ACS Medicinal Chemistry Letters

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Letter

Discovery of Tenapanor: A First-in-Class Minimally Systemic Inhibitor of Intestinal Na^+/H^+ Exchanger Isoform 3

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Cite This: *ACS Med. Chem. Lett.* 2022, 13, 1043−1051

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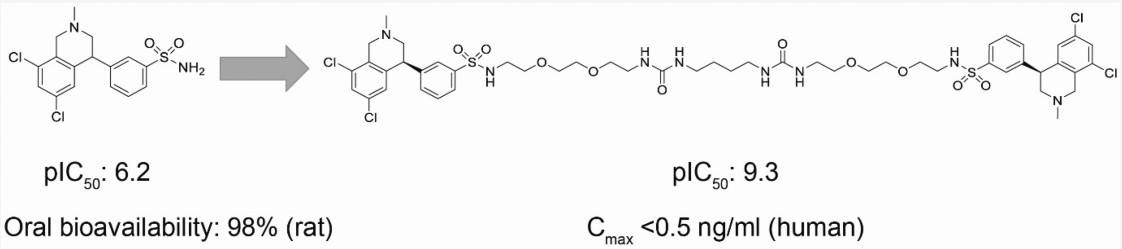


ABSTRACT: We present herein the design, synthesis, and optimization of gut-restricted inhibitors of Na^+/H^+ exchanger isoform 3 (NHE3). NHE3 is predominantly expressed in the kidney and gastrointestinal tract where it acts as the major absorptive sodium transporter. We desired minimally systemic agents that would block sodium absorption in the gastrointestinal tract but avoid exposure in the kidney. Starting with a relatively low-potency highly bioavailable hit compound (**1**), potent and minimally absorbed NHE3 inhibitors were designed, culminating with the discovery of tenapanor (**28**). Tenapanor has been approved by the U.S. Food and Drug Administration (FDA) for the treatment of irritable bowel syndrome with constipation in adults.

KEYWORDS: *Gastrointestinal tract, Intestinal targeting, Nonabsorbed drugs, Sodium−hydrogen exchanger, Tenapanor*

Irritable bowel syndrome with constipation (IBS-C) is a highly prevalent medical condition that reduces quality of life and represents a substantial economic burden on the cost of healthcare.[1] IBS-C is characterized by altered bowel habits with abdominal pain, and its global prevalence is estimated to be anywhere from 5% to 20%.[2] There are multiple proposed theories and mechanisms regarding the pathophysiology of IBS; however, the exact etiology is still unclear.[3] Treatment strategies are diverse and consist of osmotic or stimulant laxatives, motility agents, or secretagogues that act by stimulating intestinal fluid secretion, thereby accelerating gastrointestinal transit.[1]

Our treatment strategy focused on the selective inhibition of the epithelial brush border Na^+/H^+ exchanger isoform 3 (NHE3) within the gastrointestinal (GI) tract. NHE3 functions as part of neutral NaCl absorption in both the intestine and in the renal proximal tubule of the kidney, where it accounts for the majority of the total Na^+ absorbed.[4,5] In animal studies, genetic knockouts confirm that NHE3 is the dominant NHE responsible for transepithelial Na^+ absorption.[6] We reasoned that the pharmacologic inhibition of NHE3 in the gut should result in an increase in the intestinal salt and fluid content, thus promoting GI motility, and would be a viable approach for the treatment of patients with constipation-related disorders. Restricting compound exposure to the GI tract is critical because inhibition in the kidney could result in a dysregulation of the Na^+−water homeostasis and the potential perturbation of blood pressure.[7] Our discovery strategy therefore focused on the design of gut-restricted inhibitors capable of inhibiting NHE3 on the brush border membrane of the GI tract while avoiding systemic exposure and the undesired inhibition of renal NHE3.

Initial work evaluated multiple pharmacophores with reported NHE3 inhibitory activity, including tetrahydroisoquinoline (THIQ) compound classes, as candidates for hit-to-lead optimization.[8−11] Compounds were tested for their NHE3 inhibition activity using a cell deacidification assay, since the inhibition of Na^+/H^+ exchange blunts the passage of protons to the extracellular compartment. To this end, the dose-dependent inhibition of pH recovery in transfected opossum kidney cells overexpressing human (hu) and rat (r) NHE3 was assessed using a pH-sensitive dye. The pharmacokinetics (PK)

Received: January 21, 2022
Accepted: April 7, 2022
Published: April 11, 2022

https://doi.org/10.1021/acsmedchemlett.2c00037
ACS Med. Chem. Lett. 2022, 13, 1043−1051

