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Review article

Piezo1 and its inhibitors: Overview and perspectives

Nguyen Duc Thien [a], Nguyen Hai-Nam [a], Duong Tien Anh [a,**], Daniel Baecker [b,*]

[a] Hanoi University of Pharmacy, 13-15 Le Thanh Tong, Hanoi, 100000, Viet Nam
[b] Department of Pharmaceutical and Medicinal Chemistry, Institute of Pharmacy, Freie Universität Berlin, Königin-Luise-Straße 2+4, Berlin, 14195, Germany

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ABSTRACT

The cation channel Piezo1, a crucial mechanotransducer found in various organs and tissues, has gained considerable attention as a therapeutic target in recent years. Following this trend, several Piezo1 inhibitors have been discovered and studied for potential pharmacological properties. This review provides an overview of the structural and functional importance of Piezo1, as well as discussing the biological activities of Piezo1 inhibitors based on their mechanism of action. The compounds addressed include the toxin GsMTx4, Aβ peptides, certain fatty acids, ruthenium red and gadolinium, Dooku1, as well as the natural products tubeimoside I, salvianolic acid B, jatrorrhzine, and escin. The findings revealed that misexpression of Piezo1 can be associated with a number of chronic diseases, including hypertension, cancer, and hemolytic anemia. Consequently, inhibiting Piezo1 and the subsequent calcium influx can have beneficial effects on various pathological processes, as shown by many *in vitro* and *in vivo* studies. However, the development of Piezo1 inhibitors is still in its beginnings, with many opportunities and challenges remaining to be explored.

1. Introduction

Mechanotransduction, the process by which mechanical forces are transformed into electrochemical signals [1], is a key contributor to numerous biological processes, including touch and pain sensation, blood pressure regulation, and cell homeostasis [2–5]. The principal mechanism of mechanotransduction was established decades ago with the identification and characterization of mechanosensitive ion channels [6]. Still, the molecular identities of these channels remained elusive until the groundbreaking discovery of the Piezo channels by Patapoutian and co-workers [7].

Subsequent studies on two members of the Piezo family, Piezo1 and Piezo2, have provided invaluable insights into the molecular basis and biological significance of mammalian mechanotransduction [8]. While Piezo2 has so far mainly been discussed in connection with mechanosensation [9–15], Piezo1 specifically stands out as a promising therapeutic target for drug development. It is widely expressed in multiple cell types [16] and involved in various (patho)physiological processes such as vascular development [17], bone remodeling [18], and tumor progression [19], among others. Despite the absence of a fully resolved molecular structure and ligand-binding mechanism, progress has been made recently in developing novel selective inhibitors of the Piezo1 channel. However, the discovery of inhibitors is still considered in its infancy.

Studies indicate that Piezo1 inhibitors hold promise as potential candidates for treating a spectrum of human diseases, spanning from cancer to neurologic diseases [20]. Therefore, this review aims to comprehensively cover the structural features and biological activities of Piezo1, along with the latest advances regarding the discovery and pharmacological effects of selective Piezo1 inhibitors.

2. Structure of Piezo1 channel

As illustrated in Fig. 1, Piezo1 possesses a unique structure compared to other mechanically activated (MA) protein channels. It exists in a three-blade, propeller-shaped homotrimeric form, with each subunit consisting of more than 2500 amino acids (2521 in human Piezo1 and 2547 in mouse Piezo1) [22–24]. Numerous topological studies have determined the central ion-conducting pore and the three peripheral propeller-like blades as the two important regions for Piezo1 function as a mechanosensitive channel [25].

The last two transmembrane helices (TM), termed the outer helix – inner helix (OH – IH) pair, the C-terminal extracellular domain (CED), and the intracellular C-terminal domain (CTD) trimerize to form the

* Corresponding author.
** Corresponding author.
E-mail addresses: anhdt@hup.edu.vn (D.T. Anh), d.baecker@fu-berlin.de (D. Baecker).

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pore module of Piezo1 [22,26]. The CED constitutes the cap of the ion-conducting pathway and is located between the OH and the IH [22]. Because the top of the Cap is sealed, cations may access the pathway via fenestration sites located directly above the membrane [24]. The transmembrane part of the pore is lined by hydrophobic amino acid residues of the IH, which constrict to form a neck in the middle of the membrane [24]. Intriguingly, the pore has two transmembrane gates enclosed by one OH and two adjacent IHs. These gates can possibly be controlled by membrane lipids [24]. Below the transmembrane pore, cations entered the cytosol via two intracellular routes, either through the 10 Å-long constriction site or the three side portals gated by lateral plugs [23,24,27].

Each propeller blade possesses a striking 36-TM topological organization, which is grouped into nine repetitive transmembrane helical units (THU) [24]. The C-terminal THU9 is connected to the OH by a triangular α-helices arrangement, called the anchor domain [22,23]. Mutations in residues of the Piezo1 anchor are proven to cause altered mechanosensitivity and inactivation, suggesting its crucial role in Piezo1 mechanotransduction mechanisms [28]. Furthermore, three intracellular, 90 Å-long beams lever the whole structure by linking TM28 of THU7 to TM29 of THU8 [24]. Based on its position within the Piezo1 architecture, the beam might play a critical role in transducing mechanical force sensed by the distal blade to the central ion-conducting pore [22]. The N-terminal region of the blade, which contains THU1-3, is extremely flexible and yet to be resolved, thus contributing to the mechanosensitivity of the Piezo1 channel [22,24].

An unusual feature of Piezo1 is the highly curved blade that allows the protein to deform the membrane locally into a dome structure [21]. Upon opening the channel, the surrounding membrane becomes flattened, thus causing the dome to expand into an in-plane conformation [21]. This process generates free energy that might serve as the origin of the mechanical gating of Piezo1 [21]. The "membrane dome" hypothesis is evidence of Piezo channels following the force-from-lipids (FFL) model, which states that mechanosensitive channels can be directly modulated by changes in local membrane curvature and tension [29]. Another model that can explain the sensitivity of Piezo channels to long-range mechanical stimuli is the force-from-filament (FFF) model, which allows whole-cell mechanosensing via the cytoskeleton [30,31]. Indeed, Piezo channels are physically tethered to the actin cytoskeleton by the cadherin-β-catenin-vinculin complex, and the Cap domain of Piezo1 also interacts directly with the extracellular domain of E-cadherin [32]. In living cells, Piezo1 can utilize the FFF model in harmony with the FFL model, thereby enabling them to function as versatile and flexible mechanotransducers [31].

