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HIGHLIGHT

Urate transporters: Structural mechanisms and therapeutic opportunities in drug development

KEY WORDS

Urate;
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Uric acid, the end product of purine metabolism, is tightly regulated by renal transporters to maintain serum homeostasis. Dysregulation of this process leads to hyperuricemia, a precursor to gout—a debilitating inflammatory disorder with rising global prevalence. Despite clinical urgency, the field long faced critical bottlenecks: limited structural insights into urate transporters hindered mechanistic understanding of their function, and drug development relied on empirical screening, leading to off-target effects and suboptimal efficacy (*e.g.*, renal toxicity of early uricosurics).

Urate transporters, characterized by multiple conformational transitions and mutant-specific small-molecule binding profiles, particularly URAT1, have long posed significant challenges to the precise elucidation of their ligand interaction mechanisms—challenges that have recently been overcome through transformative breakthroughs, thereby reshaping the research landscape. High-resolution cryo-electron microscopy (cryo-EM) has unraveled atomic structures of key transporters: urate transporter 1 (URAT1) in complex with urate and therapeutic agents, revealing the pivotal role of the phenylalanine cage and gating residues (R477, D389) in substrate selectivity and drug binding; OAT1−probenecid complexes elucidating its broad substrate specificity *via* polar interactions; and GLUT9−urate structures uncovering a unique recognition mode devoid of a phenylalanine cage. These advances have clarified how transporter dysfunction drives metabolic disorders and provided a structural basis for drug optimization. This article reviews these mechanistic insights, analyzes the commonalities and differences in drug−transporter interactions, and discusses their implications for developing next-generation, precision-targeted gout therapeutics.

1. Inhibitory mechanisms of human URAT1 (hURAT1): Binding modes of urate and uricosuric drugs

hURAT1 is a key SLC22A subfamily member, expressed on the apical membrane of renal proximal tubular epithelial cells. Early computational predictions suggested a topology with 12 transmembrane helices and classification within the major facilitator superfamily. Enomoto et al.[1] first cloned hURAT1, confirming its localization and role in urate reabsorption. As a urate-anion exchanger, hURAT1 is vital for maintaining physiological blood urate levels. Loss-of-function mutations cause renal hypouricemia, while specific polymorphisms are linked to hyperuricemia and gout risk, highlighting its central role in urate homeostasis.

1.1. The interaction between URAT1 and urate

Urate binds to the central pocket of hURAT1, stabilized by $\pi-\pi$ stacking interactions with a phenylalanine cage composed of F241, F360, F364, F365, and F449 (Fig. 1a and d), which is a structural feature distinctive to URAT1 among urate transporters. This hydrophobic interaction may be enhanced by introducing alkyl chains to strengthen van der Waals forces. Substituting F365 or F364 with tyrosine reduces urate uptake, while mutation of F241 to tyrosine nearly abolishes transport activity, identifying it as the core residue sustaining the cage's functional integrity. This narrow hydrophobic cage (8.2 Å between F241 and F449) is key to URAT1's stringent urate specificity, in stark contrast to the broader substrate range of fellow SLC22 family members OAT1 and OAT4—likely reflecting evolutionary divergence where URAT1 specialized for urate homeostasis, while OAT1 retained promiscuous binding for xenobiotic excretion.