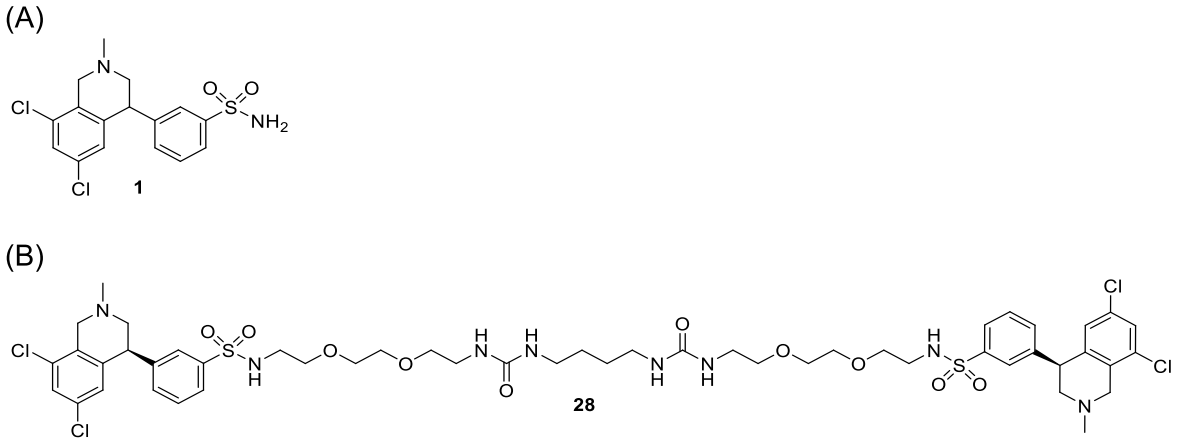
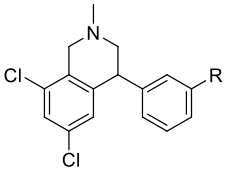
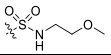
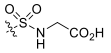
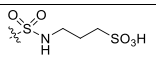
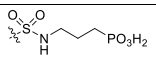
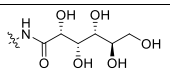
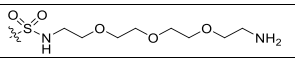
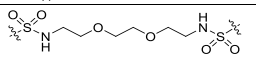


Figure 1. Structures of (A) compound **1** and (B) compound **28** (tenapanor).

Table 1. Structure–Activity Relationship (SAR) Suggesting a Solvent-Exposed Vector on the THIQ Pharmacophore



Compound	*R*	*R versus S*	pIC_{50} (hu, r)	C_{max}/AUC (dose)[a]
1		±	6.2, 6.6	159/522 (11 mpk)
5		±	6.5, 6.7	NT
6		±	7.0, 7.0	NT
7		±	5.8, 7.3	NT
8		±	5.3, 7.2	NT
2		±	7.3, 7.4	NT
3		*R*	5.6, 5.1	NT
4		*S*	7.6, 7.4	6/12 (1.5 mpk)
9		±	6.3, 6.1	30/43 (2.6 mpk)
10		±	5.3, NT	NT

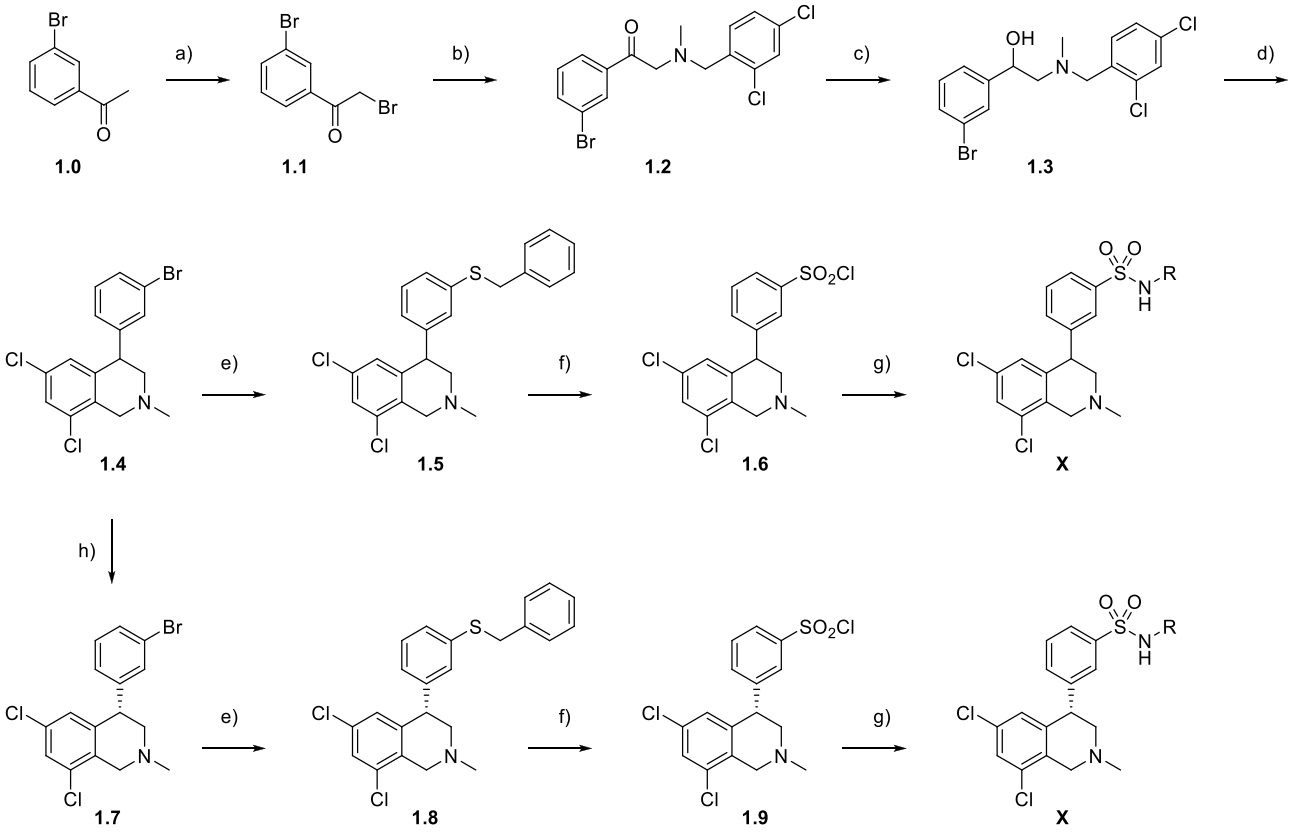
[a]Abbreviations and units used are as follows: C_{max} (ng/mL); AUC (ng·hr/mL) (Sprague–Dawley rats), mpk, milligrams per kilogram; NT, not tested; and pIC_{50} (hu, r), pIC_{50} for the human/rat NHE3 proteins. For experimental details, see the Supporting Information.

