SM-368229, a Novel Selective and Potent Non-steroidal Mineralocorticoid Receptor Antagonist With Strong Urinary Na⁺ Excretion Activity

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Abstract. Mineralocorticoid receptor (MR) antagonists, such as spironolactone (SPI) and eplerenone (EPL), are useful for the treatment of hypertension and heart failure. However, the use of these two agents has been limited due to endocrine disturbance (SPI) and poor drug action (EPL). In our search for safer and more effective MR antagonists, we identified SM-368229 as a novel non-steroidal MR antagonist. SM-368229 showed strong MR inhibitory activity with IC₅₀ values of 0.021 and 0.13 μM in the binding assay and reporter-gene assay, respectively. The selectivity of SM-368229 for MR was 18-fold higher than that for other steroid receptors, such as androgen, progesterone, and glucocorticoid receptors. SM-368229 dose-dependently increased urinary Na⁺/K⁺ ratio with an ED₅₀ value of 5.6 mg/kg in adrenalectomized rats treated with deoxycorticosterone acetate, and its efficacy was superior to that of SPI (ED₅₀ = 14 mg/kg) or EPL (ED₅₀ = 147 mg/kg). Moreover, even at high doses of 100 and 300 mg/kg, SM-368229 showed very weak anti-androgenic effect in methyltestosterone-treated male rats and no progestagenic effect in estrus cycle synchronized female rats. These findings indicate that SM-368229 may offer a new promising therapeutic option for the treatment of hypertension and heart failure.

Keywords: mineralocorticoid receptor (MR), aldosterone, spironolactone, eplerenone, steroid hormone

Introduction

Spironolactone (SPI) and eplerenone (EPL), two mineralocorticoid receptor (MR) antagonists, are useful for the treatment of hypertension, heart failure, and primary aldosteronism (1 – 4). In the randomized aldactone evaluation study (RALES) and eplerenone post-acute myocardial infarction heart failure efficacy and survival study (EPHESUS), SPI and EPL significantly reduced mortality and morbidity in patients with heart failure (5, 6). However, there are various concerns related to the safety and efficacy of these two agents. Indeed, therapy with SPI has been limited due to its induction of endocrine disturbance, mainly gynecomastia (5, 7). This undesirable effect is largely due to SPI strong affinity for the androgen receptor (AR) (8). On the other hand, EPL, which has improved selectivity for MR (9), has been shown to induce only low endocrine disturbance in humans (6). However, EPL has weak MR inhibitory activity (9), and its pharmacokinetic properties are very poor in humans and in rats (10, 11). To overcome these drawbacks, we engaged in a search for new MR antagonists with favorable safety profiles and were able to find SM-368229 (N-4,4-diethyl-2-thioxo-1,4-dihydro-2H-3,1-benzoxazin-6-yl-thiophene-2-sulfonamide) as a novel non-steroidal MR antagonist. In this study, we evaluated the pharmacological profile of SM-368229 both in vitro and in vivo and compared it to that of SPI and that of EPL.

Materials and Methods

Test compounds

The chemical structures of the test compounds used in
this study are shown in Fig. 1. SM-368229 was synthesized in our Chemistry Research Laboratories (Osaka). SPI was purchased from Sigma (St. Louis, MO, USA), and EPL was obtained from Inspra tablets (Pfizer, New York, NY, USA) by pulverization, extraction, and chromatographic purification. Test compounds were dissolved in dimethyl sulfoxide (DMSO) (Nacalai Tesque, Inc., Kyoto) for the in vitro experiments and were suspended in 0.5% methyl cellulose (MC) (Nacalai Tesque) for the in vivo experiments.

