on their own, and (ii) conjugates of drugs and compounds that possess *N*-containing groups with appropriate linkers.

Fundamentally, the design of substrates for H^+/OC antiporters can be achieved by emulating current CNS drugs that contain *N*-containing groups, such as the *N*,*N*-dimethylalkyl groups. In CNS drug development, *N*-containing groups are introduced either inadvertently or through empirical methods. In practice, drugs linked to *N*-containing transporter recognition units via cleavable linkers can be transported across the BBB or BRB by H^+/OC antiporters. After these linkers are cleaved, the delivered drugs will exhibit their activity accordingly, following the prodrug system. Strictly speaking, the parent compounds may be enzymatically generated from their respective prodrugs in capillary endothelial cells before the prodrugs permeate the basolateral membrane, either in the cerebrospinal fluid (CSF) or in the brain parenchyma after the prodrugs permeate the basolateral membrane. This process depends on the characteristics of the compounds and the design of the drugs. The mechanism by which existing CNS drugs with *N*-containing groups cross the basolateral membrane after H^+/OC antiporter-mediated internalization into capillary endothelial cells across the apical membrane is not yet understood. It is thought that they probably cross the basolateral membrane into the CSF not through passive diffusion but rather through carrier-mediated transport or exocytosis, because the normal cellular pH level is approximately 7.0, which is slightly more acidic compared with the pH of approximately 7.4 in

the blood. Nevertheless, the possibility of direct translocation, initiated by the interaction with anionic phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) at the inner lipid bilayer and cationic *N*-containing groups, such as cytosolic full-length TAT (101 amino acids) [46], cannot be firmly dismissed.

A pyrilamine derivative with the benzamide zinc-binding group as a histone deacetylase (HDAC) inhibitor has been shown to be absorbed into hCMEC/D3 cells through H^+/OC antiporter-mediated transport in an in vitro assay [Figure 8] and to successfully cross the BBB in an in situ brain perfusion assay using rat. This compound exhibited HDAC1 inhibitory activity and holds promise as a brain-penetrating HDAC inhibitor for the treatment of CNS diseases [47].

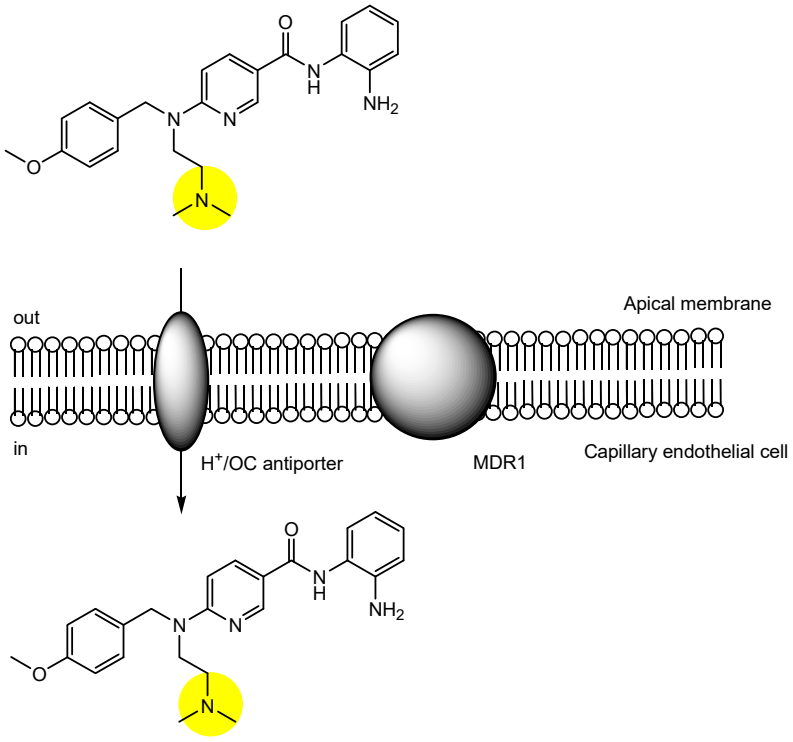

Figure 8. The mechanism of absorption mediated by the H^+/OC antiporter for a pyrilamine derivative with an *N*-containing group, acting as a histone deacetylase inhibitor.

Diphenhydramine analogs were assessed for cellular uptake via the H^+/OC antiporter using hCMEC/D3 cells. Some of these exhibited more efficient transport than the unmodified original diphenhydramine. The rank order of initial uptake rate (mL/mg Protein/min) using hCMEC/D3 cells is D (293 ± 16) > C (273 ± 26) > B (188 ± 23) > diphenhydramine (127 ± 8) > G (114 ± 23) > F (105 ± 19) > E (76.9 ± 9.8) > A (31.6 ± 5.6) > J (16.4) > H (3.70 ± 0.50) > I (2.05 ± 0.63). It has been implied that a heterocyclic amine moiety serves as a favorable transporter recognition unit [Figure 9] [39].

Chlorambucil (CHL), a chemotherapy medication, cannot penetrate the membrane due to the hydrophilic nature of the carboxyl group. The prodrug chlorambucil-scopine (CHLS), a conjugate of CHL and scopine linked by an ester bond, was observed to cross the BBB in an in vitro assay using murine brain endothelial cells and in an in situ rat brain perfusion assay. This internalized prodrug was enzymatically cleaved to trigger its activity against glioma in the brain. The *N*-containing scopine unit was identified as a substrate by the H^+/OC antiporter, as evidenced by the lack of inhibition of this transport by TEA [Figure 10] [38,48]. The parent compound chlorambucil would remain in the brain without crossing the BBB in the opposite direction.

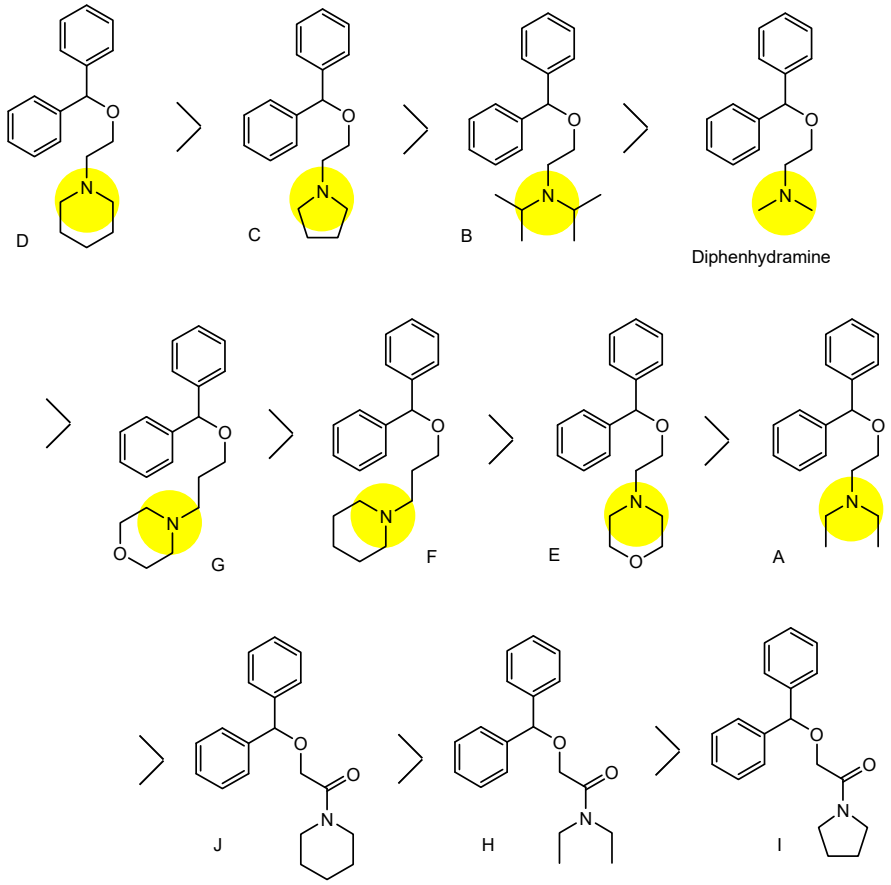

Figure 9. The rank order of transport across the membrane through H^+/OC antiporter in in vitro assay among diphenhydramine and its derivatives (**A**–**J**).

