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CONCENTRATION-DEPENDENT MODE OF INTERACTION OF ANGIOTENSIN II RECEPTOR BLOCKERS WITH URIC ACID TRANSPORTER URAT1

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Running Title:

Interaction of ARBs with URAT1

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Abbreviations: ARBs, angiotensin II receptor blockers; SUA, serum uric acid; URAT1, uric acid transporter; PZA, pyrazinecarboxylic acid

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Abstract

Serum uric acid (SUA) is currently recognized as a risk factor for cardiovascular disease. It has been reported that an angiotensin II receptor blocker (ARB), losartan, decreases SUA level, while other ARBs, such as candesartan, have no lowering effect. Since the renal uric acid transporter URAT1 is an important factor controlling the SUA level, we examined the involvement of URAT1 in those differential effects of various ARBs on SUA level at clinically relevant concentrations. This study was done by using URAT1-expressing *Xenopus* oocytes. Losartan, pratosartan and telmisartan exhibited cis-inhibitory effects on the uptake of uric acid by URAT1, while at higher concentrations, only telmisartan did so, and these ARBs reduced the uptake in a competitive kinetics. On the other hand, candesartan, EXP3174 (major metabolite of losartan), olmesartan and valsartan were not inhibitory. Preloading of those ARBs in the oocytes enhanced the URAT1-mediated uric acid uptake, showing a trans-stimulatory effect. The present study is a first demonstration of the differential effects of ARBs on URAT1 that some ARBs are both cis-inhibitory and trans-stimulatory, depending on concentration, while others exhibit either a trans-stimulatory or cis-inhibitory effect alone, which could explain the clinically observed differential effects of ARBs on SUA level. Furthermore, it was found that such differential effects of ARBs on URAT1 could be predicted from the partial chemical structures of ARBs, which will be useful information for the appropriate use and development of ARBs without an increase of SUA.

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Introduction

Many observations indicate that hyperuricemia is associated with hypertension: raised serum uric acid (SUA) levels are found in approximately 25% of hypertensive patients (Messerli et al., 1980; Cannon et al., 1966) and hypertension is present in 30% of patients with hyperuricemia or gout (Lin et al., 2000; Yu et al., 1982). Mazzali *et al.* showed that mild hyperuricemia was induced in rats given the uricase inhibitor oxonic acid, and the hyperuricemic rats developed elevated blood pressure, whereas control rats remained normotensive (Mazzali et al., 2001). Moreover, the development of hypertension was prevented by concurrent treatment with either a xanthine oxidase inhibitor (allopurinol) or an uricosuric agent (benzbromarone), both of which lowered the uric acid level (Mazzali et al., 2001). Therefore, these observations indicate that an increase of SUA level should be prevented, especially in patients with hypertension.

It was reported that the angiotensin II receptor blocker (ARB) losartan (Fig. 1) increased excretion of uric acid and decreased the SUA level in both healthy and hypertensive subjects (Nakashima et al., 1992; Burnier M et al., 1993; Fauvel et al., 1996), while the ARBs candesartan and valsartan did not affect the SUA level (Malmqvist et al.,2000; Elliott et al., 2001; Gonzalez et al., 2000). In a study of 1,161 hypertensive patients, candesartan slightly, but significantly increased the SUA level (Manolis et al., 2000). Thus, the effects of various ARBs on the disposition of uric acid might be different.

SUA levels differ significantly among individuals due to the variability in factors that contribute to the generation and/or elimination of uric acid (Johnson et al., 2003). Urinary excretion of uric acid accounts for approximately 70% of the daily production of uric acid. The so-called “four-component hypothesis”, including glomerular filtration, presecretory reabsorption, secretion, and postsecretory reabsorption, has been proposed to explain the

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renal handling of uric acid (Sica and Schoolwerth 2000). It was reported that the uricosuric effect of losartan results from its inhibitory action on the urate/anion exchanger at the brush border membranes of the renal proximal tubular epithelial cells (Enomoto et al., 2002; Roch-Ramel et al., 1997). Enomoto *et al.* found that the uric acid transporter URAT1 is involved in the reabsorption of uric acid from lumen to cytosol along the proximal tubule (Enomoto et al., 2002). In addition, they showed that the uric acid transport via URAT1 was inhibited by uricosuric benzbromarone, and that the uptake of uric acid was trans-stimulated by antiuricosuric pyrazinecarboxylic acid (PZA), which is an active metabolite of pyrazinamide (Enomoto et al., 2002). Furthermore, mutation of URAT1 is associated with idiopathic renal hypouricemia (Enomoto et al., 2005; Ichida et al., 2004). These results indicated that URAT1 plays a dominant role in uric acid reabsorption in renal tubular epithelial cells. Roch-Ramel *et al.* reported that the uptake of uric acid by renal brush-border membrane vesicles was inhibited by losartan with an IC_{50} of 13 μM (Roch-Ramel et al., 1997). Moreover, losartan at 1 mM completely blocked the uptake of uric acid via URAT1 (Enomoto et al., 2002). However, the maximum plasma total concentration of losartan was 0.65 μM after oral administration at the clinical dose of 50 mg in humans (Tamimi et al., 2005). Since plasma protein binding of losartan is 98.7% (Burnier, 2001), the maximum concentration of losartan in glomerularly filtered urine could be approximately 10 nM or less, assuming that the plasma free concentration is equal to the concentration in the glomerular filtrate. These observations indicate that the previously reported inhibitory actions of ARBs on URAT1-mediated uric acid transport may not be clinically relevant.

Therefore, in the present study, to clarify the mechanism of the variable of the effects of ARBs on SUA level, we examined the cis-inhibitory and trans-stimulatory effects of ARBs

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at more clinically relevant concentrations, focusing on URAT1. Recently, Price KL *et al.* showed that human vascular smooth muscle cells expressed URAT1 and examined the inhibitory effect of probenecid on uric acid uptake by the cells, suggesting that such mammalian-tissue derived cells would be useful for evaluation of transporters (Price et al., 2006). However, to highlight URAT1 activity alone, heterologously URAT1-expressed cells are more useful than original tissue-derived cells. Accordingly, we used *Xenopus* oocytes expressed with URAT1 as the heterologous transporter expression system. This system is useful for the study of trans-stimulatory effect of ARBs on exchange transporter URAT1, which is one of major purpose of the present study, since *Xenopus* oocyte can be directly preloaded with those test drugs into the cells by microinjection.

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Materials and Methods

Chemicals

[^{14}C]Uric acid (1.92 TBq/mmol) was purchased from Moravek Biochemicals, Inc. (Brea, CA). Losartan and telmisartan were purchased from Zhejiang Tianyu Pharmaceutical (Zhejiang, China). Valsartan was purchased from Livzon Group Changzhou Kony Pharmaceutical (Changzhou, China). EXP3174, pratosartan (Patterson et al., 2003), candesartan and olmesartan were synthesized and kindly supplied by Kotobuki Pharmaceutical (Nagano, Japan). Benzbromarone and PZA were purchased from Sigma-Aldrich (St. Louis, MO) and Wako Pure Chemical Industries (Osaka, Japan), respectively. All other reagents were purchased from Sigma-Aldrich and Wako Pure Chemical Industries.

