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Supplementary Materials for

Intestinal Inhibition of the Na^+/H^+ Exchanger 3 Prevents Cardiorenal Damage in Rats and Inhibits Na^+ Uptake in Humans

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The PDF file includes:

Figure S1

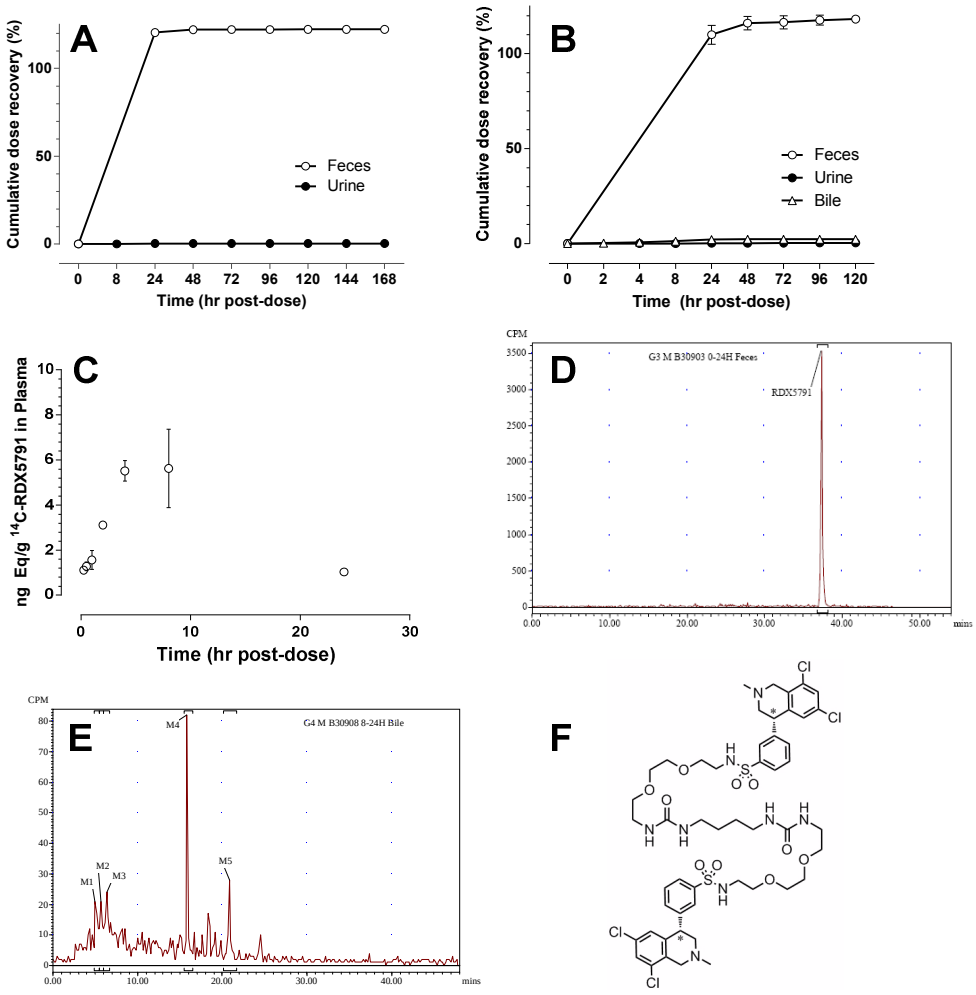

Figure S1. Summary of mass balance study of ^{14}C-tenapanor. Radiolabeled mass balance studies of ^{14}C- tenapanor. Determination of mass balance after dosing (A) normal and (B) bile duct cannulated (BDC) rats with ^{14}C- tenapanor shows the drug is excreted largely via feces in the first 24 hr after dosing. (C) Negligible ^{14}C- tenapanor -derived radioactivity was detected in plasma . (D) Detection of radioactivity in fecal extracts showed feces contained almost exclusively parent ^{14}C- tenapanor, while (E) bile contained more polar species and no detectable ^{14}C- tenapanor, which elutes at ~37.5 min in the method used in D and E. (F) The structure of ^{14}C- tenapanor. * = the labeled carbon in a non-metabolizable chiral position. Data in A-C are expressed as mean ± SEM.