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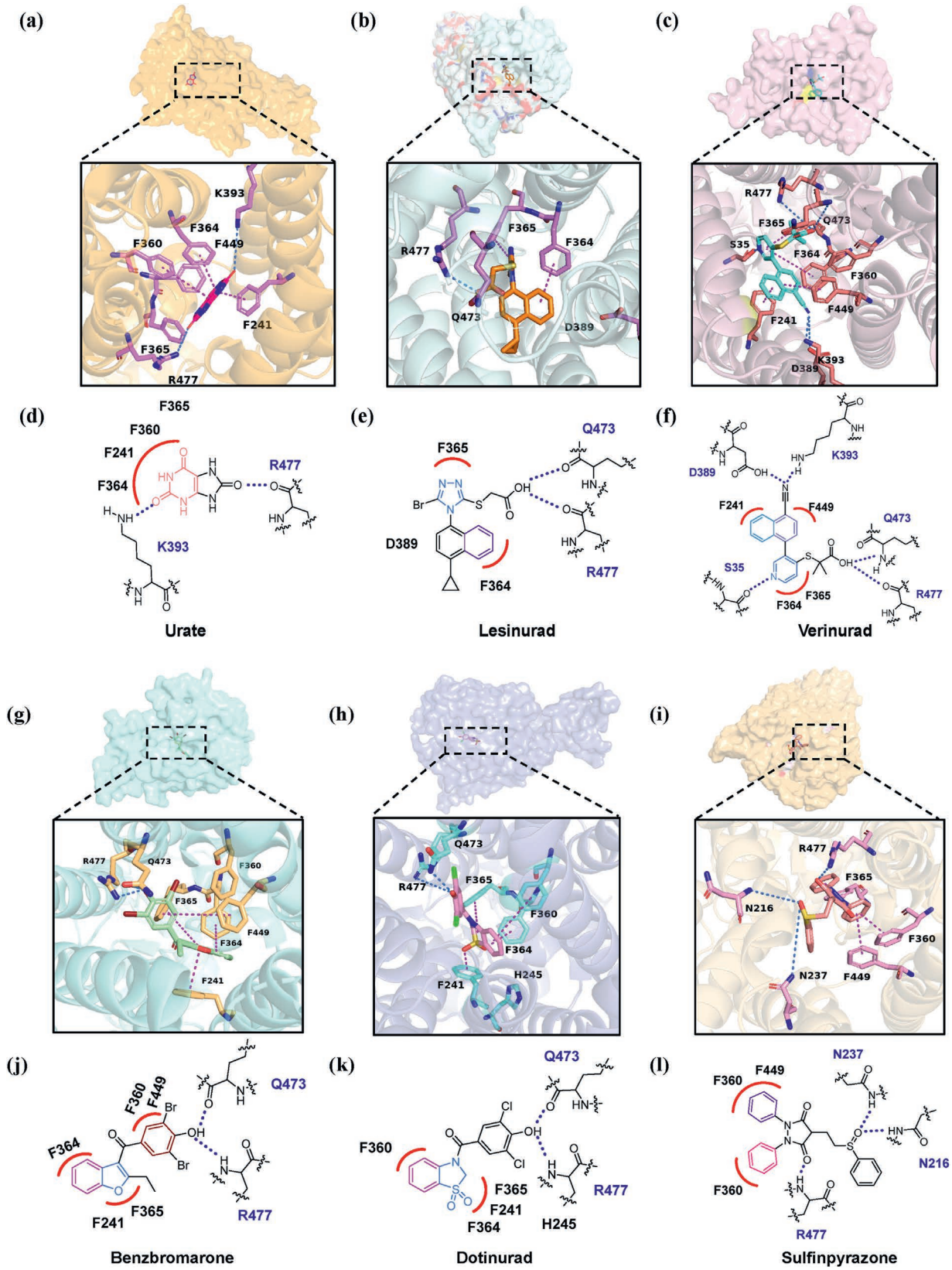

Figure 1 Inhibitory mechanisms of hURAT1 by urate and therapeutic agents. (a–c) Close-up views of binding pockets for urate (PDB code: 9JDV), lesinurad (PDB code: 9JDZ) and verinurad (PDB code: 9JDY). (d–f) Schematic representation of the interaction networks between urate, lesinurad and verinurad with URAT1 residues, depicted in 2D format. (g–i) Close-up views of binding pockets for benzbromarone (PDB code: 9JE0), dotinurad (PDB code: 9JE1), and sulfinpyrazone (PDB code: 9J76). Figure was generated by Schrödinger suite 2023-1 and PyMOL. Surrounding residues are depicted as sticks; hydrogen bonds are indicated by marine dashed lines, and hydrophobic interactions are depicted by