Uptake Experiments

Xenopus were purchased from Hamamatsu Biological Research Service, Inc., (Hamamatsu, Japan) and handled humanely according to the guidelines of the animal experimentations of Tokyo University of Science. Methods for preparation of oocytes, in vitro synthesis of URAT1 (SLC22A12)-cRNA and standard uptake experiments were described previously (Iwanaga et al., 2005). Briefly, the oocytes were injected with cRNA and cultured for 2 days, then preincubated in ND96 buffer (96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$ and 5 mM HEPES, pH 7.4) at 25 °C for 15 min. To initiate uptake of [^{14}C]uric acid, the oocytes were incubated with Cl^--free buffer (96 mM Na gluconate, 2 mM K gluconate, 1 mM Mg gluconate, 1.8 mM Ca gluconate and 5 mM HEPES, pH 7.4) containing 20 μM [^{14}C]uric acid at 25°C for the designated time. In cis-inhibitory studies, tested drugs were added simultaneously with [^{14}C]uric acid. In the trans-stimulation study,

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the oocytes were micro-injected with 50 nL of drug solution or water containing 0.1% dimethyl sulfoxide (DMSO). Immediately after the microinjection (within approximately 2 min), the oocytes were transferred to Cl^--free buffer containing [^{14}C]uric acid to initiate uptake study. In the cases of Fig. 5 and Fig. 6, cis-inhibitory effect was examined after preloading of the oocytes with PZA (50 nL of 100 mM PZA solution, pH 7.4), since PZA exhibits a strong trans-stimulatory effect on uric acid uptake, thereby eliminating almost completely the trans-stimulatory effect of ARBs added to the extracellular medium. Then, uptake of [^{14}C]uric acid was measured in the same way as described above, using Cl^--free buffer containing 20 μM [^{14}C]uric acid with or without test ARBs, within 2 min. Uptake was terminated by washing the oocytes three times with ice-cold Cl^--free buffer. The oocytes were solubilized with 5 % sodium dodecyl sulfate solution. Radioactivity was measured using a liquid scintillation counter (Perkin Elmer, Boston, MA). Artifactual cis-inhibitory and trans-stimulatory effects were checked by using 1 μM benzbromarone and 100 mM PZA, respectively, in each assay, since they have been well established as a strong inhibitor and a trans-stimulant of URAT1, respectively.

Uptake was expressed as the cell-to-medium ratio (μL/oocyte), obtained by dividing the uptake amount by the concentration of substrate in the incubation buffer (Cl^--free buffer). In this study, we repeated each experiment more than two times to confirm the results, and the typical results were shown as mean and standard error of mean value obtained from 10 oocytes. In order to estimate the kinetic parameters for the uptake by URAT1, the initial uptake rates by URAT1 (obtained after subtraction of the uptake by water-injected oocytes from that by URAT1-cRNA injected oocytes) were fitted to the following equation (1) by means of nonlinear least-squares regression analysis using the MULTI program (Yamaoka et al., 1981):

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$$v = V_{max} \times s/(K_m+s) \quad \text{.....(1)}$$

where v, s, K_m, and V_{max} are the initial uptake rate of substrate (pmol/30 min/oocyte), the substrate concentration in the medium (μM), the apparent Michaelis-Menten constant (μM), and the maximum uptake rate (pmol/30 min/oocyte), respectively. Apparent K_m value in the presence of inhibitor ($K_{m\,(+\ inhibitor)}$) was calculated by equation (1) in the same manner as described above. Then, the inhibition constant K_i value was calculated by following equation (2)

$$K_{m\,(+\ inhibitor)} = K_m(1+[I]/K_i) \quad \text{.....(2)}$$

where [I] is the inhibitor concentration (nM).

Estimation volume of oocytes

To evaluate the apparent intracellular volume of *Xenopus laevis* oocytes, antipyrine was used, since it has a high membrane permeability and negligible protein binding (Terasaki et al., 1992). A 50 nL aliquot of 100 mM antipyrine was injected into oocytes that had been injected with URAT1-cRNA or water in advance, and then the oocytes were transferred to 0.5 mL of Cl^--free buffer within 2 min to initiate the efflux of antipyrine. Antipyrine concentration in Cl^--free buffer, arising by efflux from the oocyte, was measured by high-performance liquid chromatography with Alliance 2690 and UV486 instruments detected at 245 nm (Waters Co., Milford, MA). The analysis was performed on a Mightysil C18 column (4.6 mm × 150 mm; 5 μm; Kanto Chemical, Tokyo, Japan) at a flow rate of 1 mL/min, using a mobile phase consisting of a mixture of 10 mM sodium phosphate buffer (pH 7.0) and acetonitrile (80 : 20). The apparent volume of oocytes (μL) was obtained by dividing the amount of antipyrine in one oocyte (pmol) by the antipyrine concentration in Cl^--free buffer (pmol/μL) at the steady state, on the assumption that the intracellular

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unbound concentration is equal to the concentration in Cl^--free buffer at the steady state.

Statistical analysis

Statistical significance was determined with Student's *t* test or by analysis of variance (ANOVA) followed by Dunnett's test, and a *p* value of less than 0.05 was considered statistically significant.

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Results

Cis-inhibitory effects of ARBs on URAT1-mediated uric acid uptake

Figure 2 shows the effects of various ARBs, pratosartan, including losartan, candesartan, and EXP3174, in Cl- free buffer on the uptake of [^{14}C]uric acid at 60 min. Pratosartan and losartan reduced the URAT1-mediated uptake of uric acid at concentrations from 1 to 10 nM and from 0.1 to 10 nM, respectively, while candesartan or EXP3174 had no effect at concentrations up to 100 nM. In the presence of a higher concentration (100 nM) of pratosartan or losartan, the uptake of uric acid recovered to that in the absence of ARBs. At 100 μM, EXP3174 reduced the uptake of uric acid, while candesartan did not exhibit any reduction, but rather stimulated the uptake. Interestingly, pratosartan and losartan again reduced the uptake of [^{14}C]uric acid at 100 μM. Benzbromarone significantly reduced URAT1-mediated uptake of uric acid at concentrations higher than 10 nM. Here, the uptake of [^{14}C]uric acid was evaluated routinely at 20 μM, which is less the reported K_m of URAT1-mediated transport of uric acid was 370 μM (Enomoto et al., 2002), to estimate the URAT1-mediated transport of URAT1 clearly. In separate experiments, we confirmed that similar reductions of uric acid uptake were observed at the [^{14}C]uric acid concentrations of 250 and 500 μM, which correspond to physiological concentrations of uric acid in serum, in the presence of losartan or pratosartan, but not candesartan, at 10 nM (data not shown).