Figure S2

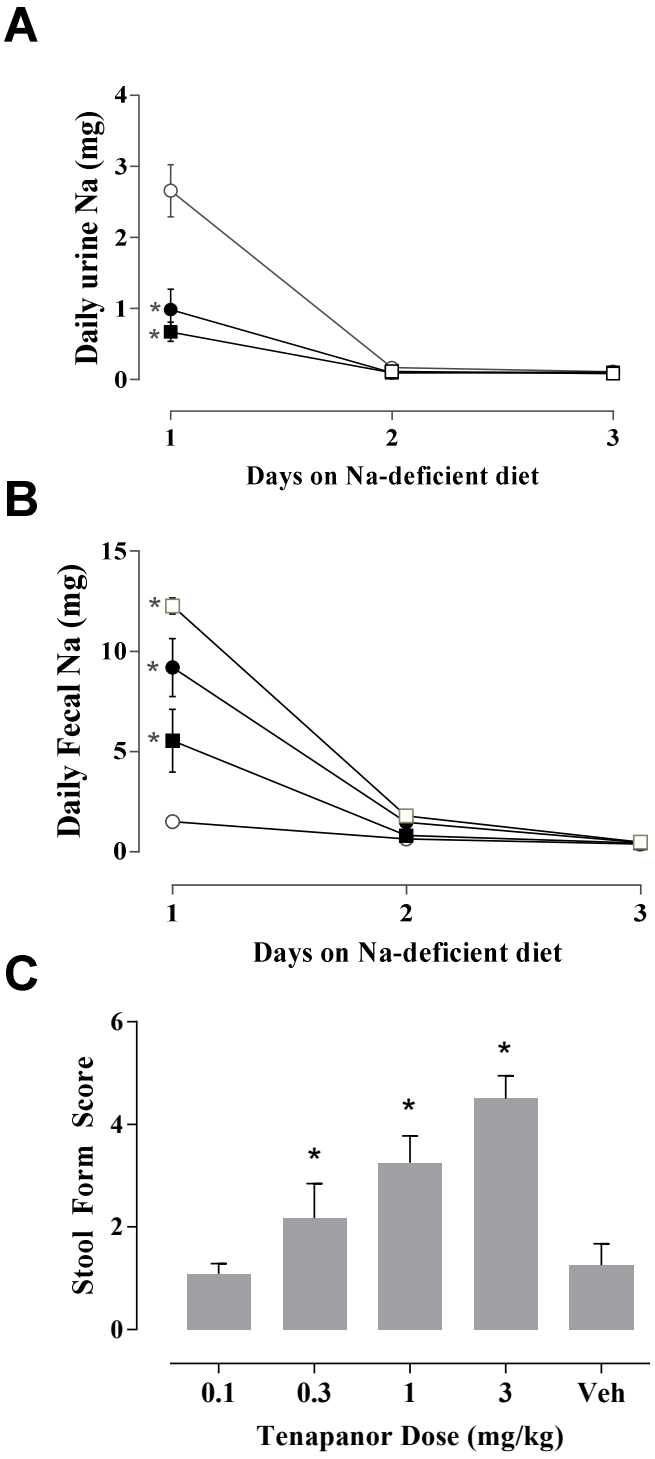
A
Daily urine Na (mg)
4
3
2
1
0
*
*
1
2
3
Days on Na-deficient diet
B
Daily Fecal Na (mg)
15
10
5
0
*
*
*
1
2
3
Days on Na-deficient diet
C
Stool Form Score
6
4
2
0
*
*
*
0.1
0.3
1
3
Veh
Tenapanor Dose (mg/kg)

Figure S2: Effect of tenapanor on fecal and urinary sodium in rats on a very low sodium diet, and dose-dependent effect of tenapanor on stool form in rats. Sprague-Dawley rats were fed a sodium deficient diet (0.01% Na^+) and dosed daily by oral gavage with vehicle (water, ○), or tenapanor at 0.3 mg/kg (■) or 1 mg/kg (●), or 3 mg/kg (□) on each of three consecutive days. Urine (A) and fecal (B) sodium were determined after 24 hour collections. (C) The effect of increasing doses of tenapanor on stool form score in rats consuming standard chow. Data are expressed as Mean ± SEM . * = $p < 0.05$ versus vehicle, 2-way ANOVA, Bonferroni's *posthoc* test.