The following biological agents, chemicals, and drugs were obtained from commercial suppliers: mifepristone, progesterone, (des-Gly10, D-Trp6)-luteinizing hormone-releasing hormone (LH-RH) ethylamide, Tris-HCl, and dextran-coated charcoal (Sigma); methyltestosterone (MT) (Tokyo Kasei Kogyo Co., Ltd., Tokyo); Opti-MEM, Dulbecco’s modified Eagle’s medium (DMEM), l-glutamine, sodium pyruvate, and MEM non-essential amino acid (MEM-NEAA) solution (Invitrogen, Walkersville, MD, USA); fetal bovine serum (FBS) (MP Biomedical, Solon, OH, USA); charcoal/dextran-treated FBS and dithiothreitol (DTT) (Thermo Fisher Scientific Inc., Waltham, MA, USA); Dual-Luciferase Reporter Assay system, plasmid Renilla Luciferase–Thymidine Kinase (pRL-TK) luciferase control reporter vectors and pGL3-mouse mammary tumor virus (MMTV) vectors (Promega, Madison, WI, USA); human PR (progesterone receptor) and AR expression plasmids based on pCMV6-XL5 (Origene, Rockville, MD, USA); deoxycorticosterone acetate (DOCA) and sucrose (Wako Pure Chemical Industries, Ltd., Osaka); sodium molybdate (Kanto Chemical Co., Inc., Tokyo); 3H-aldosterone 1 mCi/ml (37 MBq/ml) and methyltrienolone (PerkinElmer, Inc., Waltham, MA, USA); COS-7 cell line (American Type Culture Collection, Manassas, VA, USA); TransIT-LT1 transfection reagent (Mirus Bio LLC, Madison, WI, USA); and corn oil (Nacalai Tesque). Human MR and glucocorticoid receptor (GR) expression plasmids based on pcDNA3.1 (+)/zeo (Invitrogen) were constructed by our Genomic Science Laboratories (Osaka).

**Animals**

Male and female Sprague-Dawley (SD) rats were obtained from Japan SLC Co., Ltd. (Shizuoka) or from Charles River Laboratories Japan, Inc. (Kanagawa). They were housed in groups of 3 – 5 per cage in a controlled environment (23 ± 2°C, 55 ± 10% humidity) with a 12-h light/dark cycle (lights on at 8:00 AM) and allowed free access to food (CE-2; Japan Clea, Tokyo) and filtered water. All animals were quarantined and acclimatized for 1 week before use, and all procedures involving their use were reviewed and approved by the Institutional Animal Care and Use Committee of Dainippon Sumitomo Pharma Co., Ltd.

**Test compounds MR binding assay in rat kidney cytosolic fractions**

Cytosolic fractions were prepared from the kidneys of adrenalectomized rats as reported by Murase et al. (12). In brief, a few days after adrenalectomy, the rat kidneys were isolated and homogenized in TSM buffer (0.1 M Tris-HCl, 0.25 M sucrose, 0.1 M sodium molybdate, 2 mM DTT, pH 7.4), ultracentrifuged at 34,000 × g for 60 min, and the supernatants were separated and used for the MR binding assay. All procedures were carried out on ice. Test compounds MR binding affinity was evaluated as described by Grekin et al. (13) with some modifications. First, each test compound was added to a cytosolic fraction, and the mixture was pre-incubated with the GR antagonist mifepristone (final concentration of 2 μM). 3H-Aldosterone (final concentration of 2 nM) was then added (n = 2), and the new mixture was incubated for 18 h at 4°C. To remove non-MR-binding 3H-aldosterone, dextran-coated charcoal was added and the mixture was centrifuged at 700 × g for 5 min. Radioactivity in the supernatant was then measured using a liquid scintillation counter (Tri-carb 2900TR; PerkinElmer). 3H-Aldosterone specific binding was calculated by subtracting radioactivity in the supernatant from total binding activity. The final concentration of DMSO in the buffer was 2% (v/v). IC50 values were calculated as concentrations of test compounds that inhibit 50% 3H-aldosterone specific binding. Data are shown as means ± S.D. of 3 independent experiments.
Evaluation of the effects of SM-368229, SPI, and EPL on transcriptional activities of MR and other steroid receptors in COS-7 cells

The effects of test compounds on transcriptional activities of MR and other steroid receptors were evaluated using the method reported by Hu et al. (8) with some modifications. In brief, COS-7 cells were suspended in DMEM supplemented with MEM-NEAA, 2 mM L-glutamine, 1 mM sodium pyruvate, and 10% FBS and maintained at 37°C in a humidified atmosphere containing 5% CO₂ until confluence. For reporter-gene assay, the cells were transferred into 24-well plates at the density of $6.4 \times 10^5$ cells/ml in DMEM containing 5% charcoal/dextran treated FBS ($n = 3$). COS-7 cells (60% – 70% confluent) were then transiently transfected using TransIT-L1 with one of the MR, AR, PR, and GR expression vectors, pGL3-MMTV and phRL-TK. The phRL-TK plasmid was used as the internal control. Following incubation for 6 h, both test compounds and ligands were added, and the mixture was incubated for 18 h. The final ligand concentration for each steroid receptor was as follows: MR: 1 nM aldosterone, AR: 0.1 nM methyltrienolone, PR: 1 nM progesterone, and GR: 1 nM dexamethasone. On the next day, the cells were collected and lysed, and luciferase activity was measured using a luminometer (LB96V; Berthold Technologies, Bad Wildbad, Germany). The final DMSO concentration was 0.2% (v/v). IC₅₀ values were calculated as concentrations of test compounds that inhibited 50% luciferase activity as compared to untreated cells. Data are shown as means ± S.D. of 3 – 4 independent experiments.

