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## Discovery and characterization of verinurad, a potent and specific inhibitor of URAT1 for the treatment of hyperuricemia and gout

Philip K. Tan<sup>1</sup>, Sha Liu<sup>1</sup>, Esmir Gunic<sup>2</sup> & Jeffrey N. Miner<sup>1</sup>

Gout is caused by elevated serum urate levels, which can be treated using inhibitors of the uric acid transporter, URAT1. Here, we characterize verinurad (RDEA3170), which is currently under evaluation for gout therapy. Verinurad specifically inhibits URAT1 with a potency of 25 nM. High affinity inhibition of uric acid transport requires URAT1 residues Cys-32, Ser-35, Phe-365 and Ile-481. Unlike other available uricosuric agents, the requirement for Cys-32 is unique to verinurad. Two of these residues, Ser-35 and Phe-365, are also important for urate transport kinetics. A URAT1 binding assay using radiolabeled verinurad revealed that distinct URAT1 inhibitors benzbromarone, sulfapyrazone and probenecid all inhibit verinurad binding via a competitive mechanism. However, mutations made within the predicted transporter substrate channel differentially altered the potency for individual URAT1 inhibitors. Overall, our results suggest that URAT1 inhibitors bind to a common site in the core of the transporter and sterically hinder the transit of uric acid through the substrate channel, albeit with vastly different potencies and with differential interactions with specific URAT1 amino acids.

Gout is a metabolic disease caused by chronically elevated serum uric acid (sUA) levels (hyperuricemia), leading to deposition of urate in the joints and acute bouts of painful inflammatory arthritis<sup>1–3</sup>. Urate homeostasis is balanced by urate production and elimination, and homoinds and certain monkeys have relatively high sUA levels due to the presence of multiple inactivating mutations in the uric acid degrading enzyme uricase and mutations in URAT1 that increase affinity for uric acid<sup>4–6</sup>. Elimination of urate occurs primarily in the urine; however, in the kidneys approximately 90% of the urate filtered by the glomerulus is reabsorbed back into the bloodstream so that just 10% of the filtered urate is renally excreted<sup>4–6</sup>. Compared to individuals with normal sUA levels, most patients with gout exhibit reduced fractional excretion of uric acid (FEUA), leading to hyperuricemia<sup>7</sup>. URAT1 is a transporter critical for renal reabsorption of urate. Inactivating mutations of URAT1 lead to high FEUA and hypouricemia (abnormally low sUA levels)<sup>7</sup>. In patients with gout, we previously hypothesized that reduced FEUA could be due to altered URAT1 transport kinetics that increase renal urate reabsorption<sup>7</sup>. However, the reduced FEUA in gout patients could also be due to reduced renal urate secretion<sup>8–9</sup>.

Benzbromarone, sulfapyrazone, probenecid and lesinurad (Figure S1) are among a class of gout therapeutics that lower sUA levels by inhibiting URAT1 and enhancing FEUA. However, the first three are not widely used due to various safety and availability issues<sup>10</sup>. Lesinurad was recently approved for the treatment of hyperuricemia associated with gout, in combination with a xanthine oxidase inhibitor<sup>11–13</sup>. In this report, we describe the molecular pharmacology of a novel, highly potent and specific URAT1 inhibitor, verinurad (also known as RDEA3170; Figure S1), that is currently under evaluation for the treatment of gout and asymptomatic hyperuricemia. Verinurad-mediated inhibition of URAT1 is highly dependent on human URAT1 Phe-365 and Ser-35, both of which are located in the substrate channel. The high affinity of verinurad allowed development of a novel URAT1 binding assay, and results from this assay showed that all the inhibitors bind to the same site within

<sup>1</sup>Department of Biology, Andea Biosciences, Inc. (A member of the AstraZeneca Group), San Diego, CA, USA.

<sup>2</sup>Department of Chemistry, Andea Biosciences, Inc. (A member of the AstraZeneca Group), San Diego, CA, USA. Correspondence and requests for materials should be addressed to P.K.T. (email: [ptphiltan@gmail.com](mailto:ptphiltan@gmail.com))

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**Shourong Shi, Wei Zhai**



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