of the compounds were also determined. From this initial screen, THIQ compound **1** was selected for further study (Figure 1). While compound **1** possessed only moderate *in vitro* potency, it was essentially equipotent against human and rat NHE3 (pIC_{50} = 6.2 hu and 6.6 r) and had a relatively low molecular weight (371 Da; Table 1). Substituted THIQ compounds such as **1** are also modular and relatively facile to synthesize. Challenges with the development of compound **1** included the absence of a pharmacodynamic (PD) effect in PK studies and high (98%) oral bioavailability in Sprague–Dawley rats, both significant challenges in an optimization effort focused on eliminating systemic exposure while greatly enhancing biological activity. Additional requirements for target compounds in this program also included GI stability and minimal metabolism by GI microflora, which could potentially release bioavailable active metabolites.

The THIQ core found in **1** is conveniently synthesized, as shown in Scheme 1. Commercially available 3-bromoacetophenone **1.0** is α-brominated with bromine in acetic acid to afford bromide **1.1**, which is then used to alkylate 1-(2,4-dichlorophenyl)-*N*-methylmethanamine to afford intermediate **1.2**. The reduction of **1.2** with sodium borohydride in methanol afforded amino alcohol **1.3**, which underwent cyclodehydration via treatment with concentrated sulfuric acid to yield racemic tetrahydroisoquinoline **1.4**. Compound **1.4** could either be used directly for derivatization via electrophilic aromatic substitution or Suzuki-type cross-couplings or converted to amine or carboxylate intermediates using established procedures.[12,13] Thus, intermediate **1.4** was sulfurized with benzyl mercaptan to give thioether **1.5**, and oxidative chlorination with chlorine gas in acetic acid and water yielded sulfonyl chloride **1.6**. Sulfonamide derivatives of

https://doi.org/10.1021/acsmedchemlett.2c00037
ACS Med. Chem. Lett. 2022, 13, 1043–1051

Scheme 1. Synthesis of THIQ Sulfonyl Chloride[a]



[a]The following reagents and conditions were used: (a) Br_2 and AcOH at 60 °C; (b) 1-(2,4-dichlorophenyl)-*N*-methylmethanamine, dioxane, and Et_3N at 25 °C; (c) $NaBH_4$ and MeOH at 0°C; (d) H_2SO_4 and CH_2Cl_2 from 0 °C to room temperature; (e) benzylthiol, K_2CO_3, $Pd_2(dba)_3$, and Xantphos at 140 °C; (f) *N*-chlorosuccinimide, AcOH, and H_2O at room temperature; (g) RNH_2, TEA, or DIPEA or an equivalent solvent; and (h) D-(+)-DBTA and $EtOH/H_2O$.

sulfonyl chloride **1.6** were prepared from amines using either Schotten−Baumann conditions or organic bases to afford all compounds described herein, with the exception of the amides (**2**, **3**, and **4**). These amide derivatives were synthesized from intermediates prepared following the procedures described in Scheme 1; however, bromophenyl **1.1** was replaced with the corresponding protected amine to afford the aniline version of intermediate **1.4**. Acetylation with activated carboxylic acids afforded the desired amides (experimental details are provided in the Supporting Information).

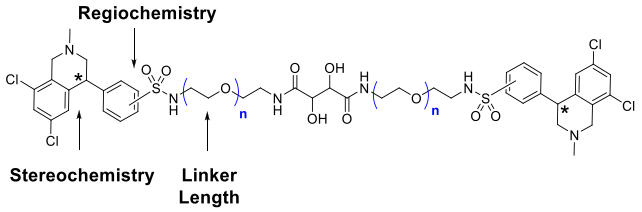
Hit optimization activities were initially focused on probing regions on compound **1** tolerant to chemical modification and determining where the activity could be improved. This work also aimed to locate regions that could tolerate the addition of functional groups predicted to reduce systemic availability. For example, the identification of solvent-exposed regions on the pharmacophore would allow the overall physical properties of the compound to be modulated by increasing polarity, increasing the number of hydrogen bond donors or acceptors, and increasing rotatable bonds, all functionality traditionally thought to hinder oral availability.[14]

In our initial studies, derivatives of **1** were prepared using functionalities to increase the polarity (neutral hydrophilic, polar ionic, and constitutively charged), and the spacing between the THIQ core and the terminal functionality was systematically varied in an effort to determine a region of solvent exposure. As shown in Table 1, the *meta* (*m*) position of the THIQ core tolerated a diverse range of functionalities. Neutral hydrophilic (**5**) and ionic groups (**6**) were accommodated with minimal impact on the *in vitro* potency, as were functional groups constitutively charged at physiological pH (sulfonate (**7**) and phosphonate (**8**)). The carbohydrate derivative (**2**) was particularly potent (pIC_{50} = 7.3 hu and 7.2 r), suggesting that this region of the compound was not simply solvent exposed but also capable of forming productive binding interactions with NHE3.