Trans-stimulatory effects of ARBs on URAT1-mediated uric acid uptake

Trans-stimulatory effect of ARBs on uric acid uptake via URAT1 was examined by measuring the uptake of uric acid by oocytes preloaded with pratosartan. Uptake of [^{14}C]uric acid by URAT1-expressing oocytes that were preloaded with pratosartan (0.1 pmol, 2 μM × 50 nL) at 15 min was significantly greater than that by oocytes preloaded with the

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same volume of water (see Supplemental data I). Figure 3 shows the concentration dependence of trans-stimulatory effect of ARBs on the uptake of uric acid via URAT1. Immediately after the microinjection of ARB solution into oocytes, the uptake of [^{14}C]uric acid was measured for 15 min. The results are shown as the uptake relative to that without ARB preloading. The tested ARBs increased the uptake of uric acid when preloaded at concentrations higher than 5 μM, except for EXP3174 (10 μM). The increment of [^{14}C]uric acid uptake in the presence of pratosartan and losartan disappeared at injected concentrations higher than 50 μM, whereas the uptake was significantly increased by preloaded candesartan and EXP3174 at injected concentrations higher than 5 or 10 μM, respectively. Figure 4 compares the trans-stimulatory effect of ARBs on the uptake of uric acid by oocytes expressed with URAT1. Each oocyte was preloaded with 50 nL of 10 μM of ARB solution and the uptake of [^{14}C]uric acid was evaluated for 15 min. Candesartan, EXP3174, losartan, olmesartan, pratosartan, and valsartan enhanced the uptake of uric acid, while telmisartan and benzbromarone showed no effect. Candesartan showed the highest enhancement of uptake.

Estimation of intracellular ARB concentration after preloading of *Xenopus* oocytes

To estimate the concentration of ARBs in oocytes after microinjection, the intracellular volume of oocytes was estimated from the distribution volume of antipyrine. The efflux of antipyrine from oocytes was evaluated in terms of the amount of antipyrine in the extracellular incubation medium. By 150 min after antipyrine was injected, the efflux of antipyrine had attained a steady state and the concentrations of antipyrine in the incubation medium (500 μL) were 81.2 and 80.3 μM for water-injected oocytes and for URAT1-cRNA-injected oocytes, respectively. Residual antipyrine in an oocyte amounted

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to 68.3 and 64.9 pmol, respectively. If antipyrine has a negligible intracellular binding and the intracellular free concentration of antipyrine is equal to that in the extracellular incubation medium, the intracellular volumes of the oocytes can be estimated to be 0.841 ± 0.067 and 0.808 ± 0.073 μL/oocyte, respectively; no significant difference was observed. Furthermore, we estimated the recovery ratio of antipyrine after microinjection (approximately 70%). Based on this result, it is possible to estimate the concentrations in oocytes after microinjection of drugs; when the injected amount was 0.5 pmol (10 μM × 50 nL), the ARB concentration in the oocyte was approximately 430 nM.

Cis-inhibitory effects of ARBs on URAT1-mediated uric acid uptake preloaded with PZA

The complicated effects of ARBs on uric acid transport shown in Fig. 2 may be explained by the occurrence of a cis-inhibitory effect at low concentrations and a trans-stimulatory effect at higher concentrations. Since PZA exhibits a strong trans-stimulatory effect on URAT1 (Enomoto et al., 2002), preloading of PZA should eliminate the apparent trans-stimulatory effect of ARBs and make the evaluation of cis-inhibitory effect of ARBs clearer. Accordingly, the cis-inhibitory effects of ARBs were examined in oocytes preloaded with PZA. Microinjection of 5 nmol (100 mM × 50 nL) of PZA resulted in higher uptakes of uric acid by oocytes as compared with oocytes without preloading of PZA or with preloading of 0.5 pmol (10 μM × 50 nL) of pratosartan (see Supplemental data II). As shown in Fig. 5, the uptake of uric acid at 30 min was reduced by 10 nM pratosartan and losartan to be less than half, showing clearer effects than those in Fig. 2. Telmisartan and benzbromarone also reduced the uptake, while the other ARBs did not cause a significant reduction. Furthermore, the inhibition kinetics of losartan,

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pratosartan, and telmisartan were analyzed and are shown by Eadie-Hofstee plots in Fig. 6 A-C. All of three ARBs increased K_m from 357.6 ± 8.2 to 822.1 ± 146.7, 895.1 ± 101.3, and 554.0 ± 35.5 μM, respectively, but did not change V_{max} (from 750.0 ± 8.3 to 783.2 ± 54.9, 775.2 ± 44.7, and 736.6 ± 23.9 pmol/30 min/oocyte, respectively). Accordingly, these ARBs were shown to inhibit URAT1 in a competitive manner and their K_i values were 7.7, 6.7, and 18.2 nM, respectively. In addition, to clarify the mechanism of trans-stimulatory effect of PZA, kinetic parameters of URAT1-mediated uric acid uptake were compared with or without microinjection of PZA. As a result, microinjection of PZA increased V_{max} from 73.1 ± 2.6 to 750.0 ± 8.3 pmol/30 min/oocyte of uric acid uptake via URAT1, while only a little change in K_m values are observed (288.2 ± 16.9 to 357.6 ± 8.2 μM) (Fig. 6D).

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Discussion

It is well known that some ARBs affect SUA level, and an increased SUA level is clinically undesirable, since it is considered to be a risk factor for cardiovascular disease (Manolis et al., 2000). An elevation of 1 mg/dL SUA level has been suggested to be comparable to an elevation of 10 mgHg systolic blood pressure (Alderman et al., 1999). Several ARBs have been reported to increase the SUA level, while losartan decreases it (Nakashima et al., 1992; Burnier et al., 1993; Fauvel et al., 1996; Manolis et al., 2000). Accordingly, it is important to clarify the mechanism of the changes of the SUA level by ARBs. SUA level is mainly controlled by the production of uric acid in liver, for which the rate-limiting enzyme is xanthine oxidase, and by renal handling, such as reabsorption by the uric acid transporter URAT1 and secretion via other transporters. Among these factors that affect SUA level, the effects of ARBs on URAT1 were examined in the present study, since URAT1 predominantly contributes to the renal reabsorption of uric acid across the apical membrane of proximal tubular epithelial cells (Enomoto et al., 2002), and previous studies suggested that renal uric acid transporter is involved as the mechanism for the effect of ARBs on SUA level (Enomoto et al., 2002; Roch-Ramel et al., 1997). Furthermore, since URAT1 is an anion/uric acid exchanger and compounds such as PZA and lactic acid stimulate the reabsorption of uric acid, modulation of URAT1 may explain both the reduction and increment of SUA level by cis-inhibition and trans-stimulation of URAT1, respectively, by ARBs. However, no studies on the mechanism of differential effects of various ARBs on the SUA level via URAT1 have been reported. Accordingly, in the present study, we examined the cis-inhibitory and trans-stimulatory effects of ARBs on the uptake of uric acid via URAT1 at clinically relevant concentrations.