Figure S3

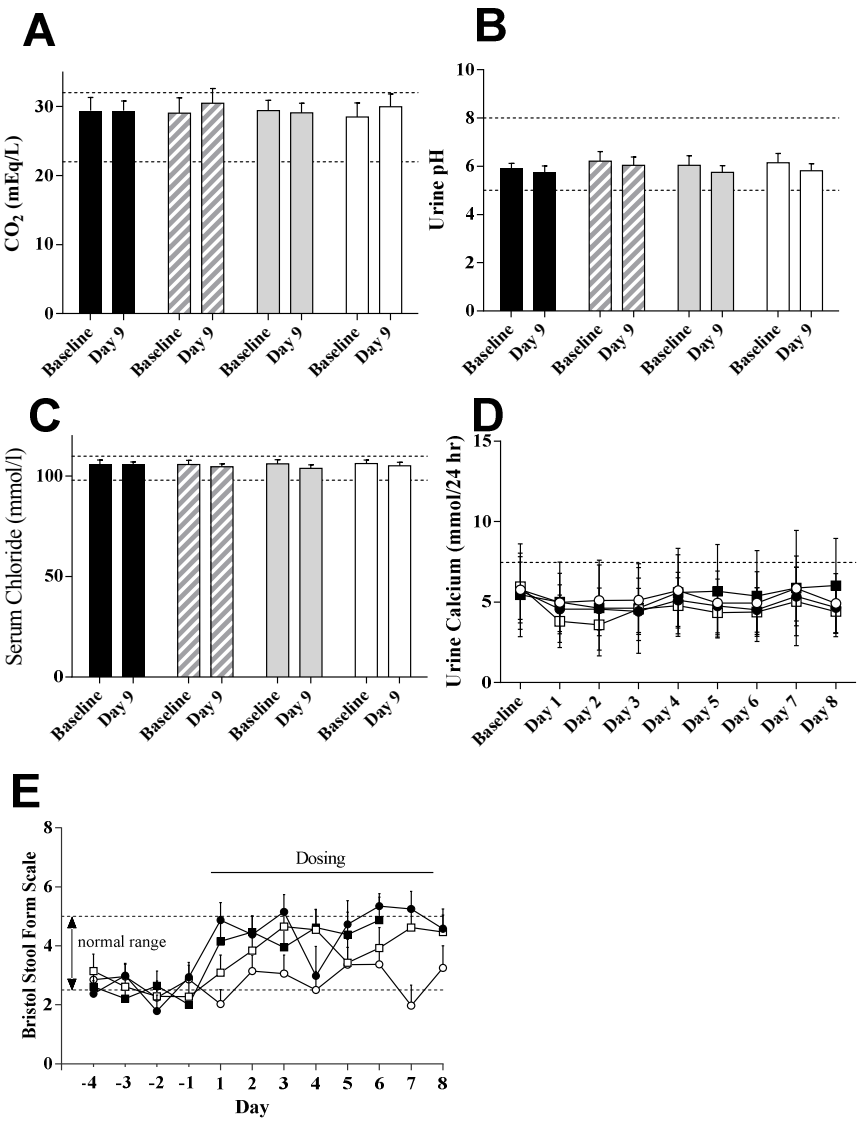
A
CO_2 (mEq/L)
0
10
20
30
Baseline
Day 9
B
Urine pH
0
2
4
6
8
10
C
Serum Chloride (mmol/l)
0
50
100
D
Urine Calcium (mmol/24 hr)
0
5
10
15
Baseline
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Day 8
E
Bristol Stool Form Scale
normal range
Dosing
-4
-3
-2
-1
1
2
3
4
5
6
7
8
Day

Figure S3: Measurements of serum bicarbonate and chloride, urine pH and urinary calcium, and stool form in humans after 7-day repeated, twice-daily dosing of placebo and 15 to 60 mg of tenapanor. Serum bicarbonate (A), urine pH (B), serum chloride (C), urinary calcium (D), or stool form (E). In A-C, white = placebo; black = 15 mg bid; hatched = 30 mg bid; grey = 60 mg bid. In D-E, ○ = placebo; ● 15 mg bid; □ = 30 mg bid; ■ = 60 mg bid. Dotted lines indicate normal ranges; in D, the lower bound of the normal range is zero. Data are expressed as mean ± SEM.