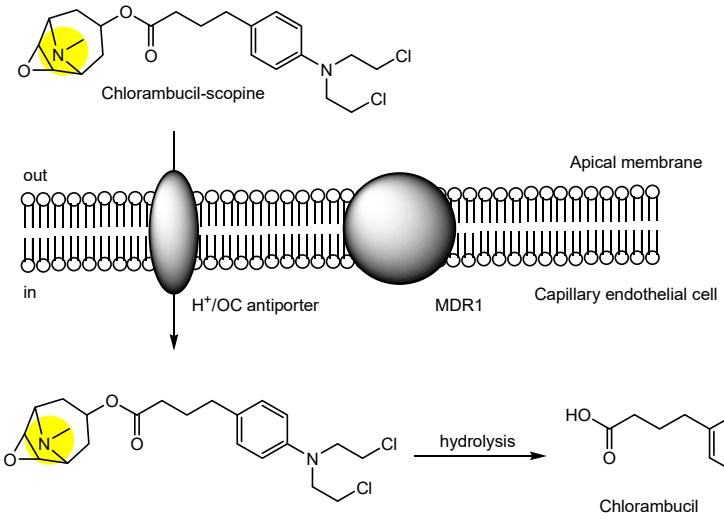

Figure 10. The mechanism of H^+/OC antiporter-mediated absorption and metabolism of the prodrug chlorambucil-scopine with an *N*-containing group.

N-[3,4-bis(pivaloyloxy)dopamine]-3-(dimethylamino)propanamide (PDDP) is a double prodrug of dopamine, consisting of the conjugation of dopamine with pivaloyl groups on the phenolic hydroxy groups and the 3-(dimethylamino)propanoyl group serving as the transporter recognition unit. PDDP was distributed in the brain in an in vivo assay based on intravenous injection in rats. PDDP was likely internalized into cells via the

H^+/OC antiporter and subsequently hydrolyzed to form dopamine in an in vitro assay using bEnd.3 cells [Figure 11]. This transport was inhibited by pyrilamine, propranolol, and imipramine, but not by choline, L-carnitine, and TEA [49].

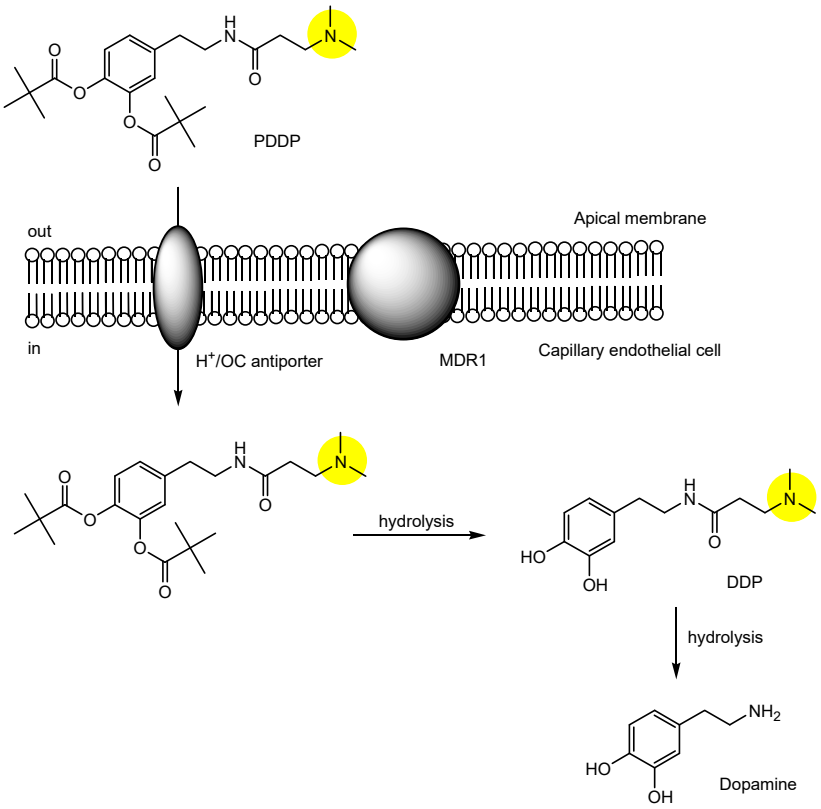

Figure 11. The mechanism of H^+/OC antiporter-mediated absorption and metabolism of the prodrug PDDP with *N*-containing group, which was subject to enzymatic hydrolysis to form the parent compound DDP and eventually dopamine.

The transport evaluation of dexibuprofen and its prodrugs, including prodrug I with the (*N*, *N*-dimethylamino)ethyl group, prodrug II with the (*N*, *N*-diethylamino)ethyl group, prodrug III with the (*N*-methylamino)ethyl group, and prodrug IV with the aminoethyl group, was conducted [Figures 12 and 13]. The rank order of transport across the membrane through H^+/OC antiporter in an in vitro assay using capillary endothelial cells was prodrug I > prodrug II > prodrug III > prodrug IV > dexibuprofen. The cellular uptakes of prodrug I, prodrug II, prodrug III, prodrug IV, and dexibuprofen in the right brain hemisphere at 37 °C were 117.69 ± 20.66, 99.59 ± 9.88, 66.51 ± 16.85, 50.03 ± 11.76, and 9.24 ± 1.50 (nmol/g), respectively, in an in vivo rat brain perfusion assay [50]. The (*N*, *N*-dimethylamino)ethyl group was identified as a potent H^+/OC antiporter recognition unit.

Currently, research in low-molecular-weight drug delivery aimed at crossing the BBB through carrier-mediated transport is being conducted, targeting characteristically identified transporters such as glucose transporter 1 (GLUT1), probably because of the ease of rational drug design [51]. Furthermore, GLUT1 is expressed on both the apical and basolateral membranes of capillary endothelial cells, facilitating the successive transport of its substrate from the bloodstream into the brain. Thus, it is important to identify the H^+/OC antiporter as early as possible to advance research in drug delivery, although its features have already been investigated [52,53]. The pharmacophore for the H^+/OC cation antiporter inhibitor was calculated using computer software based on data obtained from in vitro competitive permeation assays using labeled substrates in hCMEC/D3 cells [54]. Similarly, the chemophore, serving as a transporter recognition unit, can be obtained through calculation [4,55]. If the three-dimensional structural information of the

H^+/OC cation antiporter is utilized, transporter-conscious drug design targeting it will be conducted with high accuracy. Nevertheless, the design of compounds to cross the BBB has often been undertaken empirically, unconsciously, and fortuitously by incorporating *N*-containing groups as transporter recognition units, without making use of identified transporters such as GLUT1 [Figure 3]. This is highly significant in CNS or eye drug development because it indicates the ease of H^+/OC cation antiporter-mediated transport.

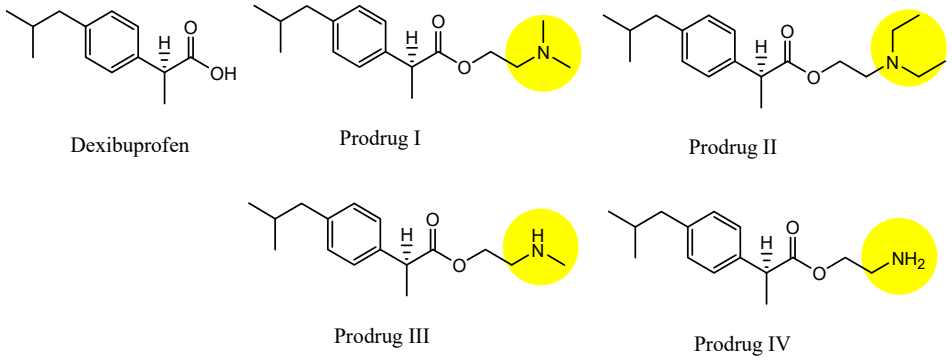

Figure 12. The structures of dexibuprofen and its prodrugs.

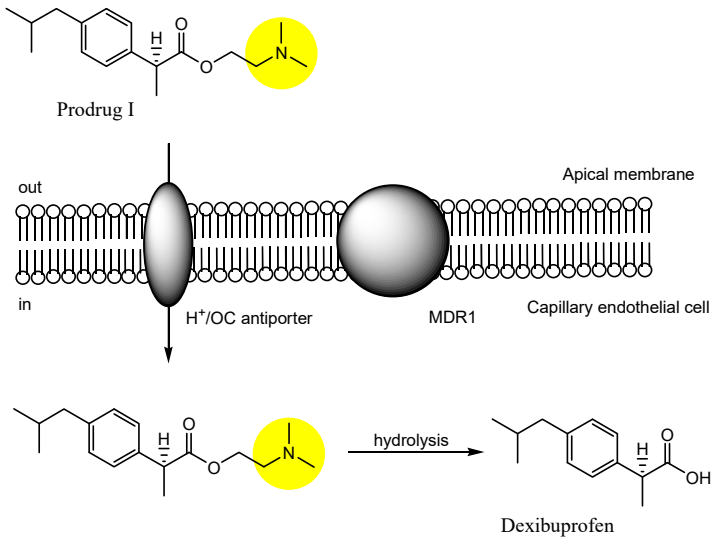

Figure 13. The mechanism of H^+/OC antiporter-mediated absorption and metabolism of the dexibuprofen prodrug, which possesses an *N*-containing group as a transporter recognition unit.