SM-368229 plasma concentration–time curve

Non-fasted SD rats aged 7 weeks were orally administered SM-368229 (10 mg/kg) and blood samples were collected from the jugular vein at 0.25, 0.5, 1, 2, 4, 6, and 24 h after SM-368229 administration. The collected blood was centrifuged (4°C, 2,000 × g, 15 min) to obtain plasma samples. Drug concentration in each plasma sample was measured using a liquid chromatography / tandem mass spectrometer (LC/MS/MS) [Alliance 2690 (Waters Corporation, Milford, MA, USA) and API3000 (Applied Biosystems Inc., Foster City, CA, USA)]. Data are shown as means ± S.D. ($n = 3$).

Evaluation of the effects of SM-368229, SPI, and EPL on urinary Na⁺/K⁺ ratio in adrenalectomized rats

The effects of test compounds on urinary Na⁺/K⁺ ratio were evaluated using the method reported by Kagawa et al. (14, 15) with some modifications. In brief, male SD rats aged 7 weeks were adrenalectomized and maintained under standard food and 0.9% NaCl drinking water for a few days. On the day of the experiment, animals, under the fasting state, were orally administered test compounds (5 ml/kg). Thirty minutes later, the animals were given 0.9% NaCl (20 ml/kg, p.o) and 1 mg/ml DOCA (1 ml/kg, s.c.) dissolved in corn oil. Vehicle animals were administered corn-oil instead of DOCA. After drug administration, the animals were maintained in metabolic cages for 23 h without food, but 0.9% NaCl drinking water was available ad libitum. Urine samples were collected and urinary Na⁺ and K⁺ concentrations were measured using a spectrophotometer (JCA-BM 1650; Japan Electron Optics Laboratory Co., Ltd., Tokyo). Urinary Na⁺/K⁺ ratio was also calculated. ED₁₅ values were calculated as doses of test compounds that increased urinary Na⁺/K⁺ ratio by 15% compared to the DOCA. Data are presented as means ± S.D. ($n = 3 – 5$).

Evaluation of the effects of SM-368229, SPI, and EPL on seminal vesicle and ventral prostate weights in methyltestosterone-treated rats

The effects of test compounds on seminal vesicle and ventral prostate weights were evaluated according to the Hershberger assay (16, 17) with some modifications. In brief, male SD rats aged 4 weeks were orally administered test compounds and subcutaneously administered 1 mg/kg MT once a day for 5 days. MT was dissolved in corn oil, and vehicle animals were administered corn oil instead of MT. After drug administration, the animals were euthanized and the weights of the seminal vesicle and ventral prostate were measured. Seminal vesicle weight (SVW) ID₃₀ values were calculated as the doses of test compounds that decreased SVW by 30% compared to MT. Data are presented as means ± S.D. ($n = 4 – 5$).

Evaluation of the effects of SM-368229, SPI, and EPL on estrus cycle in female rats

The effects of test compounds on the estrus cycle in female rats were evaluated according to the method of Zhang et al. (18). In brief, mature female SD rats aged 8 – 10 weeks had their estrus cycle synchronized with LH-RH (2 μg/rat) administered subcutaneously at 9:00 AM and again at 4:00 PM. The animals were allowed to rest for 9 days before administration of test compounds. After confirmation that the estrus cycle was in the ovulation phase by a vaginal smear test, the animals were grouped and administered test compounds once a day for 4 days. At 24 h of the last treatment, animals whose estrus cycle was in the ovulation period were regarded as having a normal estrus cycle and the percentage of animals with normal estrus cycle was calculated. The animals were then euthanized, and the oviducts were removed, placed between two glass slides, and viewed under a
dissecting microscope to count the ova. Ova ID50 values were calculated as doses of test compounds that decreased ova number by 50% compared to the vehicle. Data are presented as means ± S.D. (n = 7 – 8).