Figure S4

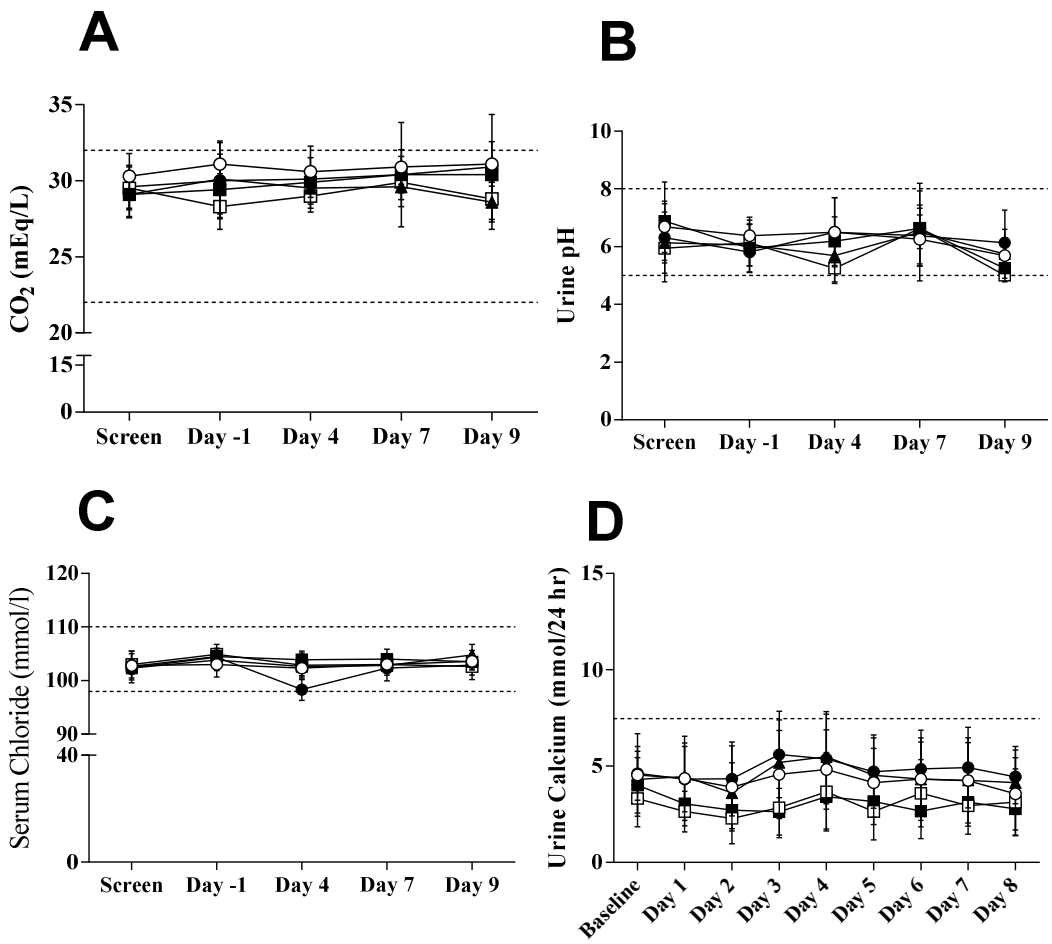

Figure S4: Measurements of serum bicarbonate and chloride, urine pH, and urinary calcium in humans after 7-day repeated, once-daily dosing of placebo and 3 to 100 mg of tenapanor. Tenapanor was dosed as a capsule at 3 mg qd, 10 mg qd, 30 mg qd, 100 mg qd, or placebo. There were no changes in serum bicarbonate (A), urine pH (B), serum chloride (C), or urinary calcium (D). ○ = placebo; ● 3 mg qd,; □ = 10 mg qd; ■ = 30 mg qd; ▲ = 100 mg qd. Dotted lines indicate normal ranges; in D, the lower bound of the normal range is zero. Data are expressed as mean ± SEM.

Figure S5

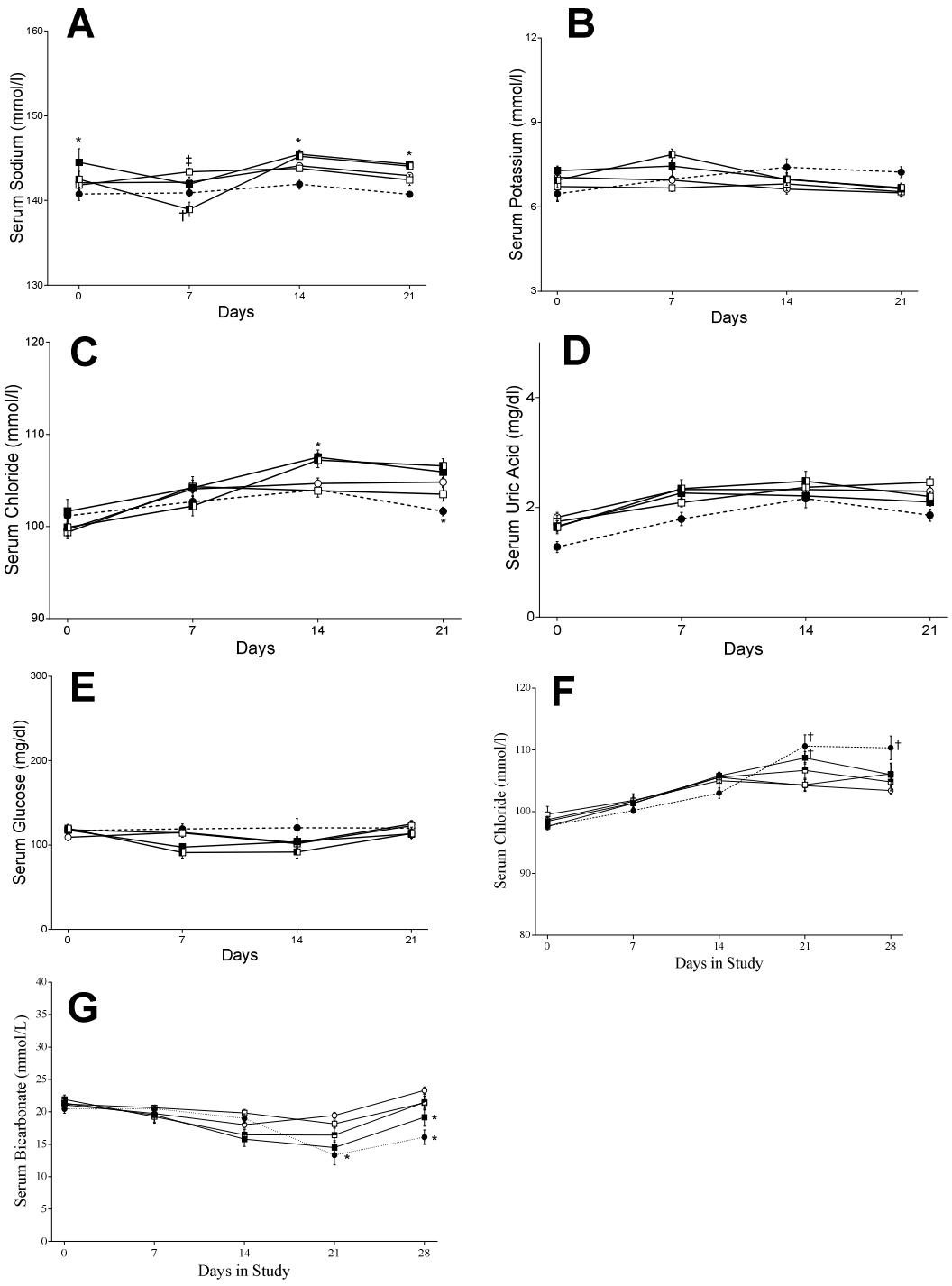
A
Serum Sodium (mmol/l)
Days
B
Serum Potassium (mmol/l)
Days
C
Serum Chloride (mmol/l)
Days
D
Serum Uric Acid (mg/dl)
Days
E
Serum Glucose (mg/dl)
Days
F
Serum Chloride (mmol/l)
Days in Study
G
Serum Bicarbonate (mmol/L)
Days in Study