First of all, losartan and pratosartan exhibited inhibitory effects on the uptake of uric

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acid by URAT1 in the clinically relevant concentration range (0.1 to 10 nM), while others, such as candesartan and EXP3174, did not. These results suggested that losartan and pratosartan are potent inhibitors of URAT1, which would explain the decrease of the SUA level by these compounds in the clinic. The lack of inhibitory effect of candesartan, valsartan and EXP3174 on URAT1 may be the reason why these ARBs do not decrease the SUA level. An interesting observation was the recovery of the uptake of [^{14}C]uric acid at higher concentrations of losartan and pratosartan, as shown in Fig. 2. Since the recovery might be explained by a trans-stimulation of those ARBs at higher concentrations, their trans-stimulatory effects were examined, as shown in Figs. 3 and 4. Except for telmisartan, all of the ARBs preloaded into oocytes enhanced the uptake of [^{14}C]uric acid. These results demonstrated that some ARBs exhibit both cis-inhibitory and trans-stimulatory effects on URAT1-mediated uptake of uric acid, depending on the concentration, while other ARBs have only a trans-stimulatory or a cis-inhibitory effect. Such differences in the effects on URAT1 among ARBs may explain the differential clinical observation of ARBs on SUA levels.

Quantitative estimation of the inhibitory action of ARBs on URAT1 is important to assess the clinical relevance. However, because of the dual effects of ARBs on URAT1, as shown in Fig. 2, the apparent cis-inhibitory effect might be underestimated. Therefore, we evaluated the actual cis-inhibitory effect of these ARBs on URAT1-mediated uric acid uptake by using PZA as a trans-stimulant of the uptake of [^{14}C]uric acid via URAT1. Since PZA preloaded in the oocytes exhibited a stronger trans-stimulatory effect on uric acid uptake than ARBs (see Supplemental data II), the apparent trans-stimulatory effect of ARBs observed in experiments such as those in Fig. 2 should be negligible in the presence of preloaded PZA. Furthermore, since the increment of URAT1-mediated uptake of uric acid

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by PZA is due to the increase of V_{max} without change its K_m (Fig. 6D), characteristics of binding site of uric acid and inhibitors on URAT1 should be maintained, and PZA can be used for further studies about the effect of ARBs on URAT1. As shown in Fig. 5, the cis-inhibitory effects of losartan, pratosartan and telmisartan in PZA-preloaded oocytes were stronger than the effects shown in Fig. 2, while other ARBs did not show any effect.

The maximum plasma concentration of losartan and pratosartan are approximately 600 nM (Tamimi et al., 2005) and 2330 nM (unpublished observation), respectively, and their plasma protein bindings are 98.7% (Burnier, 2001) and 98.0% (unpublished observation), respectively. Accordingly, their concentrations in glomerularly filtered urine are estimated to be 8 nM and 47 nM, which are higher than their observed K_i values, 7.7 and 6.7 nM for losartan and pratosartan, respectively, suggesting that they could exhibit cis-inhibitory effect on uric acid transport via URAT1 in the clinical situation. However, they are also trans-stimulatory as shown in Fig. 3. We roughly estimated the intracellular concentrations of preloaded ARBs in oocytes by measuring the effective intracellular volume of the oocytes based on the distribution volume of antipyrine (0.841 and 0.808 μL/oocyte for water-injected and URAT1-cRNA-injected oocytes, respectively). The estimated ARB concentration in oocyte was approximately 430 nM after the microinjection of 10 μM solution. Since the tissue-to-plasma concentration ratios (*Kp* value) of losartan and pratosartan are 1.44 and 1.27 in kidney (unpublished observation), their concentrations in renal tubular epithelial cells could be about 860 nM and 3000 nM, respectively. At these concentrations, trans-stimulatory effects could be possible. However, in the case of pratosartan, trans-stimulatory effect was disappeared when microinjected concentration was increased up to 100 μM (estimated intracellular concentration of oocyte was 4.3 μM), and at this condition, observed extracellular pratosartan that was effluxed from the oocytes was 8 nM

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(data not shown). Therefore, although pratosartan exhibits trans-stimulatory effect at clinical dose, it is considered that cis-inhibitory effect is more potent. Similar explanation can be applicable for losartan.

Candesartan, EXP3174, olmesartan, and valsartan did not exhibit a cis-inhibitory effect (Fig. 5), but showed a trans-stimulatory effect (Fig. 4). Such trans-stimulatory effects may lead to an increase of SUA level. The maximum plasma concentration of candesartan is 125 nM after oral administration of the clinical dose of 4 mg in humans (Suzuki and Yano 1996). Since the *Kp* value of candesartan is approximately 0.6 in kidney (Kondo et al., 1996), the candesartan concentration in renal tubular epithelial cells could be 80 nM. Since this concentration is close to the estimate at which candesartan caused a trans-stimulatory effect, trans-stimulation of URAT1 by candesartan may at least partly explain the elevation of SUA level, while contribution of other mechanism cannot be excluded. Other ARBs that we examined, such as olmesartan and valsartan, might cause a trans-stimulatory effect through this mechanism, since their concentrations in renal tubular epithelial cells were estimated to 1400 nM and 3900 nM, respectively, assuming the same *Kp* values as for candesartan (Schwocho and Masonson, 2001; Kimura et al, 2002).

It is interesting that telmisartan exhibited only a cis-inhibitory effect, but not a trans-stimulatory effect. Although the inhibitory effect of telmisartan on URAT1 was potent, the urinary excretion of telmisartan is less than 0.02% of the dose, which is less than that of losartan (3.7%), candesartan (11%), or valsartan (11.5%). Accordingly, a uricosuric effect of telmisartan might not be observed clinically. To our knowledge, there is no clinical report concerning the effect of telmisartan on SUA level.

The present observation provided an idea of structure-activity relationship on the point of cis-inhibitory and trans-stimulatory effects of ARBs, namely, including anionic moieties

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in carboxyl and tetrazolyl groups is important to determine the differential effects among ARBs (Fig. 1). Losartan, pratosartan and telmisartan that have one anionic moiety exhibited stronger cis-inhibitory effects than trans-stimulatory effects. Others, candesartan, olmesartan and valsartan with two anionic moieties did not show cis-inhibitory effects, but showed trans-stimulatory effects. Furthermore, telmisartan that has no tetrazolyl moiety was not trans-stimulatory. So, prediction of the effects of ARBs on URAT1 activity may be possible by focusing on the acidic moieties of them.

In conclusion, although several mechanisms may be involved in the variable effects of ARBs on SUA levels, in the present study we found that different ARBs appear to have different effects (cis-inhibition, trans-stimulation, or both) on uric acid transport via URAT1, depending on their concentrations. Some of those ARB effects should occur at the clinically relevant concentration range. To fully understand the interaction between uric acid and ARBs, we further need to examine the possible effects of these drugs on uric acid-related enzymes and other transporters.

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Legends for figures

Fig. 1

Chemical Structures of Angiotensin Receptor Blockers

Dotted circles indicate their anionic groups as described in Discussion.