3. Physiological and pathological importance of Piezo1

The first study about Piezo1-associated human diseases was published in 2012, two years after the discovery of Piezo channels as an essential contributor to mammalian mechanotransduction. In this study, gain-of-function mutations of human Piezo1 at the amino acids 2225 and 2456 are linked to hereditary xerocytosis, a rare genetic defect that causes erythrocyte dehydration [33]. Conversely, the loss-of-function variant G2029R is believed to induce congenital lymphatic dysplasia and lymphoedema [34]. Further investigations revealed that Piezo1 not only regulates red blood cell volume homeostasis [35], but also plays an indispensable role in vascular development [17,36], hypertension-induced arterial remodeling [37], and neuronal blood pressure control [38]. Also, several molecular mechanisms have been proposed to elucidate the effects of Piezo1 on cardiovascular systems, including flow-induced release of ATP from erythrocytes [39], and renin downregulation via increased PGE_2 levels in juxtaglomerular cells [40].

Piezo1 is also widely expressed in skeletal systems, where it is involved in many physiological and pathological processes regarding bone formation and homeostasis [18,41–44]. Reduced expression of Piezo1 is closely associated with defective osteogenesis and bone

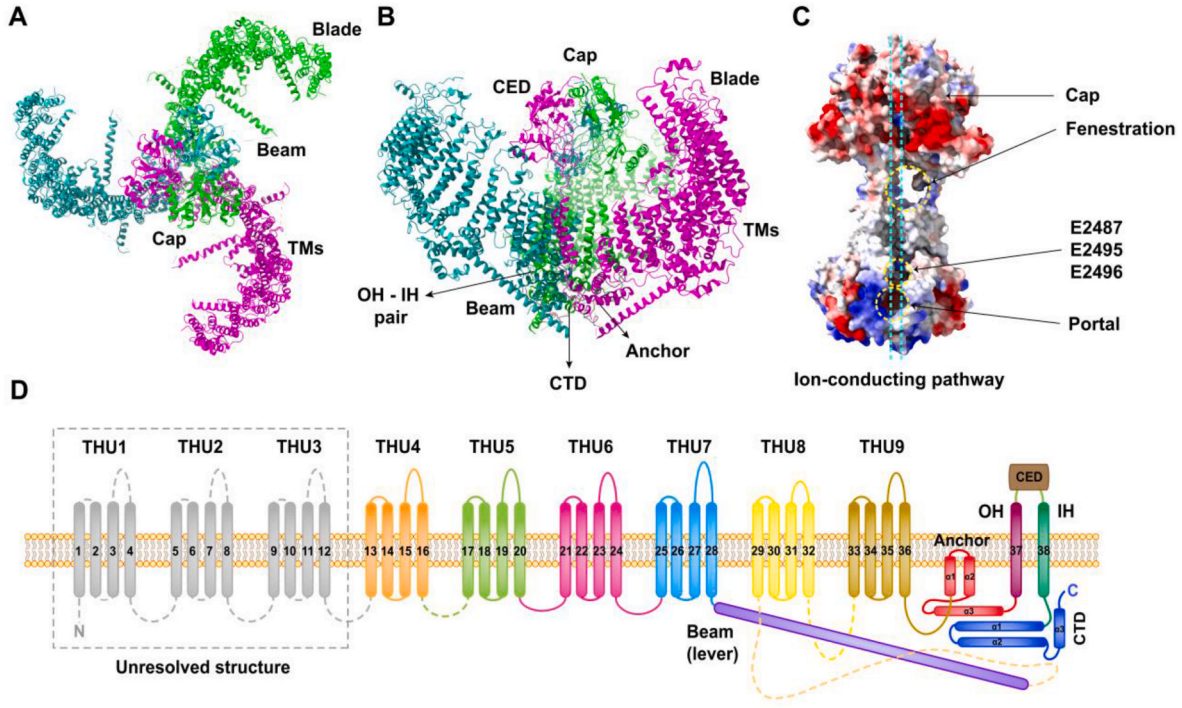

Fig. 1. Structural and topological model of mouse Piezo1 (mPiezo1) protein (adapted from Ref. [21], PDB code 6B3R). (A) Extracellular view of Piezo1, showing the three-bladed, propeller-like structure. (B) Side view of Piezo1, with major domains labeled to reveal the transmembrane helices (TMs), anchor, beam, C-terminal domain (CTD), C-terminal extracellular domain (CED), and the outer helix – inner helix (OH – IH) pair. The CED trimerizes to form the extracellular cap of the ion-conducting pathway. (C) The pore module represented as a surface electrostatic potential map. The ion-conducting pathway is marked with azure dashed lines. The two fenestration sites and the intracellular side portals are indicated with yellow-dashed circles. (D) The 38-TM topological model of Piezo1, showing the featured domains as demonstrated in (B). The dashed lines indicate unresolved structure.

integrity, possibly via increased Wnt1 production [45] or down-regulation of the Wnt signaling inhibitor Sost [46]. Knockout Piezo1 in mice also blunts the response of osteoblasts to mechanical force, thus promoting osteoclast-regulated bone resorption [45]. Taken together, Piezo may represent a novel therapeutic target for treating bone-related skeletal disorders, namely mechanical-unloading-induced bone loss and osteoporosis.

Although overexpression of Piezo1 is found in a variety of malignancies, including gastric [47,48], bladder [49], prostate [50], and breast cancers [51], its role in regulating different stages of tumor cell development remains rather elusive. As stress-activated non-selective cation channels, Piezo1 can allow Ca^{2+} permeation into the intracellular space, which in turn triggers several downstream signaling pathways involved in cell proliferation and metastasis, including YAP [52,53], AKT-mTOR [50,54], and integrin-FAK [47]. Furthermore, Piezo1 binds to and co-expresses with trefoil factor family 1 (TFF1), which is an important mediator of cell mobility and migration [47]. In colon cancer cells, Piezo1 promotes angiogenesis through upregulation of the hypoxia inducible factor 1 subunit alpha (HIF-1α), a transcriptional factor of vascular endothelial growth factor (VEGF) [55]. Interestingly, activation of Piezo1 by fluid shear stress and the agonist Yoda1 sensitizes cells to TRAIL-mediated apoptosis, as demonstrated in the case of prostate cancer [56] and glioblastoma cells [57]. Therefore, Piezo1 can function as a dual-regulator in different types of cancer, a hypothesis that requires further studies to confirm.