Figure S5: Effect of tenapanor, or tenapanor and enalapril, on serum sodium, potassium, chloride, uric acid, glucose, and bicarbonate in NPX rats. Significance by 1-way ANOVA: * = $p < 0.05$ vs. naïve controls; † = $p < 0.05$ vs. NPX vehicle controls; ‡ = $p < 0.05$ vs. NPX + enalapril. A-E: Open circle = vehicle; open square = 10 mg/kg enalapril; closed square = 1.0 mg/kg tenapanor; split square = 10 mg/kg enalapril + 1 mg/kg tenapanor; closed circle = naïve controls. F-G: Open circle = vehicle; open square = 0.3 mg/kg tenapanor; split square = 1.0 mg/kg tenapanor; closed square = 3.0 mg/kg tenapanor prophylactic setting; closed circle and dotted line = 3.0 mg/kg tenapanor treatment setting. Data are expressed as mean ± SEM.

Table S1 Potency of tenapanor activity against NHE1, NHE2, NHE3, NaPiIIb, TGR5, ASBT, and Pit-1.

Target	IC50	Result
Human NHE1	>10 μM	No detectable inhibition of human NHE1
Human NHE2	>10 μM	No detectable inhibition of human NHE2
Human NHE3	5 ± 4 nM (n>50)	~5 nM inhibitor of human NHE3
Rat NHE3	10 ± 7 nM (n>50)	~10 nM inhibitor of rat NHE3
Human NaPi2b	>10 μM	No detectable inhibition of human NaPi2b
Human TGR5	>10 μM	No detectable agonism of human TGR5
Human ASBT	>10 μM	No detectable inhibition of human ASBT
Human PiT1	>10 μM	No detectable inhibition of human PiT1

Table S2 PK parameters of tenapanor in rats, dogs, and humans.

Dose (mg/kg)	Species[D]	Diet	Co-Dosed	Route	C_{max} (ng/mL)	AUC (ng•h/mL)	% Unbound[E]
30	Rat (n=3)	0.49% Na^+	-	PO gavage	<3	BQL	<0.001 **rat plasma**
10	Rat (n=5)	0.49% Na^+	-	PO gavage	BQL	BQL	
1	Rat (n=5)	0.49% Na^+	-	PO gavage	BQL	BQL	
1	5/6th NPX Rat (n=3)	4.0% NaCl	-	PO gavage	<1	BQL	
1	5/6th NPX Rat (n=4)	4.0% NaCl	Enalapril	PO gavage	<1	BQL	
10	Dog (n=4)	0.49% Na^+	-	PO gavage	BQL	BQL	<0.001 **dog plasma**
1	Dog (n=4)	0.49% Na^+	-	PO gavage	<1	BQL	
3 mg qd[A,B]	Human (n=8)	1200 mg Na^+	-	PO capsule	BQL	BQL	<0.001 **human plasma**
10 mg qd[A,B]	Human (n=8)	1200 mg Na^+	-	PO capsule	BQL	BQL	
30 mg qd[A,B]	Human (n=8)	1200 mg Na^+	-	PO capsule	BQL	BQL	
100 mg qd[A,B]	Human (n=8)	1200 mg Na^+	-	PO capsule	BQL	BQL	
150 mg qd[A,C]	Human (n=6)	1500 mg Na^+	-	PO capsule	BQL	BQL	
450 mg qd[A,C]	Human (n=6)	1500 mg Na^+	-	PO capsule	<1	BQL	
900 mg qd[A,C]	Human (n=6)	1500 mg Na^+	-	PO capsule	<1	BQL	

A = Human doses are expressed in total milligrams of the bis-hydrochloride salt of tenapanor.