To this point, all compounds made were prepared as racemic mixtures. Compound **2** was resolved via chiral supercritical fluid chromatography (SFC) to afford **3** and **4**, which were later assigned *R*- and *S*-configurations, respectively. While **3** possessed weak *in vitro* activity (pIC_{50} = 5.6 hu and 5.1 r), **4** was potent (pIC_{50} 7.6 hu and 7.4 r), had a promising (low) exposure in rat PK studies (maximum concentration (C_{max}) of 6 ng/mL and area under the curve (AUC) 12 ng·hr/mL at 1.5 mg/kg), and was active (10 mg/kg dose) in a rat PD assay, consistent with luminal NHE3 inhibition (Supplementary Figure S1). The PD assay at this point in the program consisted of an analysis of the intestinal sodium content within the lumen of the cecum or the colon of sacrificed rats (see Supporting Information); changes in urinary sodium were not yet detected. The finding of *in vitro* potency $pIC_{50} > 7$, reduced systemic exposure, and a PD response in a relevant animal model satisfied our "hit" criteria.

https://doi.org/10.1021/acsmedchemlett.2c00037
ACS Med. Chem. Lett. 2022, 13, 1043−1051

Table 2. Effects of Linker Length, Regiochemistry, And Stereochemistry on SAR of THIQ Dimers[a]



compound	*R versus S*	*m/p*	*n*	pIC_{50} (hu, r)	C_{max}/AUC (dose)[b]	PD (3 mpk) fecal form[c]	PD (3 mpk) fecal water[d]	PD (3 mpk) urine Na^{+}[e]
11	±, ±	*m*	3	7.1, 7.6	6/6 (9.4 mpk)	2	56%	24%
12	±, ±	*m*	2	7.2, 6.9	<1/<4 (9 mpk)	1.5	53%	33%
13	±, ±	*m*	1	6.5, 5.8	NT	NT	NT	NT
14	±, ±	*m*	0	6.0, 6.3	<4/<10 (11 mpk)	NT	NT	NT
15	±, ±	*p*	3	6.8, 7.2	<5/<19 (10 mpk)	3	61%	16%
16	±, ±	*p*	2	8.4, 7.0	NT	2.5	59%	11%
17	*S, S*	*m*	3	8.9 (r)	NT	NT	NT	NT
18	*S, R*	*m*	3	6.7 (r)	NT	NT	NT	NT
19	*R, R*	*m*	3	<4.5 (r)	NT	NT	NT	NT

[a]Abbreviations used are as follows: *m, meta*; mpk, milligrams per kilogram; NT, not tested; *p, para*; and pIC_{50} (hu, r), pIC_{50} for the human/rat NHE3 proteins. For experimental details, see the Supporting Information. [b]C_{max} (ng/mL) and AUC (ng·hr/mL). [c]Stool form scale (1 = "hard pellet" and 5 = "watery"). [d]Percent water by weight. [e]Percent control.

Insights from **5**, a simple ether analog with a modestly increased potency, suggested that the oxygens in **2** and **4** may serve as H-bond acceptors rather than donors. Therefore, short polyethylene glycols (PEGs) were considered to determine whether activity similar to that of the carbohydrate derivatives could be maintained without the added stereochemistry of a polyol. Thus, **9** was prepared, and it had both a reasonable pIC_{50} (6.3 hu and 6.1 r) and a similarly low bioavailability (C_{max} = 30 ng/mL and AUC = 43 ng·hr/mL at 2.6 mg/kg; Table 1). The aminopolyethylene glycol of **9** was also a convenient vector for exploring the limits of chemical space in this region of the molecule. Although unconventional, dimerization via the aminopolyethylene glycol was investigated to determine the maximum reduction in systemic exposure that could result from such analogs, which have a relatively high molecular weight, possess a significant number of H-bond acceptors, have a high topological polar surface area, and possess a large number of rotatable bonds. The first dimer, **10**, was prepared by directly sulfonylating **9** with sulfonyl chloride **1.6** and was essentially inactive (pIC_{50} < 5.3 hu; Table 1). The additional THIQ moiety was likely making unproductive contact with NHE3, so significantly longer dimers were designed. Tartaric acid was used as a "core" to dimerize two **9** molecules; **9** was chosen because its polyol backbone bore an architecture similar to that of **2** and **4**. The resulting compound, **11**, represented a breakthrough in the program (Table 2). While *in vitro* potency (pIC_{50} = 7.1 hu and 7.6 r) was similar to that of **4** (with a pure enantiomer of **11** expected to be more potent still), systemic exposure was significantly attenuated (C_{max} = 6 ng/mL and AUC = 6 ng·hr/mL at 9.4 mg/kg). Critically, a robust PD response was observed in rats (10 mg/kg dose) in the form of a pronounced increase (56%) in stool water (Table 2). This observation is consistent with the phenotype of NHE3-null mice[15] and the activity of another intestinal NHE3 inhibitor[16] published after our initial reports.[17−20] Since wet feces made accurate stool collection in a metabolic cage impractical, the analysis of urinary Na^{+} became a primary PD biomarker, since blocking Na^{+} absorption in the GI tract would result in the reduced fractional excretion of Na^{+} in the urine (see the Supporting Information). Compound **11** robustly reduced the excretion of Na^{+} in the urine in a dose-dependent manner (Figure 2),