Fig. 2

Cis-Inhibitory Effects of ARBs on URAT1-Mediated Uric Acid Uptake by URAT1-Expressing Oocytes

Uptake of [^{14}C]uric acid (20 μM) by water-injected or URAT1-cRNA-injected oocytes was measured in the presence or absence (control) of inhibitors (0.01 - 100 μM), including losartan (open triangle), pratosartan (closed circles), candesartan (closed squares), EXP3174 (closed diamonds) and benzbromarone (open circles), for 60 min. URAT1-mediated uptake was determined by subtracting the uptake by water-injected oocytes from that by URAT1-cRNA-injected oocytes, and the values obtained were divided by the control value in each assay. Drugs were dissolved with Cl^--free buffer containing 0.1% DMSO. Each point represents the mean ± S.E. from 10 oocytes from one individual experiment. An asterisk (*) indicates a significant difference from the control by Dunnett's test ($p<0.05$).

Fig. 3

Concentration Dependence of Trans-Stimulatory Effects of Preloaded ARBs on URAT1-Mediated Uptake by URAT1-Expressing Oocytes.

Aliquots of 50 nL of losartan (A), pratosartan (B), candesartan (C) and EXP3174 (D) solutions at various concentrations from 1 to 100 μM were preloaded, and uptake of

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[^{14}C]uric acid (20 μM) was measured for 15 min. The URAT1-mediated uptake was determined by subtracting the uptake by water-injected oocytes from that by URAT1-cRNA-injected oocytes, and the values obtained were divided by the control value in each assay. Drugs were dissolved with water containing 0.1% DMSO, and adjusted to pH 7.4 with KOH. Each point represents the mean ± S.E. from 10 oocytes from one individual experiment. An asterisk (*) indicates significant difference from the control (no inhibitor) by Dunnett's test (p<0.05).

Fig. 4

Trans-Stimulatory Effect of Various ARBs on Uptake of Uric Acid by URAT1-Expressing Oocytes.

The uptake of [^{14}C]uric acid (20 μM) by water-injected or URAT1-cRNA-injected oocytes was measured after preloading 50 nL of water (control), ARB solution (10 μM) or benzbromarone solution (10 μM) for 15 min. URAT1-mediated uptake was determined by subtracting the uptake by water-injected oocytes from that by URAT1-cRNA-injected oocytes. Drugs were dissolved in water containing 0.1% DMSO, and adjusted to pH 7.4 with KOH. Each point represents the mean ± S.E. from 10 oocytes from one individual experiment. An asterisk (*) indicates a significant difference from the control by Dunnett's test (p<0.05).

Fig. 5

Inhibitory Effect of Various ARBs on Uptake of Uric Acid by URAT1-Expressing Oocytes Preloaded with PZA.

The uptake of [^{14}C]uric acid (20 μM) by water-injected or URAT1-cRNA-injected

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oocytes was measured in the presence of ARBs (10 nM) or in the absence (control) of them for 30 min. PZA was preloaded by microinjection (100 mM × 50 nL, 5 nmol), and then uptake reaction was initiated. URAT1-mediated uptake was determined by subtracting the uptake by water-injected oocytes from that by URAT1-cRNA-injected oocytes. Drugs were dissolved in Cl^--free buffer containing 0.1% DMSO. Each point represents the mean ± S.E. from 10 oocytes from one individual experiment. An asterisk (*) indicates a significant difference from the control by Dunnett's test ($p<0.05$).

Fig. 6

Kinetic Analysis of Inhibitory Effect of ARBs on Uptake of Uric Acid by URAT1 Expressed in Oocytes Preloaded with PZA.

The uptakes of [^{14}C]uric acid (20, 50, 200, 500, 1000, and 2000 μM) by water-injected or URAT1-cRNA-injected oocytes were measured in the absence (control; closed circles) or presence of 10 nM of losartan (A; closed triangle), pratosartan (B; closed diamonds), and telmisartan (C; closed squares) of them for 30 min after preload of PZA by microinjection (100 mM × 50 nL, 5 nmol). The uptake of [^{14}C]uric acid by oocyte was measured after preload of PZA (closed circles) by microinjection (100 mM × 50 nL, 5 nmol) or with Cl^- (open circles) by preincubation with ND96 buffer for 15 min (D). URAT1-mediated uptakes were determined by subtracting the uptake by water-injected oocytes from that by URAT1-cRNA-injected oocytes, and are shown by Eadie-Hofstee Plot Analysis. Drugs were dissolved in Cl^--free buffer containing 0.1% DMSO. Each point represents the mean ± S.E. from 10 oocytes from one individual experiment.

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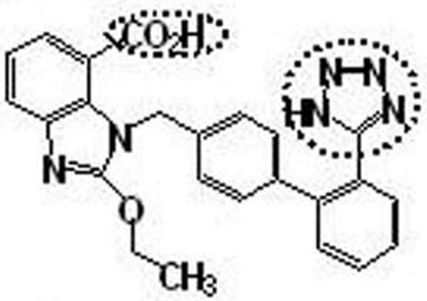
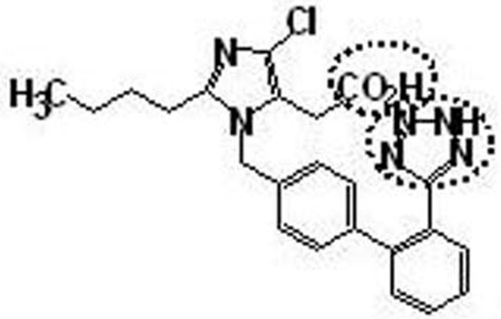
Fig. 1



Candesartan



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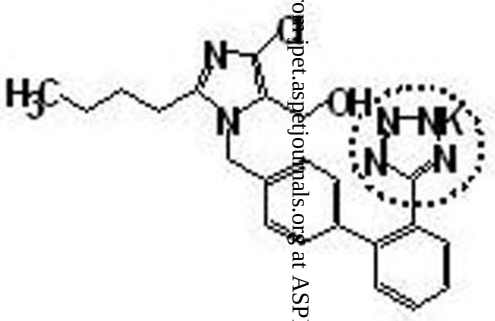

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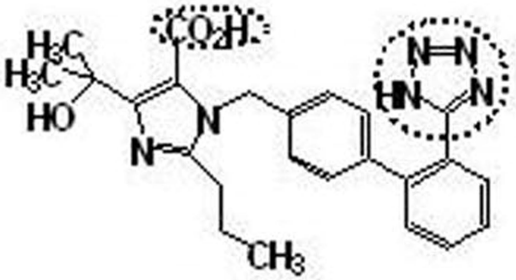
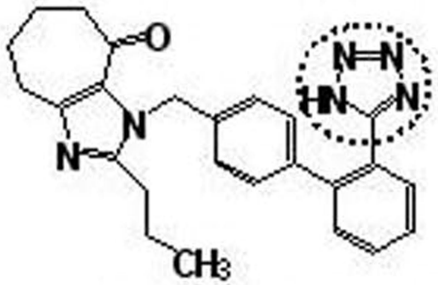
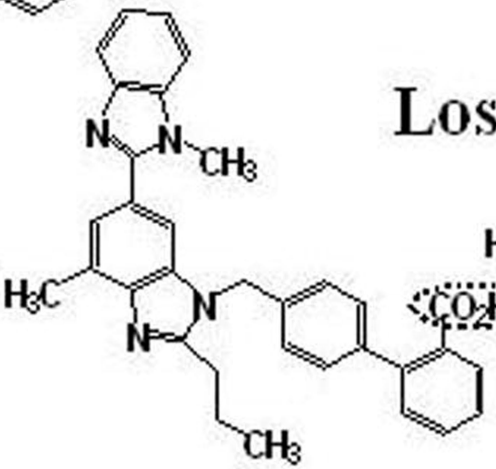