Moreover, nanoparticles are commonly employed as carriers for the transendothelial delivery of not only low-molecular-weight drugs but also middle- and high-molecular-weight drugs [3,56].

2.7. H^+/OC Cation Antiporter-Mediated Transport of PROTACs across the BBB for CNS Diseases

Targeted protein degradation [57] has garnered much recent attention because it can irreversibly and catalytically degrade target proteins, some of which were previously considered undruggable by other methods. Structurally, targeted protein degraders consist of a ligand for the target protein and a ligand for an E3 ligase (ubiquitin ligase) connected via a linker. After the target protein and E3 ligase come into proximity due to ligand binding, the target protein undergoes polyubiquitination and is subsequently degraded through the ubiquitin–proteasome system [Figure 14]. Several technologies have developed, including proteolysis targeting chimera (PROTAC) [58], which uses ligands to von Hippel–Lindau (VHL) as an E3 ligase; degronimid [59], which uses ligands to cereblon (CRBN) as an E3 ligase; and specific and nongenetic inhibitor of apoptosis protein (IAP) dependent protein

eraser (SNIPER) [60], which uses methyl-bestatin (MeBS) targeting cellular inhibitor of apoptosis protein (cIAP) as an E3 ligase. Nonetheless, the technology of targeted protein degradation with a chimera and bi-functional structure is currently collectively referred to as PROTAC, and includes PROTAC, degronimid, and SNIPER. However, VHL, CRBN, and cIAP are ubiquitously expressed in various tissues. To avoid off-target side effects, E3 ligases that are specifically expressed in particular tissues or cells should be utilized, as there are approximately 600 different types of E3 ligases. Otherwise, tissue- or cell-specific internalization across the cell membrane should be pursued, even when targeting ubiquitous E3 ligases.

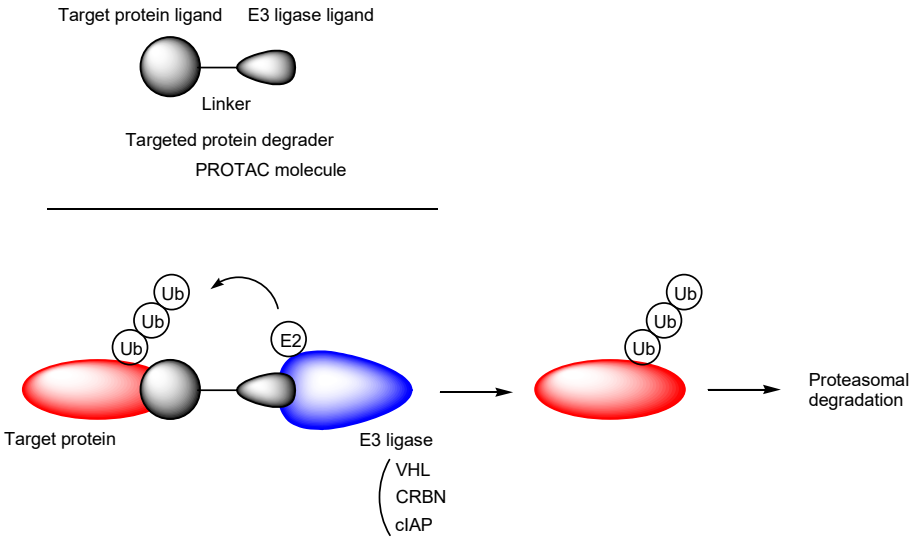

Figure 14. The canonical structure of targeted protein degraders, such as PROTAC molecules, and their degradative pathway for target proteins. As PROTAC molecules have a target protein ligand and an E3 ligase ligand connected via a linker, the target protein and E3 ligase are brought into proximity by the binding of the PROTAC molecule. The target protein undergoes ubiquitination and is subsequently degraded through the ubiquitin–proteasome system. Abbreviations: PROTAC, proteolysis targeting chimera; Ub, ubiquitin; E2, ubiquitin conjugating enzyme; E3 ligase, ubiquitin ligase; VHL, von Hippel–Lindau; CRBN, cereblon; cIAP, cellular inhibitor of apoptosis protein.

Immunomodulatory drugs (IMiDs) such as thalidomide, pomalidomide, and lenalidomide [Figure 15], which can penetrate the blood–brain barrier (BBB), are recognized as CRBN modulators. Interestingly, pomalidomide is used in PROTACs as an E3 ligase ligand. Thalidomide is not a substrate of MDR1, whereas pomalidomide and lenalidomide are substrates of MDR1. The rank order for BBB penetration is thalidomide > pomalidomide > lenalidomide. Transporters responsible for the transportation of thalidomide, pomalidomide, and lenalidomide have not been identified yet [61]. The introduction of the amino group altered the affinities for transporters between thalidomide and pomalidomide. The H^+/OC antiporter might be a candidate for mediating the BBB permeation of pomalidomide due to its *N*-containing group. Thus, PROTACs with pomalidomide as an E3 ligase ligand might act as substrates for the H^+/OC antiporter. The competitive inhibitory tests using substrates and non-substrates of the H^+/OC antiporter will clarify this.

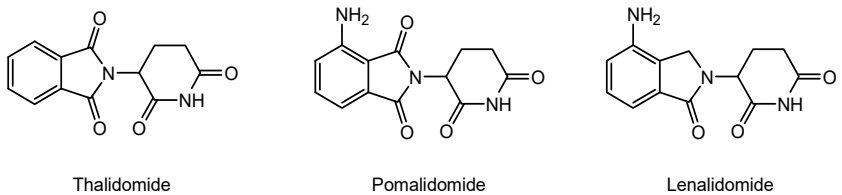

Figure 15. The structures of representative immunomodulatory drugs (IMiDs).

The radical cure for AD has not been established due to its complex pathobiology. Although many etiologies for AD have been proposed, amyloid β (Aβ) and tau are typical disease biomarkers [8]. Thus, it is suggested that Aβ and tau are significantly associated with the pathobiology of AD, ultimately leading to progressive neurodegeneration and subsequent dementia. Recently, the anti-Aβ monoclonal antibody aducanumab [62] was approved by the FDA in 2021. Moreover, the anti-Aβ protofibril monoclonal antibody lecanemab [63] was clinically approved by the FDA in 2023. The anti-Aβ monoclonal antibody donanemab [64] completed a phase 3 clinical trial with favorable results for early AD in 2023 (NCT04437511). Therefore, it is believed that the so-called amyloid hypothesis is prominent in AD drug development. Although Aβ and tau pathologies initially progress independently, Aβ pathology eventually becomes involved in enhancing the pathological process driven by tau pathology. It has been revealed that the population of neurofibrillary tangles (NFTs) containing tau, rather than Aβ plaques, is correlated with the pathogenesis of AD dementia [8]. Thus, the clearance of tau species can be a promising medical treatment for AD. PROTACs have been developed for clearing tau in the brain [65]. The tau species are found in both the extracellular and intracellular regions, although NFTs are formed within neuronal cells [8]. Intracellular tau can be captured by PROTACs that cross the BBB and subsequently cross the neuronal cell membrane. The ubiquitin–proteasome system acts within cells.

QC-01-175 [Figure 16], a PROTAC composed of a tau ligand (^{18}F-T807 derivative) and a CRBN ligand (pomalidomide) connected with a linker, has been shown to induce the clearance of aberrant tau in neuronal cell models derived from frontotemporal dementia (FTD) patients in an in vitro assay [66]. QC-01-175 crossed the neuronal cell membrane, though it is uncertain whether QC-01-175 was able to cross the BBB or not. QC-01-175 could potentially be a substrate for H^+/OC antiporter due to the presence of *N*-containing groups. On the other hand, C004019 [Figure 16], a PROTAC composed of a tau ligand (ID220149 [67]) and a VHL ligand (VHL Ligand 1 [68]) connected by a linker, reduced tau levels in the brains through subcutaneous administration in an in vivo assay using wild-type, hTau-transgenic, and 3xTg-AD mice, leading to improvements in synaptic and cognitive functions [69]. This finding suggests that C004019 crossed both the BBB and the neuronal cell membrane. Although it is unknown which transporters recognize ID220149 or VHL Ligand 1 as transporter recognition units, both units contain *N*-containing groups. C004019, capable of penetrating the brain, might potentially serve as a substrate for H^+/OC antiporter.