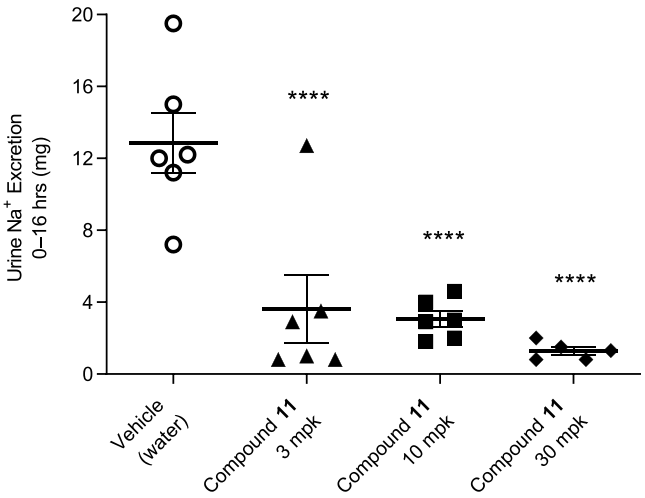
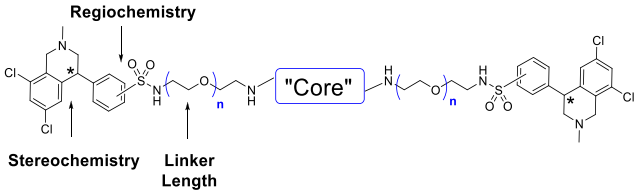


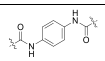
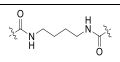
Figure 2. Activity of the THIQ dimer **11** in an *in vivo* pharmacodynamic urinary sodium excretion assay in rats. ****p < 0.0001 versus the vehicle. Data shown are the mean (±SEM) reduction in urine sodium (mg) 16 h after the oral administration of compound **11** at 3, 10, and 30 mg/kg (n = 6 per group). A one-way ANOVA test with Dunnett's multiple comparison test was used to determine the statistical significance.

consistent with blockade of intestinal NHE3. In polarized Madin−Darby canine kidney (MDCK) cells, the bidirectional apparent permeability (P_{app}) of **11** was <0.2 × 10^{-6} cm/s, indicating poor absorption across the epithelium. In contrast, the P_{app} of **1** was ≥10 × 10^{-6} cm/s, indicating extensive absorption. These results were consistent with the plasma C_{max} values of these compounds after oral administration in rats (Tables 1 and 2). The combination of *in vitro* potency,

https://doi.org/10.1021/acsmedchemlett.2c00037
ACS Med. Chem. Lett. 2022, 13, 1043−1051

Table 3. SARs of THIQ Dimers with Different Core Regions[e]



Compound	*R versus S*	*m/p*	n	Core	pIC_{50} (hu, r)	C_{max}/AUC[a] (dose)	PD (3 mpk)		
							Fecal Form[b]	Fecal Water[c]	Urine Na^{+}[d]
36	+/-	*m*	3		7.3, 7.6	<4/<12 (7.2 mpk)	1	53%	87%
20	+/-	*m*	2		<5.5, 5.2	<2/<8 (22 mpk)	2	58%	24%
37	+/-	*p*	3		7.4, 7.9	<3/<9 (19 mpk)	1.5	56%	50%
21	+/-	*p*	2		<5.5, 6.4	<3/<8 (23 mpk)	2	56%	45%
38	+/-	*m*	3		6.7, 7.0	<20/<75 (1.8 mpk)	1	50%	87%
22	+/-	*m*	2		5.7, <5.0	<5/<19 (11 mpk)	1	50%	100%
35	+/-	*p*	3		7.2, 7.1	<5/<19 (5.3 mpk)	3	60%	25%
23	+/-	*p*	2		<5.5, <5.0	<2/<8 (17 mpk)	1	49%	107%
39	+/-	*m*	3		NT, 5.2	NT	2	54%	42%
27	+/-	*m*	2		NT, 8.1	NT	3	60%	32%
40	+/-	*p*	3		NT, NT	NT	NT	NT	NT
41	+/-	*p*	2		NT, NT	NT	3	58%	29%
25	+/-	*m*	3		7.4, 7.5	NT	3	57%	50%
29	+/-	*m*	2		NT, 8.5	NT	4.5	64%	22%
42	+/-	*p*	3		NT, NT	NT	NT	NT	NT
43	+/-	*p*	2		NT, NT	NT	3	60%	14%
44	+/-	*m*	3		NT, NT	<2/<8 (28 mpk)	1.5	54%	56%
33	+/-	*p*	2		7.2, 7.4	NT	3.5	60%	23%
45	+/-	*m*	3		NT, NT	NT	NT	NT	NT
31	+/-	*p*	2		7.5, 7.4	NT	2.5	59%	29%
46	+/-	*p*	2		NT, NT	NT	3	57%	45%

[a] C_{max} (ng/mL) and AUC (ng·hr/mL). [b] Stool form scale (1 = "normal" and 5 = "watery"). [c] Percent water by weight. [d] Percent control. [e] Abbreviations used are as follows: *m, meta*; mpk, milligrams per kilogram; NT, not tested; *p, para*; and pIC_{50} (hu, r), pIC_{50} for human/rat NHE3. For experimental details, see the Supporting Information.

favorable (minimally systemic) PK properties, and a robust PD dose−response satisfied our "lead" criteria, and the optimization of **11** became a focus of the program.