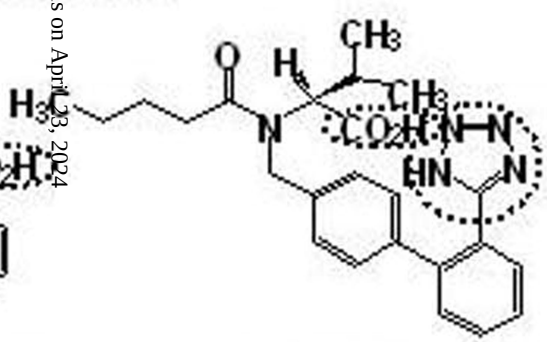
Olmesartan



Pratosartan



Telmisartan



Valsartan

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Fig. 2

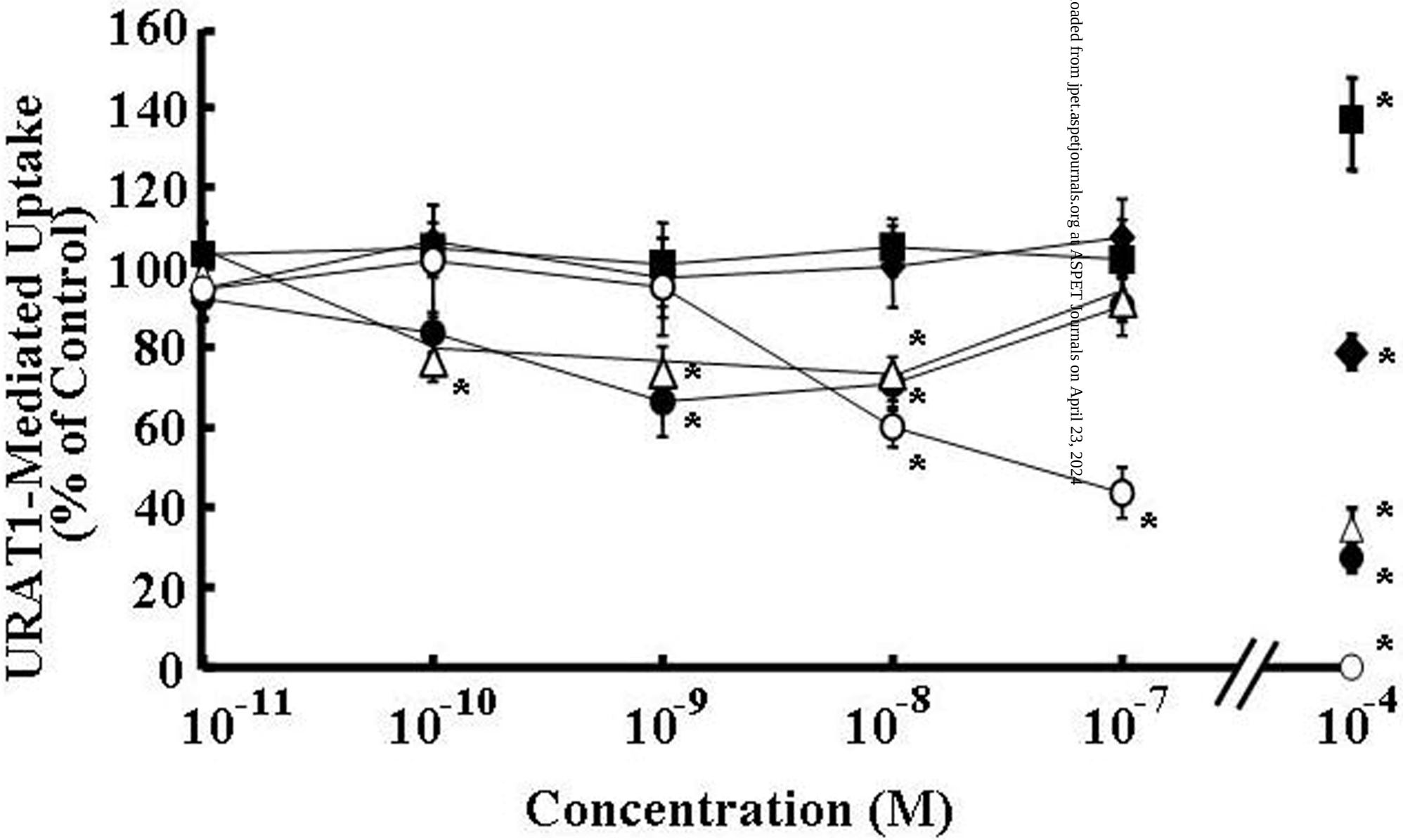