B = Doses were administered on each of seven consecutive days.

C = Doses were administered on a single day.

D = All human subjects were healthy volunteers

E = % Unbound is the percentage of unbound plasma tenapanor determined by in vitro equilibrium dialysis experiments using the rapid equilibrium dialysis (RED) device (Pierce)

BQL = below the quantitative limit of 0.5 ng/mL

Note: Typically tenapanor concentrations were below the limit of quantification (0.5 ng/mL). C_{max} and AUC values are reported as <*x* to reflect that rare samples in some individuals had low concentrations of tenapanor within the quantitative range.

Table S3 MDCK and PAMPA permeability data of tenapanor.

Compound	MDCK Monolayer Permeability							
	A(pH 7.4) → **B**(pH 7.4)		**B**(pH 7.4) → **A**(pH 7.4)		**A**(pH 6.5) → **B**(pH 7.4)		**B**(pH 7.4) → **A**(pH 6.5)	
	P_{app}	**Recovery (%)**	**P_{app}**	**Recovery (%)**	**P_{app}**	**Recovery (%)**	**P_{app}**	**Recovery (%)**
Atenolol	0.5 ± 0.1	102 ± 2%	1 ± 0.1	107 ± 4	0.5 ± 0.04	102 ± 10	2 ± 0.5	107 ± 9
Propranolol	28 ± 1	95 ± 6%	18 ± 3	95 ± 6	7 ± 0.1	99 ± 1	26 ± 0.4	104 ± 1
Labetalol	6 ± 0.3	96 ± 0.6%	14 ± 2	104 ± 0.5	1 ± 0.1	99 ± 2	14 ± 0.1	104 ± 0.3
Colchicine	0.3 ± 0.1	101 ± 3.7%	3 ± 0.2	104 ± 2	0.3 ± 0.04	100 ± 1	3 ± 0.2	104 ± 2
Tenapanor[A]	<0.01 ± <0.01	86 ± 10	<0.01 ± <0.01	64 ± 5†	<0.01 ± <0.01	83 ± 1	0.02 ± 0.03	59 ± 2†
Tenapanor[B]	0.04 ± 0.003	102 ± 0.3	0.7 ± 0.1	100 ± 0.4	0.04 ± 0.01	98 ± 2	0.6 ± 0.001	102 ± 0.3

†The low recovery observed when tenapanor was added on the basolateral side of the monolayer improved to ~100% when additives like bovine serum albumin (2%) or Tween-80 (0.1%) were employed. The general behavior of tenapanor in the presence of additives was not significantly different (i.e., low P_{app}), but permeability artifacts for control compounds confounded interpretation of data gathered in the presence of additives.

[A] = < are values that were below the limit of quantitation

[B] = Tween-80 (0.1%) was added to transport buffer to reduce the potential interaction of tenapanor with the assay plate wells and Transwell inserts.

P_{app} = Apparent permeability rate in 10^{-6} x (cm/s)

Table S4 Radiolabeled ADME and QWBA study design.

Group	Rat Strain	Dose	Samples Analyzed	Collection Times
1	Sprague-Dawley	90-110 μCi/kg	Blood	0.25-72 h
2	Sprague-Dawley & Long Evans	90-110 μCi/kg	Blood, Carcasses for QWBA	0.5-504 h
3	Sprague Dawley	23-33 μCi/kg	Urine, Feces	0-168 h
4	Sprague Dawley	23-33 μCi/kg	Urine, Feces, Bile	0-120 h

Table S5 QWBA Data for ^{14}C-tenapanor in Long-Evans rats.