The initial optimization of **11** explored the refinement of the linker length and the regiochemistry of the sulfonamide (*meta versus para*; Table 2). A methodology had not been established for preparing sulfonyl chloride intermediate **1.6** (Scheme 1) in an enantiomerically pure form (intermediate **1.9**; Scheme 1), so compounds were prepared as diastereomeric mixtures. While the tartaric acid core was maintained, *meta*-sulfonamide analogs were prepared with PEG lengths of 3 (**11**), 2 (**12**), 1 (**13**), and 0 (1,2-diaminoethane; **14**) ethylene glycol units, which revealed minimal spacing requirements. Compounds **11** (PEG-3) and **12** (PEG-2) were essentially equipotent both *in vitro* (pIC_{50} = 6.9−7.6 r) and in the PD response (urinary Na^{+} excretion reduced to 24−33% that of the vehicle controls; 3 mg/kg), while **13** (PEG-1) and **14** (PEG-0) were approximately 1−1.5 orders of magnitude less potent *in vitro* (Table 2). Analysis of the *para*-sulfonamide regiochemistry (**15** (PEG-3) and **16** (PEG-2)) suggested that the PEG-2 length was preferred in this framework (**16**, pIC_{50} = 8.4 hu and 7.0 r; urinary Na^{+} was 11% that of the control). All tested compounds in this series had very low exposure in rats (C_{max} < 10 ng/mL and AUC < 20 ng·hr/mL at 10 mg/kg). Note that, due to the robust PD response of **11**, the screening dose for PD studies was reduced to 3 mg/kg, while the PK dose was maintained at 10 mg/kg, with plasma concentrations approaching the detection limits (typically 0.5−5 ng/mL) of our routine PK screening methodology.

Stereochemical preferences within the chemotype **11** were then reconfirmed (Table 2). PEG intermediate **9** was purified by chiral SFC into what were later assigned as the *R*- and *S*-enantiomers via single-crystal X-ray analysis of the D-(+)-dibenzoyltartaric acid (DBTA) salt of intermediate **1.7** (see the Supporting Information). These enantiomers were used to prepare diastereomers **17** (*S,S*), **18** (*S,R*), and **19** (*R,R*). Note that **17** and **18** were prepared using L-(+)-tartaric acid, while **19** was prepared from racemic tartaric acid. The *S*-configuration was strongly preferred, with nearly two orders of magnitude separating the *in vitro* potency of **17** (*S,S*; pIC_{50} = 8.9 r) from that of **18** (*S,R*; pIC_{50} = 6.7 r), which had an activity similar to that of monomer **9** (Table 2). Compound **19** was inactive (*R,R*; pIC_{50} < 5.0 r). Modulating the stereochemistry in this fashion preserved the identical molecular mass and nearly identical functionality but changed the bioactive conformation of the THIQ pharmacophore. This had a dramatic impact on the *in vitro* potency, suggesting a synergistic binding mode for the dimeric pharmacophore. Although the chemical series exemplified by **11** satisfied most of our lead criteria, the recovery of the intact parent compound from rat fecal samples was variable (57−97% within 16 h at 10 mg/kg) and hydrolysis products were observed (especially at the tartrate "core"). The hydrolytic instability of the amide at this position was also observed during some synthetic conditions, perhaps due to anchimeric assistance of the vicinal hydroxyl groups. As these fragments presented a risk of systemic exposure and the possible inhibition of renal NHE3, further optimization was performed within the linker and core region.

https://doi.org/10.1021/acsmedchemlett.2c00037
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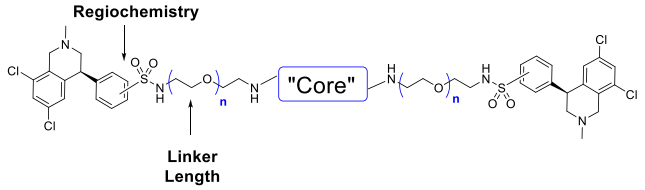
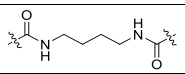
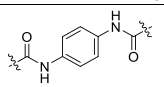
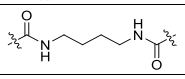
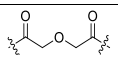
Table 4. Summary of Activity for the Six Preferred THIQ Dimers and the Final Assessment Criteria[c]



Compound	*m/p*	n	"Core"	Stool water dose-response	Increase in stool water	Stool recovery[a]	pIC_{50} (hu, r)	C_{max} at 30 mpk[b]
Assessment criteria	—	—	—	Yes	10% increase from control	>70%	-	low
24	*m*	3		No	No	96 ± 14% No metabolites detected	8.7, 8.2	<15 ng/mL No metabolites detected
26	*m*	2		Yes	Yes	93 ± 16% No metabolites detected	8.8, 8.6	<2 ng/mL No metabolites detected
28	*m*	2		Yes	Yes	92 ± 2% 2 metabolites detected	9.3, 7.8	<3 ng/mL No metabolites detected
30	*p*	2		Yes	Yes	117 ± 6% No metabolites detected	8.1, 8.0	<2 ng/mL No metabolites detected
32	*p*	2		Yes	Yes	46 ± 8% No metabolites detected	8.8, 8.0	<2 ng/mL No metabolites detected
34	*p*	3		Yes	Yes	82 ± 20% 3 metabolites detected	8.1, 7.9	<5 ng/mL No metabolites detected

[a]Data are the mean ± standard deviation (n = 30, 10 replicates from three rats for **26**, **28**, and **32**; n = 9, three replicates from three rats for **24**, **30**, and **34**. Stool recovery could be greater than 100% due to sample inhomogeneity. [b]C_{max} (ng/mL). [c]Abbreviations used are as follows: *m, meta;* mpk, milligrams per kilogram; *p, para*; and pIC_{50} (hu, r), pIC_{50} for the human/rat NHE3 proteins. For experimental details, see the Supporting Information.