Accordingly, brain-penetrating PROTACs show promise as CNS agents, both pharmacodynamically and pharmacokinetically. Non-brain-penetrating PROTACs could potentially cross the BBB when *N*-containing groups, such as the (dimethylamino)ethyl group (MW 73.14), are introduced into them as the recognition unit for H^+/OC antiporters, without interfering with target protein binding and E3 ligase binding. Moreover, after crossing the endothelium, PROTAC molecules are internalized into cells across the membrane via passive diffusion and/or carrier-mediated transport involving specific transporters. The internalization mechanisms depend on the features of PROTAC molecules, particularly in terms of hydrophobicity and their potency as transporter substrates. If PROTACs demonstrate poor membrane permeability, the introduction of vectors such as transporter recognition units, cell-penetrating peptides, or antibodies can facilitate their entry into cells.

2.8. Eye-Specific Drug Therapy

It is worth noting that there are some issues, such as selectivity, because H^+/OC antiporters are expressed at both the BBB and BRB. Probabilistically, drugs containing *N*-groups would likely be preferentially delivered to the brain due to its larger size compared with the eye. Tissue-selective pharmaceuticals can be achieved by employing prodrugs containing *N*-containing groups, which can be activated into the respective active form by tissue-specific enzymes present in either the brain or the retina. Consequently, this approach helps in avoiding off-target side effects.

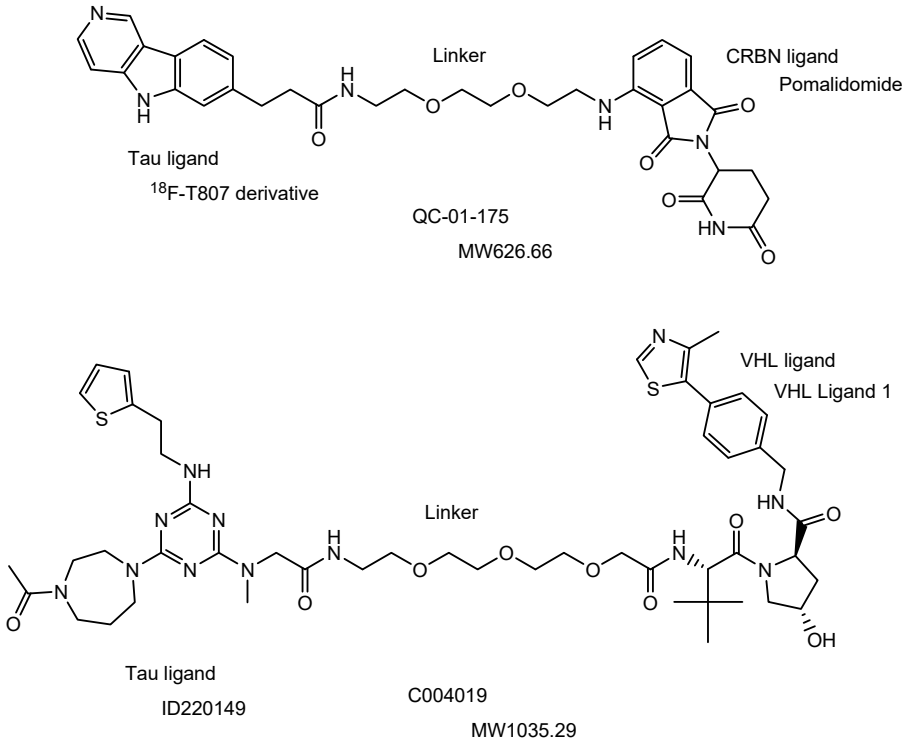

Figure 16. The structures of QC-01-175 and C004019. These contain *N*-containing groups that could potentially act as recognition units for proton-coupled organic cation (H^+/OC) antiporters. ^{18}F-T807 is an imaging compound that targets tau. Therefore, labeling such as ^{18}F is not necessary for the treatment of Alzheimer's disease. A derivative of ^{18}F-T807 simply needs to bind to the tau protein.

There are several differences between the H^+/OC antiporter at the BBB and the BRB. The population of H^+/OC antiporters at the BBB is much larger than that at the BRB. H^+/OC antiporters at the BRB are exposed to light, whereas those at the BBB are not. Blue light, with wavelengths ranging from 380 to 500 nm, carries intense light energy within the visible light spectrum (wavelengths from 400 to 800 nm), allowing it to reach the retina without absorption by the cornea and lens. On the other hand, blue light cannot penetrate the brain enclosed within the bony skull. Therefore, tissue selectivity between the eyes and the brain can be regulated by differences rooted in the biophysical structuralism advocated by Dr. Lévi-Strauss [70,71]. Specially designed low-molecular-weight drugs containing *N*-groups could be transported by H^+/OC antiporters and potentially exhibit an eye-specific drug effect. This could be achieved without off-target side effects in the brain, through photoactivation using a prodrug system with blue light. The *N*-methyl-7-hydroxyquinolinium (*N*-Me-7-HQm) caging chromophore, serving as the photosensitive protecting group, undergoes cleavage when exposed to blue light at 458 nm [Figure 17] [72]. Prodrugs, covalently conjugated between a pharmacologically active compound with an *N*-containing group and *N*-Me-7-HQm as the photosensitive protecting group [Figure 18], could cross the endothelium at the BRB via H^+/OC antiporters. Subsequently, they would be activated by blue light, exposing their active sites within the molecule. Caged prodrugs distributed in the brain would remain inactive in the absence of light and would eventually undergo metabolism. Modifications may be necessary to protect the conjugate linkage from enzymatic hydrolysis in serum during intravenous or oral administration. Alternatively, in some cases, modifications to enhance absorption in the small intestine, through both the paracellular and transcellular routes after oral administration instead of intravenous administration, might be needed.

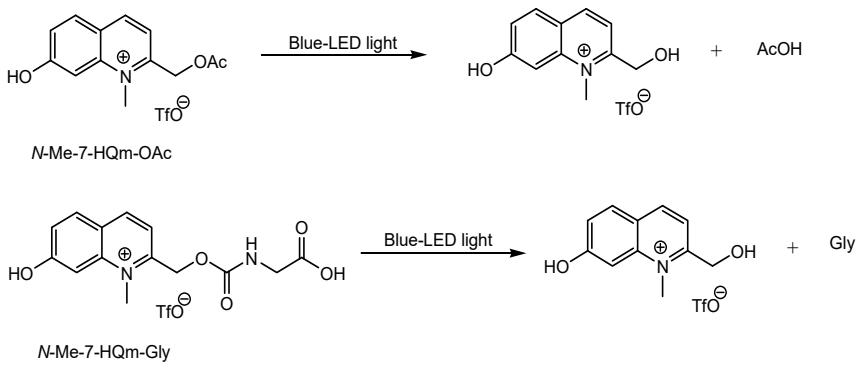

Figure 17. The structure of an *N*-methyl-7-hydroxyquinolinium (*N*-Me-7-HQm) caging chromophore and its cleavage process through visible light activation.

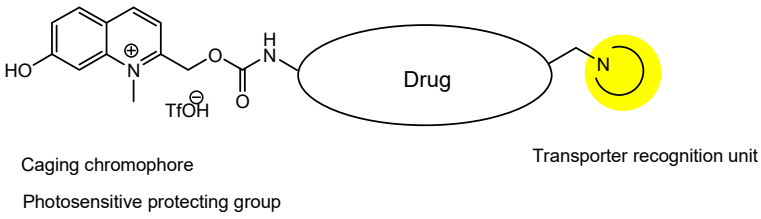

Figure 18. The structures of potential eye-specific prodrugs, covalently conjugated between a pharmacologically active compound with an *N*-containing group and *N*-Me-7-HQm as the photosensitive protecting group.

Enzymes specific to the retina include guanylate cyclase-activating protein (GCAP) [73], rhodopsin kinase (Rk or GRK1) [74], esterases [75], and retinal dehydrogenase. Enzymes specific to the brain comprise brain-specific aminopeptidase [76], γ-glutamyl transpeptidase [77,78], and glutamyl aminopeptidase [79–81]. I anticipate that inspired readers will design drugs capable of demonstrating either retina-specific or brain-specific activity upon activation of prodrugs by tissue-specific enzymes.

3. Conclusions

The impermeability of drugs at the BBB or the BRB presents a challenge in drug discovery and development. At present, neurodegenerative diseases such as AD and PD, as well as eye diseases such as diabetic retinopathy and age-related macular degeneration, represent significant unmet medical needs. Hence, an innovative therapeutic strategy needs to be devised to deliver drugs effectively and selectively to target sites across these barriers. Transporters exhibit tissue-specific expression. It has been revealed that CNS drugs are transported into the brain by H^+/OC antiporters [Table 2]. Transporter-conscious designed drugs with *N*-containing groups as transporter recognition units can cross the endothelium at the BBB or the BRB through carrier-mediated transport using the H^+/OC antiporter.