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Fig. 3

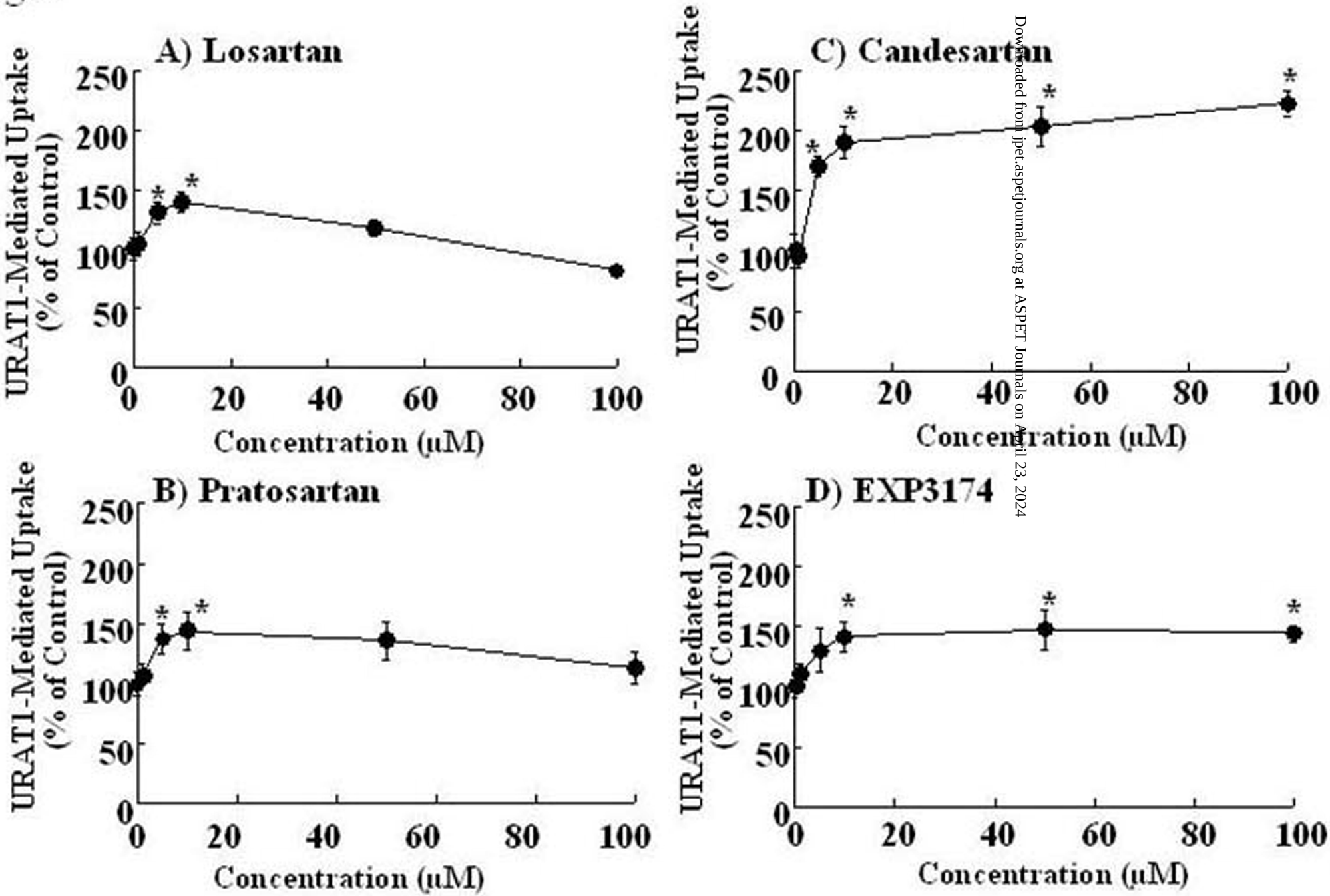

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Fig. 4

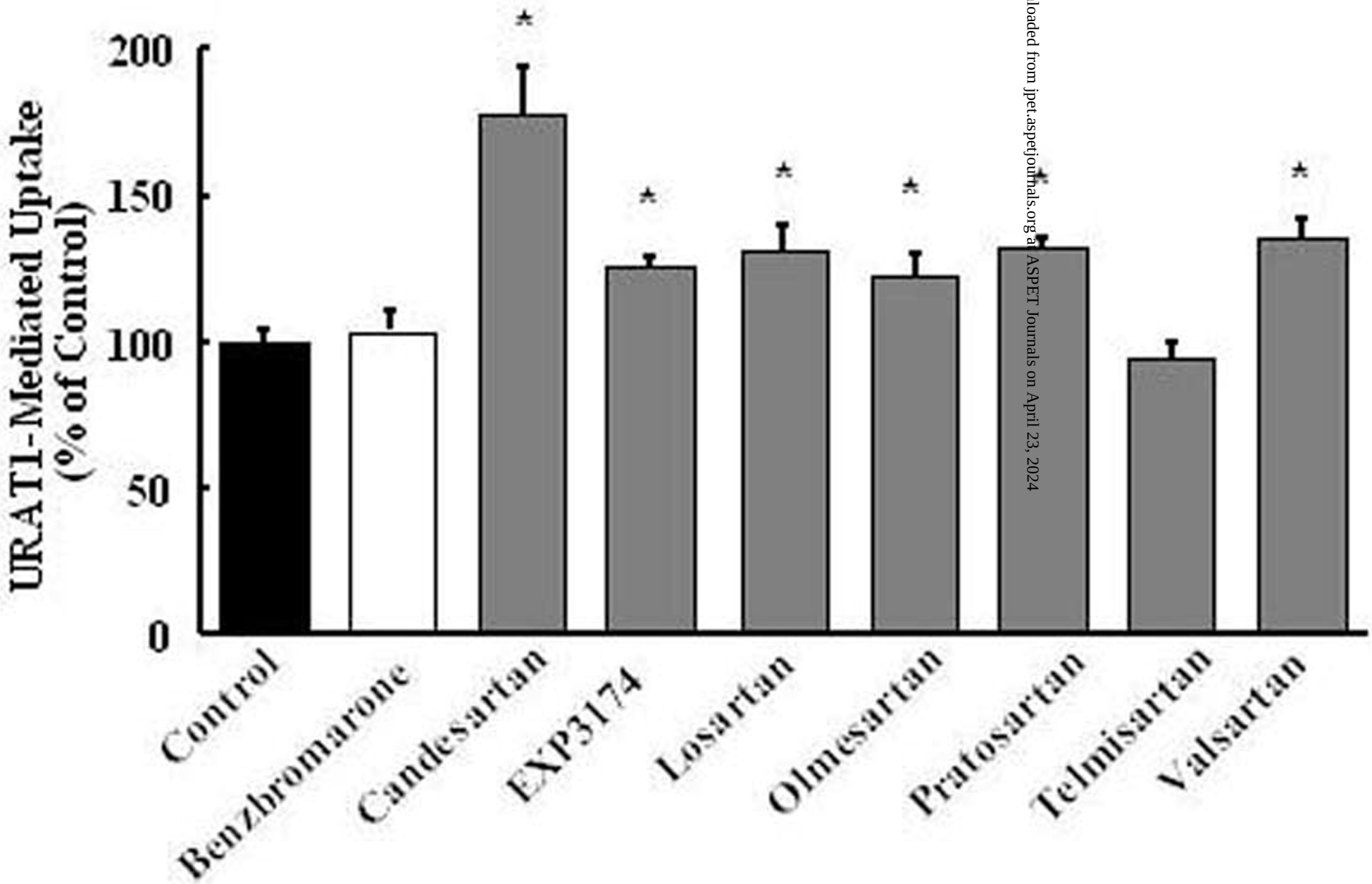

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Fig. 5