Additionally, urate engages in polar interactions with charged residues K393 and R477 *via* hydrogen bonds (Fig. 1a and d)[2]. Among them, R477 is particularly critical. Dai et al. showed it mediates hURAT1's outward-to-inward conformational transition and coordinates urate transport[3], and its high conservation across SLC22 transporters (*e.g.*, OAT1, OAT4) suggests it may represent a shared conformational gating module in the family. Complementing this, Guo et al. identified that M36 (on the extracellular side of TM1) partially occludes the entrance channel[4], creating an "outward partial closure" state that restricts non-specific urate entry—this structural checkpoint, paired with the phenylalanine cage, synergistically enhances transport efficiency and specificity, traits tailored to URAT1's role as the primary apical urate reabsorber in renal tubules.

Species-specific variations further refine this functional specialization. Yu et al.[5] expressed a brown rat URAT1 mutant (N35S/Y365F) with 74% sequence identity to human URAT1, which retained comparable substrate affinity but showed altered binding when Y365 (rat) was substituted with F365 (human). Their 3.5 Å cryo-EM structure of the rat URAT1−urate complex revealed an inward-facing conformation with a positively charged central cavity, where hydrophobic residues (M214, F360, F365, F449) and electrostatic interactions with R477 mirror human URAT1's core binding mode—yet the Y365F substitution disrupted affinity, underscoring how subtle residue differences tune urate recognition across species. Conformational flexibility of F241 and functional perturbations from R477S mutation also indicate structural plasticity, allowing URAT1 to adapt to varying urate concentrations while maintaining specificity.

1.2. Mechanisms underlying the uricosuric drug-mediated inhibition of URAT1

Uricosuric drugs inhibit the activity of URAT1 by competing with urate for binding sites and stabilizing the transporter's inward-open conformation. This "conformational locking" ensures sustained blockade of urate reabsorption, a trait likely shared with other high-potency URAT1 inhibitors but distinct from simpler competitive binders. Lesinurad and its derivatives verinurad, benzbromarone and its derivatives dotinurad and sulfinpyrazone not only compete with the substrate but also achieve high inhibitory potency by hijacking the gating residues. The revelation of the binding modes and key interaction sites of these drugs provides a structural basis for further optimizing drug design.

1.2.1. Lesinurad

Lesinurad, a uricosuric agent approved in 2015, was discontinued in the United States and withdrawn from the European market due to commercial reasons, with no launch yet in China. At the molecular level, through hydrophobic interactions and $\pi-\pi$ stacking with aromatic and methionine residues, lesinurad effectively blocks uric acid reabsorption. Specifically, the thiazole ring interacts with the phenylalanine cage, the bromine-substituted 1,2,4-triazole ring contacts F364/F365, and the carboxyl group forms polar interactions with Q473 (Fig. 1b and e)[2].

Mutation studies further illuminate how lesinurad's binding integrates URAT1's structural features while revealing residues that may underpin species or individual differences in drug response. The hURAT1 F365Y mutation significantly reduces lesinurad's inhibitory efficacy, highlighting the phenylalanine cage's role in both urate and drug recognition. Meanwhile, gating-related residues are critical for conformational stabilization: the R477N mutation weakens lesinurad binding, and D389 mutations (D389A/D389E) disrupt interactions with the cyclopropyl group, drastically lowering affinity[5]. It reflects how transporter-specific structural traits dictate drug design strategies. Integrating lesinurad's binding with URAT1's intrinsic structure-function logic advances the understanding of both this drug and the broader principles governing uricosuric drug-transporter interactions.

1.2.2. Verinurad

Verinurad (RDEA3170), a lesinurad derivative engineered to mitigate renal toxicity, functions as a second-generation selective URAT1 inhibitor (IC_{50} = 24 nmol/L). Its potent activity stems from transporter-specific structural interactions, representing an advancement over lesinurad through refined engagement with URAT1's core functional modules. It binds hURAT1's central cavity, sharing lesinurad's "conformational locking" strategy but gaining enhanced selectivity through additional polar interactions. Key contacts include: naphthalene ring $\pi-\pi$ stacking with F241 (phenylalanine cage); pyridine ring T-shaped $\pi-\pi$ interactions with F364/F365 and polar contacts with S35; cyano group hydrogen bonding with K393; and carboxyl group binding to Q437/Q473 and gating residue R477[4] (Fig. 1c and f).