Tissue	ng Eq ^{14}C-Tenapanor/g in Long Evans Rats								
	0.5 h	1 h	2 h	4 h	8 h	24 h	48 h	72 h	168 h
Adrenal gland(s)	ND	ND	ND[a]	15.0[b]	BLQ	ND	ND	ND	ND
Arterial wall	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bile	ND	ND	ND	ND	ND	ND	ND	ND	ND
Blood	ND	ND	ND	BLQ	BLQ	ND	ND	ND	ND
Bone	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bone marrow	ND	ND	ND	ND	ND	ND	ND	ND	ND
Brain cerebellum	ND	ND	ND	ND	ND	ND	ND	ND	ND
Brain cerebrum	ND	ND	ND	ND	ND	ND	ND	ND	ND
Brain medulla	ND	ND	ND	ND	ND	ND	ND	ND	ND
Brain olfactory lobe	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bulbo-urethral gland	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cecum	ND	ND	13.6	26.3	41.7	BLQ	38.9	ND	ND
Contents, cecum	ND	ND	279	13900	4480	218	668	ND	ND
Contents, esophageal	4220	550	1330	38.9	31.8	ND	ND	ND	ND
Contents, large intestine	ND	NR	ND	13800	17400	1190	642	BLQ	ND
Contents, small intestine	12700	20000	31100	1310	2500	37.5	63.1	BLQ	ND
Contents, stomach	41700	53100	23800	7310	6420	ND	45.9	ND	ND
Diaphragm	ND	ND	ND	BLQ	ND	ND	ND	ND	ND
Epididymis	ND	ND	ND	ND	ND	ND	ND	ND	ND
Esophagus	BLQ	BLQ	36.2	BLQ	BLQ	ND	ND	ND	ND
Exorbital lacrimal gland	ND	ND	ND	ND	ND	ND	ND	ND	ND
Eye lens	ND	ND	ND	ND	ND	ND	ND	ND	ND
Eye uveal tract	ND	ND	19.6	47.2	63	34.5	34.8	34.3	ND
Eye(s)	ND	ND	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	ND
Fat (abdominal)	ND	ND	ND	ND	ND	ND	ND	ND	ND
Fat (brown)	ND	ND	ND	ND	ND	ND	ND	ND	ND
Harderian gland	ND	ND	ND	ND	ND	ND	ND	ND	ND
Intra-orbital lacrimal gland	ND	ND	ND	ND	ND	ND	ND	ND	ND
Kidney cortex	ND	12.4	20.4	35.5	39.4	ND	ND	ND	ND
Kidney medulla	ND	BLQ	BLQ	14.2	11.2	ND	ND	ND	ND
Kidney(s)	ND	10.8	17.3	31.6	31	ND	ND	ND	ND
Large intestine	ND	ND	ND	BLQ	10.6	BLQ	15.2	ND	ND
Liver	31.2	22.7	40.5	77.8	100	21.2	14.1	BLQ	ND
Lung(s)	ND	ND	BLQ	BLQ	11.6	ND	ND	ND	ND
Lymph node(s)	ND	ND	ND	ND	ND	ND	ND	ND	ND
Meninges	ND	ND	ND	ND	ND	ND	ND	ND	ND
Muscle	ND	ND	ND	ND	ND	ND	ND	ND	ND
Myocardium	ND	ND	ND	ND	ND	ND	ND	ND	ND
Nasal turbinates	ND	ND	ND	ND	ND	ND	ND	ND	ND
Pancreas	BLQ	BLQ	BLQ	BLQ	BLQ	ND	ND	ND	ND
Pituitary gland	ND	ND	ND	ND	ND	ND	ND	ND	ND
Preputial gland	ND	ND	ND	ND	BLQ	ND	ND	ND	ND
Prostate gland	ND	ND	ND	ND	ND	ND	ND	ND	ND
Salivary gland(s)	ND	ND	ND	ND	ND	ND	ND	ND	ND
Seminal vesicle(s)	ND	ND	ND	ND	ND	ND	ND	ND	ND
Skin (non-pigmented)	ND	ND	ND	ND	ND	ND	ND	ND	ND
Skin (pigmented)	ND	ND	ND	ND	ND	ND	ND	ND	ND
Small intestine	233	ND[a]	61.5	93.4	29.9	48.3	12.8	ND	ND
Spinal cord	ND	ND	ND	ND	ND	ND	ND	ND	ND
Spleen	ND	ND	BLQ	10.4	ND	ND	ND	ND	ND
Stomach	75.4	32.2	35.4	38.1	BLQ	ND	ND	ND	ND
Testis(es)	ND	ND	ND	ND	ND	ND	ND	ND	ND
Thymus	ND	ND	ND	ND	ND	ND	ND	ND	ND
Thyroid	ND	ND	ND	ND	ND	ND	ND	ND	ND
Urinary bladder	ND	NR	BLQ	ND	ND	ND	ND	ND	ND
Urine	ND	NR	25.4	44.5	ND	ND	ND	ND	ND

Footnotes Table S5

h = Hours

BLQ = Below limit of quantitation (<10.2 ng Eq ^{14}C- tenapanor /g)

ND = Not detectable (sample shape not discernible from background or surrounding tissue).

NR = Not represented (tissue not present in section)

a = Tissue appeared to be fat soaked.

b = Tissue ND due to flare of gastrointestinal contents.

c = ≥1 sample above the upper limit of quantitation (>107000 ng Eq ^{14}C- tenapanor /g).

Table S6 QWBA Data for ^{14}C-tenapanor in Sprague-Dawley rats.