Immune cells also utilize Piezo1 as sensors of mechanical cues to optimize immune responses depending on the microenvironment they are exposed to [58]. Indeed, when cyclical pressure is applied on myeloid cells, subsequent Piezo1 activation leads to activation of activating protein 1 (AP-1), transcription of endothelin 1, and stabilization of HIF-1α, thus initiating a pro-inflammatory response [58]. It has also been shown that Piezo1 is critically involved in the activation of T-cells [59,60] and macrophages [61,62] through separate mechanisms [59,61]. Finally, Yoda1, a Piezo1 agonist, increases the production of inflammatory cytokines IL-6 and tumor necrosis factor-α (TNF-α) in dendritic cells [63], further illustrating the important roles of Piezo1 in mediating innate immunity against many types of cancer and infectious diseases [64].

4. Pharmacology of Piezo1 inhibitors

Given that Piezo1 plays a pivotal role in many disease-associated biological pathways, targeting this mechanosensitive protein would constitute a novel strategy towards the management of certain pathological conditions, such as dehydrated hereditary xerocytosis (DHS), osteoporosis, cancers, and some inflammatory processes [65]. Despite the fact that Piezo1 lacks a fully-resolved molecular architecture and specific ligand-binding mechanisms, substantial progress has been made in the discovery of its inhibitors, with many candidates demonstrating propitious *in vitro* and *in vivo* results. The inhibitors can be classified according to whether they are small molecules or larger molecules such as natural compounds or biomolecules (peptides or lipids). Another distinction is based on whether the inhibitors have a non-competitive or competitive effect. The latter differentiation was followed in this review. Generally, the inhibitors include the neurotoxin GsMTx4 obtained from tarantulas, the amyloid peptides known from the amyloid plaques in

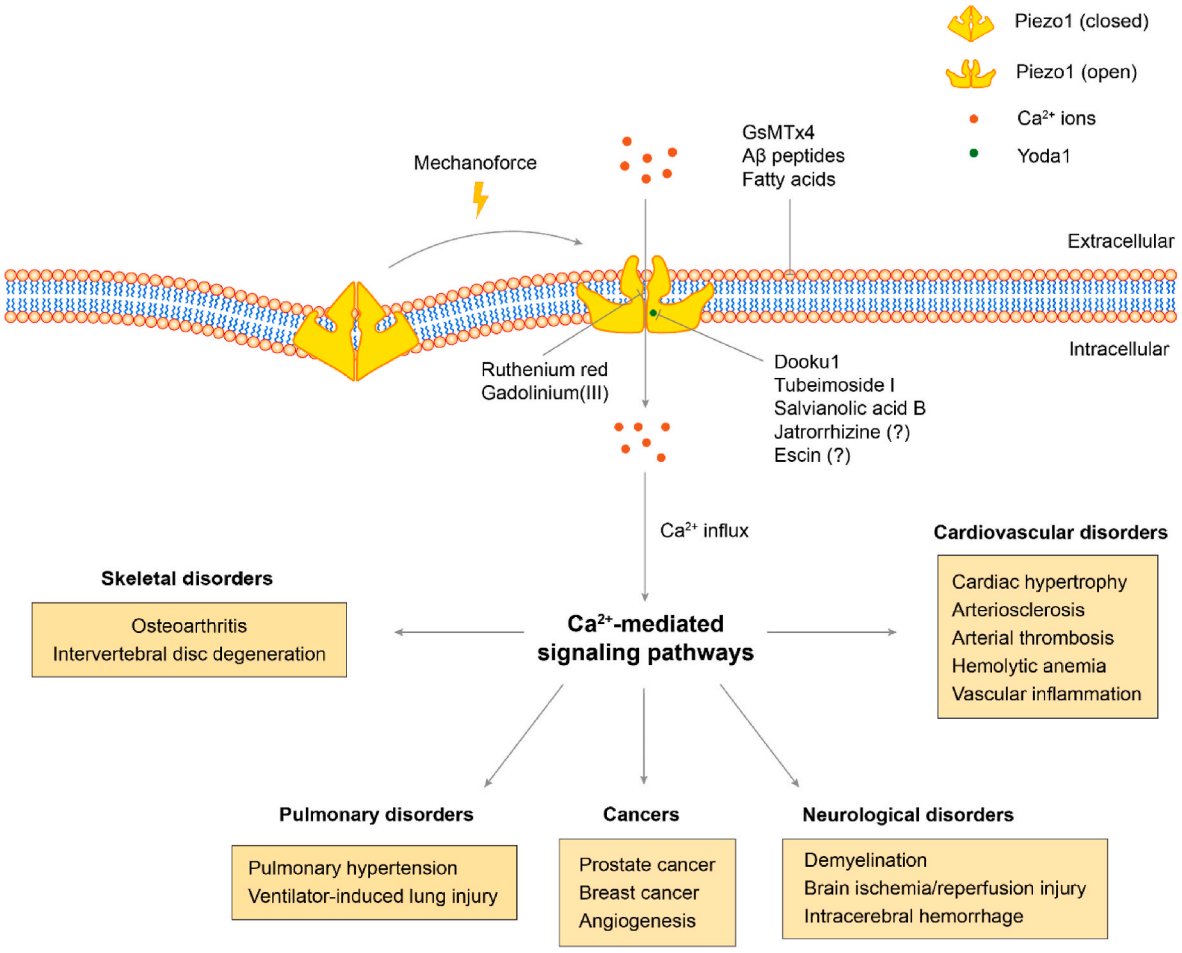

Fig. 2. Schematic overview of the disorders affected by the pharmacological activities resulting from the inhibition of Piezo1 and the sites of action of the inhibitors known to date.