Statistical analyses
Statistical significance was determined by Student’s t-test or Dunnett’s multiple comparison test. A P-value less than 0.05 was considered as statistically significant. All statistical calculations were performed with Stat Preclinica software (Takumi Information Technology, Inc., Tokyo).

Results

Test compounds MR binding affinity in rat kidney cytosolic fractions
SM-368229 dose-dependently displaced ['H]-aldosterone specific binding with an IC50 value of 0.021 μM. SPI and EPL also inhibited ['H]-aldosterone specific binding with IC50 values of 0.0051 and 0.12 μM, respectively (Fig. 2).

Effects of SM-368229, SPI, and EPL on MR transcriptional activity in COS-7 cells
SM-368229 showed potent MR antagonistic activity with an IC50 value of 0.13 μM. On the other hand, SM-368229 (10 μM) showed partial MR agonistic activity (12%). SPI and EPL showed full MR antagonistic activity with IC50 values of 0.012 and 0.42 μM, respectively (Table 1).

Effects of SM-368229, SPI, and EPL on transcriptional activities of other steroids receptors in COS-7 cells
SM-368229 showed AR, PR, and GR antagonistic activity with IC50 values of 5.0, 2.3, and 30 μM, respectively. On the other hand, SM-368229 (10 μM) showed partial PR agonistic activity (22%). Although SPI had AR, PR, and GR antagonistic activity with IC50 values of 0.011, 0.35, and 2.2 μM, respectively, it also had AR and PR agonistic activity. EPL, on the other hand, showed full AR, PR, and GR antagonistic activity with IC50 values of 4.5, 24, and over 100 μM, respectively (Table 1).

SM-368229 plasma concentration–time curve
SM-368229 (10 mg/kg, p.o.) showed sustained systemic exposure in rats (Cmax: 8027 ng/ml, t1/2: 4.5 h, area under the curve (AUC): 107233 ng·h/ml) (Fig. 3).

Effects of SM-368229, SPI, and EPL on urinary Na+/K+ ratio in adrenalectomized rats
Compared to the vehicle group, DOCA-treated rats

Table 1. In vitro selectivity of SM-368229, spironolactone and eplerenone for MR and other steroid receptors

<table>
<thead>
<tr>
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<th>IC50 (μM)</th>
<th>Selectivity for MR</th>
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<tr>
<td></td>
<td>MR</td>
<td>AR</td>
</tr>
<tr>
<td>SM-368229</td>
<td>0.13 ± 0.05 (†12)</td>
<td>5.0 ± 2.2</td>
</tr>
<tr>
<td>Spironolactone</td>
<td>0.012 ± 0.004 (†36)</td>
<td>0.011 ± 0.008 (†36)</td>
</tr>
<tr>
<td>Eplerenone</td>
<td>0.42 ± 0.07</td>
<td>4.5 ± 1.5</td>
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COS-7 cells were transiently transfected with one of the MR, AR, PR, and GR expression vectors, pGL3-MMTV, and phRL-TK. Following incubation for 6 h, both test compounds and a ligand were added. On the next day, luciferase activity was measured. Final ligand concentration for each steroid receptor was as follows: MR: 1 nM aldosterone, AR: 0.1 nM methyltrienolone, PR: 1 nM progesterone, and GR: 1 nM dexamethasone. † indicates agonistic activity at a concentration of 10 μM. Data are shown as means ± S.D. of 3 – 4 independent experiments. MR: mineralocorticoid receptor, AR: androgen receptor, PR: progesterone receptor, GR: glucocorticoid receptor.
showed significant decrease in Na⁺ excretion and significant increase in K⁺ excretion over the 23-h experimental period. Based on these findings, we used urinary Na⁺/K⁺ ratio as an index for the anti-mineralocorticoid effect of the test compounds. SM-368229 dose-dependently increased DOCA-induced decrease in urinary Na⁺/K⁺ ratio with an ED15 value of 5.6 mg/kg. On the other hand, the ED15 values of SPI and EPL were 14 and 147 mg/kg, respectively (Fig. 4).