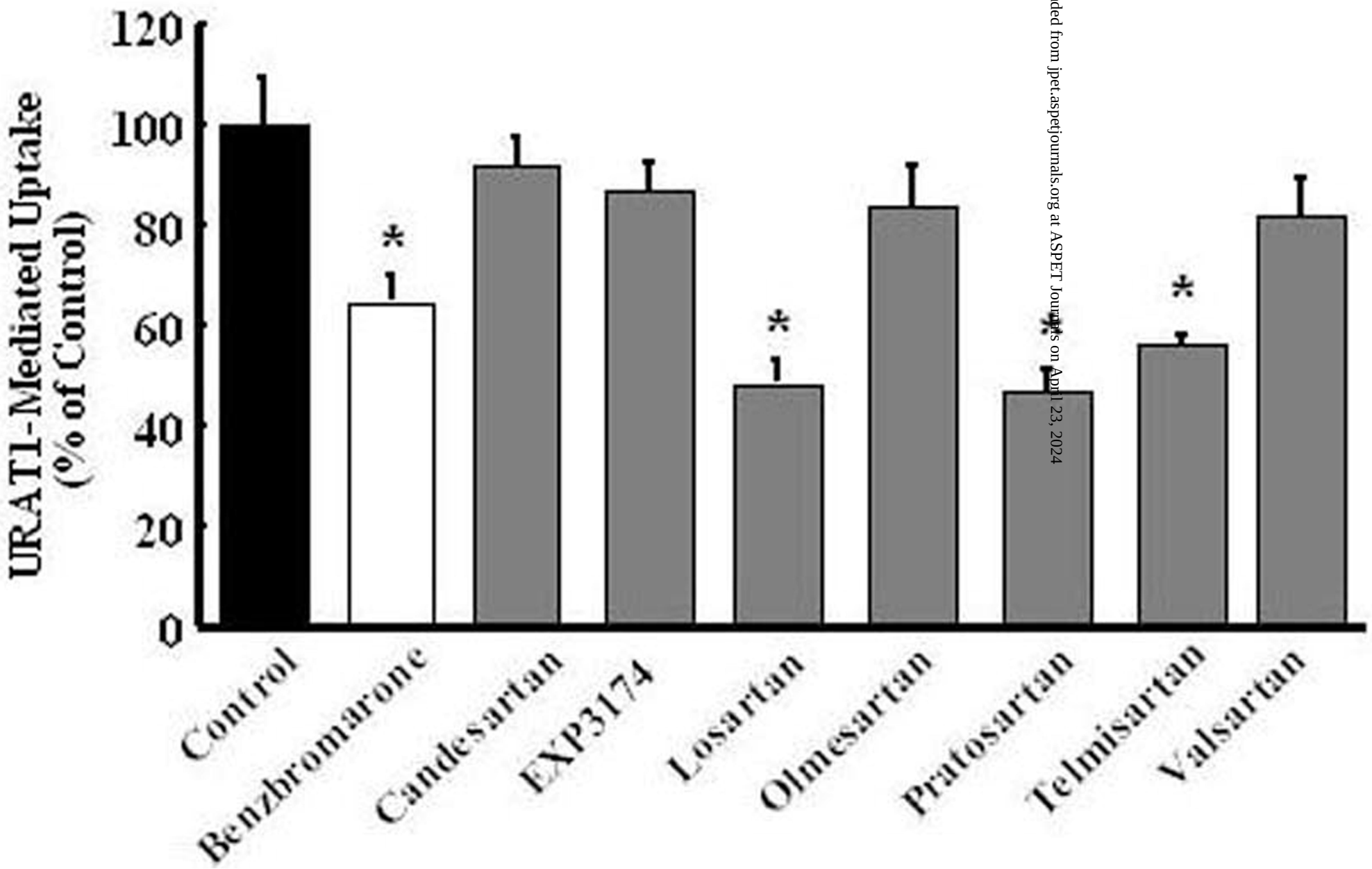

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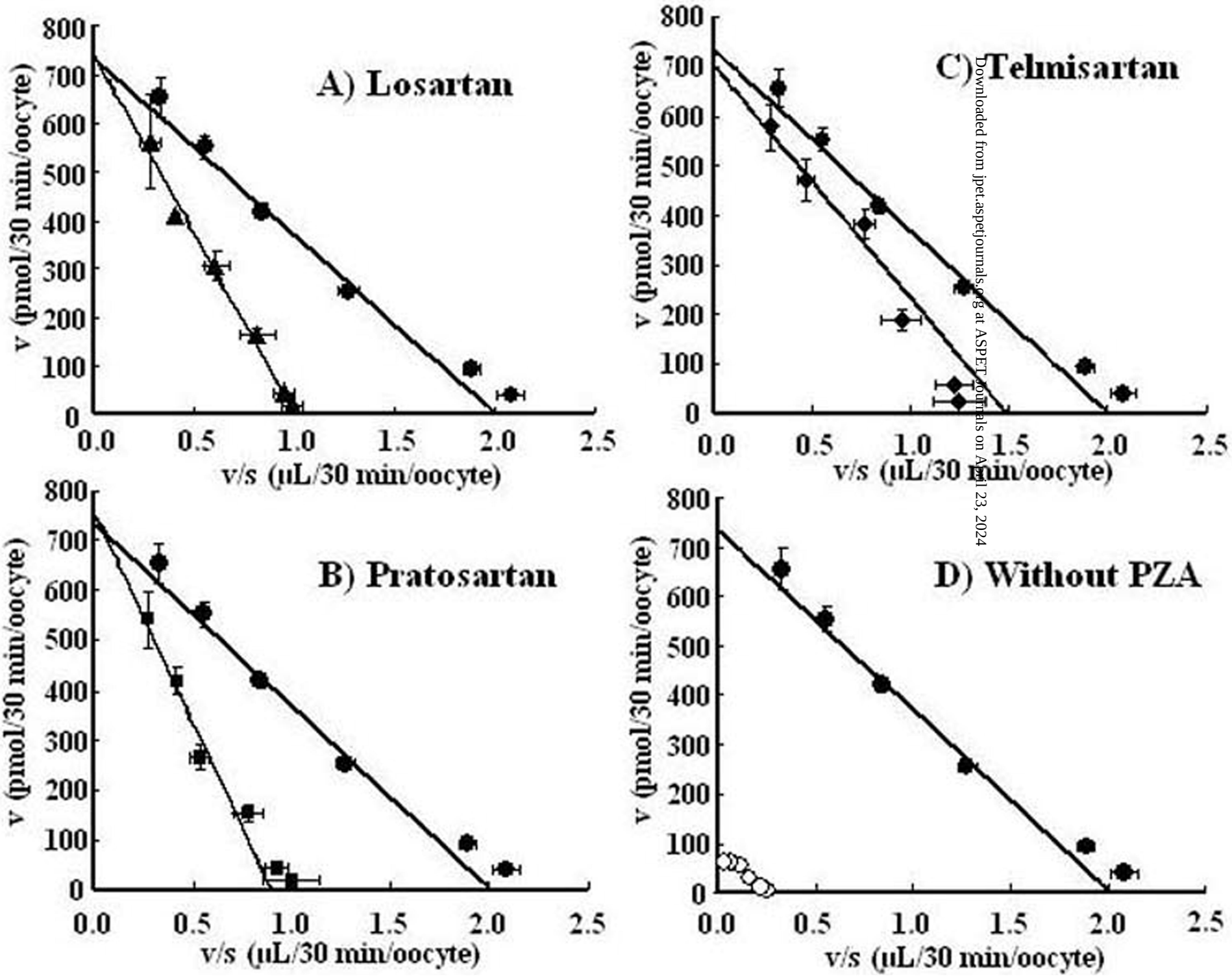
Fig. 6



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