Currently, clinically approved drugs available for treating age-related macular degeneration and diabetic retinopathy are antibody fragments targeting vascular endothelial growth factor (VEGF), including aflibercept, ranibizumab, and brolucizumab. Thus, alternative low-molecular-weight drugs specific to the eyes should be developed, considering cost and patient-friendly administration routes other than intravitreal injection. Therefore, a low-molecular-weight drug delivery system targeting the eyes must be promptly established based on carrier-mediated transport across the BRB. Low-molecular VEGF inhibitors, both with *N*-containing groups and *N*-Me-7-HQm, are potent drug candidates. On the other hand, low-molecular-weight CNS drugs for the treatment of neurodegenerative diseases, including AD and PD, should also be developed.

Table 2. Summary of the potential substrates for the proton-coupled organic cation (H^+/OC) antiporter discussed in this review.

#	Compounds	The Barrier to Cross	Tissues to Be Absorbed	Status	References
(1)	1–10	BBB	Brain	Basic research	Figure 3, [21–30]
(2)	Clonidine	BBB, Inner BRB	Brain, eyes	Launched	Figure 4, [33,45]
(3)	Quinidine	BBB, Inner BRB	Brain, eyes	Launched	Figures 4 and 5, [33–36,45]
(4)	*p*-Chloroamphetamine	BBB	Brain	Launched	Figure 4, [33]
(5)	Cocaine	BBB	Brain	Launched	Figure 4, [33]
(6)	Codeine	BBB	Brain	Launched	Figure 4, [33]
(7)	Diacetylmorphine	BBB	Brain	Launched	Figure 4, [33]
(8)	Diphenhydramine	BBB	Brain	Launched	Figures 4 and 5, [33–36]
(9)	MDMA (3,4-methylenedioxymethamphetamine)	BBB	Brain	Basic research	Figure 4, [33]
(10)	Methadone	BBB	Bain	Launched	Figure 4, [33]
(11)	Morphine	BBB	Braun	Launched	Figure 4, [33]
(12)	Nalbuphine	BBB	Brain	Launched	Figure 4, [33]
(13)	Nicotine	BBB	Brain.	Launched	Figure 4, [33]
(14)	Oxycodone	BBB	Brain	Launched	Figure 4, [33]
(15)	Tramadol	BBB, Inner BRB	Brain, eyes	Launched	Figure 4, [33,45]
(16)	Pyrilamine	BBB, Inner BRB	Brain, eyes	Launched	Figure 5, [34–36,45]
(17)	Oxycodone	BBB	Brain	Launched	Figure 5, [34–36]
(18)	Amantadine	BBB, Inner BRB	Brain, eyes	Launched	Figure 5, [34–36,45]
(19)	Memantine (MEM)	BBB	Brain	Launched	Figure 5, [31,34–36]
(20)	Aconitine	BBB	Brain	Basic research	Figure 5, [34–36]
(21)	Desipramine	Inner BRB	Eyes	Launched	[45]
(22)	Propranolol	Inner BRB	Eyes	Launched	[45]
(23)	Verapamil	Inner BRB	Eyes	Launched	[45]
(24)	Imipramine	Inner BRB	Eyes	Launched	[45]
(25)	Pyrilamine derivative with benzamide	BBB	Brain	Basic research	Figure 8, [47]
(26)	Diphenhydramine analogs	BBB	Brain	Basic research	Figure 9, [39]
(27)	Chlorambucil-scopine (CHLS)	BBB	Brain	Basic research	Figure 10, [38,48]
(28)	*N*-[3,4-bis(pivaloyloxy)domapine]-3-(dimethylamino)propanamide (PDDP)	BBB	Brain	Basic research	Figure 11, [49]
(29)	Dexibuprofen prodrugs	BBB	Brain	Basic research	Figures 12 and 13, [50]
(30)	QC-01-175	BBB	Brain	Basic research	Figure 16, [66]
(31)	C004019	BBB	Brain	Basic research	Figure 16, [69]
(32)	PROTACs with vectors	BBB	Brain	Under analysis in Tashima lab	-
(33)	Prodrugs with *N*-containing group and *N*-Me-7-HQm	Inner BRB	Eyes	Under analysis in Tashima lab	-

Drug discovery and development should take into account the structures based on the biological system at the BBB or BRB, following the principles of structuralism advocated by Dr. Lévi-Strauss [70,71]. In particular, transporter-conscious designed drugs will be systematically and comprehensively regulated to ensure delivery, metabolism if necessary, and activation at their intended sites, based on structuralism. The urgent need lies in the identification of the H^+/OC antiporter, enabling the design of substrates with strategically positioned *N*-containing groups that can interact effectively with each other and be subsequently transported through its pore. The SLC transporters constitute a superfamily comprising over 400 membrane transport proteins. The H^+/OC antiporter might be discovered within the orphan SLC transporters. Alternatively, the H^+/OC antiporter protein could be captured using affinity-based probes through affinity labeling methods, such as photo-affinity labeling. This process involves the use of modified clonidine with an azide group on the benzene ring, either in the absence of TEA or in the presence of excess TEA to saturate other cation transporters. Furthermore, AMG-595, an anti-EGFR (epidermal growth factor receptor) antibody-drug conjugate with DM1 (mertansine) linked via a non-cleavable maleimidomethylcyclohexane-1-carboxyl (MCC) linker, has been shown to be catabolized into Lys-MCC-DM1 in lysosomes. The lysosomal membrane transporter SLC46A3, which transports Lys-MCC-DM1 to the cytoplasm, was identified through shRNA analysis [82]. The H^+/OC antiporter can be identified using a similar

strategy. Living organisms are composed of biological and physical systematic structures, such as cells and tissues, regulated by structuralism [Figure 19] [70,71]. However, even within such constrained structures, new systems can be created based on existentialism, as advocated by Dr. Jean-Paul Sartre. Based on the principles of structural pharmaceutical science and existentialism, medicinal chemists and pharmaceutical scientists should strive to develop novel CNS or eye drugs that act as substrates for the H^+/OC antiporter, aiming to provide effective treatments for patients.

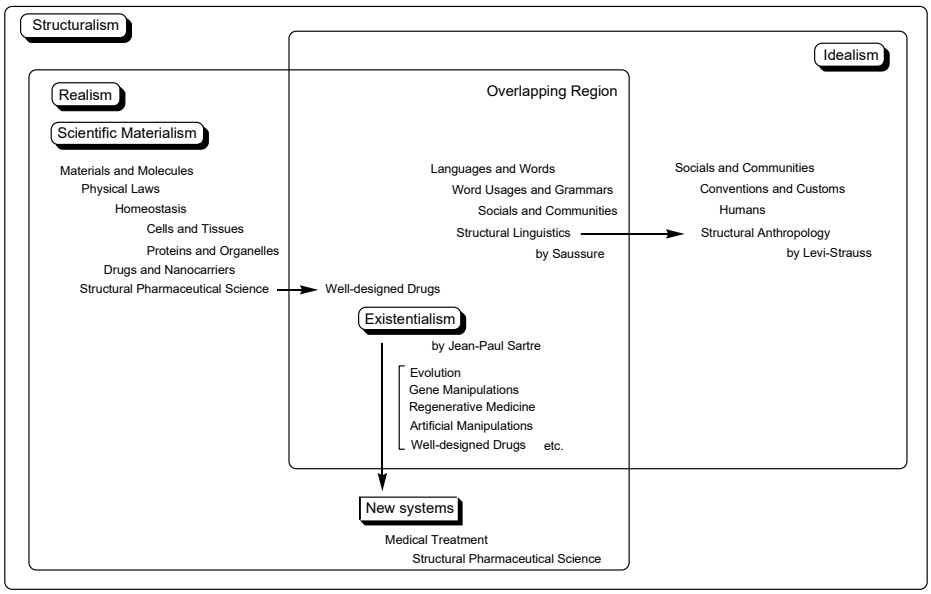

Figure 19. The correlations of events regulated by systematic structures based on structuralism.

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Conflicts of Interest: The author declares no conflict of interest.