Effects of SM-368229, SPI, and EPL on seminal vesicle and ventral prostate weights in methyltestosterone-treated rats

Compared to the vehicle group, MT-treated rats exhibited significant increases in seminal vesicle and ventral prostate weights. High doses of SM-368229 (100, 300 mg/kg) produced only a slight inhibition of MT-induced increase in seminal vesicle and ventral prostate weights with a SVW ID₃₀ value of 228 mg/kg. On the other hand, SPI strongly and significantly inhibited the MT-induced increase in both seminal vesicle and ventral prostate weights with a SVW ID₃₀ value of 58 mg/kg. EPL induced slight inhibition of MT-induced increase in both seminal vesicle and ventral prostate weights with a SVW ID₃₀ value of 896 mg/kg (Fig. 5).

Effects of SM-368229, SPI, and EPL on estrus cycle in female rats

We confirmed that rats in the vehicle group had a normal estrus cycle with many ova in the oviducts. High doses of SM-368229 (100, 300 mg/kg) or EPL (1000 mg/kg) had no negative effect on the rat estrus cycle or ovulation. On the other hand, only 38% of the animals treated with SPI showed both a normal estrus cycle and ovulation. Ova ID₅₀ values of SM-368229, SPI, and EPL were over 300, 76, and over 1000 mg/kg, respectively (Fig. 6).

Discussion

In this study, we evaluated the pharmacological profile of SM-368229, a novel non-steroidal MR antagonist, both in vitro and in vivo and compared it to that of SPI and that of EPL. SM-368229 showed strong MR inhibitory activity in the binding and reporter-gene assays. The selectivity of SM-368229 for MR was more than 18-fold that for other steroid receptors in vitro and more than 41-fold in vivo. The low selectivity of SM-368229 for other steroid receptors is very important, considering the undesirable endocrine related side-effects of SPI (5, 7). Indeed, it has been reported that EPL, which has selectivity for MR, does not cause endocrine disturbance in humans (6). We found in this study that SM-368229 selec-
activity for MR in vivo was superior to that of SPI (4.1-fold) or EPL (6.1-fold). Based on this finding, it can be assumed that SM-368229 would not induce any endocrine-related disturbance in humans.

SM-368229 showed 11-fold weaker or 3-fold stronger mineralocorticoid inhibitory activity than SPI or EPL in the reporter-gene assay, respectively. However, the anti-mineralocorticoid efficacy of SM-368229 in adrenalectomized rats was 2.5-fold or 26-fold superior to that of SPI or EPL, respectively. This superior effect in vivo may be due to SM-368229 sustained systemic exposure. SM-368229 given at the dose of 10 mg/kg resulted in an AUC value of 107233 ng·h/ml. This value is 45-fold larger than that obtained with EPL at the dose of 15 mg/kg (2380 ng·h/ml) (11). Making the same comparison with SPI is rather difficult due to the complexity of the SPI metabolic pattern and the resulting numerous active metabolites, including canrenone and 7α-thiomethylspironolactone (19, 20).

It has been reported that EPL-induced increase in urinary Na⁺/K⁺ ratio in adrenalectomized rats treated with aldosterone is 3-fold superior to that of SPI (9). However, our results show that the increase in urinary Na⁺/K⁺ ratio produced by EPL is 11-fold inferior to that of SPI. This discrepancy is probably due to the difference in urine collection time between our study and that of Gasparo (23-h experimental period vs. 4-h experimental period) (9). When developing anti-hypertensive drugs, it is important to favor long-acting drugs with convenient regimen due to compliance, high trough/peak ratio, and stable drug action. Based on this, our experimental period for measurement of urinary Na⁺/K⁺ ratio (23 h after drug administration) may reflect the effects of the test compounds on blood pressure. It would therefore be interesting to evaluate the anti-hypertensive and anti-heart failure effects of SM-368229 and compare them to those of SPI and EPL.