1.2.3. Benzbromarone

Benzbromarone, launched in 1976 and widely used in China/Japan for urate lowering (yet withdrawn from Europe in 2003 over hepatotoxicity, unapproved by FDA), is a high-affinity hURAT1 inhibitor (IC_{50} = ~200 nmol/L). It binds hURAT1's urate pocket in the inward-open conformation, with its ethylbenzofuran group engaging the phenylalanine cage *via* hydrophobic interactions, and dibromo-hydroxyphenyl group creating steric hindrance with F449 (Fig. 1g and j). Guo et al. and Wu et al.[4] clarified it also forms polar interactions with Q473/N237 and R477 (gating residue), locking hURAT1 in the inhibited state. F360A/F449A mutations abolish efficacy, linking drug action to URAT1's core transport module and guiding safer, mutation-tolerant derivatives.

1.2.4. Dotinurad

Dotinurad, approved in Japan (2020) and China (2024) for hyperuricemia, delivers marked improvements over benzbromarone: it exhibits minimal hepatorenal impact, stronger URAT1 inhibition (IC_{50} = 8 *vs.* ~200 nmol/L), and fewer off-target effects—advantages rooted in its refined adaptation to URAT1's structural modules. Its benzothiazole core occupies the phenylalanine cage (rotated 90° relative to benzbromarone's ethylbenzofuran), forming $\pi-\pi$ stacking with F241/F360/F365 and anion$-\pi$ interactions with F241/F364 *via* the sulfonyl group (Fig. 1h and k)[2,4]. More importantly, this structural modification diminishes the non-specific binding of drug molecules to hepatic enzymes, including CYP2C9. Analogous to verinurad, it uses a hydroxyl group to form hydrogen bonds with gating residue R477 and K393/Q473—directly linking drug binding to the coupling of URAT1's transport cycle.[4]

magenta dashed lines). (j–l) Schematic representation of the interaction networks between benzbromarone, dotinurad, and sulfinpyrazone with URAT1 residues, depicted in 2D format.

1.2.5. Sulfinpyrazone

Sulfinpyrazone, an FDA-approved gout therapeutic, exhibits specific URAT1 inhibition (IC_{50} = 32 μmol/L), with its binding mechanism characterized by a dynamic-stable synergy that adapts to URAT1's structural modules[4]. Yu et al. revealed its diphenylpyrazolidine group maintains stable interactions: its oxygen forms a potential hydrogen bond with gating residue R477, while the diketone moiety engages in extensive hydrophobic contacts with the phenylalanine cage (F360/F364/F365/F449) and W357. In contrast, the phenylsulfinyl group shows high dynamism, stabilizing binding *via* hydrophobic interactions with methionine residues (M215/M222/M234/M466) and forming polar contacts with N237 (Fig. 1i and l).

Notably, R477/N237 mutations drastically reduce binding affinity—tying its action to URAT1's core functional residues, similar to dotinurad and verinurad. Unlike dotinurad's fixed benzothiazole-cage engagement, sulfinpyrazone's dynamic phenylsulfinyl group represents an alternative adaptation strategy, while its reliance on R477 aligns with shared URAT1 inhibition principles. These insights enrich the structural blueprint for next-gen anti-gout drugs.

1.3. Commonalities and differences in binding modes of uricosuric drugs to URAT1

All five uricosuric drugs (lesinurad, verinurad, benzbromarone, dotinurad, and sulfinpyrazone) share a core mechanism: they competitively bind to the central cavity of hURAT1, overlapping with the urate binding site, and inhibit urate reabsorption by stabilizing the transporter in an inward-open conformation. Key common interactions include hydrophobic engagements with the phenylalanine cage (F241, F360, F364, F365, F449) and polar contacts with conserved residues like R477 and Q473, which are critical for conformational stability.