References

1. Stimulus package. *Nat. Med.* **2018**, *24*, 247. [CrossRef] [PubMed]
2. Angermann, R.; Rauchegger, T.; Nowosielski, Y.; Casazza, M.; Bilgeri, A.; Ulmer, H.; Zehetner, C. Treatment compliance and adherence among patients with diabetic retinopathy and age-related macular degeneration treated by anti-vascular endothelial growth factor under universal health coverage. *Graefe's Arch. Clin. Exp. Ophthalmol.* **2019**, *257*, 2119–2125. [CrossRef] [PubMed]
3. Tashima, T. Smart Strategies for Therapeutic Agent Delivery into Brain across the Blood-Brain Barrier Using Receptor-Mediated Transcytosis. *Chem. Pharm. Bull.* **2020**, *68*, 316–325. [CrossRef] [PubMed]
4. Tashima, T. Intriguing possibilities and beneficial aspects of transporter-conscious drug design. *Bioorg. Med. Chem.* **2015**, *23*, 4119–4131. [CrossRef] [PubMed]
5. Tashima, T. Intelligent substance delivery into cells using cell-penetrating peptides. *Bioorg. Med. Chem. Lett.* **2017**, *27*, 121–130. [CrossRef] [PubMed]
6. Tashima, T. Effective cancer therapy based on selective drug delivery into cells across their membrane using receptor-mediated endocytosis. *Bioorg. Med. Chem. Lett.* **2018**, *28*, 3015–3024. [CrossRef]
7. Tashima, T. Shortcut Approaches to Substance Delivery into the Brain Based on Intranasal Administration Using Nanodelivery Strategies for Insulin. *Molecules* **2020**, *25*, 5188. [CrossRef] [PubMed]
8. Tashima, T. Delivery of Intravenously Administered Antibodies Targeting Alzheimer's Disease-Relevant Tau Species into the Brain Based on Receptor-Mediated Transcytosis. *Pharmaceutics* **2022**, *14*, 411. [CrossRef]

9. Tashima, T. Brain Cancer Chemotherapy through a Delivery System across the Blood-Brain Barrier into the Brain Based on Receptor-Mediated Transcytosis Using Monoclonal Antibody Conjugates. *Biomedicines* **2022**, *10*, 1597. [CrossRef]
10. Tashima, T. Delivery of Drugs into Cancer Cells Using Antibody–Drug Conjugates Based on Receptor-Mediated Endocytosis and the Enhanced Permeability and Retention Effect. *Antibodies* **2022**, *11*, 78. [CrossRef]
11. de Mora, F.; Balsa, A.; Cornide-Santos, M.; Carrascosa, J.M.; Marsal, S.; Gisbert, J.P.; Abad, M.-A.; Duarte, R.F.; Wiechmann, M.; Martínez, R. Biosimilar and interchangeable: Inseparable scientific concepts? *Br. J. Clin. Pharmacol.* **2019**, *85*, 2460–2463. [CrossRef]
12. Zamek-Gliszczynski, M.J.; Taub, M.E.; Chothe, P.P.; Chu, X.; Giacomini, K.M.; Kim, R.B.; Ray, A.S.; Stocker, S.L.; Unadkat, J.D.; Wittwer, M.B.; et al. Transporters in Drug Development: 2018 ITC Recommendations for Transporters of Emerging Clinical Importance. *Clin. Pharmacol. Ther.* **2018**, *104*, 890–899. [CrossRef] [PubMed]
13. Jaramillo, A.C.; Saig, F.A.; Cloos, J.; Jansen, G.; Peters, G.J. How to overcome ATP-binding cassette drug efflux transporter-mediated drug resistance? *Cancer Drug Resist.* **2018**, *1*, 6–29. [CrossRef]
14. Hu, C.; Tao, L.; Cao, X.; Chen, L. The solute carrier transporters and the brain: Physiological and pharmacological implications . *Asian J. Pharm. Sci.* **2020**, *15*, 131–144. [CrossRef] [PubMed]
15. Gotfryd, K.; Boesen, T.; Mortensen, J.S.; Khelashvili, G.; Quick, M.; Terry, D.S.; Missel, J.W.; LeVine, M.V.; Gourdon, P.; Blanchard, S.C.; et al. X-ray structure of LeuT in an inward-facing occluded conformation reveals mechanism of substrate release. *Nat. Commun.* **2020**, *11*, 1005. [CrossRef]
16. Kumar, S.; Athreya, A.; Gulati, A.; Nair, R.M.; Mahendran, I.; Ranjan, R.; Penmatsa, A. Structural basis of inhibition of a transporter from Staphylococcus aureus, NorC, through a single-domain camelid antibody. *Commun. Biol.* **2021**, *4*, 836. [CrossRef]
17. Roberts, A.G. The Structure and Mechanism of Drug Transporters. *Methods Mol. Biol.* **2021**, *2342*, 193–234. [CrossRef]
18. Kadry, H.; Noorani, B.; Cucullo, L. A blood-brain barrier overview on structure, function, impairment, and biomarkers of integrity. *Fluids Barriers CNS* **2020**, *17*, 69. [CrossRef]
19. Liu, S.; Agalliu, D.; Yu, C.; Fisher, M. The Role of Pericytes in Blood-Brain Barrier Function and Stroke. *Curr. Pharm. Des.* **2012**, *18*, 3653–3662. [CrossRef] [PubMed]
20. Díaz-Castro, B.; Robel, S.; Mishra, A. Astrocyte Endfeet in Brain Function and Pathology: Open Questions. *Annu. Rev. Neurosci.* **2023**, *46*, 101–121. [CrossRef] [PubMed]
21. Luo, G.; Chen, L.; Kostich, W.A.; Hamman, B.; Allen, J.; Easton, A.; Bourin, C.; Gulianello, M.; Lippy, J.; Nara, S.; et al. Discovery and Optimization of Biaryl Alkyl Ethers as a Novel Class of Highly Selective, CNS-Penetrable, and Orally Active Adaptor Protein-2-Associated Kinase 1 (AAK1) Inhibitors for the Potential Treatment of Neuropathic Pain. *J. Med. Chem.* **2022**, *65*, 4534–4564. [CrossRef] [PubMed]
22. May-Dracka, T.L.; Gao, F.; Hopkins, B.T.; Hronowski, X.; Chen, T.; Chodaparambil, J.V.; Metrick, C.M.; Cullivan, M.; Enyedy, I.; Kaliszczak, M.; et al. Discovery of Phospholipase D Inhibitors with Improved Drug-like Properties and Central Nervous System Penetrance. *ACS Med. Chem. Lett.* **2022**, *13*, 665–673. [CrossRef]
23. Tanaka, Y.; Seto, M.; Kakegawa, K.; Takami, K.; Kikuchi, F.; Yamamoto, T.; Nakamura, M.; Daini, M.; Murakami, M.; Ohashi, T.; et al. Discovery of Brain-Penetrant Glucosylceramide Synthase Inhibitors with a Novel Pharmacophore. *J. Med. Chem.* **2022**, *65*, 4270–4290. [CrossRef] [PubMed]
24. Hartz, R.A.; Ahuja, V.T.; Nara, S.J.; Kumar, C.M.V.; Manepalli, R.K.V.L.P.; Sarvasiddhi, S.K.; Honkhambe, S.; Patankar, V.; Dasgupta, B.; Rajamani, R.; et al. Bicyclic Heterocyclic Replacement of an Aryl Amide Leading to Potent and Kinase-Selective Adaptor Protein 2-Associated Kinase 1 Inhibitors. *J. Med. Chem.* **2022**, *65*, 4121–4155. [CrossRef]
25. He, Y.; Schild, M.; Grether, U.; Benz, J.; Leibrock, L.; Heer, D.; Topp, A.; Collin, L.; Kuhn, B.; Wittwer, M.; et al. Development of High Brain-Penetrant and Reversible Monoacylglycerol Lipase PET Tracers for Neuroimaging. *J. Med. Chem.* **2022**, *65*, 2191–2207. [CrossRef] [PubMed]
26. Keylor, M.H.; Gulati, A.; Kattar, S.D.; Johnson, R.E.; Chau, R.W.; Margrey, K.A.; Ardolino, M.J.; Zarate, C.; Poremba, K.E.; Simov, V.; et al. Structure-Guided Discovery of Aminoquinazolines as Brain-Penetrant and Selective LRRK2 Inhibitors. *J. Med. Chem.* **2022**, *65*, 838–856. [CrossRef]
27. Peschiulli, A.; Oehlrich, D.; Van Gool, M.; Austin, N.; Van Brandt, S.; Surkyn, M.; De Cleyn, M.; Vos, A.; Tresadern, G.; Rombouts, F.J.R.; et al. A Brain-Penetrant and Bioavailable Pyrazolopiperazine BACE1 Inhibitor Elicits Sustained Reduction of Amyloid β In Vivo. *ACS Med. Chem. Lett.* **2022**, *13*, 76–83. [CrossRef] [PubMed]
28. Jones, J.H.; Xin, Z.; Himmelbauer, M.; Dechantsreiter, M.; Enyedy, I.; Hedde, J.; Fang, T.; Coomaraswamy, J.; King, K.W.; Murugan, P.; et al. Discovery of Potent, Selective, and Brain-Penetrant Apoptosis Signal-Regulating Kinase 1 (ASK1) Inhibitors that Modulate Brain Inflammation In Vivo. *J. Med. Chem.* **2021**, *64*, 15402–15419. [CrossRef]
29. Machauer, R.; Lueoend, R.; Hurth, K.; Veenstra, S.J.; Rueeger, H.; Voegtle, M.; Tintelnot-Blomley, M.; Rondeau, J.M.; Jacobson, L.H.; Laue, G.; et al. Discovery of Umibecestat (CNP520): A Potent, Selective, and Efficacious β-Secretase (BACE1) Inhibitor for the Prevention of Alzheimer's Disease. *J. Med. Chem.* **2021**, *64*, 15262–15279. [CrossRef]
30. Feng, Y.; Park, H.; Ryu, J.C.; Yoon, S.O. N-Aromatic-Substituted Indazole Derivatives as Brain-Penetrant and Orally Bioavailable JNK3 Inhibitors. *ACS Med. Chem. Lett.* **2021**, *12*, 1546–1552. [CrossRef] [PubMed]
31. Mehta, D.C.; Short, J.L.; Nicolazzo, J.A. Memantine transport across the mouse blood-brain barrier is mediated by a cationic influx H^+ antiporter. *Mol. Pharm.* **2013**, *10*, 4491–4498. [CrossRef] [PubMed]