Alzheimer's disease, fatty acids (margaric acid, arachidonic acid, eicosapentaenoic acid), metal-containing compounds such as gadolinium (III) and ruthenium red, the Yoda1 derivative Dooku1, as well as the natural compounds tubeimoside I, salvianolic acid B, jatrorrhizine, and escin. These known inhibitors are presented in more detail below, taking into account their targets in the pharmacological pathways controlled by Piezo1 (Fig. 2).

4.1. Non-competitive inhibitors of Piezo1

4.1.1. Ruthenium red and gadolinium

Tetradecaammine-1κ^5*N*,2κ^5*N*,3κ^4*N*-di-μ-oxidotriruthenium hexachloride (Fig. 3), known as ruthenium red (RR), is a cationic dye dated from the nineteenth century and has been extensively utilized in biological research as a non-selective inhibitor of calcium channels [66]. Together with the discovery of the Piezo protein family, Coste et al. demonstrated that RR is a blocker of Piezo1-induced mechanosensitive current in C2C12 cells, with an IC_{50} value of 5.4 ± 0.9 μM at −80 mV [7]. Furthermore, when applied from the extracellular side, RR inhibits inward, but not outward, MA currents of mPiezo1, indicating a pore-blocking mechanism [67]. Given that RR is a cationic molecule, one might expect its binding to be mediated through ionic interaction with negatively charged residues in the central pore region. Indeed, mutating the acidic residues E2495 and E2496 to alanine rendered mPiezo1 insensitive to RR blockade [26]. Intriguingly, these residues are located in the intracellular side portal [24], which cannot be readily accessed by RR from the extracellular side. Instead, due to the wide ion-conducting pathway and the lack of charge-filtering residues of Piezo1, RR may penetrate through the pore to reach its putative binding sites located in the intracellular vestibule [4].

Piezo1 is also inhibited by gadolinium (Gd^{3+}), a trivalent lanthanide well-known for blocking many types of MA cation channels [68]. It had been demonstrated that 30 μM of Gd^{3+} blocked about 80 % of Piezo1-induced MA currents [7]. Regarding the general mechanism of inhibition, Gd^{3+} can effectively bind to anionic phospholipids in the membrane, such as phosphatidylserine (PS), with high affinity. The resulting compaction of PS causes the membrane lateral pressure to increase, which further stabilizes the channel toward the closed state [69,70]. Though the specific mechanism of Gd^{3+} on the Piezo1 channel has yet to be elucidated, it is reasonable to conclude that Gd^{3+} blocks Piezo1 by interfering with the adjacent membrane lipids rather than directly occluding the channel pore. It is important to note that both Gd^{3+} and RR are non-specific inhibitors of Piezo1 channel with many off-targets and limited therapeutic applications [71]. Instead, Gd^{3+} and RR are now mainly utilized as pharmacological tools to probe for Piezo1 functions in different types of cells and tissues [72–76].

4.1.2. Grammostola mechanotoxin #4

Grammostola mechanotoxin #4 (GsMTx4), extracted from the venom of the tarantula spider *Grammostola spatulata*, is the first identified specific inhibitor of mechanosensitive channels [77]. It is a peptide consisting of 35 amino acids (GCLEFWWKCNPNDDKCCRPK LKCSKLFKLCNFSSA) [78]. The peptide possesses a unique structure named inhibitor cysteine knot (ICK), which contains three disulfides formed by cysteine pairs (C_1–C_4, C_2–C_5, and C_3–C_6) enveloping a

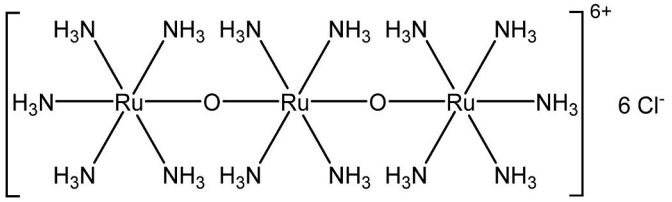

Fig. 3. Chemical structure of ruthenium red, a metal-based pore-blocker of the Piezo1.

triple-stranded β-sheet core [79]. GsMTx4 is an amphipathic molecule, with aromatic and lysin residues forming the hydrophobic domain and the hydrophilic surface, respectively [80]. In 2011, Bae et al. discovered that GsMTx4 functions as a gating modifier of Piezo1, with a K_D value of 157 nM. When applied to the extracellular face of outside-out patches, GsMTx4 induces an approximate 30 mmHg rightward shift in the pressure-response curve. Interestingly, both the enantiomeric D- and L-forms of GsMTx4 can equally block the Piezo1 channel activity, suggesting a non-canonical mechanism of inhibition [81].

The current model of GsMTx4 inhibition proposes that it acts by changing the local membrane tension instead of binding directly to the gating elements of the Piezo1 channel [82]. Molecular dynamic simulations suggested that, in the unstressed membrane, GsMTx4 can occupy a small area on the bilayer surface, where it is stabilized by positive charges of lysine residues. When applied pressure forces the lipids to stretch, GsMTx4 can physically sink deeper into the membrane, creating an "area reservoir" to "clamp" the tension applying to the outer monolayer. Consequently, the efficiency of stimulus transmission to the Piezo1 channel is disrupted [83]. Of note, GsMTx4 primarily exerts its activity on closed channels, as perfusion of the peptide in advance of mechanical stimulation almost completely blocks the Piezo1 channel [81].

Due to its specificity as an inhibitor of cationic mechanosensitive channels, GsMTx4 has been used extensively to investigate the physiological and pathological roles of the Piezo1 channel. Consistent with this trend, numerous studies have explored the therapeutic effects of GsMTx4 in relation to Piezo1 inhibition. In an *in vitro* model of neonatal rat cardiomyocytes (NRCMs), GsMTx4 can ameliorate cardiac hypertrophy mediated by Piezo1 activation [84]. The protective effects of GsMTx4 in vitamin D-induced atherosclerosis and calcification of mice vascular endothelial cells were also reported [85]. Furthermore, inhibition of Piezo1-induced Ca^{2+} influx by GsMTx4 not only effectively attenuates the severity of arterial thrombosis, but also reduces the infarct area of stroke and improves the prognosis of ischemic stroke in hypertensive mice [86].