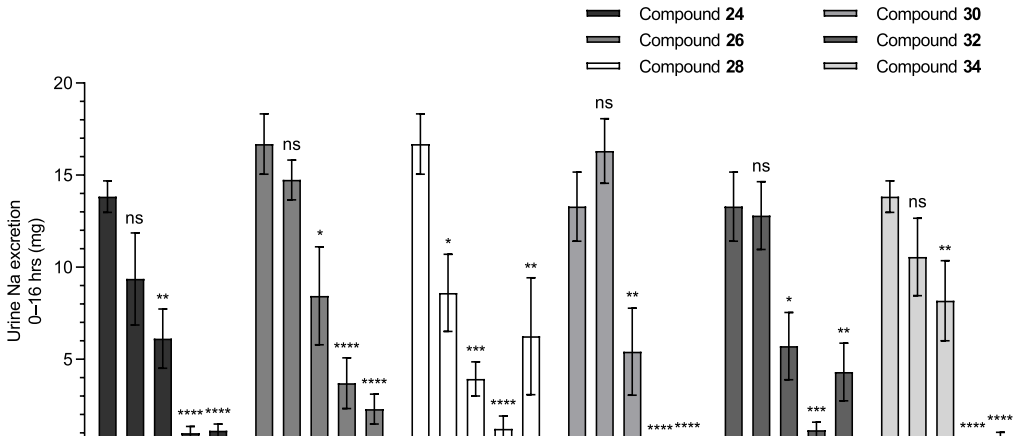


Figure 3. Dose response of urinary sodium excretion for the six THIQ dimer NHE3 inhibitor compounds in rats. Data shown are the mean (±SEM) reduction in urine sodium (mg) 16 h after the oral administration of each compound at 0.1, 0.3, 1.0, and 3.0 mg/kg (n = 6 per group). A one-way ANOVA test with Dunnett's multiple comparison test was used to determine the statistical significance (*P < 0.05; **P < 0.01; ***P < 0.001; and ****P < 0.0001), and ns stands for not significant.

The pharmacophore was optimized by systematically probing combinations of the sulfonamide regiochemistry, the linker length, and the central core. Variations of the sulfonamide regiochemistry were *meta versus para*; the *para*-sulfonyl chloride analog of compound **1.6** was analogously prepared from 4-bromoacetophenone following the procedures in Scheme 1. The linker length was PEG-2 *versus* PEG-3, and the central core was amide *versus* urea connection chemistry; aliphatic, aromatic, ether, and amine cores are shown in Table 3. Each compound was prepared as a mixture of diastereomers. Compound evaluation included *in vitro* potency, exposure (primarily C_{max} since AUC was typically not calculable due to insufficient quantifiable plasma concentrations), PD effect (percent reduction in urinary Na^+), fecal excretion (percent of the dose recovered as the intact parent compound), and pH/solubility profile. Solubility was typically high in simulated

https://doi.org/10.1021/acsmedchemlett.2c00037
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gastric fluid (>5 mg/mL; data not shown), consistent with the presence of two tertiary amines in each analog. When measured, systemic exposure throughout the series was very low, even when the dose was increased to 20 mg/kg (e.g., compound **20**, C_{max} < 2 ng/mL at a 22 mg/kg dose, and compound **21**, C_{max} < 3 ng/mL at a 23 mg/kg dose). The *in vitro* potency (pIC_{50}) of these diastereomeric mixtures was generally >7, which was expected to improve to ∼8 when the compounds were prepared in the stereochemically pure form. Analogs with a shorter PEG length and a smaller core (**22** and **23**) had poor *in vitro* activity ($pIC_{50} \leq 5.7$), which was consistent with prior SARs showing the dependence of the optimal activity on the appropriate spacing between the two THIQ pharmacophores.

Six compounds that appeared to satisfy preliminary development candidate criteria were prepared in the stereochemically pure form, either by chiral SFC or via intermediate **1.9**, and carefully evaluated (Table 4). These included compounds **24** (*S,S*-diastereomer of **25**), **26** (*S,S*-diastereomer of **27**), **28** (*S,S*-diastereomer of **29**), **30** (*S,S*-diastereomer of **31**), **32** (*S,S*-diastereomer of **33**), and **34** (*S,S*-diastereomer of **35**). These compounds also represented a cross-section of sulfonamide regiochemistry, linker length, and core motifs. In addition to established *in vitro* and *in vivo* tests, compound evaluation activities were expanded to include the PD dose−response (urine Na^+ at 0.1−3.0 mg/kg), the value of C_{max} at a high dose (30 mg/kg), the increase in fecal water content (≥10% increase over control animals), fecal recovery of the intact parent (≥70%), human ether-à-go-go-related gene (hERG) inhibition (<50% at 10 μM), and Ames (negative).

Both hERG inhibition and Ames were negative for all six compounds. As shown in Table 4, the stool water content increased by >10% for all compounds except **24**. Fecal recovery of the intact compound was >70% the administered dose for all analogs except **32** (46% ± 8%). pIC_{50} values were quite potent for these pure diastereomers (pIC_{50} = 8.1−9.3 hu and 7.9−8.2 r), and exposure (C_{max}(rat) = 30 mg/kg) was below the limit of detection for all compounds (C_{max} < 5 ng/mL for most analogs). All compounds had a robust PD dose response, and the activity was clearly titratable (Figure 3). In some instances, there appeared to be an increase in the fractional excretion of Na^+ in urine at higher doses of compound **32** and **28** at 3.0 mg/kg, but this is likely due to the incidental diversion of sodium-rich loose stools into the urinary collection tube in the metabolic cages at high compound doses. Compound **24** did not demonstrate a satisfactory pharmacodynamic dose−response effect on stool water, while **32** showed unsatisfactory GI stability (fecal recovery of 46% ± 8%). Compounds **30** and **34** had comparatively lower *in vitro* activity against human NHE3 (pIC_{50} = 8.1) compared to the other analogs. The totality of data supported compounds **28** and **26** as potential development candidates.