Gynecomastia or breast pain was observed in 10% of patients that suffered heart failure and were treated with a low dose of SPI (25 mg/day) (5). The same symptoms were also observed in 52% of patients with essential hypertension treated with high doses of SPI (150 – 300 mg/day) (7). These negative effects were related to the relatively high affinities of SPI and canrenone for AR (8, 9). Our results in this study show that SPI produced an anti-androgenic effect (SVW ID₃₀ = 58 mg/kg) at a dose relatively similar to the dose that induced the anti-mineralocorticoid effect (14 mg/kg). These findings are in agreement with those of our in vitro experiments and with the results in Gasparo’s report (9). Compared to SPI, EPL showed 11-fold selectivity for AR/MR in vitro and 6.1-fold selectivity for AR/MR in vivo (Tables 1 and
In addition, the anti-androgenic adverse effect of EPL was reported to be the same as that of the placebo in humans (6). Based on these findings, it is believed that SM-368229, which has higher selectivity for AR/MR in vitro (38-fold) and in vivo (41-fold), will not induce anti-androgenic adverse effects (gynecomastia or lack of libido) in humans.

Compounds with PR agonistic or antagonistic activity are known to affect the estrus cycle (21, 22). Clinical reports have shown menstrual irregularities in patients under SPI treatment (23 – 26). These irregularities have also been observed in animals treated with SPI (9), including those used in this study. Therefore, considering the progestagenic effect of SPI, it was important to use this drug for comparison in this study. SM-368229 showed weak PR agonistic (22% at 10 μM) and antagonist (IC50 = 2.3 μM) activity in the reporter-gene assay. However, administration of high doses of SM-368229 (100, 300 mg/kg) for 4 consecutive days did not have any effect on rat estrus cycle or ovulation. Since it remains unclear whether SM-368229 selectivity for PR/MR is good enough, additional long-term reproduction and development experiments in rodents or non-rodents are needed to confirm the safety of SM-368229.

SM-368229 in vitro selectivity for GR/MR (231-fold) was superior to that of SPI (183-fold) (Table 1). Although we found in a preliminary study that high dose of SM-368229 (1000 mg/kg for 14 days) had no effect on thymus gland weight in male rats (data not shown), our investigation was not detailed. Therefore, additional experiments are needed to evaluate the effects of SM-368229 on thymus gland weight in rats treated with dexamethasone.

We evaluated in this study the effect of SM-368229, SPI, and EPL on the MR transcriptional activity (genomic action of aldosterone) by reporter-gene assay. On the other hand, EPL has been reported to have a more potent inhibitory effect on the non-genomic action of aldosterone than SPI (27 – 29). However, the effects of non-genomic action of aldosterone have not been fully clarified, and there is no clear evidence showing a difference in the inhibitory effects of SPI and EPL on the action of aldosterone. It would be interesting to examine the effect of SM-368229 on the rapid non-genomic action of aldosterone and compared it to those of SPI and EPL.

In conclusion, we have shown in this study that SM-368229, a novel selective and potent orally active non-steroidal MR antagonist, has strong anti-mineralocorticoid activity superior to that of SPI or EPL in vivo. SM-368229 selectivity for other steroid receptors is also superior to that of SPI or EPL in vivo. These findings indicate that SM-368229 may offer a new therapeutic option for the treatment of hypertension and heart failure.

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**Table 2.** In vivo selectivity of SM-368229, spironolactone, and eplerenone for MR and other steroid receptors

<table>
<thead>
<tr>
<th></th>
<th>Na+/K⁺ ED₁₅</th>
<th>SVW ID₃₀</th>
<th>Ova ID₅₀</th>
<th>Selectivity for MR</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>MR</td>
<td>AR</td>
<td>PR</td>
<td>AR/MR</td>
</tr>
<tr>
<td>SM-368229</td>
<td>5.6</td>
<td>228</td>
<td>&gt;300</td>
<td>41</td>
</tr>
<tr>
<td>Spironolactone</td>
<td>14</td>
<td>58</td>
<td>76</td>
<td>4.1</td>
</tr>
<tr>
<td>Eplerenone</td>
<td>147</td>
<td>896</td>
<td>&gt;1000</td>
<td>6.1</td>
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</table>

Na+/K⁺ ED₁₅ values were calculated as doses of test compounds that increased urinary Na+/K⁺ ratio by 15% compared to the deoxycorticosterone acetate–treated rats (n = 3 – 5). Seminal vesicle weight (SVW) ID₃₀ values were calculated as doses of test compounds that decreased SVW by 30% compared to methyltestosterone-treated rats (n = 4 – 5). Ova ID₅₀ values were calculated as doses of test compounds that decreased ova number by 50% compared to the vehicle (n = 7 – 8). MR: mineralocorticoid receptor, AR: androgen receptor, PR: progesterone receptor.
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