GsMTx4 also demonstrates potential in the treatment of lung diseases [87–89], particularly pulmonary hypertension, and ventilator-induced lung injury (VILI). In 2019, Friedrich et al. showed that the blockade of Piezo1 by GsMTx4 can reduce lung capillary hyperpermeability caused by increased blood pressure [90]. In accordance with this result, GsMTx4 treatment in mice with chronic pulmonary hypertension (PH) results in retrogression of pulmonary vascular remodeling and partial reversal of the established PH [91]. Furthermore, inhibition of Piezo1 by GsMTx4 is sufficient to alleviate the histological abnormalities and the inflammatory response observed in VILI, as well as significantly reduce the mortality rate in rats model [92].

The importance of Piezo 1 in skeletal systems [18,93–97], as noted earlier, prompted various studies about the relationship between GsMTx4 and bone-related issues. Targeting Piezo1 by GsMTx4 has been illustrated to alleviate the severity of osteoarthritis [98–100] and protect chondrocytes from cell death after mechanical injury [101]. Immunofluorescence and Western blot analysis indicated that GsMTx4 can block the Piezo1/Calcineurin/NFAT1 signaling pathway, thus contributing to its anti-osteoarthritis effect [100]. In degenerated human intervertebral discs, the upregulation of Piezo1 decreases the synthesis of the extracellular matrix but increases the production of pro-inflammatory cytokines (IL-6, IL-1β, and TNF-α) and Nod-like receptor protein 3 (NLRP3) inflammasome, leading to an imbalance between apoptosis and autophagy process. However, GsMTx4 can partially reverse these effects [102,103].

GsMTx4 also serves as a promising candidate in the treatment of neurological disorders [104]. Using an organotypic slice culture of murine cerebellum, Velasco-Estevez et al. demonstrated that GsMTx4 is able to rescue demyelination caused by psychosine *ex vivo*, as well as ameliorate astrocyte cell death and microglial reactivity *in vivo* [105]. The neuroprotective properties of GsMTx4 are also exhibited in brain

ischemia/reperfusion injury (IRI). Oxygen-glucose deprivation/reoxygenation (OGD/OGR) is an *in vitro* model that can imitate the ischemia/reperfusion condition experienced by neurons during IRI [106]. Inhibition of Piezo1 by GsMTx4 partly rescue neuron-like PC12 cells from OGD/OGR-induced apoptosis via decreased intracellular Ca^{2+} concentration and downstream calpain activity [107]. GsMTx4 also plays a role in the treatment of intracerebral hemorrhage (ICH), as it can downexpress proinflammatory cytokines and enhance behavioral outcomes in rats with ICH [108]. Most recently, Ma et al. showed that administration of GsMTx4 to the basal forebrain area can alleviate both short and long-term fear memory impairments in sleep-deprived rats [109].

The possibility of GsMTx4 being a novel strategy for cancer treatment has also been explored [51,110,111]. In prostate cancer cells, blockade of Piezo1-induced Ca^{2+} inward current by GsMTx4 leads to inhibition of Ca^{2+}-mediated Akt/mTOR pathway [50], which plays a crucial role in tumor development [112]. Moreover, in the presence of GsMTx4, both the migration and invasion of breast cancer cells are effectively abrogated [51,113]. Another hallmark of cancer, angiogenesis, was also markedly reduced when human umbilical vein endothelial cells (HUVECs) were incubated with GsMTx4 [114].

To summarize, the *in vitro* and *in vivo* studies outlined above have highlighted the potency of GsMTx4 in counteracting a plethora of Piezo1-associated diseases. With that being said, the effects of GsMTx4 in other cationic mechanosensitive channels, either inhibition or potentiation, somewhat hamper its further development and clinical trial as a drug candidate [82]. Further research is required to design more Piezo1-selective derivatives of GsMTx4, as well as to elucidate the relationship between GsMTx4 and the management of Piezo1-related diseases.

4.1.3. *Aβ peptides*

The amyloid beta (Aβ) is a group of peptides that serve as key players in the pathogenesis of Alzheimer's disease. Similar to GsMTx4, the amphipathic nature of Aβ peptides is indicative of them being able to modulate the membrane structure. Considering that Piezo1 channels are responsive to alterations in membrane tension and stiffness, Maneshi et al. investigated whether Aβ peptides can affect cellular mechanotransduction by inhibiting Piezo1 activity [115].

Treatment of a Piezo1-overexpressing cell line (hP1-CL) with 10 μM of Aβ(1–40) or Aβ(1–42) peptides resulted in inhibition of fluid shear stress response [115]. However, these two peptides have no effect on Piezo1 currents in the outside-out patch, even though the same concentration blocking shear stress was used. A possible explanation is that the pressure inside the patch may induce modifications to the membrane properties, thus hindering the binding and penetration of Aβ peptides [115]. As is also the case with GsMTx4 [83], a membrane-associated mechanism of inhibition is suggested for Aβ peptides, based on two observations: 1) both the D- and L-form of Aβ peptides are equally effective on Piezo1, and 2) the peptide can co-localized with the channel in particular regions of the membrane [115]. Titration of Aβ response indicated that the monomeric Aβs are 10^3-fold–10^6-fold more potent than the oligomer counterparts, with the K_i ranging from femtomolar to picomolar concentrations [115].

The efficacy of Aβ peptides prompted further investigations of their involvement in Piezo1-mediated physiological and pathological processes. Indeed, low concentration (10 pM) of Aβ peptides significantly reduces cell motility by 10-fold, meanwhile increasing F-actin cytoskeleton stress [115]. In addition, removal of extracellular Ca^{2+} is followed by a marked increase in the resting actin stress [115]. This further supports the idea that Piezo1-mediated Ca^{2+} entry plays a critical role in regulating cytoskeletal reorganization and consequently cell migration. In contrast, the accumulation of Aβ peptides is closely linked with Piezo1 upregulation in reactive astrocytes [116] and microglia [117,118]. Taken together, further studies are necessary to fully elucidate the regulation of Piezo1 activity by Aβ peptides, as well as the accompanying therapeutic implications.