32. Sachkova, A.; Doetsch, D.A.; Jensen, O.; Brockmöller, J.; Ansari, S. How do psychostimulants enter the human brain? Analysis of the role of the proton-organic cation antiporter. *Biochem. Pharmacol.* **2021**, *192*, 114751. [CrossRef]
33. André, P.; Debray, M.; Scherrmann, J.-M.; Cisternino, S.J. Clonidine transport at the mouse blood–brain barrier by a new H^+ antiporter that interacts with addictive drugs. *Cereb. Blood Flow Metab.* **2009**, *29*, 1293–1304. [CrossRef] [PubMed]
34. Shimomura, K.; Okura, T.; Kato, S.; Couraud, P.-O.; Schermann, J.-M.; Terasaki, T.; Deguchi, Y. Functional expression of a proton-coupled organic cation (H^+ /OC) antiporter in human brain capillary endothelial cell line hCMEC/D3, a human blood–brain barrier model. *Fluids Barriers CNS* **2013**, *10*, 8. [CrossRef] [PubMed]
35. Higuchi, K.; Kitamura, A.; Okura, T.; Deguchi, Y. Memantine transport by a proton-coupled organic cation antiporter in hCMEC/D3 cells, an in vitro human blood-brain barrier model. *Drug Metab. Pharmacokinet.* **2015**, *30*, 182–187. [CrossRef] [PubMed]
36. Cong, J.; Ruan, Y.; Lyu, Q.; Qin, X.; Qi, X.; Liu, W.; Kang, L.; Zhang, J.; Wu, C. A proton-coupled organic cation antiporter is involved in the blood-brain barrier transport of Aconitum alkaloids. *J. Ethnopharmacol.* **2020**, *252*, 112581. [CrossRef] [PubMed]
37. Poller, B.; Gutmann, H.; Krähenbühl, S.; Weksler, B.; Romero, I.; Couraud, P.O.; Tuffin, G.; Drewe, J.; Huwyler, J. The human brain endothelial cell line hCMEC/D3 as a human blood-brain barrier model for drug transport studies. *J. Neurochem.* **2008**, *107*, 1358–1368. [CrossRef] [PubMed]
38. Wang, X.; Qi, B.; Su, H.; Li, J.; Sun, X.; He, Q.; Fu, Y.; Zhang, Z. Pyrilamine-sensitive proton-coupled organic cation (H^+/OC) antiporter for brain-specific drug delivery. *J. Control. Release* **2017**, *254*, 34–43. [CrossRef] [PubMed]
39. Tega, Y.; Tabata, H.; Kurosawa, T.; Kitamura, A.; Itagaki, F.; Oshitari, T.; Deguchi, Y. Structural Requirements for Uptake of Diphenhydramine Analogs into hCMEC/D3 Cells Via the Proton-Coupled Organic Cation Antiporter. *J. Pharm. Sci.* **2021**, *110*, 397–403. [CrossRef]
40. Kato, Y.; Sugiura, M.; Sugiura, T.; Wakayama, T.; Kubo, Y.; Kobayashi, D.; Sai, Y.; Tamai, I.; Iseki, S.; Tsuji, A. Organic Cation/Carnitine Transporter OCTN2 (Slc22a5) Is Responsible for Carnitine Transport across Apical Membranes of Small Intestinal Epithelial Cells in Mouse. *Mol. Pharmacol.* **2006**, *70*, 829–837. [CrossRef] [PubMed]
41. Díaz-Coránguez, M.; Ramos, C.; Antonetti, D.A. The inner blood-retinal barrier: Cellular basis and development. *Vision Res.* **2017**, *139*, 123–137. [CrossRef] [PubMed]
42. Constable, P.A.; Lawrenson, J.G.; Dolman, D.E.; Arden, G.B.; Abbott, N.J. P-Glycoprotein expression in human retinal pigment epithelium cell lines. *Exp Eye Res.* **2006**, *83*, 24–30. [CrossRef] [PubMed]
43. Gyawali, A.; Kang, Y.S. Blood-to-Retina Transport of Imperatorin Involves the Carrier-Mediated Transporter System at the Inner Blood-Retinal Barrier. *J. Pharm. Sci.* **2019**, *108*, 1619–1626. [CrossRef] [PubMed]
44. Tyagi, A.; Sharma, P.K.; Malviya, R. Role of Blood Retinal Barrier in Drug Absorption. *Pharm. Anal. Acta* **2018**, *9*, 5. [CrossRef]
45. Kubo, Y.; Tsuchiyama, A.; Shimizu, Y.; Akanuma, S.; Hosoya, K. Involvement of Carrier-Mediated Transport in the Retinal Uptake of Clonidine at the Inner Blood–Retinal Barrier. *Mol. Pharm.* **2014**, *11*, 3747–3753. [CrossRef]
46. Debaisieux, S.; Rayne, F.; Yezid, H.; Beaumelle, B. The Ins and Outs of HIV-1 Tat. *Traffic* **2012**, *13*, 355–363. [CrossRef]
47. Hiranaka, S.; Tega, Y.; Higuchi, K.; Kurosawa, T.; Deguchi, Y.; Arata, M.; Ito, A.; Yoshida, M.; Nagaoka, Y.; Sumiyoshi, T. Design, Synthesis, and Blood-Brain Barrier Transport Study of Pyrilamine Derivatives as Histone Deacetylase Inhibitors. *ACS Med. Chem. Lett.* **2018**, *9*, 884–888. [CrossRef] [PubMed]
48. Wang, X.; Li, J.; Xu, C.; Li, Y.; Gong, T.; Sun, X.; Fu, Y.; He, Q.; Zhang, Z. Scopine as a novel brain-targeting moiety enhances the brain uptake of chlorambucil. *Bioconjug. Chem.* **2014**, *25*, 2046–2054. [CrossRef]
49. Li, Y.; Zhou, Y.; Qi, B.; Gong, T.; Sun, X.; Fu, Y.; Zhang, Z. Brain-Specific Delivery of Dopamine Mediated by *N*,*N*-Dimethyl Amino Group for the Treatment of Parkinson's Disease. *Mol. Pharm.* **2014**, *11*, 3174–3185. [CrossRef] [PubMed]
50. Li, Y.; Zhou, Y.; Jiang, J.; Wang, X.; Fu, Y.; Gong, T.; Sun, X.; Zhang, Z. Mechanism of brain targeting by dexibuprofen prodrugs modified with ethanolamine-related structures. *J. Cereb. Blood Flow Metab.* **2015**, *35*, 1985–1994. [CrossRef] [PubMed]
51. Huttunen, J.; Adla, S.K.; Markowicz-Piasecka, M.; Huttunen, K.M. Increased/Targeted Brain (Pro)Drug Delivery via Utilization of Solute Carriers (SLCs). *Pharmaceutics* **2022**, *14*, 1234. [CrossRef]
52. Kawase, A.; Chuma, T.; Irie, K.; Kazaoka, A.; Kakuno, A.; Matsuda, N.; Shimada, H.; Iwaki, M. Increased penetration of diphenhydramine in brain via proton-coupled organic cation antiporter in rats with lipopolysaccharide-induced inflammation. *Brain Behav. Immun. Health* **2021**, *10*, 100188. [CrossRef] [PubMed]
53. Kawase, A.; Kazaoka, A.; Shimada, H.; Iwaki, M. Increased brain penetration of diphenhydramine and memantine in rats with adjuvant-induced arthritis. *Brain Res.* **2021**, *1768*, 147581. [CrossRef]
54. Chapy, H.; Goracci, L.; Vayer, P.; Parmentier, Y.; Carrupt, P.A.; Declèves, X.; Scherrmann, J.M.; Cisternino, S.; Cruciani, G. Pharmacophore-based discovery of inhibitors of a novel drug/proton antiporter in human brain endothelial hCMEC/D3 cell line. *Br. J. Pharmacol.* **2015**, *172*, 4888–4904. [CrossRef]
55. Smirnova, M.; Goracci, L.; Cruciani, G.; Federici, L.; Declèves, X.; Chapy, H.; Cisternino, S. Pharmacophore-Based Discovery of Substrates of a Novel Drug/Proton-Antiporter in the Human Brain Endothelial hCMEC/D3 Cell Line. *Pharmaceutics* **2022**, *14*, 255. [CrossRef] [PubMed]
56. Lombardo, S.M.; Schneider, M.; Türeli, A.E.; Günday Türeli, N. Key for crossing the BBB with nanoparticles: The rational design. *Beilstein J. Nanotechnol.* **2020**, *11*, 866–883. [CrossRef] [PubMed]
57. Schapira, M.; Calabrese, M.F.; Bullock, A.N.; Crews, C.M. Targeted protein degradation: Expanding the toolbox. *Nat. Rev. Drug Discovery* **2019**, *18*, 949–963. [CrossRef] [PubMed]