To further characterize these candidates, a seven day repeat-dose exploratory (i.e., non-good laboratory practice (non-GLP)) rat toxicity study was carried out. Gross and microscopic pathologies were within normal limits for all compounds, and no biologically significant abnormal clinical pathology was observed (i.e., alanine aminotransferase/aspartate aminotransferase >3× the upper limit of normal; data not shown, see the Supporting Information). Dehydration was observed due to the exaggerated PD response (i.e., diarrhea) at the studied dose (30 mg/kg). Compound **26** was ultimately rejected because an examination of its inhibition in the cell assay showed it never reached 100% (data not shown) and the potential risk of reactive quinone imine formation due to its diaminobenzene core should a fragment become systemic.[21] Key compound **28** (tenapanor) satisfied all development candidate criteria and was advanced into clinical development.

In clinical trials, tenapanor (**28**) softened stool consistency in a dose-dependent manner and increased stool frequency and weight *versus* the placebo.[22] Importantly, tenapanor plasma concentrations were below the quantification limit (0.5 ng/mL) in >95% of all plasma samples analyzed, demonstrating the successful achievement of the goal of minimal systemic absorption in humans. Tenapanor (**28**) was approved for the treatment of adult patients with IBS-C by the U.S. Food and Drug Administration (FDA) in September of 2019.[22−25] During clinical development, it was also observed that tenapanor (**28**) lowered serum phosphorus levels in dialysis patients with hyperphosphatemia, a major comorbidity in this patient population, by preventing the intestinal absorption of phosphate.[22] In mechanistic studies, the on-target inhibition of NHE3 causes a decrease in the intracellular pH (pH_i) of intestinal epithelial cells. This decrease in pH_i is thought to modulate the tight junctions between the epithelial cells, resulting in an increase in the transepithelial electrical resistance that restricts the paracellular permeability of phosphate, the major pathway for intestinal phosphate absorption.[26,27] The primary and most secondary end points were met in three pivotal clinical trials of tenapanor for the treatment of hyperphosphatemia in adult patients with chronic kidney disease (CKD) on dialysis.[28,29]

In summary, tenapanor (**28**) is a novel, potent, and minimally absorbed inhibitor of intestinal NHE3 that functions in the gastrointestinal tract to block the absorption of dietary sodium and phosphate. Systematic optimization of the regioisomer and spatial relationships within this series resulted in a potent and minimally absorbed chemotype suitable for clinical development. Data from multiple completed clinical trials led to tenapanor being approved by the U.S. FDA for the treatment of adult patients with IBS-C.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.2c00037.

Water content from the cecum and the colon; experimental procedures, including materials and methods; and chemical characterization of compounds (PDF)

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https://doi.org/10.1021/acsmedchemlett.2c00037
ACS Med. Chem. Lett. 2022, 13, 1043−1051

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Author Contributions

The manuscript was written by J.W.J. with assistance from Oxford Pharmagenesis and Ashfield MedComms, an Ashfield Health company. Chemistry and compound design was contributed by M.R.L., N.B., and J.W.J. *In vitro* assay development and execution were performed by C.W.C. and S.K.M. *In vivo* study design and execution were performed by M.N., E.D.L., J.K., K.K., L.H., A.G.S., D.C., and J.W.J. M.R.L., C.W.C., K.K., E.D.L., M.N., A.G.S., D.C., and J.W.J. analyzed or interpreted data. D.C. and J.W.J. conceived of and initiated the project. All authors contributed to manuscript review and revision, approved the final draft for submission, and are accountable for accuracy and integrity of any part of the work.

Funding

This work was funded by Ardelyx, Inc.

Notes

The authors declare the following competing financial interest(s): J.W.J., M.R.L., L.H., K.K., M.N., and A.G.S. were employed by and own stock in Ardelyx, Inc. N.B., S.K.M., C.W.C., J.K., E.D.L., and D.C. were employed by Ardelyx, Inc.

■ ACKNOWLEDGMENTS

The authors thank Pharmaron, Beijing, China, for assistance with compound preparation.

■ ABBREVIATIONS

ANOVA, analysis of variance; AUC, area under the curve; CKD, chronic kidney disease; C_{max}, maximum concentration; DBTA, dibenzoyltartaric acid; FDA, U.S. Food and Drug Administration; GI, gastrointestinal; hERG, human ether-à-go-go-related gene; hu, human; IBS-C, irritable bowel syndrome with constipation; *m*, meta; MDCK, Madin−Darby canine kidney; mpk, milligrams per kilogram; NHE3, Na+/H+ exchanger isoform 3; non-GLP, non-good laboratory practice; NT, not tested; *p*, para; Papp, apparent permeability; PD, pharmacodynamic; PEG, polyethylene glycol; pHi, intracellular pH; PK, pharmacokinetic; r, rat; SAR, structure−activity relationship; SEM, standard error of the mean; SFC, supercritical fluid chromatography; THIQ, tetrahydroisoquinoline

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ACS Med. Chem. Lett. 2022, 13, 1043−1051