4.1.4. *Saturated and polyunsaturated fatty acids*

Dietary fatty acids serve as crucial building blocks of plasma membranes and essentially contribute to their mechanical properties, such as bending stiffness and viscosity. Hence, it is predictable that they can regulate the activity of many integral membrane proteins as well, including the mechanosensitive Piezo1 channel. Romero et al. were the first to demonstrate that the saturated margaric acid (*n*-heptadecanoic acid, Fig. 4) inhibits the Piezo1 channel in a concentration-dependent manner ($IC_{50} = 28.3 \pm 3.4$ μM), with negligible effect on inactivation time constants [119]. However, this fatty acid has no effect on the Piezo2-induced current [120]. This intriguing specificity may result from a lack of Piezo2 sensitivity to membrane stiffness, which is further attributed to the force-transducing blade region of the channel [120]. In contrast, the inhibitory effect of margaric acid is mainly due to an increase in membrane order and binding stiffness, which further stabilizes the closed state of the Piezo1 channel [119].

Several polyunsaturated fatty acids (PUFAs) are also negative modulators of Piezo1 [119]. Supplementing N2A cells with either arachidonic acid (AA, Fig. 4) or eicosapentaenoic acid (EPA, Fig. 4) significantly accelerated Piezo1 inactivation by about 2-fold compared to control [119]. The authors proposed that AA and EPA can achieve this effect by either mediating the allosteric coupling between the inner pore helix (IH) and the C-terminal extracellular domain (CED) or disrupting the regulation of Piezo1 by other membrane proteins. Of note, combining EPA and margaric acid had a synergistic effect on Piezo1 activity, as demonstrated in both wild-type and gain-of-function R2456H mutant channels [119]. Given that this mutation is closely linked to red blood cell dehydration and hemolysis, monotherapy or combination therapy of dietary fatty acids could represent a novel strategy to counteract the deleterious effects observed in Piezo1-linked hemolytic anemia [119].

4.2. *Competitive inhibitors of Piezo1*

4.2.1. *Dooku1*

In an attempt to elucidate the structure-activity relationship of the Piezo1 agonist Yoda1, Evans et al. synthesized a number of Yoda1 derivatives and tested their effects on Piezo1 activity. Among these analogues, the derivative Dooku1 (Fig. 5) was a selective antagonist of Piezo1 ($IC_{50} = 1.3$ μM) without agonist activity [121]. Of note, Dooku1 possesses a pyrrole-2-yl oxadiazole moiety in substitution for the pyrazin-2-yl thiadiazole in Yoda1 (Fig. 5), suggesting that these structural features may play a crucial role in its inhibitory effects [121]. Regarding the mechanism of action, Dooku1 can act as a competitive inhibitor to Yoda1, given the structural similarities between these two compounds [121]. Wijerathne et al. further confirmed this hypothesis by demonstrating that Dooku1 is a "silent binder", having no effect on channel kinetics and opening probability [122]. Surprisingly, a study by Hatem et al. found that Dooku1 can simultaneously activate and inhibit Piezo1 activity on red blood cell membrane [123]. Therefore, the pharmacology of Dooku1 might not be *prima facie* straightforward, creating a knowledge gap for future studies to fulfill.

The pharmacological activities of Dooku1 are also gradually gaining the attention of researchers in recent years, with special emphasis on the cardiovascular system. In HbS-mutated erythrocytes, Dooku1 can reduce the level of membrane-exposed PS via decreasing Piezo1-induced Ca^{2+} influx, thus may prevent thrombosis and red cell death associated with sickle-cell anemia [124]. Activation of Piezo1 facilitates Ca^{2+} entry in enlarged cardiomyocytes and exacerbates the conditions of cardiac hypertrophy, which could be ameliorated by Dooku1 [84]. Furthermore, experiments in human aortic smooth muscle cells [125] and mice aortic valves [126] revealed that Piezo1 upregulation led to an increase in osteogenic responses and subsequent cell calcification, while application of Dooku1 partially reversed these adverse effects.

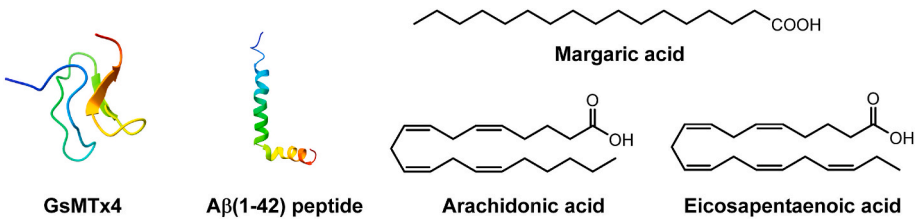

Fig. 4. The toxin GsMTx4, Aβ(1–42) peptides, and fatty acids as inhibitors of Piezo1 by modulating local membrane mechanics.