Notably, their divergent binding features provide actionable templates for overcoming the clinical limitations of existing uricosuric agents. For affinity optimization, verinurad builds upon the phenylalanine cage observed in lesinurad by incorporating additional polar groups (K393, S35), thereby enhancing inhibitory activity. In terms of toxicity reduction, dotinurad rotates its benzothiazole core by 90° relative to benzbromarone, enabling anion−π interactions (sulfonyl-F241/F364) and hydrogen bonding (hydroxyl-K393/R477), thereby improving affinity and reducing hepatotoxicity. Moreover, sulfinpyrazone exhibits a dual binding mode combining dynamic flexibility and stability: its diphenylpyrazolidine moiety stably anchors R477, while the phenylsulfinyl group flexibly engages M215 and W357. It suggests a strategy for mutation tolerance—designing inhibitors that stably bind gating residues could circumvent F365Y-mediated resistance to lesinurad. Collectively, these structural insights bridge the gap between mechanistic understanding and clinical requirements, providing a rational framework for developing next-generation uricosurics with optimized potency, safety, and broad therapeutic applicability.

2. Inhibitory mechanisms of OAT1 and GLUT9

2.1. The interaction between OAT1 and probenecid

OAT1, a key SLC22 family member, is primarily located on the basolateral membrane of renal proximal tubules and is involved in the excretion of metabolic wastes and xenobiotics. Its dysfunction can cause urate imbalances and drug toxicity accumulation, impairing kidney function. OAT1 is a target for many clinical drugs, and its inhibition can modulate drug elimination or retention, impacting efficacy and toxicity.

Dou et al.[6] reported the cryo-EM structure of rat OAT1 (also known as SLC22A6) and its complex with probenecid. They found that the aromatic part of probenecid interacts hydrophobically with Tyr230 and F438. Specific interactions include a salt bridge between its carboxyl group and K382, and a hydrogen bond between its sulfamoyl oxygen and Tyr354. The propyl groups of probenecid are near R466 and residues from TM7, TM10, and TM11, stabilizing binding (Fig. 2a and c). Replacing propyl with a shorter alkyl increases renal clearance, highlighting the importance of these interactions for binding strength and pharmacokinetics. These findings reveal the conserved substrate binding mechanism in SLC22 transporters, clarifying OAT1's broad substrate specificity and drug interactions.

2.2. The interaction between GLUT9 and urate

GLUT9 is a high-capacity urate transporter and a potential gout treatment target. Initially identified as a glucose transporter due to its sequence similarity with GLUT1-4, GLUT9 primarily functions as a unidirectional urate transporter, with urate transport activity 45 to 60 times higher than that for glucose. The N-terminal region of GLUT9 is crucial for its transport function. Full-length GLUT9 is located on the basolateral membrane, transporting urate from cells to the bloodstream, while the N-terminal-deleted GLUT9 is on the renal tubule apical membrane, involved in urate reabsorption.

Shen et al.[7] revealed the cryo-EM structure of the human GLUT9−urate complex. Urate extensively interacts with central cavity residues, forming hydrogen bonds with W336, N333, and Y327, and hydrophobic interactions with L75, I209, and L332 (Fig. 2b and d). Mutations in these residues decrease the activity and urate binding of GLUT9. Unlike URAT1, GLUT9 lacks a phenylalanine cage and does not use charged residues for urate coordination. This contrast emphasizes the distinct evolutionary paths (SLC22 versus SLC2A) that shaped different urate recognition mechanisms. The cage-driven specificity of URAT1 supports targeted reabsorption, while the hybrid site of GLUT9 enables high-capacity transport across the basolateral membrane. Although URAT1 and GLUT9 differ in structure and function, they exhibit a synergistic role in uric acid transport. URAT1 facilitates efficient cellular uptake of uric acid through conformational changes, whereas GLUT9 responds to elevated intracellular uric acid concentrations *via* structural remodeling. This interplay establishes a "substrate concentration-driven structural coupling" mechanism, which not only ensures sustained efficiency in transcellular uric acid transport but also effectively prevents intracellular uric acid accumulation. These insights advance understanding of GLUT9's mechanism and aid in designing specific inhibitors for gout and hyperuricemia treatment.