58. Wang, C.; Zhang, Y.; Wang, J.; Xing, D. VHL-based PROTACs as potential therapeutic agents: Recent progress and perspectives. *Eur. J. Med. Chem.* **2022**, *227*, 113906. [CrossRef]
59. Barankiewicz, J.; Salomon-Perzyński, A.; Misiewicz-Krzemińska, I.; Lech-Marańda, E. CRL4^{CRBN} E3 Ligase Complex as a Therapeutic Target in Multiple Myeloma. *Cancers* **2022**, *14*, 4492. [CrossRef]
60. Ma, Z.; Ji, Y.; Yu, Y.; Liang, D. Specific non-genetic IAP-based protein erasers (SNIPERs) as a potential therapeutic strategy. *Eur. J. Med. Chem.* **2021**, *216*, 113247. [CrossRef]
61. Chen, M. Permeability Characterization and Potential Transporter(s) Identification for Immunomodulatory Drugs (IMiDs) and Application of Pharmacokinetic Modeling in Resistance in Multiple Myeloma. Doctoral Dissertation, Ohio State University, OhioLINK Electronic Theses and Dissertations Center, Columbus, OH, USA, 2022. Available online: https://rave.ohiolink.edu/etdc/view?acc_num=osu1641214592550088 (accessed on 7 July 2023).
62. Pardridge, W.M. Blood-Brain Barrier and Delivery of Protein and Gene Therapeutics to Brain. *Front. Aging Neurosci.* **2020**, *11*, 373. [CrossRef]
63. van Dyck, C.H.; Swanson, C.J.; Aisen, P.; Bateman, R.J.; Chen, C.; Gee, M.; Kanekiyo, M.; Li, D.; Reyderman, L.; Cohen, S.; et al. Lecanemab in Early Alzheimer's Disease. *N. Engl. J. Med.* **2023**, *388*, 9–21. [CrossRef] [PubMed]
64. Rashad, A.; Rasool, A.; Shaheryar, M.; Sarfraz, A.; Sarfraz, Z.; Robles-Velasco, K.; Cherrez-Ojeda, I. Donanemab for Alzheimer's Disease: A Systematic Review of Clinical Trials. *Healthcare* **2023**, *11*, 32. [CrossRef]
65. Inuzuka, H.; Liu, J.; Wei, W.; Rezaeian, A.-H. PROTAC technology for the treatment of Alzheimer's disease: Advances and perspectives. *Acta Mater Med.* **2022**, *1*, 24–41. [CrossRef] [PubMed]
66. Silva, M.C.; Ferguson, F.M.; Cai, Q.; Donovan, K.A.; Nandi, G.; Patnaik, D.; Zhang, T.; Huang, H.T.; Lucente, D.E.; Dickerson, B.C.; et al. Targeted degradation of aberrant tau in frontotemporal dementia patient-derived neuronal cell models. *eLife* **2019**, *8*, e45457. [CrossRef] [PubMed]
67. Vagrys, D.; Davidson, J.; Chen, I.; Hubbard, R.E.; Davis, B. Exploring IDP–Ligand Interactions: Tau K18 as a Test Case. *Int. J. Mol. Sci.* **2020**, *21*, 5257. [CrossRef] [PubMed]
68. Soares, P.; Lucas, X.; Ciulli, A. Thioamide substitution to probe the hydroxyproline recognition of VHL ligands. *Bioorg. Med. Chem.* **2018**, *26*, 2992–2995. [CrossRef] [PubMed]
69. Wang, W.; Zhou, Q.; Jiang, T.; Li, S.; Ye, J.; Zheng, J.; Wang, X.; Liu, Y.; Deng, M.; Ke, D.; et al. A Novel Small-molecule PROTAC Selectively Promotes Tau Clearance to Improve Cognitive Functions in Alzheimerlike Models. *Theranostics* **2021**, *11*, 5279–5295. [CrossRef]
70. Laughlin, C.D.; D'Aquili, E.G. *Biogenetic Structuralism*; Columbia University Press: New York, NY, USA, 1974.
71. Leavy, S.A. Biogenetic Structuralism. *Yale J. Biol. Med.* **1976**, *49*, 420–421.
72. Narum, T. Novel Visible Light Photoactivatable Caged NeurotransmittersBased on a N-Methyl Quinolinium Chromophore. *Yakugaku Zasshi* **2019**, *139*, 263–271. [CrossRef]
73. Subbaraya, I.; Ruiz, C.C.; Helekar, B.S.; Zhao, X.; Gorczyca, W.A.; Pettenati, M.J.; Rao, P.N.; Palczewski, K.; Baehr, W. Molecular characterization of human and mouse photoreceptor guanylate cyclase-activating protein (GCAP) and chromosomal localization of the human gene. *J. Biol. Chem.* **1994**, *269*, 31080–31089. [CrossRef] [PubMed]
74. Young, J.E.; Vogt, T.; Gross, K.W.; Khani, S.C. A short, highly active photoreceptor-specific enhancer/promoter region upstream of the human rhodopsin kinase gene. *Investig. Ophthalmol. Vis Sci.* **2003**, *44*, 4076–4085. [CrossRef]
75. Hammid, A.; Fallon, J.K.; Lassila, T.; Salluce, G.; Smith, P.C.; Tolonen, A.; Sauer, A.; Urtti, A.; Honkakoski, P. Carboxylesterase Activities and Protein Expression in Rabbit and Pig Ocular Tissues. *Mol. Pharm.* **2021**, *18*, 1305–1316. [CrossRef] [PubMed]
76. Hui, K.-S. Brain-specific aminopeptidase: From enkephalinase to protector against neurodegeneration. *Neurochem. Res.* **2007**, *32*, 2062–2071. [CrossRef]
77. Frey, A.; Meckelein, B.; Weiler-Güttler, H.; Möckel, B.; Flach, R.; Gassen, H.G. Pericytes of the brain microvasculature express γ-glutamyl transpeptidase. *Eur. J. Biochem.* **1991**, *202*, 421–429. [CrossRef] [PubMed]
78. Risau, W.; Dingler, A.; Albrecht, U.; Dehouck, M.-P.; Cecchelli, R. Blood-brain barrier pericytes are the main source of γ-glutamyltranspeptidase activity in brain capillaries. *J. Neurochem.* **1992**, *58*, 667–672. [CrossRef] [PubMed]
79. Bausback, H.H.; Churchill, L.; Ward, P.E. Angiotensin metabolism by cerebral microvascular aminopeptidase a. *Biochem. Pharmacol.* **1988**, *37*, 155–160. [CrossRef] [PubMed]
80. Wilk, S.; Healy, D.P. Glutamyl aminopeptidase (aminopeptidase A), the BP-1/6C3 antigen. *Adv. Neuroimmunol.* **1993**, *3*, 195–207. [CrossRef]
81. Song, L.; Wilk, E.; Wilk, S.; Healy, D.P. Localization of immunoreactive glutamyl aminopeptidase in rat brain. I. Association with cerebral microvessels. *Brain Res.* **1993**, *606*, 286–294. [CrossRef] [PubMed]
82. Hamblett, K.J.; Jacob, A.P.; Gurgel, J.L.; Tometsko, M.E.; Rock, B.M.; Patel, S.K.; Milburn, R.R.; Siu, S.; Ragan, S.P.; Rock, D.A.; et al. SLC46A3 is required to transport catabolites of noncleavable antibody maytansine conjugates from the lysosome to the cytoplasm. *Cancer Res.* **2015**, *75*, 5329–5340. [CrossRef] [PubMed]