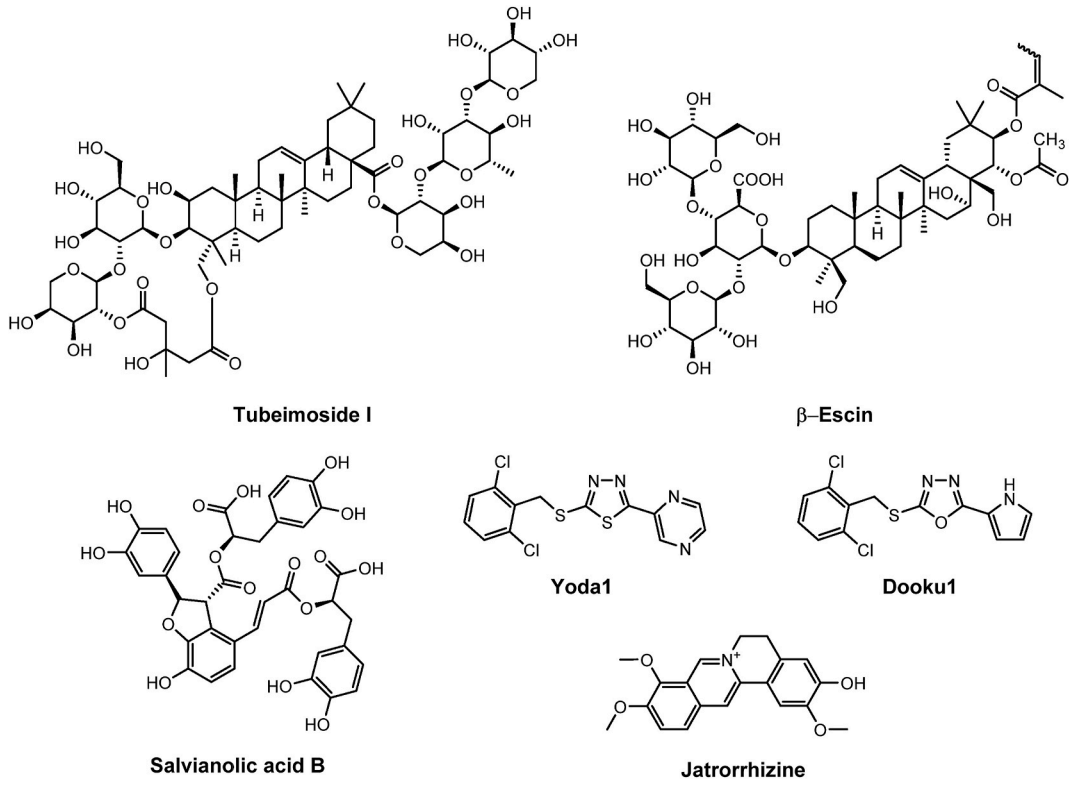

Fig. 5. Chemical structures of the competitive inhibitors of Piezo1, namely tubeimoside I, Dooku1 (Piezo1 agonist Yoda1 for comparison), salvianolic acid B, jatrorrhizine, and β-escin.

The therapeutic applications of Dooku1 in other Piezo1-associated disorders were also investigated. Kenmochi et al. observed that Dooku1 exerts inhibitory effects on the Yoda1-induced suppression of brown adipocytes differentiation [127], which itself is an important target for the treatment of metabolic diseases including obesity [128]. Dooku1 is also involved in alleviating neurological dysfunctions followed by intracerebral hemorrhage, namely brain edema, myelin shedding, neural tissue degeneration, and oligodendrocyte apoptosis [129]. Collectively, the above-mentioned discoveries emphasize the potentials of Dooku1 as a selective and versatile Piezo1 inhibitor. Nonetheless, given the poor solubility in body fluids that somewhat precludes Dooku1 *in vivo* activities, there is an urgent need for the development of novel analogues with enhanced pharmacological and pharmacokinetic profiles.

4.2.2. *Tubeimoside I*

Tubeimoside I, a triterpenoid saponin (Fig. 5) extracted from the Chinese herbal medicine *Bolbostemmatis Rhizoma*, is a renowned candidate for the treatment of various tumor diseases [130] including, but not limited to, gastric cancer [131], breast cancer [132], lung cancer [133], and glioma [134]. Recently, by conducting a screen of 92 natural compounds for potential Piezo1 regulators, Liu et al. found that tubeimoside I exerted profound inhibitory effects on Yoda1-induced Ca^{2+} responses in endothelial cells (HUVECs and MLECs), macrophages (THP-1 and RAW264.7), and Piezo1-overexpressing HEK 293T cells [135], with IC_{50} values ranging from 1.11 to 6.97 μM. Tubeimoside I also has relative selectivity towards Piezo1, as it did not block Ca^{2+} currents induced by other mechanosensitive channels including TRPC5, TRPM2, and TRPV4 [135]. Since there is a negative correlation between the concentration of Yoda1 and the inhibitory response of tubeimoside I, these two compounds may reversibly compete with each other for Piezo1 in the Yoda1 binding sites [135]. Physiologically, tubeimoside I can reduce aortic relaxation induced by Yoda1, which is consistent with a previous result by Evans et al. [121]. Nevertheless, given the ubiquitous effects of tubeimoside I in malignant diseases, more efforts are required to further establish the roles of this natural compound in Piezo1-mediated pathological processes, as well as to explore potential therapeutic applications.

4.2.3. *Salvianolic acid B*

The cardioprotective effects of Danshen (*Salvia miltiorrhiza* Bge.) and its main active component salvianolic acid B (SalB, Fig. 5) were established centuries ago in traditional Chinese medicine [136]. SalB is a polyphenolic compound and is the most common, along with salvianolic acid A (SalA). However, there are other salvianolic acids (e.g., SalC, SalD, SalE, SalF, SalG), which all share the core of (*R*)-3-(3,

4-dihydroxyphenyl)-2-hydroxypropanoic acid. SalB is composed of three of these cores and one molecule of caffeic acid [137]. In the continuing search for novel inhibitors of Piezo1, Pan et al. showed that SalB is also a selective antagonist of the Yoda1-activated Piezo1 channel, with IC_{50} values of 1.37 and 2.20 μM against HUVEC and BMDM cell lines, respectively [138]. Similar to tubeimoside I, SalB presumably exerts its effect on Piezo1 activity via competing with the Yoda1 binding site, and this inhibition is reversible [138].

Subsequent *in vitro* and *in vivo* studies revealed that SalB exhibits protective effects against atherosclerosis, which is dependent on Piezo1 activity [138]. Moreover, SalB inhibits the formation of macrophage-derived foam cells and prevents atherosclerotic plaques from developing in mice models [138]. Considering the traditional application of SalB in the treatment of vascular-related disorders including atherosclerosis [136], these findings may serve as a foundation for developing SalB-based novel cardiovascular drugs that specifically target the Piezo1 channel [138].

In addition, SalB promotes endothelial differentiation of human induced pluripotent stem cells. Piezo1 is involved in this mechanism. MAPK/Erk1/2 signaling is enhanced by triggering the Ca^{2+} flow. This activity is promising for the field of regenerative medicine [139].

4.2.4. Jatrorrhizine

The protoberberine alkaloid jatrorrhizine (Jat, Fig. 5), mainly derived from Chinese plants such as *Coptidis Rhizoma* and *Phellodendri Cortex*, possesses extensive pharmacological profiles [140,141]. In addition to its traditional use as an anti-inflammatory agent [142], recent studies have highlighted that Jat exerts beneficial effects on neurological impairments [143], cancers [144], diabetes mellitus [145,146], and microbial infections [147].