3. Conclusions and outlooks

This highlight article synthesizes recent breakthroughs in the structural biology of urate transporters, particularly URAT1, OAT1, and GLUT9. Against this structural backdrop, targeted drug design strategies can be formulated: to enhance URAT1's binding affinity with the phenylalanine cage, hydrophobic aromatic groups (such as naphthyl or biphenyl) can be introduced to strengthen π−π stacking

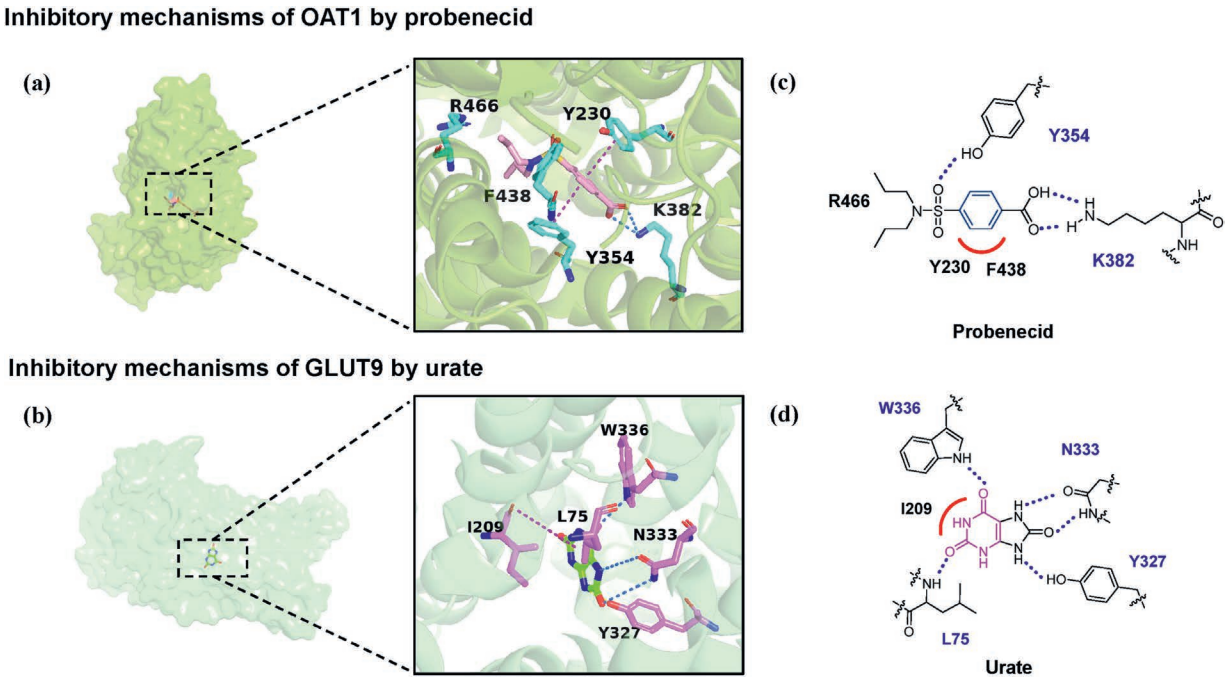

Figure 2 Binding mechanisms of OAT1 with probenecid and GLUT9 with urate. (a) Detailed view of the probenecid binding pocket in rat OAT1 (PDB code: 8SDZ). (b) Detailed view of the urate binding pocket in GLUT9 (PDB code: 8ZXM). Surrounding residues are depicted as sticks; hydrogen bonds are indicated by marine dashed lines, and hydrophobic interactions are indicated by magenta dashed lines. (c) Schematic representation of the interaction networks between probenecid with OAT1 residues, depicted in 2D format. (d) Schematic representation of the interaction networks between urate with GLUT9 residues, depicted in 2D format.