Tissue	ng Eq ^{14}C-Tenapanor/g in Sprague Dawley Rats							
	0.5 h	1 h	2 h	4 h	8 h	24 h	48 h	72 h
Adrenal gland(s)	ND	ND	BLQ	BLQ	BLQ[a]	ND	ND	ND
Arterial wall	ND	ND	ND	ND	ND	ND	ND	ND
Bile	ND	ND	ND	ND	ND	ND	ND	ND
Blood	ND	ND	BLQ	ND	ND	ND	ND	ND
Bone	ND	ND	ND	ND	ND	ND	ND	ND
Bone marrow	ND	ND	ND	ND	ND	ND	ND	ND
Brain cerebellum	ND	ND	ND	ND	ND	ND	ND	ND
Brain cerebrum	ND	ND	ND	ND	ND	ND	ND	ND
Brain medulla	ND	ND	ND	ND	ND	ND	ND	ND
Brain olfactory lobe	ND	ND	ND	ND	ND	ND	ND	ND
Bulbo-urethral gland	ND	ND	ND	ND	ND	ND	ND	ND
Cecum	ND	ND	72.2	48	ND[b]	65.6	BLQ	ND
Contents, cecum	ND	ND	706	6420	9370	1510	26.9	ND
Contents, esophageal	2930	304	141	7350	33.9	17.3	ND	ND
Contents, large intestine	ND	ND	ND	240	25600	3590	51.6	BLQ
Contents, small intestine	24700	22700	21900	32100	1960	769	BLQ	ND
Contents, stomach	49800	43900	62000	98100[c]	401	624	ND	ND
Diaphragm	ND	ND	BLQ	BLQ	BLQ	ND	ND	ND
Epididymis	ND	ND	ND	ND	ND	ND	ND	ND
Esophagus	20.1	17.6	BLQ	ND	BLQ	ND	ND	ND
Exorbital lacrimal gland	ND	ND	ND	ND	ND	ND	ND	ND
Eye lens	ND	ND	ND	ND	ND	ND	ND	ND
Eye uveal tract	ND	ND	ND	ND	ND	ND	ND	ND
Eye(s)	ND	ND	ND	ND	ND	ND	ND	ND
Fat (abdominal)	ND	ND	ND	ND	ND	ND	ND	ND
Fat (brown)	ND	ND	ND	ND	ND	ND	ND	ND
Harderian gland	ND	ND	ND	ND	BLQ	ND	ND	ND
Intra-orbital lacrimal gland	ND	ND	ND	ND	ND	ND	ND	ND
Kidney cortex	ND	BLQ	20.2	34.1	24.7	ND	ND	ND
Kidney medulla	ND	BLQ	19.1	17.9	11	ND	ND	ND
Kidney(s)	ND	BLQ	19.5	29.3	21.2	BLQ	ND	ND
Large intestine	ND	ND	ND	BLQ	ND[b]	12.8	BLQ	ND
Liver	13.3	18.7	36.3	55.1	59.1	31	BLQ	BLQ
Lung(s)	ND	BLQ	BLQ	BLQ	11	ND	ND	ND
Lymph node(s)	ND	ND	ND	ND	ND	ND	ND	ND
Meninges	ND	ND	ND	ND	ND	ND	ND	ND
Muscle	ND	ND	ND	ND	ND	ND	ND	ND
Myocardium	ND	ND	ND	ND	ND	ND	ND	ND
Nasal turbinates	ND	ND	ND	ND	ND	ND	ND	ND
Pancreas	ND	BLQ	BLQ	BLQ	ND	ND	ND	ND
Pituitary gland	ND	ND	ND	ND	ND	ND	ND	ND
Preputial gland	ND	ND	ND	ND	ND	ND	ND	ND
Prostate gland	ND	ND	ND	ND	ND	ND	ND	ND
Salivary gland(s)	ND	ND	ND	ND	ND	ND	ND	ND
Seminal vescicle(s)	ND	ND	ND	ND	ND	ND	ND	ND
Skin (non-pigmented)	ND	ND	ND	ND	ND	ND	ND	ND
Small intestine	18	17.7	20.7	21.8	122	19.4	BLQ	ND
Spinal cord	ND	ND	ND	ND	ND	ND	ND	ND
Spleen	ND	ND	BLQ	10.4	ND	ND	ND	ND
Stomach	53.8	BLQ	25.2	13.6	BLQ	BLQ	ND	ND
Testis(es)	ND	ND	ND	ND	ND	ND	ND	ND
Thymus	ND	ND	ND	ND	ND	ND	ND	ND
Thyroid	ND	ND	ND	ND	ND	ND	ND	ND
Urinary bladder	ND	ND	83	BLQ	BLQ	65.8	ND	ND
Urine	BLQ	ND	28.6	55.4	41.4	22.1	ND	ND

Footnotes for Table S6

h = Hours

BLQ = Below limit of quantitation (<10.2 ng Eq ^{14}C- tenapanor /g)

ND = Not detectable (sample shape not discernible from background or surrounding tissue).

a = Tissue appeared to be fat soaked.

b = Tissue ND due to flare of gastrointestinal contents.

c = ≥1 sample above the upper limit of quantitation (>107000 ng Eq ^{14}C- tenapanor)