In a previous study by Pan et al., Jat was found to be an efficient inhibitor of Piezo1, with an inhibitory ratio of approximately 75 % [138]. To further investigate the interplay between Jat and Piezo1 activity, Hong et al. utilized a vascular inflammation model induced by partial artery ligation in mice. The findings showed that Jat can reduce the expression of IL-1β, IL-6, and VE-cadherin while upregulating the expression of TGF-β, thereby alleviating the severity of ligation-induced vascular inflammation [148]. *In vitro* studies on H_2O_2-induced HUVECs inflammation further corroborated these results [148]. Interestingly, treatment of endothelial-specific Piezo1-knockout mice with Jat exhibited the same effect with the EC-Piezo1-flox control group [148]. Consistent with these observations, Jat could effectively block the Piezo1-induced Ca^{2+} entry in a concentration-dependent manner [148]. Additionally, the potential therapeutic effects of Jat in vascular inflammation may result from its inhibition of endothelial-mesenchymal transition (EndMT), which is closely related to Piezo1 activation [148]. Taken together, Jat is a prospective candidate for the management of vascular-related diseases. However, the inhibition mechanisms and selectivity of this compound towards Piezo1 remain to be discovered.

4.2.5. Escin

Escin is a mixture of triterpenoid saponins isolated from extracts of the seeds of horse chestnut (*Aesculus hippocastanum* L.). Escin consists of more than 30 components. However, there are two major forms, namely α-escin and β-escin, which are formed when an aqueous solution is heated. The β-escin precipitates, while the α-escin remains in solution. The most important active components include escin A and escin B (also summarized as β-escin) as well as escin C and escin D (α-escin). These derivatives differ in their acetylation pattern and the E/Z-configuration of the exocyclic double bond [149]. Escin itself was used traditionally to counteract various disorders, including anorexia, diarrhea [150], and hemorrhoids [151]. Today, the main clinical indication of escin is to treat chronic venous insufficiency (CVI) and post-operative edema [152,153]. The venotonic effects of escin are largely due to its anti-edematous and anti-inflammatory properties in endothelial cells [152]. However, the precise molecular basis by which escin exhibits its therapeutic effects remains unidentified.

Wang et al. were the first to demonstrate that Piezo1 plays an important role in escin-mediated anti-inflammatory responses [154]. When HUVECs, MLECs, and bEnd.3 endothelial cells (ECs) were treated with escin, Yoda1-induced Ca^{2+} transients were strongly suppressed, with IC_{50} values varying between 1.43 and 1.74 μM depending on the cell type used [154]. Moreover, activating Piezo1 channel either by cyclic stretch or shear stress induced the alignment of ECs, which was inhibited by application of escin [154]. Likewise, escin can suppress the Piezo1-induced expression of IL-1β and IL-6 after ECs are subjected to cyclic stretch to induce an inflammatory response [154]. These results could potentially be linked to the inhibition of NF-κB signaling pathways [154], which in turn are closely regulated by Piezo1 activation [72,103,155]. Therefore, one possible direction for future pharmacological studies of escin is to focus on the vascular protective effects in relation to Piezo1 inhibition.

5. Conclusion and perspectives

Piezo1 is a key mechanotransducer in various organ systems throughout the human body. It can sense mechanical forces and convert them into electrochemical responses embedded in complex signaling pathways. The implications of Piezo1 in many physiological and pathological procedures have also been firmly established, with many instances pertaining to bone homeostasis, cardiovascular functions, and tumor development. Nevertheless, the discovery of drugs targeting Piezo1 is still in its infancy, with only a small number of agonists and antagonists being available thus far. In this review, we summarized the essential pharmacology of Piezo1 inhibitors regarding their underlying mechanisms of action, as far as elucidated, and discussed the accompanied therapeutic potentials of these agents.

In general, targeting the Piezo1 channel by both non-specific and specific inhibitors occurred via three principal molecular mechanisms: (1) direct blockade of the channel ion-conducting pore (Gd^{3+}, ruthenium red), (2) modulations of the proximal lipid bilayers (GsMTx4, Aβ peptides, fatty acids), and (3) competitive inhibition with the Piezo1 activator Yoda1 in its binding sites (Dooku1, tubeimoside I, salvianolic acid B, jatrorrhizine, escin). Consequently, Piezo1 inhibitors can suppress the entry of Ca^{2+} ions into the intracellular space, triggering a network of complex signaling pathways that are critical determinants in many physiological and pathological processes. Indeed, several antagonists (such as GsMTx4, Dooku1, and salvianolic acid B) have demonstrated potential for counteracting a plethora of disorders related to Piezo1 dysfunctions, including, but not limited to, cardiovascular, cancer, musculoskeletal, neurological, and pulmonary diseases. However, further design and optimization of current Piezo1 antagonists is hindered by the absence of a fully resolved protein architecture, as well as proof-of-concept studies to validate the underlying mechanisms and therapeutical significance of Piezo1 inhibition. Therefore, a thorough understanding of the structural basis and essential biological profiles of Piezo1 would facilitate the discovery of more potent and druglike Piezo1 inhibitors.

Nevertheless, it is important to note that inhibition of Piezo1 can simultaneously exert the intended positive and less desired effects, depending on the specific cell types and subtle changes in the external environment. For example, inhibition of Piezo1 by GsMTx4 can induce proliferation, migration, invasion, and prevent apoptosis in lung adenocarcinoma cell lines [110]. In a mouse osteoprotic fracture model, ultrasound-induced new bone formation and fracture healing was impaired by GsMTx4 [156]. Similarly, Dooku1 attenuated the osteogenic functions of SCP-1 cells exposed to extremely low frequency electromagnetic fields (ELF-PEMF) [157]. Combined with the ubiquitous cellular expression of this channel and the non-specific nature of most Piezo1 antagonists, the off-target effects associated with Piezo1 inhibition comprise a major challenge that needs to be addressed to progress towards targeted and risk-free therapy. However, this is the

case with nearly most drug developments but does not weaken the general potential of Piezo1 as a promising drug target. There is no doubt that the design of novel Piezo1 inhibitors will be a rewarding yet challenging journey that remains to be explored.

CRediT authorship contribution statement

Nguyen Duc Thien: Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Nguyen Hai-Nam:** Writing – review & editing, Supervision. **Duong Tien Anh:** Writing – original draft, Visualization, Investigation, Formal analysis, Data curation, Conceptualization. **Daniel Baecker:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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