with F241/F365, while polar sulfonamide or hydroxyl groups can be added to form anion−π interactions with F364 or hydrogen bonds with nearby polar residues (such as K393), as demonstrated in the structural optimization of dotinurad. To improve selectivity, we emphasize that the gating residue R477 is unique to URAT1 (absent in homologous transporters like OAT1), so modifying drug molecules to form hydrogen bonds with R477 can reduce off-target binding (a major source of nephrotoxicity). For GLUT9 inhibitors, given that their urate binding relies on W336/N333/Y327, small molecules can be designed to include indole or pyrrole rings to enhance $\pi-\pi$ stacking with W336 and incorporate amide groups to form hydrogen bonds with N333/Y327, thereby increasing selectivity for GLUT9.

For drug development, the aforementioned structural data underpin several integration strategies. First, multi-omics combined with artificial intelligence (AI) supports target validation. For instance, identifying hyperuricemia-associated genetic mutations and protein expression differences to reinforce URAT1's role as a pivotal drug target. Second, integrating cryo-EM with AI enables resolution of dynamic transporter states (*e.g.*, URAT1's M36-mediated closure), shedding light on conformational changes during urate transport and informing inhibitor design. Third, structure-based design targeting allosteric sites or protein−protein Interaction (PPI) interfaces, augmented by some deep learning models (such as KarmaDock)[8] and generative scaffold design (*e.g.*, fusing apigenin's GLUT9-binding motifs with URAT1-directed structures to create novel molecules), enhances precision in inhibitor development. Additionally, structural characterization of natural product binding sites provides strategies to optimize natural product-derived inhibitors *via* structural modification, improving efficacy and pharmacokinetic properties[9]. Complementing these, designing precise inhibitors targeting clinically relevant mutations and post-translational modifications, alongside leveraging real-time probes and data standardization technologies, accelerates translation from basic research to clinical application.

Notably, current progress faces critical challenges: cryo-EM's limitations in capturing dynamic transporter-inhibitor interactions may lead to mismatches between designed inhibitors and physiological conformational changes; significant species and individual differences (*e.g.*, URAT1 F365Y mutation) render wild-type structure-based inhibitors ineffective in some patients; and limited structural data on human transporters in diverse functional states hinders AI model training and prediction accuracy. To address these, future efforts should focus on advancing cryo-EM techniques to resolve dynamic interactions, expanding structural databases of transporter variants, and developing broad-spectrum inhibitors targeting both wild-type and common mutant forms. These integrated endeavors may enable precise metabolic regulation, heralding a new era of gout therapy.

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Author contributions

Peng Zhan conceived the idea. Qian Yang wrote the draft manuscript. Peng Zhan, Ting Wu, Jianxin Pang, Shujing Xu, and Xiaoyu Shi revised the manuscript. All authors have read and approved submission of the final manuscript.

Conflicts of interest

The authors declare that they have no competing of interests.

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Qian Yang[a], Shujing Xu[a], Xiaoyu Shi[a], Jianxin Pang[b], Ting Wu[b,*], Peng Zhan[a,c,d,*]

[a]*Department of Medicinal Chemistry, Shandong Key Laboratory of Druggability Optimization and Evaluation for Lead Compounds, School of Pharmaceutical Sciences, Shandong University, Jinan 250012, China*

[b]*NMPA Key Laboratory for Research and Evaluation of Drug Metabolism &Guangdong Provincial Key Laboratory of New Drug Screening & Guangdong−Hongkong−Macao Joint Laboratory for New Drug Screening, School of Pharmaceutical Sciences, Southern Medical University, Guangzhou 510515, China*

[c]*State Key Laboratory of Natural and Biomimetic Drugs, Peking University, Beijing 100191, China*

[d]*State Key Laboratory of Druggability Evaluation and Systematic Translational Medicine (Tianjin Institute of Pharmaceutical Research), Tianjin 300301, China*

*Corresponding authors.
E-mail addresses: wutingmervyn@126.com (Ting Wu), zhanpeng1982@sdu.edu.cn (Peng Zhan).

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